B04A APPLICATION NOTE

Dynamic Live Cell Imaging of Bacteria

INTRODUCTION

The investigation of bacterial cell biology is important for a number of fields, including: genetic engineering and biotechnology, synthetic biology, infectious disease research, antibacterial development, molecular and systems biology, and clinical screening. Current methods in microbiology address bacteria on a population level, with measurements averaged over millions or billions of individual cells. The recent trend towards single cell analysis reflects the demand for more detailed information such as cell variation, dynamic response profiles, intergenerational relationships, spatial information, and morphological features.[1,2] Advances in flow cytometry and high resolution microscopy enable quantification of individual bacteria cells,[3,4] but neither approach is optimal for tracking cells over time. The small size (~1 μm) and non-adherent nature of most bacteria make live cell imaging experiments difficult with conventional methods.

CellASIC has developed the B04A microfluidic plate for spatially localizing bacteria to a single monolayer for long term, high quality live cell microscopy. The microfluidic chamber traps cells using an elastic ceiling (0.7-2.0 μm height) without preventing perfusion flow or hindering cell growth. Five upstream fluidic channels allow controlled exposure of the cells to different solutions during live imaging. The cells are in contact with a #1.5 thickness (170 μm) optical glass surface, enabling high quality imaging using an inverted microscope.

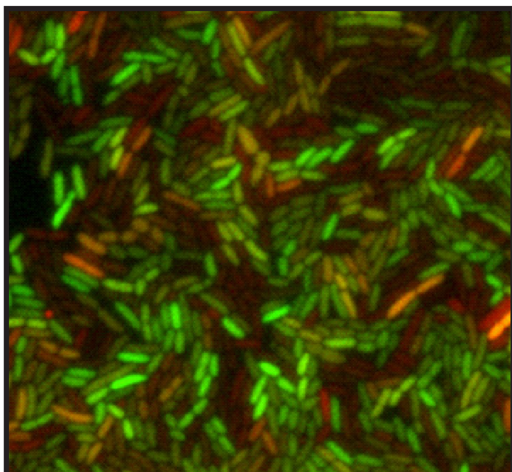

Figure 1. *E. coli* expressing GFP and mCherry cultured in the B04A microfluidic plate for 24 hours. The cells were perfused with LB medium and inducers with imaging on an 100X objective. Cells courtesy of Tim Lu, MIT.

The operation of the B04A microfluidic plate was demonstrated using *Escherichia coli*, *Caulobacter*, and *Mycobacterium*. These cells are typical species used in laboratory research, and represent a variety of cell shapes, cell cycle times, and motility. In all cases, the B04A was suitable for performing long term live cell imaging experiments with solution exchange. In addition, the wide range of chamber heights in the culture chamber is designed to accommodate most types of bacteria cells.

PLATE DESIGN

The B04A 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 B04A has 4 independent units (A-D), with each unit containing 8 wells (5 inlets, cell outlet, waste, and cell inlet). The four cell culture chambers are centralized under a single large imaging window (see figure 2). The chamber to chamber distance is 3.25 mm, reducing objective travel time and focus drift. The bottom surface of the plate is a #1.5 thickness (170 um) 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. Additionally, a gas line allows control of the environment within the microchambers through a network of gas permeable air diffusion channels.

CELL TRAPPING

Each culture chamber has 6 cell trap regions. The traps are 0.2x1.95 mm in area, with heights of 0.7, 0.9, 1.1, 1.5, 2.0, and 4.0 μm. Square support pillars hold the trap ceiling in place and prevent collapse into the floor. Each pillar has a marking to indicate the trap position (1-6). Cells are loaded in a two-step process. First, flow from well 8 to 6 transports the cell suspension into the bottom flow channel. Second,

