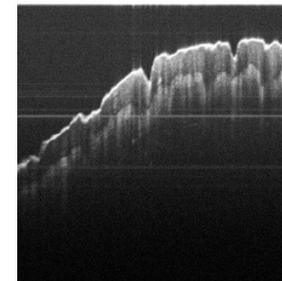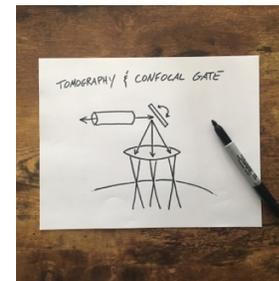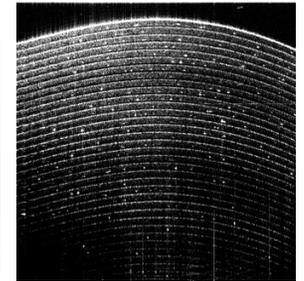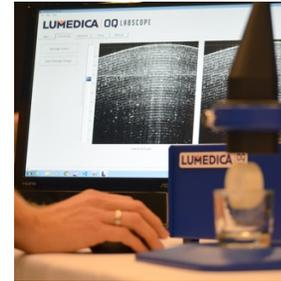# TEACHING PRINCIPLES & APPLICATIONS OF

OCT

OPTICAL COHERENCE TOMOGRAPHY
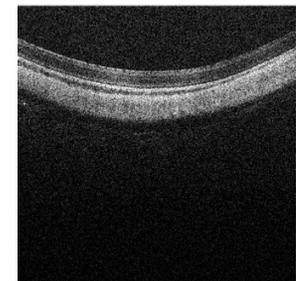
DEVELOPED AND PUBLISHED BY

LUMEDICA **Edmund** optics | worldwide

By W. Brown PhD, K. Chu PhD, Designed by S. Whitney

What is OCT?

If ultrasound can be thought of as the smaller scaled version of sonar, optical coherence tomography (OCT) is the miniature equivalent of radar. Like radar, OCT bathes its target in electromagnetic waves and measures the depths from which the "echoes" originated to form a depth-resolved image. Like ultrasound, these targets are frequently biological samples. But unlike radar, sonar, and ultrasound, OCT cannot rely on simply measuring the echoes quickly enough to form a 3D image. A typical human cell is approximately 10 μm in size, a distance traveled by light in 0.000033 nanoseconds – much too fast for today's fastest detectors to resolve. Instead, OCT uses principles of interference to determine from what depth a photon was reflected inside a sample. Ultrasound can see centimeters into tissue with millimeter resolution; OCT sees a few millimeters into tissue, but with μm level resolution. In transparent or translucent samples, OCT can see even deeper.

OCT images are formed one column at a time. A beam is focused to one point on the surface of a sample, and the depths of the photon reflections that occur beneath that point are aggregated into an image column called an A-scan. As the beam is scanned sideways, adjacent A-scans are compiled into a 2D image, called a B-scan. If multiple 2D images are acquired as neighboring planes, a 3D image, a C-scan, can be constructed. The Greek word "tomos" means "section" — our tomography images and volumes are, sensibly enough, acquired in sections.
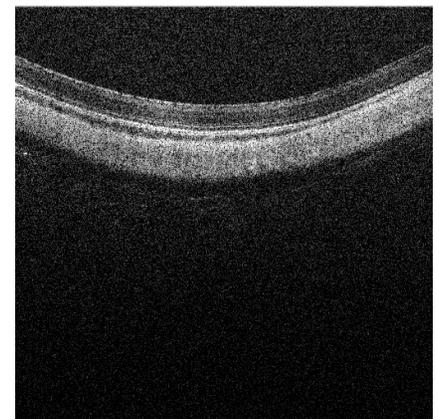

Roll of Scotch Tape
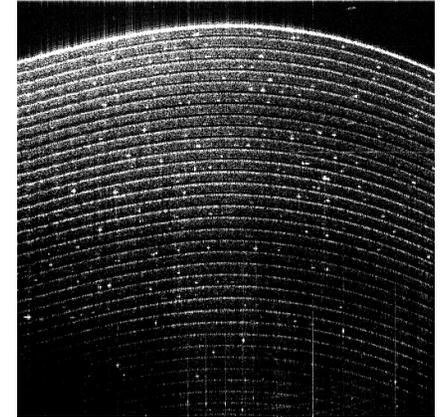
Skin on the back of a hand
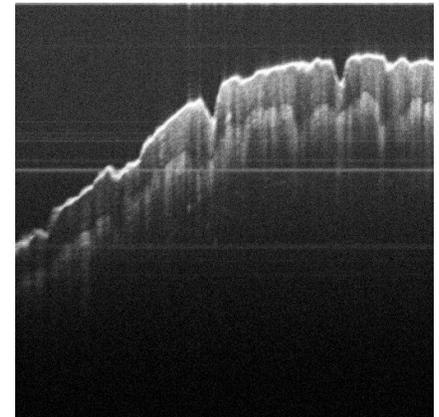
Retinal image from a rabbit

OCT vs. Other Image Modalities

Optical imaging modalities generally offer higher resolution than other medical imaging technologies, but are more limited in terms of penetration. Whereas OCT is capable of resolving on the order of 10 μm at depths up to a few millimeters. X-ray (and CT), MRI, and PET can see through the entire human body, but with resolution that is similar to ultrasound.

Given OCT's performance (depth resolving capability, ~10 μm resolution, ~ 1 mm penetration), it is particularly well-suited for diagnostic applications in which the layered organization of tissue may be disrupted by pathology. It is logical that the first widespread application for OCT has been in retinal imaging, where 9 distinct layers are densely packed into a 500 μm thickness, with all layers important to functioning vision. Furthermore, OCT light can pass readily through the eye to the retina; the eye is, after all, an organ meant to transmit light efficiently.

Imaging Modalities

Depth of Penetration (log scale)

1 m — 10 cm — 1 cm — 1 mm — 100 μm — 10 μm — 1μ

PET

CT & MRI

Ultrasound

OCT

Confocal Microscopy

1μ   10 μm   100 μm   1 mm   1 cm

Resolution (log scale)
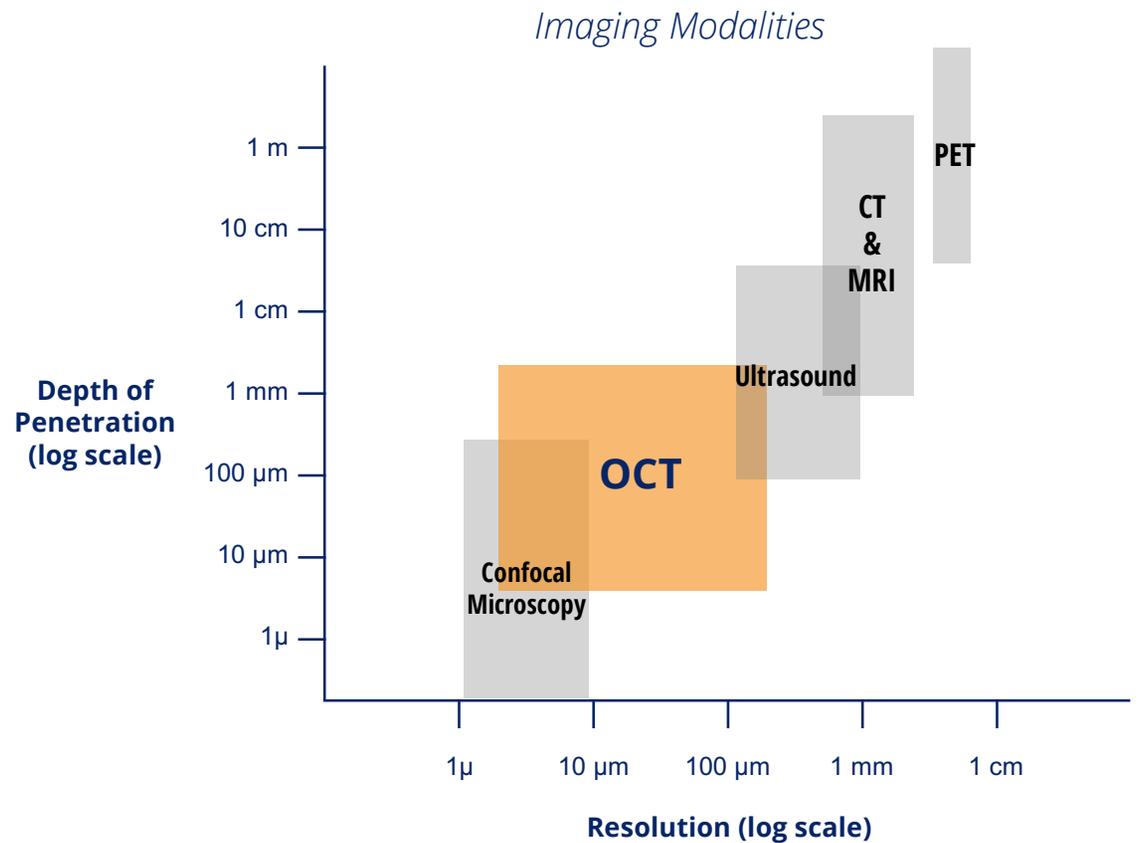
Low Coherence Interferometry

At the heart of OCT is the principle of interferometry. Imagine a light source that outputs a pure, single frequency, which can be represented in space and time as a sine wave. If we split that light into two paths, which may or may not be the same length, and then recombine the two paths, we will observe interference.

