Quantitative BioImaging Utilizing Phase Microscopy

Katherine Creath[1,2] and Goldie Goldstein[3,2]

[1]Optineering, Tucson, Arizona,
[2]College of Optical Sciences, The University of Arizona,
and [3]4D Technology Corporation, Tucson, Arizona

Abstract

Quantitative BioImaging enables measuring and following processes in cells and biological samples. This work shows results of "label-free," real-time, quantitative measurements with a recently developed phase microscopy technique providing instantaneous, dynamic measurements of live cells. This system utilizes a pixelated wire grid polarizer mask in front of the camera sensor to capture phase and polarization in a snapshot. Images of cell optical thickness (OT) topography are generated from quantitative phase data and processed to obtain relative optical volume (OV = OT * A), quantify morphological changes, and determine changes in dry cell mass (DCM α OV). Live cells were prepared on #1 coverslips or coated slides. These results show a number of different applications of this technology. Be sure to see the video files on the iPad.

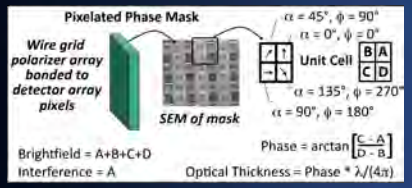
Looking for Collaborators

Video Examples on iPad

Applications

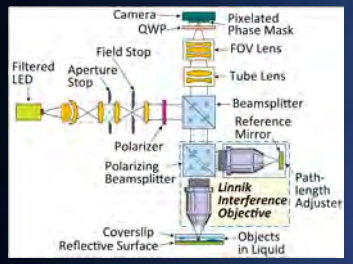
- Morphological Studies
- Mechanistic Studies
- Tissue Dynamics
- Quantify & Track Cellular Motion
- Process Monitoring
- Quantify Cellular Changes with Treatment

Enabling Technology



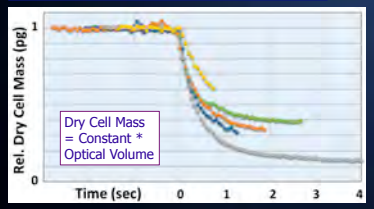
Like a color camera, this sensor sees in phase and polarization. It captures multiple relative phase shifts simultaneously to determine image phase as well as brightfield, phase contrast, dark field and DIC images. Fast data acquisition using short exposure times with a pixelated phase mask enables measurement of moving samples without blurring or scanning.

Optical Layout



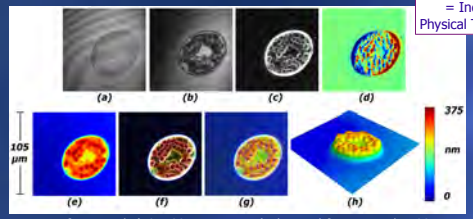
Microscope schematic for epi-illumination with a Linnik interference objective. Transparent samples in liquid are imaged in double-pass under a coverslip on a reflective surface. This system measures relative integrated optical thickness (OT) [or optical path difference (OPD)]. OT is proportional to physical thickness and index of refraction variations.

Dry Cell Mass of Histamine



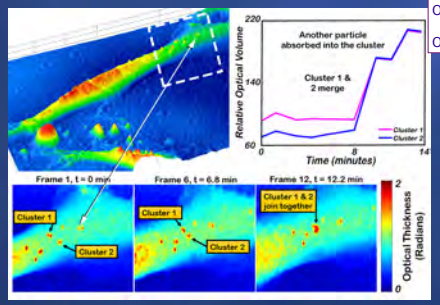
Plots showing the changes in relative dry cell mass (α to optical volume) as a function of time for 5 different mast cells in the process of releasing histamine. These preliminary plots have been normalized to show the relative changes. [Research Partner: Jason Reed, Virginia Commonwealth University, Richmond, VA]

Image Types



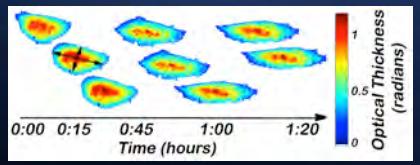
Images of a *nassula* (50 x 80μm protozoa) obtained from a single 100 μs snapshot at 50X with a 660nm source. (a) Brightfield (irradiance or intensity). (b) Phase contrast (interference – a single interferogram). (c) Simulated darkfield (phase gradient magnitudes). (d) Simulated DIC (x-gradient). (e) Pseudo-colored OT (optical thickness determined from phase). (f)-(g) Enhanced OT images. (h) 3D topographic OT plot. Plots in bottom row have same color scale.