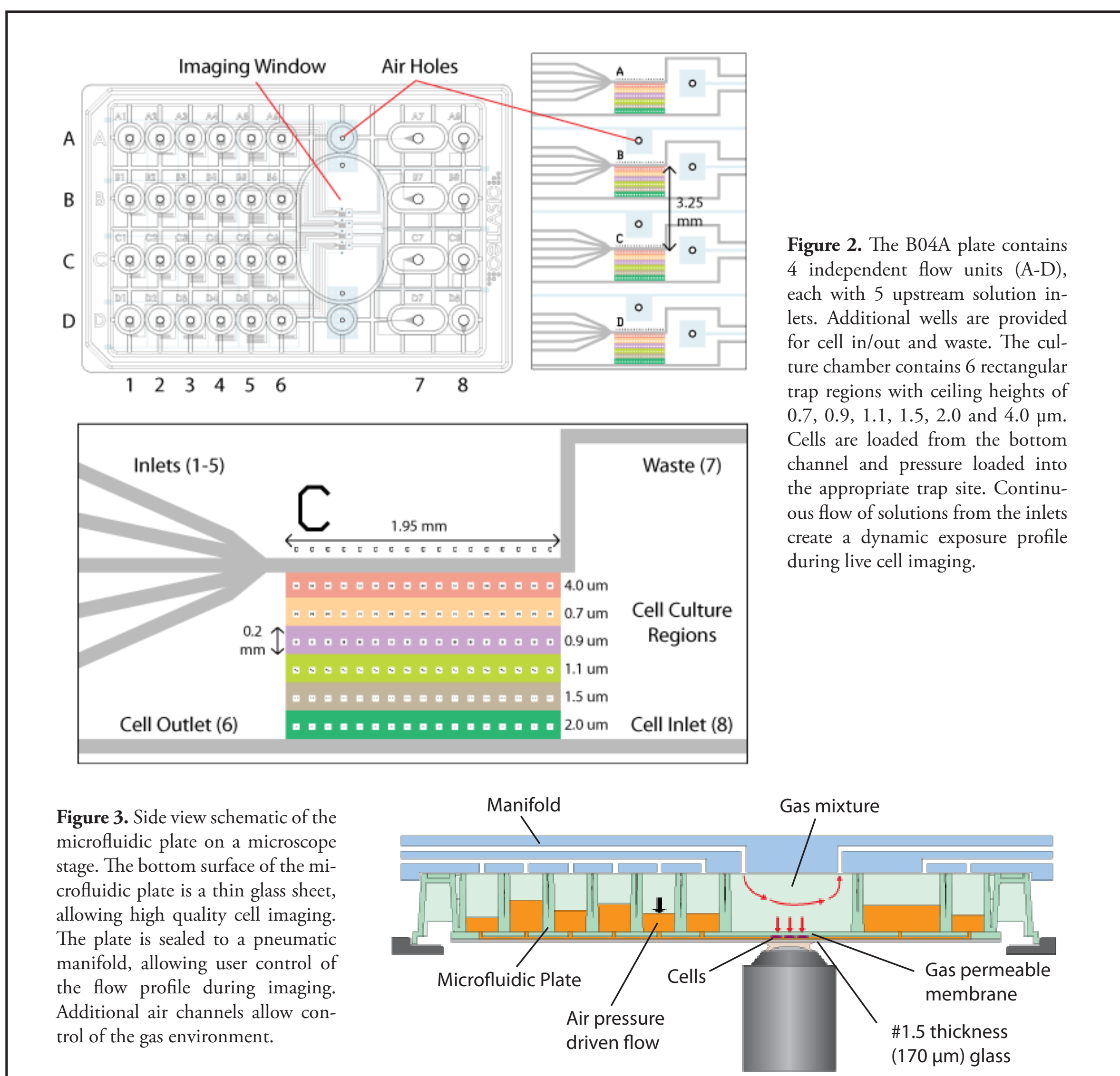

Figure 2. The B04A plate contains 4 independent flow units (A-D), each with 5 upstream solution inlets. Additional wells are provided for cell in/out and waste. The culture chamber contains 6 rectangular trap regions with ceiling heights of 0.7, 0.9, 1.1, 1.5, 2.0 and 4.0 µm. Cells are loaded from the bottom channel and pressure loaded into the appropriate trap site. Continuous flow of solutions from the inlets create a dynamic exposure profile during live cell imaging.

Figure 3. Side view schematic of the microfluidic plate 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.

both well 6 and well 8 are pressurized to drive cells into the 6 trap regions. This pressure also expands the elastic ceiling to allow cell entry. Depending on their size, cells will become trapped at varying distributions in the 6 trap locations. For *E. coli*, it is typical to see cells initially localized in the 0.7 µm region. Trapped cells are held against the glass floor by the elastic ceiling when it returns to its rest position (see figure 4). Because the ceiling never touches the floor, there is adequate space for solution to flow past the cells. Adjusting the pressure and duration of the pressure loading step can modify the loading profile. Loading cells at different initial densities and loading pressures is the best way to alter the number and distribution of cells loaded.

SOLUTION SWITCHING

Exposure solutions are introduced from the 5 inlet wells and flow down the channel on the top side of the trap chambers. As the solution flows by the chambers, cells are rapidly exposed via convection and diffusion into the trap areas. Typically, full exposure will occur 1-4 minutes after a switch is made, depending on the flow rate and position in the chambers (cells in the 0.7 µm trap will be exposed more quickly than those in the 2.0 µm region). There is little to no x-position dependence on exposure, as the entire top channel is filled in a few seconds. When cells become overgrown in the chambers, there will be some effect on the flow rate and exposure profile, as the cell bodies come to occupy a majority of the volume in the chamber. However, in most cases, the nutrient transport is sufficient to maintain cell viability even when fully confluent. With certain bacterial cells, a thick film of cells begins to form within the flow channel, which can also be studied via live cell imaging.

