Northeastern University
College *of* Engineering

Universidad de los Andes

Biomedical Imaging
Optical Imaging

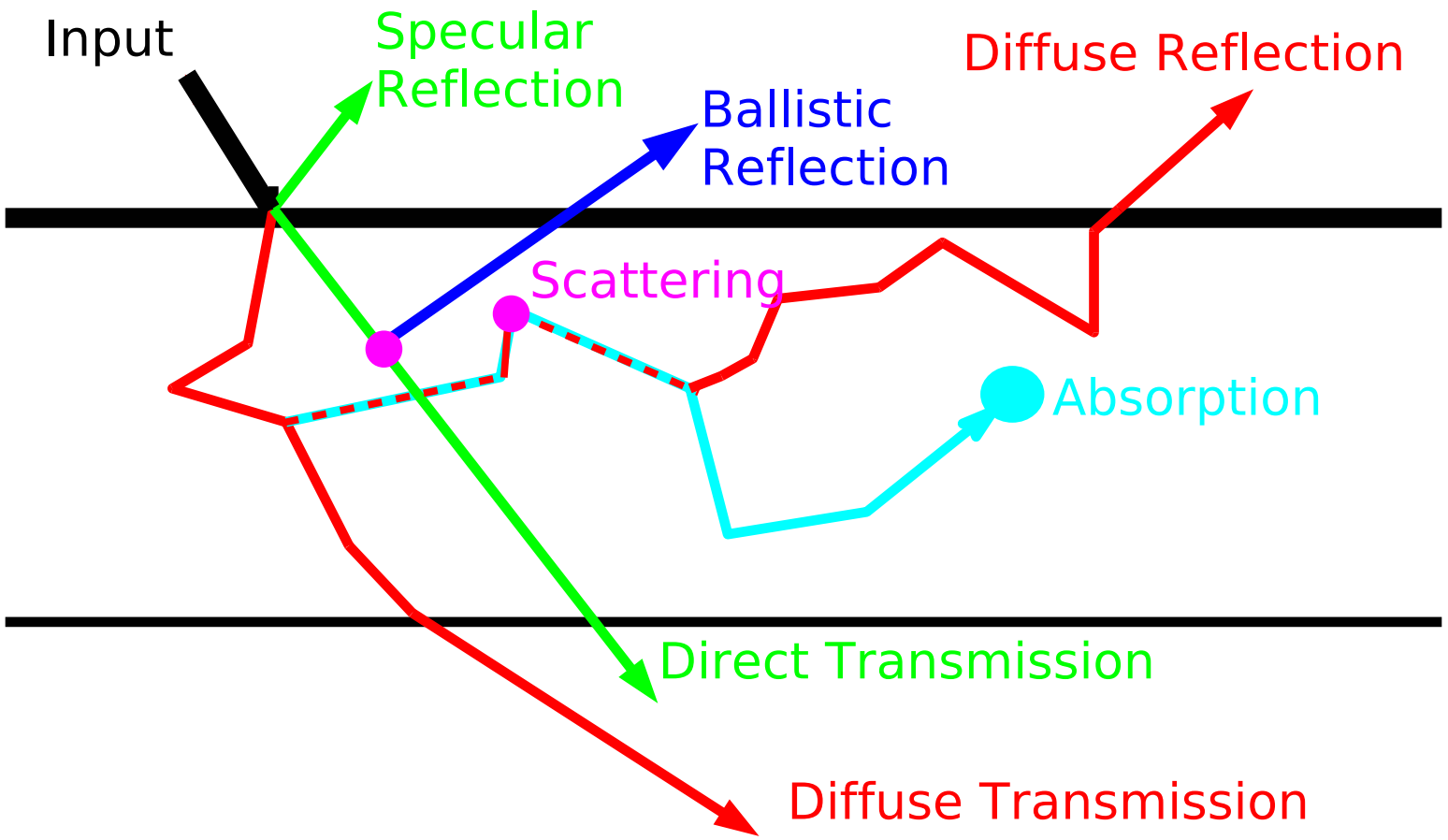
Charles A. DiMarzio and Eric Hall
EECE–4649
Northeastern University &
Universidad de los Andes

May 2023

Optical Imaging

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

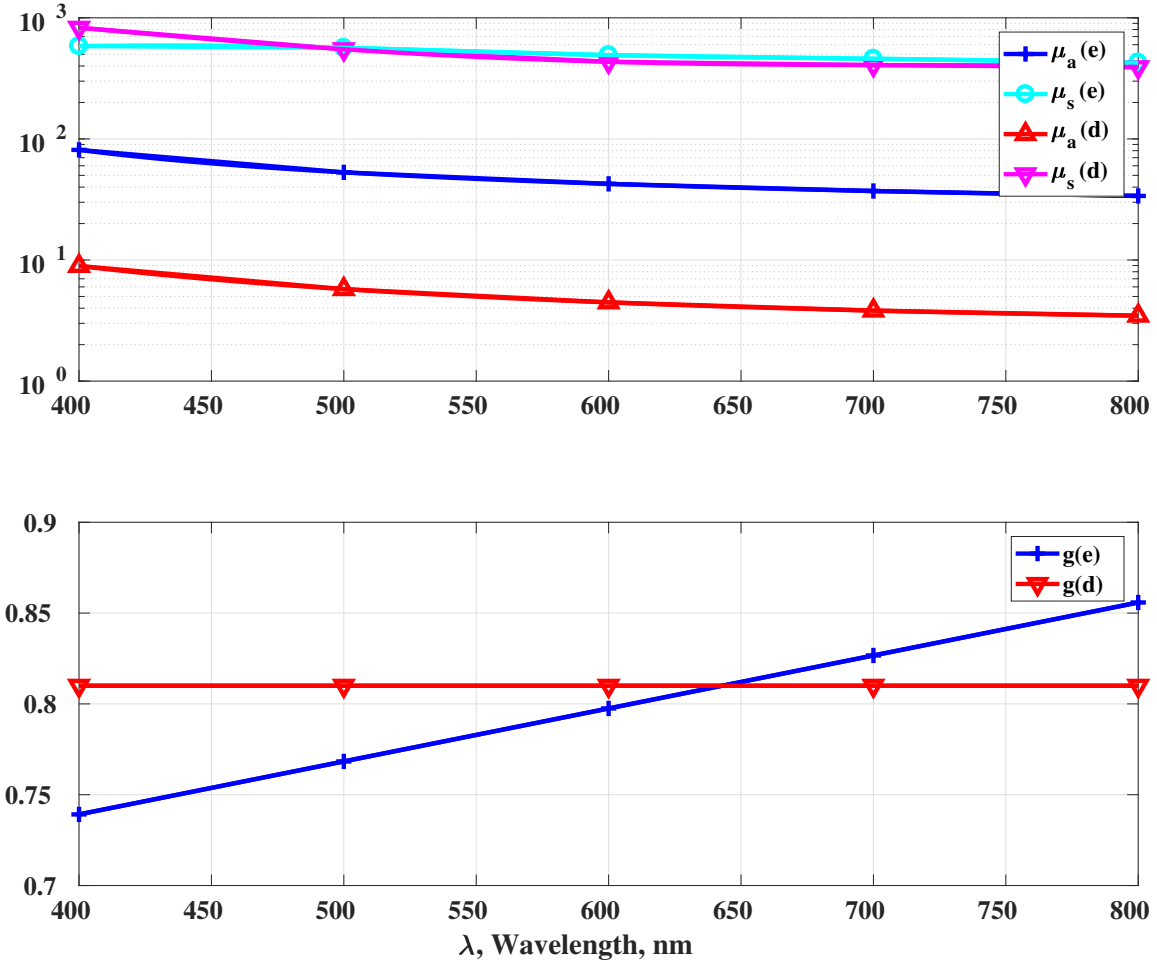
Waves Interactions



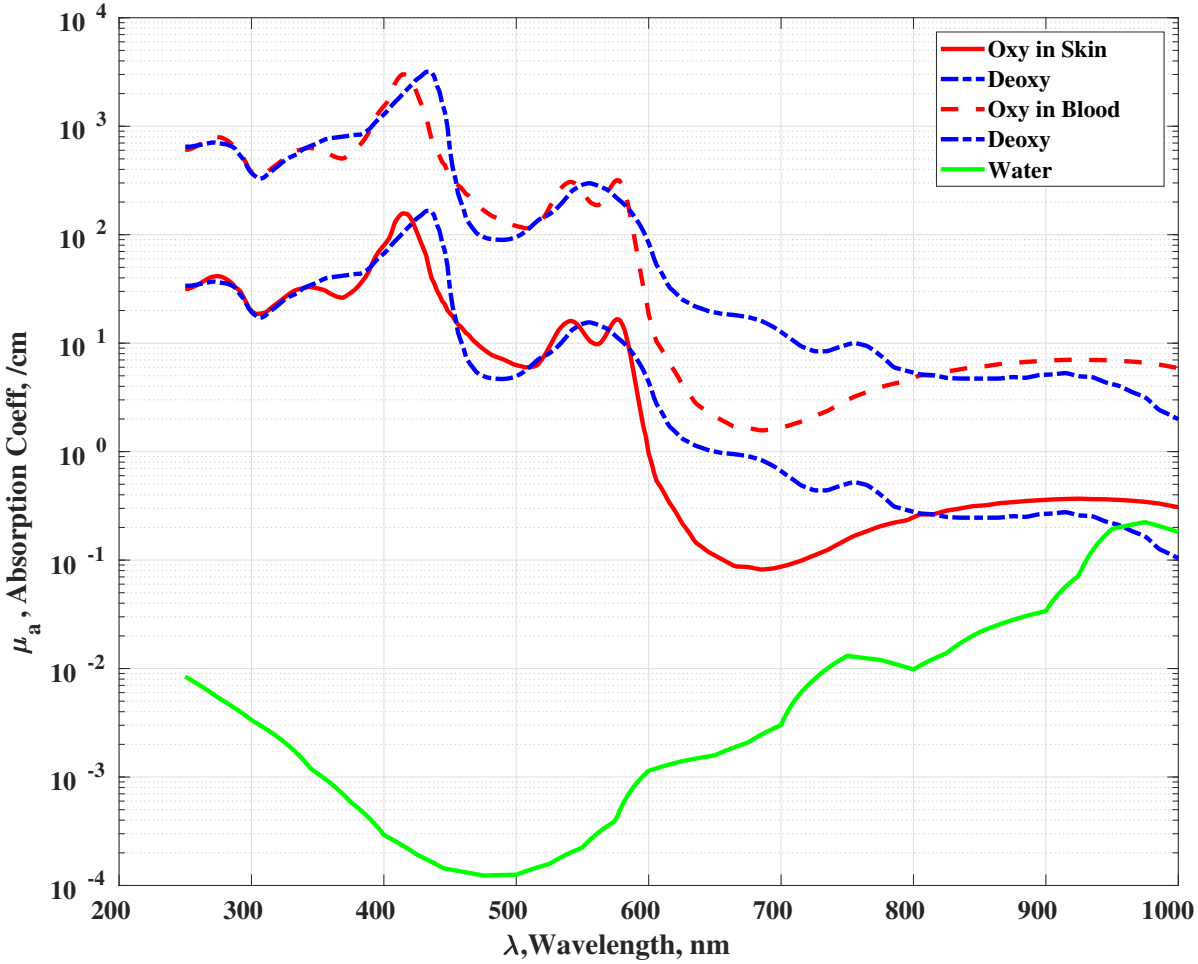
(and of course, emission)

