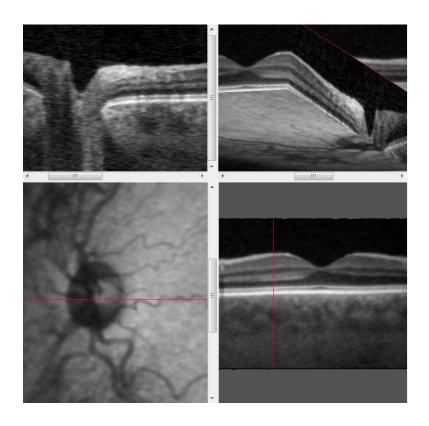
University of Cambridge Engineering Part IB Paper 8: Selected Topics

Bioengineering Imaging the Eye 1: Optical



Data from B. Povazay et. al, reconstructed using Stradview

Graham Treece April 2024

Imaging the eye: Syllabus

1. 2D Optical imaging of the eye

- Introduction
- Fundus imaging and confocal optics
- The Scanning Laser Ophthalmoscope

2 & 3. Optical Coherence Tomography (OCT)

- Time-domain OCT systems
- Spectral OCT systems

3 & 4. Ultrasonography of the eye

- Basic principles of ultrasonography
- System resolution and relationship to OCT

4. 3D Display

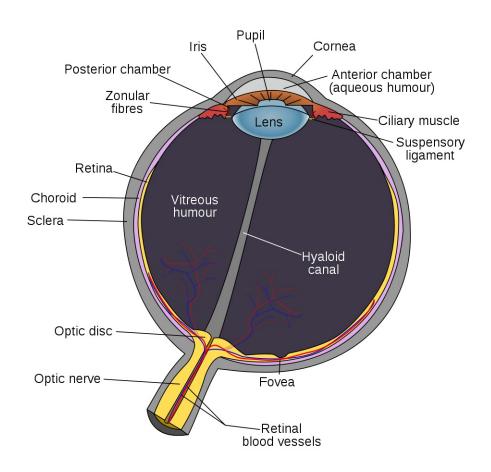
Display from 3D data

Lots of online interactive tutorials in optics:

http://zeiss-campus.magnet.fsu.edu/

https://www.olympus-lifescience.com/en/microscope-resource/

What are we looking at?



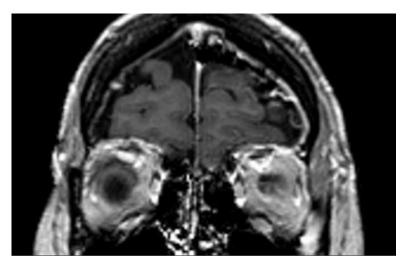
We are interested in imaging for *Ophthalmology* (the medical specialty concerned with all aspects of eye care), rather than *optometry* (examination of the eye for corrective lens prescription and some diseases).

We will concentrate on imaging the *posterior segment* (back of the eye or *fundus* including the retina) rather than the *anterior segment* (front of the eye including the pupil and cornea).

What sort of imaging techniques?

We will look at various optical techniques and ultrasonic imaging of the eye.





You can also image the whole head, including the eyes, using Computed Tomography (left) or Magnetic Resonance Imaging (right). This is useful in some cases, but much lower resolution than the techniques we will be looking at.

We will be paying particular attenuation to imaging techniques which acquire 3D rather than 2D data.

What are we looking for?

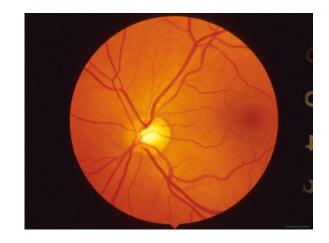
- Glaucoma A structural or functional disorder of the optic nerve, leading to visual field defects or blindness. Often (but not always) linked to increased intra-ocular pressure.
- Age-related macular degeneration (AMD) The leading cause of irreversible blindness in the developed world. Primarily caused by dysfunction of the retinal pigment epithelium leading to atrophy and drusen formation.
- Choroidal neovascularisation A characteristic of one form of AMD, in which new blood vessels grow from the choroid into the sub-retinal pigment epithelium.
- Retinal detachment Where fluid builds up underneath the retina, perhaps due to previous damage, causing the retina to come loose from the retinal pigment epithelium.
- **Diabetic retinopathy** The most common diabetic eye disease in which the retinal blood vessels swell, or abnormal new blood vessels grow.

Ophthalmoscope

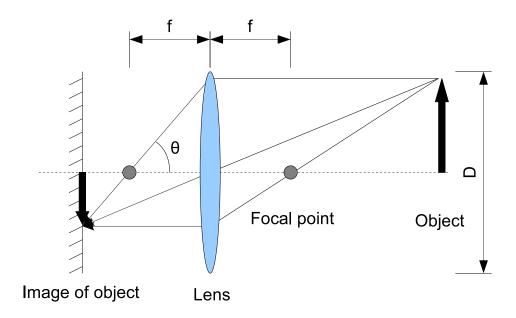


The ophthalmoscope is probably the most common device for looking in the eye. It is a simple manual device with lots of different lenses: you turn a dial to select the correct lens given the patient's eyesight. It requires a bit of practice (ask any medical student!) to see good images with it.

This is what you would see if it actually generated image *data*, and you could focus it successfully. The bright spot is the optic disc, the dark spot the macula and the red lines are blood vessels.



Imaging with a lens



This is the basic configuration for imaging with a single thin lens. Light at infinity all passes through the focal point to form an inverted image on the image plane (usually a CCD array).

