Supplementary Information (SI) for Lab on a Chip.


Supporting Information

for

# A Customizable, Low-cost 3D-Printed Device for Live Cell Confinement Imaging

Hunter Richman, Jin Ou, Manpreet Khera, Yan Yu

**Content:**

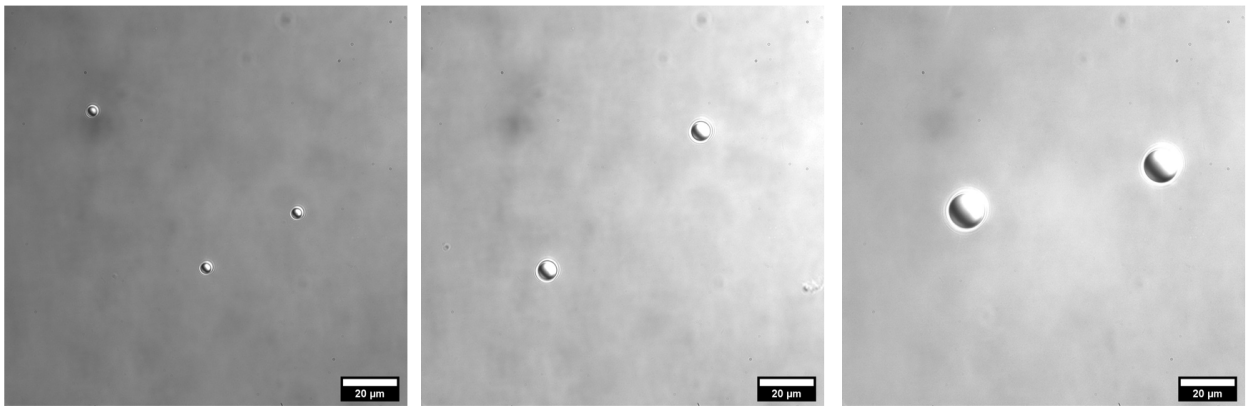
Table S1

Supplementary Figures S1-S5

Caption for Video S1

**Table S1: Comparison of current cell confinement methods**

| Confinement type | Advantages | Disadvantages | References |
|---|---|---|---|
| AFM | • Measure confinement force<br>• Adjustable confinement | • Single cell confinement<br>• Requires specialized instrumentation | 23, 28, 30 |
| Agarose pads | • Cheap/accessible | • Inconsistent confinement<br>• Unquantified confinement | 26, 27, 34 |
| Microfluidic chambers | • Extremely customizable<br>• Multicell confinement | • Requires microlithography | 12, 32 |
| Microporous scaffolds | • 3D confinement<br>• Multicell confinement | • Distribution of pore sizes<br>• Requires gel synthesis<br>• Requires characterization | 16,19 |
| Micropillar arrays | • Confines to set height<br>• Multicell confinement | • Requires microlithography | 31, 33 |




