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Quantitative bio-imaging in widefield microscopy: problems and solutions

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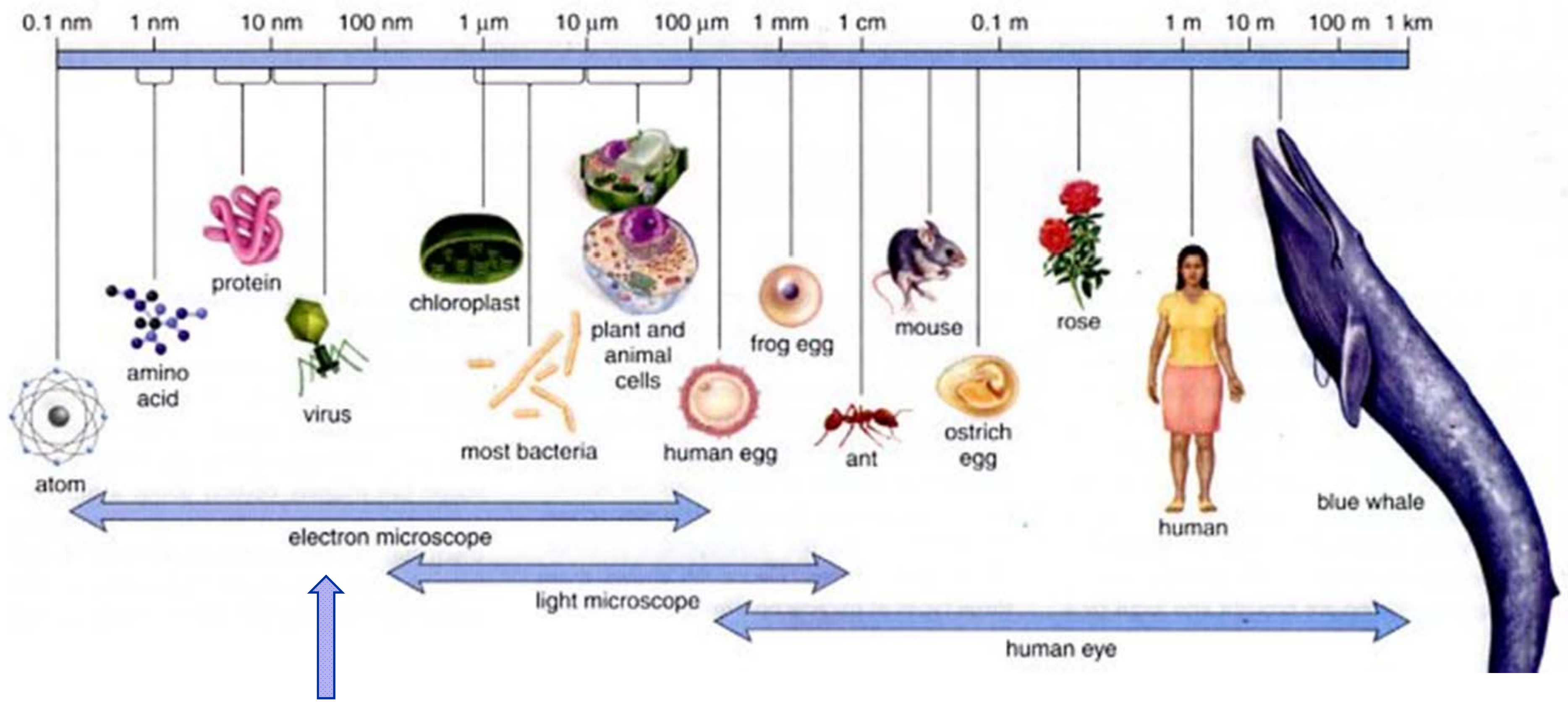
What we can see with and without a microscope



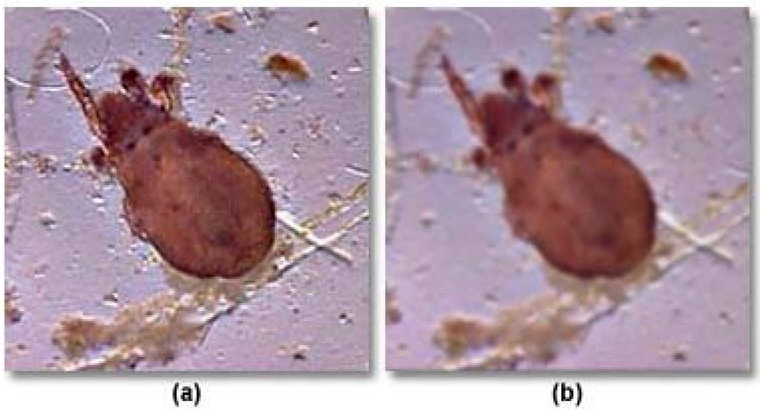
- Light microscope with superresolution techniques

X ray techniques

Image from Internet: unknown author

What we need to get a good image

- Magnification of the image



Same magnification but $NA_a > NA_b$

Image from wcssamland.weebly.com/book-c-ch-1

- Large NA of the objective
- Correct illumination
- Sufficient dynamic range of image detector
- Good optics and alignment

Outline

- Coherent versus partially coherent Illumination
- Quantitative imaging (QI)
- Phase retrieval methods for QI in microscopy
- Different approaches for 3D QI
- QI with partially coherent illumination
- Illumination coherence engineering
- Concluding remarks

Partially coherent light

- Approximation: scalar monochromatic field, Gaussian statistics

Coherent field (2D):
complex field amplitude

$$u(\mathbf{r}) = |u(\mathbf{r})| \exp[i\varphi(\mathbf{r})]$$

Partially coherent field (4D)
mutual intensity (MI)

$$\Gamma(\mathbf{r}_1, \mathbf{r}_2) = \langle u(\mathbf{r}_1) u^*(\mathbf{r}_2) \rangle$$

- Only intensity can be measured directly

$$\Gamma(\mathbf{r}, \mathbf{r}) = \langle |u(\mathbf{r})|^2 \rangle$$

Microscope illumination

- August Köhler illumination proposal (1893)
- Coherence description:
  Van Cittert-Zernike theorem

$$\Gamma(\mathbf{r}_1,\mathbf{r}_2) = u_s(\mathbf{r}_1)u_s^*(\mathbf{r}_2)\Gamma_{il}(\mathbf{r}_1-\mathbf{r}_2)$$

MI in the sample plane after passing through the object

$$\Gamma_{il}(\mathbf{r}_1,\mathbf{r}_2) \propto \int I_S(\mathbf{r}')\exp\left[-ik\left(\mathbf{r}_1-\mathbf{r}_2\right)\mathbf{r}'/\,\mathrm{f}_c\right]d\mathbf{r}'$$

MI of the illumination field

$I_S(\mathbf{r})$

Intensity distribution of incoherent illumination source

- Coherence parameter
  $S=NA_c/NA_o$

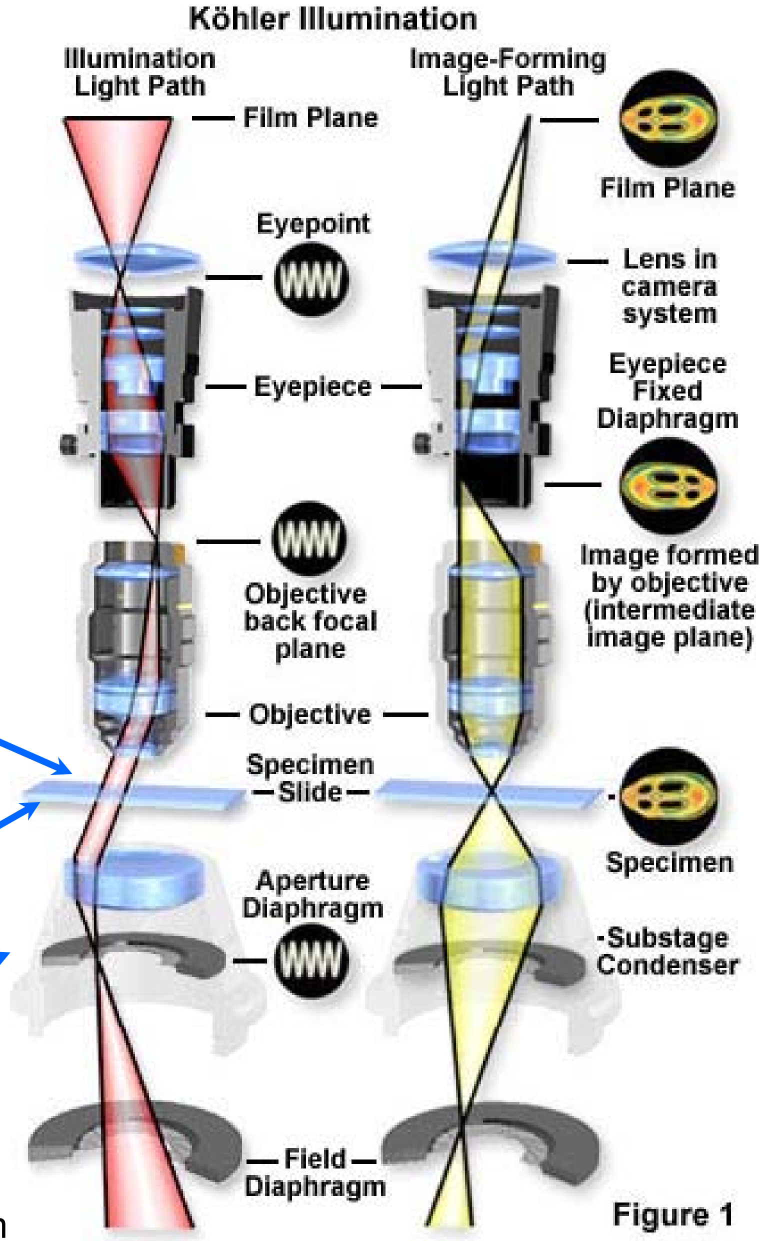
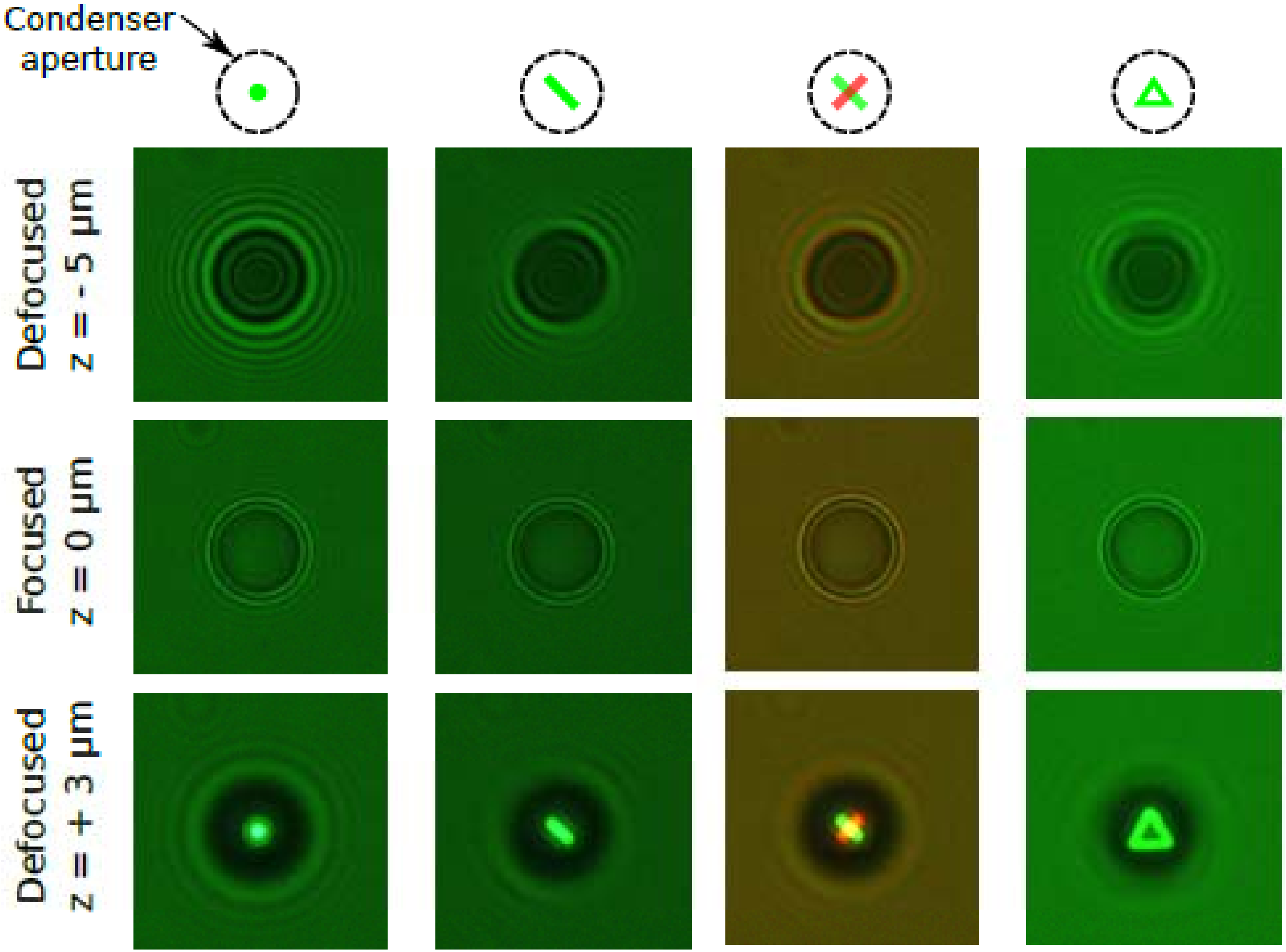


