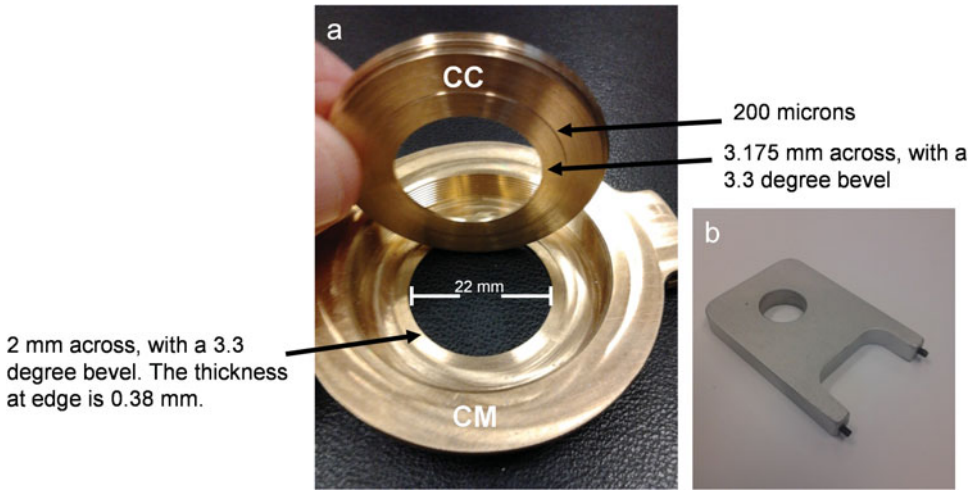
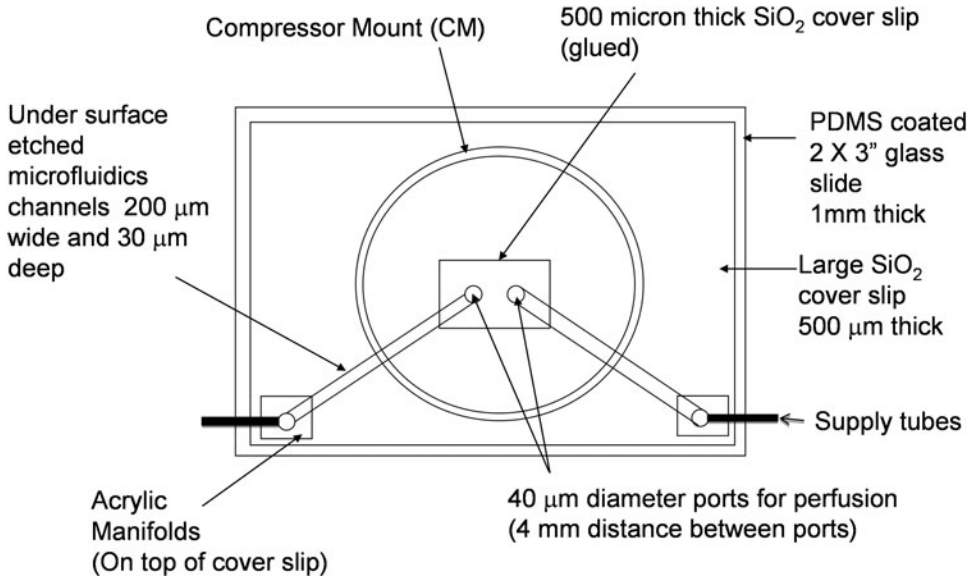
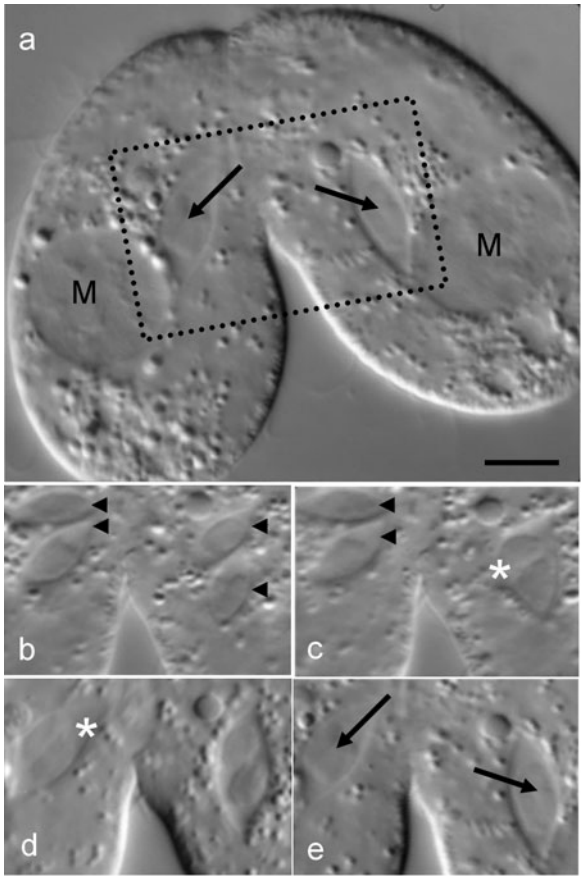


