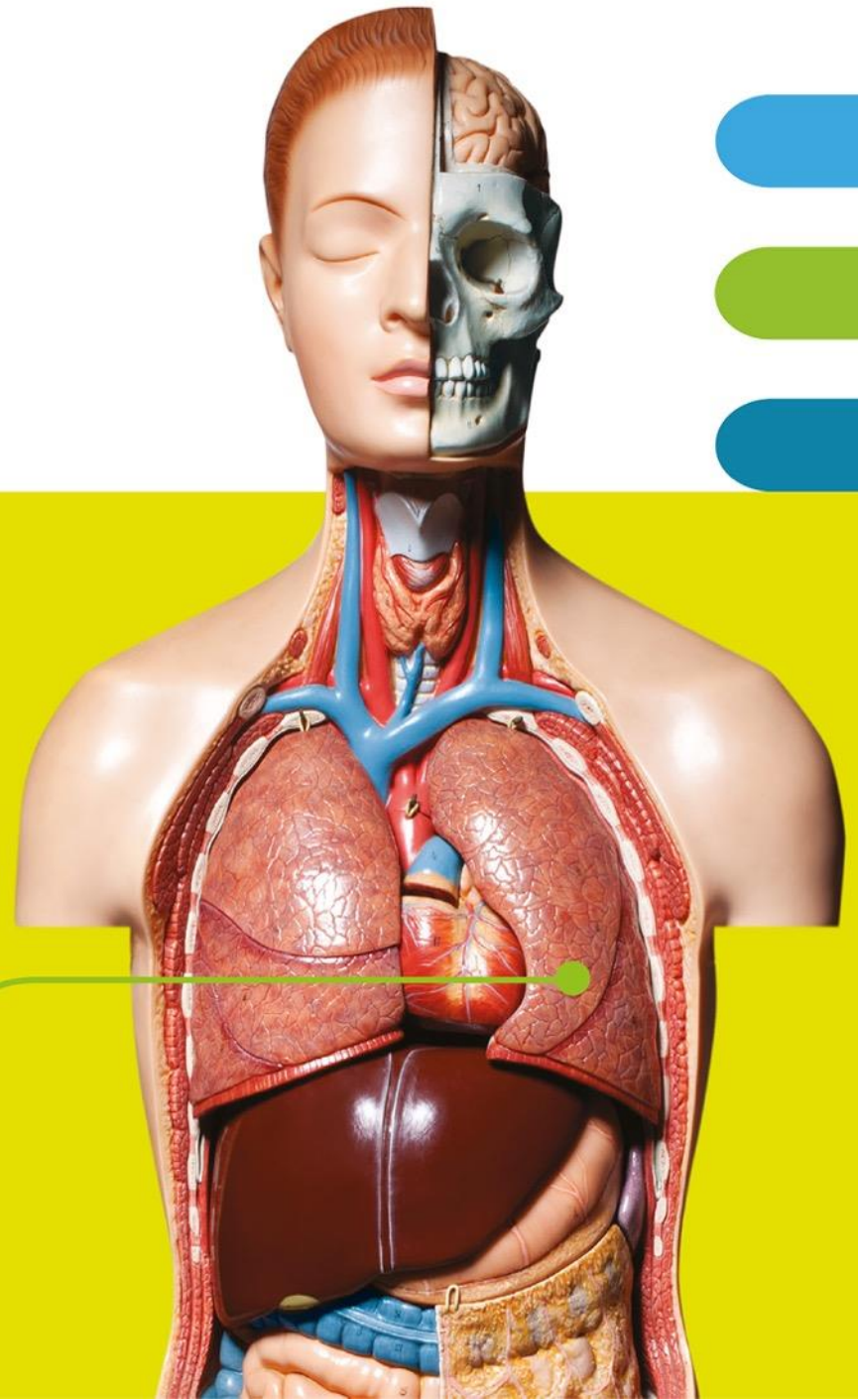


Organ-on-a-chip: practical applications & challenges

Remko van Vught

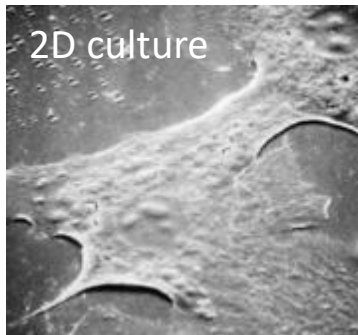
10-4-2018



A leap forward in physiological relevance

The challenge of reductionism:

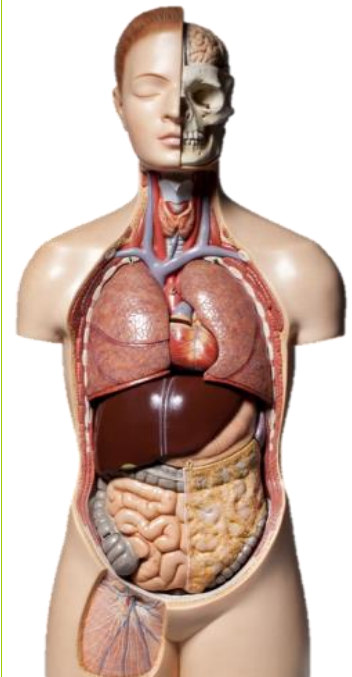
“Make things as simple as possible, but not simpler”, Albert Einstein

