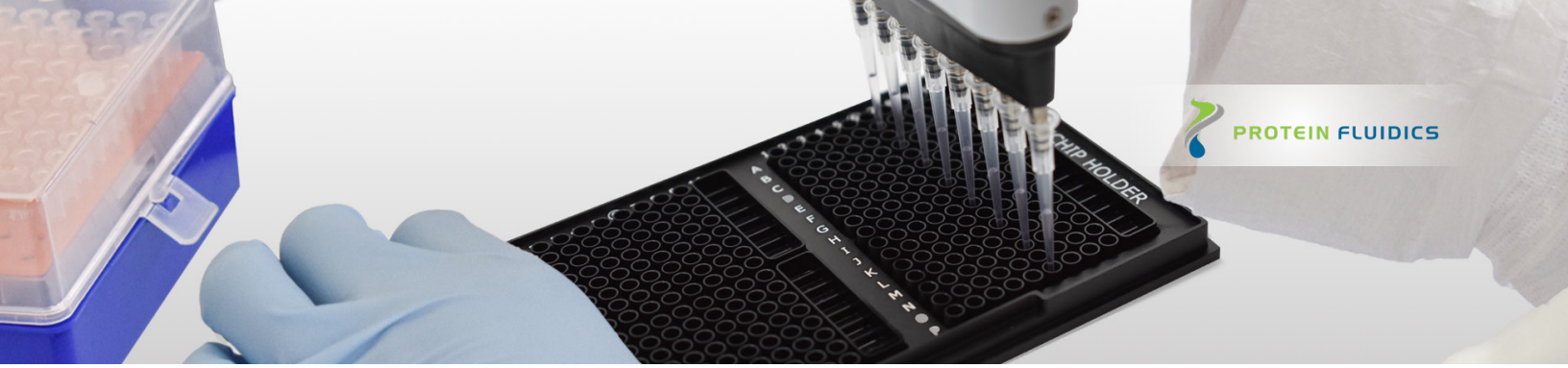


TECHNICAL NOTE

Transfer, Automated Staining, and Imaging of Organoids in Matrigel

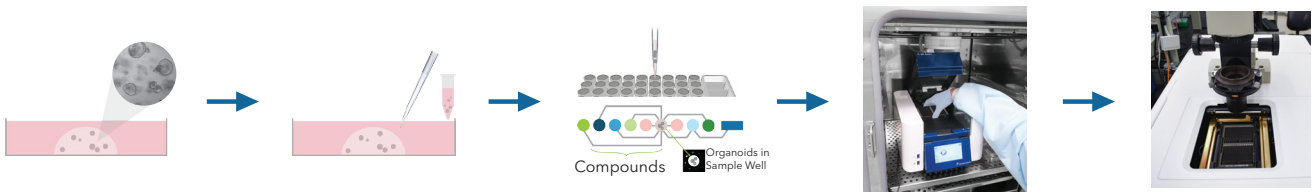
Introduction

Organoid technology attracts an enormous interest for personalized medicine, drug screening and cell therapy[1]. In most cases, organoid formation is dependent on extracellular matrices (ECM). Matrigel is an ECM-based hydrogel matrix, that is often used to generate organoids. It provides physical support and molecules that are important for cell function, attachment, and differentiation[2]. Assays with fragile organoid structures rely on manual handling which limits the ability to perform complex assays easily, quickly, and reliably. Automation of the assay workflow using Pu·MA System® eliminates the need for labor intensive manual processing of organoids which is normally prone to disruption or loss of sample[3]. It enables the streamlining of complex multi-step procedures like drug treatment and immunofluorescence staining for imaging.

This technical note describes the procedure of retrieving pulmonary organoids from a Matrigel dome and transferring them to a Flowchip in 25% Matrigel to perform automated assays using Pu·MA System. A concentration range of 20% to 30% Matrigel was experimentally determined as optimal to support and maintain organoids inside the protected chamber of the flowchip. This concentration had no effect on the performance of microfluidic channels and liquid transfers within the flowchip. Transferred organoids were stained for viability using a Pu·MA System and imaged within the flowchip.

