Quantitative Phase Microscopy of Live Biological Cell Dynamics

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Abstract. Interferometric phase microscopy of biological cell dynamics has the potential to provide a label-free quantitative tool for cell biology, as well as for medical diagnosis and monitoring. The current state of the art of this field, the open questions, and specific solutions developed in our laboratory will be presented.

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INTRODUCTION

Biological cells are microscopic dynamic entities, continuously adjusting their three-dimensional sizes, shapes and other biophysical features. Dynamic processes in biological cells last from days to less than milliseconds. For recording rapid cell dynamics, wide field microscopy should be preferred to scanning microscopy due to the fact that in the latter, the dynamics recorded might be faster than the scanning rates. If successful, wide-field acquisition of rapid dynamic cell phenomena can provide a powerful cell-biology research tool. In addition, correlating the variations in the cell dynamic response with cell function changes can identify breakdowns in the feedback pathways that can arise from dysfunction due to a disease. Thus, wide-field recording of cell dynamics can also provide a tool for medical diagnosis and monitoring of diseases.

Nevertheless, biological cells are mostly-transparent objects, and thus conventional intensity-based light microscopy fails in providing enough contrast between the cell and its environment. Exogenous contrast agents such as fluorescent dyes are widely used to solve this problem. However, contrast agents are partially cytotoxic and there is a possibility they will influence the cellular behavior. In addition, fluorescence dyes particularly tend to photobleach, potentially limiting the imaging time.

An alternative solution to the contrast problem of imaging biological cells is phase microscopy. Using this approach, one can obtain information on the cellular structure and dynamics noninvasively, meaning without using any exogenous labeling. Detectors are sensitive to intensity only, and thus to be able to record the phase of the light that has interacted with the cells, one needs to use a method that converts phase into intensity variations. Widely-used methods to perform this phase recording are the phase contrast microscopy (PhC) and differential interference contract microscopy (DIC) [1]. However, these approaches are not inherently quantitative and present distinct imaging artifacts that make it hard to correctly interpret the resulting phase images in terms of optical path delays.

Wide-field digital interferometry (WFDI), on the other hand, is able to record the entire complex wavefront (amplitude and phase) of the light interacted with the sample. Using a holographic approach, the digital camera records the interference (intensity) pattern composed of a superposition of the light field which has interacted with the sample and the mutually-coherent reference field. From the recorded complex field, one can digitally reconstruct the quasi-three-dimensional distribution of the sample field, without the need for mechanical scanning. Several experimental results obtained in our laboratory by using specially-designed WFDI techniques are presented in Figures 1-4 [2-4].

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In practice, in spite of its many attractive advantages, WFDI has been less-commonly used for recording dynamic behaviors of live cells compared to other label-free phase microscopy methods such as PhC and DIC. This situation might change in the near future by careful consideration of the WFDI limitations and their possible solutions. These special considerations are shortly elaborated next.

TIME RESOLUTION – CAMERA BANDWIDTH CONSUMPTION TRADEOFF

As mentioned above, the interference pattern recorded in WFDI is composed of a laser beam that has interacted with the sample and a mutually-coherent beam that arrives directly from the laser source, typically without interacting with the sample. This way, although the digital camera is sensitive to intensity only, the sample field, that includes

