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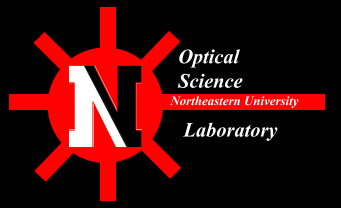

Sectioning with Single–View Structured Illumination

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With Help From Zac Hoffman, Mahsa Azizi, Kivanc Kose
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Agenda

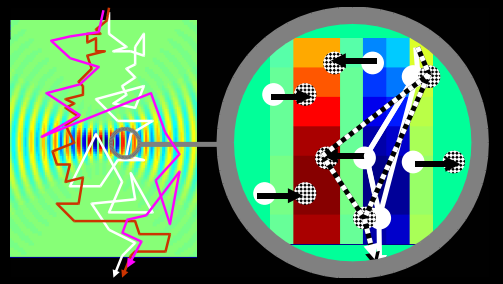
Optical Science Laboratory
Northeastern University

- Research Overview
- Modern Microscopy
- Structured Illumination (SIM)
- Random Patterns
- Sectioning with SIM
- SISIM: Single–Image SIM

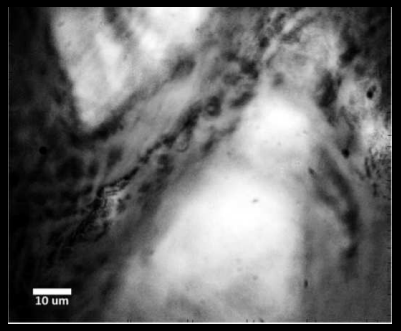
Our Current Research

- Multi–Modal Microscopy
- Light and Sound
- Structured Illumination
- Collagen Orientation
- Stepwise 3–PEF in Melanin
- Lidar

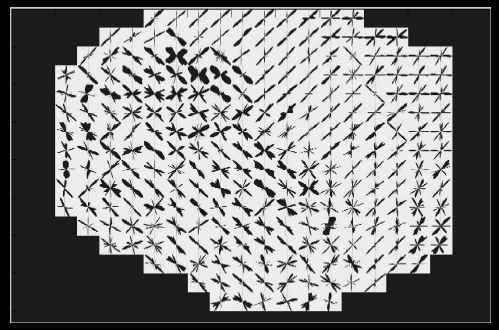
Multi–Modal



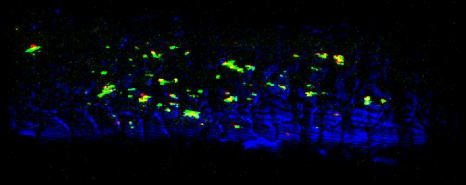
Light and Sound



SIM



Collagen



Melanin

Lidar

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

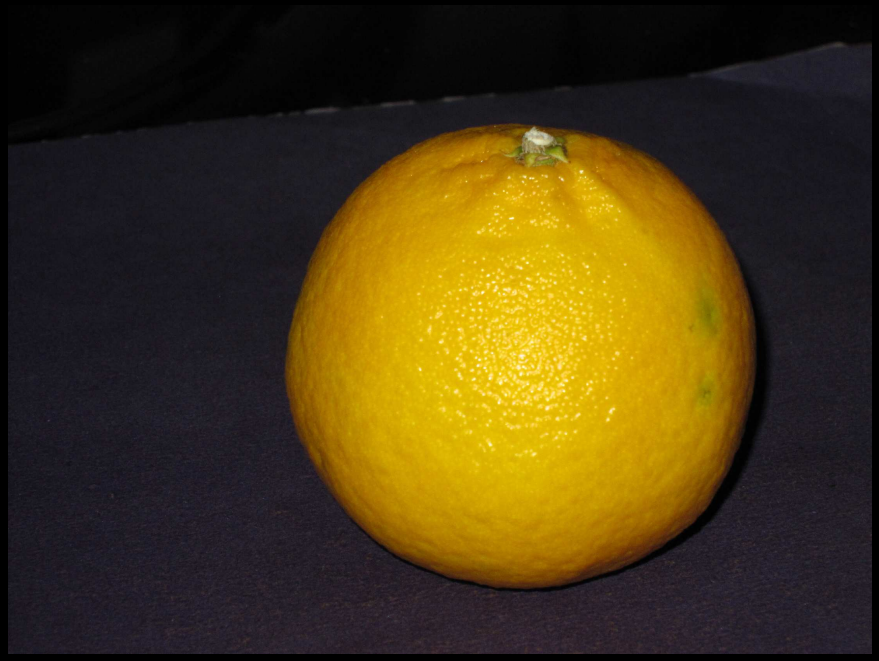
- What's so Modern?
  Microscopy has been around since 1590. . .

- . . . 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)

What Is Sectioning?

Defocusing a camera makes an object blurry;
Δz depends on Variation with x, . . .
but contrast is an issue.

