

CellASIC:B2 Microfluidic Plate Operating Instructions

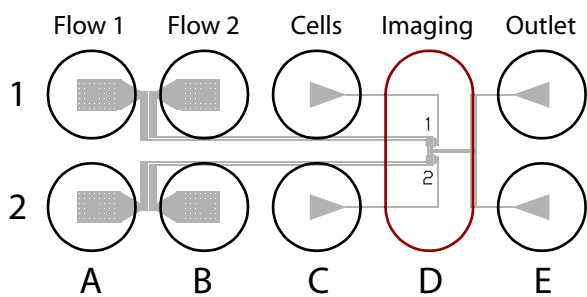


Figure 1 Well Layout

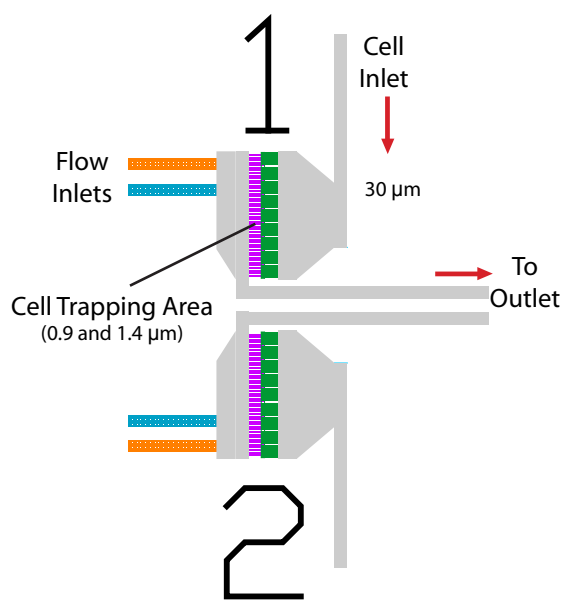


Figure 2 Trap Area

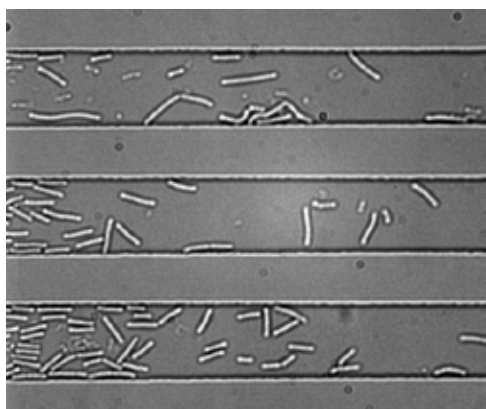


Figure 3 Trapped *E. coli*

1. The B2 microfluidic unit consists of 2 independent flow units as depicted in Figure 1. Each unit has 2 inlets, a cell loading, an imaging area, and an outlet. The plate is shipped pre-primed with sterile dl water which can be replaced with a buffer of choice prior to experiment.
2. Fill the two sets of flow inlet wells with up to 300 μ l of solution.
3. Fill the two cell inlet wells with 10-50 μ l of cell suspension. A density of $1-20 \cdot 10^6$ cells/ml is recommended depending on desired trapping density.
4. Seal the microfluidic plate to the manifold: Place the microfluidic plate on a flat surface. Align and set the manifold over the wells of the plate. Turn on the vacuum through the control panel and push down on the manifold with slight force for ~ 5 seconds to ensure uniform contact during sealing. When a proper seal is formed, the plate and manifold cannot be easily separated by hand. **Make sure a proper seal is formed before proceeding.** Leave the vacuum on during the course of the experiment.
5. Place assembly on an inverted microscope. Focus on the center of the "imaging" area. The trapping region has 48 parallel 10x100 micron regions with a 0.9 micron ceiling height, followed by 9 parallel 100x100 micron traps with a 1.4 micron ceiling height (Figure 2).
6. (Optional) Prime flow channels by turning on both flow switches for ~ 2 minutes at 4 psi to flush out shipping solution. It is recommended that this be followed by a 1 minute wash with only the first solution.
7. Turn on "cell load" switch to transport cells into the trapping region. The suggested loading protocol is at 6-8 PSI for 10 seconds. The loading profile will depend on cell density, cell size, and desired trapping density. When loaded, the trapping region will look similar to Figure 3.
8. Turn on flow to the first exposure solution. This will remove cells that are not trapped. After ~ 5 minutes of flow, the remaining cells will be those firmly held in x,y,z for imaging.
9. Flow rates and switching times are given in Figure 4.

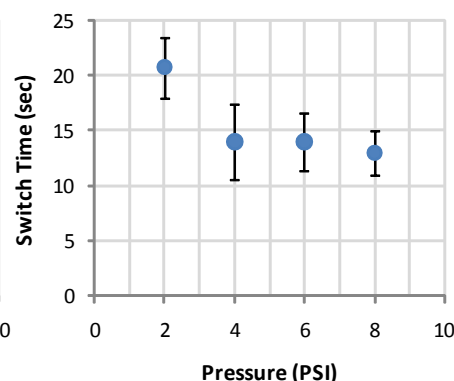
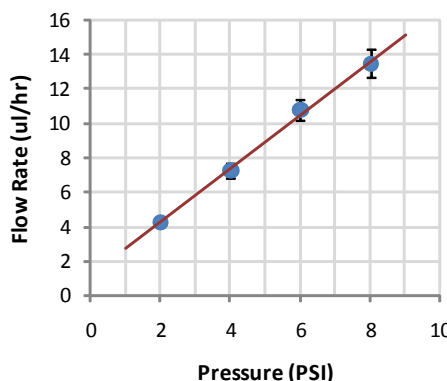


Figure 4 Flow Properties