IMPLANTABLE BIOSENSORS FOR NEURAL IMAGING: A
STUDY OF OPTICAL MODELING AND LIGHT SOURCES

by

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We aim to develop an implantable, optical neural imaging device by fabricating lasers and photodiodes onto a gallium arsenide substrate. Some studies suggest that lasers exhibit higher noise than light emitting diodes (LEDs) due to coherence effects – my studies aim to quantify this noise and to guide device development. To this end, I developed a model of a fluorescent imaging device which agreed with experiment. Noise analysis performed in phantom showed that laser sources exhibit temporal and spatial noise up to 10x higher than LED sources, and in vivo noise analysis also demonstrated this trend. I studied a neural injury model called cortical spreading depression in vitro in mouse brain slices and in vivo in the rat brain using laser and LED sources. Signal magnitudes in vitro are on the order of 10% and in vivo results are inconclusive. Future work will aim to reduce coherence related noise.
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Chapter 1  Introduction

Until recently, the overarching trend in scientific research has been to compartmentalize disciplines and to conduct research under one strictly defined theme such as neuroscience, electrical engineering, or physics. Of late, interesting new research has emerged from the overlapping interfaces of different disciplines, and many researchers have chosen to take a new path away from their respective areas, learning about related disciplines in order to drive new and exciting multidisciplinary research. The subject of this research thesis follows these same principles, and covers a wide range of topics knitted together under the common theme of optical biomedical imaging.

Current biomedical imaging modalities, including functional magnetic resonance imaging, computed tomography scan, and positron emission tomography, are mature technologies that produce undeniably useful data both for medical diagnostics and basic biomedical research. However, as with all technologies, there is room for improvement over the current gold standards. To begin with, mature imaging modalities require the person or animal (to be referred to as “the subject”) being imaged to be immobile. For adult human subjects, this is easily achieved. However, in infants or animal models, immobilization often requires the use of a sedative or anaesthetic. Due to the negative physiological effects of these drugs, especially in immunocompromised subjects, frequent imaging can have physiologically detrimental or even fatal consequences. The desire to take frequent or continuous images in unanaesthetized subjects is especially pronounced in basic biomedical research, where multiple time points in an imaging series could be used to identify modes of disease progression, drug efficacy, or responses to particular stimuli. The usefulness of a continuous imaging modality in a clinical setting is also undeniable, as it could be used to create personalized treatment plans based on up-
to-the-minute disease progression and drug efficacy monitoring. Another common but widely accepted problem with mature imaging devices are the size and cost. Due to bulky, fixed implementation, subjects must be brought to the device – it cannot be brought to them. This would not be a problem if the devices were affordable, easily obtained, and widespread. However, due to the high cost of implementation as well as maintenance and technical support, these imaging devices are not readily available in all regions. Even within a developed nation such as Canada, there are currently only 12 CT scanners and 6 MRI machines per million people [1], according to the Canadian Institute for Health Information. The need for a lower cost, portable imaging device is clear.

![Figure 1-1: Our vision of a low cost, portable, implantable imaging device. [2]](image)

The vision of our research group is to design such a device: a low cost, portable imager that can be implanted into a subject, allowing the acquisition of images in a long term, continuous modality (refer to Figure 1-1 for a cartoon of this device). In order to achieve this goal, we work on the interface of electrical and biomedical engineering. The devices are small (< 1mm) arrays of photodiodes and lasers fabricated onto the same semiconductor substrate which can be attached to wireless telemetry to allow for implantation and readout. The focus of this thesis work is to investigate both the feasibility of using these imaging devices in specific
neural applications, as well as to guide device development by examining signal strengths, temporal and spatial characteristics, and light source variability.

This report will first go over the necessary background information, including relevant areas of tissue optics and optical modeling, fundamentals of neural optical imaging, as well as the semiconductor physics behind device operation and fabrication. It will then discuss the studies carried out to evaluate feasibility and guide biosensor development, including optical modeling of the devices and their interaction with both tissue and fluorescence, *in vitro* studies of light source variability and feasibility of optical stroke imaging, and finally *in vivo* evaluation of light source variability and evaluation of a neural disease model. This report will then conclude with recommendations for biosensor design, as well as an outline of future work and applications.
Chapter 2  Background

2.1  Tissue Optics and Monte Carlo Modeling

The interaction of electromagnetic waves, or light, with a substance can be described by a set of optical parameters intrinsic to that substance, including the index of refraction (n), absorption coefficient (\( \mu_a \)), scattering coefficient (\( \mu_s \)), and anisotropy (g). For many materials, including air, water and glass, scattering and absorption are negligible, and to first order light propagation can be described using only the index of refraction. In tissue, however, scattering and absorption become prominent factors in the description of light propagation, and must be modeled with care in order to predict light distributions.

There are four relevant topics of tissue optics which will be discussed in the following sections. The first is the basic definition of the tissue optical properties (n, \( \mu_a \), \( \mu_s \) and g) which are necessary to understanding how light propagates through tissue. The second is the Beer-Lambert law, which describes the attenuation of light as it moves through an absorbing substance. The third is the diffusion approximation, which is a simplified model of light transport that has analytical solutions, valid in regimes where scattering is dominant. Finally, Monte Carlo modeling of light propagation will be discussed for cases in which the diffusion approximation is not valid. This final section will include an overview of the Monte Carlo framework used for the modeling done in this dissertation, including statistical significance of modeling results.

2.1.1  Optical Tissue Properties

The index of refraction of a substance is a dimensionless, complex number, which is often represented as:
\[ \tilde{n}(\lambda) = n_R(\lambda) - i n_I(\lambda). \quad \text{E 2-1} \]

The real part of the index, \( n_R(\lambda) \), is a scaling factor to the speed of light in that substance – essentially the impedance which the material gives to light propagation. The imaginary part of the index of refraction, \( n_I(\lambda) \), is a measure of the attenuation of light as it passes through the material, due to scattering and absorption. The scattering and absorption coefficients are proportional to the scattering and absorption cross sections (\( \sigma \)) and density (\( \rho \)) of scatterers and absorbers in the medium, respectively.

\[ \mu_s = \rho \sigma_s \quad \text{E 2-2} \]
\[ \mu_a = \rho \sigma_a \quad \text{E 2-3} \]

Both coefficients have the dimension of inverse length, and as such, their reciprocals represent the path length between scattering and absorption events. The total attenuation is a summation of the attenuations from scattering and absorption events, and is represented by an attenuation coefficient \( \mu_t \).

\[ \mu_t = \mu_a + \mu_s \quad \text{E 2-4} \]

The scattering phase function (SPF) is a function which describes the amount of light incident from a given direction, \( \hat{s} \), which is scattered in the \( \hat{s}' \) direction. The value can be difficult to calculate in tissue optics, and so an approximate function called the Henyey-Greenstein function is normally used. The SPF is not a regularly quoted value. The anisotropy coefficient, or cosine of scatter, \( g \), indicates the amount of light scattered in a forward direction after a scattering event. In tissue, \( g \) is conventionally taken to be 0.9. For a complete mathematical derivation of this coefficient using the scattering phase function, please see reference [3].
If we consider light moving through a substance which is strongly absorbing, we can calculate the light’s attenuation as a function of distance based on the number of absorptive events [3], [4],

$$I_\lambda(z) = I_\lambda(0)10^{-\varepsilon_\lambda C_B z} = I_\lambda(0)e^{-\mu_a z},$$  \hspace{1cm} (E 2-5)

called the Beer-Lambert law. \(I_\lambda\) is the light intensity, \(C_B\) is the concentration of absorbing molecules, \(z\) is the distance travelled, and \(\varepsilon_\lambda\) is called the molar absorption coefficient, molar absorptivity or molar extinction coefficient. It is a parameter commonly quoted for different absorbing substances like dyes. Care must be taken when using this parameter, as its definition is sometimes given using a natural logarithm [3], and sometimes using a logarithm of base 10 [4]. Here, the convention used in the latter reference will be followed and the base 10 logarithm will be used. As such, the relation between the extinction coefficient and absorption coefficient is given by

$$\frac{\mu_a}{2.303} = \varepsilon_\lambda C_B.$$ \hspace{1cm} (E 2-6)

Another useful relation in tissue optics is between the absorption coefficient \(\mu_a\) and the imaginary index of refraction, given by

$$n_l = \frac{\alpha\lambda}{4\pi} = \frac{\mu_a\lambda}{4\pi},$$ \hspace{1cm} (E 2-7)

where \(\alpha\) is the product of the molar extinction coefficient and the absorber concentration,

$$\alpha = 2.303\varepsilon_\lambda C_B.$$ \hspace{1cm} (E 2-8)

### 2.1.2 The Diffusion Approximation

Light is well known to exhibit wave particle duality – that is, it can be modeled as a propagating electromagnetic wave, or as a ballistic photon. Within the framework of tissue optics, both these
descriptions of light propagation can become cumbersome due to the high number of scattering events that take place. Luckily, in cases where scattering is the dominant transport mechanism, light can be modeled as a diffusing substance. In order for the diffusion model to be valid, light must move through a substance with no preferential direction, with each scattering event contributing to a random walk. In order for the diffusion approximation to be valid, the general rule of thumb is as follows:

$$\frac{\mu_s (1 - g)}{\mu_a} > 10.$$  \[E-2-9\] [5]

When this condition is satisfied, standard physical laws for the diffusion of substances along a concentration gradient can be used to describe the motion of light. For diffusing substances, the flux is $J [W/cm^2]$, $\chi$ is the diffusivity $[cm^2/s]$ and $C$ is the concentration. For light, the diffusivity is given by $cD$ (where $c$ is the speed of light and $D$ is the diffusion length) and the concentration is given by $\phi/c$ (where $\phi$ is the fluence rate) [5]. See equation E 2-10.

$$J = -\chi \frac{\partial C}{\partial z} = -D \frac{\partial \phi}{\partial z}.$$  \[E-2-10\]

In regimes where the diffusion theory is valid, the scattering of light is angularly dependent due to the anisotropy, and can be described by a reduced scattering coefficient [3]

$$\mu_s^' = (1 - g) \mu_s.$$  \[E-2-11\]

The total attenuation coefficient can then be written as

$$\mu_t = \mu_a + \mu_s^' = \mu_a + (1 - g) \mu_s.$$  \[E-2-12\]

For a comprehensive discussion of diffuse light transport, refer to [5].
2.1.3 Monte Carlo Modeling

In circumstances where the diffusion approximation is not valid, or when system geometry is so complex that boundaries make it impractical to apply diffusion theory, Monte Carlo modeling can be used to generate light distributions. Monte Carlo simulations treat light as ballistic photons, and use probability to determine the direction and intensity of a photon after a scattering event. Monte Carlo codes are generally computationally intensive, as they must calculate the path of many photons through a system in order to obtain a statistically significant distribution.

There are numerous types of Monte Carlo simulations, many optimized to a particular type of geometry. We choose to use a Monte Carlo framework called Advanced Systems Analysis Program (ASAP), by Breault Research Organization, Tucson, Arizona. This particular Monte Carlo code is surface based. Each surface is assigned an INTERFACE definition specifying the type of boundary (how much reflection and transmission), as well as two MEDIA definitions, which include the four parameters discussed in Section 2.1.1: \( n, g, \mu_a \) and \( \mu_s \). As a ray is propagated through the system, at each surface it passes from its current MEDIA to the second MEDIA specified by the INTERFACE definition, with its flux dependent on the amount of reflection and transmission specified at the boundary. In the normal ray tracing mode, rays are allowed to split at interfaces into specular and scattered components. This increases the number of rays propagating through the model and can result in extremely long simulation times if not used with care. In tissue models, the number of rays can increase dramatically due to the high number of scattering events. In order to speed up simulation time, the command SPLIT MONTECARLO can be issued, whereby no additional specular or scattered rays are created at interfaces or scattering events. In Monte Carlo mode, each ray is assigned one direction after an event, taken from the set of all possible directions it could acquire through reflection, refraction,
transmission or scattering. The probability of a given direction is proportional to the flux that would actually reflect, refract or scatter in that direction [6] (see Figure 2-1).

**Figure 2-1 Example of scatter in ASAP using conventional ray splitting and Monte Carlo analysis. In the conventional case, 10% of the power is absorbed during scattering. [7]**

**Statistics in ASAP**

In order to estimate the error in a simulation, the simulation is considered to be a Bernoulli trial where success is defined by a ray reaching the object specified as the detector, and where failure is any other case where the ray does not reach the detector. The signal to noise ratio of a Bernoulli trial is given by [8]

$$\frac{\text{Noise}}{\text{Signal}} = \frac{\sigma}{<m>} = \frac{\sqrt{(1-p)}}{\sqrt{Np}} \frac{\sqrt{(1-p)}}{\sqrt{n}},$$  \quad \text{E 2-13}

where $\sigma$ is the square root of the variance (the standard deviation), $<m>$ is the average value, $N$ is the total number of rays traced, $p$ is the probability of a ray arriving at the detector, and $n$ is the number of rays which get to the detector. In the limiting case, when the probability of a ray reaching the detector is small, the equation reduces to

$$\frac{\text{Noise}}{\text{Signal}} = \frac{\sigma}{<m>} = \frac{1}{\sqrt{n}}.$$  \quad \text{E 2-14}
Since calculating the probability of a ray reaching a detector in a complicated geometry is challenging, it is often beneficial to assume that the probability will be small, and to take the limiting case in each simulation.

When other effects are taken into consideration, such as Fresnel reflection and transmission losses, the noise to signal ratio can be written in terms of energy fluxes as [9]

\[
\frac{\text{Noise}}{\text{Signal}} = \frac{\Phi_{\text{max}}}{\sqrt{\Phi_{\text{total}}}},
\]

where \(\Phi_{\text{max}}\) is the maximum flux of an individual ray on the detector, and \(\Phi_{\text{total}}\) is the total flux of rays on the detector.

When ASAP creates rays, it uses a random number generator to determine the sequence in which the rays are created. The random number generator is initiated by a large, non negative integer, specified by the SEED command. If the SEED is not changed from simulation to simulation, the default value of 2000000001 is used, and there will be no trial to trial variation. In order to improve the statistics of the simulations, a looping function was used which changed the SEED value at every simulation run. Sets of results were obtained for each simulation, allowing a mean and standard deviation to be calculated for each simulation point. The standard deviation between trials was used as a metric to determine whether enough rays were being traced through the system. We chose to specify that if the standard deviation was more than 10% for \(n=100\) trials, then more rays were necessary to generate a statistically significant result.

### 2.1.4 Measuring optical properties using spectrally constrained, diffuse reflectance

Our collaborators in Dr. Brian Wilson’s Biophotonics group at Princess Margaret Hospital have developed a small fibre optic probe based on spectrally constrained steady state diffuse reflectance, in order to measure \textit{in vivo} tissue optical properties. The first spectral constraint of
this model is the absorption spectrum, assumed to be a linear combination of contributions from principle chromophores, as seen in E 2-16. Here, \( c_{Hb} \) is the volume fraction of hemoglobin in the blood, \( StO_2 \) is the oxygenation fraction, and \( \mu_a^{\text{oxy}} \) and \( \mu_a^{\text{deoxy}} \) are the absorption coefficients of oxygenation and deoxygenated hemoglobin, respectively.

\[
\mu_s(\lambda) = c_{Hb} [StO_2 \mu_a^{\text{oxyHb}}(\lambda) + (1 - StO_2) \mu_a^{\text{deoxyHb}}(\lambda)]
\]

E 2-16

The second constraint is the reduced scattering spectrum, assumed from bulk tissue fits to be a mono exponential function (see E 2-17). Here, \( A \) is referred to as the scattering magnitude, and \( b \) as the scattering power.

\[
\mu_s'(\lambda) = A \lambda^{-b}
\]

E 2-17

The probe records reflectance over a spectrum of wavelengths at three discrete values of source-detector separation. The parameters of interest, \( \mu_a \) and \( \mu_s' \) are then extracted by fitting the experimental reflectance to the diffusion theory reflectance model. For a full description of this method, please refer to [10]. This method is advantageous because it can be used to measure tissue optical properties in the middle of an experiment, ensuring that animal to animal variability is accounted for.

### 2.2 Brain Imaging

As discussed in the introduction to this dissertation, mature brain imaging modalities do exist and do provide useful research information. This section will provide an overview of the commonly used brain imaging methods, focusing on their spatial and temporal resolution, and the neurophysiologic signals which they detect. The imaging modality we chose, intrinsic optical signal imaging, will be described, and reasons for this choice will be elaborated. I will
then describe the physiological basis of the intrinsic optical signals seen in the four important types of functional imaging.

### 2.2.1 Functional Brain Imaging Modalities

**Functional Magnetic Resonance Imaging (fMRI)** is sensitive to changes in tissue concentrations of deoxyhemoglobin, which is paramagnetic, but insensitive to changes in oxyhemoglobin, which is diamagnetic [11]. The observed signal, called the blood oxygen level dependent (BOLD) signal, has three observable phases. When neurons are first activated, there is an initial dip where oxygen consumption is high, but blood flow has not yet increased to compensate for the higher metabolic demands. Detection of this transient signal, lasting 2-3 seconds, requires high temporal resolution. Cerebral blood flow then increases and overcompensates for the neural activity, leading to a decrease in deoxyhemoglobin. Finally, the levels return to baseline, often exhibiting a ‘post-stimulus undershoot’ (slight increase in deoxyhemoglobin) and slow return to basal levels.

**Positron Emission Tomography (PET)** makes use of radioactive tracers made of positron-emitting isotopes. These isotopes can be incorporated into different biological molecules, including water, neurotransmitters, or glucose, and injected into a subject’s bloodstream, where they will circulate preferentially to areas which have a high demand for the molecule they’re attached to. As an isotope decays, an unstable positron is emitted which will quickly combine with an electron to produce two gamma photons traveling in opposite directions. The subject is placed within a ring of detectors, and when two photons arrive at detectors ‘simultaneously’ (within nanoseconds), they are labelled as a coincidence, and their path is extrapolated [12].

**Optical Coherence Tomography (OCT)** is an interferometric technique in which a coherent light source is scanned across a tissue, and the backscattered light is compared with a reference arm to produce depth based cross sectional images. It can achieve an impressive spatial...
resolution on the micrometre scale, and has a penetration depth of up to 1-3mm in highly
scattering tissues [13]. OCT can be used not only for structural mapping of a tissue, but also to
extract functional information about blood flow from Doppler measurements, changes in blood
oxygenation, and tissue spectroscopic properties [14].

**Optical dyes** are often used as a contrast agent for short term camera-based imaging studies, in
order to increase the signal to noise ratio in images. There are several types of dyes commonly
used *in vivo*, depending on the type of imaging being done and the structures being examined.
For functional imaging of neural activity, voltage sensitive dyes (VSD) are often used. These are
dye molecules which bind to the external membrane of cells, and have an increase in
fluorescence when membrane potential changes [15]. VSD imaging is advantageous because of
its high spatial resolution and ability to measure fast changes in neuron membrane potential.
However, the lifetime of the dye in the brain limits the duration of imaging sessions, there are
negative phototoxic effects on tissue, and the dyes are not effective in all neuronal populations
in the brain.

Calcium sensitive dyes (CaSDs) are also used for functional imaging of neural activity.
These dyes are compounds whose fluorescence increases when calcium concentrations increase
[16]. During an action potential, intracellular calcium increases, and so if the cell contains
CaSDs, the increase in fluorescence can be correlated to neural activity.

Fluorescent protein dyes have become a widespread tool in neural imaging. Their
excitation and emission generally lie within the UV to visible spectrum, with the dye “mPlum”
being the closest to the near infrared (NIR) window at excitation of 595nm and emission at
655nm [17]. Their advantages are clear in that using molecular genetics, a fluorescent dye can
be incorporated exclusively into a cell population of interest, facilitating the monitoring of
progression or functionality. However, usefulness is limited in that the visible wavelengths of
light do not have a large penetration depth into tissues due to increased attenuation in biologically relevant fluids, to be discussed in the following sections.

A particular dye of interest for our research is Cy 5.5, which absorbs and emits in the near infrared window (see Figure 2-2). This dye can be conjugated with arginine-glycine-aspartic acid to form (RGD)-Cy5.5. RGD is a peptide which targets integrin receptors, and integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ have been shown to play a role in tumour growth and metastasis [18]. As a result, (RGD)-Cy5.5 accumulates preferentially in tumour tissues and can be used as a tumour contrast agent.

![Figure 2-2: (Left) Emission and excitation spectra for Cy 5.5. (Right) Dissected organs of a mouse sacrificed 4 hours after 0.5nM intravenous injection of RGD-Cy5.5. 1: U87MG tumour; 2: muscle; 3: pancreas; 4: liver; 5: kidney; 6: spleen; 7: lung. [18]]

Finally, collaborators at Princess Margaret Hospital use an endogenous fluorophore, protoporphyrin IX (PpIX), which, in the presence of excess aminolevulinic acid (ALA), has been shown to accumulate preferentially in certain tumour cells. Both PpIX and ALA compounds are produced within the heme synthesis pathway (refer to [19] for full description of this pathway). Of note, the accumulation of PpIX is due to the slow conversion of PpIX to heme by ferrochelatase (the addition of a ferrous ion to the PpIX ring structure). PpIX exhibits peak absorption at 408nm and peak emissions at 630nm and 690nm [20].
**Two Photon Fluorescence Imaging** exploits multiphoton absorption in dyes. When two photons arrive at a dye molecule within ~0.5fs of each other, their energies can combine to promote the molecule to an excited energy state. When the molecule relaxes, it emits one photon of less than double the energy (or more than half the wavelength) of the excitatory light. In order for two photon absorption to occur, photon densities must be high both spatially and temporally, requiring the use of an expensive laser exciting source. However, this method is advantageous when using dyes which absorb in the visible regime (notably the fluorescent protein dyes which can be used in genetically modified animals), because they can be excited by near infrared light ($\lambda \approx 700$-1000nm) which has greater depth penetration and less phototoxic effects in most biological tissues. Another advantage is that since the excitation wavelength is almost double the emission wavelength, rejection of the excitation light using a filter is much easier. For a complete review of two photon imaging techniques, please see [21].

**Intrinsic Optical Signal Imaging** (IOSI) uses an external light source to illuminate the tissue of interest, and a detector such as a charged coupled device (CCD) camera to record changes in light reflectance. The optical changes in an intact tissue are caused by fluctuations of blood volume and intrinsic chromophore levels. A chromophore is a substance which absorbs light at one wavelength and emits or reflects light at a different wavelength. The most commonly exploited intrinsic chromophores are oxygenated and deoxygenated hemoglobin, cytochromes, and metabolites [16]. Changes in concentrations of these substances occur during increased metabolic activity, which is a direct correlate of neuronal activation. IOS is strongly related to the BOLD signals used in fMRI. While signals from injected dyes and fluorescent molecules are often brighter and more easily resolved, IOS imaging is a less invasive approach that promises to be of use in clinical investigations.
Different groups of researchers interpret IOSIs in different ways. Within the neurophysiology community, researchers often calculate the magnitude of light intensity changes and use that number as a correlate of neural activity [22], [23]. Other researchers choose to do what they call diffuse optical tomography (DOT), in which diffuse, frequency modulated light is shone at a sample, and the changes in light intensity are fitted to extract tissue properties including scattering and absorption. A tomographic image of the optical properties of a section of tissue is reconstructed [24], [25]. Finally, some researchers choose to do what they call near infrared spectroscopy (NIRS), in which light attenuation between several sources and detectors (which are on the order of centimetres apart) are measured, and blood deoxygenation is extracted using the modified Beer-Lambert law [26]. The advantage of this technique is that it can achieve a large depth penetration, and can be done through the intact skull in humans. However, it lacks depth selectivity, and a good model of optical geometry of the subject is required to extract information.

We choose to use intrinsic optical signal imaging in our studies, and so it will be described in greater detail in the following sections.

2.2.2 **Intrinsic Optical Signal Imaging**

Intrinsic optical signal imaging (IOSI) is an imaging technique first became popular in 1986 when Grinvald et al. published a paper in Nature [27] showing that reflectance changes in live brain tissue could be correlated to neural activity. Prior to this pioneering work, maps of cortical activation were obtained using electrophysiological recording or imaging of voltage sensitive dyes. The former method is highly invasive, and since the electric potential is a local field, it can only be used to interrogate small volumes of tissue. As discussed above, voltage sensitive dyes produce fast optical signals related to changes in membrane potential associated with action potentials. At the time, Grinvald found that these fast signals were often difficult to resolve due
to their mixing with slower intrinsic signals. This problem led him to the discovery that the intrinsic signals could be used for functional imaging.

![Figure 2-3: (a) Absorption and scattering coefficients of biologically relevant fluids in the visible wavelengths. (b) Optical parameters in the near infrared (NIR) window. Both taken from [16].](image)

Intrinsic optical signals are strongly dependent on the wavelength of light chosen for imaging, due to different absorption and scattering coefficients in biologically relevant fluids (see Figure 2-3), and the absorption and emission properties of intrinsic chromophores. Near infrared wavelengths were chosen for this research due to their low absorption coefficient in water, oxygenated and deoxygenated hemoglobin. As well, these wavelengths offer relatively high scattering coefficient and low auto fluorescence in biological tissues. Due to the low absorption at these wavelengths, it is possible to do IOSI of the brain through the skull (intact skull of a mouse, thinned down for larger subjects like rats). The advantages of an imaging technique which does not require surgery (a craniotomy) are obvious – a simplified imaging protocol, less risk of infection and fatal complications, less chance of alerting the brain physiology prior to imaging.
There are three main types of functional imaging which have been done using intrinsic optical signals: somatosensory cortex stimulation using whisker deflection [28], somatosensory cortex stimulation using forepaw stimulus [29], and visual cortex stimulation using a visual stimulus [22]. An advantage of these types of stimuli is that they are trial average methods. On a given subject, many trials using identical stimuli can be imaged, and the trials can be averaged to extract a higher signal to noise ratio. In a rat, typical somatosensory cortex IOSI responses (ΔI/Io) are between 10^{-4} and 10^{-3} [30], while visual cortex signals are usually studied in mice, and are on the order of 10^{-4} [22]. Visual cortex studies are also commonly done in cat models, but a rodent model was used due to its simplicity in obtaining animal approvals, and also due to the preliminary nature of these studies. See Figure 2-4 for typical examples of the principal types of IOSI experiments. Lately, there has been increasing interest in using IOSI for motor cortex studies [31] [32]. However, due to the difficulty in performing these studies in animal models (a rat cannot be instructed to squeeze a tennis ball, but monkeys can be trained to reach for objects), results are progressing slowly.
Of late, IOSI has been used to image stroke and a phenomenon associated with stroke called peri infarct depolarizations (PIDs). While we are interested in the signals discussed above, we choose to first work with a stroke model due to the large optical signal magnitude changes generated. In contrast to the optical signals discussed above, cortical spreading depression (CSD), which is a model of PIDs, has been shown to generate IOSI responses on the order of $10^{-2}$ (see Figure 2-5b) in a rat in individual trials. It is important to note that CSD is not a trial averageable stimulus, and so the analysis done on the signal is more analogous to a motor cortex functional study than to a visual or somatosensory study. Further detail on CSD and stroke imaging will be provided in the following sections.
2.2.4 **Physiological basis of optical changes during stroke**

Ischemic stroke is by definition a transient or permanent reduction in blood flow in a major brain artery. The area of the brain in which blood flow is completely blocked, and cells have lost their membrane potentially terminally, is referred to as the ‘core’. Surrounding the core, and separating it from healthy brain tissue, is an area which retains some blood perfusion, called the ‘penumbra’. Studies have found that cells often depolarize in waves within the penumbra, and that over time, the core can spread into the penumbra and increase the region of cell death. The penumbra has consequently become a therapeutic target for stroke researchers, who wish to reduce the spread of the ischemic core into the penumbra after a stroke event.

