Optical Biopsy

- Definition: The in situ imaging of tissue microstructure with a resolution approaching that of histology, but without the need for tissue excision and processing.

OCT in non-invasive diagnostics

- Ophthalmology
  - diagnosing retinal diseases.
- Dermatology
  - skin diseases,
  - early detection of skin cancers.
- Cardio-vascular diseases
  - vulnerable plaque detection.
- Endoscopy (fiber-optic devices)
  - Gastrology,
- Functional imaging
  - Doppler OCT,
  - spectroscopic OCT,
  - optical properties,
  - PS-OCT.
- Guided surgery
  - delicate procedures
  - brain surgery,
    - knee surgery,
  - …
Optical Ranging in Biological Tissue

Optical Imaging in Light Scattering Tissue

- Is it easy to make high-resolution images with light in tissues?
- White light from a projector is shone through a hand
  - you cannot identify any fine structure (bone etc.) although the wavelength of the light is shorter than typical cell sizes
  - only red light passes through – why?
- in X-ray of a hand and we can identify bone structure
- X-rays are ionising whereas light is non-ionising
- The following presentation aims at introducing an imaging modality that can image in biological tissues with micrometer resolution
  - albeit the penetration depth is limited the clinical impact and relevance is huge

Optical Coherence Tomography

- Imaging technique
- Based on intensity differences in backscattered light from tissue
- Similar to ultrasound, but higher resolution
- Superficial tissue structures
- Time of flight?

General characteristics

- Perform cross-sectional measures of tissue in situ similar to US
  - time for a pulse to travel
- Detection is based on low coherence interferometry
  - reflected infrared light
General characteristics

• Penetration depth ≈ 2-3 mm
  • wavelength dependent
• Resolution axial ≈ 4-20 µm
  • detector dependent
• Resolution lateral ≈ 5-20 µm
  • spot size

OCT based on coherence measurement

• Signals are only detected when the optical path length in the sample and reference arm are within the coherence length of the source “coherence gate”

Coherence and Source Requirements

• Temporal coherence
• Spatial coherence
• Partial Coherence
• Coherence time
• Coherence Length

Spatial coherence

• Spatial coherence is measured by a wave front with a constant phase all over space. It means that between any two points in space the phase difference is constant with time.
  • A laser light has spatial coherence when the phase difference between two points in space is constant in time.
Spatial coherence, cont

• When a laser operates in a single basic transverse mode (TEM$_{00}$), it has the maximum spatial coherence.

• The spatial coherence is the cause of the high directionality of the laser beam.
  - Spatial coherence is important in applications where all the power is needed in a single spot (diffraction limited focusing).

Spatial Coherence

• Spatial coherence is the dependence of fringe visibility on the spatial extent of the source.

• The spread in wavelengths among the sources is small enough so that the coherence length is much greater than the range of optical path distance (OPD) in the measurement.

• Therefore, fringe visibility is not affected by the bandwidth of the power spectrum.

Temporal coherence

• Temporal Coherence means that the light has a single wavelength (or a single frequency), and this is the property of mono-chromaticity

• A wave has complete temporal coherence when the phase of the wave at a certain instant of time ($\Delta t$) along the travelling wave front, is equal to the phase of the wave after it advance a distance $L$ at a time $L/c$ for every $L$.

Temporal coherence, cont

• It means that in a time ($\Delta t$), while the wave advances a distance of $c(\Delta t)$ it keeps its shape as the original wave

• The narrower the linewidth ($\Delta \nu$) of the light source, the better is its temporal coherence

• The temporal coherence is a measure of the ability of the radiation to perform interference, as a result of differences in path lengths between the two beams.

