Electronic Supplementary Material (ESI) for Lab on a Chip.

Modified inverted selective plane illumination microscopy for sub-micrometer imaging resolution in polydimethylsiloxane soft lithography devices

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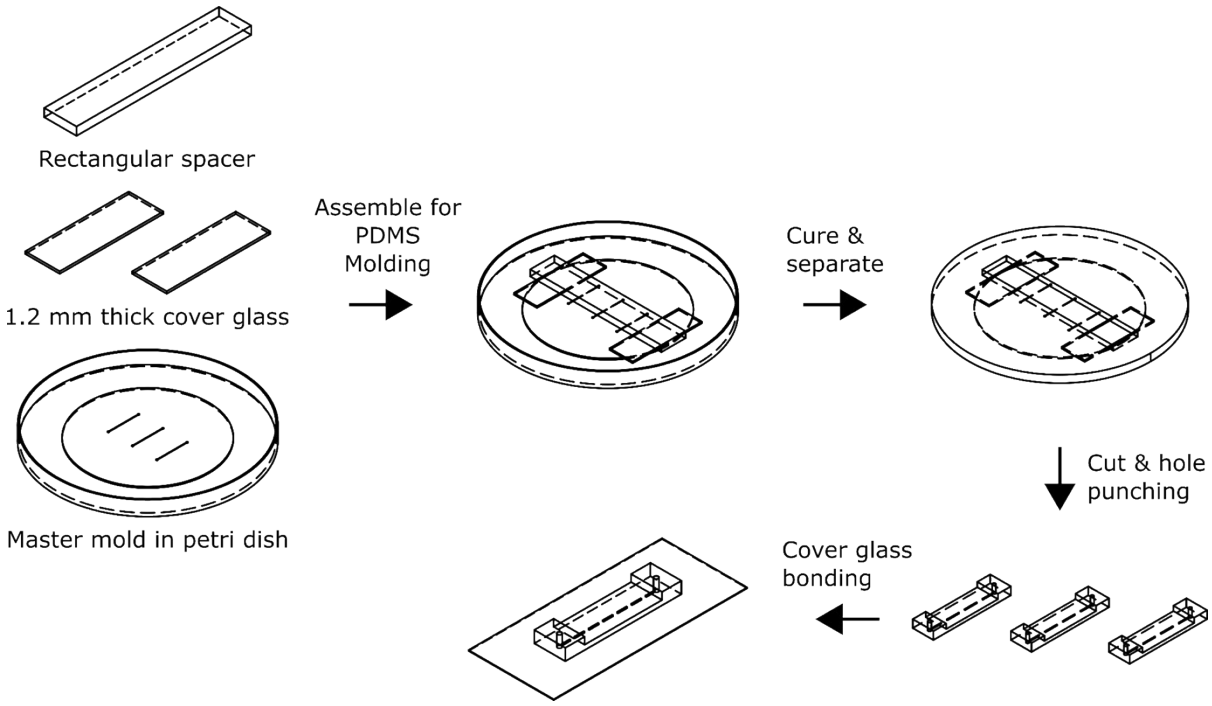


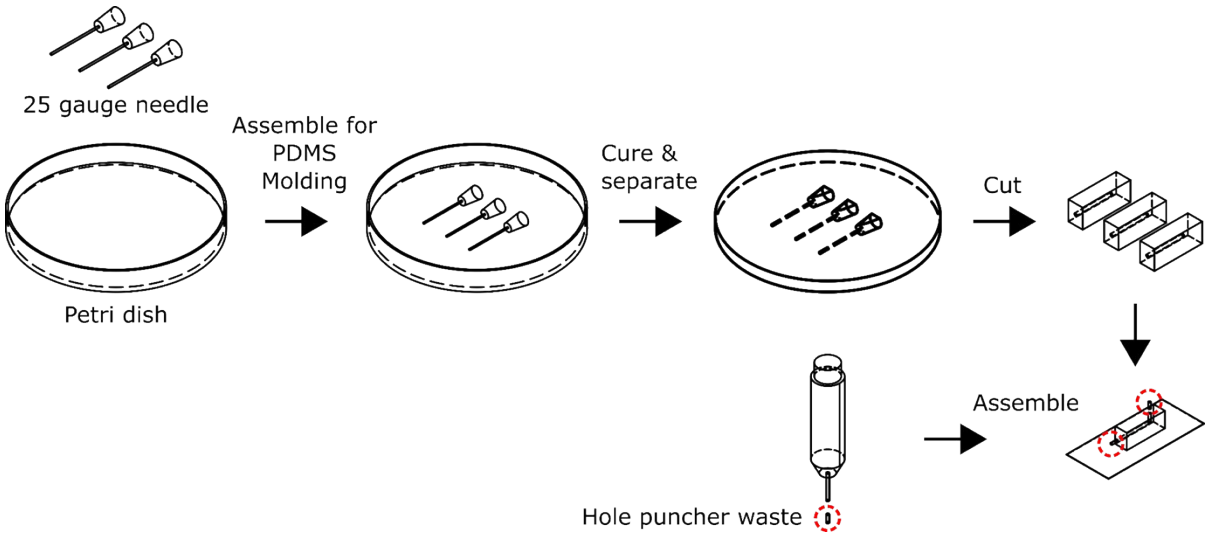
Supplementary Figure 1 – Protocol for manufacturing m-iSPIM compatible PDMS microchannel device.

