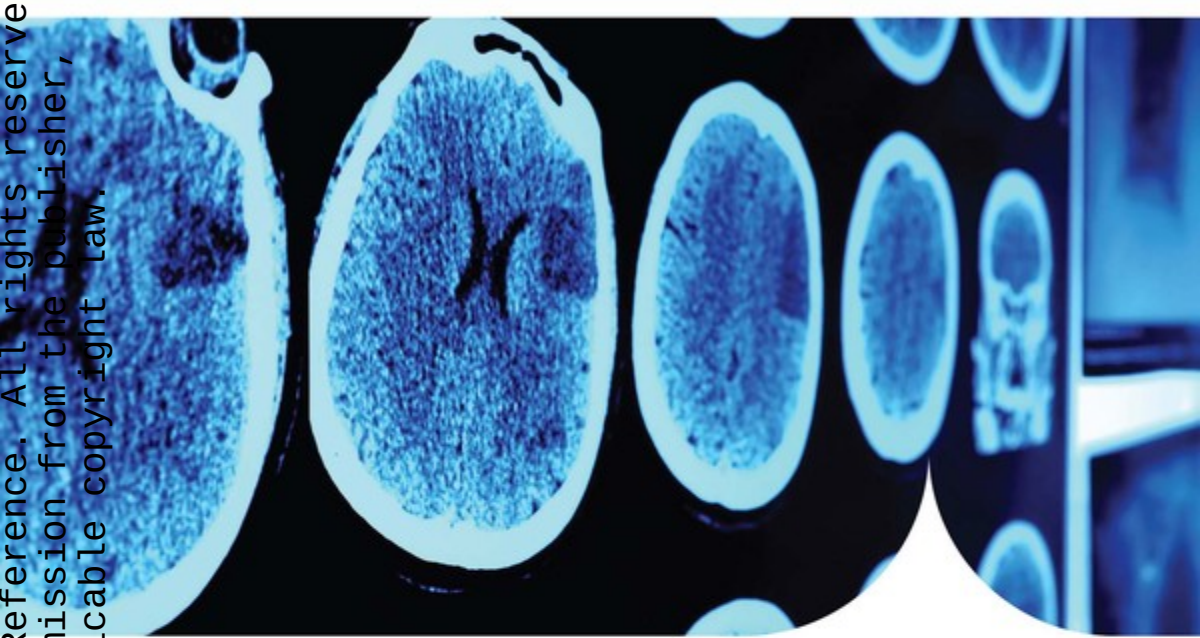

# Emerging Developments and Practices in Oncology

Issam El Naqa

# Emerging Developments and Practices in Oncology

Issam El Naqa
*University of Michigan, USA*

A volume in the Advances in Medical Diagnosis, Treatment, and Care (AMDTC) Book Series

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# Detailed Table of Contents

systems. In this chapter are explored potential applications of these technologies in the cytopathology laboratory including: data storage, laboratory information systems, population screening programs, quality control and assurance, education and proficiency testing, e-learning, tele-consultation, primary diagnosis and research. The impact of their adoption on the daily workflow is highlighted, possible shortcomings especially for security and privacy issues are identified and future research directions are presented.



A major challenge in the latest computer-aided detection (CADe) of polyps in CT colonography (CTC) is to improve the false positive (FP) rate while maintaining detection sensitivity. Radiologists prefer CADe system produce small number of false positive detections, otherwise they might not consider CADe system improve their workflow. Towards this end, in this study, we applied a nonlinear regression model operating on CTC image voxels directly and a nonlinear classification model with extracted image features based on support vector machines (SVMs) in order to improve the specificity of CADe of polyps. We investigated the feasibility of a support vector regression (SVR) in the massive-training framework, and we developed a massive-training SVR (MTSVR) in order to reduce the long training time associated with the massive-training artificial neural network (MTANN) for reduction of FPs in CADe of polyps in CTC. In addition, we proposed a feature selection method directly coupled with an SVM classifier to maximize the CADe system performance. We compared the proposed feature selection method with the conventional stepwise feature selection based on Wilks' lambda with a linear discriminant analysis classifier. The FP reduction system based on the proposed feature selection method was able to achieve a 96.0% by-polyp sensitivity with an FP rate of 4.1 per patient. The performance is better than that of the stepwise feature selection based on Wilks' lambda (which yielded the same sensitivity with 18.0 FPs/patient). To test the performance of the proposed MTSVR, we compared it with the original MTANN in the distinction between actual polyps and various types of FPs in terms of the training time reduction and FP reduction performance. The CTC database used in this study consisted of 240 CTC datasets obtained from 120 patients in the supine and prone positions. With MTSVR, we reduced the training time by a factor of 190, while achieving a performance (by-polyp sensitivity of 94.7% with 2.5 FPs/patient) comparable to that of the original MTANN (which has the same sensitivity with 2.6 FPs/patient).

# Preface

Cancer remains a leading cause of death worldwide in both men and women, with about a yearly 14 million new cases and 8 million cancer related deaths (15% of all deaths) according to the latest global statistics by the World Health Organization (WHO, 2014). Cancer affects people at all ages and many of them will die of cancer with dire socioeconomic consequences on patients, their relatives and the healthcare system. Cancer is a diverse family of diseases (about 100 known cancers) that is characterized by an abnormal growth of cells with acquired ability of uncontrolled progression and invasion of surrounding tissues disrupting their vital functions and leading to patient death (Hanahan & Weinberg, 2011). Luckily, the number of cancer survivors has more than doubled in recent years compared to decades ago, thanks mainly to advances in early detection and personalized treatment of the disease, however, much still needs to be done to mitigate its lethal effects (American Cancer Society, 2016).

Patients diagnosed with cancer have multiple treatment options to choose from depending on their disease type and its status. Typically, patients diagnosed with localized and early stage disease would receive surgery, benefit from watchful waiting or active surveillance as in prostate cancer, or the use of medication for management purpose, rather than curative purpose, as in certain types of leukemia. Therefore, there is strong emphasis in oncology on early detection and diagnosis using the latest advances in medical imaging and biotechnology hardware and software tools. However, patients at more advanced stages of cancer disease would receive chemoradiotherapy, molecular targeted therapy, and more recently immunotherapy, or a combination of these treatment modalities. In particular, immunotherapy by checkpoint blockade and in combination with traditional treatments have emerged as a promising therapeutic alternative for cases where the tumors have spread to other organs providing new hopes for patients otherwise were considered terminal in the recent past (Zou et al., 2016). Moreover, many current and new technologies are being repurposed to aid in combating cancer (Pantziarka et al., 2014).

Recent years have witnessed tremendous advancement in technologies for aiding in the detection, diagnosis, and treatment of cancer that ranges from new hard detectors, nanotechnologies, to new imaging and biotechnology techniques. Covering any of these topics would be worthy of a separate volume by itself. Therefore, in this monograph we focus on two main areas: imaging, bioinformatics, and their interlinks. In the era of evidence-based and precision medicine there has been specific interest in improving the two main frontiers of cancer care: early detection and personalized treatment. New cancer detection tools continue to be developed with the purpose to avoid the overwhelming socioeconomic burdens associated with cancer disease detection and management. Previous cancer treatment strategies have been limited to population based type regimens according to their staging information with mixed results. However, with the exponential growth in patient-specific information treatment personalization has become more feasible than ever allowing each patient to receive the kind of care that is tailored best to their own needs (Schilsky, 2014). In this monograph, the contributors touch on many these issues by presenting current and new technologies. For instance, early cancer detection and diagnosis using breast cancer as a case study with its known challenges is presented in Chapter 1. More recent biotechnology advances using epigenetic signatures are subsequently discussed in Chapter 2 as new promising biomarker and possibly a target for therapeutic intervention. The tremendous growth of patient specific-information and the emergence of so called big-data era (El Naqa, 2016) requires capabilities to integrate clinical and laboratory information as presented in Chapter 3. The big data promise potential is examined in more details in Chapter 4 in the context of radiotherapy escalated regimen known as stereotactic body radiotherapy (SBRT), which is currently considered the front runner in radiation treatments due to shortened times and more effective outcomes. However, generating and sharing large amount of data require the use of more advanced information technologies as presented by the cloud and mobile computing technologies highlighted in Chapter 5. The application of bioinformatics in the era of big data is driven by advances in data analytics and in particular machine learning algorithms in oncology (El Naqa et al., 2015) as demonstrated in recent computer-aided detection of polyps in computed tomography colonography of Chapter 6. Quantitative imaging techniques is a new -omics area of research that aims to make better use of the wealth information embedded in medical images beyond current subjective radiological assessments. Analogous to genomics in bioinformatics, this field of quantitative imaging is termed radiomics (Aerts et al., 2014; Avanzo et al., 2017) and is presented in Chapter 7. Concluding with this imaging theme, new advances in imaging of cancer treatments side effects including endomicroscopy techniques are presented in Chapter 8. Endomicroscopy is highlighted as a new promising tool to enable tracking of stem cell therapy to mitigate side effects in radiotherapy.

## TARGET AUDIENCE OF THE BOOK

The monograph presents emerging and recent advances in oncology aiming to improve early detection and personalized treatment of cancer. These advancements span many diverse areas that go beyond the scope of such text. Therefore, the focus has been on two key areas: medical imaging and bioinformatics. The target audience of this book will be composed of trainees, professionals and researchers working in the field of oncology, imaging, and bioinformatics from various disciplines. It is expected that the presented material would appeal to trainees in the oncology field (residents) or in the fields of imaging and computer sciences (graduate students) whom would be interested to learn about the latest trends and applications of imaging and bioinformatics technologies to cancer. The topics presented in the book are expected to be of interest also to active practitioners and researchers in these fields who are seeking to apply their domain knowledge to oncology or for oncology practitioners and clinician scientists whom are interested in the latest trends and developments in oncology as it pertains to applying new imaging and bioinformatics technologies.

## ORGANIZATION OF THE BOOK

The book is organized into eight chapters. A brief description of each of the chapters follows:

Chapter 1 provides a broad and comprehensive review of early detection techniques in breast cancer from self-examination awareness by patients at risks to improving proficiency of clinical examination using laboratory tests (e.g., genetic screening of the BRCA1 and BRAC2 genes), medical imaging, and biopsy procedures. The chapter focuses on the different imaging modalities available for breast cancer screening including traditional diagnostic methods (X-ray mammography, ultrasound scans, magnetic resonance imaging [MRI], X-ray Computed Tomography [CT Scans]) and new emerging imaging techniques (Infrared thermography, electrical impedance mammography [EIM], breast ductography, optical imaging tests, and the recent Positron Emission Mammography [PEM]). The chapter also discusses the different available biopsy procedures once a cancer is suspected (Fine Needle Aspiration biopsy [FNA], core needle biopsy, vacuum-assisted core biopsies, surgical biopsy, and lymph node biopsy). Although the focus of the chapter has been on breast cancer many of the described technologies could be extended to other solid cancers too.

Chapter 2 takes upon the challenge of addressing the heterogeneity of cancer response to different therapeutic interventions through examining the epigenetic signatures in breast cancer resistance. Epigenetics are similar to gene expression profiles as being inheritable molecular pattern alterations of a cellular phenotype

but they do not involve changes of the DNA sequence itself rather they are mediated by several molecular mechanisms (histone modifications, DNA methylation and acetylation). The chapter further examines the emerging interlinks between epigenetics, microRNAs and mitochondrial control in cancer signaling and treatment response. The chapter concludes by providing an optimistic view of the possibilities offered by better understanding of epigenetics for developing new drugs/inhibitors in combination with current regimens to improve cancer treatment. This is an area of active research in bioinformatics and pharmacology to repurpose existing drugs or design new ones as guided by experimental and computational findings.

Chapter 3 addresses the missing opportunity and the need in biomedical research laboratories for integrating huge amounts of complex and heterogeneous data generated by molecular experiments and relating these with clinical endpoints. This is a current challenge in this era of Big data that is shared across most industrial and academic institutions. The chapter presents an institutional experience in developing and implementing a Laboratory Assistant Suite (LAS) software tool that provides graphical user tools to support decision-making tasks and building complex analyses on integrated data. The software tool links biological samples analyzed by labs with their patient records and their clinical history. The tool also tracks molecular experiments and allows automatic annotation of biological samples with their molecular results. It uses semantic web technologies (graph-based representation) of relevant concepts (ontologies) from the genomic domain. The chapter presents two use cases (Patient enrollment and aliquot collection, Derivation of aliquot for Sanger Sequencing experiment) to demonstrate the developed software tool with its usage statistics. The authors are also considering expanding the software tool beyond their own institution into other academic centers, which would allow for integrating more data and provide new opportunities for more collaborative work.

Chapter 4 examines Big data and its potential in promising dose escalation studies in radiotherapy known as stereotactic body radiotherapy (SBRT). SBRT has demonstrated impressively-high local control and cure rates rivaling the efficacy of surgical resection in several cancers. This has been made possible by the innovative developments in image-guided radiotherapy and advanced delivery radiation systems. Moreover, there is a strong interest in combining SBRT with immunotherapeutic agents that could complement or even synergize with local therapy to limit distant failures. However, this success has presented challenging uncertainties shared by many regimens in cancer including: identifying optimal prescription doses from a wide pool of diverse clinical practices; understanding the underlying tumor and normal tissues biology at such doses; knowing how to combine new treatments with other therapeutic agents and in designing better clinical trials. The chapter discusses the challenges and prospects of applying Big data analytics to SBRT as a case study of how such data-centric approach can overcome limitations in the

current clinical trial system to understand the intricate of new promising treatment options by leveraging existing day-to-day clinical data.

Chapter 5 continues with this theme of Big data but from the perspective of how new information technologies based on cloud and mobile computing could aid in its realization and development. The chapter highlights the latest technological developments in cloud computing, big data and mobile devices for cytopathology laboratories as an example of applying these technologies to solve data access and integration issues. The chapter reviews the main cloud and mobile systems (public, private, hybrid, community) in terms of applications, services, storage and computing powers. It presents application issues related to quality control and assurance, whole pathology slide imaging, e-learning, and tele-cytology. It also discusses new research areas in cytogenetics and proteomics further enabled by cloud computing but also highlights the security challenge of patient confidential data being stored remotely and being sent and received through the internet. However, the chapter weighs the benefits of reduced cost, flexibility, and mobility versus privacy risks that would need further research from the internet security sector to improve secure transfer of clinical information and limit potentials for such breach risks.

Chapter 6 presents the use of machine learning methods as main the driving force behind image and data analytics for CAD systems. Specifically, the chapter examines the example of massive-trained support vector regression (SVR) algorithms to polyps in CT colonography (CTC). The chapter presents first the CTC database used. Then, reviews the general framework for massive-training of nonlinear regression methods and its application in support vector machines. The process of feature selection extraction using sequential forward floating selection (SFFS) is also described. This is followed by evaluation of results using free-response receiver-operating characteristics (FROC) curves. The results show very high accuracy compared to neural networks but at a higher computational efficiency.

Chapter 7 continues with the quantitative medical image analysis theme but with a broader perspective covering both diagnostic and therapeutic applications as part of the new emerging radiomics field. Radiomics aims to unravel hidden knowledge embedded in medical imaging for comprehensive mining and identify image features association with observed clinical endpoints and/or underlying biological processes. The chapter reviews recent advances and discuss current challenges for using radiomics in radiation oncology as an example field of application. The chapter discusses issues related to image acquisition, image registration, structure contouring, image feature (static and dynamic) extraction and fusion of single and multiple modalities, statistical outcome modeling (traditional statistics and machine learning algorithms), and combination with other imaging modalities and other 'omics' (radiogenomics) for developing robust models of treatment outcomes. Several radiomics/radiogenomics examples are drawn from brain cancer treatments are used

to highlight potentials and challenges in this new field including: tumor grading and subtypes identification and associations of imaging with genomics biomarkers.

Chapter 8 presents on the imaging of side effects that are associated with cancer treatments and may limit their efficacy and/or reduce the quality of life of cancer patients' lives post-treatment. The chapter focuses on radiation-induced lung injury (RILI), a major limiting factor in breast and lung radiotherapy that hinders extension of promising dose escalation studies such as SBRT, discussed, in chapter 4 to more advanced stages of disease. The chapter discusses the different available imaging modalities for RILI including: anatomical (CT and MRI), functional (Scintigraphy and Single Photon Emission Computed Tomography [SPECT]), and more recent molecular imaging (Fluorodeoxyglucose [18F] Positron Emission Tomography [PET] and optical techniques). The chapter further presents emerging imaging developments for RILI detection and monitoring including: functional (Hyperpolarized MRI, Dynamic Contrast enhanced perfusion MRI, and CT perfusion imaging [CTPI]) and molecular (molecular MRI, molecular scintigraphy and SPECT, PET, and Fluorescence endomicroscopy). In particular, fluorescence endomicroscopy (FE) imaging has gained special interests due to its high contrast and resolution particularly when tagged with specialized imaging probes to target certain molecules (such as collagen I/II in the case of RILI fibrosis). The chapter further highlights FE role in RILI, which has been recently demonstrated for tracking and monitoring mesenchymal stem cells efficacy in a preclinical model of fibrosis, as a new promising intervention that is being investigated for RILI treatment.

## CONCLUSION AND OUTLOOK

In spite of recent progress and advances in early detection, monitoring, and personalized treatment, cancer remains a leading cause of death of men and women worldwide, possibly only second to heart diseases. This monograph highlights recent emerging developments and technologies being deployed in the fight against cancer. The book is primarily focused on applications and advances in medical imaging, bioinformatics, the interlinks between them. It is expected that advances in early cancer detection technologies using imaging and genomics will contribute to better diagnosis, prognosis, and better treatment outcomes. However, in an era of data-centric knowledge and personalized treatment enables by new emerging oncology technologies that would improve the integration of huge amounts of complex and heterogeneous imaging and molecular data. It is recognized that data resulting from clinical trials remains inadequate to cover the heterogeneity of the general population and fulfill the needs of modern personalized oncology, therefore, a large emphasis has been put on big data technologies and analytics. This is addressed by this book in

the case of radiation dose escalation studies and its combination with other promising modalities. However, this example could be extended to other cancer treatments to including surgery, chemotherapy, and more recently immunotherapy. On the technology side, one can take advantage of existing and upcoming communication infrastructures enabled by modern cloud and mobile computing resources. While on the analytic side, one can apply advanced machine learning techniques to interrogate large amounts of heterogeneous data. In the case of medical imaging in particular, big data or -omics analytics has led to emergence of the field of radiomics as analogous to genomics in the field on bioinformatics. Another field that is resulting from the merger of medical imaging and bioinformatics is called radiogenomics, which is still an evolving field that aims to further reduce the gap between these two important areas. In addition, the study of cancer won't be completed without the ability also understand the side the effects that can result of current treatment regimens and how to limit their adverse effects while amplifying their potentials. Different technologies that allow quantification of toxicities using new imaging modalities are discussed in book in the context of lung injury and may be extended to other inflammatory diseases associated with cancer treatment. It is expected that the technologies and techniques presented in this book will help enrich our current understanding and knowledge of emerging research to combat and defeat cancer by improving early detection capabilities, increase treatment options while limiting risks, and guiding decision making. These emerging technologies will most likely contribute to better treatment outcomes and improvement in quality of care for cancer patients soon, if not curing at least turning cancer from a lethal to a chronic disease that a patient can still manage while enjoying their lives to the fullest.

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# Chapter 1
# Breast Cancer Detection and Diagnosis

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## ABSTRACT

*Breast cancer in women persist to be one of the primary reason of death in the world. Since the exact causes are not completely known, the most important approach is to reduce this mortality by early detection and treatment. Cancer is very difficult to diagnose in its early stages and patients only experience the symptoms when cancer has fully developed. As yet there are no effective cancer detection techniques that can detect and cure cancer at an early stage. Early cancer detection challenges very much rely on diagnostic imaging techniques at the screening stage. Newer diagnostic techniques in imaging has potential to detect timely and classify women at high possibility of the ailment. There are a several investigations that can assist in the identification of cancer, as well as blood tests, physical checkups and a several of imaging techniques including of ultrasound, MRI, mammograms and chest x-rays. This chapter focuses on the current detection techniques, discusses the shortcomings, and identifies the need for new, safer and cheaper detection techniques.*

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## IMPORTANCE OF EARLY BREAST CANCER DETECTION

Breast cancer is at times detected at the later stages after symptoms appear as early breast cancer has no symptoms. It is important to detect this disease at the initial stages when there are no visible changes occurring. Premature detection incomes uses a method that lets breast cancer get spotted prior than might have happened. Breast cancers identified with initiating symptoms are more likely to be bigger and most probably have advanced further than the breast. Whereas, breast cancers identified through screening tests are expected to be minor in size and still restricted to the breast. The dimension of a breast cancer and exactly how much it has extended are the main critical aspect in anticipating the identification of the patient with this ailment. Cancer if diagnosed at the initial stages can save thousands of lives each year and many more could be avoided by doing regular self-examination and taking early screening tests. The objective of screening investigations for breast cancer is to discover in advance earlier they start to source tumor that can be sensed.

## DIFFERENT SCREENING INVESTIGATION METHODS

Screening tests can be classified as

1. **Physical Screening and History:** Checking body for lumps, swelling or anything appearing unusual. Patient's past and current sicknesses history should also be taken in order to decide the patient management care plan.
2. **Laboratory Tests:** Medical procedures that examine specimen of blood, urine, gene mutations or tissues in the body to identify cancer.
3. **Imaging Procedures:** Techniques defining the regions inside the body by creating pictures.

Some cancers not ever show symptoms or turn into life-threatening, but if established by a screening investigation, the cancer may be treated. There is no approach to identify if treating the cancer would help the individual live longer than if no treatment were given. There is an increased possibility of an individual committing suicide in the first year after the cancer is being diagnosed.

Also, cancer treatments have effects and can lead to depression. For certain cancers, discovering and cancer treatment at the initial level does not increase the chance of a cure or help an individual survive long. False positive diagnosis, test result indicating cancer when there is no cancer can cause anxiety and depression. False positives are followed by further tests and procedures.

# EARLY DETECTION: BREAST SELF-EXAMINATION (BSE)

## Review

The intention of breast self-examination is to know the configuration of breast, identify how breasts generally sense and capable to detect modifications in the breast. In many cases the main way in which breast cancer is first detected is self-examination by the patient. Physical changes in or on the breast may be detected by the woman herself. The routine comprises of the woman herself observing at and sense each breast for potential swellings, tumor or lump under their armpit. It is recommended to have breast self-examination in aid with mammogram and clinical breast examination, besides not as a replacement for one or the other method.

Breast self-examination on its own can reduce the mortality rate is at present a causing disagreement (Ancelle-Park & Nicolan, 1999; IARC handbooks of cancer prevention, 2002). There has been a study concluding that there is not enough support that self-examination of the breast might decrease fatality from breast cancer (Ancelle-Park & Nicolan, 1999). Although this is the effortless way of detecting; it also the nominal accurate. Self-examination of the breast comprises of physical and visual examination of the breasts.

## Physical Examination

### Overview

Various approaches and configurations are followed in breast self-assessment. Many approaches advise that the woman position straight facing the mirror with the upper body visible in sight. Inspect for lumpiness, inflammation on or region surrounding the breasts. This is frequently redone in several different positions, for instance with arms held above head and while having hands on hips (Kösters & Gøtzsche, 2003).

### Breast Sensing Procedures

The breast is sensed with the finger pads to feel the tumor (both deeper in the tissue or on the skin) or pain. There are a number of collective outlines, which are aimed to make sure thorough analysis.

The perpendicular strip configuration involves touching the fingers in the vertical direction on the breast.

The pie-wedge configuration begins from the nipple and slowly shifts in the outer direction.

The circular configuration implicates sensing with the fingers in circular motion from the nipple directing outside shown respectively in Figure 1 (a), (b) & (c).

## Examination Position

Physical investigation of the breast can be carried out horizontally (lying down) or vertically (standing up in the shower), subject to the woman's choice (see Figure 2).

1. **Lying Down and Standing Straight:** Some recommendations conceptually distributes the breast into four regions and investigates each region discretely. The breast sensing procedure covers the whole breast including the armpit region. This is normally carried out initially while standing forward-facing the mirror followed by in lying down position.
2. **In the Shower:** Swellings and tumors can be felt easily with the damp lathery condition as it reduces the roughness of the skin. Smaller breast woman would use one arm to examine breast while other hand resting on head. For the larger breasts, it is recommended to support the breast from below with the hand and pressing the breast using other hand from the top

## Pre and Post-Menopause Sensing Condition

Non-lactating women are required to softly squeeze every nipple to observe any discharge.

*Figure 1. Breast self-examination sensing procedures: (a) pie-wedge configuration; (b) perpendicular configuration; (c) circular configuration*
*Khatib & Oussama, 2006.*

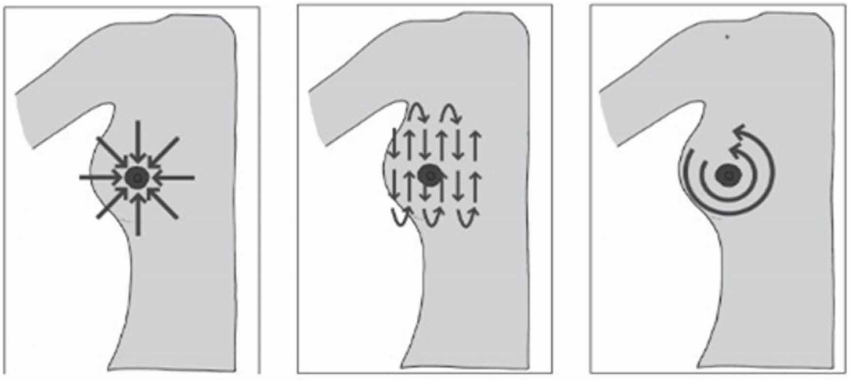
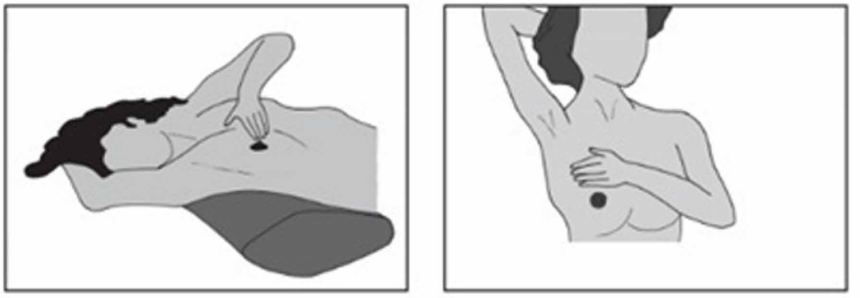


*Figure 2. Examination positions: (a) lying down; (b) in the shower*
*Khatib & Oussama M.N, 2006.*



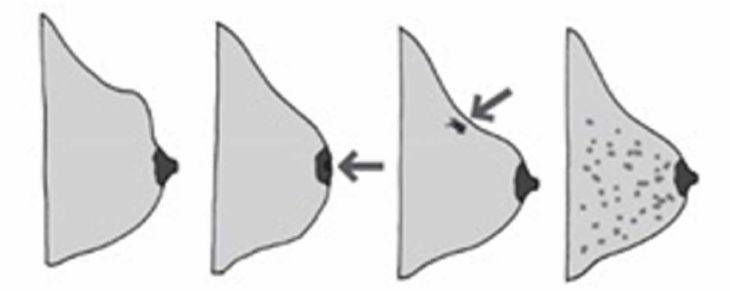
Recommended approach for pre-menopausal women is to do self-examination after menstrual cycle as changes can occur in breasts due to hormone variations and breasts are less puffed-up and tender. Postmenopausal women having uneven periods can do self-exam every month irrespective of their menopause. Proper breast self-inspections usually gain seven to ten minutes by an experienced professional (Aronowitz & Robert, 2007).

## Modifications to Be Taken Into Account

In probing the breast, modifications or abnormalities (shown in Figure3) that ought to be looked for and informed to the physician are:

1. Tumor or hard lump detected in or around breast including armpit region.
2. Tumor or solidifying of the soft tissue that do not disappear or reduces after succeeding menopause.
3. Variation in the breast architecture, volume or evenness.
4. Breast stiffening or puffiness.
5. Lumpiness or wrinkling in the breast.
6. Lumpiness, skin inflammation or other modification in the nipple or breast skin.
7. Soreness or tenderness of the nipple or breast skin.
8. Nipple discharge, mainly if the release is colorless and gluey, murky or takes place without pressing the nipple.
9. Nipple twisted or pointing inward.
10. Any breast modifications that may possibly raise concern.

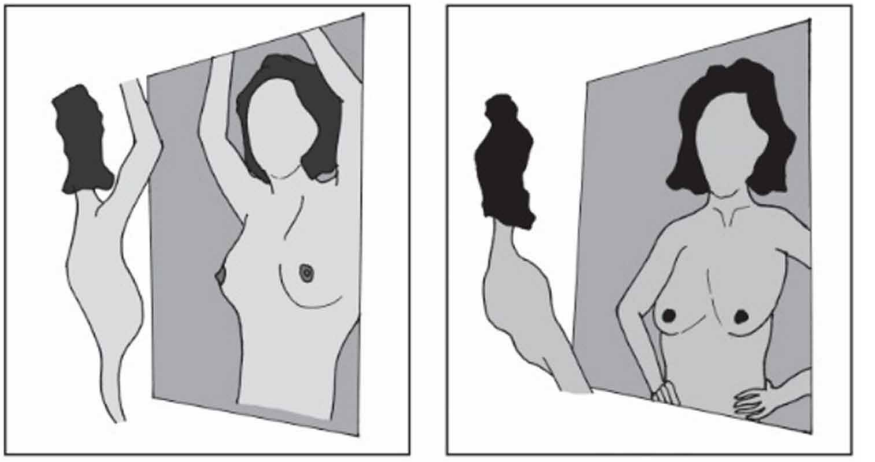
*Figure 3. Breast modifications to be taken into account: (a) swelling; (b) nipple inversion; (c) wrinkling; (d) skin appearance*
*Khatib & Oussama, 2006.*



## Visual Examination

The visual examination is additional aid in detecting potential breast ailment. For the visual inspection, the woman have to position facing mirror with bare upper body (see Figure 4).The woman must inspect breast with raised arm (Figure 4a) and with palms resting on hips (Figure 4b).Alterations in the shape or area surrounding the breasts, variations in color and shape, nipple discharge (Figure 3)must be looked for carefully. It must be considered that two breasts are not exactly similar. Yet, a woman is aware of her breast, what they look like and is capable to recognize any alterations more easily. The concerns can later be discussed with the physician.

*Figure 4. Visual examination position: (a) raised arm; (b) arms resting on hips*
*Khatib & Oussama, 2006.*



## Mechanisms for Improving Breast Self-Examination

It is suggested through surveys and studies that self-breast examination is an initial aid for the early breast cancer detection. Obtainability of flyers, leaflets and bath cards to women are significant as an indication to practice self-examination of the breast regularly. Informing people about the helpfulness and significance of self-examination can play a key role in the early cancer detection.

Though performing self-analysis escalates a woman's despair, distressing, and nervousness (Baxter, 2001). Women are expected to carry out self-examination of the breast if they have been mistakenly informed that breast cancer possibly exist, when the woman is in fact cancer free (Sofer & Antonovsky, 1984).

# EARLY DETECTION CLINICAL BREAST EXAMINATION

## Review

Breast checkup in clinics is an investigative checkup done by a physician or a nurse. It comprises of visual checkup and feeling of the breast. The whole breast is inspected including each under arm and the below collarbone area. Clinical breast examination used in conjunction with mammography is thought necessary to decrease fatality from breast cancer. Clinical breast checkup is considered as a successful initial stage in discovering the potential existence of the ailment. Yet, it cannot be exclusively employed independently, without the facts given by diagnostic mammogram and fine needle aspiration. The effectiveness of breast examination performed in clinics dependents on various issues: correct positioning, thorough examination, vertical strip method is used, right placing and motion of the fingers, and a checkup time for each breast is 5 minutes (Barton, Harris & Fletcher, 1999)

## Examination Procedure

The first stage of this examination is done in three various vertical standup positions: arms stress-free at the sides, hands squeezed tightly on the waist and inclined forward, and arms above the head. Abnormalities and variations in the appearance of the breasts is detected through this examination. Total region of the breast inspected in both the seated and flat positions. At each palpation region three intensities of pressure is applied. Initial applied pressure in minimal then average followed by

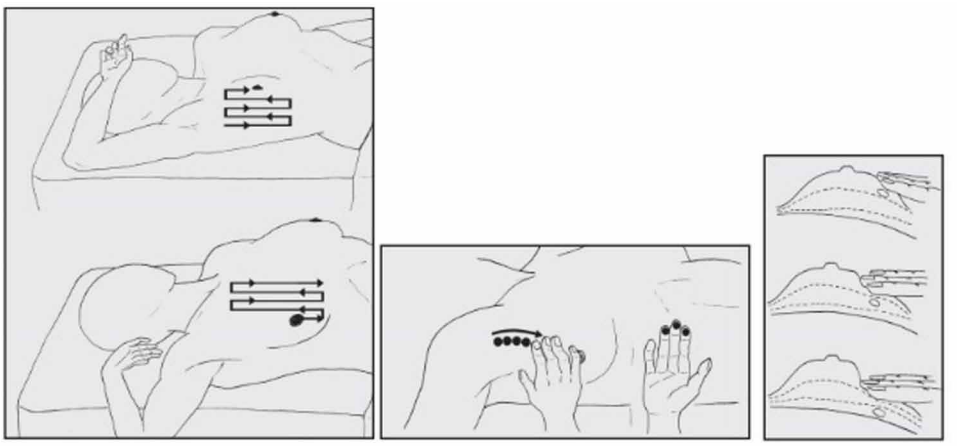
an intense force. Scrutinization is carried out with the middle fingers finger pads, and force in circular movements is applied at every region indicated in Figure 5. In case on any irregularity, the respective region of the other breast is inspected. If the outcome is not mutual, further examination is needed (Barton, Harris & Fletcher, 1999). A breast examination in clinics must be included in women's regular check-ups. Commencing from 20 years, clinical examination should be performed every alternate year, to yearly from the age of 40.

## Clinical Breast Examination Proficiency

Several clinical studies are done to establish the helpfulness of clinical breast examination in comparison with, and together with further screening procedures. Studies show that breast examination carried out in clinics has only identified 3%–5% of malignant tumors that are overlooked via screening mammography above 50 years of women, and 10% or more in women 40–49 years. Clinical breast examination identifies breast cancers of size of 1–2 cm or more. In a randomized trail, it is established that detection of small breast cancer by mammograms do not help in reducing the morality rate (Miller, 2000).

A Research group on Cancer has decided that there is not sufficient information to support the fact that mortality rate has decreased with clinical breast examination alone or in aid with mammography (IARC handbooks of cancer prevention, 2002).

*Figure 5. Clinical breast examination procedure: (a) position of sensing breasts; (b) sensing techniques; (c) pressure levels*
*Khatib & Oussama, 2006.*



## LABORATORY TESTS

The aim of breast cancer testing is to recognize genetic threat in women with higher risk, identify and establish breast cancer in its initial phase. The tissue samples required for the tests may include a needle biopsy or by surgically removing breast tissue. Genetic risk test is usually carried out to find out gene mutation BRCA1 or BRCA2 in women having history early breast or ovarian cancer among her blood relatives.

## IMAGING PROCEDURES

### X-Ray Mammography

Mammography has been the generally practiced Imaging procedure for breast cancer screening. Numerous clinical studies have revealed decrease in the mortality rate for mammography (Shapiro, Strax, & Venet, 1971; Thurfjell & Lindgren, 1996; Hendrick, Smith, Rutledge et al., 1997, Tabar, Vitak, Chen et al., 2001; Boone, 2002; Hammsterstein, Miller & White, 1979). It is carried out to detect the tumor that cannot be felt followed by further examinations like biopsy to confirm the ailment. Mammograms itself doesn't detect the lump, it only aids in detecting the lump that cannot be seen and felt. X-ray imaging involves passing a beam of X-rays from the body and onto a plate containing a film which is sensitive to X-rays shown in Figure 6. Due to the varying density of human tissues the resulting image on the film will be related to the tissue density within the part of the body being imaged. Extensive research on amount of radiation given to the region of interest has been conducted in detail (Boone, 2002; Hammsterstein, Miller & White, 1979). In case of breast cancer, calcification is normally a preliminary indication, mainly if the soft tissue hardening is minor or irregular in shape. This works very well with bones and other high-density organs since the difference in density compared with surrounding tissues produces a high contrast image. There are limitations with mammography. Mammography screening is more challenging with younger women denser breasts. Implants or major surgical scratches are also complicated to screen.

Mammography can be classified as diagnostic and/or screening.

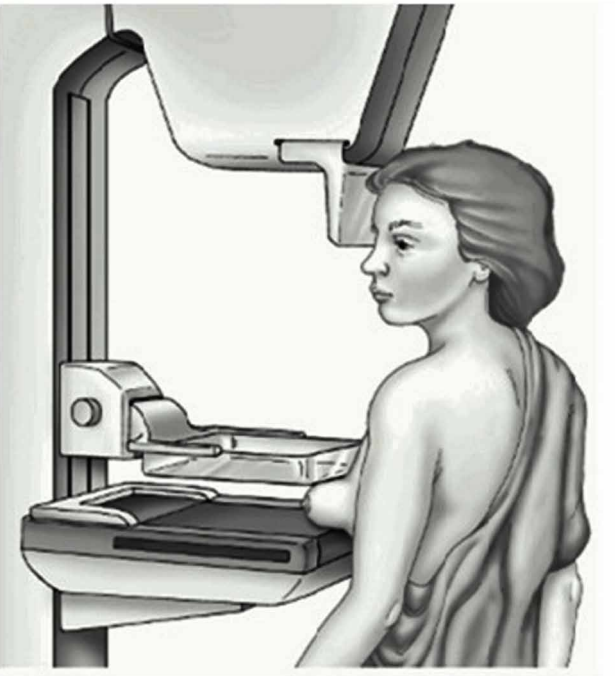
1. **Screening Mammography:** It is an investigation recommended to woman having no indication of breast cancer. The aim of screening mammogram is to locate cancer at the initial stage and too small to be sensed. Initial discovery of minor breast cancers by screening mammography encouragingly increases probability of effective cancer management. Screening mammography is

suggested every year or two years for women once they are 40 years and every year as soon as they are 50 years. Screening mammography is recommended before 40 years if a woman has a hereditary breast cancer history.

2. **Diagnostic Mammography:** It is investigation suggested in situation of an irregularity established during screening mammography or has a breast lump or secretion discharge from nipple. Diagnostic mammography is used to define position and size of breast deformities and to image the adjacent tissues and lymph nodes.

However, Mammograms restricts women with denser breasts. Denser breast tissues can make it difficult to detect any changes on the mammogram since normal breast tissue is of similar density to cancerous tissue. Dense breasts can hide a lump. Younger, expectant and lactating women are considered to have denser breasts. Therefore, X-ray mammography is generally used for women above the age of 35 (Health Highlights, 2014).

*Figure 6. Compressing breast between the two plates for mammography*
*Early Detection, Diagnosis and Staging Topics, 2015.*



## Ultrasound Scans

These scans are normally used to access breast irregularities screening or mammography. One of the benefits of ultrasound imaging is its ability to diagnose whether a lump is solid or fluid-filled (i.e. a cyst). Ultrasound scan followed by biopsy is frequently used in deciding if a tumor is benign (non-cancerous) or a malignant (cancerous). Ultrasound imaging works by transmitting high frequency acoustic energy into the body via an ultrasound transmitter and recording the reflected wave fronts shown in Figure 7.

Ultrasound is used to look at an irregularity revealed by mammography or through a breast checkup. At present just mammography is the procedure use to look for breast cancer in women with not any cautionary indication of breast cancer.

## Magnetic Resonance Imaging (MRI)

Scanning women with greater threat for breast cancer is generally done using MRI. It also supports malignancy management by evaluating treatment response and can aid in determining suitable procedure by offering statistics on the degree of the growth. MRI is at times used to scan doubtful regions establish by mammography or to examine breast already identified with breast cancer.

*Figure 7. Ultrasound procedure*
*Source: (http://www.imaginis.com/ultrasound/ultrasound-imaging-of-the-breasts-2).*

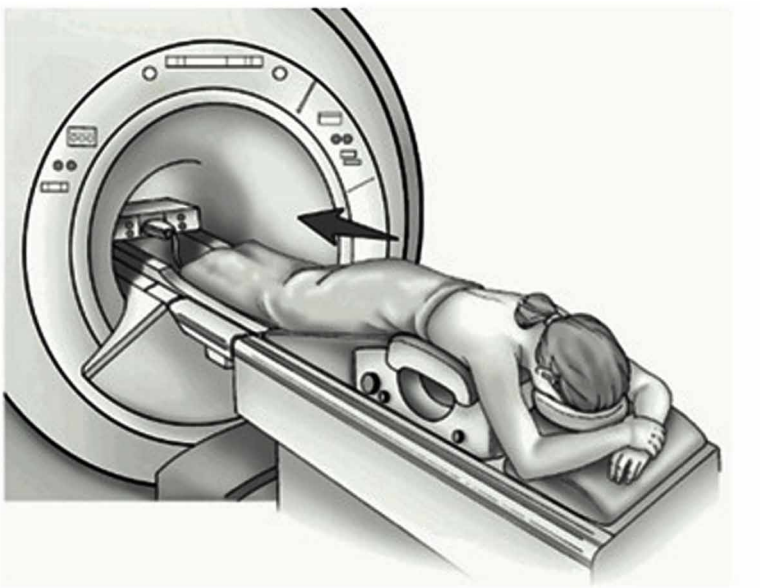
MRI scans are carried out by lying in a narrow cylindrical opening, face down, on a platform mainly planned for the checkup. The platform comprises of sensors has openings for each breast that allow them to be imaged without being compressed. It is recommended and important not be move and be very still throughout the scan.

MRI is not suggested to women at normal likelihood of breast malignancy, as it would result in unwanted biopsies and other tests. Although MRI can discovery some cancers not been able to be picked up by mammogram, it also detects benign tumors. Benign tumors need further checking to confirm that cancer is there or not, which means getting back for more tests and/or biopsies.

## X-Ray Computed Tomography (CT Scans)

CT scan improves the abrasion-background dissimilarity and provides improved sensitivity, and it is likely to obtain information from the denser tissues. This aids in scanning denser breast women. Mammography is at present the preferred checkup for breast cancer, however, CT scans may offer additional description of a breast abrasion when done with mammography because of its better distinctive resolution, better field of visualization, and cross-sectional proficiency (Chang, Sibala & Fritz, 1978; Chang, Nesbit & Fisher, 1982).

*Figure 8. Patient lying face down for breast magnetic resonance imaging (MRI)*
*Early Detection, Diagnosis and Staging Topics, 2015.*



Lump in a denser breast can be scanned better with CT in contrast to mammography. As breasts are not the principal center of attention of most chest scans, abnormal identifications not unusual (Chang, Nesbit & Fisher, 1982; Goldberg, White & McAvoy, 1994; Kim & Park, 2003).

## EMERGING DETECTION TECHNIQUES

Nowadays, there are diverse practices for diagnosis: ultrasound, mammography, MRI, biopsies, and, lately, thermography (Ng, 2009; Bonnema, Van Geel & Van Ooijen, 1997; Schnall, Blume & Bluemke, 2005; Geller, Kerlikowske & Carney, 2003). In reality, thermography emerged in 1956 (K. R. Foster, 1998) but was rejected due to the low quality thermal images (Wishart, Campisi & Boswell, 2010). Yet, with the improvement of novel thermal imaging, thermography is well thought-out as a corresponding aid for the breast cancer identification (Arora, Martins & Ruggerio, 2008). The current diagnosis procedures are deficient in early detection, especially for younger women. New emerging imaging techniques EIM, PET and Infrared Thermography have limitations and advantages. Advantages in the discovery of breast cancer includes timely identification of abnormal tissue such as cancerous from normal healthy tissue, effective tool for scanning younger women as dense breast tissue may make X-ray mammography more difficult to read and interpret. Also keeping in mind that they do not expose to harmful radiation and are non-invasive. Their limitations in the clinical diagnosis is the poor spatial resolution. The image quality is mainly related to two factors: the accuracy of the data collection system and the image reconstruction technique.

### Infrared Thermography

It is also known as thermal imaging. Breast thermography is another diagnostic procedure to detect cancer from infrared breast images. Breast thermography is entirely non-invasive and no radiative. Its working principle involves above complete zero emit infrared energy. It is essential to reiterate that thermography does not substitute mammograms. It is used in conjunction with mammograms and contributes in making decisions with as much information as possible.

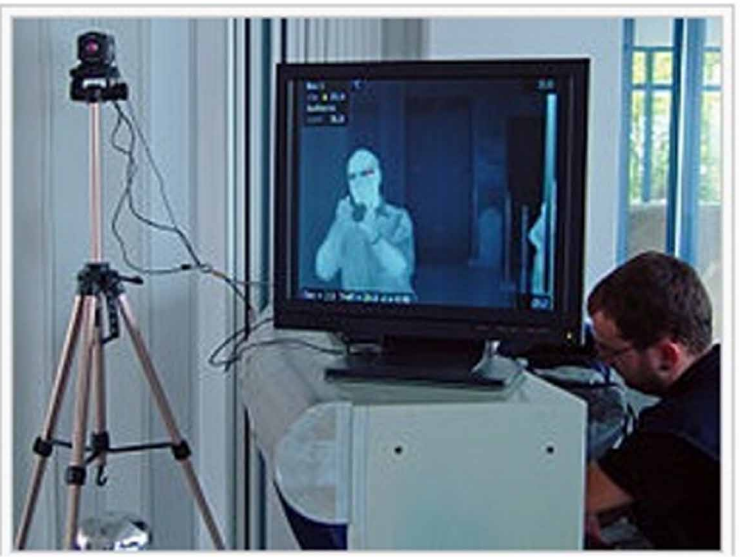
In thermography, special thermal cameras are used to sense and map the temperature of the breast surface shown in Figure 9. Breast tumor will ingest extra nutrients than the neighboring tissue to aid its development causing the rise in temperature in areas with higher circulation and metabolism (Ng & Kee, 2008). Cancer tumor skin temperature is about 1 and 3° Celsius higher than the adjacent skin (Amri, Saidane & Pulko, 2011). A cancerous tumor can be the source of different

thermal distribution, creating temperature asymmetry among breasts (Ng, Ung, Ng et al., 2001; Köşüş, Köşüş, Duran et al., 2010). Thermal regularity has been seen in the human body and so as the any thermal variation in either of the breasts should be same (Ng, Fok, Peh et al., 2002).

## Electrical Impedance Mammography (EIM)

Electrical Impedance Mammography images the electrical conductivity of the breast. It is established on the knowledge that cancer cells pass electricity in an altered way than common cells. The procedure permits a very tiny electrical current from the breast and then senses it on the breast skin. Procedure involves using small electrodes in contact to the skin. EIM is not radioactive or involves breast compression. These potentials are then used to reconstruct the conductivity of the imaged region. With a prior knowledge of the conductivity of various components comprising the image region, the composition of the structure can be determined. The image resolution dependents on the number of electrodes. EIT measurements are noted by injecting minor currents and measuring the resultant potentials between the couple of electrodes around the area to be scanned. EIM technique can be used as diagnostic aid but cannot be used in breast cancer screening. The usage of EIM for

*Figure 9. Infrared thermal imaging*
*Thermographic camera & screen, photographed in the airport terminal of Ioannina, Greece. Source: (https://commons.wikimedia.org/wiki/File:Airport_Thermographic_Camera.jpg).*



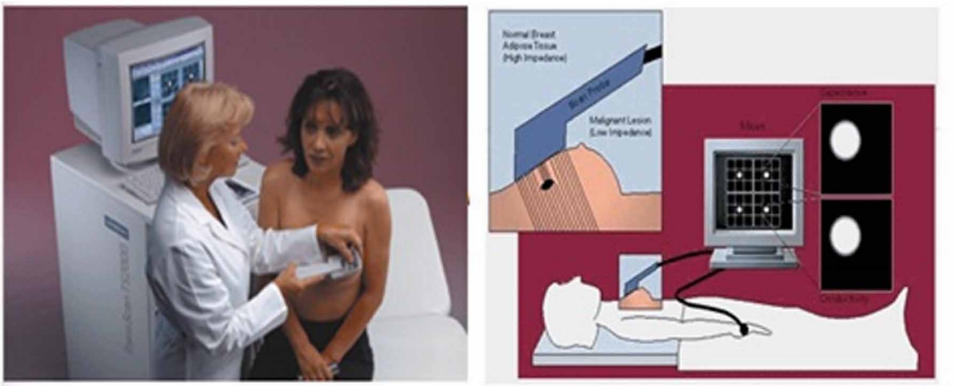
breast cancer identification is non-invasive and low-cost imaging technology. EIM has the potential in detecting early stage cancer, however there are still challenges that hindering EIM to be provided as a routine health care system (Polk & Postow, 1986; Faes, van der Meij, de Munck et al., 1999; Fricke & Morse, 1926).

The only commercially available EIM system is Siemens Trans Scan TS2000 for finding electrical breast impedance measurements. The images are acquired through a square shaped sensor squeezed against the breast. The scans are processed to yield a defined image on the display. There have been various studies to identify breast cancer using Trans-Scan TS2000. There was a study carried out to assess the performance of the Trans-Scan TS2000 as adjunct modality. The outcome of this study was the ability of Trans-Scan TS2000 to identify small lumps. The smallest lump established by the Trans-Scan TS2000 was 3 mm in diameter (Melloul, Paz, Ohana et al., 1999).

