Fundamentals in Biophotonics

Interaction of Light With Tissues-

Endoscopic techniques

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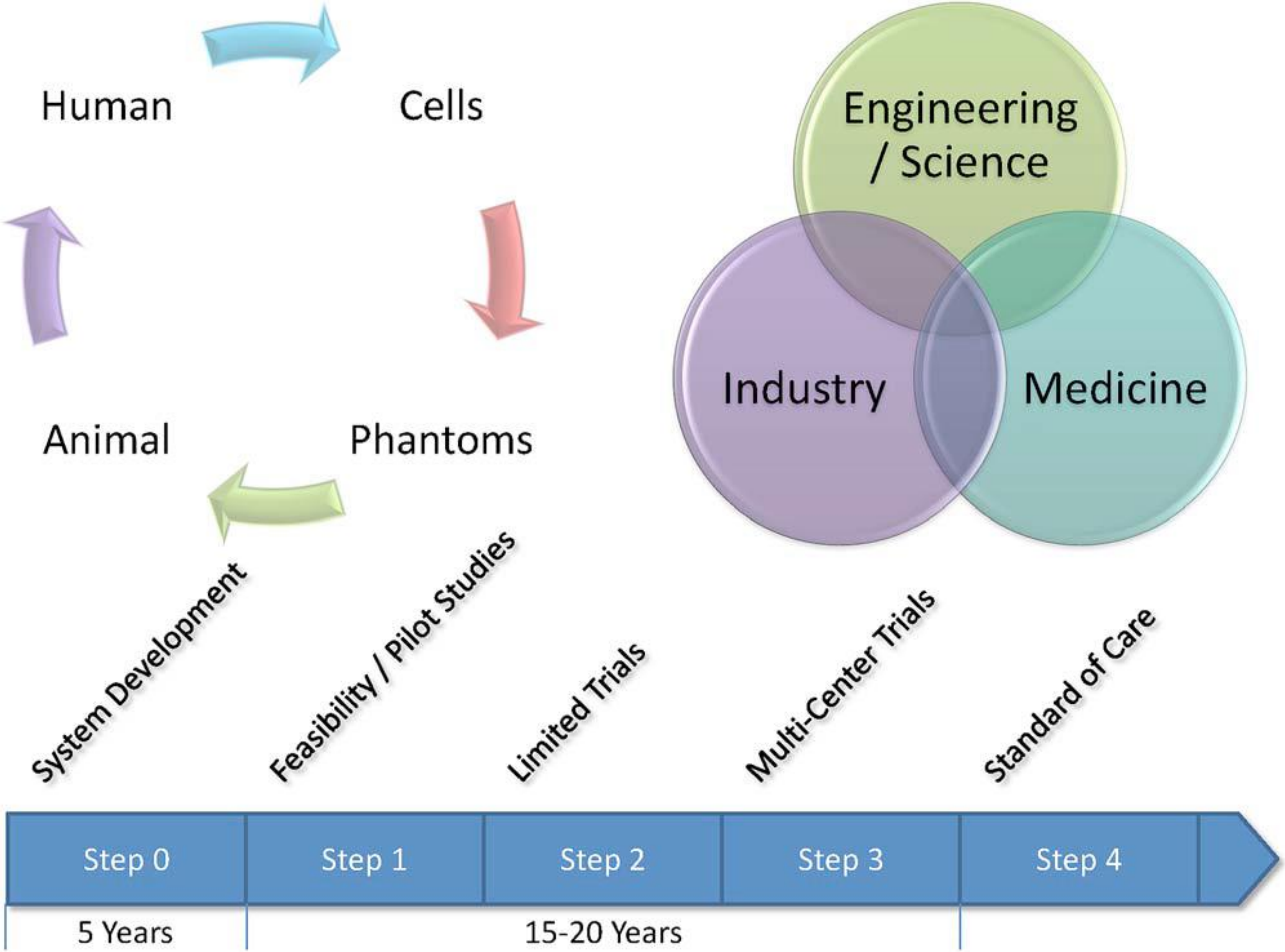
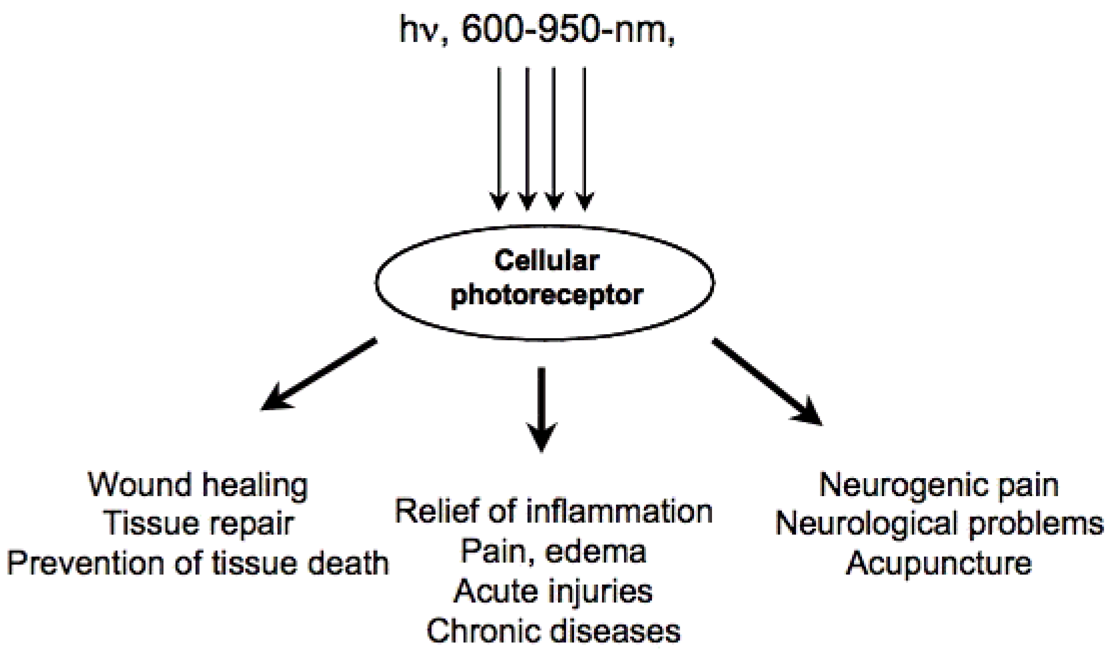
Steps for development from Bench to Bedside



Steps of Development from the Bench to Bedside

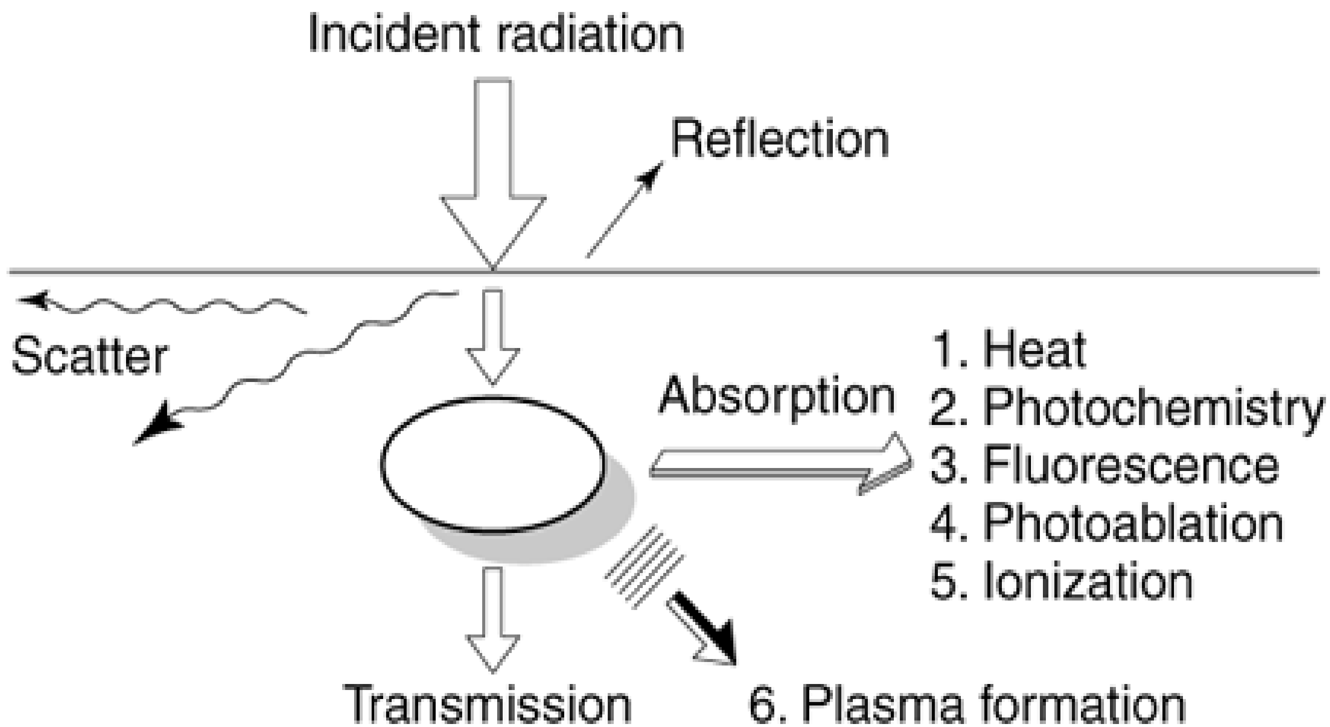
- In 1967, a few years after the first working laser was invented, Endre Mester in Semmelweis University, Budapest, Hungary wanted to test if laser radiation might cause cancer in mice . He shaved the dorsal hair, divided them into two groups and gave a laser treatment with a low powered ruby laser (694 nm) to one group. They did not get cancer, and to his surprise the hair on the treated group grew back more quickly than the untreated group. This was the first demonstration of "laser biostimulation".

Endre Mester, MD



Interaction of Light With Tissues

- A tissue is a self-supporting bulk medium light propagation produces
- Absorption
- Scattering
- Refraction
- Reflection
- Plasma formation



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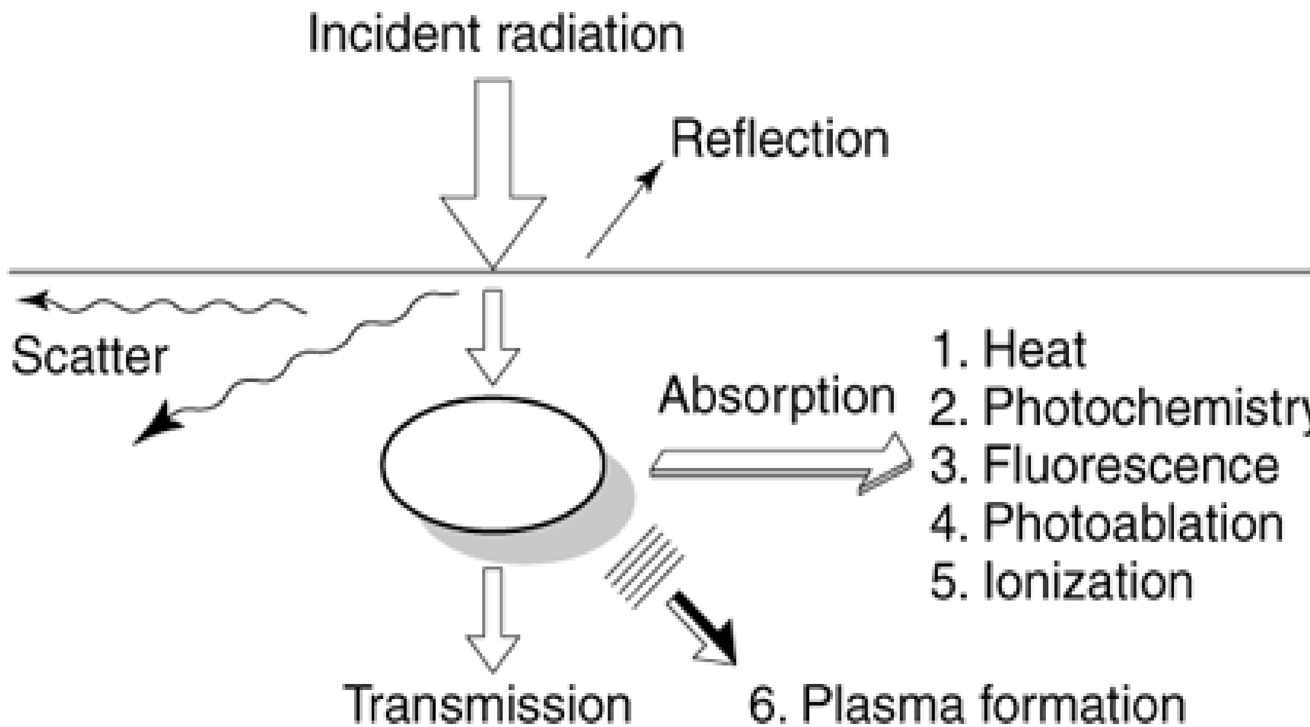


Light Scattering Processes

- Most pronounced effect in a tissue
- Turbidity – measurement of submicron particles that obscure light rays, or apparent nontransparency of a tissue results in a loss of the initial directionality of a collimated beam as well as in defocusing of the light beam spot

Light Scattering

Elastic Scattering

Incident and scattered photons are of the same frequency

Rayleigh scattering

- Scattering by particles of size smaller than the wavelength of light.
- Scattering depends on λ^{-4}, hence significantly more for blue compared to red light.
- Forward and backward scattering is the same

Mie scattering

- Scattering by particles of size comparable to λ.
- Weaker wavelength dependence: λ^{-X} with $0.4 \leq X \leq 0.5$.
- Preferably forward scattering.

Inelastic Scattering

Incident and scattered photons are of different frequencies

Brillouin Scattering

The difference in energy generates acoustic phonons.

Raman Scattering

The difference in energy generates a vibrational excitation in the molecule.