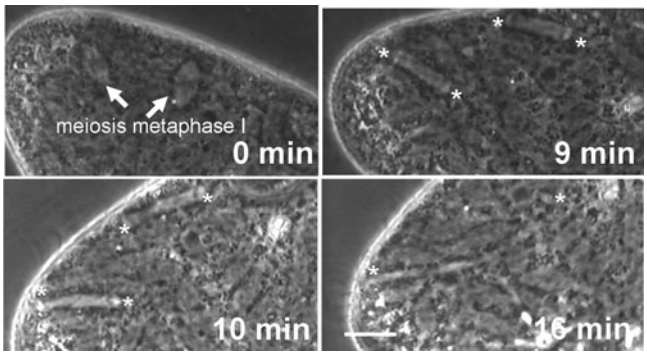
Supplementary Figure 1. Image of compressor mount (CM) and bottom side of coverslip compressor (CC). **a:** The CC will thread into the CM and will push down on a 25 mm coverslip that is nestled within the CM. The CC is machined with a 1/8 inch (3.175 mm) bevel that angles downward toward the center of the device. This will contact the glass coverslip (GC) and push down on it, bending it. The threading on outside of CC and inside of CM is 40 threads per inch (15.75 threads per cm). The CM then drops into the inner ring (Fig. 1) and locks into place with a gentle twist. The compressor mount is 50 cm across. The inner ring is threaded into the outer ring that is glued to the glass slide. The threading on the inner and outer ring is 48 threads per inch (18.9 threads per cm). **b:** We have constructed an aluminum tool that has pins that fit into the two holes seen on the top side of the CC. This is used to screw the CC into the CM.



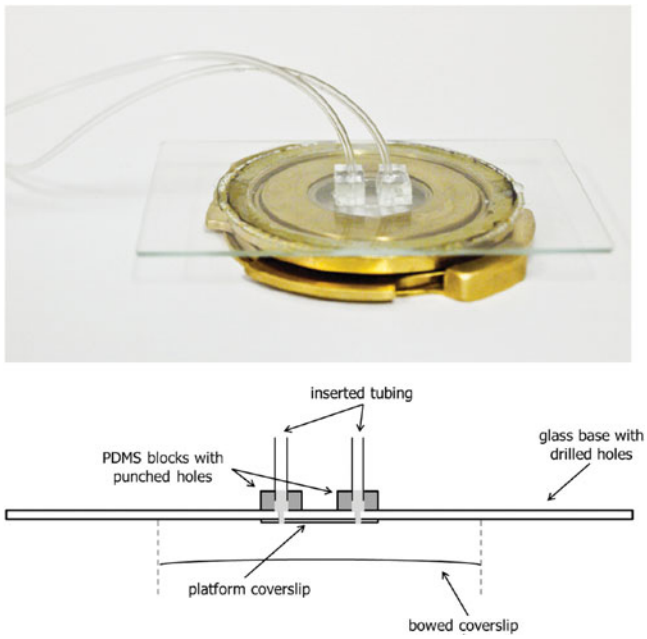
Supplementary Figure 2. Schematic of the outer ring and glass base incorporated with glass microfluidics.