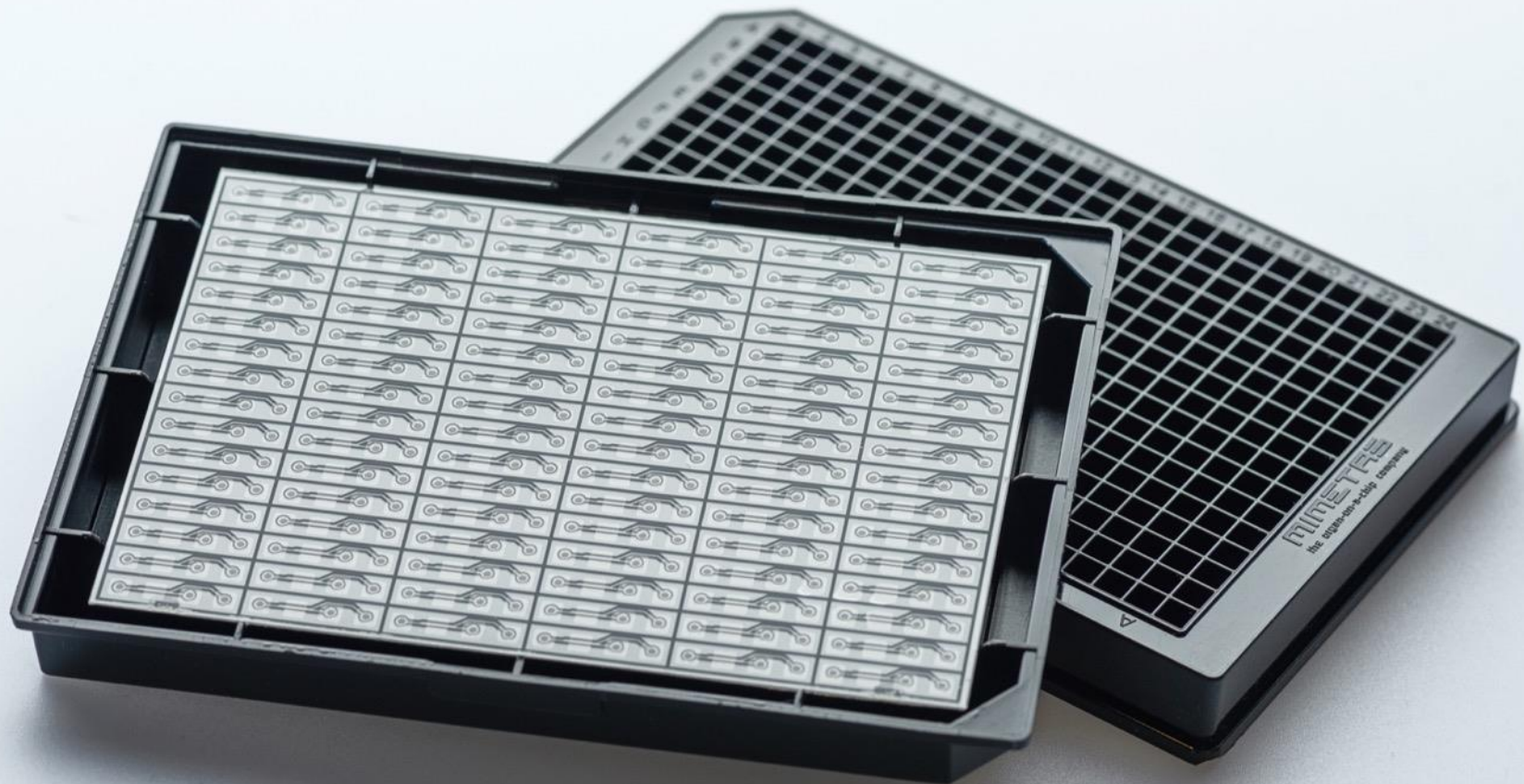


Animal testing

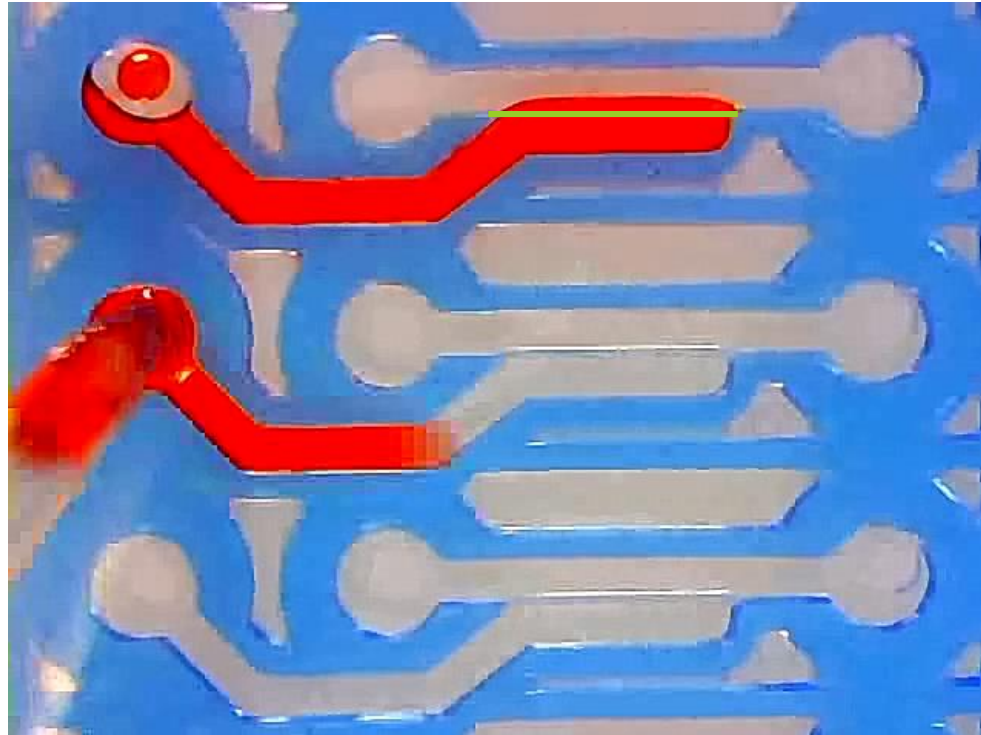


Human tissues
Co-culture
Perfused
ECM embedded
Tubes & vessels



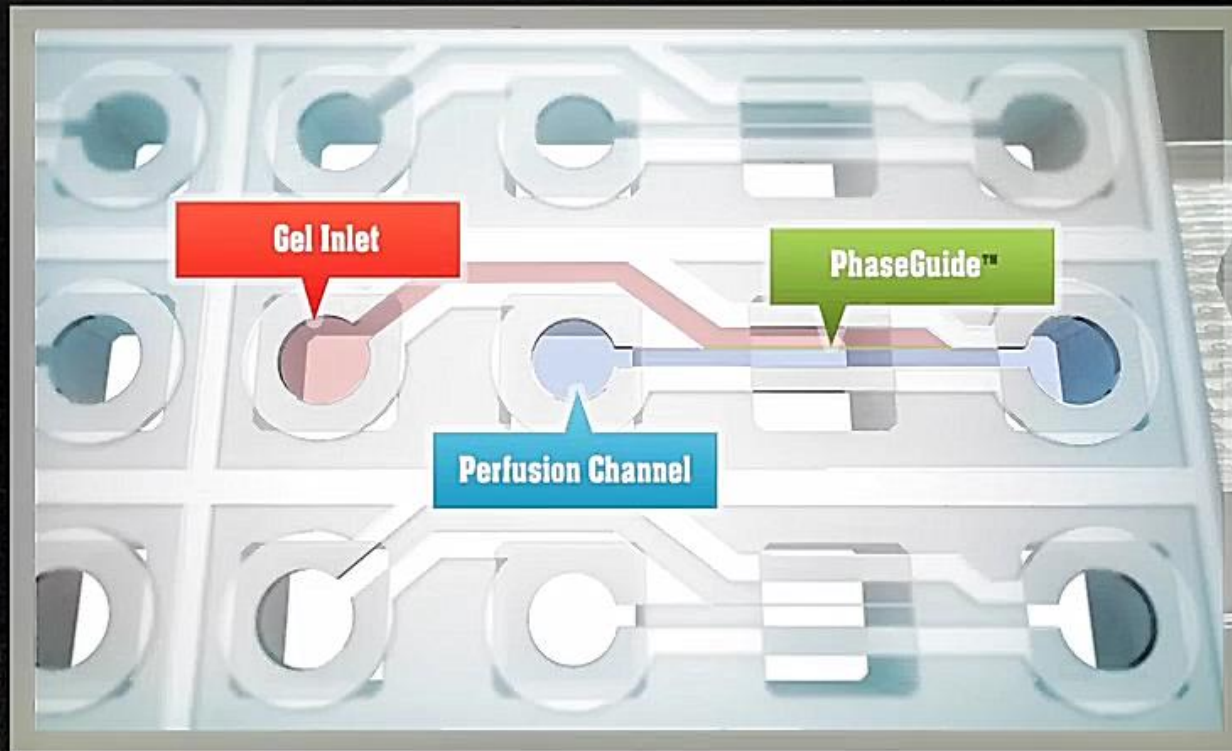


PhaseGuide™

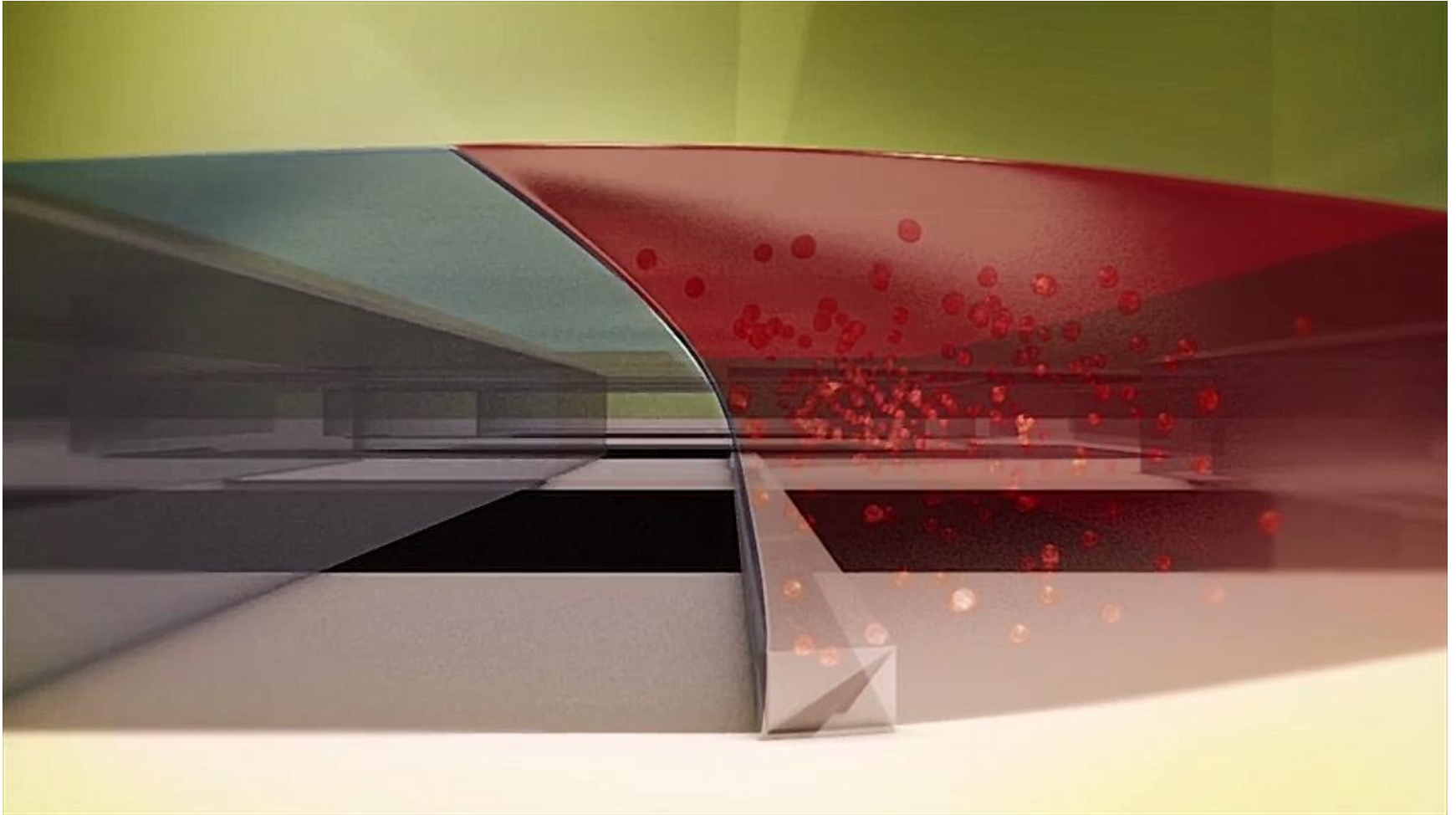


Vulto et al 2011 Phaseguides: a paradigm shift in microfluidic priming and emptying. *Lab Chip* 11(9) 1596-1602

Mimetas BV Leiden, 2018

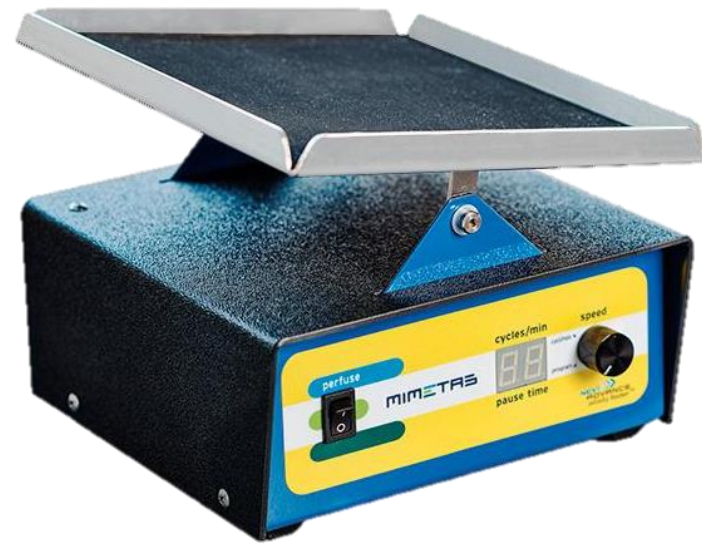
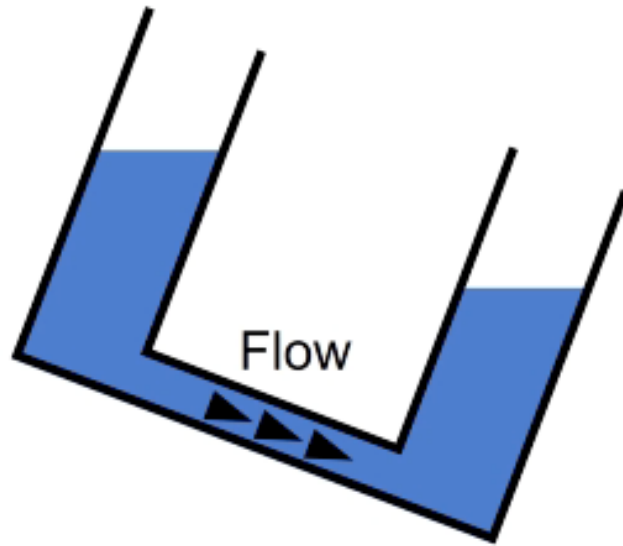