Skin Optical Properties

e=epidermis, d=dermis



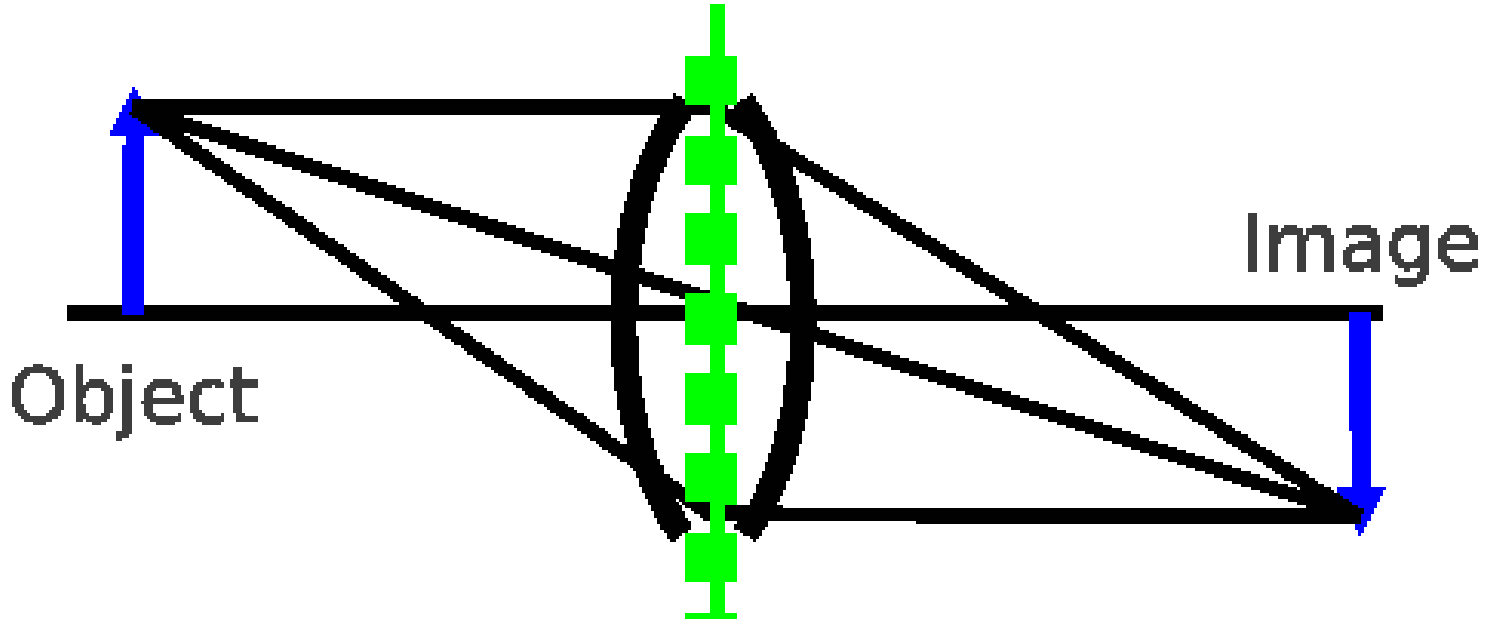
Blood and Water



Light Penetration

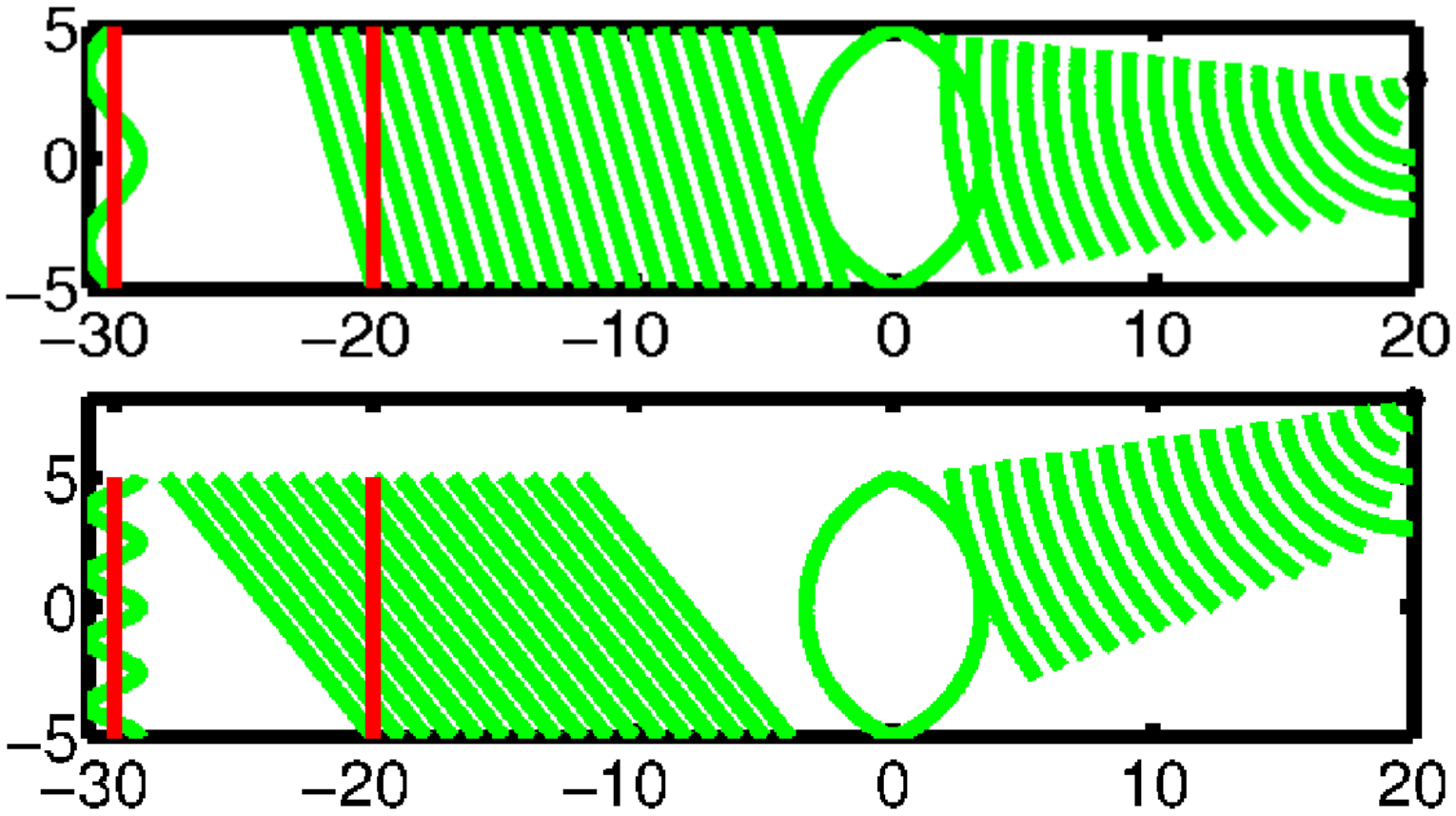
- Best in Near–IR Window
- Ballistic to 100s of micrometers
- Except in the Eye
- Diffuse to centimeters in Near–IR

Lenses

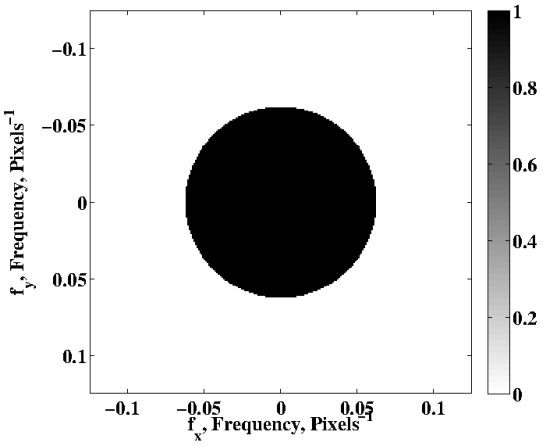


$$\frac{1}{s}+\frac{1}{s'}=\frac{1}{f} \qquad m=\frac{x'}{x}=-\frac{s'}{s}$$

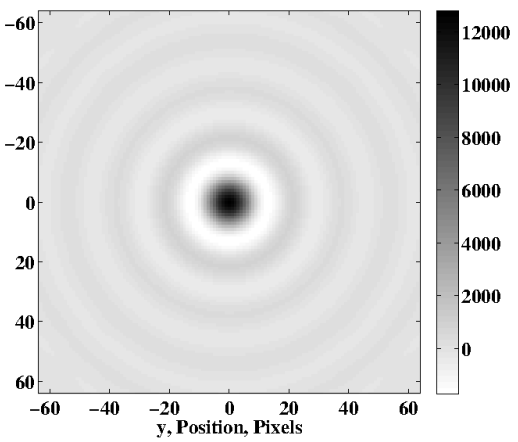
Optical Fourier Transform



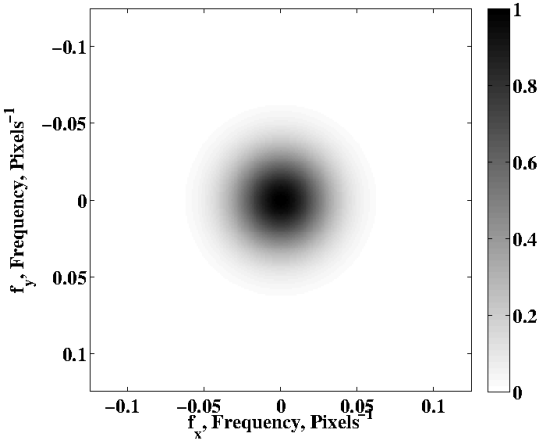
2–D Fourier Transform Pairs



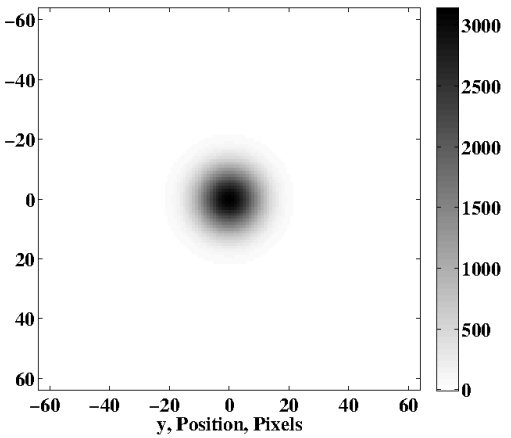
A. Aperture



B. Airy Function PSF

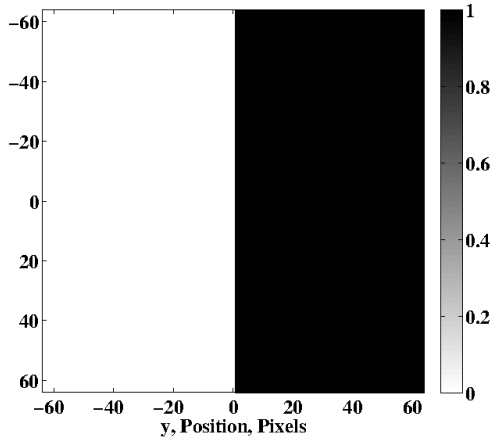


C. Gaussian Apodization

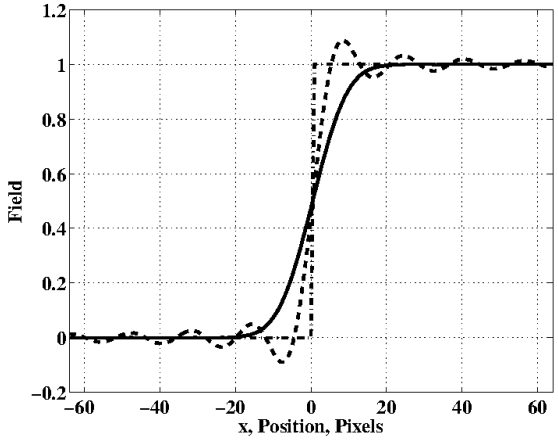


D. Gaussian PSF

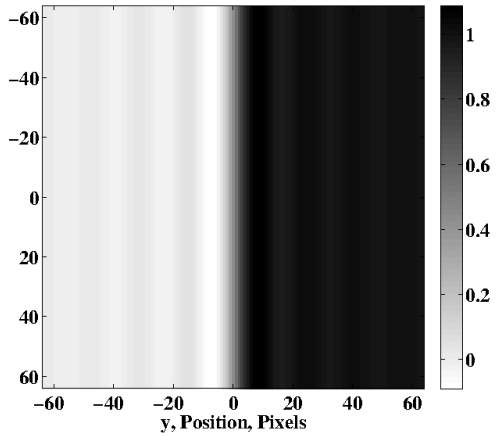
Pupil as Low–Pass Filter



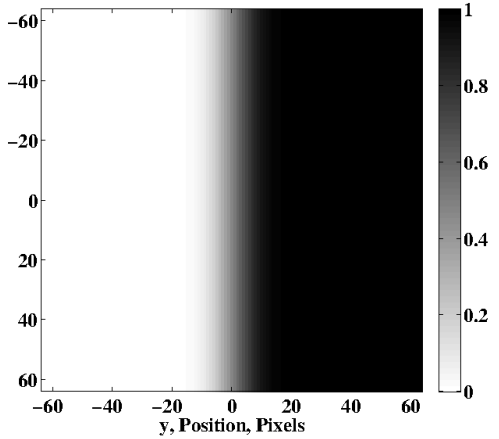
A. Knife Edge Object



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 $\quad \rightarrow \quad 3.2\ \mu$m $\qquad f_{max} = 312/\text{mm}$

