L&L Biosystems

RASTRUM™ technology overview

INVENTIA LIFE SCIENCE

The RASTRUM™ Technology

RASTRUM™ Workflow Overview

Advanced Cell Models consist of a specific arrangement of RASTRUM™ 3D Structure, RASTRUM Matrix and your cells. This arrangement mimics the *in vivo* environment, enabling you to recapitulate the desired biology *in vitro*. The RASTRUM Platform integrates seamlessly into your existing workflow, making it possible to create Advanced Cell Models with ease.

The RASTRUM™ Technology

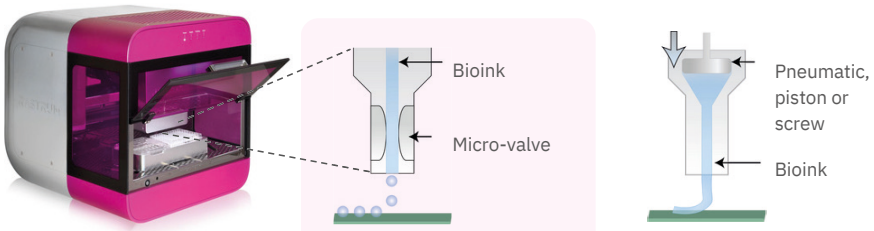
RASTRUM™ Drop-On-Demand Technology

Matrix-embedded 3D cell models are more physiologically relevant than 2D cultures (1). Despite their utility, the generation of 3D cell models via manual methods is time-consuming and low-throughput (2). Therefore, there is a clear and unmet need for consistent and reproducible production of 3D cell culture models for research applications.

Bioprinting technologies have emerged as a viable approach for the high-throughput generation of 3D cell models. There are various approaches to bioprinting, including extrusion-based and stereolithography (3-4). Inventia's unique drop-on-demand technology is akin to inkjet printing, whereby instead of depositing pixels of colour onto a page, the RASTRUM™ Platform deposits cells and matrix components onto the surface of a well plate, and builds these components layer-by-layer to form a desired 3D structure (5). Notably, drop-on-demand technology achieves high cell viability by reducing shear stresses on cells, as the pressure required to eject a droplet from the printhead is lower than that used in extrusion printing (6-7), or that of regular manual air displacement pipetting.

Nanoliter droplet printing achieves a homogeneous distribution between droplet-to-droplet and within each print across entire well plates (compatible with 24, 96 and 384 well plates). The printing process enables a predictable average number of cells per droplet with low variance, resulting in a coefficient of variation below the industry standard of 20% per print.

However, droplet-based printing is well suited to the production of 3D cell models as it enables rapid printing into well plates and simultaneous placement of multiple extracellular matrix components (8).

