Multi-Plane Tomographic Phase Retrieval for 4D Cell Microscopy

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IMA Workshop Series: Phaseless Imaging in Theory and Practice
University of Minnesota, August 18, 2017
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University of California
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Stanford University

EPFL

École Polytechnique Fédérale de Lausanne
Bright-field image of a HeLa cell
Intensity Profile

Bright-field image of a HeLa cell
Cells and soft tissues are phase-only objects
- Highly transparent
- Weakly scattering
- Weakly absorbing

Low-contrast images under the conventional light microscope
Bright-field image of a HeLa cell
Zernike phase contrast image

[Zernike, 1942]
Differential interference contrast (DIC) image

[Nomarski, 1955]
“Looking at” Unstained Cells

- Dedicated phase imaging modalities: Interferometry-based microscopes
  + Quantitative phase maps
  - Higher cost

[Marquet et al., 2005]
[Cotte et al., 2013]
[Kim et al., 2016]

Courtesy of Phasics
Courtesy of Lyncée tec
Courtesy of TomoCube
Courtesy of Nanolive

Denis Gabor
Defocus Microscopy
Consider a propagating field $U(x, z)$ in the $+z$ direction:

$$U(x, z) = U_0(x, z)e^{jkz},$$

at an axial plane $z \in \mathbb{R}$, and over some lateral domain $x = (x, y) \subset \mathbb{R}^2$. 
Phase Retrieval in Bioimaging

- Paraxial wave equation for $U_0$:

$$\frac{\partial}{\partial z} U_0(x, z) - \frac{j}{2k} \nabla^2 U_0(x, z) = 0,$$
Phase Retrieval in Bioimaging

- Paraxial wave equation for $U_0$:

$$\frac{\partial}{\partial z} U_0(x, z) - \frac{j}{2k} \nabla_\perp^2 U_0(x, z) = 0,$$

- Transport-of-intensity equation:

$$-k \frac{\partial}{\partial z} I = \nabla_\perp \cdot I \nabla_\perp \phi$$

[Teague, 1983]

- Transport-of-phase equation:

$$2k l^2 \frac{\partial}{\partial z} \phi = \frac{1}{2} I \nabla_\perp^2 I - \frac{1}{4} \nabla_\perp^2 I^2 - l^2 \nabla_\perp^2 \phi^2 + kl^2$$

- $\nabla_\perp$: transverse gradient
- $\nabla_\perp^2$: transverse Laplacian

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Transport-of-Intensity Approach

- Object plane
- Image plane
- Illumination source
- Imaging system
- Defocus planes
- In-focus plane
- Phase Object

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Transport-of-Intensity Equation (TIE)

\[- \frac{k}{l(x, z)} \frac{\partial}{\partial z} l(x, z) = \nabla^2_{\perp} \phi(x, z)\]

\[\frac{\partial}{\partial z} l(x, z) \approx \frac{l(x, z + \Delta z) - l(x, z - \Delta z)}{2\Delta z}\]
Transport-of-Intensity Approach

TIE Forward Model (in Fourier domain)

\[ \hat{b}(\omega) = 4\pi \Delta z \| \omega \|_2^2 \hat{h}_{\text{TIE}} \hat{\phi}(\omega) \]
PHASE CONTRAST, A NEW METHOD FOR THE MICROSCOPIC OBSERVATION OF TRANSPARENT OBJECTS

by F. ZERNIKE, Groningen

PART I

Every microscopist knows that transparent objects show light or dark contours under the microscope in different ways varying with change of focus and depending on the kind of illumination used.
Under the paraxial approximation, the intensity is expressed as

$$I(x, z) = |U_0(x) \ast p_F(x, z)|^2$$
Contrast Transfer Function

- Under the paraxial approximation, the intensity is expressed as

\[ I(x, z) = |U_0(x) * p_F(x, z)|^2 \]

**First-order assumptions:**

Absorption is negligible, \( U_0 = e^{i\phi} \)

Weak-phase object, \( e^{i\phi} \approx 1 + j\phi \)

**measured image at distance z**

\[
I(x, z) = |(1 + j\phi) * p_F(x, z)|^2 \\
= ((1 - j\phi) * p_F^*(x, z)) ((1 + j\phi) * p_F(x, z))
\]

We ignore the second-order terms as phase is small.

