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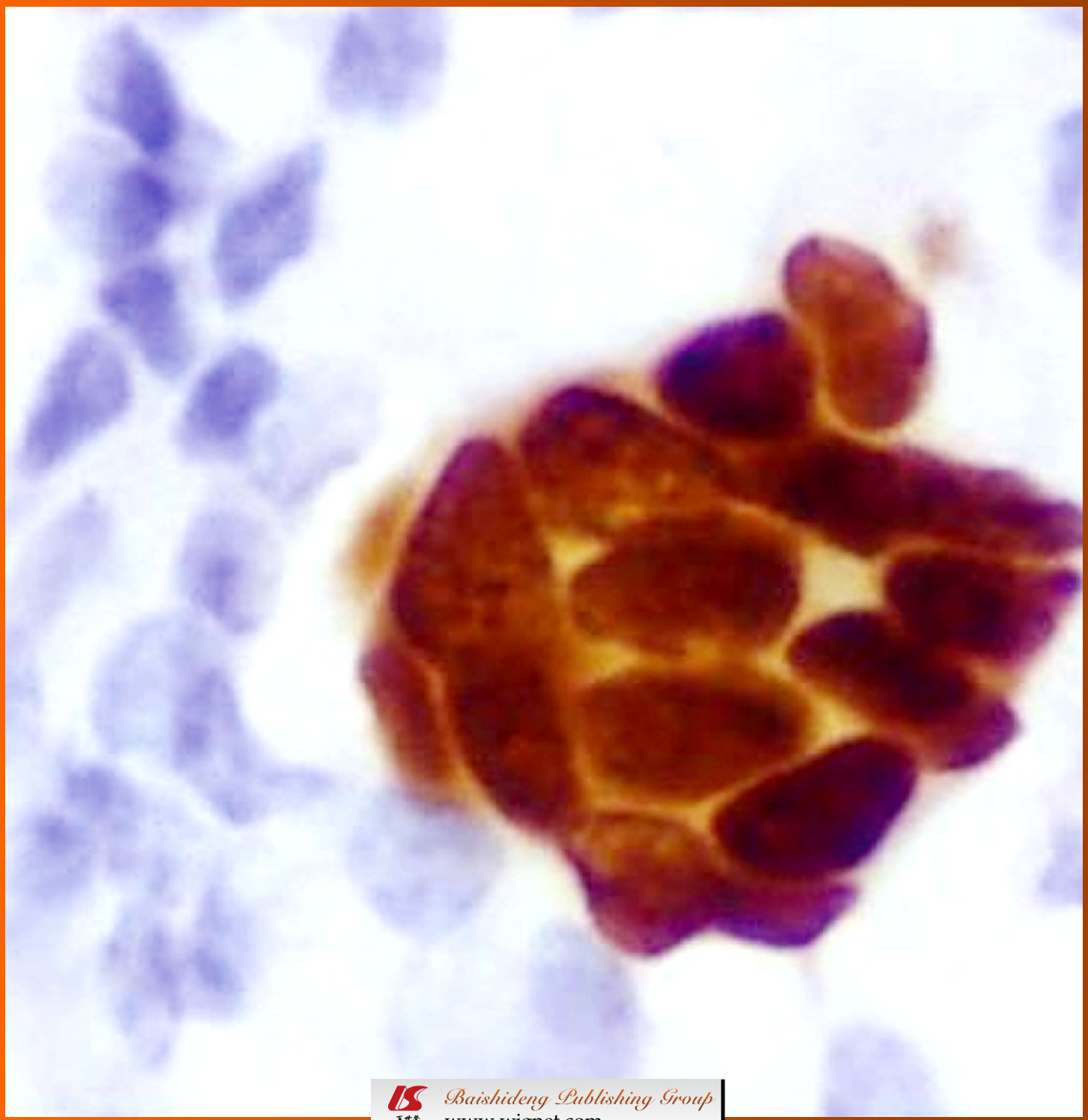

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Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-8538-1892
Fax: +86-10-8538-1893
E-mail: baishideng@wjgnet.com
http://www.wjgnet.com

EDITING
Editorial Board of *World Journal of Clinical Oncology*,
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-5908-0036
Fax: +86-10-8538-1893
E-mail: wjco@wjgnet.com
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Fax: +852-3115-8812
Telephone: +852-5804-2046

E-mail: baishideng@wjgnet.com
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SUBSCRIPTION
Beijing Baishideng BioMed Scientific Co., Ltd.,
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-8538-1892
Fax: +86-10-8538-1893
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Lin Tian, Director
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Beijing 100025, China
Telephone: +86-10-8538-1892
Fax: +86-10-8538-1893
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EDITORIAL

Imaging as a diagnostic and therapeutic tool in clinical oncology

Eddie Yin-Kwee Ng, Rajendra U Acharya

Eddie Yin-Kwee Ng, School of Mechanical and Aerospace Engineering, College of Engineering, Nanyang Technological University, 50, Nanyang Avenue, Singapore 639798, Singapore
Rajendra U Acharya, School of Engineering, Ngee Ann Polytechnic, Singapore 599489, Singapore
Author contributions: Ng EYK and Acharya RU contributed equally to this editorial.
Correspondence to: Eddie Yin-Kwee Ng, PhD, PGDTHE, Associate Professor, School of Mechanical and Aerospace Engineering, College of Engineering, Nanyang Technological University, 50, Nanyang Avenue, Singapore 639798, Singapore. mykng@ntu.edu.sg
Telephone: +65-65-67904455 Fax: +65-65-67911859
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Abstract

According to the WHO report published in 2010, about 13% of all deaths are due to cancer. Of these, lung, liver, stomach, colon and breast cancer are the most prevalent. It was also reported that about 30% of the deaths due to cancer can be avoided, if diagnosed and treated early. Hence, there is an urgent need to diagnose these cancers efficiently. Various imaging and therapeutic methods have been proposed and used to accurately detect cancer. In this special two issues, there are eight papers covering different aspects of oncology using various imaging or therapeutic methods.

Key words: Imaging; Clinical oncology

Ng EYK, Acharya RU. Imaging as a diagnostic and therapeutic tool in clinical oncology. *World J Clin Oncol* 2011; 2(4): 169-170 Available from: URL: http://www.wjgnet.com/2218-4333/full/v2/i4/169.htm DOI: http://dx.doi.org/10.5306/wjco.v2.i4.169

Medical imaging and diagnostics have been established techniques over the last 50 years. Various endoscopic models have been designed and developed which differ in their ability to inspect the inner surfaces of the body cavities. Furthermore, the rigid nature of such endoscopes and the need for high resolution imaging has enabled researchers to develop more sophisticated flexible probes for medical and single cell imaging for early disease diagnosis. In this special two issues, there are eight papers covering different aspects of oncology using various imaging or therapeutic methods.

Paper 1[1] introduces the background of optical spectroscopy in cancer management, which includes the advantages of this technique compared with other established techniques, the principle of optical spectroscopy and the typical instrumentation setup. Current progress in optical spectroscopy for the diagnosis of cancer in the brain, breast, cervix, lung, stomach, colon, prostate and skin are reviewed. A few commercially available clinical instruments based on optical spectroscopy techniques are presented. Several technical challenges and standard issues are also discussed.

A preliminary system to perform online rendering side-by-side with laser scanning confocal endomicroscopic imaging is presented in Paper 2[2]. By having an immediate knowledge of the dataset quality as well as the biological tissue conditions, alterations can be made on the spot. This will introduce the opportunity to change imaging conditions or medical decisions according to the online rendering results. This work is also motivated by the need to realize the quality of the captured datasets in real time to reduce excessive time required for offline rendering.

Imaging of gastroenteropancreatic neuroendocrine tumors can be broadly divided into anatomic and functional techniques. Anatomic imaging determines the local extent of the primary lesion, providing crucial information required for surgical planning. Functional imaging not only determines the extent of metastatic disease spread, but also provides important information with regards to the biologic behavior of the tumor, allowing clinicians to decide on the most appropriate forms of treatment. Paper

3[3] reviews the current literature on this subject, with an emphasis on the strengths of each imaging modality.

Existing imaging modalities for breast cancer screening, diagnosis and therapy monitoring, namely X-ray mammography and magnetic resonance imaging (MRI), have been proven to have limitations. Diffuse optical imaging, a set of non-invasive imaging modalities using near-infrared light, may be an alternative, if not a replacement, to those existing modalities. Paper 4[4] covers the background knowledge, recent clinical trial outcome, and the future outlook of this newly emerging medical imaging modality.

Paper 5[5] presents a technique for automation in the diagnosis of breast cancer from immunohistochemically stained biopsy specimens. It has been demonstrated through this work that manual evaluation introduces a number of variations, whereas automated analysis provides objective evaluation and is repeatable. Such automation can facilitate fast and efficient diagnosis of breast cancer cases and eliminate human errors to a large extent.

Efforts have been made to improve the accuracy of breast cancer diagnosis using different imaging modalities. Ultrasound and MRI have been used to detect breast cancers in high risk patients. Recently, electrical impedance imaging and nuclear medicine techniques are also being widely used for breast cancer screening and diagnosis. Paper 6[6] discusses the capabilities of various breast imaging modalities.

Paper 7[7] proposes techniques to investigate the strength of support vector regression in cancer prognosis using imaging features. In this work, the authors have used the combinational methods of Support Vector Classification-Regression, feature selection, and sampling that can improve cancer prognosis. More significant features may further enhance cancer prognosis accuracy.

The clinical outcomes of high intensity focused ultrasound (HIFU) ablation applied to cancers are discussed. The current challenges in the application of HIFU in tumor treatment, such as HIFU-mediated drug delivery, vessel occlusion, and soft tissue erosion ("histotripsy") are discussed in paper 8[8].

We hope that the papers in this special issue will help to disseminate knowledge in the field of clinical oncology.

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TOPIC HIGHLIGHT

Eddie Yin-Kwee Ng, PhD, PGDTHE, Associate Professor, *Series Editor*

Breast imaging: A survey

Subbhuraam Vinitha Sree, Eddie Yin-Kwee Ng, Rajendra U Acharya, Oliver Faust

Subbhuraam Vinitha Sree, Eddie Yin-Kwee Ng, School of Mechanical and Aerospace Engineering, Nanyang Technological University, Singapore 639798, Singapore
Rajendra U Acharya, Oliver Faust, Department of Electronics and Computer Engineering, Ngee Ann Polytechnic, Singapore 599489, Singapore
Author contributions: All the authors have equally contributed to writing the text and formatting the paper.
Correspondence to: Eddie Yin-Kwee Ng, PhD, PGDTHE, Associate Professor, School of Mechanical and Aerospace Engineering, Nanyang Technological University, Singapore 639798, Singapore. mykng@ntu.edu.sg
Telephone: +65-6790-4455 Fax: +65-6791-1859
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Abstract

Breast cancer is the second leading cause of death in women. It occurs when cells in the breast start to grow out of proportion and invade neighboring tissues or spread throughout the body. Mammography is one of the most effective and popular modalities presently used for breast cancer screening and detection. Efforts have been made to improve the accuracy of breast cancer diagnosis using different imaging modalities. Ultrasound and magnetic resonance imaging have been used to detect breast cancers in high risk patients. Recently, electrical impedance imaging and nuclear medicine techniques are also being widely used for breast cancer screening and diagnosis. In this paper, we discuss the capabilities of various breast imaging modalities.

Key words: Breast cancer; Breast magnetic resonance imaging; Breast ultrasound; Mammography; Thermography

Peer reviewer: Gary M Tse, MD, Department of Anatomical and Cellular Pathology, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong, China; Ali Syed Arbab, MD, PhD, Associate Scientist and Director, Cellular and Molecular Imaging Laboratory, Department of Radiology, Henry Ford Hospital, 1 Ford Place, 2F, Box 82, Detroit, MI 48202, United States; Slimane Belbraouet, PhD, Professeur Titulaire, Directeur, École des sciences des aliments, de nutrition et d'études familiales, Université de Moncton, Moncton, NB, E1A 3E9, Canada

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INTRODUCTION

Breast cancer starts in the breast cells of both women and men. Worldwide, breast cancer is the second most common type of cancer after lung cancer (10.9% of cancer incidence in both men and women)[1] and the fifth most common cause of cancer death[2]. The National Breast Cancer Foundation has estimated around 200 000 new breast cancer cases and 40 000 deaths every year in women. In men, these statistics are 1700 and 450, respectively[3]. According to the National Cancer Institute, an estimated 207 090 new cases and 39 840 deaths from breast cancer (only women) are expected to occur in the United States, despite recent advances in treatment[4]. Given such circumstances, early diagnosis of breast cancer is considered vital, because statistics have shown a five-year survival rate of 96% for those whose cancer was detected in the early stages[3].

The breast is composed of identical tissues in both men and women, and hence, breast cancer also occurs in men. Breast cancer incidence in men is approximately 100 times less than in women, but men with breast cancer are considered to have the same statistical survival rates as women[5-7].

