CHAPTER 2

LITERATURE SURVEY

2.1 INTRODUCTION

To understand the existing techniques for breast cancer, research articles from IEEE transactions, Elsevier journals, ACM journals and also from International conference are studied. This study will form the groundwork for this thesis is given below.

2.2 STUDIES RELATED TO BREAST CANCER

Soft Computing techniques play an important role for decision in applications with imprecise and uncertain knowledge. The application of soft computing disciplines is rapidly emerging for the diagnosis and prognosis in medical applications. Among the various soft computing techniques, fuzzy expert system takes advantage of fuzzy set theory to provide computing with uncertain words. In a fuzzy expert system, knowledge is represented as a set of explicit linguistic rules. Diagnosis of breast cancer suffers from uncertainty and imprecision associated with imprecise input measures and incompleteness of knowledge of experts. However, there are several technology-oriented studies reported for breast cancer diagnosis, few studies has been reported for the breast cancer prognosis. Fatima. B., et al in [1] describe a fuzzy expert system for breast cancer prognosis to further support of the process of breast cancer diagnosis. This approach is capable enough to
capture ambiguous and imprecise information prevalent in characterization of breast cancer. For this, the paper utilizes a fuzzy reasoning model, which has high interpretability for interacting with human experts during prognosis process and consequently early diagnosis of the diseased. The performance results on real patients dataset reveal the accuracy of the system with an average 95% which shows the superiority of the system in the prognosis process compared to other related works.

Breast Cancer Diagnosis and Prognosis were two medical applications, which pose a great challenge to the researchers. The use of machine learning and data mining techniques have revolutionized the whole process of breast cancer Diagnosis and Prognosis. Breast Cancer Diagnosis distinguishes benign from malignant breast lumps and Breast Cancer Prognosis predicts when Breast Cancer are likely to recur in patients that had their cancers excised. Thus, these two problems were mainly in the scope of the classification problems. This study paper summarizes various reviews and technical articles on breast cancer diagnosis and prognosis. Shelly Gupta., et al in [2] describes an overview of the current research being carried out using the data mining techniques to enhance the breast cancer diagnosis and prognosis.

The objective of this research are to explore the implementation of a Bayesian Belief Network for an automated breast cancer detection
support tool. That is intuitive that Bayesian network is employed as one viable option for computer-aided detection by representing the relationship between diagnoses, physical findings, laboratory test results and imaging study findings [3]. This work brings important entities such as Radiologists, Image Processing Scientists, Data Base Specialists and Applied Mathematicians on a common platform. A brief background concerning causal networks, probability theory and Bayesian networks are given available computational tools and platforms were described. That is explained that, by exploiting conditional independencies entailed by influence chains, it is possible to represent a large instance in a Bayesian network using little space and that is often possible to perform probabilistic inference among the features in an acceptable amount of time. The next steps towards realizing a Bayesian Belief Network Implementation is described. Bayesian network has an unparallel advantage of being able to exploit the explicit structure of the domain model to derive a graphical representation for learning. The encoding of independencies in the network topology admits the design of efficient procedures for performing computations over the network. For the application of computer-aided detection in mammography, the researchers intend to design an interface between the project’s Bayesian network learning algorithm and the radiologists, so that the radiologists can have interaction with the system by labeling only a small number of informative images presented by the active learning algorithm.
In [6] Ashraf, M., et al. propose a new approach for breast cancer diagnosis using a combination of an Adaptive Network based Fuzzy Inference System (ANFIS) and the Information Gain method. In this approach, the ANFIS are to build an input-output mapping using both human knowledge and machine learning ability and the information gain method are to reduce the number of input features to ANFIS. An experimental result shows 98.23% accuracy which underlines the capability of the proposed algorithm.

Diagnosis of diseases are a well known problem in the medical field. Past research shows that medical database of disease can be trained by using various neural network models. Many medical problems face the problem of curse of dimensionality due to the excessively large number of input attributes. Breast cancer are one such problem. Vazirani., et al in [7] propose the use of modular neural network for effective diagnosis. In the proposed methodology four modules were made; each module gets half the problem attributes which is trained and tested by two neural network models, Back Propagation Neural Network (BPNN) and Radial Basis Function (RBFN). Integration are done using a probabilistic sum rule. The modular neural network gave an accuracy of 95.75% over training data and 98.22% over testing accuracy, which was experimentally determined to be better than monolithic neural networks.
In [9] Karabatak M., et al. proposes an automatic diagnosis system for detecting breast cancer based on Association Rules (AR) and Neural Network (NN). In this study, AR are used for reducing the dimension of breast cancer database and NN are used for intelligent classification. The proposed AR + NN system performance are compared with NN model. The dimension of input feature space are reduced from nine to four by using AR. In test stage, 3-fold cross validation method is applied to the Wisconsin breast cancer database to evaluate the proposed system performances. The correct classification rate of the proposed system are 95.6%. This research demonstrated that the AR can be used for reducing the dimension of feature space and proposed AR + NN model can be used to obtain fast automatic diagnostic systems for other diseases.

Even though cancer research has traditionally been clinical and biological in nature, in recent years data driven analytic studies have become a common complement. In medical domains where data and analytics driven research are successfully applied, new and novel research directions were identified to further advance the clinical and biological studies. Dursun Delen., et al in [10] used three popular data mining techniques (decision trees, artificial neural networks and support vector machines) along with the most commonly used statistical analysis technique logistic regression to develop prediction models for prostate cancer survivability. The data set contained
around 1, 20, 000 records and 77 variables. A k-fold cross-validation methodology was used in model building, evaluation and comparison. The results showed that support vector machines were the most accurate predictor (with a test set accuracy of 92.85%) for this domain, followed by artificial neural networks and decision trees.

An Artificial Neural Network (ANN) is an information-processing paradigm inspired by the way the densely interconnected, parallel structure of the mammalian brain processes information. The key element of the ANN paradigm are the novel structure of the information processing system. Learning in ANN typically occurs by example through training, or exposure to a set of input/output data where the training algorithm iteratively adjusts the connection weights (synapses). These connection weights store the knowledge necessary to solve specific problems. Sudhir D., et al. in [12] proposed, neural networks. Support Vector Machine method for diagnosis of breast cancer. SVMs can only be used for classification, not for function approximation. The Support Vector Machine (SVM) is implemented using the kernel Adatron algorithm. The kernel Adatron maps inputs to a high-dimensional feature space and then optimally separates data into their respective classes by isolating those inputs, which fall close to the data boundaries. The proposed neural network model holds promise for radiologists, surgeons and patients with information, which was previously available only through biopsy, thus substantially
reducing the number of unnecessary surgical procedures. For training and testing the neural network various databases available on the internet as well as gathered information from hospitals is used.