• Temporal coherence is the dependence of fringe visibility on the power spectrum of the source.
Coherence properties

• Light is assumed as electromagnetic radiation
  \[ E = E_0 e^{i(k \cdot r - \omega t + \phi)} \]

• Consists of wave-trains with different lifetimes

• Average lifetime, \( \tau_0 \), is called coherence time
  \[ \tau_0 = \tau_{\text{ave}} = 1/\Delta \nu \]

Coherence lengths of light sources

<table>
<thead>
<tr>
<th>Source</th>
<th>Mean wavelength ( \lambda ) (nm)</th>
<th>Linewidth ( \Delta \lambda ) (nm)</th>
<th>Coherence length ( \Delta L_c )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermal IR (8000-12000 nm)</td>
<td>10000</td>
<td>=4000</td>
<td>=25000 nm = 2.5 ( \lambda )</td>
</tr>
<tr>
<td>Mid-IR (500-5000 nm)</td>
<td>4000</td>
<td>=2000</td>
<td>=8000 nm = 2( \lambda )</td>
</tr>
<tr>
<td>White light</td>
<td>550</td>
<td>=300</td>
<td>=900 nm = 1.6( \lambda )</td>
</tr>
<tr>
<td>Mercury arc</td>
<td>546.1</td>
<td>=1.0</td>
<td>=0.03 cm</td>
</tr>
<tr>
<td>Stabilized He-Ne laser</td>
<td>632.8</td>
<td>=10 ( \lambda )</td>
<td>=400 m</td>
</tr>
<tr>
<td>Special He-Ne laser</td>
<td>1153</td>
<td>8.9*10^{-7}</td>
<td>15*10^{-7} m</td>
</tr>
</tbody>
</table>

Coherence length

• Coherence Length (\( L_c \)) is the maximum path difference possible for a specific temporal coherence, which still shows interference.

• This specific temporal coherence is related to specific linewidth (\( \Delta \lambda \)) by the formula: \( L_c = c/(\Delta \nu) \)
  - \( \Delta \nu = \) Frequency linewidth of radiation, in units of Hertz [Hz]
  - \( c = \) Speed of light in vacuum, in units of [m/s]

• Narrowing the frequency linewidth, increase coherence length

• This is the reason why it is recommended to use a single (longitudinal) mode laser in applications related to interference (such as holography)

The Temporal Coherence Time and the Spatial Coherence Length

The temporal coherence time is the time the wave-fronts remain equally spaced. That is, the field remains sinusoidal with one wavelength:

The spatial coherence length is the distance over which the beam wave-fronts remain flat:

Since there are two transverse dimensions, we can define a coherence area.
Spatial and Temporal Coherence

Beams can be coherent or only partially coherent (indeed, even incoherent) in both space and time.

The coherence time is the reciprocal of the bandwidth

The coherence time is given by:

\[ \tau_c = \frac{1}{\Delta \nu} \]

where \( \Delta \nu \) is the light bandwidth (the width of the spectrum).

Sunlight is temporally very incoherent because its bandwidth is very large (the entire visible spectrum).

Lasers can have coherence times as long as about a second, which is amazing; that's \( >10^{14} \) cycles!

The spatial coherence depends on the emitter size and its distance away

The van Cittert-Zernike Theorem states that the spatial coherence area \( A_c \) is given by:

\[ A_c = \frac{D^2 \lambda^2}{\pi d^2} \]

where \( d \) is the diameter of the light source and \( D \) is the distance away.

Basically, wave-fronts smooth out as they propagate away from the source.

Starlight is spatially very coherent because stars are very far away.

Irradiance of a sum of two waves

Different colors

\[ I = I_1 + I_2 \]

Same colors

\[ I = I_1 + I_2 + c e \text{Re} \{E_1 \cdot E_2^*\} \]

Interference only occurs when the waves have the same color and polarization.
The irradiance when combining a beam with a delayed replica of itself has fringes

Irradiance is given by:

\[ I = I_1 + c \varepsilon \text{Re} \left\{ E_1 \cdot E_2^* \right\} + I_2 \]

Suppose the two beams are \( E_0 \exp(i \omega t) \) and \( E_0 \exp(i \omega (t - \tau)) \), that is, a beam and itself delayed by some time \( \tau \):

\[
I = 2I_0 + c \varepsilon \text{Re} \left\{ E_0 \exp[i \omega t] - E_0 \exp[-i \omega (t - \tau)] \right\}
\]

\[
= 2I_0 + c \varepsilon \exp[i \omega \tau]
\]

\[
= 2I_0 + c \varepsilon \cos(\omega \tau)
\]

\[
I = 2I_0 + 2I_0 \cos(\omega \tau)
\]

The Michelson Interferometer

The Michelson Interferometer splits a beam into two and then recombines them at the same beam splitter.

