Lens-free holographic imaging of microscopic objects using photonic structures and CMOS imagers

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Abstract

Current techniques to capture images of microscopic objects often rely on conventional microscopes, which are typically lens-based, resulting in bulky systems. While providing high spatial resolution, these systems have small field of view, limited throughput and because of high-precision optics tend to be quite expensive. However, several applications in industrial inspection (e.g. flat panel inspection) and biomedical applications (e.g. point of care devices) require an imaging system that is compact, high resolution, high throughput, large field of view and cost effective. Lens-free holographic imaging is a promising technology that has the potential to meet all these challenging requirements. We have developed two prototype systems, one targeting a large field of view imaging systems achieving high resolution (1.23um) over a large field of view (29mm^2). In another prototype configuration, we are able to image single cells passing by one-by-one inside a micro-fluidic channel at a resolution of 550nm. Furthermore, in an effort to miniaturize our implementations we are investigating the use of photonic structures as our next generation coherent light source.

1. Approach to Lens Free Holographic Imaging

In the Lens-free imaging technique coherent light gets diffracted on an object with microscopic features and interferes with the illumination wavefront. This interference is recorded by a standard image sensor and in software the in-focus amplitude and phase image of the original object is computed. The technique is based on in-line holography invented by Dennis Gabor [1]. With the advances in CMOS technology scaling, Gabor's photographic plate can be replaced with a fast high-resolution digital image sensor, and computers are fast enough to do a real-time numerical reconstruction.

The key system parameters in this context are the illumination, the image sensor and the reconstruction/post-processing algorithms. By configuring all these parameters the lens free imaging system can be optimized for specific applications in terms of field of view, resolution, through-put and compactness/cost.

A microscope based on the in-line holography configuration, needs to eliminate the inherent twin-image distortion [5, 6] to achieve quality and resolution comparable to conventional microscopes. Our main contribution is a unique fast iterative phase-retrieval method in combination with multi-wavelength image acquisition to overcome twin image distortions while still achieving high resolution. This multi-wavelength iterative phase retrieval technique is the result of a co-optimization of the illumination optics, imaging hardware and reconstruction software resulting in a robust lens-free image acquisition system [2]. An example showing the progressive suppression of the twin-image distortion in images of cell colonies is shown in Figure 1.

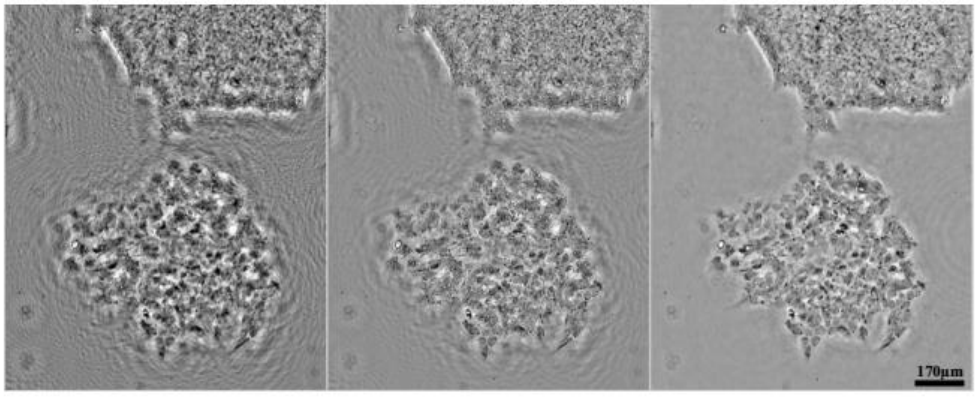

Figure 1: Multi-wavelength iterative phase retrieval used for twin image suppression on images of cell colonies. More iterations result in better suppression of the inherent twin image distortion, and hence cleaner images.