Watch full movie https://youtu.be/L_VEJAZ5J6U

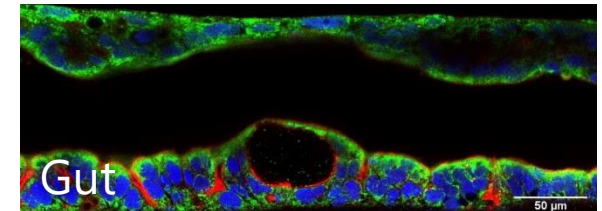
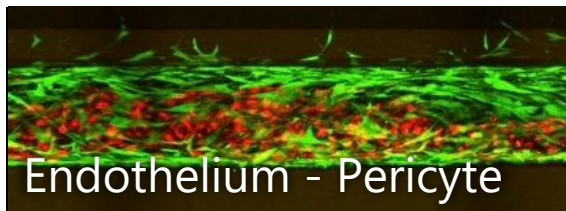
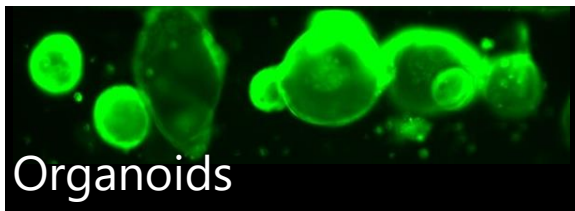
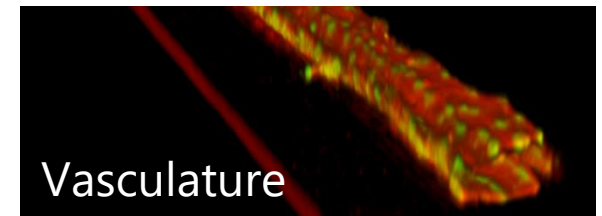
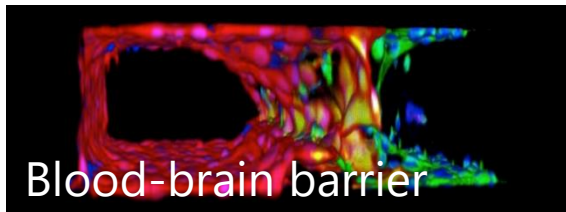
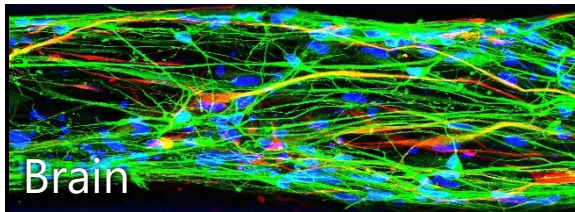
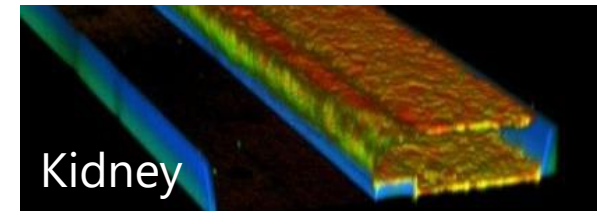
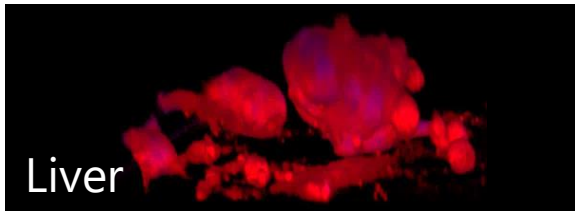
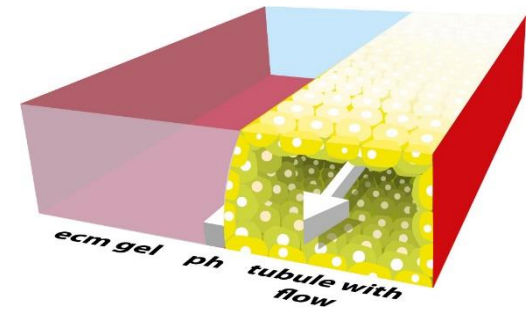
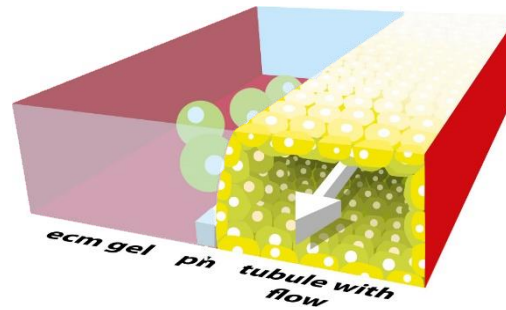
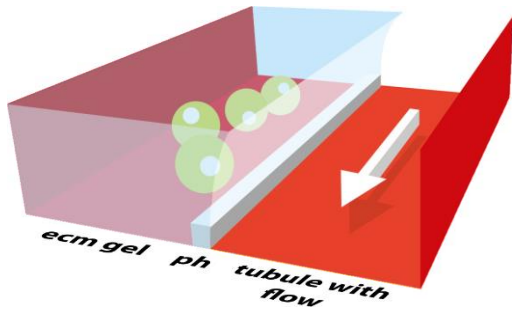


Watch full movie https://youtu.be/L_VEJAZ5J6U

Pump-free continuous perfusion



Organ models in the OrganoPlate®



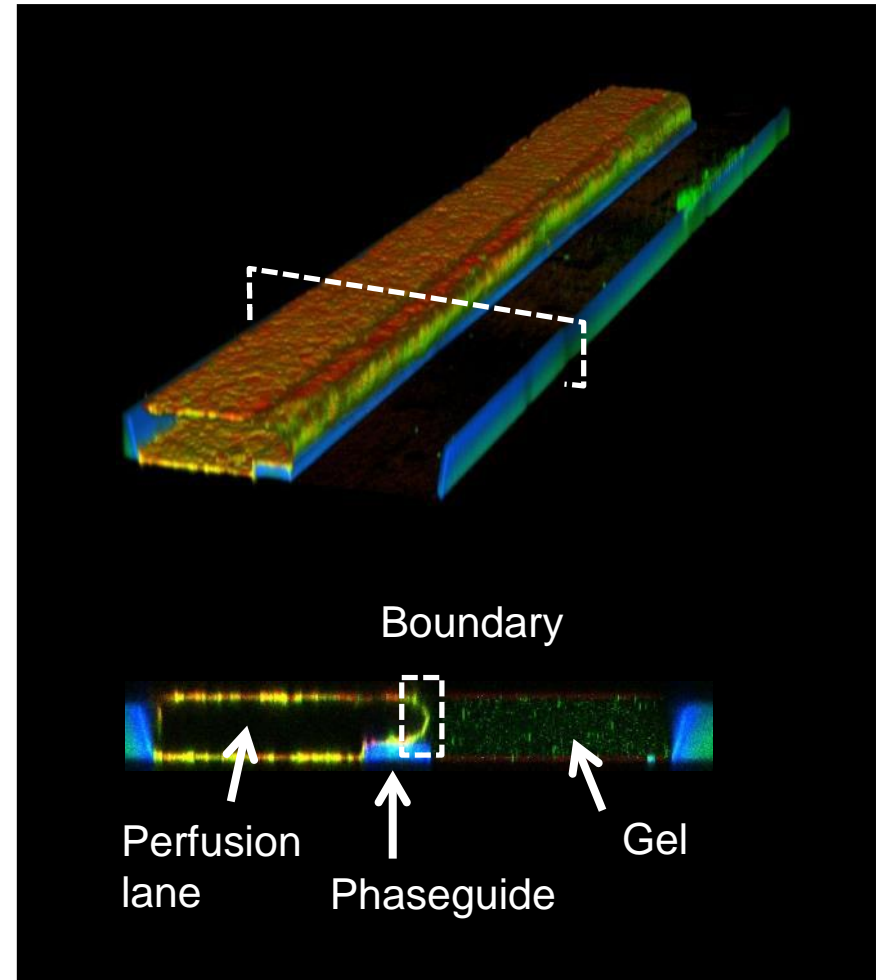
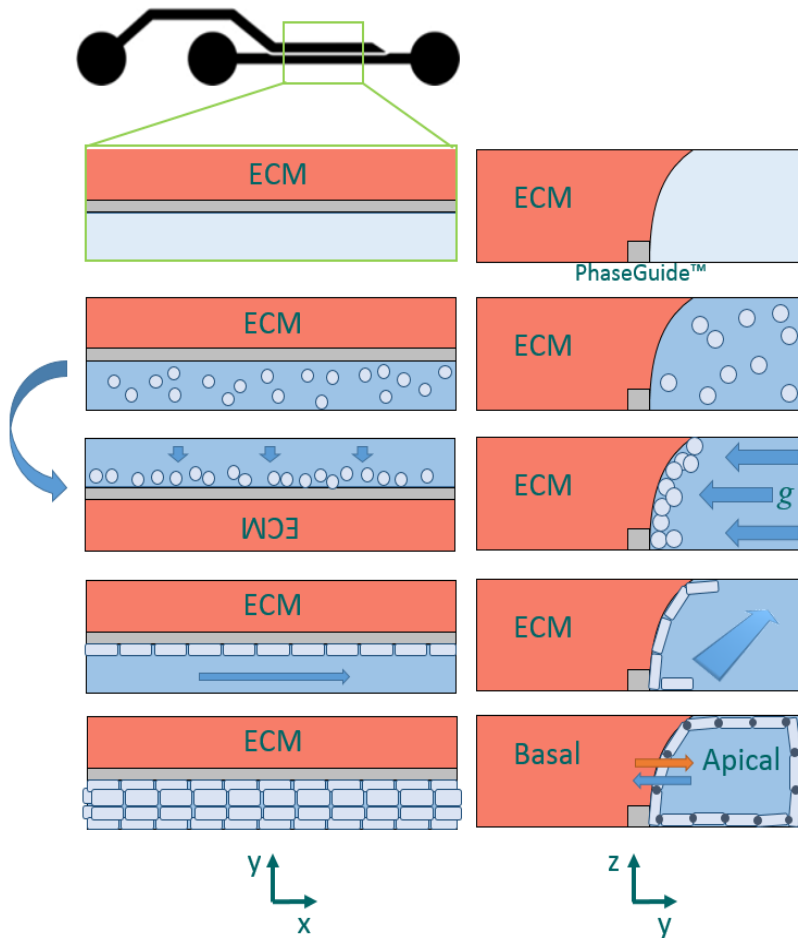
Solid tissues