There are two ways of describing the lens, either numerical aperture NA or f-number (written f/#) N:

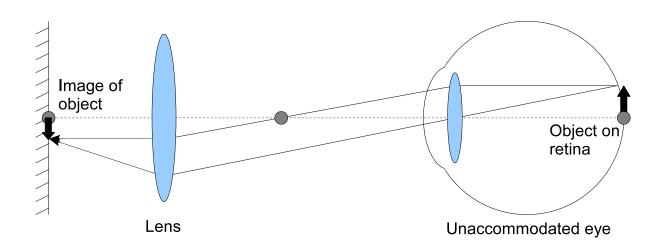
$$NA = n\sin\theta, \qquad N = \frac{f}{D} \tag{1}$$

where n is the refractive index (for air, n = 1). But:

$$NA = n \sin \tan^{-1} \frac{D}{2f}$$

$$\approx \frac{n}{2N}$$
(2)

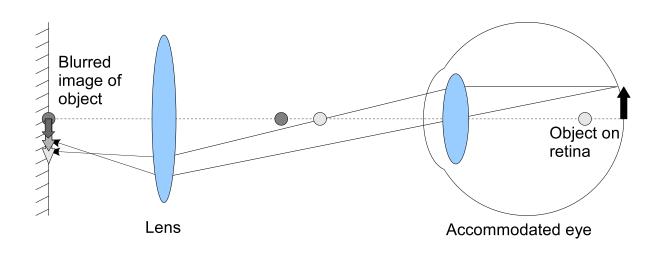
Focusing on the fundus



If we want to image the fundus (back of the eye) we have to look through the eye lens which has its own focusing effect. If the eye is *unaccommodated* — i.e. focused at infinity — we can set up the camera to also be focused at infinity, and we will end up with the image we want of the back of the eye.

Effectively the eye does half the job for us!

Focusing on the fundus



However, if the patient is not looking at infinity, for instance if they are looking at the camera rather than a far wall, then the eye will be *accommodated* — i.e. focused at a near point — and we will end up with a blurred image of the retina.

In general we need to adjust the camera for each patient according to their own optical properties. Ophthalmoscopes have a set of lenses so you can pick whichever gives you a clear image. In this case adjustment also has to be made for the clinician's optics.

Fundus camera

So why can't we just focus a normal digital camera at infinity, set the zoom reasonably high, turn the flash on and point it at an eye¹?



Clearly something interesting is happening - there is a much more in focus circular region in the middle which is showing *something* from the inside of the eye. But it is not very useful!

We are missing at least two things here: most importantly, the incoming light (flash) is reflecting off the cornea, so that is all we end up seeing. But in addition the resolution is not very good.

¹This is not to be regarded as a recommendation . . .

Fundus camera



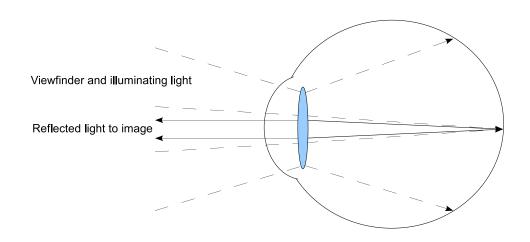
Modern fundus cameras record digital images: the previous ophthalmoscope image was actually from a Fundus Camera. On the right is another, taken using green light.

The fundus (posterior region of the inside of the eye) includes the retina, optic disc and macula. This is a modern fundus camera, which is much more sophisticated than the manual ophthalmoscope.



You can use light filters and also various dyes (for angiography) to show specific anatomical features.

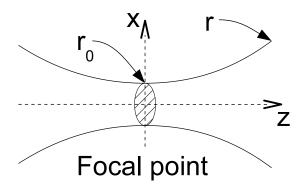
Illuminating the fundus



To avoid reflections off the cornea, the illumination is through an annulus around the outer circumference of the dilated pupil, which generally needs to be at least 3 mm diameter for a successful image. The reflection is then observed through the centre of the pupil.

Because the observed light is not coincident with the illuminating light on the cornea, reflections are considerably reduced. However, we can only see about one sixteenth of the illuminating light, which limits image quality given there is a sensible maximum illumination.

Lens resolution



We assume that a light beam has a circular cross-section, and at the focus, the intensity varies with x as a Gaussian function:

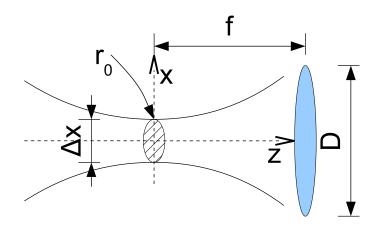
$$I(x) = I(0) \exp^{-\frac{2x^2}{r_0^2}} \tag{3}$$

It can be shown that the radius of this beam has the following form as the beam diverges:

$$r^{2}(z) = r_{0}^{2} \left(1 + \left(\frac{\lambda z}{\pi r_{0}^{2}} \right)^{2} \right)$$
 (4)

where λ is the wavelength of light, and $r = r_0$ at the focal point z = 0.

Radial (lateral) resolution



The *radial resolution* is the focal spot size $\Delta x = 2r_0$. At the lens, z = f, and $r \approx \frac{D}{2}$, hence from (4):

$$\left(\frac{D}{2}\right)^2 = \left(\frac{\Delta x}{2}\right)^2 \left(1 + \left(\frac{4\lambda f}{\pi \Delta x^2}\right)^2\right)$$

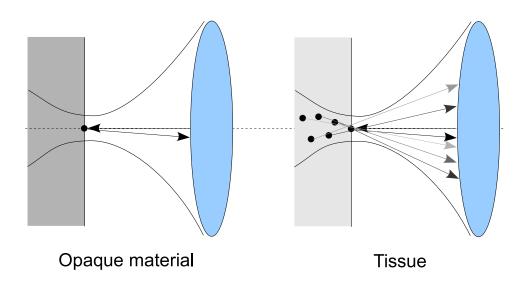
But $D \gg \Delta x$, so we ignore the 1 then square root:

$$D = \frac{4\lambda f}{\pi \Delta x} \quad \Rightarrow \quad \Delta x = \frac{4\lambda f}{\pi D}$$

Using (1) and (2), we can hence express Δx in terms of either numerical aperture NA or f/# N:

$$\Delta x = \frac{4\lambda N}{\pi} \approx \frac{0.64\lambda}{\text{NA}} \text{ (for air)}$$
 (5)

Scattering in real tissue

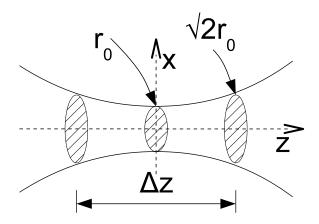


If a lens is focused on a single point which scatters (reflects) the light back, then the image of that point will have a lateral extent given by Δx .

