Optical imaging with the use of multiple scattering

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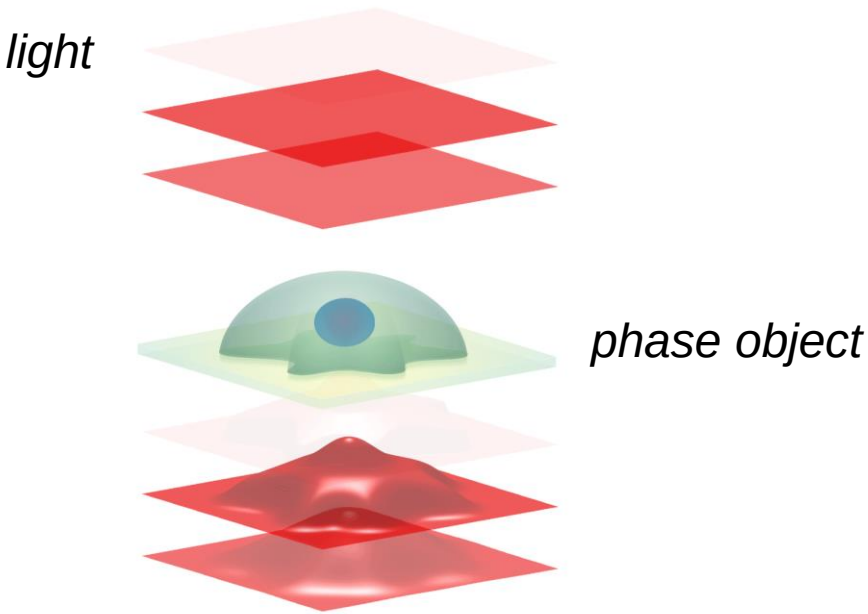
Phase object

- Phase object: *transparent* under light

- How can we perceive the existence of the clear globe?

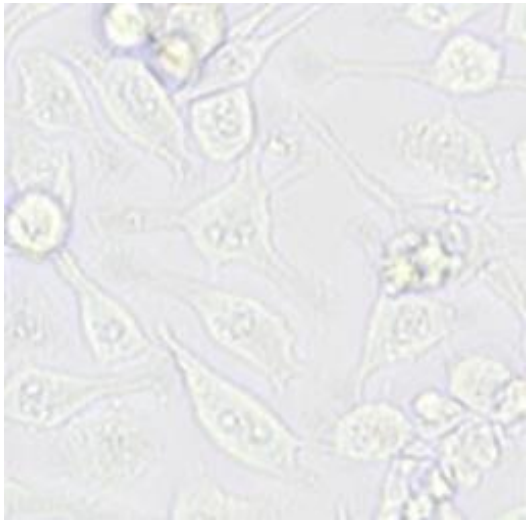
Phase object



The phase of the incident light is *delayed* due to the presence of the sample.

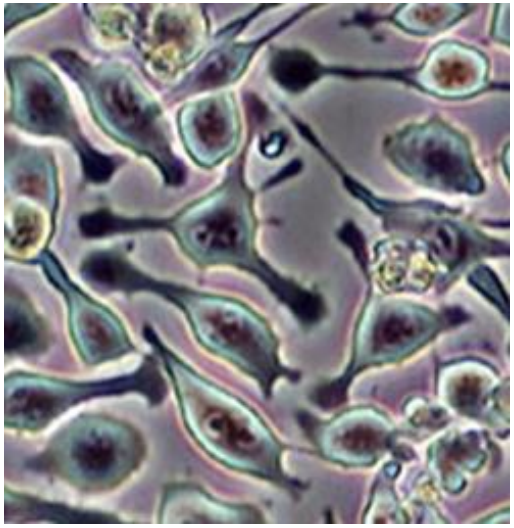
Phase: label-free imaging contrast

Bright field



Nikon website

Phase contrast

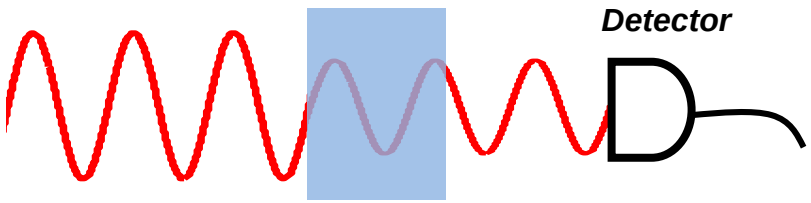


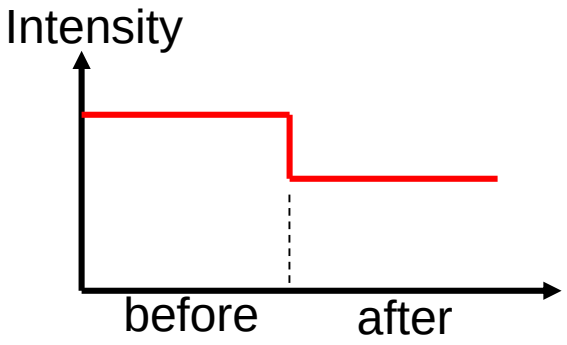
- Frits Zernike 1934, Nobel prize for physics in 1953
- Phase visualizes transparent structures with better contrast

Not quantitative !!

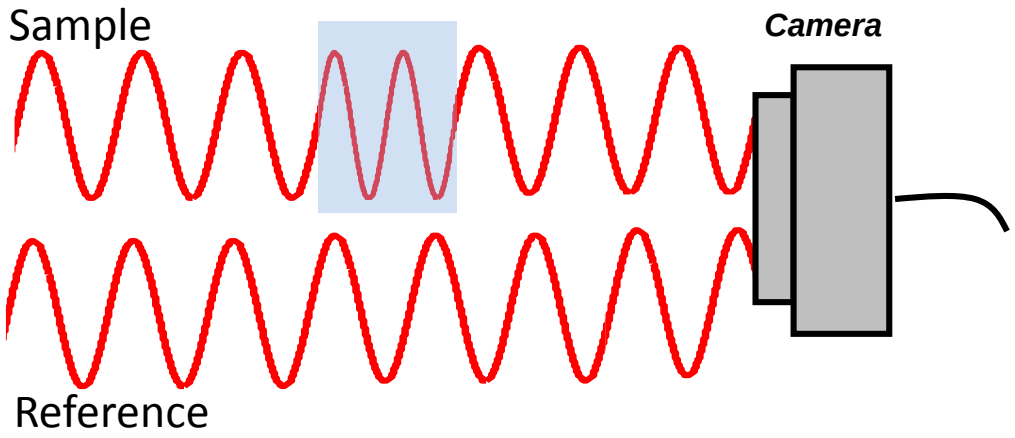
Intensity vs. phase detection

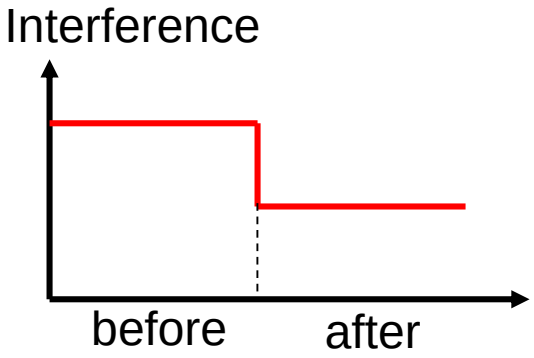
- For an absorptive object





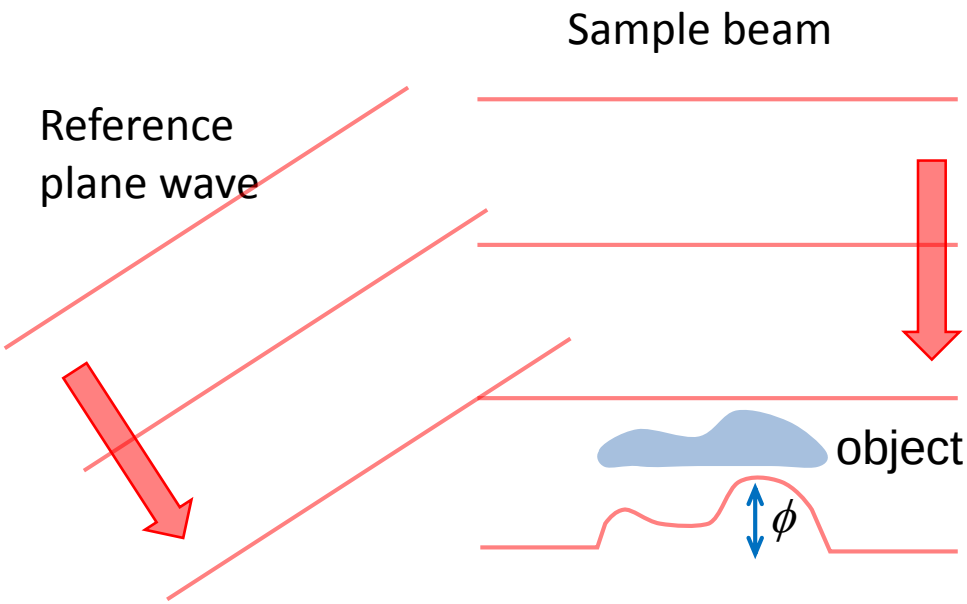
- For a transparent object





A phase measurement will be efficient for visualization of transparent biological samples.

Interferometry quantifies a phase



Illumination Schemes

- Point-illumination
- Line-field
- Wide-field

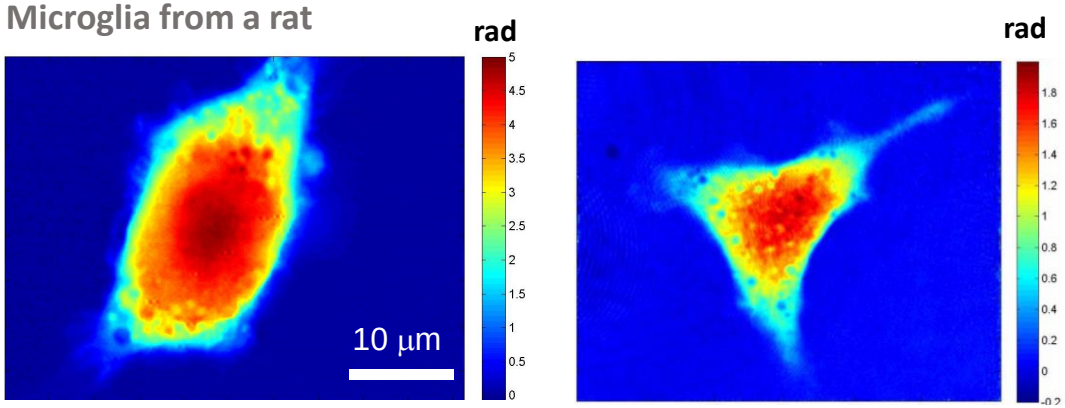
Detection Methods

- Phase shifting interferometry
- Off-axis interferometry
- In-line holography

Functional imaging

- Dry mass
- Volume
- Refractive index
- Membrane fluctuations

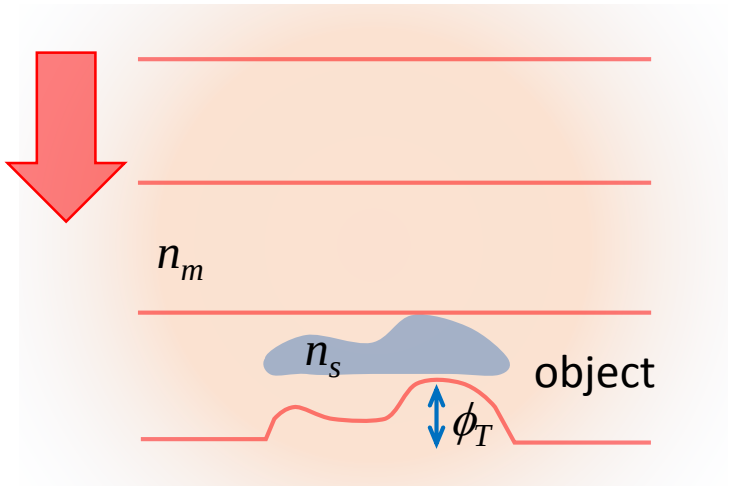
Microglia from a rat

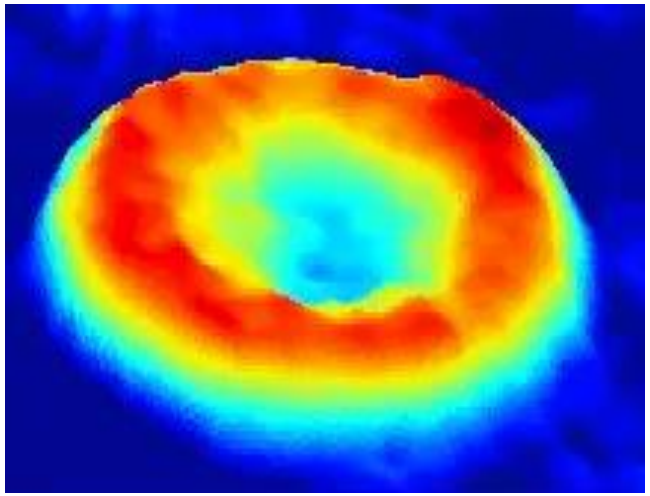


Y. Choi *et al.*, Opt. Lett., **36** 2465 (2011)

Phase measures a thickness

Homogeneous sample

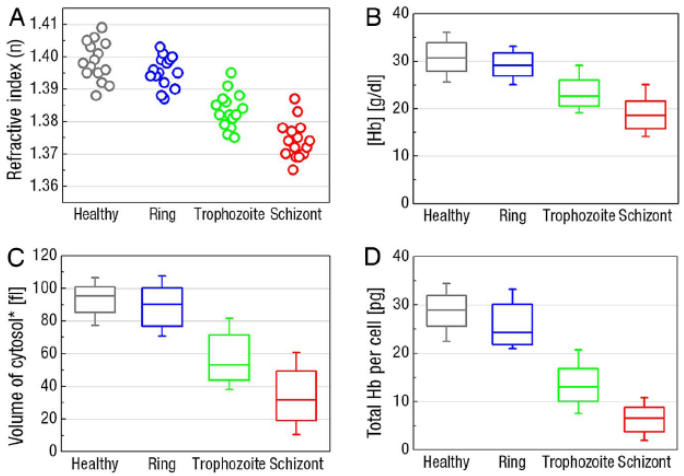


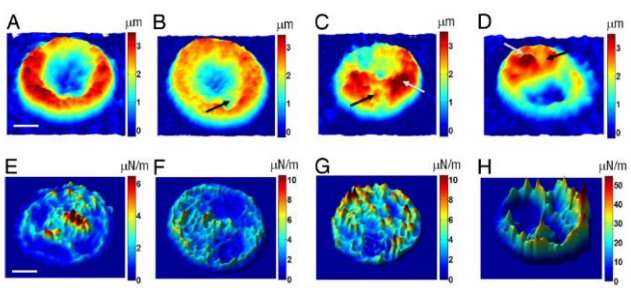


$$\Delta\varphi_T(x,y) = \frac{2\pi}{\lambda}\Delta n\, h(x,y)$$

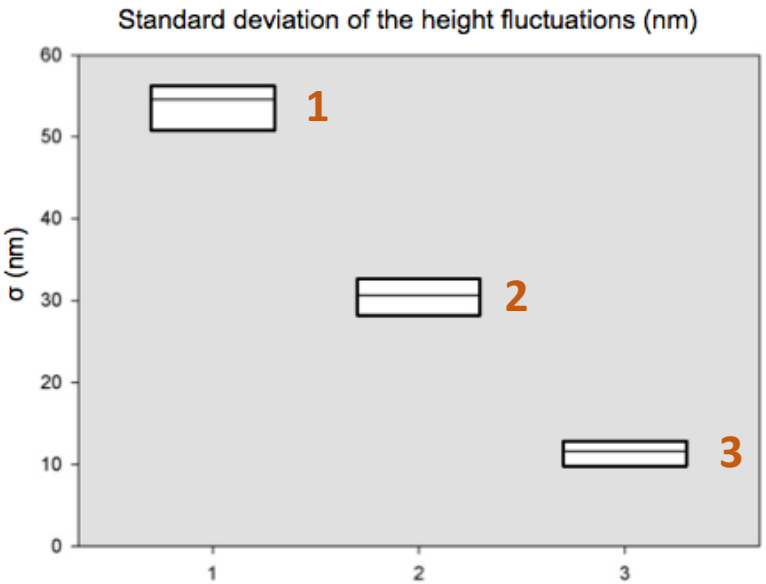
The transmission phase is proportional to the sample thickness.

