

C04A Microfluidic Chlamydomonas Trap Plate

C04A-01

Description: The C04A plate contains 4 independent chambers, each with 6 upstream inlet channels. Each chamber has a series of microfluidic pocket traps of 4.0 micron in height to hold cells stationary during long term, high magnification, perfusion imaging.

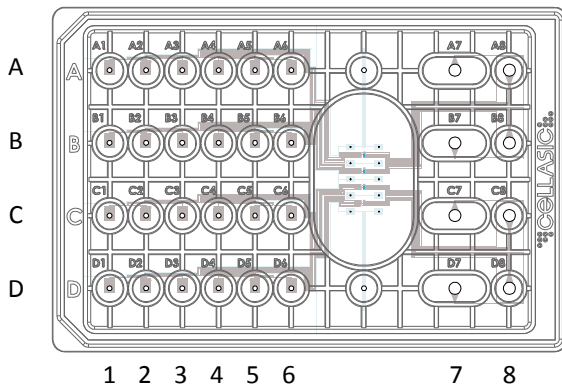
Applications:

- Time-lapsed, high magnification imaging of Chlamydomonas cells
- Long term continuous perfusion experiments (3 days typical, up to 14+ days)
- Solution exchange experiments (induction, inhibition, drug dosing, etc.)
- Comparing up to 4 different cell types or exposure conditions in parallel
- Flagella growth studies
- Temperature, gas atmosphere, and light control (temperature shift, anoxic, etc.)

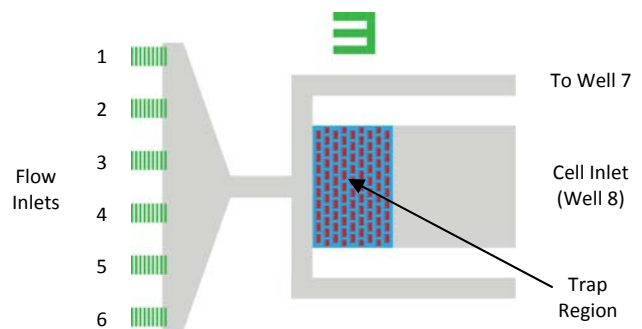
Product Specifications:

- Use with ONIX-262 system and F84 manifold
- SBS standard footprint frame (fits to typical 96 well stage holders)
- #1.5 thickness (170 μm) glass slide bottom (in contact with cells)
- 274 single cell traps per chamber (10x8x5 micron each)
- Laminar flow rates of 1-100 $\mu\text{l/hr}$ (typical chamber refresh times of <5 seconds)
- Microfluidic gas exchange channels

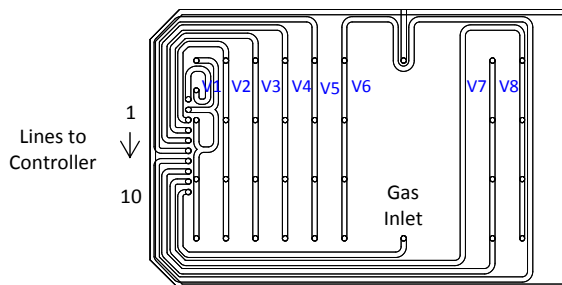
Plate Design:



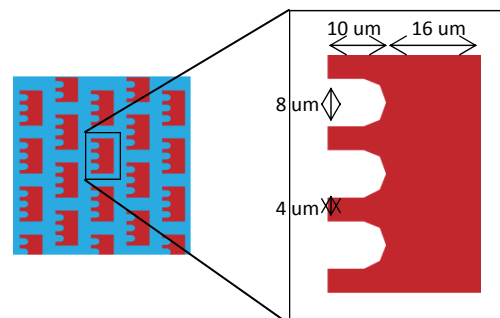
The C04A has 4 independent units (A-D), each with 6 inlet wells (1-6), a cell inlet (8), and a cell outlet well (7). All four culture chambers are located under a single imaging window.



The culture chambers are located under a large imaging window for high magnification phase objectives and to minimize travel distance. All channels are resistance matched for uniformity.



