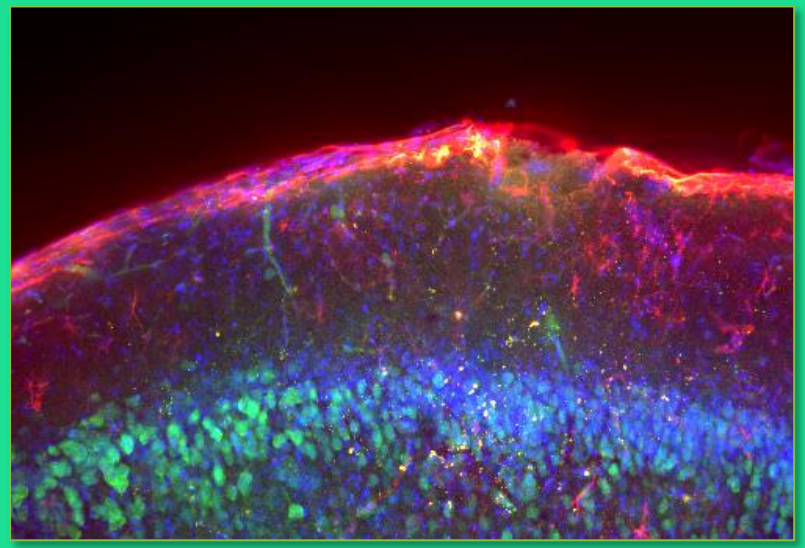

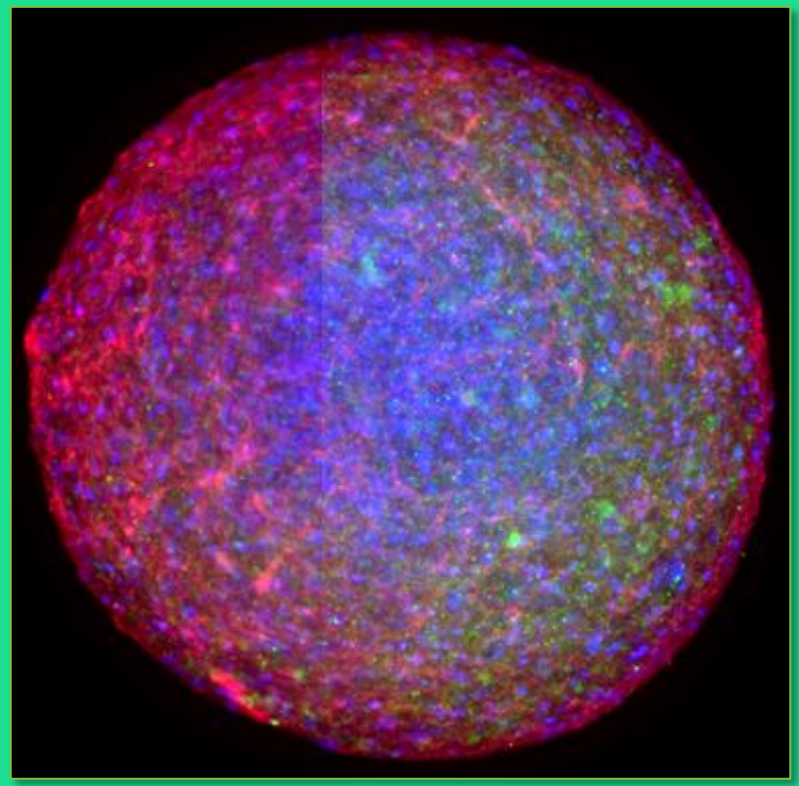

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Precision Cut Liver Slices (PCLS) for use in NASH and Dili Studies

Overview

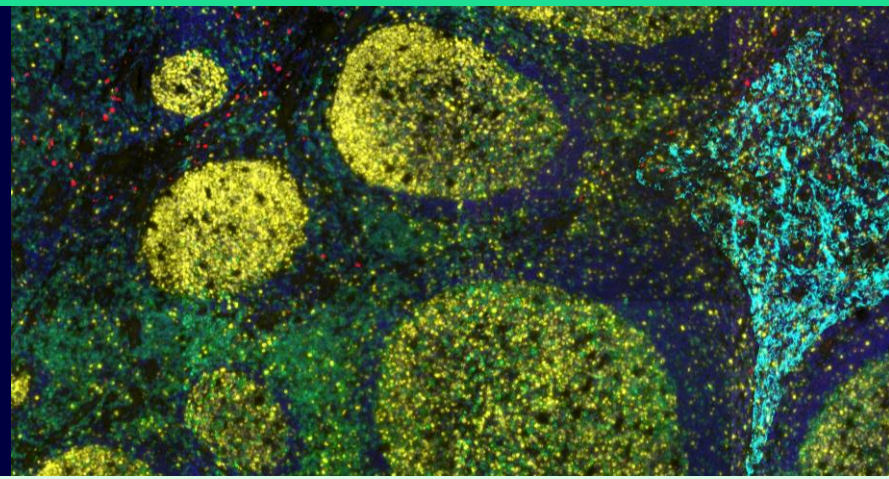
- Introduction to Visikol and BICO
- Introduction to Advanced Cell Culture Models
- Precision Cut Liver Slices (PCLS)
  - Variations of model
  - Standard assay format

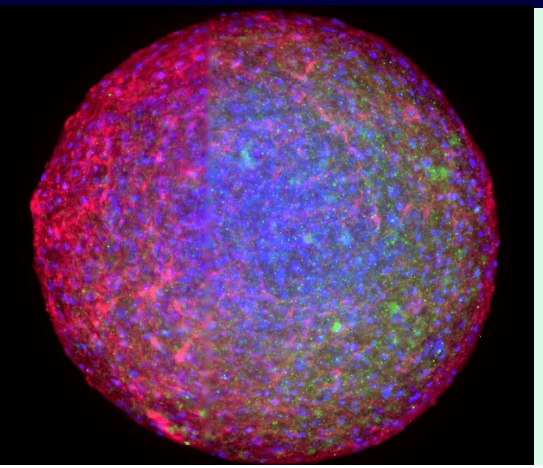
About Visikol

Industry leader in advanced models and tissue imaging focused on improving and accelerating drug discovery and development through contract research services

Worldwide customer base of startups, small, medium, big pharma, and prestigious research institutions.

Expertise in Drug Discovery, Assay Design, Oncology, Immunology, Inflammation, and Liver Disease.





Advanced Imaging

3D Tissue Imaging, Multiplex IF, Digital Pathology, Histology, and Slide Scanning

Advanced Cell Culture

2D and 3D Cell Culture Models and Assays, Ex Vivo Tissue Assays, High Content Imaging, Custom Models and Assays

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BICO: The Bio Convergence Company

Employees: 234
Offices: 9
CELLINK A BICO COMPANY

Employees: 87
Offices: 2
MATTEK A BICO COMPANY

Employees: 32
Offices: 1
VISIKOL A BICO COMPANY

Employees: 5
Offices: 1
ADVANCED BIOMATRIX A BICO COMPANY

Employees: 80
Offices: 3
nanoscribe A BICO COMPANY

Employees: 75
Offices: 1
BIOSERO A BICO COMPANY

Employees: 75
Offices: 1
CYTENA A BICO COMPANY

Employees: 18
Offices: 1
CYTENA BPS A BICO COMPANY

Employees: 77
Offices: 2
DISPENDIX A BICO COMPANY

Employees: 46
Offices: 1
ECHO A BICO COMPANY

Employees: 158
Offices: 4
SCIENION A BICO COMPANY

Employees: 34
Offices: 1
CELLENION A BICO COMPANY

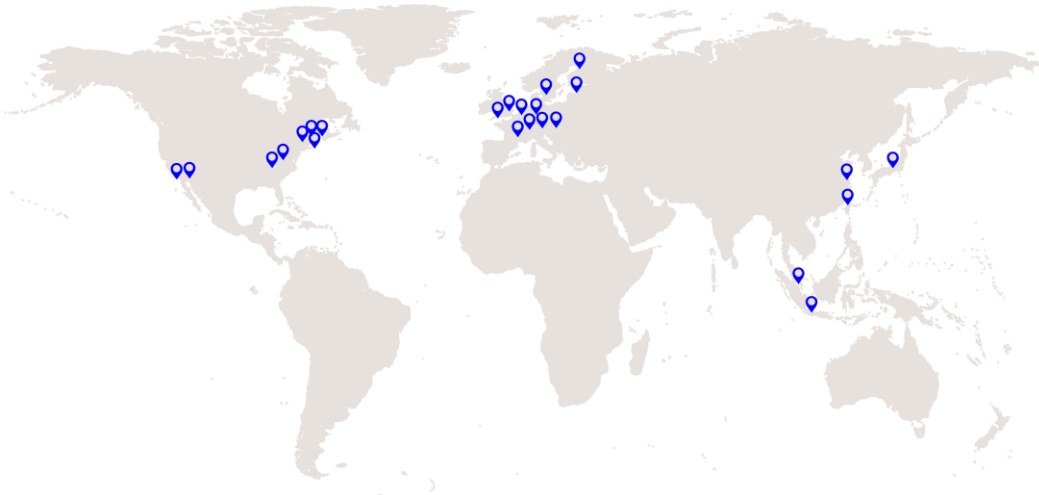
Employees: 107
Offices: 8
GINOLIS A BICO COMPANY