Another challenge, aiming to reduce the overall dimensions of the lens free imaging system is related to the illumination design. Our current design is still using external illumination devices (typically laser sources) coupled with optical fibers to illuminate the objects. Such a design however does not scale towards extreme miniaturization. Inspired by prior research in photonic phased arrays [8, 9] and their capability to replicate any wave-front, we propose to use a photonic phased array as a scalable on-chip (large-area) illumination source. A light source, consisting of a raster of out-of-plane out-coupling gratings connected by a distribution network based on single mode waveguides laid out in a fractal or matrix layout and processed with standard silicon fabrication accuracy. This solution eliminates the need for large vertical distances enabling a highly miniaturized microscope solution [4].

2. Novelty of our approach and related work

More than half a century ago Dennis Gabor proposed to capture the interference between an illuminating wavefront and the diffraction of an object using a photographic plate. This idea marked the birth of holography [1]. Today we are still using the same inline holographic configuration but the photographic plate has been replaced with a fast high resolution digital image sensor. The result is a lens-free holographic microscope (LHM) that can yield images with a high spatial resolution over a large field of view. Moreover, the image reconstruction step can be performed completely in the digital domain, eliminating mechanical focusing. Due to this unique combination of features, the field of LHM has been rather active over the last few years [10].

With respect to the suppression of twin image distortions, numerous techniques have been proposed to eliminate or reduce this inherent distortion. Some use an iterative support-based approach, others rely on an a-priori knowledge / assumption about the imaged objects (Inverse Filtering Method). Yet other techniques are based on an iterative recombination of a multitude of input images resulting in elimination of the twin-image signal. These techniques are known as Iterative Phase Retrieval techniques [6, 7]. Recording multiple images of the same (microscopic) scene can be achieved by varying the effective optical path-length between object and image sensor. This can be implemented in a practical setup in a number of ways, for example by changing the refractive index of the media or by changing the physical distance between the objects and the imaging sensor [10]. The latter approach however places rather strict requirements on the light source and the overall alignment of the hardware: a perfectly planar incident light is required and the consecutive holograms have to be pixel-aligned to enable the iterative recombination. Such pixel alignment is often challenging in conditions where motion is involved, e.g. vibrations in scanning systems. Pixel misalignment errors tend to accumulate in the iterative phase-retrieval process, resulting in a reduced final image quality, which is in many cases unusable for the targeted application. Alternatively, high-complexity software algorithms can be used to compensate for the hardware imperfection, yet their computational complexity makes application of such systems impossible for real-time imaging.

In contrast, our approach of using an in-line multi-wavelength lens-free imaging technique does not suffer from the above-mentioned limitations. The main advantage of the wavelength-varying or “multi-wavelength” technique (in which the object is sequentially illuminated at different wavelengths) compared to the “multi-depth” technique is the fact that it does not require any mechanical/geometrical changes to the imaging setup during image capture. Thus, the image capture can even happen at high speeds limited only by the speed of the illumination and imaging electronics. Furthermore, the multi-wavelength image sequences can be captured in burst mode with minimal delay between the consecutive frames, thus enabling for example high-speed live imaging of biological processes.

3. Prototype Results

We have developed prototypes that demonstrate the capabilities of this technology, achieving high resolution (1.23um) over a large field of view (29mm^2) [2]. The resulting system is also compact compared to conventional microscopes. In Figure 2 a reconstructed image of a stem-cell colony is depicted which has a large field of view and high resolution compared to a high resolution image obtained with a bulky phase contrast microscope with a 10x objective. When we compare against traditional objective based microscope systems, our system has 19x more field-of-view for the same resolution as a 10x objective lens. Moreover, compared to objective based systems, our lens-free microscope approach is a scalable solution, even suited for large area industrial inspection systems like quality inspection of thin films or surface coatings.