A key feature of the B04A 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 B04A allows 5 different solutions to be switched during the course of the experiment. As one example, 4 concentrations of a fluorescent dye (25%, 50%, 75%, 100% of dextran conjugated fluorescein, 3kDa, Invitrogen) were switched at 20

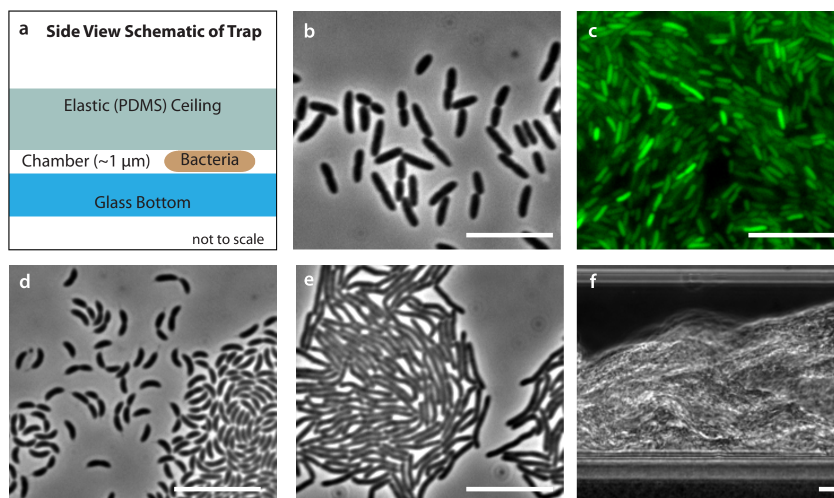

Figure 4. Imaging of bacteria in the microfluidic trap. (a) Side view schematic of the cell trap chamber shows the glass bottom floor, a trapped rod-shaped bacteria, and the elastic PDMS ceiling. (b) Phase contrast image (100X objective) of *E. coli* in the 0.9 µm trap region immediately after loading. (c) After 24 hours of perfusion culture and staining for viability (Invitrogen Bac-Light), cells remain in a fixed monolayer. (d) *Caulobacter* trapped and cultured in the 0.9 µm region. (e) *Mycobacterium smegmatis* cultured in the 0.9 µm region. (f) Bacterial film (*E. coli*) in the flow channel after 24 hours of perfusion culture. Image taken with a 40X objective. All scalebars are 10 µm.

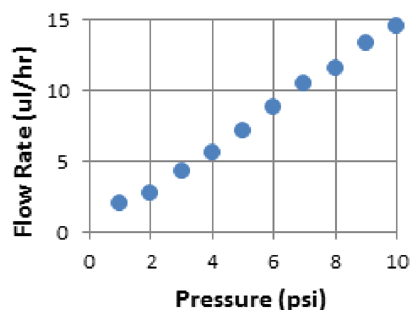

Figure 5. Flow rate as a function of pressure applied by the ONIX manifold to the inlet wells. Flow rates of 1-15 µl/hr allow long term continuous flow experiments to be performed on the microscope stage. The inlet wells hold 300 µl of solution, allowing a single experiment to run for over 24 hours. In general, a flow pressure of 2-4 psi is suitable for bacterial cell culture.

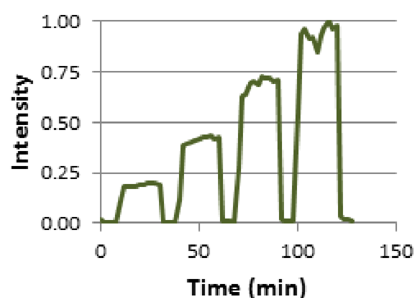

Figure 6. Sequential switching between four concentrations of fluorescent dye with intermediate wash steps. Intensity was measured in the 0.7 µm trap region every 2 minutes with flow at 2 psi. Data was plotted normalized to the max/min of intensity images. The fluctuation in readout is a result of instability in the fluorescence bulb on the microscope.

minute intervals with 10 minute wash steps in between (see figure 6). Fluorescence intensity was measured in the 0.7 µm trap region with a 100X objective. Note the rapid and complete response of the solution, creating a clean "step function" in the culture region. Since all five channels converge near the culture chamber, the B04A plate minimizes the dead volume during switching to a few nanoliters. Even with this small dead volume, there will be a brief interval (generally a few seconds) immediately after a switch where the old solution is washed out before the selected solution flows in. 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 B04A chamber allows long term maintenance of bacteria under well controlled conditions. Since the cells are trapped in x,y,z space, it is possible to track cell responses over time on the same group of cells. This allows collection of kinetic response data on live cells not possible with other approaches. Moreover, as the cells are prevented from moving, intracellular dynamics can also be monitored with high resolution. We observed cell growth in the trap chamber to be equivalent to expected values, indicating there is no detrimental effect of the microfluidic culture configuration (see figure 7). Interestingly, *E. coli* monitored in the microfluidic chambers over many generations exhibit a number of morphologies, such as "normal" rod-shaped cells, smaller fast swimming cells (in the 1.1+ µm regions), filamentous strings of cells, and 3D bio-film structures in the flow channel.

