Phase (Contrast) Imaging

Quantification of 3D refractive index (RI)

Group 3
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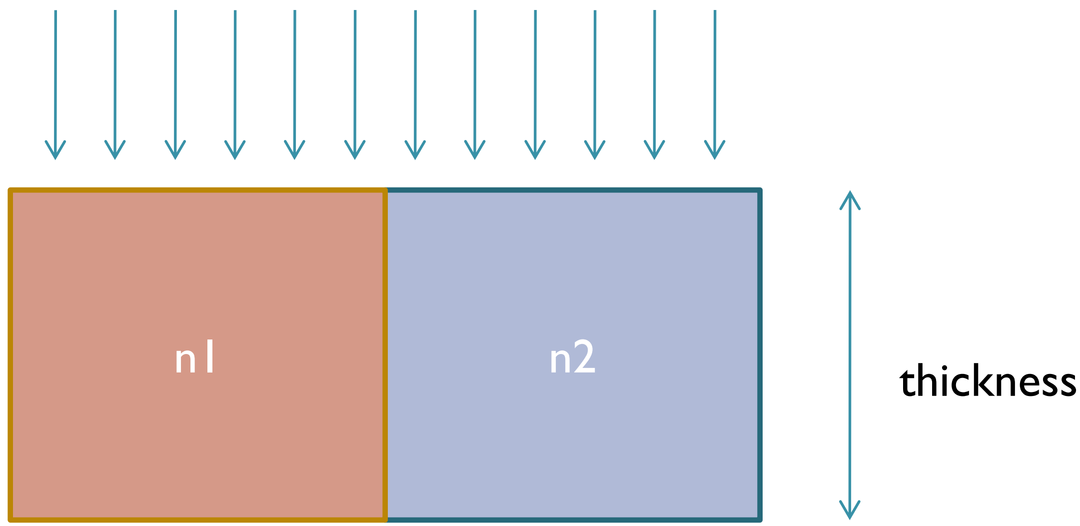
Outline

- A. Phase imaging
- B. Algorithm of holography
- C. 3D experimental result
- D. Pros and cons