Image from
http://www.olympusmicro.com/primer/anatomy/kohler.html

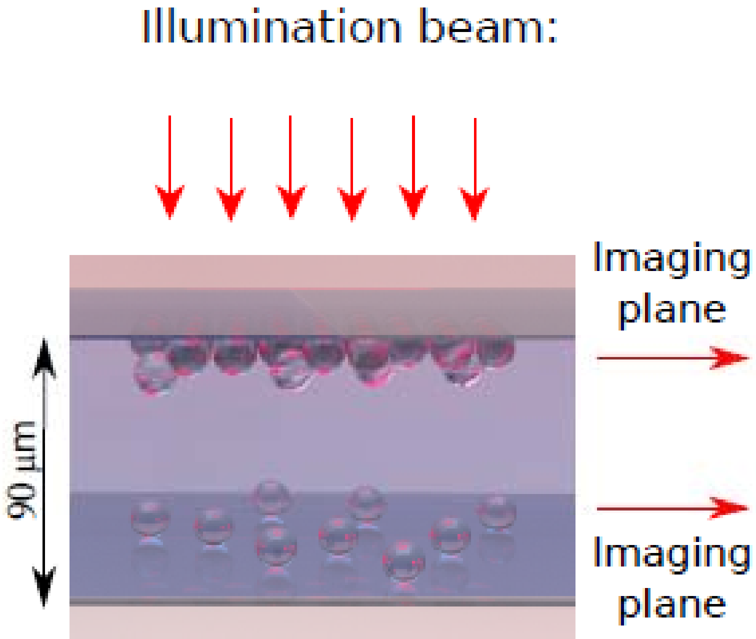
Image depends on condenser aperture

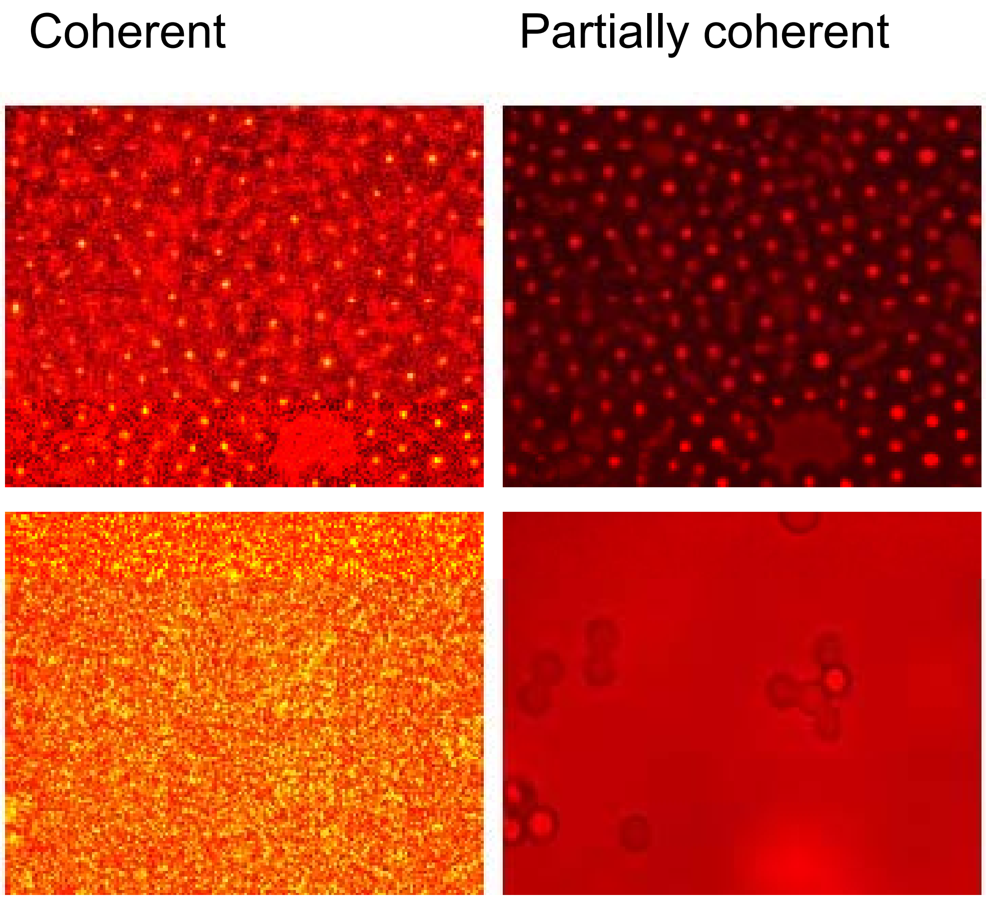


[J. A. Rodrigo & T. Alieva, Opt. Letters, 39 (2014)]

Partially coherent versus coherent illumination

- Speckle suppression



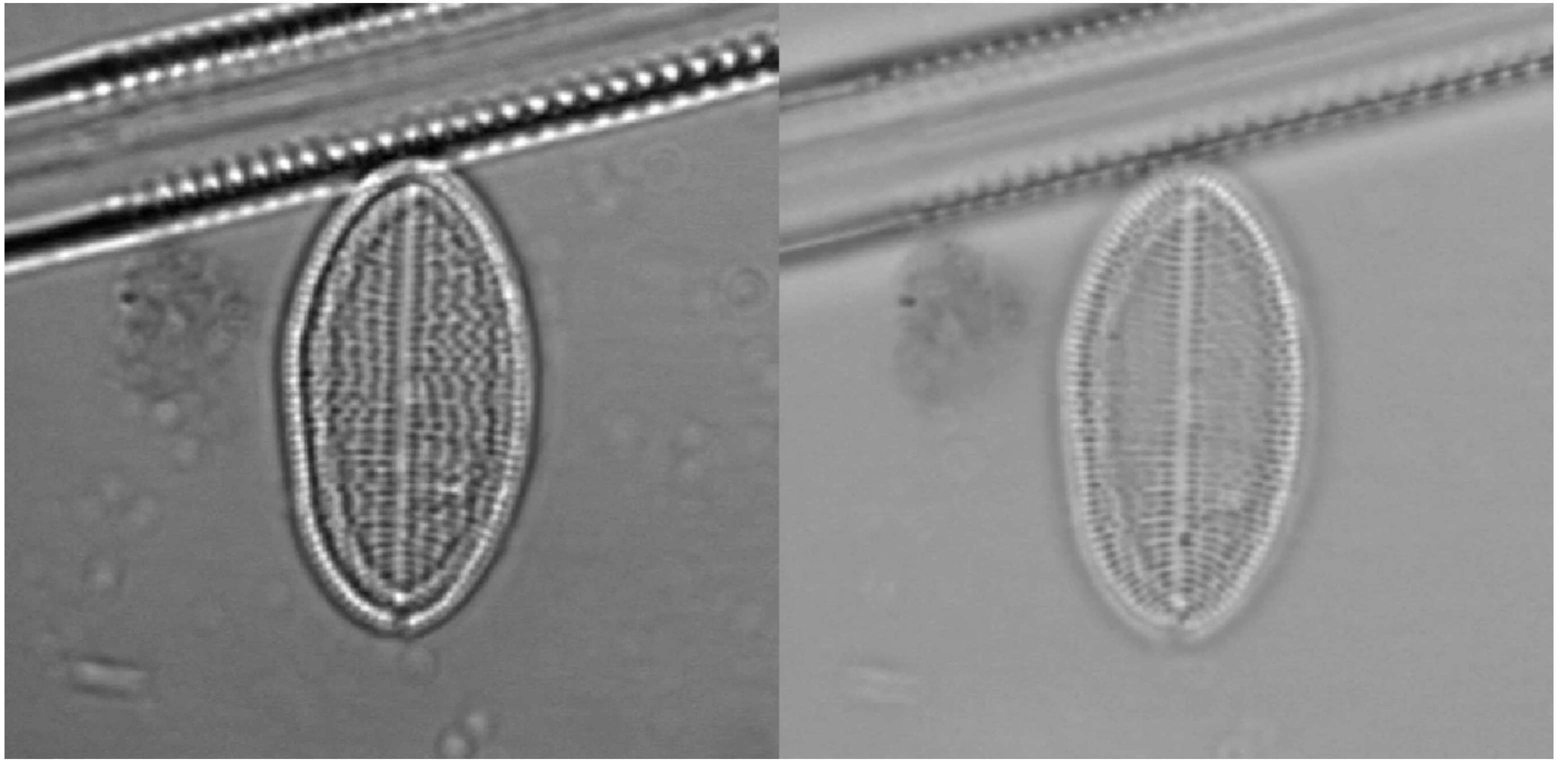


[J. Rodrigo and T. Alieva, Opt. Express 22 (2014)]

Partially coherent versus coherent illumination

- Optical sectioning: Diatom focusing

Coherent S=0.3 | Partially coherent S=0.7



[Courtesy J. Rodrigo and J. M. Soto , 2017]

What information we want to get from an image?

- Object form

  +

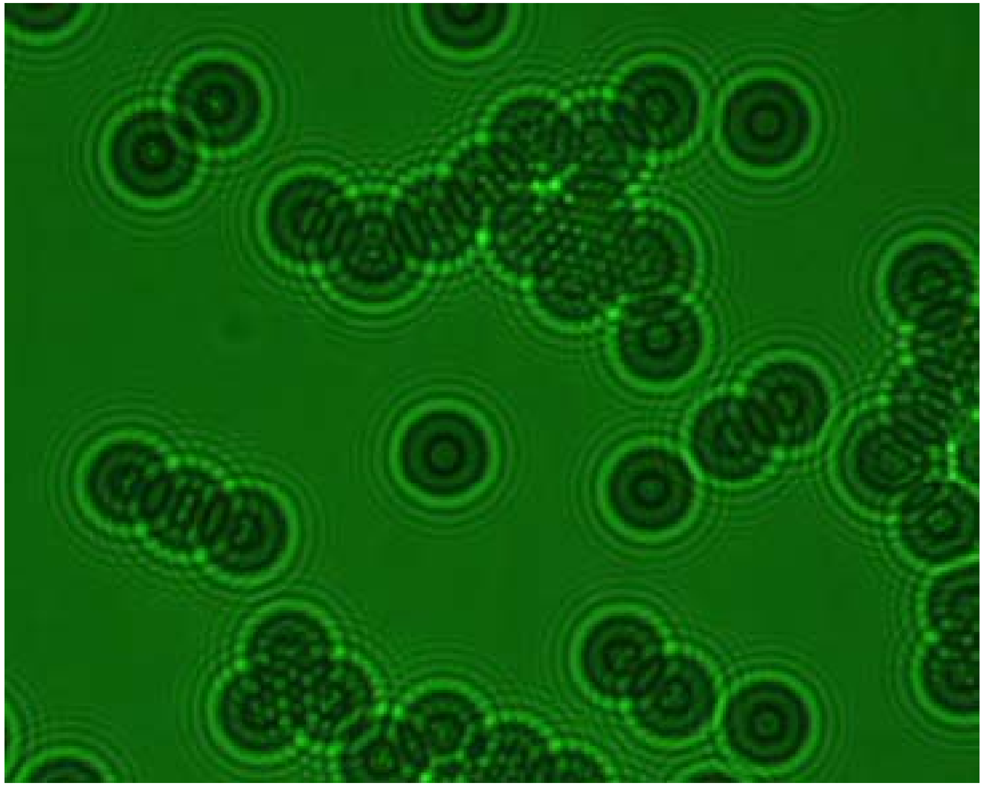
- Object size

  +

- Object composition

  =

- Quantitative imaging



Movie: 4,7 μm polystyrene spheres focusing
[J. A. Rodrigo & T. Alieva, Opt. Lett., 39 (2014)]

- Does an image of biological object provide directly this information?

Quantitative phase imaging

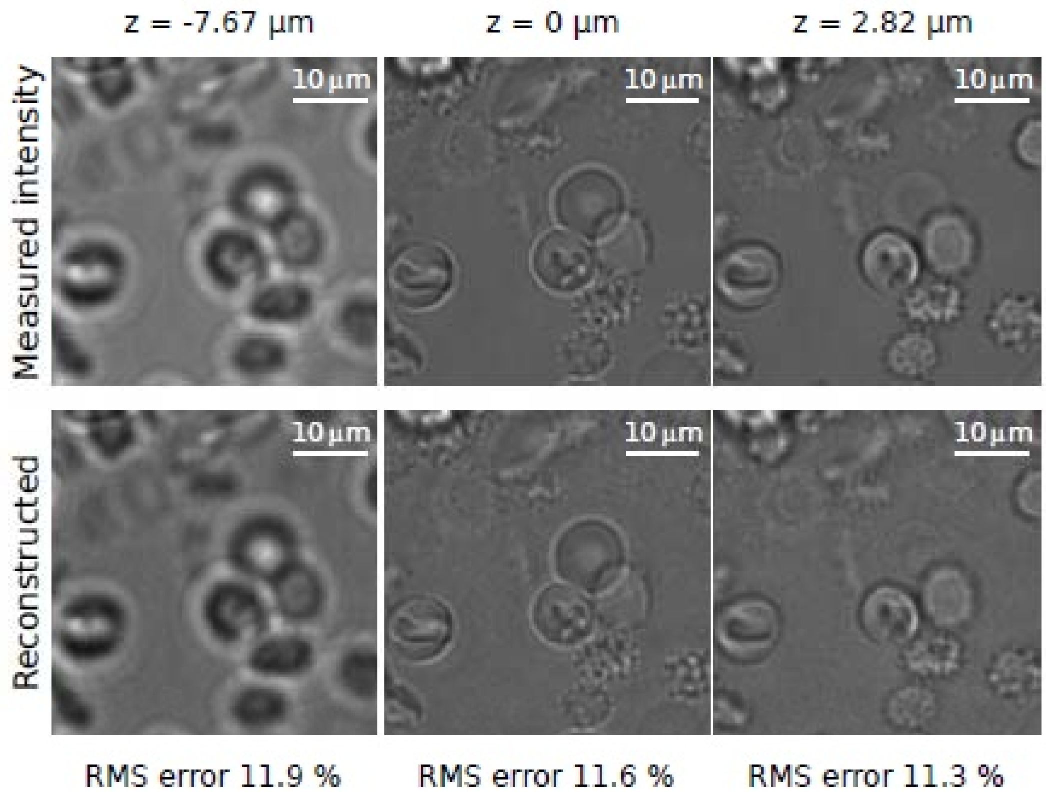
- Biomedical microscopic imaging: usually **bad absorption contrast**
- Specimen may be often treated as a **phase only object**
- But **only intensity distribution** is detectable
- How to recover the image phase?

  Using computational methods for its recovery!

- Application of image phase retrieval:
  - Digital refocusing
  - Information about specimen refractive index (thickness, optical potential, etc.)

Digital refocussing and object thickness

- Recovered phase of the image in one plane 3D Image is recovered calculating back/forward field propagation



J. A. Rodrigo & T. Alieva, Opt. Express, 22 (2014)

- Thickness of object

$$t(\mathbf{r}) = \int n_s(\mathbf{r}, z)dz$$

is recovered from phase (optical path difference profile) using eikonal approximation

$$\varphi_s(\mathbf{r}) = \int k(n_s(\mathbf{r}, z) - n_m)dz$$

➔ the object size >>λ

Phase retrieval methods

Coherent light

$$u(\mathbf{r}) = \left|u(\mathbf{r})\right| \exp\left[i\varphi(\mathbf{r})\right]$$

Measurements: intensity distributions

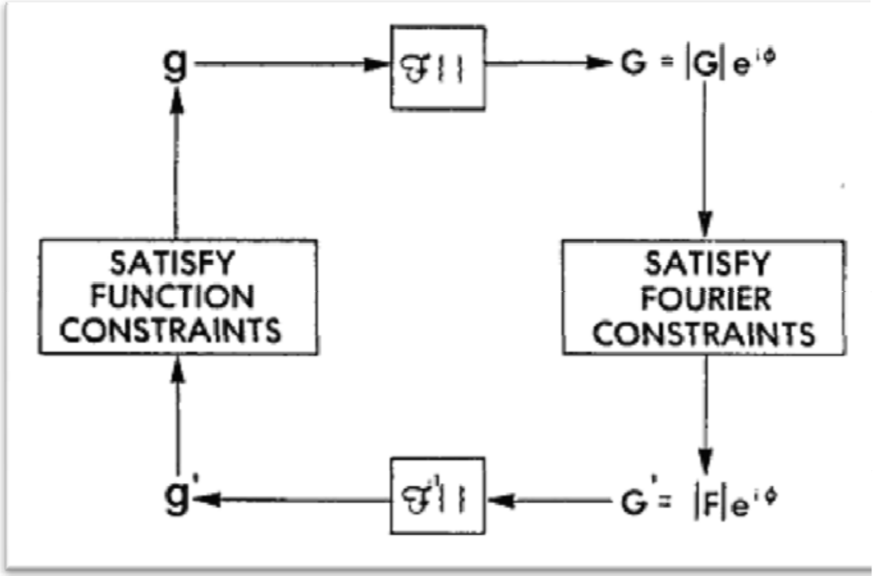
$$\left|u(\mathbf{r})\right|^2, \left|\tilde{u}_j(\mathbf{r})\right|^2, j = 1, 2, \ldots N$$

HOW TO RECOVER THE PHASE?