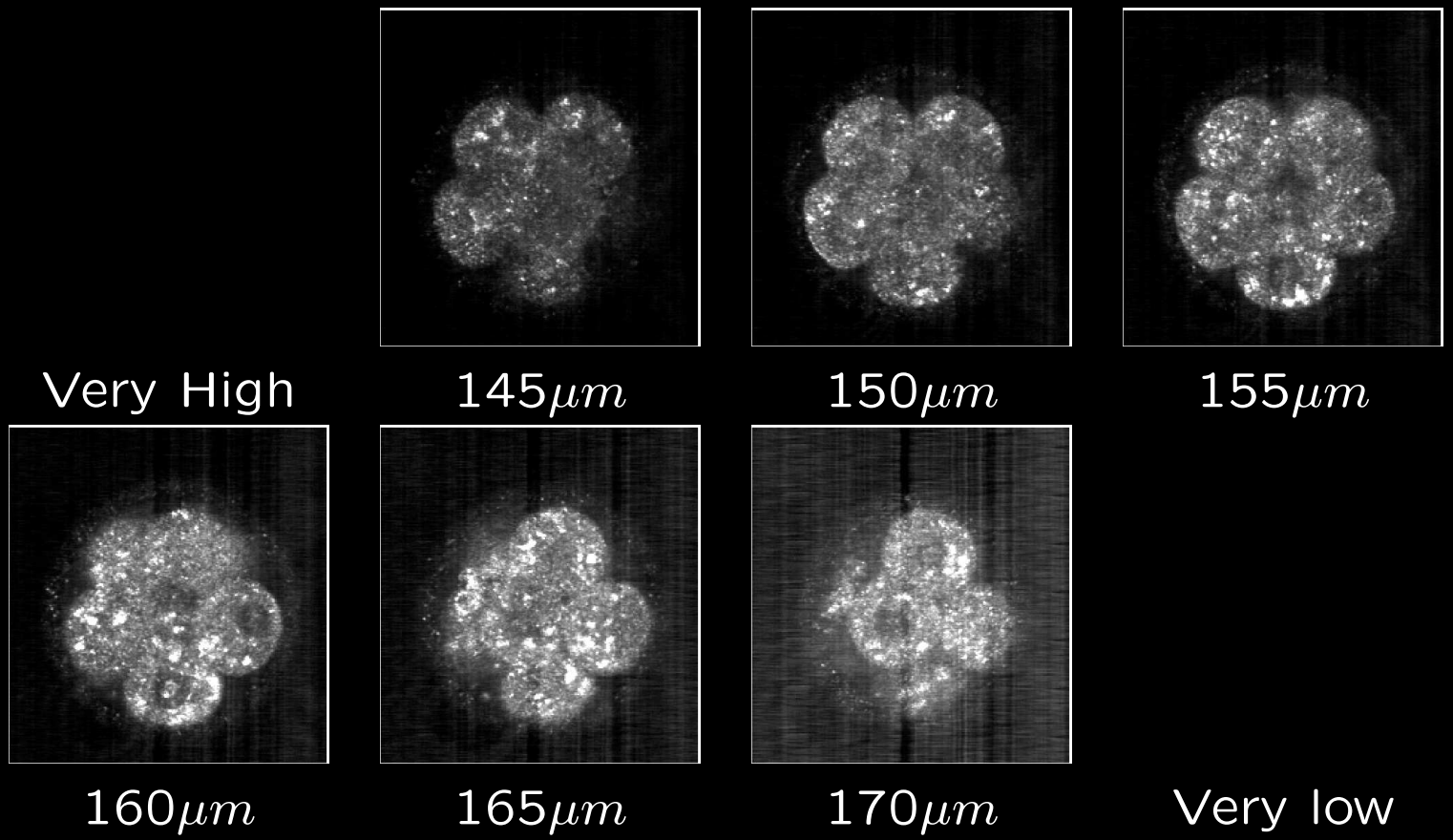


In–Focus Image

Out–Of–Focus Image

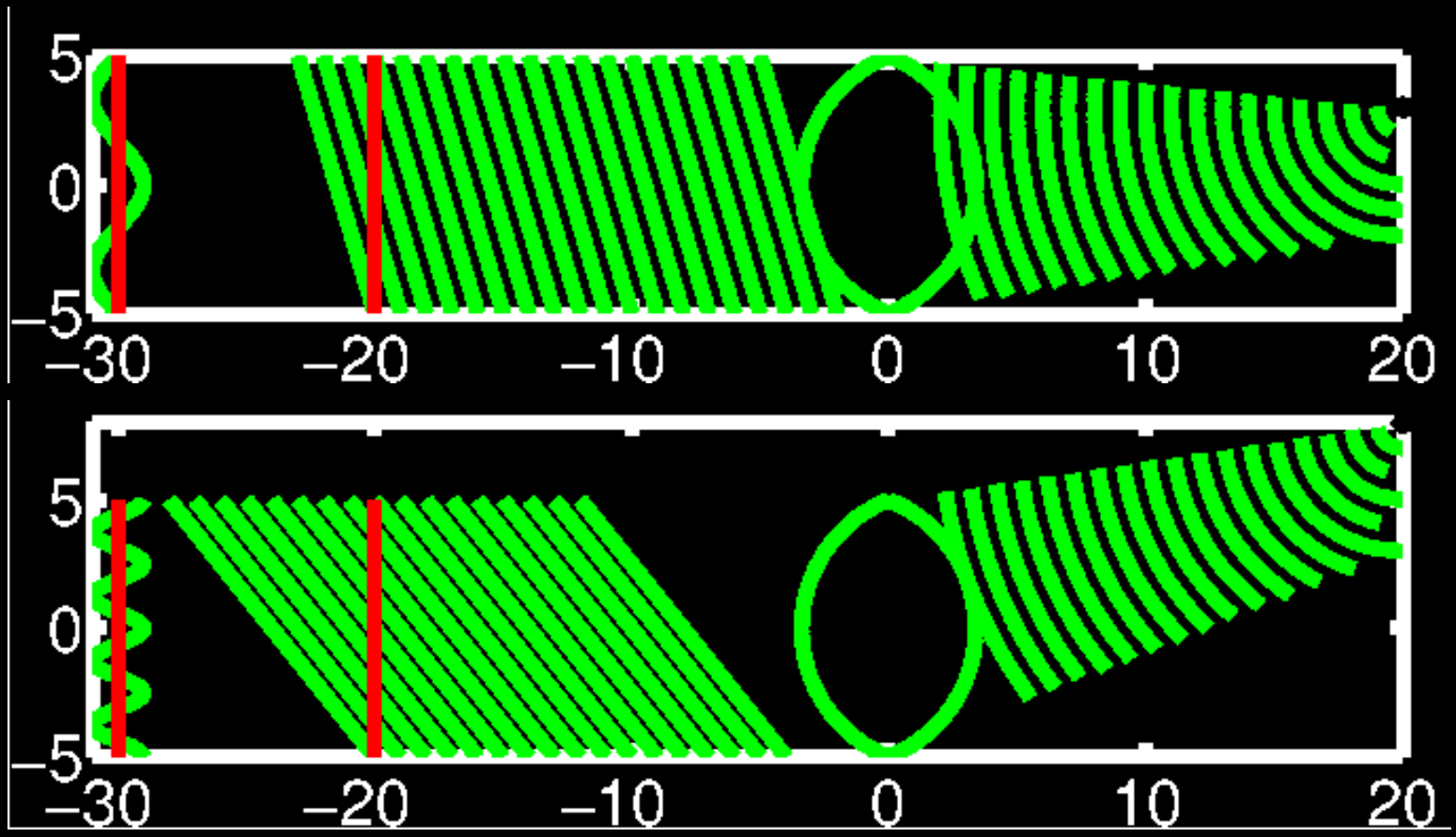
What Is Sectioning?

Defocusing a confocal microscope makes an object disappear



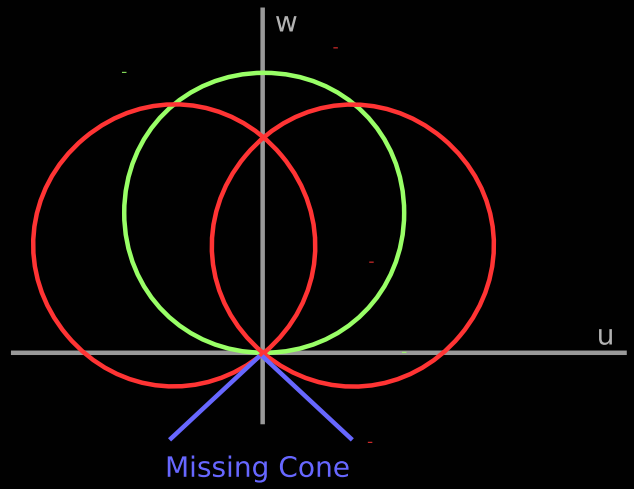
Judy Newmark (Warner Group), Bill Warger