Materials: 1) 1 master mold with negative channel structure; 2) 1 petri dish (150×25 mm); 3) 2× 1.2 mm thick microscope slides (75×25 mm); 4) 1 rectangular acrylic bar with suitable size (100×15×10 mm); 5) 1:10 PDMS mixture 6) 500 μm hole puncher

Procedure: The master mold, microscope slides, and then the spacer are stacked on an empty the petri dish as shown. The dish is filled with PDMS mixture, where it may be necessary to press down the components as they may dislodge with the mixture. The PDMS is cured, cut to shape and then inlets and outlets hole punched with a 500 μm hole puncher, and then bonded to a coverslip after plasma treatment.

Notes:

1. The master mold used is a silicon wafer that contains an array of 100×25 μm, 20 mm long (end-to-end) microchannel structures generated by photolithography using SU-8 2002 photoresist.
2. The surface of the rectangular spacer should be smooth for the top surface of the device to remain optically flat and the width of the spacer should not cover the master mold's inlets or outlets such that there is sufficient thickness for a stable tube connection. The spacer dimensions used in this study are 100×15×10 mm.



Supplementary Figure 2 – Protocol for manufacturing m-iSPIM compatible PDMS microchamber device.

Materials: 1) 25-gauge needles; 2) 1 petri dish (150×25 mm); 3) 500 µm hole puncher; 4) 1:10 PDMS mixture

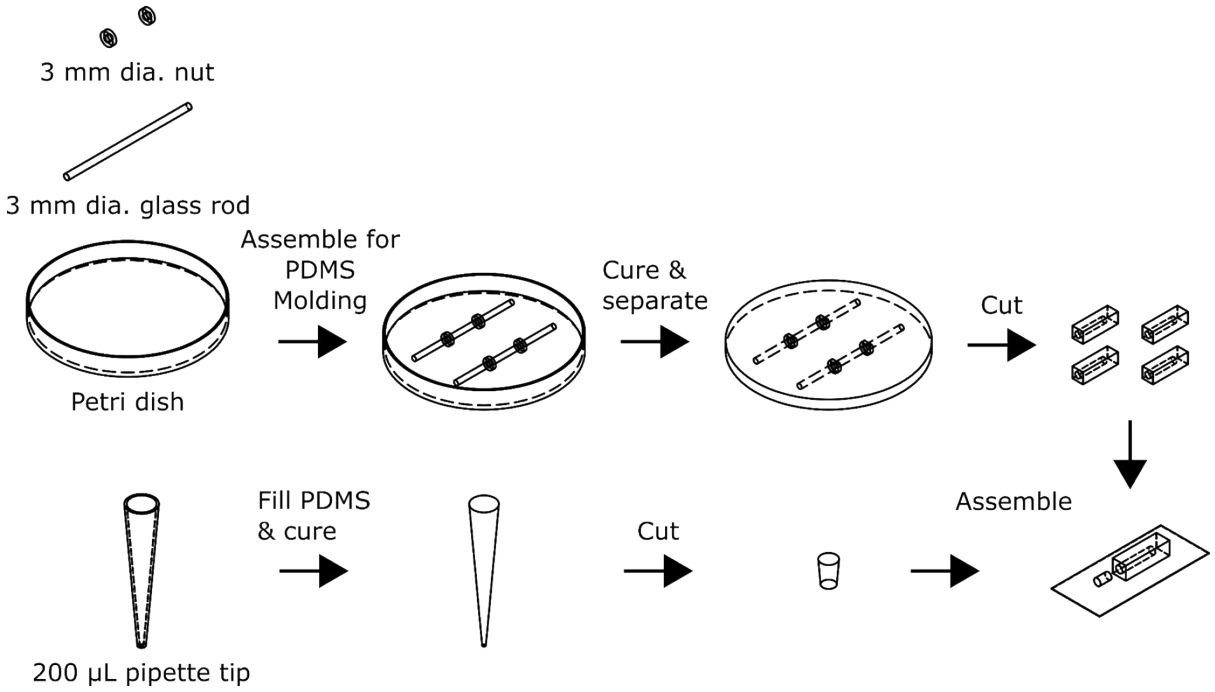
25-gauge needles are positioned in the petri dish and serve as a mold. Upon pouring of premixed PDMS, the needles tips become elevated from the petri dish surface. The PDMS is then cured, cut, and bonded to coverslip.