## Breast Ductograpghy

Breast Ductography or Galactography is an x-ray analysis of the inside of the milk ducts to detect the cause of nipple discharge. Discharge from nipple is a common indication and is generally linked by a benign lump. Discharge due to benign source is cloudy and does not require follow-up. Whereas discharge from a cancerous source may be bloody, clear serious or milky (Fiorica, 1994; Jardines, 1996). Identification of the discharge is generally vague and does not aid in preventing a tumor to spread (Tabar, Dean & Pentek, 1983; Leis, 1989). Ductography is frequently employed to determine and distinguish ductal deformities. Ultrasound, Magnetic resonance imaging (MRI) and Mammography are to image the breast but they do not picture the inside of the breast's milk ducts to the similar manner as ductography.

*Figure 10. Scanning using hand held Trans Scan probe*
*Source: (http://www.imaginis.com/t-scan/how-does-t-scan-imaging-of-the-breast-work).*



## Optical Imaging Tests

Optical imaging is another emerging imaging method that is likely to play a significant part in breast cancer discovery. Optical properties of the breast tissues are evaluated using infrared light by means of light propagation. Light is transmitted through the optical imaging device, where it is being reflected back and absorbed by the tissue. Afterwards the remaining light is recorded by detectors and reconstructed images are attained using advanced imaging programs (Arridge & Schweiger, 1997; Schweiger, Nissilä, Boas et al., 2007). The procedure does not use radioactive emissions, not involving compressing the breast, comparatively low-cost and easily available.

## Molecular Breast Imaging (MBI)

Also known as nuclear medicine breast imaging Scintimammography or breast specific gamma imaging (Scintimammography, 2014). This relatively new nuclear medicine imaging technique identifies tissue metabolism change. Minor dose of radioactive constituents is introduced into the body and specially designed cameras are used to see where they go (Nass, Sharyl, Henderson et al., 2011). While other imaging investigations establishes tumor changes in the body structure. MBI involves compact semiconductor γ-cameras for imaging breast at higher resolution. MBI technique is like mammography however applies less compression strength. Breast cancer is identified with the help of a radioactive tracer. A small amount of radioactive tracer is introduced in the blood stream which attaches to the breast malignant cell and is spotted by a special γ-camera. MBI corresponds to human body imaging procedures such as mammography and ultrasound. It is being considered in parallel to mammograms for denser breast women (Sardanelli, Podo & D'Agnolo, 2007; Lehman & Magn, 2006). In addition, as follow up routine for a tumor or an unusual mammogram (Berg, Blume & Cormack, 2008; Saslow, Boetes & Burke, 2007).

## Positron Emission Mammography (PEM)

A lately developed imaging technique that visualizes the breast metabolism. PEM scanning is a nuclear medicine procedure that images the flow of molecules in the body. Glucose having a radioactive atom is introduced into the blood (Marti-Climent, Dominguez-Prado, Garcia-Velloso et al., 2014). As malignant cells speedily grow, they absorb higher radioactive sugar. The principal advantage of PEM imaging is that diseases such as cancer is first evident as disordered metabolism before anatomic modifications can be seen (Shimada, Setoguchi, Yokouchi et al., 2014; Pires, Borges, Lopes-Costa et al., 2014). Anatomic techniques, ultrasound, MRI and mammography for denser or scarring breasts are not much specified. In such circumstances glucose

containing a radioactive atom can be critical in the determination of proper medical management (García Vicente, Cruz Mora, León Martín et al., 2014; Ogino, Nakajima, Kakuta et al., 2014).Image of radioactivity regions in the body is produced by special cameras. PEM cameras utilize two small movable flat detectors that are pressed directly against the breast like mammography. The camera technology used by PEM is more sensitive than entire body CT imaging in finding the breast cancers (Eo, Chun, Paeng et al., 2012). PEM cameras have the detector several centimeters away from the body surface, which limits scan resolution. Also, they are unable to differentiate between cancerous and non-cancerous tumors.

## GENETIC TESTS

Also, commonly known as predictive genetic testing checks for the genes raising the risk for developing cancer cells. Generally cancer is not hereditary, but breast (McClain, Palomaki, Nathanson et al., 2005; John, Miron & Gong, 2007; Malone, Daling & Doody, 2006), ovarian (King, Marks, Mandell et al., 2003), pancreatic and prostate cancer can strongly be supported by genes and can run in families.

A healthy person has certain genes that are generally defensive against cancer, they correct any DNA mutilation that naturally happens when cells split. Receiving damaged types or "variants" of these genes considerably increases risk of developing malignant cells, since the transformed genes are unable restore the defected cells, which can build up and form a malignant tumor.

BRCA1 (BReast CAncer genes 1) and BRCA2 (BReast CAncer genes 2) are two most common genes that increase cancer possibility if they become altered. Possibility of having breast and ovarian cancer considerably increases in women having BRCA gene.

BRCA genes are not the only ones contributing towards increased cancer risk (Antoniou, Pharoah & Narod, 2003). Researchers lately recognized further around 100 new gene variants related with higher possibility of prostate, ovarian and breast cancer. Exclusively, these new gene variants marginally increase the possibility of cancer, but a combination could mean a great threat generally. An altered version of BRCA1 gene can be passed on to the off springs if either of parents are affected.

The BRCA gene blood test follows DNA studies to spot alterations in each of the two breast cancer at risk BRCA1 and BRCA2 genes. Probability of having ovarian and breast cancer is higher in women inheriting BRCA1 and BRCA2 mutated genes as compared with the general people.

The BRCA gene blood test is referred only to women having family background of breast cancer. It is rare to have a BRCA gene variations.

There are usually two steps to genetic testing:

1. A family member with malignancy has a diagnostic blood test to see if they have a cancer risk gene.
2. If family member test is positive, one can have the predictive genetic test to see if the similar defective gene has.

### Mastectomy

Also known as Risk-reducing surgery means removing the breasts or ovaries that could become cancerous. Carriers of a defective BRCA gene may require to consider a preventative mastectomy. Women opting for risk-reducing mastectomies decrease the possibility of having breast cancer by nearly 90% (Thompson & Easton, 2002; Kadouri, Hubert & Rotenberg, 2007). However, a mastectomy is a main operation and recovering from it can be physically and emotionally not easy. Surgery can also lower ovarian cancer threat. Women who have their ovaries removed before the menopause not only radically reduce possibility of developing ovarian cancer, but also reduce breast cancer up to 50%. Nonetheless, this will prompt an early menopause, meaning not able to have own children unless you store eggs or embryos (Daly, Axilbund & Buys, 2010; Balmaña, Díez & Castiglione, 2009). The possibility of ovarian cancer in women carrying a BRCA mutated gene does not arise significantly till about the age of 40. Thus, carriers of the defective gene younger than 40 generally wait to have this risk reducing surgery.

## NEEDLE TESTS AND BIOPSIES

Biopsies are the only way and plays a vital role in the concluding diagnostic of the ailment. Surgical procedures are required to determine the exact nature of the abnormality after imaging screening. Pathological investigation is carried out to conclude the tumor is benign or malignant. Cancerous (Malignant) cells demonstrate abnormalities as compare to healthy cells. Indications include modifications in the size, nature, and appearance of cell nuclei. Malignant cells can also alter the healthy organized of cells in breast. Cancer can be diagnosed established upon the witnessed alterations, conclude how irregular the cells seem, and see either there is a single change or a combination of changes. These results help in breast cancer treatment.

Cancer can be diagnosed using physical checkup, blood tests and various imaging procedures including Magnetic resonance imaging (MRI), Ultrasound, Computed tomography and X-ray Mammography. Investigating a sample of abnormal tissue, known as biopsy aids in final diagnostic. Also it helps in concluding the type of malignant, how fast it is likely to develop. These facts are essential in deciding the suitable treatment.

## Fine Needle Aspiration Biopsy (FNA)

Fine needle aspiration is comprised of a small needle being placed into the abrasion to take out fluid, in case of a tumor, or a small quantity of cells for histological investigation. Local anesthesia is used and the removed cells are similarly analyzed. In cases where the lump is too small to feel through the skin, the needle may be guided with the help of X-ray or ultrasound images into the site of the abnormality. The needle for an FNA biopsy is thinner than the blood test needles.

Though FNA biopsy is the easiest at times miss a cancer if the needle is not positioned between the malignant cells. And if cancer cells are established, it is generally not likely to decide if the cancer is invasive. In certain circumstances, there may not be sufficient cells to carry out some of the additional lab investigations that are normally done on breast cancer samples (Sausville, & Longo, 2005).

## Core Needle Biopsy

Core (large needle) biopsy (FNA) procedure is identical as to that of Fine needle aspiration (FNA) except that the procedure is carried out using a much wider bore needle indicated in Figure 12.

*Figure 11. Fine needle aspiration using ultrasound*
*Early Detection, Diagnosis and Staging Topics, 2015.*

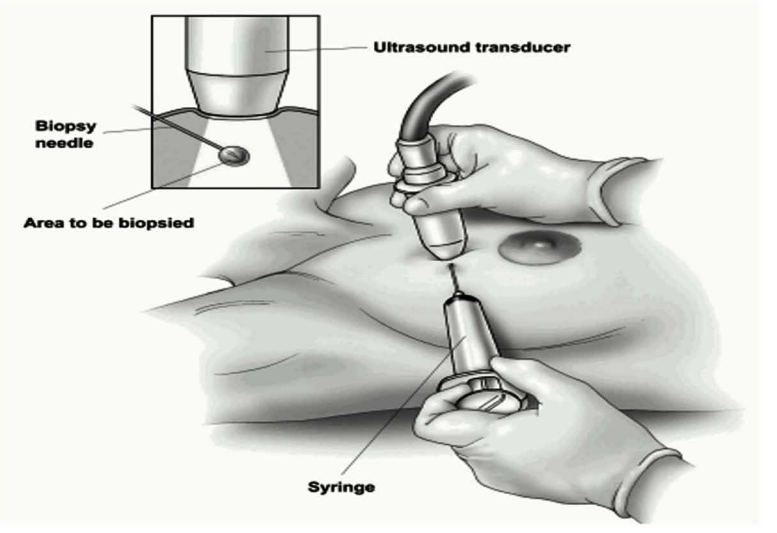
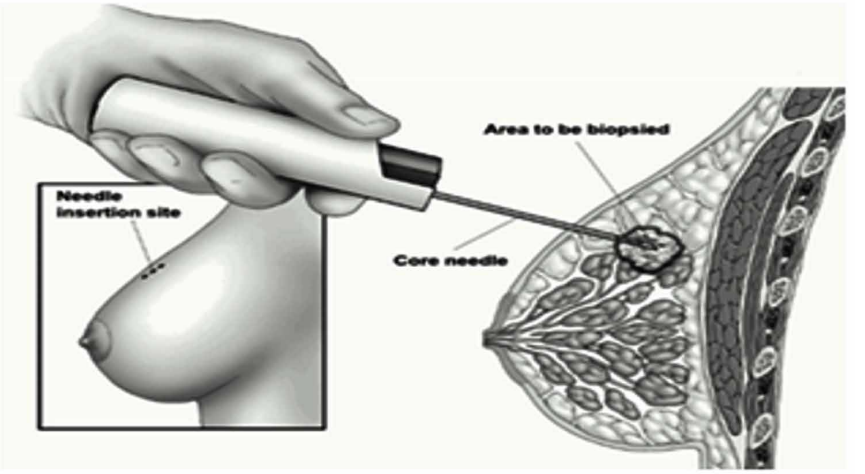


*Figure 12. Core needle biopsy*
*Early Detection, Diagnosis and Staging Topics, 2015.*




Stereotactic core needle biopsy is guided by mammograms from changed angles to locate the area of interest. This biopsy can be directed by Mammography or a MRI scan.

## Vacuum-Assisted Core Biopsies

Core biopsy can also be done vacuum-assisted. The hollow probe is directed using mammography, ultrasound or MRI to the abnormal breast tissue. Multiple samples can be taken from the same incision. This method usually removes more tissue than a regular core biopsy.

## Surgical Biopsy

Surgical or Open biopsy is rarely the carried out to take away part of all of the lump for microscopic investigation. Generally, breast cancer is established by means of needle biopsy. Surgical biopsy can further be classified as Incisional and Excisional biopsy. Incisional biopsy is to remove part of the abnormal mass if the whole mass is difficult to remove whereas Excisional biopsy is extracting the whole abnormal are. Core needle biopsy is generally adequate for an identification, but at times an open biopsy may be required subject to the lesion position, or if a core biopsy is not certain.

## Lymph Node Biopsy

Lymph node biopsy is done to check the cancer if under arm area is enlarged. This widening can be sensed or identified by mammography or ultrasound. Even if no lymph nodes are enlarged, the lymph nodes under the arm are generally examined for cancer when the breast tumor is removed at surgery.

## CONCLUSION

To summarize, breast cancer can be diagnosed using a physical checkup, blood tests and various imaging procedures including: Magnetic resonance imaging (MRI); Ultrasound; Computed Tomography; and X-ray Mammography. Investigating a sample of abnormal tissue, known as a biopsy, aids in the final diagnosis. Also, the biopsy helps in concluding the type of malignancy and so how fast it is likely to develop. These facts are essential in deciding a suitable treatment.

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# Chapter 2
# Epigenetic Signature in Breast Carcinoma, a Hidden Language to Dictate Against Genomic Insults

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## ABSTRACT

*The bottleneck in breast carcinoma treatment regimen is actually contributed from inherent genetic and epigenetic signatures present in heterogeneous clonal populations. Epigenetic changes are viewed as permanent and inheritable molecular pattern alterations of a cellular phenotype such as the gene expression profile but do not involve changes of the DNA sequence itself. Epigenetic phenomena are mediated by several molecular mechanisms comprising of histone modifications, DNA methylation and microRNA (miRNA) guided tools. Epigenetic reprograming may help in protective adaption to environment insults as chemotherapy and radiation therapy either enhance epigenetic tag or erase the epigenetic tag. Such epigenetic tools are being preferably used by several cancer types including breast carcinoma to achieve distinctive proliferation, metastasis and resistance in the wake of genomic insults. In this book chapter, we highlight the summarized findings on implications of epigenetic landscape in breast carcinoma occurrence and presenting as promising avenues for therapeutic intervention.*

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## INTRODUCTION

Notwithstanding, deepened insights about the genetic, biochemical, signaling and epigenetic implications in breast carcinoma, still this heterogeneous and most complex diseases are elusive in their nature. It is a fact that noticeable threatening aspects of breast carcinoma namely including metastasis, relapse and multidrug resistance are less understood and clinical reasons behind success in treatment and diagnosis (Feinberg & Tycko, 2004; Jeggo et al., 2016). Along with the existing regimen of chemotherapy and radiation therapy approaches, which mainly centered on the creating disturbances in the genomic world of breast carcinoma and ultimately forcing breast carcinoma to death destiny, there is need to extend our ways and observations culminating into new ideas and out of box approaches (Feinberg & Tycko, 2004; Jeggo et al., 2016; Montenegro et al., 2015; Ribezzo et al., 2016). The current regimen of genotoxic drug and radiation therapy based treatment shown promises in some cases, at the same time failures have been widely perceived in the area of breast cancer treatment. As per the literature and scientific evidence, it is true that several carcinomas including breast cancer are highly manipulative for their protective umbrella in the form of dedicated pool of DNA repair protein employing several pathways (Feinberg & Tycko, 2004; Jeggo et al., 2016). The efforts to utilize the knowledge about DNA repair proteins and their selfish act in breast carcinoma will provide opportunities for cocktails of precise drugs/inhibitors directed precisely against one or more aberrant pathways providing protective shield (Montenegro et al., 2015; Ribezzo et al., 2016). The recent findings pointed out the possibilities that a certain breast carcinoma cells may not be initially resistant against genotoxic drug treatment, however in due course of time due to epigenetic heterogeneity accumulation; these cells eventually will acquire drug resistance capability (Jovanovivc et al., 2010; Suvà, Riggi & Bernstein, 2013; Basse & Arock, 2015; Wright, 2013; Shinjo & Kondo, 2015). In recent time, overwhelming attentions are being thrown to epigenome alterations as “Epimutations” specifically centered to DNA repair proteins engaged in creating protective umbrella in case of carcinoma survival strategy to thwart genomic insults caused due to genotoxic drugs and radiation therapy (Brown & Strathdeeemail, 2015; Hamm & Costa, 2015; Lv et al., 2016; Bansal, David, Farias & Waxman, 2016). Here, objectives of this book chapter are to encompass the most recent summarized information on the potential of epigenetic mark in the perspectives of breast carcinoma prognosis and treatment.

## BACKGROUND

In recent times, focus is being placed on broaden our emerging understating about the treatment of several cancer types including breast carcinoma highlighting the bridging the gap between treatment strategies and patients genetic/epigenetic variable tag, which is critical for better outcomes in the perspectives of individualized/ personalized therapy (Egger, Lian, Aparicio & Jone, 2004; Suvà et al., 2013; Brown & Strathdeeemail, 2015; Hamm & Costa, 2015; Lv et al., 2016; Bansal et al., 2016). The success of breast carcinoma treatment regimen encompassing genotoxic and radiation therapy hinges upon the two critical alterations genetic or epigenetic signature. Essentially, such genetic or epigenetic manipulations among breast carcinoma cells substantiate the earlier common view that normal healthy cells response against a type of drug by one defined pathways, on the other hand each carcinoma cells adopt plethora of manipulative strategy. Such diverse routs to defend against a type of drugs are supposedly may be one of reason behind elusive cover held by these heterogeneous breast carcinoma cells (Wright, 2013; Brown & Strathdeeemail, 2015; Hamm & Costa, 2015; Lv et al., 2016; Bansal et al., 2016). In recent time, the epigenetic changes have been witnessed as the forefront research strategies across the cancer research community. The scientific findings supporting the epigenetics at the center to link the trio mechanisms of gene expression, internal disturbances and external factors have garnered a lot of attention and created wave of interest among scientific community (Brown & Strathdeeemail, 2015; Hamm & Costa, 2015; Lv et al., 2016; Bansal et al., 2016). In recent times, new insights surface in the form of epigenome package and pattern, this has been suggested to be means survival and proliferation tools in the hand of breast carcinoma community (Brown & Strathdeeemail, 2015; Hamm & Costa, 2015; Lv et al., 2016; Bansal et al., 2016). Therefore, understanding the molecular players engaged in preferential adaptations in epigenetic landscape may pave the way for a new class of drugs/ inhibitors which may be used in the form of cocktails of drug regimen. It is being conjectured that there is certain distinctions between healthy and carcinoma cells in terms of ability to manipulate epi-genome and their landscape through the various guiding sticks including cell-cell communications, cell-niches exchanges, molecular messengers, regulatory players, extra as well as intracellular signaling cascades (Brown & Strathdeeemail, 2015; Jeggo et al., 2015; Hamm & Costa, 2015; Lv et al., 2016; Bansal et al., 2016).

## MAIN FOCUS OF THE CHAPTER

### About Breast Carcinoma Pathophysiology and Heterogeneity

According to Darwinian Theory of evolution every tumor cell dynamically adapts to changing microenvironment. There are numerous of genetic and epigenetic aberrations that generate tumor heterogeneity (Ng, 2012; Chen & Wang, 2016). Breast tumor heterogeneity is can be well explained by two different theories that are cancer stem cell (CSC) hypothesis and the clonal evolution/selection model. Fundamental principle behind both the theories is origination of multiple aberrations in single cell that initiates proliferative action (Martelotto, Ng, Piscuoglio, Weigelt & Reis-Filho, 2014; Chen & Wang, 2016). The CSC theory suggests that there exist little inhabitants inside the tumor that culminates cancer development such population of cells is known as cancer stem cells whereas major residents are non-tumorigenic (Meacham & Morrison, 2013). Therefore, CSCs are believed to responsible for cancer heterogeneity. CSCs can be identified on the basis of cell surface markers such as $CD^{44+}CD^{24-//}$low in breast cancer cells (Al-Hajj, 2003). Source of heterogeneity in CSC model is attributed to aberrant differentiation program and mutations (Martelotto et al., 2014). Whereas on the contrary the clonal evolution model depicts clonal expansion of mutations in oncogene or tumor suppressor gene in every new round along with new set of mutations leading to Darwinian selection. It has been reported that new mutations continuously fuel tumor formation. The clonal expansion is heritable change in gene expression due to epigenetic alterations like DNA methylation, histone modifications, acetylation (Marusyk & Polyak, 2010). During early stage of breast cancer progression activation of oncogene is significantly observed due to hypermethylation of CpG islands. Around 100 of hypermethylated gene are reported to be found during breast cancer progression (Wu, Sarkissayan & Vadgama, 2015). Hypermethylation of tumor suppressor genes like MINT17, RARβ2, RASSF1A, MINT31 are confirmed to play critical role in breast cancer development (Van Hoesel et al., 2013). The purpose behind the epigenetic control inside normal as well as breast carcinoma cells may be different. There are several dedicated team of enzymes working to create a on and off switches for gene expression without altering the actual coded four alphabets gene sequence (Jeggo et al., 2016).

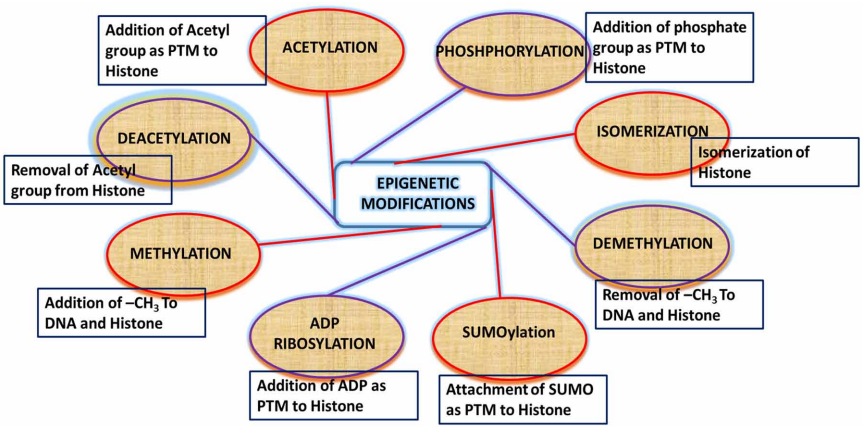
### Epigenetic Signature and Their Platform to Manipulate

In broad sense, epigenetics is the science that helps us to delineate mechanisms about the combinatorial interactions between internal cellular wiring and external clues will shape up the structural and functional tags within the cellular genome. In last decades, the evidences have accumulated to corroborate that Epigenetic

being seated on driver seat to guide normal physiological processes such as aging, development, death and metabolisms, further it is being perceived as a pivotal player in many diseases such as cancer, Alzheimer's, and diabetes (Feinberg & Tycko, 2004; Jeggo et al., 2016; Montenegro et al., 2016; Ribezzo et al., 2016). The following idea is also being perceived that breast carcinoma cells may utilize their ability to alter epigenome landscape to hijack the communication wiring in their niches, which plausibly may help them to survive during genomic insulting threats. Based on the evidence and possible available tools to alter epigenome is being depicted as illustrating model (Figure 1). Figure 1 is an example of a figure caption within a chapter.

Epigenetics is the change in protein associated with DNA or modification to DNA instead of mutation in the DNA sequence itself that is heritable and reversible. According to growing work of evidence, various mechanisms are in place within the cellular world to mark an epigenetic landscape including methylation acetylation, ubiquitylation, somoylation, phosphorylation etc. During the increase or decrease in the epigenetic tags, we find several critical ramifications being noticed within any type of human body cells including stem cell or non-stem cells and cancer cell or healthy cells. Therefore, epigenetic tag/markers are features and tools in the hand of cellular machinery to manipulate at the time of need (Brown & Strathdeeemail, 2015; Jeggo et al., 2016; Hamm & Costa, 2015; Lv et al., 2016; Bansal et al., 2016) cascades (Brown & Strathdeeemail, 2015; Jeggo et al., 2016; Hamm & Costa, 2015; Lv et al., 2016; Bansal et al., 2016).

*Figure 1. This illustration depicts the several epigenetic mechanisms associated to bring about epigenome portfolio within the normal as well as cancer cells. These modifications comprise of methylation, demethylation, acetylation, deacetylation, phosphorylation, ADP ribosylation, sumoylation and isomerization.*

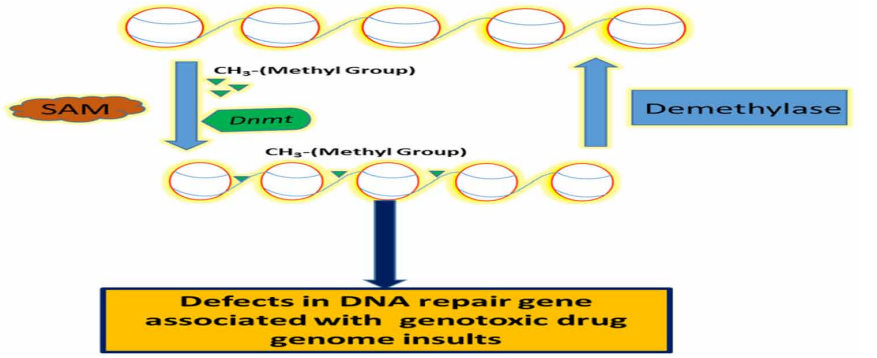


Since methylation is carried out on both DNA and chromatic associated histones player. It has been concluded that methylation is of two types of hypomethylation and hypermethylation. In case of hypomethylation, there is demethylation of previously methylated DNA resulting into the expression of certain genes which was kept silence during the development process cascades (Brown & Strathdeeemail, 2015; Jeggo et al., 2016). Hypermethylation is the methylation of certain promoters of the genes leads to the repression of the expressed genes. It has been confirmed that methylation of DNA bases is carried out by the DNA methyltransferases (DNMTs). The most common DNA bases to be methylated are cytosine. There are three classes of DNMTs, which are involved in the methylation of DNA base as DNMT1, DNMT3a and DNMT3b. DNMTs are reported to contain conserved cysteine residue in its catalytic loop which attack as a nucleophile on the sixth carbon of the cytosine ring leading to covalent bond between the enzyme and DNA. Consequently, the $C^5$ of the cytosine is strongly activated, and attacks the methyl group of the cofactor SAM. The reaction produces a methylated C5 with a proton attached cascades (Hamm & Costa, 2015; Lv et al., 2016; Bansal et al., 2016). The epigenetic changes in the genome sequence have been reported to be inheritable as well as inheritable. The genetic and epigenetic changes which has a major role in the histone conformation change causes the deflection in the chromatin structure further resulting in the suppression or activation of the genes (Terry, McDonald, Wu, Eng & Santella, 2016). These changes include the DNA methylation, hypermethylation, the remodeling of nucleosome during the post translational modification, regulating the coding region by the junk DNA (Paska & Hudler. 2015). Recent study depicts that the epigenetic signature can be used as biomarkers for the early detection of the breast carcinoma as assessing at the cellular level which can measure the DNA methylation from the DNA circulating in the tumor microenvironment (Terry et al., 2016).

The DNA methylation of CpG rich sequences blocks the DNA binding and other transcription factors sensing ability. Some friendly protein subunits that support the transcriptional suppression binds the methylated CpG regions thereby enhancing the repression for example MeCP2 protein which helps in engaging the histone deacetylase proteins to further bind the gene (Handy, Castro & Loscalzo, 2011). The outline of methylation and demethylation process is illustrated in (Figure 2). Figure 2 is an example of a figure caption within a chapter.

There is growing evidence to support that lysine and arginine residues are the target residue in histone that is subjected to methylation modification. In this milieu, methylation of lysine is done by histone lysine methyltransferases. There are eight classes of histone lysine methyltransferases based on their substrate protein domain or sequence homology next to the target lysine. These classes are represented as HKMT1s methylate H3K9, HKMT2 methylate H3K4, HKMT3 methylates H3K36 and H4K20, HKMT4 methylates H3K79, HKMT5 methylates H4K20, HKMT6

*Figure 2. This work flow shows about the one of possible epigenetic mechanisms in the form of methylation of target DNA sequence. In this process of epigenetic modifications, S-Adenosylmethione (SAM) serves as donor of Methyl group and enzymatic transfer steps are acted by group enzyme family DNA methyl transferases (DNMT) and the reverse epigenetic modifications carried out by the demethylases.*



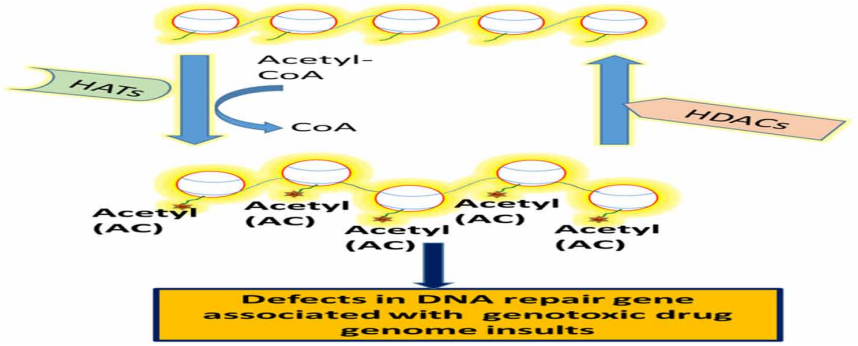

methylates H3K27, HKMT7 methylates H3K4 and some non-histone proteins, such as p53, DNA methyltransferase 1 (Dnmt1) and nuclear factor kappa B (NF$\kappa$B), HKMT8 methylates H3K9cascades (Lv et al., 2016; Bansal et al., 2016). In the same line of target amino acid residue, methylation of arginine is finished by histone arginine methyltransferases. They are classified into four groups namely type I HRMTs di-methylate only one guanidine nitrogen of target arginine by asymmetrical modification. The Type II HRMTs transfer one methyl group to each of the guanidine nitrogen atoms cascades (Brown & Strathdeeemail, 2015). The type III HRMTs is having similar activity as Type II but have additional ability to mono-methylate the internal nitrogen atom $\omega$–N of arginine residues in a peptide. Type IV HRMTs only modify $\omega$–N with mono-methylation. In view of common appreciative thought about the lysine modification of Histones as a post translational modification tools to create epigenetic tags are stretched to potential non-histone target protein which may be playing crucial role in gene regulation during normal and diseases condition (Zhang, Huang & Shi, 2015; Bansal et al., 2016).

Demethylation of lysine is done by two classes of histone lysine demethylases; lysine demethylase 1 removes mono or di -methylation with the help of the co-factor FAD which oxidizes the substrate amine to an imine through a hydride transfer mechanism. The other class of lysine demethylation is Jumonji C (JmjC) C-terminal domain family of histone demethylases have the ability to remove all types of methylation with the help of cofactors $Fe^{2+}$ and alpha ketoglutarate cascades (Brown & Strathdeeemail, 2015; Jeggo et al., 2016). It acts by oxidizing $Fe^{2+}$ to $Fe^{3+}$ by molecular oxygen that leads to the production of superoxide radical species,

which attacks the cofactor alpha ketoglutarate and leads to the release of a $CO_2$ and the generation of a $Fe^{4+}$oxointermediate which oxidizes the substrate methyl group and generates a carbinolamine that spontaneously releases formaldehyde to complete the demethylation on the substrate lysine cascades (Lv et al., 2016; Bansal et al., 2016). Acetylation generally leads to the expression of genes by adding acetyl group to the proteins associated with the DNA. The addition of acetyl group to the proteins leads to the change in their confirmation and result in the exposure of certain genome sequences which get transcribed and leads to the abnormality; or more precisely leading to the abnormal behavior of healthy cells and reflected in the form of several cancer types including breast carcinoma. The transfer of acetyl group to the target chromatin protein is being facilitated by dedicated set enzymes histone acetyl transferases (HATs) (Brown & Strathdeeemail, 2015; Jeggo et al., 2016; Hamm & Costa, 2015; Lv et al., 2016; Bansal et al., 2016). HATs are further categorized into five major families namely as HAT1, Gcn5/PCAF, MYST, p300/CBP, and Rtt109, and some additional proteins also display HAT activity. More precisely, HATs perform their action using acetyl coenzyme as a cofactor attaches an acetyl group to lysine residue of the target histone protein cascades (Brown & Strathdeeemail, 2015; Jeggo et al., 2016; Hamm & Costa, 2015; Lv et al., 2016; Bansal et al., 2016). On the flip side of acetylation is the deacetylation job enacted by the group of enzymes HDACs that act antagonistically to the HATs as it removes acetate group setting to the deacetylation and repression of expressed genes. They have been categorized into five classes namely, classes I, IIa, IIb, III and IV. It is not compulsory that only there is methylation or acetylation at a place it can be both or more precisely we can say that these modifications occur at histone tail as histone tails are more accessible to these enzymes than the histone itself so for example methylation or acetylation of lysine residue present at tail may leads to the change in gene expression (Brown & Strathdeeemail, 2015; Jeggo et al., 2016; Hamm & Costa, 2015; Lv et al., 2016; Bansal et al., 2016). The brief flow diagram of acetylation process in epigenetic modifications is being presented in Figure 3. Figure 3 is an example of a figure caption within a chapter.

SUMOylation is a post-translational modification strategy that has been implicated in several cellular processes including epigenetics. Small Ubiquitin-like Modifier (SUMO) proteins are attached to and detached from the target histone proteins in cells to manipulate the chromatin paradigm. It has also been viewed that reverse of sumoylation as ubiquitinylation could be a potential way to alter the epigenetic scenario (Geiss-Friedlander & Melchior, 2007; Vranych et al., 2014; Basse & Arock, 2015). Protein ADP-ribosylation has been considered as one of posttranslational modification mechanisms dedicated to altering the functions of key proteins which may be key to the important cellular governing process including epigenetics. ADP-ribosylation as a process is ruled by ADP-ribosyltransferases and sirtuins family

*Figure 3. This outline describes about the one of possible epigenetic mechanisms in the form of acetylation of target chromatin component histone. In this process of epigenetic modifications, Acetyl -CoA serves as donor of Acetyl group and enzymatic transfer steps are acted by group of enzyme family Acetylase and the reverse epigenetic modifications carried out by the Histone Decetylases (HDACs).*




proteins as writer, is sensed by proteins that contain binding modules (readers) that recognize specific parts of the ADP-ribosyl posttranslational modification, and is erased by the action of ADP-ribosylhydrolases (erasers). Based on their complexities, ADP ribosylation has been associated with several molecular decision-making process and differentiation and epigenetic process (Hottiger, 2015; Basse & Arock, 2015). It is being established that histone post translation modification being a critical event carried out by several processes including phosphorylation is actually a highly wired network of cellular epigenetic regulation (Rossetto, Avvakumov & Côté, 2012; Basse & Arock, 2015). Most recent investigations into cancer etiology have identified a key role played by epigenetics. Specifically, aberrant DNA and histone modifications which silence tumor suppressor genes or promote oncogenes have been demonstrated in multiple cancer models. In breast cancer, DNA methylation profiles have been linked to hormone receptor status and tumor progression cascades (Brown & Strathdeeemail, 2015; Jeggo et al., 2016; Hamm & Costa, 2015; Lv et al., 2016; Bansal et al., 2016).

## Epigenetic, MicroRNA, and Mitochondrial Control in Cancer Signaling

There is an emerging trend in harnessing the highlighted potential of MicroRNAs (miRNAs) in cellular gene regulation steps in both normal as well as cancer cell types. It has been suggested that epigenetic mark and microRNAs (miRNAs) are interlinked with each to produce a pattern of gene expression. Their inherent

regulatory role is commonly manipulated in several types of cancer. It is believed that epigenetic factors may be able to dictate miRNome (defined as the full spectrum of miRNAs for a specific genome) profile witnessed in several cancer types (Di Leva & Croce, 2010; Fabbri & Calin, 2010; Choudhry & Catto, 2011; Lin & Gregory, 2015). On the other hand, players of the epigenetic signature (such as DNA methyltransferases, histone deacetylases etc.) may be placed under the guidance from a dedicated group of miRNAs (defined as epi-miRNAs). Besides, certain microRNAs can indirectly manipulate the expression of tumor suppressor genes associated with epigenetic player. The inclusive investigation sees the cross talk between miRNAs and epigenetic agent opens new avenues to deal with human carcinogenesis and to find new regiment drugs/inhibitor cocktails (Fabbri & Calin, 2010; Choudhry & Catto, 2011). Further, exploratory research has been conducted to confirm the DNA hypermethylation within the 3 and 10 kb of a CpG island may be able to regulate miRNAs expression cancer. In recent time, several studies have been designed to study cross talk between microRNA and epigenetic landscape. The authors have produced insights for detailed picture of a molecular mechanism involving miRNAs and 5hmC that contribute to a large role in the aggressive nature of breast cancer (Song et al., 2013). MicroRNAs (miRNAs) are small noncoding RNAs being elucidated as gene expression regulatory player by representing as post transcriptional blocker or arrestor and widely reported in several organisms ranging from plant to animal (Choudhry & Catto, 2011; Lin & Gregory, 2015). A widespread role for miRNAs in diverse molecular processes driving the initiation and progression of various tumor types has recently been described. The authors have discussed about the ramifications of the aberrant expression of miRNAs in human cancers and their implications in tumor metastasis, which might elucidate miRNAs as oncogenes or tumor suppressors (Di Leva & Croce, 2010; Fabbri & Calin, 2010; Choudhry & Catto, 2011; Lin & Gregory, 2015). In addition, there is emphasis that the genomic/epigenetic alterations and transcriptional/post-transcriptional mechanisms linked with the abnormal expression of miRNAs in cancer. In recent times, there are comprehensive understanding about miRNA biology that might ultimately yield further understanding into the molecular mechanisms of tumorigenesis and new therapeutic strategies against cancer (Di Leva & Croce, 2010; Fabbri & Calin, 2010; Choudhry & Catto, 2011; Lin & Gregory, 2015).

The association of epigenetic perturbations and landscape are highly acknowledged in plethora of cellular ups and downs leading to human diseases. In growing evidence, in spite of dominance of epigenome signature of nuclear origin, it is being perceived that defined role of mitochondria in epigenetic changes are inevitable (White et al., 2015; Feeley et al., 2015; Shaughnessy et al., 2015). As knowledge is available regarding mitochondria, is the being a tiny cellular organelle resident, they are power house of cell and in addition engaged in myriad of cellular decision

process. Notwithstanding the overwhelming evidences to link autophagy and cancer, unresolved questions such driving ability of autophagy in epigenetic modifications of cancer is being pursued (Sui et al., 2015; White, Mehnert & Chan, 2015). These mitochondria mostly borrow protein player from nuclear encoded system and at the same time keep own identity to express mitochondrial genome encoded consisting 37 genes. In the light of epigenetic perspectives, it is also being construed that mitochondrial world as an extra-epigenetic player and system besides the well-known nuclear even epigenetic events such as methylation, acetylation and phosphorylation, etc. (Smiraglia, Kulawiec, Bistulfi, Gupta & Singh, 2008; Feinberg & Tycko, 2004).

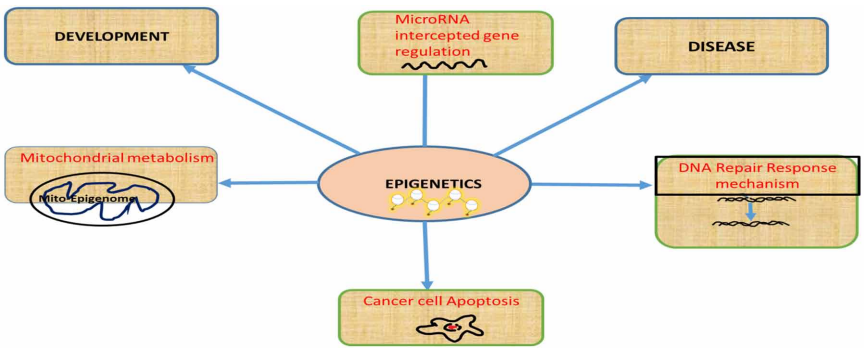
Therefore, growing trends to visualize the presence of mitochondria and nucleus within the cellular compartment as give and take system creating a sound two way retrograde signaling mechanisms (Shock, Thakkar, Peterson, Moran & Taylor, 2011; Iacobazzi, Castegna, Infantino & Andria, 2013; Verdin & Ott, 2014; Facobazzi et al., 2015; White et al., 2015; Feeley et al., 2015; Shaughnessy et al., 2015). It is also well-known fact that mitochondria are considered as a hub for important cellular bioenergetics actors in the form of ATP, Acetyl Co-A, Succinate, fumarate etc. It is also known that to create an epigenetic picture, there should be close crosstalk between nuclear epigenome and environmental clue, which is essentially driven by bioenergetics agent a product of mitochondrial world. In a growing evidence of proven experiments indicated that mitochondria may be able to modulate the nuclear epigenetic imprint through the folate metabolism. Further, it is suggested that folate may contribute in generation of S-adenosylmethionine (SAM), an important methyl donor in the process of methylation target protein and genomic sequences (Shock et al., 2011; Iacobazzi et al., 2013; Verdin & Ott, 2014; Facobazzi et al., 2015). In addition, there is experimental observation that mitochondrial copy number reduction may be able to drive discernible alterations in the methylation pattern of certain nuclear gene. Such studies accentuate and forward the importance of coherence among mitochondria, epigenetic pattern and cancer disease outcome (Smiraglia et al., 2008; Wallace, 2010; Feeley et al., 2015; Shaughnessy et al., 2015). Besides, the use of retrograde signaling tools by mitochondria to facilitate nuclear epigenetic changes, there has been emerging attention towards the potential of epigenetic phenomena platform within this tiny organelle. As the experimental being emerged out, it has been convinced that mitochondria may harbor histone like other proteins and may be able to contribute to the epigenetic like changes in the mitochondrial genome, for convenience referred as mito-epigenome (Shock et al., 2011; Minocherhomji, Tollefsbol, & Singh, 2012; White et al., 2015; Feeley et al., 2015; Shaughnessy et al., 2015). Experimental data pointed out that the presence of mitochondrial DNA methyltransferase enzyme 1 (mtDNMT1) and ensuing cytosine methylation in the mitochondria that lead to the comprehension that mtDNA methylation may be used a tool by mitochondria to reflect their intriguing regulation intervention in cancer

(Shock et al., 2011; Iacobazzi et al., 2013; Verdin & Ott, 2014; Facobazzi et al., 2015; White et al., 2015; Feeley et al., 2015; Shaughnessy et al., 2015). Overall, cellular bridges among the epigenetic landscape and other cellular events such as microRNA, development, apoptosis, mitochondrial deregulation etc. are presented in (Figure 4). Figure 4 is an example of a figure caption within a chapter.

## Achievement in Prognosis/Diagnosis Success From Epigenetic Signature Study

So far clonal heterogeneity has widely been exploited for therapeutic purpose especially in case of breast cancer. Epigenetic changes serve as biomarkers for cancer diagnosis and risk estimation. Further identification of such biomarker may be helpful in identifying individual response to therapy thereby ensuring prolonged survival (Verma, 2015). Hypermethylation has been widely investigated by silencing of BRCA1in breast cancer (Esteller et al., 2000). Histone modifications studies have become popular in epigenetics as histone proteins are in involved in regulating chromosome packaging and genome stability via process of acetylation, phosphorylation, and methylation (Wu et al., 2015). In recent past, presence of Ras association domain family 1 isoform A(RASSF1A) methylation in patient serum has been used as marker for early detection of disease as well it helps in understanding response to Tamoxifen drug (Jovanovic, Ronneberg, Tost & Kristensen, 2010). MSDK (methylation-specific digital karyotyping) and SAGE (serial analysis of gene expression) techniques has contributed in genome wide methylation analysis of breast cancer tissues which revealed DNA methylation differentiation and gene expression pattern in breast tumor (Bloushtain-Qimron, 2008; Jovanovic, 2010).

*Figure 4. This flow diagram describes about the possible linkages among epigenetic alterations, DNA repair response mechanisms, mitochondrial metabolism, cellular apoptosis, microRNA guided gene regulation, development and diseases.*



Epigenetic changes can serve as a biomarker and can indicate the tumor severity and also depicting the pattern of the modification. The major cause of the relapse or the cancer progression is the epigenetic modifications caused in the tumor suppressor genes which help the cancer cell to proliferate selfishly without any botheration of barrier. The MGMT (O6-methylguanine-DNA methyltransferase) gene being a tumor suppressor gene, in various tumors has been found to be hypermethylated in case of breast carcinoma and various other cancer types thereby leading to accumulation of A to G mutations and rapid progression of the tumor (Asiaf et al., 2015).

The therapy designed for carcinoma treatment to overcome the carcinogenesis, radio-resistance, chemotherapy resistance problem is using the inhibitors like histone deacetylase inhibitors (HDACis) and DNA methyltransferase inhibitors (DNMTIs) (Oronsky et al. 2014; Subramaniam, Thombre, Dhar, & Anant, 2014). The role of HDACis is that it inhibits the histone deacetylase enzyme which is responsible for the removal of the acetyl groups thereby causing the tight bond formation between the histone proteins and the DNA which inhibits the access of the gene transcription. Recently, research group has provided sufficient data to link the epigenetic pattern in the form of silencing of MGMT gene expression due to the excessive hypermethylation of MGMT promoter in breast cancer. Further, they also suggested the potential of such study in prognosis and therapeutic intervention in breast tumor patients (Asiaf et al., 2015). As discussed in this section, the life style related change in the epigenetic landscape is tightly linked with the breast cancer prognosis and treatment. In one of the experimental evidence, they have validated about the obesity associated hypermethylation type of epigenetic changes. Such epigenetic alterations may be associated with the gene expression alterations in breast tumor, which may be considered as a clue for the treatment (Hair et al., 2015). In recent experimental approach, evaluation of genetic and epigenetic alterations as $O^6$-methylguanine-DNA methyltransferase methylation status in the blood plasma samples from breast tumor patients was made to find a close association with the DNA repair capacity and breast tumor response against chemotherapy. Such study prudentially warrants a future monitoring system for prognosis and drug treatment response monitoring in case of breast tumor patients and also highlights that epigenetic signature as a potential target in breast tumor implications (Fumagalli et al., 2014). In a clinical study, the blood plasma sample from breast tumor patients analyzed for the study of methylation epigenetic signature, they suggested that there is significant correlation between promoter methylation platform and DNA repair capacity. Such observations could be extended in the future for treatment and diagnosis approaches in future (Guerrero-Preston et al., 2014).

In addition to a number of experimental evidence linking epigenetic alterations with the progression and outcomes of breast tumor, protein arginine methyltransferases (PRMTs) has been verified for their association with the cell-cycle regulation, DNA

repair circuits, breast cancer survival and carcinogenesis (Oh et al., 2014).The epigenetic alterations have been considered as being used as on and off switches and in the same directions, these research group have verified that BRCA1 epigenetic shut off predicts about the sensitivity to platinum-based chemotherapy in breast and ovarian cancer (Stefansson, Villanueva, Vidal, Marti & Esteller, 2012). In an experimental approach to delineate the association between epigenetic changes and breast carcinoma response cascade, it has been suggested that progressive epigenetic alterations in advancing breast tumors result in aberrant DDR-apoptotic pathway in this manner pushes tumor development. Further, it was proposed that epigenetic events related with of DDR-apoptotic genes could be of reversible in nature and may be considered as an option for therapeutic interventions and prognosis scope (Pal et al., 2010).

## Therapeutic Prospects in Breast Carcinoma Epigenetic Signature

The genetic and epigenetic landscape within the breast carcinoma comprised of plethora of molecular cascade events including DNA modification and histone protein alterations (Chiappinelli, Zahnow, Ahuja & Baylin, 2016). It is widely believed that epigenetic alterations may be able to contribute during the breast carcinoma genomic insults scenario (Nowsheen et al., 2014; Jeggo et al., 2016; Chiappinelli et al., 2016). According to literature, methylation pattern of gene and their promoter has been recognized as a tool in epigenetic alterations. To understand the drug resistance and epigenetics, authors have identified that in two breast cancer cell line models of aromatase inhibitors (AI) resistance mechanisms may be due to widespread DNA hyper- and hypomethylation, with enrichment for promoter hypermethylation of developmental homeobox gene HOXC10 (Pathiraja et al., 2014). In recent time, the intended and precise pattern of Histone post translational modifications being construed as aberrant strategy being used as tool by several types of carcinoma. Thus, the vision and insights to think about the potential epi-drug development and their impact in cancer treatment regiments is highly warranted (Audia & Campbell, 2016).