- Gerchberg-Saxton type algorithms
- Interferometry / Holography
- Transport-of-intensity equation
- Phase-space tomography

Iterative methods of phase retrieval

- Gerchberg - Saxton algorithm:
  Image intensity $|g(\mathbf{r})|^2$
  +
  Fourier power spectrum $|F(\mathbf{r})|^2$
  (= image intensity in conjugated plane)



- The process converges when

$$\|g - g_n\|^2 \approx \|g - g_{n-1}\|^2$$

R. W. Gerchberg and W. O. Saxton, Optik 35, 237 (1972);
J. R. Fienup, Appl. Opt. 21, 2758 (1982)

Generalized Gerchberg-Saxton methods

Other possible constraints provided phase diversity:

- Fresnel diffraction patterns
- Defocused images
- Diffraction patterns in asymmetric systems
- Object information (size, form, etc.)

Z. Zalevsky et al, Opt. Lett. 21, 842 (1996); L. Camacho et al, Opt. Exp. 18, 6755 (2010); J. A. Rodrigo, et al, Opt. Express 18,1510 (2010)

Paraxial approximation of Helmholtz equation

- Helmholtz equation: $\Delta E(\mathbf{r}) + k^2 E(\mathbf{r}) = 0$

- z is a beam propagation direction $E(\mathbf{r}) = u(\mathbf{r})\exp(ikz)$

$$\left[\Delta_\perp + i2k\frac{\partial}{\partial z}\right]u(\mathbf{r}) + \frac{\partial^2}{\partial z^2}u(\mathbf{r}) = 0$$

$$\left|\frac{\partial^2}{\partial z^2}u(\mathbf{r})\right| \ll \left|k\frac{\partial}{\partial z}u(\mathbf{r})\right| \qquad \left[\Delta_\perp + i2k\frac{\partial}{\partial z}\right]u(\mathbf{r}) = 0$$

- The angle between the wave vector $\mathbf{k}$ and z is small

Generalized Fresnel transforms

= Canonical integral transforms = ABCD transforms

- Propagation through a paraxial system is described by

$$u_o(\mathbf{r}_o) = \int u_i(\mathbf{r}_i) K_{\mathbf{T}}(\mathbf{r}_i, \mathbf{r}_o) d\mathbf{r}_i$$

$$\Gamma_o(\mathbf{r}_{1o}, \mathbf{r}_{2o}) = \iint \Gamma_i(\mathbf{r}_{1i}, \mathbf{r}_{2i}) K_{\mathbf{T}}(\mathbf{r}_{1i}, \mathbf{r}_{1o}) K_{\mathbf{T}}^*(\mathbf{r}_{2i}, \mathbf{r}_{2o}) d\mathbf{r}_{1i} d\mathbf{r}_{2i}$$

with kernel parameterized by real symplectic ray transformation matrix **T**

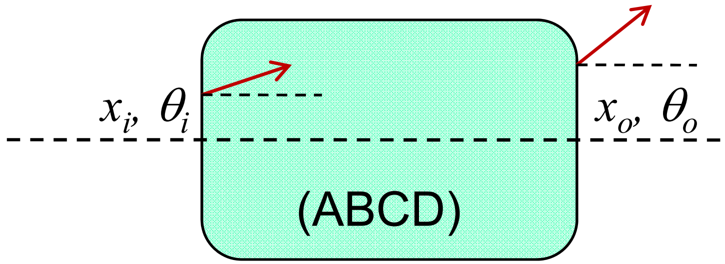
$$K_{\mathbf{T}}(\mathbf{r}_i, \mathbf{r}_o) = \begin{cases} \dfrac{1}{\sqrt{\det i\mathbf{B}}} \exp\left(i\pi\left[\mathbf{r}_i^t \mathbf{B}^{-1} \mathbf{A} \mathbf{r}_i - 2\mathbf{r}_i^t \mathbf{B}^{-1} \mathbf{r}_o + \mathbf{r}_o^t \mathbf{D} \mathbf{B}^{-1} \mathbf{r}_o\right]\right), & \det \mathbf{B} \neq 0 \\ \dfrac{1}{\sqrt{|\det \mathbf{A}|}} \exp\left(i\pi \mathbf{r}_o^t \mathbf{C} \mathbf{A}^{-1} \mathbf{r}_o\right) \delta\left(\mathbf{r}_i - \mathbf{A}^{-1} \mathbf{r}_o\right), & \mathbf{B} = 0 \end{cases}$$

S. A. Collins, J. Opt. Soc. Am. 60,1168 (1970);
M. Moshinsky and C. Quesne, J. Math. Phys. 12, 1772 (1971).

Ray transformation matrix

- Ray transformation matrix $\mathbf{T}$ connects the position $\mathbf{r}$ and the direction $\mathbf{p}$ of the ray in the input and output planes of an optical system

$$\begin{pmatrix} \mathbf{r}_o \\ \mathbf{p}_o \end{pmatrix} = \begin{bmatrix} \mathbf{A} & \mathbf{B} \\ \mathbf{C} & \mathbf{D} \end{bmatrix} \begin{pmatrix} \mathbf{r}_i \\ \mathbf{p}_i \end{pmatrix} = \mathbf{T} \begin{pmatrix} \mathbf{r}_i \\ \mathbf{p}_i \end{pmatrix}$$

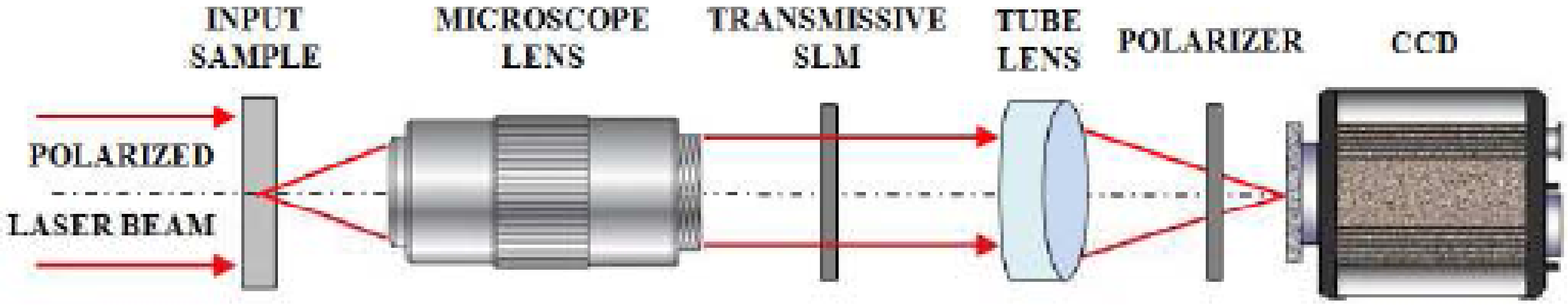


- Matrix $\mathbf{T}$ is symplectic and is characterized by only 10 parameters, det $\mathbf{T}$=1

$$\mathbf{J} = \mathbf{T}^t \mathbf{J} \mathbf{T}, \qquad \mathbf{J} = \begin{pmatrix} \mathbf{0} & \mathbf{I} \\ \mathbf{-I} & \mathbf{0} \end{pmatrix}$$

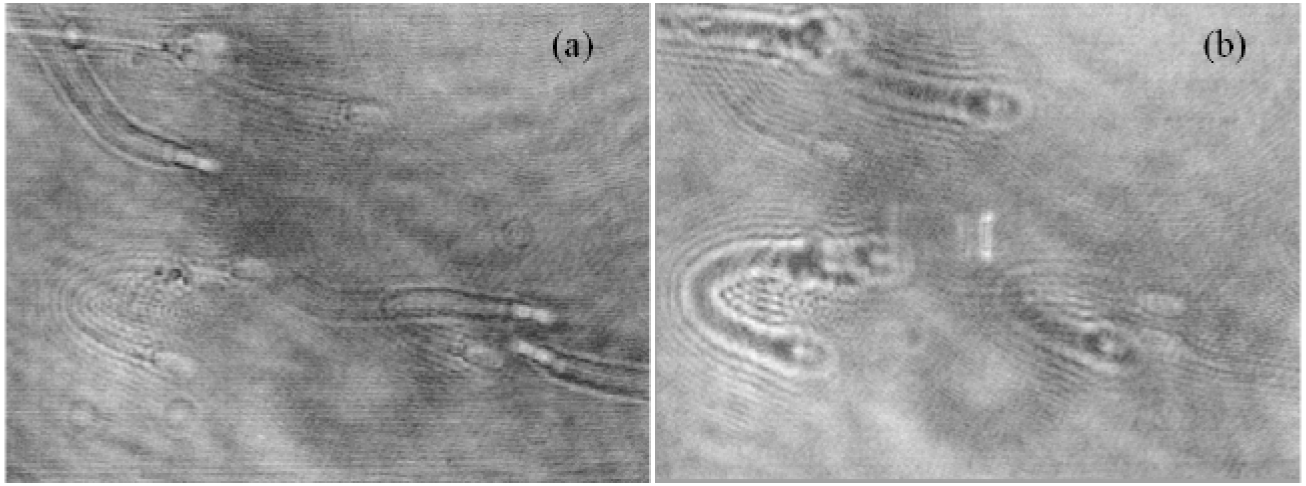
Quantitative phase microscopy using defocusing

- Spatial light modulator is used for implementation of digital lenses responsible for defocusing

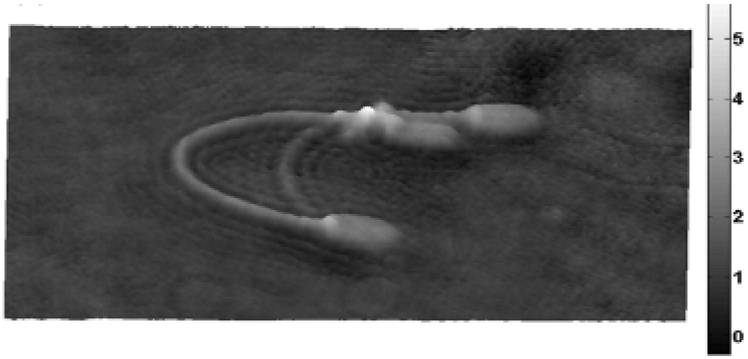


L. Camacho, V. Micó, Z. Zalevsky, and J. García, Opt. Exp. 18, 6755 (2010)

QPM using defocusing: Results



Two from 9 intensity images used for phase reconstruction



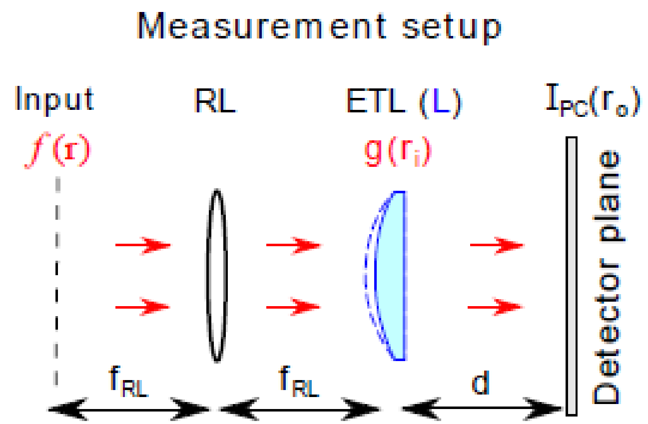
3D representations of swine sperm cells from unwrapped phase distribution (15 iteration cycles)

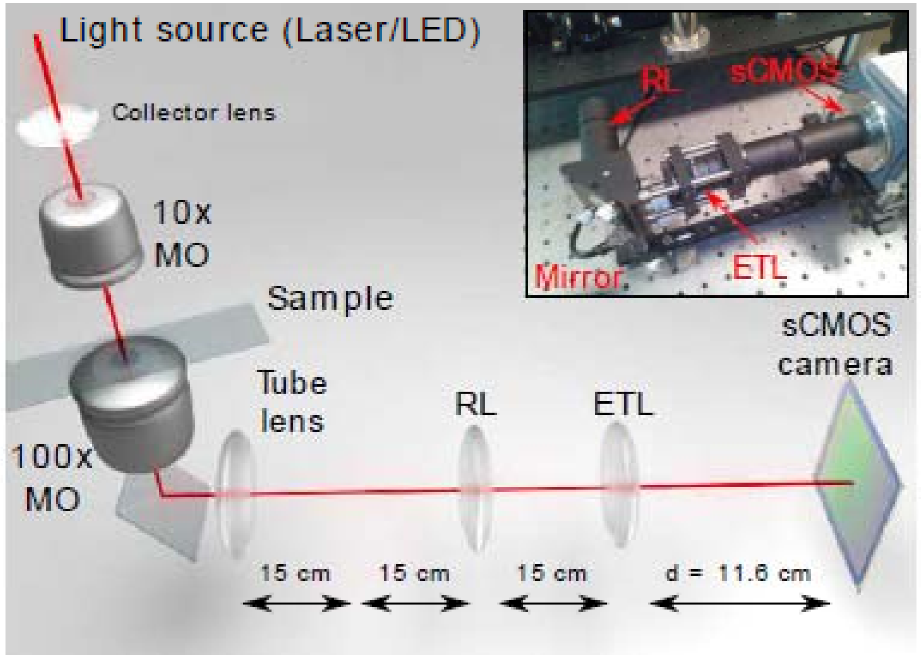
L. Camacho, V. Micó, Z. Zalevsky, and J. García, Opt. Exp. 18, 6755 (2010)

QPM using defocusing and partially coherent illumination: setup

- Electro-tunable lens (ETL) is used for defocusing
- Each image is measured in 10 ms
- Object's wavefield reconstruction in < 20 s

