Tomographic diffractive microscopy: principles and applications

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Intensity Microscopy

Dark-field
Oblique illumination
Rheinberg illumination
Hoffman modulation
Polarized microscopy
Phase contrast (Zernike)
Differential Phase Contrast (Nomarski)
Ultramicroscope (Siedentopf & Zsigmondy)

:-) Some control of the illumination
:-) A certain comprehension of the light specimen interaction phenomenon
:-( Inherently 2-D
Phase Microscopy

:-) Usually, one records intensity only images

Gabor Holography
Phase Stepping Holography
Off-Axis Holography
Front Wave Analyser

=> One can (now easily) measure amplitude and phase of the diffracted field
Principle of Holography

0-Order  Order 1  Order -1

How to get the object wave (order 1) from the fringe pattern?

\[ I(x, y) = |u(x, y)|^2 + |u_r(x, y)|^2 + u(x, y)u^*(x, y) + u^*(x, y)u_r(x, y) \]

Nobel Prize 1971 Physics
Denis Gabor
"for his invention and development of the holographic method".
Phase Stepping Holography

4 holograms, with 4 different phases of the reference wave

\[ I_k(x, y) = |u(x, y)|^2 + |u_r(x, y)|^2 + u(x, y)u^*_r(x, y)e^{-jk\pi/2} + u^*(x, y)u_r(x, y)e^{jk\pi/2} \]

\[ \mu = +u(x, y)u^*_r(x, y) \]

\[ I_0 = |u(x, y)|^2 + |u_r(x, y)|^2 + \mu + \mu^* \]
\[ I_1 = |u(x, y)|^2 + |u_r(x, y)|^2 - j\mu + j\mu^* \]
\[ I_2 = |u(x, y)|^2 + |u_r(x, y)|^2 - \mu - \mu^* \]
\[ I_3 = |u(x, y)|^2 + |u_r(x, y)|^2 + j\mu - j\mu^* \]

One obtains:

\[ I_0 - I_2 = 4\Re(\mu) \]
\[ I_3 - I_1 = 4j\Im(\mu) \]

\[ u = \frac{(I_0 - I_2) + (I_3 - I_1)}{4u_r} \]
Off-Axis Holography

The reference wave is angularly shifted => périodic modulation

Fourier transform of the hologram splits the different orders:

\[
\mathcal{I}(x, y) = |u(x, y)|^2 + |u_r(x, y)|^2 + u(x, y)u_r^*(x, y) + u^*(x, y)u_r(x, y)
\]

Spatial filtering: selection of order 1
Commercial DHM

Phase Holographic Imaging

lyncée tec DHM
Wavefront Analyser

Phasics SID4Bio

Measurement of the integral refractive index and dynamic cell morphometry of living cells with digital holographic microscopy

B. Rappaz, et al.,
Coherent Light Diffraction

Weakly diffracting/diffusing/absorbing object
1st Born approximation
The diffracted wave is interpreted as a part of the 3-D Fourier 3D transform of \(<n>\)

Semi-transparent object reconstruction from holographic data
Image Space / Fourier space

Spatial domain

Frequency domain

- Objective numerical aperture ⇒ Limitation of the detection angle
Holographic Microscopy: Results

1 angle

Limited 3-D resolution

Profilometry

Integral measurements
Illumination Control: Consequences

A radical solution:

1 unique illumination direction!

Cellule CD34 - Image Georges Jung, Laboratoire d’Hématologie Centre Hospitalier Régional Emile Muller - Mulhouse

\[ R_{\text{trans}} = \frac{\lambda}{2NA_{\text{cond}} \cdot NA_{\text{obj}}} \]
Tomography by Illumination Rotation

- Different angles of illumination $\Rightarrow$ other object frequencies
- Large number of angles
  $\Rightarrow$ Extended and filled frequency support

- Objective numerical aperture $\Rightarrow$ Limitation of the detection angle
- Condenser numerical aperture $\Rightarrow$ Limitation of the illumination angle
Tomographic Microscopy: Transmission

Object Reconstruction

2-D FT → Hologram → Wave front → PSH

New angle → Object frequencies → projection

3-D image n+ik → 3-D FT → 3-D frequency support → addition
Holography / Tomography

1 angle

372 angles
High-resolution three-dimensional tomographic diffractive microscopy of transparent inorganic and biological samples

Tomography => Index of Refraction

Epithelial cells

10 µm

High resolution tomographic diffractive microscopy of biological samples
Tomography => Index of Refraction

Granulocytes

False colour rendering:
Red : absorption
Blue : refraction
Commercially available!

See their website for interesting applications
Several active groups in the world (Korea, Poland, Taiwán, France, Germany, Italy…). Workshop MiFobio 2018
Applications http://nanolive.ch

- Cellular morphological changes induced by drug treatment
- Nanodiamonds internalization & 3D distribution in living cells
Applications http://nanolive.ch

Fission yeast (Schizosaccharomyces pombe) during division

Fibroblast reticular cell seeded on glass nanopillars
Applications http://www.tomocube.com

Cell apoptosis

Bacterial growth

HeLa cell

Microalgae
Tomography by Wavelength Variation

- No moving part
- Low gain in resolution
- Wide spectrum coherent sources

Recorded frequency support in transmission
Phase Contrast Microscopy

Annular diaphragm

Source

Condenser aperture

Phase plate

Objective back focal plane

Image plane

Nobel Prize 1953 Physics
Frits (Frederik) Zernike
"for his demonstration of the phase contrast method, especially for his invention of the phase contrast microscope".

www.microscopyu.com
White-light diffraction tomography

White-light diffraction tomography of unlabelled live cells
Commercially available!

