

MIICROFLUIDIC-BASED OIL-IMMERSION LENSES FOR HIGH RESOLUTION MICROSCOPY

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ABSTRACT

Optical imaging systems with submicron resolution typically require the use of expensive, high numerical aperture (NA) microscope objective lenses. Here, we describe a novel Microfluidic-based Oil-Immersion Lens (μ OIL) chip that can perform submicron and wide field of view imaging. Key element of the chip design is the use of high index of refraction, oil-immersion ball mini-lenses that have high NA (~ 0.8) and therefore very good optical resolution ($0.6\text{--}0.7\ \mu\text{m}$). The μ OIL chip can be part of a lab-on-chip system or it can be used as an add-on module in low cost stereoscopes to enable high resolution imaging of biological samples in the lab, in the doctor's office or in the field.

KEYWORDS

Microfluidics, Lab-on-chip, Doublet Lenses, Oil-Immersion Lens, Optical Chip, Microfabrication, Numerical Aperture, Optical Imaging, Microscopy.

INTRODUCTION

Optical microscopy is the gold standard for the visualization of biological entities as well for the identification, diagnosis and monitoring of many diseases such as sickle-cell disease, malaria, and tuberculosis. High-end optical systems that achieve submicron resolution imaging rely on multi-element objective lenses that are expensive and bulky. In recent years, there has been an increased interest in integrating optical components on-chip [1][2]. The development of micro-optical modules typically involves the challenging task of lens miniaturization if superb imaging quality needs to be maintained [3][4][5].

This work demonstrates a novel microfluidic-based approach for obtaining a low-cost, high-NA array of miniaturized doublet lenses for imaging biological samples. The proposed lenses are made out of high index of refraction sapphire lenses. Those lenses are placed on top of an array of oil-filled microwells that are microfabricated on a silicon chip. Not only the Microfluidic-based Oil-Immersion Lens (μ OIL) array provides optical performance (resolution, NA) equivalent to the performance of a conventional microscope objective but it also enables wide field of view imaging as its size can be easily scaled up.

DESIGN

The μ OIL chip consists of 25 sapphire ball mini-lenses (1 mm in diameter, refractive index of 1.77) integrated on top of a 5x5 array of oil-filled, microwells (Figure 1 A). We use the term 'mini-lenses' to indicate that those lenses are off-the-shelf, non-microfabricated lenses. The microwells are connected through an open-air microfluidic network that distributes the oil equally to each microwell in the entire array. The ball mini-lenses have a diameter slightly larger than the diameter of the microwells and therefore they sit on top of them. When the microwells are filled with oil, the mini-lenses are half immersed in oil and half exposed to air. As a result, the oil/mini-lens assembly acts as a doublet lens, resembling the hemispherical front lens of a high numerical aperture oil-immersion microscope objective.

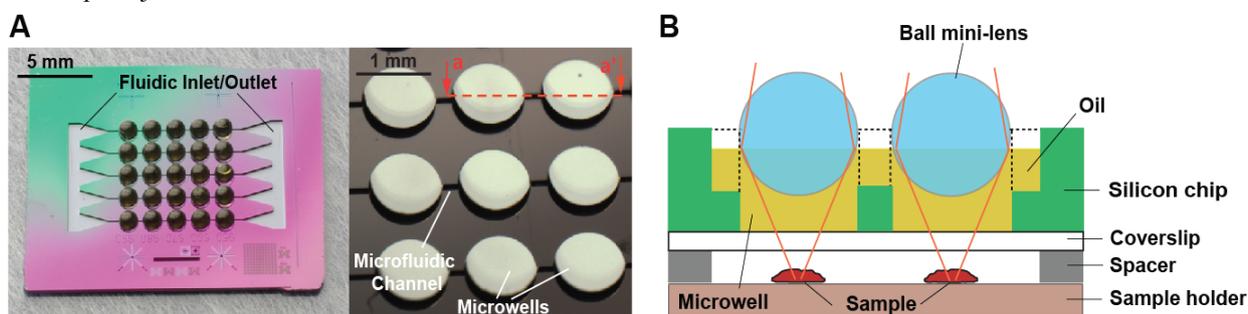


Figure 1: (A) The μ OIL chip containing 25 oil-immersion ball mini-lenses. A close-up view of the microwell array is shown in the right optical micrograph. (B) Schematic of the cross sectional view (indicated as a-a' in A) of the μ OIL architecture. The microwells and mini-lenses are $970\ \mu\text{m}$ and $1\ \text{mm}$ in diameter respectively.

The sample of interest is placed underneath the μ OIL chip at a fixed distance from the doublet array and it is imaged through a $100\ \mu\text{m}$ thick glass coverslip (Figure 1 B). The distance between the ball mini-lens and the sample which affects the magnification and the NA, is accurately defined by the thickness of a spacer – a photoresist film that is patterned on the backside of the glass coverslip.

