emulate

Protocol for Emulate Organ-Chips:

Fluorescence Imaging

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EP126 v1.0

TITLE	DOCUMENT	VERSION
Fluorescence Imaging	EP126	1.0
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Goals:	Key Steps:	Other Required Materials:
Visualize fluorescent probe in an Emulate Organ-Chip using fluorescence microscopy	• Prepare microscope • Image chip	• Stained chips • Glass slides • Lint-free wipes • Fluorescence microscope

1. Overview

All fluorescent or confocal imaging of cells can be done directly in the Organ-Chip. You do not need to disassemble the chip, or isolate the membrane in order to image cells. The membrane is located 0.8 mm from the bottom of the chip and is visually accessible by most objectives on most microscopes, although the use of a long-working distance objective lens is recommended for optimal results. The chip is made of an optically transparent material that will not cause any significant distortion of your signal or image; it does not auto fluoresce.

2. Method

1. Turn on fluorescence microscope prior to imaging to ensure adequate setup and readiness.
   - Note: For live imaging, set the live imaging chamber to 37°C, 5% CO_2, at least 30–60 minutes before imaging.
2. Securely place a clear glass slide onto the stage of the microscope.
3. Carefully remove the chip carrier from the Pod™ module and remove the chip from the chip carrier.
4. Place the chip onto the glass slide.
5. For imaging of fixed samples, clean the chip surface with a lint-free wipe moistened with 70% ethanol prior to placing the chip lengthwise onto the glass slide. For live imaging, do not use ethanol. A lint-free wipe moistened with sterile water can be used to gently clean the central area of the chip.
6. Using the brightfield setting and starting with the 10x objective, locate the focal plane of the membrane pores, which are distinguished by their hexagonally-packed arrangement (see Fig. 1). Once the correct focal plane has been found, move the stage until the channel region of the chip is located.
7. Once the channel region is found, adjust magnification as desired, refocusing on the chip membrane. Switch off the brightfield and switch on the desired fluorescent signal. Focus on the cells of interest in either the top or bottom channel.

8. Starting at either the inlet or outlet, capture representative images along the length of the entire channel. For quantitative analysis, we recommend acquiring a minimum of 3 images per channel, spaced evenly along the channel length. Both fluorescent and brightfield images should be captured as needed.
9. Repeat steps 7 and 8 to image cells in the other channel.
10. Write down wavelength and exposure time used for every fluorescence signal of interest. Ensure that these parameters remain consistent between all chips within a given experiment for downstream semi-quantitative analysis.
11. Save the images using a format that allows for integration of acquisition information (metadata, such as CZI, VSI, LSM) in the designated study folder for further image analysis.

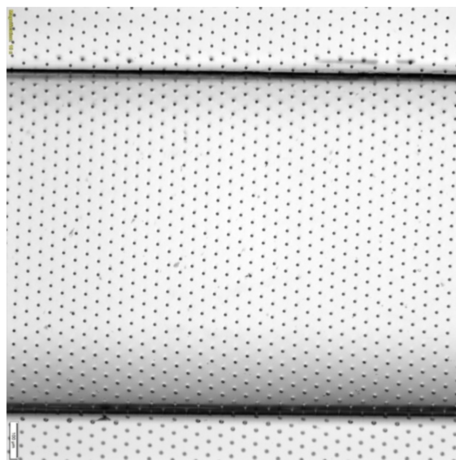

Figure 1: Membrane pores visualized at 10x magnification; Chip-S1™ channel sidewalls are clearly visible.

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