Optical Thickness and Optical Volume Measurement of Dynamic Cellular Motion

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This paper describes new and recent research related to quantifying optical thickness and determining optical volume dynamically using a quantitative phase imaging microscope. With this microscope, it is possible to quantify relative optical thickness and volume changes at fast time scales not possible by other techniques. The microscope developed for this research utilizes a Linnik interference microscope with epi-illumination as described in a recent Biomedical Optics Express article [1]. The source is a diode laser at 660nm coupled into the microscope through a multi-mode optical fiber after passing through a rotating diffuser. Koehler illumination is used to image the aperture stop in the entrance pupil of the microscope objectives. The Linnik interference objective uses two identical microscope objectives where one is imaged on a flat reference surface and the other on the sample (see Figure 1). Nikon polarization microscope objectives at 20X NA 0.45 and 50X NA 0.8 are utilized. Additional magnification is obtained with a flip-in 2X field of view (FOV) lens to provide better sampling at the camera. The camera is an Illunis XMV-2020 with a Moxtek pixelated phase mask [1, 2].

Optical phase is determined by imaging in polarized light with a pixelated phase mask where the object is compared to a flat reference surface. For the examples in this paper, objects are viewed in double pass on a reflective surface as shown in Figure 1. This method yields quantitative depth/thickness values that are relative to the total optical thickness (actual integrated physical thickness times index of refraction). Areas that have higher index of refraction are more optically dense and appear thicker [1, 3].

A major feature of this microscope is the ability to take phase image snapshots at 15 fps (frames per second) to study changes in optical topography caused by cellular changes and motion. Because this technique utilizes spatial phase measurement techniques rather than temporal techniques, short exposures are possible (<1ms) to freeze motion while being insensitive to vibration [2, 3]. At the poster session examples of movies will be presented. For this summary representative stills are shown.

Figure 2 shows different types of images of a paramecium imaged at 50X obtained simultaneously at 15fps. Figure 2(a) shows one of the interferograms used to determine optical thickness (equivalent to phase contrast). Optical thickness is directly proportional to the optical phase difference between the reference and sample beams [1, 3]. (b) shows simulated differential interference contrast (DIC) calculated by finding the x gradient of the optical thickness. (c) shows simulated darkfield determined by calculating the gradient magnitudes of the optical thickness. (d) shows the optical thickness (OT) in pseudocolor. (e) shows a composite image of the OT over DIC. (f) shows a 3D surface topographic map of the OT. Cilia motion is quantifiable from time series image sequences.

As an example of dynamic cellular measurements, cardiac myocytes from 1-2 day old neonatal Sprague Dawley (Harlan, Indianapolis, IN) rats were prepared at the University of Arizona and plated onto #1 round coverslips. After 2 weeks of incubation the cells were measured in a Bioptechs FCS3 perfusion

chamber with an aluminized base plate and a 100µm gasket. Cells were kept at 37°C with an HBSS (Hank's balanced salt solution) fluid bath.
Figure 3 shows OT maps of an area of cells imaged at 40X (20X with 2X FOV lens) before and after pushing IPHC (isoproterenol hydrochloride – a beta adrenergic agonist). These images are the first in two time series of 200 datasets taken at 15 fps over a 13.3 sec time period. These images are the same 349 x 326 pixel subarea of 1050x1200 images. The optical volume of the subareas is determined by summing all the OT values and then scaling so that both traces have the same mean and same relative scaling. The actual physical volume is not obtainable from these data because the thickness of the cell culture and the index of refraction data are unknown.

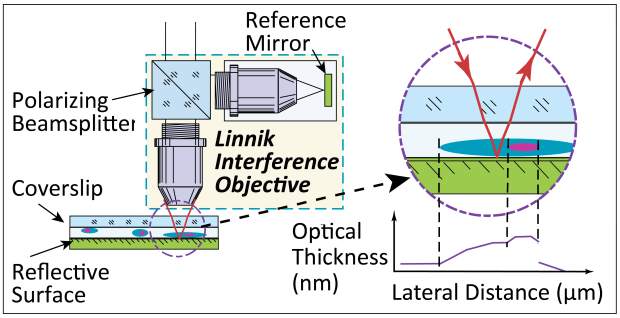

Figure 1. Sample configuration for measurements. Liquid gap is ~100µm with #1 coverslips (~150µm).