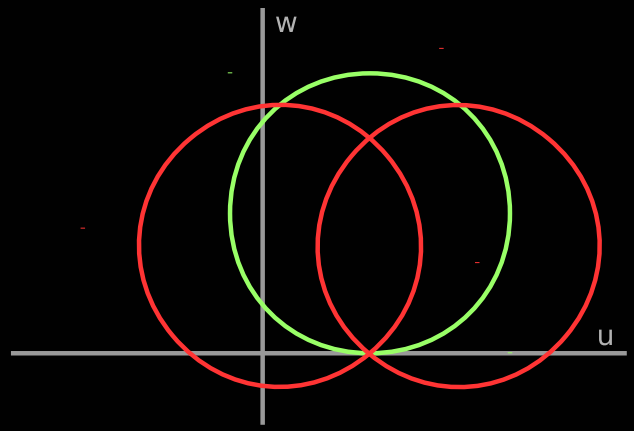
Varying Spatial Frequencies



SIM

- SIM = Structured Illumination Microscopy
- Uses an Optical Fourier Transform
- Can Improve Resolution by 2X
- Can Also Provide Sectioning





Structured Illumination

- Image as a Product in the Field (Spatial) Plane

$$\text{Image} = \text{Illumination} \times \text{Transmission}$$

- Low Pass Filter

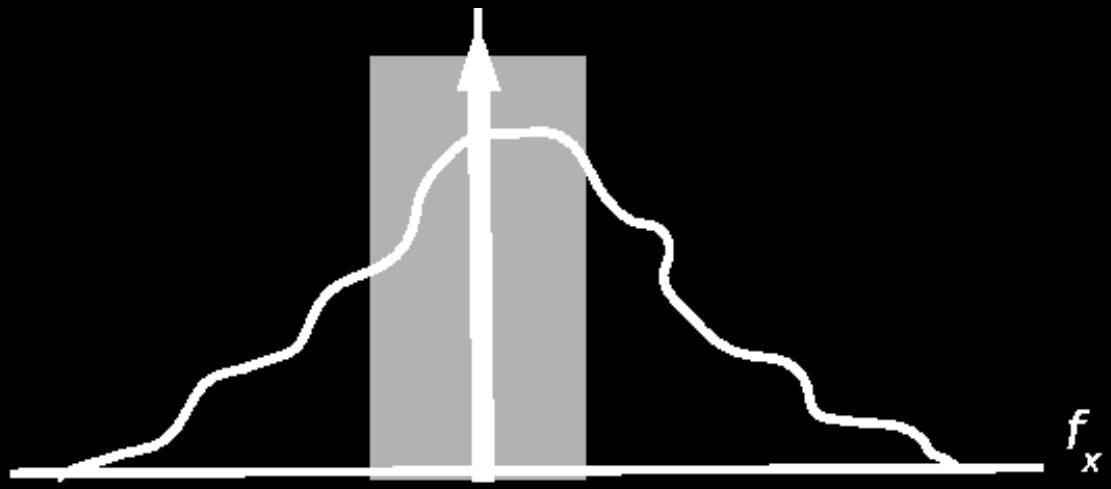
$$f_x < \frac{NA}{\lambda}$$

$$\text{Image} = (\text{Illumination} \times \text{Transmission}) * \text{Filter}$$

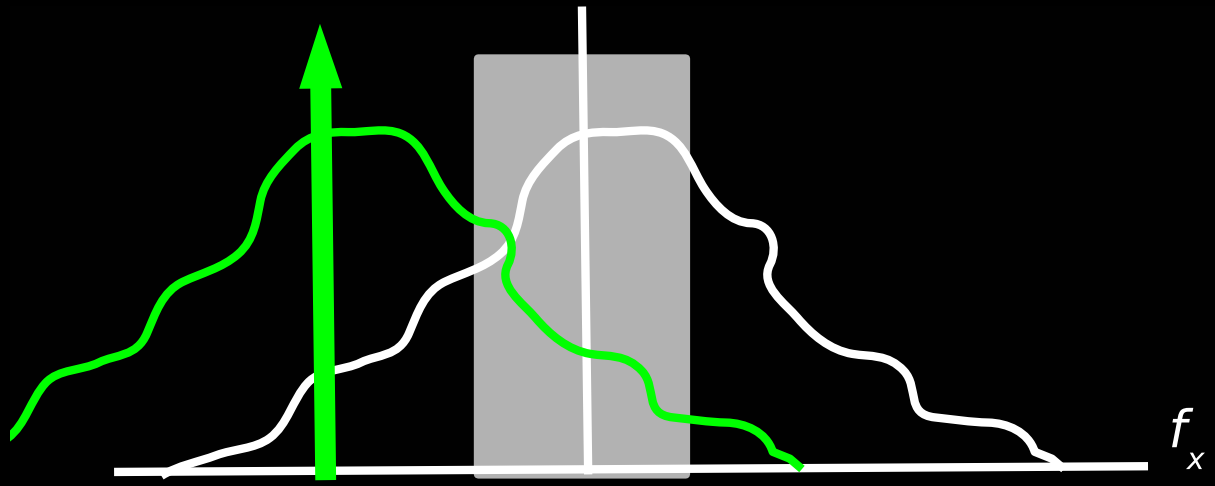
- Pupil (Spatial Frequency) Domain

$$\text{IMAGE} = (\text{ILLUMINATION} * \text{TRANSMISSION}) \times \text{FILTER}$$

How It Works



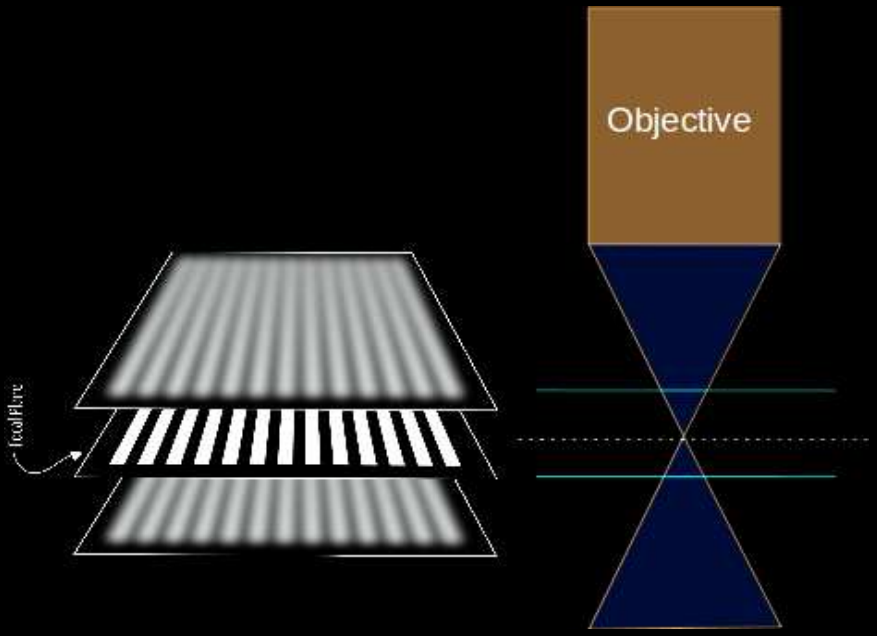
"DC" or Plane–Wave; Pupil as a Low–Pass Filter