The F84 manifold has 8 pressure channels (V1-V8) to control flow rates through the microfluidics. A vacuum line is used to seal the plate to the manifold, and a gas line enables atmosphere control.



The trap chamber is 0.6 x 0.3 mm in area with heights of 4.0 (red) and 5.0 (blue) micron. Each single cell trap pocket is 10 x 8 μm in size. Each block has 3 traps with 15 μm gaps between blocks.

Cell Trapping Mechanism

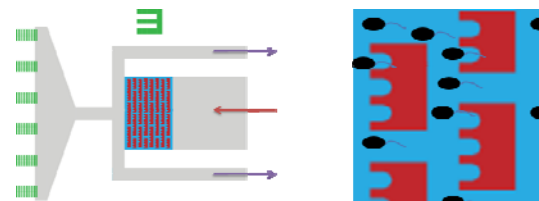
The microfabricated chamber gently holds cells against a silicone pocket for long term perfusion imaging experiments. The trap chamber has a height of 5.0 micron (blue), with 4.0 micron height in the pocket regions (red). Flagella will extend under the 4 um gap to the right of the pocket during perfusion.

- Rounded pocket design holds a single cell for long term tracking.
- Staggered block pattern enables free flow of cells around traps to increase loading efficiency.
- 5 micron chamber height keeps cells in a single focal plane without hindering flagella movement.
- Up to 274 cells trapped per chamber

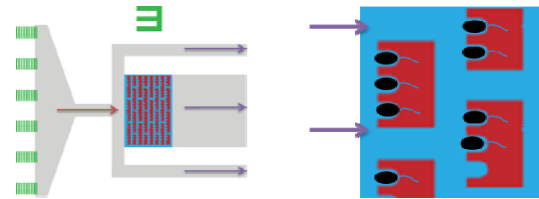
(Top) Cells are loaded from suspension (right to left) to fill the chamber with cells.

(Middle) Flow of medium (left to right) pushes cells into the trap pockets, with the flagella pointing to the right. Continuous perfusion (left to right) keeps the cells in the pockets during imaging.

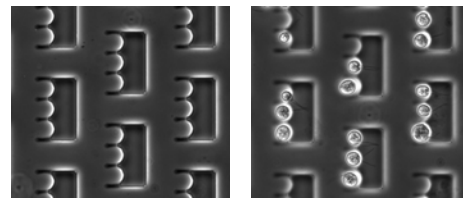
(Bottom) Microfluidic region before and after cell loading viewed with a 40X phase contrast objective.



Flow Cells into Chamber
(Right to left flow)



Flow Cells into Pockets
(Left to right flow)

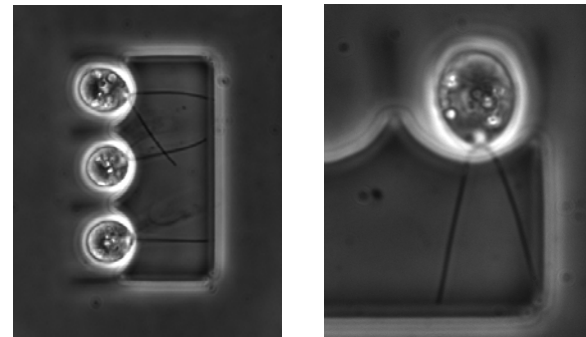


Chlamydomonas Cell Loading into
Microfluidic Pockets

Imaging Quality

The C04A plate is designed for optimal image quality for high end microscopes.

- The cells are held against a #1.5 thickness (170 um) glass coverslide floor, making the plate compatible with immersion and high NA objectives.
- The large imaging window allows light to pass freely for optimal phase contrast quality.
- The four microchambers are located within a 1.5 cm area, minimizing stage travel and focus drift.



Chlamydomonas cells in the microfluidic pocket at 100X and 150X phase contrast. Note the flagella pointing against the flow.

Solution Switching

Our advanced perfusion control enables experiments not possible with existing instrumentation.

- Six switchable inlet wells for each chamber
- Highly laminar flow provides uniform exposure profiles and sharp transition boundaries
- Low shear ensures minimal stress on cells
- Rapid solution exchange rates and <10 nL dead volume for fine time resolution experiments
- Perfusion rates of ul/hr allow continuous multi-day experiments on your microscope



Solution exchange profile at 2 psi between a fluorescent dye and buffer solution in a 0.25x0.2 mm field of view (20X objective).

