Electronic Supplementary Information (ESI)

Title: Dynamic nano-imaging via microsphere compound lens integrated microfluidic device with 10× objective lens

Guangxing Wu, ‡[a] Serene Wen Ling Ng, ‡[a] Yan Zhou §[a] and Minghui Hong* [a, b]

a. Department of Electrical and Computer Engineering, National University of Singapore, 4 Engineering Drive 3, Singapore 117576, Singapore.
b. Pen-Tung Sah Institute of Micro-Nano Science and Technology, Xiamen University, 33 Qunxian Road, Xiamen 361005, China.
‡ These authors contributed equally to this work.
§ The current address of Yan Zhou is Peng Cheng Laboratory, Shenzhen 518055, China

This Text file includes:

Section 1: The design of the MCL;

Section 2: The experimental measurements of the depth-of-field of a single microsphere and MCL coupled with different objective lenses

Other supplementary materials for this manuscript include the following:

Movie S1: 200 nm polystyrene nanoparticles imaged by an MCL with 10× objective lens (MP4);
Movie S2: 200 nm polystyrene nanoparticles imaged by a single microsphere with 20× objective lens (MP4);
Movie S3: 200 nm polystyrene nanoparticles imaged by an MCL with 20× objective lens (MP4);
Movie S4: 100 nm polystyrene nanoparticles imaged by an MCL with 10× objective lens (MP4);
Movie S5: Particle tracing by an MCL with 10× objective lens (MP4);
Movie S6: Yeast cells imaged by an MCL with 10× objective lens (MP4);

Section 1: The design of the MCL

In this work, the MCL consists of two vertically stacked microspheres. The target imaging resolution of the MCL integrated microfluidic device is to discern 100 nm transparent polystyrene nanospheres in water environment. The target magnification of the MCL is ~10× so that the low-power objective lens with a long depth-of-field (DOF) can be employed. In the design of the MCL, the refractive indices and sizes of the two microspheres were selected to meet these requirements. Besides, the MCL should work in virtual-real imaging mode. Specifically, the bottom and upper microspheres should work in virtual and real imaging modes, respectively. Such imaging mode combination has been demonstrated to be advantages over other imaging modes combinations owing to its large light energy collection angle and an extensive operation space between the MCL and the objective lens [1].

In the microsphere nano-imaging area, barium titanate glass (BTG) microspheres with refractive indices between 1.9-2.2 are most frequently used in the PDMS immersion environment. On one hand, low refractive index microspheres, such as the silica microsphere and the polystyrene microsphere, cannot effectively achieve nano-imaging in liquid or PDMS immersion environment due to the small refractive index contrast [2,3]. On the other hand, BTG microspheres with refractive indices between 1.9-2.2 are commercially available from Cospheric Company in a wide diameter range, which facilitates the design of microsphere compound lenses. Therefore, in this work, the BTG microspheres with refractive indices between 1.9-2.2 are selected to compose the MCL. Detailed analysis about how to determine the specific sizes and refractive indices of the bottom and upper BTG microsphere is given in parts (a) and (b) below, respectively.

(a) The size and refractive index of the bottom microsphere

The reason to select the bottom microsphere with a diameter of ~50 μm is clarified in the main text and will not repeat here. In terms of the refractive index, a large refractive index of the bottom BTG microsphere is preferred because microsphere with a larger refractive index exhibits higher magnification ability in the virtual imaging mode, which indirectly affects the imaging resolution of the whole setup. According to the geometric optics theory, when the bottom microsphere almost contacts with the sample in microfluidic channels for nano-imaging, its magnifications is calculated by the formula below [1]:

$$\beta = \frac{n_1}{2n_0 - n_1},\tag{1}$$

where n_0 and n_1 are refractive indices of the environment and microsphere, respectively. In our case, the refractive index of the PDMS immersion medium (n_0) is ~1.44. The BTG microspheres to be selected have refractive indices between 1.9-2.2. According to the above formula, the magnification of the bottom microsphere increases with its refractive index. Therefore, if a high magnification is required, the bottom microsphere should have a large refractive index. A high magnification of the bottom microsphere is critical to the imaging resolution of the MCL. The imaging principle of the MCL involves two cascaded magnification processes. Firstly, the bottom microsphere produces an enlarged virtual image of the object in the microfluidic channel. Secondly, the upper microsphere takes the enlarged virtual image as

the imaging target and magnify it further. If the magnification of the bottom microsphere is insufficient and the magnified feature size of the object is still beyond the resolution limit of the upper microsphere in the compound lens, the upper microsphere cannot well resolve the enlarged virtual image and thus the imaging resolution of the whole setup is restricted. As such, the refractive index of the bottom microsphere should be as large as possible to achieve a large magnification and thereby realize optimal imaging resolution. Therefore, commercially available ~50 μm BTG microspheres with the largest refractive index of 2.2 are selected as the bottom microspheres.