Suppose the input beam is a plane wave:

\[
I_{\text{in}} = I_1 + I_2 + c \varepsilon \text{Re} \left\{ E_0 \exp[i(\omega \tau - 2kL_2)] - E_0 \exp[-i(\omega \tau - 2kL_2)] \right\}
\]

\[
= I + 2I \exp[-2i k(L_2 - L_1)]
\]

\[
= 2I \{1 + \cos(k \Delta L)\}
\]

where \( I = I_1 = I_2 = (c \varepsilon_0) / 2 \) and \( \Delta L = 2(L_2 - L_1) \)

The OCT setup

The OCT (TD – principle)
Operating Principles of OCT
Reference Beam Path Length

Optical Tomographic Imaging of Tissue Structure and Physiology

**Challenge:** Scattering of photon destroy localization

Mean free scattering path:
- Skin tissue: $1/\mu_s \approx 50 \mu m$
- Blood: $1/\mu_s \approx 8 \mu m$

- Technology:
  - Time of flight (only ballistic photons or minimally scattered photons are selected)
  - Photon migration (amplitude and phase of photon density wave are measured)
  - Optical coherence tomography (coherence gating is used to select minimally scattered photons)
Probing depth

- Multiple scattered light does not contribute to the object’s Fourier spectrum it forms a disturbing background.
- Its effects are a reduction of imaging contrast, a reduction of resolution, and a reduction of penetration depth.

Sampling and probing beam

Interference of monochromatic light

- Light is assumed as electromagnetic radiation
  \[ E = E_0 e^{i(k \cdot r - \omega t + \phi)} \]
- Interference: Superposition of waves
  \[ E = E_1 + E_2 = E_1 e^{i(k \cdot r - \omega t + \phi_1)} + E_2 e^{i(k \cdot r - \omega t + \phi_2)} \]
- Detection of light waves:
  \[ I \propto \langle E^2 \rangle \]
Correlation function

- Defining a normalized correlation function

\[ \gamma_{12}(\tau) = \frac{\Gamma_{12}(\tau)}{\sqrt{I_1 I_2}} \]

- We get

\[ I_p(\tau) = I_1 + I_2 + 2\sqrt{I_1 I_2} \gamma_{12}(\tau) \cos(2\pi v_0 \tau) \]

Interference of monochromatic light

Detection of light waves:

\[ I \propto \langle E^2 \rangle = \langle (A_1 \cos(\omega t + \phi_1) + A_2 \cos(\omega t + \phi_2))^2 \rangle \]

\[ I = I_1 + I_2 + 2\sqrt{I_1 I_2} \cos(\phi) \quad \phi = \phi_2 - \phi_1 \]

If \( I_1 = I_2 = I_0 \), \( I = 2I_0(1 + \cos(\phi)) \)

In phase \( \phi = 0, \pi, 2\pi, ..., I = 4I_0 \)

Out of phase \( \phi = \pi, 3\pi, 5\pi, .... \quad I = 0 \)

Photon sources

Atoms or molecules radiate wavetrains of finite length

- More than one wavelength (spectral bandwidth)
- Fixed phase relation only within individual wavetrain

Coherence

Correlation of light wave at two points in space-time:

\[ \Gamma(r_1, t_1; r_2, t_2) = \langle E(r_1, t_1)E^*(r_2, t_2) \rangle \]

Temporal Coherence (longitudinal)

\[ \Gamma = \langle E_a(t)E^*_b(t) \rangle \]

Spatial Coherence (lateral)

\[ G = \langle E_a(t)E^*_d(t) \rangle \]
Temporal coherence

\[ \Gamma = \langle E_a(t)E_{b*}(t) \rangle = \langle E(t+t_{\text{coh}})E^*(t) \rangle \]

- Coherence time: 
  The time for the elementary wavetrain to pass a single point.

- Coherence length: 
  The length of the wavetrain where there is definite phase relation.

Temporal Coherence

Temporal coherence is a measure of spectral bandwidth.
A high (good) temporal coherence gives a narrow spectral bandwidth ("pure" light of single wavelength (color)).

