Near-Infrared Fluorescence Spectroscopy and Imaging for Cancer Detection and Evaluation

Jain S.\(^1\) and Huang Z.\(^2\)

Division of Bioengineering, Faculty of Engineering, National University of Singapore, Singapore, 117576

ABSTRACT

Tissue autofluorescence has been studied extensively over the last decade for non- or minimal-invasive medical diagnosis. It is determined by tissue morphology and biochemical constituents in tissue and, therefore, can be used for characterizing tissue in healthy or diseased states. Autofluorescence emission is also critically dependent on the wavelength of incident light being used to excite the effect. The aim of the project is to study an imaging system based on the excitation of the tissue using Near Infrared Imaging (NIR) with a wavelength between 700-1000 nm and study the spectrum obtained. The major objective of this work is to determine the usefulness of the NIR excited fluorescence and imaging for cancer diagnosis and evaluation. The autofluorescence of the tissue has been studied which allows the imaging and cancer detection without incorporation of any external agents that might prove harmful for the body in the long run. A new setup has been studied to excite the tissue using NIR to qualitatively and quantitatively describe the metabolic changes in the tissue.

INTRODUCTION

Cancer is a general term that defines an uncontrolled growth of cells in the body. Any harmful mutations that affect the genetic makeup of the cells might lead to a loss of function in the housekeeping genes which in-turn results in the production of proteins that are either faulty or non-functional. These essential proteins are important checkpoints in the cell-cycle and loss of any check points leads to a subsequent malfunctioning of the cell cycle and an uncontrolled growth of the cells.\(^{[Dollinger et al., 2002]}\) They have the ability to invade tissues apart from host tissue, are self sufficient in growth and can avoid potential death signals to cause programmed cell death.\(^{[Chance et al., 2005]}\) Despite the availability of various medications and therapies to treat and control cancer effectively, it continues to be the cause of death for millions every year due to inability to effectively detect cancer at early stages.\(^{[Chen et al., 2005]}\) The American Cancer Society itself projected that a total of 1,437,180 new cancer cases and 565,650 deaths can occur from cancer in the United States in 2008. Death from cancer is expected to continue to rise to about 9 million in the year 2015.\(^{[Jemal et al., 2008]}\)

So the effective treatment in cancer involves early diagnosis and detection. Several mechanisms are currently available for detection and diagnosis of cancer. These include use of imaging techniques namely X-Ray scan, Computed Tomography (CT Scan), Magnetic Resonance Imaging (MRI), Single Photon Emission Computed Tomography (SPECT) and

\(^1\) Student
\(^2\) Assistant professor and UROP Supervisor
Positron Emission Tomography (PET) out of which the last three are capable of providing 3-D imaging of the body parts. [Pierce et al., 2008] But they all suffer from significant limitations. Although the techniques described here have been very successful in detection of tumours in various parts of the body, the limitations remain in the minimum size of the tumour that can be detected. Since the cancer basically arises from a single progenitor cell, which would require at least a detection ability of $10^{-14}$. [Frangioni, 2008; Saisho and Yamaguchi, 2004] This is not possible with the current available technologies and hence has now shifted to developing techniques that can allow for the possibility of detection of cancer at the earliest stages and smallest sizes. [Frangioni, 2008; Chen et al., 2005; 2005]

The molecular mechanisms of the cells are greatly altered in cancer and they provide a key to detect any variability in the molecular components as a key to detection. Furthermore, all tissues contain certain components that can absorb radiation at a particular wavelength and reach an excited state. [Demos et al., 2009; Kondepati et al., 2008] Returning to the normal state, the tissues emit characteristic radiation with a particular spectrum which is a source of understanding the conditions of cells in the tissues. However, all wavelengths of light are not suitable for the tissues and some may be harmful due to their heating or other ionisation effects. [Frangioni, 2008; Kondepati et al., 2008] Near Infrared Spectroscopy (NIRS) or use of near infrared radiation for auto fluorescence is the use of light wavelength of 700-1000 nm [Keilhauer and Delori, 2006; Shao et al., 2009; Kondepati et al., 2008] which is used for tissue excitation and emission. NIRS is a minimally invasive technique for detection. It is being applied in various areas of biomedical research including brain imaging, radiology, formation and quality controls. [Kondepati et al., 2005; Nioka and Chance, 2005; Pavlova et al., 2008]

