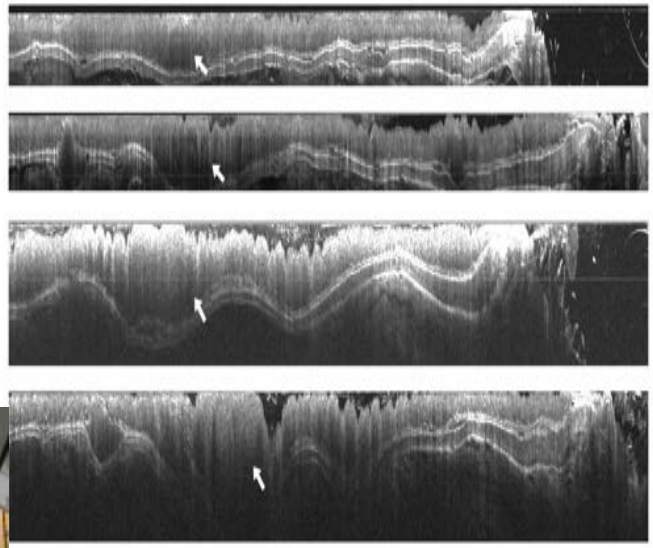
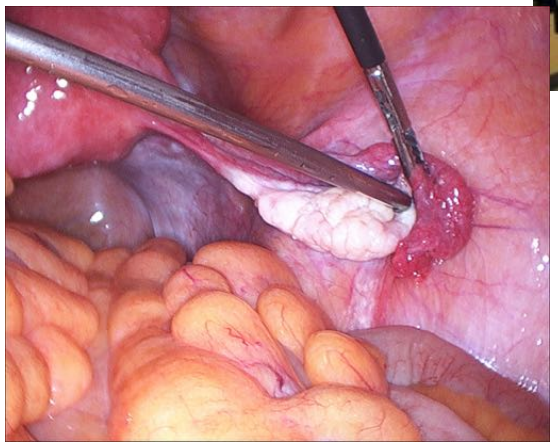
# Biomedical Optics



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# What is Biomedical Optics?

- A consideration of the unique ways in which light interacts with living matter.

Dr. Bruce Tromberg, flashlight in mouth

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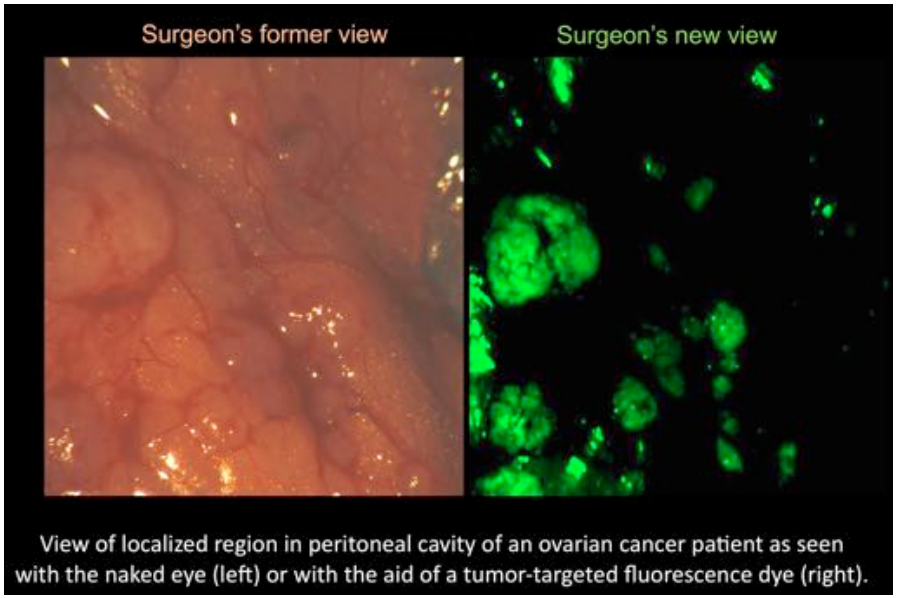
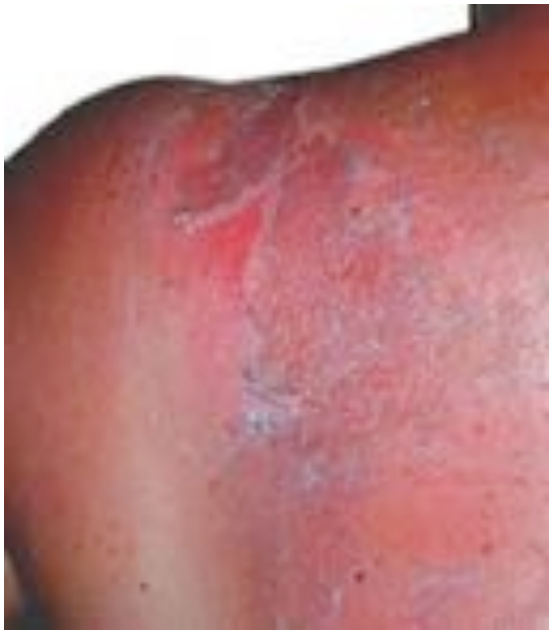
# What is Biomedical Optics

- A consideration of the unique needs and constraints of working with living matter.




Purdue



hikeandsurvive.com

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# Outline

1. Tissue Optics
   - Scattering
   - Absorption
   - Fluorescence
2. Light propagation in tissue
   - Beer's law
   - Monte Carlo implementation of the Radiative Transport Equation
   - Diffusion Approximation to the RTE
3. Diagnostic optical methods
   - Scattering (and absorption)-based
   - Fluorescence-based
4. Therapeutics
   - Laser treatment of port wine stains as an example

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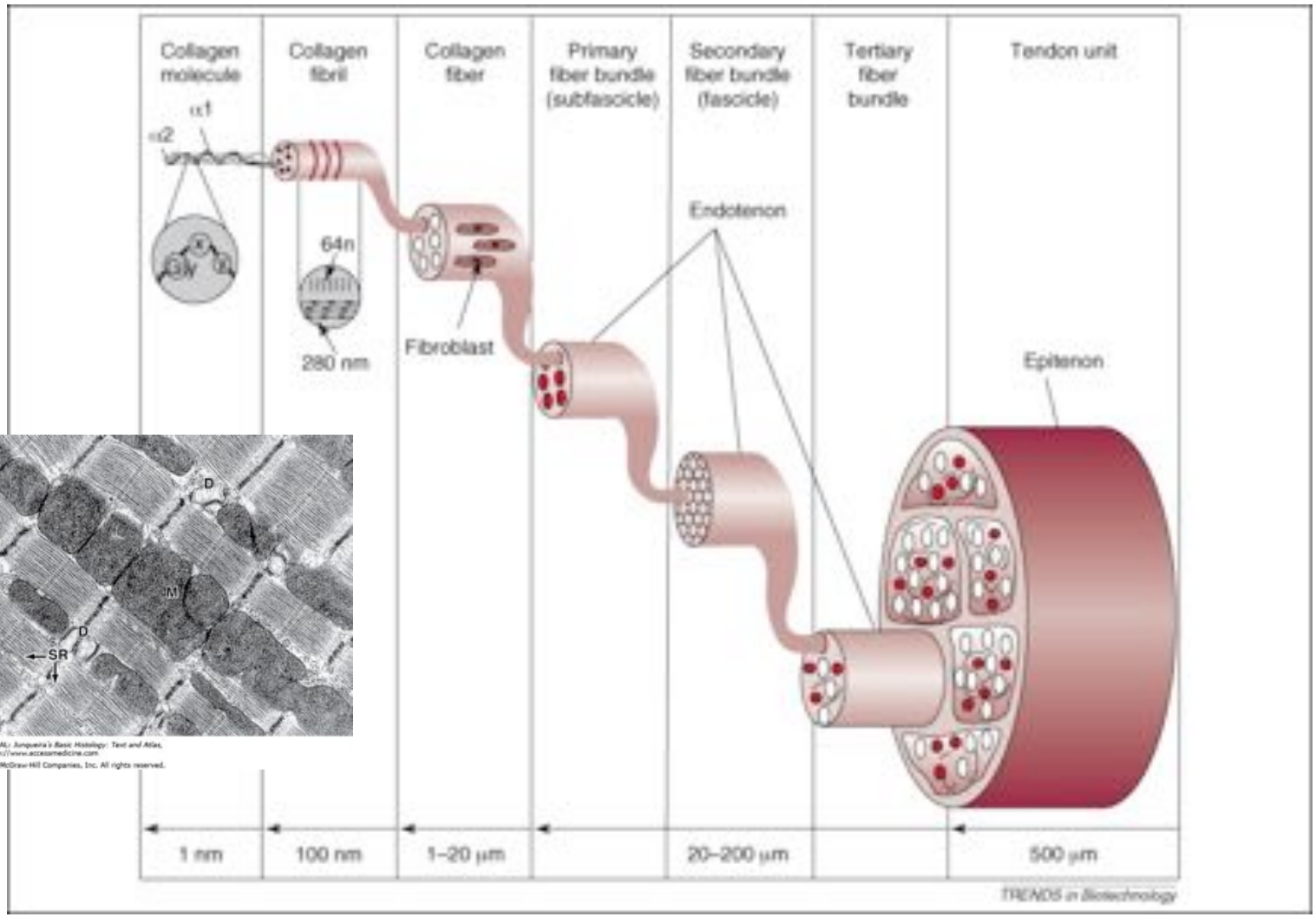
# Tissue Optics- Scattering

- Tissue is composed of structures from nm to m scales







Source: Mescher AL: Junqueira's Basic Histology: Text and Atlas, 12th Edition: http://www.accessmedicine.com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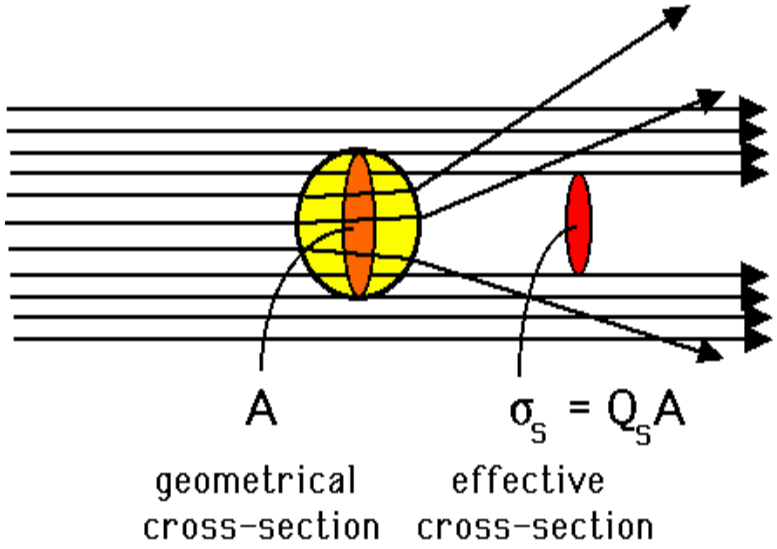
# Tissue Optics- Scattering

- The deviation of light rays at every index of refraction mismatch in tissue is too complex to follow exactly.
- A bulk tissue parameter called the *scattering coefficient* μs [1/cm] is used to define the scattering properties.

  $\mu s = \sigma_s * Ns$

  $\sigma_s$ = atomic scattering cross section [$cm^2$]

  $N_s$ = # of scattering molecules/unit volume [$cm^{-3}$]




Steve Jacques, OMLC

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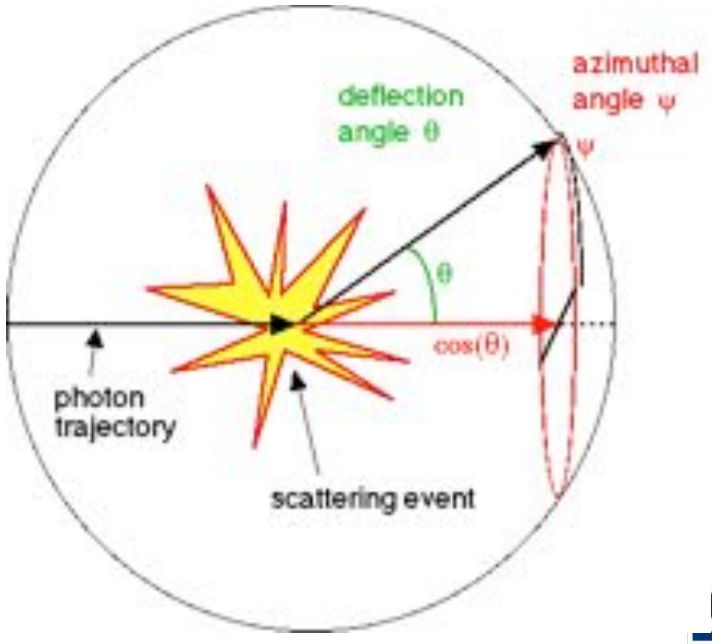
# Tissue Optics- Scattering

- 1/ the scattering coefficient $\mu_s$ says how far a photon is likely to travel before being scattered (the *scattering length*). This is 10-100 μm (0.001-0.01 cm) in most tissues at commonly-used wavelengths.
- However, it doesn't say to what <u>angle</u> the photon will be scattered. That is defined by the *anisotropy* g.

  $g = \langle \cos\theta \rangle$

- There is also some *phase function* defining the shape of the scattering curve.
- Azimuthal scattering is assumed to be uniform.

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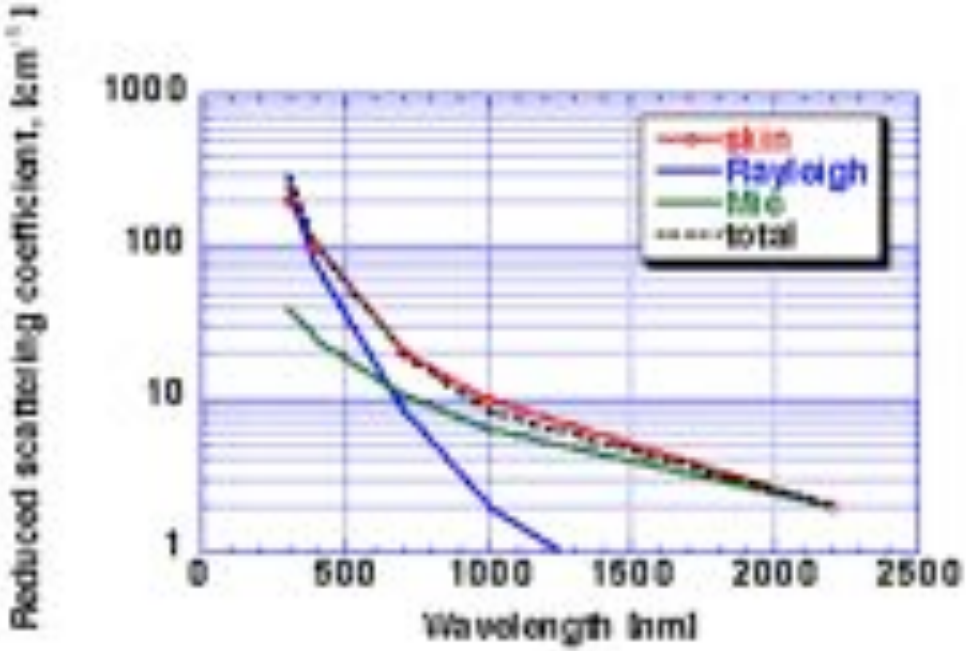
# Tissue Optics- Scattering

- How light spreads out in tissue is a function of both $\mu_s$ and g, and often the two are combined into the *reduced scattering coefficient* $\mu'_s = \mu_s(1-g)$ [1/cm]
- g is about 0.7 to 0.95 so $\mu'_s$ is lower than $\mu_s$.
- Scattering monotonically decreases with wavelength.

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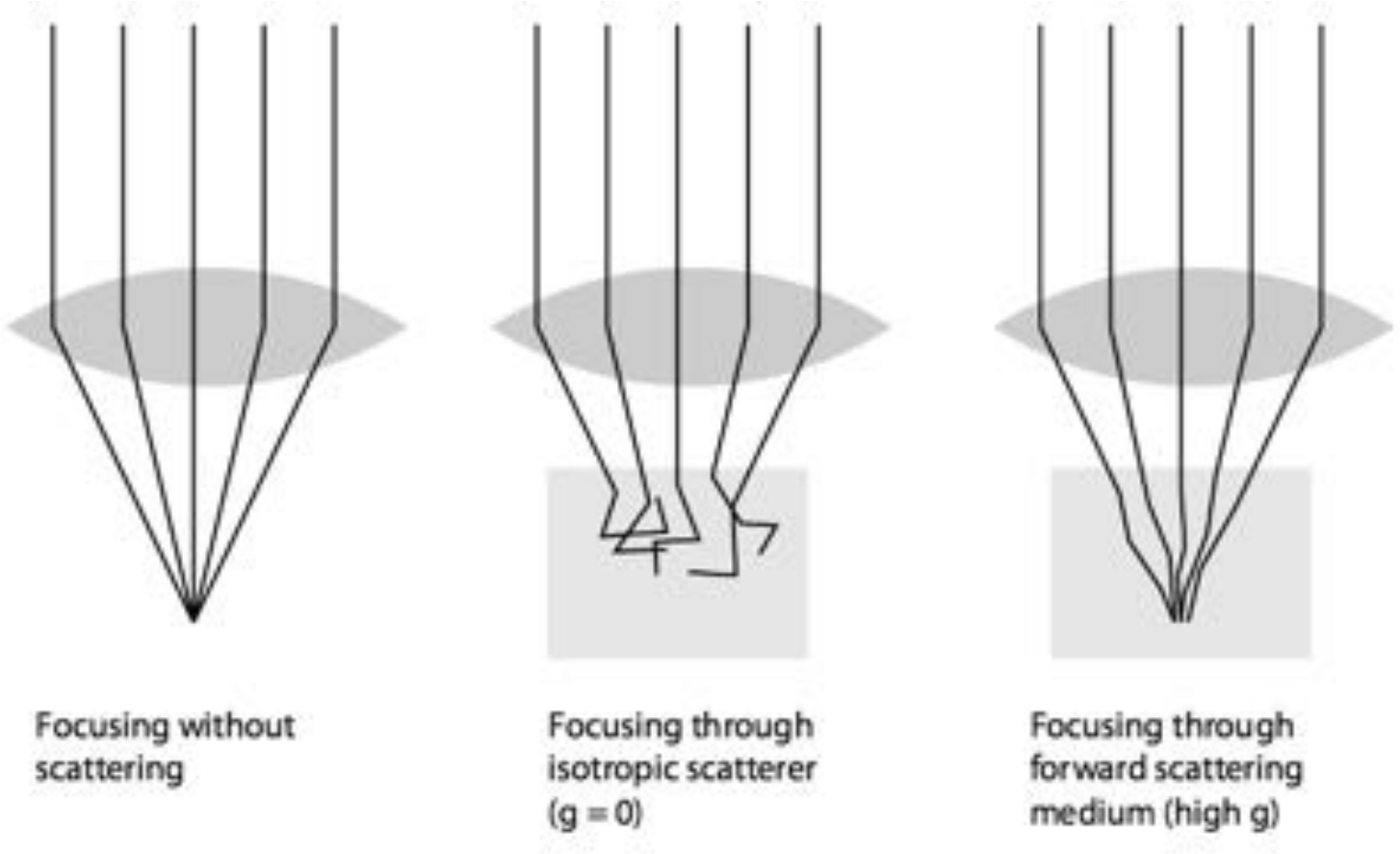
# Tissue Optics- Scattering

- Tissue high g contributes to ability to image




Judkewitz, Cal Tech

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# Tissue Optics- Scattering

- In many tissues, scattering is so high that over moderate distances (mm's), light behaves as a diffusing wave.

Regine Choe, URMC

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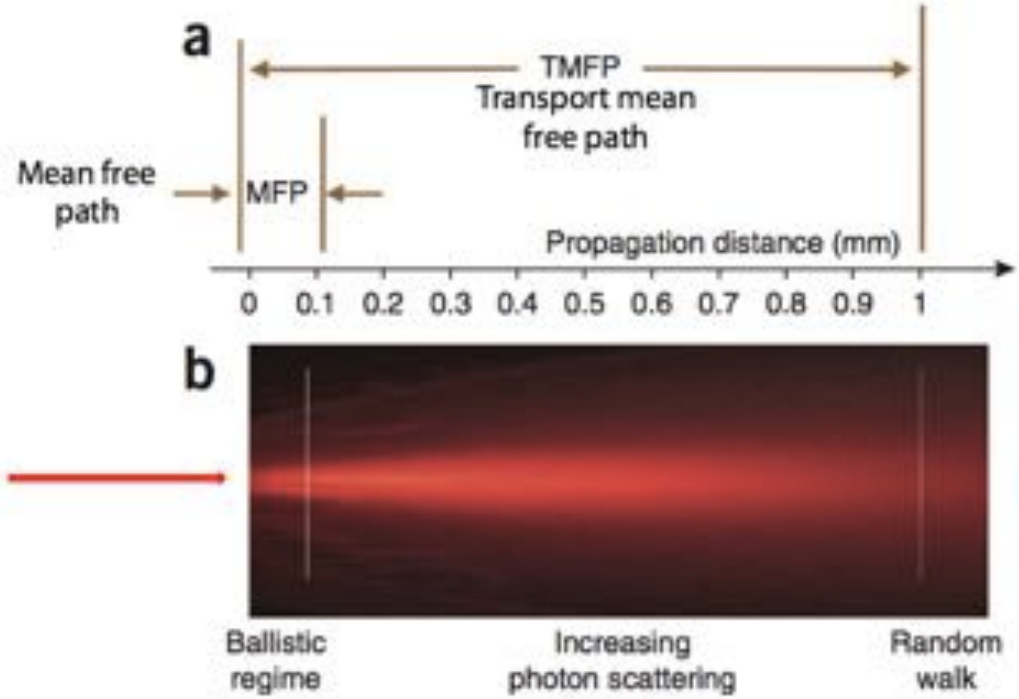
# Tissue Optics- Scattering

- Optical imaging instruments tend to work in either the "single scattering" (ballistic) mode (microscopy, optical coherence tomography), or the "diffuse" (random walk) mode (optical diffuse tomography, bioluminescence , fluorescence spectroscopy).




Ntziachristos, 2010

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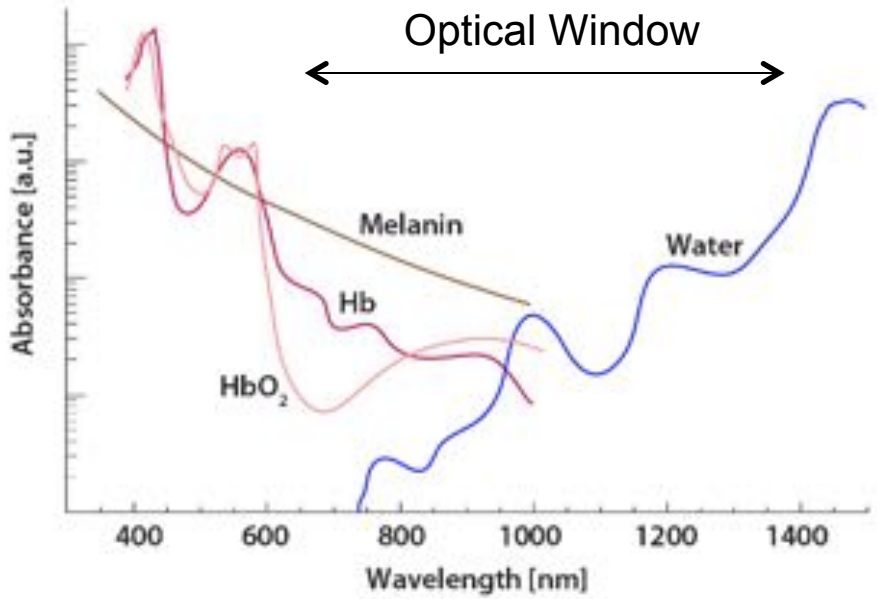
# Tissue Optics- Absorption

- Many chromophores exist in tissue, which absorb light.

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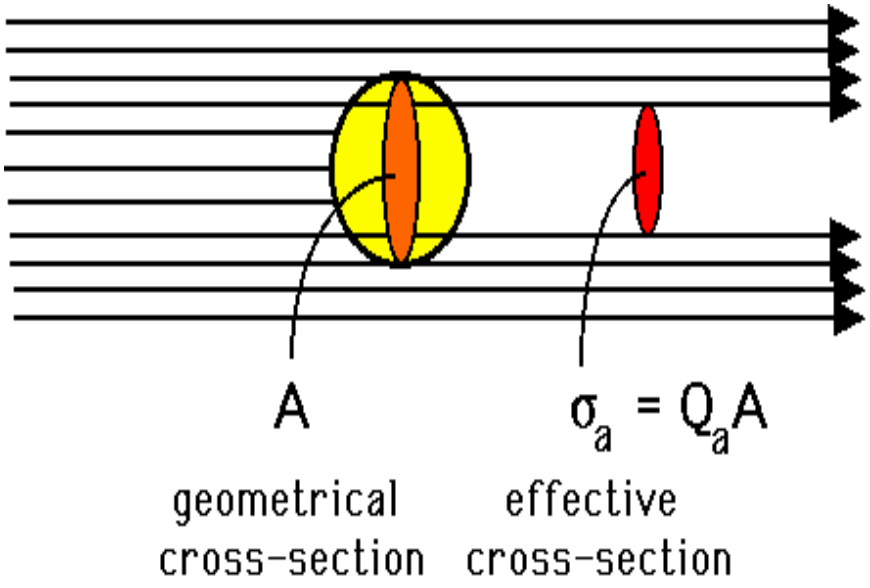
# Tissue Optics- Absorption

- A bulk tissue parameter called the *absorption coefficient* μa [1/cm] is used to define the absorption properties.

  $\mu_a = \sigma_a * N_a$

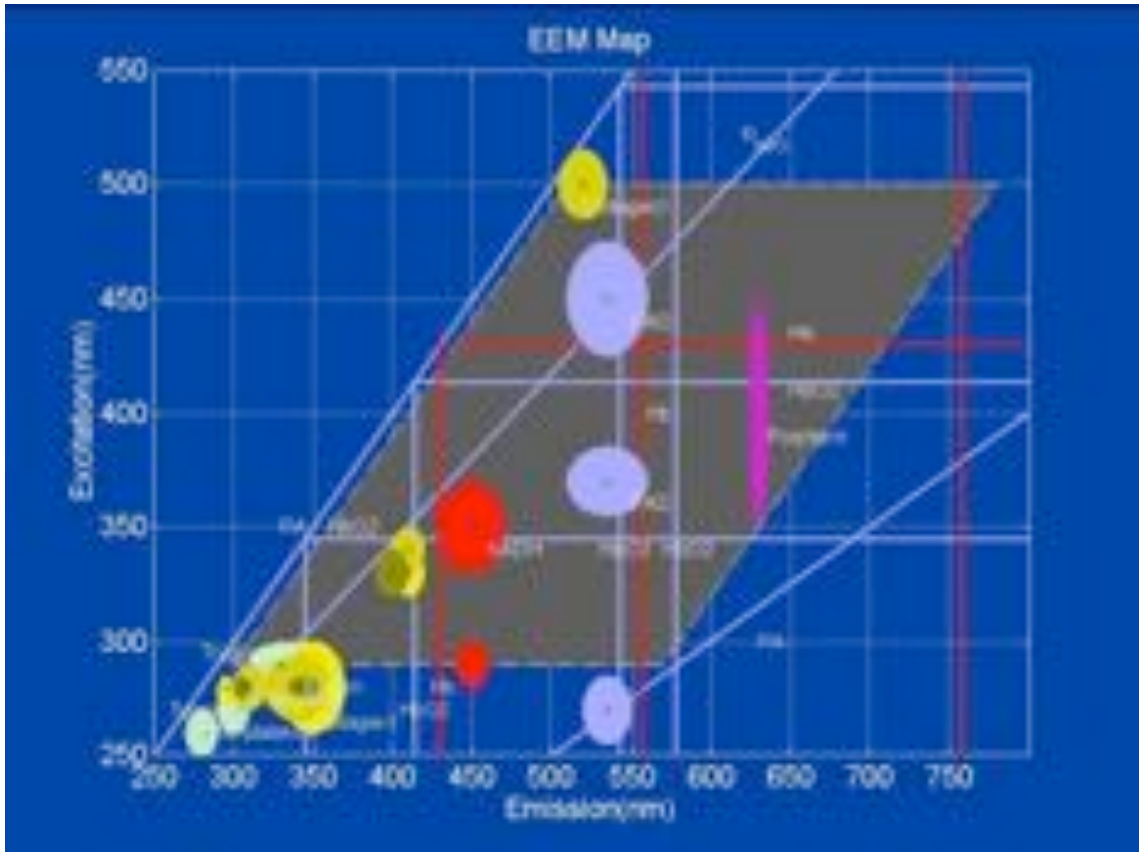
  $\sigma_\alpha$ = atomic absorption cross section ($cm^2$)

  $N_a$ = # of absorbing molecules/unit volume ($cm^{-3}$)



# Tissue Optics- Fluorescence

- Light absorbed by some molecules can be re-emitted at longer wavelengths.