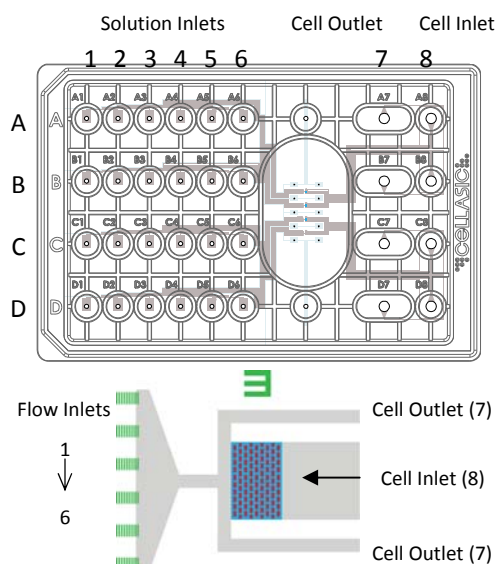


Figure 1. Plate Layout



Figure 2. Plate with Manifold on Stage

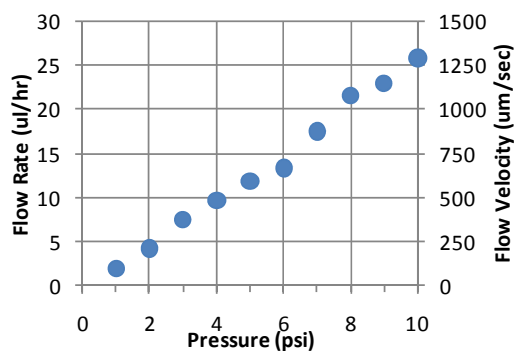


Figure 3. Flow Rate

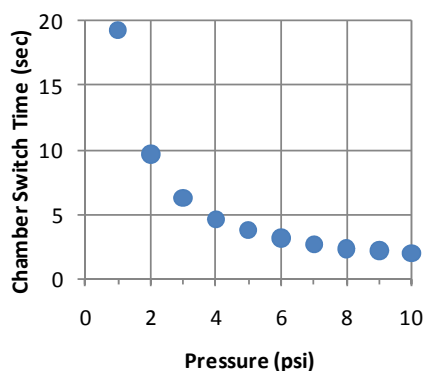


Figure 4. Chamber Switch Time

Operation Instructions

1. The C04A microfluidic plate contains 4 parallel chambers, each with 6 solution inlets (Figure 1). Each row of wells (A-D) addresses the corresponding chamber. The plate is shipped pre-primed with sterile dl water containing penicillin/streptomycin, which can be replaced with a buffer of choice prior to experiment. Plate performance guaranteed up to the 6 month expiration date.
2. Fill the six sets of flow inlet wells (col 1-6) with up to 300 μ l of solution. If less than 4 units will be used, fill the unused inlet wells with buffer. Empty the cell outlet well (col 7).
3. Fill the cell inlet wells (col 8) with 50 μ l of cell suspension, making sure to cover the hole at the bottom of the well. A density of $1-20 \cdot 10^6$ cells/ml is recommended depending on desired trapping density.
4. Seal the microfluidic plate to the F84 manifold: Clean the manifold gasket with 70% ethanol and blot dry. Place the microfluidic plate on a flat surface. Align and set the manifold over the wells of the plate. Turn on the vacuum switch on the ONIX box and push down on the manifold with slight force for \sim 5 seconds to ensure uniform contact during sealing. When a proper seal is formed, the green "sealed" light will be lit. Make sure a proper seal is formed before proceeding. Leave the vacuum on during the course of the experiment. (If the seal is unsuccessful, turn off the vacuum switch and wait for the blue "ready" light to turn back on. Repeat step 4 above. If a seal cannot be formed, please contact CellASIC.)
5. Place assembly on an inverted microscope (Figure 2). Focus on the center of the imaging area. The trapping region is 0.6 x 0.4 mm in size with an array of cell trapping pockets.
6. Open the ONIX FG software on a computer attached to the USB line from the control box. Select the "C4" tab from the front page. If you do not see the tab, make sure you have an updated version of the software (www.cellasic.com/Products-downloads) or contact CellASIC.
7. On the control window (see figure 6), set "Cell Load" parameters to 1 psi and 30 seconds (flow 8) and 1 psi and 5 seconds (flow 7). Click the "Load Cells" button. Flow from 8 will bring cells into the chamber. Reverse flow from 7 will populate the pockets. Repeat as necessary. Manual controls can also be used.
8. Wash un-trapped cells by flowing one or more inlet solutions at 5psi for 5 minutes. This will also serve to prime the specified inlet channel by flushing out the shipping solution. Cells remaining in the trap regions are now firmly held in x,y,z for imaging.
9. Flow properties are given in Figures 3 and 4. Figure 3 shows the total flow rate out of each inlet well at the specified pressures. The flow velocity is of the laminar front moving through the chamber, or the local switch rate for cells in the chamber. Figure 4 gives the time for the laminar front to move through the entire chamber. Multiple inlet wells can be run at the same time; the total flow rate is the sum of the individual channels.