The basic mechanism of NIRS involves the travelling of an excited photon through the tissue. The absorption of the photon by any of the biochemical component of the tissue will depend on the properties of the tissue that include tissue’s scatter, anisotropy and refractive indices. [Fournier et al., 2006] The major absorbers in any tissue are basically lipids, water, and oxyhemoglobin and deoxyhemoglobin with the total absolute absorption depending on the molar concentration of each component. [Lin et al., 2007; Kondepati et al., 2007] The NIR Spectra can be mostly assigned to the overtones arising out of the absorption and emission by the C-H, N-H and O-H groups which are part of the basic body building molecules in the body. [Pavlova et al., 2008] Chemical, histological and biochemical assays of the cancer tissues have shown in many studies that the compositions of the lipids, fatty acids, carbohydrates as well as proteins are greatly altered in the cancerous tissue due to the different demands of the tissue. [Weissleder and Ntziachristos, 2003] The spectra obtained provide both qualitative and quantitative information concerning the blood flow to the tissue and oxygen saturation due to increased rates of metabolism in the cancerous tissue. [Fournier et al., 2006; Pavlova et al., 2008]

NIR autofluorescence focuses on use of endogenous fluorophores present in the body tissues. [Weissleder and Ntziachristos, 2003; Pierce et al., 2008] Although administration of the external fluorescent agents have been used and studied with some of them receiving FDA approval as well, it is always better to carry out imaging without introduction of any fluorophores as it would prevent any changes in the compositions of the tissues and prevent occurrence of any side effects that may accompany along with the fluorophores.
The current project aims at understanding the setup that has been arranged and designed by the laboratory and studies the mechanism of autofluorescence and the expected outcomes from cancerous tissue. Due to limitations in the availability of the cancerous tissues, the project has focussed on the literature studies and the comparisons of the new setup with the conventionally used NIRS setup.

MATERIALS AND METHODS

Patient Samples for Experiment

Samples of tissues, malignant colon cancer and normal tissue were obtained from a patient on 24th March 2009 from the Mount Elizabeth Hospital Singapore. The samples were stored at 4°C. The tissues were allowed to thaw before the experiment but were not exposed to the outside environment to minimize any changes in the chemical composition of the tissues which might affect the fluorescence and the data collection.

Experimental Set Up

The wavelength of the laser beam used is 785 nm. Before beginning the experiment the power of the laser was checked at 785 nm without inserting a polarizer in the pathway and it was found to be 525 mW which was in comparison with the previous data measurements. When not in use the laser was switched off to maintain the life time of the laser beam. The polarizer was then inserted in the path and the power of the laser was checked again. It was found to be 117 mW. The polarizer was removed and an anti-filter with different filtering components was inserted and was rotated till the point where the power of the laser was found to be the same as that when the polarizer is present in the path.

Experiment

The tissues were mounted on a normal glass slide covered with aluminium foil which helps prevent any background fluorescence which may be present in the presence of glass. The normal tissue was first mounted on the slide after dipping in normal saline so as to provide a similar internal body environment. Since the slide had to be mounted vertically, as much as possible the liquid was removed so that tissue does not slide down. The cancerous tissue was also placed in close proximity with the normal tissue following the same protocol forming a boundary so as to mimic a similar pathological situation where the cancerous tissue is surrounded by normal body tissue.