In a recent effort to look into the implications of cyclin D-cyclin dependent kinase (CDK) 4/6-inhibitors in breast cancer, they reported that CDK4/6 inhibitors as palbociclib (PD-0332991), ribociclib (LEE011), and abemaciclib (LY2835219) may be able to bring about desirable results in breast cancer treatment by modulating several mechanisms such as including gene amplification or rearrangement and epigenetic alterations (Hamilton & Infante, 2016). Therapeutic resistance and genomic instability in breast carcinoma have been suggested to be attributed due to their metabolic reprogramming. To further dissect out the effects of cancer metabolisms and epigenetic aberration, they have elucidated that targeting downstream effectors of

histone methylation and demethylation pointed out the PRC1/2 polycomb complexes as the ultimate targets for metabolic regulation and further strongly substantiating the contribution of polycomb group proteins in non-homologous end-joining DSB repair (Efimova et al., 2016). In pursuance to harnessing the potential of certain drugs to interfere with the cancer epigenetic pattern, 5-aza-2'-deoxycytidine (DAC) acting as demythylating agent which is currently used to treat certain cancer types and sensitize against to chemotherapy and immunotherapy. Here, they have reported that aberrant hypermethylated sites in breast carcinoma may be reversed by the treatment of histone demethylase agent DAC and opens up opportunity to see the potential of drugs/inhibitors to intervene in the epigenetic modification system (Bell et al., 2016). It is a well-known fact that BRCA1 gene expression reduction in certain breast tumor type is driving agent behind deficient double strand break repair pathway. The genomic and epigenomic based study clearly showed that epigenetic signature in the form of methylations of MLH1 and PAX5 added with the unmethylations of CCND2 and ID4 may be considered as reliable predictors for BRCA1 associated breast tumors. Such studies lead us to strong perceptions that epigenetic landscape could be a potential therapeutic drug target in case of BRCA1 associated breast tumor type (Branham et al., 2016). In an effort to establish the link between BRCA1 inactivation and epigenetic modification to BRCA1 promoter methylation, clinical patient tissue sample from TNBC breast tumor sample revealed that BRCA1 promoter methylation suggested being considerably associated with reduced BRCA1 expression leading to functional loss of BRCA1. Thus, to suggest targeting the demethylation of BRCA1 promoter and screening BRCA1 promoter methylation signature may be added potential to TNBC cancer patient prognosis and therapeutic approaches (Yamashita et al., 2015). In a recent era of genomics and epigenetics, vast knowledge is being gathered to substantiate that environmental factors in the form of life style including our nutrients are responsible for modifications in our epigenetic picture. In a recent experimental result, resveratrol and pterostilbene has been found to modulate DNA damage response by affecting SIRT1 and DNMT epigenetic player expression. This study along with other once again flashes the attention to consider the environmental factors such as nutrients, life style etc. as a factor for the treatment approaches in case of cancer types including breast carcinoma (Kala, Shah, Martin & Tollefsbol, 2015). Currently, efforts are being made to understand the genetic and epigenetic switches in several types of tumors including breast carcinoma. In one of such attempt, it has been suggested that epigenetic alterations in set of 22 genes may be responsible for aberrant response in key cellular process including DNA damage response and cellular proliferation pathway. Such investigations dedicated to unearthing the implications of epigenetic switches in tumor response mediated by key cellular responses may create new settings for cancer treatment (Mukherjee et al., 2016). It has also been suggested that the use of epigenetic silencing

mediated by DNMT1 may lead to the transcriptional repression of microRNA-200 family members. Further, repression of microRNA-200 family regulatory player is actively engaged in several tumor types development and genotoxic agents mediated drug resistance (Ning et al., 2015). Based on the emerging understanding, DNA hypermethylation has been linked with the alterations in DNA repair capacity and apoptosis mechanisms in certain cancer types including breast carcinoma. Therefore, the efficacy of 5-aza-2-deoxycytidine was tested by facilitating DNA demethylation, which will serve as reverse of DNA hypermethylation. Further, they suggested that DNA demethylataion may be able to restore back the DNA repair defects and promoting cell death (Singh, Treas, Tyagi & Gao, 2012). In a study, Silibinin a class of phytochemical drug has been reported to downregulate miRNA-21 and miRNA-155 to bring apoptosis in in MCF-7 cell line (Zadeh, Motamed, Ranji, Majidi & Falahi, 2016). Recently, there is report on a combined therapy using dipyridamole in the presence of a new synthetic antifolate, 3-O-(3,4,5-trimethoxybenzoyl)-(-)-catechin that leads to the blockage of both the folic cycle and the methionine cycle in breast cancer cells and sensitized these cells to radiotherapy. They have suggested that drugs sensitization may be mediated by the recruitment of BRAC1 and 53BP1to the chromatin regions flanking DNA double-strand which prevent the double strand repair system to carry out fixing the lesions. In breast carcinoma, the driving player behind the metastasis problem is still unnoticed and less surfaced in the perspectives of genetic and epigenetic coding programs. In an effort to elucidate the epigenetic reprograming strategy in breast carcinoma metastasis, there are conceived idea that differential methylation pattern may be driving reason behind the genetic control of set of genes dedicated to fork on behalf of metastasis. This research group have highlighted that RON receptor tyrosine kinase and macrophage stimulating protein (MSP) dictated signaling cascades are responsible for the aberrant expression of G:T mismatch-specific thymine glycosylase MBD4. Further, they concluded that MBD4 guided augmented DNA methylation will be driving force behind the breast cancer metastasis. Such investigation along with other path-breaking report envision about the potential therapeutic interventions in future to block either the signaling cascades protein player or methylating protein such as MBD-4 to interrupt the metastasis of carcinoma (Cunha et al., 2014).

## Therapeutic Targeted to Histone Code Alterations

Recently, views are growing to establish the link among tumor microenvironment, hormone responsive and epigenetic landscape in breast carcinoma. In case of potent antitumor drug Trichostatin A has been reported to modulate the gene ESR1 gene expression and such gene modulation is strongly associated with the epigenetic marker such as HDAC mediated histone modification (Gameiro et al., 2016). In

current scenario, the triple-negative breast cancer (TNBC) is well known for highly aggressive nature and poor outcome, therefore recent experimental approaches have utilized the various approaches together including gene silencing, epigenetic modulation and DNA repair protein inhibitors. In this direction, histone deacetylase inhibitors (HDACi) a type of epi-drugs and link with homologous recombination pathway repair such as HDAC inhibitor suberoylanilidehydroxamic acid (SAHA) and HDAC1/2-specific inhibitor romidepsin (ROMI) revealed capability to augmenting TNBC sensitivity to PARPi via HR DNA repair gene modulation. Such experimental data provide a treatment options to breast cancer patient by hammering the genetic and epigenetic points concomitantly (Wiegmans, Yap, Ward, Lim & Khanna, 2015). Further, authors revealed that the growth inhibitory effects of IN-2001 were related to the cell cycle arrest and induction of apoptosis (Joung, Min, Kim & Sheen, 2012). A group of enzymes-lysine acetyltransferases was widely recognized responsible for their regulation of contribution in transcription as cofactors and by acetylation of histones and other proteins. Tip60, an acetyltransferase catalytic subunit of human NuA4 complex and reportedly this HAT enzyme type has a clearly evident participation in cellular signaling and DNA damage repair. The aberrant tendency of this enzyme Implicated in several cancer types survival and death mechanisms. Thus, it has been proposed that such enzyme may serve as promising therapeutic target in several cancer types including breast carcinoma (Judes et al., 2015). In recent report, human MOF (males absent on the first), a MYST (Moz-Ybf2/Sas3-Sas2-Tip60) family of histone acetyltransferases (HATs) if silenced may lead to DNA damage defects and genomic instability. Therefore, it has been pointed out that MOF unspeculated expression in several tumor types including breast carcinoma may work as culprits for the observed genotoxic drug resistance and treatment complications (Su, Wang, Cai & Jin, 2016). In current understanding of mechanisms behind cancer aberrant proliferation and survival linked with the epigenetic alterations in addition to the established facts about genetic and signaling pitfalls. Therefore, to strike with hammer ascending of idea by inhibiting molecular players such as DNA methyltransferase (DMT), histone methylase (HMT) and histone decetylase (HDAC) that are responsible for favorable epigenome landscape in breast carcinoma (Basse & Arock, 2015). In recent, the chromatin reprogramming in the face of histone modification is being extensively investigated to provide evidence for gene regulation alterations in breast carcinoma. With such interest, C-terminal binding protein (CtBP) is a NADH-dependent transcriptional repressor has been provided with evidence to link carbohydrate metabolism to epigenetic regulation by changing the chromatin face. The surfaced data from such study delineate comprehensive knowledge in establishing the role of CtBP and chromatin landscape in breast carcinoma pharmacologic and metabolic intervention (Di et al., 2013).

The authors demonstrate that ectopic expression of histone acetyltransferases and histone deacetylases (HDAC) will push breast carcinoma towards their survival and proliferation. The results marked that HDAC9 is central in breast carcinoma gene regulatory network and play protective role in response to HDAC inhibitors in breast carcinoma treatment (Lapierre et al., 2016). There are sufficient evidence to emphasize that epigenetic modification may be playing a crucial role in the immune evading ability of certain cancer types including breast carcinoma. In experimental results, research group have validated that in case of breast carcinoma cells epigenetic drugs such as the pan-HDAC inhibitor vorinostat as well as the class I HDAC inhibitor entinostat may be able to reverse the ability of breast carcinoma to evade the mounting attack from T lymphocyte lysis action. They have also concluded that during these inhibitors treatment options, most prominent and determinant player identified as HDAC1. That may be able to mediate the immune response modulation in case of breast carcinoma (Adams & Eischen, 2016; Gameiro, Malamas, Tsang et al., 2016).

## SOLUTIONS AND RECOMMENDATIONS

Despite clear evidence on drug resistance in breast cancer treatment, a well precision inhibitors and agents are recommended to bring normalcy from aberrant epigenetic settings. The therapeutic solutions are perceived in the form of peptide mimetic, miRNA mimetic, inhibitors to epigenetic modifiers and epigenetic profiling as biomarkers for individualized cancer therapy.

## FUTURE RESEARCH DIRECTIONS

In view of convincing evidence to show the aberrant epigenetic landscape in breast cancer, several molecular inhibitors towards epigenetic modifiers, modulators, epigenetic mediators and miRNA mimetics are expected to get appreciated at basic science, pre-clinical and clinical stage. Hence, personalized medicine based upon the epigenetic profile could be recommended to the individual breast carcinoma patients as well as other cancer types.

## CONCLUSION

In conclusion, the epigenetic phenomena within normal or breast carcinoma cells are interpreted as the opportunity of turning gene on and off in perspectives of prognosis and therapeutic intervention more precisely genomic insults strategy.

However, curiosity become bigger to know whether dancing tune of gene on or off can be manipulated for the sake of breast tumor patient's benefit. During the extensive summary to explore ways to mend the epigenetic landscape, it provides a hope and promise for better drugs/inhibitors combination along with the current regimen of genomic insults approaches in breast cancer treatment.

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## KEY TERMS AND DEFINITIONS

**Cancer:** A disease in which abnormal cells divide uncontrollably and destroy body tissue.

**Carcinoma:** A cancer arising in the epithelial tissue of the skin or of the lining of the internal organs.

**DNA Damage:** DNA damage is defined changes in the chemical structure of DNA, such as a break in a strand of DNA, a base missing from the backbone of DNA, or a chemically changed base such as 8-OHdG. Damage to DNA that occurs naturally can result from metabolic or hydrolytic processes.

**Epigenome:** The epigenome is called as landscape of chemical compounds that can guide genome what to do.

**Gene:** A unique sequence of nucleotides within a chromosome, which code for a protein, non-coding regulatory RNA etc.

**Genome:** It represents the complete set of genes or genetic material present in a cell or organism.

**Histone:** These classes of proteins are chief protein components of chromatin, acting as spools around which DNA winds, and playing a role in gene regulation.

**microRNA:** A microRNA (abbreviated miRNA) is a small non-coding RNA molecule (containing about 22 nucleotides) found in plants, animals and some viruses, that functions in RNA silencing and post-transcriptional regulation of gene expression.

**Mutation:** Mutation is called as the permanent alteration of the nucleotide sequence of the genome of an organism, virus, or extrachromosomal DNA or other genetic elements.

**Stem Cell:** An undifferentiated cell of a multicellular organism which is capable of giving rise to indefinitely more cells of the same type, and from which certain other kinds of cell arise by differentiation.

# Chapter 3
# LAS:
## A Bio-Clinical Integrated Laboratory Information System for Translational Data Management

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## ABSTRACT

*Rapid technological evolution is providing biomedical research laboratories with huge amounts of complex and heterogeneous data. The LIMS project Laboratory Assistant Suite (LAS), started by our Institution, aims to assist researchers throughout all of their laboratory activities, providing graphical tools to support decision-making*

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*tasks and building complex analyses on integrated data. Thanks to a clinical data management module, linking biological samples analysed by translational research with the originating patients and their clinical history, it can effectively provide insight into tumor development. Furthermore, the LAS tracks molecular experiments and allows automatic annotation of biological samples with their molecular results. A genomic annotation module makes use of semantic web technologies to represent relevant concepts from the genomic domain. The LAS system has helped improve the overall quality of the data and broadened the spectrum of interconnections among the data, offering novel perspectives to the biomedical analyst.*

## INTRODUCTION

In the last years, the advent of automation and high-throughput technologies in biomedical research laboratory activities has introduced numerous issues related to the amount and diversity of the data produced, the selection of robust procedures for sample tracking, and the management of computer-based workflows needed to process and analyze the raw data generated. For these reasons, the adoption of a Laboratory Management Information System (LIMS) can no longer be overlooked as a means to achieve good levels of quality control over laboratory activities and efficiently handle massive volumes of data.

A LIMS aims at helping researchers in their daily laboratory practice by providing different quality control strategies, improving the accessibility of the instruments, and tracking biological samples and their related information. In particular, more and more laboratories resort to LIMS to improve sample management, which usually involves sample handling, registering and locating (e.g. to retrieve where a particular sample is stored). Moreover, these systems can enforce security policies to restrict data access only to authorized users, by complying with current regulations, such as the ISO/IEC 17025 standard.

Core functionalities commonly found in most LIMS systems include the following:

- Sample tracking
- Stocks management
- Support to the implementation of experimental workflows/protocols
- Management of heterogeneous data
- Built-in features and interfaces that assist researchers throughout laboratory activities.

Nowadays a wide choice of LIMSs is available, both open-source projects and proprietary software. Commercial solutions are large, complex and feature-rich

products designed to be sold to large laboratories. Their license fees are often prohibitive, and each extra feature or module they provide might come at additional costs (Wood, 2007). To reduce these costs, the last generation of commercial LIMSs adopt web-oriented software technologies, and in particular the Software as a Service (SaaS) distribution model, lowering the end user's total expenditure on licensing fees, IT assets and maintenance. Examples of commercial solutions are STARLIMS (Abbott, 2013), Exemplar LIMS (Sapio, 2010), LABVANTAGE SAPPHIRE (Labvantage, 2011).

Some institutions rather opt to invest in the development of in-house solutions and/or to adapt open-source projects to their own requirements. Even though costs may turn out very similar to those of commercial products, laboratories may still prefer in-house development in favor of software functionalities that meet the specific needs of their researchers. Indeed, many of these solutions target specific sub-domains, such as molecular experiments, and tracking of in vivo and/or in vitro experiments. The very graphical interfaces, as well as any data entry strategies, are usually designed to track all the details of the experimental procedures defined by the laboratory technicians, and to simulate the laboratory environment. From a developer's point of view, in-house solutions also permit to explore and adopt new technologies, to define new and complex data models, and to fine-tune the overall system performance. Last, the developer team may design new software functionalities to keep up with how research needs evolve within the laboratory.

In some cases, the adoption of an available LIMS may present issues, since each product is usually focused on sub-fields of cancer research, manages only few omics technologies and may force some constraints on the experimental procedures. For these reasons, in 2011, our institution started to implement its own LIMS, named the Laboratory Assistant Suite (LAS) platform (Baralis et al., 2012). The main purpose of the platform is to assist researchers in different laboratory and research activities, allowing management of different kinds of raw data (e.g., biological, molecular), tracking experimental data, supporting decision-making tasks and integrating heterogeneous data for complex analyses. To the best of our knowledge, the procedures adopted in our experimental pipeline are largely standardized, and they reflect common practice in the oncological research field. Thus, we believe that a big fraction of functionalities offered by the system we are developing could be useful to other research institutes. During the last years, we also included a clinical data management module, targeted at linking biological samples analyzed by translational research with the originating patients and their clinical history. In this way, scientists can gain better insight into tumor development by jointly studying the clinical evolution of the disease and the experimental results derived from in vivo/in vitro experiments. Furthermore, we improved the genomic annotation module in an effort to build a common repository for molecularly annotated samples, generated

by different technologies and described with standardized terminology. For this purpose, we exploit semantic web technologies to represent relevant concepts from the genomic domain, drawn from various publicly available databases and resources, and interlinked with annotated samples.

The chapter presents the main characteristics of Laboratory Assistant Suite (LAS) and its adoption in the research laboratories of the Institute for Cancer Research at Candiolo and its research partners. The Background section provides an overview of LIMS solutions presented in the literature and distributed by commercial companies, while the Laboratory Assistant Suite section analyzes the context in which the LAS project has been developed. Next, the basic concepts of each domain addressed by LAS are described. The Use cases section presents some real usage scenarios, to show how the system can effectively support daily activities, and how different domains (e.g., clinical and biological) can indeed be handled in a closely related fashion by the LAS platform. Moreover, the Usage statistics section discusses statistics about the data and the activities currently tracked by LAS. Finally, future developments and research directions are presented.

## BACKGROUND

Laboratory Information Management Systems (LIMS) are information systems used to support, and increase, research and laboratory activity efficiency and automation (Prasad & Bodhe, 2012; Moumtzoglou et al., 2015). Both commercial and free solutions are currently available for deployment.

Many companies are currently investing effort in the development of biological information systems. For instance, ATGC Labs provides both Freezer Web Access (2015) and Lab Inventory (2015). The former is a web-based application designed to organize the laboratory workflows, track research, development and data. The latter is designed to track and manage laboratory inventories, place orders and generate inventory reports. BioMatters (2015) is a company which develops useful commercial genomics tools. For instance, it provides Geneious, a desktop-based comprehensive suite of molecular biology tools; it also provides cloud solutions for genomics, such as a remotely managed Genome Profiler. BIOVIA CISPro (2015) delivers all the necessary tools in order to track and report chemicals and supplies. It also meets safety and regulatory requirements while monitoring reagents and cross-laboratory material sharing. Another commercial solution is Clarity LIMS (2015), which comes in four different editions: Run Manager (free), Silver, Gold and X editions (priced licenses). Clarity Run Manager provides sample tracking, sample sheet generation, single user access and visual pooling & adapter assignment. Support is forum-only. Differently, both Silver, Gold and X editions provide Quality Control tracking,

a collaboration portal for different users, reagent and control tracking, overview reporting and a set of pre-configured workflows. In this case, the system may be available on-premise or hosted in a remote facility, and phone support is available. Further, Gold and X edition provide more configurability, extended support and automation. LabCollector (2015) is a versatile LIMS composed of different modules and enhanced by several add-ons. In particular, LabCollector manages reagents, chemicals and supplies, strains and cells, plasmids, and sequences. It provides a storage system, security and integrity features (such as users and permissions), barcoding, data exchange capabilities and reporting tools. Similarly, LabGuru (2015) is a web-based LIMS featured with a laboratory notebook, sample organization and collection, materials inventory and collaboration tools. LabLinx Inc. (2015) provides three different LIMS products. ELab, formerly known as LabLinx LIMS, eases tasks such as sample logging, barcoding, workload management and scheduling. It also provides sample analysis, quality control and data exchange. webLIMS is distributed as SaaS (software as a service), and it is proposed as a more scalable solution because it is hosted and updated on the cloud. sciCloud.net is a suite of tools for LIMS applications, hosted in the cloud, and accompanied by social networking tools to enhance collaboration between researchers. LABVANTAGE Solutions Inc. (2011) provides a large set of features, such as sample and batch management, Quality Control, advanced storage and logistics, and task scheduling. Further, it offers interfaces for data retrieval, integration with other software, and security. In contrast, a specific solution for mouse colonies is mLIMS (2015) which tracks rodent colonies. Qualoupe LIMS (2015) provides a suite of over 30 applications, which can be fully or partially accessed by operators, depending on their role (e.g. Laboratory Technician, Laboratory Manager, etc.). It provides several features such as sample registration, workflow allocation, reports and data exchange between different applications. RURO Inc. (2015) proposes several products: Limfinity, LIMS engine which powers all company solutions; FreezerPro, for frozen laboratory samples management; ezColony, used to manage transgenic facilities on any scale; and Sciency ELN, an electronic notebook to organize research and share data. StrainControl (2015) is a software used to manage strains, proteins, plasmids, oligos, chemicals and inventories, providing roles and users support. It provides both a free version for individual researchers and professional priced licenses.

Besides commercial solutions, several tools have also been proposed within the research community, which are mostly open-source and web-based. BIKA LIMS (2015), is an open-source project with sample management and batching, data analysis, validation and query capabilities, results reporting, data export and a web-based client.

Several LIMS also provides collaboration utilities. For instance, LabTrove (2015) is web-based and provides a collaboration framework for researchers to share their

experiments, thoughts, observations and achievements. It is also accompanied by a chronological diary associated with each research flow. Galaxy LIMS (Giardine et al., 2005; Blankenberg et al., 2010; Goecks et al., 2010; Scholtalbers et al., 2013) provides several features such as operations on genomic sequences (integration, functional annotation), workflows, analysis inspection, and collaboration between users. It also has a history system, which records the sequence of user operations. It is web-based (and cloud-based in the public version) and, therefore, cross-platform. Galaxy also introduces the use of annotation for each analysis (for instance, to enrich the metadata with the dataset description, algorithm version, etc.), ensuring the reproducibility of the results. OpenSpecimen (2015), formerly known as caTissue Plus, is a free open-source biobanking system. It provides a set of features such as: role-based access, multisite support, storage containers, rapid data entry and annotations. Further, it supports data exchange and programmatic access (API). Quartzy (2015) is a reliable, easy-to-use and free laboratory management system. It is a secure web-based service which is backed by marketing partnership with life-science companies. It tracks laboratory order requests, manages inventory, provides a shared repository for documents, and schedules equipment usage.

There are also additional tools focused on NGS technologies: MendeLIMS (Grimes et al., 2014) is used to manage clinical genomic studies, in particular for sample and NGS process management. It is web-based and provides the following features:) enrollment of patients and acquisition of samples; ii) sample assessment and processing; iii) genomic analysis; and iv) DNA sequencing with Quality Control. Personalized Oncology Suite (2015) (Dander, Baldauf et al., 2014) is a web-based open-source platform which integrates clinical and Next Generation Sequencing data, and whole-slide biological images from tissue sections. It also provides integration with public data available on COSMIC and filtering queries on data. SMITH (Venco et al., 2014) is a web application developed to manage information from Next Generation Sequencing experiments. In particular, it stores the description of the protocol and the algorithm used in each experiment. It provides several features such as sample management, quality control, role-based access and reagent storage. The EnzymeTracker (Triplet & Butler, 2012) is a web-based open-source LIMS for sample tracking. It consists of a collection of online spreadsheets, and it eases data entry and visualization. It also provides a library of shared records such as experimental protocols, and a set of reporting and administration tools. adLIMS (Calabria et al. 2014) is an open-source customization of ADempiere ERP, available both as a web-based and a Java fat application. It provides sample management, data standardization and exchange, workflow traceability and roles management. Onco-STS (Gavrielides et al., 2014) is a web-based sample tracking system for oncogenomic studies. It allows more efficient and secure sample data handling, simultaneous remote access to the system to ease collaboration between researchers,

and analysis details (e.g. Quality Control). QTREDS (Palla et al., 2014) is a web-based LIMS for sequencing and genotyping. It provides several features such as sample, workflow and user management, inventory and experimental protocol definition. SeqBench (Dander, Pabinger et al., 2014) is another web-based application, used to manage and analyze exome sequencing data. It provides access to the analysis pipeline SIMPLEX and it presents results accompanied by functional annotations. LabIS (Blazek et al., 2015) is an open-source LIMS developed at the University of Hradec Králové (Czech Republic). It is developed as a SaaS with a web-based interface, and it consists of four main modules: Security (authentication), Groupware (collaboration tool), eLearning (electronic learning materials), and LabApp. The latter module takes into account a standard analysis process cycle, composed of definition of the study purpose, experiments, data analysis and result publication. SaDA (Singh et al., 2015) is a web application LIMS used for storing, retrieving and analyzing data from microarrays. It provides sample and filter tracking, experimental metadata management and microarray analysis. It also provides data exchange with common data formats.

A useful resource to check for updates on LIMS availability is the LIMSWiki (2015).

## LABORATORY ASSISTANT SUITE

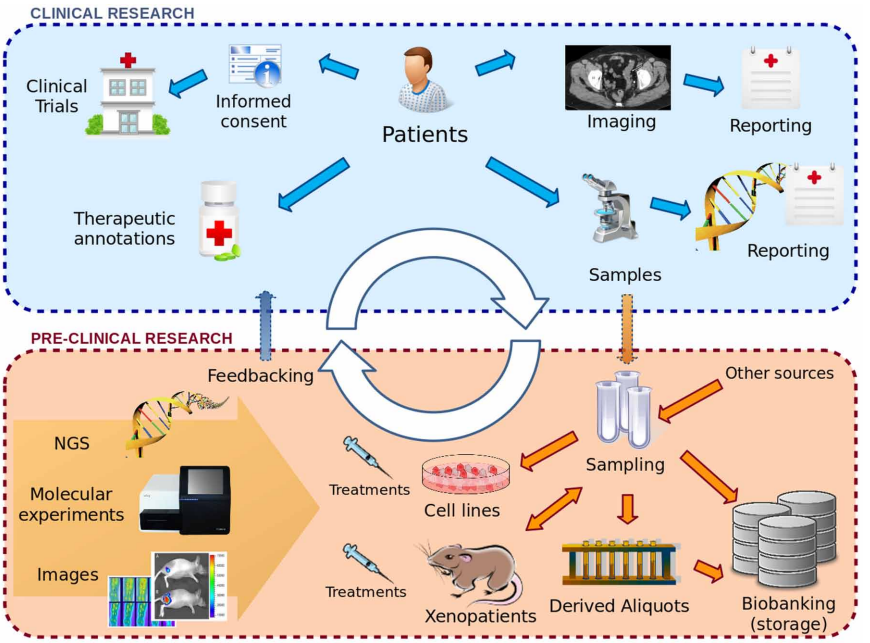
### Context

Translational research aims at enhancing patient care, transferring scientific discoveries from the laboratory to a real clinical context. It is a kind of metaphorical scientific cycle from bench to bedside and back again through complex iterative processes, operating between (i) laboratory (i.e., pre-clinical research) and (ii) clinic (see Figure 1).

Pre-clinical researchers perform their laboratory activities and then provide clinical investigators with relevant scientific information. Those scientists can use that information on the clinical side for designing new trials and calibrating new cancer therapies. However, a reverse flow also exists in the translational process, whereby a subset of patient specimens collected during clinical trials is donated to research for pre-clinical experimentation. Besides, clinical researchers provide feedback to laboratory scientist about therapies, patient health conditions and other significant issues.

Such a chain of processes produces huge data collections with complex schema and dynamic relationship patterns. Those data need to be properly managed by an information system devoted to tracking materials and information flows between laboratory and clinic in an integrated fashion.

*Figure 1. The translational data flow*




## Pre-Clinical Research

Every day several procedures are performed in research laboratories to analyze different biological and medical aspects of tumors, with the aim of discovering new knowledge and improving the therapies. We started to analyze a subset of procedures developed and adopted in the research laboratories of our institution to model our environment. At the beginning, we focused our attention on the procedures that are involved in the xenopatient experimental pipeline (Bertotti et al., 2011). This approach is based on the serial transplantation of human tumor specimens in immunocompromised animals. The aim is to help in translating the correlative information emerging from data integration into clinically relevant and functionally validated biomarkers. After completing the main modules addressing the management of tumor specimen and xenopatient life cycle, we started to include additional elements in our environment in order to manage several research activities and exploit collected data. In the following, we describe the main activities of a research study including the xenopatients approach.

Tumor specimens are initially collected from surgical interventions. From the individual patient-derived material, a set of aliquots is generated (i.e., vital, RNA later and snap-frozen). According to the characteristics of the aliquots and the purpose of the research study in which they are collected, different operations can be performed: storage in a dedicated container (e.g., freezers), extraction of derived aliquots such as DNA and RNA, implantation in immunocompromised animals (i.e., xenopatients) and derivation of cell lines. Researchers can apply different experimental treatments on the implanted animals and monitor them. For instance, researchers can monitor the evolution of the tumor mass in an animal by means of measurements, and evaluate the response to drugs according to well-defined treatment protocols. Moreover, new tissue samples from these animals can be generated for further analyses. Indeed, all the (derived) aliquots can be exploited for experimental analyses with different technologies. For instance, the expression values of thousands of genes can be analyzed by means of the microarray technology, while Sanger sequencing experiments allow the identification of genetic mutations in target sequences. Besides, Next-Generation Sequencing techniques have become available in the last few years, reducing analysis cost and increasing data throughput hugely.

## Clinical Research

Clinical research is a fundamental step to the improvement of the state of the art of cancer medicine. This stage involves voluntary patients enrolled in clinical trials for the evaluation of the effectiveness of new drugs or treatments. Each patient signs an informed consent to allow clinical researchers to track their data anonymously and, in many cases, to provide pre-clinical researchers with her/his own specimens collected during the clinical trial. In that medical stage, patients follow experimental protocols and their health condition is continuously supervised, generating collections of clinical information.

We analyzed the main processes of clinical research in our organization to investigate the information flow. There are different types of clinical data, for instance: tumor description, tumor assessment, therapies, surgical interventions, diagnostic events, etc. In addition, recent diagnostic techniques produce data at the molecular level. Therefore, clinical research also deals with complex genetic information.

If the patient agrees, a part of her/his collected biological specimens (e.g., fresh tissue or blood) can be made available to the research laboratories. That bio-material flow is the one of the main starting points of laboratory research. Clinical data are bound to specimens collected, since the clinical history of a specimen represents a fundamental source of knowledge for laboratory researchers during the design of experiments and the analysis of results.

## Pre-Clinical and Clinical Integration

To the aim of managing and integrating pre-clinical and clinical information, a robust but flexible data management platform is needed. In particular, different types of information (e.g., biological data, molecular data, procedure tracking data, sample tracking data), some of which can be highly complex, should be independently managed by the platform but, at the same time, interconnected to permit integrated analyses. User interfaces should on the one side be practical and intuitive, and perfectly fit the actual procedures on the other, in order to avoid hindering the experimental pipeline. This is particularly relevant when working with biological samples, which implies that data should be entered by the user in a hostile environment (e.g., while working with gloves on in sterile conditions with potentially infectious samples).

## Architecture

The LAS platform has been developed using the Model-View-Controller paradigm (Leff & Rayfield, 2001). With this architecture there is a separation of software components based on one of the three roles that they can play.

Regarding data modeling, LAS uses different database technologies to fit the needs of the application and to treat the heterogeneous data characteristics in a suitable way. In particular, LAS uses a relational database in order to track entities and their properties. This database also stores information about the various experimental procedures. Besides the relational database, (which could be thought of as the "operating" database), a non-relational document database is exploited for storing complex data and files generated by laboratory instruments. A graph database (Neo4j, 2015) is also employed for three main purposes:

- **Representation of Biological Entities and Their Relationships:** The biological entities generated in LAS are inherently hierarchical. Reconstructing the genealogical tree of each entity, performing ad hoc queries and isolating all entities related to the same patient are an essential part of clinical data management, and ensure appropriate action can be taken when there is a change in the conditions defined by the informed consent.
- **Knowledge Base and Ontologies:** The domains covered by the LAS modules are vastly heterogeneous. They are represented in the graph database, thus forming a knowledge base exploited throughout the LAS universe, and can be easily interconnected with one another. The knowledge base is structured into several "layers", corresponding to different levels of abstraction (e.g. Genomic-protein, Clinical, etc.), and can be augmented with new domains.

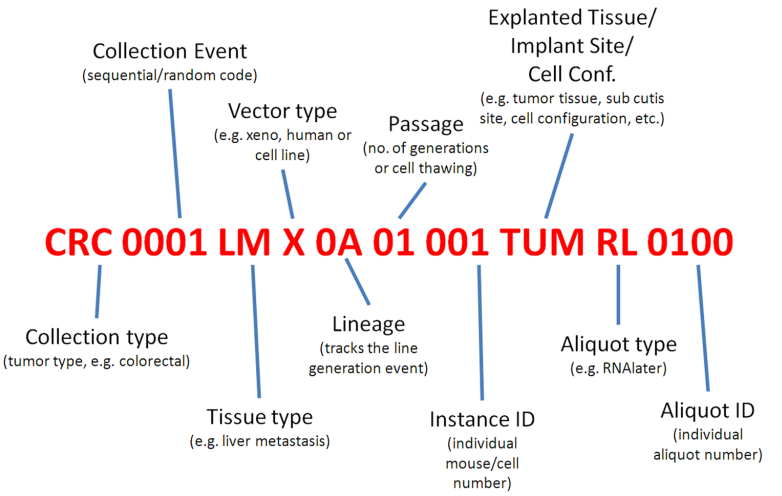
- **Social Network, Data Access Control:** LAS has been conceived for use by different research groups. Thus, data should carry information about their owners. By modeling ownership as a social network on the graph (i.e., nodes representing users and research groups), it is easy to manage data access permissions. We can also take advantage of this layer to represent collaborations between different research groups or users and manage the sharing of data permissions.

The following entity types are defined by LAS to properly manage clinical and pre-clinical data:

- **Patient:** This entity represents a person having signed an informed consent (IC) to allow the collection of specimens for research purposes.
- **IC:** It represents the signed informed consent for a study. Without a signed IC, no specimens or information whatsoever about the patient may be collected.
- **Study:** The study can be for instance a clinical trial or a research study approved by one or more institutions. Each study has a set of associated rules that define the constraints for collecting biological samples and performing research experiments.
- **Collection:** It represents the collection of several specimens sharing homogeneous characteristics or a common source event. For instance, a collection can pertain to all of the tissues collected from one patient, following a surgical intervention. Different surgical interventions on the same patient usually translate to separate collections.
- **Aliquot:** The collected specimens split according to their intrinsic characteristics. The system is capable of managing several kinds of aliquots (e.g. viable, RNALater, SNAP Frozen).
- **Biomouse:** It represents the biological complex generated by the implant of an aliquot into a given animal. If multiple aliquots are implanted in the same physical mouse at different sites, different biomice are generated.
- **Cell Line:** This entity represents in vitro experiments. Cell lines can be generated from viable aliquots by means of a generation and thawing process, or from other cell lines by means of expansion procedures.

Each collection and biological entity (i.e., aliquot, biomouse, cell Line) is identified by a unique and mnemonic key, named GenealogyID, which encodes relevant information regarding the history of the entity. This key is automatically generated by the LAS platform through formal rules. In Figure 2 the structure of the GenalogyID is reported. The first part of the GeneaologyID (up to the instance

*Figure 2. Genealogy ID structure*




ID field) summarizes information about the ancestors of the bioentity, while the last part is used to describe some specific features of the current bioentity (e.g., aliquot type for aliquots, implant site for biomice).

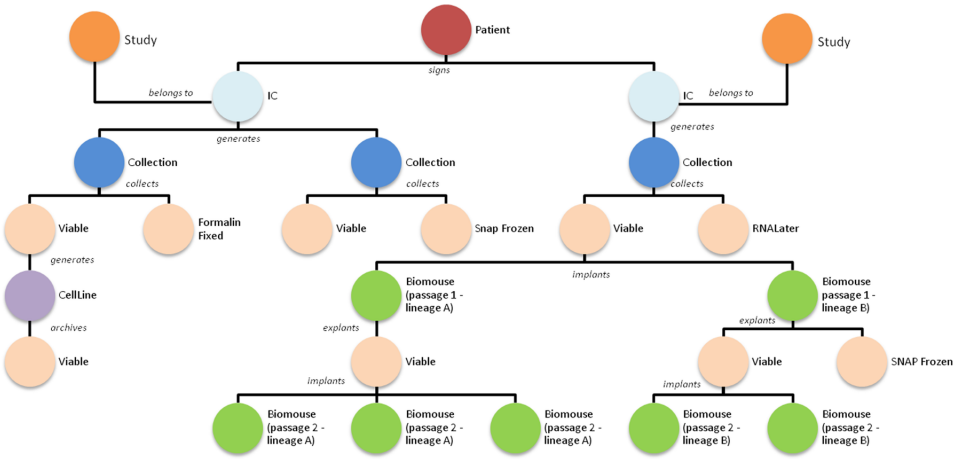
In Figure 3 we report a graph representation of the relationships among the LAS entities. It may be noticed that one patient can generate one or more collections by means of the same informed consent, or through different ICs related to different studies. Each collection can be composed of different aliquot types and several instances of the same aliquot type. If a viable specimen is implanted into animals, it generates one or more biomice. First-generation (or "first-passage") biomice are also labeled with a different lineage identifier (e.g. A, B). Each biomouse can generate aliquots that can in turn be implanted into other animals, so as to generate second-passage biomice. The implantation/explant process can be repeated several times according to the purpose of the research study. Viable aliquots can also be used to generate cell lines, which can be expanded and/or archived to produce other generations.

All such relationships are stored in the graph database, while detailed information about each entity and the description of the procedures applied are collected in the relational database.

## Social Layer

The management of data produced by different people and/or groups requires that access to functionalities and information be limited according to several aspects such

*Figure 3. Graph representation (genealogy tree) of the relationships among LAS entities. The nodes belonging to the same macro-category are represented with the same color. The edges are labeled according to the procedure exploited to generate the children.*




as group and/or project membership, and user role. For these reasons, the platform manages users and their privileges according to the following concepts.

- **Working Group:** A Working Group (WG) is a set of users in the LAS platform that work together towards a specific goal (e.g., project, research activity). The data produced by the users of the same group are private, unless they are intentionally shared with other groups.
- **User Profiles:** The users belonging to a WG can be divided into two main categories:
  - **Principal Investigator (PI):** He/she is the manager of one or more WGs. PIs can add or remove users from their WGs, and grant or revoke permission to access functionalities for every user in their WGs. During the registration process, PIs can select one or more activities they wish to handle within LAS. Their registration will be evaluated by the system administrator.
  - **LASUser:** He/she is a standard user, whose activities depend on the choices of the PI. During the registration process, LASUsers should select the PI they report to. They are also asked to specify one or more roles, according to the task(s) they need to carry out, so as to help the PI in assigning appropriate functionalities during the registration acceptance phase.

Users of both categories (i.e., PI and LASUser) can only access the LAS functionalities they have been enabled to use, and they can only manage data that belong to their WGs. Only some roles are allowed to share data with another WG. Data sharing can be performed either through a transfer operation, or directly through the Query Module when the query results include bioentities such as aliquots, biomice or cell lines.

To properly manage the different entities, Laboratory Assistant Suite includes a set of fully-fledged applications, or modules. Each module addresses the management of a specific type of entity and its associated experimental procedures. We can catalogue the modules included in the current version of LAS under the categories described in the following.

## Clinical

The Clinical Manager Module is devoted to the management of patient clinical information, collected during trials and follow-ups. This module tracks the patient data entity and related clinical activity. Since a patient could be enrolled in more than one trial, we centralized the management of patient information in a single module, irrespective of the trial context, in order to relate all data referring to the same patient to the same data entity.

In the clinical module, we track for each patient both context information (i.e., personal data, medical center of the trial, etc.) and relevant clinical events through the LAS Case-Report-Form (CRF). All data are linked to the corresponding informed consent. This module allows clinical investigators to assess cancer status over time also at the lesion level, if necessary, by tracking the lesion type (i.e., primary or metastasis) and its anatomic position. In clinical trials, therapy information is essential, therefore we designed the clinical module to manage and classify therapeutic data and their relationships with pathology or target lesion, in case of local treatments (e.g., radiotherapy). A similar approach has been taken to tracking surgical interventions and related data. The clinical module has is also capable of managing the flow of bio-materials (e.g., fresh tissues). These specimens play a pivotal role in translational research, since in many cases they are collected and provided to a laboratory, where researchers can use them for bench activities along with clinical information collected during a trial. Patient personal data are not disclosed in this phase, since laboratory researchers can use clinical information only anonymously.

Clinical data types are very heterogeneous and are characterized by dynamic and complex schema and relationship patterns (e.g., an MRI image could be related to the observed lesions, to the medical center where it has been performed and to its reporting). The module tackles this complexity by modeling data in a dynamic, flexible and scalable way.

## Biobank

With the rapid advances in biomedical and genetic technologies, collections of biological materials have attracted increasing attention from the researcher community since they represent a fundamental resource for the research and the diagnosis of different pathologies, and the study of possible therapeutic applications. Such collections, named biobanks, are commonly divided into tissue and genetic biobanks according to the types of biological materials they store. Our platform addresses both issues. In particular, the module named BioBanking Management Module manages a wide range of activities, including management of biological samples and associated pathological information, as well as support to a number of laboratory-related procedures. For instance, the module can currently handle: (i) the collection of biological material from surgical intervention and acquisition of aliquots from external laboratories. Aliquots stored in the system are characterized by features such as tumor type (e.g., colorectal), tissue type (e.g., liver metastasis), source hospital or laboratory, and pathological information; (ii) measurement of aliquot physical characteristics, such as volume, concentration, purity and quality; (iii) derivation of new biological materials (e.g., DNA, cDNA); (iv) planning of molecular experiments.

The biological material used in our laboratories is stored by means of several types of containers (e.g., freezers, racks, plates, tubes). Their mutual interactions (i.e., which container can host another one) can change according to characteristics such as the layout and the laboratory procedure. For instance, a plate of a given manufacture and model may be able to host only some kind of tubes. Similarly, a research group may like to assign only one type of aliquot (e.g., RNA Later) to some plates. We developed a dedicated module, named the Storage Management Module, which allows managing any kind of container by defining and applying different rules to them.

## In Vivo Experiments

Studies that are "in vivo" (Latin for "within the living") are those in which the effects of various biological entities are tested on whole, living organisms – usually animals (e.g., xenopatients). This kind of experiments are normally exploited to test drug therapies and expand the collection of biological samples. We based our development on the model described in Bertotti et al. (2011) to manage immunocompromised animals and monitor the xenopatient life cycle, from their acquisition by the research institute to their death. In particular, during the acquisition of the animals, we track several features such as status, strain, age, and source. To speed up the identification of the animal and the retrieval of related information, the system promotes the usage

of barcode readers when mice are equipped with RFid tags. Furthermore, our platform manages the implants/explants of tumor tissue into/from the xenopatients. To perform these operations, the bank and storage applications are involved in retrieving the tumor aliquots stored in the containers (e.g., plate, tube). Since in vivo experiments are usually aimed at testing treatments, we provide interfaces to define the characteristics of treatments and track tumor growth. Treatments can be composed of different phases, each associated with several pieces of information related for instance to the drug, the administration mode, the dose, and the administration frequency; the measures associated with the tumor mass can be categorized in qualitative and quantitative. Finally, scientists are supported along the decision process by means of ad-hoc graphical utilities that allow monitoring of all experimental features (e.g., tumor growth) and planning of activities.

## In Vitro Experiments

Unlike in vivo experiments, "in vitro" studies are performed with cells or biological molecules studied outside their normal biological context. The purpose of these experiments can be the manipulation of tumor cells in a controlled, artificial environment in order to test the effects of different drug therapies on the cells without the bias effects induced by a whole organism. The LAS platform defines a cell line as the set of bioentities that are generated from the same biological entity and are under the same experimental conditions. The experimental conditions are defined by the protocols that describe the type of process (i.e., adherent, suspend, organoid) and the set of culturing conditions applied. The culturing conditions are made up by a set of items categorized into the following groups: (i) nutrients and chemicals, (ii) hormones/growth factors, (iii) antibiotics, (iv) serum, and (v) media. In addition to the definition of experimental protocols, the platform allows the management of the generation/thawing procedures of cell lines. In particular, the researcher can generate/thaw several cell lines by selecting one or more generation protocols and, similarly to the implantation of xenografts, the generation can be handled by using the Biobank and Storage modules for retrieving the aliquots of interest, by means of their container barcode. The generated cell lines will inherit the characteristics of the selected protocols. During the cell line life cycle, scientists can perform a set of operations: (i) trash one or more plates, (ii) expand the cell line by defining dilution parameters, expansion protocols and the number of output plates for each protocol, (iii) send a set of plates to experiments, and (iv) plan archival procedures. The archival procedure generates different aliquot types with their associated parameters (e.g., volume [ml], count [cell/ml]) and positions them within the destination container.

## Molecular Experiments

Different types of molecular analyses can be conducted on biological samples, to investigate various aspects of their genetic constituents that may have an impact on the development of oncogenic behavior. For instance, biologists may be interested in analyzing mutations for a target gene which is involved in tumor proliferation. For this purpose, several techniques have been developed over the years, and are nowadays largely exploited in research laboratories. In an effort to closely track the translational research pipeline from the collection of samples to their analysis, Laboratory Assistant Suite provides support to tracking the most frequently used techniques in our institution: Sanger sequencing, Real-time PCR and Sequenom.

Sanger sequencing is a method of DNA sequencing based on the selective incorporation of chain-terminating dideoxynucleotides, named primers, by DNA polymerase during in vitro DNA replication. This technique was developed by Frederick Sanger and colleagues in 1977, and it is one of the most widely used sequencing methods. Since this approach can retrieve only a small sequence of the target region delimited by the primers, in the last years next-generation sequencing techniques have become increasingly popular, due to their powerful method of sequencing the entire genome. Nonetheless, Sanger sequencing is often still preferred, due to its lower costs, when only a few, relevant genes need to be analyzed.

The real-time polymerase chain reaction (RT-PCR) is a laboratory technique of molecular biology based on the polymerase chain reaction (PCR). It is used to amplify and simultaneously quantify a targeted DNA molecule. The quantity can be either an absolute number of copies (CN) or a relative amount when normalized to DNA input or additional normalizing genes. Unlike classical PCR, the amplified DNA is detected during the reaction progress (i.e., in "real time") and not only at the end.

Sequenom SNP genotyping uses a bead-less and label-free primer-extension chemistry to generate allele-specific products with distinct masses. Allele discrimination is conferred by primer extension with a high-fidelity polymerase enzyme across the SNP site, leading to differences in mass of extended products. Differentiation of genotypes for each of the SNPs multiplexed in one assay is a result of unique mass ranges for the extension primers.

All of these technologies are specifically handled by dedicated LAS modules, which track every phase of the molecular experiments according to a common pattern: sample validation, definition of the experimental design, upload of any raw data files generated by the instruments, definition of the analytical pipeline (if any), and upload of structured result data. Each molecular module resorts to a specific set of ad-hoc APIs, made available by the Genomic Annotation Manager and allowing an appropriate description of the experimental context. For instance, the Sanger

Sequencing and the Real-Time PCR modules need to connect to the GAM to retrieve the description of all amplicons (of the appropriate technology type) as well as of all possible alterations (e.g. sequence alterations, gene copy number variations) known in the literature, to allow both the experiment definition and the result submission phases. The results of each analysis are ultimately submitted to the GAM, serving as a common repository of molecular and genomic data annotations.

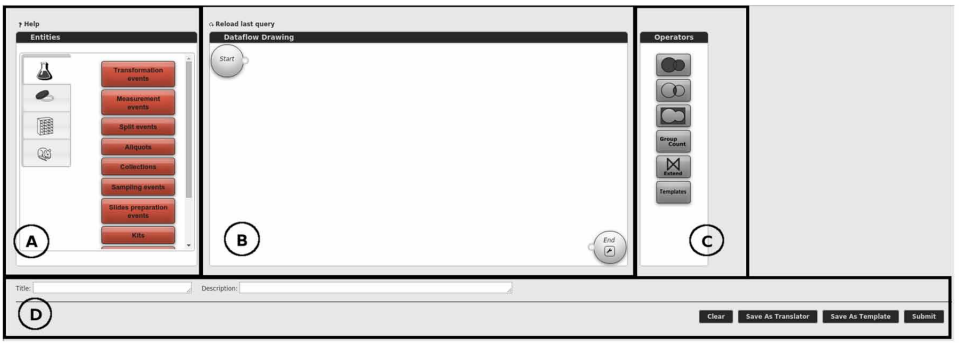
## Query

In addition to tracking experimental procedures and recording all the data related to biological entities, the integration of heterogeneous information is fundamental to discovering new knowledge related to tumors. The Query module, named Multi-Dimensional Data Manger (MDDM), can extract all information of interest from the databases in a uniform way by exploiting a graphical tool, named the query generator (Figure 3). Queries are generated by defining a workflow (block B in Figure 4) composed of one or more blocks, named query blocks, which are shown on the left-hand side of the editor (block A) and categorized according to the module from which the data are drawn (e.g., the flask icon for biobank data, the mouse icon for xenopatient data). Each query block defines the object that will be retrieved (e.g., aliquot, xenopatient, container), its related information of interest and the filtering conditions. Set operators (union, intersection, difference) and special operators (group-count, extend, template blocks), listed in block C, can also be used in the workflow. Before retrieving the data from the corresponding modules, the workflow is analyzed to detect improperly defined operations (e.g., intersections among disjoint sets of objects) and define an optimal execution plan on the distributed databases. Once the workflow has been defined, a title and a description may be assigned to the query (block D) to reuse it in the future for different purposes. A query may also be designed, saved as a template and provided to unexperienced users, for use by means of wizards. Finally, the system allows enriching the result set with additional information, by means of predefined templates.

## Genomic Annotation Manager

Molecular experiments can easily generate huge amounts of data, with disparate representation formats. Whereas the LAS Molecular Experiments modules are specifically targeted at generating quantitative data from a variety of experimental sources and techniques, the Genomic Annotation Manager (GAM) provides a higher-level, qualitative insight into the genomic features of biological samples. This information is shaped in the form of annotations, i.e., a set of semantical labels attached to a sample, pointing out some of its relevant features.

*Figure 4. Query generator interface*




To ensure semantical coherence and adopt a somewhat standardized nomenclature, all relevant concepts from the genomic and biological domains used for labeling samples have been drawn from a number of public, freely accessible databases and ontologies (COSMIC, 2015; Forbes et al., 2014; SO, 2015; Eilbeck et al., 2005; dbSNP, 2015; Sherry et al., 2001). This information has been structured into a knowledge base, modeled as a graph and stored in a graph database (Neo4j, 2015). Concepts are interlinked with one another, according to both general-purpose semantical relationships such as containment ("part of") and generalization ("is a"), and domain-specific relationships (e.g. pointing out an underlying biochemical process, as in "is transcribed from"). New concepts and relationships, as well as new domains of interest, may be added as needed, to account for novel findings and broaden the spectrum of investigation.

