Automated Workflow for 3D Cell Model Generation and Immunofluorescence Phenotyping using Microfluidic Pu·MA System EC (Environmental Control)

Booth #903

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INTRODUCTION

Three-dimensional cell models have gained popularity, compared with traditional 2D models, because they better reproduce key aspects of human tissues and are amenable to a wide range of applications from basic research to pharmaceutical drug safety and efficacy testing. Immunofluorescence (IF) staining and high-content imaging of 3D cell models are important tools that allow evaluation of the expression and localization of specific proteins within cells, as well as the distribution and interaction of different cell types, in response to treatments. Image-based phenotypic profiling is a validated strategy by which data-rich biological images are analyzed for patterns, revealing disease-associated screenable phenotypes. This process helps to understand disease mechanisms and to assess novel therapies more effectively. However, the transition from 2D to 3D cell models has resulted in challenges related to sample handling and assay development and requires more sophisticated protocols and instrumentation.

Here we present our novel **automated workflow** for **phenotypic profiling of 3D models** using **microfluidic flowchips** and the **new Pu·MA System EC (Environmental Control).** The Pu·MA System EC can precisely control temperature & carbon dioxide levels and maintain high relative humidity in the flowchip chamber. The system enables generation of 3D cell models combined with automated 3D cell-based assays.

The Pu·MA System EC workflow consists of

- **3D cell model formation from a cell suspension with automated media exchanges**
- **Incubation with compounds**
- **Sample fixation and automated washes**
- **Automated IF staining**

The samples are then imaged in the flowchip and analyzed on confocal microscopes or high-content imaging systems.

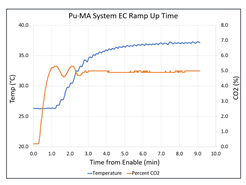
Pu·MA SYSTEM EC (ENVIRONMENTAL CONTROL)

- Control of temperature, CO_2 and relative humidity in the flowchip chamber
- Protected Sample Chamber technology prevents sample loss and damage
- Scheduled automated media exchanges to support 3D culture
- Scheduled automated collection of supernatants for secreted factors analysis
- Automated workflow solutions for IF Staining and other 3D cell assays

CO_2 1% to 10%

+5 °C above ambient to 40 °C

> 80% humidity with reservoir



Short Term Stability (10 min)

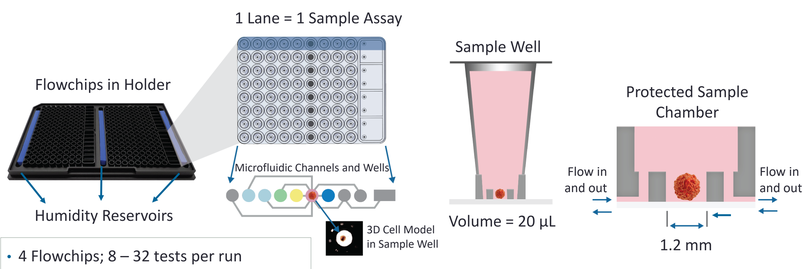
	Avg	StDev	CV
Temp	37.1	0.10	0.3%
CO_2	5.0	0.05	1.0%

Long Term Stability (24 hr)

	Avg	StDev	CV
Temp	36.9	0.16	0.4%
CO_2	4.9	0.07	1.4%

MICROFLUIDIC FLOWCHIP

Each Pu·MA System flowchip contains eight lanes of reagent wells connected by microfluidic channels. Four flowchips are placed in holder that locates all wells in a 384 multiwell plate format providing for 32 samples per assay. Each flowchip lane is designated to one 3D sample and consists of a sample well connected to 8 reagent wells. Flowchip wells can be filled with reagents (media, compounds, stains, etc.) depending on the assay. Organoids/spheroids are loaded into the sample well and located in a protected chamber at the bottom of the well. This allows reagents to be transferred in and out of the sample well without disturbing or drying out the microtissue. The bottom of the flowchip is a thin cyclic olefin copolymer (COC) film which makes it compatible with high resolution imaging (Fig 1).

