Northeastern University
College *of* Engineering

Biomedical Imaging

Optical Imaging

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EECE–4649
Northeastern University

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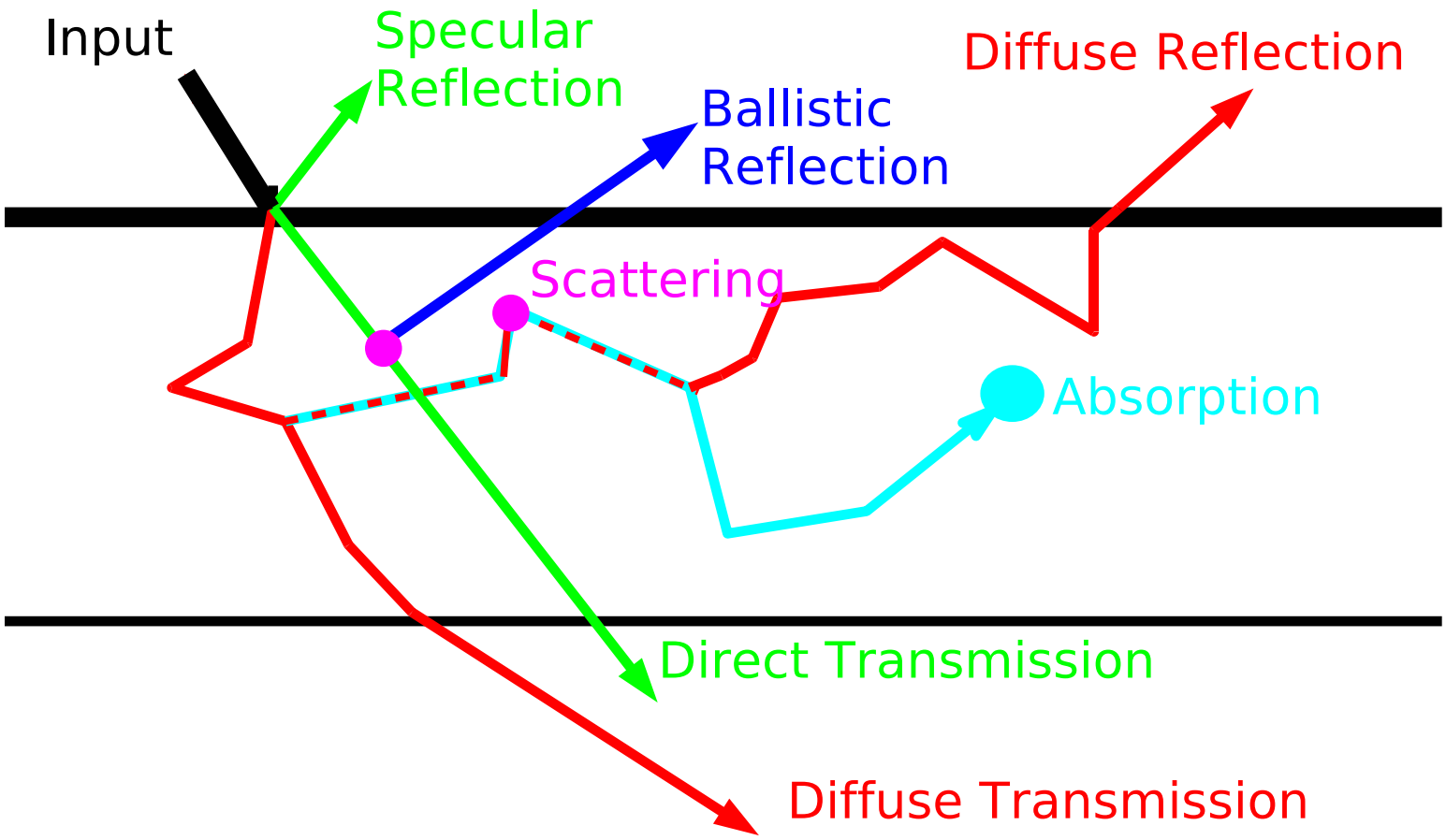
Optical Imaging

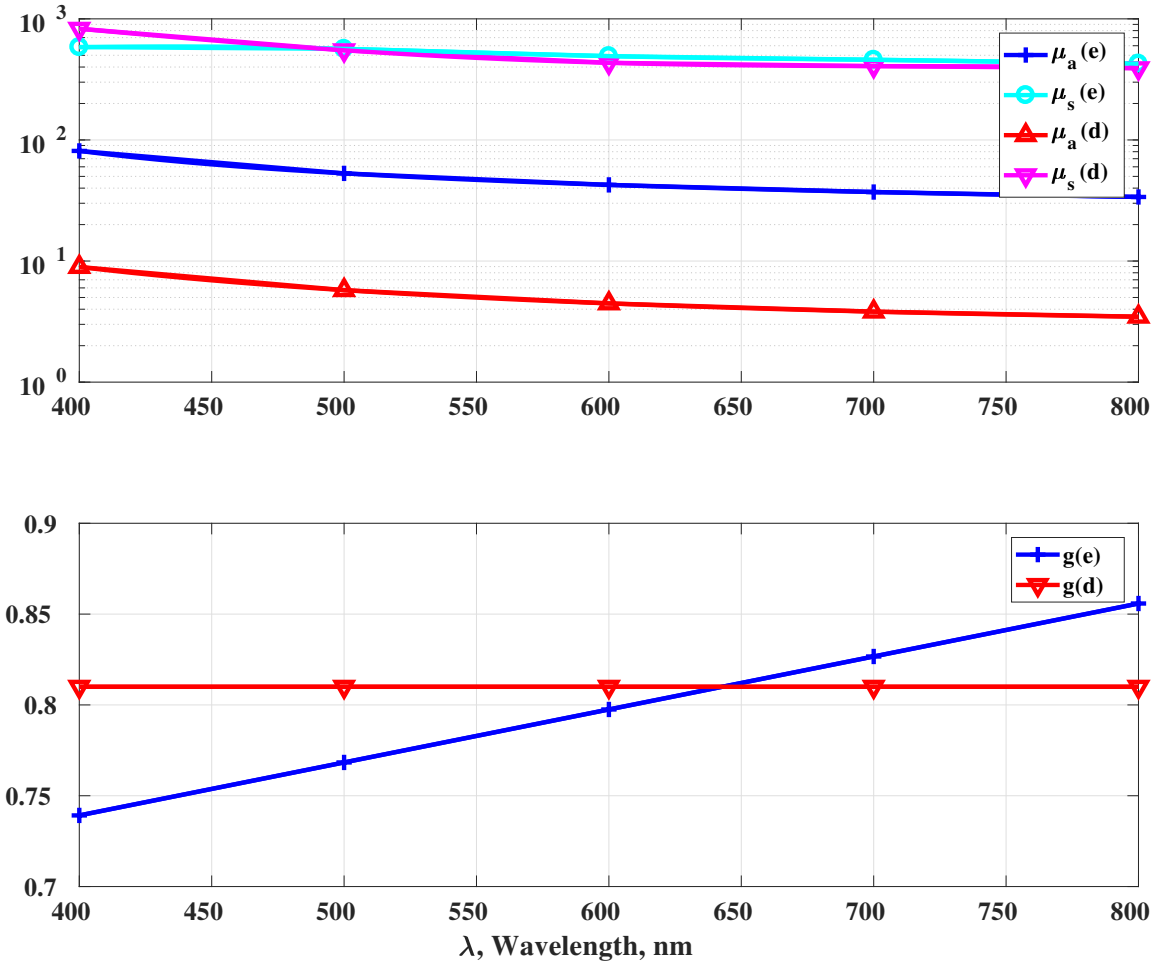
- Basics; μ_s, μ_a, n
- Optical Instruments: Lens Equation, Magnification
- Fourier Transform: NA, and more
- Sources and Detectors
- Microscopy
  - Brightfield Microscopy
  - Fluorescence
  - Phase Contrast
  - Confocal Microscopy
  - Multi–Photon and Harmonic Microscopy
- Optical Coherence Tomography
- Diffusive Optical Tomography

Waves Interactions

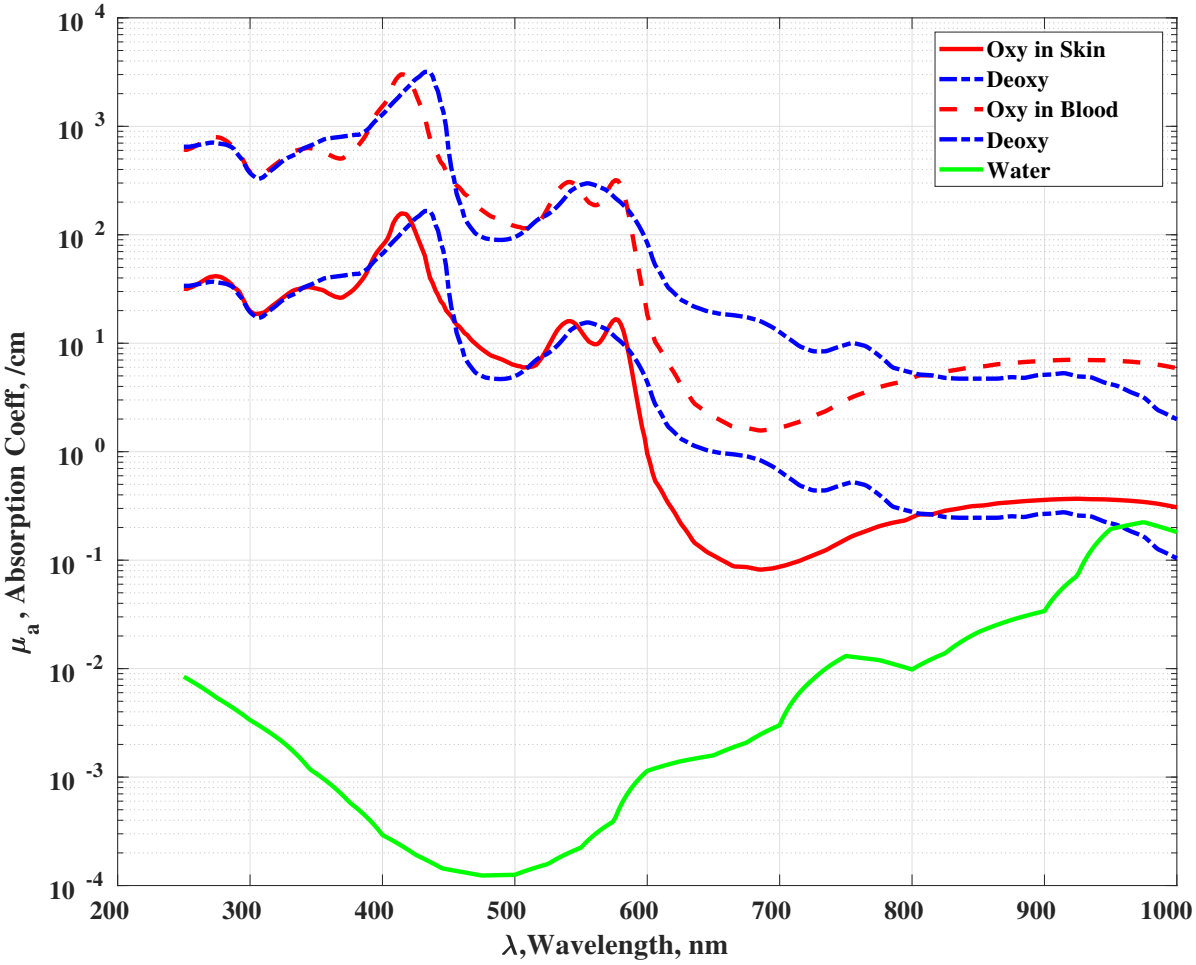


(and of course, emission)

