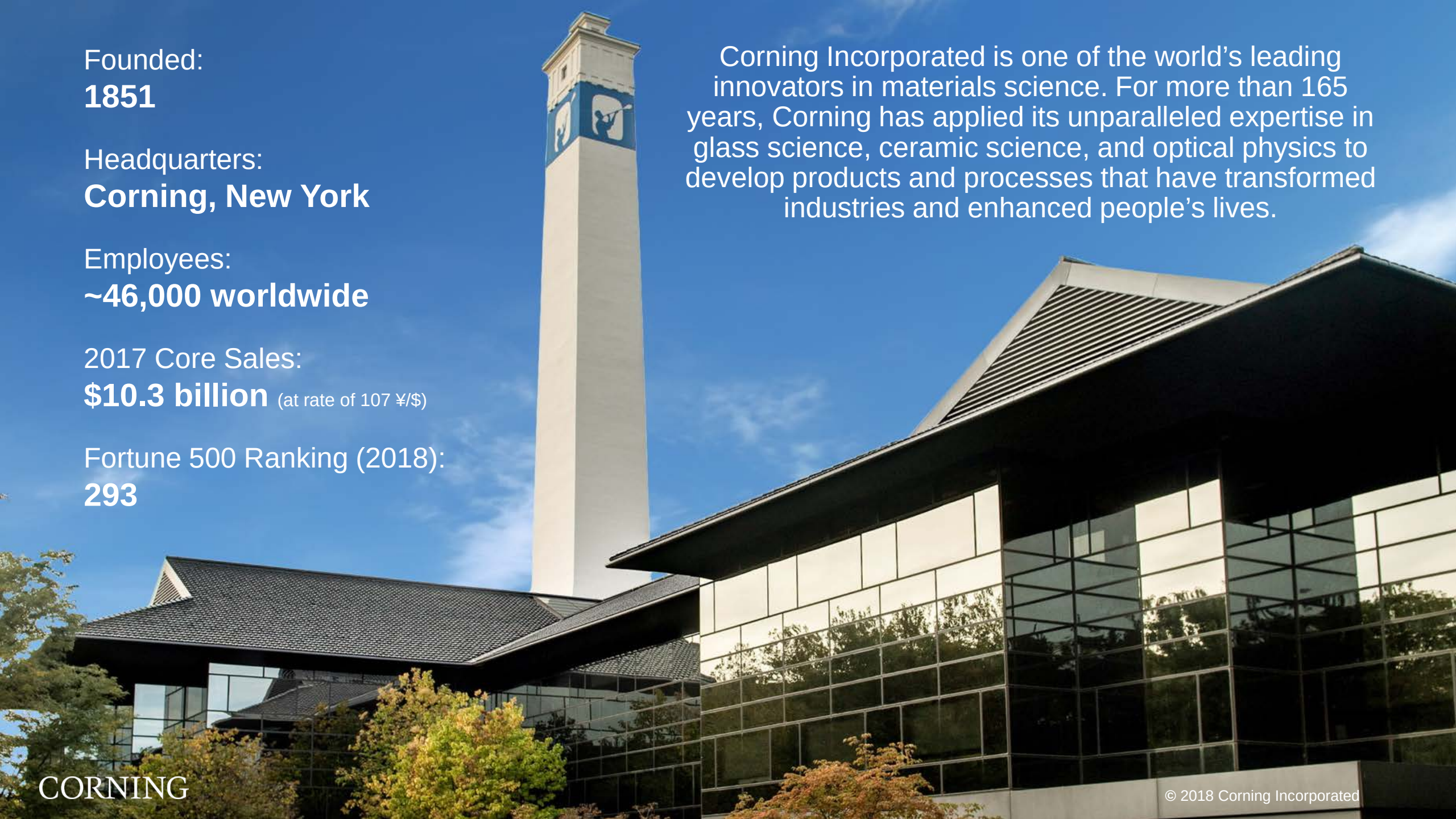
CORNING

Advances in three-dimensional cell culture in drug research, discovery and biologic manufacture

Richard M. Eglen, PhD
VP & GM
Corning Life Sciences



Founded:
1851

Headquarters:
Corning, New York

Employees:
~46,000 worldwide

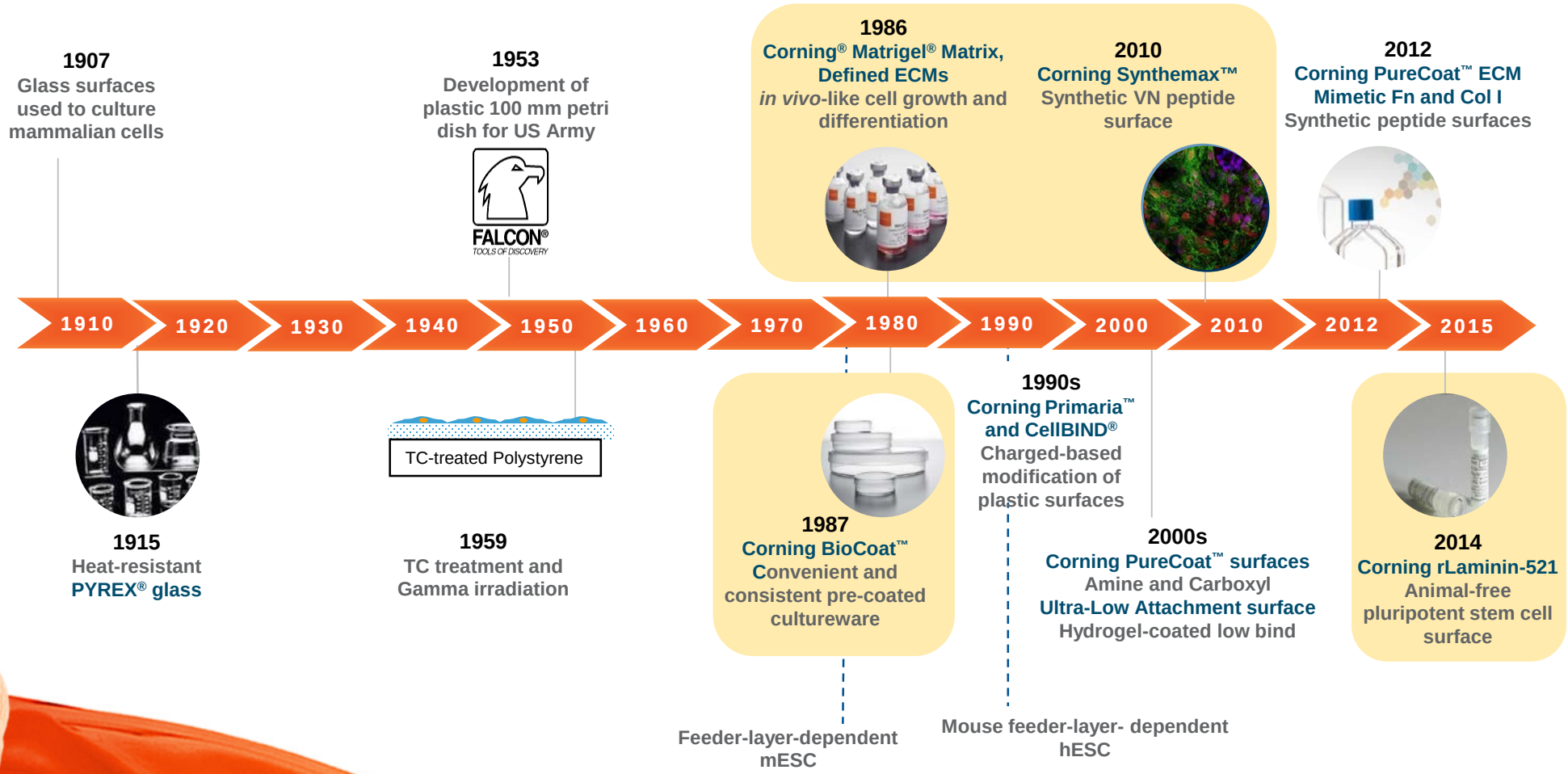
2017 Core Sales:
$10.3 billion (at rate of 107 ¥/$)

Fortune 500 Ranking (2018):
293

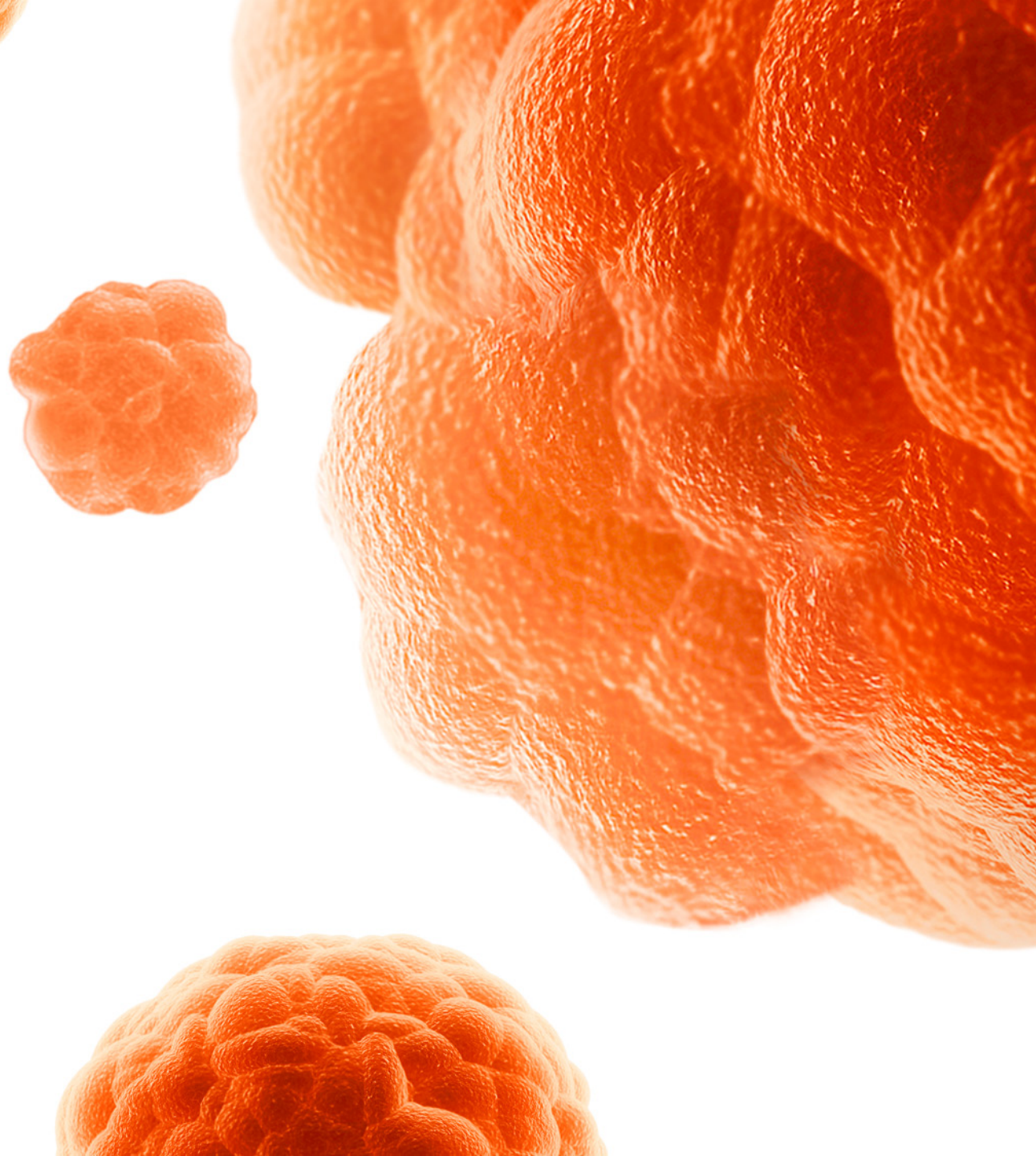
Corning Incorporated is one of the world's leading innovators in materials science. For more than 165 years, Corning has applied its unparalleled expertise in glass science, ceramic science, and optical physics to develop products and processes that have transformed industries and enhanced people's lives.

CORNING

We have a rich history of driving innovation in cell culture surfaces, all critical to *ex vivo* cell growth

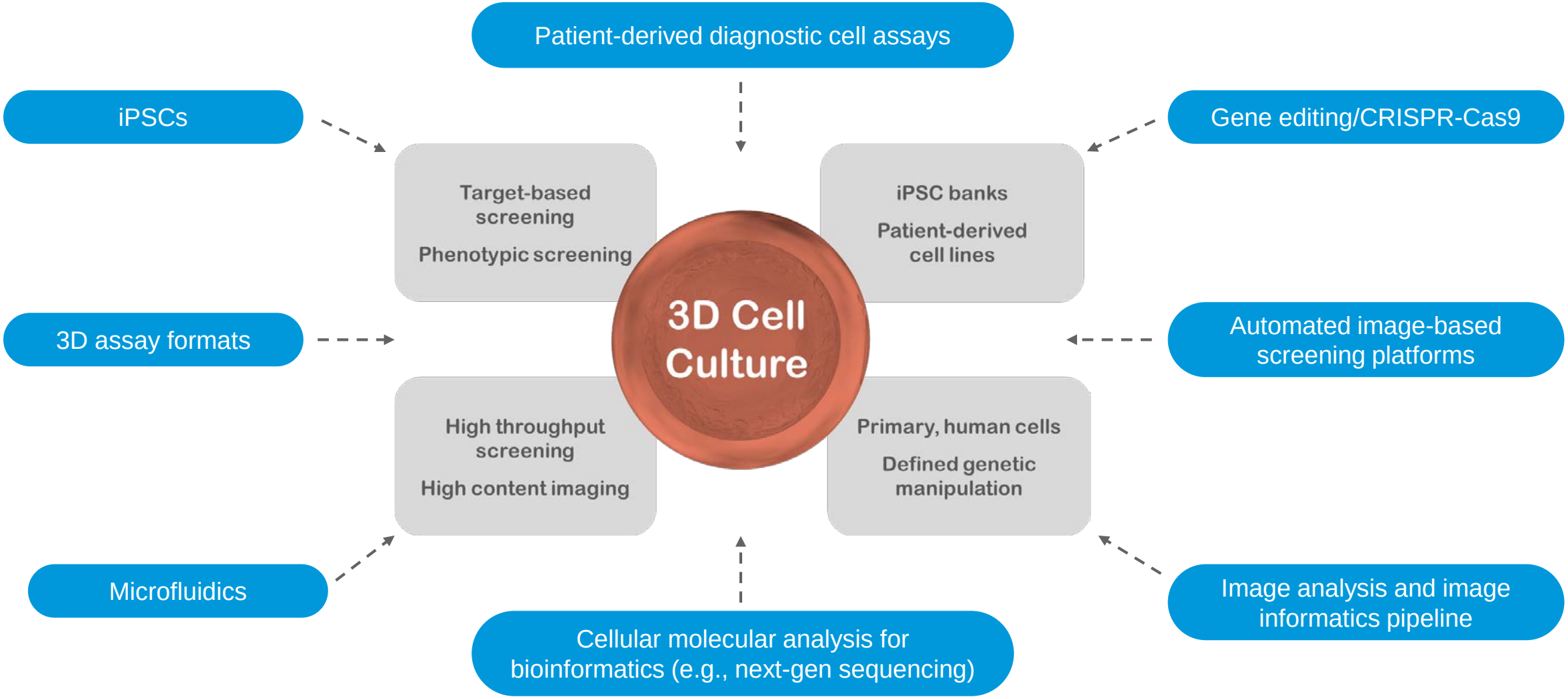


Overview of 3D cell culture



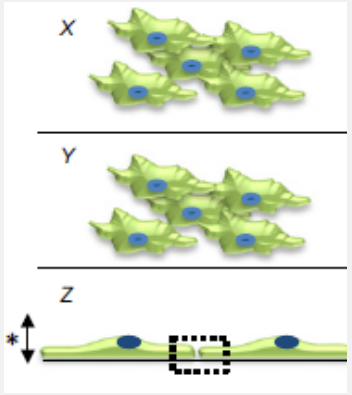
Increased biological relevance:

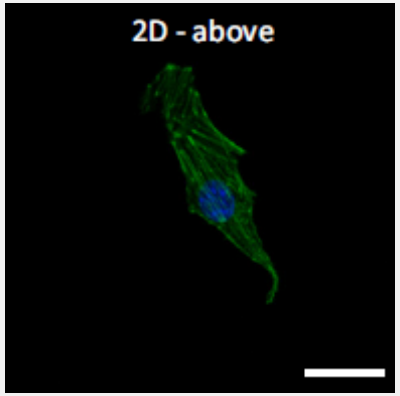
A major goal for research & drug discovery technologies