If the two paths were exactly equal in distance, then the two beams would recombine constructively; the crests and troughs from both halves would be arriving at the same time in perfect sync, and the output wave will be twice as strong as either half. But if the two paths were different by exactly half a wavelength, the crest of one wave would now be synchronized with the trough of the other wave. These two waves would destructively interfere and cancel each other out, resulting in no output. A device that accomplishes this wave splitting and recombination is an interferometer and is extraordinarily sensitive to differences in distance between its two paths.

But this type of interferometry has its limits. What if the difference between two paths was one whole wavelength? We would still observe constructive interference. We could not distinguish the one-wavelength difference from zero difference, or for that matter from ten wavelengths or a hundred. Any integer wavelength difference would produce constructive interference and thus be indistinguishable under this single-frequency interferometry. The problem is that the wave we are using is too periodic; there is no way to tell one period from the next.

Instead, let us now imagine a light source that outputs a multitude of frequencies (wavelengths) such that the field amplitude at any point in time is almost random. This light source is not periodic. Should we send this light through our interferometer, we will see constructive interference if the two paths are exactly matched, and at no other point. Coherence refers to the ability of light to interfere with other light; since this broad bandwidth light cannot interfere with itself at any point except an exact match of distance, it has very low coherence. Hence, interferometry with this type of light is low-coherence interferometry. The margin of error – the amount of path length difference that causes coherence to be lost – is called the coherence length and is inversely related to the bandwidth of the light source.

The centerpiece of an OCT system is a low-coherence interferometer, which splits broadband light into two paths. One path, known as the reference, uses a mirror to reflect the light at a known path length. The other path is the sample to be imaged. The presence or absence of light reflected from a depth corresponding to the path length set by the reference mirror determines the presence or absence of detected interference.

Constructive Interference


Deconstructive Interference

Tomography & Confocal Gate

Determining the depth of reflections is only half of OCT; we must also resolve objects in the lateral dimension. The lateral resolution in the OCT system is determined solely by the shape of the beam produced by the imaging optics and is independent of the axial resolution. As in all imaging systems, a higher numerical aperture (NA) results in a tighter focus and better lateral resolution. However, in OCT, we must be sensitive to a trade-off between lateral resolution and depth of field (or depth of imaging). The smaller the lateral spot size, the shorter the depth over which that focus can be maintained. Despite the capability of OCT to distinguish depths, that ability is wasted if the beam does not maintain its focus over a long enough range. It is for this reason that OCT systems generally have somewhat reduced lateral resolution when compared to confocal microscopy. The NA must be chosen to maintain a useful depth-of-focus, which limits the lateral resolution to 10-30 µm, depending on the exact configuration and application.

To create a tomographic image, the beam can be scanned across the sample to build a 2D and then a 3D image. This is typically accomplished with a pair of galvanometer (galvo) scanning mirrors. Each mirror scans in one axis and the two mirrors are configured orthogonally to each other to provide 2 dimensional scanning. Since the mirrors must be offset from each other, the scanning point is slightly different for each axis, which may result in some distortion of the scan pattern. More recently, OCT systems have begun using MEMS (micro-electromechanical system) mirrors where one mirror can scan in both axes. These mirrors are limited in size, but also consume less power.

Components of Tomography & Confocal Gate
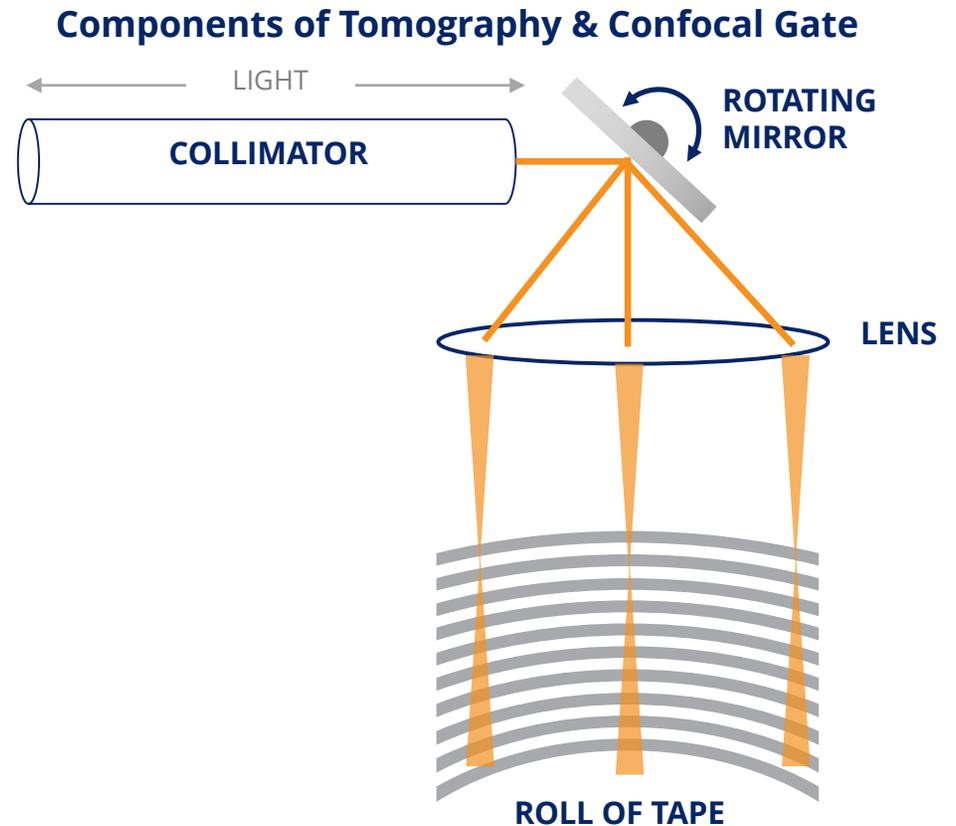
There are several types of

OCT

Time Domain

Fourier Domain

Spectral Domain

Swept Source

Advanced

Time Domain OCT

The first OCT systems were based on what is now known as a "time domain" architecture. Low-coherence interferometry was used as described above to determine how much light was being reflected from a depth corresponding to the reference mirror position. As the reference mirror swept through a range of depths, at any particular time, any light from the sample arm that was within one coherence length of the present reference position would generate interference. This signal would be observed at the detector as an AC signal with a frequency that corresponded to the speed of the reference arm and an amplitude proportional to the strength of the scatterers in the sample at a given depth. As the reference arm was scanned, a time series of the AC amplitude was recorded, giving an A-scan. This A-scan would then be repeated as the beam was scanned across the sample and consolidated into a B-scan.

TIME DOMAIN OPTICAL COHERENCE TOMOGRAPHY
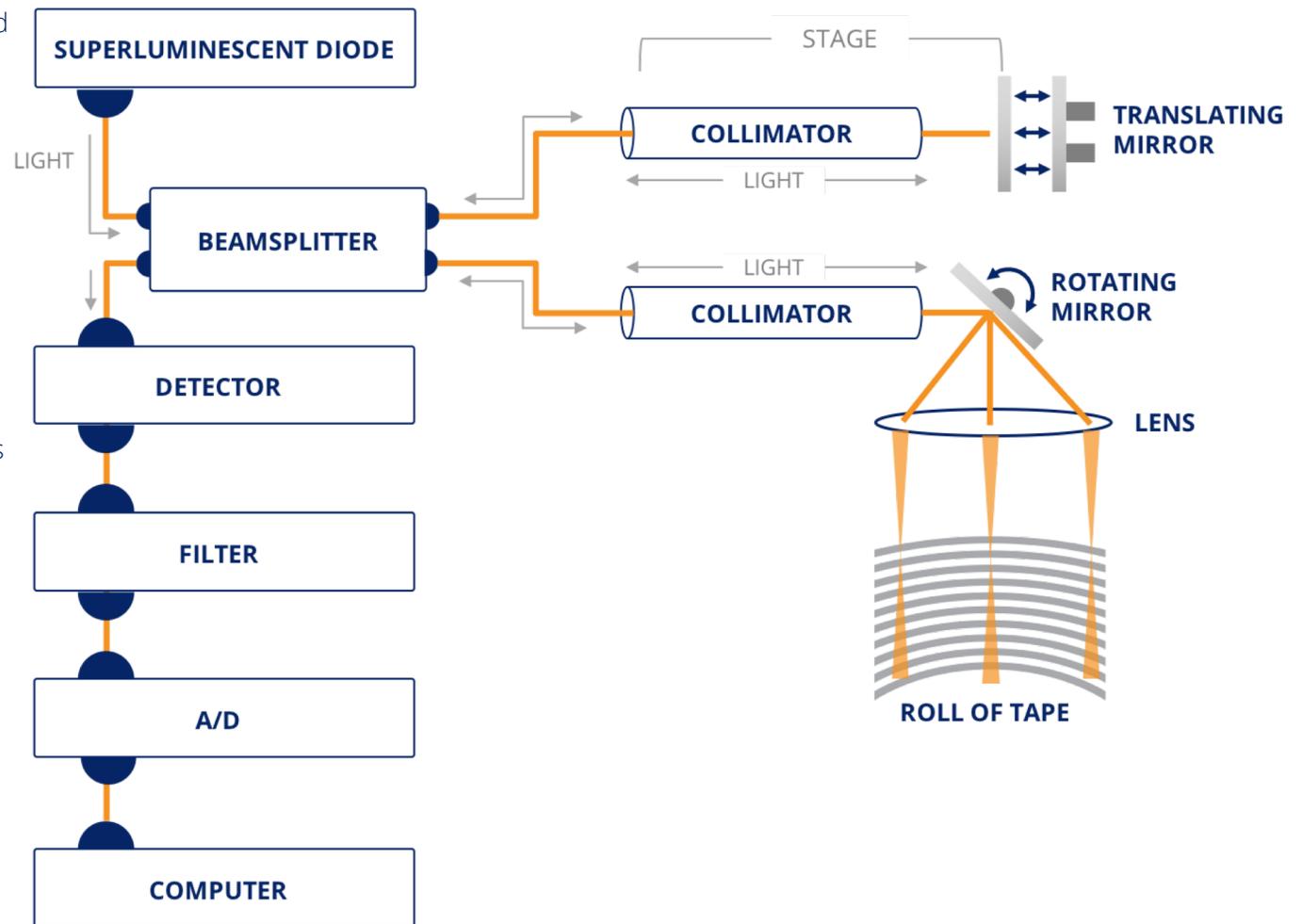
Fourier Domain OCT