Employees: 28
Offices: 1
QINSTRUMENTS A BICO COMPANY

15
Companies

38
offices

1,200+
Employees

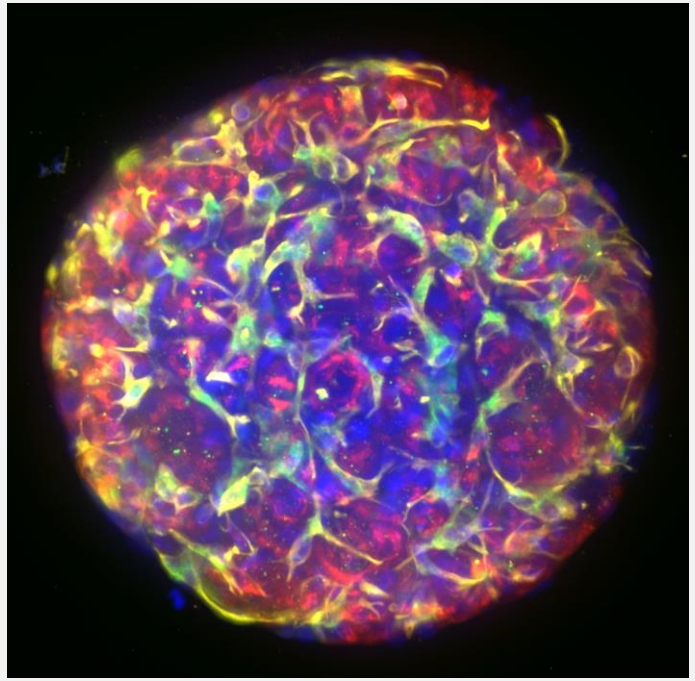


Employees: 30
Offices: 1
BICO

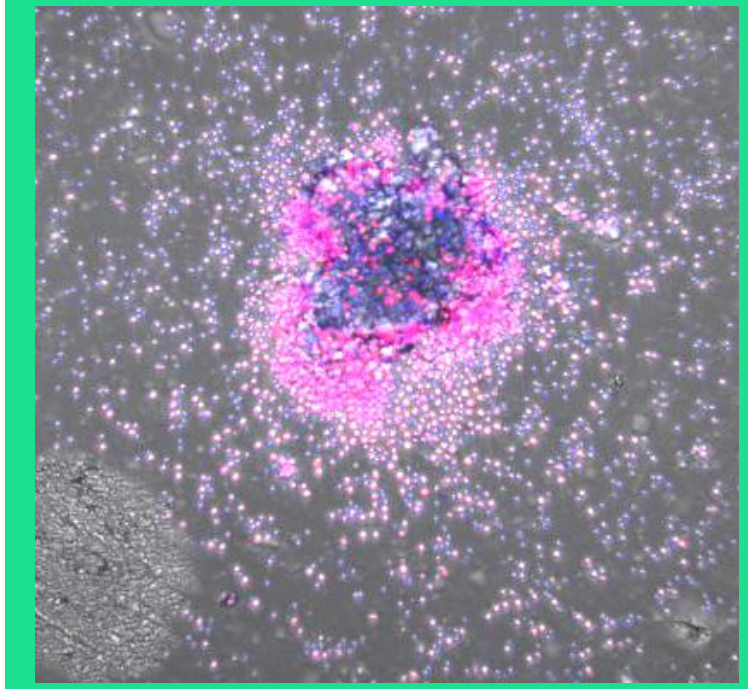
Advanced Cell Culture

Core focus in liver and oncology models as well as custom assay and model development.

Liver Cell Culture Models and Assays



Oncology Models and Assays



Custom Cell Culture Assays

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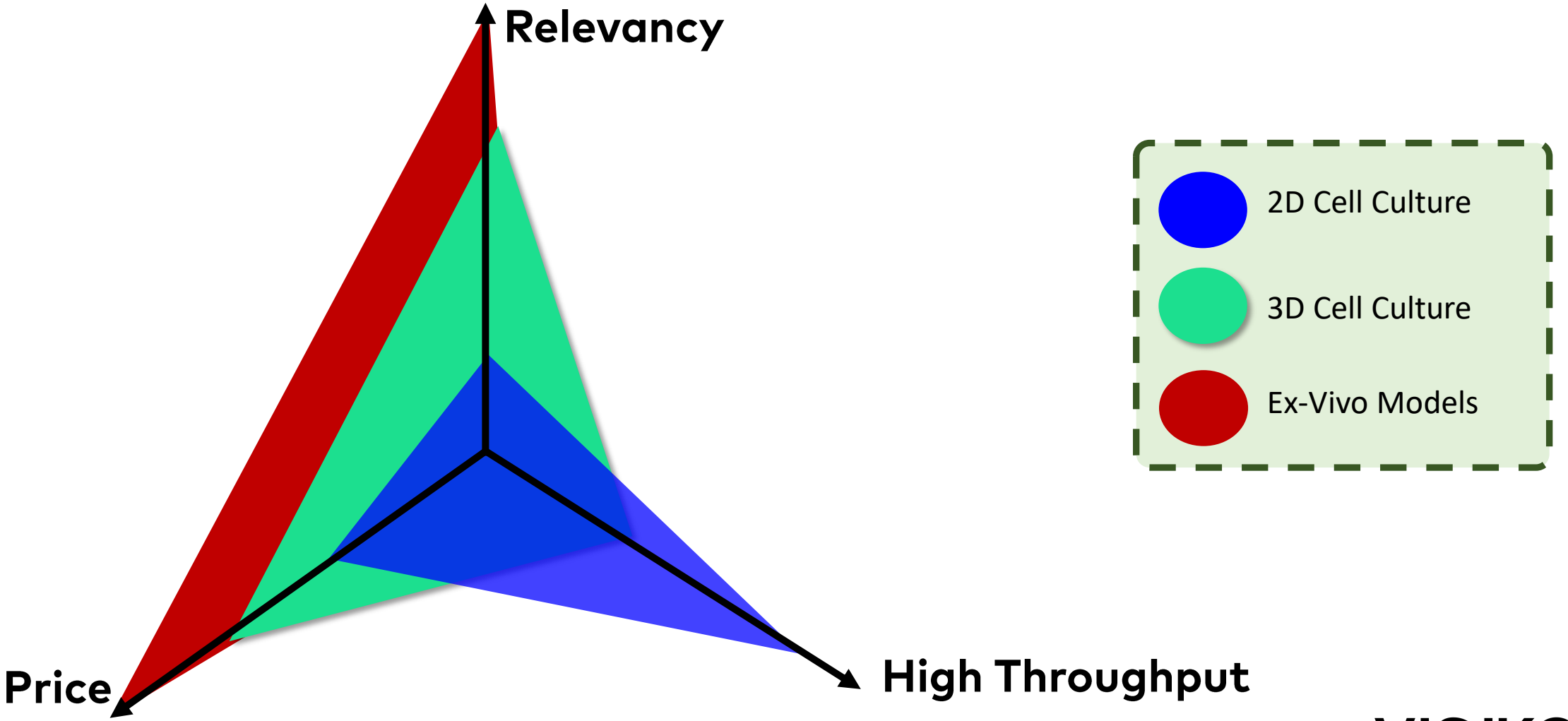
Liver Cell Culture Assays and Models

Diverse suite of models to balance price, throughput and in vivo relevancy



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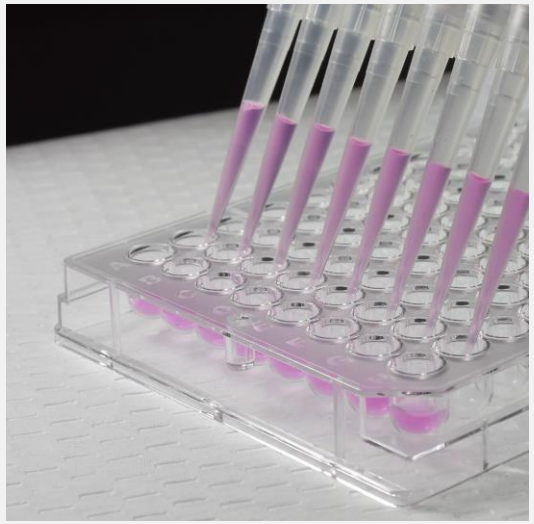
Liver Cell Culture Assays and Models

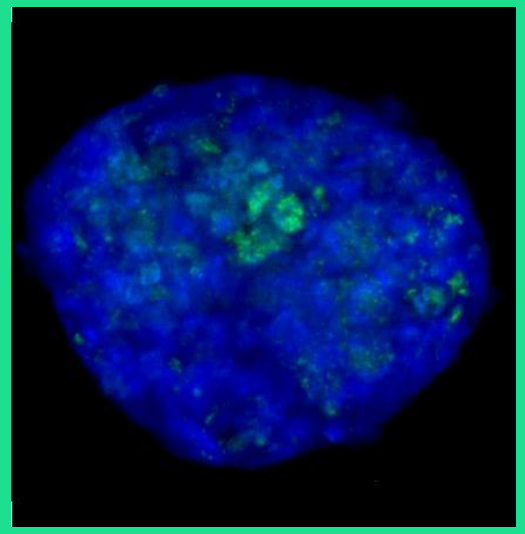
- Hepatoxicity/DMPK Studies: mitochondrial, metabolic, immune infiltration and efficacy
- Liver disease models: NAFLD, Steatosis, NASH, Fibrosis