Skin Optical Properties



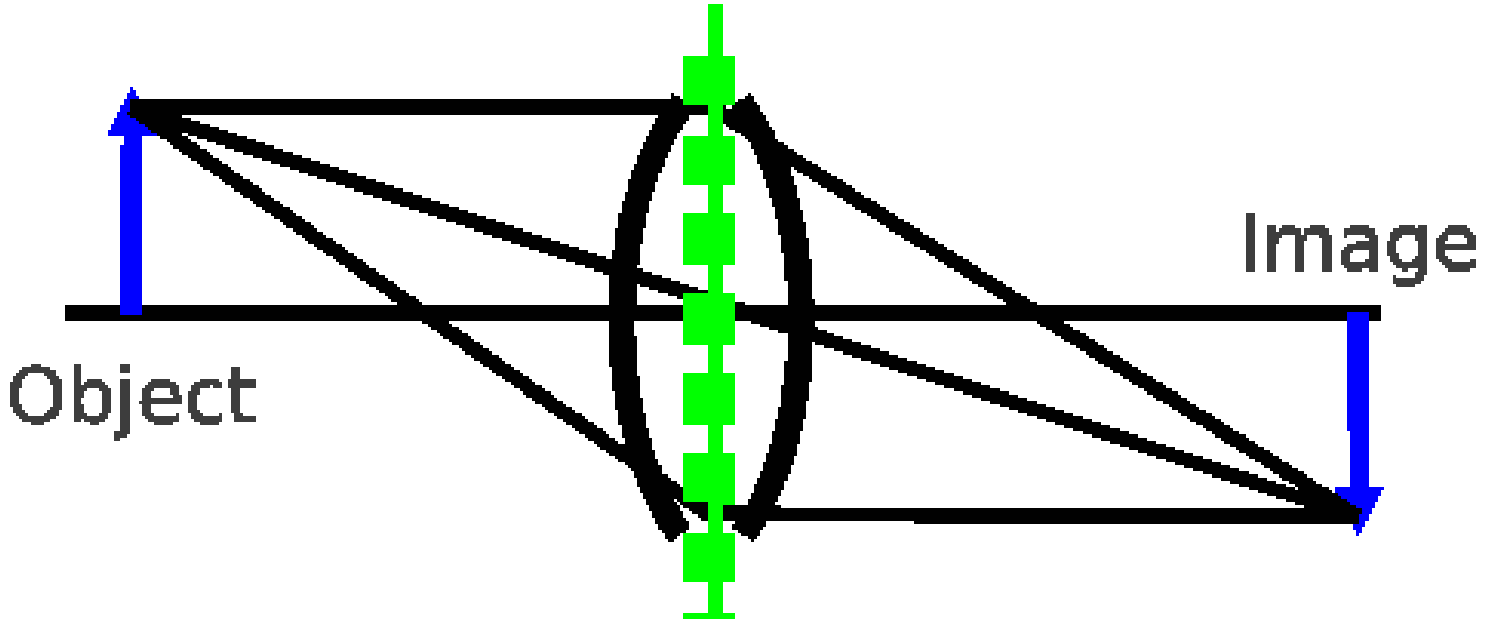
Blood and Water



Light Penetration

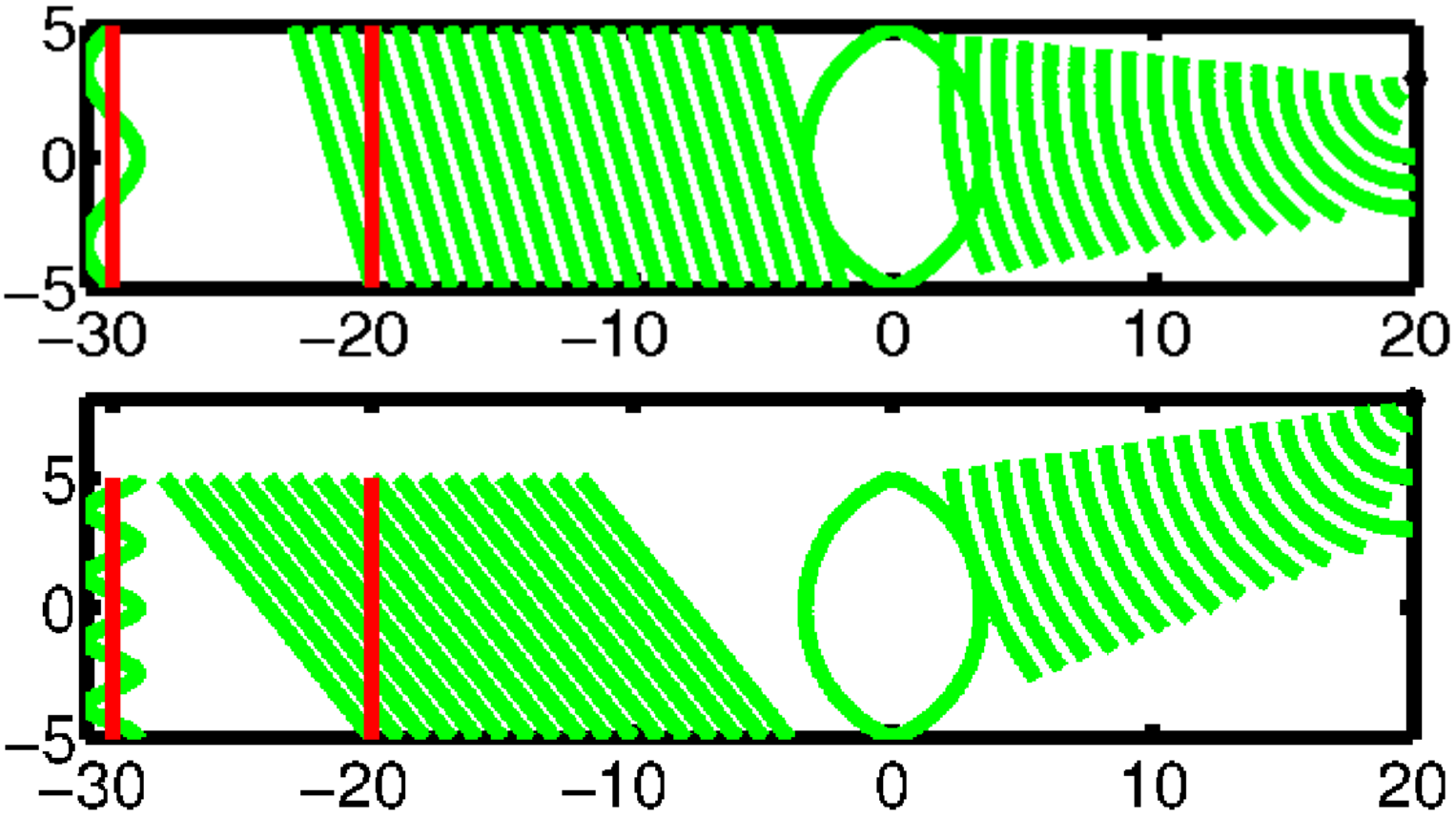
- Best in Near–IR Window
- Ballistic to 100s of micrometers
- Diffuse to centimeters
- Except in the Eye

Lenses

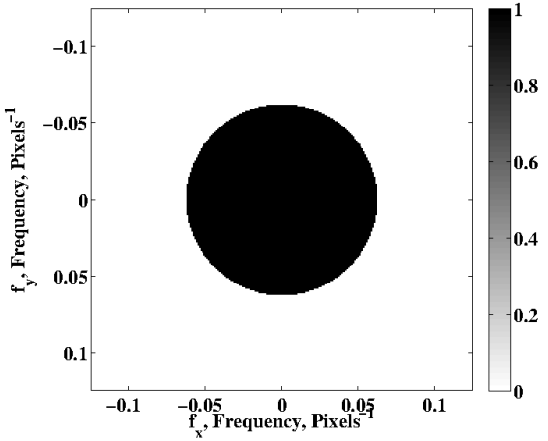


$$\frac{1}{s} + \frac{1}{s'} = \frac{1}{f} \qquad m = \frac{x'}{x} = -\frac{s'}{s}$$

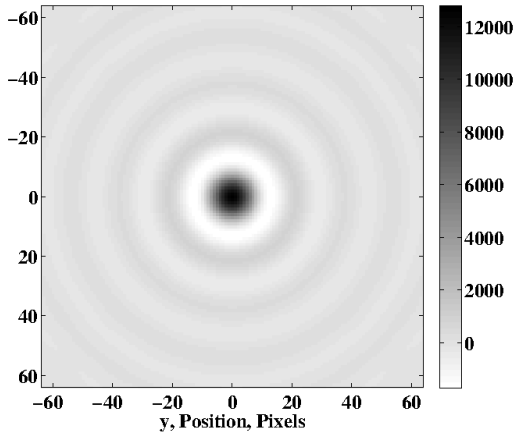
Optical Fourier Transform



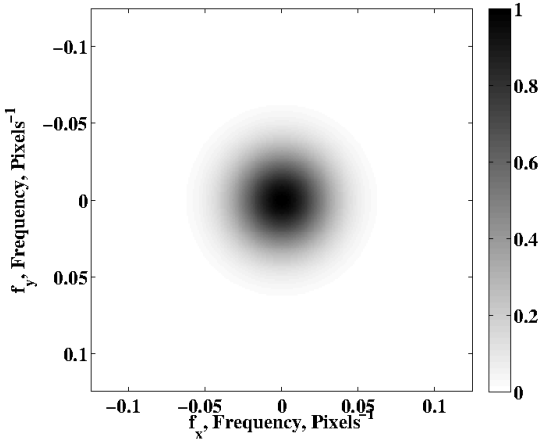
2–D Fourier Transform Pairs



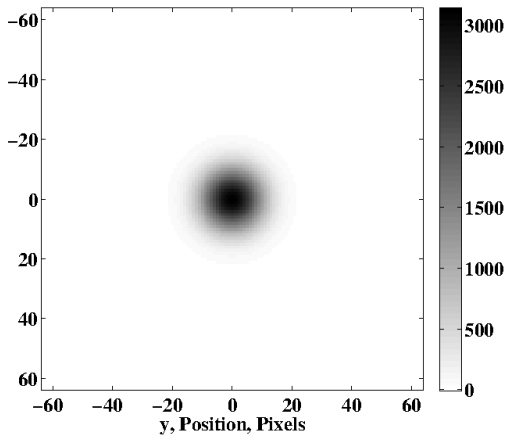
A. Aperture



B. Airy Function PSF

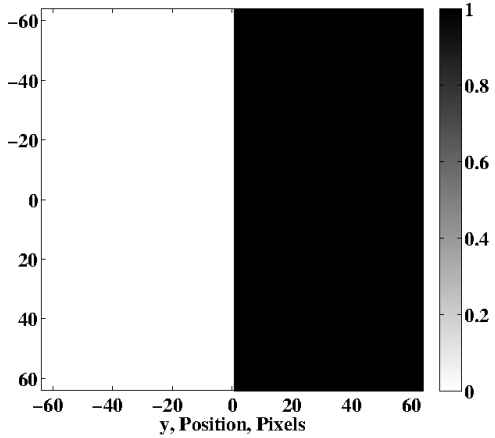


C. Gaussian Apodization

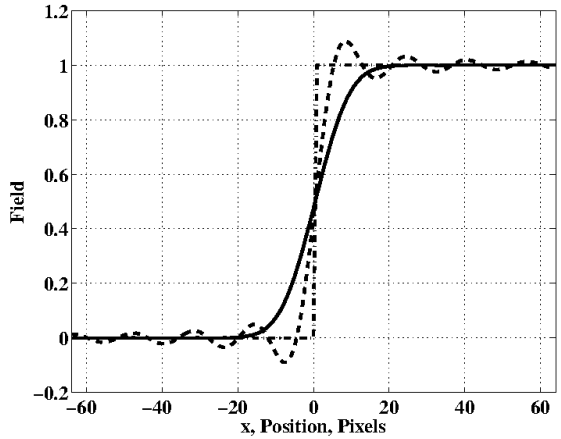


D. Gaussian PSF

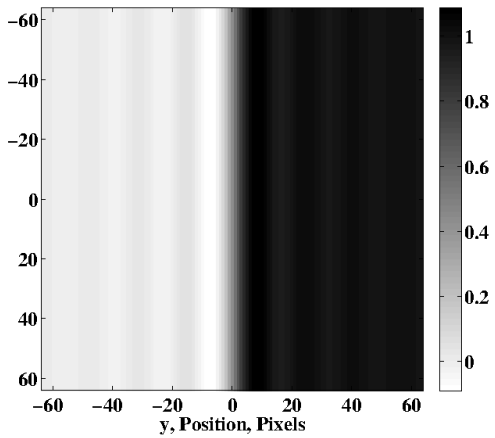
Pupil as Low–Pass Filter



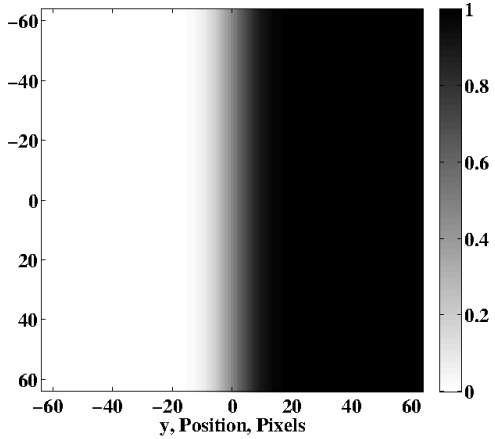
A. Knife Edge Object



B. Image Slices



C. Image with Aperture



D. Image with Gaussian

Resolution

- Transverse

$$f_x = \frac{u}{\lambda} = \frac{\sin\theta\cos\zeta}{\lambda} \qquad MAX = \frac{NA}{\lambda}$$

$$\delta = \frac{\lambda}{NA}$$

- Axial

$$\delta z = \frac{\lambda}{NA^2}$$

- Examples

$NA = 0.95 \quad \lambda = 500$ nm $\quad \rightarrow \quad$ 526 nm $\qquad f_{max} = 1900/\text{mm}$

$NA = 0.25 \quad \lambda = 800$ nm $\quad \rightarrow \quad$ 3.2 μm $\qquad f_{max} = 312/\text{mm}$

Light Sources

- Tungsten Lamp
- Quartz–Halogen–Tungsten Lamp
- Mercury Lamp
- Light–Emitting Diode
- Laser (Pulsed, CW)

Detectors

- Photon Detectors vs. Thermal Detectors
- Some Vacuum Photomultipliers
- Mostly Silicon Photon Detectors
- Arrays
  - Slower
  - Massively Parallel
  - Pixel Size Choices (Resolution, Full Well, *etc.*

Early Microscopes

- Compound Microscope (Jansen, 1590)
- Simple Microscope (m=300) (Leeuwenhoek, early 1600s)
- Physiological Observation (Hooke 1665)
- Diffraction Theory (Abbe, 1860)
- Diffraction–Limited Imaging (Spencer, mid 1880s)

