Drop-on-Demand Printing Technology of the RAISTRUM 3D Bioprinter

Highlights
The drop-on-demand technology of the RAISTRUM™ 3D bioprinter enables:

- Quick and efficient printing of matrix components and cells
- Precise control of droplet deposition to build complex 3D structures
- Preservation of cell viability post-printing

RAISTRUM combines drop-on-demand bioprinting with synthetic modifiable matrix systems to make the creation of matrix-embedded 3D cell models simple, reproducible and efficient. This application note will detail the key printing features offered by RAISTRUM.

Introduction
Matrix-embedded 3D cell models are more physiologically relevant than 2D cultures. Despite their utility, the generation of 3D cell models via manual methods is time-consuming and low-throughput. 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. 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 matrix components.

The drop-on-demand bioprinting 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. Notably, drop-on-demand bioprinting 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.

RAISTRUM makes it possible to print multiple matrix components in a single pass of the printhead. This multiplexing capability makes it quicker and more efficient to build complex 3D cell models. RAISTRUM allows precise control over in-well placement of matrix components, spacing and volume of the ejected matrix (Figure 2). The 2-axis linear motion control

Figure 1: Representative droplet visualisation images showing bioink droplet formation after ejection from a RAISTRUM printer nozzle.

Figure 2: The 2-axis linear motion control
system enables the placement of droplets at a resolution of 20 μm along each axis.

**Figure 2:** Demonstration of printing control using RASTRUM. 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).

### Printing 3D Hydrogel Structures

**RASTRUM** creates 3D structures by printing tiny droplets of a PEG-based bioink and an activator solution from separate nozzles, which combine to instantly form a hydrogel at room temperature (**Figure 3**).

**Figure 3:** Drop-on-drop gel formation using RASTRUM. Bioink and activator droplets combine to instantly form a hydrogel structure.

By precisely controlling the deposition of matrix components onto the surface of a well plate, **RASTRUM** can be used to build complex 3D structures. An example of a commonly printed structure containing cells is shown below (**Figure 4**). The small hydrogel plug structure has a volume of approximately 300 nL and can be printed into a 96-well plate, with the whole plate printed in less than 10 minutes. For further information about this 3D cell model, see our application note ‘High-Throughput Production of **Matrix-Embedded Multicellular Tumour Spheroids by Drop-on-Demand 3D Bioprinting’.

**Figure 4:** Consistent printing of small hydrogel structures into the wells of a 96-well plate using RASTRUM.

### Printing Cells

The deposition of matrix components by **RASTRUM** is not only precise, but ejection from the printer is also gentle on cells. Various cell types have been successfully printed using **RASTRUM**, including a variety of established cancer cell lines, keratinocytes, dendritic cells, primary cells lines and patient-derived cancer cells. The viability of SK-N-BE(2) (neuroblastoma), H460 (lung cancer) and U87vIII (glioblastoma) cells before and after printing is shown below (**Figure 5**).

**Figure 5:** Viability of SK-N-BE(2), H460 and U87vIII cells before and after printing using RASTRUM, measured via Trypan Blue exclusion. Data are presented as the mean ± standard deviation (n = 3 per group).
RASTRUM can also print sensitive cell types, such as induced pluripotent stem cells (iPSCs), with cells maintaining viability and pluripotency markers after printing (Figure 6). 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 6: iPSCs maintain viability and pluripotency markers after printing with RASTRUM onto a layer of Matrigel™ and culturing for 48 hours. Confocal image shows cells stained for pluripotency markers OCT4 and SSEA-4.

**Summary and Conclusions**

In this application note, we have highlighted the ability of the RASTRUM 3D bioprinter to enable precise droplet control of matrix components and gentle deposition of cells. This demonstrates the utility of RASTRUM in the high-throughput bioprinting of hydrogel structures and 3D cell models for research applications.

**References**

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