Fourier domain OCT stems from a mathematical theorem that, at first glance, seems to have little applicability to OCT. The Wiener-Khinchin theorem states (approximately) that the autocorrelation of a signal is given by the Fourier transform of its spectrum. If we wanted to compute the autocorrelation of an electrical signal, for example, we could send that signal through a spectrum analyzer and then take the Fourier transform of that measured spectrum. But how does that have any relevance to OCT?

Low-coherence interferometry is, in essence, a correlation operation. When we integrate the two paths of an interferometer and feed it to a detector, we are essentially measuring the cross-correlation between the two waves; in OCT, we are specifically interested in the cross-correlation between the known reference and the unknown sample.

Now let us consider the reference R and the sample S following their recombination in the interferometer (R + S). An autocorrelation of this signal is simply the cross-correlation of (R + S) with itself. That operation distributes in the same manner as multiplication and becomes simply the sum of the autocorrelation of R, the autocorrelation of S, and the cross-correlation of R and S – our target signal. R originates from a single reflector, so the autocorrelation of R is a delta function centered at zero displacement. Sample reflectance is typically much lower in amplitude than the reference reflector, so the autocorrelation of S is often negligible. That leaves our target signal as the key ingredient in the autocorrelation of R + S.

We now have a way of extracting our desired cross-correlation signal from the autocorrelation of the interference. In turn, the Wiener-Khinchin theorem prescribes a way to determine the autocorrelation: by taking the Fourier transform of the spectrum of the signal. **This means that we can perform OCT by obtaining the spectrum of the interference signal and taking its Fourier transform.** All that is needed is a means of measuring the spectrum; the two primary methods are detailed in the following two sections.

The reference need not be scanned in depth to generate an A-scan in Fourier domain OCT; all depths are measured at once from a single acquisition of the spectrum of interference light, compressing hundreds or thousands of sequential measurements into one shot. The inefficiency of wasting photons while probing individual depths is thus also avoided.

The astute reader may question how a reference mirror can be used to interrogate low-coherence light with a path length difference of several millimeters if the coherence length is only a few μm long. Isn't the point of low-coherence interferometry to make these long-range interferences impossible?

The key lies in separating the analysis of the low-coherence light into its constituent wavelengths. Each wavelength is individually narrow-band and therefore long in coherence and can therefore be used to interrogate longer distances. The weakness of long coherence interferometry (the inability to resolve ambiguities from periodic behavior) is overcome by the simultaneous detection of many other frequencies; aggregating all that information together via the Wiener-Khinchin theorem allows us to have the best of both worlds.

Spectral Domain OCT

Spectral domain OCT uses the same broadband light source as time domain OCT. As described in the previous section, Fourier domain OCT relies on measuring the optical spectrum of the interference light. In spectral-domain OCT, this is accomplished by a high-resolution spectrometer in the detector arm of the interferometer. The spectrometer is typically matched to the wavelength range of the source and has from 512 up to 4096 pixels across the spectrum. The pixels are part of a line scan camera that integrates for the duration of an A-scan. The contents of each pixel are then digitized, read out, and sent to the processor. The processor performs the Fourier transform, typically a Fast Fourier Transform (FFT), and then generates the 2D B-scan image from a set of A-scans.

Because OCT images are formed via Fourier transform in FD-OCT, a number of Fourier transform properties inform the performance-limiting factors in SD-OCT. When performing a Fourier transform, the sampling frequency in the spectral domain determines length of the spatial domain signal; conversely, the bandwidth in the spectral domain corresponds to the resolution in the spatial domain. In SD-OCT, this means the number of resolvable wavelengths, which is most dependent on the number of pixels in the spectrometer, sets the OCT scan range available, i.e. the maximum depth over which OCT can be obtained. (Scan range should not be confused with penetration depth, which is limited by the loss of photons in a scattering tissue, or with depth-of-focus, which is limited by the loss of focus due to diffraction.) The combined bandwidth of the light source and the spectrometer determines the axial resolution available, a familiar property of OCT that is carried over from time-domain.

Spectral domain OCT systems have been typically built around either silicon or InGaAs sensors. Silicon detectors come in either CCD or CMOS implementations and have spectral responsivity from 450 nm to 1000 nm. Few OCT systems utilize visible light (400 to 700 nm), owing to the poorer penetration compared to infrared; in applications such as ophthalmology, the visible light would be seen by the patient during the scan and can also cause the patient to react and blink whereas infrared light does not. In the eye, 850 nm light provides good resolution and sufficient penetration to visualize the layers of the retina, while wavelengths beyond 1000 nm are increasingly absorbed by the water in the eye and do not provide sufficient retinal signal.

InGaAs is sensitive from about 900 nm out to 2600 nm. 1310 nm has been used, particularly for imaging of skin since it can penetrate much deeper than 850 nm light. The InGaAs components are generally move expensive than silicon components, particularly arrays with large numbers of pixels (> 1,000), which has limited the use of long wavelength SD-OCT systems.

The light sources used for SD-OCT are typically superluminescent diodes (SLDs), which are devices with the spatial coherence of a laser (allowing them to be focused to small spots and propagated through single-mode fiber optics) but with a much larger spectral bandwidth that confers low coherence properties. Research SD-OCT systems may employ more exotic or specialized broadband sources, including supercontinuum lasers or ultrafast pulsed lasers.

