Optical Coherence Tomography Laboratory

Location:
Chesterfield Building
701 W. Main St., Suite 410

LABORATORY RULES

Laser Safety

You will be using a low-power (<5 mW) an infrared superluminescent diode. While not a laser, this light source is considered a laser for the purposes of safety because it is spatially coherent, and can thus be focused to small spots (e.g., on your retina!) Because of the low power, eye protection (i.e. goggles) will not be required. However, because the beam is not in the visible wavelengths, we must take care to exercise common sense precautions, since you cannot tell if the beam is hitting your eye. DO NOT LOOK DIRECTLY INTO THE BEAM, and DO NOT POINT THE BEAM AT ANYONE ELSE. That means leave the scanner unit on its stand.

Equipment Safety

This concerns the safety of our lab equipment, which is actually in much greater jeopardy than you are. You should know that research optics components are in general very expensive. Even the simplest components in the room, such as simple 1" diameter first-surface mirrors, cost on the order of $100 each. Please read and understand the following rules which apply when working in any research optics laboratory:

1. DO NOT TOUCH OPTICAL SURFACES WITH ANYTHING, INCLUDING YOUR FINGERS AND/OR BREATH. An optical surface is any surface of a lens, mirror, beamsplitter cube, etc. which has been polished flat to very high tolerance and is usually specially coated with a hair-thin coating of metal or dielectric. Even very clean human fingers have enough oil on them to contaminate such a surface, ruining both it and your experiment. All optics you will be using are in black aluminum mounts, which is the only part you should ever touch.

2. DO NOT BREAK OPTICAL ELEMENTS. Most optical elements are made of glass or similarly fragile material, and yet are held in heavy metal mounts which can easily be tipped over or knocked into one another. Move them around very carefully.

3. DO NOT BREAK OPTICAL FIBER. You will be using optical fiber which is encased in a flexible plastic protective housing. The fiber can easily be broken by bending it sharply, dropping or sitting something on it, or pinching it between you and something hard (like the edge of a table).

4. DO NOT FORCE MECHANICAL COMPONENTS. You will be using mechanical translators and mirror aiming mounts which have fine (read: expensive) micrometer positioners. They are all relatively new and in good shape; if one of them will not turn, that means it is at the end of its range of motion. There are also some computer-controlled motion devices, which have very

accurate, but fragile mechanisms. Please do not put any force on anything that isn't obviously a handle.

5. BE CAREFUL ABOUT CHANGING ELECTRONIC SETTINGS. Some of the electronics control sensitive elements. Please understand what you are doing before changing settings.

1. INTRODUCTION

For this lab we will work in teams of two. Teams will be assigned and scheduled ahead of time. Each team will need to bring a USB stick with at least 200 MByte of free space.

In this laboratory, you will be investigating Optical Coherence Tomography (OCT), which makes use of optical interferometry, to perform tomographic biomedical imaging with spatial resolution approaching the wavelength of light. OCT has already been demonstrated to perform imaging in the eye and retina with spatial resolution an order of magnitude superior to competing techniques, making it very useful for ophthalmic diagnosis.

OCT uses a fiber optic interferometer to perform highly accurate and sensitive measurements of the spatial distribution of light scattering in tissue. OCT is analogous to pulse-echo imaging techniques such as ultrasound, in that two-dimensional images of internal tissue microstructure are built up from a series of laterally displaced longitudinal scans of tissue reflectivity versus depth. In any pulse-echo imaging technique, the spatial resolution is determined by the length of the incident pulse of radiation, which is in turn limited by its wavelength. In OCT, light is used rather than ultrasonic waves, and thus the potential imaging resolution is on the order of optical wavelengths (< 0.001 mm) rather than ultrasonic ones (~0.1-1.0 mm). Unfortunately, ultrashort light pulses that are only a few waves long are much harder and more expensive to produce than correspondingly short ultrasonic pulses. In order to circumvent this problem, we use a special technique called *low-coherence interferometry* (LCI). LCI makes measurements based on the *coherence length* of a continuously emitting light source, rather than the *pulse length* of a pulsed light source.

The system used in this lab is a research spectral domain OCT (SD-OCT) system. SD-OCT uses a broadband light source and a spectrometer to collect the LCI data. Since the data is collected as a function of wavelength, it must be Fourier transformed to provide data as a function of depth in the sample. For this reason, SD-OCT systems and swept-source OCT systems (SS-OCT) are often collectively referred to as Fourier Domain OCT (FD-OCT). This is in contrast to early OCT systems that were time domain (TD-OCT) and directly measured light scattered from the sample as a function of the pathlength of the reference arm.

The first part of this laboratory concentrates on collection of OCT data from the system used both a phantom (roll of tape) and human skin. In the second part of the lab, you will take the raw data from the spectrometer and write the data processing routine to generate OCT images.

