Spheroids *versus* Scaffolds: development of 3D liver cultures

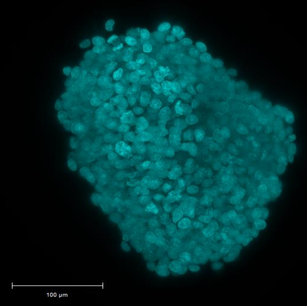
Jonathan Temple, Raphaël Lévy, Gary Allenby
Department of Biochemistry, Institute for Integrated Biology, University of Liverpool L69 7ZB (j.temple@liverpool.ac.uk)

Why 3D culture models?

- Better represent the *in-vivo* environment by exposing the whole surface of the cell, increasing the activation of signalling pathways, as well as other cellular processes.
- Drug induced liver injury is a common adverse drug reaction that is costly for both the health and pharmaceutical industries. There is therefore, a large demand for an *in-vitro* system that better predicts drug toxicity.
- Scaffolds are Mimetix® scaffold made from Poly-L-lactic acid (PLLA) and cut to 1000 µm x 50 µm with 4 µm diameter fibres and an overall porosity of app. 80%.
- Here we use stably transduced HepG2 cells which express a fluorescent marker in the nucleus (RFP-H2B) and express Luciferase enzyme to compare two forms of 3D cell culture to determine which, if any, have improved function and enable better predictivity of the toxicity of drugs and other compounds.

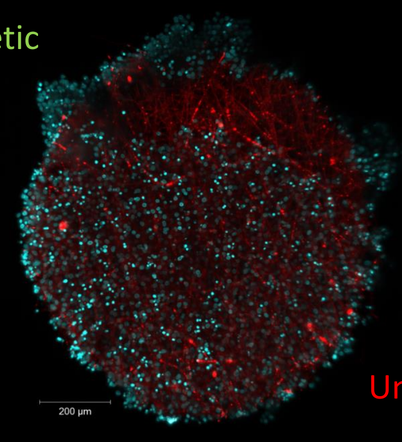
Spheroids

Cheap
Easy to culture
Hypoxic/necrotic core
Produce own ECM
Imaging difficulties
Mimic solid tissue and avascular tumours
Low porosity
High-throughput
Uniform
Poor mechanical stability



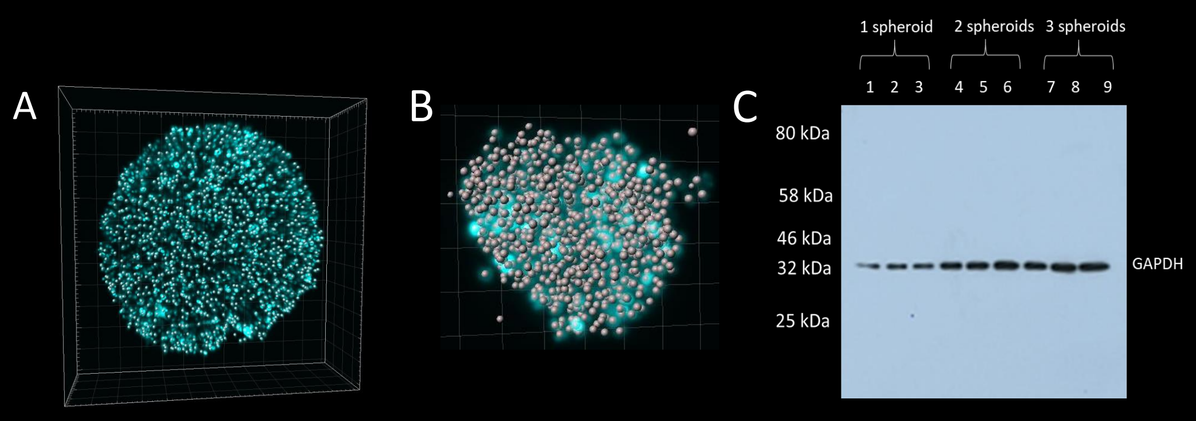
Scaffolds

High porosity
Magnetic
Fluorescent
Mechanically stable
Customisable
Manufactured from a variety of materials
Highly variable
Difficult to culture
Interfere with analysis/imaging
Cells truly in 3D?
Uneven cell distribution