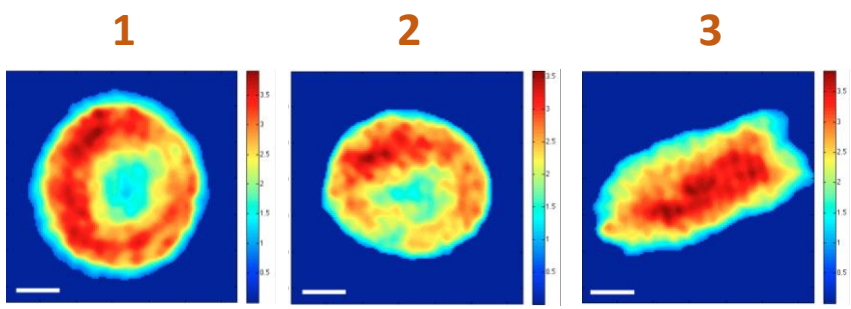
Measurement of membrane fluctuation





Y. Park et al., PNAS (2008).



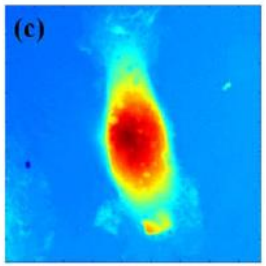


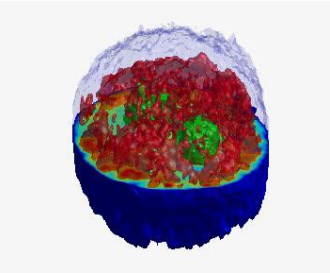
RBCs from a patient of sickle cell anemia

Pros and cons of the phase imaging

Pros:

- Label-free imaging of transparent samples with higher contrast
- Quantitative measurement of physical properties of samples



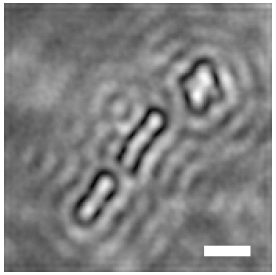


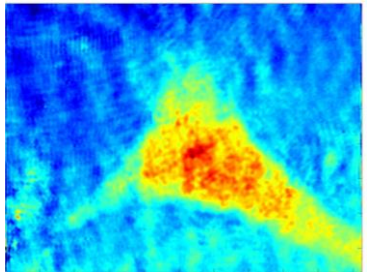
M. Kim et. al, *Optics Letters* **36** 148 (2011)

W. Choi et. al, *Nature Methods* **4** 717(2007)

Cons:

- Poor imaging resolution
- Noise from coherent light sources





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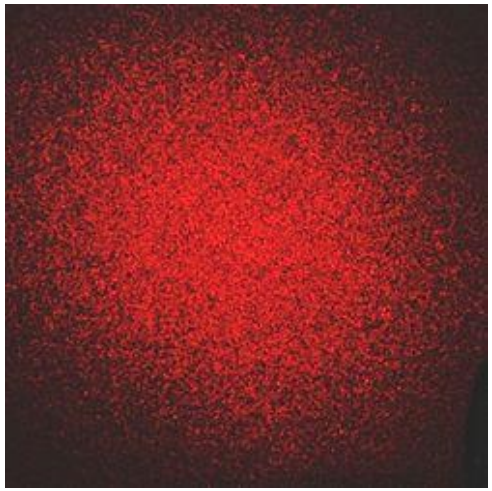
Light scattering

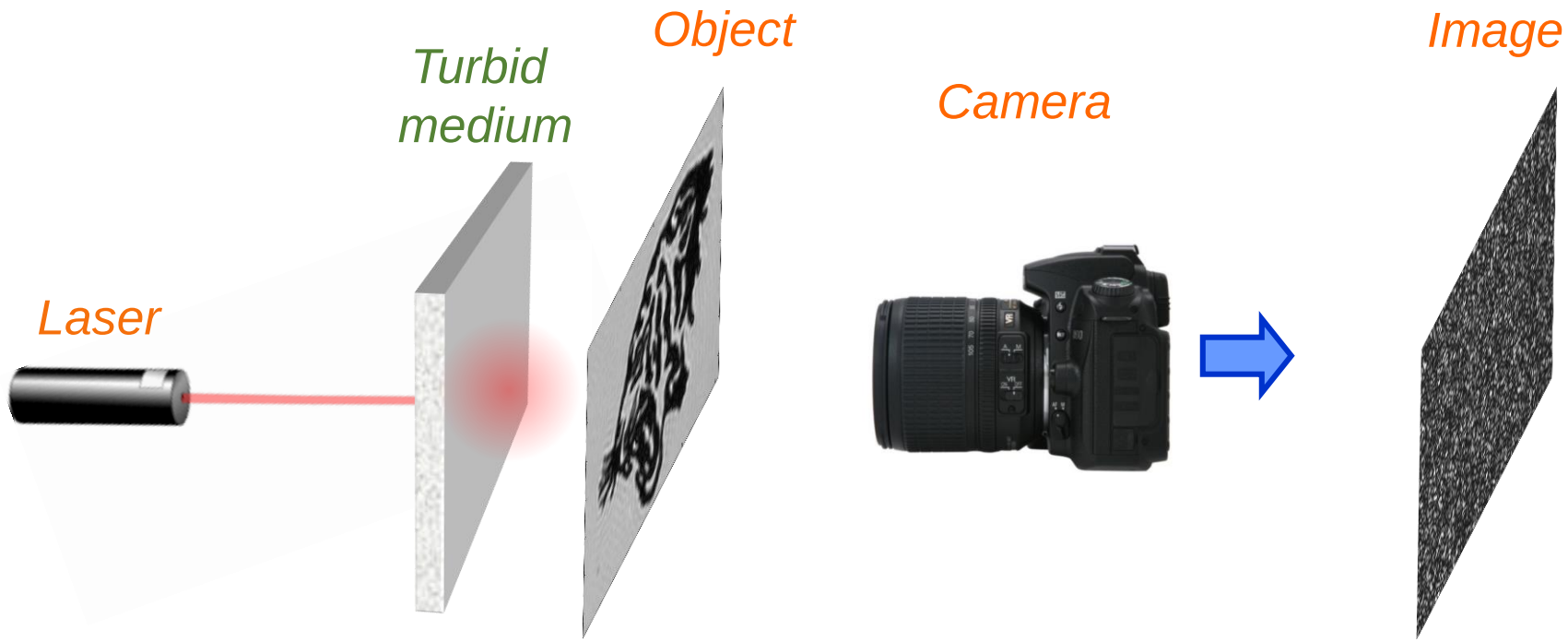
Speckle: a consequence of multiple scattering

http://f64.org.uk/news.htm

Speckle pattern

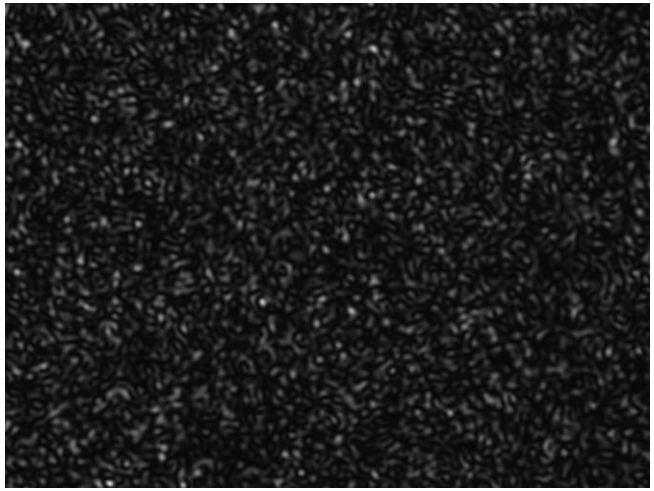


Speckle illumination

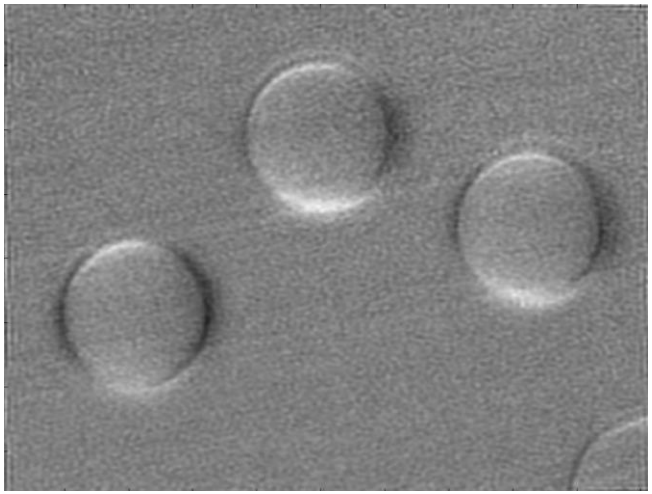


- The image information lies on top of the speckle pattern.

Dynamic speckle illumination (DSI)



Static speckle illumination



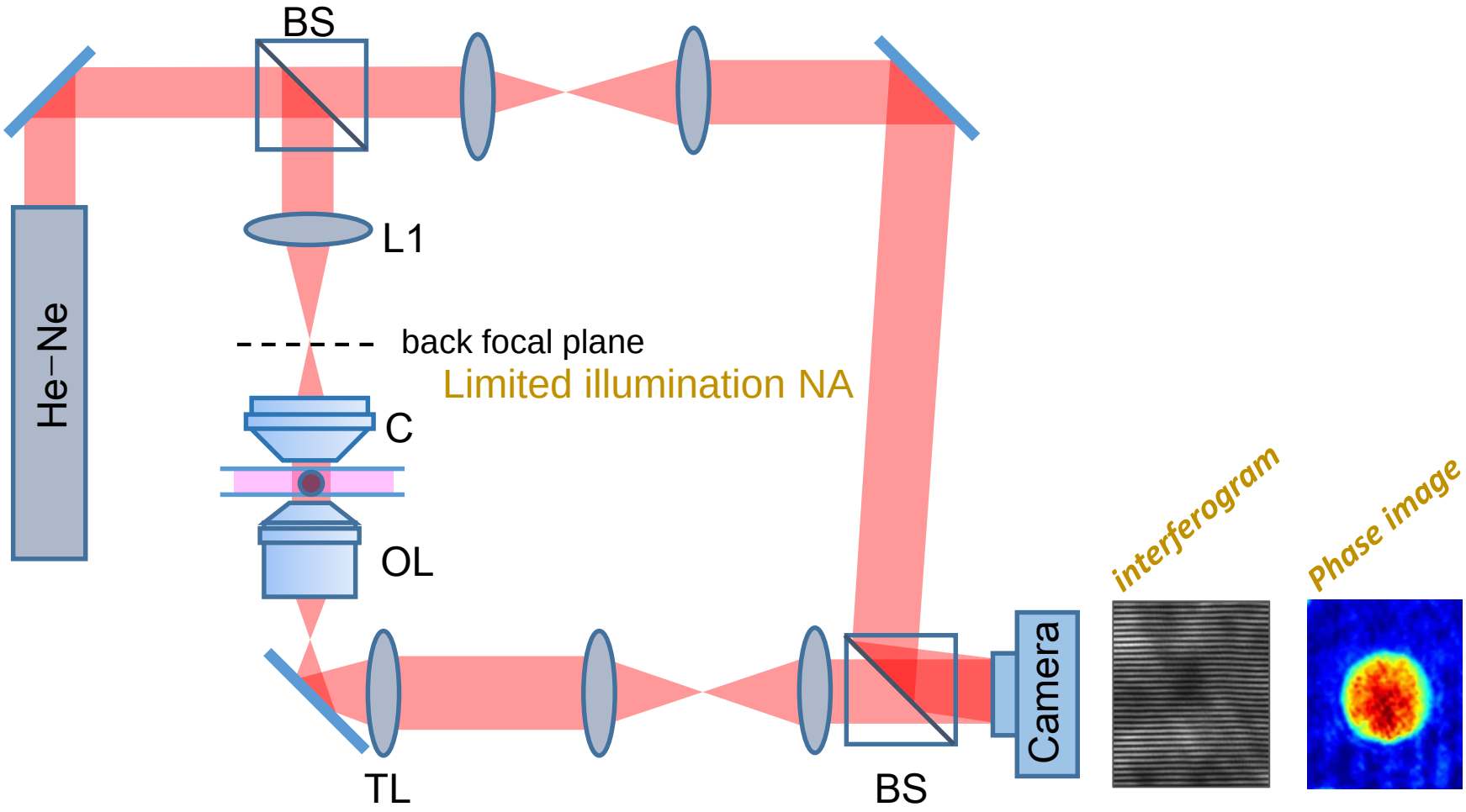
Dynamic speckle illumination

Speckle illumination :

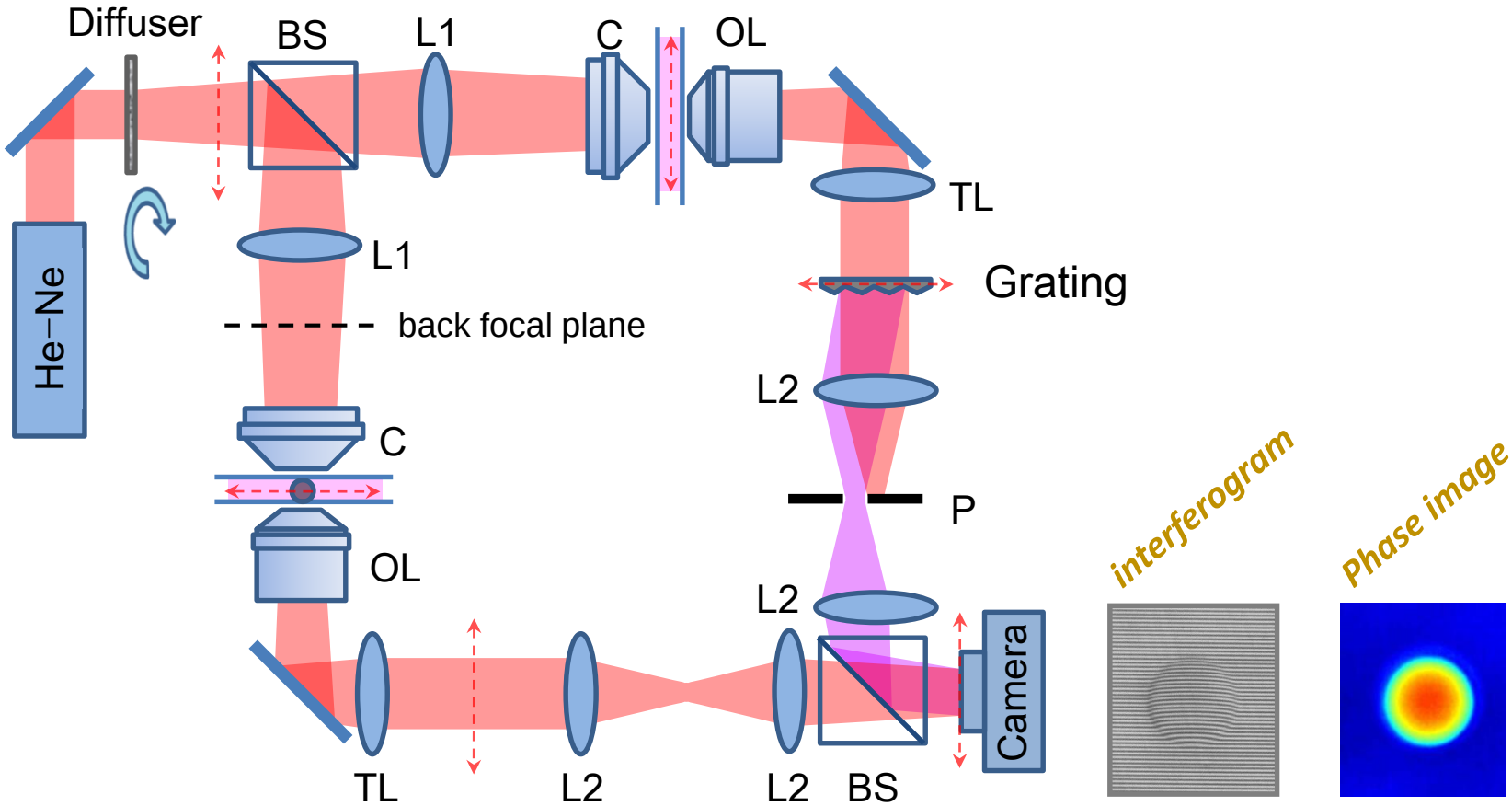
removes the fixed pattern noise → improves image quality
uses all the illumination NA → resolution enhancement

Take the other two advantages!!

Off-axis digital holography

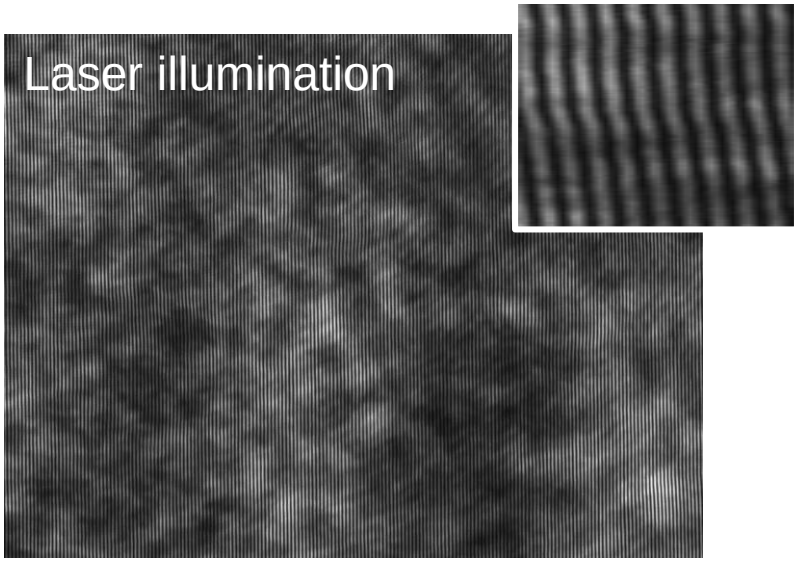


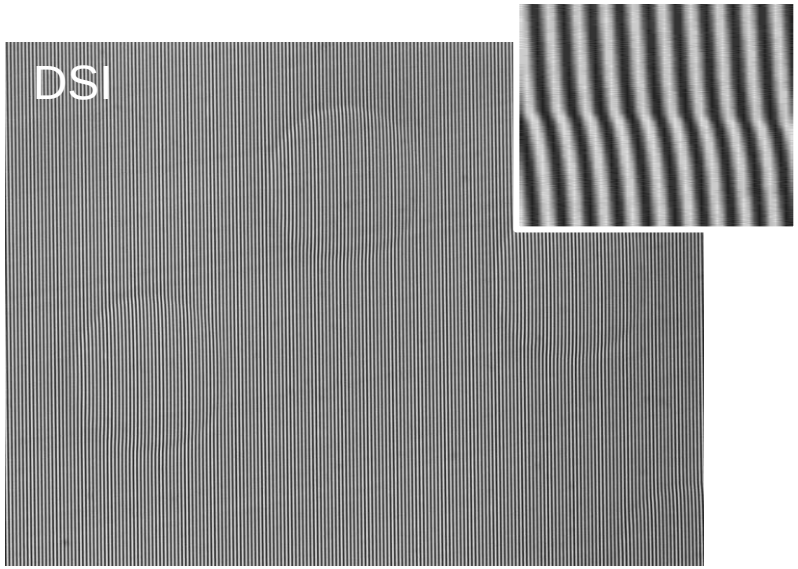
Quantitative phase microscope (QPM) with DSI

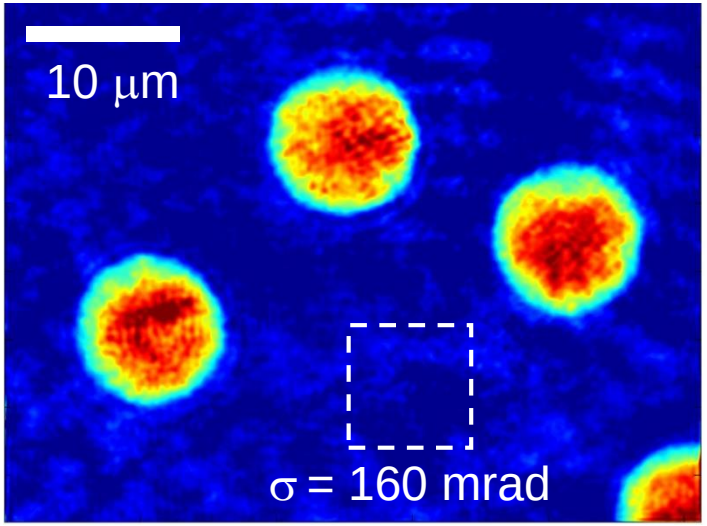


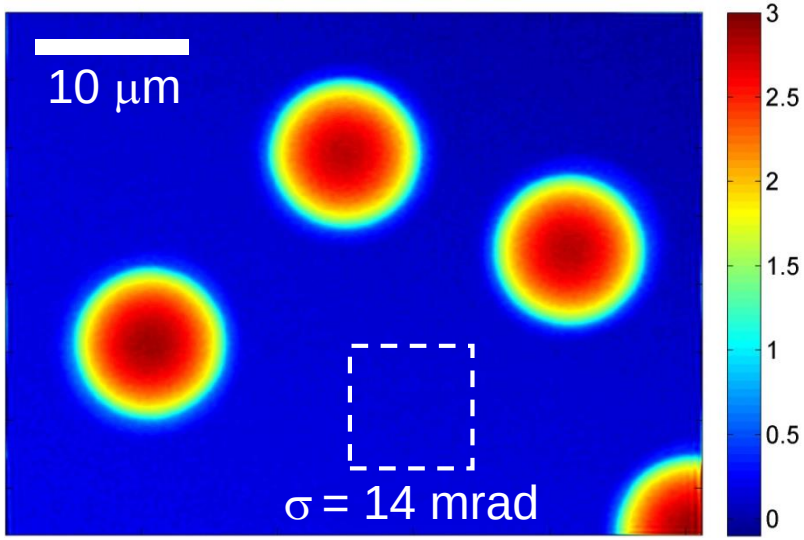
Use the similar optical configuration in both arms for delivering the same speckle pattern to the image plane.