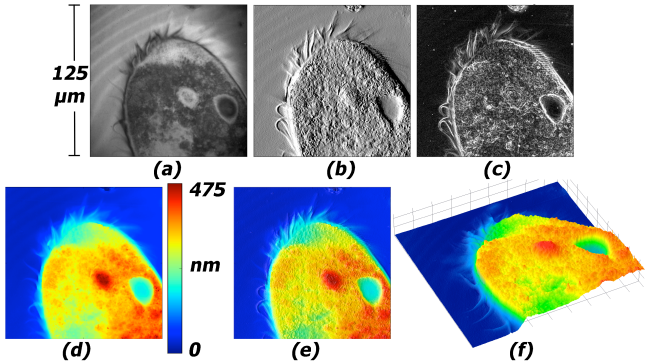

Figure 2. Images of paramecium in water. (a) Phase contrast. (b) DIC. (c) Dark field. (d) OT. (e) OT/DIC Composite. (f) 3D OT topography.

A major advantage of this technique is that relative physical changes can easily be measured. Figure 4 shows the relative optical volume for these two time series before and after pushing IPHC. These cells spontaneously beat (or twitch) about once every 4 seconds when in the HBSS at 37°C. After the IPHC is pushed, the beating frequency increases by a factor of 8 to twice a second and the flexion is about 3 times stronger. The optical volume is getting smaller in this subarea when the cells beat because the cells are noticeably stretching out and expanding during the beats.

These measurements show how quantitative optical phase and optical volume measurements can be used to study a group of cells over time. Data movies for these and other examples will be presented.

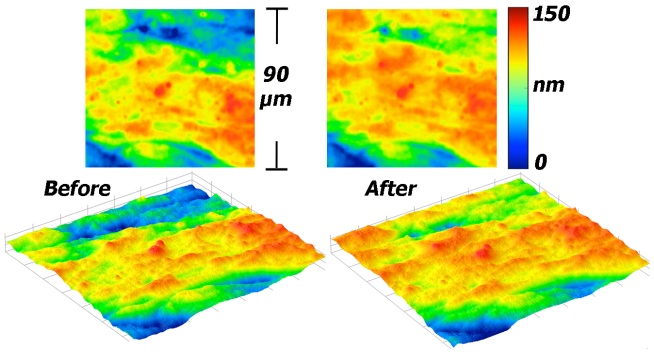

Figure 3. Rat cardiomyocyte OT maps (left) before and (right) after pushing IPHC.

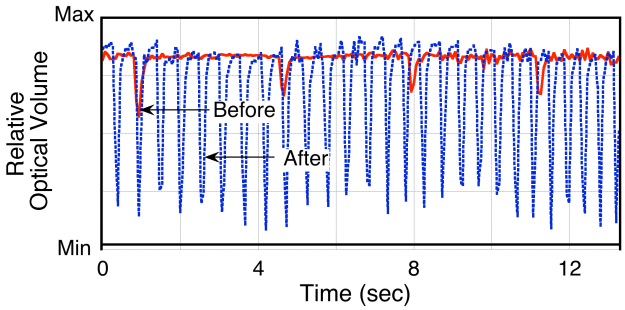

Figure 4. Relative optical volume over time series of 200 datasets. The same area of cells is compared (left) before and (right) after pushing IPHC.

References:

[1] K. Creath and G. Goldstein, *Biomedical Optics Express,* **3** (2012) p. 2866-2880.
[2] B. T. Kimbrough, *Appl. Opt.,* **45** (2006) p. 4554.
[3] K. Creath, *Proc. SPIE,* **7782** (2010) p. 77820B.
[4] The authors acknowledge Charles Crandall, Craig Weber, Ron Lynch, and Jordan Lancaster for their assistance in this project. This work partially supported by NIH/NCRR 1R43RR028170-01, 2R44RR028170-02, and NIH/NIGMS 8R44GM103406-03.