Neurons use adenosine triphosphate (ATP), which is created in an oxygen dependent process called oxidative phosphorylation, as their source of energy. As blood flow is reduced or blocked during an ischemic stroke, the oxygen required to create ATP is unavailable, and it is estimated that existing ATP is consumed within two minutes of an ischemic event [34]. Energy dependent processes within the core, notably the functioning of sodium/potassium pumps on the plasma membrane of neurons, which normally consume 70% of the energy supplied to the brain, are unable to continue. As a result, the intracellular ionic balances of neurons as well as glia are disrupted, membrane potential is lost, and cells depolarize, leading to an influx of sodium and efflux of potassium into the extracellular space. Water flows into cells with the sodium ions, causing swelling. As a result of ionic imbalances, voltage dependent calcium channels are activated [35], and intracellular levels of calcium can increase to 50-100μM (compared to normal levels of 0.1-1μM) [34]. Excitatory amino acids are released, and since energy-dependent mechanisms for reuptake are non functional, there is an accumulation of neurotransmitters, notably glutamate, in the extracellular space. The cells within the core die by various necrotic pathways, including lipolysis, proteolysis, and disaggregation of microtubule
networks [35]. As cells within the core die, they release molecules into the extracellular space that diffuse into the surrounding penumbra, and are hypothesized to lead to waves of spreading depression and cellular damage there.

For hours and up to days after an ischemic event, cellular damage can occur in the penumbra by mechanisms which are not fully understood [36]. Within the penumbra, researchers have identified waves of neuronal and glial cell depolarizations which have been shown to increase infarct size as well as spread into healthy tissue [35]. A model used to study this mechanism of cell damage and death is the cortical spreading depression (CSD), which can be induced either *in vitro* in brain slices or *in vivo* in whole brain in three principle ways: by inducing stroke conditions [37], [38], by applying a drop of high potassium solution [39], or by using a pin prick [40].

**In vitro IOSI of cortical spreading depression**

In vitro, CSD is studied in the brain slice model, which has been used to study brain physiology since the 1950s [41]. Animals are euthanized and decapitated, and the brain is removed to a solution of artificial cerebral spinal fluid (ACSF) and perfused with oxygen in as little time as possible. Substances essential to cell survival in the brain, notably glucose and oxygen, diffuse from the ACSF into the brain slice. This ensures that the tissue remains viable, that is, neurons retain their membrane potential and can propagate action potentials and subsequently repolarize. Soon after removal of the whole brain to solution, a microtome is used to cut the brain into thin (on the order of $10^2 \mu m$) slices, which in solution have much better access to diffusing nutrients. Carefully prepared slices can remain viable for several hours.

Slice preparations allow the researcher to control the tissue’s chemical environment. By changing the concentration of components of the perfusing ACSF, different physiologically relevant environments can be simulated, and cell response can be monitored optically by
monitoring changes in tissue optical properties, or electrically by measuring the DC potential of electrodes inserted into the slice.

Optically, a camera and light source can be set up to measure light transmitted through the slice or light reflected from the slice. The primary cause of optical changes in a slice is cell swelling [37]. If a group of cells depolarize, the sodium channels in the cells open allowing sodium and chloride, which are normally found in higher extracellular concentrations, to flow into the cell. Water follows this change in concentration gradient by osmosis, causing the cells to swell. As a cell swells, its elastic membrane stretches, reducing kinking or folding that can scatter light. As well, the index of refraction of the intracellular environment approaches that of the extracellular environment, reducing scattering at the boundary of the cell. These reductions in cell scattering lead to an increase in light transmitted through the slice, or correspondingly, a decrease in light reflected from the slices.

**In vivo IOSI of cortical spreading depression**

Recently, several groups have begun to do optical imaging of stroke *in vivo*. This is an obvious choice, as during stroke, there are changes in blood volume as well as oxygenation which are large in magnitude. For example, Abookasis et al. used white light in periodic spatial patterns to illuminate the cortex of a rat during middle cerebral artery occlusion (MCAo) [42]. The reflectance images were then fit using steady-state diffusion to extract chromophore concentrations (see Figure 2-5a). In addition, these authors have shown that optical changes during cortical spreading depression are on the order of $10^{-2}$ *in vivo* (see Figure 2-5b).
Figure 2-5: (a) Concentrations of deoxyhemoglobin (Hbr) pre (left) and post (right) middle cerebral artery occlusion [42]. (b) Preliminary data showing optical changes during cortical spreading depression in a rat. Shown during a presentation at the OSA Biomedical Optics conference in 2006. Used with permission from the author. [43]

2.3 Semiconductor Physics of Implantable Biosensors

An implantable biosensor is a device which can be surgically implanted into a test subject and left there. In order for this to be feasible, the biosensor must be very compact. We are working in collaboration with the Harris group at Stanford on biosensors which are composed of a laser light source (vertical cavity surface emitting laser), and a low noise detector (a PIN photodiode), fabricated onto a semiconductor substrate. The possible arrangement of these components into an individual sensor is not unique; there are several different architectures which could be used for a biosensor, seen in Figure 2-6 (a). Proximity architecture is chosen because with it, one sensor can be repeatedly used to study several different samples. As well, because all of the sensor components are on one substrate, surgical implantation is easier. Novel fabrication techniques developed at Stanford allow these components to be monolithically integrated onto a single substrate, as seen in Figure 2-6 (b).
2.3.1 PIN Photodiodes

Photodetectors are made by exploiting the contact properties of a junction between different types of doped semiconductors. The most common photodiode is a simple pn junction, in which a p-type semiconductor is abutted to an n-type semiconductor. As discussed in McKelvey’s treatment of contacts [45], a depletion layer is formed between the two regions. Diode behavior is observed in pn junctions as a result of the depletion layer (see Figure 2-7). The Fermi levels of the two regions will align with or without a bias, causing a band bending as depicted in Figure 2-8 (a). If a photon of sufficient energy is incident on the p-type region of the diode (point A, in Figure 2-8 (a)), an electron and hole pair will be formed. If this happens close (within one diffusion length) of the depletion region, it is probable that the electron will enter the depletion region and drift across the junction (following the downward bending band). Similarly, if a photon is absorbed in the n-type region (C), close to the depletion region, then the hole will diffuse across the junction before recombining. Both of these processes result in a current, as charges are moving across the junction. The most optimal place for a photon to strike the photodetector is in the depletion region itself (C). When this happens, the electron will drift towards the n-type region and the hole will drift towards the p-type region, and both will contribute to the current. If the pn photodiode is modified to contain an intrinsic region (p-i-n)
then the potential drop will occur primarily across the intrinsic region, and so most of the incident photons will be absorbed in that region and produce holes and electrons which contribute to the current. The photodiode can further be enhanced by applying a reverse bias, which widens the depletion region, encouraging newly formed electron hole pairs to separate and diffuse across the depletion region (see Figure 2-8 (b)).

Figure 2-7 PN junctions [46].

Figure 2-8 (a) Band bending in a PN junction [47]. (b) Material structure and band bending in a PIN reverse biased junction [46].

2.3.2 Vertical cavity surface emitting lasers

A vertical cavity surface emitting laser (VCSEL) operates on the same basic principles which govern the operation of all diode lasers. PIN diode lasers are structured like waveguides. The p-type and n-type regions have higher index of refraction than the intrinsic region, and act as the cladding of the waveguide with the core intrinsic region between them. This creates a confining
structure. Electrons and holes created in the p-type and n-type regions under forward bias will be captured and confined together, increasing the probability that they recombine. When current is injected, stimulated emission is produced, leading to coherent light output.

A VCSEL is characterized by having a very short cavity length, on the order of 1-3\(\lambda\). The cavity is made from two highly reflective mirrors called distributed Bragg reflectors (DBRs). DBRs are dielectric stacks of thin layers of materials with different index of refraction. Light is forbidden from propagating within this material, leading to a reflection coefficient of greater than 99%. The high reflection coefficient significantly increases photon lifetime within the cavity, meaning that the photon will pass back and forth through the cavity many times. This increases the probability that each photon will generate stimulated emission.

The operation of a VCSEL can be understood by examining the rate equations in the active region of the laser. The active region is defined as the region where recombining carriers contribute to useful gain and photon emission [48] - usually referring to the lowest bandgap region within the depletion region of the pin junction. The rate equations govern the change in carrier and photon densities, respectively, due to various recombination and generation rates, and will be of the general form

\[
\frac{dN_x}{dt} = \sum R_{\text{generation}} - \sum R_{\text{recombination}}, \quad \text{E 2-18}
\]

For the change in carrier density, four terms must be considered: the fraction of terminal current that provides carriers for recombination in the active region, the spontaneous recombination rate, the non radiative recombination rate, and the stimulated recombination rate (which is dominant in laser systems). The rate equation is given by

\[
\frac{dN}{dt} = \eta \frac{I}{qV} - R_{sp} - R_w - g_{\text{nr}} N_p, \quad \text{E 2-19}
\]
where in the first term, $\eta_i$ is the injection efficiency, $I$ the terminal current, $q$ the electronic charge, and $V$ the active region volume [49]. In the final term for stimulated recombination, $g$ is the optical gain of the active region (which will be discussed later) and $v_g$ is the group velocity of the mode. A similar equation can be written for the photon density which includes three terms: the rate of stimulated emission, the rate of spontaneous emission, and the rate of photon loss

$$\frac{dN_p}{dt} = \Gamma g v_g N_p + \Gamma \beta_{sp} R_{sp} - \frac{N_p}{\tau_p},$$

where $\Gamma$ is the three dimensional mode confinement factor (the overlap between the optical mode and distribution of material gain created by the carriers); $\beta_{sp}$ is the spontaneous emission factor; and $\tau_p$ is the photon lifetime in the cavity [49], which, as we will see later, is increased by high reflectance distributed Bragg reflectors (DBRs).

A laser operates on the principle that photons can be absorbed in the active region and amplified through stimulated emission to create a coherent beam. The amplification of the photons is described by the gain $g$ of the active region, which is usually exponential due to a population inversion at the junction. Gain is defined as

$$g = \frac{\text{Power generated per unit length}}{\text{Power carried by the beam}}.$$  

For a complete description of VCSEL gain, please see [49].

Threshold is the point at which the gain is equal to the losses in a laser. In order for a laser to reach threshold, the gain in the active region must compensate for all propagation and reflection losses [27], so that the electric field density of the photon is constant after each successive trip across the cavity. The current needed for a laser to reach threshold is referred to as the threshold current.
VCSELs can be driven to generate single transverse mode or multimode emission. Modes occur because of the boundary conditions imposed at the edge of the quantum well, where the electric field must go to zero due to Maxwell’s equations. As a result, modes are quantized within the active region of a laser. Single mode emission is the lowest energy emission state. All photons created in this state are within one mode, a Gaussian beam, and have the same phase \[50\]. This is analogous to the even quantum ground state, or the s-orbital of an electron. In contrast, when the laser is pumped with a higher current, at a certain point the laser will support a multimode emission pattern. This transition can be clearly seen when examining a laser spot shape: in single mode operation it is circular and evenly illuminated, and when it transitions to a multimode operation, it develops lobes, and often has a slightly darker cross shape in the middle. The adjacent modes in a multimode laser are \(\pi\) out of phase with each other \[51\]. While calculable in theory, the description of a multimode beam is often a complex superposition of the different modes, which depends on the fine structure and gain medium of the particular laser cavity.

2.3.3 Material Considerations

The biological requirements discussed in section 2.2 clearly elucidate the need for low signal to noise ratio of the biosensor. A key source of noise which must be reduced for optimal biosensor function is the dark current of the detector. The dark current is the signal present in the detector when it is not illuminated. Dark current is caused by intrinsic carrier recombination, as well as defects within the material of the detector mesa, which can originate during material growth or processing. GaAs was chosen as a material for the biosensors due to its low dark current. The dopant atom used in p-type layers is carbon, and the dopant used in the n-type layer is silicon.
The material used for the VCSEL structures includes $\text{Al}_x\text{Ga}_{1-x}\text{As}$ layers for the DBRs. The atomic lattice constant is a key consideration when designing the layers of a semiconductor substrate. Epitaxial growth of a lattice mismatched layer onto a substrate results in defects in the material. The closer the lattice constants of the layers, the thicker the layers can be grown with negligible defects in the material. As seen in Figure 2-9 (a), the lattice constant does not appreciably change as the fraction x changes between GaAs and AlAs. As well, GaAs has a much lower density of intrinsic carriers than Si. As a result, $\text{Al}_x\text{Ga}_{1-x}\text{As}$ devices have a very low dark current in comparison to silicon based devices, as can be seen in Figure 2-9 (b).

In addition to dark current considerations, we desire an integrated device with lasers in the optical window (650nm – 850nm). $\text{Al}_x\text{Ga}_{1-x}\text{As}$ devices are ideal for this wavelength of laser, due to their energy band gap (see Figure 2-9), which ranges from 1.424eV (GaAs) to 2.168eV (AlAs). The equivalent photon energies for these band gaps are calculated using $E$ 2-22 to be 871nm and 572nm, respectively. The laser wavelengths can be varied between these two values by changing the amount of aluminum and gallium in the quantum well. The specific material used in the quantum well and DBRs of the VCSELs is proprietary, and as such will not be explicitly given in this discussion.

Figure 2-9 (a) Energy gap versus lattice constant for ternary compounds and binary compounds. [52] (b) Comparison of dark current for $1\text{mm}^2$ photodetectors at room temperature. [53]
\[ \lambda = \frac{hc}{E_{\text{gap}}} \]

The substrates used for the biosensor were grown by molecular beam epitaxy (MBE). During MBE, atoms are evaporated from sources within an ultrahigh vacuum (UHV) chamber, and condense onto the substrate in uniform layers. MBE is highly effective in growing layers of III-V semiconductors, due to the different chemical properties of each respective group of elements. Group V elements are much more volatile than group III elements, and so they will not adhere to the substrate unless there is a group III element present with which they can form a compound. This ensures that stoichiometry is conserved during the growth process.

The quality of MBE grown materials depends on how long the MBE machine has been in operation. Figure 2-10 shows the difference in pinhole defects between two different MBE machines, and two different runs done on the same machine at Stanford University. The detectors fabricated on the first two substrates naturally had a much higher dark current than the detectors fabricated on the latter substrate.

![Figure 2-10 Dark field microscopy of pinhole defects in (a) Growth from system 1 (b) First run done on system 4 (c) Subsequent run done on system 4 [54]](image)

Another source of detector defects is in the micro fabrication stages of the device. High temperature processes and oxygen plasmas are known to lead to a higher overall dark current [55]. Excessive handling of the devices also leads to increased dark current, as defects can be formed if the material is scratched, jarred or dropped. Processing related defects can be minimized, but are ultimately unavoidable. Oxygen plasmas must be used to clean organic
materials off of the surface prior to metallization, and high temperatures must be used in annealing ohmic contacts.

At the time of writing this dissertation, MBE and MOCVD (metalorganic chemical vapour deposition) growth and fabrication techniques had been optimized to generate detectors with a dark current as low as 2pA.

### 2.3.4 Fabrication Steps

Standard optical photolithography was used in each step to create a template for deposition of films or metals, or to protect parts of the device during etching. The templates seen in Figure 2-11 are for individual sensors, however, the masks were for a sample size of 1cm x 1.5cm – an array of 5x10 sensors. I helped develop the photodiode fabrication process described below for three months at the Stanford Nanofabrication Facility in California.

![Figure 2-11 Mask set [54] used for device fabrication](image)
In the first step, a layer of nitride was deposited over the whole sample using plasma enhanced chemical vapour deposition (PECVD). Optical photolithography and plasma dry etching (using an SF$_6$/O$_2$ based recipe) were used to remove the nitride from all areas except for the contact pad. The nitride offers isolation between contacts, as well as a solid surface for probing and wire bonding.

Next, electron beam metallization was done to form top side N-contacts. A bilayer of photoresist was used during all metallization processes. Two resists with different development rates are used, so that an undercut is formed around the edges where the contacts will be formed. The bilayer prevents the contacts from being attached to different parts of the sample during electron beam metallization, ensuring that during lift-off, the contacts are not removed with the resist and overlaying coating of metal.

As a third step, the detector mesa area was defined using an electron-cyclotron resonance microwave plasma etching system. The mask set unfortunately did not cover the nitride pad during this step, and so it was etched away. The nitride pad was then re-deposited and re-etched as in step one. Upon analysis of detector performance, no significant changes were seen due to nitride on the sidewalls of the detector. This error was corrected when a new mask set was designed.

Next, p-contact metallization was done in a similar manner to step 2. Backside metallization was also done at this stage, to facilitate future electrical characterization.

Penultimately, thick resist was used for sidewall coverage of the detector mesa. The thick resist prevents light from striking the side of the detector (causing errors in photo detection) and also provides a slope for metallization of the vias.

Finally, metallization of the contact pads and vias was performed. Final results are seen in Figure 2-12. For a more complete description of fabrication procedures and challenges, please see [55].
2.3.5 Light Emitting Diodes

Semiconductor light emitting diodes (LEDs) have a similar structure to a laser diode, in that they are forward biased semiconductor p-n junction. However, unlike a laser, they lack the highly reflective mirrors on the edge of the resonant cavity. As a result, photons created do not pass back and forth within the cavity many times before emission, and are not multiplied in the gain material. The light emitted from an LED is isotropic and largely incoherent as a result.

2.4 Speckle noise in coherent light sources

Since the 1960s, optics researchers have identified a phenomenon called speckle which occurs when partially or full coherent light sources interact with a rough surface. To understand speckle, one should consider each reflective point on the rough surface to be a point source. In places where the phase of the reflected light matches that of the incoming coherent light, constructive interference occurs, and correspondingly, where the phase is 180° out of phase with...
the incoming light, destructive interference occurs. These random patterns of destructive and constructive source interference are called speckle, and are especially pronounced in circumstances where the roughly reflecting surface is moving over time.

Speckle can occur due to surface scattering or volume scattering, but is usually a combination of both types. For surface scattering, for example from a rough metallic surface, differences in path length which cause constructive or destructive interference are due primarily to the varying surface height. On the other hand, in volumetric scattering substances like tissue, the speckle interference is due primarily to optical path length differences caused by multiple scattering events which change the photon’s path. As a result, for surface scatterers, the primary variable is surface height fluctuation due to roughness. For volumetric scatterers, the primary variable is scattering length, commonly represented in tissue by $\mu_s$.

The metric used to measure speckle is called the contrast, $C$, and is defined as the ratio of the standard deviation over the mean value of intensity levels [56], $C = \frac{\sigma}{<I>}$. For a fully developed speckle pattern, in which coherent, single wavelength light is reflected from a surface with a Gaussian distribution of roughness, the contrast is equal to one [57], [58].

Speckle can result in degraded image quality with low signal to noise ratio. As the popularity of using lasers for projection applications, laser Doppler, or imaging such as optical coherence technology (OCT) has increased in the past few years, many research groups have worked to develop solutions to reduce speckle. Physical methods aimed at spatially de-cohering the laser light prior to imaging, such as placing rotating diffusers or liquid crystal displays in the path of the laser beam, have shown some success [59] especially in the field of projection system optics. However, these solutions are large scale and may not be possible to miniaturize and integrate into an implantable device. Digital filtering techniques, which can be applied
during post processing of images, have become powerful methods used by many groups who study OCT [60], [60]. In particular, researchers have found a dramatic reduction in speckle noise by incoherently compounding angularly resolved images. The first description of this method reported up to 8dB increase in signal to noise ratio when compounding over an angular range of 32° [61]. This work is promising for OCT studies, but is most likely not be a viable solution for a miniaturized device.

Most recently, studies have shown that vertical cavity surface emitting lasers driven with pulsed current will emit in a spatially incoherent regime, but only in the far field of the source [56]. This is a promising step towards reducing speckle for other applications.

While initially, speckle was seen as an entirely detrimental laser property, researchers have recently begun to use speckle to their advantage in imaging applications. Because speckle patterns change due to motion of individual scattering particles, the way in which a speckle pattern changes over time can be used to extract information about the movement of a sample. For a full review of using time varying speckle to measure flow, see [57].
Chapter 3 Motivation and Problem Statement

The overall goal of our research group is to develop implantable, optical biosensors for neural imaging, as discussed in the introduction. We chose to use lasers in biosensor design due to their high power per device footprint, focused spot size, and directionality. As a result, it is important to examine the noise differences between our VCSELs and standard LEDs, and attempt to reduce the coherence noise, which is most likely due to speckle (as discussed in section 2.4).

Recently, researchers at Washington State University rigorously compared incoherent (light emitting diode), partially coherent (superluminescent diode) and fully coherent (laser diode) light sources in both in vitro and in vivo neural imaging paradigms [62]. They found in both cases that the incoherent light emitting diodes exhibited significantly lower signal to noise ratio than coherent or partially coherent source, and suggested that researchers doing optical neural imaging use only incoherent light source.

The first step in comparing coherent and incoherent light sources was a source of additional motivation to investigate this issue. Work was done with researchers in Dr. Michael P. Stryker’s neurophysiology group at the University of California, San Francisco (UCSF) where researchers were performing intrinsic optical signal imaging of the mouse visual cortex in order to evaluate visual plasticity in the UB3A mouse (a model of Angleman syndrome). Angleman syndrome can cause epilepsy, mental retardation, and movement as well as sleep disorders. Collaborators at UCSF were studying visual plasticity as a model of experience. Their protocol was to image the visual cortex of both wildtype and UB3A mice who had had one eye sewn shut for several days. The mouse was presented a visual stimulus which varied in contrast at a frequency of 0.1Hz. Visual cortex responses were then measured for the contralateral and
ipsilateral eyes, and the image analyses were done in the frequency domain in order to extract the signal response only at 0.1Hz, the frequency of the stimulus. This ensured that the breathing and heart rate components of the signal were filtered out.

Figure 3-1: (Left) Contralateral eye response to visual stimulus, imaged using an incoherent light emitting diode. The top image is of the amplitude of response. The bottom image indicates the phase of the response, that is, which part of the stimulus signal resulted in the largest change in amplitude. (Right) Corresponding maps of amplitude and phase, imaged using a VCSEL.

A high powered LED was normally used for brain illumination, and images were captured with a CCD camera controlled by custom acquisition software. VCSELs were brought to UCSF so that we could compare the images obtained with each type of light source. The
results we saw were irrefutable: the LED generated clear maps of visual cortex activation, and the VCSEL produced maps that looked like white noise (see Figure 3-1).

We had until this point expected that the strong laser intensity would compensate for any additional noise due to coherence effects, and consequently, we were not expecting that the speckle would cause such a low signal to noise ratio.

![Figure 3-2](image)

**Figure 3-2:** *(Left)* Top map represents the amplitude of visual cortex signal obtained with both eyes open, using a laser with two diffusing elements in front of it, and averaging 100 stimulus cycles. The bottom map is again the phase, representing the part of the stimulus cycle which generated the strongest visual cortex response. *(Right)* These maps were taken immediately after the maps in the lefthand images, with the stimulus monitor turned off.

In order to evaluate the effects of coherence on image quality, we went back to UCSF to try using modified laser light sources to image. The laser light was passed through an optically
diffusing element (a lens composed of small scattering elements), in an attempt to decohere the light prior to its interaction with the cortex. Many more stimulus cycles were averaged, collecting images for 16 minutes as opposed to the usual 4 minutes that our collaborators use. These experiments showed promising results, seen in Figure 3-2. The left hand set of images were taken on a mouse with both its eyes open, using a laser with two diffusing elements in front of it. The spatial binning was increased, and 100 stimulus cycles were averaged. In order to ensure that the concentration of signal seen in the top right corner of the phase map was not an artefact, a subsequent series of images was taken using the same setup, but with the stimulus monitor turned off. As there was no signal seen in these maps, we concluded that the experimental setup using a laser and two diffusers was able to resolve some visual cortex signal, though not as clearly as when using a light emitting diode.

The experiments conducted at UCSF provided the motivation to study the interaction of coherent and incoherent light sources with tissue, in order to identify both the cause of the additional noise in coherent sources, and also to explore ways of quantifying and reducing the noise, by careful selection and evaluation of noise metrics. A large part of the work in this dissertation has been towards achieving these goals.
Chapter 4   Optical Modeling

Effective optical modeling can be used to plan experiments as well as to design devices, saving a researcher both time and resources. To this end, an optical model of a rodent body, including the brain, would be a valuable tool for any group of researchers who exploit light tissue interactions in rodents. The following sections outline the progress made towards this goal, and the challenges that have been identified in creating a rodent optical model.

4.1  Body organs from magnetic resonance imaging

In order to accurately represent the geometry of a mouse body, magnetic resonance imaging (MRI) data of a mouse body, segmented volumetrically into different organs, was obtained from collaborators at the Mouse Imaging Facility (MICe, Toronto Center for Phenogenomics, Toronto, ON). As discussed in Section 2.1.3, the optical modeling program used, ASAP, is a surface based Monte Carlo code. As such, the first step in using the MR data was to convert it from labelled voxels (volume data) to surface based objects. This was accomplished most efficiently using the program Amira®, software designed to visualize and manipulate biomedical data. Amira® was able to create a mesh along the surface of a labelled volume object, and that mesh could be exported from Amira® and brought into a CAD program (Rhinoceros by McNeel). Within Rhino, commands exist to create surfaces from the mesh by creating one surface from each triangle of the mesh. As the MR data used were very high resolution, this resulted in a body model containing millions of surfaces. Since ASAP has a 9999 object limit, coupled with the obvious fact that so many objects would dramatically increase Monte Carlo calculation time, efforts were made to reduce the number of surfaces needed to represent the organs. The first option for facet reduction was to reduce the resolution of the data. However,
this option led to significant information loss, as well as the creation of some overlapping edges between adjacent organs (see Figure 4-1).

![Figure 4-1: (Right) Original resolution mouse stomach. (Left) Resolution required to have less than ASAP’s limit of 9999 objects in the whole mouse body.](image)

The second route for facet reduction was using drape commands in Rhino, which covered the top half of an approximately spherical object with one curving surface. The full surface was represented by draping it from opposite ends, and then cutting the drapes in the middle and creating a seam to merge them together. This generated a full resolution object composed of three surfaces (see Figure 4-2). While this methodology works for approximately spherical objects, it will not work for complex structures like pelvic bones or blood vessels, or discontinuous tissues like muscle.

![Figure 4-2: Bladder composed of three surfaces, constructed using a drape command in Rhino.](image)
4.2 **Biosensor Models**

In order to guide biosensor development, optical models of biosensor geometry were constructed and validated using *in vitro* dye well experiments. Some challenges remain in the modeling, which will be discussed in this section.

4.2.1 **Fluorescent biosensor sensitivity**

The work discussed in this section has been published in the conference proceedings of Photonics West [63]. I modeled the biosensor geometry in the optical modeling program ASAP. Together with collaborators at Stanford, the optical model was validated by simulating and experimentally determining collection efficiency $C_{\text{sensor}}$, defined as the amount of fluorescence emission light collected at the photodetector (and thus photocurrent measured) relative the optical excitation power for a particular sensor and target (dye) geometry. In other words, this parameter is a geometrical factor where 100% means that all the emitted light over $4\pi$ steradians is collected. The collection efficiency takes into account the dimensions of the sensor including: the photodetector shape and location relative the laser, the roughly reflecting package cap and top aperture (to restrict light at oblique angles from reaching the detector), and the aspheric lens.

