

M04S APPLICATION NOTE

Dynamic Live Cell Imaging Chamber

INTRODUCTION

The analysis of living cells *in vitro* is critical to understanding basic biology, signaling pathways, drug effects, and disease models. Current methods provide excellent means to interrogate living cells via biomolecular probes, fluorescence microscopy, and genetic manipulation.¹ However, technology for environment control of living cells during analysis has not advanced significantly since the Petri dish. There is a growing body of evidence to indicate that the cellular environment, or “niche,” is just as important (or even more critical) than genetic factors for determining cell phenotype.² Therefore, a method for providing more accurate, dynamic control of living cells has the potential to drastically advance the state-of-the-art for live cell analysis.^{3,4}

CellASIC has developed the M04S microfluidic plate for perfusion based microenvironment control for long term, high quality live cell microscopy. The microfluidic chamber recreates the physiologic mass transport condition for optimized cell health. Four upstream fluidic channels allow controlled exposure of the cells to different solutions during live imaging. The plate can also be cultured in a standard incubator using a dedicated gravity driven flow channel. The cells are in contact with a #1.5 thickness (170 μm) optical glass surface, enabling high quality imaging using an inverted microscope. An integrated micro-incubator system delivers temperature and gas control to the microfluidic chambers.

The operation of the M04S microfluidic plate was demonstrated by long term culture of adherent cells, creating

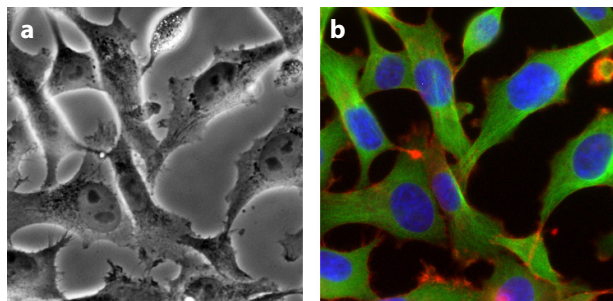


Figure 1. Cells cultured in the M04S microfluidic chamber. (a) HT-1080 cells imaged under phase contrast and (b) immunostained for nuclei, actin, and microtubules. Fixing and staining performed within the microfluidic chamber. Images taken with a 40X objective lens.

dynamic solution profiles (media switching and spatial gradient), and fixation/immunostaining of cells within the microfluidic chamber. The microfluidic chamber was designed to be compatible with standard culture methods, including surface coating, 3D culture, and co-culture. The bottom surface of the chamber is plasma-activated glass, which supports most standard cell lines without coating, including HeLa, NIH3T3, MCF7, MCF10A, HT1080, HUVEC, etc.

PLATE DESIGN

The M04S microfluidic plate is built on the ONIX platform developed by CellASIC (www.cellasic.com/ONIX). The plate has a SBS standard 96 well footprint to fit to typical microscope stage holders. The custom well layout was designed to maximize live cell imaging capabilities. The M04S has 4 independent units (A-D), with each unit containing 8 wells (1 gravity inlet, 4 switching inlets, 1 cell inlet, 2 wastes). The four cell culture chambers are centralized under a single large imaging window (see figure 2). The chamber to chamber distance is 5.2 mm, reducing objective travel time and focus drift. The bottom surface of the plate is a #1.5 thickness (170 μm) optical glass slide to maximize quality of high resolution, high numerical aperture imaging. The plate houses all experiment solutions allowing control with an external pneumatic manifold (see figure 3). The manifold lets the user direct flow rates and select exposure solutions without perturbing the microscope stage. A gas line allows control of the environment within the micro-chambers through a network of gas permeable air diffusion channels. Temperature is regulated through an on-board heater/chiller on the manifold.