However, tissue is translucent and contains lots of scatterers. All the scatterers in the light path might contribute to the returned light and hence the image is effectively blurred over the *axial* or *depth* direction as well.

There are also more complex light interactions with tissue, and light is attenuated as it passes through, so responses from deeper in the tissue are less. Typically we can see about 1 mm depth in skin.

Axial (depth) resolution



The axial resolution Δz is the range in z over which the beam area is within twice the size of the focal spot. Hence at $z = \frac{\Delta z}{2}$, $r = \sqrt{2}r_0$, so from (4) (remembering $r_0 = \frac{\Delta x}{2}$):

$$\left(\frac{\Delta x}{\sqrt{2}}\right)^2 = \left(\frac{\Delta x}{2}\right)^2 \left(1 + \left(\frac{4\lambda \frac{\Delta z}{2}}{\pi \Delta x^2}\right)^2\right)$$

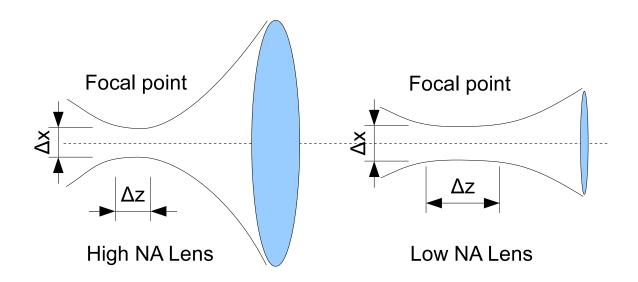
re-arranging gives:

$$\frac{\Delta x}{2} = \frac{\lambda \Delta z}{\pi \Delta x} \quad \Rightarrow \quad \Delta z = \frac{\pi}{2\lambda} \Delta x^2$$

Using (5), we can hence also express Δz in terms of numerical aperture NA or f/# N:

$$\Delta z = \frac{8\lambda N^2}{\pi} \approx \frac{0.64\lambda}{(\text{NA})^2} \text{ (for air)}$$
 (6)

Resolution summary



The *resolutions* Δx and Δz of the lens indicate how close two points at the focus can be and still be distinguishable.

The values are dependent on the numerical aperture (NA) and also the wavelength of light λ :

$$\Delta x \approx \frac{0.64\lambda}{\text{NA}}$$

$$\Delta z \approx \frac{0.64\lambda}{(\text{NA})^2}$$

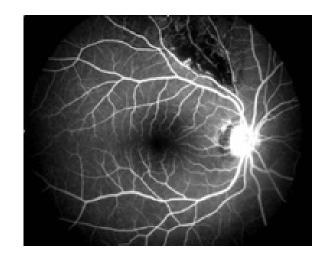
In practice, this is further limited by the pixel spacing in the CCD array and also the optics of the eye. Achievable values are in the range $2\mu m$ to $15\mu m$.

Scanning Laser Ophthalmoscope



A scanning laser ophthal-moscope (SLO) is a tomo-graphic technique which can also image with more precision than a fundus camera. It doesn't take one picture immediately, but has to scan a laser ('raster scan') over the retina to produce an image.

This is a fluorescence image from an SLO showing the retinal blood vessels very clearly. The dark regions at the edge and in the middle are because this is a *cross-section* not a conventional picture.



The Scanning Laser Ophthalmoscope

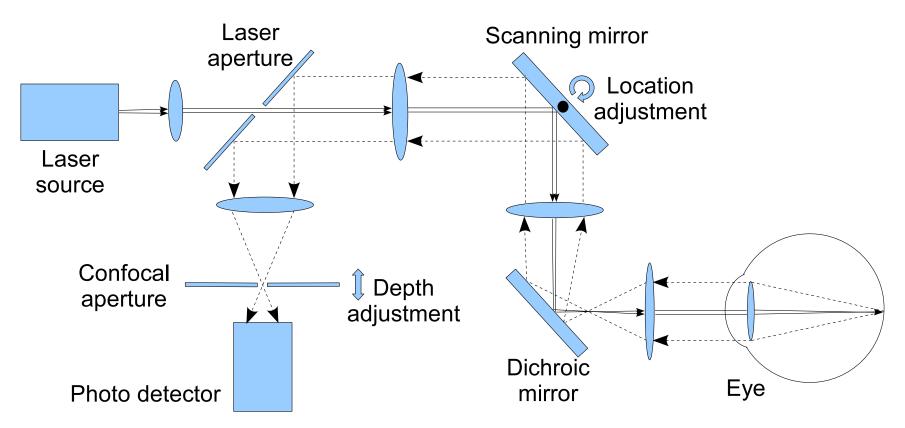
The Scanning Laser Ophthalmoscope (SLO)² overcomes some of the restrictions of the Fundus Camera. In particular an SLO:

- Uses a very small spot of focused laser light to illuminate a particular bit of the fundus. This improves the radial (*x*) resolution slightly, but greatly improves the illumination efficiency.
- Uses *confocal* optics to reduce the depth range for a given focus. This allows *tomographic* imaging, i.e. sectioning, which means we can produce 3D image data.
- Requires scanning mirrors to gradually sweep the laser spot over the eye to form the whole image, and hence is slower than the fundus camera which uses conventional *widefield* imaging.
- Uses laser centre wavelengths from 400 nm to 1300 nm to look at different features in the retina.

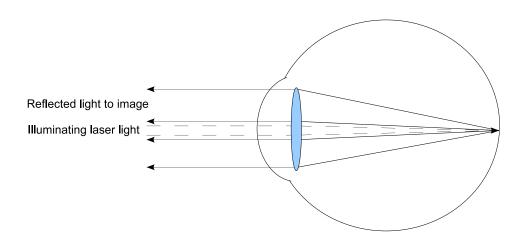
A conceptual schematic is shown on the next page.

²We will be considering the more commonly used *confocal* SLO or cSLO.