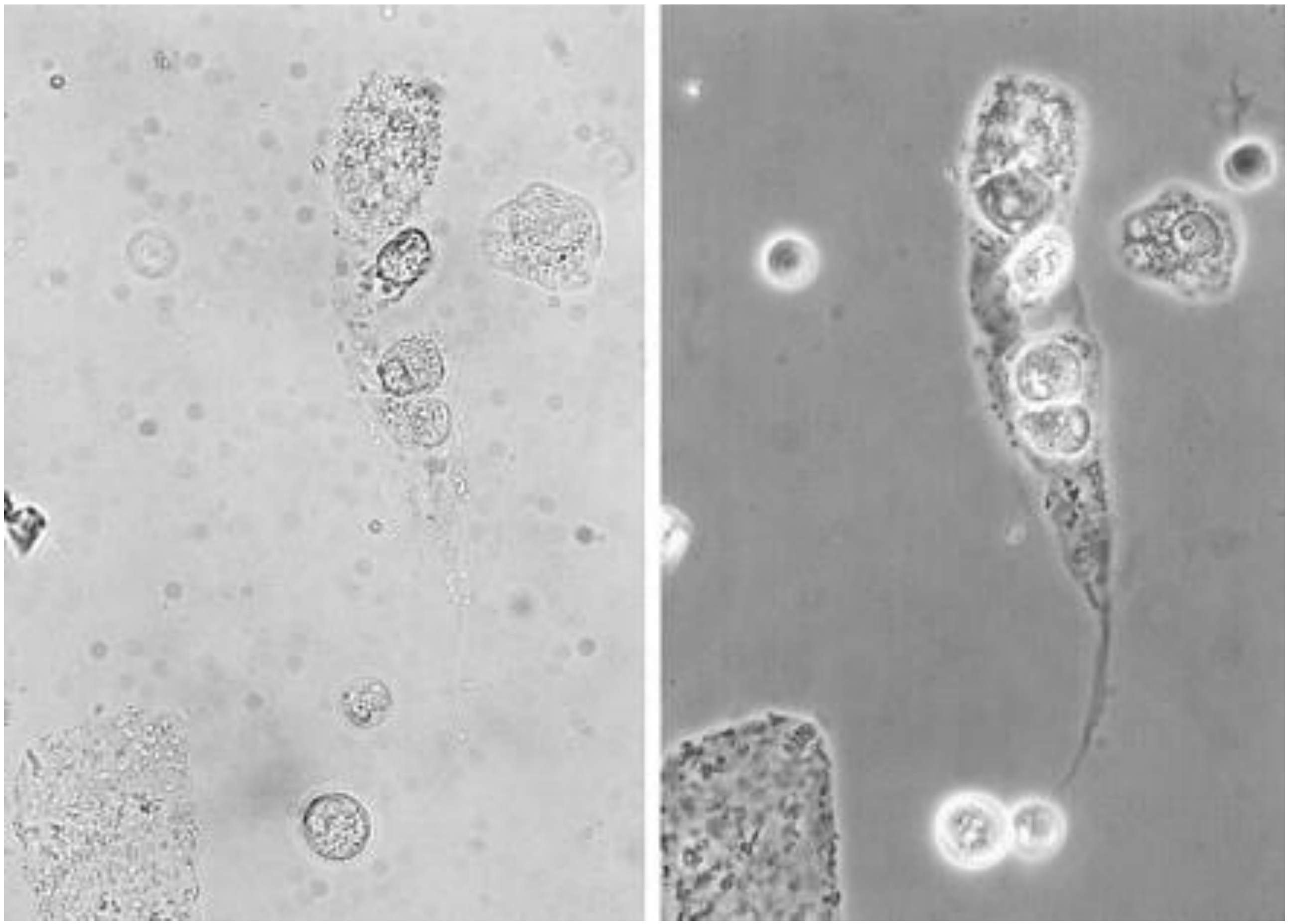
Simple review



Phase difference $\phi = 2\pi/\lambda * (n2-n1) * \text{thickness}$

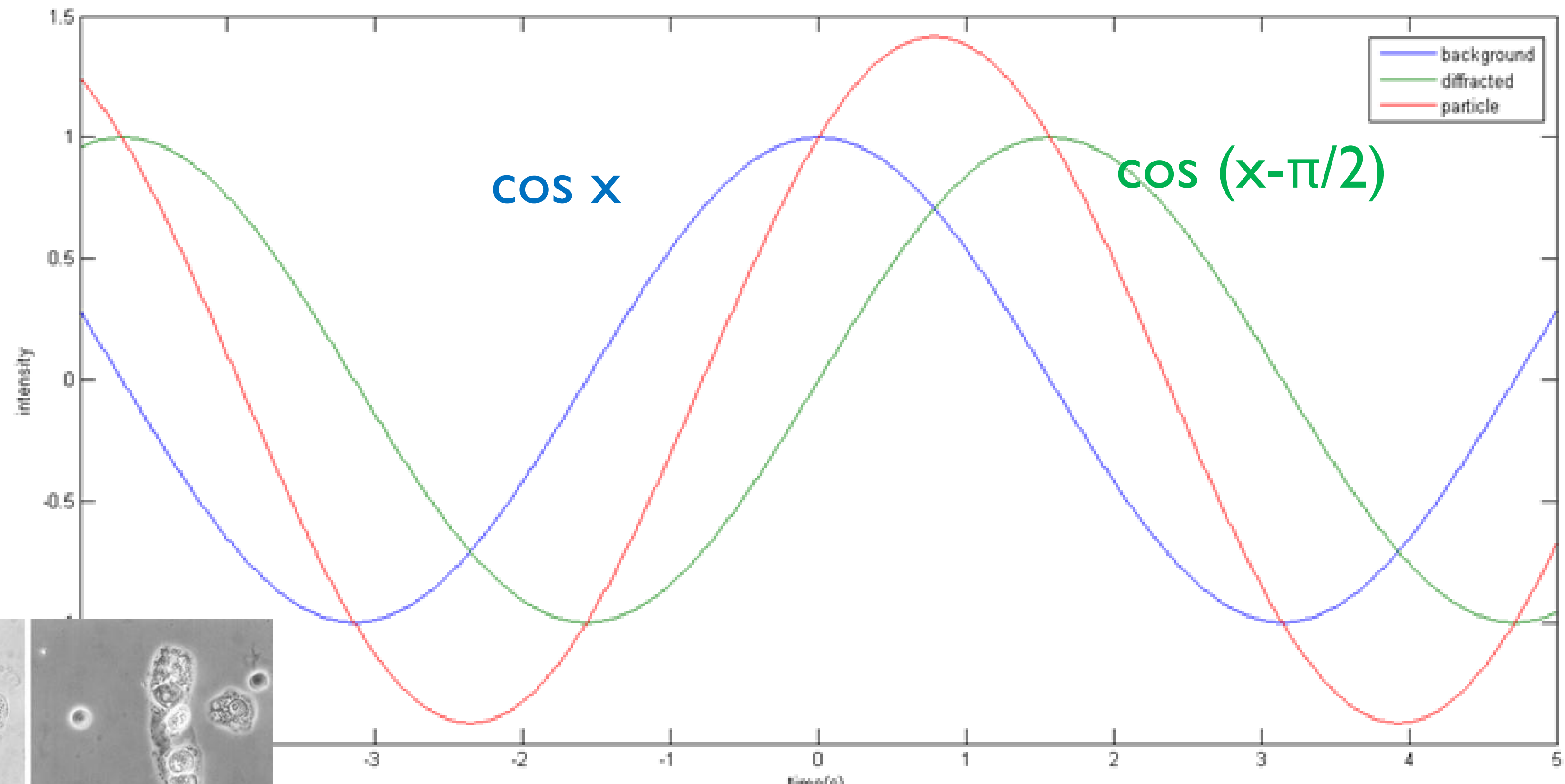
Bright field and phase contrast

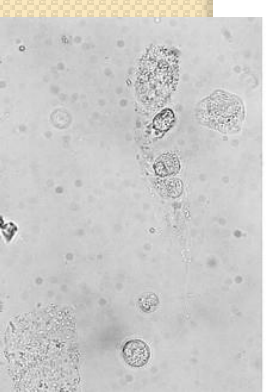
(left) (right)