Improvement of imaging quality



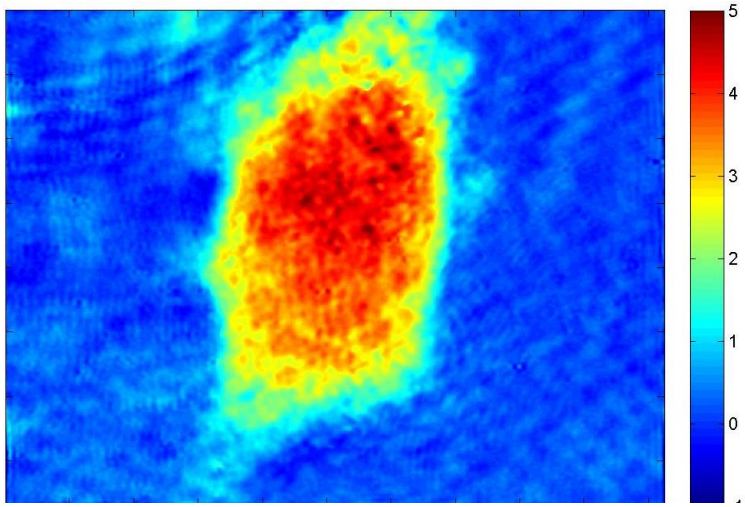




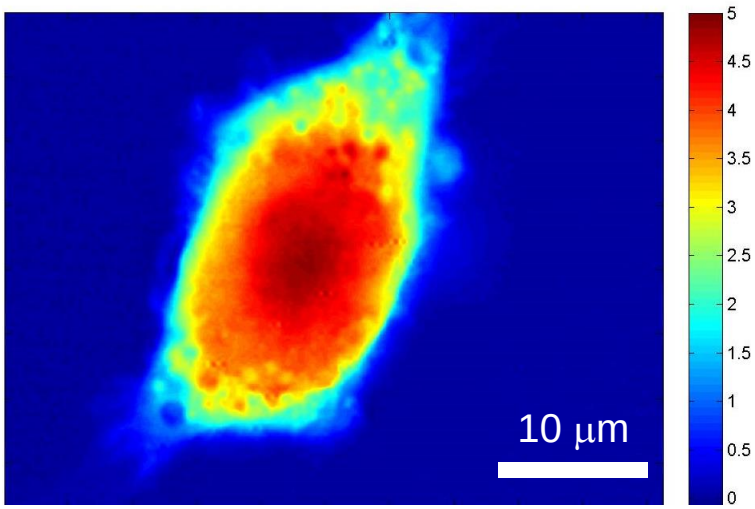


Improvement of imaging quality

Laser illumination



DSI

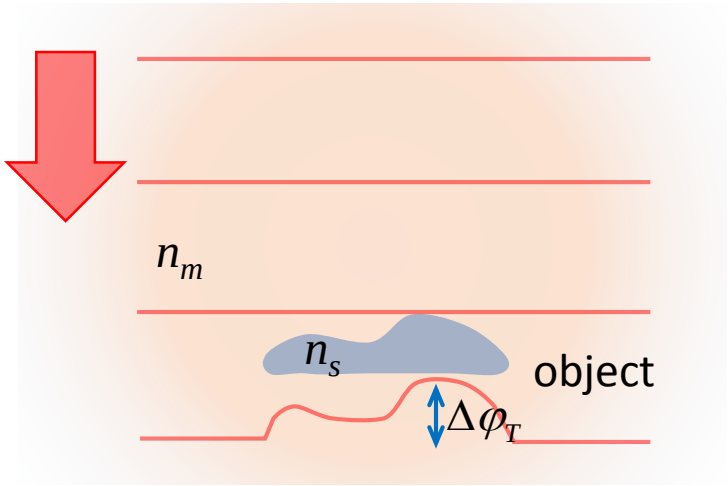


Microglia from a rat

Y. Choi *et al.*, Opt. Lett., **36** 2465 (2011).

Interpretation of a phase

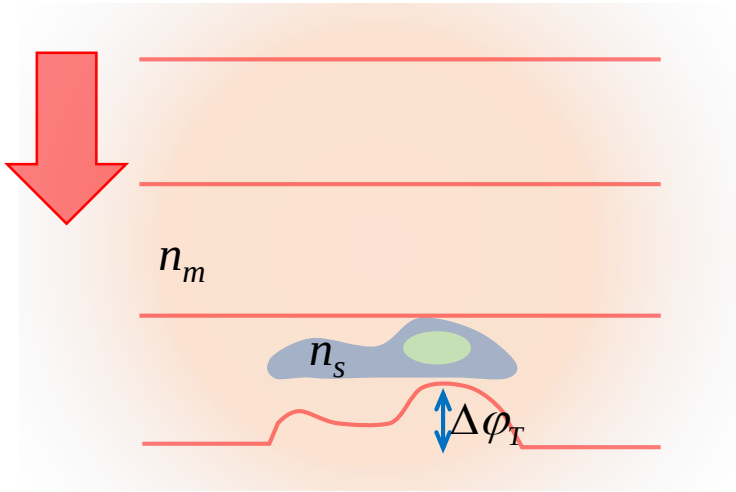
Homogeneous sample



$$\Delta\varphi_T(x,y) = \frac{2\pi}{\lambda}\Delta n\, h(x,y)$$

"Sample thickness" is directly proportional to the measured phase.

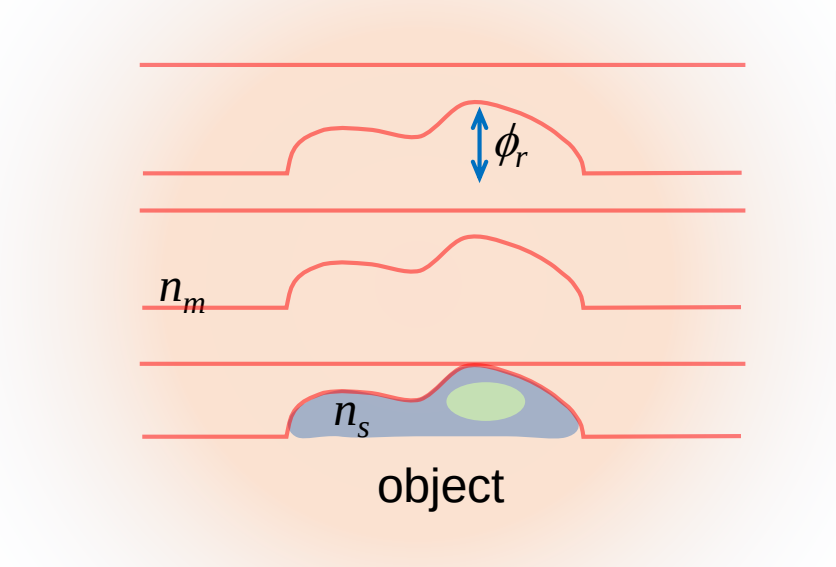
Inhomogeneous sample



$$\Delta\varphi_T(x,y) = \frac{2\pi}{\lambda}\int \Delta n(x,y,z)\,dz$$

Calculation of "sample thickness" requires the knowledge of 3-D refractive index of the sample.

Reflection phase as an alternative



Easy interpretation

Reflection phase depends *only* on sample's morphology

$$\phi_r = \frac{2\pi}{\lambda} n_m 2h$$

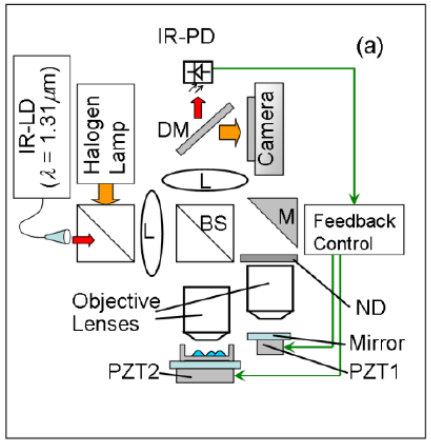
Higher phase sensitivity

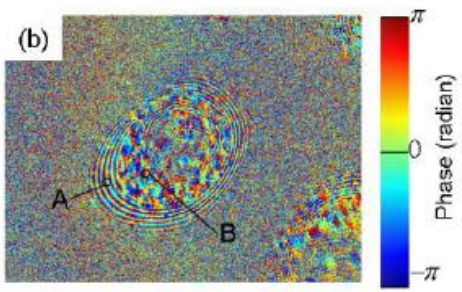
$2n_m/\Delta n$ ~ 90 for eukaryotic cells

Prerequisites:

- **Depth selectivity** for distinguishing each surface
- **High speed** for studying fast dynamic motions

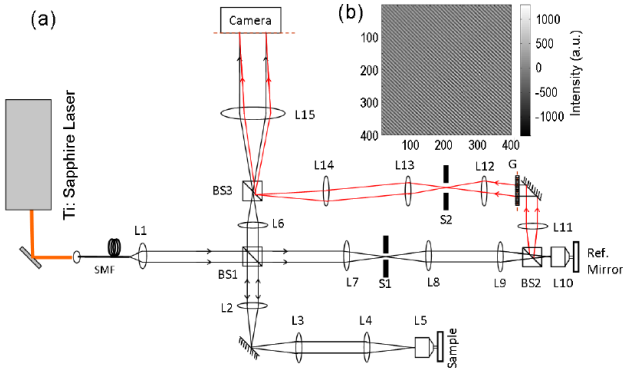
Previous approaches: using temporal gating

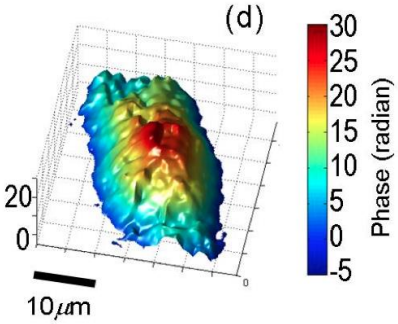




- Thermal light source
- z-sectioning : 0.93 μm
- Lateral resolution : 0.56 μm
- 7-step phase shifting
- Speed: 0.8 fps

Yamauchi *et al*., Opt. Express **19** 5536 (2011)

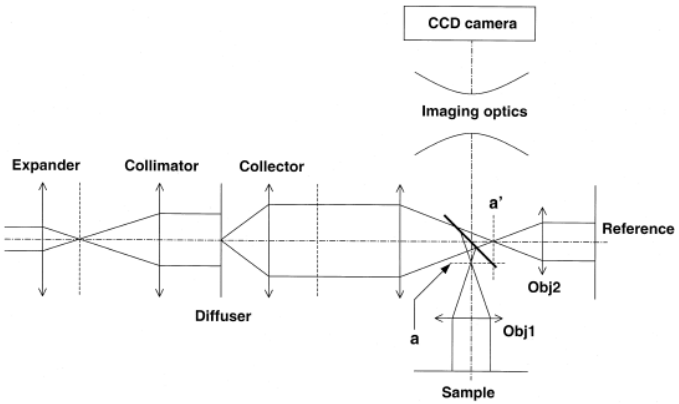


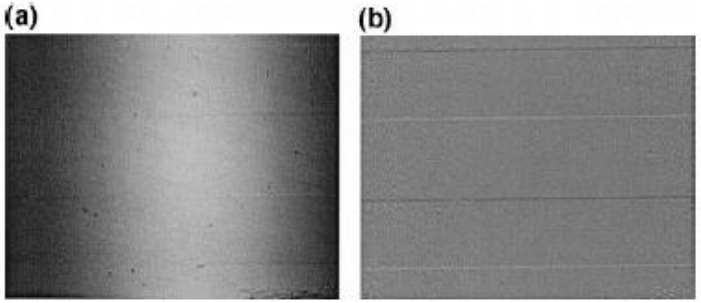


- Mode-locked Ti:sapphire laser
- Single shot
- Speed: 1K fps
- z-sectioning : ~ 4 μm

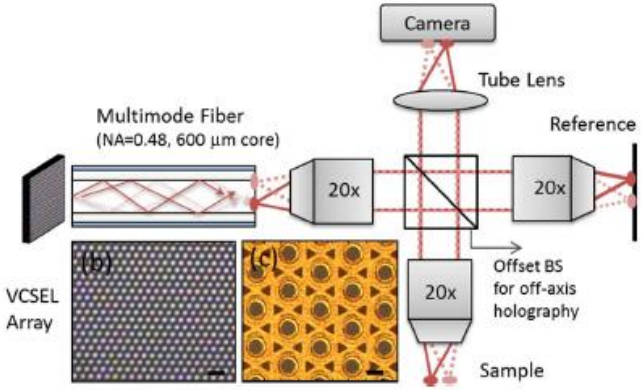
Yaqoob *et al*., Opt. Express **19** 7587 (2011)

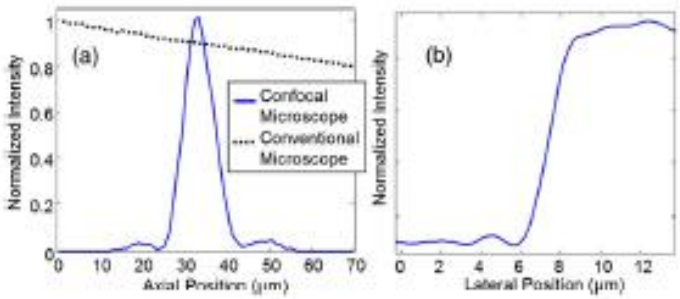
Previous approaches: using spatial coherence





Somekh *et al*., Opt. Commun. **174** 75 (2000)

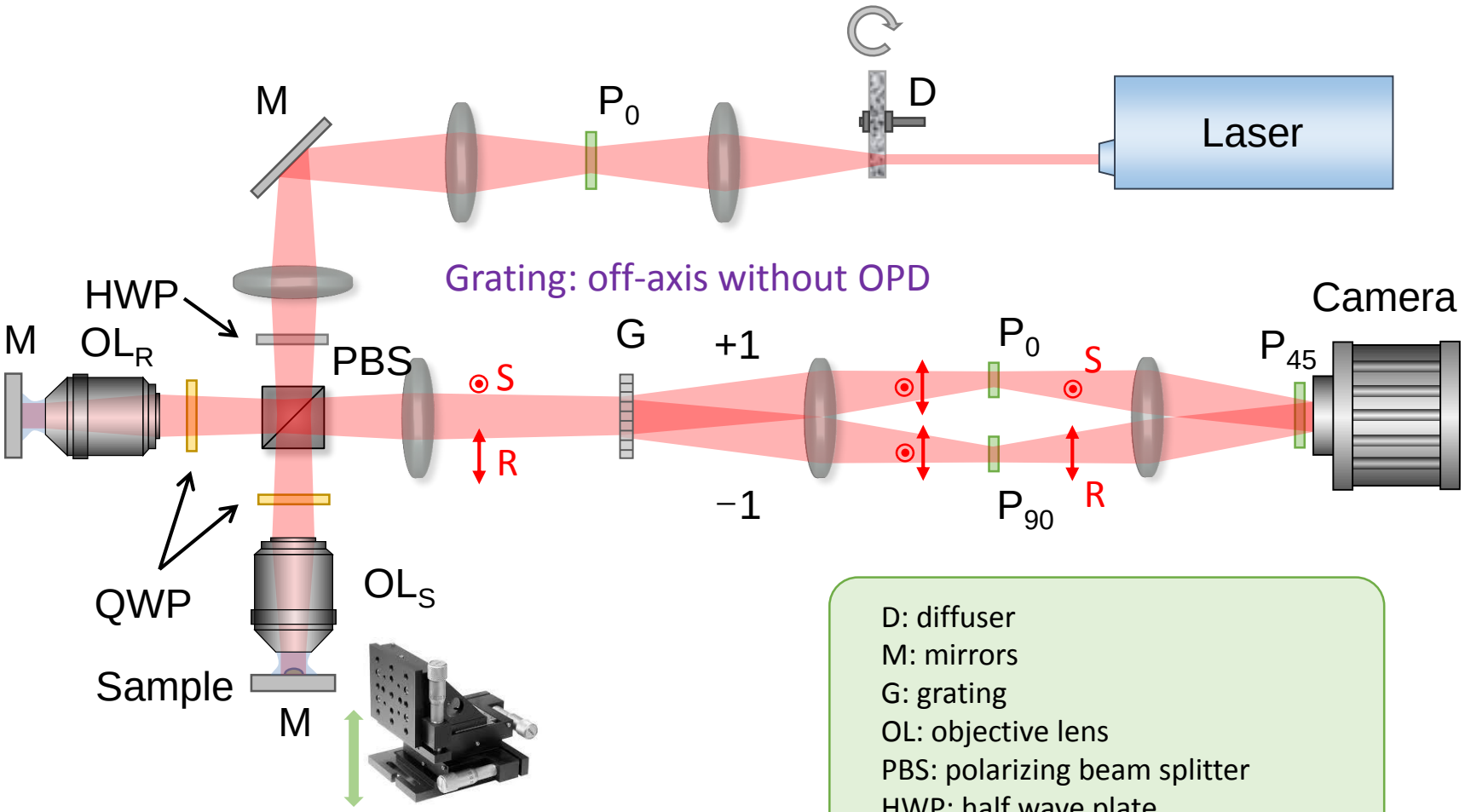




Redding *et al*., Opt. Lett. **39** 4446 (2014)

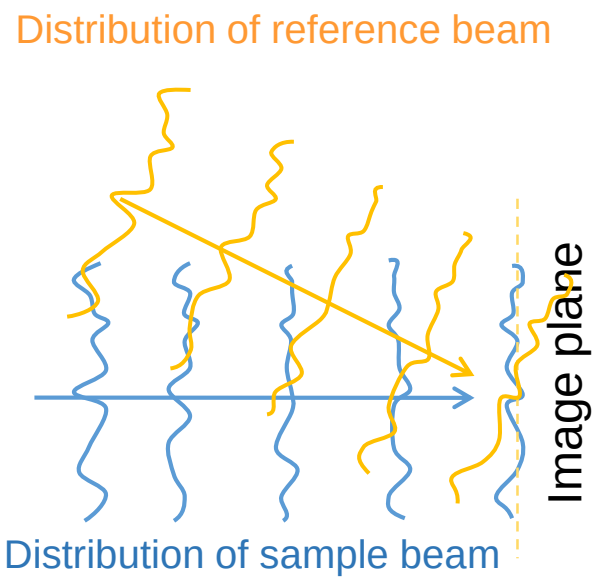
- Off-axis configuration (tilting the reference mirror)
- Numerical aperture: (0.3 – 0.4)
- Depth selectivity: ~8 µm

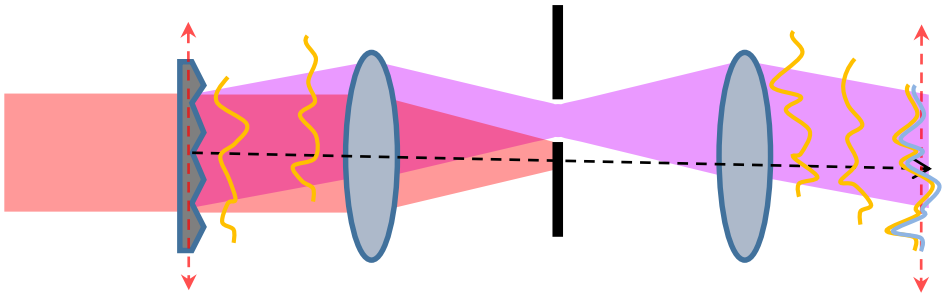
Dynamic speckle reflection phase microscopy