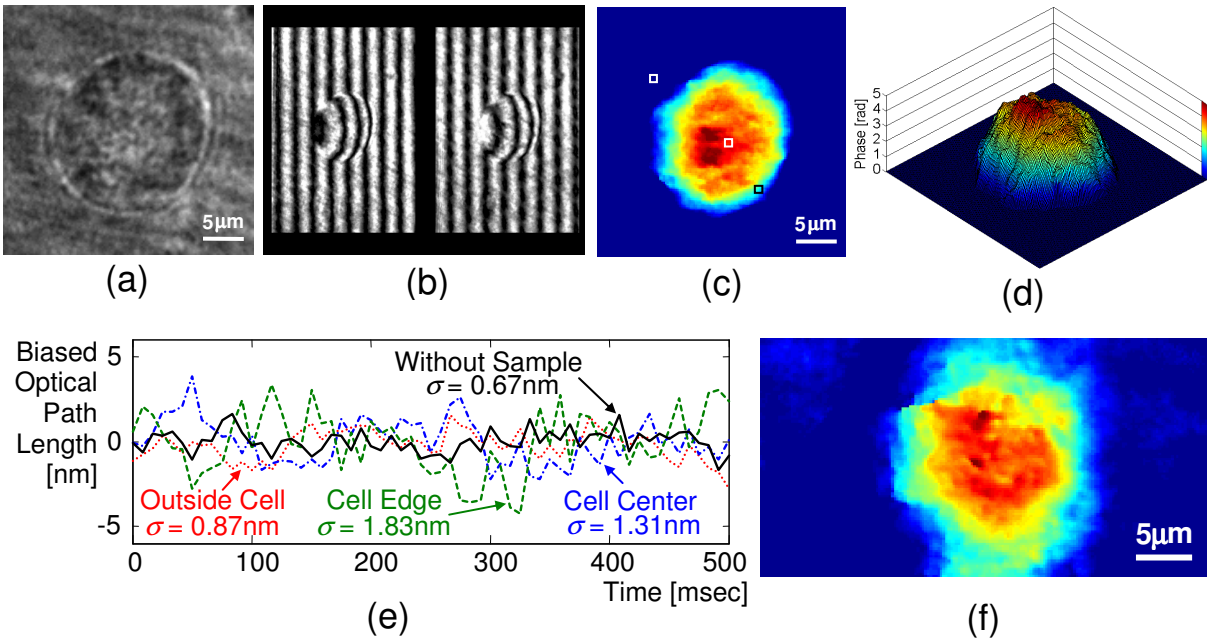

FIGURE 1. (a-e) WFDI quantitative phase microscopy of a human breast cancer (MDA-MB-468) cell in growth media: (a) Intensity image through the system (low visibility); (b) Two phase-shifted interferograms of the sample captured in a single camera exposure; (c) Final unwrapped phase profile; (d) Surface 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 [2]. Video demonstrations are available in www.opticsinfobase.org/ol/abstract.cfm?URI=ol-34-6-767.

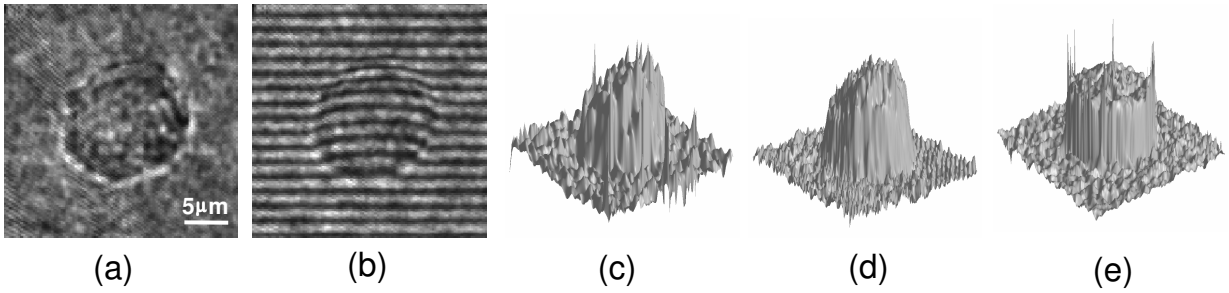

FIGURE 2. WFDI quantitative phase microscopy of live human skin cancer (A431, epidermoid carcinoma) cell in growth media: (a) Intensity image through the system (low visibility); (b) Single off-axis interferogram; Surface plots of the final unwrapped phase obtained by: (c) traditional off-axis geometry (old method), (d) slightly-off-axis geometry (proposed method), and (e) traditional on-axis geometry (old method) [3].