CELL LOADING

Cells are loaded into the culture chamber using a capillary driven method. This allows the user to load cells using a pipette, and can be done in a sterile hood without any external systems. Cell suspension is pipetted into the cell inlet well (6) and liquid is aspirated out of the waste wells (7 & 8). This creates a surface tension force that pulls the cell suspension into the chamber. As the cells enter the chamber,

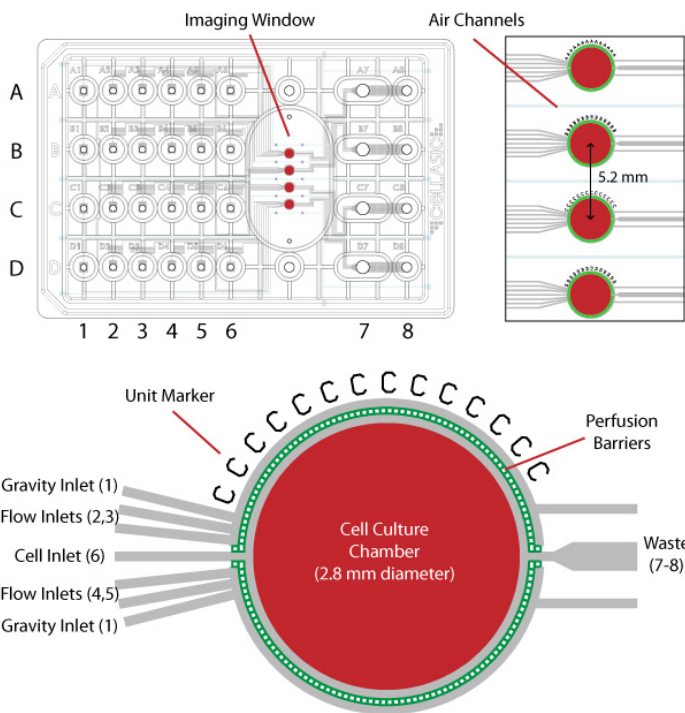
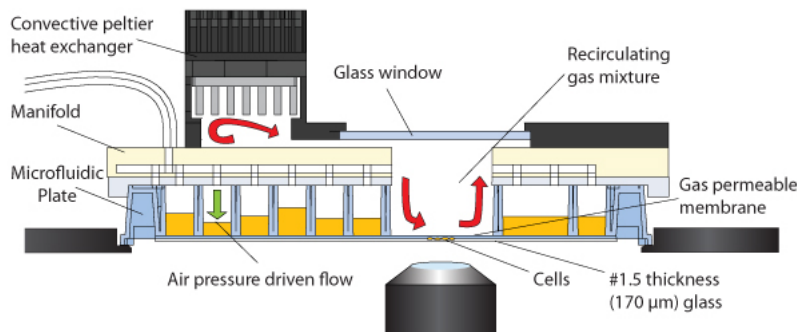


Figure 2. The M04S plate contains 4 independent flow units (A-D), each with 4 upstream solution inlets, a gravity flow inlet, a cell inlet, and 2 waste wells. The culture chamber is 2.8 mm in diameter (100 μm height) and is surrounded with a microfabricated perfusion barrier. Inlet 1 is a gravity flow well, allowing long term cell culture in a standard incubator without a pressure system. Continuous flow of solutions from the inlets creates a dynamic exposure profile during live cell imaging.

Figure 3. Side view schematic of the microfluidic plate with micro-incubation manifold on a microscope stage. The bottom surface of the microfluidic plate is a thin glass sheet, allowing high quality cell imaging. The plate is sealed to a pneumatic manifold, allowing user control of the flow profile during imaging. Additional air channels allow control of the gas environment.



the flow profile allows them to settle to the floor without any stress. Typically, loading is completed in 3 minutes. If more cells are desired, higher concentrations (or repeated loading cycles) can be implemented (figure 4). After the cells settle to the bottom of the chamber, continuous flow of medium enables them to attach and grow (figure 6). Surface coating can be performed prior to cell loading by flowing the coating solution (e.g. poly lysine, collagen, fibronectin) into the chamber using the same method. Alternatively, 3D culture can be achieved by mixing the cell suspension with the 3D gel, and loading together, allowing the cells/gel to polymerize within the chamber. The design of the flow channels allows continuous perfusion even in the presence of 3D gel.

