TEXTURE ANALYSIS OF OPTICAL COHERENCE TOMOGRAPHY SPECKLE FOR THE DETECTION OF TISSUE VARIABILITY

by

Andras Adam Lindenmaier, H.B.Sc.

A thesis submitted in conformity with the requirements for the degree of Masters of Science
Department of Medical Biophysics
University of Toronto

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Abstract

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Andras Adam Lindenmaier

Master of Science
Graduate Department of Medical Biophysics
University of Toronto
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About 50% of cancer patients are treated with X-ray radiation therapy; however, with current treatment feedback, the effects and the efficacy of the treatment are generally detected several weeks/months after treatment completion. This makes the adjustment of the treatment based on early response, and identification of non-responding patients, nearly impossible.

In this thesis a novel method combining optical coherence tomography and a gamut of image analysis methods is explored as a potential approach to detecting tissue variability. Applying texture analysis to the optical coherence tomography images may allow for the tracking of radiation therapy induced cell microstructural changes in cancer patients and help in the adjustment of treatment based on early response.
Acknowledgements

Over the course of the past two years, there have been numerous people who helped me along the way. I would like to take these few lines to thank everyone in the PMH community, all of my Dept. of Medical Biophysics friends, professors, staff, and co-workers.

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# Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>2D</td>
<td>two dimensional</td>
</tr>
<tr>
<td>3D</td>
<td>three dimensional</td>
</tr>
<tr>
<td>BL</td>
<td>basal layer</td>
</tr>
<tr>
<td>BV</td>
<td>blood vessels</td>
</tr>
<tr>
<td>CNRS</td>
<td>Le Centre national de la recherche scientifique, France</td>
</tr>
<tr>
<td>CT</td>
<td>computed tomography</td>
</tr>
<tr>
<td>c/v</td>
<td>ratio of two parameters of generalized Gamma distribution</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribo nucleic acid</td>
</tr>
<tr>
<td>F</td>
<td>fat</td>
</tr>
<tr>
<td>FdOCT</td>
<td>Fourier domain OCT</td>
</tr>
<tr>
<td>FT</td>
<td>Fourier Transform</td>
</tr>
<tr>
<td>IL</td>
<td>intermediate layer</td>
</tr>
<tr>
<td>IR</td>
<td>infrared</td>
</tr>
<tr>
<td>kθ</td>
<td>product of the two parameters and mean of the Gamma distribution</td>
</tr>
<tr>
<td>LP</td>
<td>lamina propria</td>
</tr>
<tr>
<td>MLE</td>
<td>maximum likelihood estimation</td>
</tr>
<tr>
<td>NRC</td>
<td>National Research Council of Canada</td>
</tr>
<tr>
<td>NRC_OCT</td>
<td>OCT system on loan from the National Research Council of Canada (2D)</td>
</tr>
<tr>
<td>Nu</td>
<td>nude</td>
</tr>
<tr>
<td>OCT</td>
<td>optical coherence tomography</td>
</tr>
<tr>
<td>PSF</td>
<td>point spread function</td>
</tr>
<tr>
<td>ROI</td>
<td>region of interest</td>
</tr>
<tr>
<td>RT</td>
<td>radiation therapy</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SGLDM</td>
<td>spatial grey level dependence matrix</td>
</tr>
<tr>
<td>SL</td>
<td>superficial layer</td>
</tr>
<tr>
<td>SNR</td>
<td>signal to noise ratio</td>
</tr>
<tr>
<td>sdOCT</td>
<td>spectral domain OCT</td>
</tr>
<tr>
<td>ssOCT</td>
<td>swept source OCT</td>
</tr>
<tr>
<td>tdOCT</td>
<td>time domain OCT</td>
</tr>
<tr>
<td>TOR_OCT</td>
<td>OCT system built in the Vitkin Lab in Toronto (3D)</td>
</tr>
<tr>
<td>WC</td>
<td>window chamber</td>
</tr>
</tbody>
</table>
Chapter 1: Introduction

1.1. Cancer, prevalence, and treatment

Cancer is loosely defined as an uncontrolled and abnormal collection of cells that are free to divide, show decreased response to the immune controls of the body, and spread to neighbouring tissue to cause further disease. As described by Hanahan and Weinberg in their definition of the "Hallmarks of Cancer" [1], to be able to call a group of cells a tumour, ten criteria have to be satisfied, as shown in the figure below: 1) they sustain their own growth by sending proliferation signals, 2) they avoid inhibitory signals and control by evading growth suppressors, 3) they can reproduce indefinitely through replicative immortality, 4) they invade other cells/tissues and metastasize to other organs; 5) they grow their own blood supply through angiogenesis, 6) they resist pre-programmed cell death: and more recently discovered: 7) they can reprogram the cells' metabolic pathways to allow for increased growth rates, 8) they can avoid the body's normal immune responses, 9) they usually induce tumour promoting inflammation, and 10) they have unstable DNA.

Figure 1: Hallmarks of Cancer (adapted from [1])
According to the Canadian Cancer Society [2], about 30% of deaths in Canada are caused by cancer and 41% of females and 46% of males are expected to develop cancer at some point in their lifetime.

As a way to control the disease, about 50% of cancer patients are treated with X-ray radiation therapy (RT) [3]; however, the effects and the efficacy of the treatment are generally detected several weeks/months after treatment completion. This makes the adjustment of the treatment based on early response, and identification of non-responding patients, nearly impossible. Since radiation therapy uses ionizing radiation to create differential tissue damage between tumour and normal tissue, it is not side-effect free and has quite a few detrimental consequences. Ionizing radiation not only breaks DNA bonds directly, it also interacts with molecules in the body (primarily water) to create free radicals that cause DNA damage as an indirect effect [4]. Normal tissue generally receives lower doses, can recover more readily, and so it is somewhat less affected; however, some permanent damage is always also caused. It would prove beneficial for patients if tumour status and its response to radiation therapy early on in the process could be measured, so that different treatment options could be explored without causing negative side effects through inadvertent normal tissue irradiation.
1.2. Radiation therapy response assessment and monitoring

Ionizing radiation is known to cause tissue damage by affecting cells, connective tissues, as well as vasculature. Radiation therapy (RT) relies on the fact that slowly dividing normal cells can repair DNA damage more readily than rapidly dividing tumour cells can. As a result, radiation differentially damages both types of cells: quickly dividing tumour cells are often damaged irreversibly, while slowly/not dividing normal cells are given a chance to repair themselves.

Radiation is known to induce many of the different forms of cell death in different proportions, all of which follow a specific pathway [5]. Early types of cell death induced by radiation are apoptosis, senescence, autophagy and necrosis, while mitotic catastrophe is considered a type of late cell death [5]. Apoptosis is the process by which cells undergo death either due to internal or external stresses that signal the cell that it needs to terminate. It is a normal process that limits the existence of damaged cells in the body. Senescence is a similar, self-control process, but does not result in immediate cell death. Senescent cells simply stop replicating; however, they may be present for a while in the body. Autophagy is another type of cell death which is more dramatic and fast. The cell engulfs parts of its cell membrane and breaks down the components into small macromolecules and units of energy that can be reused by other cells. This way some of the cell is recycled. Finally, necrosis is also a type of early cell death and it is characterized by a disorganized process that the cell does not have control over and is usually due to unfavourable conditions (for example lack of oxygen) that do not allow the cell to survive.

Whether the death is late or early is determined by whether the cells have already entered mitosis. Mitotic catastrophe is caused by the cell's inability to divide properly and, as such, it becomes trapped in a half-divided state from which it can not revert and it dies. The shorter the interval from RT delivery to cell division, the more likely it is that the chromosomes will pair incorrectly and cause cell death.

RT can induce more than one type of radiation response and can cause more than one type of damage in solid tumours. As such, the responsiveness of the tumour to the therapy needs to be assessed very thoroughly. Currently, there are no good methods to assess tumour response to radiation: most involve measurements of tumour volume changes. While tumour shrinkage
has shown some correlation with positive outcomes of treatment [6,7], the correlation is not very good and there are many shortcomings of the method [8]. The dead cell and connective tissue mass that is usually present in solid tumours can not quickly disintegrate after RT, so even though the tumour cells may be completely eliminated, the tumour size will not change for weeks to months. Furthermore, shrinkage of the tumour by even as much as 75% does not guarantee that there are no more tumour initiator cells present. This, unfortunately, leaves the possibility that they are still viable and could cause tumour recurrence soon after RT is completed.

A more dynamic feedback system that can provide a reliable measure of the early response of the tumour to radiation could ensure much better treatment plan adaptation, may be able to predict the chance of recurrence, and would provide the option of forgoing treatment if the benefits are not clear. Adaptive therapies would not only allow for temporal control of the delivered RT, but also spatial control. With the information provided by high resolution real-time imaging, very precise doses could be delivered to the areas with highest probability of clonogenic cells and reduced doses to those areas with less probability of harboring cells of tumorigenic capabilities. This would further help decrease the amount of normal tissue being damaged during RT procedures for patients who are indeed responsive to treatment.

1.3. Motivation of the study: detection of tissue changes due to RT

A potentially more sensitive alternative to bulk tumour volume assessment is through the determination of what fraction of cells undergo specific types of cell death as well as measuring the real time of their death and not a delayed signal. Cell death results in cellular changes that cause the tissue optical properties to change, and so the tissue will likely exhibit a different signal in an OCT image depending on what its composition is. Highly condensed DNA material and cell membrane blebbing will increase the amount of scatter returning from the tissue, while cellular swelling and phagocytosis will likely give off yet a different optical signature. Table 1 shows some of the cellular microenvironment changes that are expected when cells die and tissue is RT altered.
Table 1: Cancer cell death types and the cellular and microenvironmental changes that they are associated with that may affect the way the tissue will interact with light. (adapted from [5])

<table>
<thead>
<tr>
<th>Radiobiological Event</th>
<th>Cellular Micro-environment Changes</th>
</tr>
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<tbody>
<tr>
<td>Necrosis</td>
<td>- Cellular swelling</td>
</tr>
<tr>
<td></td>
<td>- DNA digestion</td>
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<tr>
<td></td>
<td>- Lysis of cellular and organelle membranes</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>- Cell membrane blebbing</td>
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<tr>
<td></td>
<td>- Cell shrinkage</td>
</tr>
<tr>
<td></td>
<td>- Nuclear fragmentation</td>
</tr>
<tr>
<td></td>
<td>- Chromatin condensation and fragmentation</td>
</tr>
<tr>
<td>Senescence</td>
<td>- Flattening and granularity of cell</td>
</tr>
<tr>
<td>Autophagy</td>
<td>- Cell membrane blebbing</td>
</tr>
<tr>
<td></td>
<td>- Chromatin condensation</td>
</tr>
<tr>
<td></td>
<td>- Formation of vesicles</td>
</tr>
<tr>
<td>Mitotic Catastrophe</td>
<td>- Multiple micro-nuclei</td>
</tr>
<tr>
<td></td>
<td>- Nuclear fragmentation</td>
</tr>
<tr>
<td></td>
<td>- Dicentric chromosomes</td>
</tr>
<tr>
<td>Connective Tissue Remodeling</td>
<td>- Stromal remodeling</td>
</tr>
<tr>
<td></td>
<td>- Fiber shortening</td>
</tr>
</tbody>
</table>

The hypothesis of this thesis is that optical coherence tomography (OCT), a high resolution imaging modality, can distinguish between different tissue types, with the potential to track RT induced changes in tissues. As shown in Table 1, RT induced cell death can follow very different paths depending on the processes by which the cells break down. As such, each may produce a very unique signature in the OCT signal that will likely be extractable using advanced image analysis methods.