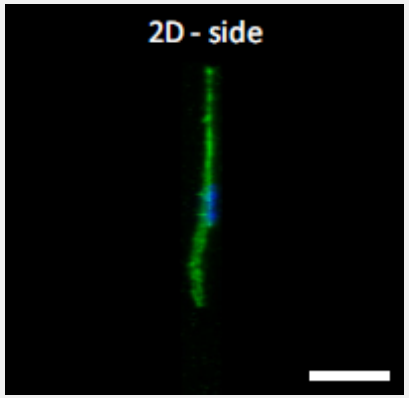


3D culture drives cells to a natural phenotype

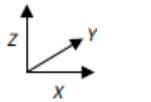
2D culture

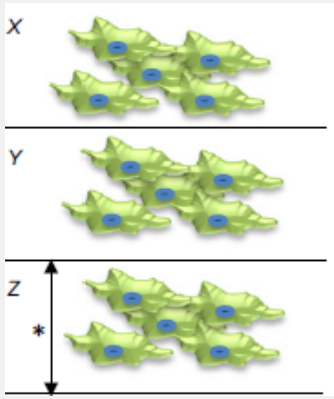


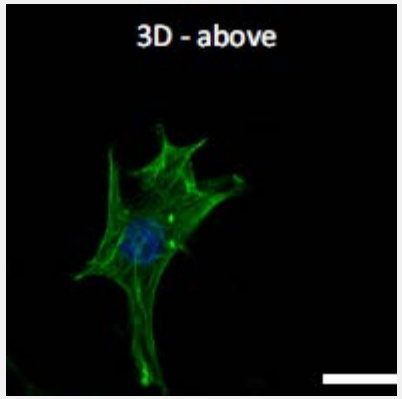


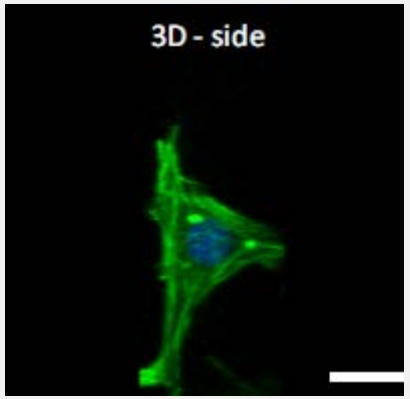


3D culture



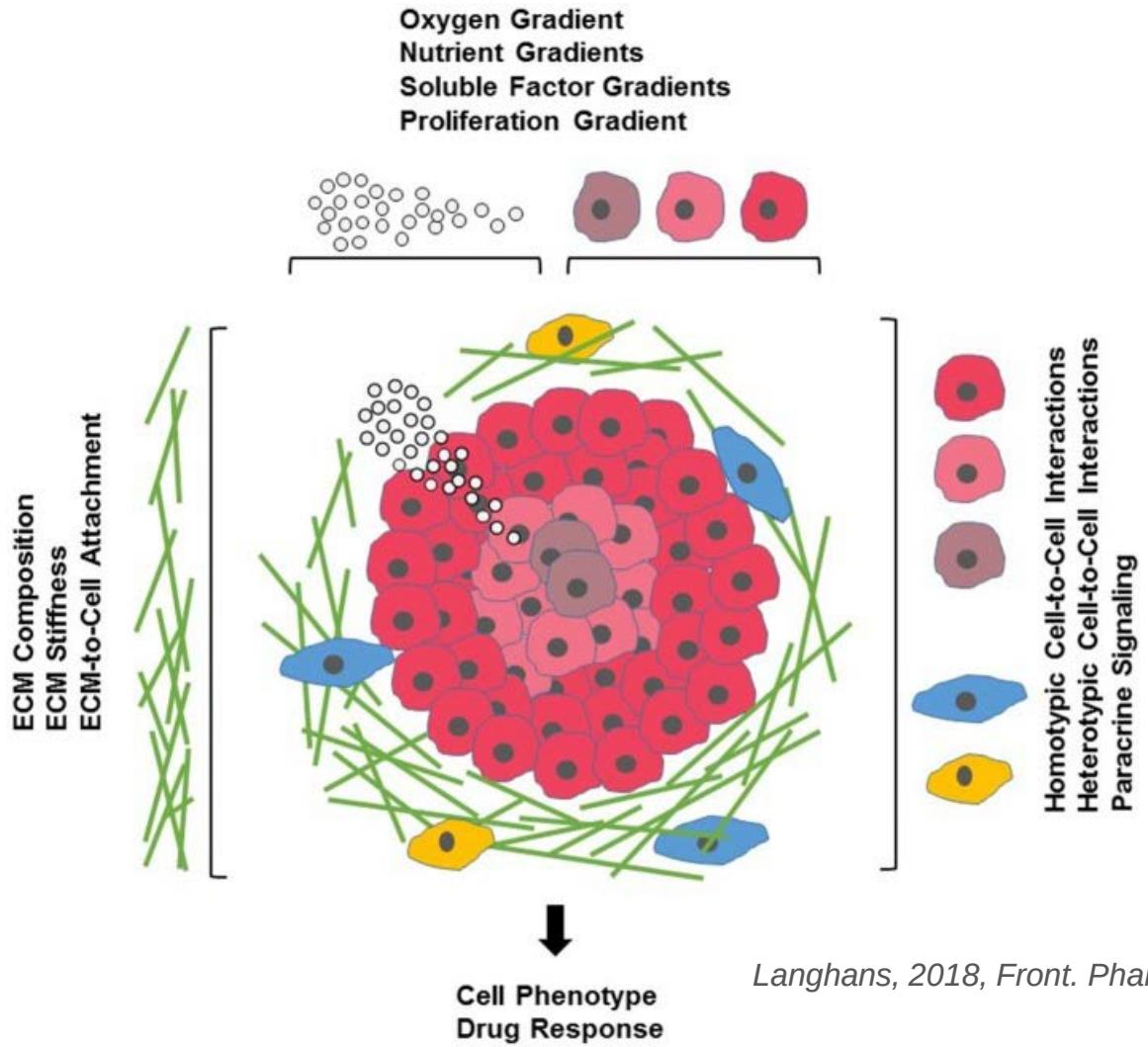






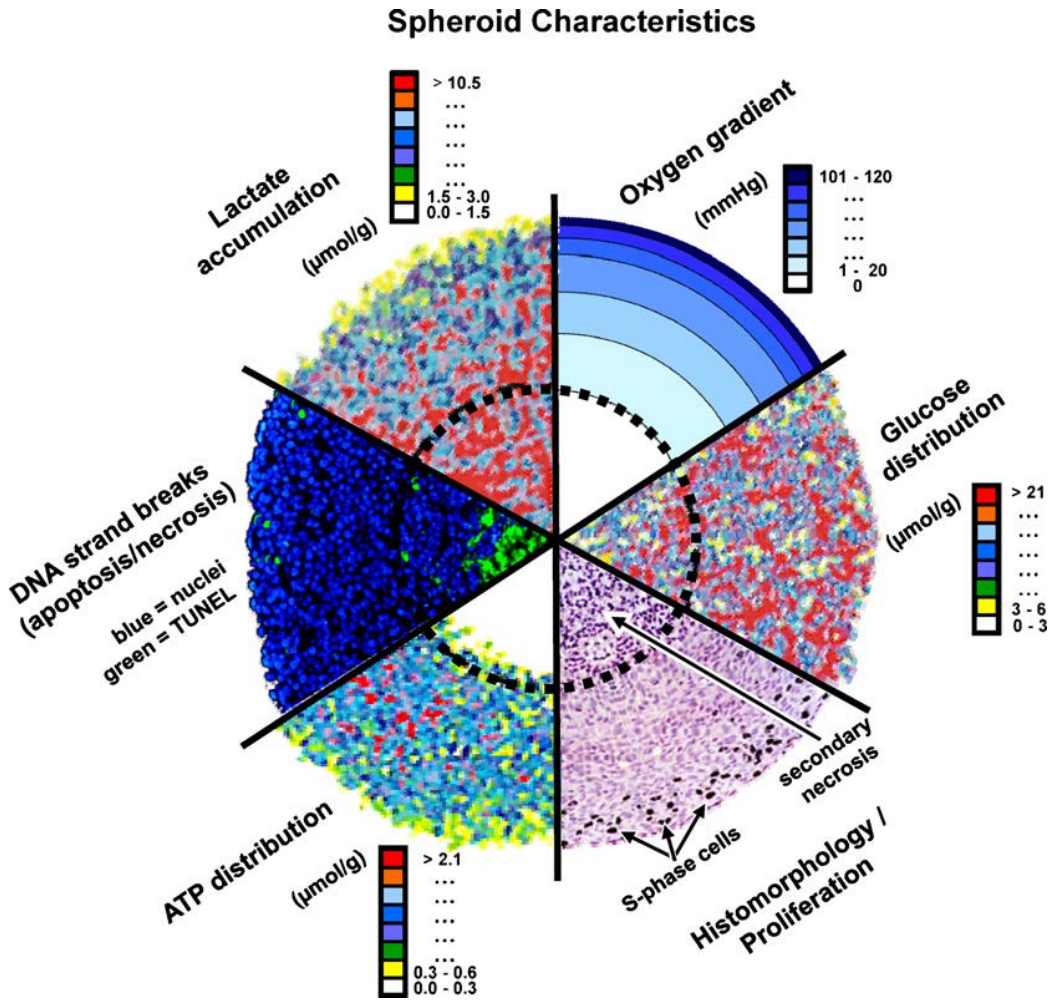
Knight and Przyborski, 2015, J. Anat. 227:746.

Cells and their microenvironment



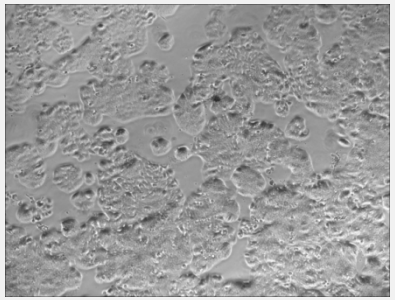
Langhans, 2018, Front. Pharmacol. 9:1.

3D cell spheroids: reflect cell microenvironment *in vivo*

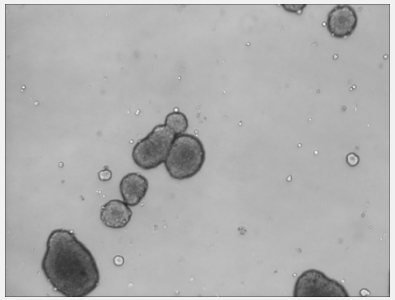


Hirschhaeuser, et al., 2010, J. Biotechnol. 148(1):3-15.

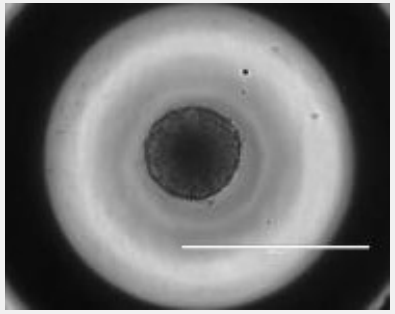
HT-29 monolayer



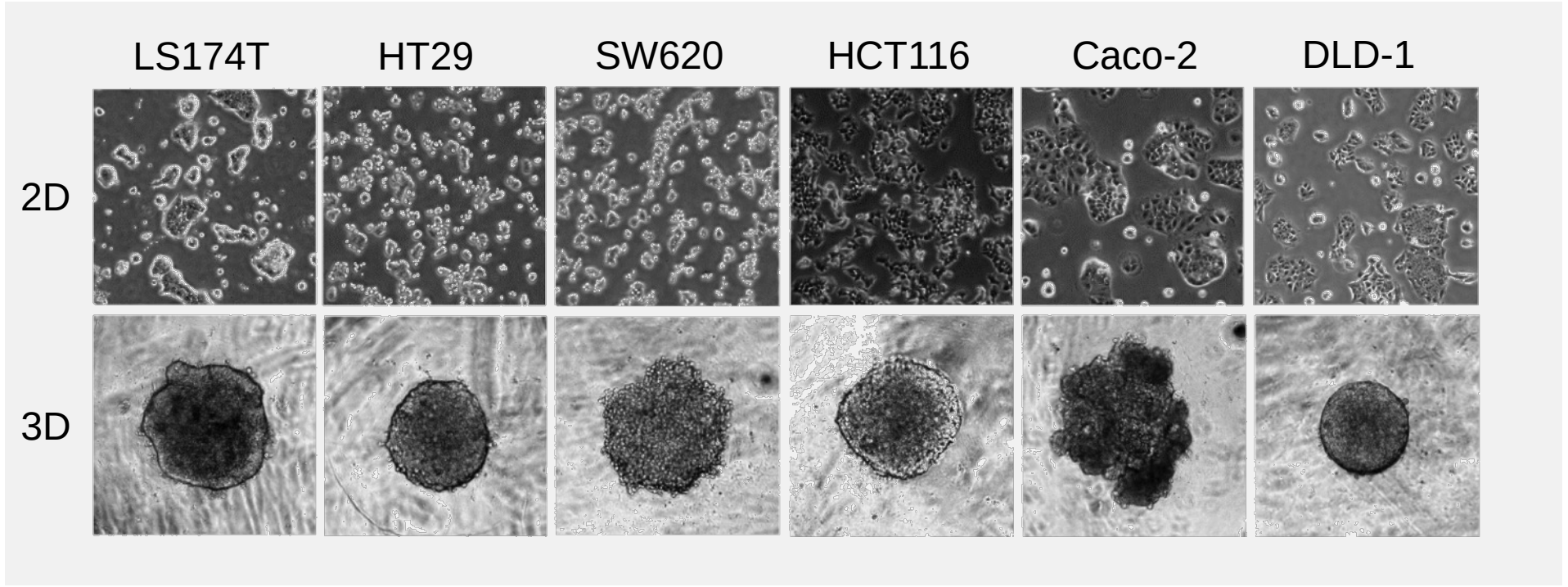
HT-29 multicellular spheroids



HT-29 single spheroid per well



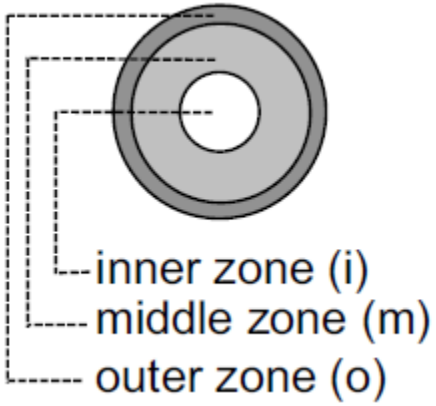
Colon cancer cells in 2D and 3D culture

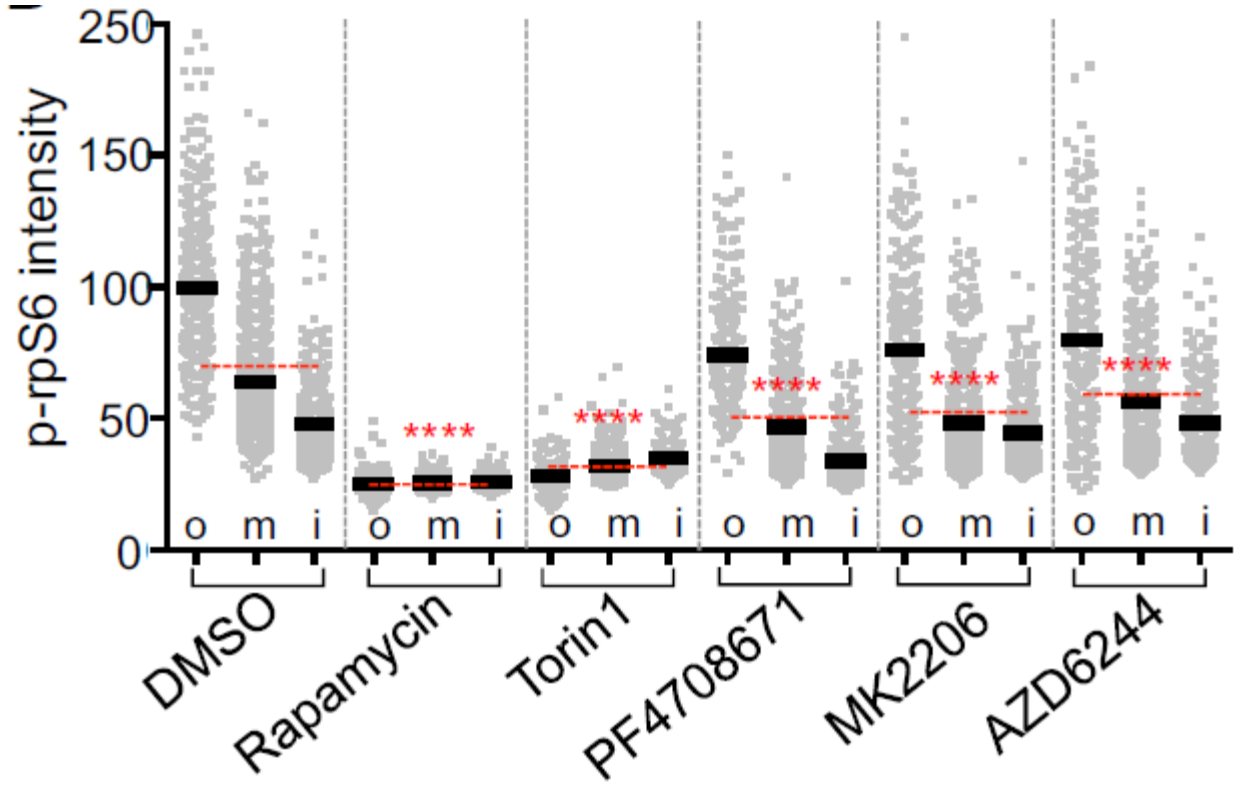


LS174T, HT29, SW620, HCT116, Caco-2, and DLD-1 colon cancer cells were grown as monolayers or spheroids (3000 cells/spheroid). 48 h, cells and spheroids were analyzed.