Co-culture

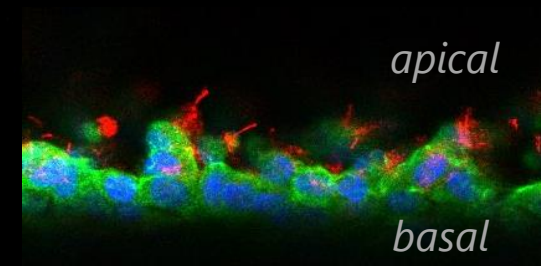
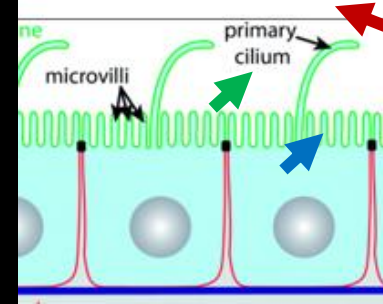
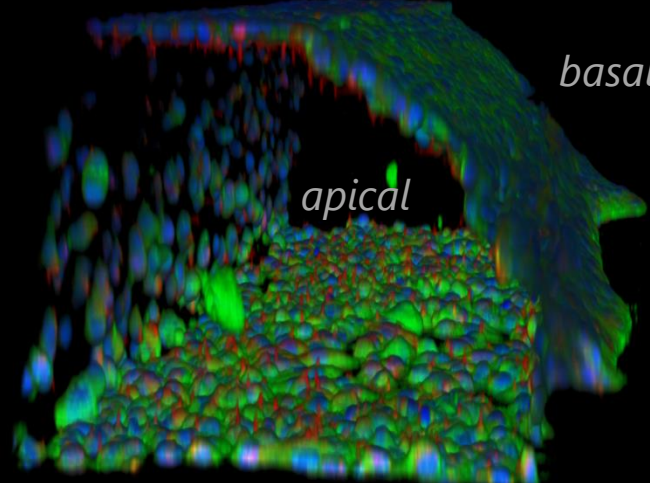
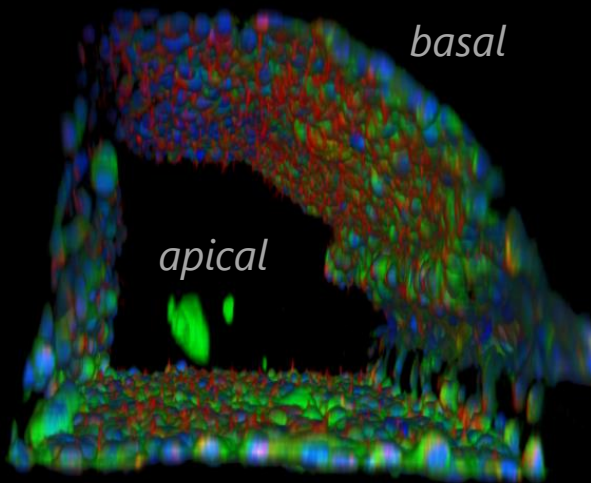
Barrier tissues



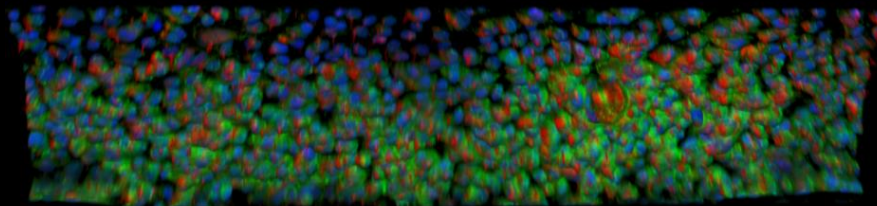
Tube seeding in OrganoPlates®



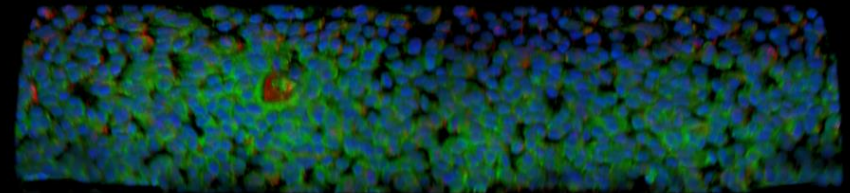
Human Proximal Tubule Model



Acetylated tubulin ZO-1 DNA



apical perspective



basal perspective

- Human Renal Proximal Tubular Epithelial Cells form a tube in the 3-lane OrganoPlate®
- RPTECs are polarized (cilia on apical surface) and express tight junction markers

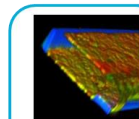


NephroScreen: toxicity screening with a proximal tubule-on-a-chip

NephroScreen assay availability

	ciPTEC-OAT1		Sigma-RPTEC	
	2D	OrganoPlate®	2D	OrganoPlate®
Viability				
WST		S		
Calcein				
PI/YoPro®			ND	ND
Secreted/leaky enzymes				
LDH		S		S
NAG		S	ND	ND
GGT		ND		ND
Secreted proteins				
KIM-1	(↓)	(↓)	ND	ND
NGAL/Lcn2			ND	ND
Expressed Proteins (IF)				
Claudin-2			ND	ND
RNA-Transcripts				
Claudin-2		S		S
Heme Oxygenase 1		S		S
TNF-α		S		S
NGAL/Lcn2		S		S
KIM-1				ND
Secreted miRNAs				
miRNA1 (confidential ID)		S	ND	ND
miRNA2 (confidential ID)		S	ND	ND
miRNA3 (confidential ID)		S	ND	ND
miRNA4 (confidential ID)		S	ND	ND
Functional Assays				
Inulin-FITC leakage	NA		NA	
Barrier integrity assay	NA		NA	S
TEER	NA		NA	
Mitochondrial potential		ND	ND	ND
ROS production		ND	ND	ND
Pgp drug-interaction		S		
MRPs drug-interaction		S		
OAT drug-interaction				
OCT2 drug-interaction			ND	ND

NephroScreen Validation Screen 2017



3D tubules



1 tubule/chip
40 chips/plate



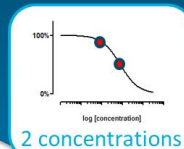
2 cell lines



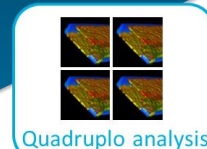
12 compounds



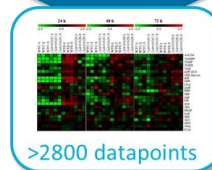
15 biomarkers



2 exposure times



Quadruplo analysis



A range of nephrotoxicity read outs were implemented in the 3D NephroScreen and are undergoing validation using 4 benchmark compounds and 8 blinded compounds with known clinical effects supplied by the Sponsors.

Partners:

Radboudumc

Mimetas BV Leiden, 2018

MIMETAS
the organ-on-a-chip company

n|w Fachhochschule
Nordwestschweiz



Universiteit Utrecht

Funding:



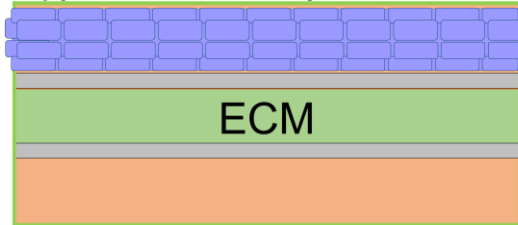
Sponsors:



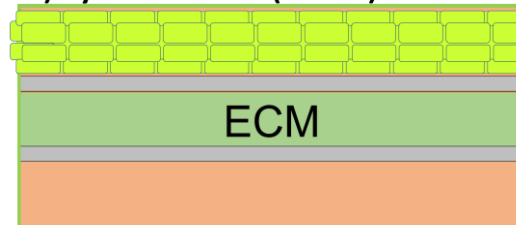


Real-time Barrier Integrity Assay

A) perfused boundary tissue



B) dye in medium (lumen)



C) not exposed, leak-tight barrier



D) toxicant exposed tubule, non leak-tight



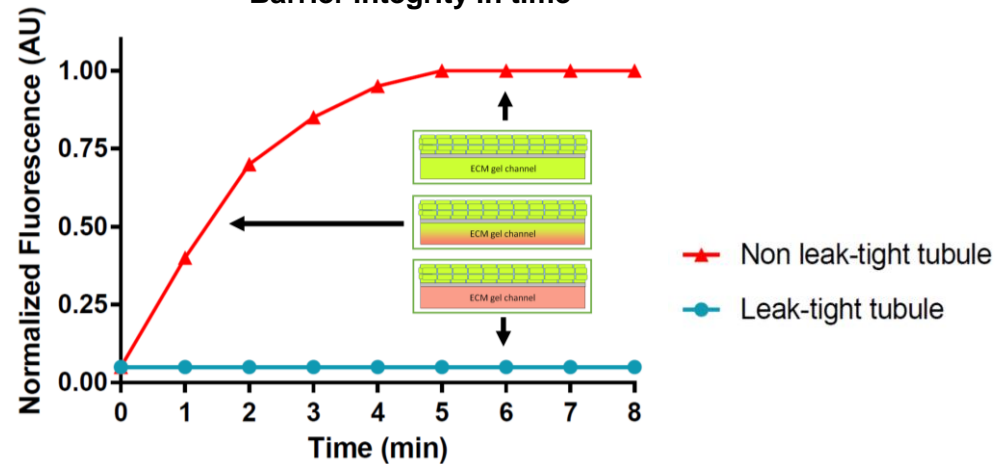
No cells (NC)