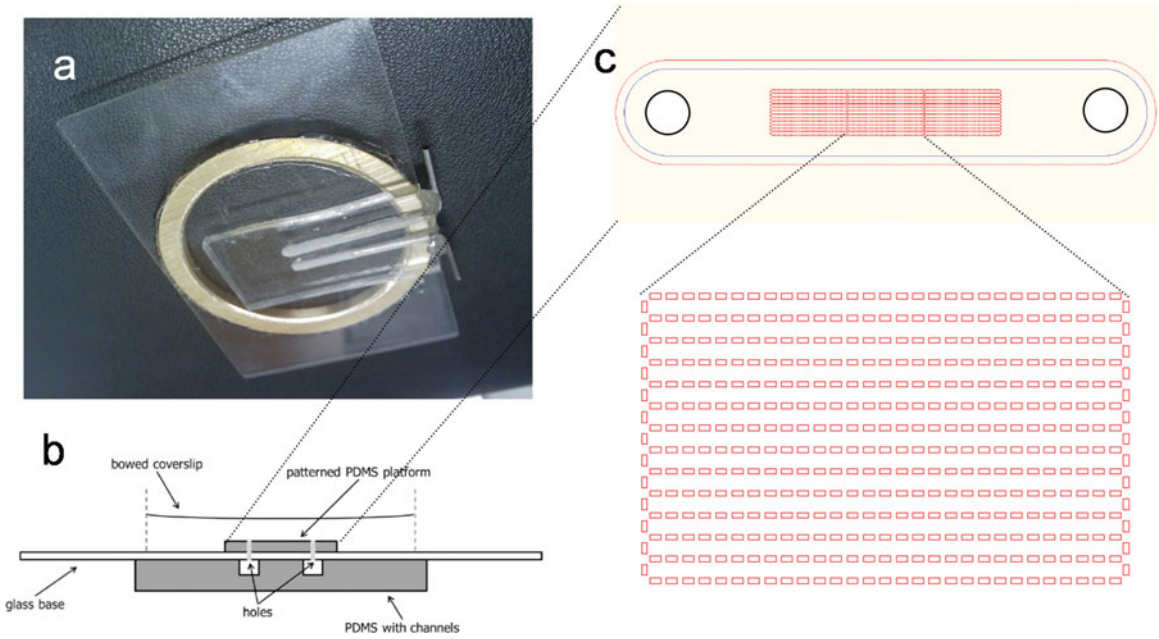
Supplementary Figure 3. Fertilization in the ciliate *T. thermophila*. **a:** Conjugating cells captured at the moment of fertilization using DIC optics and a 100 × 1.35 NA lens, double oil. Pronuclear chromosomal bundles have migrated together and are fusing (arrows). The square area delineated by the dotted lines is shown under higher magnification in Supplementary Figures 2b–2e, with Figure 2e being a cropped image of Supplementary Figure 2a. Bar is 5 μm. **b:** Pronuclei have formed spindle-like structures. The top pronucleus in each cell was exchanged at the conjugation junction and came from the other cell. **c:** The two pronuclear membranes have fused in the cell on the right (asterisk). **d:** The two pronuclear membranes have now fused in the cell on the left (asterisk). **e:** The chromosomal bundles in both fertilization nuclei migrate towards each other and intermingle (arrows). The time course of events in Figures 2b–2e took approximately 2 min.