Light Sources

- Tungsten Lamp (3200K)
- Quartz–Halogen–Tungsten Lamp (3500K - Melts at 3683K)
- Mercury Lamp (Some Useful Narrow Lines)
- Light–Emitting Diode ($\approx$ 20 nm Linewidth)
- Laser (Pulsed, CW, Narrow, Strong Lines)

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

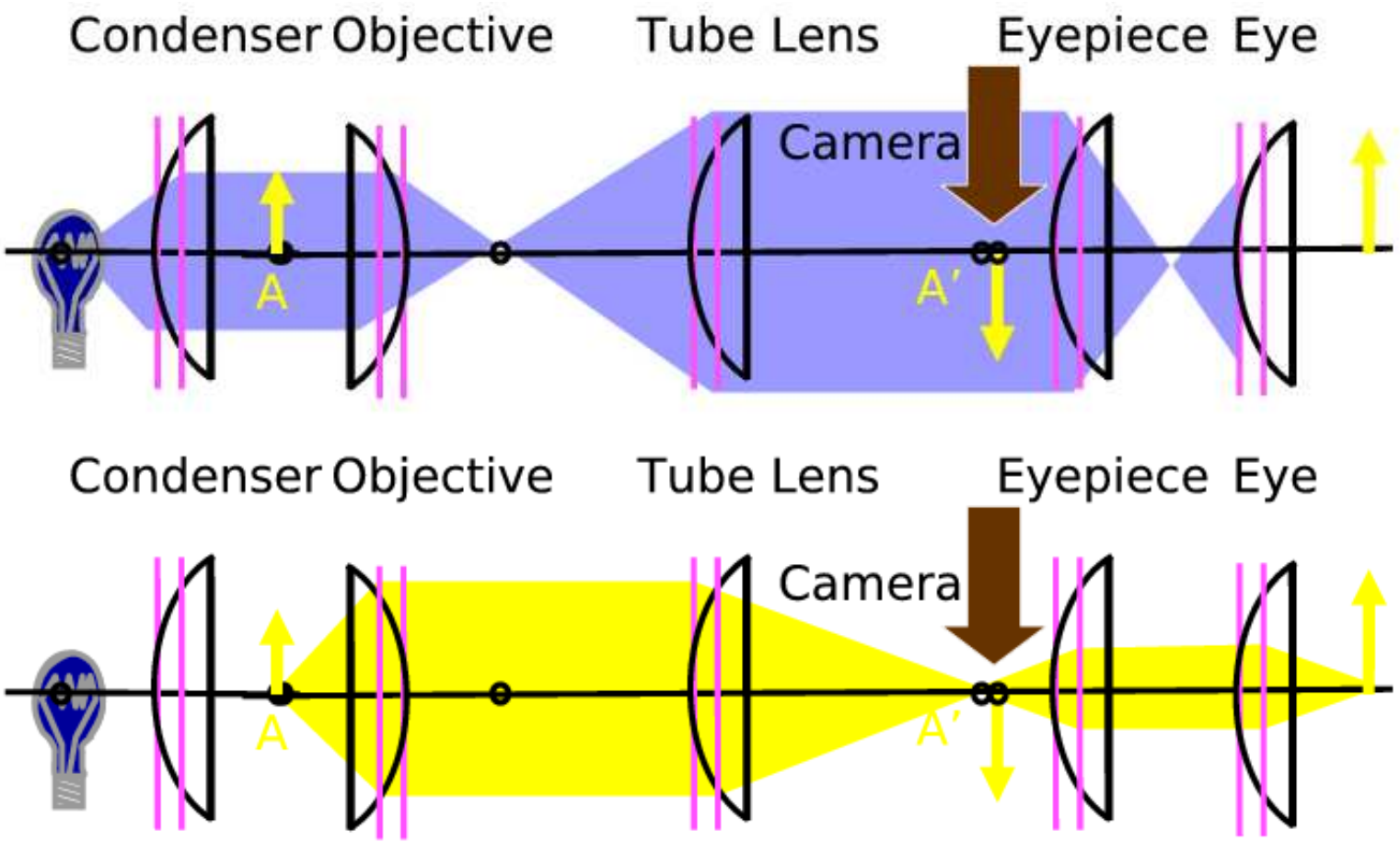
- What's so Modern?
  Microscopy has been around since 1590. . .

- . . . 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

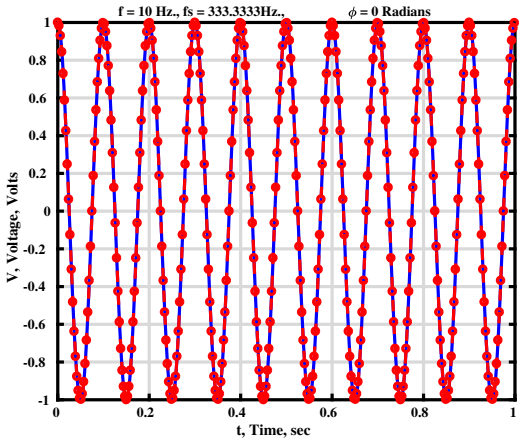
- Point–Spread Function Covers 4 Pixels

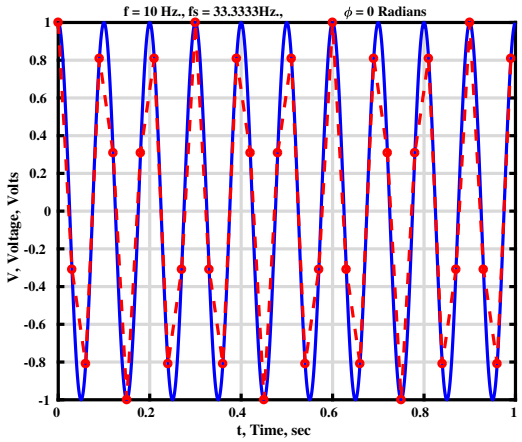
Sampling with an Array

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

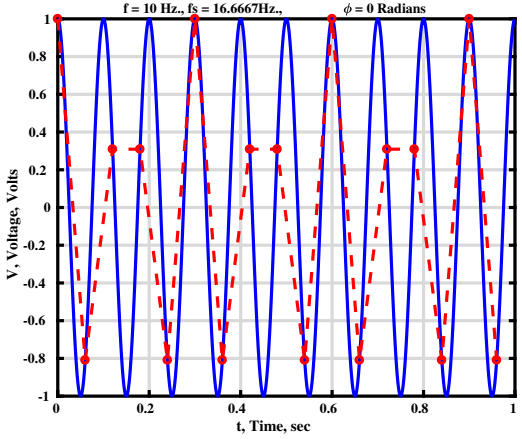
Sampling Example

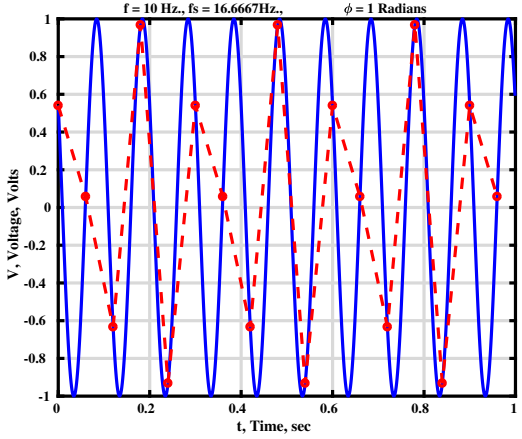
Keeping Nyquist Happy . . .