Modern Microscopy

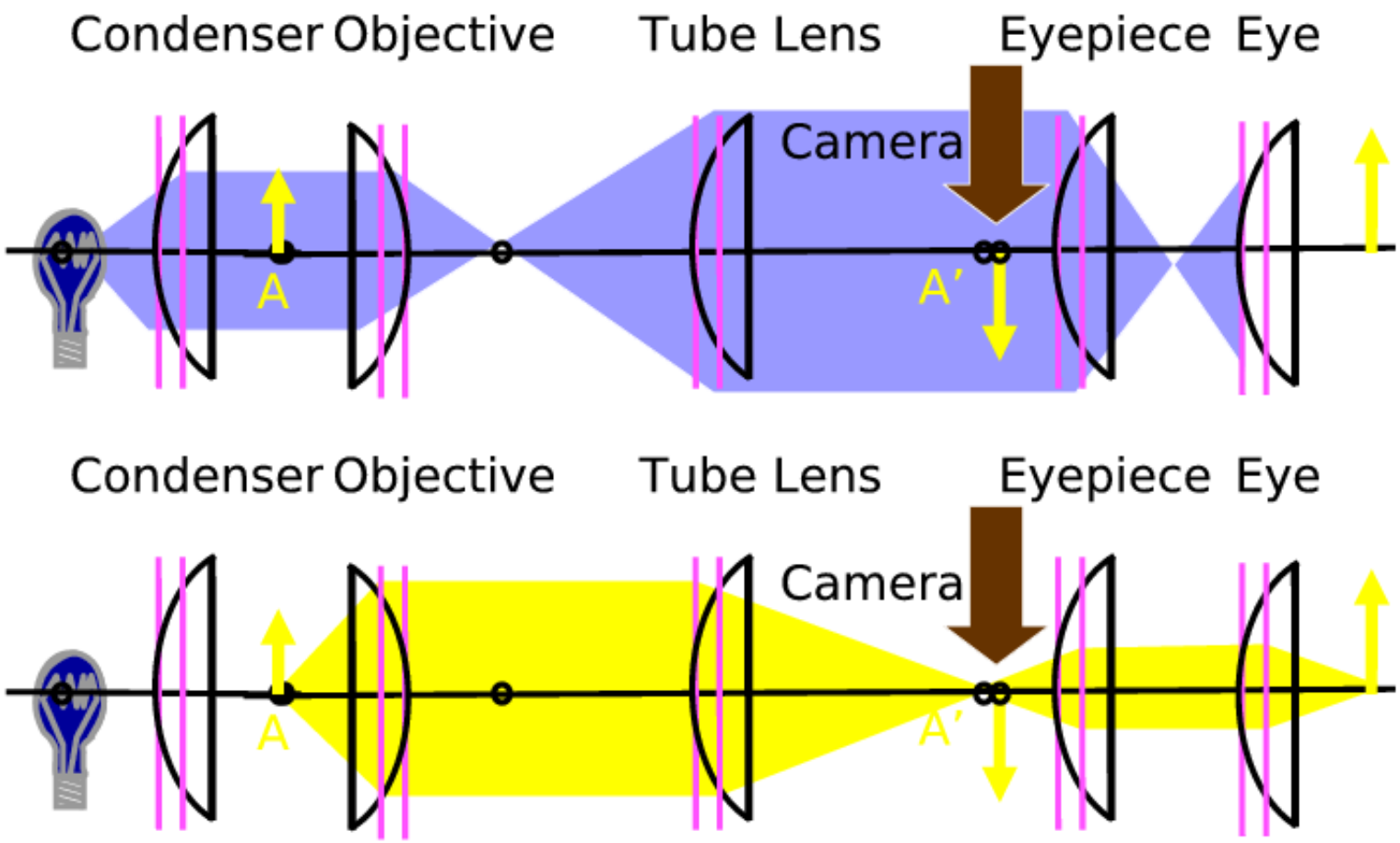
- What's so Modern?
  Microscopy has been around since 1590. . .

- . . . But a Lot Has Happened in the Last Few Decades

- Three Reasons why the Time is Right
  - Illumination Sources (From Tungsten to Lasers, LEDs)
  - Fast, Low–Cost Computers (and Cameras, *etc.*)
  - Chemistry (Molecular Tags)

Microscope Layout

Fourier Transform Between Field Planes and Pupil Planes



Example

- 10X 0.25 Objective with Green Light

$$NA = 0.25 \quad \lambda = 500 \text{ nm} \quad \rightarrow \quad 2\ \mu\text{m}$$

- Resolution on Camera

$$2\ \mu\text{m} \times 10 = 20\ \mu\text{m}$$

- Camera Pixel 5 micrometers

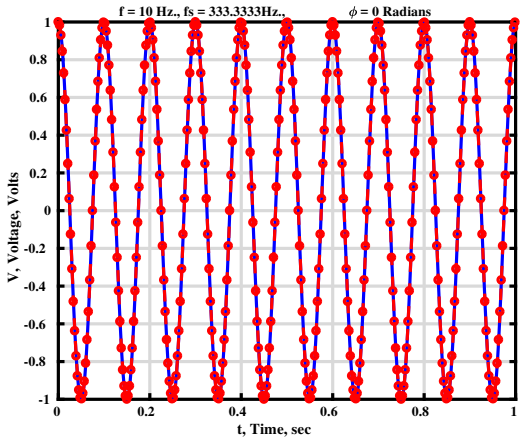
- Point–Spread Function Covers 4 Pixels

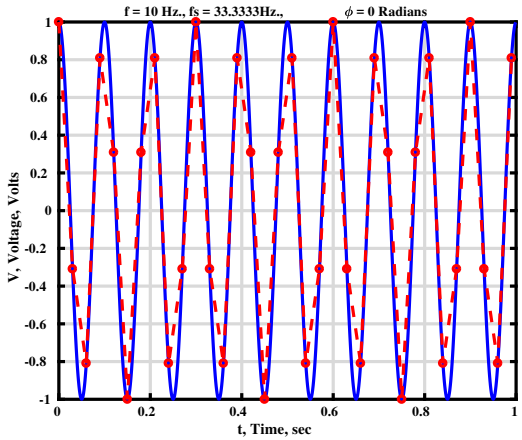
Sampling with an Array

- Pixel Pitch *vs.* Pixel Size
- Pixel Pitch *vs.* Object Size
- Blurring
- Aliasing
- Nyquist
- Anti–Aliasing Filter

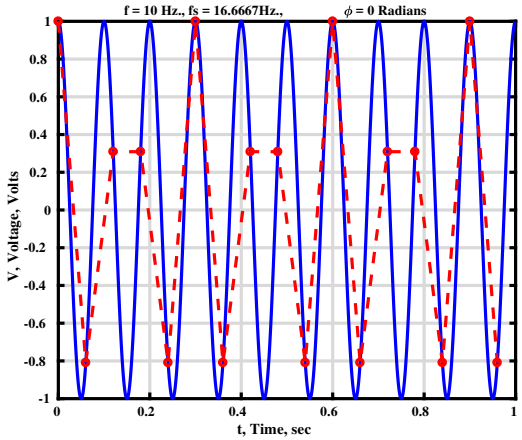
Sampling Example

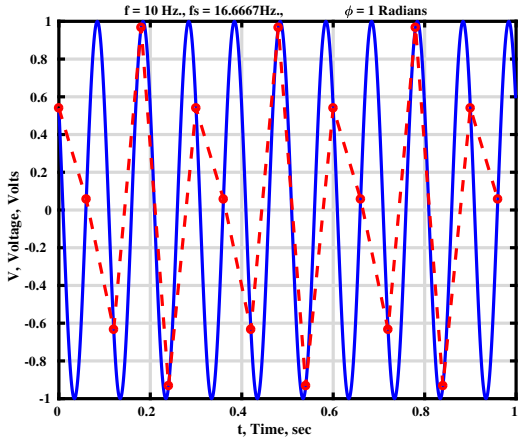
Keeping Nyquist Happy . . .



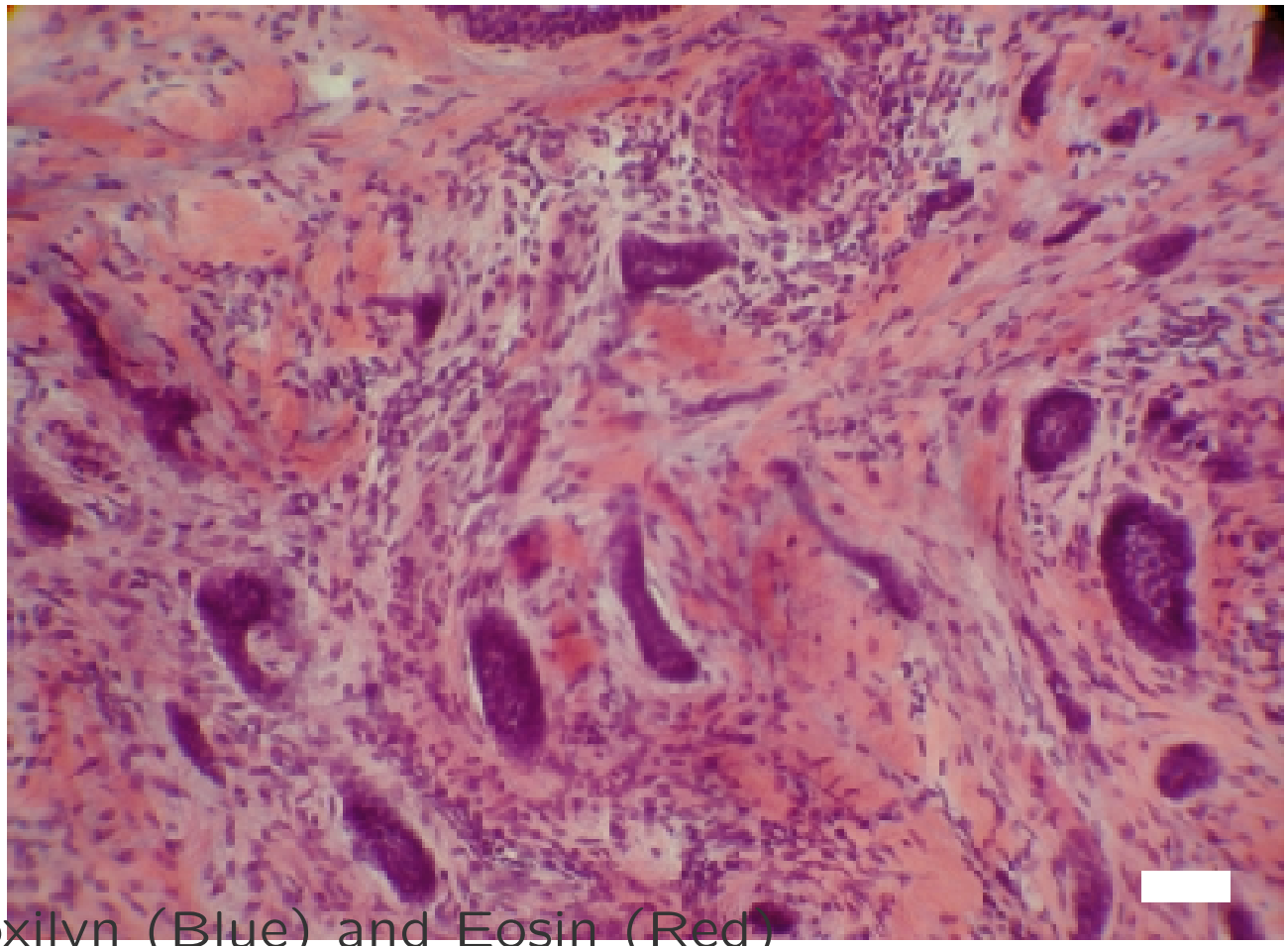


. . . or Not





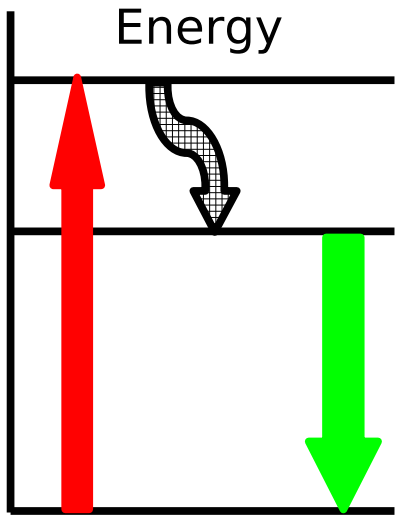
Pathology Slide



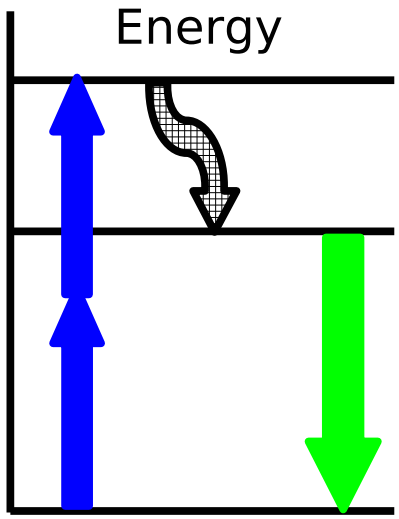
Hematoxilyn (Blue) and Eosin (Red)