D: diffuser
M: mirrors
G: grating
OL: objective lens
PBS: polarizing beam splitter
HWP: half wave plate
QWP: quarter wave plate
P_α: polarizer with α–degree rotation

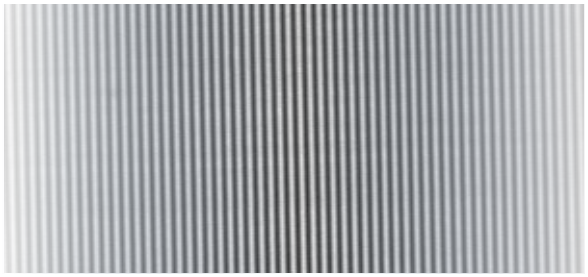
Grating: the key idea for off-axis interferometry





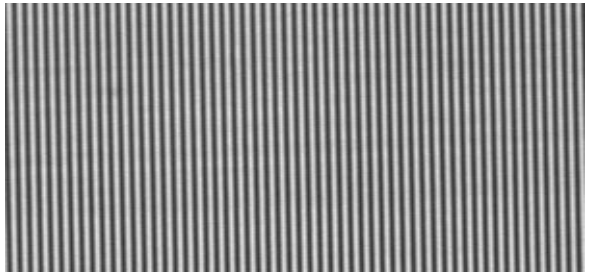
Tilting the reference beam:

- physical rotation of wavefront
- reduction of fringe contrast

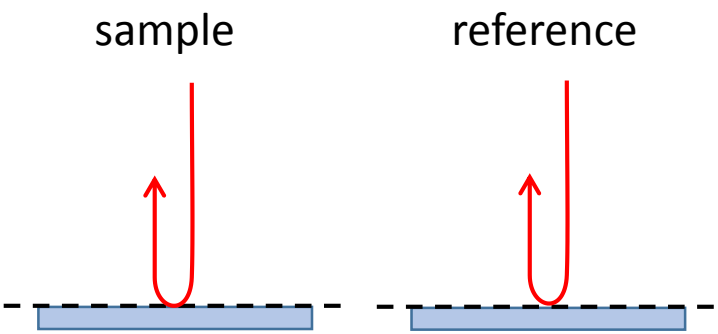


Grating:

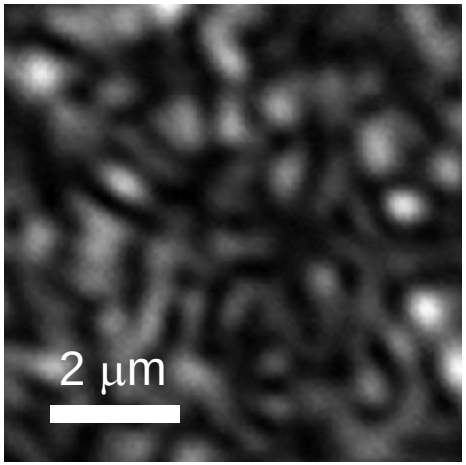
- maximum overlap of two wavefronts
- uniform fringe contrast



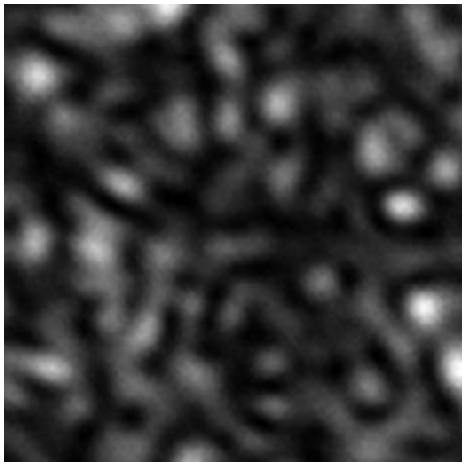
Interference when no optical path difference



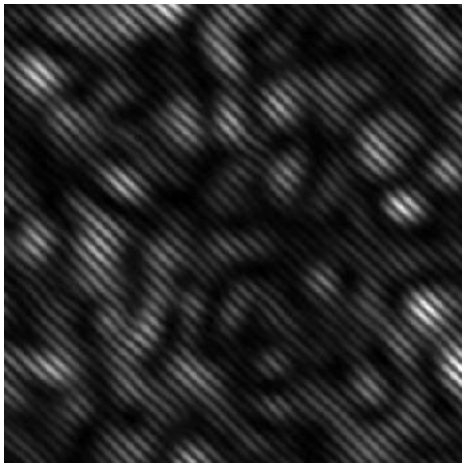
Reference speckle



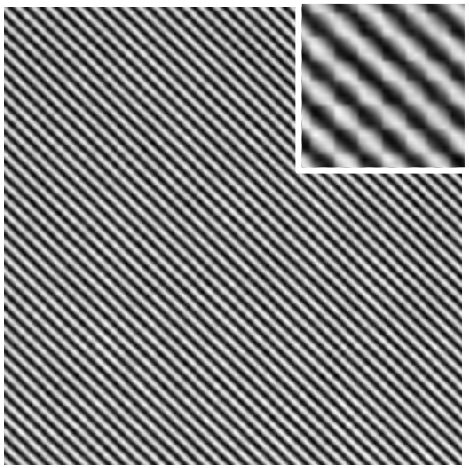
Sample speckle



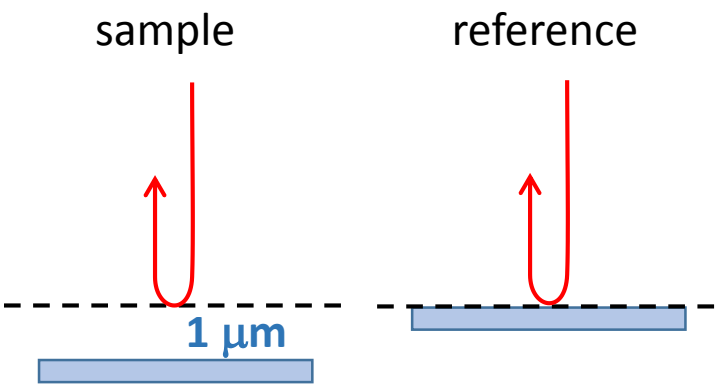
Static interference



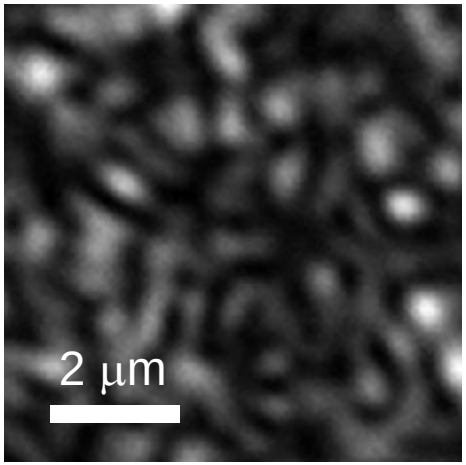
Dynamic interference



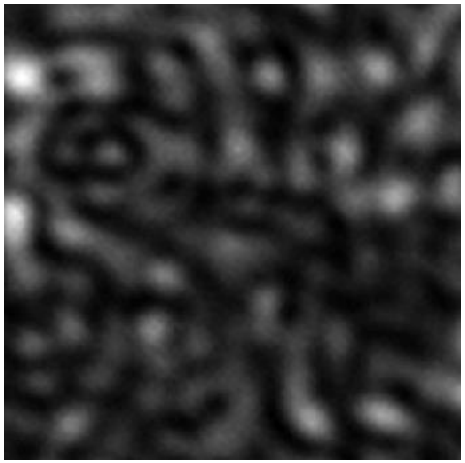
Interference when 1 µm-shift of the sample plane



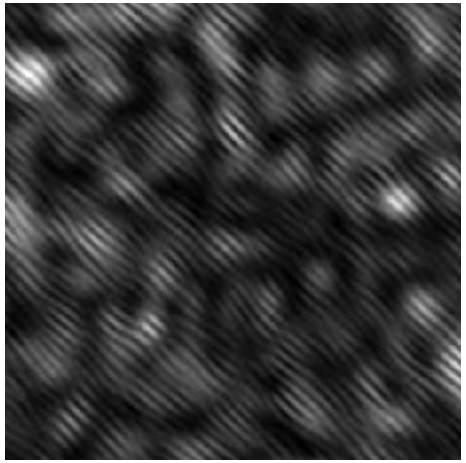
Reference speckle



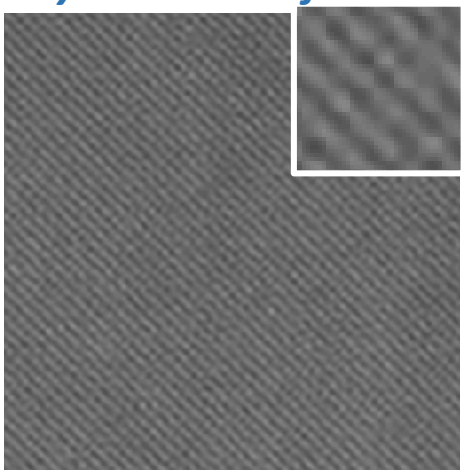
Sample speckle



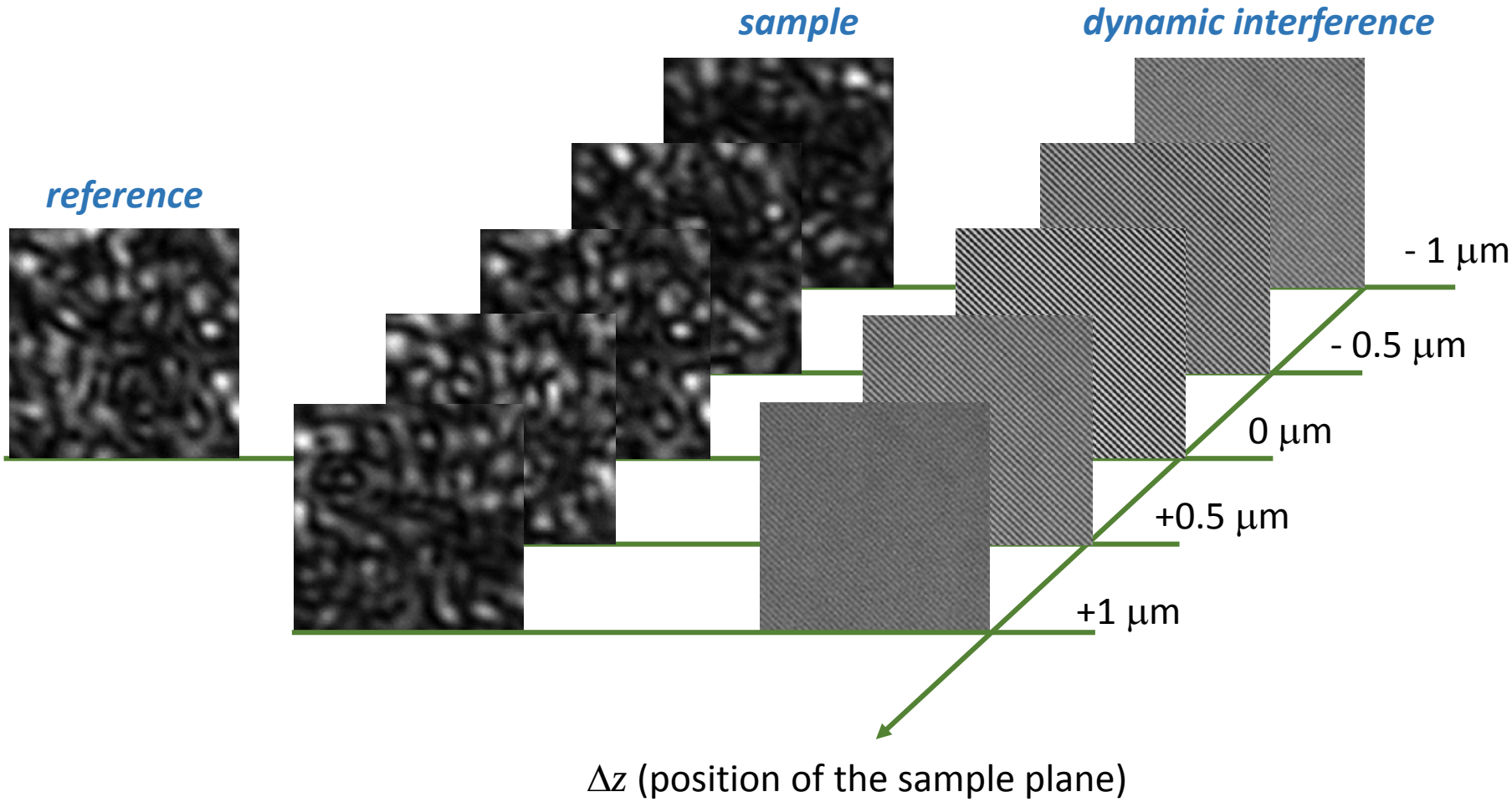
Static interference



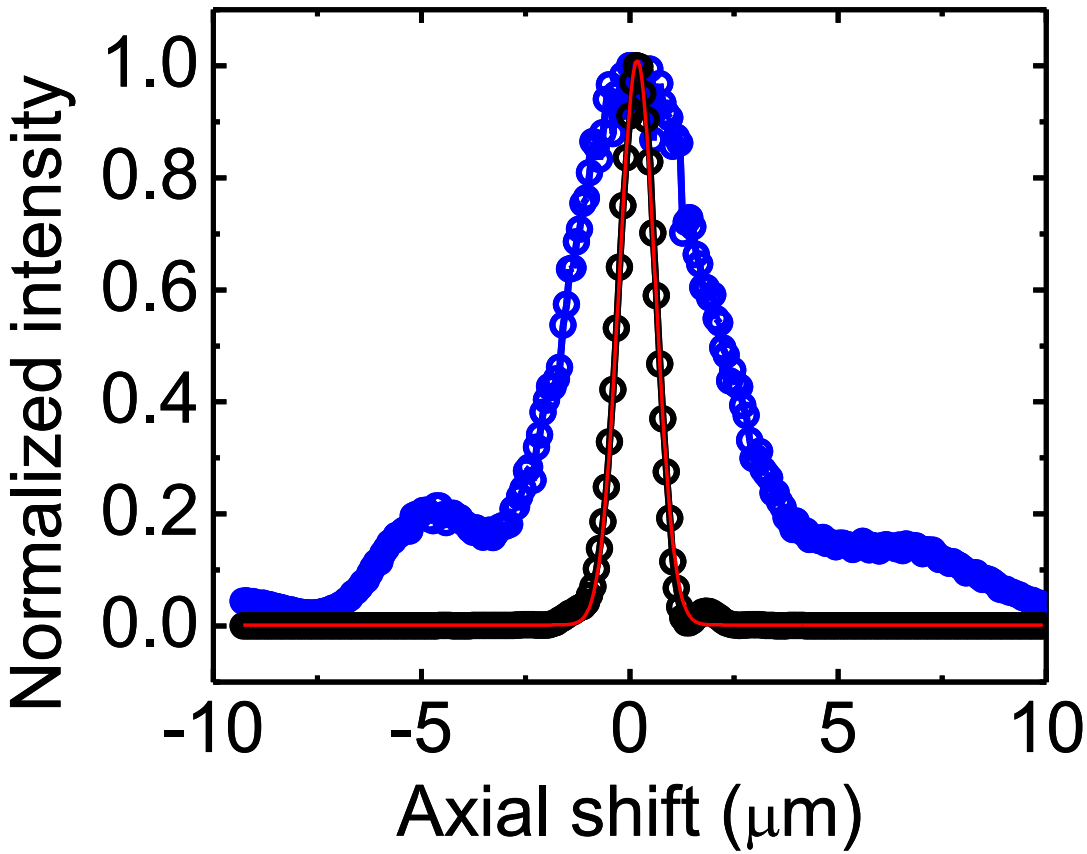
Dynamic interference



Measurement of axial response



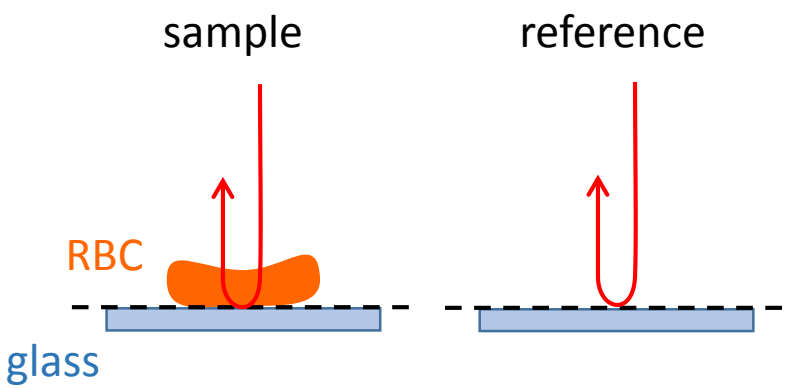
Axial response: depth selectivity

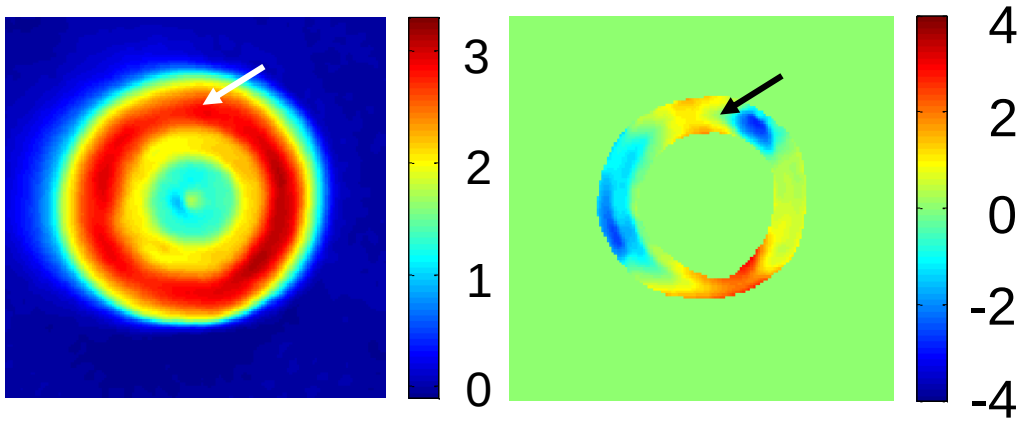


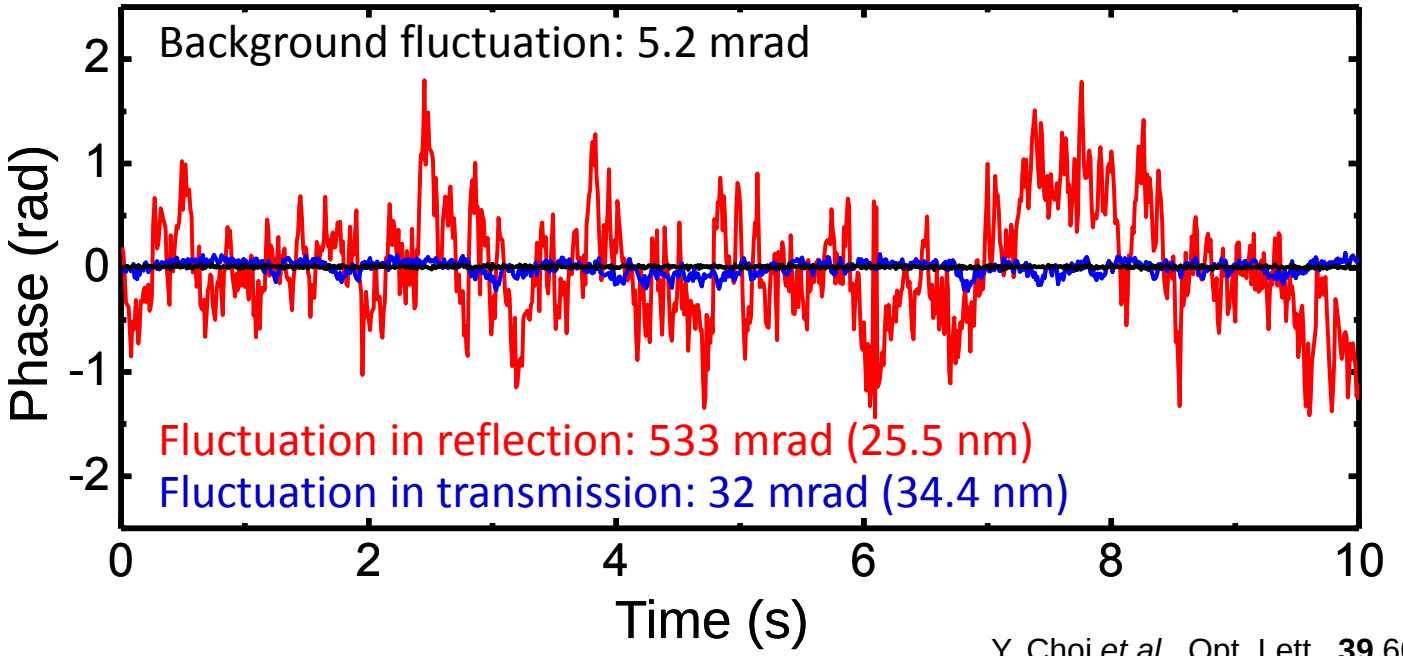
~6 μm without DSI

~1 μm with DSI

RBC fluctuations measurements (@ 100 fps)



