A different view of turbidity: Elastic scattering analysis

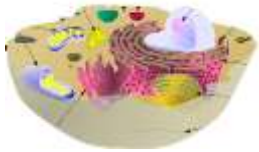
Andrew Berger

Abbe lecture #5

28.01.2014

Road map for today

- Why scattering (as opposed to absorption)?



Scattering you may have already heard about

Fundamentals of elastic scattering

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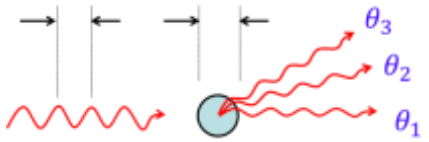
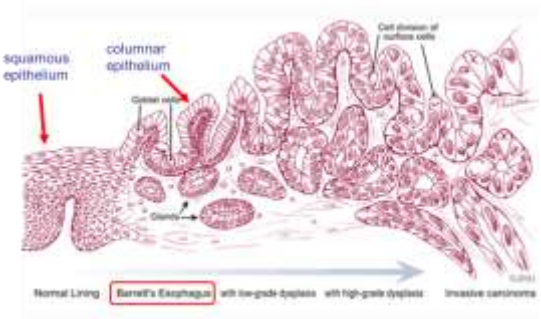


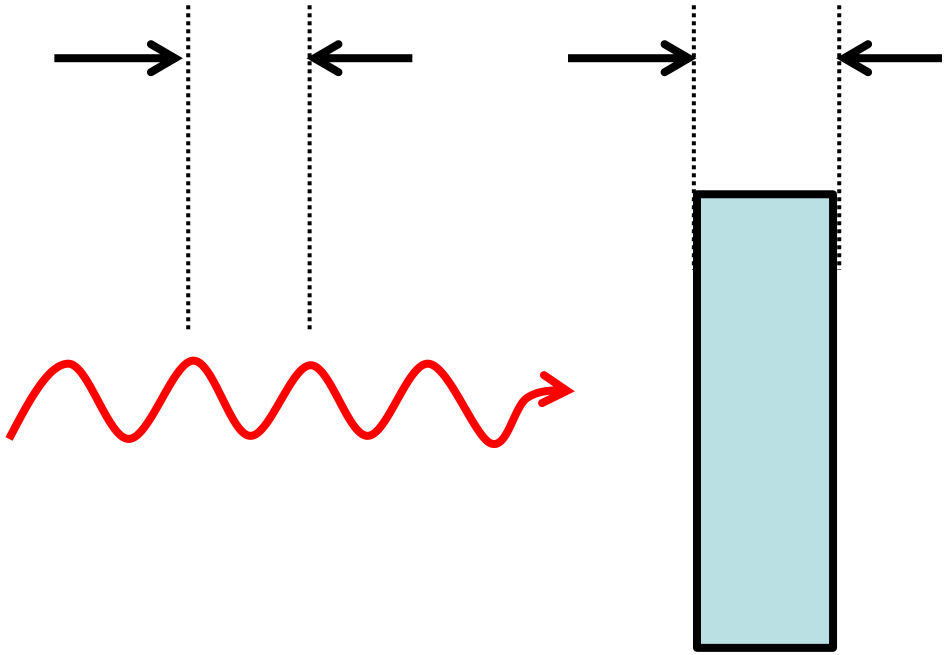
wavelength-resolved
angularly-resolved

Experiments and applications

spectral domain
angular domain

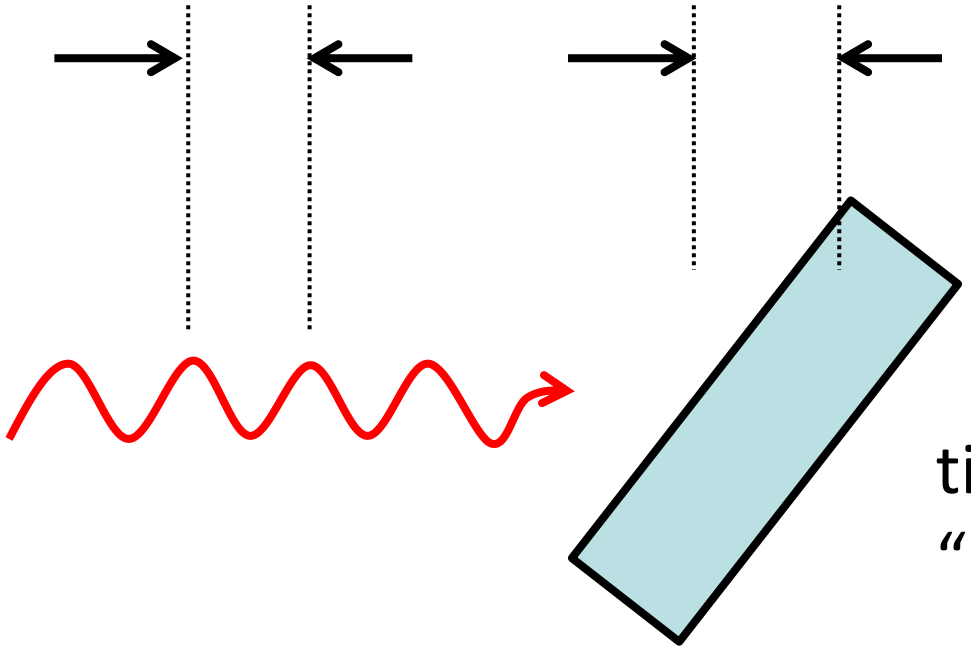


Size-dependent elastic scattering



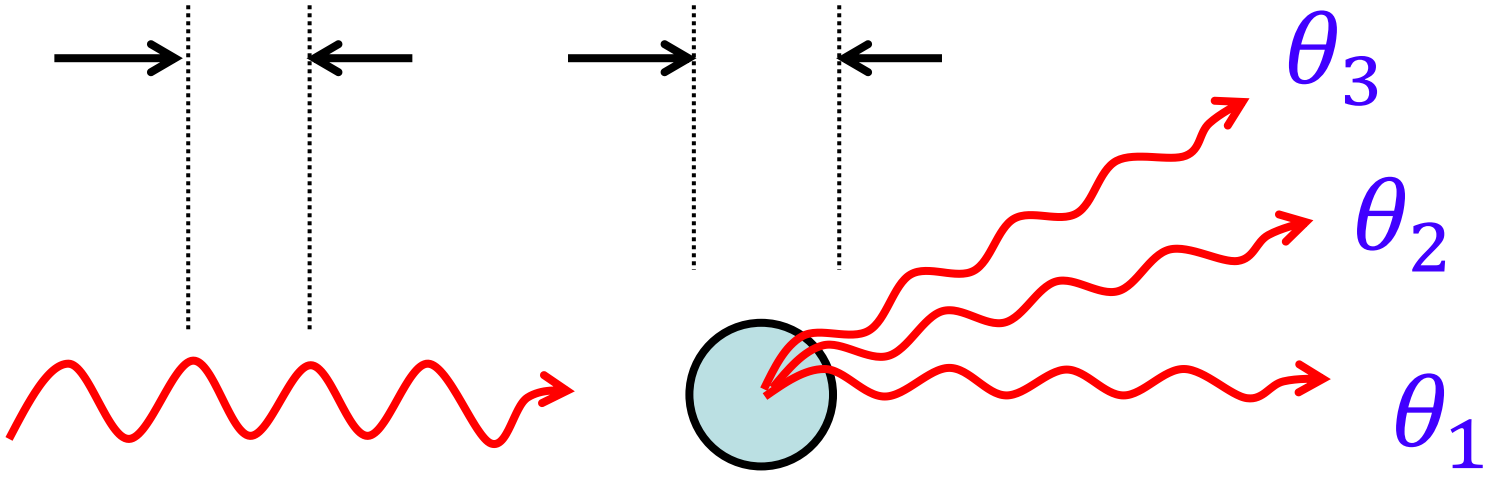
resonant wavelengths "tunnel through"

Size-dependent elastic scattering



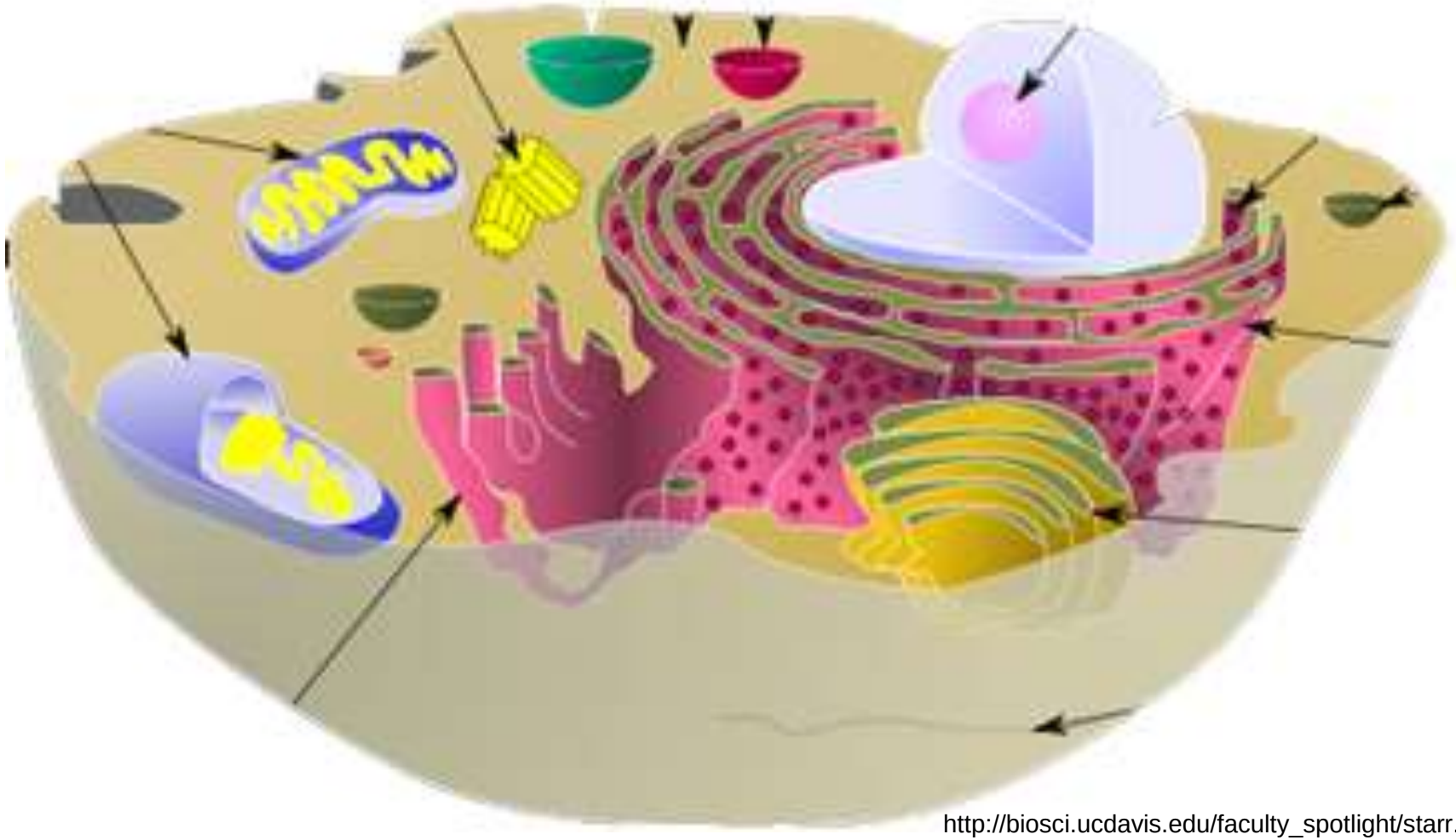
tilting changes the
“resonant wavelength”

Size-dependent elastic scattering



sphere: angle-dependent resonance

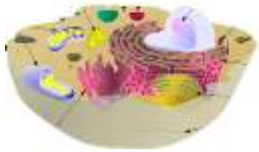
Sensitivity to organelle size



http://biosci.ucdavis.edu/faculty_spotlight/starr.html

Road map for today

Why scattering (as opposed to absorption)?



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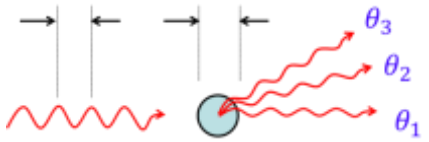

wavelength-resolved
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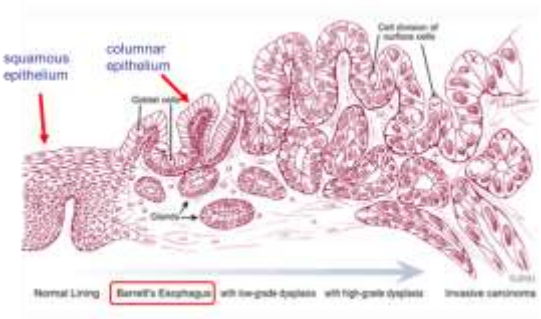
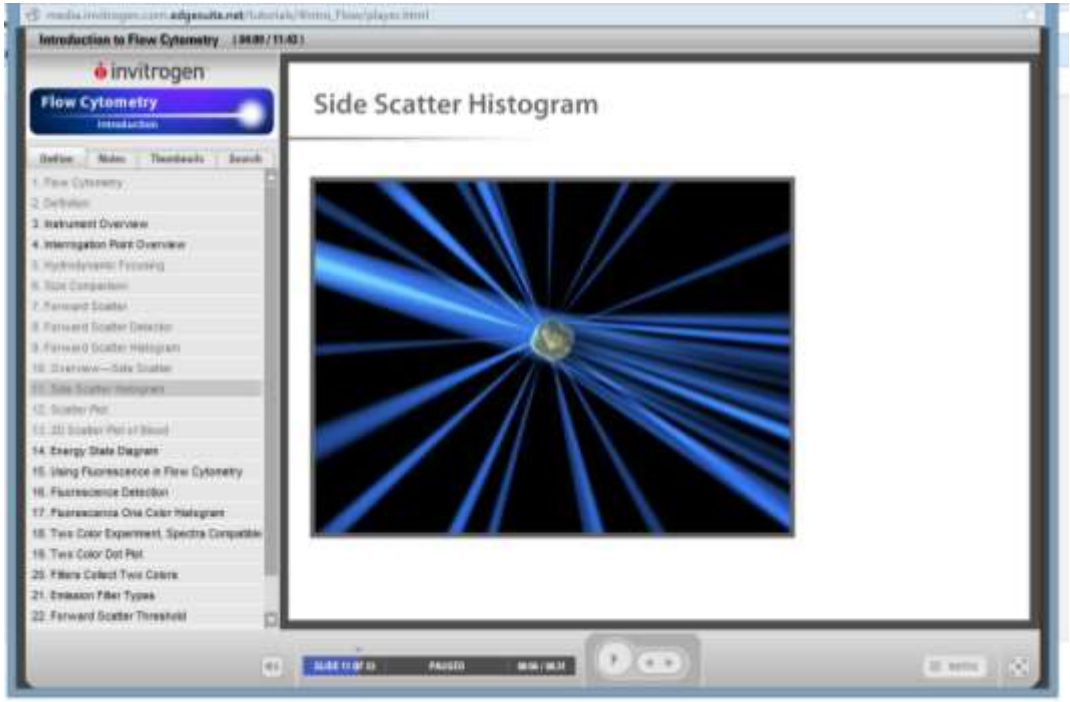


Flow cytometry

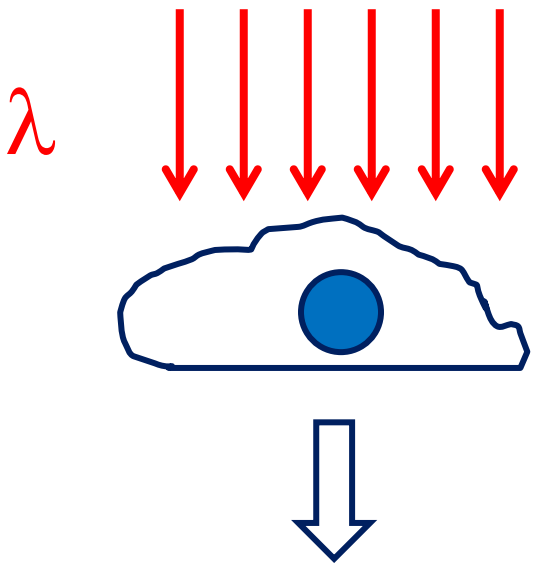
Tutorial on forward and side scatter for flow cytometry:

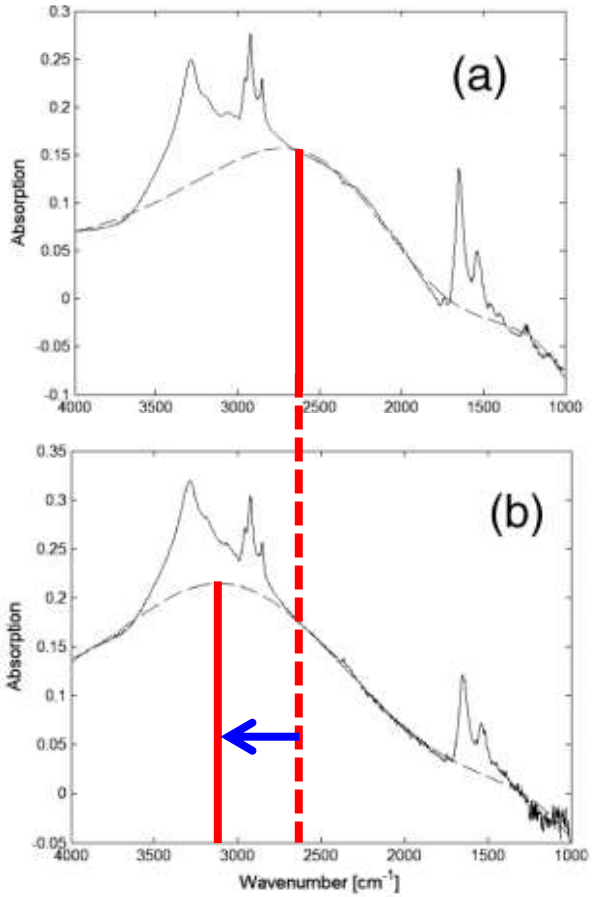


http://media.invitrogen.com.edgesuite.net/tutorials/4Intro_Flow/player.html

Comment:
Larger cells are typically more strongly forward-peaked, in addition to scattering more overall.