Milind Rajadhyaksha

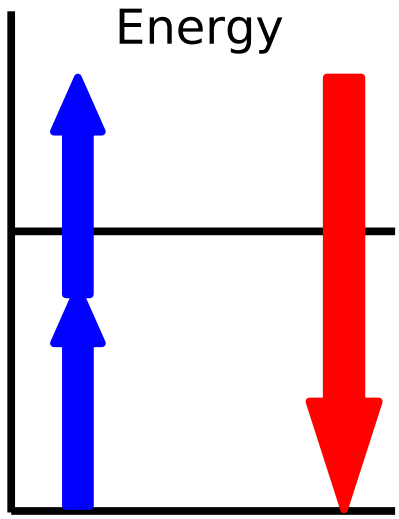
Wavelength–Changing Processes



Fluorescence

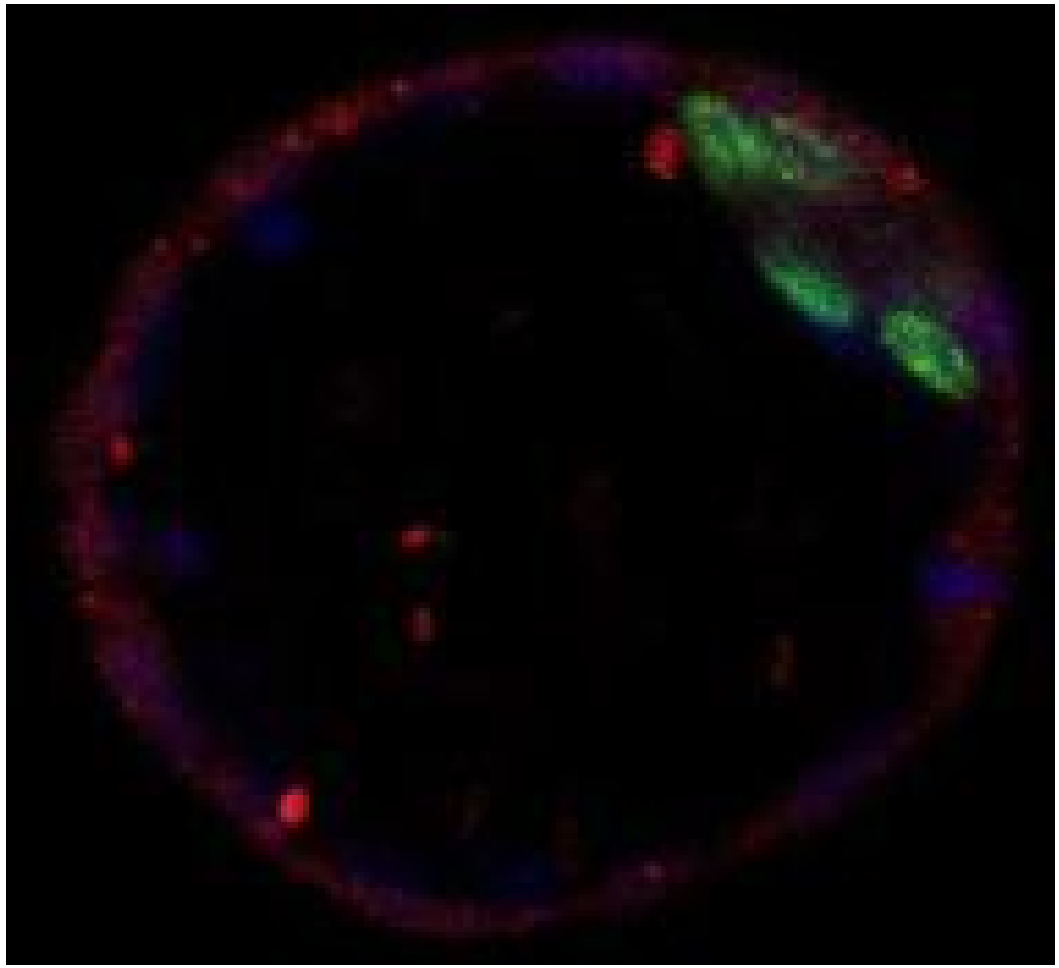


2–Photon Fluorescence



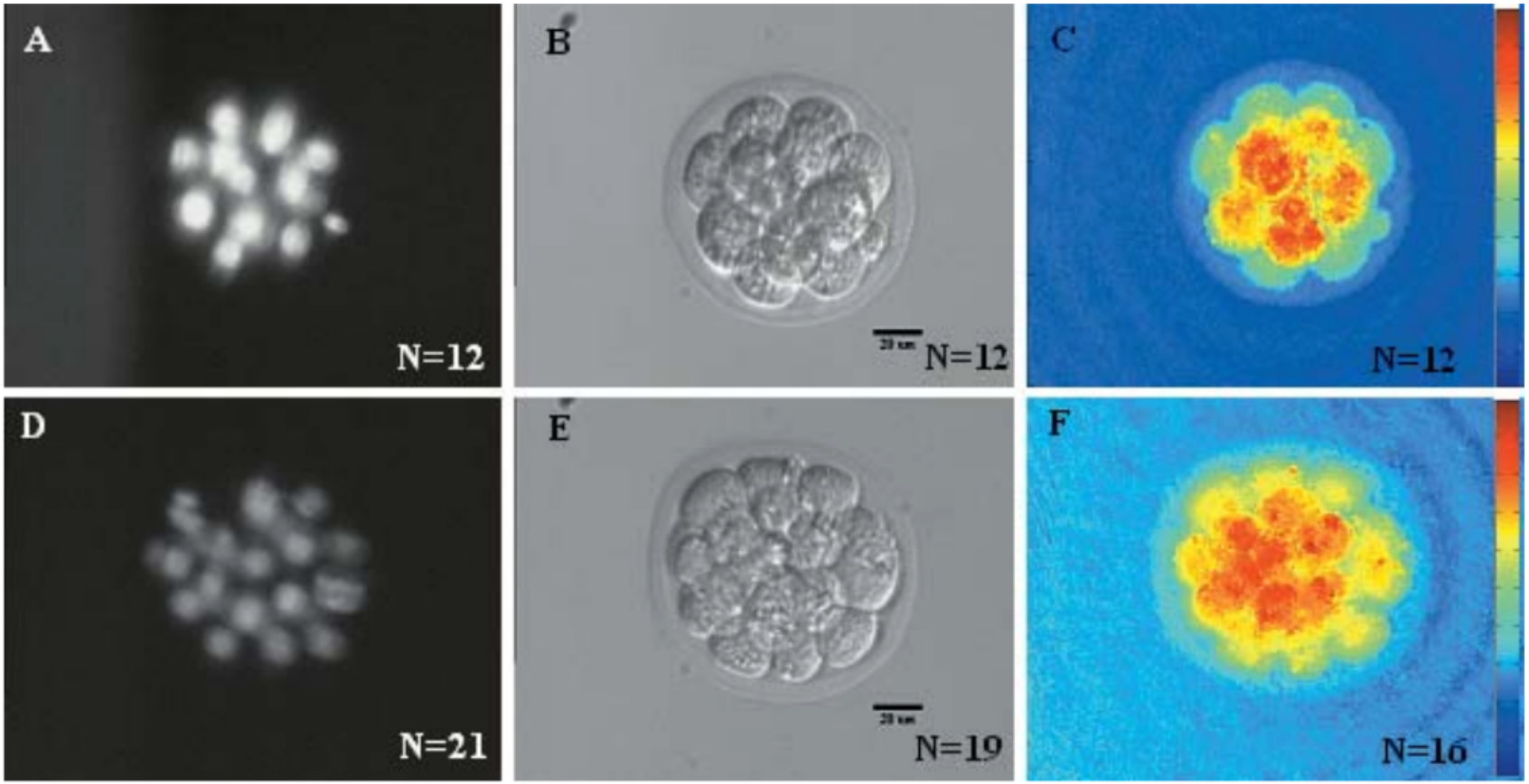
Second Harmonic

Fluorescence Imaging



Gal, OCT4, Dapi
http://www.mediacy.com/index.aspx?page=UManchester_stemcellanalysis

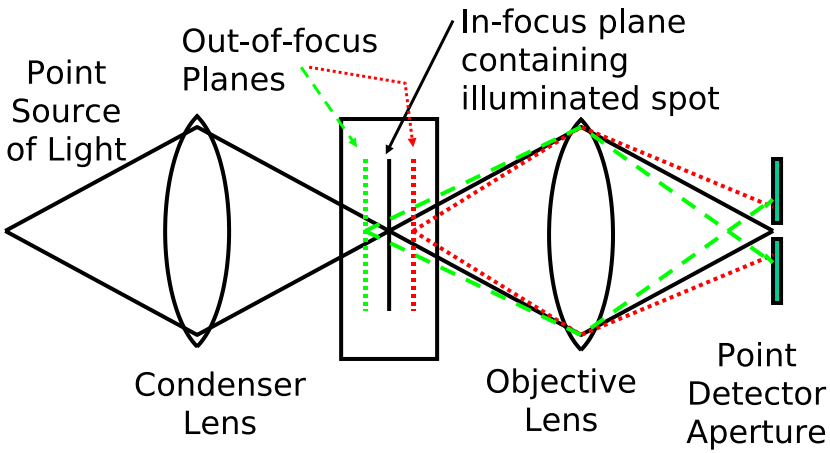
DIC and Phase



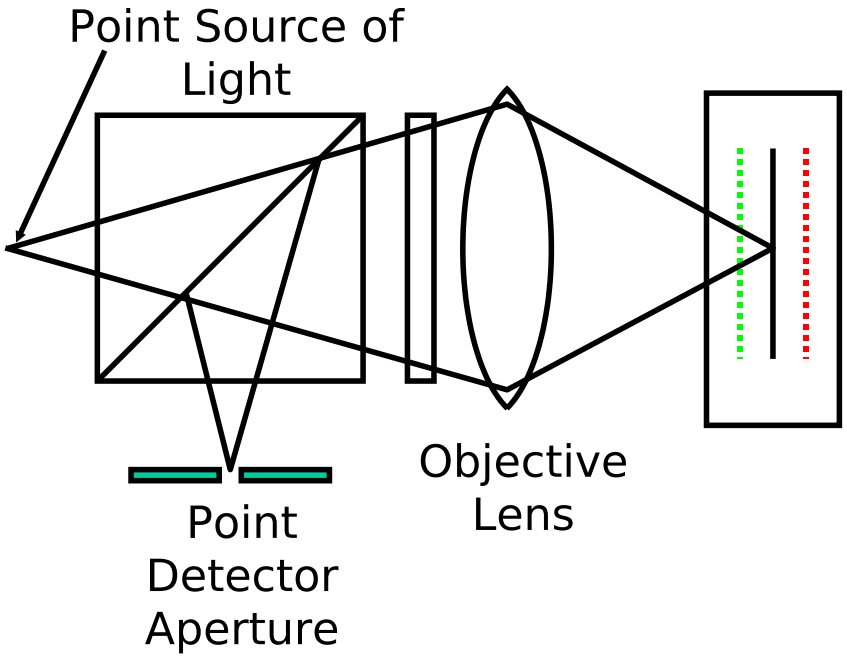
Epi–Fluorescence with Hoechst Dye, *vs.* DIC and OQM

Newmark *Microscopy and Microanalysis*, 2007

Confocal Microscopy



Trans–Illumination

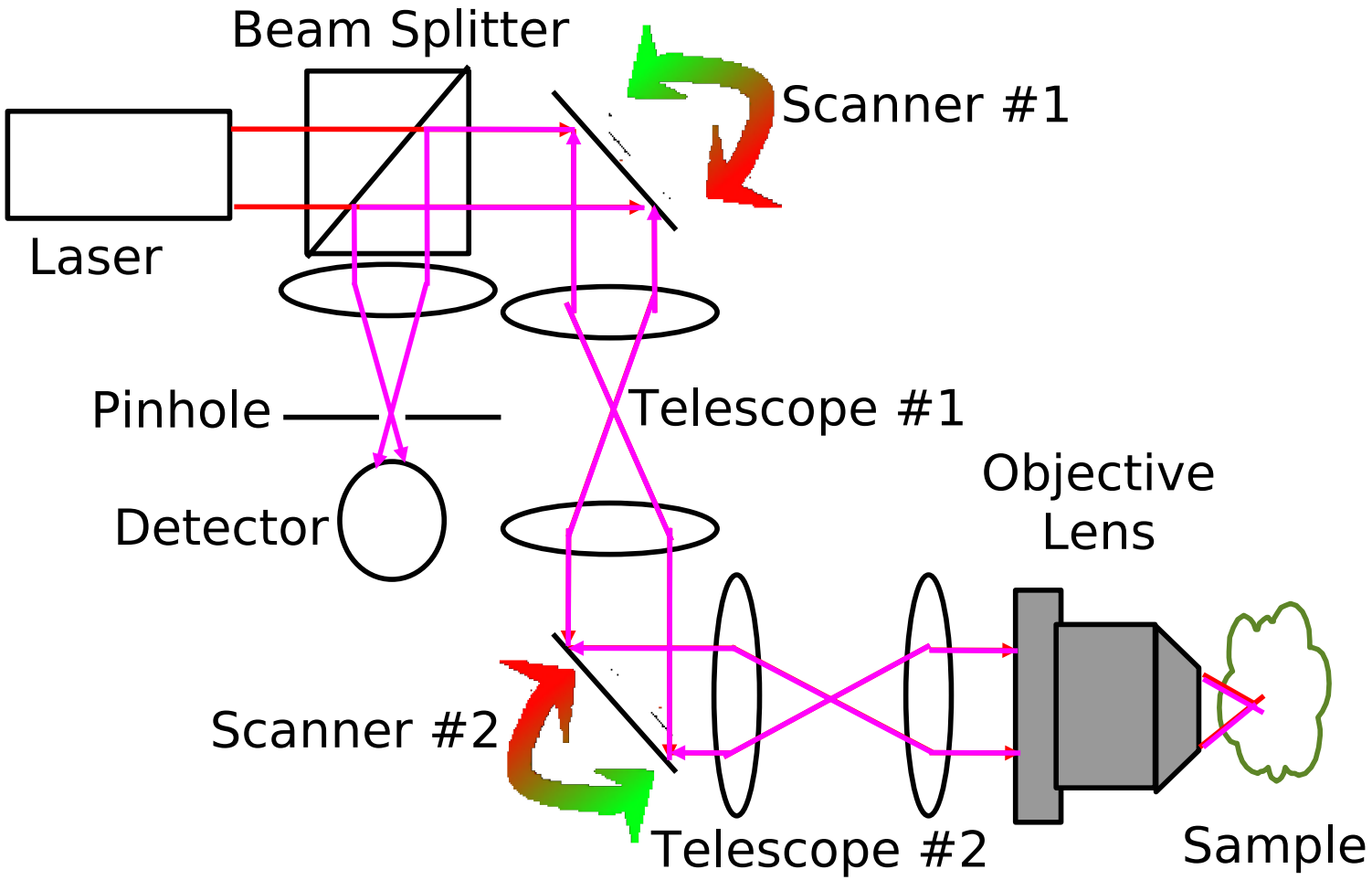


Epi–Illumination (Usual)