J. A. Rodrigo & T. Alieva, Opt. Express, 22 (2014)





QPM using defocusing and partially coherent illumination: Technique

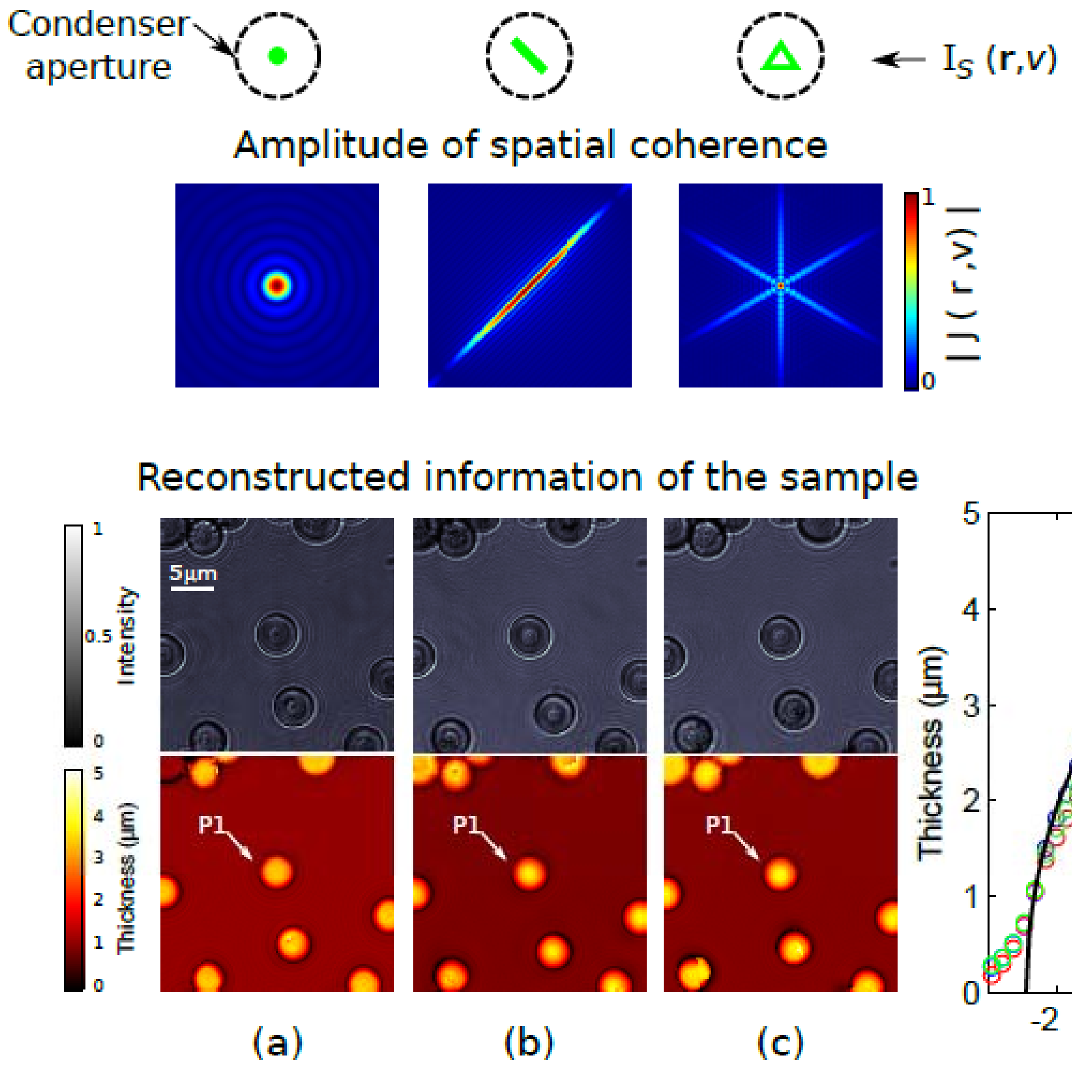
- The technique is based on

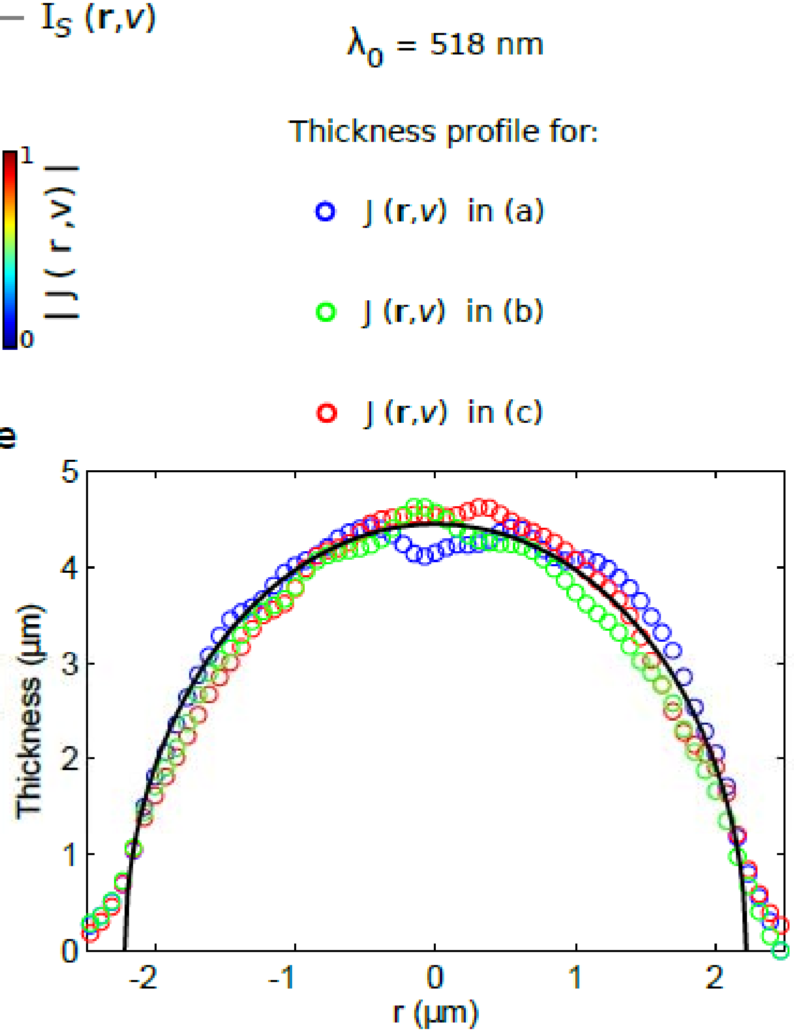
$$I_m^{PC}(\mathbf{r}) \propto \int I_m^{C}(\mathbf{r}') I_S\left[(\mathbf{r}-\mathbf{r}') s_m\right] d\mathbf{r}'$$

- $I_m^{PC}(\mathbf{r})$ is the measured image given for a given ETL focal distance f_m
- $I_m^{C}(\mathbf{r})$ is the intensity distribution for an ideal (speckle-free) spatially coherent illumination
- $I_S(\mathbf{r})$ is the light intensity distribution at the condenser back focal plane
- s_m is a scaling factor

J. A. Rodrigo & T. Alieva, Opt. Express, 22 (2014)

QPM with partially coherent illumination: Results





J. A. Rodrigo & T. Alieva, Opt. Letters, 39 (2014)

Transport-of-intensity equations (TIE)

- Paraxial approximation for Helmholtz equation, $\mathbf{r}=(x,y)$:

$$\left[i\frac{\partial}{\partial z}+\frac{\nabla_{\mathbf{r}}^{2}}{2k}\right]u(\mathbf{r},z)=0$$

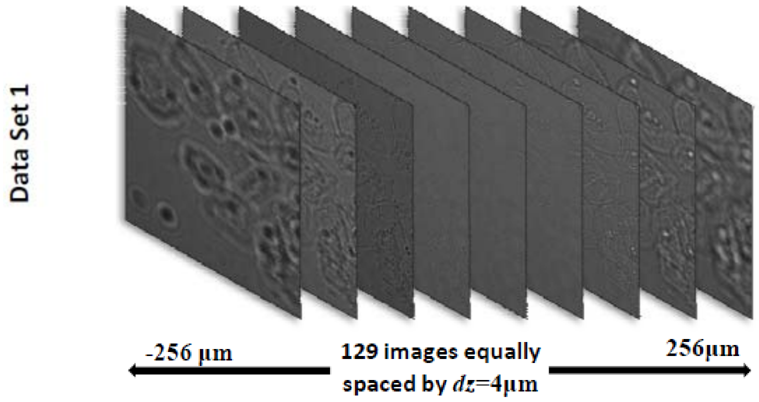
- Phase reconstruction of $u(\mathbf{r},z)=\sqrt{I(\mathbf{r},z)}\exp(i\varphi(\mathbf{r},z))$ from close Fresnel diffraction patterns

$$k\frac{\partial}{\partial z}I(\mathbf{r},z)=-\nabla_{\mathbf{r}}\cdot\left[\nabla_{\mathbf{r}}\varphi(\mathbf{r},z)I(\mathbf{r},z)\right]$$

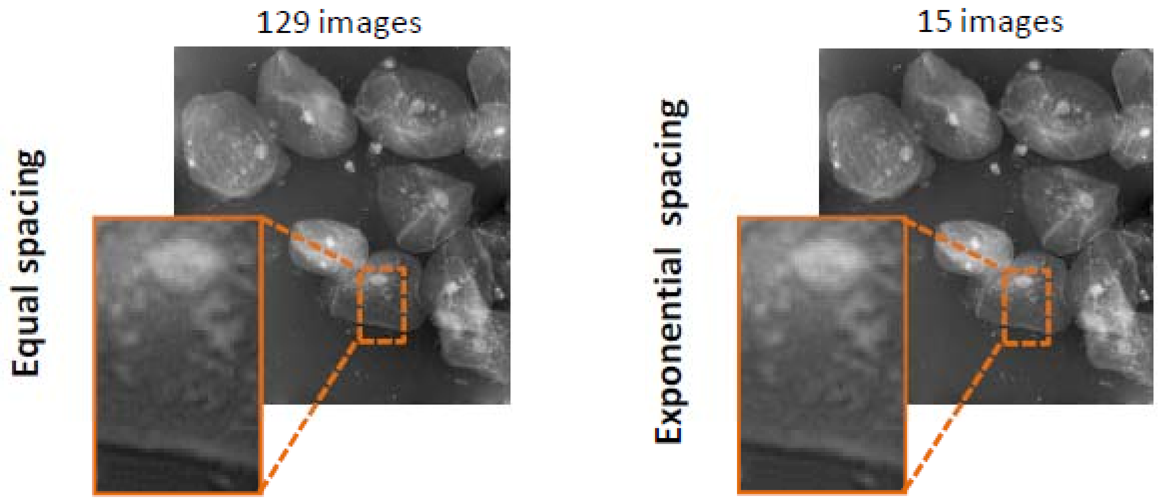
- In conventional optical microscopy several defocused images are used

M. R. Teague, J. Opt. Soc. Am. 73, 1434 (1983); N. Streibl, J. Opt. Soc. Am. A 2, 121 (1985); T. E. Gureyev, A. Roberts, K. A. Nugent, J. Opt. Soc. Am. A 12, 1942 (1995); A. Barty et al, Opt. Lett. 23, 817 (1998); D. Paganin & K. A. Nugent, Phys. Rev. Lett. 80, 2586 (1998).

TIE phase retrieval: Results

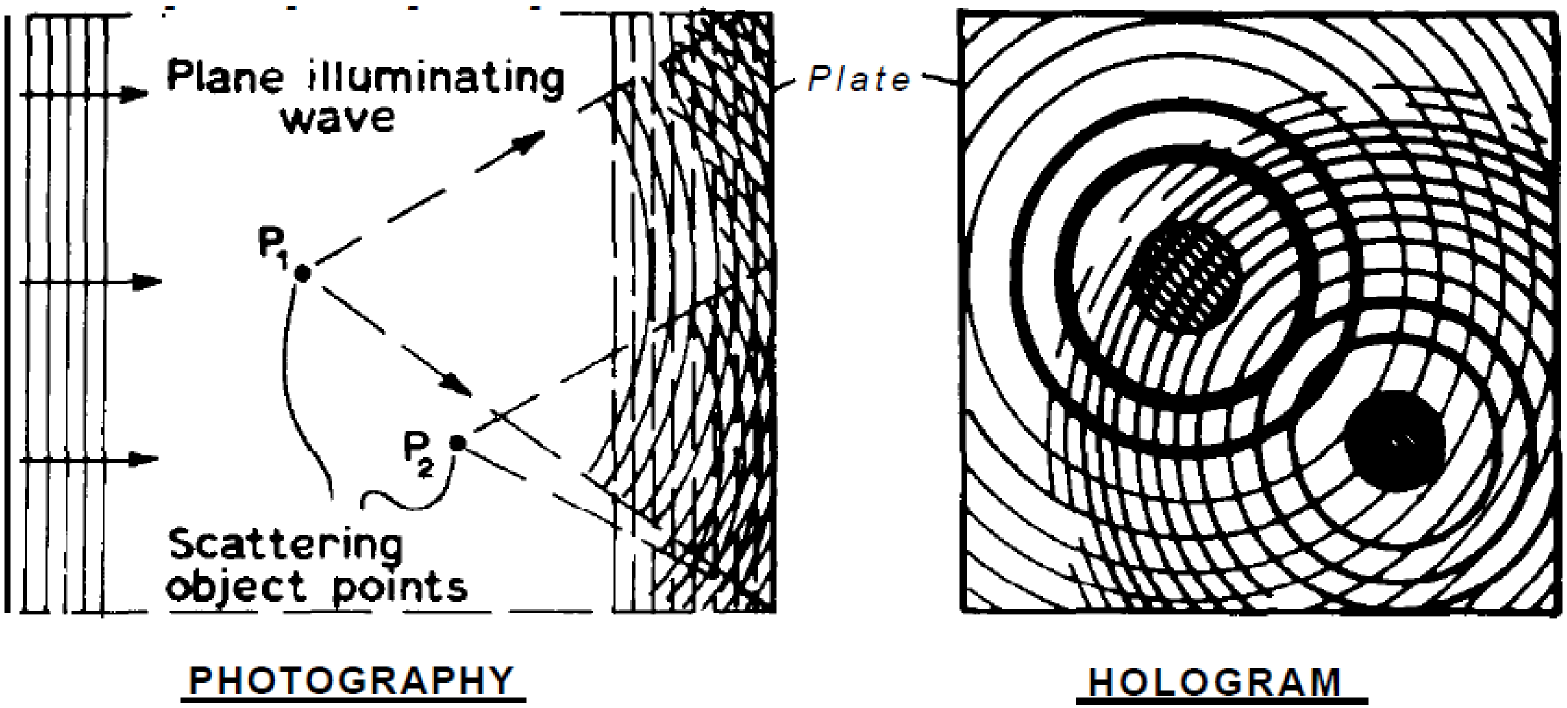


Human cheek cells, 20x, NA=0.5
λ=650nm, $\Delta\lambda$=10nm



Images from Z. Jingshan el al. Opt. Express 22, 10661 (2014);
L. Tian, J. C. Petruccelli, and G. Barbastathis, Opt. Lett. 37, 4131 (2012); J. C. Petruccelli et al, Opt. Express 21, 14430 (2013); L. Waller, et. al, Opt. Express 18, 12552 (2010).