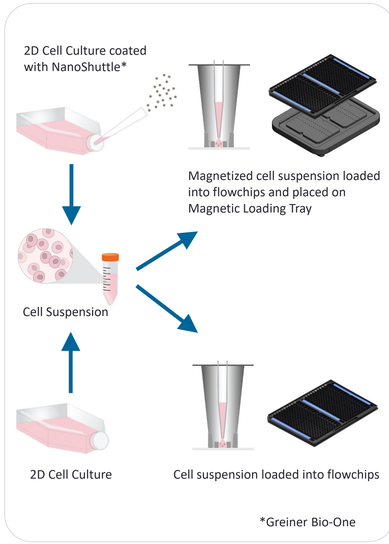


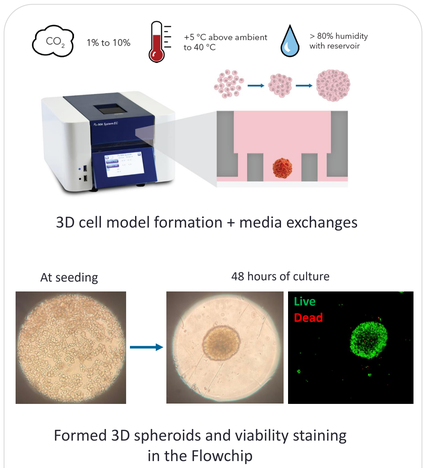
- 4 Flowchips; 8 – 32 tests per run
- Wells are on 384-plate spacings
- Thin optically clear COC bottom

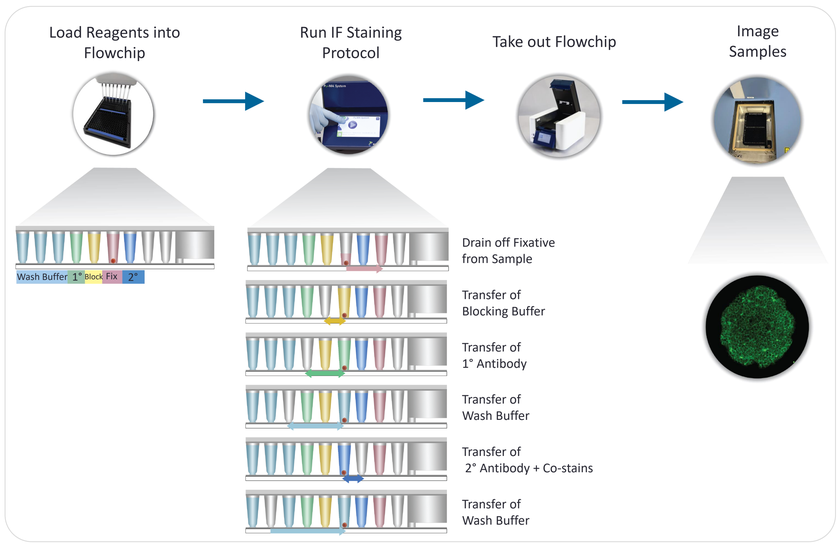
Figure 1. Schematic of flowchips showing microfluidic channels layout and sample well with proprietary protected sample chamber. The diameter of the sample well clear aperture is 1.2 mm and compatible with high-content imaging.

WORKFLOW FOR 3D CELL MODEL FORMATION AND IF STAINING IN Pu·MA SYSTEM EC

Cell Preparation and Loading into Flowchip → 3D Model Formation and Media Exchanges → Automated IF Staining and Imaging







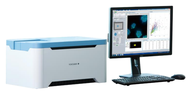
CONFOCAL IMAGING OF EPITHELIAL AND MESENCHYMAL MARKERS IN BREAST CANCER 3D MODELS

3D Cell Models

MCF7 Spheroids
- ER Positive
- Non-aggressive and non-invasive
- Low metastatic potential

