Decoupled and simultaneous three-dimensional imaging and optical manipulation through a single objective

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The combination of optical manipulation and microscopy is of paramount importance in medicine, biology, chemistry, and physics [1–8]. Optical manipulation experiments generally require simultaneous imaging for data acquisition, and usually the same objective lens is used for optical trapping and imaging. This leaves space around the sample free for techniques such as microfluidics, holographic microscopy, rheology, or atomic force microscopy [4,2–12]. However, it does pose a fundamental restriction on which part of the sample may be imaged while trapping, since the imaging and trapping planes are inherently decoupled.

To overcome this restriction, two objectives have been positioned nose-to-nose, on opposite sides of the sample, with one providing the optical trapping, and the other the imaging [13–15]. In this way the trapping and imaging planes are mechanically decoupled, which allows for independent imaging of optically trapped particles in three dimensions. However, this configuration requires thin samples and limits access to the sample. Another approach to decouple the imaging and trapping planes is to use wavefront engineering and computational feedback. Mechanical or digital wavefront engineering of either the imaging or trapping wavefront can offset its relative axial position [16–18], though aberrations in the refocused plane are inevitable. In the case of mechanical refocusing the axial depth is limited ∼10 μm [19]. In digital refocusing, aberration corrections can increase this depth to ∼30 μm for optical trapping [20,21], though the low optical efficiency [22] limits its applicability in simultaneous three-dimensional (3D) imaging. Digital holographic microscopy has become an increasingly popular technique for 3D tracking and is easily combined with optical trapping [10,23]. Nevertheless, the imaging and trapping planes are not decoupled and the sample still needs to be accessible from both sides. In addition, it is prohibitively difficult to apply holographic microscopy to dense and turbid samples due to the complexity of the resulting holograms.
In this Letter, we present a unique approach that facilitates 3D holographic optical trapping and simultaneous, decoupled imaging using a single microscope objective at the sample. By remotely refocusing the imaging plane [19] it is optically decoupled from the trapping plane, and we are able to image in 3D while simultaneously optically manipulating the system, without the need for any digital feedback or aberration correction. Figure 1(a) shows the schematic of our setup, which is composed of the remote refocusing component, the CMOS camera or confocal scan head, holographic optical trapping, and a single objective inverted microscope (see Supplement 1). Importantly, a wide variety of other imaging and trapping techniques can be easily incorporated in our setup, which underlines the general applicability of our approach. Our setup is designed to optically manipulate the sample while simultaneously imaging the volume around the manipulated region using a single objective at the sample.

The key to optically decoupling the imaging and trapping planes is the aberration-free remote refocusing component [19], which is highlighted in Fig. 1(b) for brightfield and confocal modes. In brightfield mode, the light from the sample, collected by O1, is relayed to O2, which produces a low magnification 3D image of the sample. This 3D image is then reimaged with a high magnification onto the CMOS camera by O3. In this way we remotely image our sample, where the axial displacement of objective O3 controls the position of the imaging plane within the sample. Importantly, the remote refocusing component fully retains the properties of the chosen imaging mode. When used in confocal mode, the translation of O3 dynamically changes the axial position of the confocal illumination and imaging planes, thereby retaining the optical sectioning of the sample, as shown in Fig. 1(b). The maximum axial depth in our system is 66 μm and is limited by the travel of O3 (see Supplement 1). We characterize the remote refocusing by measuring the point spread functions generated at the sample for different axial positions of objective O3 (see Supplement 1). Figure 1(c) shows the intensity distributions at O1 when O3 is positioned at an axial position of z = –50, 0, and +50 μm, respectively. In each case the intensity distribution is that of a diffraction limited spot, which demonstrates the aberration-free remote refocusing in our system [19].

Figure 2 shows the remote refocusing microscope operating in brightfield and confocal mode, respectively. Figure 2(a) presents brightfield images of a 10-μm grid, while Fig. 2(b) shows confocal images of a colloidal monolayer (see Supplement 1). In each image O3 is positioned at z = –50, 0, and +50 μm, from left to right, respectively, which refocuses the imaging plane in the sample to an axial depth of +33, 0, and −33 μm relative to the nominal focal plane of O1. At each position of O3, the sample stage is then adjusted to bring the grid or colloidal monolayer back into focus. Both imaging modes show excellent contrast and resolution for the full range of remote refocusing. A minor reduction in the collected fluorescence can be seen at z = ±33 μm due to the reduced collection of the objectives when operating away from their focal planes. This has little effect in the brightfield images.