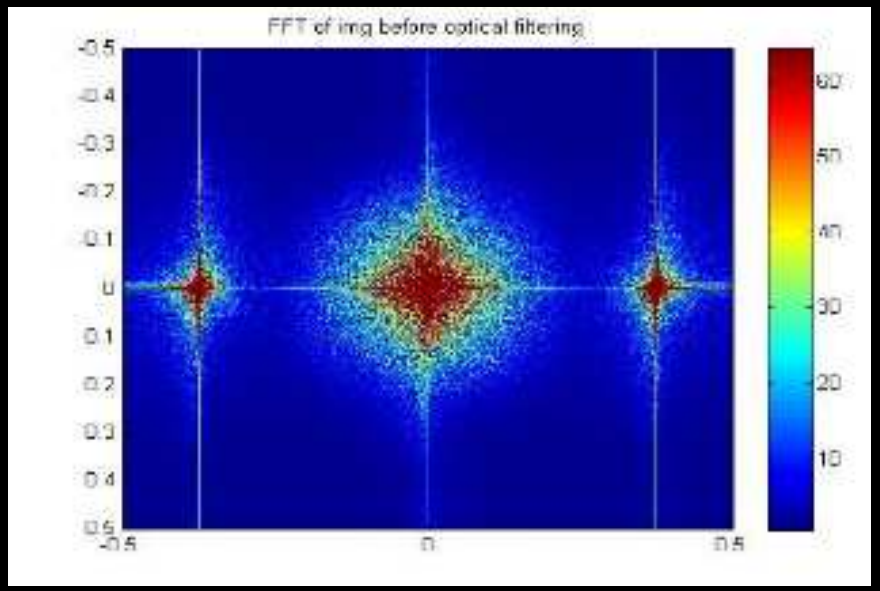
Offset Illumination: Multiply in the Image; Convolve in the Pupil

SIM Sectioning Concept

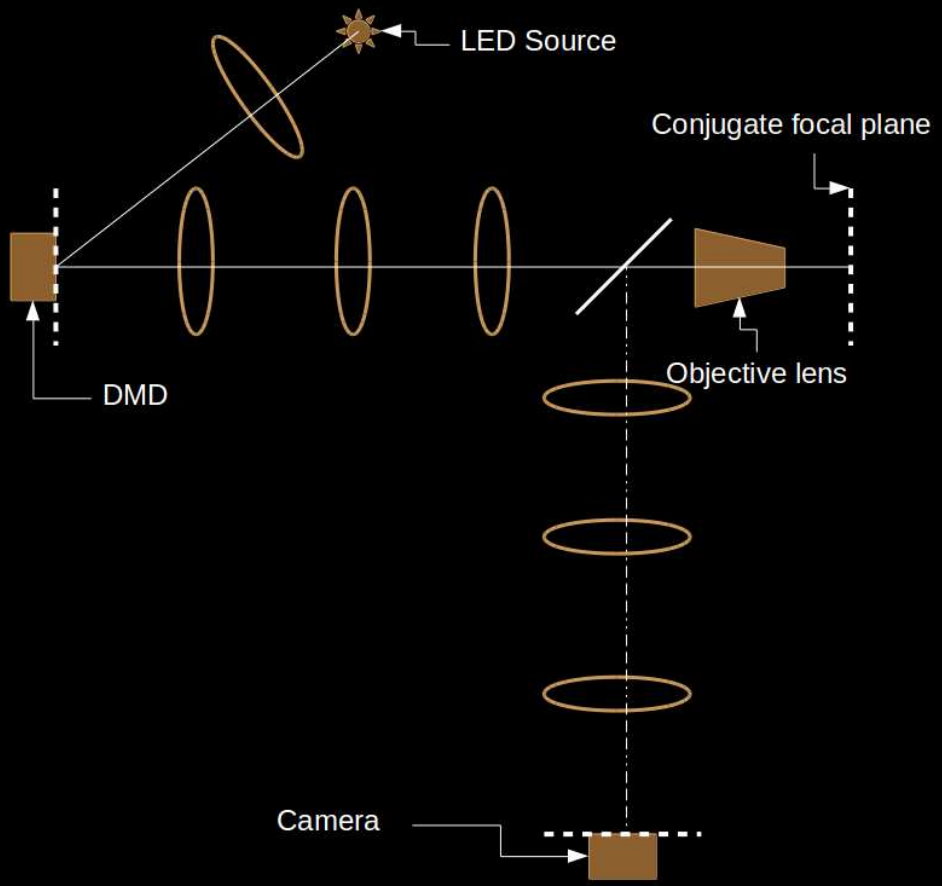
- High frequency modulation pattern in focal plane
- Blurred pattern above and below
- Resolution dependent on frequency and NA



Fourier Domain



Layout



635nm, 18X, 0.4 NA

Random Modulation

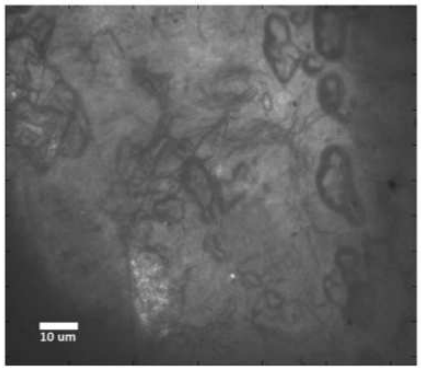


Fig. 9 Wide-field *in vivo* image at the surface.

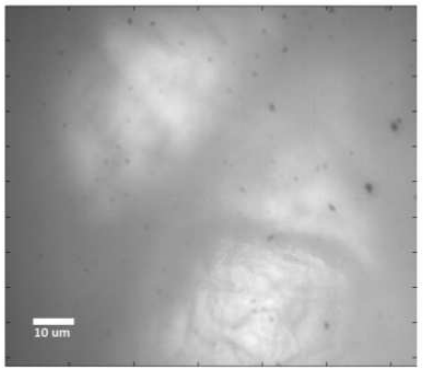


Fig. 11 Wide-field *in vivo* image at depth.

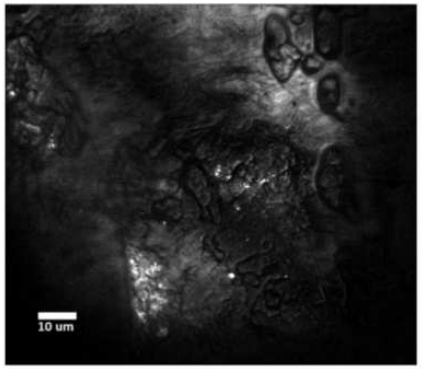


Fig. 10 CRII *in vivo* image showing the stratum corneum.