Leak tight tube (PC)

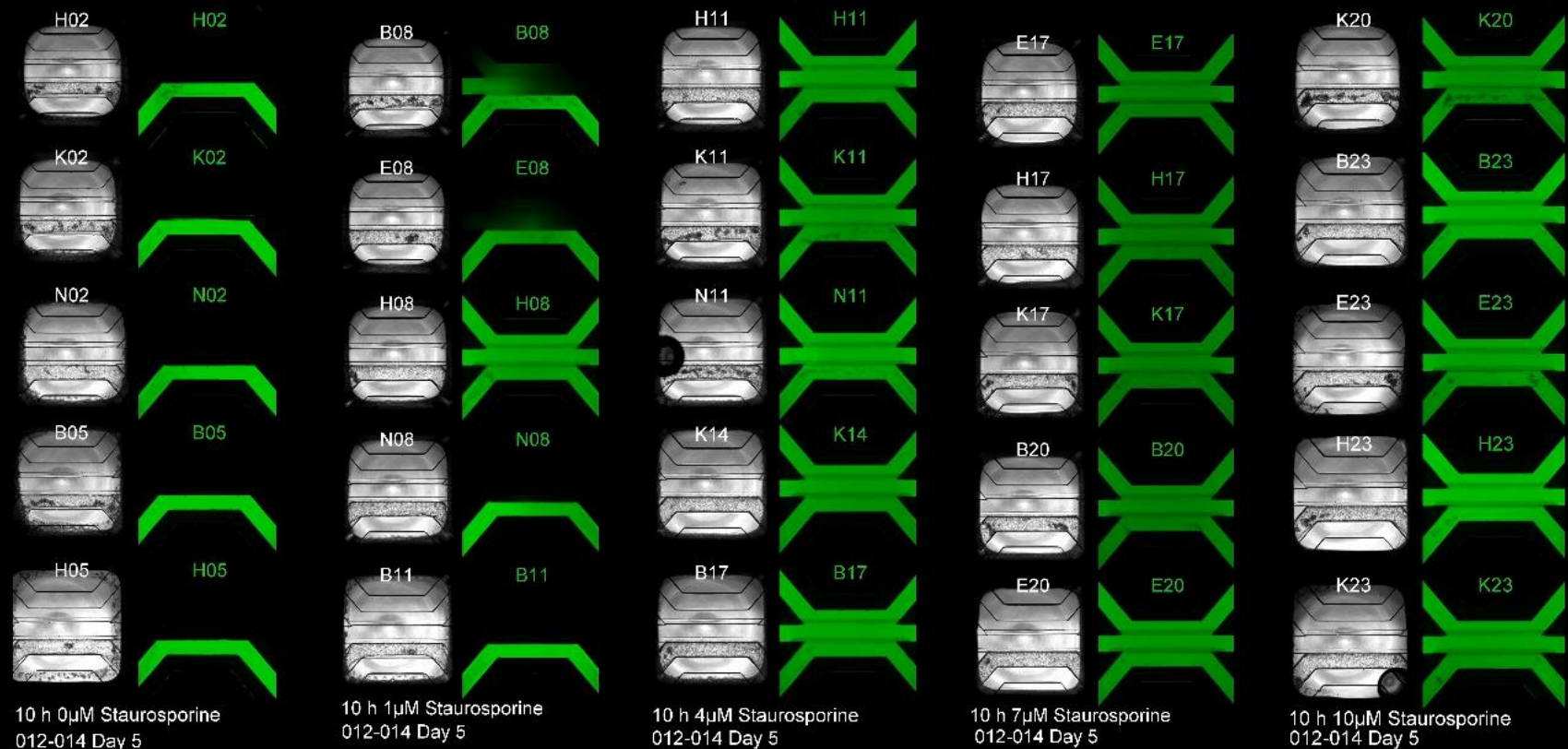


Barrier integrity in time



Real-time barrier integrity assay

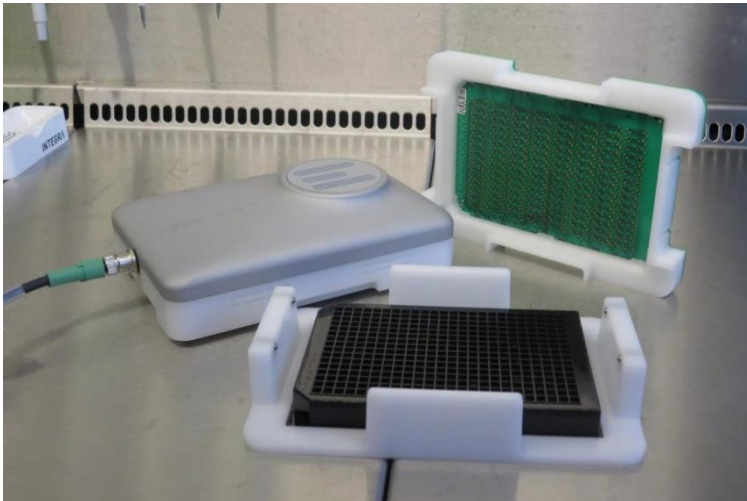
MDCK (canine distal tubule cell line) in 3-lane OrganoPlate®
Day 5: Staurosporine exposure (15h)



- Dose dependent loss of barrier integrity measured in real time on tubular model



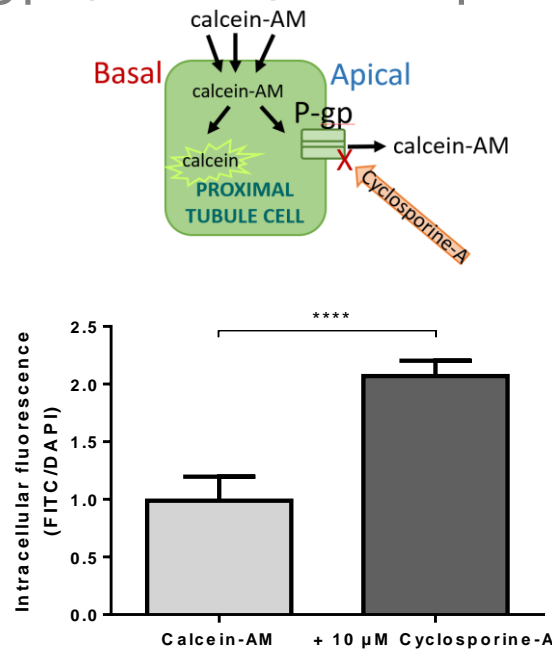
- Barrier assessment
- 120 channels
- High data quality
- Realtime and long-term
- Non invasive
- Flow
- Incubator



- Launch Q3 2018
- **Beta open now!**

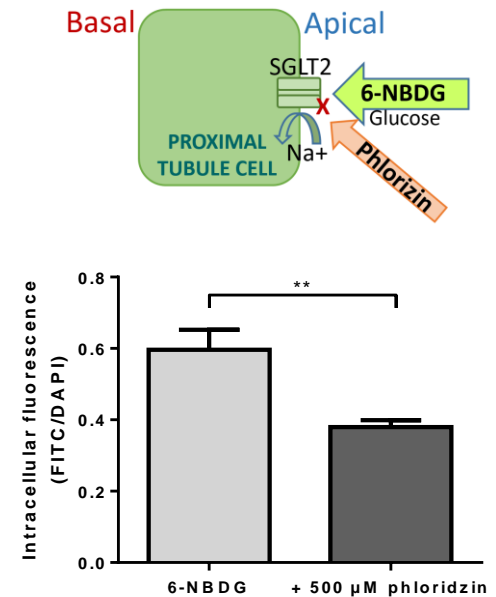
Efflux- and Influx Inhibition

- Efflux Inhibition of the P-gp (MDR1) Transporter



- Significant inhibition of calcein-AM efflux by cyclosporine-A

- Influx Inhibition of Glucose Analog 6-NBDG



- Significant inhibition of 6-NBDG influx by phlorizin

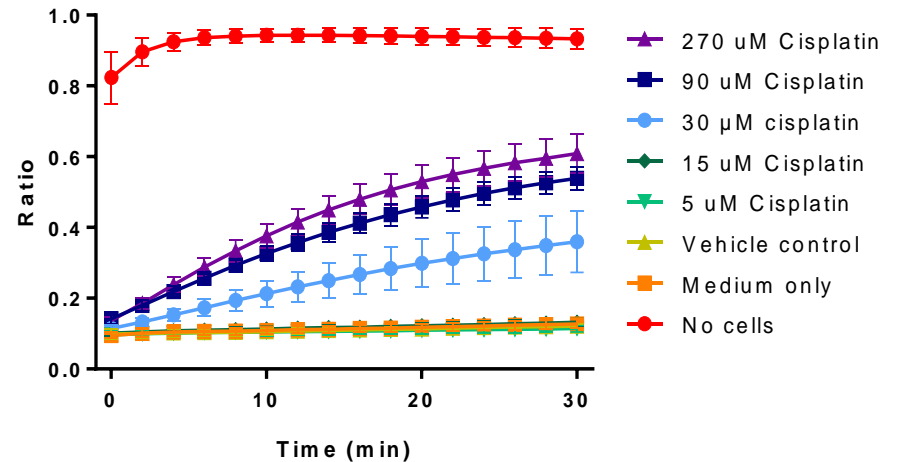
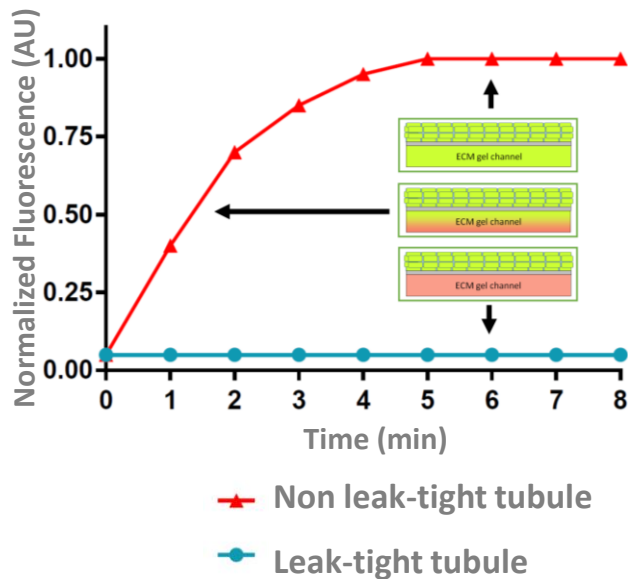