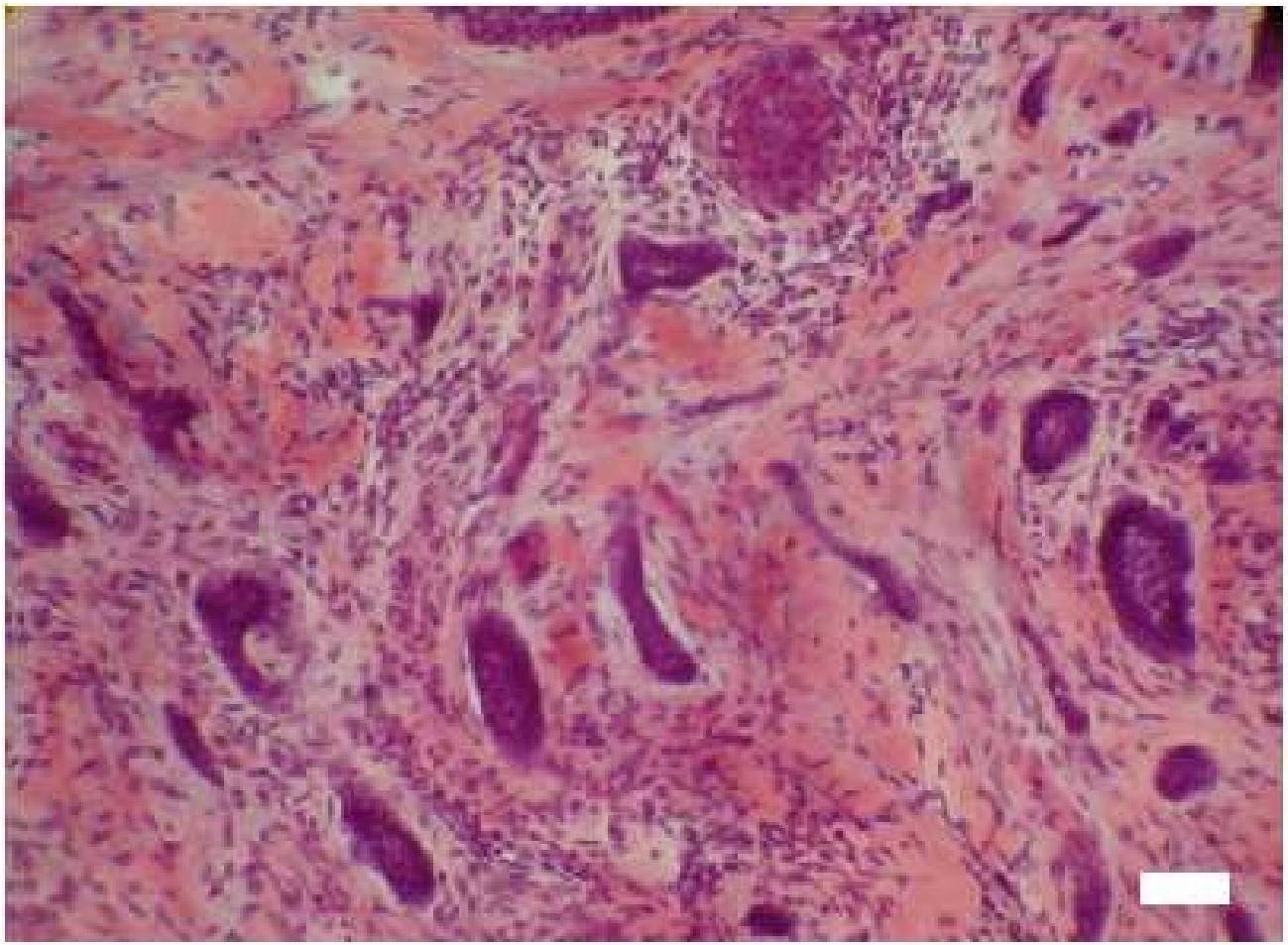


. . . or Not





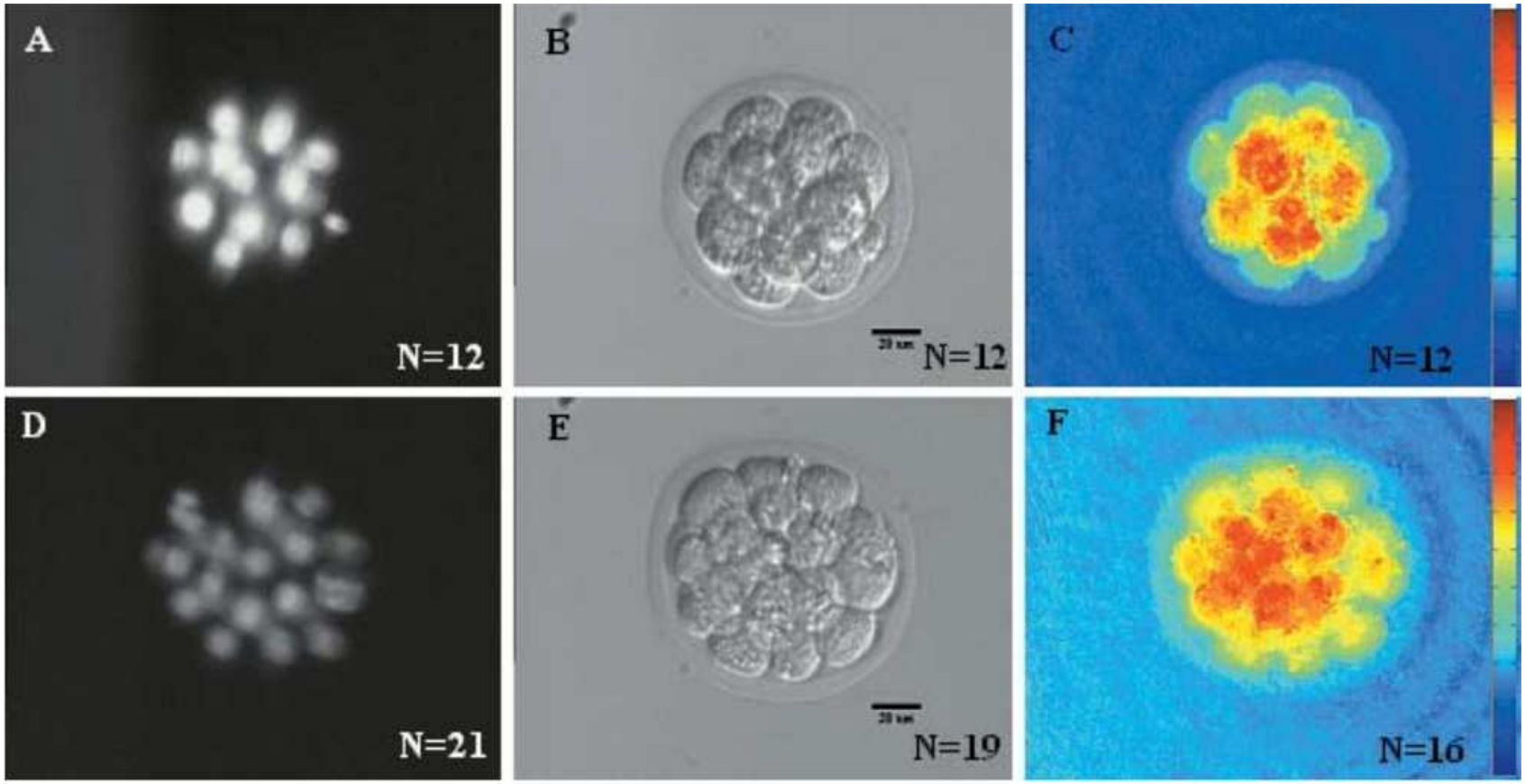
Pathology Slide



Hematoxilyn (Blue) and Eosin (Red)

Milind Rajadhyaksha

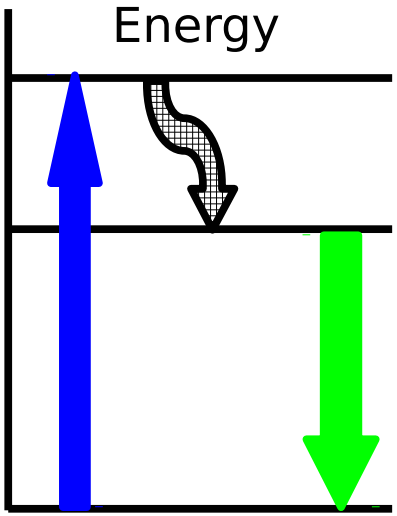
DIC and Phase



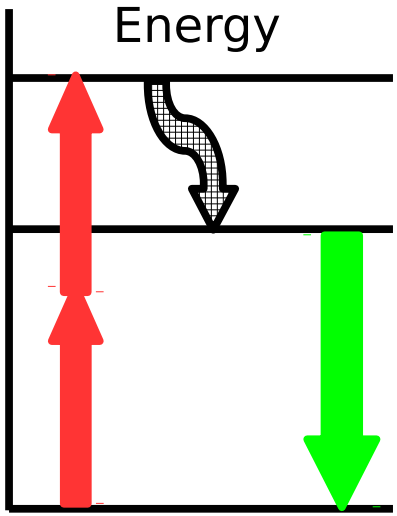
Epi–Fluorescence with Hoechst Dye, *vs.* DIC and OQM

Newmark *Microscopy and Microanalysis*, 2007

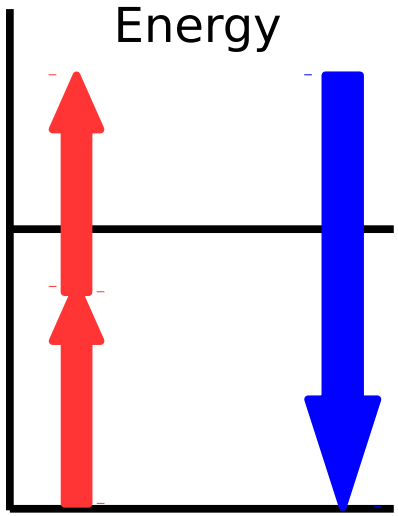
Wavelength–Changing Processes



Fluorescence

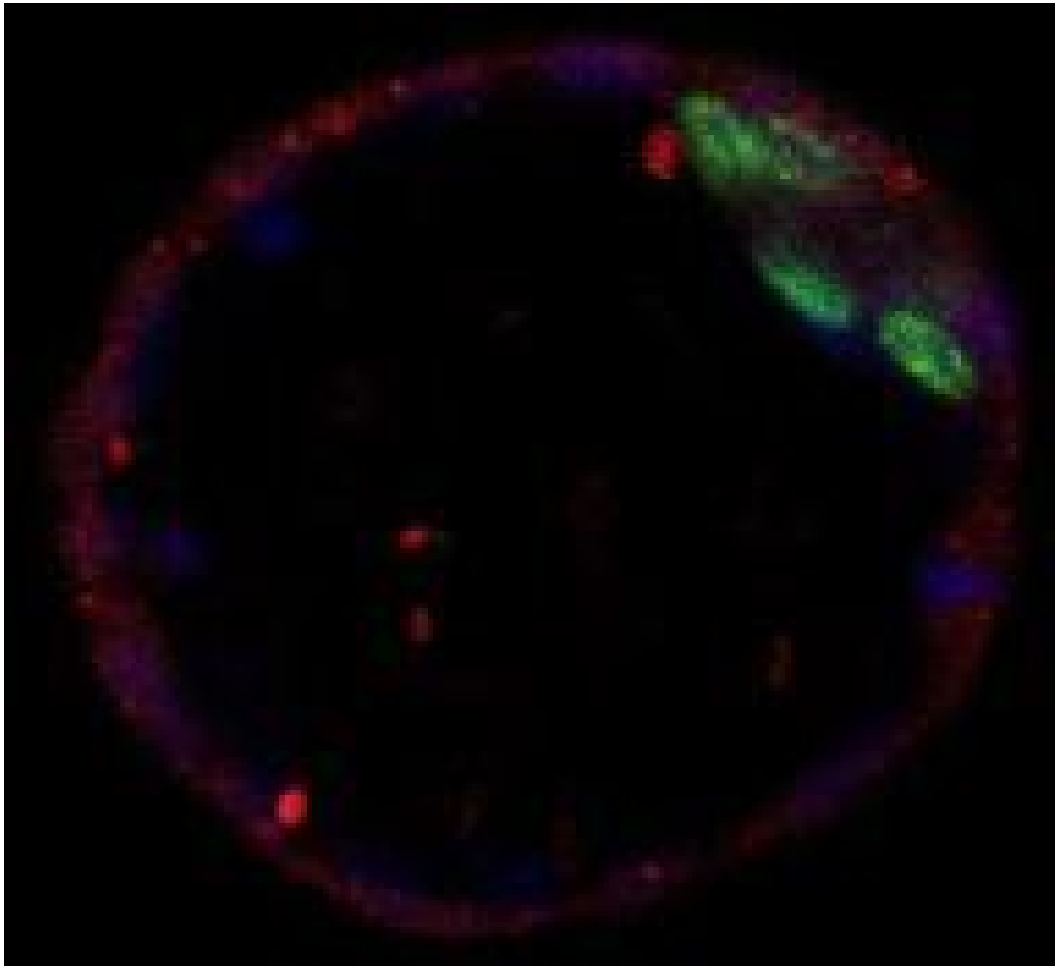


2–Photon Fluorescence



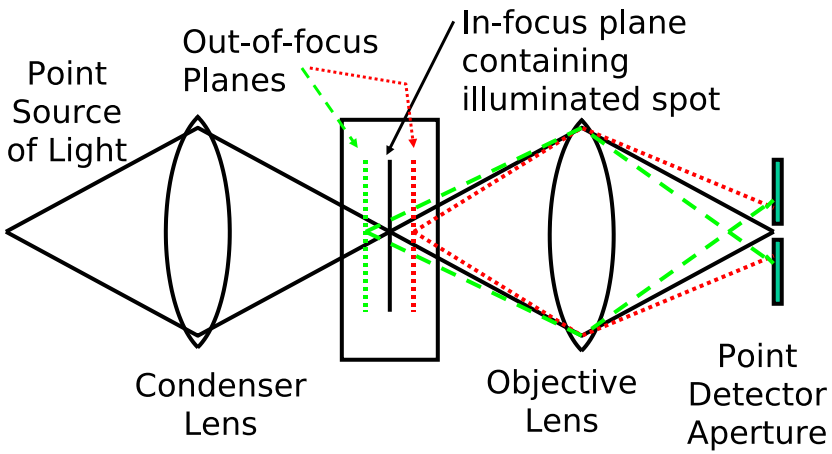
Second Harmonic

Fluorescence Imaging

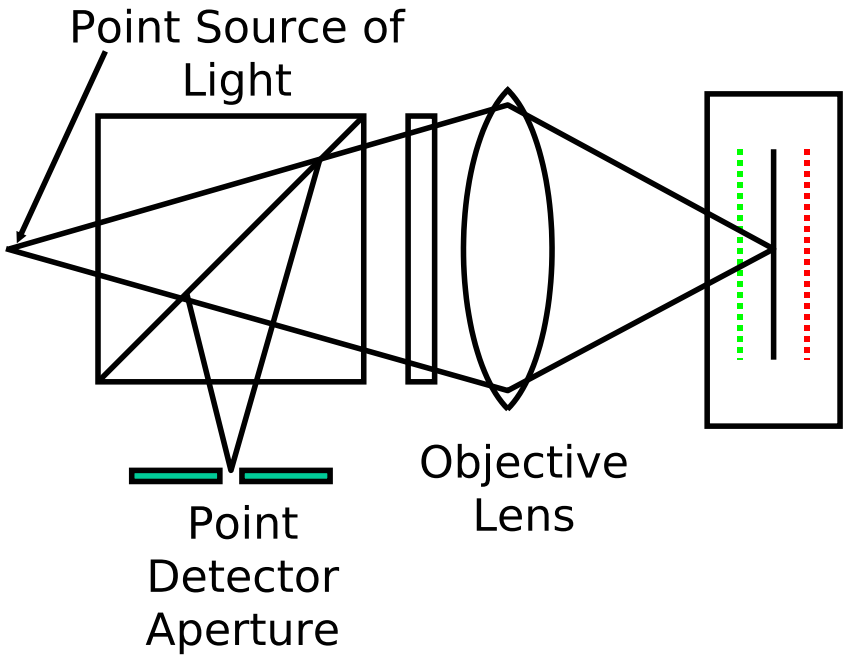


Gal, OCT4, Dapi
http://www.mediacy.com/index.aspx?page=UManchester_stemcellanalysis