Within the GAM, every annotation is thus a semantical statement establishing a relationship (by means of a predicate) between a biological sample (the subject of the statement) and a concept (the object of the statement), such as a genetic mutation. It is represented within the graph database as a node of type "annotation" with a pair of incoming and outgoing edges – one linking the biological sample to the annotation node by means of a "has_annotation" relationship, and the other linking the annotation node to the reference node in the knowledge base by means of a "has_reference" relationship. The annotation node is often linked to other nodes, such as the process that produced the annotation or the raw experimental data.

The GAM is organized into multiple components, and includes the following.

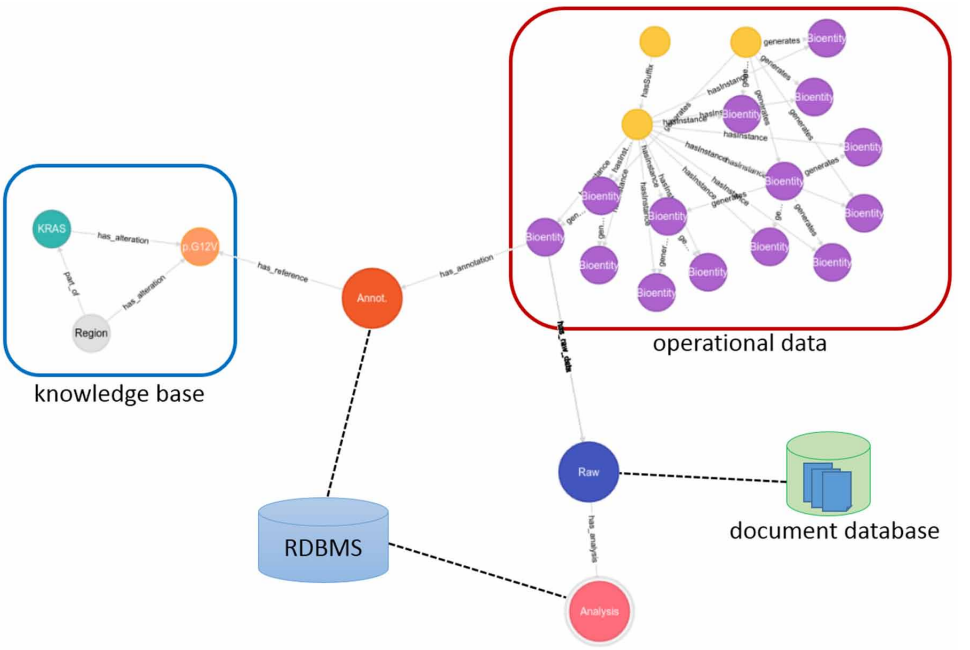
- **Genome/Transcript Reference:** The GAM embeds a graph-based representation of the latest human genome assembly with annotated features, such as genes, exons/introns, and relevant transcripts, drawn from the Gencode project (Gencode, 2015). Many of the concepts and relationships

used to describe the genomic domain have been borrowed from the Sequence Ontology (SO, 2015).

Much like with popular genomic browser utilities (UCSC Genome Browser, 2015), every object that becomes a part of the GAM needs to be identified by means of its coordinates within the human genome. To this aim, the GAM offers an alignment facility, exploiting the BLAT alignment tool (Kent, 2002). For instance, many of the specific reagents employed by the LAS Molecular modules, such as primers, amplicons, or probes, are also modeled within the GAM in terms of the genomic region(s) they are designed to inspect, and stored inside the reference database. This allows some automatic reasoning to take place, such as inferring a list of candidate alterations that may be spotted in a given molecular experiment, based on the reagents used.

- **Domain-Specific Knowledge Base:** This component includes information from a number of public databases, modeled in graph form. It provides the concepts and the relationships used for annotating biological samples. This information, as well as the genome/transcript reference, may also be accessed by other LAS modules to provide context, through a set of APIs.
- **Normalized Molecular Data Repository:** Whenever a molecular experiment is created from one of the LAS Molecular modules, the corresponding results are automatically submitted to the GAM as a new analysis batch. The data are stored in a relational database in a normalized and self-contained format.
- **Validated Annotation Database:** The normalized molecular data may be inspected by the researcher to identify meaningful events and correlations. A multi-dimensional querying engine is available to this aim, allowing the user to choose the type(s) of events she wishes to inspect, the gene(s) affected, and the biological samples. The results of the query may be exported to a spreadsheet for off-line review, or they may be validated to generate a set of relevant annotations. In the latter case, the molecular data may be automatically categorized as appropriate (for instance, by selecting the ontological concept that best describes the numerical value of the data), and new annotations are created in the graph database. The original molecular data are linked to the corresponding annotations, and may be accessed at any time. Annotations may also be queried and used in turn to generate further annotations. The core idea somehow mimics the roll-up and drill-down operations of the dimensional fact model leveraged by data warehousing systems (Golfarelli & Rizzi, 2009): by

*Figure 5. Semantic annotation model*




navigating both the biological sample hierarchy and the conceptual ontology, annotations may be generated with different granularities and varying levels of conceptual abstraction. In addition, cross-domain links (e.g. between the gene copy number and the drug treatment response of a specific sample) may be established and annotated.

## USE CASES

The following sections illustrate how the Laboratory Assistant Suite platform can concretely support laboratory research. We present two real application scenarios we encountered in our laboratories, and describe how the LAS tools address these issues. Even though the idea and the design of such tools were based on the functional requirements of our laboratories, we believe they reflect common practice in the oncological research field and are general enough to be useful to other researchers from other institutions.

## Use Case 1: Patient Enrollment and Aliquot Collection

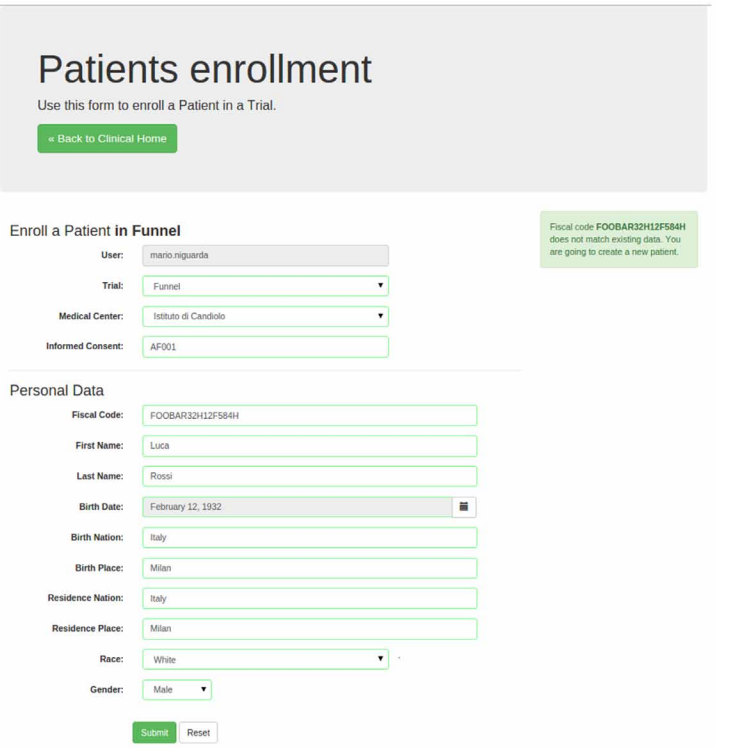
The clinical module provides clinical investigators with a user-friendly front-end to enroll a new patient. In the example in Figure 6 the form dedicated to the use case is shown.

In the first part of the form, the user has to fill in the trial data and the name of the medical center where the patient is going to be enrolled. Next, the code of a new informed consent is requested.

The second part of the form is dedicated to the patient's personal data. If the clinical module binds the new patient data to an existing patient, it appends the new enrolling information to the existing patient entity. Once the form has been accurately filled out, the user can submit the enrollment request to the system. When the patient is properly enrolled in a trial, clinical investigators can start inserting data in the case-report-form (CRF) and registering samples belonging to that patient.

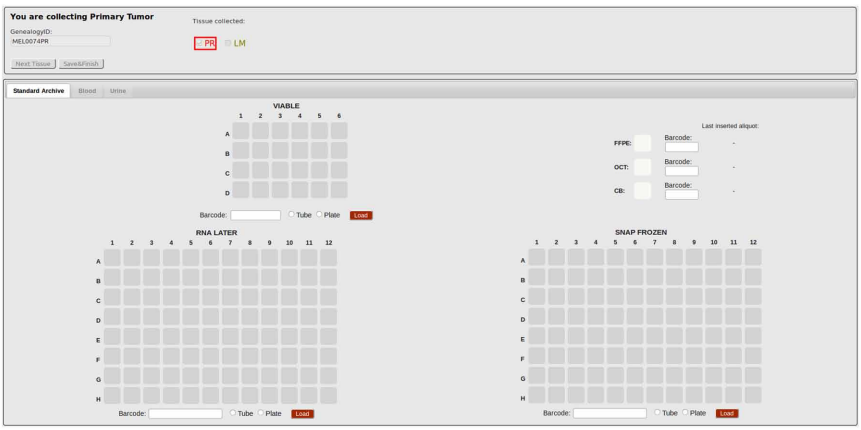
In some cases, sample registration could happen before the corresponding enrollment event. That is a case of event-flow reversal and, to tackle that problem,

*Figure 6. Patient enrollment interface*



the BioBanking module has been designed to communicate with the Clinical module. In this scenario, the procedure starts in the BioBanking module with a sample collection operation, consisting of two phases. In the first one, the researcher inputs preliminary information about the collection event (e.g. informed consent) and selects one or more tissue types. Besides, it is possible to insert an identifier for the patient and several kinds of clinical data (categorized according to an internal ontology) correlated with the surgical intervention. In the next phase, the researcher can add one or more samples for each of the tissue types chosen. Figure 7 depicts the interface for the collection of biological samples, which is designed to closely match the physical working environment of the researcher. Every tab holds the containers for viable, FFPE, RNA later, and snap-frozen aliquots that are usually collected for in-vivo and in-vitro experiments. To speed up data entry procedures, most pieces of experimental equipment (i.e., clinical folders, plates, tubes) are bar-coded by the institution prior to their use, and can be identified by means of barcode readers. The user may thus load or create plates or tubes, and she can insert an aliquot in a given position by simply clicking on the corresponding cell. This operation can be comfortably achieved on a touch-screen device without resorting to a mouse. This results in a reduced number of lean interactions with the system, allowing the researcher to focus on the experimental task at hand, rather than on data entry operations. At the end, the BioBanking module sends the informed consent code to the clinical module, and a new patient is created, with an attached sample collection, but without any personal information, since laboratory researchers are not allowed to manage personal data. Later, a clinical investigator will fill out the patient information using the Clinical module.

*Figure 7. Collection interface*



## Use Case 2: Derivation of Aliquot for Sanger Sequencing Experiment

To perform molecular experiments, researchers need to create derivative aliquots compatible with the target analysis. For Sanger sequencing, DNA aliquots are required, so first of all it is necessary to track their creation using the BioBanking module. In this case, the researcher will plan a derivation for the selected aliquots and assign this task to a user (e.g., a technician). The user in charge has to select the aliquots, the derivation protocol and the corresponding kit involved in the process. Then, she proceeds to the last part of the procedure that is managed by the interface depicted in Figure 8. In the top side (block A), information related to the derivation protocol and the output are visualized. In block B some parameters related to the preparation of new aliquots are reported. Some values (e.g., volume and concentration of generated aliquots) can be changed at will by the user for each aliquot, and the system will automatically update the values of the others to match the protocol rules. Once the technician has prepared the aliquots, she has to position them in some containers (block C). User inserts the corresponding barcode to load the containers and then positions the newly generated aliquots in the available places using drag and drop. When all aliquots have been processed, a final report is shown.

Once DNA aliquots have been derived, a Sanger Sequencing experiment can be executed. The procedure starts in the BioBanking module with the choice of the type of experiment (among the many available), the samples involved, and the user responsible for carrying out the operation. These data are sent to the Sanger Sequencing module, that handles every part of this type of experiment, and the web browser is automatically redirected to the Sanger experiment request page. After filling in some basic information, such as a title and a description for the experiment, the operator executing the sequencing task follows a procedure guiding her throughout the various steps of the experiment. The procedure may always be interrupted at a given step and resumed later, allowing the user the time to carry out the actual experimental procedures.

In the first step, each physical sample collected by the user must be validated by scanning the corresponding barcode, to ensure samples are not mistaken. Next, the user may proceed to the experiment definition page. The main purpose of this step is to select the set of amplicons that will be used to perform Sanger sequencing. Recall that each amplicon will amplify an interval of nucleotides lying on a specific gene (or in a gene region, such as a given exon). Thus, a combination of multiple amplicons, named an assay, is typically used with the same samples, to investigate possible alterations in several genomic regions. The selection of amplicons from the Sanger experiment definition page relies on the interaction with the Genomic Annotation Manager (GAM), whose knowledge base already includes the descriptions

of all available amplicons. To select the desired amplicons, the user must enter a valid gene symbol. A list of all amplicons matching the specified gene will be returned by the GAM and shown to the user, who may so assemble her own assay. A 'Preferred targets' list is also available, recommending amplicons that have often/recently been exploited. The user may also permanently save the current assay for later re-use, by assigning it a name.

After defining the experimental setting, and once the Sanger sequencing experiment has been performed, results must be entered into the system in two ways. First, one or more raw data packages may be selected from disk and uploaded to the LAS Repository for permanent storage. These are normally data files generated by the sequencing device. As in most cases such files are analyzed once by external ad-hoc software and never accessed again, the user may choose to ignore this step, in which case no files will be stored in the repository for the current experiment. Next, structured results, obtained from the analysis of the raw data, must be entered. The corresponding user interface is shown in Figure 9. Depending on the experimental technology, different genomic or transcriptional features of the samples may be assessed, such as possible sequence alterations, structural variants, copy number variations, or gene expression levels. In the case of Sanger sequencing, the system currently only supports sequence mutation calling.

The interface shows a table, listing all analyzed samples, together with the genes targeted by the analysis. Each cell at the intersection between a gene and a sample displays the current mutational status of the sample for that gene. Note that, when multiple amplicons have been used to amplify different regions of the same gene, their results will be grouped under the same gene column.

All cells are initially set to "WT" (wild type), signifying that no mutation has been detected. The mutational status can be changed by clicking on the pencil icon in the cell. A pop-up window will open, showing a list of all known sequence alterations relative to the gene. This list is automatically populated by the GAM to include only alterations falling within the regions amplified by the amplicons used in the experiment. New alterations not currently in the knowledge base may also be added. For each detected alteration, an allele frequency (defaulting to 0.5) must be provided. Both the alterations and the corresponding allele frequencies will be displayed in the mutational status cell, which will also change its color from green to red. As an alternative to manual entry, mutational data may also be read from a file; this is useful when a mutational report is exported from the analysis software.

The wrench button beside each sample allows specifying the outcome of the experiment (in terms of "failure" or "success") for each amplicon, since sequencing errors ("badseq") may need to be recorded as well as detected alterations. When an amplicon has been marked as "failed" for a specific sample, the alterations

encompassed within its amplified region will no longer be shown in the mutational status pop-up window.

Once the mutational status for each sample has been properly described, the user may click the “Save measures” button. The data will be saved locally in the Sanger Sequencing database, and sent to the GAM, originating a new analysis event. From then on, this data will be part of the annotation database, and may be queried from the GAM interfaces.

*Figure 8. Derivation interface*

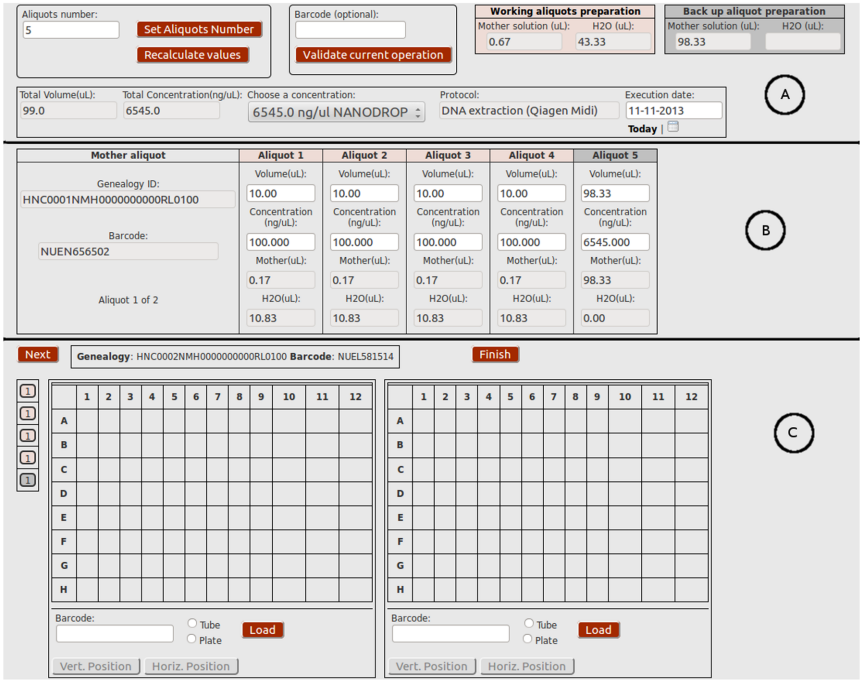
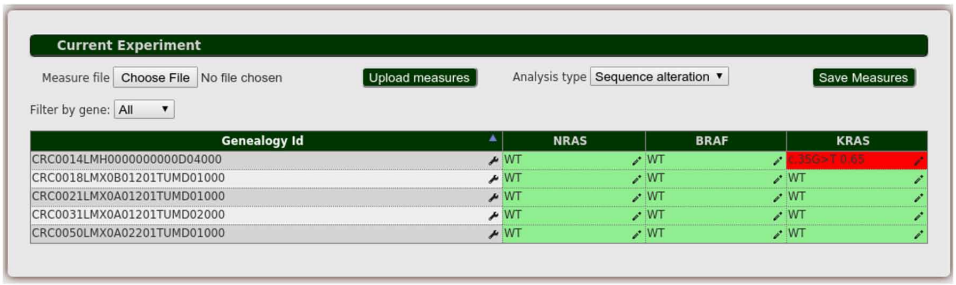



*Figure 9. Sanger sequencing experiment results interface*



## USAGE STATISTICS

The Laboratory Assistant Suite platform has been actively employed for research in our institution since March 2012. Only a few modules (e.g., BioBanking, Storage) have been available in the first release, with functionalities similar to those of the current version. Instead, the module addressing user authentication and data privileges, and the MDDM initially provided only a limited set of basic functionalities. During the first year, new functionalities have been deployed, and existing ones have been improved based on user feedback. In March 2013, the Sanger and the Real-Time PCR modules have been deployed, while all other modules included in the current suite have been released in June 2013.

Within our institution, a number of research groups are currently using the platform, and a large amount of data has already been produced and stored. In particular, as of October 2015, 4,467 collections, each one including all biological entities (i.e., aliquots, xenopatients) that share a common origin (i.e., the same collection event), are stored in the database. Each month, approximately 77 new collections are started. The BioBanking module currently includes approximately 160,000 aliquots, as pointed out in Table 1. On average, 2,800 aliquots are generated each month, including aliquots coming both from surgical interventions on human patients, and from xenopatients and derivation procedures. Since the most active user group works with xenopatients, approximately 76% of the aliquots stored in the BioBaningk are generated from mice. A detailed categorization of aliquots, based on their source, is reported in Table 2. Derived aliquots represent approximately 24% of the BioBanking content, and most of them are DNA. On average, out of 648 derived aliquots extracted each month, 508 are DNA aliquots. Statistics on the derived aliquot distribution is reported in Table 3. The BioBanking module also tracks aliquot consumption according to the types of experiments. Over 62% are sent to external laboratories to perform special analyses, while other molecular experiments are performed in our institution. As depicted in Table 4, the LAS modules cover a wide range of experiments performed within the institution. Figure 10 plots the number of performed experiments over time. On average, 248 aliquots are used each

*Table 1. BioBank aliquots*

| | Stored | | Available | |
|---|---|---|---|---|
| | # | % | # | % |
| Human | 36,988 | 22.92 | 33,009 | 22.19 |
| Xenopatients | 124,398 | 77.08 | 115,746 | 77.81 |
| Total | 161,386 | | 148,755 | |

*Table 2. Categorization of stored and available aliquots according to the source*

| | Human | | | | Xenopatients | | | |
|---|---|---|---|---|---|---|---|---|
| | Stored | | Available | | Stored | | Available | |
| | # | % | # | % | # | % | # | % |
| RNALater | 5,851 | 15.82 | 4,518 | 13.69 | 36,381 | 29.25 | 34,679 | 29.96 |
| Snap frozen | 519 | 1.40 | 513 | 1.55 | 32,518 | 26.14 | 32,177 | 27.80 |
| DNA | 12,218 | 33.03 | 11,731 | 35.54 | 18,418 | 14.81 | 17,264 | 14.92 |
| Viable | 3,474 | 9.39 | 2,562 | 7.76 | 16,463 | 13.23 | 11,721 | 10.13 |
| Formalin fixed | 439 | 1.19 | 365 | 1.11 | 12,240 | 9.84 | 12,120 | 10.47 |
| RNA | 170 | 0.46 | 155 | 0.47 | 6,070 | 4.88 | 5,709 | 4.93 |
| Plasma | 14,303 | 38.67 | 13,155 | 39.85 | 79 | 0.06 | 79 | 0.07 |
| cRNA | 14 | 0.04 | 10 | 0.03 | 1,446 | 1.16 | 1,418 | 1.22 |
| OCTfrozen | 0 | 0.00 | 0 | 0.00 | 158 | 0.13 | 148 | 0.13 |
| cDNA | 0 | 0.00 | 0 | 0.00 | 625 | 0.50 | 431 | 0.37 |
| Total | 36,988 | | 33,009 | | 124,398 | | 115,746 | |

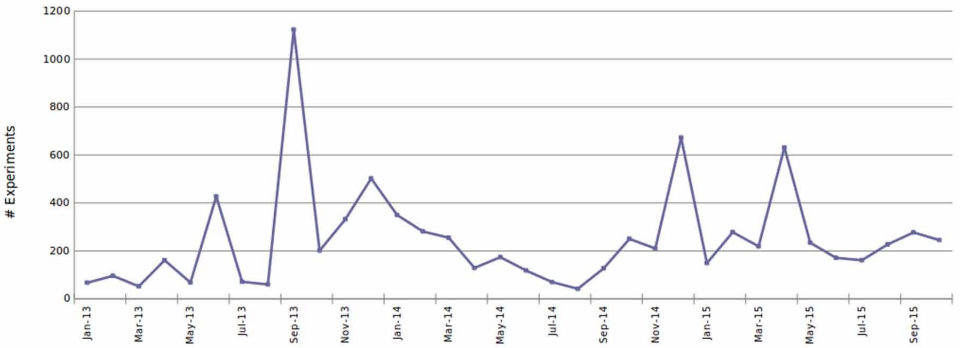
*Table 3. Derivations*

| | # Derivatives Created | DNA | RNA | cDNA | cRNA | # Derivation Events |
|---|---|---|---|---|---|---|
| Total | 37,570 | 29,467 | 6,030 | 625 | 1,448 | 10,462 |
| Average | 648 | 508 | 104 | 11 | 25 | 180 |

*Table 4. Experiments*

| Experiment | # Aliquots | % Aliquots |
|---|---|---|
| Collaboration | 5,406 | 62.44 |
| Real time PCR | 1,552 | 17.93 |
| Histology-IHC | 519 | 5.99 |
| Sequenom | 443 | 5.12 |
| Sanger Sequencing | 409 | 4.72 |
| Microarray | 276 | 3.19 |
| WesternBlots | 53 | 0.61 |

*Figure 10. Number of experiments per month*




month for experiments, except for September 2013 where over 1,000 aliquots were sent to an external laboratory for a particular analysis.

The Storage module manages all containers used by the groups registered with the LAS system. About 160,000 containers belonging to different categories and with different characteristics are tracked. In Table 5, the categories of containers, with the current number of available units, are reported.

The module of in vivo experiments is one of the most currently used ones, since it manages the core research activity of most active users. At the time of writing, more than 22,000 mice have been tracked. Most of them have already undergone programmed explant, while the remaining part are currently under treatment with experimental drugs. A detailed categorization of mice statuses is reported in Figure 11. On average, each month 368 implants and 200 explants are executed, where each explant generates approximately 10 aliquots.

Regarding experimental treatments performed on mice, to date, more than 7,000 treatments have been applied, and 153 are still under execution. On average, about 160 treatments are started each month, and as many are ended. In Figure 12, the number of monthly treatments tracked since the deployment of the LAS system is reported.

*Table 5. Containers*

| Container Type | # Units |
|---|---|
| Freezers | 26 |
| Racks | 370 |
| Plates | 2,132 |
| Tubes full or empty | 157,336 |

*Figure 11. Mice status*

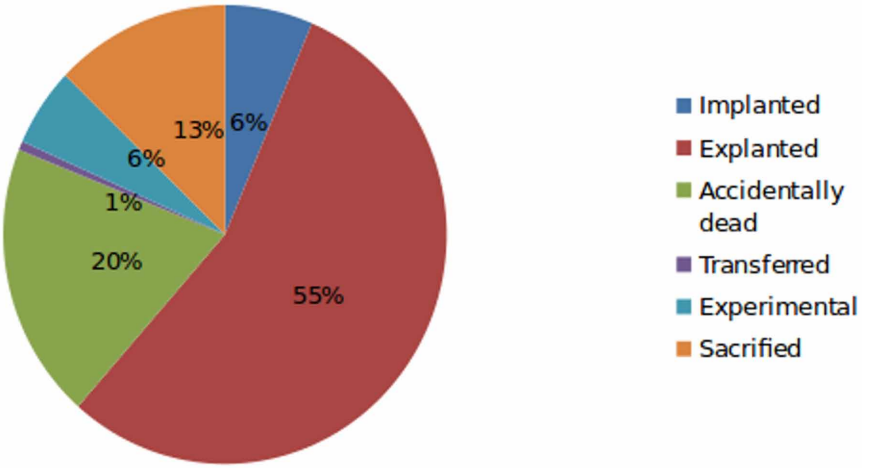
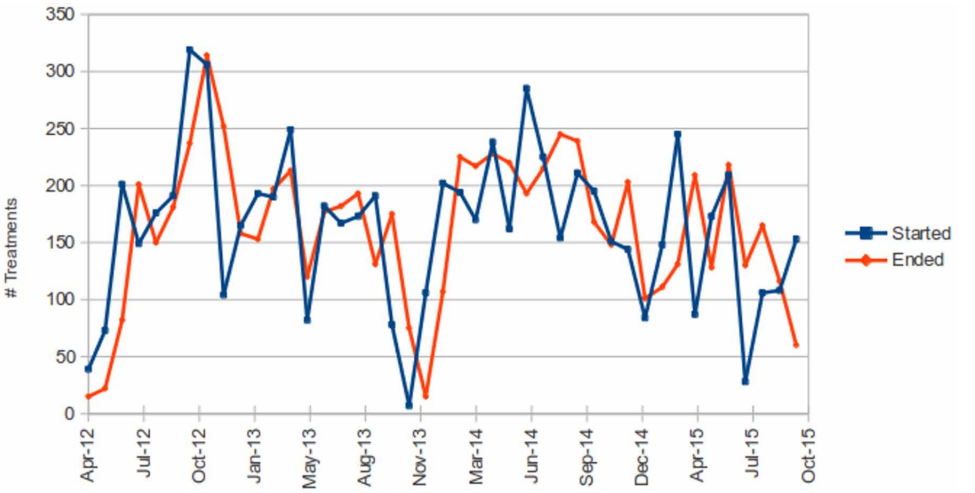



*Figure 12. Number of treatments per month*




## FUTURE RESEARCH DIRECTIONS

Laboratory Assistant Suite is a relatively young project that needs improvements and polishing to meet user requirements and expectations, and to be able to compete with commercial LIMS under some aspects.

We are currently evaluating a possible re-engineering of the LAS architecture. One of the main critical issues is the fact that, in the context of a research laboratory, requirements evolve rapidly, due to the frequent introduction of new experimental procedures. It would be desirable to reduce the amount of time devoted to the development of new software features implementing such procedures. Hence, the new design will focus on the development of generic components that can be reused in different contexts. Another goal is to use pattern-based programming in order to build workflows in a semiautomatic way. With regard to this issue, we are considering the use of new technology solutions that improve the responsiveness of the system and its flexibility. In particular, we are directing our attention to the adoption of the MVC programming pattern also on the client side, and to use of non-relational databases (Pokorny, 2013).

Finally, we aim to ease cooperation between different research groups, potentially in a geographically distributed context. The solution is to have various installations of LAS, whose coherence will be maintained by a single central node. For this purpose, the graph structure should play a central role in the co-existence of multiple installations. Thanks to the use of non-relational databases, workloads could also be managed at the database level, through features like replica sets and sharding.

## CONCLUSION

The Laboratory Assistant Suite (LAS) platform is designed to track several laboratory activities and properly manage heterogeneous biological entities. Researchers are supported in decision-making tasks and building complex analyses on integrated data by means of graphical tools. The exploitation of semantic web technologies to represent relevant concepts from the genomic domain allows effectively annotating biological samples with their molecular results. Furthermore, in the last release, the system allows linking biological samples analyzed by translational research with the originating patients and their clinical history, in order to effectively provide insight into tumor development. The real use-cases presented in this chapter show the effectiveness of the proposed approach in modeling the research environments, managing laboratory-related procedures and linking pre-clinical and clinical data. We believe that future releases of the LAS system will improve the overall reliability and usability of the platform, helping research laboratories deliver high-quality research.

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## KEY TERMS AND DEFINITIONS

**Biobank:** A type of biorepository that stores biological samples for use by research.

**Laboratory Information Management System (LIMS):** Software-based laboratory and information management system that offers a set of key features that support a modern laboratory's operations.

**Molecular Oncology:** A branch of oncology that leverages recent advances in molecular biology to define the changes affecting the control of cell growth, responsible for the rise and development of tumors, in molecular terms, and to trace the steps by which certain tumors evolve.

**Pre-Clinical Research:** A stage of research that begins before clinical trials, during which human patients are not involved but investigation is performed on animal or cellular models.

**Real-Time Polymerase Chain Reaction (RT-PCR):** Method for the detection and quantization of an amplified PCR product monitored at each cycle, "in real time".

**Sanger Sequencing:** Method of DNA sequencing based on the selective incorporation of chain-terminating dideoxynucleotides by DNA polymerase during in vitro DNA replication.

**Xenopatient:** Immunocompromised animal implanted with one or more tumor tissue samples, used for research purposes in the oncological field.

# Chapter 4
# Big Data Approaches to Improve Stereotactic Body Radiation Therapy (SBRT) Outcomes:
## Big Data for SBRT

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## ABSTRACT

*'Big data' approaches carry promise for advancing our understanding of stereotactic body radiation therapy (SBRT) (also termed stereotactic ablative radiotherapy, SABR) and is guiding the design of clinical trials using hypofractionated radiotherapy. However, the field of big data in radiotherapy, or in combination with other therapies, is still in its infancy and will likely benefit from multidisciplinary collaborative teams including physicians, physicists, radiobiologists, biostatisticians, bioinformaticists and other data scientists analyzing shared data. We herein review opportunities to use the Big data (including dosimetry, clinical factors, imaging and biomarkers/ genomics) to improve SBRT outcomes.*

## INTRODUCTION

Achieving an understanding of the radiobiology of hypofractionation has been the subject of intense interest, and has been driven by recent clinical successes, and resulting comparative effectiveness considerations, favoring shortened treatment courses. A flagship example is SBRT for stage I non-small cell lung cancer (NSCLC)- where multiple studies report impressively-high local control and cure rates (Timmerman et al., 2010), rivaling the efficacy of surgical resection (Chang et al., 2016). Similarly, excellent local control has been reported following SBRT for other malignancies; e.g. oligometastases to organs such as the liver (Timmerman & Cho, 2014). This has been made possible by the innovative developments in image-guided radiotherapy and advanced delivery systems. Moreover, there is a strong interest in combining SBRT with immunotherapeutic agents that could complement or even synergize with local therapy to limit distant failures (Zeng et al., 2014; Rekers et al., 2014). However, this success has presented challenging uncertainties including: identifying optimal SBRT prescription doses from a very wide pool of diverse clinical practices; understanding the underlying tumor and normal tissues radiobiology at such doses; knowing how to combine SBRT with other therapeutic agents and in designing clinical trials using hypofractionation; the use of SBRT for retreatment; and adopting criteria to support decision making in a multi-modality treatment clinic (Navarria & Ascolese, n.d.; Salama & Chmura, 2014; Amini et al., 2014).

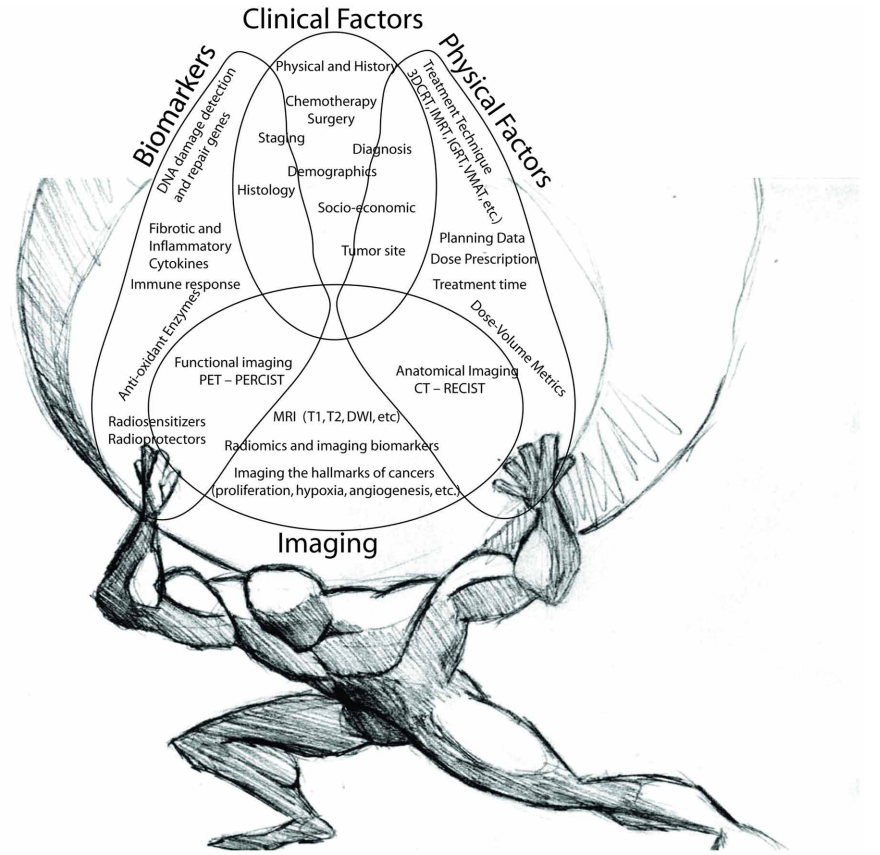
The available SBRT data, including that from prospective/randomized trials, are somewhat limited, and reports are often missing relevant elements that can affect outcomes. In addition, different analytic approaches might interpret the same set of data differently. Computer technology now allows for much more information to be acquired and processed, enabling so-called "big data" analyses. This approach perhaps can be leveraged to accelerate research related to SBRT.

In the era of data-driven decision making, it is hoped that many of the challenges in optimizing the efficacy, and minimizing toxicity of SBRT and stereotactic radiosurgery (SRS), could be addressed through advances in biotechnology, quantitative imaging, and computational modeling. Notably, in 2010, authors from the QUANTEC (Quantitative Analyses of Normal Tissue Effects in the Clinic) effort supported a data-pooling culture of retrospective and prospective data (Deasy et al., 2010). Moreover, the QUANTEC introductory papers emphasized that, in addition to dose-volume metrics, endogenous biological markers (biomarkers) (Bentzen et al., 2010) and computer extracted imaging features could act as predictors of radiation side effects and surrogate endpoints for clinical outcomes (Jeraj et al., n.d.). Recently, the potential role of '-omics,' whether biological (genomics, proteomics, metabolomics, etc.) (Rosenstein et al., 2014; Andreassen et al., 2012; Abazee et al., 2013; Coates et al., 2015) or imaging (radiomics) (Lambin et al., 2012; Aerts et al., 2014; El Naqa, 2014) has been recognized. Efforts have been directed towards integrative systems-based approaches that would include both data types as part of a radiotherapy 'pan-omics' framework (El Naqa, 2014) towards a better decision support system (Lambin et al., 2013). The radiotherapy data carries the five Vs (volume, velocity, variety, veracity, and value) hallmarks of big data as depicted in Figure 1. Probably the volume part of this data is still evolving but the need of particularly the analytical part of big data methodology to dealing with valuable heterogeneous datasets with high veracities is indispensable. In recognition of these opportunities, ASTRO, in association with the NIH, organized a workshop (Bethesda, MD, Aug 2015) to "explore opportunities for radiation oncology research, quality assessment, and clinical care in the era of big data" (Benedict, El Naqa & Klein, n.d.).

The clinical outcomes following radiation therapy appear to be determined by many clinical, physical, and biological factors (see Figure 1); e.g. disease stage, age, comorbid conditions, time/fractionation/dose/volume parameters, concurrent/sequential therapies, inherent radiation sensitivities. Further, these many factors likely interact in a complex manner in determining outcome, and sorting out these relationships is challenging. Nevertheless, national and institutional protocols have been developed to provide treatment guidance, for specific groups of patients, based on these types of factors. Further, many cooperative groups and institutions have built computer databases to organize and retain much of this type of information along with outcome data. Indeed, these types of datasets have been helpful in defining our current treatment recommendations. However, published protocols and outcomes are often conflicting, confusing, and incomplete.

This problem might be particularly acute for SBRT as it is recognized that the technology for SBRT has spread faster than the early adopters are able to define good dose/volume guidelines, and that SBRT-based technologies are being applied in broader groups of patients (e.g. now in patients with both localized and advanced

*Figure 1. The atlas of big data bringing together the silos of radiotherapy data (physical, clinical and biological together) for better decision making in SBRT*




stages of cancer). It is hoped and expected that big data analytics can accelerate our development of more data-driven dose/volume guidelines (both for desired target doses as well as normal tissue limits) for treatment planning as well as provide more insights into underlying complex radiobiology that determines outcomes.

## ANSWERED AND UNANSWERED QUESTIONS ON THE RADIOBIOLOGY OF SBRT

SBRT has been successful for small/localized tumors where tumorcidal doses can be given with acceptable normal tissue risks (Timmerman & Cho, 2014). To safely apply the same strategy with large tumors, or tumors near unavoidable critical

normal structures, requires exquisite target localization and immobilization and a better understanding of the radiobiology of the tumor control/complication tradeoffs.

Classical radiobiology principles predict that biological effect is very sensitive to dose per fraction, with lower $\alpha/\beta$ values indicating higher sensitivities. Despite over a hundred years of experience in radiotherapy outcomes, there are still major uncertainties regarding the most accurate $\alpha/\beta$ values to be used for a given endpoint (or even if the $\alpha/\beta$ model is the optimal means to describe these effects). Nonetheless, cancers with estimated low $\alpha/\beta$ values (e.g., melanoma, liposarcoma, prostate and some breast cancers) may benefit from hypofractionation (Orton, 2012), provided that this $\alpha/\beta$ value is lower than that of the critical associated normal tissues (e.g. typically assumed to be $\approx$ 3 Gy for many late responding normal tissues). Conversely, the apparent majority of cancers with a high $\alpha/\beta$ (e.g. $\geq$ 10 Gy) might be more-optimally treated with conventional or hyper-fractionation regimens in order to achieve an acceptable therapeutic ratio and minimizing the risks to late-reacting normal tissue (i.e. following the 5 Rs principles and the linear quadratic (LQ) model) (Brown, Carlson & Brenner, 2014). It should be noted that available data are primarily thought to support application of the standard LQ model in the range of 1-5 Gy per fraction (Joiner, 2009). Alternative models have been proposed to more accurately predict biological response to doses typical of SBRT (i.e., $\geq$5 Gy). However, these models are based on the assumption that reproductive cell death is ultimately responsible for biological response at both conventional and typical of SBRT doses per fraction. The uncertainty in how to translate the hypofractionated SBRT doses to conventionally fractionated dose equivalents has ignited the debate between proponents of the 5Rs and advocates of "new" radiobiology that may involve additional mechanisms related to stem cells, vascular damage, and immune-mediated effects (Orton, 2012; Brown, Carlson & Brenner, 2014; Kirkpatrick, Meyer & Marks, 2008; Sheu et al., 2013; Song et al., 2013). While considered "new" radiobiology, the concept of radiation-mediated immune response dates back decades (Milas 1976). Nevertheless, recent technological advances have enabled a reduction in normal tissue dose-volume exposure such that differences between the $\alpha/\beta$ of normal tissue and tumor are less critical (i.e. when the irradiated volumes are small, the impact of these factors is reduced). In particular, intensity modulation, image guidance, immobilization and motion management allowed exploitation of volume dependence governing normal tissue response (i.e. minimizing normal tissue exposure to therapeutic doses). The high dose gradients commonly used with SBRT also enable effective sparing of critical organs. Early clinical trials that demonstrated the safety of SBRT/SABR transferred principles of cranial stereotactic radiosurgery to extra-cranial tumor sites and emphasized selecting optimal dose-fractionation regimens for tumor control based on radiobiological principles (Blomgren et al, 1995). However, application of

the 5Rs of radiobiology and LQ to higher doses per fraction has been challenging in concept and in light of recent SBRT/SABR results, that have demonstrated excellent local control, but far less normal tissue effects than what maybe would be predicted from the LQ model (Glastein, 2011, 2008), however, this remains a subject of debate (Fowler et al., 2004).

Current SBRT dose-fractionation regimens utilized in clinical practice and trials are based on earlier preclinical studies, with some based on LQ modeling, and others developed from Phase I trials or other clinical experiences. Therefore, there is a need to develop a better rationale for current practice and future hypofractionation clinical trials, particularly, when involving systematic targeted agents, by reflecting an evidence-based understanding of tumor control and normal tissue tolerances at higher doses per fraction. This will likely need to incorporate classical and new radiobiology, and appropriately upgrade our modeling schemes using knowledge-driven approaches. Possible non-dosimetric variables that could feasibly impact dose response of tumor and normal tissue include things such as tumor histology, tumor/target site and microenvironoment, tumor genetic factors, host (patient) genetic factors, environmental factors and patient comorbidities (Lambin et al., 2013). Big data approaches might be well suited to consider these large number, and likely-interacting, variables.

## BIOMARKERS OF SBRT

In general, a biomarker is defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathological processes, or pharmacological responses to a therapeutic intervention" (Group BDW, 2001). Biomarkers can be categorized based on the biochemical source of the marker of interest into endogenous or exogenous biomarkers.

### Endogenous Biomarkers

These are typically based on molecular biology laboratory measurements from tissue or fluid specimens and can be classified into (1) 'expression biomarkers,' measuring changes in gene expression or protein levels or (2) 'genetic biomarkers,' based on variations, for tumors or normal tissues, in the underlying DNA genetic code.

#### Pre-Treatment

For example, Cannon et al. reported that pre-treatment neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) are correlated with clinical outcomes

after stereotactic radiation (Cannon et al., 2015). Specifically, the overall survival was 23 months for patients with higher NLR or PLR and nonlocal tumor failure rates were 11% for patients with PLR less than 250 and 58% for PLR greater than 250 ($p < 0.001$). In a Japanese cohort of 117 SBRT lung patients, Yamashita et al. found correlations between incidence of RP and higher blood serum concentration of Krebs von den Lungen-6 (KL-6) and surfactant protein D (SP-D) levels. Moreover, an interstitial pneumonitis shadow in patient's CT was also found to correlate with RP, suggesting an interstitial pneumonitis shadow and high value of serum KL-6 & SP-D before SBRT predict for a higher likelihood of severe RP, independently of dose volume histogram parameters (Yamashita et al., 2010, 2014).

Initially, the addition of a new biomarker could raise more questions than it answers, but the big data approach can lead to a better understanding of those issues over time. For example, in a 2004 Japanese study of 16 patients in whom 3 developed grade 3 RP after single fraction SBRT, a rise in KL-6 from pre-treatment levels was significantly correlated with RP risk (Hara et al., 2004). A 2011 study (from another Japanese institution) of 100 patients, showed that KL-6 levels increase with the onset of RP and then decrease after the administration of steroids; the pre-treatment KL-6 levels was significantly correlated with grade 2-3 RP risk, with a hazard ratios of ~4 using a cutoff of 500 U/ml (Iwata et al., 2011). Other than these 2 studies, very little has been published on KL-6 as a prognostic marker after radiotherapy (a January 2016 PubMed search of "KL-6" AND "radiation" yielded 20 studies), begging the question as to why such compelling data has not resulted in more investigations, or why such a potentially potent biomarker was not discovered sooner. Could the combination of KL-6 and another biomarker be more predictive? Again, this is purely speculation until more institutions include these metrics in their follow up and published data. In the big data approach, if many institutions track and share these and other potentially important indicators, these questions may be answered and a more complete understanding can be achieved.

## Post-Tx Marker of Response

Moré et al. analyzed exhaled breath for predicting onset of radiation pneumonitis (RP) after SBRT, potentially allowing for an adaptive treatment approach. They reported that acute changes in exhaled nitric oxide (eNO) and exhaled carbon monoxide (eCO) concentrations, defined as percent changes between each pre-fraction and post-fraction measurement, were significantly smaller in RP versus non-RP cases ($p = 0.022$ and 0.015, respectively). In an exploratory analysis, a combined predictor of baseline eNO greater than 24 ppb and acute decrease in eCO less than 5.5% strongly correlated with RP incidences ($p = 0.0099$)(More et al., 2014).

## Imaging Biomarkers

These are based on introducing a substance into the patient's body such as those used in molecular imaging.

### Pre-Treatment

As an example of using imaging as a biomarker of SBRT outcome, Shultz *et al.* showed that higher standard-uptake value (SUVmax) on FDG-PET imaging was associated with poor disease distant progression in stage I NSCLC patients treated with SBRT (Shultz et al., 2014). Iizuka *et al.* (2014) showed that a low apparent diffusion coefficient (ADC) value ($\leq 1.05 \times 10^{-3}$ mm$^2$/s) on pre-treatment diffusion-weighted MRI and a high SUVmax ($\geq$7.9) on FDG-PET scans were each associated with a greater risk of disease progression in a small cohort of 15 NSCLC patients treated with SBRT, but these results did not reach statistical significance. However, the combination of both metrics was statistically significant.

### Post-Tx Marker of Response

Huang et al. conducted a systematic review of 26 studies reporting radiological changes post-SABR on CT and PET images and suggested that recurrent disease versus fibrosis should be suspected if high-risk CT changes are seen with SUVmax $\geqslant$5 on PET (Huang et al., 2013). The group also suggested that advanced quantitative feature analysis using textural features as part of the radiomics arsenal could be effective in distinguishing recurrence from post-SBRT fibrosis (Mattonen et al., 2014).

## Genetic Factors

It has been recognized that metastatic progression accounts for most of the cancer recurrence after SBRT for early stage NSCLC. In a study of 91 patients, distant metastasis accounted for almost half the recurrences and was the only significant prognostic factor for overall survival on multivariate analysis (Bradley et al., 2010). A signature of 144 genes was suggested to predict metastasis in stage I lung cancer patients following surgery or SBRT (Kim et al., 2014). Interestingly, it has been demonstrated that radiation effects go beyond the depopulation of viable cancer cells and may cause modifications of the tumor microenvironment. The latter can stimulate a host immune response and mediate indirect cell kill by the activation of adaptive anti-tumor immunity, primarily acting like a vaccine (Formenti, n.d.). There is experimental and clinical data in certain cancers (*e.g.,* lymphoma, melanoma

(Postow et al., n.d.) to suggest an immune triggered role of local radiation on distant tumor growth, referred to as the abscopal effect. Moreover, Dewan et al. showed in a breast cancer mouse model that an 8 Gy×3 and antibody combination yielded the best results (Dewan et al., 2009). There are several ongoing clinical trials in multiple sites of combining SBRT with immunotherapeutic agents, including checkpoint inhibitors targeting CTLA-4, PD-1 and PD-L1 (Rekers et al., 2014; Tywman-Saint et al., 2015).

## INFORMATICS APPROACHES FOR SBRT BIG DATA

Utilizing advances in informatics and biotechnology, a promising alternative approach to simple modifications to the LQ model is to explore systems-based solutions that can integrate a clinical, physical, and biological data to create a more robust evidence-based model. It is noted that big data analytics is not only about data size but also about heterogeneity and violation of the p-omics versus the pan-omics in such data (El Naqa, n.d.). Often, it has been a challenge to see distinctions between using a particular model, e.g., LQ for reporting purpose versus making predictions. However, in a systems radiobiology approach, intra-radiotherapy changes and post-radiotherapy treatment outcomes could be optimized through using *top-down* approaches based on complex systems analyses (e.g., machine learning methods) (El Naqa, 2013; Torres-Roca, 2012; Oh et al., 2012) or *bottom-up* approaches based on first principles of radiation physics and biology to model cellular damage temporally and spatially (e.g., multi-scale modeling with Monte Carlo techniques) (Torres-Roca, 2012; Oh et al., 2012; El Naqa & Seuntjens, 2012;Prokopiou et al., 2015).