Procedure

In this protocol we used pulmonary organoids that were derived from primary human lung epithelial cells (ScienCells, Co.). They were grown in Matrigel domes using reagents and protocol from StemCell Technologies. Cells were expanded in 2D, mixed with GF reduced 90% Matrigel and seeded into Matrigel domes in 24-well plates. The workflow of this technote is shown in Figure 1 and the detail of steps are described below.



Organoids grown in Matrigel dome

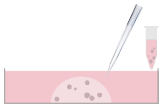
Add Cell Recovery Solution & collect released organoids

Transfer organoids & reagents into flowchips

Run assay in Pu·MA System

Image organoids in flowchip

Fig 1. Workflow for transfer of Matrigel grown organoids into flowchips for automated Pu·MA System assay.



Retrieval of organoids from the Matrigel dome

1. Remove as much of culture medium as possible from the 24-well plate taking care not to disturb organoids.

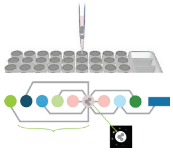
2. Add pre-chilled Cell Recovery Solution (CRS) (2X volume of Matrigel volume). Pipette the CRS and organoids up and down gently to carefully break up the Matrigel without damaging organoids.

3. Incubate the plate at 4°C for 20 minutes.

4. Check the organoids under the microscope to see if the Matrigel has been depolymerized and organoids are free-floating.

5. Transfer CRS and organoids into an Eppendorf tube and centrifuge briefly.

6. Remove the CRS without disturbing the pellet with organoids and wash with cold PBS by short centrifugation (400 G for 2 minutes).



Loading organoids to the sample protected well of the Flowchip.

Recommendation: To help with successful transfer, we use an additional light source (A4 Ultra-Thin Portable LED) to illuminate the flowchips from the bottom. It helps for the correct positioning of the pipette tips for dispensing organoids.

1. Remove the PBS wash carefully and resuspend organoids in 25% Matrigel in media on ice. (Note: Matrigel concentrations between 20% and 30% can be used.)

2. The loading organoids procedure dispenses approximately 1-2 µL of 25% Matrigel to form a dome with organoids within the protected sample chamber of the flowchip. There are two options for loading organoids into the flowchips:

(A) Transfer of multiple organoids:

· Aspirate 4 µL of the organoids in 25% Matrigel from the Eppendorf tube. Hold the pipet tip vertically to allow organoids to sediment closer to the end of the tip. It is recommended to use a P20 or P100 pipette.

· Position the pipette tip vertically close to the bottom of the protected sample chamber. It is acceptable to touch the bottom of the sample chamber.

· Dispense organoids by slowly pushing the plunger. There might be some residual Matrigel in the tip. DO NOT PUSH plunger to the second stop to prevent introducing bubbles.

· Carefully remove the pipette tip from the well before allowing the plunger to return to its rest position and without touching the sides of the well, otherwise you risk aspirating organoids back into the tip.

(B) Transfer of single organoid:

· Transfer organoids in 25% Matrigel into a U-bottom plate.

· Aspirate single organoid in 4 µL. Hold the pipet tip vertically to allow organoid to sediment closer to the end of the tip. Follow the same steps as described above in 2A for multiple organoids.

3. After organoids are transferred, incubate the flowchips at room temperature for 10-15 min to allow the Matrigel to solidify.

4. Add 18 µL of appropriate media on top of the Matrigel by placing the pipette tip on the bottom side of the well and gently press the plunger. Figure 2 shows bright field images of organoids in the protected sample chamber in 25% Matrigel.