Mie theory in single-cell FT-IR spectroscopy



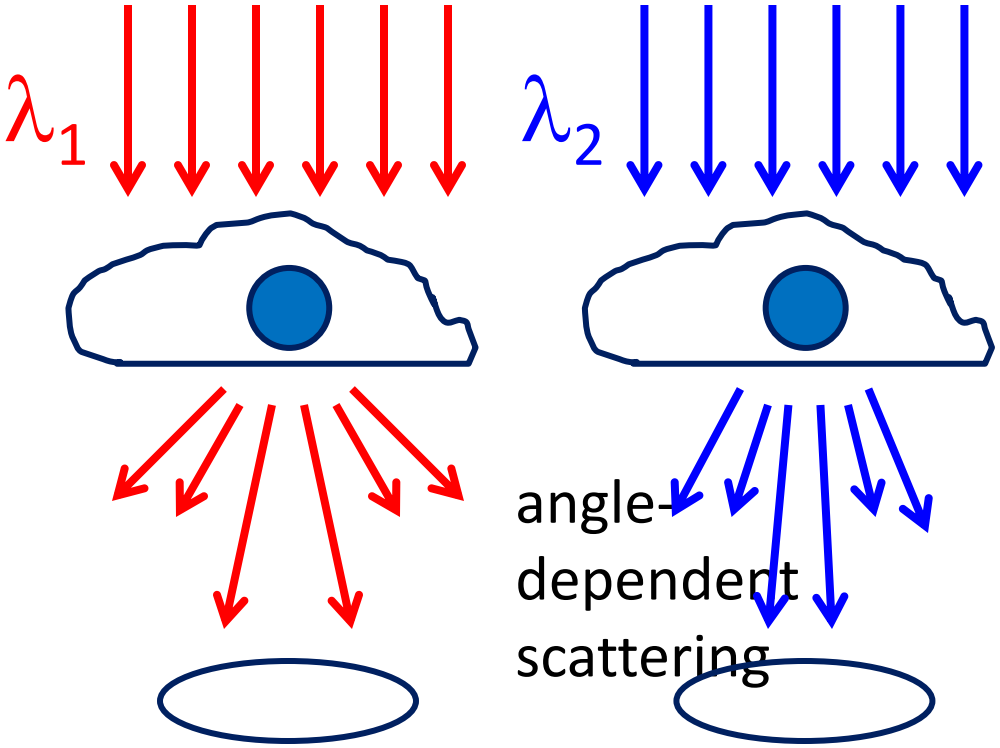


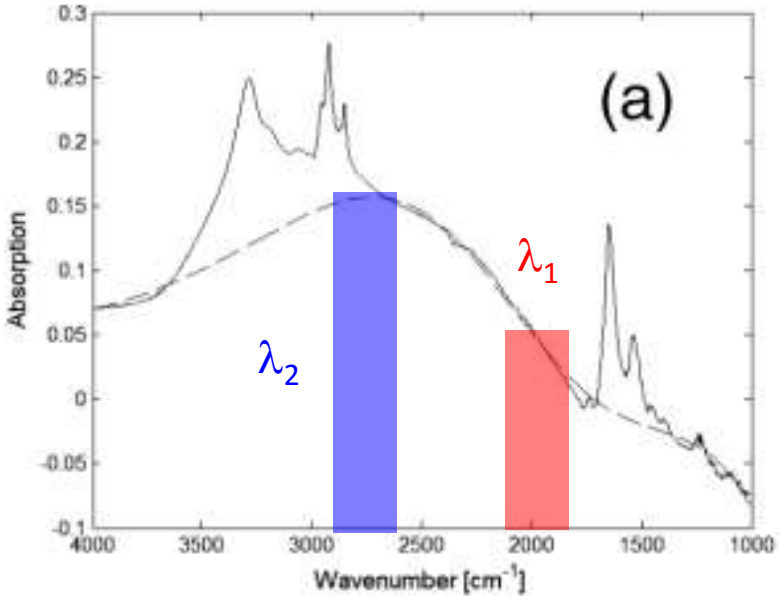
cell #1

cell #2

A. Kohler et al., "Estimating and Correcting Mie Scattering in Synchrotron-Based Microscopic Fourier Transform Infrared Spectra by Extended Multiplicative Signal Correction," Applied Spectroscopy **62**(3), 259-266 (2008)

Mie theory in single-cell FT-IR spectroscopy





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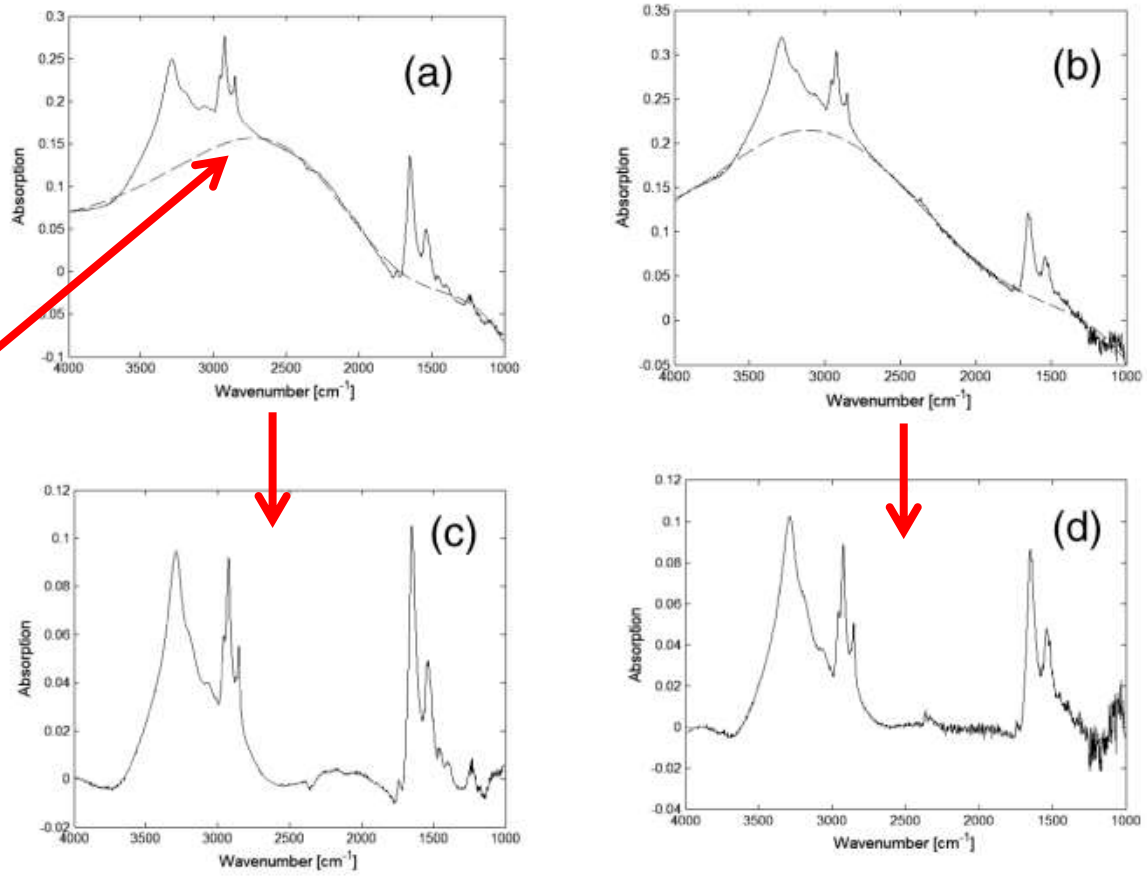
Mie theory in single-cell FT-IR spectroscopy

model using Mie theory!



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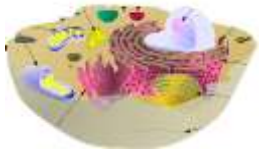

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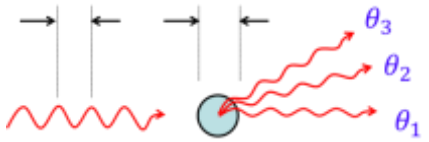
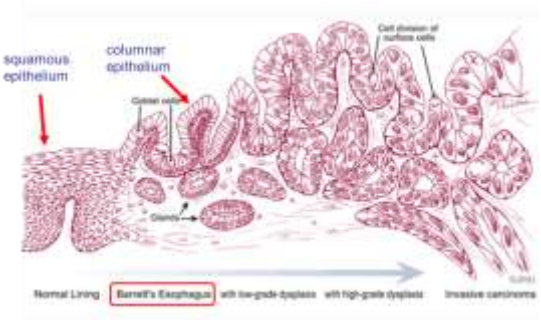


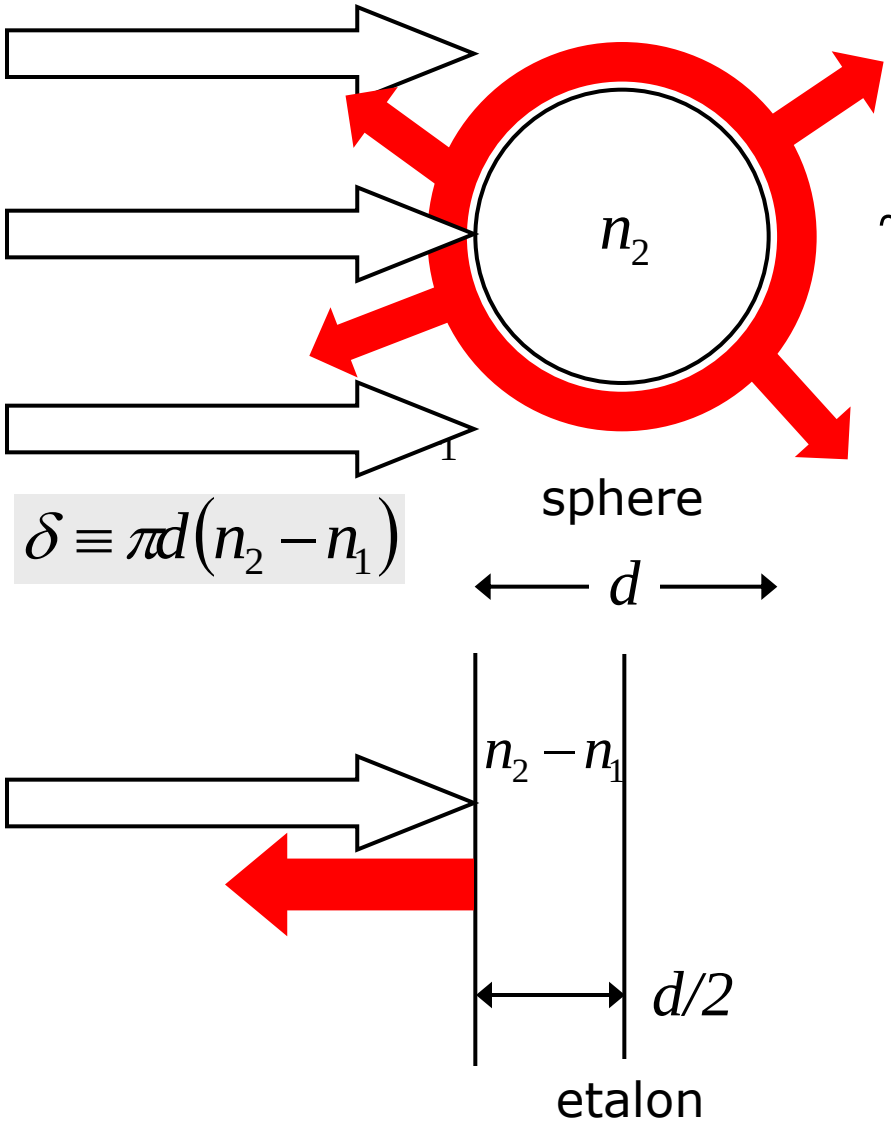
wavelength-resolved
angularly-resolved

Experiments and applications

spectral domain
angular domain



Spectral dependence of scattering



$$\sim 1 - \frac{\sin(2\delta/\lambda)}{\delta/\lambda} + \left[\frac{\sin(\delta/\lambda)}{\delta/\lambda}\right]^2$$

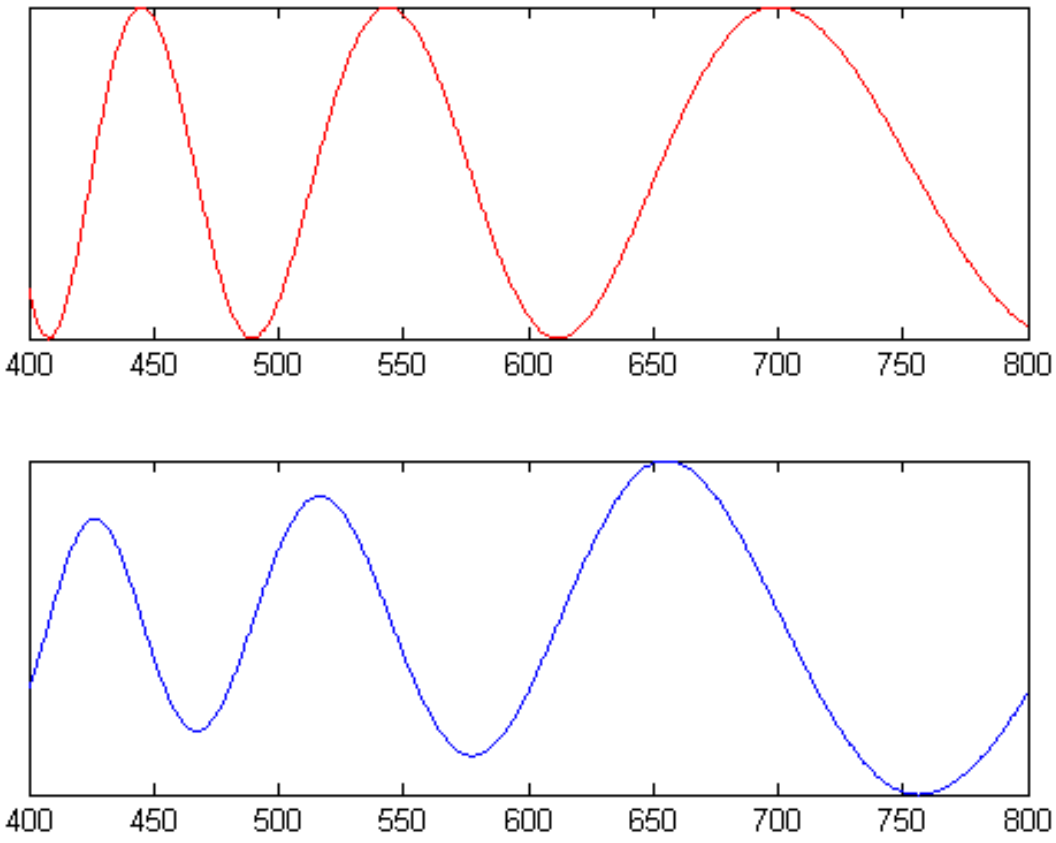
van de Hulst approximation to Mie theory

$$\sim \frac{F\sin^2(\delta/\lambda)}{1 + F\sin^2(\delta/\lambda)}$$

(F = cavity finesse)

Spectral dependence of scattering

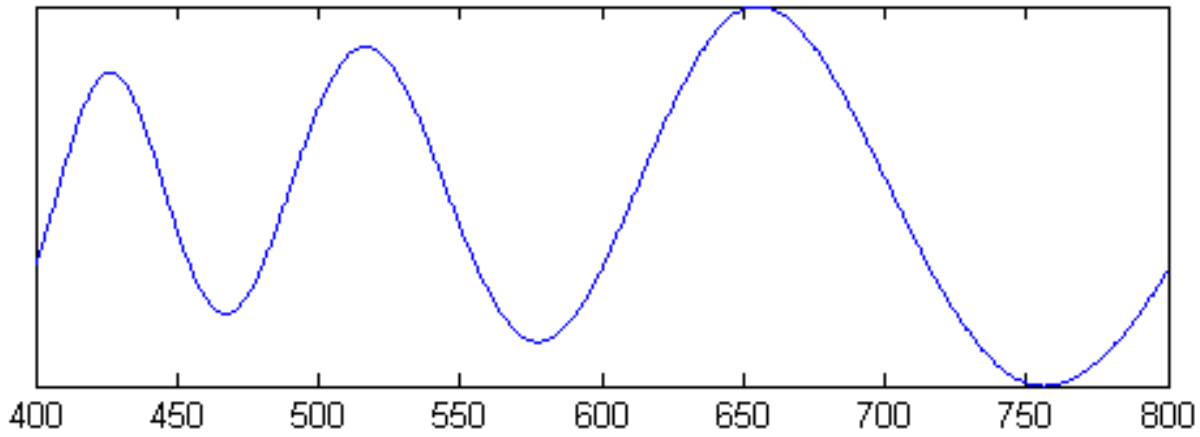
- d=5 microns
- n_1 = 1.36
- n_2/n_1 = 1.06



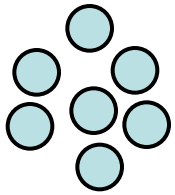
1-D etalon

3-D sphere

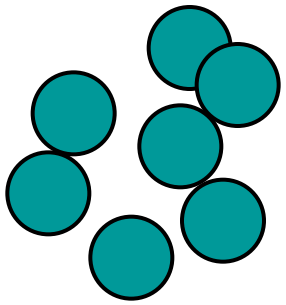
Scattering spectroscopy



$$\sim d^2\left\{1-\frac{\sin(2\delta/\lambda)}{\delta/\lambda}+\left[\frac{\sin(\delta/\lambda)}{\delta/\lambda}\right]^2\right\}$$



δ_1

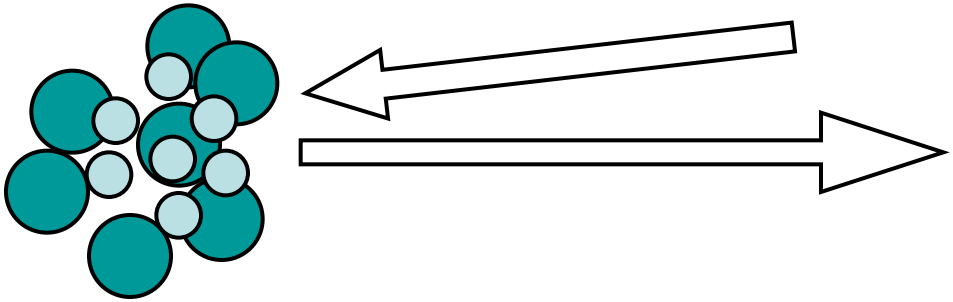


$\delta_2 > \delta_1$

different spacing of scattering peaks

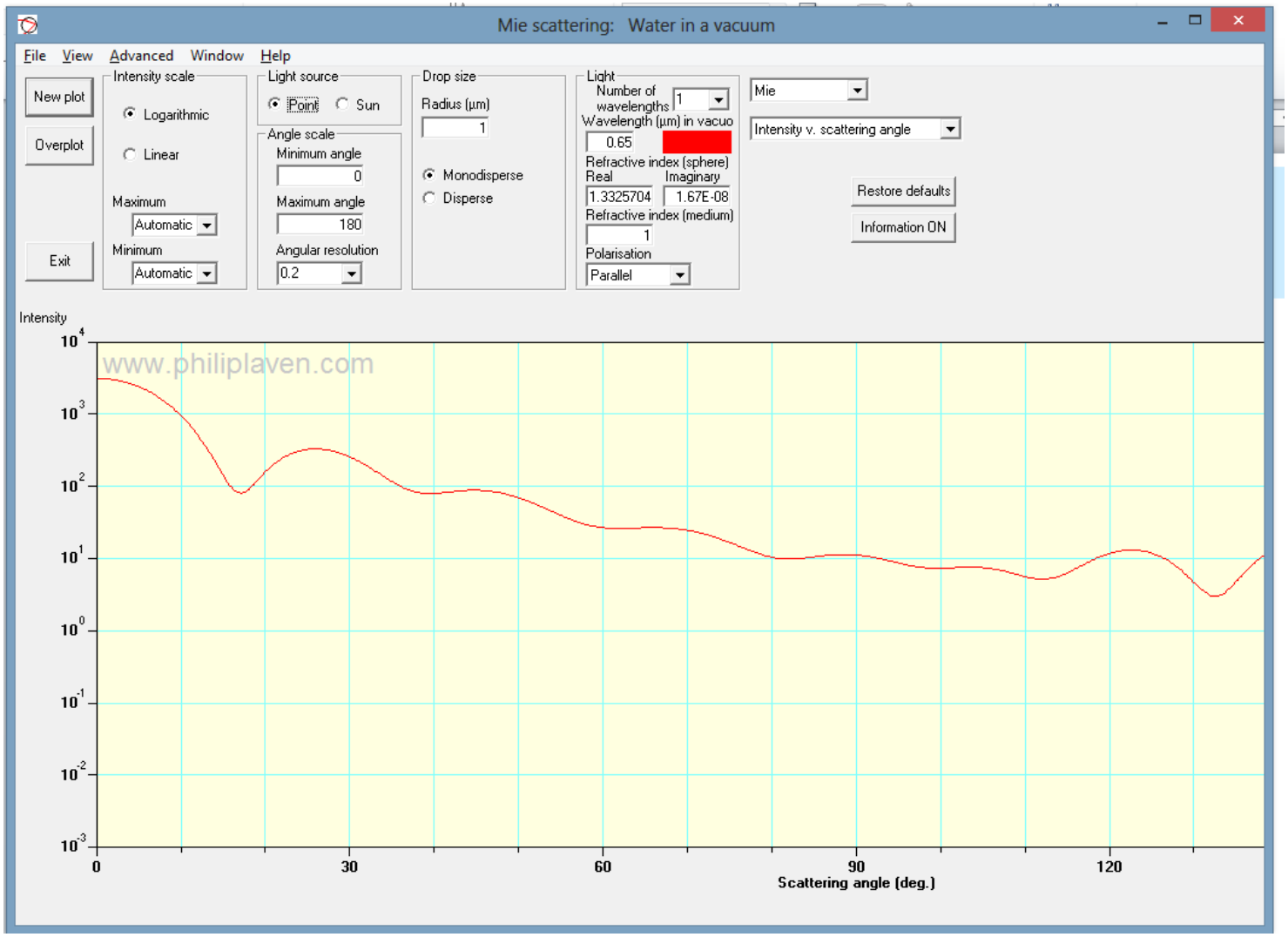
- spacing of peaks: size of scatterer
- depth of modulation: number of such scatterers

mixture



superposition of spectra

MiePlot program: calculation of scatter patterns



http://www.philiplaven.com/mieplot.htm

Road map for today

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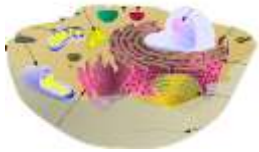