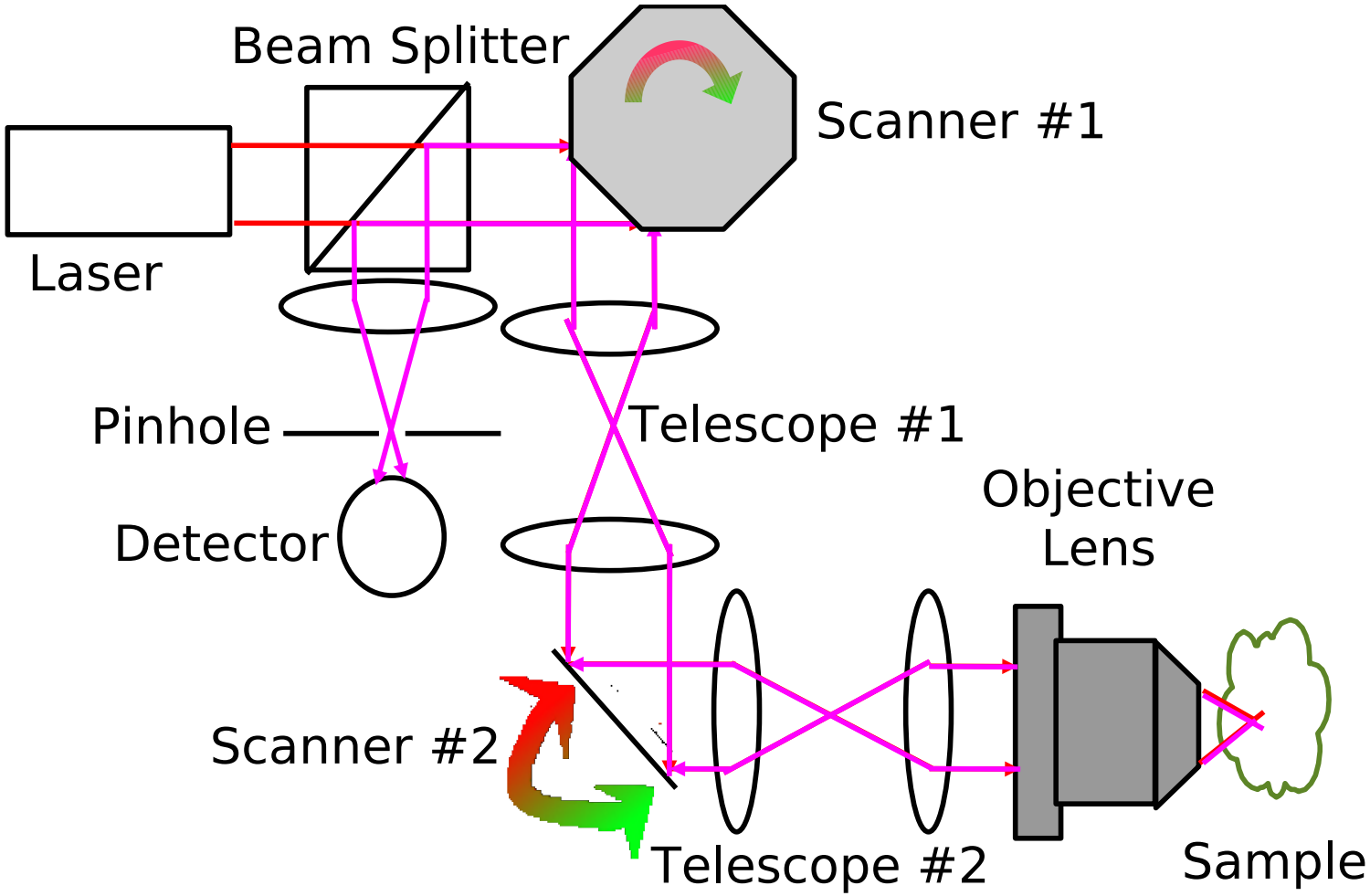
Reflectance or Fluorescence

Adapted from Milind Rajadhyaksha

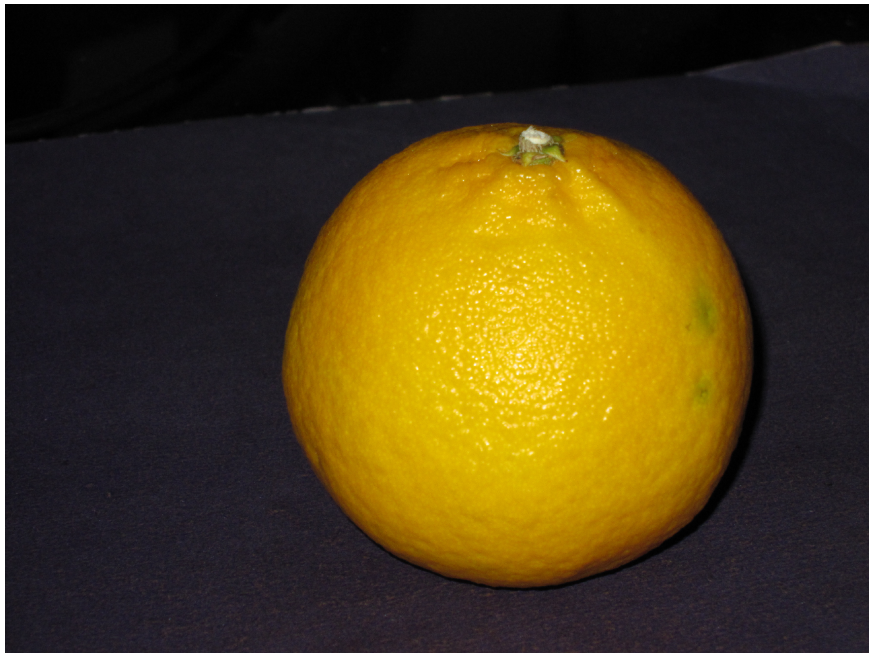
2–Galvo System



Polygon/Galvo System



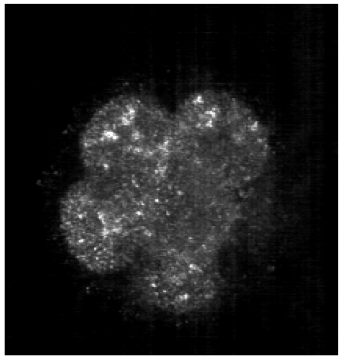
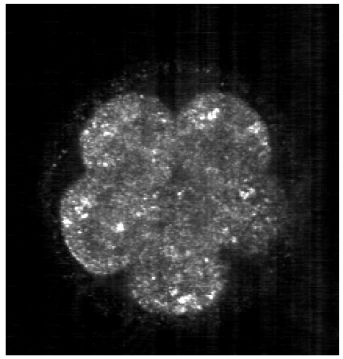
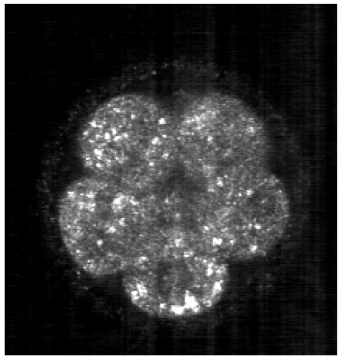
Brightfield Focusing



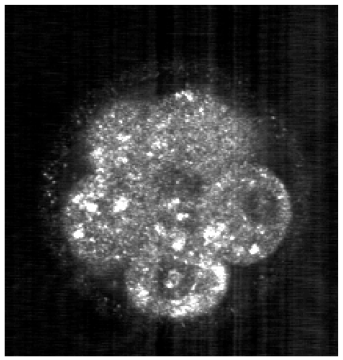
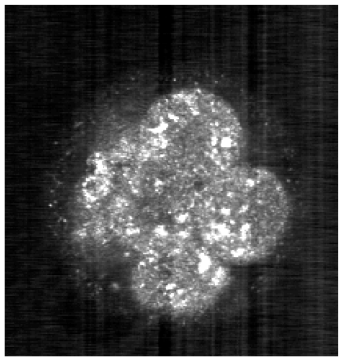
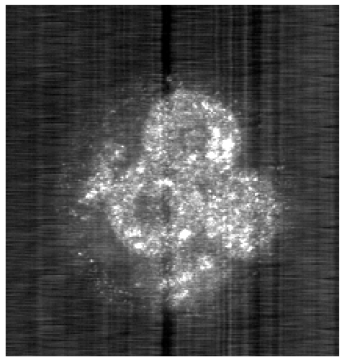
In–Focus Image

Out–Of–Focus Image

Confocal Focusing

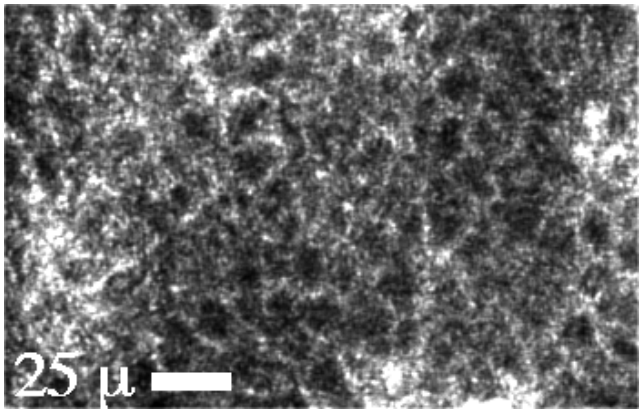
Very High | 145μm | 150μm | 155μm

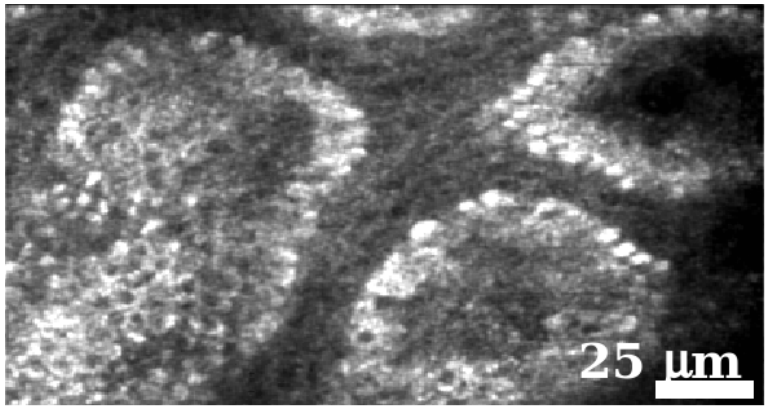
160μm | 165μm | 170μm | Very low

Judy Newmark (Warner Group), Bill Warger

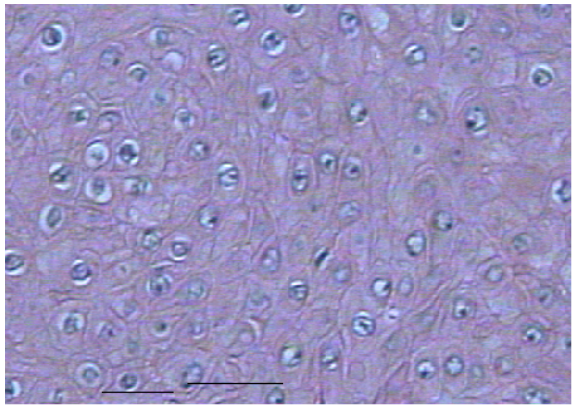
Normal Skin



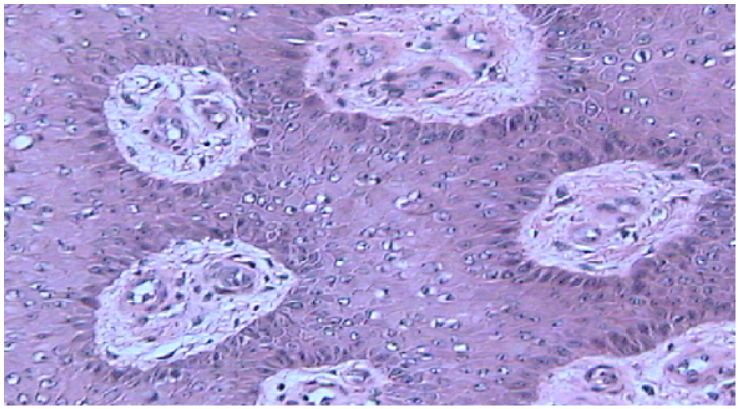
CRM, Spinous Layer



Basal Layer



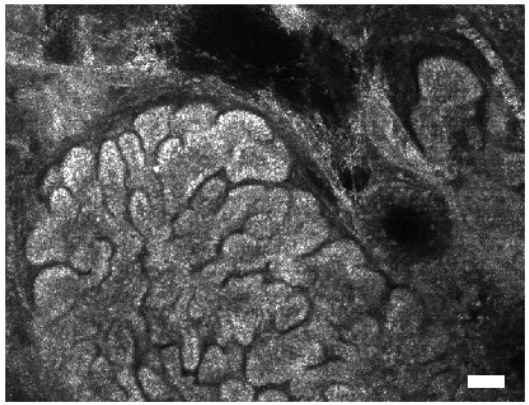
H&E, Spinous Layer



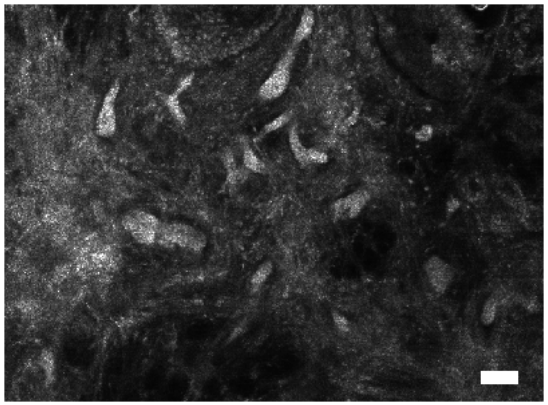
Basal Layer

Milind Rajadhyaksha

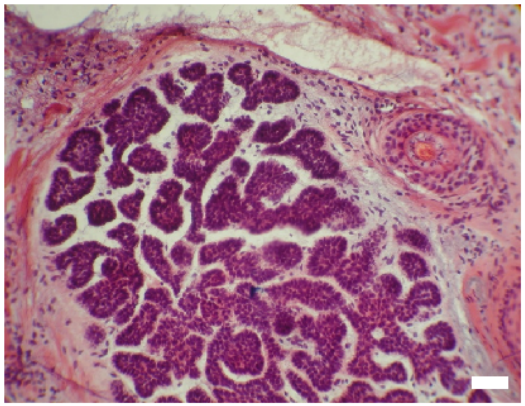
Skin Cancers



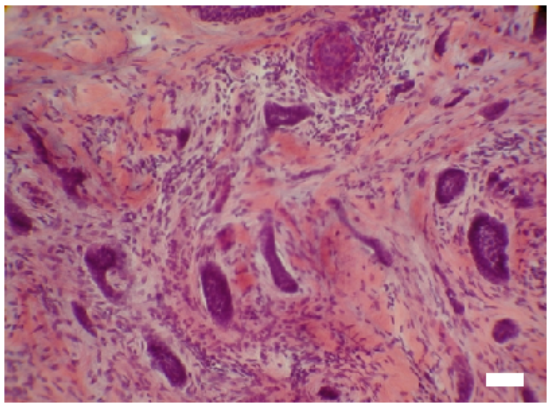
CRM, Nodular BCC



Infiltrative BCC



H&E, Nodular BCC



Infiltrative BCC

Milind Rajadhyaksha

Large 3–D Mosaics

Mouse Embryo at Day 9

Z–Stack from Confocal Reflectance Microscopy

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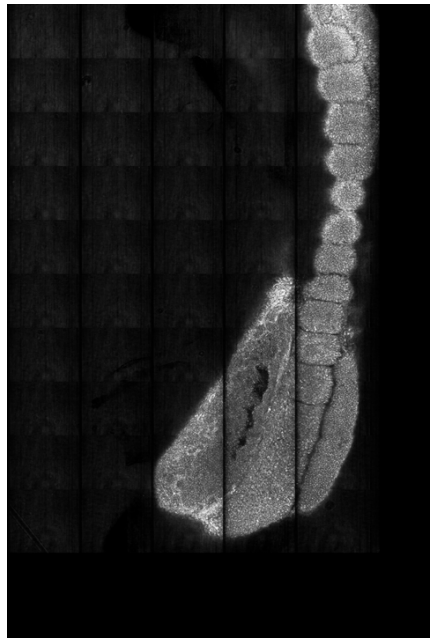