In particular, I modeled an *in vitro* sensitivity experiment, in which a solution of Cy5.5 was sensed from the bottom side of a clear well. For the simulation, a flat, isotropically radiating disk was modeled in place of the Cy5.5 solution with the same dimensions as the solution in the well. Rays were traced from the radiating disk through the biosensor geometry. The amount of flux incident on the detector/filter mesa surface was recorded as a percentage of the initial flux. The collection efficiency of the present design was simulated to be 0.055%. In order to validate this simulation, the experimental collection efficiency was calculated using E 4-1 [64].
\[ I_{\text{det}} = \frac{q \cdot P_{\text{laser}} \cdot Q_E \cdot T_{\text{filter}} \cdot C_{\text{sensor}}}{\hbar c} \left( 1 - 10^{-\text{dM}} \right) \]  \hspace{1cm} \text{E 4-1}

\( I_{\text{det}} \) represents the amount of photocurrent that should be measured from a uniform thickness of fluorescent molecules. The terms and assumed values are listed below in Table 4-1. \( T_{\text{filter}} \) quantifies the overlap between the emission filter and the Cy5.5 emission spectra (Figure 4-3).

The values for the molar extinction coefficient \( \varepsilon \) and quantum efficiency \( Q_E_{\text{dye}} \) of the dye were taken from the manufacturer’s data sheet and verified in literature for aqueous solution [65], but were not measured experimentally. Using these values, the experimental collection efficiency, \( C_{\text{sensor}} \) was measured to be to be 0.060%; the value is in agreement with the simulated collection efficiency of 0.055%. For conceptual comparison, if equation E 4-1 is used with the simulated collection efficiency to calculate a theoretical current response from a 100\( \mu \)L of 1\( \mu \)M dye, the value is 690pA which compares favourably with experimental value of 750pA.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda_{\text{em}} )</td>
<td>Emission wavelength</td>
<td>750nm</td>
</tr>
<tr>
<td>( P_{\text{laser}} )</td>
<td>Laser intensity</td>
<td>0.385mW</td>
</tr>
<tr>
<td>( Q_E_{\text{dye}} )</td>
<td>Fluorescence quantum yield of the dye</td>
<td>30%</td>
</tr>
<tr>
<td>( Q_E_{\text{det}} )</td>
<td>Quantum efficiency of the detector</td>
<td>77% (measured)</td>
</tr>
<tr>
<td>( T_{\text{filter}} )</td>
<td>Filter transmission</td>
<td>16%</td>
</tr>
<tr>
<td>( E )</td>
<td>Molar extinction coefficient of dye</td>
<td>250,000 cm(^{-1})M(^{-1})</td>
</tr>
<tr>
<td>( d )</td>
<td>Thickness of dye</td>
<td>0.275cm (100( \mu )L)</td>
</tr>
<tr>
<td>( M )</td>
<td>Concentration of dye</td>
<td>1( \mu )M</td>
</tr>
</tbody>
</table>
Figure 4-3 Sensor characteristics (excitation wavelength and detector response for normally incident light) overlaid with Cy5.5 excitation and emission spectra. For greater incident angles (up to 30 degrees), the detector response is blue-shifted while still providing 5 (measured) orders of magnitude suppression at the excitation wavelength.

It is important to note that the collection efficiency of the sensor geometry is small due to the large sample volume we wish to interrogate. A similar integrated fluorescence sensor designed for micro fluidic applications exhibits a collection efficiency of 2.5% [64] - almost 50 times higher than this device. The collection efficiency is an area that can be optimized to increase biosensor sensitivity, by using a different lens or sensor geometry.
4.2.2 Dye well at distances

Figure 4-4: Schematic of biosensor model, including the biosensor (detector mesa structure), a coherent, divergent source, the collimating lens, and an absorbing strip of dye embedded in a scattering tissue.

The biosensor model can be divided into three distinct parts: (1) the creation of a highly divergent Gaussian source, (2) the propagation of this source through the biosensor geometry and its absorption into the sample, (3) the re-emission of isotropic light from the sample (fluorescence) and its propagation back through the biosensor geometry to the detector. Each of these aspects of the model will be discussed in the following sections, along with the associated results and challenges.

High divergent Gaussian source

The creation of a highly divergent Gaussian source in ASAP is possible only through the use of coherent beam tracing. In ASAP’s coherent mode, each ‘beam’ is composed of a base ray, which carries the flux and travels in the direction of beam propagation, as well as four parabasal rays. Two parabasal rays describe the beam waist (two are necessary if the beam is not circularly symmetric, that is, if it has a different waist size in the x versus y direction). The other two parabasal rays describe the divergence, or far field angle of the beam [66]. Again two rays are necessary for non-circularly symmetric beams. All four parabasal rays must be paraxial to
the base ray, so that each can be traced using geometrical optics, to recover a Gaussian after propagation through an optical system.

In order to create complex light sources, ASAP creates a superposition of multiple weighted Gaussian beams which represents, through constructive and destructive interference, the characteristics of the source being modeled. As described above, each beam is composed of a base ray and four parabasal rays, which can all be propagated through a system by geometrical means. If at any point an optical element causes the parabasal rays to become not paraxial to the base ray, ASAP issues an error or a warning, and the source can be remade before the element in question in order to avoid this problem.

The summative nature of coherent beam optics in ASAP results in a much longer computation time than incoherent ray optics. In addition to long computation times, ASAP does not accurately keep track of flux during coherent scattering. As a result, in the biosensor model, the highly divergent Gaussian source must first be created coherently, propagated until it is nearly collimated (right before the lens element), and then recreated as an incoherent source so that flux tracking can be done as it propagates through a scattering medium like tissue. In order to do this, the program was scripted to create the Gaussian source, propagate it to a plane immediately before the lens, and record the source flux and direction on that plane. These data was saved to a distribution file, since it is computationally intensive both to create the coherent source and also to calculate its distribution on a plane. The program was then told to switch to incoherent mode, and the plane was scripted to emit incoherent rays specified by the distribution file. The justification for performing this coherent to incoherent transition before the lens is that by this point, the source has moved into the far field regime where coherence effects should be negligible. The far field regime is defined to be approximately five times the Rayleigh range, $z_o$ [67]:

46
where $\omega_o$ is the beam waist, equal to 2.5$\mu$m for the VCSELs we use, and $\lambda$ is the VCSEL wavelength, equal to 675.5nm. Since the lens is 3.5mm from the beam waist, it is in the far field regime.

This method of transitioning from coherent to incoherent light propagation does work, but has a few challenges associated with it. The first is that when the coherent beam data is being recreated as incoherent ray data, the parabasal rays are stripped and only the base ray is left. This generates what Breault terms ‘drop outs’ in the source profile – points where the flux of the source is not smooth but goes to zero (see Figure 4-5). The drop outs should not affect the model results, as the integrated flux of the source is normalized to 100 prior to tracing through the system. The second challenge is that the number of rays cannot be adjusted. If there were x number of beams, there can be no more than x number of rays after the conversion. The rebuilt, incoherent source with drop outs is shown in Figure 4-6, overlaid on a MATLAB based propagation of a Gaussian beam.
Figure 4-5: Gaussian source profile in ASAP after coherent to incoherent conversion. As can be seen, the source envelope is Gaussian, but contains numerous drop outs due to parabasal ray stripping.

Figure 4-6: Gaussian beam propagation done in MATLAB (blue line) versus ASAP (red dots) for a beam with 2.5 μm waist, propagated a distance of 3.53 mm. Red dots along the x-axis are due to source dropouts created during the coherent to incoherent conversion.
**Propagation of the source outwards and absorption in the sample**

In order to track the absorption within the volume of the model representing the fluorescent dye well, the command `VOXELS ABSORBED x x' y y' z z' [ n n' n'' ]` was issued, where the first six variables specify the volume to be tracked, and the last three variables specify the number of voxels in the x, y and z directions, respectively. The flux of all absorption events that occur within each of these voxels was tracked and saved to a distribution file that was later called by the program. The incident source (to be referred to as the ‘excitation source’), is of a particular wavelength and as such, the dye and sample both have optical properties ($n$, $g$, $\mu_a$ and $\mu_s$) specific to the excitation wavelength. Once the program was finished tracing, and all rays have been absorbed or have scattered outwards into free space, the system reset, all existing ray fluxes were cleared, and the geometry was rebuilt with optical properties at the emission wavelength.

In order to verify that enough rays were being traced to obtain a statistically significant result, this second segment of the simulation was traced 100 times using different SEED values for each run (see section 2.1.3 for a discussion of SEEDing in ASAP). The flux absorbed in an individual voxel in the middle of the dye well was tracked and its absorbed flux (from an excitation source normalized to 100) and standard deviation were calculated to be $(2.69\pm0.05)\times10^{-4}$, which is a deviation of less than 2%.
As a second verification, the attenuation of light as it passes through a tissue or dye sample was estimated using the Beer-Lambert law, and then calculated in ASAP. For the absorbing, non-scattering dye case, an imaginary index of refraction of \( N_k = 1.34 \times 10^{-6} \) (representing the dye Cy 5.5 in a 1μM concentration) was used, and a wavelength of 675.5nm. Then, as seen in E 2-7 of section 2.1, the attenuation is given by the Beer-Lambert law as:

\[
I = I_0 \exp(-\mu_a z) = I_0 \exp\left(-\frac{4\pi N_k}{\lambda} z\right). \tag{E 4-3}
\]

**Emission from the dye**

The distribution of absorbed flux discussed in the previous section is now recalled by the program, and each voxel emits (throughout the volume in an isotropic distribution) the amount of flux it absorbed, multiplied by the conversion efficiency of the dye, at the wavelength of emission of the dye. The rays are then propagated through the system, and the flux incident on
the detector mesa is tracked. This section of the model already showed good agreement with theory, as discussed in the fluorescent biosensor sensitivity section (4.2.1).

4.2.3 Results at distances

In order to verify that the dye well simulation was working properly, experimental trials were conducted by collaborators at Stanford, using different sensor packages and dye wells filled with Cy 5.5 dye, at different distances from the sensor lens. The first data from these trials did not fit with the theoretical predictions, as it all showed peaks in the flux on the detector when the dye well was pressed against the lens (see Figure 4-8).

![Signal vs distance (max normalized to 1)](image)

*Figure 4-8: First iteration of dye well experiments. Simulation data is overlaid.*
Further trials with different sensor packages found one detector/laser combination that exhibited the same trend as the simulation data (see Figure 4-9). This indicates that the collection efficiency of the sensor is highly dependent on sensor geometry. The current sensors are hybrid devices, meaning that the detector and VCSEL are not integrated onto one substrate, but are instead diced and packaged onto the same pin arrangement. As such, small variations in detector-laser geometry can be expected. However, once the sensor is fully integrated, the geometry will be specified by the fabrication mask set, and theoretical to experimental comparisons are expected to be more consistent.

**4.3 Modeling Shapes at Depth**

Collaborators at Princess Margaret Hospital in Dr. Brian Wilson’s Biophotonics group are working on using different wavelengths of light to determine the depth of fluorescing objects. Their vision is a portable device which could be used on the spot by surgeons to identify both the shape and depth of tumours before a surgical procedure. As a feasibility analysis, work was
done to develop a model in ASAP showing the light flux at the surface of a block of tissue from three fluorescing 3D objects embedded at different depths. To calibrate the model, and ensure that ASAP was providing an accurate representation of the experimental conditions, a capillary channel submerged in a tissue phantom was simulated at four different wavelengths (405nm, 495nm, 546nm, and 625nm), with dye emission at 700nm. See Table 4-2 for the scattering and absorption coefficients of the homogenized tissue phantom, rabbit brain and fluorophore at these wavelengths.

Table 4-2: Optical parameters of the tissue phantom and fluorophore at different excitation wavelengths as well as the emission wavelength (700nm) of the fluorophore.

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Homogenized Tissue Phantom</th>
<th>Rabbit Brain (n=3) Fluorophore (PpIX)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\mu_a$ (cm$^{-1}$)</td>
<td>$\mu_s'$ (cm$^{-1}$)</td>
</tr>
<tr>
<td>405</td>
<td>47</td>
<td>37</td>
</tr>
<tr>
<td>495</td>
<td>2.9</td>
<td>31</td>
</tr>
<tr>
<td>546</td>
<td>5.7</td>
<td>28</td>
</tr>
<tr>
<td>625</td>
<td>0.13</td>
<td>25</td>
</tr>
<tr>
<td>700</td>
<td>0.039</td>
<td>23</td>
</tr>
</tbody>
</table>

Note: For the rabbit brain, the number of significant digits is based on one standard deviation of the data, calculated from trial averaging statistics. For homogenized tissue phantom and fluorophore, the measurements are taken to be accurate to 5% and significant figures are adjusted accordingly.

Results are shown in Figure 4-10. Very good agreement is shown in the three lowest wavelengths, with a slight discrepancy in the higher 625nm data. This discrepancy is believed to be a result of the dimensions of the capillary channel. At 625nm, the 400$\mu$m channel is optically thin due to lower scattering. We believe that the model may not be accurately addressing scattering and absorption in the optically thin geometry.
Figure 4-10: Experiment (blue squares) versus simulation (coloured lines with dots) values of the peak flux in a cross section taken at the surface of a dye capillary embedded in a tissue phantom.

Figure 4-11: (Left) ZX plane geometry of buried fluorescing objects. Spot (blue) is 0.1 mm from the tissue surface. Pyramid (orange) is 0.8mm deep, and rectangle (green) is 2mm deep. (Right) YX plane geometry.
After ASAP showed good agreement between model results and experiment for the capillary buried in homogenized tissue, I created a model of three dimensional fluorescing objects buried at different depths within rabbit cortical tissue – a cylinder, a box, and a pyramid, (see Figure 4-11 for specific geometry). Simulations were then done for each of the four excitation wavelengths discussed above, and the flux pattern at the surface of the tissue was determined.

Figure 4-12: Magnitude and distribution of flux on the surface of the tissue from embedded, fluorescing shapes. White outline of embedded shapes has been overlaid for clarity.
At the lowest wavelength, 405nm, the high absorption coefficient ($\mu_a=22.31\text{cm}^{-1}$) was generating ‘NaN’ (not a number) errors in ASAP. Technical support suggested that this may be caused by the fluorophore and tissue absorbing nearly 100% of the incident light. In order to avoid this issue, a lower absorption coefficient of $\mu_a=15\text{cm}^{-1}$ was used at this wavelength.

As can be seen in Figure 4-12, when light attenuation in the tissue is high (most pronounced in the 405nm plot), the surface light distribution is dominated by objects which are close to the surface. In contrast, at lower light attenuation (for example, the 625nm plot), fluorescence from deeper objects is much more pronounced. This indicates that different wavelengths of light will be useful for identifying the depth of fluorescing objects, most notably tumours.
Chapter 5  Light Noise Analysis

As discussed in the motivation section, a large part of this thesis work has been dedicated to evaluating the differences in noise between coherent and incoherent sources, in order to both quantify and reduce the noise by modifying the laser. The following sections discuss the noise measurements done both \textit{in vitro} and \textit{in vivo}.

5.1  \textit{In Phantom Noise Analysis}

In order to evaluate the stability of the light sources (VCSELs and LEDs), the power sources driving the light sources, as well as the camera switching mechanism, extensive imaging trials were done prior to \textit{in vivo} studies. Image processing methods were evaluated on these data, and methods of image sorting, processing, and metric determination were optimized. In addition, due to the nature of speckle, slight motions in surface height are highly impacting on the resulting speckle pattern. As a result, it is important to analyze the noise of each source first in a phantom, in order to eliminate the movement of imaging surfaces due to physiological effects such as breathing and heart rate. In order to quantify noise a flexible silicone phantom with negligible absorption and uniform scattering due to embedded titanium dioxide particles was used (see Table 5-1 for measured optical properties of the phantom at relevant wavelengths).

5.1.1  Methods

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure51.png}
\caption{Block diagram of experimental imaging setup.}
\end{figure}
A QImaging Retiga 4000R monochrome, 12-bit, cooled CCD camera was used for all experiments. This camera has a pixel size of 7.4 μm x 7.4μm, and was attached to the image acquisition computer (Dell Precision 5400, Xeon® CPU @2.33GHz, 4 Gb of RAM) via IEEE 1394 firewire connection. The chamber containing the CCD chip was filled with nitrogen gas to prevent any condensation from forming, and the glass was coated with an antireflective coating to avoid coherent etalon effects when imaging with a laser. The camera was suspended from a custom built camera rig, composed of mostly 80/20 parts. The camera rig allows coarse motion in two directions (18 inches of travel in x, 18 inches of travel in z), as well as fine focus in the z direction (20mm in 1μm steps), and ±20° of tilt. Light was captured using two Nikon 50mm f/1.4 lenses coupled nose-to-nose, producing a one to one sized imaging field of 15.15mm x 15.15mm.

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Absorption Coefficient $\mu_a'$ (cm$^{-1}$)</th>
<th>Reduced Scattering Coefficient $\mu_s'$ (cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>625</td>
<td>negligible</td>
<td>6.10±0.01</td>
</tr>
<tr>
<td>660</td>
<td>negligible</td>
<td>5.95±0.01</td>
</tr>
<tr>
<td>675</td>
<td>negligible</td>
<td>5.89±0.01</td>
</tr>
</tbody>
</table>

Two light sources evaluated: a laser in various modes of operation, and a super bright LED. The first source was a vertical cavity surface emitting laser (VCSEL) from Honeywell, operating at 675nm (see section 2.3.2 for a description of VCSEL operation). The laser performance was evaluated in single and transverse multi mode operation, as well as using diffusing optical elements, which make the beam more uniform and potentially reduce speckle. For single mode operation, a current of 4.9mA was chosen, based on examination of the laser spot size and shape. For multi mode operation, the VCSEL was driven at 14.5mA, which was
determined to have the highest optical power using a Newport power meter. For certain trials, the multimode laser was first passed through one or two diffusers from Thorlabs.

The second source was a super bright LED from Luxeon [68], operating at 625nm. This LED is packaged with a collimating lens above the source, and an aluminum submount on the back, which acts as a heat sink for additional thermal stability. This LED can be driven at upwards of 350mA, but was typically drawing between 6mA and 30mA of current to create a comparable intensity to the VCSEL.

Two power sources were used to drive the light sources, a low noise current source from Keithley (6221 DC and AC current source), and a power supply from Agilent (E3612A DC power supply). A low noise switch (Keithley 7001 switch system, with two 7158 ten channel cards), was used to control the camera acquisition and to switch between light sources. The switch has two cards, each of which can be attached to a power supply. Card one was attached to the Agilent power supply and then to the LEDs. Card two was attached to the Keithley current source, and then to the VCSELs. During image acquisition, the switch channel list was programmed to contain the LED channel and the VCSEL channel (see Figure 5-1 for a cartoon of this setup). Once armed, it waits for a 5V TTL pulse out from the camera (once it acquires a frame), and then switches channels, and sends a 5V pulse to the camera, triggering it to take another image. In this manner, each even image is taken using the same light source, and each odd image is taken using the other light source.

For imaging trials, each of the sources were attached to magnetic, jointed holders, and aimed such that the center of the light spot was on the middle of the phantom. The camera was aligned above the phantom, and focused 100μm below the surface of the phantom, to avoid pixel to pixel grey level variation due to surface inhomogeneities. The camera exposure was set such that the average grey level was around 3500 for the VCSEL, and then LED current was
adjusted such that its grey level was comparable at the same exposure time. A small (<1mm²) piece of aluminum foil was placed in the center of the phantom, and aligned such that it caused saturation from one source, and dark pixels from the other source. This was later used in post processing to identify which images were taken using which source, and also for sorting the images.

### 5.1.2 Results

In order to evaluate noise and frequency fluctuations in each source, the following series of images were taken: LED versus single mode laser, LED versus multimode laser, LED versus multimode laser plus one diffuser, LED versus multimode laser plus two diffusers. The first noise metric used to evaluate the sources was the grey level standard deviation over time. Two regions of interest (ROIs) of interest were used to examine the effects of spatial averaging: 1x1 pixels and 20 x 20 pixels. These areas were chosen in a uniformly illuminated region of each image series – usually the centre of the phantom. The mean value of the ROI was calculated for each frame, and the standard deviation of this average value was calculated over 4000 images. The standard deviation was then divided by the mean value of the ROI, in order to normalize the results since some sources were slightly brighter than others. In addition, temporal averaging of every 10 frames was conducted. See Figure 5-2 for a schematic of the algorithm.

The second noise metric evaluated was the spatial contrast of the images. The contrast is the standard deviation of the values of pixels in a ROI divided by the mean value of all the pixels in the ROI, and is the commonly used metric for measuring speckle (as discussed section 2.4). As the phantom is approximately stationary, we expect the contrast to fluctuate around the same value over a time series of images. The average contrast in a 20x20 pixel region of interest over 1000 frames was calculated for no temporal averaging, and 10 frame temporal averaging. See Figure 5-5 for a schematic of the analysis algorithm.
Figure 5-2: Block schematic of temporal image analysis algorithm. The standard deviation of the 4000-entry “ROI Mean” array is taken and divided by the mean value of the “ROI Mean” array. For temporally averaged data, the standard deviation of the 400-entry “ROI Mean, 10 temporal average” array is taken and then divided by the mean value of that array.

Figure 5-3: Spatial contrast noise calculations. (Top) No temporal averaging. Contrast is calculated on each region of interest, and the average contrast of 1000 frames is then calculated. (Bottom) 10 frame temporal averaging. Each pixel in the ROI is averaged with 10 other frames. The contrast of the average pixel values is calculated, and the average value of the contrast is then calculated.
Several image acquisition trials were done for each type of source. Between trials, each of the following were varied slightly: the source distance from the phantom (1.5cm – 9.0cm), source angle to the phantom (30° – 80° evaluated visually), and camera exposure (3.5ms – 50ms). The camera frame rate was recorded for each imaging trial as it varied with exposure. The noise metric for each trial was calculated separately, and then the trial results for each point were averaged and the standard deviation calculated for each point. The temporal noise results for the different sources are shown in Figure 5-4, and the spatial noise results are shown in Figure 5-5 (see Appendix B for tabulated data).

**Figure 5-4: In vitro temporal noise analysis for different light sources.** SM = single mode VCSEL, MM = multimode VCSEL, MM20d = multimode VCSEL with a 20° optical diffuser, MM20d50d = multimode VCSEL with a 20° and 50° diffuser. N=4000 images.
In vitro speckle contrast of light sources

Figure 5-5: In vitro spatial noise analysis of a 20x20 pixel region of interest for the following light sources: SM = single mode VCSEL, MM = multimode VCSEL, MM20d = multimode VCSEL with a 20° optical diffuser, MM20d50d = multimode VCSEL with a 20° and 50° diffuser. N= 1000 images.

The image series were also evaluated in the frequency domain, in order to investigate any prominent frequency components of the noise. Each image trial acquired 8200 sequential images, so that when sorted, each light source image series would have 4096 (a multiple of $2^N$) images for fast Fourier transform (FFT) calculation. The amplitude of the FFT was normalized according to Parseval’s theorem by dividing through by the length of the original vector being transformed. As well, the lower bound on the frequency was taken, by Nyquist’s theorem, to be half of the sampling frequency of each series. The series frequency in turn was half of the camera frame rate, due to the alternation between sources during image acquisition. Representative results are shown in Figure 5-6.
5.1.3 Discussion

In each type of analysis conducted, the single mode laser noise is significantly higher than any of the other sources. This result is expected, as in multimode operation, mixing between modes of different phase (see section 2.3.2 for an explanation of multimode versus single mode laser operation) reduces the coherence of the source. The multimode laser with diffusers shows a less clear trend. In all cases, the addition of diffusers leads to a reduction in noise from the multimode case. However, two diffusers are rarely better than one diffuser. This indicates that there is either an optimal thickness of scattering medium for reducing speckle, or that the angular dependence of the scatterers is an important factor.

In the temporal analysis, each source showed a reduction in noise after spatial (1x1 ROI versus 20x20 ROI) or temporal (no averaging versus averaging 10 frames). Spatial averaging simulates a larger pixel size, which is crucial to these studies, as the size of our pixels will be the size of the photodiode mesas: approximately 200μm x 200μm. As mentioned previously, the size of each pixel in the camera is 7.4μm x 7.4μm. A 20x20 ROI represents a 148μm x 148μm area, which is a much better approximation of a biosensor pixel. Focusing on these data, one can
see that the single mode laser still has very high noise when no spatial averaging is done, but drops to within a factor of three of the other light sources when spatially averaged. The trade off in spatial averaging is a reduction in spatial resolution, which must be considered for each individual physiological phenomenon. The desired signal to noise ratio of the light source must be balanced with the temporal (as well as spatial dynamics) of the signal of interest. This point will be discussed further in the next chapter, where the neural model of cortical spreading depression is examined.

As a verification of our noise analysis, we compare our LED noise with reported results from the Frostig group at UC Irvine, who do trial average imaging of rat somatosensory cortex. They report an LED which is stable to $2.5 \times 10^{-4}$ over time when 64 trials are averaged [23]. Our LED temporal noise in a 20x20 pixel region of interest was calculated to be $4.1 \times 10^{-3}$. Noise should decrease by a factor of $\frac{1}{\sqrt{N}}$ due to the statistics of averaging, and so dividing our LED noise by 8 results in a theoretical LED noise of $5 \times 10^{-4}$ for 64 trials. This is in agreement to within a factor of two with the Frostig group’s results.

In the spatial analysis, the single mode laser showed a much higher contrast than any of the other light sources, as expected due to mode considerations. As well, the contrast was not reduced after taking a 10 frame temporal average. This indicates that the speckle pattern was not changing significantly over time, as is expected since the silicone phantom is relatively stable. In an *in vivo* case, we expect that the speckle contrast would be reduced by averaging several frames, due to physiological movement causing a dynamic speckle pattern over time.

The frequency domain graphs of the LED showed curious results: sharp peaks in each series of images taken, which were not seen in any of the laser trials. The peak location was seen to shift with camera acquisition rate. The peaks were also seen to disappear when the LED was
driven with the low noise Keithley current source instead of the Agilent power source. Upon examination of many sets of data, the peak was found to always be located near a multiple of the wall plug frequency, 60Hz. This indicates that the Agilent source is coupling the wall plug frequency into the driving source and causing fluctuations which, due to the slower sampling rate of our camera (less than 30Hz), are seen through aliasing at lower frequencies. Due to equipment considerations (only one Keithley current source is available), it is necessary to continue to drive the LED with the Agilent source when using the switching mechanism. Happily, even with this sharp peak in the frequency domain, the Agilent-driven LED exhibited, within a factor of two, the same magnitude of noise as the Keithley-driven LED, indicating that the integral of noise over all frequency components is still large in comparison to the noise seen in that one peak.

Comparisons between in vitro and in vivo light source noise will be discussed in section 5.2.3.

5.2 In vivo light source analysis

After performing a thorough in vitro noise analysis (discussed in the previous section), the temporal noise analysis was repeated for each light source in an in vivo, exposed cortex preparation. Due to the nature of speckle noise, the need to evaluate the noise dynamics when the sample is vibrating due to heart rate and breathing is evident.

5.2.1 Methods

Image acquisition was similar to the in vitro noise studies. All animal studies were carried out in accordance with the University of Toronto Animal Care Committee standards.
**Surgical Protocol**

The procedures we used are similar to those used by Dr. Michael P. Stryker at UCSF [69]. Anaesthesia was administered via a mask placed over the snout to deliver isofluorane (induction at 5%, 2-2.5% maintenance) mixed with 100% oxygen, to ensure proper oxygenation of the animal throughout the procedure. The animal was monitored continuously to ensure that it remained at a surgical plane of anaesthesia. The corneas were protected with ophthalmic lubricant ointment (artificial tears). Throughout the surgery and imaging, respiration was monitored visually, heart rate was felt, and hind limb withdrawal reflex was tested, all at regular intervals. Following the onset of surgical anaesthesia, as judged by the cessation of hind limb withdrawal and corneal blink reflexes, the animal's head was shaved. Topical lidocaine was applied to incision margins and tips of ear bars; the animal was positioned in a stereotaxic head holder. The scalp was incised and retracted to expose the right hemisphere of the skull.