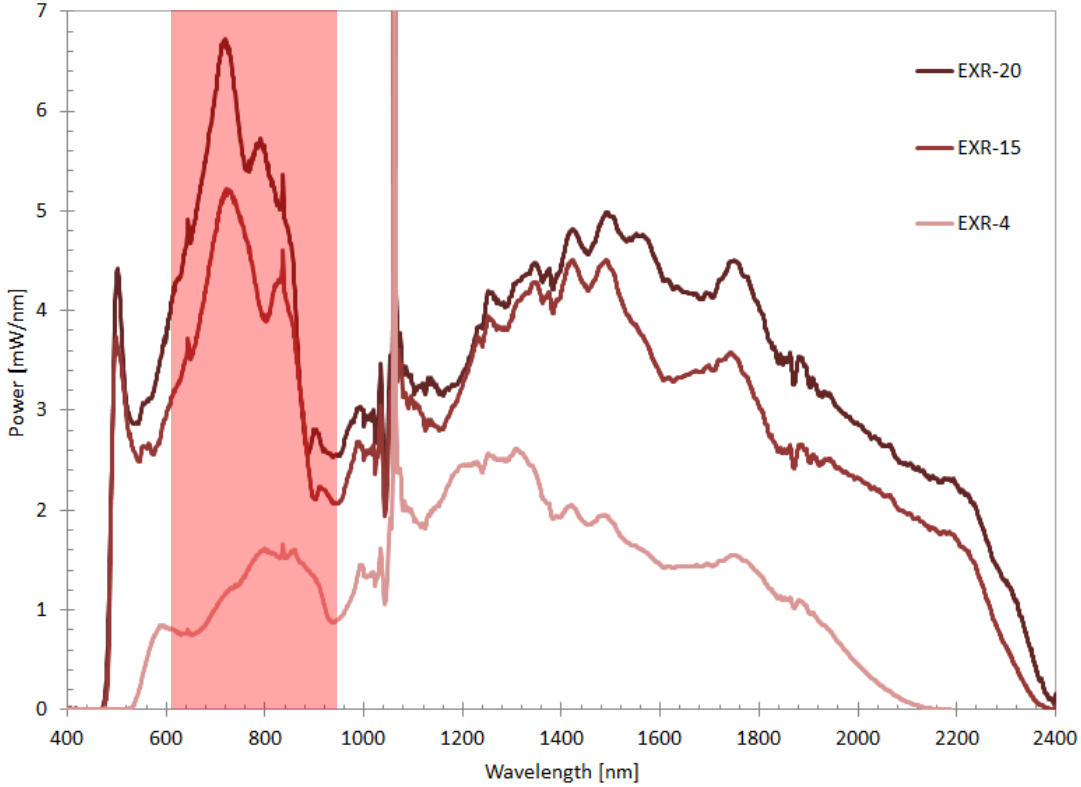




Y. Choi *et al*., Opt. Lett., **39** 6062 (2014).

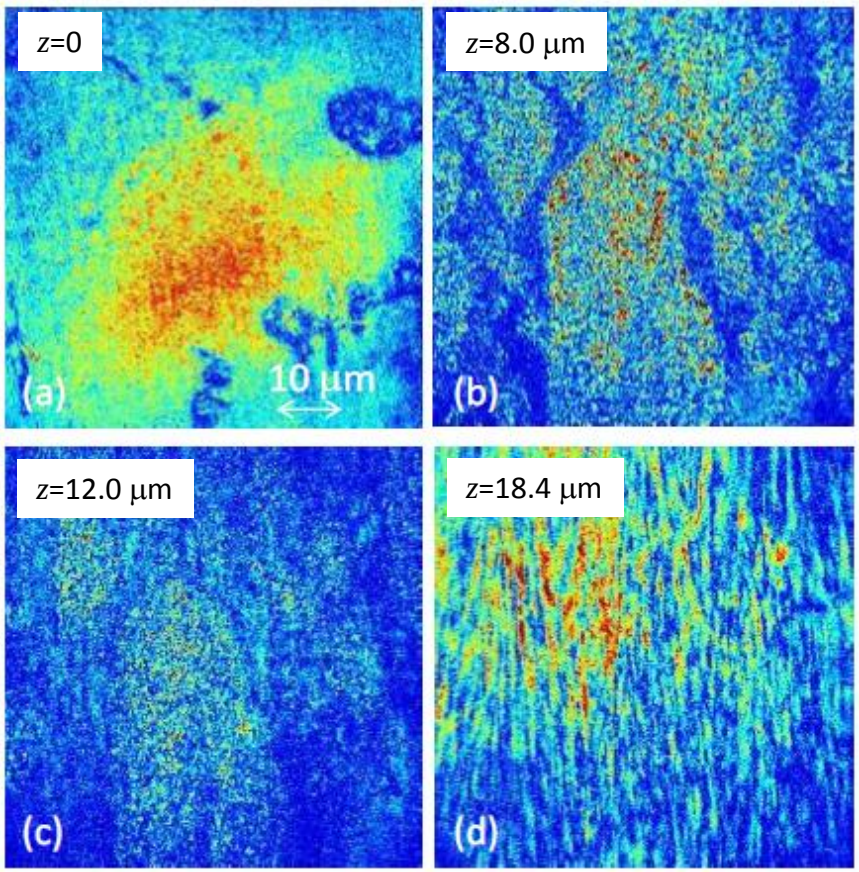
For better depth selectivity

Spectra of super continuum lasers



Applications for non-biological samples

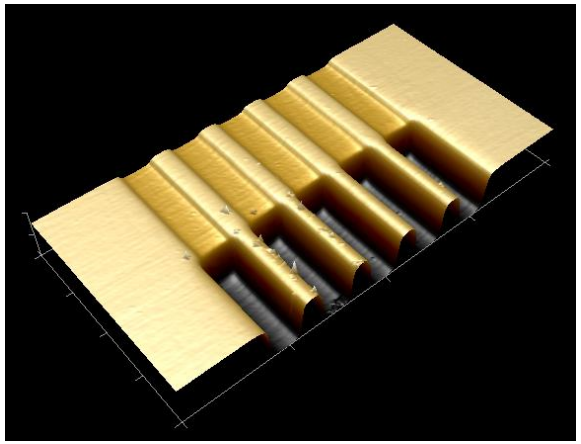
- **Depth-selective measurement for UV-responsive polymer**

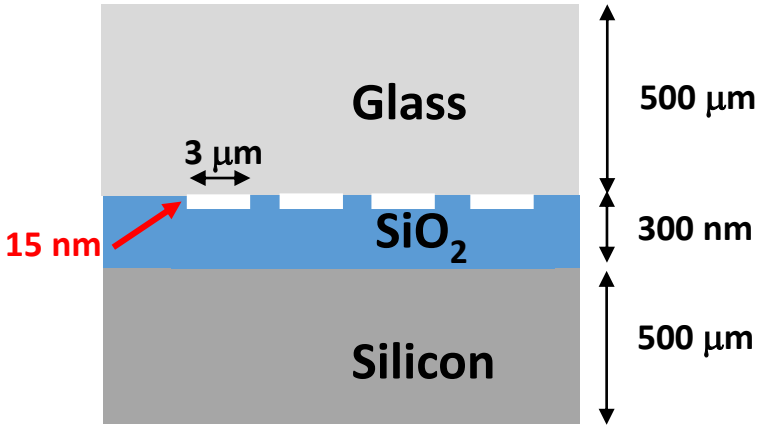


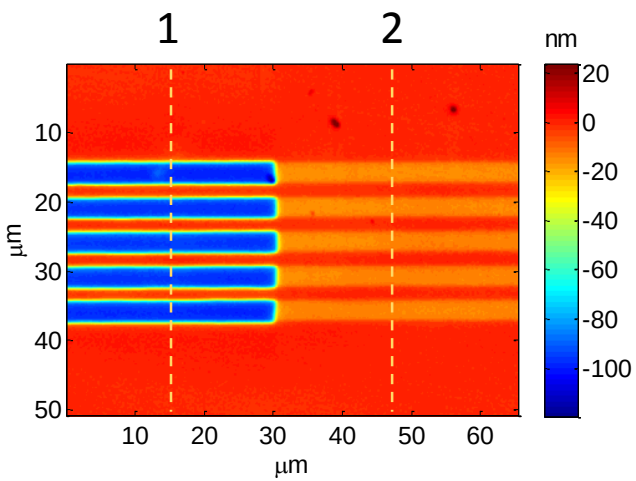
Katayama *et al.*, Phys. Chem. Chem. Phys. (2014)

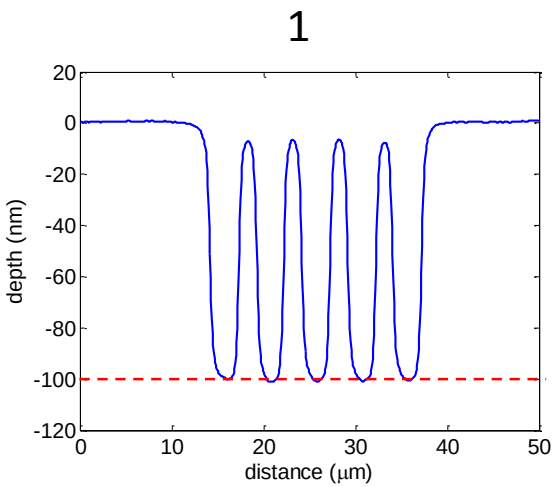
Applications for non-biological samples

- **Profilometry**









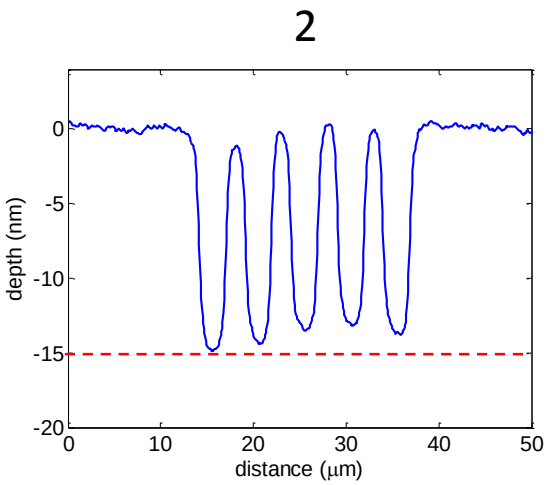


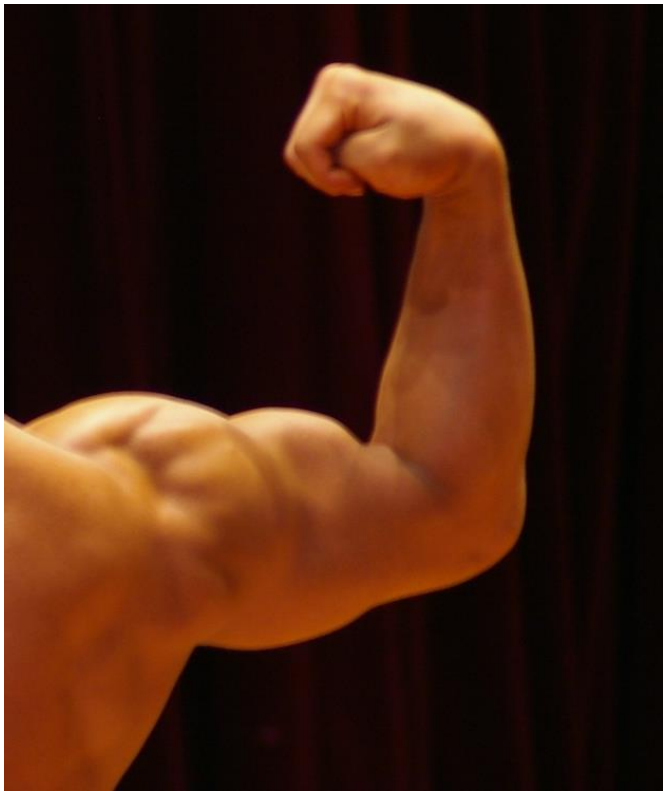
Table of contents

Light scattering in tissues

Glass fish

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

Human skin



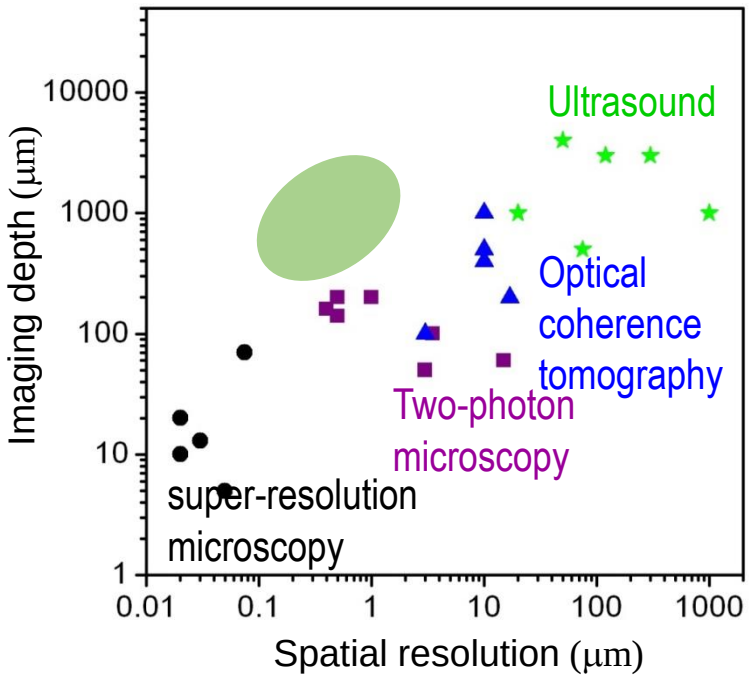
- Human tissue is opaque for visible light
- Optical microscopes can image only superficial layers of human tissues

Opaque human tissues

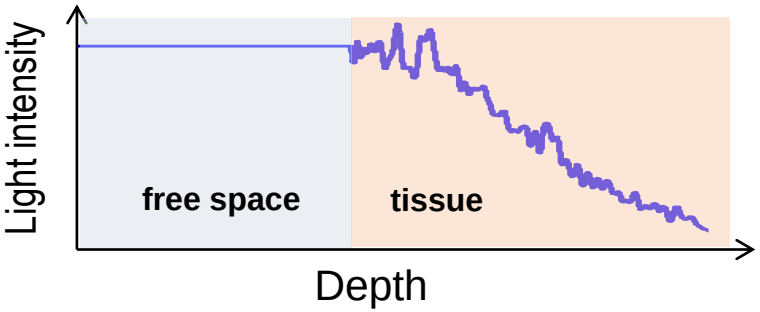
- Individual cells are almost transparent, but tissues are not.

Because *scattering* is ~100 times higher than *absorption*.

Light scattering causes problems



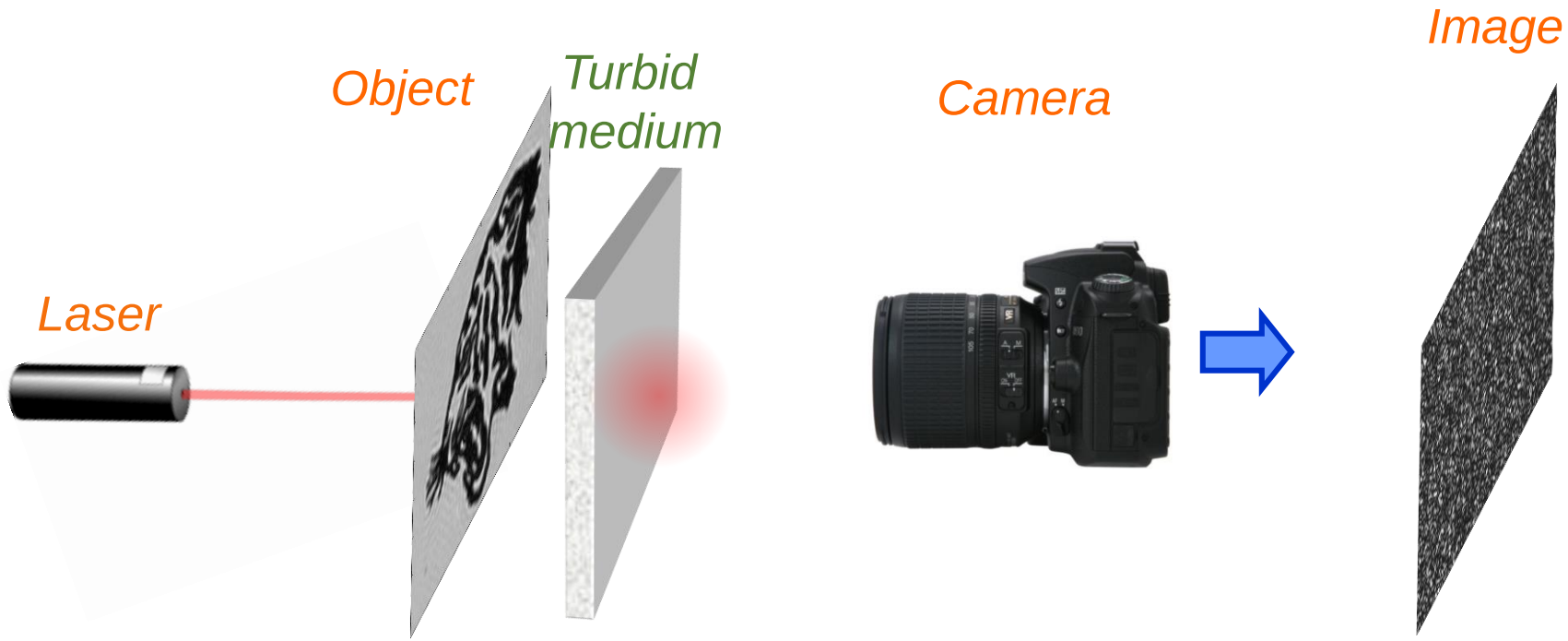
Limited imaging depth



Inefficient energy delivery

How to control and suppress the effect of scattering?