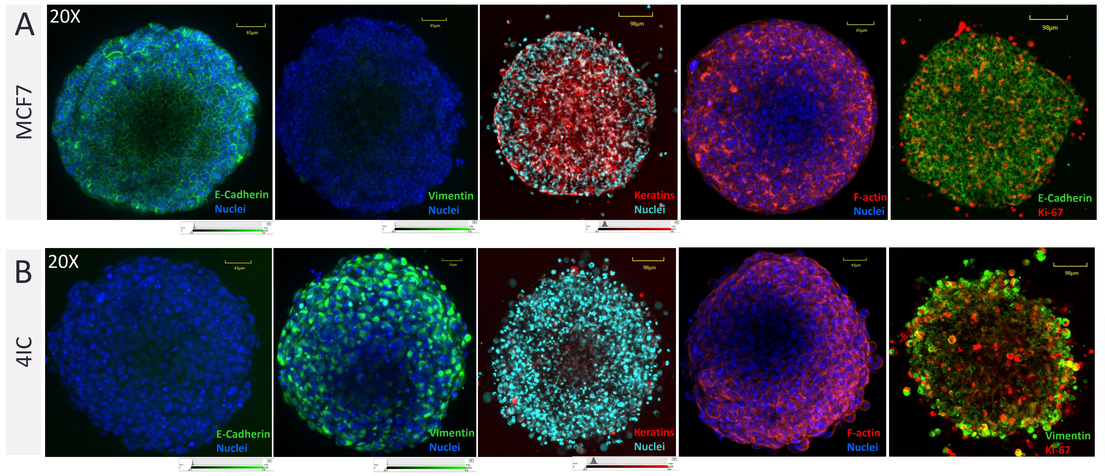
TU-BcX-4IC Tumoroids
- Derived from a primary TNBC tumor
- Highly metastatic, rapid growth
- Aggressive and resistant to therapy

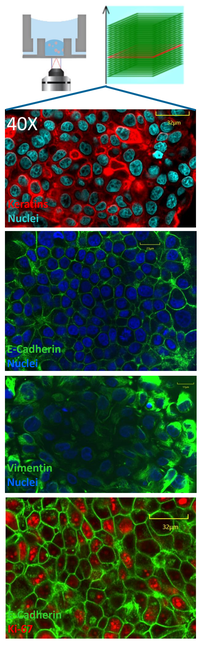
Imaging: CellVoyager CQ1 Benchtop High-Content Analysis System



- Confocal spinning disc technology
- High precision stage incubator and low phototoxicity
- Four fluorescence channels + transmitted light
- Integration with CellPathfinder high content analysis software

MCF7 spheroids and 4IC tumoroids were created by seeding 5,000 cell/well into the flowchip and culturing over 48 hours. Automated media exchanges was done in the system every 12 hours. Formed spheroids and tumoroids were fixed, washed, and stained. All the steps were performed in the Pu·MA System EC.





Confocal Z-Stack images were acquired between 70 – 120 µm range at 2.12.1 µm Z-step. Maximum projection images (MIP) were constructed from the Z-stacks (Fig 2).

We analyzed the expression of cell markers for proliferation and epithelial-mesenchymal transition (EMT) phenotype in two triple-negative breast cancer models: MCF7 spheroids (Fig 2A) and patient-derived tumoroids, created from primary cells isolated from a patient-derived highly aggressive tumor explant, TU-BcX-4IC (Fig 2B).

MCF7 spheroids showed strong signals for intense epithelial markers (E-Cadherin, cyto-keratins).

4IC patient-derived tumoroids showed marked downregulation of epithelial markers with intense Vimentin expression, suggesting that these highly aggressive cells display mesenchymal phenotype, which could be associated with highly metastatic and drug resistant profile of this model.

Figure 2. MIP images from confocal Z-stacks of MCF7 spheroids (A) and 4IC Patient-derived tumoroids (B). High resolution images of individual Z-slice (C).

CONCLUSIONS

- The completely automated Pu·MA System EC workflow for 3D cell model formation, media exchanges and IF staining eliminated sample transfer, disturbance and manual handling errors.
- Automated IF staining combined with high-content imaging provides consistent and reproducible high-quality comprehensive phenotypic data.
- This platform is a valuable tool in wide range of applications including disease modeling, drug discovery and personalized medicine.

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