New Directions in Interferometric Phase Microscopy of Biological Cell Dynamics

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Abstract: Interferometric phase microscopy has the potential of becoming a widely-used tool for quantitative measurements of biological cells. We introduce the current state of the art, the open questions, and solutions experimentally developed in our laboratory.

OCIS codes: (090.1995) Digital holography, (090.2880) Holographic interferometry, (170.3880) Medical and biological imaging, (180.3170) Interference microscopy.

Rapid, dynamic processes in biological cells can occur over millisecond to second time scales. Membrane fluctuations, cell swelling, neuronal dynamics, and movement-related phenomena are only a few examples of dynamic processes of interest in cell biology. Visualizing these fast dynamic phenomena requires developing wide-field microscopy techniques that can achieve high data acquisition rates, while retaining resolution and contrast to enable the measurements of fine cellular features. However, biological cells are mostly-transparent three-dimensional objects that are very similar to their surrounding in terms of absorbance and reflection, and thus conventional intensity-based light microscopy lacks the required contrast. As a solution, exogenous contrast agents such as fluorescent dyes are used. However, fluorescent contrast agents tend to photobleach, reducing the available imaging time. Other concerns include potential cytotoxicity and the possibility that the agents will influence cellular behavior. As an alternative, phase microscopy can provide label-free information on cellular structure and dynamics. Traditional phase microscopy methods, such as phase contrast and differential interference contrast microscopy, are widely used today. However, these approaches are not inherently quantitative and present distinct imaging artifacts. Thus, they do not enable interpretation of the resulting phase images in terms of quantitative optical path delays. Wide-field digital interferometry (WFDI), on the other hand, has the potential to provide a powerful, nondestructive tool for quantitative phase measurements of biological cell dynamics [1-3].

WFDI is based on a holographic approach in which one measures the interference pattern composed of the superposition of the light field interacted with the sample and the mutually-coherent reference field. With this approach, the entire wavefront describing the sample is captured. From the recorded complex field, one can digitally reconstruct the quasi-three-dimensional distribution of the sample field, without the need for mechanical scanning. Figures 1-4 show experimental results obtained in our laboratory by using specially-designed WFDI techniques.

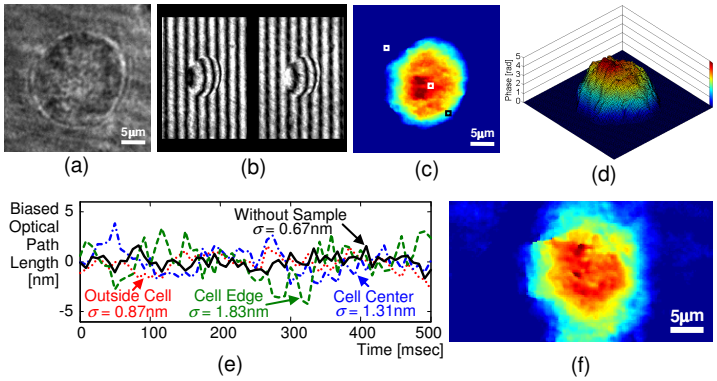

Fig. 1. (a-e) WFDI quantitative phase microscopy of human breast cancer (MDA-MB-468) cell in growth media: (a) Intensity image through the system (low visibility); (b) Two phase-shifted interferograms of the sample captured in a single camera exposure; (c) Final unwrapped phase profile; (d) Surface plot of the phase profile shown in (c); (e) Temporal phase stability without the sample, and with the sample in the three points marked in (c); (f) The final unwrapped phase profile of a rat beating myocyte [1]. Video demonstrations are available in www.opticsinfobase.org/ol/abstract.cfm?URI=ol-34-6-767.

