

Imaging of Cellular Fluorescence on Corning® Transwell® Permeable Supports

CORNING

Application Note

Audrey Bergeron and Ann Rossi, Ph.D.
Corning Incorporated, Life Sciences
Kennebunk, ME USA

Introduction

Corning 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 μm , 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 μm , 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 μm .

Materials and 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 1×10^5 cells/cm² in Dulbecco's Modified Eagle's Medium (DMEM; Corning 10-013-CM) supplemented with 10% Fetal Bovine Serum (FBS; Corning 35-010-CV). Transwell permeable support membranes tested included 0.4, 1.0, and 3.0 μm PET as well as 0.4 and 3.0 μm PC. The cells were cultured overnight in a 37°C, 5% CO₂ humidified incubator.

The next day, cells were stained live with 5 μM Calcein AM (Corning 354216), 350 nM MitoTracker® Orange CMTMRos (Thermo Fisher M7510), and 10 $\mu\text{g/mL}$ Hoechst 33342 (Thermo Fisher 62249) in Hank's Balanced Salt Solution with calcium and magnesium (HBSS; Corning 21-023-CM) for 45 minutes in a 37°C, 5% CO₂ 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 μm steps taken for each representative composite image.

Results and Discussion

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.

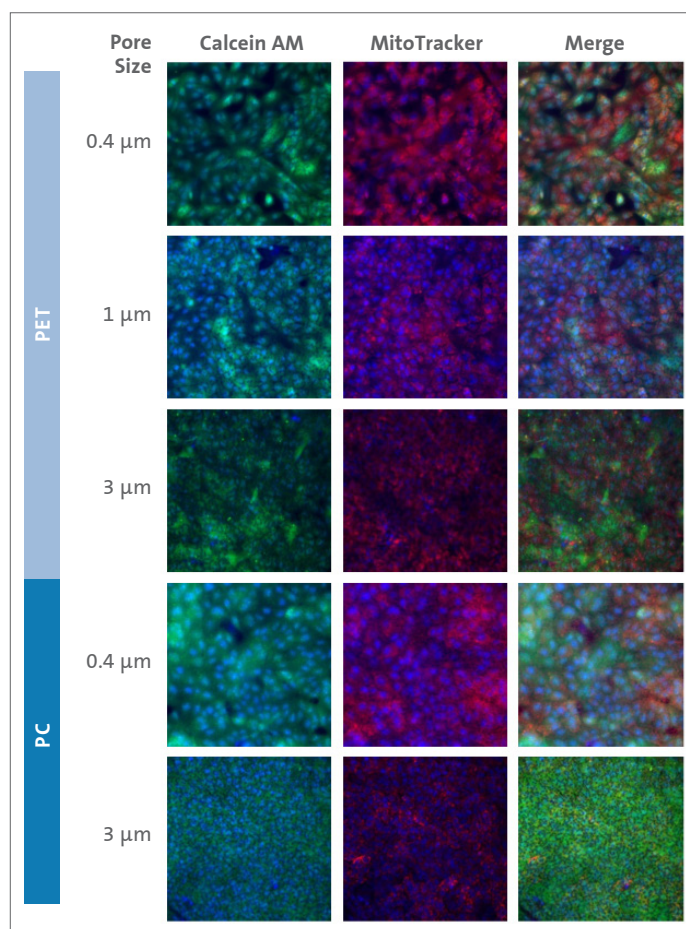


Figure 1. Representative composite confocal images of MDCKII/MDR1 cells stained live with Calcein AM (green), MitoTracker Orange CMTMRos (red), and Hoechst 33342 (blue). Cells were stained and imaged directly in Transwells on a variety of membrane types.

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 Corning® 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.

How to Purchase: For specific availability in your region and purchasing options, terms and conditions of sale, customer/product support, and certificates, visit www.corning.com/how-to-buy.

Warranty/Disclaimer: Unless otherwise specified, all products are for research use or general laboratory use only.* Not intended for use in diagnostic or therapeutic procedures. Not for use in humans. These products are not intended to mitigate the presence of microorganisms on surfaces or in the environment, where such organisms can be deleterious to humans or the environment. Corning Life Sciences makes no claims regarding the performance of these products for clinical or diagnostic applications. *For a listing of US medical devices, regulatory classifications or specific information on claims, visit www.corning.com/resources.

Corning's products are not specifically designed and tested for diagnostic testing. Many Corning products, though not specific for diagnostic testing, can be used in the workflow and preparation of the test at the customers discretion. Customers may use these products to support their claims. We cannot make any claims or statements that our products are approved for diagnostic testing either directly or indirectly. The customer is responsible for any testing, validation, and/or regulatory submissions that may be required to support the safety and efficacy of their intended application.

CORNING

Corning Incorporated
Life Sciences

www.corning.com/lifesciences

NORTH AMERICA

t 800.492.1110

t 978.442.2200

ASIA/PACIFIC

Australia/New Zealand

t 61 427286832

Chinese Mainland

t 86 21 3338 4338

India

t 91 124 4604000

Japan

t 81 3-3586 1996

Korea

t 82 2-796-9500

Singapore

t 65 6572-9740

Taiwan

t 886 2-2716-0338

EUROPE

CSEurope@corning.com

France

t 0800 916 882

Germany

t 0800 101 1153

The Netherlands

t 020 655 79 28

United Kingdom

t 0800 376 8660

All Other European Countries

t +31 (0) 206 59 60 51

LATIN AMERICA

grupolA@corning.com

Brazil

t 55 (11) 3089-7400

Mexico

t (52-81) 8158-8400

The information contained within is accurate as of the date of publication and subject to change without notice.

For a listing of trademarks, visit www.corning.com/trademarks. All other trademarks are the property of their respective owners.

© 2019, 2025 Corning Incorporated. All rights reserved. 6/25 CLS-AN-521 REV1