The top-down approach has gained steam in recent years, particularly in the areas of radiogenomics and genome-wide association studies (GWAS) of single-nucleotide polymorphisms (SNPs). For example, there are several ongoing SNPs genotyping initiatives to better link genetic variants to radiation-associated toxicities, e.g. pan-European GENEPI project (Baumann & Begg, 2003), British RAPPER project (Burnett et al., 2006), Japanese RadGenomics project (Iwakawa et al., 2006), and the US Gene-PARE project (Ho et al., 2006). An international consortium has also been established to coordinate and lead efforts in this area (West, 2010). A review of the progress and potentials of radiogenomics in the big data era have been recently summarized (Rosenstein et al., 2014). Moreover, a complementary alternative to using SNPs is the use of copy number variation (CNVs), which were shown using a candidate gene approach to improve toxicity risk prediction in prostate cancer patients treated with hypofractionated radiotherapy, by acting as dose-modifiers, using analytical and data-driven models (Coates et al., 2015). Nevertheless, an

important consideration in radiogenomics modeling is the clinical and dosimetric confounding issues, that is best undertaken by multidisciplinary teams including radiation oncologists, physicists, radiobiologists, molecular biologists, geneticists, epidemiologists and biostatisticians (Andreassen et al., 2012). Big data approaches raise new challenges of archiving, ontology, visualizing, and analyzing tremendous heterogeneous datasets of clinical characteristics, dosimetry, imaging, and molecular data in a clinical setting. Furthermore, issues related to data sharing protocols across multiple institutions, which is vital in the big data era, have been a major impediment to scientific progress for technical and non-technical reasons (Sullivan et al., 2012) and may require a cultural change to attain its goals (Mayo et al., n.d.). This sentiment was echoed in Vice President Biden's call for a "moonshot" approach to cure cancer. A possible solution suggested by QUANTEC is to adopt a policy of anonymizing clinical trials data and making these data publicly accessible after publishing of primary work (Deasy et al., 2010). An alternative approach is to apply rapid learning in which, innovative information technologies are developed that support semantic interoperability and enable distributed learning and data sharing without the need for the data to leave the hospital (Lambrin et al., 2013). This further raises the issue of properly protecting confidentiality and patient privacy by utilizing advanced secure telecommunication and networking technologies.

A key challenge is to obtain appropriate information regarding patient outcomes that can complete the information feedback loop to drive model improvements. One new and attractive tool for providing this information is patient reported outcomes (PROs) for both on acute and late toxicity. Typically, questionnaires are used, so in this regard this approach is widely available. However, this approach can be logistically challenging; some of the questionnaires are cumbersome, it requires patient's active participation, and symptoms are often non-specific (e.g. rectal toxicities can be attributed to either the rectum or anus (Thor et al., 2015), or fatigue following chest RT that is due to anemia from the chemotherapy might be attributed to the lung irradiation).

To leverage the "Big Data" revolution in radiation treatment, a prospective data collection system has been developed for Gamma Knife stereotactic radiosurgery (Gamma Knife Patient Registry sponsored by Elekta). The goal of this registry is to gather and analysis of high quality clinical data. The registry architecture is scalable and suitable for any aspect of neurosurgical practice. The Big Data elements include clinical (e.g. demographics; diagnostic items), physical (e.g. treatment parameters), and follow-up information for clinical and imaging endpoints. Analytical tools use varied filters to generate customized outcomes charts (e.g., survival, local control, neurologic function and complications etc.). The initial testing over 3 years has proved that the system is reliable, robust and dynamic and comprehensive process of clinical information.

## DISCUSSION AND RECOMMENDATIONS

- We are at the dawn of an era when the underlying genomic characterization of a given tumor and its host patient is obtainable, along with an increased ability to use imaging to characterize both the tumor phenotype and treatment response. Combining this information with other clinical factors (e.g. age, gender, chemotherapy, life-style, etc) as well as the temporal and spatial delivered dose distribution, will allow for an unprecedented ability to both predict the likelihood of treatment success and to flag unexpected outcomes for further analysis. The rational addition of new information to outcomes analyses should build on reliable, well-established (radiobiological) principles. Thus, the era of Big Data is not a post-radiobiological era – rather, the Big Data approach will complement classic approaches.
- Radiotherapy outcome predictions have suffered from silos: silos of single-study data and silos of dose-driven versus biomarker-driven approaches over the past decades that have been impediment to coping with rapid advances in treatment technology (van den Bent & Cairncross, 2003; Bibauuult et al., 2013; Ree & Redalen, 2015). Big data holds the promise of facilitating integration of these data as shown in Figure 1. This is particularly important to address current challenges in SBRT range from selecting the appropriate prescription to combination with targeted therapy. More than ever, this requires construction of high quality multi-institutional databases including multiple aspects of SBRT: dose distributions, clinical factors (age, gender, relevant co-morbidities, chemotherapy, biomarker, imaging markers and outcomes. Ideally, these would be linked to pre- and post-treatment biospecimen collections, as local tumor control of small tumors has become relatively easy to achieve with SBRT, and discovery of the optimal fractionation schedules for SBRT to enhance systemic therapy will be facilitated with biomarker studies (Almo & Guha, 2014).
- Systems should be put in place to readily pool data from multiple settings/ institutions such that enough data is available to adequately explore the impact of large number of variables. Approaches based on federated databases and rapid learning are promising in this regard and may enable a realistic data sharing schemes. It is important to note that complex modeling techniques cannot compensate for poor data aggregation.
- As suggested in many journals' instructions, it is recommended that studies reporting on biomarkers follow the Reporting recommendations for tumor MARKer prognostic studies (REMARK) guidelines (Altman et al., 2012; McShane et al., 2005). The aim of these guidelines is to enhance reporting and reproducibility of results and subsequent utilization in the clinic.

- In addition, the confounding effects of dosimetric and clinical variables need to be included in the dataset analysis as illustrated in Figure 1. However, there is a large pool of data that could be useful for SBRT/SRS studies that is rapidly growing given the success of SBRT and the pressures of the changing healthcare environment.
- It is also important to notice that successful application of advanced big data methodologies such as complex system analysis or machine-learning methods needs to account for the intricacies of radiobiology to be successful. Big data is not about building a black box but rather a tool to help improve our understanding of radiobiological mechanisms, generate new hypotheses and support better clinical decision-making. The success of which will hinge on building multidisciplinary collaborative teams of all stakeholders: physicians, physicists, radiobiologists, biostatisticians, bioinformaticists and other data scientists in order to achieve the promise of big data and realize its potential in daily clinical practice.

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# Chapter 5
# Emerging Technologies Serving Cytopathology: Big Data, the Cloud, and Mobile Computing

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## ABSTRACT

*Cytopathology became a popular since George Papanicolaou proposed the famous test Pap 60 years ago. Today cytopathology laboratories use the microscope as primary diagnostic device; however modern laboratories host numerous modalities for molecular tests and exchange data via networks; additionally, there are imaging systems producing pictures and virtual slides at enormous sizes and volume. The latest technological developments for cloud computing, big data and mobile devices has changed the way enterprises, institutions and people use computerized systems. In this chapter are explored potential applications of these technologies in the cytopathology laboratory including: data storage, laboratory information*

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*systems, population screening programs, quality control and assurance, education and proficiency testing, e-learning, tele-consultation, primary diagnosis and research. The impact of their adoption on the daily workflow is highlighted, possible shortcomings especially for security and privacy issues are identified and future research directions are presented.*

## INTRODUCTION

The term "cloud services" (also known in modern technology jargon as "the cloud") refers to a network of servers connected by the Internet or any other type of network that enables users to combine and use computing power on an as-needed basis. Cloud computing is a novelty that rapidly showed tremendous opportunities for applications in medicine and health care improvement (Eugster, Schmid, Binder, & Schmidberger, 2013; Fernandez-Llatas, Pileggi, Ibanez, Valero, & Sala, 2015; Glaser, 2011; Kuo, 2011; Lupse, Vida, & Stoicu-Tivadar, 2012; Mirza & El-Masri, 2013; Patel, 2012; Rosenthal et al., 2010; Waxer, Ninan, Ma, & Dominguez, 2013). It is expected that by 2018 there will be approximately a 27% increase in the US cloud computing market for medical images at a Compounded Annual Growth Rate (CAGR). This is mainly due to the growing volume of medical images and the increasing costs of ownership for maintaining Picture Archiving and Communication Systems (PACS) (GlobalData, 2012). To deal with this challenge, analysis techniques, especially suitable for the laboratory environment, have been developed for future application (A. Pouliakis, Archondakis, Karakitsou, & Karakitsos, 2014; A. Pouliakis, Spathis, et al., 2014).

In parallel to cloud computing there are new developments for mobile computing. Especially in the health sector Mobile Health (mHealth); which is defined as the practice of medicine and public health supported by mobile devices; is nowadays evolving. Available mHealth applications are nowadays used for collecting community and clinical health data, delivering healthcare information of patient vital signs in real-time, as well as direct healthcare provisioning. Today there are available handheld computing applications for: ambulatory medicine (Banitsas, Perakis, Tachakra, & Koutsouris, 2006; Kiselev, Gridnev, Shvartz, Posnenkova, & Dovgalevsky, 2012; Pavlopoulos, Kyriacou, Berler, Dembeyiotis, & Koutsouris, 1998; Rosales Saurer, Mueller-Gorchs, & Kunze, 2009; Zerth, Besser, & Reichert, 2012), diabetes management (Ribu et al., 2013; Skrovseth, Arsand, Godtliebsen, & Hartvigsen, 2012; Spat et al., 2013), asthma management (Finkelstein, Hripcsak, & Cabrera, 1998; Gupta, Chang, Anyigbo, & Sabharwal, 2011), control of obesity

(Patrick et al., 2009), smoke cesation (Ghorai, Akter, Khatun, & Ray, 2014; Ybarra, Holtrop, Prescott, & Strong, 2014), seizure management (Pandher & Bhullar, 2014), stress management (Clarke et al., 2014) and treatment of depression (Burns et al., 2011) among others.

However the majority of mHealth of applications are related to fitness (43%) followed by health resource (15.0%) and diet/caloric intake (14.3%,). User engagement has the form of self-monitoring and training (74.8%) (Sama, Eapen, Weinfurt, Shah, & Schulman, 2014); Despite the fact that do exist applications targeting patients, currently, there are rather limited applications targeting physicians and doctor-patient interactions (Martin, 2012). Pioneering field seems to be radiology consultation for X-rays and mostly Computer Tomography (Choudhri et al., 2013; Johnson et al., 2012; Toomey et al., 2010) and ECG transmission (Vaisanen, Makijarvi, & Silfvast, 2003). mHealth applications are very limited in the fields of pathology and even less in cytopathology; despite both specialties deal with images. In relation to pathology, the most reported uses of handhelds, are limited to experimental endeavors in education and telemedicine (Park, Parwani, Satyanarayanan, & Pantanowitz, 2012). For the field of cytopathology, even after a thorough search, there are found rather limited articles or reports . However, pathology and cytopathology share many common characteristics. Actually, in most countries, cytopathology is considered as a subspecialty of pathology. Thus concepts and ideas can be useful to both specialties; therefore applications can be transferred from one domain to the other.

Within this chapter, we analyze the state of the art related to the application of cloud computing services and infrastructure and mobile computing for cytopathology, identify and propose potential applications, explore possible solutions for potential problems and finally promote the benefits of transforming traditional application of the cytopathology laboratory into cloud based services with mHealth where appropriate. The main application areas include: 1) storage of data emphasizing on image archiving and access, 2) shifting of the traditional Laboratory Information Systems from laboratory or hospital hosting to cloud hosting and access of these services from the mobile device, 3) cloud based services supporting population screening and especially for cervical cancer screening, representing the vast work load of cytopathology laboratories, 4) applications of cloud to support Quality Control and Quality Assurance (QC&QA) 5) shifting the traditional proficiency testing and the need for continuous medical education to e-services and mobile access using cloud infrastructure 6) primary diagnosis via tele-cytology and tele-consultation on the basis of cloud computing and the mobile device 7) cytological image analysis, cytogenetics and genome analysis using the cloud computing power and 8) virtual slides for cytopathology and how the mobile device can be useful.

## BACKGROUND

A cloud system is a network of servers offered as a service that can be easily adapted to users' needs. More specifically, it is designed to be flexible, scalable, secure and robust. In most cases cloud systems provide software, access to data, storage and processing power; through this interconnected grid of computers resource sharing through the Internet is permitted. The services are usually priced on a pay-per-use model.

Cloud systems are categorized into three different groups, according to the offered service type:

- Infrastructure as a Service (IaaS), in which case hardware, storage and physical devices are offered.
- Software as a Service (SaaS), where software and hosted applications are available to users.
- Platform as a Service (PaaS), which offers the capability to deploy applications created using programming languages, libraries, services, and tools owned and supported by the provider.

Users do not have physical access to or control over the underlying cloud infrastructure, but have control over the deployed applications and the general services. According to the hosting location, clouds can be public, private, hybrid, or community. In particular, when employed in the medical field they can be described as:

- **Public Clouds:** For general use. The cloud owners are responsible for information hosting; public clouds are rarely used in the field of medicine and in case of their use, data are encrypted.
- **Private Clouds:** Which are only for in-hospital use and are dealing with confidential patient data. The owners or the hospital premises are responsible for hosting the equipment.
- **Hybrid Clouds:** Which are hosting non-confidential information on public Clouds and confidential information in a private domain, i.e. split sensitive from non-sensitive data to ensure security.
- **Community Clouds:** Which are hosting information among members of the same community. Laboratories and hospitals may create a community Cloud in order to share the infrastructure and software applications.

Cloud computing applications are not well established in the field of cytopathology, however they could provide important web-based services, which may be incorporated in the Laboratory Information System and become part of a web-based Electronic

Health Record; the end users are: cytopathologists, other medical, para-medical and non medical personnel, clinical doctors and patients.

The context of Cloud computing can be formulated as follows:

- **Applications:** Cloud based applications can be hosted on remote servers and operate in real time from a thin client or via a web browser. Browser based access has many advantages: no installation, no maintenance, support issues are resolved in one place as application software is hosted on dedicated (real or virtual) servers, so there is no risk that the thin client could influence the system. The applications may operate as software as a service (SaaS), software plus service or data as a service. Especially in the cytopathology arena, users may take advantage of some kind of "Software as a Service" for image reviewing, creating diagnostic reports, or billing.
- **Client:** A Cloud client, is the medium which cytopathologists use to access the applications via Internet. Cloud clients may be a desktop, laptops, tablets and Smartphones. Usually applications are exposed via a web browser.
- **Infrastructure:** Cloud infrastructure includes all hardware (computing, networking, power supplies, cabling) and the buildings containing it. Hardware is inexpensive mass produced servers. The server environment may be running partial or complete virtualization, grid computing or paravirtualization technologies. Cloud infrastructure, in the Cytopathology arena includes computer hardware and servers used for software operation and data storage.
- **Platform:** The cloud platform is referring to the way that applications are deployed. In the field of Cytopathology, a well designed platform is an essential parameter for efficient application of laboratory's applications.
- **Services:** Service refers to what users can reap from their cloud experience. Today there is a great amount of services for users wishing to take advantage of cloud infrastructures. Some of these services are unique, while others enhance services that are already available. A cloud service, in the field of Cytopathology, can be, without having to be limited to, either a web-based image archiving system or a web-based image gallery among others.
- **Storage:** Storage devices are the most common failing computer component . Via cloud technology, organizations assure data safety. In cases of emergency situations the chances of complete failure of all systems is almost negligible, because in the cloud there are many computers operating simultaneously and complementing each other. For cytopathology laboratories, the cloud is an enabling technology for Big Data, enables, for example, the storage of large medical laboratory databases in the form of documents and image libraries,

instead of physical storage at site which is much more expensive and difficult to maintain without specialized personnel.

- **Processing Power:** Clouds can offer almost unlimited processing power. Organizations using the cloud can scale up their infrastructure as needed, and when there is a real need for more processing power. Thus Cloud computing, in the field of Cytopathology, can provide infinite processing power at a very low cost.

The Cloud is the locomotive for the access to services from mobile devices, because it offers infrastructure, processing power and storage that is not available on handheld computers. Mobile telephony is in use more than 20 years. Advances of the latest decade have contributed to the conversion of mobile telephones into smart telephones with the aid of sophisticated operating systems. Especially, in the last five years, hardware components enhancing the performance of mobile phones have become available: processing power (4 or 8 processors), cameras (more than 10Mpixel nowadays) and large resolutions and displays (more than 1500x2500). The 4G and WiFi connectivity have created an always open, rather inexpensive channel for continuous connection to Internet. Nowadays, mobile phones are not designed to be smaller in size, in contrast manufacturers design them so that they are comparable to tablets in terms of size and weight. Their software environment is user friendly and provides functions similar to those released for computer software five years ago. It seems that today mobile devices have the maturity of computers used in the health sector in previous years and could be implemented. Some of the most important characteristics of mobile devices are:

- Battery which powers the device for days
- CPU with processing power competing desktop and portable computers
- Excellent high resolution graphics on various display sizes
- Processor memory counted today in gigabytes
- Storage memory of 32 and more Gigabytes via flash interchangeable memories
- GPS for global positioning
- 3G and 4G connectivity
- WiFi connectivity (150Mbps and even 300 Mbps) that competes the 1,000 Mbps offered by cable based LANs
- Bluetooth connectivity for personal area networks allowing users to have many connected devices and gadgets in co-operating mode
- USB connectivity for coupling the handheld to other devices
- Accelerometer
- Gyroscope

- Compass
- Thermometer
- One or more high-resolution cameras
- Capacitive touch screen allowing a rich user interaction
- Connectors to interface with displays on high-end models

In summary, the Cloud combined with the always connected smart phone is a couple that has the potential to offer important applications in the cytopathology ecosystem, capable to exploit big data to every user.

## MAIN FOCUS OF THE CHAPTER

### Potential Applications of Cloud and Mobile Computing in Cytopathology

Cytopathology laboratories deal with a lot of image analysis, because routine diagnosis is performed via glass slides analyzed by specialists via the microscope. However, modern cytopathology laboratories perform additional examinations based on molecular biology methods and immunocytochemistry. Nowadays, a modern cytopathology laboratory is equipped with numerous modalities allowing scientists to perform medical tests and exchange data via networks; they also employ imaging systems to create digital pictures of the glass slides and even virtual slides (complete slides in electronic format), a method known as Whole Slide Imaging (WSI). Therefore, the volume of data in cytopathology labs nowadays is enormous. More specifically, it is not only the images, but also the examination results produced by analyzersthat require immediate safe storage. Cytological image capture and transmission, known as telecytology, has been made possible by new cameras and microscopes connected to computers (Archondakis et al., 2009; Pantanowitz, Hornish, & Goulart, 2009; Pinco, Goulart, Otis, Garb, & Pantanowitz, 2009). The wide implementation of such telemedicine systems became necessity dictated by the need of real-time results for therapeutic decisions (Briscoe et al., 2000; Raab et al., 1996; Yamashiro et al., 2004) as well as for physician training (Stergiou et al., 2009b). Telediagnosis and training are not the sole applications, other applications, include but are not limited to the everyday routine tasks. Specifically, there are interesting applications for results reporting, day to day laboratory management, WSI storage and viewing, interactions between patient and doctor, doctor to doctor and between members of the same laboratory, in addition there are applications relevant to quality control and assurance. Consequently, we analyse various applications considering

the Cloud and mobile computing, and try, wherever possible to pinpoint potential future applications.

## Data Storage

Storing, archiving, sharing and accessing data from the cloud allows organizations to manage data more efficiently and cost-effectively while overcoming a lot of the legal, regulatory and technical challenges that data requirements pose (AT&T, 2012). The Cloud enables hospitals to:

- Efficiently handle large size images
- Use non-proprietary, standards-based, vendor-neutral architecture
- Expand or contract storage capacity easily
- Manage authentication, encryption and security protocols
- Conduct efficient system-wide application upgrades
- Extend the life of existing infrastructure/investments

Cytopathology laboratories require storage of images and patient information, files are obliged to be in one or more servers and accompanying disk arrays. In general cytopathology laboratories have poor or no experience in maintaining them. A server crash may result in severe data losses. Additionally, servers require constant upgrades. These two reasons are good enough for a modern cytopathology laboratory to shift its data to the Cloud. By doing this, the laboratory reduces dramatically all costs related to server software and hardware, as well as the costs for maintenance and licenses. Here the cloud acts as a LIS, telecytology software, and billing unit. A patient may perform a cytological examination at a hospital or a private laboratory, representative images of this case may be stored on a hybrid Cloud. If the patient performs another cytologic examination after some time, in another (and possibly distant) laboratory, she/he can provide the reporting cytopathologists direct access to the images stored in the Cloud. The cytopathologists can retrieve and merge the images on their workstation and make the final diagnosis after having reviewed the images of the previous cytological[s] examination[s].

## Laboratory Information Systems

Laboratory management requires accurate and within specific and strict time-frame transmission of critical laboratory results to the caregiver; thus, interventions can be timely provided and adverse outcomes are prevented. These operations are linked to the provisioning of quality health services (A Pouliakis, Athanasiadi, et al., 2014; A Pouliakis, Margari, et al., 2014; Shen & Yang, 2001). Laboratory information

systems (LIS) come to supervise and facilitate these many varieties of inpatient and outpatient medical tests and laboratory operations (Pearlman, Wolfert, Miele, Bilello, & Stauffer, 2001). The major features of LISs include management of sample check in, order entry, specimen processing, result entry and patient demographics as well as medical history. A LIS tracks and stores all the information related to the patient from arrival until he/she leaves the facilities, and stores the data for future retrieval. LISs produce as well reports for the tests that handle, and statistics related to various aspects of the laboratory such as time for execution of examinations, turnaround time, sample volumes etc. Therefore, a LIS is defined as a package of computer programs used to process, store and manage data from all stages of medical processes and tests. Modern LISs are networked, as the various modalities (biomedical analyzers) have networking capabilities as well as can be configured to automatically release examination results for storage; barcode technology automates sample and patient identification processes as well.

Large cytopathology laboratories employ information technology (IT) personnel to manage hardware, software, provide technical expertise and support, handle infrastructure failures, as well as optimize the systems to run mission critical applications. Cloud computing can change this landscape. Nowadays, laboratories need to use virtualization techniques that are included in the basic characteristics of cloud computing; virtualization provides convenient and on-demand network access to a shared pool of configurable resources such as networks, servers, storage and applications, and definitely share the cost for owning and managing in-house equipment and personnel. In addition to cost reduction, the set-up and configuration time for a laboratory wishing to adopt new cloud hosted LISs becomes minimal. Therefore, a cytopathology laboratory that does not have a LIS, but wants to start using a Cloud-based LIS may purchase such a system, configure it according to its specific needs, train the users and launch the application within a short time frame. Using a community Cloud, many hospitals can share standard software and thus save large amounts of resources.

The Cloud comes as an enabling technology to put handheld devices in the LIS arena. For instance a significant process of cytopathology laboratory operations is risk management (Sciacovelli, Secchiero, Zardo, D'Osualdo, & Plebani, 2007), during the pre analytical phase (Vacata, Jahns-Streubel, Baldus, & Wood, 2007; Westbrook, Georgiou, & Rob, 2008), during sample analysis, or in the post analytical phase. Mobile devices, fit perfectly in such applications because they are always connected and at the side of the users. In a published study (Saw, Loh, Ang, Yip, & Sethi, 2011), was reported the experience with a setting using SMSs in reporting critical lab values. SMSs were used to notify critical laboratory results in a teaching hospital; this application provided services and was employed to meet the documentation and audit requirements of critical result reporting (as posed by

regulatory agencies and/or ISO 15189). The team employed a text messaging system (Critical Reportable Result Health care Messaging System [CRR-HMS]) allowing receivers to acknowledge or reject a critical result by replying to the SMS. When there was no response within 10 minutes, procedures of escalation were activated to alternative physicians, according to a predefined roster. The result was that the required time for physician response was decreased from 7.3 minutes to 2 minutes. The CRR-HMS was a useful tool to communicate critical results from the laboratory to physicians, eventually it enabled rapid and timely information transmission and therapeutic or patient management interventions.

## Screening Programs Using the Cloud and the Mobile Device

Cervical cancer (CxCa) is one of the major women mortality reasons in the world (Jemal et al., 2011). However, it can be prevented; the key is the regular examination of all women fulfilling specific criteria with a test Papanicolaou; this process is referred to as population based CxCa screening program. As the population that needs to be screened is very large, the organization, the quality control and assurance are three critical aspects for the successful realization of the program. Unavoidably, computerized systems supporting the program must be in place (A. Pouliakis, Iliopoulou, & Karakitsou, 2012; A. Pouliakis & Karakitsos, 2011). There are several important issues related to the software systems supporting screening programs where the application of cloud computing can be a solution:

### Availability

It is the ability to cope with, and if necessary recover from failures of the host server either due to hardware reasons or due to failure of the operating system and the application software, and to cope with hardware and maintenance activities that may cause downtime. The system availability is crucial for the program smooth operation, if the system is not operational the consequences range from missing appointments to canceled, treatments. Hosting of such systems in a cloud ensures the high availability of servers, moreover as cloud services providers have multisite hosting and multiple internet connections, the availability of connectivity is ensured as well.

### Scalability

Refers to the ability to spread both the system software and the load across multiple servers. Hosting the application in a cloud provider ensures that there will be plenty of CPU power available. By design, the virtual machines offered by cloud services providers are fault tolerant and utilize many servers that appear to the end user as a

single machine, therefore the escalation of the application is easy to be performed via the use of additional servers either for hosting the application or for expanding the database.

### Deployment and Problem Resolution

Definitely deploying traditional applications to end user computers is not a choice for IT systems supporting screening programs, the chosen solution is central hosting the applications, and preferably to use web based applications hosted in the cloud. The benefits from this architecture are evident: the maintenance is easier and applied in a single place, bug fixes and improvements when applied are immediately available to the end users.

A mobile device connected to the cloud based services can be an extremely useful tool for CxCa screening. It can be used to send informative messages to women thus arrange and proceed for regular control (i.e. to perform their scheduled Pap test), they can be used to inform women for the Pap test outcome, in cases of normal results or to proceed for additional tests, when required. Additionally, they can be used to send informative material on the benefits for such examinations and therefore to motivate women to perform the test; therefore the screening program can have more impact and effectiveness.

## Quality Control and Assurance via the Cloud and the Mobile Device

There are many aspects related to the quality control and quality assurance (QC&QA) in cytology and how to control and assess. One of the fundamental methods is the correlation of the cytologic and histologic answers (Izadi-Mood, Sarmadi, & Sanii, 2013). The concept is to evaluate the discrepancies, determine the reason of their occurrence, and also ensure the appropriate patient care. The way cytologic and histologic correlation can be performed, has changed over time. Previously, every few months all the cases dealt with would be gathered and examined. Nowadays, this method is no longer acceptable, as it would take months for a potential mistake to be discovered, made known to the patients and being corrected(Renshaw, 2011). The improvement of information systems and LISs in most cytologic and histologic laboratories, allows such correlations to be performed immediately after the release of the examination results. Data mining either on-line or in a later stage may produce important knowledge for trends and problematic areas in the processes and therefore initiate corrective actions for quality improvement. Additionally image-based systems to evaluate the quality of classification systems such as the Bethesda System 2001 for reporting the results of cervical cytology (Solomon et al., 2002) have already

been reported in the literature (Sherman, Dasgupta, Schiffman, Nayar, & Solomon, 2007). The Cloud obviously can be of help for QC&QA as the related applications may be developed and operated for such an environment, storage and application load will not be an issue and additional benefits may be obtained as the QC&QA application can be shared among numerous laboratories and rare cytological cases can be used by all participating labs.

ISO 15189:2012 is in the EU the recommended standard for medical laboratories and requires successful participation in proficiency testing programs. According to this standard, one of the challenges facing cytopathology laboratories today is the implementation of board certified external quality assessment (EQA) schemes (proficiency testing). The purpose of the adopted EQAs is to ensure that microscopic (cytological) findings are correctly identified and interpreted by laboratory personnel, additionally that they are stored and communicated properly (Archondakis, 2013, 2014; Friedman & Wyatt, 2006; Lee et al., 2003; Nagy & Newton, 2006; Vooijs et al., 1998). Today there are EQA schemes available as telecytologicy applications, for instance using virtual slides, cytopathologists under certification use their computers to remotely diagnose them and be certified. The Cloud can not only host the large volume of virtual slides but facilitates their use on mobile devices, thus enables immediate quality control at any time any place. In the next section the efforts made so far for Whole Slide Imaging (WSI) and the role of the Cloud and mobile devices are presented.

## Whole Slide Imaging in the Cloud and the Mobile Device

Digital imaging in pathology has undergone a period of exponential growth and expansion catalyzed by changes in imaging hardware and gains in computational processing. Today, digitization of entire glass slides at near the optical resolution limits of light can occur in a few minutes additionally whole slides can be scanned by fluorescence or by multispectral imaging systems. WSI or Virtual Slides have been successfully used in surgical pathology, but its usefulness and clinical application have been limited in cytology for several reasons, mainly the lack of availability of z-axis depth focusing; as cytological samples in contrast to histological have a 3D structure. However, nowadays there are available systems capable for whole slide imaging with z-axis control. This has boosted the application of digital slides for cytology (Diller & Kellar, 2015; Fonseca, Santos-Silva, Lopes, Almeida, & Vargas, 2015; Gutman et al., 2013; Higgins, 2015; Krishnamurthy et al., 2013; Mancino et al., 2015; Shakeri, Hulsken, van Vliet, & Stallinga, 2015; Wright et al., 2013). Digital whole slide imaging seems to be the future of cytopathology, probably in the future glass slides will be replaced by whole slide scanned images.

Virtual slide sizes are enormous, their size is in the range of hundreds of Mega bytes, depending on the magnification, the scanned slide area and z-axis scans. The Cloud can provide an affordable storage space, easily expandable and additionally offer data storage assurance. Moreover, no local maintenance by laboratory technicians is required. However, transfer of such data volumes from/to the laboratory and the Cloud can present challenges. Nowadays, the revolution in telecommunication networks, may provide the required bandwidth, and automated slide scanning workstations (see Figure 1) may operate overnight and store the virtual slide data without requiring physical attendance. Storing the virtual slides outside of the cytopathology laboratory premises facilitates exposing of data to a broad range of users, not only for desktop computers but for the mobile device as well.

A PubMed search for whole slide imaging or digital slides reveals that there are about 200 references. However, there is no single publication that is relevant to cytopathology and the mobile device. To the authors' opinion, this technology seems to be ready to support WSI and it seems only a matter of time before it can be implemented. Today, there are map and navigation applications for the mobile device. For example, Google Maps, and Microsoft Bing Maps. Functionally these applications are similar to WSI. For example, are employed pyramidal images broken up to chunks/tiles served to the viewer in real-time. In some applications is used the publically available map Application Programming Interface (APIs) from Google and Microsoft to create WSI viewers for the desktop (Triola & Holloway, 2011). Obviously this approach can be transferred to the mobile device as already happens for map and navigation applications. The second aspect of WSI images is depth or z-axis. This feature is required because in cytopathology doctors focus the microscope to different depths, and as mentioned earlier modern WSI systems

*Figure 1. Automated slide scanner capable or unattended operation (left), slide loader for 200 glass slides (right)*

produce multilayer (z-axis) scans. The capability of the APIs, to present different information layers can be of use to fulfill this requirement. Finally, the multi-touch user interface of the mobile device screen is an additional advantage capable to provide a rich user experience.

WSI is not the application but the tool. In reality applications are related to the purpose that clinical doctors use WSI. Applications of WSI include: archiving, diagnosis, consultation, providing the examination media to the patient and training among others. Actually, there is available an application for the iPad device, this is related to the online distribution of WSIs, as teaching sets especially for countries with low resources (Fontelo, Faustorilla, Gavino, & Marcelo, 2012). The study had a setting involving iPad tablet devices and fifty medical students. There were used two web servers, providing digital pathology virtual slides via a web interface. One server was remote while the second mirrored the content on the local network. The results indicated that the speed of serving the WSIs via the local server was much faster, thus, it was preferred by the students. This study revealed the critical role of networking and storage infrastructures in the acceptance of mHealth applications and the role of the cloud as well.

## E-Learning

Over the past decades the evolution of information technologies, and telecommunications, made the World Wide Web (WWW) a low cost and easily accessible tool for the dissemination of information and knowledge, education could not be left outside. Various researchers have proved that the traditional learning theories can be applied in a web-based learning system and moreover web-based distance education, may improve education and support new educational systems, radically changing training in comparison to traditional learning (Garrison, Schardt, & Kochi, 2000). Medical science and education is a major category of lifelong learning, as it is one of the most rapidly evolving sciences; new media offer an advantage for improvement, therefore the new developments and the constant growth of existing knowledge make continuous education vital for continuous improvement. In the field of cytopathology, teaching usually takes place in front of the microscope, supplemented by real-case presentations, didactic lectures and audiovisual materials. However, it is not possible for all teaching facilities to provide microscopes dedicated for educational purposes, a smaller number of them possess multi-user microscopes, permitting simultaneous access to a high number of trainees and is rather unlikely to have one microscope for each trainee.

Cloud and web based training programs appears to be a really promising solution; as the financial barriers, workplace and time restrictions, experienced by the traditional educational methods in cytopathology, can be eliminated. Additionally, web-based

learning systems can involve traditional learning methods and skills such as decision making, reasoning and problem solving, can be developed (Casebeer et al., 2003; Stergiou et al., 2009a). Such skills are critical for the everyday medical practice of professionals. Cloud computing can become the means to give additional value, to the already available digital age education. Rich information becomes crucial for training; still images captured by the microscope do have limitations. The trainee experiences only a small part of the glass slide, and areas selected by experts. This constitutes a bias for the trainees of the digital era. The solution is obvious: complete digitized slides (WSI). However, the storage and transmission of such large amount of data, despite being possible nowadays, faces a lot of difficulties. The rise and spread of cloud computing facilitate the elimination of both barriers, as storage is virtually unlimited, has low price and telecommunication infrastructure has a large capacity and is cheap because it is shared. Thus cloud computing and virtual slides seem to be the future of continuous medical education and eLearning for cytopathologists.

The mobile device has been already employed in this training process. In 2006, a group of reasearchers, reported on the use of mobile phone cameras as a method of remote teaching in undergraduate pathology education (Sharma & Kamal, 2006), by allowing the use of mobile devices during examinations as an aid to memory. Five years later, another researcher (Collins, 2011), reported on the usage of iPads for the online distribution of digital textbooks for cytopathology. The major benefits of this cytopathology e-book was the searchable content, the interactive text and references with high resolution cytopathology photographs, the capability to bookmark a page for future reference, the video embedding for multimedia rich content and the instant access to medical references with a single touch. The 64 GB capacity of the mobile device was considered as adequate storage space. Nowadays, the cloud based storage and the always connected capability provides virtually unlimited storage. Since 2011, there are several commercial applications online, these include handbooks (Elsevier, 2015), atlases, for example the Johns Hopkins Atlas of Pancreatic Cytopathology (Meszaros, 2014) and the majority of cytopathology related scientific journals. Five years earlier mobile applications were designed taking to account the special characteristics of the mobile device, however modern mobile phone browsers, have turned all those pioneering efforts almost obsolete, as any web page can today be viewed on standard mobile phone browsers and the high resolution of screens enhances this experience.

## Tele-Cytology

Telecytology is the interpretation of cytological material at distance via the use of digital images. Historically there are numerous attempts to implement telecytology (Khurana, 2012; Thrall, Pantanowitz, & Khalbuss, 2011; Tsilalis et al., 2012a). It

is ideally suited for cases that encounter transportation difficulties, such as cases of patients residing in countries with lots of mountains, islands or many dispersed cities and villages. In these situations, because of weather conditions, vehicles, ships or planes are unable to travel and in many cases due to extreme weather conditions (snow, rain, winds) cities and villages are cut-off. However, telecytology has still limited applications and acceptance. This is mainly due to the challenges in making digital images of acceptable quality, able to reproduce the biological material seen on the glass slides, therefore, minimizing diagnostic errors due to the poor quality of the digital representation.

Cytopathologists traditionally perform diagnostic tasks with minimal information; thus they are using other sources of information in order to improve the accuracy of their diagnoses (the triple test in breast fine-needle aspiration cytology, immunocytochemistry, or molecular tests such as flow cytometry for cervical cancer detection). The Cloud represents a network of interconnected information systems, therefore, disconnected medical data sourcescould easily be integrated. As the medical records of more patientscould be accessible, the amount of information available to cytopathologists will increase. Cloud computing has the potential to make the entire medical record of a patient, accessible for review by a cytopathologist. However, the obligation of the cytopathologist to review such records is questionable and a subject for discussion (Renshaw, 2011), because at present there is no standard or a recommendation. Some questions are: should cytopathologist review admission notes for all cases? Should cytopathologists seek prior material from other laboratories that are mentioned in these notes if they were not sent for review by the clinicians? Should cytopathologists review imaging material? For example, reviewing mammograms to ensure that lesions under examination, appear as well in the radiographic material, the ultimate goal is to ensure that cytological material is from the tissue appearing in the mammogram.

Despite the questions mentioned in the previous paragraph, it is a fact that there will be increasing access to more and more information, obviously by means of cloud computing technology. Accessing all these big data is obviously time consuming and in the most of the cases of no value, and this is a major reason for the previous questions related to the obligations of cytopathologists in the digital era. The answer is not clear; however, cloud systems have the capability to exploit advanced and intelligent search engines that may automatically identify important information and alert cytopathologists. These capabilities will remove the time barriers for the adoption of extensive health record examination. As a result, further evaluation and development of intelligent cloud based search engines exploiting artificial intelligence appears worthwhile. Computing power intensive algorithms seem to be easily a reality with cloud based services on demand.

Digital cytopathology has undergone a period of growth and expansion; catalyzed by changes in imaging hardware and computing. Telecytology (Della Mea, Cataldi, Pertoldi, & Beltrami, 2000; Markidou, Karakitsos, & Pouliakis, 1999; Williams, Mullick, Butler, Herring, & O'Leary T, 2001) is the most obvious application having the potential to be transferred directly into the mobile device. In the past, telecytology has been used for reproducibility assessment (Archondakis, 2013; Tsilalis et al., 2012b), in the field of remote diagnosis there is a plethora of applications as well (Briscoe et al., 2000; Ribu et al., 2013; Spat et al., 2013; Yamashiro et al., 2004), but in all cases via desktop or laptop computers. The advances on mobile devices: first on transmission speed and second on display resolution, seem to allow mobile cytopathology. Applications can be extremely simple and sometimes is not even required development of specialized software, for instance images can be send by e-mail or uploaded on a web place, additionally image viewers are already embedded into the mobile devices due to photography enhancements.

In the scientific bibliography related to histopathology, in 2009, two independent groups (Bellina & Missoni, 2009) reported the use of mobile phone cameras, to take static digital microscopy images through the microscope and used them for telepathology. Moreover, telemedical applications are valuable tools for cytopathologists in order to promote interlaboratory collaboration for obtaining a second opinion.

## Research

The potential applications of cloud computing for research in laboratory medicine (A. Pouliakis, Spathis, et al., 2014) and cytopathology (A. Pouliakis, Archondakis, et al., 2014) seems endless, it is not only the endless storage but mainly the enormous processing power of the cloud. The major research fields are:

### Cytogenetics

the large comparative genomics studies require increasing computational power as the number of available genome sequences constantly rises. Local infrastructures are becoming not capable to supply the demand for increased computational power. Parallel computing architectures, toolboxes (Baudis, 2006; Drozdov, Ouzounis, Shah, & Tsoka, 2011; Shannon et al., 2003) and in particular cloud computing systems, seem to be the solution to alleviate this increasing pressure. Nowadays many efforts and implementations have been reported: comparative genomics algorithms (specifically the Reciprocal Smallest Distance-RSD algorithm) from local computing infrastructures have been redesigned for cloud environments in order to exploit their speed and flexibility (Wall et al., 2010). The results indicated that

cloud computing environments may provide a substantial boost for the algorithm execution time and problem solving with a manageable cost. Technological advances for DNA sequencing have lowered the price for a personal genome sequence (the 3 billion letters in our DNA) towards under $1,000 (Davies, 2010). This will create a new challenge: the analysis of cohorts for cancer research and treatment. Large distributed databases are required due to the increased data volume (Big Data) and obviously extreme CPU power for their analysis. Cloud computing could be a solution for both issues. A side effect of low costs in DNA sequencing comes from the offered opportunity that allows scientists to collect and analyze whole genomes for genome-wide association studies. As such genetic data do have the potential to be more informative than standard medical records. In addition the capability offered by cloud computing to access and analyze such data sets, being supplied by scientific groups around the planet, necessitates either a paradigm shift in the way that science is done, and/or revised understandings of privacy and informed consent. A proposal by some researchers is to promote both shifts (Greenbaum & Gerstein, 2011).

## Proteomics

Proteomic techniques can be used to identify markers for cancer diagnosis because the proteome reflects both the intrinsic genetic program of the cells, as well as the impact of the environment (Bhat, Dakna, & Mischak, 2015; Godovac-Zimmermann, 2015; Perez-Riverol, Alpi, Wang, Hermjakob, & Vizcaino, 2015; Yin, Levy, Willinger, Adourian, & Larson, 2015). Proteome analysis has been used in cytopathology for the identification of tumors in various organs: thyroid (Torres-Cabala et al., 2006), breast (Li, Zhao, & Cui, 2013; Sohn et al., 2013), gastric system (Fowsantear, Argo, Pattinson, & Cash, 2013; Uppal & Powell, 2013) and cervix (Yim & Park, 2006) as well as for response to treatment (Madden et al., 2009). One of the most promising developments from the study of human proteins is the identification of potential new drugs for disease treatment.

Proteome research is mainly based on the assignment of unidentified spectra to peptides. These methods, including tag-based and de novo searches, have a high computational cost and involve processing of large volumes of experimental spectra thus requiring computers with large storage capacity. These exhaustive identification attempts can rarely be carried out in laboratories, due to the lack of computational power, and limited support by information technology specialists to run specialized algorithms. Cloud computing is the ideal environment to perform such type of research. As a result, several attempts have been proposed and conducted by various researchers (Halligan, Geiger, Vallejos, Greene, & Twigger, 2009; Leprevost et al., 2013; Mohammed et al., 2012; Muth, Peters, Blackburn, Rapp, & Martens, 2013;

Slagel, Mendoza, Shteynberg, Deutsch, & Moritz, 2015). Cloud computing allows laboratories to pay for compute time as per requirements, rather than investing on local server clusters and IT personnel.

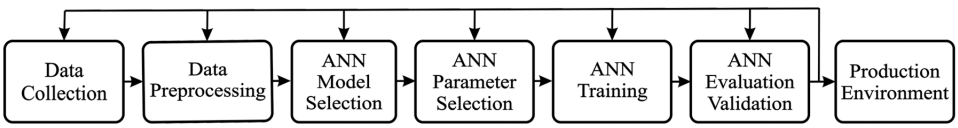
## Artificial Intelligence

The application of artificial intelligence in cytopathology is not new (Karakitsos, Stergiou, et al., 1996). During the last decades, various classification techniques have been used in medicine and especially in diagnostic cytology, involving either classical statistical models or more advanced techniques, such as artificial neural networks (ANNs) (Astion & Wilding, 1992; Cochand-Priollet et al., 2006; Karakitsos, Cochand-Priollet, Guillausseau, & Pouliakis, 1996; Marchevsky, Tsou, & Laird-Offringa, 2004; C. Markopoulos et al., 1997). More specifically, concerning cytology, ANNs have been applied to various organs, among others stomach (Chien et al., 2008; Karakitsos et al., 2004; Yamamura et al., 2002), breast (Dey, Logasundaram, & Joshi, 2013; Ljung, Chew, Moore, & King, 2004; Ch Markopoulos et al., 1997; Wolberg, Tanner, Loh, & Vanichsetakul, 1987), urinary system (Karakitsos et al., 2005; Schaffer, Simon, Desper, Richter, & Sauter, 2001; Vriesema et al., 2000), cervix (Giovagnoli, Cenci, Olla, & Vecchione, 2002; Karakitsos et al., 2011; Kok, Habers, Schreiner-Kok, & Boon, 1998) thyroid (Haymart, Cayo, & Chen, 2009; Karakitsos, Cochand-Priollet, Pouliakis, Guillausseau, & Ioakim-Liossi, 1999; Rorive et al., 2010; Varlatzidou et al., 2011) and endometrium (Abraham Pouliakis et al., 2013).

A typical cycle for the creation of a useful ANN system (see Figure 2) involves several but typical steps (A. Pouliakis, Archondakis, et al., 2014)

- **Data Collection:** Obtaining the data that will be used to create the intelligent system
- **Data Preprocessing:** Depending on the type of the ANN system and algorithm, data preprocessing may be required
- **ANN Model Selection:** Choice of a suitable ANN type for the problem
- **Parameter Selection:** Configuration of the selected ANN parameters
- **ANN Training:** Identification of the ANN parameters that actually compose the solution to the problem

*Figure 2. Typical cycle for ANN system production*



- **Evaluation:** Check the performance of the produced system on known and unknown data

If the performance of the system is satisfactory then the produced ANN system can be used in a production environment otherwise the procedure should start again from an earlier stage. This complexity and the requirement to run numerous systems and combinations of ANN types, architectures and parameters increases exponentially the number of experiments and the computing resources and computing time. Additionally the training procedure for several ANN types is computing power "hungry"; it is not unusual that training of some ANN types can take weeks of computer time. Cloud computing can provide a pool of shared resources available on demand, thus can facilitate the production of ANN systems both by increasing the number of experiments that can run and by decreasing the time of each experiment. Parallelization comes to contribute in this arena as ANNs can be programmed to be trained in parallel machines, thus reduction of training time, if appropriate computing resources are available, is possible within the cloud environment.

## Impact of Cloud Computing on Cytopathology Daily Workflow

Cloud computing allows administrators of cytopathology departments to take advantage of virtualization capabilities and avoid maintenance or hardware and eventually software. In summary the benefits in the routine work include:

- Cytopathologists may focus on their diagnostic practice and not how or where the service is hosted or processed.
- Cloud computing permits cytopathology end users to use hardware and software stored remotely over the Cloud, without having to purchase them, but rather to apply a pay-per-use model.
- By means of cloud computing, various cytology and medical applications are delivered as a service over the Internet, which is named software as a service (SaaS software).
- Cloud computing can provide a software platform for laboratory information systems, remote image review software (telecytology) and billing software to cytopathologists, having remote access to all applications by using computers or handhelds over the Internet.
- Cloud facilitates access of data through Internet and via mobile devices, thus information is available on any-time, any-place, any-manner, this enhances the turnaround time and the quality of services offered, eventually for the benefit of the patients.

## Shortcomings of Cloud and Mobile Computing

As happens for all emerging technologies, early adopters encounter unforeseen problems. In the field of cloud and mobile cytopathology there may be numerous issues. The major is security, because patient confidential data is stored remotely and is sent and received through Internet. This makes the data vulnerable to security violations. When considering WSI viewing on smartphones; a major issue is the physically small screen Because a physically large display is a requirement for viewing and interpreting WSIs. A second problem related to WSI is the vendor specificity data formats, which impede viewing in any handheld device and desktop. The third issue related to WSI is the size of the virtual slide; which poses requirements for broadband communications and device memory.

# SOLUTIONS AND RECOMMENDATIONS

Security issues can be caused by the explosion of data in the air (wireless networks) and the installation of databases and servers in publicly accessible networks. Solutions to tackle these problems are proposed subsequently. Data encryption during storage and transmission and while connecting with the services is one choice; specifically, the solution includes encrypted storage in the databases as well as encryption during data transfer in communication channels. More advanced methods for the cloud environment have been proposed as well, for example to employ a multi-cloud approach with sharing mechanisms for keys (Mouli & Sesadri, 2013) or identifying patients using cross reference numbers (Kondoh, Teramoto, Kawai, Mochida, & Nishimura, 2013), both methods separate sensitive from non-sensitive information.

Passwords and password control mechanisms or mandatory biometric checks can be used for unauthorized access prevention. Today many smartphones and laptops have embedded hardware for biometrics based security such as fingerprints. These can be exploited to enhance and ensure user and/or device authentication.

Long term archival of data, load balancing and fail over can be offered by the cloud service providers by 1) maintaining mirror servers 2) providing multiple communication channels connected to numerous Internet service providers 3) expansion of processing power by employing additional virtual servers.

For WSI access issues from the handheld device and in relation to the three identified problems in the previous section, there possible workarounds: (screen size) screens can be expanded if needed, for example by external monitors of foldable screens, see for example the prototype proposed by Sony 2010 (SONY, 2010). Projectors of very small size are commercially available and may be integrated in mobile devices in the future, thus digital slides can be projected on walls. In relation

to the multitude of incompatible WSI formats, there are some efforts performed as well: actually it is proposed a vendor neutral open source library (Goode A, 2008), named OpenSlide; this library is functional on desktop computers, however, porting in the mobile environment is feasible, because OpenSlide is implemented as device driver. The third issue of WSI was related to the large size of data; here the cloud can provide affordable and expandable storage space with no requirements for local storage. This comes at the cost of transferring large data volumes from/to the cloud. Fortunately, telecommunication networks may today provide the required bandwidth as 4G, 5G and WiFi networks are continuously expanding.

## FUTURE RESEARCH DIRECTIONS

Cloud computing is expected to be further exploited by cytopathology laboratories wishing to modernize their services, enhance activities and reduce costs. One challenge is to resolve possible problems regarding data safety and security under a real environment that will reveal issues not foreseen during the design phase. Evaluation of existing applications, such as e-mail or cloud based storage and access of images and WSIs is required as well. Especially for the mobile device, such evaluation should be performed in a multiuser environment, by using different handheld models and according to well-structured questionnaires and evaluation metrics. Definitely, electronics and industry products to enhance display sizes or facilitate access to large displays is an issue requiring more research, efforts and especially experimental products to be tested in practice seems to be a serious necessity.