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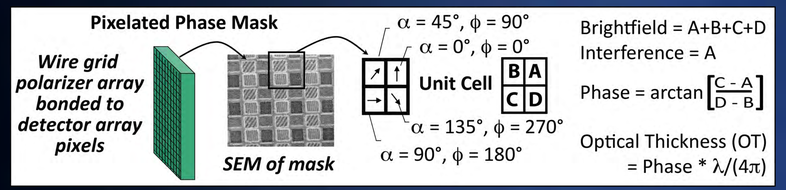
Abstract

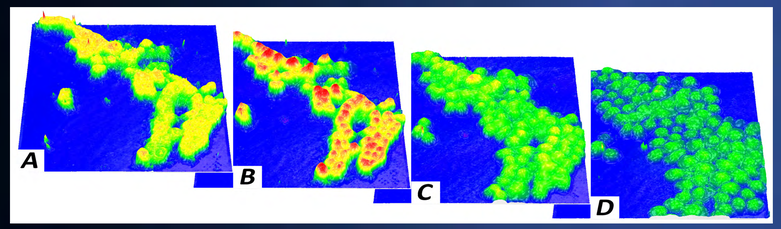
This microscope system obtains "Label-free" measurements of biological objects in epi-illumination using harmless light levels without the need for scanning and vibration isolation. 4-dimensional video measurements of dynamic motions of live cells are used to track cells and changes in morphology. A pixelated phase mask utilizing polarization properties of light enables simultaneous measurement of brightfield, phase contrast, phase, simulated DIC and darkfield images. Background surface shape is automatically removed to quantify changes. The examples shown here highlight various types of image processing and the monitoring of different biological processes.

Enabling Technology



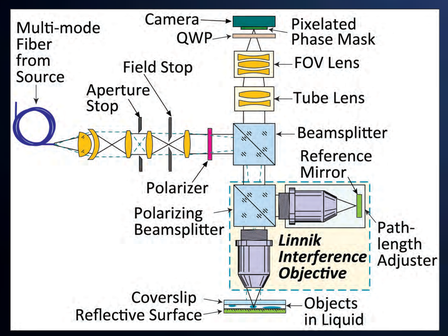
Like a color camera that sees in phase (or polarization), four relative phase shifts are obtained simultaneously to determine many different types of images. Fast data acquisition using short exposure times with a pixelated phase mask enables measurement of moving samples without blurring or scanning.

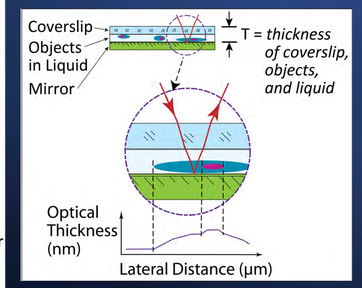
Quantify Dynamic Cellular Processes



Time series of 3D phase images of a breast cancer cell culture on a #1 coverslip. All images have the same optical thickness pseudocolor scale from 0 to 550 nm. (A) Cells in original media. (B) After contact with purified water. (C) After more purified water. (D) After further contact with NaOH.

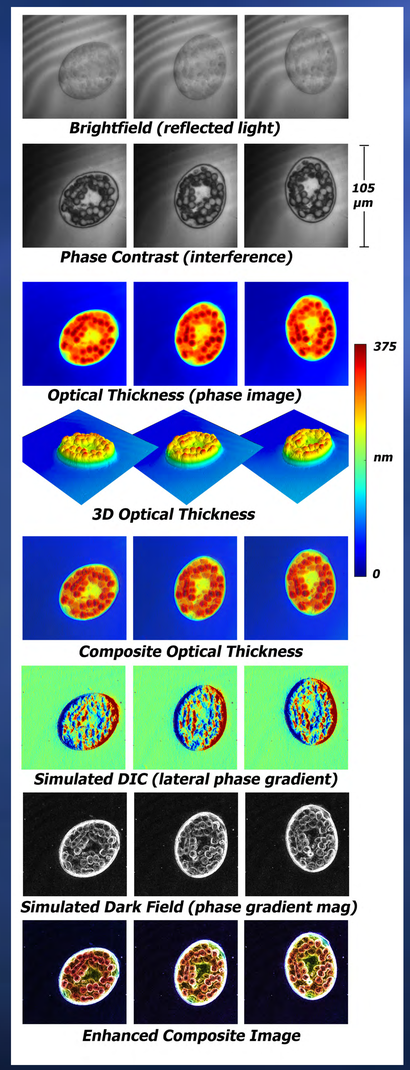
Optical Layout



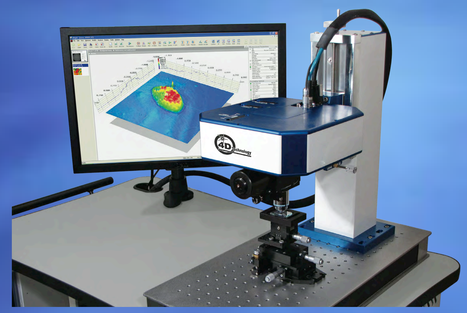


(Left) Microscope schematic for epi-illumination with a Linnik interference objective. **(Right)** Transparent samples in liquid are imaged under a coverslip on a reflective surface. This system measures relative integrated optical thickness (OT) [or optical path difference (OPD)]. OT is related to both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