A. Phase imaging (theory)

Optical path difference

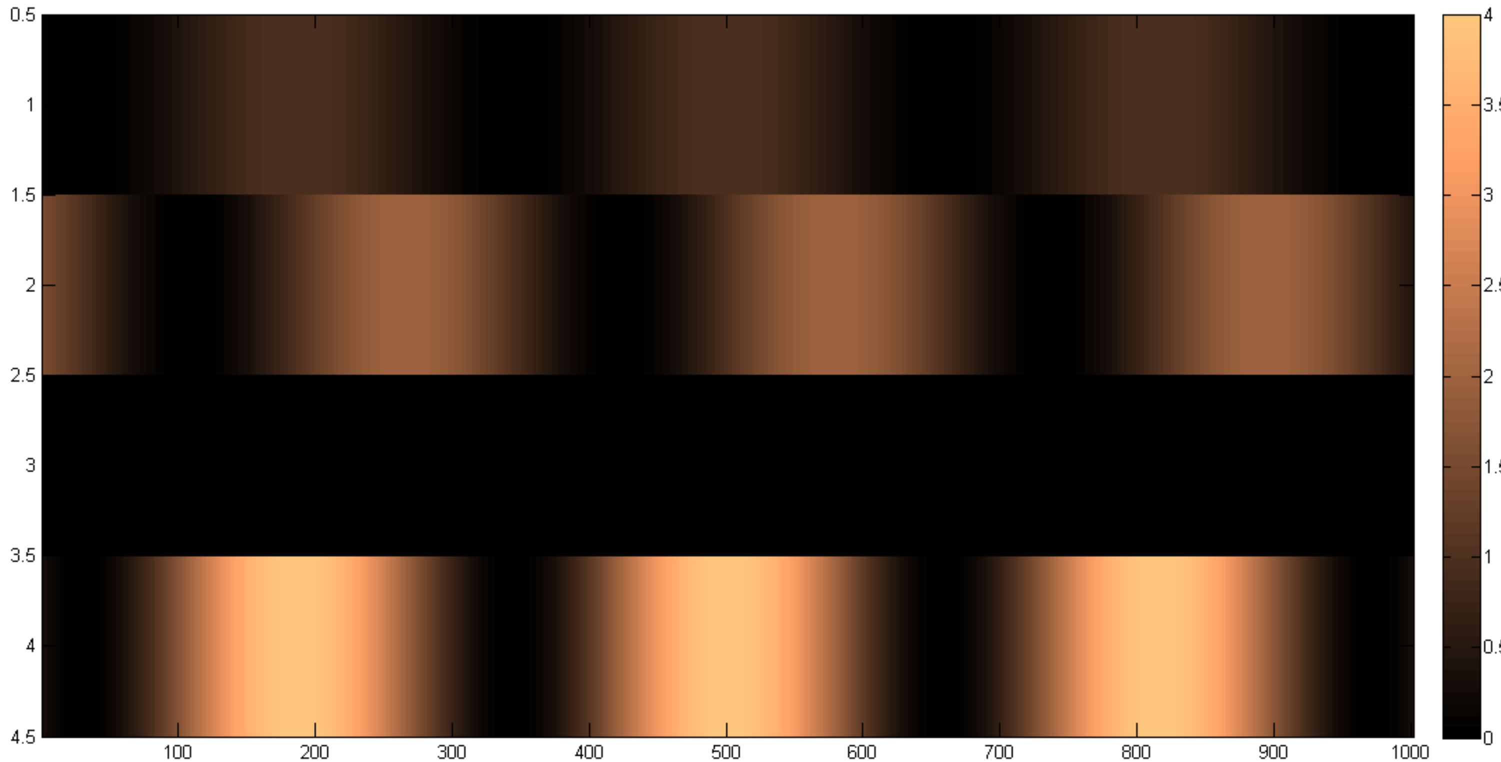




一般通過細胞的光，會有 π/2 的波程差

$\phi = 2\pi/\lambda * (n2-n1) * \text{thickness}$

一般通過細胞的光，會有 π/2 的波程差，亦即第二列之情況。



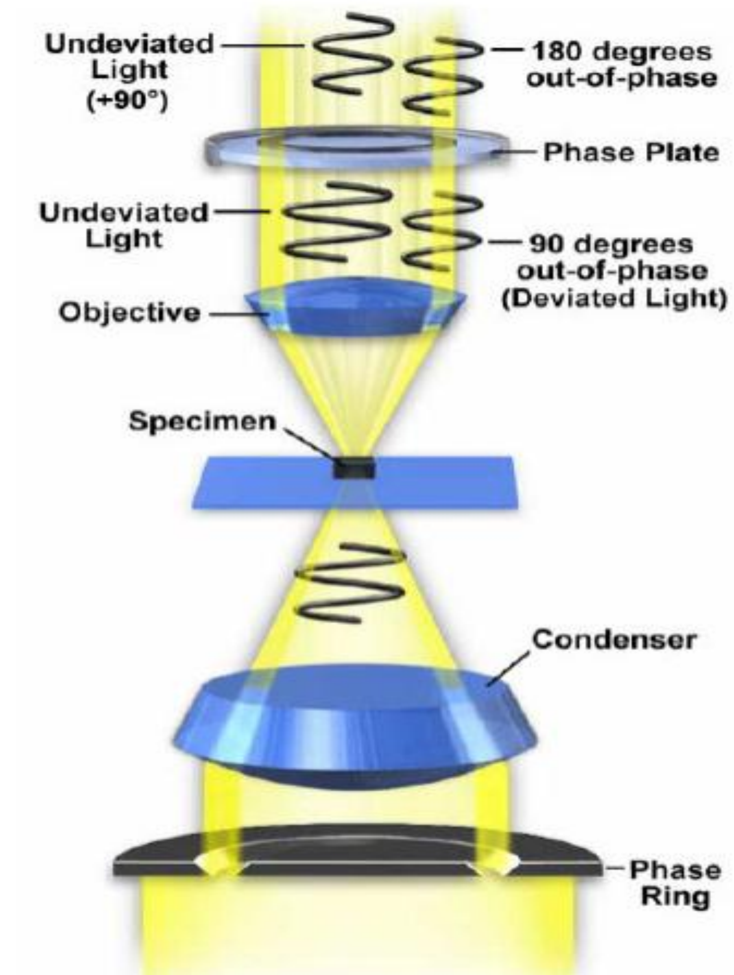
First row: cos x

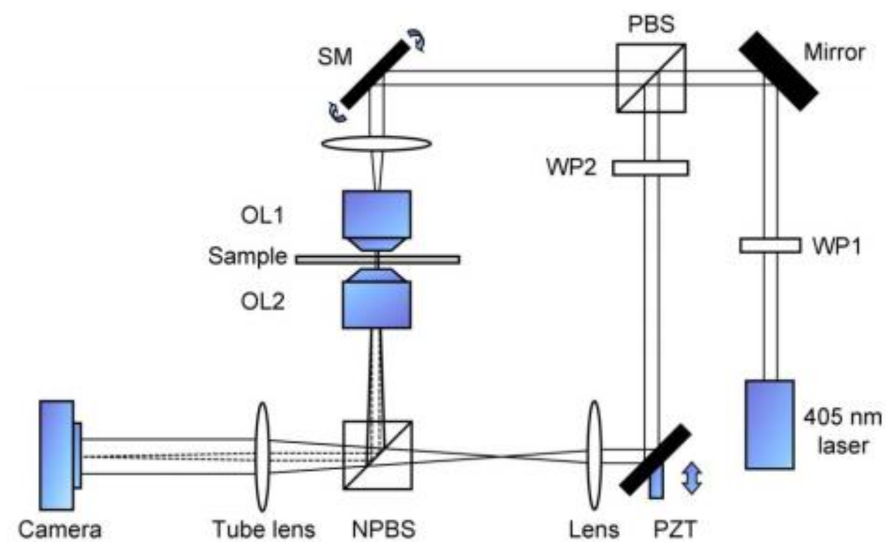
Second row: cos x + cos(x+π/2) → Bright field

Third row: cos x + cos(x+π) (destructive) → Phase imaging

Forth row: cos x + cos(x+2π) (constructive) → Phase imaging

Instrument





Not common path

Common path

Positive and negative phase contrast

(constructive) (destructive)