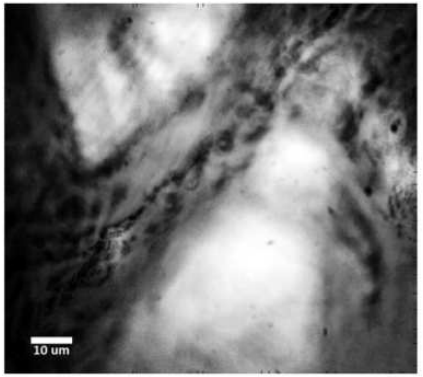


Fig. 12 CRII *in vivo* image showing the stratum granulosum.

Hoffman, *JBO*, 2012

SIM Sectioning Experiment

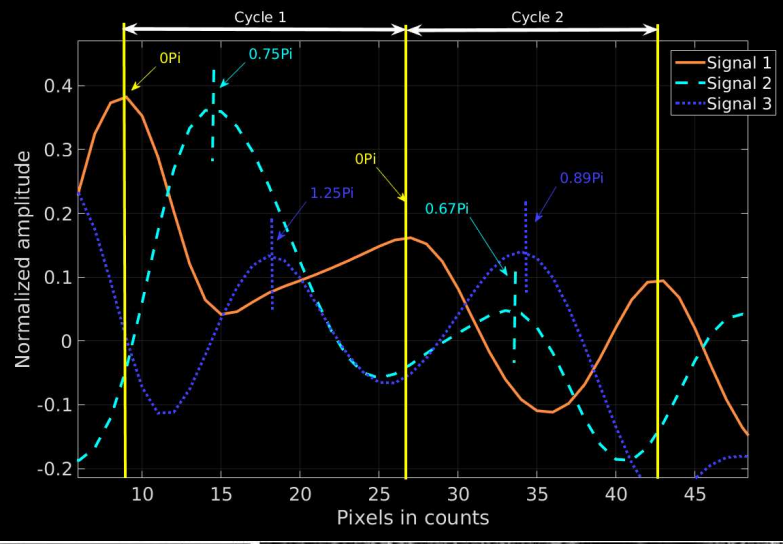
Phases = $[0^{\text{deg}}, 120^{\text{deg}}, 240^{\text{deg}}]$

$I_{sec} =$

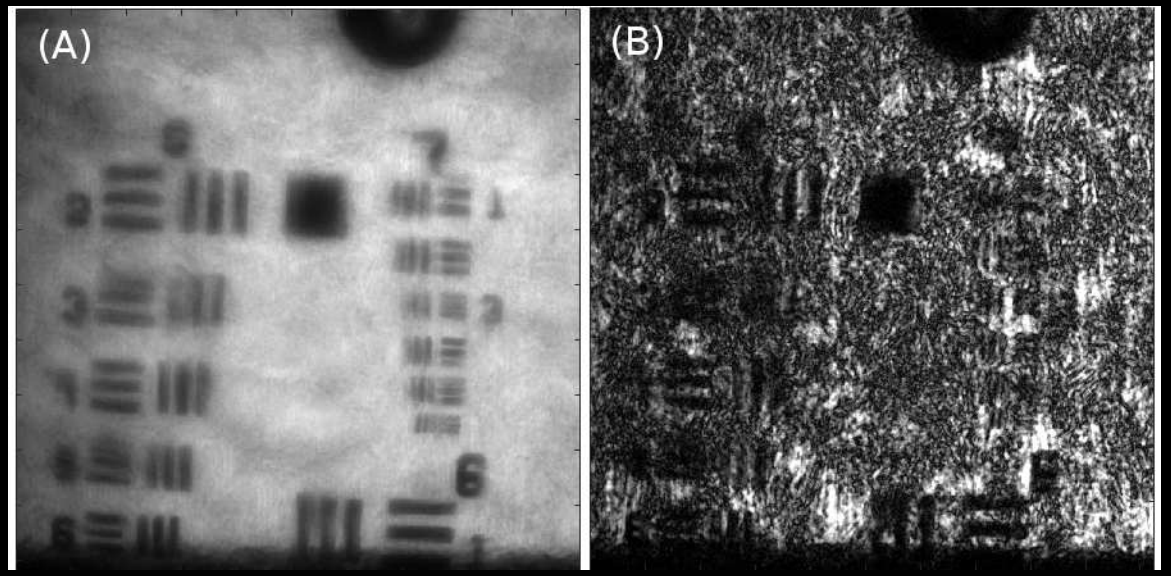
$$\sqrt{(I_1 - I_2)^2 + (I_1 - I_3)^2 + (I_2 - I_3)^2}$$