(b) The size and refractive index of the upper microsphere

When the bottom microsphere is determined, the refractive index and size of the upper microsphere are selected to meet the real imaging mode requirement and achieve the desired magnification (~10×) for the whole MCL. Basically, all the BTG microspheres with refractive indices between 1.9-2.2 can satisfy the above two demands by changing the diameters of microspheres. Formulas to calculate the magnification β_{MCL} and imaging distance l'_{MCL} of the MCL can be found in reference and are reproduced here [1]:

$$
\begin{cases}
l'_1 = \dfrac{(2n_0 - n_1)\, r_1 l - 2n_0 r_1^2}{2(n_1 - n_0)l + 2n_0 r_1 - n_1 r_1} + 2r_1 \\[4mm]
l'_{\mathrm{MCL}} = \dfrac{(2n_0 - n_2)\, r_2\left(l'_1 + g - 2r_1\right) - 2n_0 r_2^2}{2(n_2 - n_0)\left(l'_1 + g - 2r_1\right) + 2n_0 r_2 - n_2 r_2} + 2r_2
\end{cases}
, \qquad (2)
$$

$$
\begin{cases}
\beta_1 = \dfrac{n_1 r_1}{2l(n_1 - n_0) + r_1(2n_0 - n_1)} \\[4mm]
\beta_2 = \dfrac{n_2 r_2}{2(n_2 - n_0)\left(l'_1 + g - 2r_1\right) + r_2(2n_0 - n_2)} \\[4mm]
\beta_{\mathrm{MCL}} = \beta_1 \times \beta_2
\end{cases}
, \qquad (3)
$$

where β_1 and β_2 are the magnification factors of the bottom and upper microspheres, respectively. r_1 and r_2 are the radius of the bottom and upper microspheres, respectively. n_0, n_1 and n_2 are the refractive indices of the environment, the bottom microsphere and the upper microsphere, respectively. l and l'_1 indicate the object distance and image distance of the bottom microsphere, respectively. g is the gap between two microspheres. According to their convention, object distance l and gap g should take a negative value for calculation. When the upper microsphere working in the real imaging mode and the magnification of the MCL is 10×, β_{MCL} calculated from formulas (2) and (3) should be -10. The negative sign of value represents the image is an inverted real image. When $n_1 = 2.2$ and $r_1 = 50$ μm, the diameters (Dc) of the upper BTG microspheres that make corresponding MCLs achieve $\beta_{\mathrm{MCL}} = -10$ are calculated for different n_2 as displayed in Fig S1(a) below. To realize the real imaging mode and 10× magnification, the diameter of the upper BTG microsphere should be hundreds of micrometers. The largest diameter of commercially available n~2.2 BTG microsphere is 85 μm, which cannot meet the design requirements. Finally, n~1.93 BTG microspheres, which are commercially available in a wide diameter range from 60 μm to 600 μm, are employed as the

upper microsphere in the MCL.
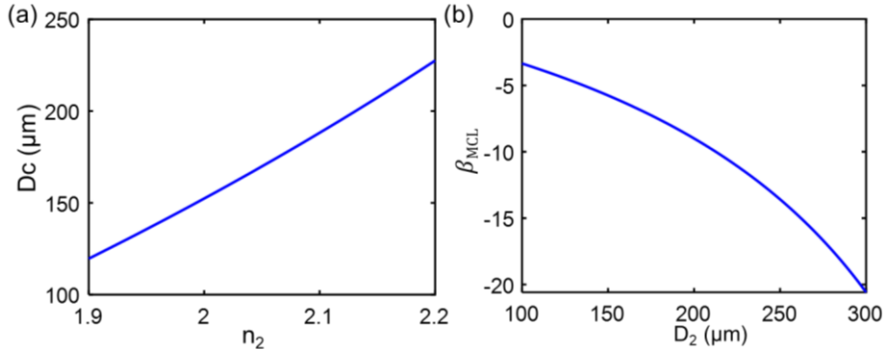

Fig. S1. (a) Diameters (Dc) of the upper BTG microspheres that make corresponding MCLs achieve 10× magnification versus their refractive indices. (b) The magnification of MCL versus the diameter (D_2) of the upper microsphere when the refractive indices of the bottom and upper microsphere are 2.1 and 2.4, respectively.

