Supplementary Materials for

Microscopic scan-free surface profiling over extended axial ranges by point-spread-function engineering

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Published 28 October 2020, Sci. Adv. 6, eabc0332 (2020)
DOI: 10.1126/sciadv.abc0332

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- Figs. S1 to S4
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Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/44/eabc0332/DC1)

- Movies S1 to S4
Fig. S1. 3D localization precision for the inflatable membrane. (A) Single frame of fluorescent beads on the inflatable membrane (after subtracting the background). Scale bar: 200 µm. (B) Close-up images of four out of the 60 localized beads. Scale bar: 10 µm. (C) The 3D positions of bead number 1, over the 120 movie frames. The blue line depicts the localized positions, the orange line depicts a 12-order polynomial fit. (D) To account for the bead motion, the polynomial fit was...
subtracted from the localized positions to attain bead 1's residuals. The same procedure was applied to all 60 beads. The localization precision (both lateral and axial) was estimated as the mean of the standard deviation values of the residuals (for all of the 60 beads). The histograms of the axial localizations residuals for the four beads from (B) are presented, together with the obtained standard deviations values. Red are the fitted Gaussian curves.

**Fig. S2.** Digital holography microscopy line profile of the inflatable membrane using a 2.5X 0.07NA objective. The three curves show a full view of three inflation states of the membrane. In the concave case, the arrows highlight steps where the reconstruction failed to reproduce the membrane shape.

**Fig. S3.** Digital micro-mirror device (DMD) illumination pattern. Seventy spots in a 7×10 array were projected onto the reflective surfaces using the illumination-engineering module depicted in Fig. 1A. The spots were separated by 150 DMD pixels and each spot is 5×5 pixels. The DMD pixel size is 7.56 µm.
**Fig. S4. Tilted reflective plane profilometry by PSF engineering.** (A) Captured frames of the same field of view (FOV) presented in Fig. 3C, here with 500 ms exposure time. Left: imaging with the standard PSF, i.e. no mask is displayed on the spatial-light modulator (SLM). Right: imaging with the Tetrapod PSFs, i.e. a phase-mask is displayed on the SLM. Scale bar: 10 µm. (B) Dictionary PSFs used to reconstruct the 3D surface. Scale bar: 2 µm. (C) Reconstructed 3D tilted surface. The color-map represents the deviation from a fitted plane.

**Note S1: Lateral sampling frequency on the inflatable membrane.**

The mean lateral sampling was calculated according to:

\[
\text{emitter density} = \frac{\text{number of emitters localized}}{\text{membrane area}} = \frac{60}{\pi \cdot \left(\frac{1.25}{2}\right)^2} = 48.89 \frac{1}{\text{mm}^2}
\]

(1)

The highest possible lateral sampling, without PSF overlap, is defined by the lateral size of the Tetrapod PSF, ~54 µm in the first and last dictionary elements (which corresponds to the largest recorded axial displacements).

**Note S2: Localization precision.**

For the dynamic PSF profilometry based on fluorescent beads, we have quantified the axial precision based on all of the 60 localized fluorescent beads over the 120 movie frames (where four of them are presented in fig. S1). The mean signal counts of the 60 beads is distributed exponentially with a mean of 16,710 ADU and a background of 105 ADU. The corresponding mean axial precision is 5.3 µm, and the mean lateral precision is 188 nm in x and 179 nm in y. When plotting the obtained lateral and axial precisions for each of the 60 localized beads as a function of their
corresponding signal counts and fitting a power-law decay curve to the data, the law’s exponent values obtained are -0.4 for both x and y precisions and -0.7 for z precision (where -0.5 would correspond to the signal-limited case). This serves as an indication that in the fluorescence case, the main source of localization error is the signal level.

For the second mode of implementation, based on illumination arrays, we reconstructed the tilted plane described in section 2.1 based on frames with two different exposure times: 10 ms and 500 ms. Both measurements gave similar axial precisions (33 nm and 27 nm, respectively), suggesting that in the reflective measurement, precision is mostly limited by model mismatch, rather than signal to noise.

Note S3: Illumination-engineering module.

1. Demagnification of the digital micro-mirror device (DMD) pattern on the sample.

As shown in Fig. 1A, the DMD pattern is first magnified by a factor of 1.5 by lenses L4 and L5. Then, it is demagnified by L6 lens and the objective lens, by a factor that is equal to twice the magnification of the objective. To verify the final demagnification of the DMD pattern, the distance between two projected focused spots in the sample plane was measured, using the standard PSF on the reflective stepped surface presented in Fig. 3G, yielding a spot separation of 208 µm. Compared to the distance of the spots in the DMD pattern (equal to 1134 µm, see fig. S3), the total demagnification factor obtained is 0.183. That is in a good accordance with the expected value which is equal to:

$$\text{Total demagnification} = \frac{1.5}{2M} = \frac{1.5}{8} = 0.1875$$

(2)

2. Depth of field of illumination spots.

Here we show that the varying distances between the sample and the objective do not result in a substantial broadening of the illumination spots. This broadening is due to the finite numerical aperture (NA) of the illumination system.