Partially coherent sources

- Coherent source:
  - Monochromatic: same wavelength
  - Constant phase relation

- Incoherent source:
  - Broad spectrum band \( P(v) \)
  - Random Phase

- Partially coherent source:
  - Broad spectrum band (\( \Delta \lambda = 10-100 \text{ nm} \)), \( P(v) \)
  - Definite phase relation within coherence length \( L_{\text{c}} \) (2-15 \( \mu \text{m} \))
    - If \( \Delta L_{\lambda} < L_{\text{c}} \), interference observed
    - If \( \Delta L_{\lambda} > L_{\text{c}} \), interference disappeared

Interference with partially coherent light

\[ I_1(v_1) = 2 \sum I_0(v_i) [1 + \cos(2\pi \Delta L v_i)] \]
\[ I_2(v_2) = 2 \sum I_0(v_i) [1 + \cos(2\pi \Delta L v_j)] \]
\[ I_1(v_1,v_2) = \frac{1}{2} \sum I_0(v_i) \]
\[ \Gamma(\Delta L, v_1,v_2) = 2 \sum I_0(v_i) \cos(2\pi \Delta L v_i) \]
Interference with two light sources of different frequency

\[ I_1(n_1) = 2I_0(n_1)[1 + \cos(2\pi D n_1)] \]

\[ I_2(n_2) = 2I_0(n_2)[1 + \cos(2\pi D n_2)] \]

\[ \Gamma(\Delta L, \nu_1, \nu_2) = 2I_0(\nu_i)\cos(2\pi \Delta L \nu_i) \]

Interference with Partial Coherence Light Source

\[ I_1(n_1) = 2I_0(n_1)[1 + \cos(2\pi D n_1)] \]

\[ I_2(n_2) = 2I_0(n_2)[1 + \cos(2\pi D n_2)] \]

\[ I_3(n_3) = 2I_0(n_3)[1 + \cos(2\pi D n_3)] \]

\[ I_m(n_m) = 2I_0(n_m)[1 + \cos(2\pi D n_m)] \]

\[ \Gamma(\Delta L, \nu_1, \nu_2, \nu_3, ..., \nu_m) = 2\sum_{i=1}^{m} I_0(\nu_i)\cos(2\pi \Delta L \nu_i) \]

Gamma function for light with continuous spectra given by the spectral density of \( S(\nu) \):

\[ \Gamma(\Delta L) = 2I_0 \int_0^{\infty} S(\nu)\cos(2\pi \Delta L \nu) d\nu \]

For light with discrete wavelengths \( I(\nu_i) \):

\[ \Gamma(\Delta L, \nu_1, \nu_2, ..., \nu_m) = 2\sum_{i=1}^{m} I(\nu_i)\cos(2\pi \Delta L \nu_i) \]
Interference with partial coherence light source

For discrete light with different wavelength:

\[ \Gamma(\Delta L, \nu_1, \nu_2, \ldots, \nu_m) = 2 \sum_{i=1}^{m} I_i(\nu_i) \cos(2\pi\Delta L \nu_i) \]

For continuous spectra with spectral density of \( S(\nu) \):

\[ \Gamma(\Delta L) = 2L_0 \int S(\nu) \cos(2\pi\Delta L \nu) d\nu \]

Interference of partially coherent light

If the time delay \((\tau)\) between light in reference and sample paths is changed by translating the reference mirror, total power detected at the interferometer output is given by a time-average of the squared light amplitude:

\[ I(t) = \left| \left[ E_r(t) + E_s(t) \right] \right|^2 = I_s + I_r + \Gamma_s(\Delta L) \]

\[ \Gamma_s(\Delta L) = 2 \int K_s K_r S(\nu) \cos(2\pi\Delta L \nu) d\nu \]

Assuming that there is no spectral modulation in the reflectivity of both the sample and reference arms:

\[ \Gamma_s(\Delta L) = 2K_s K_r \int S(\nu) \cos(2\pi\Delta L \nu) d\nu \]

If the source spectral distribution is a Gaussian function:

\[ S_0(\nu) \propto e^{-\left(\frac{\nu - \nu_0}{\Delta \nu}\right)^2} \]

\[ \Gamma_s(\tau) \propto e^{-\left(\frac{\nu - \nu_0}{\Delta \nu}\right)^2} \cos(2\pi\Delta L \nu) \]