Optical Penetration depth

- Scattering creates a loss of intensity as the light propagates through a tissue
- Described by an exponential function

$$I(z) = I_0 e^{-(\alpha + \alpha_s) z}$$

$z =$ Depth in the tissue

$I_0 =$ Intensity entering the tissue

$\alpha =$ Absorption coefficient

$\alpha_s =$ Scattering Coefficient

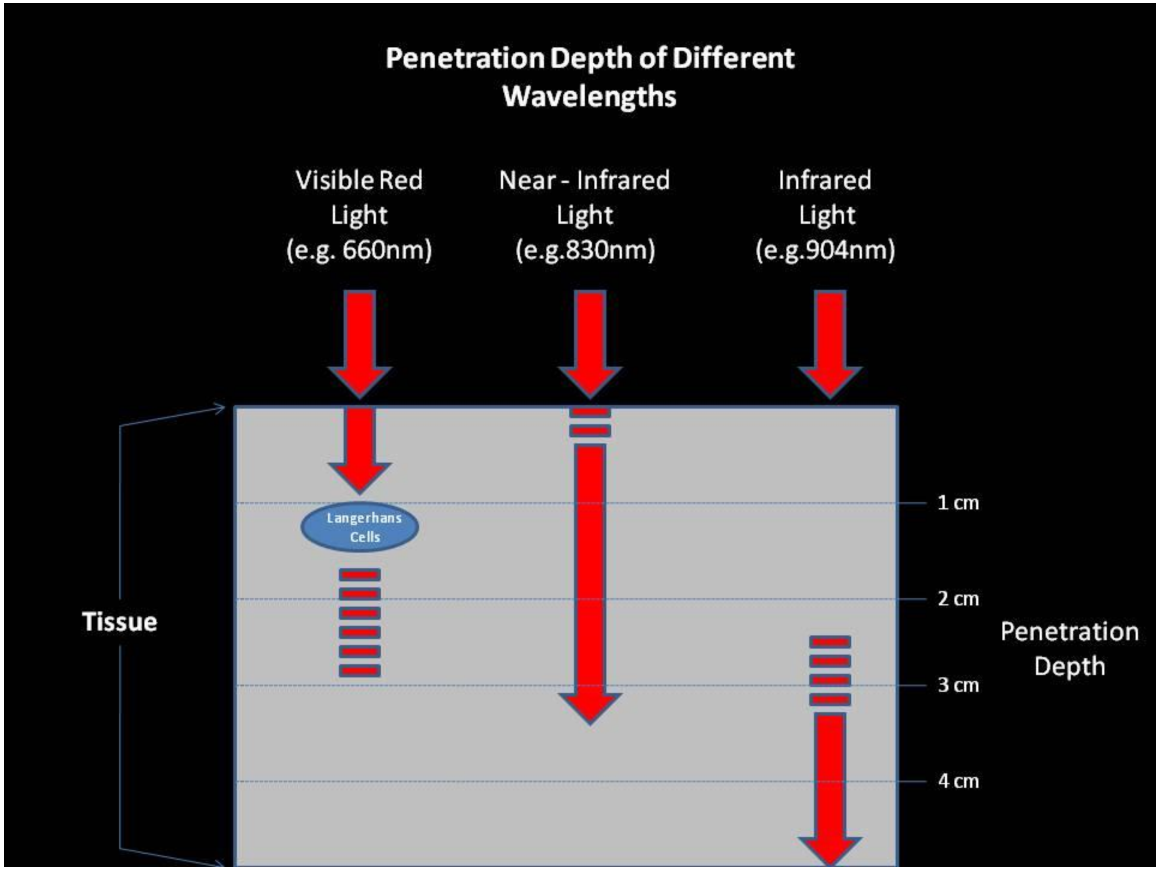
Scattering occurs as photons of light interact with and pass through tissue structures.

Most information obtained from studying the bulk scattering component of reflectance represents information about the presence of large particles, such as the presence of collagen in the lamina propria and submucosa

Optical Penetration depth δ

- Measurement of how deep light can penetrate into a tissue
- Initial intensity is reduced by 90% at a depth of 2δ
- δ decreases with the vascularity (blood content) of a tissue
- δ is less for blue light than for red light
- Largest in the region of 800 – 1300 nm

$$\delta = 1/(\alpha + \alpha_s)$$



Measurement of Optical Properties of a Tissue

- Typical transmission experiment – measure transmission of a beam through a tissue of a finite length
- Gives us a total attenuation coefficient
- Double-Integrating spheres
- Simultaneously determines the reflectance, absorption, and scattering

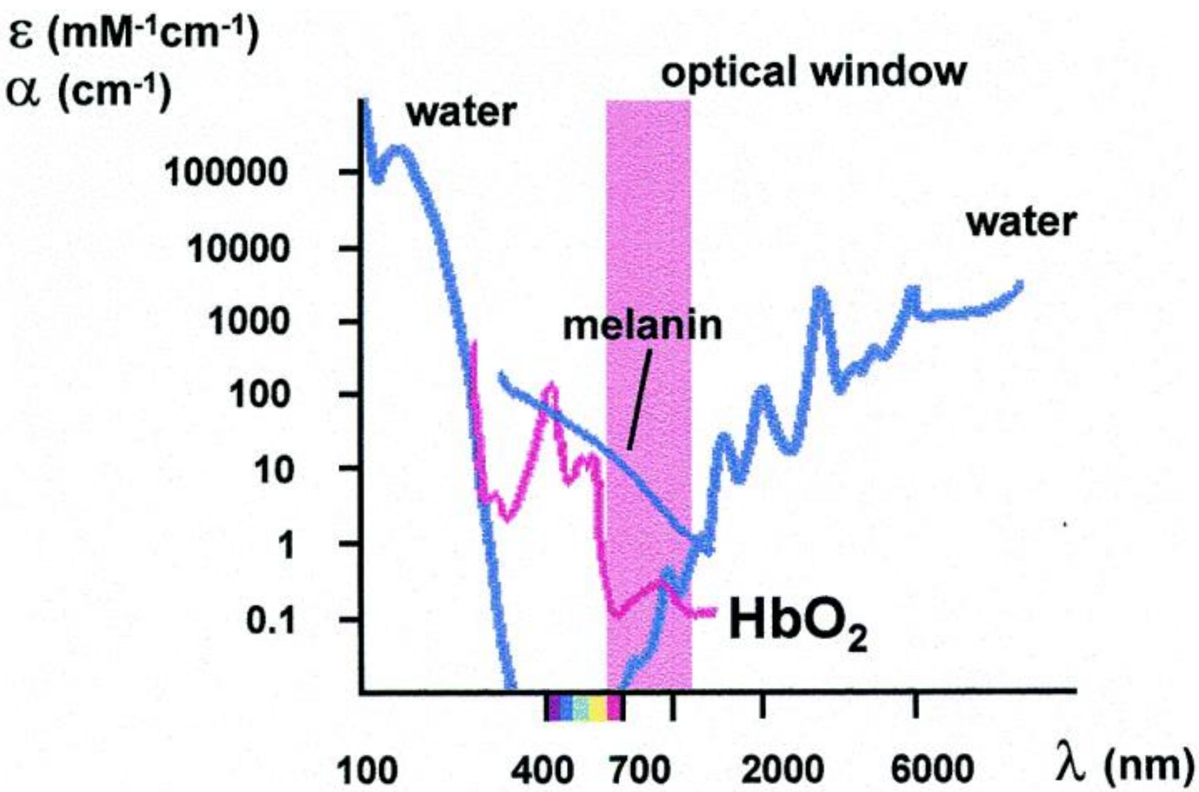
Measures the intensity of light in the forward direction of propagation

Two nearly identical spheres are located in front of and behind the tissue sample

Light detectors collect light from all angles (hence the term integrating spheres)

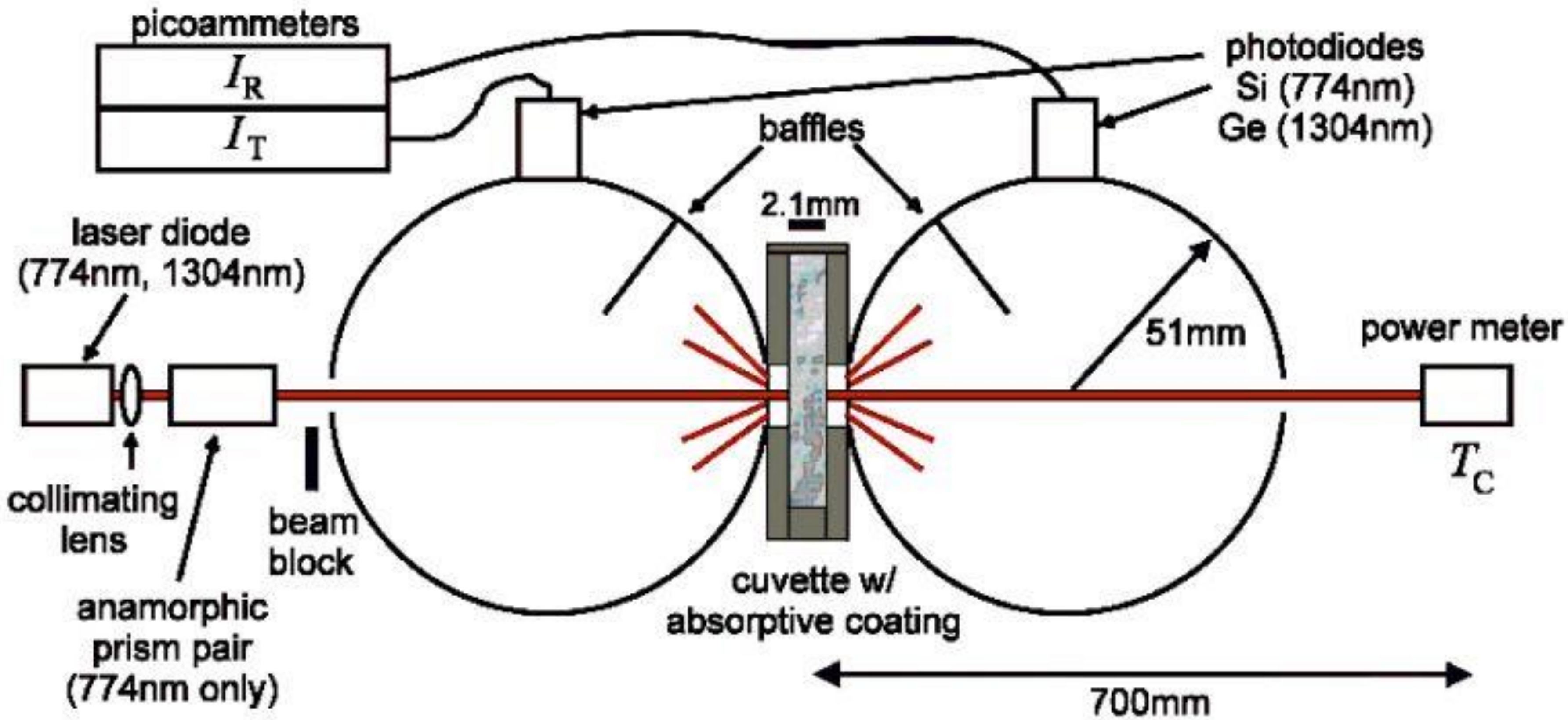
Absorption of Light in Tissue

- The tissue molecules that absorb the light are usually referred to as pigments. Hemoglobin and water are two common body constituents that can function as pigments. Hemoglobin has a very high absorption in the violet and blue/green portions of the visible spectrum. Absorption declines in the red region of the spectrum, which is why hemoglobin is red - it does not absorb red light.



The graphic shows absorption spectra of major intracellular absorbers. The molecular exctinction coefficients of oxygenated haemoglobin and melanin and the absorption coefficient of water are shown.

Double-Integrating Sphere



Light-Induced Processes

Tissue-light interaction

Radiative

Tissue autofluorescence

Fluorescence from various constituents of the tissue

Nonradiative

Photochemical

- Excited state reaction.
- Occurs even at low optical power density.

Thermal

- Light absorption converted to heat.
- Can produce coagulation, vaporization, carbonization, and melting.

Photoablation

- Direct breaking of cellular structure.
- Performed by high-energy UV radiation.

Plasma-Induced Ablation

- Induced by high-intensity short pulse.
- Dielectric breakdown creates ionized plasma that interacts with light to produce ablation.

Photodisruption

- Shockwave generation at high pulse intensity.
- Fragmentation and cutting of the tissue by mechanical force of shockwave.

Autofluorescence-tissue

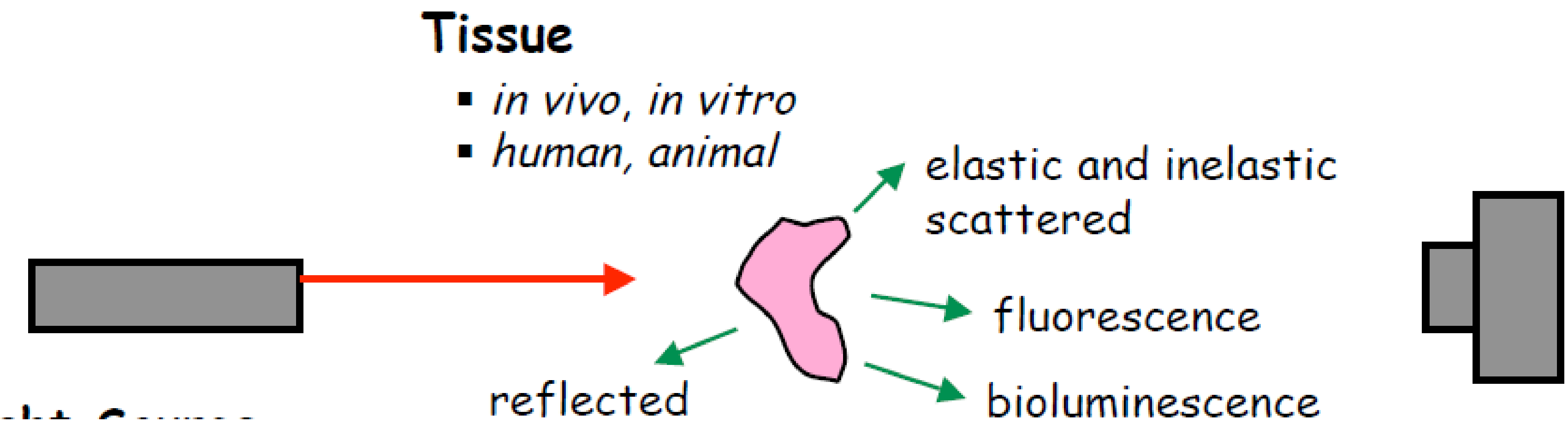
- Any given tissue has a nonuniform distribution of may fluorophores
- The amount of fluorophores may vary as a function of depth below the tissue surface
- Autofluorescence may be different from a premalignant or malignant tissue compared to a normal tissue

- Development of cancer involves a series of changes some of which can be probed by fluorescence

  - protein expression (Trp)
  - metabolic activity (NADH/FAD)
  - nuclear morphology
  - organization
  - structural integrity (collagen)
  - angiogenesis