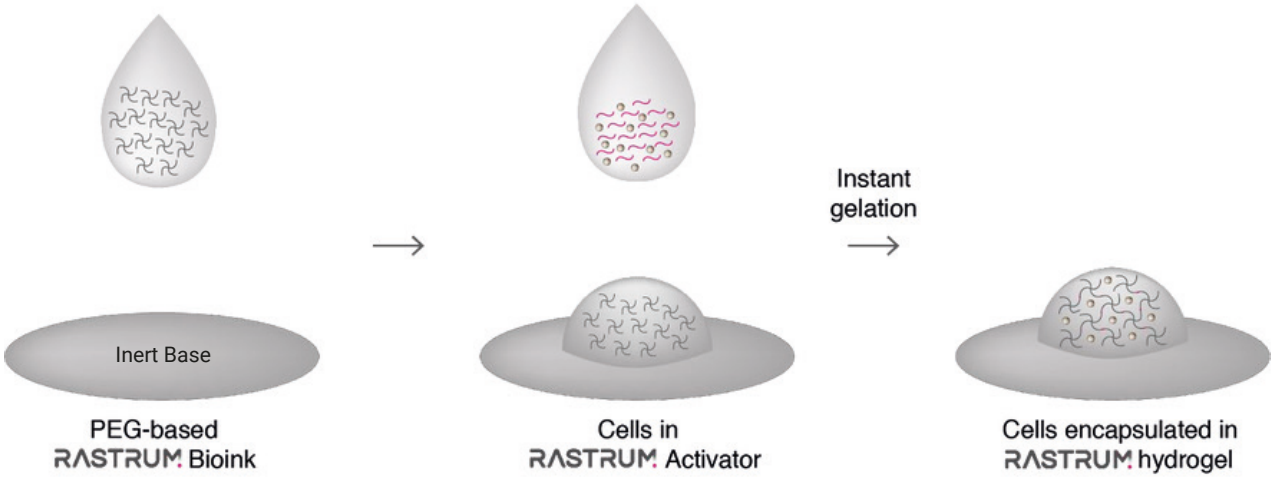


Property	Drop-on-demand	Extrusion
Throughput	High	Low
Cell Viability	>80%	40 - 80%
Sensitive Cell Types	Yes	No
Structure Size	Small (0.5 - 3 mm)	Large (>10mm)
Working Volume	nL	µL

The RASTRUM™ Technology

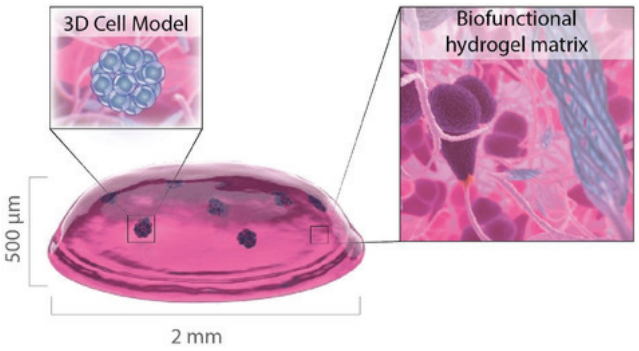
RASTRUM™ Drop-on-Demand Technology

Gelation occurs instantly upon the combination of one nanolitre drop of the RASTRUM™ Bioink and one nanolitre drop of RASTRUM Activator deposited from a printhead with multiple, independent reusable dispensing valves to create the rich RASTRUM Matrix. First, an inert base layer of hydrogel is printed to prevent cells attaching to the bottom of the well. The RASTRUM Biofunctional Bioink is printed on top of the inert base layer. Finally, your cells are added to the RASTRUM Activator, which when combined with RASTRUM Bioink, results in the immediate encapsulation of your cells in a 3D Matrix environment.



RASTRUM Matrices are tunable in their stiffness and biofunctional properties, enabling the creation of optimal environments for any cell type.

Growth factors and treatments can be added to the media to freely diffuse through the hydrogel structures



The RASTRUM™ Technology

RASTRUM™ Matrices

RASTRUM™ Matrices offer unparalleled versatility and customisation options. Featuring a Polyethylene Glycol (PEG) backbone, these matrices can be finely tuned to a variety of stiffnesses. Moreover, they can be functionalised with a diverse array of biological additives, including cell adhesion peptides, full-length proteins and glycosaminoglycans. This exceptional flexibility empowers researchers to faithfully mimic the complex *in vivo* cellular microenvironment of any tissue of interest.

All of our matrices are synthetic, chemically defined and animal-free, making them ideal for drug discovery and development applications. They are strictly quality-controlled to ensure consistency batch-to-batch and are fully permeable to antibodies, growth factors and small molecules. The RASTRUM Matrix has a consistent scaffold porosity that enables free cell migration, Matrix metalloproteinases (MMP)-driven remodelling and diffusion of media and drug treatment. These aspects enable migration for spontaneous contact, and chemotaxis-driven self-assembly into multicellular structures, including multi-cell type structures from mixed cell suspensions or stem cell sources.

