Supplementary information

Light-driven micro-tool equipped with a syringe function

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Fabrication of micro-tools

Our micro-tools are fabricated using a commercial two-photon polymerization (2PP) setup (Nanoscribe Photonic Professional). The photoresist (IP-L 780) is prepared over a microscope cover slip by drop casting. A pulsed laser (780 nm, 140 mW average power, 100 fs pulse duration) is used to induce twophoton absorption in the photoresist and creates a solid voxel. The laser or the stage can be scanned in 3D in order to "write" intended structures within the photoresist. In our particular setup, we use a piezo stage to do the scanning. The scanning trajectory can be set by the Cartesian coordinates that define our structures. In a raster scanning approach, these coordinates are extracted by slicing volumetric structure data from CAD software and then writing the structure line by line, layer by layer, from bottom to top, until the structure is fully printed. An alternative approach is to use directional scanning where the trajectory is the contour of the structure itself. We find that it is simpler to define our structure based on parametric equations that give the coordinates in a sequential manner based on some monotonically increasing parameters. The hollow body of our micro-tool is based on the surface of revolution of the socalled teardrop curve, which we parametrized as $x = A\cos\theta$

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x = A\cos\theta
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y = \left[B\sin\theta\sin^{m}\frac{1}{2}\theta + r\right]\cos\phi
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$$
z = \left[C\sin\theta\sin^{m}\frac{1}{2}\theta + r\right]\sin\phi
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\n(S1)

where $A = 20 \mu m$, $B = 12 \mu m$, and $C = 8 \mu m$. The parameters $\theta = 0, \pi$ and $\phi = 0, 2\pi$ are sampled at 100 points within their respective range. The sampling interval is related to the hatching and slicing distance in the traditional raster scanning approach mentioned above. The radius of the spout is set to $r = 3 \mu m$ and the taper towards the spout is determined by the parameter *m* which we set equal to 5. The spherical handles for optical trapping have diameters of 8 μm. In Figure S1a, we show a schematic of the micro-tool as it is being fabricated on top of the glass substrate. Thin supporting feet below each spherical handle ensure that the micro-tools are anchored firmly to the glass substrate. These feet are made by fabricating closely spaced vertical lines until they merge into a solid square bar with cross-sectional area of 1 μ m \times 1 μm and has height of 9 μm. A hole with a diameter of 8 μm is left open on top of each micro-tool body for the subsequent deposition of thin gold disk on the bottom inner wall by electron beam vapor deposition. A mask with matching hole (see Figure S1b) is fabricated on top of the micro-tool, held by support posts anchored to the substrate, to expose only the target region while shielding the rest of the micro-tool during the deposition process.

The sequence of our fabrication starts with making the mask where we set the laser power at 70% and the scan speed to be 100 μm s-1. The schematic diagram in Figure S1 only shows few support posts for visual clarity but more are actually added for structural integrity and to minimize warping during development (see the SEM image in Fig. S1D). The mask is fabricated 20 µm from the substrate to have sufficient gap from the micro-tool since we want the mask and the micro-tool to be separated during collection. When the mask is finished, the micro-tool is fabricated underneath starting with feet, the spherical handles and finally the main body. For the micro-tool the laser power is set to 60% while the scan speed is lowered to 50 μ m s⁻¹ as a good compromise between fabrication time and accuracy. We are able to fabricate a 6 \times 6 array of micro-tools and mask in about five hours. Figure S1c shows a brightfield image of the fabricated

micro-tools and Figure S1d shows a SEM image where we approximate the thickness of the wall of the micro-tool to be around 1.5 μm at its thickest and 0.5 μm at its thinnest side. The SEM image also shows more details in the structure of the mask. After two-photon exposure, the written structures are developed in a bath of isopropyl alcohol for 15 minutes. A second alcohol bath ensures that no photoresist remains inside the hollow body of each micro-tool.

Figure S1| Fabrication of micro-tools. a) Schematic diagram of the 2PP fabrication of masks and microtools. They are fabricated on top of a microscope cover slip. An offset is made to ensure that the structure is anchored properly. **b)** The hole on the mask is seen when viewing from a different angle. This hole exposes the target region where deposition will take place. **c)** Brightfield and **d)** SEM images of the fabricated structures.