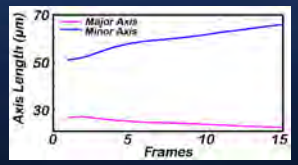
Optical Volume of Vesicle Transport



(upper left) 3D plot of myoblast optical thickness showing vesicles taken at 40X with a 511nm source. (lower images) Sample images showing tracking of vesicle clusters over 14 minutes. (upper right) Plot showing how the optical volume of these clusters changes with time. Note that as clusters merge, the optical volumes sum with agreement to within 0.4%. [Research Partner: Sanofi-Aventis, Tucson, AZ]

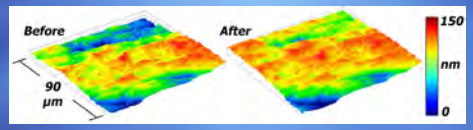
Quantify Morphological Changes





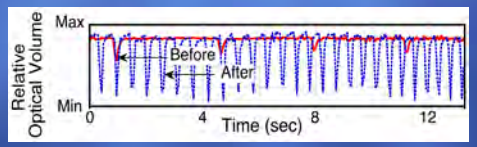
Myocyte morphology measured over 1:20 hours taken at 40X with 511nm source. (left) shows a single isolated cell as it changes. (right) plots variations in the major and minor axis of the cell. (15 frames over 1:20 hrs). [Research Partner: Sanofi-Aventis, Tucson, AZ]

Beating Rat Cardiac Myocytes



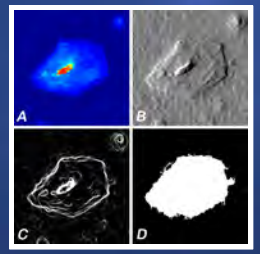
3D topographic maps showing relative optical thickness of embryonic rat cardiac myocyte cells cultured on a #1 coverslip. Measured in a Bioptechs FCS3 perfusion chamber at 37°C with 40X at 660nm. [See Movies]

Optical Volume Changes w/Treatment



Relative optical volume over time series of 200 datasets of cell culture above. The same area of cells is compared before and after pushing IPHC (isoproterenol hydrochloride). Note changes in both strength of contractions and speed of contractions. These are indicative of changes in the force of the contractions. Contraction strength cannot be determined without quantitative volumetric data.

Cell Boundaries

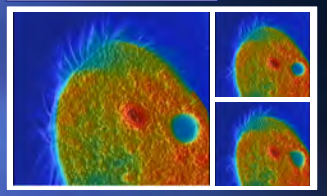


Optical thickness of a single epithelial cell imaged at 40X with a 511nm source. (A) Pseudocolored optical thickness. (B) Simulated DIC. (C) Cell gradient magnitudes. (D) Cell membrane boundaries.

Microscope Specifications

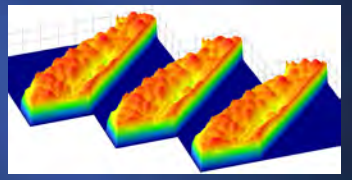
- Objectives: 20X (NA 0.45) & 50X (NA 0.8)
- 1X or 2.25X FOV lenses
- Source wavelength: 511 or 660 nm
- Fast data acquisition – no scanning
- Real-time processing – 15 fps
- Vibration insensitive

Paramecium Cilia



Paramecium cilia motion in real-time. This 125mm area was phase imaged at 50X with 660nm source. The OT scale has a 475nm range. Note variations in cilia and vacuole. [See Movie]

Zebrafish Blood



Blood flow through a 44 x 33 μm area in the cardinal vein of a live, anesthetized 3-day old zebrafish at 20X with 511nm source. [See Movie]

Further Information

1) Creath, K., and Goldstein, G., "Dynamic quantitative phase imaging for biological objects using a pixelated phase mask," Biomedical Optics Express **3**(11), 2866-2880 (2012).
2) Goldstein, G., and Creath, K., "Quantitative Phase Microscopy: How to make data meaningful," Proc. SPIE **8949**, 89481C (2014).

Research Partners

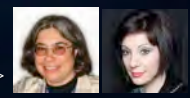
James Millerd, Neal Brock, Charles Crandall and Erik Novak, *4D Technology Corporation, Tucson, AZ*
Andy Rouse, Ron Lynch, Craig Weber, Jordan Lancaster, and Maki Niihori, *University of Arizona, Tucson, AZ*
Jane Peppard, Joy Prisco, Erica Harnish, and Elaine Powers *Sanofi-Aventis Research Center, Tucson, AZ*
Albert Kellner, *Sanford Burnham Medical Research Institute, San Diego, CA*
Jason Reed, *Virginia Commonwealth University, Richmond, VA*
Adam Wax, *Duke University, Durham, NC*
James McGrath, *University of Rochester, Rochester, NY*

Funding

This work was partially supported by the National Institutes of Health (NIH/NCRR 1R43RR028170-01, 2R44RR028170-02, NIH/NIGMS 8 R44 GM103406-03) and 4D Technology Corporation.

Contact Info

EMAIL: Kathy Creath <kcreath@ieee.org>, Goldie Goldstein <goldie.goldstein@4dtechnology.com>
BLOG: 4dbiocam.blogspot.com
WEB: http://www.u.arizona.edu/~kcreath



June 2014