Riedl, et al., 2017, J Cell Sci. 130:203.

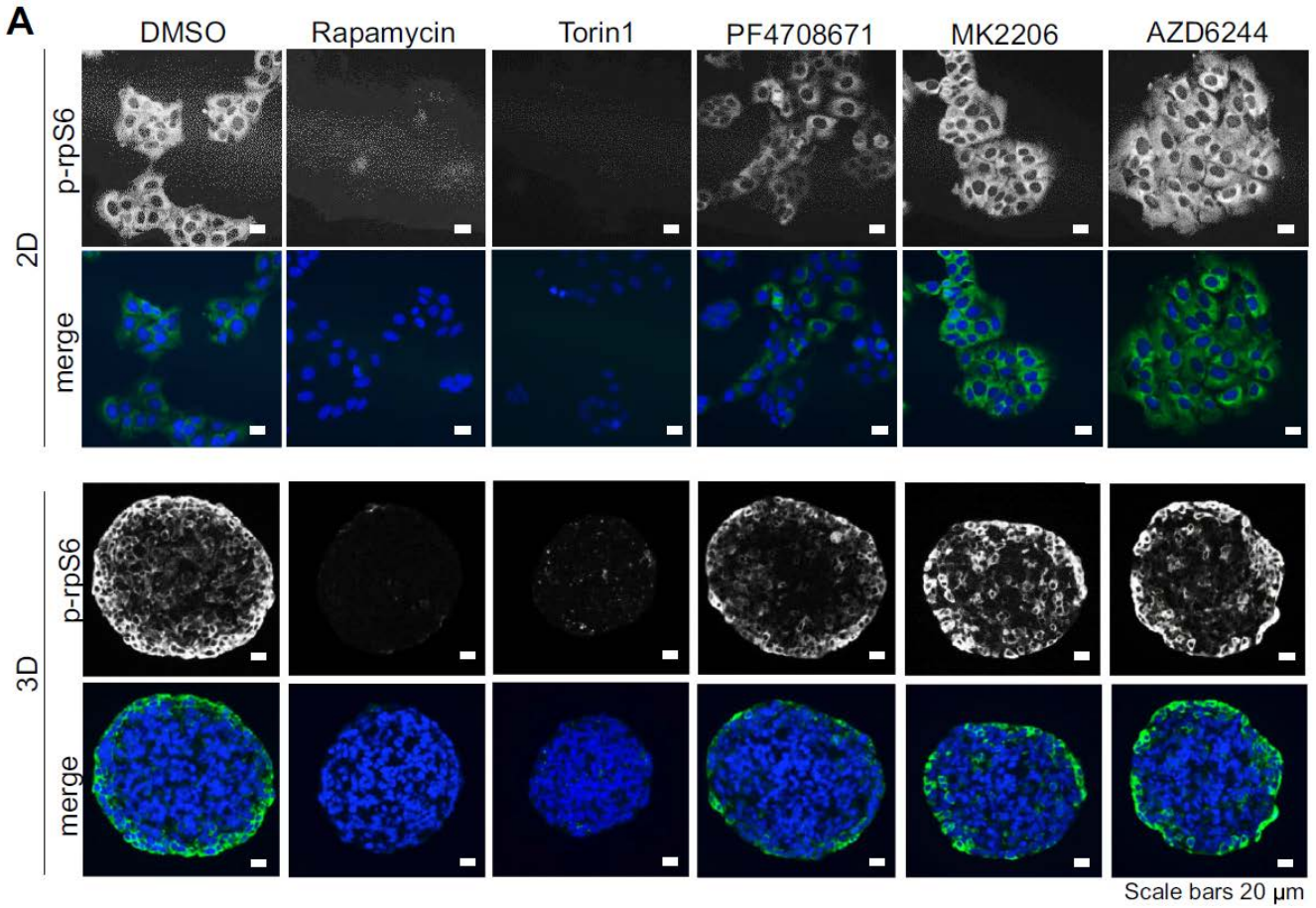
Decrease of RPS6 phosphorylation from outside to inner core of DLD-1 spheroids





Riedl, et al., 2017, J Cell Sci. 130:203.

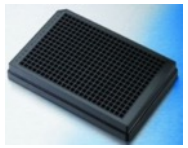
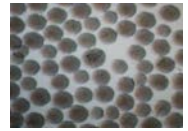
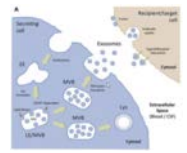
Colon cancer cells in 2D and 3D culture



Riedl, et al., 2017, J Cell Sci. 130:203.

3D cell spheroids and screening assays

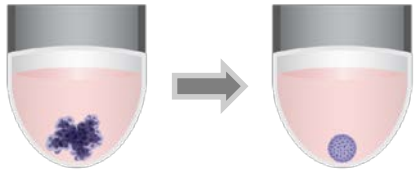
3D cell culture: emerging applications

	Cell Analysis	Provides large relevant materials for genetic and cellular research
	Compound Screening	Anti-oncologic compound screening against patient tumor tissue; preclinical drug ADME/Tox
	3D Printing & Biobanking	Building blocks for 3D printing of multicellular tissues, organoids; tissue regeneration
	Cell Therapy	Optimize therapeutic effects delivered as spheroids/cell aggregates (e.g. MSC, islets)
	EV Therapy	Therapeutics released by cells, patient treatment (e.g. exosomes)

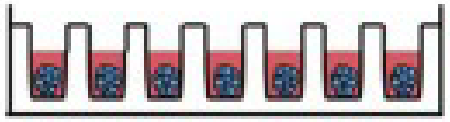
3D cell culture methods

Anchorage-independent (Non-scaffold-based)

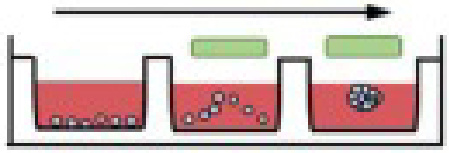
Hanging Drop



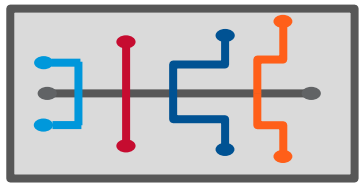
Low Attachment Plate



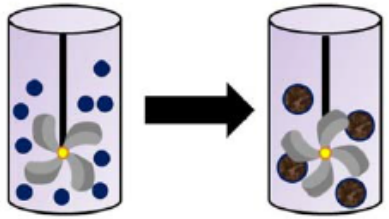
Magnetic Levitation



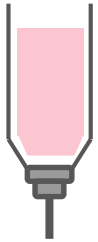
Microfluidic Devices



Centrifuge / Agitation



3D Bioprinting



Anchorage-dependent (Scaffold-based)

Biological Hydrogels

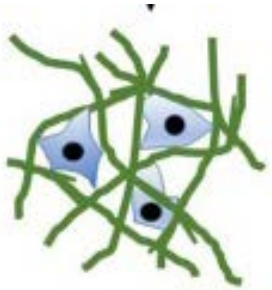
Animal-derived
- Collagen
- Corning® Matrigel® matrix
- Gelatin

Plant-derived
- Alginate

Synthetic Hydrogels

Non-natural
- Polyethylene Glycol (PEG)
- Polylactic Acid (PA)
- Polyglycolic Acid (PGA)

Natural
- Hyaluronic Acid (HA)
- Peptides

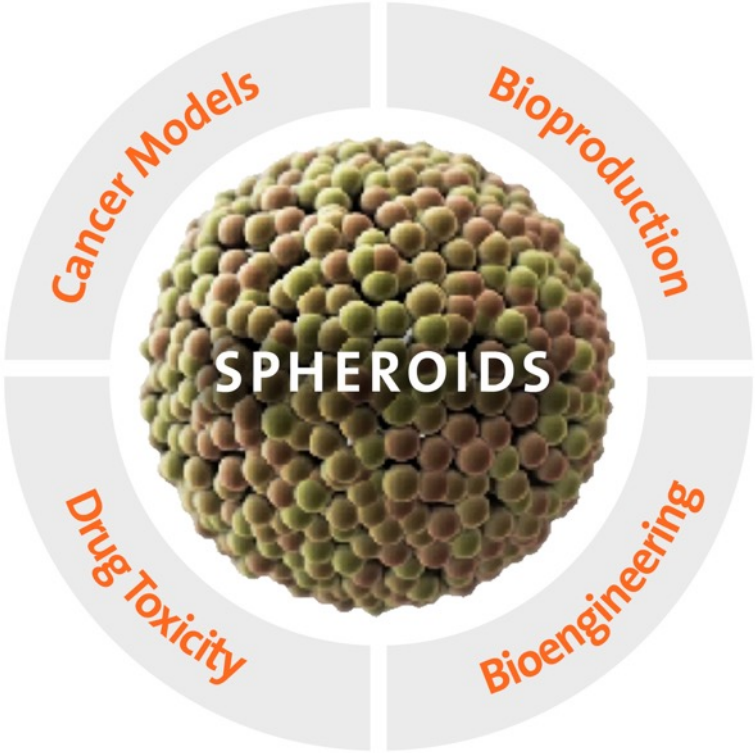


3D cell culture: cell spheroid applications

Microplates (96-, 384-, 1536-well)

- Drug discovery
- Drug development

***In vitro* toxicity models** (increased *in vivo* predictivity)



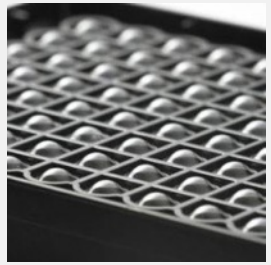
Large scale vessels

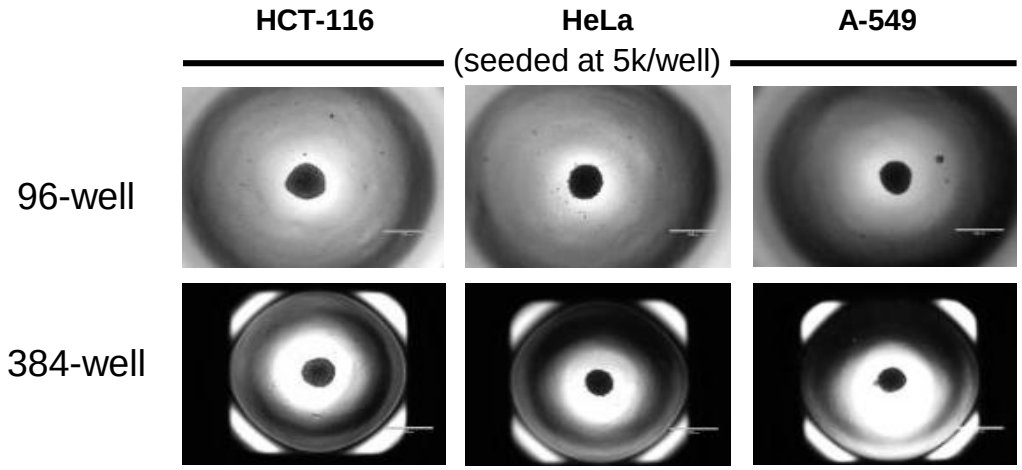
- Vaccine production
- Biologics production

- **Embryonic bodies**
- **Tissue engineering**
- **MSC therapy**

3D cell culture: spheroid microplates

- Culture and assay in same well
- Ultra-Low Attachment surface coating
- Single, uniform-sized spheroids per well
- HTS scalable formats:
  96-, 384-, 1536-well



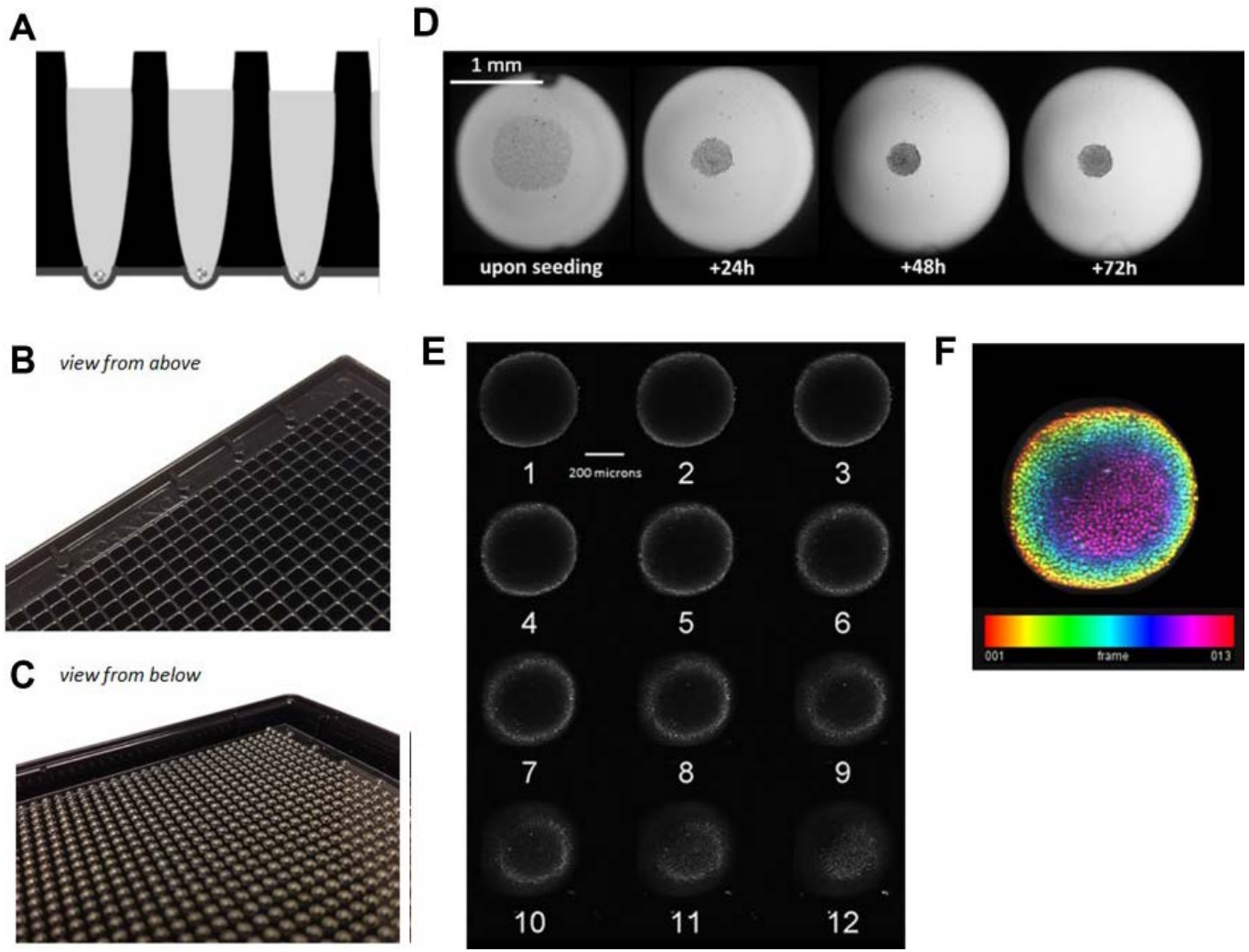


>40 cell types demonstrated

Cell line	ATCC Cat. No.	Tumor Type	Medium	Spheroid Morphology
BT-474	HTB-20	Human breast/duct carcinoma	RPMI/10% FBS	Tight
A549	CCL-185	Human lung carcinoma	F-12K/10% FBS	Tight
HEK-293	CRL-1573	Human embryonic kidney	DMEM/10% FBS	Tight
5/9m alpha 3-18	CRL-10154	Hamster (CHO-K1 derived), M-CSF production	DMEM/10% FBS	Aggregate
DU-145	HTB-81	Human prostate carcinoma	DMEM/10% FBS	Tight
IMR-32	CCL-127	Human brain neuroblastoma	DMEM/10% FBS	Aggregate
Detroit 562	CCL-138	Human pharynx, SCC	DMEM/10% FBS	Tight
MCF7	HTB-22	Human breast adenocarcinoma	DMEM/10% FBS	Aggregate
PANC-1	CRL-1469	Human pancreatic carcinoma	DMEM/10% FBS	Tight
Hep G2	HB-8065	Human hepatocellular carcinoma	DMEM/10% FBS	Aggregate
U-2 OS	HTB-96	Human bone osteosarcoma	McCoy's 5a/10% FBS	Tight
HCT 116	CCL-247	Human colon carcinoma	McCoy's 5a/10% FBS	Tight
HT-29	HTB-38	Human colon adenocarcinoma	McCoy's 5a/10% FBS	Tight
PC-3	CRL-1435	Human prostate adenocarcinoma	F-12K/10% FBS	Loose
MDA-MB-231	HTB-26	Human breast adenocarcinoma	L-15/10% FBS	Aggregate