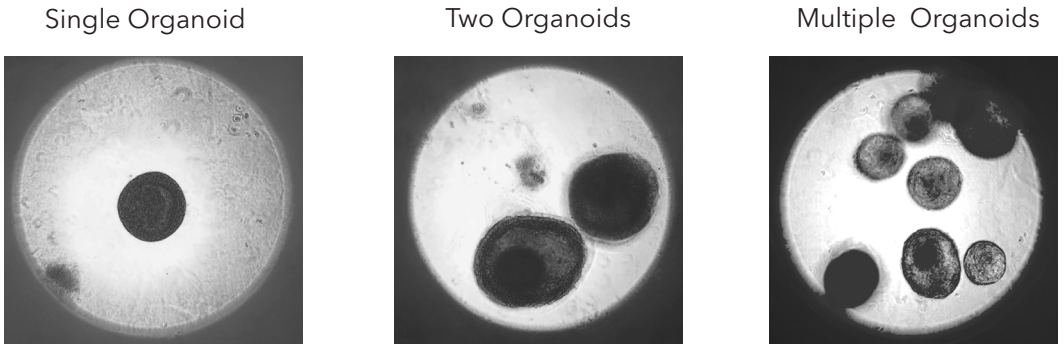
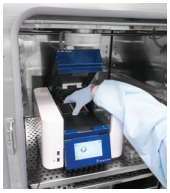


Fig 2. Bright field images of organoids in the protected sample chamber of flowchips in 25% Matrigel. Organoids shown range between 100 to 500 µm in diameter.



Automated Assay using Organoids in Matrigel

The Pu·MA System was used in this technote for running the automated protocol for staining the lung organoids in 25% Matrigel.

1. In wells adjacent to the sample wells, a cocktail of stains was added at 2X concentration. Stains used: CyQuant™ Direct Cell Proliferation Assay (as per instructions fromThermofisher Scientific) for viable cells, and Ethidium Homodimer-1 (EthD, 3 µM final concentration).

2. At the end of the incubation an optional wash step can be performed. The flowchip holder was removed and organoids were imaged (Figure 4).

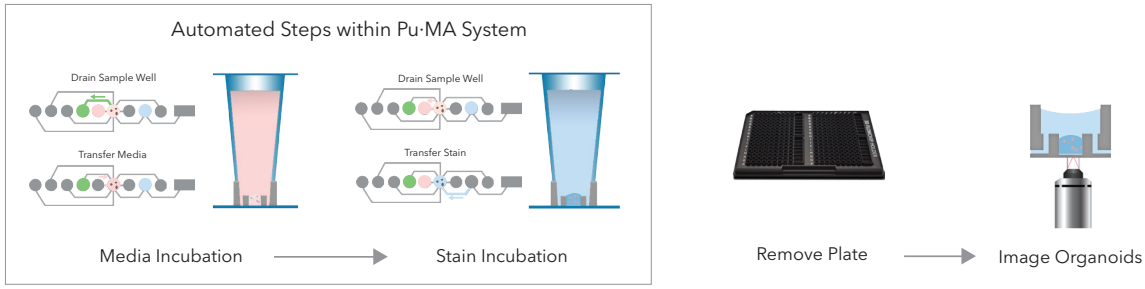
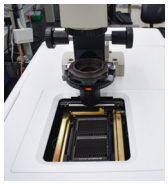


Fig 3. Automated assay workflow steps within the Pu·MA System.



Imaging of Stained Organoids in Matrigel

1. Images were acquired with the ImageXpress MicroConfocal Imaging System (Molecular Devices) using the 10X Plan Apo air objective and 20X water immersion objective.

2. 3D Z-stack images were taken with the confocal option (60 µm pinhole spinning disc), capturing images 5 – 10 µm apart depending on objective magnification. Based upon sample type, the Z-stack acquisitions covered a range of 120 to 250um deep.

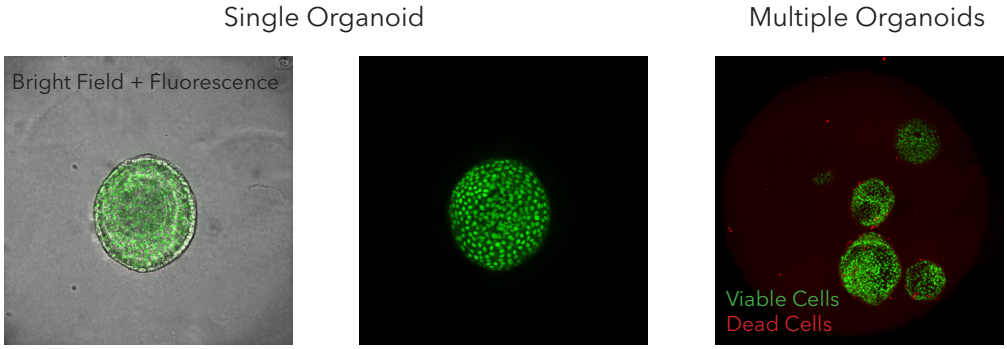


Fig 4. Confocal images of a single organoid (A) and multiple organoids (B) stained with CyQuant (viable cells) and EthD (dead cells) in 25% Matrigel.