RASTRUM™ Matrices

Source / Origin	**Synthetic, Xeno-free**
ECM Environment	**Tunable**
Matrix Properties	**Permeable (pore size 10-30 µm)** **Optically clear** **No auto-fluorescence**
Handling	**Room temperature stable**
Bioink & Activator Gelation Time	**~ 1 sec**
Batch-to-Batch Consistency	**High**
Cells Retrievable	**Yes**
Compatibility	**Standard readouts, imaging, end-point assays***

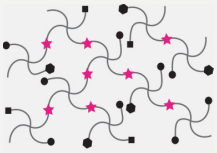
*See Appendix B

The RASTRUM™ Technology

RASTRUM™ Matrices

RASTRUM™ Matrices are synthetic, xeno-free, highly biocompatible, and tunable to recapitulate any extracellular matrix environment. The core RASTRUM Matrix Library comprises over 50 diverse combinations of adhesion peptides, full length proteins, and glycosaminoglycans, closely mimicking the complex *in vivo* cellular microenvironment, whether you are working with cell lines, primary cells, or iPSCs.

The RASTRUM platform fits seamlessly into your existing workflow (see Appendix B), enabling fundamental exploratory biology, high throughput drug discovery or personalised screening. Due to the optically-clear and permeable matrix properties, in situ analysis from drug dosing to nucleic acid extraction are easy. Cells can be easily recovered using the Cell Retrieval Solution without impacting the viability of the cells for any downstream application. The applications with RASTRUM are extensive as RASTRUM Advanced Cell Models are compatible with standard readouts, imaging and end-point assays. Incorporating your automated image analysis, existing plate reader, or drug dosing systems can be done with ease.

Stiffness Selection	Peptide Selection	RASTRUM Plus additives
Storage Modulus Px01 0.7 kPa Px02 1.1 kPa Px03 3.0 kPa Px06 4.8 kPa 	**Adhesion Peptides** RGD + YIGSR + GFOGER **or** RGD + YIGSR + NYYSNS RGD: Fibronectin YIGSR: Laminin, β chain GFOGER: Collagen I NYYSNS: Collagen IV	**Glycosaminoglycan** Hyaluronic Acid (HA) **Full length Proteins** Laminin-211 Laminin-521 Fibronectin

The RASTRUM™ Technology

RASTRUM™ Model Architecture

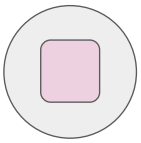
INERT BASE

When culturing cells in 3D gels, a common issue is that cells can migrate out of the gel and grow on the plastic surface underneath, forming a monolayer.

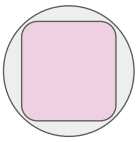
To stop monolayer formation, we have developed a unique Inert Base Layer, a non-functionalised and non-degradable PEG based layer of gel, that is directly printed on top of the plastic well surface, effectively forming an inert barrier. This Inert Base Layer is designed specifically to prevent cell interaction and growth on its surface. By implementing this layer, we create a pure 3D environment where cells remain confined within the biofunctional matrix, enabling more accurate and representative experimental conditions.

RASTRUM™ STRUCTURES

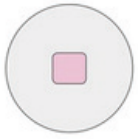
RASTRUM™ Structures are predefined, requiring no additional optimisation or changing of printing parameters to get started.



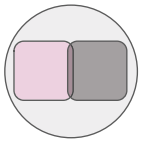
Imaging - enables simple imaging and minimal z-distribution



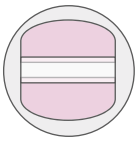
Large Plug - enables the culture of a large number of cells per well with reproducible 3D encapsulation



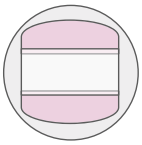
Immunohistochemistry - enables easy removal of the 3D models from the well



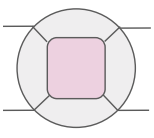
Dual-Matrix - enables two adjacent models with a small area of overlap in between



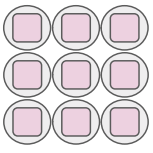
Triple-Matrix (Small Gap) - three adjacent touching gels, with a central gel creating a gap of ~0.75 mm between the top and bottom gels



Triple-Matrix (Large Gap) - three adjacent touching gels, with a central gel creating a gap of ~1.5 mm between the top and bottom gels



Mematix - enables printing into a membrane insert to create an interface between cells and the surrounding environment