Spectral Domain OCT

**Spectral Domain Optical Coherence Tomography**
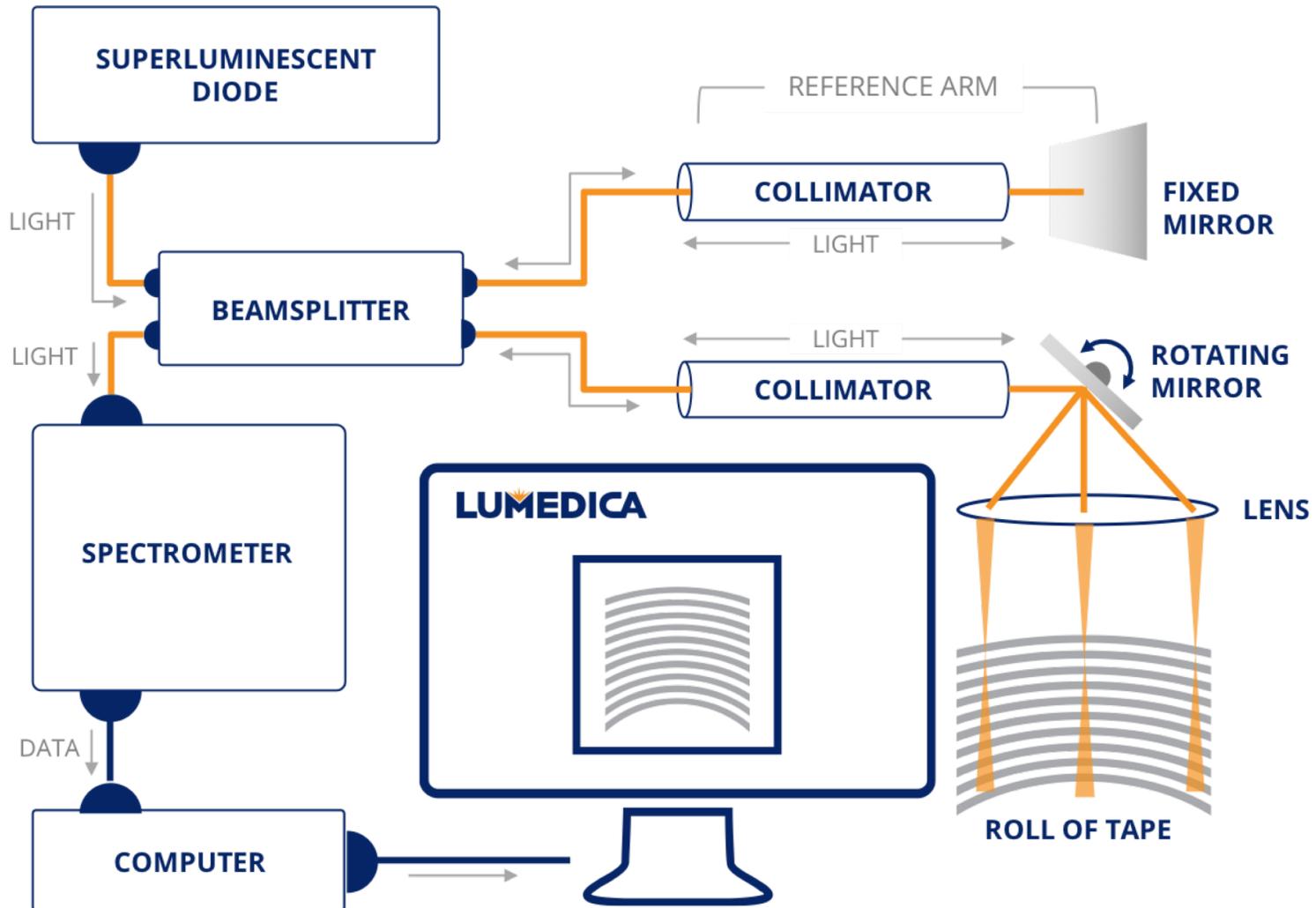
Swept Source OCT

An alternative to spectrally decomposing a broadband source is to temporally sweep the wavelength of a narrowband source. Swept source OCT (SS-OCT) uses a tunable laser, i.e. a laser where the wavelength can be changed in time. Like spectral domain OCT, the reference arm has a fixed path length. The detector arm now has a single high speed photodiode. As each point in time is matched to one wavelength of the swept source, a spectrum can be generated by measuring the interference signal over time. One wavelength sweep of the laser generates one A-scan. The photodiode and associated electronics must be fast enough to digitize the entire spectrum as the laser sweeps. The digitized spectrum is then Fourier transformed by the processor to generate a depth profile of the scattered light. In some implementations a 2nd photodiode is added in the source arm and used to provide balanced detection of the interferogram. This configuration provides background subtraction and also increases the signal to noise ratio.

Swept source OCT systems have been built at wavelengths from 1050 up to 1700 nm. This was partially driven by the work in tunable sources from 1530 to 1590 nm for telecom applications. Swept source systems typically have a larger overall imaging depth since the lasers intrinsically have a narrow linewidth, the equivalent of spectrometer resolution in SD-OCT, which sets the full imaging range. The axial resolution is often less, since it is more difficult to get a laser to sweep over a longer range of wavelengths.

Swept Source Optical Coherence Tomography
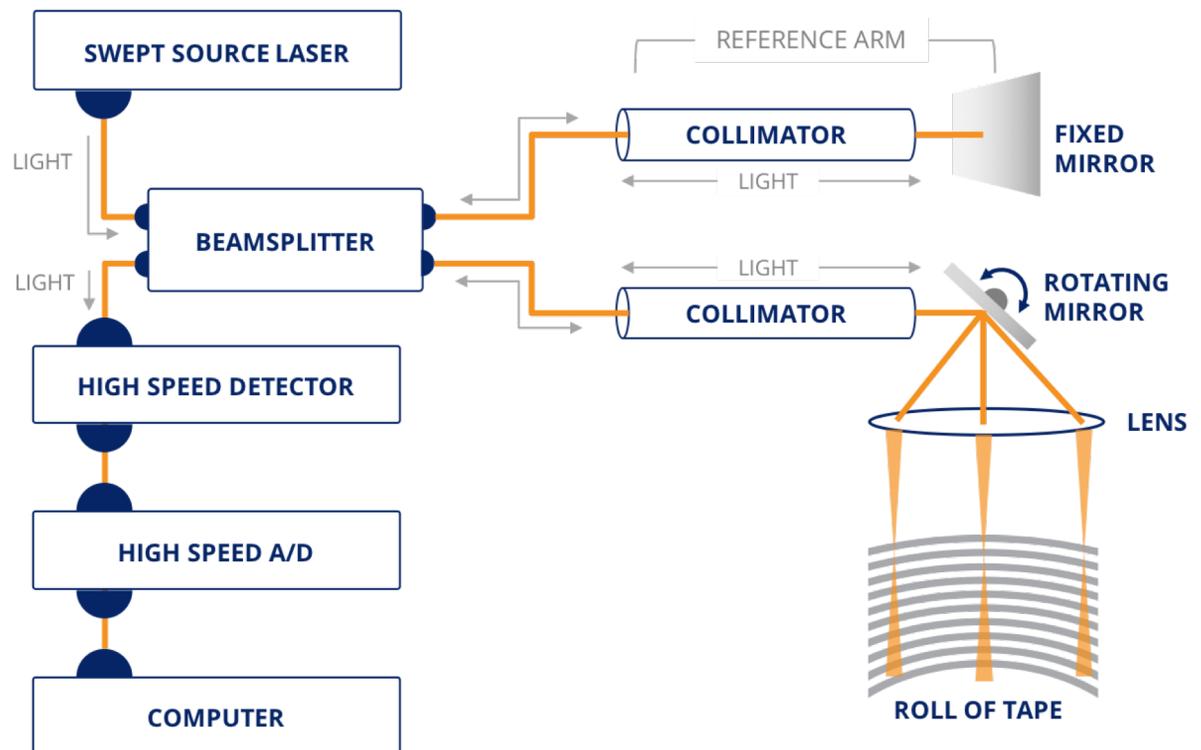
Swept source lasers and OCT systems are offered by multiple companies. They are used clinically in intravascular and dermatology OCT systems. There are 1050 nm swept source systems used for retinal imaging.

Variations on OCT

High Resolution OCT

As previously noted, the axial resolution of an OCT system is inversely related to the bandwidth of the source. So increasing the source bandwidth will provide smaller (better) axial resolution. The axial resolution also goes as one over the square of the center wavelength of the source, so shorter wavelengths provide better resolution for a given source bandwidth.

Typical retina OCT systems operate around 840 nm with an optical bandwidth of 50 – 60 nm, which gives an axial resolution around 5 µm in most samples. By increasing the source bandwidth above 100 nm, the axial resolution is now less than 3 µm. The figure shows a side by side comparison of a roll of Scotch™ tape imaged with an OQ PathScope system (3 µm resolution) and again using the PathScope with half of its bandwidth digitally blocked (~ 6 µm resolution).

Roll of Tape
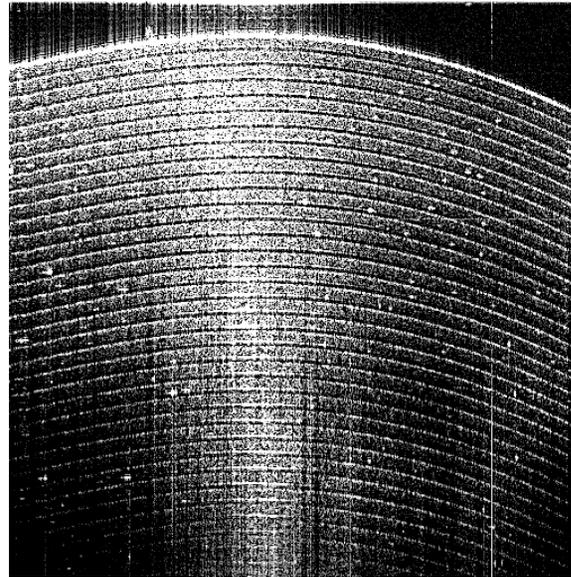
Standard Resolution Image
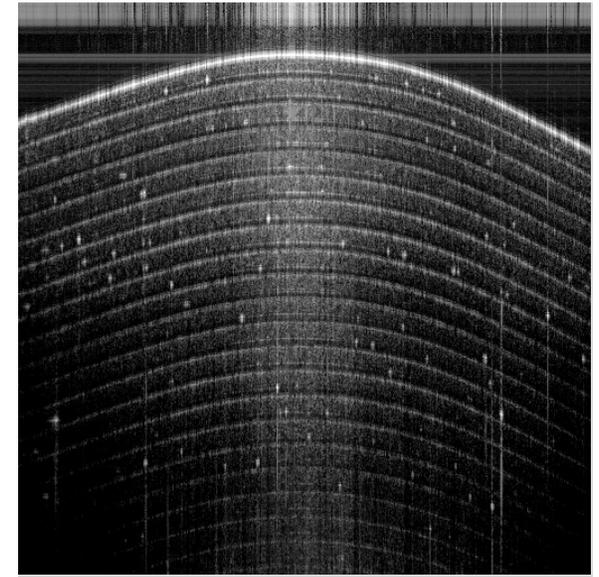
High Resolution Image

Variations on OCT

Advanced OCT

Several advanced variants are available to supplement the structural information provided by standard OCT imaging.