In this paper, our focus is on breast cancer detection modalities which use breast images obtained by various techniques for analysis and subsequent detection. For

better survival odds and reduced use of treatments and therapies and, therefore, fewer side-effects, many imaging modalities are continually being developed to diagnose this disease as early as possible. Some of these modalities are used for screening purposes, some for diagnostic purposes, and a few others for adjunctive evaluation. Techniques that enable mass level screening should be cost-effective and efficient enough to reach the masses. Once breast cancer has been detected in screening tests, more detailed evaluations are usually performed using diagnostic modalities which may also be used for initial diagnosis. Adjunctive modalities are used to provide the doctors and clinicians with additional confidence in their initial diagnosis. The currently used modalities include mammography, breast ultrasound, thermography, magnetic resonance imaging (MRI), positron emission tomography (PET), scintimammography, optical imaging, electrical impedance based imaging, and computed tomography (CT).

Since cancer is a complex disease with varied pathology, many variations of the basic detection technique used in each of these modalities have been carried out over the years in order to improve the detection efficiency[8]. The main aim of this paper is to provide a discussion on the capabilities of each of these modalities, which are presented in the following sections.

VARIOUS MODALITIES USED FOR BREAST CANCER DETECTION

This section presents a review of the various modalities used for breast cancer detection.

Mammography

Mammography is the most common method of breast imaging. It uses low-dose amplitude-X-rays to examine the human breast. Cancerous masses and calcium deposits appear brighter on the mammogram. This method is good for detecting Ductal Carcinoma In Situ (DCIS) and calcifications. Currently, mammography is the gold standard method to detect early stage breast cancer before the lesions become clinically palpable. Mammography has helped to decrease the mortality rate by 25%-30% in screened women when compared with a control group after 5 to 7 years[9]. Randomized trials of mammographic screening have provided strong evidence that early diagnosis and treatment of breast cancer reduces breast cancer mortality[10].

It is very difficult to detect cancer in the early stage using mammographic screening. However, additional screening tests may reduce the death rate from breast cancer. The mammography screening test has been shown to lower the death rate in randomized controlled trials conducted with the general population[11-13]. Mammographic imaging has proved to be scientifically more suitable for screening, and hence, may be used for general screening[12]. Patients with abnormal breast findings were screened using mammography, sonography and magnetic resonance (MR) mammography[14]. Carcinoma *in situ* was diagnosed in 78.9% and 68.4% of patients using mammography and MR mammography, respectively. A combination of all three diagnostic methods performed better in detecting invasive cancer and multifocal disease. However, the sensitivity of mammography and sonography combined was identical to the performance of MR mammography (i.e. 94.6%).

In digital tomosynthesis mammography, the basic mammography technique has been modified to acquire 3D views of the breast[15]. In another variation called ductography, contrast agents are used to determine the presence of a mass within the ducts. A recent development of mammography is contrast-enhanced digital mammography (CEDM) which uses an intravenous injection of an iodinated contrast agent in conjunction with a mammography examination[16]. Diekmann *et al*[17] evaluated the diagnostic benefits of CEDM over conventional mammography. They found an increase in sensitivity from 0.43 to 0.62 on using CEDM, and also observed better sensitivity in the case of dense tissues. This is a potentially useful benefit as it is known that conventional mammography is not very sensitive in detecting cancer in dense breast tissues.

Breast ultrasound

Ultrasound imaging is used to detect breast lesions and it is used as an adjunct tool for detecting the location of the suspicious lesion. The ultrasound transducer directs high-frequency sound waves into the breast tissues and detects the reflected sound waves. These detected waves are used to display 2D images. As the sensor is moved over the breast, continuous real-time images can be captured. Ultrasound can be used as an adjunct to mammography for clinical examination in the assessment of both palpable and impalpable breast abnormalities. Ultrasound screening in asymptomatic women causes unacceptable false positive and false negative outcomes[18]. Hence, there is little evidence to support the use of breast ultrasound in breast cancer screening.

Mammography alone misses many cancers in dense-breasted women. The diagnostic yield of mammography with an automated whole breast ultrasound (AWBU), for women with dense breasts and/or at elevated risk of breast cancer, is better[19]. A study by Kelly *et al*[19] showed that 87% of cancer detections added by AWBU were found in the 68% of studies in women with dense/very dense breasts. Hence, AWBU resulted in significant cancer detection improvement compared with mammography alone. Kopans[12] has suggested that sonography should always be used with mammography or other imaging techniques. It alone will not be able to detect lesions accurately. Another study that supports the use of mammography and ultrasound together is the ACRIN 6666 trial. The results of this study indicated that incorporating a screening ultrasound with mammography would detect an additional 1.1 to 7.2 cancers per 1000 high-risk women, however, at the expense of an increased false positive rate[20]. Breast cancer is common among Japanese women in their late 40s with small and dense breasts. It was shown that the performance of ultrasound was similar to

that of mammography in detecting breast cancers in these women[21]. The authors of this study have also suggested that the combination of mammography and ultrasound is a suitable method for breast screening in Japan. A very recent specific study which was conducted to evaluate the efficiency of whole breast ultrasound based on BI-RADS final assessment categories in women with mammographically negative dense breasts[22] has reported that ultrasound is useful for dense breast evaluations.

Advancements in ultrasound technology include 3D ultrasound that formats the sound wave data into 3D images[23], automated ultrasound for a good overall view of the breast[24], Doppler Ultrasound[25], and sonoelastography[26].

Breast thermography

Cancerous and pre-cancerous tissues have a higher metabolic rate resulting in growth of new blood vessels supplying nutrients to the fast growing cancer cells. As a consequence, the temperature of the area surrounding the pre-cancerous and cancerous breast tissue is higher when compared to the normal breast tissue temperature. The breast has been recognized to exhibit a circadian rhythm, which reflects the physiology. There is evidence to indicate that these rhythms, associated with malignant cell proliferation, are non-circadian[27,28]. The relationship between breast skin temperature and breast cancer has been examined[29,30]. Measurable changes were observed in skin temperatures between clinically healthy and cancerous breasts. The cyclic variation in temperature and vascularization of the normal breast thermograms under a controlled environment were studied[31]. The results of this study help in the analysis of normal and abnormal breast thermography.

Nowadays, breast thermograms are widely used for the accurate detection of breast cancer[32-38]. Thermography is a promising screening tool because it is able to diagnose breast cancer at least ten years in advance. However, both analysis and interpretation of thermograms depends on analysts.

MRI

MRI uses the hydrogen nucleus (single proton) for imaging purposes because this nucleus is abundant in water and fat. The magnetic property of the hydrogen nucleus is used to produce detailed images from any part of the body. The patient who is examined using MRI is placed in a magnetic field and a radio frequency wave is applied to create high contrast images of the breast. In dynamic contrast enhanced-MRI (DCE-MRI)[39], a contrast agent is injected before the images are captured. This technique has been found to be more sensitive than mammography[40].

Application of state-of-the-art imaging modalities, namely MRI, magnetic resonance spectroscopy (MRS), nuclear imaging, and optical imaging, for precise identification of human breast tumors and their use in monitoring chemotherapeutic responses has been discussed[41]. MRI helps in investigating vascular changes associated with neoangiogenesis[42]. It is popular in diagnosis, and is now being used to assess tumor response to treatment. It is predicted that new contrast agents and improvements in measurement and analytical methods will help the use of MRI in investigating the vascular dependence of tumor growth and the activity of vascular-directed therapies.

Breast MRI is a widely used imaging modality for the early detection of breast cancer[43]. Early results suggest that MRI can dramatically improve the yield of screening certain at-risk populations. Further work may be performed to clarify the role of breast MRI in the early detection of breast cancer. Recent work on breast MRI with 3 Tesla magnets, showed that MRI had a higher spatial and temporal resolution and a better signal to noise ratio[44].

Numerous studies have demonstrated that malignant tissues have elevated levels of choline-containing compounds, suggesting that these compounds may serve as non-invasive markers for detecting malignancies[45]. *In vivo* non-invasive MRSI uses equipment that is almost identical to the normal MRI apparatus but with specific sequences for spectroscopic signal acquisition to visualize the total choline content in the breast. MRS improves the specificity of MRI further, and it can predict response to therapy and/or evaluate very early response to chemotherapy. In a study using MRS, the specificity was observed to be 87.5% which was significantly higher than that obtained using MRI (62.5%)[46]. Novel contrast agents are being developed to provide more sensitive and more specific discrimination of benign from malignant lesions. MRS and MRI are rapidly becoming standard capabilities of clinical MR systems with magnets 1.5 Tesla or stronger[47]. The promising results from multiple institutions reported so far suggest that MRS, along with MRI, can improve the clinical assessment of breast cancer in the future. Numerous multicenter trials may still be needed before these new techniques can be widely used to guide diagnostic decisions and to predict response to therapy. Brain and prostate cancers also exhibit increased choline levels, and hence, MRS is suitable for assessing these cancers[48].

In another version of MRI, namely diffusion weighted imaging (DWI), image contrast arising out of the differences in the motion of water molecules between tissues is utilized for imaging. No external contrast agents are needed. The Apparent Diffusion Constant (ADC) parameter was found to be higher in tumor tissues compared to normal tissues, and hence, this ADC has been used in the assessment of metastatic breast cancer response to chemoembolization[49]. DWI-MRI has also been used for evaluating a variety of other cancers including liver, prostate and pancreatic carcinomas[48].

In MRI based elastography, a periodic motion is generated by a mechanical shaker to one side of the breast and the resulting displacement field inside the breast is captured by MRI to determine the elasticity parameters[50]. This technique relies on MRI's ability to detect slight motion. MRE studies have also been tried to assess prostate cancers[48].

MRI is useful for women with a higher risk of breast cancer, has good image resolution, is effective for evaluating dense breasts, helps to evaluate inverted nipple, allows the simultaneous evaluation of both breasts, helps to de-

termine whether lumpectomy or mastectomy is the best treatment, and it has no side effects as there is no radiation[51]. The limitations of this technique are that it is not good at diagnosing ductal carcinoma *in situ* (DCIS), may lead to many false positives, is slow (30 min to one hour), more expensive, and may not show all calcifications. Recently, an analysis was conducted to study the correlation between film mammography and MRI in screening breast cancer in high-risk women[52]. The authors found no significant correlation, and suggested that using both modalities for screening is likely to improve the odds of detecting early stage cancers.

Positron emission tomography

Positron emission tomography (PET) is a nuclear medicine imaging technique which is used to produce three dimensional images. It detects a pair of γ rays, which are emitted from the radionuclide that is introduced into the human body. Malignant tumors are characterized by increased glucose metabolism compared with normal cells. This produces a good contrast between cancerous and normal cells in PET images. It provides information about the chemical functions inside organs and tissues. However, PET is very expensive and yields poor resolution images. Furthermore, the patient is subjected to radiation exposure. PET has been used frequently to predict treatment response in several cancers[48].

Single photon emission computed tomography (SPECT) and PET use radiolabeled isotopes[53]. Both imaging modalities provide unique opportunities to study animal models of breast cancer with direct application to human imaging. MRI and PET are complementary and valuable in monitoring response and assessing residual disease of locally advanced breast cancer treated with neoadjuvant chemotherapy[54]. Their study suggested that the combined use of MRI and PET were complementary and offered advantages over clinical breast examination. PET was more accurate in predicting pathologic non-response, and the response evaluated using MRI correlated well with macroscopic pathologic complete response.

Scintimammography (SMM), SPECT and PET can be used as adjunct imaging tools for detecting and staging breast cancer, however, they cannot replace invasive procedures, due to insufficient sensitivity to detect small (less than 1 cm) tumor deposits[55]. SMM is useful for assessing palpable breast masses in women with dense breasts. Several enzymes and receptors have been targeted for imaging breast cancers with PET. Fluorodeoxyglucose is useful in the detection and staging of recurrent breast cancer and assessing its response to chemotherapy.

PET used to complement mammography is known as positron emission mammography (PEM), and it has been reported that PEM may not be adversely affected by breast density, hormone replacement therapy, and menopausal status of the patient[56].

Scintimammography

The scintimammography imaging technique uses a radioisotope to visualize lesions of the breast. It is difficult to detect breast cancer in dense breast tissue using mammography. As a result, mammogram-based breast cancer detection techniques yield a high number of false positives. Scintimammography with technetium tetrofosmin (Tc-99 tetrofosmin) provides better precision in the diagnosis of women with dense breasts. It is suitable for dense breasts, can image breasts with implants, can image large and palpable abnormalities, and it can be used when multiple tumors are suspected[57]. A high-resolution breast-specific gamma camera was used to evaluate the occult breast cancer in women at high risk of breast cancer[58]. The authors found that high-resolution breast-specific scintimammography was able to detect small (< 1 cm), mammographically occult, nonpalpable lesions not otherwise detected by mammography or physical examination in women with increased risk for breast cancer. The joint use of mammography and 99mTc-methoxy isobutyl isonitrile (MIBI) scintimammography to reduce the number of biopsies required in patients with suspected breast cancer has been studied[59]. The total number of biopsies performed was reduced by 34%. In scintimammography with Tc99m compounds, the value of planar Tc99m sestamibi scanning for auxiliary lymph node evaluation was presented[60]. Their work confirmed that non-tomographic Tc99m sestamibi scintimammography had a very low detection rate for auxiliary lymph node involvement and may not be suitable for clinical assessment of breast cancer. The sensitivity and specificity of Breast-Specific Gamma Imaging (BSGI) for the detection of breast cancer by using pathologic results as the reference standard was determined[61]. BSGI showed high sensitivity (96.4%) and moderate specificity (59.5%) in the detection of breast cancers.