High Throughput - enables high throughout applications in a 384 well-plate format

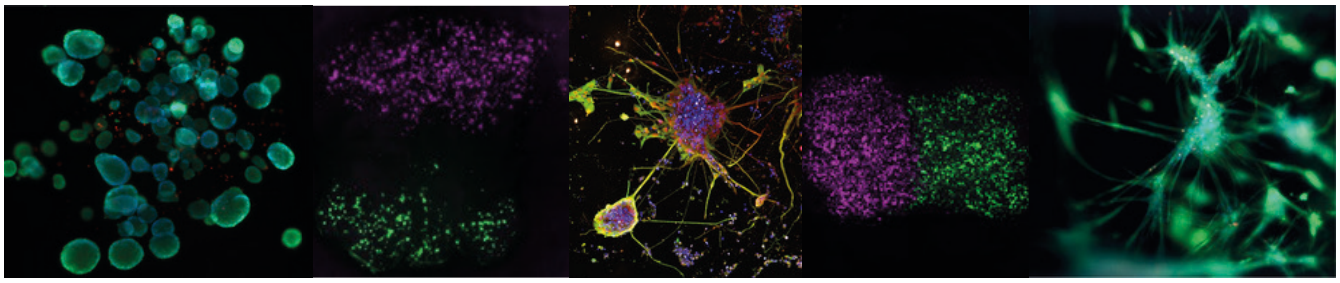
The RASTRUM™ Technology

RASTRUM™ Model Architecture

Characteristics of Various RASTRUM™ Advanced Models

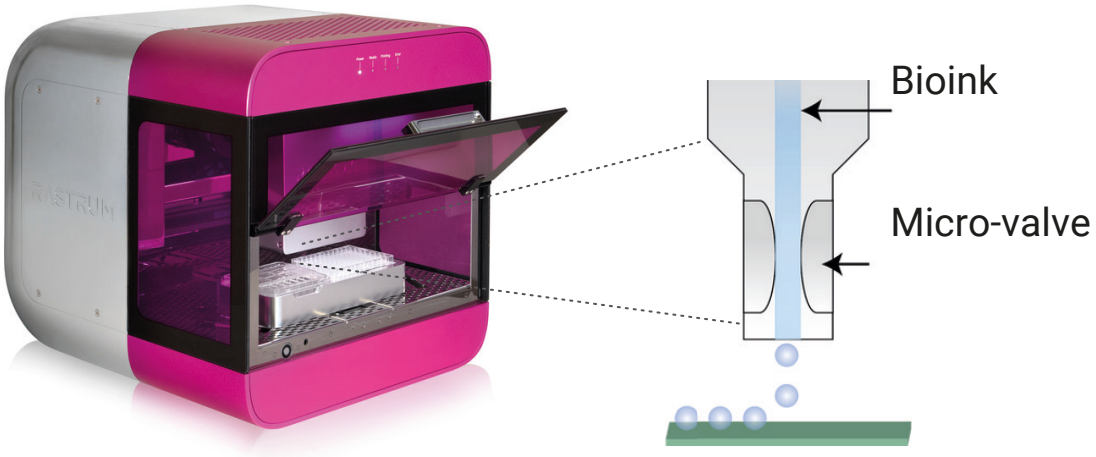
Model	Plate	Approx. gel volume (µl)	Gel height (mm)	Gel width (mm)	Matrix Cell Density* (M/mL)	Compatibility	Application
Imaging	96	1.1	0.5	2.2	20	All classes	Image-based analysis (Brightfield and fluorescent)
Large Plug	96	7.2	0.5	Full well	20	All classes	Proteomics, DNA / RNA seq, organoids / tumouroids, direct co-cultures
Immunohistochemistry	24	1.9	1.1	2,2	20	All classes	Histology, Spatial transcriptomics, Spacial biology
Dual-Matrix	96	1.9**	0.5	3.7-4.3	20	Px01, Px02	Migration / invasion, immuno-oncology, neurite outgrowth, co-cultures
Mematix	96	-	-	Full well	5	Px01	Chemotaxis, paracrine signalling, metabolic activity, invasion
Triple-Matrix - Small Gap ***	96	1.1	0.5	2.2	20	Px01, Px02****	Migration / invasion, immuno-oncology, neurite outgrowth, co-cultures, metastasis
Triple-Matrix - Large Gap	96	6.7	0.5	Full well	20	Px01, Px02, Px03****	Migration / invasion, immuno-oncology, neurite outgrowth, co-cultures Dose-
High Throughput	384	2.9	0.25	Full well	20	All classes	Response curves, IC50, High content screening