2D Cell Culture



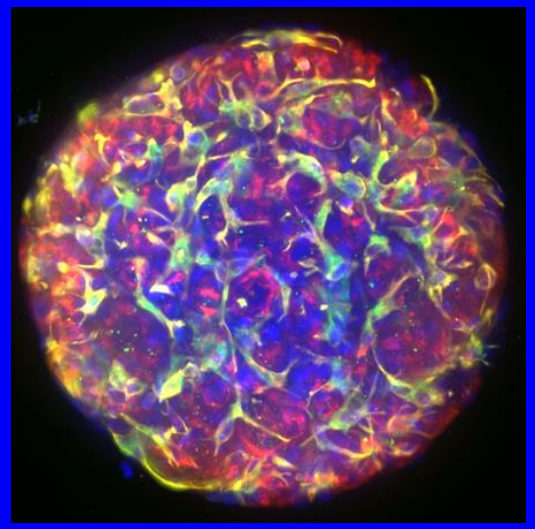
HUREL® Micro Livers
10 Species

Simple Spheroids



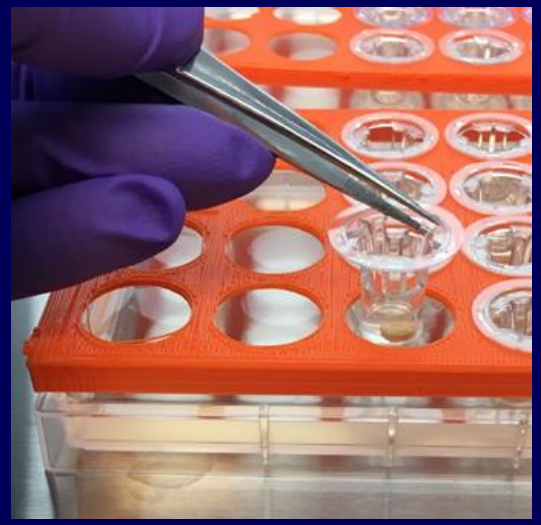
Primary Human
Hepatocytes or HepaRG

Complex Spheroids



PHH or HepaRG with
Non-Parenchymal Cells

***Ex vivo* Tissue Slices**



Human, Rodent, Feline,
Canine Assays

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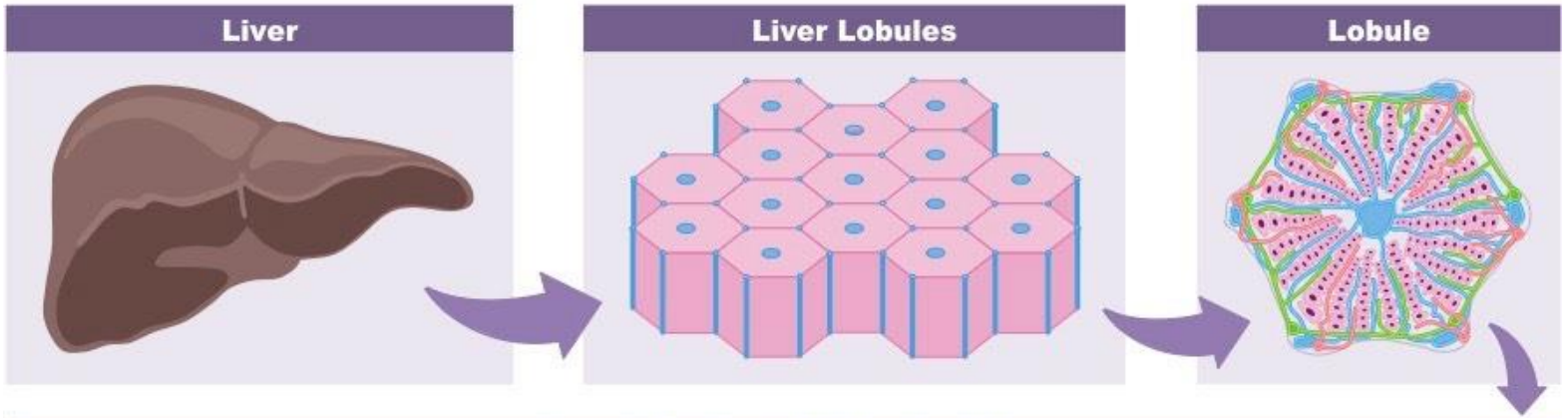
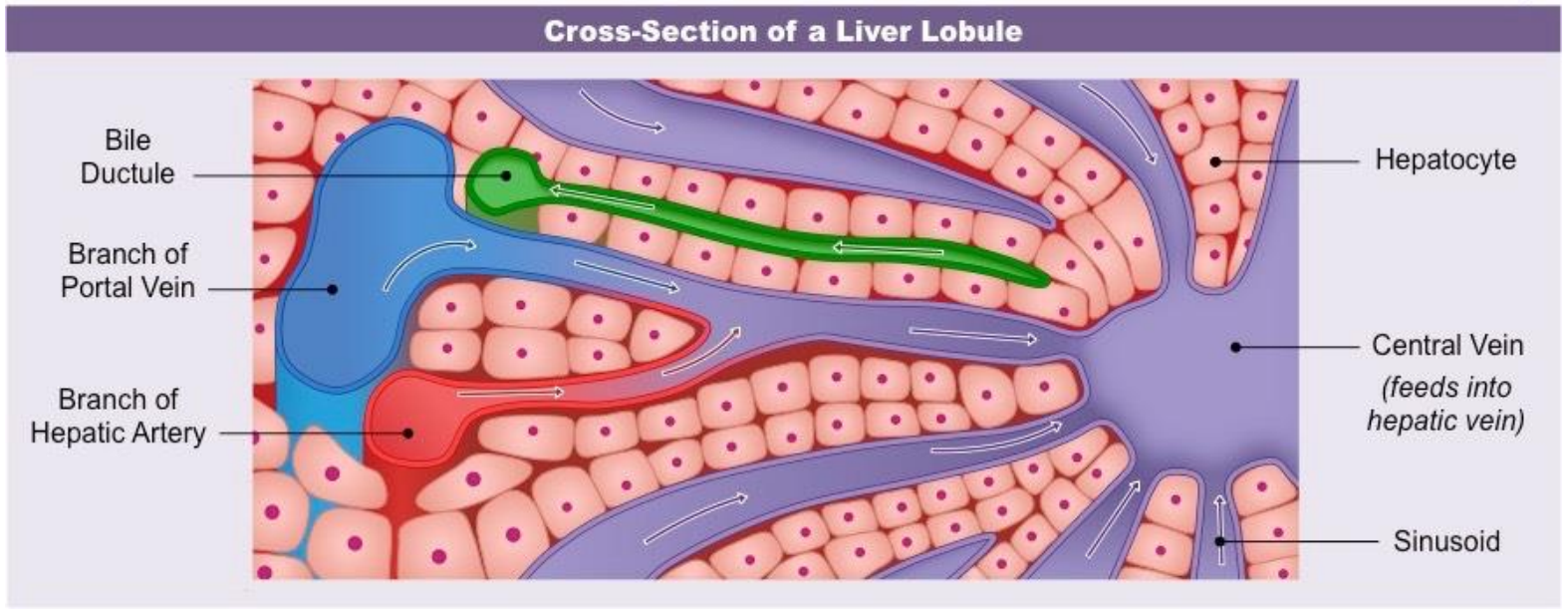
Liver Anatomy & Physiology





Gaskell, et al. Toxicol. Res., 2016

Structure

- Hepatocytes, nonparenchymal cells
- Sinusoids
- Canaliculi (→ bile ductule)

Select Liver Functions

- Filter blood
- Produce bile for digestion
- Produce plasma proteins
- Store glycogen
- Breakdown/clear drugs and toxic substances
- Vitamin storage

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The Need for *In Vitro* Liver Models: Liver Disease…