Statistical learning theory was introduced in the late 1960’s. Until the 1990’s it was a purely theoretical analysis of the problem of function estimation from a given collection of data. In the middle of the 1990’s new types of learning algorithms called support vector machines based on the developed theory are proposed. This made statistical learning theory not only a tool for the theoretical analysis but also a tool for creating practical algorithms for estimating multidimensional functions. This article presents a very general overview of statistical learning theory including both theoretical and algorithmic aspects of the theory. The goal of this overview are to demonstrate how the abstract learning theory established conditions for generalization which is more general than those discussed in classical statistical paradigms and how the understanding of these conditions inspired new algorithmic approaches to function estimation problems. A more detailed overview of the theory (without proofs) can be found in Vapnik (1995). In [14] Vapnik., one can find a detailed description of the theory (including proofs).

In this work a correspondence are derived between regularization operators used in regularization networks and support vector kernels. Smola A.J., et al. in [15] prove that the Green’s
Functions associated with regularization operators were suitable support vector kernels with equivalent regularization properties. Moreover, the paper provides an analysis of currently used support vector kernels in view of regularization theory and corresponding operators associated with the classes of both polynomial kernels and translation invariant kernels. The latter is also analyzed on periodical domains. As a by product we show that a large number of radial basis functions, namely conditionally positive definite functions, may be used as support vector kernels.

In [13] Minami Y., et al. investigated the risk of breast cancer development in women with Benign Breast Disease (BBD); 387 screen-detected BBD women and 1,489 normal women, taken from participants in the breast cancer screening program during 1978-1986, are followed through 1991. While 2,811 person-years in the BBD group and 11,018 person-years in the normal group are accumulated, 5 women in the BBD group and 6 women in the normal group developed breast cancer. Using the Mantel-Haenszel method, Relative Risks (RR) are estimated for all women with BBD and women in some BBD types. Significantly elevated risk of breast cancer is observed in all women with BBD (RR = 3.26, 95% Confidence Interval (CI) 1.08-9.83). Women with proliferative BBD were at high risk of breast cancer (RR = 8.48, 95% CI 2.99-24.10), but no increased risk is observed for women with non-proliferative BBD (RR = 0.93, 95% CI
0.11-7.66). These results were consistent with those in high-risk countries for breast cancer. In the management of women with BBD, histopathological diagnosis of the breast lesion are essential and women with proliferative BBD should be followed up carefully.

Breast cancers are the most frequently diagnosed cancer and the leading cause of cancer death in females worldwide. In [14] Helmrich SP., et al. identified the extent to which selected demographic, hormonal and reproductive factors influence the cause of breast cancer using the logistic regression technique to determine the risk of getting the disease. Two thousand three hundred and ninety seven (2397) women were sampled for the study from the Korle-bu Teaching Hospital, of which 1022 (42.64%) are diagnosed with breast cancer between the periods January 2002 to December 2008. Breast feeding, late menarche, contraceptive usage, and time interval between age at menarche and age at menopause all decreased the risk of breast cancer development (OR = 2.306 <0.0001). Later age at menopause on the other hand increased the risk of breast cancer development. It is recommended that governmental or nongovernmental organizations improve on health education/campaigns about breast cancer to create awareness and reduce mortality.

A number of genes were known to be involved in inherited susceptibility to breast and/or ovarian cancer. In the context of high-risk families the most important genes were BRCA1 on chromosome
17q, which are associated with a high penetrance of both breast and ovarian cancer and BRCA2 on chromosome 13q, which causes a high risk of breast cancer but a lower risk of ovarian cancer. Other high-risk cancer genes that confer increased risks of breast or ovarian cancer in addition to other cancers include the hereditary non-polyposis colorectal cancer genes and the TP53 gene, which causes breast cancer as part of the Li-Fraumeni syndrome. The predisposing mutations in these genes were relatively rare in the population. More common genes which is associated with an increased, but lower risk of breast cancer is the ataxiatelangiectasia gene and the HRAS1 gene. In [18] [20] Ford D., et al reviews recent progress in mapping and cloning of these susceptibility genes and provides estimates of the cancer risks associated with each gene and the frequency of predisposing mutations.

Previous report has indicated that Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) for Cyto Keratin 19 (CK-19) may be useful in the management of patients with breast cancer. However, the specificity of this technique is low, principally because of a high rate of false-positive results. To improve the specificity of this assay, they developed a quantitative RT-PCR methodology that enables an estimate to be made of the number of CK-19 transcripts in blood and bone marrow samples [38]. They examined 45 peripheral-blood samples and 30 bone marrow samples from patients with a variety of
non neoplastic conditions using nested RT-PCR for CK-19. They also examined bone marrow and peripheral-blood samples from 23 patients with primary breast cancer and peripheral-blood samples from 37 patients with metastatic breast cancer. The number of CK-19 transcripts was estimated in positive specimens by competitive PCR and normalized to the number of ABL transcripts as an internal control for the quality and quantity of cDNA. RT-PCR results are compared with the number of CK-19-positive cells detected by immunocytochemistry. Analysis of samples from patients without cancer enabled them to define an upper limit for the background ratio of CK-19 to ABL transcripts (1:1,000 for blood samples and 1:1,600 for bone marrow samples). Using these figures as cut-off points, elevated CK-19: ABL ratios are detected in peripheral-blood samples of 20 of 37 (54%) patients with metastatic breast cancer and in bone marrow samples of 14 of 23 (61%) patients with primary breast cancer. Only three of 23 (13%) primary breast cancer peripheral-blood samples and none of the control samples are positive by these criteria. Only two of 23 patients (9%) with primary breast cancer showed immunocytochemically detectable cells in the blood; 10 of 23 (43%) showed immunocytochemically detectable cells in the bone marrow. Of 36 patients with metastatic breast cancer, eight (22%) showed positive events. Quantitative RT-PCR for CK-19 detects a percentage of patients with breast cancer and may enable the progression or regression of the disease to be monitored.
One thousand, four hundred and forty-six patients with carcinoma of the breast treated with Halsted mastectomy (167), Patey mastectomy (732), and conservative surgery with axillary dissection, either at the same time (340), or separately (207), were evaluated with regard to the number and distribution of axillary lymph nodes [39]. A total of 29,378 were removed and examined, on average 20.3 per patient. The average number of nodes was 13.5 at the first level, 4.5 at the second and 2.3 at the third. The same number of nodes were removed in patients treated with extensive surgery, such as Halsted mastectomy and limited surgery such as lumpectomy and in independent axillary dissection. In 839 cases metastases were found in the axilla. The average number of involved nodes was 6.4. Out of 839 patients, the first level was the site of metastases in 828, the second level in 364 and the third in 187. When a single lymph node was involved, it was nearly always at the first level. In only 11 cases, were the second and/or third levels invaded without metastases at the first level. Therefore, the percentage of cases with skipping metastases was very low (1.3%). It appears from the present data that the spread of breast cancer to the axilla follows a regular pattern. The first level is invaded first, while in most cases, the second and third levels are involved only when the first is substantially affected.