Instrumentation for clinical tissue fluorescence measurements

- can be very simple, compact and relatively cheap



Light Source

lasers, LEDs, lamps

wavelength selectors

cw, oscillatory, pulsed

Image Acquisition

wide-field

point measurements+scanning

endoscopic

proximity or remote

Light Detector

CCD camera

photodiode, phototube

streak camera

single frame, video

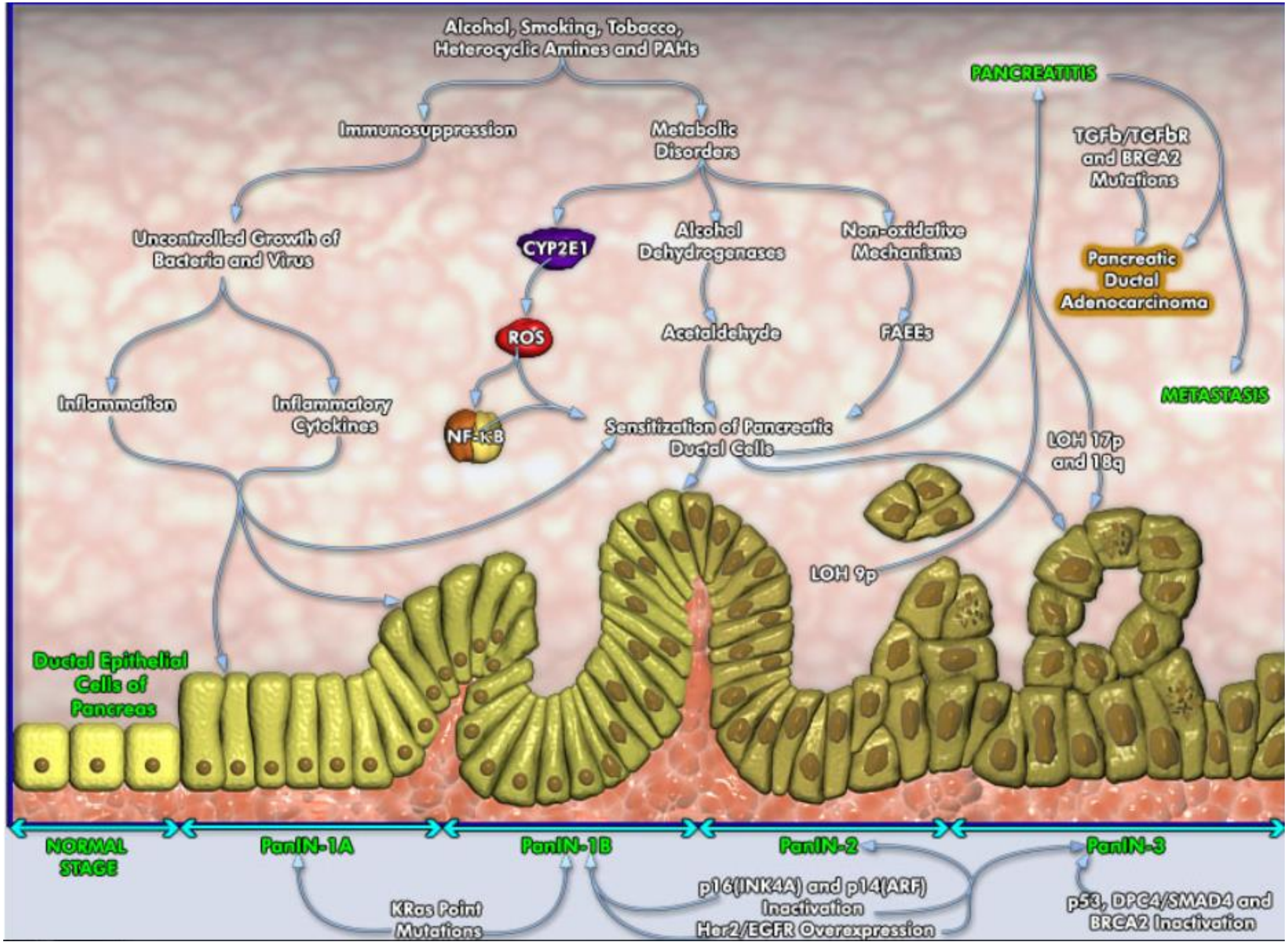
time gated

wavelength selector

- An imaging technique that uses the interactions of light with cells and tissues to diagnose and treat abnormalities.
- Ex. When light shines on cells, the particles of light are scattered by atoms in the molecules of the cells. A special imaging device records these scatter patterns. The molecules in abnormal cells create different scatter patterns than normal cells
- Pros: view tissues deep in the body using light
- Endoscope: a thin flexible tube that has bright light and video cameras a its end.
- Surgical techniques: endoscope has surgical tools at its end so it can be used with minimal small incisions and the doctor can perform any surguries by using an endoscope such as repair knees, gallbladder removal and more

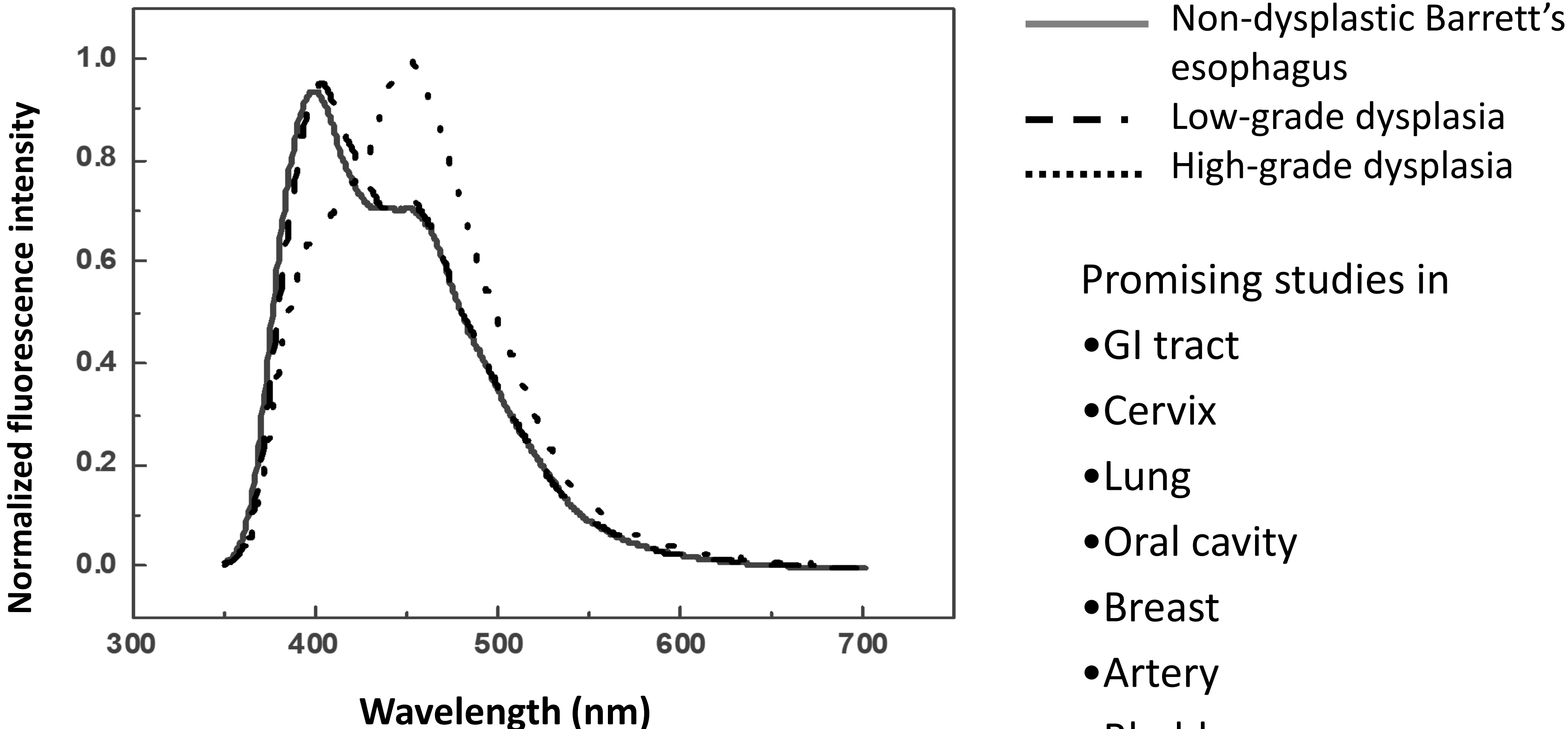
Neoplasia –pancreatic cells

- . Neoplasia ("new growth" in Greek) is the abnormal proliferation of cells.



Consistent autofluorescence differences have been detected

- between normal, pre-cancerous and cancerous spectra



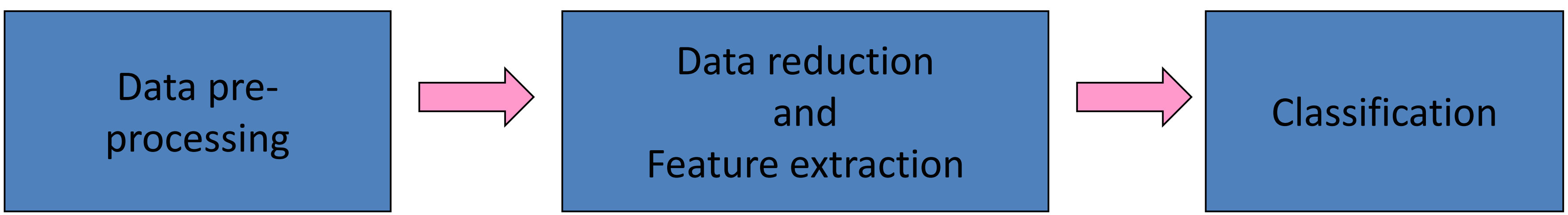
Promising studies in

- GI tract
- Cervix
- Lung
- Oral cavity
- Breast
- Artery
- Bladder

Methods of data analysis

- Main goal for fluorescence diagnostics: Identify fluorescence features that can be used to identify/classify tissue as normal or diseased.
- Main approaches
  - Statistical
  - Empirical
  - Model Based

Empirical and statistical algorithms



Normalization

Principal Component Analysis
Ratio methods

Detection of cervical pre-cancerous lesions using fluorescence spectroscopy

- During the natural lifetime of a woman, **squamous** epithelium which lines the ectocervix gradually replaces the **columnar** epithelium of the endocervix, within an area known as the **transformation zone**. The replacement of columnar epithelium by squamous epithelium is known as squamous metaplasia.

- Most pre-cancerous lesions of the cervix develop within the transformation zone.
- The Papanicolaou (Pap) smear is the standard screening test for cervical abnormalities
- If a Pap smear yields atypical results, the patient undergoes a colposcopy, i.e. magnified (typically 6X to 15X) visualization of the cervix.

- 3-6% acetic acid is applied to the cervix and abnormal areas are biopsied and evaluated histo

- 4-6 billion dollars are spent annually in the US alone for colposcopic evaluation and treatment

- Major disadvantage colposcopic evaluation is its wide range of sensitivity (87-99%) and specificity (23-87%), even in expert hands.

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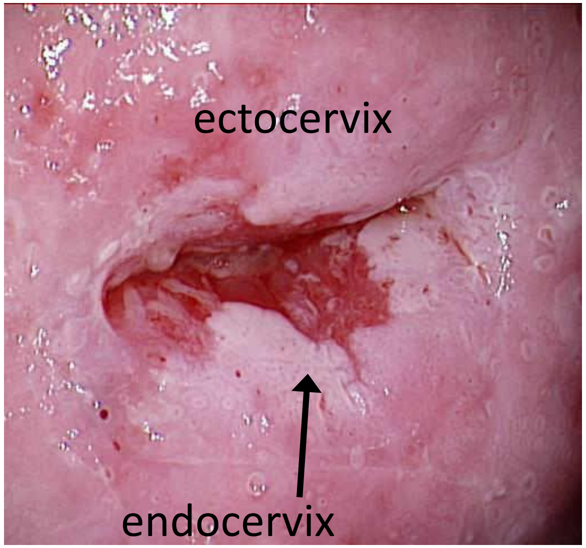
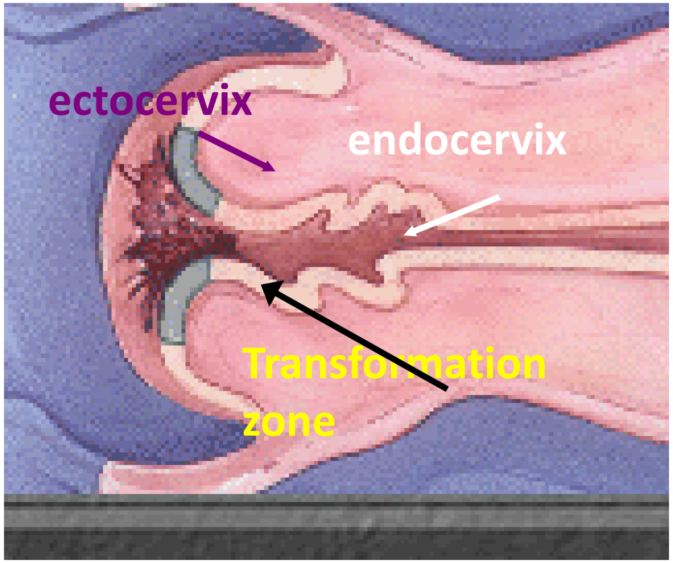
Detection of cervical pre-cancerous lesions using fluorescence spectroscopy



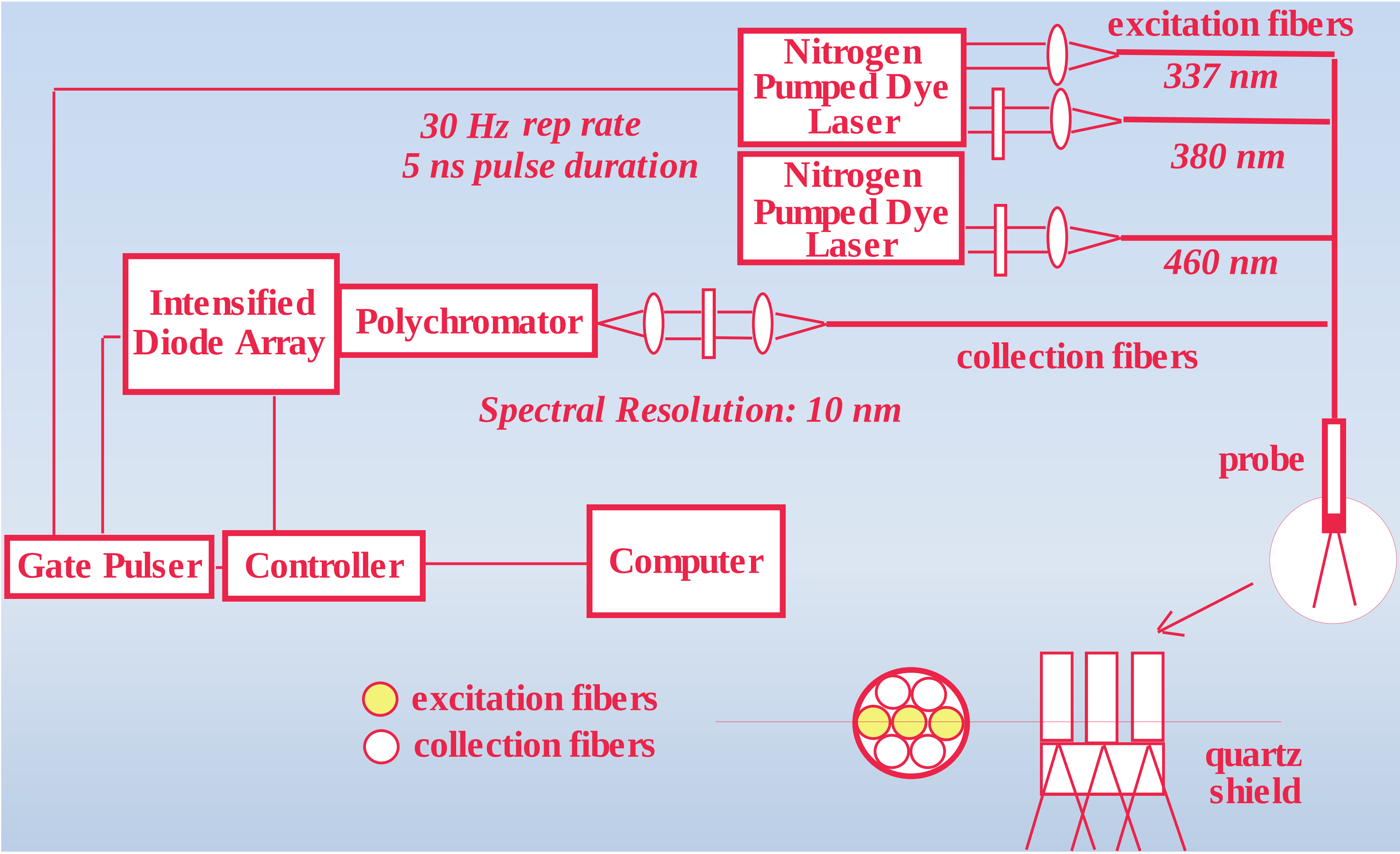
Colposcopic view of uterine cervix



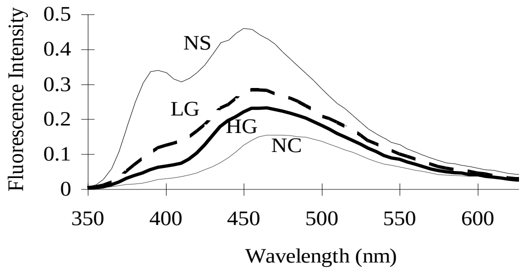
Major tissue histopathological classifications