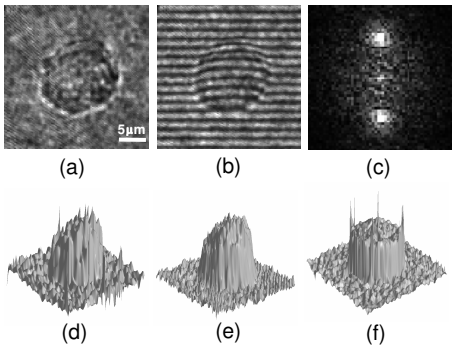

Fig. 2. WFDI quantitative phase microscopy of live human skin cancer (A431, epithelial carcinoma) cell in growth media: (a) Intensity image through the system; (b) Single off-axis interferogram; (c) Spatial spectrum of this interferogram. Surface plots of the final unwrapped phase obtained by: (d) traditional off-axis geometry (old method), (e) slightly-off-axis geometry (new proposed method), and (f) traditional on-axis geometry (old method) [2].

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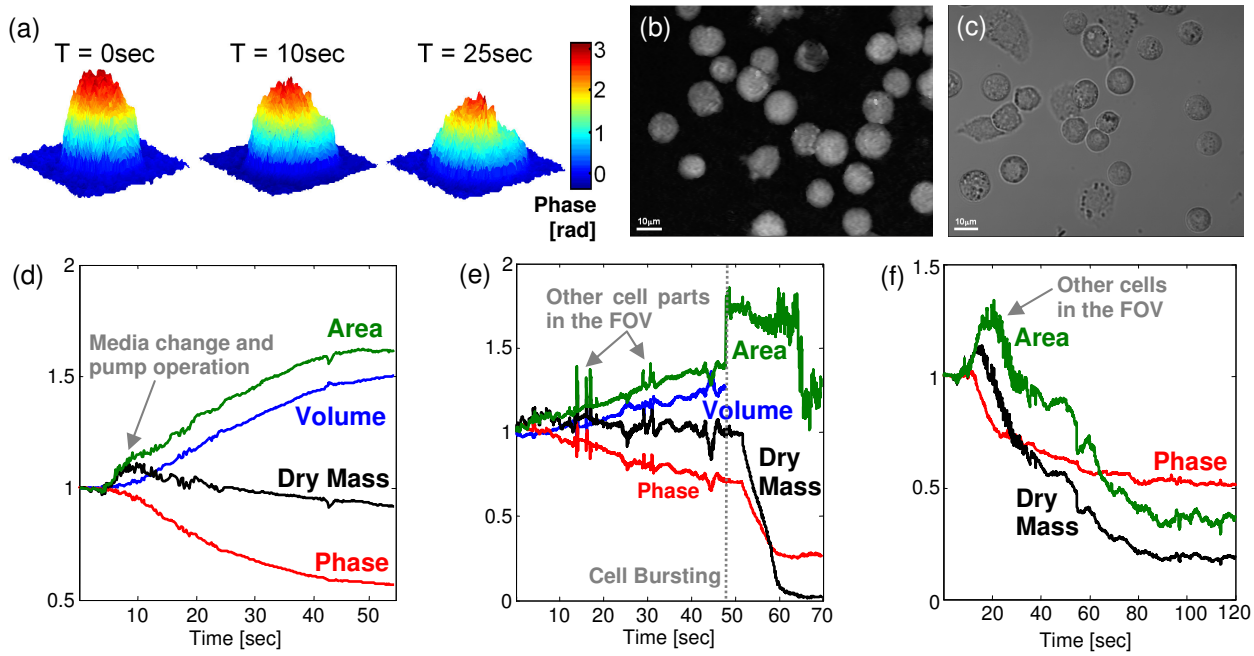

Fig. 3. Phase microscopy of articular chondrocyte fast dynamics due to hypo-osmotic pressure: (a) Surface plots of WFDI-based phase profiles of a chondrocyte in several different time points; (b) WFDI-based phase profile of the cell monolayer, acquired at 120 frames per second (fps); (c) Phase image of the cell monolayer obtained by confocal DIC microscopy, acquired at 0.75fps which was the maximal frame rate possible for this field of view with the confocal microscope used (brought here for comparison to fast acquisition capability of WFDI). Video demonstrations are available in [3]; WFDI-based graphs of the relative change in various cell morphological parameters during: (d) single-cell swelling (partially visualized in (a)), (e) single-cell swelling and bursting, and (f) cell monolayer dynamics (partially visualized in (b)).