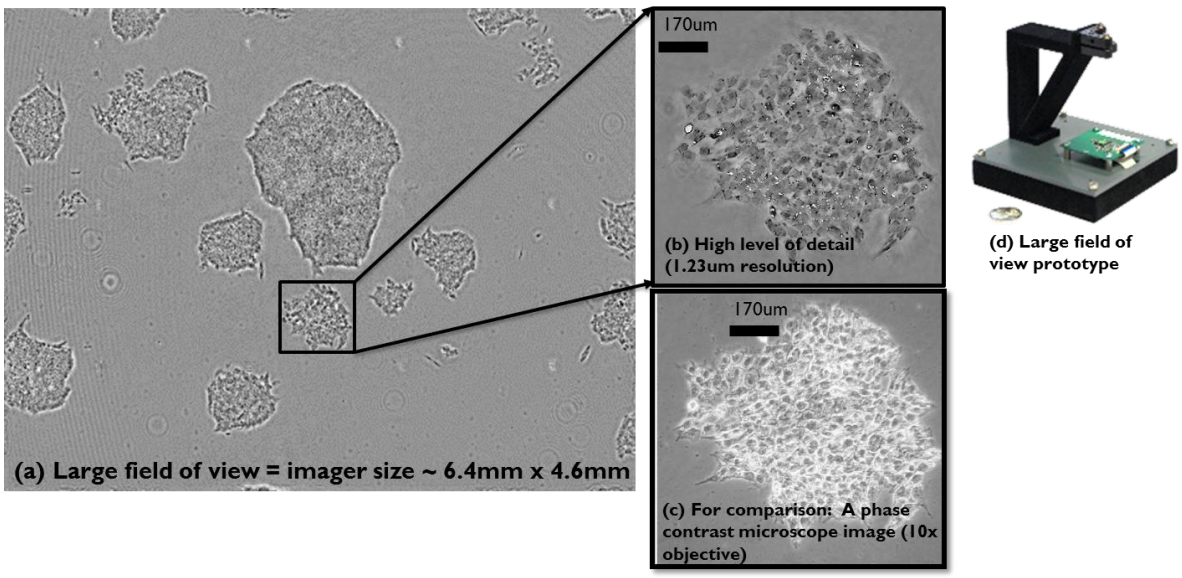

Figure 2: (a) Full field of view of a stem cell colony culture using our prototype
(b) detailed view of a small region illustrating more than single cell resolution
(c) phase-contrast image of the same region for reconstruction quality comparison (d) Large field of prototype

In a different lens-free microscopy configuration, we are able to image single cells passing by one-by-one inside a micro-fluidic channel (see Figure 3). In this prototype, we have achieved a resolution of 550nm, enough to differentiate different types of white blood cells [3]. Here, similar to conventional lens based microscopes, we have traded field of view for high resolution. However, we are able to make the lens free microscope extremely compact; the total microscope is only a few mm^3 in volume. These results illustrate that this technique is very well suited for future very compact point-of-care devices.