- Normal squamous epithelium
- Squamous metaplasia
- Low-grade squamous intraepithelial lesion
- High-grade squamous intraepithelial lesion
- Carcinoma

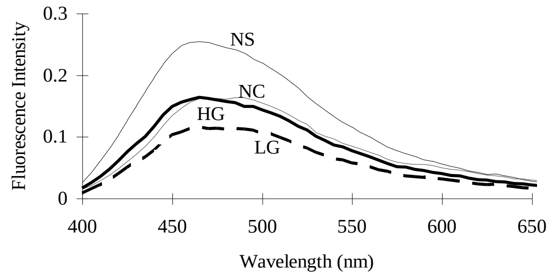
Instrumentation



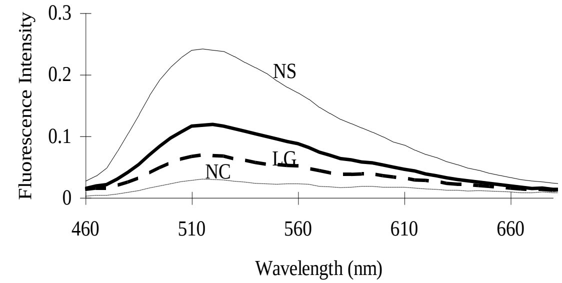
Data processing



337 nm Excitation



380 nm Excitation



460 nm Excitation

PRE-PROCESSING

Normalized Spectra at Three Excitation Wavelengths

Normalized, Mean-scaled Spectra at Three Excitation Wavelengths

DIMENSION REDUCTION: PRINCIPAL COMPONENT ANALYSIS

SELECTION OF DIAGNOSTIC PRINCIPAL COMPONENTS: T-TEST

CLASSIFICATION: LOGISTIC DISCRIMINATION

Constituent Algorithm 1

Constituent Algorithm 3

Constituent Algorithm 2

Posterior Probability of being NS or SIL

Posterior Probability of being LG or HG

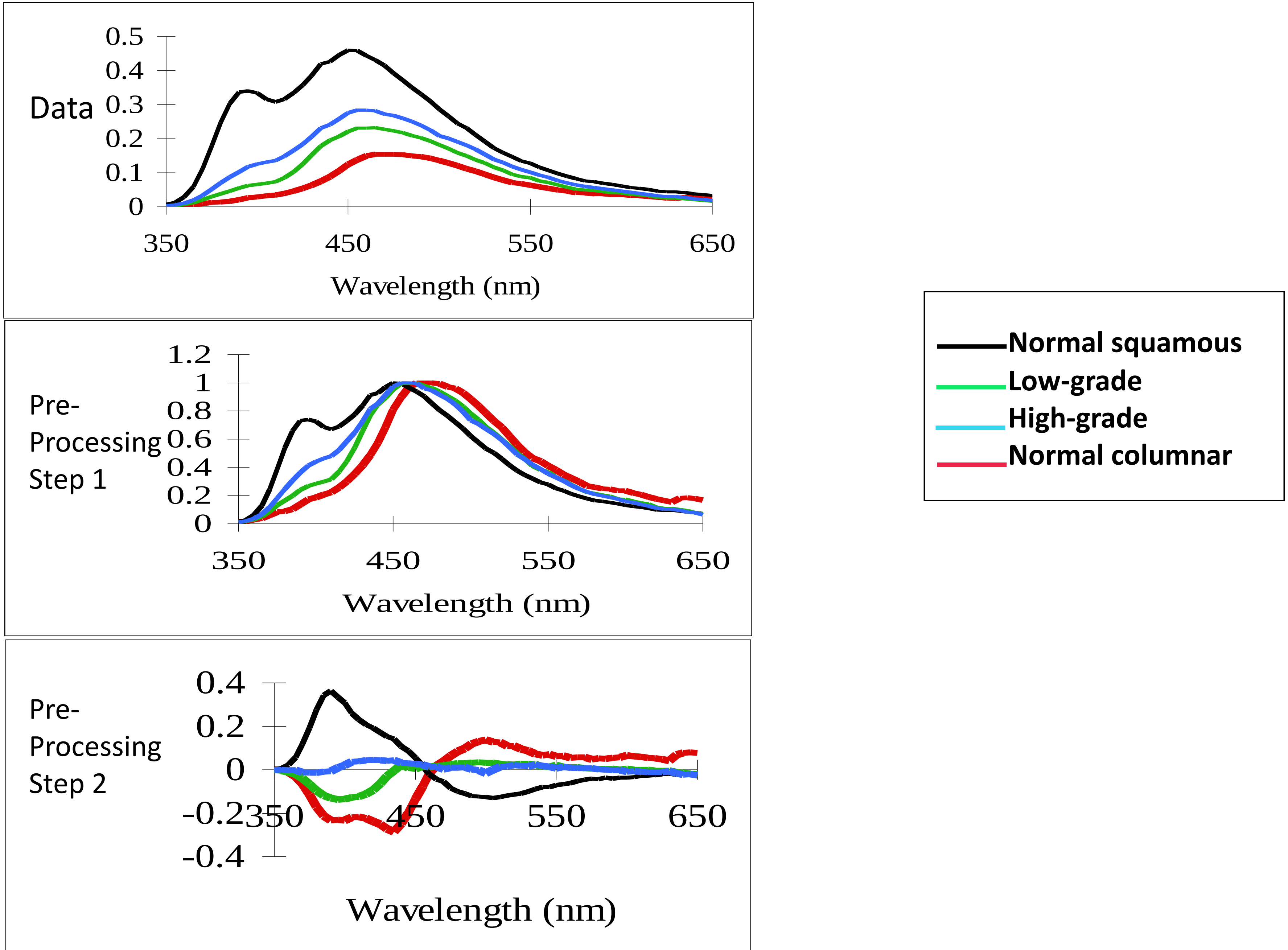
Posterior Probability of being NC or SIL

DEVELOPMENT OF COMPOSITE ALGORITHMS

Composite Screening Algorithm (1,2)

(1,,3) *Composite* Diagnostic Algorithm

Data processing

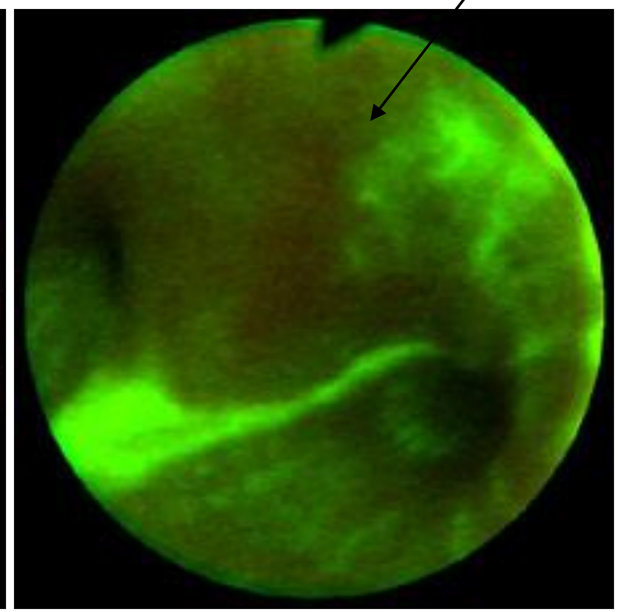


Detection of lung carcinoma in situ using the LIFE imaging system

- an area of abnormal brownish red fluorescence in the sub-carina. The area (image #2) measured 2 to 3 millimeters in width and was confirmed by biopsy as carcinoma-in-situ with small foci of microinvasion.

Carcinoma in situ

White light bronchoscopy



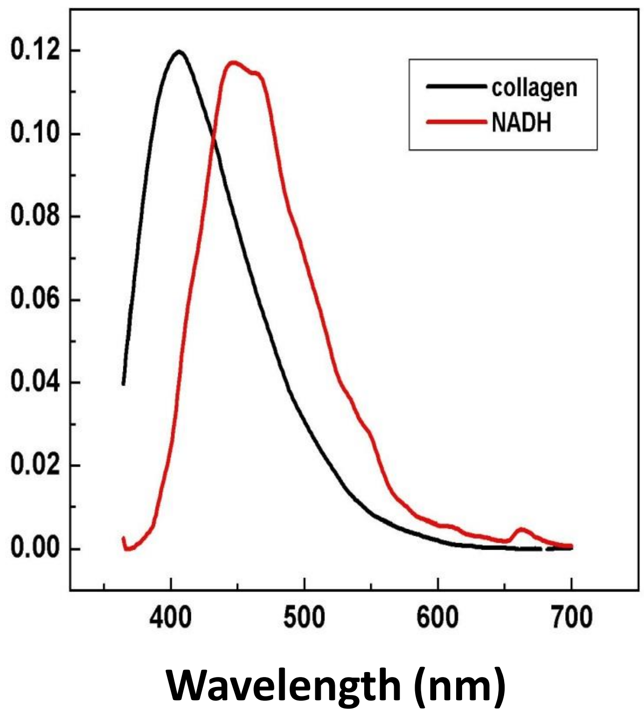
Autofluorescence ratio image

Fluorescence imaging based on ratio methods

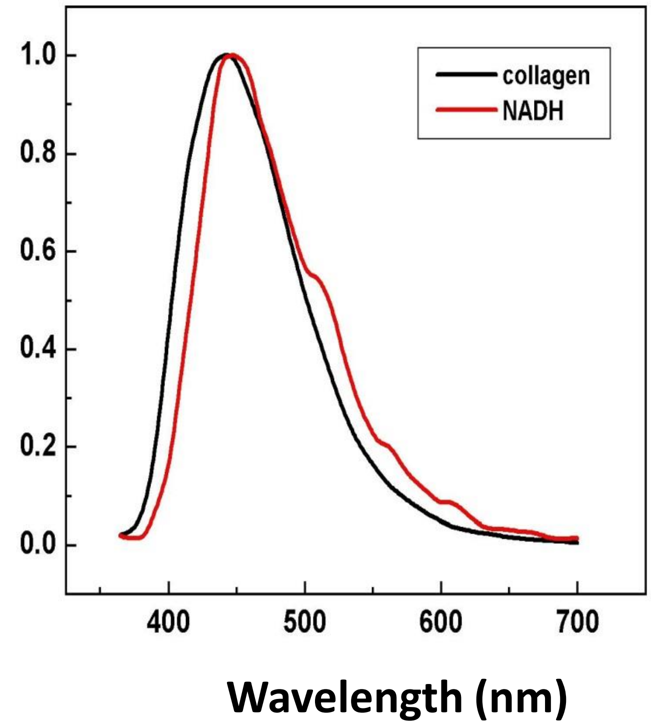
- Wide field of view (probably a huge advantage for most clinical settings)
- Eliminates effects of distance and angle of illumination
- Easy to implement
- Provides no intuition with regards to origins of spectral differences

Collagen and NADH spectra are sufficiently distinct only for some excitation wavelengths