Procedure: 1) place the needles in the petri dish as shown, our needles are able to stand (having the needle part positioned horizontally without touching the petri dish); however, if they do not it is necessary to glue them on the petri dish to maintain needle tip in elevated position; 2) fill the dish with pre-mixed, degassed 1:10 PDMS mixture, it may be necessary to reposition the components as they may slightly shift as the mixture is added (no need if glued); 3) cure the mixture with preferred protocol, here we cured at 80^{o}C for 2 hours; 4) separate the PDMS replica from the petri dish; 5) cut out microchambers each with an approximate width of 5 mm and desired length, there should be only one opening; 6) use the hole puncher to punch out an opening near the sealed end of the channel, keep the hole puncher wastes; 7) bond the device on a cover slip for easy handling; 8) (after sample loading) insert hole punch wastes into the microchamber opening to achieve sealing.

Notes:

1. The waste from the hole punching of inlets and outlets are retained to be used as stoppers to prevent leakage of solution into and out of the microchamber.

2. The PDMS mixture volume should be carefully measured such that the overall height covers just over the needle, which reduces device height for better iSPIM compatibility.



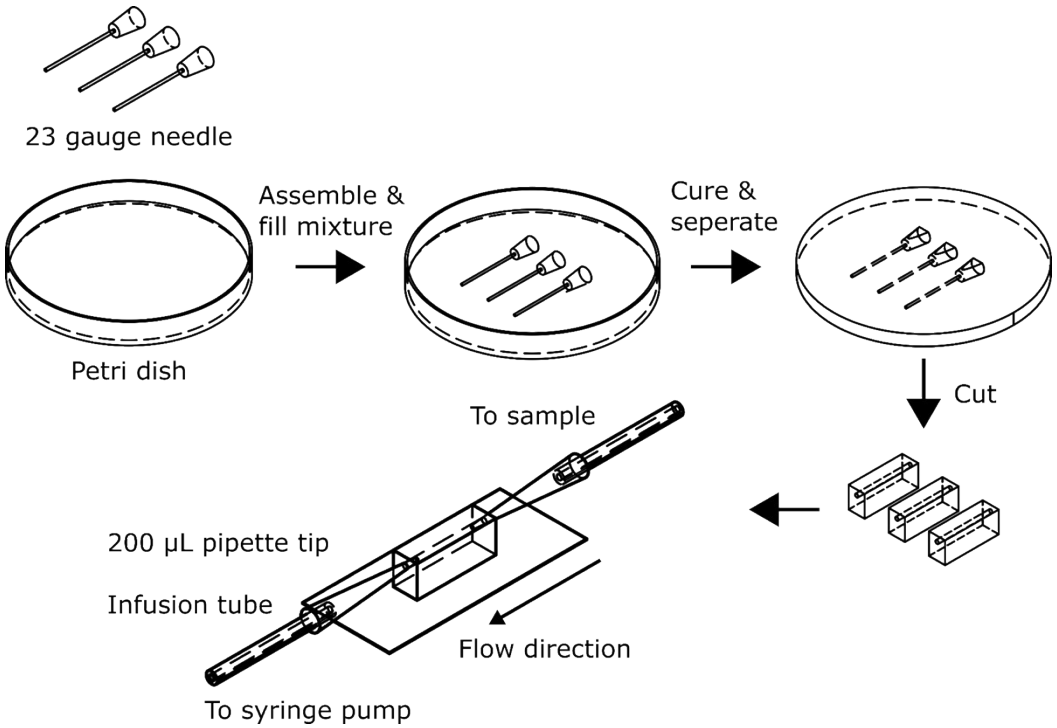
Supplementary Figure 3 – Protocol for manufacturing m-iSPIM compatible PDMS chamber device.

