Chapter - 1

Introduction

Chapter 1

Introduction

The astonishment and wonder underlying, "What a wonderful and complex machine a human body is!", is better appreciated from the fact that, the functioning of the human body, in all its complexities, is still far from being completely understood. Recent research indicates that every human body produces carcinogenic cells quite frequently. In simpler words, human bodies produce singular or multiple cancer cells on any given day at any time; however the body's defence mechanism, or immunization system destroys this random growth of carcinogenic cells. This phenomena has been observed, while recording the optical resonance of cancer cells in human blood through lasers. The systematic destruction of these cells by the human body ensures that cancer as a disease is not as widespread. It is the failure of this immunization system, that results in an unprecedented growth of cancer cells. It is believed that it takes almost 8 years for the cancer growth to become detectable, since it starts as a single cell. This provides, in principle, a long time to detect cancer, if the tools employed are sophisticated. It is evident that, the method must be very sensitive since it has to carry out cell level discrimination. Recent research in this field is towards employing light to do this differentiation.

The largest sufferers of cancer are, 12 % of the women above the age of 45 years. They suffer from breast cancer as well cervical cancer. They not only suffer from disease but social stigma and loss of dignity attached with it.

1.1 Breast cancer

Breast cancer is the fifth most common cause of cancer-deaths after lung, stomach, liver, and colon cancers. In 2005, breast cancer caused 502,000 deaths (7 percent of cancer-deaths, almost 1 percent of all deaths) worldwide (1). It is the second most fatal cancer in women after lung cancer(2; 3). It is estimated that there will be 80,000 new cases of breast cancer in India each year. Lippman reported that Asian women have one-fifth to one-tenth the risk of women in North America or Western Europe. Because the breast is composed of identical tissues in males and females, breast cancer also occurs in males, though it is less common. Breast cancer is an abnormal growth and uncontrolled division of cells in the breast. Cancer cells can invade and destroy surrounding normal tissue and can spread throughout the body via lymph fluid and rarely via blood. In reality there are numerous types of breast cancer. Some are benign, some modestly malignant while others are extremely serious(2). Breast cancer can begin in different areas of the breast the ducts, the lobules, or in some cases, the tissue in between. The breast tissues investigated in this study were malignant and benign tumors and their normal counterparts.

The chances of cure in women who develop the disease is related to early diagnosis.

1.1.1 Diagnostic Techniques for Detection of Breast Cancer

There are three methods for early detection of breast cancer.

•First is mammography i.e. X-ray of the breast, done at regular intervals, say every 2 years. However, mammography is expensive, technology driven and requires stringent quality control and extensive experience on the part of technicians and doctors involved. If these are not available, mammography can do more harm than good by falsely diagnosing cancer or missing it when it is actually present.

•The second method is for a woman to get herself examined clinically by a breast specialist. It appears that if clinical examination is done properly it may be as effective as mammography.

•The third method is self-examination.

The huge uncertainty factor in detection of cancer by the existing methods, keep researchers busy, for the development of new detection methods which can conform the detection with certainty.

1.1.2 Malignant tumor

The two most common types of breast cancer are named after the parts of the breast in which they start.

Ductal Carcinoma: It starts in the cells which line the breast's ducts, beneath the nipple and areola. The ducts supply milk to the nipple. Between 85% and 90% of all breast cancers are ductal. If the cancer is DCIS (ductal carcinoma in situ), it is well contained, not invasive, and can be very successfully treated. Usually removed during a lumpectomy, if the tumor margins are clear of cancer, follow-up treatment may include radiation. If ductal cancer has broken into nearby breast tissue (invasive cancer) then a mastectomy may be needed, and the doctor may also recommend chemotherapy.

Lobular Carcinoma: It begins in the lobes, or glands which produce milk in the breast. The lobes are located deeper inside the breast, under the ducts. About 8% of breast cancers are lobular. If the cancer is LCIS (lobular carcinoma in situ) that means the cancer is limited within the lobe and has not spread. It may be removed during a lumpectomy, if the tumor margins are clear of cancer, follow-up treatment may include radiation. If lobular cancer has spread into nearby breast tissue (invasive cancer) then a mastectomy may be needed, and the doctor may also recommend chemotherapy.