The geometry of the μ OIL array (e.g. microwell and ball mini-lens diameter, glass coverslip thickness) was selected such that the focal length of the ball mini-lens ($\sim 620 \mu\text{m}$, taken from its center) is located at the backside of the glass coverslip. In this case, optical rays originating from the sample always converge as they exit the chip.

MICROFABRICATION OF THE μ OIL CHIP

The microfabrication of the μ OIL chip is completed in 6 steps (Figure 2): 1) a 200 nm thick silicon dioxide film is deposited and patterned on a 400 μm thick silicon wafer to define the microfluidic network, 2) a photolithographic step is then performed on a photoresist layer to define the microwell array, 3) a time-controlled deep reactive ion etching (DRIE) step is carried out to etch the wafer through, 4) a 100 μm thick glass coverslip, having a 10 μm thick SU-8 layer on its backside, is attached to the silicon wafer using epoxy, 5) the microfluidic network and the array are filled with immersion oil (refractive index of ~ 1.516) and finally, 6) the ball mini-lenses are manually assembled on the oil-filled array.

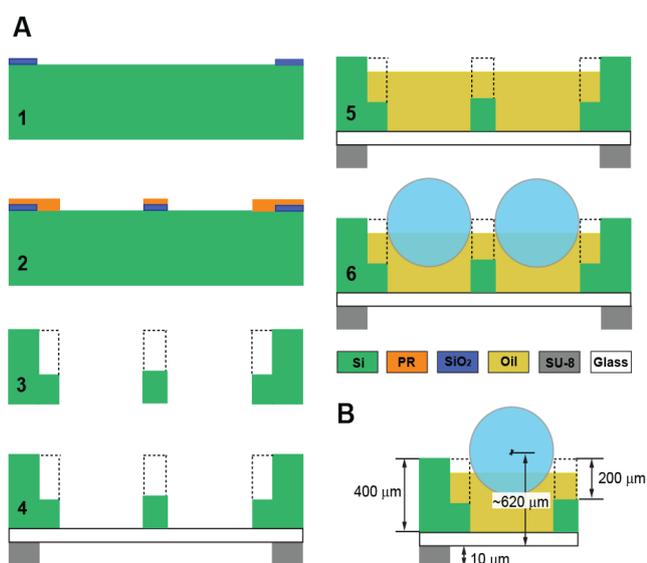


Figure 2: Microfabrication process (A) and critical dimensions (B) of the μ OIL chip. The dotted lines indicate the microwell sidewalls that are not visible in the highlighted cross section. The focal plane is located $\sim 620 \mu\text{m}$ below the center of the ball mini-lens and coincides with the backside of the glass coverslip.

RESULTS AND DISCUSSION

We performed optical simulations to calculate the NA and magnification of the μ OIL chip as a function of the distance of the sample from the focal plane of the array (Figure 3). That distance coincides with the spacer thickness as the focal plane of the array is located at the backside of the glass coverslip. Assuming a minimum spacer thickness of 5-10 μm (e.g. when imaging a cell), the magnification and NA reach a maximum value of $\sim 72\times$ and ~ 0.8 respectively. As the spacer thickness increases, there is a dramatic reduction in the magnification while the NA decreases almost linearly.

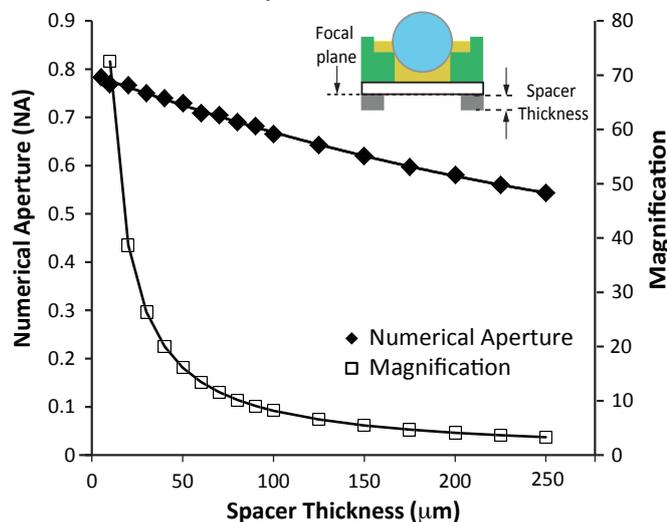


Figure 3: Numerical Aperture (NA) and magnification versus spacer thickness.

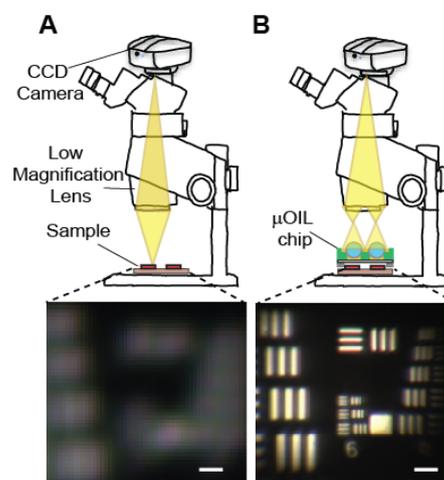


Figure 4: A low-magnification stereoscope cannot image line patterns (A) from a resolution chart (St 1951 USAF). The lines become visible when imaged through the μ OIL chip (B). Scale bar, 10 μm .

