

# Imaging of Cellular Fluorescence on Corning's Transwell® Permeable Supports

## Application Note

CORNING

Audrey Bergeron and Ann Rossi, Ph.D.

Corning Life Sciences  
Kennebunk, ME USA

### Introduction

Corning's Transwell permeable supports can be used for a variety of cell culture applications to better mimic the *in vivo* environment. Transwell inserts with larger pore sizes, such as 3.0, 5.0, or 8.0  $\mu\text{m}$ , are typically used for migration, chemotaxis, and invasion assays, while Transwell inserts with smaller pore sizes, such as 0.4, 1.0, or 3.0  $\mu\text{m}$ , can be used for co-culture applications and cell polarization studies. In co-culture and cell polarization applications, fluorescent imaging of cells cultured on the permeable membrane may be required to confirm cell membrane integrity or the presence of differentiated cell types. Traditionally, fluorescent imaging of cells cultured on Transwell inserts requires the membranes to be cut out of the Transwell insert and placed onto a slide or onto the bottom of a multiwell plate. However, with improvements in the capabilities and resolution of current imaging systems, especially confocal imagers, this may no longer be necessary. Here, we demonstrate fluorescent imaging of MDCKII/MDR1 cells directly on Transwell permeable supports in their cell culture receiver plate. This study utilized Transwell permeable supports with both polyester (PET) and polycarbonate (PC) membranes with pore sizes ranging from 0.4 to 3.0  $\mu\text{m}$ .

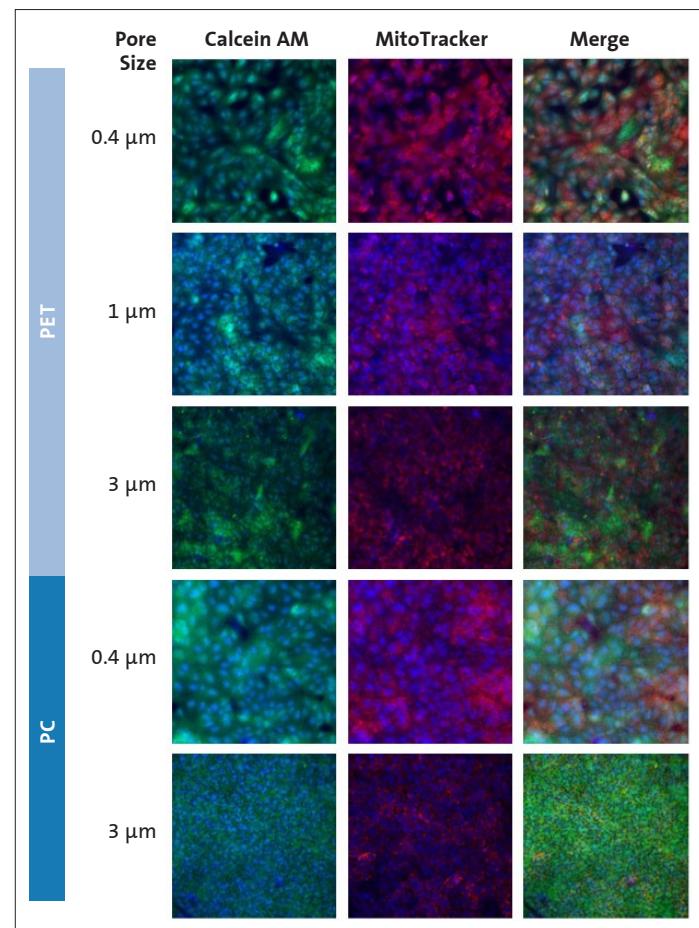
### Materials/Methods

MDCKII/MDR1 cells were obtained from Dr. Piet Borst (Netherlands Cancer Institute) and seeded into HTS 96-well Transwell permeable supports and 6.5 mm Transwell permeable supports at  $1 \times 10^5$  cells/cm $^2$  in Dulbecco's Modification of Eagle's Medium (DMEM; Corning Cat. No. 10-013-CM) supplemented with 10% Fetal Bovine Serum (FBS; Corning Cat. No. 35-010-CV). Transwell permeable support membranes tested included 0.4, 1.0, and 3.0  $\mu\text{m}$  PET as well as 0.4 and 3.0  $\mu\text{m}$  PC. The cells were cultured overnight in a 37°C, 5% CO<sub>2</sub> humidified incubator.