A 3mm diameter craniotomy was performed 1mm right of the midline, and 1mm posterior to the bregma [70] [71], over top of the right parietal cortex. Once the skull was removed, exposed brain was rinsed with warm (40°C) saline, and covered with a piece of gauze soaked in saline. The dura was left intact. In one case, a burr hole was drilled 2mm posterior to the imaging field, just posterior to the bregma, and covered in saline soaked gauze. The brain was left to stabilize for 30 minutes (dripping on fresh warm saline at regular intervals). A 10cc syringe was filled with petroleum jelly and used to build a wall around the exposed brain. This was then filled with warm (40°C) agarose (3.5% in sterile saline), and covered with a glass cover slip.

The entire procedure was conducted under continuous anaesthesia and was terminal. Animals were euthanized (0.2mL/kg of T61 delivered intracardiac) without regaining
consciousness after several hours (expected maximum duration 10 hours) of biophysical measurement, which will be described in the following sections.

![Figure 5-7: (Right) Green light (530nm) image of the exposed rat cortex showing surface vasculature, taken from rat 3. White square demarks ROI location for noise analysis. (Left) Same type of image taken from rat 4.](image)

**Imaging methods**

The imaging setup discussed in section 5.1.1 was used. Of note, the camera tilt is important *in vivo*, as the exposed cortex on the right hemisphere is normally slightly sloped. A consistent focus on or a few hundred micrometers below the surface dictates the need for a tilted camera when the rat is in a stereotactic frame.

The camera was focused on the surface of the brain, and a few green light (530nm) images were taken in order to identify highly vascularized areas in the data analysis (see Figure 5-7). The camera was then defocused between 300-600μm below the cortical surface, to maximize the signal from upper cortical layers and minimize the signal from surface vasculature. Any time the field of view was altered by moving the rat or the camera, another green light image was taken so that vasculature geometries could be identified during post processing.
The switch was turned on and set to alternate between two light sources. The following series of data were taken: LED versus single mode VCSEL, LED versus multimode VCSEL, LED versus multimode VCSEL with diffuser. In each case, the exposure was set such that the laser source grey levels were peaked around 3500, and the LED current was adjusted so that it showed similar brightness to the laser.

5.2.2 Results

For in vivo noise analysis, regions of interest (ROIs) were selected from areas of the cortex which were minimally vascularized, as seen in the green LED images. The red light images were then manually aligned with the green LED images, in order to determine coordinates of the ROIs on the red light series. This was done to ensure that any slight variations in camera positioning, or rescaling of the images between QImaging Pro, where the green light images were captures, and the C++ API, where the red light images were captured, were eliminated.

Temporal noise was calculated by methods described in section 5.1.2, using 1000 frames for each light source with no temporal binning. Results are shown graphically in Figure 5-8 (tabulated results for each rat are given in Appendix B). Note that the image series in rat 3 using the multimode laser with two diffusers showed some saturation in the exposed cortical regions, and so no data points were available.
In vivo noise analysis of two rats, over 1000 images

**Figure 5-8: In vivo temporal noise analysis of four different sources used in two rats.**

The spatial noise metric of contrast was not calculated on the *in vivo* images due to the high degree of physiological movement, particularly due to blood flow. This particular noise metric would not provide useful information about light source noise, but in future could be used to extract information about blood flow. This type of analysis is beyond the scope of this report, but will be discussed further in section Chapter 8: Future work and applications.

### 5.2.3 Discussion

These results do not show a strong trend between all types of light sources. Consistently, the LED has a lower temporal noise than the single mode or multimode bare lasers. However, the effect of the diffusers is not clear from these results. This may be because biological noise is the dominant factor in pixel variation. When examining sequential images of the brain, it is evident that the heart rate is causing a shifting of the brain in each frame. This shifting can be filtered...
digitally. For an example of this type of algorithm, which was developed for imaging of the rat spinal cord directly over top of the lungs, see [72].

In addition, the temporal noise results between the two animals differ significantly for the single mode laser. When examining the series of images visually, it is evident that rat 3’s brain was moving much more from frame to frame due to breathing and heart rate. This could be due to a lower effectiveness of the anaesthetic, or a less effective placement of the stereotactic earbars. Based on the spatial results from the in vitro noise analysis (see Figure 5-5), it is expected that the single mode laser would be most affected by extra motion, due to the higher contrast speckle pattern moving from frame to frame.

A striking feature of the in vivo temporal noise analysis is that it produces in all cases lower noise values than the in vitro cases. We hypothesize that this is due to an integration of different speckle patterns caused by physiological vibration of the cortex during the camera frame rate. Again as a benchmark, we compare our LED temporal noise to published results from a leading intrinsic optical signal imaging research team. In the in vivo case, the LED exhibited a temporal noise of approximately $2.5 \times 10^{-3}$ in both rodents measured. This would correspond to a 64-trial noise of $3.1 \times 10^{-4}$, also in good agreement with the Frostig group’s findings.
Chapter 6 Models of Cortical Spreading Depression

6.1 In vitro feasibility: Hippocampal brain slice studies

In order to evaluate the preliminary feasibility of imaging stroke phenomenon in vivo using optical biosensors, studies were done in mouse hippocampal brain slices which were exposed to stroke like conditions – low oxygen and glucose. As researchers normally use transmission geometry to shine light through a brain slice and measure changes with a camera, investigations were started to determine if optical changes were still visible when using a reflection geometry by shining a light source at a brain slice and measuring changes in the reflected light with a camera (Cascade 512:B digital imaging system from Photometrics).

6.1.1 Methods

![Brain slice imaging setup](image)

*Figure 6-1: Brain slice imaging setup. In transmission geometries, the broad spectrum source is used, and the bottom of the brain slice dish is transparent. In reflection geometries, a 660nm red LED is used, and the bottom of the brain slice dish is covered with black tape.*

As discussed in section 2.2.4, mouse hippocampal brain slices were obtained and perfused in artificial cerebral spinal fluid. Once stabilized, images were recorded every four seconds for a few minutes, and oxygen and glucose deprivation (OGD) conditions were induced
by switching the perfusing solution to low glucose ACSF bubbled with nitrogen instead of oxygen. This procedure was repeated using different brain slices for both transmission and reflection geometries, as the cells in a particular brain slice cannot recover from the damage done during one session of OGD. After images had been acquired, they were post processed by simply subtracting a baseline image (the first image in the series) from the rest of the images.

6.1.2 Results

In all brain slices examined, waves of spreading depression could be seen clearly once the background subtraction has been done (see Figure 6-2, enhanced for printed clarity). In order to compare signal magnitudes, regions of interest (ROIs) were identified in the stratum radiatum layer of the hippocampus. This is a dendritic layer containing ‘en passant’ excitatory synapses, glia and interneurons. Figures showing ROI location for each brain slice, as well as change in grey level in the ROI over time can be seen in Figure 6-3.

The inversion in percent change between transmission and reflection geometries is expected. In the brain slice, as transmission increases during a spreading wave, reflection should correspondingly decrease, since they are inversely proportional to each other.

The main peaks of optical change, which occurred as a spreading wave moving through the region of interest, are seen to be on the order of 20% for transmission geometries, and 10% for reflection geometries. Prior to each sharply peaking CSD event, the optical signal is seen to vary slowly. We believe that this occurs as the cells’ energy production begins to decrease, preventing them from maintaining a resting potential. As sodium potassium pumps slow down, the change in ionic balance causes an increase in intracellular sodium, which in turn draws water into the cell, causing swelling. Swelling, in turn, would account for the slow increase in transmission and slow decrease in reflection. This can also be noted in the montage of images seen in Figure 6-3.
Figure 6-2: (Top) Montage of images from mouse hippocampal brain slice in transmission geometry. Shown: every 6 images (24 seconds), starting at $t=204s$, OGD began at $t=180s$.
(Bottom) Mouse hippocampal brain slice in reflection geometry. Shown: every 6 images (24 seconds), starting at $t=240s$. OGD began at $t=120s$. All images have had background subtracted from them to emphasize changes, and the contrast is increased fourfold.
6.1.3 Discussion

The results obtained in studying cortical spreading depression in hippocampal brain slices provide insights into designing in vivo experiments. The first conclusion obtained from these data is that the optical changes which occur during a CSD are smaller when imaged in reflectance geometry than they are when imaged using transmission geometry. However, both signal magnitudes are significant, and both camera and biosensor setups should have no technical issues in resolving the optical changes.

The second conclusion is that these waves of spreading depression do not move in straight lines, and their origin sites appear to be random. This is in part due to the method used
of inducing CSD – a global oxygen glucose deprivation of the brain slices. Hopefully, when inducing CSD in vivo using a cortical pin prick, the origin sites will be more localized to the pin prick location. However, it will still be challenging to determine which direction the waves will travel outwards from the pin prick. Directionality is a concern due to the geometry of the proposed biosensors. If an array of detectors and lasers is placed over top of the brain to image a CSD event, the field of view is in essence directly below the array. Consequently, a method is needed to induce a CSD in that location and ensure that it travels into an area being imaged by the array.

6.2  **Cortical spreading depression in vivo**

In order to study brain function, an animal subject is required that possesses a neuroanatomy similar enough to human neuroanatomy that results may be relevant to future application in humans. Of the suitable mammals that meet this criterion, the lowest order is the rodent, with the two most common laboratory animals being the rat and the mouse. The rat was chosen as the *in vivo* animal model over a mouse model because the cortex is larger in a rat, and previous researchers have shown that many neural signals of interest are 10 times larger in magnitude in a rat. Had a mouse been used as the animal model, further cleanroom work would have been necessary to reduce the dark current of the PIN photodiodes by an order of magnitude.

While the rat’s neuroanatomy has many similarities to human neuroanatomy, one fundamental difference is that cerebral blood flow in mammals is inversely related to their body weight [35]. As such, glucose and oxygen metabolism, as well as blood flow in rats are three times as high as in humans. There are also some important differences in gross cerebral vascular anatomy (the rat has more collateral blood flow pathways between large cerebral vessels than humans), and neuronal and glial densities differ [35]. We acknowledge these differences and
choose to use the rat as our model, and consider these differences when thinking about the extension of our imaging work to human brain imaging.

We chose to compare signal to noise ratio of the different light sources for an in vivo optical phenomenon. As preliminary in vitro studies indicated that cortical spreading depression causes a large (on the order of 10%, see Section 6.1) optical change in reflectance, we chose to try this model in vivo in the rat. We of course expect a different signal magnitude in vivo due to hemodynamics, and also due to the cells being more confined and having less room to swell in a whole brain preparation.

6.2.1 Methods

The surgical procedure discussed in section 5.2.1 was followed. Attempts to induce CSD were made after the series of images with no stimulus were taken to evaluate in vivo light source noise. Images were then acquired as discussed in section 5.2.1.

There are several well documented methods of inducing cortical spreading depression. In an attempt to determine the best protocol for producing reliable CSD events, different methods were attempted on different rats.

The first attempted method was cortical pin prick. Generally, researchers use micromanipulators to insert a very fine needle a few millimetres into the cortex [40]. However, the exposed cortex in our rats was covered with agarose and a glass cover slip for imaging purposes, and a manipulator could not be found that worked with our geometry. Pinprick was thus administered by hand. The switching mechanism was turned on, and image acquisition began a few seconds prior to pinprick, in order to have a baseline series of images for post processing. A 27 gauge needle (outer diameter of 0.41mm) was inserted at an angle under the glass cover slip and into the cortex, and care was taken to insert the tip only far enough so that it
could be seen entering the cortex. This method caused excessive brain bleeding in several cases, which was hard to note until the end of the imaging series due to red light illumination of the cortex. In one trial, no bleeding was noted, and a wave of reflectance change was noted just after cortical pin prick.

The second method used was to drill an additional 1mm diameter burr hole through the skull [73], 2mm anterior of the craniotomy (1mm anterior of the bregma). A syringe was filled with 1M KCl in water, and a 27 gauge needle was attached. Once imaging had begun and a few seconds of baseline frames had been taken, the needle was inserted into the burr hole and 0.1mL of 1M KCl was injected into the burr hole by hand.

### 6.2.2 Results

![Figure 6-4: (Left) LED image (525nm) of the exposed rat cortex, showing pin prick location and region of interest analyzed (Right) Image of exposed cortex taken before camera was able to tilt. Image was taken with no agarose or cover slip, leading to saturation effects from reflections off of the skull.](image)

Out of the four rat surgeries performed, only the first generated a series of images that appear to contain a cortical spreading depression event. During other surgeries and trials, there was often bleeding associated with the cortical pin prick, which ruined the images series. The trials in
which we attempted to use KCl injection were inconclusive due to large amounts of physiological motion in the brain.

As the only potential CSD event occurred during the first surgery conducted, the imaging protocols were not yet perfected. The first 25 images in the laser series taken during the potential CSD event is shadowed by my hand (which was holding the needle for the pinprick). Consequently, there is no real baseline image that can be used in the laser series. I chose to use frame 26 as the ‘baseline’ in the analysis, as at this point the pinprick had occurred, but the spreading wave was not yet visible in the region of interest in which I chose to do the analysis. For continuity, I also used image 26 from the LED series as the ‘baseline’ image.

Figure 6-5: (Left) Full time course of potential CSD event. Traces are normalized by a ‘baseline’ image taken after pinprick but before CSD, at frame 26. (Right) Shorter time course of potential CSD event.

6.2.3 Discussion

A comparison of signal magnitudes between the light sources is difficult, as the ‘baseline’ image used was not actually a baseline before pinprick. We believe that the LED trace does not return to the frame-26 baseline after the potential CSD event due to a slight shift in reflection of the
LED due to the cover glass settling after it had been disturbed during pinprick. However, it is evident that the signal magnitudes from both light sources are high: on the order of several percent, which is several orders of magnitude better than the signals we would expect from a somatosensory or visual cortex response.

I calculated the speed of the potential CSD event by determining at what time the peak of the wave moved through an ROI a distance away from the primary ROI of analysis. The speed calculated was 15.86 mm/min. The values commonly quoted in literature are 3-5 mm/min [40], indicating that this may not have been a cortical spreading depression event despite the expected magnitude in intensity change.

In order to obtain concrete results from these studies, a reliable method of inducing cortical spread depression, as well as verifying that a spreading depression has occurred, is required. The author suggests that the best method of induction is by pressure injection of potassium chloride (KCl) using a micromanipulator controlled glass electrode, into a burr hole located within 2mm of the imaging field. The electrode should be inserted after the burr hole is drilled, and the brain should be allowed to stabilize for half an hour in case the electrode insertion itself causes cortical spreading depression. This method ensures that the imaging field of view is not shadowed or altered by objects moving within it, and also ensures that the imaged surface of the brain is not damaged. As well, any bleeding which may occur when the electrode is inserted, will not alter the image sequences. In order to verify that cortical spreading depression has occurred, a recording electrode should be inserted at the edge of the field of view, under the cover glass slip, prior to the half hour brain stabilization. Then, a DC shift recorded by that electrode would confirm that a CSD event has occurred.

The incorporation of a light source identification method into image acquisition would also greatly improve results. Currently, once the images have been sorted using a thresholding
method, the two series are examined by eye, and the series which appears to exhibit changing speckle patterns is labelled the ‘laser’ series. However, in cases where the switch is alternating between two lasers (for example, one in multimode and one in single mode operation), it is necessary to have a reliable method of identifying which light source produced which series of images. This could be solved by incorporating a tiny angled mirror into the field of view, tilted such that light from one source is reflected directly into the camera (causing a saturated spot) and light from the second source is shone away from the camera (causing a dark spot), similar to the tin foil labelling approach discussed in section 5.1.1. If the addition of a mirror is not possible due to experimental setup considerations, the researcher should take single baseline images before turning the switch on and label them as source 1 or source 2, so that series can later be compared and identified. It is noteworthy that this problem would be greatly simplified if the switch could be improved so that it never misses a trigger.
Chapter 7  Conclusions and Recommendations

In this section, I will give a brief overview of the accomplishments, results, and remaining questions from each chapter of this dissertation, and I will summarize my recommendations for an implantable optical biosensor.

I first explored the use of the optical modeling program ASAP in predicting biosensor function. I created a model of a highly divergent Gaussian source, which was converted to an incoherent source after the lens collimated the beam to make it nearly paraxial. Each component of the model was verified independently, and the total model was shown to agree with one hybrid biosensor experiment using a fluorescent dye well. Other sensor/laser combinations exhibited a different trend with distance indicating that the flux on the detector is highly sensitive to biosensor architecture – this will not be a factor in the modeling once an integrated sensor is produced. The model remains to be verified with a scattering tissue sample, which can be done by modeling the geometry of a subcutaneous tumour and comparing model results to those obtained experimentally at Stanford.

I next developed two noise metrics for the light sources, and evaluated them in vitro using a silicone phantom and in vivo in the exposed rat cortex. The first metric is a temporal noise, which evaluates how much the mean value of a ROI changes over time. This is important in optical imaging setups, because the stability of an illumination over time dictates the achievable signal to noise resolution of the system. For example, if the fluctuations of the light source itself are too high, they will obscure any small signals in the images. The single mode laser was found to have the highest temporal noise, and the LED the lowest. In particular, the LED temporal noise in a 20x20 pixel region of interest was calculated to be 4.1x10^{-3}. This is comparable to within a factor of two with the results published by the Frostig group at UC.
Irvine, who claim to use an LED which is stable to $2.5 \times 10^{-4}$ over time when 64 trials are averaged [23] (statistically, the noise should decrease by a factor of $\frac{1}{\sqrt{N}}$, which would give us a theoretical LED noise of $5 \times 10^{-4}$ for 64 trials). In the \textit{in vivo} case, I found that the temporal noise was less than in the \textit{in vitro} analysis. We believe this occurs due to an integration of light source fluctuations over the regular movement of the exposed cortex during the exposure time of the camera. In the \textit{in vivo} case, the LED exhibited a temporal noise of approximately $2.5 \times 10^{-3}$ in both rodents measured. This would correspond to a 64-trial noise of $3.1 \times 10^{-4}$, also in good agreement with the Frostig group’s findings.

The second noise metric is the spatial contrast of a region of interest. This metric was evaluated only in the stationary \textit{in vitro} case, as it is expected to fluctuate constantly due to physiological motion (in particular blood flow) \textit{in vivo}. The contrast is a measure of how much speckle the light source and surface produce, and as such, is a direct reflection of the coherence of the light source being used. Theoretically, a fully developed speckle pattern – the pattern produced by a single frequency coherent source reflecting off of a surface with a Gaussian distribution of surface roughness – produces a contrast of one. I found that the contrast of the single mode laser on the silicone phantom was 0.092 – over four times larger than the contrast of the multimode laser. It makes sense that the contrast is around a tenth of a fully developed speckle pattern, as the f-number (relative aperture) of the camera was set to two, allowing it to collect and average light from several different angles.

In both temporal and spatial metrics, temporal averaging of ten frames did not significantly improve the noise. This indicates that large fluctuations do not occur on a 10-frame, or 2.5s time scale. The mean values of ROIs were seen to fluctuate slowly over time for the VCSEL light sources, indicating a possible thermal drift. This makes sense for the laser, as it is uncooled and does not have any kind of heat sink. In the future, I would recommend
incorporating some active cooling or passive heat sinking in order to provide greater source stability. The LED, on the other hand, varied much less over time, and correspondingly, is attached to a large piece of metal acting as a heat sink.

The higher noise levels in the coherent VCSEL versus the incoherent LED are hypothesized to be due to speckle. This theory is supported by the decrease in noise seen when moving from a single mode operation to multi mode operation, as well as slight decreases seen when passing the light through a diffusing element. The former causes a reduction in coherence due to mixing of adjacent modes which are out of phase and the latter causes a reduction in coherence due to slight differences in photon path length and direction. In addition, when viewing the image sequences, speckle patterns can be seen to move from frame to frame in all coherent source series, while the illumination remains relatively uniform in LED series. In order to further reduce laser source noise, the coherence of the VCSEL must be reduced even more, to a level comparable with the LED.

Finally, I investigated cortical spreading depression (CSD) in an in vitro mouse brain slice model. I found that reflection geometries produced slightly smaller changes in intensity during a CSD wave than transmission geometries, but that the reflection changes were still large (on the order of 10%) and easily discernable with a camera and basic image processing algorithms. The in vitro slice model showed that waves of CSD start at unpredictable foci, and move in unpredictable directions. This led us to examine CSD in vivo, in order to determine if a more focal induction would cause a more controlled wave. The location and direction of travel of a CSD event are important to us because if we wish to image a CSD event using implantable biosensors, we must be able to implant the biosensor over top of the region of the brain through which a CSD will travel. The in vivo results are inconclusive, and indicate that a reliable method
of inducing CSD as well as verifying that a CSD event has occurred are required before quantifiable data can be obtained.

As a final recommendation, I propose that the best biosensor design for the time being is to use an integrated VCSEL and photodetector with an LED glued adjacently on the package. With this device design, the high coherence of a single mode VCSEL operation can be exploited to measure blood velocities, while the low coherence, low noise of the LED can be used to obtain images with a high signal to noise ratio. Ongoing work should be focused on improving the multimode VCSEL so that it can exhibit noise as low as an LED, and also in miniaturizing any solution developed to reduce the laser coherence. A more detailed description of future work generated by this project, as well as potential applications of implantable optical biosensors, will be discussed in the final chapter of this dissertation.
Chapter 8  Future Work and Applications

Collaborators at Sunnybrook hospital in Toronto are working to create a 125\textmu m-resolution geometry of a rat brain using a 7T MR scanner. We have discussed the interface issues challenges present in ASAP, and they are working to segment the brain in such a way that slices of it can be taken which are layered in structure. This would allow future students to use a draping method to create full resolution surfaces which are not touching. In addition, alternate Monte Carlo ray tracing programs such as Light Tools by Optical Research Associates, are being explored. Optical properties of different sections of the brain (notably the skull and cortex) have been measured in collaboration with researchers at Princess Margaret Hospital, and will be used in the optical brain model. Once this model is fully functional, theoretical light distributions will be predicted for neural models by altering the properties of blood vessels to reflect increased or decreased oxygenation, and these models will be used to optimize biosensor architecture.

While optical modeling is progressing, future students should works towards developing a reliable method of inducing cortical spreading depression and verifying that it has occurred, as discussed in section 6.2.3. In conjunction, image processing algorithms should be developed to remove brain motion due to breathing and heart rate. Once surgical, imaging, and signal processing methods have been optimized for CSD models, more difficult imaging preparations can be studies, such as visual, somatosensory, or motor cortex.

A mask set for an array of neural biosensors is currently being developed. Once biosensor fabrication is complete, they should be used in conjunction with camera studies in order to compare gold standard images to biosensor images directly. This can be done by modifying the current imaging setup with a beam splitter that directs some of the light from the
sample to a biosensor detector, so that it can be recorded and read out using the biosensor technology.

The applications of this technology are widespread, but key points will be addressed in this section. An important consideration is that for each potential application, studies must first be conducted in order to optimize biosensor design for that particular biological signal of interest. As fabrication of these sensors requires many hours of expensive clean room time, it is imperative that the final design be optimized prior to constructing the sensor.

The first application of interest is the use of these sensors in brain machine interfaces (BMIs). These technologies translate neural activity in the motor cortex of the brain to results on a computer. The current method of obtaining neural signals is through the use of implantable electrode arrays. Our collaborator at Stanford University, Dr. Krishna Shenoy, believes that optical sensor technology could be used in conjunction with and maybe eventually replace existing electrode arrays. Currently, his research group is conducting studies in which an electrode array is implanted into a monkey’s motor cortex, and transmits signals to a computer wirelessly [74]. The spike patterns are recorded and analyzed on the fly.

Another direction that could be taken with this technology is to combine it with the current Optogenetics work being done by researchers like Karl Deisseroth at Stanford University. His group has developed a mouse model in which populations of neurons can be genetically altered by incorporating light sensitive opsin proteins: channelrhodopsin-2 to activate neurons, and halorhodopsins to inhibit neurons [75]. One can imagine using an integrated biosensor to activate the opsins while simultaneously imaging.

A final application of interest lies in optical imaging of the brain prior to and during epileptic attacks. Recently, researchers have shown that optical changes occur in the brain minutes to seconds before the cascading, detrimental electrical activity of an epileptic seizure
If optical biosensors could be implanted above an epileptic center in the brain, they could be used for early detection of epileptic attacks.

It is important to note that the above applications are not comprehensive: optical, implantable biosensors promise to be widely applicable to many different disease models, and are not limited to neural imaging applications.
References


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Appendix A  ASAP Codes

A.1  Fluorescent biosensor model with dye well

**************************************************************************DYE_WELL.INR**************************************************************************

!! MODEL OF A FLUORESCENT BIOSENSOR INCLUDING
!! DETECTOR MESA, CAP WITH AN APERTURE DRILLED IN IT,
!! ASPHERIC LENS, AND POLYSTYRENE DYE WELL SAMPLE.

!! CALLS A PREVIOUSLY MADE GAUSSIAN DISTRIBUTION CALLED
!! 'TEST_35HR.DIS' AS AN INCOHERENT SOURCE
!! CONSTRUCTED USING MAKE_GAUSSIAN.INR

!! SPONTANEOUS EMISSION CAN BE MODELLED BY UNCOMMENTING THE
!! SOURCES EMITTING FROM THE VCSEL MESA/SIDEWALLS
!! BUT THIS MUST BE NORMALIZED

!! WRITTEN BY ELIZABETH MUNRO, 2009

$DO 1 100       !! LOOPS THROUGH 100 TIMES TO GENERATE STATISTICS
{                   
**************************************************************************SYSTEM UNITS**************************************************************************
    SYSTEM NEW
    RESET

    UNITS MM
    WAVELENGTH 675.50 NM
    PIXELS 501
    HALT 500000

    VALUE=20000001+(2*?)
    SEED (VALUE)

    !!!!DEFINE RAY SPLIT AND SCATTER CHARACTERISTICS
    SPLIT 1500 MONTECARLO
    LEVEL 1000
    FRESNEL AVE

    !!CREATE VOLUME SCATTER MODELS
    !! FOLLOWS FORM VOLUME G MU_S’MU_A OBSCURATION RATIO
MODEL
  VOLUME 0.9 1E-9’0.025 1

!! DEFINE SYSTEM MEDIA
MEDIA
  !1.4 SCATTER 1 'DYE'
  1.56 'GLASS'  !! INDEX OF REFRACTION OF POLYSTYRENE
  1.59908 'EC0550'
  3.7 'DET_MEDIA'  !! GAAS INDEX AT 670NM
  1.41.34285E-6 'DYE'  !! CY 5.5 ABSORPTION AND INDEX IN SUBCUTANEOUS
  SPOT FOR 1UM CONCENTRATION

COATINGS PROPERTIES
  0.009913  0.990087 'AR'  !! COATING AT 675 NM
  0 1 'TRANSMIT'
  0 0 'ABSORB'
  1 0 'REFLECT'
  1 0 'CAP'

!!GEOMETRY MACRO
!!MAKE_GEOMETRY {

!!!!!!!!!!SYSTEM VARIABLES!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!