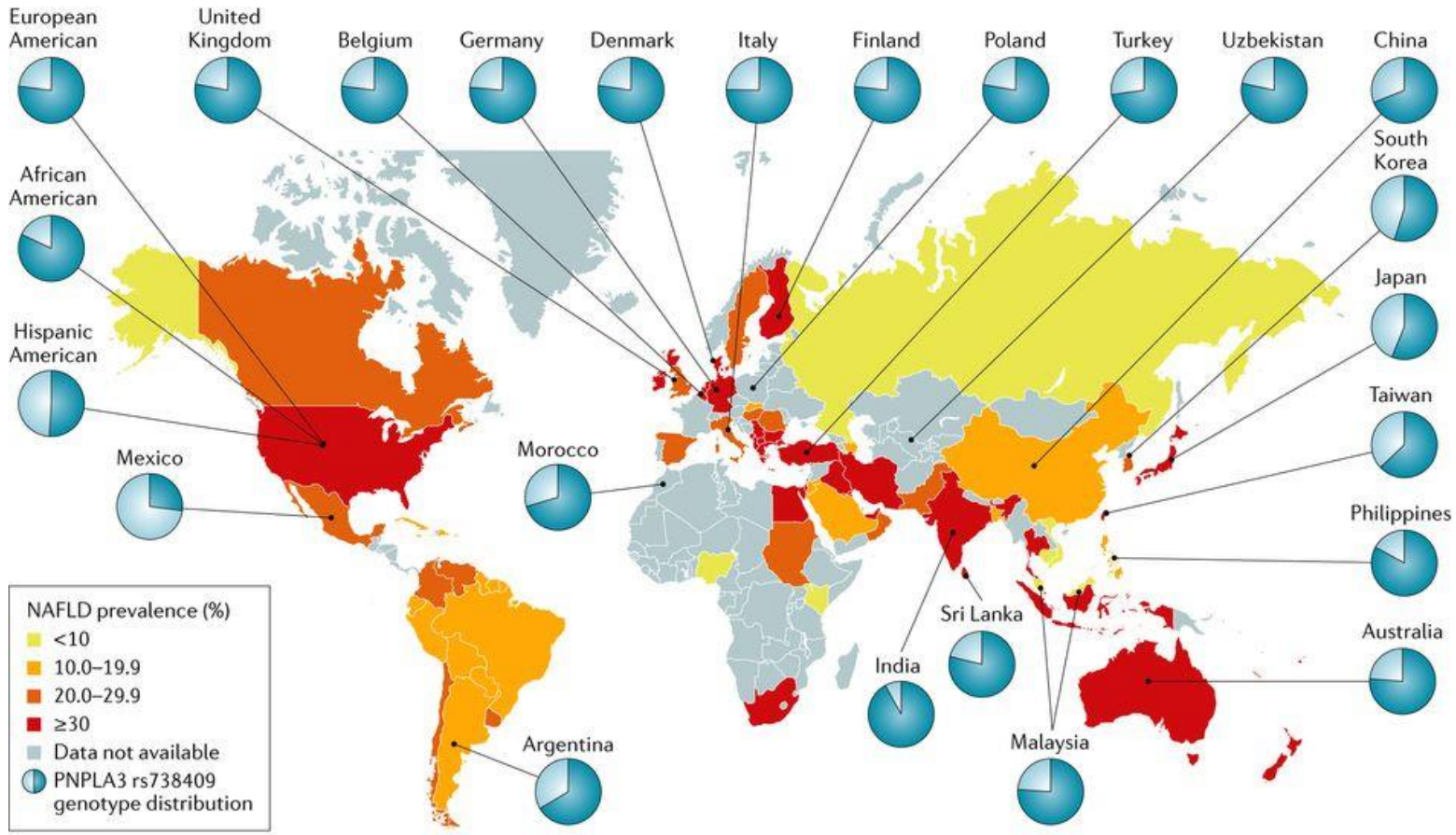
Non-alcoholic fatty liver disease (NAFLD)

64 m
Affected in US

24%
Global prevalence

$103b
direct medical costs

Can lead to steatohepatitis (NASH), fibrosis, cirrhosis, liver-related mortality, hepatocellular carcinoma



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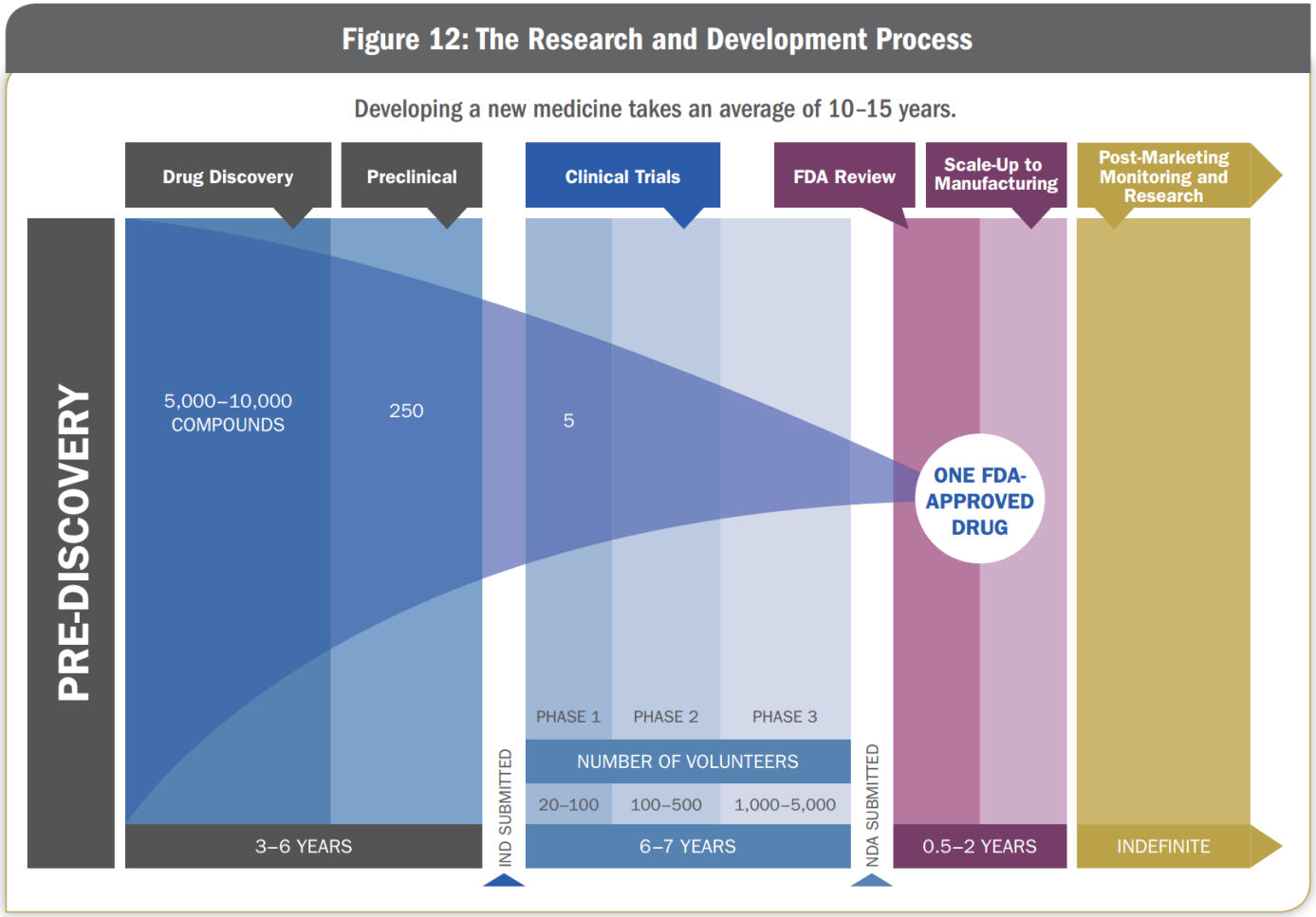
The Need for *In Vitro* Liver Models: … and Liver Injury!

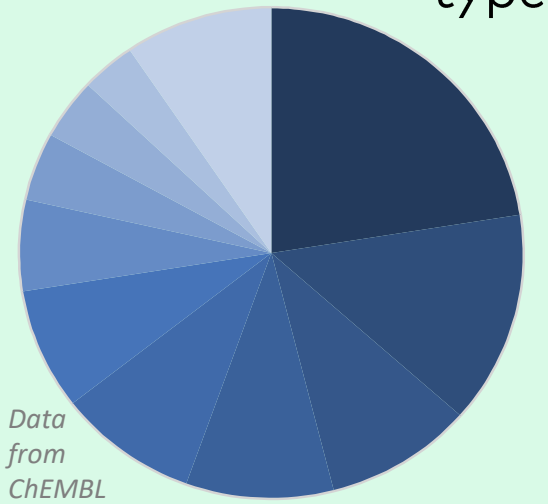


SOURCE: Pharmaceutical Research and Manufacturers of America, Drug Discovery and Development: Understanding the R&D Process, www.innovation.org.

Hepatotoxicity testing

High cost; risk → need for high-throughput, low-cost models for more effective early-stage screening

Post-market withdraw toxicities by type



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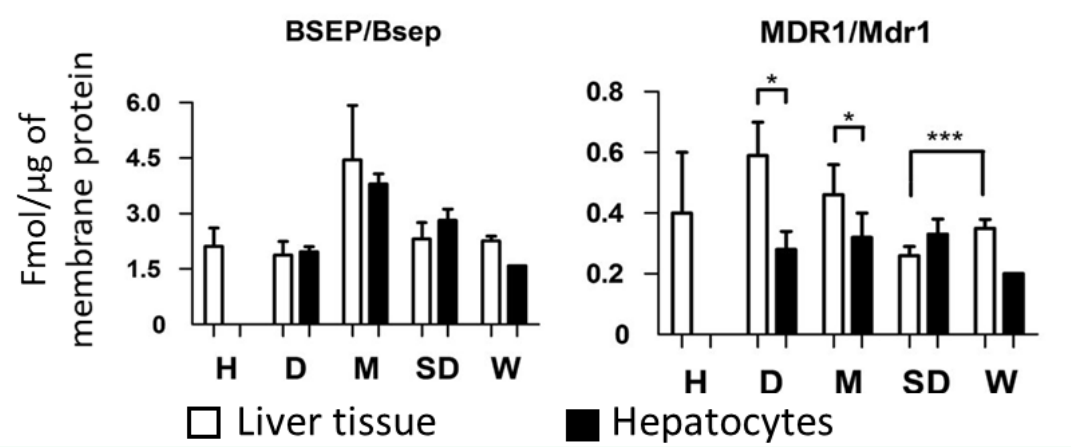
The Need for *In Vitro* Liver Models: *Why In Vitro?*

In comparison with in vivo animal models, in vitro models…

- Are less expensive
- Can be higher throughput
- Minimize ethical concerns
- Can enable greater translational relevance

Hepatobiliary transporter expression



Wang, et al. *Drug Metabolism and Disposition*, 2015.