Confocal Microscopy



Trans–Illumination



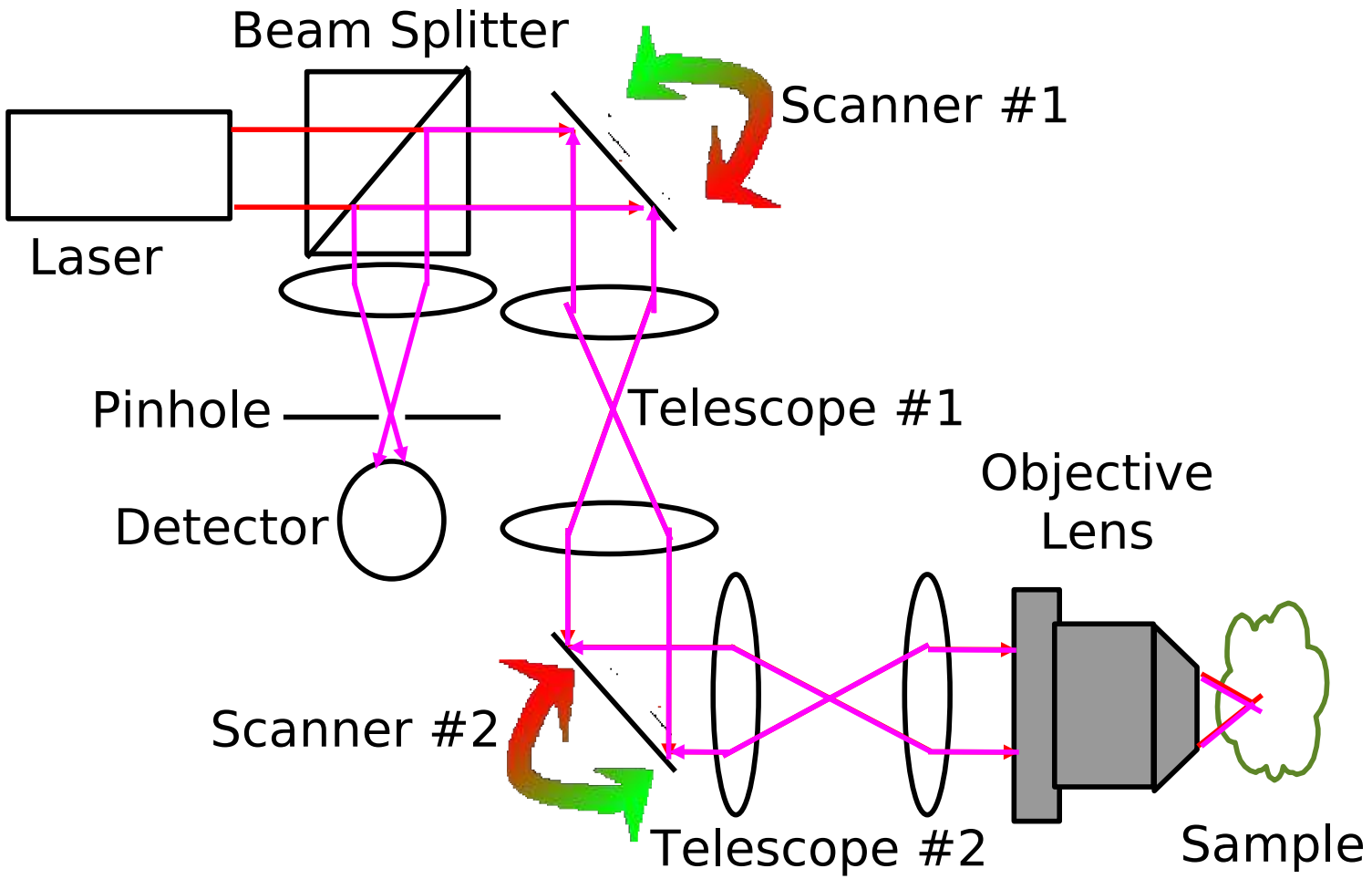
Epi–Illumination (Usual)

Reflectance or Fluorescence

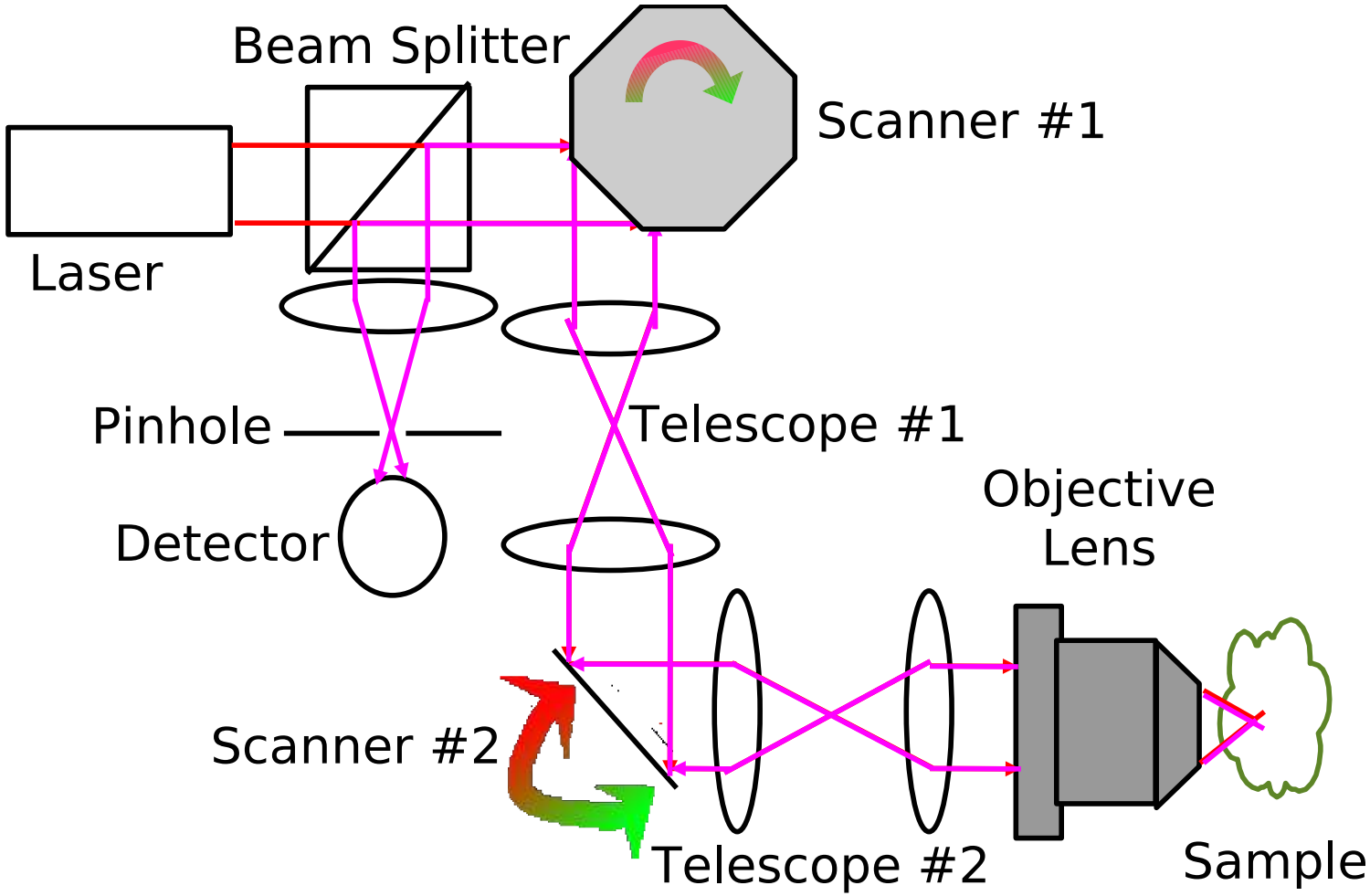
Adapted from Milind Rajadhyaksha

2–Galvo System

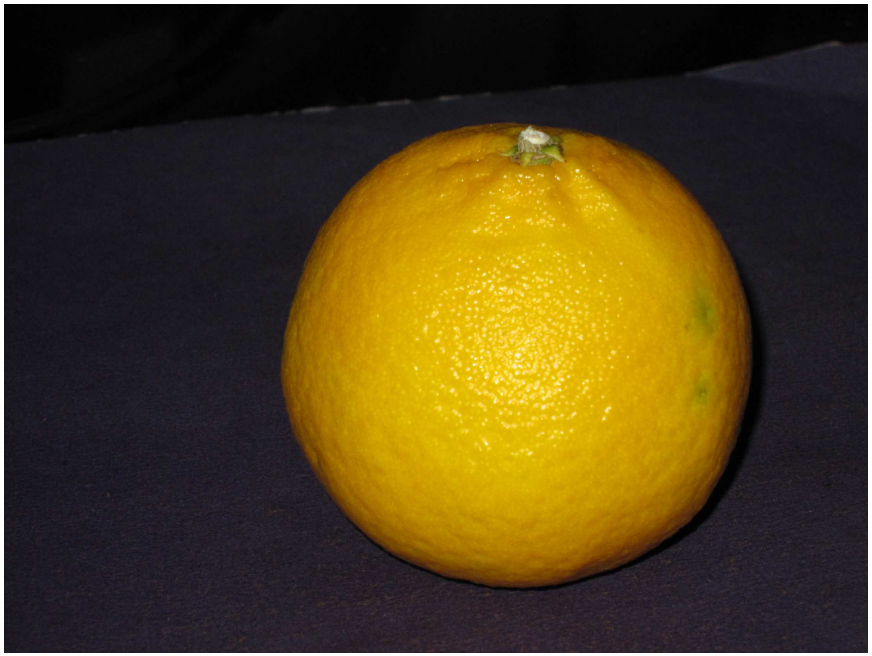
Scan in the Pupil!