Summary

In this tech note we outline a simple transfer method of organoids in 25% Matrigel into the Pu·MA System flowchips to perform automated assays. We demonstrated that you could perform viability staining without disturbing the organoids. A concentraition range of 20% to 30% Matrigel is compatible with our Pu·MA System flowchips and does not disrupt fluid transfers within the microfluidic channels. The optically clear bottom of the flowchip makes it easy to acquire high resolution confocal images[3] including the use of water immersion objectives. The compatibility of the Pu·MA System with Matrigel makes it possible to perform automated organoid assays within the physiologically relevant conditions to study various drug discovery applications with minimum perturbations to the sample.

Acknowledgments

This study was conducted in collaboration with Dr. Oksana Sirenko and Mr. Matthew Hammer (Molecular Devices, Inc.).

References

1. Clevers, Hans. "Modeling development and disease with organoids." Cell 165.7 (2016): 1586-1597.

2. Hughes, Chris S., Lynne M. Postovit, and Gilles A. Lajoie. "Matrigel: a complex protein mixture required for optimal growth of cell culture." Proteomics 10.9 (2010): 1886-1890.

3. Cromwell, E. F., Leung, M., Hammer, M., Thai, A., Rajendra, R., & Sirenko, O. (2021). Disease Modeling with 3D Cell-Based Assays Using a Novel Flowchip System and High-Content Imaging. SLAS technology, 24726303211000688.

Materials

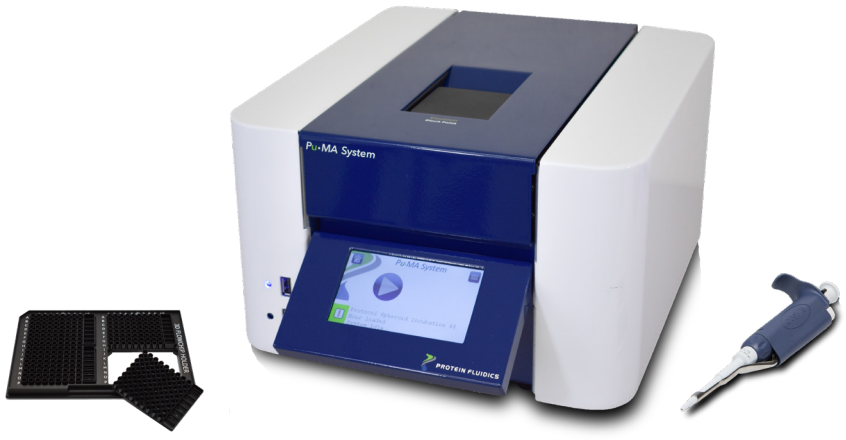
Matrigel (Corning #356234)
Cell Recovery Solution (Corning #354253)
Pipet tips for the transfer of organoids (regular bore, Eclipse UE-200G)
Wide bore tips (1 -1.2 mm, Rainin Mettler Toledo PN 30389247)
CyQuant Assay Kit Thermofisher Scientific C35011
Ethidium Homodimer Thermofisher Scientific E1169
A4 Ultra-Thin Portable LED Light Panel
Pu·MA System (Protein Fluidics)
Pu·MA System Flowchips (Protein Fluidics)
ImageXpress MicroConfocal Imaging System (Molecular Devices)
Pipettes (P20 or P100)

Instrument Compatibility

Light microscope
Automated Confocal Imaging System
Plate Reader (384 well spacing)

Contact Us

Protein Fluidics, Inc.
650-529-5080
support@proteinfluidics.com



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