Before conducting the experiment, background images were taken using the CCD camera mounted opposite the tissue in order to be later subtracted from the actual images so as to prevent any image artefacts due to presence of background radiation. The entire experiment was carried out in a dark room with the experimental set up being enclosed in a dark chamber to minimize any external light interference. The background images were acquired at different exposure times (0.01s, 0.02s, 0.03s and 5s) since short exposure times are good for white light, long exposure times are necessary for fluorescence.

The slide was then mounted on to the stand. Real time images were first acquired using the CCD camera so as to make sure the tissues are in focus with the light source and clear sharp images are obtained. The background was then subtracted from the image while doing this. Once the tissue had been properly aligned in the focus of the white light, different images are
obtained using different exposure times with the set up being enclosed in the dark chamber to prevent any interference from outside light sources. These measurements were taken in absence of any polarizer.

The next set of images are taken in the presence of laser during which the precaution should be taken that the laser should be switched on only during the time of taking the images as the laser may damage the tissue due to long exposure. The same procedure was followed as in the case of white light and the images are taken with long exposure time of 5 s. The respective background image is subtracted that had been taken for long exposure times. These measurements are taken in the absence of any polarizer.

Next the polarizer was inserted in to the path along with an analyzer in front of the CCD. Different angle combinations of analyzer and polarizer are used for getting different images in presence of laser beam. The same process is repeated with the white light as well.

For the acquisition of the spectrum the same tissue samples were used. However in this case one tissue sample was analyzed one at a time. The slides were first cleaned using isopropyl alcohol and the tissues were dipped in saline again in order to prevent any damage to the tissue. The fibre optic channels for the spectrum acquisition were analyzed using a fibroscope to check the presence of any dust particles that may damage the equipment like the spectrometer to which it is connected to. They were cleaned and then connected to get a complete circuit. The laser probe was cleaned with alcohol as well since the laser probe is in contact with the tissue this time. The power of the laser was measured again and it was found to be 205 mW which is in agreement with previous data measurements. The entire measuring system was again enclosed in a dark chamber and background spectral radiation is taken in order to check for the presence of any background noises that may interfere. The system is set to global dark and the spectrum that may be present is zeroed. This would eliminate any background noises from the measurements.

The tissue is mounted on to the slide however all the water is not dried since this time the slide is kept horizontal and the laser beam is now in contact with the tissue which may cause heat generation that may damage the tissue but in presence of water the heat is absorbed and tissue is protected. The spectral measurements were taken several times and the same process is repeated for another part of the tissue (same sample on the slide). The entire process is repeated for the cancerous tissue as well. The measurements were recorded and later analyzed. The tissues are stored back in -80°C so that they could be used later.

RESULTS AND DISCUSSION

All images were analysed using the image processing software (Matlab®, Version 7.4.0.287). It was desired to get a good contrast at the boundary of the normal and the cancerous tissue which is the same situation as observed in a physiological environment. Under different conditions of white light or fluorescence, exposure times were analysed. The position of maximum brightness in the image versus the pixel distance was plotted for different exposure times. A clear boundary between the two tissues was obtained when the image was obtained using the NIR laser. However, in case of white light, no difference between the two tissues could be detected. Furthermore, the size of the tissue was not a constraint in detection of the tissue boundary since it was purely dependent on the difference in the fluorescence between the
two tissues due to the different biochemical compositions in the cancerous tissues. Figure 1 displays an image taken under white light at an exposure time of 0.01 s while figure 2 displays an image taken at an exposure of 5 s using NIR laser. Figures 3 to 7 display the boundary obtained between the normal and cancerous tissue under different exposure times and different combinations of polariser and analyser. Figure 8 displays an overlying image and boundary between the tissues.