*Refers to maximum cell density value that is tested.
**Gel volume: 0.96 µL/environment.
***Gel volume: 0.24 - 0.48 - 0.24 µL/environment.
****The same matrix class in all environments.



The RASTRUM™ Technology

RASTRUM™ Platform



The RASTRUM instrument is based on cutting edge drop-on-demand technology which allows for very fast and precise model creation that is gentle on cells. The printing process occurs at a step change compared to manual methods and is compatible with standard microtiter well plates, including 96 and 384-well plates. RASTRUM™ Advanced Cell Models are built layer-by-layer when the two component matrix fluids come into contact with each other on the well plate surface. This enables the creation of simple or complex structures depending on the research application.

The drop-on-demand technology approach is akin to inkjet printing, whereby instead of depositing pixels of colour onto a page, the bioprinter deposits cells and matrix components onto the surface of a well plate, and builds these components layer-by-layer to form a desired 3D structure (5).

Inkjet bioprinting is a high-throughput and reproducible method for creating complex 3D cell models that would not be possible using manual methods or other 3D bioprinting technologies (9). Compatibility with a wide range of synthetic ECM materials ensures reproducibility of generated 3D cell models (10), and the ability to print multiple cell and matrix types as part of the same 3D structure enables a high level of customisation of the models that can be produced (11). These features, in addition to the high printing speed and cost-effectiveness of inkjet bioprinting, makes the technology amenable to upscaling, which supports its application in high-throughput drug screening. (12)

The RASTRUM™ Technology

RASTRUM™ Platform

The RASTRUM™ combines drop-on-demand technology with synthetic modifiable matrix systems to make the creation of matrix-embedded Advanced Cell Models simple, reproducible and efficient. Multiple extracellular matrix components are printed in a single pass of the printhead.

This multiplexing capability makes it quicker and more efficient to build complex 3D cell models. RASTRUM allows precise control over in-well placement of matrix components, spacing and volume of the ejected matrix (Figure 1). The 2-axis linear motion control system enables the placement of droplets at a resolution of 20 µm along each axis.

RASTRUM can also print sensitive cell types, such as neural progenitor cells (NPCs) with cells maintaining viability and their differentiation potential (Figure 2) after printing. These results highlight the ability of RASTRUM to generate 3D cell cultures without negatively affecting the viability of the cells during the printing process.

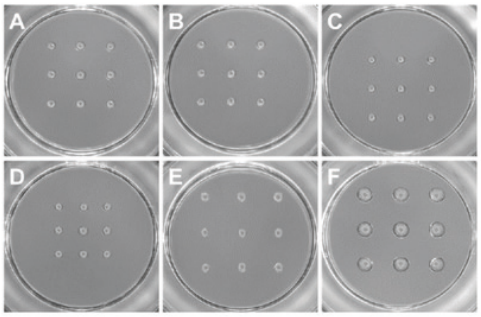


Figure 1: Demonstration of printing control using **RASTRUM**. Printed in a 96-well plate with an approximate area of 0.32cm . By changing the printing parameters, a series of bioink droplets printed in the centre of the well (A) can be shifted to the left (B), shifted downwards (C), printed closer together (D), printed farther apart (E), or printed at a greater volume (F).