Second most common is a group of breast cancers that invade nearby tissue:

Invasive (Infiltrating) Breast Cancer: Invasive, or infiltrating, breast cancer has the potential to spread out of the original tumor site and invade other parts of your breast and body. There are several types and subtypes of invasive breast cancer.

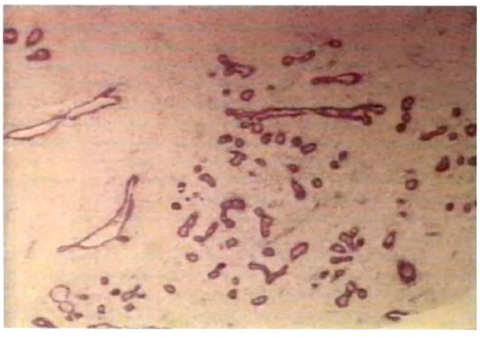
Inflammatory Breast Cancer: It is the least common, but most aggressive of breast cancers, taking the form of sheets or nests, instead of lumps. It can start in the soft tissues of the breast, just under the skin, or it can appear in the skin. Unlike ductal and lobular cancers, it is treated first with chemotherapy and then with surgery. When detected early, inflammatory breast cancer can be a manageable disease, and survival rates increase.

Paget's disease of the nipple/areola: It often looks like a skin rash, or rough skin. It resembles eczema, and can be itchy. The itching and scabs (if scratched) are signs that cancer may be under the surface of the skin, and is breaking through. Paget's is usually treated with a mastectomy, because the cancer has by then invaded the nipple, areola, and the milk ducts.

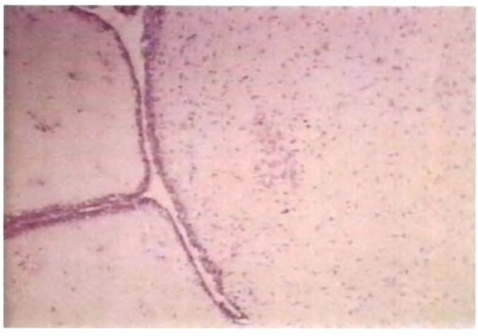
1.1.3 Benign tumor

A tumor that is not cancer is called benign. They do not grow and spread the way cancer does. Fibroadenoma is the most common benign breast tumors seen in women. Fibroadenoma means a tumor composed of glandular and fibrous tissues. The fibroadenomas investigated are of the following types.

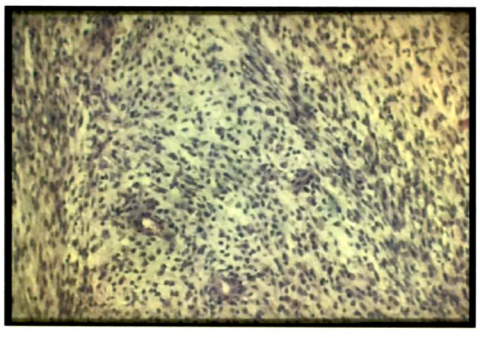
Pericanalicular fibroadenoma: Fibroadenomas consists of a delicate fibroconnective tissue stroma. Within the stroma, if the glandular structure, uncompressed by the stroma, have a round to oval lumen, it is called pericanlicular type fibroadenoma.



(a)Pericanalicular type fibroadenoma



(b)Intracanalicular type fibroadenoma



(c)Cystosarcoma phyllodes

Figure 1.1: Three different types of fibroadenoma breast tissues.(4; 5)

Intracanalicular fibroadenoma: If the glandular structure have a slit like appearance, then it is called an intracanalicular fibroadenoma.

Mixed type: If the tissue shows variation in structure from pericanalicular to intracanalicular type, then it is classified as a mixed type.

Cystocarcoma phyllodes: The malignant counterpart of fibroadenoma is cystocarcoma phyllodes, in which the epithelial elements are benign, but the stromal tissue is malignant. It results from malignant degeneration of fibroadenoma, estimated to occur in 1% patients, who have fibroadenoma for many years.

Fibrocystic disease: This disease consist principally of cysts due to overgrowth of fibrous stroma and clinically often produces palpable lump.