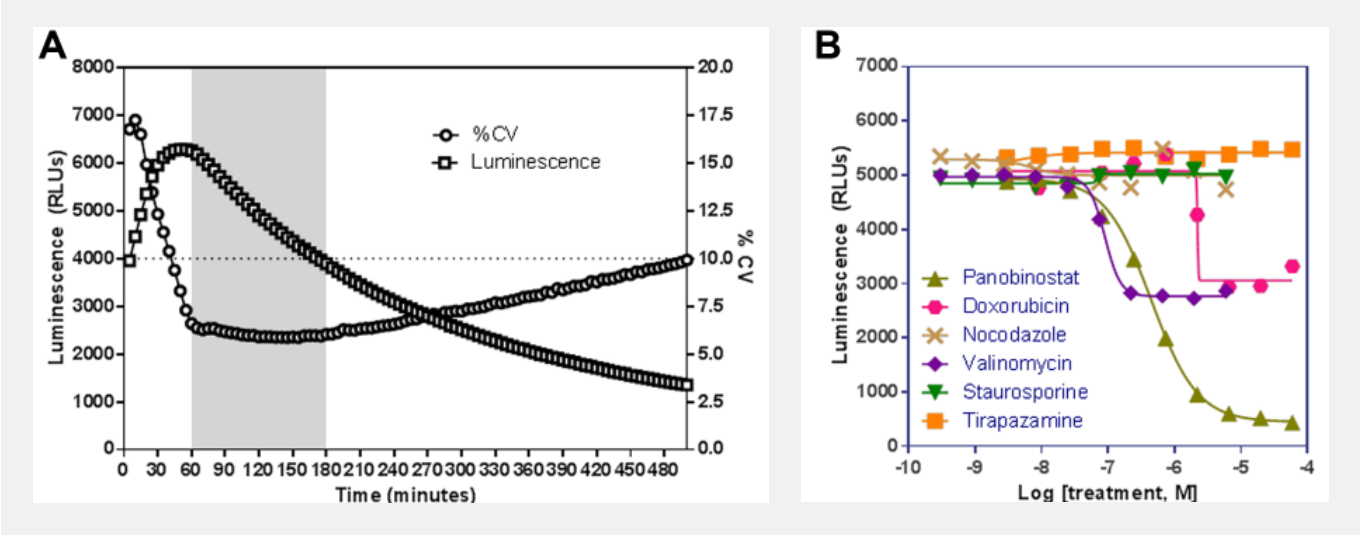
ultraHTS goes 3D:

3D spheroid microplates in 1536-well format



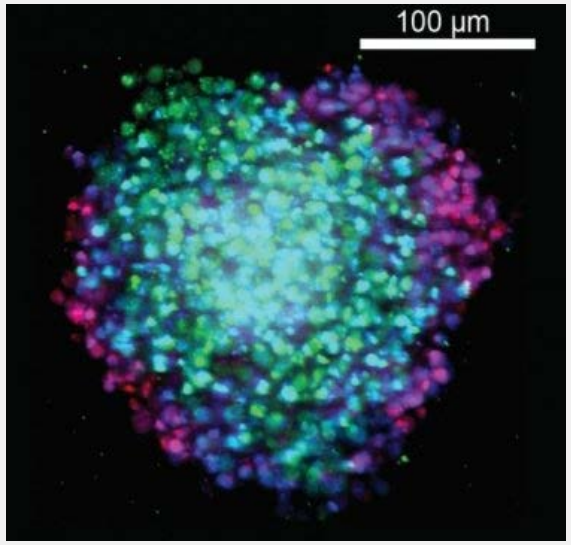
Madoux, et al., 2017 SLAS Disc. 22:516.

3D spheroid cytotoxicity assay characterization



Madoux, et al., 2017 SLAS Disc. 22:516.

Identification of cardiac glycosides inhibiting adenocarcinoma cells carrying oncogenic KRAS mutations



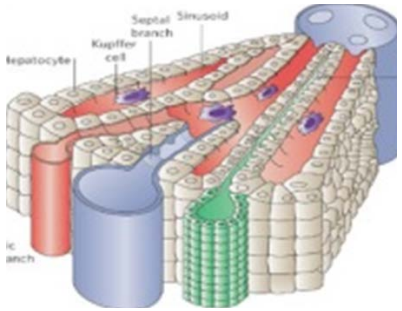
BxPC-3-KRASWT cell line

- Previous screening efforts relied on 2D cell culture, which oversimplified *in vivo* conditions
- 3D spheroid-based primary screening assay for HTS small molecule screening
- 3D screening approach enables assay conditions more closely reflecting cell environment *in vivo*

"Identified a cardiotonic glycoside, Proscillaridin A, as a potent and selective inhibitor of KRAS mutant cells...

…Proscillaridin A would not have been identified as a selective hit in a 2D assay, illustrating the utility of the spheroid-based 3D platform to uncover new biology".

Kota, et al., 2018, Oncogene online May 2018.

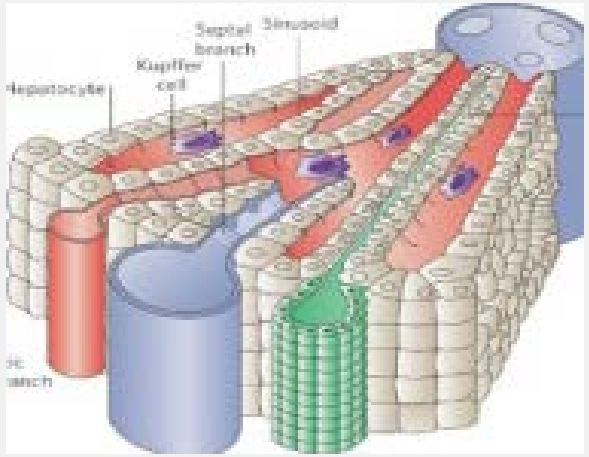


3D heptocell spheroids and screening assays

2D vs. 3D primary human hepatocyte (PHH) cell culture

2D PHH monolayer

3D architecture of liver tissue

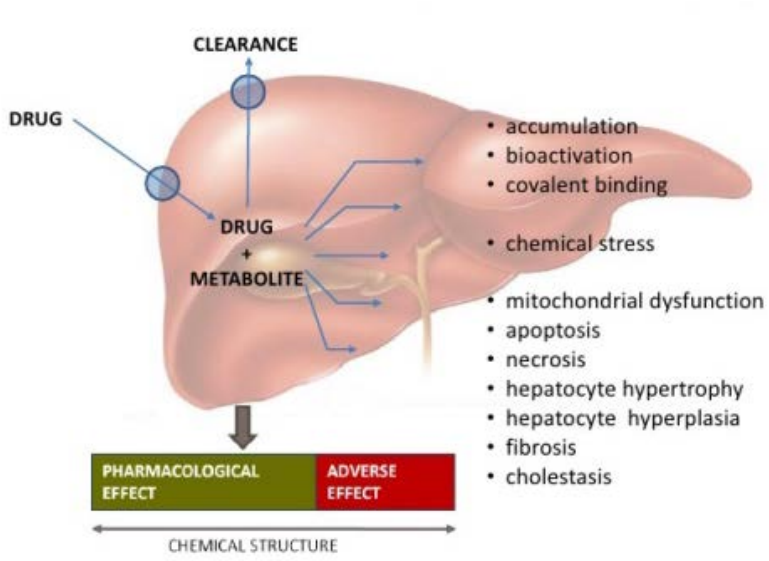


- 2D culture systems play a pivotal role in research; however, classical 2D culture systems do not reflect liver complexity.
- 3D culture systems sustain the cell viability, maintain *in vivo* phenotypes, genomic & proteomic expression profiles.
- Compared to other 3D systems, spheroids require fewer cells, are technically easier, and adaptable to HTS.

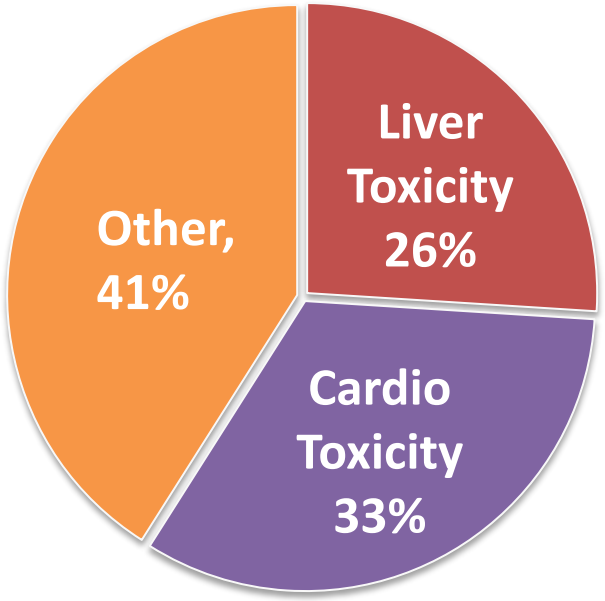
DILI - a leading cause for drug attrition & clinical failure

Among drugs withdrawn due to toxicity, 26% are attributed to DILI

Complex mechanisms for DILI

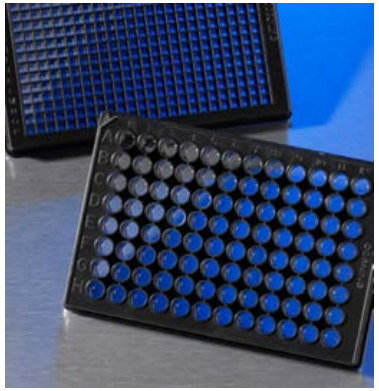


Drugs Withdrawn Due to Toxicity (1990-2010)



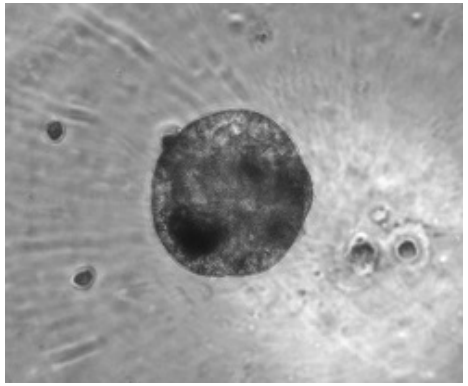
Building a 3D liver cell spheroid model

Corning® Spheroid Microplates



Cryopreserved PHH

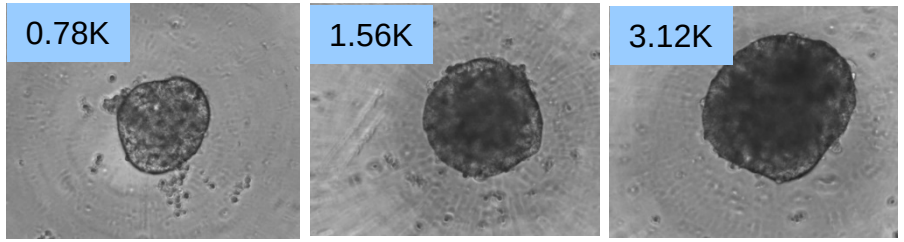
3D Liver Spheroid



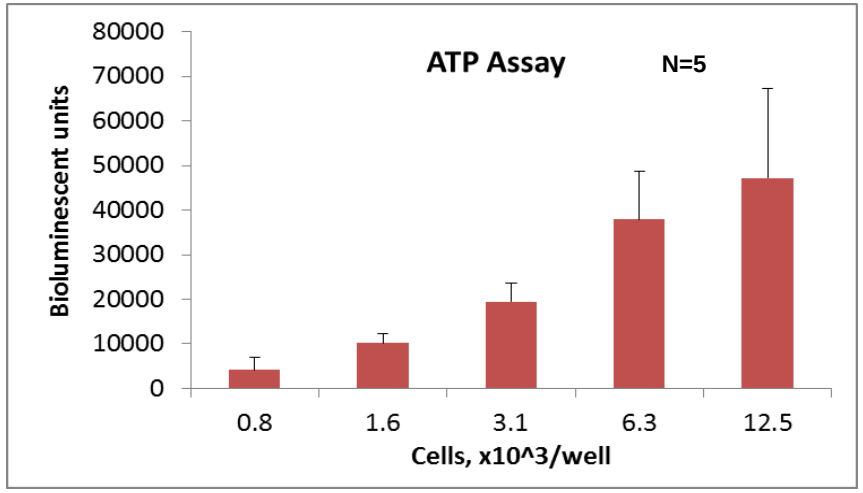
- Establish PHH spheroid culture protocol
- Develop 3D liver toxicity assay

Seeding density, spheroid size, and ATP levels

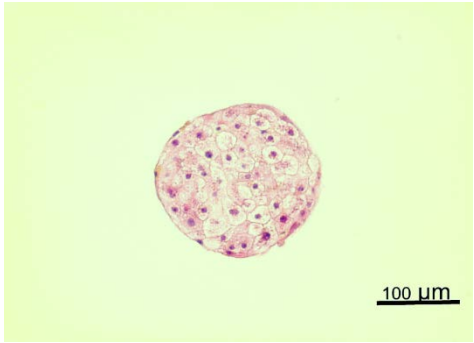
Day 7 Spheroid



Spheroid Viability Measurement



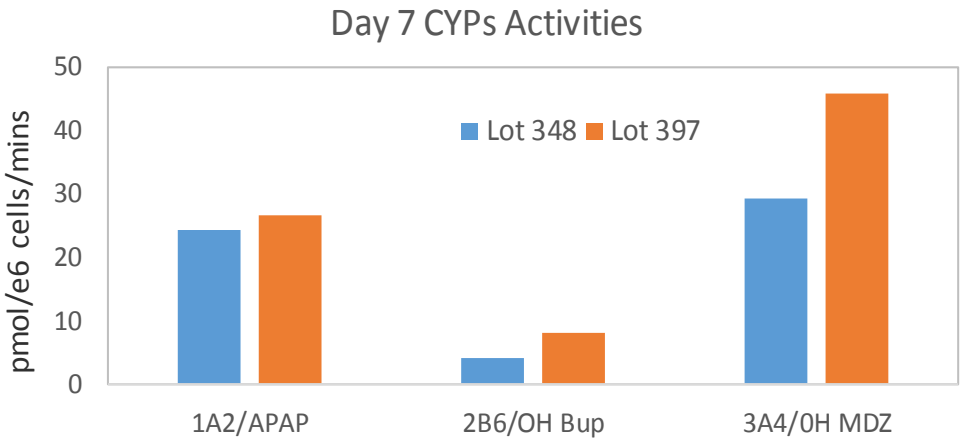
H&E Staining of a Day 8 PHH Spheroid (1000 cells/ spheroid)

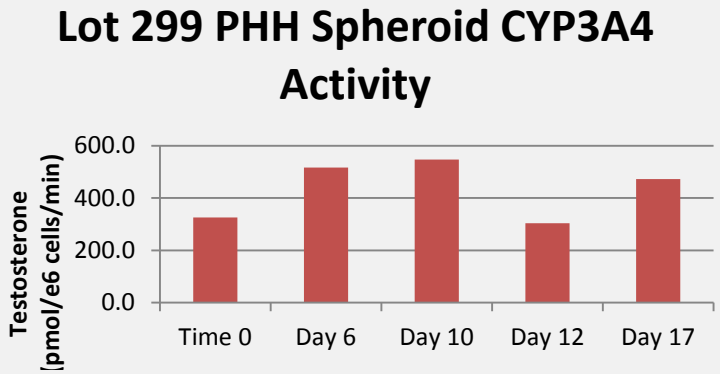


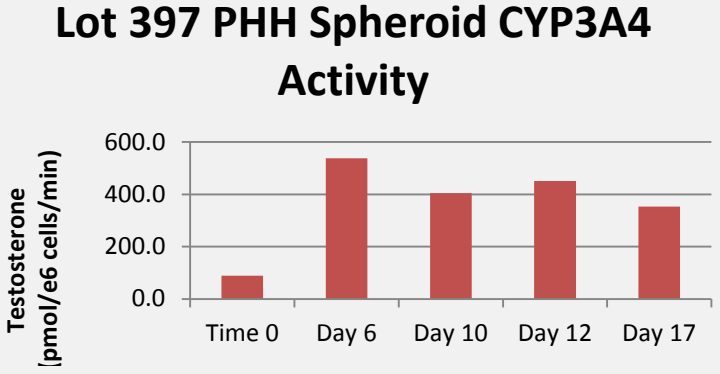
- Spheroid morphology & sizes routinely monitored.
- Bioluminescent ATP assay for viability performed in same spheroid microplates.
- Single spheroids form with seeding densities <5K cells/well on 96-well spheroid microplate.