!! ASPHERIC LENS COEFFICIENTS
ASP1_R=19.92
ASP1_K=0
ASP1_A=-4.23710E-3
ASP1_B=6.34840E-4
ASP1_C=-3.46536E-5
ASP1_D=0
ASP1_E=0

ASP2_R=-2.94
ASP2_K=-0.481104
ASP2_A=2.89094E-5
ASP2_B=3.76282E-5
ASP2_C=-3.26442E-6
ASP2_D=1.17572E-6
ASP2_E=0

LENS_POS1=0
LENS_POS2=2.94
FLAT_POS2=1.57

D=2.25  !!SEMIWIDTH OF ASP1 IN MICRONS
E=2.655  !!SEMIWIDTH OF ASP2 IN MICRONS
LENS_FLAT_RAD=3.165  !!WIDTH OF FLAT PART IN MICRONS
CT=2.94  !! CENTER THICKNESS OF LENS
CAPR=4.1  !! CAP RADIUS
CAPH=3.431
APER=1.40 !! RADIUS OF THE APERTURE IN THE CAP
DIST=-2.93
WAIST=0.0000025
TOPR=0.02  !! RADIUS OF THE VCSEL MESA
TESTD=2.945

!! DYE WELL VARIABLES
INNERR=6.8072/2  !! INNER RADIUS OF THE DYE WELL
OUTERR=8.255/2    !! OUTER RADIUS OF THE DYE WELL
WIDTH=1.778      !! THICKNESS OF BOTTOM GLASS
DYEH=4          !! HEIGHT OF THE DYE IN THE WELL
WELLZ=2.94+4.01 !! Z LOCATION OF BEGINNING OF THE WELL
                 !!CHANGE THIS IN THE BOTTOM HALF OF THE CODE TOO!!!!

WELLH=10        !! TOTAL HEIGHT OF THE WELL
QMEFF=0.28      !! QUANTUM EFFICIENCY OF CY5.5 DYE

RAYNUM=500000  !! NUMBER OF RAYS IN THE DYE EMISSION
SP1=5000001    !! NUMBER OF RAYS IN SPONTANEOUS EMISSION FROM ACTIVE REGION
SP2=50000000   !! NUMBER OF RAYS IN SPONT EMISSION FROM VCSEL MESA BEFORE APODIZATION

**************************ASPHERIC LENS*******************************

!! TUBE
SURFACE
  TUBE Z (LENS_POS1+0.08)-0.001 (D) (D) (LENS_POS1+0.08)+0.001 (D) (D)
  OBJECT 'TUBE1'
  INTERFACE COATING AR EC0550 AIR
  REDEFINE COLOR 18

SURFACE
  PLANE Z (LENS_POS1+0.08) ELLIPSE (LENS_FLAT_RAD) (LENS_FLAT_RAD)
  OBJECT 'LENS_FLAT_S1'
  BOUNDS +.2
  INTERFACE COATING AR EC0550 AIR
  REDEFINE COLOR 18

SURFACE
  TUBE Z (FLAT_POS2)-0.001 (E) (E) (FLAT_POS2)+0.001 (E) (E)
  OBJECT 'TUBE2'
  INTERFACE COATING AR EC0550 AIR
  REDEFINE COLOR 18

SURFACE
  PLANE Z (FLAT_POS2) ELLIPSE (LENS_FLAT_RAD) (LENS_FLAT_RAD)
  OBJECT 'LENS_FLAT_S2'
BOUNDS +.2
INTERFACE COATING AR EC0550 AIR
REDEFINE COLOR 18

SURFACE
  TUBE Z (LENS_POS1)-1 (LENS_FLAT_RAD) (LENS_FLAT_RAD) (FLAT_POS2)+1
  (LENS_FLAT_RAD) (LENS_FLAT_RAD)
  OBJECT 'TUBE3'
  BOUNDS +.4 -.2
  INTERFACE COATING AR EC0550 AIR
  REDEFINE COLOR 18

!! ASPHERIC LENSES
SURFACE
  OPTICAL Z (LENS_POS1) (ASP1_R) (ASP1_K) (ASP1_A) (ASP1_B) (ASP1_C)
  (ASP1_D) (ASP1_E) ELLIPSE (D) (D)
  OBJECT 'ASP1'
  BOUNDS -.5
  INTERFACE COATING AR EC0550 AIR
  REDEFINE COLOR 18
SURFACE
  OPTICAL Z (LENS_POS2) (ASP2_R) (ASP2_K) (ASP2_A) (ASP2_B) (ASP2_C)
  (ASP2_D) (ASP2_E) ELLIPSE (E) (E)
  OBJECT 'ASP2'
  BOUNDS +.5
  INTERFACE COATING AR EC0550 AIR
  REDEFINE COLOR 18

*********************DETECTOR MESA GEOMETRY*********************
$case upper
TITLE =Import of GTX file C:\Documents and Settings\Elizabeth\My
Documents\Masters\ASAP\Model_TDO\detector_mesa_newdims.GTX
$ECHO NONE
!!
CURVES
  POINTS -.1100000E-3  0.000000    0.2500000E-3 REL; $FAST 12 5
  0.0001100  0. -.0002500  1. 0.0000367  0. -.0002500  1. -.0000367  0. -.0002500  1.
  -.0001100  0. -.0002500  1. 0.0001100  0. -.0000833  1. 0.0000367  0. -.0000833  1.
  -.0000367  0. -.0000833  1. -.0001100  0. -.0000833  1. 0.0001100  0. 0.0000833  1.
  0.0000367  0. 0.0000833  1. -.0000367  0. 0.0000833  1. -.0001100  0. 0.0000833  1.
  0.0001100  0. 0.0002500  1. 0.0000367  0. 0.0002500  1. -.0000367  0. 0.0002500  1.
  -.0001100  0. 0.0002500  1.
  PATCH 3 3
  OBJECT =DETECTOR.Extrude1.Face0

INTERFACE COATING "REFLECT" "AIR" "DETECTOR"
REDEFINE COLOR 2
SHIFT Z -0.00293
CURVES
POINTS 0.000000 0.4000000E-3 0.2500000E-3 REL; $FAST 12 5
0.0004000 -0.0002500 1. 0.0001333 -0.0002500 1. 0. -0.0001333 -0.0002500 1.
0. -0.0004000 -0.0002500 1. 0.00004000 -0.0000833 1. 0. 0.0001333 -0.0000833 1.
0. -0.0001333 -0.0000833 1. 0. -0.0004000 -0.0000833 1. 0.00004000 0.0000833 1.
0. 0.0001333 0.0000833 1. 0. -0.0001333 0.0000833 1. 0. -0.0004000 0.0000833 1.
0. 0.0004000 0.0000833 1. 0.0001333 0.0000833 1. 0.0001333 -0.0002500 1.
0. -0.0004000 0.0002500 1.
PATCH 3 3
OBJECT = DETECTOR.Extrude1.Face1

INTERFACE COATING "REFLECT" "AIR" "DETECTOR"
REDEFINE COLOR 2
SHIFT Z -0.00293

CURVES
POINTS -0.4600000E-3 0.8000000E-3 0.2500000E-3 REL; $FAST 12 5
-0.0004600 0.00002500 1. -0.0004600 0.00000833 1. -0.0004600 0. -0.0000833 1.
0.0004600 0. -0.0002500 1. -0.0001533 0.0000833 1. -0.0001533 0. -0.0000833 1.
-0.0001533 0. -0.0000833 1. -0.0001533 0.00002500 1. 0.0001533 0.0000833 1.
0.0001533 0.0000833 1. 0.0001533 0. -0.0000833 1. 0.0001533 0. -0.0002500 1.
0.0004600 0.00002500 1. 0.0004600 0.00000833 1. 0.0004600 0. -0.0000833 1.
0.0004600 -0.0002500 1.
PATCH 3 3
OBJECT = DETECTOR.Extrude1.Face2

INTERFACE COATING "REFLECT" "AIR" "DETECTOR"
REDEFINE COLOR 2
SHIFT Z -0.00293

CURVES
POINTS -0.9200000E-3 0.4000000E-3 0.2500000E-3 REL; $FAST 12 5
0. -0.0040000 -0.0002500 1. 0. -0.001333 -0.0002500 1. 0.0001333 -0.0002500 1.
0.0004000 -0.0002500 1. 0. -0.0004000 -0.0000833 1. 0. -0.0001333 -0.0000833 1.
0.0001333 -0.0000833 1. 0.00004000 -0.0000833 1. 0. -0.0004000 0.0000833 1.
0. -0.0001333 0.0000833 1. 0.00001333 0.0000833 1. 0.00004000 0.0000833 1.
0. -0.0004000 0.0002500 1. 0. -0.0001333 0.0002500 1. 0.0001333 0.0002500 1.
0.0004000 0.0002500 1.
PATCH 3 3
OBJECT = DETECTOR.Extrude1.Face3

INTERFACE COATING "REFLECT" "AIR" "DETECTOR"
REDEFINE COLOR 2
SHIFT Z -0.00293

CURVES
POINTS -.8100000E-3 0.000000 0.2500000E-3 REL; $FAST 12 5
-.0001100 0. -.0002500 1. -.0001100 0. -.0000833 1. -.0001100 0. 0.0000833 1.
-.0001100 0. 0.0002500 1. -.0000367 0. -.0000833 1. -.0000367 0. 0.0002500 1.
-.0000367 0. 0.0000833 1. -.0000367 0. 0.0002500 1. 0.0000367 0. -.0002500 1.
0.0001100 0. -.0002500 1. 0.0001100 0. -.0000833 1. 0.0001100 0. 0.0000833 1.
0.0001100 0. 0.0002500 1.
PATCH 3 3
OBJECT = DETECTOR.Extrude1.Face4

INTERFACE COATING "REFLECT" "AIR" "DETECTOR"
REDEFINE COLOR 2
SHIFT Z -0.00293

CURVES
POINTS -.7000000E-3 0.7000000E-4 0.2500000E-3 REL; $FAST 12 5
0. -.0000700 -.0002500 1. 0. -.0000700 -.0000833 1. 0. -.0000700 0.0000833 1.
0. -.0000700 0.0002500 1. 0. -.0000233 -.0002500 1. 0. 0.0000233 -.0000833 1.
0. 0.0000233 .0000833 1. 0. 0.0000233 0.0002500 1. 0. 0.0000233 0.0002500 1.
0. 0.0000700 -.0002500 1. 0. 0.0000700 -.0000833 1. 0. 0.0000700 0.0000833 1.
0. 0.0000700 0.0002500 1.
PATCH 3 3
OBJECT = DETECTOR.Extrude1.Face5

INTERFACE COATING "REFLECT" "AIR" "DETECTOR"
REDEFINE COLOR 2
SHIFT Z -0.00293

CURVES
POINTS -.4600000E-3 0.3800000E-3 0.2500000E-3 REL; $FAST 12 5
-.0002400 -.0002400 0.0003000 1.0000000 -.0002400 0.0002400 0.0003000 0.3333333
0.0002400 0.0002400 0.0003000 0.3333333
0.0002400 -.0002400 0.0003000 1.0000000 -.0002400 -0.0002400 0.0001000 1.0000000 -
.0002400 0.0002400 0.0001000 0.3333333
0.0002400 0.0002400 0.0001000 0.3333333 0.0002400 -.0002400 0.0001000 1.0000000 -
.0002400 -.0002400 -.0001000 1.0000000
-.0002400 0.0002400 -0.0001000 0.3333333 0.0002400 0.0002400 -.0001000 0.3333333
0.0002400 -.0002400 -.0003000 1.0000000 -.0002400 0.0002400 -.0003000 0.3333333
0.0002400 0.0002400 -.0003000 0.3333333

98
0.0002400 -.0002400 -.0003000 1.0000000
PATCH 3 3
OBJECT =DETECTOR.Extrude1.Face6
CURVES
LINE  1.000000     1.916667     0.000000     1.000000     1.083333     0.000000
LINE  1.000000     1.083333     0.000000     2.000000     1.083333     0.000000
LINE  2.000000     1.083333     0.000000     2.000000     1.916667     0.000000
LINE  2.000000     1.916667     0.000000     1.000000     1.916667     0.000000
COMPOSITE GAP -1 -1
UVSPACE .1
OBJECT .1; BOUND -.1

INTERFACE COATING "REFLECT" "AIR" "DETECTOR"
REDEFINE COLOR 2
SHIFT Z -0.00293

CURVES
POINTS -.2200000E-3 0.7000000E-4 0.2500000E-3 REL; $FAST 12 5
0. 0.0000700 0.0002500 1. 0. -0.0000700 0.0000833 1. 0. -0.0000700 -0.000833 1.
0. -0.0000700 -0.0002500 1. 0. -0.0000233 0.0002500 1. 0. -0.0000233 0.000833 1.
0. -0.0000233 -0.000833 1. 0. -0.0000233 -0.0002500 1. 0. 0.0000233 0.0002500 1.
0. 0.0000233 0.0000833 1. 0. 0.0000233 -0.000833 1. 0. 0.0000233 -0.0002500 1.
0. 0.0000700 0.0002500 1. 0. 0.0000700 0.0000833 1. 0. 0.0000700 -0.000833 1.
0. 0.0000700 -0.0002500 1.
PATCH 3 3
OBJECT =DETECTOR.Extrude1.Face7

INTERFACE COATING "REFLECT" "AIR" "DETECTOR"
REDEFINE COLOR 2
SHIFT Z -0.00293

CURVES
POINTS -.4600000E-3 0.4000000E-3 0.5000000E-3 REL; $FAST 12 5
-0.0004600 0.004000 0. 1. -0.001533 0.004000 0. 1. 0.0001533 0.004000 0. 1.
0.0004600 -0.004000 0. 1. -0.004600 0.001333 0. 1. -0.001533 0.001333 0. 1.
0.0001533 0.001333 0. 1. 0.004600 0.001333 0. 1. -0.0004600 0.0001333 0. 1.
-.0001533 0.0001333 0. 1. 0.0001533 0.0001333 0. 1. 0.0004600 0.0001333 0. 1.
0.0004600 0.0004000 0. 1. -0.0001533 0.0004000 0. 1. 0.0001533 0.0004000 0. 1.
0.0004600 0.0004000 0. 1.
PATCH 3 3
OBJECT =MESA
CURVES
LINE  1.760870     1.000000     0.000000     2.000000     1.000000     0.000000
LINE  2.000000     1.000000     0.000000     2.000000     2.000000     0.000000
LINE  2.000000     2.000000     0.000000     1.000000     2.000000     0.000000
LINE  1.000000  2.000000  0.000000  1.000000  1.000000  0.000000
LINE  1.000000  1.000000  0.000000  1.239130  1.000000  0.000000
LINE  1.239130  1.000000  0.000000  1.239130  1.175000  0.000000
POINTS  1.500000  1.325000  0.000000  REL; $FAST 12 5
-.2608696 -.1500000  0.  2. -.2608696 -.0903263  0.  1. -.2410121 -.0351950  0.  2.
-.2211545 0.0199363  0.  1. -.1844626 0.0621320  0.  2. -.1477707 0.1043277  0.  1.
-.0998305 0.1271639  0.  2. -.0518902 0.1500000  0.  1. 0.0000000 0.1500000  0.  2.
 0.0518902 0.1500000  0.  1. 0.0998305 0.1271639  0.  2. 0.1477707 0.1043277  0.  1.
 0.1844626 0.0621320  0.  2. 0.2211545 0.0199363  0.  1. 0.2410121 -.0351950  0.  2.
 0.2608696 -.0903263  0.  1. 0.2608696 -.1500000  0.  0.
LINE  1.760870  1.175000  0.000000  1.760870  1.000000  0.000000
COMPOSITE GAP -1 -1
UVSPACE .1
OBJECT .1; BOUND -1

INTERFACE COATING "ABSORB" "AIR" "DETECTOR"
REDEFINE COLOR 2
SHIFT Z -0.00293

CURVES
POINTS -.4600000E-3 0.4000000E-3  0.000000  REL; $FAST 12 5
-.0004600 -.0004000  0.  1. -.0001533 -.0004000  0.  1. 0.0000000 0.0000000  0.  1.
 0.0004600 -.0004000  0.  1. -.0000133 -.0004000  0.  1. -0.0001533 -.0000133  0.  1.
 0.0001533 -.0001333  0.  1. 0.0000460 -.0001333  0.  1. -.0000460 0.0001333  0.  1.
 0.0001533 0.0001333  0.  1. 0.0000460 0.0001333  0.  1. 0.0000460 0.0000400  0.  1.
 0.0000460 0.0004000  0.  1. 0.0001533 0.0000400  0.  1. 0.0001533 0.0004000  0.  1.
 0.0004600 0.0000400  0.  1.
PATCH 3 3
OBJECT =DETECTOR.Extrude1.Face9
CURVES
LINE  2.000000  1.000000  0.000000  2.000000  2.000000  0.000000
LINE  2.000000  2.000000  0.000000  1.000000  2.000000  0.000000
LINE  1.000000  2.000000  0.000000  1.000000  1.000000  0.000000
LINE  1.000000  1.000000  0.000000  1.239130  1.000000  0.000000
LINE  1.239130  1.000000  0.000000  1.239130  1.175000  0.000000
POINTS  1.500000  1.325000  0.000000  REL; $FAST 12 5
-.2608696 -.1500000  0.  2. -.2608696 -.0903263  0.  1. -.2410121 -.0351950  0.  2.
-.2211545 0.0199363  0.  1. -.1844626 0.0621320  0.  2. -.1477707 0.1043277  0.  1.
-.0998305 0.1271639  0.  2. -.0518902 0.1500000  0.  1. 0.0000000 0.1500000  0.  2.
 0.0518902 0.1500000  0.  1. 0.0998305 0.1271639  0.  2. 0.1477707 0.1043277  0.  1.
 0.1844626 0.0621320  0.  2. 0.2211545 0.0199363  0.  1. 0.2410121 -.0351950  0.  2.
 0.2608696 -.0903263  0.  1. 0.2608696 -.1500000  0.  0.
LINE  1.760870  1.175000  0.000000  1.760870  1.000000  0.000000
LINE  1.760870  1.000000  0.000000  2.000000  1.000000  0.000000
COMPOSITE GAP -1 -1
UVSPACE .1
OBJECT .1; BOUND -1
INTERFACE COATING "REFLECT" "AIR" "DETECTOR"
REDEFINE COLOR 2
SHIFT Z -0.00293

$ECHO
GROUP -10
SHIFT 0 -0.00092 -0.0005
SCALE FROM M
RETURN
$CASE

*******************************************************************
**OTHER GEOMETRY**
*******************************************************************

!!CAP - ROUGH, REFLECTING SURFACE
SURFACE
  PLANE Z 0 ELLIPSE (CAPR) (CAPR) (APER)/(CAPR)
OBJECT 'CAP.TOP'
ROUGHNESS !!MODEL 1
INTERFACE COATING "CAP" "AIR" "DET_MEDIA"
REDEFINE COLOR 17

!!BOTTOM 10 PIN HOLDER
SURFACE
  PLANE Z -(CAPH) ELLIPSE (CAPR) (CAPR)
OBJECT 'CAP.BOTTOM'
ROUGHNESS !!MODEL 1
INTERFACE COATING "REFLECT" "AIR" "DET_MEDIA"
REDEFINE COLOR 17

!! CAP CYLINDER
SURFACE
  TUBE Z 0 (CAPR) (CAPR) -(CAPH) (CAPR) (CAPR)
OBJECT 'CAP.EDGE'
ROUGHNESS !!MODEL 1
INTERFACE COATING "CAP" "AIR" "DET_MEDIA"
REDEFINE COLOR 17
BOUNDS -.3 +.2

!! VCSEL TOP
SURFACE
  PLANE Z (DIST) ELLIPSE (TOPR) (TOPR)
OBJECT 'TOP'
INTERFACE COATING "REFLECT" "AIR" "DET_MEDIA"
REDEFINE COLOR 15

!! VCSEL SIDE
SURFACE
TUBE Z (DIST)-0.0035 (TOPR) (TOPR) (DIST)-0.0035-0.00005 (TOPR) (TOPR)
OBJECT 'VCSEL_SIDE1'
INTERFACE COATING "REFLECT" "AIR" "DET_MEDIA"
REDEFINE COLOR 6

*************************DYE WELL GEOMETRY*************************

!! OUTTER CYLINDER
SURFACE
PLANE Z (WELLZ) ELLIPSE (OUTERR) (OUTERR)
OBJECT 'OUTER.Z1'
INTERFACE COATING "BARE" "AIR" "GLASS"
REDEFINE COLOR 14

SURFACE
TUBE Z (WELLZ) (OUTERR) (OUTERR) (WELLZ)+(WIDTH)+(WELLH) (OUTERR)
(OUTERR)
OBJECT 'OUTER.EDGE'
INTERFACE COATING "BARE" "AIR" "GLASS"
REDEFINE COLOR 14
BOUNDS +.2

!! DYE IN CYLINDER
SURFACE
PLANE Z (WELLZ)+(WIDTH) ELLIPSE (INNERR) (INNERR)
OBJECT 'DYE.Z1'
INTERFACE COATING "ABSORB" "DYE" "GLASS"
!!INTERFACE COATING "BARE" "GLASS" "DYE"
REDEFINE COLOR 13

!! AIR IN CYLINDER
SURFACE
PLANE Z (WELLZ)+(WIDTH)+(DYEH) ELLIPSE (INNERR) (INNERR)
OBJECT 'AIR.Z1'
INTERFACE COATING "BARE" "DYE" "AIR"
REDEFINE COLOR 12

SURFACE
TUBE Z (WELLZ)+(WIDTH) (INNERR) (INNERR) (WELLZ)+(WIDTH)+(DYEH)
(INNERR) (INNERR)
OBJECT 'DYE.EDGE'
INTERFACE COATING "BARE" "GLASS" "DYE"
REDEFINE COLOR 13
BOUNDS +.3 -.2

SURFACE
TUBE Z (WELLZ)+(WIDTH)+(DYEH) (INNERR) (INNERR)  
(WELLZ)+(WIDTH)+(WELLH) (INNERR) (INNERR)  
OBJECT 'AIR.EDGE'  
INTERFACE COATING "BARE" "GLASS" "AIR"  
REDEFINE COLOR 12  

**SPONT EMISSION**

**EMITTING OBJECT VCSELSIDE1 (SP1) ISO**  
**FLUX TOTAL 50**  
**USERAPOD ANGLES Z -15 15 0 360**  
**EMITTING OBJECT TOP (SP2) ISO**  
**SELECT ONLY SOURCE 2**  
**FLUX TOTAL 50**  
**SELECT ALL**  
**WINDOW X Z**  
**PLOT FACETS 15 15 OVERLAY**  
**TRACE PLOT 100000**  
**VIEW**  
**WINDOW X Y**  
**CONSIDER ALL!!ONLY DETECTOR.EXTRUDE1.FACE8**  
**SPOTS POSITION**  
**DISPLAY**  
**PICTURE**  
**RETURN**  
**STATS**  

**SOURCE**

IMMERSE AIR  
CONSIDER ALL

WINDOW X -2 2 Z -3 12  
**PLOT FACETS OVERLAY**  

BEAMS INCOHERENT GEOMETRIC

**VOXELS ABSORBED -(INNERR)/201 (INNERR)/201 -(INNERR)/201 (INNERR)/201  
(WELLZ)+(WIDTH) (WELLZ)+(WIDTH)+(DYEH)/201 2 2 1**  
**VOXELS ABSORBED -(INNERR) (INNERR) -(INNERR) (INNERR) (WELLZ)+(WIDTH)  
(WELLZ)+(WIDTH)+(DYEH) 201 201 201**
EMITTING DATA TEST_35HRS.DIS
SHIFT Z -0.5
FLUX TOTAL 100

TRACE !!PLOT 1000 COLOR 24 !! Show ray entry and direction

WINDOW X Y
CONSIDER ONLY DYE.Z1
SPOTS POSITION
DISPLAY
PICTURE
RETURN

******************************************************************************
***ABSORB IN DYE******************************************************************************
$GRAB 'VOXELS' FLUX_ABSORBED !!GRAB TOTAL FLUX ABSORBED
&REG FLUX_ABSORBED        !!SHOW AT THE CONSOLE OUTPUT
$COPY 9 VOXL.DIS          !!COPY DEFAULT BRO009.DAT TO NAMED FILE

RAYS 0          !!CLEAR PREVIOUS SOURCE DATA

******************************************************************************
**********REBUILD SYSTEM AT NEW WAVELENGTH OF EMISSION************
******************************************************************************
SYSTEM NEW
$IO VECTOR REWIND
RESET

UNITS MM
WAVELENGTH 694 NM
PIXELS 501
HALT 500000

!!!!DEFINE RAY SPLIT AND SCATTER CHARACTERISTICS
  SPLIT 1500 MONTECARLO
  LEVEL 1000
  FRESNEL AVE

!!CREATE VOLUME SCATTER MODELS
!! FOLLOWS FORM VOLUME G MU_S’MU_A OBSCURATION RATIO

!! DEFINE SYSTEM MEDIA
MEDIA
  1.56 'GLASS'      !! INDEX OF REFRACTION OF POLYSTYRENE
1.59908 'EC0550'
3.7 'DET_MEDIA' !! GAAS INDEX AT 670NM
1.4 'DYE' !! CY 5.5 NOT ABSORPTIVE AT 694NM

COATINGS PROPERTIES
0.006103 0.993897 'AR' !! COATING AT 694 NM
0 1 'TRANSMIT'
0 0 'ABSORB'
1 0 'REFLECT'
0.1 0 'CAP'

**************************SYSTEM VARIABLES**************************
!! ASPHERIC LENS COEFFICIENTS
ASP1_R=19.92
ASP1_K=0
ASP1_A=-4.23710E-3
ASP1_B=6.34840E-4
ASP1_C=-3.46536E-5
ASP1_D=0
ASP1_E=0

ASP2_R=-2.94
ASP2_K=-0.481104
ASP2_A=2.89094E-5
ASP2_B=3.76282E-5
ASP2_C=-3.26442E-6
ASP2_D=1.17572E-6
ASP2_E=0

LENS_POS1=0
LENS_POS2=2.94
FLAT_POS2=1.57

D=2.25   !! SEMIWIDTH OF ASP1 IN MICRONS
E=2.655   !! SEMIWIDTH OF ASP2 IN MICRONS
LENS_Flat_RAD=3.165   !! WIDTH OF FLAT PART IN MICRONS
CT=2.94   !! CENTER THICKNESS OF LENS
CAPR=4.1   !! CAP RADIUS
CAPH=3.431

APER=1.40   !! RADIUS OF THE APERTURE IN THE CAP
DIST=-2.93
WAIST=0.0000025
TOPR=0.02   !! RADIUS OF THE VCSEL MESA
TESTD=2.945

!! DYE WELL VARIABLES
INNERR=6.8072/2   !! INNER RADIUS OF THE DYE WELL
OUTERR=8.255/2   !! OUTER RADIUS OF THE DYE WELL
WIDTH=1.778 !! THICKNESS OF BOTTOM GLASS
DYEH=4 !! HEIGHT OF THE DYE IN THE WELL
WELLZ=2.94+4.01 !! Z LOCATION OF BEGINNING OF THE WELL
WELLH=10 !! TOTAL HEIGHT OF THE WELL
QMEFF=0.28 !! QUANTUM EFFICIENCY OF CY5.5 DYE
RAYNUM=500000 !! NUMBER OF RAYS IN THE DYE EMISSION
SP1=5000001 !! NUMBER OF RAYS IN SPONTANEOUS EMISSION FROM ACTIVE REGION
SP2=50000000 !! NUMBER OF RAYS IN SPONT EMISSION FROM VCSEL MESA BEFORE APODIZATION

ASPHERIC LENSES

!! TUBE
SURFACE
  TUBE Z (LENS_POS1+0.08)-0.001 (D) (D) (LENS_POS1+0.08)+0.001 (D) (D)
  OBJECT 'TUB1'
  INTERFACE COATING AR EC0550 AIR
  REDEFINE COLOR 18

SURFACE
  PLANE Z (LENS_POS1+0.08) ELLIPSE (LENS_FLAT_RAD) (LENS_FLAT_RAD)
  OBJECT 'LENS_FLAT_S1'
  BOUNDS +.2
  INTERFACE COATING AR EC0550 AIR
  REDEFINE COLOR 18