or

$$I_{AC} = I_1 e^{i0} + I_2 e^{i2\pi/3} + I_3 e^{i4\pi/3}$$

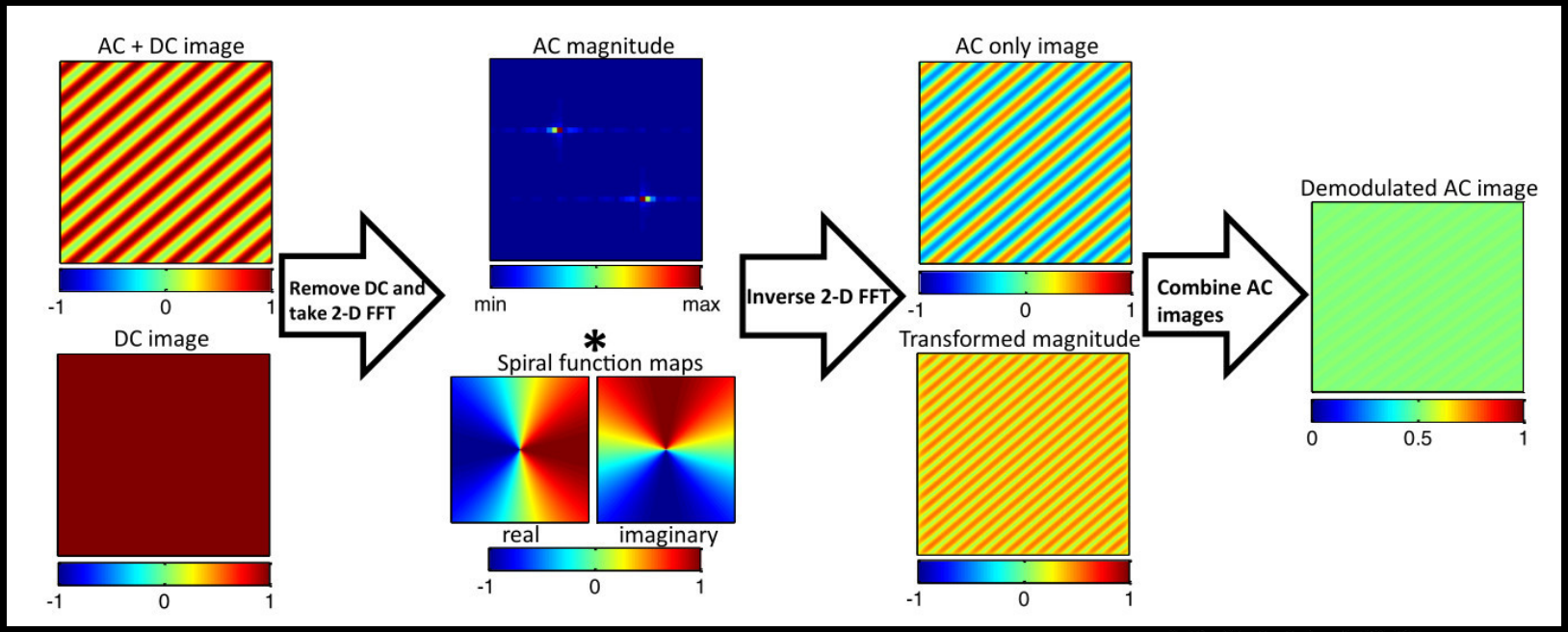


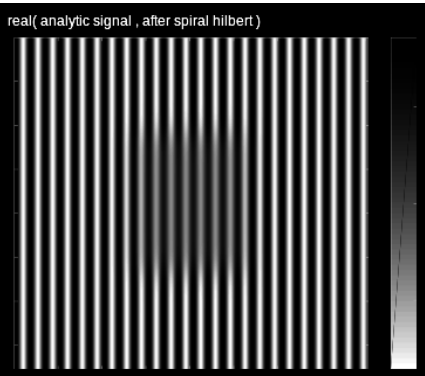
Layer 1:
Ground Glass
Layer 2:
Gel ($n \approx 1.33$)
Layer 3:
Resolution Chart

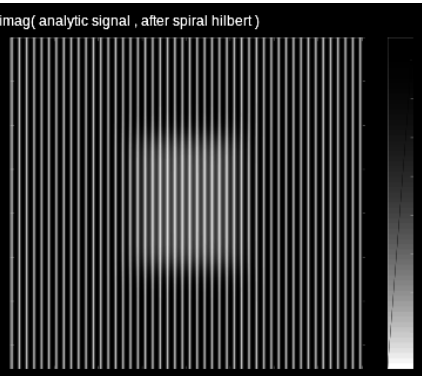


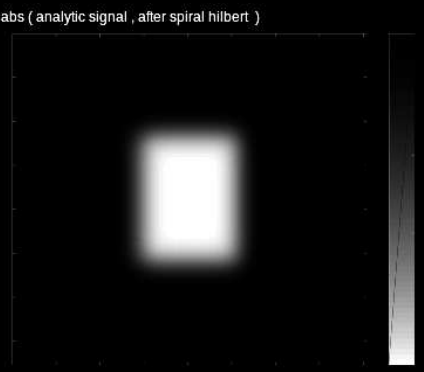
Neil, *Optics Letters* 1997

Spiral Hilbert Transform



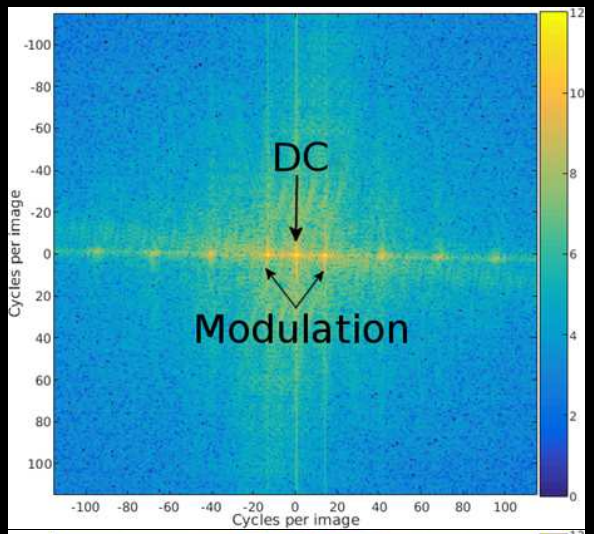


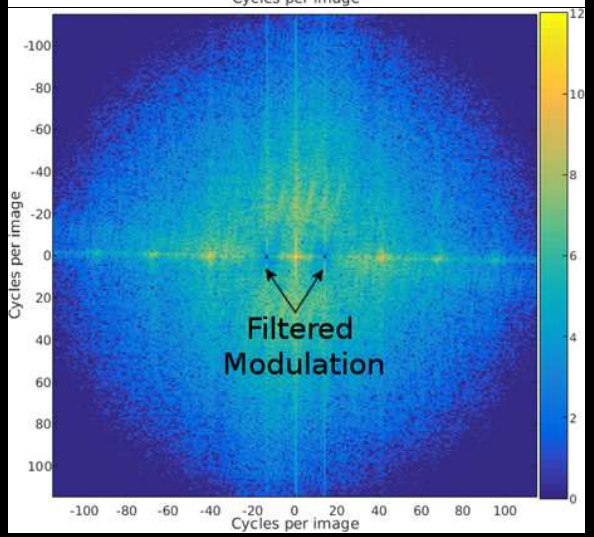




Nadeau, *JBO* 2014

Processing





CCD Captures Modulated Image, $\Gamma(x, y)$.

↓

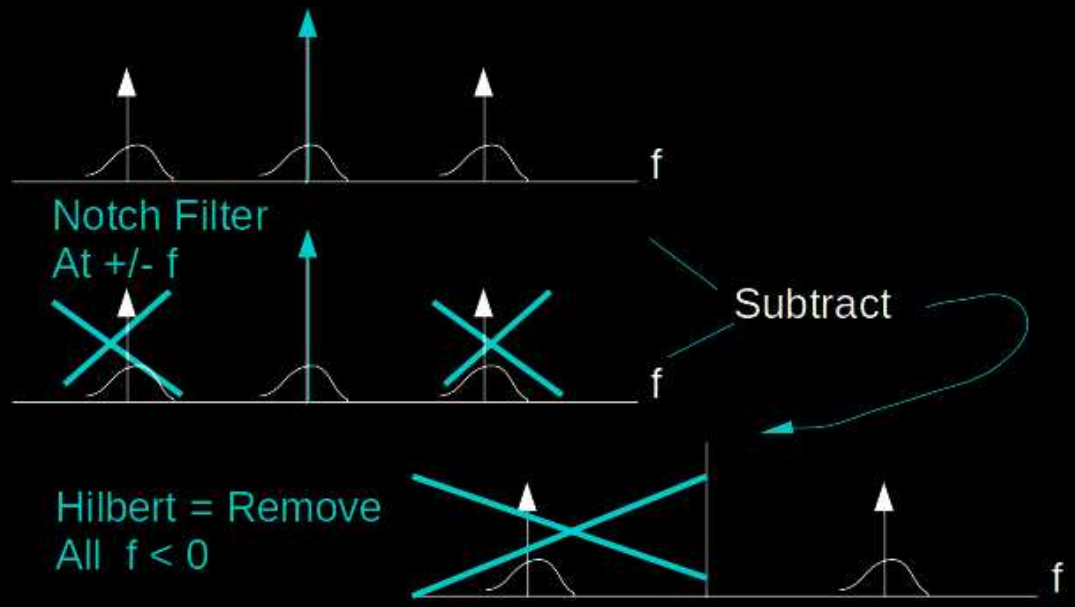
Estimate Unmodulated Image by Notch Filtering AC Components, $R(x, y)$.