The aim of this project is to first develop an image analysis algorithm that can detect these cellular and microenvironment changes, and to correlate these metrics with the different types of tissue responses induced by RT. There is a wide array of cells, of which only a small proportion possess tumourigenic capabilities. The differential treatment of these would prove invaluable as a tool for tumour control. These newly developed analysis methods should report on previously undetectable functional and structural tissue changes in-vivo, with potential impact.
for improving the optimization/delivery of radiation therapy as well as having fundamental scientific value in “shedding light” on radiotherapy.
Chapter 2: Optical Coherence Tomography

Optical coherence tomography is a high-resolution, contrast-free, and relatively fast imaging modality that can be used for the imaging of microscopic structures in vivo. It uses laser light as a probe and, as such, is able to resolve tissue structures the size of a few microns [9]. The contrast in OCT is due to the changes in the refractive index of the medium or sample being imaged and, therefore, OCT is highly sensitive to detecting boundaries and different layers without the need for potentially toxic contrast agents. Due to the scattering and absorption of tissue, OCT can usually image up to ~2 mm into it, though this number is both wavelength, power, and tissue dependent.

Figure 3: OCT imaging capabilities as compared to other modalities. The sizes of the disks show the resolution, while the lengths of the pendulums show the penetration depths of each modality. (adapted from [10])

Various types of OCT imaging exist; the most common are structural, speckle variance, and Doppler flow imaging. In Figure 4 below, structural, speckle variance and Doppler OCT images of a healthy normal patient's oral cavity can be seen in panels a), b), and c) respectively. In brief, structural imaging, as the name suggests, is a 2D (slice) or 3D (slab) intensity rendering of a sample that the laser light penetrates into and is scattered back from. The result is an image in which the structures appear as bright or dark objects compared to the surrounding medium depending on whether they are more or less scattering, respectively. Speckle variance imaging
takes advantage of the fact that parts of a sample that are more fluid or have higher thermal motion have a more uniform backscattered signal than parts that are more solid or have less thermal motion. Since the scatterers (as in the case of blood vessels and red blood cells) can more readily move in a liquid than in a solid, the signal is also more variable in time. By taking multiple images of the same location and comparing the temporal variability of the signal from image to image, it is possible to trace out where the sample is more fluid and where it is more solid [11]. This method is mainly used for detecting blood vessels. Finally, using a slightly different imaging setup (just like in ultrasound), OCT can be used for the measurement of flow. If the sample that is imaged has vessels that have flow in them, then by looking at the backscattered signal changes with time and using the Doppler formulas, the blood flow rate can be calculated, with the one condition that the flow direction and imaging axis are not perpendicular.

In this thesis, the signal in structural OCT images will be analyzed for the detection of tissue changes that are smaller than the resolution of the system. As such, these changes are not readily visible in the acquired images, but can be detected with advanced image analysis statistics/signal processing.

Figure 4: a) Sample structural image of the labial tissue of a normal human volunteer with corresponding histology showing some common features of the oral mucosa: IL - intermediate layer, BL - basal layer, SL - superficial layer, LP - lamina propria, BV - blood vessels, F - fat. Scale bars 500 µm b) Sample speckle variance images showing microvasculature. Scale bars 200 µm. c) Doppler flow images of human labial tissue. 1mm x 1mm regions shown. (adapted from [12] and appendix B).
2.1. OCT Fundamentals

OCT is an interferometric imaging modality in which a laser beam is split into two components: part of the signal goes to a reference arm and part goes to a sample arm. The reference arm is reflected back by a mirror, while the sample arm interacts with a tissue sample placed into the beam and collects the light back-scattered to bring it to the beam splitter. Here the two returning waves combine to form an interference signal which can be measured by a detector [9].

The image can be formed in many ways: the simplest is to move the reference mirror back and forth, which causes the reference arm to interfere constructively with different parts of the returning sample arm signal, thus probing different depths of the tissue sample [10]. By plotting the intensity of the light at the detector as a function of the reference mirror's position, an A-scan line can be formed, which is just a representation of the amount of coherent light that is scattered back from the tissue. If the sample is then also translated, many different A-scans can be combined to form a B-mode image which is a typical OCT image showing the changes in refractive index, or the scattering boundaries as light is traveling in depth into the tissue. Figure 5 below shows the laser shining onto a beam splitter with half of the signal going to the reference mirror and half hitting the sample. The mirror reflects the light back, while the sample scatters some of the light back as well. The two signals form a readable intensity value at the detector when the two waves interfere constructively and by moving the reference mirror, the entire depth can be probed. The A-scan is simply the intensities sensed by the detector as a function of different depths (different positions of the reference mirror). To obtain the B-scan, the sample or the laser beam is incrementally translated in one direction. This can be further expanded by translating the sample in the other (perpendicular) direction as well, thus, forming a 3D intensity image.
In the following, it will be discussed how exactly the image is formed when the reference mirror moves. This type of OCT is called time domain OCT (tdOCT) as there is a time delay between the sample and reference arms. In OCT, low coherence light is used, which means that the laser light is only in phase with itself for a short period of time or over a short physical distance. The laser shines onto the beam splitter and is split into two separate beams. One beam always goes to the sample and interacts with the tissue where the light is scattered back from various depths. The other beam goes to the reference mirror and is reflected back. However, since this mirror is moving, the light takes a little longer to get back to the beam splitter. This time difference is proportional to twice the path difference between the sample and reference arms. From all the backscattered light that comes from the sample arm, only the small part that is still coherent (within that path difference), is what interferes with the reference beam to form a signal. As such, the shorter the coherence length -- or the distance over which the temporal
coherence of the laser light is maintained [13] -- the smaller the region of tissue that is being probed. The coherence length is chosen as small as possible to increase the axial resolution of the system. The resolution also depends on the bandwidth of the laser beam, as the bandwidth is inversely proportional to the coherence length [10]. The coherence length of the OCT system is given by equation (1) below [13], where $l_c$ is the coherence length/resolution of the system, n is the refractive index of the medium being imaged, $\lambda_o$ is the central wavelength, and $\Delta\lambda$ is the spectral bandwidth of the laser source. Coherence length is used synonymously with the axial resolution of the system [13].

$$l_c = \frac{2 \ln(2) \lambda_o^2}{n\pi \Delta\lambda}$$  

(1)

Moving a mirror back and forth is not very optimal or fast: mobile components introduce unnecessary vibrations and noise. As such, with advances in technology, a much more convenient method was devised, in which instead of moving the mirror, the laser beam is swept through a range of wavelengths. This sweeping, while keeping the reference mirror in place, results in a slightly different depth imaged at every wavelength, since with increasing wavelength a different depth is probed. The sample reflectivity as a function of depth (i.e. the A scan) is still recoverable via an inverse Fourier transform (FT). This type of OCT imaging provides a faster and better signal to noise ratio (SNR) and has become the standard for OCT systems. It is commonly referred to as “swept source OCT (ssOCT)” and it is a type of Fourier domain OCT (FdOCT).

The actual physics behind ssOCT is a little less intuitive and it is detailed below. As the name suggests, it uses the fact that the signal detected is a function of the wavelength of the laser and is encoded in frequency space. The signal can be Fourier transformed to find the depth resolved intensity profile of the sample. Unlike tdOCT, the reference mirror stays fixed and the signal is detected over a range of wavelengths that are emitted with a very narrow spectral line width. As such, at any one time the signal detected continuously is also collected from a very narrow spectral width and is given by [14]:

$$I_D = |E_D|^2 = \langle E_D|E_D^*\rangle = (E_R + E_S)(E_R + E_S^*) = 2E_0^2 + 2E_0^2 \cos(2kn\Delta z)$$  

(2)
where the reference and sample arms have the input power split equally. In the above equation, $I_D$ is the intensity detected at the detector, $I_0 = |E_0|^2$ is the initial intensity, $E_D, E_R, E_S, E_0$ are the electric fields at the detector, coming from the reference arm, coming from the sample arm, and the initial fields, respectively. The last equality requires that the reference and sample arm electric fields be treated as real vectors. $k = 2\pi/\lambda$ is the particular wavenumber detected, $n$ is the refractive index and $\Delta z$ is the path length difference between the reference and sample arms. Now the backscattered signal comes from multiple depths and so the total intensity needs to be integrated over depth to give:

$$I_D(k) = 2I_0[1 + \int_0^{\infty} r(z)\cos(2knz) \, dz] + \int_0^{\infty} \int_0^{\infty} r(z')r(z)e^{-i2kn(z-z')} \, dzdz'$$

(3)

where $I_0$ is again the initial intensity, $r(z)$ is the reflectivity at depth $z$, $n$ is the index of refraction, $k$ is the wavenumber, and $i$ is the complex number equal to $\sqrt{-1}$.

The second term in the above equation describes the mutual interference of waves scattered within the sample, which, due to their random nature, is very small and is usually filtered out at the OCT detection end. For the many different wavelengths that the laser sweeps through, the signal detected becomes:

$$I_D(k) = 2I_0(k)[1 + \int_0^{\infty} r(z)\cos(2knz) \, dz]$$

(4)

where the second term of equation (3) has been dropped for simplicity. $I_0(k)$ is the intensity of the laser source at wavelength $k$, which is not perfectly even across all the emitted wavelengths of the laser source, due to the design limitations of it.

The meaning of $I_D(k)$ in frequency domain is a little less intuitive, so to transform the intensity profile from k-space into the spatial domain, the inverse FT of equation (4) is taken:

$$FT^{-1}[I_T(k)] \approx FT^{-1}[I_0(k)] \otimes [\delta(z) + \hat{r}(z)]$$

(5)

Where the intensity with depth is now the convolution ($\otimes$) between the point spread function (PSF) and the sum of the Dirac delta function and the depth dependent reflectivity of the sample.
It is clear that the greater the spread (bandwidth) of $I_0(k)$, the more sharp the $\text{PSF} = \text{FT}^{-1}[I_0(k)]$ will be and so the higher the axial resolution of the system, as discussed before.

### 2.2. Speckle and texture analysis

Due to the interferometric nature of OCT, light that is backscattered comes from more than one scatterer in the tissue sample, resulting in speckling of the image. In simple terms, speckle can be thought of as the graininess of the typical OCT image. It arises from the random interference of waves scattered by sub-resolution scatterers in the sample. In tissue, these are the cellular organelles, nuclei, and other features that have high scattering coefficient mismatch (i.e. spatial variation in the refractive index). The speckle texture does not correspond to resolvable scattering structures, but the brightness of the speckle pattern does reflect the local distribution and average optical properties of the underlying scatterers. As such, speckle can be used to infer more information than what the direct structural image has to offer, but advanced image analysis algorithms are needed to extract this information. Image analysis of the OCT image speckle texture is one such approach.