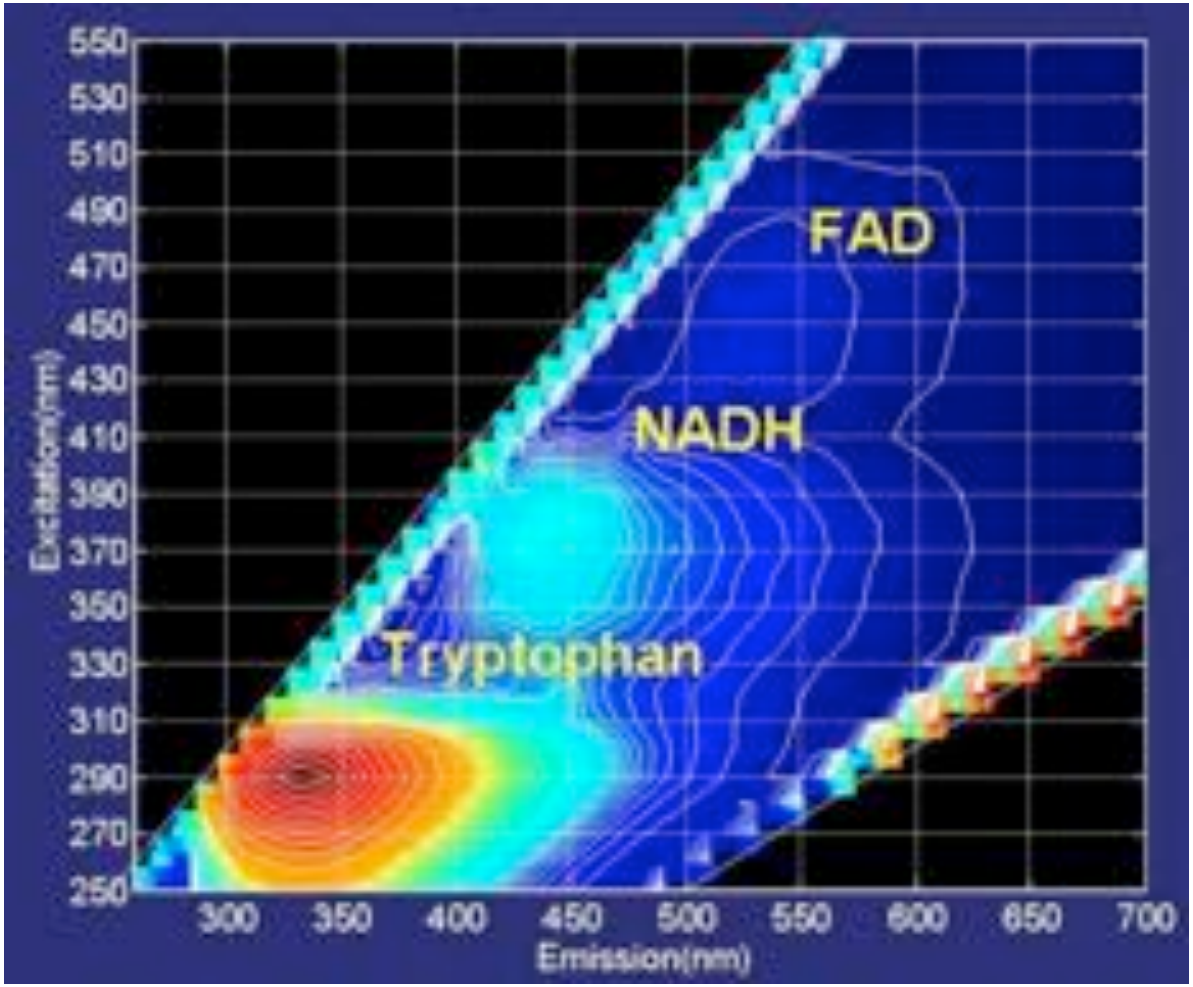

Panaworks.com

| Endogenous fluorophores | Excitation maxima (nm) | Emission maxima (nm) |
|---|---|---|
| Amino acids | | |
| Tryptophan | 280 | 350 |
| Tyrosine | 275 | 300 |
| Phenylalanine | 260 | 280 |
| Structural proteins | | |
| Collagen | 325 | 400, 405 |
| Elastin | 290, 325 | 340, 400 |
| Enzymes and coenzymes | | |
| FAD, flavins | 450 | 535 |
| NADH | 290, 351 | 440, 460 |
| NADPH | 336 | 464 |
| Vitamins | | |
| Vitamin A | 327 | 510 |
| Vitamin K | 335 | 480 |
| Vitamin D | 390 | 480 |
| *Vitamin $B_6$ compounds* | | |
| Pyridoxine | 332, 340 | 400 |
| Pyridoxamine | 335 | 400 |
| Pyridoxal | 330 | 385 |
| Pyridoxic acid | 315 | 425 |
| Pyridoxal 5'-phosphate | 330 | 400 |
| Vitamin $B_{12}$ | 275 | 305 |
| Lipids | | |
| Phospholipids | 436 | 540, 560 |
| Lipofuscin | 340–395 | 540, 430–460 |
| Ceroid | 340–395 | 430–460, 540 |
| Porphyrins | 400–450 | 630, 690 |

FAD, flavin adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; AND(P)H, reduced nicotinamide adenine dinucleotide phosphate.

# Tissue Optics- Fluorescence

- Typical EEM of cells.

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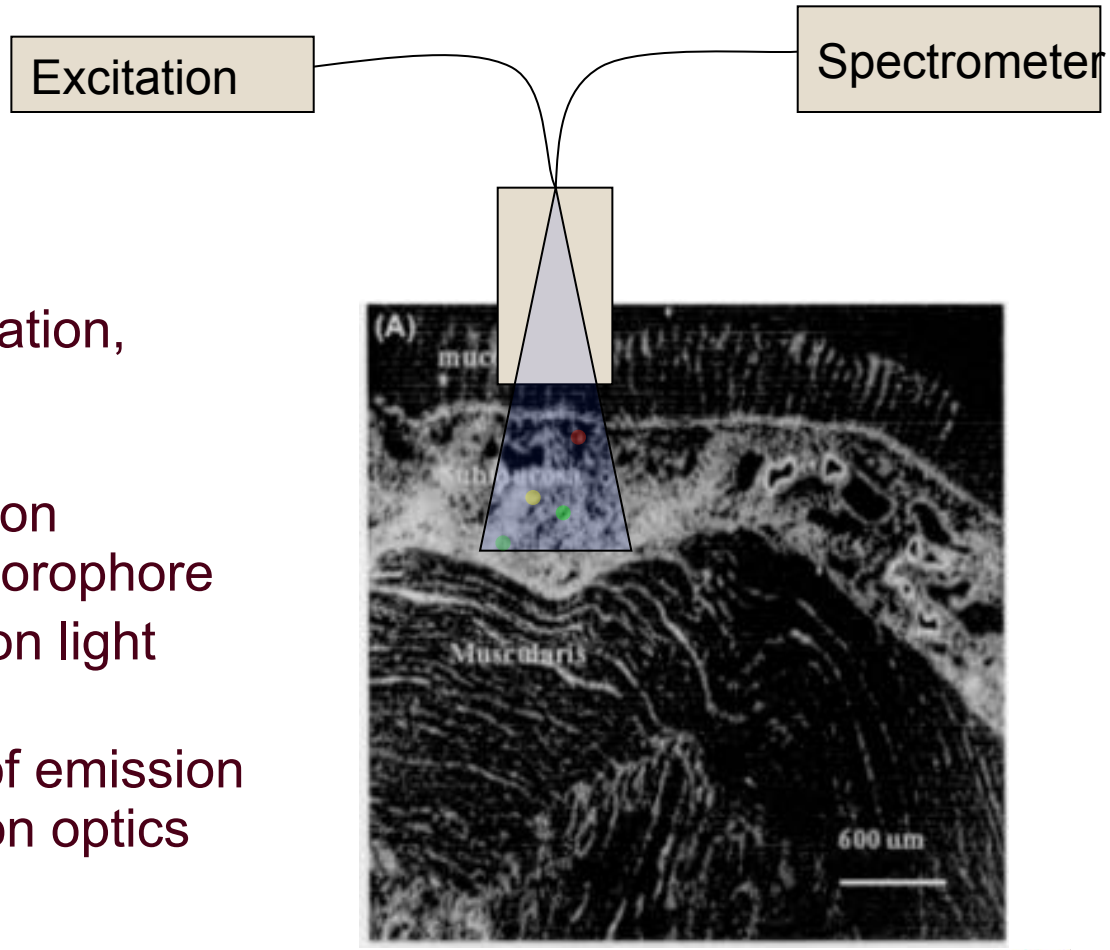
# Tissue Optics- Fluorescence

- In tissue, emission spectra are a superposition of fluorophores, and dependent on:
  - Fluorophore concentration, quantum yield at the excitation wavelength
  - Attenuation of excitation light to positions of fluorophore
  - Attenuation of emission light back to surface
  - Collection efficiency of emission light by instrumentation optics




Huang, Oncology 2004

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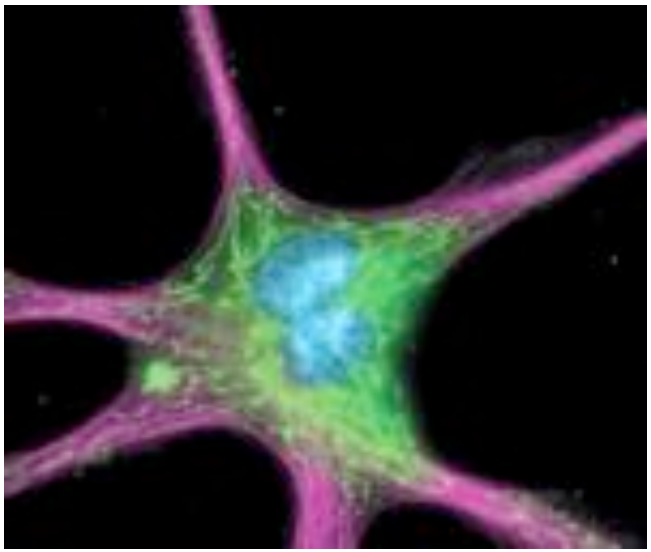
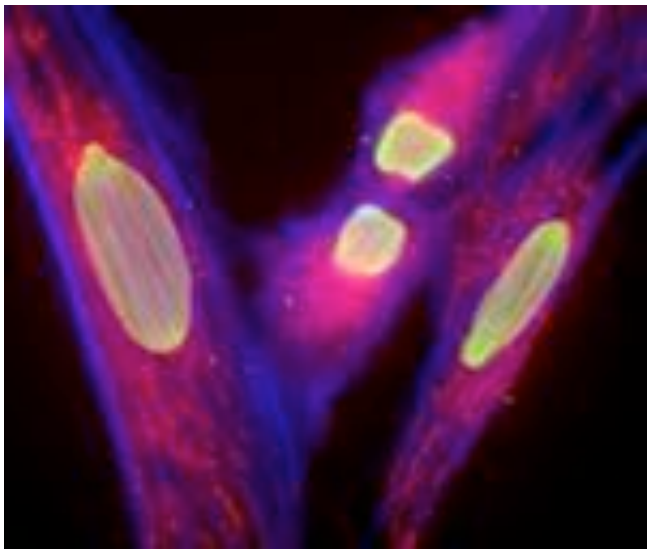
# Tissue Optics- Fluorescence

- Many exogenous fluorophores can be used that are either non-selective (FDA approved fluoroscein, indocyanine green, and methylene blue) or selective (none are FDA approved)





ThermoFisher

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# Light Propagation- Beer's Law

- Beer's law can be used to approximate light intensity in tissue in a one-dimensional case:

$$I = I_o e^{-\mu_t x}$$

- What is $\mu_t$?

  Often, $\mu_t = (\mu_a + \mu'_s)$, for ballistic photons, $\mu_t = (\mu_a + \mu_s)$

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# Light Propagation- Beer's Law

- Beer's law can be used to estimate light levels through homogeneous tissue.

  Optical Coherence Tomography of the colon

  - x = 2 x 0.1 cm
  - μa ~ 0 at 1300 nm
  - μs = 100 1/cm at 1300 nm

  So light is attenuated by factor of $e^{-20}$ or $10^{-9}$

  (i.e. almost no ballistic photons are detected at this depth)

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# Light Propagation- Radiative Transport Equation

- A beam of light loses energy through
  - Divergence
  - extinction (absorption and scattering)
- A beam of light gains energy from
  - scattering directed towards the beam
  - light sources in the medium (wikipedia)

$$\frac{\partial L(\vec{r},\hat{s},t)/c}{\partial t} = -\hat{s}\cdot\nabla L(\vec{r},\hat{s},t) - \mu_t L(\vec{r},\hat{s},t) + \mu_s \int_{4\pi} L(\vec{r},\hat{s}',t) P(\hat{s}'\cdot\hat{s}) d\Omega' + S(\vec{r},\hat{s},t)$$

L = Radiance (energy flow per unit normal area per unit solid angle per unit time

r = position

s = unit direction vector

P = probability of scattering into beam

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# Light Propagation- Diffusion Approximation

- In most biological tissues $\mu_s >> \mu_a$, simplifications can be made and the RTE reduced to the diffusion equation

$$\frac{1}{c}\frac{\partial \Phi(\vec{r},t)}{\partial t} + \mu_a \Phi(\vec{r},t) - \nabla \cdot [D \nabla \Phi(\vec{r},t)] = S(\vec{r},t)$$

$$D = \frac{1}{3(\mu_a + \mu_s')}$$

$\Phi$ = fluence rate (energy flow at all angles per unit normal area per unit time)

D = diffusion constant

- For a constant point source in an infinite homogeneous medium, the solution is

$$\Phi(\vec{r}) = \frac{1}{4\pi D r}\exp(-\mu_{\text{eff}} r) \qquad \mu_{\text{eff}} = \sqrt{\frac{\mu_a}{D}}$$

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# Light Propagation- Monte Carlo

- The diffusion approximation fails for cases where absorption is significant compared to scattering, or generally near boundaries.
- MC method can be used to simulate photon transport. This works well, for example, simulating the fluence rate that will reach a therapeutic target such as a blood vessel.
- MC included in some optical design software packages.

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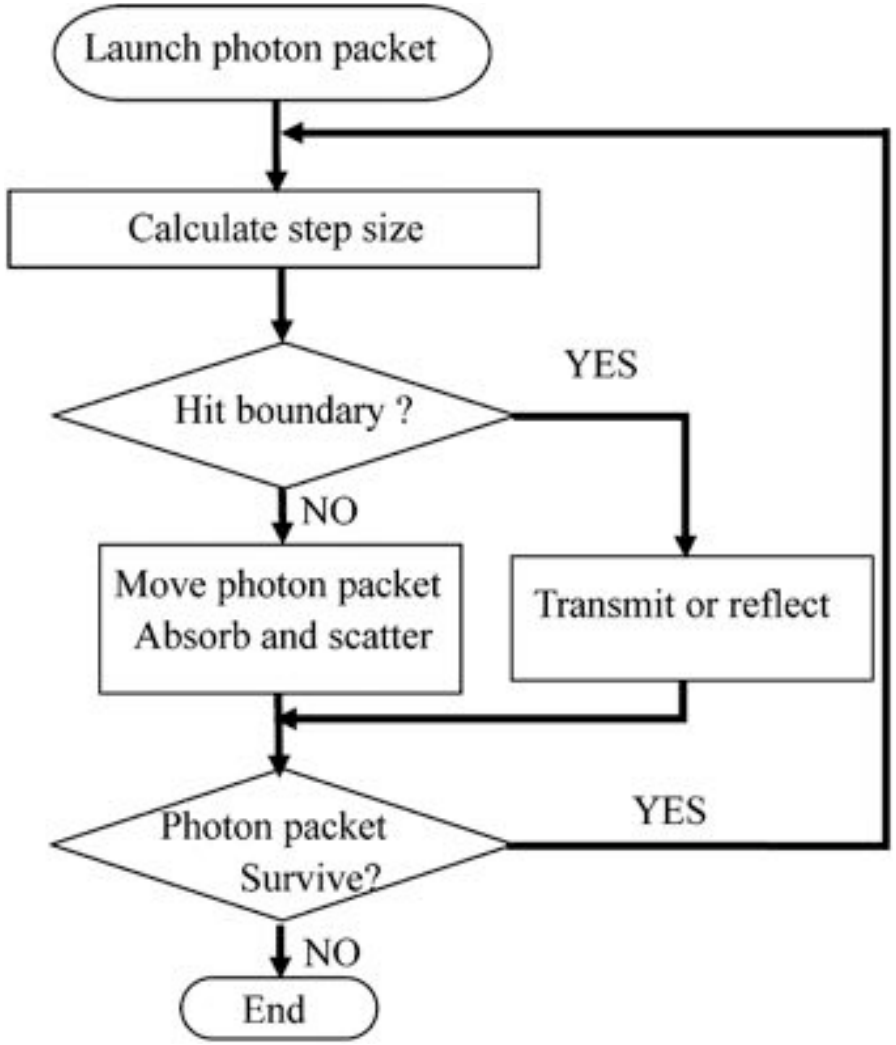
# Light Propagation- Monte Carlo

- MC is simple to implement, and can be used with any arbitrary geometry with arbitrary optical properties.
- Generally, many millions of photon packets are required to create an accurate solution

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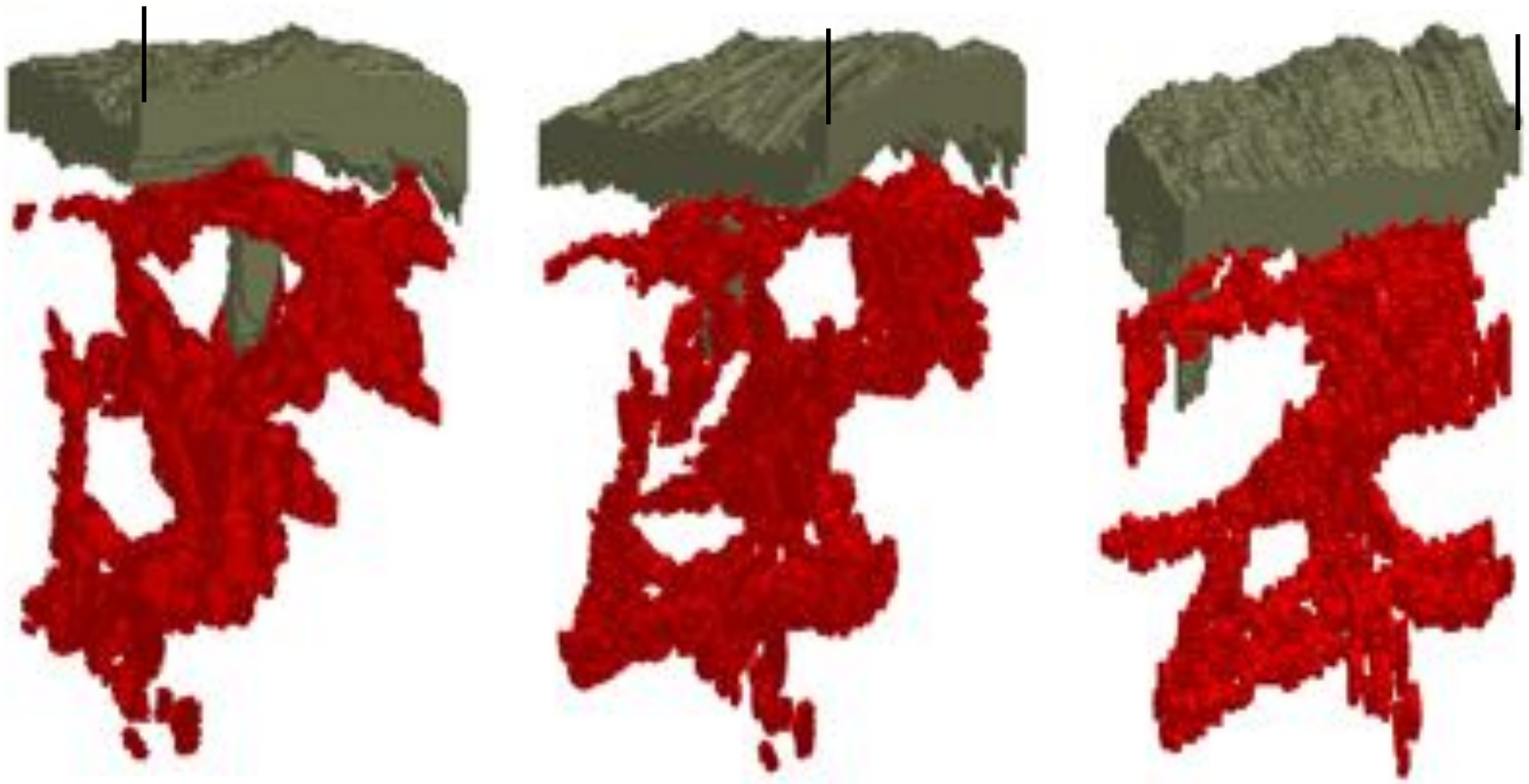
# True Reconstruction of a Skin Biopsy



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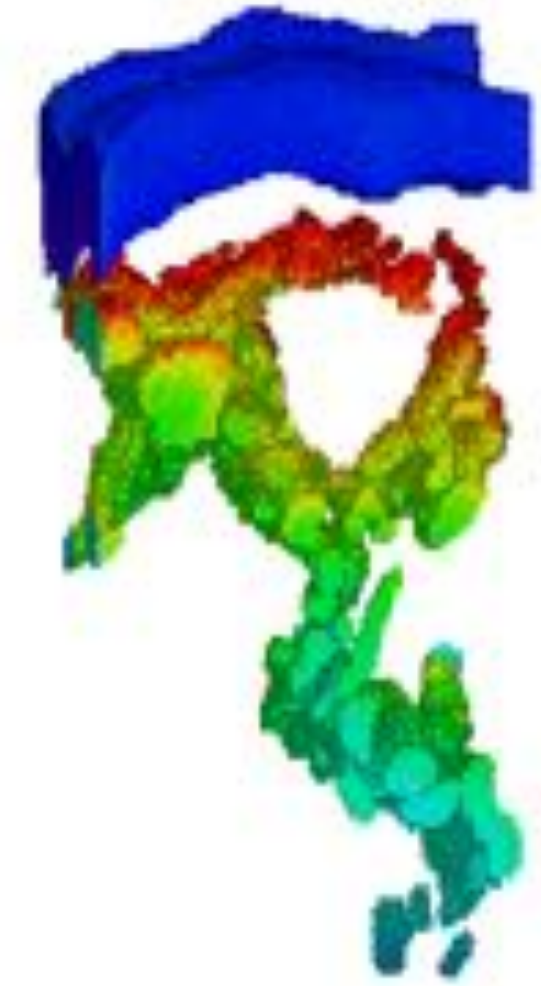
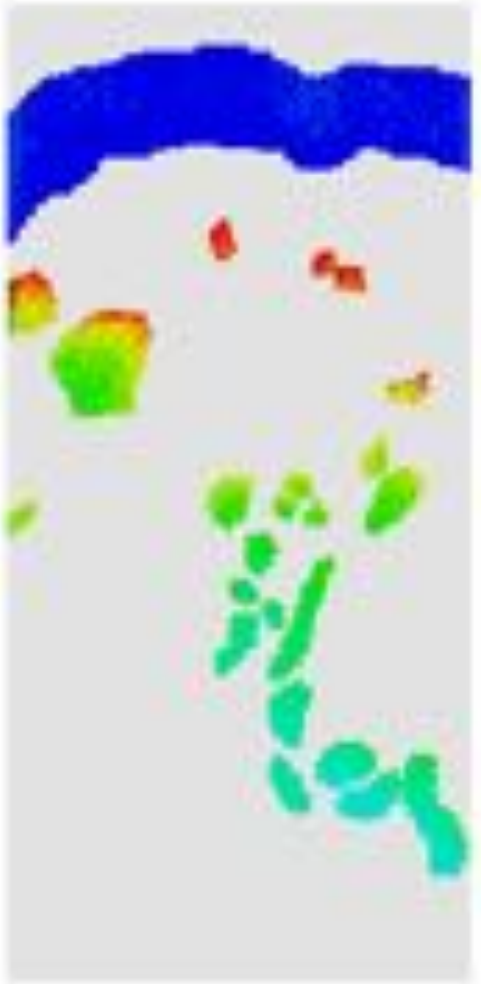
# Example Light Source Term