Metabolizing enzyme inducibility

The effect of rifampin, dexamethasone and phenobarbital on human, minipig, beagle dog and rat hepatocyte CYP3A activity in vitro

Species	*Rifampin*	*Dexamethasone*	*Phenobarbital*
Man	++++	+	+++
Minipig	++	–	??
Beagle dog	++	–	??
Rat	(+)	+++	++

(–) no induction; (+) marginal induction; (++) medium induction; (+++) strong induction; (++++) very strong induction; (??) not studied.

Pasanen, et al. Real Academia Nacional de Farmacia, 2004.

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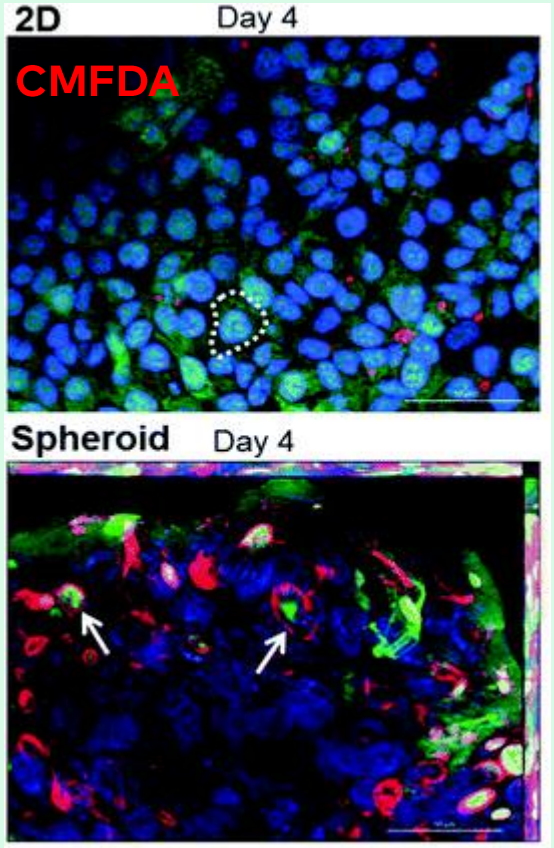
The Need for *In Vitro* Liver Models: *Why 3D?*

Bile Canaliculi Formation

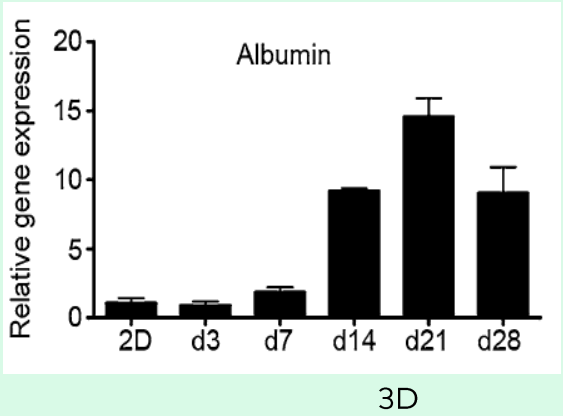


Gaskell, et al. *Toxicol. Res.*, 2016

2D vs 3D In Vitro Liver Models

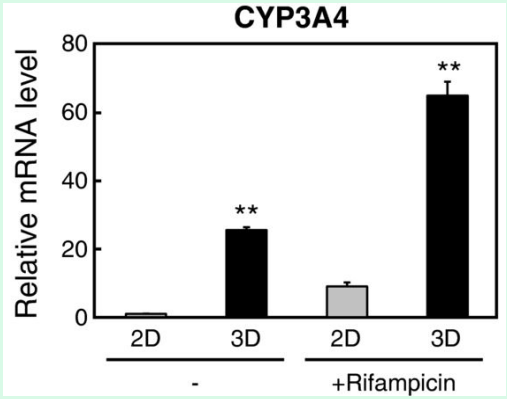
Despite throughput differences, cell-cell and cell- extracellular matrix contact can facilitate many functional differences

Albumin Secretion



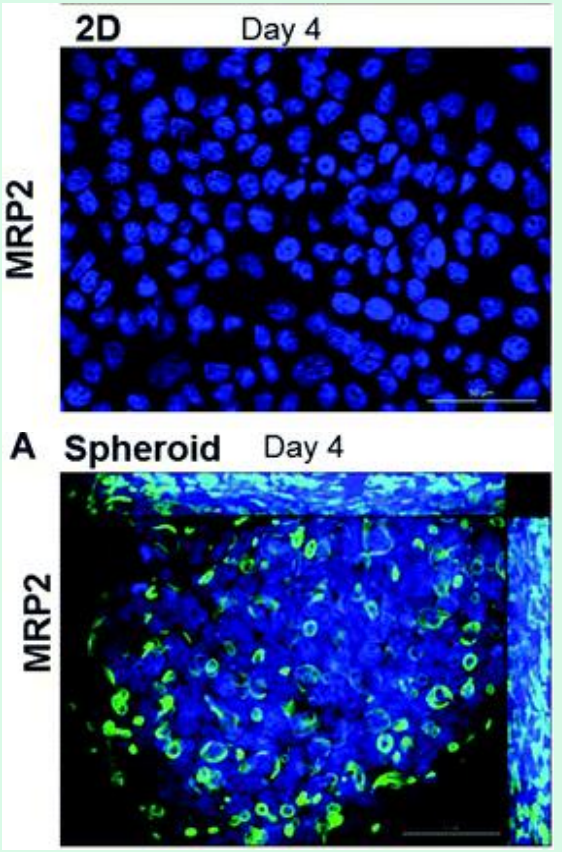
denBraver, et al. *Arch. of Toxicol.* 2014

P450 Inducibility



Takahashi, et al. *Bioscience Reports*, 2015.

Transporter Expression



Gaskell, et al. *Toxicol. Res.*, 2016

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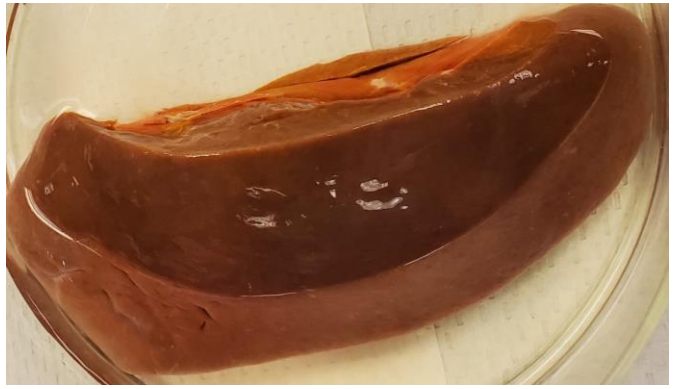
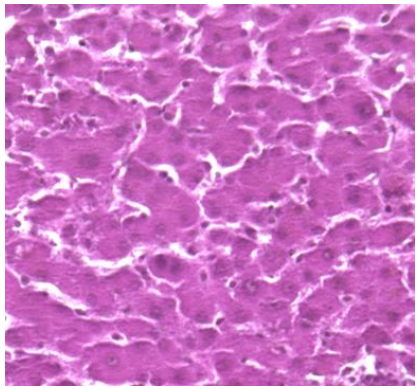
Precision Cut Liver Slices

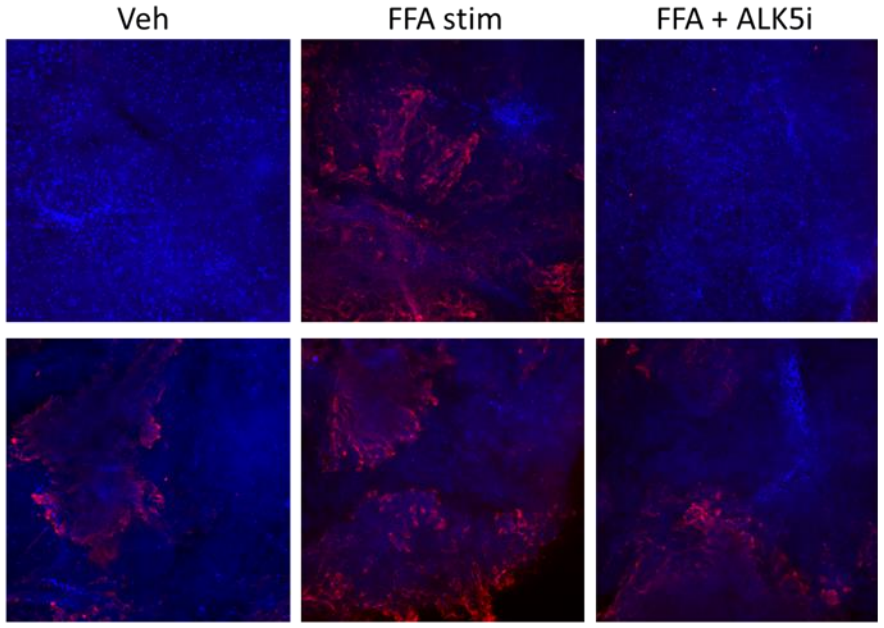
- Precision cut liver slices as an alternative in vitro model for evaluating anti-fibrotic agents
  - 3 approaches:
    - Normal human tissue, ex vivo disease state induction and therapeutic evaluation
    - Diseased human tissue, ex vivo therapeutic evaluation
    - Mouse disease model, ex vivo therapeutic evaluation

