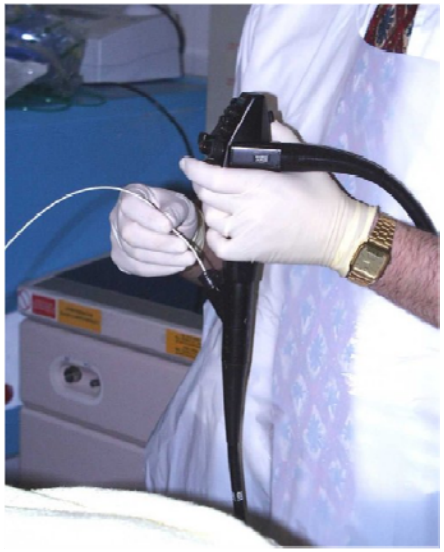
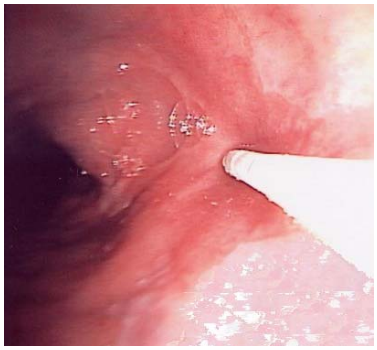
Light Scattering Spectroscopy

Introduction

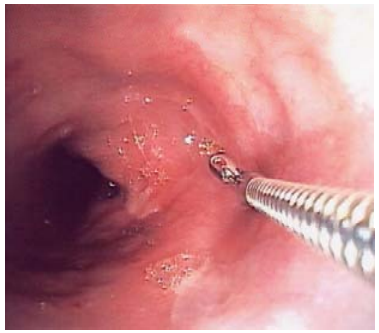
LSS – How did it start?

- **What is the problem?**
  - Gastroenterologist: “Problem: need real-time in situ cancer detection”
  - Want compatibility with endoscopes
  - Prefer non-invasive technique (optical detection of cancer with “magic” laser?)
  - Must be affordable, user friendly
- **Optical Biopsy:**
  - Noninvasive Detection of Cancer with light





Optical “biopsy”

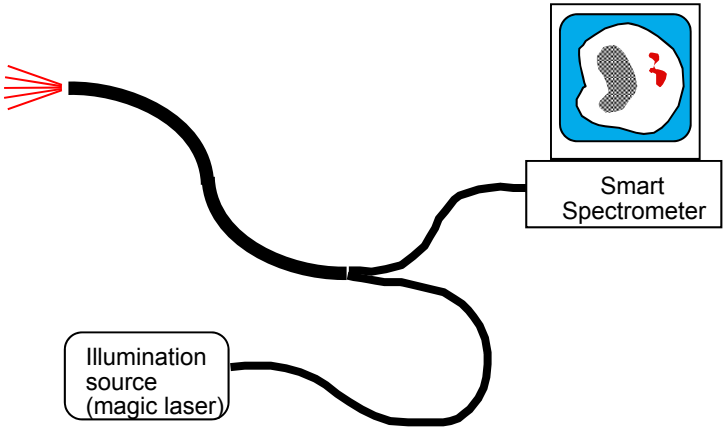


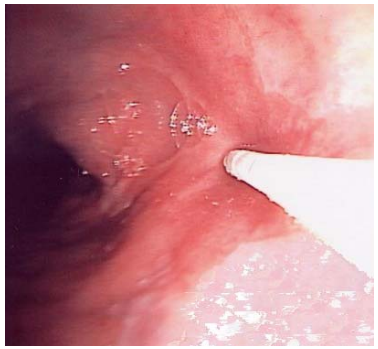
Surgical biopsy

Introduction

- **Optical Biopsy**
  - Noninvasive → Optical detection
  - Endoscopic compatibility → Fiber-optic mediation
  - Diagnosis → Optical spectroscopy
- **Various spectroscopies can be used for optical tissue diagnostics**
  - Auto-fluorescence
  - Exogenous-drug fluorescence
  - Raman
  - Absorption & FTIR
  - Elastic scattering

The internist's dream: smart colonoscope



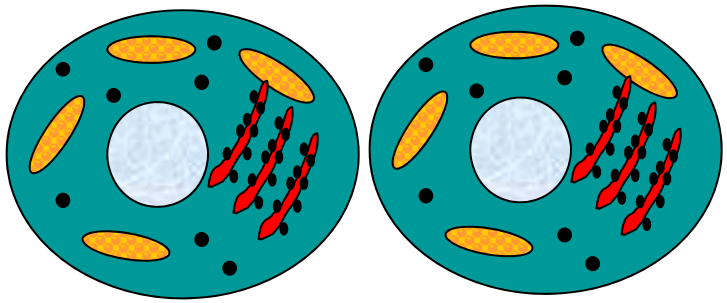


Optical “biopsy”

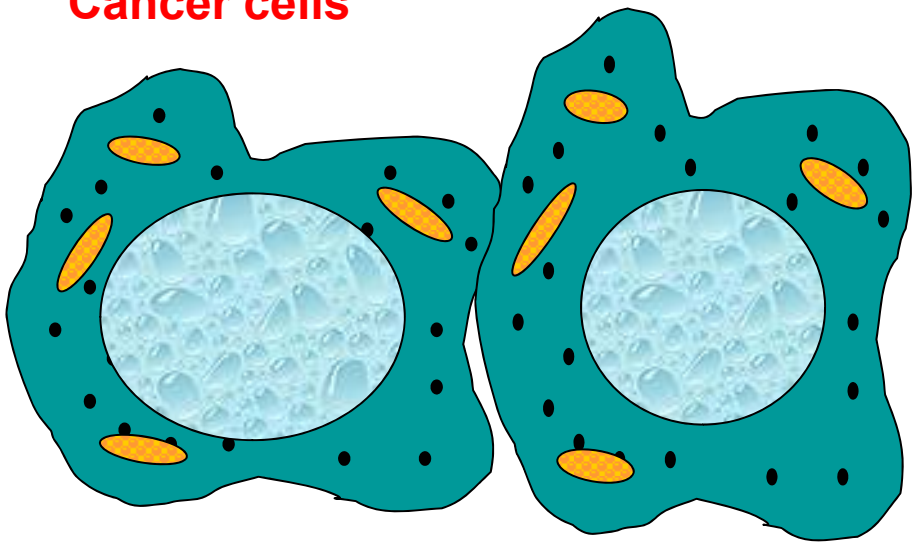
But what does a pathologist look for?

- **But what does a pathologist look for?**
  - Architectural changes
  - Cancer Characteristics
    - Shape of cell
    - Shape of nucleus
    - Ratio of nucleus to total cell volume
    - Chromatin distribution
    - Structure of organelles
    - PLEOMORPHISM (variations in nuclear size and DNA density)
    - Cell density and distribution
- **This information is available from elastic optical-transport data**
  - Elastic scattering
  - Reflection / transmission
  - Absorption
- **Elastic Scattering**
  - Spectral and angular patterns of light scattering depend on the size and structure of the scattering particle

Normal cells

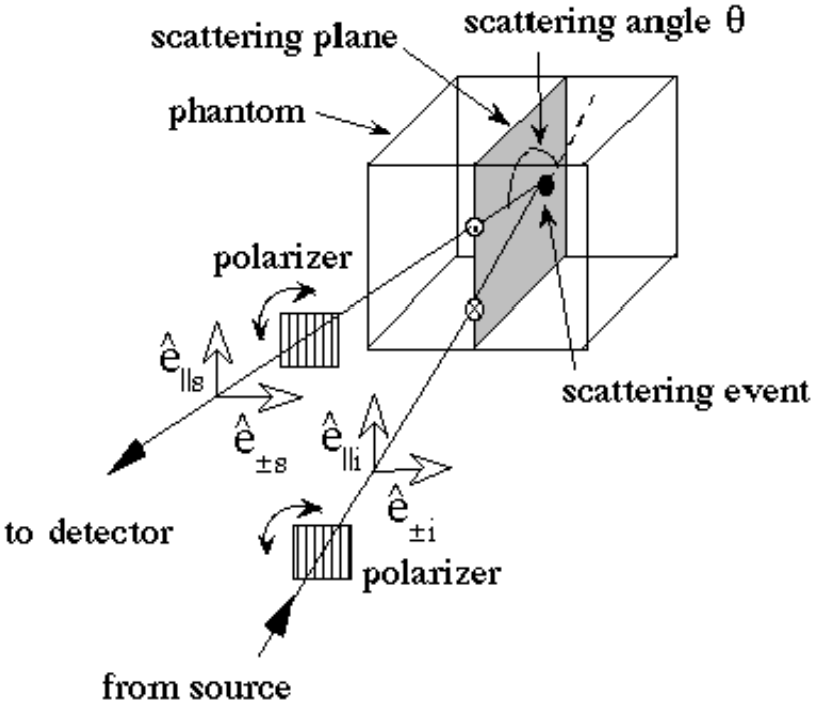


Cancer cells



Mie Theory

- **Gustav Mie, 1908**
- **Particles comparable or larger than the wavelength**
- **Why does wavelength dependence of scattering change with variations in microscopic tissue morphology?**