The Scanning Laser Ophthalmoscope



SLO Illumination



Compared to the conventional Fundus Camera, in the SLO only a small aperture is used for the laser illumination, the remaining large area of the pupil being used for collection of the light reflected from the retina. This improves the optical efficiency, and hence enables the use of lower light power levels.

It has been estimated that for typical scanning conditions, with an exposure of 75 ns per spot, and 26 ms per frame, the maximum safe power without damaging the retina was 30 mW.

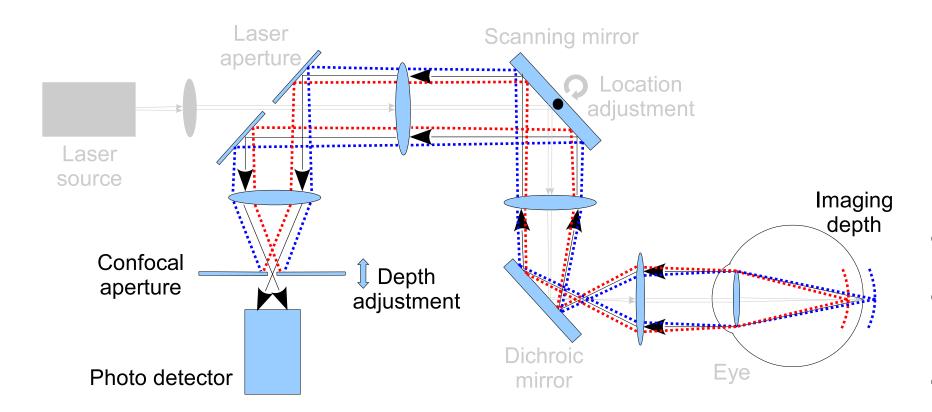
Confocal Scanning

(See diagram on the next page)

- An aperture (plate with a small hole) is placed at or near the focus just before the photo detector, so that only light passing very close to the focus passes through.
- Most of the light reflected from greater or lesser depths into the tissue hits the plate and does not pass through to the photo detector: this reduces the depth of tissue over which the instrument is sensitive.
- A smaller aperture increases the depth resolution (i.e. a narrower tissue section) but it also decreases the amount of light reaching the detector. Conversely larger apertures increase the amount of light but reduce the depth resolution.
- The scanning depth can be changed by moving the confocal aperture axially (along the optical axis). Ideally we want the lens before the aperture to have low NA so there is a reasonable range of depths with good optical efficiency.

Engineering Part IB: Paper 8 Bioengineering

Confocal Scanning

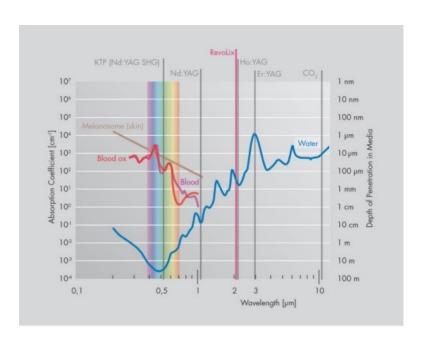


SLO Summary

The Scanning Laser Ophthalmoscope:

- Is a *tomographic* technique, which means it produces cross-sections at a given depth.
- Has a lateral resolution of about $15 \, \mu m$, mainly due to achievable sizes of the laser spot on the retina, given we have to illuminate through the cornea and lens. This is slightly better than the Fundus Camera.
- Has a depth resolution which varies with confocal aperture size. The resolution is system-dependent: for example an aperture of $40 \, \mu m$ might have a depth of focus of $400 \, \mu m$ whereas an aperture of only $10 \, \mu m$ would have a depth of focus of $100 \, \mu m$.
- Uses lower light power levels than the Fundus Camera.
- Typically has a 10 ms or so scan time for one line, more for acquiring 2D or 3D data. This is slower than the Fundus Camera.

Light sources and absorption

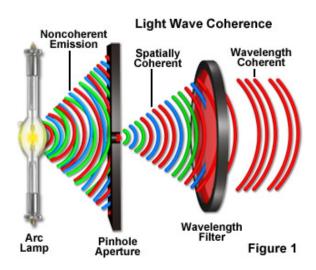


http://info.lisalaser.de

Both absorption and reflectivity are important material properties.

Tissue responds differently to laser light of different wavelengths. The optic nerve head is particularly reflective at 500 nm, 600 nm wavelength illumination gives good contrast images of the fundus generally. Higher, near-infrared, wavelengths such as 800 nm are better for imaging deeper structure, since they have lower absorption coefficients.

Coherent and incoherent light

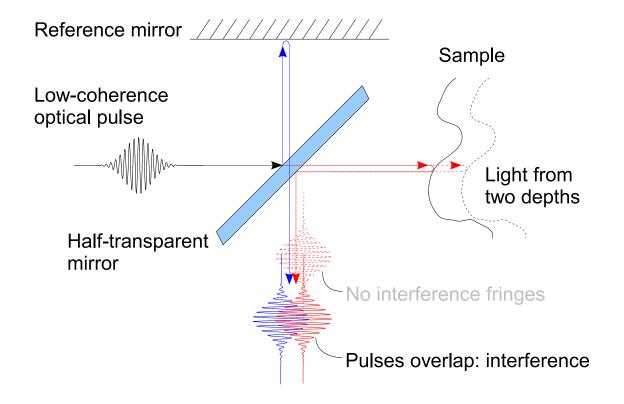


Olympus Microscopy Resource Centre

The Scanning Laser Ophthalmoscope does not take advantage of the fact that lasers can produce *co-herent* light. This is light which retains the same shape (waveform) as it travels. As long as coherent light is consistently polarized, it can be combined to produce *interference fringes*.

Such fringes are annoying in normal optical imaging (they produce patterns at sharp edges of structures) but they can be put to very good use in Optical Coherence Tomography (OCT), which makes use of the Michelson Interferometer, first used in 1887.

Low-coherence optical interferometry



An interferometer combines a reference signal with a signal reflected off the sample being imaged. This is achieved by splitting the signal into two: half is sent to a reference mirror and half to the sample. They are then re-combined after reflection.