SOLUTION SWITCHING

Exposure solutions are introduced from the 4 inlet wells and flow through the chambers to the waste (well 7). Wells 7 and 8 are connected as a shared outlet for increased vol-

ume. Well 1 is a gravity flow well, with a perfusion rate of approximately 80 $\mu\text{l/day}$. This is used for pre-culture of cells in the M04S plate in a standard incubator when solution exchange or imaging is not necessary, for example to expand or differentiate cells over a period of a few days. Wells 2-5 are the pressure driven wells. The flow rate and exchange times are given in figure 5. The highly laminar flow profile means that when the input solution is changed, a sharp fluid interface is created that moves across the culture area from left to right. The velocity of this front is given in figure 5a. The time it takes for the entire front to reach the end of the culture chambers is plotted in figure 5b. The actual local exchange time (the transition from solution 1 to 2 around the cells) happens much more quickly, typically in a few seconds. The small volume of the culture chamber enables fast solution exchange at flow rates from 5-80 $\mu\text{l/hour}$. This means that a typical imaging experiment (with 300 μl per inlet well) can run for well over 24 hours.

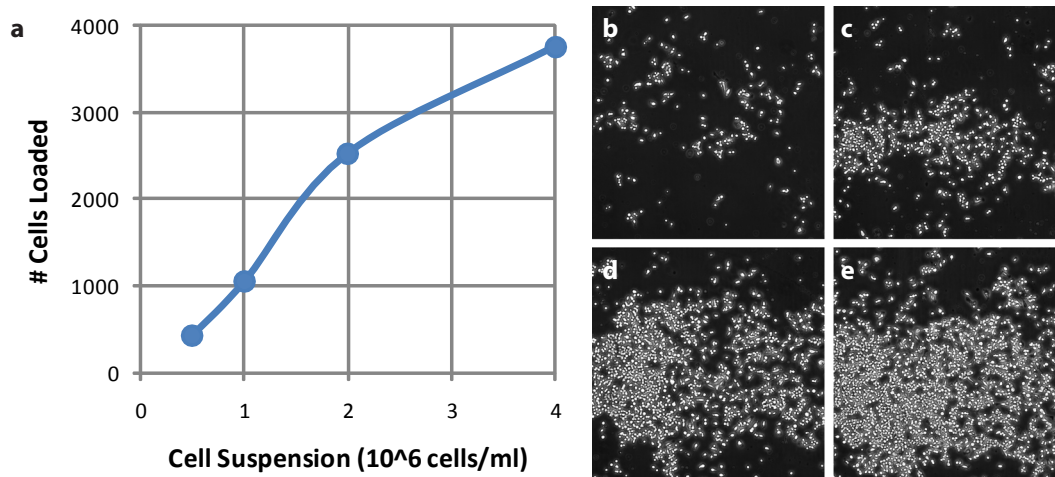


Figure 4. Cell loading in the microfluidic chamber. (a) Each chamber can be seeded with up to a few thousand cells by varying the cell density during capillary flow loading. Images taken with a 4X objective of HT-1080 cells loaded at (b) 0.5, (c) 1.0, (d) 2.0, and (e) 4.0 million cells/ml.