337 nm excitation



358 nm excitation

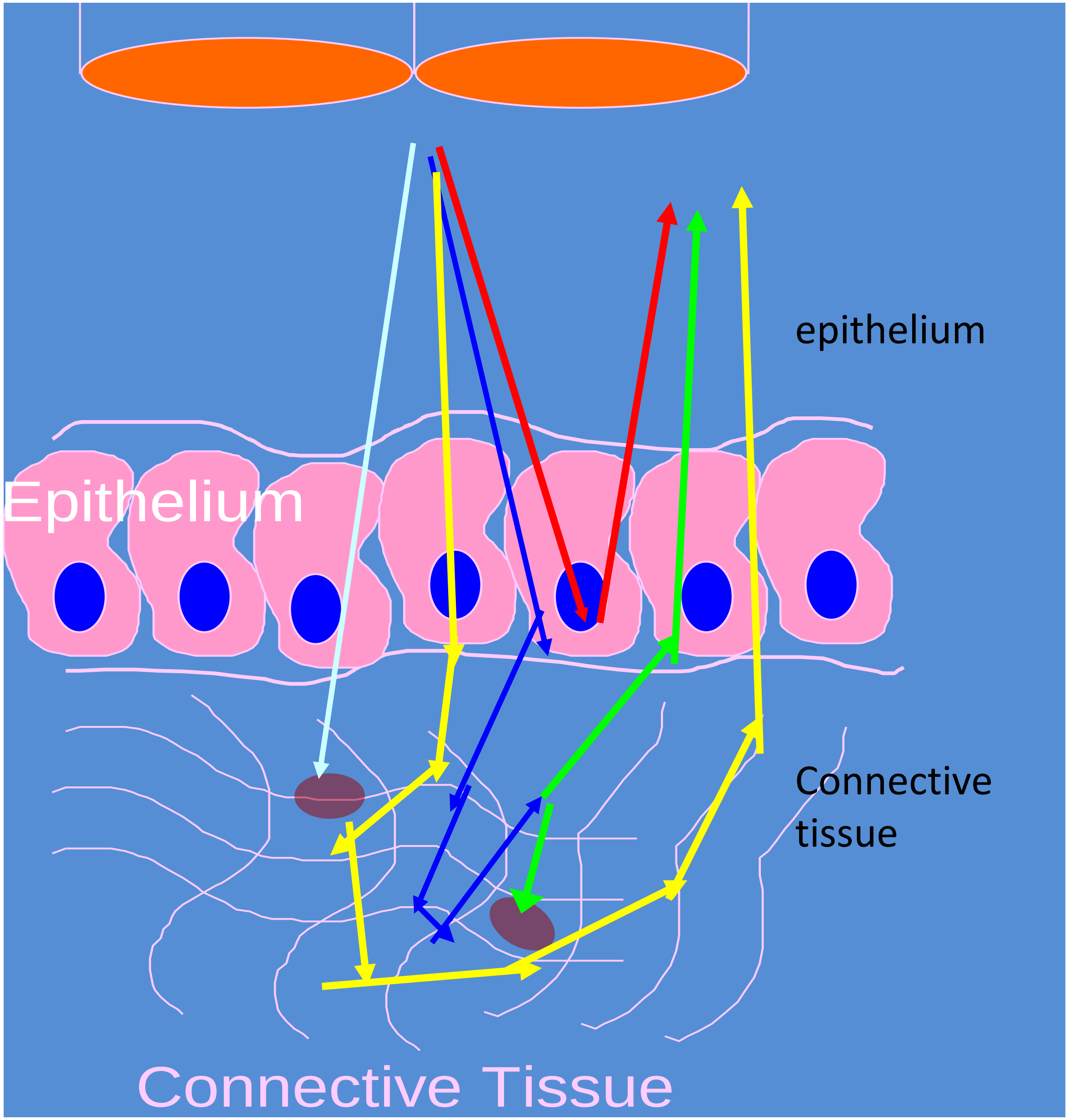


Tissue absorption and scattering may affect significantly tissue fluorescence

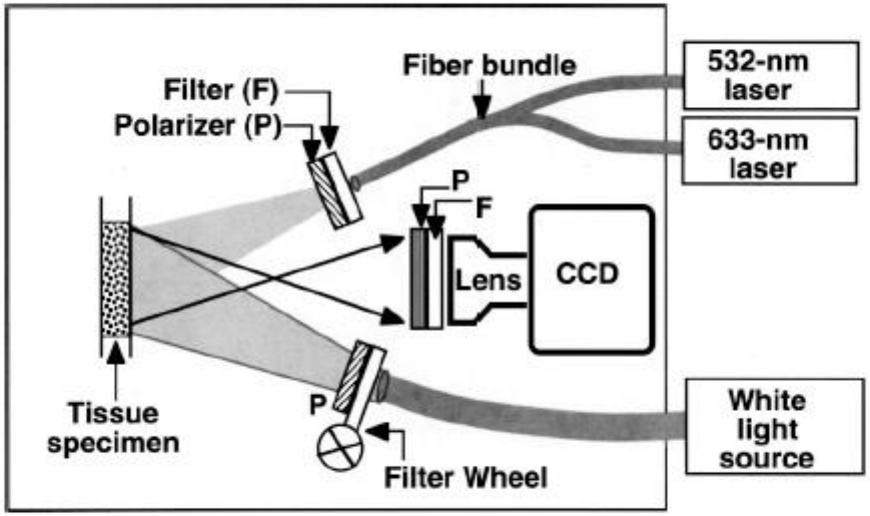
- single scattering

- absorption
  - Hemoglobin, beta carotene

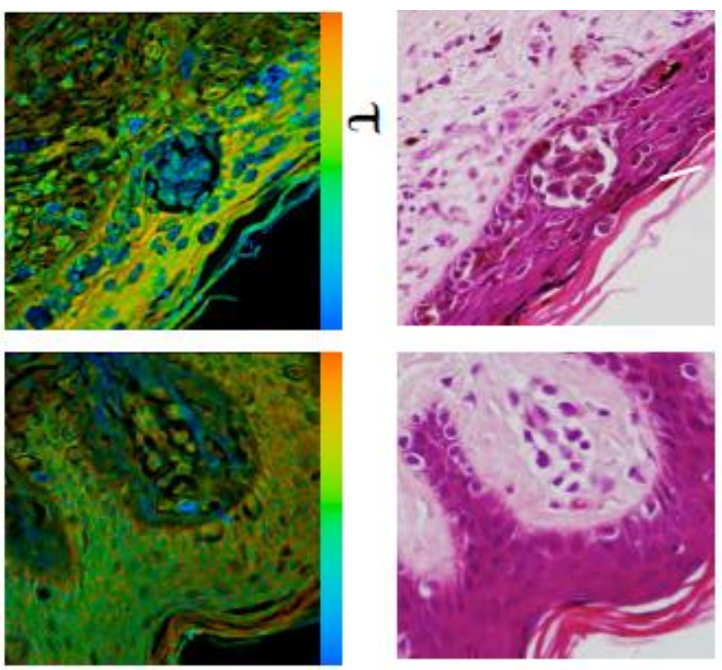
- fluorescence



Fluorescence and Fluorescence Lifetime Imaging



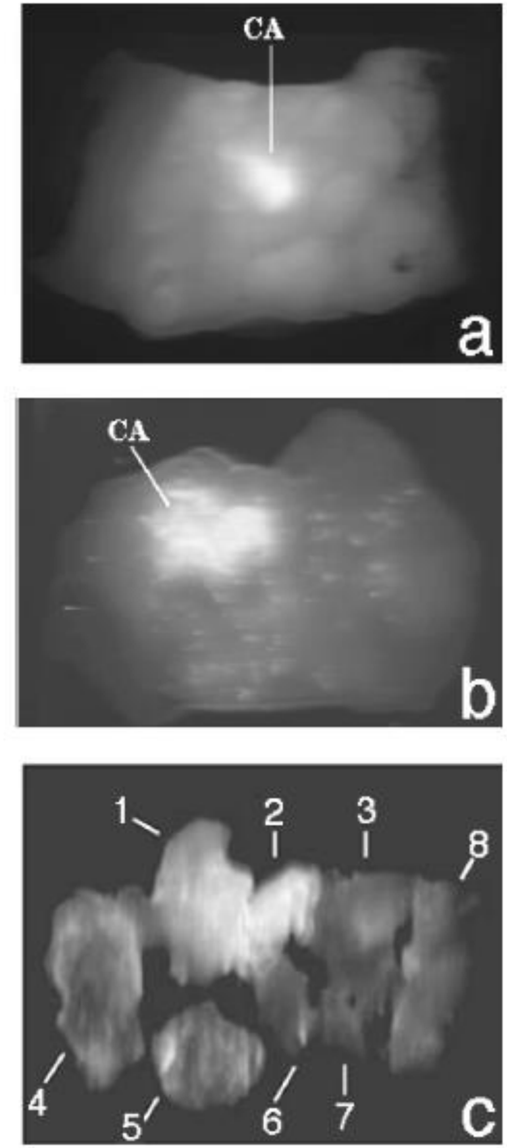
FLIM and H&E images of melanoma cells



Pancreas

Prostate

Bladder



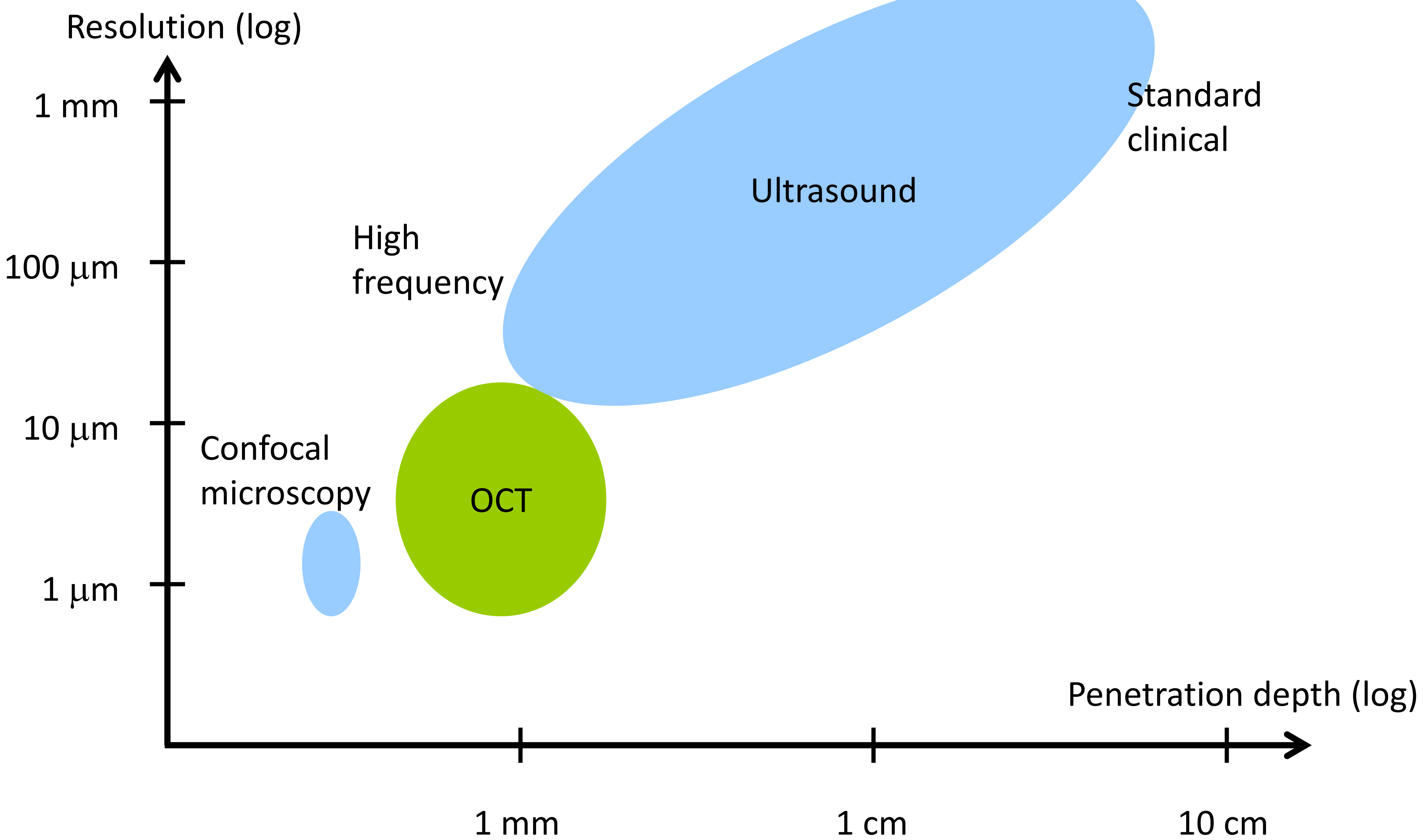
633 nm excitation NIR autofluorescence Images showing cancer (CA) lesions.

Optical coherence tomography

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

Imaging Technology	Typical resolution (mm)	Imaging Depth (mm)	System Cost	Speed	Features
Ultrasound	150	150	Low	Vide rate	Contact
MRI	1000	Unlimited	High	Video rate	Non-Invasive
CT	1000	Unlimited	High	Video rate	Non-Invasive, Radiation
OCT	1-15	1.5	Low	Video rate	Non-Invasive

OCT vs. standard imaging



Optical coherence tomography

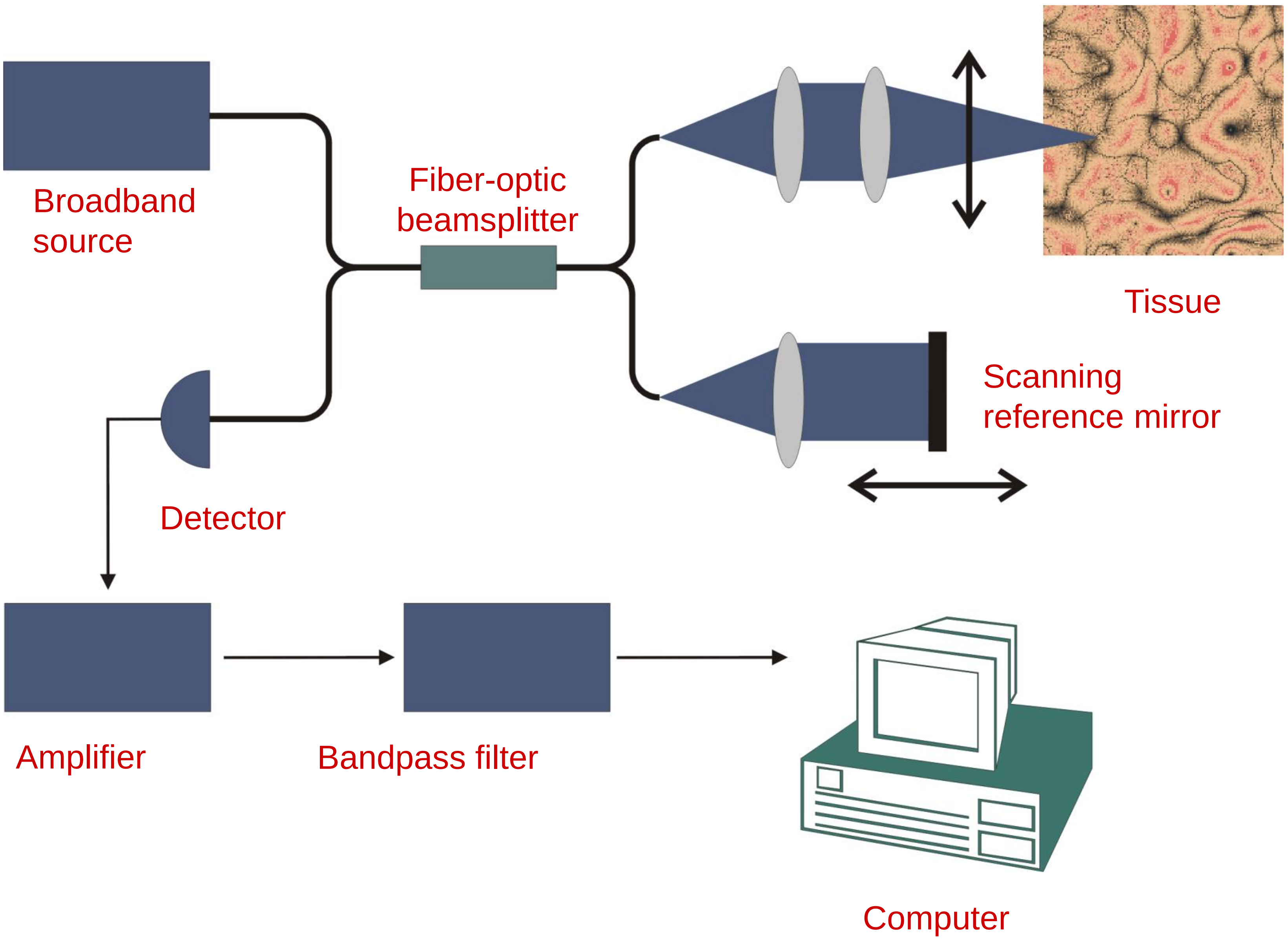
- Three-dimensional imaging technique with ultrahigh spatial resolution even in highly scattering media
- Based on measurements of the reflected light from tissue discontinuities
  - e.g. the epidermis-dermis junction.
- Based on interferometry
  - involves interference between the reflected light and the reference beam

- 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.