Breast cancers are one of the leading cancers for women in developed countries including India. That is the second most common
cause of cancer death in women. The high incidence of breast cancer in women have increased significantly in the last years. In [41] Sudhir D., et al. discussed various data mining approaches that has been utilized for breast cancer diagnosis and prognosis. Breast Cancer Diagnosis distinguishes benign from malignant breast lumps and Breast Cancer Prognosis predicts when Breast Cancer are to recur in patients that had their cancers excised. This study summarizes various review and technical articles on breast cancer diagnosis and prognosis. Also they focused on current research being carried out using the data mining techniques to enhance the breast cancer diagnosis and prognosis.

In [46] Verma, B., et al. propose the creation of an ensemble neural network by incorporating a k-means classifier. This technique are designed to improve the classification accuracy of a multi-layer perceptron style network for mass classification of digital mammograms. The proposed techniques have been tested on a benchmark database and the result has been contrasted with current research. The experimental results demonstrate that the accuracy of the proposed technique is comparable with the existing systems.

There are two competing methods for improving the accuracy of a radiologist interpreting screening mammograms: Computer Aids Diagnosis (CAD) or independent second reading [47]. Bibliographic databases are searched for clinical trials. Meta-analyses estimated
impacts of CAD and double reading on odds ratios for cancer detection and recall rates. Sub-group analyses considered double reading with arbitration. Ten studies compared single reading with CAD to single reading. Seventeen compared double to single reading. Double reading increases cancer detection and recall rates. Double reading with arbitration increases detection rate (Confidence Interval (CI): 1.02, 1.15) and decreases recall rate (CI: 0.92, 0.96). CAD does not have a significant effect on cancer detection rate (CI: 0.96, 1.13) and increases recall rate (95% CI: 1.09, 1.12). However, there are considerable heterogeneity in the impact on recall rate in both sets of studies. The evidence that double reading with arbitration enhances screening is stronger than that for single reading with CAD.

An intelligent computer-aided diagnostics system may be developed to assist the radiologists to recognize the masses/lesions appearing in breast in different groups of benignancy/malignancy. In present work we have attempted to develop a computer assisted treatment planning system implementing Genetic algorithm-based Neuro-fuzzy approaches [48]. The boundary based features of the tumor lesions appearing in breast has been extracted for classification. The shape features, represented by Fourier Descriptors, introduce a large number of feature vectors. Thus, to classify different boundaries, a standard classifier needs a large number of inputs and simultaneously to train the classifier a large number of training cycles
were required. This may invite the problem of overlearning, followed by chance of misclassification. In the proposed methodology, Genetic Algorithm (GA) have been used for searching of significant input feature vectors. Finally adaptive neuro fuzzy-based classifier have been introduced for classification of tumor masses in breast.

The application of Artificial Neural Networks (ANNs) for prognostic and diagnostic classification in clinical medicine have become very popular. In particular, feed-forward neural network has been used extensively, often accompanied by exaggerated statements of their potential. In [50] G. Schwarzer., et al describe essentials of feed-forward neural networks and their statistical counterparts that are, logistic regression models were reviewed. They point out that the uncritical use of ANNs may lead to serious problems, such as the fitting of implausible functions to describe the probability of class membership and the underestimation of misclassification probabilities. In applications of ANNs to survival data, further difficulties arise. Finally, the results of a search in the medical literature from 1991 to 1995 on applications of ANNs in oncology and some important common mistakes were reported. It is concluded that there are no evidence so far that application of ANNs represents real progress in the field of diagnosis and prognosis in oncology.

ANN is nonlinear regression computational devices that has been used for over 45 years in classification and survival prediction in
several biomedical systems, including colon cancer. In [51] F. E. Ahmed., et al. described three-layer free forward artificial neural networks with backpropagation error, which are widely used in biomedical fields, and a methodological approach to its application for cancer research, as exemplified by colon cancer. Review of the literature shows that applications of these networks has improved the accuracy of colon cancer classification and survival prediction when compared to other statistical or clinicopathological methods. Accuracy, however, must be exercised when designing, using and publishing biomedical results employing machine-learning devices such as ANNs in worldwide literature in order to enhance confidence in the quality and reliability of reported data.

Breast cancer are the second leading cause of cancer deaths in women worldwide and occurs in nearly one out of eight women. In [52] Janghel R., et al developed a hybrid intelligent system for diagnosis, prognosis and prediction for breast cancer using SANE (Symbiotic, Adaptive Neuro-evolution) and compared with ensemble ANN, modular neural network, Fixed architecture Evolutionary Neural Network (F-ENN) and Variable architecture Evolutionary Neural Network (V-ENN). While the monolithic neural and fuzzy system has been extensively used for diagnosis, the individual limitations of the various models put a great threshold on prediction accuracies, which may be overcome with the use of SANE. The SANE system evolves a population of
neurons that cooperate to form a functioning neural network. Breast cancer database from the University of Wisconsin available at UCI Machine Learning Repository are used for conducting experimental work.