Tuberculosis mastitis: It is a rare benign breast disease, which usually affects young women during reproductive period.

Figure 1.1 shows the slides of the three types of fibroadenoma described above.

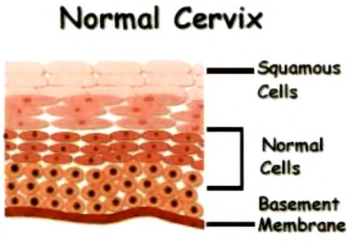
Cervical cancer is the third most common cancer in women worldwide (6) and is the leading cause of death in women, exceeded by breast cancer. It is the most neglected and hidden by the women patients because of the social stigma and the shame attached to it. It robs the dignity as well as the status of a woman. Also it is the most painful one and traumatic for the women to have it. To fight effectively against the disease, the front line attempt should be to detect it as early as possible so it can be treated effectively and lives can be saved along with women's dignity.

1.2 Cervical cancer

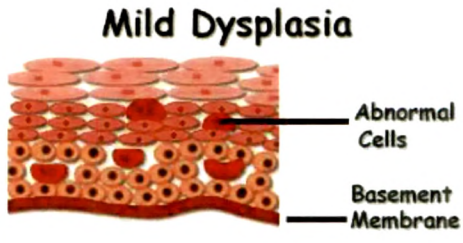
Cervical cancer begins in cells on the surface of the cervix. Over a period of time, the cervical cancer can invade more deeply into the cervix and nearby tissues. The cancer cells can spread by breaking away from the original (primary) tumor. They enter blood vessels or lymph vessels, which branch out into all the tissues of the body. The cancer cells may attach to other tissues and may grow to form new tumors that may damage those tissues as well. The spread of cancer is called metastasis. Dysplasia is an early form of cancer. There are two main types of cancer of the cervix. About 80% to 90% are squamous cell carcinomas. The other 10%-20% are adenocarcinomas. If the cancer has features of both types it is called mixed carcinoma.(7)

1.2.1 Cervical Dysplasia

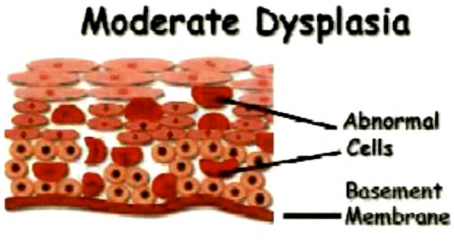
The term dys means abnormal, while plasia means growth. Cervical dysplasia means abnormal growth of cervical cells. Cervical dysplasia is also called cervical intraepithelial neoplasia, or CIN. Dysplasia is not cancer, but it can develop into



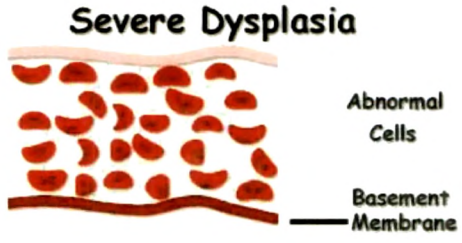
(a) Normal cervix



(b) Mild dysplasia (CIN I)



(c) Moderate dysplasia (CIN II)



(d) Severe dysplasia (CIN III)

Figure 1.2: Normal cervical tissue and stages of its cancer

cancer of the cervix, if not treated early.

Cervical dysplasia is classified as:

•**Mild (CIN I):** In mild dysplasia (CIN I) only a few cells are abnormal. Mild cervical dysplasia sometimes goes away without treatment. However many doctors will treat it at this early stage to prevent it from progressing.

•**Moderate (CIN II):** In moderate dysplasia (CIN II) the abnormal cells involve about one-half of the thickness of the surface lining of the cervix.

•**Severe (CIN III):** In severe dysplasia (CIN III), also called carcinoma-in-situ, the entire thickness of cells is abnormal, but the abnormal cells have not yet spread below the surface or basement membrane. Carcinoma-in-situ literally means "cancer in place". This severity of dysplasia MUST BE TREATED because it will most often develop into invasive cancer.