Specimens in Positive and Negative Phase Contrast

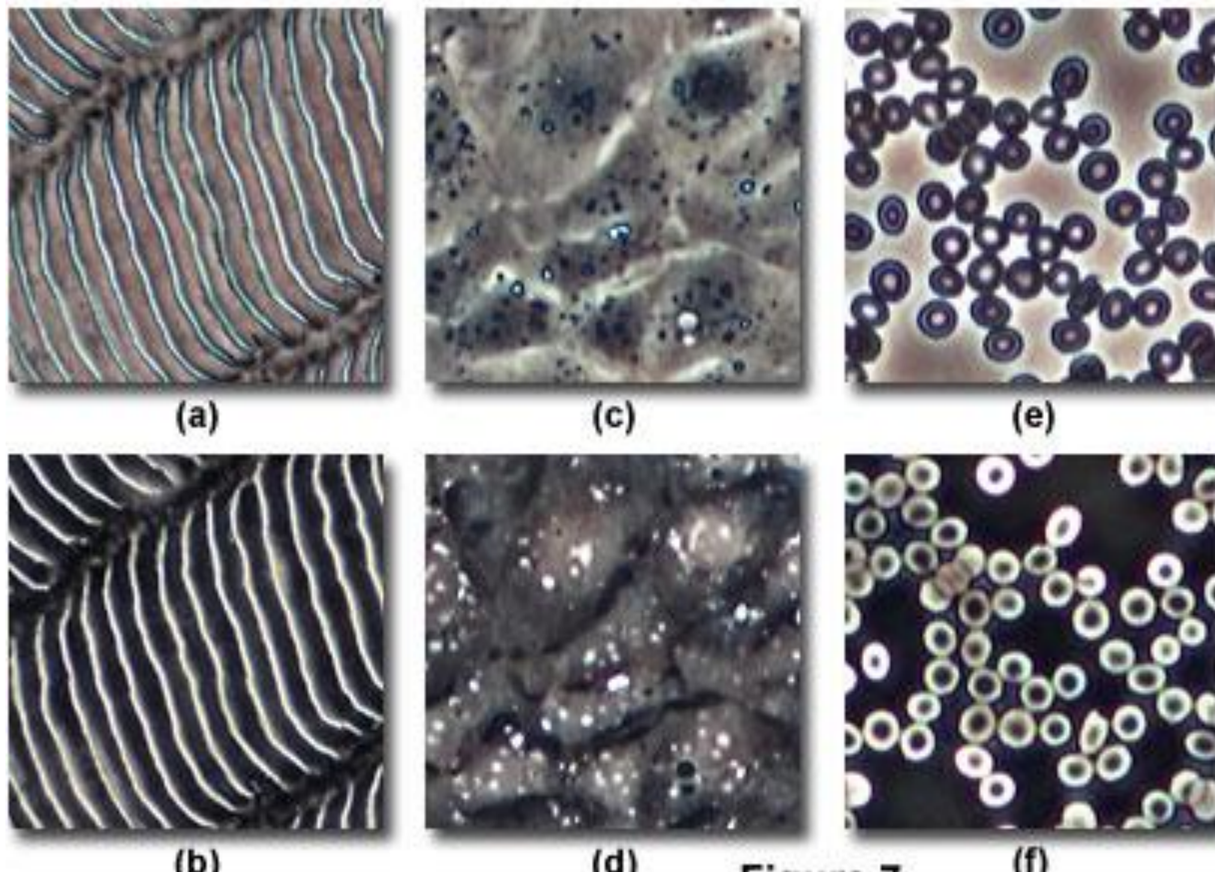
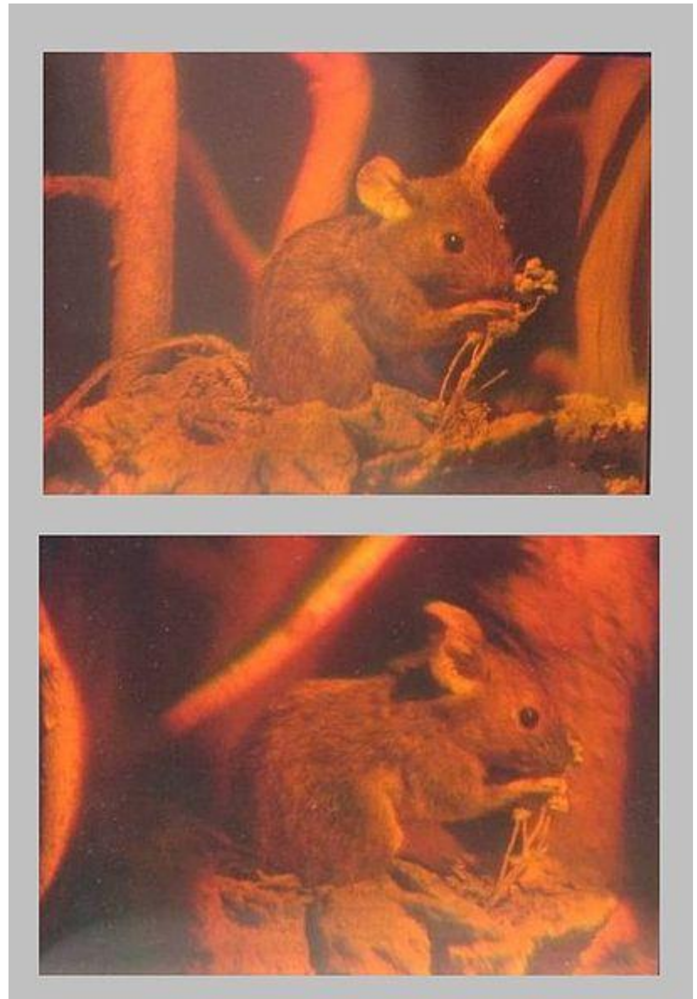