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**Fig. 1.** Experimental setup combining 3D remote refocusing microscopy and optical manipulation. (a) Schematic diagram of the experimental setup, which consists of four main units: a single objective inverted microscope, remote refocusing, holographic optical trapping, and a CMOS camera or a confocal scan head containing the resonant scanner, the excitation laser, and the photo-multiplier tube (PMT). The flip mirror in front of the CMOS camera and the confocal scan head determines which imaging mode is used. (b) Simplified schematic of the optical remote refocusing technique in brightfield and confocal modes. In brightfield, a low magnification 3D image of the sample is created by O2, which is subsequently reimaged by O3 onto the CMOS camera. In confocal mode, the translation of O3 changes the axial position of the illumination and imaging focal planes, resulting in optical sectioning of the sample. (c) xz profiles of the intensity distribution of a focused laser beam formed by O1 at the sample plane. The three panels correspond to O3 being positioned at z = –50 (top), 0 (middle), and +50 μm (bottom), respectively. Each intensity profile is normalized to the maximum pixel value.
To illustrate the simultaneous and decoupled 3D imaging and optical trapping capabilities, we image four particles that are trapped at the vertices of a tetrahedron. Three vertices of the tetrahedron are placed in the same xy plane, separated by 20 μm. The fourth vertex is positioned 17 μm above this plane. Figure 3(a) shows a sequence of five brightfield images with axial increments of 5 μm and starting 5 μm below the base of the tetrahedron. Figure 3(b) presents confocal images at the same resolution, field of view, and axial position as the brightfield images in Fig. 3(a). The tetrahedral geometry is obvious from both the brightfield and confocal images, where the optical sectioning of the confocal imaging is reflected by the absence of the out-of-focus contributions that are present in the brightfield images. Figure 3(c) presents a 3D view of the tetrahedron as observed in confocal mode, which shows the capability of our system to image around a static optically trapped network using only a single objective at the sample.

More challenging, we directly image the 3D flow field around optically trapped particles using our remote refocusing microscope in confocal mode. As the flow profile around a sphere is well known \([24,25]\) and relevant for active systems such as swimming bacteria \([26]\), it forms an ideal proof-of-principle case study for our setup. First, we trap a single 10 μm diameter sphere in a dilute dispersion of small ~500 nm diameter fluorescent tracer particles and subsequently flow the tracer suspension past the trapped sphere.

![Figure 2](image-url)

**Fig. 2.** Remote refocusing microscopy. (a) Brightfield images of a 10 μm grid positioned at axial depth of \(z = \pm 33\), 0, and \(-33\) μm relative to the nominal focal plane of O1. The images are then brought into focus by positioning O3 at \(z = -50, 0,\) and \(+50\) μm, respectively. (b) Confocal images of a colloidal monolayer for the same axial positions as in (a).

![Figure 3](image-url)

**Fig. 3.** Decoupled and simultaneous 3D imaging and optical manipulation. Colloids optically trapped at the vertices of the tetrahedron. (a) Brightfield images with axial increments of 5 μm and starting 5 μm below the base of the tetrahedron. (b) Corresponding confocal images at the same resolution, field of view, and axial position. (c) 3D image of the tetrahedron as observed in confocal mode. (d) Confocal image showing the flow profile of ~500 nm diameter particles around a single 10 μm diameter optically trapped sphere. The image is averaged over 5000 frames (the inset shows a region of 19 μm x 19 μm of a single frame) with the measured velocity field overlaid. For clarity we normalize the velocity component in x by the bulk flow velocity. (e) and (f) Flow fields around three optically trapped spheres, which are trapped either (e) in the same plane (\(z = 0\) μm) or (f) in three different planes (\(z = -10, 0,\) and 10 μm), as detailed in the illustrations.
For clarity, we normalize the velocity component in the $x$ direction by the bulk flow velocity, i.e., far away from the trapped sphere. In Figs. 3(e) and 3(f) we show the flow profiles around three optically trapped spheres, where they are trapped either at the same height (e) or at three different heights (f). These flow profiles corroborate the capability of our system to simultaneously and independently perform quantitative imaging and optical manipulation in 3D while also exhibiting how our system may be used to address fundamental hydrodynamic problems. While the Stokes flow around a single sphere is well established, the flow profile around a network of particles in the limit of small particle–particle separation is less obvious as it may not be described by the superposition of the Stokes flow around single spheres [28]. In addition, the complex 3D flow profiles in active systems could be elucidated directly, for example, by trapping motile bacteria such as Chlamydomonas and simultaneously imaging the flow field [26].

In summary, we have developed a unique method to optically decouple 3D imaging from optical trapping. By remotely refocusing the sample, only a single objective at the sample is required, which allows access for further experimental techniques such as rheology or microfluidics. Our system is capable of aberration-free 3D imaging with an axial range of 66 $\mu$m, while simultaneously manipulating the sample with optical tweezers. Because our simple yet powerful technique is compatible with other optical manipulation and imaging techniques, we believe it is directly relevant to the wide range of experiments that use optical manipulation and simultaneous imaging.

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See Supplement 1 for supporting content.

**REFERENCES**