As a demonstration of monitoring cell growth response over time, we cultured *E. coli* in normal LB medium for 1

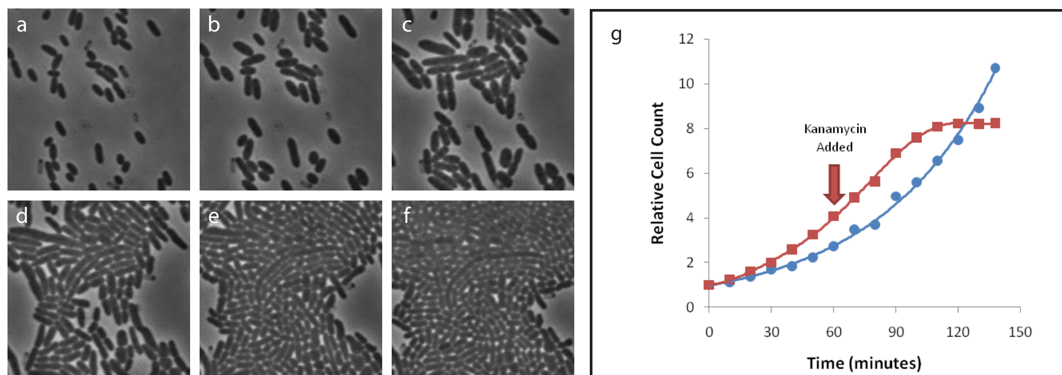

Figure 7. (a-f) Time lapsed images of *E. coli* growing in the B04 plate. Cells were cultured at 37°C with flow of LB medium at 2 psi with imaging on a 100X phase contrast objective. Photos were captured at **(a)** 0, **(b)** 30, **(c)** 60, **(d)** 90, **(e)** 120, and **(f)**150 minutes. **(g)** Cell growth response to addition of kanamycin during live cell imaging. *E. coli* were monitored in 2 parallel chambers with (red squares) and without (blue circles) addition of kanamycin after 1 hour and individual cells were counted to create the growth curves.

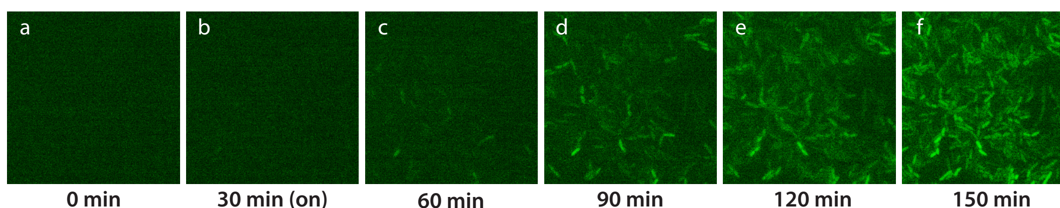

Figure 8. Induction of GFP expression in *E. coli*. Cells were induced at 30 minutes, leading to increased expression of GFP over 2 hours. Note the cells are maintained in fixed position during the flow exchange. Cells courtesy of the Tim Lu Lab, MIT.

hour, then switched to LB/kanamycin medium. These cells were not resistant to kanamycin, an antibiotic that interferes with ribosome function. Two chambers were imaged simultaneously with a 100X phase contrast objective and monitored for growth rate. As expected, after exposure to kanamycin, *E. coli* stopped growth (see figure 7). In a similar experiment, arabinose was used to induce GFP expression in *E. coli* in the microfluidic chamber and tracked over time (figure 8).

SUMMARY

The ability to track individual bacteria cells with time lapse imaging is beneficial to a variety of applications. Current approaches are not able to maintain non-adherent bacteria cells in a single focal position for time-lapse imaging and long-term monitoring of response to solution changes. CellASIC has developed the innovative B04A microfluidic perfusion chamber specifically designed to trap bacteria cells in a single focal plane without limiting solution exchange or cell growth properties. This design has been demonstrated for monitoring *E. coli* for long term culture, fluorescence quantification, solution exchange response, and time-varying inputs. Further, the ease-of-use, flexibility, and accessibility of this advanced technology platform should prove beneficial to a wide range of bacterial cell biology applications.

REFERENCES

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