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Contrast Transfer Function

measured image at distance z (in Fourier domain)

\[ \hat{I}(w, z) = I(\delta(w) + 2 \sin(\pi \lambda z \|w\| \frac{2}{\lambda} \phi(w))) \]

[Joachim, 1996]
CTF gives us a TIE-like equation:

\[ \hat{b}(\omega) = 4 \sin(\pi \Delta z \|\omega\|_2^2) \hat{\phi}(\omega) \]

Finite Differences

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Analysis of Spatial Frequencies

CTF Forward Model (in Fourier domain)

$$\hat{b}(\omega) = 4 \sin(\pi \Delta z \|\omega\|_2^2) \hat{\phi}(\omega)$$

$$\hat{h}_{CTF}$$

Large defocus

Small defocus

$$\hat{h}_{TIE}$$  $$\hat{h}_{CTF}$$
Regularized Phase Imaging

We propose to use an improved data fidelity term:

\[
\Phi^* = \underset{\Phi}{\text{arg min}} \quad \frac{1}{2} \| H_1 \Phi - b_1 \|_W^2 + \frac{1}{2} \| H_2 \Phi - b_2 \|_W^2 + \tau \sum_{k \in \Omega} \| [L \Phi]_k \|_2
\]

TV regularization

We can recover existing methods:

- [Paganin et al., 2004]
- [Chou et al., 2007]
- [Tian et al., 2012]
- [Zuo et al., 2013]
- [Bostan et al., 2014]
- [Carranza et al., 2015]
Regularized Phase Imaging

-300 µm -50 µm 0 50 µm 300 µm

Tikhonov regularization

TV regularization

Small defocus measurements

Large defocus measurements

Small and large defocus measurements

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Experimental Results
Experiment Settings

- We imaged paraformaldehyde-fixed and unstained HeLa cells.
  - Zeiss Axio Observer Z1 microscope
  - Leica HCX PL Fluotar 40×0.75 NA objective
  - Camera pixel size is 6.5 microns

- Measurements are acquired at
  - 2 micron defocus
  - 10 micron defocus

- The maximum frequency is set in accordance with the diffraction limit

- Regularization parameter $\tau$ is manually tuned
Experiment Results

Bright-field image (infocus)

TIE-Tik Reconstruction (using 3 images)
TIE-Tik Reconstruction (using 5 images)
TIE-TV Reconstruction (using 3 images)
Proposed method

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Validation Results

DHM image

Bright-field image (infocus)

DIC image (infocus)

[Bostan et al., 2015]

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Segmentation Results

Bright-field image (infocus)  TIE Reconstruction  Delineation Result
Towards Multi-Modal Cell Imaging...
Multi-Modal Cell Imaging

- Super-resolution fluorescence microscopy:
  + High specificity
  + Unprecedented insight into subcellular structures
  - Complete morphology is harder to observe

- Phase (label-free) microscopy:
  + Complete morphology is observable
  - No specificity!

[Hell and Wichmann, 1994]
[Betzig et al., 2006]
[Rust et al., 2006]
Multi-Modal Cell Imaging

SLOW DATA ACQUISITION!

- Super-resolution fluorescence microscopy:
  + High specificity
  + Unprecedented insight into subcellular structures
    - Complete morphology is harder to observe

- Phase (label-free) microscopy:
  + Complete morphology is observable
  - No specificity!

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Multi-Modal Cell Imaging

Super-resolution optical fluctuation imaging (SOFI) already uses multi-plane system.

[Geissbuehler et al., 2014]
[Deritzer et al., 2009]

Multi-plane Imaging for Phase + Fluorescence

Sample stability!

Partial coherence for 3D objects!
Multi-Modal Cell Imaging

PRISM (Phase Retrieval Instrument with Super-resolution Microscopy)

https://arxiv.org/abs/1705.05766
Multi-Modal Cell Imaging

- Defocus Phase Imaging
- Super-Resolution Optical Fluctuation Imaging

+ Simultaneous 8 defocus measurements are acquired
+ The image acquisition up to 200 Hz
+ Quantification of membrane fluctuations
Phase Image

Fluorescence Image

Courtesy of P. Odermatt
3D Forward Model

Monochromatic scattering (under Born approximation)

\[ \Gamma(\omega, t = 0) = \int_{0}^{\infty} \langle U_s(\omega, \nu), U_i^*(\omega, \nu) \rangle d\nu \]

Cross-spectral density between the scattered and the incident field

\[ \Gamma(\omega) = jF(\omega)H(\omega) \]

Single-scattering object assumption

\[ I(x) = I_{DC} + \Gamma(x) + \Gamma^*(x) \]

[Sheppard et al., 1994]
[McCutchon, 2002]
Conclusion

4D cell imaging is feasible via multi-plane model.

Linearized models can be valid for single cell imaging.
Acknowledgements
Thanks!