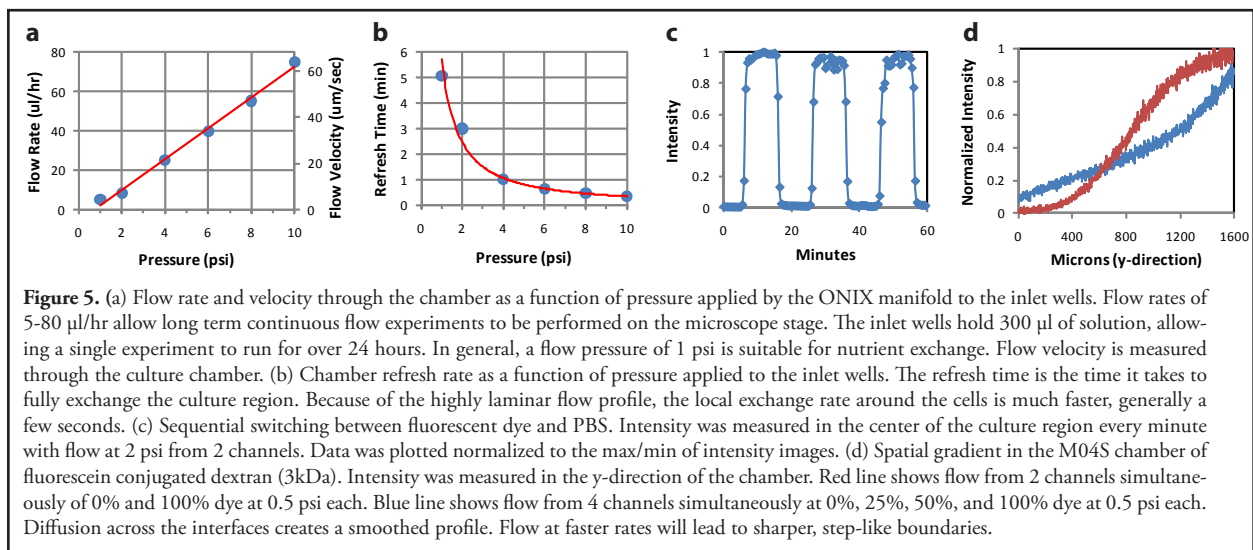


Figure 5. (a) Flow rate and velocity through the chamber as a function of pressure applied by the ONIX manifold to the inlet wells. Flow rates of 5-80 $\mu\text{l/hr}$ allow long term continuous flow experiments to be performed on the microscope stage. The inlet wells hold 300 μl of solution, allowing a single experiment to run for over 24 hours. In general, a flow pressure of 1 psi is suitable for nutrient exchange. Flow velocity is measured through the culture chamber. (b) Chamber refresh rate as a function of pressure applied to the inlet wells. The refresh time is the time it takes to fully exchange the culture region. Because of the highly laminar flow profile, the local exchange rate around the cells is much faster, generally a few seconds. (c) Sequential switching between fluorescent dye and PBS. Intensity was measured in the center of the culture region every minute with flow at 2 psi from 2 channels. Data was plotted normalized to the max/min of intensity images. (d) Spatial gradient in the M04S chamber of fluorescein conjugated dextran (3kDa). Intensity was measured in the y-direction of the chamber. Red line shows flow from 2 channels simultaneously of 0% and 100% dye at 0.5 psi each. Blue line shows flow from 4 channels simultaneously at 0%, 25%, 50%, and 100% dye at 0.5 psi each. Diffusion across the interfaces creates a smoothed profile. Flow at faster rates will lead to sharper, step-like boundaries.

A key feature of the M04S plate design is that solutions can be changed during live cell imaging without perturbing the plate or microscope. This enables tracking of cell responses to changing solution environments. The M04S allows 4 different solutions to be switched during the course of the experiment. As one example, 2 solutions (PBS and dextran conjugated fluorescein, 3kDa, Invitrogen) were switched at 10 minute intervals (see figure 5c). Note the rapid and complete response of the solution, creating a clean “step function” in the culture region. Since all 4 channels converge near the culture chamber, the M04S plate minimizes the dead volume during switching to a few nanoliters. Another feature is the ability to create spatial gradients (see figure 5d). When more than one channel is flowed simultaneously, laminar flow and diffusion across the interface creates a stable spatial gradient. For sensitive kinetic experiments, it is recommended that a tracer dye be used to accurately follow solution flow profiles.

TIME LAPSE IMAGING

The favorable cell culture environment in the M04S chamber allows long term maintenance of adherent cells under well controlled conditions. This enables enhanced live cell tracking of cellular events in response to changes in media, cell cycle, drug dosing, and other stimuli. We demonstrated 2 common methods for high resolution cell analysis using microscopy: 1) immunostaining, and 2) transfection. To facilitate immunostaining of cells cultured in the M04S plate, an automated flow protocol was set up to sequence fixing, permeabilizing, blocking, primary antibody, secondary antibody, and all wash steps. This allows the unique benefit of monitoring live cells in the M04S plate, and then subsequently fixing the same cells and analyzing by immunofluorescence. In a second example, live cells cultured in the M04S plate were transfected with GFP-tubulin (Invitrogen Cellular Lights reagent). After exposure to the