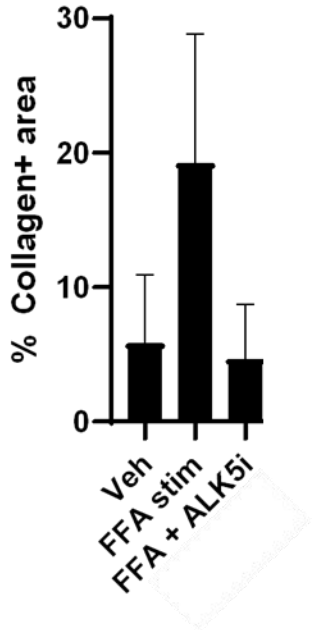
Normal Human PCLS Stimulated to Induce Disease State

- F0-F1 human liver
- Precultured for 5h
- Stimulated for 48h









- FFA stimulation induced collagen deposition
- ALK5 inhibitor treatment reduced FFA-induced collagen deposition
- Advantages:
  - Relatively easy to acquire normal liver tissue
  - Can induce disease state to relatively similar extent by using same induction protocol each experiment
- Disadvantages
  - Disease state that is induced may exhibit non-physiological architecture or pathway

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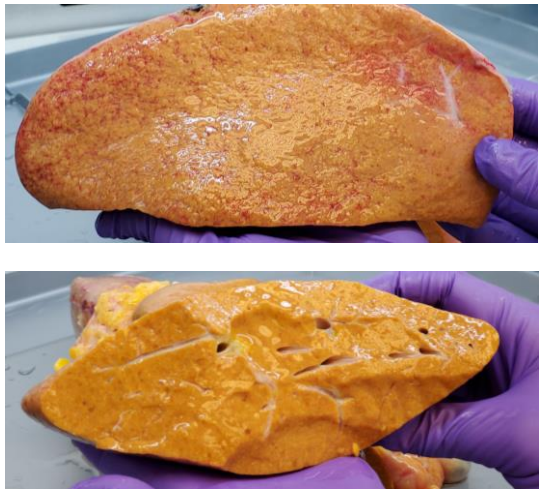
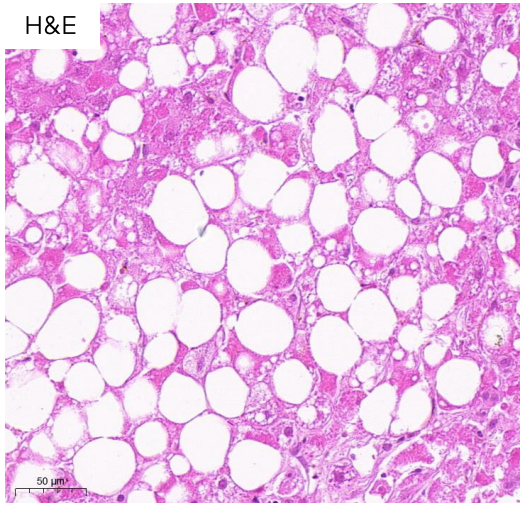
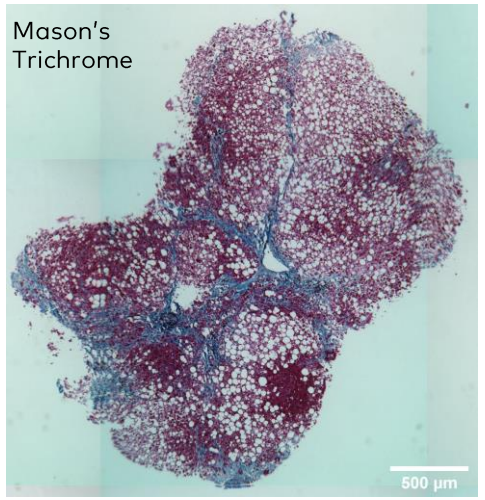
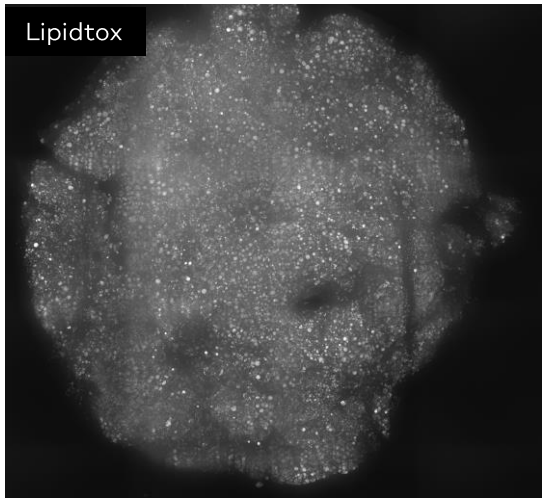
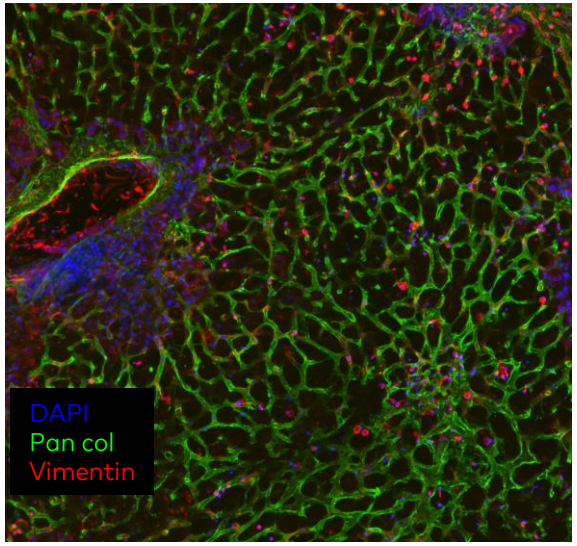
Diseased Human Liver PCLS

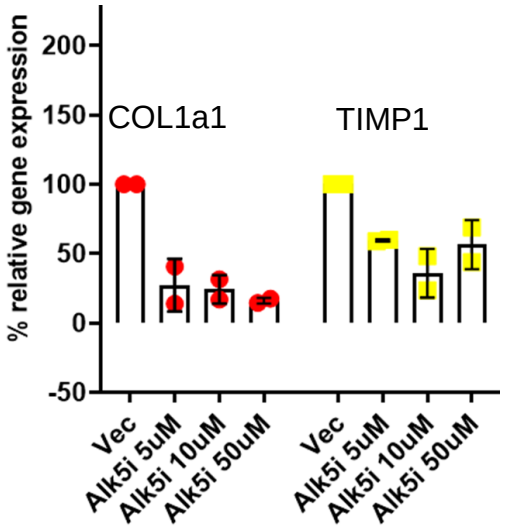


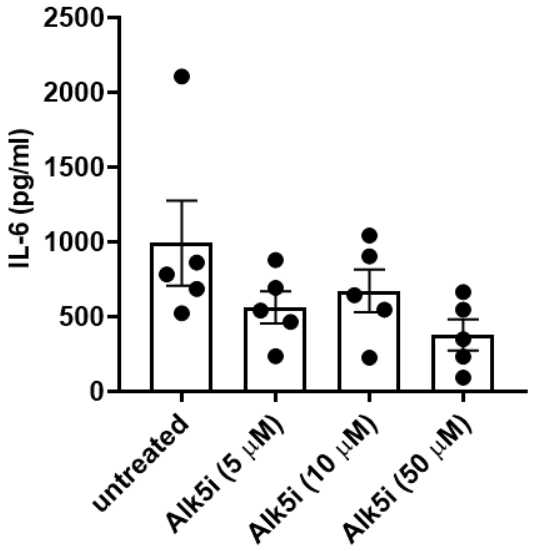












- F3 human liver, Precultured for 5h, treated for 72h with ALK5 inhibitor
- Treatment reduces expression of collagen and TIMP1 (MMP inhib)
- Advantages
  - Most physiologically relevant disease state
- Disadvantages:
  - Difficult to attain diseased liver tissues (usually acquired as explants rather than non-transplantable donor tissue)
  - Disease state variability