When imaging living samples that include blood circulation, it is often useful to quantify the velocity of flow. Just as the pitch of a train whistle rises as it approaches and drops as it recedes, the frequency of light is affected by the Doppler effect when it interacts with a moving medium. Light reflected from blood flowing away from the illumination is said to be red-shifted (longer wavelengths), while flow towards the light source causes it to be blue-shifted (shorter wavelengths). OCT instrumentation designed to be sensitive to these shifts can produce Doppler OCT images, in which the relative motion of the objects in the image can be color-coded.

The flow of blood is not always towards or away from the direction of illumination, however; in the retina, for example, the blood vessels that supply the retina run parallel to the surface, making it difficult for Doppler techniques to detect any flow. Other methods have been developed, generally termed OCT angiography, that are designed to detect flow in an arbitrary direction. These techniques generally rely on the dynamics of speckle. In all coherence imaging systems, including OCT, speckle is the result of rays scattered within tissue interfering with each other, causing bright and dark spots on the image. Generally, the appearance of speckle is undesirable, since it can obscure the true structure of the sample. However, OCT angiography methods use speckle advantageously. Because the pattern of speckle is determined by the interference of essentially random paths through the scattering tissue, minute changes, including the flow of blood, is enough to alter the pattern. OCT angiography works by measuring the speed at which new speckle patterns are generated, which is a marker for how quickly the blood is flowing.

Other advanced techniques have been developed to provide chemically specific information from OCT. One such method is spectroscopic OCT. In FD-OCT, the spectrum of the interference signal is already measured, and for standard OCT processing, objects in the sample are assumed to interact with all wavelengths of light equally. However, the spectrum can be divided into windows in order to examine the relative intensity of interference in different spectral regions. This analysis typically comes at the cost of resolution; dividing the spectrum into sections reduces the effective bandwidth.

Another type of functional augmentation is polarization sensitive OCT. Light, as a transverse wave, is characterized by a polarization vector in addition to an amplitude. Some materials, including many proteins, are birefringent, interacting differently with light depending on its polarization. Birefringence effectively rotates the polarization of the light that returns to the detector. Polarization sensitive OCT systems use two detectors to separately detect the two orthogonal orientations of the sample arm, then reconstruct the birefringence properties of the tissue based on the difference in polarization states of adjacent reflections.

Applications of OCT: Clinical

The largest commercial markets for OCT systems have been in the clinical space, primarily in ophthalmology. Numerous ophthalmic equipment companies sell OCT systems. Most ophthalmology offices throughout the U.S., Europe and Japan have an OCT system, and about half of Optometry practices also have one.

Ophthalmology

OCT first became available in the late 1990s and is now the standard of care for many diseases in ophthalmology. Numerous layers can be identified including the nerve fiber layer, ganglion cell layer, layer of rods and cones, retinal pigment epithelium, and others. The dip in the center of the image is the fovea where the highest concentration of cones are, which generate color vision. The larger hole on the left of the image where the optic nerve and blood vessels enter the eye.

OCT can be used to identify most retinal pathologies and diseases. OCT images will show retinal holes and detachments. Diseases such as diabetic retinopathy, diabetic edema, macular degeneration, and glaucoma can be diagnosed.
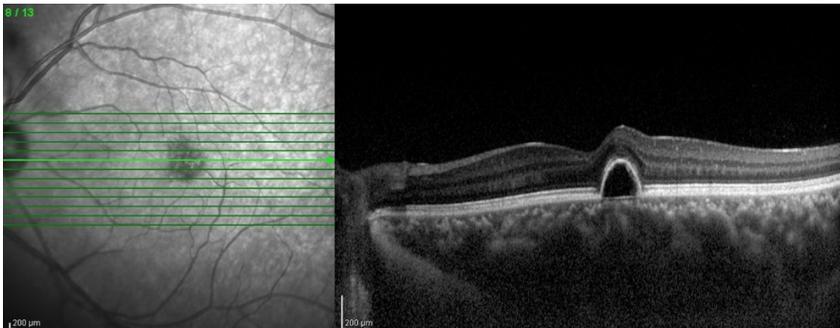
An OCT image of a normal human retina.

Intravascular

OCT is used to image the inside of blood vessels, primarily the arteries around the heart. Unlike ophthalmic systems, which can operate non-invasively, intravascular OCT is obtained by inserting a fiber-optic imaging probe via a catheter from the leg to the heart. A beam is projected sideways, out of the catheter sheath, to acquire A-lines that penetrate the wall of the vessels. The fiber-optic probe rotates to generate a B-scan that is circular, rather than rectangular, as the probe completes a full circle. 3D volumes can be obtained by pulling the probe back through the vessel as the rotational B-scans are acquired; this scanning configuration is known as a helical scan because of the spiral path of the OCT beam as it both rotates and retracts.

Intravascular OCT can image plaques and help to determine if they are stable or are likely to erupt and cause a heart attack or stroke. OCT also can be used for stent placement and to confirm that stents are properly expanded. It may also be used to follow up on stents after they have been implanted for some time.

Other Clinical Applications

Other clinical areas where OCT is being used include dental, dermatology, pathology, and intra-surgical. In dental market, the goal is to replace X-rays with infrared light, thus eliminating the health concerns around ionizing radiation. OCT also provides a much better image of soft tissue, such as the gums, compared to X-rays. In dermatology the goals include seeing deep enough into skin to help identify clinical conditions such as melanoma, or to access burn severity and thus determine needed therapeutic path.

Other Applications of OCT

Research

OCT, and in particular retinal OCT, is being used in research for imaging small animals such as mice and rats. Research areas include development of ophthalmic drugs and toxicity testing of all types of drugs. OCT has reduced the number of animals needed in many tests, since it can be performed longitudinally on living animals instead of needing tissue extraction at multiple time points.

Other research areas include ex vivo and in vivo tissue imaging of various types of animals.

Porcine cornea angle   *Rabbit retina*   *Mouse ear*   *Human fingernail*
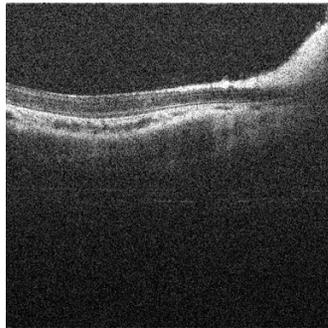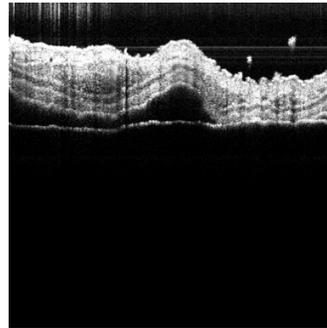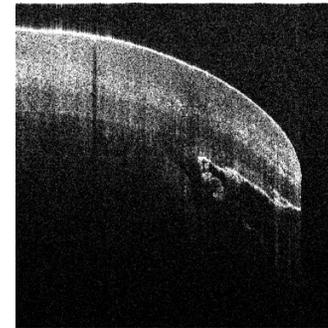
Non-Destructive Testing

Non-destructive testing has emerged recently as a new application of OCT. This uses OCT as a form of 3D imaging for samples with important features in the µm to millimeter range. It works particularly well with transparent or translucent samples. Some applications include imaging contact lens, imaging pill coatings, etc.
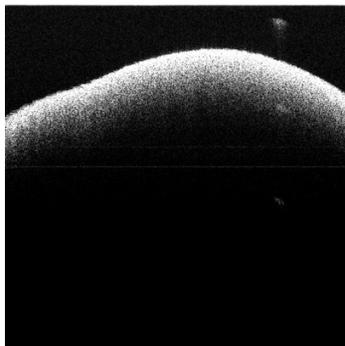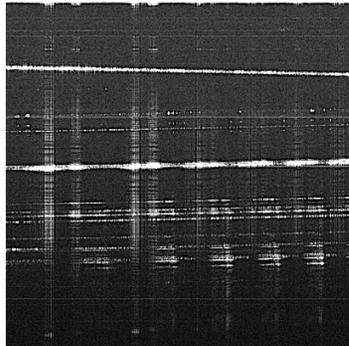
Ibuprofren Pill   *Smartphone Touchscreen*

Labs

with the OQ LabScope OCT system

These labs are designed to be conducted with the Lumedica OQ LabScope, available from Edmund Optics® [Stock Number #37-139]. For sales and product support please visit www.edmundoptics.com/OCT

Lab #1: Seeing Fringes

Summary: *In this exercise, you will observe interference fringes between the built-in reference arm and a mirror-like reflection from a glass slide. A glass slide is used instead of a mirror because the reflection from a mirror is typically so strong that it overwhelmingly saturates the spectrometer camera. You will observe the interference fringes with the glass surface placed at a shallow position and compare them with the fringes seen with the glass moved to a deeper position.*

Objective: *Understand low coherence interferometry. Measure light source coherence.*

Equipment needed:

- *Glass blank, such as a microscope slide*
- *Software, such as Matlab®, to analyze acquired data*

Steps

1. Start the LabScope software, and begin a scan.

2. Adjust the vertical position of the LabScope scan head so that it is about two inches away from the imaging platform. On the screen, there may be some white lines that appear across the field of view, but these should not move when the vertical position of the LabScope is adjusted. If the lines DO move up and down as the LabScope moves up and down, raise the LabScope position until the moving lines no longer appear.