As it was stated earlier in the materials and methods, the cancerous tissue had been mounted on to the normal tissue. Even without image analysis, it is quite clear in the image itself that there an increased fluorescence in the cancerous tissue as it appears brighter as compared to the normal tissue. Even without analysing the boundary detection between the two tissues it becomes quite clear from the image taken that cancerous tissue can be identified from the normal tissue with changed physiochemical compositions which can be attributed to the change in the chemical compositions of the tissue. It can be clearly seen from data obtained that use of NIR laser beam allows the excitation of the endogenous fluorophores that results in striking contrast between the cancerous and the normal tissues. This technique allows the detection of the boundary in a normal environment without the requirement of any specific size of the tissue. It is especially important for detection of cancer at early stages where the techniques like MRI, CT scan pose limitations due to size constraints.

The development of cancer is a long process and its detection is the best way to conquer it. However, physical discomfort in the affected part of the body may take long time to surface before the cancer can be detected in a measurable size. Furthermore, even if the discomfort is detected the size of the cancer tissue is not in the detectable range of the scan. This technique allows us to utilise the changes at the molecular level in the cells of the tissue to detect the presence of the cancer. Development of imaging technologies that allow for detection at early stages without introduction of any external agents that can be harmful for the body, is a great step forward in medical imaging. Changes in molecular mechanisms that could earlier be only detected using genetic technologies now have a way of external appearance and detection due to change in fluorescence properties of the cancerous tissue.[Zeng et al., 2004]

Table 1 lists the various diagnostic markers that act as endogenous fluorophores for different cancer detection. Studies have shown that there is an increase in phospholipids and total cholesterol in the cancerous tissues. [Kondepati et al., 2005; Kondepati et al., 2008] Furthermore, the difference in the scattering and absorption spectra of the normal and cancerous tissue can be attributed specifically to the changes in the haemoglobin and the oxyhemoglobin which itself corresponds to the increased blood volume in the tissues. There is also a decrease in the tissue haemoglobin oxygen saturation indices that can be attributed to metabolically active tissues in the cancerous tissue. There is higher water content due to increased cellularity and results in oedema. [Kondepati et al., 2005; Kondepati et al., 2008]
<table>
<thead>
<tr>
<th>Tissue</th>
<th>Diagnostic Markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>Oxyhemoglobin and deoxyhemoglobin, or combination of these with water and lipid concentrations, and scattering</td>
</tr>
<tr>
<td>Skin</td>
<td>Combinations of the bands for oxyhemoglobin and deoxyhemoglobin, water and lipid bands</td>
</tr>
<tr>
<td>Prostate</td>
<td>Water bands</td>
</tr>
<tr>
<td>Brain</td>
<td>Total blood volume and oxygen saturation</td>
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<tr>
<td>Pancreas</td>
<td>Lipid bands</td>
</tr>
<tr>
<td>Colorectal</td>
<td>Lipid bands</td>
</tr>
</tbody>
</table>

Several advancements have been made in the use of NIRS for the detection and diagnosis of cancer. Suggestions have been made for the use of the exogenous fluorophores for imaging and detection. [Adams et al., 2007; De Grand et al., 2006; Pierce et al., 2008; Cerussi et al., 2009] A variety of targeted (NIR fluorochrome attached to affinity ligand) [Demos et al., 2009] and activatable image probes based on fluorescence energy transfer have been developed. In addition use of quantum dots [Gao et al., 2004] and nanoparticles for targeted cancer detection imaging has also been suggested but then they all involve the administration of external substances which is not the case in NIR imaging using endogenous endophores. [Pierce et al., 2008; De Grand et al., 2006; Weissleder and Ntziachristos, 2003]

It has also been shown that NIRS can be used not only in diagnostic applications but therapeutic applications also. [Kondepati et al., 2007] These include photodynamic treatment, radiation therapy and neoadjuvant therapy. Studies have shown that NIRS can be used to monitor the progress of the cancer when using various therapies. [Cuenca et al., 2006] Optical surgical methods for treatment of cancers that includes reflectance NIR fluorescence and tomographic NIR fluorescence is one of the potential applications of NIRS. [Weissleder and Ntziachristos, 2003]

CONCLUSION

The entire experience was targeted to study the cancer detection and evaluation using Near Infrared Imaging. Using the tissue samples obtained a series of images were taken under different exposure times and subsequently analysed to understand the advantages that NIR has over currently available imaging techniques. The experience was a rewarding experience where I have gained insight into biomedical imaging and the techniques available.

