

Supplementary Information

Rapid Spheroid Clearing on a Microfluidic Chip

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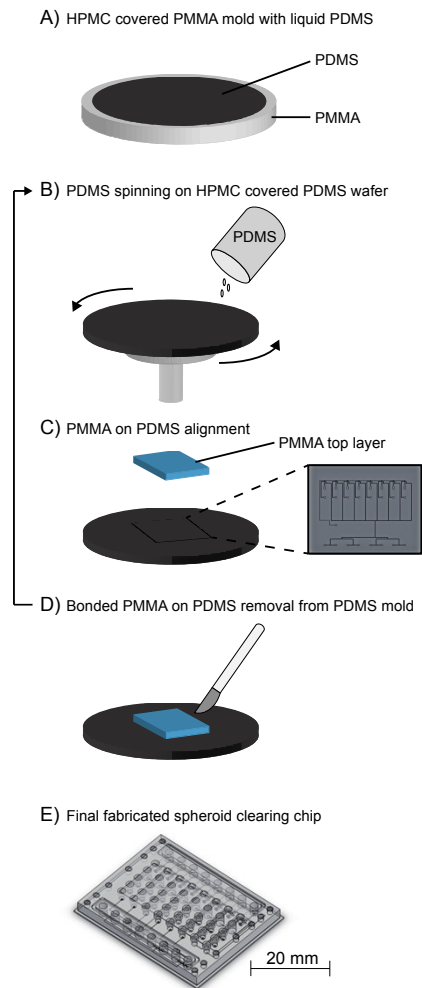


Figure S1. Fabrication workflow for the mClear-Chip. **A)** A PMMA master mold is fabricated through micro milling to achieve semi-round channels of $> 200 \mu\text{m}$. A negative PDMS wafer is cast from the HPMC-treated PMMA mold. The PDMS wafer is blackened to make the structures on it visible. **B)** A thin PDMS layer is spun onto the blackened PDMS wafer, and then **C)** a 4 mm PMMA adapter is bonded onto the thin PDMS layer, **D)** cut off with a scalpel and removed from the wafer. **B)–D)** The same procedure is performed a second time with the second PDMS layer on the control PDMS wafer to obtain a two-layer PDMS fluidic network bonded to PMMA on top. **E)** The resulting three-layer hybrid is then bonded onto a glass slide to obtain the complete chip platform for spheroid clearing.

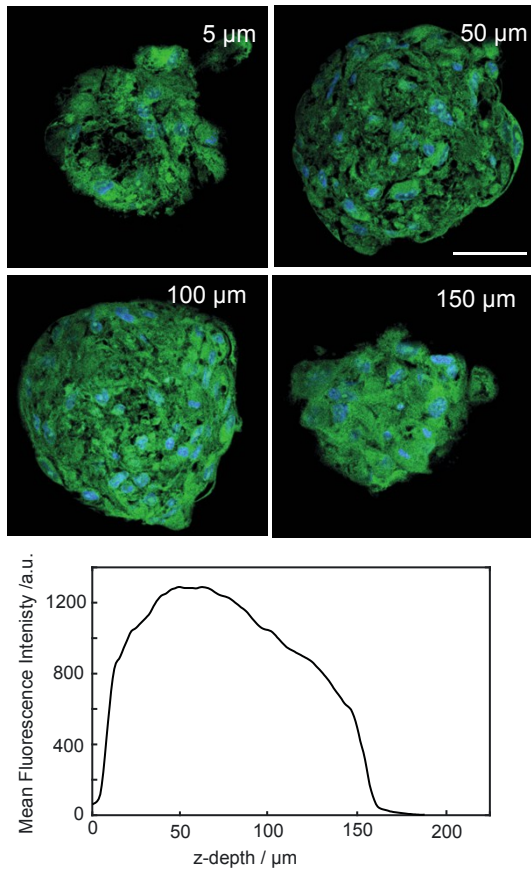


Figure S2. Representative fluorescence image sections through a hASC spheroid stained with anti-GAPDH (Atto488). Lower panel shows the average fluorescence intensity of all image sections of the spheroid in dependence of the imaging depth. While the brightness of the images in B were gradually adjusted, the laser power during acquisition not.

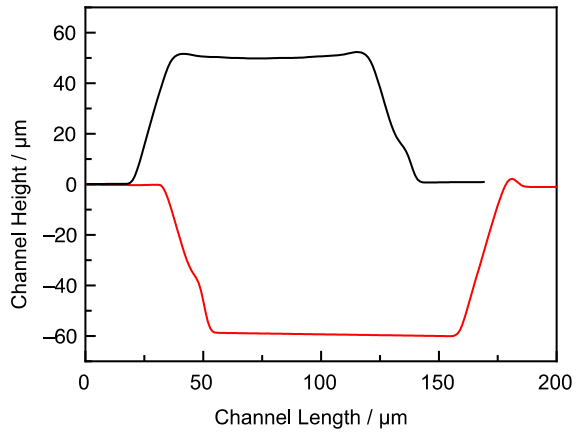


Figure S3. Comparison of microchannel profiles from a milled (red line) and photolithography mold (black line). The profiles were taken from microchannels on the lower and upper PDMS molds of the mClarity Chip. The surface roughness of the microchannel surfaces defined as arithmetic mean deviation of the line profile (R_a) was on both molds $> 0.8 \mu\text{m}$.