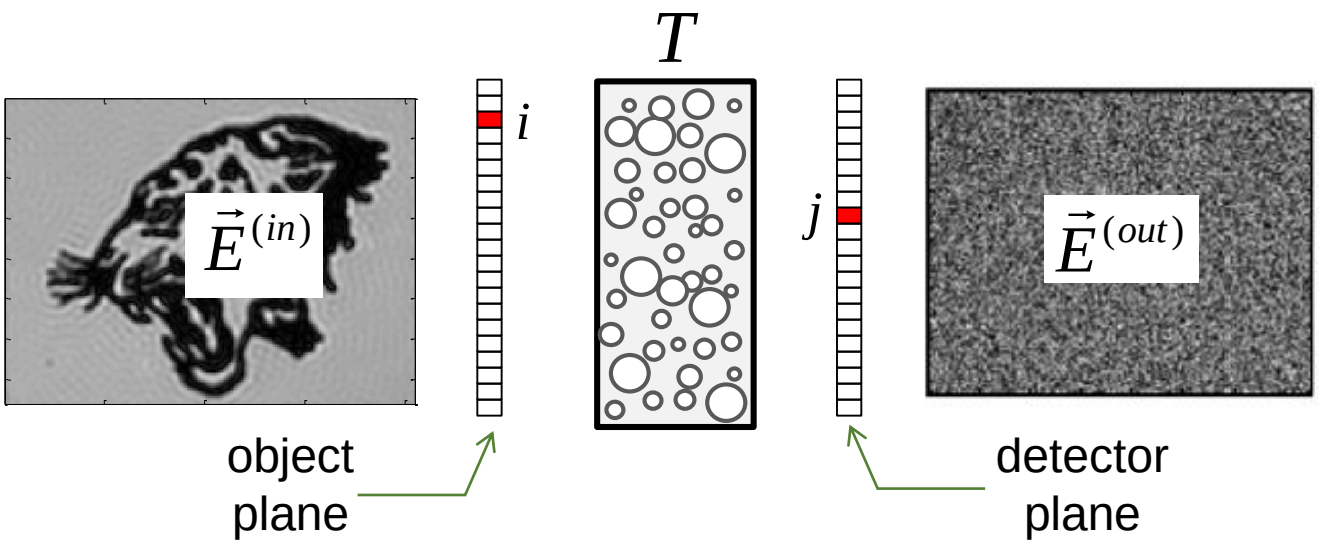
Task: seeing through turbidity



- **Multiple scattering washes the image information out !**

Transmission matrix

- Linear optical response → Linear operator → Matrix representation
- Transmission matrix: scattering characteristic in a forward direction

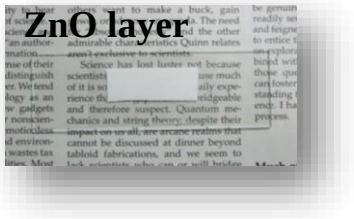


$$\vec{E}^{(out)} = T\vec{E}^{(in)}$$

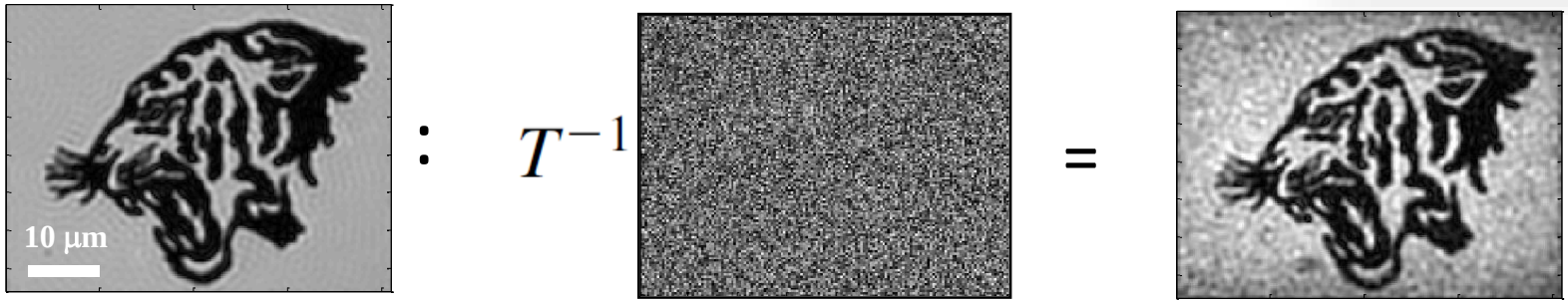
- Image reconstruction $$T^{-1}\vec{E}^{(out)} = \vec{E}^{(in)}$$

→ The effect of multiple scattering can be inverted.

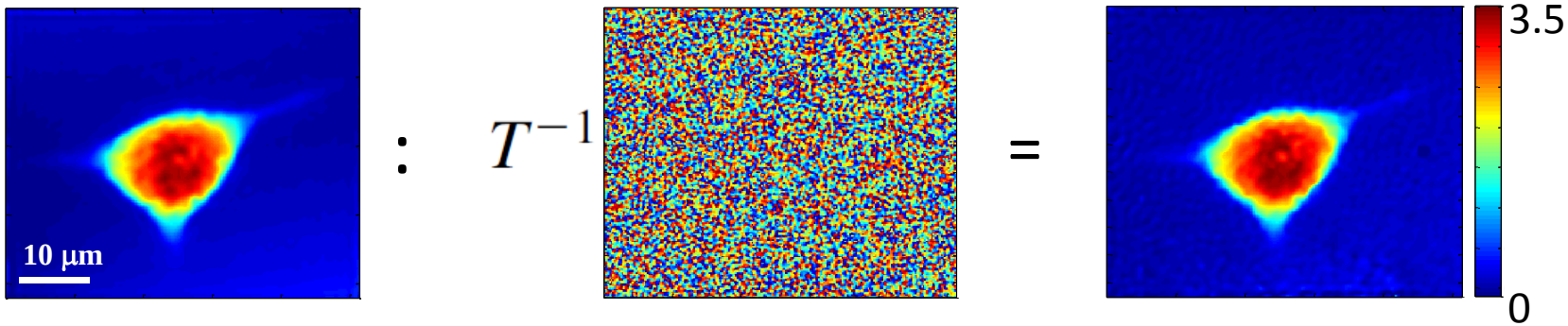
Turbid lens imaging (TLI)



Imaging a test object through a ZnO nanoparticle layer (25 μm thick)



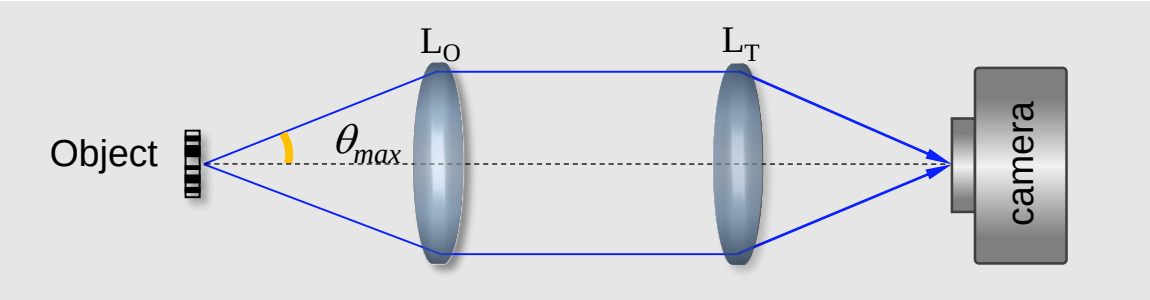
Imaging a living cell through a skin tissue (0.45 mm thick)



Y. Choi *et al*., Phys. Rev. Lett. **107**, 023902 (2011).
Y. Choi *et al*., Opt. Lett. **36**, 4263 (2011).

Benefits of a scattering lens #1

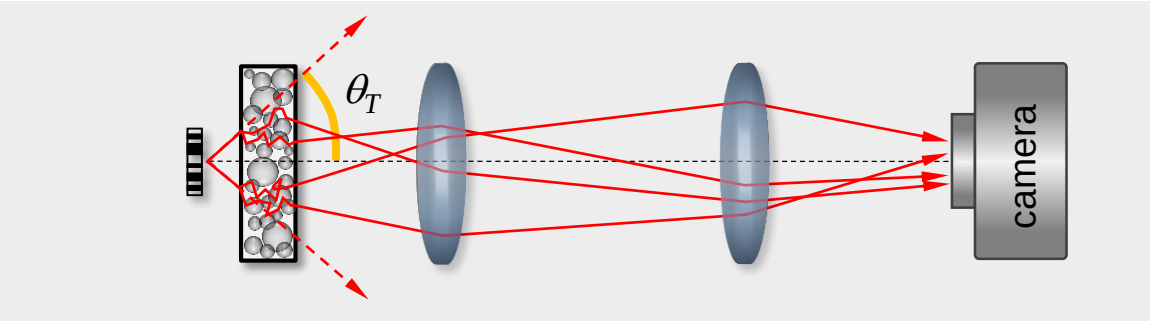
- **Conventional imaging:**



Resolution limited by the NA of an objective lens

$$\Delta = 1.22 \frac{\lambda}{n \sin \theta_{\max}}$$

- **Scattering lens imaging:**



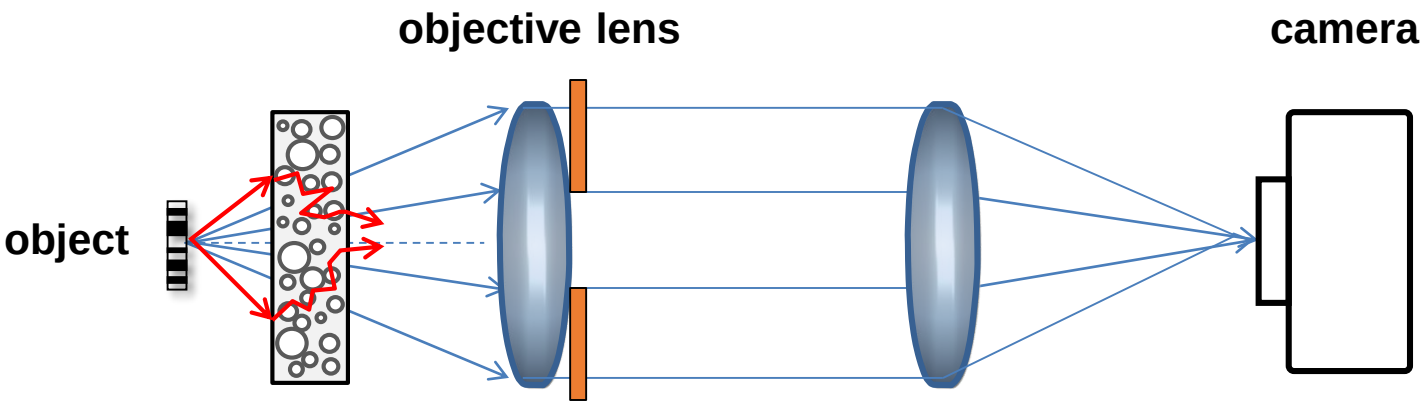
turbid medium

Resolution enhancement by multiple scattering

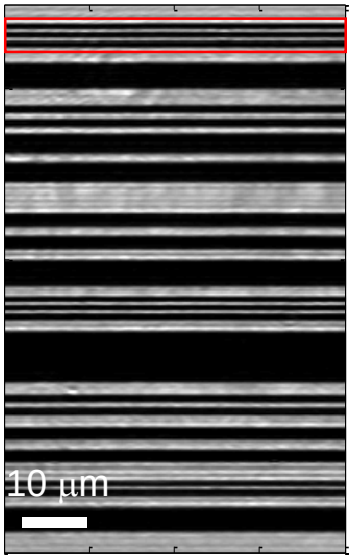
$$\Delta = 1.22 \frac{\lambda}{n \sin \theta_T}$$

θ_T: range of T-matrix

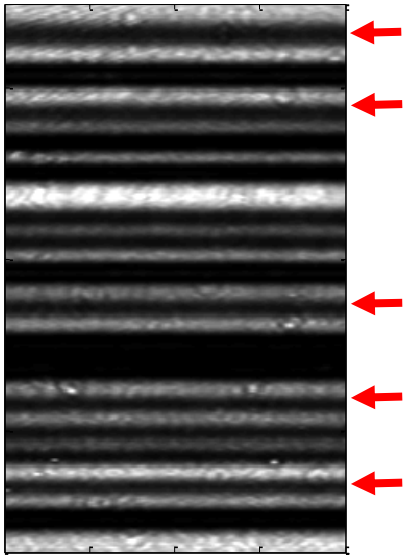
Conventional vs. scattering lens imaging



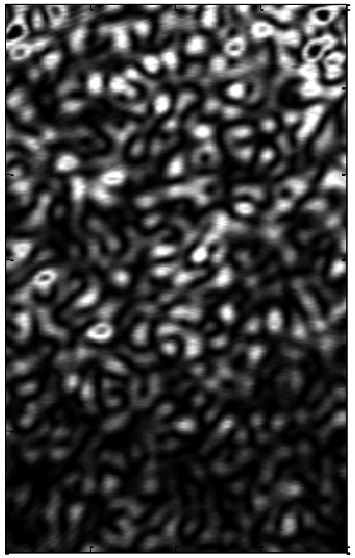
NA = sin θ_{max} = 1.0



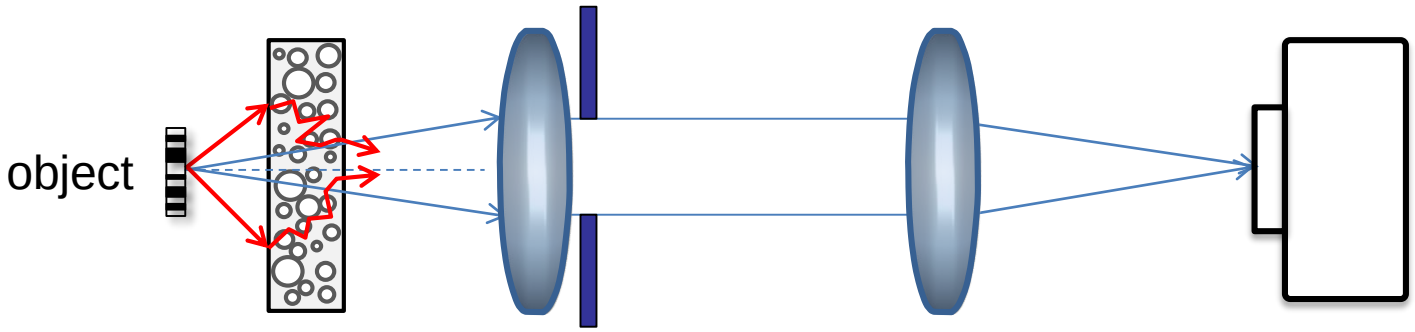
NA = 0.15



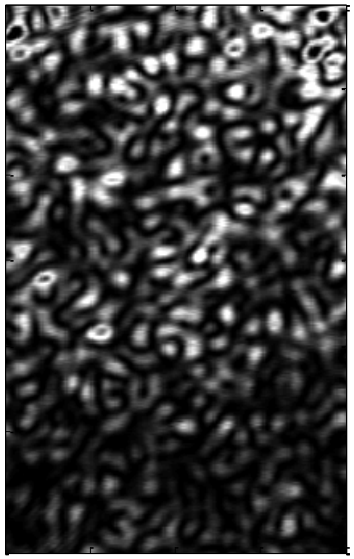
scattering lens with
low NA objective lens



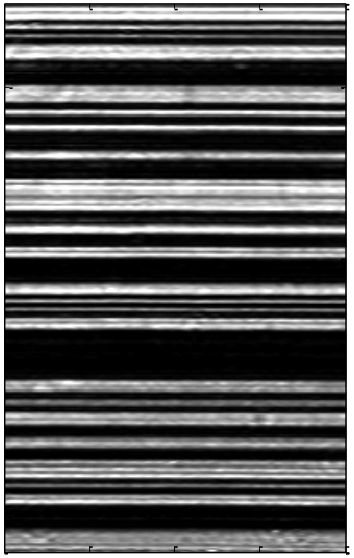
Scattering lens imaging system



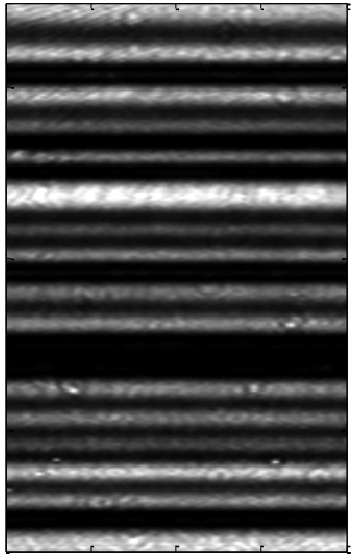
distorted image



recovered image

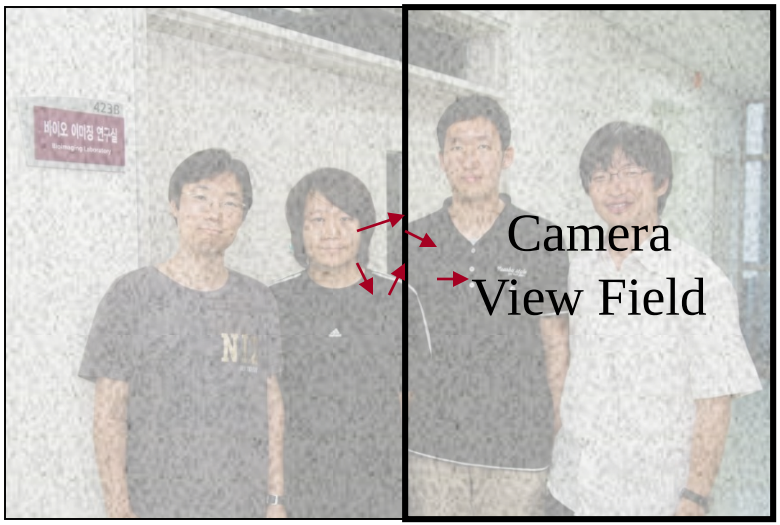


low NA image (0.15)