Breast cancer continues to be a significant public health problem in the world. Approximately, 1, 82, 000 new cases of breast cancer were diagnosed and 46,000 women die of breast cancer each year in the United States. Even more disturbing are the fact that one out of eight women in US will develop breast cancer at some point during her lifetime [54]. Primary prevention seems impossible since the causes of this disease still remain unknown. Early detection are the key to improving breast cancer prognosis. Mammography are one of the reliable methods for early detection of breast carcinomas. There is some limitations of human observers and it is difficult for radiologists to provide both accurate and uniform evaluation for the enormous number of mammograms generated in widespread screening. The presence of Micro Calcification Clusters (MCCs) is an important sign for the detection of early breast carcinoma. An early sign of 30–50% of breast cancer detected mammographically are the appearance of clusters of fine, granular microcalcification and 60–80% of breast carcinomas which reveal MCCs upon histological examinations. The high correlation between the appearance of the microcalcification clusters and the diseases shows that the CAD
(Computer Aided Diagnosis) systems for automated detection/classification of MCCs will be very useful and helpful for breast cancer control. In [55] H.D. Cheng., et al summarize and compare the methods used in various stages of the computer-aided detection systems. In particular, the enhancement and segmentation algorithms, mammographic features, classifiers and their performances were studied and compared. Remaining challenges and future research direction is also discussed.

In [56] Dehghan, F., et al. present a Computer-Aided Diagnosis (CAD) system for automatic detection of clustered Micro Calcifications (MCs) in digitized mammograms. The proposed system consists of two main steps. First, potential microcalcification pixels in the mammograms were segmented out by using 4 mixed features consisting of two wavelet features and two gray level statistical features and then the potential microcalcification pixels were labeled into potential individual microcalcification objects by their spatial connectivity. Second, MCs were detected by using a set of 17 features extracted from the potential individual microcalcification objects. The classifier which are used in the first step are the multilayer feed forward Neural Network (NN) classifiers but for the second step they used three different classifier which are multilayer feed forward Neural Network (NN), SVM with polynomial kernel and SVM with Gaussian RBF kernel and the result of each classifier is obtained separately. The
method was applied to a database of 40 mammograms (Nijmegen database) containing 105 clusters of MCs. A Free-Response Operating Characteristics (FROC) curve are used to evaluate the performance of CAD system with each classifier. Results show that the proposed system gives quite satisfactory detection performance. In particular, 89.55% mean true positive detection rate are achieved at the cost of 0.782, 1 and 0.95 false positive per image for neural network, Support Vector Machine (SVM) with polynomial kernel and SVM with Gaussian RBF kernel classifiers, respectively.

Breast cancer are the most frequently diagnosed malignancy and the second leading cause of mortality in women. In the last decade, ultrasound along with digital mammography have come to be regarded as the gold standard for breast cancer diagnosis. Automatically detecting tumors and extracting lesion boundaries in ultrasound images are difficult due to their specular nature and the variance in shape and appearance of sonographic lesions. Past work on automated ultrasonic breast lesion segmentation have not addressed important issues such as shadowing artifacts or dealing with similar tumor like structures in the sonogram. Algorithms that claim to automatically classify ultrasonic breast lesions rely on manual delineation of the tumor boundaries. In [57] A. Madabhushi., et al. present a novel technique to automatically find lesion margins in ultrasound images, by combining intensity and texture with empirical
domain specific knowledge along with directional gradient and a deformable shape-based model. The images were first filtered to remove speckle noise and then contrast enhanced to emphasize the tumor regions. For the first time, a mathematical formulation of the empirical rules used by radiologists in detecting ultrasonic breast lesions, popularly known as the "Stavros Criteria" are presented in this paper. They have applied this formulation to automatically determine a seed point within the image. Probabilistic classification of image pixels based on intensity and texture are followed by region growing using the automatically determined seed point to obtain an initial segmentation of the lesion. Boundary points were found on the directional gradient of the image. Outlier is removed by a process of recursive refinement. These boundary points were then supplied as an initial estimate to a deformable model. Incorporating empirical domain specific knowledge along with low and high-level knowledge makes it possible to avoid shadowing artifacts and lowers the chance of confusing similar tumor like structures for the lesion. The system is validated on a database of breast sonograms for 42 patients. The average mean boundary error between manual and automated segmentation was 6.6 pixels and the normalized true positive area overlap was 75.1%. The algorithm was found to be robust to 1) variations in system parameters, 2) number of training samples used and 3) the position of the seed point within the tumor. Running time
for segmenting a single sonogram was 18 s on a 1.8-GHz Pentium machine.

Ultra Sound are the useful diagnostic tool to distinguish benign from malignant masses of the breast. It is a very convenient and safe diagnostic method. However, there are considerable overlap benignancy and malignancy in ultrasonic images and interpretation are subjective. A high performance breast tumors Computer-Aided Diagnosis (CAD) system can provide an accurate and reliable diagnostic second opinion for physicians to distinguish benign breast lesions from malignant ones. The potential of sonographic texture analysis to improve breast tumor classifications have been demonstrated. However, the texture analysis is system-dependent. The disadvantages of these systems which use texture analysis to classify tumors were they usually perform well only in one specific ultrasound system. While Morphological based US diagnosis of breast tumor will take the advantage of nearly independent to either the setting of US system and different US machines. In this study [58], the tumors were segmented using the newly developed level set method at first and then six morphologic features were used to distinguish the benign and malignant cases. The Support Vector Machine are used to classify the tumors. There were 210 ultrasonic images of pathologically proven benign breast tumors from 120 patients and carcinomas from 90 patients in the ultrasonic image database. The database contains only
one image from each patient. The ultrasonic images were captured at the largest diameter of the tumor. The images were collected consecutively from August 1, 1999 to May 31, 2000 the patient’s ages ranged from 18 to 64 years. Sonography are performed using an ATL HDI 3000 system with a L10-5 small part transducer. In the experiment, the accuracy of SVM with shape information for classifying malignancies are 90.95% (191/210), the sensitivity are 88.89% (80/90), the specificity are 92.5% (111/120), the positive predictive value are 89.89% (80/89) and the negative predictive value are 91.74% (111/121).

In [59] Maryellen L., et al. present a computationally efficient segmentation algorithm for breast masses on sonography that are based on maximizing a utility function over partition margins defined through gray-value thresholding of a preprocessed image. The performance of the segmentation algorithm are evaluated on a database of 400 cases in two ways. Of the 400 cases, 124 are complex cysts, 182 are benign solid lesions and 94 were malignant lesions. In the first evaluation, the computer-delineated margins are compared to manually delineated margins. At an overlap threshold of 0.40, the segmentation algorithm correctly delineated 94% of the lesions. In the second evaluation, the performance of our computer-aided diagnosis method on the computer-delineated margins is compared to the performance of their method on the manually delineated margins.
Round robin evaluation yielded Az values of 0.90 and 0.87 on the manually delineated margins and the computer-delineated margins, respectively, in the task of distinguishing between malignant and nonmalignant lesions.