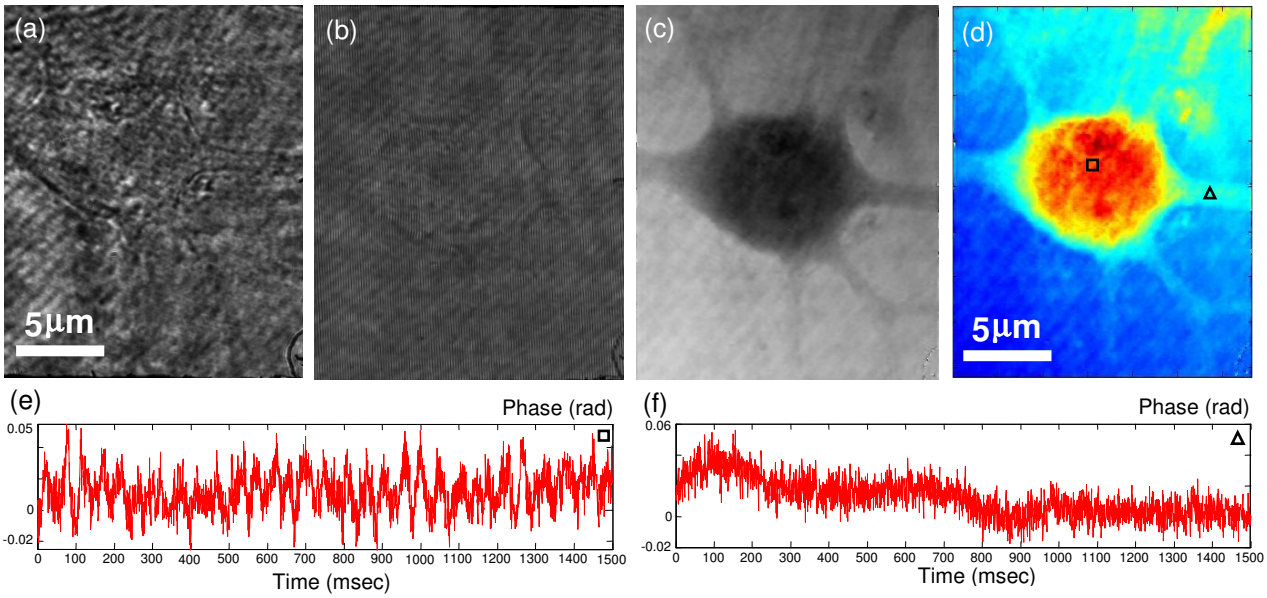

Fig. 4. WFDI quantitative phase microscopy of hippocampal neuron dynamics, recorded at 2000 frames per second: (a) Regular intensity image through the system (low visibility, only edges are seen); (b) Neuron interferogram; (c) Final unwrapped phase (optical-path-delay) profile obtained by the digital interferometric processing; (d) The phase profile with digital coloring. No exogenous contrast agents were used; (e) Neuronal phase dynamics on the neuron body (marked by a square in (d)); (f) Neuronal phase dynamics on the dendrites (marked by a triangle in (d)). Different dynamic behaviors are seen. Similar results were obtained by checking many other similar points on the neuron body and dendrites.