↓

Subtract Unmodulated Image from Modulated, $\Gamma(x, y) - \Gamma(x, y)$

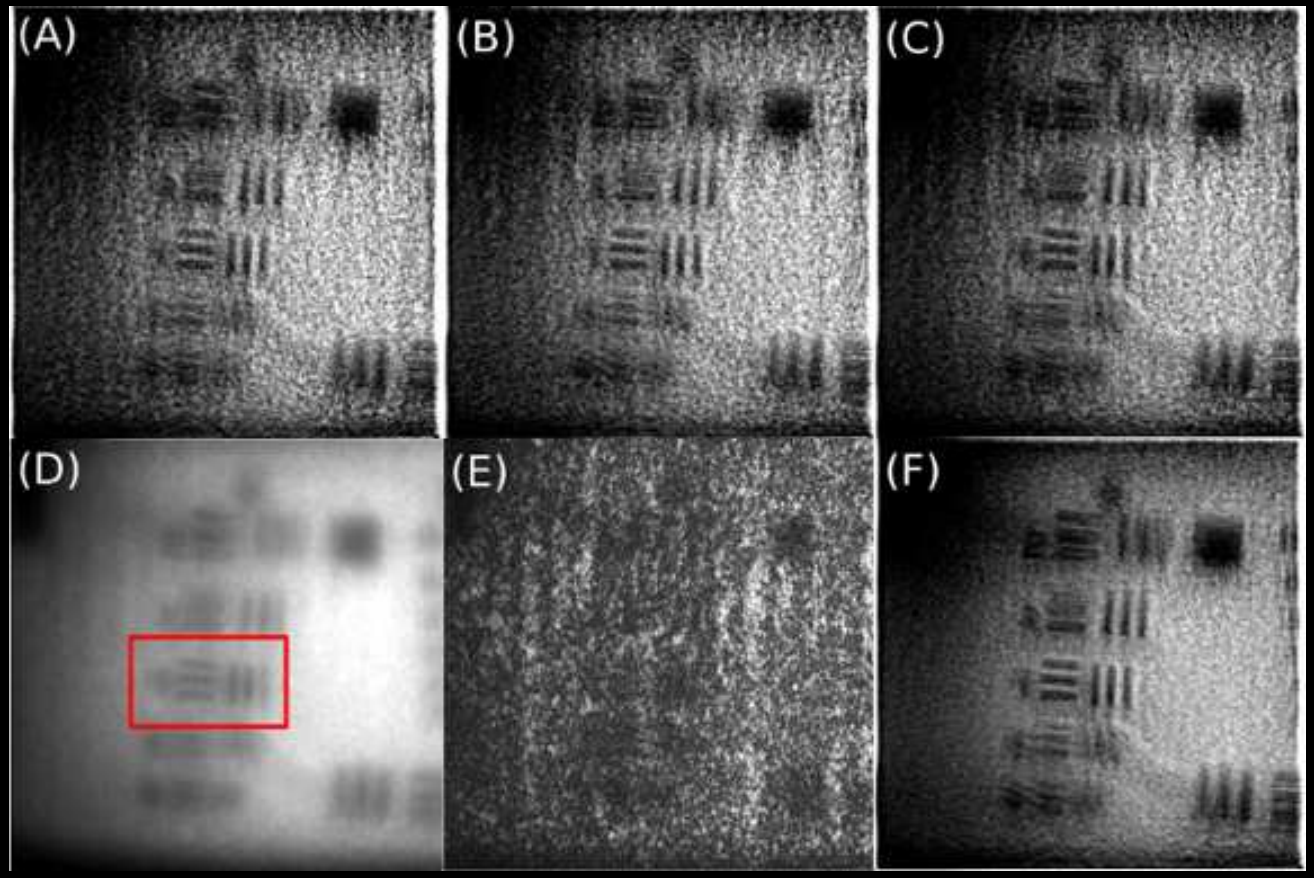
↓

Demodulate Using Spiral Hilbert Transform to Produce Sectioned Image $R_{IF}(x, y)$.



