

Dynamic Phase Imaging Interference 4D Microscope System

Phase I SBIR NIH NCRR

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Overview

We have developed an innovative, highly flexible, dynamic 4D interference microscope system prototype capable of creating 4-dimensional, quantitative, full-field, phase-image movies of live cells (Figs. 1-2). A primary use of this optical system is to enable instantaneous and time-lapse measurements of dynamic motions within and among live cells without the need for contrast agents. This "label-free," vibration insensitive, imaging system enables measurement of biological objects in reflection over a field of view ranging from a few hundred micrometers to several millimeters with NAs ranging from 0.05 to 0.95, wavelengths from 400-1000nm, and time scales of fractions of seconds to days.

At the core of the instrument is a novel phase-measurement camera (PMC) enabling simultaneous measurement of multiple interference patterns utilizing a pixelated phase mask taking advantage of the polarization properties of light. This patented technology, originally invented for testing large telescopes in situ, is a unique and innovative means of capturing phase images. Utilizing this technology with application-specific software enables the creation of a phase image movies in real time at video rates so that dynamic motions can be tracked.

Highlights

- Dynamically measures optical thickness (Fig. 3)
- Live objects do not need to be still
- No contrast media necessary
- Watch processes in real time
- Get quantitative bright field, phase contrast and phase data all at once.

Goals

We are now completing Phase I and are preparing our Phase II SBIR Proposal. We are looking for applications and research partners for Phase II that can showcase this technology and demonstrate the commercial viability of this technology.

Possible applications

- | | |
|--|------------------------|
| • Morphological and volumetric studies | • Flow cytometry |
| • Mechanistic studies | • Tissue dynamics |
| • Quantify cellular motion | • Track cell migration |
| • Process monitoring (e.g. apoptosis) | • Nerve transmission |
| • Monitor and track cellular damage | • Photodynamic therapy |
| • Quantify cellular changes with treatment | |

Contacts

If you would like more information or have a good application for this technology, please contact the PI's Kathy Creath kcreath@ieee.org and James Millerd james.millerd@4dtechnology.com.

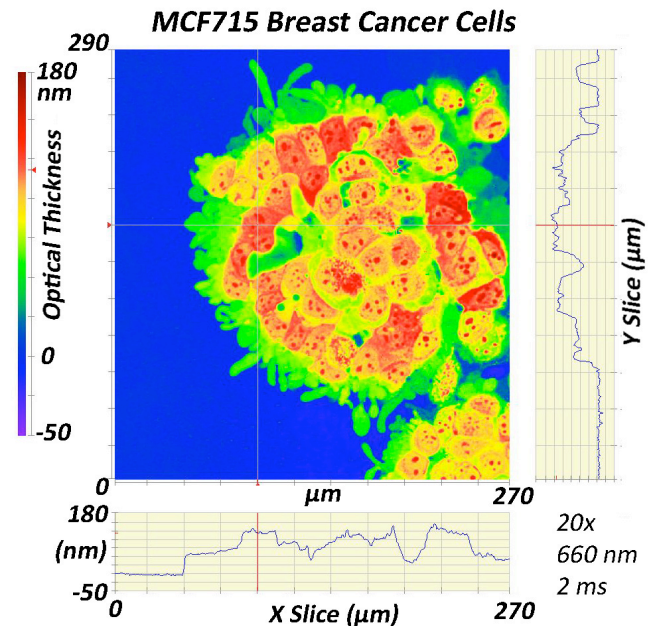


Figure 1. Phase image of breast cancer cell culture taken at 20X with a 660 nm wavelength and 2 ms exposure. No stain or labeling used. Notice detail of organelles within cells and intercellular matrix.

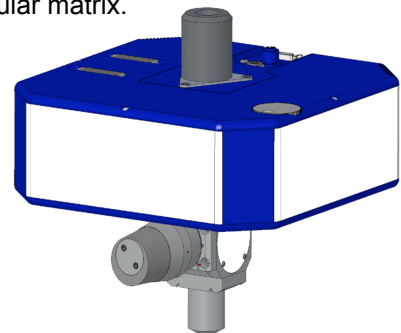


Figure 2. Conceptual drawing of prototype 4D phase imaging microscope with Linnik interference microscope objective on bottom.

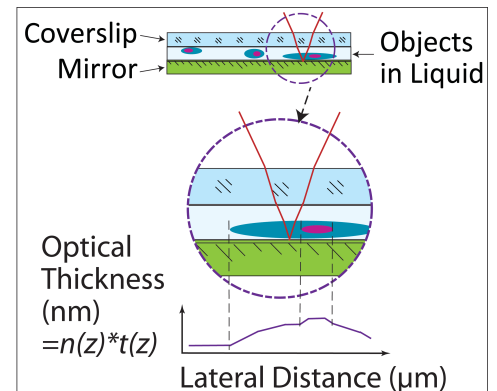


Figure 3. Objects are sandwiched in water or media between a highly reflective mirror and a coverslip (top). This microscope measures optical thickness (OT) which is the index of refraction $n(z)$ times physical thickness $t(z)$. Denser objects have a larger OT.

Examples

Figure 1 shows a phase image of a breast cancer cell culture grown on a coverslip and placed on top of a highly reflective mirror (Fig. 3). The pseudocolor scale represents the optical thickness which is the index of refraction times the physical thickness as a function of depth.

After the cells in Fig. 1 were exposed to AZCC purified water and then KCL we see that there are changes that are quantifiable in terms of optical thickness and cell volume (Fig. 4). Fig. 4A shows that the cells are not even visible in brightfield illumination while Fig. 4B shows one of the four interferograms used to calculate the optical thickness shown in Fig. 4C-D. The differences between Figs. 1 and 4 indicate quantifiable differences in cell morphology as a function of perturbation.