In [60] N. Patrick., *et al.* investigated the classification of Regions of Interest (ROI’s) on mammograms as either mass or normal tissue using a Convolution Neural Network (CNN). A CNN are a backpropagation neural network with two-dimensional (2-D) weight kernels that operate on images. A generalized, fast and stable implementation of the CNN is developed. The input images to the CNN are obtained from the ROI’s using two techniques. The first technique employed averaging and subsampling. The second technique employed texture feature extraction methods applied to small subregions inside the ROI. Features computed over different subregions are arranged as texture images, which are subsequently used as CNN inputs. The effects of CNN architecture and texture feature parameters on classification accuracy are studied. Receiver Operating Characteristic (ROC) methodology is used to evaluate the classification accuracy. A data set consisting of 168 ROIs containing biopsy-proven masses and 504 ROI’s containing normal breast tissue is extracted from 168 mammograms by radiologists experienced in mammography. This data set is used for training and testing the CNN. With the best combination of CNN architecture and texture feature parameters, the
area under the test ROC curve reached 0.87, which corresponded to a true-positive fraction of 90% at a false positive fraction of 31%. The author’s results demonstrate the feasibility of using a CNN for classification of masses and normal tissue on mammograms.

In [63] Biennial breast cancer screening for women ages 50-69 years are recommended by the World Health Organization. It have been claimed that the cumulative risk of a false-positive recall are a significant disadvantage in breast cancer screening programs. The primary objective of this study is to estimate the cumulative risk of a false-positive recall during a screening period of 20 years in women ages 50-51 years who were screened biennially in a population-based screening program. A secondary objective is to estimate the cumulative risk of undergoing fine-needle aspiration cytology, core needle biopsy, and open biopsy with benign morphology in the same group of women. The Norwegian Breast Cancer Screening Program invites all women aged 50-69 years who reside in the country to a 2-view mammography biennially. A nationwide database that covers all of the invited women includes individual information about all screening activity. Results from three screening rounds in four countries are the basis for this study. False-positive recalls due to abnormal mammograms among 83,416 women who participated all the 3 screening rounds are the basis for the estimation. It is calculated that women aged 50-51 years who participate in biennial screening run a cumulative risk of 20.8%
for a false-positive recall during a screening period of 2 decades. The cumulative risk of undergoing fine-needle aspiration cytology is estimated at 3.9% and the risk of undergoing core needle biopsy or open biopsy with benign morphology is 1.5% and 0.9%, respectively. False-positive recalls were disadvantages in a breast cancer screening programs, but the cumulative risk seemed to be acceptable in the Norwegian Breast Cancer Screening Program. That is important to communicate the existence and extent of this risk to the target group.

In [66] single micrometastatic tumor cells encased in mesenchymal tissues, such as Bone Marrow (BM), were regarded as suitable targets for adjuvant immunotherapy since they were easily accessible for both immunoglobulins and immune effector cells. However, the antigen profile of such cells, to which antibody therapy might be targeted, cannot be deduced from the antigen pattern of the primary tumor. To evaluate the antigen profile of disseminated cells found in BM aspirates from 20 breast cancer patients, they applied a quantitative immuno-cytochemical double-marker assay and typed for 4 common tumor-associated cell-surface antigens (c-erbB-2, CO17-1A, MUC-1, LewisY). Individual breast cancer cells are identified by F(ab) fragments of the pan-CytoKeratin (CK) Monoclonal Antibody (MAb) A45-B/B3, directly conjugated with alkaline phosphatase, which identified cancer cells as sensitively as the standard APAAP procedure (r = 0.998; p < 0.0001). CK+ cells co-expressed c-erbB-2, CO17-1A,
MUC-1 and LewisY in 87%, 78% and 79% of patients, respectively; however, the frequency of double-positive cells per sample varied considerably. The mean percentage of double-positive cells per total number of CK+ cells is 41% for c-erbB-2 (range 0-92%), 47% for CO17-1A (range 0-75%), 49% for MUC-1 (range 0-67%) and 32% for LewisY (range 0-59%). In 14 of these patients, they used an antibody cocktail to type CK+ cells for the combined expression of all 4 antigens. The antibody cocktail labeled significantly more CK+ cells than each of the single MAbs alone, resulting in a mean of 71% double-positive tumor cells (34-100%). They concluded that expression of tumor-associated cell-surface antigens on micrometastatic cancer cells in BM are heterogeneous, which may limit the efficacy of monovalent immunotherapeutic strategies directed against only one particular antigen. Thus, defining target antigens expressed by the actual target cells emerges as a crucial first step in selecting appropriate therapeutic targets.

In [72] tumor cells can reach every anatomic district, organ and tissue through the peripheral blood circulation. Tumor cell shedding is considered an early event in the multi-phase process of metastasis and the possibility of detecting tumor cells in the bloodstream and/or bone marrow before clinical evidence of distant metastases needs to be explored. The use of new sophisticated diagnostic and investigative techniques have boosted the study of tumor cell contamination of bone
marrow and peripheral blood. Molecular techniques, such as reverse-transcriptase polymerase chain reaction, may be useful tools to reach this target, but, today immunocytochemistry is still considered the gold standard to assess new techniques to detect isolated tumor cells in hematopoietic tissue. Little is known about the biology of isolated tumor cells in the peripheral blood or bone marrow. Two crucial points need to be evaluated: the identification of specific marker of breast cancer cells with clonogenic potential and their biologic properties and the prognostic impact of detection of the isolated tumor cells in the bone marrow or peripheral blood stem cell collections.

In [75] although Prostate Specific Antigen are the most valuable tumor marker for the diagnosis and management of prostate carcinoma, it is widely accepted that PSA are not prostate specific. Numerous studies has shown that PSA is present in some female hormonally regulated tissues, principally the breast and its secretions. In this review, they summarized the findings of PSA in the breast, and focused on its potential for clinical applications in breast disease. PSA are produced by the majority of breast tumors and are a favorable indicator of prognosis in breast cancer. Low levels of PSA is released into the female circulation and while the level of serum PSA are elevated in both benign and malignant breast disease, the molecular form of circulating PSA differs between women with and without breast cancer. These findings indicate that PSA may has potential diagnostic
utility in breast cancer. PSA may also have a clinical application in benign breast disease, as both the level and molecular form of PSA differ between Type I and II breast cysts. High levels of PSA has been reported in Nipple Aspirate Fluid (NAF) and recent studies has shown that the concentration of PSA in NAF are inversely related to breast cancer risk, indicating that NAF. PSA may represent a clinical tool for breast cancer risk assessment. Thus, PSA represents a marker with numerous potential clinical applications as a diagnostic and/or prognostic tool in breast disease.

