Holographic 3-D Imaging Flow Cytometry

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Holography

Digital holographic microscopy can record the wavefront distortion by a transparent cell.

In combination with tomographic data acquisition, digital holography can record the 3-D refractive index distribution of a sample.
Digital holographic tomography provides the refractive index that can be connected to important biochemical parameters.

\[ n(\lambda) = n_0(\lambda) + \sum_i \alpha_i(\lambda)C_i \]

Volume, Mass, Density, Composition…

All at a single-cell level

Refractive index measurement

→ Observing normal and pathophysiology of single cells in the least invasive manner

→ Sorting cells for regenerative medicine

Existing scanning-based methods for digital holographic tomography

A. Sample rotation

Sample rotation

Illumination

Objective lens

Tube

Water or immersion oil


B. Beam rotation

Illumination

Cells in a petri dish

Objective lens

V. Lauer, J Microsc 205, 2002;

✓ Imaging throughput: < 10 cells/sec
Digital holographic tomography meets imaging flow cytometry

Collimated laser beam
Cylindrical lens
Object
Focused beam = Many plane waves propagating in different directions

N. Lue, et al., Opt Express 16, 2008;

✓ Imaging throughput: ~ 10 cells/sec
Label-free 3-D imaging of refractive index

Spatial resolution: 2X diffraction limit (~100 nm @532 nm)
Refractive index accuracy: 0.001
Cell size homeostasis studied with picogram-level cell mass measurement

A. Cells divide asymmetrically.

B. To compensate for the size difference, the growth of larger cells slows after reaching a certain point.

There must exist an internal mechanism to monitor the size of a cell and coordinate its growth and division.

Chondrocyte enlargement studied with single-cell-level volume & density measurement

Multiple phases of chondrocyte enlargement

Mammalian chondrocytes undergo three distinct phases of volume increase, including a phase of massive cell swelling in which the cellular dry mass is significantly diluted.”

Label-free detection of hematologic malignancies

Hypergranular promyelocytic leukemia with heavily stained granules and single or bundles of Auer rods (arrow)

Morphologic features of acute myeloid leukemia with cup-like nuclear phenotype or nuclear invaginations (arrow)


Quantification of stem cell differentiation by refractive index

Promyelocytic leukemia cell line (HL-60)

- Biochemical methods
  - Western blot
  - Immunochemistry
  - Real-time PCR

- Physical changes
  - Structures in the cytoskeleton and nucleus
  - Mechanics of cells and nuclei

Finding biological/clinical implications of the changes in measured refractive index continues...

Summary

• Digital holographic tomography allows studying complex physical and biological systems in a minimally-invasive manner.
• We are developing new methods that will allow high-throughput, label-free 3-D imaging of samples in a flow cytometry configuration and imaging-based active sorting.
Development of high-throughput, multi-scale imaging tools to advance biomedicine

Biophysics at a nanometer scale

Monitoring radioisotope uptake at a cellular level

High-throughput 3-D imaging flow cytometry

Realistic medical imaging simulation
- Sub-organ structures
- Physiological models
- Light-matter interaction

Next-generation X-ray imaging
Thank you