See their website for interesting applications
Applications http://phioptics.com

SEM

Confocal

Spiculated RBC

HT29 cell

Live neuron
Tomography / Fluorescence Comparison

Snowdrop pollen

Tomography
red: index n > index immersion medium

Fluorescence
Non-Isotropic Resolution

Absorption

Index of refraction

Autofluorescence
Tomography by Specimen Rotation

- Sample rotation may be difficult
- low NA => quasi-isotropic, but rather low resolution
Tomography by Specimen Rotation

Problems and Solutions in 3-D Analysis of Phase Biological Objects by Optical Diffraction Tomography
M. Kujawińska, et al., Int. J. Optomechatronics 8, p. 357 (2014)

Tomographic phase microscopy with 180° rotation of live cells in suspension by holographic optical tweezers
Tomography by Specimen Rotation

Rapid 3D Refractive-Index Imaging of Live Cells in Suspension without Labeling Using Dielectrophoretic Cell Rotation
M. Habaza, et al.,

Tomographic flow cytometry by digital holography
F. Merola, et al.,
Light: Science & Applications 6, paper e16241 (2017)
Missing Frequencies

Recorded frequencies  Missing part  “Missing apple core”

“Diffraction microtomography with sample rotation: influence of a missing apple core in the recorded frequency space”
Multiview Tomography
Multiview Tomography

Improved and isotropic resolution in tomographic diffractive microscopy combining sample and illumination rotation
Towards High NA, IsoResolution

\[ \lambda = 633\text{nm or } 475\text{nm}, \quad \text{NA}_{\text{obj}} = 1.4, \quad \text{NA}_{\text{cond}} = 1.4 \]

Fast acquisition (less than 10s for 1 object orientation / 400 illuminations)
Real-time reconstruction for each object orientation (1 volume of data each 3s)
Optical Fiber Tip ($\lambda=475$ nm $R_{\text{predicted}}=95$nm)

Size $x$-$y$-$z$: 150 nm
Zeolith microcrystal

(a): 1 view  
(b-e) fusion of 8 views

1 view  
1 view deconvolved  
fusion of 8 views
Betula Pollen

http://www.vcbio.science.ru.nl
Betula Pollen

Integrated dual-tomography for refractive index analysis of free-floating single living cell with isotropic superresolution
Low-cost microscopy/tomography

Lens-free optical tomographic microscope with a large imaging volume on a chip
S. O. Isikman, et al., PNAS 1015638108 (2011)

Aydogan Ozcan’s group
UCLA
Low-cost microscopy/tomography

Comparative study of fully three-dimensional reconstruction algorithms for lens-free microscopy
Drawback: Speed

Intensity Microscopy: Transmission, Phase contrast, DIC…

Diffractive Tomographic Microscopy

Incoherent Parallel ⇒ Ultrafast 😊

Coherent Sequential => Slow 😞

Not-quantitative Low-resolution

Quantitative High-resolution

CCD
Fast 1-D Scanning

“Tomographic phase microscopy”
1-D Scanning

“The overall shape takes a form of what we might call a “peanut.”

“Image formation in holographic tomography”
Acquisition  Reconstruction  Display

1.4 ms for 1 angle

Projection

Off-axis
2-D FT
GPU

Object frequencies

Addition

New angle

1 hologram
Camera->CPU

CPU->Mirror

Data preparation
+ 3-D FT
GPU
240 ms

3-D frequency support

Display
(±1ms)
GPU Reconstruction

Nvidia Tesla C2075, Cuda, FFTW : 3.5 3D images/s

Tomographic diffractive microscopy: towards high-resolution 3-D real-time data acquisition, image reconstruction and display of unlabeled samples
Other possible approach

Conventional QPI

- Spatially coherent illumination
- \( \mu bead \)
- Limited axial resolution

(\( xy \) plane)

(\( xz \) plane)

- 3D resolution ↔ fluorescence

New approach

- Spatially incoherent illumination
- Bon et al., Optics Express 2014
3D live cell imaging

Living COS-7 cell

$OPD$ with spatially Incoh. Illum. + 3D deconvolution

3D shape of the cell
Fast acquisition, compatible with live imaging (just a z-stack!)
Fixed tissue imaging (1/2)

Skin mouse (15 µm thick)

Cell layer visualization without labeling
Fixed tissue imaging (2/2)

Brain slice (90 µm thick)

Axons or Dendrites

100 µm

0 µm

18 nm (OPD)

z = 5 µm

z = 20 µm

z = 50 µm

z = 70 µm

✓ Thin structures visible even after few tens of microns
Multimode Imaging

- Refraction Microscopy
- Wide-Field Microscopy
- High-NA Dark-Field Microscopy

Synthetic reconstruction

Wavefront

Compute Intensity ($I^2$)

FT ($I^2$)
Conclusion

Unprepared samples => use of a new kind of information <n>

High resolution imaging: $\lambda/(3.5\text{NA})$ lateral experimentally demonstrated

Challenges:
- RT acquisition/reconstruction/display
- polarimetric TDM
- “true” superresolution?