$-3\mu m$ $27\mu m$ $84\mu m$ $114\mu m$

Selected Sample Z Locations from Mosaic

3200 wide by 4800 high by 160 deep, Decimated for Display

Irina Larina (Baylor), Kirill Larin (Houston), Joe Kerimo

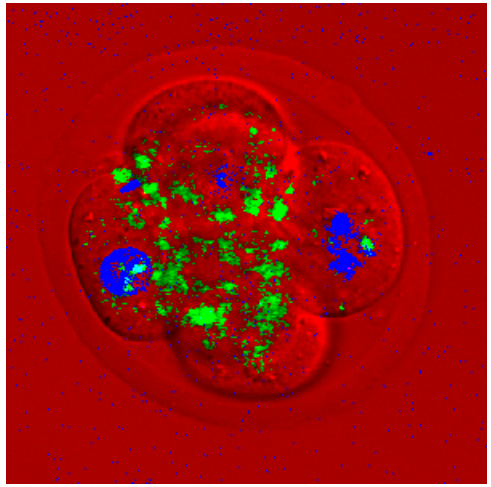
Multi–Modal Slices

Inverted Microscope
Red: DIC
Blue: Hoechst CFM
Green: CRM

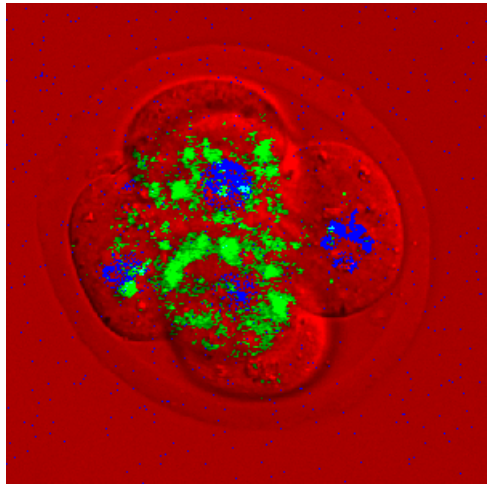
Hoechst Confocal shows nuclei

Weak CRM deep suggests lack of ballistic light.

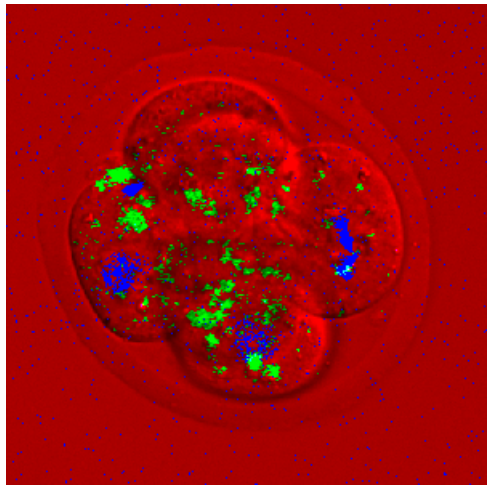
1. Top (Deep)



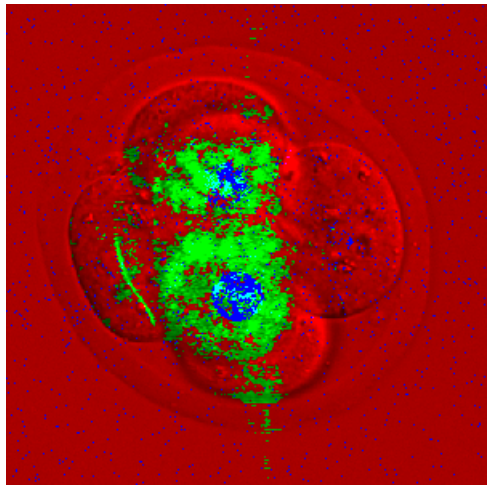
2.



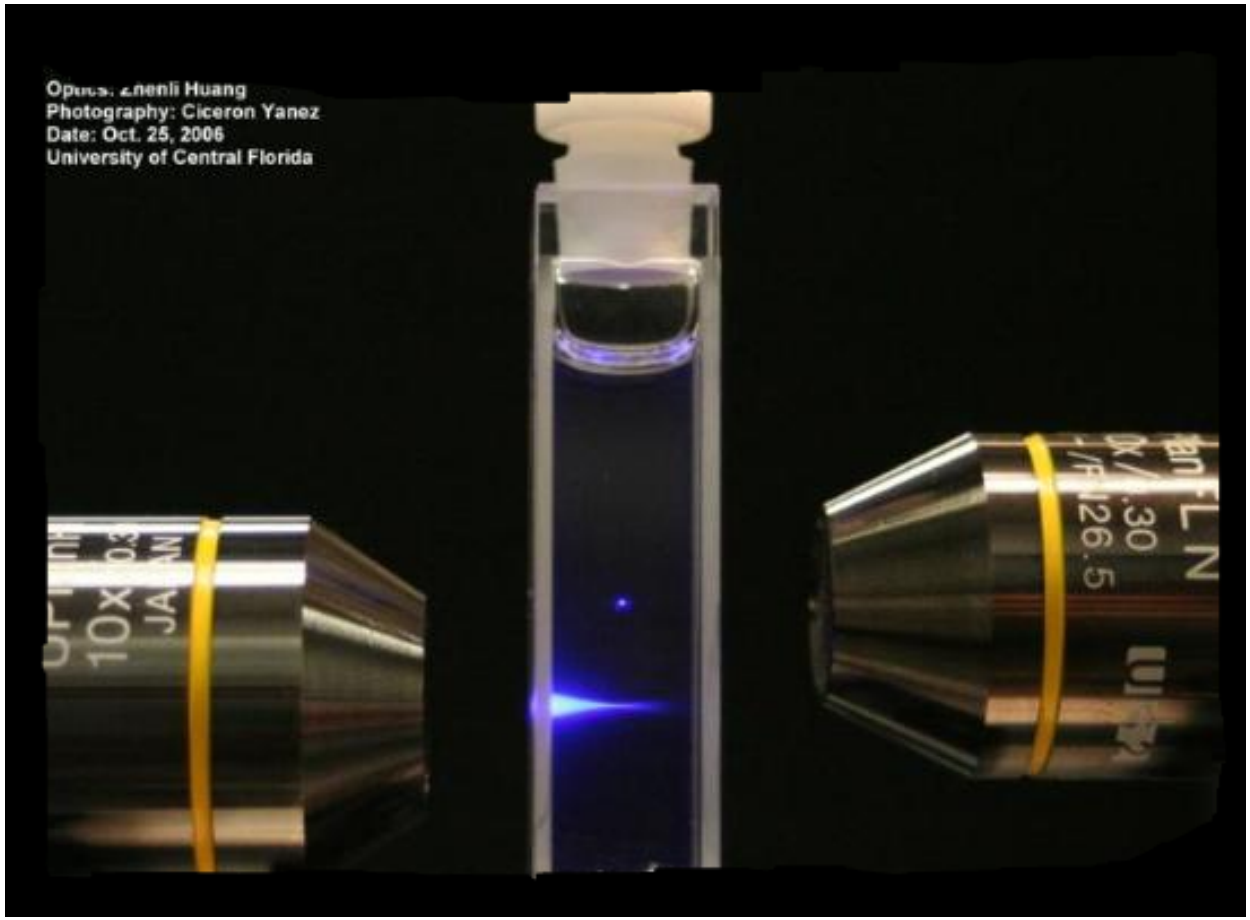
3.



4. Bottom



2–Photon Microscopy



Huang, UCF

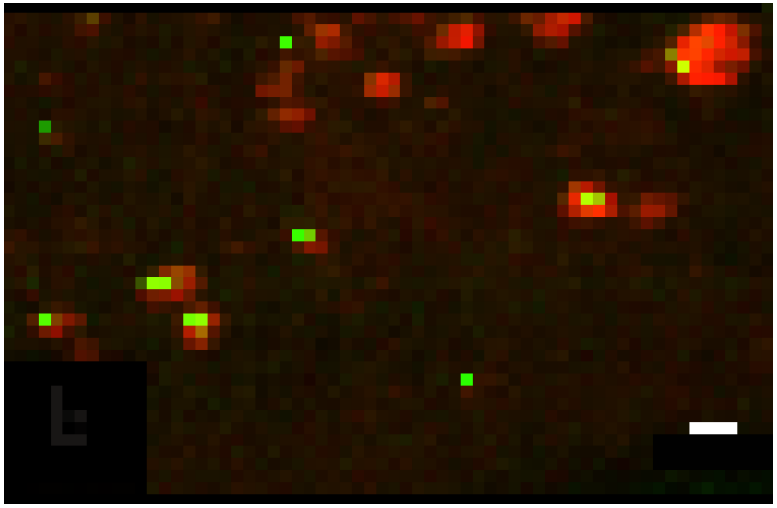
2–P Advantages

- IR Light to Reduce Photodamage
- Nonlinearity to Reduce Photodamage
- IR Light to Increase Penetration
- No Pinhole (Better Alignment, Better Sectioning)
- Wide Detector (Collects All Light, including Scattered)
- Easier Filtering