Materials: 1) 3 mm diameter glass rod; 2) petri dish; 3) Two 3 mm diameter nuts for each rod; 4) 200 µL pipette tips; 5) 1:10 PDMS mixture

Procedure: Glass rods are fitted with two 3mm diameter nuts each to elevate the rod above the petri dish and then assembled on the petri dish as PDMS molds. Premixed PDMS is poured in and cured. The chambers are then cut out from both ends of the rod to an approximate width of 5 mm. To generate stoppers, 200 µL pipette tips are filled with the PDMS mixture and cure accordingly and removed from the pipette tips by pulling from the open end. The stopper is then cut to a suitable length.

Notes:

1. While other materials can be used for the rods, glass is ideal for its smooth surface, reducing aberrations stemming from the inner chamber wall.
2. PDMS mixture volume should be carefully measured to cover just over the height of the rods, which reduces the overall device height for better iSPIM compatibility.



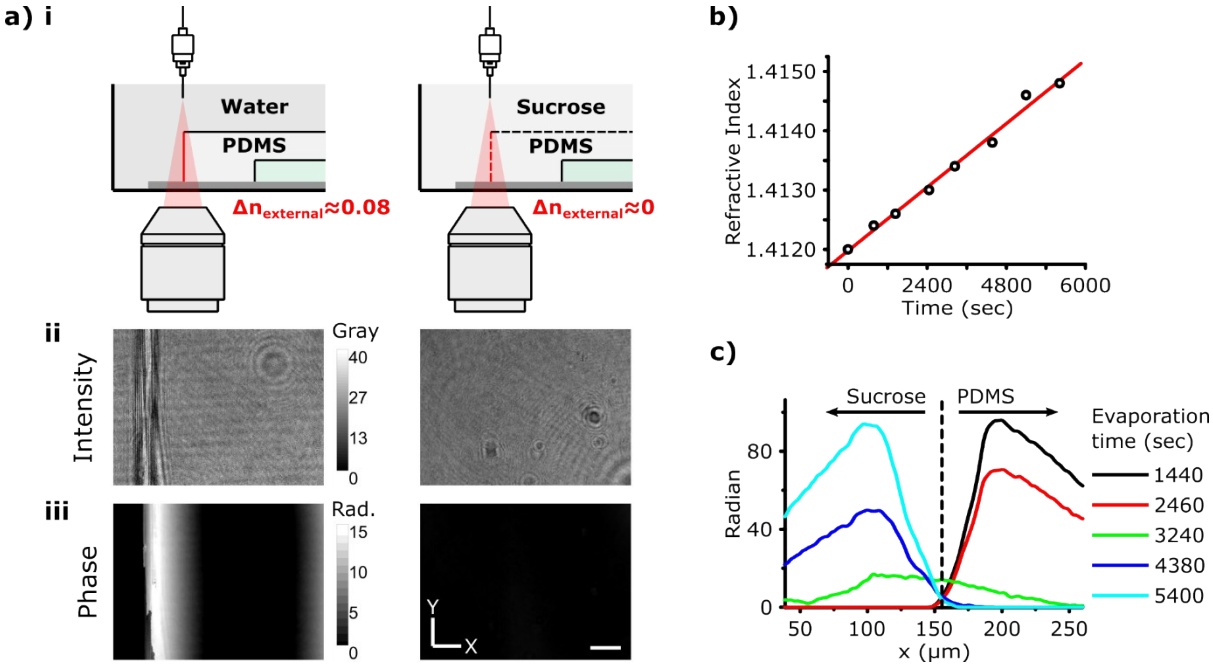
Supplementary Figure 4 – Protocol for manufacturing flow-enabled m-iSPIM compatible PDMS channel device.

Materials: 1) 23-gauge needles; 2) petri dish; 3) 1:10 PDMS mixture; 4) 200 µL pipette tips; 5) infusion tubes

23-gauge needles have an outer diameter of approximately 600 µm. The overall procedure for manufacturing the microchamber device is equivalent to the previous microchamber device protocol but requires through holes at both sides as inlet and outlet. Here, a customized connection using 200 µL pipette tips and infusion tubes and Parafilm as sealing is demonstrated. Again, PDMS mixture volume should be carefully measured to that the overall height is just over the rods, which reduces device height for better iSPIM compatibility.