Steven Jacques, Oregon Graduate Center

$$\begin{bmatrix} E_{\|s} \\ E_{\perp s} \end{bmatrix} = \frac{e^{-jk(r-z)}}{-jkr} \begin{bmatrix} S_2 & S_3 \\ S_4 & S_1 \end{bmatrix} \begin{bmatrix} E_{\|i} \\ E_{\perp i} \end{bmatrix}$$

At some distance away from the particle:

$$\begin{bmatrix} I_{\|s} \\ I_{\perp s} \end{bmatrix} = \text{constant} \begin{bmatrix} |S_2|^2 & 0 \\ 0 & |S_1|^2 \end{bmatrix} \begin{bmatrix} I_{\|i} \\ I_{\perp i} \end{bmatrix}$$

$$S_1 = \sum_q \frac{2q+1}{q(q+1)} (a_q \pi_q + b_q \tau_q)$$

$$S_2 = \sum_q \frac{2q+1}{q(q+1)} (a_q \tau_q + b_q \pi_q)$$

a_q and b_q are complex expressions invoking spherical Bessel functions

π_q and τ_q are defined as

$$\pi_n = \frac{P_n^1}{\sin\theta} \quad \text{and} \quad \tau_n = \frac{dP_n^1}{d\theta}$$

where P_n^1 is the Legendre polynomial

Mie Theory

- **If you really love the math …**

$$a_n = \frac{\mu m^2 j_n(mx)[xj_n(x)]' - \mu_1 j_n(x)[mxj_n(mx)]'}{\mu m^2 j_n(mx)[xh_n^{(1)}(x)]' - \mu_1 h_n^{(1)}(x)[mxj_n(mx)]'},$$

$$b_n = \frac{\mu_1 j_n(mx)[xj_n(x)]' - \mu j_n(x)[mxj_n(mx)]'}{\mu_1 j_n(mx)[xh_n^{(1)}(x)]' - \mu h_n^{(1)}(x)[mxj_n(mx)]'},$$

j_n = spherical Bessel functions

$h_n^{(1)}$ = spherical Hankel functions

$x = \alpha = 2\pi n_o a/\lambda$ the "size parameter"

$m = N_i/N_o = n_i/n_o$ if no absorp.

[F(x)]' means differentiation with respect to the argument

Bohren & Huffman, Absorption and Scattering If Light by Small Particles, Wiley Science

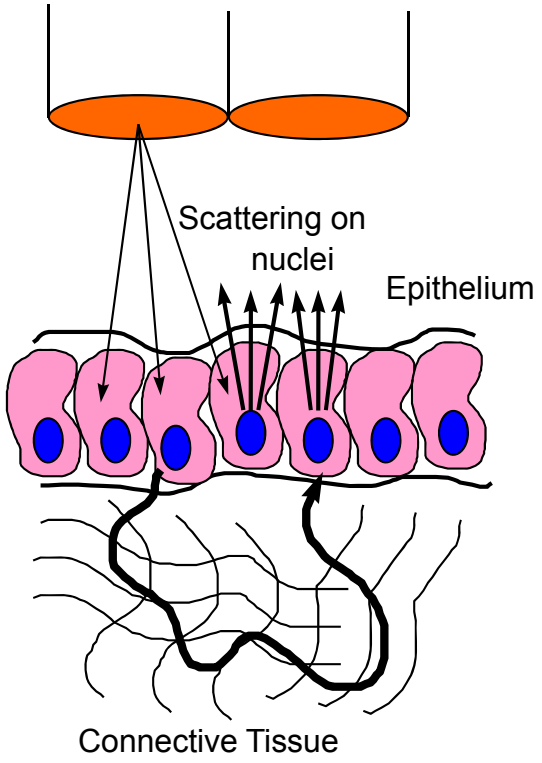
Light Scattering Spectroscopy

- **Wavelength Dependence**
  - The scattering cross section of the nuclei exhibits a periodicity with wavelength

$$\sigma_s(\lambda, l) = \frac{\pi}{2} l^2 \left(1 - \frac{\sin(2\delta/\lambda)}{\delta/\lambda} + \left(\frac{\sin(\delta/\lambda)}{\delta/\lambda} \right)^2 \right)$$

$$\delta = \pi l (n_n - 1) n_c$$

where
n_c is the refractive index of cytoplasm
n is the refractive index of the nuclei relative to that of cytoplasm.



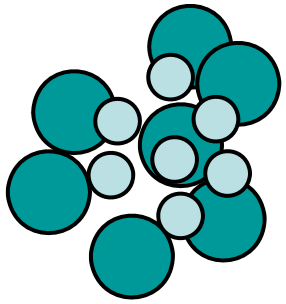
Perelman et al. Phys Rev Let 80: 627-630 (1998); Backman et al. Nature 406: 35-36 (2000)

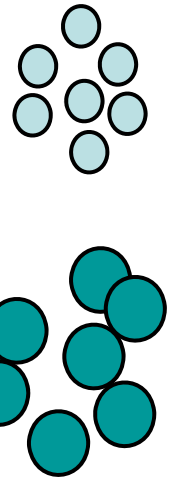
Light Scattering Spectroscopy

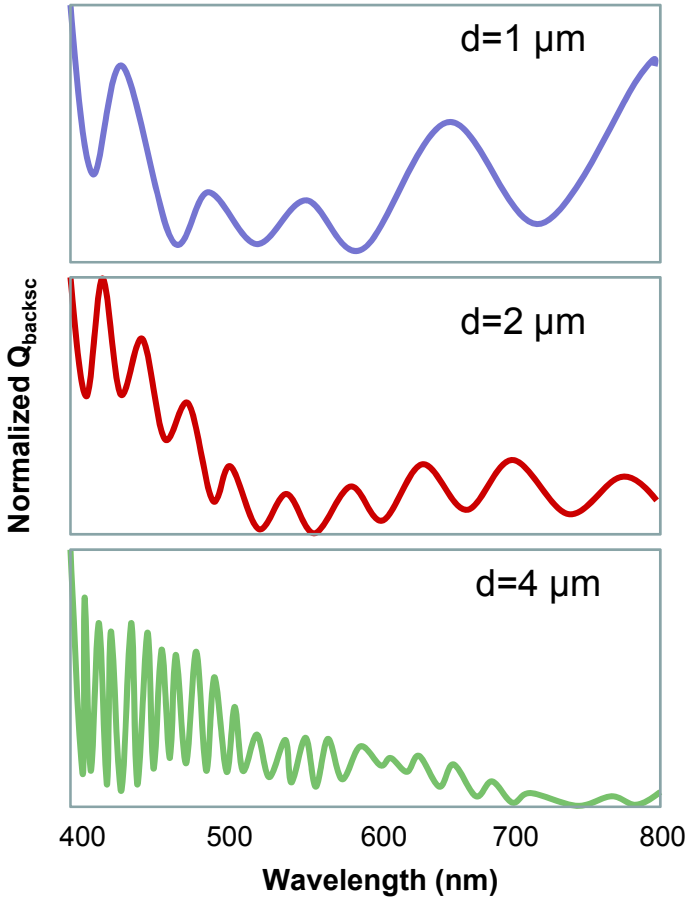
- **Wavelength dependence**
  - With diffuse reflectance, all particle sizes look white

Light Scattering Spectroscopy

- **Wavelength Dependence**
  - As d ↑
    - more rapid oscillations
  - Characteristics
    - spacing of peaks →size of scatterer
    - depth of modulation →number of such scatterers
  - More often → mixture of scatterers
    - Superposition of spectra