PHH spheroids maintain drug metabolic activities

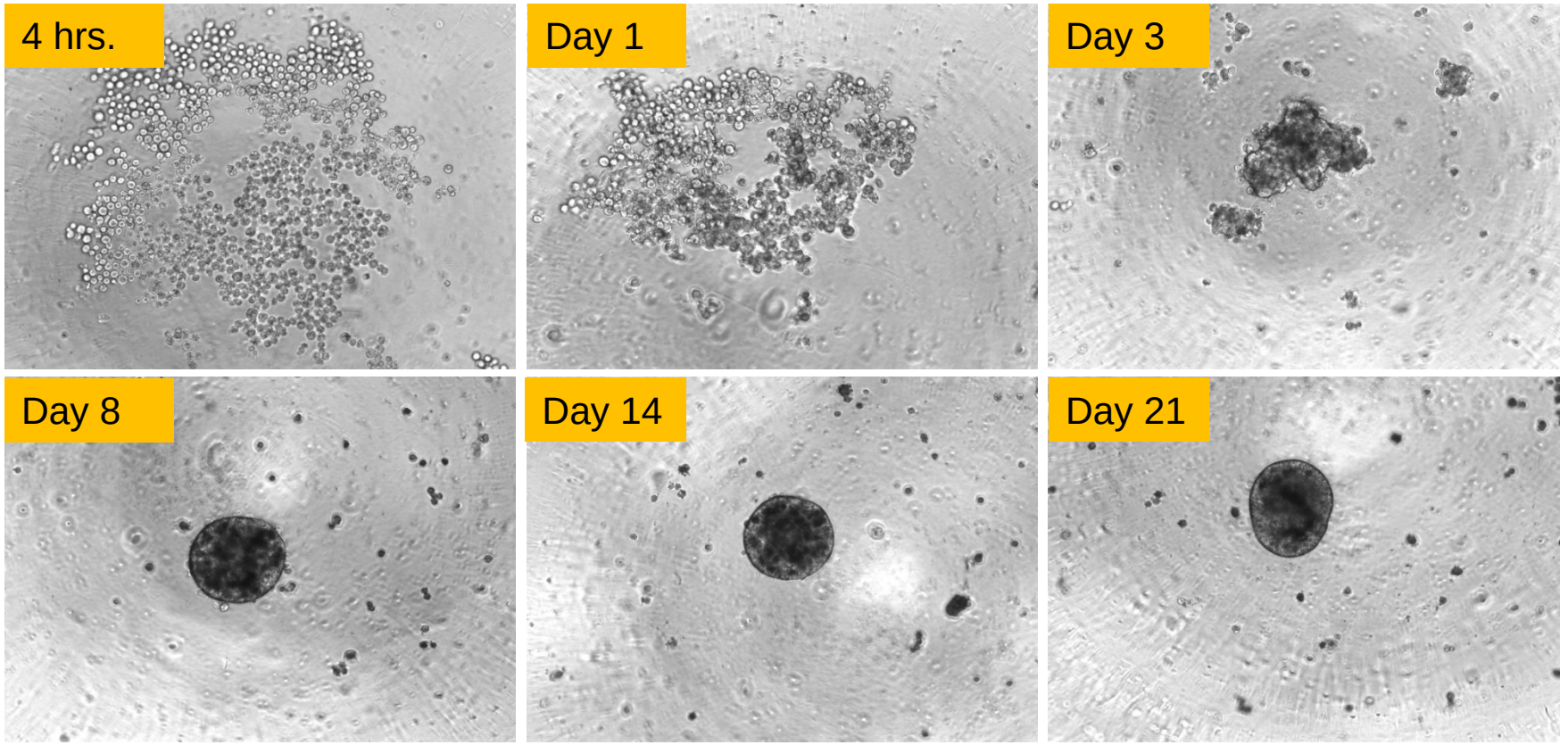
10 spheroids = 10K cells



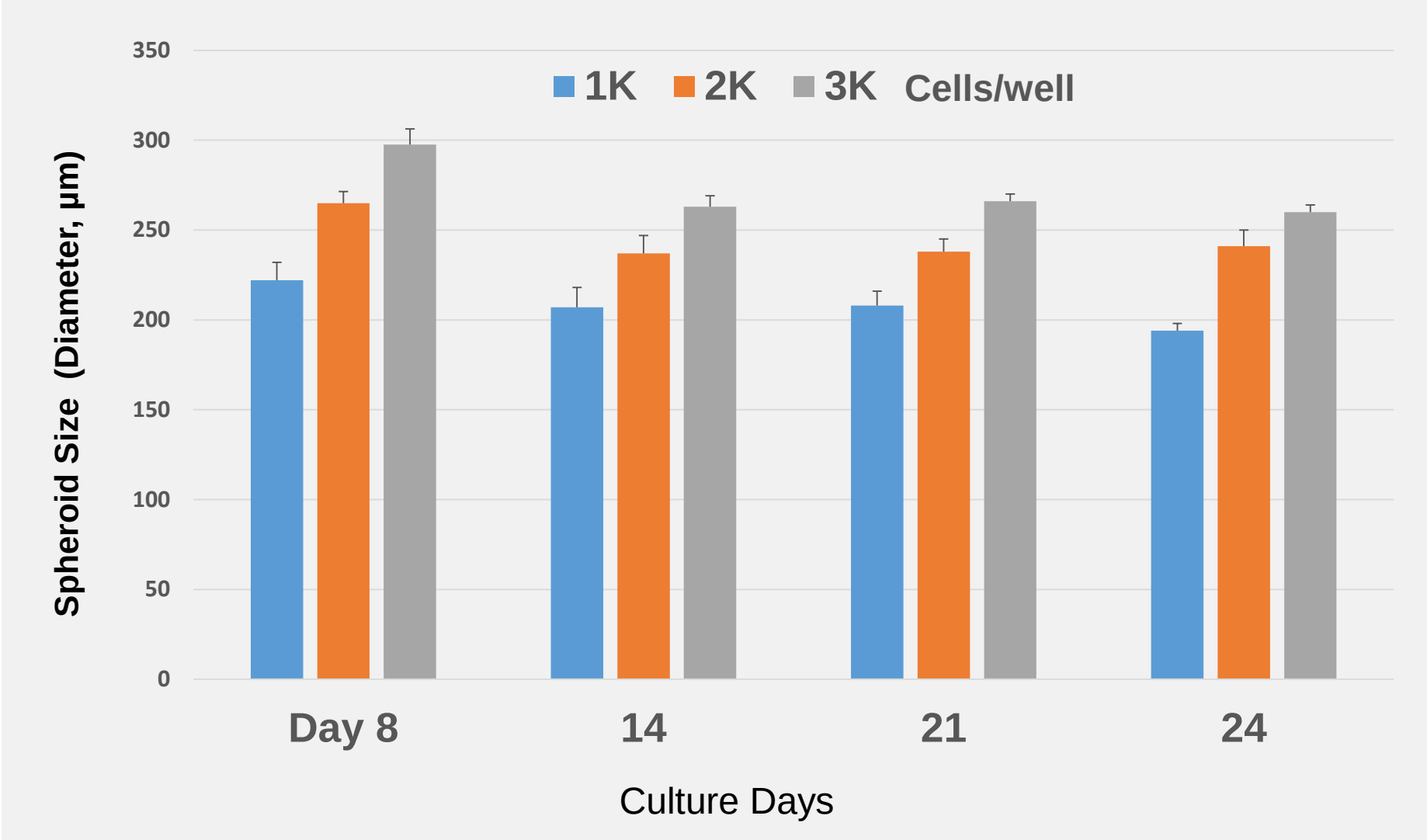




Spheroid size & morphology of liver spheroids is stable during long-term culture



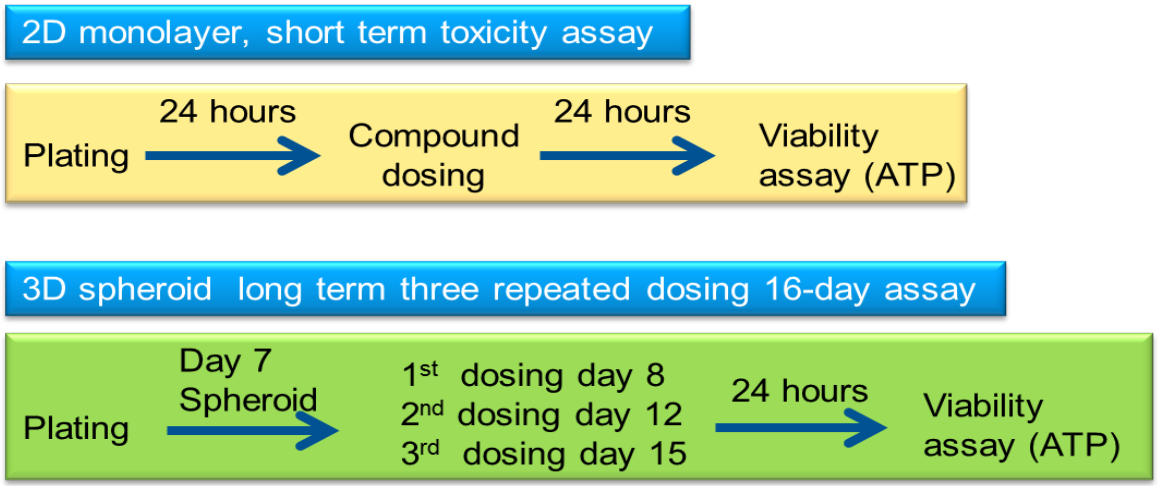
Spheroid size & morphology of liver spheroids is stable during long-term culture



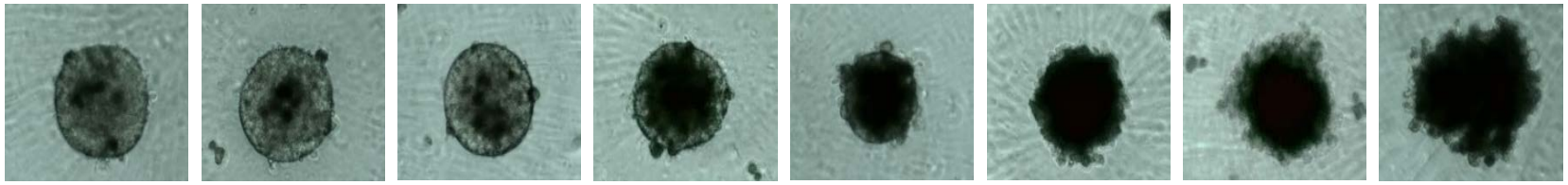
Testing DILI compounds:
3D PHH spheroids vs. 2D PHH monolayer

DILI Severity Category	Compound	C_{max} (µM)
1. Severe	Amiodarone	5.3
	Bosentan	7.4
	Nefazodone	4.3
	Tolcapone	47.6
	Troglitazone	6.4
	Trovafloxacin	5.0
	Valproic Acid	693.4
2. High concern	Diclofenac	10.1
	Rosiglitazone	1
3. Low concern	Acetaminophen	165.4
	Chlorpromazine	0.9
	Pioglitazone	2.95
4: Enzyme elevations	Dexamethasone	0.2
5. No DILI	Flumazenil	1.1

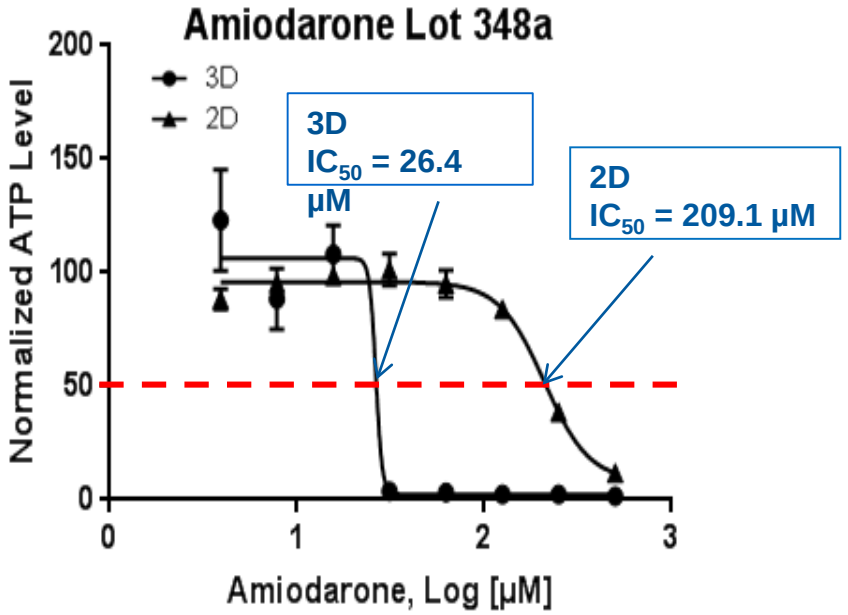
- Hepatocytes in 2D culture are short-lived for ~7 days.
- 3D spheroid culture supports long-term hepatocyte viability in culture for up to 4 weeks.
- 3D spheroid culture is capable of testing chronic liver toxicity with repeated dosing.



Example: DILI compound (amiodarone) pharmacology

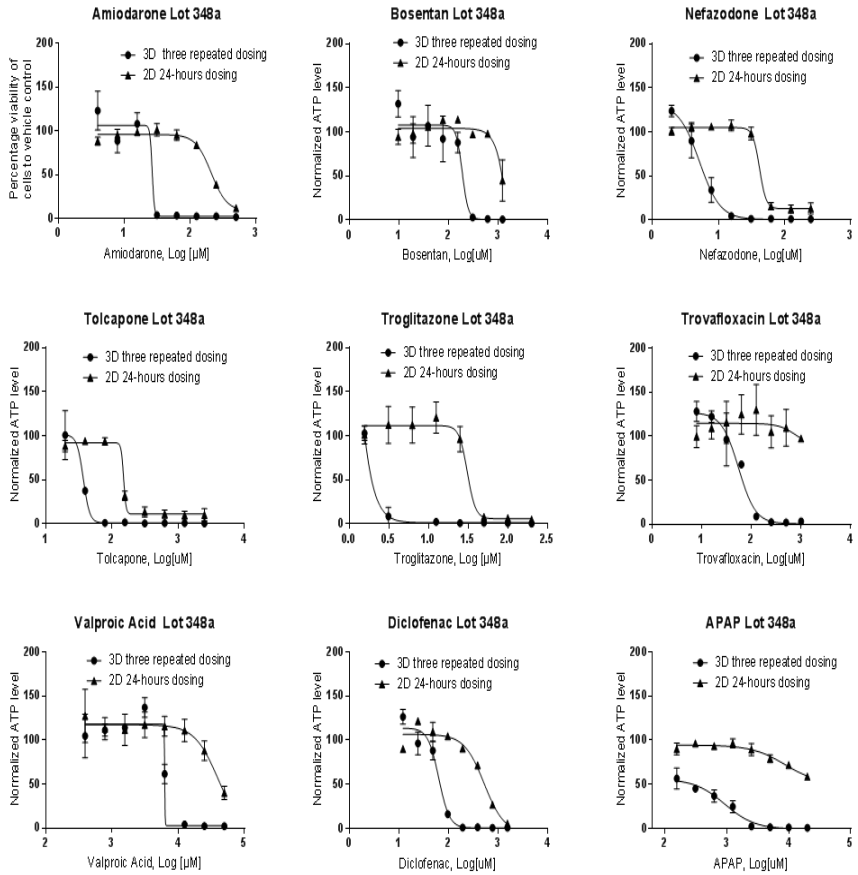


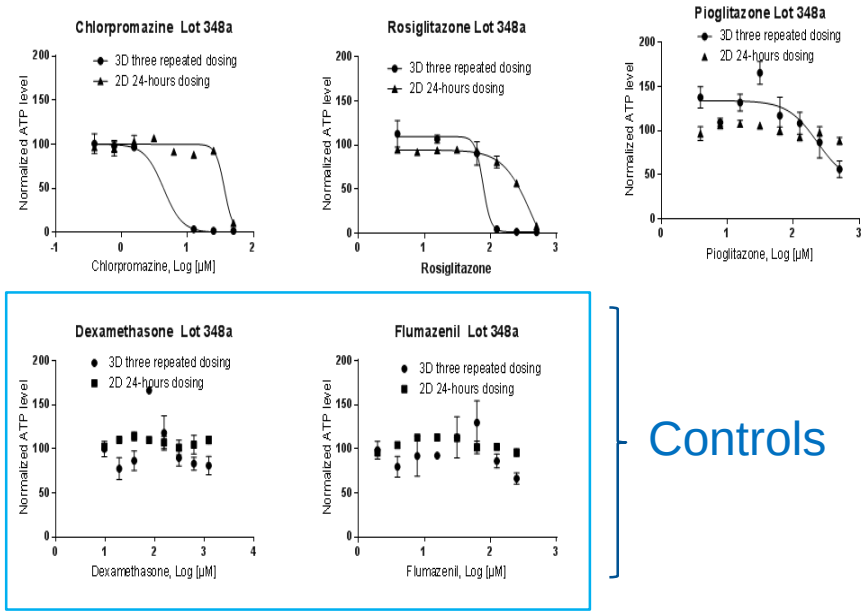
Conc. (µM) 3.9 500.0



Spheroids show increased sensitivity to DILI compound treatment vs. 2D cultures.