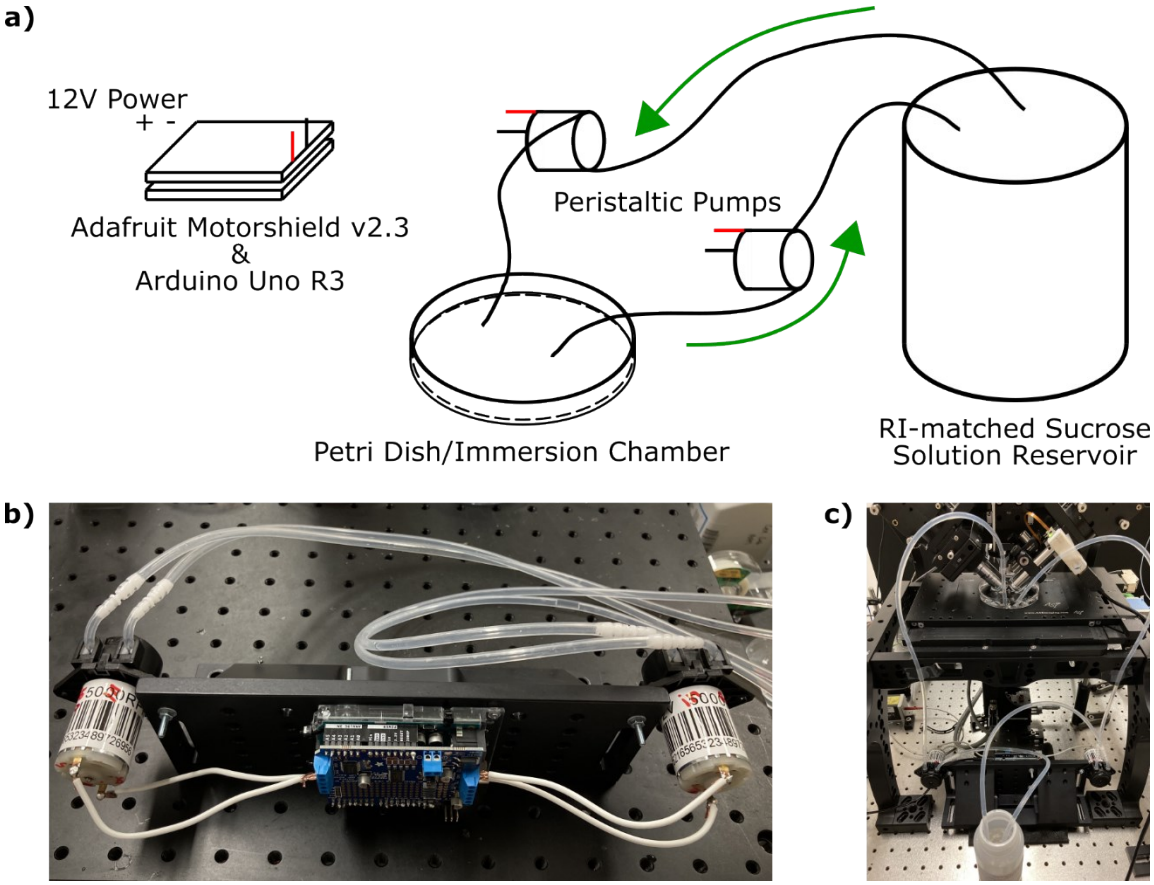
Procedure: 1) place the needles in the petri dish as shown, our needles are able to stand (having the needle part positioned horizontally without touching the petri dish); however, if they do not it is necessary to glue them on the petri dish to maintain needle tip in elevated position; 2) fill the dish with pre-mixed, degassed 1:10 PDMS mixture, it may be necessary to reposition the components as they may slightly shift as the mixture is added (no need if glued); 3) cure the mixture with preferred protocol, here we cured at 80^{o}C for 2 hours; 4) separate the PDMS replica from the petri dish; 5) cut out channels each with an approximate

width of 5 mm and desired length; 6) trim channel inlet and outlet, make sure they are fully opened without blockage; 7) bond the device on a cover slip for easy handling; 8) insert infusion tube as deep as possible into the pipette tip and seal with Parafilm; 9) carefully insert pipette tip into channel inlet and outlet to form a sealed channel.



Supplementary Figure 5 – Assessing external interface RI matching quality of a PDMS device

a) i. Illustration of imaging outer edge of PDMS device using DHM fiber illumination. **ii.** Reconstructed brightfield intensity images and **iii.** phase images showing the water-PDMS and sucrose-PDMS interface. Scale bar: 100 μm. **b)** Time-dependent changes to RI of the sucrose solution as it undergoes evaporation ($\Delta n/sec > 5x10^{-6}$). **c)** OPD profile retrieved at different timepoints during evaporation.



Supplementary Figure 6 – Fluidic feedback system for maintaining RI and sucrose solution homogeneity in petri dish

a) Schematic showing the fluidic feedback system with labelled components. **b)** Prototype device of the fluidic feedback system. **c)** The device connected to the petri dish immersion chamber for m-iSPIM.