3. Stop scanning and save a Raw B-scan. This is a reference scan. It contains no contribution from the sample, and shows only the light returning from the internal reference arm.

4. Resume scanning and switch to the Setup tab on the software. This shows the raw spectrum being collected by the spectrometer. This is also called a "raw A-scan," from just one of the 512 total scan positions covered in a B-scan. The raw B-scan (reference scan) you just collected was the raw A-scan you see on this screen, bundled together with the 511 other A-scan positions.

5. Switch back to the Main tab of the software. Place the glass blank on the imaging platform.

6. Lower the LabScope stand until the glass blank appears as a line across the field of view, near the top of the image. If the line appears fuzzy and appears to move down in the screen as you lower the LabScope, you have gone too far! You are looking at the complex conjugate of the glass surface. Raise the LabScope until the line appears sharp, and also gets lower on the screen as you raise the labscope.

7. Adjust the focus so that the glass surface line appears bright, but not so bright that it saturates the camera. The indications of saturation are multiple ghost lines of the glass surface appearing on the screen, or an excessive amount of white on the screen such that no lines are observable at all.

8. Stop the scan and save a Raw B-scan of your glass slide.

9. Resume the scan and switch to the Setup tab. Note the sinusoidal signal that is now superimposed on the spectrum. This sinusoidal signal represents the interference fringes between the sample (glass surface) and the reference.

10. Switch back to the Main tab. Raise the LabScope until the glass surface appears approximately in the middle of the vertical field of view. You may need to adjust the lens focus as you go to maintain visibility of the line.

11. Stop the scan and save another Raw B-scan of your glass slide.

12. Resume the scan and switch to Setup again. Now note the appearance of the interference fringes. They should now be smaller in width, i.e. higher frequency.

Data collection:

You should have collected:
- A raw B-Scan of only the reference light (no sample).
- A raw B-Scan of the glass surface as it appears near the top of the image.
- A raw B-Scan of the glass surface as it appears near the middle of the image.

Lab #1: Seeing Fringes (continued)

Analysis: The raw B-scans saved are uncompressed TIF files that can be opened with software such as ImageJ, Fiji, or Matlab®.

Open the raw reference spectrum you saved. Each of the 512 rows correspond to raw A-scans taken at different scan positions on the sample. Since there was no sample present in the reference spectrum, this image is 512 repeated measurements of the reference. Try averaging these 512 rows together for a less noisy version of the reference spectrum.

Now load the spectrometer calibration provided by Lumedica with your system. This gives the wavelength of light seen by each pixel on the spectrometer, i.e. each of the columns in your raw B-scans.

Using your reference scan and the calibration data, determine the center wavelength and the bandwidth of the LabScope light source. Once those parameters have been determined, calculate the coherence length of the LabScope light source.

Now load the spectrometer calibration provided by Lumedica with your system – you will need to compute the wavelength of each spectrometer pixel. The spectrometer calibration is saved as a set of 4 polynomial coefficients in your Lumedica software directory as "machine.Rev2.xml." Open this file in a text editor such as Notepad. Look for "CalibrationCoefficientA" through "CalibrationCoefficientD." Copy down these numbers. Create a linear array n from 1 to 1024. You can compute the wavelength of each pixel according to the following formula:

$$\lambda = A \cdot n^3 + B \cdot n^2 + C \cdot n + D$$

This gives the wavelength of light in nanometers seen by each pixel on the spectrometer, i.e. each of the columns in your raw B-scans.

Using your reference scan and the calibration data, **determine the center wavelength and the bandwidth** of the LabScope light source. Once those parameters have been determined, **calculate the coherence length** of the LabScope light source.

Questions:

1. In which case (glass surface near top of image, glass surface near middle of image) did the frequency of the interference fringe appear higher?

2. In this procedure, you were instructed to adjust liquid lens focus to prevent saturation. Is this a good way to prevent saturation for imaging biological samples?

3. What effect would doubling your light source bandwidth have on your coherence length?

Lab #2: Measuring Resolution

Summary: *In this exercise, you will measure both the lateral (horizontal) and axial (vertical) resolution of the LabScope system. You will cause an intentional defocus and analyze the effect on these two performance parameters.*

Objective: *Measure axial and lateral resolution of OCT system*

Equipment needed:

* *Glass blank, such as a microscope slide*
* *Software, such as Matlab®, to analyze acquired data*

Steps

1. Start the LabScope software, and begin a scan.

2. Adjust the vertical position of the LabScope scan head so that it is about two inches away from the imaging platform. On the screen, there may be some white lines that appear across the field of view, but these should not move when the vertical position of the LabScope is adjusted. If the lines DO move up and down as the LabScope moves up and down, raise the LabScope position until the moving lines no longer appear.

3. Press the "Update background." The remaining lines should disappear.

4. Place the glass blank on the sample stage and bring it into focus by lowering the LabScope until the surface of the glass appears near the top of the OCT image. You will likely need to tilt the glass to reduce its intensity on the OCT image to prevent saturation.

5. Shift the glass blank towards you (as you face the LabScope stand) until the edge of the glass appears in the middle of the field of view. The surface of the glass should look like a cliff, with a sudden plunge towards the bottom at its edge. (see figure on Page 20)

6. Stop the scan and save a B-scan (NOT a Raw B-scan).

7. Resume the scan. Shift the liquid lens focus until the brightness of the line representing the slide surface is noticeably less bright.

8. Stop the scan again and save another B-scan.

Data collection:

* A B-scan image of the glass slide surface. The surface of the slide should appear at the top of the image for about half of its width. The image of the slide edge should appear about in the center of the lateral field of view.

* A B-scan image of the glass slide surface at a sub-optimal liquid lens focus. The same features as the image above should still be observable, although image quality (both brightness and sharpness) may be decreased.

Lab #2: Measuring Resolution (continued)

Analysis: Open the properly focused B-scan in ImageJ or Fiji. Use the Line Selection tool to draw a line following the surface of the glass slide. Extend the line selection past the edge of the slide surface such that the selected line includes a significant portion without the slide surface.

Use the Plot Profile tool to generate a line profile of the selection. You should see something resembling a step function: a low value that jumps to a high value plateau. Zoom into the plot and determine the distance in pixels over which the plot transitions from 10% above the low end value to 10% below the high end value. This is known as the 90-10 knife edge response, and is one measure of lateral resolution.

Now select a line that runs vertically through the surface of the glass slide. Again, use the Plot Profile tool to generate a line profile. This time, you should see a low value for most of the field, punctuated by a sharp peak corresponding to the slide surface. Measure the number of pixels over which the peak is greater than or equal to half of its maximum value, relative to the low value that surrounds it. This is known as the full-width half-maximum.

Repeat these steps for the B-scan saved with intentional defocus caused by the liquid lens.

Questions

1. Did the defocus cause a more pronounced resolution change in the lateral or axial dimension? Why?

2. The saved images are processed and recorded on a logarithmic scale. How does this impact the measurements made in this exercise?

3. What units are the measurements made in this exercise? How would you convert them to distance units?

4. Would the axial resolution change if imaging an object submerged in water?

Surface of glass
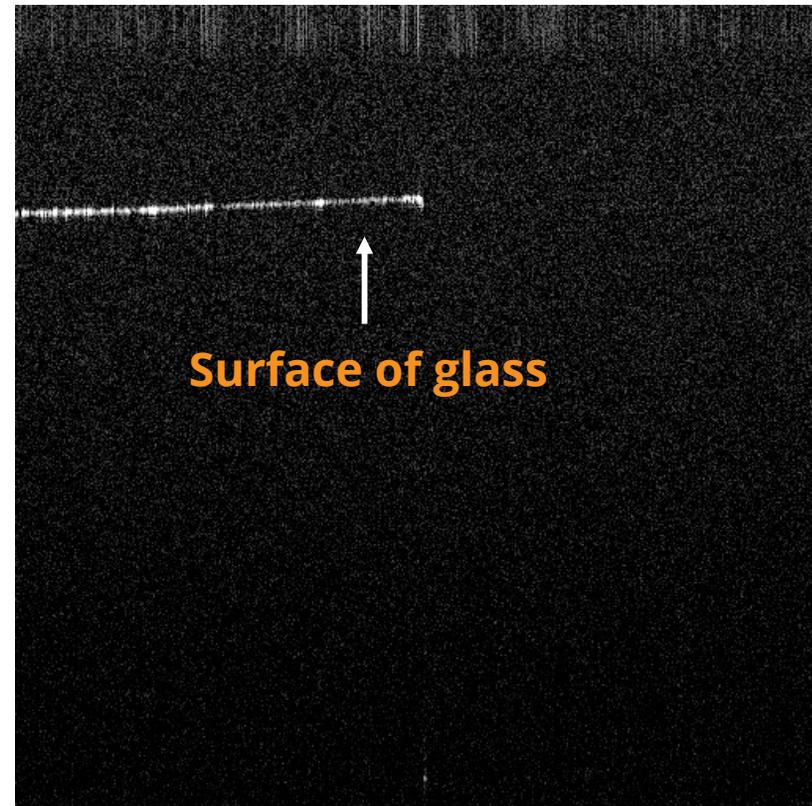
Lab #3: OCT Processing

Summary: *You will learn the OCT signal processing flow by processing your own raw OCT data into images. Although Fourier domain OCT relies on the simple principle of depth encoded by frequency, processing a FD-OCT image is not quite as simple as taking a Fourier transform. We must subtract the components of the spectrometer signal that are not related to interference, linearize the data in k-space, manage any uncorrected system and sample dispersion, and scale the output to maximize dynamic range.*