In OCT, speckle is actually caused by two phenomena: one component is the random, deterministic interference of the backscattered waves, as described above, and the other is the noise in the system, that is due to electrical noise as well as multiply scattered light. As such, OCT images contain both signal-carrying and signal-degrading speckle [15]. Over the years, many OCT speckle removal algorithms [16-18] have been developed in an attempt to create smoother images with higher structural contrast, but potentially with reduction of the signal-carrying speckle.

More recently, interest was again stirred in trying to understand OCT speckle and to try and extract its signal-carrying information. With this approach, different tissue types may be differentiable in non-processed, raw OCT images. Based on Gossage et al [19] three main types of texture analysis can be explored: statistical, spectral and structural. The statistical methods rely on region histograms and usually can quantify coarseness and contrast in 2D images. Spectral methods (analyze the frequency domain $I_0(k)$ signal) use the autocorrelation function of a region to quantify the periodicity and the orientation/organization of the texture. Finally,
structural methods based on pattern primitives employ positioning rules to describe and identify the texture.

A few recent studies have shown that texture analysis of speckle can indeed be used to differentiate tissue types. Sullivan et al [20] explored the possibility of differentiating types of tissue derived from breast cancer patients and using fractal analysis (statistical) categorized them into adipose, stroma, and carcinoma. Flueraru et al [21] differentiated the various layers of an ex-vivo rabbit artery using metrics derived from the scattering coefficients of the tissue as well as fractal analysis. Neither of these methods quantified the tissue differences in-vivo however.

In this thesis, some of the same as well as other, both statistical and structural methods are tested for their ability to detect tissue variability in OCT images of in-vivo normal and tumour tissues.
Chapter 3: Methods

3.1. Mouse window chamber model

The experiments described in below used mice for in-vivo experimentation of cancers. A mouse window chamber model was used that has been previously described briefly in [22], but for completeness it is again discussed here. Due to availability, suppressed immune system, and lack of hair, the NCr Nu (Taconic) nude mice were used for window chamber implantation. All mice were female of age ~10 weeks old. They underwent surgery for the implantation of the window chamber as well as xenograft induction. The Me 180 human cervical cancer tumour xenografts were created from ~500,000 cells transfected with DsRed2 dye received from Dr. Richard Hill, Princess Margaret Hospital, Toronto, Canada. The tumours were allowed to grow 7 days before being treated with radiation, after which they were monitored with fluorescence microscopy (Leica) for tumour cellular status monitoring and OCT for tissue microscopic changes.

In the experiments described in the following, a set of 5 is represented: 2 controls that had tumours but were not treated with radiation and 3 mice that received 8, 15 and 30 Gy of radiation. The radiation was delivered as a single dose using the small animal micro-irradiator available in the Princess Margaret Hospital animal facility (X-Rad 225Cx Precision Xray Inc., Toronto, Canada), as further described in the next section.

All experiments were conducted under general anesthesia using a mixture of 5mg/mL ketamine and 1mg/mL xylazine. The mass of the mouse in grams was multiplied by 18 to get the amount of anesthetic administered in µL. The mice were implanted with the window chamber and the tumour xenograft during the same procedure. Seven days later they received radiation therapy, and after were imaged using OCT and fluorescence microscopy. All experiments were performed according to the University Health Network Animal Resource Center guidelines for the care and use of laboratory animals.

3.2. Animal micro-irradiator

The technical specifications of the X-Rad 225Cx are available from Precision Xray inc. The settings used in this experiment are shown in the table below:
<table>
<thead>
<tr>
<th>Setting</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irradiation Energy</td>
<td>100 kVp</td>
</tr>
<tr>
<td>Tube Current</td>
<td>6.4 mA</td>
</tr>
<tr>
<td>Dose rate</td>
<td>2.35 Gy/min</td>
</tr>
<tr>
<td>Filter</td>
<td>aluminum</td>
</tr>
<tr>
<td>Collimator</td>
<td>2.5 mm</td>
</tr>
<tr>
<td>Source to surface distance</td>
<td>~10 cm</td>
</tr>
</tbody>
</table>

Table 2: X-Rad 225Cx settings used for the focal irradiation of the tumours.

The mice were anesthetized as described above and were placed onto a custom stage as shown in Figure 6.

![Figure 6: Animal micro-iradiator setup with a) mouse window chamber visible with radiochromic film in place (radiation area shown by the blue colour change), scale bar 1 cm b), c) two side views of custom stage with mouse in place under the collimator and X-ray tube. Scale bars 5 cm.](image)

An aluminum filter was used in conjunction with a 2.5mm lead collimator to focally deliver the radiation exactly to the tumour area with the mouse window chamber placed at ~3.5
cm from the collimator (giving an irradiation spot of 4mm in diameter). Radiochromic film was used to check the alignment of the X-ray beam with the tumour location before treatment delivery and to verify dosimetry. RT was then delivered as a single dose of 8, 15, and 30 Gy to three of the 5 mice (M1, M2, and M3 respectively) discussed here, while the other two control mice (C1 and C2) were also in the room and received the same handling as the ones treated (but received no RT). The doses were chosen so as to reflect the typical cumulative fractionated external beam dose, or brachytherapy dose used for the treatment of cancer patients.

3.3. Two OCT systems: advantages and disadvantages

After irradiation, the mice underwent imaging for 21 days at 1, 2, 4, 7, 10, 14, 17, and 21 days post treatment. They were first imaged using the Leica stereomicroscope and, once the location of the tumour was determined from the fluorescence, both the normal, tumour, and in between (transition) areas were imaged using OCT.

In this study, two OCT systems were tested out as a means of possibly detecting cellular microstructure level changes. As discussed in chapter 1.2., the radiation induces biological changes that are sub-resolution (sub-micron) in size but that should affect the speckle in OCT images. To this end, systems with greater sensitivity would allow for easier extraction of the radiation effects.

The two systems used are the swept source OCT system on loan from the National Research Council of Canada (NRC) in Ottawa that has a swinging ball lens fiber at the sample end and allows for 2D imaging only (NRC_OCT) [23] and the swept source OCT system that has galvo mirrors at the sample arm and allows for 3D imaging (TOR_OCT), developed in the Vitkin lab [11]. The probing ends of the two systems are shown in Figure 7.

Both OCT systems have a similar resolution; however, the detection is different. The NRC_OCT system offers better depth of penetration due to a novel differential detection that allows for a better signal to noise ratio [23]. The two systems were used in parallel. To take advantage of its higher sensitivity, the 2D NRC_OCT system was used to image all the mice. The less sensitive but 3D imaging capable TOR_OCT system was used to image only one control (C1) and the 30Gy (M3) irradiated mouse to save time and avoid exposing the mice to unnecessary cumulative doses of anesthesia.
With the current procedure, each mouse was asleep for ~25 minutes/procedure/time point resulting in increased resistance to the ketamine/xylazine anesthetic in the second half of the experiment (days 10, 14, 17, 21). The 2D OCT data was collected in such a way that 5 regions were tested in both the normal and tumour tissue with 5 B-mode images taken at each location, as well as one to two B-mode images acquired in what was considered a normal to tumour transition area of unknown normal/tumour status. As such, at every time point and for every mouse there are a total of 51-52 images. The schematic below shows where in the dorsal skin-fold window chamber (WC) the imaging was performed.
Using the TOR_OCT system, a 6x6x2 mm 3D volume was imaged covering most of the window chamber of the 30Gy mouse and of one of the controls. Due to the penetration limit of the light, and increased attenuation with depth, only ~1 mm of the tissue was actually being probed.

In the 2D NRC_OCT system, a laser of $\lambda_0=1320$ nm, and bandwidth of $\Delta \lambda = 110$ nm was used, which had an axial resolution of ~6 µm and a lateral resolution of 14 µm (in the y direction). As mentioned before, the NRC_OCT's differential balance detection system allowed for better SNR and thus slightly better effective penetration depth.

Similarly, in the 3D TOR_OCT system a 36kHz swept source laser of center wavelength $\lambda_0=1310$ nm, and bandwidth of $\Delta \lambda = 112$ nm was used in a Mach-Zehnder interferometer configuration with 90/10 sample/reference arm power division. The system had an axial resolution of ~8 µm and a lateral resolution of 14 µm in both the x and the y directions.

The specifications of the two OCT systems are summarized in Table 3.

<table>
<thead>
<tr>
<th></th>
<th>NRC_OCT</th>
<th>TOR_OCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central wavelength</td>
<td>1320 nm</td>
<td>1310 nm</td>
</tr>
<tr>
<td>Bandwidth</td>
<td>110 nm</td>
<td>112 nm</td>
</tr>
<tr>
<td>Axial resolution</td>
<td>7 µm</td>
<td>8 µm</td>
</tr>
<tr>
<td>Lateral resolution</td>
<td>14 µm (x and y)</td>
<td>14 µm</td>
</tr>
<tr>
<td>Imaging capabilities</td>
<td>2D</td>
<td>2D, 3D</td>
</tr>
<tr>
<td>Sweep frequency</td>
<td>20 kHz</td>
<td>36 kHz</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>~85 dB</td>
<td>107 dB</td>
</tr>
</tbody>
</table>

Table 3: Specifications for the two OCT systems used.

3.4. Texture analysis

To be able to detect the sub-resolution tissue changes caused by ionizing radiation, texture analysis of the OCT speckle was used. As discussed in section 2.2., the speckle has information about the underlying tissue's average optical properties that can be used to differentiate tissue types and detect response to radiation therapy. Which texture analysis method is best at differentiating specific tissue changes is, however, not known and needs further study. The following methods are a few of the more commonly seen texture analysis methods that have shown some success in differentiating tissue types, though none have been reported to be the
most optimal for OCT speckle analysis. For this project, each was tested extensively on the OCT data obtained using the more sensitive NRC_OCT system. The Gamma distribution based metrics correlated best with the tissue type and were able to differentiate with high sensitivity and specificity what was considered normal tissue from Me180 tumour tissue.

### 3.4.1. Gamma distribution metrics

The Gamma distribution based metrics use the experience from high frequency ultrasound, applying it to OCT images [25,26]. In a study published by Raju and Srinivasan in 2002 [27] it has been shown that the Gamma distribution is best describing the shape of the speckle distribution in high frequency ultrasound images. The study compared the Rayleigh, Rician, K, Nakagami, Weibull, and Generalized Gamma distributions, with the K, Weibull and Generalized Gamma performing the best at fitting experimental data. They however also found that the Generalized Gamma distribution derived parameters showed a larger variability when human skin was imaged. Since the speckling seen in OCT is very similar in nature and the underlying physics to that in ultrasound, the Gamma distribution was used to fit the OCT speckle distribution discussed in this thesis as well, with the hopes that it will provide both a good fit of the data as well as a parameter that can differentiate many tissue types. Speckle in ultrasound arises from the random interference of sound waves that are scattered back from sub-resolution scatterers just as in OCT, but at different length scales. This allows the Gamma distribution to model OCT and optics data quite well too.