2. MATERIALS:

Please identify that the following materials have been provided before proceeding.

- OCT System - Lumedica OQ Labscope 2.0 SX
- Workstation - 1080P monitor, wireless mouse and keyboard.
- Thor Labs IR Detector Card
- Roll of gift-wrapping tape

2. EXPERIMENT 1: OCT SYSTEM

In the first part of the laboratory, you will use a research SD-OCT system.

2.1 OCT Apparatus

The apparatus for this experiment is a new research OCT system made by Lumedica.

Identify all of the elements and trace the fiber paths of the interferometer as they are described below:

- The central component of the interferometer is the FIBER COUPLER, which is a small metal tube with 4 OPTICAL FIBERS coming out of it (two out of each end). Like the cube beamsplitter used in the Michelson interferometer lab, you can think of the fiber coupler as a 50/50 beamsplitter with one input port, two intermediate ports leading to SAMPLE AND REFERENCE ARMS of the interferometer, and one output port.

- Connected to the input port is a fiber leading from a SUPERLUMINESCENT DIODE (SLD), which is similar to a laser diode but emits over a broader range of wavelengths.

- The reference arm fiber of the fiber coupler is connected to a FIBER COLLIMATOR, which is a specially coated and mounted lens that collimates (makes parallel) the light expanding from the fiber tip. The collimated reference arm light passes through a lens and is then reflected back by a mirror. By adjusting the focal position of the lens, the power transmitted through the reference arm and back to the fiber optic can be adjusted. The lens and mirror are on manual adjustors and should not be touched.

- Light from the sample arm fiber is also collimated using a fiber collimator, and then is coupled into the optical pathway of a custom designed microscope. There is a MEMS mirror that scan the light in the x and y dimensions, under control of the OCT software. This is all enclosed within the scanning unit on the stand. Put the THOR LABS IR DETECTOR CARD on the stage under the microscope; you should see the sample arm OCT light on it.

- The output port of the fiber coupler goes to the spectrometer. The spectrometer includes a collimator assembly, a turning mirror, a transmission diffraction grating, an imaging lens set, and

a visible to near-infrared line scan array. All of these are packaged in 3D-printed housing which maintains alignment of the optics. The detector array is a one-dimensional CMOS line scan array made from silicon, which is near-infrared-sensitive material, and contains 2048 pixels. The near-infrared pixels can be read at 10 million times/second, but for this experiment will be run at approximately 5,000 lines (or A-scan)/second.

2.2 Methods

2.2.1 Set-up

1. Make sure you have identified all parts of the OCT apparatus and understand their basic function. You may wish to do the first part of the analysis, drawing a schematic of the LCI setup in your lab notebook, now.
2. All parts should be powered up and ready to go when you get to the lab.
3. Insert your USB stick into the USB hub connected to the computer. Set up a folder to save your data on your USB stick.
4. The software on the computer should already be running. Ask for help from an instructor if it is not. *Note: For this device to run correctly, the system's date needs to be set to May 2019.*
5. Locate the "data" folder in the quick access panel of a file explorer window. Raw OCT from the software will save to this directory.

2.2.2 Data Acquisition

Your main job in part I of this experiment is to obtain enough information to generate an OCT image of the phantom (roll of tape) and of human skin (from a finger and fingernail).

Each group may share the same roll of tape dataset, but each individual member should take a turn at the controls to acquire an image of his or her own finger to process independently.

1. Create a folder name in the first blank field in the top right corner. This will create a new directory in the "data" folder.
2. Press "Start Scan" in the main tab.
3. Place a stack of Scotch tape under the scanner. Adjust the stage so that you can see the top of the roll of tape at the top of the OCT image.
4. You may notice some horizontal line artifacts on the image. This is due to an improper background subtraction. Update the background by doing the following:
    a. Use the focus knob to move the OCT scanner far out of range of the tape.
    b. Click "Update Background."

c. You will also save a raw copy of the background. Enter a file name (*e.g.* tape_BG) in the second blank field in the top right corner. Press "Save raw b-scan."

5. Move the tape back into focus.
6. Rename the file (*e.g.* tape_RAW) and save the raw B-scan of the tape, and again copy the raw file to your personal drive.
7. Let the tape continue to be imaged for a few seconds, then press Stop Scan. This will enable the Save Image option.
8. Click Save Image. This will save a processed version of the image you are viewing.
9. Find the ".tif" image you just saved in the "Data" folder. Rename it to include your name and what the sample was (e.g., "Ken Adam Tape").
10. Click on the "Advanced" tab. This is the window in which you can view averaged images.
11. Click "Average Scans." The last 30 frames acquired in the buffer during Step 6 will be averaged and shown on the right panel. Click "Save Average Image" to save this image.
    a. Locate and copy/rename the file from the Data folder.
12. Collect images of the skin on your fingertip (for each lab partner).
13. Save the following, as performed above for the tape:
    - The averaged image
    - At least 1 of the processed OCT images
    - The corresponding raw image for the processed OCT image.
    - The background image.
7. Collect images of human finger nail
8. Save the following:
    - The averaged imaged
    - At least 1 of the processed OCT images
    - The corresponding raw image for the processed OCT image.
    - The background image.
9. Verify that you have all data saved on the USB drive at this point.
10. Remove USB stick.