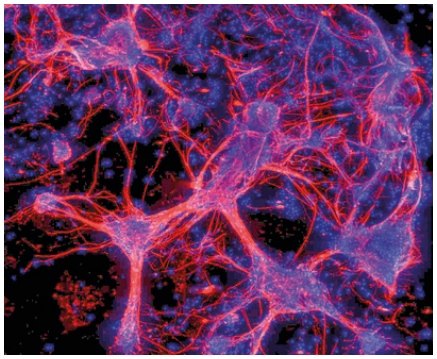


Figure 2: Immunofluorescent stain for BIII-tubulin and DAPI in neural progenitor cells printed in a 384-well plate using **RASTRUM**. Credit: Dr Chloe Whitehouse, Merck Sharp & Dohme (MSD).

The RASTRUM™ Technology

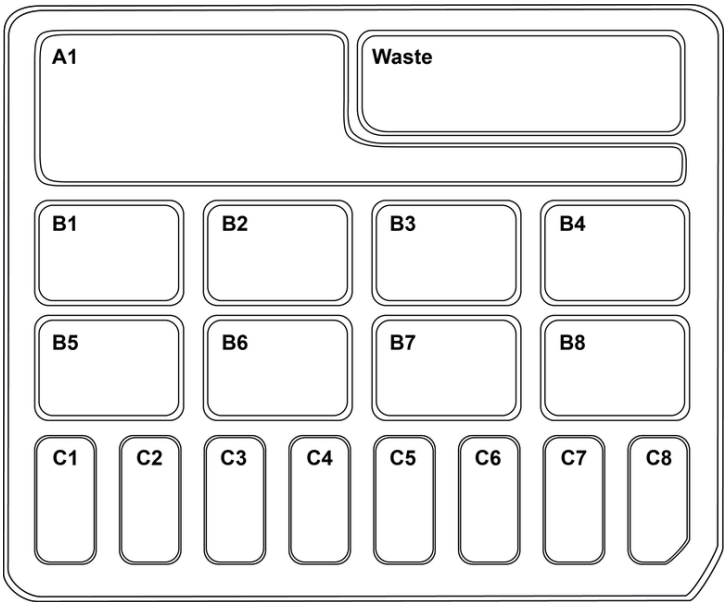
RASTRUM™ Consumables

RASTRUM™ Bioinks and Activators

All RASTRUM™ Bioinks and Activators are provided frozen, in a ready-to-use format.

RASTRUM™ Cartridges

The RASTRUM Cartridge is an all-in-one consumable that contains multiple reservoirs, with each reservoir serving a different purpose. All RASTRUM processes - from experimental to maintenance - can be executed with the RASTRUM Cartridge.



Compatible with standard 24, 96, 384 well plates (not provided by Inventia) - see Appendix A for a complete list of compatible plates