Within the B-mode images of 400x900 pixels (height x width), a region of interest (ROI) of 64x190 pixels was chosen at a 100 pixel depth in the center of the images corresponding to a physical area of ~210x630µm. Since all OCT images suffer from attenuation of the signal with depth, this was done to keep the attenuation effect a minimum and not a factor in the analysis. The assumption being made is that the tissue in the 100 pixel depth attenuated evenly. The pixel intensity distribution in the chosen ROI was then represented as a histogram with both a dynamic binning algorithm as well as a constant bin width determined by trial and error. The dynamic binning algorithm was adapted from [28] and used to calculate the optimal bin width for each histogram. Once the histograms were created, they were fitted with the three parameter
Generalized Gamma distribution (eq. (6)) or with the two parameter Gamma distribution (eq. (7)) [29]:

$$f(x; c, v, a) = \frac{c}{a^{cv}} \frac{1}{\Gamma(v)} x^{cv-1} e^{-\left(\frac{x}{a}\right)^c}$$  \hspace{1cm} (6)

$$f(x; k, \theta) = \frac{1}{\theta^k} \frac{1}{\Gamma(k)} x^{k-1} e^{-\frac{x}{\theta}}$$  \hspace{1cm} (7)

where in the context of ultrasound $c$, $v$ and $k$ are shape parameters and are believed to be related to the scatterer number density, while $a$ and $\theta$ are scale parameters and they are thought to describe the scatterer cross section and organization [25]. Equations (6) and (7) are related, with (7) being the special case of (6) when $c = 1$, making $v = k$ and $a = \theta$.

The Gamma distribution is a special case of the Generalized Gamma distribution and as such, the main difference between the two is the fitting algorithm used and the extra parameter that allows for better fitting. The Generalized Gamma distribution uses a Maximum Likelihood Estimation (MLE) to fit the histogram and is highly dependent on the initial values determined from moments (descriptors of the basic shape such as the mean and the variance of a set of points). The Gamma distribution employs a Least Squares estimate that is less sensitive to the initial input parameters and is less computationally intensive. Both programs were written in Matlab R2009b with a free curve-fitting algorithm plug-in called Ezyfit developed by Frederic Moisy [30], University Paris Sud, University Pierre et Marie Curie, CNRS. Ezyfit allows for the definition of the functional form of the curve to be fit to the data and performs the fitting. Both types of Gamma function were assessed for their ability of differentiating tissue types in data sets containing both normal and Me180 tumour tissue. They performed equally well; however, the Gamma distribution was more likely to find a good fit solution than the Generalized Gamma, and therefore it was adopted as the best functional form to fit the experimental data. This was likely due to the greater uncertainty of the initial guesses in the case of the Generalized Gamma distribution and its influence on the occasional lack of proper convergence of the solution.
Various metrics were also explored using combinations of the $k$ and $\theta$ parameters as well as the goodness of fit. The same was done for the Generalized Gamma Distribution. In both cases, the $k\theta$ mean was the parameter that best classified normal and tumour tissue.

In both cases, the dynamic binning based on the Shimazaki algorithm [28] was also evaluated, but showed little to no effect on the quality of the fits and was therefore removed. This way the comparison of one image/ROI to the next was not confounded by the variable binning.

3.4.2. Box counting/fractal dimension

Another texture analysis method that has previously been shown to be able to differentiate different tissue types is that based on box-counting [31]. Very much like in the algorithms used by cartographers to determine the jaggedness of a shore, box-counting determines the jaggedness of a single A-scan line. When the A-scan -- a single depth scan -- intensity is plotted as a function of depth, a jagged curve is seen. Box-counting then divides the A scan profile into equal sized squares and determines how many of them have part of the curve in them. This process, called box counting, is then repeated for many box sizes. The number of boxes with part of the curve in them as a function of the box size gives a relation that on a log-log plot is a measure of the fractal dimension [21] of the image. The more jagged the A scan profile is, the higher the fractal dimension will be. The box dimension has to be larger than the axial resolution of the OCT system. To this end, the same ROI as discussed above was chosen and every one of the 190 A-scans was analyzed for its fractal dimension. These values were then plotted and fitted with a Gaussian curve, the mean of which gives an average fractal dimension. This mean was calculated for all the images in the set and tested as a metric for the differentiation of normal and tumour tissue but, unfortunately, the method showed very little success. Below is a schematic of how the fractal dimension of an ROI is determined:
Figure 9: Schematic diagram of the box counting method for measuring the 1D fractal dimension of A scans in an ROI. An A scan from the ROI is plotted on an intensity vs. depth curve and a grid of set box side length is overlaid. This then is repeated for several box side lengths. The logarithm of the number of full boxes (i.e. boxes containing a portion of the signal trace) as a function of the logarithm of the box length shows a linear relationship, the fit of which has a slope equal to the fractal dimension of that A scan. If this is then repeated for all the A scans in the ROI, the distribution of fractal dimensions and the mean fractal dimension can be determined for every ROI, and used as a metric for tissue differentiation. The more speckled the image is, the higher the variations in the A scan will be and so a correspondingly higher mean fractal dimension will be determined.

Traditionally box-counting algorithms divide the A-scan profile into a square with dimensions that are the multiples of 2. This makes it easy to perform the box-counting as an A-scan of 64 pixels in depth is plotted with a maximum number of grey levels of 64 values and so the 64x64 box can be divided into $2^2$, $2^3$, $2^4$, $2^5$, and $2^6$. Using a 64x64 area, however, is not a requirement of box-counting, it is just an easy implementation programmatically in a binary system and has thus prevailed. This suffers from the fact that when the log-log plot is fitted, there are only 3-4 data points (the first two are ignored since the box length is shorter than the coherence length of the OCT system) to fit. The realization that multiples of other numbers could
be included in the custom box counting algorithm changed the protocol: instead of using the 6 values of 2, $2^2$, $2^3$, $2^4$, $2^5$, and $2^6$, a 60 pixel region of the original ROI was selected and the intensity values scaled to fit to 60 as well. In this way a 60x60 area was created which could now be divided up into 10 box sizes of 2, 3, 4, 5, 6, 10, 15, 20, 30, 60. This gave an additional 4 data points, practically doubling the number of points for the log-log plot described above, thus yielding a more robust fractal dimension value. Unfortunately even with the new algorithm, the fractal dimension did not work too well for the differentiation of tumour and normal tissue and was, therefore, not pursued extensively.

3.4.3. Spatial grey level dependence matrix (SGLDM) based metrics

SGLDM is another texture analysis method that has been mentioned for the analysis of either OCT [19] or CT images [32, 33] but has not been extensively studied for either. In simple terms, it is an algorithm that takes every pixel (for example, in the same pre-defined ROI of 64x190 pixels) and compares it to all its neighbours. SGLDM is a second-order statistic because it takes into account the position of each pixel relative to its neighbours and does not rely only on a statistical distribution like the Gamma distribution method described above, which is a first-order approach. First-order statistics calculate the probability of a grey-level pixel value to be present in a sample whereas second-order statistics calculate the probability of a grey-level pixel value as well as a neighbour in a predetermined direction occurring simultaneously in the sample [34]. As such second-order statistics are more sensitive to directionality in images.

To create the SGLDM of an ROI, the intensity of each pixel is compared to the intensity of all the others. An SGLDM then has as many elements as there are grey-levels in the image being analyzed. In the following, all the ROIs were analyzed using a grey-level colour map of 256x256 values. As such, each SGLDM is 256x256 in size. To find the SGLDM, the algorithm developed by Gossage et al. [35,36,37] was used and implemented for the analysis of OCT images. Each pixel was compared to the ones next to it, in the directions of 0,45,90, and 135 degrees, clockwise starting from the right, respectively, as shown in Figure 10.
The SGLDM is then a probability matrix that gives the probability of each grey level happening in a certain position in the ROI. The following are a few of the parameters calculated from the SGLDM:

\[
\text{local homogeneity} = \sum_{i=0}^{L-1} \sum_{j=0}^{L-1} \frac{1}{1 + (i - j)^2} p(i,j|\theta) \tag{8}
\]

\[
\text{energy} = \sum_{i=0}^{L-1} \sum_{j=0}^{L-1} p(i,j|\theta)^2 \tag{9}
\]

\[
\text{entropy} = - \sum_{i=0}^{L-1} \sum_{j=0}^{L-1} p(i,j|\theta) \log (p(i,j|\theta)) \tag{10}
\]

\[
\text{inertia} = \sum_{i=0}^{L-1} \sum_{j=0}^{L-1} (i - j)^2 p(i,j|\theta) \tag{11}
\]

where \(p(i,j|\theta)\) is the probability that a pixel with gray level \(i\) in the image is a distance of 1 pixel away from a pixel with gray level \(j\) in the \(\theta\) direction. The SGLDM already has the directionality in the above discussed four angles, so \(p(i,j) = \text{SGLDM}(i,j)\).

3.4.4. Simple first-order image analysis metrics

Since the above metrics are all fairly elaborate and dependent on a lot of variables, there is the possibility that the subtle variations in tissue types are lost in the complexity of the methods. As such, simple, first order metrics were also tested for their ability to differentiate
different tissue types. Using the same sized ROI, the skewness and the kurtosis were also calculated. As the names suggest, skewness is a measure of the lack of symmetry in the distribution of the image pixel intensities, while kurtosis is a measure of how peaked or flat the distribution is [29]. They are defined by the following two formulas:

\[
skewness = \frac{\sum_{i=1}^{N} (I_i - \bar{I})^3}{(N - 1)\sigma^3}
\]  \hspace{1cm} (12)

\[
kurtosis = \frac{\sum_{i=1}^{N} (I_i - \bar{I})^4}{(N - 1)\sigma^4}
\]  \hspace{1cm} (13)

where \(I_i\) is the intensity of pixel \(i\) and \(\bar{I}\) is the average intensity over the ROI. \(N\) is the total number of pixels and \(\sigma\) is the standard deviation. As an example, the normal distribution has a skewness of 0 and kurtosis of 3.
Chapter 4: Using the various metrics to differentiate tissue types

As discussed in the previous chapters, the goal is to differentiate tissue types using one, or a combination of, the texture analysis parameters/metrics extracted from the OCT images. The results of tissue differentiation attempts using the various metrics is discussed next, and then followed up with a discussion of their limitations and routes for improvement.

4.1. Generalized Gamma distribution

While the Generalized Gamma distribution showed a good differentiation between the histograms of tumour and normal tissue, it was seemingly not too effective at picking up the effects due to the RT. The c/v parameter showed the most potential at differentiating tissue types, however all combinations of a,c, and v were tested. Here only the results from the Generalized Gamma c/v parameter fits are shown. As the error bars in Figure 11 suggest, there is a large variability within a single mouse/time point which stays relatively consistent throughout.