Optical imaging

Optical imaging uses near infrared (NIR) wavelength light to detect lesions inside the breast. Diffuse optical imaging (uses NIR light to penetrate into the breast), diffuse optical tomography (uses NIR light of wavelength 700 to 1000 nm), and optical mammography (uses laser light) are the different types of optical imaging which use different wavelengths of light to detect breast lesions.

Diffuse optical imaging (DOI) is a noninvasive optical technique which uses NIR light to quantitatively characterize the properties of thick tissues[62]. Factors affecting the DOI performance are intrinsic and extrinsic contrast mechanisms, quantitation of biochemical components, and image formation/visualization. Currently, the new direction is to develop standardized DOI platforms that can be used as stand-alone devices or in conjunction with MRI, mammography, or ultrasound which can provide new insights for detecting disease in mammographically dense tissue, distinguishing between malignant and benign lesions, and understanding the impact of neoadjuvant chemotherapies.

Optical imaging offers complementary features to radiologic imaging techniques, primarily the quantitative imaging of hemoglobin saturation and concentration, and the selective imaging of specific gene expression with high sensitivity, because background signals can be suppressed using enzyme-activated fluorescence probes[63]. This meth-

od can also characterize vascularization, permeability, and a plethora of contrast agents with high sensitivity, without using harmful radiation, and probably at less cost.

Electrical impedance based imaging

Our body tissues offer impedance to the flow of electric current. Studies have shown that cancerous breast tissues have lower impedance when compared to normal tissues. Electrical impedance tomography (EIT) and electrical impedance scanning (EIS) are the two types of electrical impedance based imaging techniques available. In EIT, 2D or 3D images are reconstructed from a large number of impedance values which are captured by placing electrodes around the breast surface in a circular fashion. However, in EIS or electrical impedance mapping (EIM), a planar electrode array is used and there is no need for complicated reconstruction algorithms which are used for EIT.

Zou *et al*[64] presented a review of the noninvasive impedance imaging techniques for breast cancer detection, such as EIT and EIM. They suggested that an invasive impedance technique can be more effective by combining it with other cancer indicators. They have proposed the possibility of improving EIM using a pair of electrode arrays, one for exciting the breast surface and the other for measuring the impedance. They concluded that magnetic induction tomography and other magnetic induction based impedance imaging techniques are promising. The T-SCANTM technology and its use as a diagnostic tool for breast cancer detection was discussed by Assenheimer *et al*[65]. They used theoretical models with simplified geometries to show that the display of planar two-dimensional maps of the currents detected at the breast's surface related to the electric field distribution within the breast. The differences in the distribution of the various tissue types can be used to discriminate between various pathological states. They also suggested that low frequency impedance measurements can be used in breast cancer diagnosis. EIS has been found to provide a rather high sensitivity for the verification of suspicious breast lesions[66].

The possibilities of using electrical impedance mammography for the investigation of mammary gland state in women with different hormonal status was studied[67]. They found that electrical impedance mammograms from different groups had clear visual distinctions and statistically significant differences in mammary gland conductivity. Further investigations on histomorphological characteristics of false negative and false positive lesions may be needed to gain further knowledge about the bioelectric characteristics of breast lesions.

CT

CT uses X-rays to capture 2D images or slices of the examined body parts. Subsequently, different algorithms are used to generate corresponding 3D images which provide anatomical information such as the location of lesions. Usually CT has low contrast, and hence, iodinated contrast media is injected intravenously to increase the contrast of the CT images. The iodine contrast injection dramatically enhances the visualization of tumors. The diagnostic accuracy of CT perfusion in differentiating metastatic from inflammatory enlarged axillary lymph nodes in patients with breast cancer was evaluated[68]. They showed that CT perfusion may be an effective tool for studying enlarged axillary lymph nodes in patients with breast cancer. The study presents information on vascularization of lymph nodes, helping to understand the changes occurring when neoplastic cells implant in lymph nodes. The lifetime attributable risk (LAR) of cancer incidence associated with radiation exposure from 64-slice computed tomography coronary angiography (CTCA) was studied and the influence of age, sex, and scan protocol on cancer risk was evaluated[69]. These estimates, which were derived from simulation models, suggest that the use of 64-slice CTCA was associated with a non-negligible LAR of cancer. This risk varies markedly and was considerably greater for women, younger patients, and for combined cardiac and aortic scans.

A hybrid technique combining PET and CT is useful for staging potential metastatic cancers[70]. This technique has the combined advantages of both CT and PET: tumor location is better captured by CT and PET indicates a metabolically active or malignant tumor based on glucose uptake. CT often incidentally identifies lung nodules during exams for other lesions in the thorax. Therefore, recently, a dedicated breast CT prototype that has a high-resolution, isotropic, rotating detector was developed. Subjective evaluation of breast CT images revealed excellent anatomical detail, good depiction of microcalcifications, and exquisite visualization of soft tissue components which belong to the tumor when contrasted against adipose tissues[71].

BIOMARKERS

A disease cannot be easily and straight-forwardly diagnosed based on symptoms as the initial symptoms may point to a group of diseases with similar features. Moreover, in the case of cancer, it will be too late to make a diagnosis based on symptoms, as symptoms appear when the tumor is relatively large. Therefore, for early detection, the modality must be capable of detecting cancer in asymptomatic women. By the time a tumor is detected by most of the current imaging modalities, molecular changes would have already occurred in the suspected area. Detection of such molecular changes, therefore, would be our best bet to capture the presence of cancer at its earliest stages. A biomarker is a measurable phenotypic parameter that characterizes an organism's state of health or disease, or a response to a particular therapeutic intervention[72]. Diagnostic assays using such biomarkers have good potential in early cancer detection.

Studies have demonstrated the utility of direct examination of the cytomorphology of exfoliated cells in detecting breast cancer[73]. The molecular analysis of tumor biomarkers in nipple aspirate fluid (NAF) or in ductal lavage has also been found to be useful for the detection of breast cancer[74]. However, the cytomorphology-based analysis is subjective, and most women may not produce NAF. Lipids, carbohydrates, polyamines, proteins, and nucleic acids have also been studied as potential biomarkers for early breast

cancer detection. A detailed review of the biomarkers used for early detection can be found in[75]

Response to therapy is first observed at the molecular and cellular level and then at the anatomical level. Therefore, biomarkers are useful in predicting treatment response. In current clinical practice, the standard markers used for general prognosis assessment and the prediction of therapy response are the hormone receptor (ER and PR) status, HER-2/neu status, and the labeling of Ki-67 antigen.

ER and PR testing have been used as markers for the prognosis and the prediction of response to anti-estrogen therapy[76-77]. The HER2/neu is a protein that has higher aggressiveness in breast cancers. Amplification of the *HER2/neu* gene and over-expression of the HER2/neu protein have been observed in 10%-34% of invasive breast cancers[78]. The human Ki-67 protein expression is associated with cell proliferation. The Ki-67 labeling index, which is a fraction of Ki-67-positive tumor cells, is often correlated with the clinical course of cancer. A detailed review of the emerging biomarkers used for breast cancer management (prognosis and treatment response prediction) has been carried out by Ross *et al*[79].

The term molecular imaging was defined by the Commission on Molecular Imaging of the American College of Radiology as "the spatially localized and/or temporally resolved sensing of molecular and cellular processes *in vivo*." Molecular imaging explores either changes in metabolic rate, cell proliferation rate, hormone expression, gene expression, or protein production. The main modalities for molecular imaging are PET, SPECT, MRS, and optical imaging; PET imaging being the most widely used modality. PET has been used for the in vivo quantification of ER. Since [18F]-16α-[fluororestradiol] (FES) has shown most promise in quantifying the functional ER status of breast cancer, it has been used as a tracer in PET-ER imaging[80]. PET-ER imaging (FES-PET) can therefore predict the likelihood of a patient's response to hormonal therapy, and thereby, determine the suitability of the patient for this type of treatment. The results of some related studies can be found in[81]. Jeraj *et al*[82] have presented a comprehensive review of the various functional and molecular imaging techniques used in oncology. They have presented the effectiveness of such imaging techniques for a variety of cancers.

CONCLUSION

Current breast imaging modalities play a vital role in assisting clinicians in the primary screening of cancer, in the diagnosis and characterization of lesions, staging and restaging, treatment selection and treatment progress monitoring and in determining cancer recurrence. In this paper, we have discussed the capabilities of the different breast imaging techniques that are currently used in clinical setups. It is evident from the material presented in this paper that no single modality is completely useful in all areas of breast cancer management. Therefore, research is continually being carried out to improve the existing modalities and develop new modalities based on the physical, chemical, and biological properties of cancerous breast tissue that differentiates it from normal and benign tissues. Cancer is a disease with no specific cure, and its treatment involves a wide variety of side-effects. Moreover, the survival rate is largely dependent on early detection. A disease with such disturbing and life-threatening factors warrants a huge amount of research to develop modalities (screening, diagnostic, adjunct, standalone, and hybrid) that help in early detection and in finding a possible cure. Currently, research on modality development is moving towards imaging at the molecular level. This type of imaging will also help in understanding the nature of cancer growth and development which in turn might lead us closer to finding a possible cure for this disease. Moreover, the use of computer-aided diagnosis techniques has been widely advocated for the improvement of cancer detection efficiency and for reducing the inter-observer variability that is associated with the subjective human interpretation of the images obtained.

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TOPIC HIGHLIGHT

Eddie Yin-Kwee Ng, PhD, PGDTHE, Associate Professor, *Series Editor*

Online volume rendering of incrementally accumulated LSCEM images for superficial oral cancer detection

Wei Ming Chiew, Feng Lin, Kemao Qian, Hock Soon Seah

Wei Ming Chiew, Feng Lin, Kemao Qian, Hock Soon Seah, School of Computer Engineering, Nanyang Technological University, Singapore 639798, Singapore
Author contributions: Chiew WM, Lin F, Qian K and Seah HS performed research; Ming CW and Lin F wrote the paper.
Supported by The grant SBIC RP C-010/2006 from A*Star Biomedical Research Council
Correspondence to: Feng Lin, Associate Professor, School of Computer Engineering, Nanyang Technological University, Singapore 639798, Singapore. asflin@ntu.edu.sg
Telephone: +65-67906184 Fax: +65-67926559
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Peer reviewer: Brendan Curran Stack, JR, MD, FACS, FACE, Chief, McGill Family Division of Clinical Research, Department of Otolaryngology-HNS, University of Arkansas for Medical Sciences, 4301 West Markham, Slot 543, Little Rock, AR 72205, United States

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Abstract

Laser scanning confocal endomicroscope (LSCEM) has emerged as an imaging modality which provides non-invasive, *in vivo* imaging of biological tissue on a microscopic scale. Scientific visualizations for LSCEM datasets captured by current imaging systems require these datasets to be fully acquired and brought to a separate rendering machine. To extend the features and capabilities of this modality, we propose a system which is capable of performing realtime visualization of LSCEM datasets. Using field-programmable gate arrays, our system performs three tasks in parallel: (1) automated control of dataset acquisition; (2) imaging-rendering system synchronization; and (3) realtime volume rendering of dynamic datasets. Through fusion of LSCEM imaging and volume rendering processes, acquired datasets can be visualized in realtime to provide an immediate perception of the image quality and biological conditions of the subject, further assisting in realtime cancer diagnosis. Subsequently, the imaging procedure can be improved for more accurate diagnosis and reduce the need for repeating the process due to unsatisfactory datasets.

INTRODUCTION

A variety of medical imaging modalities such as computed tomography (CT) scanning[1], ultrasound imaging[2] and magnetic resonance imaging (MRI)[3] are used to capture digital images of biological tissue and structures. The laser scanning confocal endomicroscope (LSCEM)[4] is an emerging modality used to perform non-invasive, *in vivo* scanning at a microscopic scale beneath the tissue surface. This technique is also useful to capture volumetric datasets by capturing slices at progressive depth levels. The LSCEM which is an extended variation of the laser scanning confocal microscope (LSCM)[5,6], has a miniature probe to perform *in vivo* imaging on live tissue in hard to reach areas.