Melanin 3–P

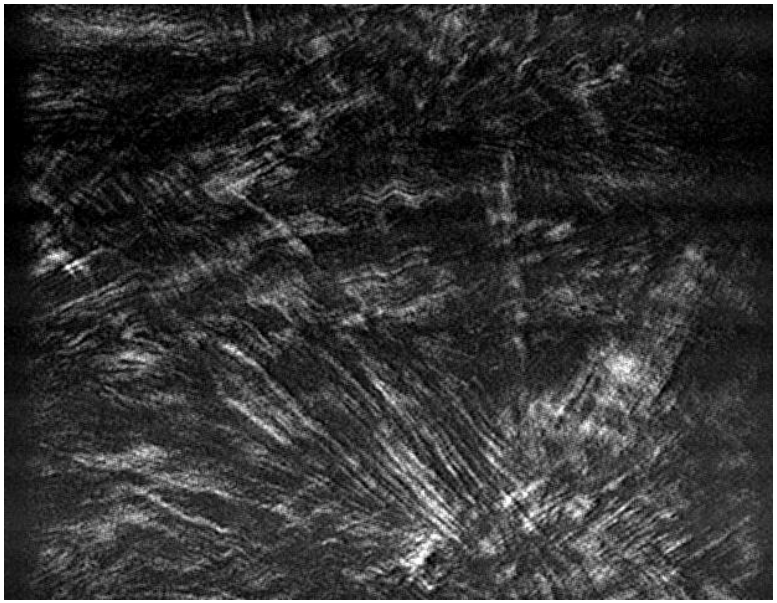
Before Activation

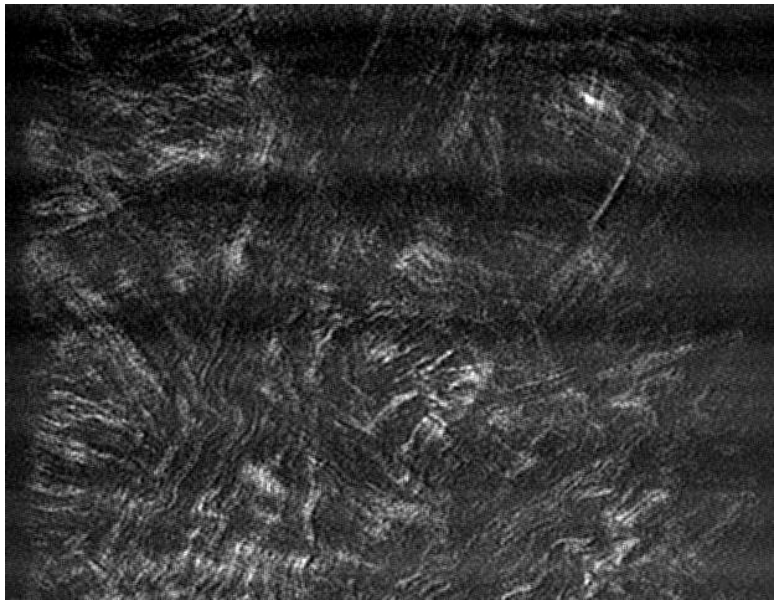
After Activation



Kerimo *Photochemistry and Photobiology*, 2011

Collagen Fibrils in SHG



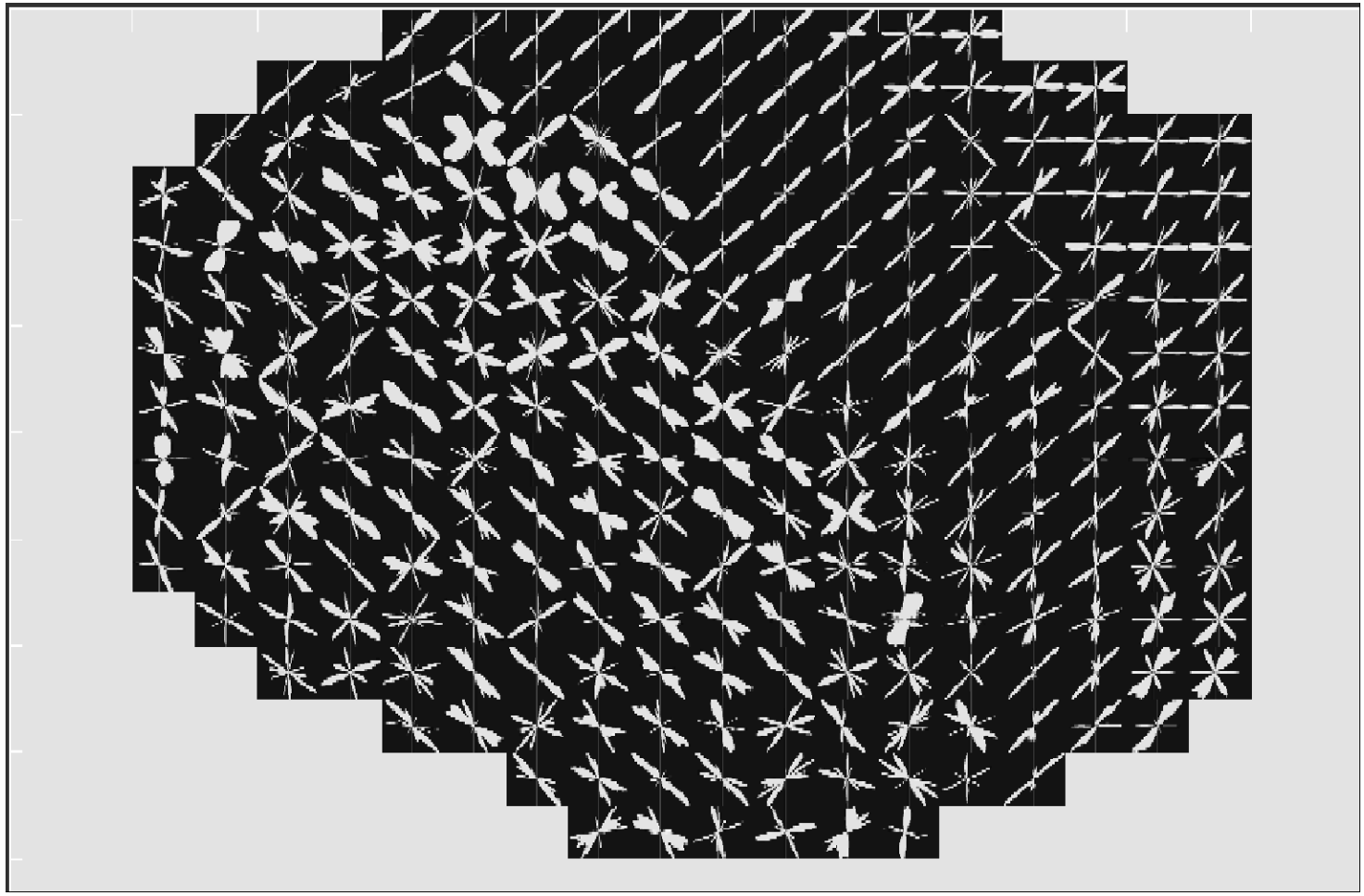


- Long–Range Goal: Understand Organization Under Load
- Current Goal: Measure Organization in Cornea

Thanks to Yair Mega, Mike Robitaille, Ramin Zareian

Collaboration with Kai–Tak Wan and Jeff Ruberti

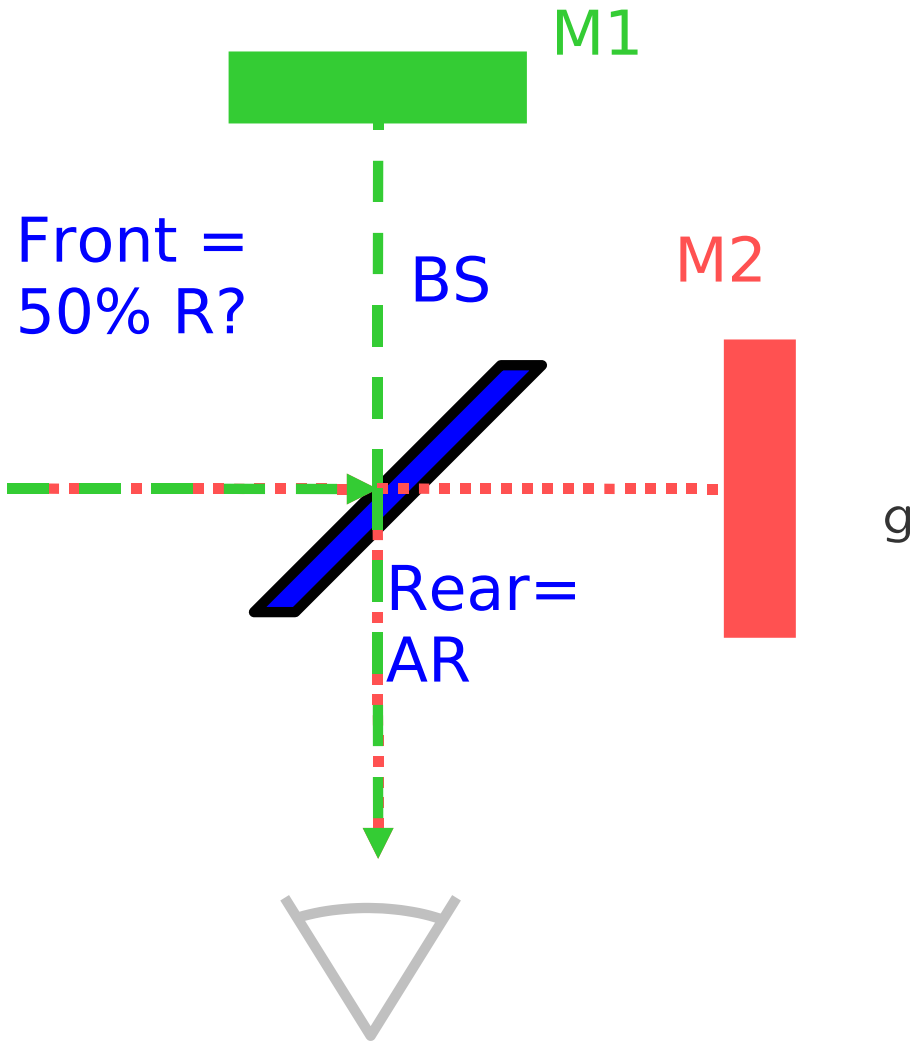
Collagen Fibril Organization



Optical Coherence Tomography

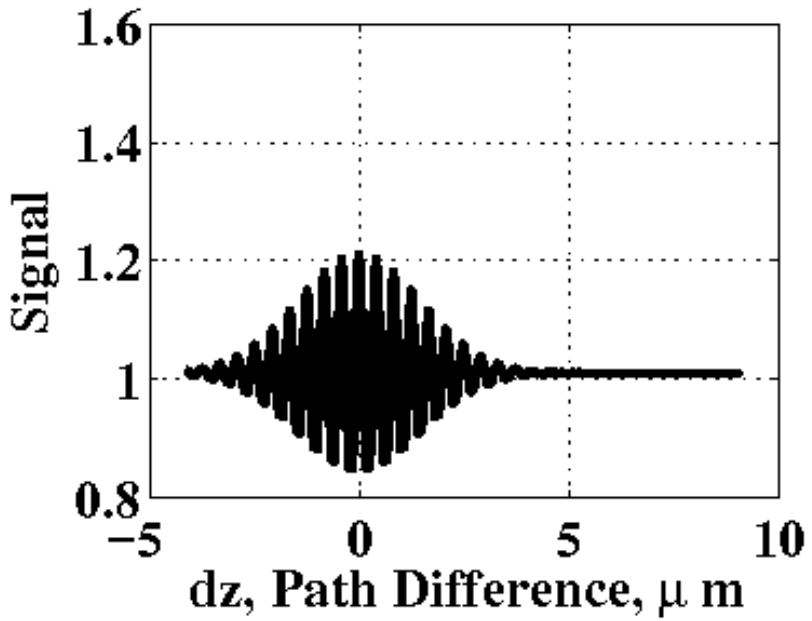
- Short Coherence Source
  - Super–Luminescent Diode
  - Ti:Sap Laser
  - Other
- $M1$ is Reference
- Moving Reference Mirror
- $M2$ is Target
- Interference? Compare...
  - Path Difference
  - Coherence Length

- Michaelson Interfereometer

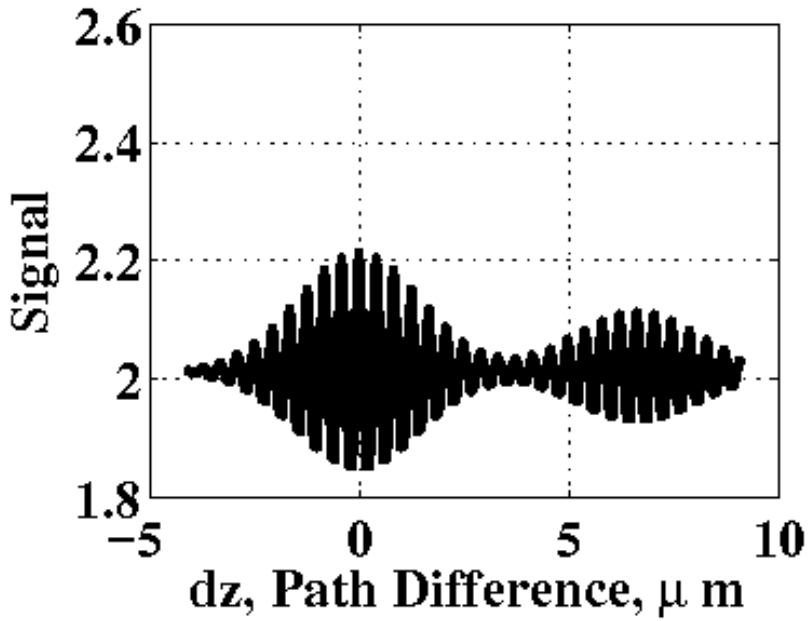


OCT Signals

- Examples with Partial Reflectors
- Air–Glass Interfaces (Simulated Signals)
- Idea Extends to Thick "Distributed" Targets

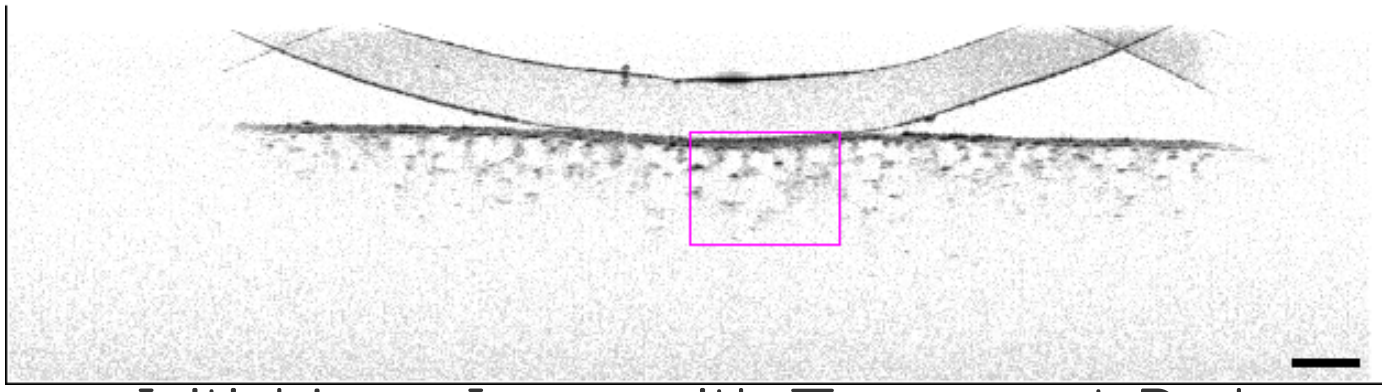


A. Target at Zero

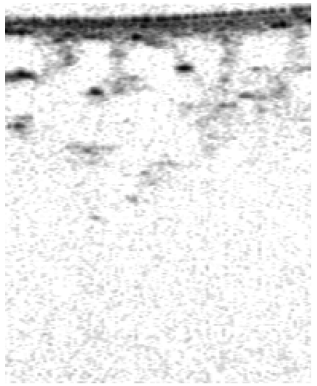


B. Added target at 8 μm

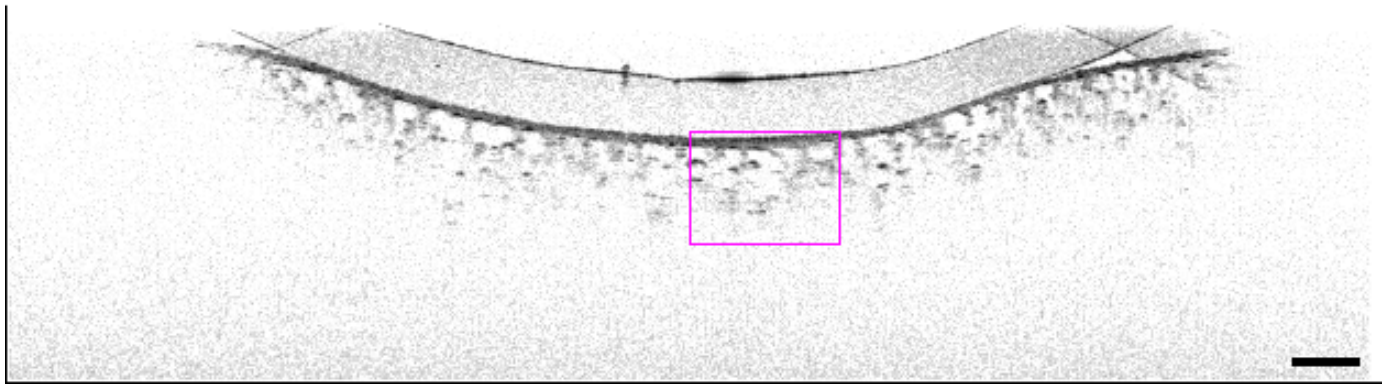
Lung Images (OCT)