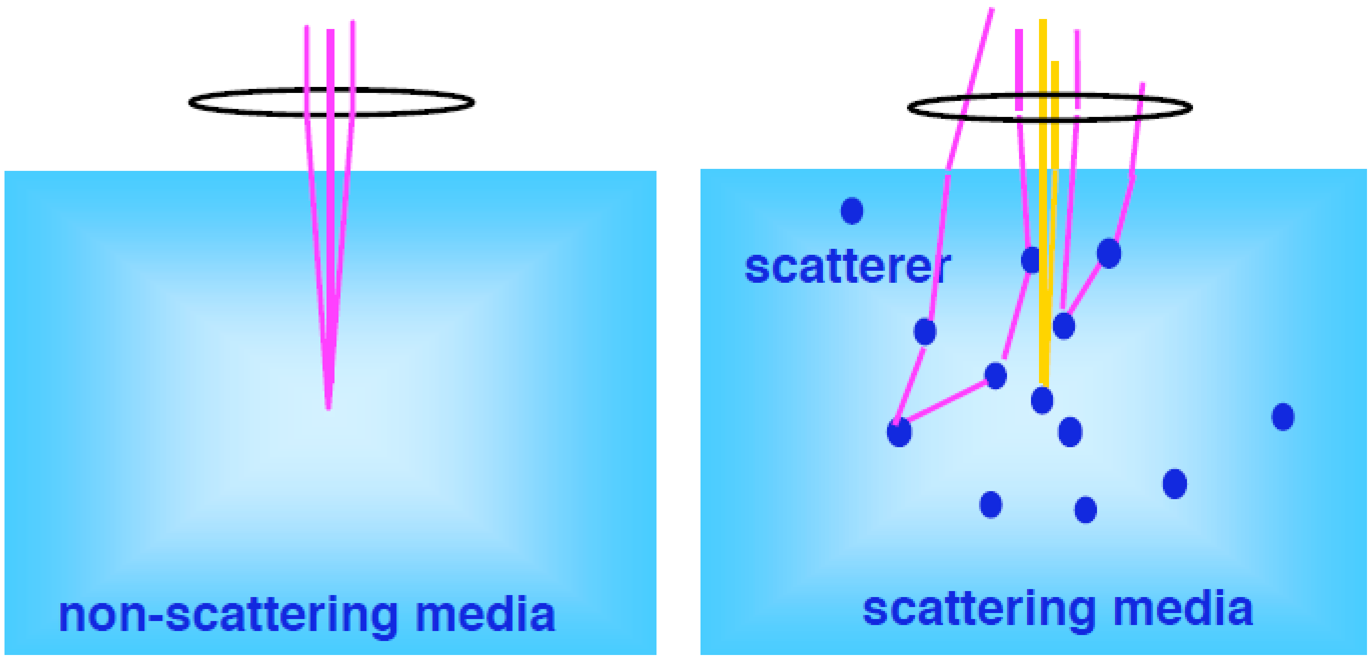
- Guided surgery
  - delicate procedures
  - brain surgery,
    - knee surgery,
    - …

OCT setup


Broadband source
Fiber-optic beamsplitter
Tissue
Scanning reference mirror
Detector
Amplifier
Bandpass filter
Computer

Optical coherence tomography

- **Challenge: scattering of photons destroys localization**



System perspective

Light sources
- Superluminescent diodes
- Semiconductor amplifiers
- Femtosecond lasers
- …

Beam delivery and probes
- Hand-held probe
- Catheter
- Ophthalmoscope
- Microscope

OCT imaging engine
- Resolution
- Reference delay scanning
- Doppler/polarization/spectroscopy
- Detection
- Frequency domain

continue

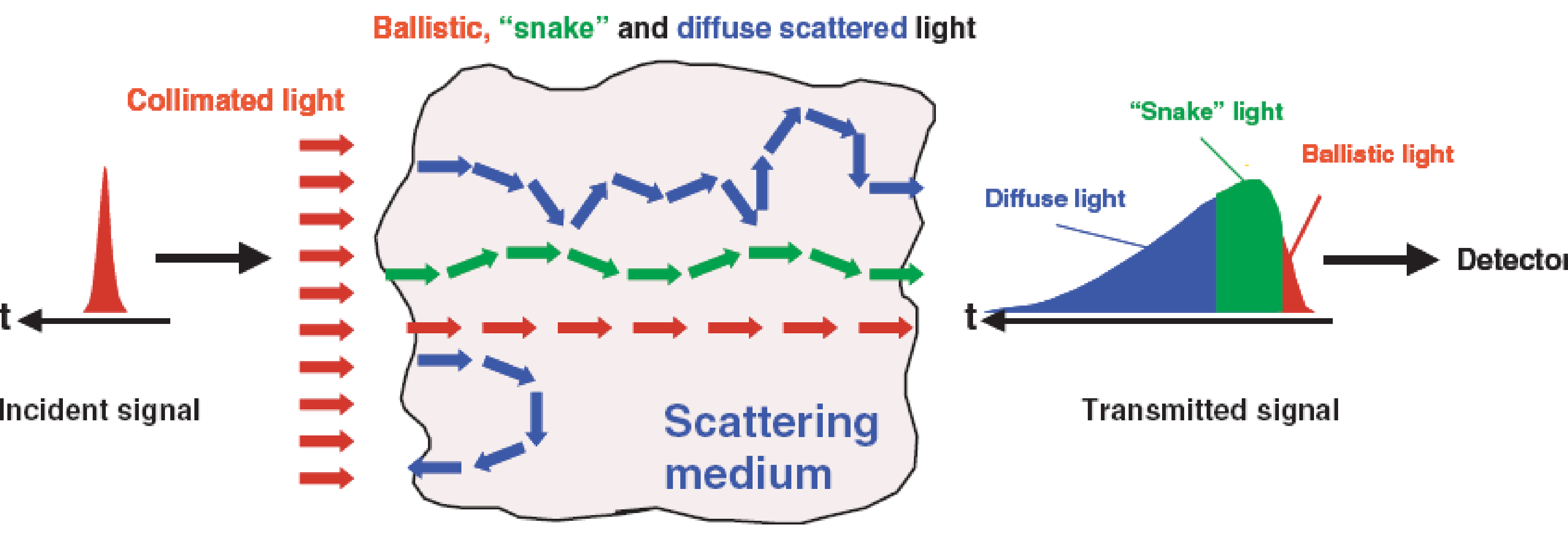
Computer control
- Drive system
- Real-time display
- Data management

Image & signal processing
- Motion reduction
- Speckle reduction
- Image enhancement
- Rendering algorithms
- …

Choosing the light source

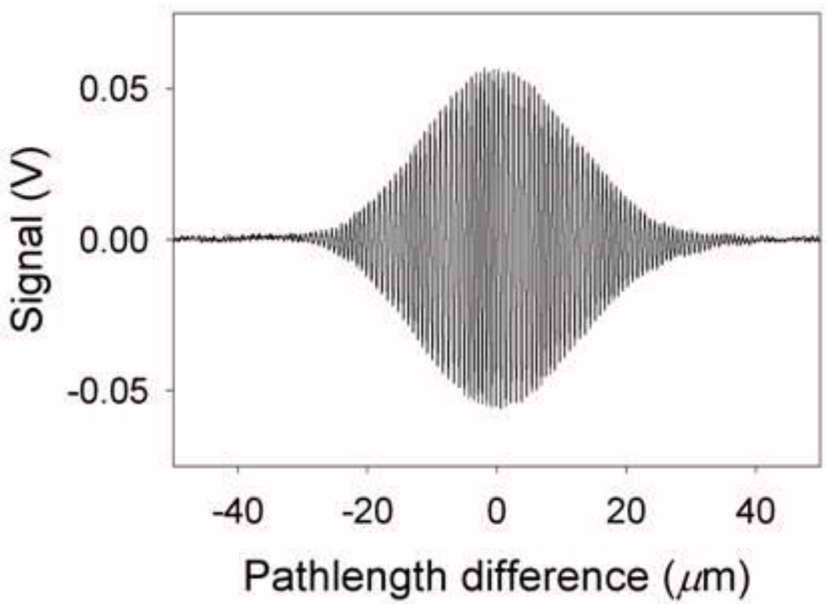
- Four primary considerations
  - wavelength,
  - bandwidth,
  - power (in a single-transverse-mode),
  - stability;

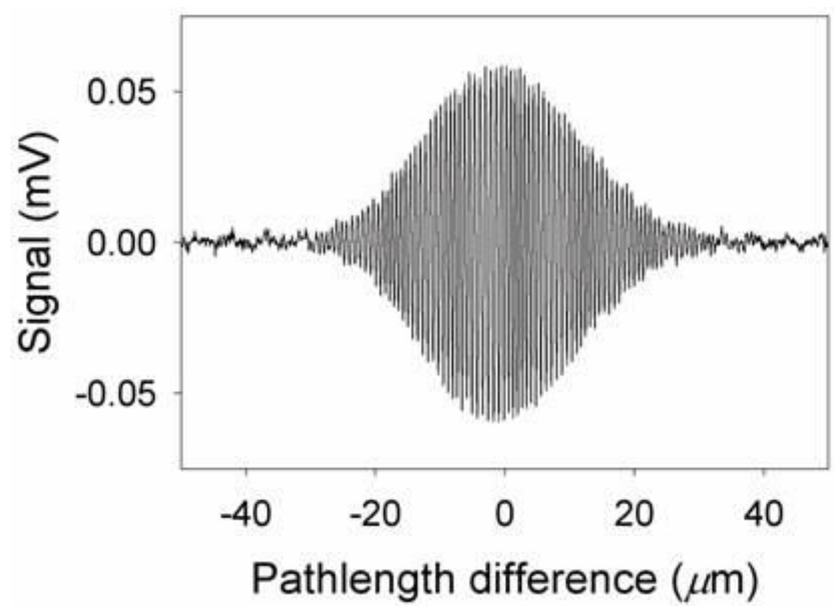
    portability, ease-of-use, etc.
- Light propagation (Monte Carlo simulation)



Choosing the light source

- Scattering mainly attenuates the signal

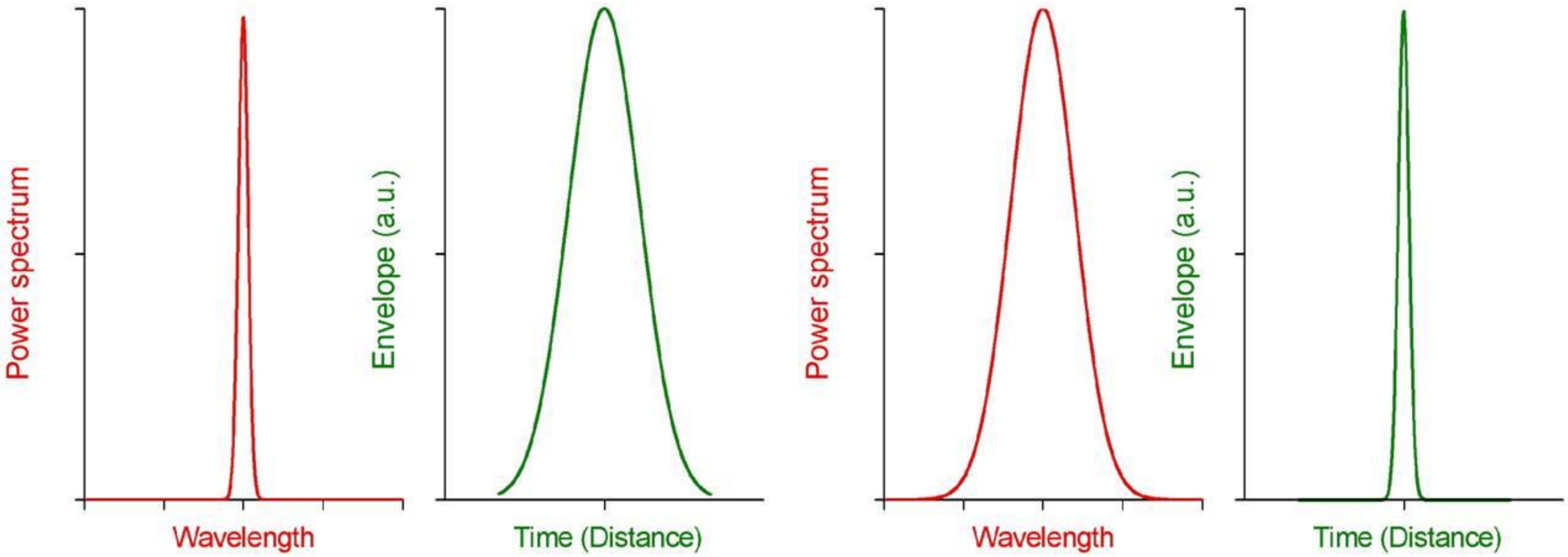




Basic property

- the temporal coherence envelope function $G(\tau)$ is related to the power spectral function $S(\nu)$ through
  $G(\tau) = FT\{S(\nu)\}$
  - Wiener-Kinchine theorem
- broadband source ⇔ high axial resolution

Source spectrum and envelope

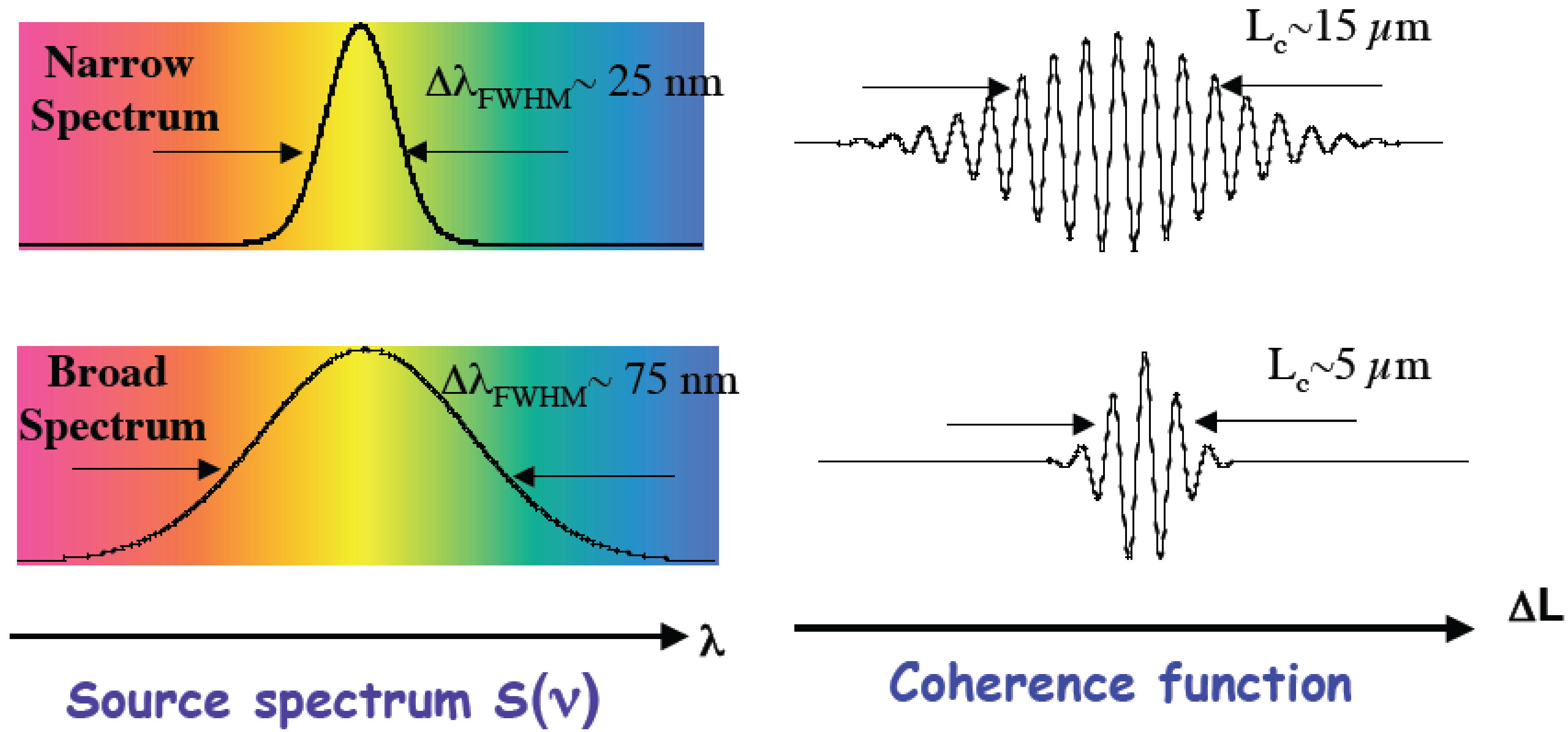


- The axial resolution is

$$l_c = \frac{2c\ln 2}{\pi}\frac{1}{\Delta\nu} = \frac{2\ln 2}{\pi}\frac{\lambda_0^2}{\Delta\lambda} \approx 0.44\frac{\lambda_0^2}{\Delta\lambda}$$

  – notice that $\Delta\lambda$ is the 3dB-bandwidth!