Typically, LSCM is able to penetrate and section a specimen up to 50 μm or more[7], which enables image acquisition at different depths, subsequently permitting volumetric dataset capturing. The image quality is much improved compared to other widefield microscopy techniques[6] due to the isolation from background fluorescence signals. Furthermore, this modality enables live *in vivo* imaging[8], minimizing patient's agony of biopsy and

avoiding flaws arising due to physical tissue cutting and specimen staining.

However, the procedural tasks for undergoing LSCEM imaging involve consumption of valuable time and resources. Firstly, current LSCEM imaging systems require manual control fully operated by the user. Secondly, the presence of typical imaging flaws such as poor dataset quality, which can only be realized after imaging ceases, will require the procedure to be re-performed. Apart from that, the lack of a realtime volume visualization system limits the flexibility for changes on-the-spot during imaging.

Performing diagnoses for diseases and medical abnormalities is a complex operation which requires trained expertise to provide an accurate outcome. However, having experienced diagnosticians alone is not enough, as the help of additional imaging and computing systems are also crucial. There has been discussion about the need for interactive visualization of LSCM datasets[9]. Consequently, it is meaningful to explore various ideas and methods proposed to view 3D datasets as 2D images. Scientific visualization of medical datasets has always been important to aid practitioners relate changes across acquired data images and perceive them visually for more precise analyses. These relations and changes are normally difficult or impossible to realize under traditional methods of reading separate data. It is also clinically useful if the captured slices can be perceived from arbitrary viewing angles or highlight desired features. These functions introduce user interaction and further promote the effectiveness of visualization.

In view of that, we present an online visualization system which performs volume rendering on-the-fly while LSCEM imaging is being carried out. In our aim to improve the imaging procedure, this system should perform the required tasks without alterations to its pre-existing features and performances.

PREVIOUS WOKE

The LSCEM is a useful tool used to visualize microscopic tissue structures to a high level of detail. Since the advent of LSCEM for medical-based applications, many procedures have used this modality to obtain volume datasets[4,10-11]. Further improvements on the obtained datasets involved work including image processing methods. Some of the works include deconvolution methods[12-14] to mitigate image degradation[15], and volume reconstruction[16] for the misalignment problem. Recently LSCM has also been combined with reconfigurable computing to provide realtime imaging for instant diagnosis and tissue assessment[17].

Initial attempts to visualize LSCM datasets using volume rendering is presented by Sakas *et al*[18]. The authors discussed methods to render LSCM datasets, including surface reconstruction[19], maximum intensity projection (MIP)[20], and using illumination models. A specially developed visualization system known as proteus[21] is used to visualize time dependent LSCM data, focusing on the analysis of chromatin condensation and decondensation during mitosis. From the conducted experiments the authors concluded that there was no all around visualization technique at the time of publishing, and that various techniques should be offered, each with its own strengths and weaknesses.

Fast visualization of LSCM datasets using the ray casting algorithm has shown the capability to provide interactive examination of datasets, improving the understanding of LSCM imaging. Further enhancement using pre-processing for intensity compensation and image deconvolution to enhance the dataset is also useful.

Volume rendering is the study of displaying 3D objects onto a 2D screen. A generalized rendering equation[22] is initially used to model physical properties of light transport. This equation describes the way objects are viewed in reality, enabling computing systems to simulate these phenomena. Subsequently, this equation is used as a theoretical basis for varying model derivations[23]. One of the most commonly used methods for rendering objects is the ray tracing algorithm. The algorithm can be used to model different objects, including polynomial surfaces[24], fractal surfaces and shapes[25], as well as volumetric densities[26].

Initially, CT scanned datasets are visualized using multiplanar reconstruction[27], which provides cross sectional representations using arbitrary configurations. The VolVis framework[28-29] is used to visualize MRI and confocal cell imaging datasets. This system is further extended to include global illumination and shadow casting[30]. A multidimensional transfer function[31] is used to render the human head and brain datasets into meaningful imaging, enabling the human user to intuitively identify different tissue material and boundaries. An open source toolkit known as the medical imaging interaction toolkit (MITK)[32], promotes interactivity and image analysis specifically for visualization of medical datasets. This system is subsequently included in the development of a software platform for medical image processing and analysis[33], with the aim of incorporating medical image processing algorithms into a complete framework.

MATERIALS AND METHODS

Overview

The involved imaging device in our project is the Optiscan FIVE1 Endomicroscope (Optiscan Imaging Ltd., Victoria, Australia). This device is capable of scanning an area of 475 μm × 475 μm with up to 1024 × 1024 resolution. The scanning time for each slice with the highest resolution is approximately 1.4 s. Operating the LSCEM device requires at least one person. Functional interfaces include: (1) directing the probe to the desired imaging area; (2) controlling image scanning using a footswitch control panel; and (3) managing the dataset through the imaging software on a PC.

The conventional imaging procedure on the device is commenced by applying fluorescent material to the tissue. After absorption, the tissue will emit light signals when laser light is applied. Capture trigger and imaging depth are fully operated manually by the user. To capture a full volumetric dataset, the operator has to repetitively increment depths and capture slices individually. This

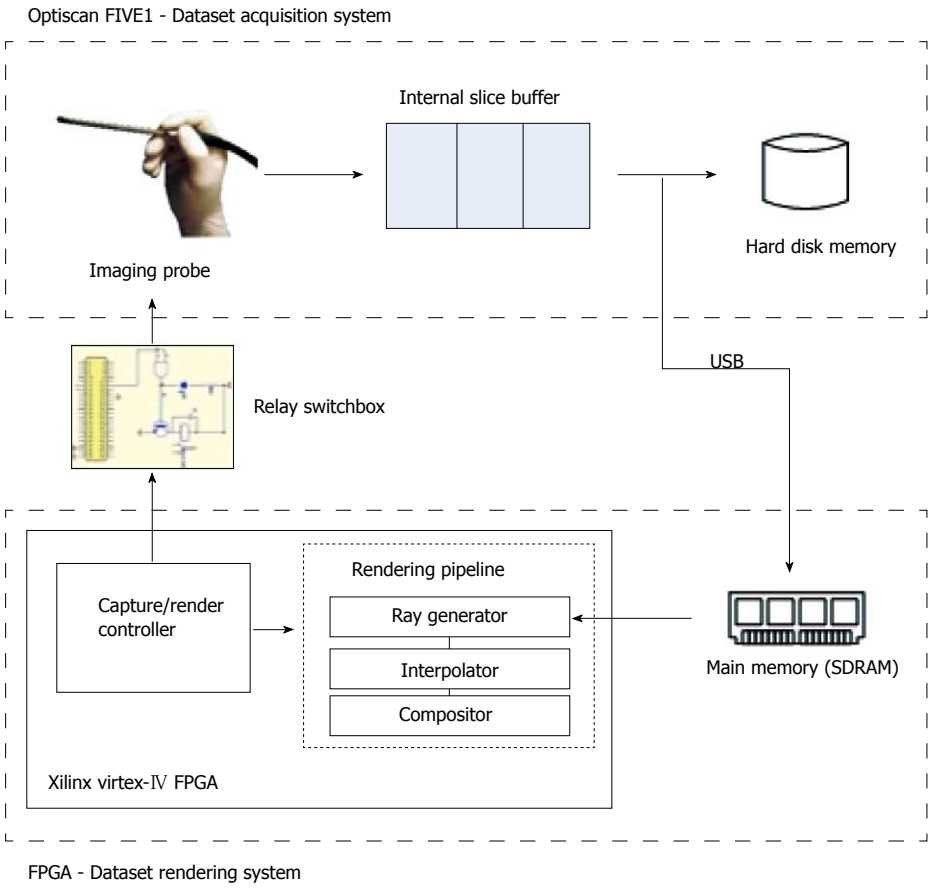

Figure 1 The system architecture.

results in a prolonged imaging session, where tissue or probe movements may be introduced to compromise the dataset accuracy. Also, such controls are tedious and repetitive, so an automated system is desired.

We built our prototype system on a field-programmable gate array (FPGA) chip. The Celoxica RC340 FPGA Development board is the main platform we use to program the FPGA. FPGAs are future oriented building bricks that allow logic circuits to be implemented in any fashion. System-on-Chip designs can thus be built on a single device using FPGAs. FPGAs are also well-known for the reconfigurable feature, which allows different designs to be implemented on the same chip. This promotes lower design costs and ease of prototyping. In our system, we integrate automated imaging control, realtime rendering and task allocation into a single device, which makes FPGAs suitable as they do not adhere to any fixed computational or programming models.

The FPGA is responsible for running three core tasks in parallel. Firstly, to efficiently couple the imaging and rendering systems reliably, task allocation and synchronization is maintained by the FPGA. Secondly, the FPGA acts as an automated controller to replace the existing footswitch for dataset acquisition. Finally, the entire volume rendering system is built on the FPGA due to support for full algorithm flexibility.

The image-ordered ray casting algorithm is used for rendering. This algorithm casts rays from each pixel on the screen towards the dataset and is an iteration process which visits each pixel on the viewing screen. Along each ray, points of a constant distance are sampled. Finally sampling points on each ray are combined in a compositing module and the output is used as the display pixel. Various compositing methods are present, commonly MIP, averaging, nearest-point and alpha compositing.

System design

We designed the imaging-rendering system as shown in Figure 1. We developed the novel volume renderer using reconfigurable hardware for experimentation flexibility and cheaper cost for replicating prototypes. Through a handheld probe the Optiscan FIVE1 imaging device captures endomicroscopic images at incremental steps within the tissue depth to build up volumetric data. This is achieved by detecting the light signals emitted from the fluorescent chemical applied to the biological tissue. These signals, when captured by confining to a single focal plane, form an image slice. Consecutive slices captured across increasing depth will thus form a volume dataset.

Default operations involve using the provided footswitch for depth control, calibration and triggering the capture process. The supplied configuration requires the user to increment capture depths slice-by-slice and send instructions to manually progress to the next slice and trigger a capture. This poses disadvantages which include prolonged capture time and complications that arise, due

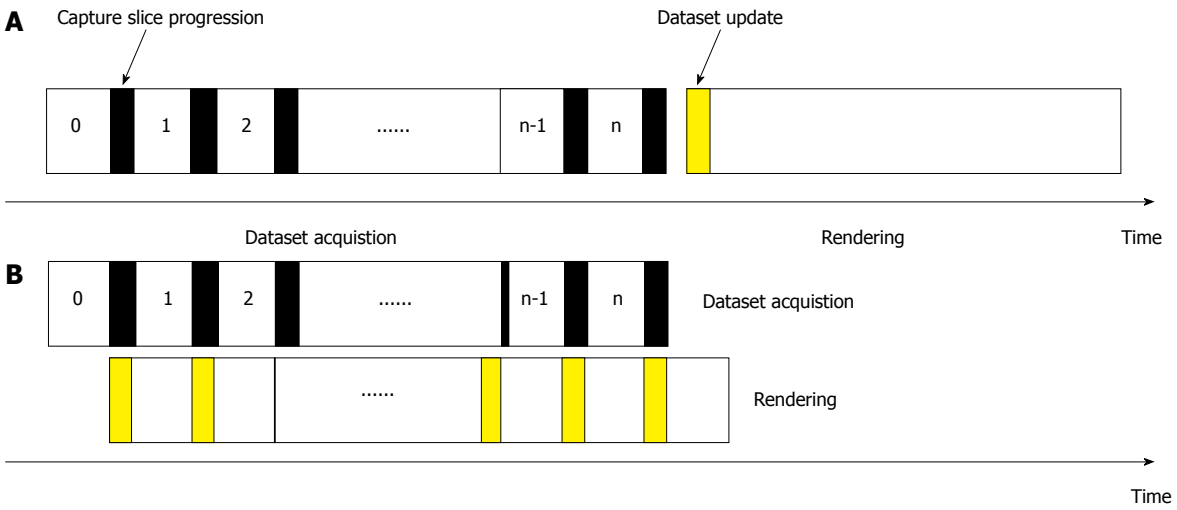

Figure 2 Imaging-rendering allocation. A: Conventional procedure; B: Simultaneous dataset capture and rendering.

to change of state and condition of the tissue. Requiring the user to hold the probe while performing stepping controls on the footswitch will introduce distortion due to movements, which is significant at a microscopic scale.

Apart from that, to perform continuous online rendering, scanning along the z-depth direction must be continuous and progress incrementally deeper into the tissue. In response, we developed an alternative hardware to substitute the footswitch[34]. The main task of the relay switchbox hardware is to replace the manual controls of the footswitch by sending automated signals at constant intervals to increment the z-depth value every time a slice capture is complete. The user is no longer required to manually increment plane depths and initiate capture with this substitute.

For this design, the FPGA is required to fulfill the following requirements: (1) capability to perform all the rendering computations; (2) provide realtime control signals over the imaging device; and (3) support automated scanning and capturing controls.

In the LSCEM device, the captured data are buffered in an internal memory while the scanning point moves across the subject in a raster scanning fashion. We forward an entire slice after each completed capture *via* the USB to our rendering device, ensuring that the rendered result is continuously updated as soon as the slice is acquired. Once the first slice is received, rendering is initiated. An electronic controller manages the rendering and imaging tasks, so the operations of the imaging device do not affect the renderer and vice versa. Rendering is performed in conjunction with acquisition, where after each capture the relay switchbox instructs the probe to progress to the next slice automatically.