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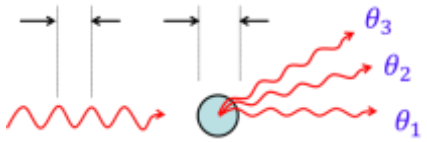
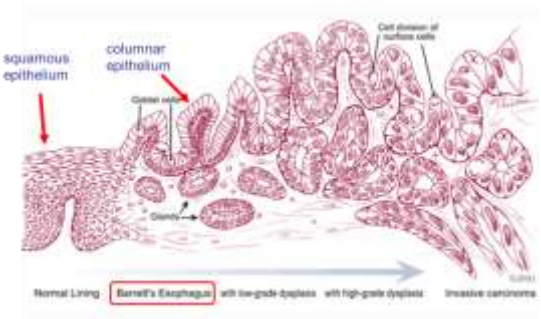


→ Experiments and applications

spectral domain
angular domain



Spectroscopic light scattering system

IEEE JOURNAL OF SELECTED TOPICS IN QUANTUM ELECTRONICS, VOL. 5, NO. 4, JULY/AUGUST 1999

Polarized Light Scattering Spectroscopy for Quantitative Measurement of Epithelial Cellular Structures *In Situ*

Vadim Backman, Rajan Gurjar, Kamran Badizadegan, Irving Itzkan, Ramachandra R. Dasari, Lev T. Perelman, and Michael S. Feld

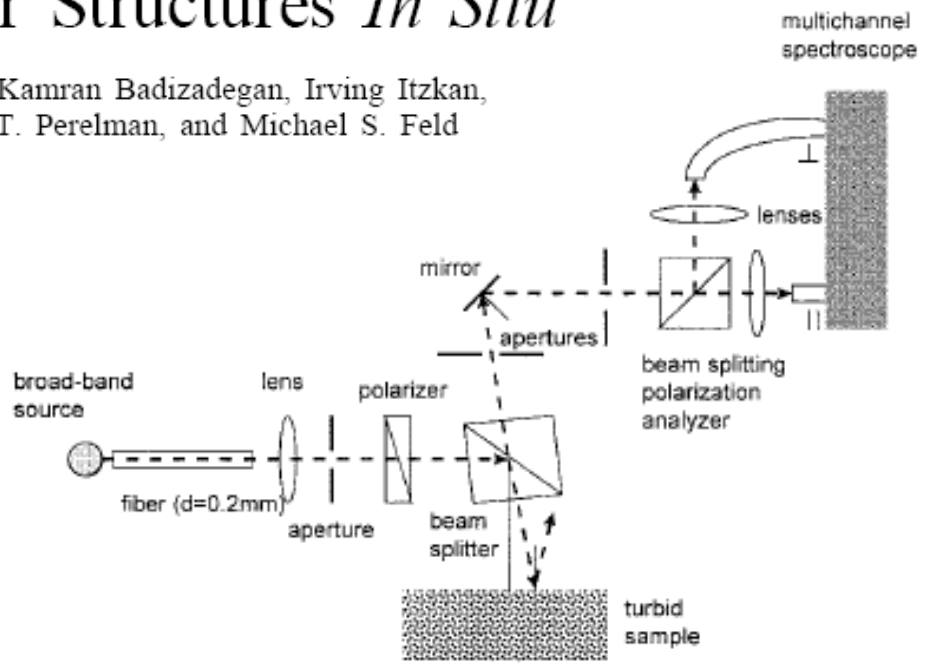
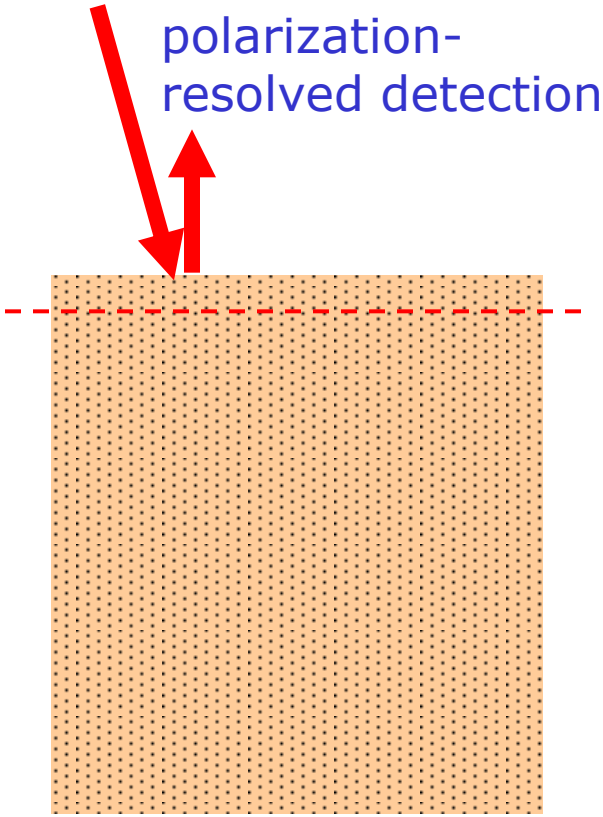


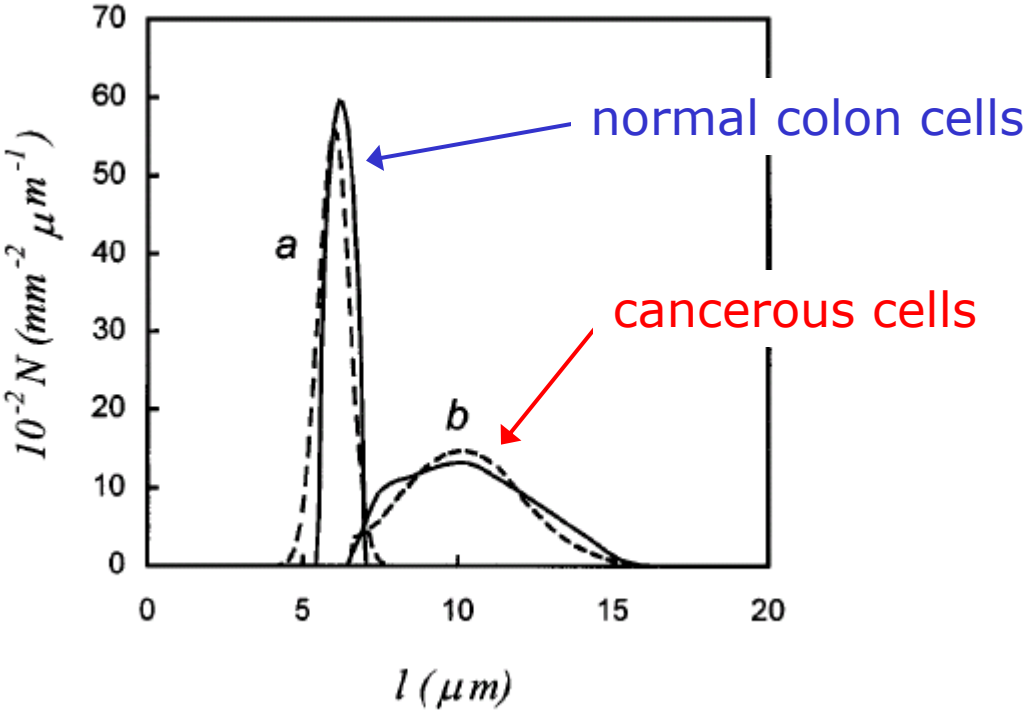
Fig. 1. Schematic diagram of polarization LSS system.

Scattering spectroscopy

broadband polarized illumination

polarization-resolved detection





Perelman et al., *Phys Rev Lett* **80:**627 (1998) and following.

Method reports on pre-cancerous, subcellular alterations

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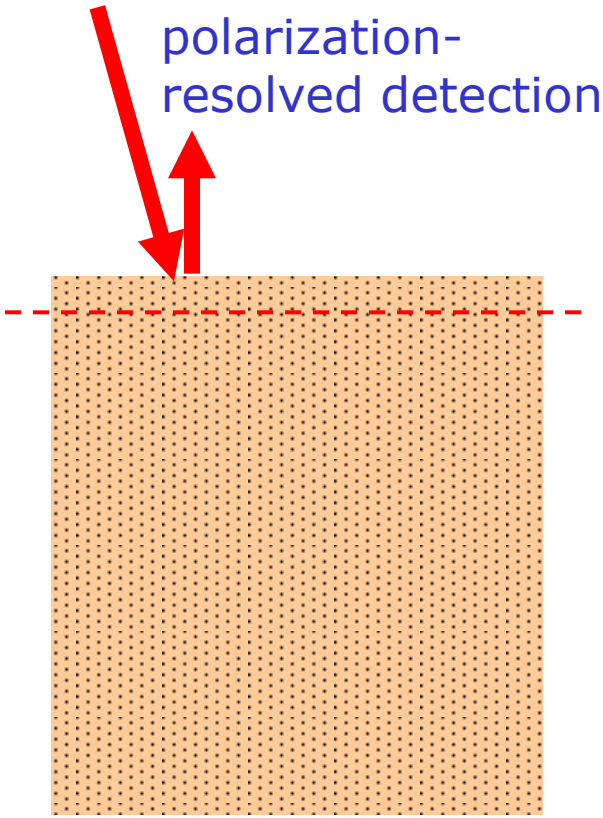
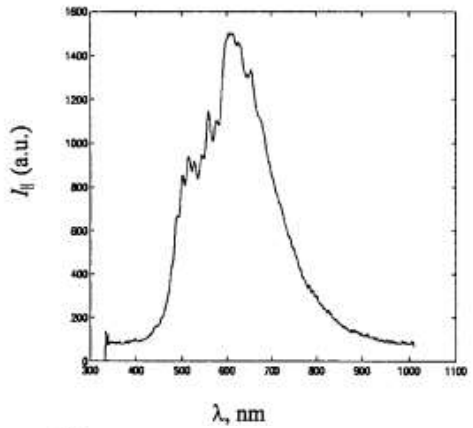
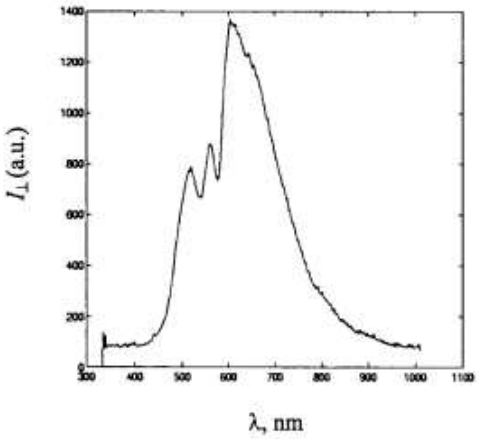




parallel



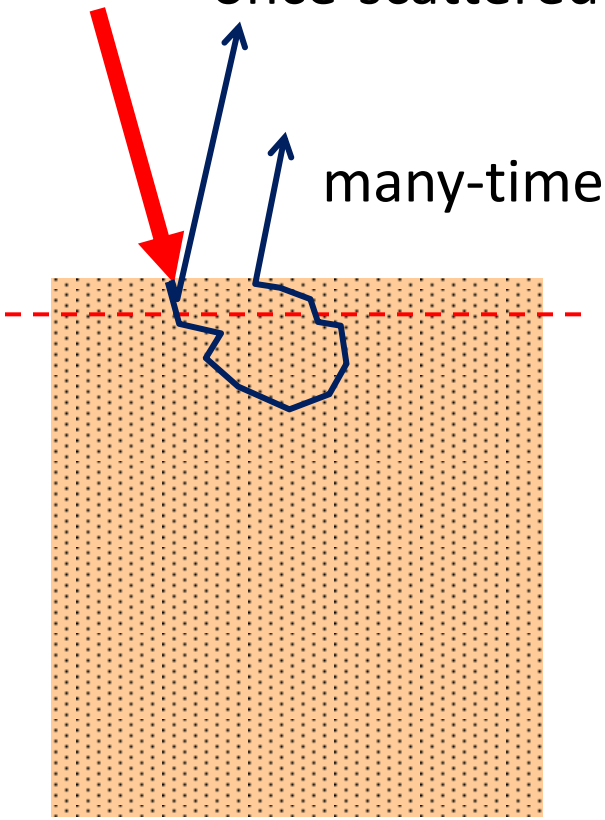
perpendicular

Backman et al., *IEEE J. Sel. Top. Quant. Electr.* **5(4):**1019-1026.

Method reports on pre-cancerous, subcellular alterations

The polarization/surface “trick”

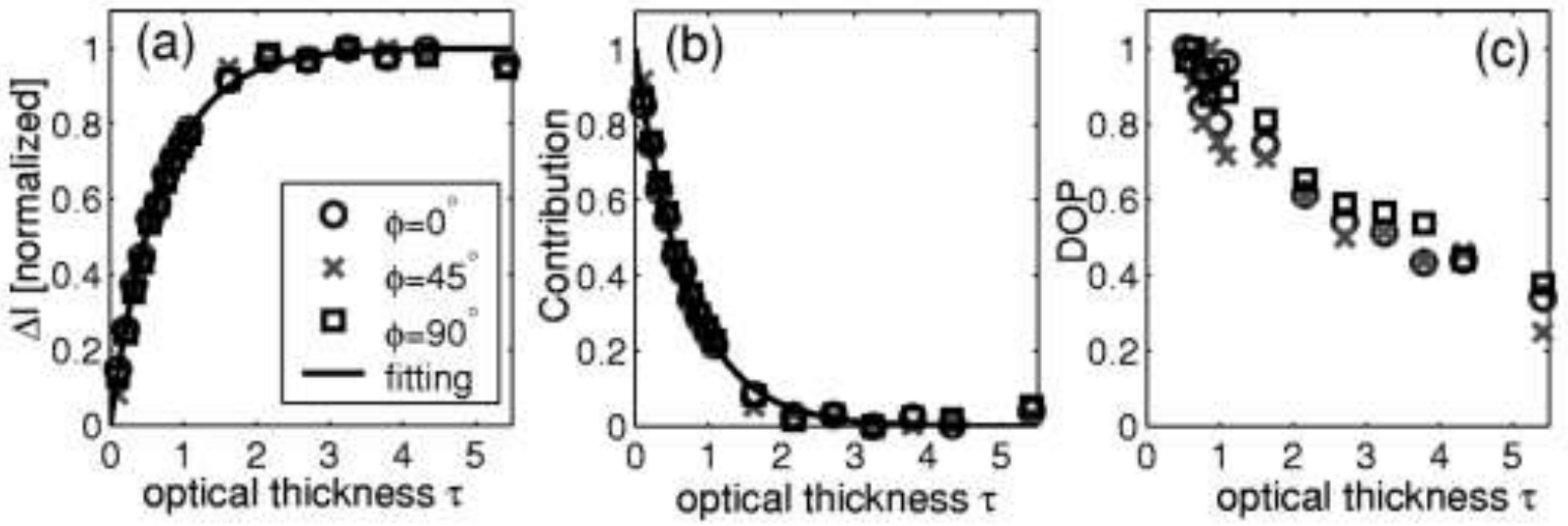
once-scattered light: retains polarization



many-times scattered light: “forgets” polarization

$$S_{par} = (1/2)S_{diffuse} + S_{surface}$$
$$S_{per} = (1/2)S_{diffuse}$$

Difference signal is mostly superficial in origin



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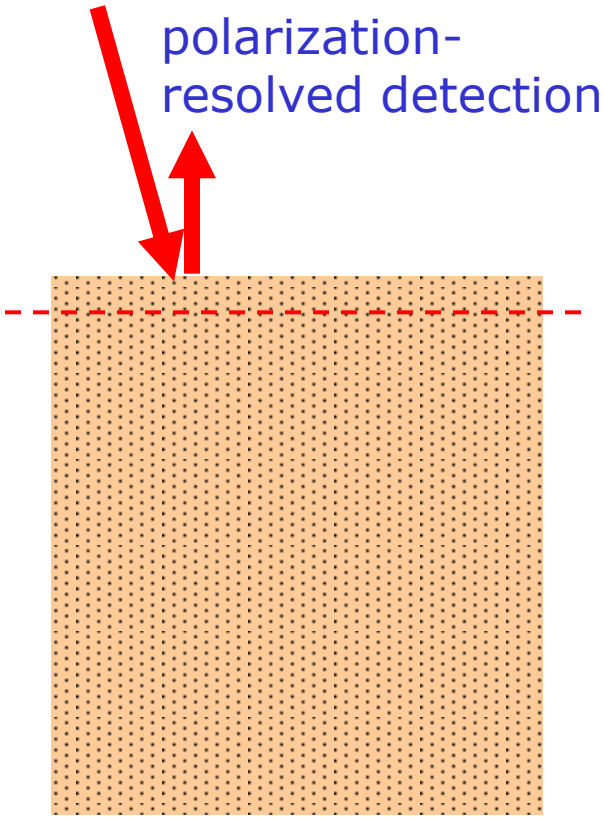
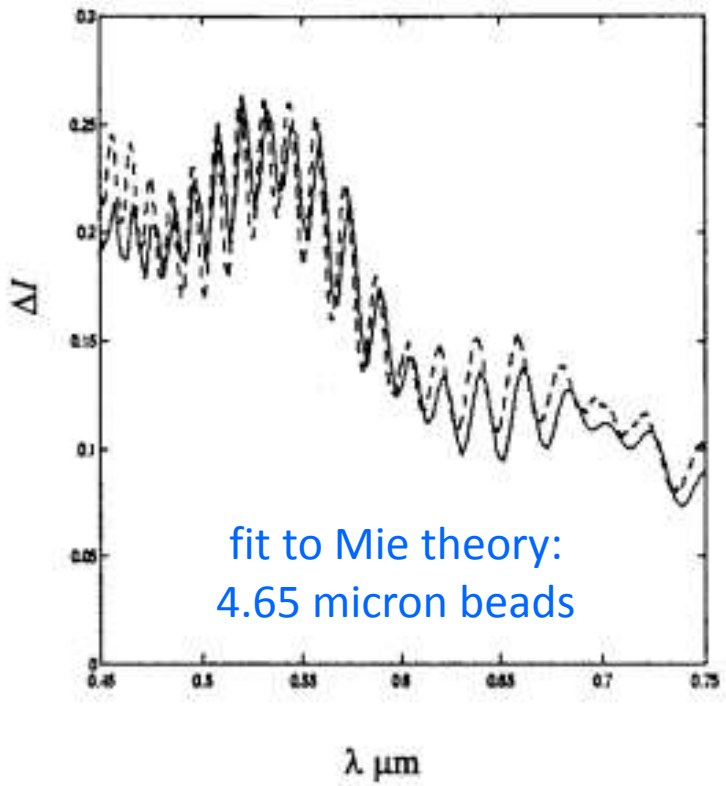