The outside of the cervix and the vagina are covered by a layer of flat cells called squamous or skin-like cells. There are many of these cell layers before the

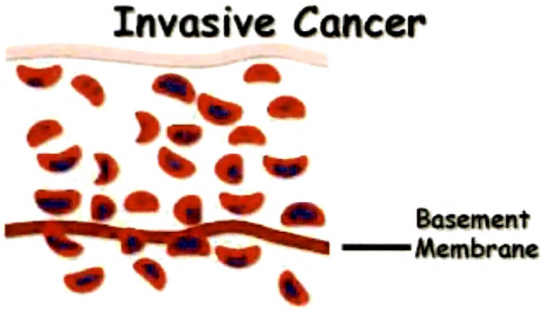


Figure 1.3: Cervix with invasive cancer

first flattened layer of cells with a nuclei or center. Normally at the bottom of the cell layers are the round, younger cells. As the cells mature, they rise to the surface and become flat. The skin-like covering is separated from the underlying structures by a basement membrane.

In all grades of dysplasia and carcinoma-in-situ all of the abnormalities are confined to the cells ABOVE the basement membrane of the cervix.

With invasive cancer, the cells are not only abnormal throughout the entire thickness from the top to the basement membrane, but they invade the basement membrane. Invasive cancer is treated entirely differently than dysplasia and usually involves extensive surgery. The depth of invasion past the basement membrane is an important piece of information that will help make the decision on the proper treatment needed. Micro - invasive cancer, invasion less than 3 mm, may be treated differently and more conservatively than invasive cancer that goes deeper.

1.2.2 Conventional Diagnostic Techniques for Detection of Cervical Cancer

Routine screening for cervical abnormalities can detect early-stage cancer and precancerous conditions that could progress to invasive disease. The process begins with a Pap test, also known as a Pap smear.(8)

•ThinPrep Pap Test:

The ThinPrep Pap Test, is a liquid-based test that employs a fluid medium to collect and preserve cervical cells. A woman who receives a ThinPrep Pap Test will not experience any difference in the procedure used to collect the sample of cervical cells.

•Speculoscopy:

This procedure involves the use of a magnifier and a special wavelength of light. Speculoscopy allows the physician to see cervical abnormalities that would otherwise be undetectable when performing a Pap test.

•Schiller Test And Colposcopy:

In the Schiller test physician applies a vinegar-like solution to the cervix and then coats it with iodine. Next, the physician performs a colposcopy, which is an examination of the cervix with a magnifying instrument called a colposcope. Healthy cervical cells look brown, whereas abnormal cells appear white or yellow. The Schiller test and colposcopy are painless office procedures that have no side effects. If colposcopy reveals abnormal areas on the cervix, the physician will order a biopsy - the removal of tissue for examination under a microscope by a cytopathologist (a specialist who studies cells in order to diagnose disease).

•Coloposcopic-Directed Biopsy:

In this the physician uses forceps to remove small pieces of cervical tissue from areas of the cervix where abnormal-looking tissue has been detected. Local anesthetic is sometimes used to numb the cervix, and a woman who undergoes this office procedure may briefly experience pain, mild cramping, or light bleeding.

•Endocervical Curretage:

This procedure, which is generally performed at the same time as colposcopic biopsy, removes cells from the endocervix (that part of the cervix which is closest to the body), which cannot be seen during colposcopy. A woman who has endocervical curettage may experience menstrual-type cramping or light bleeding for a short time afterward.

•Cone Biopsy:

This procedure consists of removing a cone-shaped piece of tissue from the cervix. Tissue is removed from the area between the ectocervix and the endocervix, where cancerous and precancerous conditions most often develop. The

two methods commonly used to perform cone biopsy are:
(a) Loop electrosurgical excision procedure (LEEP) and
(b) Cold knife cone

•**Dilation And Curettage (D&C):**

If a Pap test doesn't clearly indicate whether abnormalities are caused by problems in the cervix or in the endometrium (lining of the uterus), the physician may conduct a dilation and curettage (D&C). During a "D&C", the physician enlarges the cervix (dilation) and scrapes the inside of the uterus and cervical canal (curettage) to remove tissue for microscopic analysis.

•**Cervicography:**

This diagnostic procedure enables a physician to obtain and examine a photographic image of the cervix. Cervicography may clarify abnormal Pap test results in women at above-average risk of developing cervical cancer. It could eventually reduce the need for colposcopy.