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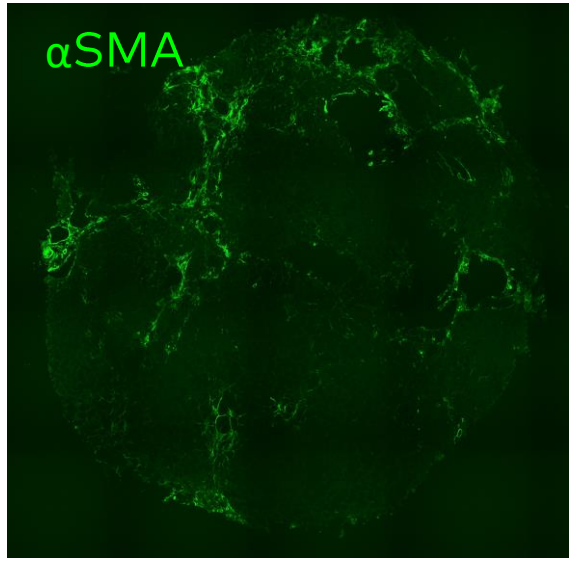
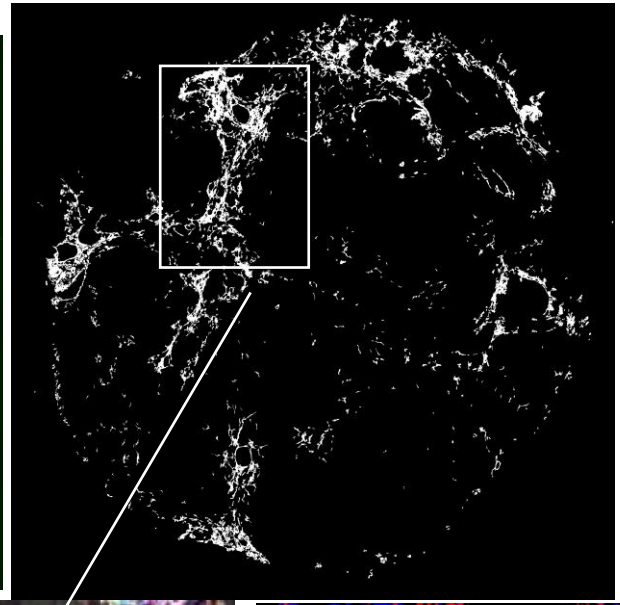
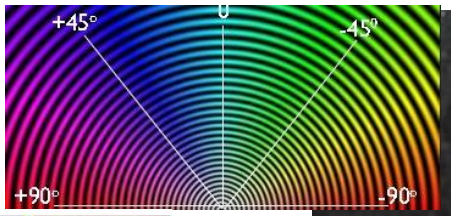
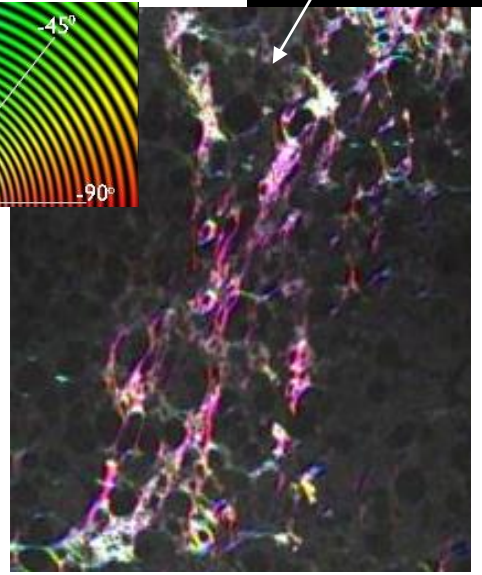
Diseased Human Liver PCLS

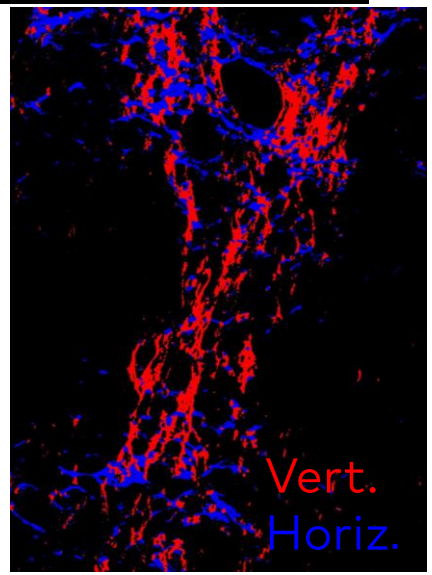
- Can leverage immunofluorescence labeling, imaging, and image analysis to quantify changes to disease-relevant features
- Example:







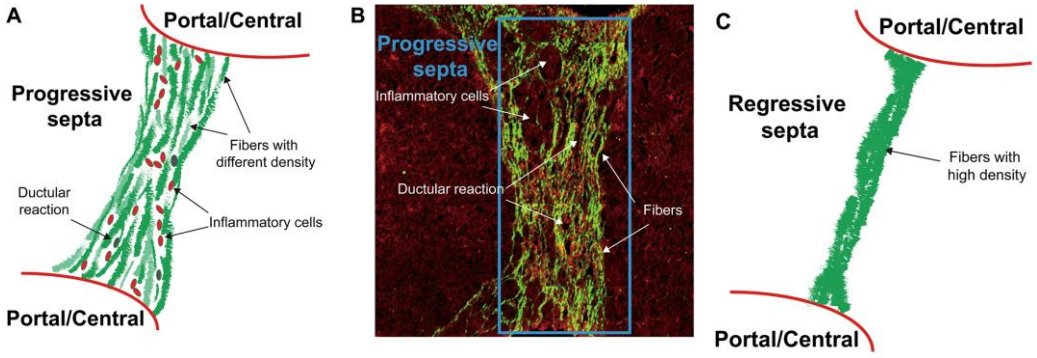




Advanced septa size quantitation determines the evaluation of histological fibrosis outcome in chronic hepatitis B patients

Bingqiong Wang, Yameng Sun, Jialing Zhou, Xiaoning Wu, Shuyan Chen, Shanshan Wu, Hui Liu, Tailing Wang, Xiaojuan Ou, Jidong Jia & Hong You

Modern Pathology **31**, 1567–1577(2018) | Cite this article



"Septal width was the most consistent indicator of prognosis in patients with fibrosis."

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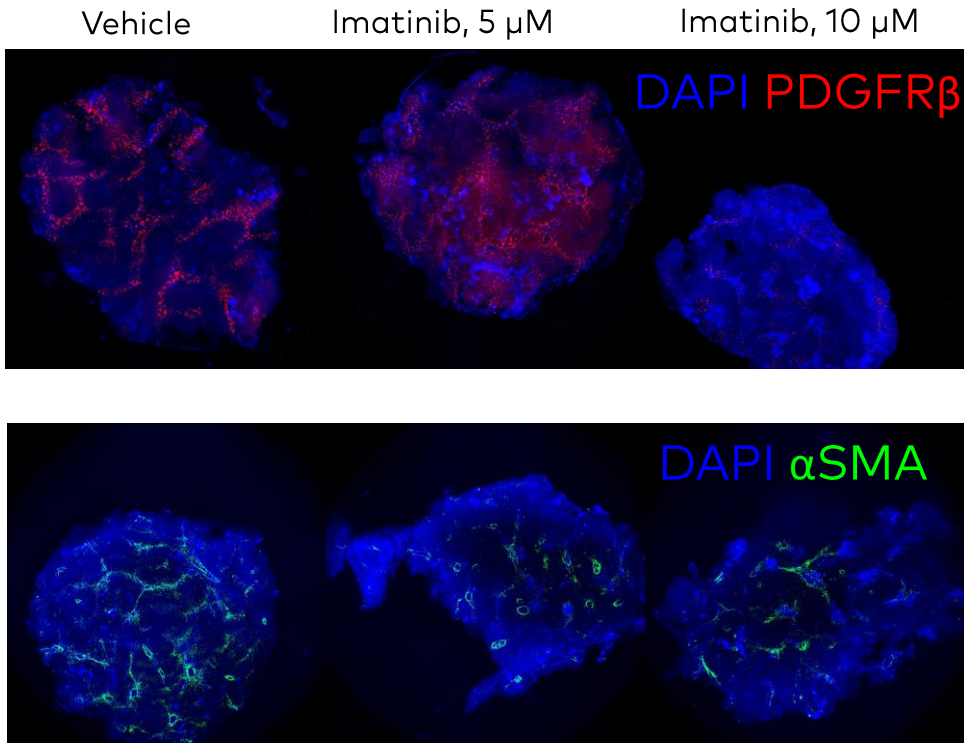
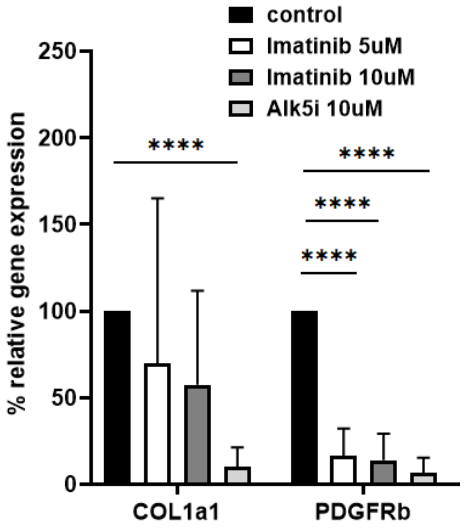
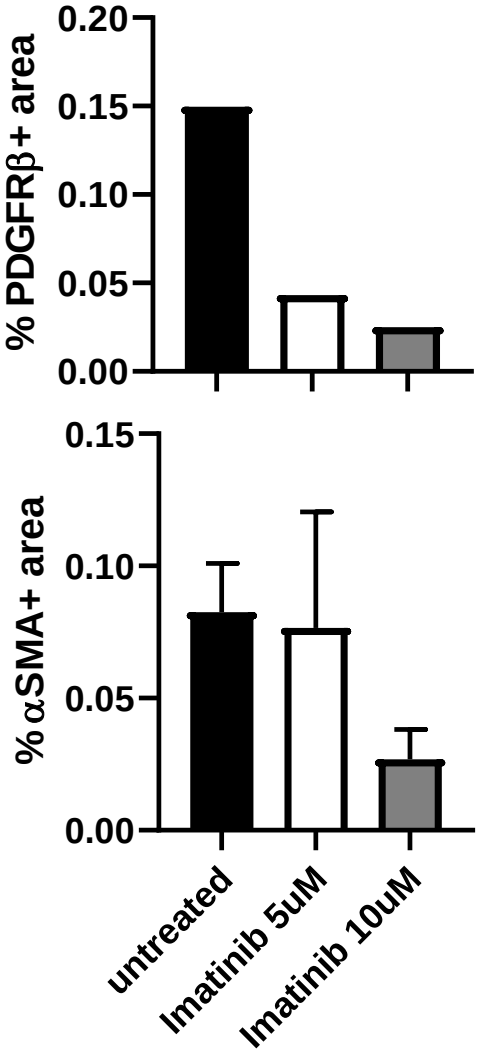
Mouse CCl_4 PCLS