Sectioning at Depth

SISIM Phase 1 SISIM Phase 2 SISIM Phase 3



Widefield SIM 3 Phase SISIM Sum

Human Skin

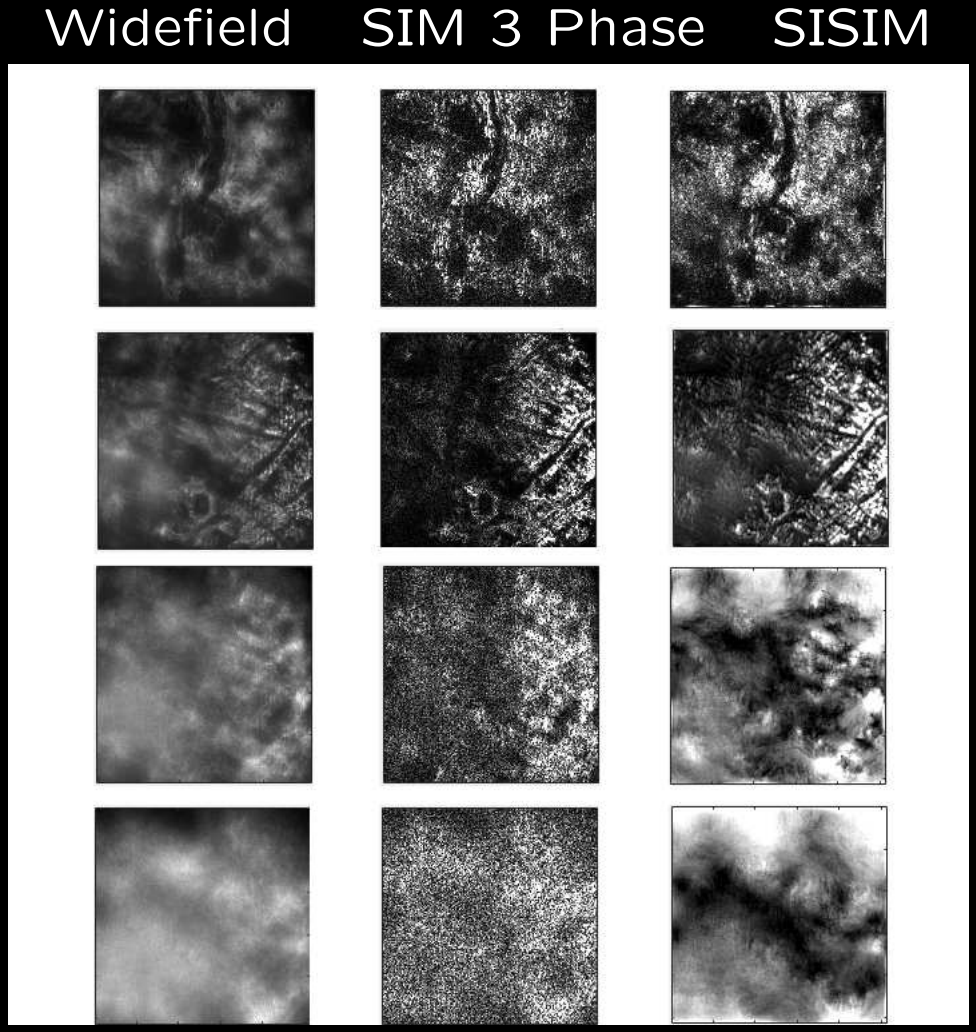
All Images
750μm Square

0 to 25μm deep

25 to 50μm deep

50 to 75μm deep

75 to 100μm deep

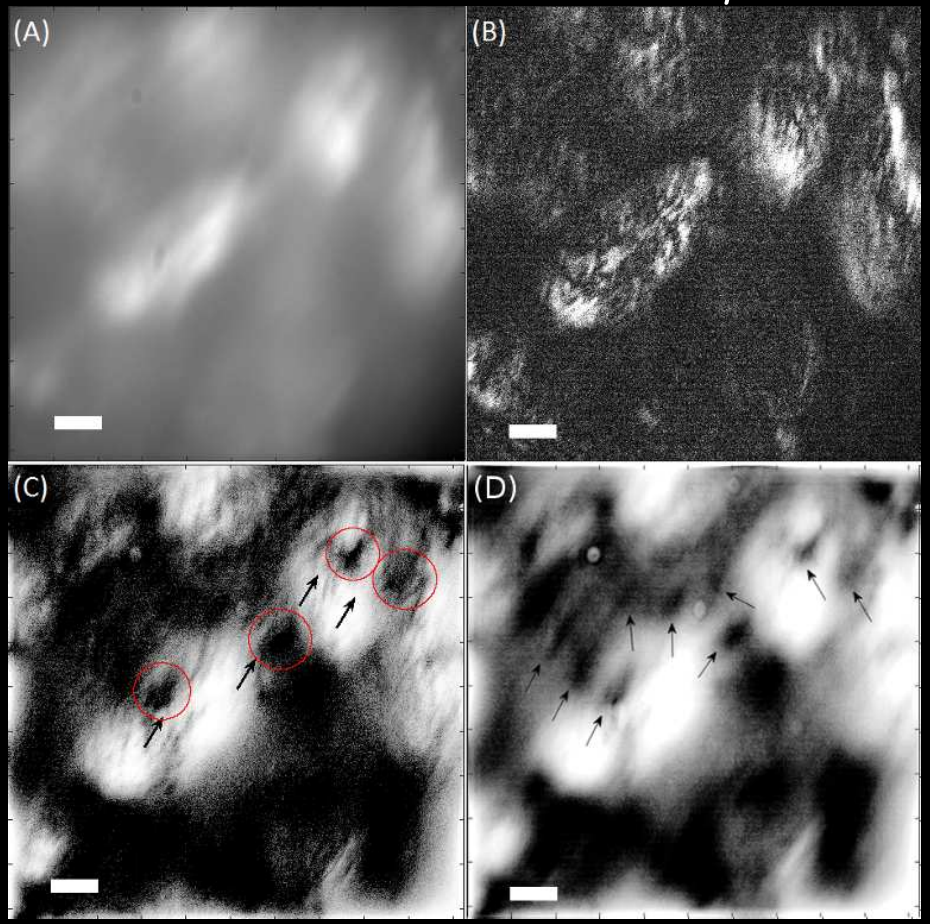


Motion Artifacts

Human Skin at $\approx 50\mu$m

Widefield

SIM 3 Phase



SISIM

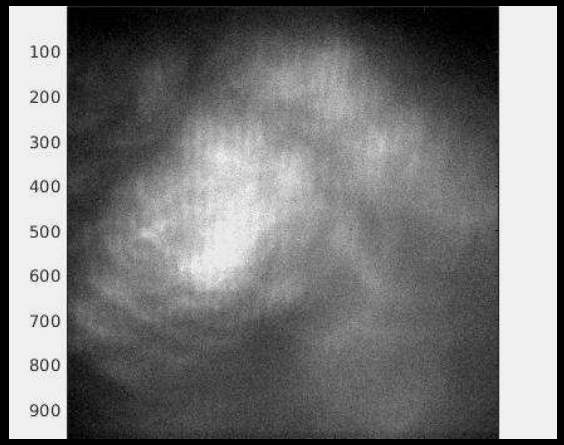
SISIM Sum Registered

Scale Bar $\approx 10\mu$m

Registration Algorithm

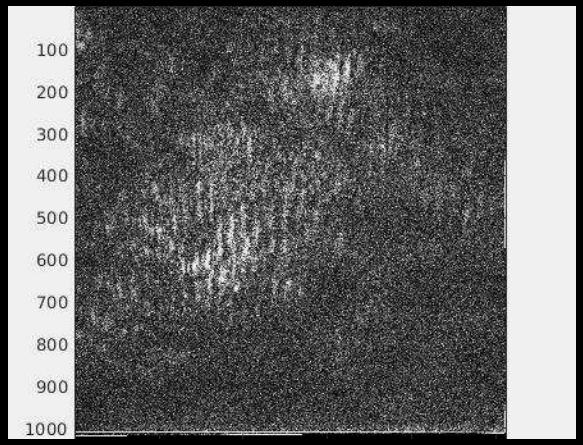
- Removes modulation pattern first.
- Apply registration to just the specimen.
- Align all images before sectioning.
- Add three phases after they are aligned and sectioned
- images to produce high-quality sectioning.

Regstration Results

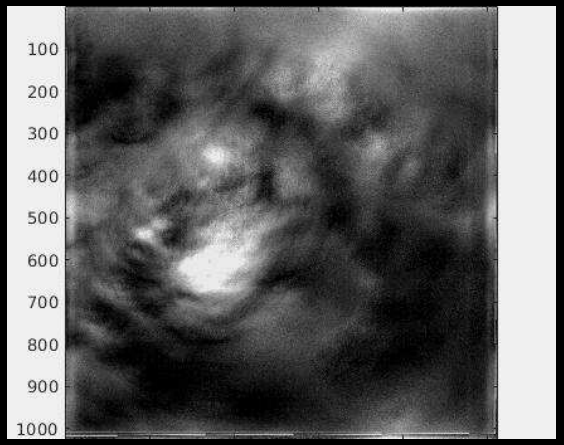


Widefield

All Images
$\approx 750\mu$m Square
See Videos



SIM 3 Phase



Registered SISIM

Summary

- SIM Sectioning Artifacts Using 3 Phases
  - Refraction
  - Motion
- SISIM Uses a Single Image
- Adding Images Recovers SNR
- Registration on Image Allows Recovery of SNR

Thanks

- Funding: NSF CBET-1510281

- Graduate Students
  - Dr. Zachary Hoffman, Ph.D. 2018
  - Ms. Mahsa Azizi, Ph.D. Student

- Collaborators
  - Dr. Kivanc Kose (MSKCC)
  - Dr. Milind Rajadhyaksha (MSKCC)