The imaging-rendering arrangement is illustrated in Figure 2. Conventional practices require the full dataset to be completed and imaging to be ceased before rendering in an alternative engine. We allocate these two processes to be performed concurrently, so that immediate results can be shown as soon as a slice is captured. The rendering and imaging operations are performed in parallel, and the imaging always captures an additional slice to be rendered for the next cycle.

The process is initiated with a slice capture by the imaging system, while the renderer is on standby as the dataset is empty. After a complete slice is captured, it is forwarded and stored in the SDRAM of the FPGA system. Rendering proceeds by retrieving required voxels for computation from the SDRAM. The SDRAM as a shared resource between imaging and rendering must be allocated to be mutually exclusive to avoid access conflicts.

The operations repeat until the final slice as determined by the user, when capturing is ceased. Rendering can still be performed on the existing dataset until it is terminated by the user.

OPTIMIZATIONS

To accommodate for the realtime requirements of the system, we have also employed several custom designed optimizations on the FPGA rendering system.

Pre-computing ray parameters for ray casting traversal

This optimization eliminates repetitive matrix transformation computations for sampling coordinates. In this case, matrix multiplications are reduced to simple arithmetic addition and subtraction operations. The ray parameters are represented by a coordinate for the ray origin and a vector for the ray direction.

Cubic memory organization for dataset storage

We store the dataset voxels according to a customized arrangement in the SDRAM as shown in Figure 3. This is due to trilinear interpolation for sampling voxels, which requires eight memory accesses to the SDRAM. Storing dataset voxels in a cubic fashion enables fast retrieval of these points for computations in a single cycle due to the 64-bit data bus. Using this technique, sampling points require less memory access for more efficient operations.

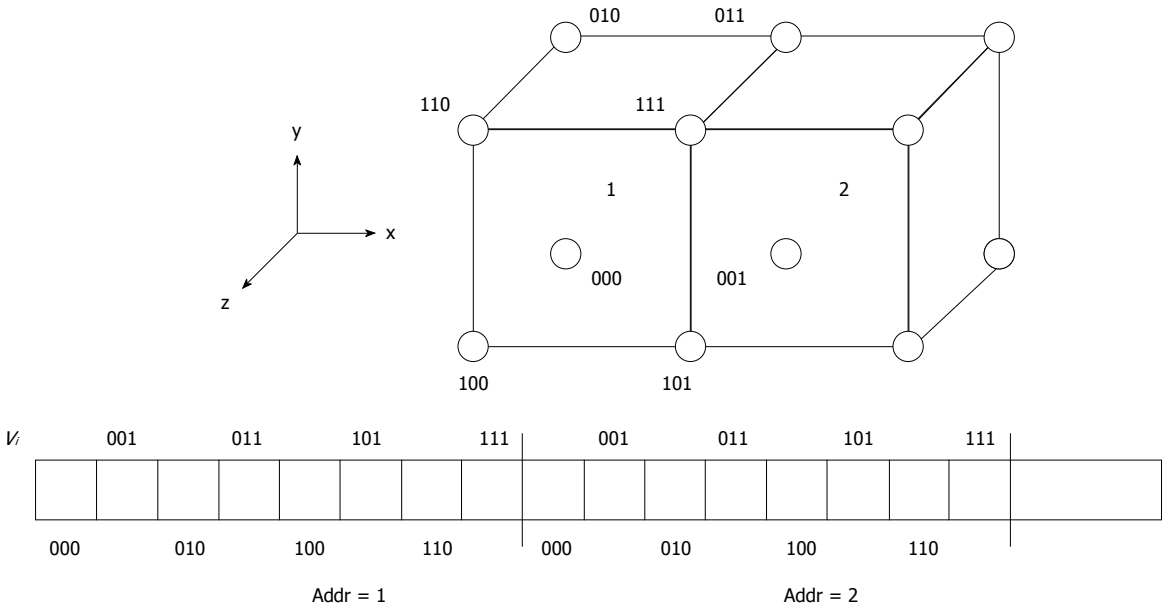

Figure 3 Cubic memory organization: Voxels are arranged in memory as cubes.

Accumulative rendering mode

We introduce the accumulated rendering mode in this system. Rather than revisiting the whole dataset when a new slice is updated, the previous rendering output is directly combined with the new slice instead. Through this computation, load is limited to only two slices, regardless of the size of the dataset. Modifications to the existing compositing methods will be required, and we would anticipate further refinement and utilize it to our advantage. We perform compositing for accumulative rendering using the following formula.

To accommodate the accumulative rendering mode, we use four framebuffers to store intermediate slices. This novel design is illustrated in Figure 4. We use four SRAM blocks as the framebuffers, two of which facilitate compositing and the remaining two as display buffers. This is used to overcome the problem of simultaneous read/write accesses, prohibited by the use of a single data bus.

During operation, the first slice only involves memory writing, where one SRAM from each framebuffer is accessed to store the acquired slice. In all other stages, the SRAM blocks interchange between memory reading and memory writing to ensure continuous flow, providing simultaneous reading and writing of rendering results. This is significant in the case of parallel pipelined coding, where memory read and write operations are continuous at each cycle, while the operations are only repeated once for each pixel. In single data placement systems, the read and write operation has to be time-multiplexed, resulting in longer time required for processing.

Optimization limitations

The introduction of these optimization techniques speeds up the rendering computation, but at the expense of several limitations. In online mode, a constant viewing configuration is employed. Viewing angles and distances cannot be adjusted in this mode, as pre-computation of ray parameters assumes that the rays are always fixed in such a manner. This limitation is further reinforced with the method of combined slices, as a view change will require the whole dataset to be re-traversed instead of only visiting the existing rendering and the new slice.

Rendering is also performed only for parallel projections, which assume that all casted rays have the same direction. Further extensions can be done to include perspective projection ray casting through modification of the system and additional memory requirements to store different ray parameters for each pixel.

Also, cubic memory scheme organizes memory cubes continuously instead of interleaved. This introduces duplications of the voxels in memory, thus increasing the memory requirements to approximately eight times dataset size. However, the capacities supported by modern hardware and our system of 256 Mbytes on-board memory can easily satisfy these requirements.

Accumulative rendering mode is currently supported only for orthographic projection rendering. The viewing direction is always assumed to be perpendicular to the slices for accumulative rendering. Further refinement of this design can enable views from arbitrary directions. As this technique only renders two slices, cubic memory is not employed for rendering under this mode, but is used for rendering under arbitrary view configurations.

EXPERIMENTAL RESULTS AND DISCUSSION

The prototype system has been built and deployed with our collaborators at the National Cancer Center of Singapore (NCCS) for clinical experiments. A photo of the system is shown in Figure 5. This system includes the Optiscan FIVE1 LSCEM imaging device, the Celoxica

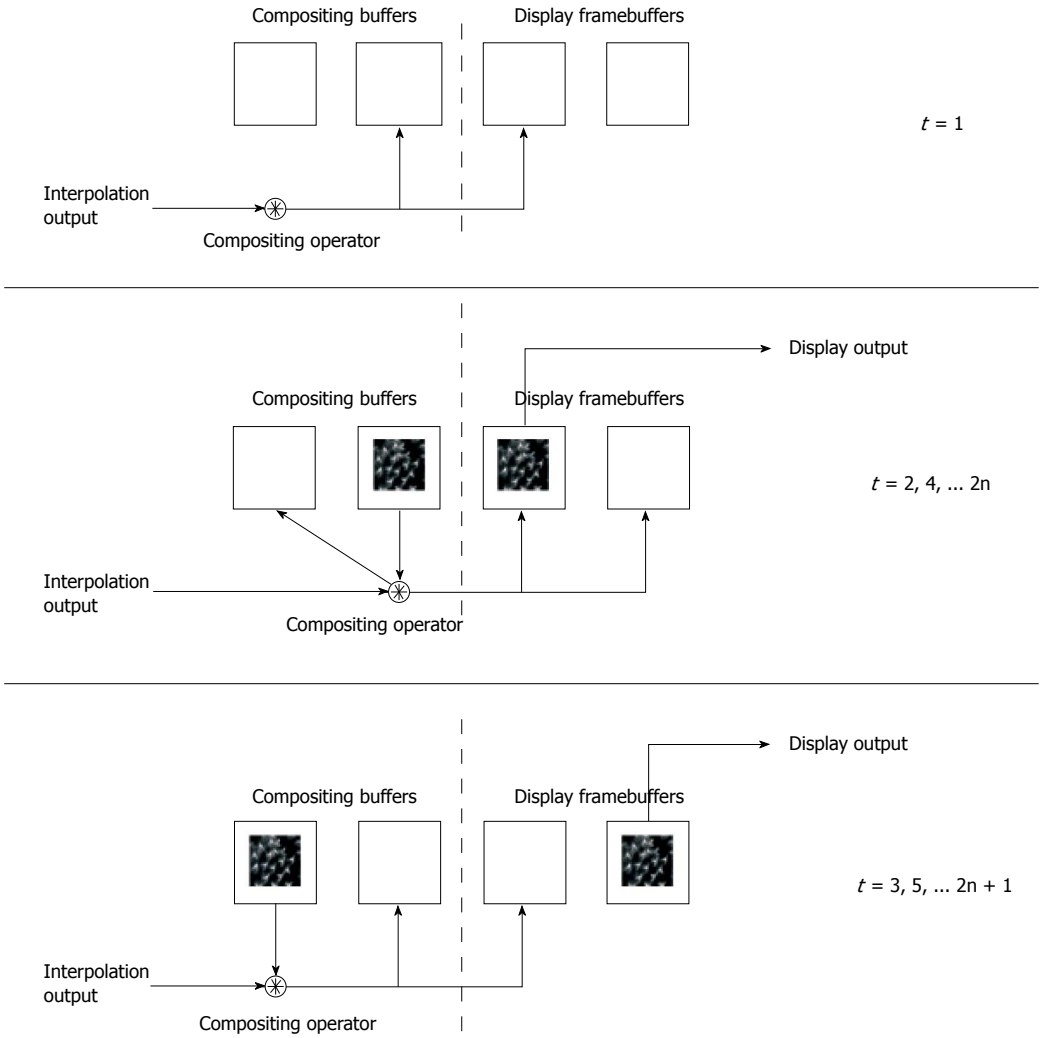

Figure 4 Framebuffers for accumulative rendering mode.

RC340 FPGA Development board, the relay switchbox hardware, and user interactivity tools.

We show the realtime online rendering results for a mouse tongue dataset in Figure 6. The rendering results are live results obtained during the imaging experiment. For rendering performance, the rendering system has to at least be able to perform faster than the rate slices are acquired in order to be meaningful. This rate is approximately 1.4 s per slice. The maximum number of slices is dependent on the significance of signal degradation as the focal plane advances deeper into the tissue. For our case, the maximal is around 40 slices.

The rendering results in Figure 6 show two different views. The orthographic projection renders the dataset by setting the viewing plane parallel to the acquired slices, under accumulative rendering mode. This shows perceived results viewed through consecutive depths. At the bottom right corner is a parallel projection with a specific viewing configuration, which shows an overview of the current dataset. Observations can be made through these different views across increasing thicknesses to the dataset.

The rendering will provide immediate perception of the acquired datasets at high quality. This is aimed towards improving the imaging procedure and further enhancing the features and capabilities of LSCEM imaging. With this current platform, further extensions can be included, such as image processing techniques to provide automated diagnosis of cancer stages[17].

The entire imaging procedure is now simplified, and can be fully operated by a single user. The user can adjust the probe either by a handheld device or through a fixed stand. Capture controls are fully automated, where the user is only required to initiate and terminate imaging controls. This enhances the imaging time as well as requiring less attention from the user to operate different controls at the same time.