•**Histopathology:** Histopathology is gold standard process for determining the cancer and precancers stages in cervical tissue. For histopathology the vertical cross section of tissue, having thickness around 5m has been cut and stained. This stained slide is examined under microscope.

1.2.3 Limitations of these Diagnostic Techniques:

•Mammography quantitatively probes density changes in breast tissue, however, theses changes are not uniquely correlated with the probability of breast cancer. Because of this, it serves as a screening technique rather than as a diagnostic tool. This is evidenced by the fact that only 10-25% of mammographically detected lesions are found to be malignant upon needle biopsy. As a consequence of the limitations of current techniques, each year a large number of breast biopsies are performed on benign lesions. The desirability of reducing the number of benign biopsies performed, patient trauma, time delay, and the high medical costs associated with biopsy has motivated researchers to explore minimally invasive optical methods for diagnosing malignant lesions in the breast.

•Since the Pap smear has an average sensitivity of 58% and specificity of 69%, many lesions are missed or overcalled (Sensitivity is defined as the probability that

a test is positive in the presence of disease; specificity is the probability that a test is negative in the absence of disease)(8). The transition from the intraepithelial neoplasia to cancer averages several years; thus, few cancers are missed in women who receive annual screening. However, many women are unnecessarily referred for colposcopy, resulting in considerable anxiety and economic cost.

•Colposcopy has an average sensitivity of 96% and specificity of 48%; thus, biopsy is required to confirm diagnosis. This sensitivity allows for the detection of most cancers, but the specificity suggests that many lesions are overcalled. Many unnecessary biopsies are performed. Alternatively, those patients with high-grade lesions wait 2 weeks for confirmation and may be lost for follow-up. Finally, considerable controversy exists over the evaluation and management of low-grade disease because we do not know which lesions will progress to cancer or regress to normal (8).

•Some of the above methods are painful to the patient undergoing test and experience complications such as bleeding, infection, etc. and the patient is advised to take rest for few days.

To overcome the short comings of the present methods or scenario, the basic need is to develop some novel imaging techniques for early detection, screening, diagnosis, and image-guided treatment of life-threatening diseases and cancer.

Optical principles have been used in medical diagnosis since the dawn of the medical sciences. Changes in skin colours are typical symptoms for a number of infectious diseases. Well known examples include the pallor of an anaemic patient or the yellowish skin of a baby with jaundice.

The human body responds with skin rashes when confronted with infectious diseases such as chickenpox or measles. Infected wounds develop a red-purple-bluish colour. Thus, the spectral information is very important for the correct diagnosis of disease; a knowledge that has been utilized by physicians for hundreds of years.

Every tissue type emits fluorescent radiation (autofluorescence). Studies of this radiation reveal that tumors and pathological tissues have an altered autofluorescence pattern, which creates possibilities for new diagnostic principles.

The interest for diagnosis based on optical principles has increased tremendously in past few years, largely as a result of the availability of new optical

components and technology. For instance, tiny optical fibres can be inserted into muscles, introduced into blood vessels and into most cavities of the human body. This means that we can extract diagnostic information from most part of the human body with little or no discomfort to the patient. Thus "optical biopsies" can be taken, by studying reflected and absorbed light from the interior of an organ without the painful or risky removal of tissue sample for post-operative laboratory analysis.

The field in which optical principles are applied to solve diagnostic or therapeutic problems is known as Bio-optics.(9)

Developments in the optics and optical instrumentation have given new dimensions to the optics for biological studies. Especially the inventions of lasers have made revolution. Now-days lasers are used in the biological and medical sciences for diagnostic purposes. Such applications of optics and optics based instrumentation in biology and medical science have brought up an interdisciplinary area known as *"Biomedical optics"*. Biomedical optics involves the fusion of optics (photonics) with biology and medical science. The use of photonics, particularly for optical diagnostics, as well as for light-activated and light guided therapy, has a major impact on health care. It offers great hope for the early detection of diseases and for new therapeutic modalities. The need for novel materials and technologies to detect diseases at early stages, to provide more effective targeted therapies and to restore impaired biological function is constantly increasing.