Figure 7

B. Algorithm of holography

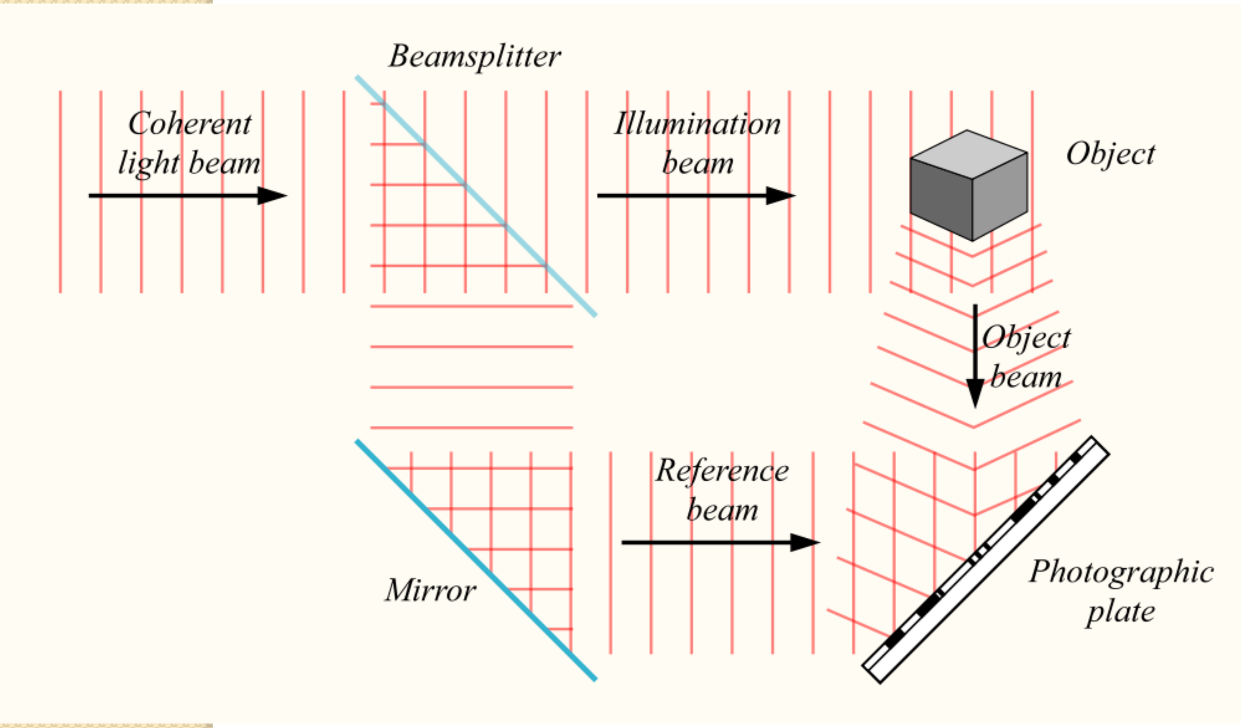


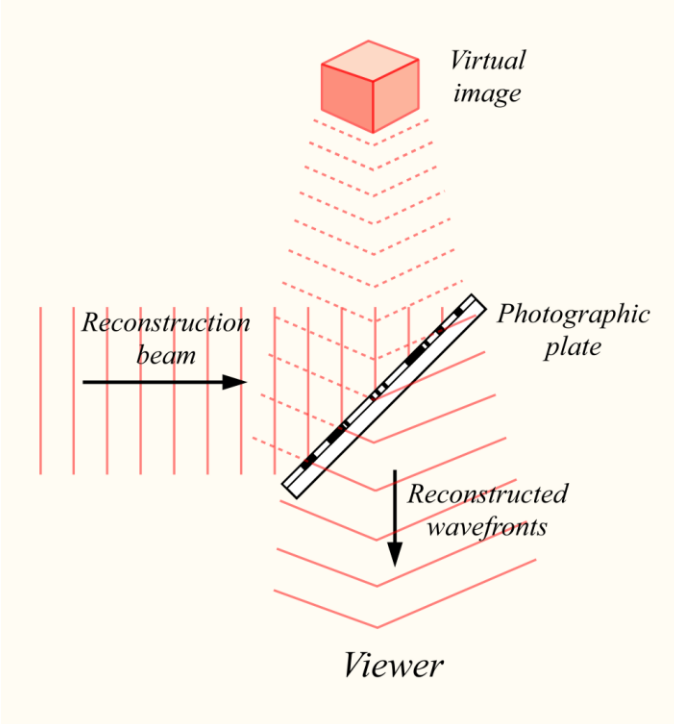
Holography

$$|U_O + U_R|^2 = U_O U_R{}^* + |U_R|^2 + |U_O|^2 + U_O{}^* U_R$$

$$T = k|U_O + U_R|^2$$

$$U_H = TU_R = k[U_O|U_R|^2 + |U_R|^2 U_R + |U_O|^2 U_R + U_O{}^* U_R{}^2]$$





(digital)

B. Algorithm of holography

Equations (4) yield

$$\{X_j\} = [A]^{-1}\{B_j\}, \quad (5)$$

where

$$[A] = \begin{bmatrix} M & \sum_{i=1}^{M} \cos \delta_i & \sum_{i=1}^{M} \sin \delta_i \\ \sum_{i=1}^{M} \cos \delta_i & \sum_{i=1}^{M} \cos^2 \delta_i & \sum_{i=1}^{M} \cos \delta_i \sin \delta_i \\ \sum_{i=1}^{M} \sin \delta_i & \sum_{i=1}^{M} \sin \delta_i \cos \delta_i & \sum_{i=1}^{M} \sin^2 \delta_i \end{bmatrix}, \quad (6)$$

$$\{X_j\} = \{a_j \quad b_j \quad c_j\}^T, \quad (7)$$

$$\{B_j\} = \left\{ \sum_{i=1}^{M} I_{ij} \quad \sum_{i=1}^{M} I_{ij} \cos \delta_i \quad \sum_{i=1}^{M} I_{ij} \sin \delta_i \right\}^T. \quad (8)$$