Y. Choi *et al.*, Phys. Rev. Lett. **107**, 023902 (2011).

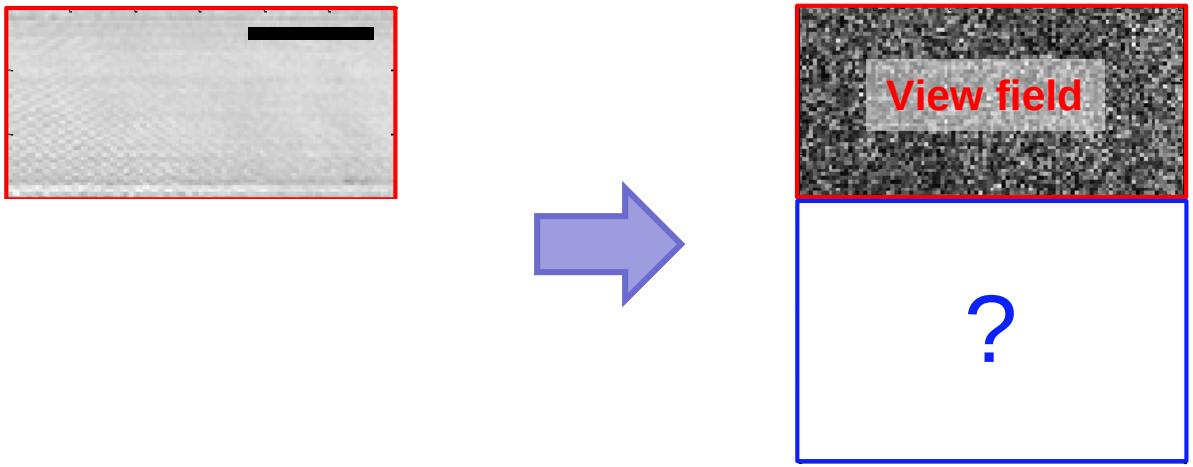
Benefits of a scattering lens #2

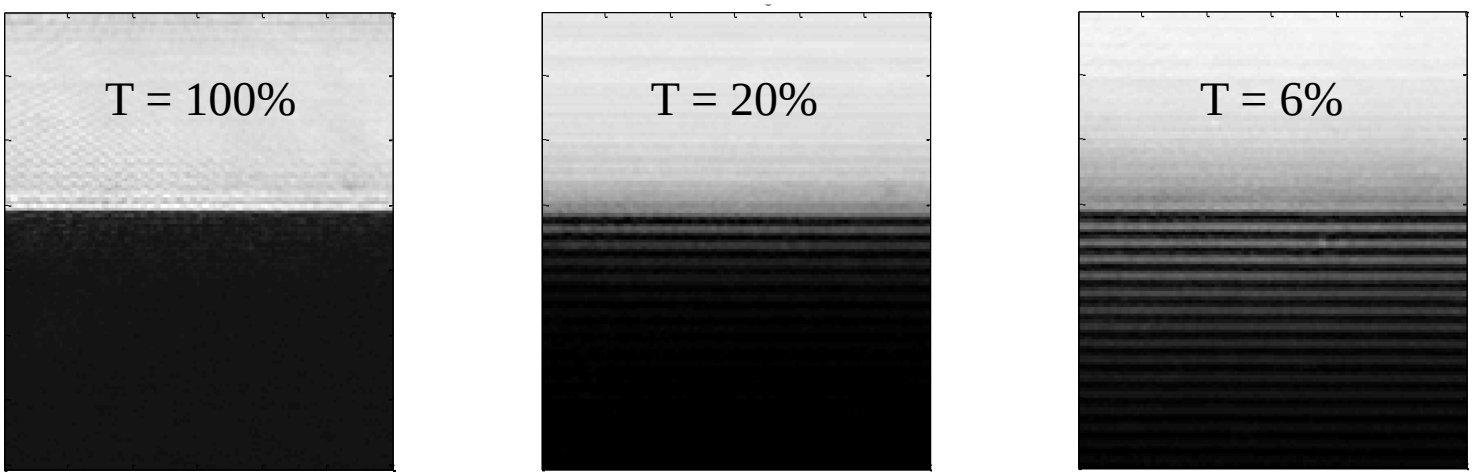


Photons travel along the lateral directions via *multiple scattering*.

Extended field of view of TLI

target object

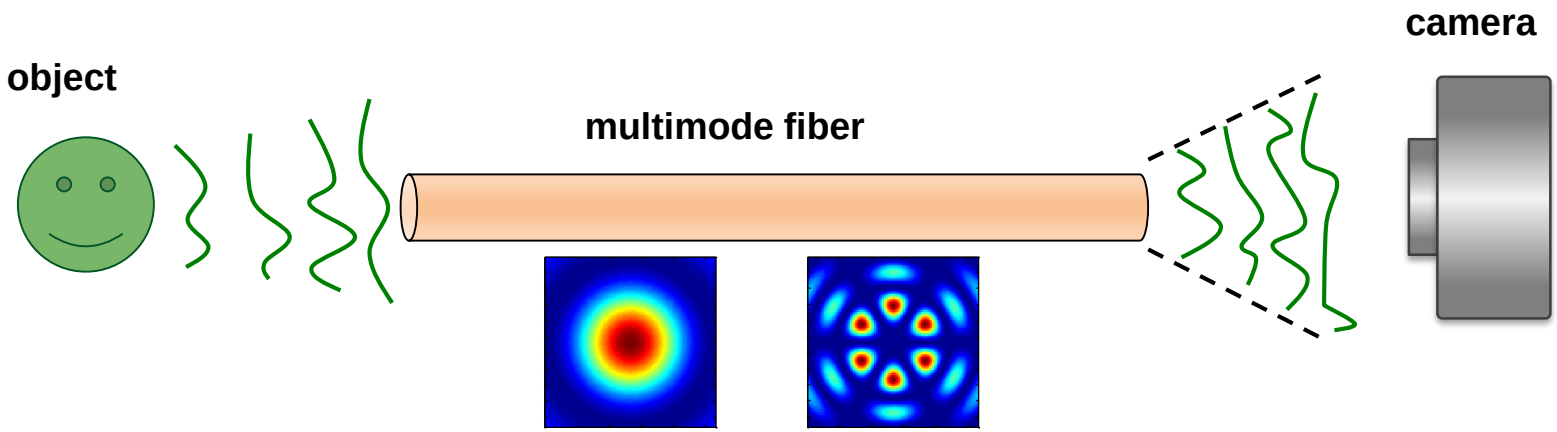




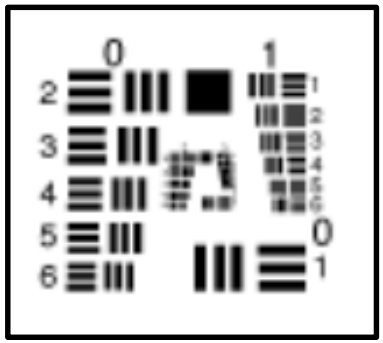
With a scattering lens, the range over which the image can be seen is extended beyond the normal field of view.

Single multimode optical fiber

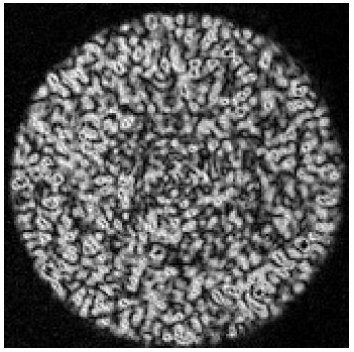
- A multimode optical fiber carries power of light.



Input image



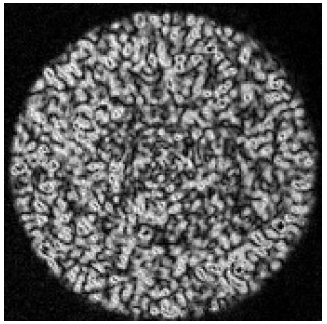
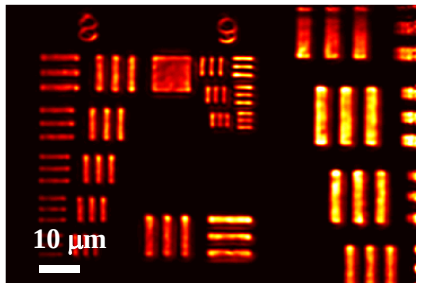
Output image

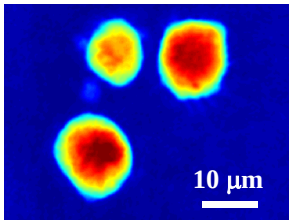


Mode dependent dispersion distorts the incident wave.

Single multimode optical fiber: just a lens

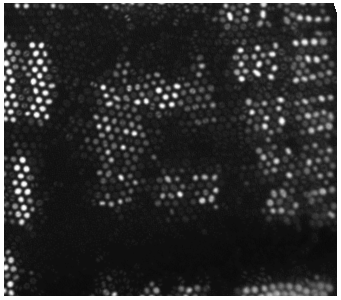
TLI can convert a multimode fiber into a flexible lens

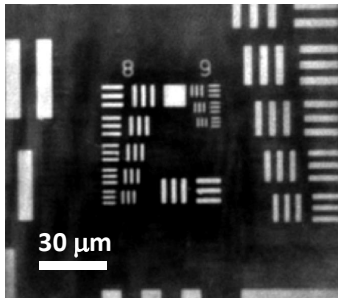
T^{-1}  = 



Y. Choi *et al*., Opt. Lett. **38**, 2253 (2013).

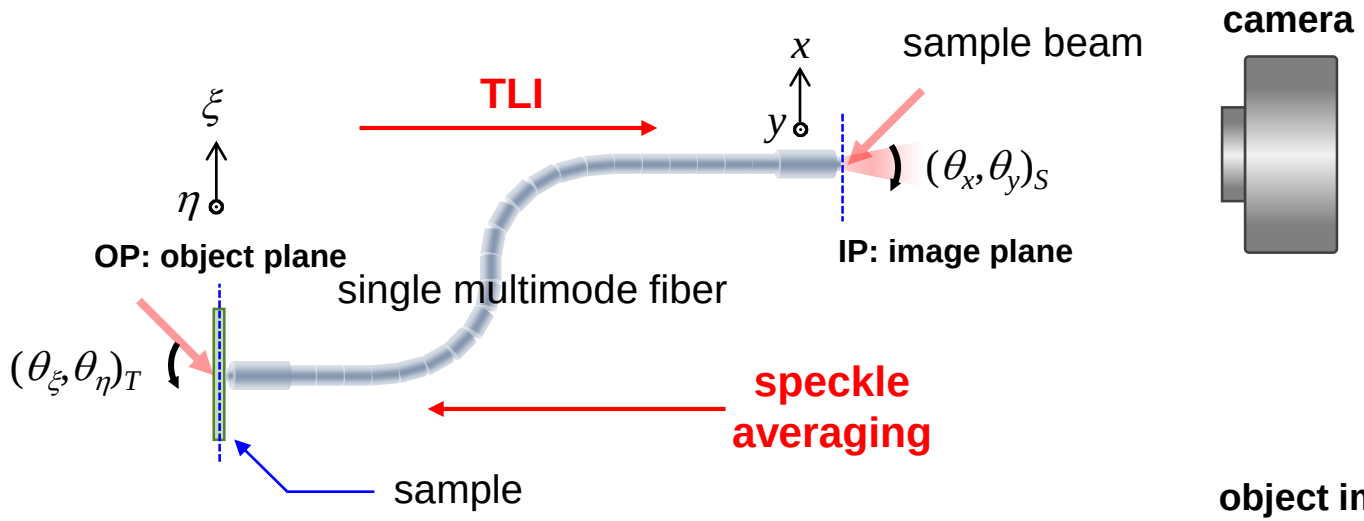
Removing pixilation of a fiber bundle

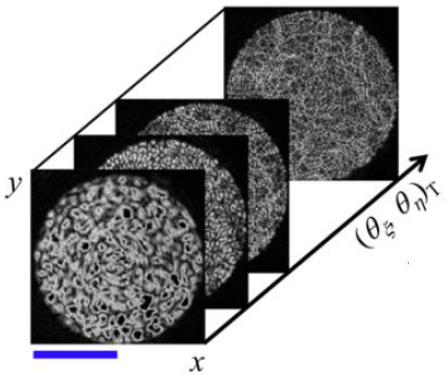


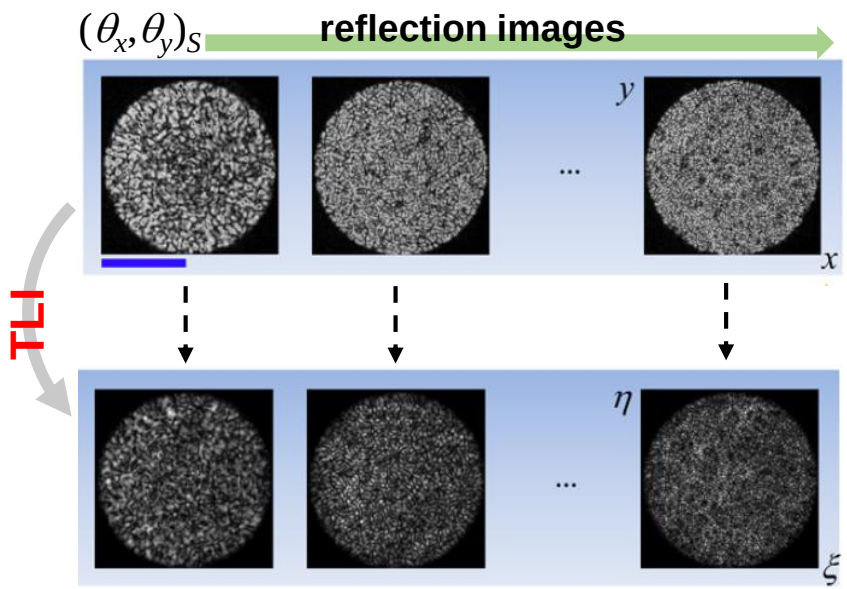


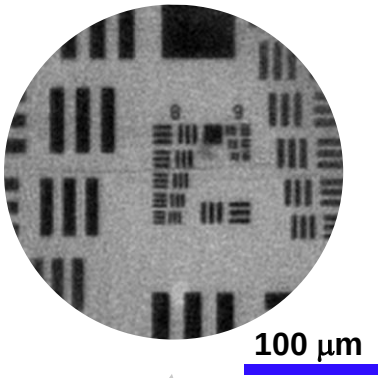
Y Choi *et al*., IEEE J. Sel. Top. Quant., **20** 6800213 (2014).

Lensless microendoscopy by a single fiber (LMSF)



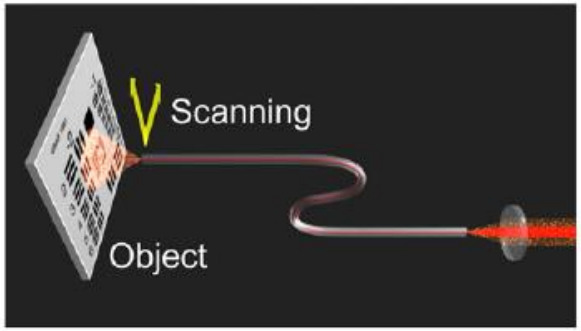


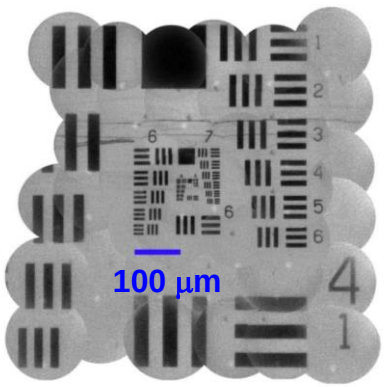




speckle averaging

The thinnest endoscope in the world



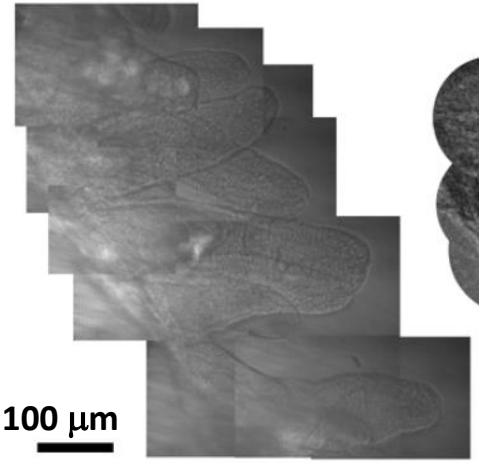


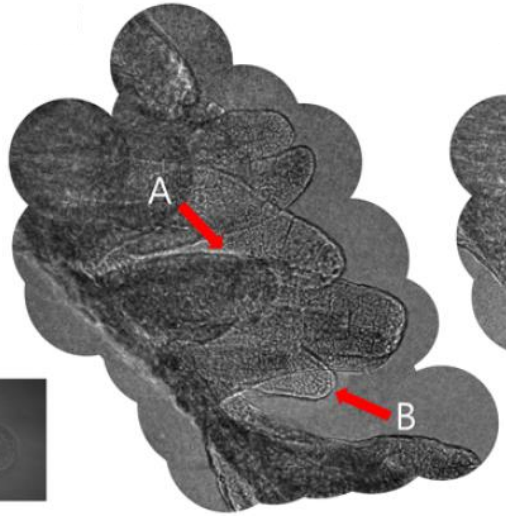
composite image

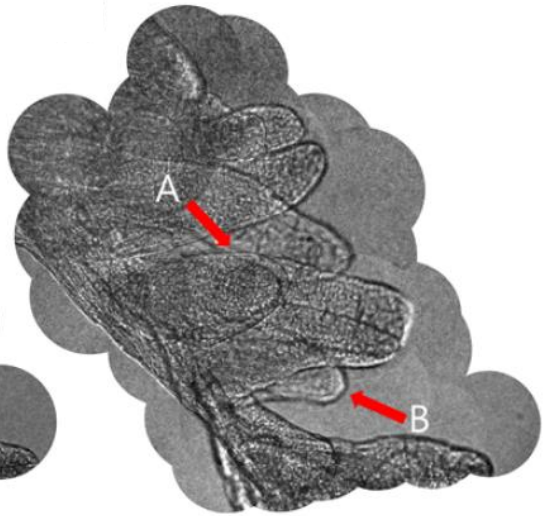
conventional bright field image

LMSF at original focus

LMSF after refocusing





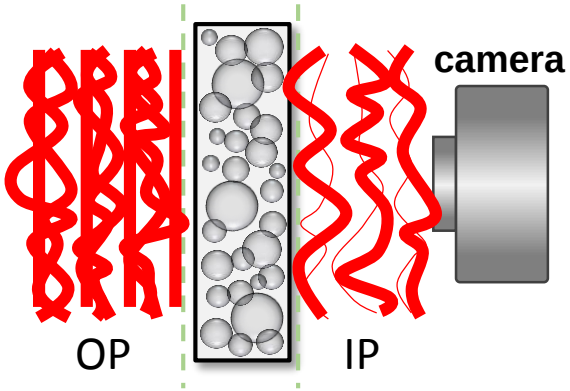


ex-vivo image of villi in an intestine tissue of a rat

Y. Choi *et al*., Phys. Rev. Lett. **109**, 203901 (2012).

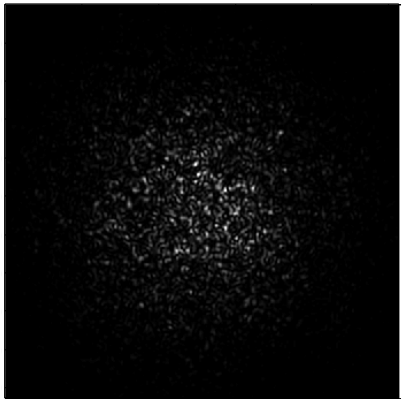
(Selected as an Editor's suggestion, highlighted in PRL (Viewpoint) and Nature Photonics)