CONCLUSION

We have presented our preliminary system to perform online rendering side-by-side with LSCEM imaging. By having an immediate knowledge of the dataset quality as well as the biological tissue conditions, alterations can be made on the spot. This will introduce the opportunity to change imaging conditions or medical decisions according to the online rendering results. This work is also mo-

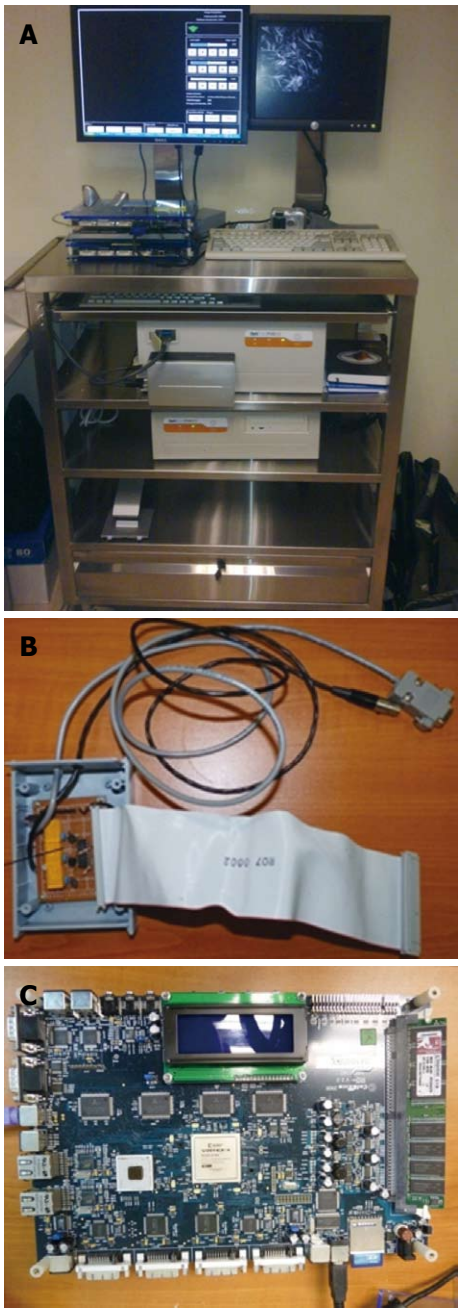

Figure 5 The Laser scanning confocal endomicroscope imaging-rendering prototype system.

tivated by the need to realize the quality of the captured datasets in realtime to reduce excessive time required for offline rendering.

The LSCEM procedure is also simplified and only one user is required to fully operate the system. Using electronic automation to substitute the footswitch as manual controls, the user is not required to repetitively control the imaging sequences to capture a full volumetric dataset. This reduces the time and attention required to perform LSCEM imaging.

We have also shown the FPGA as a viable solution for designing a system which can run the required tasks in parallel. Providing automated controls to substitute the prevailing manual capture, we make use of our custom built hardware to interface with the imaging system. Also the FPGA is able to provide synchronized controls between imaging and rendering, which is crucial in ensuring a stable operation. The full flexibility offered by FPGAs is important, considering future developments for embedded rendering systems to co-exist with imaging devices.

We would extend this work further by incorporating more sophisticated rendering procedures such as perspective projection rendering and interactive view changes in realtime.

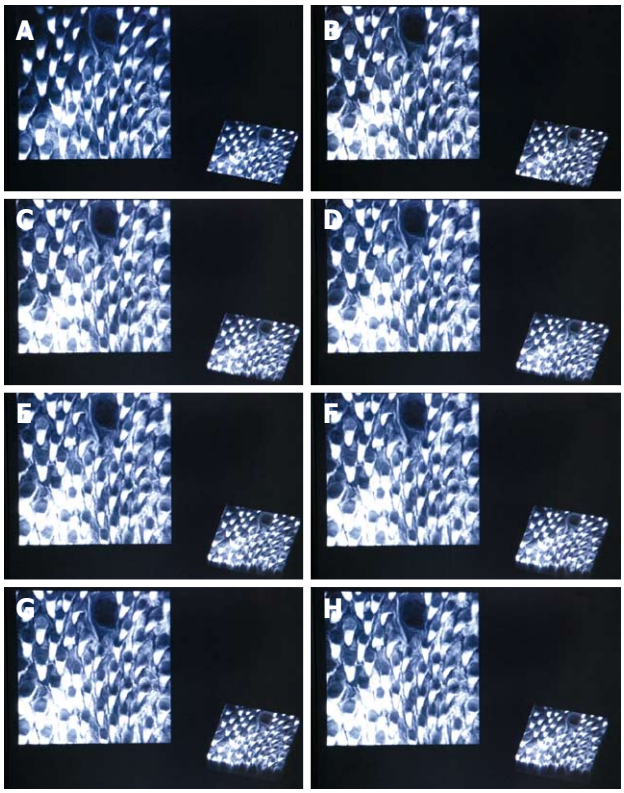

Figure 6 Online rendering of the mouse tongue surface across increasing number of slices. A: 2 slices; B: 10 slices; C: 15 slices; D: 20 slices; E: 25 slices; F: 30 slices; G: 35 slices; H: 38 slices.

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ORIGINAL ARTICLE

Automation of immunohistochemical evaluation in breast cancer using image analysis

Keerthana Prasad, Avani Tiwari, Sandhya Ilanthodi, Gopalakrishna Prabhu, Muktha Pai

Keerthana Prasad, Manipal Centre for Information Science, Manipal University, Manipal 576104, Karnataka, India
Avani Tiwari, Muktha Pai, Department of Pathology, Kasturba Medical College, Manipal University, Manipal 576104, Karnataka, India
Sandhya Ilanthodi, Department of Pathology, A.J Institute of Medical Sciences, Manipal 576104, Karnataka, India
Gopalakrishna Prabhu, Department of Biomedical Engineering, Manipal Institute of Technology, Manipal 576104, Karnataka, India
Author contributions: Prasad K designed and carried out this research; Tiwari A carried out the sample collection, tissue processing and acquisition of images; Tiwari A, Pai M and Ilanthodi S provided the readings for the manual evaluations; Prabhu G supervised this research; all authors contributed to the writing of this paper.
Correspondence to: Keerthana Prasad, Assistant Professor, Manipal Centre for Information Science, LG02, Academic Block 5, M.I.T Campus, Manipal 576104, Karnataka, India. keerthana.prasad@manipal.edu
Telephone: +91-984-5308499 Fax: +91-820-2925033
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Abstract

AIM: To automate breast cancer diagnosis and to study the inter-observer and intra-observer variations in the manual evaluations.

METHODS: Breast tissue specimens from sixty cases were stained separately for estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER-2/neu). All cases were assessed by manual grading as well as image analysis. The manual grading was performed by an experienced expert pathologist. To study inter-observer and intra-observer variations, we obtained readings from another pathologist as the second observer from a different laboratory who has a little less experience than the first observer. We also took a second reading from the second observer to study intra-observer variations. Image analysis was carried out using in-house developed software (TissueQuant). A comparison of the results from image analysis and manual scoring of ER, PR and HER-2/neu was also carried out.

RESULTS: The performance of the automated analysis in the case of ER, PR and HER-2/neu expressions was compared with the manual evaluations. The performance of the automated system was found to correlate well with the manual evaluations. The inter-observer variations were measured using Spearman correlation coefficient r and 95% confidence interval. In the case of ER expression, Spearman correlation r = 0.53, in the case of PR expression, r = 0.63, and in the case of HER-2/neu expression, r = 0.68. Similarly, intra-observer variations were also measured. In the case of ER, PR and HER-2/neu expressions, r = 0.46, 0.66 and 0.70, respectively.

CONCLUSION: The automation of breast cancer diagnosis from immunohistochemically stained specimens is very useful for providing objective and repeatable evaluations.

Key words: Automation; Breast cancer diagnosis; Computer aided diagnosis; Image analysis; Immunohistochemical study

Peer reviewer: Songsak Petmitr, PhD, Associate Professor, Department of Tropical Nutrition and Food Science, Mahidol University, 420/6 Ratchawithi Road, Bangkok 10400, Thailand

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INTRODUCTION

Cancer of the breast is the second most common human

neoplasm and accounts for approximately one quarter of all cancers in females after cervical carcinoma[1]. The accurate diagnosis of cancer plays a very important role in the treatment of patients with neoplastic breast disease. Immunohistochemical evaluation of hormone receptor expressions in tumor cell nuclei is an integral part of routine breast cancer diagnosis and provides important information for prognosis and choice of therapeutic approach.

Estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER-2/neu) over-expression as a predictor for herceptin therapy are crucially important in the biology of breast carcinoma. ER and PR expressions are the only predictive factors with proven usefulness in selecting patients who are likely to respond to adjuvant endocrine therapy. Patients lacking these receptors tend to have a shorter disease-free survival and earlier recurrence than those expressing these receptors[2]. In around 20%-30% of breast carcinoma, HER-2/neu is amplified and over-expressed. It is associated with an adverse prognosis independent of other prognostic factors in most cases and appears to be stronger in node-positive carcinoma. Immunohistochemical reactivity of tumor cells to ER, PR, and HER-2/neu helps the clinician to establish the mode of therapy and indicates the survival and recurrence rates of the tumor. The recurred tumor or metastatic tumor may not show the same immunoreactivity, and the unstable status of HER-2/neu in breast cancer is clinically significant[3]. Receptor status in recurred or metastasized breast cancer can be different from the original tumor. It was reported that ER status changed in around 33% of cases, and HER-2/neu status changed in around 10%.

In addition, ER-positive and ER-negative breast cancers have distinct disease-specific patterns[4]. A molecular classification of breast cancer is also performed based on their reactivity. The treatment protocol varies with the pattern of reactivity and is based on the molecular classification. Immunohistochemistry (IHC) is expected to play an increasingly important role in the clinical management of breast cancer[5].

The main challenges that pathologists are currently facing are productivity, accuracy and objective evaluation. Manual evaluation takes more time and resources. It is less accurate and is also highly subjective. Qualitatively, the immunohistochemically stained specimen can be evaluated visually as the presence of a specific color. However, to perform a quantitative evaluation, the number of stained cell nuclei and/or the amount of specimen that has been stained needs to be measured. For this purpose, computerized image analysis based methods are needed.

Traditionally, pathologists distinguish between positive and negative results based on visual judgment of the percentage of positive tumor cells, the cutoff being arbitrarily defined between 5% and 45%[6-9]. Some studies report the use of semiquantitative scores to assess nuclear staining intensity as a marker of the number of receptors per cell[10-13]. Diverse computerized image analysis systems have also been employed to provide more standardized data for quantification and were found to correlate well with semiquantitative scoring methods[14-16]. A large number of studies have reported the use of image analysis as a means of evaluating histological staining. Substantial efforts have been made to correlate the evaluations made by experienced pathologists with quantitative values[17-26]. Initial studies on the use of computerized image analysis were limited to evaluation of images based on gray levels[27,28]. Recently, studies have used the color spectrum of histological stains rather than gray levels for analysis of the images to discriminate cellular details[29,30]. The receptors can be accurately quantified by measuring the strength of expression[13]. Hence, automation of quantification could be very useful for the evaluation of histological staining for the diagnosis of breast cancer. However, their use in the routine diagnostic laboratory is limited due to the high cost and the complexity of the image analysis systems[17].

The goals of the present study were to establish the validity of the in-house developed image analysis system (TissueQuant version 1.0) for classification of the images for the diagnosis of breast cancer and to determine data variability due to investigator bias by calculating inter- and intra-observer variability in the case of manual evaluations. Each case was subjected to immunohistochemical evaluation along with the image analysis system and validation was performed by comparing visual and computer analysis of the same tissue fields.

MATERIALS AND METHODS

Specimens from sixty patients were subjected to immunohistochemistry separately with antibodies for ER, PR and HER-2/neu. The patient's name, age, sex and clinical data were recorded. Out of a total of 60 cases studied 23 (38%) were younger than 50 years while the remaining 37 (62%) were older than 50 years of age. The youngest patient was 30 years and the oldest was 72 years old. The mean age of the patients was 52.5.

The specimens were received in 10% formalin and were sampled after fixation for less than 24 h. Care was taken not to over fix the tissue, as this would interfere with the receptor analysis. The specimens were examined grossly for ulceration, peau d' orange, and retraction of nipple. The deeper resected margin was stained with India ink. Adequate numbers of sections were taken from the nipple and areola, the tumor proper, all the margins with and without tumor, adjacent breast parenchyma and other relevant areas.

Staining protocol

Hematoxylin and eosin (HE) was used as a routine stain to establish the histopathological diagnosis and for general study of the tissue; the markers ER, PR and Her-2/neu were assessed using immunohistochemistry; image analysis was performed using TissueQuant software for each immunohistochemically stained slide.

Immunohistochemistry staining for ER, PR and HER-2/neu was carried out by the polymer labeling 2-step method using the Super Sensitive™ Polymer- HRP IHC detection system (Biogenex).