Equation (5) requires at least three different phase shifts δ_i to ensure that $[A]$ is nonsingular. From Eqs. (5)–(8) the unknowns a_j, b_j, and c_j can be solved and the phase ϕ_j can be determined from

$$\phi_j = \tan^{-1}(-c_j/b_j). \quad (9)$$

It is worth noting that $[A]^{-1}$ has to be assembled only once because its components depend only on δ_i. Therefore this step requires the same order of calculation time as that of the conventional phase-shifting algorithms.[8]

For the known ϕ_j, the least-squares criterion yields

$$\{X_i'\} = [A']^{-1}\{B_i'\}, \quad (12)$$

where

$$[A'] = \begin{bmatrix} N & \sum_{j=1}^{N} \cos \phi_j & \sum_{j=1}^{N} \sin \phi_j \\ \sum_{j=1}^{N} \cos \phi_j & \sum_{j=1}^{N} \cos^2 \phi_j & \sum_{j=1}^{N} \cos \phi_j \sin \phi_j \\ \sum_{j=1}^{N} \sin \phi_j & \sum_{j=1}^{N} \sin \phi_j \cos \phi_j & \sum_{j=1}^{N} \sin^2 \phi_j \end{bmatrix}, \quad (13)$$

$$\{X_i'\} = \{a_i' \quad b_i' \quad c_i'\}^T, \quad (14)$$

$$\{B_i'\} = \left\{ \sum_{j=1}^{N} I_{ij} \quad \sum_{j=1}^{N} I_{ij} \cos \phi_j \quad \sum_{j=1}^{N} I_{ij} \sin \phi_j \right\}^T. \quad (15)$$

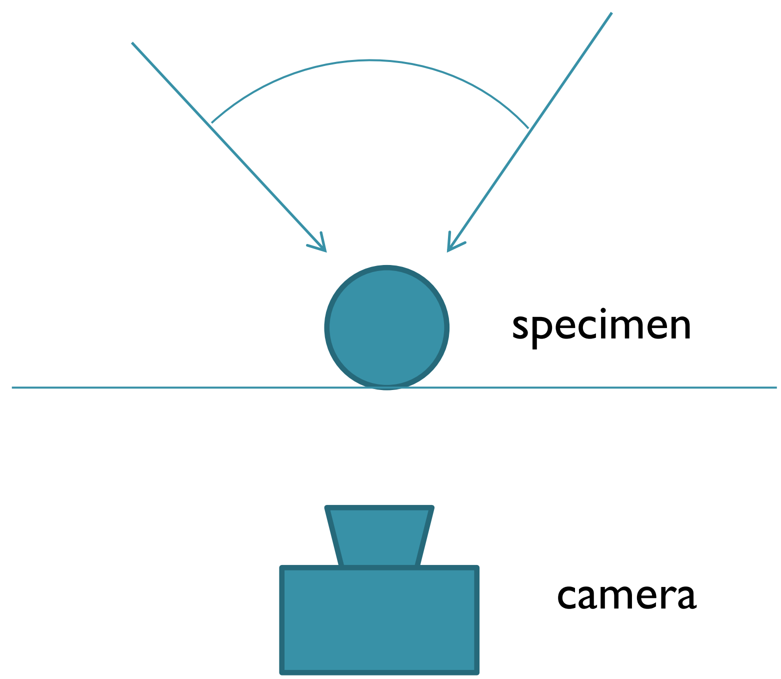
The unknowns a_i', b_i', and c_i' can be solved from Eqs. (12)–(15). Then the amount of phase shift in each frame can be determined from