Laser energy density = 1 J/cm$^2$



250+ J/cm$^3$
200 J/cm$^3$
100 J/cm$^3$
35 J/cm$^3$
1-5 J/cm$^3$



100 μm

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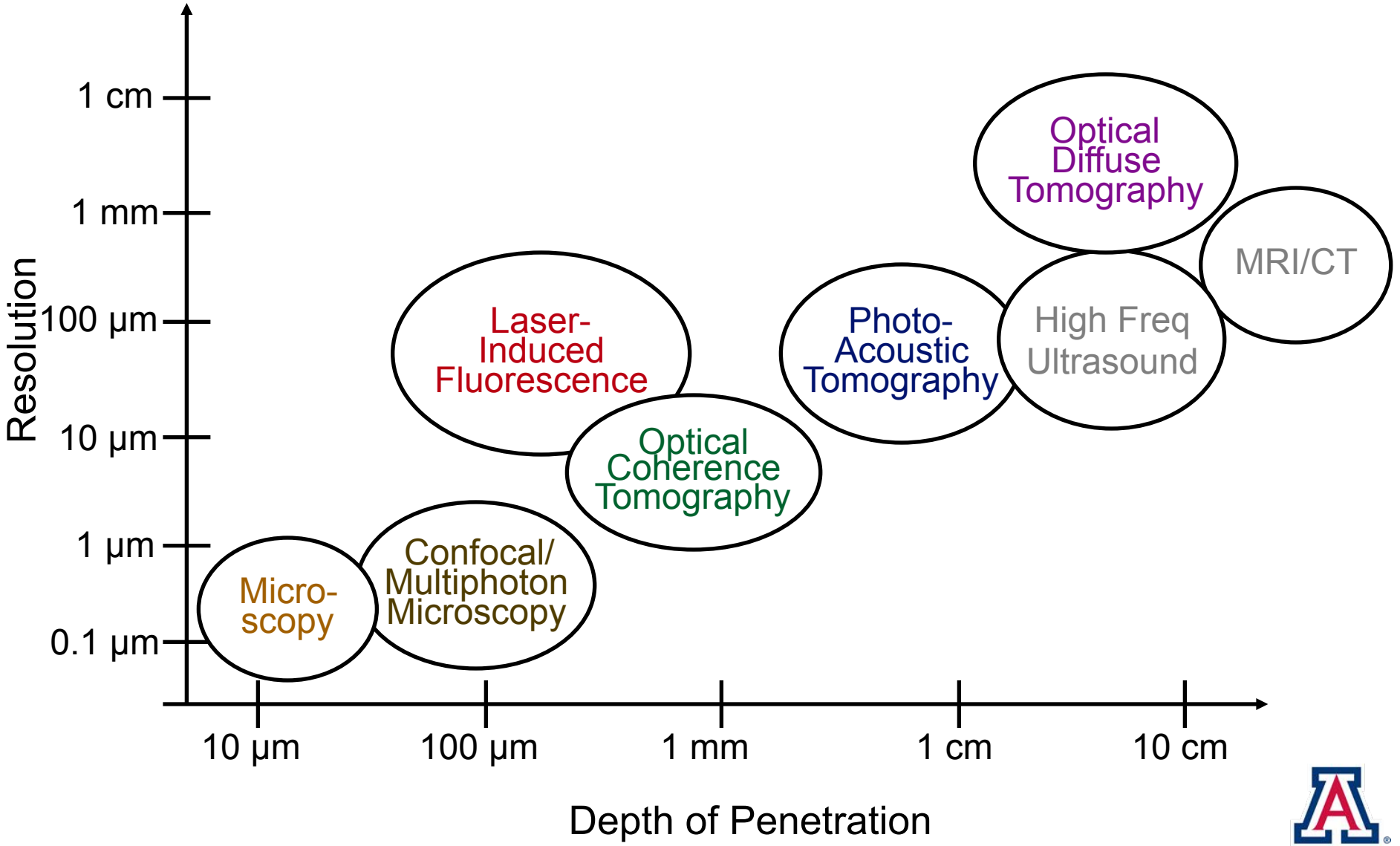
# Imaging Methods

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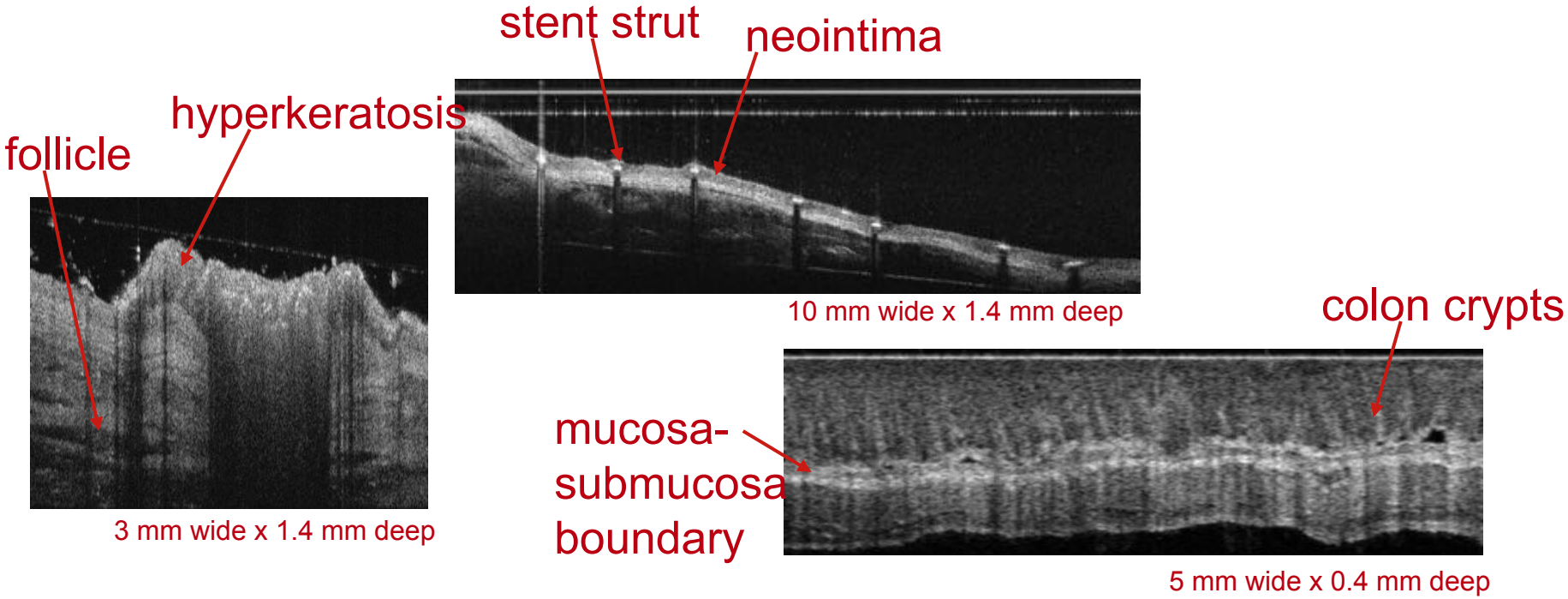
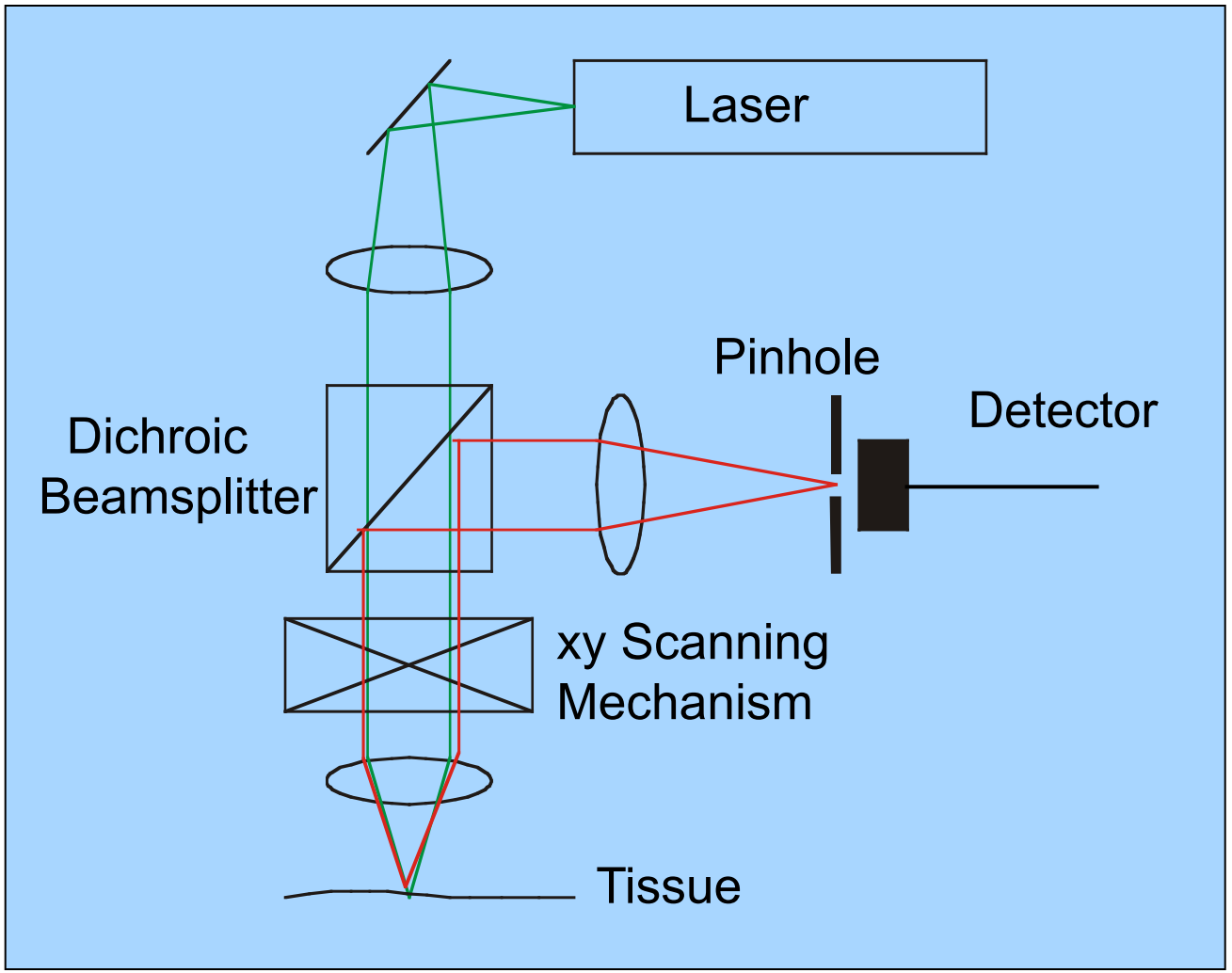
# Optical Coherence Tomography

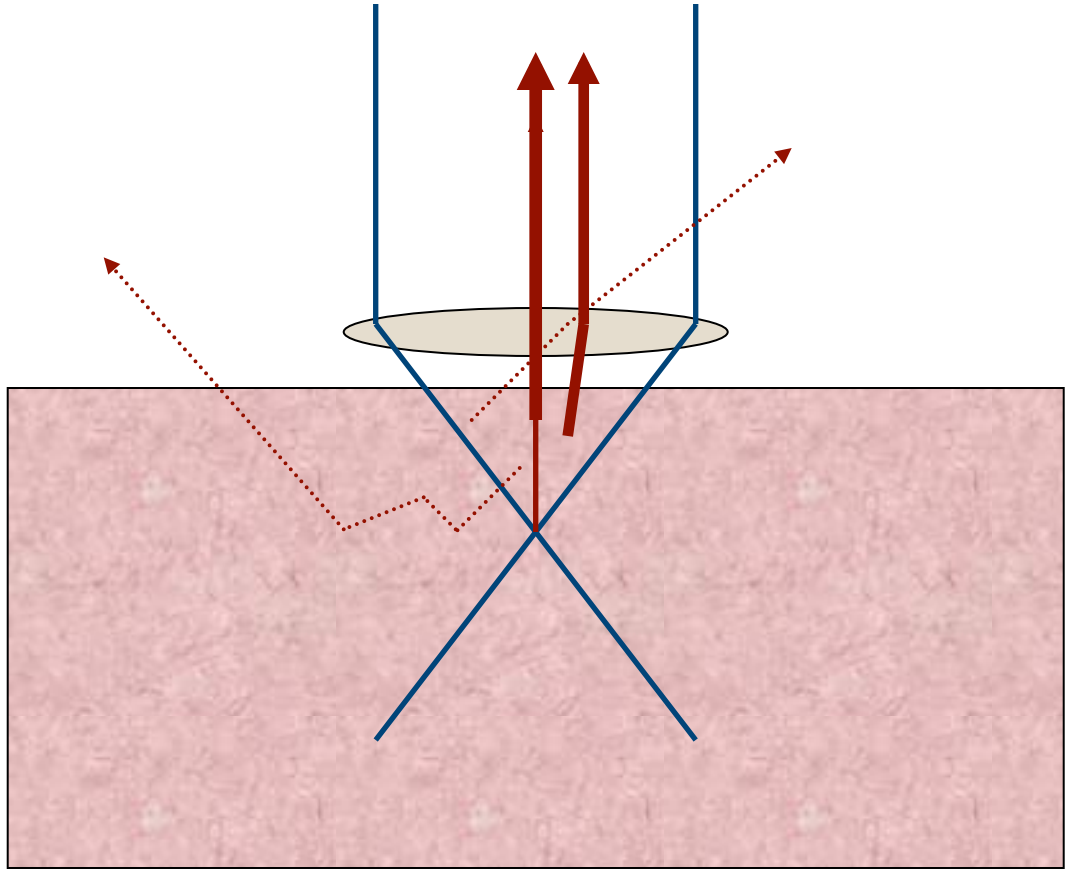
- A portion of an electromagnetic wave incident on tissue is reflected from index of refraction mismatches. Timing indicates depth of mismatch, amplitude indicates magnitude of mismatch.
- OCT is outstanding for mapping the microstructure of tissue (10's of microns scale). This capability is available in robust, simple configurations.



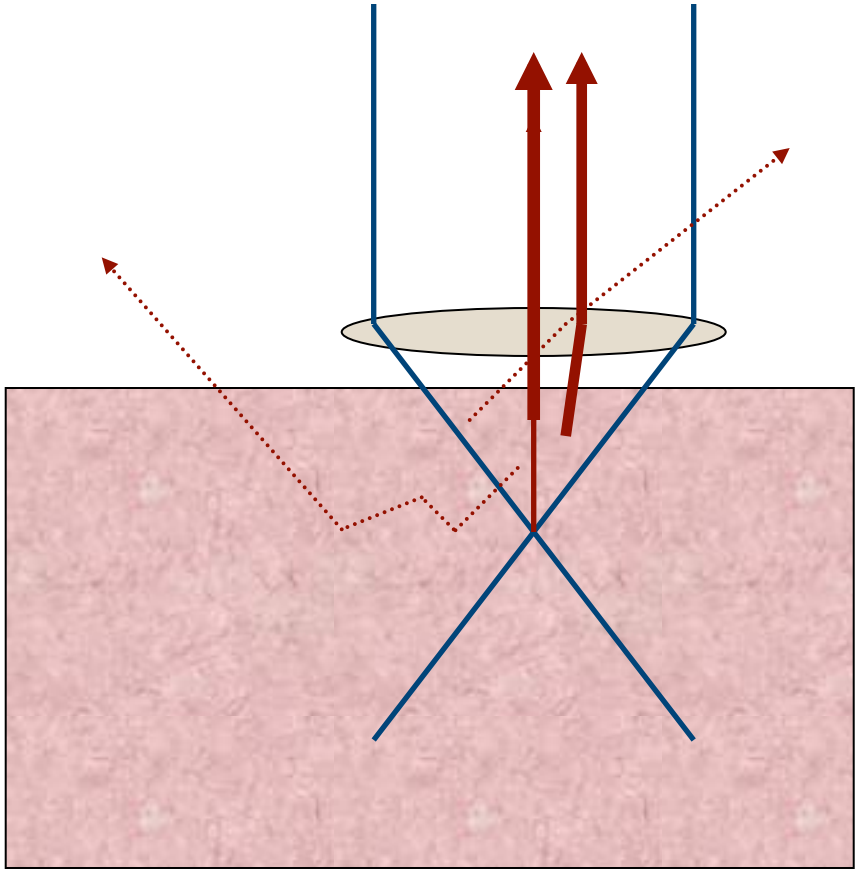
# OCT is Based on Confocal Microscopy



# Spatial Gating



# Spatial Gating



Example:

Focus at 300 μm depth

$\mu_s$ = 100 $cm^{-1}$

6 optical depths in and out

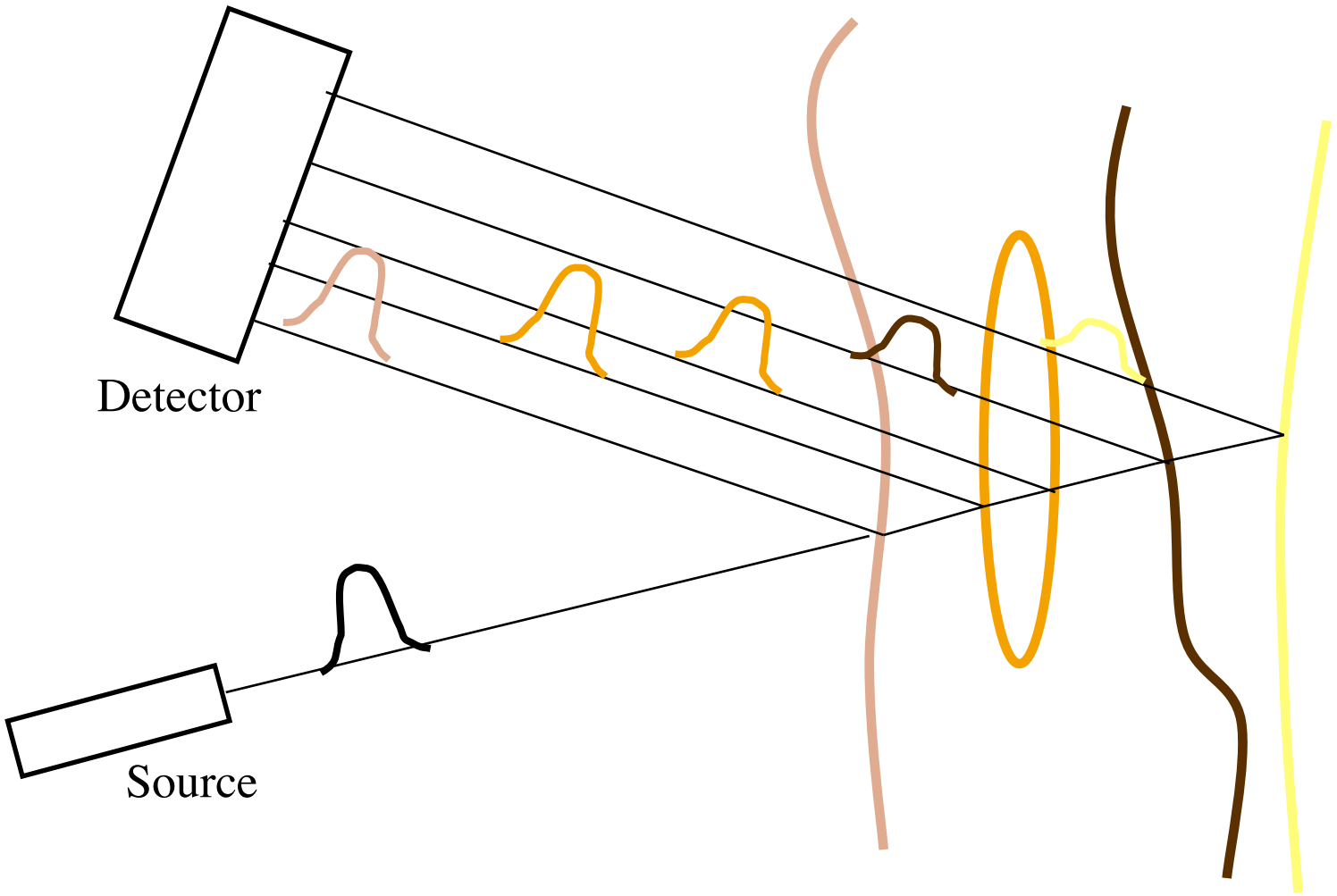
$$\frac{I_{300}}{I_o} = e^{-\mu_s \ell(2)} = e^{-6} = 0.0026$$

Signal is less than -50 dB

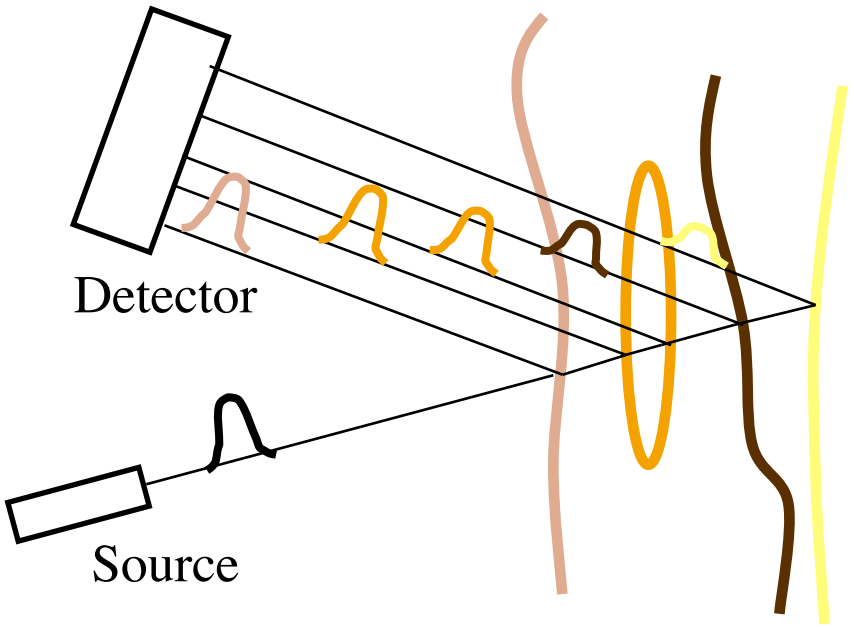
Confocal systems only have about 40-50 dB rejection at 300 μm from focus

Scatter from surface overwhelms signal!

# What can we add? Temporal gating



# What can we add? Temporal gating




This works well for ultrasound:

$c\Delta t = \Delta z$

$c_u = 1500$ m/s

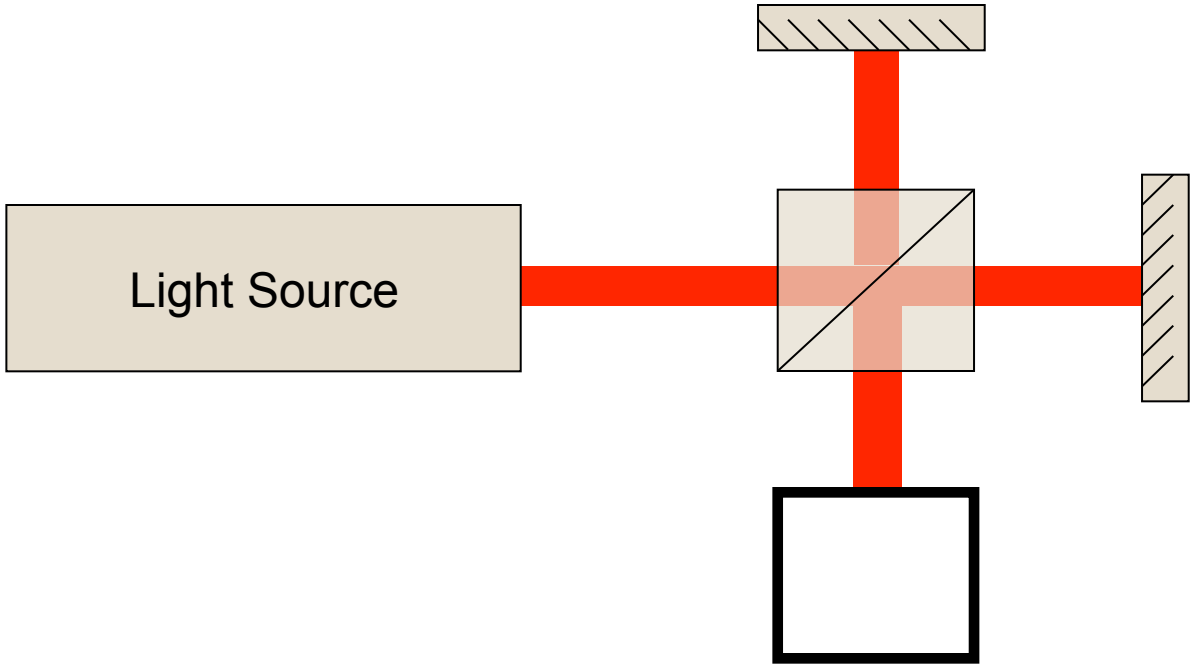
For light:

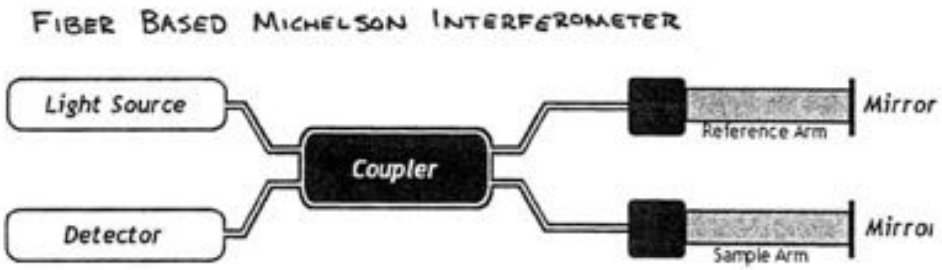
$c_l = 3 \times 10^8$ m/s

For 10 μm resolution

$\Delta t = 3.3 \times 10^{-14}$ s = 33 fs

# An Easier Way




The signal seen at the detector is a function of the optical pathlength between the reference and sample arms

# Broadband Interference and Axial Resolution

Suppose we interfere broadband light in a Michelson interferometer

- In the sample arm we have a mirror (an impulse function)
- We move the reference mirror while the sample remains in place (this is TIME DOMAIN OCT)

Result: we only see destructive and constructive interference when the optical path lengths in both arms are approximately equal. This region where we see interference corresponds to the coherence length of the source.