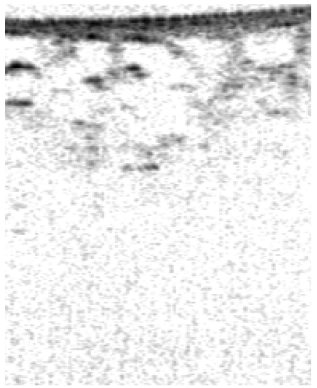
Initial Lung Image with Transparent Probe



(Detail)



Partially Indented Lung Image

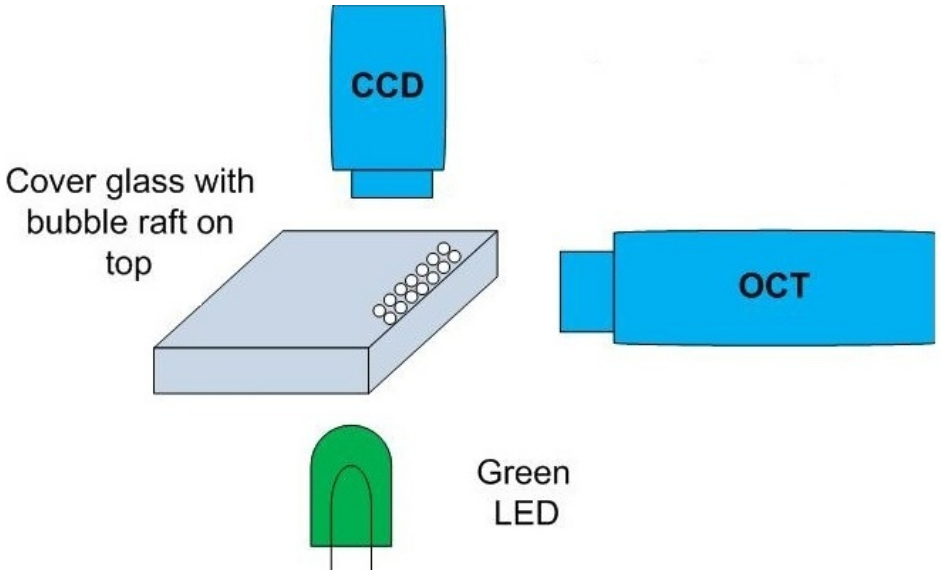


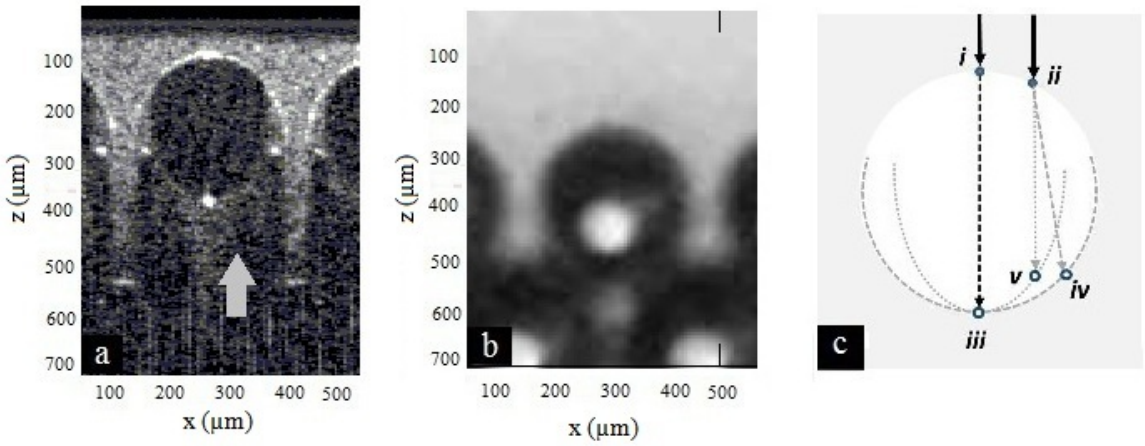
(Detail)

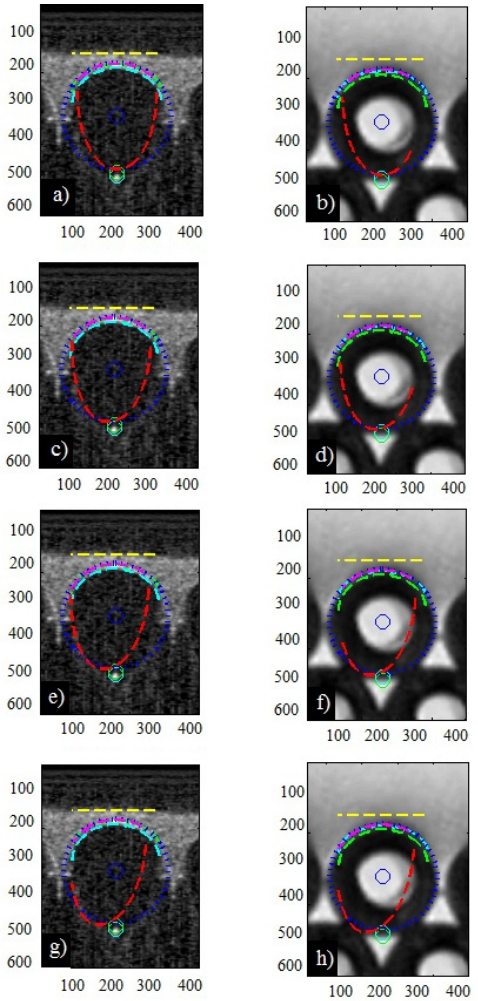
Scale Bar 300 μm

Andrew Gouldstone, Maricris Silva, MIE Ph.D. 2011

Bubble Phantom

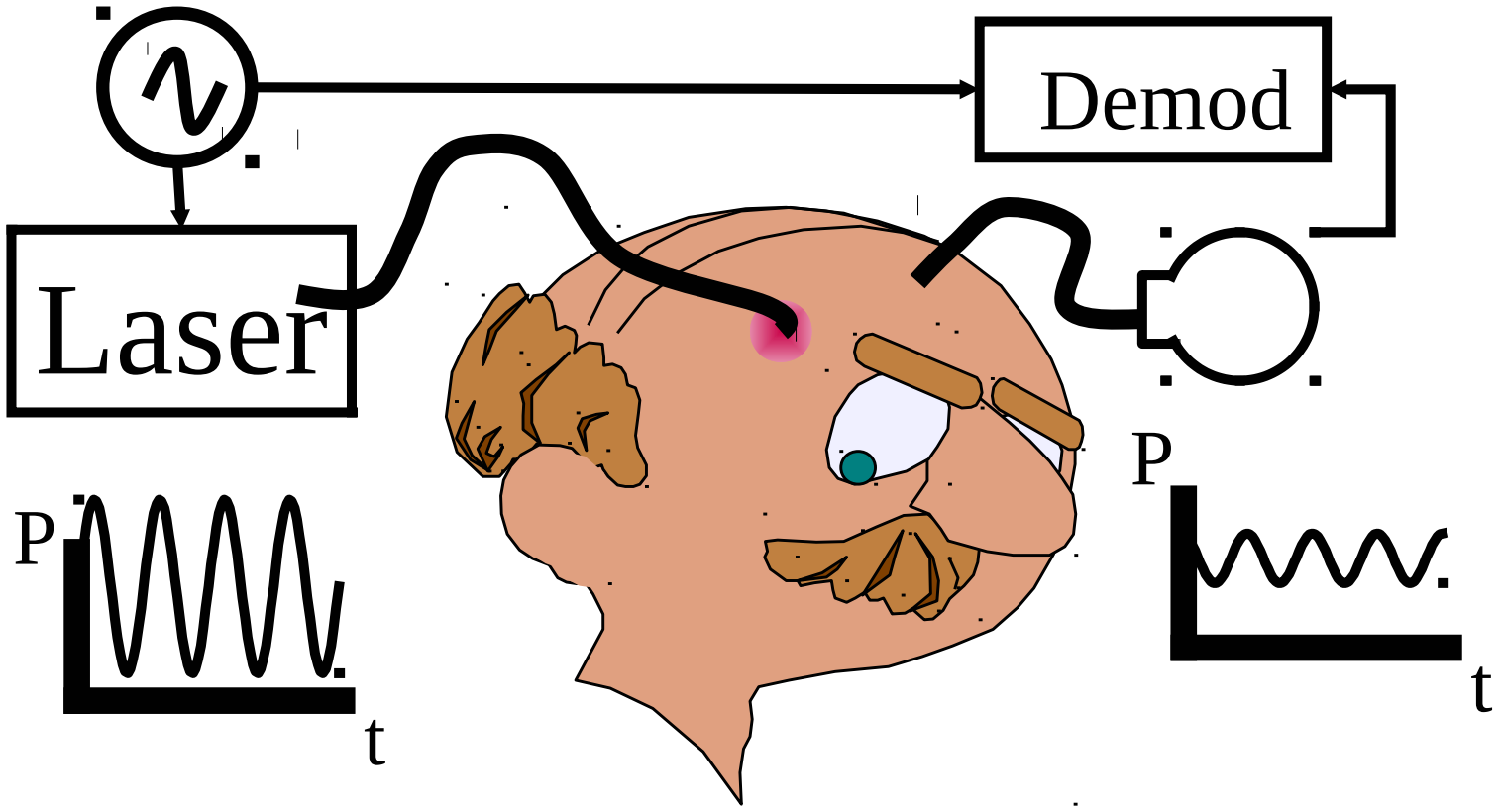




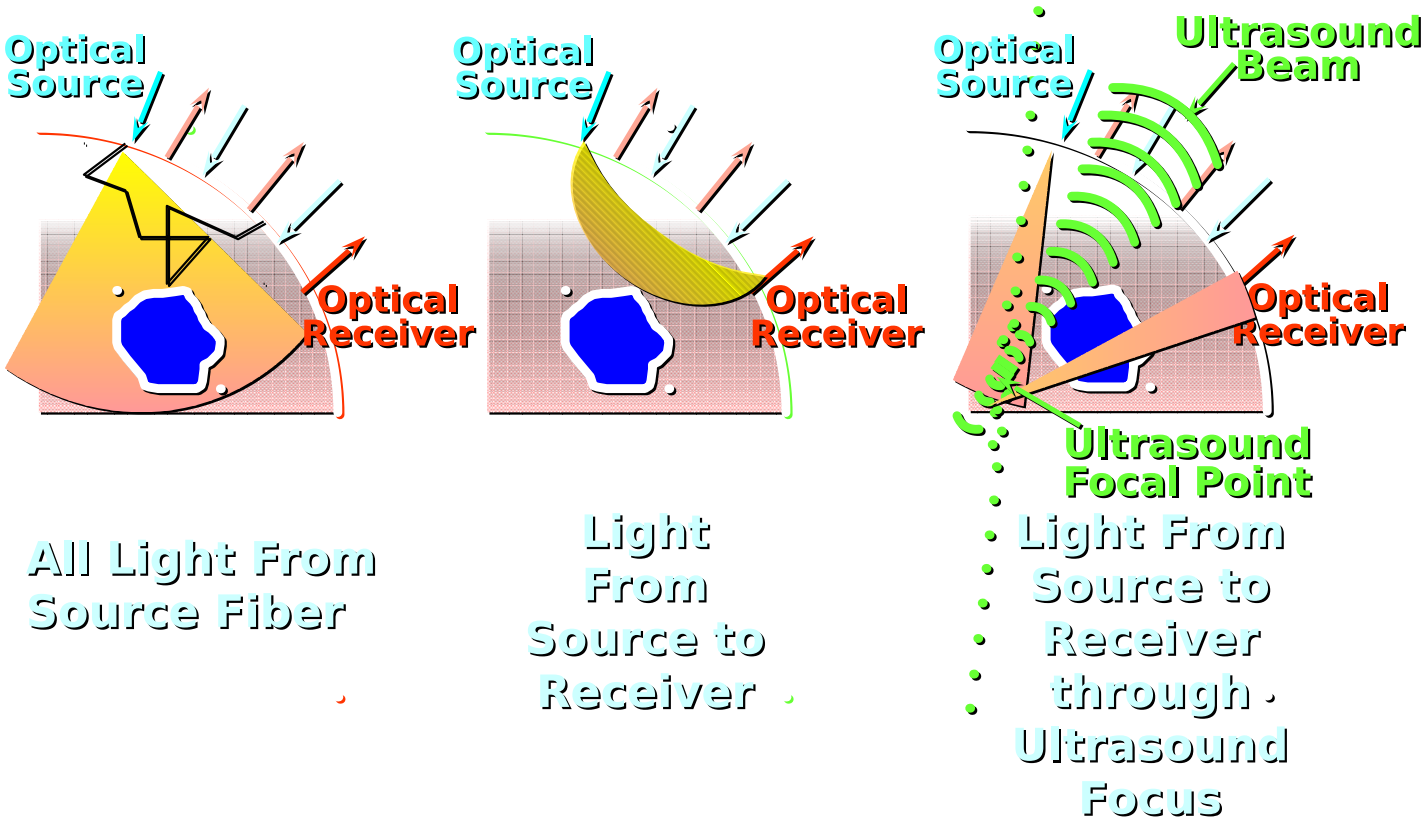


Golabchi, *Biomedical Optics Express*, 2012

Diffusive Imaging



DOT and Ultrasound



Some Safety Issues

- Chemical Toxicity
- Light Toxicity
  - Photochemical
  - Thermal
- Issues for Patient and Operator

Summary

- Imaging with Light Offers
  - Imaging Deep in the Body
  - Imaging with Sub–Micrometer Resolution
  - Non–Invasive Imaging

Summary

- Imaging with Light Offers
  - Imaging Deep in the Body
  - Imaging with Sub–Micrometer Resolution
  - Non–Invasive Imaging
- Pick Any Two