Polygon/Galvo System



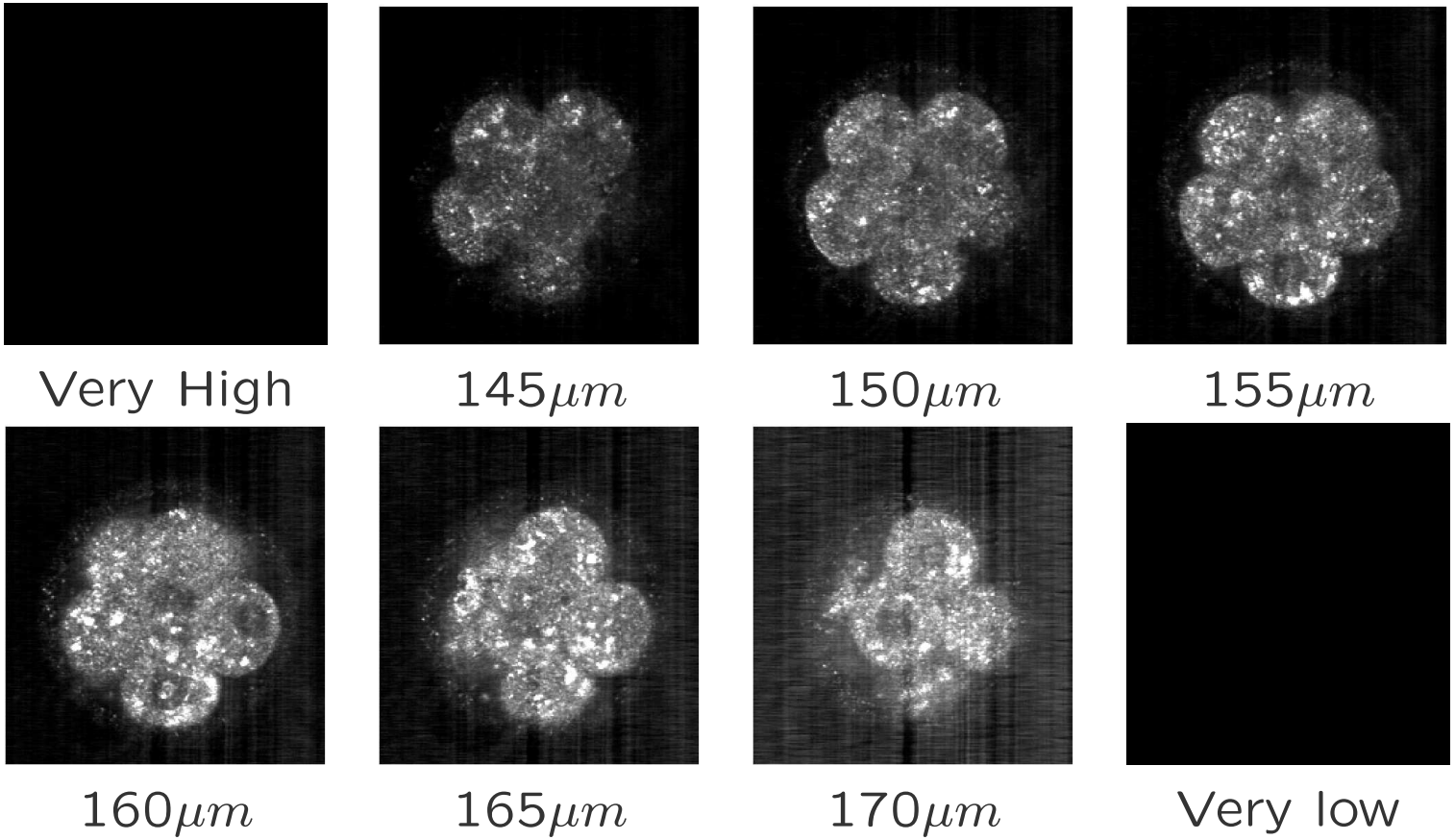
Brightfield Focusing



In–Focus Image

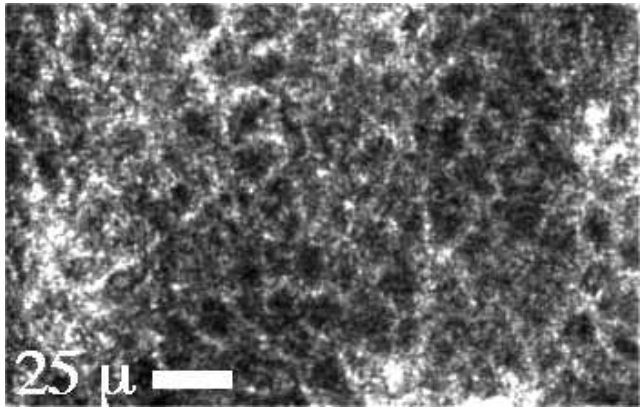
Out–Of–Focus Image

Confocal Focusing

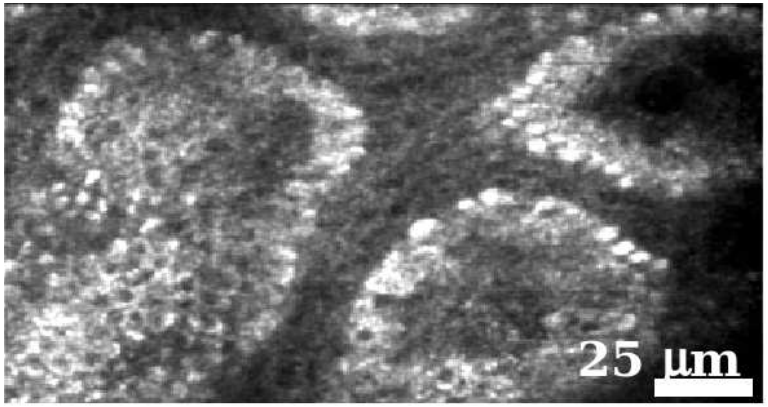


Judy Newmark (Warner Group), Bill Warger

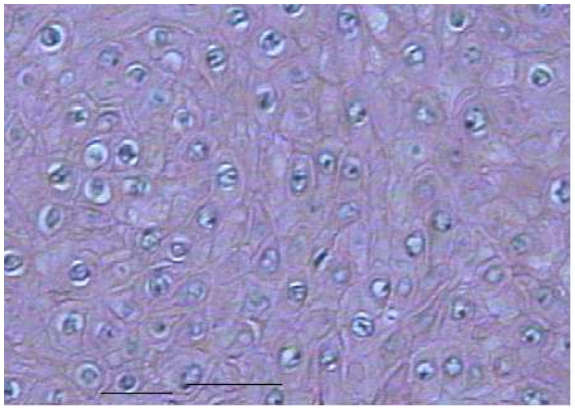
Normal Skin



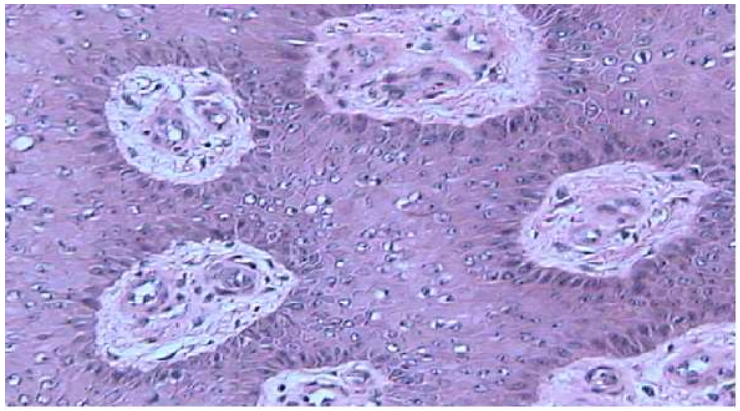
CRM, Spinous Layer



Basal Layer



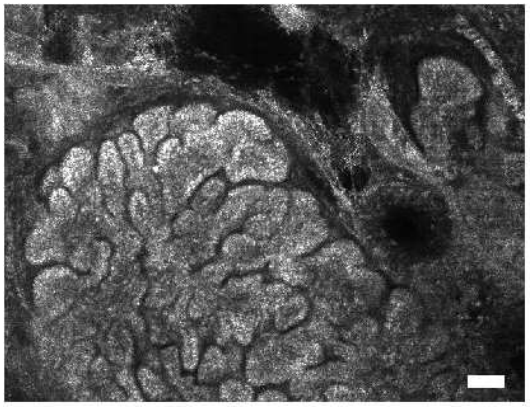
H&E, Spinous Layer



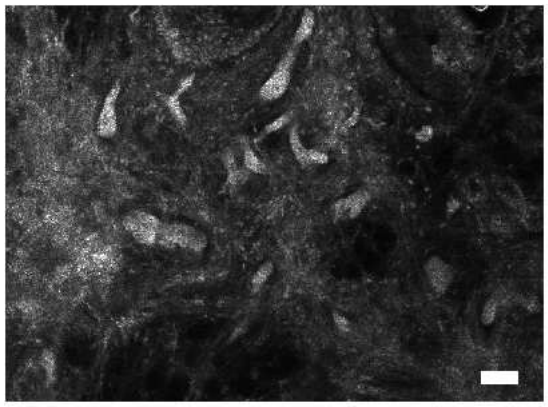
Basal Layer

Milind Rajadhyaksha

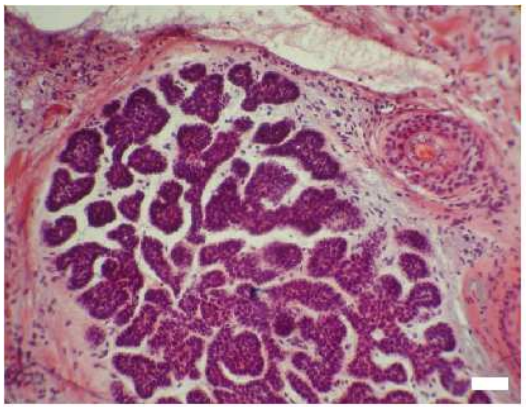
Skin Cancers



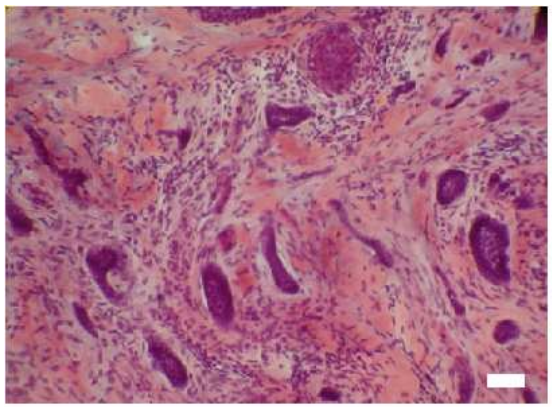
CRM, Nodular BCC



Infiltrative BCC



H&E, Nodular BCC



Infiltrative BCC

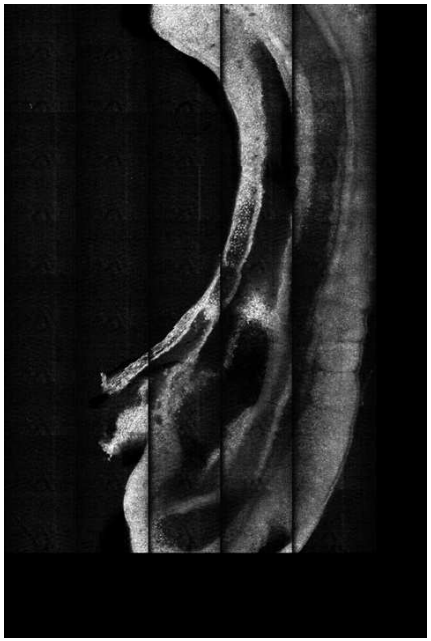
Milind Rajadhyaksha

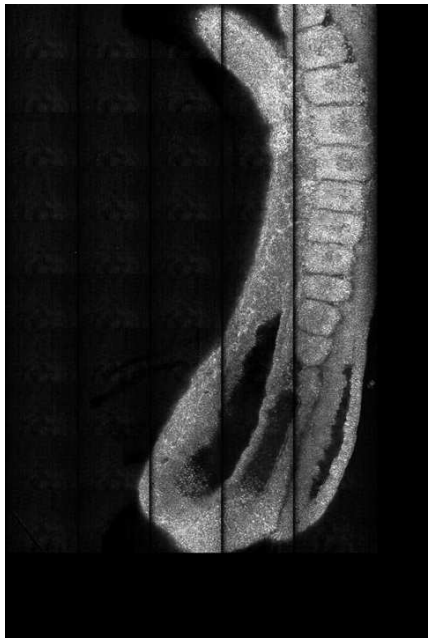
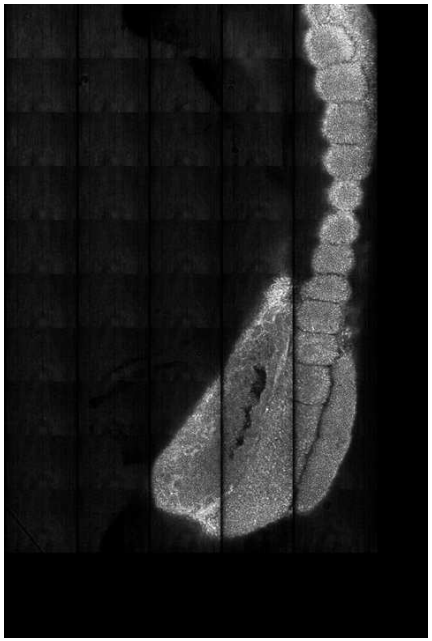
Large 3–D Mosaics

Mouse Embryo at Day 9

Z–Stack from Confocal Reflectance Microscopy

.