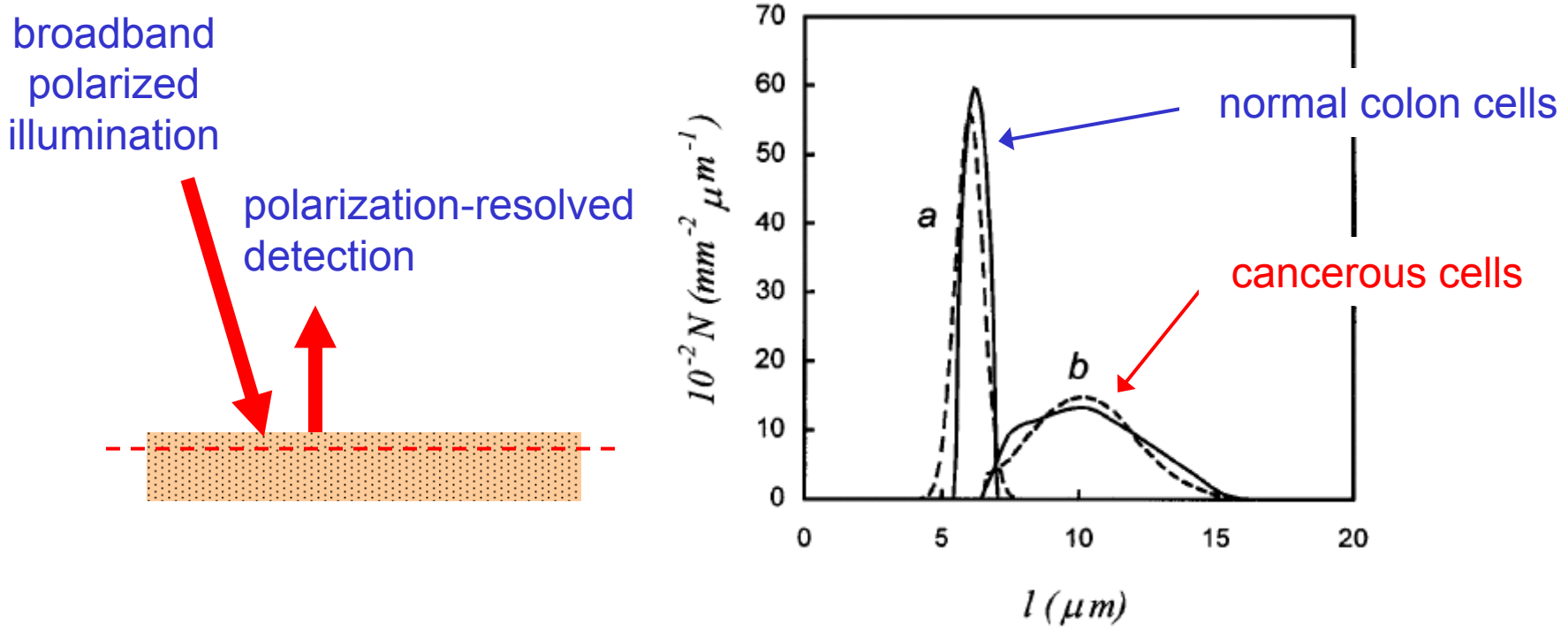






Light Scattering Spectroscopy

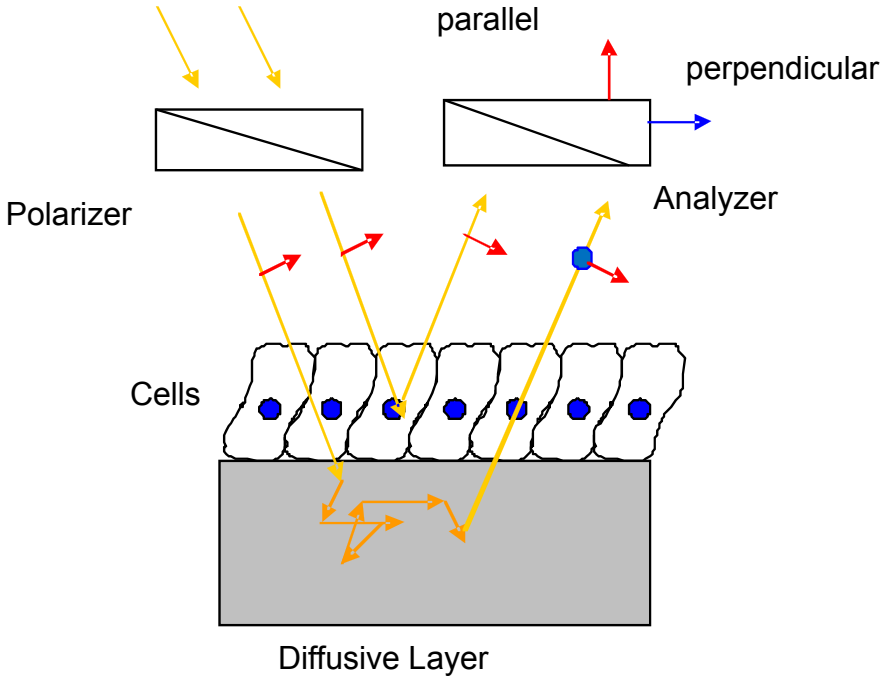
- **Wavelength Dependence**

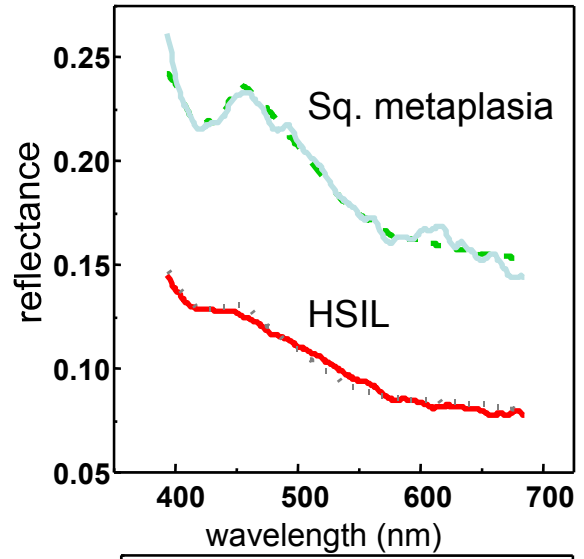


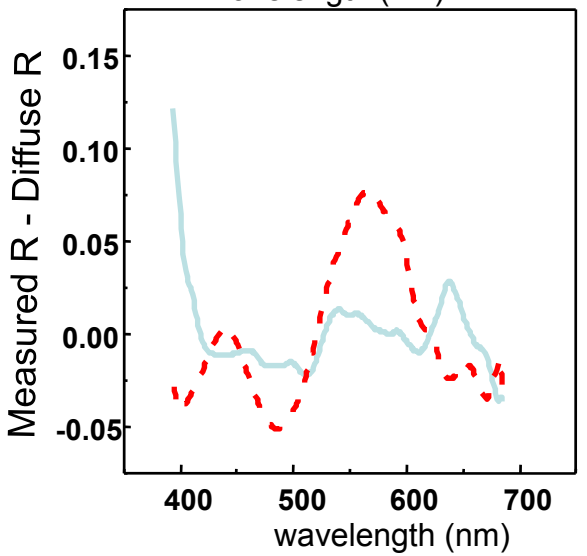
Perelman et al. Phys Rev Let 80: 627-630 (1998); Backman et al. Nature 406: 35-36 (2000)