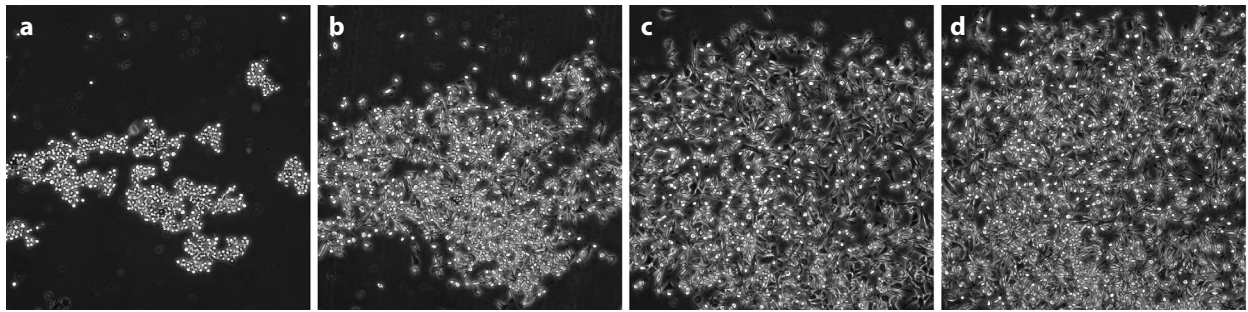


Figure 6 Growth of MDA-MB-231 cells in the microfluidic chamber with continuous perfusion after (a) 1 hour, (b) 1 day, (c) 2 days, and (d) 3 days. Images taken with a 4X objective lens.

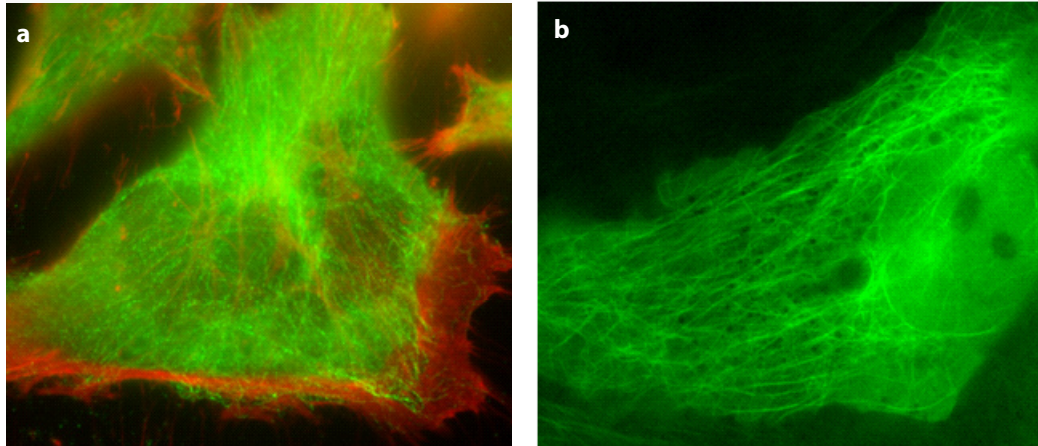


Figure 7. On-chip staining of cells cultured in the M04S chamber. (a) HeLa cell cultured in the microfluidic chamber immunostained via flow exposure. Red stained for actin, green stained for tubulin. (b) MCF10A cell cultured in the microfluidic chamber and transfected with GFP-tubulin via flow exposure. Images taken with a 100X objective lens.

GFP reagent, the cells expressed the protein of interest and could continue to be tracked in the microfluidic system (see figure 7).

SUMMARY

The ability to control and monitor living cells is critical for understanding signaling networks and complex phenotypes in response to stimuli. CellASIC has developed the innovative M04S microfluidic perfusion chamber to optimize cell microenvironment control while facilitating long term high quality microscopy. This design has been demonstrated with a wide range of cell lines for fluorescence quantification, solution exchange response, and time-varying inputs. Existing cell analysis methods such as immunostaining, transfection, fluorescent probes, and more, are easily adapted to the microfluidic format. Further, the ease-of-use, flexibility, and accessibility of this advanced technology platform should prove beneficial to a wide range of cell biology applications.

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