3 µm spacers
$6.75 \times 10^6$ particle / mL

7 µm spacers
$2.60 \times 10^5$ particles / mL

12 µm spacers
$7.95 \times 10^4$ particles / mL

**Fig. S1**: DIC images of spacer bead distribution on coverslips.

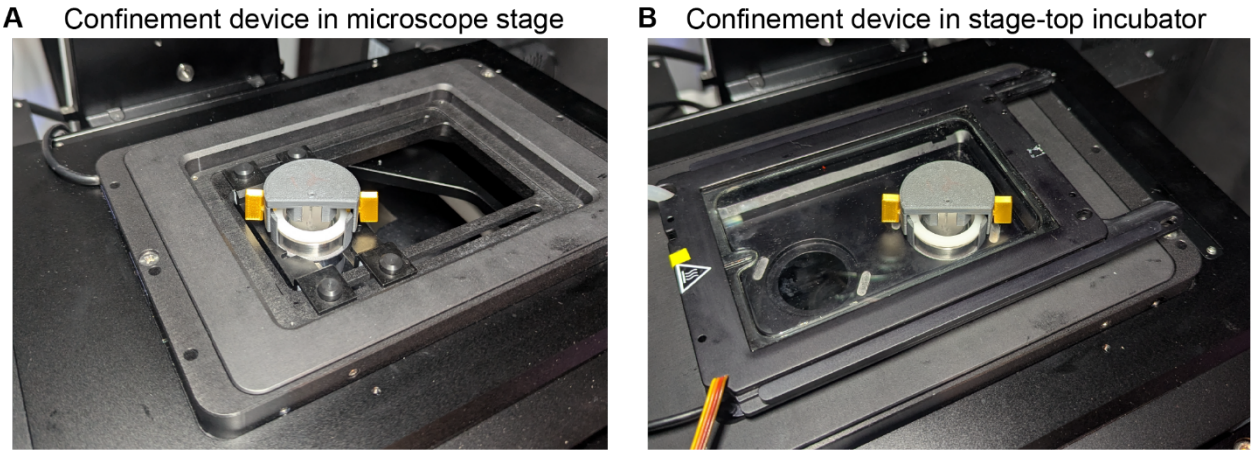



**Fig. S2**: Photographs of the confinement device positioned on an inverted microscope stage without a stage-top incubator (left) and enclosed within a stage-top incubator (right).