The RASTRUM™ Technology

RASTRUM™ Software

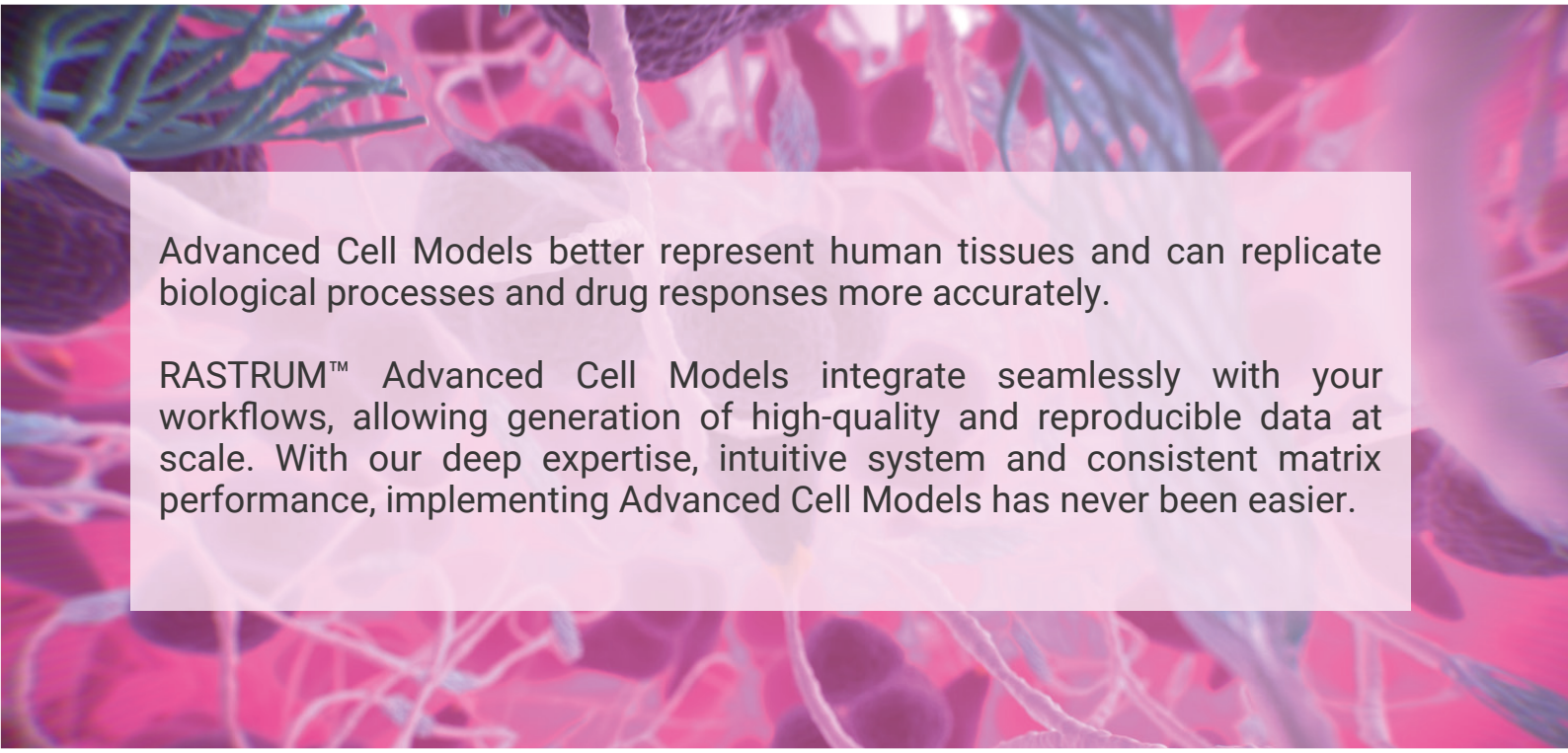
Included with the purchase of your RASTRUM™ Platform, RASTRUM Cloud and the RASTRUM App make designing & producing Advanced Cell Models easy. RASTRUM Cloud is a **no-code solution** that allows you to effortlessly design your RASTRUM Advanced Cell Model platform experiments. You can **fully customise** your PrintRuns by having control over your Well Plate configuration, Advanced Cell Models, RASTRUM Matrices and even more variables. Accessible online, the Cloud-enabled software means you can **plan your experiment from anywhere at any time.**

RASTRUM Cloud is used to design your Advanced Cell Models. Simply select your model, input your cell density and a protocol will be automatically generated.

Our RASTRUM App is the gateway to your RASTRUM Instrument. The RASTRUM App drives the execution of your designed PrintRun to generate plates of Advanced Cell Models using RASTRUM Platform.

The RASTRUM App monitors your RASTRUM Instrument health, notifies you if maintenance is needed, and allows you to conduct routine maintenance activities to ensure that your RASTRUM Instrument is in tip-top condition.

The RASTRUM™ Solution



Advanced Cell Models better represent human tissues and can replicate biological processes and drug responses more accurately.

RASTRUM™ Advanced Cell Models integrate seamlessly with your workflows, allowing generation of high-quality and reproducible data at scale. With our deep expertise, intuitive system and consistent matrix performance, implementing Advanced Cell Models has never been easier.

AUTOMATED & FAST

The automated platform is designed for speed and simplicity, which requires minimal user steps.

Our unique drop-on-demand technology allows the printhead to precisely deposit nanolitre droplets to accurately create complex structures at speed while being gentle on the cells.