Case study: Cisplatin induced toxicity

- Renal toxicity has been noted in 28% to 36% of patients treated with a single dose of 50 mg/m²
- The dose is reduced when the patient's creatinine clearance (a measure of renal function) is reduced
- The maximum human plasma concentrations (C_{max}) for free cisplatin is:
 - 4.5 ± 1.6 µM (at dosing of 30 mg/m²)
 - 14.3 ± 2.3 µM (at dosing of 100 mg/m²)
- Evaluation of a single, 48 hour exposure in Human Proximal Tubule Model using multiple end-points

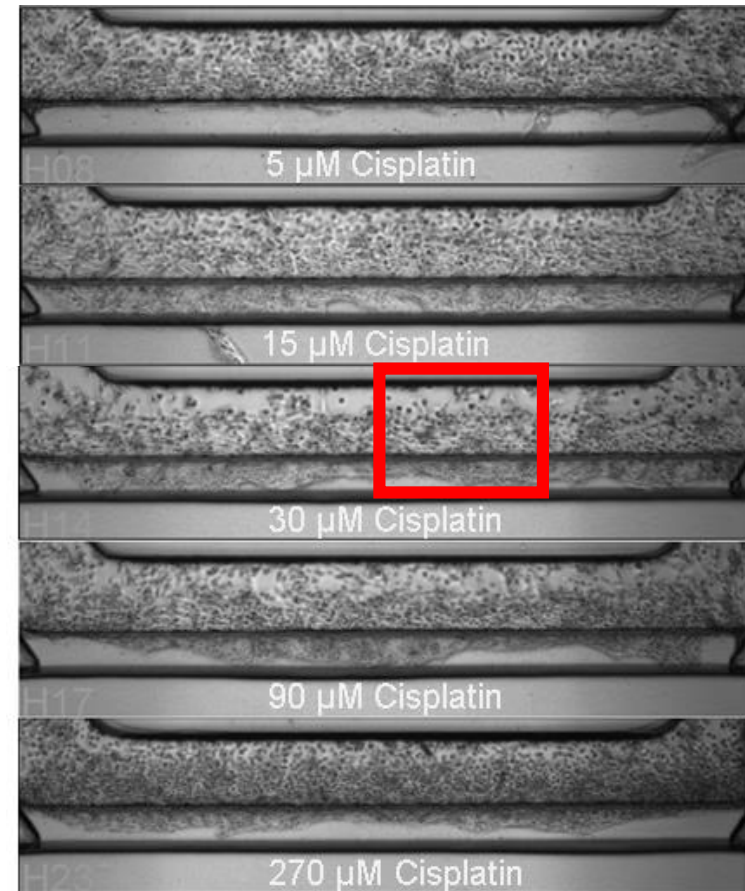
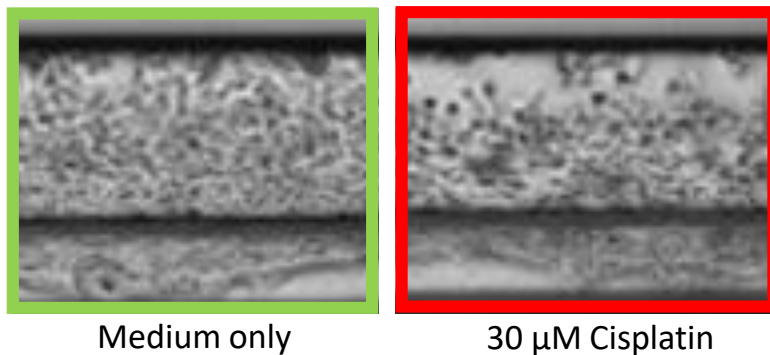
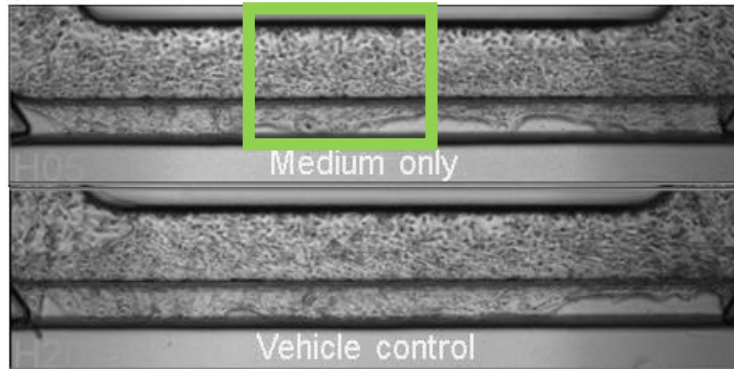


Toxicity assessment: barrier integrity



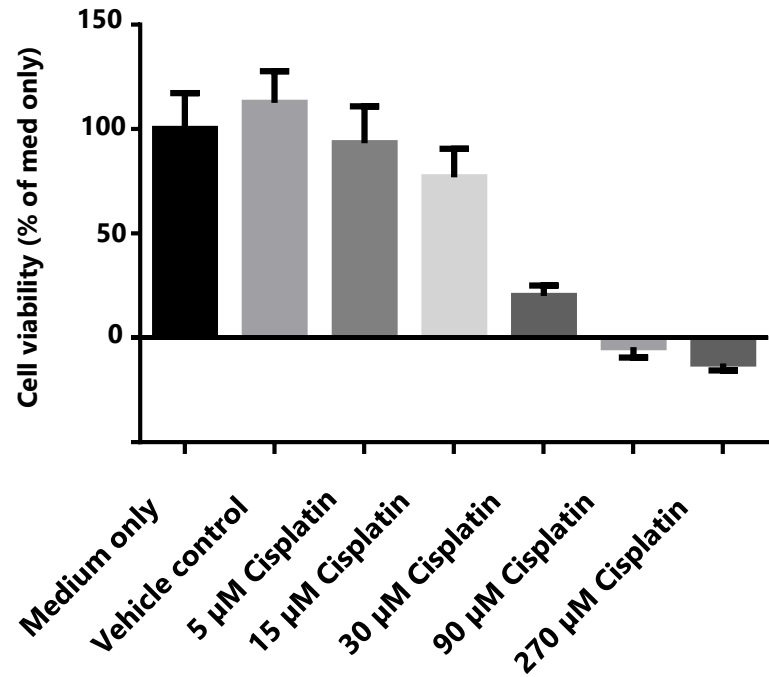
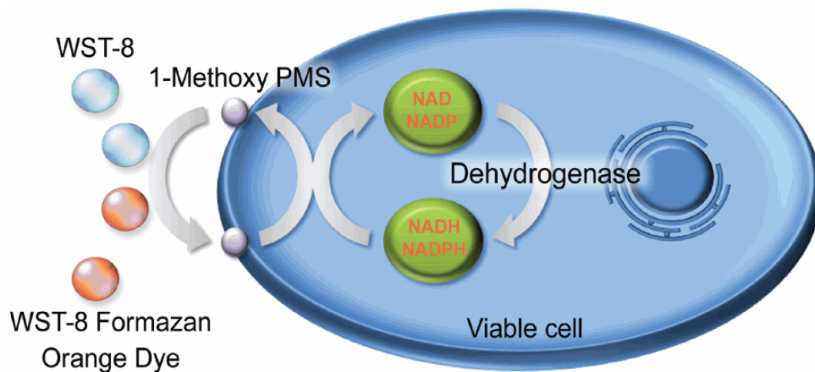
- Dose-dependent decreased barrier integrity of proximal tubules can be measured after 48h exposure to cisplatin

Toxicity assessment: morphological



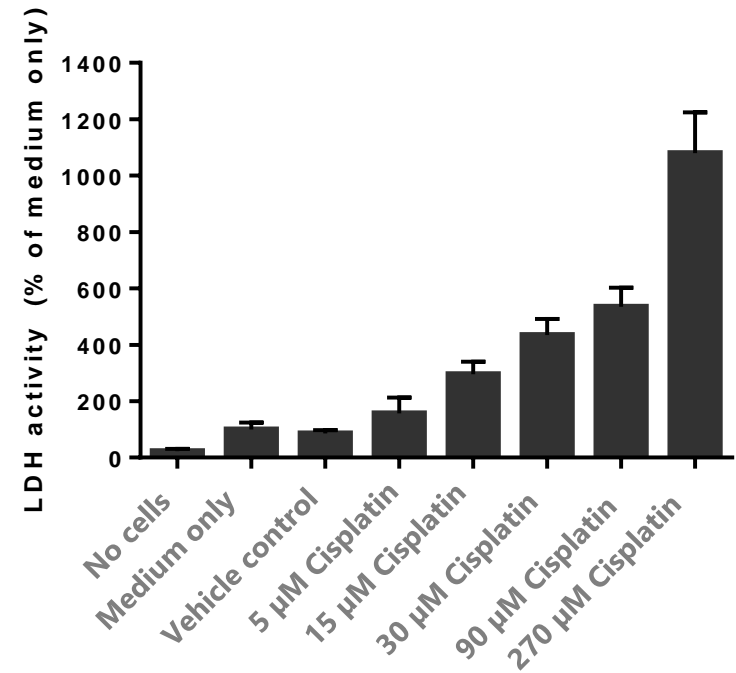
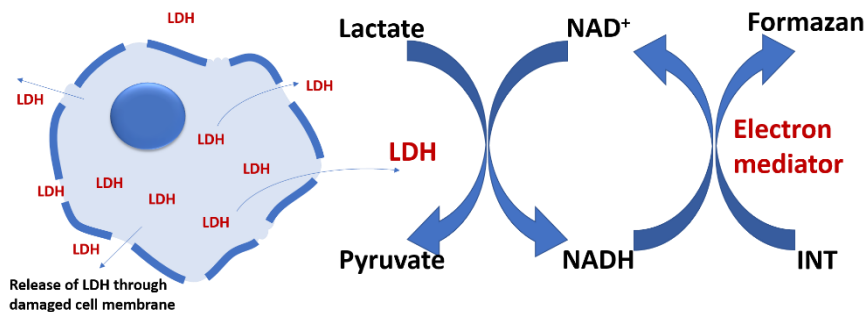
- Loss of proximal tubule viability can be observed by phase contrast imaging