Where \( L_0 \) is the coherence length of the partial coherence source given by:

\[ L_0 = \frac{\nu_0}{\Delta \nu} \]

Assuming light coupled equally into reference arm and sample arm with spectral amplitude of \( A_o(\nu) \). The light coupled back to the detector from the sample and reference arm is given by:

\[ A_r(\nu) = e^{j2\pi \nu L} K_r A_o(\nu) \]

\[ A_s(\nu) = e^{j2\pi \nu L} K_s A_o(\nu) \]

Interference of partially coherent light

\[ E(t) = \int_{-\infty}^{\infty} A(\nu) e^{j2\pi \nu t} d\nu \]

Where: \( E(t) \) is electrical field amplitude emitted by a low coherent light source; \( A(\nu) \) is the corresponding spectral amplitude at optical frequency \( \nu \).

Because phase in each spectral component are random and independent, cross spectral density of \( A(\nu) \) satisfies:

\[ < A^*(\nu) A(\nu') > = S(\nu) \delta(\nu - \nu') \]

Where: \( S_o(\nu) \) is the source power spectral density [W/Hz]; \( \delta(\nu - \nu') \) is the Dirac delta function satisfying:

\[ \delta(\nu - \nu') = 0 \quad \text{if} \quad \nu \neq \nu' \]

\[ \int_{-\infty}^{\infty} f(\nu) \delta(\nu - \nu') d\nu = f(\nu') \]

and:

\[ \int_{-\infty}^{\infty} S(\nu) \delta(\nu - \nu') d\nu = S(\nu') \]
Coherence function

Narrow Spectrum
\[ \Delta \lambda_{\text{FWHM}} \approx 25 \text{ nm} \]
\[ L_c \approx 15 \mu m \]

Broad Spectrum
\[ \Delta \lambda_{\text{FWHM}} \approx 75 \text{ nm} \]
\[ L_c \approx 5 \mu m \]

Source spectrum \( \lambda \) → Coherence function \( \Delta L \) → Fourier Transformation

Optical Coherence Tomography

Interference with Partial Coherence Light Source

Interference fringes observed only when optical path lengths are matched within coherence length of the source

Light sources for OCT

- Continuous sources
  - SLD/LED/superfluorescent fibers,
  - center wavelength;
    - 800 nm (SLD),
    - 1300 nm (SLD, LED),
    - 1550 nm, (LED, fiber),
    - power: 1 to 10 mW (c.w.) is sufficient,
  - coherence length;
    - 10 to 15 mm (typically),
- Example
  - 25 nm bandwidth @ 800 nm
  - 12 mm coherence length (in air).

Superluminescent diodes (SLDs)

- Definition: broadband semiconductor light sources based on superluminescence
  - (Acronym: SLD)
- Superluminescent diodes (also sometimes called superluminescence diodes or superluminescent LEDs) are optoelectronic semiconductor devices which are emitting broadband optical radiation based on superluminescence.
  - Superluminescence: amplified spontaneous emission
Light sources for OCT

- Pulsed lasers
  - mode-locked Ti:Al2O3 (800 nm),
  - 3 micron axial resolution (or less).
- Scanning sources
  - tune narrow-width wavelength over entire spectrum,
  - resolution similar to other sources,
  - advantage that reference arm is not scanned,
  - advantage that fast scanning is feasible.

Demodulation of OCT signals

Construction of image

Time domain OCT

Source of contrast: refractive index variations
Image reconstructed by scanning
OCT Imaging System

Ex Vivo Imaging of Colon Cancer

High Speed OCT Imaging System

Fourier domain OCT
Resolution of OCT

\[ L_c = \frac{4 \ln 2}{\pi} \frac{\lambda^2}{\Delta\lambda} \]

\( L_c \) = coherence length
\( \lambda \) = center wavelength
\( \Delta\lambda \) = optical bandwidth

\[ \Delta z = \frac{L_c}{2n_g} \]

\( \Delta z \) = longitudinal resolution
\( n_g \) = group index

\( n_g = n - \frac{\lambda}{2\Delta\lambda} \frac{dn}{d\lambda} \)

Resolution of OCT, cont

\[ \Delta x = \frac{4\lambda}{\pi} \cdot \frac{f}{d} \]