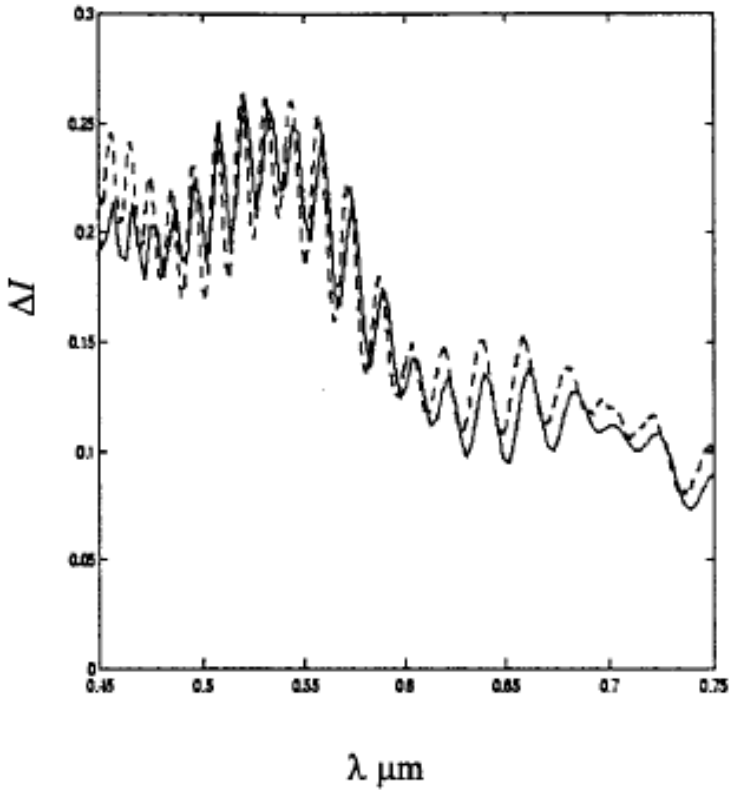


difference

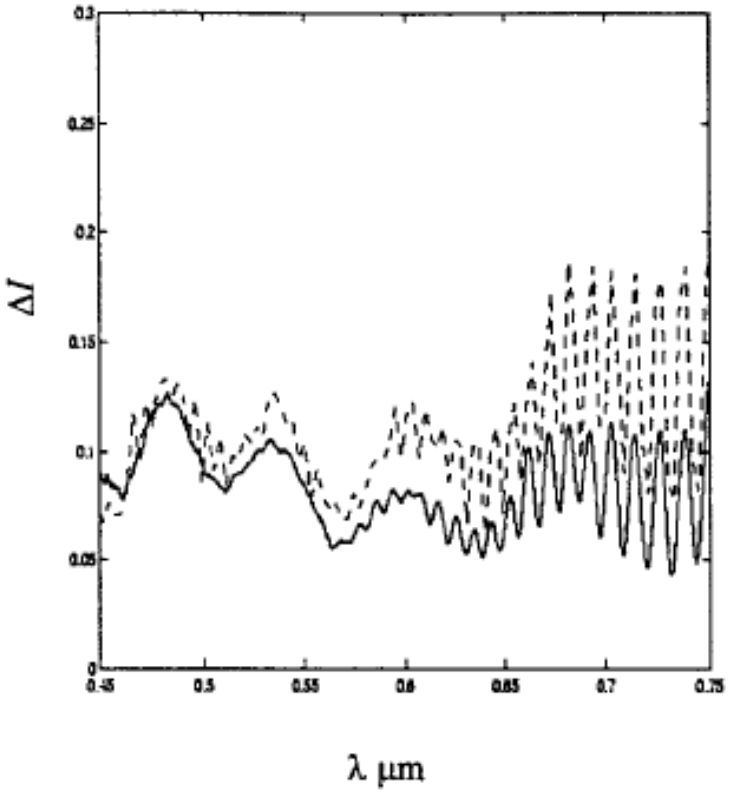
Backman et al., *IEEE J. Sel. Top. Quant. Electr.* **5(4):**1019-1026.

Method reports on pre-cancerous, subcellular alterations

Good agreement for bead layers placed above absorbing/scattering substrates



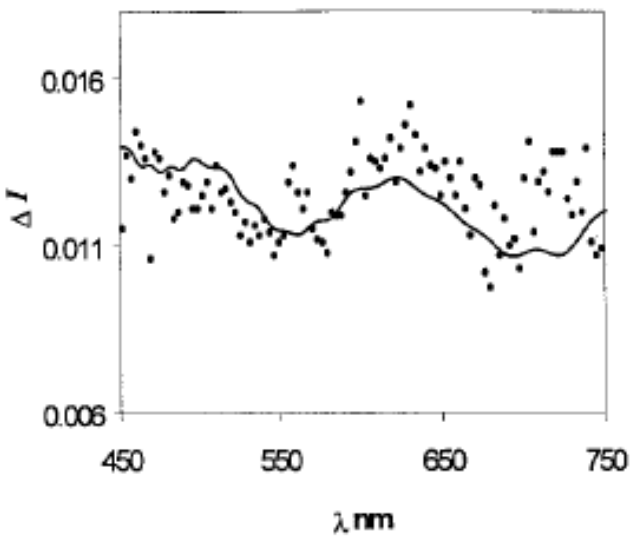
4.65 micron beads in water, n=1.19



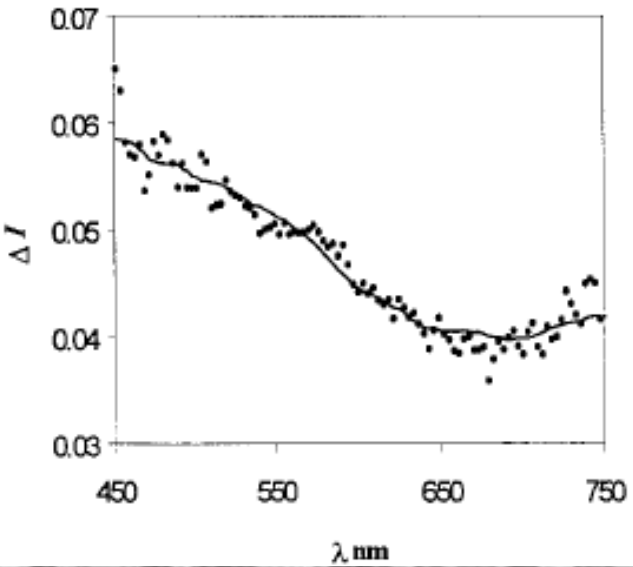
9.5 micron beads in water, n=1.19

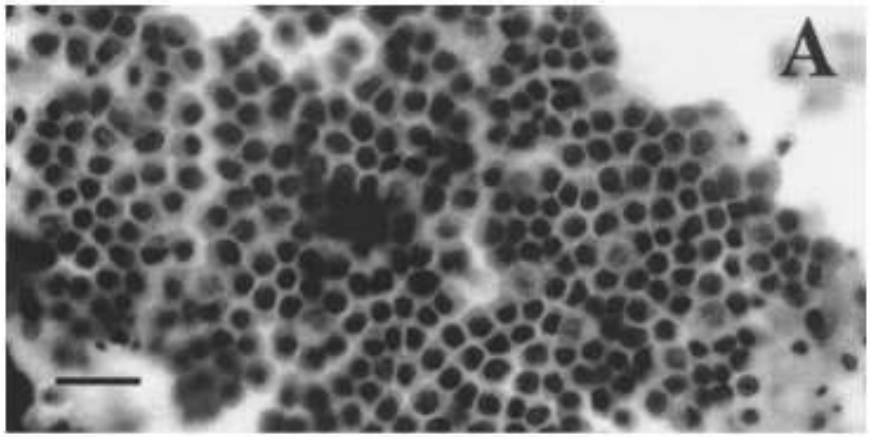
Different nuclear sizes = different scattering vs. wavelength

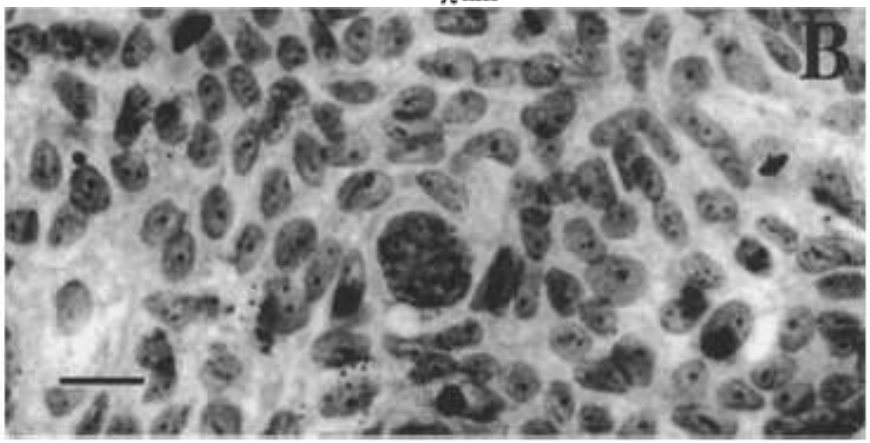
Normal



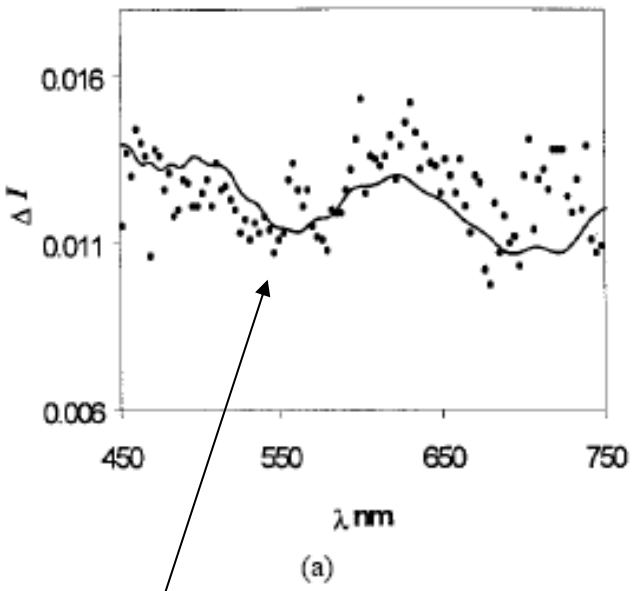
Malignant



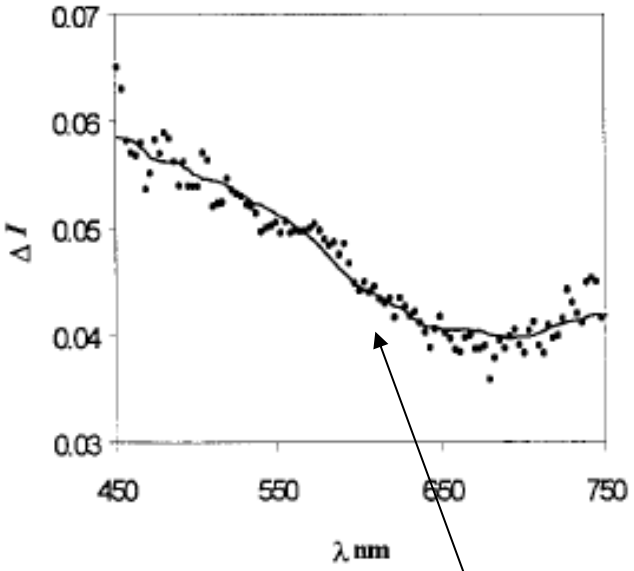




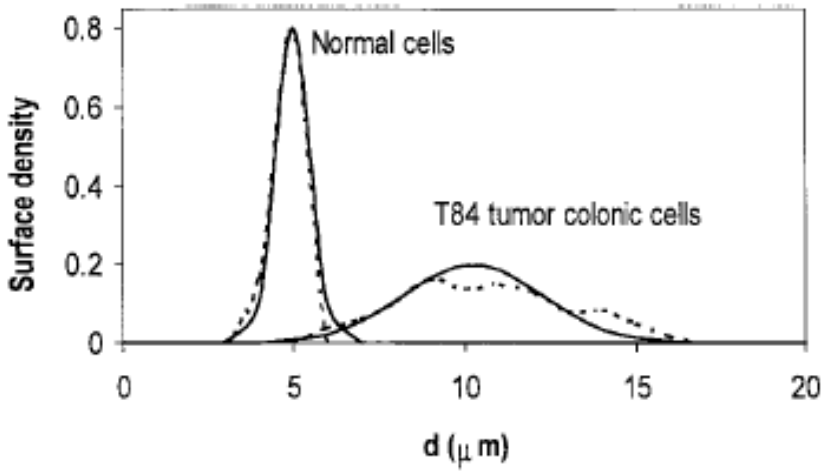
Size distributions correctly estimated!



Normal cells



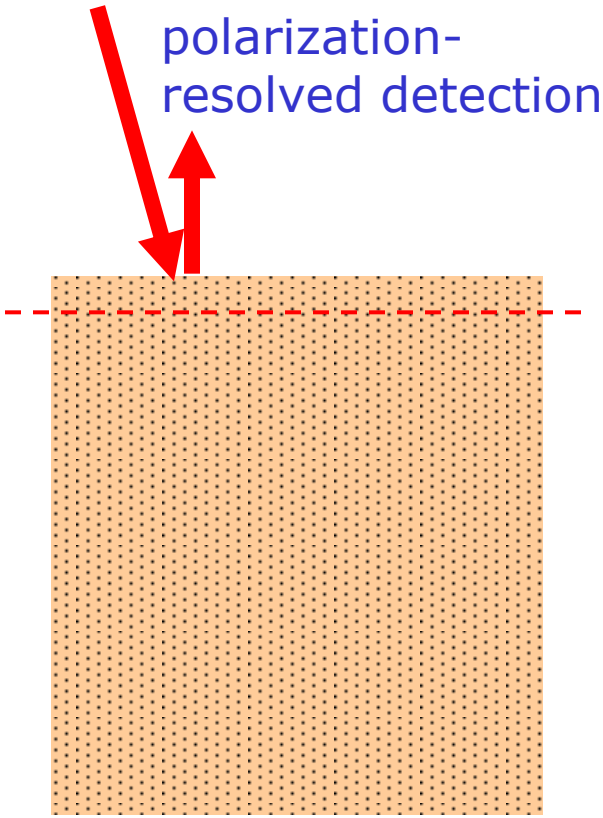
Tumor cells

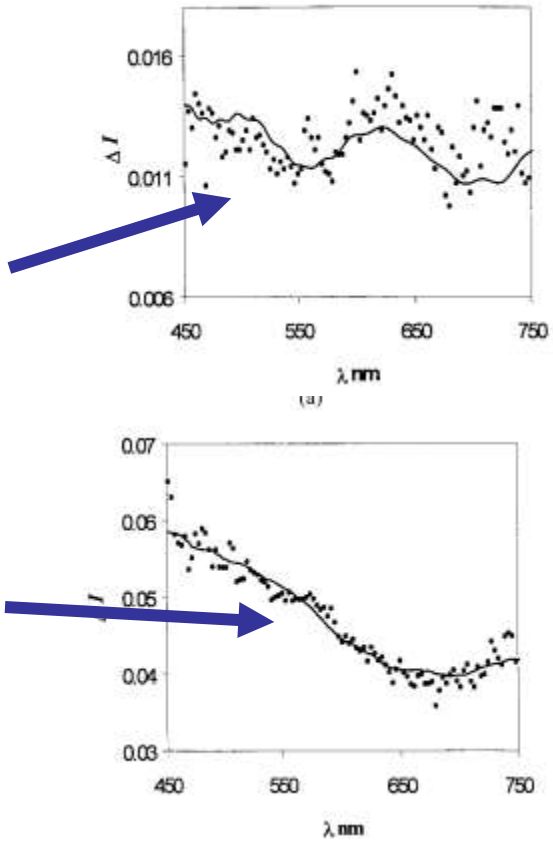


Scattering spectroscopy

broadband polarized illumination

polarization-resolved detection





Backman et al., *IEEE J. Sel. Top. Quant. Electr.* **5(4):**1019-1026.

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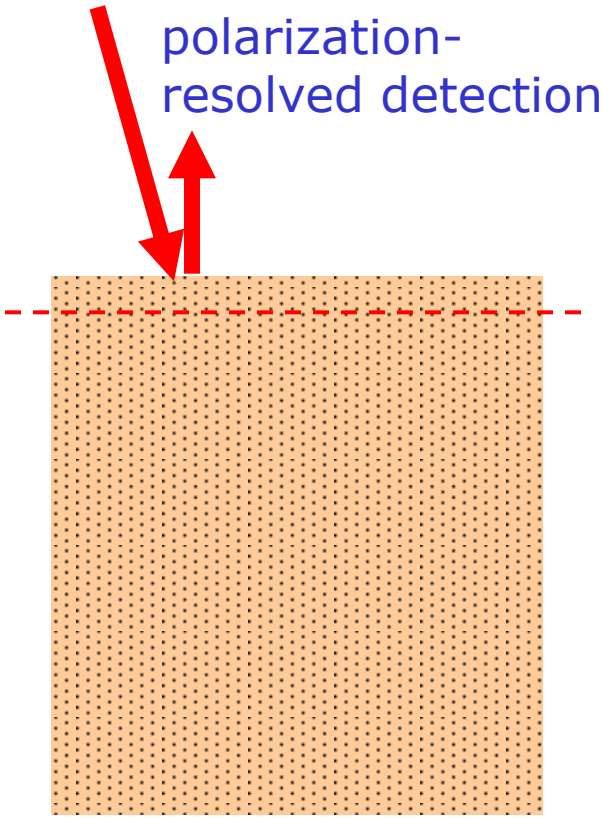
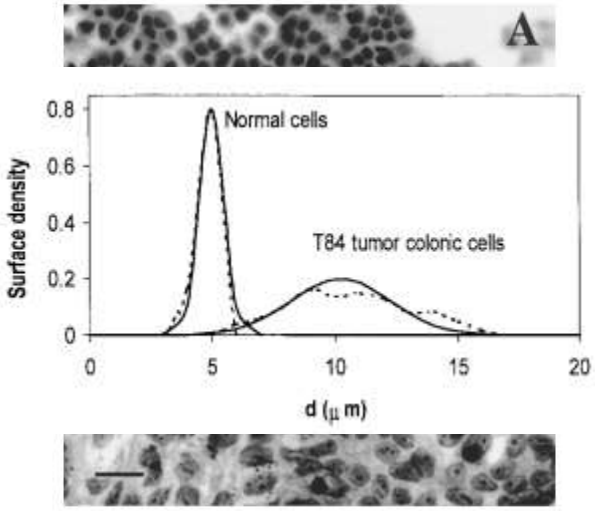
broadband polarized illumination

polarization-resolved detection





Backman et al., *IEEE J. Sel. Top. Quant. Electr.* **5(4):**1019-1026.

Method reports on pre-cancerous, subcellular alterations

Using light scattering to detect early cancerous transformations

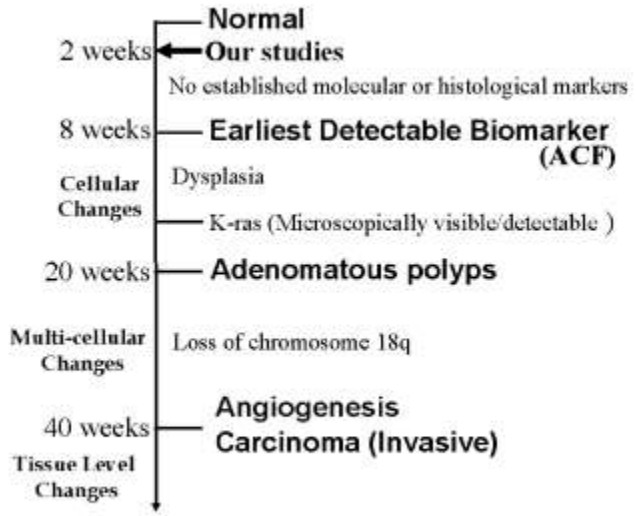
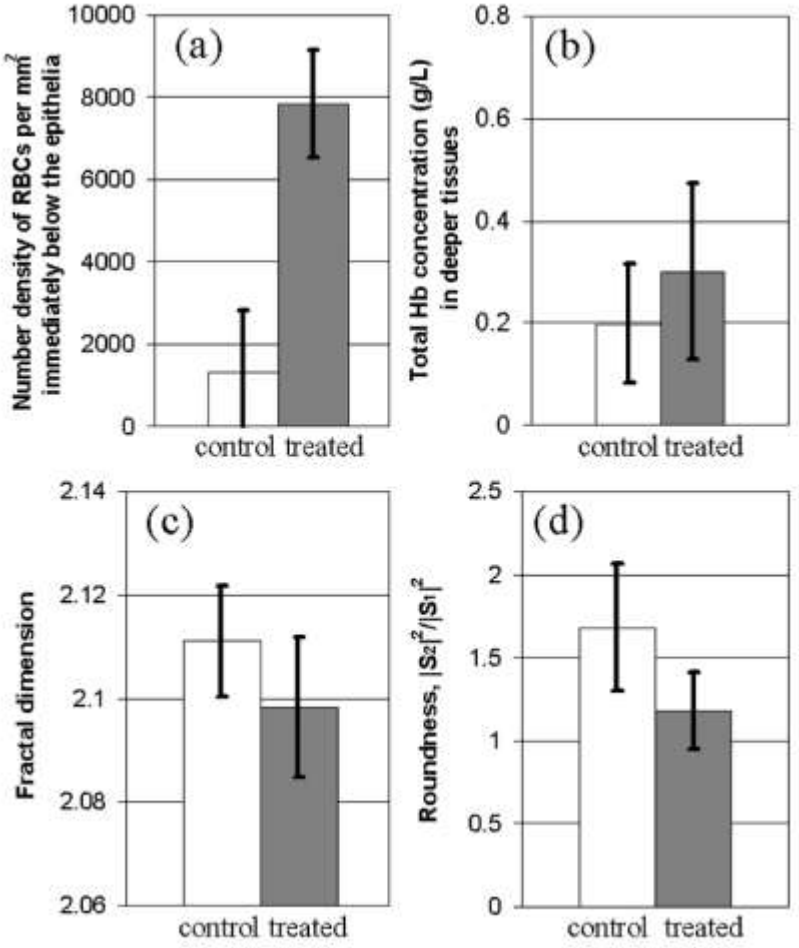


Fig. 2. Development process of rat-carcinogenesis model.