2.3 Analysis

1. Draw a schematic of the LCI and label all of the components.

2. Plot a single spectrum from the spectrometer. Calibrate using the spectrometer info in item 3 in section 3.2.1

3. Calculate the central wavelength λ_0, the FWHM wavelength bandwidth $\Delta\lambda$, and the coherence length l_c from your measurements of the spectrum. Report all these measurements in μm.

3. EXPERIMENT 2: OCT DATA PROCESSING

In the second part of the laboratory, you will use the raw data collected in the first part of the lab to feed into a data processing routine that you will write in Matlab and generate OCT images.

3.1 Apparatus

The system is the same one used in the first part of the lab. You should have the data that was saved during the imaging session. This should include raw files, a background spectrum file and a processed OCT image. The goal of this part of the lab is to use the raw file and the background spectrum file to generate an image similar to the processed OCT images from the software on the OCT system.

3.2 Methods

3.2.1 Data Processing

1. **Load files from storage.**

   Load or read in the files acquired with the OCT system. For each sample this should include the processed OCT image, the raw OCT image, and a background image. The files are .tif's and should have 1024 pixels (from the spectrometer) for each of the 512 A-scans. Each pixel is saved at 2 bytes, but there is only data in the lowest 12 bits.

2. **Background subtraction.**

   In this step the background image is subtracted from the raw image, leaving just the fringe. Average across all of the A-scans in the background and then use this averaged A-scan to subtract from each A-scan in the raw file.

3. **Wavelength calibration**

   This spectrometer has 1024 pixels. The wavelength calibration for the pixels is available on Sakai as a MAT file. Load this vector into Matlab; each element is the wavelength in nm of the corresponding pixel.

4. **Resampling, wavelength to wavenumber**

   The spectrometer is calibrated in wavelength, but the FFT needs data that is linearly spaced in wavenumber as the input. The wavenumber $k = 2\pi / \lambda$, so we need to match the endpoints of the spectrometer and then generate a linear spacing between the endpoints. You can change the number of points in the array at this step, but we will resample into 1024 points.

   So

   $$k_1 = 2\pi / \lambda_{1024}$$

   and $k_{1024} = 2\pi / \lambda_1$

   Various types of resampling can be used, however for this exercise linear resampling is fine.

5. **Dispersion compensation**

   We will use empirical dispersion compensation for this processing. There are methods to both physically determine the dispersion in an OCT system and methods to numerically optimize the dispersion compensation, but for this exercise, trying numbers until the image "looks good" will be sufficient.

   The dispersion compensation takes the form of

   $$D_{1..1024} = e^{-i\,(E2 * n * n + E3 * n * n * n)}$$

   Where

   n is an array of 1024 numbers running linearly from -1 to 1

   E2 is an empirically derived number

   E3 is an empirically derived number

   This array D is multiplied by each A-scan, thereby adjusting the phase at each wavenumber (or the effective pathlength seen by that wavenumber).

6. **FFT**

   Each A-scan is now FFT (fast Fourier transformed). The output will be an array of complex numbers, but we only want the magnitude of each complex number for this processing

7. **Image scaling**

   The dynamic range of the OCT images is approximately 60 dB. In order to see contrast across this entire range, log plots are typically used. We will use 20 * log 10 on our data.

8. **Image display**

   Plot the image. Set the upper and lower limits to provide maximum contrast on the screen (i.e. match the upper limit to the maximum signal in the image) while minimizing the noise (i.e. set the lower limit so that the noise is reduced, but you don't lose the lower signal levels).

3.4 Analysis / Writeup

1. Plot, from your saved data, your best GREY SCALE B-Scan image of the tape phantom that illustrates the various terms and artifacts, and label them on your image. Make sure your image includes distance scales on both axes.
2. Plot, from your saved data, your best COLOR SCALE B-scan images of human skin. Include distance scales, lookup table, and note the imaging depth achieved. Point out any anatomical features in your image that you can identify.
3. Provide all Matlab functions you wrote to process and/or display data.

Questions to answer in your lab report:

4. What ways can you think of to increase the accuracy of the OCT image and to decrease the various artifacts? Can you think of a method to remove the complex conjugate artifact?
5. Describe how the raw data and processed OCT image would be affected by each the following errors. How can they be fixed?
    a. Excessive dispersion
    b. Excessive sample reflection
    c. Wrong reference path length