![Graph showing variation of c/v parameter of the Generalized Gamma Distribution function with days after the delivery of RT. M3 received 30 Gy of ionizing radiation, while C1 received none. The error bars are the SD of the signal over all the tumour or normal images acquired at each time point (a total of ~25 images). The lines have been added to guide the eye.](image-url)

Figure 11: Variation of the c/v parameter of the Generalized Gamma Distribution function with days after the delivery of RT. M3 received 30 Gy of ionizing radiation, while C1 received none. The error bars are the SD of the signal over all the tumour or normal images acquired at each time point (a total of ~25 images). The lines have been added to guide the eye.
the experiment. This is potentially due to the fact that the WC model itself is variable and that there are significant changes in the tissue optical properties when translating through the WC, suggesting a heterogeneous tissue that will have an effect on all measurements. Since there was no way of ensuring that the same area is imaged from one time point to the next, the variation of the error bar sizes between time points is justified. The size of the error bars could be a representation of both the tissue heterogeneity in the WC, as well as the variability in the imaging method and the analysis of the images. If the OCT system is unstable it will result in variations in the signal. Moreover, if the fitting of the pixel intensity distributions is not consistent due to high dependence on initial guesses of the MLE based optimization algorithm, artificial tissue variation can also be created. These concerns are further addressed in sections 4.8.1. and 4.8.3. Due to the high dependence of the Generalized Gamma distribution on the initial values in the MLE optimization process, this method yielded slightly less consistent results than the Gamma distribution and so further development of metrics focused more on those derived from the Gamma distribution.

4.2. Gamma distribution

The Gamma distribution based results are in many ways similar to those seen in the case of the Generalized Gamma. After careful examination of all the different fit derived parameter combinations, the mean $k\theta$ parameter proved to be the best at differentiating different tissue types. As shown in Figure 12, the relative error bars are smaller on average than those seen previously, but the general trends are the same. Both Figures 11 and 12 show the results of texture analysis performed on the exact same OCT images and ROIs within them, so the variation is not due to between sample variability and therefore this can not be too large. As discussed in the methods section, the imaging was performed at 5 different locations in each tissue type and at each location, 5 repeat images were taken without repositioning of the sample. When comparing the $k\theta$ mean value of one image to that of the next image in the same position, the variation is minimal as visible in Figure 13. This suggests that the OCT system is stable enough to provide consistent data and the texture analysis method used is also relatively consistent. The data points for the 5 repeat images taken in the same location group together very consistently both in the tumour and the normal tissue, thus showing a stable imaging system.
Figure 12: Variation of the $k\theta$ mean parameter of the Gamma distribution function with time after the delivery of RT. M3 received 30 Gy of ionizing radiation, while C1 received none. The error bars are the SD of the signal over all the tumour or normal images acquired at each time point (a total of ~25 images). The lines were added to guide the eye. The difference between normal and tumour tissue is clearly discernable, however the differences between the irradiated and non-irradiated tumour tissue are not as clear.

Figure 13: Variability of the $k\theta$ mean parameter of the Gamma distribution function within the single WC of a control mouse at 17 days after window chamber implantation. The two points between the tumour and normal tissue are from what was considered the in-between "transition" region of unknown pathological status. "Islets" of the repeat images taken in the same area are noticeable, showing strong repeatability and stability of the imaging and analysis process. The values for the tumour images are lower, while those for the normal tissue images are higher. The data points are in the order that they were acquired in (both within normal tissue as well as tumour tissue as identified based on the fluorescence) and so the order does not represent physical position within the WC.
Interestingly, the tumour tissue seems to be more homogeneous than the normal tissue (and hence the smaller error bars), something that is likely due to the more scattering, but also more absorbing, nature of the tumour. The consistency of the data however, does not remove the question of inhomogeneous tissue within the WC and the possibility of error due to the positioning of the area being imaged at different time points. As visible in Figure 13, there is considerable variation in both the normal and tumour tissue when imaging in different areas.

### 4.3. Normal and tumour tissue differentiation

As discussed in the article reproduced in Appendix A, it is possible to use the Gamma distribution derived $k\theta$ mean parameter as a metric to differentiate between normal and tumour tissue. In the figure below, the striking difference between the pixel intensity distribution of the tumour and normal tissue can be seen.

Figure 14: Comparison of the pixel intensity distribution of the normal and tumour tissue within the same mouse as well as between an untreated control and RT treated mouse at four days after RT. The $k\theta$ parameter reflects the shape changes seen. The differences due to the radiation are not as obvious as those between each of the normal to tumour comparisons which are noticeable both in the $k\theta$ parameter values as well as the shapes of the histograms. While only showing a single mouse, the same trends are visible in all the other mice analyzed with this method, but not all discussed in this thesis ($n = \sim 12$).
This large difference can be exploited, for example in the context of digitally aided pathology or tissue classification. This find is very exciting, as texture analysis has not been previously shown to detect tissue differences with such high sensitivity and specificity from OCT images *in-vivo*.

![Graph showing differences in kθ parameter between non-irradiated normal and tumour tissue of a single mouse at 11 days after tumour implantation.](image)

Figure 15: Differences of the kθ parameter between non-irradiated normal and tumour tissue of a single mouse at 11 days after tumour implantation, with the transition areas shown as falling somewhere in-between the two (normal regions were identified as those with no fluorescent signal, tumour regions were red fluorescent, while transition regions were considered as those on the edge of the fluorescent tumour region). The mean values are different statistically with a p-value of >10⁻²⁰, analyzed by an unpaired two-tailed t-test. While only showing data of a single mouse, similar differentiation is seen with all other mice analyzed, with some variation in the center of the kθ parameter cluster locations but still complete differentiation between the normal and tumour tissue clusters. The error bars show the SD of the data points between the 25 normal and 25 tumour images acquired. (adapted from [38] and Appendix A)

While the error bars are relatively large due to the tissue inhomogeneities, it is clear that the normal and tumour tissue values of the kθ parameter are significantly different statistically. The difference when quantified by an unpaired two-tailed t-test was found to have a p-value of greater than 10⁻²⁰ at the 95% confidence level.

In Figure 16, a systematic texture analysis of an entire B scan is shown. Unlike in the previous methods discussed, here the ROI is translated across the B scan image and at every position the kθ mean parameter is calculated. This sliding box algorithm developed, allows for the visualization of the continuous variation of the tissue in space. The consecutive translations of the box are such that there is a 90% overlap. The figure shows a gradual decrease in the kθ mean parameter as the box moves from a mostly normal tissue (on the left) to a mostly tumour tissue (on the right). This is consistent with previous observations of the tumour having a lower kθ mean parameter than normal tissue.
Figure 16: Variation of the kθ parameter as a function of translation of the ROI along the B-mode image at a constant depth, with a step size of 90% ROI overlap. The transition from the mostly normal (left of image) to mostly tumour (right of image) tissue is shown by the decrease in the kθ values with position. As noted before, normal tissue has a higher kθ parameter value than tumour. (adapted from [38] and Appendix A)

### 4.4. Fractal dimension

Unfortunately the fractal dimension of the different tissue regions varies so much that it is impossible to tell the signal and noise apart. Most probably, this is due to errors in fitting the few data points (3-4) in the log-log plots and, as a result, the variation is too great from one A scan to the next as well as between different images. Goodness of fit statistics were also tested as a potential metric, but again the trends were not discernable.
Fractal dimension was not optimal for differentiating even between the relatively clear tumour and normal tissue boundaries. Even after the analysis with non-multiples of 2, the results were inconclusive and this type of analysis was not pursued further.

Figure 17: Variation of the mean fractal dimension with time in the untreated control and 30 Gy treated mice. The variability in the box-counting method of log-log fits to determine fractal dimension overshadows any RT effects there may be. This method was also unable to differentiate normal from tumour tissue. The error bars correspond to the SD of all the mean fractal dimension values calculated over the 25 normal and 25 tumour tissue images collected at each time point. The lines were added to guide the eyes.

4.5. Simple metrics: skewness and kurtosis

Skewness and kurtosis were also not very successful at differentiating tissue types. The ROI size may not have been optimal for the large-scale statistics that both of these metrics rely on. A smaller ROI might have helped in identifying tissue heterogeneities and perhaps trends in them. They were also unable to differentiate normal and tumour tissues as visible in Figure 18. Comparing skewness and kurtosis, it is clear that both follow very similar trends and are likely highly dependent on the mean intensity value as well as related to each other. While attenuation may be larger in the tumour relative to normal tissue, giving a good contrast mechanism, the same argument can not be made for RT effects. The Gamma distribution based metrics seemed
to have less variability with WC imaging position changes, and were thus chosen as the analysis/quantification method of choice. Further analysis of the effect of ROI size on skewness and kurtosis may reveal that they are more sensitive metrics to tissue variability and that is why the error bars are so large in Figure 18. However, due to the high correlation between the two metrics, it is highly unlikely that tissue variability is what is biasing the measurements, as both metrics are too dependent on the average intensity in the ROI (see equations (12) and (13)).

Figure 18: Variation of the skewness and kurtosis with time in the untreated control and 30 Gy treated mice. Note the interdependence of the two metrics. The error bars show the SD of the skewness and kurtosis values within the 25 tumour and 25 normal images and are evidence for relatively high variability between the different readings. The lines were added to guide the eyes.
Perhaps an algorithm that also factors in differences due to attenuation may reveal more subtle tissue changes in conjunction with these metrics.

4.6. Spatial grey level dependence matrix based metrics

SGLDM was also tested as an alternate approach to detect some changes due to radiation. Since the SGLDM also takes into account the position of the different pixels, it may provide additional information when compared to the other statistical methods. Here the same ROI dimensions were used as before (64x190 pixels or 210 µm x 630 µm). In Figure 19, the variability of the four SGLDM based metrics with time is shown, but there are no clear trends for the differentiation of normal and tumour tissue or the differentiation of irradiated and non-irradiated tissue.