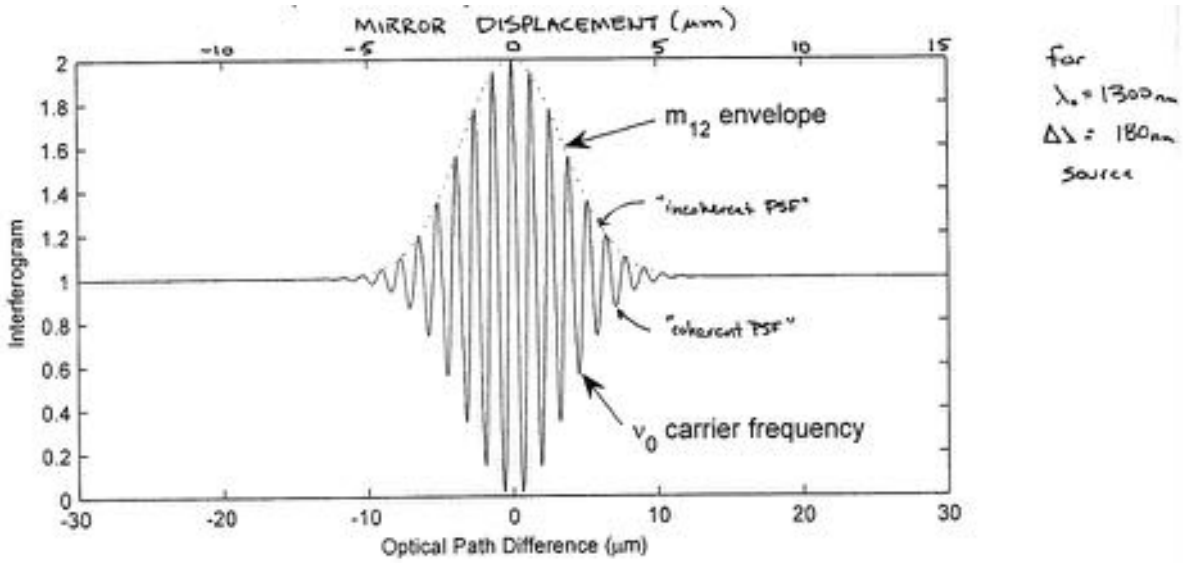
# Broadband Interference and Axial Resolution

The equation for this interference and a simulation are below.

$$I(\Delta t) = I_o + I_o m_{12}(\Delta t)\cos(2\pi \upsilon_o \Delta t + B_{12}(\Delta t))$$

$$m_{12}(\Delta t) = \left| \frac{\Im_{\Delta t}\{I_o(\upsilon)\}}{I_o} \right|$$

$$B_{12} = ang\left[\Im_{\Delta t}\{f(\upsilon)\}\right]$$



# Broadband interference and Axial Resolution

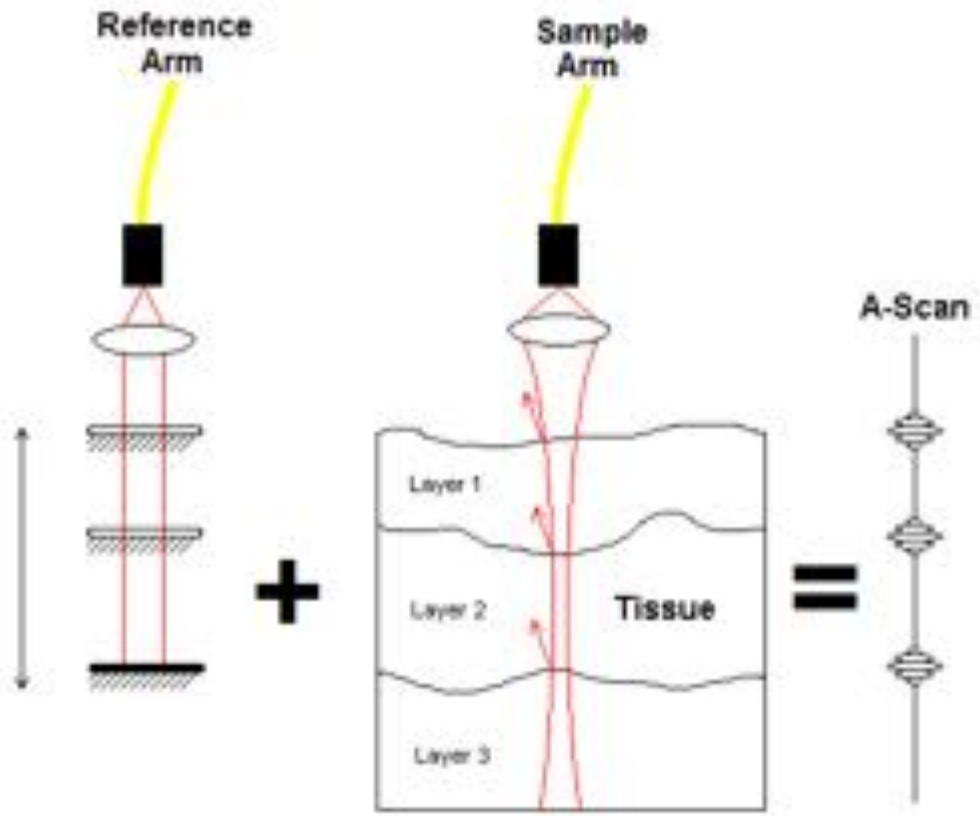
To find the theoretical best axial resolution of an OCT system

1. Determine the FT of the source spectrum
2. Determine the FWHM of this transformation in terms of displacement in the sample arm (remember since light is retroreflected, a mirror displacement of $\Delta z$ corresponds to an OPD of $2\Delta z$.

For a Gaussian distributed light source, the axial resolution of OCT is:

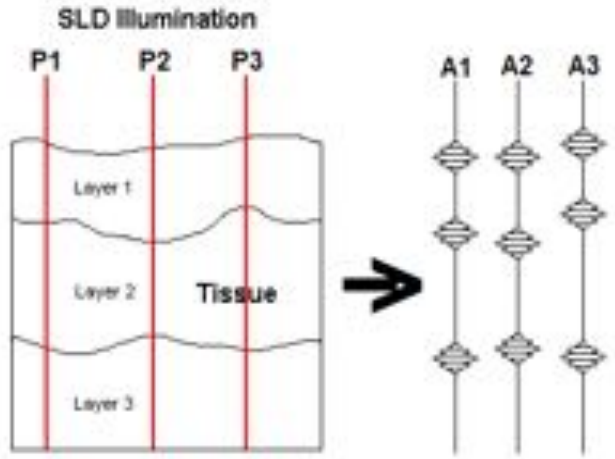
$$\Delta z_{FWHM} = 2\frac{\ln(2)}{\pi}\frac{\lambda_o^2}{\Delta\lambda}$$

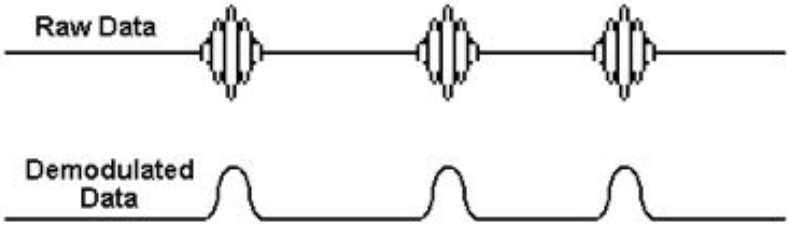
# Detector signal



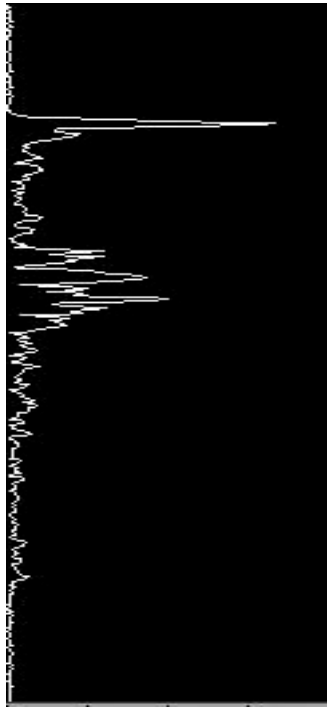
# Optical Coherence Tomography

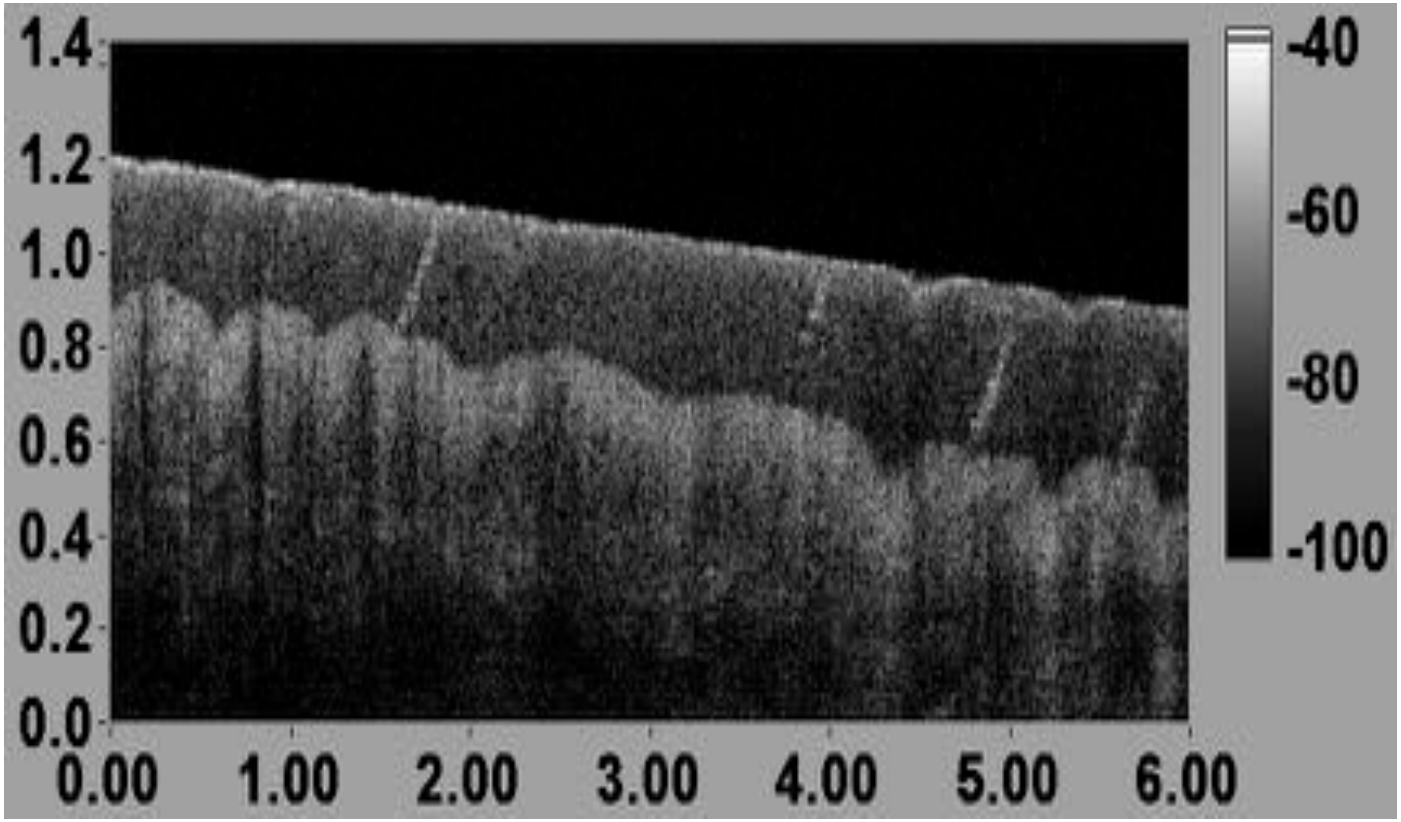
- The beam is scanned laterally across the sample to collect many A-scans (50-500/mm is typical)
- This gives a B-scan, or two dimensional image.
- The raw data from each A-scan is demodulated to measure the amplitude and phase of the signal.



- Most tissues have many index of refraction mismatches. The amplitude of the demodulated A-scan is roughly a depth-resolved indication of the backscattering properties of the tissue.

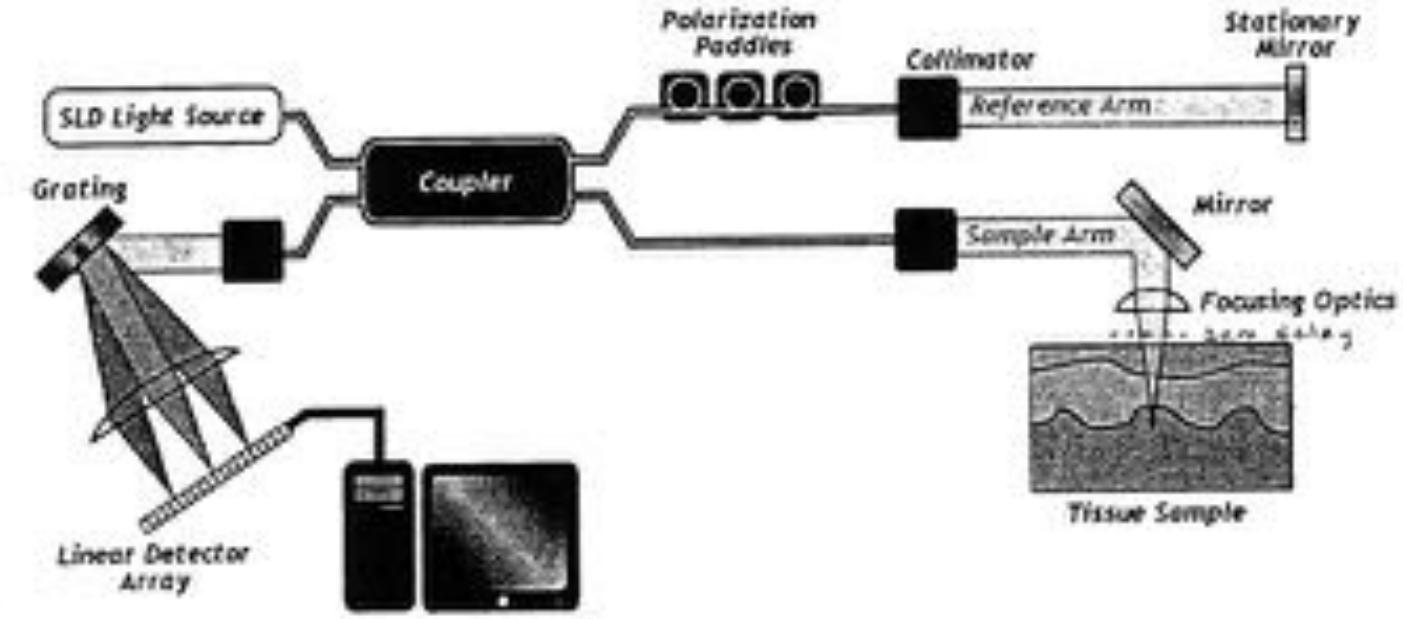




# Fourier Domain OCT

- Collect the signal in the Fourier domain, that is as a function of wavenumber or temporal frequency (k or $\nu$).

$$I(\nu) = I_o(\nu)\left\{1 + \int_0^\infty a(z)\cos(2\pi\left(\frac{2\nu}{c}\right)z)dz\right\}$$



# Fourier Domain OCT

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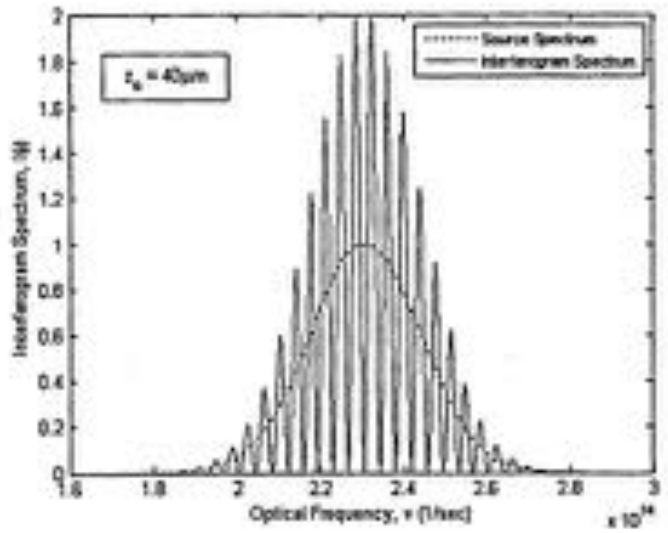
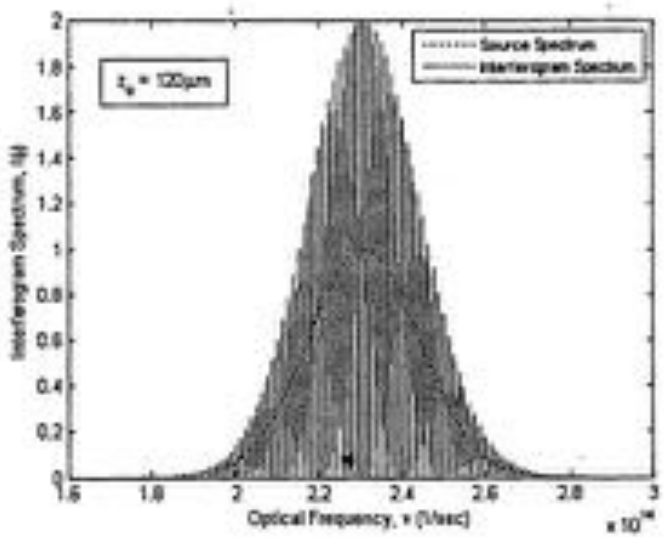
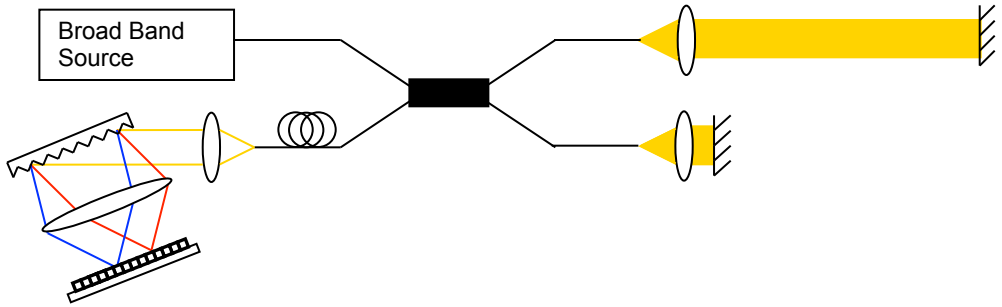
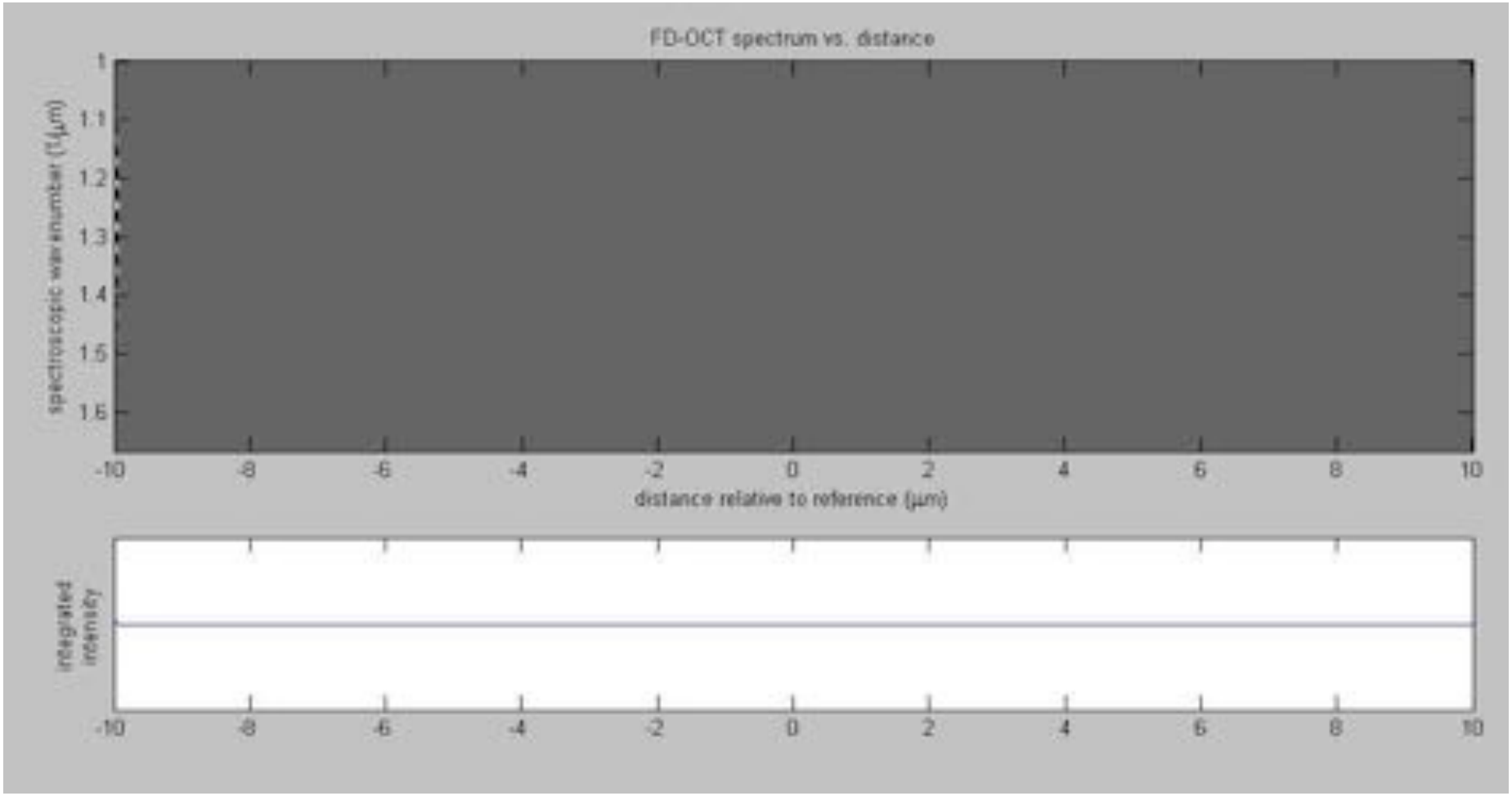
- Below are examples for a reflector at 40 μm and 120 μm from zero delay- note change in frequency.



# Fourier Domain OCT



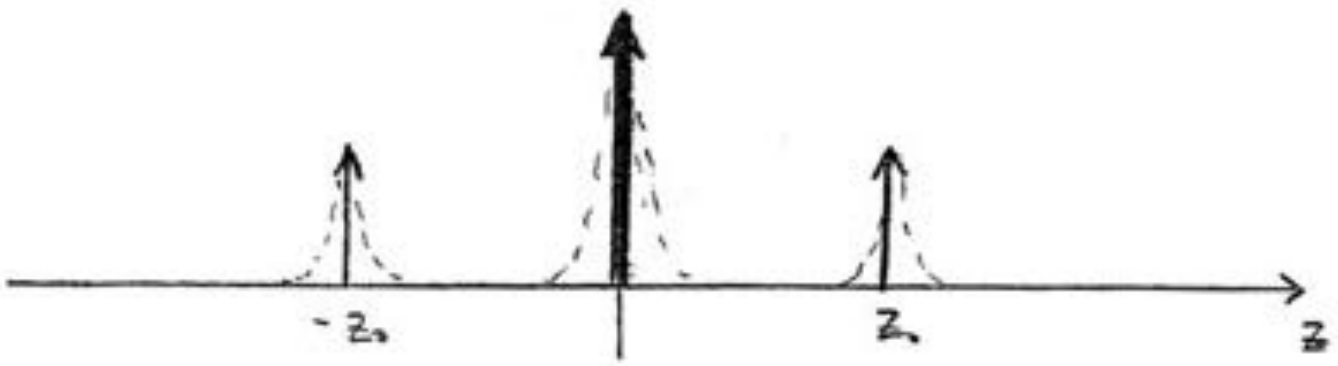



Example: a mirror located at $z_o$ will produce the following spectrum

$$I(\nu) = I_o(\nu) + I_o(\nu)\cos(2\pi\nu(\frac{2z_o}{c}))$$

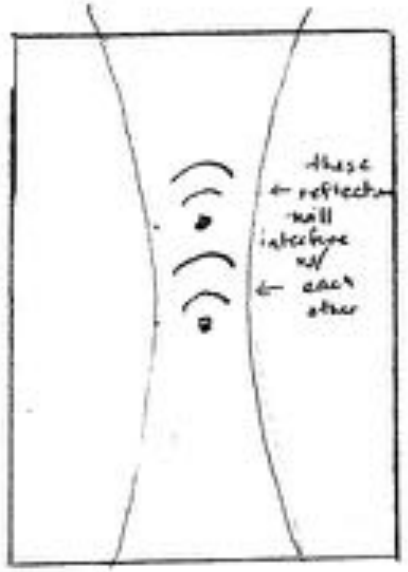
$$\frac{2\nu}{c} \xrightarrow{FT} z$$

$$\hat{a}(z) \propto \left(\delta(z) + \frac{\delta(z-z_o)+\delta(z+z_o)}{2}\right) \otimes I_o(z)$$

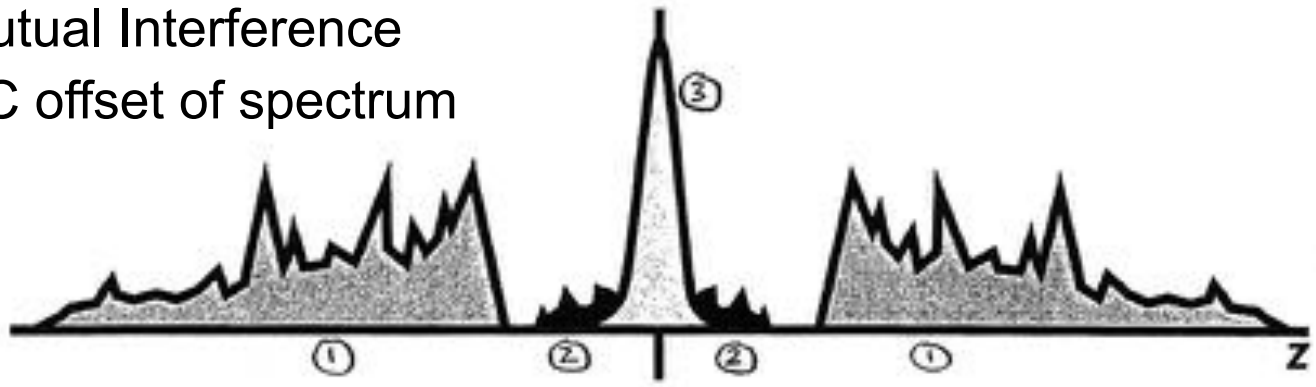


For real tissues, must also consider the fact that there will be interference between waves reflected from tissue scatterers

The mutual interference of these scatterers occurs around z=0. The magnitude of this mutual interference is much weaker (usually) than the real signal because the signal term is weighted by the strong reflectance amplitude.



1) The signal
2) Mutual Interference
3) DC offset of spectrum

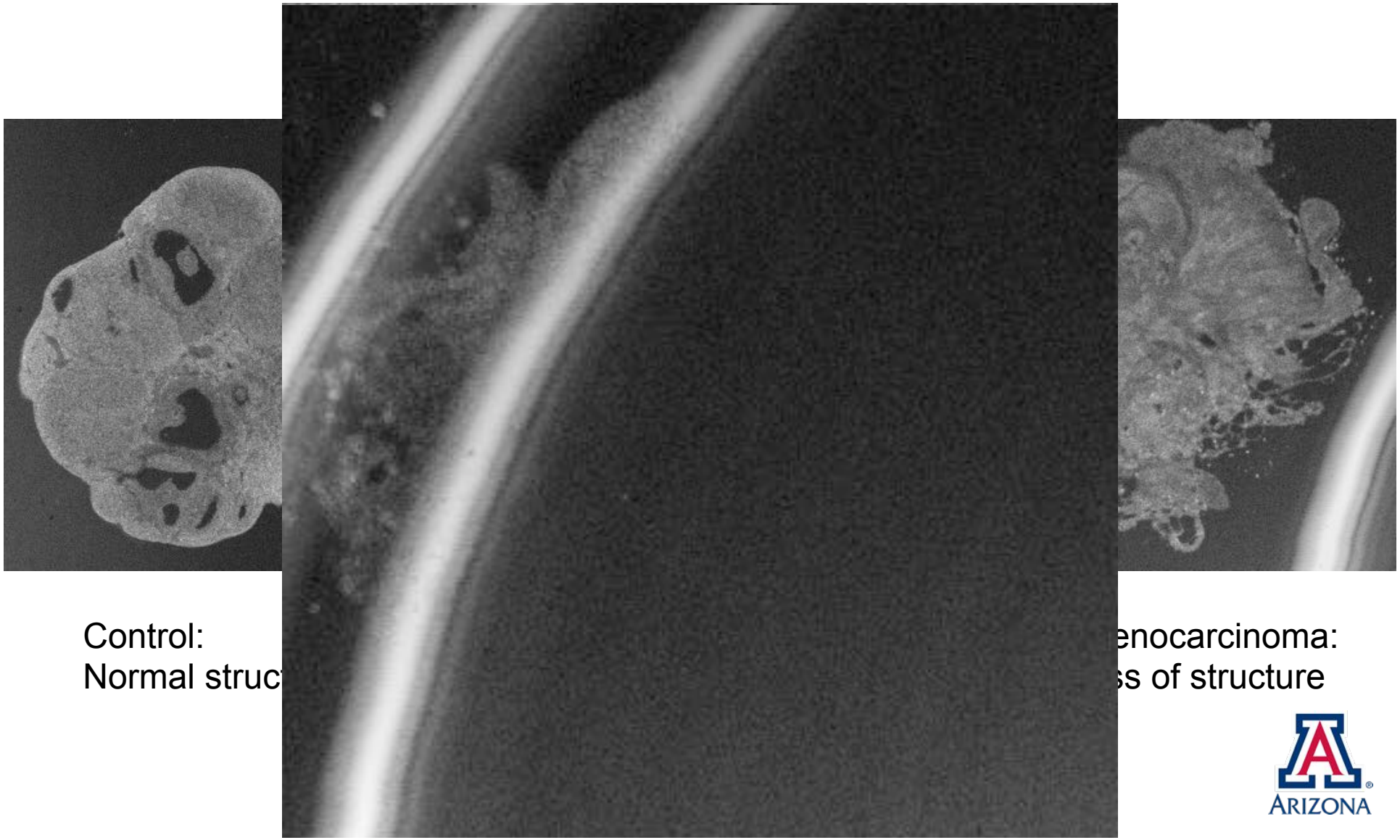


# Advantages and Disadvantages

- FDOCT has two advantages over time-domain OCT:
  - Fast acquisition speed
    - no scanning elements needed to acquire a-scan data
    - All a-scan data acquired in one exposure of linear detector array
  - SNR increase
    - Since all N a-scan data acquired at once, for the same imaging speed each element is exposed N times longer. For shot-noise limited operation, the SNR increases by sqrt(N).
- FDOCT has some disadvantages:
  - SNR fall-off with depth
    - Imaging depth can be limited by the resolution of the spectrometer (TDOCT only limited by attenuation of tissue)
  - Additional Data processing
    - Must interpolate spectrum acquired as a function of wavelength into function of $\nu$, then Fourier transform data prior to displaying
  - Technology limitations
    - Detector (CCD) arrays noisier than single element photodetectors. *Swept source FDOCT* gets around this.