In the field of biomedical applications, optical diagnosis techniques are now emerging as viable tools for tumor detection. Of these, fluorescence spectroscopy has been used fruitfully for diagnosis as well as for therapeutic purposes (10; 11; 12; 13; 14; 15). Fluorescence techniques are being increasingly employed to investigate both morphological and biochemical changes inside a tissue. Morphological changes prevalent in tumors, such as enlargement and hyperchromasia of nuclei, overcrowding and irregular cellular arrangement are known to alter light propagation and scattering properties in such media and hence affect the fluorescence spectra. Due to disease, the fluorophores inside the tissue fluoresce differently, as compared to their normal counterparts. A number of fluorophores ranging from structural proteins to various enzymes and coenzymes, some of which participate in the cellular oxidation-reduction processes, are present in the human

tissue and can be excited by ultraviolet and visible light. Due to its sensitivity to minute variations, fluorescence spectroscopy can provide quantitative biochemical information about the state of the tissue, which may not be obtained using standard pathology. Over other light-based investigation methods, fluorescence spectroscopy is often preferred because of its high sensitivity, high speed, and safety.

1.2.4 Importance of Optical Spectroscopy

Optical spectroscopy offers several benefits over traditional diagnostic methods. It non-invasively probes the endogenous absorption, scattering and fluorescence of a large number of biological molecules already present in the tissue, thus providing a wealth of biochemical information related to disease progression without the need for tissue removal. Additionally, advances in sensitive detectors and optical fibers make it possible to measure optical spectra rapidly and remotely from human tissues in vivo. This diagnostic tool can potentially improve the accuracy of current diagnostic procedures, reduce unnecessary biopsies, and permit early detection of pre-cancerous lesions. A large number of clinical investigations carried out on a variety of tissue sites including the cervix, skin and oral cavity demonstrate that optical spectroscopy provides sensitive and specific detection of epithelial pre-cancers and early cancers (16). Optical techniques such as Fluorescence, Raman, and light scattering can be used to distinguish cancerous from non-cancerous state of the tissue. The laser-induced fluorescence from tissues is shown to contain information regarding the nature of the tissue and hence serves as a very efficient and cheap way for cancer diagnosis. Of the above mentioned methods, fluorescence techniques have been one of the most widely used in light-based diagnostic systems. Over the past 15 years its diagnostic potential has been tested in different organs of the body, including the mouth (17), breast (18), esophagus (19) and bladder (20). In the early 1980's, Alfano and Yao (21) first introduced laser spectroscopy for tooth decay detection. They extended their works to distinguish rat and human normal tissues from cancerous tissues (10). This technique was then used by Kittrel et. al. for diagnosis of atherosclerotic plaque (22), which was extended further by Deckelbaum (23). Barbara et al.

(24) reported ultraviolet laser induced fluorescence for the detection of cancer in human stomach tissues. Nirmala Ramanujam and co-workers demonstrated the use of laser induced fluorescence (LIF) for the detection of cervical intraepithelial neoplasia (CIN). In 1999, Majumdar et al. studied the tissues from the human oral cavity to suggest that the collagen/ NADH concentrations is higher in normal tissue sites in comparison with cancerous tissue sites and were able to discriminate cancerous from normal oral tissues (17).

1.3 Focus of the thesis

Early diagnosis is still not possible through conventional diagnostic techniques. If diagnosed early, these cancers are the most treatable forms of cancer. The requirement of continuous monitoring for malignancy of a significant percentage of women population has led to an intense search for safe, reliable and fast diagnostic methods. This fact motivates my study and I have used Wavelet Transform and Singular Value Decomposition (SVD) to get better insights into the problem. Wavelet transform, Singular Value Decomposition and Wavelet based Autocorrelation of polarized fluorescence spectroscopic data of human breast and cervical tissues are found to reliably differentiate normal, benign and malignant tissue types and isolate characteristic biochemical signatures of cancerous tissues, which can possibly be used for diagnostic purpose. A number of parameters capturing spectral variations and subtle changes in the diseased tissues in the visible wavelength regime are clearly identifiable in the wavelet domain.

The ultimate goal of my study is to establish a method that is more accurate and efficient than conventional method for early detection of cancer.