Light Scattering Spectroscopy

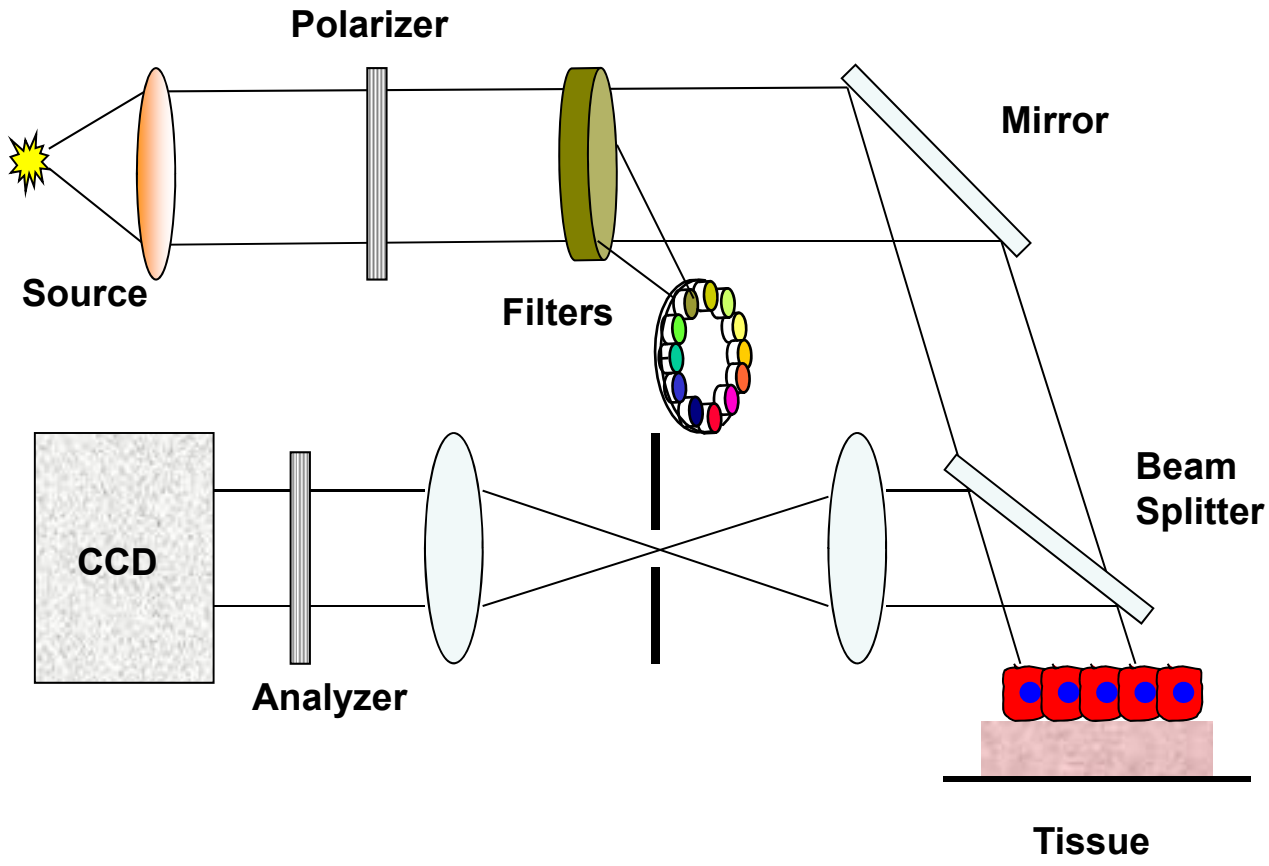
- **Polarization Contrast**
  - Separate single vs. multiple scattering



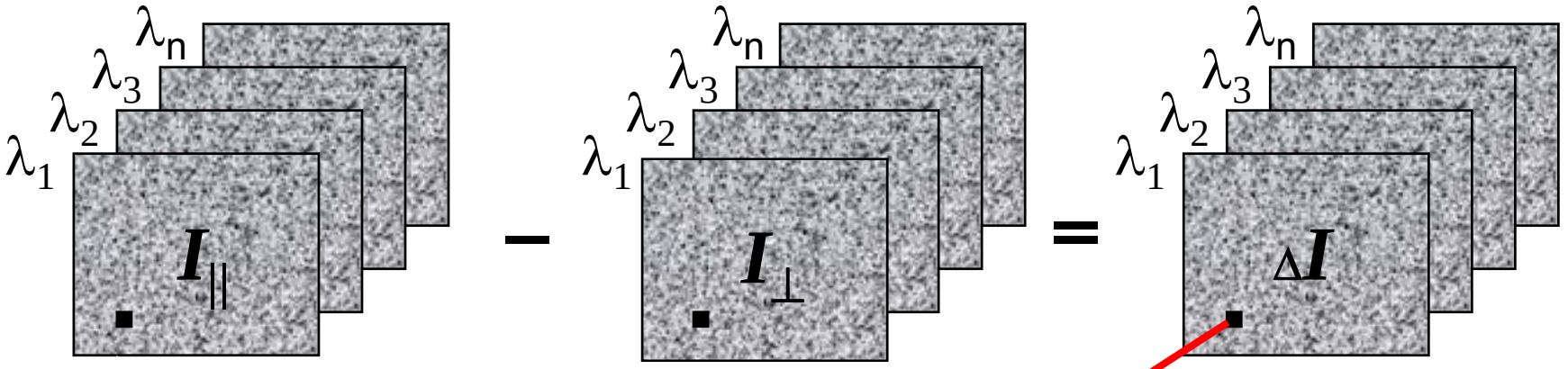




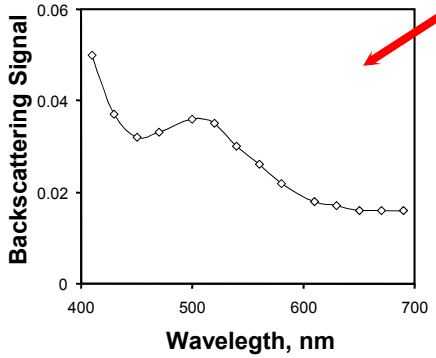
Principles of LSS Imaging



Principles of LSS Imaging



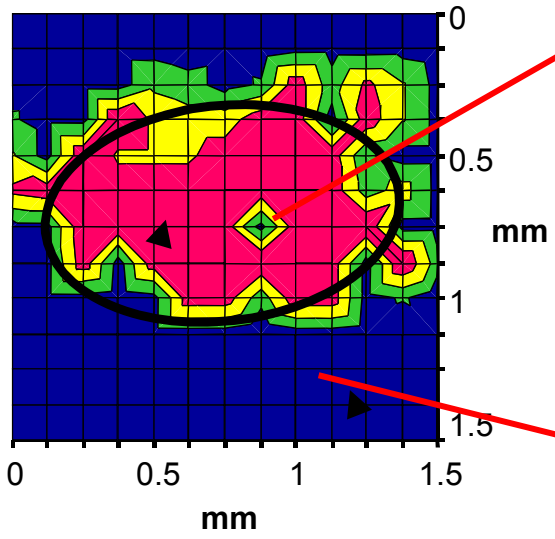
pixel 25 x 25 μm



→ *d*, σ, *n*

Principles of LSS Imaging

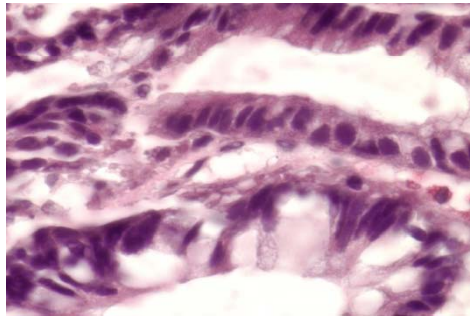
- **LSS Imaging of Colon Adenoma: Nuclear enlargement**