Figure 19: Variation of the local homogeneity, entropy, energy and inertia with time in the untreated control and 30 Gy treated mice. No clear trends are visible for differentiating irradiated and non-irradiated tumour tissue. Trends for differentiating normal and tumour tissue are also not noticeable. The entropy and energy are related quantities and show very similar trends, while the local homogeneity and inertia are seemingly reciprocals of each other. The lines were added to guide the eyes.
4.7. Variability of the signals in tissue - 3D analysis

As mentioned in the previous sections, there is a need to understand tissue heterogeneity in order to be able to accurately quantify the variation due to cancer, treatment, or other factors. Figures 13 and 16 above clearly show that there is some variability in the WC which is seen as gradual (as detected by the large ROI of the kθ metric which averages the signal and smoothes the sharp edges). This variation has the potential of masking subtle modulations of tissue optical properties if not accounted for thoroughly. One way of dealing with the decrease in resolution due to a large ROI will be discussed in the next section, but here we focus on the tissue variability itself. Figure 20 shows the variation in the kθ parameter of the Gamma distribution, but unlike in Figure 13, here the analysis was "3D" and a more "continuous" sample is used. Similar to the sliding box method of Figure 16, but analyzing all the B scan slices in the 3D OCT data set, a parametric map of the variation of the kθ parameter is calculated over the entire imaged region of the WC. It is clear from the plot that the parameter can vary up to 0.5x10^{-3} which is as much as 20% of the signal. The region imaged in this data set is not large enough to see the spatial variability of tissue (and part of it is tumour tissue), but by comparing the same normal area at each data point and looking at the signal variation it is possible to determine how much the tissue changes over time. The 3D datasets allow for easier co-registration of the images and so potentially better comparison of "identical areas" between time points, without the uncertainty of sample positioning.
Figure 20: Variation of the kθ parameter with position in a control (unirradiated) mouse. The deep blue well corresponds to the location of the tumour as identified using fluorescence microscopy. The registration was performed using white light images and OCT speckle variance images of the vasculature in the WC. The area of fluorescence and the blue well co-register with ~98% overlap. The scales of the x and y direction axes are in pixels, with the x direction pixels having a size of ~8 µm while the y direction pixels have a size of 210 µm due to the averaging effect of the sliding box ROI shown in Figure 16.

4.8. Quantification of the signal variability

The need for quantifying the signal fluctuations due to the different sources of variability are obvious and therefore an attempt is made to address the issue in this section. The variability sources were identified as those due to intra-mouse tissue heterogeneity, inter-mouse heterogeneity (even though mice are identical genetically, their skin tissue is not necessarily the same, this may be due to WC implantation procedure as well as physiological variability), OCT system variability (i.e. laser power fluctuations, alignment of optics due to temperature fluctuations, etc.), and OCT image acquisition location. Of course, there is a time-dependent variability of the mouse WC tissue due to physiological changes.
4.8.1 Intra-mouse variability (tissue heterogeneity)

To address the concern of "intra-mouse" variability, both the spatial and temporal variation needed to be assessed. For the spatial heterogeneity, the variation of the kθ parameter was measured within the same mouse at a single time point in the normal and tumour tissue (25 images each) and repeated for all five mice. The relative standard deviation is the same as the relative variance and is calculated by dividing the standard deviation with the mean and representing the quantity as a percentage:

\[
\text{rel. SD} = \frac{\text{mean}}{\text{SD}} \times 100 \%
\]  

(14)

The relative standard deviation of the different tissue types at the three time points is as shown in Figure 21. The data suggests that the variability is much higher in the normal tissue than it is in the tumour tissue. This is contrary to the expectation that tumour tissue is more heterogeneous than structured epithelial tissue, so the issue was further investigated. The higher variability in the kθ parameter of normal tissue is actually due to the poor fitting of the histograms. As visible in Figure 22, normal tissue is usually significantly thinner than tumour tissue and therefore is not always thick enough for the size of the ROI. As a result, the ROI sometimes is not fully filled by the normal tissue and the fits of the functions to the pixel intensity distributions are quite poor. Because of this, the tumour tissue (which is always thick enough for the ROI used) variability was used to estimate the tissue heterogeneity in general. This has an average value of 9% (range of 3.3-16%) when averaged over all five mice and 3 time points ~1 week apart (chosen to sample the entire temporal duration of the experiment). The maximum of this range, so 16% was used as the variability threshold for all further calculations. That is to say that if the variability within the set of images acquired for a mouse at a time point was larger than this value, there had to have been some error that is causing an unrealistic increase in variability across the WC.
Figure 21: Relative standard deviation of the $k\theta$ parameter within the tumour and normal tissue of each mouse at three time points. The mean and standard deviation of the normal and tumour tissue (25 images each) at every time point was determined. The relative standard deviation (defined as the percent standard deviation over the mean or the relative variance) was then calculated from these. The normal tissue is seemingly highly variable due to an imaging artifact caused by improper fitting of the narrow slivers of normal tissue as shown in Figure 22.

Figure 22: Sample OCT images with the standard ROI selected in a) thin normal skin tissue and b) tumour tissue. The size here is shown as physical distance, with the 200 µm cover-slip clearly visible in image a). As seen for normal tissue a), the ROI overfills its spatial extent, thus the histograms are distorted; this yields poorer fits to the Gamma distribution and scattered $k\theta$ parameter values.
4.8.2. Temporal tissue variability

To estimate tissue variability over time, the average kθ parameters within each mouse over all readings in tumour and normal tissue were determined. The fluctuation of these average kθ parameter values over time in each mouse was then quantified using the relative standard deviation. Only data that were below the maximum of 16% tissue heterogeneity threshold mentioned above were included, resulting in some incomplete results. Nonetheless, Figure 23 shows a consistent trend of a variability of approximately 25-35%. It is important to keep in mind, that this value also includes the 9% average tissue heterogeneity already calculated. Moreover, the natural changes of both the normal and tumour tissue over time will affect these results.

![Figure 23: Relative standard deviation of the average kθ parameter over all time points within the tumour and normal tissue of each mouse. The normal tissue is still highly variable due to an imaging artifact caused by the ROI size and so those data points were not used in this calculation. As a result, the C1 and C2 normal tissue data points are not representative of any real variation since they had too few data points.](image)

4.8.3. System variability

To quantify the system variability, a standard IR card (a card with a thin, uniform IR sensitive coating) was imaged over the period of 20 days, a time span comparable to that of the animal experiments. Unlike the mouse data however, only 1-2 images per time point were recorded, for a total of 12 images and 12 kθ parameter values. During the 20 days, between
readings, the system was actively being used, the IR card sample moved and the laser source switched on and off many times, as required per normal operation. These conditions mimicked what happened during the mouse imaging experiments. After analyzing the IR card images using the same ROI and Gamma fit parameters as for the animals, the variability of the system was determined to be ~17%, though using a small sample size. The plot below shows the variation from the beginning to the end of the experiment.

![kθ parameter variation of a standard IR card over a period of 20 days](image)

Figure 24: kθ parameter variation of a standard IR card over a period of 20 days. The results show a system fluctuation of ~17%.

This 17% value is however quite high and is due not only to system fluctuations but also sample placement. Since the method is so sensitive, and the IR card not completely homogeneous at the micron scale, imaging different areas of it likely results in a slightly different kθ parameter value. But the same issue is relevant for tissue imaging as well, where the tissue area imaged from one time point to the next is not the same; thus, this fluctuation estimate is a realistic estimate of the error associated with each measurement in tissue.

Another way to estimate real system fluctuation is to compare the values of the repeat images acquired in the same sample and location consecutively. It is important to note that while the images are acquired consecutively, the ROI selection and analysis is done independently on every image. By calculating the relative standard deviation of the groups of 5 images in a set of 50 images, it was determined that the fluctuation is between 0.5-3.5% with an average of 1.9%.
4.8.4. Inter-mouse variability

Ideally, to quantify the mouse to mouse variability, the normal tissue of all the mice would be compared. Unfortunately, due to the fact that the normal tissue is too thin for the ROI size, the tumour tissue heterogeneity was estimated amongst the five mice before radiation treatment. The average $k\theta$ parameter value of each mouse on the day before RT was used to calculate the relative standard deviation between them. This yielded 11.6%, consistent with the other variability observations.

4.8.5. Normal to tumour tissue variation and how it compares to all other sources of variation

The normal to tumour tissue variation was again calculated using the relative standard deviation. By taking the $k\theta$ parameter of every mouse and time point where the tissue homogeneity did not surpass the aforementioned 16% threshold in both the normal and tumour tissue each and calculating the relative standard deviation between the two, the average normal to tumour tissue variability was determined to be ~40%. To see how the normal to tumour variability compares to the other calculated variability values, they are all summarized in Figure 25.

It is clear from summary figure (Figure 25) that the normal to tumour tissue variability, while dependent on all the other variations, is larger, and as a result allows for the tumour and normal tissue differentiation. While the difference is not clear, it is expected that both the normal and tumour tissue changes during the 2-3 week time period of the experiment. This shows promise for potentially seeing the more subtle RT effects, given that they cause a change in the $k\theta$ parameter values of at least 25%, which seems to be the limit for the combined system/acquisition-area selection and analysis variation (the sum of average system and average tissue homogeneity values).
Figure 25: Summary of all % variability due to the different factors. The first four values are tissue, position and imaging setup dependent, while the last two are also time dependent, hence their larger values. These values were quantified as described in the different sections of 4.8. It is clear that the difference between normal and tumour tissues causes much higher variation in the $k\theta$ parameter than any of the other variations/sources of error do. It is plausible that the RT effects are more subtle and are thus lost in this "background" variability, and perhaps why the presented method does not readily detect/quantify them.

4.9. Limitations of the methodology

The Gamma distribution based method that uses an ROI which the pixel intensity histogram properties are quantified suffers from some limitations. Specifically, the large size of the ROI results in an averaging of the signal over a large area of 210 x 630 µm, thus reducing the effective resolution compared to the actual resolution of the OCT. Unfortunately this can not be reduced significantly as the Gamma Distribution function analysis needs many data points for a smooth enough histogram fit to be created and strong enough fit statistics to be obtained. The current method uses 64x190 pixels, so a total of ~12000 pixels (optimized by trial and error) to create the statistics necessary for the histograms. A potentially better way of generating enough pixels for 3D analysis is to replace the currently used 2D planar ROI selection with a 3D slab of a similar number of pixels as shown in Figure 26. This way the distance between any two pixels will also be reduced (as the maximum distance will be the diagonal of a slab of much shorter side length and not a large plane). The method would thus improve the resolution in all three dimensions proportionally, without any directional bias introduced. Currently, as visible in
Figure 20 for example, there is bias where the direction between adjacent B scans is sampled very densely, while the direction between adjacent A scans is very scarcely sampled.

![Figure 26: Suggested ROI selection for 3D texture analysis. The sample on the left shows the current imaging method where planar ROIs are translated along a single vertical plane through the sample. The sample on the right shows an improved analysis design where a 3D slab is translated that has a volume encompassing as many pixels as the planar ROI in the current method, but with a smaller pixel to pixel distance. Each side shows 4 "equivalent" ROIs.](image)

Furthermore, mouse to mouse variability needs to be better addressed. Although genetically identical mice were used in this study, tissue characteristics as detected from the structural OCT images (using the image analysis algorithms presented) vary from mouse to mouse. One way to deal with this is to compare only within subject, using a method suggested in the "future work" section (5.3.1.). This may reduce the errors due to mouse-to-mouse variability, but does not address within-WC (intra-mouse) inhomogeneities. Just like any dynamic biological system, there are intrinsic tissue variations that will influence the way light will interact with tissue. These variations need to be accounted for in the image analysis algorithm. Once properly correlated with tissue types, the metrics and the range of values they take on can be better understood in the context of actual values. As with any new imaging modality, a look-up table style database needs to be generated that will allow for the assessment of whether the signal is a real biological variation or an imaging artifact.
Chapter 5: Conclusion

5.1. Impact of work and its applicability

In this thesis, the possibility of detecting tissue variation using OCT combined with advanced speckle texture analysis methods was discussed in detail. There is a need for tumour treatment tracking and OCT may be one of the modalities that can solve this problem.