Bibliography

[1] WHO (February 2006). Fact sheet No. 297: Cancer. Retrieved on 2007-04-26. 2

[2] M. E. Lippman, Harrison's Principles of Internal Medicine (Mc Graw Hill, 1998) 562. 2

[3] P. H. Pestonjamasp, I. Mittra, *J. Biosc.* **25(1)**, 19, (2000). 2

[4] From Bristol biomedical image archieve, www.brisbio.ac.uk xi, 5

[5] From www.breastdiseases.com/slides/ben46.htm xi, 5

[6] R. R. Kortum, R. Drezek, K. B. Engquist, C. B. Scott, "Cervical dysplasia diagnosis with Fluorescence Spectroscopy", *Lasers Surg. Med.*, **19**, 63-74 (1996). 6

[7] http://www.mjbovo.com/Women/Dysplasia.htm 6

[8] http://www.ehealthmd.com/library/cervicalcancer/CC_diagnosis.html 8, 11

[9] Medical & Biomedical Engineering & Computing, vol.**41**, 241, (2003). 12

[10] R. R. Alfano, G. C. Tang, A. Pradham, W. Lam, D. S. J. Choy, and E. Opher, "Fluorescence spectra from cancerous and normal human breast and lung tissues", *IEEE Journal of Quantum Electronics*, **23**, 1806, (1987). 12, 13

[11] G. C. Tang, Asima Pradhan, Wenling Sha, J. Chen, C. H. Li, S. J. Wahl and R. R. Alfano, "Pulsed and cw laser fluorescence spectra from cancerous,

normal and chemically treated normal human breast and lung tissues", *Appl. Opt.*, **28**, 2337, (1989). 12

[12] G. C. Tang, A. Pradhan and R.R. Alfano, "Spectroscopic differences between human cancer and normal lung and breast tissues", *Lasers Surg. Med.*, **9**, 290, (1989). 12

[13] R. R Kortum and E. Sevick Muraca, "Quantitative optical spectroscopy for tissue diagnosis", *Ann. Rev. Phys. Chem.*, **47**, 555, (1996). 12

[14] G. A. Wagniers, W. M. Star and B. C. Wilson, "In vivo fluorescence spectroscopy and imaging for oncological applications", *Photochem. Photobiol.*, **68**, 603, (1998). 12

[15] Recent references on various applications of lasers in biology and medicine can be found in IEEE Journal of Selected Topics in Quantum Electronics **9**, 140, (2003). 12

[16] N. Ramanujam, "Fluorescence spectroscopy of neoplastic and non-neoplastic tissues", *Neoplasia*, **(1-2)**, 89, (2000). 13

[17] S. K. Majumder and P. K. Gupta" Synchronous Luminescence Spectroscopy for Oral Cancer Diagnosis", *Lasers in the Life Science*, **9**, 143, (2000). 13, 14

[18] B. V. Laxmi, R. N. Panda, M. S. Nair, A. Rastogi, D. K. Mittal, A. Agarwal and A. Pradhan, "Distinguishing normal, benign and malignant human breast tissues by visible polarized fluorescence", *Laser Life Sci.*, **9**, 229, (2001). 13

[19] G. A. Wagnieres et al., *Appl. Opt.*, **36**, 5608, (1997). 13

[20] M. S. Soloway, "Bladder-cancer", *J. Urol.*, **48(4)**, 631, (1996). 13

[21] R. R. Alfano and S. S. Yao, "Human teeth with and without caries studied by visible luminescent spectroscopy", *J. Dent. Res.*, **60**, 120, (1981). 13

[22] C. Kittrel, et al., "Diagnosis of arterial atherosclerosis using laser-induced fluorescence", *Appl. Opt.*, **24**, 2280, (1985). 13

[23] L. I. Deckelbaum, J. K. Lam, H. S. Cabin, K. S. Clubb, and M. B. Long, "Discrimination of normal and atherosclerotic aorta by laser-induced fluorescence", *Lasers Surg. Med.*, **7**, 330, (1987). 13

[24] B. W. Chwirot, Z. Michniewicz, M. Kowalska, and J. Nussbeutel, "Detection of Colonic Malignant Lesions by Digital Imaging of UV Laser-Induced Autofluorescence", *Photochemistry and Photobiology*, **69(3)**, 336, (1999). 14