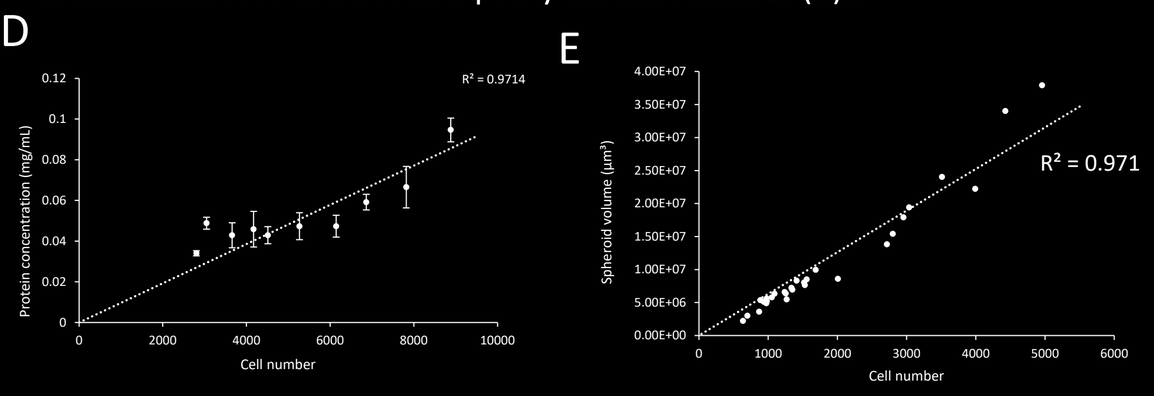


The Quantification Challenge

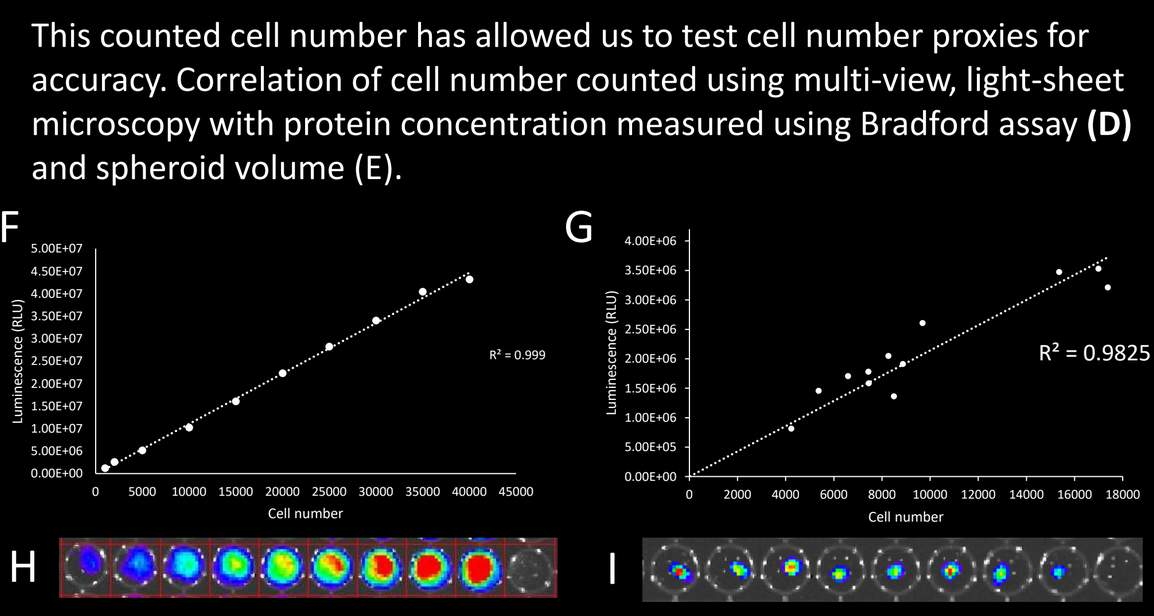
One of the largest problems that face 3D cultures comes when performing and normalising biochemical readouts. Several normalisation methods are being implemented in the field with many papers using a cell number proxy like total protein/DNA. Here we use light-sheet imaging to allow us to image deep within the 3D cultures and count the number of cells.



Counting cells grown in 3D cultures using IMARIS; Scaffold **(A),** Spheroid **(B)** Western blot for GAPDH as a proxy for cell number (C) **.**



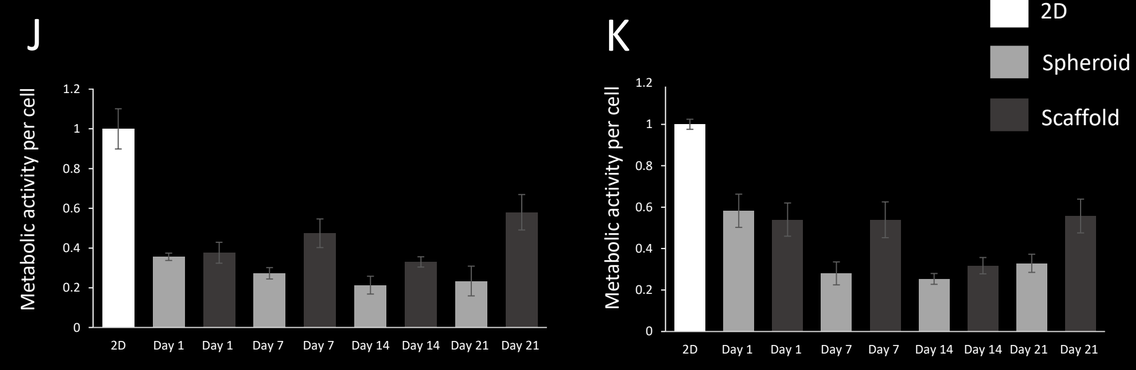
This counted cell number has allowed us to test cell number proxies for accuracy. Correlation of cell number counted using multi-view, light-sheet microscopy with protein concentration measured using Bradford assay **(D)** and spheroid volume (E).