In [77] Jenssen TK., et al. analyzed associations between gene expression in breast cancer and patient survival for 8024 genes from a previously published microarray data set. Analysis of survival, by using the logrank test, is performed automatically for each gene. After correcting for multiple testing, they identified 95 genes whose expression is significantly associated with patient survival. The independent prognostic value of the genes ranking the highest in univariate analysis, together with clinical parameters was assessed by Cox multivariate regression analysis. The P-values from these logrank tests were also mapped to chromosomal positions and compared with previously reported amplicon regions. They used PubGene web tools to identify groups of genes that has co-occurred in the literature and whose expression patterns are associated with survival. Our analyses demonstrate the comprehensiveness of the microarray technology with
respect to measuring gene expression and indicate that the technology may be used to screen for potential clinical markers.

In [78] genetic changes underlie tumor progression and may lead to cancer-specific expression of critical genes. Over 1100 publications has described the use of Comparative Genomic Hybridization (CGH) to analyze the pattern of copy number alterations in cancer, but very few of the genes affected were known. Here, they performed high-resolution CGH analysis on cDNA microarrays in breast cancer and directly compared copy number and mRNA expression levels of 13,824 genes to quantitate the impact of genomic changes on gene expression. They identified and mapped the boundaries of 24 independent amplicons, ranging in size from 0.2 to 12 Mb. Throughout the genome, both high and low-level copy number changes has a substantial impact on gene expression, with 44% of the highly amplified genes showing over expression and 10.5% of the highly over expressed genes being amplified [74]. Statistical analysis with random permutation tests identified 270 genes whose expression levels across 14 samples are systematically attributable to gene amplification. These included most previously described amplified genes in breast cancer and many novel targets for genomic alterations, including the HOXB7 gene, the presence of which in a novel amplicon at 17q21.3 is validated in 10.2% of primary breast cancers and associated with poor patient prognosis. In conclusion, CGH on cDNA
microarrays revealed hundreds of novel genes whose overexpression are attributable to gene amplification. These genes may provide insights to the clonal evolution and progression of breast cancer and highlight promising therapeutic targets.

In [78] the most appropriate systemic therapy for a population of patients with breast cancer are determined from clinical trial data. However, the heterogeneity of breast cancer are such that within a population individual patients derive variable benefit. There are therefore a need for predictive molecular factors in order that treatment can be individualised. This review describes the roles of HER-2, Epidermal Growth Factor Receptor (EGFR), Estrogen Receptor (ER)/Progesterone Receptor (PgR), Ki67, Bcl-2, p53 and gene expression profiling in predicting responses to endocrine, cytotoxic and biological therapies. ER and PgR remain the only well-established predictive markers of responses to endocrine therapy, although HER-2/neu has an emerging role in this area and in choice of adjuvant chemotherapy [73]. There is considerable methodological difficulties in identifying useful predictive factors but on the basis of current evidence other biomarkers add little additional information. The development of targeted therapies means that the molecular targets themselves may become useful predictive factors for directing use of these therapies. HER-2 already have an established role in this area, but the role of EGFR requires further elaboration. The use of DNA
microarrays to assess gene expression profiles may revolutionise our ability to predict responses to therapy [76] [79].

This study is performed to evaluate the potential of specific mRNA markers to detect micrometastases by Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR) and Southern blot analysis of Sentinel Lymph Nodes (SLNs) and blood from patients with breast cancer [37]. They assessed the specificity of Carcino Embryonic Antigen (CEA), Cyto Keratin-19 (CK-19), CK-20, Gastrointestinal Tumor-Associated Antigen-733.2 (GA733.2), and mucin-1 (MUC-1) in the blood of healthy donors (n = 13) and lymph nodes from patients without cancer (n = 3) by RT-PCR assay [80]. The sensitivity of the RT-PCR assay for the target mRNA markers is assessed in breast cancer cell lines (n = 4), primary breast tumors (n = 8), and the frozen sections of SNs (n = 22) from 22 patients with American Joint Committee on Cancer (AJCC) stages I to IIIA breast cancer. CK-20 is the only mRNA marker not detected in lymph nodes or blood from patients without cancer. Both the blood and lymph nodes from patients without cancer expressed CEA, CK-19, GA733.2, and MUC-1 mRNA. All four breast cancer cell lines and six of eight primary breast tumors expressed all five mRNA markers. Expression of mRNA by the RT-PCR assay in the frozen-section SNs (n = 12) without metastases by conventional histopathology ranged from 8% (CK-20) to 92% (GA733.2). Detection of RT-PCR cDNA products in frozen-section SNs
is increased with Southern blot analysis compared with Ethidium Bromide (EtBr) Gel Electrophoresis for all mRNA markers except CK-19. CEA, CK-19, GA733.2, and MUC-1 show no diagnostic value as mRNA markers for the detection of micrometastases by the RT-PCR assay because they were expressed in the blood and lymph nodes of patients without cancer. Further studies were needed to assess the sensitivity of CK-20 to detect micrometastases by the RT-PCR assay in the blood and frozen-section SNs of patients with breast cancer.

This study is undertaken to assess a multiple-marker RT-PCR and Southern blot assay for detection of metastases in frozen sections of sentinel lymph nodes from breast cancer patients. Sentinel lymphadenectomy is performed in 41 AJCC (American Joint Committee on Cancer) stage I-IIIA breast cancer patients and 57 Sentinel Nodes (SNs) are excised. The SN, which are the first node in the lymphatic basin to receive metastases from the primary tumor, is identified using isosulfan blue dye. Hematoxylin and Eosin (H&E), Immuno-HistoChemistry (IHC) and RT-PCR are performed on adjacent sections of the SN. Six consecutive 12-microm frozen sections of each SN are obtained for the RT-PCR assay to determine expression of mRNA tumor markers C-Met, beta1 --> 4GalNAc-T and P97. [81] Metastatic breast cancer is detected by H&E in 10 of 57 (18%) SNs and by IHC in an additional 7 (12%). Only 1 of 17 (6%) SNs with metastases did not express any of the 3 tumor mRNA markers. C-Met,
beta1 --> 4GalNAc-T and P97 tumor mRNA markers are expressed in 31 (78%), 21 (53%) and 25 (63%) of 40 SNs without metastases respectively. At least 2 mRNA tumor markers are expressed in 25/40 (63%) histo-pathologically tumor-free SNs, whereas all 3 mRNA tumor markers are expressed in 17/40 (43%) SNs. Expression of all 3 mRNA tumor markers in a SN is significantly higher in patients with a family history of breast cancer (p = 0.05), prior history of breast cancer (p < 0.05), infiltrating lobular carcinoma (p = 0.06), estrogen receptor-negative (p = 0.04) tumor or a higher Bloom Richardson score (p = 0.04). The multiple-marker RT-PCR and Southern blot assay improves the detection of occult metastases in the SN when compared to conventional H&E and IHC analysis. Expression of all 3 tumor mRNA markers in the SN correlated with poor prognostic clinico-pathologic factors compared to expression of 0 to 2 markers.