\( f \) = focal length
\( d \) = spot size of objective lens

\[ b = 2z_R = \frac{\pi (\Delta x)^2}{2\lambda} \]

\( b \) = focus depth
\( z_R \) = Rayleigh range

Axial Resolution vs Bandwidth

Lateral resolution: Decoupled from axial resolution

Lateral resolution similar to that in a standard microscope
Light source Parameters

Wavelength and bandwidth determine the axial resolution \( (l_c) \):

\[
l_c = \frac{2 \ln 2 \lambda^2}{\pi \Delta \lambda}
\]

Typical resolutions are:

<table>
<thead>
<tr>
<th>Light Source</th>
<th>( \lambda_0 )</th>
<th>( \Delta \lambda )</th>
<th>( \Delta L )</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLD</td>
<td>1300 nm</td>
<td>50 nm</td>
<td>15 ( \mu )m</td>
</tr>
<tr>
<td>Ti:Sapphire laser</td>
<td>800 nm</td>
<td>125 nm</td>
<td>2 ( \mu )m</td>
</tr>
</tbody>
</table>

Lateral resolution is determined by the size of the focal spot

Optical Coherence Tomography

- Peripapillary area
- Nerve fiber layer thickness

Dermatology

Cardiology: Vulnerable Plaque
Calcified human aorta

Plaques with fine structures

Plaque Imaging: 1300 nm, 15 µm

Plaque Imaging: 800 nm, 5 µm

- Human thoracic aorta
- Human femoral artery

L: Lumen
T: Thrombus
I: Intima
M: Media
**Image depth vs wavelength**

- 1300 nm (upper)
- 850 nm (middle)
- histology (lower)

**OCT signal analysis: flow**

- The interferometric detector current, generated by a moving scatterer in the sample is given by:

  \[
  \tilde{i}_d(t) = A(t)\cos[2\pi(f_r - f_s)t + \Phi(t)]
  \]

  \(A(t)\) is the amplitude of the reflectivity as a function of depth \(\Phi(t)\) is a phase term.
Coherent demodulation

- Complex envelope of the interferogram
- Short time Fourier transform (STFT)

\[ i_d(t) = A(t) \exp[-j\{2\pi f_s t + \Phi(t)\}] \]

\[ V_s = \frac{f_s \lambda_0}{2n_t \cos \theta} \]

- \( V_s \) is the mean velocity
- \( n_t \) is the mean tissue index of refraction
- \( \theta \) is the angle between the incident beam and direction of motion of scatterers within the sample

Color Doppler OCT (CD OCT)

OCT radar

Spectroscopic OCT Imaging

Spectroscopic OCT

Wavelet Transform:
\[ W(\omega, \tau) = \left| \int_0^{\infty} (t + \tau) e^{\frac{-\omega^2 t^2}{2}} e^{i\omega t} dt \right|^2 \]

Center of gravity of the spectrum:
\[ \nu(\tau) = \frac{\sum_{\nu} \nu |W(\nu, \tau)|^2}{\sum_{\nu} |W(\nu, \tau)|^2} \]

In vivo Spectroscopic OCT

Statistics on all center frequencies \( \nu_c \): Average, rms
\[ \frac{1}{N} \sum_{x,y} \nu_c(x,y) \Rightarrow \bar{\nu}, \Delta \nu \]

HSL color space
Luminance = const.

Spectroscopic OCT Imaging

Xenopus laevis (African frog)

Amplitude tomogram
Spectroscopic tomogram

Longer wavelengths penetrate deeper
Areas of wavelength-dependent backscattering are visible
OCT modalities

Time Domain
- Scanning reference mirror
- Broad-band light source
- Photo detector
- Interference signal (TD)

Spectrometer-based
- Broad-band light source
- Diffraction grating
- 1-D detector
- Interference signal (FD)

Fourier Domain
- Swept source based
- Narrow line width
- Interference signal
- Mirror image
- DC
- Depth (nm)

Swept source based
- Broad-band light source
- Swept light source
- Photo detector
- Interference signal

Fig. 21 Schematic of different OCT modalities. OCT systems can be classified into time domain (TD) and Fourier domain (FD) systems. FD OCT systems can be divided into spectrometer based and swept source based systems.