WFDI has several limitations that have prohibited it from being a widely-used tool for quantitative measurements of biological cell dynamics, in spite of its many attractive advantages. The main limitations are shortly elaborated in the following.

a. Time resolution – camera bandwidth consumption tradeoff: The interferometric signal contains, except for the desired sample field, unwanted dc and twin-image diffracted waves. Off-axis interferometry copes with this problem by imposing a large angle between the reference and sample beams, which creates a spatial separation between the desired and undesired waves at the digital camera plane. However, this approach comes at the expense of ineffective use of camera bandwidth, which, for an image interferogram, means that high spatial frequencies in the sample field might be lost. For acquiring the phase profiles of certain dynamic biological processes, the requirement for the digital camera frame-rate might be demanding and such frame-rates are frequently obtained by using less camera pixels per frame, while further narrowing the camera spatial bandwidth.

An alternative approach to more effectively use the camera spatial bandwidth is the on-axis interferometry. In this approach one sets the angle between the sample and reference beams to zero. This results in a required camera bandwidth that is the same as that needed for acquiring the sample intensity image alone but, on the other hand, also causes the undesired diffracted waves to occlude the desired sample field. The traditional solution to this problem is to acquire three or four phase-shifted on-axis interferograms of the same sample and to digitally separate the sample field through digital signal processing. However, for dynamic processes, the sample may change between the acquisitions of the multiple frames. In addition, phase noise may increase due to system fluctuations between the frames. Methods for acquiring all on-axis interferograms in a single camera exposure have been proposed. However, since at least 3 interferograms are needed, the camera bandwidth is not effectively utilized. By optimizing this tradeoff, one can achieve both high time resolution and an effective use of the camera bandwidth, enabling phase profile acquisition of biological cells containing fine details across as a large field of view as possible, while avoiding the loss of time resolution that is required for imaging fast biological cell phenomena.

b. Stability and sensitivity of the interferometric system: Interferometric optical systems usually contain beam splitters that divide the beam into reference and sample arms and then combine these two beams at the detector. The two beams usually pass through different paths and thus collect different area-dependent noise features. For example, air perturbations can be different in the sample and reference arm paths. Furthermore, these area-dependent noise features might change temporally, which negates the possibility of measuring them offline before the recoding period in order to cancel them later. Speckle noise, laser power stability, detector noise, and mechanical stability of the optical system are additional problems that one has to cope with when imaging dynamic processes by WFDI. In addition, when imaging fast dynamic phenomena, the detector integration time might have to be short, yielding low intensities at the detector plane and a low signal-to-noise-ratio phase profile. It is not always possible to simply increase the laser power to solve this problem, since this might damage the biological sample. The goal is thus to design the interferometric system so that the signal of interest level will always be kept above the noise level.

c. Specificity inside cells: Many biological cells are at the range of 5-20 micron thickness. Cells contain internal organelles that might be of specific interest for medical and biomedical studies. For example, the cell nuclear characteristics have been recognized as histological markers for the genetic and epigenetic changes leading to cancer [4]. However, WFDI acquires quasi-three-dimensional profiles, which does not yield complete sectioning through the image since out-of-focus light coming from different axial layers in the sample is also detected. WFDI provides whole-cell information that lacks the specificity for identifying subcellular components. Solving this limitation can offer additional information that is currently missing with the presently-used WFDI techniques.

d. From phase profiles to quantitative-functional analysis: WFDI is a quantitative recoding technique. However, simple quasi-three-dimensional visualization should not be the end of the process. A quantitative analysis should permit extraction of numerical parameters which are useful for cytology or medical diagnosis. If acquired by a transmission-mode interferometric setup, the resulting phase profiles represent the multiplication between the index of refraction differences and the geometrical path delays. Local changes in the index of refraction may occur during action potential for example or whenever there is an ion flux inside the cell. Independently or not, geometrical path changes can occur due to movement of intracellular components. Thus, these conjugated parameters, the index of refraction differences and the geometrical path delays, may not be distinct when acquiring the phase profile of a dynamic cell. This fact should be considerred during the system development and the following data analysis.

Our laboratory is currently developing new approaches for coping with each of the problems described above. These approaches, the related interferometric microcopy systems, and experimental demonstrations on various types of biological cell dynamic behaviors will be discussed in this invited lecture.

References

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