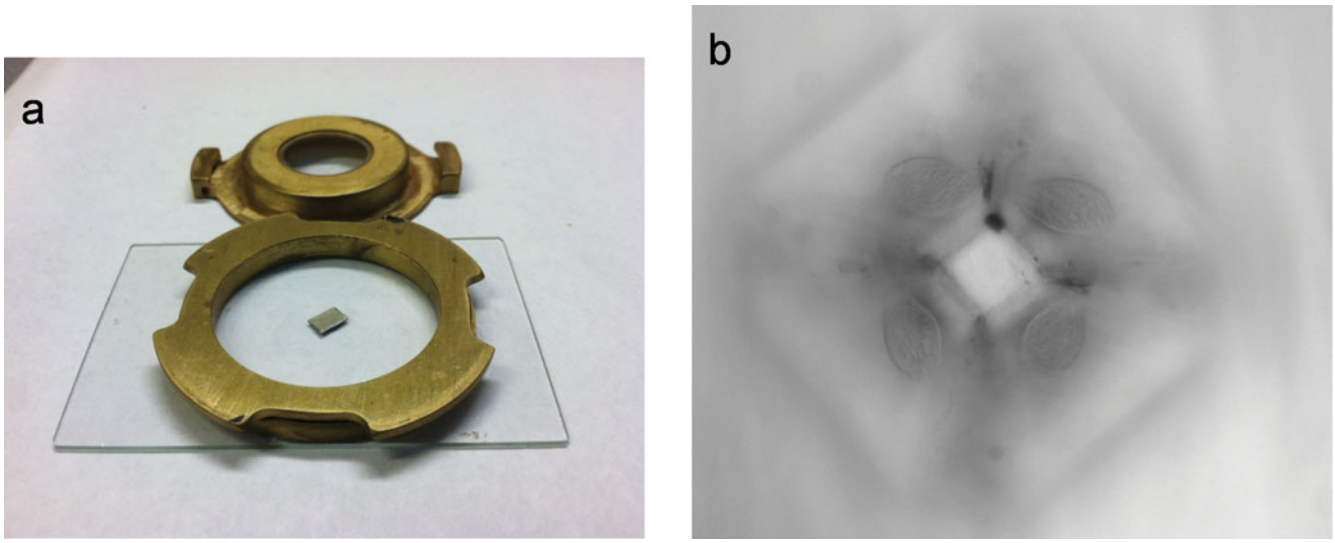
Supplementary Figure 4. Two meiotic metaphase I spindles in the ciliate *P. tetraurelia*. Cells were captured with phase contrast optics using a 100 × 1.35 NA lens. Chromosomes can be seen lined up at the start of metaphase and at the poles in the 9 and 10 min images. The spindles become difficult to see as they elongate and traverse the periphery of the cell at 16 min (ends of spindles labeled with asterisks). The cells moved slightly between the second and third time point. The bar is approximately 5 μm.



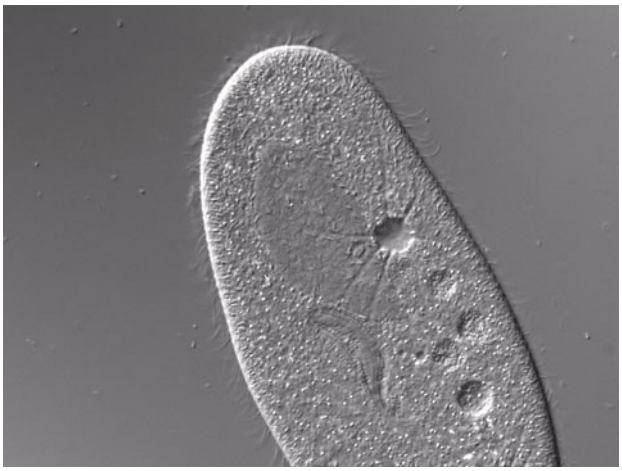
Supplementary Figure 5. Perfusion-enabled microcompressor with integrated PDMS platform. **a:** PDMS base with horizontal channels for fluid flow plasma bonded to the bottom of the glass slide. **b:** Diagram of device from side view showing the two PDMS components in this device. **c:** The entire PDMS platform design, including the pill shaped boundary wall. A series of posts with a 10 μm × 20 μm cross section formed the lining of each 830 μm × 30 μm slot. The post capping the end of each slot was of the same size. The distance separating adjacent posts was 5 μm. Holes for perfusion are punched into the PDMS at either end of the device inside the outer wall (black circles). **d:** Detail of a subset of the grid structure, designed to fit one *C. elegans* worm into any one of the long rectangular spaces lined with posts.