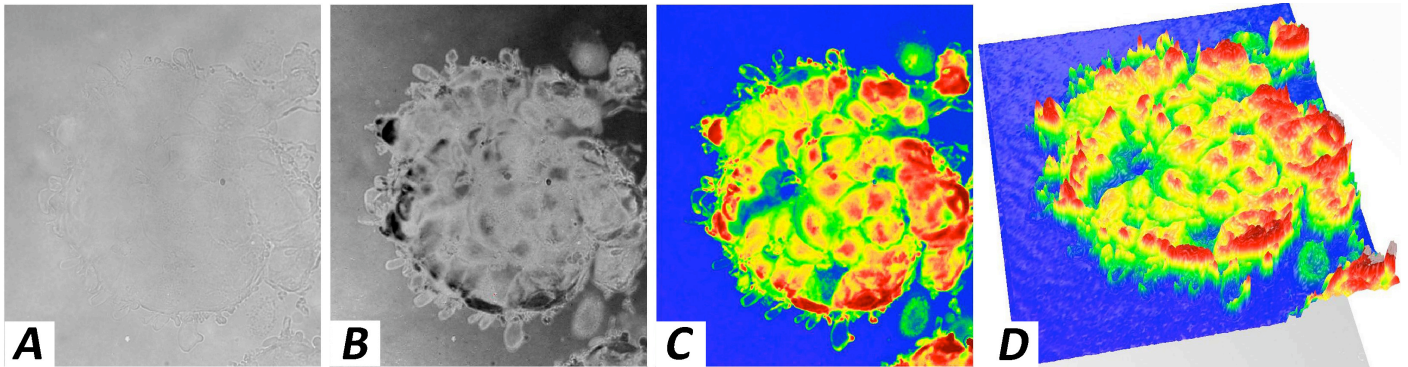


Figure 4. (A) Bright field image of breast cancer cell culture of Fig. 1 taken after contact with AZCC purified water and then KCL. (B) One of four interferogram channels measured showing raw data. This is similar to what you'd see with DIC or phase contrast. (C) Phase image with pseudo-color representation of optical thickness scaled from -50 to 220 nm. This is calculated from a combination of all 4 interferogram channels. Notice swelling of cells and shriveling of cilia compared to Fig. 1. (D) 3D plot of (C).

Figure 5 shows a time series of another group of breast cancer cells swelling and breaking down after being exposed to AZCC purified water followed by NaOH taken again at 20X. QuickTime movies of these data can be viewed at <http://www.optics.arizona.edu/creath/files/phasemovies.html>.

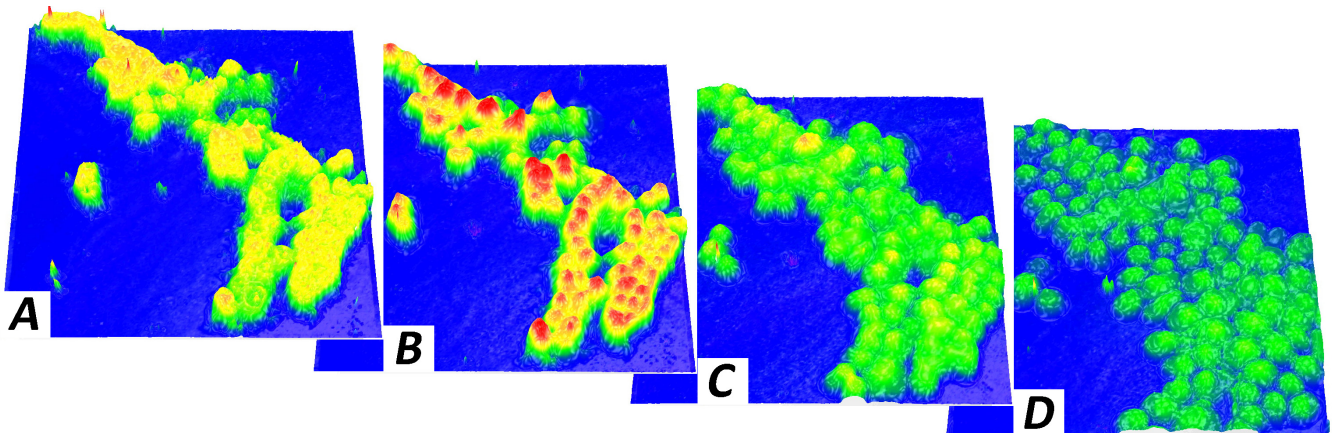


Figure 5. 4D Time series of 3D phase images of another breast cancer cell culture. All images have same optical thickness pseudocolor scale from -100 to 450 nm. (A) Cells in original media. (B) After contact with AZCC purified water the cells swell. (C) After more purified water the cells swell and flatten. (D) After contact with NaOH the cells are beginning to break down. Processes can be monitored with specified time delays as short as 30 frames / second. These are a sampling of movies taken with sampling times of a few seconds over several minutes.

Figure 6 shows measurement of a rotifer, which is a more complex object having about a thousand cells. The tail is at the lower right and the mouth at the upper left. Note the detail in the internal organs. Dynamic microscopic phase imaging enables measurement of quantitative optical thickness for volumetric, mechanistic, and morphological studies of cells and simple organisms. Movies and images of the rotifer can be viewed at <http://www.optics.arizona.edu/creath/files/phasemovies.html>.

Figure 6. Phase image showing optical thickness of a rotifer taken at 10X with a 785 nm source.