Slide preparation

Slides were washed in soapy water and then washed three times with distilled water. Thereafter, they were rinsed in methanol and dried at room temperature. Poly-L-lysine solution was applied to the slides which were dried overnight at room temperature. Sections were treated as follows: (1) 5 μ thick sections were cut and mounted on the slides coated with poly-L-lysine. The sections were deparaffinized with 3 changes in xylene, 2 changes in methanol and then a decreasing concentration of isopropyl alcohol (i.e. 90%, 70%, 50% alcohol) and finally in distilled water for 5 min each; (2) The slides were then immersed in 3% hydrogen peroxide for 20 min to quench the endogenous peroxidase; (3) Antigen retrieval and unmasking was carried out by immersion in citrate buffer and incubation in a pressure cooker with 250 mL of water at 100℃ for 15-20 min; (4) After cooling at room temperature, the slides were washed in buffer (0.1 mol/L Tris-HCl, 0.15 mol/L NaCl, pH 7.5) with the washing procedure carried out in a jar containing Tris buffer and immersing the slides for two 5 min periods (total of 10 min); (5) This was followed by incubation for 20 min in the Power block (buffered casein solution with sodium azide) to suppress non-specific binding of subsequent reagent; (6) The slides were then incubated in mouse primary antibody for 75 min at room temperature, after which they were washed in Tris buffer. For the ER study, mouse monoclonal antibody diluted with HK941-YAK in buffered glycine phosphate pH 7.1, 6% protein and 0.09% sodium azide was used. For the PR study, mouse monoclonal antibody in phosphate buffered saline pH 7.6 containing 1% bovine serum albumin (BSA) was used. For the HER-2/neu study, mouse monoclonal antibody from tissue culture supernatant diluted in phosphate buffered saline pH 7.6 containing 1% BSA and 0.09% sodium azide was used; (7) The slides were then immersed in Super Enhancer for 30 min, after which the washing procedure was repeated; (8) The slides were immersed again in Poly HRP (horseradish peroxidase) for 20 min, after which they were washed with Tris buffer followed by distilled water for 5 min each; (9) The slides were then treated with diaminobenzidine (DAB) chromogen for 5 min to develop the brown color; (10) Thorough washing in Tris buffer and distilled water was then performed; and (11) Counter staining with Meyer' s hematoxylin was performed for 1 min and then the slides were washed in tap water. The slides were dried and mounted with a cover slip and mounting media.

Immunohistochemistry scoring

ER and PR expressions: According to the International Breast Cancer Study Group, ER and PR were graded as: (1) None (grade 0): none of the tumor cells showed nuclear staining; (2) Low (grade 1): 1%-9% of cells showed nuclear positivity; and (3) High (grade 2): ⩾ 10% of the cells showed nuclear positivity.

When considering the hormone receptor status, grade 1 and 0 were considered hormone receptor negative, and grade 2 was considered positive.

HER-2/neu expression: According to US FDA panel findings, (1) Grade 3: cell surface protein expression-positive: defined as uniform intense membrane staining of > 30% of invasive tumor cells; (2) Grade 2: cell surface protein expression- equivocal: defined as complete membrane staining that is either non uniform or weak in intensity but with obvious circumferential distribution in at least 10% of cells; and (3) Grade 0 or 1: cell surface protein expression- negative: no staining or weak, incomplete membrane staining in any proportion of the tumor cells.

When considering the HER-2/neu membrane status, grade 0, 1 and 2 were considered negative and grade 3 was considered positive.

Image analysis

Images were analyzed using the in-house developed software (TissueQuant). The facility for choosing the color representing the maximum density of hormone expression as a reference color is provided in the software. Using this facility, the color setting was used for analysis purposes. The software assigns scores to the various shades of the color represented by each pixel of the image, based on how close the shade is to the reference color. Using these values, the total hormone expression in the image is quantified. For this, the image is represented in the HSI color model. Gaussian weighting functions are used for scoring the shades. The widths of the Gaussian weighting functions are decided by the different ranges for the hue, saturation and intensity components. These values decide the range of shades of the color which should be considered as positive staining. These weighting functions provide the flexibility of fine adjustments of the color shades to be included.

Considering two parameters based on the color scores, a classification system was developed to classify a particular case as positive, negative and strongly positive. There were two parameters assigned-Mean and Mean-Max. Mean was the main criterion on which the decision was made. It represents the average hormone expression present in the image. Mean-Max was used as a helpful parameter for decision-making. This represents the maximum depth of the color shade present in the image. This was useful when the expression was concentrated in a small area. A grade was calculated based on the above two scores.

A screenshot of the software is shown in Figure 1. The user is provided with a facility to open the image and click on the region of the image with the reference pixel color. A set of sliders are provided to adjust the color parameters on the lower mid panel. The centers and widths of the Gaussian weighting functions can be adjusted with these sliders. The color parameters are set this way to calculate the color score for each pixel which is mapped from 0 to 255 for the purpose of display as a grayscale image in the right upper panel of the user interface. The color score and the color settings for the particular study are displayed just below the resulting image. This facilitates user interaction to select the appropriate color settings for the quantification. A "Save"button is also provided which

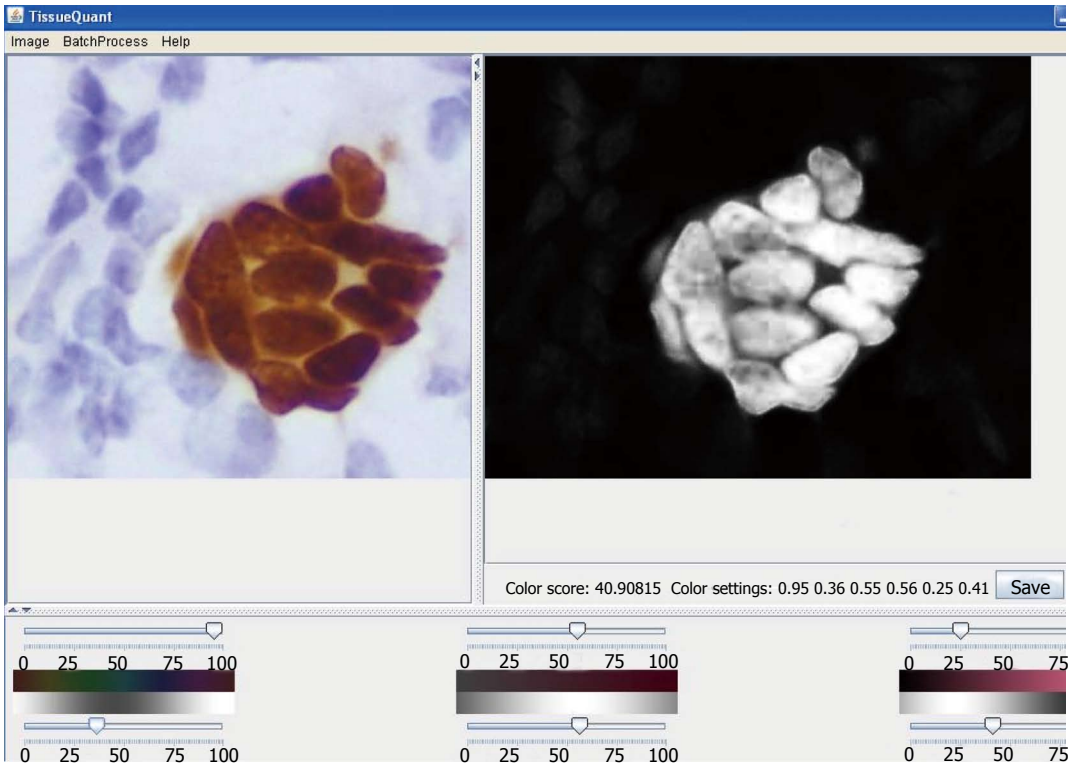

Figure 1 Screenshot of the TissueQuant software. On the left panel of the main window the original image is opened. The right panel displays the color score representation of the image. In the lower panel are the sliders which could be adjusted to select the color shade of interest.

facilitates saving of the resulting image. We could also save the color settings by specifying a name for the particular study. These color settings can be used for batch processing of image sets with the same color settings in an automated manner. To work on huge sets of images with the same color setting directly, the "Batch Analysis" option is used. The directory in which the images are saved, the color setting and the image type used for the batch analysis need to be specified by the user. All images in the specified directory are processed and the resulting images are stored in the 'result-images' directory, which is generated under the specified directory. The mean color score and the image name are stored in an MS Excel file in the same directory.

Manual method and image analysis

The specimens from all sixty cases were stained separately for ER, PR and HER-2/neu. All cases were assessed by manual grading as well as image analysis. The manual grading was carried out by an experienced expert pathologist. To study inter-observer and intra-observer variations, we obtained readings from another pathologist as the second observer from a different laboratory who has a little less experience than the first observer. We also took a second reading from the second observer after 30 d to study intra-observer variations.

For statistical analysis of the results, we used SPSS 11.5 for Windows and GraphPad Prism 4.03. The inter-observer and intra-observer variations were evaluated with SPSS software. The Spearman correlation coefficient was obtained using GraphPad Prism software. A ROC curve was drawn using Microsoft Excel software using the sensitivity and specificity of the algorithm for each of the expressions.

RESULTS

A comparison of the grading of the cases by the expert and the image analysis software TissueQuant for ER, PR and HER-2/neu expressions are shown in Tables 1-5 and Figure 2.

ER

All cases were assessed by manual grading as well as image analysis. Thirty cases with grade 0 on manual grading showed a similar grade on image analysis. Out of 12 cases with a manual grading of 0, 6 showed grade 1 and 6 showed grade 2 on image analysis. Two cases with manual grade 1 showed grade 2 on image analysis. All cases with manual grade 2 showed a similar grade on image analysis. These findings were statistically highly significant (Table 1).

The ER expression evaluation performed by the expert was compared with the evaluation by the second pathologist. Evaluation by both pathologists in 24 cases of grade 0 out of 42 cases, 2 cases of grade 1 out of 4 cases and 8 cases of grade 2 out of 14 cases matched correctly. The ER expression evaluation was repeated by the second pathologist and was compared to the first evaluation. The evaluation in both readings matched in 19 cases of grade 0 out of 26 cases, 12 cases of grade 1 out of 21 cases and 6 cases of grade 2 out of 13 cases.

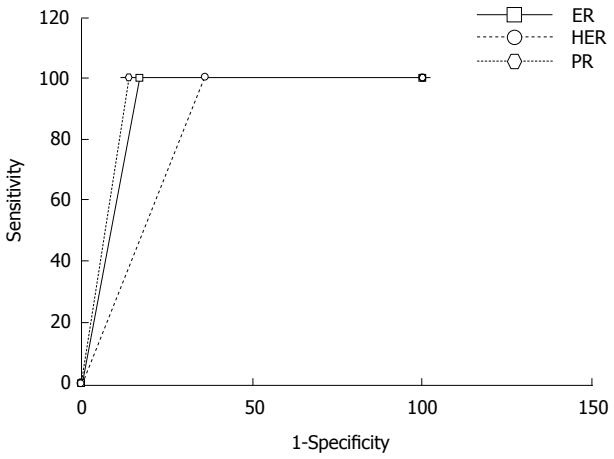

Figure 2 ROC curves for the automated evaluation of estrogen receptor, progesterone receptor and human epidermal growth factor receptor-2 expression images using TissueQuant. For all three expressions the sensitivity of 100% is maintained, the specificity for PR expression is best at, 86%, for ER the specificity is 82.3% and for HER-2/neu expression the specificity is least at, 64.3%. ER: Estrogen receptor; PR: Progesterone receptor; HER-2/neu: Human epidermal growth factor receptor-2.

Table 1 Comparison of manual grading and image analysis of estrogen receptor

Manual grading	Image analysis			Total
	Grade 0	Grade 1	Grade 2	
Grade 0	30	6	6	42
Grade 1	0	2	2	4
Grade 2	0	0	14	14
Total	30	8	22	60

PR

PR status was also assessed by manual grading as well as image analysis. Thirty five cases with grade 0 on manual grading showed a similar grade on image analysis. Out of 8 cases with a manual grading of 0, 6 showed grade 1 and 2 showed grade 2 on image analysis. Five cases with manual grade 1 showed grade 2 on image analysis. All cases with manual grade 2 showed a similar grade on image analysis. These findings were statistically highly significant (Table 2).

The PR expression evaluation performed by the expert was compared with the evaluation by the second pathologist. Evaluation by both pathologists in 32 cases of grade 0 out of 43 cases, 6 cases of grade 1 out of 7 cases and 6 cases of grade 2 out of 10 cases matched correctly.

The PR expression evaluation was repeated by the second pathologist and was compared to the first evaluation. The evaluation in both readings matched in 28 cases of grade 0 out of 34 cases, 8 cases of grade 1 out of 18 cases and 3 cases of grade 2 out of 8 cases.

HER-2/neu

For HER-2/neu status, 6 cases with manual grade 0 showed a similar grade on image analysis. One case with a manual grade of 0 showed grade 2 on image analysis. None of the cases showed grade 1 on manual as well as image analysis. Five cases with manual grade 2 showed grade 3 on image analysis. All 46 cases with manual grade 3 showed a similar grade on image analysis. These findings were highly significant (Table 3).

Table 2 Comparison of manual grading and image analysis of progesterone receptor

Manual grading	Image analysis			Total
	Grade 0	Grade 1	Grade 2	
Grade 0	35	6	2	43
Grade 1	0	2	5	7
Grade 2	0	0	10	10
Total	35	8	17	60

Table 3 Comparison of manual grading and image analysis of human epidermal growth factor receptor-2

Manual grading	Image analysis				Total
	Grade 0	Grade 1	Grade 2	Grade 3	
Grade 0	6	0	1	0	7
Grade 1	0	0	0	0	0
Grade 2	0	0	2	5	7
Grade 3	0	0	0	46	46
Total	6	0	3	51	60

HER-2/neu expression evaluation performed by the expert was compared with the evaluation by the second pathologist. Evaluation by both pathologists in 6 cases of grade 0 out of 7 cases, 3 cases of grade 2 out of 7 cases and 29 cases of grade 3 out of 46 cases matched correctly.