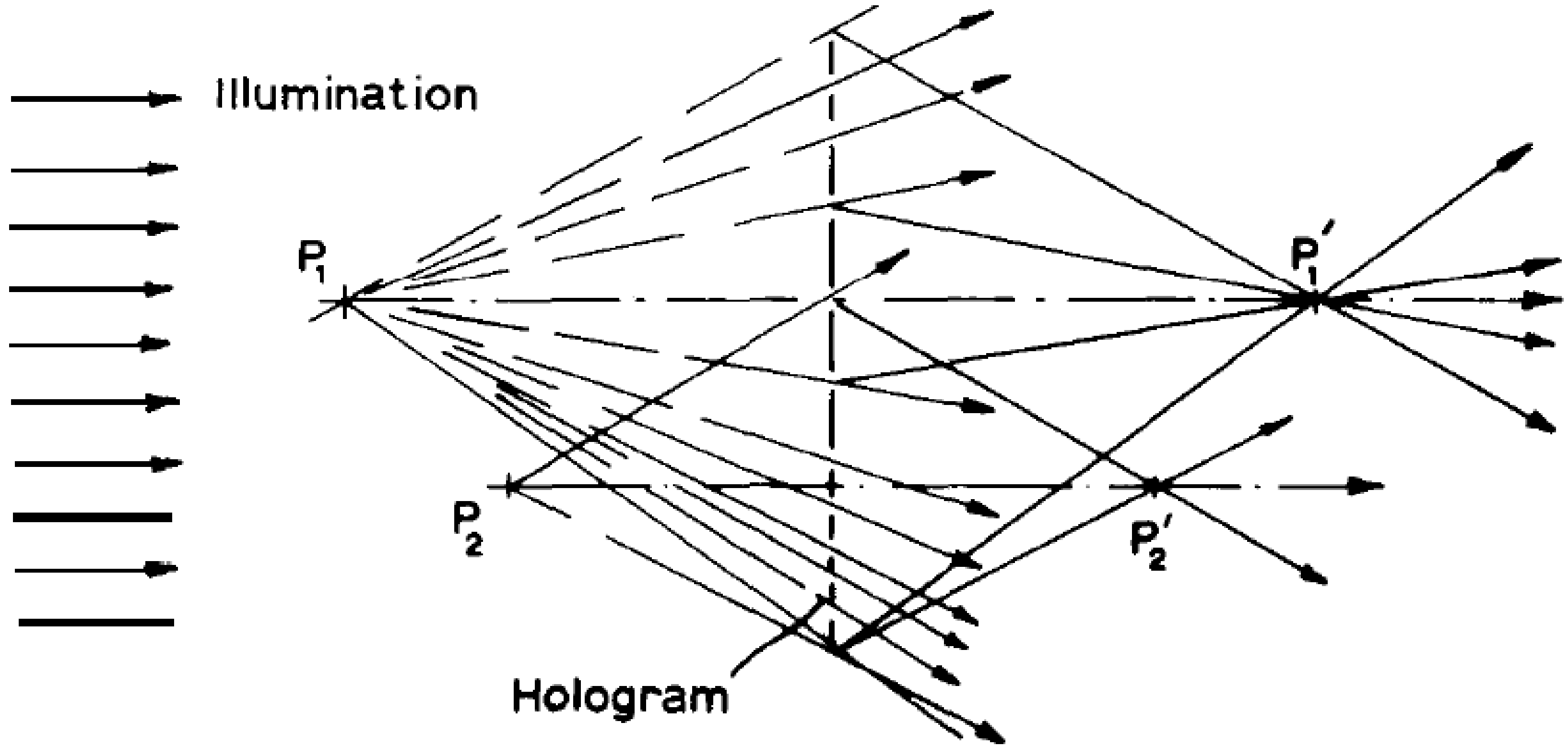
Gabor picture of image formation



From Dennis Gabor Nobel Prize Lecture, December 11, 1971

$$I(\mathbf{r}) = |u(\mathbf{r})|^2 = \left(u_O(\mathbf{r}) + u_R(\mathbf{r})\right)\left(u_O(\mathbf{r}) + u_R(\mathbf{r})\right)^*$$
$$= |u_R(\mathbf{r})|^2 + |u_O(\mathbf{r})|^2 + u_O^*(\mathbf{r})u_R(\mathbf{r}) + u_O(\mathbf{r})u_R^*(\mathbf{r})$$

Gabor picture of object wave recovery

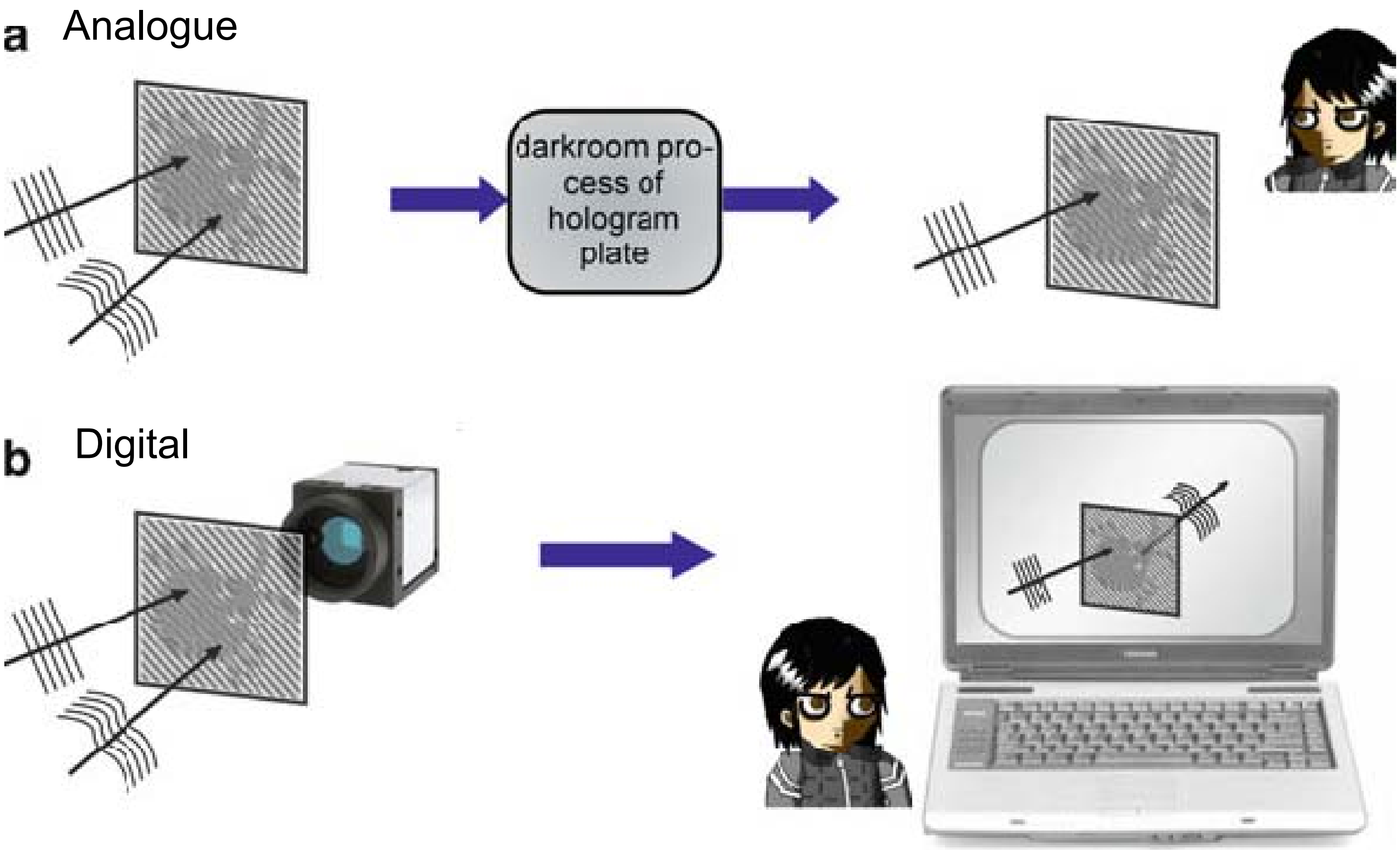


From Dennis Gabor Nobel Prize Lecture, December 11, 1971

$$u_R(\mathbf{r})I(\mathbf{r}) = u_R(\mathbf{r})\left|u_R(\mathbf{r})\right|^2 + u_R(\mathbf{r})\left|u_O(\mathbf{r})\right|^2 + u_O^{*}(\mathbf{r})u_R^2(\mathbf{r}) + u_O(\mathbf{r})\left|u_R(\mathbf{r})\right|^2$$

Digital holography

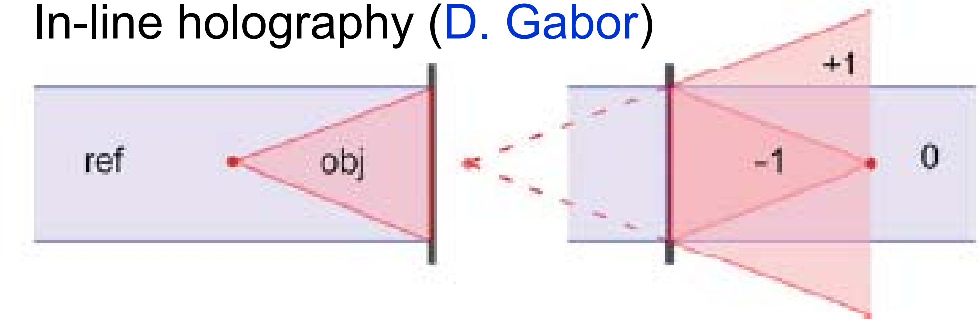
- Numerical hologram reconstruction



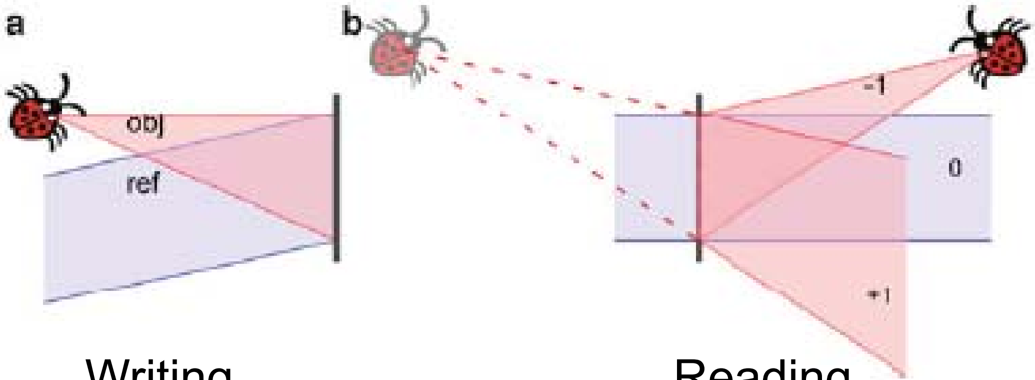
Images from M. K. Kim, Digital Holographic Microscopy, Springer (2011)

In-line and Off-line holography

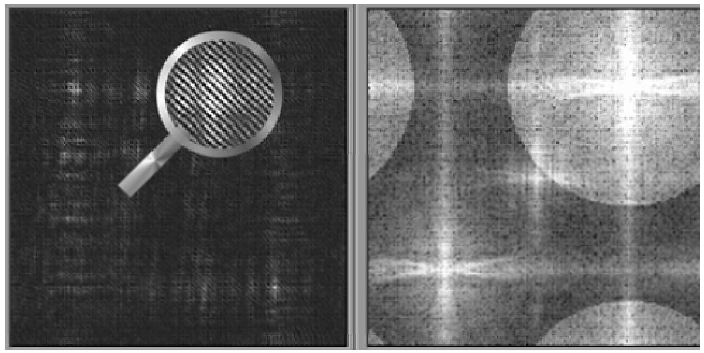
In-line holography (D. Gabor)



Off-line holography (E. Leith and J. Upatnieks)



Writing Reading



Images from M. K. Kim, Digital Holographic Microscopy, Springer (2011)

Digital holographic microscopy research:

P. Marquet et al, Opt. Lett. 30, 468 (2005); F. Charrière et al , Opt. Lett. 31, 178 (2006); G. Popescu et al, Opt. Lett. 31, 775 (2006); B. Kemper & G. von Bally, Appl. Opt. 47, A52 (2008); V. Micó et al, Opt. Express 16, 19260 (2008).