Alternatively the μ OIL chip can be used as an add-on module in a low-cost, low NA stereoscope to achieve high resolution imaging (Figure 4). Such a configuration can be useful in a resource-limited setting or in the doctor's office where a high-end microscope is not available.

We furthermore experimentally obtained the Modulation Transfer Function (MTF) of a stereoscope (Olympus sz61)/ μ OIL system by imaging line pairs from a resolution test chart (Figure 5) using a 10 μm spacer. Even the smallest line pairs of 0.78 μm line width were clearly distinguishable. A maximum resolution value of $\sim 0.6\text{-}0.7 \mu\text{m}$ is anticipated (depending on the extrapolation method used) when the image contrast approaches zero. We should point out that the theoretical maximum resolution estimated from the maximum NA value (NA=0.77, see figure 3) is $\sim 450 \text{ nm}$. That deviation can be attributed to optical aberrations of the combined system that were not taken into

account during the optical simulations as well as imperfections of the experimental setup (e.g. the light source of the stereoscope is not collimated).

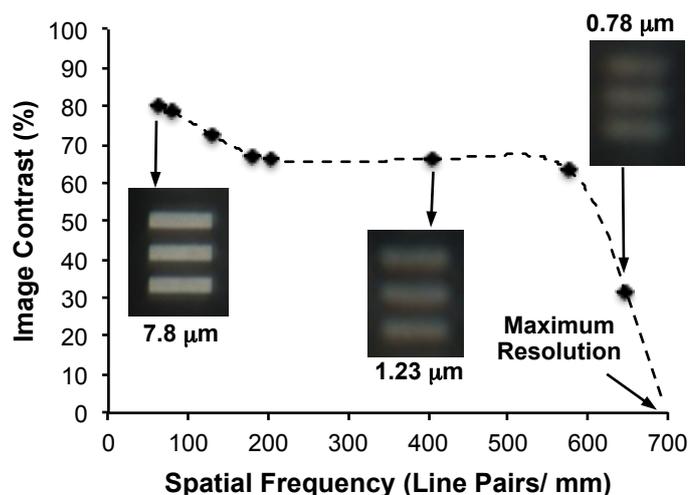


Figure 5: Image contrast versus spatial frequency of a resolution line pattern. The obtained curve represents the MTF of the combined system. The micrographs highlight line pairs of three different widths (7.8 μm , 1.23 μm and 0.78 μm).

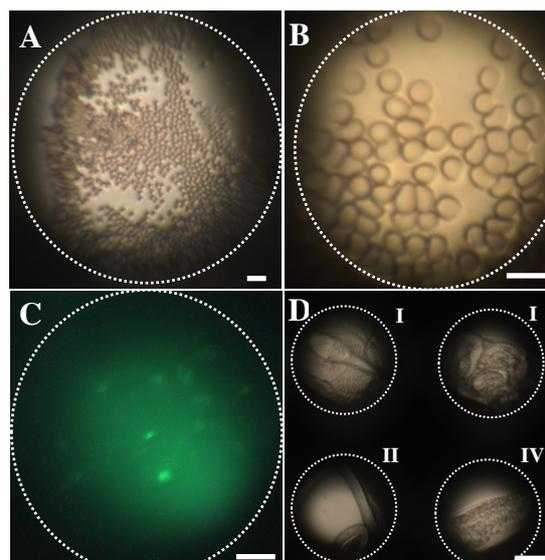


Figure 6: **A & B.** Brightfield images of microspheres (A) and blood cells (B), **C.** Fluorescence image of white blood cells. **D.** *C. elegans* eggs in various developmental stages (I, II and III). Part of the worm body is shown in IV. A, B, C: Scale bar, 10 μm . D: Scale bar, 100 μm .

The ability of the combined system to image microspheres and cells was demonstrated in brightfield (transmission) and epi-fluorescence modes using a 10 μm spacer (Figure 6 A-C). In both cases, individual 2- μm in diameter microspheres and blood cells were clearly visible. Finally, to demonstrate wide field of view imaging, we used the μOIL chip to image *in vivo* the nematode *C.elegans* at different developmental stages (Figure 6 D). The field of view was $\sim 200 \mu\text{m}$ in diameter for each lens in the array. In this case, the spacer thickness was adjusted to $\sim 210 \mu\text{m}$ thick in order to compensate for the thick worm body.

CONCLUSIONS

We developed a microfluidic-based oil-immersion lens chip with high NA, submicron resolution and wide field of view imaging capabilities. We believe that the proposed μOIL chip can be used as a stand-alone unit in lab-on-chip systems as or as an add-on accessory in low cost stereoscopes for various research and diagnostic biomedical applications.

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