SURFACE
  TUBE Z (FLAT_POS2)-0.001 (E) (E) (FLAT_POS2)+0.001 (E) (E)
  OBJECT 'TUB2'
  INTERFACE COATING AR EC0550 AIR
  REDEFINE COLOR 18

SURFACE
  PLANE Z (FLAT_POS2) ELLIPSE (LENS_FLAT_RAD) (LENS_FLAT_RAD)
  OBJECT 'LENS_FLAT_S2'
  BOUNDS +.2
  INTERFACE COATING AR EC0550 AIR
  REDEFINE COLOR 18

SURFACE
  TUBE Z (LENS_POS1)-1 (LENS_FLAT_RAD) (LENS_FLAT_RAD) (FLAT_POS2)+1
  (LENS_FLAT_RAD) (LENS_FLAT_RAD)
  OBJECT 'TUB3'
  BOUNDS +.4 -.2
  INTERFACE COATING AR EC0550 AIR
  REDEFINE COLOR 18

!! ASPHERIC LENSES
SURFACE
OPTICAL Z (LENS_POS1) (ASP1_R) (ASP1_K) (ASP1_A) (ASP1_B) (ASP1_C)
(ASP1_D) (ASP1_E) ELLIPSE (D) (D)
OBJECT 'ASP1'
BOUNDS -.5
INTERFACE COATING AR EC0550 AIR
REDEFINE COLOR 18

SURFACE
OPTICAL Z (LENS_POS2) (ASP2_R) (ASP2_K) (ASP2_A) (ASP2_B) (ASP2_C)
(ASP2_D) (ASP2_E) ELLIPSE (E) (E)
OBJECT 'ASP2'
BOUNDS +.5
INTERFACE COATING AR EC0550 AIR
REDEFINE COLOR 18

++++++++++++++++++++++DETECTOR MESA GEOMETRY++++++++++++++++++++++

$case upper
TITLE =Import of GTX file C:\Documents and Settings\Elizabeth\My
Documents\Masters\ASAP\Model_TDO\detector_mesa_newdms.GTX
$ECHO NONE
!!
CURVES
POINTS -.110000E-3  0.000000  0.2500000E-3 REL; $FAST 12 5
0.0001100 0. -.0002500 1. 0.0000367 0. -.0002500 1. 0.0000367 0. -.0002500 1. 
-.0001100 0. -.0002500 1. 0.0001100 0. -.0000833 1. 0.0000367 0. -.0000833 1. 
-.0000367 0. -.0000833 1. 0.0001100 0. 0.0000833 1. 0.0000367 0. 0.0000833 1. 
0.0001100 0. 0.0002500 1. 0.0000367 0. 0.0002500 1. -.0000367 0. 0.0002500 1. 
-.0001100 0. 0.0002500 1.
PATCH 3 3
OBJECT =DETECTOR.Extrude1.Face0

INTERFACE COATING "REFLECT" "AIR" "DETECTOR"
REDEFINE COLOR 2
SHIFT Z -0.00293

CURVES
POINTS 0.000000 0.4000000E-3 0.2500000E-3 REL; $FAST 12 5
0. 0.0004000 -.0002500 1. 0. 0.0001333 -.0002500 1. 0. -.0001333 -.0002500 1. 
0. -.0004000 -.0002500 1. 0. 0.0004000 -.0000833 1. 0. 0.0001333 -.0000833 1. 
0. -.0001333 -.0000833 1. 0. -.0004000 -.0000833 1. 0. 0.0004000 0.0000833 1. 
0. 0.0001333 0.0000833 1. 0. -.0001333 0.0000833 1. 0. -.0004000 0.0000833 1. 
0. 0.0004000 0.0002500 1. 0. 0.0001333 0.0002500 1. 0. -.0001333 0.0002500 1. 
0. -.0004000 0.0002500 1.
PATCH 3 3
OBJECT =DETECTOR.Extrude1.Face1
INTERFACE COATING "REFLECT" "AIR" "DETECTOR"
REDEFINE COLOR 2
SHIFT Z -0.00293

CURVES
POINTS -.4600000E-3 0.8000000E-3 0.2500000E-3 REL; $FAST 12 5
-.0004600 0.0002500 1. -.0004600 0.0000833 1. -.0004600 0. -.0000833 1.
-.0004600 0. -.0002500 1. -.0001533 0.0002500 1. -.0001533 0.0000833 1.
-.0001533 0. -.0000833 1. -.0001533 0.0002500 1.0.0001533 0.0002500 1.
0.0001533 0.0000833 1.0.0001533 0. -.0000833 1.0.0001533 0. -.0002500 1.
0.0004600 0.0002500 1.0.0004600 0.0000833 1.0.0004600 0. -.0000833 1.
0.0004600 0. -.0002500 1.
PATCH 3 3
OBJECT =DETECTOR.Extrude1.Face2

INTERFACE COATING "REFLECT" "AIR" "DETECTOR"
REDEFINE COLOR 2
SHIFT Z -0.00293

CURVES
POINTS -.9200000E-3 0.4000000E-3 0.2500000E-3 REL; $FAST 12 5
0. -.0004000 -.0002500 1. 0. -.0004000 -.0000833 1. 0. -.0004000 0.0000833 1.
0.0004000 -.0002500 1. 0. -.0004000 -.0000833 1. 0. -.0004000 0.0000833 1.
0.0004000 -.0000833 1.0.0004000 -.0000833 1.0.0004000 0.0000833 1.
0. -.0004000 0.0002500 1. 0. -.0004000 0.0002500 1. 0.0.0004000 0.0002500 1.
0.0004000 0.0002500 1.
PATCH 3 3
OBJECT =DETECTOR.Extrude1.Face3

INTERFACE COATING "REFLECT" "AIR" "DETECTOR"
REDEFINE COLOR 2
SHIFT Z -0.00293

CURVES
POINTS -.8100000E-3 0.000000 0.2500000E-3 REL; $FAST 12 5
-.0001100 0. -.0002500 1. -.0001100 0. -.0000833 1. -.0001100 0. 0.0000833 1.
-.0001100 0.0002500 1. -.000367 0. -.0002500 1. -.000367 0. -.0000833 1.
-.0000367 0.0000833 1. -.0000367 0.0002500 1.0.0000367 0. -.0002500 1.
0.0000367 0. -.0000833 1.0.0000367 0.0000833 1.0.0000367 0. -.0002500 1.
0.0001100 0. -.0002500 1.0.0001100 0. -.0000833 1.0.0001100 0.0000833 1.
0.0001100 0.0002500 1.
PATCH 3 3
OBJECT =DETECTOR.Extrude1.Face4
INTERFACE COATING "REFLECT" "AIR" "DETECTOR"
REDEFINE COLOR 2
SHIFT Z -0.00293

CURVES
POINTS -.700000E-3 0.700000E-4 0.250000E-3 REL; $FAST 12 5
0. -.0000700 -.0002500 1. 0. -.0000700 -.0000833 1. 0. -.0000700 0.0000833 1.
0. -.0000700 0.0002500 1. 0. -.0000233 -.0002500 1. 0. -.0000233 -.0000833 1.
0. -.0000233 0.0000833 1. 0. -.0000233 0.0002500 1. 0. 0.0000233 -.0002500 1.
0. 0.0000233 0.0002500 1.
PATCH 3 3
OBJECT =DETECTOR.Extrude1.Face5

INTERFACE COATING "REFLECT" "AIR" "DETECTOR"
REDEFINE COLOR 2
SHIFT Z -0.00293

CURVES
POINTS -.460000E-3 0.380000E-3 0.250000E-3 REL; $FAST 12 5
-.0002400 -.0002400 0.0003000 1.0000000 -.0002400 0.0003000 .33333333 0.0002400 .33333333
0.0002400 0.0002400 0.0003000 1.0000000 -.0002400 0.0003000 .33333333 0.0002400 .33333333
0.0022400 -.0002400 0.0003000 1.0000000 -.0002400 0.0003000 .33333333 0.0002400 .33333333
0.0002400 0.0002400 0.0001000 1.0000000 -.0002400 0.0001000 .33333333 0.0002400 .33333333
-.0002400 0.0002400 0.0001000 .33333333 0.0002400 0.0001000 .33333333 0.0002400 .33333333
0.0002400 -.0002400 -.0001000 1.0000000 -.0002400 -.0001000 .33333333 0.0002400 .33333333
-.0002400 .0002400 -.0003000 1.0000000 -.0002400 .0002400 -.0003000 .33333333
0.0002400 .0002400 -.0003000 .33333333 0.0002400 .0002400 -.0003000 1.0000000
PATCH 3 3
OBJECT =DETECTOR.Extrude1.Face6
CURVES
LINE 1.000000 1.916667 0.000000 1.000000 1.083333 0.000000
LINE 1.000000 1.083333 0.000000 2.000000 1.083333 0.000000
LINE 2.000000 1.083333 0.000000 2.000000 1.916667 0.000000
LINE 2.000000 1.916667 0.000000 1.000000 1.916667 0.000000
COMPOSITE GAP -1 -1
UVSPACE .1
OBJECT .1; BOUND -.1
SHIFT Z -0.00293

CURVES
POINTS -.2200000E-3 0.7000000E-4 0.2500000E-3 REL; $FAST 12 5
 0. -.0000700 0.0002500 1. 0. -.0000700 0.0000833 1. 0. -.0000700 -.0000833 1.
 0. -.0000700 -.0002500 1. 0. -.0000233 0.0000833 1. 0. -.0000233 -.0002500 1.
 0. 0.0000233 0.0000833 1. 0. 0.0000233 -.0000833 1. 0. 0.0000233 -.0002500 1.
 0. 0.0000700 0.0002500 1. 0. 0.0000700 0.0000833 1. 0. 0.0000700 -.0000833 1.
 0. 0.0000700 -.0002500 1.
PATCH 3 3
OBJECT =DETECTOR.Extrude1.Face7

INTERFACE COATING "REFLECT" "AIR" "DETECTOR"
REDEFINE COLOR 2
SHIFT Z -0.00293

CURVES
POINTS -.4600000E-3 0.4000000E-3 0.5000000E-3 REL; $FAST 12 5
 -.0004600 -.0004000 0. 1. -.0001533 -.0004000 0. 1. 0.0001533 -.0004000 0. 1.
 0.0004600 -.0004000 0. 1. -.0001533 -.0001333 0. 1. -.0001533 -.0001333 0. 1.
 0.0001533 -.0001333 0. 1. 0.0004600 -.0001333 0. 1. -.0004600 0.0001333 0. 1.
 -.0001533 0.0001333 0. 1. 0.0001533 0.0001333 0. 1. 0.0004600 0.0001333 0. 1.
 -.0004600 0.0004000 0. 1. -.0001533 0.0004000 0. 1. 0.0001533 0.0004000 0. 1.
 0.0004600 0.0004000 0. 1.
PATCH 3 3
OBJECT =MESA

CURVES
LINE  1.760870     1.000000     0.000000     2.000000     1.000000     0.000000
LINE  2.000000     1.000000     0.000000     2.000000     2.000000     0.000000
LINE  2.000000     2.000000     0.000000     1.000000     2.000000     0.000000
LINE  1.000000     2.000000     0.000000     1.000000     1.000000     0.000000
LINE  1.000000     1.000000     0.000000    1.239130     1.000000     0.000000
LINE  1.239130     1.000000     0.000000    1.239130     1.175000     0.000000
POINTS  1.500000     1.325000     0.000000 REL; $FAST 12 5
 -.2608696 -.1500000 0. 2. -.2608696 -.0903263 0. 1. -.2410121 -.0351950 0. 2.
 -.2211545 0.0199363 0. 1. -.1844626 0.0621320 0. 2. -.1477707 0.1043277 0. 1.
 -.0998305 0.1271639 0. 2. -.0518902 0.1500000 0. 1. 0.0000000 0.1500000 0. 2.
 0.0518902 0.1500000 0. 1. 0.0998305 0.1271639 0. 2. 0.1477707 0.1043277 0. 1.
 0.1844626 0.0621320 0. 2. 0.2211545 0.0199363 0. 1. 0.2410121 -.0351950 0. 2.
 0.2608696 -.0903263 0. 1. 0.2608696 -.1500000 0. 0.
LINE  1.760870     1.175000     0.000000    1.760870     1.000000     0.000000
COMPOSITE GAP -1 -1
UVSPACE .1
OBJECT .1; BOUND -.1

110
INTERFACE COATING "ABSORB" "AIR" "DETECTOR"
REDEFINE COLOR 2
SHIFT Z -0.00293

CURVES
POINTS -.460000E-3 0.400000E-3 0.000000 REL; $FAST 12 5
-.0004600 -.0004000 0. 1. -.0001533 -.0004000 0. 1. 0.0001533 -.0004000 0. 1.
0.0004600 -.0004000 0. 1. -.0004600 -.0001333 0. 1. -.0001533 -.0001333 0. 1.
-.0001533 .0001333 0. 1. .0001533 .0001333 0. 1. .0004600 .0004000 0. 1.
0.0004600 .0004000 0. 1. -.0004600 .0001333 0. 1. -.0001533 .0001333 0. 1.
0.0001533 .0001333 0. 1. .0004600 .0001333 0. 1. .0001533 .0001333 0. 1.
-.0001533 .0001333 0. 1. -.0001533 .0001333 0. 1. -.0004600 .0004000 0. 1.
-.0004600 .0004000 0. 1. -.0001533 .0004000 0. 1. .0001533 .0004000 0. 1.
0.0004600 .0004000 0. 1.

PATCH 3 3
OBJECT =DETECTOR.Extrude1.Face9
CURVES
LINE 2.000000 1.000000 0.000000 2.000000 2.000000 0.000000
LINE 2.000000 2.000000 0.000000 1.000000 2.000000 0.000000
LINE 1.000000 2.000000 0.000000 1.000000 1.000000 0.000000
LINE 1.000000 1.000000 0.000000 1.239130 1.000000 0.000000
LINE 1.239130 1.000000 0.000000 1.239130 1.175000 0.000000
POINTS 1.500000 1.325000 0.000000 REL; $FAST 12 5
-.2608696 -.1500000 0. 2. -.2608696 -.0903263 0. 1. .2410121 -.0351950 0. 2.
-.2211545 0.0199363 0. 1. .1844626 0.0621320 0. 2. .1477707 0.1043277 0. 1.
.0998305 0.1271639 0. 2. .0518902 0.1500000 0. 1. 0.0000000 0.1500000 0. 2.
.0518902 0.1500000 0. 1. 0.0998305 0.1271639 0. 2. 0.1477707 0.1043277 0. 1.
.1844626 0.0621320 0. 2. .2211545 0.0199363 0. 1. .2410121 -.0351950 0. 2.
.2608696 -.0903263 0. 1. .2608696 -.1500000 0. 0.
LINE 1.760870 1.175000 0.000000 1.760870 1.000000 0.000000
LINE 1.760870 1.000000 0.000000 2.000000 1.000000 0.000000

COMPOSITE GAP -1 -1
UVSPACE .1
OBJECT .1; BOUND -.1
INTERFACE COATING "REFLECT" "AIR" "DETECTOR"
REDEFINE COLOR 2
SHIFT Z -0.00293

$ECHO
GROUP -10
SHIFT 0 -0.00092 -0.0005
SCALE FROM M
RETURN
$CASE

******************************************************************************OTHER GEOMETRY******************************************************************************
!!CAP - ROUGH, REFLECTING SURFACE
SURFACE
   PLANE Z 0 ELLIPSE (CAPR) (CAPR) (APER)/(CAPR)
OBJECT 'CAP.TOP'
ROUGHNESS !!MODEL 1
INTERFACE COATING "CAP" "AIR" "DET_MEDIA"
REDEFINE COLOR 17

!!BOTTOM 10 PIN HOLDER
SURFACE
  PLANE Z -(CAPH) ELLIPSE (CAPR) (CAPR)
OBJECT 'CAP.BOTTOM'
ROUGHNESS !!MODEL 1
INTERFACE COATING "REFLECT" "AIR" "DET_MEDIA"
REDEFINE COLOR 17

!! CAP CYLINDER
SURFACE
  TUBE Z 0 (CAPR) (CAPR) -(CAPH) (CAPR) (CAPR)
OBJECT 'CAP.EDGE'
ROUGHNESS !!MODEL 1
  INTERFACE COATING "CAP" "AIR" "DET_MEDIA"
  REDEFINE COLOR 17
  BOUNDS -.3 +.2

!! VCSEL TOP
SURFACE
  PLANE Z (DIST) ELLIPSE (TOPR) (TOPR)
OBJECT 'TOP'
  INTERFACE COATING "REFLECT" "AIR" "DET_MEDIA"
  REDEFINE COLOR 15

!! VCSEL SIDE
SURFACE
  TUBE Z (DIST)-0.0035 (TOPR) (TOPR) (DIST)-0.0035-0.00005 (TOPR) (TOPR)
OBJECT 'VCSELSIDE1'
  INTERFACE COATING "REFLECT" "AIR" "DET_MEDIA"
  REDEFINE COLOR 6

******************************************************************************DYE WELL GEOMETRY******************************************************************************

!! OUTER CYLINDER
SURFACE
  PLANE Z (WELLZ) ELLIPSE (OUTERR) (OUTERR)
OBJECT 'OUTER.Z1'
  INTERFACE COATING "BARE" "AIR" "GLASS"
  REDEFINE COLOR 14

SURFACE
  TUBE Z (WELLZ) (OUTERR) (OUTERR) (WELLZ)+(WIDTH)+(WELLH) (OUTERR) (OUTERR)
OBJECT 'OUTER.EDGE'
INTERFACE COATING "BARE" "AIR" "GLASS"
REDEFINE COLOR 14
BOUNDS +.2

!! DYE IN CYLINDER
SURFACE
  PLANE Z (WELLZ)+(WIDTH) ELLIPSE (INNERR) (INNERR)
  OBJECT 'DYE.Z1'
  INTERFACE COATING "BARE" "GLASS" "DYE"
  REDEFINE COLOR 13

!! AIR IN CYLINDER
SURFACE
  PLANE Z (WELLZ)+(WIDTH)+(DYEH) ELLIPSE (INNERR) (INNERR)
  OBJECT 'AIR.Z1'
  INTERFACE COATING "BARE" "DYE" "AIR"
  REDEFINE COLOR 12

SURFACE
  TUBE Z (WELLZ)+(WIDTH) (INNERR) (INNERR) (WELLZ)+(WIDTH)+(DYEH)
  (INNERR) (INNERR)
  OBJECT 'DYE.EDGE'
  INTERFACE COATING "BARE" "GLASS" "DYE"
  REDEFINE COLOR 13
  BOUNDS +.3 -.2

SURFACE
  TUBE Z (WELLZ)+(WIDTH)+(DYEH) (INNERR) (INNERR)
  (WELLZ)+(WIDTH)+(WELLH) (INNERR) (INNERR)
  OBJECT 'AIR.EDGE'
  INTERFACE COATING "BARE" "GLASS" "AIR"
  REDEFINE COLOR 12

!!PATCH OF DYE - SMALLER TO BOUND NEW SOURCE AND PREVENT WRONG SIDE ERRORS

!! DYE IN CYLINDER
SURFACE
  PLANE Z (WELLZ)+(WIDTH)+0.001 ELLIPSE 0.999*(INNERR) 0.999*(INNERR)
  OBJECT 'DYES.Z1'
  INTERFACE COATING "BARE" "DYE" "DYE"
  REDEFINE COLOR 13

!! AIR IN CYLINDER
SURFACE
  PLANE Z (WELLZ)+(WIDTH)+(DYEH)-0.001 ELLIPSE 0.999*(INNERR) 0.999*(INNERR)
  OBJECT 'AIRS.Z1'
INTERFACE COATING "BARE" "DYE" "DYE"
REDEFINE COLOR 12

SURFACE
TUBE Z (WELLZ)+(WIDTH)+0.001 (INNERR)*0.999 0.999*(INNERR)
(WELLZ)+(WIDTH)+(DYEH)-0.001 0.999*(INNERR) 0.999*(INNERR)
OBJECT 'DYES.EDGE'
INTERFACE COATING "BARE" "DYE" "DYE"
REDEFINE COLOR 13
BOUNDS +.3 -.2

GROUP -3 'DYES'

TITLE 'EMISSION FROM DYE'
WINDOW X -2 2 Z -3 12
PLOT FACETS OVERLAY

OBJECT 0
BOUND -.1 !!MAKE SURE THIS IS ACTUALLY CLIPPING THE VOLUME
EMITTER??
RETURN

IMMERSE DYE
BEAMS INCOHERENT GEOMETRIC

EMITTING DATA VOXEL (RAYNUM)
  FLUX TOTAL (FLUX_ABSORBED)*QMEFF
SPOTS POSITION EVERY 100 COLOR 17 OVERLAY

IMMERSE AIR
VOXELS OFF
TRACE !!PLOT 1000 COLOR 7

TITLE 'SPOTS POSITION ON DETECTOR SUCORFACE'
WINDOW X Y
CONSIDER ONLY MESA
SPOTS POSITION

!!DISPLAY
!!AVERAGE 2 2
!!PICTURE
!!RETURN

$IO OUTPUT SIMTEST_D4.01.TXT +FILE(11) !! Opens file for outputing data
STATS
$IO OUTPUT CLOSE !! CLOSES FILE

}
A.2 Gaussian distribution creation

*********MAKE_GAUSSIAN.INR**************

!! CREATES A GAUSSIAN SOURCE WITH WAIST AT (DIST),
!! AND RAYS TRACED STARTING AT Z = -0.5.
!! SUMS THE COHERENT CONTRIBUTIONS AND DUMPS
!! THE RAY INFORMATION INTO A "LASTDUMP.DIS" IN THE
!! WORKING DIRECTORY OF ASAP

!! WRITTEN BY ELIZABETH MUNRO, 2009

*********SYSTEM UNITS**************

SYSTEM NEW
RESET

UNITS MM
WAVELENGTH 675.50 NM
PIXELS 501

!!!!DEFINE RAY SPLIT AND SCATTER CHARACTERISTICS
  SPLIT 1
  LEVEL 1
  FRESNEL AVE

!!CREATE VOLUME SCATTER MODELS
!! FOLLOWS FORM VOLUME G MU_S'MU_A OBSCURATION RATIO
MODEL
  VOLUME 0.898 30.6'0.033 1

!! DEFINE SYSTEM MEDIA
MEDIA
  1.4 SCATTER 1 'TISSUE'
  1.59908 'EC0550'
  3.75 'DET_MEDIA'   !!GAAS INDEX AT 670NM
  3.75 'DETECTOR'
  1.4'2.6857E-4 'DYE'   !!CY 5.5 ABSORPTION AND INDEX IN SUBCUTANEOUS SPOT

COATINGS PROPERTIES
  0.00979  0.99021 'AR'     !!COATING AT 675.5 NM
  0 1 'TRANSMIT'
  0 0 'ABSORB'
  1 0 'REFLECT'

*********SYSTEM VARIABLES**************
WAIST=0.886*0.0025 !! BEAM WAIST RADIUS IN ASAP IS DEFINED AS
SQRT(PI/4)*WO
DIST=-2.93 !! Z WAIST LOCATION
ZEE=0 !! REBUILD PLANE LOCATION
TP=1.1 !! LOCATION OF TEST PLANE WHERE PROFILE IS GRAPHED

**********************GEOMETRY***************************************

!! PLANE FOR DISTRIBUTION CALCULATIONS
SURFACE
   PLANE Z (ZEE) ELLIPSE 6 6
OBJECT 'REB1'
   INTERFACE COATING 0 0 "AIR" "AIR"
   REDEFINE COLOR 17

!! TEST PLANE WHERE PROFILE IS GRAPHED
SURFACE
   PLANE Z (TP) ELLIPSE 10 10
OBJECT 'TEST1'
   INTERFACE COATING ABSORB "DET_MEDIA" "AIR"
   REDEFINE COLOR 17

*************************SOURCES****************************************

IMMERSE AIR
BEAMS COHERENT DIFFRACT
PARABASAL 8
WIDTHS 1.6

GAUSSIAN Z -0.5 (DIST) 201 (WAIST)

*******************REBUILD SOURCE AS INCOHERENT ************************
TITLE 'BIOSensor FLUORESCENCE MODEL'
WINDOW X -2 2 Z -3 12
PLOT FACETS 15 15 OVERLAY
TRACE STEP 1 PLOT COLOR 3 OVERLAY

WINDOW X Y
CONSIDER ONLY REB1
SPREAD NORMAL (ZEE)
!!$GRAB 'Beams' 1 2 FLUXTOTAL

DUMP

CONSIDER ONLY TEST
SPOTS POSITION
DISPLAY
GRAPH 0.5
RETURN
A.3 Fluorescent three dimensional objects buried in tissue

**********************************************************************
!! MODEL OF THREE FLUORESCING OBJECTS
!! EMBEDDED IN RABBIT CORTICAL TISSUE AT VARIOUS DEPTHS.
!! WITH OPTICAL PROPERTIES OF 4 DYES AND EXCITATION WAVELENGTHS
!! TRACKS ABSORBED FLUX IN THE DYE VOLUMES AND THEN REBUILDS
!! THE SYSTEM AT THE EMISSION WAVELENGTH, AND EMITS
ISOTROPICALLY
!! FROM THE DYE VOLUMES

!! WRITTEN BY ELIZABETH MUNRO, 2009

**********************************************************************

SYSTEM NEW
RESET

!! SYSTEM DEFINITIONS
BEAMS INCOHERENT GEOMETRIC
UNITS MM

PIXELS 511

COATING PROPERTIES
0 1 'TRANSMIT'
1 0 'REFLECT'
0 0 'ABSORB'

!!!!DEFINE RAY SPLIT AND SCATTER CHARACTERISTICS
SPLIT 1500 MONTECARLO
LEVEL 1000
FRESNEL AVE

WAVELENGTH 546

!!CREATE VOLUME SCATTER MODELS
!! Follows form volume g mu_s`mu_a OBSCURATION RATIO
MODEL
MODEL
VOLUME 0.9 19.41`1.5 1  !! EXCITATION AT 405
   !! Mu_a should be 2.231, but this causes NaN errors
   !! Using Mu_a = 1.5 fixes this, but isn't 100% valid!!
VOLUME 0.9 19.41`1.379 1  !! FLUOROPHORE EXCITATION AT 405

!! VOLUME 0.9 17.98'0.153 1 !! EXCITATION AT 495
!! VOLUME 0.9 17.98'0.16548 1 !! FLUOROPHORE EXCITATION AT 495
!! VOLUME 0.9 17.31'0.376 1 !! EXCITATION AT 546
VOLUME 0.9 17.31'0.1379 1 !! FLUOROPHORE EXCITATION AT 546
!! VOLUME 0.9 16.45'0.022 1 !! EXCITATION AT 625
!! VOLUME 0.9 16.45'0.0469 1 !! FLUOROPHORE EXCITATION AT 625
!!