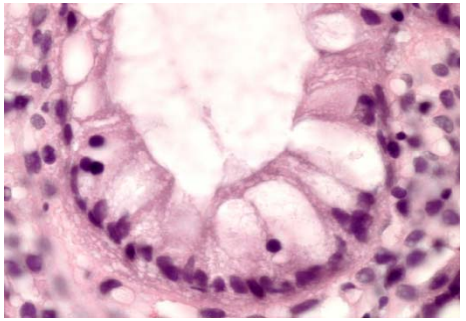
Enlarged Nuclei, %

- 40-50
- 30-40
- 20-30
- 10-20

Adenoma



Normal

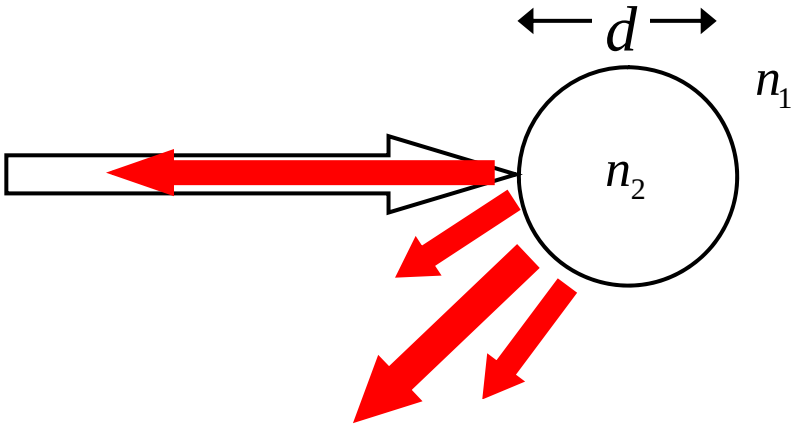


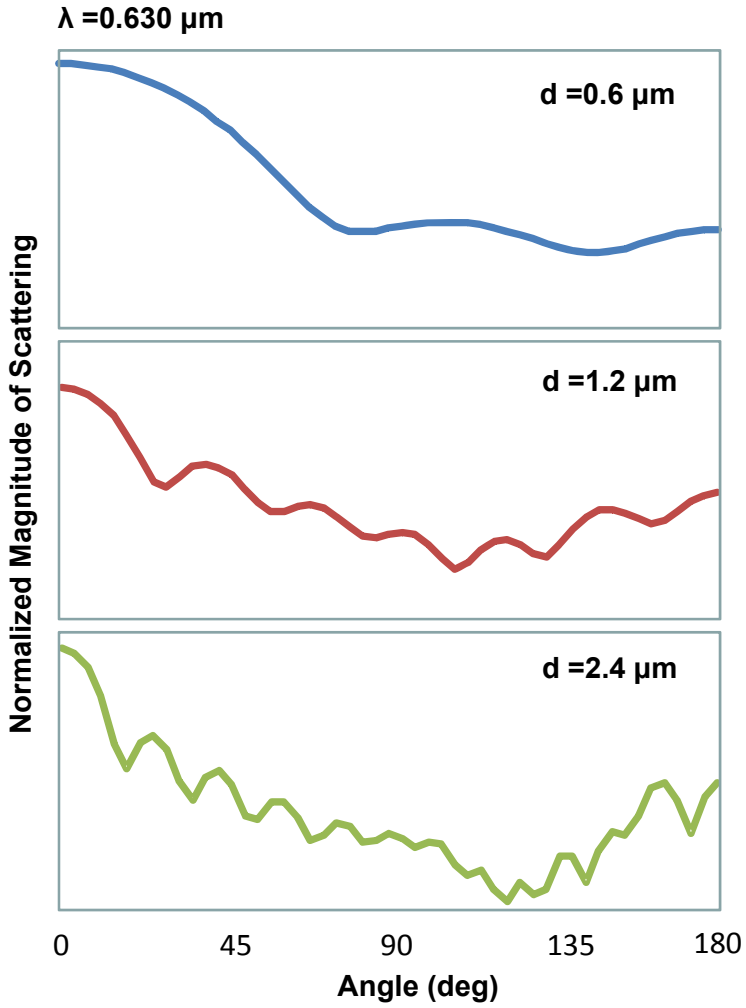
	Morphometry	*LSS*
d, normal	5.6 ± 0.20	5.7 ± 0.13
σ, normal	1.01	0.82
d, adenoma	7.44 ± 0.23	7.67 ± 0.40
σ, adenoma	1.59	2.19

Backman et al. *Nature Medicine* 7: 1245-1248, 2001

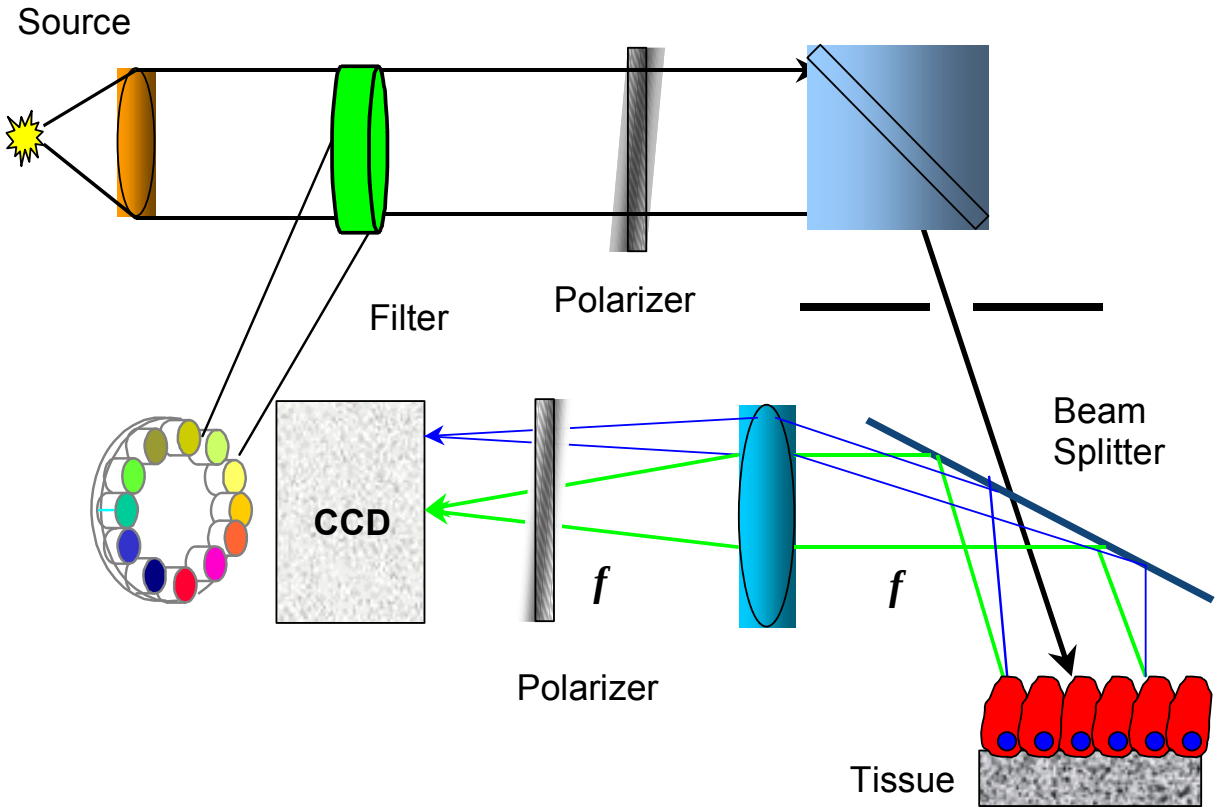
Light Scattering Spectroscopy

- **Angularly-resolved scattering**
  - Angular distribution has interferometric (oscillatory) behavior as well
  - As d ↑
    - more rapid oscillations



