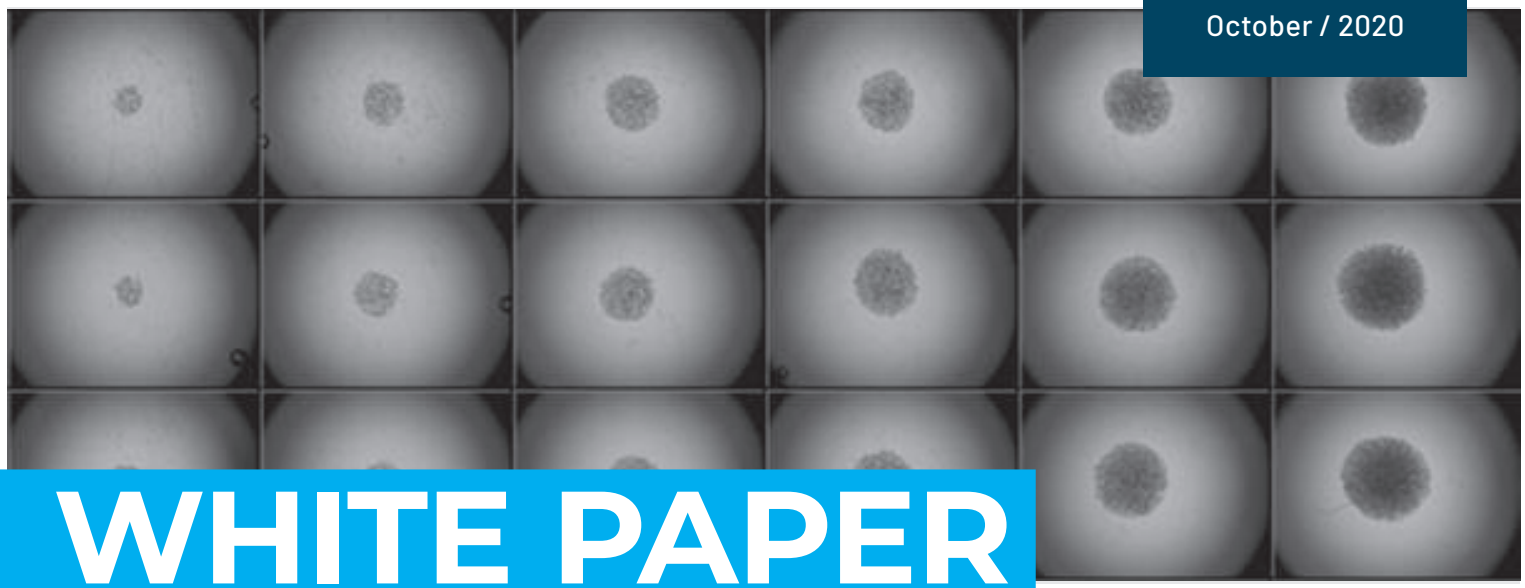
October / 2020



WHITE PAPER

TECHNICAL NOTES & APPLICATIONS FOR LABORATORY WORK

MAGNETIC 3D BIOPRINTING - HIGH-THROUGHPUT SPHEROID IMAGING AND ANALYSIS USING PERKINELMER ENSIGHT™ MULTIMODE PLATE READER

CONTENT

1/ INTRODUCTION

The main flaw of traditional cell culture methods is the use of two-dimensional (2D) monolayers of cells, which are not a good representation of *in vivo* tissue environments. Today, there are various options for growing cells in 3D, including round-bottom surfaces, hydrogels or scaffold-based methods. However, for 3D cell culture to be assimilated at a faster rate by laboratories, there is a need for tools that enable 3D cell culture to be performed using routine 2D cell culture workflows. E.g. Tasks such as media exchanges and imaging cells can be challenging when working with 3D cell culture because cells are neither attached to plastic or grown on a flat surface. Magnetic 3D cell culture is the solution to perform 3D cell culture as easily as in 2D.