!!CREATE MEDIA USING VOLUME SCATTER MODELS
  MEDIA
    1.40 SCATTER 1 'TISSUE'
    1.40 SCATTER 2 'FLU'
    1.5 'GLASS'

!! SYSTEM VARIABLES

SD=5 !! SOURCE DISTANCE FROM SURFACE OF TISSUE
DX1=5 !! HALF WIDTH IN X OF SHAPE 1
DY1=4 !! HALF WIDTH IN Y OF SHAPE1 (BOX)
DZ1=0.5 !! HALF THICKNESS OF SHAPE1 (BOX)
D=2 !! DEPTH OF SHAPE1 (BOX)
Y1=1 !! SHIFT FACTOR OF BOX

DX2=2.5 !! HALF WIDTHS OF TRIANGLE
DY2=SQRT(2.75)/2
DZ2=0.25

X2=-6 !! SHIFTS OF TRIANGLE
Y2=1
Z2=-0.8

DX3=0.5 !! DIAMETER OF SURFACE SPOT
X3=-8
Y3=-8
Z3=-0.1
TH=0.1 !! THICKNESS OF SURFACE SPOT

C=25 !! HALF WIDTH OF BOX OF TISSUE WHICH HOLDS THE SHAPES
EFF=0.25 !! FLUOROPHORE CONVERSION EFFICIENCY

VB=10.1 !! Cube for tracking VOXELS ABSORBED - contains all three objects
VBZ=3.1 !! Z DIRECTION BOUNDING

!! GEOMETRY
SURFACE
PLANE X (C) RECTANGLE (C) (C)  
OBJECT 'TISSUE.FACE.1'  
INTERFACE COATING BARE AIR TISSUE  
REDEFINE COLOR 1  
SHIFT Z -(C)

SURFACE  
PLANE -X -(C) RECTANGLE (C) (C)  
OBJECT 'TISSUE.FACE.2'  
INTERFACE COATING BARE AIR TISSUE  
REDEFINE COLOR 1  
SHIFT Z -(C)

SURFACE  
PLANE Y (C) RECTANGLE (C) (C)  
OBJECT 'TISSUE.FACE.3'  
INTERFACE COATING BARE AIR TISSUE  
REDEFINE COLOR 1  
SHIFT Z -(C)

SURFACE  
PLANE -Y -(C) RECTANGLE (C) (C)  
OBJECT 'TISSUE.FACE.4'  
INTERFACE COATING BARE AIR TISSUE  
REDEFINE COLOR 1  
SHIFT Z -(C)

SURFACE  
PLANE Z 0 RECTANGLE (C) (C)  
OBJECT 'TISSUE.TOP'  
INTERFACE COATING BARE AIR TISSUE  
REDEFINE COLOR 1

SURFACE  
PLANE -Z -2*(C) RECTANGLE (C) (C)  
OBJECT 'TISSUE.BOTTOM'  
INTERFACE COATING BARE AIR TISSUE  
REDEFINE COLOR 1

GROUP -6 'TISSUE'

!!CUBE GEOMETRY  
SURFACE (CUBXPOS=101)  
PLANE X (DX1) RECTANGLE (DY1) (DZ1)  
OBJECT 'SHAPE1.FACE.1'  
INTERFACE COATING "BARE" "TISSUE" "FLU"  
REDEFINE COLOR 13  
SHIFT Z -(DZ1)-(D)  
SHIFT Y Y1
SURFACE (CUBXNEG=102)  
PLANE X -(DX1) RECTANGLE (DY1) (DZ1)  
OBJECT 'SHAPE1.FACE.2'  
  INTERFACE COATING "BARE" "TISSUE" "FLU"  
REDEFINE COLOR 13  
SHIFT Z -(DZ1)-(D)  
SHIFT Y Y1  

SURFACE (CUBYPOS=103)  
PLANE Y (DY1) RECTANGLE (DZ1) (DX1)  
OBJECT 'SHAPE1.FACE.3'  
  INTERFACE COATING "BARE" "TISSUE" "FLU"  
REDEFINE COLOR 13  
SHIFT Z -(DZ1)-(D)  
SHIFT Y Y1  

SURFACE (CUBYNEG=104)  
PLANE Y -(DY1) RECTANGLE (DZ1) (DX1)  
OBJECT 'SHAPE1.FACE.4'  
  INTERFACE COATING "BARE" "TISSUE" "FLU"  
REDEFINE COLOR 13  
SHIFT Z -(DZ1)-(D)  
SHIFT Y Y1  

SURFACE (CUBZNEG=105)  
PLANE Z -2*(DZ1)-(D) RECTANGLE (DX1) (DY1)  
OBJECT 'SHAPE1.BOTTOM'  
  INTERFACE COATING "BARE" "TISSUE" "FLU"  
REDEFINE COLOR 13  
SHIFT Y Y1  

SURFACE (CUBZPOS=106)  
PLANE Z -(D) RECTANGLE (DX1) (DY1)  
OBJECT 'SHAPE1.TOP'  
  INTERFACE COATING "BARE" "TISSUE" "FLU"  
REDEFINE COLOR 13  
SHIFT Y Y1  

GROUP -6 'SHAPE1'  

********************START TRIANGLE GEOMETRY**************************  

$case upper  
TITLE =Import of IGES file C:\Documents and Settings\Elizabeth\My Documents\Masters\ASAP\Wilson Group\shapes at depth\triangle.IGS  
$ECHO NONE  
!!SolidWorks IGES file using analytic representation for surfaces
CURVES (TRI1=201)
LINE  0.000000    -1.657049     0.000000     0.000000    -1.657049    0.5000000
LINE  0.000000    -1.657049    0.5000000     2.500000     0.000000    0.5000000
LINE  2.500000     0.000000     0.5000000     2.500000     0.000000     0.000000
LINE  2.500000     0.000000     0.  0.000000    -1.657049     0.
COMPOSITE GAP -1 -1
OBJECT =SHAPE2.1
INTERFACE COATING "BARE" "TISSUE" "FLU"
REDEFINE COLOR 4
SHIFT Z -0.5
SHIFT (X2) (Y2) (Z2)

CURVES (TRI2=202)
LINE  2.500000     0.000000     0.000000     2.500000     0.000000    0.5000000
LINE  2.500000     0.000000     0.5000000     -2.500000     0.000000    0.5000000
LINE -2.500000     0.000000     0.5000000     -2.500000     0.000000     0.000000
LINE -2.500000     0.000000     0.  2.500000     0.000000     0.
COMPOSITE GAP -1 -1
OBJECT =SHAPE2.2
INTERFACE COATING "BARE" "TISSUE" "FLU"
REDEFINE COLOR 4
SHIFT Z -0.5
SHIFT (X2) (Y2) (Z2)

CURVES (TRI3=203)
LINE -2.500000     0.000000     0.000000    -2.500000     0.000000     0.5000000
LINE -2.500000     0.000000     0.5000000     0.000000    -1.657049    0.5000000
LINE  0.000000    -1.657049     0.5000000     0.000000    -1.657049     0.000000
LINE  0.000000    -1.657049     0.  -2.500000     0.000000     0.
COMPOSITE GAP -1 -1
OBJECT =SHAPE2.3
INTERFACE COATING "BARE" "TISSUE" "FLU"
REDEFINE COLOR 4
SHIFT Z -0.5
SHIFT (X2) (Y2) (Z2)

CURVES (TRI4=204)
LINE  0.000000    -1.657049     0.5000000    -2.500000     0.000000     0.5000000
LINE -2.500000     0.000000     0.5000000     2.500000     0.000000     0.5000000
LINE  2.500000     0.000000     0.5000000     0.000000    -1.657049    0.5000000
COMPOSITE GAP -1 -1
OBJECT =SHAPE2.4
INTERFACE COATING "BARE" "TISSUE" "FLU"
REDEFINE COLOR 4
SHIFT Z -0.5
SHIFT (X2) (Y2) (Z2)

CURVES (TRI5=205)
LINE  2.500000     0.000000     0.  0.000000    -1.657049     0.
LINE  0.000000  -1.657049  0. -2.500000  0.000000  0.
LINE -2.500000  0.000000  0.  2.500000  0.000000  0.
COMPOSITE GAP -1 -1
OBJECT =SHAPE2.5
INTERFACE COATING "BARE" "TISSUE" "FLU"
REDEFINE COLOR 4
SHIFT Z -0.5
SHIFT (X2) (Y2) (Z2)

$ECHO
GROUP -5 'SHAPE2'
SCALE FROM MM
RETURN
$CASE

**************************END TRIANGLE GEOMETRY**************************

SURFACE
TUBE Z (Z3)-(TH) (DX3) (DX3) (Z3) (DX3) (DX3)
OBJECT 'SPOT.TUBE'
INTERFACE COATING "BARE" "TISSUE" "FLU"
REDEFINE COLOR 11
SHIFT (X3) (Y3) 0

SURFACE
PLANE Z (Z3) ELLIPSE (DX3) (DX3)
OBJECT 'SPOT.Z1'
    INTERFACE COATING "BARE" "TISSUE" "FLU"
    REDEFINE COLOR 11
    SHIFT (X3) (Y3) 0
    BOUNDS -.2

SURFACE
PLANE Z (Z3)-(TH) ELLIPSE (DX3) (DX3)
OBJECT 'SPOT.Z2'
    INTERFACE COATING "BARE" "TISSUE" "FLU"
    REDEFINE COLOR 11
    SHIFT (X3) (Y3) 0
    BOUNDS +.3

!! SOURCE
IMMERSE AIR
GRID RECT Z (SD)+(D) -10 10 -10 10 1001 1001
    SOURCE DIRECTION 0 0 -1
FLUX TOTAL 100.0
VOXELS ABSORBED -(VB) (VB) -(VB) (VB) -2*(VBZ) 0 301 301 101
WINDOW Z Y
PLOT FACETS 15 15 OVERLAY

TRACE !!PLOT 1000

!!$VIEW

$GRAB 'VOXELS' FLUX_ABSORBED   !! GRAB TOTAL FLUX ABSORBED
$REG FLUX_ABSORBED              !! SHOW AT THE CONSOLE OUTPUT
$COPY 9 VOXEL.DIS               !! COPY DEFAULT BRO009.DAT TO NAMED FILE
STATS

RAYS 0 !! CLEAR PREVIOUS SOURCE RAYS
******************************************************************
******************************************************************
******************************************************************
********************REMAKE SYSTEM GEOMETRY************************
******************************************************************
******************************************************************
******************************************************************
SYSTEM NEW
SIO VECTOR REWIND

PIXELS 511
UNITS MM

COATING PROPERTIES
  0 1 'TRANSMIT'
  1 0 'REFLECT'
  0 0 'ABSORB'

!!!!DEFINE RAY SPLIT AND SCATTER CHARACTERISTICS
  SPLIT 1500 MONTECARLO
  LEVEL 1000
  FRESNEL AVE

!! REDEFINE TISSUE PROPERTIES AT THE NEW WAVELENGTH Emitted BY THE
FLUOROPHORE
!!CREATE VOLUME SCATTER MODELS
!! FOLLOWS FORM VOLUME G MU_S'MU_A OBSCURATION RATIO

WAVELENGTH 700
MODEL
  VOLUME 0.9 15.75'0.0072 1   !! EMISSION PROPERTIES OF TISSUE
  VOLUME 0.9 15.75'0.00138 1   !! EMISSION PROPERTIES OF DYE
MEDIA
1.4 SCATTER 1 'TISSUE'   !! NEW SCATTER MODEL FOR NEW WAVELENGTH
1.4 SCATTER 2 'FLU'
1.5 'GLASS'

!! SYSTEM VARIABLES

SD=5       !! SOURCE DISTANCE FROM SURFACE OF TISSUE
DX1=5      !! HALF WIDTH IN X OF SHAPE 1
DY1=4      !! HALF WIDTH IN Y OF SHAPE1 (BOX)
DZ1=0.5    !! HALF THICKNESS OF SHAPE1 (BOX)
D=2        !! DEPTH OF SHAPE1 (BOX)
Y1=1       !! SHIFT FACTOR OF BOX

DX2=2.5    !! HALF WIDTHS OF TRIANGLE
DY2=SQRT(2.75)/2
DZ2=0.25

X2=-6      !! SHIFTS OF TRIANGLE
Y2=1
Z2=-0.8

DX3=0.5    !! DIAMETER OF SURFACE SPOT
X3=-8
Y3=-8
Z3=-0.1
TH=0.1     !! THICKNESS OF SURFACE SPOT

C=25       !! HALF WIDTH OF BOX OF TISSUE WHICH HOLDS THE SHAPES
EFF=0.25   !! FLUOROPHORE CONVERSION EFFICIENCY

VB=6.1     !! Cube for tracking VOXELS ABSORBED - contains all three objects
VBZ=3.1    !! Z DIRECTION BOUNDING

!! GEOMETRY

SURFACE
PLANE X (C) RECTANGLE (C) (C)
OBJECT 'TISSUE.FACE.1'
   INTERFACE COATING BARE AIR TISSUE
   REDEFINE COLOR 1
   SHIFT Z -(C)

SURFACE
PLANE -X -(C) RECTANGLE (C) (C)
OBJECT 'TISSUE.FACE.2'
   INTERFACE COATING BARE AIR TISSUE
   REDEFINE COLOR 1
SHIFT Z -(C)

SURFACE
PLANE Y (C) RECTANGLE (C) (C)
OBJECT 'TISSUE_FACE_3'
INTERFACE COATING BARE AIR TISSUE
REDEFINE COLOR 1
SHIFT Z -(C)

SURFACE
PLANE -Y -(C) RECTANGLE (C) (C)
OBJECT 'TISSUE_FACE_4'
INTERFACE COATING BARE AIR TISSUE
REDEFINE COLOR 1
SHIFT Z -(C)

SURFACE
PLANE Z 0 RECTANGLE (C) (C)
OBJECT 'TISSUE_TOP'
INTERFACE COATING BARE AIR TISSUE
REDEFINE COLOR 1

SURFACE
PLANE -Z -2*(C) RECTANGLE (C) (C)
OBJECT 'TISSUE_BOTTOM'
INTERFACE COATING BARE AIR TISSUE
REDEFINE COLOR 1

GROUP -6 'TISSUE'

!!CUBE GEOMETRY
SURFACE (CUBXPOS=101)
PLANE X (DX1) RECTANGLE (DY1) (DZ1)
OBJECT 'SHAPE1_FACE_1'
INTERFACE COATING "BARE" "TISSUE" "FLU"
REDEFINE COLOR 13
SHIFT Z -(DZ1)-(D)
SHIFT Y Y1

SURFACE (CUBXNEG=102)
PLANE X -(DX1) RECTANGLE (DY1) (DZ1)
OBJECT 'SHAPE1_FACE_2'
INTERFACE COATING "BARE" "TISSUE" "FLU"
REDEFINE COLOR 13
SHIFT Z -(DZ1)-(D)
SHIFT Y Y1

125
SURFACE (CUBYPOS=103)
  PLANE Y (DY1) RECTANGLE (DZ1) (DX1)
  OBJECT 'SHAPE1.FACE.3'
  INTERFACE COATING "BARE" "TISSUE" "FLU"
  REDEFINE COLOR 13
  SHIFT Z -(DZ1)-(D)
  SHIFT Y Y1

SURFACE (CUBYNEG=104)
  PLANE Y -(DY1) RECTANGLE (DZ1) (DX1)
  OBJECT 'SHAPE1.FACE.4'
  INTERFACE COATING "BARE" "TISSUE" "FLU"
  REDEFINE COLOR 13
  SHIFT Z -(DZ1)-(D)
  SHIFT Y Y1

SURFACE (CUBZNEG=105)
  PLANE Z -2*(DZ1)-(D) RECTANGLE (DX1) (DY1)
  OBJECT 'SHAPE1.BOTTOM'
  INTERFACE COATING "BARE" "TISSUE" "FLU"
  REDEFINE COLOR 13
  SHIFT Y Y1

SURFACE (CUBZPOS=106)
  PLANE Z -(D) RECTANGLE (DX1) (DY1)
  OBJECT 'SHAPE1.TOP'
  INTERFACE COATING "BARE" "TISSUE" "FLU"
  REDEFINE COLOR 13
  SHIFT Y Y1

GROUP -6 'SHAPE1'

********************************************START TRIANGLE GEOMETRY********************************************
$case upper
TITLE =Import of IGES file C:\Documents and Settings\Elizabeth\My Documents\Masters\ASAP\Wilson Group\shapes at depth\triangle.IGS
$ECHO NONE
!!SolidWorks IGES file using analytic representation for surfaces

CURVES (TRI1=201)
  LINE 0.000000 -1.657049 0.000000 0.000000 -1.657049 0.5000000
  LINE 0.000000 -1.657049 0.5000000 2.500000 0.000000 0.5000000
  LINE 2.500000 0.000000 0.5000000 2.500000 0.000000 0.000000
  LINE 2.500000 0.000000 0. 0.000000 -1.657049 0.
  COMPOSITE GAP -1 -1
  OBJECT =SHAPE2.1
  INTERFACE COATING "BARE" "TISSUE" "FLU"
  REDEFINE COLOR 4
  SHIFT Z -0.5

126
SHIFT (X2) (Y2) (Z2)

CURVES (TRI2=202)
LINE  2.500000     0.000000     0.000000     2.500000     0.000000    0.5000000
LINE  2.500000     0.000000    0.5000000 -2.500000     0.000000     0.5000000
LINE -2.500000     0.000000    0.5000000 -2.500000     0.000000     0.000000
LINE -2.500000     0.000000     0.  2.500000     0.000000    0.
COMPOSITE GAP -1 -1
OBJECT =SHAPE2.2
INTERFACE COATING "BARE" "TISSUE" "FLU"
REDEFINE COLOR 4
SHIFT Z -0.5
SHIFT (X2) (Y2) (Z2)

CURVES (TRI3=203)
LINE -2.500000     0.000000     0.000000    -2.500000     0.000000    0.5000000
LINE -2.500000     0.000000    0.5000000     0.000000    -1.657049    0.5000000
LINE  0.000000    -1.657049    0.5000000     0.000000    -1.657049    0.000000
LINE  0.000000    -1.657049    0. -2.500000     0.000000    0.
COMPOSITE GAP -1 -1
OBJECT =SHAPE2.3
INTERFACE COATING "BARE" "TISSUE" "FLU"
REDEFINE COLOR 4
SHIFT Z -0.5
SHIFT (X2) (Y2) (Z2)

CURVES (TRI4=204)
LINE  0.000000    -1.657049    0.5000000    -2.500000     0.000000    0.5000000
LINE -2.500000     0.000000    0.5000000     2.500000     0.000000     0.5000000
LINE  2.500000     0.000000    0.  0.000000    -1.657049     0.
COMPOSITE GAP -1 -1
OBJECT =SHAPE2.4
INTERFACE COATING "BARE" "TISSUE" "FLU"
REDEFINE COLOR 4
SHIFT Z -0.5
SHIFT (X2) (Y2) (Z2)

CURVES (TRI5=205)
LINE  2.500000     0.000000     0.  0.000000    -1.657049     0.
LINE  0.000000    -1.657049     0. -2.500000     0.000000     0.
LINE -2.500000     0.000000     0.  2.500000     0.000000     0.
COMPOSITE GAP -1 -1
OBJECT =SHAPE2.5
INTERFACE COATING "BARE" "TISSUE" "FLU"
REDEFINE COLOR 4
SHIFT Z -0.5
SHIFT (X2) (Y2) (Z2)
$ECHO
GROUP -5 'SHAPE2'
SCALE FROM MM
RETURN
$CASE

************************************************ END TRIANGLE GEOMETRY ************************************************

SURFACE (SPOTUB=501)
TUBE Z (Z3)-(TH) (DX3) (DX3) (Z3) (DX3) (DX3)
OBJECT 'SPOT.TUBE'
INTERFACE COATING "BARE" "TISSUE" "FLU"
REDEFINE COLOR 11
SHIFT (X3) (Y3) 0

SURFACE (SPOTPOSZ=502)
PLANE Z (Z3) ELLIPSE (DX3) (DX3)
OBJECT 'SPOT.Z1'
   INTERFACE COATING "BARE" "TISSUE" "FLU"
   REDEFINE COLOR 11
   SHIFT (X3) (Y3) 0
   BOUNDS -.2

SURFACE (SPOTNEGZ=503)
PLANE Z (Z3)-(TH) ELLIPSE (DX3) (DX3)
OBJECT 'SPOT.Z2'
   INTERFACE COATING "BARE" "TISSUE" "FLU"
   REDEFINE COLOR 11
   SHIFT (X3) (Y3) 0
   BOUNDS +.3

CONSIDER ALL
WINDOW Y -10 10 Z -10 0
PLOT FACETS 15 15 OVERLAY
CONSIDER ALL

IMMERSE FLU

!!THREE SOURCES
OBJECT 0  !! THE SOURCE TO BE CREATED
   BOUNDS -(TRI2) -(TRI5)
EMITTING DATA VOXEL 10000001 COLOR 1

OBJECT 0
   BOUNDS +(CUBXNEG) +(CUBYNEG) +(CUBZNEG) -(CUBYPOS) -(CUBZPOS) -(CUBXPOS)
EMITTING DATA VOXEL 10000001 COLOR 2

OBJECT 0
BOUNDS -(SPOTUB) -(SPOTPOSZ) +(SPOTNEGZ)
EMITTING DATA VOXEL 1000001 COLOR 3

FLUX TOTAL (FLUX_ABSORBED)

!! TRACE RAYS FROM FLUOROPHORE EMISSION
WINDOW Y -10 10 Z -10 0
SPOTS POSITION COLOR 4 OVERLAY

CONSIDER ALL
TITLE '705 EMISSION'
VOXELS OFF !! SAVES TIME BY NOT TRACKING VOLUME FLUX
TRACE !!PLOT 1000
!!$VIEW

!! LOOK AT FLUX ON SURFACE
WINDOW X Y
CONSIDER ONLY TISSUE.TOP
SPOTS POSITION OVERLAY
DISPLAY
AVERAGE 4 4 !! TO SMOOTH DATA
PICTURE
RETURN
A.4 Collection efficiency calculation for sensor geometry

******************************************************************************COLLECTION_EFFICIENCY_LENS.INR******************************************************************************
!! This file traces isotropic rays from a distance away from the lens
!! The flux captured on the detector mesa is recorded, accounting for
!! losses in light due to geometry considerations

!! The looping function changes the SEED value for statistical purposes,
!! and also alters the distance of the source from the lens

!! Written by Elizabeth Munro, 2009

******************************************************************************

$DO 1 71
{
******************************************************************************SYSTEM UNITS******************************************************************************
SYSTEM NEW
RESET
UNITS MM
WAVELENGTH 675.50 NM
PIXELS 101

!!!!DEFINE RAY SPLIT AND SCATTER CHARACTERISTICS
   SPLIT 1500 MONTECARLO
   LEVEL 1000
   FRESNEL AVE

!! DEFINE SYSTEM MEDIA
MEDIA
   1.56 'GLASS'   !! INDEX OF REFRACTION OF POLYSTYRENE
   1.59908 'EC0550'  !! INDEX OF LENS MATERIAL
   3.7 'DET_MEDIA'   !! GAAS INDEX AT 670NM
   1.4 1.34285E-6 'DYE'  !! CY 5.5 ABSORPTION AND INDEX, 1UM CONCENTRATION

COATINGS PROPERTIES
   0.009913 0.990087 'AR'   !! LENS COATING AT 675 NM
   0 1 'TRANSMIT'
   0 0 'ABSORB'
   1 0 'REFLECT'
   1 0 'CAP'

******************************************************************************SYSTEM VARIABLES******************************************************************************
!! ASPHERIC LENS COEFFICIENTS
ASP1_R=19.92
ASP1_K=0
ASP1_A=-4.23710E-3
ASP1_B=6.34840E-4
ASP1_C=-3.46536E-5
ASP1_D=0
ASP1_E=0

ASP2_R=-2.94
ASP2_K=-0.481104
ASP2_A=2.89094E-5
ASP2_B=3.76282E-5
ASP2_C=-3.26442E-6
ASP2_D=1.17572E-6
ASP2_E=0

LENS_POS1=0
LENS_POS2=2.94
FLAT_POS2=1.57

D=2.25 !SEMIWIDTH OF ASP1 IN MICRONS
E=2.655 !SEMIWIDTH OF ASP2 IN MICRONS
LENS_FLAT_RAD=3.165 !WIDTH OF FLAT PART IN MICRONS
CT=2.94 ! CENTER THICKNESS OF LENS
CAPR=4.1 ! CAP RADIUS
CAPH=3.431

APER=1.40 ! RADIUS OF THE APERTURE IN THE CAP
DIST=-2.93
WAIST=0.0000025
TOPR=0.02 ! RADIUS OF THE VCSEL MESA
TESTD=2.945

!! DYE WELL VARIABLES
INNERR=6.8072/2 ! INNER RADIUS OF THE DYE WELL
OUTERR=8.255/2 ! OUTER RADIUS OF THE DYE WELL
WIDTH=1.778 ! THICKNESS OF BOTTOM GLASS
DYEH=4 ! HEIGHT OF THE DYE IN THE WELL

WELLH=10 ! TOTAL HEIGHT OF THE WELL
QMEFF=0.28 ! QUANTUM EFFICIENCY OF CY5.5 DYE

$IF ? LT 11 THEN
  VALUE=20000007+(2*?)
  SEED (VALUE)
  WELLZ=(CT)+0.01 ! WELLZ IS THE LOCATION OF THE START OF THE DYE WELL
$ELSEIF 11 LE ? AND ? LT 21 THEN
  VALUE=20000007+(2*?)
  SEED (VALUE)
  WELLZ=(CT)+0.51
$ELSEIF 21 LE ? AND ? LT 31 THEN
VALUE=20000007+(2*?)
SEED (VALUE)
WELLZ=(CT)+1.01
$ELSEIF 31 LE ? AND ? LT 41 THEN
  VALUE=20000007+(2*?)
  SEED (VALUE)
  WELLZ=(CT)+1.51
$ELSEIF 41 LE ? AND ? LT 51 THEN
  VALUE=20000007+(2*?)
  SEED (VALUE)
  WELLZ=(CT)+2.01
$ELSEIF 51 LE ? AND ? LT 61 THEN
  VALUE=20000007+(2*?)
  SEED (VALUE)
  WELLZ=(CT)+2.51
$ELSEIF 61 LE ? AND ? LT 71 THEN
  VALUE=20000007+(2*?)
  SEED (VALUE)
  WELLZ=(CT)+3.01
ENDIF

SWID=5*(LENS_FLAT_RAD)  !! SEMIWIDTH OF TISSUE SLAB
DYE=(WELLZ)    !! DEPTH OF DYE
DYER=0.72          !! RADIUS OF DYE DISK
DYEH=2.748          !! HEIGHT OF DYE DISK
PI=101            !! PIXELS
REB_PI=201        !! PIXELS USED IN REBUILDING THE SOURCE. 201 = 28,357 RAYS, 501 = 176,207 RAYS, ETC
RAYNUM=500001      !! NUMBER OF RAYS EMITTED FROM THE DYE AFTER REBUILD

********************ASPHERIC LENS***********************************
!! TUBE
SURFACE
  TUBE Z (LENS_POS1+0.08)-0.001 (D) (D) (LENS_POS1+0.08)+0.001 (D) (D)
  OBJECT 'TUBE1'
  INTERFACE COATING AR EC0550 AIR
  REDEFINE COLOR 18

SURFACE
  PLANE Z (LENS_POS1+0.08) ELLIPSE (LENS_FLAT_RAD) (LENS_FLAT_RAD)
  OBJECT 'LENS_FLAT_S1'
  BOUNDS -.2
  INTERFACE COATING AR EC0550 AIR
  REDEFINE COLOR 18

SURFACE
  TUBE Z (FLAT_POS2)-0.001 (E) (E) (FLAT_POS2)+0.001 (E) (E)
  OBJECT 'TUBE2'
INTERFACE COATING AR EC0550 AIR
REDEFINE COLOR 18

SURFACE
  PLANE Z (FLAT_POS2) ELLIPSE (LENS_FLAT_RAD) (LENS_FLAT_RAD)
  OBJECT 'LENS_FLAT_S2'
  BOUNDS +.2
INTERFACE COATING AR EC0550 AIR
REDEFINE COLOR 18

SURFACE
  TUBE Z (LENS_POS1)-1 (LENS_FLAT_RAD) (LENS_FLAT_RAD) (FLAT_POS2)+1
  (LENS_FLAT_RAD) (LENS_FLAT_RAD)
  OBJECT 'TUBE3'
  BOUNDS +.4 -.2
INTERFACE COATING AR EC0550 AIR
REDEFINE COLOR 18