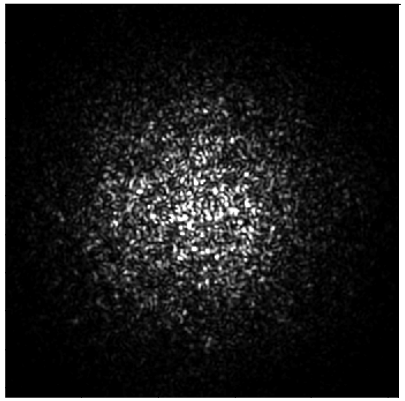
Enhancement of light transmission



energy transmittance

before



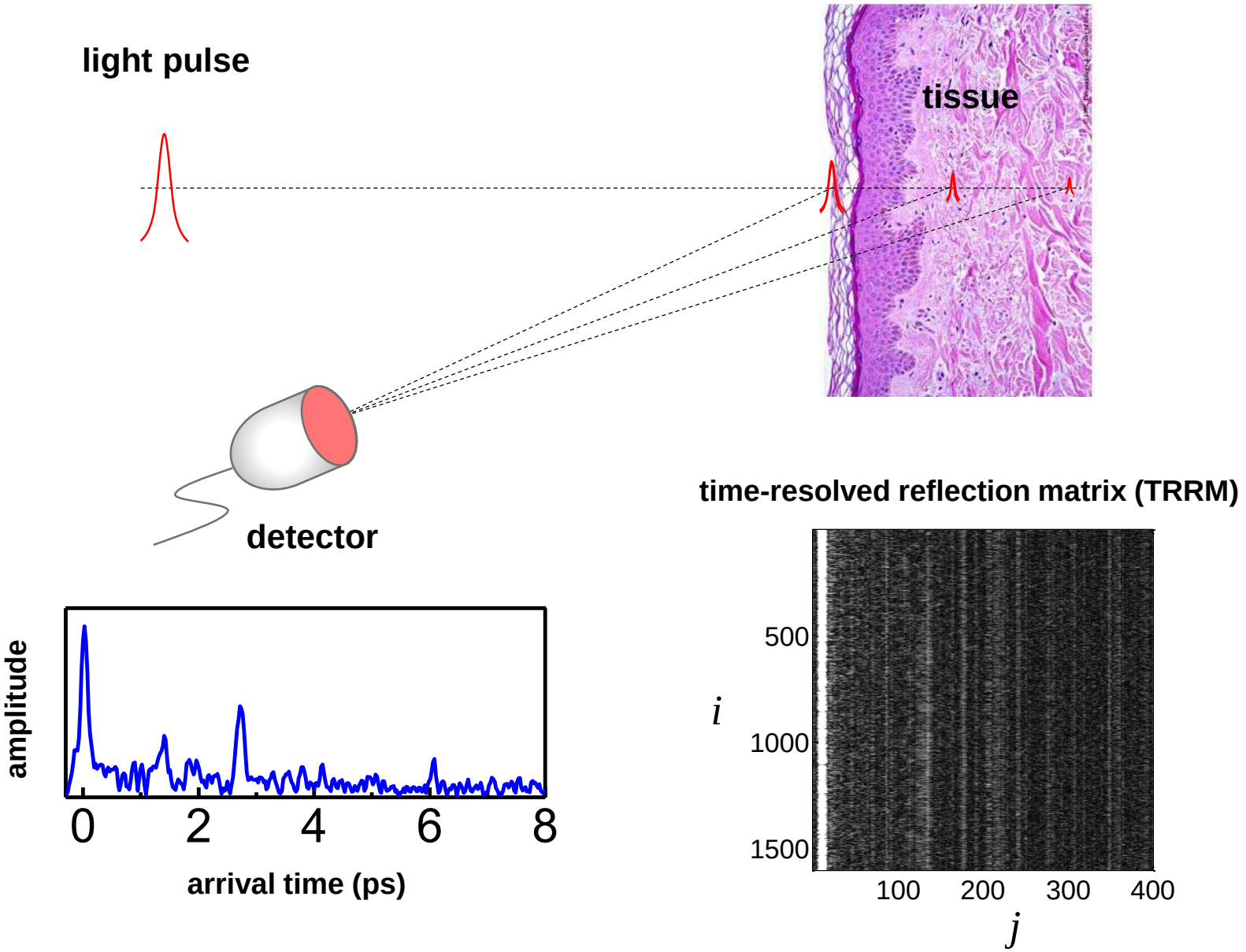


after

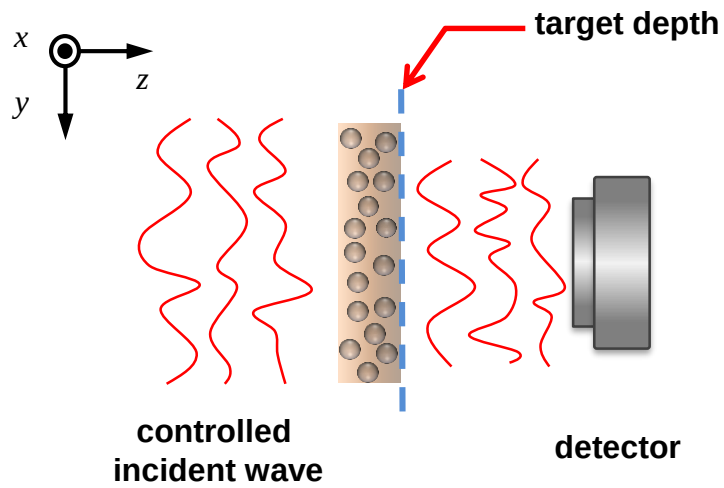
400 % transmission enhancement with the eigenchannel

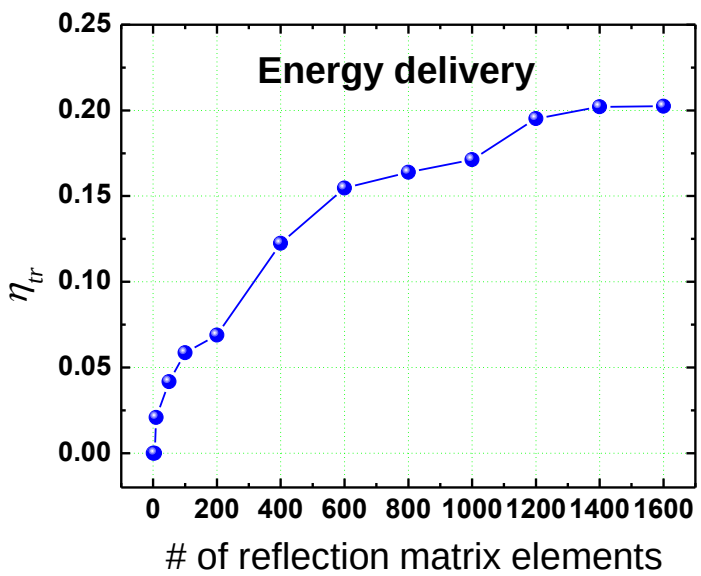
M. Kim, Y. Choi *et al*., Nature Photon. **6**, 583 (2012).

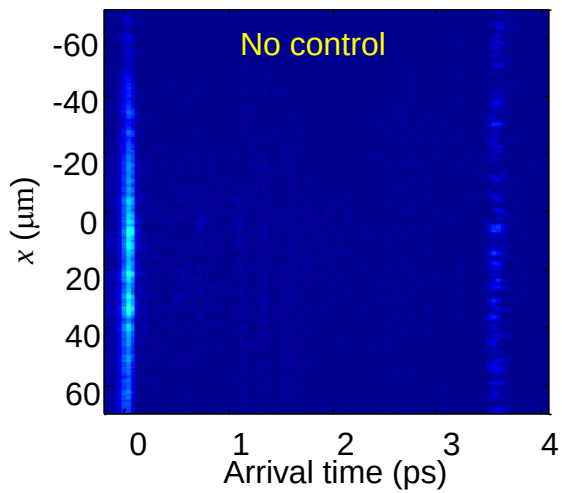
Reflection matrix

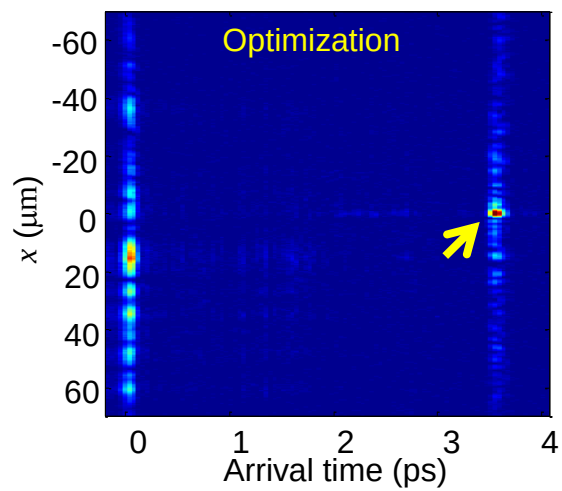


Enhancement of energy delivery in reflection mode









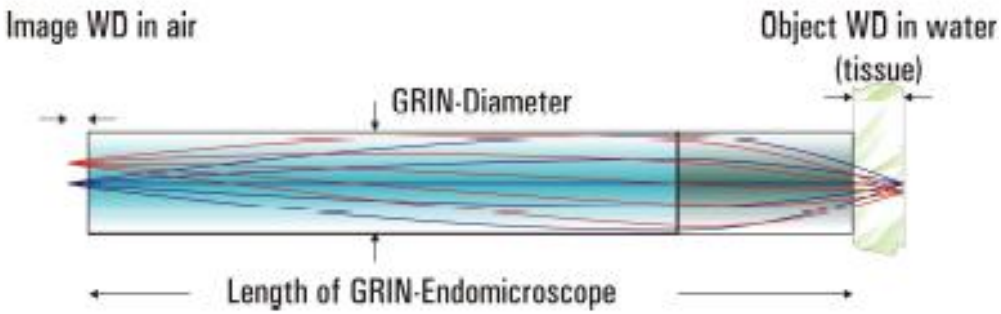
Y. Choi *et al.*, Phys. Rev. Lett., **111** 243901 (2013).

Table of contents

High-resolution rigid endoscope

Key element: Graded index lens (GRIN) lens

GRIN lens



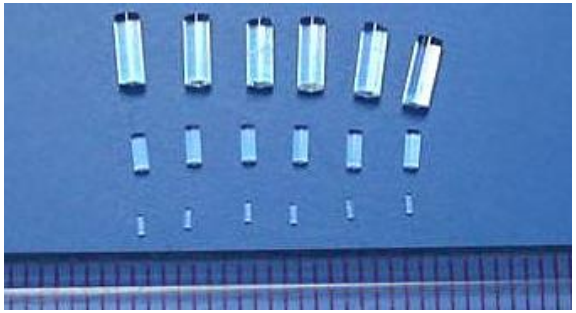
- Developing high-resolution endomicroscopy using a thin GRIN lens

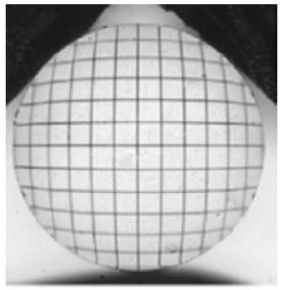
Properties of GRIN Lenses

Advantages:

- Delivers an image like a normal lens
- Wide view available
- Compact size and easy modification

GRIN lens

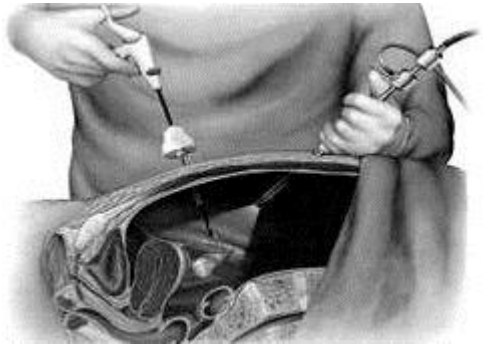




Drawback:

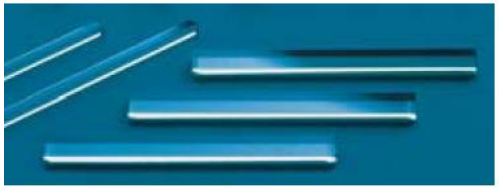
- Image distortion
- Poor resolution

Final destination

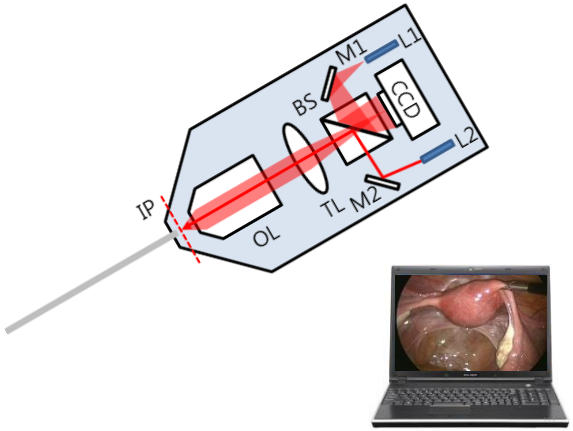


Laparoscope

Extended view field



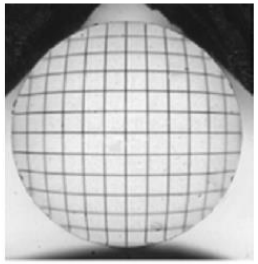
GRIN rod lens



Arthroscope

System calibration

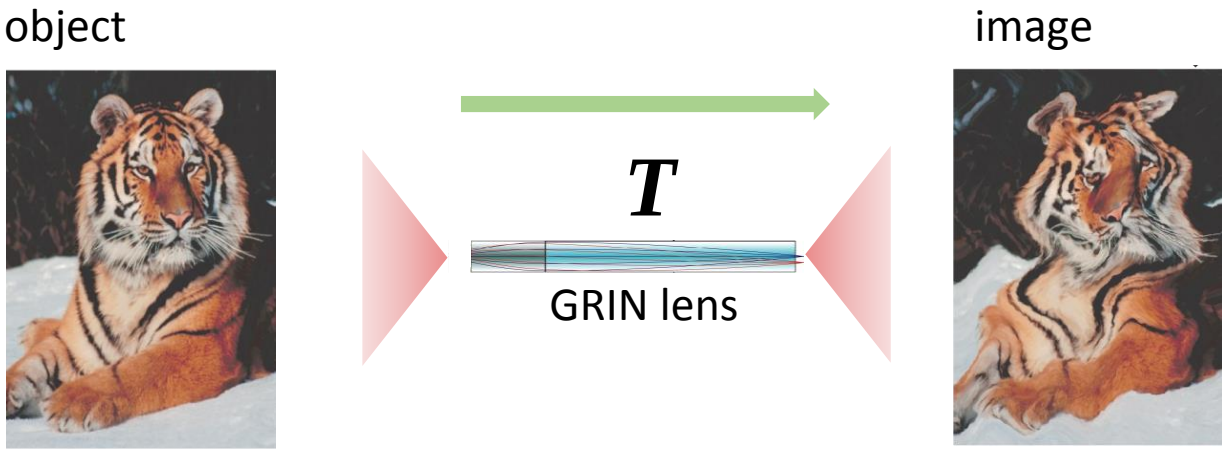
Aberration correction



In-vivo system

Wide-field
Aberration-fee
High resolution

T removes aberration for a GRIN lens



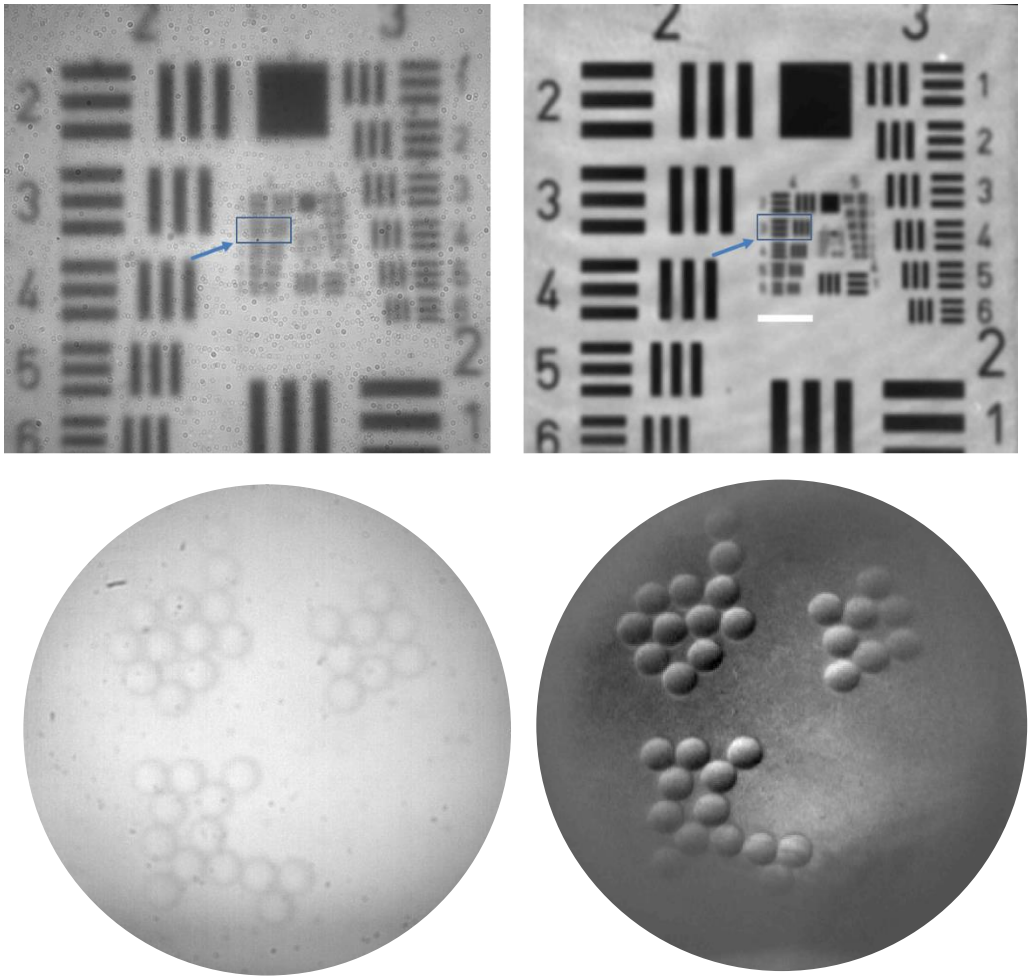
$\boldsymbol{T}$: input-output relation of a GRIN lens

- **reconstruction**

$\boldsymbol{T}^{-1}$

=

High resolution imaging through a GRIN lens



LED **Corrected**

Summary

Achievements

- Highly sensitive phase microscope using dynamic speckle illumination
- Non-contact, wide-field and single-shot measurement
- Measurement of nuclear membrane fluctuation of a complex cell
- High-resolution image reconstruction through a scattering medium
- Enhanced light energy delivery through a scattering medium
- The thinnest endoscope using a single multimode fiber
- Development of ultra-thin endoscopy using a GRIN lens

Plans

- Improvement of reflection phase microscopy
  - Effective collection of diffused light, phase stabilization
- Development of high resolution rigid endoscopy
  - Aberration correction

Acknowledgements

Group members

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