In conjunction with the new speckle texture analysis techniques presented above, OCT can now allow for the probing of any tissue in the body where a catheter probe, needle, or endoscope can be delivered, and has the potential to provide tumour tracking \textit{in-situ}. While the experiments of this project were conducted in a small group of mice, there is potential for the achievement of radiation response monitoring \textit{in-vivo} using OCT. In the following few sections, some of the steps to further development towards this possibility will be discussed.

5.2. Limitations

Before further experiments are planned, the limitations of the current approach need to be considered. OCT has a high resolution and, with the new methods presented in this thesis, it has sensing capabilities of tissue variation, however, due to the limit of penetration of light into tissue, it can not probe bulk tumours that are large. Theoretically, at any one time the OCT sensor would be able to probe a \(2\times2\times2\) mm volume without having to be translated or removed and reinserted in a different orientation/position. This is a limitation imposed by the physics of the OCT (the focusing optics) and of the depth to which the light can penetrate in tissue. Also, since the resolution of the analysis method is degraded by the large ROI size, the method is limited by the area/volume it can use as an input. To provide the Gamma fit a large enough sample size to form the distribution function, enough points need to be present in the ROI. Currently that size is \(200\times600\ \mu\text{m}\) (not optimal), but this could potentially be reduced by using a 3D ROI instead of a 2D plane as discussed in 4.8.

The number of conclusions that can be drawn from the above results is limited as all the data are in a relatively small sample of 5 mice. Moreover, all the mice were genetically identical, as was the tumour cell line. OCT tissue differentiation that worked in this mouse/cell line combination might not be as sensitive for a different combination. Additional work needs to be
done to explore different tissue as well as tumour types. While it is not possible to try all combinations, the method may be tested for a specific application in mind, such as prostate cancer treatment response monitoring and further customized for it. Once proven in that specific setting, additional applications could be explored.

The final step in any pre-clinical experiment is of course the transfer to the clinic. While this combination of OCT imaging and analysis works well for differentiating tissue types in mice, the transition to humans might not be as straightforward as hoped for. The applicability of these findings in a clinical setting needs further study, but may really impact clinical care in the future.

5.3. Further directions

5.3.1. Half window irradiation platform

To eliminate the variability introduced by comparing one mouse to another, a limitation that the above experiments suffer from (as discussed in 4.9), a new experiment was designed that allows for the comparison of a given mouse to itself, as both irradiated treated and unirradiated control. To this end, a custom collimator was designed that can irradiate half a window chamber, instead of the previously described focal spot size. This allows for the creation of four distinct areas: unirradiated normal, irradiated normal, unirradiated tumour and irradiated tumour as shown on the schematic below. The collimator is equipped with two alignment lasers that are used for the orientation of the half-crescent according to where the tumour is located in the WC. This is important for being able to irradiate half of the two types of tissues. With this new approach, the errors due to comparing two different mice are eliminated. It is however incorrect to assume that the four areas are completely "isolated" from one another. Molecular signals and other environmental factors [39] may be transmitted from one region to the next either through the circulatory system or by simple diffusion which may also confound results. Experiments have already been successfully performed to test the feasibility of this setup on 6 mice, but with 2D and 3D OCT image data yet to be analyzed using the methods discussed in this thesis.
5.3.2. Brain imaging

To test out a significantly different biological model, an *in-vivo* mouse brain and human glioblastoma model has been developed with a custom window chamber on the mouse skull (Dr. Gelareh Zadeh's group at the Hospital for Sick Children, Toronto, Canada) as well as a convenient head mount that allows for the immobilization of the heads and the imaging of the brains longitudinally (Dr. Alex Vitkin's group and the contribution of the author). Due to the very small brain window chamber and a much more sensitive organ being exposed to the outside world, the longitudinal OCT imaging of the mouse brain was quite cumbersome. The potential benefits are quite significant: radiation therapy is one of the treatment modalities for glioblastoma, a disease with which patients have a median survival without treatment of only ~5 months [40], and so early monitoring of the efficacy of RT is of great importance (RT extends the median survival to ~12 months [41], so there is still clearly much room for improvement). Due to the much smaller window chamber size, 2D imaging is difficult in this setting, but the 3D...
approach has been tested and shown to work. The speckle texture analysis methods will be tested on this new model in the coming months.

5.3.3. Other texture analysis methods

The above described texture analysis methods are not the only possible methods for the extraction of speckle properties. The study is not an exhaustive testing, but rather a small sample of the many methods that are possible. There is certainly a need to extract the (so far underappreciated/untapped) information in the OCT speckle to potentially add another layer of useful information to the already wide spectrum of parameters that OCT measures.

5.3.4. Modeling of OCT speckle

While the approach described in this thesis worked in the determination of a parameter that measures tissue differences, how this metric relates to tissue microstructural changes is unknown. To determine this, a better understanding of the interaction between light and tissue in the context of OCT imaging, and means to model this interaction, are needed. There have been a few recent endeavours in this direction by OCT theoreticians [42,43], however progress has been modest. Due to the stochastic interaction of light with tissue, the OCT signal, and specifically its speckle features are very hard to predict. If, however, this will one day be possible, the modeling of OCT speckle will allow for the possibility of correlating speckle derived parameters with the underlying tissue microstructural and compositional changes.

5.3.5. Phantoms

To better understand the correlation between speckle derived parameters and corresponding tissue properties, basic tissue mimicking phantoms were developed containing different concentrations of 1 µm diameter silica microspheres embedded in polyacrylamide. More work needs to be done in order to be able to make the phantoms more homogeneous, but by creating multiple concentrations of different sphere sizes and shapes, tissue differences can be experimentally imitated. This has been tried by other researchers [44] using polystyrene beads
suspended in solution who have shown that the different bead sizes as well as concentrations result in modulations of the a, c, and v parameters of the Generalized Gamma distribution. Silica is a better model of tissue scatterers than polystyrene (n = 1.59) as its refractive index of n = ~1.37 is a lot closer to that of average tissue at n = ~1.38. However, the beads are also a lot heavier than polystyrene and so there is a need for the optimization of the solid polyacrylamide recipe to allow for a quick curing before the beads can settle. Homogeneous silica bead polyacrylamide solid phantoms are yet to be built for tissue mimicking. Nonetheless, even with the inhomogeneous phantoms, some trends are visible. With beads of 1 µm diameter, the larger the concentration, the larger the derived kθ mean parameter value. The graph below shows k, θ, and the kθ mean parameter with increasing concentration.

Figure 28: Variation of the a) k, b) θ, and c) the kθ mean parameter with increasing 1 µm diameter silica bead concentration.

5.4. Conclusions

In this thesis, the possibility of using OCT combined with speckle texture analysis algorithms was investigated with the goal of detecting tissue variability and early RT response. The Gamma distribution using a Least Squares optimization was determined to be the most
optimal at differentiating tissue types. It can accurately differentiate normal and tumour tissue with high in mouse WC models of Me180 cervical cancer cell lines. The variation of the kθ parameter of the Gamma distribution from normal to tumour tissue is approximately 40%, allowing for its detection. Having quantified the variation in signal due to the system, animal model, and experimental procedure, it can be stated that any variation higher than approximately 25% in the kθ parameter would be detected by the presented method. While the RT effects are subtle and thus likely less than the ~25% kθ parameter limit above, it is plausible that the OCT imaging and analysis method discussed in this thesis may pick up on them. To further test this, experiments have been proposed and initiated that allow the elimination of some of the sources of variation sources, to further improve the efficacy of the model.
References:


Appendices:

Appendix A

The results of the normal to tumour tissue comparison were published in Optics Letters 38, 1280-1282 (2013) a copy of which is included. The same methods were then further developed for the extraction of other tissue differences.
Texture analysis of optical coherence tomography speckle for characterizing biological tissues in vivo

Andras A. Lindenmaier, Leigh Conroy, Golnaz Farhat, Ralph S. daCosta, Costel Flueraru, and I. Alex Vitkin

1Department of Medical Biophysics, University of Toronto, 610 University Ave., Toronto, Ontario M5G 2M9, Canada
2Imaging Research, Sunnybrook Health Sciences Centre, 2075 Bayview Ave., Toronto, Ontario M4N 3M5, Canada
3Ontario Cancer Institute, University Health Network, 610 University Ave., Toronto, Ontario M5G 2M9, Canada
4Institute for Microstructural Sciences, National Research Council, 1200 Montreal Road, Ottawa, Ontario K1A 0R6, Canada
5Department of Radiation Oncology, University of Toronto, 610 University Ave., Toronto, Ontario M5G 2M9, Canada

Corresponding author: Alex.Vitkin@uhn.ca

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We demonstrate a method for differentiating tissue disease states using the intrinsic texture properties of speckle in optical coherence tomography (OCT) images of normal and tumor tissues obtained in vivo. This approach fits a gamma distribution function to the non-log-compressed OCT image intensities, thus allowing differentiation of normal and tumor tissues in an ME-180 human cervical cancer mouse xenograft model. Quantitative speckle intensity distribution analysis thus shows prospect for identifying tissue pathologies, with potential for early cancer detection in vivo. © 2013 Optical Society of America

OCIS codes: 100.2990, 110.0500, 170.3880, 170.0335.

Cancer is a prevalent disease with terrible social and economic consequences. Early detection of cancer is crucial for timely intervention, which can lead to better outcomes [1]. Imaging methods that use light-tissue interactions have been shown to be sensitive to many subtle diseased tissue alterations [2]. As such, optical coherence tomography (OCT) is a promising technology that may play a role in the differentiation between normal and diseased tissue states in vivo. It is particularly attractive for clinical application because of its high resolution, subsurface imaging capability up to ~2 mm in tissue and fiberoptic implementation that permits minimal invasive access to many anatomically restricted areas. While OCT’s impressive resolution is on the micrometer scale, this is generally insufficient for direct visualization of cellular organelles and cellular level changes, as these are submicron in size. There is, however, some evidence that image analysis of OCT speckle patterns can extract additional tissue information [3,4]. Speckle in OCT images results from the interference of backscattered light from the subsresolution scatterers in the tissue being imaged. Speckle is known to give a good measure of the underlying scatterers’ average properties, such as size, shape, and distribution, without individually resolving them [3].

In this study, we use the local variation in OCT speckle to obtain a measurement of the average changes in the speckler properties of the tissue being imaged. The metric is then used to differentiate between normal and tumor tissues in vivo. Specifically, the spatial variation of OCT speckle intensities is quantified by fitting the un-compressed OCT data to a gamma distribution function, similar to methods used in previous high-frequency ultrasound tissue characterization studies and an OCT apoptosis study [5,6]. The pixel intensity distribution is approximated by the gamma distribution using a simple least squares optimization algorithm. The OCT speckle variation is quantified through the fit parameters of this distribution. To test this, in vivo OCT imaging was performed on NCr nude mice with dorsal skin-fold transparent window chambers (WCs) to allow for direct imaging of normal and solid tumor tissues [7]. Fluorescence labeled DiSCRed-Me180 cervical cancer xenografts were implanted in the mouse dorsal skin-fold WCs and monitored using OCT starting 7 days after implantation. This animal model permitted OCT imaging of tissues for up to 26 days from tumor cell implantation (which usually coincides with WC implantation). All animal studies were performed in accordance with University Health Network Animal Resource Center guidelines for the care and use of laboratory animals.

