Computational illumination for high-resolution 3D phase imaging

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Computational imaging

1) Modify optical design  2) Take picture(s)  3) **Crunch Data**  4) Final result

The computer becomes a part of the imaging system

5) Start company
Optics Abstractions

- Quantum optics
- Electromagnetic optics
- Wave optics
- Geometrical optics (ray tracing)
- Paraxial ray optics (matrix methods)

B. Saleh, *Photonics.*
Phase imaging: seeing the invisible

- light is a wave, so has intensity and phase
- optical phase is too fast to capture directly
Applications: biological microscopy

Intensity

Endothelial cells

Human cheek cells

Phase

E-coli

HeLa cells

Intensity Phase

-1

-0.5

0

0.5

1

rad
Applications: X-ray and EUV phase imaging

Photomask phase edges

LBNL tomography beamline

Absorption  Phase contrast


» bioimaging
» materials characterization
» lithography


2.5um
Phase retrieval is a nonlinear problem

**Optical System Design**

- Complex function $x$
- Optical system $A$
- Detector (measures intensity)

**Algorithm**

Find $x$ such that $\text{Intensity} = |Ax|^2$
Phase from defocus

\[ \text{Intensity}(z) = |A_z x|^2 \]

Leverages existing hardware
→ software add-on only

Phase from defocus with many images

Intensity stack + noise (SNR~0.5)

High noise causes most techniques to break down!

Kalman filter [1-3] provides optimal noise performance

Covariance matrix is impractically large

N=number of pixels > 1 Mpxl

Kalman filter must store and manipulate covariance matrix of size $N^2 \rightarrow \text{Terabytes of data!}$

Sparsity of covariance matrix can be exploited:

<table>
<thead>
<tr>
<th></th>
<th>Complexity</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kalman filter</td>
<td>$O(N^3)$</td>
<td>$O(N^2)$</td>
</tr>
<tr>
<td><strong>Sparse Kalman filter</strong></td>
<td>$O(N\log N)$</td>
<td>$O(N)$</td>
</tr>
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For our data, we achieve $10^{11}$ speed-up

Focus step positions can be optimized

equally spaced
129 images

Recovered phase (radians)

-45.6 μm     Intensity stack     45.6 μm

Focus step positions can be optimized

-45.6 μm  
Intensity stack  
45.6 μm

non-equally spaced
5 images

Recovered phase (radians)


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Intensity $I = \text{detector}$ (measures intensity)

Optical System Design

find such that $I_{\text{Intensity}} = |Ax|^2$

Algorithm
Computational illumination with an LED array

LED array

Camera

3D imaging

Gigapixel phase imaging

Real-time multi-contrast

Brightfield

Darkfield

Phase contrast


Multi-contrast in an LED array microscope

- Replace illumination unit with LED array
- Each LED corresponds to illumination by a different angle

**Brightfield**

**Darkfield**

**Phase Contrast**

![Image of microscope setup](image_url)
Real-time multi-contrast imaging

- Brightfield
- Dark field
- Phase contrast

20x, NA~0.4, 30 Hz
C. Elegans on NGM petri plate (Dillin lab, UCB)

Differential phase contrast

contrast is proportional to the derivative of phase!\(^{[1-3]}\)

Transfer function for differential phase contrast

» Amplitude information is symmetric in Fourier space

» **Phase** information is asymmetric

20µm
Transfer function for differential phase contrast

- Similar to derivative model within NA
- 2x better resolution from partial coherence

Quantitative phase from DPC measurements

LED array

existing microscope

Sample

Pupil space

Camera

Phase transfer function

DPC image

Phase

20µm

rad

1

-1
It’s easy to change the DPC angle
2-axis DPC is good for accuracy & speed

Reconstructed phase

Phase transfer function

Top-bottom DPC

Left-right DPC

2-axis DPC

20µm
Computational illumination with an LED array

LED array

200µm
Gigapixel phase imaging
25µm

Real-time multi-contrast
brightfield
darkfield
phase contrast


3D imaging
380 µm


Darkfield images represent sub-resolution features

Can we stitch together all of Fourier space?

Scanning through all LEDs provides full coverage in Fourier space + overlap redundancy

\[ NA_{eff} = NA_{obj} + NA_{illumin} \]
\[ = 0.1 + 0.6 \]
\[ = 0.7 \]
Fourier Ptychography achieves resolution beyond the diffraction limit of the objective.

Our version of ideas in:
Digital Aberration removal

4x 0.1NA Nikon, Synthesized NA = 0.6

Raw data, central LED illumination

Reconstructed image

Our version of ideas in:
Reconstruction algorithm is nonlinear optimization

1. Start with low-resolution object initial guess
2. Check estimate against data – forward problem
   - 1) Impose intensity constraint
   - 2) Fourier support constraint – inverse problem
3. Update object estimate

Comparing to other phase imaging methods

Full 4x FoV phase reconstruction (synthetic 0.7 NA)

Phase from TIE 40x 0.65 NA

40x 0.65 NA Phase contrast

unstained human breast basal epithelial cell MCF10A

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Unstained Human Bone Osteosarcoma Epithelial U2OS (phase) sample

二十微米

视野~2.1毫米 x 1.7毫米

分辨率~400纳米

13千 x 11千像素

重建
We collect a lot of data

» For current resolution/FoV:
  » ~300 images (16 bit, 5.5 Mpxl each)
  » Full frame rate of camera = 50 fps
  » Speed limit = 0.55 Gb/s!

» Phase retrieval requires ~10x more data collected than reconstructed (overlap in Fourier space)

How can we use data more efficiently?
Coding strategies for phase?

» Illuminate sample from multiple LEDs

» Want:
  1) LEDs far apart
  2) Asymmetry for phase contrast

Random coding is good!

Multiplexed illumination improves acquisition speed

sample's Fourier space


low-resolution image

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Multiplexing reduces acquisition time and data

Sequential 1 LED
293 images
T = 586s

Multiplexed 8 LEDs
293 images
T = 293s

Multiplexed 8 LEDs
40 images
T = 40s

Only uses 14% of the data!

synthesized NA = 0.6

Time-lapse phase (in vitro)

Hela Cells

~0.4µm resolution (0.7 NA)
3D intensity and phase imaging

LED array

camera
Scanning illumination angles relates to depth

LED array

scan illumination in \((\theta_x, \theta_y)\)

camera

thick sample

\[ z_i \]

\[ \Delta x \]

\[ \Delta z \]
Light field dataset gives depth information

Our version of ideas in:
Digital refocusing for phase contrast

LED pattern

100µm

3D intensity

100µm

3D phase contrast

10x, NA = 0.25
Refocusing range: -100µm-100µm
C. Elegans on NGM petri plate (Dillin lab)

Diffraction effects cause error in light field results

Physical focus

Light field refocus

We need phase to correct diffraction errors!

Diffraction effects cause error in light field results.

Our method improves the focus and refocus of images compared to physical focus and light field refocus.

Multi-slice model for 3D

[Image of multi-slice model with LED array and 3D sample]
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varying illumination angle

OR? AND?
Can we get enhanced resolution + 3D?

Low-resolution full FoV image from 4× 0.1 NA objective

Resolution depends on defocus distance

Resolution depends on defocus distance

3D reconstructions in phase and amplitude


Synthetic NA 0.7, Spirogyra algae
Current work: mobile microscopy
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Find us on GigaPan: WallerLab_Berkeley
Twitter: @oprickster

Open-source code & datasets: www.laurawaller.com