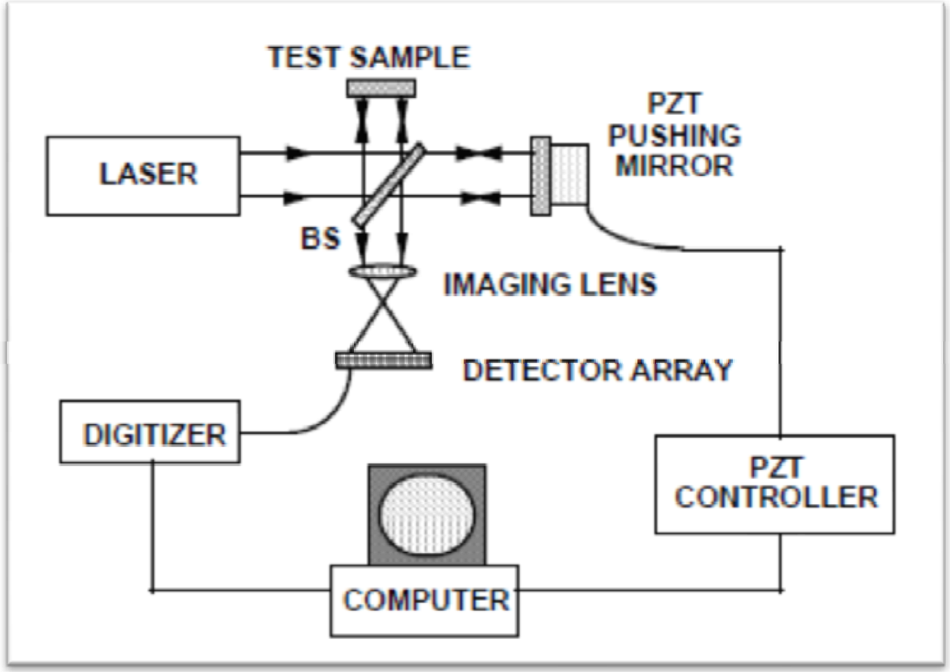
Phase-shifting digital holography

- Superposition of sample beam

  $$u_O(\mathbf{r}) = |u_O(\mathbf{r})| \exp[i\varphi_O(\mathbf{r})]$$

  with reference one

$$I_n(\mathbf{r}) = |u_O(\mathbf{r})|^2 + |u_R(\mathbf{r})|^2 + 2|u_O(\mathbf{r})u_R(\mathbf{r})|\cos[\varphi_O(\mathbf{r}) + \alpha_n]$$



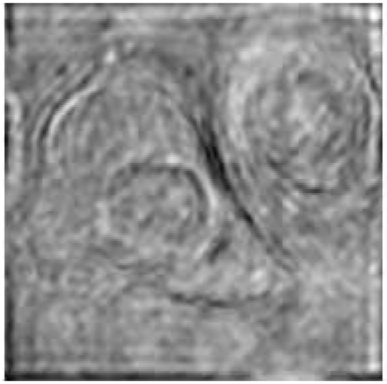
- Controlable change of the reference beam phase

$$\alpha_n = \pi n / 2 \rightarrow I_n(\mathbf{r})$$

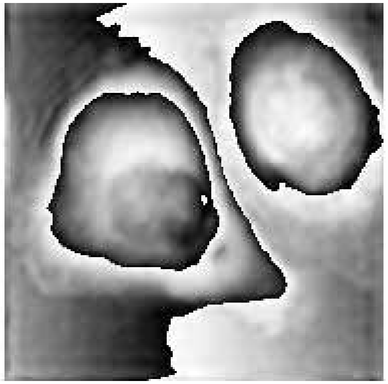
$$\tan[\varphi_O(\mathbf{r})] = \frac{I_4(\mathbf{r}) - I_2(\mathbf{r})}{I_1(\mathbf{r}) - I_3(\mathbf{r})}$$

I. Yamaguchi and T. Zhang, Opt. Lett. 22, 1268 (1997)

Digital holographic microscopy: example

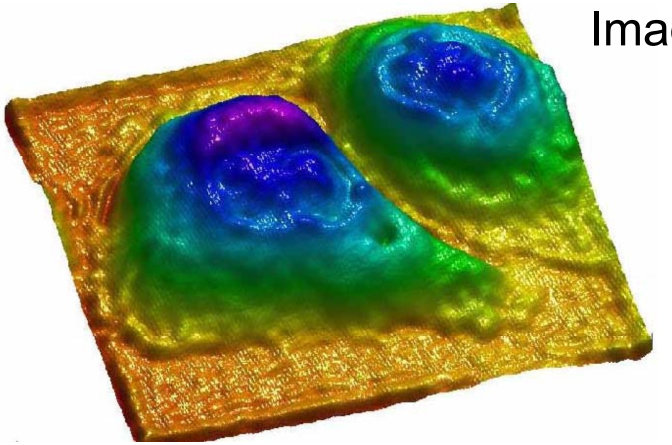


Hologram



Phase image

Unwrapped phase Image

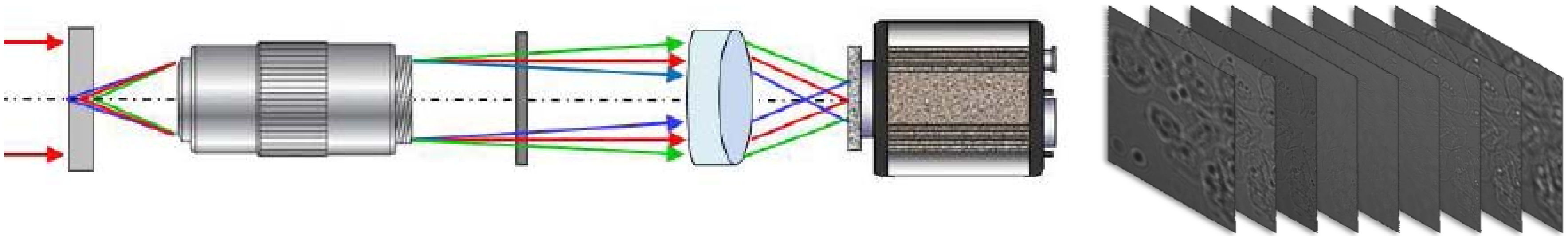


DHM (off-line hologram) of SKOV3 ovarian cancer cells (60 x60 μm^2, 404 x404 pixels). The phase profile is accurate to about 30 nm of optical thickness.

Images from C. J. Mann et al, Opt. Express 13, 8693 (2005).

Image - object relation

- Does image phase recovery resolve the QI problem?
- Phase of the image contains entanglement data of 3D object: Every 2D image from a series obtained by refocusing contains information from other slices



- Microscope transfer function has to be taken into account in image interpretation
- There are different approaches for 3D object information recovery

Mathematical formalizm of 3D imaging

- Helmholtz equation (scalar cuasi-monochromatic approximation)

$$\Delta u(\mathbf{r}) + k^2 n^2(\mathbf{r}) u(\mathbf{r}) = 0$$

$$\Delta u(\mathbf{r}) + k^2 n_0^2 u(\mathbf{r}) = k^2 \left[n_0^2 - n^2(\mathbf{r}) \right] u(\mathbf{r})$$

- Optical potential: $V(\mathbf{r}) = k^2 \left[n_0^2 - n^2(\mathbf{r}) \right]$
- Propagation in homogeneous medium:

$$\Delta u(\mathbf{r}) + k^2 n_0^2 u(\mathbf{r}) = 0$$

- Micro-objects are treated as perturbations of refractive index $n(\mathbf{r})$ which is a complex-valued function, $n_0(\mathbf{r})$ is a refractive index of surrounging medium, $k=\omega/c$, $\mathbf{r}=(x,y,z)$

Several approximations for Helmholtz equation solution

- Paraxial approximation
- Eikonal approximation
- Born (Rayleigh) approximation (small perturbation method):

  $$u = u_0 + u_1 + u_2 + \ldots$$

  is linear with respect to complex field amplitud

- Rytov approximation (slow (smooth) perturbation method, multiple forward scattering):

  $$u = \exp(\psi_0 + \psi_1 + \psi_2 + \ldots)$$

  is nonlinear and multiplicative with respect to complex field amplitud

Geometric optics approximation

- Conditions:
  - Smoth changes on the wavelength $\lambda \left| \nabla n^2(\mathbf{r}) \right| \ll n^2(\mathbf{r})$
  - Does not take into account diffraction

- Debay approximation

$$u(\mathbf{r}) = \left(A_0(\mathbf{r}) + \frac{A_1(\mathbf{r})}{ik} + \frac{A_2(\mathbf{r})}{(ik)^2} + \dots \right) \exp\left[ik\varphi(\mathbf{r}) \right]$$

- Solution:

Eikonal approximation:

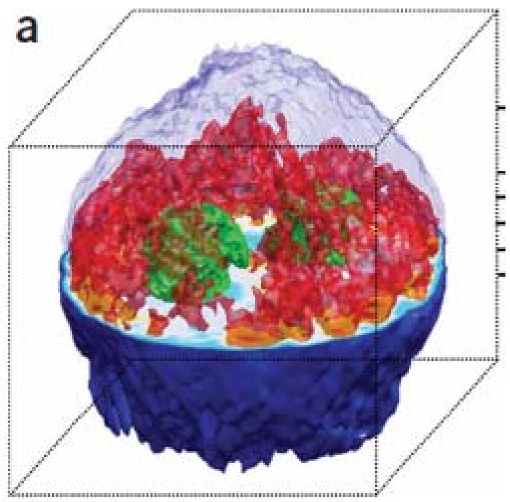
$$\nabla \varphi = n(\mathbf{r})$$

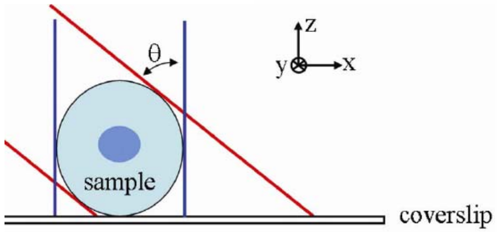
$$\Rightarrow \quad \varphi = \int_0^z n(z)dz$$

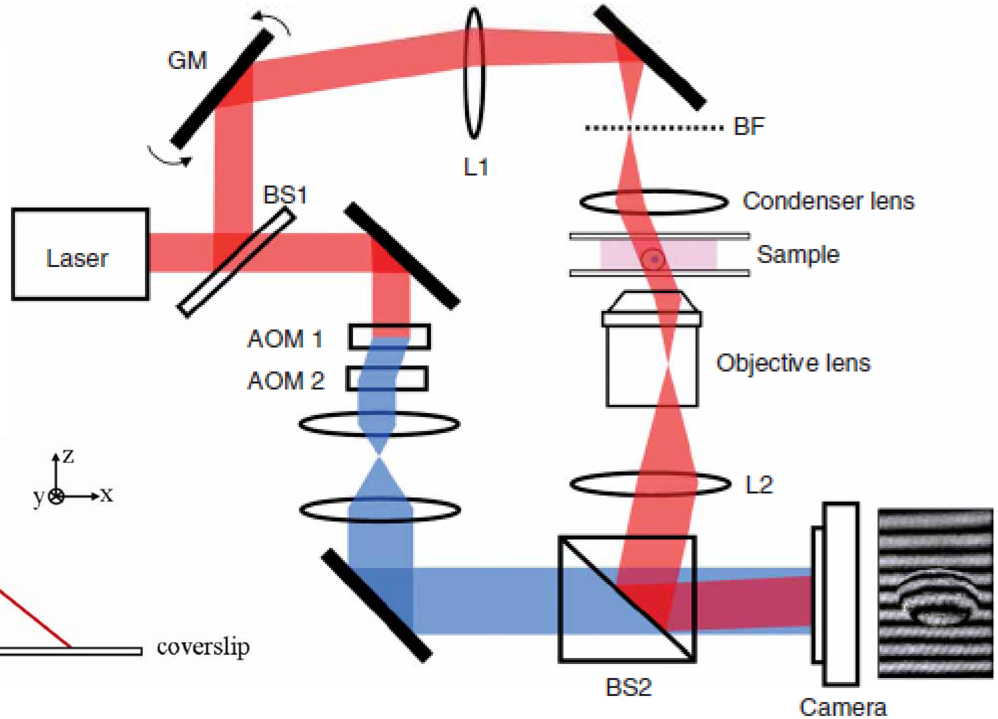
$$2\nabla \varphi \nabla A_0 + A_0 \Delta \varphi = 0$$
$$2\nabla \varphi \nabla A_1 + A_1 \Delta \varphi = -\Delta A_0$$
$$\dots$$
$$2\nabla \varphi \nabla A_n + A_n \Delta \varphi = -\Delta A_{n-1}$$

Eikonal approximation: Applications

- Object thickness estimation
- Phase tomography (similar to CT)







$n(\mathbf{r})$ of a HeLa cell. Nucleoli are colored green n =1.375–1.385 and parts of cytoplasm with n>1.36 are colored red. The side of the cube is 20 μm.

81 phase images (4 holograms per image) are recorded for sample illumination angles θ = -60 to +60 degrees in steps of 1.5 degrees

Images from W. Choi et al Nature Meth. 1 (2007)

First order Born approximation

$$u(\mathbf{r}) = u_0(\mathbf{r}) + u_1(\mathbf{r})$$

$$u_1(\mathbf{r}) = -\int G(\mathbf{r},\mathbf{r}')V(\mathbf{r}')u_0(\mathbf{r}')d\mathbf{r}'$$

where $G(\mathbf{r},\mathbf{r}')$ is a Green's function (3D field distribution from a point source). $\Delta G(\mathbf{r},\mathbf{r}') + k^2 n_0^{\,2} G(\mathbf{r},\mathbf{r}') = \delta(\mathbf{r},\mathbf{r}')$

- Conditions for the first order approximation:
  - Weak scattering (magnitude of the scattering light << magnitude of the incident light)
  - Only the undiffracted light and its interference with once-diffracted light are considered.
- The calculation of u_1 is similar to the problem of calculation of field created by independent sources.

Coherent diffraction tomography (2π-DHM)

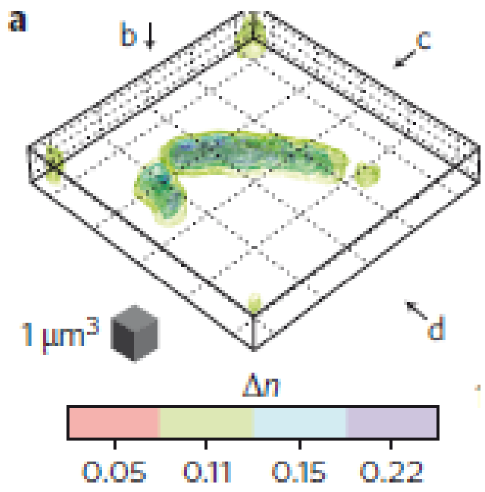
- For shift-invariant systems, $G(\mathbf{r}-\mathbf{r}')$, the $V(\mathbf{r})$ is recovered by deconvolution

$$u_1(\mathbf{r}) = -\int G(\mathbf{r}-\mathbf{r}')V(\mathbf{r}')u_0(\mathbf{r}')d\mathbf{r}'$$

$$\mathrm{FT}[u_1(\mathbf{r})] = -\mathrm{FT}[V(\mathbf{r})u_0(\mathbf{r})] \times \mathrm{CTF}$$

- FT stands for 3D Fourier Transform; CTF=FT[$G(\mathbf{r})$] is a coherence transfer function

- Deconvolution is a challenging task: different regularization methods are applied



$\Delta n(\mathbf{r})$ of bacteria E. coli

NA=1.4, 240 holograms in 18 s were recorded. Phase images were calculated using Fresnel reconstruction. Experimental CTF was applied for deconvolution.