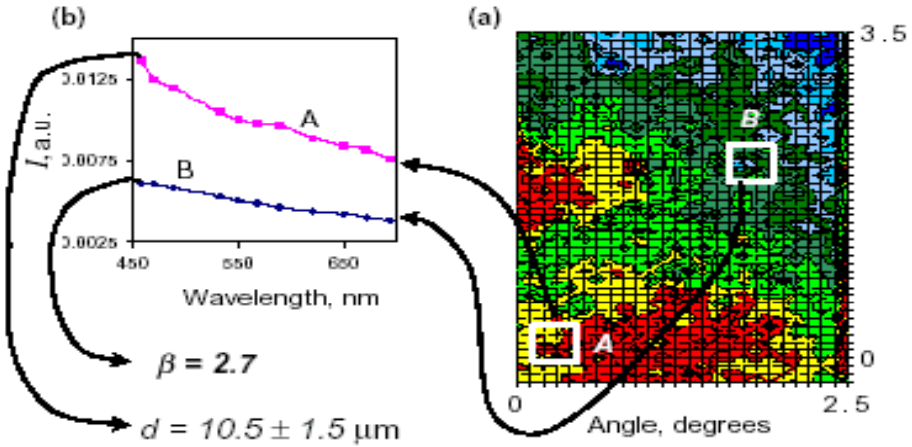


Angular LSS Imaging



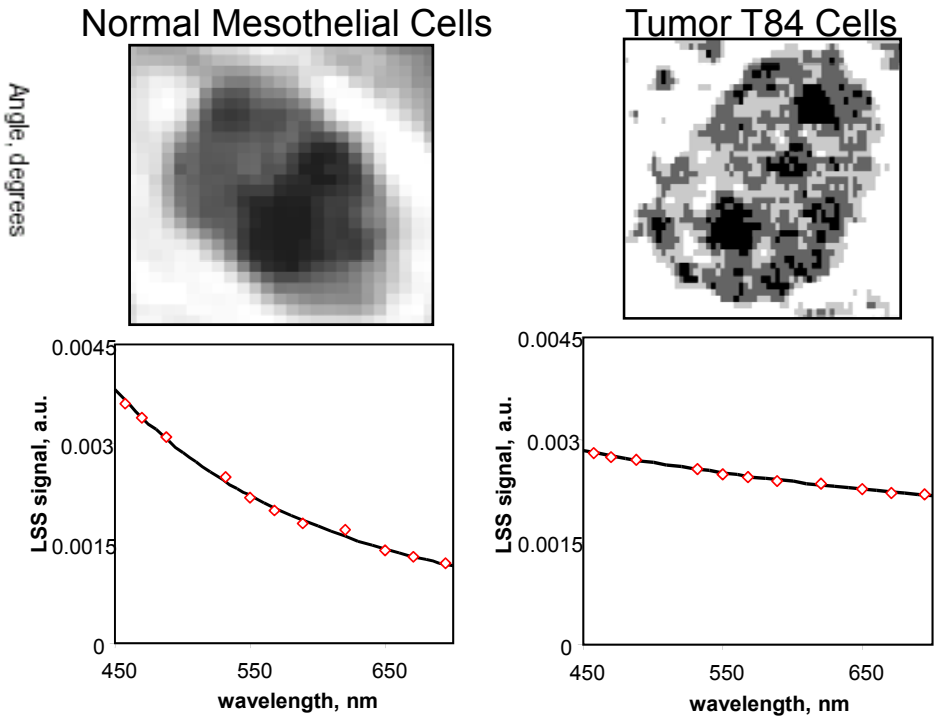
Angular LSS Imaging

- **Angular LSS Studies: Experiment with T84 Cells**



$N(d) \sim d^{-\beta}$, $\beta = 2.7$ vs. 2.2

Cell Nuclei	Morphometry	LSS
Mean diameter, μm	10.4	10.5
Standard deviation, μm	1.35	1.52



Fitting parameter β = 2.7

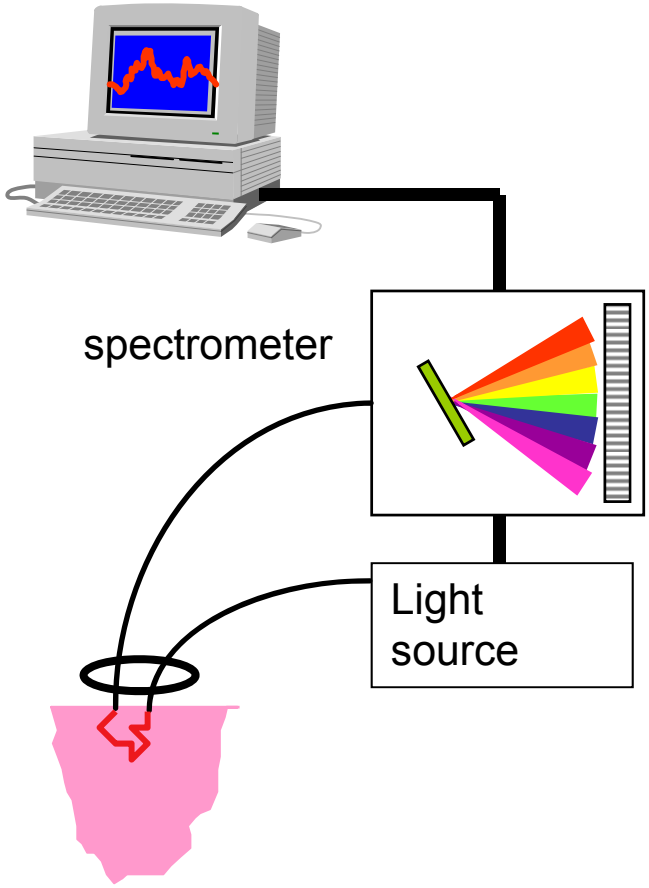
Fitting parameter β = 2.2

Backman et al. IEEE J Sel Top Quant Electron, 7: 887:893, 2001

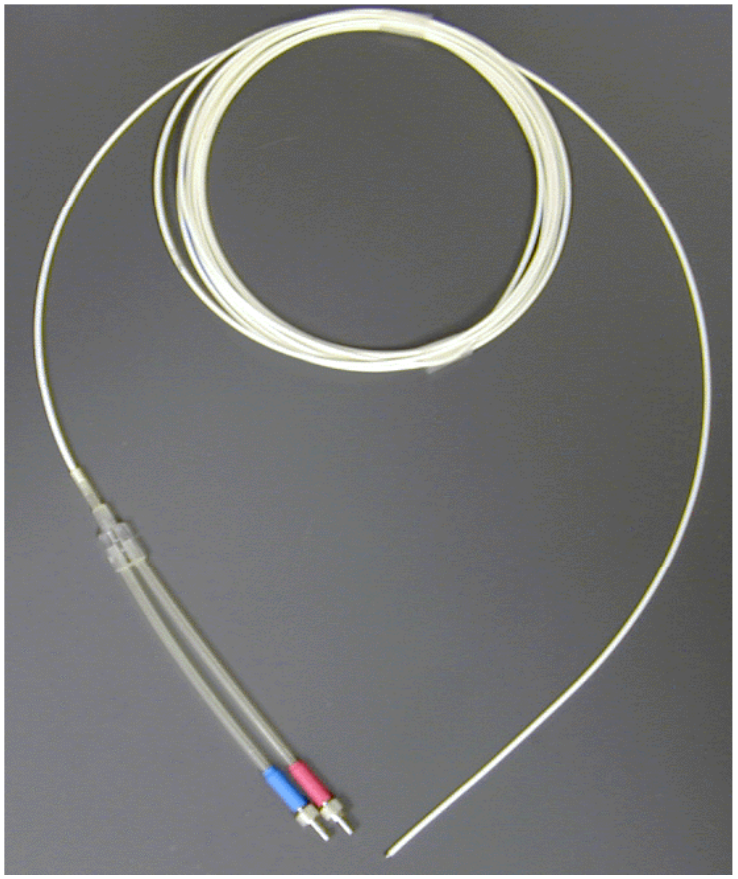
Summary of LSS

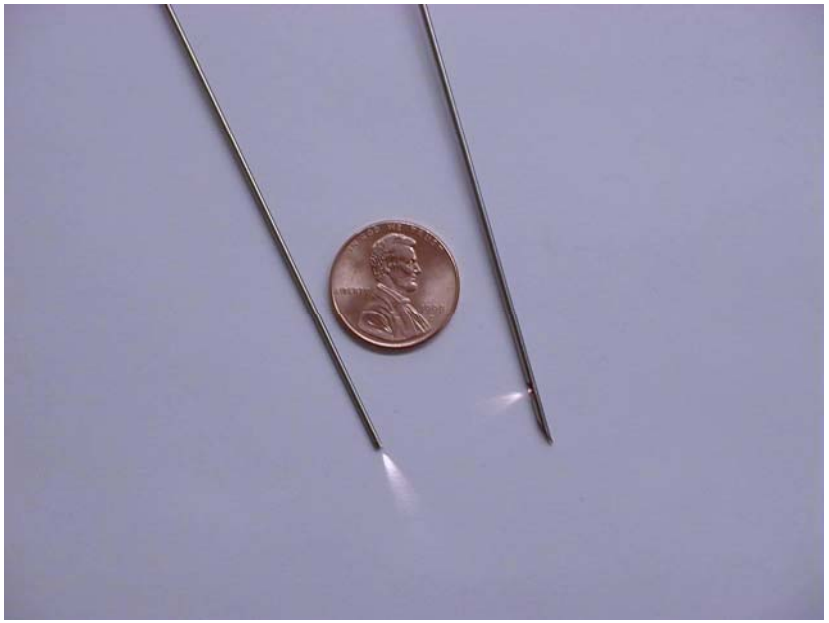
- **LSS contrasts:**
  - Polarization: single vs. multiple scattering
  - Angle: small vs. large particles
  - Spectrum: size and refractive index
- **Advantages:**
  - Strong signal - allows use of lower cost components.
  - Sensitive to important chromophores that are not fluorescent: e.g., hemoglobin.
  - Sensitive to both tissue structure and biochemistry.
    - can distinguish different normal tissues.
- **Disadvantages:**
  - May not be sensitive to subtle biochemical changes preceding structural changes.