Detection of micrometastases in the regional tumor-draining lymph nodes are critical for accurate staging and prognosis in melanoma patients. They hypothesized that a Multiple-Mrna Marker (MM) Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) assay would improve the detection of occult metastases in the Sentinel Node (SN), compared with Hematoxylin and Eosin (H&E) staining and Immune Histo Chemistry (IHC), and that MM expression are predictive of disease relapse [82]. Seventy-two consecutive patients with clinical early-stage melanoma underwent sentinel lymphadenectomy. Their
SNs are serially sectioned and assessed for MAGE-3, MART-1, and tyrosinase mRNA expression by RT-PCR, in parallel with H&E staining and IHC, for melanoma metastases. MM expression in the SNs is correlated with H&E and IHC assay results, standard prognostic factors, and disease-free survival. In 17 patients with H&E and/or IHC-positive SNs, 16 (94%) expressed two or more mRNA markers. Twenty (36%) of 55 patients with histopathologically negative SNs expressed two or more mRNA markers. By multivariate analysis, patients at increased risk of metastases to the SN had thicker lesions (P = .03), are 60 years of age or younger (P < .05), and/or are MM-positive (P < .001). Patients with histopathologically melanoma-free SNs who are MM-positive, compared with those who are positive for one or fewer mRNA markers are at increased risk of recurrence (P = .02). Patients who are MM-positive with histopathologically proven metastases in the SN are at greatest risk of disease relapse (P = .01). H&E staining and IHC underestimate the true incidence of melanoma metastases. MM expression in the SN more accurately reflects melanoma micrometastases and are also a more powerful predictor of disease relapse than were H&E staining and IHC alone.

In [83] Fleming TP., et al describe a novel cDNA isolated from a primary human breast adenocarcinoma and differentially expressed in several breast carcinoma cell lines. The protein encoded by this cDNA, which we had named mammaglobin, are homologous to a family of
secreted proteins that includes rat prostatic steroid-binding protein subunit C3, human Clara cell 10-kilodalton protein and rabbit uteroglobin. Expression of the mammaglobin gene are restricted to the adult mammary gland. More significantly, in an analysis of 35 breast tumor biopsies, mammaglobin mRNA levels are increased at least 10-fold relative to normal breast tissue in 23% of cases. The breast-specific expression of this potentially secreted protein and its frequent overexpression in primary human breast tumors suggest that mammaglobin may be a novel marker for the management of breast cancer.

In [84] Sentinel Lymph Node Biopsy (SLNB) are evaluated in breast cancer patients to improve detection of metastases and to guide therapy with minimal morbidity. The use of reverse transcription-PCR analysis to increase detection of tumor cells in SLN of breast cancer patients are hampered by the lack of specific markers. In this study, seven markers are evaluated by reverse transcription-PCR for expression in human Breast adeno Carcinoma lines (BrCa) and in normal nodes from non-cancer patients. Two markers yielded exceptional results; mammaglobin and carcinoembryonic antigen transcripts are detected in 100 and 71% BrCa, respectively, and are absent from all normal lymph nodes. These markers will be used as components of a multimarker panel to evaluate sentinel nodes in an on-going, multicenter clinical trial.
In [85] Real-time RT-PCR are the relatively new technology that uses an online fluorescence detection system to determine gene expression levels. It have the potential to significantly improve detection of breast cancer metastasis by virtue of its exquisite sensitivity, high throughput capacity and quantitative readout system. To assess the utility of this technology in breast cancer staging, they determined the relative expression levels of 12 cancer-associated genes (mam, PIP, mamB, CEA, CK19, VEGF, erbB2, muc1, c-myc, p97, vim and Ki67) in 51 negative-control normal lymph nodes and in 17 histopathology-positive ALNs. We then performed a Receiver Operating Characteristic (ROC) curve analysis to determine the sensitivity and specificity levels of each gene. Areas under the ROC curve indicated that the most accurate diagnostic markers are mam (99.6%), PIP (93.3%), CK19 (91.0%), mamB (87.9%), muc1 (81.5%) and CEA (79.4.0%). mam is over expressed in 16 of 17 lymph nodes known to contain metastatic breast cancer at levels ranging from 22 to 2.8 x 10(5)-fold above normal mean expression, whereas PIP is over expressed from 30 to 2.2 x 10(6)-fold above normal in 13 lymph nodes.

Real-time RT-PCR analysis of pathology-negative LN from breast cancer patients revealed evidence of over expression of PIP (6 nodes), mam (3 nodes) and CEA (1 node) in 8 of 21 nodes (38%). Our results provide evidence that mam, PIP, CK19, mamB, muc1 and CEA can be applied as a panel for the detection of metastatic and occult micrometastatic disease [74].
In [86] Prostate-Specific Antigen (PSA) is recently found in 30% of female breast tumours. In this study they examined if PSA circulates in the blood of breast cancer patients and if serum PSA have any clinical application. They compared serum PSA levels between women with and without breast cancer, between woman with PSA-positive and PSA-negative breast cancer and between woman with breast cancer before and after surgical removal of the tumour. They found that for women > or = 50 years, there are no difference in serum PSA between normal or breast cancer patients. We also could not find any difference in presurgical or postsurgical serum PSA between women who has PSA-positive or PSA-negative breast cancer. We found no correlation between PSA concentrations in matched presurgical and postsurgical sera, between presurgical sera and tumour cytosols and between post-surgical sera and tumour cytosols. High-performance liquid chromatography have shown that PSA in normal male serum consists mostly of PSA bound to alpha 1-antichymotrypsin (molecular weight approximately 1,00,000) and PSA in breast tumours and presurgical and post-surgical serum consists mostly of free PSA (molecular weight approximately 33,000). These data suggest that female serum PSA are not associated with tumour PSA levels. We speculate that most of the circulating PSA in women originates from the normal breast. It appears that serum PSA in women does not have potential for breast cancer diagnosis or monitoring but our previous
data is consistent with the view that tumour PSA concentration are a favourable prognostic indicator in women with breast cancer.