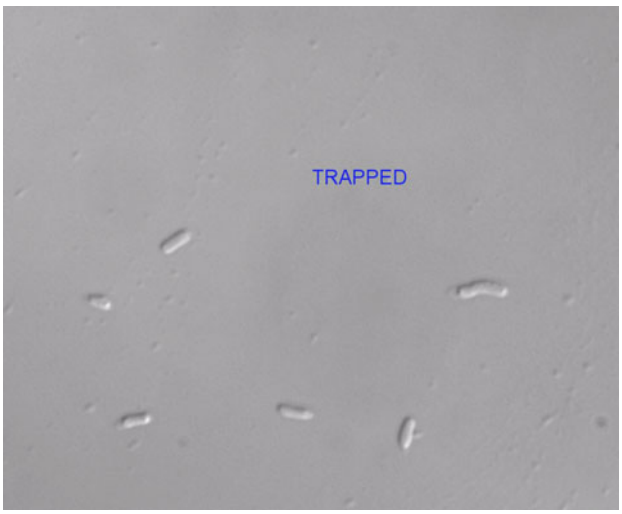
Supplementary Figure 6. Perfusion-enabled microcompressor with integrated PDMS platform. Similar in nature to the device in Supplementary Figure 5, but the channels through the PDMS manifold are vertical. Actual device is shown inverted on top (**a**) and schematic of the microfluidics from the side is shown below (**b**). A higher magnification of the platform from above showing PDMS design is depicted in (**c**).



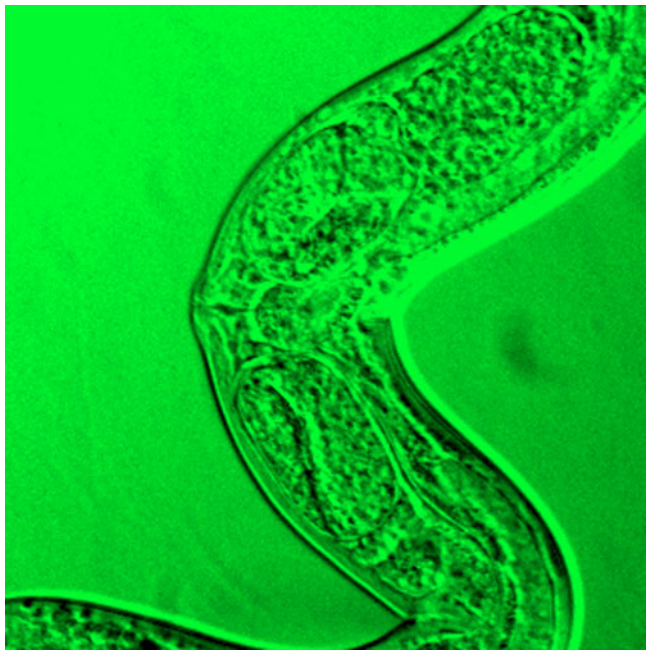
Supplementary Figure 7. Multiperspective well mounted as the specimen base and used to trap a swimming *T. thermophila* cell. **a:** The specimen base (center of device) is an array of chemically etched 80 μm diameter pyramidal wells in a silicon wafer. **b:** The same cell is imaged from four different perspectives. *T. thermophila* reflections (real image is in middle and out of focus) as cell swims and is trapped in the 3D volume of the reflective walls.