# Higher Resolution 3D OCT (en face)

- Mouse model of ovarian cancer



Control:
Normal struc

enocarcinoma:
ss of structure

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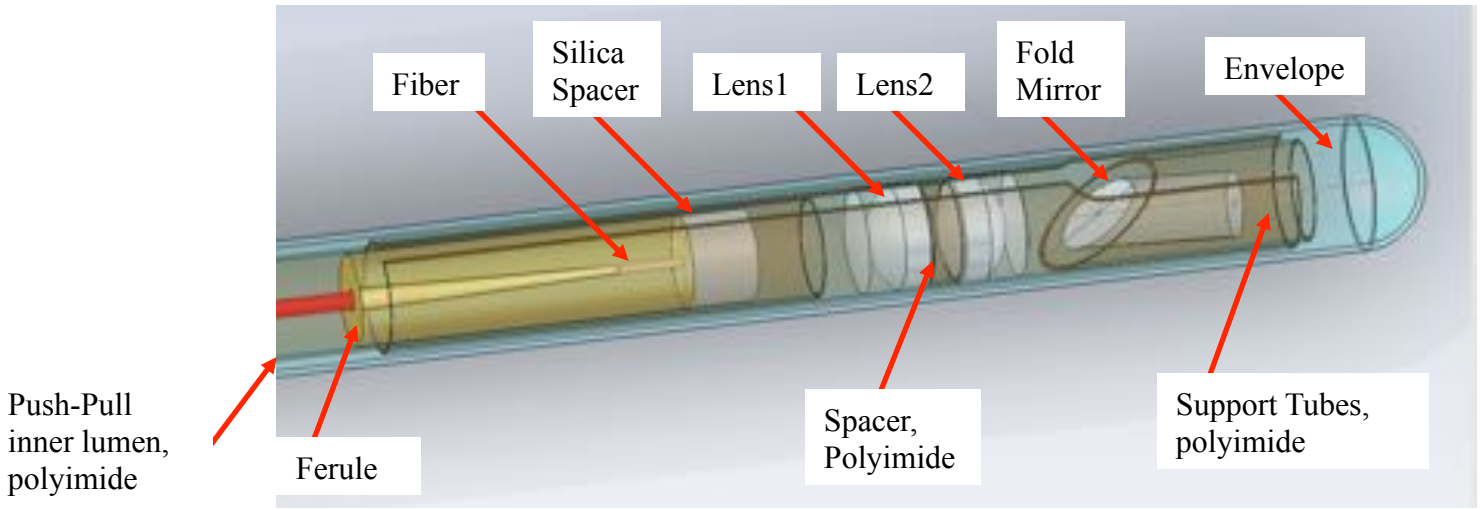
# Endoscopic OCT

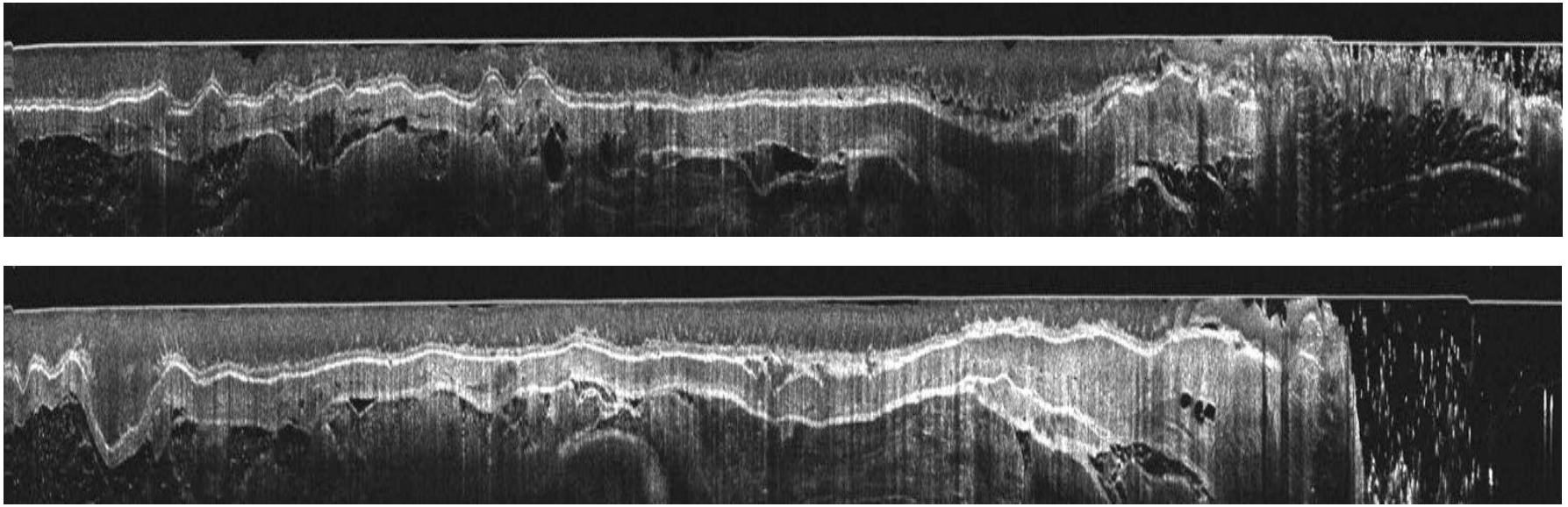
- Easy to create a variety of delivery optics. Can make very small endoscopes to reach internal organs, blood vessels/



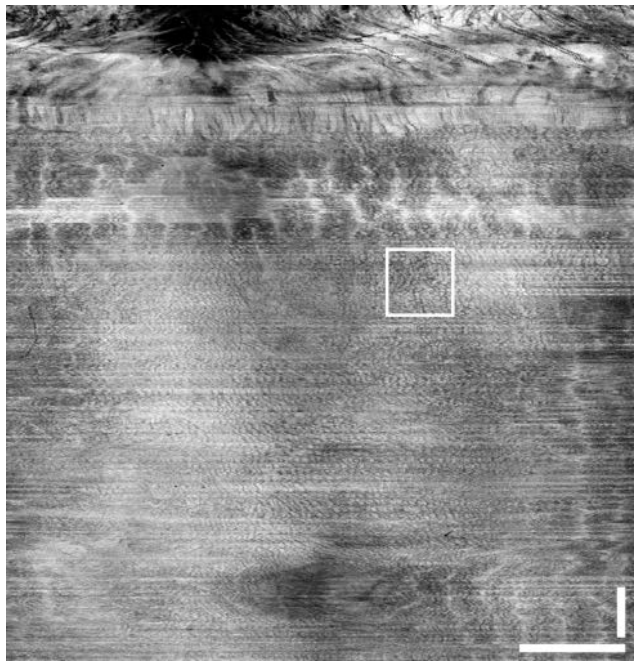

- 2 mm in diameter, exotic glass doublet and mirror- based
- Supports very large bandwidth for high axial resolution
- High numerical aperture for high lateral resolution
- 5 μm lateral spot size (1:1 imaging of fiber core)

# In vivo endoscopic mouse colon

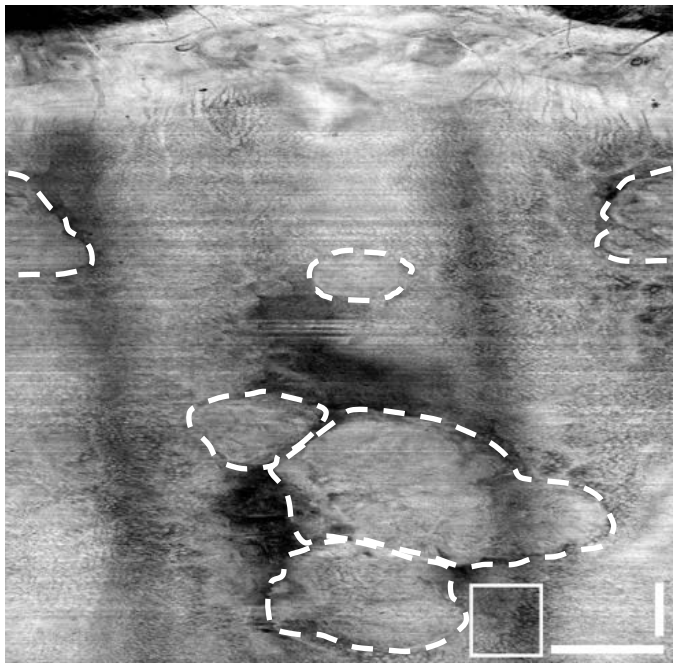
proximal Vertical distortion. Images 30mm X 0.5mm distal



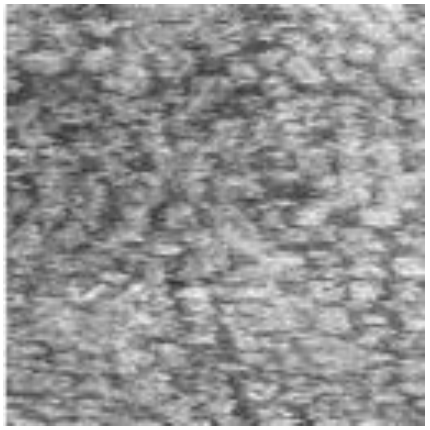
# Projection 3D OCT (spiral scan, virtually flattened)



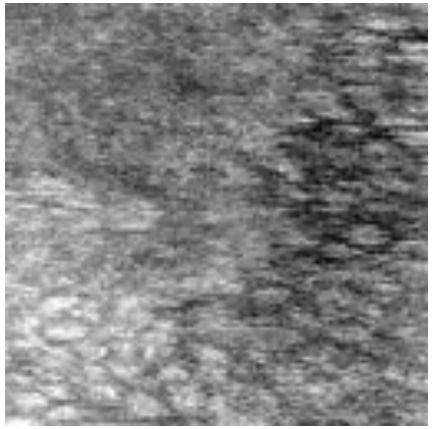
10 mm x
6.3 mm



Healthy



Adenomatous



# OCT Translation to the Operating Room

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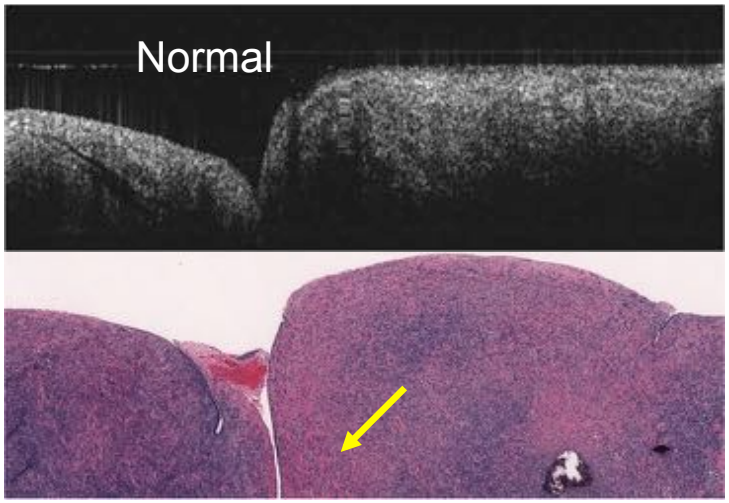
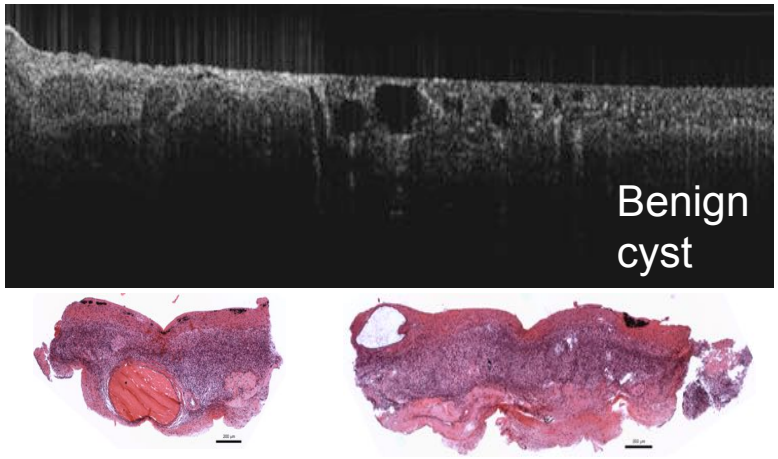
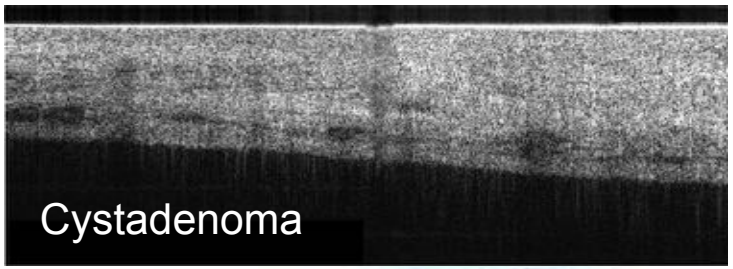
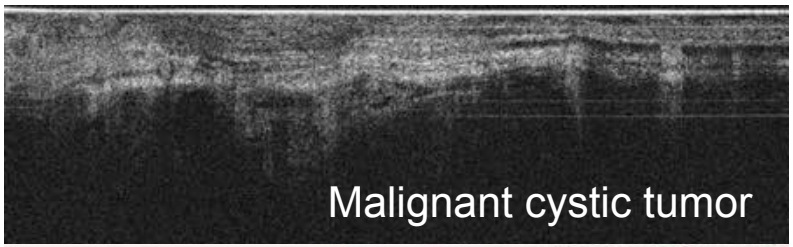
# *In vivo* OCT images




**4 mm x 2 mm**

# Diffuse Optical Tomography

- The imaging modality in which the spatially inhomogeneous optical properties are inferred from surface fluence measurements (often at multiple wavelengths) is called Diffuse Optical Tomography
- Advantages:

  functional information:

  $\mu_a \Rightarrow$ Hb, $Hb0_2$, $H_20$, fat concentration

  $\mu_s' \Rightarrow$ structural information

  cost-effective, "portable" (compared to MRI, CT)
- Disadvantages

  ill-posed problem $\rightarrow$ reconstruction difficult (optical properties and tumor dimension to be determined)

  low resolution- several mm

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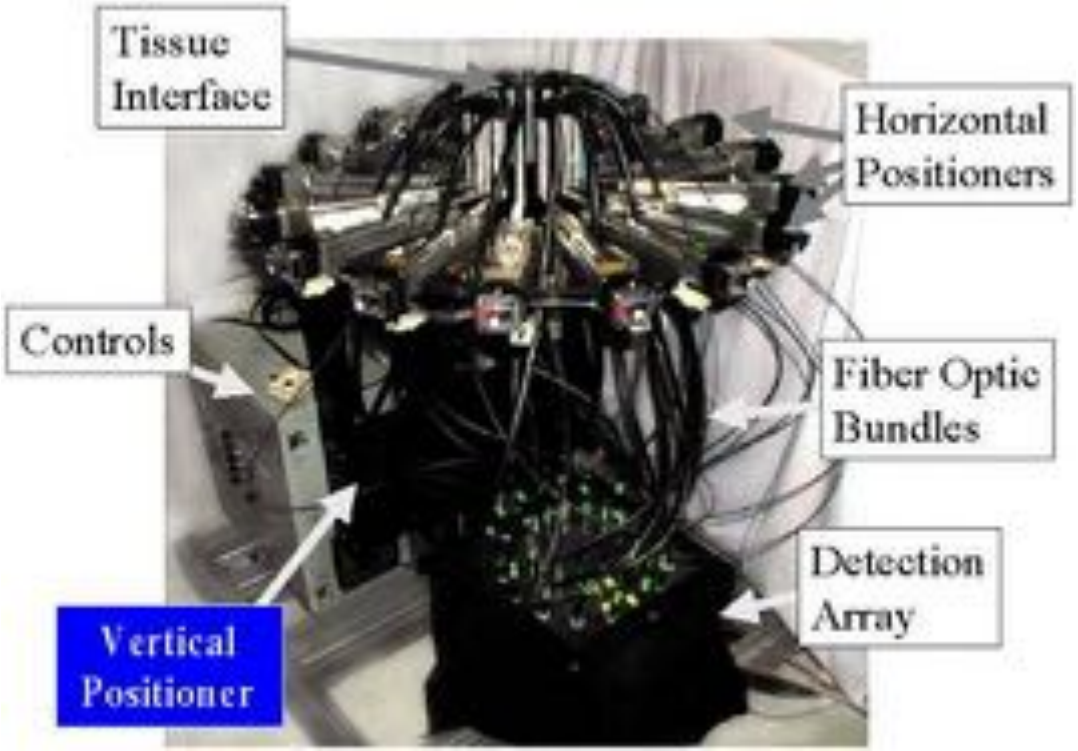
# Instrumentation

- Have multiple sources and multiple detectors in various geometries: ring, slab, array
- Ring geometry breast imagers shown




http://www-nml.dartmouth.edu/nir/index.html

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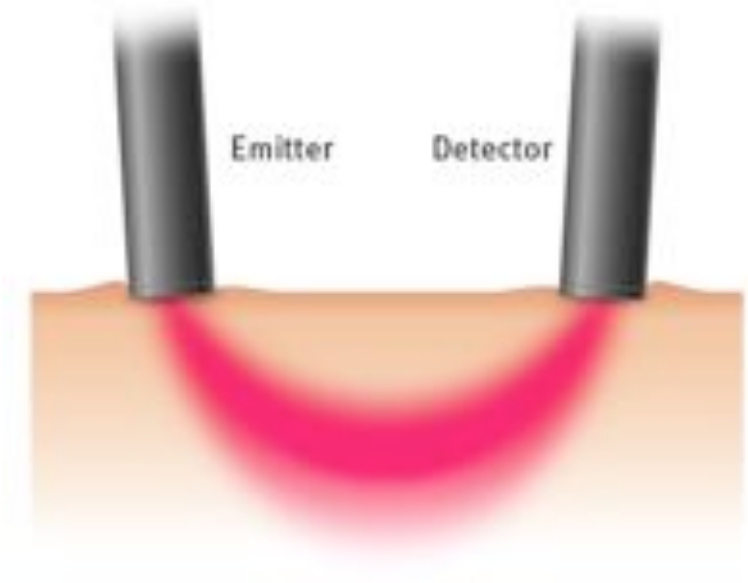
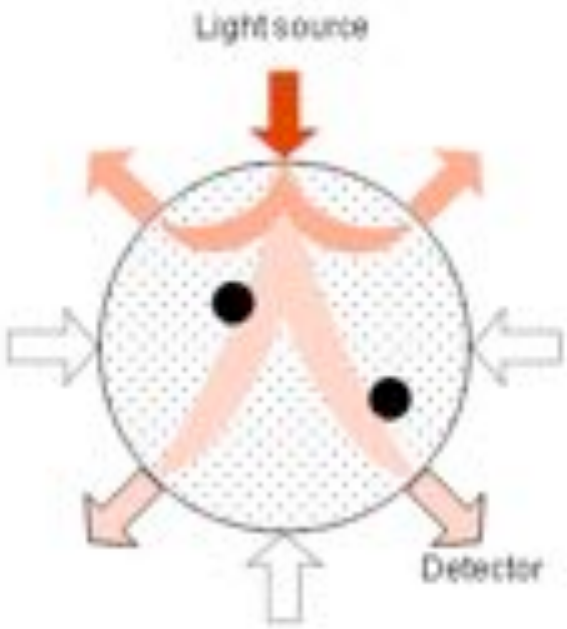
# Photon Path is Probabalistic

- Do not know the path the photon took from source to detector, but know probability.




iss.com




Dutta

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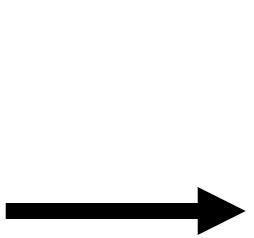
# Forward Problem



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# Inverse Problem

?

?

?

?

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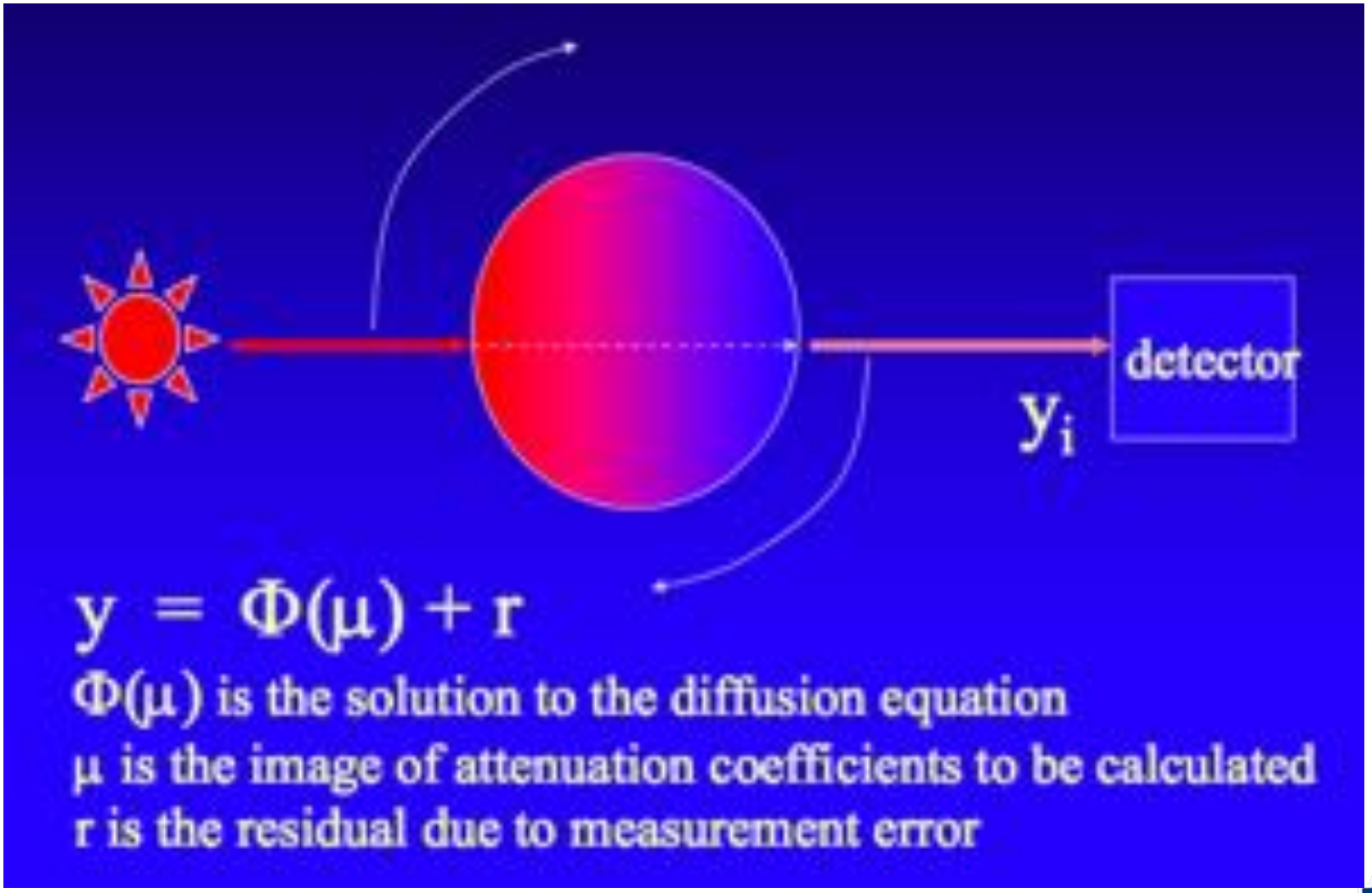
# DOT forward problem

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# DOT inverse problem

- No accurate analytic solution exists (some do with simplifications). Typical methods:
  - Filtered backprojection, taking into account probabilistic trajectory of photons.
  - Iterative approach. Assume an initial set of attenuation coefficients, compute fluences (forward problem). Adjust guess of attenuation coefficients based on difference between computed and measured fluences, Jacobian.