2/ GREINER BIO-ONE MAGNETIC 3D BIOPRINTING TECHNOLOGY

The principle of magnetic 3D bioprinting relies on the magnetization of cells with NanoShuttle™-PL, a biocompatible nanoparticle assembly of gold nanoparticles, ironoxide, and poly-L-lysine (PLL). The NanoShuttle™-PL magnetizes cells by electrostatically attaching to the cellmembranes via PLL. The reproducible formation of a single spheroid per well in an F-bottom plate with cell-repellent surface is induced by the magnetic forces of one magnet below each well within 15 min (Fig. 1). Then, spheroids are generally formed within hours, depending on the cell type. These structurally and biologically representative 3D models formed *in vitro* are ready to use for various downstream experiments to facilitate continuous assessment of cell viability and other functions.

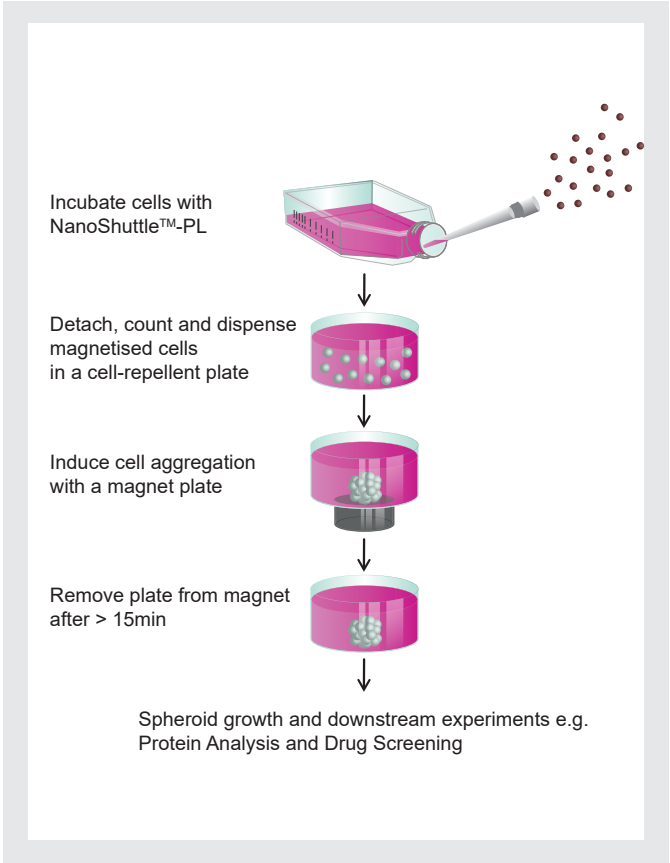


Figure 1: Magnetic 3D bioprinting process. Cells are magnetized overnight with NanoShuttle™-PL (NS), resuspended in media, and added to a cell repellent microplate. By placing this plate on top of a magnet plate, cells aggregate at the bottom of the flat well within 15 min to a few hours to form spheroids.

3/ PERKINELMER ENSIGHT™ PLATE READER

The PerkinElmer EnSight™ multimode plate reader (Fig. 2) is capable of rapid well imaging alongside conventional multimode detection technologies, including ultra-sensitive luminescence. The instrument is modular and can be equipped with up to 6 different detection technologies (plus Epic® label free outside Europe). Microwell image acquisition and analysis are fast: a 96 or 384-well plate can be imaged and analyzed in less than 5 minutes. For optimal imaging, this instrument requires flat surfaces. Hence magnetic 3D culture in flat bottom plates assures ideal imaging conditions.