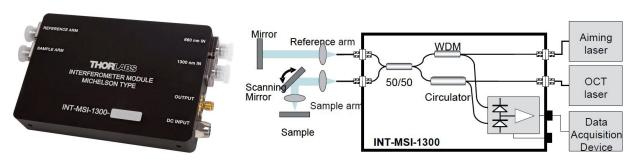
If the optical path length to reference mirror and object is the same, the signals interfere to give twice the amplitude. If they are exactly half a wavelength apart, they combine to give nothing at all. This leads to interference signals.

Low-coherence optical interferometry

If the path length is completely different there is no interference at all.



Interferometers can be constructed in a variety of ways. This is a Michelson Interferometer using mirrors and a half-reflecting mirror at the centre



They can also be constructed using optical fibres which are deliberately designed to transfer light between each other rather than keeping all the light contained in the fibre.

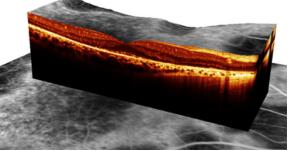
Optical Coherence Tomography



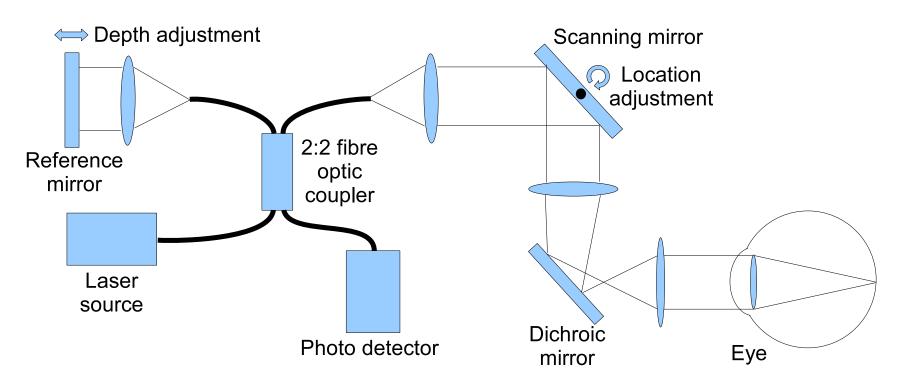
Optical Coherence Tomography (OCT) also uses a laser, but takes advantage of the coherence of laser light to create a very high resolution 3D volume to a depth of 1-3 mm in tissue.

These are some examples — we can now see 'into' the retina and look at the various retinal layers, as well as measuring thickness or geometry.





Optical Coherence Tomography



OCT Interferometer Signal

If we model the electric field E_l from the laser simply as:

$$E_l(\omega) = E_0(\omega) \ e^{j\omega t} \tag{7}$$

then the reference signal E_r (assuming an ideal mirror) is:

$$E_r(\omega) = E_0(\omega) \ e^{j\left(\frac{\omega}{c}l_r - \omega t\right)} \tag{8}$$

where l_r is the round trip distance from the mirror.

The signal E_s from the object comprises contributions from many scatterers, and is given by:

$$E_s(\omega) = E_0(\omega) \int_{-\infty}^{\infty} r_s(l_s) \ e^{j\left(\frac{\omega}{c}l_s - \omega t\right)} \ dl_s \tag{9}$$

where l_s is the round trip distance to a scatterer, and $r_s(l_s)$ is the *amplitude reflectivity density function* — this tells us the optical reflectivity with depth, and is what we really want to be able to see.

The output of the interferometer is the sum of E_r and E_s . However, the photo diode responds to light *intensity* $I(\omega)$:

$$I(\omega) = |E_r + E_s|^2$$

= $|E_r|^2 + |E_s|^2 + 2\Re\{E_r E_s^*\}$ (10)

OCT Interferometer Signal

If we define the power spectrum $S(\omega) = |E_0|^2$, then substituting (8) and (9) into (10) gives:

$$I(\omega) = S(\omega) + S(\omega) \left| \int_{-\infty}^{\infty} r_s(l_s) e^{j\frac{\omega}{c}l_s} dl_s \right|^2 + 2\Re \left\{ S(\omega) \int_{-\infty}^{\infty} r_s(l_s) e^{j\frac{\omega}{c}(l_r - l_s)} dl_s \right\}$$
(11)

The first term results purely from the reference signal, the second term from the scatterers in the object being imaged, and the third is the interference term we are interested in.

In time-domain OCT, a single photo diode is used, which measures total intensity integrated across all frequencies, i.e.:

$$I = \int_{-\infty}^{\infty} I(\omega) \, d\omega \tag{12}$$

But if we move the mirror (change l_r) only the third term in (11) changes. Hence we can reject the other terms by high-pass filtering I to find the component which varies as the mirror is moved.

Time-domain OCT

Hence if we substitute the third term in (11) into (12), also replacing the frequency ω with the centre frequency and deviation $\omega_0 + \omega_b$, we have:

$$2\Re\left\{\int_{-\infty}^{\infty} S_0(\omega_b) \int_{-\infty}^{\infty} r_s(l_s) e^{j\frac{\omega_0 + \omega_b}{c}(l_r - l_s)} dl_s d\omega_b\right\}$$
(13)

where S_0 is just the spectrum S centred about ω_0 .