!! ASPHERIC FACES OF LENSES
SURFACE
  OPTICAL Z (LENS_POS1) (ASP1_R) (ASP1_K) (ASP1_A) (ASP1_B) (ASP1_C)
  (ASP1_D) (ASP1_E) ELLIPSE (D) (D)
  OBJECT 'ASP1'
  BOUNDS -.5
INTERFACE COATING AR EC0550 AIR
REDEFINE COLOR 18

SURFACE
  OPTICAL Z (LENS_POS2) (ASP2_R) (ASP2_K) (ASP2_A) (ASP2_B) (ASP2_C)
  (ASP2_D) (ASP2_E) ELLIPSE (E) (E)
  OBJECT 'ASP2'
  BOUNDS +.5
INTERFACE COATING AR EC0550 AIR
REDEFINE COLOR 18

**********************DETECTOR MESA GEOMETRY**********************
!! This geometry is imported from the SolidWorks CAD file I drew it in

$case upper
TITLE =Import of GTX file C:\Documents and Settings\Elizabeth\My
Documents\Masters\ASAP\Model_TDO\detector_mesa_newdims.GTX
$ECHO NONE
!!
CURVES
  POINTS -.1100000E-3  0.000000  0.2500000E-3 REL; $FAST 12 5
  0.0001100  0. -.0002500  1. 0.0000367  0. -.0002500  1. -.0000367  0. -.0002500  1.
  -.0000367  0. -.0000833  1. 0.0001100  0. -.0000833  1. 0.0000367  0. -.0000833  1.
  -.0000367  0. -.0000833  1. 0.0001100  0. -.0000833  1. 0.0000367  0. -.0000833  1.
  0.0000367  0. 0.0000833  1. -.0000367  0. 0.0000833  1. -.0000367  0. 0.0000833  1.
  0.0001100  0. 0.0002500  1. 0.0000367  0. 0.0002500  1. -.0000367  0. 0.0002500  1.
-0.001100 0. 0.0002500 1.
PATCH 3 3
OBJECT =DETECTOR.Extrude1.Face0

INTERFACE COATING "REFLECT" "AIR" "DETECTOR"
REDEFINE COLOR 2
SHIFT Z -0.00293

CURVES
POINTS 0.000000 0.400000E-3 0.2500000E-3 REL; $FAST 12 5
 0. 0.0004000 -.002500 1. 0. 0.001333 -.002500 1. 0. -.001333 -.002500 1.
 0. -.0004000 -.002500 1. 0. 0.0004000 -.000833 1. 0. 0.001333 -.000833 1.
 0. -.001333 -.000833 1. 0. -.0004000 -.000833 1. 0. 0.0004000 0.000833 1.
 0. 0.001333 0.000833 1. 0. -.001333 0.000833 1. 0. -.0004000 0.000833 1.
 0. 0.0004000 0.0002500 1. 0. 0.001333 0.0002500 1. 0. -.001333 0.0002500 1.
 0. -.0004000 0.0002500 1.
PATCH 3 3
OBJECT =DETECTOR.Extrude1.Face1

INTERFACE COATING "REFLECT" "AIR" "DETECTOR"
REDEFINE COLOR 2
SHIFT Z -0.00293

CURVES
POINTS -.4600000E-3 0.8000000E-3 0.2500000E-3 REL; $FAST 12 5
-.0004600 0. 0.0002500 1. -.0004600 0. 0.0000833 1. -.0004600 0. -.0000833 1.
-.0004600 -.0002500 1. -.0001533 0. 0.0002500 1. -.0001533 0. 0.0000833 1.
-.0001533 0. -.0000833 1. -.0001533 0. -.0002500 1. 0.0001533 0. 0.0002500 1.
0.0001533 0. 0.0000833 1. 0.0001533 0. -.0000833 1. 0.0001533 0. -.0002500 1.
0.0004600 0. 0.0002500 1. 0.0004600 0. 0.0000833 1. 0.0004600 0. -.0000833 1.
0.0004600 0. -.0002500 1.
PATCH 3 3
OBJECT =DETECTOR.Extrude1.Face2

INTERFACE COATING "REFLECT" "AIR" "DETECTOR"
REDEFINE COLOR 2
SHIFT Z -0.00293

CURVES
POINTS -.9200000E-3 0.4000000E-3 0.2500000E-3 REL; $FAST 12 5
 0. -.0004000 -.002500 1. 0. -.0001333 -.002500 1. 0. 0.0001333 -.002500 1.
 0. 0.0004000 -.002500 1. 0. -.0004000 -.000833 1. 0. -.0001333 -.000833 1.
 0. -.0001333 -.000833 1. 0. 0.0004000 -.000833 1. 0. -.0004000 0.000833 1.
 0. -.0001333 0.0000833 1. 0. 0.0001333 0.0000833 1. 0. 0.0004000 0.0000833 1.
 0. -.0001333 0.0000833 1. 0. 0.0001333 0.0000833 1. 0. 0.0004000 0.0000833 1.
134
INTERFACE COATING "REFLECT" "AIR" "DETECTOR"
REDEFINE COLOR 2
SHIFT Z -0.00293

CURVES
POINTS -.8100000E-3 0.000000 0.2500000E-3 REL; $FAST 12 5
-.0001100 0. -.0002500 1. -.0001100 0. -.0000833 1. -.0001100 0. 0.0000833 1.
-.0001100 0. 0.0002500 1. -.0000367 0. -.0002500 1. -.0000367 0. -.0000833 1.
-.0000367 0. 0.0000833 1. -.0000367 0. 0.0002500 1. 0.0000367 0. -.0002500 1.
0.0001100 0. -.0002500 1. 0.0001100 0. -.0000833 1. 0.0001100 0. 0.0000833 1.
PATCH 3 3
OBJECT =DETECTOR.Extrude1.Face4

INTERFACE COATING "REFLECT" "AIR" "DETECTOR"
REDEFINE COLOR 2
SHIFT Z -0.00293

CURVES
POINTS -.7000000E-3 0.7000000E-3 0.2500000E-3 REL; $FAST 12 5
0. -.0000700 -.0002500 1. 0. -.0000700 -.0000833 1. 0. -.0000700 0.0000833 1.
0. -.0000700 0.0002500 1. 0. -.0000233 -.0002500 1. 0. -.0000233 -.0000833 1.
0. -.0000233 0.0000833 1. 0. -.0000233 0.0002500 1. 0. 0.0000233 -.0002500 1.
0. 0.0000233 -.0000833 1. 0. 0.0000233 0.0000833 1. 0. 0.0000233 0.0002500 1.
0. 0.0000700 -.0002500 1. 0. 0.0000700 -.0000833 1. 0. 0.0000700 0.0000833 1.
0. 0.0000700 0.0002500 1.
PATCH 3 3
OBJECT =DETECTOR.Extrude1.Face5

INTERFACE COATING "REFLECT" "AIR" "DETECTOR"
REDEFINE COLOR 2
SHIFT Z -0.00293

CURVES
POINTS -.4600000E-3 0.3800000E-3 0.2500000E-3 REL; $FAST 12 5
-.0002400 -.0002400 0.0003000 1.0000000 -.0002400 0.0002400 0.0003000 0.3333333
0.0002400 0.0002400 0.0003000 0.3333333
INTERFACE COATING "REFLECT" "AIR" "DETECTOR"
REDEFINE COLOR 2
SHIFT Z -0.00293

CURVES
POINTS -.2200000E-3 0.7000000E-4 0.2500000E-3 REL; $FAST 12 5
  0. -.0000700 0.0002500 1. 0. -.0000700 0.0000833 1. 0. -.0000700 -.0000833 1.
  0. -.0000700 -.0002500 1. 0. -.0000233 0.0002500 1. 0. -.0000233 0.0000833 1.
  0. -.0000233 -.0000833 1. 0. -.0000000 -.0000833 1. 0. 0.0000000 -.0000833 1.
  0. 0.0000700 0.0002500 1. 0. 0.0000000 0.0000833 1. 0. 0.0000000 -.0000833 1.
  0.0000700 -.0002500 1.
PATCH 3 3
OBJECT =DETECTOR.Extrude1.Face7

INTERFACE COATING "REFLECT" "AIR" "DETECTOR"
REDEFINE COLOR 2
SHIFT Z -0.00293

CURVES
POINTS -.4600000E-3 0.4000000E-3 0.5000000E-3 REL; $FAST 12 5
-.0004600 -.0004000 0. 1. -.0001533 -.0004000 0. 1. 0.0001533 -.0004000 0. 1.
  0.0004600 -.0004000 0. 1. -.0001533 -.0001333 0. 1. -.0001533 -.0001333 0. 1.
  0.0001533 -.0001333 0. 1. 0.0004600 -.0001333 0. 1. -.0004600 0.0001333 0. 1.
  -.0001533 0.0001333 0. 1. 0.0001533 0.0001333 0. 1. 0.0004600 0.0001333 0. 1.
-0.004600 0.0004000 0. 1. -0.001533 0.0004000 0. 1. 0.0001533 0.0004000 0. 1. 0.0004600 0.0004000 0. 1.

PATCH 3 3

OBJECT =MESA

CURVES
LINE 1.760870 1.000000 0.000000 2.000000 1.000000 0.000000
LINE 2.000000 1.000000 0.000000 2.000000 2.000000 0.000000
LINE 2.000000 2.000000 0.000000 1.000000 2.000000 0.000000
LINE 1.000000 2.000000 0.000000 1.000000 1.000000 0.000000
LINE 1.000000 1.000000 0.000000 1.239130 1.000000 0.000000
LINE 1.239130 1.000000 0.000000 1.239130 1.175000 0.000000

POINTS 1.500000 1.325000 0.000000 REL; $FAST 12 5
-.2608696 -.1500000 0. 2. -.2608696 -.0903263 0. 1. -.2410121 -.0351950 0. 2.
-.2211545 0.0199363 0. 1. -.1844626 0.0621320 0. 2. -.1477707 0.1043277 0. 1.
-.1098305 0.1271639 0. 2. -.0518902 0.1500000 0. 1. 0.0000000 0.1500000 0. 2.
-.02410121 -.0903263 0. 1. -.2608696 -.0903263 0. 0.

LINE 1.760870 1.175000 0.000000 1.760870 1.000000 0.000000

COMPOSITE GAP -1 -1

UVSPACE .1

OBJECT .1; BOUND -.1

INTERFACE COATING "ABSORB" "AIR" "DETECTOR"

REDEFINE COLOR 2

SHIFT Z -0.00293

CURVES
POINTS -.4600000E-3 0.4000000E-3 0.000000 REL; $FAST 12 5
-.0004600 -.0004000 0. 1. -.0001533 -.0004000 0. 1. 0.0001533 -.0004000 0. 1.
0.0004600 -.0004000 0. 1. -.0001533 -.0004000 0. 1. 0.0001533 -.0004000 0. 1.
0.0004600 -.0004000 0. 1. -.0001533 -.0004000 0. 1. 0.0001533 -.0004000 0. 1.
0.0004600 -.0004000 0. 1. -.0001533 -.0004000 0. 1. 0.0001533 -.0004000 0. 1.
0.0004600 -.0004000 0. 1. -.0001533 -.0004000 0. 1. 0.0001533 -.0004000 0. 1.
0.0004600 -.0004000 0. 1. -.0001533 -.0004000 0. 1. 0.0001533 -.0004000 0. 1.

PATCH 3 3

OBJECT =DETECTOR.Extrude1.Face9

CURVES
LINE 2.000000 1.000000 0.000000 2.000000 2.000000 0.000000
LINE 2.000000 2.000000 0.000000 1.000000 2.000000 0.000000
LINE 1.000000 2.000000 0.000000 1.000000 1.000000 0.000000
LINE 1.000000 1.000000 0.000000 1.239130 1.000000 0.000000
LINE 1.239130 1.000000 0.000000 1.239130 1.175000 0.000000

POINTS 1.500000 1.325000 0.000000 REL; $FAST 12 5
-.2608696 -.1500000 0. 2. -.2608696 -.0903263 0. 1. -.2410121 -.0351950 0. 2.
-.22211545 0.1271639 0. 1. -.1844626 0.0621320 0. 2. -.1477707 0.1043277 0. 1.
-.0998305 0.1271639 0. 2. -.0518902 0.1500000 0. 1. 0.0000000 0.1500000 0. 2.
0.0518902 0.1500000 0. 1. 0.0998305 0.1271639 0. 2. 0.1477707 0.1043277 0. 1.
0.1844626 0.0621320 0. 2. 0.2211545 0.0199363 0. 1. 0.2410121 -.0351950 0. 2.
0.2608696 -.0903263 0. 1. 0.2608696 -.1500000 0. 0.
LINE 1.760870 1.175000 0.000000 1.760870 1.000000 0.000000
LINE 1.760870 1.000000 0.000000 2.000000 1.000000 0.000000
COMPOSITE GAP -1 -1
UVSPACE .1
OBJECT .1; BOUND -.1
INTERFACE COATING "REFLECT" "AIR" "DETECTOR"
REDEFINE COLOR 2
SHIFT Z -0.00293

$ECHO
GROUP -10
SHIFT 0 -0.00092 -0.0005
SCALE FROM M
RETURN
$CASE

******************************OTHER GEOMETRY******************************

!!CAP - ROUGH, REFLECTING SURFACE
SURFACE
  PLANE Z 0 ELLIPSE (CAPR) (CAPR) (APER)/(CAPR)
OBJECT 'CAP.TOP'
ROUGHNESS
  INTERFACE COATING "CAP" "AIR" "DET_MEDIA"
REDEFINE COLOR 17

!!BOTTOM 10 PIN HOLDER
SURFACE
  PLANE Z-(CAPH) ELLIPSE (CAPR) (CAPR)
OBJECT 'CAP.BOTTOM'
ROUGHNESS !!MODEL 1
  INTERFACE COATING "REFLECT" "AIR" "DET_MEDIA"
REDEFINE COLOR 17

!! CAP CYLINDER
SURFACE
  TUBE Z 0 (CAPR) (CAPR) -(CAPH) (CAPR) (CAPR)
OBJECT 'CAP.EDGE'
ROUGHNESS !!MODEL 1
  INTERFACE COATING "CAP" "AIR" "DET_MEDIA"
REDEFINE COLOR 17
BOUNDS -.3 +.2

!! VCSEL TOP
SURFACE
PLANE Z (DIST) ELLIPSE (TOPR) (TOPR)
OBJECT 'TOP'
INTERFACE COATING "REFLECT" "AIR" "DET_MEDIA"
REDEFINE COLOR 15

!! VCSEL SIDE
SURFACE
  TUBE Z (DIST)-0.0035 (TOPR) (TOPR) (DIST)-0.0035-0.00005 (TOPR) (TOPR)
OBJECT 'VCSELSIDE1'
  INTERFACE COATING "REFLECT" "AIR" "DET_MEDIA"
  REDEFINE COLOR 6

******************DYE WELL GEOMETRY******************************

!! OUTER CYLINDER
SURFACE
  PLANE Z (WELLZ) ELLIPSE (OUTERR) (OUTERR)
OBJECT 'OUTER.Z1'
  INTERFACE COATING "BARE" "AIR" "GLASS"
  REDEFINE COLOR 14

SURFACE
  TUBE Z (WELLZ) (OUTERR) (OUTERR) (WELLZ)+(WIDTH)+(WELLH) (OUTERR) (OUTERR)
  OBJECT 'OUTER.EDGE'
  INTERFACE COATING "BARE" "AIR" "GLASS"
  REDEFINE COLOR 14
  BOUNDS +.2

!! DYE IN CYLINDER
SURFACE (NEGZPLANE=101)
  PLANE Z (WELLZ)+(WIDTH) ELLIPSE (INNERR) (INNERR)
OBJECT 'DYE.Z1'
  INTERFACE COATING "BARE" "GLASS" "DYE"
  REDEFINE COLOR 13

!! AIR IN CYLINDER
SURFACE (POSZPLANE=102)
  PLANE Z (WELLZ)+(WIDTH)+(DYEH) ELLIPSE (INNERR) (INNERR)
OBJECT 'AIR.Z1'
  INTERFACE COATING "BARE" "DYE" "AIR"
  REDEFINE COLOR 12

SURFACE (DYETUBE=103)
  TUBE Z (WELLZ)+(WIDTH) (INNERR) (INNERR) (WELLZ)+(WIDTH)+(DYEH)
  (INNERR) (INNERR)
  OBJECT 'DYE.EDGE'
  INTERFACE COATING "BARE" "GLASS" "DYE"
  REDEFINE COLOR 13
**BOUNDS +.3 -.2**

**SURFACE**
- TUBE Z (WELLZ)+(WIDTH)+(DYEH) (INNER) (INNER)
- TUBE Z -(WELLZ)+(WIDTH)+(DYEH) (INNER) (INNER)

**OBJECT 'AIR.EDGE'**

**INTERFACE COATING "BARE" "GLASS" "AIR"**

**REDEFINE COLOR 12**

****************************SOURCE******************************

!! I used to make the emitting sources as several disks. Better way is below.

!! EMITTING DISK -Z (DYE) (DYER) (RAYNUM) ISO
!! EMITTING DISK Z (DYE)+0.01 (DYER) (RAYNUM) ISO
!! EMITTING DISK -Z (DYE)+0.5*(DYEH) (DYER) (RAYNUM) ISO
!! EMITTING DISK Z (DYE)+0.01+0.5*(DYEH) (DYER) (RAYNUM) ISO
!! EMITTING DISK -Z (DYE)+(DYEH) (DYER) (RAYNUM) ISO
!! EMITTING DISK Z (DYE)+0.01+(DYEH) (DYER) (RAYNUM) ISO

IMMERSE DYE !! The source is being made inside the dye, so you get wrong side errors
!! unless you specify that it is immersed in dye

!! Bounds object 0 (by default, the source to be created) by the dye cylinder
**OBJECT 0**

**BOUNDS -(DYETUBE) +(NEGZPLANE) -(POSZPLANE)**

**EMITTING SPHEROID 0 0 (WELLZ)+0.5*(DYEH) 1 1 0.6*(DYEH) 5000000**

**FLUX TOTAL 100**

**TITLE 'COLLECTION EFFICIENCY'**

**WINDOW Y -4 4 Z -5 (DYE)+(DYEH)+1**

**PLOT FACETS OVERLAY**

**SPOTS POSITION OVERLAY**

**TRACE PLOT 1000**

**WINDOW X Y**

**CONSIDER ONLY MESA**

**SPOTS POSITION** !! Important command! Calculates the flux on the mesa.

!! Output the flux on the mesa into text files
!! These can be imported into Excel and sorted
!! so that they can be made into vectors in MATLAB
!! for plotting.

$IF ? LT 11 THEN

$IO OUTPUT SIMTEST_D0.01.TXT +FILE(11) !! Opens file for outputing data

STATS

$IO OUTPUT CLOSE !! CLOSES FILE
$ELSEIF 11 LE ? AND ? LT 21 THEN
$IO OUTPUT SIMTEST_D0.51.TXT +FILE(11)  !! Opens file for outputing data
   STATS
$IO OUTPUT CLOSE !! CLOSES FILE
$ELSEIF 21 LE ? AND ? LT 31 THEN
$IO OUTPUT SIMTEST_D1.01.TXT +FILE(11)  !! Opens file for outputing data
   STAT
$IO OUTPUT CLOSE !! CLOSES FILE
$ELSEIF 31 LE ? AND ? LT 41 THEN
$IO OUTPUT SIMTEST_D1.51.TXT +FILE(11)  !! Opens file for outputing data
   STAT
$IO OUTPUT CLOSE !! CLOSES FILE
$ELSEIF 41 LE ? AND ? LT 51 THEN
$IO OUTPUT SIMTEST_D2.01.TXT +FILE(11)  !! Opens file for outputing data
   STAT
$IO OUTPUT CLOSE !! CLOSES FILE
$ELSEIF 51 LE ? AND ? LT 61 THEN
$IO OUTPUT SIMTEST_D2.51.TXT +FILE(11)  !! Opens file for outputing data
   STAT
$IO OUTPUT CLOSE !! CLOSES FILE
$ELSEIF 61 LE ? AND ? LT 71 THEN
$IO OUTPUT SIMTEST_D3.01.TXT +FILE(11)  !! Opens file for outputing data
   STAT
$IO OUTPUT CLOSE !! CLOSES FILE
$ENDIF
}


A.5 Simple light attenuation test

********************ATTENUATION_TESTS.INR********************
!! This code tests the attenuation of a light source as it passes through
!! an absorbing (dye) or absorbing and scattering (tissue) medium
!! Intensities are output to a text file so that they can be compared
!! to back of the envelope Beer-Lambert calculations in MATLAB

!! Written by Elizabeth Munro 2009

$DO 1 26
{
**********************************SYSTEM UNITS**********************************
SYSTEM NEW
RESET
UNITS MM
WAVELENGTH 625 NM     !! FOR DYE CASE
!!WAVELENGTH 675.5 NM    !! FOR TISSUE CASE
PIXELS 501
HALT 500000

!!!!DEFINE RAY SPLIT AND SCATTER CHARACTERISTICS
SPLIT 1500 MONTECARLO
LEVEL 1000
FRESNEL AVE

!!CREATE VOLUME SCATTER MODELS
!! FOLLOWS FORM VOLUME G MU_S’MU_A OBSCURATION RATIO
MODEL
  VOLUME 0.9 22.85'0.019 1     !! SCATTERING MODEL FOR TISSUE AT 625NM

!! DEFINE SYSTEM MEDIA
MEDIA
  1.40 SCATTER 1 'DYE'
  !1'1.34285E-6 'DYE'    !! DYE: CY 5.5 ABSORPTION AND INDEX FOR 1UM
CONCENTRATION

COATINGS PROPERTIES
  0 1 'TRANSMIT'
  0 0 'ABSORB'
  1 0 'REFLECT'

C=25     !! WIDTH OF TISSUE OR DYE BOX
W=?*0.2-0.2     !! DEPTH OF TISSUE OR DYE
***************TISSUE OR DYE BOX GEOMETRY***************

SURFACE
PLANE X (C) RECTANGLE (C) (C)
OBJECT 'TISSUE.FACE.1'
   INTERFACE COATING BARE AIR DYE
   REDEFINE COLOR 1
   SHIFT Z -(C)

SURFACE
PLANE -X -(C) RECTANGLE (C) (C)
OBJECT 'TISSUE.FACE.2'
   INTERFACE COATING BARE AIR DYE
   REDEFINE COLOR 1
   SHIFT Z -(C)

SURFACE
PLANE Y (C) RECTANGLE (C) (C)
OBJECT 'TISSUE.FACE.3'
   INTERFACE COATING BARE AIR DYE
   REDEFINE COLOR 1
   SHIFT Z -(C)

SURFACE
PLANE -Y -(C) RECTANGLE (C) (C)
OBJECT 'TISSUE.FACE.4'
   INTERFACE COATING BARE AIR DYE
   REDEFINE COLOR 1
   SHIFT Z -(C)

SURFACE
PLANE Z 0 RECTANGLE (C) (C)
OBJECT 'TISSUE.TOP'
   INTERFACE COATING BARE AIR DYE
   REDEFINE COLOR 1

SURFACE
PLANE -Z -(W) RECTANGLE (C) (C)
OBJECT 'TISSUE.BOTTOM'
   INTERFACE COATING ABSORB AIR DYE   !! Absorbing interface to act as a detector
   REDEFINE COLOR 1

GROUP -6 'DYE'

*************SOURCE*************

IMMERSE AIR
CONSIDER ALL
WINDOW X -2 2 Z -3 12
PLOT FACETS OVERLAY

IMMERSE AIR
GRID RECT Z 5 -4@1 2@101
   SOURCE DIRECTION 0 0 -1
FLUX TOTAL 100.0

TRACE PLOT 1000 COLOR 24  !! Show ray entry and direction

WINDOW X Y
CONSIDER ONLY TISSUE.BOTTOM
SPOTS POSITION

$IO OUTPUT ATTENUATION_TISSUE.TXT +FILE(11) !! Opens file for outputing data
   $REG W
   STATS
$IO OUTPUT CLOSE !! Closes file

}
### Appendix B  Results of light source noise analysis

**Table B-1: In vitro temporal noise analysis**

<table>
<thead>
<tr>
<th>Light Source</th>
<th>1x1 ROI</th>
<th>20x20 ROI</th>
<th>10 frames temporally</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single mode VCSEL</td>
<td>0.05±0.01</td>
<td>0.011±0.001</td>
<td>0.05±0.01 0.011±0.002</td>
</tr>
<tr>
<td>Multi mode VCSEL</td>
<td>0.018±0.005</td>
<td>0.006±0.002</td>
<td>0.017±0.006 0.006±0.002</td>
</tr>
<tr>
<td>Multi mode + 20° diffuser</td>
<td>0.012±0.003</td>
<td>0.006±0.001</td>
<td>0.011±0.004 0.006±0.001</td>
</tr>
<tr>
<td>Multi mode + 20° + 50° diffusers</td>
<td>0.015±0.001</td>
<td>0.006±0.004</td>
<td>0.014±0.001 0.006±0.004</td>
</tr>
<tr>
<td>LED (average between trials)</td>
<td>0.009±0.005</td>
<td>0.004±0.004</td>
<td>0.005±0.004 0.004±0.002</td>
</tr>
</tbody>
</table>

Note: Significant digits are based on trial averaging statistics. The error is taken to be one standard deviation of the data.

**Table B-2: In vitro spatial noise analysis**

<table>
<thead>
<tr>
<th>Light Source</th>
<th>1 frame temporally</th>
<th>10 frames temporally</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20x20 ROI, 1000 frames</td>
<td>20x20 ROI, 1000 frames</td>
</tr>
<tr>
<td>Single mode VCSEL</td>
<td>0.092±0.006</td>
<td>0.090±0.005</td>
</tr>
<tr>
<td>Multi mode VCSEL</td>
<td>0.025±0.003</td>
<td>0.024±0.003</td>
</tr>
<tr>
<td>Multi mode + 20° diffuser</td>
<td>0.014±0.005</td>
<td>0.012±0.005</td>
</tr>
<tr>
<td>Multi mode + 20° + 50° diffusers</td>
<td>0.014±0.002</td>
<td>0.011±0.004</td>
</tr>
<tr>
<td>LED (average between trials)</td>
<td>0.008±0.002</td>
<td>0.006±0.004</td>
</tr>
</tbody>
</table>

Note: Significant digits are based on trial averaging statistics. The error is taken to be one standard deviation of the data.
<table>
<thead>
<tr>
<th>Light Source</th>
<th>Rat 3 1x1 ROI</th>
<th>Rat 3 20x20 ROI</th>
<th>Rat 4 1x1 ROI</th>
<th>Rat 4 20x20 ROI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single mode VCSEL</td>
<td>0.0296</td>
<td>0.0238</td>
<td>0.0126</td>
<td>0.0044</td>
</tr>
<tr>
<td>Multi mode VCSEL</td>
<td>0.0117</td>
<td>0.0061</td>
<td>0.0124</td>
<td>0.0065</td>
</tr>
<tr>
<td>Multi mode + 20° diffuser</td>
<td>0.0075</td>
<td>0.0006</td>
<td>0.0063</td>
<td>0.0019</td>
</tr>
<tr>
<td>Multi mode + 20° + 50° diffusers</td>
<td>Saturated – no data</td>
<td>Saturated – no data</td>
<td>0.0077</td>
<td>0.0039</td>
</tr>
<tr>
<td>LED (average between trials)</td>
<td>0.0060±0.0007</td>
<td>0.0021±0.0009</td>
<td>0.0066±0.0007</td>
<td>0.0026±0.0007</td>
</tr>
</tbody>
</table>

Note: Significant digits for LED are based on trial averaging statistics. The error is taken to be one standard deviation of the data. For other sources, no trial averaging is available, and four significant figures are assumed.