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# Types of DOT instruments

- Modulation of source
  - Steady-state
  - Time-domain (take advantage of differing paths of early and late photons
  - Frequency-domain (diffusing waves)

  (latter two can distinguish absorption and scattering)
- Geometry of measurement
- Free-form or constrained (with MRI data)
- Intrinsic contrast or contrast-enhanced

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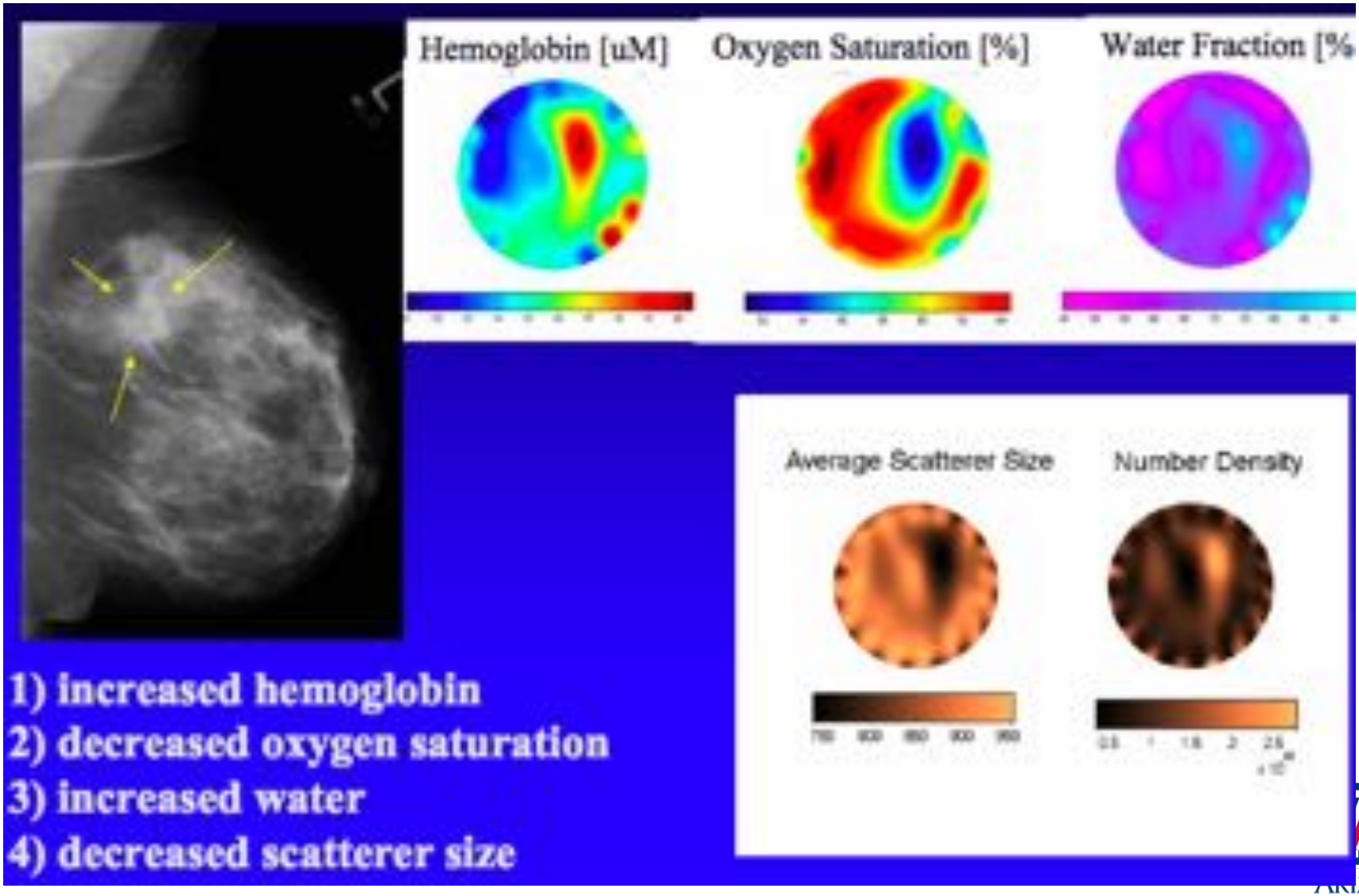
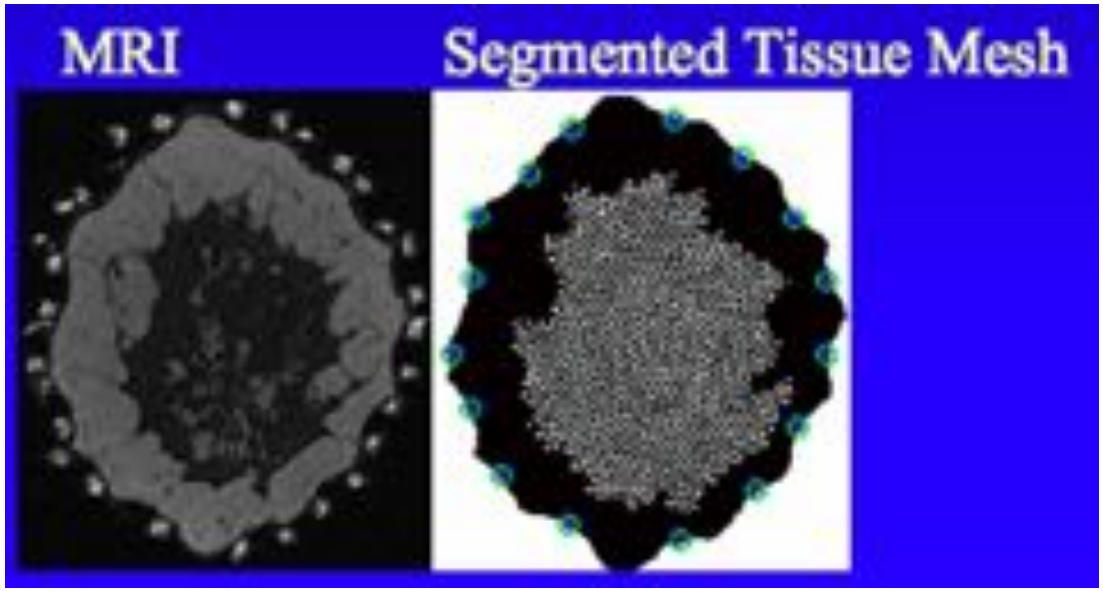
# Multi-spectral imaging of breast



# Prior information can greatly improve results

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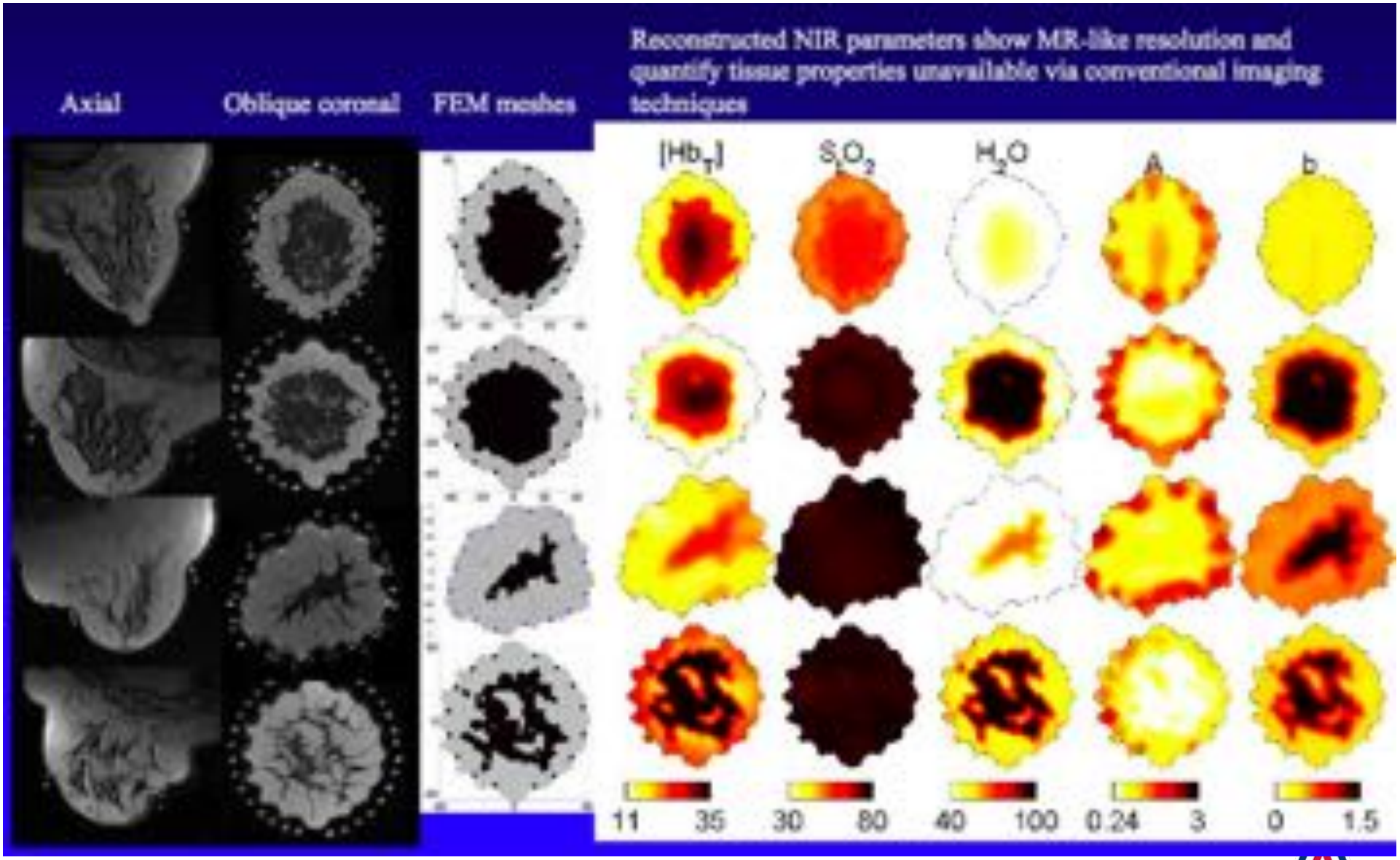
Axial
Oblique coronal
FEM meshes
Reconstructed NIR parameters show MR-like resolution and quantify tissue properties unavailable via conventional imaging techniques
[Hb_T]
S_tO_2
H_2O
A
b
11
35
30
80
40
100
0.24
3
0
1.5

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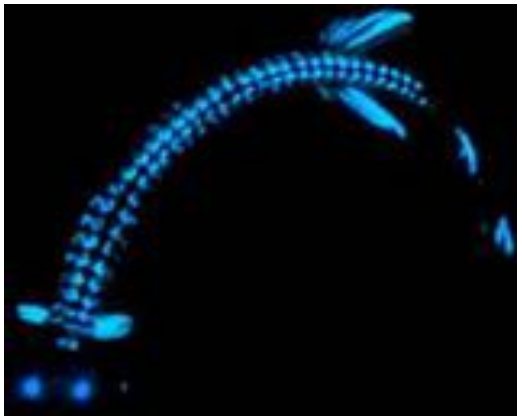
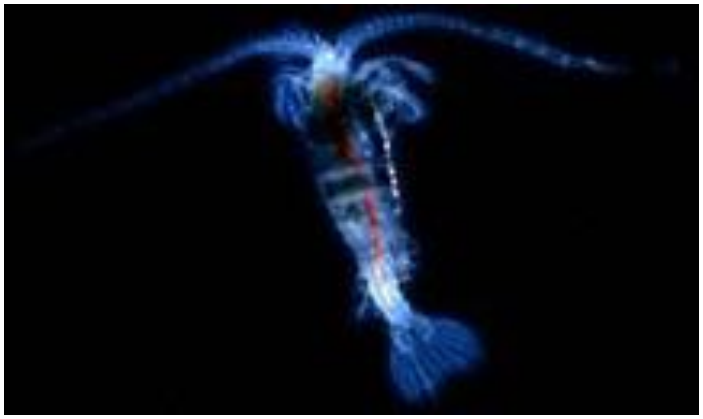
Visible light produced by living organisms

# Bioluminescence

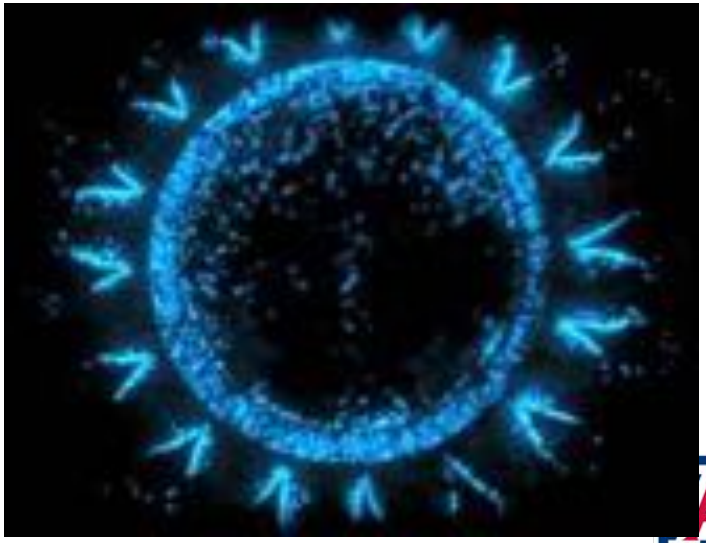


Black dragonfish



crustaceans

bacteria



jellyfish

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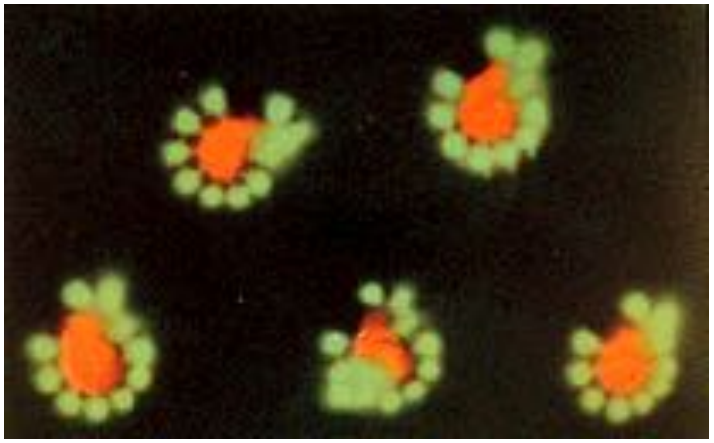
Mushroom



Railroad worm

Glow worm

Firefly

# Bioluminescence

- Visible light made by living creatures
  - Land: fireflies, earthworms, centipedes, fungi (usually red, yellow, or green)
  - Sea: bacteria, plankton, jellies, crustaceans, fish (usually blue or green)
- We will discuss two types of bioluminescence
  - Luciferin/luciferase reaction (chemical production of light, requires organism's energy, e.g. fireflies)
  - Fluorescent proteins (fluoresce in the presence of suitable excitation light, passive on organism's part, e.g. jellyfish)

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# Luc reaction

- Luciferin- any material that emits light when it loses electrons in the presence of luciferase
- Luciferase- and enzyme that must be present to facilitate oxidation of luciferin
- There are many different forms of luciferin and luciferase, but all require the presence of oxygen and ATP

luciferin + ATP ---> luciferyl adenylate + PPi

luciferyk adenylate + O2 ---> oxyluciferin + AMP + light

This reaction is 90% efficient in producing light from energy stored in ATP

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# *in vivo* Use of luc Reaction

- Cells can be transfected with the genetic sequence to produce luciferase. This sequence can be fused behind a promoter- luciferase will only be produced with the gene of interest is being expressed.
- Bacteria that naturally produce luciferase and luciferin can be introduced into a host- e.g. to track infection. Nothing additional is needed except to collect light from the bacteria in a light-proof box.
- For cells expressing luciferase, luciferin must be injected. *There must also be sufficient oxygen and ATP for the reaction to proceed*.

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# Fluorescent proteins

- Cells can also be transfected with the genetic sequence to produce fluorescent proteins. Green fluorescent protein is most widely used, but they are available from blue to red emission wavelengths.
- External excitation light must be provided.
- JAX sells hundreds of mouse strains that produce fluorescent proteins either ubiquitously, in certain cell types, or conditionally upon expression of a promoter.

http://kumikae01.gen-info.osaka-u.ac.jp/tg/00%20GreenMice.mpg

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# Problem with fluorescence

- Animals themselves are relatively highly fluorescent, making detection of fluorescently labeled cells (if they are small in number and/or deep) difficult.

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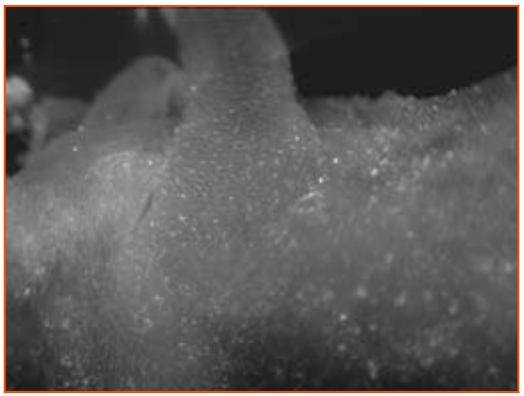
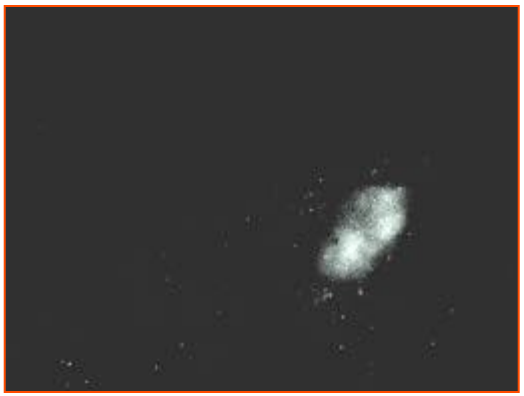
# Spectral Unmixing

Live mouse with GFP-labeled tumor in lung

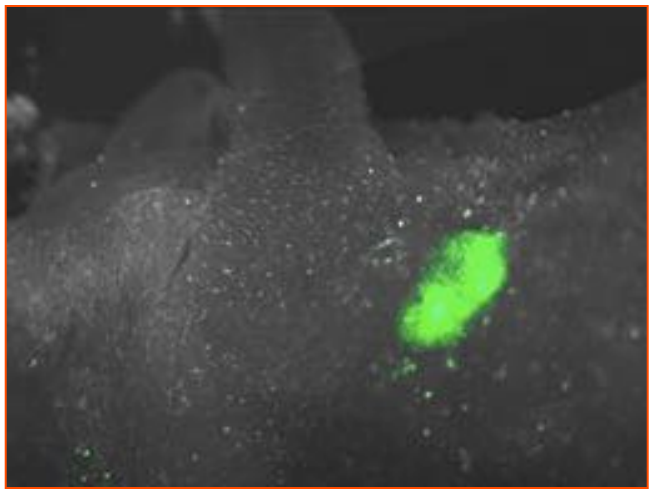
Autofluorescence signal only—after spectral unmixing





GFP signal only—after spectral unmixing

Color composite of GFP and autofluorescence signals



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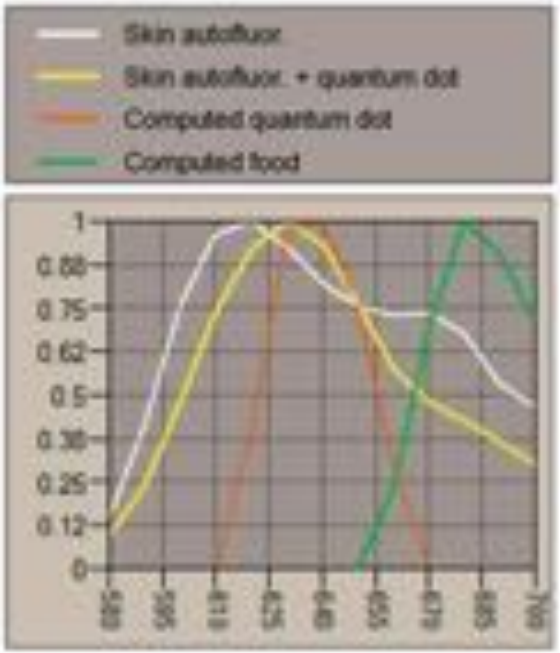
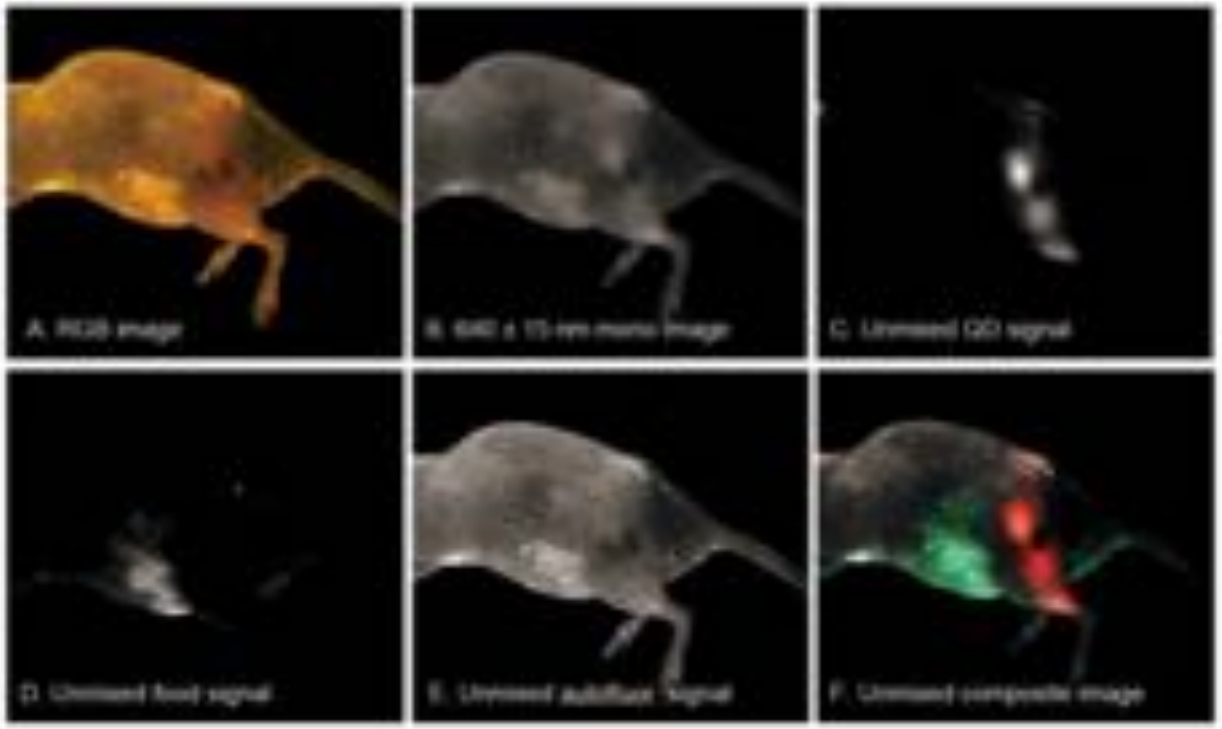
# Spectral Unmixing



Mansfeld et al., 2005, J Biomed Opt

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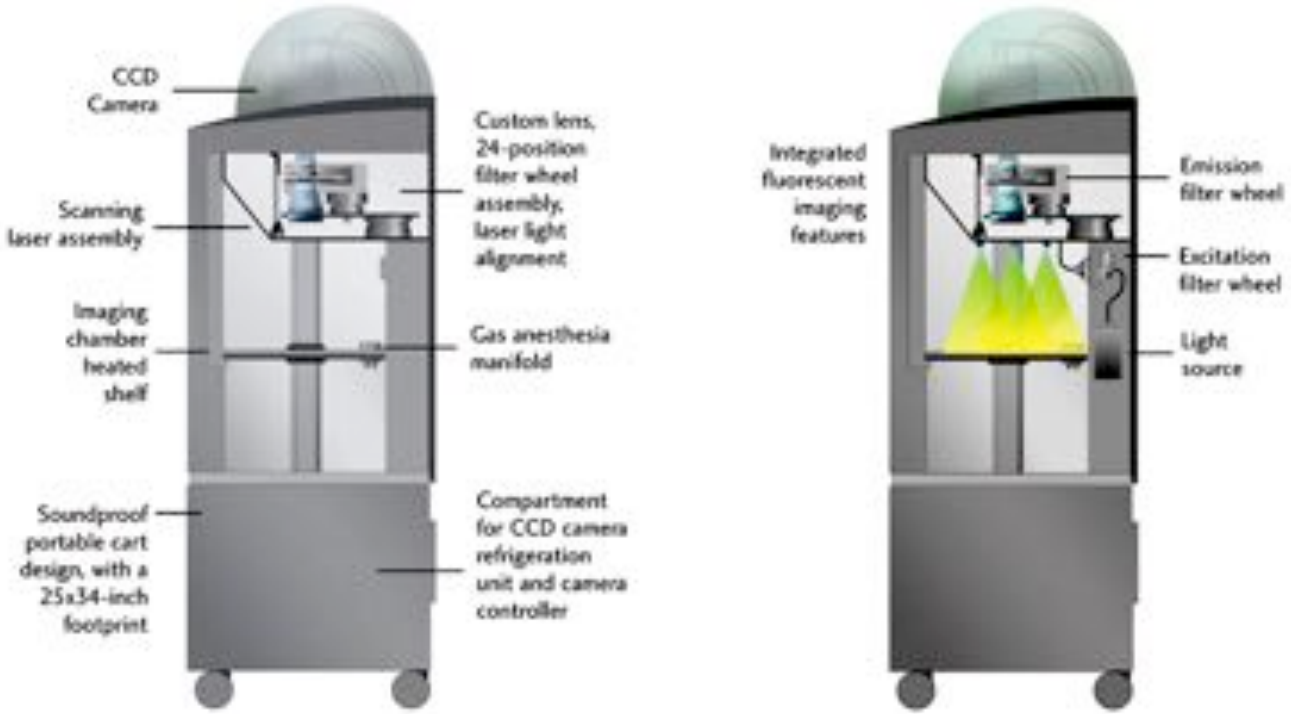
# Measurement Optics

- Dark box imagers (luc and fluorescence)
  - Have a very sensitive CCD and a dark box. Suitable for *in vivo* or culture plates.




Xenogen

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# Comparison of GFP vs. Luc

| | GFP | Luciferase |
|---|---|---|
| Signal-to-noise ratio | Low | High |
| Earliest tumor detection in experiment | Day 7 | Day 1 |
| No. of mice that can be imaged | Few (one mouse for our system; depends on excitation light source design) | Many (up to 10 mice demonstrated in our system) |
| Substrate | None | Luciferin |
| Volume vs. imaging correlation | 0.99 | 0.97 |
| Biomass vs. imaging correlation | 0.81 | 0.93 |
| Image acquisition time | Milliseconds (200–300 ms) | Minutes (10 min) |
| Special requirements | none | ATP plus oxygen required for sufficient light production |

Choy et al. 2003, Biotechniques

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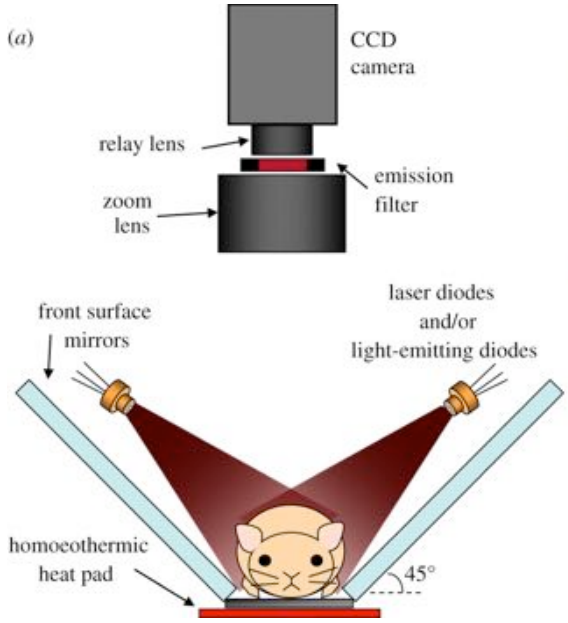
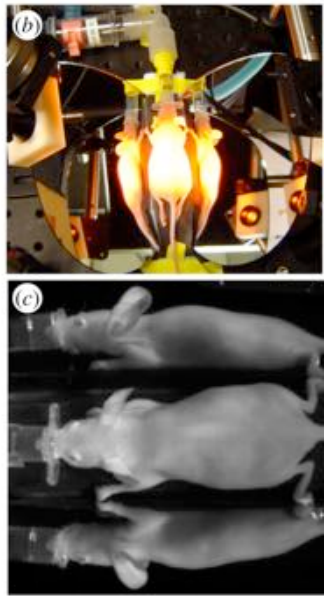
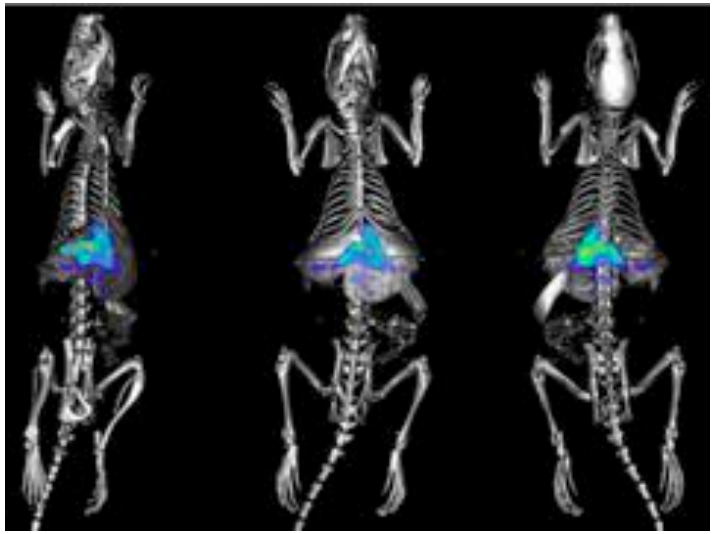
# Fluorescence Molecular Tomography

- Can use principles of diffuse optical tomography to gain 3D images
- Easier in mice (small) than humans. CT often added to provide anatomical reference



# Laser-Tissue Interaction

- So far, we have been talking about light-tissue interactions where (hopefully) the tissue is not altered by the interaction
  - Photobleaching, DNA damage, heating not desirable
- What about interactions where we WANT to alter the tissue
- Generally a matter of increased *irradiance* ($W/cm^2$), often achieved by short pulse durations, and choice of *wavelength* that interacts strongly with the tissue chromophores
- There are many applications- example given of Port Wine Stain.