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

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

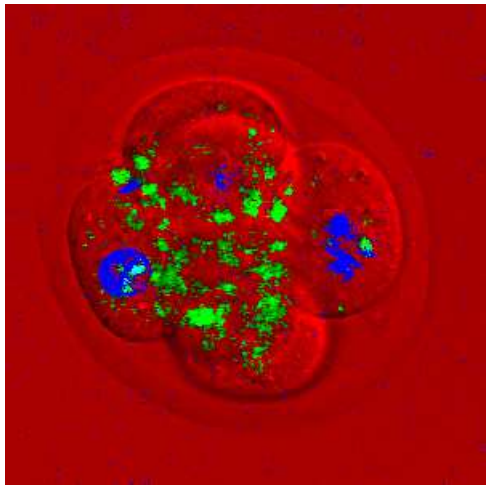
Multi–Modal Slices

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

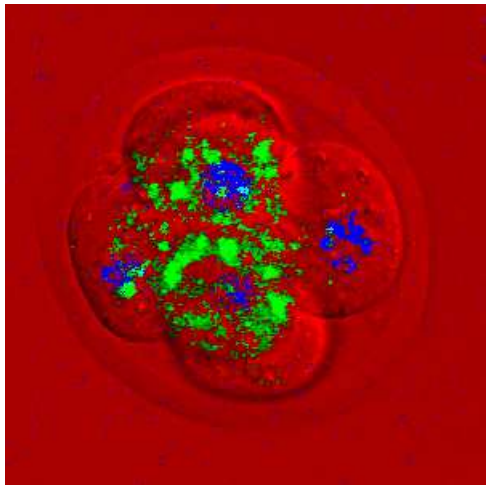
Hoechst Confocal shows nuclei

Weak CRM deep suggests lack of ballistic light.

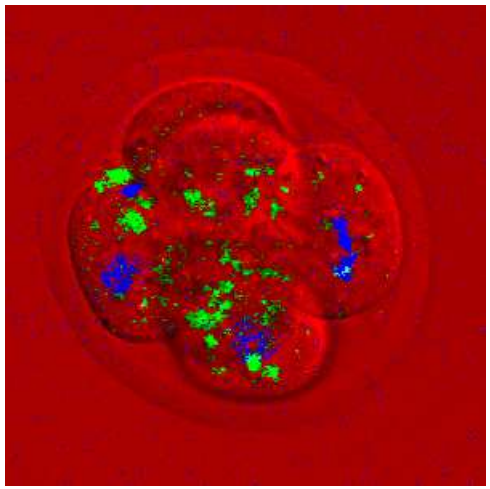
1. Top (Deep)



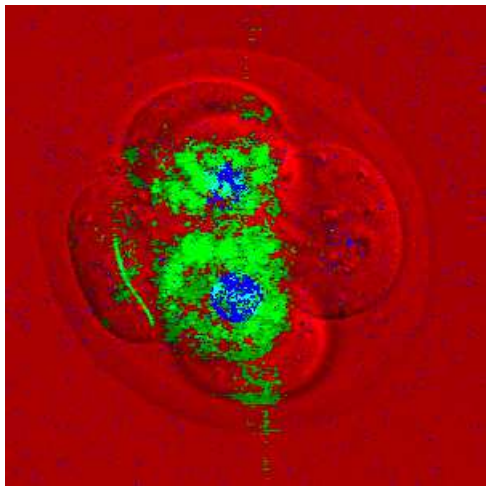
2.



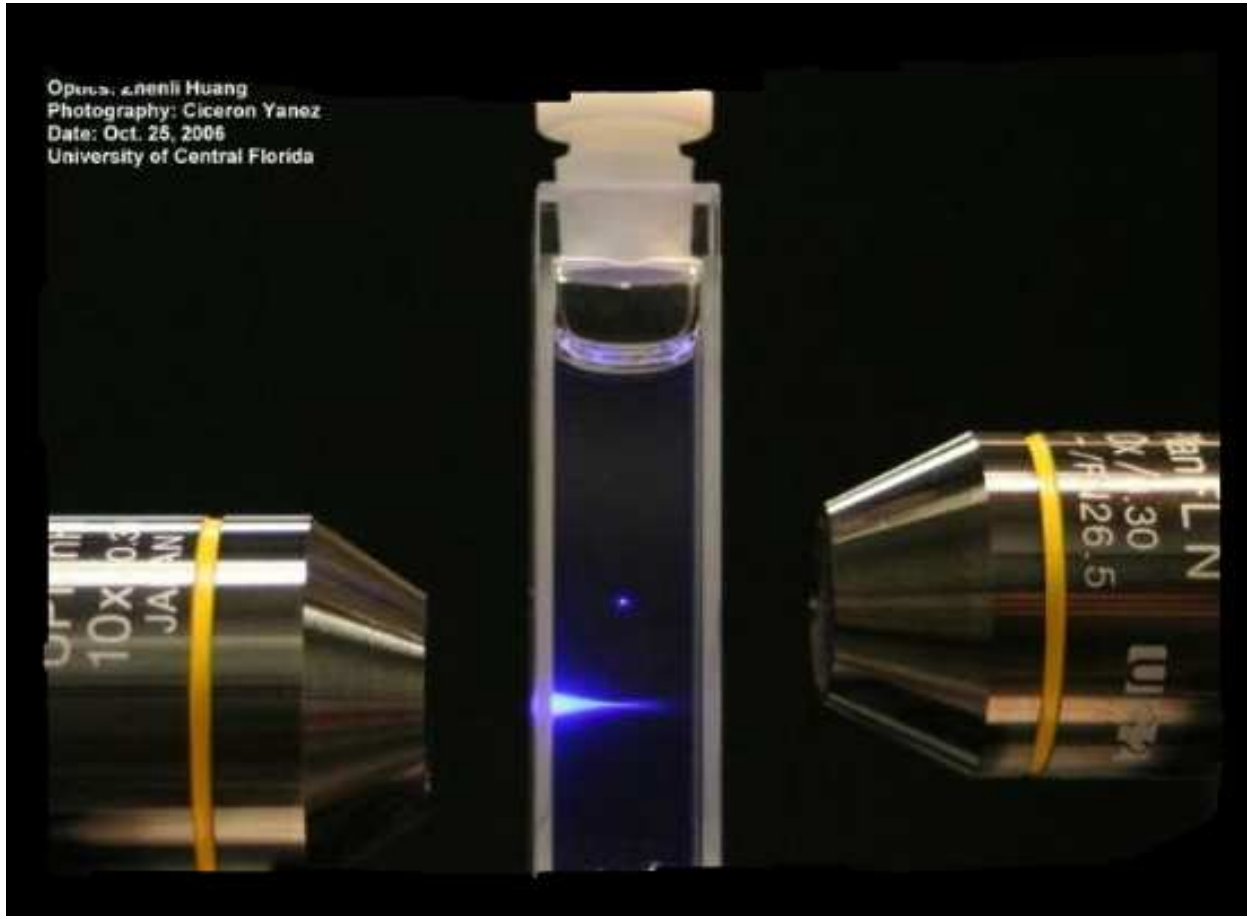
3.



4. Bottom



2–Photon Microscopy



Huang, UCF

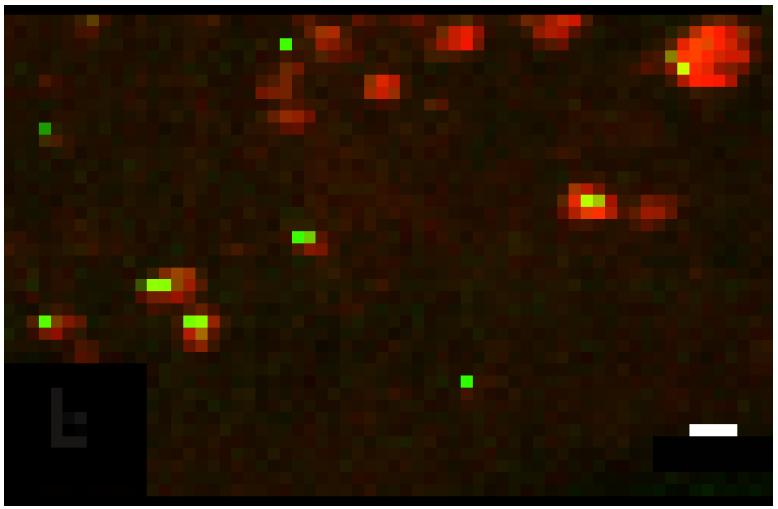
2–P Advantages

- 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

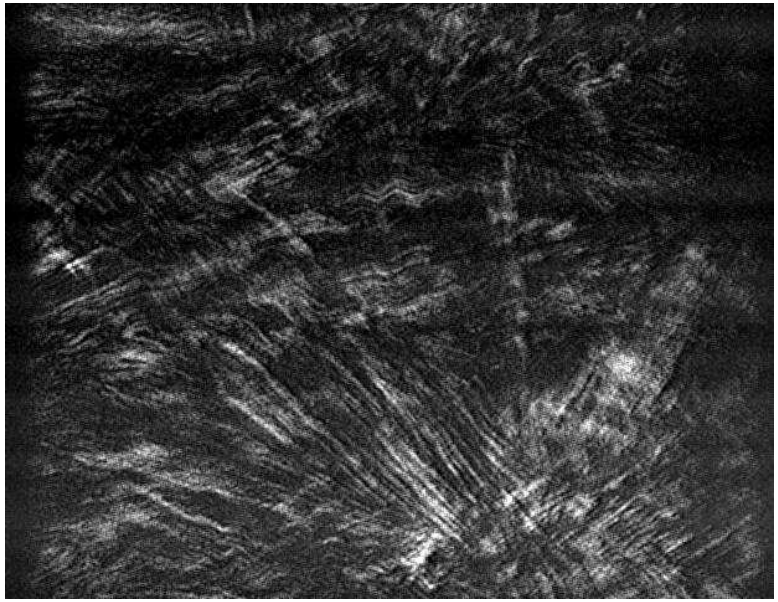


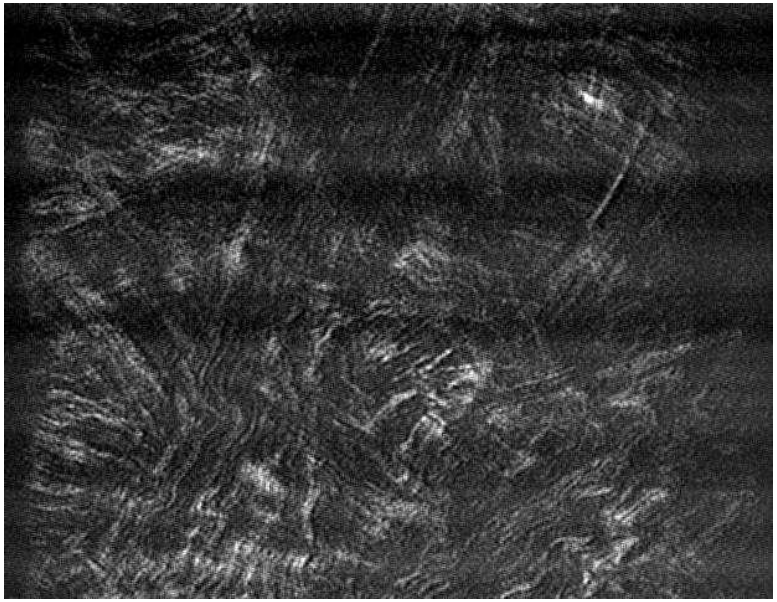
Kerimo *Photochemistry and Photobiology*, 2011