Re-arranging the order of integration:

$$2\Re\left\{e^{j\frac{\omega_0}{c}l_r}\int_{-\infty}^{\infty}r_s(l_s)\ e^{-j\frac{\omega_0}{c}l_s}\int_{-\infty}^{\infty}S_0(\omega_b)\ e^{j\frac{\omega_b}{c}(l_r-l_s)}\ d\omega_b\ dl_s\right\}$$
(14)

The inner integral looks like a Fourier Transform of $S_0(\omega_b)$, and can be replaced (by the Wiener-Khinchin theorum) with the *autocorrelation* of the electric field $R_{ee}(t)$, where $t = \frac{l_r - l_s}{c}$:

$$I_{\rm ac} \approx 2\cos\frac{\omega_0}{c}l_r \Re\left\{ \int_{-\infty}^{\infty} r_s(l_s) e^{-j\frac{\omega_0}{c}l_s} R_{ee}(\frac{l_r - l_s}{c}) dl_s \right\}$$
(15)

Time-domain OCT

There are several important points to note:

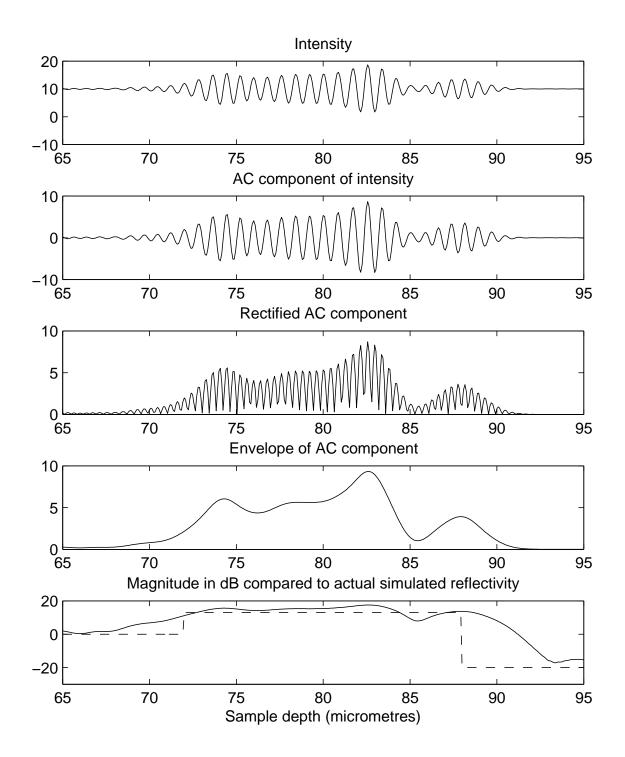
- The cosine term means that moving the mirror (changing l_r) over the sample depth will modulate the intensity with an AC signal over depth.
- If we measure the amplitude or *envelope* of the oscillations, we will recover the integral within the $\Re \{\}$.
- The inner integral includes $r_s(l_s)$ (the term we actually want) over all depths. But the autocorrelation function R_{ee} is only non-zero close to zero, i.e. where $l_r \approx l_s$. So we end up recording r_s close to the reference mirror position l_r .
- The depth *resolution* is determined by the extent of the autocorrelation R_{ee} , which is entirely a function of the source power spectral density $S_0(\omega)$.

Time-domain OCT summary

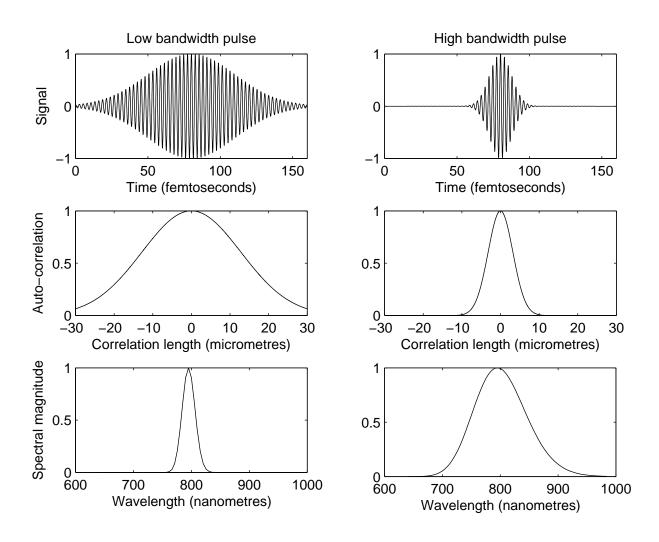
This is a summary of the imaging process:

- Move the reference mirror so the reference scans over the imaging depth in the sample.
- As the mirror is being scanned, send out laser pulses, and record the intensity of the interference signal at the photodiode.
- High-pass filter these measurements to remove the reference and sample-only intensities, and leave the depth-varying AC component.
- Rectify and low-pass filter to give the amplitude (envelope) of the AC signal. This is the sample reflectivity over depth.
- Move the laser beam to the next location and repeat.

Time-domain OCT summary



Resolution of time-domain OCT



Depth resolution can be better than $5 \mu m$, but this depends on the bandwidth of the pulse. The larger the bandwidth, the shorter the pulse, and the better the resolution.

Resolution of time-domain OCT

The axial (depth) resolution in this case is given by the correlation length l_c of the laser pulse. For a Gaussian-modulated envelope (as on the previous page), this is:

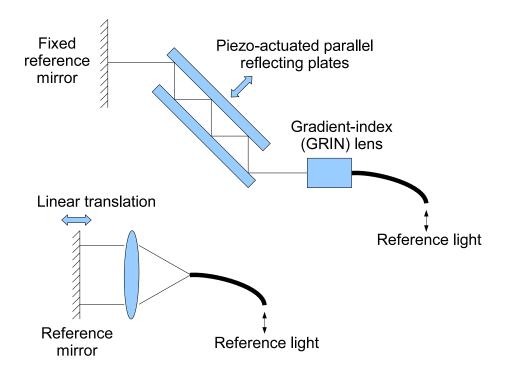
$$l_c = \frac{2\ln 2}{\pi} \frac{\lambda_0^2}{\Delta \lambda}$$

$$\propto \frac{\lambda_0^2}{\Delta \lambda} \tag{16}$$

where λ_0 is the centre wavelength and $\Delta\lambda$ the bandwidth in wavelength. This equation is often used to give an estimate of resolution even when the pulse does not have exactly this shape.

The lateral resolution is given by the optics to be $\approx 15 \mu m$ — generally not quite as good as in Scanning Laser Ophthalmology since a lens with a lower NA is generally necessary to give good results across a range of scanning depths. Systems with adaptive optics can achieve lateral resolutions up to $\approx 2 \mu m$.