Interference with Partial Coherence Light Source



$$L_c = 0.44\lambda^2/\Delta\lambda$$

Fourier Transformation

Light sources for OCT

- **Continuous sources**
  - SLD/LED/superfluorescent fibers,
  - center wavelength;

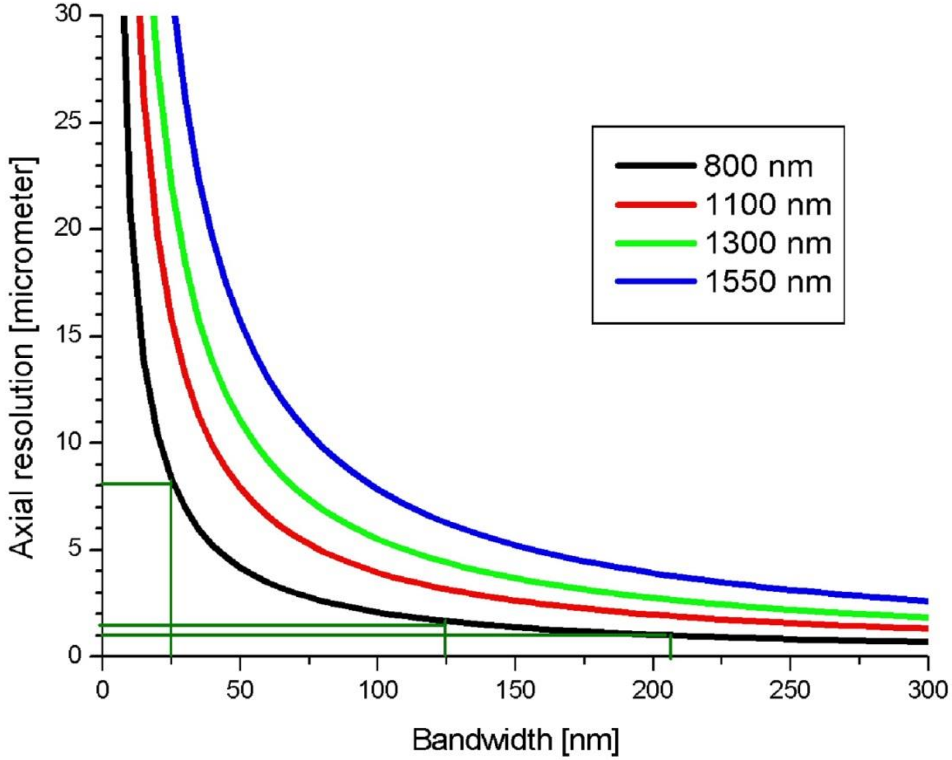
    800 nm (SLD),

    1300 nm (SLD, LED),

    1550 nm, (LED, fiber),

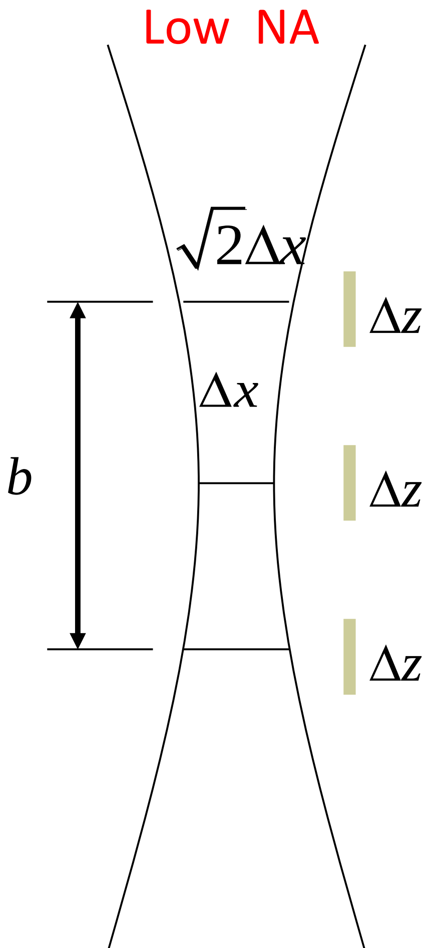
    power: 1 to 10 mW (c.w.) is suffi
  - coherence length;

    10 to 15 m (typically),
- **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.



OCT spatial resolution

- Axial and lateral resolutions are decoupled

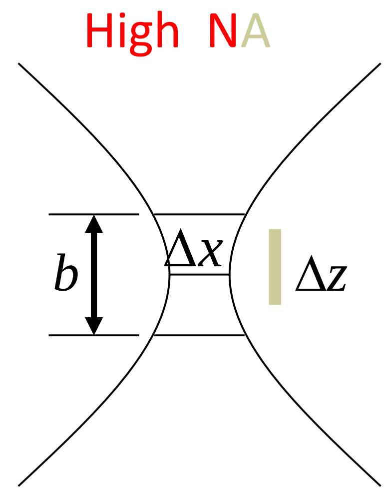


Lateral resolution

$$\Delta x = \frac{4\lambda}{\pi}\left(\frac{f}{d}\right)$$

Depth of focus

$$b = 2z_R = \pi\frac{\Delta x^2}{2\lambda}$$



- The lateral resolution is determined by the focusing conditions
  - optics
  - dynamic vs. static focusing

High resolution OCT

- Broad bandwidth sources
  - solid-state lasers,
  - sub-5 fs pulse;
    Ti:Al2O3 (Spectral bandwidth: 350 nm demonstrated),
  - other lasers/wavelengths available or needed.
- Special interferometers and fiber optics
  - support for broad spectral range,
  - dispersion balanced,
  - current system used for OCT: 260 nm bandwidth, ~1.5µm resolution.
- Chromatically corrected optics
  - aberrations can decrease resolution and SNR.
- Broad bandwidth detectors and electronics
  - dual balance detection,
  - low noise circuitry necessary

Scanning devices

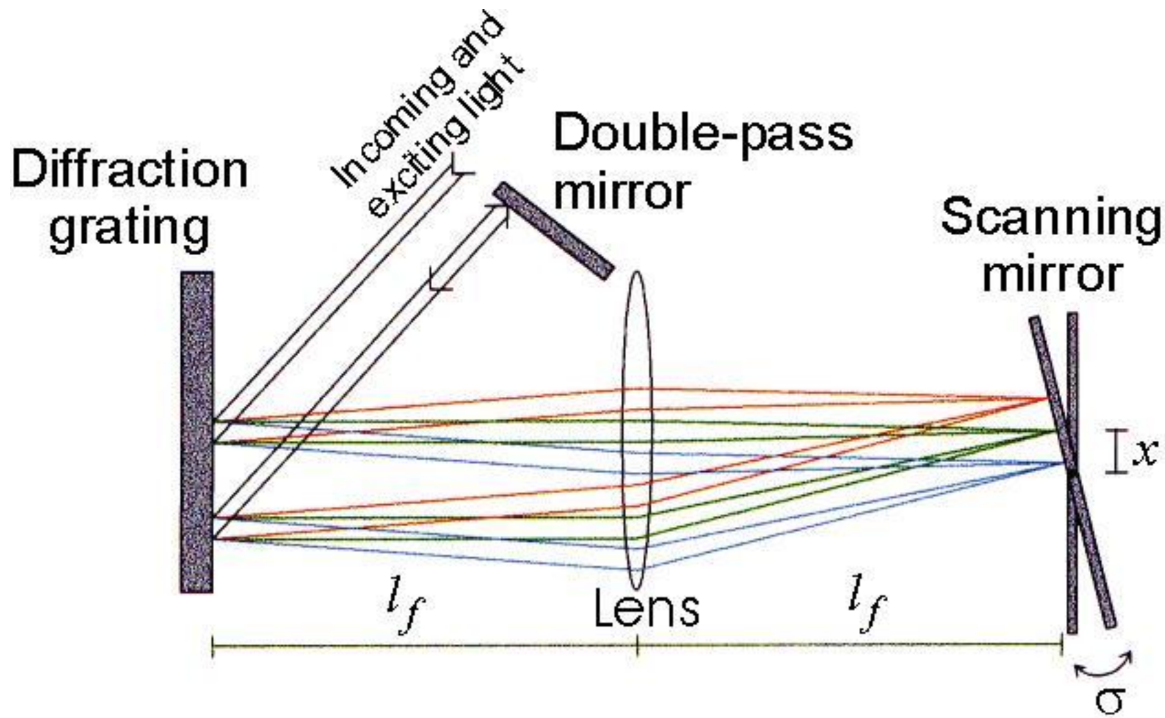
- Piezo or motorized scanning devices
  - ideal for both longitudinal and lateral scanning.
- Galvanic mirrors
- Resonance scanners
- Helical mirrors
  - longitudinal scanning.
- Fiber stretcher
  - longitudinal scanning

Fourier domain rapid scanning optical delay line (RSOD)

- The technique was originally developed for femtosecond pulse measurements
  - based on Fourier-transform pulse shaping techniques.
- Relies on the basic property of the Fourier transform

$$x(t-t_0) \overset{\Im}{\longleftrightarrow} X(\omega)\exp\{-j\omega t_0\}$$

- phase ramp in
- the Fourier domain
- corresponds to a group delay in the time domain.



RSOD in the lab

- Free-space group pathlength
  - p: the grating pitch,
  - f: focal length,
  - s: mirror angle.

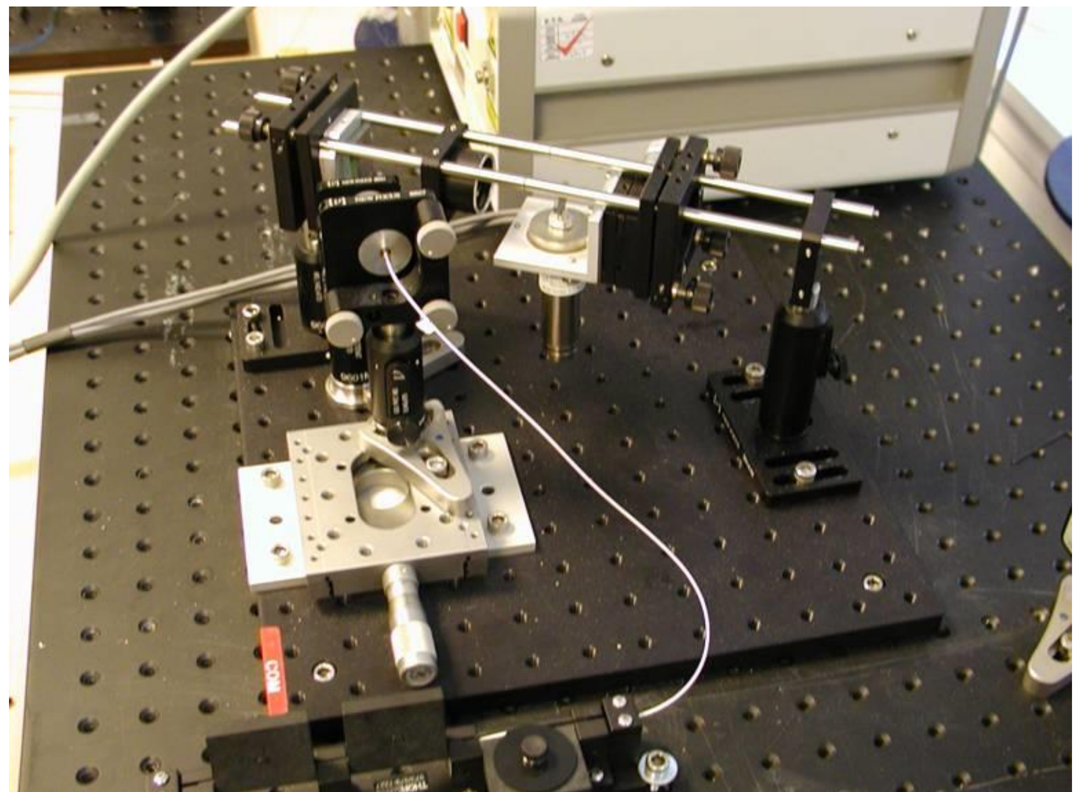
$$\Delta l_g = 4\sigma x - \frac{4\sigma l_f \lambda_0}{p}$$

- Interferogram central frequency

$$f_0 = \frac{4x}{\lambda_0}\frac{\partial \sigma}{\partial t}$$

- Bandwidth

$$\Delta f = \frac{2\Delta\lambda}{\lambda_0^2}\left(2x - \frac{2l_f \lambda_0}{p}\right)\frac{\partial \sigma}{\partial t}$$



RSOD

- Scanning capabilities (galvo)
  - ~ 200 Hz,
  - ~ 5 mm.
- Scanning capabilities (resonant)
  - ~ 4-8 kHz,
  - ~ 5 mm.
- Advantages
  - dispersion compensation feasible since phase and group delays may be separated,
  - center frequency of interferogram is adjusted through axial position

OCT in non-invasive diagnostics

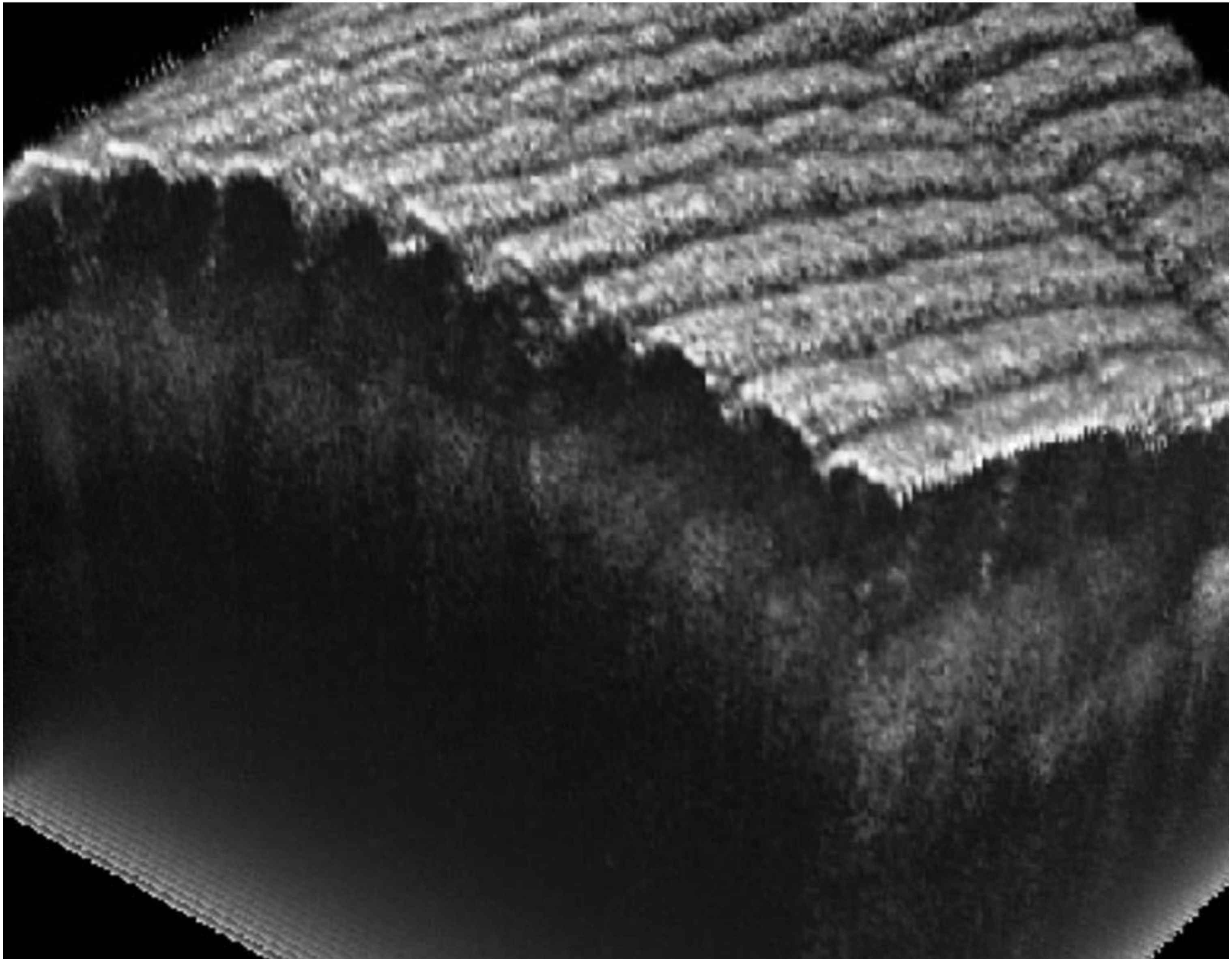
- Ophthalmology
  - diagnosing retinal diseases.
- Dermatology
  - skin diseases,
  - early detection of skin cancers.
- Cardio-vascular diseases
  - vulnerable plaque detection.
- Endoscopy (fiber-optic devices)
  - gastrology,
  - …

- Functional imaging
  - Doppler OCT,
  - spectroscopic OCT,
  - optical properties,
  - PS-OCT.