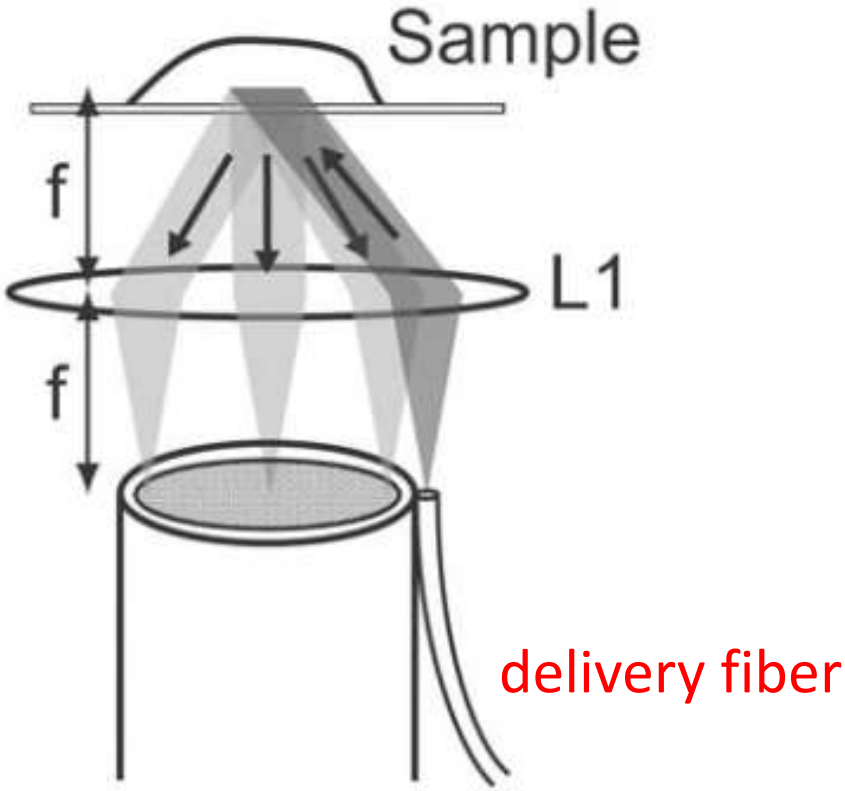


Fourier-domain angle-resolved low coherence interferometry through an endoscopic fiber bundle for light-scattering spectroscopy

John W. Pyhtila, Jeffrey D. Boyer, Kevin J. Chalut, and Adam Wax
Department of Biomedical Engineering and the Fitzpatrick Center for Photonics and Communication Systems, Duke University, Durham, North Carolina 27708

772 OPTICS LETTERS / Vol. 31, No. 6 / March 15, 2006

Angle-resolved scattering, combined with low-coherence depth ranging



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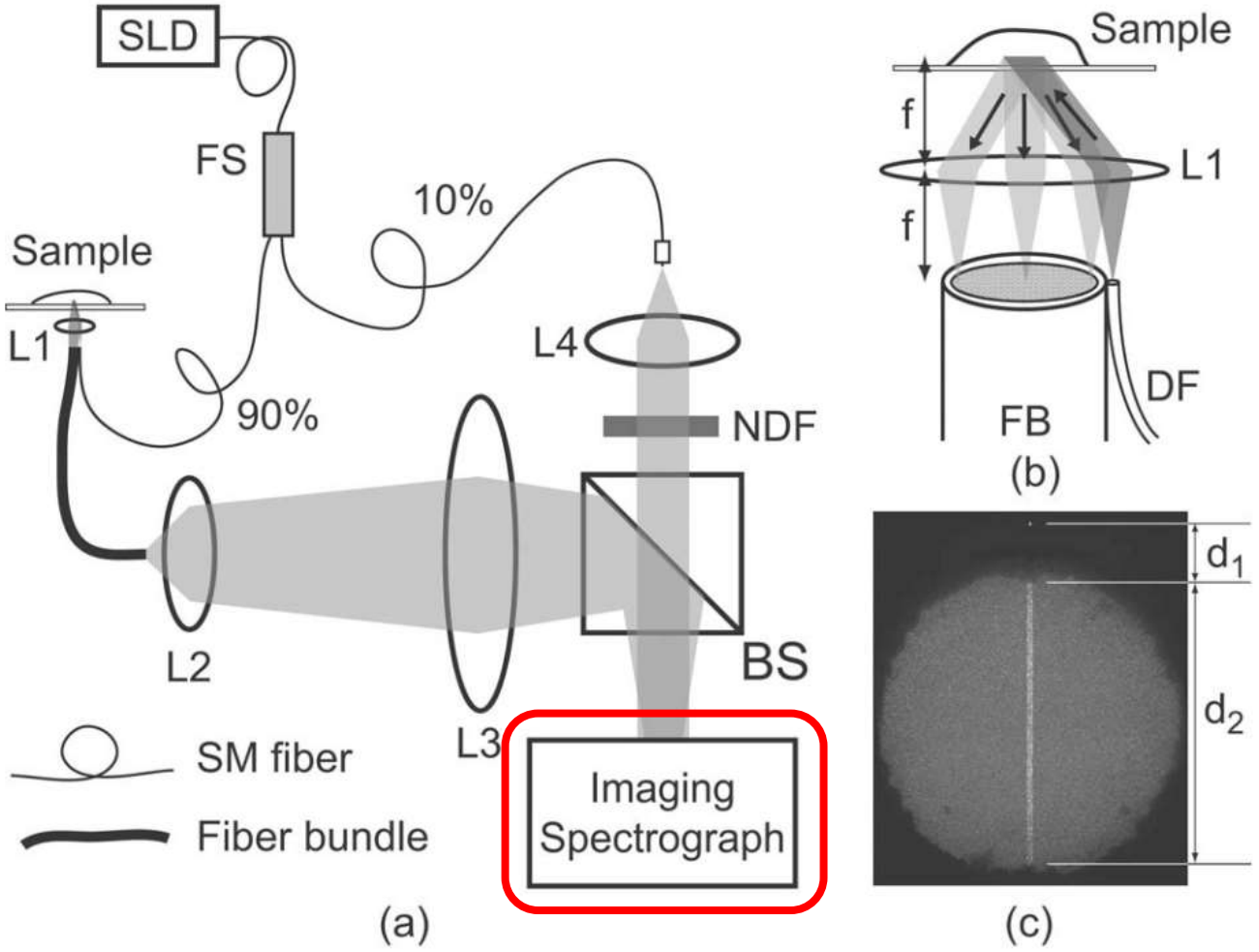
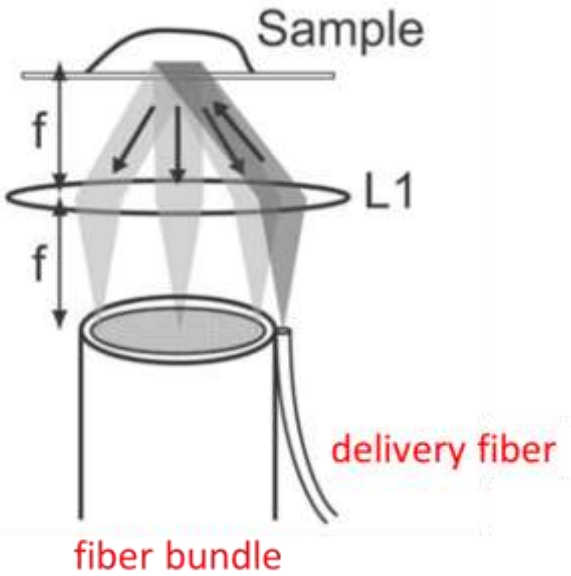
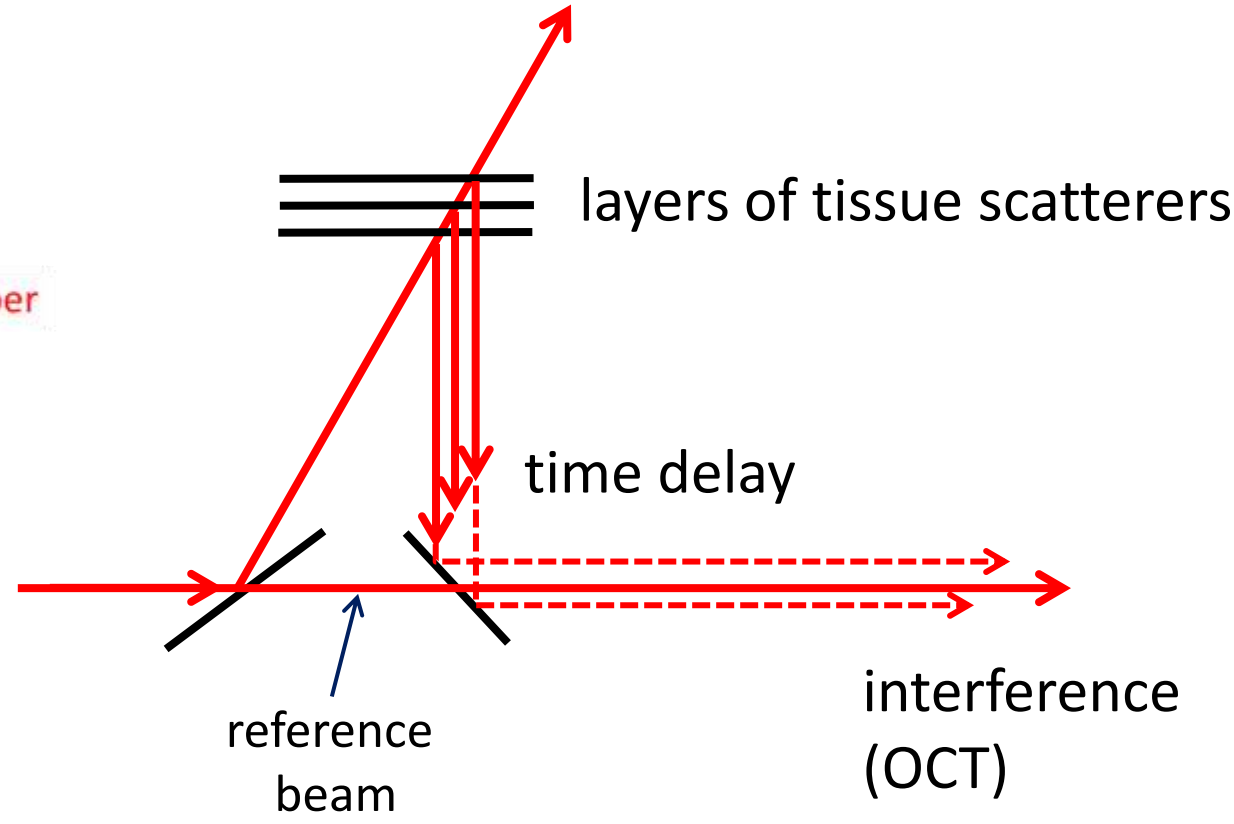
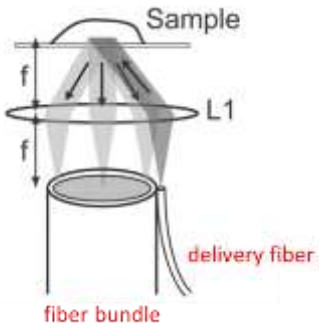


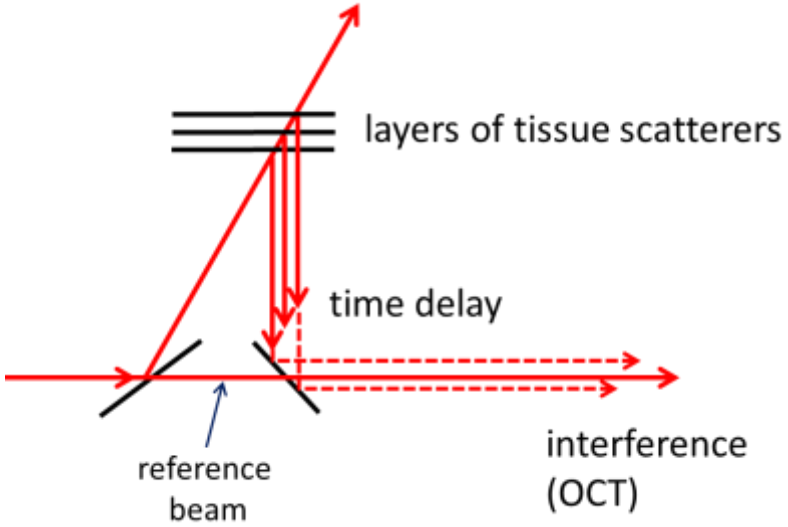
Measuring at different depths

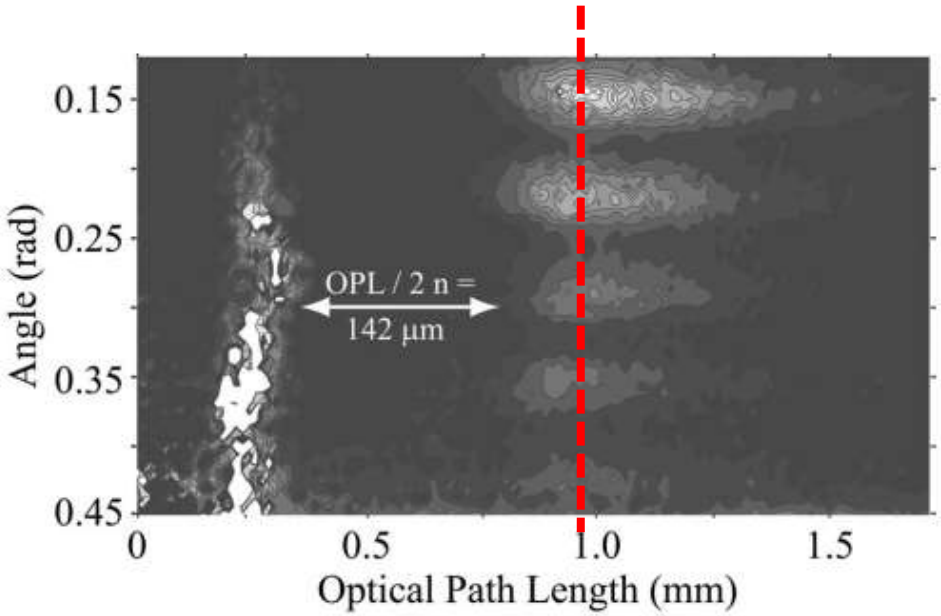


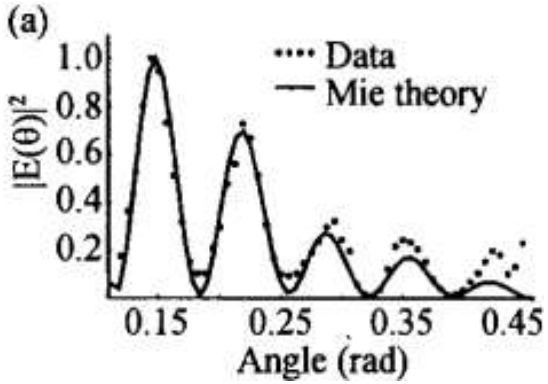


Measuring at different depths









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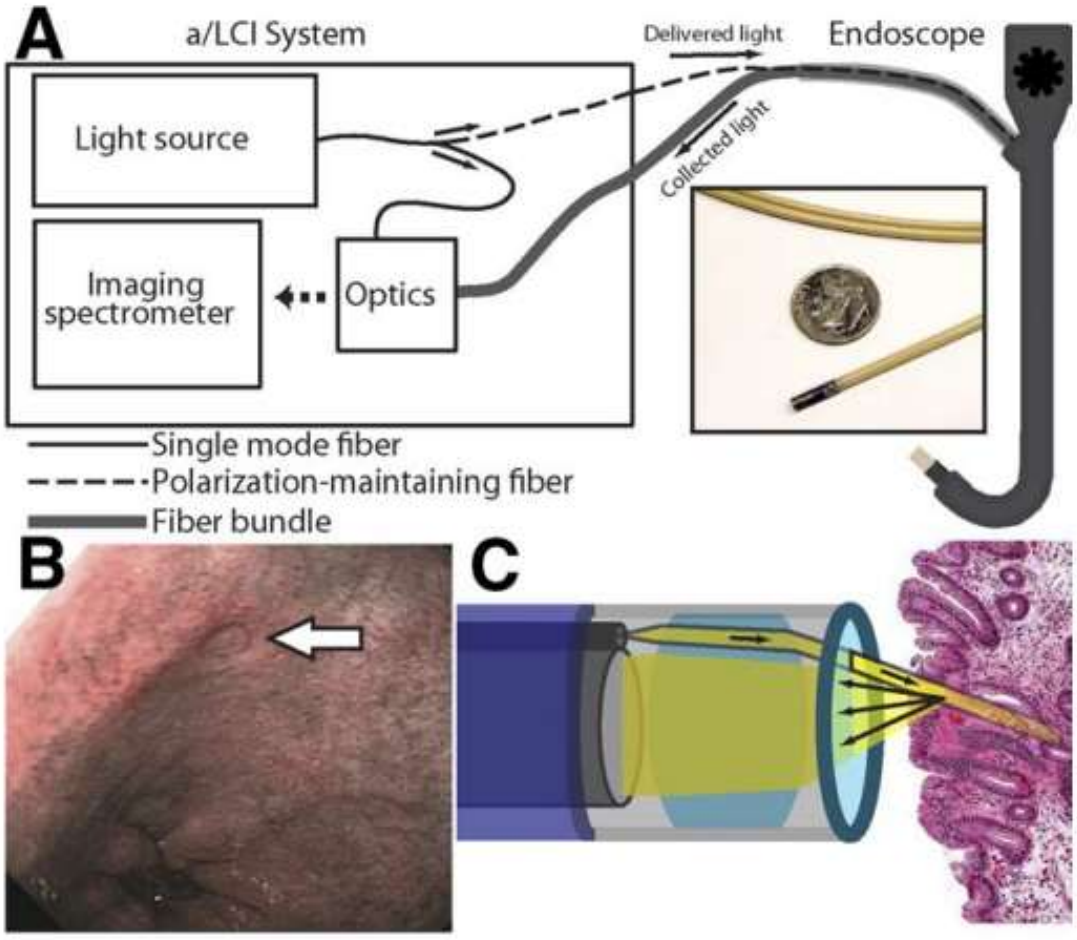
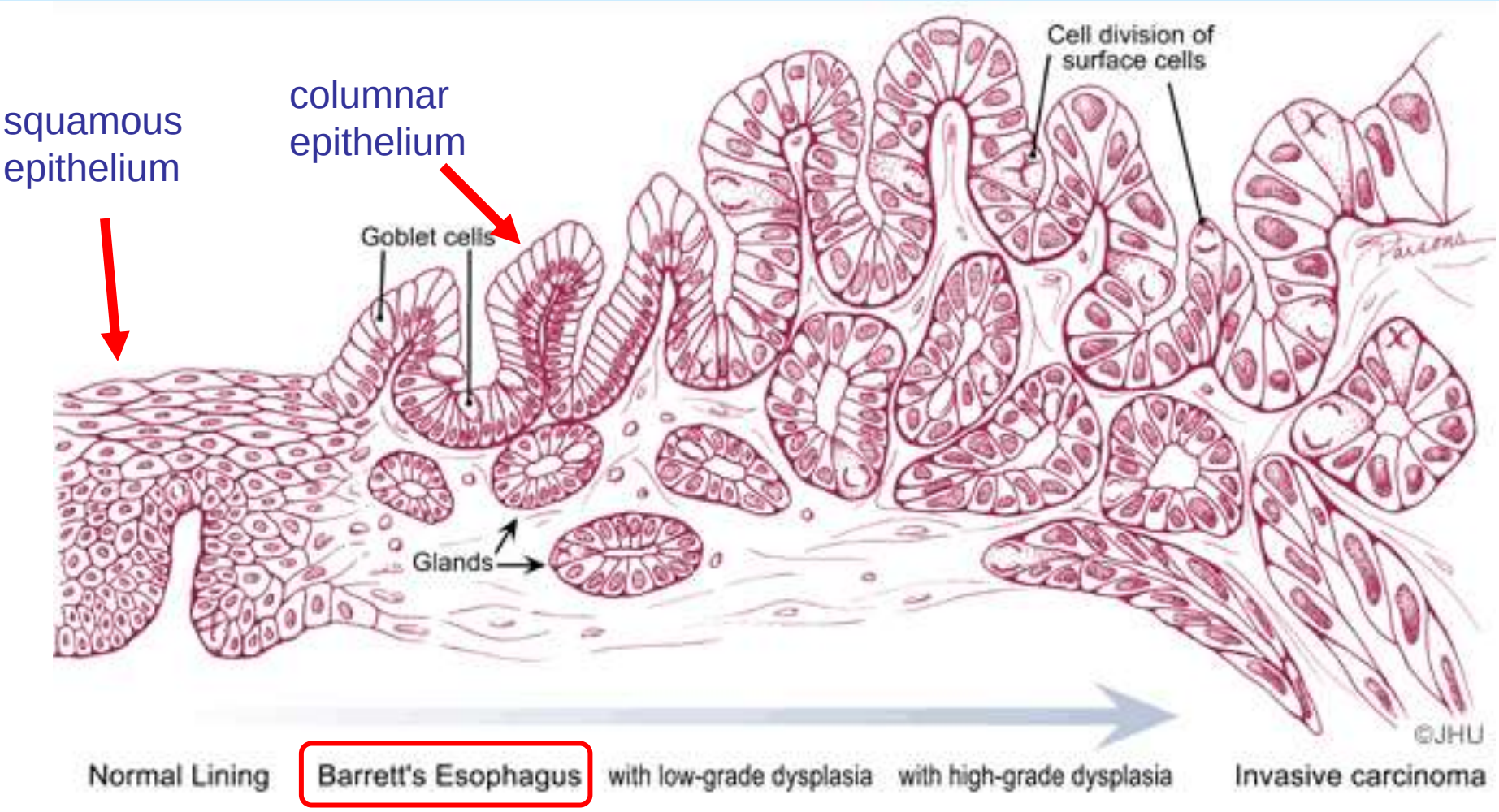
Clinical instrument for Barrett's esophagus