In the research arena, it is known that DNA sequencing costs are continuously decreasing. Today it is possible and extremely easy for genomics researchers to have large amounts of data either accessible through the Cloud or produced in their laboratory. The increasing availability of computational power according to Moore's law and the falling price of storage, allow scientists of cytopathology to create and store large genetic data sets for their research and additionally employ hungry for computing power algorithms to process them. Lack of in house software and computational power to experiment on exhaustive search algorithms, is expected to lead researchers towards cloud computing services in order to conduct their research. Otherwise large amounts of information that could be used for knowledge extraction remains untouched, underutilized and unexplored. Cloud computing, as a bonus, will provide methods and tools for data sharing among scientists, a field that more research seems to be required as well.

## CONCLUSION

Cloud computing promotes the concept of bedside cytopathology, point-of-care cytopathology, and instant cytopathology. It provides cytopathologists, pathologists, physicians, and even patients with the possibility to view medical data on any display and device using simply an Internet connection. The cloud based systems have the potential to improve the telecytology field by providing a model to save resources and improve patients control and management. Also, having an electronic clinical history would save paper, physical space and would improve the efficiency of clinicians and cytopathologists. The use of shared infrastructure would result in increasing medical data homogeneity, facilitating correlations and data mining. Nowadays we have reached a point where both the software and the hardware of the mobile device, coupled with the connection to the cloud, where vast amounts of data can be stored, are powerful enough to be leveraged in cytopathology. The main advantage is that it gives all patients equal access to medical services, irrelevant of geographical and time barriers. This is especially important when it comes to specialists and other services usually inaccessible outside of large urban centers.

Perhaps the most technically elegant and challenging application is mobile WSI. However, it is not the most wanted application, more demand appear to be on simple services, such as notifications, results transmission, education and perhaps laboratory management and the interactions among the various "actors" in the cytopathology ecosystem.

Despite the security and privacy risks; that modern technology seems to be able to resolve, the cloud and mHealth technology shows major benefits that the public sector and government IT organizations could take advantage of. In brief:

- Reduced cost, mainly for the hardware but for shared software as well
- Flexibility, as mHealth technology offers much more flexibility than desktop based computing
- Mobility, uses can access information wherever they are, rather than having to remain at their desks and with appropriate freedom from time and space

Work on cloud, handheld and mobile computing in cytopathology is scarce today, but, there is great potential for new applications. Nowadays, there are clear niches and several experimental successes with handhelds in cytopathology, for education, telecytology, and care delivery via various communication interactions; however development of standards, experimentation with more studies, practicing and guidelines for validation are required. The couple of the Cloud and mobile computing seems to have the potential to change the landscape of cytopathology in the future.

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## KEY TERMS AND DEFINITIONS

**Cloud Computing:** A large number of computers connected through a network, capable to run an application on many computers and to configure virtual servers. Virtual servers do not have physical presence and can be moved around and scaled up or down without to be noticed by end users, like a cloud.

**Cytopathology:** A specialty of medicine related to the study and diagnosis of diseases by the examination of human (and animal) cells.

**e-Health:** The healthcare supported by electronics, informatics and telecommunications.

**e-Learning:** A broad concept referring to the application of information and communication technologies (ICT) for learning purposes.

**Laboratory Information System (LIS):** Or Laboratory Information Management System (LIMS) is a software-based system for the support of operations of the modern laboratory, such as workflow, sample tracking, data exchange interfaces. LISs are often capable to be connected with medical analyzers for automated extraction and storage of measurements.

**Mobile Health (mHealth):** The practice of medicine and public health supported by mobile devices.

**Quality Control:** The set of processes by which entities review the quality of all factors involved in product, service or activity, during QC processes, the products, services and activities are tested or validated in order to reveal defects and problems, before their release.

**Screening:** In the medical world is the examination of a group of persons in order to identify healthy persons from those who have an undiagnosed disease or have high risk to get sick.

**Telecytology:** The application of cytopathology from distance.

# Chapter 6
# Massive-Training Support Vector Regression With Feature Selection in Application of Computer-Aided Detection of Polyps in CT Colonography

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## ABSTRACT

*A major challenge in the latest computer-aided detection (CADe) of polyps in CT colonography (CTC) is to improve the false positive (FP) rate while maintaining detection sensitivity. Radiologists prefer CADe system produce small number of false positive detections, otherwise they might not consider CADe system improve their workflow. Towards this end, in this study, we applied a nonlinear regression model operating on CTC image voxels directly and a nonlinear classification*

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*model with extracted image features based on support vector machines (SVMs) in order to improve the specificity of CADe of polyps. We investigated the feasibility of a support vector regression (SVR) in the massive-training framework, and we developed a massive-training SVR (MTSVR) in order to reduce the long training time associated with the massive-training artificial neural network (MTANN) for reduction of FPs in CADe of polyps in CTC. In addition, we proposed a feature selection method directly coupled with an SVM classifier to maximize the CADe system performance. We compared the proposed feature selection method with the conventional stepwise feature selection based on Wilks' lambda with a linear discriminant analysis classifier. The FP reduction system based on the proposed feature selection method was able to achieve a 96.0% by-polyp sensitivity with an FP rate of 4.1 per patient. The performance is better than that of the stepwise feature selection based on Wilks' lambda (which yielded the same sensitivity with 18.0 FPs/patient). To test the performance of the proposed MTSVR, we compared it with the original MTANN in the distinction between actual polyps and various types of FPs in terms of the training time reduction and FP reduction performance. The CTC database used in this study consisted of 240 CTC datasets obtained from 120 patients in the supine and prone positions. With MTSVR, we reduced the training time by a factor of 190, while achieving a performance (by-polyp sensitivity of 94.7% with 2.5 FPs/patient) comparable to that of the original MTANN (which has the same sensitivity with 2.6 FPs/patient).*

## INTRODUCTION

Colorectal cancer is the second leading cause of mortality due to cancer in the United States (Jemal et al., 2009; Mamonov, et al., 2014). Evidence has shown that the risk of colon cancer death could be reduced with early detection and removal of colonic polyps (Winawer et al., 1997). Fiberoptic (or optical) colonoscopy is considered the gold-standard diagnostic test as it offers direct biopsy or removal of suspicious colonic polyps (Winawer et al., 1997). However, optical colonoscopy is invasive, i.e., it has risks of complications such as perforation; it is expensive, and it requires a long examination time and creates high patient discomfort. Therefore, medical centers are seeking alternative techniques as population screening tools. CT colonography (CTC), also known as virtual colonoscopy, has been proposed as an alternative, less invasive technique for detecting colorectal neoplasms (Chaoui, Blake, Barish, & Fenlon, 2000; Coin et al., 1983; Johnson & Dachman, 2000; McKenna, et al., 2012; van Wijk, et al., 2010; Vining, 1997), which requires a lesser examination time and causes less patient discomfort. However, the sensitivity of CTC can be lower for inexperienced readers because there is a long learning curve for CTC reading. This

limitation begs for a CADe approach as a "second reader" to assist radiologists in detecting polyps from CTC images (Yoshida & Dachman, 2005).

There has been great interest in the development of automated or semi-automated CADe schemes (Giger & Suzuki, 2007; Suzuki, 2014) for the detection of polyps in CTC in the past decade (Gokturk et al., 2001; Summers et al., 2001; Summers et al., 1998; Suzuki, 2012b; Suzuki, 2013; Suzuki, 2014; Kenji & Suzuki, 2012; Vining, Ge, Ahn, & Stelts, 1999; Yoshida & Nappi, 2001). A CADe scheme for polyp detection is typically composed of candidate detection followed by supervised classification. The task of candidate detection is to achieve a high sensitivity in detecting polyps by including as many suspicious lesions as possible. After the polyp candidate detection stage, feature extraction and analysis are performed on the objects detected in CTC. Based on these features, various classifiers have been applied that classify the candidates into polyps and non-polyps so that FP detections can be reduced while a high level of sensitivity is maintained (Wu et al., 2013). Linear and quadratic discriminant analysis were used by Yoshida et al. (Yoshida & Nappi, 2001), as well as by Jerobko et al. (A. Jerebko, Lakare, Cathier, Periaswamy, & Bogoni, 2006), as simple and effective classifiers. Acar et al. also applied a linear classifier based on edge-displacement field features (Acar et al., 2002). Gokturk *et al.* employed a support vector machine (SVM) to distinguish between polyps and normal tissue (Gokturk et al., 2001). Song et al. used receiver operating characteristic curve to guide SVM based polyp detection (Song, et al., 2014). To improve the discriminant ability of SVMs, a committee of SVMs has been proposed to take advantage of combining multiple classifiers (Jerebko, Malley, Franaszek, & Summers, 2005). Another popular classifier is the artificial neural network (ANN) (A. K. Jerebko, Summers, Malley, Franaszek, & Johnson, 2003). Logistic regression has also been employed for reducing FP detections where features were ordered according to their relevance (van Ravesteijn et al., 2010). Yao et al. employed a topologic height map for FP reduction (Yao, Li, & Summers, 2009). Zhu et al. developed two-dimensional projection features for distinction between FP and true-positive (TP) detections (Zhu et al., 2010). Devi et al. used k-means clustering and SVM for feature extraction and classification of true polyps from false positives (K. Gayathri Devi, 2013). A distance weighted based classifier was proposed for CADe of polyps by Hu et al. (Yifan Hu, 2015). In summary, all of these proposed classifiers operated on extracted geometric, texture, morphologic, and other features from segmented polyp candidates in CTC images. However, the extracted features might be noisy (with errors) due to CTC image reconstruction errors, segmentation errors, and other factors. Moreover, it requires not only domain knowledge for design of the set of features to be extracted, but also advanced feature selection methods for choosing the most discriminant ones.

Feature selection has long been an active research topic in the CADe research field (Ong & Seghouane, 2011; Fu et al., 2014). Several intensive surveys have been conducted on the topic of feautre subset selection (Chandrashekar & Sahin, 2014; Dash & Liu, 1997; Guyon & Elisseeff, 2003; Liu & Motoda, 1998; Huan Liu, Yu, Member, Yu, & Member, 2005). A hybrid feature selection technique was used for detection of prostate cancer in a trance-rectal ultrasound image-based CAD system (Maggio et al., 2010). An ensemble classifier based on the AdaBoost learning algorithm was coupled with a sequential feature selection (SFS) technique to discriminate breast tumors in ultrasonic images (Takemura, Shimizu, & Hamamoto, 2010). To improve the correct classification rate (CCR) in grading for prostatic carcinoma in pathological images, a sequential forward floating selection (SFFS) approach was coupled with the Bayesian, k-NN, and SVM (Huang & Lee, 2009). A wrapper feature selection technique based on the Particle Swarm Optimization (PSO) was proposed for prostate cancer analysis in transrectal ultrasound (TRUS) images (Mohamed & Salama, 2008). One of the most popular feature selection methods is the stepwise feature selection based on Wilks' lambda coupled with linear discriminant analysis (LDA) (Draper & Smith, 1998). The method has been applied in FP reduction in CADe systems for CTC because of its simplicity and effectiveness (Yoshida & Nappi, 2001). However, the Wilks' lambda criterion only measures group separation, but not classification performance directly (Draper & Smith, 1998). Moreover, classification between polyps and non-polyps is very difficult because of noisy and overlapping image features. The performance of stepwise feature selection based on LDA begs more advanced feature selection methods and classifiers.

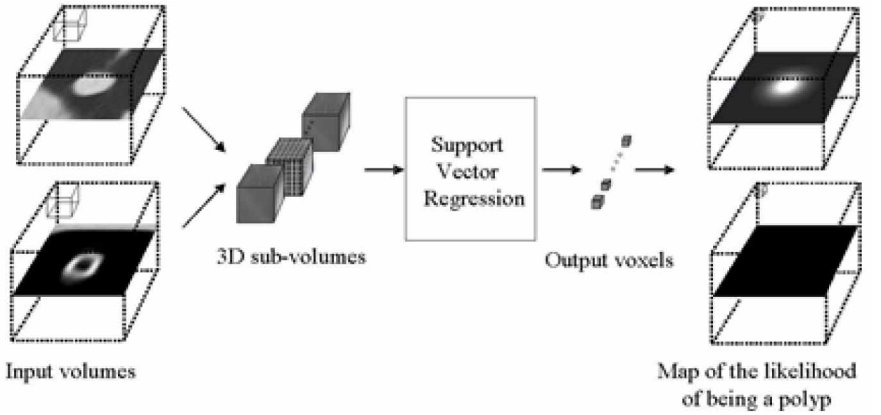
Recently, Suzuki et al. presented a different approach to the reduction of FP detections of polyps, in which an ANN was used as a regression technique instead of a classifier (Kenji Suzuki, Hiroyuki Yoshida, Janne Nappi, & Abraham H. Dachman, 2006). The inputs to the ANN regression model were voxel values of CTC images rather than computed features from segmented polyp candidates. The ANN was trained with a massive number of subvolumes extracted from 3D CTC volumes together with "teaching" volumes containing the distribution for the "likelihood of being a polyp", therefore termed *Massive-Training ANN* (MTANN) (Chen & Suzuki, 2013, 2014; Chen, Suzuki, & MacMahon, 2011; K. Suzuki, 2012a; Suzuki, Abe, MacMahon, & Doi, 2006; Suzuki & Doi, 2005; Suzuki, III, Li, Sone, & Doi, 2003; Hang, et al., 2013). As a nonlinear regression technique, the MTANN is able to learn to differentiate between the underlying structures of polyps and non-polyp regions. Therefore, the trained MTANN is able to enhance polyps and suppress non-polyps so that the score for a polyp is higher than that for a non-polyp. The promising performance of the MTANN has been demonstrated in the reduction of FP detections in CTC CADe (Suzuki, Rockey, & Dachman, 2010; Suzuki, Yoshida, Nappi, Armato, & Dachman, 2008; Kenji Suzuki et al., 2006), computerized detection

of lung nodules in low-dose CT (Arimura et al., 2004; Li et al., 2005; Suzuki, 2009; Kenji Suzuki et al., 2003), and CADe for detecting nodules in chest radiographs (Suzuki, Shiraishi, Abe, MacMahon, & Doi, 2005). However, the computational cost of the training of an MTANN is very high, given the large number of training samples extracted from 3D CTC images. For example, the training of a 3D MTANN with ten polyps and ten FPs took 38 hours on a personal computer (Intel, Xeon, 2.7 GHz) (Suzuki, Yoshida, Nappi et al., 2006). The training time increases much more when a mixture of expert MTANNs is used for reducing a large variety of FPs. It took 244 hours to train a mixture of six MTANNs. This drawback hinders the development of a CADe scheme. Recently, a dimension reduction technique based on a Laplacian eigenmap has been used in the MTANN framework to reduce the training time by a factor of 9.5 while maintaining a comparable performance (Suzuki, Zhang, & Xu, 2010).

In this study, we investigated the feasibility of one state-of-the-art nonlinear regression technique, namely, support vector regression (SVR), as an alternative to improve the efficiency of training of the massive-training framework for reducing FPs in the computerized detection of polyps in CTC. Unlike ANNs, SVR is a memory-based method that stores a part of or the entire training data for testing. Therefore, it is generally fast to train and is able to improve the efficiency of the massive-training methodology. Moreover, SVR is a kernel-based nonlinear regression technique, where a kernel function is used for implicitly transforming the original image data into a high-dimensional reproducing kernel Hilbert space (RKHS). The transformation is able to capture the inherent nonlinearity underlying the CTC images by enhancing polyps and suppressing non-polyp objects. Rooted in a maximum margin property, SVR offers excellent generalization ability and robustness to outliers. In this study, we applied SVR as a volume-processing technique in the distinction of polyps from FP detections in a CTC CADe scheme. The MTSVR model was trained directly with voxel values from CTC images. A 3D scoring method based on a 3D Gaussian weighting function was applied to the outputs of MTSVR for distinction between polyps and non-polyps.

On the other hand, we also applied the support vector machine as a classifier operated on the extracted features. To this end, we proposed a novel feature selection method based on an SFFS procedure to maximize the area under the receiver-operating-characteristic curve (AUC) value. The maximal AUC SFFS method was directly coupled with an SVM classifier. The AUC value has been used extensively to measure how a CADe system performs (Tan et al., 2014; Wang et al., 2015). Therefore, our proposed feature selection method is able to choose a subset of features for directly maximizing the performance of a CADe system, particularly, in our case, reducing the FP detections in the CADe of polyps in CTC.

*Figure 1. Architecture and training of a 3D MTSVR. The input CTC volumes including a polyp or a non-polyp are divided voxel by voxel into a large number of overlapping 3D subvolumes.*




We tested the proposed feature selection method in terms of FP reduction performance by comparing it to the popular stepwise feature selection based on Wilks' lambda with an LDA classifier. In order to demonstrate the merit of the proposed MTSVR model, we compared it with the MTANN technique in terms of the training time reduction and FP reduction performance. The novelty of this work is twofold. First, it provided an alternative, i.e., SVR, to the core of the massive-training framework, other than dimension reduction techniques, to improve the efficiency of the training while maintaining a comparable performance in reducing FPs in the computerized detection of polyps in CTC. Second, this study presented a novel feature selection method that was able directly to maximize the AUC value of the CADe system.

## MATERIALS AND METHODS

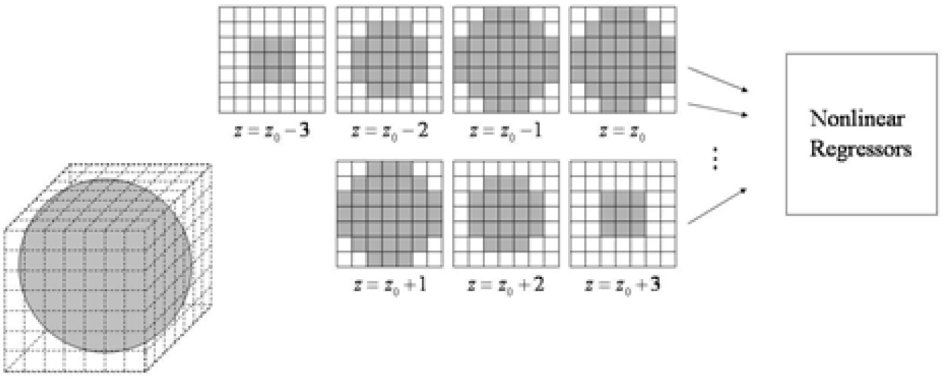
In this section, we depict the database used in this study and the performance of a previously reported CADe scheme (Yoshida & Nappi, 2001). We describe the feature extraction and maximal AUC SFFS feature selection method. The general massive-training framework of a nonlinear regression technique as a classifier system to differentiate polyps from FP detections is also presented. We provide the technical background of support vector machines both as a classifier and a regression model.

## CTC Database

We retrospectively collected the CTC cases used in this study. The database was acquired at the University of Chicago Medical Center. It consisted of 240 CTC datasets obtained from 120 patients. Each patient followed the standard CTC procedure with pre-colonoscopy cleansing and colon insufflations with room air or carbon dioxide. Oral contrast was not administered. Both supine and prone positions were scanned with a multi-detector-row CT scanner (LightSpeed QX/i, GE Medical Systems, Milwaukee, WI) with collimations between 2.5 and 5.0 mm, reconstruction intervals of 1.0-3.0 mm, and tube currents of 60-120 mA with 120 kVp. The detailed reconstruction intervals were as follows: 1 mm (2 patients), 1.25 mm (2 patients, 7 polyps), 1.5 mm (64 patients, 12 polyps), 2.5 mm (51 patients, 10 polyps), and 3 mm (1 patient). Each reconstructed CT section had a matrix size of 512×512 pixels, with an in-plane pixel size of 0.5-0.7 mm. Optical colonoscopy was also performed for all patients. In this study, we used 5 mm as the lower limit on the clinically important size of polyps. The locations of polyps were confirmed by an

expert radiologist based on CTC images and on pathology and colonoscopy reports. Seventeen patients had 29 colonoscopy-confirmed polyps, 15 of which were 5-9 mm and 14 were 10-25 mm in size. The shapes of the polyps were pedunculated and sessile (i.e., there were no flat lesions; refer to (Lostumbo, Suzuki, & Dachman, 2009) for the definition). The whole database was divided into a training set and a testing set. The training set consisted of 27 patients, 10 of which had 10 polyps. We selected 10 non-polyps (i.e., FP sources) from the training set. These 10 polyps and

*Figure 2. The spherical-input subvolume and the slice-by-slice representation of the digital quasisphere in a 7 × 7 × 7 voxel cube. Each 7× 7 square stands for a certain image slice in the subvolume, where $z_0$ is the middle slice. The input voxels to the nonlinear regression models are the gray squares in each matrix.*



10 non-polyps were used to train the proposed models. The testing set contained 93 patients including 19 polyps in seven patients and 86 negative patients.

An initial CADe scheme for detection of polyps in CTC was applied to the database. The CADe scheme was composed of 1) colon segmentation based on centerline tracing (Nappi, Dachman, MacEneaney, & Yoshida, 2002), 2) detection of polyp candidates based on the shape index and curvedness of the segmented colon (Yoshida & Nappi, 2001), 3) calculation of 3D pattern features of the polyp candidates (Nappi et al., 2002; Nappi & Yoshida, 2003; Yoshida & Dachman, 2005), and 4) classification of the polyp candidates as polyps or non-polyps based on quadratic discriminant analysis (QDA) without any feature selection method. The initial CADe scheme yielded a 94.7% (18/19) by-polyp sensitivity with 5.1 (474/93) FPs per patient for the testing set. The major sources of FPs included rectal tubes, stool, haustral folds, colonic walls, and the ileocecal valve. This dataset would be used for training and testing of the MTSVR and MTANN. The MTSVR and MTANN were applied after the initial classification with a QDA classifier.

On the other hand, we chose a subset of the database for the experiment on feature selection. We selected 103 patients. The candidate detection algorithm in the initial CADe scheme missed one polyp, and two polyps were detected only in one view (supine or prone), yielding 24 true–positive polyp detections (TPs) in 46 supine and prone views, and 2,624 FPs. Therefore, the candidate detection algorithm in the initial CADe scheme achieved a 96% (24/25) by-polyp sensitivity with 25.5 (2624/103) FPs per patient. This database would be used for training and testing the proposed maximal AUC SFFS feature selection. The feature-based classification was applied directly after the candidate detection algorithm.

## General Framework of Massive-Training Nonlinear Regression (Xu & Suzuki, 2011)

The basic idea of using nonlinear regression techniques to distinguish polyps from non-polyp objects is to learn the distinctive underlying image structures of different classes. Unlike a classifier that is based on features extracted from segmented objects, a regression model uses individual voxel values as input. It is thus able to differentiate subtle characteristics of different classes in a local scale. For example, the shape index has been proposed as a feature to detect polyps and has also been retained for classification (Yoshida & Nappi, 2001). However, the shape index values for a polyp and for part of a rectal tube would be very close because both have cap-like shapes. Therefore, a classifier failed to separate rectal tubes from polyps based on the shape index (K. Suzuki, H. Yoshida, et al., 2006). However, the underlying image structures are very different. Rectal tubes are hollow in the center, whereas polyps are solid.

The general framework of massive-training nonlinear regression is illustrated in Fig. 1. The input volumes are 3D CTC images. Usually, the pixel size within a CT image is different from the reconstruction interval across CT sections. Moreover, the reconstruction intervals might vary among different institutions under different protocols. In order to mitigate such variations, we converted the original CTC images into isotropic volume data. The voxel values were normalized to a range between 0 and 1, where 1 corresponded to 1000 Hounsfield Units (HU) and 0 was matched to –1000 HU. Each input volume contained 64 CT slices, and each image slice was of 64 by 64 pixel size. The center of the input volume is the detected location of suspicious polyps from the original CADe scheme. If we input each volume directly to the nonlinear regression models, the dimension is prohibitively large (64×64×64 = 262,144). Therefore, we divide the input volumes into multiple subvolumes. Each subvolume is a cube of 7×7×7 dimension. We scan the entire input volume with the subvolume voxel by voxel. Because the average shape of polyps is close to a sphere, we can further reduce the number of input voxels for the nonlinear regression model. Figure 2 demonstrates the scheme for extracting a quasi-sphere from a subvolume cube. The number of voxels in the digital quasi-sphere is 171, compared to 343 in the original subvolume cube. The gray square in each matrix is the input voxel to the nonlinear regression model. Therefore, the computational cost is reduced dramatically while the essential image information of polyps is preserved.

The inputs to the nonlinear regression model are the voxel values in the quasi-spherical subvolume, $V_S$. The output of the nonlinear regression model is a continuous scalar value that corresponds to the center voxel in the subvolume, which is defined as

$$O\left(x,y,z\right) = SVR\left\{I\left(x-p,y-q,z-r\right)\middle|\left(p,q,r\right)\in V_s\right\} \tag{1}$$

where $I\left(x-p,y-q,z-r\right)$ is the normalized input voxel to the nonlinear regression model, *x, y,* and *z* are the global coordinates, *p, q,* and *r* are local coordinates, and *SVR*{•} is the output of the nonlinear regression model. The entire output volume is obtained by scanning of the entire input CTC volume with the input subvolume voxel by voxel.

The nonlinear SVR model enhances polyps and suppresses non-polyp objects in the output 3D images. In order to distinguish between the two classes, we present a 3D scoring method that translates the output volume into a single scalar value. The score is defined as

$$S = \sum_{\left(x,y,z\right)\in V_E} f_G\left(x,y,z;\sigma_w\right)\times O\left(x,y,z\right) \tag{2}$$

where $V_E$ is a volume for evaluation that is large enough to cover a polyp or a non-polyp object. The criterion for choosing a suitable $V_E$ is determined by the standard deviation of a Gaussian weighting function in the scoring method given in Eq. (3). We chose a volume of 21x21x21 voxels for $V_E$, because it is large enough to cover the Gaussian weighting function. $O(x, y, z)$ is the output voxel value from the trained nonlinear regression model, and $f_G\left(x, y, z; \sigma_w\right)$ is a 3D Gaussian weighting function with standard deviation $\sigma_w$, which is described as

$$f_G\left(x, y, z; \sigma_w\right) = \frac{1}{\sqrt{2\pi}\sigma_w} e^{-\frac{x^2+y^2+z^2}{2\sigma_w^2}} \tag{3}$$

The purpose of weighting of the output volume with the 3D Gaussian function is to combine the individual voxel values into a single score. The score is a weighted summation of the output voxel values. Because the 3D Gaussian weighting function is centered at the origin of the volume, the higher the score value, the more likely it is that the candidate is a polyp. Classification between polyps and non-polyps is made by thresholding of the scores.

## Support Vector Machines

SVMs are a machine learning technique that maximizes the margin of separation between positive and negative classes. The SVMs achieve this desirable property by implementing the method of structural risk minimization. The principle of structural risk minimization states that the generalization error rate of an SVM on unseen testing data is bounded by the sum of the error rate on training data and an extra term that depends on the *Vapnik-Chervonenkis (VC) dimension* (Vapnik, 1998). Therefore, an SVM is able to provide a good generalization performance. An SVM was first invented by Vapnik as a powerful tool for pattern recognition (Christopher J. C. Burges, 1998; Vapnik, 1998), and has been successfully applied in handwritten digit recognition (Chris J.C. Burges & Schölkopf, 1997), face detection (Osuna, Freund, & Girosi, 1997), text categorization (Joachims, 2002), and many other applications (Christopher J. C. Burges, 1998). On the other hand, an SVM as a memory-based learning method can be trained very fast to train because a part of the training data, called *support vectors*, is stored after the training phase. In this study, we present the SVM as a classifier and a nonlinear regressor. SVR is a regression version of SVM by incorporating a quantitative response. This is one of the main motivations for the use of SVR in the massive-training framework for improvement of the efficiency.

Given a set of *N* training data points $\left\{(x_i, y)\right\}_{i=1}^{N}$ where $x_i$ is the feature vector with $x_i \in \Re^L$ and $y_i$ is the class label with $y_i \in \left\{-1, \quad 1\right\}$, the decision function for the SVM classifier can be written as

$$f(x) = \sum_{i=1}^{N} \alpha_i y_i K(x_i, x) + \alpha_0 \tag{4}$$

The parameters $\alpha_i \geq 0$ are called Lagrange multipliers that are optimized through quadratic programming. $K(x_i, x_j)$ is a symmetric nonnegative inner-product kernel function that is defined in the reproducing kernel Hilbert space (RKHS) with Mercer's theorem (Vapnik, 1998):

$$K(x_i, x_j) = \Psi(x_i)^{\mathbf{T}} \Psi(x_j) \tag{5}$$

The solution depends on the input data samples through the inner product kernel function. Therefore, even though we might not know the explicit formulation of each nonlinear function $\Psi(x)$, we can still obtain the optimal solution via the inner product kernel function. In the applications of SVMs, popular kernel functions include:

Linear kernel function: $K(x_i, x_j) = x_i^{\mathbf{T}} x_j$ (6)

*d*th degree polynomial function: $K(x_i, x_j) = \left(1 + x_i^{\mathbf{T}} x_j\right)^d$ (7)

Gaussian kernel function: $K(x_i, x_j) = \exp\left(-\left\|x_i - x_j\right\|^2 / 2\sigma^2\right)$ (8)

Sigmoid kernel function: $K(x_i, x_j) = \tanh\left(a x_i^{\mathbf{T}} x_j + b\right)$ (9)

The optimal Lagrange multipliers $\alpha_i \geq 0$ in the optimal decision boundary (4) are computed through the maximization of the following objective function:

$$\max_{\alpha_i} \quad \sum_{i=1}^{N} \alpha_i - \frac{1}{2} \sum_{i=1}^{N} \sum_{j=1}^{N} \alpha_i \alpha_j y_i y_j K(x_i, x_j) \tag{10}$$

subject to the following constraints:

$$\sum_{i=1}^{N} \alpha_i y_i = 0 ,$$

$\alpha_i \geq 0$ for *i =1, 2,..., N.*

As the SVM can be reformulated through the regularized function estimation problem with a *hinge* loss criterion (Vapnik, 1998), it can be shown that the SVM has the property of large margin and is robust against outliers. The kernel functions play an important role in the optimal decision boundary of the SVM (4). The RKHS is induced by the kernel functions which control the data distributions in the feature space.

An SVM has also been adapted for a nonlinear regression problem with a quantitative response (Smola & Schölkopf, 2004). Unlike the conventional square loss function that is sensitive to the presence of outliers, the SVR model employs an $\varepsilon$-*insensitive* loss function that is robust to outliers. The $\varepsilon$-*insensitive* error measure ignores any errors of size less than $\varepsilon$. It is defined as

$$V_{\varepsilon}(e) = \begin{cases} 0 & if \ |e| < \varepsilon, \\ |e| - \varepsilon & otherwise \end{cases} \tag{11}$$

Therefore, any error falling into the $\varepsilon$-band is not counted toward the loss. This is analogous to the SVM classifier, where data samples on the correct side of the decision boundary and far away from it are ignored in the optimization.

Consider a nonlinear regression model where the dependence of a scalar $d$ on a vector $\mathbf{u}$ is given by

$$d = f(\boldsymbol{u}) + \nu \tag{12}$$

The function $f(\bullet)$ and the statistics of noise $\nu$ are unknown except that the additive noise $\nu$ is statistically independent of the input vector u. In the massive-training framework, $d$ is the continuous voxel value $T(x,y,z)$ from the corresponding "teaching" 3D Gaussian function in Eq. (18). The goal of the nonlinear regression model is to

estimate the dependence of *d* on u, provided there is a set of training data $\left\{\left(u_i, d_i\right)\right\}_{i=1}^{N}$, where $\left(u_i, d_i\right)$ are the sample values of the input vector u and the model output *d*, respectively. In SVR, an estimate of *d*, denoted as *g*, is expanded in terms of the nonlinear functions in the rich RKHS as follows:

$$g = \sum_{j=0}^{L} w_j \phi_j(u) = w^T \Psi(u) \tag{13}$$

where

$$\Psi(u) = \left[\phi_0(u), \phi_1(u), \ldots, \phi_L(u)\right]^T$$

are the nonlinear functions associated with RKHS, *L* is the dimension of the feature space, which might be infinite, and $w = [w_0, w_1, \ldots, w_L]^T$ are the weights we aim to estimate. SVR achieves this goal by minimizing the following empirical risk:

$$\min_{w} \sum_{i=1}^{N} V_\varepsilon(d_i - g_i) + \frac{\lambda}{2}\|w\|^2 \tag{14}$$

where $V_\varepsilon(\bullet)$ is the *ε-insensitive* error function defined in Eq. (11), *N* is the total number of training samples, and λ is the regularization parameter which controls the VC dimension of the model. Because the cost function in Eq. (14) is not differentiable at the points $\pm\varepsilon$, the optimization problem can be reformulated by introduction of nonnegative *slack variables* (Smola & Schölkopf, 2004). If w is the minimizer of the criterion in Eq. (14), then the solution can be shown to have the form

$$w = \sum_{i=1}^{N} (\alpha_i - \alpha_i')\Psi(u_i) \tag{15}$$

where $\alpha_i$ and $\alpha_i'$ are positive Lagrange multipliers that maximize the dual objective function

$$\max_{\alpha_i, \alpha_i'} \sum_{i=1}^{N} d_i(\alpha_i - \alpha_i') - \varepsilon \sum_{i=1}^{N} (\alpha_i + \alpha_i') - \frac{1}{2}\sum_{i=1}^{N}\sum_{j=1}^{N} (\alpha_i - \alpha_i')(\alpha_j - \alpha_j')K(u_i, u_j) \tag{16}$$

subject to the following constraints:

$$\sum_{i=1}^{N}(\alpha_i - \alpha_i') = 0\,,$$

$$0 \le \alpha_i, \alpha_i' \le 1/\lambda\,.$$

$K\left(u_i, u_j\right)$ is a symmetric nonnegative inner-product kernel function defined in the RKHS as defined in Eq. (5).

Note that only a subset of the solution values $(\alpha_i - \alpha_i')$ is nonzero, and the corresponding data points are called *support vectors*. The two free parameters, $\varepsilon$ and $\lambda$, impact the VC dimension of the optimal nonlinear function

$$g(v) = \sum_{i=1}^{N}(\alpha_i - \alpha_i')K(u_i, v) \tag{17}$$

$\varepsilon$ is the width that controls the tolerance of error measure. If the response, $d$, is scaled such that we use $V_\varepsilon(e/\sigma)$ instead, then we might consider using a preset value for $\varepsilon$. The regularization parameter $\lambda$ can be estimated, for example, by cross-validation. Given any unseen testing data sample **v**, the prediction is obtained by plugging of **v** into Eq. (17)

In MTSVR, the goal is to estimate an optimal nonlinear function $g(v)$ so that it is able to characterize the underlying nonlinear image structures. In the testing stage, we need to obtain the output of MTSVR *O(x,y,z)*. To this end, we set the variable **v** in Eq. (17) as the input

$$\left\{I\left(x-p, y-q, z-r\right)\middle|\left(p,q,r\right) \in V_s\right\}.$$

## Training of Nonlinear Regression Models

We used 10 polyps and 10 non-polyps as training cases, which had been used in our previous studies. If we scan the whole 64x64x64 volume with a 7x7x7 cube voxel by voxel, there will be 195,112 (58x58x58) subvolumes. Because most of the subvolumes overlap and are away from the polyps, we focus only on the subvolumes extracted from a 15x15x15 cube (i.e., a training volume) centered at the center of

the volume object. To reduce the number of subvolumes further, we selected those subvolumes sampled at every other voxel. Therefore, 512 (8x8x8) subvolumes were extracted from each volume object. In total, we extracted 5,120 (512x10) subvolumes for each class, with 171 dimensions for each subvolume, according to the methodology described in Section 2.2. Therefore, there are 10,240 training examples in total. Hence, we called the proposed nonlinear regression models a massive-training SVR (MTSVR).

The "teaching" response *T* in the MTSVR for training examples extracted from polyps used a 3D Gaussian weighting function whose peak was located at the center of the polyp. On the other hand, we used all zero values as the desired response for non-polyp training examples. Therefore, both models were able to learn the underlying image structures by enhancing polyps with a 3D Gaussian weighting function and suppressing non-polyps with zeros. The desired response is described as follows:

$$T(x,y,z) = \begin{cases} \dfrac{1}{\sqrt{2\pi}\sigma_T} \exp\left\{-\dfrac{x^2+y^2+z^2}{2{\sigma_T}^2}\right\} & for\ a\ polyp \\ 0 & for\ non\text{-}polyps \end{cases} \tag{18}$$

The standard deviation $\sigma_T$ controls the size of the Gaussian weighting function. The coordinate $(x, y, z)$ is consistent with the one used in Eq. (1). Training of the MTSVR involves a large number of subvolume-voxel pairs. The input sample is a vector of length 171, and the "teaching" response *T* is a scalar, either a voxel value extracted from the 3D Gaussian weighting function or zero. The center voxel from the corresponding teaching sub-volume is also extracted as the teaching value. The 3D MTSVR is massively trained by use of each of a large number of input sub-volumes together with each of the corresponding teaching single voxels. After training, the 3D MTSVR is expected to output a higher value for a polyp and a lower value for a non-polyp.

The MTSVR used quadratic programming for solving the maximization problem in Eq. (16) and for obtaining the optimal solution in Eq. (17). SVR is memory-based because a compact kernel function is used in characterizing the data structure in Eq. (5). Therefore, some training examples were stored after the training process. In the MTSVR, only a part of the training examples was retained. These are called support vectors. This is very different from the MTANN approach, where all of the training data were discarded after the linear-output back-propagation algorithm (Suzuki, Horiba, & Sugie, 2003; Suzuki, Horiba, Sugie, & Nanki, 2004). The relevant information was represented in the optimal weights in the linear-output ANN regression model. We will explore different kernel functions in Equations

(6) – (9) used in the MTSVR. We used grid search to find the best kernel function with optimal parameters.

## Feature Extraction

Feature extraction is one of the most important steps in the FP reduction system. Features from the polyp candidates were required to have sufficient discriminatory power to distinguish lesions from non-lesions. Given the lesion candidates detected from CT images, we extracted seventy-five morphologic, gray-level-based, and texture features to form the set of feature candidates. Both 2D and 3D features were computed. 2D features were calculated in the slice where the segmented candidate region had the largest area. 3D features were computed in the overall segmented volume.

In order for features such as contrast between segmented candidate regions and the outside to be computed, the outside region must be determined. For this purpose, we created a ring structure for a 2D case and a shell structure for a 3D case surrounding the detected candidate, denoted the band region, as the outside. To this end, we performed binary dilation operations on the detected candidates with a square structuring element of 21x21 pixels and 11x11 pixels (a cube of 21x21x21 and 11x11x11 voxels for a 3D case) as the structuring elements (L.Vincent, 1991). The difference between the output dilated regions would be the final band regions. Therefore, the outside region was defined as either a ring or a shell with a width of 5 pixels and 5 pixels away from the boundary of the detected candidate. We denote the set of pixels/voxels inside the segmented candidate region as $I$, the set of pixels/voxels on the contour as $C$, the set of pixels/voxels in the band region as $O$, and the fraction of pixels/voxels in set $X$ with intensity value $i$ as $H(X,i)$.

Table 1 lists all of the extracted features with brief explanations. These features are aimed at serving as discriminatory descriptors for differentiating between true lesions and FP detections. Features 1-11 in the table describe the 3D gray-level information of regions $I$ and $C$, as HCCs and polyps often have higher gray values than do FPs. Features 12-19 characterize the 3D shape information of the segmented regions because polyps have round shapes. Features 16-19 calculate the radial and tangential gradient indices inside the candidate regions and also in the band regions. The indices measure how much of a gradient of an image is directed radially and tangentially. In order to make these features meaningful and discriminant, the delineation of the candidates is required to correspond closely to the real object boundaries. This, in turn, requires the accuracy of the clustering method employed in the detection of polyps (Yoshida & Nappi, 2001).

Besides gray-level and shape-based features, we also computed histogram-based features (Features 20-41). In order to highlight the edge information of the

*Table 1. Extracted 2D and 3D features*

| Feature # | Feature Notation | Brief Explanation |
|---|---|---|
| **3D Features** | | |
| 1-10 | Gray_level_max/min/mean/median/ std_inside/contour | Maximum, minimum, mean, median, and standard deviation of gray levels inside and on the contour of the candidate |
| 11 | Outline | Voxel summation over perimeter value of each slice |
| 12 | Sphericity | Ratio of the surface area of a sphere (with the same volume as the given candidate) to the surface area of the candidate |
| 13 | Volume | Segmented region volume |
| 14 | SA | Surface area of the candidate |
| 15 | SphereIrr | Ratio of the overlapped volume between the candidate and a sphere (of same volume) to the overall volume of the candidate |
| 16-19 | RGI/TGI_inside/outside | Radial / tangential gradient indices (measure of how much of the gradient of an image is directed radially / tangentially) inside and outside (band region) the candidate |
| 20-23 | Top/bottom_10%_inside/outside | Thresholds of top or bottom 10% histogram inside and outside (band region) the candidate |
| 24-27 | Gray_max/maxRange_inside/outside | Maximum and maximum range of the histogram of gray level images inside and outside (band region) the candidate |
| 28-31 | Sobel_max/maxRange_inside/ outside | Maximum and maximum range of the histogram of Sobel images inside and outside (band region) the candidate |
| 32-35 | Gray_FullWidth_five/one_inside/ outside | Full width at half and 0.1 maximum of the histogram of gray level images inside and outside (band region) the candidate |
| 36-39 | Sobel_FullWidth_five/one_inside/ outside | Full width at half and 0.1 maximum of the histogram of Sobel level images inside and outside (band region) the candidate |
| 40-41 | Histogram_overlap_gray/Sobel | Overlapped histograms of inside and outside (band region) the candidate based on gray level and Sobel images respectively |
| 42-48 | Gray_level_constrast(1-7) | Seven contrast features based on gray level images |
| 49-54 | Sobel_constrast(1-6) | Six contrast features based on Sobel images |
| 55-58 | Sobel_mean/relStd_inside/outside | Mean and relative standard deviation inside and outside (band region) the candidate based on Sobel images |
| 59 | Sobel_power_3D | Average Sobel power value inside the candidate |
| **2D Features** | | |
| 60-63 | Gray_level_mean/std_inside/outside | Mean and standard deviation of pixel values inside and outside (band region) the 2D contour of the candidate |
| 64 | Area | Area of the 2D contour |
| 65 | Perimeter | Perimeter of the 2D contour |
| 66 | Circularity | Area * 4 * 3.14 / perimeter / perimeter |
| 67 | CircleOverlap | Ratio of overlapped area between the 2D contour and a circle (of same area) to the overall are of the 2D contour of the candidate |
| 68 | CircleIrr | Ratio of perimeter of a circle (of same area as the 2D contour) to the 2D contour |
| 69-72 | RGI/TGI_inside/outside | Radial and tangential gradient indices inside and outside (band region) the 2D contour of the candidate |
| 73 | Sobel_power_2D | Average Sobel power value inside the 2D contour |
| 74-75 | Haralick_inside/outside | Haralick texture features inside and outside (band region) the 2D contour of the candidate |

*Table 2. Maximal AUC SFFS feature selection method*

| |
|---|
| **Initialization:** |
| Full feature set from CT images $X$, selected feature set at step 0 $F_0=\{\emptyset\}$, predefined feature number $l=75$, $k=0$. |
| **while** $k<=l$ |
| $x^+ = \arg\max_{x \in X - F_k} J(F_k + \{x\})$ **(19)** |
| $F_{k+1} = F_k + \{x^+\}$ |
| $k=k+1$ |
| **if** $k>2$ |
| $x^- = \arg\max_{x \in F_k} J(F_k - \{x\})$ **(20)** |
| **while** $J(F_k-\{x\})>J(F_{k-1})$ **and** $k>2$ |
| $F_{k-1}= F_k - x^-$ |
| $k=k-1$ |
| **if** $k>2$ |
| $x^- = \arg\max_{x \in F_k} J(F_k - \{x\})$ |
| **end** |
| **end** |
| **end** |
| **end** |
| **Output:** |
| Selected feature set $Y_l$ |

segmented candidates, we applied the Sobel operator to the original CT images to create edge-enhanced images (Gonzalez & Woods, 2007). These histogram-based features specify the range, distribution, and overlap of the voxel values in gray-level and edge-enhanced images inside and outside the delineated candidates. Features 32-39 characterize the full width at half and 10% maximum of the gray-level and edge-enhanced images inside and outside the segmented candidates.

Because polyps usually protrude to the colon lumen, the contrast between inside and outside the detected candidates provides discriminatory information. Features 42-54 calculate different contrast measures for gray-level and edge-enhanced images

*Table 3. Mean-square error (MSE) between "teaching" responses and outputs for training data samples, and AUC values and FP reduction rates without removal of TPs from different regression models with different parameters for testing data samples*

| Methods | Model Parameters | | | Training MSE | Testing AUC | FP Reduction Rate Without Removal of TPs |
|---|---|---|---|---|---|---|
| MTSVR | linear | | | 0.0419 | 0.7821 | 0.1391 |
| | Polynomial | d | 2 | 0.0363 | 0.7734 | 0.1623 |
| | | | 4 | 0.0343 | 0.7754 | 0.1656 |
| | | | 6 | 0.0347 | 0.7752 | 0.1634 |
| | | | 8 | 0.0456 | 0.7802 | 0.1367 |
| | | | 10 | 0.1458 | 0.7572 | 0.1574 |
| | Gaussian | σ | 0.1 | 0.0278 | 0.7930 | 0.4151 |
| | | | 0.35 | 0.0178 | 0.7864 | **0.5147** |
| | | | 0.7 | 0.0131 | 0.7900 | 0.4519 |
| | | | 1 | 0.0114 | 0.7941 | 0.3885 |
| | | | 5 | 0.0083 | 0.8578 | 0.2378 |
| | | | 10 | 0.0082 | **0.8608** | 0.2085 |
| | Tanh | a = 1, b = 0 | | 0.0435 | 0.7738 | 0.1428 |
| | | a = 2, b = 0 | | 0.0422 | 0.7823 | 0.1439 |
| | | a = 3, b = 0 | | 0.0420 | 0.7834 | 0.1445 |
| | | a = 3, b = 1 | | 0.0420 | 0.7835 | 0.1446 |
| MTANN | Number of hidden neurons | | 10 | 0.0239 | 0.7804 | 0.2965 |
| | | | 20 | 0.0182 | 0.7867 | 0.3476 |
| | | | 25 | 0.0162 | 0.7707 | **0.4683** |
| | | | 30 | 0.0147 | 0.8012 | 0.2822 |
| | | | 40 | 0.0117 | **0.8139** | 0.2638 |
| | | | 50 | 0.0106 | 0.8080 | 0.3905 |

(Brake, Karssemeijer, & Hendriks, 2000). The simplest contrast information is the voxel intensity difference between inside region *I* and outside region *O*, defined as

$$contrast1 = E(I) - E(O) \tag{21}$$

where *E* stands for the expectation operator. The second contrast quantifies the separation of two intensity distributions as

$$contrast2 = \frac{\Big(E(I) - E(O)\Big)^2}{Var(I) + Var(O)} \tag{22}$$

where *Var* denotes the variance operator. The third contrast value is the absolute distance between two normalized histograms which is defined as

$$contrast3 = \sum_i \Big|H(I,i) - H(O,i)\Big| \tag{23}$$

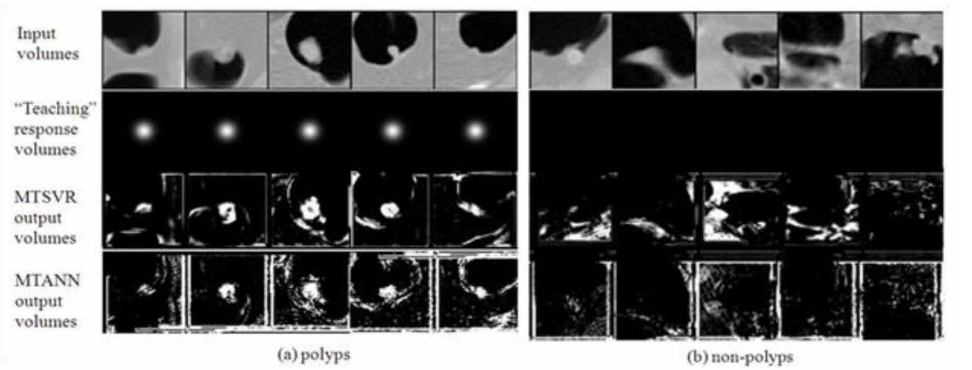
where *H(I,i)* and *H(O,i)* are the normalized histograms inside and outside the segmented object. The value of the third contrast measure ranges from 0 (total overlap) to 2 (complete separation). The other three contrast features are based on different definitions of distance. The fourth contrast is based on information theory, represented by

$$contrast4 = \sum_i \Big(H(I,i) - H(O,i)\Big) In \frac{H(I,i)}{H(O,i)} \tag{24}$$

The fifth is based on the Bhattacharyya coefficient,

$$contrast5 = -In \sum_i \Big(H(I,i)H(O,i)\Big)^{-1/2} \tag{25}$$

*Figure 3. Illustrations of the central axial slices of representative training polyp volumes (a) and non-polyp volumes (b). In the output volumes of MTSVR and MTANN, polyps are represented by bright voxels in the center, whereas non-polyps are suppressed and almost dark.*



And the sixth is based on the Matsutsita distance

$$contrast6 = \sqrt{\sum_i \left(H\left(I,i\right) - H\left(O,i\right)\right)^2} \tag{26}$$

These six contrast features are common in both gray-level and edge-enhanced images. We define the seventh contrast feature for the gray-level images as

$$contrast7 = \frac{H(I,i)^{t10\%} - H(I,i)_{b10\%}}{H(I,i)^{t10\%} + H(I,i)_{b10\%}} \tag{27}$$

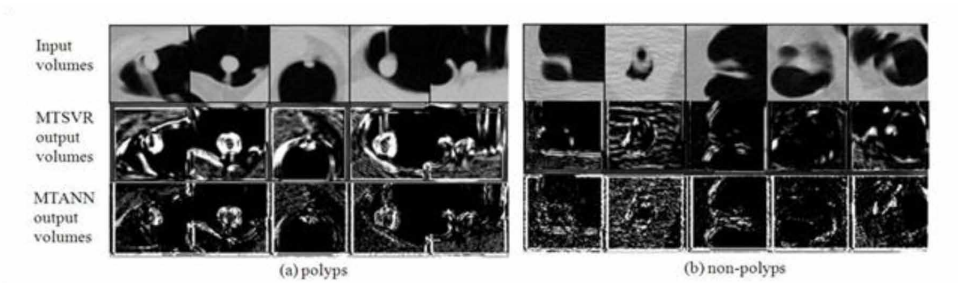
where $H(I,i)^{t10\%}$ and $H(I,i)_{b10\%}$ denote the thresholds of the top and bottom 10% of the histogram inside the segmented candidate, respectively.