Toxicity assessment: Enzymatic activity



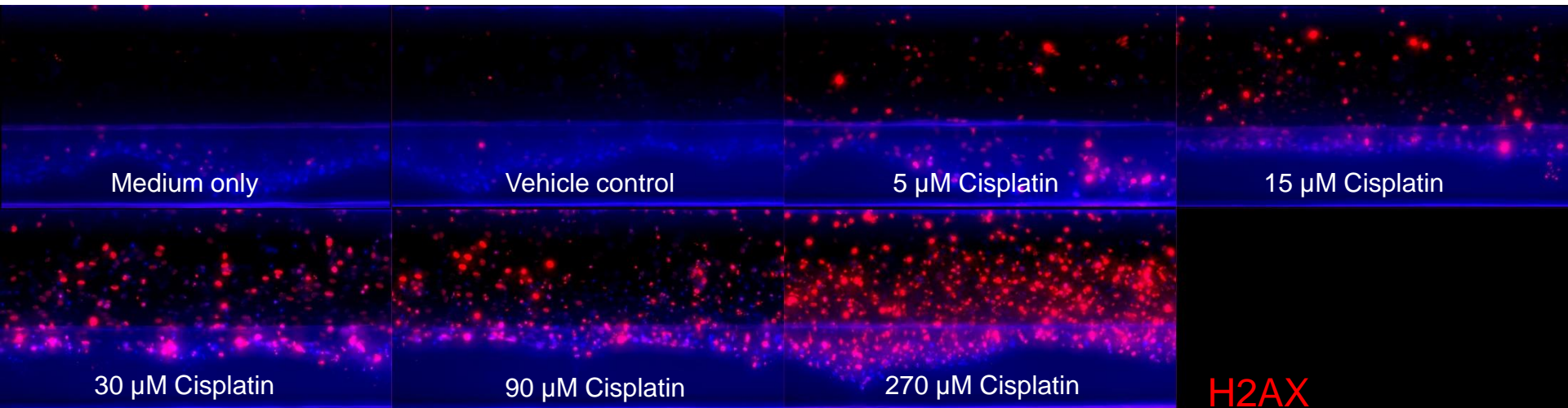
- Dose-dependent decrease in WST-8 signal can be measured after 48h exposure to cisplatin

Toxicity assessment: Enzymatic activity



- Dose-dependent increase in LDH activity can be measured in the medium of proximal tubules after 48h exposure to cisplatin

Toxicity assessment: DNA damage



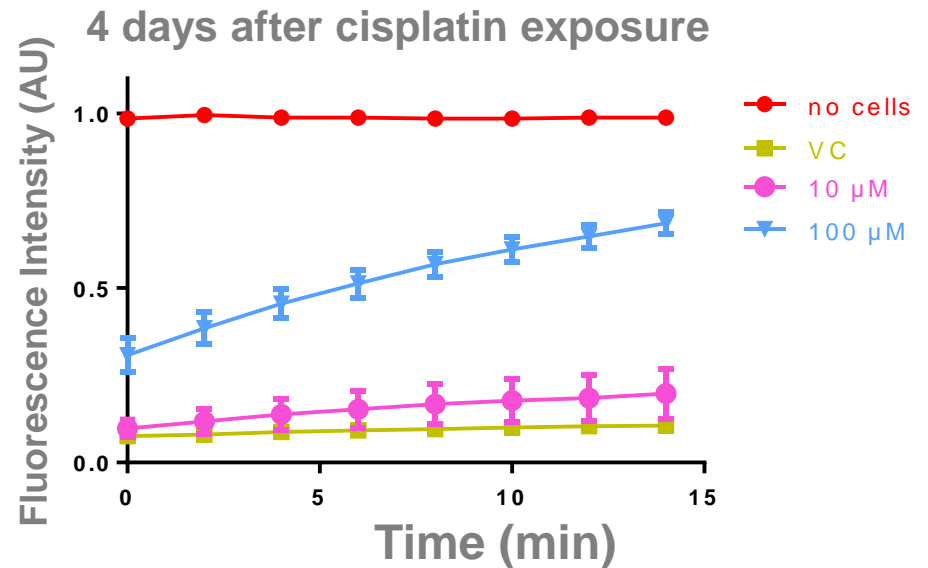
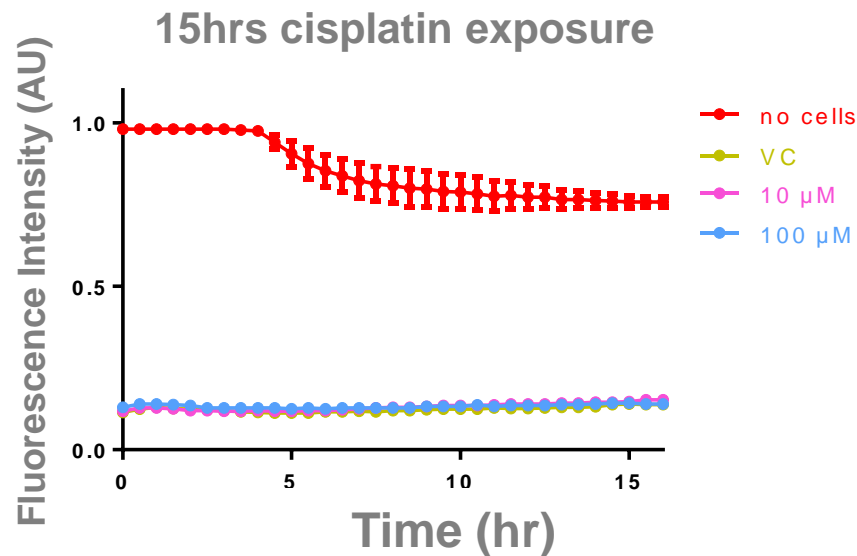
- Dose-dependant increase in H2AX expression of proximal tubules can be measured after 48h exposure to cisplatin

Overview: Cisplatin induced toxicity

	5 μ M	15 μ M	30 μ M	90 μ M	270 μ M
Barrier function	Green	Green	Red	Red	Red
Morphology	Green	Green	Red	Red	Red
Enzymatic activity (WST-8)	Green	Green	Red	Red	Red
Enzymatic activity (LDH)	Red	Red	Red	Red	Red
DNA damage	Red	Red	Red	Red	Red

- All readouts detected cisplatin induced toxicity after a single, 48 hour exposure.
- The DNA damage and LDH assays are most sensitive for the detection of cisplatin induced renal toxicity.

Time dependent toxicity

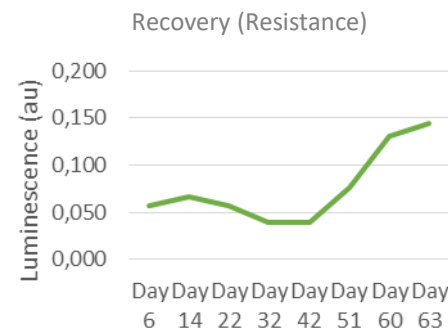
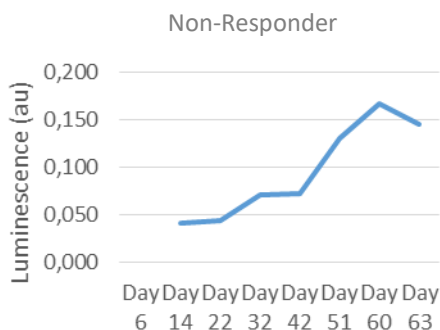
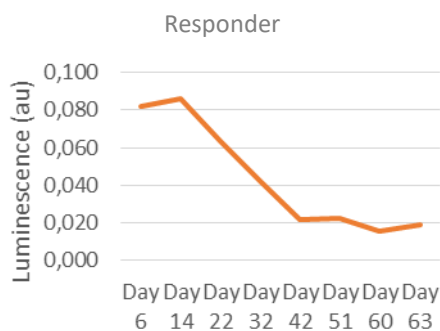
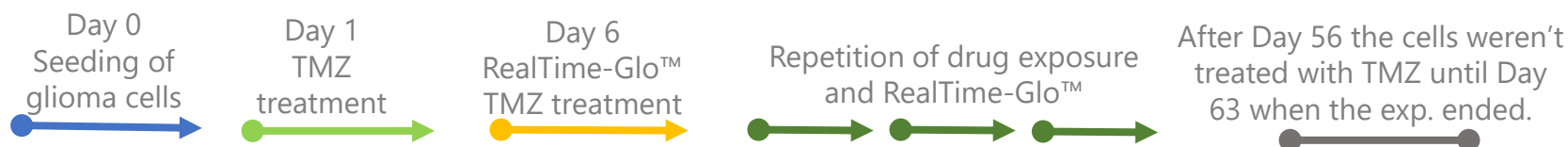


- Cisplatin influences RPTEC barrier integrity after 4 day recovery after 24h exposure of 10 and 100 μM cisplatin



Repeated dosing: Modelling Treatment Effects

- Long term treatment can select and/or induce resistance
- Modelling patient specific resistance in the Organoplate®
 - Long term culture under low drug dose
 - Determine compound sensitivity of 3D cultures at different time points





Next step: Renal clearance model

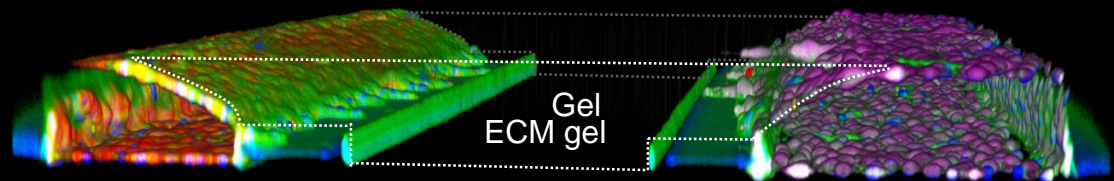
- Adding a blood vessel to the kidney tube allows creation of a functional kidney unit
- Applications: renal clearance, acute kidney injury model