The OCT system used in this study has been described previously [8]. Briefly, it uses a 20 kHz Santec HSL2000-HL laser source with center wavelength at 1320 nm and bandwidth of 110 nm, resulting in an axial resolution of 7 μm in tissue. Using a 3 x 3 Mach–Zender interferometer with a semiconductor optical amplifier in the sample arm and dual channel simultaneous detection, the system allows for higher signal-to-noise ratio than conventional systems. A heater and translation stage were used to position the ketamine and xylazine anesthetized mice under the ball lens fiber of the OCT probe. Representative B-mode OCT images were collected from within the tumor area as well as surrounding normal tissues in the WC. The tumor contour was visible directly in the OCT images, but as a definitive measure, the tumors were also imaged using a Leica MZ FLIII stereo-microscope (Leica Microsystems GmbH, Wetzlar, Germany) with a 560 nm excitation filter for localization of the Texas Red glowing tumors. This allowed each B-mode image to be categorized as being from a tissue region that is either normal, contoured tumor or in-between transition area. A region of interest (ROI) was then selected within each image (normal or tumor...
tissue images), starting at a depth of 100 pixels below the top of the coverslip of the WC. The standard depth was chosen to minimize complicating effects due to signal attenuation with depth. The ROI was 64 × 190 pixels, corresponding to a physical size of ~210 μm × 630 μm, chosen large enough to allow for good histogram statistics. The OCT pixel intensity distribution in the ROI (without log-compression) was then represented as a histogram. Various methods of optimizing the binning of the distribution, including fixed and "dynamic" binning based on the Shimazaki method [9] were tested; fixed binning was found to be optimal for histogram comparison of many images as it kept the relative histogram amplitudes constant and comparable between images. The histograms were then fitted using a least squares method with the gamma distribution function [10],

\[
f(x, \alpha, \beta) = \frac{1}{\Gamma(\alpha)} x^{\alpha-1} e^{-x/\beta}. \tag{1}\]

The parameters \( \alpha, \beta \) are known as the shape and scale parameter, respectively [5,10]. While individually they describe different aspects of the gamma distribution, the "mean" of the distribution obtained via \( \alpha/\beta \) was used here as a composite metric for the detection of tissue properties. As described by Tunis et al. [5] in the context of high frequency ultrasound, the \( \alpha/\beta \) ratio is proportional to the effective tissue scatterer number density, and can be related to cellular changes that affect the scattering properties of the tissue.

Representative histograms of nonlog-compressed OCT signal intensities are shown in Fig. 1 for a normal and a tumor region, showing clear differences in the distribution of specie intensities and the \( \alpha/\beta \) mean of the corresponding gamma fits varying accordingly. The goodness of fit was typically \( R^2 \sim 0.985 \) (range 0.950–0.990) for several hundred ROIs, demonstrating excellent quantitative description of the data. Figure 2(a) shows a representative B-scan image that encompasses the transition from a normal to a tumor region in the WC, while Fig. 2(b) shows the variation of the mean \( \alpha/\beta \) parameter across the normal-tumor boundary. This was obtained based on a sliding box algorithm, similar to the one used for fractal box counting [11]. The algorithm slides a box, equal in size to the ROI of 64 × 190 pixels, from the left to the right of the image, calculating the mean \( \alpha/\beta \) parameter in the ROI at every step. In the calculations of the data shown, the step size was 19 pixels (90% overlap of consecutive boxes) and the sampled area was at a depth of 120 pixels from the top of the image (i.e., ~100 pixels from top of the WC). The transition from normal to tumor regions is well quantified by the decrease in the \( \alpha/\beta \) mean of the gamma distribution fits. Since the OCT image being analyzed is a transition region between normal and tumor tissue, the range of values of the \( \alpha/\beta \) mean falls between the extremes of purely normal and purely tumor regions in Fig. 1. Similarly, when comparing images from different tissue regions in the WC, corresponding to both normal and tumor tissue, the strong correlation of the \( \alpha/\beta \) mean parameter with disease state is also evident. Figure 2(a) shows the separation between normal, tumor, and "transition" regions (total of 52 images from 25 normal, 25 tumor, and 2 transition regions). The \( \alpha \) versus \( \beta \) plot clearly classifies the tumor and normal tissue.

![Fig. 1.](https://example.com/fig1.png) (Color online) Histograms of the OCT image pixel intensity distribution in a 64 × 190 pixel (~210 μm × 630 μm) ROI region for (a) normal and (b) Me180 tumor tissue 17 days after tumor implantation. The mean \( \alpha/\beta \) parameter was found by fitting the distribution with the gamma function [Eq (1)], with the line of fit shown in blue. The mean \( \alpha/\beta \) parameter values were found to be 9.08 × 10⁻³ and 3.94 × 10⁻² for the normal and tumor tissue, respectively.

![Fig. 2.](https://example.com/fig2.png) (Color online) (a) B-mode image of normal to tumor tissue (left to right) transition region of a mouse 17 days post-injection of tumor cells. The 64 × 190 pixel ROI box is blue and its sliding region is shown by the white lines. The image field of view is 1.32 mm × 3 mm (depth × width). (b) The variation of the mean \( \alpha/\beta \) parameter as a function of the center of the sliding ROI in (a).

![Fig. 3.](https://example.com/fig3.png) (Color online) (a) Plot of the \( \alpha/\beta \) ratio from ROIs of 32 images of varying origin; 25 normal, 25 tumor, and 2 transition regions of a mouse 17 days post injection of tumor cells. The ROI used for the image analysis was 64 × 190 pixels and at a depth of 100 pixels below the top of the WC, as before. (b) Bar plot of the average \( \alpha/\beta \) mean parameter from normal and tumor tissue. The error bars are ±1 SD from the mean.
into two groups, while the transition regions of unknown/ mixed tissue pathology fall somewhere in between, being similar to the normal tissue.

In Fig. 3(b) the average a/b mean parameter value is shown for sets of normal and tumor images. The resultant differentiation is excellent: using an unpaired two-tailed t-test it was determined that the difference between the two groups was statistically significant at a 95% confidence level with a p-value of < 10^-30. The variation within a given pathology is quite small, as shown by the modest size of the error bars (+/− standard deviation). This suggests that the metric detects details of tissue microstructural variation that are finer than differences due to different tissue types, and is thus sensitive to subtle features of tissue heterogeneity. This quantifiable heterogeneity effect is considerably smaller than the tumor–normal tissue differences, and as such does not confound the differentiation of these tissue types.

Such the methodology enables the detection of small variations in tissue heterogeneity, the described speckle analysis technique could also be used for the detection of changes within a tissue type. For example, a common cancer treatment is radiation therapy. While the detailed radiobiology of tumors is still not completely understood [12], radiation-induced cell death, cellular structural changes, and connective tissue alterations may result in changes of the optical properties of tissues. As such, and based on the current findings, the response of tumors to radiation may potentially be monitored using the OCT speckle analysis algorithm described herein. Since OCT is noninvasive, and the mouse WC allows for focal irradiation of the tumor, the analysis pipeline could provide a valuable preclinical tool for the study of radiation response dynamics in solid tumors and adjacent normal tissues longitudinally and in vivo. In addition to fundamental insights, noninvasive detection of early radiobiological tissue alterations could be performed clinically using fiber optic technologies and thus used to guide adaptive radiation treatment delivery. Since clinical assessment of radiation response currently incurs delays of weeks to months, our proposed methodology could potentially enable earlier detection (~2–3 weeks) of tissue changes and corresponding treatment adjustments.

While the presented method was applied to 2D B-mode images, its use can be easily extended to the analysis of 3D datasets. By analyzing 3D data, the method can allow for the delineation of tumors and the sensitive volumetric mapping of tissue property variations.

A drawback of the current implementation of the method is its use of a relatively large R0 of 210 μm × 630 μm, as selected to get suitable gamma fits and statistics, which results in some spatial blurring and averaging over potentially heterogeneous tissue regions. Further systematic studies of R0 size/shape that will improve the method’s spatial resolution and minimize averaging while still yielding accurate fits will likely yield better results. Such studies are ongoing. The a/b mean parameter shows good differentiation between normal and tumor tissue types, however it is not clear exactly what biological events are causing the changes in the speckle signal and the variation in intensity distributions. Correlative studies of tissue histopathology and two-photon fluorescence microscopy will be used to link variations in the a/b mean parameter to tissue- and cellular-level changes in the tissue. While the preliminary results presented here demonstrate promise for differentiating tissue types, additional metrics may be necessary to sample the subtle and complex spectrum of tumor tissue biology and response to treatments. With a multiparametric approach, the correlation of biological events to a biophysical metric parameter space may become realistic and useful. Further, the imaging optics (i.e., the NA, illumination geometry, working distance) may influence the performance of the algorithm, effects yet to be studied. Preliminary results from a different OCT system indicate comparable algorithm performance in differentiating normal from tumor tissue (data not shown), suggesting that the methodology is fairly robust.

In summary, we describe a novel quantitative OCT speckle analysis algorithm based on gamma distribution fits. We demonstrate its performance in differentiating between normal and tumor tissues in vivo in a WC mouse xenograft setting. The method has the potential for detecting subtle tissue alterations caused by disease development (e.g., cancer progression), or stemming from minimally invasive treatments (e.g., radiation therapy). In the former context, early tumor detection and staging/margin delineation may be possible; in the latter scenario, treatment feedback/personalization are attractive options. Several avenues for further studies and methodology improvement have also been outlined.

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Appendix B

Differentiation of normal and pathological tissues in human subjects based on structural, svOCT and Doppler flow measurements was discussed in the paper published in Biomedical Optics Express 3, 826-839 (2012).

Noninvasive in vivo structural and vascular imaging of human oral tissues with spectral domain optical coherence tomography

Bahar Davoudi, Andras Lindenmaier, Beau A. Standish, Ghassan Allo, Kostadinka Bizheva and Alex Vitkin

Abstract:

A spectral domain optical coherence tomography (SD-OCT) system and an oral imaging probe have been developed to visualize the microstructural morphology and microvasculature in the human oral cavity. Structural OCT images of ex vivo pig oral tissues with the histology of the same sites were acquired and compared for correlations. Structural in vivo OCT images of healthy human tissue as well as a pathologic site (ulcer) were obtained and analyzed based on the results of the ex vivo pig study, drawing on the similarity between human and swine oral tissues. In vivo Doppler and speckle variance OCT images of the oral cavity in human volunteers were also acquired, to demonstrate the feasibility of microvascular imaging of healthy and pathologic (scar) oral tissue.