Metastasis of malignant breast cells are in part mediated through degradation of the extra-cellular matrix by proteolysis, enabling malignant cells to migrate through the surrounding stroma. Heparanase-1 (HPR1) are an endoglycosidase that specifically degrades the Heparan Sulfate (HS) moiety of proteoglycans, a component of the extracellular matrix and basement membrane [87]. Fifty-one primary breast tumors, 13 lymph node metastases, 4 ductal carcinoma in situ, 7 benign and 5 normal specimens are examined for HPR1 expression using immunohistochemical staining. The functional role of HPR1 expression is determined by examining HS deposition using immunofluorescence staining. Sixteen of 30 breast carcinomas (53%) with sentinel node metastasis expressed HPR1. In contrast, only 5 of 21 nonmetastatic primary breast carcinomas (23%) are HPR1 positive. Eighteen of 30 breast carcinomas between 1 and 5 cm expressed HPR1, compared with 3 of 21 HPR1-positive specimens in tumors < or =1 cm. Statistical analysis revealed that HPR1 expression is associated with breast tumor metastases (P =.04) and primary tumors between 1 and 5 cm (P =.002). Ninety percent of HPR1-positive tumors lacked HS deposition, suggesting an inverse correlation between HPR1 expression and HS deposition. HPR1 expression correlates with the lack of HS deposition and with the metastatic potential of breast cancers. The
frequency of HPR1 are significantly higher in breast tumors between 1 and 5 cm than in tumors < or =1 cm.

In [88] Pedersen AN., et al. investigated the association between tumor tissue level of total tissue inhibitor of metalloproteinases-1 (TIMP-1) and prognosis in patients with primary breast cancer and to analyze whether measurement of TIMP-1 in tumor extracts added prognostic information to that obtained from measurements of urokinase-type plasminogen activator and Plasminogen Activator Inhibitor type 1 (PAI-1). An established sandwich enzyme-linked immunosorbent assay is thoroughly validated for the measurement of total TIMP-1 in tumor tissue extracts and used to determine levels of total TIMP-1 in 341 detergent-extracted tumor tissue samples from patients with primary breast cancer. The median age of the patients is 56 years (range, 29-75 years) and 164 were lymph node-negative, and 177 are lymph node-positive. The median follow-up time of the patients is 8.5 years (range, 7.3-11.3 years) and during follow-up 153 patients experienced recurrence of disease, and 136 patients died. In univariate survival analysis, they found a significant association between tumor tissue TIMP-1 level and both shorter recurrence-free survival (p = 0.0004) and shorter overall survival (p = 0.03). In multivariate survival analysis, higher tumor tissue TIMP-1 levels significantly and independently predicted shorter recurrence-free survival (p < 0.05, hazard ratios >1, comparing quartiles II-IV with I).
In addition, they found that measurement of TIMP-1 levels added prognostic information to that obtained from measurement of PAI-1. In conclusion, high levels of TIMP-1 in tumor tissue extracts were significantly associated with a poor prognosis in patients with primary breast cancer. However, further validation in independent data sets are needed.

In [89] early detection of breast cancer are the key to improve survival rate. Thermogram is a promising front-line screening tool as it was able to warn women of breast cancer up to 10 years in advance. However, analysis and interpretation of thermogram is heavily dependent on the analysts, which may be inconsistent and error-prone. In order to boost the accuracy of preliminary screening using thermogram without incurring additional financial burden, Complementary Learning Fuzzy Neural Network (CLFNN), FALCON-AART are proposed as the Computer-Assisted Intervention (CAI) tool for thermogram analysis. CLFNN are a neuroscience-inspired technique that provides intuitive fuzzy rules, human-like reasoning and good classification performance. Confluence of thermogram and CLFNN offers a promising tool for fighting breast cancer.

Digital Infrared Thermal Imaging (DITI) have resurfaced in this era of modernized computer technology. Its role in the detection of breast cancer is evaluated. In this prospective clinical trial, 92 patients
for whom a breast biopsy is recommended based on prior mammogram or ultrasound underwent DITI. Three scores are generated: an overall risk score in the screening mode, a clinical score based on patient information and a third assessment by artificial neural network [90]. Sixty of 94 biopsies were malignant and 34 are benign. DITI identified 58 of 60 malignancies, with 97% sensitivity, 44% specificity, and 82% negative predictive value depending on the mode used. Compared to an overall risk score of 0, a score of 3 or greater is significantly more likely to be associated with malignancy (30% vs 90%, P < .03). ITI was a valuable adjunct to mammography and ultrasound, especially in women with dense breast parenchyma.

A fully automatic method for breast cancer diagnosis based on microscopic biopsy images are presented. The method achieves high recognition rates by applying multi-class support vector machines on generic feature vectors that is based on level-set statistics of the images [91]. They also considered the problem of classification with rejection and showed preliminary results that point to the potential benefits. Index Terms were breast cancer, biopsy, automatic diagnosis, SVM, image processing and feature extraction.

Impedance spectroscopy is an emerging tool in the analysis of cancer [1-4] [40]. Our goal is to investigate the diagnostic and prognostic capabilities of a MEMS-based micro-bioimpedance sensor. To this end, we report a unique narrow multi-branched electrode
system in a micro-cell culture chamber on a silicon chip capable of detecting metastatic human breast cancer cells in a tri-culture comprised of normal epithelial MCF10A, HS68 fibroblast and cancerous MDA-MB-231 cells to simulate a breast tumor biopsy sample [94]. A bioimpedance signature unique to the metastatic MDA-MB-231 cancer cells is elicited by a chemical stimulus, Suberoyl Anilide Hydroxamic Acid (SAHA), an experimental anti-cancer agent that exerts selective actions on the cytoskeletal organization of the cancer cells [5]. This is the first time a complex cell culture consisting of three different cell types has been analyzed using MEMS-based impedance spectroscopy.