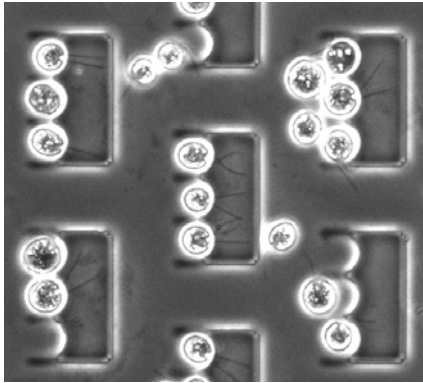


Figure 5. Chlamydomonas cells trapped into microfluidic pockets

Courtesy of the Marshall Lab, UCSF

Operation Instructions (cont.)

10. Use the tabs on the right side of the software control window to set up your experiment. Manual operation is possible by clicking on the valve buttons and dragging or typing in values on the flow bars on the left of the software control window.
11. For custom tuning of flow scheduling, use the "Protocol" tab to enter text based commands. The commands are given in Figure 6.
12. Click "Run" to run the perfusion program.
13. Acquire images using your standard microscope methods.

Software Operation

The screenshot shows the ONIX FG C4 software interface. On the left, there are two vertical flow regulators for Flow X (orange) and Flow Y (blue). Below them are two status indicators: "Vacuum is ready" and "Air pressure is ready", both with green checkmarks. The main panel displays a schematic of the microfluidic chip with 8 valves (V1-V8) and 10 columns. On the right, there are three tabs: "Load Cells", "Create Protocol", and "Run Protocol". Below the tabs is a table with columns for "Column:", "Duration (min):", and "Flow (psi):". At the bottom, there are fields for "Protocol start time:", "Program length:", "Current line:", "Current command:", and "Next step in:". Annotations with red arrows point to various parts of the interface: "Valve on/off Buttons" points to the valve controls; "Regulator Setpoints" points to the flow rate sliders; "Status Indicators" points to the green checkmarks; "Function Tabs" points to the "Run Protocol" tab; and "Program Status" points to the "Program length:" field.

Note: Flow X controls V1 and V2, Flow Y controls V3-V8

Figure 6. ONIX FG C4 Interface

Text Commands:

- setflow X n** ; where $n = 0.25-10$ (psi) ; sets flow rate on X (orange)
- setflow Y n** ; where $n = 0.25-10$ (psi) ; sets flow rate on Y (blue)
- open V1** ; V1, V2, ..., V8, all ; opens pneumatic valve
- close V1** ; V1, V2, ..., V8, all ; closes pneumatic valve
- wait n** ; n is minutes ; holds current condition until next step
- end** ; ends the program ; shuts off all valves and resets regulators
- %** ; Put at the beginning of line for comments

The screenshot shows the "Run Protocol" tab with a list of 12 text commands:

```

1 % Sample Protocol
2
3 setflow Y 2
4 open V3
5 open V5
6 wait 10
7 setflow X 4.5
8 open V1
9 wait .5
10 close all
11 end
12
  
```