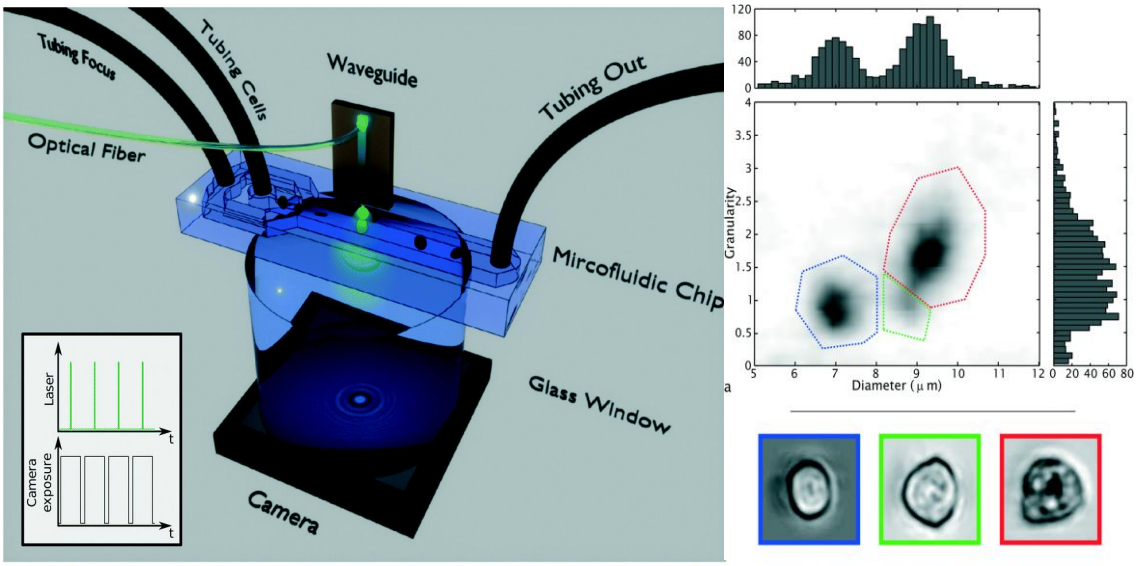

Figure 3 single cell lens-free microscope setup (left) in combination with microfluidics capable to differentiate between 3 types of white blood cells (right)

For our photonic based illumination approach, our first prototyped devices, fabricated using our in-house SiN photonic process technology show promising results for application in lens free imaging [4]. An example of such a 16x16 phased array photonic chip is shown in Figure 4. We are currently characterizing the optical performance of the prototype phased arrays and matching the results with our design and simulation models. Alternative designs for out-couplers and waveguide layout are being explored with the goal to find the best trade-off between density and out-coupler characteristic (minimizing the wave-front distortions).

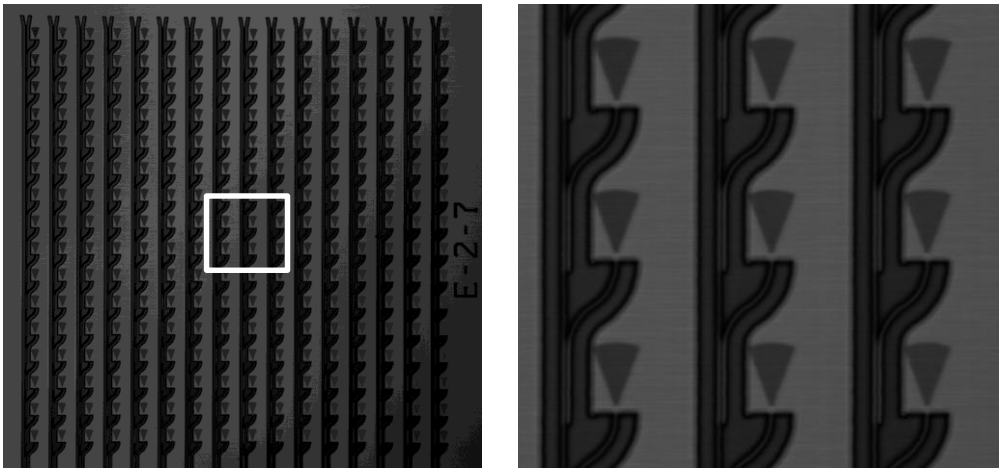

Figure 4 Bright-field microscope image of our first prototype phased array fabricated using our in-house SiN process technology: Full array of 16x16 out-coupling collimating gratings (left), detail of the evanescently coupled gratings (right).

4. Conclusions

Several applications in industrial inspection and biomedical require an imaging system that is compact, high resolution, high throughput, large field of view and cost effective. Lens-free holographic imaging is a promising technology that has the potential to meet all these challenging requirements. It does not use any lenses and nor any mechanically moving parts for focusing. Using the traditional inline holographic configuration in combination with a fast high-resolution digital image sensor we have reported results of multiple prototypes achieving high resolution over a large field of view in combination with a fast iterative multi-wavelength phase retrieval method to eliminate the inherent twin image distortions. Also a single cell inspection system capable of differentiating between three different white blood cell types. All these prototypes can be further miniaturized when we combine them with reported results from specifically designed photonic structures as our next generation light source modules.

References:

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