$$\delta_i = \tan^{-1}(-c_i'/b_i'). \quad (16)$$

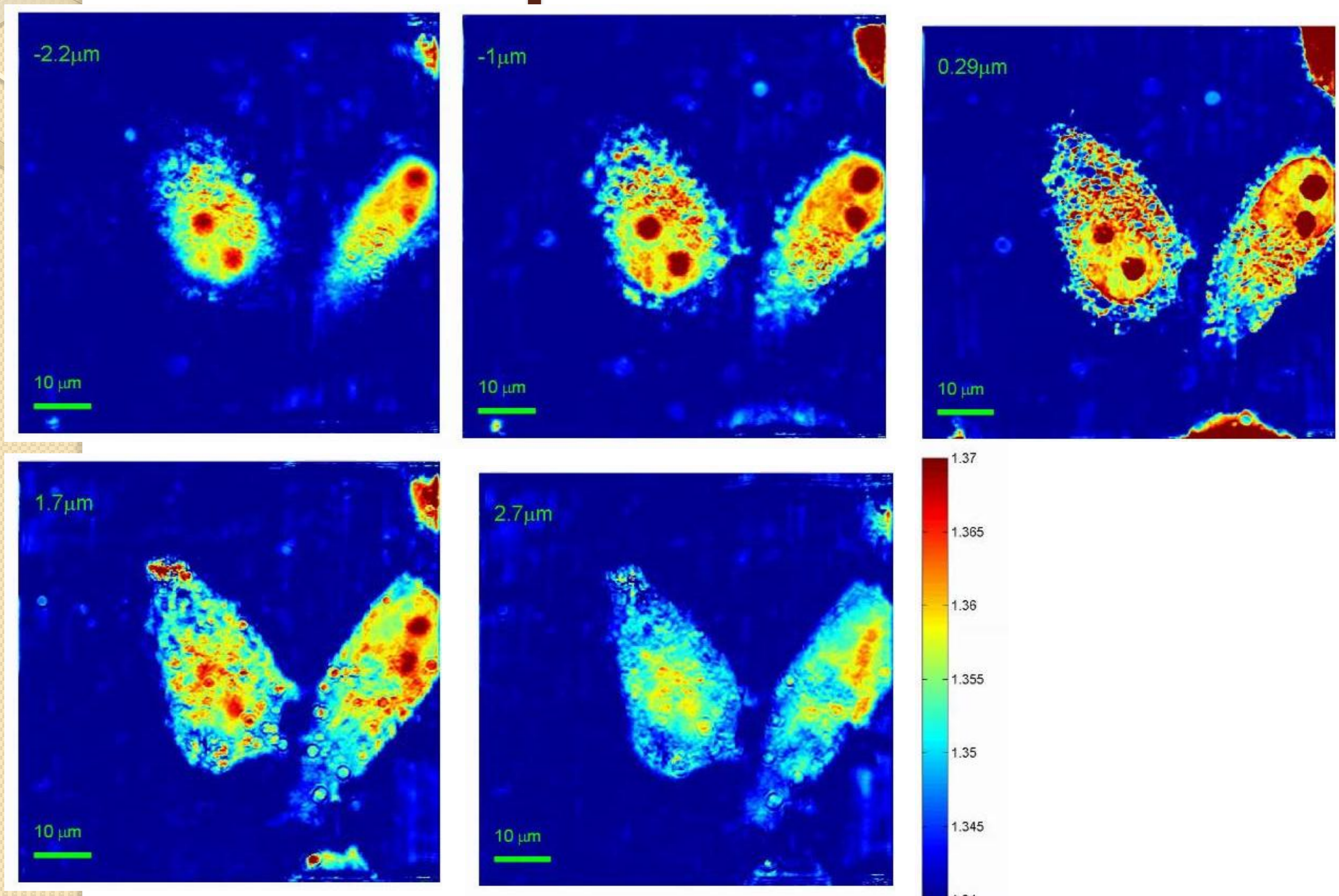
Step 3. Convergence limit: The algorithm repeats steps 1 and 2 until the phase-shift values converge. The convergence criteria for relative phase-shift amounts can be expressed as

$$|(\delta_i^{\,k} - \delta_1^{\,k}) - (\delta_i^{\,k-1} - \delta_1^{\,k-1})| < \epsilon, \quad (17)$$

scan in different angle



C. 3D experimental result



FULL ARTICLE

Digital holographic microtomography for high-resolution refractive index mapping of live cells

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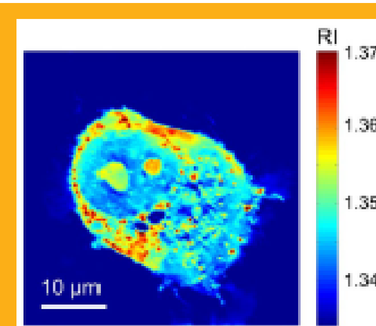
Quantification of three-dimensional (3D) refractive index (RI) with sub-cellular resolution is achieved by digital holographic microtomography (DHμT) using quantitative phase images measured at multiple illumination angles. The DHμT system achieves sensitive and fast phase measurements based on iterative phase extraction algorithm and asynchronous phase shifting interferometry without any phase monitoring or active control mechanism. A reconstruction algorithm, optical diffraction tomography with projection on convex sets and total variation minimization, is implemented to substantially reduce the number of angular scattered fields needed for reconstruction without sacrificing the accuracy and quality of the reconstructed 3D RI distribution. Tomogram of a living CA9-22 cell is presented to demonstrate the performance of the method. Further, a statistical analysis of the average RI of the nucleoli, the nucleus excluding the nucleoli and the cytoplasm of twenty CA9-22 cells is performed.



$x - y$ slice of the reconstructed RI map of a CA9-22 cell near the focal plane.

D. pros and cons

- Pros:
  - Do not hurt specimen.
  - Can see Au nanoparticle by scattering.
  - Acquire 3D information.
- Cons:
  - Sensitive to vibration.
  - Laser noise.

Reference

- 1.Digital holographic microtomography for high resolution refractive index mapping of live cells
- 2.Advanced iterative algorithm for phase extraction of randomly phase-shifted interferograms
- 3.Quantitative Phase Imaging of Nanoscale Cell Structure and Dynamicsn //Gabriel Popescu
- 4.http://www.microscopyu.com/articles/phasecontrast/

Thanks for your listening !!