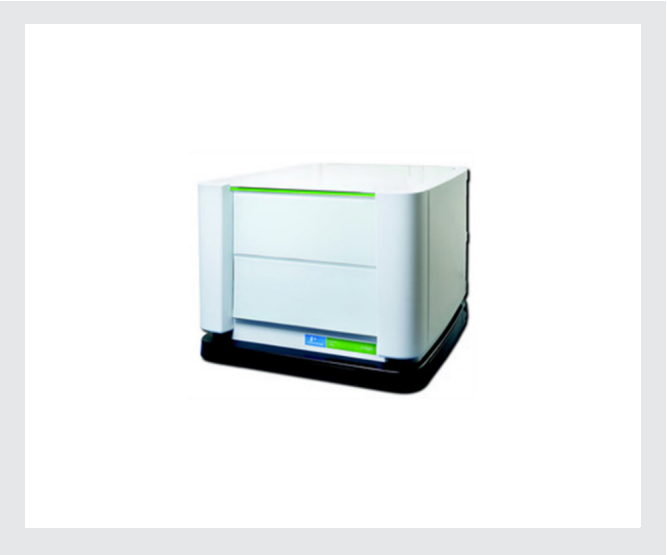


Figure 2: PerkinElmer EnSight™ multimode plate reader

4/ EXPERIMENTAL RESULTS

4.1/ REPRODUCIABLE SPHEROID SIZE WITH DIFFERENT CELL LINES

Two different human cancer cell lines, pancreatic cancer (Panc1) and ovarian cancer (A2870), were seeded in a 96-well µClear cell-repellent microplate. After magnetic 3D Bioprinting, EnSight™ was used to detect the size and number of spheroids/well, using automated image analysis (Fig. 3).

Spheroids are easy to hold down with magnetic forces while adding or removing liquids, and the magnetic forces allow centering of the spheroids in the middle of each well. Fig. 3 also shows the consistency in size obtained by seeding 6 increasing cell concentrations (from 2.5K to 50K per well). Reproducible spheroid sizes and one spheroid per well were obtained, due to cells forming spheroids around a fixed magnetic field (Fig. 3 and 4).

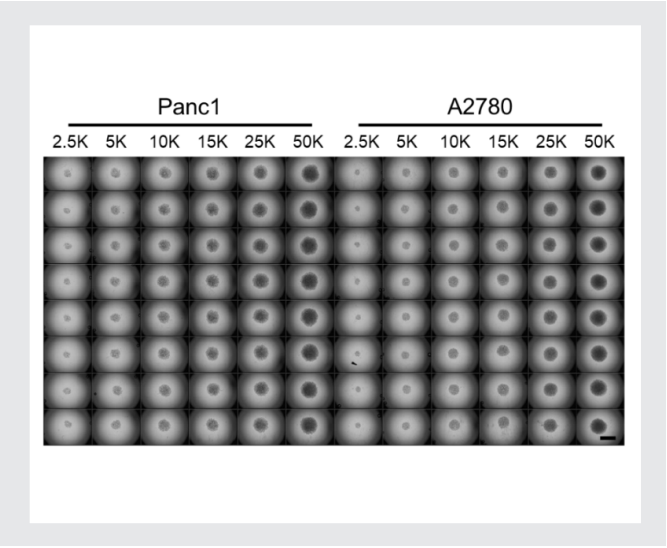


Figure 3: Images acquired with EnSight™ of pancreatic cancer (Panc1) and ovarian cancer (A2870) spheroids printed at varying cell numbers (2,500 to 50,000 cells). Images were captured 24 hours after printing. (Scale bar = 2 mm).

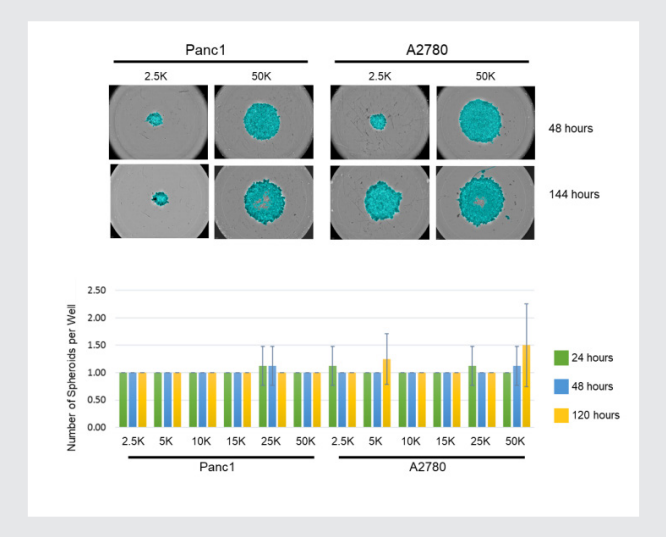


Figure 4: Image analysis showing single spheroid per well at 24 hours (green), 48 hours (blue), and 120 hours (yellow) of culture. Error bars represent the standard deviation of n=8 samples.