To ensure that the micro-tools and the masks are attached securely to the glass substrate, we start laser scanning at a 0.5 μm offset below the substrate as shown in Figure S1a. Hence, the solidified support structures are in contact with the substrate, preventing any floating structures that might be washed away. The next section will discuss the collection of the micro-tools from the substrate.

Collection of micro-tools

The two-photon fabrication of the micro-tool is followed by the electron beam physical vapor deposition (Alcatel SCM600) starting with a 1 nm layer of titanium as adhesion layer followed by a 5 nm layer of gold. After deposition the next crucial step is to remove the micro-tools from the substrate and to transfer them into a cytometry cuvette where the trapping experiments will be later performed.

We have built a separate setup for this purpose. The setup is essentially an inverted microscope with a 20x objective lens. We have added XYZ stage controlled by motorized actuators (Thorlabs, 6mm DC actuators) that hold and control a microliter syringe (Hamilton, 25 μL gas tight syringe). We fitted a fine glass capillary tube (Vitrocom, 80 μ m × 80 μ m inner channel cross section) as the needle of the syringe. A schematic of the setup and an actual microscope image of the collection process are shown in Figure S2. The transparency of the capillary tube makes the interactive collection of the micro-tool more convenient. The micrometer movement of the motorized actuators allows us to position the capillary tube near the structures and by slightly nudging the structures, the mask and the micro-tool can be safely removed from the substrate. Upon removal from the substrate, the mask and the micro-tool are floating freely and can be drawn in using the syringe. The pumping of the syringe is controlled by another motorized actuator. At this point, we are not concerned whether the mask is also collected.

Once the micro-tools are collected, the syringe is lifted from the substrate using the stage. The substrate containing the remaining micro-tools are removed and replaced with the cytometry cuvette (Hellma, 250 μm × 250 μm inner channel cross section) containing a solution of deionized water, 0.5% Tween 80 surfactant, 10% ethanol and microbeads (e.g. 2 µm silica or 1 µm polystyrene beads). Using the XYZ stage, the capillary tube is slowly inserted into the channel of the cuvette and the micro-tools are released inside using the syringe. The capillary tube is slowly pulled out and the cuvette is sealed with vacuum grease and nail polish. A video showing the collection and transfer of a micro-tool is provided as Supplementary material. Finally the cuvette is taken to the BioPhotonics Workstation for the optical trapping and material transport experiments.

Figure S2| Collection of micro-tools. A fine glass capillary tube is fitted to a microliter syringe to dislodge and collect the micro-tools. The movement and pumping of the syringe are controlled by motorized actuators. The interactive collection mechanism employed in our setup allows up to 100% transfer efficiency.

Movies accompanying the manuscript

Movie S1. Collection and transfer of micro-tools. After vapor deposition of the metal layer, the microtools are collected from the glass substrate. This is accomplished by using a fine glass capillary tube fitted to a microliter syringe. The movement of the syringe and the capillary tube is controlled by motorized actuators. The micro-tools are dislodged from the glass substrate by a gentle push from the capillary tube. The pump of the syringe is also controlled by another actuator which collects the dislodged micro-tools. Using the same capillary tube and syringe, the micro-tools are transferred to a cytometry cuvette for succeeding trapping and loading/unloading experiments. The schematic diagram for the methodology is shown in Figure S2.

Movie S2. Illustrative flow speed measurement using a 2 μm-silica bead as tracer. The metal layer inside the micro-tool is heated using a laser beam (1070 nm). The heat generated causes convection current that draws in the particle. A feature tracking algorithm is used to monitor the velocity of the tracer. The velocity plot is shown in Figure 3 in the manuscript.

Movie S3. Loading of cargo inside the micro-tool. Here we demonstrate loading of cargo inside the micro-tool and spatial control. The micro-tool is optically manipulated to pick up cargo at different locations. Snapshots of the experiment are shown in Figure 4 in the manuscript.

Movie S4. Pumping of cargo. Here we demonstrate pumping of cargo in and out of the micro-tool by moving the heating beam across the metal layer. This functionality mimics the familiar pumping action of a syringe. Snapshots of the experiment are shown in Figure 5 in the manuscript.