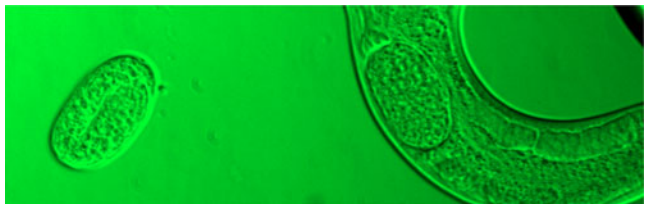
Supplementary Video 1. *P. sonneborni* cell immobilized with an Olympus 40× dry .7 NA lens and imaged with DIC optics. The video is in real time. Note the cilia beating at the periphery of the cell and at the oral apparatus (lower, center part of cell), the trichocyts in the cortical region of the cell, and the large macronucleus just to the left of the vacuole. This movie is acquired at video rate (32 frames/s).



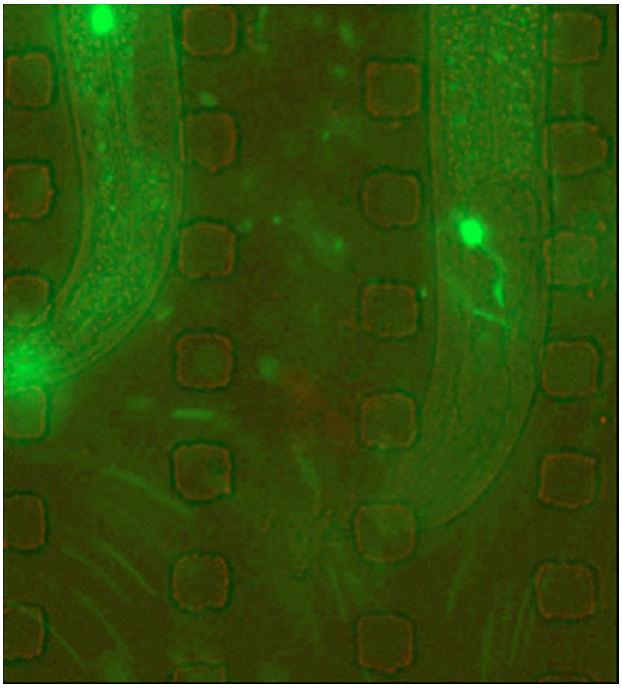
Supplementary Video 2. *K. pneumoniae* bacterial cells are shown initially trapped by gentle mechanical microcompression and then released. This movie is real time and acquired at video rate (32 frames/s) using a 100 × 1.35 NA lens and imaged with DIC optics.



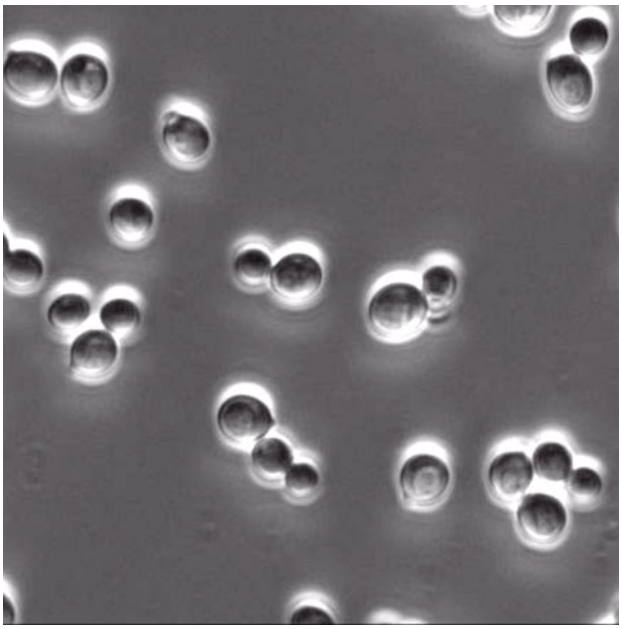
Supplementary Video 3. *C. elegans* worm immobilized on an Olympus upright BH2 microscope with a 40×, 0.65 NA dry lens. Bright field images were acquired every 5 s. Note that the worm is carrying several embryos.



