Lens-free interference microscopy for detection of minute quantity of materials

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We present a novel, low-cost and portable lens-free microscope for measuring ultrathin and highly transparent samples with sub-nanometric axial resolution. We utilized the proposed device to measure microarrays of biomarkers and its changes after binding events, providing a very rapid and efficient way of analysing fluidic biological samples for specific proteins. Thanks to the wide field-of-view of the device, many different proteins can be detected at once during one measurement. Furthermore, the performance and portability of the microscope paves the way towards a rapid analysis of biomedical samples even in remote areas.

Keywords: lens-free microscopy, biosensing, portable, point-of-care, protein detection

Introduction

Optical microscopes have been an indispensable tool in laboratories all around the world for centuries. They offer reliable imaging of samples at high magnifications, however, there are some inherent limits in terms of device size (due to the geometry and use of lenses), field-of-view (FOV) being coupled to magnification or insensitivity to very thin or transparent samples. In particular, monitoring biomedical binding events or in-line production monitoring of transparent samples is not possible. Over the past years, rapid technological development lowered the cost of both computation and image capturing devices (namely CMOS sensors). This progress gave birth to a novel kind of devices called ***lens-free microscopes***. These overcome many of the aforementioned limits, while also offering new features and possibilities.

The goal of this project is to design and realize a portable, low-cost lens-free interferometric microscope with high resolution in the axis of the beam [1]. While such microscope would undeniably be very valuable for many different scientific fields, it's primarily being developed for point-of-care biological imaging and biomolecules detection [2]. Reliable protein detection in fluids (e.g. blood) is essential for diagnosis and assessment of patients' health, however, current analysis techniques require lots of time and cannot be effectively performed outside clinical laboratory. Both issues are resolved with the proposed device.

Methodology

The proposed device imaging relies on phase-contrast, similarly to how interferometers work. Collimated monochromatic light is polarized (P1) and passes a Savart plate (SP1). This special optical element consists of two stacked layers of birefringent crystals with specific orientation and divides the beam into two parallel sheared beams of perpendicular polarization (EPx, EPy). These beams then pass through the sample. Any additional minute layer of material or different refractive index in one of the two beams introduces an optical path difference (OPD). After the sample, both beams are interfered together using an inversely placed Savart plate (SP2). Depending on the optical path (=phase) difference, the beams will interfere constructively or destructively. After a second polarizer (P2), this interferometric pattern is captured by a camera and processed in a computer.

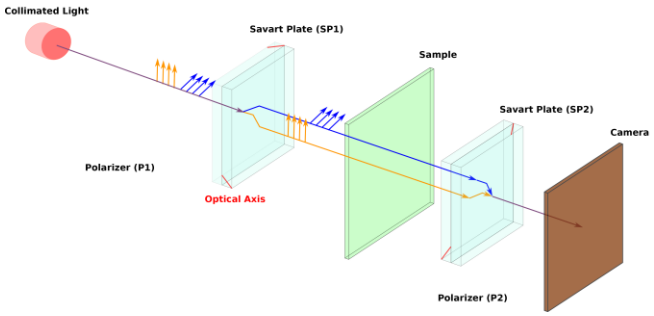

Image 1: *Schematic of the lens-free interferometric microscope. Every beam of light is split into two sheared beams and later recombined, resulting in intensity contrast depending on the sample-introduced phase-shift between the two sheared beams.*

To calculate the phase information from the interferograms, a novel modified Phase-Shifting Digital Holography (PSHD) approach is used [1]. Conventionally, a set of interferograms with specified phase variation of reference wave is needed for PSHD [3]. Here, a larger set of images is acquired with a varying tilt of the Savart plate. This extended dataset makes it possible to acquire the OPD from the most phase-sensitive position of the setup, further improving the axial resolution without the need of precise control of reference wave phase.

Results and discussion

Following the proposed design, the whole setup was constructed. Using monochromatic LED illumination, datasets were acquired with Savart plate tilting. Both the illumination and tilt during the measurement were controlled by a software, running on compact single-board computer with Linux. This single-board computer was also used to process the data.

The spatial resolution achieved was determined using the USAF1951 target, with the smallest resolved features having planar size of $\sim 25\,\mu m$. Background noise of $< 1\,nm$ was achieved for the OPD maps, essentially allowing the detection of very fine phase objects. This was successfully utilized to measure height of fabricated transparent SiO_2 dot arrays on glass with height of $\sim 2\,nm$ [1]. The height was calculated from the measured OPD map with knowledge of silica's refractive index and verified with AFM measurement.

Finally, microarrays of bovine-serum albumin $100\,\mu m$ dots attached to epoxysilane-modified glass were measured [2]. When these dots were subject to anti-BSA immunoglobulin G (which selectively binds to BSA), they were converted to bilayers of higher thickness. Thanks to the high axial resolution, it was possible to distinguish these thicker spots where the bonding of protein and antibody occurred.

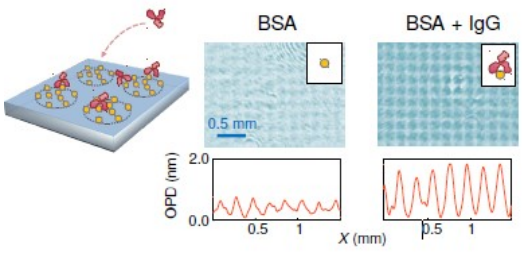

***Image 2**: OPD images and profiles for BSA and BSA+IgG dot microarrays. BSA+IgG shows significantly higher OPD profile.*

With use of specific proteins, detection arrays of multiple biomolecules can be prepared. Because the microscope uses collimated light, the field-of-view of the device is decoupled from the spatial resolution. This makes it possible to measure many spots simultaneously and paves the way for fast analysis of multiple biomarker in fluidic biological samples. The testing arrays can be prepared in advance and stored conveniently due to their small size, further promoting the idea of portable device enabling biological sample analysis in remote areas.

Conclusions

In this project, we proposed and realized a lens-free interferometric microscope with very high axial resolution. The device was successfully used to detect very thin, highly transparent inorganic and biological samples. This concept shows great potential for real-world applications in the field of biomedical detection.

Acknowledgment

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