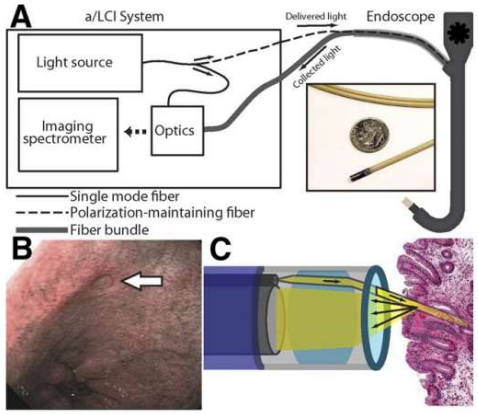
Terry et al., "Detection of Dysplasia in Barrett's Esophagus With In Vivo Depth-Resolved Nuclear Morphology Measurements," Gastroenterology **140**(1), pp. 42–50 (2011).

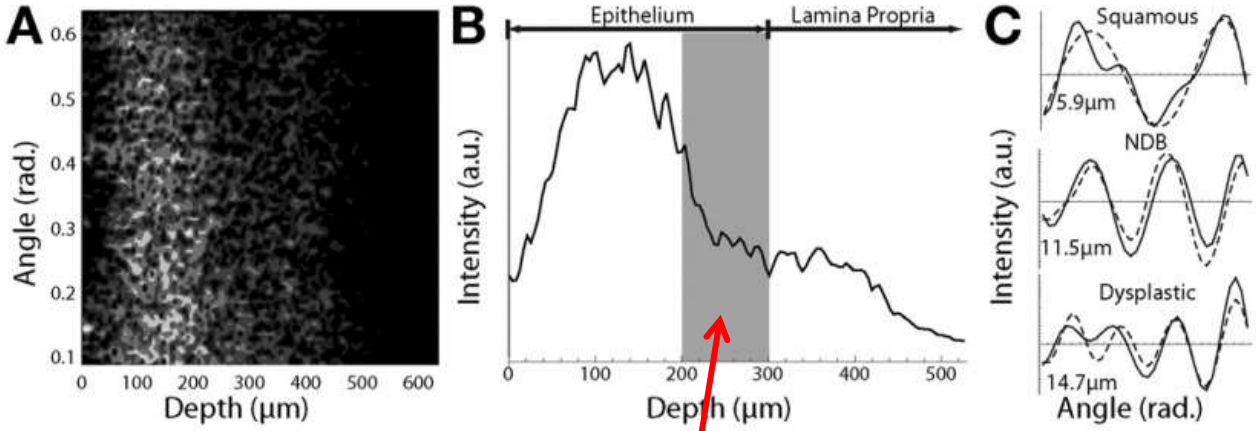
Barrett's Esophagus



http://pathology2.jhu.edu/beweb/Definition.cfm

Clinical instrument for Barrett's esophagus

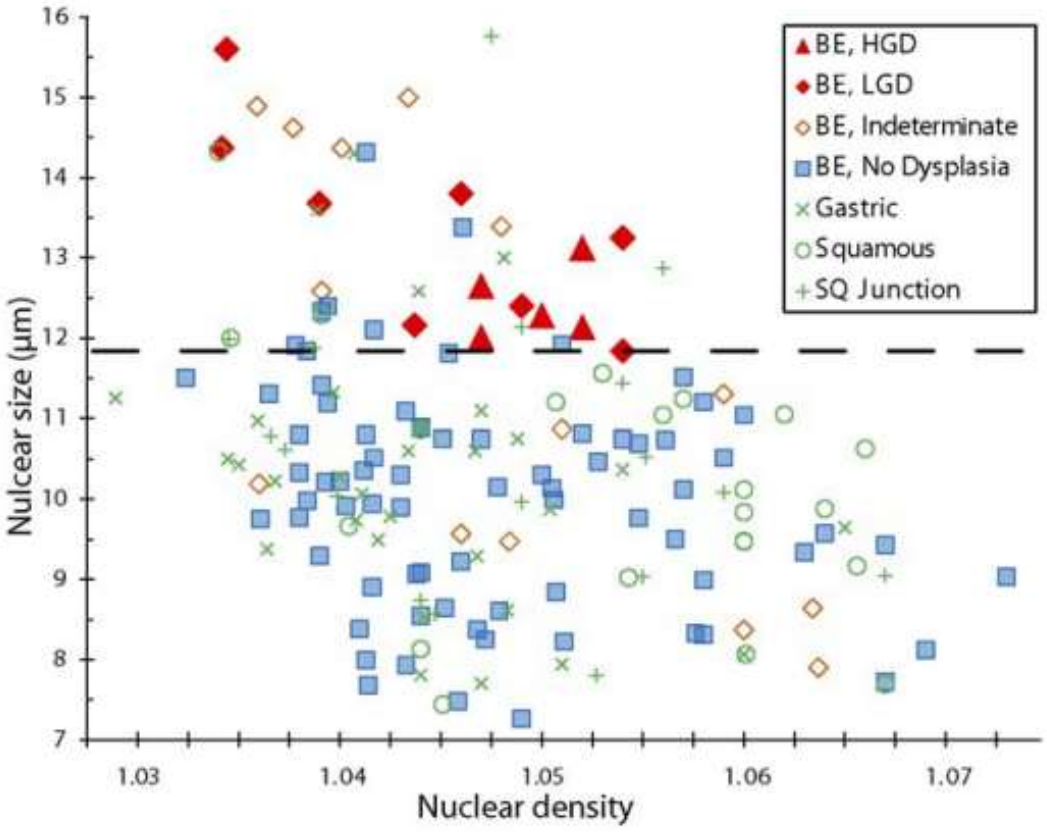




key region: 200-300 um below surface

Terry et al., "Detection of Dysplasia in Barrett's Esophagus With In Vivo Depth-Resolved Nuclear Morphology Measurements," Gastroenterology **140**(1), pp. 42–50 (2011).

Agreement with pathology

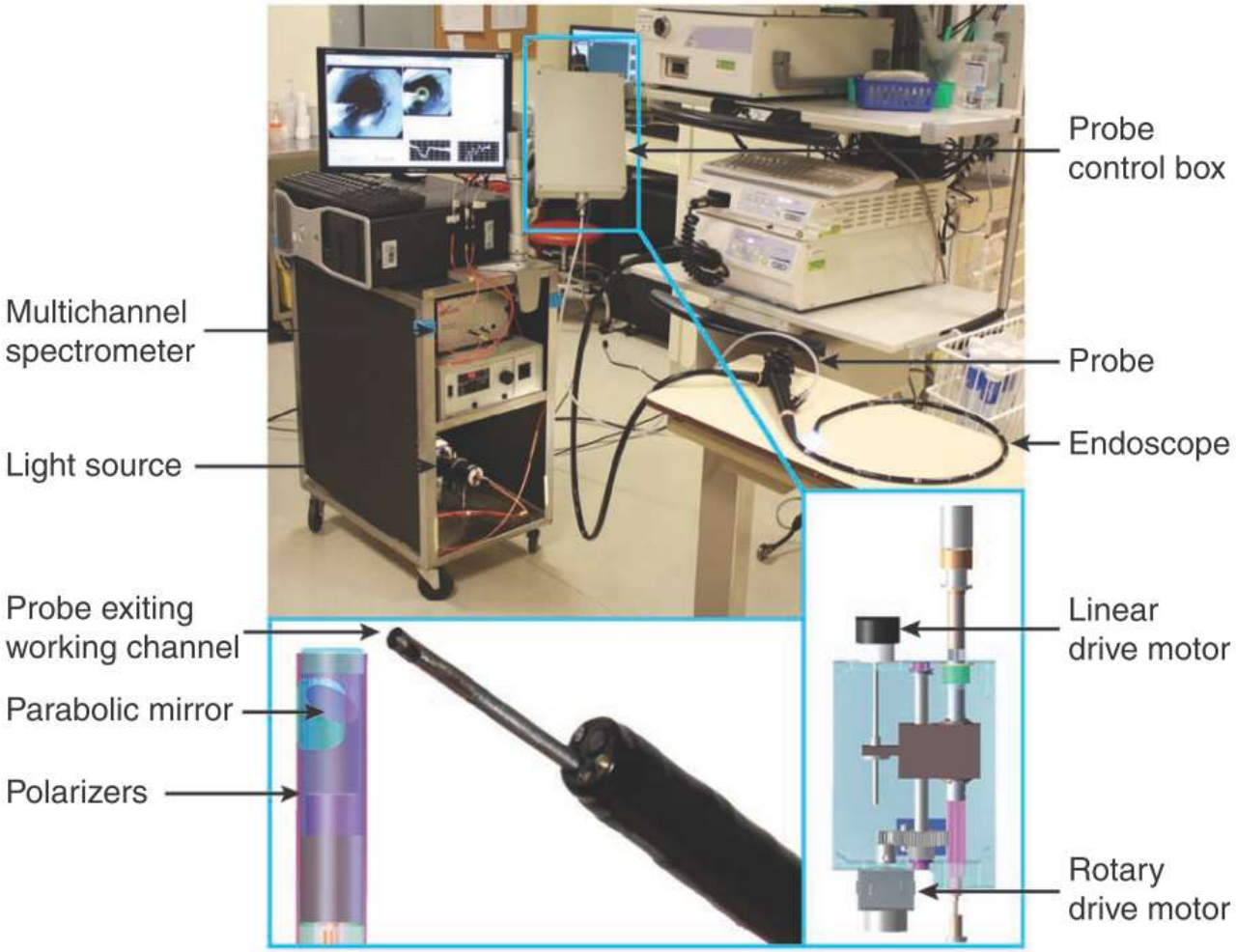


all dysplastic samples

100% sensitivity
86% specificity

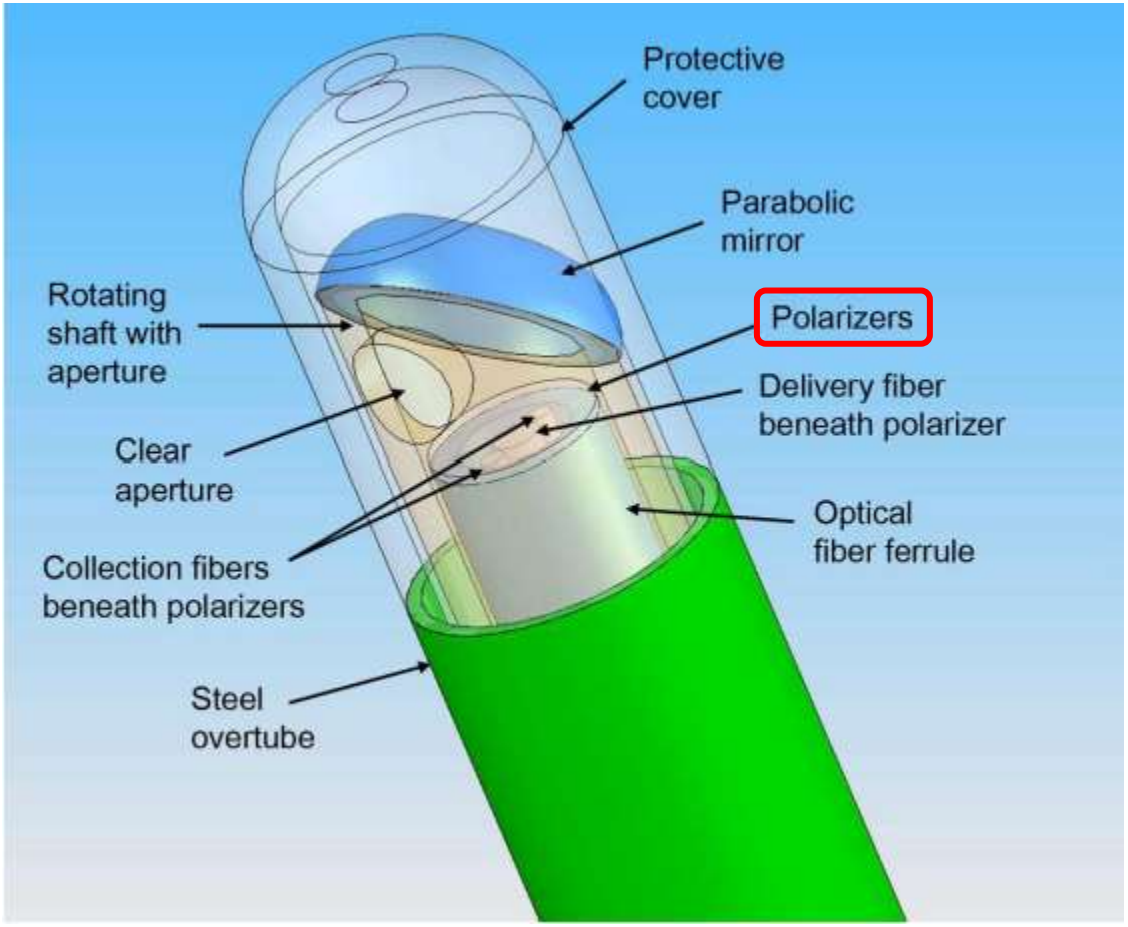
Terry et al., "Detection of Dysplasia in Barrett's Esophagus With In Vivo Depth-Resolved Nuclear Morphology Measurements," Gastroenterology **140**(1), pp. 42–50 (2011).

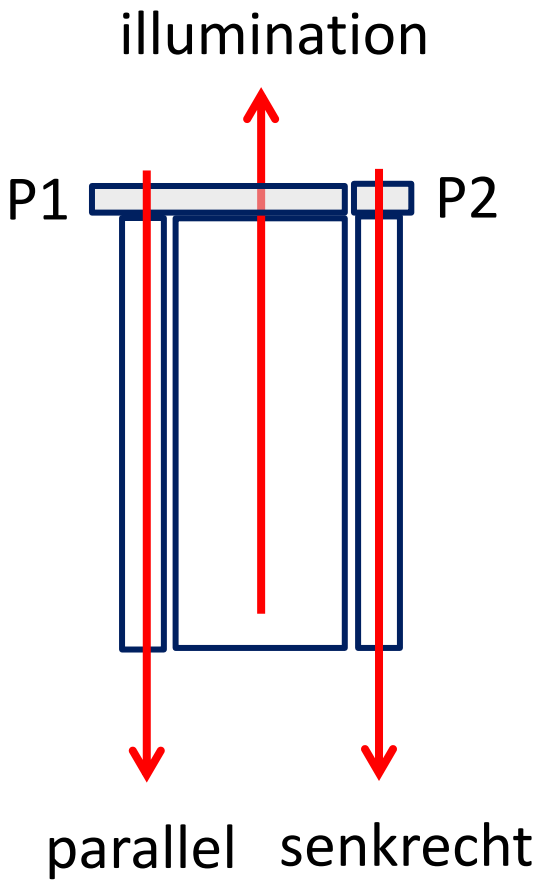
Wavelength-resolved elastic scattering



Qiu et al., "Multispectral scanning during endoscopy guides biopsy of dysplasia in Barrett's esophagus," Nature Med., ***16****(5), 603-607 (2010)*

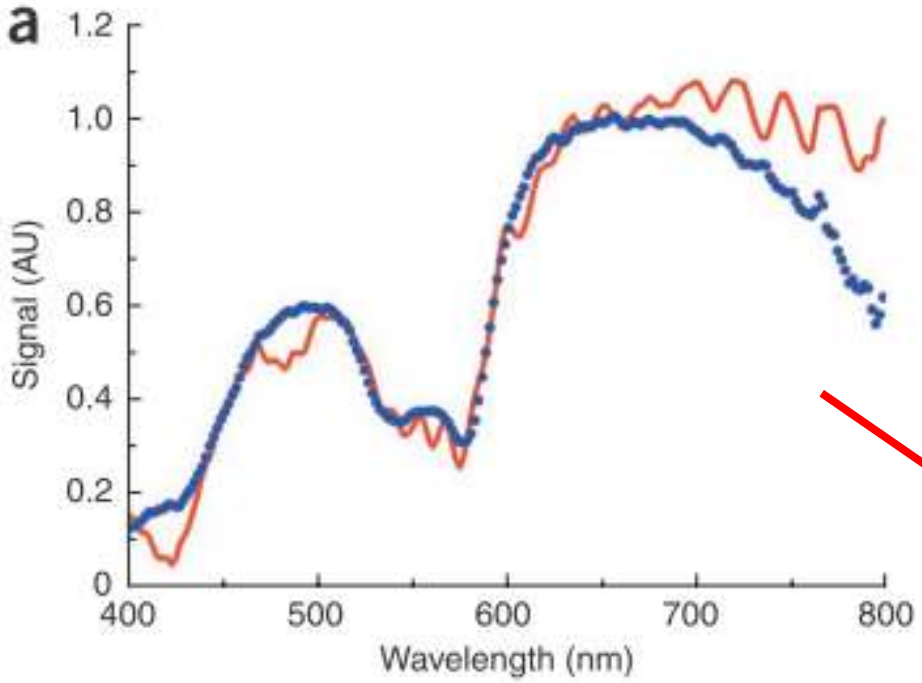
Details of esophageal probe





Qiu et al., "Multispectral scanning during endoscopy guides biopsy of dysplasia in Barrett's esophagus," Nature Med., ***16****(5), 603-607 (2010)*