Objective: *Understand processing steps to get from raw spectral data to A-scan and then to B-scan.*

Equipment needed:

- *Scotch® tape phantom, comprised of at least 10 layers of cellophane tape stacked on a business or index card*
- *Software, such as Matlab®, to analyze acquired data*

Steps:

1. Start the LabScope software, and save a raw reference scan as detailed in steps 1-3 in Lab 1.

2. Place the tape stack on the sample stage and bring it into focus by lowering the LabScope scan head.

3. Stop the scan and save a Raw B-scan as well as a standard B-scan.

Data collection:

- A raw B-scan containing only reference light (no sample).
- A raw B-scan of the cellophane tape stack.
- A standard B-scan of the cellophane tape stack.

Analysis: This analysis ideally requires Matlab® or similar software, though an adept Excel user could even manage to program OCT processing on a spreadsheet.

The general steps are as follows: 1) background subtraction, 2) interpolation to a linear wavenumber space, 3) compensation of system dispersion, 4) Fourier transformation, and 5) output image scaling. It is recommended that you write a script to perform all of these steps in a single flow, as the dispersion component step will require you to iteratively try different coefficients and evaluate the effect on image quality.

Background subtraction: Load the raw B-scans from both the reference and the tape stack. Rather than directly subtracting the background image from the tape stack image, create a substitute reference image in which each row is a duplicated copy of the mean of the 512 reference rows. Now, subtract this averaged reference image from the raw B-Scan of the tape stack. Pay attention to data types; this subtraction should produce negative numbers, so be sure to recast the data as a type that supports negative numbers.

Resampling: By design, the spectrometer is approximately equally spaced in wavelength. However, FD-OCT produces sinusoidal interference patterns not as a function of wavelength λ, but wavenumber k:

$$k = \frac{2\pi}{\lambda}$$

You already know the wavelength of each pixel of the spectrometer, which is known as the spectrometer calibration. You must now convert those wavelengths to wavenumber according to the above equation. However, you'll find that the wavenumber spacing between pixels is not constant. You must first interpolate the raw spectral data you acquired to a linearized k-space.

Find the first (highest) k-value and the last (lowest) k-value after you've completed your wavenumber conversion. Now generate a linear vector, 1024 elements long, that progresses linearly (i.e. in equal increments) from the first to last k-value. In Matlab® the command 'linspace' accomplishes this task efficiently.

Now, use an interpolation method (in Matlab®, 'interp1') to interpolate, i.e. resample, the spectrum from its original sampling to the linearized k-space sampling. You must perform this resampling for each raw A-line, although in Matlab®, when 2D data is given to interp1, the interpolation is performed independently for each row in the 2D array.

Lab #3: OCT Processing (continued)

Dispersion compensation: Although the sample and reference arms are well balanced in terms of total optical path length, it is more difficult to manufacture a system with precisely the same lengths of all materials in sample and reference arms. If a lens in the scan head, which is part of the sample arm, is not matched by an identical lens in the reference arm, then a difference in dispersion will occur. In this case, each wavelength of light experiences a slightly different optical path difference between the reference and sample. The apparent position of a reflector in the sample would appear slightly shifted depending on wavelength. When the contributions of all the wavelengths are summed during the Fourier Transform step, the reflector becomes blurred in depth.

Dispersion can be compensated by multiplying each element of the raw spectrum by the phase that is equal and opposite to the physical wavelength-dependent path length shift. Phase is shifted by multiplying by a complex exponential: exp(iφ). We will model the dispersion with a third-order polynomial in phase. First, generate a linear vector from -1 to 1 with 1024 elements (one for each resampled spectrometer pixel). Now generate a dispersion phase function φ(n):

$$\phi(n) = -(b \cdot n^2 + c \cdot n^3)$$

where b and c are linear constants. You will plug this phase into your complex exponential and multiply each spectrometer pixel by its corresponding value. You have to experiment with different values of these coefficients to determine what combination yields the best axial resolution.

Fourier transform: Now, you are finally ready to perform the eponymous step of Fourier domain OCT: the Fourier transform. Compute the magnitude (absolute value) of the 1D Fourier transform of each row of your dispersion-compensated data from above.

Image scaling: The output from the Fourier transform step will span several orders of magnitude. Unaltered, your data at this stage will be on a linear scale; an object twice as reflective would have produced an image twice as bright. However, since OCT is sensitive to weak reflections, typical OCT images are displayed on a logarithmic scale to enhance the lower magnitude reflections. Compute the 10-base logarithm of your linear image data. Display this data as an image, and experiment with the white and black point settings, such as using the Matlab® command 'imagesc'. Adjust these values to your liking until the output closely matches the OCT images processed and saved by the LabScope software.

Questions

1. The Fourier transform of a 1024-length vector remains a 1024-length vector, yet the LabScope OCT output is only 512 pixels in depth. Why is the size of the vector reduced by half?

2. What would be the consequence of not saving a reference background image?

3. Why is there no zero or first order term to the dispersion correction?

4. Let's say we wanted to upsample our OCT data in the axial direction for finer pixel spacing. (This is commonly the case when we are trying to measure the axial resolution, for example). What would be an easy way of accomplishing this?

Lab #4: Index of Refraction

Summary: *OCT measures optical path length delay, which is a product of both physical depth and index of refraction, which is the factor by which the speed of light is slowed in a medium. Up to this point, we have been using path length interchangeably with physical depth. Now, we will examine how to use the LabScope to measure both the thickness and index of refraction of a glass slide.*

Objective: *Understand index of refraction. Measure index of refraction of a glass cover using OCT system.*

Equipment needed: *Microscope cover glass, a business or index card*

Steps:

1. Start the LabScope software and begin a scan. Update the reference background as needed.

2. Place the card on the sample stage. Position the LabScope scan head so that the card is visible in OCT. The card should appear as a scattering bright surface, not as sharp a line as a glass or mirror surface.

3. Place the cover glass on top of the card such that the top surface of the glass covers about half of the lateral field of view. Adjust the vertical position of the LabScope so that the top of the slide is visible near the top of the OCT image, but clear of any artifacts near the top of the screen. Adjust the liquid lens focus to optimize sharpness and brightness of the glass surface. The card surface should be visible throughout the lateral field of view, although the glass will cause the card to appear deeper than in the region not covered by the glass (see figure — right).

4. Stop the scan and save a B-scan.

Data collection:

- A B-scan showing, on one side, the surface of the paper card, and on the other side, the surface of the glass slide overlying the surface of the paper card.

Analysis:

This analysis can be completed entirely within ImageJ or Fiji. The **physical thickness** of the glass can be directly measured as the vertical distance between the edge of the glass and the top surface of the card WITHOUT the glass. As the difference in these depths is in air, there is no index of refraction correction needed.

The **optical thickness** of the glass is measured as the vertical distance between the surface of the glass and the top surface of the card under the glass. This distance is the physical distance measured above multiplied by the index of refraction of the glass.

The index of refraction of the glass is thus the optical thickness divided by the physical thickness.

Questions:

1. The glass slide makes the card underneath appear deeper than it really is. Are there any materials that would make the card appear shallower than its true position?

2. Suppose you submerged the entire sample (card and glass) in water. How would your image change?

Card surface (no glass)

Card surface (apparent depth Increased by glass)
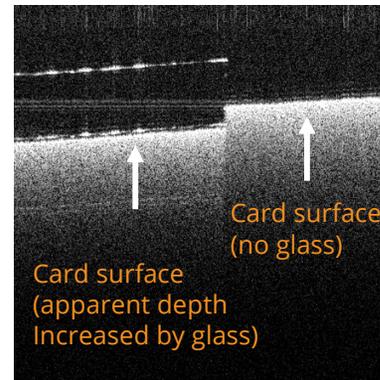
Lab #5: Measuring Sample Dispersion

Summary: *Each wavelength of light may be transmitted through a material at slightly different velocities (also known as index of refraction), an effect known as dispersion. We have previously discussed correcting the effects of dispersion introduced from the lenses and fibers of the OCT system itself. We will now examine the effect of dispersion introduced by a sample we are imaging.*

Objective: *Observe and characterize the effect of dispersion in a sample material.*

Equipment needed:

- *Plano-convex lens such as Edmund Optics ® #45-280 or similar, or a glass blank at least 3 mm in thickness*

Steps:

1. Start the LabScope software and begin a scan. Update the reference background as needed.

2. Place the plano-convex lens on the sample stage. Bring the top surface of the lens into focus by adjusting the vertical position of the LabScope.

