

## Brightfield and Fluorescent Imaging of RASTRUM 3D Bioprinted Cell Models

### Highlights

Synthetic extracellular matrices printed using the RASTRUM™ 3D bioprinting platform enable *in situ* imaging of cells within the 3D hydrogel structure for compatibility with:

- Brightfield microscopy for clear morphology
- Fluorescence microscopy without autofluorescent interference
- Small molecule and antibody-based staining by commonly used techniques
- High-throughput imaging analysis in standard well plate formats

### Introduction

While 3D matrix-embedded cell models are more physiologically relevant than 2D cell cultures,<sup>1</sup> the generation of 3D cell models via manual methods is time-consuming and low-throughput.<sup>2</sup> Many currently used models have low reproducibility due to the inherent batch-to-batch variation of animal-derived matrices.<sup>3-4</sup> Furthermore, a number of currently used matrices for *in vitro* culture, such as Matrigel® and polymer scaffolds, are plagued by background autofluorescence or loss of image resolution with hydrogel thicknesses greater than 100 µm.<sup>5-6</sup>

RASTRUM combines drop-on-demand bioprinting with synthetic modifiable matrix systems to make the creation of matrix-embedded cell cultures simple, reproducible and efficient. This application note will demonstrate the compatibility of imaging methods with 3D cell models created using RASTRUM.

### Methods

#### 3D Bioprinting

MCF-7 (human breast cancer) or NHDF (normal human dermal fibroblast) cells were resuspended in activator F41 at a cell density of  $5 \times 10^6$  cells/mL or  $1 \times 10^7$  cells/mL, respectively. Bioink F38 or F93 (for MCF-7 and NHDF, respectively) and cell-activator solution were printed into a 96-well plate to form uniform hydrogel structures in every well. Layer-by-layer printing resulted in 3D cell models with cells evenly distributed throughout the structure (Figure 1).

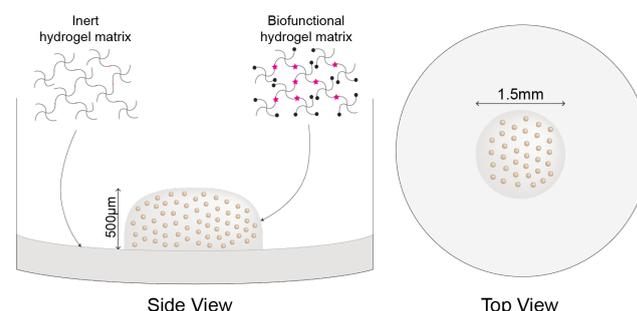


Figure 1: Representation of the RASTRUM 3D bioprinted hydrogel structure containing cells (figure not to scale).

#### Live/Dead Assay

A Live/Dead cell viability assay was performed 7 days post-printing by incubating cells with 1 µM Calcein-AM (Biotium) and 2 µM Ethidium Homodimer-III (Biotium) at 37°C for 30 minutes. Brightfield and fluorescent images of live cells were captured as z-stacks using an Observer 7 fluorescence microscope (Zeiss).

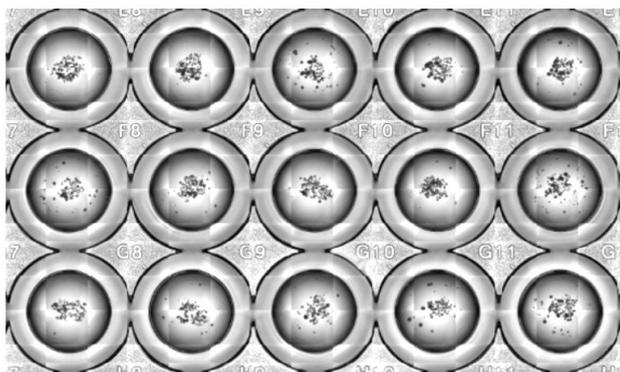
#### Antibody Staining

Matrix-embedded cells were fixed with 4% paraformaldehyde 7 days post-printing. Cells were permeabilised with 0.1% Triton-X-100 for

30 minutes at room temperature and incubated with 10% goat serum for 30 minutes at room temperature to block non-specific antibody binding. Anti-GAPDH or anti-B-actin primary antibodies (Abcam) were added at a 1:400 dilution in 0.1% BSA and incubated at 4°C for 12 hours. Gels were washed three times with PBS before the addition of secondary antibody (IgG-633 or IgG-488, Thermo Fisher Scientific) diluted 1:1000 in 0.2% BSA and incubation at 4°C for 12 hours. Gels were washed three times with PBS, followed by the addition of 1  $\mu$ M Hoechst 33342 (Invitrogen) and incubation for 15 minutes at room temperature prior to imaging. Brightfield and fluorescent images of fixed cells were captured as z-stacks using an Observer 7 fluorescence microscope (Zeiss).

## Results and Discussion

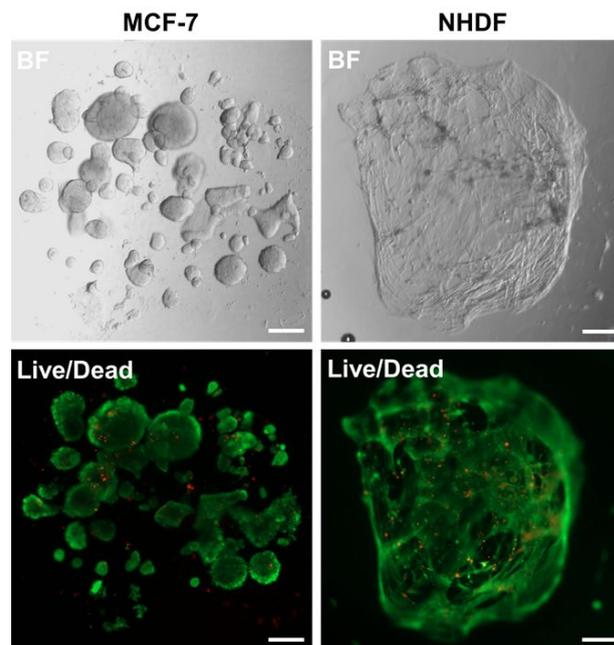
Hydrogel structures were printed in the centre of each well of the 96-well plate. Reproducible printing of the hydrogel structures allowed for high-throughput imaging with consistent X/Y positions and Z-plane focus ranges for each well across the 96-well plate (**Figure 2**). Microwell structures printed using **RASTRUM** are compatible with most imaging platforms that accommodate well plate formats.