Multi-Channel Cell Imaging



Time series of a moving protozoa (50 x 80 μm) taken at 50X with a 660 nm source and 250 μs exposures.



BioCam: Dynamic Quantitative Microscope.

Microscope Specifications

- Objectives
  - 50X (NA 0.8)
  - 20X (NA 0.45)
- 1X or 2.25X FOV lenses
- Sources: 660, 785, 532 and 460nm
- Fast data acquisition – no scanning
- Real-time processing
- Vibration insensitive
- Track temporal events

- Simultaneously obtain
  - Optical thickness (calculated from phase)
  - Phase (from all 4 channels)
  - Brightfield (average of 4 channels)
  - Phase contrast [interference] (single channel)
  - Simulated Dark field (phase gradient magnitude)
  - Simulated D.I.C. (phase gradient)

References

[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] Creath, K., and Goldstein, G., "Processing and improvements in dynamic quantitative phase microscope," Proc. SPIE **8589**, 85891A (2013).

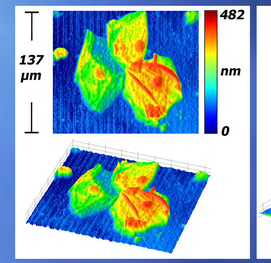
[3] Goldstein, G., and Creath, K., "Dynamic four-dimensional microscope system with automated background leveling," Proc. SPIE **8493**, 84930N (2012).

[4] Creath, K., "Dynamic quantitative phase images of pond life, insect wings, and in vitro cell cultures," Proc. SPIE **7782**, 77820B (2010).

Applications

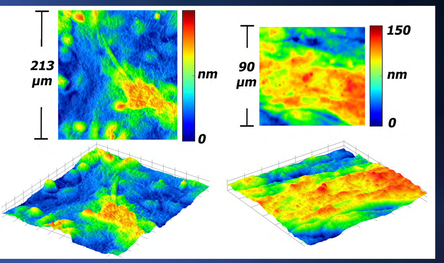
- Morphological Studies
- Mechanistic Studies
- Tissue Dynamics
- Quantify & Track Cellular Motion
- Process Monitoring
- Quantify Cellular Changes with Treatment

Epithelial Cells



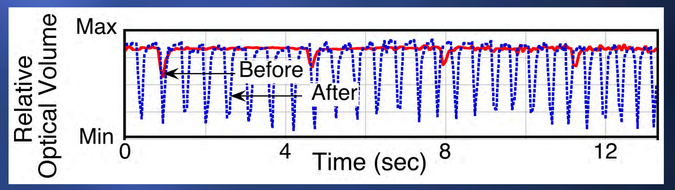
Optical thickness and 3D topographic maps of human epithelial cells taken at 40X with a 660nm source.

Beating Cardiac Myocytes



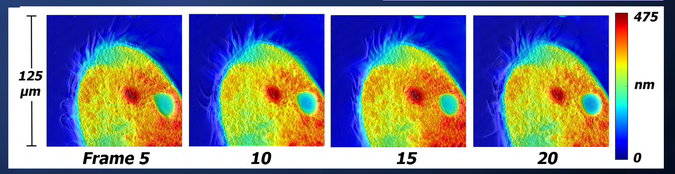
Rat cardiac myocyte cells cultured on #1 coverslip. Measured in a perfusion chamber at 37°C with 40X at 660nm. Optical thickness and 3D topographic maps of two areas. Data series taken at 15 fps for analysis below.

Optical Volume Before & After Treatment



Relative optical volume over time series of 200 datasets. The same area of cells (above right) is compared before and after pushing IPHC.

Track Motion



Time series of a ciliate taken at 50X with a 660 nm source and 250 μs exposures.

Acknowledgements

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