3. Use the liquid lens focus to optimize the brightness of the top of the lens in the image.

4. Use the dispersion control settings (2 sliders) to optimize the sharpness of the top of the lens in the image. Record the sliders settings that correspond to the best sharpness.

5. Now lower the LabScope to bring the bottom of the lens into focus. The image of the top of the lens may "wrap around" the top of the image; this is ok, but make sure the image of the bottom of the lens is not obscured.

6. Adjust the liquid lens focus and dispersion sliders again to maximize the brightness and sharpness of the bottom surface of the lens. Record the dispersion settings again.

Analysis:

1. Subtract the dispersion coefficients that yielded the best results at the top of the lens from the coefficients that yielded the best results at the bottom of the lens.

2. Write the dispersion phase equation from Lab #3 using the difference coefficients you just computed. This is the dispersion caused by the glass lens.

Question:

1. Suppose you are using the LabScope to image objects embedded in the glass you examined in this exercise. The objects you are interested in are located approximately halfway between the top and bottom surfaces. What dispersion compensation coefficients would you expect to give you your best resolution?

Lab #6: 3D OCT Imaging of Biological Sample (fingertip)

Summary: *Acquire a 3D image of your fingertip and observe its subsurface features.*

Objective: *Explore the 3D imaging capabilities of OCT technology.*

Equipment needed: *Your finger*

Steps:

1. Start the LabScope software and begin a scan. Update the reference background as needed.

2. Place your finger, with your fingerprint facing up, on the sample stage. Position the LabScope and adjust its focus to obtain a clear image of the skin on your finger. Move your finger as needed to center the highest point of your finger at the center of the image. Try to hold your finger flat against the bottom of the sample stage.

3. Go to the Configuration tab. Change the number of B-scans per C-scan to 512.

4. Return to the Main tab. Click on "Capture C-scan." You will need to hold your finger as still as you can until the scan completes, approximately 1 minute.

5. Go to the Processing tab. Each of the 512 frames you captured previously are accessible here in a buffer. You can scroll through each frame using the slider.

6. Click "Save All Image." This will save a .jpg and a .tif of each frame, sequentially numbered, to a time-stamped subfolder in the data folder.

Data Collection:

- 3D scan of your finger

Analysis:

1. ImageJ includes 3D capabilities. You can import your 3D data in ImageJ or similar software to render in 3D.

2. Create an additional subfolder in the subfolder where your 3D data was saved. Move all .tif files, but not the .jpg files, into this new folder.

3. In ImageJ, go to File ⯈ Import ⯈ Image Sequence. Navigate to the new subfolder where your .tif files are. Select any of these files and click Open. ImageJ will now open this 3D data as a "stack" of 2D images.

4. Explore the 3D visualization features in ImageJ, particularly Image ⯈ Stacks ⯈ Orthogonal views. This gives you three cross-sectional views, XZ, XY, and YZ, and the ability to scroll through these cuts with your mouse. You can also render the data in a 3D volume that you can rotate and manipulate using Plugins ⯈ 3D ⯈ 3D viewer. If you do not see this option enabled, you must install the appropriate ImageJ plugin.

Questions:

1. The lab procedure instructed you to begin your 3D image after observing the highest point on your finger. Why is that important?

2. One of the ways you may view 3D images is by viewing cut-through slices in the XY, XZ, and YZ planes. Given that the 3D imaging requires obtaining multiple 2D images in sequence, in what plane or planes would you be most likely to observe motion artifacts? (The usual B-scan plane is the XZ plane – X refers to the first lateral dimension, Z refers to the axial/depth dimension.)

Answers to Lab Questions

Lab 1: Seeing Fringes

1. The interference fringes should be higher frequency when the glass surface appears farther away from the top of the image, i.e. deeper. This is the fundamental principle of Fourier domain OCT: the frequency of the interference fringe corresponds to the depth of the object producing the interference.

2. Adjusting the liquid lens can prevent saturation because as a beam defocuses, light collection is less efficient. However, the focal spot size is also enlarged, which is NOT desirable in normal imaging circumstances, as the lateral resolution would become worse. Though this is an easy way to adjust sample power in this exercise, you should not use focal adjustments strictly to prevent saturation. Better methods include angling the sample to the LabScope so that mirror-like (specular) reflections do not come directly back to the LabScope, or to lower the spectrometer camera gain.

3. Doubling the light source bandwidth in OCT would cut the coherence length in half. This would correspond to a doubling of axial resolution (assuming a correspondingly larger bandwidth spectrometer is utilized).

Lab 2: Measuring Resolution

1. Defocusing the beam should cause a loss in lateral resolution (i.e. a larger number), while the axial resolution should be nearly unchanged. This is because the lateral resolution is determined by the shape of the beam; when defocused, the beam size is larger, and the ability to resolve sharp edges is poorer. Axial resolution in OCT is derived primarily from the coherence properties of the illumination, which is not affected by defocus, and should be relatively constant.

2. The log scale amplifies weaker signals, which causes us to overestimate the width of peaks, resulting in poorer measured resolution than in reality. The proper way to characterize resolution is to use linearly scaled output. After you learn how to process your own OCT images in Lab 3, you will have the ability to scale your output as you see fit, including on a linear scale. If you wish to accurately characterize your LabScope, you may choose to repeat Lab 2 using linearly-scaled images of your own making.

3. The units are in pixels. They may be converted to µm by dividing your fields-of-view (both lateral and axial) by 512.

4. Water has an index of refraction of approximately 1.33, meaning light travels about 33% more slowly in water. If you visualize the coherence window of the OCT window as being a small pulse that propagates through space, that pulse gets shorter as the speed of light slows. Thus, the resolution in water is actually better than in air by a factor of 1.33. This is an important factor to consider, since most biological samples are aqueous.

Lab 3: OCT Processing

1. Since the spectrum measures only real signals, the Fourier transform of that spectrum must be symmetric. The bottom half of the transformed output is a mirror image of the top half and thus contains no additional information. It is usually cropped out.

2. Without saving a reference, the reference spectrum cannot be subtracted out prior to OCT processing. Instead of containing mostly the interference term, which is an oscillatory term about zero, the unsubtracted spectrum is all positive numbers. This offset creates artifacts that usually appear as a bright band near the top of the image.

3. The zero-order term is unnecessary because multiplying an entire signal by a constant complex number prior to Fourier transformation causes its phase to change wholesale, but not its magnitude. Since our OCT image is formed from only the magnitude of the Fourier transform, there would be no effect from a constant phase offset.

4. The first-order term is also unnecessary because it is a Fourier transform property that a constant phase slope becomes a simple shift after the transform. Applying a first-order phase term would shift the depth of objects in the OCT image, but would not improve their sharpness.

5. We can upsample our output image by "zero-padding" the spectrum just before taking the Fourier transform. This means, for example, taking our 1024-length spectrum and concatenating 3072 zeros at the end to form a 4096-length spectrum. When this vector is Fourier transformed, the resulting sampling density will be 4x higher. This does NOT yield superior resolution from the imaging sense; no new information is created by zero-padding. Still, this can be a useful way of improving measurements of distance involving small numbers of pixels, such as characterization of resolution.

Answers to Lab Questions (continued)

Lab 4: Index of Refraction

1. No material can increase the speed of light faster than in vacuum. Therefore no material can make a structure underneath it appear shallower than in reality.

2. The distance between the top and bottom surfaces of the glass would remain the same if the entire sample were to be submerged in water. This apparent distance is determined entirely by the glass itself. However, because the water has an index of refraction of approximately 1.33 (compared to approximately 1.5 for glass), the image of the card surrounding the glass would be pushed downwards, but not as far as under the glass.

Lab 5: Dispersion

The dispersion correction coefficients would be approximately the average of the best coefficients you obtained for the top surface and the bottom surface. In a uniform medium, such as the glass lens or slide, dispersion accumulates linearly; at the midpoint between the surfaces, approximately half of the dispersion of the total thickness would exist.

Lab 6: 3D imaging of the fingertip

1. Recall that objects that appear too close to the imaging lens do not disappear from the image, but instead begin to wrap back around as the complex conjugate artifact. If the highest point in a 3D object is not in your starting frame, it is possible that it would wrap around and be improperly imaged when the 3D scan begins, which would disrupt your ability to view the 3D volume.

2. The X axis, along which B-scans are performed, is the fast axis – the scanning is configured to occur in this plane quickly enough on the LabScope to be near video rate. The scanning from plane to plane is comparatively slow; several seconds may elapse before the entire volume is completed. Any motion in the sample during that time will cause distortions in that slower Y direction. Therefore, the XY and YZ plane views may show evidence of motion artifacts if the sample was not completely still.