Holotomographic imaging of eukaryotic cells

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Method Article

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Abstract

Holotomography measures 3D refractive index (RI) distribution in cells and tissues without exogenous labeling. Here we describe a protocol for holotomographic imaging of generic eukaryotic cells using a standardized Tomocube holotomographic microscope. Combined with the recent advances in machine learning, holotomographic imaging enables a broad range of new biological and medical applications.

Introduction

Reagents

Equipment

- Holotomographic microscope (HT-2; Tomocube)

- Microscope control software (TomoStudio; Tomocube)

- Imaging chamber (TomoDish; Tomocube)

Procedure

1. Prepare eukaryotic cells of interest in an imaging chamber filled with appropriate media. Make sure that the cells are not too confluent.

2. Apply a drop of immersion medium (distilled water) on the inverted objective lens of the microscope.

3. Place the imaging chamber on the stage of the microscope.

4. Control and align the positions of the objective and condenser lenses using Calibration tab of the microscope control software.

5. Control the stage to position a cell of interest in the field-of-view.

6. Focus on the sample by adjusting the objective lens.

7. Control the stage to find a blank position without any cells.

8. Click Calibrate to measure multi-angle holograms of the background.

9. Control the stage to position the cell of interest at the center of the field-of-view.

10. Click 3D Snapshot to measure the holograms of the cell.

11. Click Process to reconstruct the 3D RI tomogram from the measured holograms.

12. Repeat 5-11 for all cells of interest.

13. Optional: use the collected RI tomogram datasets for downstream applications powered by machine learning.

Troubleshooting

Time Taken

- A few minutes for sample preparation

- Less than a second per tomographic measurement

- Less than a minute per tomographic reconstruction

Anticipated Results

References

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