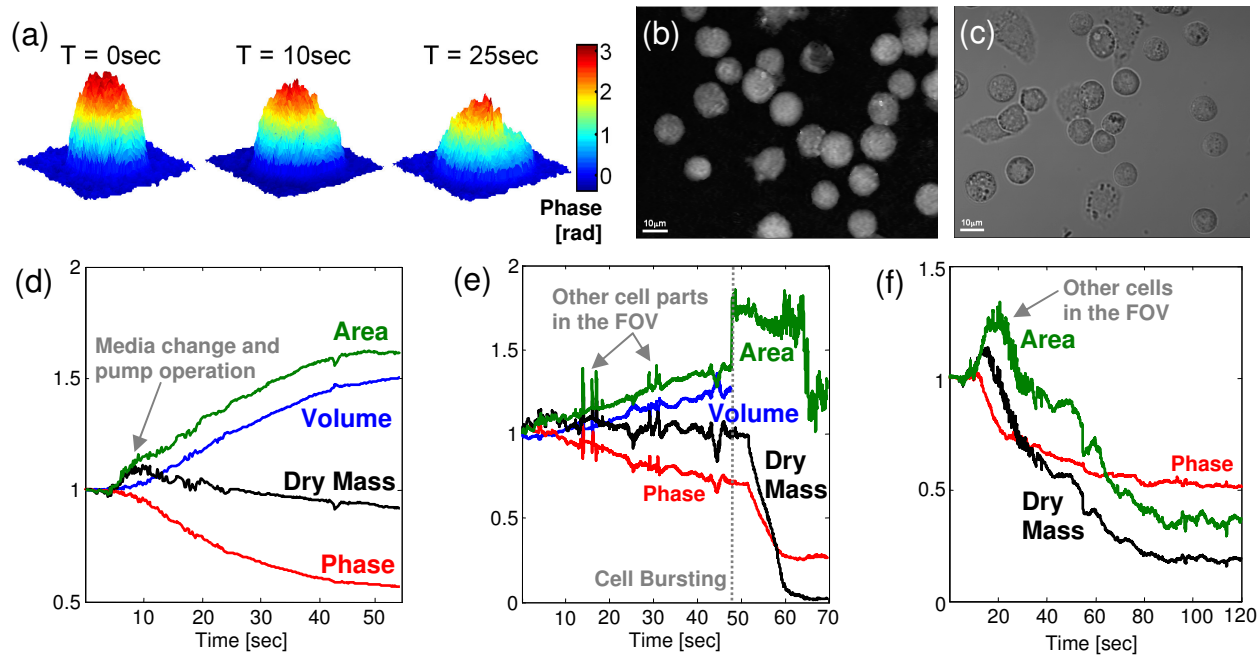

FIGURE 3. Articular chondrocyte fast dynamics due to hypo-osmotic pressure: (a) WFDI-based surface plots of the phase profiles in several different time points; (b) WFDI-based phase profile of the cell monolayer, acquired at 120 frames per second; (c) Phase image of the monolayer obtained by DIC microscopy (brought here for comparison). 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)) [4].

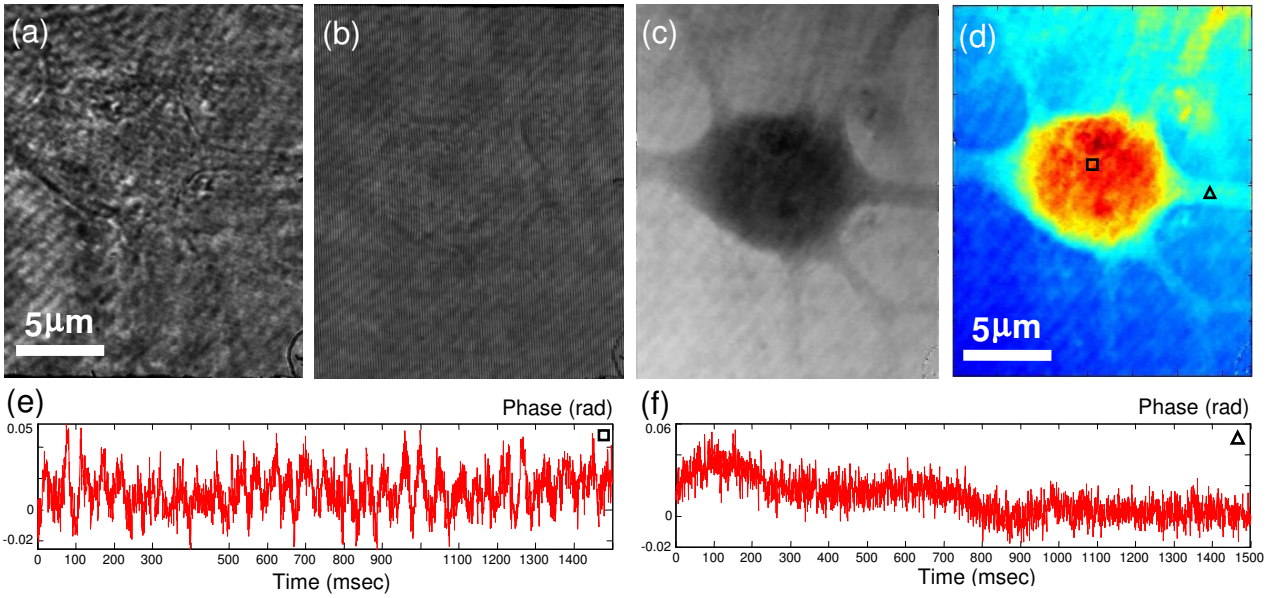

FIGURE 4. WFDI quantitative phase microscopy of a hippocampal neuron, recorded at 2000 frames per second: (a) Regular intensity image through the system (low visibility, only edges are seen); (b) Neuron interferogram; (c) Final unwrapped phase profile obtained by the digital interferometric processing; (d) The phase profile with digital coloring. No 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 inspecting many other similar points on the neuron body and dendrites.