Images from Y. Cotte, Nature Photon. 7, 113 (2013)

Coherence transfer function

- Green's function for free space

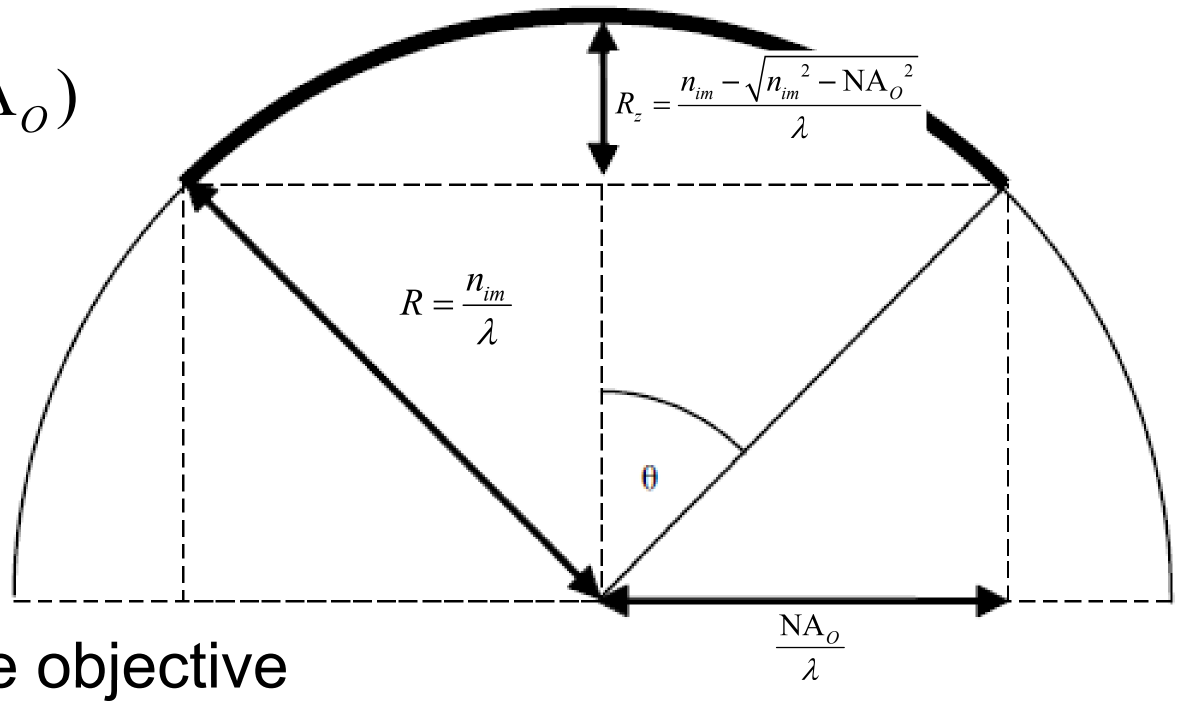
$$g(\mathbf{r}) = \frac{1}{4\pi|\mathbf{r}|}\exp(ikn_0|\mathbf{r}|)$$

- CTF approximation:

$$\mathrm{CTF}(\mathbf{R}) = G(\mathbf{R}) = \mathrm{FT}\left[g(\mathbf{r})\right](\mathbf{R}) \times P(\mathbf{R}_\perp) \times U(R_z)$$

$$P(\mathbf{R}_\perp) = \mathrm{circ}(\mathbf{R}_\perp \lambda / \mathrm{NA}_O)$$

$$U(R_z) = \begin{cases} 1 & R_z \geq 0 \\ 0 & R_z < 0 \end{cases}$$



- Oblique illumination

changes the frequency content accepted by the objective

First order Born approximation for partially coherent illumination

- Equation for mutual intensity $\Gamma(\mathbf{r}_1,\mathbf{r}_2)=\left\langle u(\mathbf{r}_1)u^*(\mathbf{r}_2)\right\rangle$

$$\begin{aligned}\Gamma(\mathbf{r}_1,\mathbf{r}_2)=\Gamma_0(\mathbf{r}_1,\mathbf{r}_2)&\\ +\int G(\mathbf{r}_1-\mathbf{r}_1')V(\mathbf{r}_1')\Gamma_0(\mathbf{r}_1',\mathbf{r}_2)d\mathbf{r}_1'&\\ +\int G^*(\mathbf{r}_2-\mathbf{r}_2')V^*(\mathbf{r}_2')\Gamma_0(\mathbf{r}_1,\mathbf{r}_2')d\mathbf{r}_2'&\end{aligned}$$

- Intensity for coherent illumination $\Gamma_0(\mathbf{r}_1,\mathbf{r}_2)=u_0(\mathbf{r}_1)u_0^*(\mathbf{r}_2)$

$$I(\mathbf{r})=\Gamma(\mathbf{r},\mathbf{r})=I_0(\mathbf{r})+u_0^*(\mathbf{r})u_1(\mathbf{r})+u_0(\mathbf{r})u_1^*(\mathbf{r})$$

- We obtain Gabor holography expression without the term

$I_1(\mathbf{r})=u_1(\mathbf{r})u_1^*(\mathbf{r})$ which is of the second-order approximation

N. Streibl, J. Opt. Soc. Am. A 2, 121 (1985)

3D imaging with partially coherent illumination

- Optical potential expansion on real (phase) P and imaginary (absorption) A parts: $V(\mathbf{r}) = P(\mathbf{r}) + iA(\mathbf{r})$

- 3D FT of the 3D intensity distribution

$$I(\mathbf{R}) = B\delta(\mathbf{R}) + A(\mathbf{R})H_A(\mathbf{R}) + P(\mathbf{R})H_p(\mathbf{R})$$

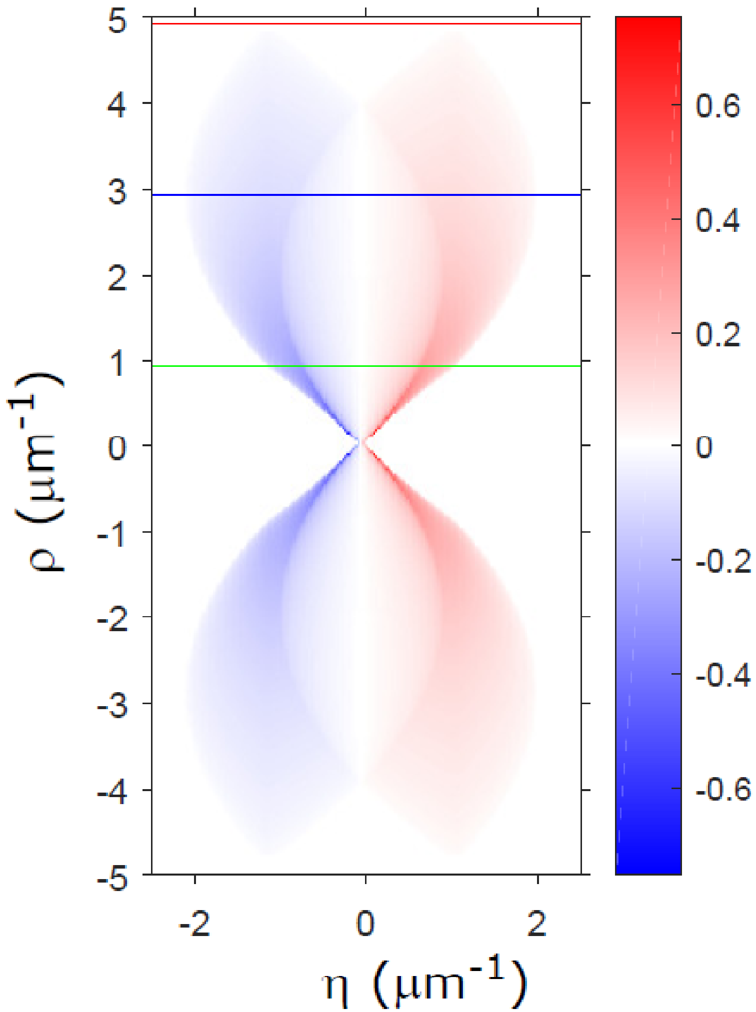
$$H_A(\mathbf{R}) = i\int S(\mathbf{R}')\left[G(\mathbf{R}+\mathbf{R}') - G^*(\mathbf{R}'-\mathbf{R})\right]d\mathbf{R}'$$
$$H_P(\mathbf{R}) = \int S(\mathbf{R}')\left[G(\mathbf{R}+\mathbf{R}') + G^*(\mathbf{R}'-\mathbf{R})\right]d\mathbf{R}'$$
$$B = \int S(\mathbf{R}')d\mathbf{R}'$$

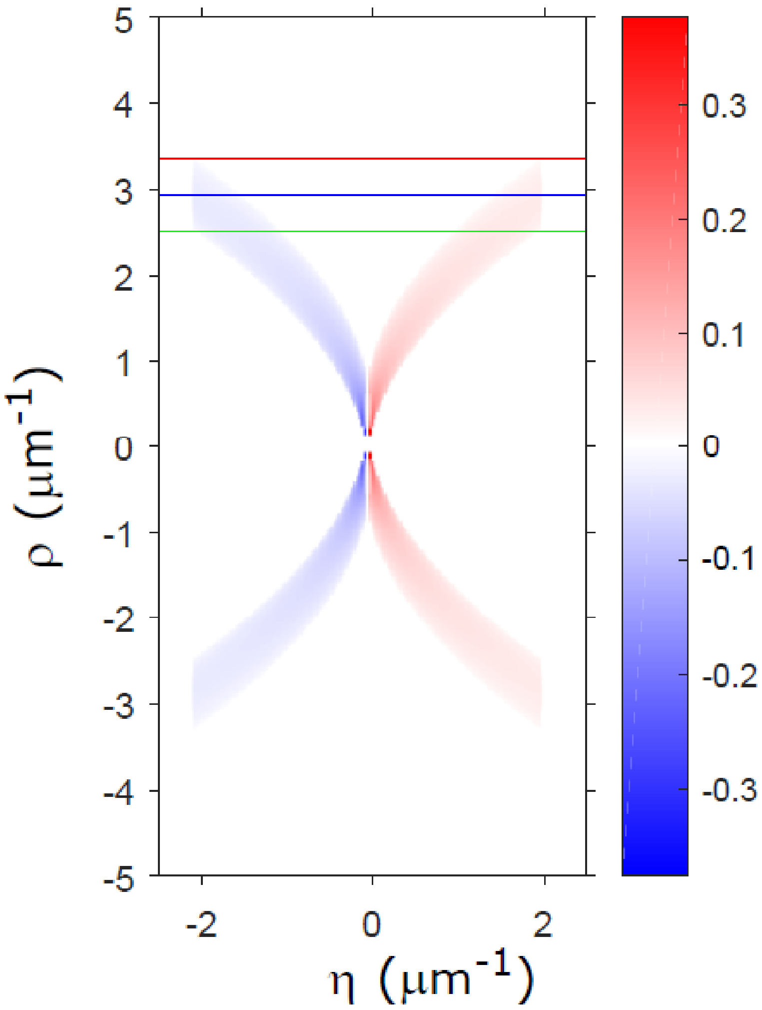
where $H_A(\mathbf{R})$ and $H_P(\mathbf{R})$ are absorption and phase transfer functions, $S(\mathbf{R})$ is intensity (incoherent source) over condenser aperture

N. Streibl, J. Opt. Soc. Am. A 2, 121 (1985); C.J.R. Sheppard & X. Mao, J. Opt. Soc. Am. A 6, 1260(1989); M. H. Jenkins & T. K. Gaylord, Appl. Opt. 54, 8566, 9213 (2015)

3D-Phase Optical Transfer Function (POTF)

$S = 0.68$ $\quad NA_O = 1.4 \quad$ $S = 0.14$





[Courtesy J. Rodrigo and J. M. Soto , 2017]

Rytov approximation

- The solution in the form

$$u(\mathbf{r}) = a(\mathbf{r})\exp\left[i\varphi(\mathbf{r})\right] = \exp\left[\psi(\mathbf{r})\right]$$

where $\psi(\mathbf{r}) = \ln\left[a(\mathbf{r})\right] + i\varphi(\mathbf{r})$

- Helmholtz equation: $\nabla^2\psi(\mathbf{r}) + \left[\nabla\psi(\mathbf{r})\right]^2 + k^2 {n_0}^2 = V(\mathbf{r})$

- Incident field: solution for $V(\mathbf{r})$=0 $\quad u_0(\mathbf{r}) = \exp\left[\psi_0(\mathbf{r})\right]$

- First approximation $\psi = \psi_0 + \psi_1$

$$\nabla^2\psi_1(\mathbf{r}) + 2\nabla\psi_0(\mathbf{r})\nabla\psi_1(\mathbf{r}) = -\left[\nabla\psi_1(\mathbf{r})\right]^2 + V(\mathbf{r})$$

$$(\nabla^2 + k^2 {n_0}^2)\left[u_0(\mathbf{r})\psi_1(\mathbf{r})\right] = \left[-\left[\nabla\psi_1(\mathbf{r})\right]^2 + V(\mathbf{r})\right]u_0(\mathbf{r})$$

S. M. Rytov, Izv. AN SSSR, 2, 223 (1937).

First order Rytov approximation

$$\psi_1(\mathbf{r}) = \frac{1}{u_0(\mathbf{r})} \int G(\mathbf{r},\mathbf{r}') \left[[\nabla \psi_1(\mathbf{r})]^2 - V(\mathbf{r}) \right] u_0(\mathbf{r}') d\mathbf{r}'$$

- Solution in the first iteration of this equation

$$[\nabla \psi_1(\mathbf{r})]^2 - V(\mathbf{r}) \approx -V(\mathbf{r})$$

$$\psi_1^{(0)}(\mathbf{r}) = -\frac{1}{u_0(\mathbf{r})} \int G(\mathbf{r},\mathbf{r}') V(\mathbf{r}) u_0(\mathbf{r}') d\mathbf{r}'$$

- First order Rytov approximation $u(\mathbf{r}) = u_0(\mathbf{r}) \exp\left[\psi_1^{(0)}(\mathbf{r}) \right]$

$$= \quad u_0(\mathbf{r}) \exp\left[\frac{u_1^{(\mathrm{Born})}(\mathbf{r})}{u_0(\mathbf{r})} \right]$$

- Conditions:
  - Slow changes of refractive index on a scale of λ
  - Multiple forward scattering is taken into account

Relations between the first order approximations

- Taking the first two terms of Taylor series of exp in the first Rytov approximation the first Born approximation is obtained

$$u(\mathbf{r}) = u_0(\mathbf{r})\left[1+\psi_1^{(0)}(\mathbf{r})\right]$$

- If the scattering angle is small ($\lambda L \ll s^2$, where s is a perturbation scale, L is the propagation distance) then Rytov approximation is reduced to

$$\psi_0(\mathbf{r}) = ikn_0 z \qquad \psi_1^{(0)}(\mathbf{r}) = -\frac{i}{2kn_0}\int_0^L V(\mathbf{r})dz \approx ikn_0\int_0^L \delta n(\mathbf{r})dz$$

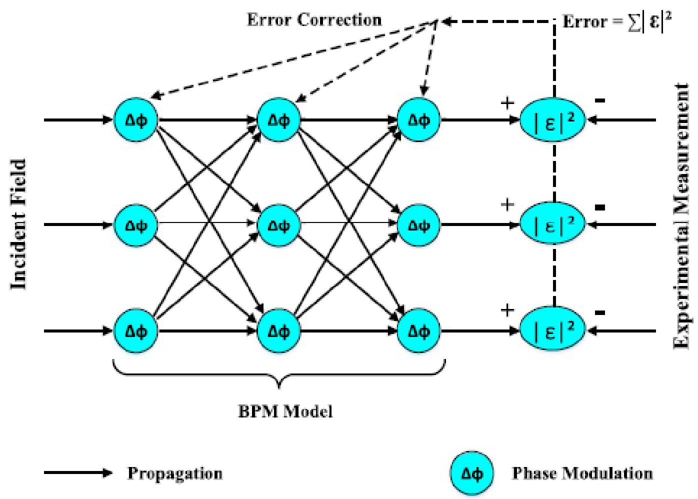
where $n(\mathbf{r}) = n_0 + \delta n(\mathbf{r}) \quad \Rightarrow \quad V(\mathbf{r}) \approx -2(kn_0)^2\,\delta n(\mathbf{r})$