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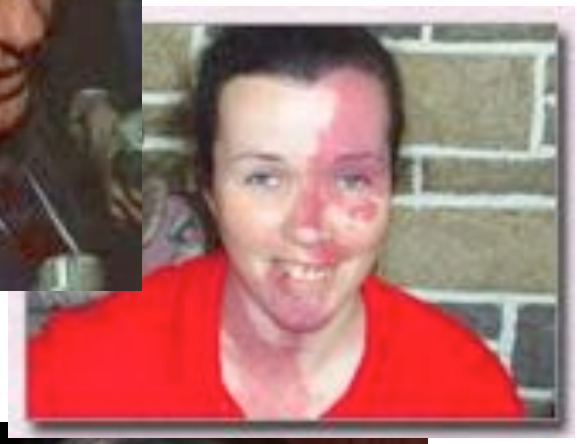
# What is Port Wine Stain?

- A congenital cutaneous vascular malformation.
- 0.3% of babies are born with PWS.
- Red or purple in color on adults.
- Can appear anywhere on the body.
- Can (rarely) indicate a serious underlying condition such as Sturge-Weber Syndrome.



http://www.loveyourmark.com/

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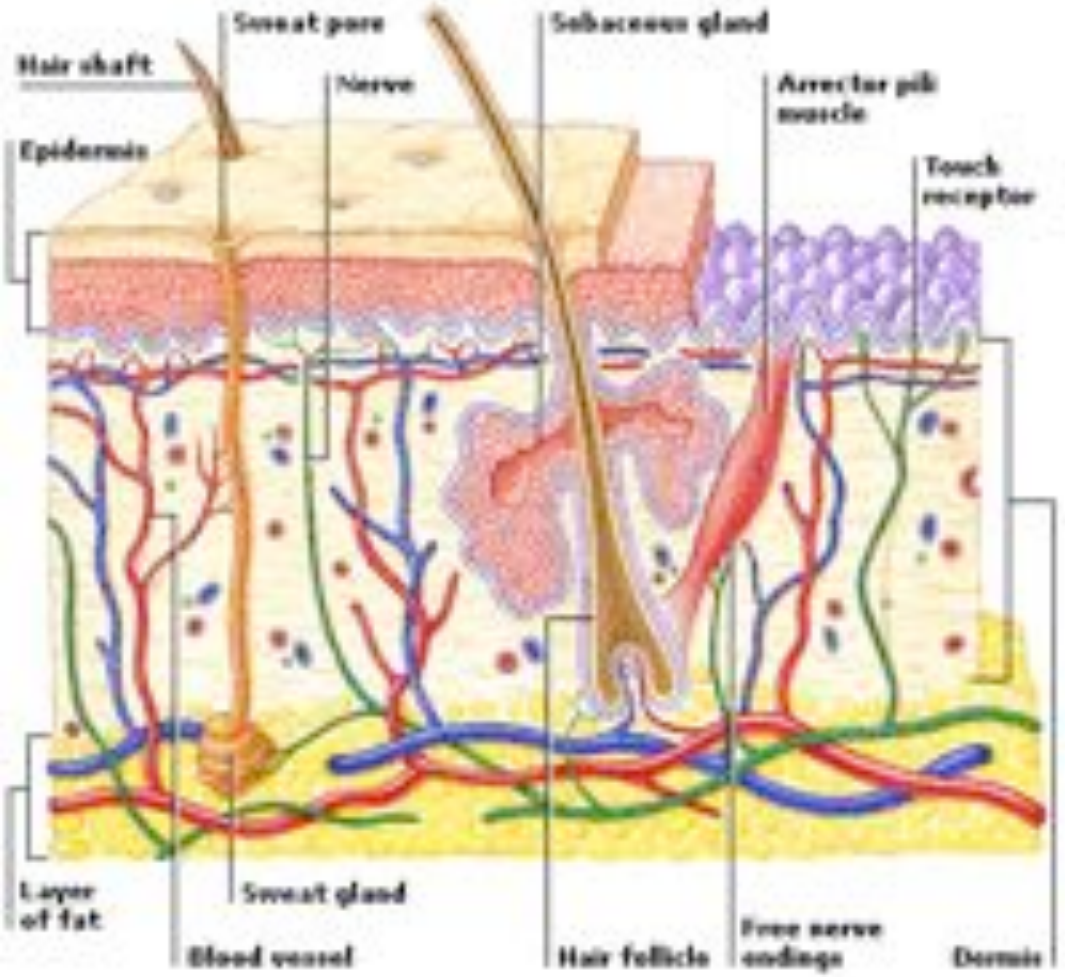
# Blood Vessels in the Skin




www.infoplease.com

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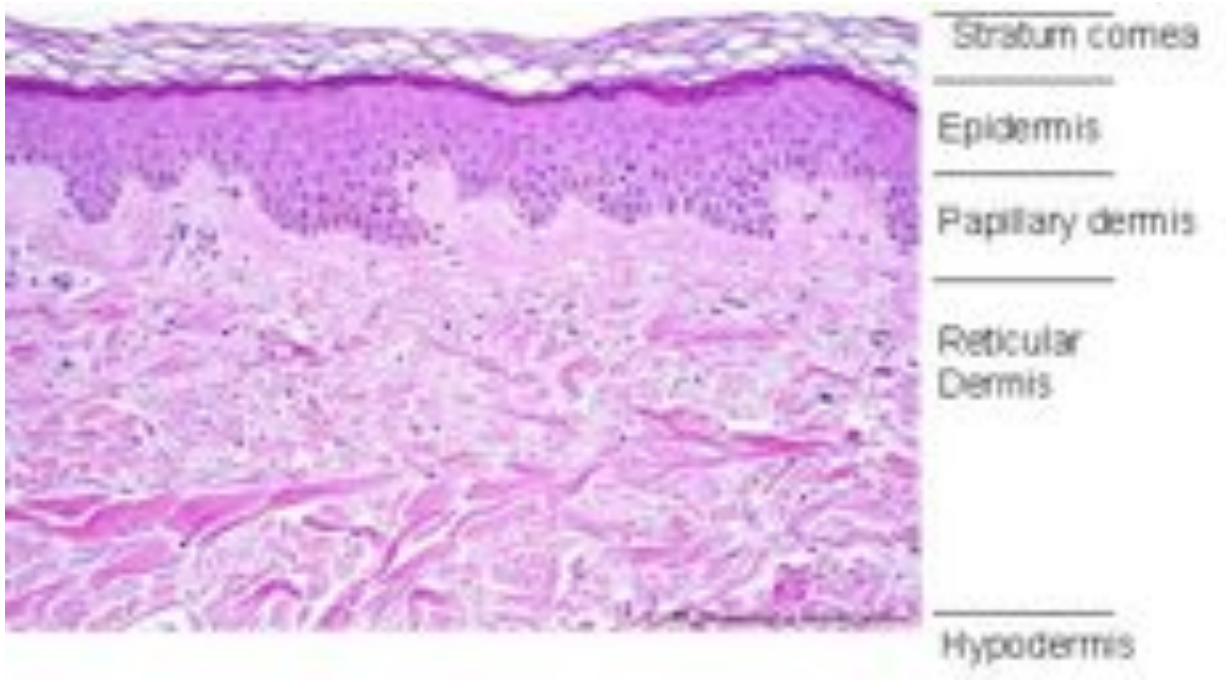
# Blood Vessels in Normal Skin

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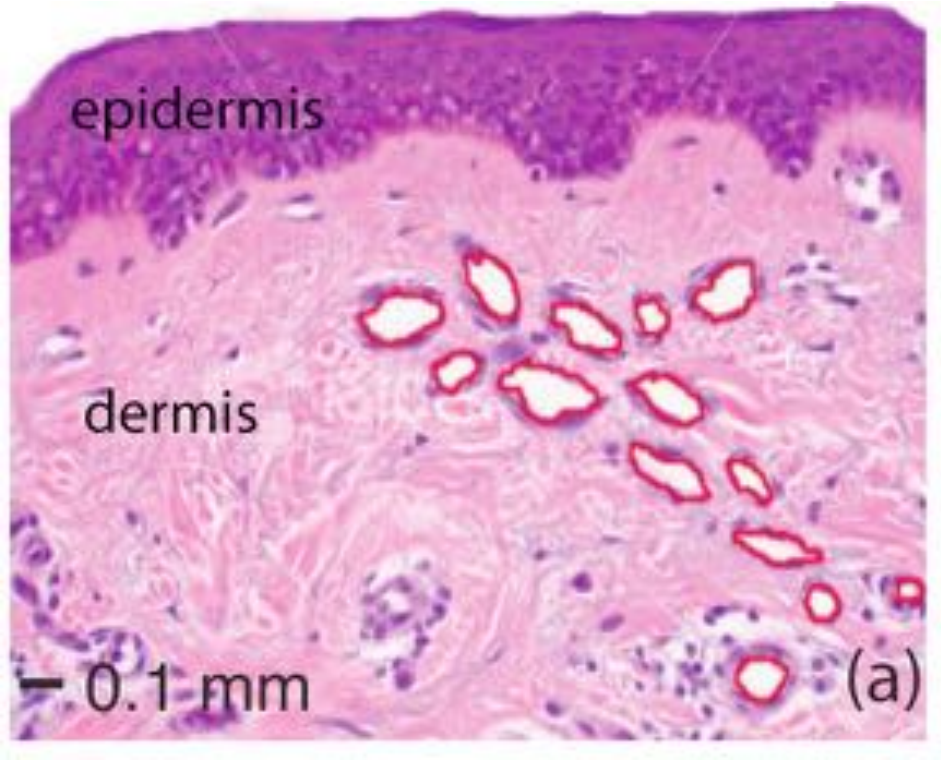
# Blood Vessels in PWS Skin




UC Irvine

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# Modern Treatment

- Laser to selectively target the hemoglobin in blood, heat up and destroy blood vessels, but spare everything else.
- Balloon pop illustration https://www.youtube.com/watch?v=iukFVUiN_rU

Laser pioneer Arthur Schawlow

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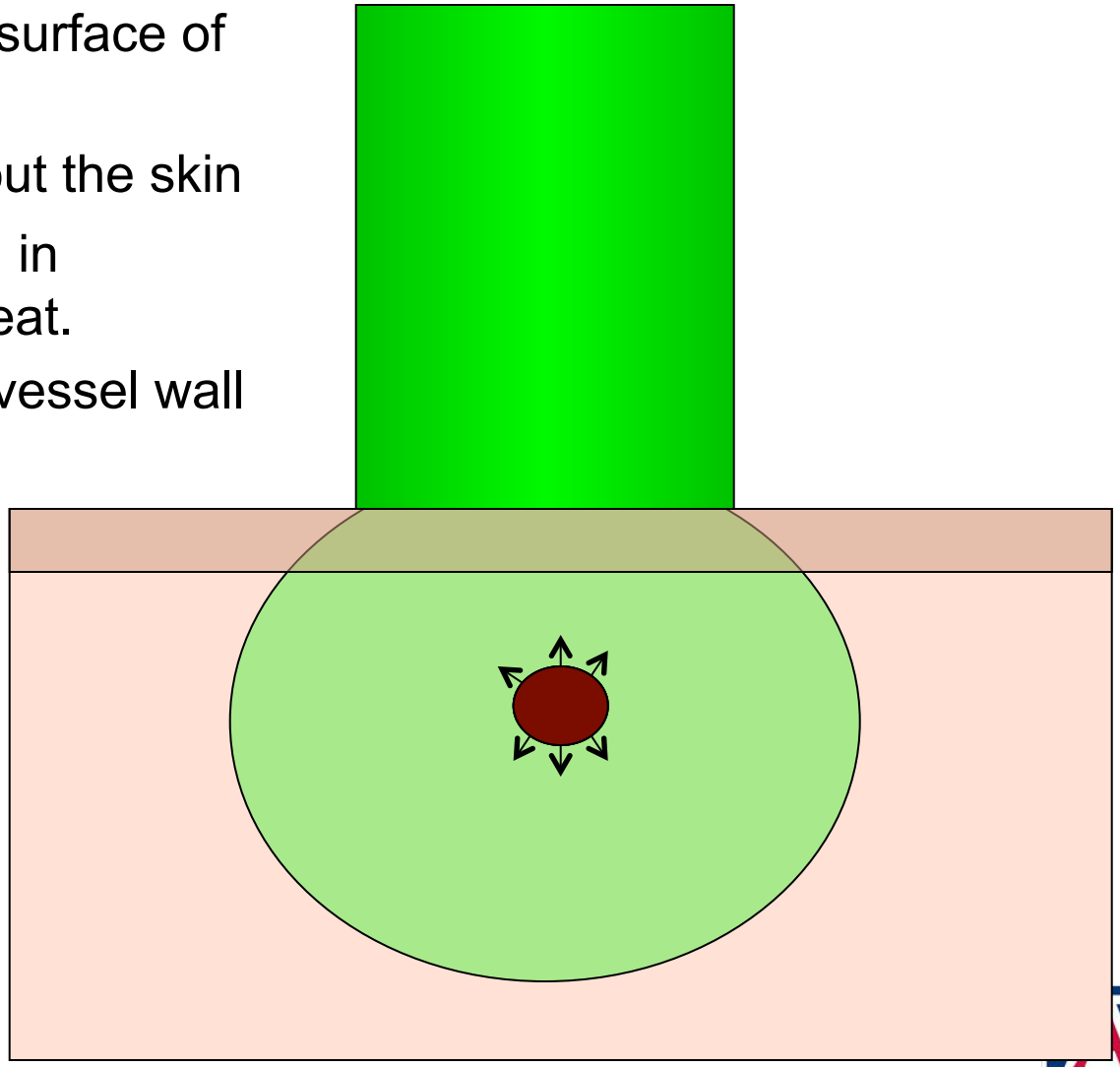
# Simplified Model: Blood Vessel in Tissue

- Laser light incident on surface of the skin
- Light scatters throughout the skin
- Assume light absorbed in hemoglobin, creates heat.
- Heat diffuses to blood vessel wall and coagulates vessel.



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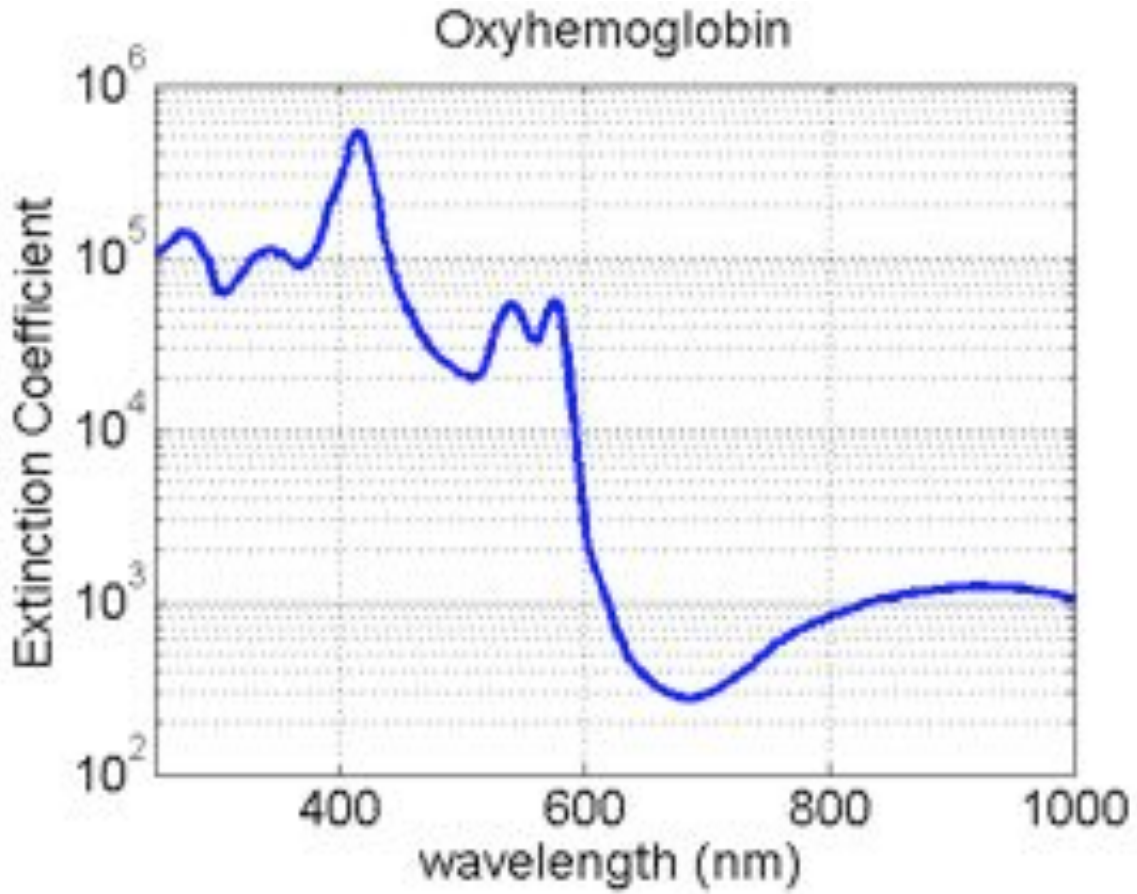
# What is the target?

- Hemoglobin- what is best absorbed wavelength?

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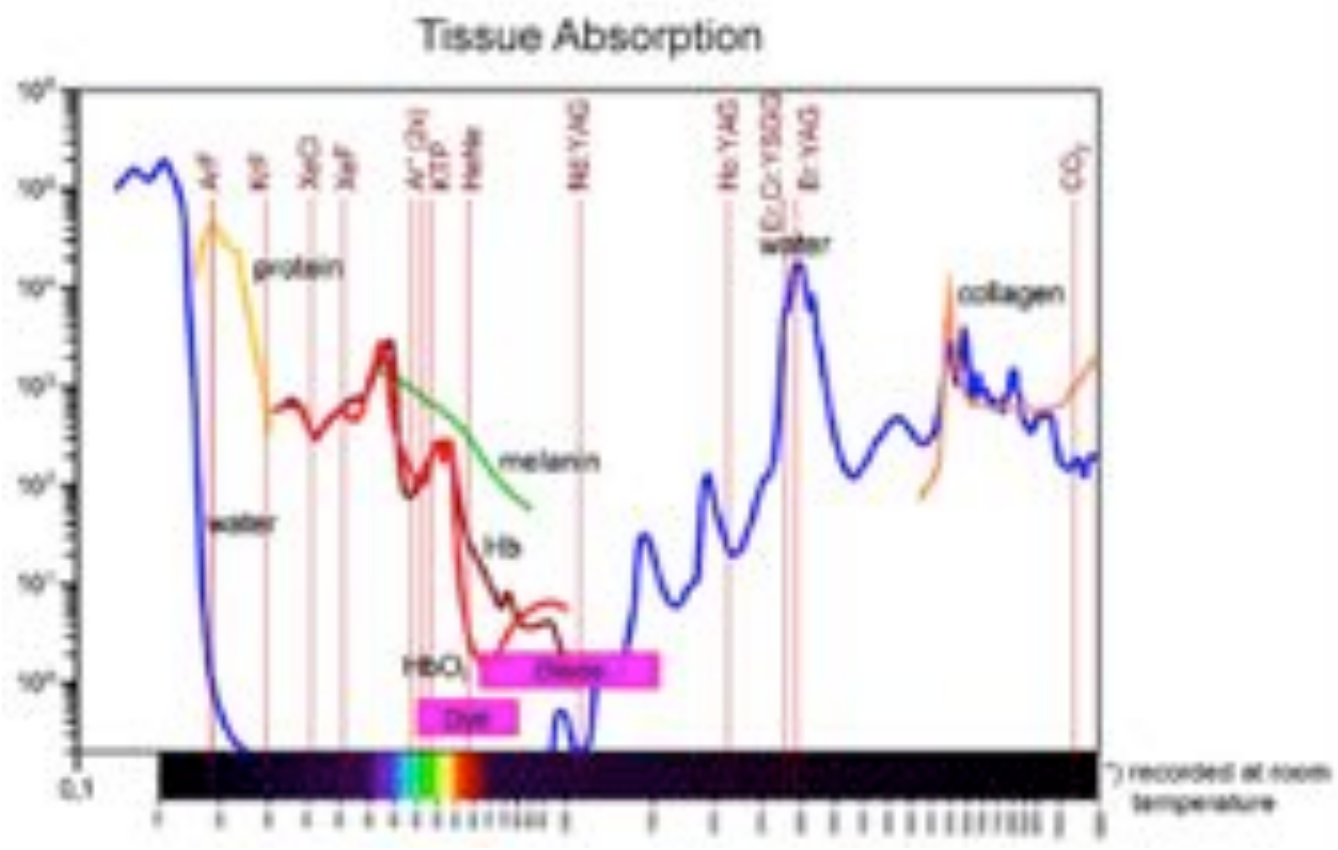
# Competing Chromophores

- What else do we need to worry about in tissue?
- What are technology limitations?
- What is the overall best wavelength to choose?




http://www.hightechlaser.be

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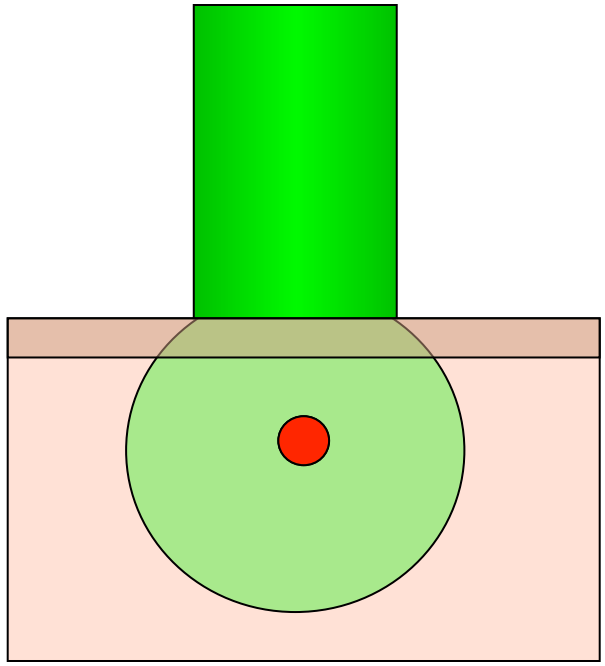
# Spot Size

- Because we are highly scattering, energy is scattered out of the laser beam as well as being absorbed as it propagates through tissue.
- Larger diameter beams are better, since in the center of the beam scatter in = scatter out.
- For practical purposes, beams ≥5 mm diameter penetrate deepest, and can coagulate blood vessels up to ~1 mm deep.



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# What About the Laser Pulse Duration?

For a given amount of total pulse energy:

- If the laser pulse is too short, the effect will be explosive.
  - Abnormal blood vessels will heal from an explosive tear

- If the laser pulse is too long (the rate is too slow), the heat will "leak" or diffuse out of the blood vessel into the surrounding skin
  - May damage surrounding skin
  - Blood vessel may not get hot enough to be destroyed

- Ideally, the pulse duration matches something called the Thermal Relaxation Time of the blood vessel:

  approximately $\tau < \delta^2/K$

  $\delta$ is blood vessel diameter

  K is diffusivity, a constant for blood (close to water)

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# Thermal relaxation time

If laser pulse is shorter than this time, in thermal confinement, can assume that energy is deposited instantaneously, minimize heat diffusion

| Time t | Thermal penetration depth $Z_{therm}$ |
|---|---|
| 1 ps | 0.7 nm |
| 1 ns | 22 nm |
| 1 μs | 0.7 μm |
| 10 μs | 2.2 μm |
| 100 μs | 7 μm |
| 1 ms | 22 μm |
| 10 ms | 70 μm |
| 100 ms | 0.22 mm |
| 1 s | 0.7 mm |

- Example sizes:
  - Capillary 10 μm
  - Abnormal capillary 50-100 μm
  - Varicose vein 1-3 mm
  - Melanosome 1 μm
  - Tattoo pigment granule 30-400 nm

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# Computing Temperature

- Temperature rise

$$dT = \frac{dQ}{mC}$$

$$\boxed{dT = \frac{S}{\rho C}}$$

Q heat content (J)

m mass (g)

C specific heat (J/gK) 4.3 for water

$\rho$ density (g/cm$^3$) 1 for water

S light source term (J/cm$^3$)

- If laser pulse is thermally confined, knowing the fluence of laser light that reaches the blood vessel times absorption coefficient of blood (source term), can compute temperature rise at the end of laser pulse.

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# Arrhenius damage equation

$$\Omega(\tau) = A\int_0^{\tau} \exp\left(-\frac{\Delta E}{RT(t)}\right)dt = \ln\left(\frac{C_o}{C_\tau}\right)$$

- A: frequency factor (e.g. $10^{44}$ 1/s)
- $\Delta$E: activation energy (e.g. 2.9 x $10^5$ kJ/mol)
- R: gas constant
- $C_o$: initial concentration of undamaged molecules
- $C_\tau$: concentration of undamaged molecules after time $\tau$
- Coagulation and irreversible tissue damage assumed to occur at $\Omega$=1
- Sometimes talk about a "critical temperature." This assumes a constant temperature exposure, and sometimes assumes a given time (few seconds). Critical temperature is not very accurate but can be a guide.