3D spheroids are more sensitive to DILI compounds vs. 2D PHH culture

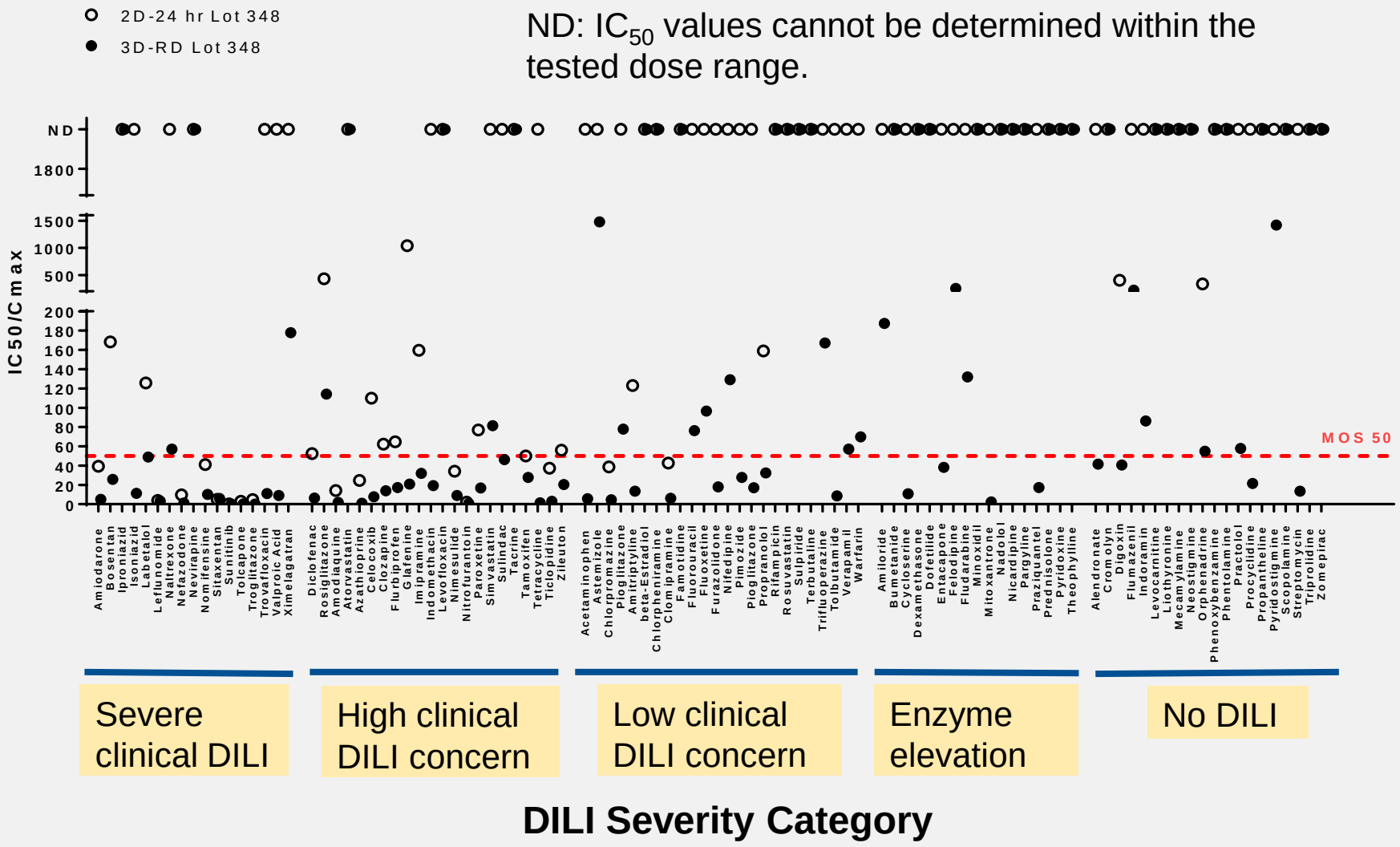




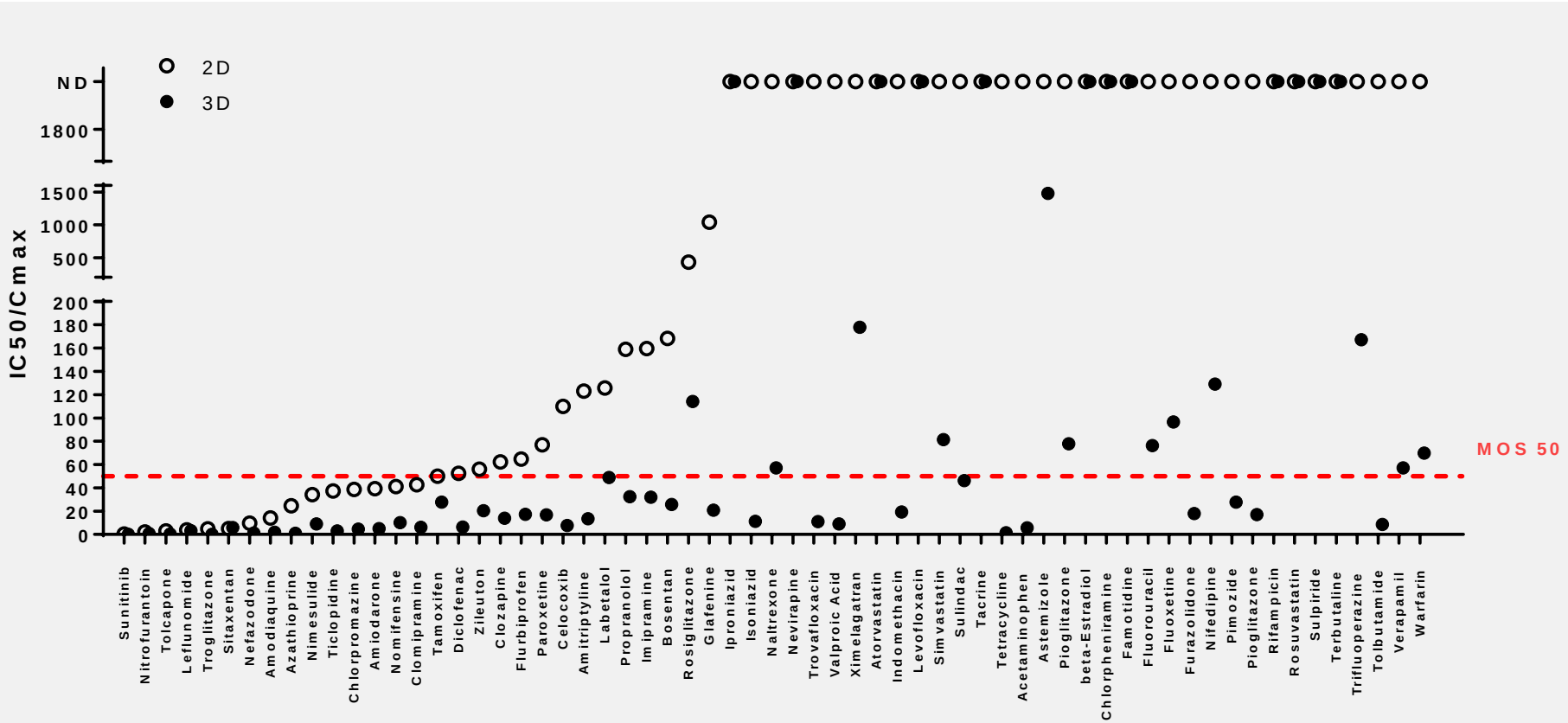
Controls

- 3 PHH lots tested in 3D vs. 2D culture
- 3D spheroids showed increased sensitivity to DILI compound treatment

3D PHH liver spheroids show superior sensitivity to DILI compound treatment

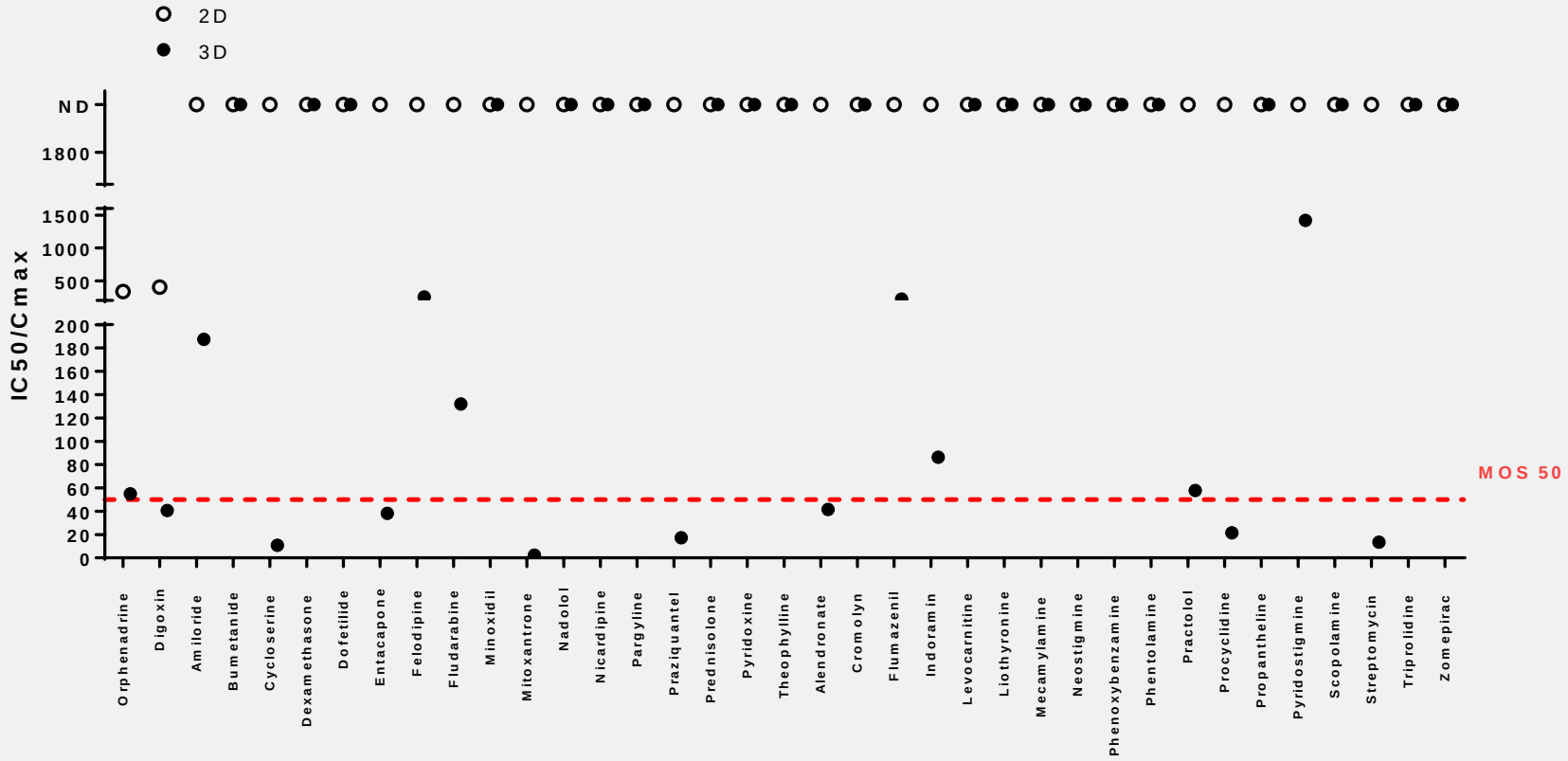


3D PHH liver spheroids show superior sensitivity to DILI compound treatment (continued)



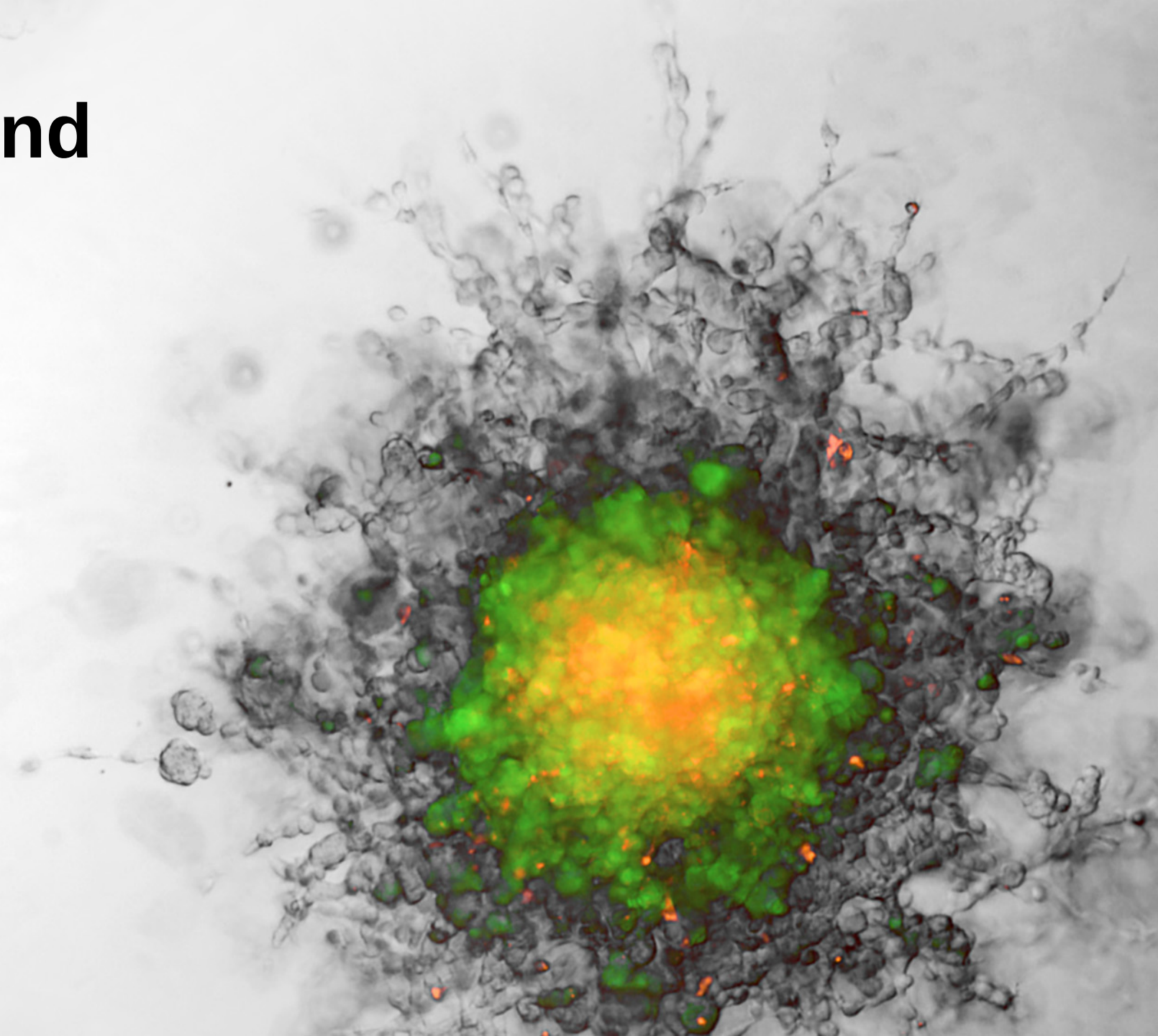
DILI compounds = Categories 1, 2, and 3

3D PHH liver spheroids show superior sensitivity to DILI compound treatment (continued)