4.2/ SPHEROID GROWTH AND ANALYSIS

A2780 spheroid diameter changes at a faster rate than Panc1 over 120 hours (Figures 5). The number of cells influenced the growth rate of the two cell lines in 3D cultures.

A2780 3D cultures with lower cell numbers (2.5K to 15K cells) showed faster growth rate over 120 hours when compared to cultures with higher cell numbers (25K and 50K cells; Fig. 5).

Panc1 did show significant change in diameter and cultures seem to become darker over time. The darker appearance of these cultures at 120 hours could suggest the increased density of the 3D cultures, change in spheroid organization, and/or viability of the 3D cultures (Fig. 5). Viability of 3D cultures can also be assessed using luminescence assays, such as Perkin Elmer's ATPlite. Luminescence measurements can be performed with the EnSight when equipped with the ultra-sensitive luminescence module (data not shown).

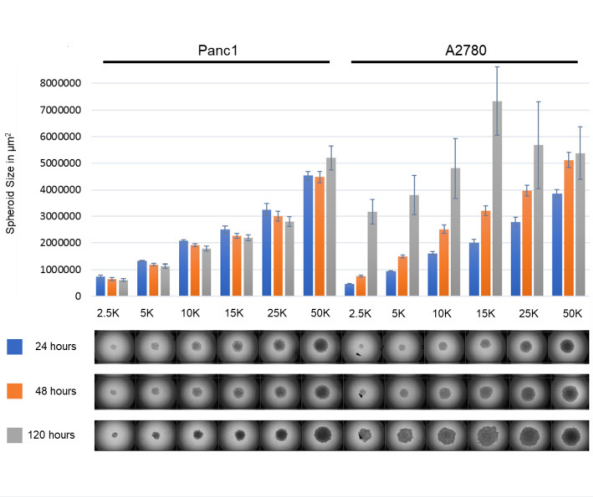


Figure 5: Spheroid size measured and analyzed with EnSight™ as a function of time (blue - 24 hours, orange – 48 hours, and gray – 120 hours).

5/ ACKNOWLEDGEMENT

We want to acknowledge Prof. Pizzimenti and her laboratory members (Dep. Clinical and Biological Sciences, Corso Raffaello 30, University of Turin, Italy) for scientific discussion, and for kindly providing cells and laboratory resources to conduct these experiments.

PRODUCT & ORDERING INFORMATION

- 3D cell culture formation within 24 hours for most cell types in a fast and easy 2D workflow
- Reproducible one spheroid per well formation
- Performed on a flat surface optimal for high-resolution microscopy and HTS
- Easy media changes and co-culture of different cell types

Greiner Bio-One

Scalable technology to form spheriods from 6 Well up to 1536 Well

Item No.	Description
655841	96 Well Bioprinting Kit black µClear®
781841	384 Well Bioprinting Kit black µClear®
657841	NanoShuttle™-PL Refill 1 Pack
657843	NanoShuttle™-PL Refill 3 Pack
657846	NanoShuttle™-PL Refill 6 Pack
657852	NanoShuttle™-PL Refill 12 Pack
655976	96 Well Cell-Repellent Plate, PS, sterile, flat bottom, black µClear, with lid, 8 pcs./bag, 32 pcs./case
781976	384 Well Cell-Repellent Plate, PS, sterile, flat bottom, black µClear, with lid, 8 pcs./bag, 32 pcs./case

PerkinElmer

www.perkinelmer.com

Item No.	Description
HH34000000	EnSight™ base unit, including filter-based absorbance
HH34000074	Well imaging module for brightfield and fluorescence imaging (4 LEDs)
6066736	ATPlite 1Step for proliferation / toxicity assays assays on 3D spheroids
6066943	ATPlite 3D, for proliferation / toxicity assays on 3D spheroids

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