Wavelength data from esophagus



parallel

perpendicular

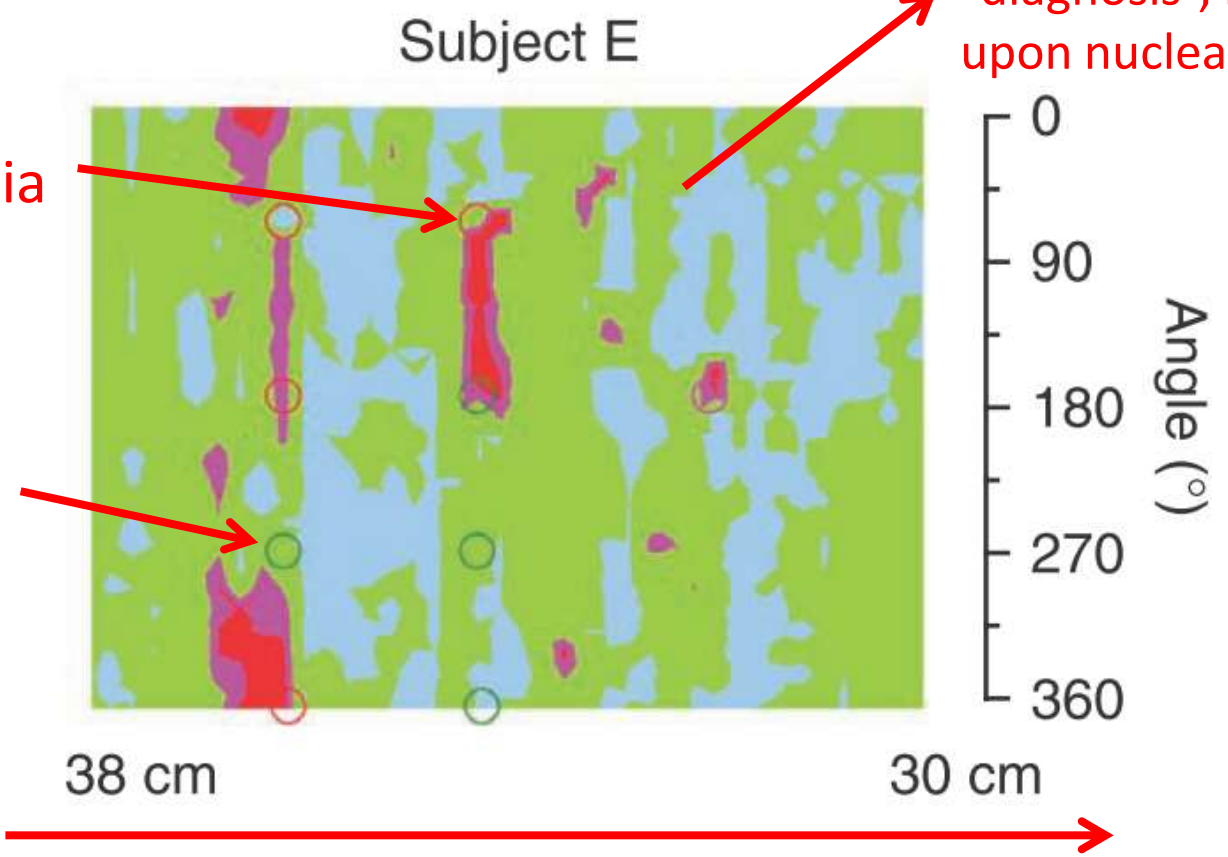
deduce nuclear size distribution

Qiu et al., "Multispectral scanning during endoscopy guides biopsy of dysplasia in Barrett's esophagus," Nature Med., ***16****(5), 603-607 (2010)*

Video from data acquisition procedure

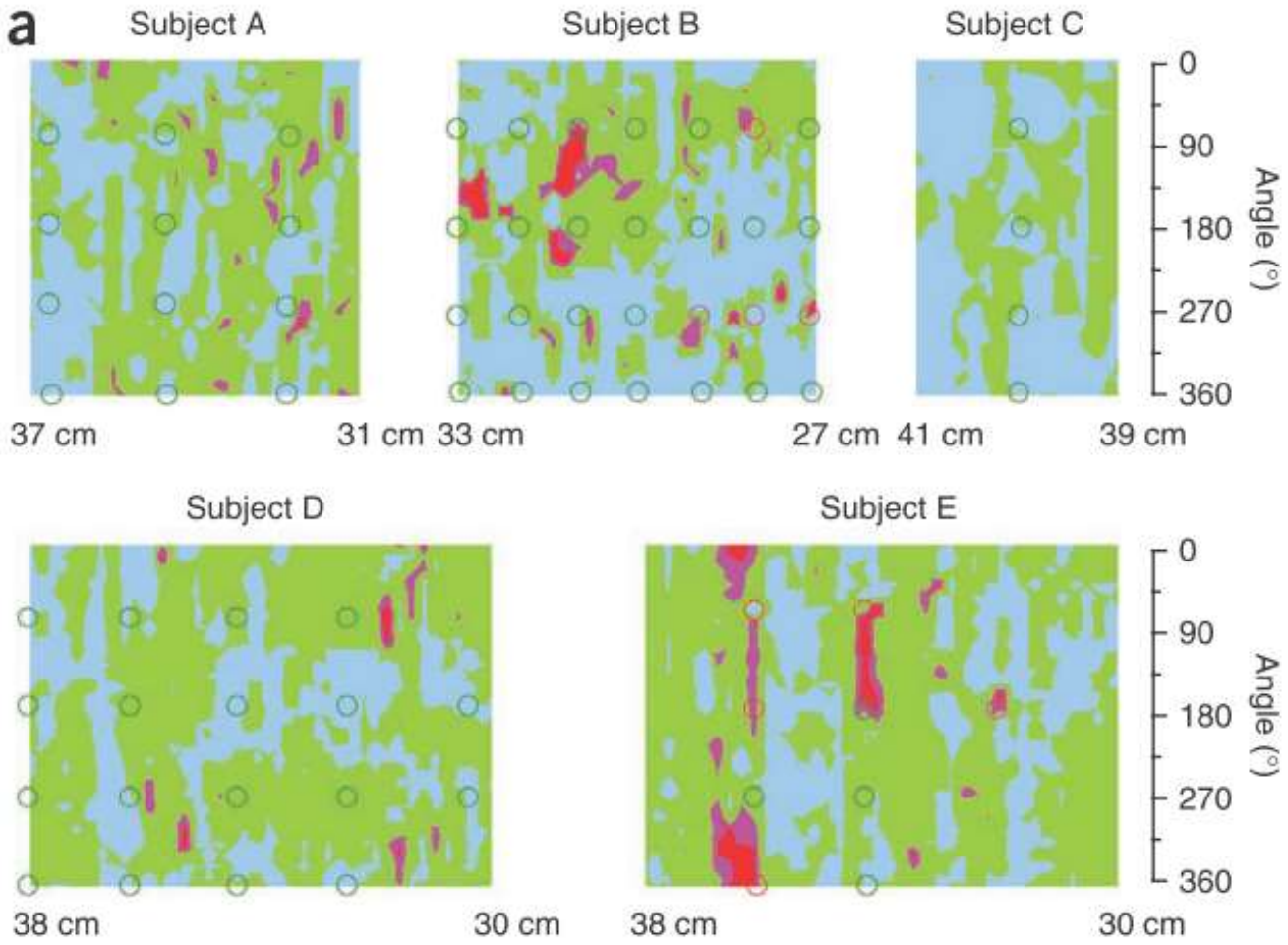
Qiu et al., "Multispectral scanning during endoscopy guides biopsy of dysplasia in Barrett's esophagus," Nature Med., ***16****(5), 603-607 (2010)*

Esophageal maps



Qiu et al., "Multispectral scanning during endoscopy guides biopsy of dysplasia in Barrett's esophagus," Nature Med., ***16****(5), 603-607 (2010)*

Esophageal maps



92% sensitivity,
96% specificity

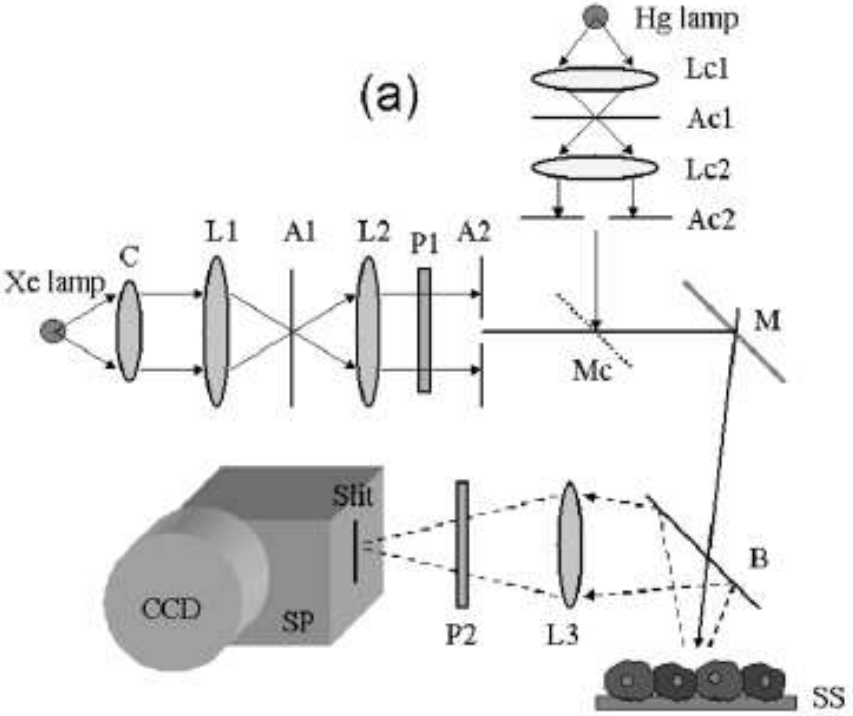
*Qiu et al., "Multispectral scanning during endoscopy guides biopsy of dysplasia in Barrett's esophagus," Nature Med., **16**(5), 603-607 (2010)*

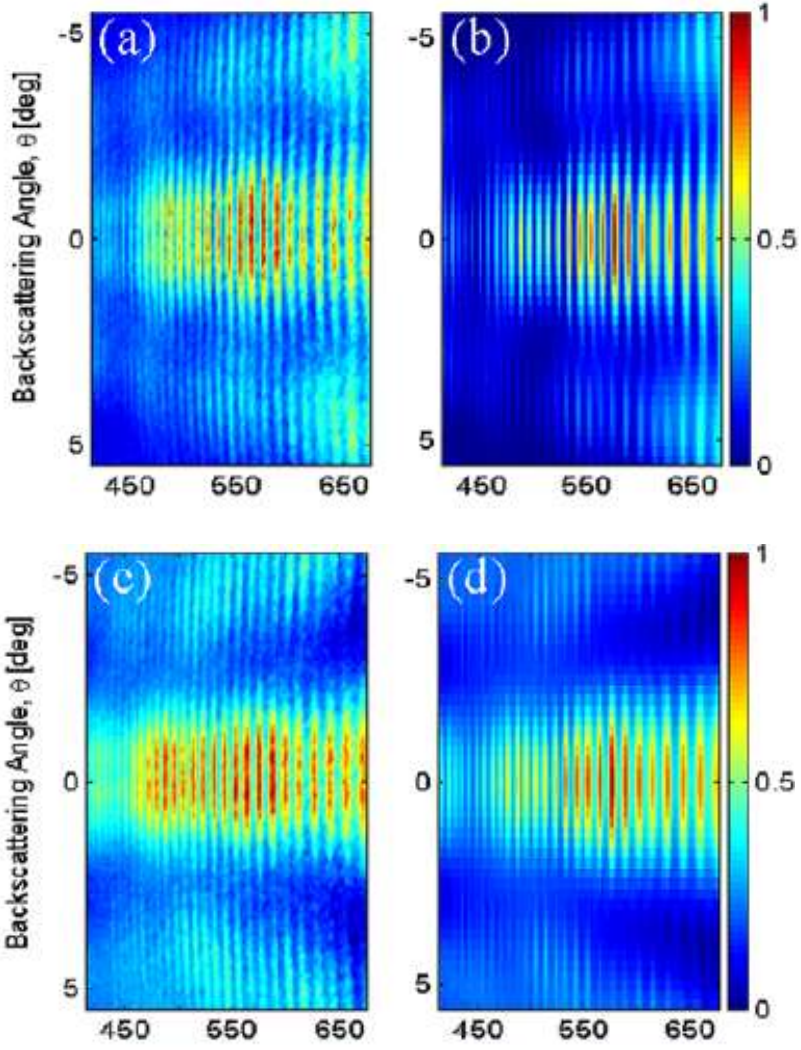
Angular AND spectral information at once!

IEEE JOURNAL OF SELECTED TOPICS IN QUANTUM ELECTRONICS, VOL. 9, NO. 2, MARCH/APRIL 2003 243

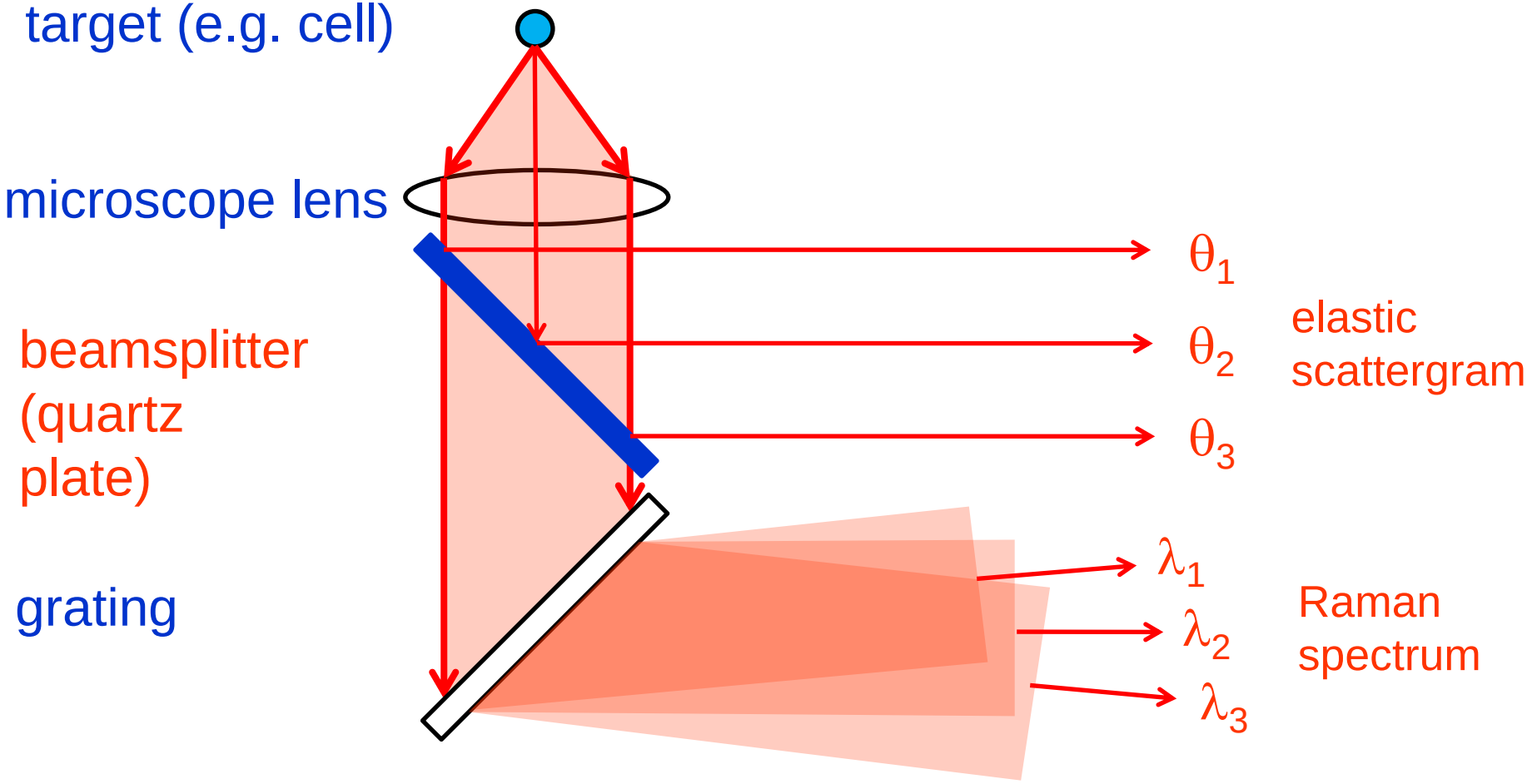
Simultaneous Measurement of Angular and Spectral Properties of Light Scattering for Characterization of Tissue Microarchitecture and Its Alteration in Early Precancer

Young L. Kim, Yang Liu, Ramesh K. Wali, Hemant K. Roy, Michael J. Goldberg, Alexey K. Kromin, Kun Chen, and Vadim Backman

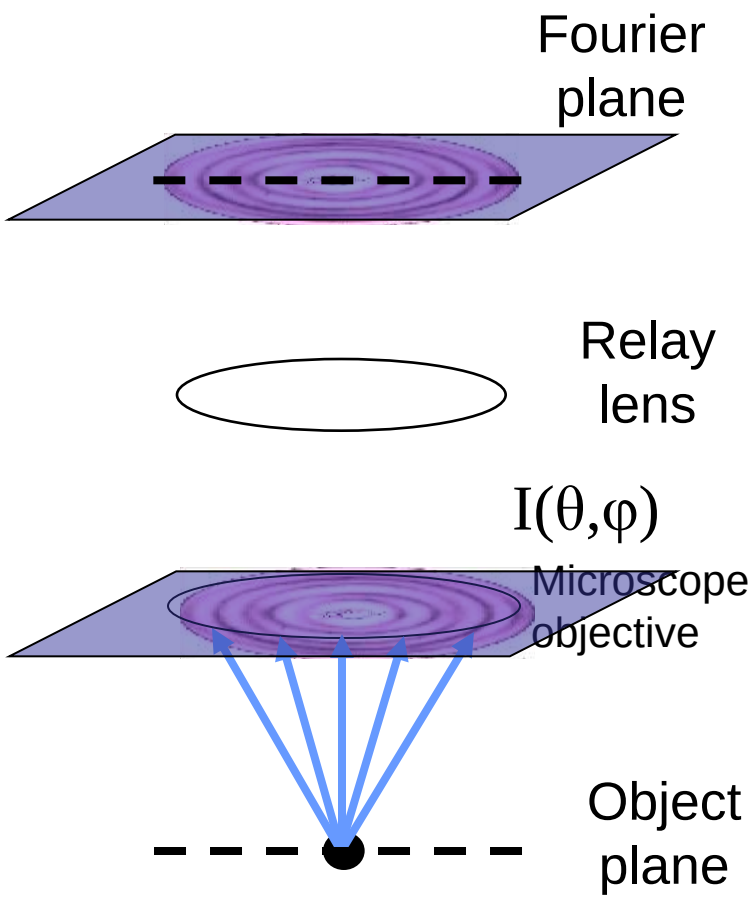




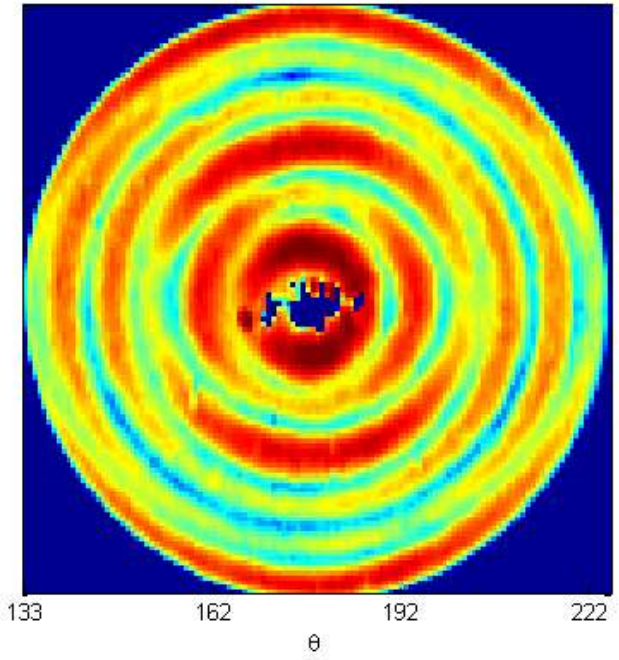
Angular and Raman at once!