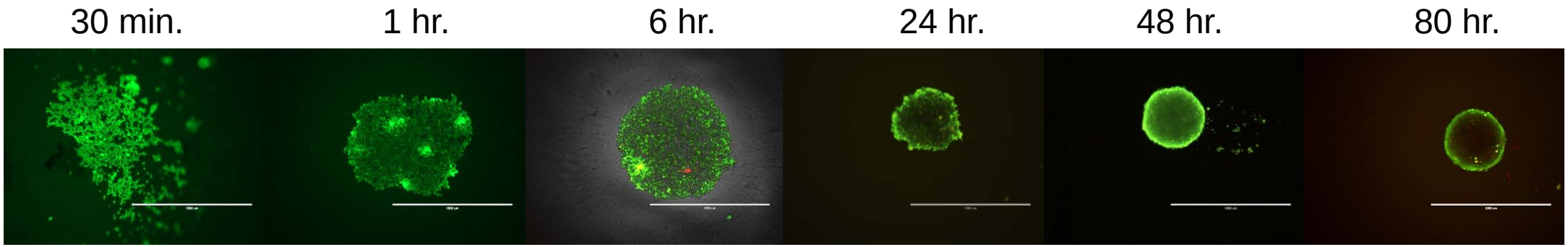
Control compounds = Categories 4 and 5

3D cell culture and imaging



Spheroid formation and growth kinetics

- Cell aggregation occurs within the first few hours of seeding
- Duration of spheroid culture is cell line-dependent
- After 48 hours of culture, HT-29 cells form well-rounded, compact spheroids with defined edges
- Begin to show initial signs of a necrotic core after ~80 hours in culture



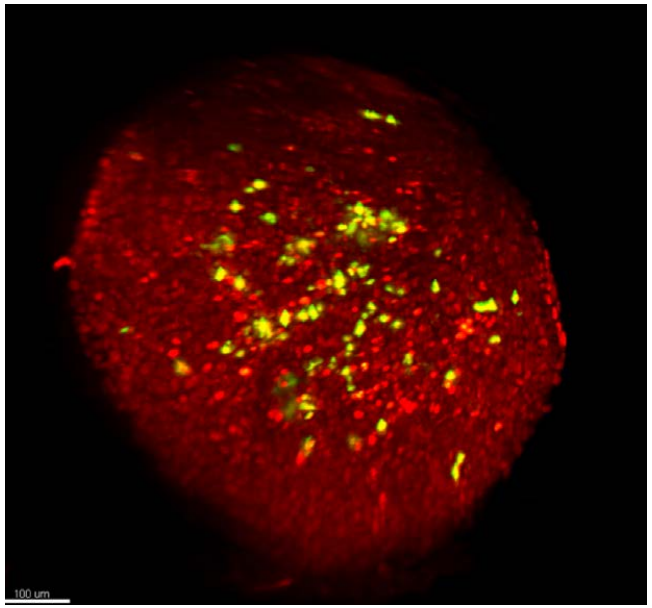
Within the first hour, cells aggregate in well center

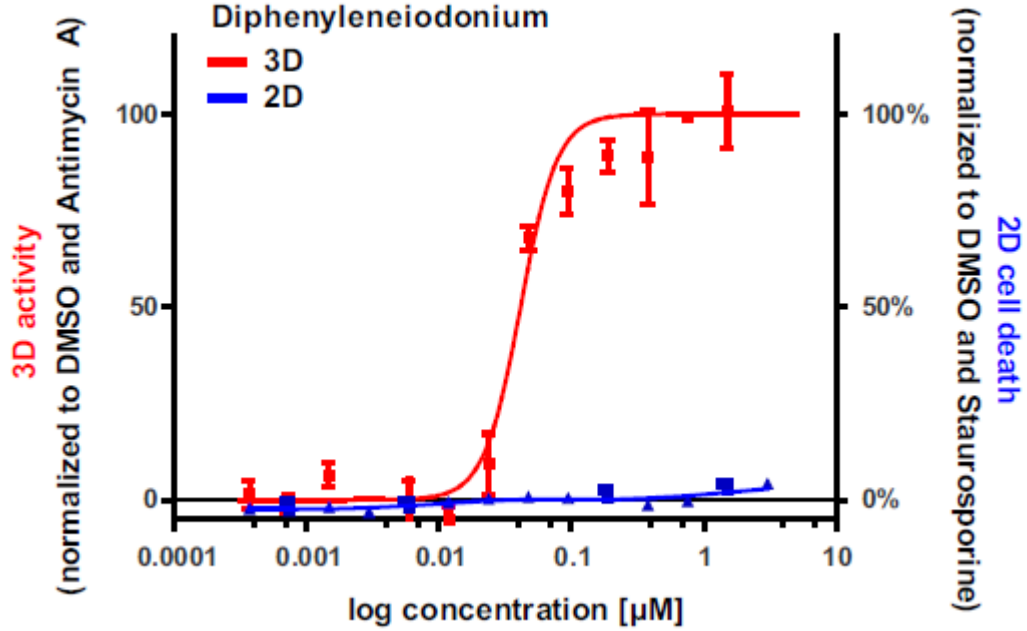
After several hours, cells form a more defined spheroid

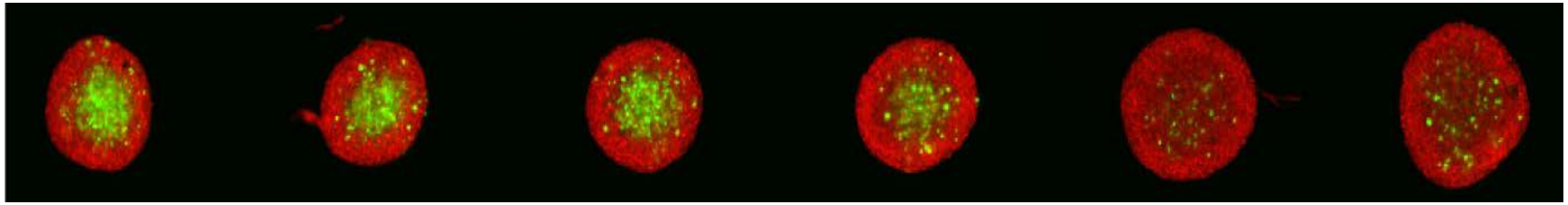
After 48 hours, spheroid is well-rounded with defined edges

By 80 hours, spheroid center shows early necrosis

3D culture and HCS

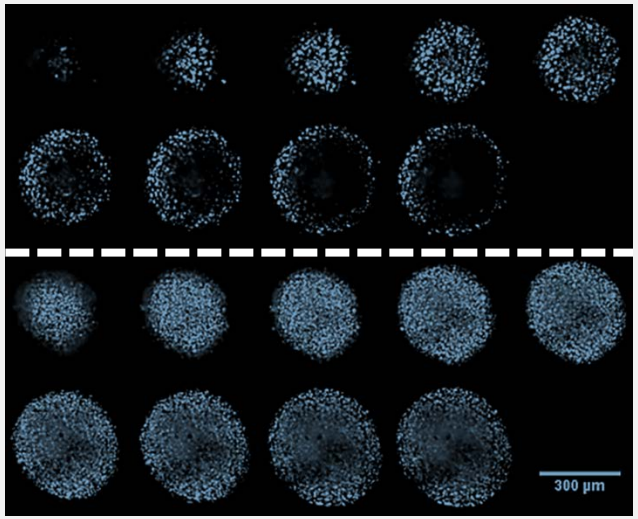






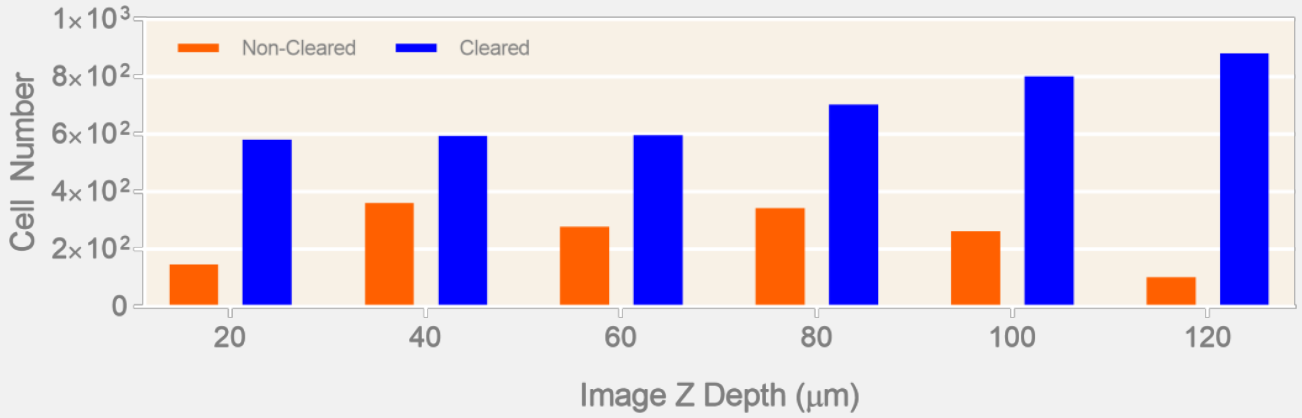
Wenzel, et al., 2014, Exp Cell Res 323:131.

Spheroid imaging and optical clearing



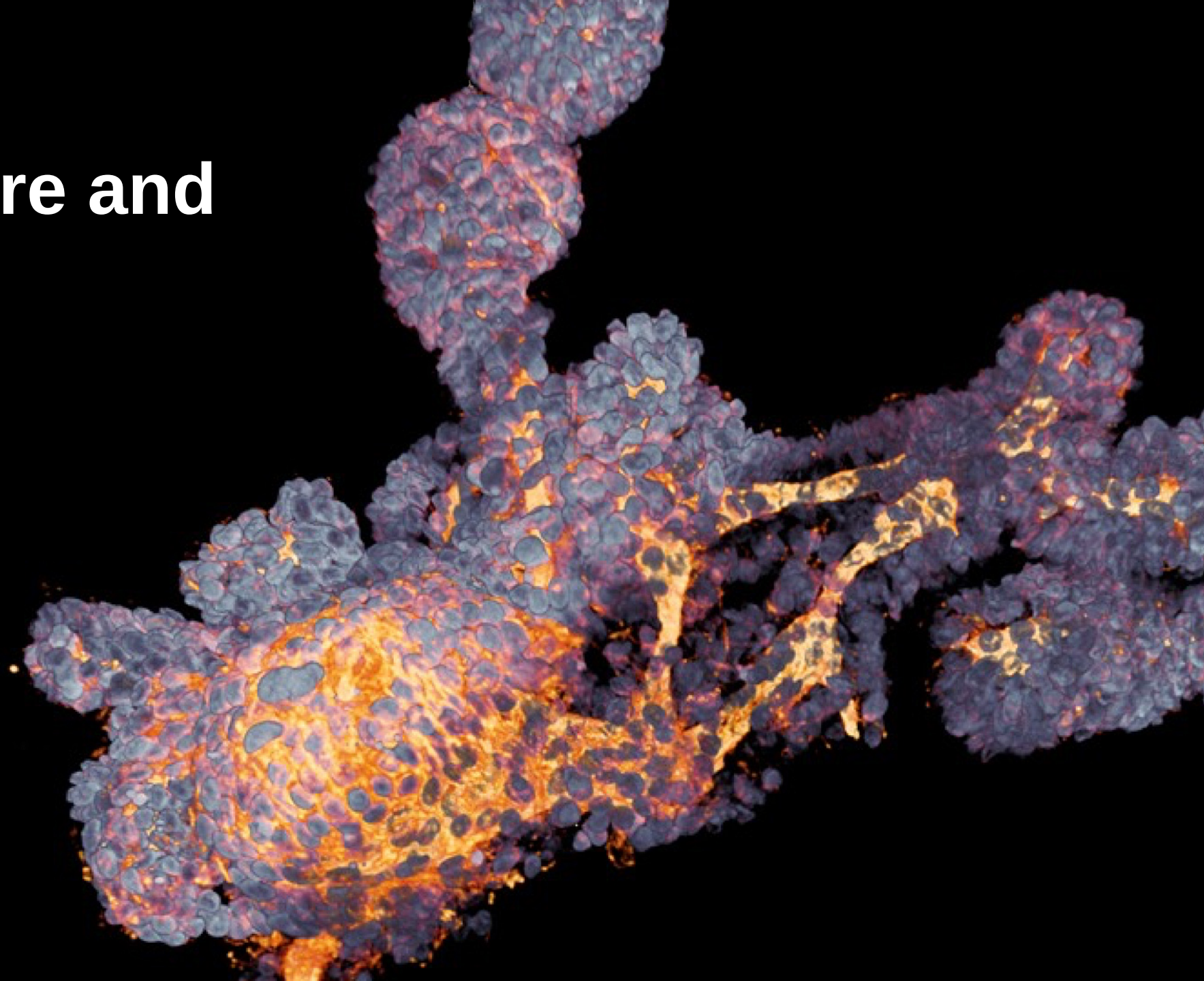
Representative montage of 20 µm slices from confocal z-stack of DAPI-stained spheroid.

Non-cleared (top rows) and cleared (bottom rows) spheroid.



Number of cells detected at various z-depth through non-cleared (orange) and cleared (blue) HepG2 spheroids.

3D cell culture and organoids

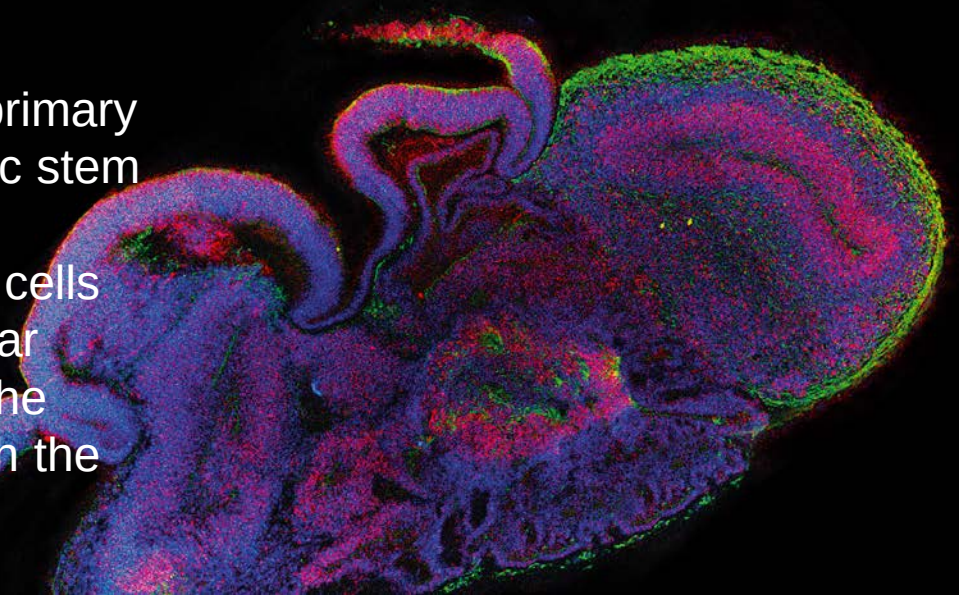


3D cell culture and organoids

Originally, “organoid” referred to primary cultures of tissue fragments separated from the stroma within 3D gels to form organ-like structures

Now encompasses a variety of tissue culture techniques that result in self-organizing, self-renewing 3D cultures