**Figure 2:** Representative brightfield images of MCF-7 spheroids encapsulated within **RASTRUM** hydrogels 7 days post-printing.

MCF-7 and NHDF cells were evenly dispersed throughout the hydrogels and proliferated to form spheroid and network-like structures, respectively. This indicates that the hydrogel matrices are able to support cell viability and growth post-printing over 7 days of culture (**Figure 3**). Live/Dead cell markers were able to penetrate the hydrogel matrices for *in situ*

staining and live cell imaging of spheroid and cell network structures, using standard imaging techniques used for 2D cell cultures, without background autofluorescent interference.

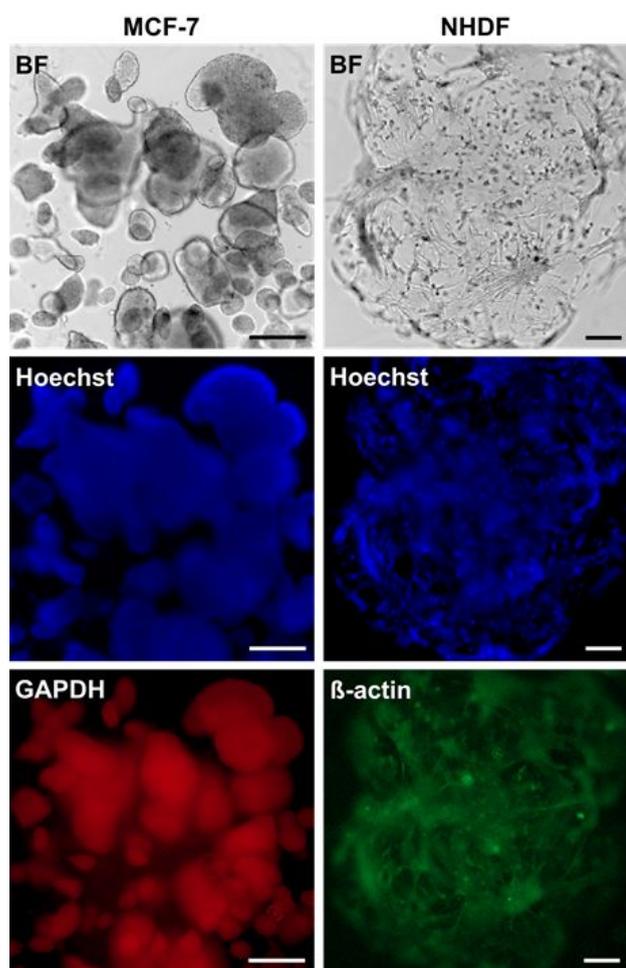


**Figure 3:** Representative brightfield (BF) and Live/Dead images of MCF-7 and NHDF cells encapsulated in the **RASTRUM** hydrogels after 7 days in culture. Scale bars are 200  $\mu$ m.

**RASTRUM** hydrogel matrices supported a two-step antibody staining protocol, in which a fluorescently tagged secondary antibody was used for visualisation. This indicates that both primary and secondary antibodies can diffuse into the hydrogel matrix and that antibody staining is not lost during the wash steps. Furthermore, the synthetic **RASTRUM** hydrogels enabled fluorescently-tagged secondary antibodies to bind cellular targets without cross-reactivity or background autofluorescence attributed to the matrix (**Figure 4**).

The ease of both small-molecule and, particularly, antibody-based staining in these 3D cell models is likely due to a combination of factors. The controlled hydrogel matrix formulation does not autofluoresce or exhibit non-specific binding. Additionally, the printed cell models are only  $\sim$ 300 nL in total volume, compared to typically  $\sim$ 20  $\mu$ L plugs used for Matrigel<sup>®</sup> and other hydrogel systems, meaning

that reagent and antibody diffusion throughout the 3D structure is very efficient.



**Figure 4:** Representative brightfield (BF), Hoechst and GAPDH or  $\beta$ -actin antibody staining images of MCF-7 and NHDF cells encapsulated in **RASTRUM** hydrogel matrices 7 days post-printing. Scale bars are 200  $\mu$ m.

## Summary and Conclusions

In this application note, we have shown that **RASTRUM**-generated 3D cell models are compatible with a range of commonly used imaging methods, allowing for high-throughput *in situ* cell visualisation using standard immunostaining techniques. Therefore, **RASTRUM** can be used in the high-throughput formation and analysis of matrix-embedded cell models for various biological research applications.

## References

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## Contact Us

**Inventia Life Science Pty Ltd**

[www.inventia.life](http://www.inventia.life)

### **Australia**

Suite 1.13 / 90-96 Bourke Road

Alexandria NSW 2015

Telephone: 1800 849 128

Fax: +61 2 8399 0304

Email: [info@inventia.life](mailto:info@inventia.life)

### **US**

Telephone: +1 833 462 5959

### **Ireland**

Telephone: +353 818 370 035

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