The next day, cells were stained live with 5  $\mu\text{M}$  Calcein AM (Corning Cat. No. 354216), 350 nM MitoTracker® Orange CMTMRos (Thermo Fisher Cat. No. M7510), and 10  $\mu\text{g}/\text{mL}$  Hoechst 33342 (Thermo Fisher Cat. No. 62249) in Hank's Balanced Salt Solution with calcium and magnesium (HBSS; Corning Cat. No. 21-023-CM) for 45 minutes in a 37°C, 5% CO<sub>2</sub> humidified incubator. Cells were rinsed with HBSS prior to imaging with a Thermo Fisher CellInsight™ CX7 High-Content Screening (HCS) platform with a 20X objective in confocal mode, with 5 z-stack images of 2  $\mu\text{m}$  steps taken for each representative composite image.

### Results

Fluorescently labeled cells were imaged directly on each membrane type. Live cell stain Calcein AM (green), mitochondrial health stain MitoTracker Orange CMTMRos (red), and nuclear stain Hoechst 33342 (blue) were imaged in confocal mode and representative composite images are shown in Figure 1. The data demonstrates that removal of the PET and PC membranes from their supports is unnecessary for imaging, thus facilitating high throughput fluorescence microscopy of cells grown in Transwell permeable supports.



**Figure 1.** Representative composite confocal images of MDCKII/MDR1 cells stained live with Calcein AM (green), MitoTracker Orange CMTMRos (red), and Hoechst 33342 (blue). Cells were stained and imaged directly in Transwells on a variety of membrane types.

## Recommendations for Protocol Optimization

- Follow fluorescent staining protocols typically used for live or fixed cell staining.
- Create a new form factor in your imager specifically for Transwell® permeable supports. Start with a Corning® 24-well or 96-well form factor and adjust the Z-height. The height may be outside of the range of auto-focus and need to be manually focused. To focus and find the correct Z-height for Transwell permeable supports, we recommend staining cells cultured on a Transwell permeable support with crystal violet or staining the cell nuclei.

- To accommodate for the increased distance between the cells and the imager, the exposure time may need to be increased compared with imaging of cells cultured on a microplate.

For more specific information on claims, visit the Certificates page at [www.corning.com/lifesciences](http://www.corning.com/lifesciences).

**Warranty/Disclaimer:** Unless otherwise specified, all products are for research use only. Not intended for use in diagnostic or therapeutic procedures. Not for use in humans. Corning Life Sciences makes no claims regarding the performance of these products for clinical or diagnostic applications.

For additional product or technical information, visit [www.corning.com/lifesciences](http://www.corning.com/lifesciences) or call 800.492.1110. Outside the United States, call +1.978.442.2200 or contact your local Corning sales office.

# CORNING

### Corning Incorporated

*Life Sciences*

836 North St.

Building 300, Suite 3401

Tewksbury, MA 01876

t 800.492.1110

t 978.442.2200

f 978.442.2476

[www.corning.com/lifesciences](http://www.corning.com/lifesciences)

### ASIA/PACIFIC

#### Australia/New Zealand

t 61 427286832

#### China

t 86 21 3338 4338

f 86 21 3338 4300

#### India

t 91 124 4604000

f 91 124 4604099

### Japan

t 81 3-3586 1996

f 81 3-3586 1291

### Korea

t 82 2-796-9500

f 82 2-796-9300

### Singapore

t 65 6572-9740

f 65 6735-2913

### Taiwan

t 886 2-2716-0338

f 886 2-2516-7500

### EUROPE

[CSEurope@corning.com](mailto:CSEurope@corning.com)

#### France

t 0800 916 882

f 0800 918 636

#### Germany

t 0800 101 1153

f 0800 101 2427

#### The Netherlands

t 020 655 79 28

f 020 659 76 73

#### United Kingdom

t 0800 376 8660

f 0800 279 1117

### All Other European Countries

t +31 (0) 206 59 60 51

f +31 (0) 206 59 76 73

### LATIN AMERICA

[grupoLA@corning.com](mailto:grupoLA@corning.com)

#### Brasil

t 55 (11) 3089-7400

#### Mexico

t (52-81) 8158-8400