HER-2/neu expression evaluation was repeated by the second pathologist and was compared to the first evaluation. The evaluation in both readings matched in 6 cases of grade 0 out of 7 cases, 2 cases of grade 2 out of 7 cases and in all 46 cases of grade 3.

DISCUSSION

Various types of solutions are available to quantify staining intensity and range from inexpensive, general purpose software to specific, expensive software. Some of the image analysis systems used for such studies are SAMBA, Image Pro Plus, Metaview, Lucia software, and BioQuant Nova Prime. Charpin *et al* used Metaview software for staining intensity quantification. This is a general purpose image processing software[31]. Suitable threshold values for the Red (R), Green (G) and Blue (B) components are selected to choose the stained area. The amount of positively stained area gives the measure of staining. Charpin *et al*[32] used the SAMBA 4000 image analysis system for quantification of hormone receptor expression. For each marker's positive cell surface, integrated and mean optical densities and IOD histograms were compared. Pauschinget *et al*[33] and Soukupova *et al*[34] made use of Lucia software for stain quantification, which uses a measure of optical density. Diaz Encarnacion *et al*[31] and Niendorf *et al*[35] used a threshold and area measurement approach. Hatanaka *et al*[20] used WinROOF with macroinstructions for analyzing each captured area. Lehr *et al*[17] used Adobe

Table 4 Results of the classification of estrogen receptor, progesterone receptor and human epidermal growth factor receptor-2 expression images

	True positive	True negative	False positive	False negative	Sensitivity (%)	Specificity (%)	PPV[1] (%)	NPV[2] (%)
ER expression	14	38	8	0	100	82.6	63.6	100
PR expression	10	43	7	0	100	86.0	58.8	100
HER-2/neu expression	46	9	5	0	100	64.3	90.2	100

[1]PPV: Positive predictive value; [2]NPV: Negative predictive value. It can be seen that the sensitivity has been maintained at 100%. The specificity of the evaluations for ER and PR is good. The specificity of HER-2/neu expression is comparatively less than the ER and PR expression evaluations. ER: Estrogen receptor; PR: Progesterone receptor; HER-2/neu: Human epidermal growth factor receptor-2.

Table 5 Spearman correlation coefficient (95% confidence interval) of the different evaluations

	Expert *vs* automated analysis[1]	Expert *vs* pathologist[2]	Pathologist reading 1 *vs* pathologist reading 2[3]
ER expression	0.73 (0.59 to 0.83)	0.53 (0.32 to 0.69)	0.46 (0.24 to 0.64)
PR expression	0.82 (0.73 to 0.90)	0.63 (0.45 to 0.76)	0.66 (0.49 to 0.78)
HER-2/neu expression	0.92 (0.88 to 0.96)	0.68 (0.52 to 0.80)	0.70 (0.54 to 0.81)

[1]Represents the correlation of the automated analysis with the expert's evaluation; [2]Represents the correlation between two pathologists; [3]Represents the correlation between readings by the same pathologists taken at different time periods. It can be seen that the Spearman correlation coefficients for evaluations by automated analysis are better for all receptor expressions.

Photoshop-based image analysis to quantify hormone receptor expression in breast cancer. The feature selection was done with the Magic Wand tool which could reliably select all immunostained nuclei. The nuclear immunostaining index was calculated as the difference between nuclear and background immunostaining intensity. Vrekoussis *et al*[36] reported the use of freeware ImageJ for the analysis of immunohistochemically stained sections of breast cancer. McCabe *et al*[37] and Chung *et al*[38] carried out quantitative analysis of hormone receptor expressions in breast cancer using the AQUA system. BioQuant Nova Prime is an advanced image analysis tool designed for biomedical research. Ariol SL 50 is an automated microscope slide analysis tool, which acquires monochrome images through three bright field filters, using cell masking templates and applies area analysis. Image Pro Plus (Media Cybernetics) and EMPIX Imaging solutions are also being used for stain quantification. Sharangpani *et al*[39] developed a semi-automatic system to quantify estrogen and progesterone receptor immunoreactivity in human breast cancer. All these applications work on the basis of threshold and area measurement or change in optical density. It is not always possible to select appropriate R, G and B thresholds to suitably select shades of a particular color. Hence, the approach of threshold and area measurement is inadequate. In addition, when a tissue section is studied for a particular substance, other components present in the section may also take up the stain, expressing different colors. In such cases, the change in optical density is not a suitable indicator to measure the amount of the substance under study. However, the in-house developed software, TissueQuant, overcomes all these drawbacks by facilitating discrimination between colors and also between depths of color. Thus, it provides a fully automated solution for more efficient quantification of staining intensity.

Automation of image analysis holds promise for improving inter- and intra-observer reproducibility which is the main problem with manual analysis. However, the lack of standards in system performance makes automation less reliable. Automation could also face problems with variations in illumination while imaging, variations in staining intensities, and section thickness. These could be solved with automated sectioning and staining systems. Standardized guidelines for transmission of baseline colors are very important since the evaluation is based on intensity. The clinical utility of automated analysis depends on strict adherence to quality assurance of the systems. Discrepancies in evaluation could be really serious; hence, automated evaluation does not eliminate the role of the pathologist. In our study, the focus was to identify all possible positive cases to ensure there were no false negative results. Once this is done, the expert pathologist can prioritize the slides to confirm the evaluation of the automated system.

The benefit of automation and computer-aided diagnosis has been demonstrated here. It was observed that there was a very good correlation between image analysis and expert opinion in evaluating the ER, PR and HER-2/neu expression images. The system was designed to avoid any false negative findings, hence, the specificity was compromised to obtain 100% sensitivity. This can be seen from the ROC curve in Figure 2. Table 5 shows the inter-observer variations and the intra-observer variations. It can be seen that the correlation between two readings by the same observer was slightly higher than the correlation of the readings by the two pathologists in the PR and HER-2/neu expression images. However, in the case of the ER expression images, intra-observer variation was greater than inter-observer variation. It was observed that in the majority of cases of mismatch, the grade 0 cases were mostly evaluated as grade 1 rather than grade 2, and similarly cases of grade 3 were mostly evaluated as grade 2 rather than grade 1. The automated analysis correlated best with the expert's opinion

in all three cases and this was significantly higher than the correlation between the two different observers and between two readings by the same observer.

This paper presents a technique for automation of the diagnosis of breast cancer from immunohistochemically stained biopsy specimens. Our goal was to provide 100% sensitivity and the software was successfully used to efficiently classify the cases. It was also demonstrated that the manual evaluation introduced a lot of variation, whereas the automated analysis provided an objective evaluation and was repeatable. Such automation could facilitate fast and efficient diagnosis of breast cancer and eliminate human errors, to a large extent. However, the results reported here could be further improved with the use of neural networks or other such classification models.

COMMENTS

Background

Cancer of the breast is the second most common human neoplasm and accounts for approximately one quarter of all cancers in females after cervical carcinoma. Immunohistochemical evaluation of hormone receptor expressions in tumor cell nuclei is an integral part of routine breast cancer diagnosis and provides important information for prognosis and choice of therapeutic approach. The main challenges that pathologists are currently facing are productivity, accuracy and objective evaluation. Pure visual estimates of immunohistochemically stained biopsy specimens provide very crude results with poor inter-observer and intra-observer reproducibility. For this purpose, computerized image analysis based methods are needed.

Research frontiers

Many studies have been carried out using computerized image analysis for the automation of evaluation. The use of various software packages such as ImageJ, the AQUA system, Image Pro Plus, and Adobe Photoshop has been reported. Another area which has seen very good advancement is the high throughput technology called tissue microarray (TMA) which generates a huge number of images in a fully automated and standardized manner which also makes it best suited for automation of evaluation.

Innovations and breakthroughs

In this article, the authors introduce the in-house developed software, TissueQuant, for automation of the evaluation of images of immunohistochemically stained biopsy specimens. The method they proposed provides a fully automated solution to these evaluations. The algorithm was designed to obtain 100% sensitivity. The inter-observer and intra-observer variations are reported. In addition, the correlation of the automated analysis with the expert's evaluation is also reported. It can be seen that the automated evaluation correlated well with the expert and overcame the problem of inter-observer and intra-observer variation.

Applications

The proposed method can be used to automate evaluations of images generated with the Tissue MicroArray technique so as to handle high throughput. The same technique can also be modified to evaluate any image where staining intensity needs to be assessed for decision-making.

Terminology

Estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER-2/neu) are specific hormone receptors which are expressed when stained with the respective immunohistochemical stains.

Peer review

Keerthana Prasad and colleagues demonstrated that the automation of breast cancer diagnosis from immunohistochemically stained specimen is a useful tool to provide objective and repeatable evaluations.

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Meetings

Events Calendar 2011

January 13-14, 2011
3rd Breast-Gynecology International Cancer Conference BGICC, Cairo, Egypt

January 15-16, 2011
Melanoma 2011: 21st Annual Cutaneous Malignancy Update, San Diego, CA, United States

January 15, 2011
Current Trends in Breast Cancer: Updates From the 2010 San Antonio Breast Cancer Symposium, Dallas, TX, United States

January 20-22, 2011
Gastrointestinal Cancers Symposium 2011, San Francisco, CA, United States

January 21-23, 2011
8th Meeting of the EAU Section of Oncological Urology, London, England, United Kingdom

January 27-28, 2011
2nd National Conference: Recent Advances in Renal and Bladder Cancer, London, United Kingdom

January 27-28, 2011
8th Annual Cancer Drugs Research & Development, San Diego, CA, United States

February 10-12, 2011
17th Annual NOCR Meeting, Las Vegas, NV, United States

February 19-22, 2011
Scripps Cancer Center's 31st Annual Conference: Clinical Hematology and Oncology, San Diego, CA, United States

February 24-26, 2011
European Multidisciplinary Conference in Thoracic Oncology (Lung 2011-EMCTO), Lugano, Switzerland

February 25-27, 2011
7th European Congress on Hematologic Malignancies: From Clinical Science to Clinical Practice, Budapest, Hungary

March 02-05, 2011
64th Society of Surgical Oncology Annual Cancer Symposium 2011, San Antonio, TX, United States

March 04-06, 2011
8th Annual Oncology Nursing Advanced Practice: Innovation through Practice, San Diego, CA, United States

March 07-09, 2011
9th International Symposium on Targeted Anticancer Therapies, Paris, France

March 09-13, 2011
16th National Comprehensive Cancer Network Annual Conference (NCCN 2011), Hollywood, FL, United States

March 11-12, 2011
12th European Congress: Perspectives in Lung Cancer, Torino, Italy

March 14-18, 2011
Oncology Imaging Update in Costa Rica, Guanacaste, Costa Rica

March 17-19, 2011
International Cancer Prevention Update Symposium, New York, United States

March 18-22, 2011
Vienna, Austria
26th Annual EAU Congress

April 02-06, 2011
AACR 102nd Annual Meeting, Orlando, FL, United States

April 08-10, 2011
Asian Oncology Summit 2011, Hong Kong, China

April 20-23, 2011
9th International Gastric Cancer Congress, Seoul, South Korea

April 29-30, 2011
Cancer Survivorship Conference, Minneapolis, MN, United States

May 23-24, 2011
4th International Conference on Ovarian Cancer Screening, London, United Kingdom

June 03-07, 2011
47th American Society of Clinical Oncology Annual Meeting, Chicago, IL, United States

June 20-23, 2011
7th EADO Congress European Association of Dermato-Oncology, Nantes, France

June 22-25, 2011
ESMO Conference: 13th World Congress on Gastrointestinal Cancer, Barcelona, Spain

June 23-25, 2011
"MASCC/ISOO 2011 International Symposium, Athens, Greece

July 03-07, 2011
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3rd World Congress of the International Academy of Oral Oncology 2011, Singapore, Singapore

August 15-17, 2011
International Conference and Exhibition on Cancer Science & Therapy, Las Vegas, Nevada, United States

September 1-3, 2011
Tri-Society Head and Neck Oncology, Singapore, Singapore

September 7-10, 2011
Hallmarks and Horizons of Cancer, Lausanne, Switzerland

September 23-27, 2011
Joint 16th ECCO and 36th ESMO Multidisciplinary Cancer Congress, Stockholm, Sweden

October 06-07, 2011
Current Status and Future of Anti-Cancer Targeted Therapies, Buenos Aires, Argentina

November 30-December 03, 2011
AORTIC 2011-Entering the 21st Century for Cancer Control in Africa, Cairo, Egypt

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1 **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World J Gastroenterol* 2007; **13**: 6356-6364 [PMID: 18081224 DOI: 10.3748/wjg.13.6356]

Chinese journal article (list all authors and include the PMID where applicable)

2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

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Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

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11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

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12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

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13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

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15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

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