Northeastern University
College *of* Engineering

Collagen Fibrils in SHG

Universidad de los Andes





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

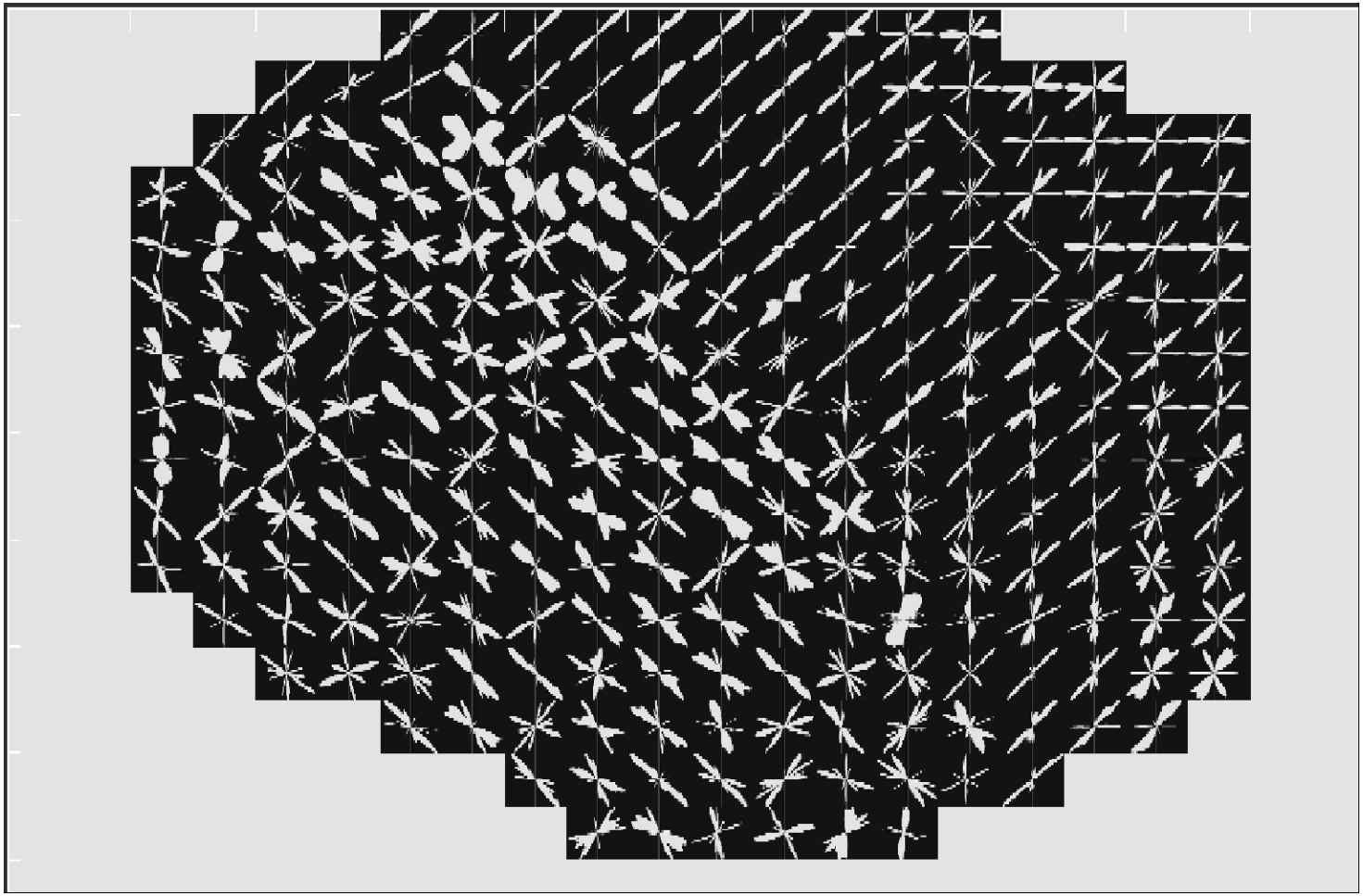
Thanks to Yair Mega, Mike Robitaille, Ramin Zareian

Collaboration with Kai–Tak Wan and Jeff Ruberti

Northeastern University
College *of* Engineering

Collagen Fibril Organization

Universidad de los Andes



Jimmy McLean (DOC Taiwan 2014, Ph.D. Columbia, Now at SpectraWave

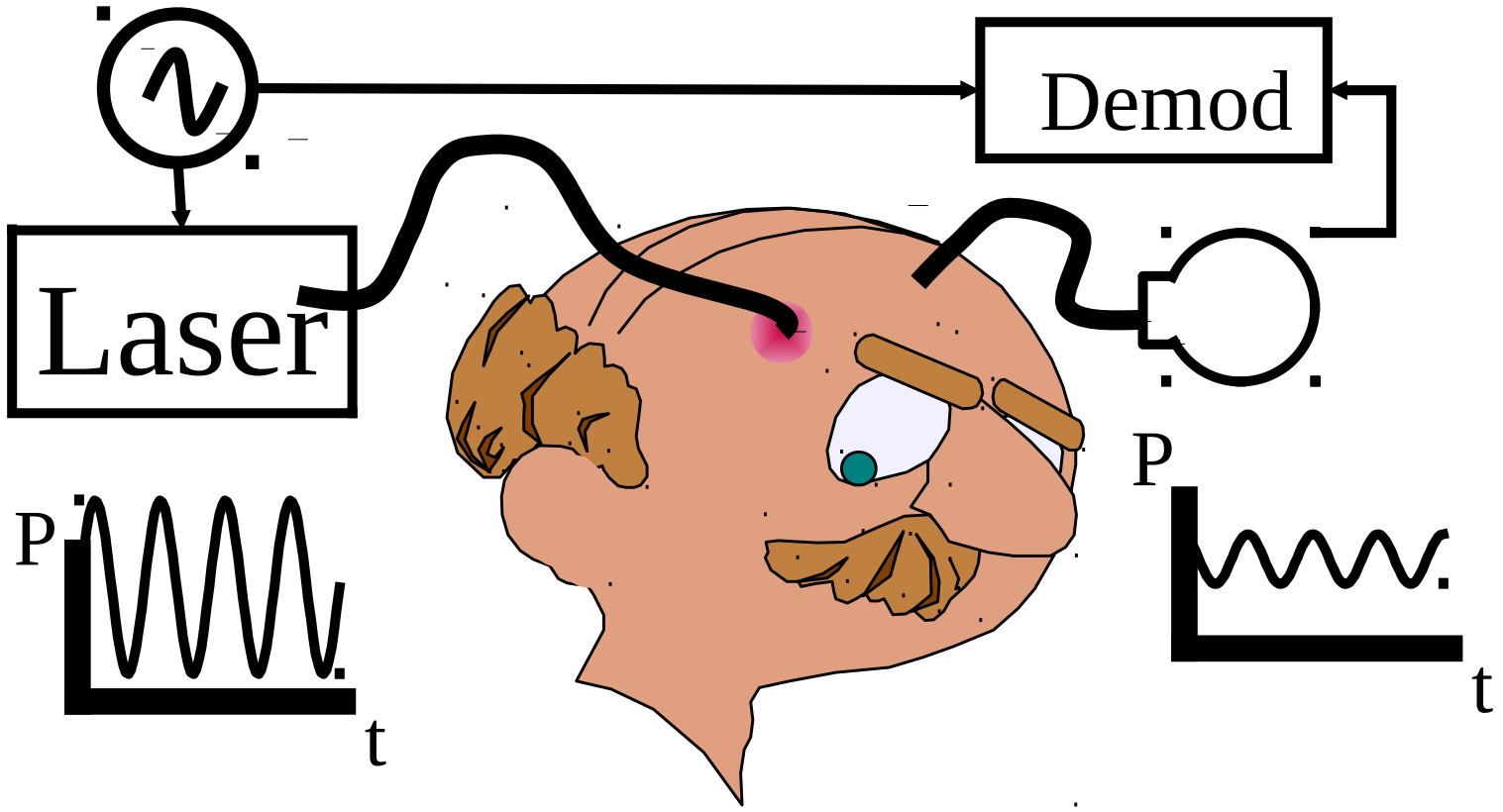
2–Photon *vs.* SHG

2–Photon	SHG
$\lambda_{em} > \lambda_{ex}/2$	$\lambda_{em} = \lambda_{ex}/2$
Depends on λ_{em}	Less Dependent on λ_{em}
Exponential Time Decay	Instantaneous
Random Direction	Forward Direction
Unpolarized (Maybe)	Polarized

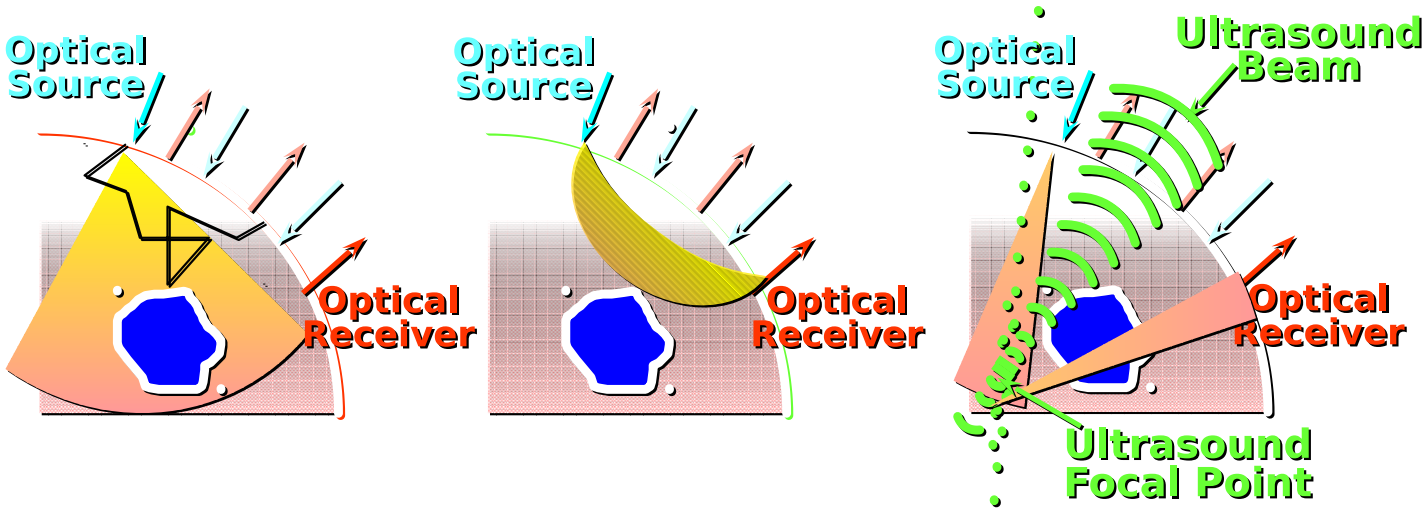
Slit Lamp

https://en.wikipedia.org/wiki/Slit_lamp

Diffusive Imaging



DOT and Ultrasound



All Light From Source Fiber

Light From Source to Receiver

Light From Source to Receiver through Ultrasound Focus

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