- Pre-validated 14-day CCl_4 model (Melior Discovery)
- No pre-culture
- Treated for 72h with ALK5 inhibitor or imatinib
- Imatinib and ALK5 inhibitor treatment reduces collagen and PDGFRβ gene expression
- Imatinib reduces PDGFRβ and αSMA expression by immunofluorescence





- Advantages
  - Lower variability because disease state is induced using identical protocol in identical mouse strain within each experiment
  - Study can be ordered "on demand" without waiting for donor or explant tissue
- Disadvantages:
  - Murine vs human
  - CCl_4 is an injury induced fibrosis model, rather than metabolic (metabolic available via high fat diet, but duration of induction is longer, riskier, and more variable)



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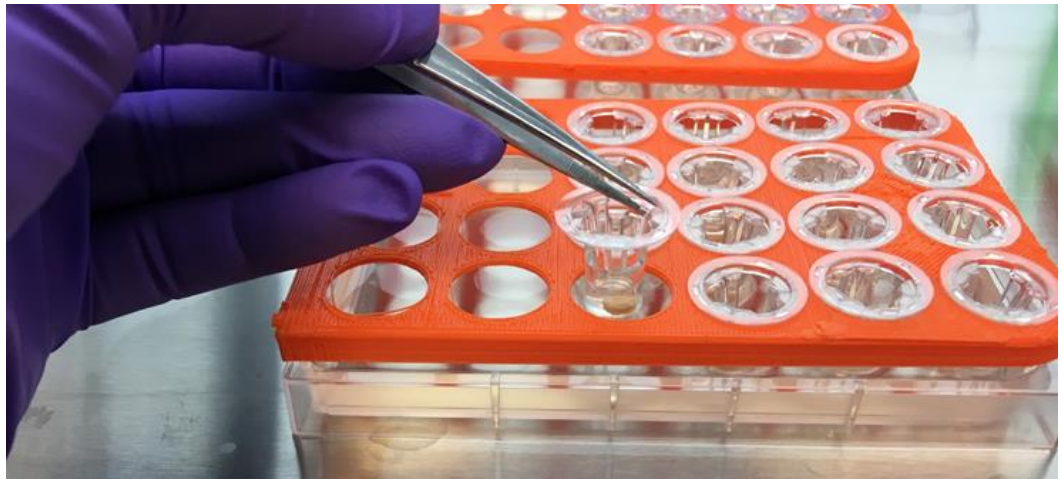
Standard Assay Format

- 432 slices – 18 24-well plates cultured for 72-hour period
- 0, 24, 48, 72 hrs – media collected
  - Analysis for PK/PD
  - ELISA for client target and albumin
- qPCR endpoints
  - First three time points for all samples
  - Client target, albumin, TIMP1, pro-collagen
- All tissues snap frozen and sent to client
- Other analysis endpoints available upon request: histology, HPLC/LCMS, high content imaging, multiplex slide imaging
- Report detailing finds delivered 2-3 weeks from completion of study



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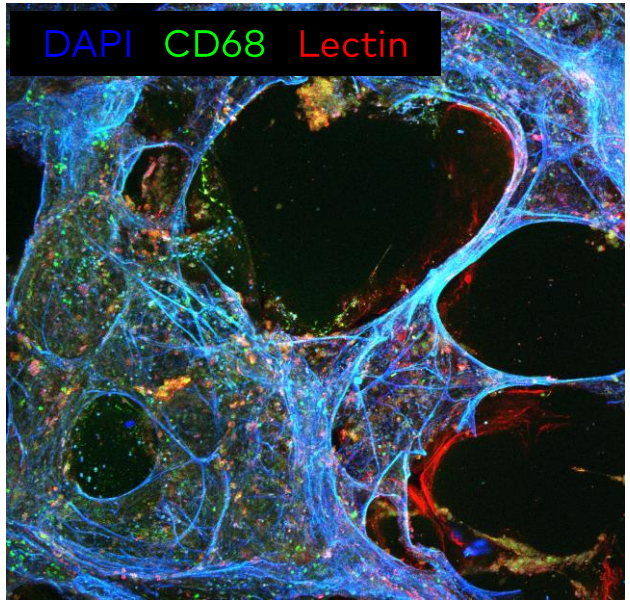
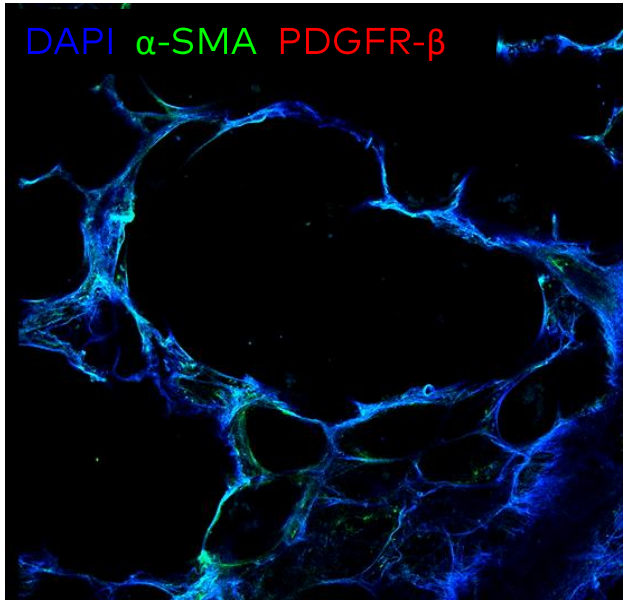
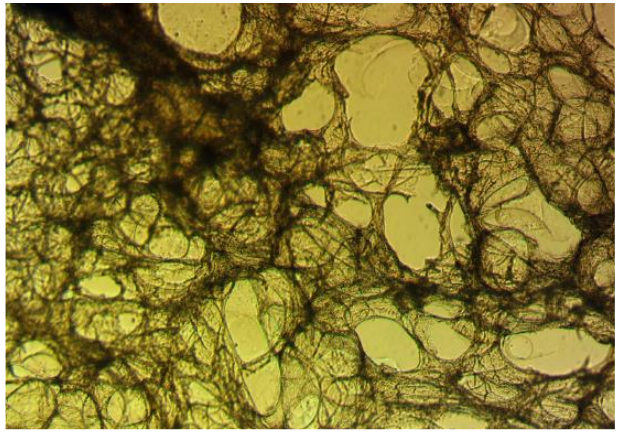
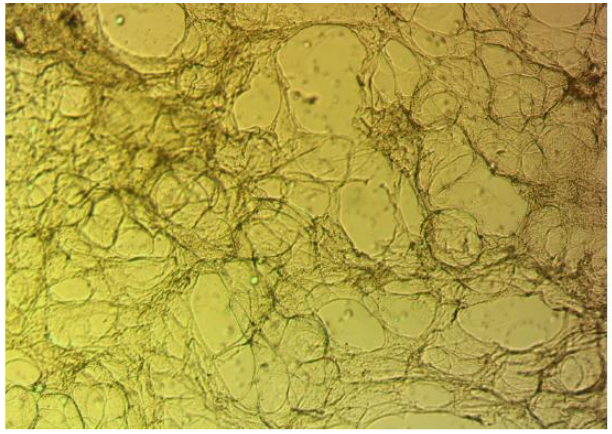
Other Assays/Tissue Types







Pre-clear PCLS



Post-clear PCLS

- Other tissue types
  - Lung (left)
  - Heart
  - Brain
  - Tumor
- Other host models
  - Human
  - Mouse, rat
  - Non-human primate
- Other endpoints
  - Traditional histology & staining (i.e. H&E, PSR, Masson's Trichrome)
  - Immunofluorescence (markers of proliferation, viability, cell subtype, cell activation, drug targets), 3D tissue imaging, and quantitative analysis
  - ELISAs on tissue digests or supernatants
  - qPCR
- More complex assays
  - Immune cell infiltration
  - Therapeutic penetration
  - Drug metabolism analysis (HPLC)

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Thanks!

Visit us at Booth #2018

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