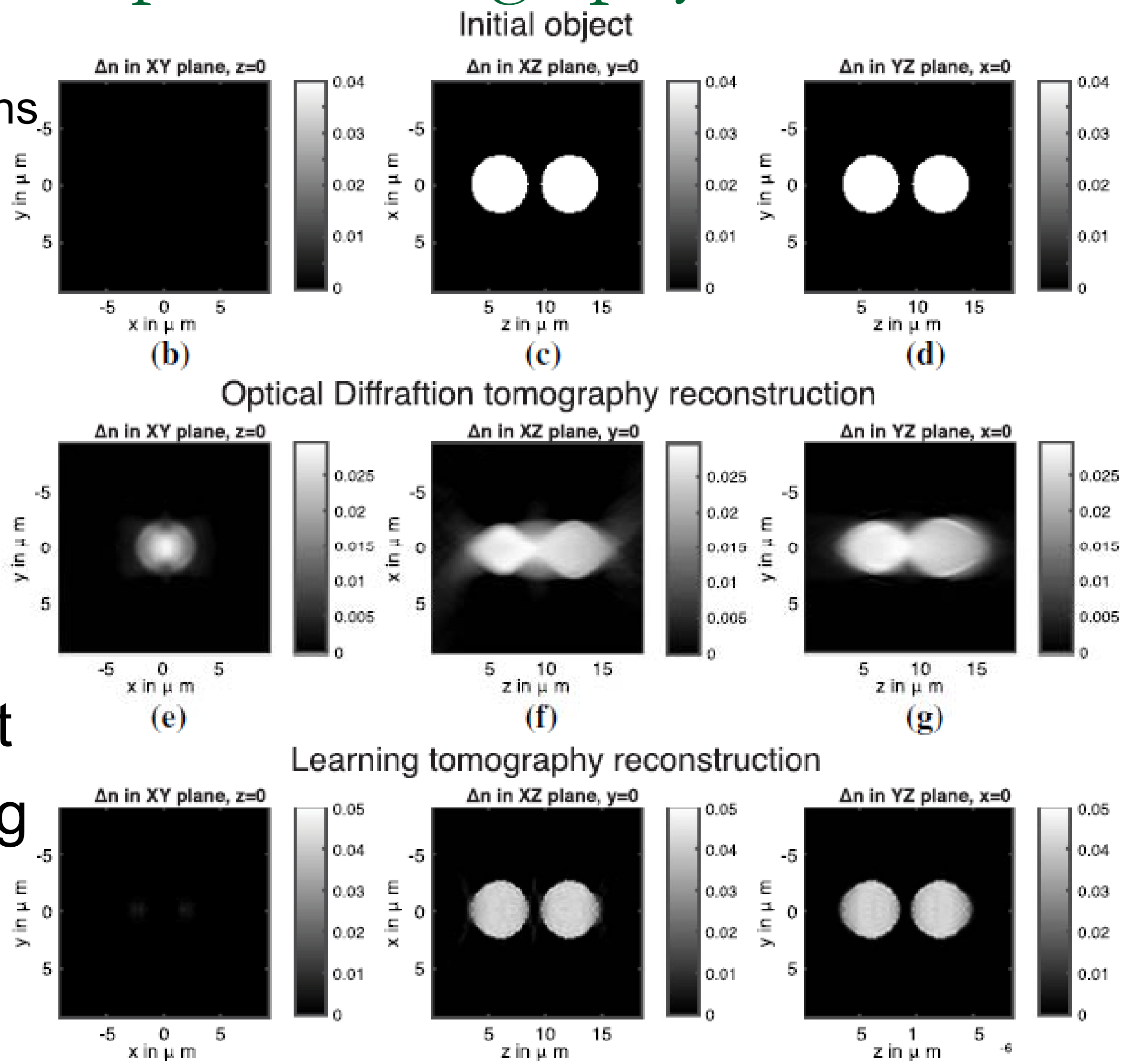
Eikonal approximation: $u(\mathbf{r}) = \exp\left[ik\left(zn_0 + \int_0^L \delta n(\mathbf{r})dz\right)\right]$

A. J. Devaney, Opt. Lett. 6, 374 (1981); M. Nieto-Vesperinas, Scattering and Diffraction in Physical Optics (1991)

Learning approach to optical tomography

420 layers, 80 angles, 100 iterations





- DHM 3D phase object reconstruction by training an artificial neural network + beam propagation method

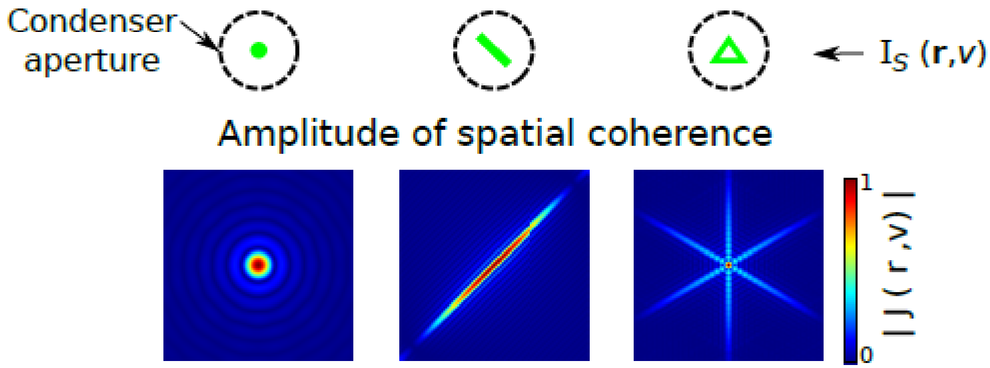
Images from U. S. Kamilov et al, Optica 2, 517(2015);L. Tian &L. Waller Optica 2, 104 (2015)

Illumination coherence engineering

- Partially coherent illumination provides larger spatial frequency acceptance, but with poor SNR

- Development of QPI microscopic techniques (iterative, TIE, holographic ones) for temporally or/and spatially partially coherent illumination requires proper coherence design

- Illumination coherence engineering = design of form, size, temporal frequency content of intensity distribution of spatially incoherent light projected on the condenser aperture.



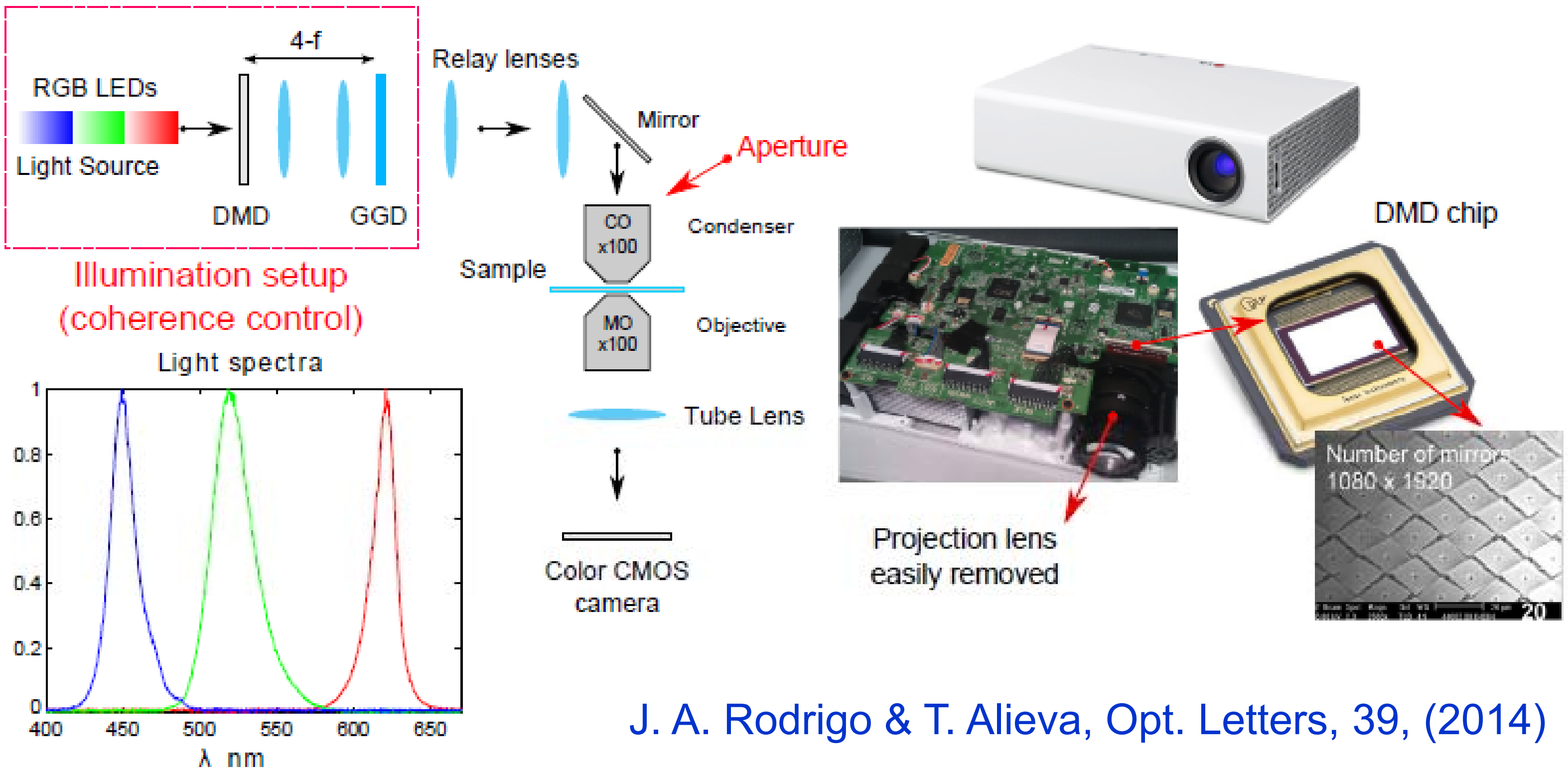
J. A. Rodrigo &T. Alieva, Opt. Lett, 39 (2014)

DPL for illumination coherence design

- RGB LEDs: Design of temporal coherence
- Digital micromirror device (DMD): fast response (milliseconds), no chromatic aberrations
- Easy programmable device
- Other applications of the DLPs in microscopy:
  - Structured illumination [J. Stirman et al, Nature Methods 8 (2011)]
  - Contrast enhancement imaging [E.C. Samson and C. M. Blanca, New Journal of Physics 9 (2007)]
- Alternative proposals: LED array illumination [G. Zheng, Opt. Lett. 36, 3987 (2011); L. Tian et al, Optica 2, 904 (2015)]

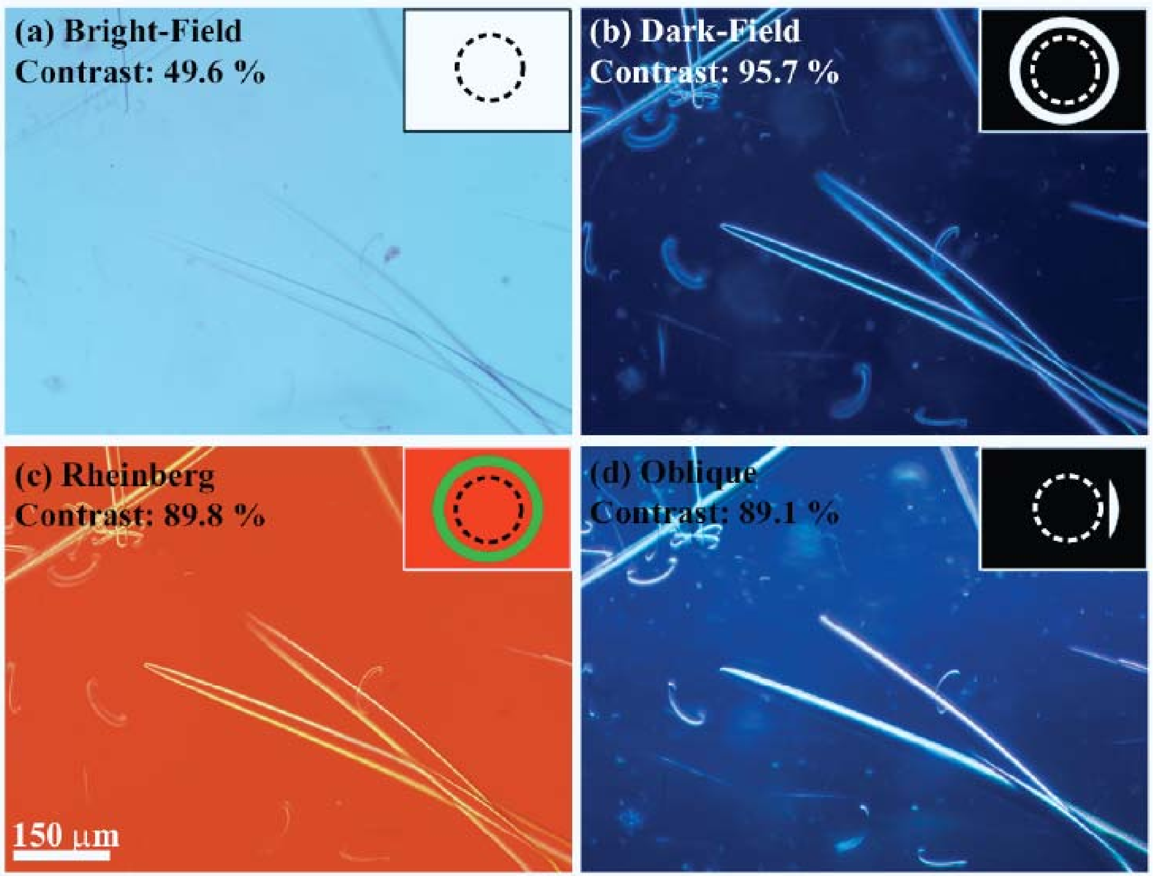
How to setup?

- DMD:
  - Up to 2 million of controlled micromirrors
  - Pattern Refresh Rate: 2kHz @ 8-bit grayscale



J. A. Rodrigo & T. Alieva, Opt. Letters, 39, (2014)

From qualitative to quantitative imaging



DLP was used
for mask projection

Widefield images of siliceous spicules of a starfish taken under
(a) bright-field, (b) dark-field, (c) Rheinberg and (d) oblique illumination.

Images from E.C. Samson & C. M. Blanca, New J. Physics 9 (2007)

Concluding remarks

- 2D and 3D QI with coherent and partially coherent illumination is an active research area. Some successful solutions have been commercialized.

- Only a small % of the research works devoted to QI in widefield microscopy has been used/cited as an example in this presentation.

- There are still a lot of problems to solve: fast data acquisition and processing, low SNR, rigorous reconstruction methods, proper sampling, correct illumination design, unwrapping, regularization, etc.

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