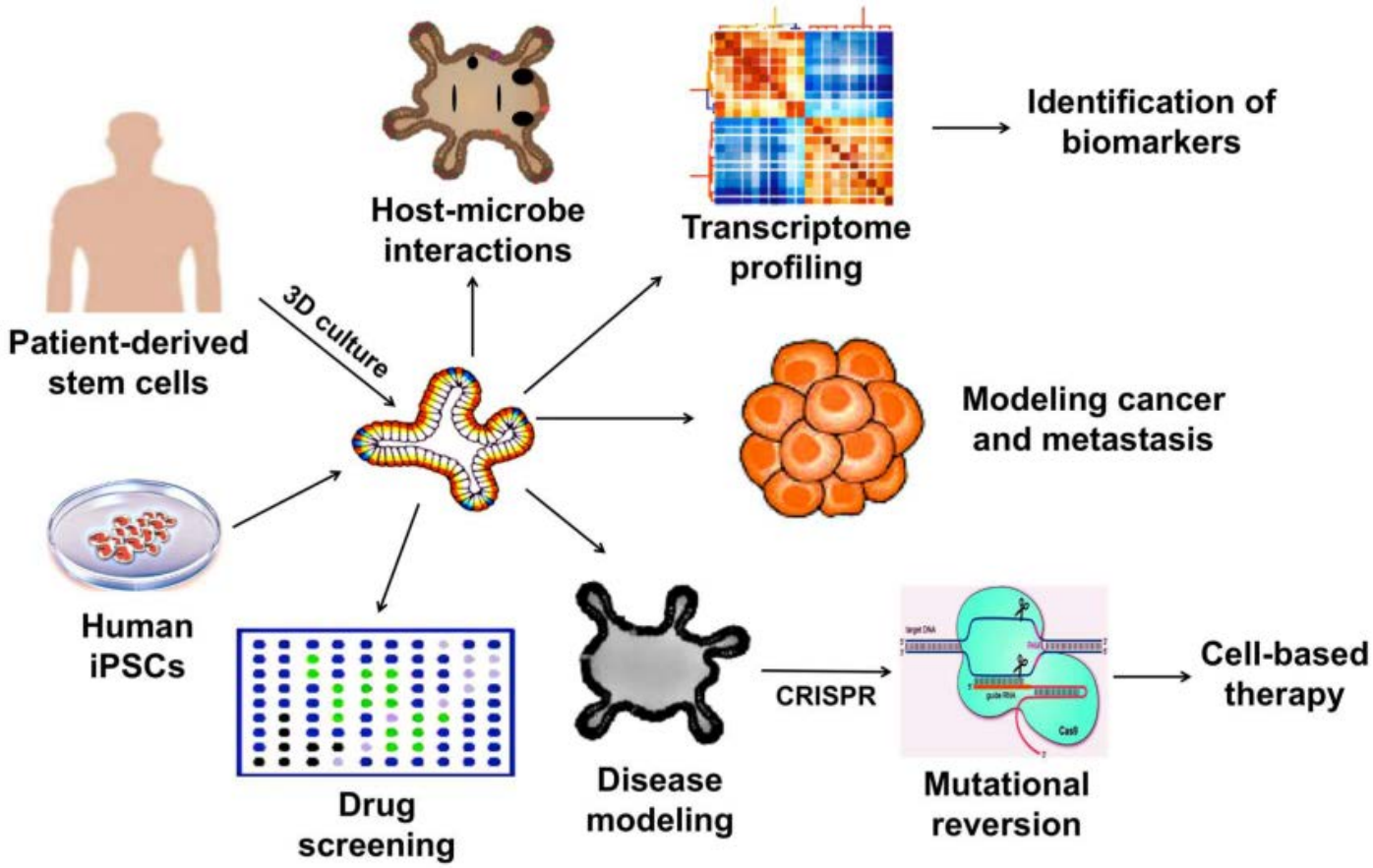
…derived from primary tissue, embryonic stem cells, or induced pluripotent stem cells that have a similar functionality as the tissue from which the cells originate



Simian M and Bissell M, 2017 J. Cell Biol. 216:31.

- Near-physiological models to study human development and diseases
- More advanced organoid cultures will allow for screening platforms for drug discovery that are:
  - More cost-effective than animal models
  - Precise models of human diseases that cannot be recapitulated in animals

iPSCs and organoids



Alhaque, et al., 2018, Trans Roy Soc B 373, 20170216, 2018

Recently established disease models involving human induced pluripotent stem cell (hiPSC)-derived organoid culture systems

Tissue/Organ	Disease Modeled	References
Brain	Zika virus and congenital brain malformations	Kelava et al., 2016 [3]; Dang et al., 2016 [11]; Garcez et al., 2016 [12]; Cugola et al., 2016 [14]
	Primary microencephaly	Kelava et al., 2016 [3]; Dang et al., 2016 [11]; Lancaster et al., 2013 [17]; Li et al., 2017 [20]
	Autism/macrocephaly Alzheimer's disease Parkinson's disease	Mariani et al., 2015 [21] Raja et al., 2016 [22] Monzel et al., 2017 [23]
Liver	Alagille syndrome A1AT deficiency Cystic fibrosis	Guan et al., 2017 [24]; Gomez et al., 2016 [25]
Pancreas	Cystic fibrosis Pancreatic ductal adenocarcinoma Diabetes mellitus	Hohwieler et al., 2017 [26] Huang et al., 2015 [27]; Baker et al., 2016 [28] Kim et al., 2016 [29]
Intestinal	Host–microbe interactions e.g., human norovirus	Finkbeiner et al., 2012 [30]; Yin et al., 2015 [31]; Ettayebi et al., 2016 [32]
	Cystic fibrosis (CF)	Dekkers et al., 2013 [33]; Schwank et al., 2013 [34]
	Colorectal cancer	Drost et al., 2015 [35]; van de Wetering et al., 2015 [36]
	Host–microbial interactions (e.g., *Helicobacter pylori*)	Finkbeiner et al., 2012 [30]; Huang et al., 2015 [27]; Amieva et al., 2016 [37]; Boj et al., 2017 [38]
Stomach	Cancer	Takasato et al., 2015 [39]
Kidney	Polycystic kidney disease Ovarian cancer	Freedman et al., 2015 [40] Yucer et al., 2017 [41]; Lawrenson et al., 2013 [42]
Urological	Prostate cancer	Gao et al., 2014 [43]; Gao et al., 2015 [44]
Lung	Fibrotic lung disease	Dye et al., 2015 [45]; Barkauskas et al., 2017 [46]; Chen et al., 2017 [47]
Retinal	Leber congenital amaurosis (LCA), Retinitis pigmentosa, Age-related macular degeneration	Wahlin et al., 2017 [48]; Llonch et al., 2018 [49]; DiStefano et al., 2018 [50]

3D cell culture: intestinal organoids

Human iPS Cell Culture

↓

iPSC embryoid body formation

6 days ↓ Spheroid microplate

Definitive Endoderm Differentiation

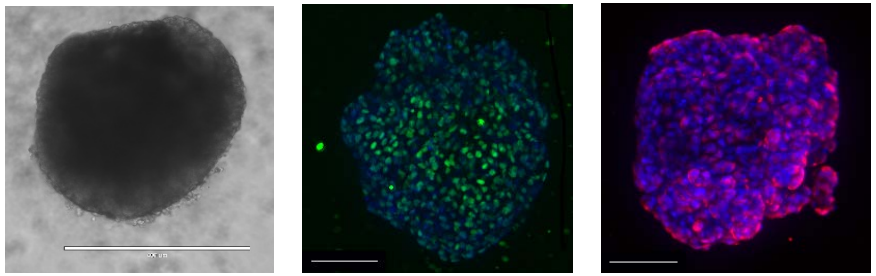
4 days ↓ Spheroid mciroplate

Mid/hindgut differentiation

28 days ↓ Spheroid microplate + Matrigel® matrix

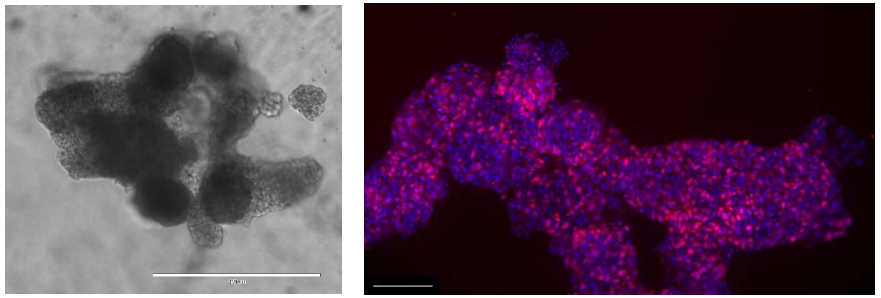
Intestinal formation

Definitive Endoderm



Representative photomicrographs of definitive endoderm spheroids. SOX17 (middle) and CXCR4 (right) expression with Hoechst nuclei counterstain.

Mid/Hindgut



Representative photomicrographs of mid/hindgut spheroids. CDX2 stained spheroid with Hoechst nuclei counterstain (right).

Screening iPSC-derived intestinal organoids in spheroid plates

iPSCs

↓ (6 days)

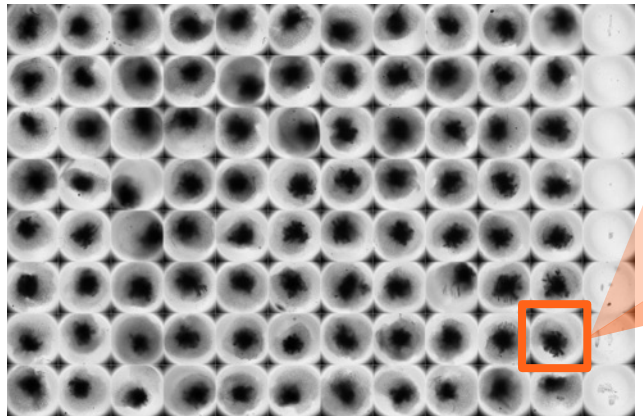
Definitive Endoderm

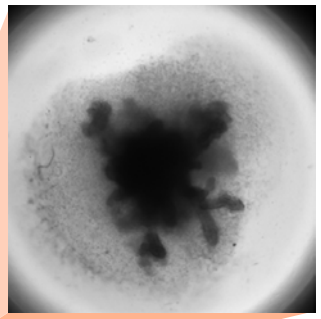
↓ (4 days)

Mid/Hindgut

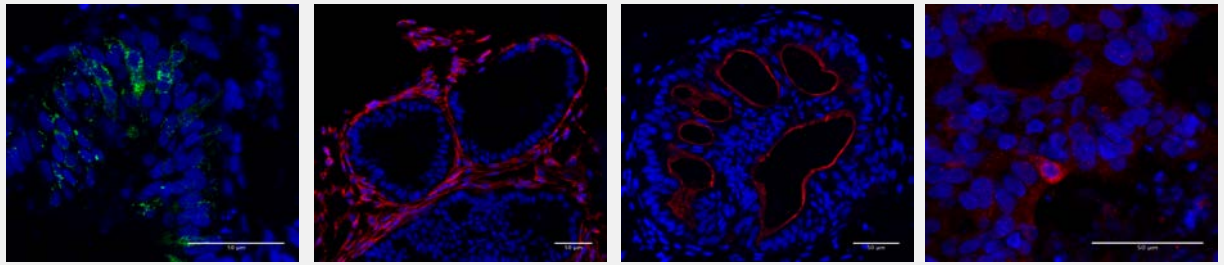
↓ (28 days)

Intestinal Organoids





- Single organoid per well
- Consistent formation
- Suitable for imaging

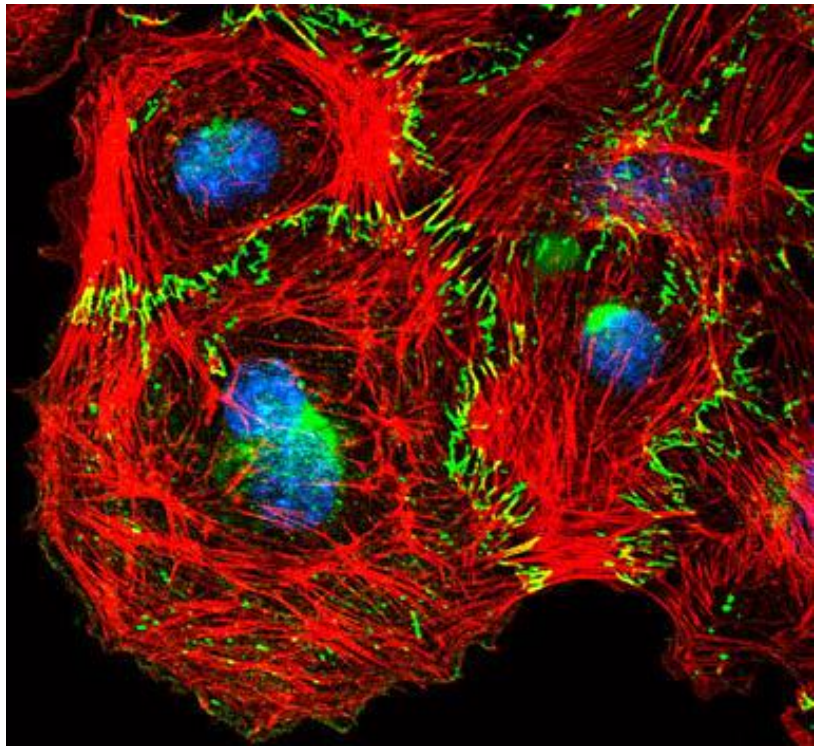


Muc2 (goblet cells) | **Vimentin (mesenchymal cells)** | **Villin (enterocytes)** | **Lysozyme (paneth cells)**

License required to perform work on intestinal organoids from Hubrecht Organoid Technology

Patent#:
US 8,642,339,B2

At our core, we are a cell biology company – leading through our expertise in innovation and manufacturing


Market Leader, Cell Culture Innovator

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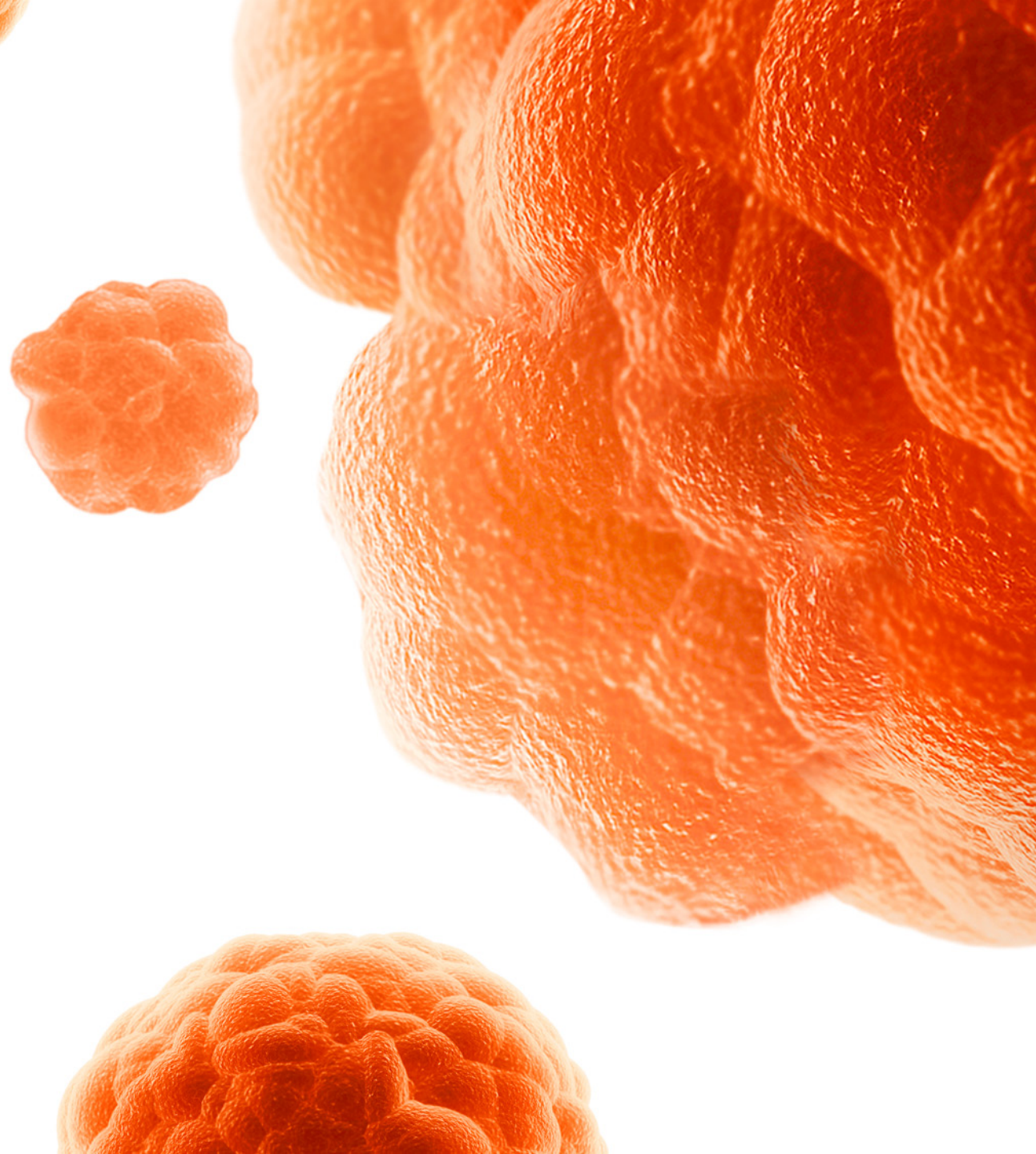
- Feng Li
- Allison Tanner
- Greg Martin
- Vasiliy Goral
- Mark Rothenberg



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