In addition to the 3D features, we also extracted 16 2D features (Features 60-75) from the slice where the segmented candidate region had the largest area. The descriptions are given in Table I, which should be self-explanatory.

## Maximal AUC SFFS Feature Selection (Xu & Suzuki, 2014)

Although the list of extracted features in Table 1 provides discriminant information to distinguish lesions from FP detections, it is important to select a subset of features in order to overcome unnecessary computations and overfitting, and to achieve a better performance. To this end, we developed a maximal AUC SFFS feature selection method to choose a subset of features to ensure a reliable SVM classifier.

*Figure 4. Illustrations of the performance of the trained 3D MTSVR and MTANN with representative polyps and non-polyps, and the corresponding output volumes. The central axial slices of the 3D volumes are shown.*



We adopted the wrapper approach in which the searching procedure was coupled with the SVM to yield the AUC value for evaluation in each step (Kohavi & John, 1997). The basic idea is to select features that can directly reflect the performance measure for a CADe system. Unlike the individual feature ranking approach, the proposed method chooses features based on the collective discriminatory power of the feature subset because it is the combination of features, not individual ones, that contributes to the performance of the classifier. It is even possible to find a subset of features that are not selected by an individual feature ranking method, but performs better in term of ROC analysis. An in-depth comparison of the wrapper- and filter-based feature selection methods in a recently published survey (Chandrashekar & Sahin, 2014) may be useful in this regards. On the other hand, we used the AUC value from the ROC curve as the selection criterion, because it directly measures how a CADe system performs in general. This is different from the class separation criterion used in the stepwise feature selection based on Wilks' lambda (Draper & Smith, 1998), classification accuracy which only focuses on one particular operating point of the ROC curve (Huang, Chung, Sheu, Kuo, & Mikulas, 2008), or any other measures (Boroczky, Zhao, & Lee, 2006; Hupse & Karssemeijer, 2010; Takemura, Shimizu, & Hamamoto, 2010). The AUC maximization criterion will maximize the performance of a CADe system by selecting relevant features. It has been shown that the AUC value corresponds to the probability of correctly identifying which of the two cases is normal and which is abnormal (Hanley & McNeil, 1982). From a statistical perspective, the AUC value is also equivalent to the well-known nonparametric Wilcoxon statistic (Hanley & McNeil, 1982). These connections provide alternative views of the AUC value and make it a suitable performance measure of a CADe system.

Table 2 outlines the main procedure of the proposed first feature selection method. It starts with an empty selected feature set $F_0$. Then it begins to include one feature at a time which would maximize the AUC value, calculated via an SVM classifier, of the selected feature subset given a subset size. This is given in Eq. (19), where the criterion $J(F_k+\{x\})$ is the AUC value of the SVM classification with the selection feature set $(F_k+\{x\})$. Therefore, Eq. (19) guarantees that the selected feature would produce the maximal AUC value with the combination of the existing features in the subset. However, this step only includes features without removing any existing ones. It might be possible to increase the AUC value by removing some features from the selected subset. This is realized in Eq. (20) and onwards. It starts with the selected feature subset, and it removes one feature at a time if the remaining feature subset performs better than the one containing the feature to be removed. The procedure

will continue until the number of the features in the selected subset reaches the total number of the available features. The feature subset with the maximal AUC value would be selected as the final output of the procedure.

## Performance Evaluation Criteria

We used a mean-square error (MSE) to evaluate the training performance of the MTSVR, as it offers a direct comparison between the "teaching" response and the output from the models. To assess the performance of the trained models in the testing data, we calculated the area under the receiver-operating-characteristic (ROC) curve (AUC) values (Metz, 1986), an FP reduction rate without removal of TPs, and free-response ROC (FROC) analysis (Egan, Greenberg, & Schulman, 1961) as performance metrics. The AUC value is calculated based on the maximum-likelihood estimation of the binormal ROC curve (Metz, Herman, & Shen, 1998). It offers the overall performance of the regression models after 3D scoring as classifiers to distinguish between polyps and non-polyps. We conducted statistical tests to determine whether the difference in AUC values from different methods is statistically significant. The FP reduction rate without removal of TPs describes the percentage of FPs that has been eliminated by selecting a threshold without sacrificing any TPs.

*Figure 5.*

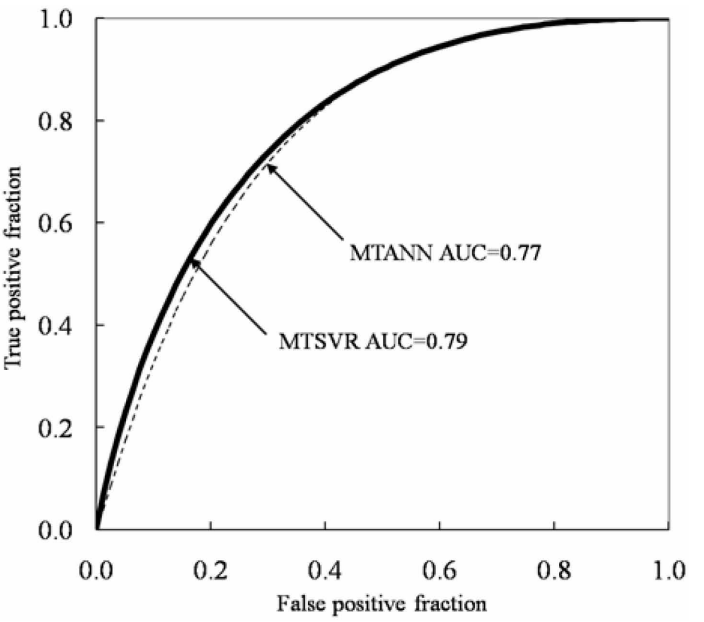
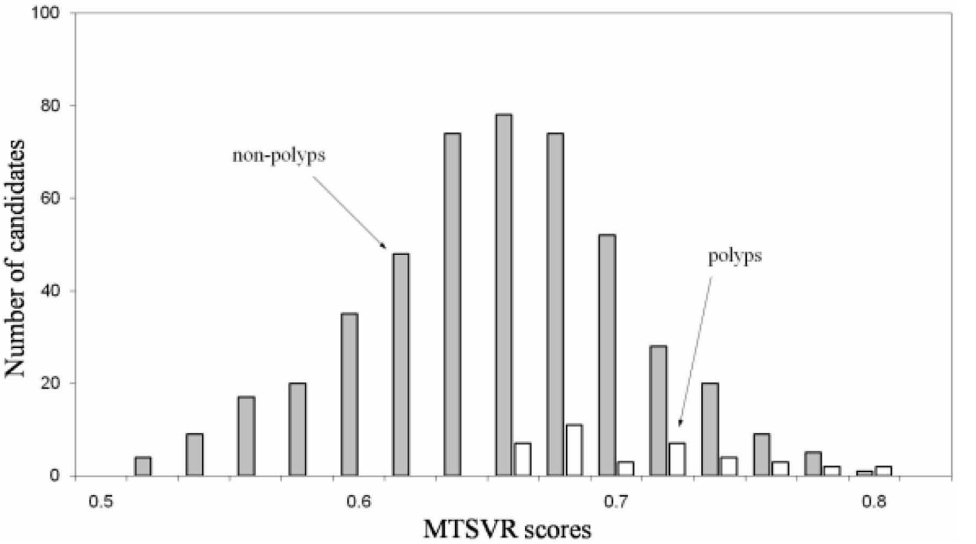


*Figure 6. Histogram of the scores from MTSVR with a Gaussian kernel function with σ=0.35 for 36 TP volumes (from 18 polyps), and 474 FPs (non-polyps) produced by the original CADe scheme for the detection of polyps in CTC*




## RESULTS

In this section, we present the performance of the proposed MTSVR and feature selection method in reducing FP detections. We also compare the results with the previous studies on MTANN (Kenji Suzuki et al., 2006) and the popular stepwise feature selection based on Wilks' lambda with a LDA classifier (Yoshida & Nappi, 2001).

### Training Performance of MTSVR

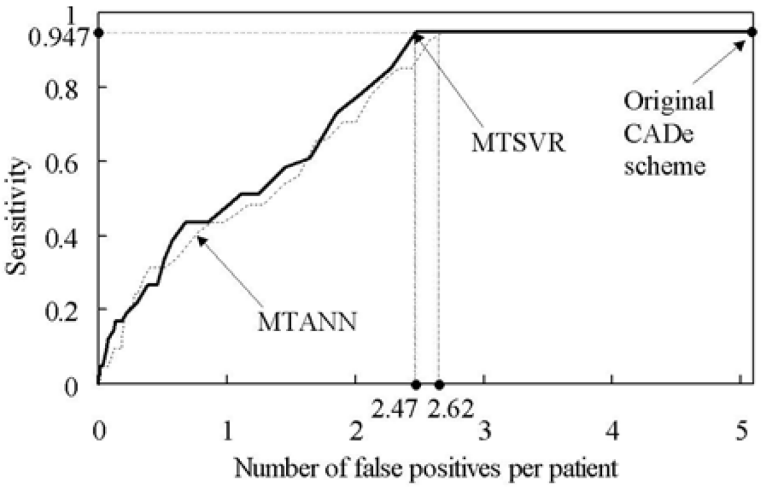
We manually selected ten representative polyps and ten non-polyps with different visual appearance such as size, shape, and contrast from the CTC datasets. The ten non-polyps covered major sources of FPs such as rectal tubes, stool, haustral folds, colonic walls, and the ileocecal valve. The purpose was to make the training data samples represent the database (ideally the whole population). The standard deviation $\sigma_T$ in the desired response volume (18) was chosen empirically as 4.5 voxels (Kenji Suzuki et al., 2006).

Table 3 presents the MSE between the "teaching" responses and the outputs from MTSVR and MTANN with different parameters. The four kernel functions in Eqs. (6) – (9) with different parameter values were studied in the MTSVR. The Gaussian

kernel function offered the best performance in terms of MSE over the other three kernel functions. Linear and polynomial kernel functions produced a much higher MSE. It is interesting to note that the tanh kernel function had a relatively similar performance for different combinations of parameters. We varied the number of neurons in the hidden layer in MTANN. Suzuki *et al.* used 25 in their study (Kenji Suzuki et al., 2006). Although the MTANN with 25 neurons in the hidden layer did not offer the best MSE for training samples, it achieved the highest FP reduction rate for testing samples (Kenji Suzuki et al., 2006).

In order further to gain qualitative insights into the difference in performance of the MTSVR and MTANN, we applied the best-trained regression models to the training polyps and non-polyps. Figure 3 presents five representative training polyps and non-polyps with corresponding output images from the MTSVR and MTANN. We used a Gaussian kernel in Eq. (8) with $\sigma$=0.35 in the MTSVR and 25 neurons in the hidden layer in the MTANN. The "teaching" response images for polyps in the training phase contain the 3D Gaussian function in Eq. (18) shown in the second row of Fig. 3 (a). The second row in Fig. 3 (b) shows the "teaching" response images for non-polyps. Both methods were able to learn the underlying CTC images by enhancing polyps and suppressing non-polyps. The output volumes for two small polyps (the farthest left and right images) from both methods are stronger and larger than the original ones in the input volumes, which demonstrates the ability of the MTSVR and MTANN to enhance small polyps. However, it should also be noted that the shapes of the output volumes for the farthest left polyp in MTSVR are rounder and larger than the one in the MTANN output volume. Figure 3 (b) presents the

*Figure 7. FROC curves indicating the performance of the MTSVR and MTANN*



case for training non-polyps. The center object is dark in the farthest left output volume of the MTSVR, whereas it remains in the output volume of the MTANN.

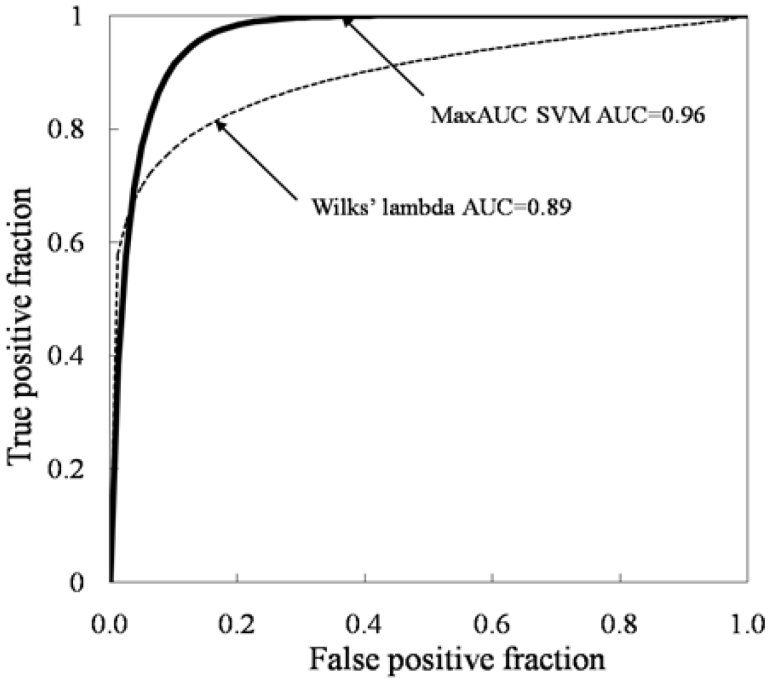
## Testing Performance of MTSVR

The main focus of this study was to reduce the number of FP detections in CTC CADe. Although MSE is a good indicator for regression, it might not be strictly correlated with FP reduction. Therefore, we applied all of the trained regression models, not only the best one in terms of MSE, to 474 testing non-polyps (FPs) and 36 TP volumes (that constitute 18 polyps). Table 3 presents the AUC values and FP reduction rates without removal of TPs for MTSVR and MTANN with different parameters for the testing CTC cases. The MTSVR with a Gaussian kernel with $\sigma$=10 produced the best AUC value. This is consistent with the lowest MSE obtained with the same parameter. However, the highest FP reduction rate was achieved with a Gaussian kernel with $\sigma$=0.35, which suggests the difference between two performance metrics. Linear, polynomial, and tanh kernel functions had a much lower performance than did the Gaussian kernel function. We also provided the performance for MTANN with different numbers of neurons in the hidden layer in Table 3. An MTANN with 25 hidden layer neurons achieved the best FP reduction rate. Because the FP reduction rate is the most important criterion in this study, we chose a Gaussian kernel function with $\sigma$=0.35 for MTSVR and 25 hidden layer neurons for MTANN in the following experiments without further mentioning the specific parameters. We selected five representative testing polyps and non-polyps to show different output volumes from MTSVR and MTANN in Fig. 4. The trained models were able to enhance the testing polyps and suppress the non-polyps. The ability of both methods to enhance small polyps can be demonstrated again in the middle of the images in Fig. 4 (a). In the farthest left and middle images of Fig. 4 (b), the testing non-polyps become dark in the output volumes of the MTSVR. However, the non-polyps can still be seen in the output volumes of the MTANN.

The AUC values obtained by the MTSVR and the MTANN were 0.79 and 0.77, respectively. However, the difference is not statistically significant (two-sided *p-value* = 0.75). Figure 5 plots the ROC curves from MTSVR and MTANN. The difference between ROC curves from MTANN and MTSVR can be further investigated by examination of the score distributions. We present the histograms of scores from the MTSVR in Fig. 6. The distribution of non-polyps in MTSVR has a bell shape centered in the middle, whereas the dynamic range of polyps is much smaller.

We evaluated the performance of the proposed MTSVR for FP reduction by using FROC analysis. Figure 7 shows FROC curves indicating the performance of the MTSVR and the original MTANN for FP reduction. The proposed MTSVR was able to eliminate 51.5% (244/474) of FPs without removal of any true-positives, i.e.,

*Figure 8. The ROC curves for the maximal AUC SFFS feature selection and stepwise feature selection based on Wilk's lambda method*




it achieved a 94.7% (18/19) by-polyp sensitivity with an FP rate of 2.47 (230/93) per patient, whereas the MTANN eliminated 47.2% (230/474) of the FPs, which resulted in the same sensitivity with 2.62 (244/93) FPs per patient. The performance comparison between MTSVR and MTANN illustrated that the proposed MTSVR offered comparable a AUC value and FP reduction rate.

## Computational Efficiency Comparison of MTSVR and MTANN

One of the main contributions of the paper is to improve the computational efficiency of the massive-training framework in the development phase of a CADe scheme by using SVR while maintaining a comparable performance. In the developmental stage of a new CADe scheme, a low training computational cost is crucial because one would change some parameters of the massive-training model, alter training cases, or optimize the parameters of an initial detection scheme.

Let $N$ be the total number of training samples, $m$ the number of *support vectors* in MTSVR, $d_l$ the dimension of training input samples, in the case where $m/N<<1$, the number of operations for MTSVR is $O(m^3+ m^2N+mNd_l)$ (Christopher J. C.

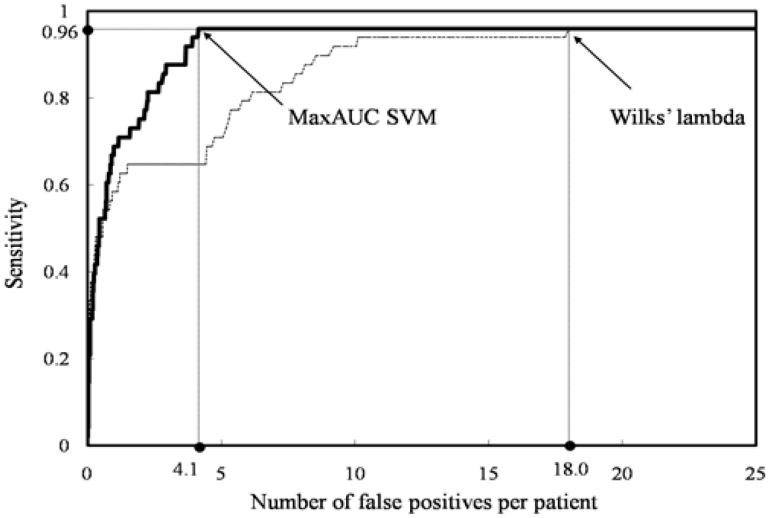
Burges, 1998). In our application, the ratio *m/N* is usually around 0.2. Therefore, MTSVR scales linearly with the number of the training samples *N.* On the other hand, the computational complexity of the MTANN depends on the dimension of training input samples dl, the number of hidden neurons $N_H$, the number of training samples *N,* and the number of iterations required for training of the ANN by using the back-propagation algorithm (Suzuki, Horiba, & Sugie, 2002). The training of an MTANN was performed 500,000 times (K. Suzuki, H. Yoshida, et al., 2006). Therefore, the computational costs of MTSVR would be lower than that of the MTANN in most situations.

We compared the computational costs of the MTSVR and MTANN on a workstation (Intel, Xeon, 2.7GHz, 1GB RAM). The training of the original MTANN took 38 hours, whereas that of the MTSVR took 12 minutes. Thus the training time was reduced by a factor of 190 with the MTSVR. Compared to the MTANN coupled with a Laplacian eigenmap for dimension reduction (Suzuki, Zhang, et al., 2010), the MTSVR offered a comparable performance in terms of AUC values and the FP reduction rate without removal of TPs while reducing the training time even more (i.e., by a factor of 20).

## Performance of Maximal AUC SFFS Feature Selection

We compared the proposed maximal AUC SFFS feature selection method with the conventional stepwise feature selection based on Wilks' lambda with an LDA

*Figure 9. FROC curves for the maximal AUC SFFS feature selection and stepwise feature selection based on Wilks' lambda method*



classifier approach in a leave-one-lesion-out cross-validation test in FP reduction in CADe of polyps. Out of 75 features extracted from the polyp candidates, the maximal AUC SFFS feature selection method chose 11 features. On the other hand, the stepwise feature selection based on Wilks' lambda selected 23 features. There were only 4 common features that were selected by both methods, namely, volume and the standard deviation of voxel values of the detected polyp candidates, the maximum value of the voxel range, and the histogram contrast between the segmented candidate and the outside band region with a fixed length.

Figure 8 plots the ROC curves for both methods. The figure clearly demonstrates the better performance of the proposed feature selection method. The maximal AUC SFFS feature selection method achieved an AUC value of 0.96, whereas the stepwise feature selection based on Wilks' lambda yielded an AUC value of 0.89. We conducted a statistical significance test. The difference between the two AUC values was statistically significant with a two-sided $p$-value of 0.03.

We tested the overall FP reduction system with the FROC curves, as shown in Fig. 9. The proposed FP reduction system with the maximal AUC SFFS feature selection method was able to eliminate 83.9% (2202/2624) of FPs without removal of any TPs, i.e., it achieved a 96.0% by-polyp sensitivity with an FP rate of 4.1 (422/103) per patient. On the other hand, the FP reduction system with the stepwise feature selection based on Wilks' lambda eliminated 29.3% (770/2624) of the FPs, which resulted in the same sensitivity, with 18.0 (1854/2624) FPs per patient. This result clearly illustrates the better performance of the proposed feature selection method as its criterion directly reflects how a CADe system performs.

## CONCLUSION

In this study, we presented an MTSVR model in the massive-training framework for FP reduction in CADe of polyps in CTC. A novel feature selection method based on the SFFS procedure for maximizing the AUC value was also proposed. The maximal AUC SFFS feature selection method was directly coupled with an SVM classifier. Therefore, we have presented the SVMs in two different approaches, namely, a regression model and a classifier. The MTSVR achieved a comparable performance in terms of FP reduction in a CTC database compared with the original MTANN method. On the other hand, the MTSVR yielded a better computational efficiency by reducing the training time by a factor of 190 compared to that for the original MTANN. We also compared the proposed feature selection method with the conventional stepwise feature selection based on Wilks' lambda with an LDA classifier. Our proposed feature selection obtained a statistically significant increase in the AUC value and a reduction of FP detections.

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# Chapter 7
# Radiomics:
## The New Frontier in Quantitative Image Modeling in Radiotherapy

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## ABSTRACT

*Recent advances in image-guided and adaptive radiotherapy have ushered new requirements for using single and/or multiple-imaging modalities in staging, treatment planning, and predicting response of different cancer types. Quantitative information analysis from multi-imaging modalities, known as 'radiomics', have generated great promises to unravel hidden knowledge embedded in imaging for mining it and its association with observed clinical endpoints and/or underlying biological processes. In this chapter, we will review recent advances and discuss current challenges for using radiomics in radiotherapy. We will discuss issues related to image acquisition, registration, contouring, feature extraction and fusion, statistical modeling, and combination with other imaging modalities and other 'omics' for developing robust models of treatment outcomes. We will provide examples based on our experience and others for predicting cancer outcomes in radiotherapy generally and brain cancer specifically, and their application in personalizing treatment planning and clinical decision-making.*

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## INTRODUCTION

kV x-ray computed tomography (kV-CT) has been historically considered the standard modality for treatment planning in 3D conformal (3DCRT) or intensity-modulated radiotherapy (IMRT) because of its ability to provide electron density information for heterogeneous dose calculations (Khan & Gerbi, 2012; Webb, 2001). However, additional information from other imaging modalities could be also used to improve treatment monitoring and prognosis in different cancer sites (El Naqa et al., 2009; Kumar et al., 2012; Lambin et al., 2012). Physiological information (tumor metabolism, proliferation, necrosis, hypoxic regions, etc.) can be collected directly from nuclear imaging modalities such as single photon emission computed tomography (SPECT) and positron emission tomography (PET) or indirectly from magnetic resonance imaging (MRI) (Condeelis & Weissleder, 2010; Willmann, Van Bruggen, Dinkelborg, & Gambhir, 2008). For instance, changes in tumor volume captured on CT images may be predictive of local control in lung cancer (Ramsey et al., 2006; Seibert et al., 2007). In addition, functional/molecular imaging and in particular 2-deoxy-2-[18F]fluoro-D-glucose (FDG) PET, a glucose metabolism analog, has shown promise as a potential prognostic factor for predicting radiotherapy efficacy or potential side effects. The primary focus in the literature to this point has been directed towards simple metrics describing $^{18}$F-FDG PET images, especially maximum standardized uptake values ($SUV_{max}$). On the other hand, dynamic contrast enhanced MRI (DCE-MRI), a perfusion surrogate, was tested in the rat brain to monitor radiation-induced adverse effects on healthy tissues (Constanzo et al., 2016) or assessing tumor physiological characteristics (Hormuth, Skinner, Does, & Yankeelov, 2014; Poulin et al., 2015), and used to assess treatment response of soft tissue sarcoma (Shapeero, Vanel, Verstraete, & Bloem, 2002; van Rijswijk et al., 2003; Vanel et al., 2004). Also, apparent diffusion coefficients (ADC) values from diffusion-weighted MRI (DW-MRI), a measure of water molecules diffusion (Brownian motion) in tissue, were significantly correlated with sarcoma response to radiotherapy (Einarsdóttir, Karlsson, Wejde, & Bauer, 2004). However, only sparse reports focused on radiation-induced grey matter damage, as for example Horská et al., who studied subcortical changes after cranial radiation therapy in children (Horská et al., 2014).

Advances in delivery and imaging technologies put a step forward into a new era of image-guided and adaptive radiotherapy (IGART), which has witnessed burgeoning interest in applying different imaging modalities, both to define the target volume and to predict treatment response. In modern IGART, there is a strong interest for using multimodal imaging in tumor staging and optimizing the treatment planning of different cancer types (Jaffray, 2012). The goal is to achieve improved target definition by incorporating complementary anatomical information

(CT, magnetic resonance (MR), ultrasound, etc.) coupled with an improved disease characterization and localization using functional and molecular imaging (positron emission tomography, functional MR, etc.). For instance, PET/CT has been utilized for staging, planning, and assessing the response to radiation therapy in lung (J. Bradley et al., 2004; J. D. Bradley, Perez, Dehdashti, & Siegel, 2004; Erdi et al., 2000; Mac Manus et al., 2003; Macmanus et al., 2003, 2003; Pandit, Gonen, Krug, & Larson, 2003; Toloza, Harpole, & McCrory, 2003; Verhagen et al., 2004), gynecological (Miller & Grigsby, 2002; Mutic et al., 2003), and colorectal cancers (Ciernik, 2004). Similarly, Yang et al. showed that the combined evaluation of contrast-enhanced CT and FDG-PET/CT predicts the clinical outcomes in patients with aggressive non-Hodgkin's lymphoma (Yang et al., 2009). Most recently, PET/MR has started to make its appearance in the field (Thorwarth, Müller, Pfannenberg, & Beyer, 2013; Zaidi, Mawlawi, & Orton, 2007). Denecke et al. compared CT, MRI and FDG-PET in the prediction of outcomes to neoadjuvant radiochemotherapy in patients with locally advanced primary rectal cancer, demonstrating sensitivities of 100% for FDG-PET, 54% for CT, and 71% for MRI and specificities of 60% for FDG-PET, 80% for CT, 67% for MRI (Denecke et al., 2005).

Quantitative imaging is of particular interest in the case of brain cancer. Currently, the World Health Organization (WHO) classification of tumors in the central nervous system (CNS-WHO) is mainly based on histologic criteria. For example, WHO classification recognizes 3 histological types of grade III gliomas: including anaplastic astrocytoma (AA), anaplastic oligoastrocytoma (AOA), and anaplastic oligodendroglioma (AO) (Louis et al., 2016). AOA is a heterogeneous group with a considerably variable survival. This heterogeneity might result from the subjectivity of pathological diagnoses of AOA. The 2016 CNS-WHO is predicated on the basis of combined phenotypic and genotypic classification, and on the generation of "integrated" diagnoses (Louis et al., 2016). Therefore, imaging modalities are of critical clinical importance in making decisions regarding initial and evolving treatment strategies, and can ultimately be applied to (i) provide more reliable differentiation, especially when the neoplasm is heterogeneous, (ii) avoid invasive procedures such as biopsy, especially in cases of brain cancer where the risks may outweigh the benefits, and (iii) expedite or anticipate the diagnosis (Zacharaki et al., 2009). Moreover, quantitative imaging coupled with genomics shows great promises as *in vivo* portraits of the spatial distribution of gene expression within tumors. For instance, activity of the hypoxia gene-expression, which contains genes implicated in angiogenesis and tumor hypoxia (Leo, Giaccia, & Denko, 2004), was associated with the contrast enhancement (CE) imaging phenotype (Diehn et al., 2008). Also, a recent study explored the possibility of using texture features extracted from images of conventional MR sequences to make predictions of mutations, grade II

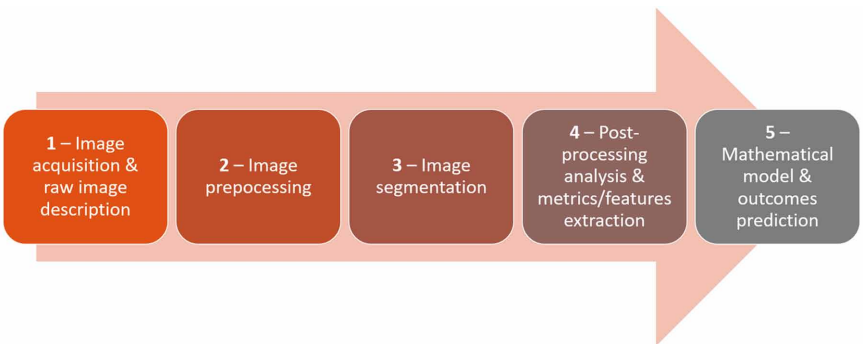
versus grade III low grade gliomas (LGG), and progression versus non-progression of LGG (Zhou et al., 2017).

In this chapter, we discuss the development of image-based (radiomics) models to predict radiotherapy outcomes from single and hybrid imaging modalities and present their application generally and in brain cancer imaging specifically.

## FROM IMAGING LEARNING PROCESS TO RADIOMICS

The process of accumulating knowledge from imaging is a multi-step process as depicted in Figure 1. The first step involves the acquisition of high quality images, preferably based on standard modalities acquisitions for diagnostic or planning purposes. From this image, the gross tumor volume (GTV) is defined, either with an automated segmentation method or alternatively by manual contouring made by experienced radiologists or radiation oncologists (steps 2 and 3). Quantitative imaging features and metrics are extracted from the previously defined tumor region (step 4). These quantitative image features are categorized based on image intensity, shape, size or volume, and texture, while derived feature metrics could be based on their magnitude, directionality, anisotropy, center of mass and maximum surface distance, for example. Then, a feature selection procedure is applied to these extracted image metrics. The most informative features are identified based on their independence from other image aspects, reproducibility and importance for predicting outcomes. The selected features are then analyzed for their relationship with treatment outcomes or gene expression. Ultimately, the imaging traits are incorporated into mathematical predictive models for treatment outcome that aimed

*Figure 1. Knowledge learning from imaging. This process starts by image acquisition and ends by understanding its content and summarizing it as predictive results. Radiomics focuses on image understanding steps 4 and 5.*



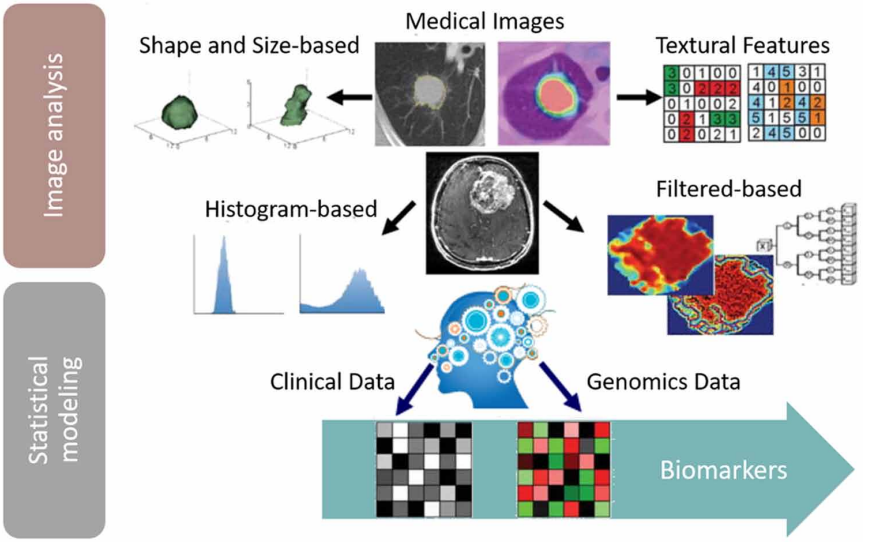
at providing added value in comparison with commonly used predictors (step 5). In summary, the extraction of quantitative information from imaging modalities and relating information to biological and clinical endpoints is a new emerging field referred to as *radiomics* (Kumar et al., 2012; Lambin et al., 2012).

## IMAGING AS A BIOMARKER OF RADIOTHERAPY RESPONSE

As aforementioned, radiomics could be summarized as two main steps: (1) extraction of relevant static and dynamic imaging features and (2) incorporating these features into mathematical model to predict outcomes as discussed in the following subsections (Figure 2).

From medical image analysis and statistical modeling, radiomics may provide predictive or prognostic biomarkers. A biomarker is formally defined as biological characteristics that can be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention (Naylor, 2003); and more practically as including tools and technologies that can aid in understanding the prediction, cause, diagnosis, progression, regression, or outcome of treatment of disease (Mayeux, 2004). Biomarkers can be divided into prognostic and predictive. A prognostic biomarker

*Figure 2. The two steps of radiomics leading to biomarkers identification*
*Modified from (Yip et al. 2016). ©Institute of Physics and Engineering in Medicine. Reproduced by permission of IOP Publishing. All rights reserved.*



informs about a patient's likely cancer outcome (e.g. disease recurrence) independent of the treatment received, e.g., Prostate-specific antigen (PSA) serum levels at the time of a prostate cancer diagnosis (Ballman, 2015). A predictive biomarker informs about the probable benefit from a therapeutic intervention. For example, MGMT (methyl-guanine methyl transferase) methylation status identifies glioblastoma multiform (GBM) patients who would most likely benefit from the addition of temozolomide (TMZ) to cranial radiation therapy (Stupp et al., 2009). Therefore, with a relevant choice of features extraction and mathematical models, radiomics may provide biomarkers useful to predict tumor radiation therapy response.

## Image Features Extraction

The features extracted from images could be divided into static (time invariant) and dynamic (time variant) features according to the acquisition protocol, and into pre- or intra-treatment features according to the scanning time point (El Naqa, 2014).

1. **Static Image Features:** Most of radiomics studies are based on conventional MRI sequences or static PET image acquisition, which provides a snapshot of enhancement at one point in time. There are five categories of static image features:
    a. **Standardized Uptake Value (SUV) or Hounsfield Unit (HU) Descriptors:** SUV is a standard image quantification method particularly used in PET analysis (Strauss and Conti, 1991), likewise HU is used in CT. SUV is the ratio between the radioactivity activity concentration [kBq/ml] measured by the PET scanner within a region of interest (ROI) and the decay-corrected amount of injected radiolabeled FDG [kBq], with respect to the weight of the patient [g]. HU is an arbitrary scale based on attenuation with water assigned a CT value of 0. In radiomics, raw intensity values are converted into SUVs/HUs and statistical descriptors such as maximum, minimum, mean, standard deviation (SD), and coefficient of variation (CV) are extracted. Similarly, in the case of MRI, proton density or other pulse sequences contrast information could be summarized.
    b. **Metabolic Tumor Volume Features:** The total lesion glycolysis (TLG) is also used in FDG-PET and is defined as the product of the metabolic tumor volume and mean SUV to measure the reduction in tumor metabolism between pre and post treatment (Benz et al., 2008; Erdi et al., 2000; Larson et al., 1999). For example, based on TLG features, a recent study demonstrated the utility of FDG PET-CT performed in the third week of primary radiation therapy to identify head and neck squamous cell carcinoma (HNSCC) patients with poor and good treatment outcome

for selection to a possible adaptive therapy (Min et al., 2015). Similarly, for brain tumor grading and surgery planning, the tumor volumes can be calculated in comparison to the background volume of interest, using a tumor-to-background ratio (TBR) and the maximum radiotracer uptake ($TBR_{max}$). FET uptake ($TBR_{max}$) and the tumor volume measured in static FET-PET (TBR > 2.0) showed a significant correlation to overall survival that could be improved in combination with the volume of the contrast-enhancing tumor part (MRI), suggesting a clinical benefit to perform both imaging modalities to assess individuals' prognosis (Bette et al., 2016).

c. **Intensity Volume Histogram (IVH):** This is analogous to the dose volume histogram (DVH) widely used in radiotherapy treatment planning in reducing complicated 3D data into a single easier to interpret curve. Each point on the IVH defines the absolute or relative volume of the structure that exceeds a variable intensity threshold as a percentage of the maximum intensity (El Naqa et al., 2009). This method would allow for extracting several metrics from images for outcome analysis such as $I_x$ (minimum intensity to x% highest intensity volume), $V_x$ (percentage volume having at least x% intensity value), and descriptive statistics (mean, minimum, maximum, standard deviation, etc.). We have reported the use of the IVH for predicting local control in lung cancer (Vaidya et al., 2012), where a combined metric from PET and CT image-based model provided a superior prediction power compared to commonly used dosimetric-based models of local treatment response.

d. **Morphological Features:** These are generally geometrical shape attributes such as eccentricity (a measure of non-circularity), which is useful for describing tumor growth directionality; Euler number (the number of connected objects in a region minus the total number of holes in the object) and the solidity (a measurement of convexity), which may be a characteristic of benign lesions (Jain, 1989; O'Sullivan *et al.*, 2005). An interesting demonstration of this principle is that a shaped-based metric based on the deviation from an idealized ellipsoid structure (i.e., eccentricity), was found to have strong association with survival in patients with sarcoma (O'sullivan, Roy, O'sullivan, Vernon, & Eary, 2005; O'sullivan, Roy, & Eary, 2003). Recently, deformation morphometry-based image analysis techniques were applied to quantitatively measure anatomical volume change in the brain as a therapy response in patients receiving whole-brain radiation for treating medulloblastoma (Fuentes et al., 2015). Using a template-based segmentation at each time point in the longitudinal study, the new volume at time $t = \tau$ may be calculated by

integrating the Jacobian of the deformation over the segmented region. Post-irradiation volume changes show promise as a quantitative imaging biomarker of the therapy.

e. **Texture Features:** Texture in imaging refers to the relative distribution of intensity values within a given neighborhood. It integrates intensity with spatial information resulting in higher order histograms when compared to common first-order intensity histograms. It should be emphasized that texture metrics are independent of tumor position, orientation, size, and brightness, and take into account the local intensity-spatial distribution (Castleman, 1979; Haralick, Shanmugam, & others, 1973). This is a crucial advantage over direct (first-order) histogram metrics (e.g., mean and standard deviation), which only measures intensity variability independent of the spatial distribution in the tumor microenvironment. Texture methods are broadly divided into three categories: statistical methods (e.g., high-order statistics, co-occurrence matrices, moment invariants), model based methods (e.g., Markov random fields, Gabor filter, wavelet transform) and structural methods (e.g., topological descriptors, fractals) (Zhang and Tan, 2002; Castellano et al., 2004). Among these methods, statistical approaches based on the co-occurrence matrix and its variants such as the grey level co-occurrence matrix (GLCM), neighborhood gray tone difference matrix (NGTDM), run-length matrix (RLM), and grey level size-zone matrix (GLSZM) have been widely applied for characterizing tumor heterogeneity in images (Chicklore et al., 2013). Four commonly used features from the GLCM include: energy, entropy, contrast, and homogeneity (Haralick et al., 1973). The NGTDM is thought to provide more human-like perception of texture such as: coarseness, contrast, busyness, and complexity. RLM and GLSZM emphasize regional effects. For example, texture features are used to provide an automated tool that may assist in the imaging evaluation of brain neoplasms by determining the glioma grade and differentiating between different tissue types, such as primary neoplasms (gliomas) from secondary neoplasms (metastases) (Zacharaki et al., 2009).

2. **Dynamic Image Features:** Dynamic features can be extracted from time-varying acquisitions such as dynamic PET or MR that would enable a fuller depiction of the wash-in and wash-out contrast agent (or radiotracer) kinetics within tumors, and thus provide more insight into the tumor microenvironment, pharmacokinetics of anticancer drugs, and response to therapy (Weber, 2006; Yankeelov et al., 2007). These features are based on kinetic analysis using tissue compartment models yielding parameters related to transport and binding rates (Tofts, 1997; Watabe, Ikoma, Kimura, Naganawa, & Shidahara, 2006).

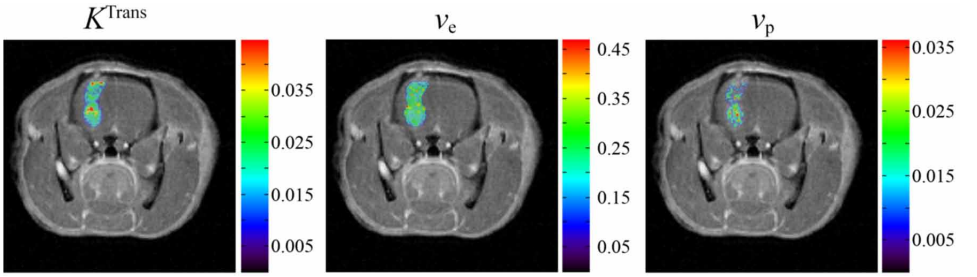
a. **Permeability and Perfusion Features ($K^{Trans}$, $\upsilon_e$ and $\upsilon_p$):** An example from DCE-MRI, assessing vascular permeability in an F98 glioblastoma rat model is shown in Figure 3, in which, a 3-compartment model is used and extracted kinetic parameters were the volume transfer rate constant ($K^{Trans}$), the extravascular-extracellular volume fraction $\upsilon_e$ (dimensionless) and the blood volume fraction $\upsilon_p$ (Poulin et al., 2015). This study demonstrated that kinetic modeling of MR images could characterize intra-tumor heterogeneity with the potential to incorporate such simple methods into clinical applications of combined or serial MRI-PET imaging systems. A rather interesting approach to improve the robustness of such features is the use of advanced 4D iterative techniques (Reader et al., 2006). Further improvement could be achieved by utilizing multi-resolution transformations (e.g., wavelet transform) to stabilize kinetic parameter estimates spatially (Turkheimer, Aston, Asselin, & Hinz, 2006).

b. **Time-Activity Curve (TAC), Peak Tumor-to-Background Ratio (pTBR) and Time-to-Peak (TTP):** These recent features evaluated the prognostic value of dynamic images $^{18}$F-FET PET for brain tumor patients (Calcagni et al., 2011; Fleischmann et al., 2017; Pyka et al., 2014). Time-activity curve (TAC) generates a plot of the mean radioactivity value in a region of interest (ROI) across a sequence of PET images, i.e., over time. As demonstrated by Calcagni et al., three types of TACs may occur: the type I, the curve can always ascending and no peak is identifiable during the study; the type II, the maximum peak is reached at a midway point (20 – 45 minutes) followed by a plateau or a slow descent; and the type III, the peak of the curve is identifiable at an early point of time (20 minutes) followed by a steep decrease (Calcagni et al., 2011). Based on the normalized TACs, the following dynamic parameters can be extracted: the peak TBR (pTBR), defined as the highest TBR with time, and the TTP that is the time from the beginning of the dynamic acquisition up to the pTBR of the tumor (Figure 4). For instance, Pyka et al. used pTBR and TTP features to investigate new prognostic factors optimizing individual treatment for glioma patients.

## Outcome Modeling for Radiomics

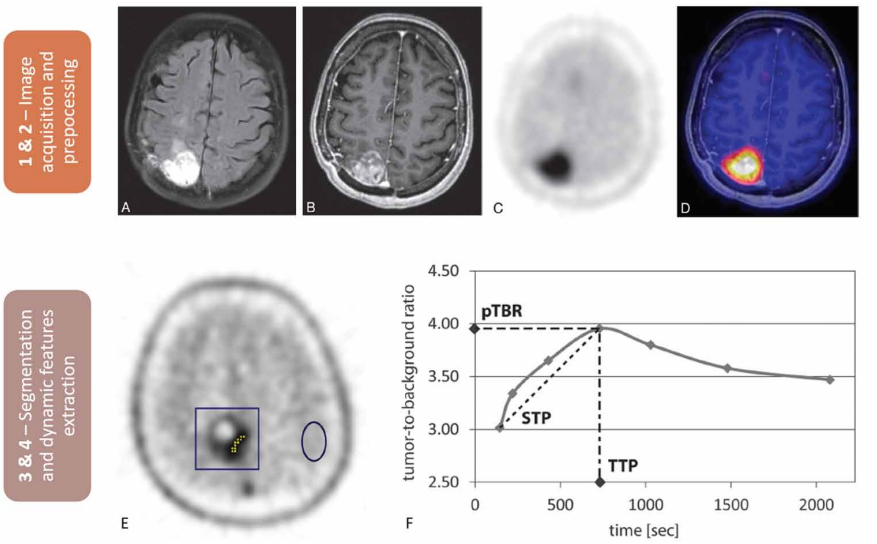
Outcomes in radiation oncology are generally characterized by two metrics: tumor control probability (TCP) and the surrounding normal tissue complication probability (NTCP) (Steel, 2002; Webb, 2001). The dose-response explorer system (DREES) is a dedicated software tool for modeling of radiotherapy outcome (El Naqa *et al.*,

*Figure 3. Dynamic features extracted from DCE-MRI in case of a F98 glioblastoma rat model. Data are presented for one slice with parameter maps of the volume transfer rate constant $K^{Trans}$ ($min^{-1}$), the extravascular-extracellular volume fraction $v_e$ (dimensionless) and the blood volume fraction $v_p$ (dimensionless) superimposed on T1-weighted images.*
*Courtesy Martin Lepage, PhD from the study of (Poulin et al., 2015).*




**For a more accurate representation see the electronic version.*

*Figure 4. Dynamic features extracted from 18F-FET PET in case of low grade glioma patients. Example of combined 18F-FET PET/MR imaging: A, T2-weighted FLAIR; B, T1-weighted image with Gd-DTPA; C, static 18F-FET PET; and D, fused image (B and C). Dynamic FET-PET anal-ysis: E, Region-of-interest definition with 90% isocontour tumor region of interest (dots) and background region of interest (circle); and F, Corresponding time-activity curve with defini-tions of peak tumor-to-background ratio (pTBR), time-to-peak (TTP), and slope-to-peak (STP).*
*Modified from (Pyka et al., 2014).*



2006c). A detailed review of outcome modeling in radiotherapy is presented in our previous work (El Naqa, 2013). In the context of image-based treatment outcomes modeling (radiomics), the observed outcome (e.g., TCP or NTCP) is considered to be adequately captured by extracted image features (El Naqa *et al.*, 2009; El-Naqa *et al.*, 2004), where complementary imaging information are built into a data-driven model such as classical logistic regression approaches or more advanced machine learning techniques.

1. **Outcome Modeling by Logistic Regression:** Logistic modeling is a common tool for multi-metric modeling. In our previous work (Deasy and El Naqa, 2007; El Naqa et al., 2006a), a logit transformation was used:

$$f\left(x_i\right) = \frac{e^{g\left(x_i\right)}}{1+e^{g\left(x_i\right)}}, i = 1,\ldots,n \tag{1}$$

where, *n* is the number of cases (patients), $x_i$ is a vector of the input variable values (i.e., image features) used to predict $f\left(x_i\right)$ for outcome $y_i$ (i.e., TCP or NTCP) of the $i_{th}$ patient, and g($x_i$) is a linear combination of *d* variables, expressed as:

$$g\left(x_i\right) = \beta_o + \sum_{j=1}^{d} \beta_j x_{ij}, i = 1,\ldots,n, j = 1,\ldots,d \tag{2}$$

where, the $\beta$'s are the set of regression coefficients of the model determined by maximizing the probability that the data gave rise to the observations. Feature selection could be done *a priori* or as part of the optimization process using shrinkage techniques by adding a penalty to the L1 norm (LASSO) or the L2 norm (ridge).

2. **Outcome Modeling by Machine Learning:** Machine learning represents a wide class of artificial intelligence techniques (e.g., neural networks, decision trees, support vector machines (SVMs)), which are able to emulate living beings' intelligence by learning the surrounding environment from the given input data. These methods are increasingly being utilized in radiation oncology because of their ability to detect nonlinear patterns in the data (El Naqa et al., 2015b). This is due to their ability to detect complex patterns in heterogeneous datasets with superior results when compared to state-of-the art in each of these disciplines. There are two common types of learning: supervised and unsupervised. Supervised learning is used to estimate an unknown (input,

output) mapping from known (input, output) samples (e.g., classification or regression). In unsupervised learning, only input samples are given to the learning system (e.g., clustering or dimensionality reduction). In image-based outcome modeling, we focus mainly on supervised learning, wherein the endpoints of the treatments such as TCP or NTCP are provided by experienced oncologists in our case. In particular, neural networks were extensively investigated to model post-radiation treatment outcomes for cases of lung injury (Munley et al., 1999; Su et al., 2005) and biochemical failure and rectal bleeding in prostate cancer (Gulliford et al., 2004; Tomatis et al., 2012). A rather more robust approach of machine learning methods is kernel-based methods and its favorite technique of SVMs, which are universal constructive learning procedures based on the statistical learning theory (Vapnik, 1998). Learning is defined in this context as estimating dependencies from data (Hastie et al., 2001).