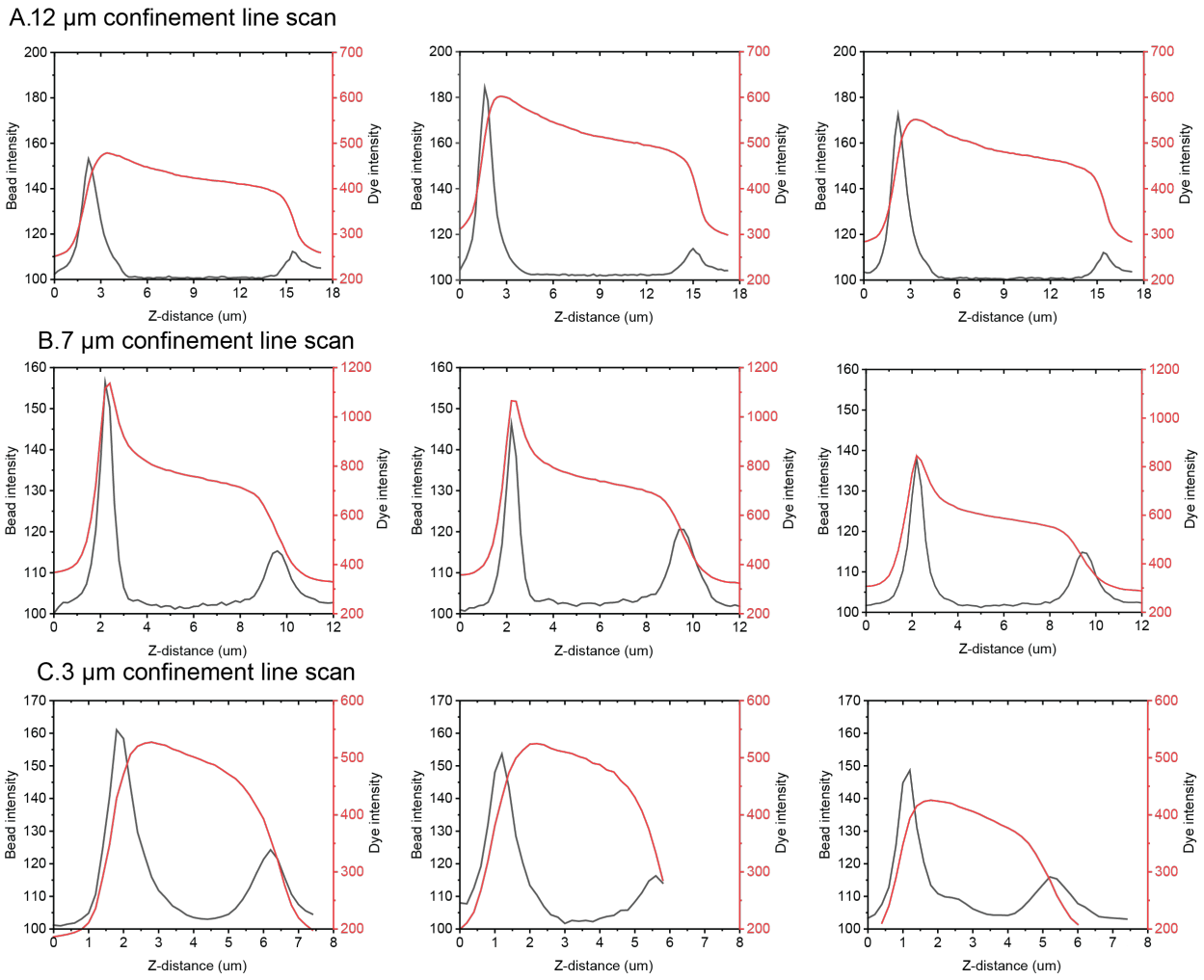



**Fig. S3:** Line-scan intensity profiles of surface-adsorbed beads on coverslips and fluorescent dye in PBS at 3 different locations across the coverslip for each confinement height.

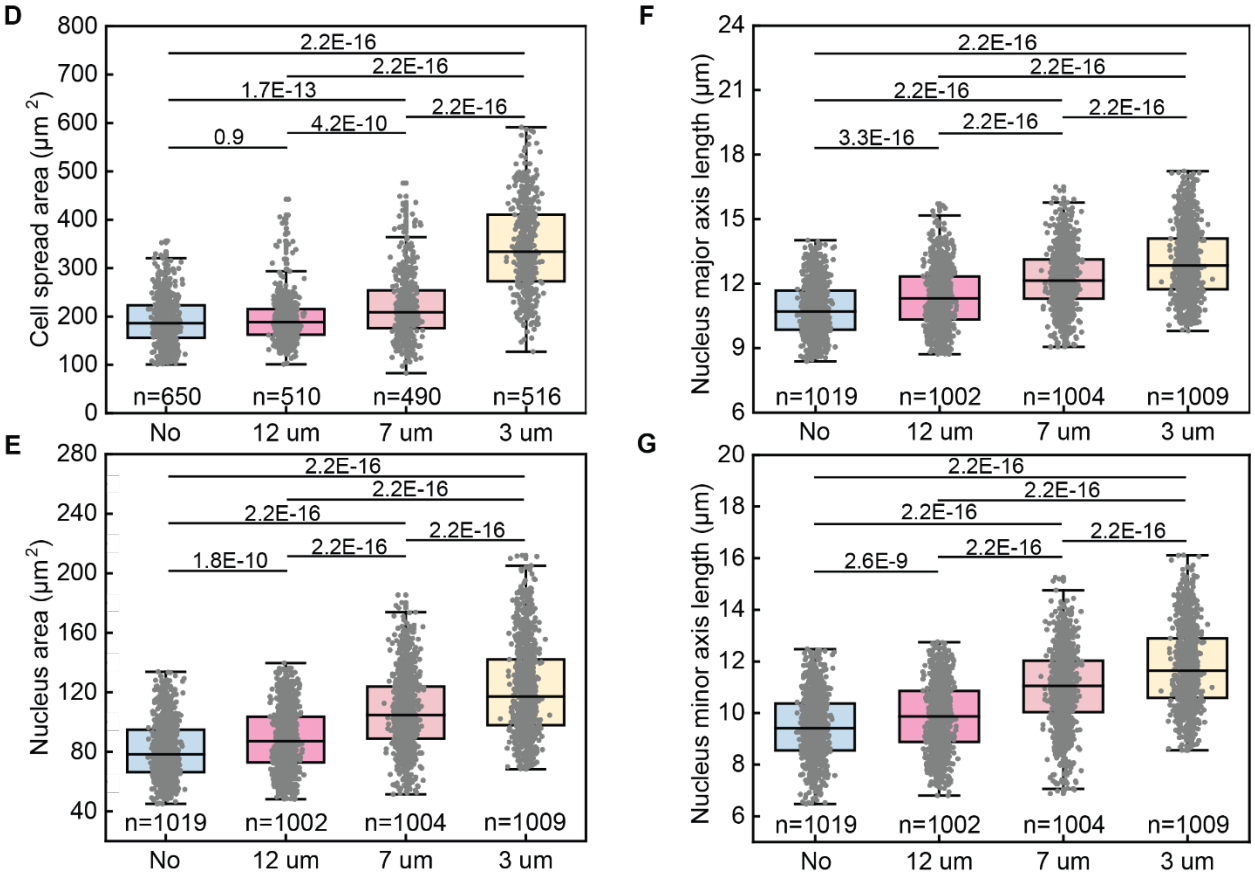



**Fig. S4**: Quantification of 2D nuclear morphology and cell spread area under confinement with exact p values, from same dataset as shown in Figure 3D-G. Here, exact p values are reported for all statistical comparisons in place of significance indicators shown in Figure 3D-G.