Top view in phase contrast



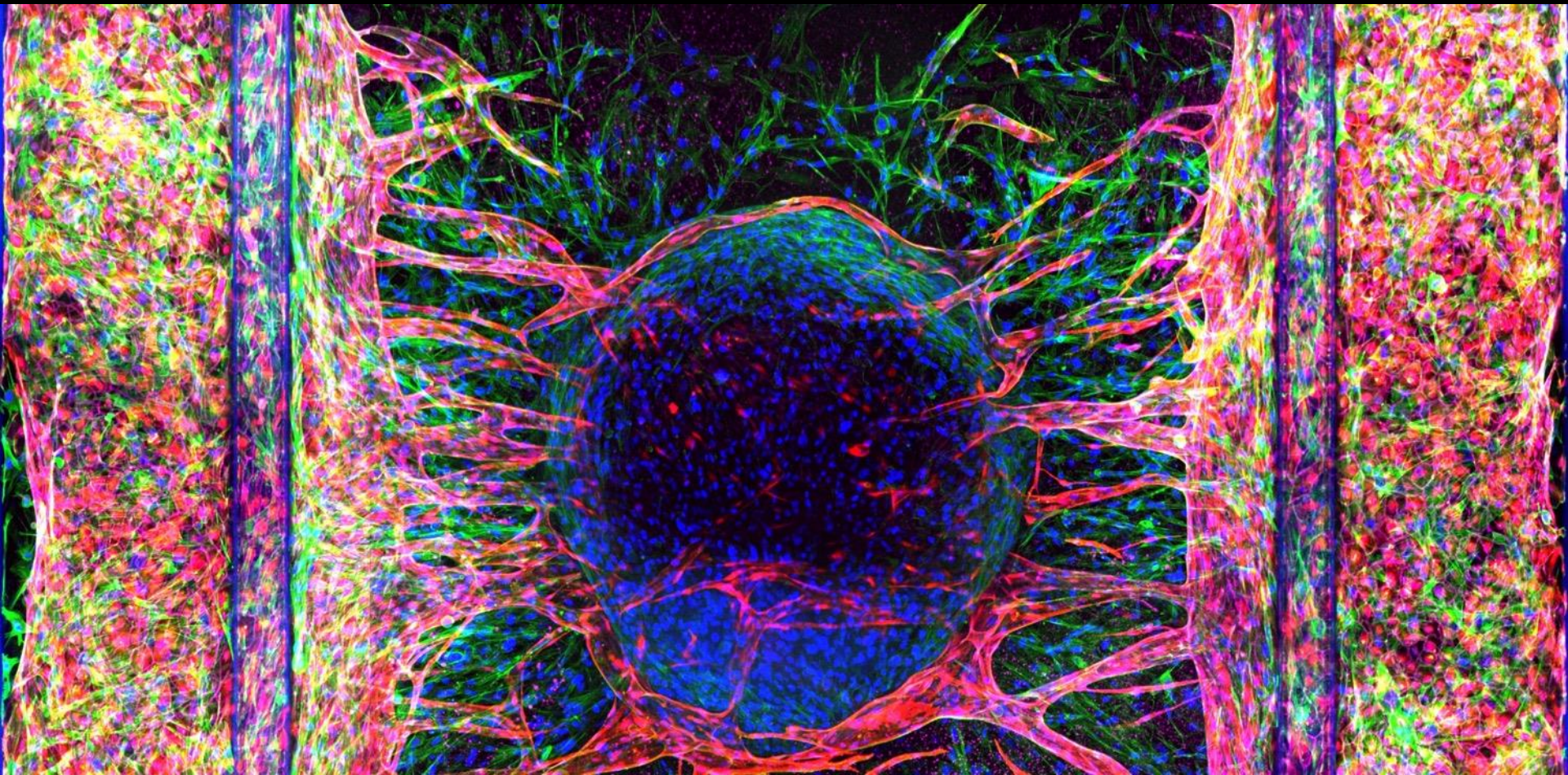
Kidney proximal tubule

Bloodvessel





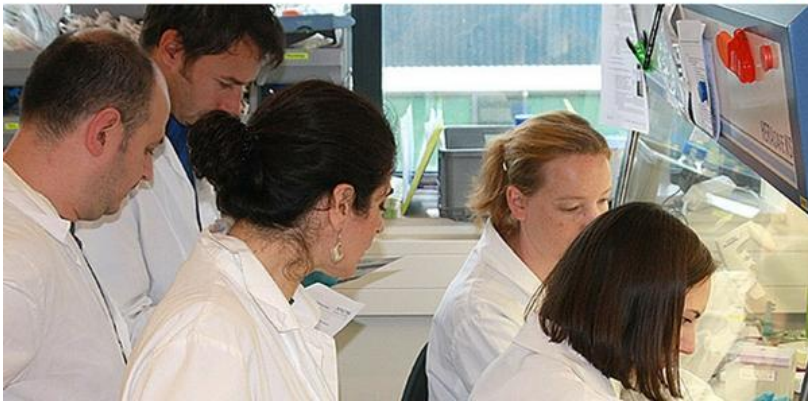
Next step: Vascularized tissues



Nucblue Actingreen RFP VE-Cadherin

- Organ-on-a-chip is ready to use for safety assessment
- Kidney validation study has been completed, results will be published soon
- Assays for: Permeance, Transport and Toxicity assessment
- Long-term and repeated dosing is possible
- Low adsorption, non-PDMS material
- Excellent inter-plate and inter-lab reproducibility

Organ-on-a-Chip Workshop



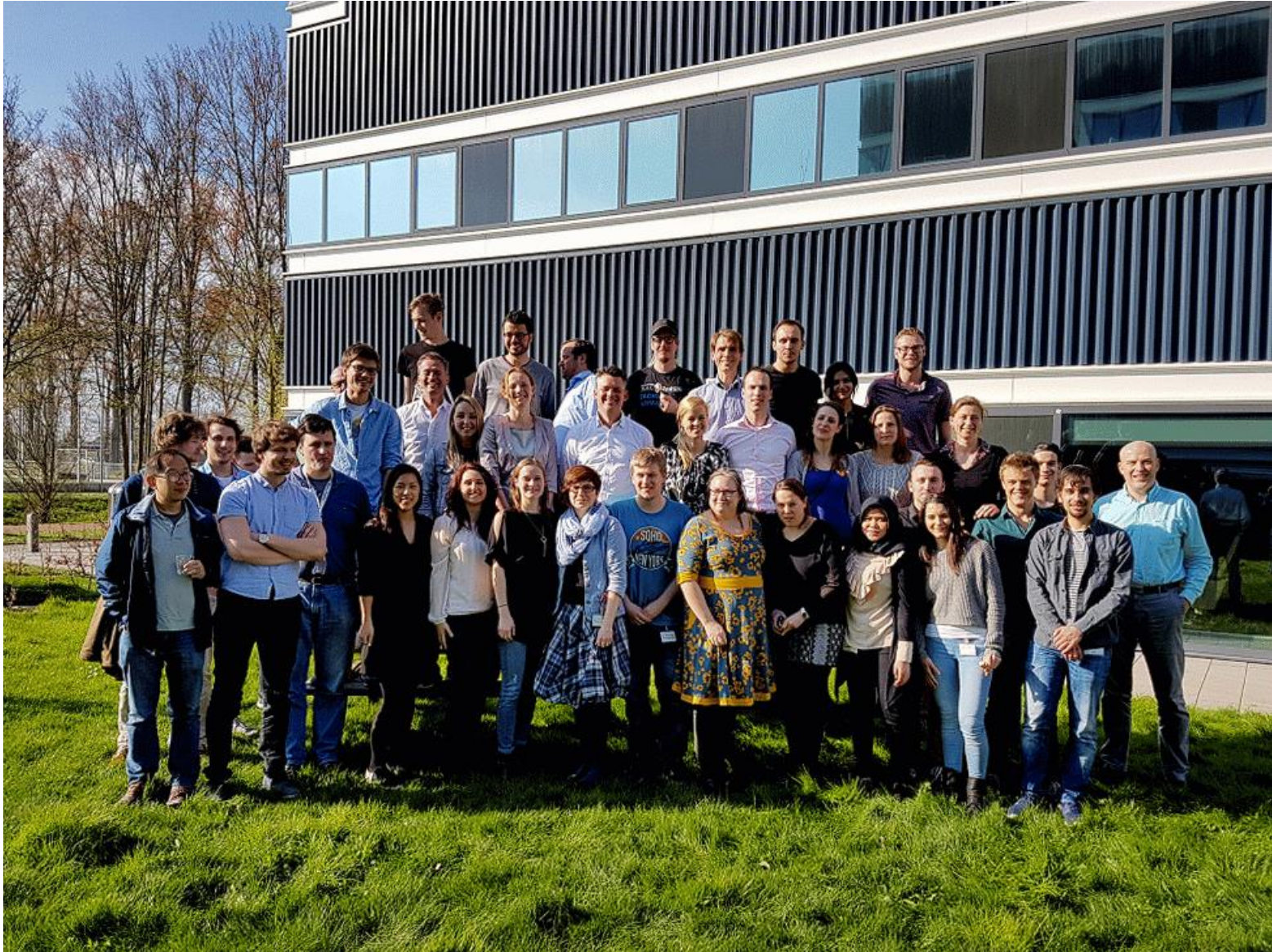
- 2-day hands-on experience
- Establish 3D cell cultures
- Take stunning high-content images
- Leiden (The Netherlands) or Gaithersburg, MD (USA)
- Free lunch included
- Get 2 OrganoPlates® for free!
- Sign-up:

<https://mimetas.com/page/workshops>

“Perfectly organized and executed”

“The hands-on sessions are great!”

Acknowledgements



MIMETAS

the organ-on-a-chip company



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www.mimetas.com

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The Netherlands