Moving the reference mirror

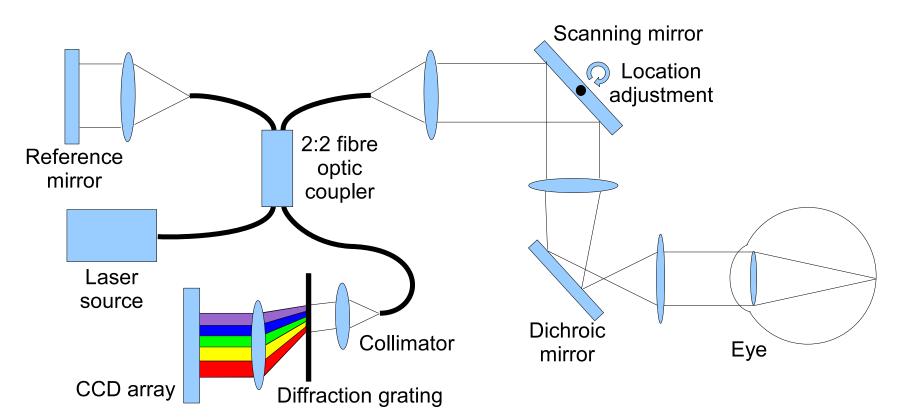


The scanning time in time-domain OCT is largely a function of how fast the reference mirror can be moved. There are many ways of doing this, two examples are shown above.

Both the systems above can move the reference mirror at sustained scanning speeds of about 40 mm/s, repeating the depth scan at 30 Hz. Faster scanning speeds are possible with other designs.

However, we would ideally like to have no moving parts at all ...

Spectral OCT



Spectral OCT

In spectral OCT we split the light from the interferometer using a diffraction grating and measure all the frequency components in parallel with a CCD array. This means we directly measure $I(\omega)$ in (11).

The spectra of the first term in (11) is measured by blocking the object arm, and the second term by blocking the reference arm. This leaves us with:

$$I_{\text{int}}(\omega) = 2\Re \left\{ S(\omega) \int_{-\infty}^{\infty} r_s(l_s) \ e^{j\frac{\omega}{c}(l_r - l_s)} \ dl_s \right\}$$
 (17)

Once again, the integral looks just like a Fourier Transform, so we can express this as³:

$$I_{\text{int}}(\omega) = 2\Re \left\{ S(\omega) \frac{1}{2} \mathcal{F} \left\{ r_s(\frac{l_s - l_r}{c}) \right\} (\omega) \right\}$$
 (18)

Re-arranging leads to:

$$r_s(\frac{l_s - l_r}{c}) = \mathcal{F}^{-1} \left\{ \frac{I_{\text{int}}(\omega)}{S(\omega)} \right\}$$
 (19)

which gives us the entire spectral reflectivity r_s at all depths in one go, whereas with time-domain OCT we need separate measurements for each depth. This makes spectral OCT much faster.

³The $\frac{1}{2}$ is because we only detect the magnitude, not phase, of the Fourier Transform

Spectral OCT

There are several important points to note:

- The division by *S* implies we can only capture useful information over the bandwidth of the original source signal, so we set the CCD array to capture this frequency range.
- $I(\omega)$ is discrete, since we capture this with a CCD array. The effective sampling frequency is the bandwidth of the laser ΔF , so the sample spacing in *time* is $\frac{1}{\Delta F}$, and in *depth* z is:

$$\Delta z = \frac{c}{\Delta F} = \frac{1}{\frac{1}{\lambda_0 - \frac{1}{2}\Delta\lambda} - \frac{1}{\lambda_0 + \frac{1}{2}\Delta\lambda}} \approx \frac{\lambda_0^2}{\Delta\lambda}$$
 (20)

where c is the speed in the medium. This has a similar form to (16).

• The *total* imaging depth *d* depends on the number of samples, i.e. the number *S* of CCD elements⁴:

$$d = S\Delta z = S\frac{c}{\Delta F} \approx \frac{S\lambda_0^2}{\Delta \lambda} \tag{21}$$

 $^{^4}$ The useful depth is actually only $\frac{1}{2}$ of this, again because we only detect the magnitude, not phase, of the Fourier Transform

Spectral OCT summary

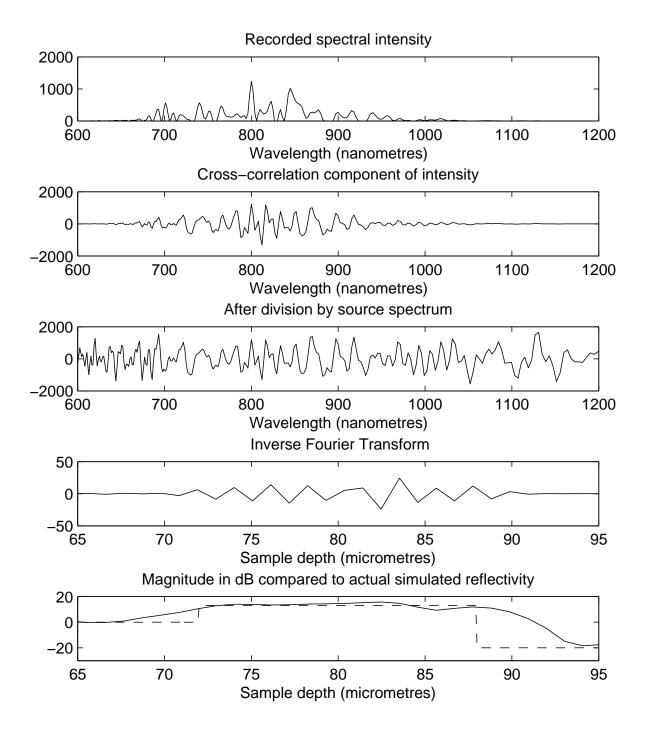
This is a summary of the imaging process:

- The reference mirror is fixed so the reference point is just outside the sample⁵.
- Record calibration spectra for the reference (by blocking the sample path) and sample (by blocking the reference path).
- Record the spectrum from the interferometer using the diffraction grating and CCD array.
- Remove the spectra due only to the reference and sample, leaving the interference spectra⁶.
- Divide this spectra by the source spectra.
- Take the inverse Fourier Transform of this new spectra, giving the reflectivity across the entire depth.
- Move the laser beam to the next location and repeat.