Schematic of simple LSS system



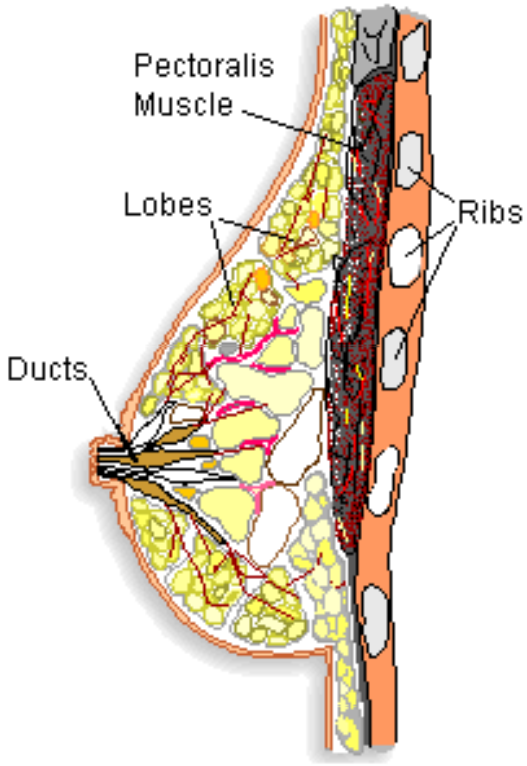
Optical fiber probes





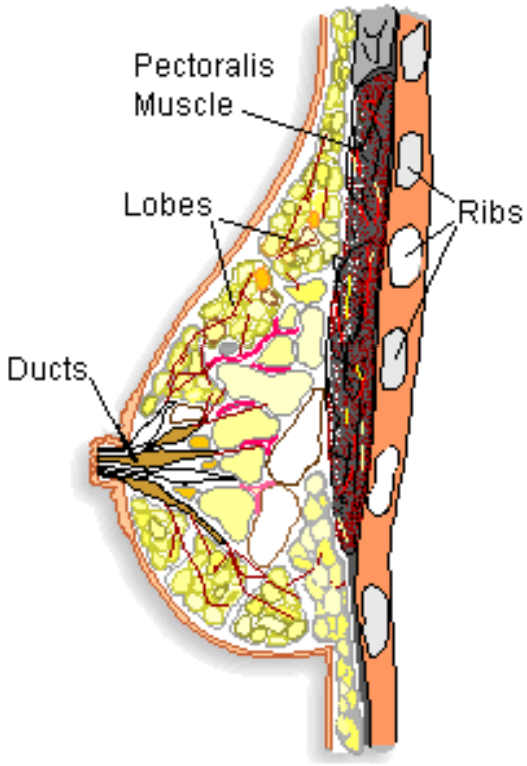
Optical biopsy for breast cancer

- **Application #1:**
  - Improve reliability and capability of transdermal needle diagnosis (increase the volume of sensitivity compared to FNA).
- **Application #2:**
  - Provide a real-time in-situ diagnostic tool for tumor margins during breast-conserving surgery.
- **Application #3:**
  - Provide a real-time in-situ assessment of the “sentinel” lymph node during surgery.



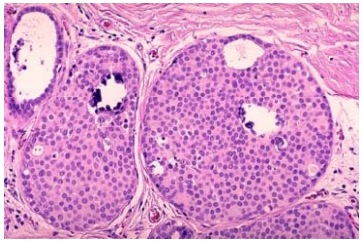
Optical biopsy for breast cancer

- **Breast Anatomy - Architecture Complex structures**
  - Heterogeneous
  - Hormonal control
  - Changes with age
  - Variety of cell types