BIOLOGICALLY RELEVANT

The library of synthetic, xeno-free Matrices are tunable in their stiffness and can be functionalised to accurately replicate the complex *in vivo* cellular microenvironment of any desired tissue.

REPRODUCIBLE & COMPATIBLE

RASTRUM enables you to create reproducible Advanced Cell Models.

With optically-clear and permeable matrix properties, RASTRUM Advanced Cell Models are compatible with standard readouts, imaging and end-point assays.

References

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Appendix A - Compatible Well Plates

RASTRUM™ Compatible Well Plates

24 Well Plates

- Corning #3524 - Corning 24 Well TC-Treated Micropplates
- Eppendorf #0030741005 - Eppendorf Cell Imaging Plates
- Greiner #662160 - Greiner CELLSTAR Multiwell Culture Plates
- ThermoFisher #142475 - Nunc™ MicroWell™ 24-Well, Nunclon Delta-Treated, Flat-Bottom Microplate

96 Well Plates

- Corning #CLS3904 - Corning 96 Well Black Polystyrene Microplate
- Corning#3599 - Corning 96-Well Clear Flat-Bottom Polystyrene TC-treated Microplate
- Corning #3595 - Corning® 96 Well TC-Treated Microplates
- Greiner #655180 - Greiner CELLSTAR® 96-Well Clear Flat-Bottom Polystyrene Cell Culture Microplate
- Greiner #655087 - Cell Culture Microplate, 96 Well, PS, F-Bottom (Chimney Well), µClear®, Black, CELLSTAR®, TC, Sterile
- PerkinElmer #6055300 - PhenoPlate™ 96-well Microplates, TC treated, Black, with lid.
- ThermoFisher #167008 - Nunc™ MicroWell™ 96-Well, Nunclon Delta-Treated, Flat-Bottom Microplate

384 Well Plates

- Corning #3764 - 384-Well TC-treated, Black Plate with Clear Flat Bottom, Low Flange with Lid
- Corning #3701 - 384-Well Clear Flat Bottom PS, TC-treated Microplates
- Corning #3985 - 384-Well, TC-treated, Black Plate with Thick, Clear Flat Bottom, with Lid
- Corning #3765 - 384-Well, TC-treated, White Plate with Clear Flat Bottom, Low Flange with Lid
- Greiner #781080 - Greiner BIO-ONE 384 Well Cell Culture Microplates, white
- Greiner #781086 - Greiner BIO-ONE 384 Well Cell Culture Microplates, black
- Greiner #781182 - Greiner BIO-ONE 384 Well Cell Culture Microplates, clear
- PerkinElmer #6057300 - PhenoPlate™ 384-well Microplates, TC-treated,, Black, With Lid, Case of 50

Well Inserts

- Corning #3384 - HTS Transwell®-96 Permeable Support with 8.0 µm Pore Polyester Membrane, 8 per Case, Sterile

Appendix B - Compatible Workflows

RASTRUM™ Compatible Workflow Examples

Advanced Cell Models produced by the RASTRUM™ platform can be utilised in many downstream applications, integrating seamlessly into existing workflows.

Real Time Analysis

Optical - Fluorescence or Brightfield Imaging
Observing motility, quantity, dimension / shape.

Chemical - Electrophysiology, Plate Reader, Assays
Observing marker expression (dye or antibody), Monitoring cell proliferation, cell viability, released substances e.g. ECM remodeling, calcium signalling

Endpoint Analysis

Optical - Fluorescence or Brightfield Imaging
Same methods as for "Real Time Analysis", using reagents that are destructive, e.g. antibody staining, live / dead analysis.

Chemical - In Situ
Same readouts as for "Real Time Analysis", before or without retrieving cells from the RASTRUM™ Matrix

Chemical - Analysis after Cell Retrieval
Sample from within the well - e.g. AlphaLISA
Protein Quantification - e.g. Antibody-based (such as Western Blot, ELISA)
RNA Quantification - e.g. qPCR
Single Cell Methods

Chemical - Tissue
Immunohistochemistry of culture slices

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