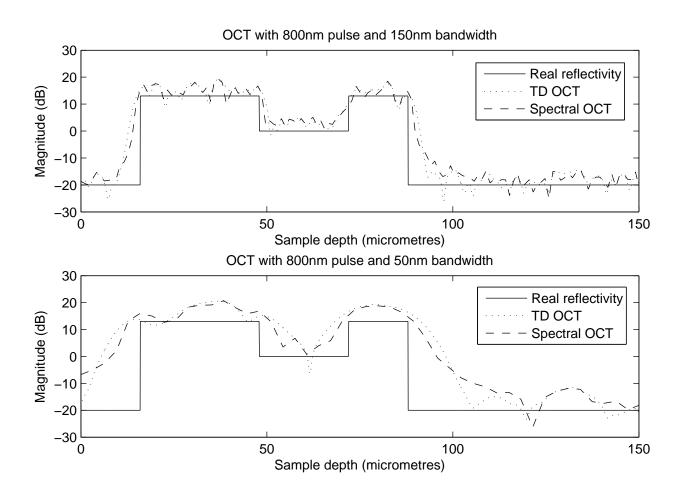
⁵There are more complex techniques which don't require this step

⁶Again, there are alternative ways of achieving this

Spectral OCT summary

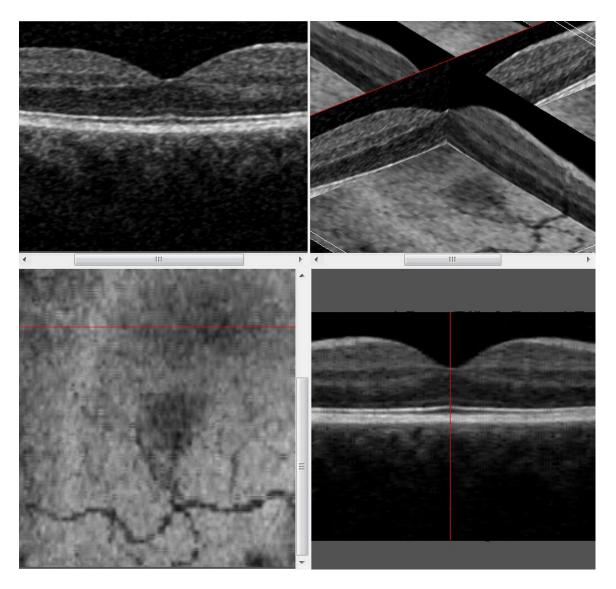


OCT comparison



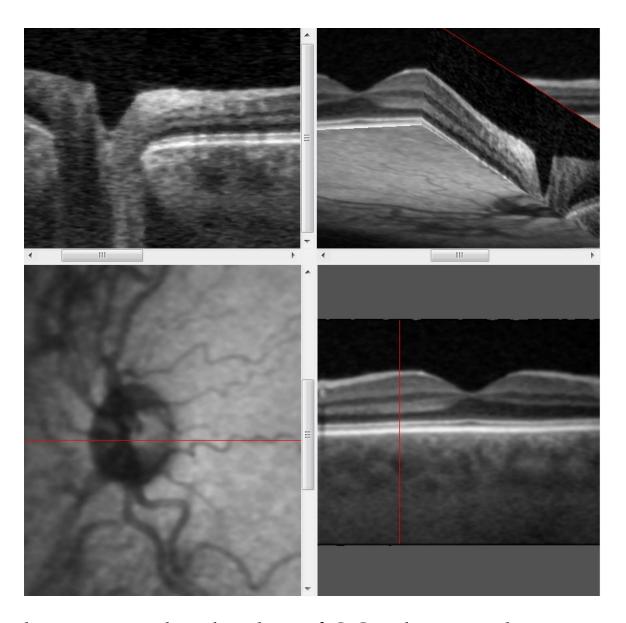
- Lateral resolution is similar for both time-domain and spectral OCT.
- Depth resolution is mainly a function of the bandwidth of the laser source, not the technique.
- Spectral OCT has slightly better depth resolution of $\approx 5\mu m$ due to the division of the measured spectra by the source spectra $S(\omega)$.

Example OCT Image at 800 nm



These are images from 3D OCT data acquired with a laser of centre wavelength 800 nm and bandwidth 50 nm. The top-left and bottom-right views show cross-sections through the macula in the retina. The bottom-left shows the view looking into the eye, and top-right displays these views in 3D.

Example OCT Image at 1060 nm



This is a similar display of OCT data at a laser centre wavelength of 1060 nm and a similar bandwidth. The optic disc is also visible, and the various retinal layers are substantially clearer.

OCT Complications

- We assumed the refractive index n was equal to 1 (air) in all optical paths. In practice the refractive index of the eye varies from 1.33 for the aqueous humor to 1.41 at the lens. We have to take this into account when matching the reference and sample distances it is the *optical path length* we want to match, not the physical distance.
- All the optical paths were assumed to be non-dispersive, i.e. the propagation constant $k = \frac{\omega}{c}$, so the light waveform doesn't change shape as it travels. In practice, the eye *is* dispersive, so the reflection path must also contain dispersive material.

Any difference in dispersion will make the reflected light slightly different from the source light. This has the effect of reducing the correlation between the two, which in turn will reduce the depth resolution.

OCT Complications

- Diffraction gratings produce spectra with uniform *wavelength* spacing, whereas, for the inverse Fourier Transform, we really want uniform *frequency* spacing. Hence we have to do some interpolation to the data from the CCD array in spectral OCT. This also means that the signal-to-noise ratio in practice varies with depth into the sample.
- Although we are apparently measuring the tissue reflectivity density, actually this is only a measure of the *amount of light received at the photo diode* with depth. Light is gradually absorbed by the tissue and the measured 'reflectivity' will be less as we get deeper into the tissue. Eventually all the light is absorbed and we can see no deeper.