   To note, the above calculations for the diameter of the upper BTG microspheres to achieve 10× magnification are not accurate and deviate from the experiments, which is caused by two factors. Firstly, the refractive index values of BTG microspheres used to compose the MCL are provided by the supplier. According to the description of supplier, the refractive index values of these BTG microspheres are measured at the wavelength of ~589 nm. However, in this work, the wavelength of illumination light used for imaging experiments is 420 nm. Due to the positive dispersion effect, the actual refractive indices should be larger than the values provided by the supplier. As an assumption, if the refractive indices of the bottom and upper microspheres increase from 1.93 and 2.2 to 2.1 and 2.4 due to the dispersion, the calculated diameter (D_2) of the upper microsphere for 10× magnification should be 213 μm as shown in Fig. S1. (b), which is close to the experimental results. Secondly, as referred in many references, microspheres exhibit significant wave-optics effects, which also induce the imaging properties deviating from the predictions of geometric optics theory. Therefore, the formulas based on geometric optics theory can only provide a rough range of the desired parameters of microspheres to compose the MCL. In this work, the specific diameter of the upper BTG microsphere is finally selected through many experimental tests. We have used n~1.93 BTG microspheres with diameters in the range of 150 μm- 300 μm to compose the MCL and confirmed that the n~1.93 BTG microsphere with a diameter of ~230 μm can satisfy the above design requirements for the upper microsphere in the MCL.

Section 2: The experimental measurements of the depth-of-field of a single microsphere and MCL coupled with different objective lenses

In the experiment to measure the DOF of a single microsphere and MCL coupled with different objective lenses, a nano-steps sample was fabricated in a silicon wafer using focused ion beam (FIB) milling. The SEM and AFM images of the fabricated nano-steps sample are displayed in Figs. S2 (a) and (b), respectively. The nano-steps sample consists of 10 steps and the distance between the lowest point and the highest point is ~790 nm as seen in Fig. S2 (c), which displays the height profile along the red dashed line in Fig. S2 (b). According to the definition, the DOF is the distance from the nearest object plane in focus to that of the farthest plane simultaneously in focus. Therefore, we can approximately quantify the DOF by counting the number of discerned nano-steps in the images captured by the single microsphere and the MCL imaging schemes when the nano-steps sample is imaged.
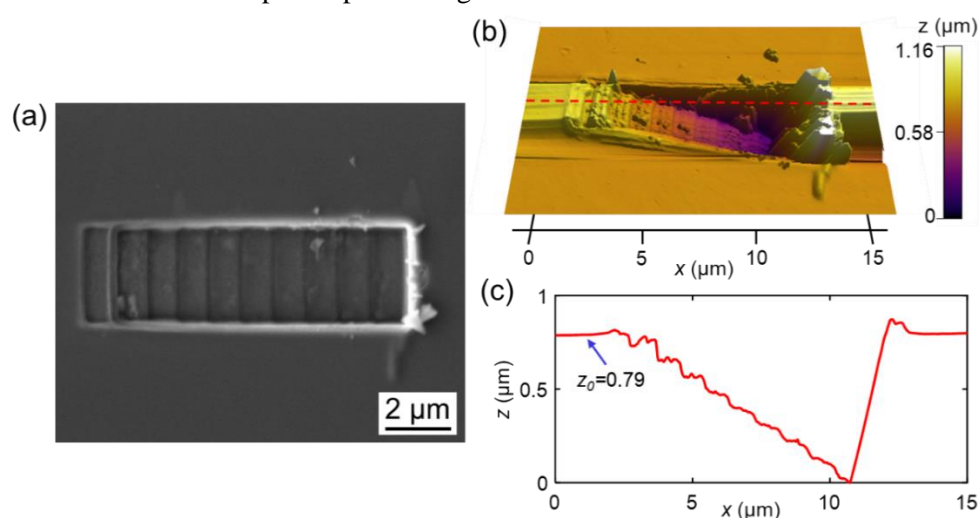

Fig. S2. (a) SEM and (b) AFM images of a nano-steps sample fabricated by FIB. (c) The height profile along the red dashed line in (b).

In the imaging experiment, two microspheres with the same refractive indices and diameters as those used in the microfluidic device are employed to compose the MCL, i.e. a n~2.2 50 μm BTG microsphere (bottom) and a n~1.93 230 μm BTG microsphere (upper). For the convenience of operation, the microsphere and MCL are immersed in oil rather than the PDMS membrane for imaging. To better reflect the imaging properties of the MCL used in the microfluidic device, the selected immersion oil has a refractive index of ~1.43, which is close to the refractive index of PDMS (n~1.44). In the experiment, the two microspheres are fixed on the tips of two metal needles, which are mounted on two three-axis moving stages. Using the high precision moving stages, the two microspheres can be assembled into an MCL, which can be conveniently relocated to the point of interest on the sample for imaging.