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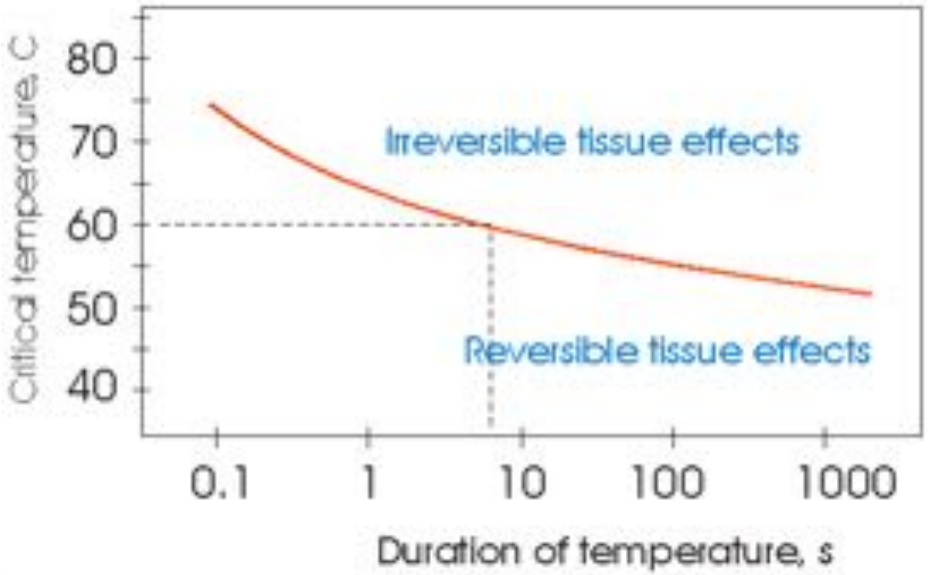
# Damage in tissue




| Temperature | Biological effect |
|---|---|
| 37 °C | Normal |
| 45 °C | Hyperthermia |
| 50 °C | Reduction in enzyme activity, cell immobility |
| 60 °C | Denaturation of proteins and collagen, coagulation |
| 80 °C | Permeabilization of membranes |
| 100 °C | Vaporization |
| > 100 °C | Carbonization |
| > 300 °C | Melting |

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# How Much Energy to Put In?

- Want to heat the blood vessel to the temperature where it will be destroyed, but no hotter to avoid surrounding damage.
- Don't want to boil or vaporize blood.

| Temperature | Biological effect |
|---|---|
| 37 °C | Normal |
| 45 °C | Hyperthermia |
| 50 °C | Reduction in enzyme activity, cell immobility |
| 60 °C | Denaturation of proteins and collagen, coagulation |
| 80 °C | Permeabilization of membranes |
| 100 °C | Vaporization |
| > 100 °C | Carbonization |
| > 300 °C | Melting |

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# Principle of Selective Photothermolysis

1. Select a laser wavelength that is preferentially absorbed by the target tissue
2. Make sure sufficient energy can penetrate through the entire tissue of interest (may need to select a wavelength not so highly absorbed in tissue)
3. Select a spot size large enough to maximize delivery of energy to target tissue, but not larger than necessary
4. Select a pulse duration that is about or slightly less than the thermal relaxation time of the target tissue
5. Select an energy that is sufficient to thermally coagulate the target of interest, not more

- Overall, selectively destroy target while minimizing collateral damage.

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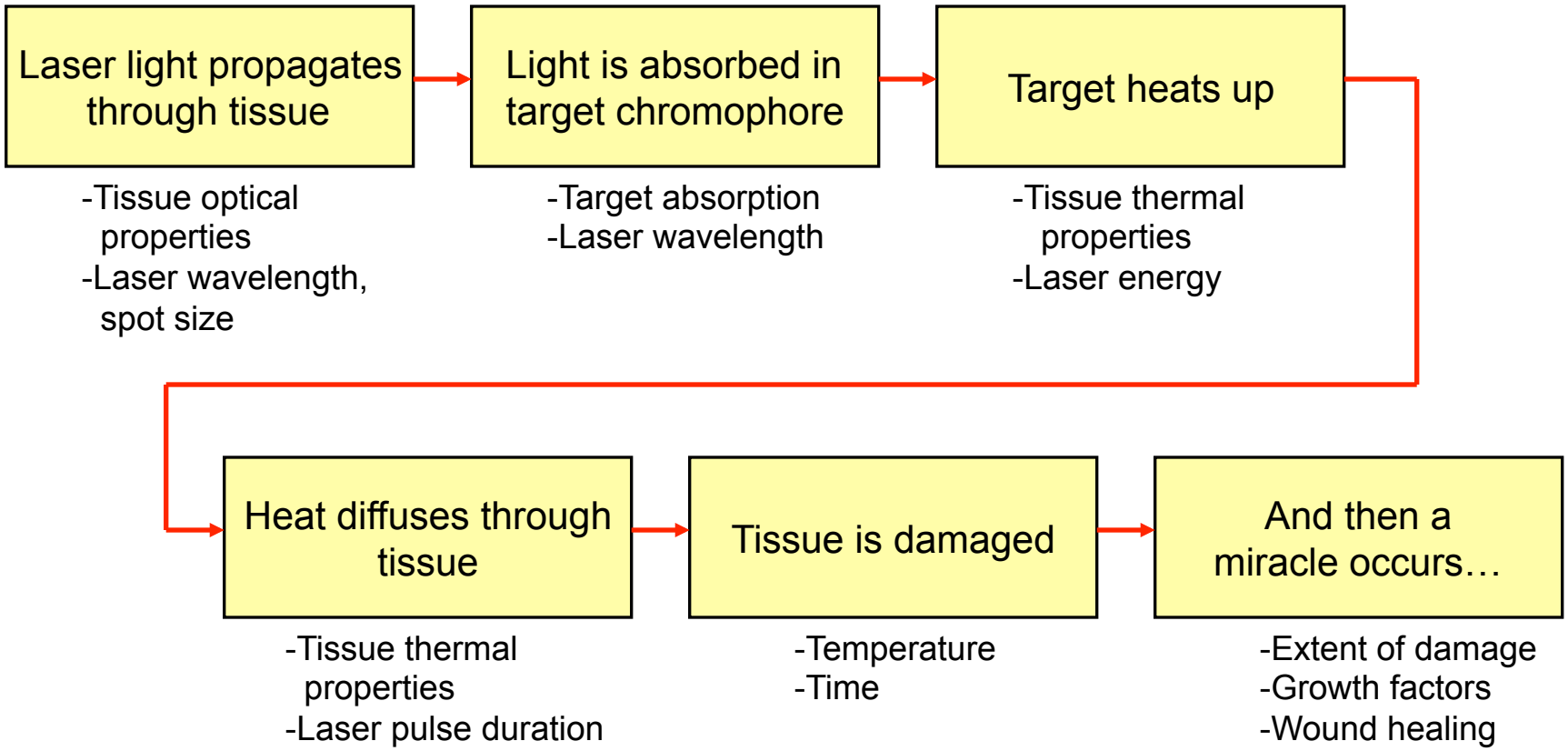
# Putting it All Together- Light to Cure

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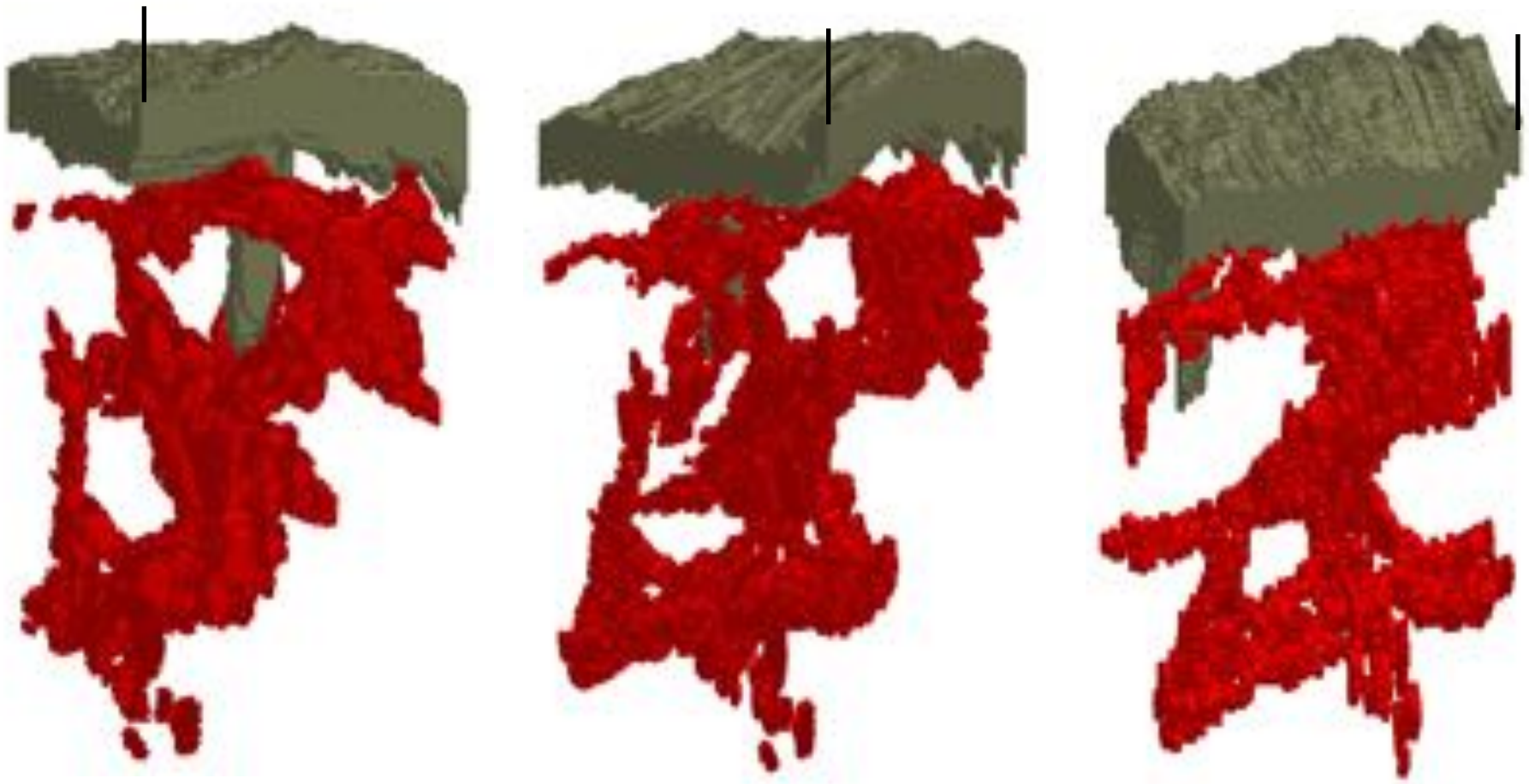
# Example in a True Reconstruction of a PWS Biopsy



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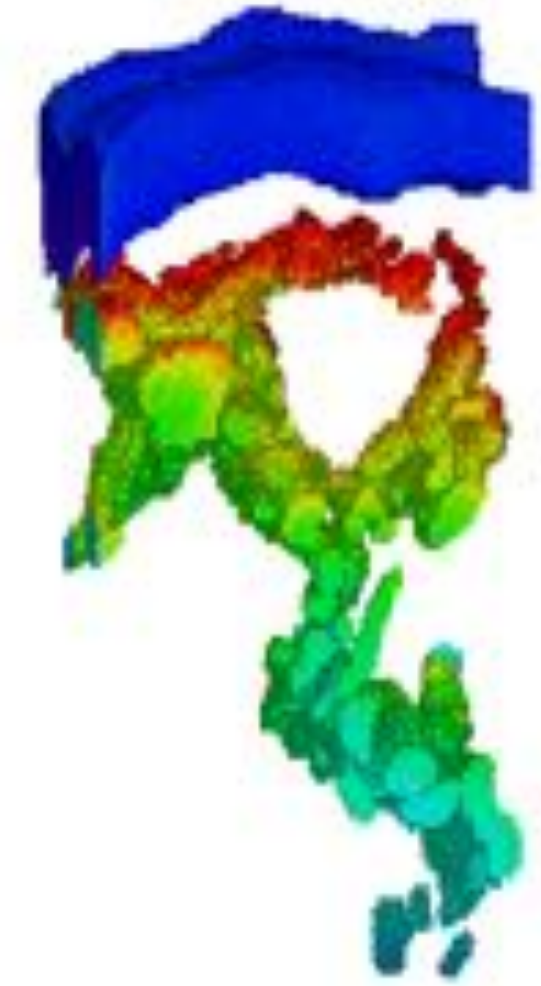
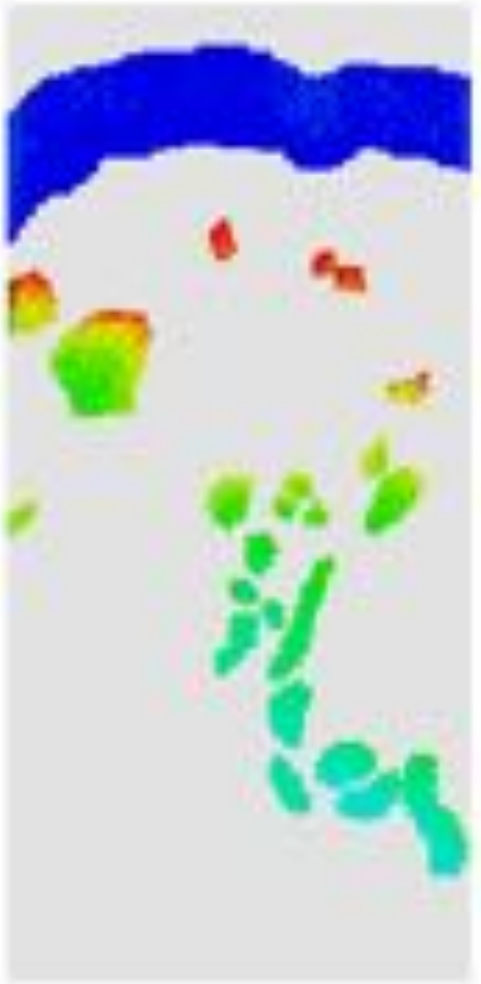
# Example Light Source Term

Laser energy density = 1 J/cm$^2$



250+ J/cm$^3$
200 J/cm$^3$
100 J/cm$^3$
35 J/cm$^3$
1-5 J/cm$^3$



100 μm

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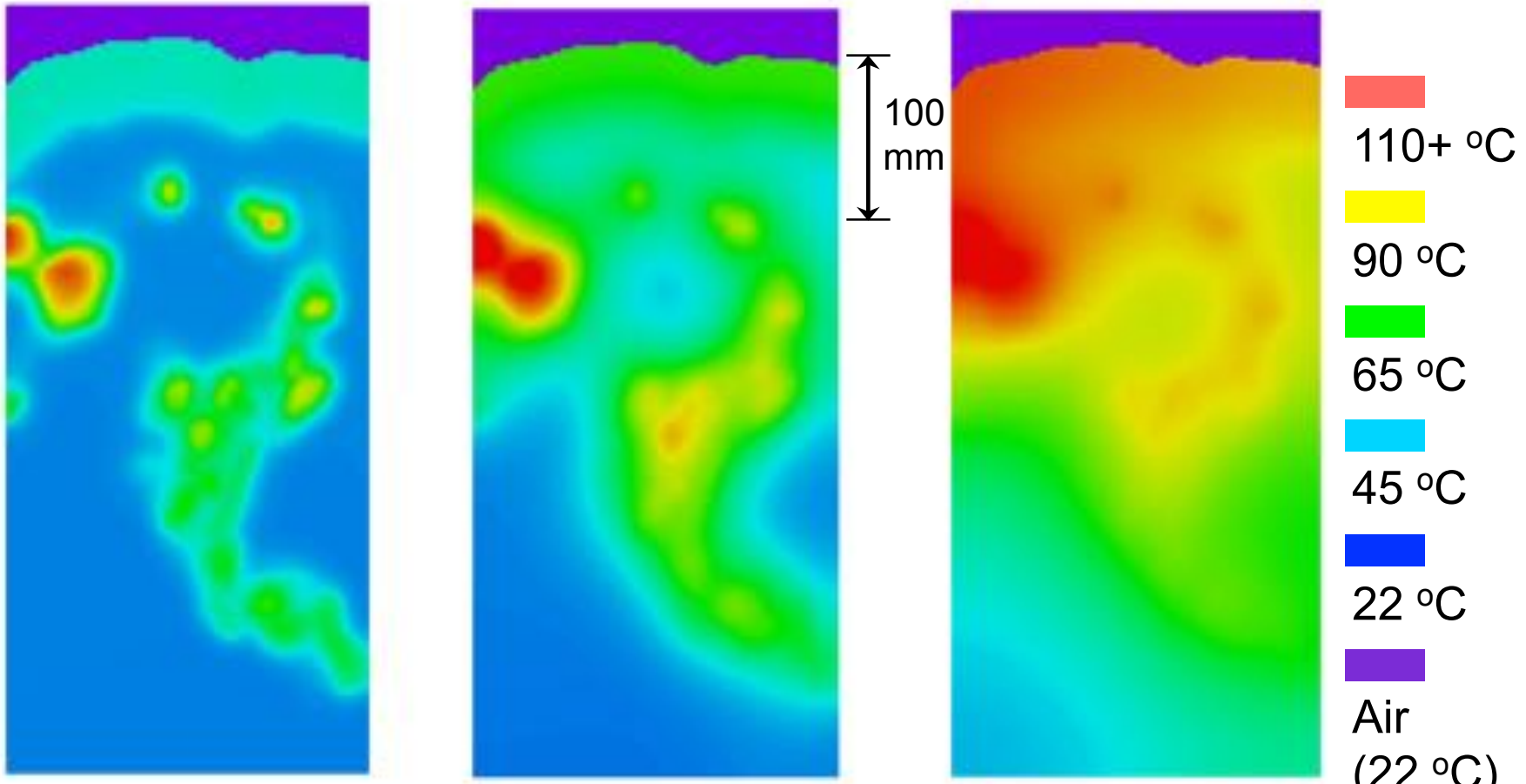
# Temperatures at End of Pulse

## (energy adjusted to same peak temp)




$\tau_p$ = 0.5 ms

$\tau_p$ = 5 ms

$\tau_p$ = 50 ms

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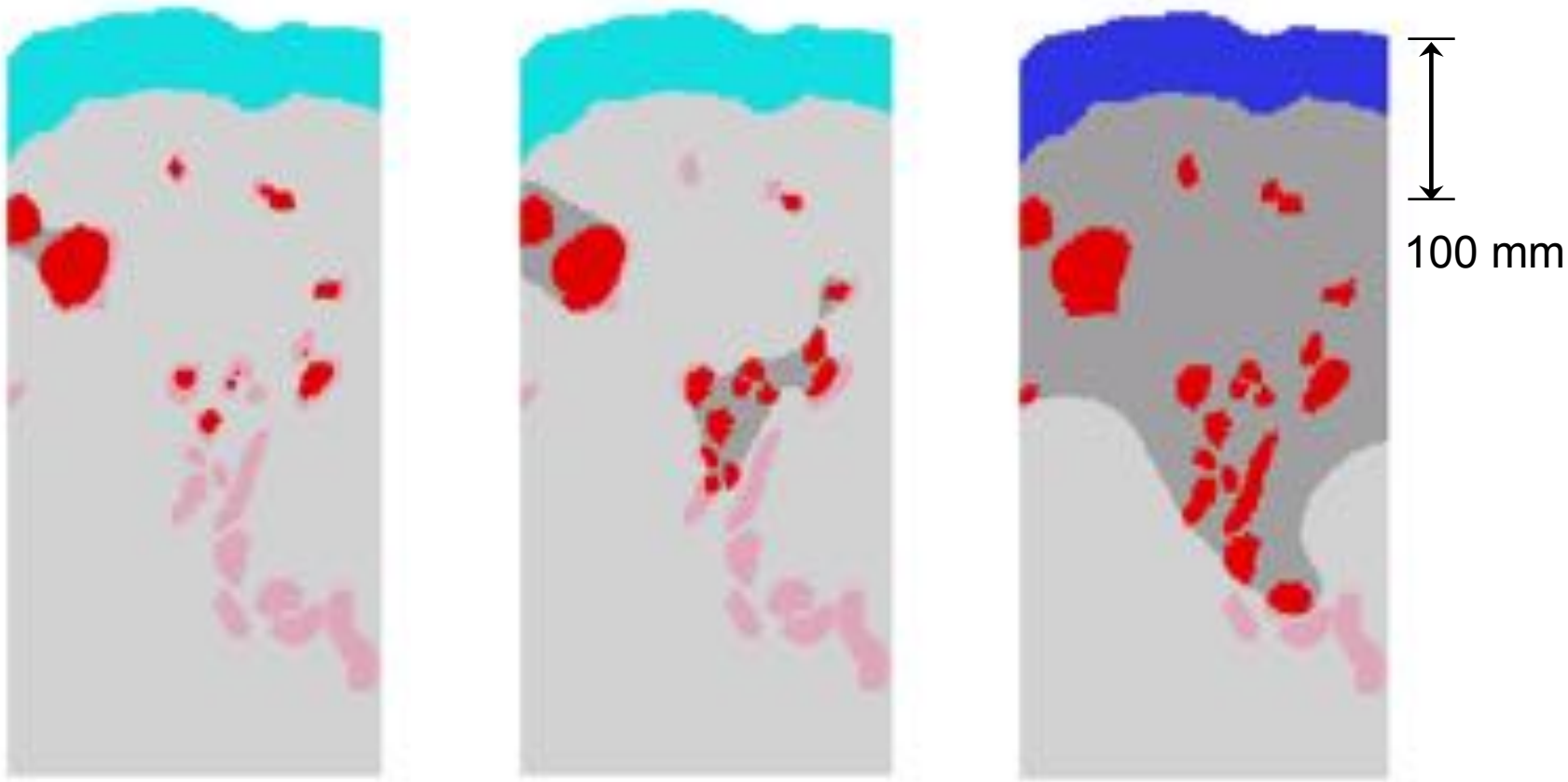
# Final Extent of Coagulation
## (after tissue has cooled)




$\tau_p$ = 0.5 ms

$\tau_p$ = 5 ms

$\tau_p$ = 50 ms

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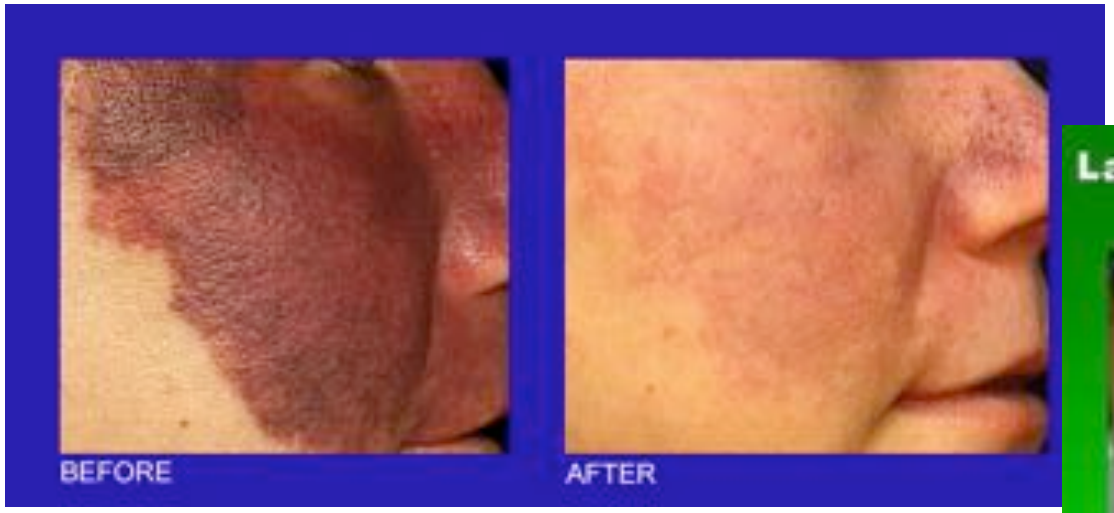
# How Well Does Laser Treatment Work?

- About 25% of patients: a cure
- About 50% of patients: incomplete but significant lightening
- About 25% of patients: no significant improvement (small incidence of side effects)
- Patients may have dozens of treatments for years.




emedicine.medscape.com/article/1120509-overview


www.kudzu.com/merchant/photos/1107823.html

# Why is Laser Treatment not 100% successful?

- Poor match of laser parameters to blood vessel anatomy.
  - Size, depth, number of blood vessels.
- Failure to understand that tissue being treated is dynamically changing *during* the laser pulse.
- Biology- healing response is poorly understood- system may not "reboot" correctly but instead redevelop old problems (abnormal blood vessels).

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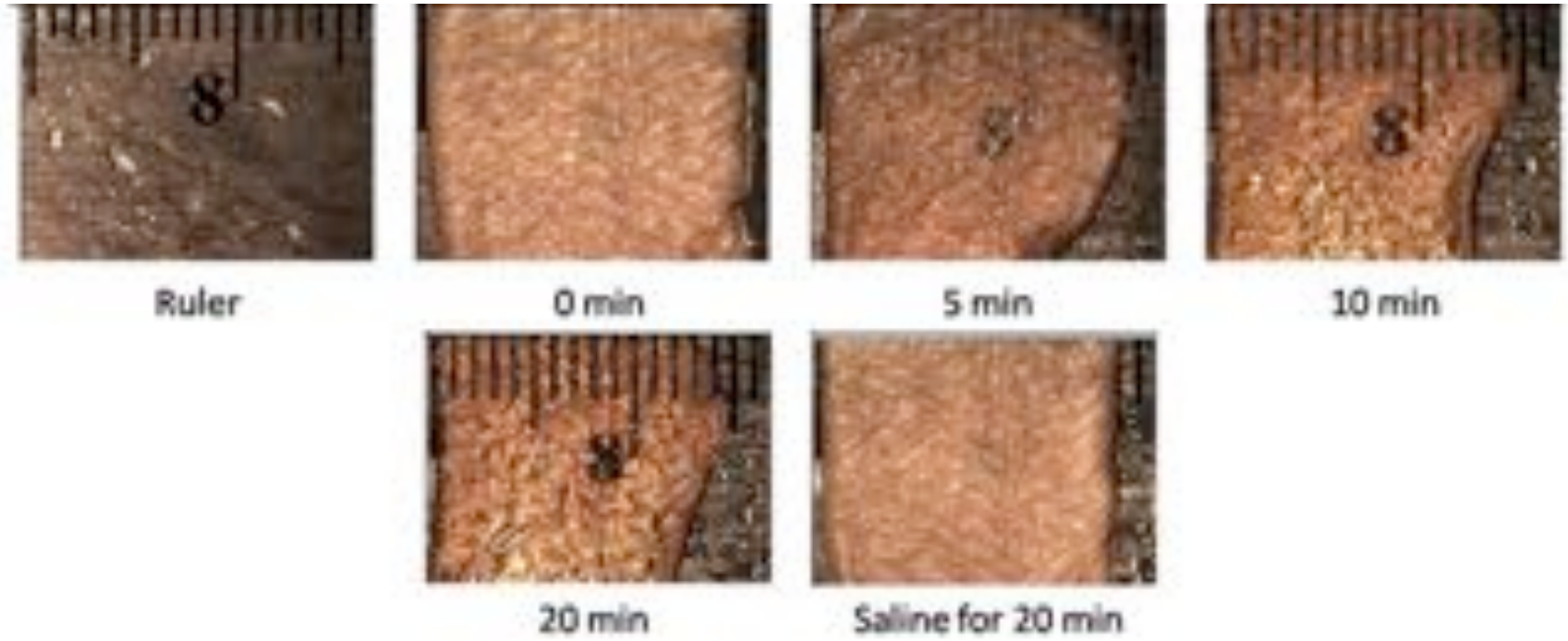
# Future of Treatments

- Tissue clearing to reduce scatter, more light to deeper blood vessels.
- However, scatter is beneficial to uniformly illuminate vessels




Bernard Choi, Ph.D. UC Irvine

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# Further Reading

- Jacques, S. “Optical properties of biological tissues: a review” Phys. Med. Biol. 58 (2013) R37–R61. http://omlc.org/news/dec14/Jacques_PMB2013/Jacques_PMB2013.pdf

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# Questions?

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