To describe the DMD back focal plane (BFP), consider a single DMD spot of $n \times n$ pixels$^2$ (equation S3).

$$DMD_{\text{spot}} = \text{rect} \left[ \frac{x}{n \cdot DMD_{\text{pixel size}}} \right] \cdot \text{rect} \left[ \frac{y}{n \cdot DMD_{\text{pixel size}}} \right]$$

(3)

To compute the corresponding BFP, a Fourier transform is applied, to obtain equation S4:

$$D_{\text{DMD BFP}} = \text{sinc} \left[ n \cdot DMD_{\text{pixel size}} \cdot \frac{\tilde{x}}{\lambda f} \right] \cdot \text{sinc} \left[ n \cdot DMD_{\text{pixel size}} \cdot \frac{\tilde{y}}{\lambda f} \right] \cdot \text{circ} \left( \frac{D_{\text{Lens}}}{2} \right)$$

(4)

Where $\tilde{x}$ and $\tilde{y}$ are the BFP coordinates, $\lambda$ is the laser wavelength, $f$ is the focal length and $D_{\text{Lens}}$ is the diameter of the lens which is located right after the DMD (L4: $f=100$ mm in Fig. 1A, with a 2 inch diameter).

Therefore, for a $5 \times 5$ pixels$^2$ spot on the DMD, the BFP diameter is 1.5 mm. As shown in Fig. 1A, the DMD BFP is further magnified by a factor of 2.67 before the BFP of the objective. Hence on the BFP of the objective, the conjugate DMD BFP size is 4 mm.

The BFP diameter of an objective is calculated according to the following equation:

$$D_{\text{Objective BFP}} = \frac{2f_{\text{tube}} \cdot NA}{\sqrt{M^2 - NA^2}}$$

(5)
For the 4X, 0.13NA air objective, the 20X, 0.75NA air objective, and the 100X, 1.45NA oil objective, the BFP diameters are 13 mm, 15 mm and 5.8 mm respectively. According to the derived BFP sizes, it can be concluded that the NA of the illumination system is ~3 /~3.7/~1.4 times smaller than the NA of the 4X/20X/100X objective, therefore the depth of field of the illumination is sufficiently larger than the one defined for the objective using Equation 1. Hence, the illumination pattern remains ‘in-focus’ longer than the microscope’s detection PSF, and there is no significant distortion of the PSF due to broadening of the illumination spots (Fig. 3 and Fig. 4).

**Supplementary Movie Captions:**

**Movie S1. Inflatable membrane profiling by fluorescence-based PSF engineering.** A PDMS membrane (1.25 mm diameter), decorated with a low density of fluorescent beads, is inflated and deflated by controlling the air pressure in a proximal cavity. The membrane was imaged at 50 Hz using a 4X 0.13NA objective (3.3×3.3 mm² FOV). Left: the captured fluorescence video frames during which the membrane was deformed (after subtracting the background). Each bead's PSF shape changes with its axial location. Right: the corresponding surface reconstruction of the membrane. The reconstructions are based on localization of 60 fluorescent beads (represented as red dots). The surface color represents the interpolated axial position of the membrane. Scale bar: 200 µm.

**Movie S2. Inflatable membrane profiling by DHM using a 10X objective.** Reflection-based digital holography microscopy (DHM) profilometer was used to reconstruct the 3D dynamics of a PDMS membrane (1.25 mm diameter), while being inflated and deflated. The membrane was imaged at 12.5 Hz with the DHM profilometer using a 10X 0.3NA objective (enabling only a partial view of the membrane, 0.66×0.66 mm² FOV). The corresponding line reconstructions (from the center of the membrane surface reconstruction) are shown. At angles above ~4.7 degrees, the DHM method incorrectly reconstructs the membrane surface (the line reconstructions of the smooth membrane exhibits non-smooth jumps).

**Movie S3. Inflatable membrane profiling by DHM using a 2.5X objective.** Reflection-based digital holography microscopy (DHM) profilometer was used to reconstruct the 3D dynamics of a PDMS membrane (1.25 mm diameter), while being inflated and deflated. The membrane was imaged at 12.5 Hz with the DHM profilometer using a 2.5X 0.07NA objective (this enabled a full view of the membrane, 2.64×2.64 mm² FOV). The corresponding line reconstructions (from the center of the membrane surface reconstruction) are shown. At angles above ~1 degree, the DHM method incorrectly reconstructs the membrane surface (the line reconstructions of the smooth membrane exhibit non-smooth jumps).

**Movie S4. Dynamic, tilting-mirror profiling by label-free PSF engineering.** A 12.7 mm diameter mirror undergoes a damped oscillatory motion, after tilting the mirror and releasing it. Using an array of projected illumination spots to illuminate the surface, the mirror is imaged at 50 Hz with a 20X 0.75NA objective (0.66×0.66 mm² FOV). Left: recorded video frames of the illumination-spot array reflected off the mirror. Top right: the corresponding surface reconstructions based on localization of the 42 illumination spots (represented as red dots). The surface color represents the deviation of the reconstruction from a fitted plane. Bottom right: the experimentally derived angles of the fitted plane, relative to the steady state resting angle, during the video acquisition time. Scale bar: 50 µm.