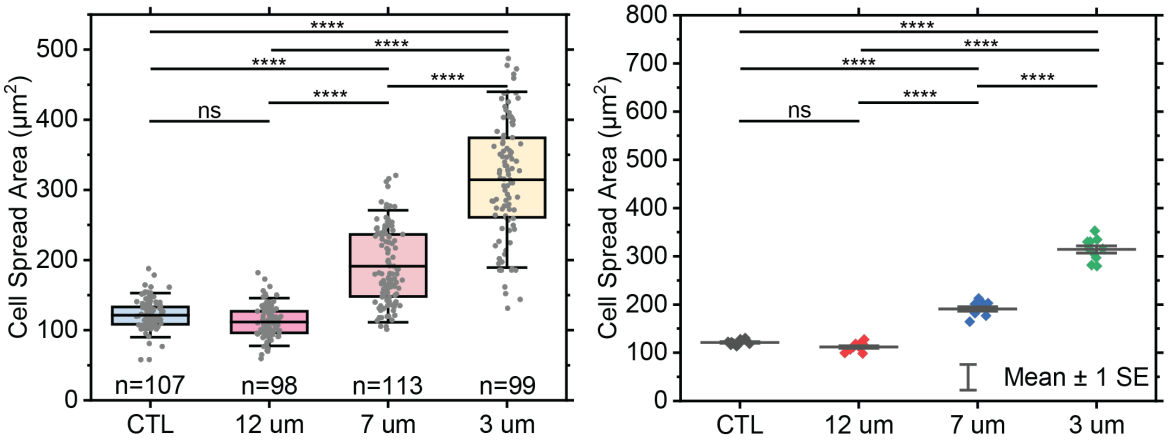


**Fig. S5**: Quantification of 2D cell spread area of RAW264.7 cells expressing RelA-GFP under confinement. (A) Each point represents single cell data. (B) Each point corresponds to the average of ~10 cells from a field of view.