Comprehensive comparisons are made for the single microsphere and MCL coupled with different objective lenses. The imaging results of the nano-steps sample captured by a single 50 μm BTG microsphere (n~2.2) and the MCL are presented in Figs. S3(a)-(b) and (c)-(d), respectively. Figs. S3(a) and (b) display the images acquired by the single BTG microsphere

coupled with 10× and 20× objective lenses, respectively. The field-of-views of the single BTG microsphere coupled with 10× and 20× objective lens both can cover the region of ~7 nano-steps. However, only ~3 and ~2 nano-steps can be observed with sharp edges in Figs. S3(a) and (b), respectively. The edges of other nano-steps within the field-of-view are blur. These results indicate that the single microsphere coupled with the 10× and 20× objective lenses only possess the DOF of ~230 nm and ~ 150 nm according to the height profile in Figs. S2(c). The imaging contrast of Figs. S3(a) is better than Figs. S3(b), which may be ascribed to the larger DOF of the 10× objective lens.
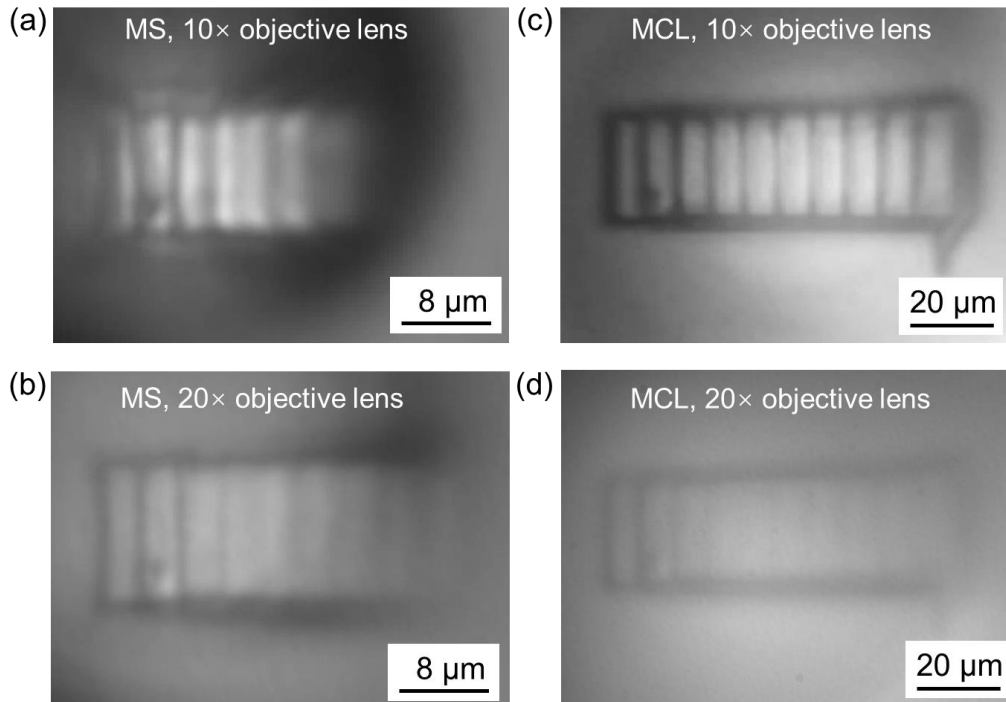

Fig. S3. The nano-steps sample imaged by a single 50 μm BTG microsphere (n~2.2) coupled with (a) a 10× objective lens, (b) a 20× objective lens and by a MCL coupled with (c) a 10× objective lens, (d) a 20× objective lens.

As a comparison, the field-of-views of the MCL coupled with the 10× and 20× objective lenses both can cover the whole nano-steps sample as displayed in Figs. S3(c) and (d), respectively. Remarkably, all the 10 nano-steps in Figs. S3(c) can be observed with sharp edges, which indicates that the DOF of the MCL coupled with a 10× objective lens is no smaller than ~790 nm. Therefore, the DOF of the MCL coupled with a 10× objective lens is indeed larger than the single microsphere coupled with a 10× or 20× objective lens. In addition, the overall image quality of Figs. S3(c) is also significantly better than those images captured by the single microsphere imaging setups. Specifically, Figs. S3(c) shows less image distortion compared with Figs. S3(a) and better image contrast than Figs. S3(b), which demonstrate the superiority of the combination of the MCL coupled with a 10× objective lens in imaging. As shown in Figs. S3(d), the image captured by the MCL coupled with a 20× objective lens shows poor contrast, which is consistent with the imaging results of 200 nm polystyrene nanoparticles displayed in Fig. 3(c) of the main text. It is largely due to the shorter DOF of the lens assembly accompanied

by the excessive total magnification coefficient (~200×). These results thus demonstrates that a large DOF of the lens system is very important for the imaging contrast.

Based on these experimental evidences, it can conclude that the combination of a MCL coupled with a low-power objective lens has a larger DOF than the single microsphere nano-imaging setup and imaging systems requiring high-power objective lenses. The MCL coupled with a low-power objective lens can collect more light signals from the object, which significantly benefits the overall imaging quality.

References

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