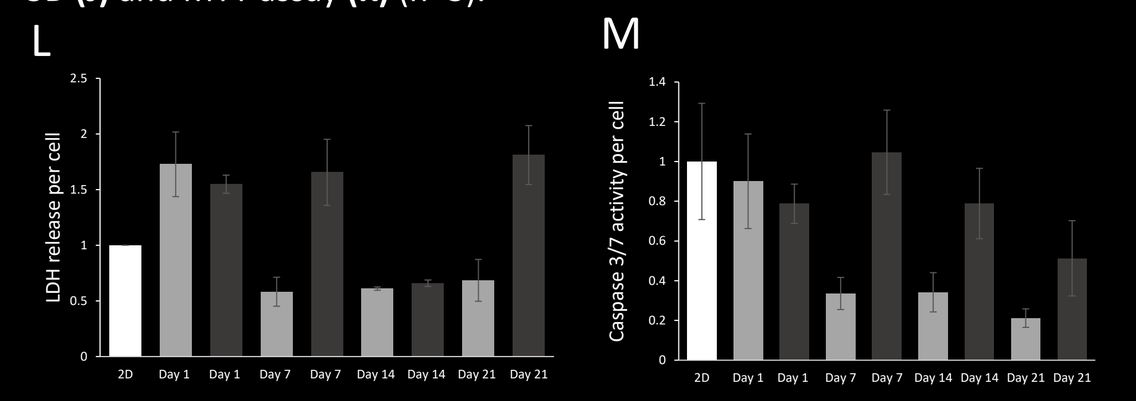
Correlation of cell number counted using multi-view, light-sheet microscopy with the luminescence values of the exogenous luciferase for cells in 2D **(F)** and the two 3D cultures **(G)** recorded using luminescent imaging **(H, I)**.

Biochemical characterisation

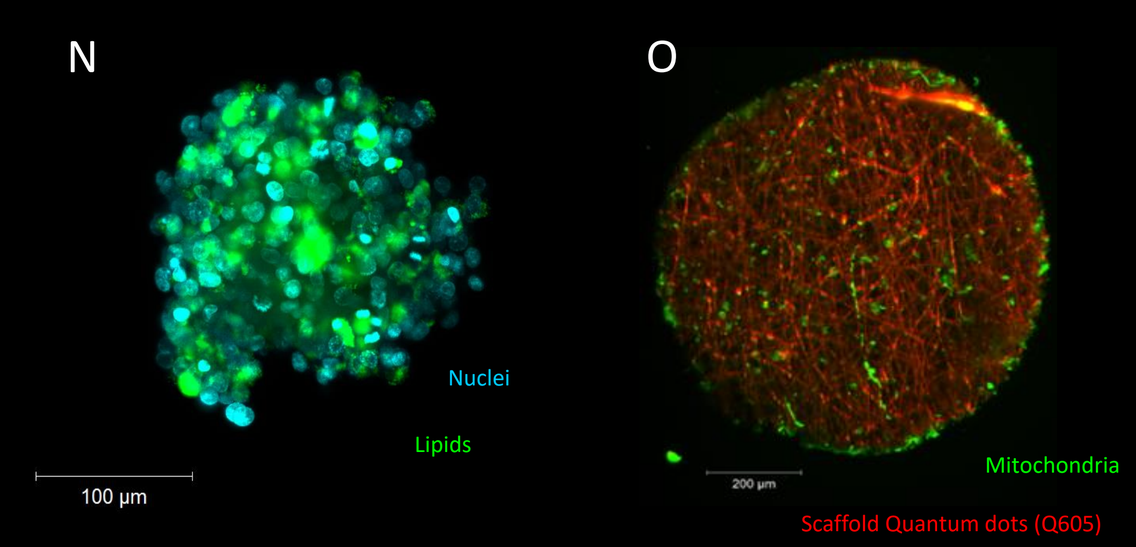
Exogenous luminescence is an accurate and non-destructive cell number proxy that allows us to measure multiple readouts from the 3D cell cultures normalised to the number of cells. This allows us to compare cellular processes between the different culture types.



Metabolic activity per cell normalised to 2D. Cell number was measured using luminescence and metabolic activity was measured using Cell-titre Glo ® 3D **(J)** and MTT assay **(K)** (n=3).



Markers for cell damage and death also normalised per cell. Measured using LDH assay **(L)** and Caspase-Glo ® 3/7 3D **(M) (n=3).**



Light sheet microscopy allows us to quantify the 3D volumes of different fluorescent markers giving indications on function including lipid production **(N)** and mitochondrial potential **(O).**

BBSRC
aurelia bioscience
ELECTROSPINNING COMPANY
UNIVERSITY OF LIVERPOOL