ACKNOWLEDGEMENTS

I would like to express my heartfelt gratitude to Dr. Huang Zhiwei under whose guidance and assistance, I was able to complete the project. I would also like to extend my regards to Xiaozhou for providing useful information whenever I needed and helping me carry out the experiments. This was a wonderful experience that has definitely helped me in building a good research background during my course of undergraduate studies.
References


Jemal, A., R. Siegel, E. Ward, Y. Hao, J. Xu, T. Murray, and M. J. Thun (2008), Cancer


Saisho, H., and T. Yamaguchi (2004), Diagnostic imaging for pancreatic cancer: computed tomography, magnetic resonance imaging, and positron emission tomography, Pancreas, 28(3), 273-278.


APPENDIX

Figure 1 displays image of the tissue taken under white light exposure.

Figure 2 displays image of the tissue taken under fluorescence light under exposure of 5s when the angle of the polariser was set to 0 and that of analyser to 90.
Figure 3 represents the pixel locations of highest intensity in the image under fluorescence light at 5 seconds exposure and angle of the polariser 0 and that of analyser 0.

Figure 4 represents the pixel locations of highest intensity in the image under fluorescence light at 5 seconds exposure and angle of the polariser 0 and that of analyser 90.
Figure 5 represents the pixel locations of highest intensity in the image under fluorescence light at 5 seconds exposure and angle of the polariser 45 and that of analyser 45.

Figure 6 represents the pixel locations of highest intensity in the image under fluorescence light at 5 seconds exposure and angle of the polariser 45 and that of analyser 135.
Figure 7 represents the pixel locations of highest intensity in the image under fluorescence light at 5 seconds exposure and no polariser.

Figure 8 represents an overlying image with the suspected boundary between the cancerous and the normal tissue under fluorescence light at 5 s exposure. It clearly demonstrates that this difference in intensity between the two tissues can be suitably used for demarcating cancerous tissue from normal tissue.
# Undergraduate Research Opportunities Programme (UROP)

## Timesheet

**Student's Name:** Swati Jain  
**Matric No.:** U075254E  
**Department:** Bioengineering  
**Supervisor’s Name:** Dr. Huang Zhiwei  
**Project Title:** Near Infrared Fluorescence for Imaging and Spectroscopy for Cancer Detection  
**Start Date:** 18/9/2009

<table>
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<tr>
<th>Date</th>
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<th>Description of Work</th>
<th>Supervisor’s Signature</th>
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<td>18/9/08-17/9/08</td>
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<td>Reading up journals for basic knowledge for NIR spectroscopy</td>
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<tr>
<td>18/10/08-19/11/08</td>
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<td>~30</td>
<td>Journal review for existing methods for NIR spectroscopy and reasons for usage of endogenous fluorophores for cancer detection</td>
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<tr>
<td>20/11/08-30/11/08</td>
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<td>Writing and submission of preliminary report collating the journal review on NIR imaging for cancer detection</td>
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Exam Break + Exams for Sem 1 for AY08 09

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<th>Date</th>
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<td>Study of instrumentation set up in the lab and compare the advantages of this set up with the current methods</td>
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<td>Performing the experiment on the tissue sample obtained from the Singapore General Hospital to study the imaging of cancer using NIR</td>
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Exam Break + Exams for Semester II AY08 09

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<td>900</td>
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<td>Analysis of results and writing final report for grading by mentor</td>
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</table>

**Total Hours:** 155

**Remarks:**

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*Note: Supervisors' signatures are not clear.*