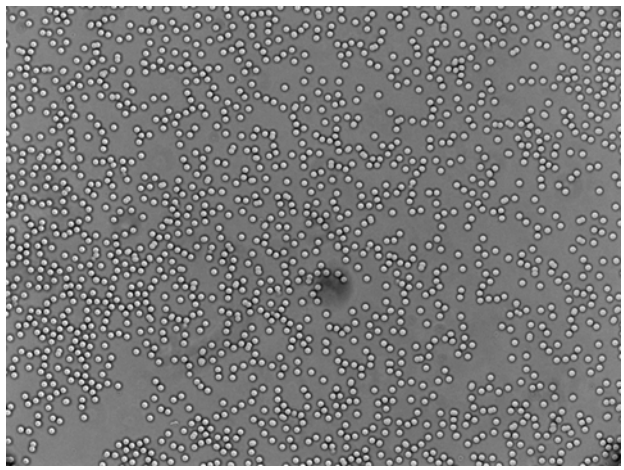
Supplementary Video 4. Same *C. elegans* worm as in Supplementary Video 3 and Figures 3c and 3d with newly released embryo. Compression was adjusted to immobilize the embryo. Bright field images were acquired every 5 s.



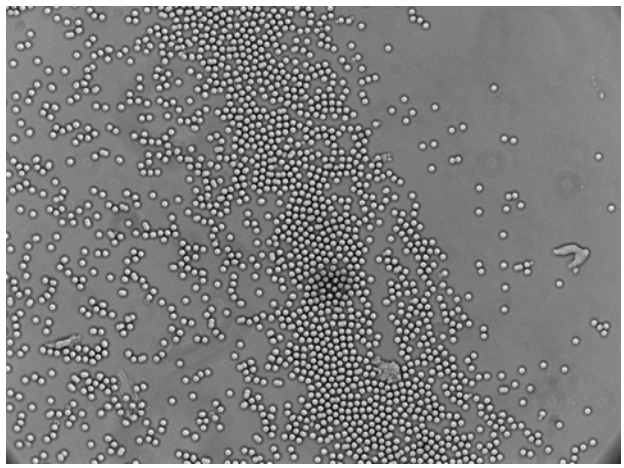
Supplementary Video 5. *C. elegans* worm compressed in a bed of PDMS posts in a perfusion-enabled microcompressor (Supplementary Fig. 6). *E. coli* expressing GFP were pumped into the device as a food source. Worm is compressed between the posts so that it unable to move laterally and by the compressor coverslip and PDMS floor so it also can't travel in the z direction. Images were acquired every 1 s.



Supplementary Video 6. Phase contrast image of compressed *S. cerevisiae* cells growing inside a perfusion enabled mechanical compressor. This device had the same manifold as Supplementary Figure 5. The manifold connected to two 1 mm holes drilled in the 12 mm coverslip platform. Yeast grew continuously throughout the 5 h video. Frames acquired every 15 s using a 40×1.35 NA lens.



Supplementary Video 7. Bright field image of 5 μm polystyrene beads compressed into a small "z" volume. Beads were not completely immobilized in this movie. This demonstrates how flat the field becomes as the compressor coverslip begins to interact with the lower coverslip platform. All of the beads seem to be fairly well confined in the same plane. Frames were acquired every 5 s.



Supplementary Video 8. Bright field image of a large field of 5 μm polystyrene beads immobilized by the mechanical microcompressor. The beads in the bottom left are still mobile, while the rest of the field is completely immobilized. In other experiments, we were able to completely immobilize an entire 1 mm $\times$ 1 mm field, suggesting that a large area of the coverslip platform could be set and positioned with a defined trapping distance, depending on the size beads used.