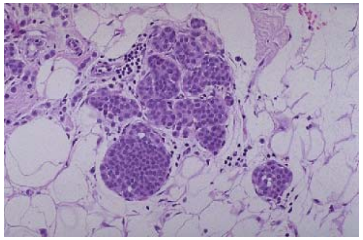


Optical biopsy for breast cancer

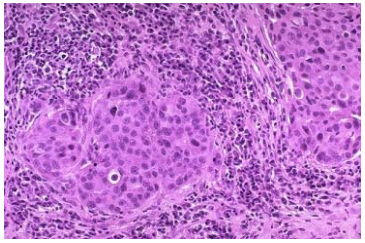
- **Malignancies of the Breast**



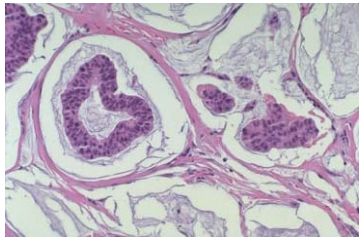
Ductal



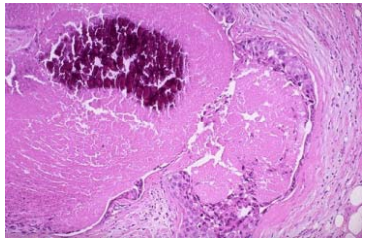
Lobular



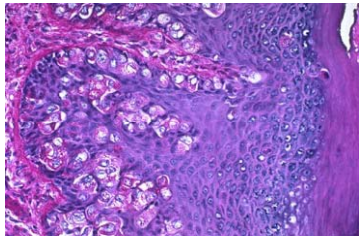
Medullary



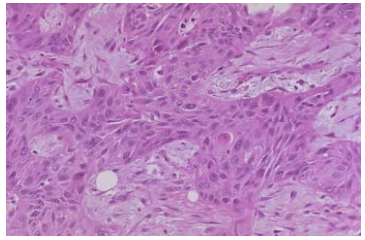
Mucinous



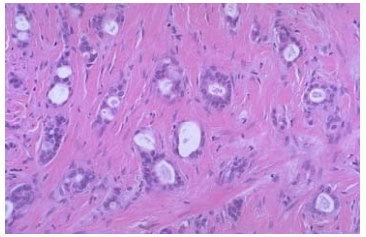
Comedo



Paget's disease



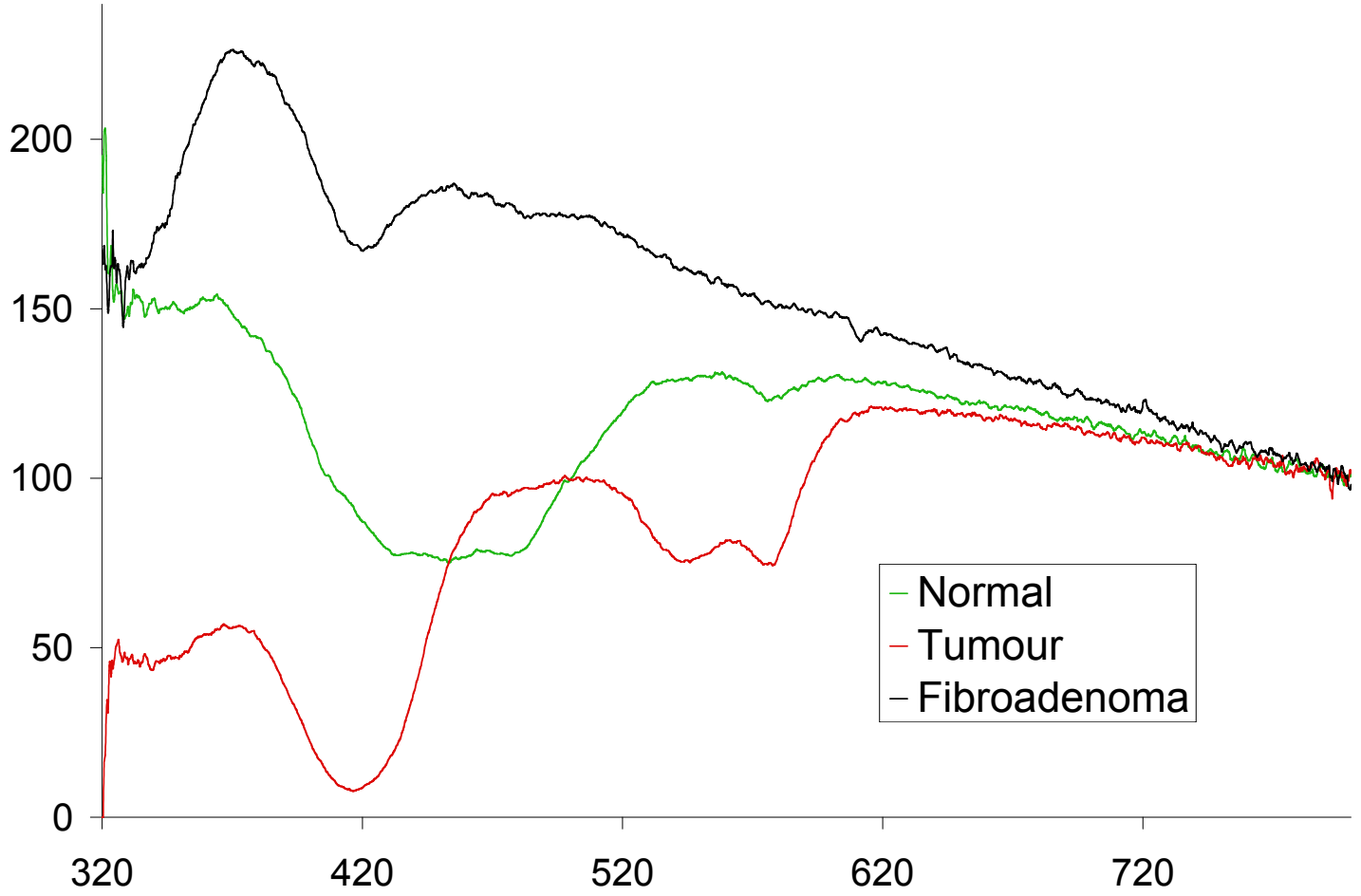
Metaplastic



Tubular

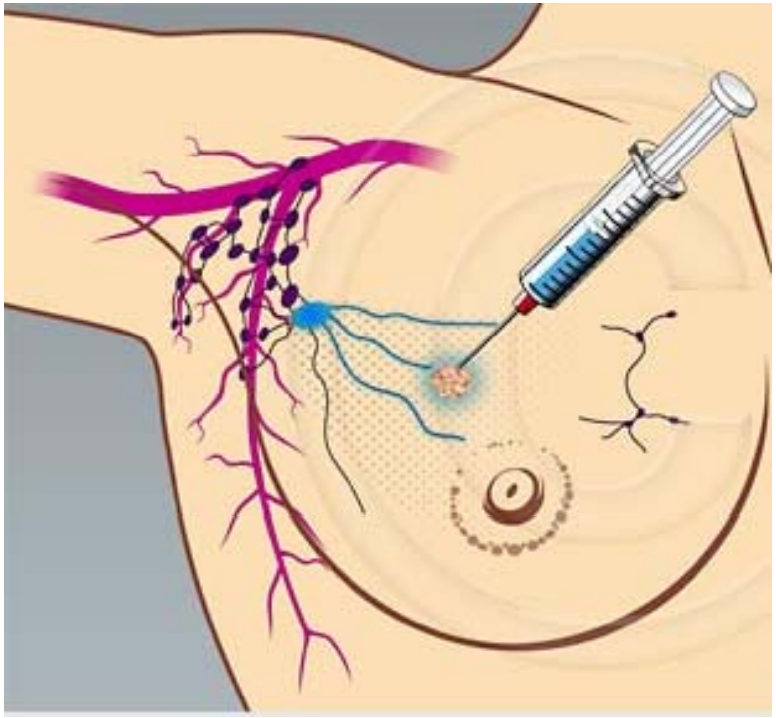
Optical biopsy for breast cancer

- **Breast Tissue Spectra**



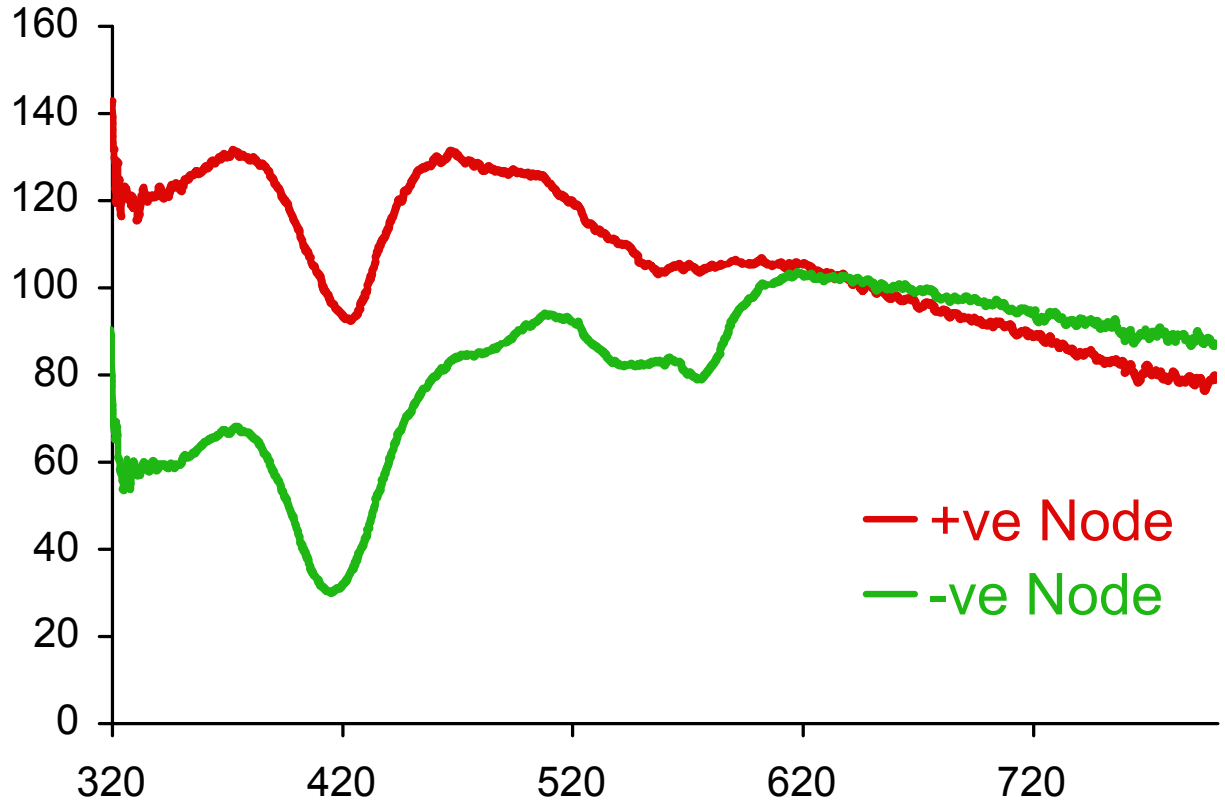
Optical biopsy for breast cancer

- **Sentinel Lymph Node**
  - SLN biopsy aims to determine the necessity of extensive surgery to the armpit
  - Patients requiring further surgery may have to undergo separate surgery
  - Current techniques to examine SLN during operation are impractical



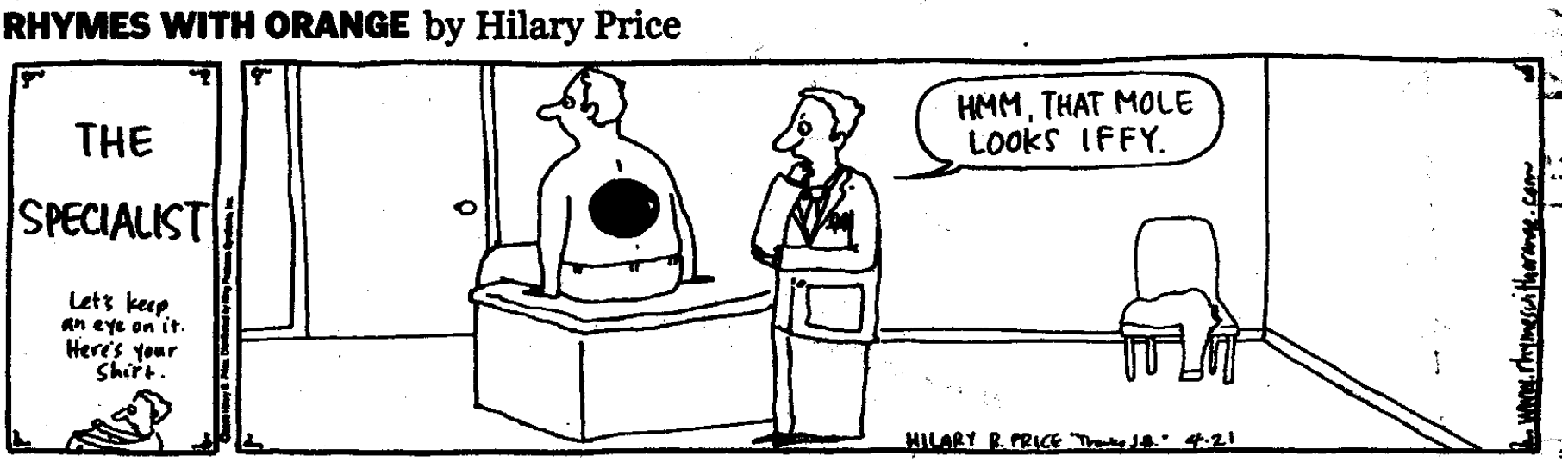
Optical biopsy for breast cancer

- SNL Spectra



Primary Care

- **Primary care physicians have poor accuracy in determining when to refer a patient to the specialist**



An engineer's chicken