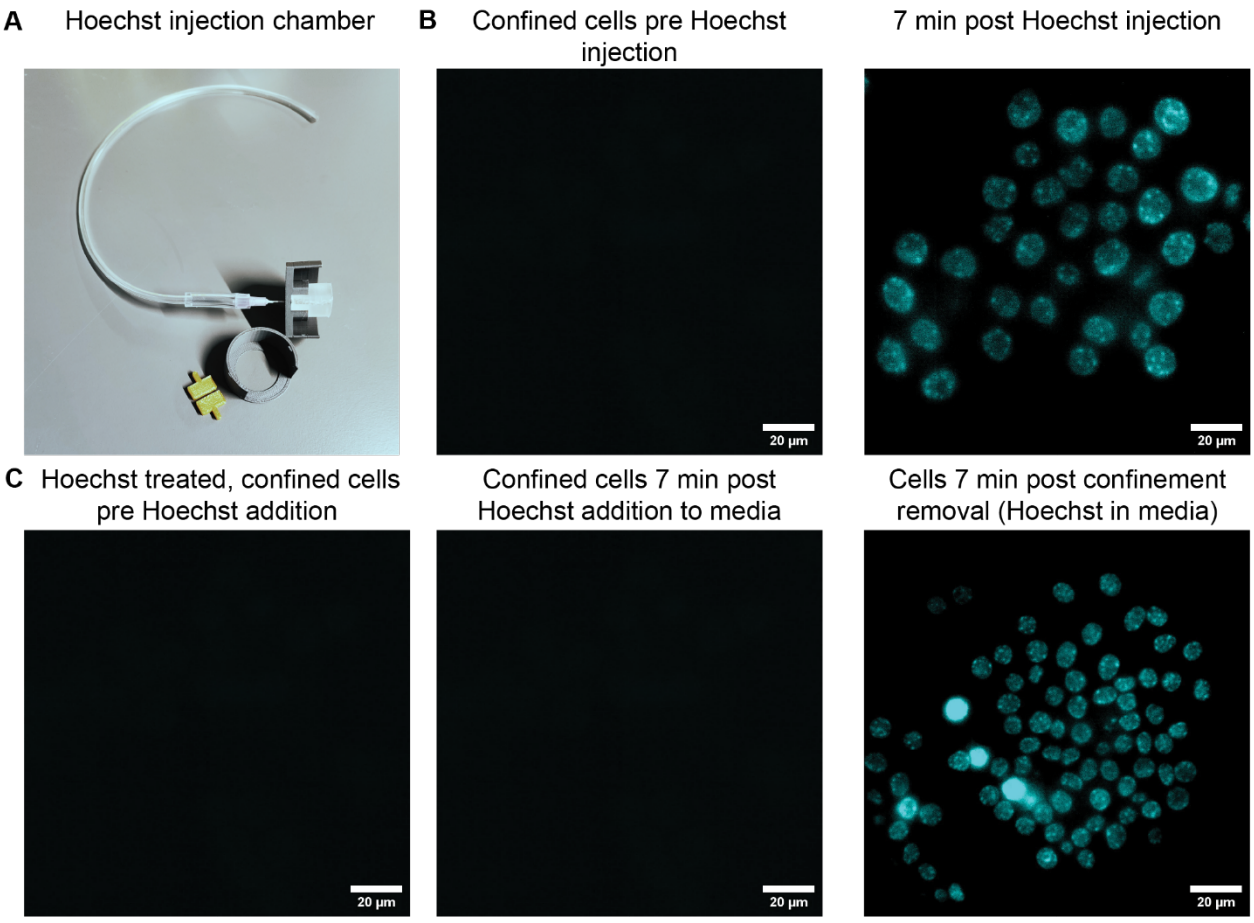



**Fig. S6**: Injection of Hoechst under confinement. (A) Confinement device with injection modification. (B) RAW264.7 cells under 7 µm confinement before and after Hoechst injection. (C) RAW264.7 cells under 7 µm confinement pre-Hoechst in media, post-Hoechst in media, and after confinement was removed. Hoechst did not stain confined cells when added to the surrounding until confinement was removed.

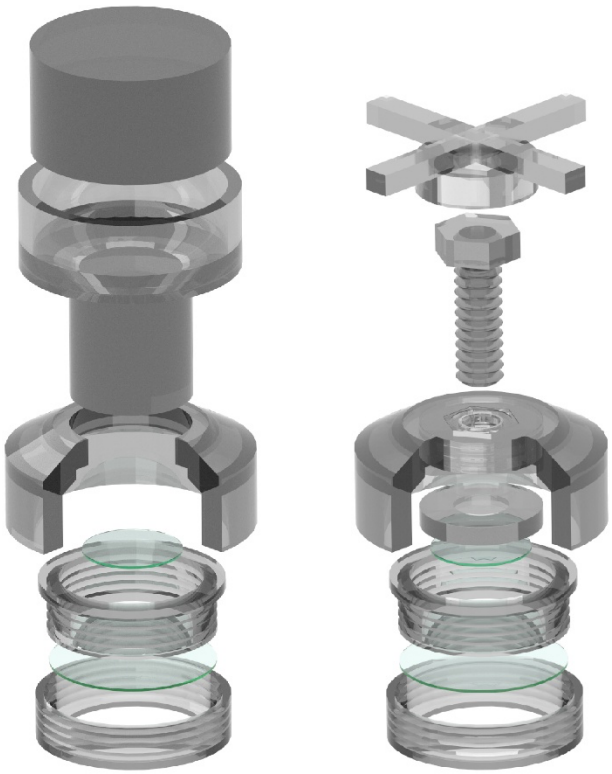


**Fig. S7**: Earlier confinement chamber designs. (Left) Weight-based confinement device. (Right) Screw-based confinement device.

**Video S1**: Assembly of cell confinement device.