the sample phase profile, is recorded by the digital camera. The problem is that the interferometric signal contains, except for the desired sample field, unwanted dc and twin-image diffracted waves. In order to reconstruct the sample phase profile, these unwanted waves have to be eliminated during the digital processing stage. Off-axis interferometry [5-8] 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. However, this approach comes at the expense of ineffective use of the camera spatial-frequency bandwidth, which means that high spatial frequencies (or alternatively the captured field of view (FOV)) of the recorded sample field might be lost. For acquiring the phase profiles of rapid 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. Thus, when the camera bandwidth is intentionally limited in order to obtain high frame rates, camera bandwidth consumption is an important factor. Therefore, although traditional off-axis interferometry requires a single exposure for acquiring the sample field, it might not always be the best choice for recoding cell dynamics, due to the fact that it is wasteful with the camera spatial bandwidth.

An alternative approach to more effectively use the camera spatial bandwidth is the on-axis interferometry [9-11]. 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 by using all acquired interferograms together. This approach may not be practical for dynamic processes, where the sample may change between the several frames of acquisition. In addition, phase noise may increase due to system fluctuations between the frames [12].

Hence, there is a clear tradeoff between the time resolution and the camera spatial bandwidth consumption and both approaches, on-axis interferometry and off-axis interferometry, have their own disadvantages for phase microscopy of cell dynamics. These disadvantages have to be carefully considered when designing the WFDI system, and finding the optimal working point, in which one can optimize both time-resolution and camera spatial-bandwidth consumption, is frequently required to obtain better dynamic phase imaging capabilities. Our laboratory has developed two different approaches [3,13] for optimizing time resolution and camera bandwidth consumption in WFDI, as will be presented in the lecture.

PHASE NOISE, STABILITY AND SENSITIVITY

Interferometric optical systems typically 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 [12]. 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. Our laboratory is developing a new type of common-path interferometer, which can help in reducing phase noise.

PHASE UNWRAPPING PROBLEM

After using the interferogram to extract the complex field of the sample, an arctangent operation is typically applied to calculate the sample phase profile. This calculation is inherently limited to a range of $-\pi$ to π, and thus one cannot directly measure the phase profile in an ambiguous way. Many algorithms have been developed to detect discontinuities of 2π in the phase profile and remove them [14]. However, the presence of noise in the wrapped phase profiles might cause the digital algorithm to incorrectly unwrap these profiles. Another complication is the case where there is a true phase changes larger than π between two adjacent points, which breaks the assumption of the digital unwrapping algorithms that the phase function is slowly varying. In addition, these digital algorithms should not be too computationally heavy, to allow working with large phase images rapidly.

An alternative approach to the solely post-processing digital unwrapping algorithm is to change the experimental setup and acquire additional information that can help one detecting and solving the unwrapping

problem. For example, the use of two or more illumination wavelengths [15-17] has been proposed an alternative approach to the unwrapping digital algorithms mentioned above. Using this method, one can extend the unambiguous phase measurement range to a level of the synthetic "beat" wavelength between the two illumination sources. As currently performed by our laboratory, it is possible to design the system to acquire the interferograms at several wavelengths simultaneously, which is well suited for cell dynamic measurements.

LACK OF SPECIFICITY INSIDE CELLS

Biological 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 [18]. 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. Hence, WFDI provides whole-cell information that lacks the specificity for identifying subcellular components. Solving this limitation can offer additional information that is missing with the presently-used WFDI techniques. Our laboratory is developing two different approaches to cope with this problem. The first one is based on the addition of tagging agents that can be detected by WFDI, where the use with these tagging agents can create specificity inside cells. The second approach is based on digital processing that is capable of rejecting out-of-focus haze at a specific axial reconstruction plane.

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 considered during the system development and the following data analysis. Our laboratory has applied quantitative analysis to articular chondrocytes that change their volume quickly due to stress [4]. Based on the acquired wide-field phase profiles, we have shown that it is possible to obtain real-time key morphological data for both a single cell and population of cells in a monolayer, while being limited only by the true frame rate of the digital camera.

CONCLUSIONS

In this manuscript, we have shortly reviewed the advantages of WFDI for quantitative phase microscopy of biological cell dynamics. We have also presented the main limitations of this technique and mentioned several solutions that are currently developed in our laboratory for coping with each of these limitations. By solving these inherent restrictions, we expect that WFDI will be much more widely used for recording biological cells dynamics, where commercial microscopes and clinical devices that are based on WFDI will be available soon.

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