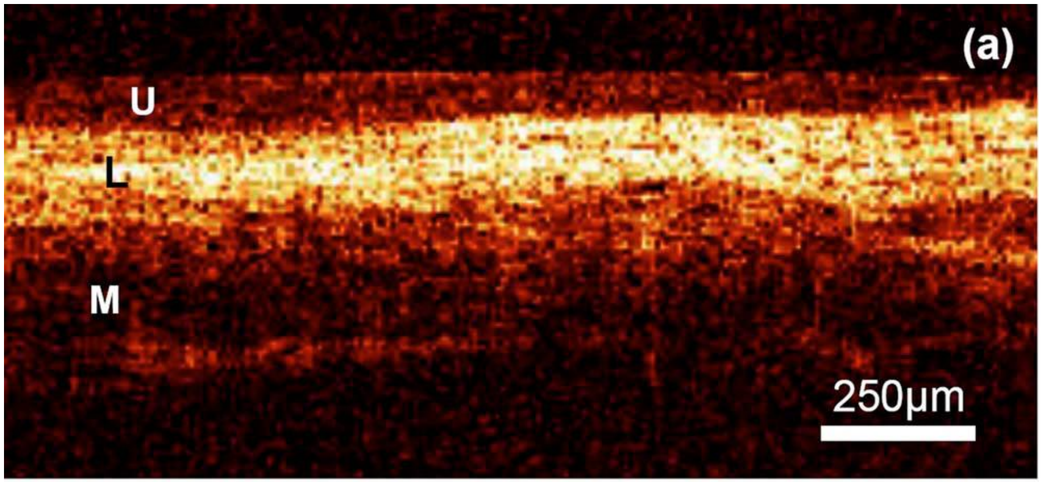
- Guided surgery
  - delicate procedures
  - brain surgery,
    - knee surgery,
    - …

OCT data volume showing the morphology of a human finger pad

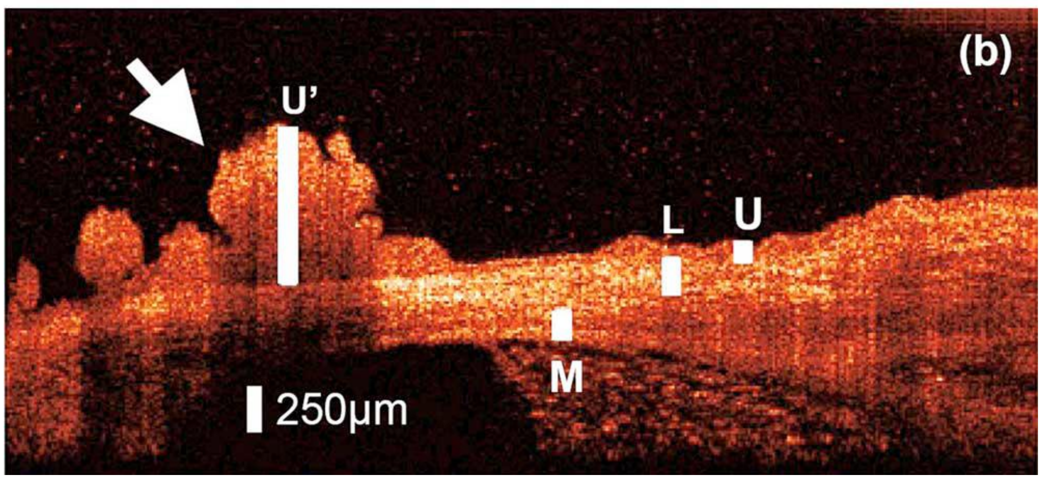
- Scanning depth is 2 mm.



OCT examples



High magnification OCT image of benign human bladder taken with a clinical system during cystoscopy. Three distinct layers are visible: urothelium U, lamina propria L, and muscularis propria M. A sharp border is visible between the urothelium and the lamina propria.



Low magnification OCT image of a neoplastic rat bladder taken with aresearch system. The diseased urothelium U', arrow is clearly visible.

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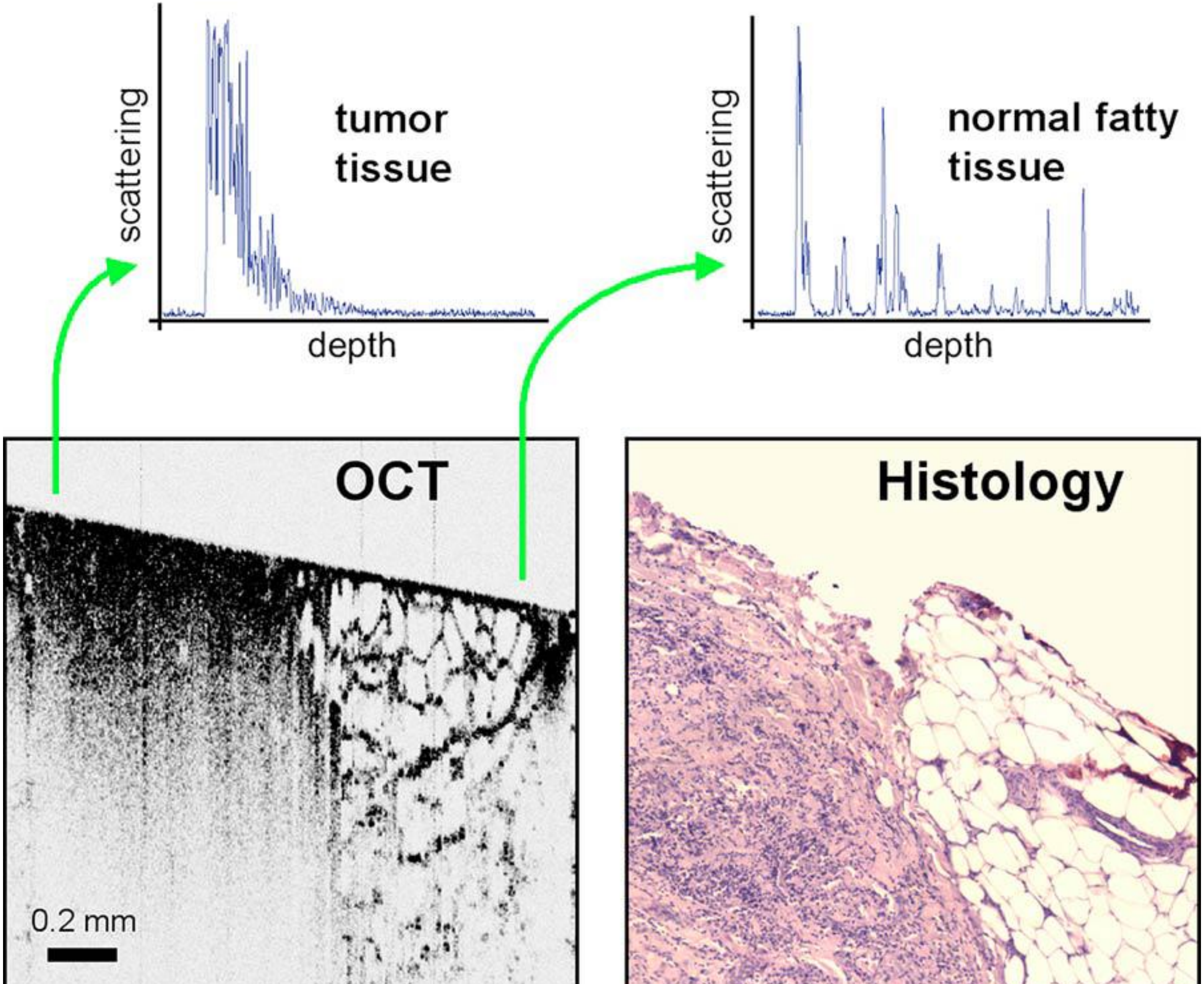
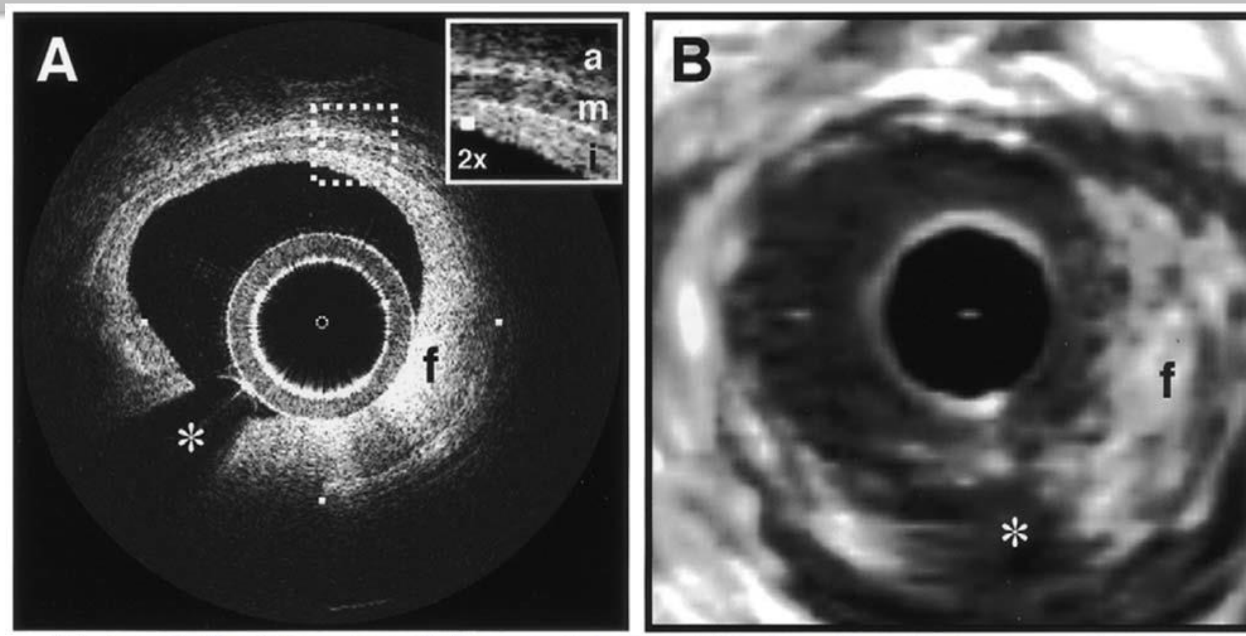
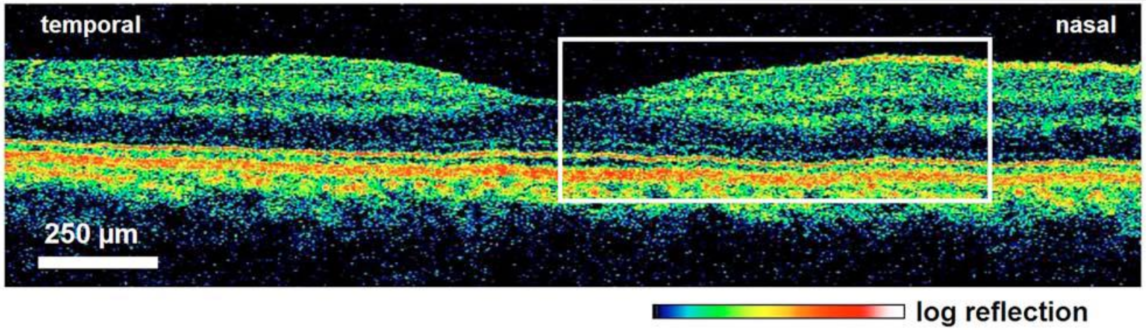
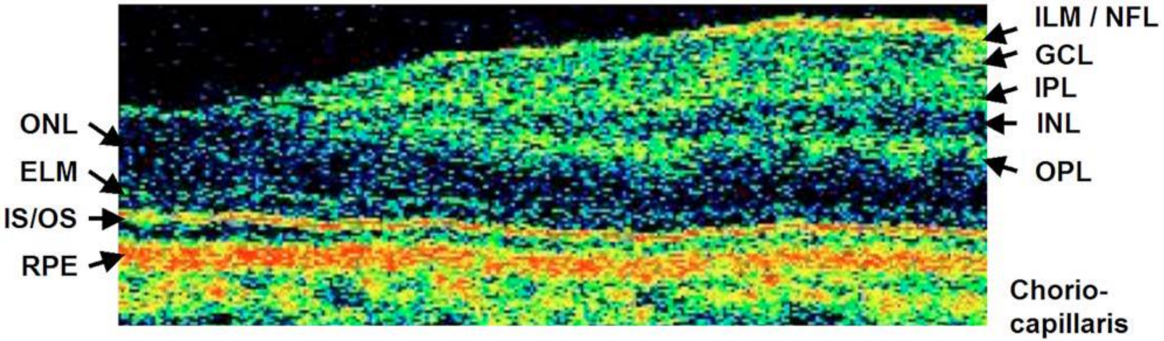
OCT imaging of the human breast



- Intravascular imaging of a fibrous coronary plaque showing: *i*—intimia with intimial hyperplasia, *m*—media, and *a*—adventitia.
- The left figure is an intravascular OCT image showing a clear delineation of layers, including the internal *.







Clinically adapted systems