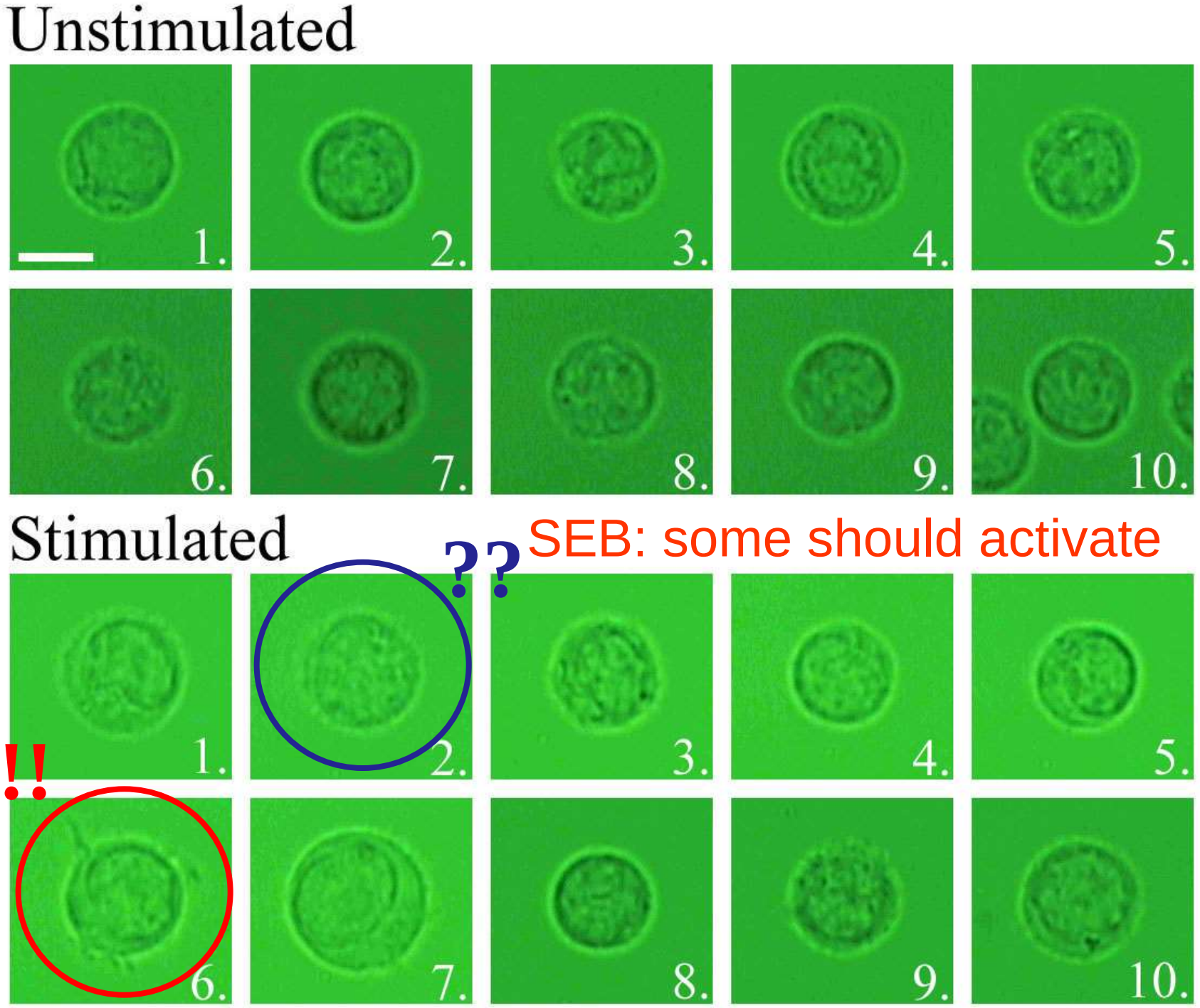
Recording the angular pattern



Angle Mapped to Position

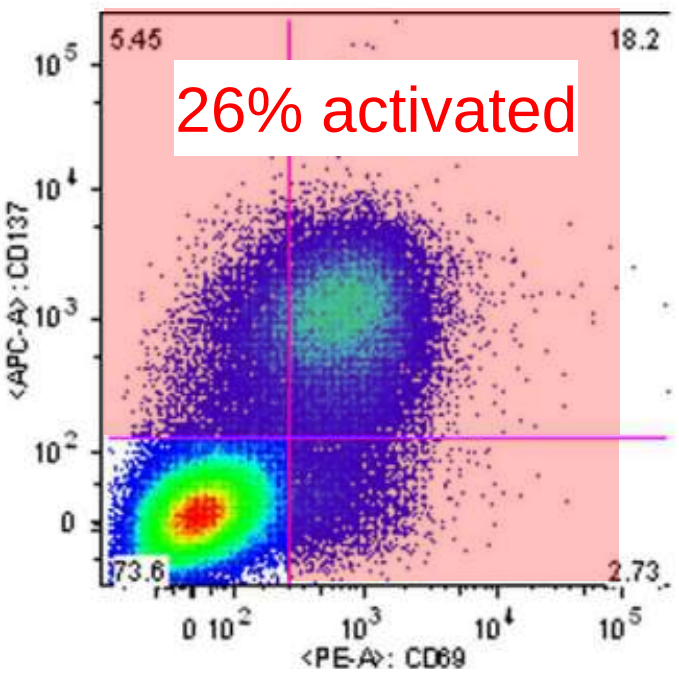


I(x,y) in Fourier plane = I(θ,φ) in object plane

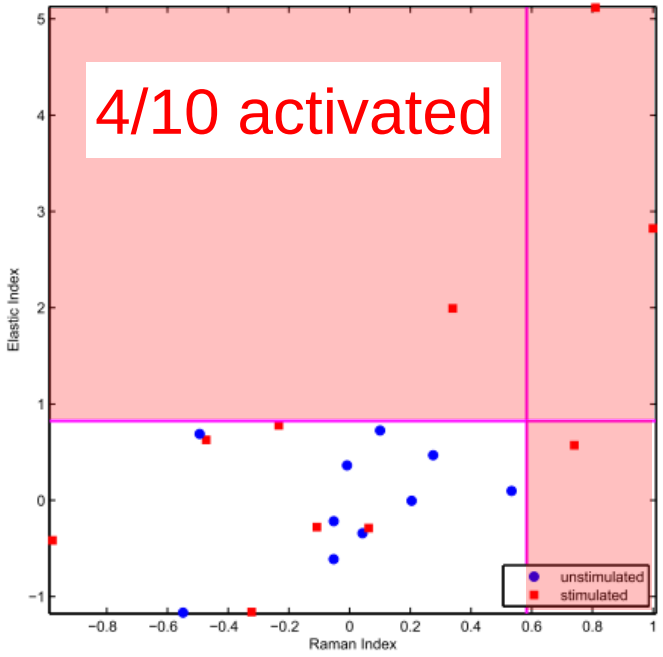

Unstimulated
1.
2.
3.
4.
5.
6.
7.
8.
9.
10.
Stimulated
??
SEB: some should activate
!!
1.
2.
3.
4.
5.
6.
7.
8.
9.
10.

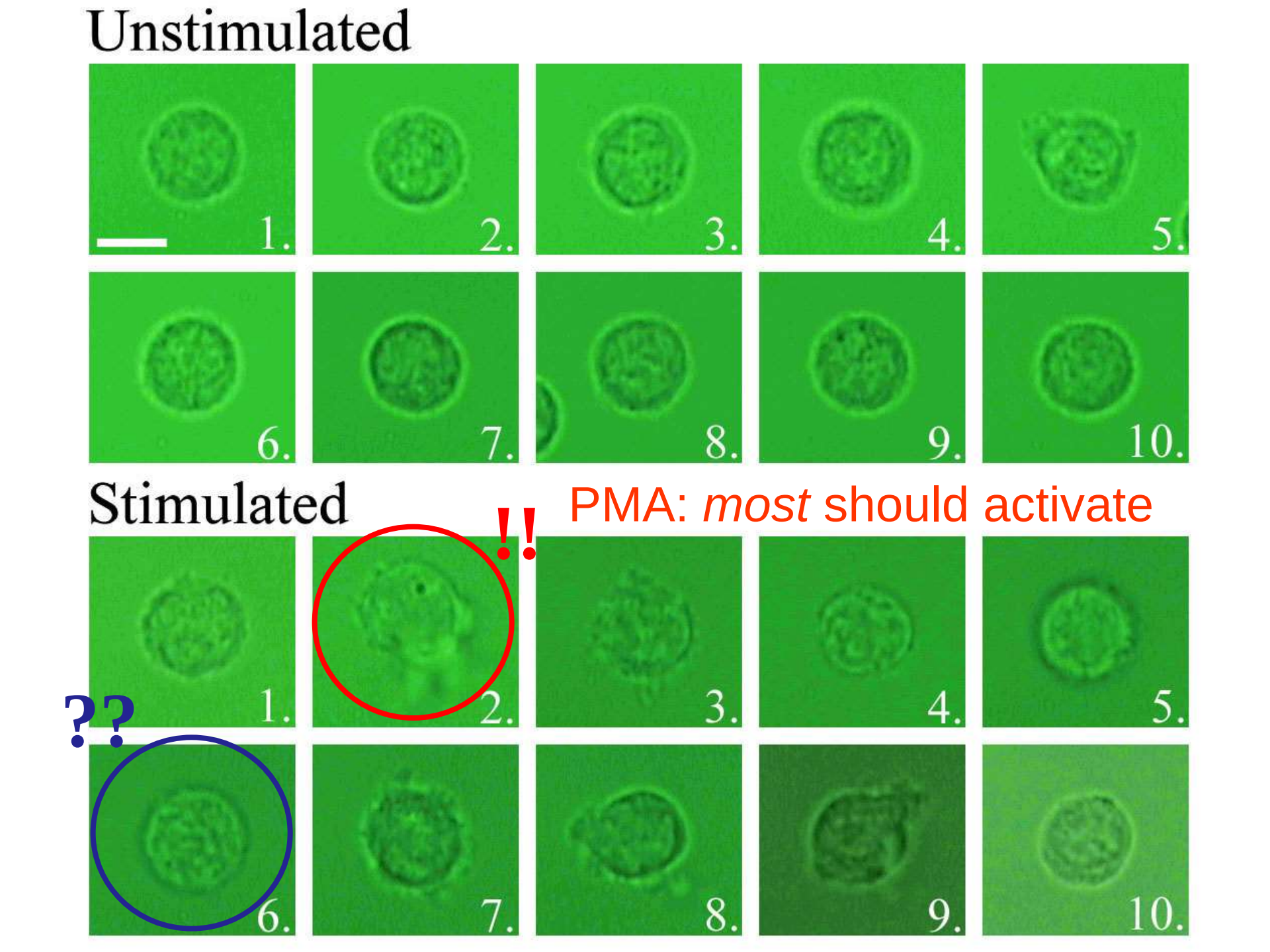
Flow cytometry comparison…

Flow cytometry

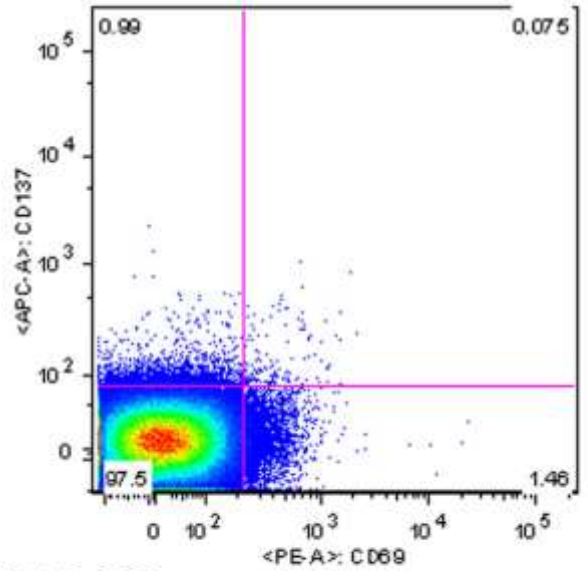


IRAM analysis

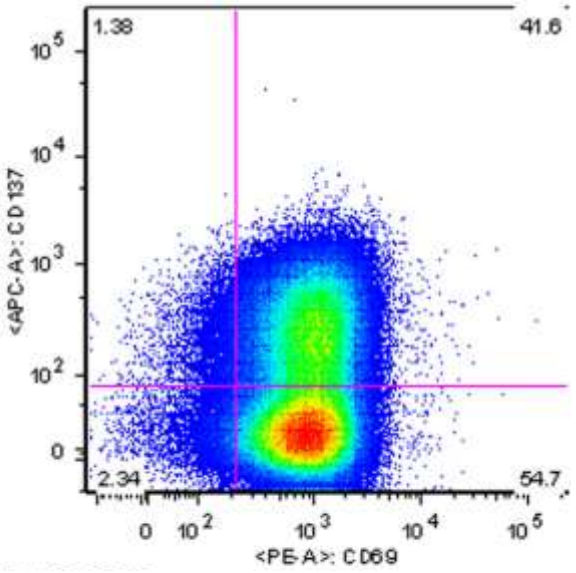



Unstimulated
1.
2.
3.
4.
5.
6.
7.
8.
9.
10.
Stimulated
!!
PMA: *most* should activate
??
1.
2.
3.
4.
5.
6.
7.
8.
9.
10.

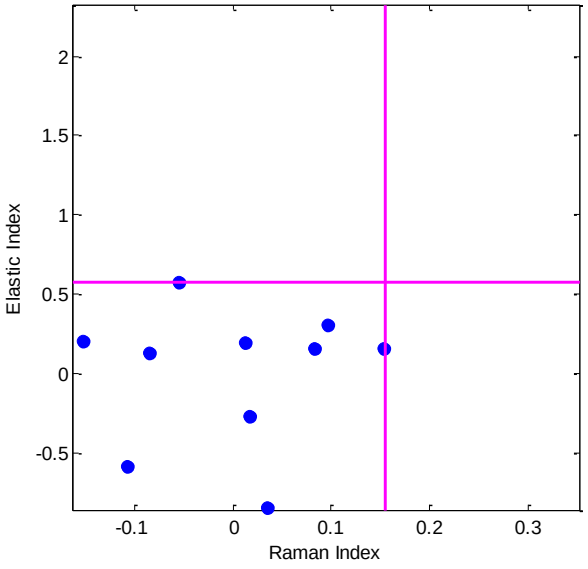
Unstimulated

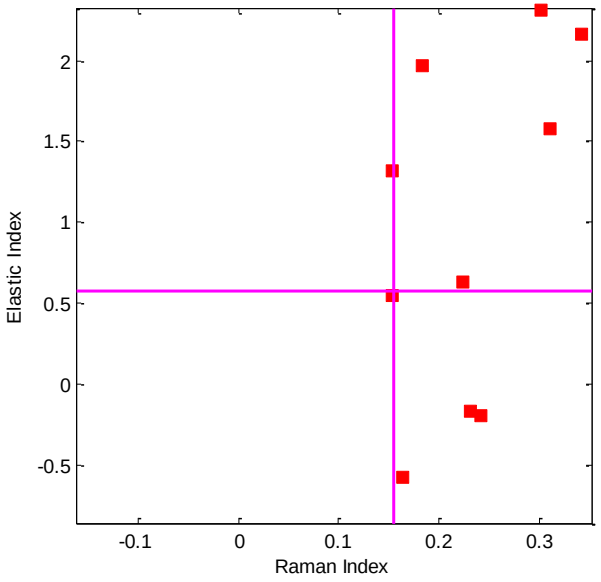


Stimulated



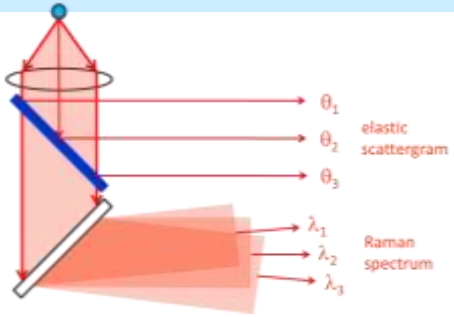
Flow cytometry (gold standard)



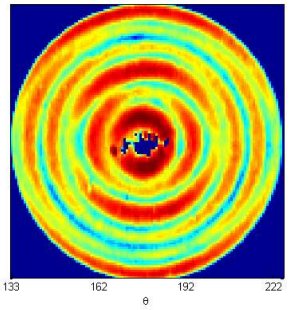


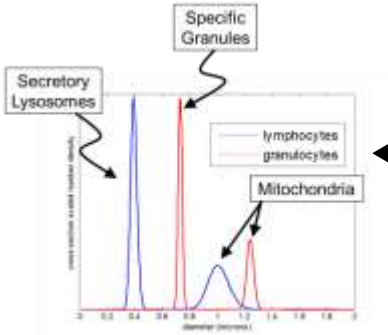
Smith et al., J. Biomed. Opt., 15(3), 036021 (June 2010).

Summary: single-cell organelle sizing



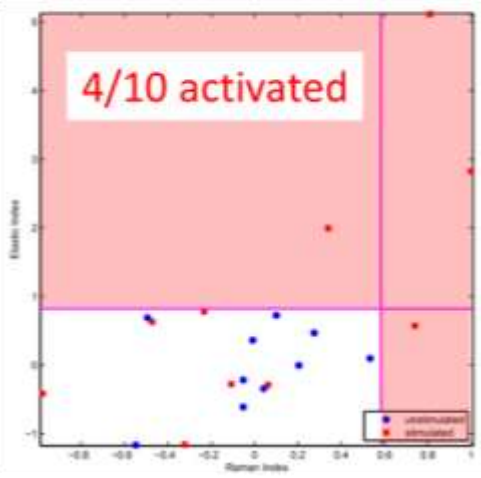
multimodal with
Raman spectroscopy





fit *angular scatter pattern*
to 2-size distribution

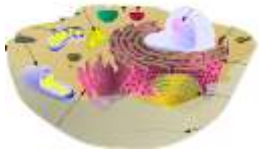
IRAM analysis



detect differences
or changes in
cellular response

Road map for today

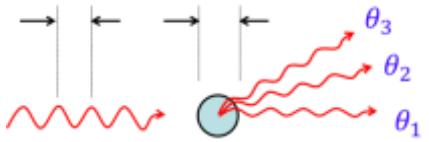
Why scattering (as opposed to absorption)?



Scattering you may have already heard about

Fundamentals of elastic scattering

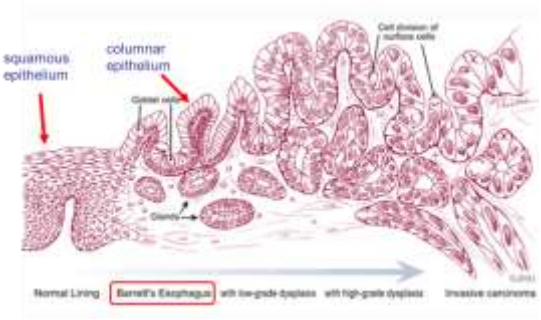
$$\sim 1 - \frac{\sin(2\delta/\lambda)}{\delta/\lambda} + \left[\frac{\sin(\delta/\lambda)}{\delta/\lambda}\right]^2$$



wavelength-resolved
angularly-resolved

Experiments and applications

spectral domain
angular domain



Review of lectures

Tuesdays in January (7.1, 14.1, 21.1, 28.1), 2:00 pm, IPHT Sitzungssaal

Lecture 2 - Turbid tissue optics I: *Introduction*
Lecture 3 - Turbid tissue optics II: *Instrumentation and measurements*
Lecture 4 - Turbid tissue optics III: *(More) Applications*
Lecture 5 - A different view of turbidity: *elastic scattering analysis*

Thank you very much!