For discrimination between patients who are at low risk versus patients who are at high risk of radiation therapy, the main idea of SVM would be to separate these two classes with 'hyper-planes' that maximizes the margin between them in the nonlinear feature space defined by implicit kernel mapping as shown in Figure 5. The objective here is to minimize the bounds on the generalization error of a model on unseen data before rather than minimizing the mean-square error over the training dataset itself (data fitting). Mathematically, the optimization problem could be formulated as minimizing the following cost function:

$$L(w,\xi) = \frac{1}{2} w^T w + C\sum_{i=1}^{n} \xi_i \tag{3}$$

subject to the constraint:

$$y_i\left(\boldsymbol{w}^T \boldsymbol{\Phi}\left(\boldsymbol{x}_i\right) + b\right) \geq 1 - \xi_i, i = 1,2,\ldots,n \tag{4}$$

$\xi_i \geq 0$ for all *i*

where, $w$ is a weighting vector each datapoint $\left(x_i, y_i\right)$, *b* the bias, *T* the transpose operator, *ξi* the error tolerance, *C* a regularization parameter, and $\Phi\left(\cdot\right)$ a nonlinear

mapping function. The $\xi i$ allowed for each sample to be on the wrong side of the margin (called hinge loss). Note that minimization of the first term in Eq. (3) increases the separation (margin) between the two classes, whereas minimization of the second term improves fitting accuracy. The trade-off between complexity (or margin separation) and fitting error is controlled by the regularization parameter *C*. However, such a nonlinear formulation would suffer from the curse of dimensionality (i.e., the dimensions of the problem becomes too large to solve) (Haykin, 1999; Hastie et al., 2001). Therefore, the dual optimization problem (using Lagrangian duality properties) is solved instead of Eq. (3), which is convex complexity becomes dependent only on the number of samples and not on the dimensionality of the feature space. The prediction function in this case is characterized by only a subset of the training data, each of which are then known as 'support vectors' $s_i$:

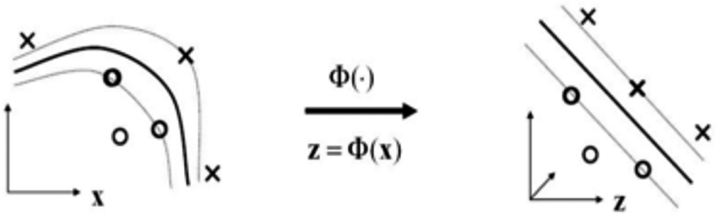
$$f\left(x\right) = \sum_{i=1}^{n_s} \alpha_i y_i K(s_i, x) + \alpha_0 \tag{5}$$

where, $n_s$ is the number of support vectors (i.e., samples at the boundary of Figure 5), $\alpha_i$ are the dual coefficients determined by quadratic programming, and $K(s_i,x)$ is the kernel function. Typical kernels (mapping functionals) include:

$$\begin{aligned} &Polynomials: && K(x,x') = \left(x^T x' + c\right)^q \\ &Radial\ basis\ function\ (RBF): && K(x,x') = \exp\left(-\frac{1}{2\sigma^2}\left\|x - x'\right\|^2\right) \end{aligned} \tag{6}$$

where, $c$ is a constant, $q$ is the order of the polynomial, and σ is the width of the radial basis functions. Note that the kernel in these cases acts as a similarity function between sample points in the feature space. Moreover, kernels enjoy closure properties, i.e., one can create admissible composite kernels by weighted addition and multiplication of elementary kernels. This flexibility allows for the construction of a neural network by using a combination of sigmoidal kernels. Alternatively, one could choose a logistic regression equivalent kernel by proper choice of the objective function in Eq. (3).

*Figure 5. Kernel-based mapping from a lower dimensional space (X) to a higher dimensional space (Z) called the feature space, where non-linearly separable classes become linearly separable.*




## RADIOMICS AND RADIOGENOMICS EXAMPLES FROM BRAIN CANCER

### Tumor Grading and Subtypes Identification

The standard reference for characterizing brain neoplasms is currently based on histopathologic analysis following surgical biopsy or resection, but this also has limitations including sampling error and variability in interpretation (Aronen et al., 1994; de Wolde, Pruim, Mastik, Koudstaal, & Molenaar, 1997).

A recent study used dynamic FET-PET imaging features with a logistic regression approach that allowed the calculation of the individual probability of a high-grade gliomas (Calcagni et al., 2011). Using 2 variables, early SUV and sum of the frame-to-frame differences (SoD), and assuming that a probability 50% indicates high-grade glioma and a probability 50% indicates low-grade glioma, they found 100% specificity (CI: 90.5–100) and 93% sensitivity (CI: 68 –99.8) due to one "false negative" (patient 23 in Table 4). Logistic regression can work well also using few variables, such as early-to-middle SUV tumor ratio because it is one of the simplest methods to obtain a reasonably accurate model. Similarly, Pyka et al. wanted to clarify the possible role of dynamic FET-PET imaging in assessing the prognosis of patients with first diagnosed glial cell tumors (Pyka et al., 2014). The data showed a correlation of several kinetic parameters and static tumor-to-background ratio (TBR) with progression-free survival. The dynamic parameter TTP (see Figure 4) proved superior to static TBR, peak TBR and slope-to-peak and yielded convincing statistical results in the overall patient collective and especially in the low-grade subgroup. In addition, magnetic resonance image features can be used to identify glioblastoma phenotypic subtypes. In a recent study, Itakura et al. have identified three distinct clusters of unilateral, solitary GBM defined by quantitative MR image features (Itakura et al., 2015). For each subject they extracted gray-value histogram statistics, textures, sharpness of lesion boundaries, and metrics of compactness and

roughness of each lesion. The imaging phenotypes of GBM subtypes were discovered in one cohort and validated in a second cohort. In addition to distinguishing imaging phenotypes, or "clusters" —which were named Pre-Multifocal, Spherical, and Rim-Enhancing—the three clusters demonstrated significant differences in survival probabilities and in associations with canonical signaling pathways. Imaging-based markers of disease phenotype may therefore offer actionable knowledge for clinical decision-making and therapeutic targeting.

## Radiogenomics

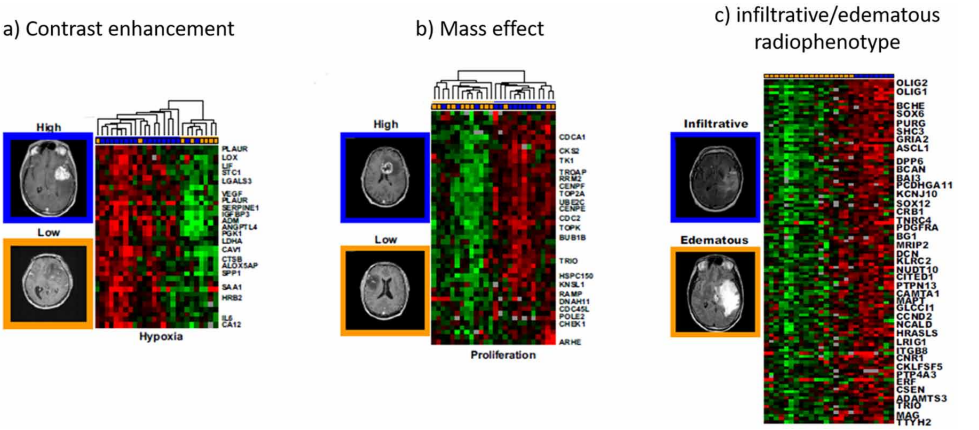
Furthermore, radiomics can be linked with the concept of radio-genomics, which assumes that imaging features are related to gene signatures. For more details, the reader can refer to a recent review article (El Naqa et al., 2017).

One of the first radiogenomics study was performed by Diehn and colleagues, who identified and associated noninvasive imaging surrogates with brain tumor gene-expression modules (Diehn et al., 2008). Most of the gene-expression signatures were captured by unique imaging traits, suggesting specific association between radiophenotypes and the underlying gene-expression signatures. For example, activity of the hypoxia module, which contains genes implicated in angiogenesis and tumor hypoxia (e.g., VEGF, ADM, PLAUR, SERPINE1, CA12), was associated with the contrast enhancement (CE) imaging phenotype ($P = 0.012$) (Figure 6a). Also, they found a strong association between the mass effect radiophenotype, which is the growth of a tumor pressing against nearby brain tissue, and the proliferation gene-expression signature ($P = 0.0017$) (Figure 6b). Indeed, clustering of tumors based solely on the expression of genes involved in proliferation and cell-cycle progression (e.g., TOP2A and CDC2) resulted in segregation of the majority of low- and high-mass-effect tumors to opposite branches of the dendrogram. Gene Ontology term analysis, i.e., concepts/classes used to describe gene function, of the infiltrative radiophenotype-associated genes (Figure 6c) revealed a significant enrichment of terms involved in CNS development, including nervous system development ($P < 0.003$) and gliogenesis ($P < 0.034$).

Similarly, Itakura et al., associated radiomics with genomics, by estimating various signaling pathway activities as they pertained to the three clusters (imaging phenotypes) mentioned above (Itakura et al., 2015). All three clusters were significantly associated with one or more regulatory pathways. For example, the Pre-Multifocal phenotype was marked by only one receptor pathway (upregulation of the c-Kit stem cell factor). The Spherical GBM phenotype was characterized by downregulation of 21 pathways, including c-Kit, VEGFR signaling PDGFR-α signaling, FOXA transcriptional networks, and angiopoietin (Ang)/Tie2. The Rim-Enhancing phenotype was differentiated by upregulation of 31 pathways,

*Figure 6. Gene-expression surrogates for MRI features. a) Expanded view of the association between the hypoxia gene-expression module and contrast enhancement. Tumor arrays were clustered by using only cDNA clones contained within the gene module. The value of the imag-ing trait for each tumor is indicated by the colored box above the expression map. Representa-tive MR images are depicted on the left. A subset of named genes is labeled. b) Expanded view of the association between the proliferation gene-expression module and mass effect. c) Expres-sion of the infiltrative radiophenotype-associated genes in the initial set of GBMs. The infiltra-tive pattern was differentiated from an edematous pattern based on the appearance of hyperin-tense signal on T2-weighted images and reflects the interface between a tumor and the adjacent normal brain.*

*Modified from Diehn et al., 2008. © Copyright (2008) National Academy of Sci-ences, U.S.A.*




**For a more accurate representation see the electronic version.*

including canonical WNT, PDGFR-β signaling, VEGFR signaling, etc. Based on these associations with canonical signaling pathways, the imaging subtypes have the potential for targeted therapy. This suggests the potential utility for patients in the Pre-Multifocal GBM phenotype of targeting c-Kit, PDGFR-β with tyrosine kinase inhibitors, such as imatinib and dasatinib, this last being used in Phase II trial RTOG 0627 (Lassman et al., 2015).

A recent radiogenomics study focused on low grade gliomas (LGG). Several molecular biomarker tend to identify LGG subtypes, as Jiang et al. who found that AOA could be divided into 2 subgroups with significantly different prognoses according to the status of 1p/19q and IDH1/2 (Jiang et al., 2013). The subgroup with 1p/19q co-deletion and/or IDH1/2 mutation had better prognosis, similar to AO, and the other subgroup without such genetic signatures had worse prognosis, similar to AA. In addition, in a pooled meta-analysis, Hu et al. demonstrated the significant protective effect chromosomal 1p/19q co-deletion has on survival of

patients with grade II and III oligodendrogliomas (Hu, Richards, & Jensen, 2016). Patients with this co-deletion showed significantly longer overall survival/progression free survival times compared to those without the co-deletion. Patients with this co-deletion also are shown to have significantly longer overall survival times when treated with radiation therapy as well as chemotherapy in comparison to radiation therapy alone. From these molecular biomarkers, Zhou et al. explored the possibility of using textural features extracted from images of conventional MR sequences to make predictions of wild-type IDH versus IDH1 mutation; IDH1 mutation with 1p/19q co-deletion versus IDH1 mutation without 1p/19q co-deletion; grade II versus grade III low grade gliomas (LGG); and progression versus nonprogression of LGGs (Zhou et al., 2017). Using logistic regression and bootstrap testing evaluations, their multivariable texture model showed great promises with areas under the receiver-operating characteristic curves of 0.86, a sensitivity of 0.74, and a specificity of 0.79 in distinguishing grade II from grade III gliomas, and predicting IDH1 mutation and 1p/19q co-deletion with high accuracy.

## DISCUSSION AND CONCLUSION

The use of imaging in outcome modeling of radiotherapy response has witnessed rapid increase in recent years adding more value to an already existing use of imaging in cancer treatment in general and radiotherapy in particular. However, there are several issues that are currently limiting its rapid progression. It is well recognized that image acquisition protocols may impact the reproducibility of extracted features from image modalities, which may subsequently impact the robustness and stability of these features for treatment prediction. This includes static features such as SUV/HU descriptors and texture features. Interestingly, texture-based features were shown to have a reproducibility similar to or better than that of simple SUV descriptors (Tixier et al., 2012). This demands protocols for standardized acquisition. In addition, factors that may impact the stability of these features also include signal-to-noise ratio (SNR), partial volume effect (PVE), motion artifacts, parameter settings, resampling size, and image quantization (Cheng, Fang, & Yen, 2013; El Naqa et al., 2009). For example, errors caused by PVEs on absolute quantification of brain perfusion and other hemodynamic parameters have been reported for dynamic susceptibility contrast-MRI (Knutsson et al., 2015) and for CT imaging (Riordan et al., 2014). Similarly, PVE from cerebrospinal fluid affects diffusion tensor imaging tractography (Baron & Beaulieu, 2015). Nevertheless, advances in hardware and software technologies will further facilitate wider application of advanced image processing techniques to medical imaging to achieve better clinical results. For instance, pre-processing methods such as denoising and deconvolution methods

already help in mitigating such artifacts (El Naqa et al., 2005; Zaidi, Abdoli, Fuentes, & El Naqa, 2012), however, more advanced image restoration methods based on nonlocality and sparsity may be more fruitful (Gunturk & Li, 2012). Outcome modeling using logistic regression has become a de facto standard, however, more advanced modeling techniques using machine learning may provide further predictive power particularly when dealing with more complex and nonlinear relationships among features and between clinical outcomes. Given the noninvasive nature of medical imaging and its wide use in clinical practice, it is likely that identification of imaging phenotypes tied to distinct molecular phenotypes will help to advance individualized patient care. Future studies with serial dynamic (Cu, FET, …)-PET/MR assessing all metabolic and anatomical parameters may be of value to further improve the predictive sensitivity and specificity of features for adaptive radiation therapy. We believe that the synergy between image analysis and machine learning (El Naqa, Li, Murphy, & others, 2015) could provide powerful tools to strengthen and further the utilization of image-based outcome modeling in clinical practice towards improved clinical decision making and personalized medicine in the future.

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# Chapter 8
# Radiation-Induced Lung Injury Imaging:
## Current Status and New Developments

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## ABSTRACT

*Radiation-induced lung injury (RILI) occurs in up to 30% of thoracic radiotherapy (RT) cases and is a major limiting factor of dose escalation to achieve tumor control and improve survival. RILI can be separated into two phases: an early inflammatory phase and a late fibrotic phase. Imaging has the potential to provide a helpful understanding of RILI for diagnosis, monitoring and treatment. Current clinical imaging methods rely on anatomical imaging and occasionally incorporate functional imaging. With the advent of molecular imaging, specific targeted probes can be designed to image RILI at every stage of the process. Molecular imaging is still in its infancy and most new RILI imaging techniques are still under development. This chapter summarizes the different imaging methods used clinically for RILI imaging and explores new developments for the future of RILI management.*

## INTRODUCTION

Lung cancer 5-year survival rates remain very low (15%) and is the leading cause of cancer deaths in the world. During the course of treatment, about 50% of cancer patients will undergo radiation therapy (RT) (Bentzen, 2006). RT uses ionizing radiation to kill tumor cells via DNA and cellular damage and treat cancer. RT dose escalation would improve tumor control but is limited by normal tissue toxicity. Like

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all cancer therapies, radiotherapy involves the risk of developing side effects that could impair the quality of life of patients and lead to severe complications. However, unlike chemotherapy, the side effects of radiotherapy are localized in the organs or tissues that have been irradiated. There is a fine balance, or therapeutic window, between achieving cure or tumor control and reducing adverse effects or toxicities associated with radiotherapy. Radiation-induced lung injury (RILI) occurs in up to 30% of patients who received thoracic irradiation. This primarily includes patients treated for lung cancer, but also includes breast, esophageal cancer or lymphoma patients (Bentzen, 2006; Kong, Haken, Eisbruch, & Lawrence, 2005).

The dose delivered to treat the tumor is limited by side effects, acute and late, to surrounding normal tissue. Imaging has the potential to monitor such effects to allow for potential prevention or mitigation strategies in a patient specific manner

(Robbins et al., 2012). Imaging can be used to diagnose patients that are at risk of developing side effects early in the course of treatment when it is still possible to change or adapt treatment or add drugs to mitigate those. Having an imaging tool to detect adverse effects allows for monitoring the response of the patient to mitigating agents or validating newly developed treatment options.

Current clinical practice focuses on anatomical imaging for diagnosis and monitoring of RILI. RILI is a complex process comprising of a myriad of molecular players acting over time to develop functional lung impairments and radiological evidence of disease. Functional lung tests are used to assess the overall lung capacity but lack spatial resolution. Functional imaging techniques can also be used to map lung function for RT avoidance or treatment monitoring. With the advent of molecular imaging and the development of targeted imaging probes, it becomes possible to gain insight into RILI at the molecular level. Most molecular imaging methods for RILI imaging, although promising, are currently being developed and are not yet routinely implemented in the clinic.

This chapter aims to review the current status of RILI imaging and highlight future developments with the potential to improve the diagnosis, monitoring and management of RILI clinically.

## BACKGROUND

### Radiation-Induced Lung Injury (RILI)

Radiation-Induced Lung Injury (RILI) **is** the damage sustained by normal tissue in the lungs following radiation exposure. The response of normal tissue to irradiation is complex, starting with an inflammatory phase, a proliferative phase and a tissue remodeling phase, each involving a cascade of cytokines and immune cells (Figure 1).

RILI consists of (1) an early inflammatory phase (reversible) that occurs within weeks of radiotherapy that includes: normal tissue damage, cytokine induction, hypoxia, macrophage accumulation and activation; and (2) a late fibrotic phase (irreversible) appearing months or years following treatment that involves tissue remodeling and fibrosis (Bentzen, 2006; Santyr et al., 2014). The early inflammatory phase is called radiation pneumonitis (RP) and the late phase with scar tissue formation is referred to as fibrosis or radiation-induced lung fibrosis (RILF). The mechanism behind the development of RP is still unclear. It involves a combination of factors such as radiation dosimetric parameters, tumor size and location, concurrent chemotherapy and patient specific factors including age or comorbidities (Kong et al., 2005). Limiting RILI risk is a major step towards dose escalation and the application of promising hypofractionated regimens in the lung.

The most common symptom of RILI is dyspnea (difficulty breathing), which can range from mild to severe. Dyspnea is often accompanied with a non-productive cough and rarely fever. Late RILI symptoms can include pulmonary insufficiency that can become chronic (Graves, Siddiqui, Anscher, & Movsas, 2010).

Clinical symptoms associated with RILI are evaluated and graded by the physician following RT. Scoring of RILI can be done using several grading systems that are slightly different. The Radiation Therapy Oncology Group (RTOG) defines a grading system for early and late effects as shown in Table 1 (Kong et al., 2005).

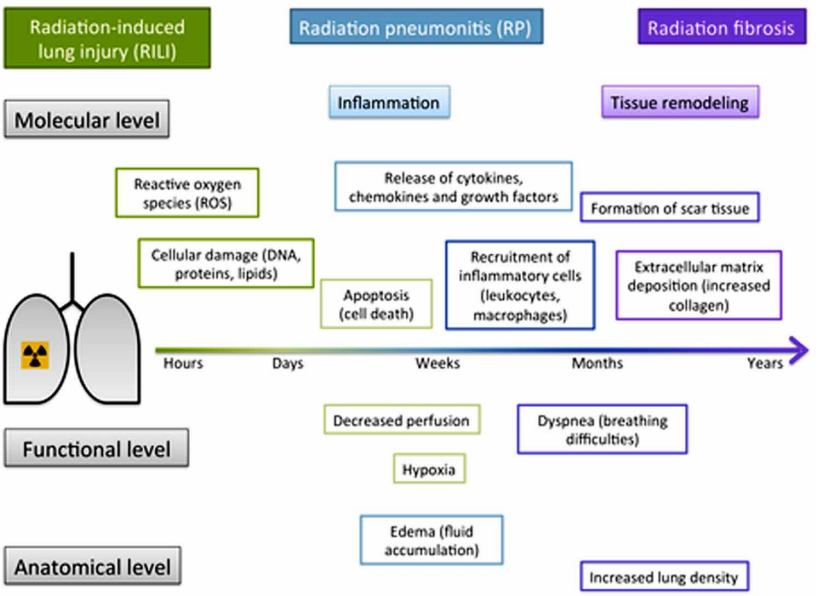
*Table 1. RILI scoring schema according to the Radiation Therapy Oncology Group (RTOG) for both early and late effects*

| RTOG Scoring | Acute Early Effects | Late Effects |
|---|---|---|
| Grade 1 | Mild symptoms of dry cough or dyspnea on exertion | • Asymptomatic or mild symptoms (dry cough)<br>• Slight radiographic appearances |
| Grade 2 | • Persistent cough requiring narcotic, antitussive agents<br>• Dyspnea with minimal effort but not at rest | • Moderate symptomatic fibrosis or pneumonitis (severe cough)<br>• Low grade fever<br>• Patchy radiographic appearances |
| Grade 3 | • Severe cough unresponsive to narcotic antitussive agent or dyspnea at rest<br>• Clinical radiological evidence of acute pneumonitis<br>• Intermittent oxygen or steroids may be required | • Severe symptomatic fibrosis or pneumonitis<br>• Dense radiographic changes |
| Grade 4 | • Severe respiratory insufficiency<br>• Continuous oxygen or assisted ventilation | • Severe respiratory insufficiency<br>• Continuous $O_2$<br>• Assisted ventilation |
| Grade 5 | Death | Death |

The lungs' response to RT is thought to follow the steps of an abnormal wound healing mechanism: injury, inflammation and repair. Reactive oxygen species (ROS) are produced upon tissue irradiation creating DNA and cellular damage, leading to cell death. Consequently, the epithelial cells lining the lungs (pneumocytes type I and II) undergo apoptosis. Cell depletion induces the secretion of growth factors and the recruitment of inflammatory cells to the injury location. The loss of endothelial cells and small vessels reduces lung perfusion and increases hypoxia. Following the wound healing process, inflammatory cells (leukocytes) are recruited to the injury site and secrete chemokines, cytokines and growth factors to recruit and activate more cells (macrophages) to assist in the process. Following RT, this process is not properly regulated and can lead to chronic inflammation, which promotes RILI. The cascade of inflammatory signaling triggers the repair phase with the secretion of interleukin (IL-1 and IL6), tumor necrosis factor (TNFα) and transforming growth factor (TGF-β), as well as the recruitment and activation of macrophages. All these factors contribute to tissue remodeling and scarring in the form of fibrosis while perpetuating the inflammatory response leading to chronic RILI (Graves et al., 2010).

Figure 1 illustrates the process of RILI over time at the anatomical, function and molecular levels.

*Figure 1. RILI process over time at the molecular, functional and anatomical level. Upon irradiation, normal lung tissue responds with a cascade of molecular events leading to functional clinical symptoms and anatomical changes. Each step in the RILI process can be a target for imaging.*



RP is a complex process and studying the cellular inflammatory infiltrate or cell recruitment can be used to understand the process and potentially design targeted probes or therapeutic agents. It was shown that there is a dramatic increase in mast cells (cell type from the immune system) infiltration in the lungs of rats following thoracic irradiation (Szabo et al., 2010). The predominant cell types recruited in RILI in mice are macrophages and lymphocytes. This acute response is thought to be due primarily to parenchymal cell injury and can therefore sensitize the lungs to a subsequent injury from another exposure like endotoxins (Johnston, Williams, Elder, Hernady, & Finkelstein, 2004).

Pulmonary function tests (spirometry) are the gold standard to clinically measure lung function by detecting changes in volumes or flow following inhalation and exhalation. However, they are non-specific, lack spatial resolution and are not sensitive to small lung function changes that can be compensated for by unimpaired lung areas. Imaging can overcome these drawbacks anatomically, functionally or molecularly for diagnosis of lung injury and monitor the potential efficacy of new therapeutic agents. Imaging needs to be validated and benchmarked with proven techniques to demonstrate safety and sensitivity, thereby improving early diagnosis and allowing for a better chance to intervene in the course of treatment. In this way, clinical and pre-clinical imaging can be complementary where one contributes to improving the other (van Echteld & Beckmann, 2011).

## IMAGING RADIATION-INDUCED LUNG INJURY: CURRENT STATUS

Current clinical methods to evaluate pulmonary disease include tissue biopsies for histological analysis or bronchoalveolar lavage analysis, both invasive methods and subjective depending on where and when the sample was taken. Lung function tests are non-specific and subtle changes can go undetected (Dharmarajan & Schuster, 2005).

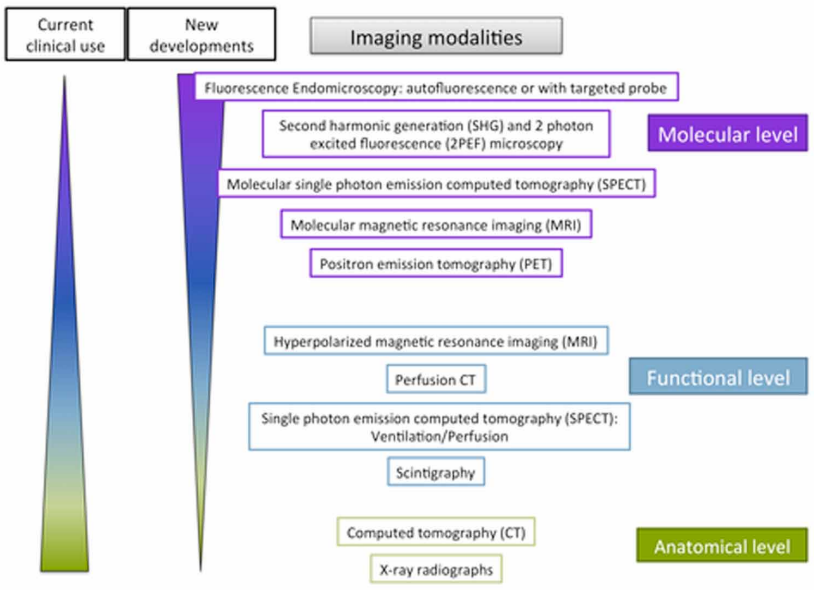
RILI is most commonly evaluated with anatomical imaging and less so with functional or molecular imaging. (Figure 2).

### Anatomical Imaging

#### X-Ray Radiographs and Computed Tomography (CT)

As mentioned earlier, anatomical imaging remains the principal method to assess RILI clinically. The most common diagnosis tool is X-Ray radiographs (2-dimensional) and its 3-dimensional equivalent, computed tomography (CT). Both methods use

*Figure 2. Imaging modalities for RILI imaging from anatomical, functional to molecular level. The current use in the clinic is inversely proportional with new developments in the field. More established techniques are more anatomical and used more clinically. The more novel the development, the more molecular and the less it is used clinically.*




X-rays traveling through the body and attenuation by tissue is measured on the other side with a detector. In the case of CT, the source and detector rotate around the patient to reconstruct a 3D image. X-Ray based imaging relies on the differences in tissue attenuation properties going from high density (bones) to low density (lung). Cell infiltration, edema or fibrosis from RILI will induce an increase in lung density (towards soft tissue density), which becomes visible on CT images. These changes are referred to as radiological evidence of RILI, be it pneumonitis or fibrosis related (Robbins et al., 2012).

Airway remodeling is commonly assessed with CT from which one can extract many features of fibrosis such as reticulation (fine network of lines from architectural distortion), honeycombing ("clustered cystic air spaces" possibly due to small airway dilation) and ground-glass opacity (fibrosis smaller than the CT resolution or active inflammation) (Kusmirek, Martin, & Kanne, 2016; van Echteld & Beckmann, 2011).

Importantly, increased CT density values in the lungs can be correlated with 3D dose-distribution maps (Robbins et al., 2012).

Pre-clinical models are key to understanding and developing techniques for RILI. With the advent of small animal imaging and RT equipment, it is now possible to compare clinical and pre-clinical results. Monitoring CT changes longitudinally in a partial lung irradiation mouse model showed a dose dependence using the same machine to image and deliver RT (Granton et al., 2014).

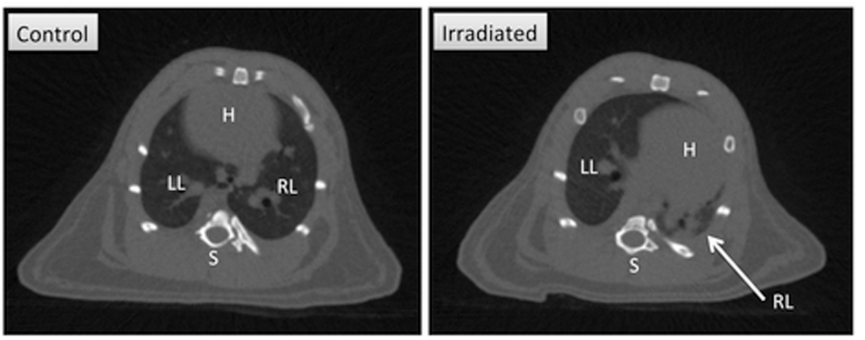
Figure 3 (Perez, Ybarra, Chagnon et al, in press) shows an example of microCT imaging in a rat model of RILI. The right lung was irradiated with 18 Gy on a clinical linear accelerator and the onset of RILI was monitored. The affected lung appears denser with opacity on CT and shows a "patchy" pattern (Figure 3, right, arrow). The heart and mediastinum shift towards the injured lung is also noticeable (Figure 3, right).

## Magnetic Resonance Imaging (MRI)

Magnetic resonance imaging (MRI) can also provide anatomical images of RILI. MRI is a technique based on magnetic fields and radiowaves. MRI signal comes from protons (hydrogen mainly from water present in the body) and therefore provides high soft tissue contrast. However, MRI is not the method of choice for the lungs due the fact that the lungs have more air space than tissue, leading to a loss of MRI signal.

Pulmonary inflammation is characterized by the presence of fluid in the lungs (edema). Edema can be visualized using MRI where an increased signal appears. Changes in MRI signal allow for monitoring the effects of anti-inflammatory drugs such as glucocorticosteroids in pre-clinical models (van Echteld & Beckmann, 2011).

*Figure 3. Pre-clinical CT image (transverse plane) of rat model of RILI 24 weeks following 18 Gy irradiation to the right lung. We observe fibrotic lung tissue in the irradiated lung appearing denser on CT imaging (arrow) compared to the control. Also visible is the shift of the heart and mediastinum towards the injured lung. Left: Control; Right: Irradiated. H: Heart; S: Spine; LL: Left Lung: RL: Right Lung*



## Functional Imaging

### Scintigraphy and Single Photon Emission Computed Tomography (SPECT)

A radioactive tracer is administered and localizes to the site of interest. The tracer emits gamma rays that can be detected using gamma cameras (2D planar scintigraphy). By detecting those gamma rays around multiple angles around the patient, a 3D image of tracer localization is reconstructed. Scintigraphy (2-dimensional) and its 3D counterpart SPECT are clinically used to image lung ventilation and perfusion. Radioactive technetium labeled macro-aggregated albumin (Tc99m-MAA) is injected intravenously in the patient. Following the radiolabeled tracer allows monitoring lung perfusion (gas exchange). Ventilation is imaged through the inhalation of radionuclides (Xenon or technetium DTPA), which mimics where air localizes in the lungs. Defects in ventilation or perfusion can be visualized and localized based on those scans.

Ventilation and perfusion (V/Q ratios) of the lungs can be obtained with $^{133}$Xe SPECT and regional defects can be identified. It is one of the most common imaging methods for pulmonary function, although it suffers from poor spatial resolution (van Echteld & Beckmann, 2011).

SPECT pulmonary function measurement of defects in perfusion is more sensitive than in ventilation, and both are more common than CT density changes (Robbins et al., 2012).

## Molecular Imaging

### Fluorodeoxyglucose ($^{18}$F) Positron Emission Tomography (FDG-PET)

In positron emission tomography (PET) imaging, a molecule of interest is labeled with a radioactive positron-emitter. The probe localizes in the region of interest to be imaged, emits a positron that annihilates with a nearby electron and emits two photons traveling in opposite directions towards a ring detector. It is then possible to reconstruct an image of where the photons came from inside the body and therefore where the radioactive imaging probe was localized. In the case of FDG, the imaging probe is a radiolabelled modified glucose molecule, which gets trapped in cells consuming glucose, and is therefore a marker of metabolism, localizing regions of high glucose intake.

Pulmonary inflammation is difficult to identify from chest X-ray and CT alone as it provides only anatomical information. Molecular imaging has the potential to visualize pulmonary inflammation by targeting inflammatory markers such as FDG-PET for the increased glucose consumption by inflammatory cells or by labeling inflammatory cells themselves (Dharmarajan & Schuster, 2005).

Lung inflammation can be imaged with FDG-PET, where a signal increase is observed due to increased tracer uptake in inflammatory cells, including neutrophils and macrophages (van Echteld & Beckmann, 2011).

Pulmonary fibrosis can be reproducibly monitored with FDG-PET by evaluating standardized uptake value (SUV) metrics in patients with idiopathic pulmonary fibrosis (Win et al., 2012). FDG-PET allows monitoring pneumonitis following RT. However, those radiological changes do not always correlate with clinical symptoms but show superior sensitivity (Robbins et al., 2012).

## IMAGING RADIATION-INDUCED LUNG INJURY: NEW DEVELOPMENTS

### Functional Imaging

#### Hyperpolarized MRI

In lung injury, gas exchange is impaired due to a thickening of the blood-gas barrier between capillaries, where red blood cells uptake oxygen, and the alveolar space (van Echteld & Beckmann, 2011).

Hyperpolarized (HP) MRI is a heavily researched area of lung imaging. HP $^{129}$Xe can be used with MRI to image gas exchange in the lungs with a single breath hold in healthy volunteers and patients with pulmonary fibrosis. After the subject inhales HP $^{129}$Xe, the gas remains mainly in the airspaces but a small fraction of it also dissolves in the lung tissue. $^{129}$Xe resonates differently when it is in interstitial tissue or plasma so it is possible to distinguish these two dissolved phases from each other. These two compartments, referred to as the barrier and the RBCs (red blood cells), differ in their chemical shift. With $^{129}$Xe behaving like oxygen, it is possible to use it as a probe for studying gas exchange. $^{129}$Xe defect maps were compared to CT images of idiopathic pulmonary fibrosis (IPF) patients and show similar regions of fibrosis. It is possible to derive defect maps based on ratios between gas, barrier and RBCs phases. RBCs to gas maps show reduced intensity and defects in gas exchange in IPF patients, whereas healthy volunteers showed homogenous maps (Kaushik et al., 2016).

HP $^{3}$He-MRI from lung cancer patients were compared before and after RT. Post-RT regions of pneumonitis present on CT images were correlated with areas of reduced ventilation obtained with HP $^{3}$He-MRI (Ireland et al., 2010).

Moreover, hyperpolarized MRI was used in RILI rat models to image lung anatomy, function and metabolism. Detected changes in imaging signal due to pneumonitis correlate with histology and could be used to monitor RILI (Santyr et al., 2014). HP $^{129}$Xe MRI combined with gas transfer modeling was also applied to RILI rat models for the detection of early and late effects post-irradiation (Fox et al., 2014; Li et al., 2016). Indeed, early gas exchange defects were further detected with HP $^{129}$Xe MRI in a rat model of RILI and showed significant differences in pulmonary tissue thickness and relative blood volume measured in irradiated animals compared to controls (Santyr et al., 2016). In another study, early RILI including inflammation and hypoxia were investigated in a rat model with hyperpolarized $^{13}$C MR spectroscopy and imaging. Mapping the lactate to pyruvate ratio signal as a surrogate of metabolic activity demonstrated an increased signal in RILI rats compared to controls (Thind et al., 2013; 2014).

### Contrast Enhanced Perfusion MRI

Contrast enhanced perfusion MRI can be used to visualize early and late effects following RT. Gadolinium-DTPA is injected in the subject and showed altered kinetics with a contrast enhancement in irradiated compared to controls (Robbins et al., 2012).

### CT Perfusion Imaging (CTPI)

CT perfusion imaging (CTPI) measures organ perfusion by injecting an iodine-based contrast agent and monitoring its transport through the organ. This is usually combined with mathematical models to extract parameters of interest. This technique was used to image patients post-RT to detect acute RILI. RILI patients showed changes in blood flow, volume and permeability surface between pre- and post-RT (Hu et al., 2014).

## Molecular Imaging

### Molecular MRI

MRI can be used as a molecular imaging modality by tagging a molecule of interest with Gadolinium to become an MR imaging probe. As the imaging probe localizes

to the site of interest (in this case the lungs), it is possible to visualize and quantify the accumulation of the probe in a specific location using MRI.

For fibrosis being characterized by an overexpression of collagen, molecular MRI was used to specifically detect pulmonary fibrosis in a mouse model using a collagen type I targeted probe (Caravan et al., 2007; 2013).

MRI in combination with nanoparticles can provide insight into the development of pulmonary fibrosis. Gadolinium-based nanoparticles were administered intratracheally in a mouse model of lung fibrosis and were monitored with MRI. Nanoparticle contrast provided an increased MRI signal in fibrotic areas of the lungs (Tassali et al., 2016).

## Molecular Scintigraphy and SPECT

SPECT can also become a molecular imaging technique using the idea of imaging probes to target a specific process in RILI.

Collagen accumulation is an important feature of pulmonary fibrosis and therefore a good target for imaging probes. A collagen targeting peptide (collagelin) was synthesized based on the Glycoprotein VI affinity for collagen and labeled with Tc-99m for in vivo scintigraphy in a lung fibrosis mouse model (Muzard et al., 2009).

αvβ6 integrin overexpression plays an important role in pulmonary fibrosis and is a potential target for drug development as well as imaging. A specific αvβ6 integrin peptide was labeled with $^{111}$In for SPECT-CT imaging in a mouse model of lung fibrosis and was successfully detected in the lungs with an increased signal in injured lungs compared to controls (John et al., 2013).

Two biomarkers for RP imaging were targeted to develop SPECT imaging probes for perfusion (macroaggregated albumin) and for apoptosis (duramycin). Two to three weeks following RT, perfusion volume decreased and duramycin uptake increased, while no changes in breathing rate were observed at this time. This suggests that SPECT imaging with new biomarkers is a good predictor of RP (Medhora et al., 2016).

Molecular SPECT for lung inflammation imaging was developed, in which annexin V (marker of cell stress) or interleukin-8 (binds neutrophils) were radiolabeled (van Echteld & Beckmann, 2011).

## PET

Activation of macrophages plays an important role in the pathogenesis of pulmonary fibrosis. Macrophages were targeted using a cysteine cathepsin probe for both optical pre-clinical and PET imaging in patients showing an increase in signal in fibrosis cases (Withana et al., 2016).

## Fluorescence Endomicroscopy (FE)

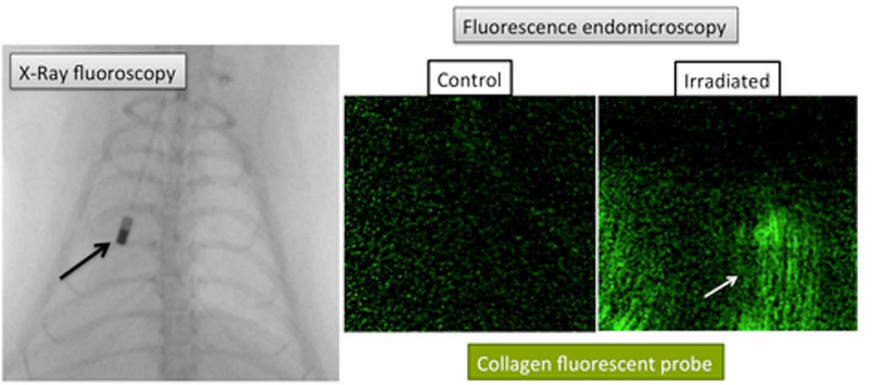
Lung being an accessible organ, it is amenable to minimally invasive imaging to obtain "optical biopsies" through bronchoscopy. FE or endoscopic confocal fluorescence microscopy is a promising new technique that allows fluorescence optical imaging at the tip of an endoscope. A laser scanning unit coupled to a bundle of optical fibers allows the excitation photons to travel through the endoscope to the tissue and the emitted fluorescence is collected by the same fibers back to give a detailed image of the field of view. It has been used in conjunction with fluorescent probes to observe a variety of cellular and architectural changes in the lungs of normal or injured rat models (Chagnon et al., 2010).

Confocal fluorescence endomicroscopy is amenable to clinical use to image the human airways as far as the alveoli, using tissue autofluorescence (elastin at 488nm) as contrast. Structural differences were observed between smokers and non-smokers (Thiberville et al., 2009).

Fluorescence endomicroscopy was used to image acute lung injury in a rat model with a fluorescent smart probe activated by an enzyme expressed by neutrophils and macrophages (myeloperoxidase). Enhanced lung enzymatic activity was observed in injured lungs compared to controls (Chagnon et al., 2015).

A fluorescent collagen probe was used with fluorescence endomicroscopy to detect pulmonary fibrosis (Figure 4, right). FE in combination with fluoroscopy

*Figure 4. Image-guided Fluorescence endomicroscopy (FE) of RILI. Left: X-ray fluoroscopy image of RILI rat model showing the location of the tip of the endoscope probe (black arrow). Right: FE images of control and irradiated lung using a fluorescent collagen probe. The white arrow shows collagen fiber structures in the irradiated lung as opposed to random patterns of dotted fluorescence in the control lungs.*



(X-Ray radiographs) (Figure 4, left) and pre-imaging CT allows for image-guided bronchoscopy where it is possible to correlate fibrosis location with FE images of collagen fibers (Perez, Ybarra, Chagnon et al, in press).

### Second Harmonic Generation (SHG) and Two Photon Excited Fluorescence (2PEF) Microscopy

Second harmonic generation (SHG) microscopy allows for the quantitative analysis of fibrillar collagen structures, a hallmark of fibrosis (Chen, Nadiarynkh, Plotnikov, & Campagnola, 2012).

Nonlinear optical microscopy combining second-harmonic generation (SGH) and two photon excited fluorescence (2PEF) was integrated in an endoscopic fiber-optic spectrometer for the exploration of the lungs. During fibrosis, the extra-cellular matrix remodels with an accumulation of collagen and elastin, which can be detected by SGH and 2PEF, respectively (Peyrot et al., 2012).

## IMAGING IN THE PREVENTION, MITIGATION, AND TREATMENT OF RILI

Radioprotectors such as free radical scavengers (amifostine, captopril or pentoxifulline) have a protective effect on tissue following RT and can be used to prevent RILI. However, with the results of clinical trials being limited, it is not currently approved for RILI prevention. Once the RILI diagnosis is established, the most common treatment is the use of corticosteroids due to their anti-inflammatory properties. It is unclear if this treatment is beneficial even after cessation. A few other potential RILI therapies are being explored including antioxidants, superoxide dismutase, nitric oxide, TGF-β, statins, matrix metalloproteinases, cytokines and growth factors (Graves et al., 2010).

### Radioprotectors

The potential of amifostine as a radioprotector was tested in a rabbit model of lung SBRT with CT, contrast-enhanced MR angiography (perfusion), $^{3}$He MRI (ventilation) and compared to histology. Decreased perfusion was observed with ce-MRA in the irradiated group but not in the irradiated with amifostine, while no changes were observed in ventilation with $^{3}$He-MRI (Mata et al., 2014).

## Normal Lung Avoidance RT Planning

Functional imaging information such as ventilation and perfusion acquired with SPECT, PET, MRI or CT can be incorporated into treatment planning. The goal of functional image guided radiotherapy treatment planning is to specifically avoid regions of highly functional lung tissue while increasing the dose to defective regions. Despite many feasibility studies of the use of functional images in treatment planning, the clinical impact has not been proven. Clinical trials are underway to incorporate MR or CT ventilation images for radiation therapy planning. Ventilation defects are less common than perfusion defects and have been shown to be a superior metric for assessing RILI. Importantly, the use of functional imaging techniques has to include accurate image registration in order to be included in treatment planning, including fiducials, reproducible patient positioning, breath hold or gated breathing scans. Thus far, the benefits of using functional image guided planning have not showed significant clinical impact and more work is needed in order to understand the underlying mechanism of RILI imaging and the effect of modifying dose delivery accordingly (Ireland, Tahir, Wild, Lee, & Hatton, 2016).

By integrating ventilation and perfusion SPECT imaging information with commonly used dosimetric values, it is possible to obtain a superior predictor of RP. This could lead to sparing highly functional lung regions during treatment planning to potentially reduce the risk of RP (Hoover et al., 2014).

Dosimetric parameters were prospectively combined with functional imaging data acquired with 4D-CT, SPECT ventilation and perfusion or both to evaluate RP. RP correlated with dosimetric parameters and functional imaging, especially when combined (Kimura et al., 2015).

## Mesenchymal Stem Cells (MSCs)

Mesenchymal stem cells (MSCs) have been proposed in the treatment of lung injury (inflammation and fibrosis) for their immune-modulatory properties and their paracrine anti-inflammatory potential (Tzouvelekis, Antoniadis, & Bouros, 2011).

Fluorescence endomicroscopy (FE) in a rat model of RILI was used to track MSCs in vivo. 3 weeks following RT, rats were injected with fluorescently labeled MSCs intravenously or endotracheally and were imaged with FE. MSCs were visible in the lungs although more numerous when injected directly in the trachea. An automated cell counting algorithm allowed for the quantification of MSCs in controls and RILI showing a slight increase in detected cells in the injured lung (Perez, Ybarra, Chagnon et al, in press).

## FUTURE RESEARCH DIRECTIONS

The emerging trend in RILI imaging and imaging in general is moving towards molecular imaging. Rather than looking at anatomical changes that take time to develop before becoming visible on anatomical images, it is now possible to image molecular targets at every stage of disease progression. Molecular and functional imaging give a better understanding of RILI and might provide useful diagnostic information early on in order to readapt RT treatment or give mitigating agents before permanent fibrosis damage occurs. These new molecular imaging techniques, although promising, require benchmarking and validation before they can be approved for clinical use. Indeed, the process from bench to bedside takes time for newly developed imaging probes and imaging devices. The power of molecular imaging with in vivo visualization of molecular processes is contributing to the assessment of new therapeutic strategies for RILI. Future research for RILI imaging will be in designing and validating new targeted imaging probes, and using these techniques to assess the potential of RILI treatments. Ultimately, the goal is to make RT more efficient with less toxicity in order to achieve better tumor control for cancer patients.

## CONCLUSION

This chapter described the molecular steps involved in RILI following thoracic irradiation as well as provided an overview of the imaging techniques used clinically and in development stage for RILI management.

RILI is a complex process composed of multiple stages of molecular events leading to pneumonitis and fibrosis. Each molecular player in the process can be used as an imaging target. Imaging of RILI is evolving from purely anatomical (CT) to functional (SPECT, MRI) and molecular (PET, SPECT, MRI, optical). Molecular imaging has the potential to contribute to RILI diagnosis, monitoring and mitigation. However, more research both clinically and pre-clinically is required in order to bring new imaging techniques from bench to bedside.

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## KEY TERMS AND DEFINITIONS

**Contrast Agent:** Substance used to enhance contrast in medical imaging.

**Cytokine:** A signaling protein secreted by immune cells that has an effect on other cells.

**Fibrosis:** The scarring process following tissue injury. Tissue remodeling takes place with the deposition of extracellular matrix (collagen).

**Growth Factor:** A substance that promotes cell growth and proliferation.

**Imaging Probe:** Substance that detects and allows the visualization of a biological process. A specific target that binds or detects the process of interest is coupled to a labeling element that can be detected with imaging.

**Inflammation:** Process by which the body defends itself from injury or infection. It involves the recruitment of cells from the immune system and the release of cytokines.

**Lung Perfusion/Ventilation:** Perfusion is the blood in capillaries that attain the alveoli for gas exchange. Ventilation is the air that attains the alveoli for gas exchange.

**Medical Imaging:** Techniques used to visualize the inside of the body for clinical diagnosis.

**Molecular Imaging:** Visualization and quantification of biological processes at the molecular level in a living organism.

**Radiation Therapy Treatment Planning:** Following cancer diagnosis, a CT scan is acquired and the tumor as well as organs at risk are contoured on the image. Based on the contours, the radiation beams are chosen and optimized to maximize dose to the tumor while minimizing the dose to the surrounding normal tissue. The dose is calculated and the best plan is chosen to be delivered to the patient.

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**Alberto Grand** holds a Master's Degree in Computer and Electrical Engineering from Politecnico di Torino and the University of Illinois at Chicago, and a Ph.D. in Computer and Systems Engineering from Politecnico di Torino. His research interests are in the fields of data management systems and data mining, with a focus on large-scale data management, data integration, and biological and molecular data annotation. Since March 2013, he has been assistant coordinator in the development of an information system for the management, integration, and analysis of biological and molecular data at the Institute for Cancer Research and Treatment of Candiolo (IRCC), Italy.

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