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Implantology SS 2018

BIOMICROFLUIDICS & ORGAN-ON-A-CHIP

EBERHARD KARLS
UNIVERSITÄT
TÜBINGEN



Fraunhofer
IGB

- ➡ Microfabrication techniques
- ➡ Organ-on-a-chip technology

- ➡ Microfabrication techniques
- ➡ Organ-on-a-chip technology

➡ Primary microfabrication

- ▶ Lithographic techniques
 - ▶ with etching and deposition of materials (wet etching, dry etching, CVD, PECVD, sputtering etc.)
- ▶ Mechanical machining (milling, drilling etc.)
- ▶ Laser ablation
- ▶ ...

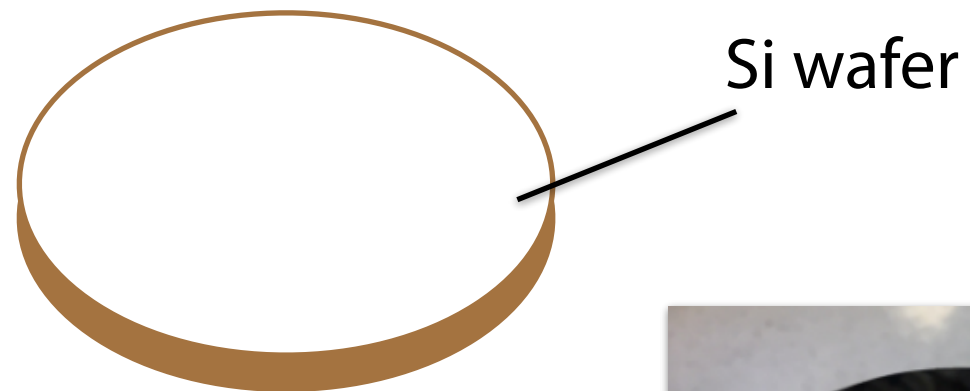
➡ Replication technologies:

Subsequent "mass production" from microstructures master tools

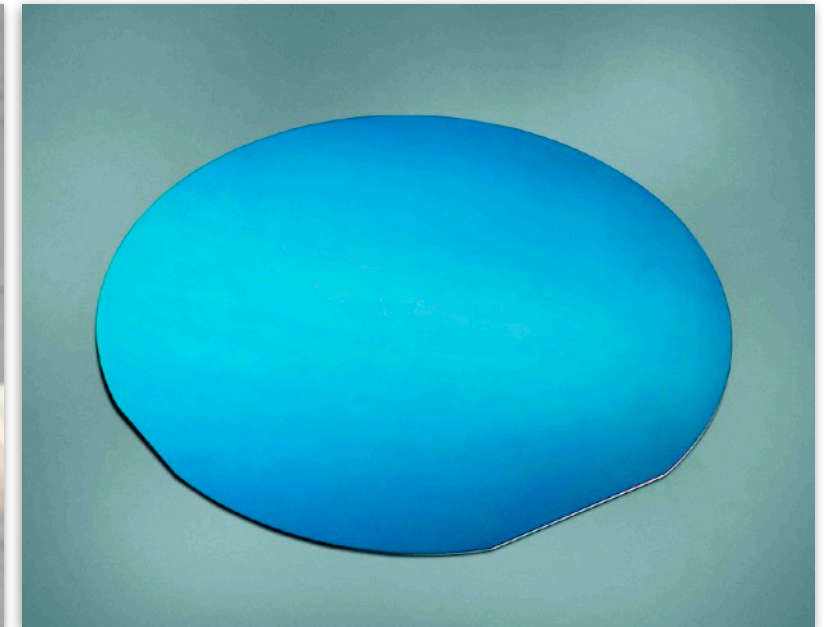
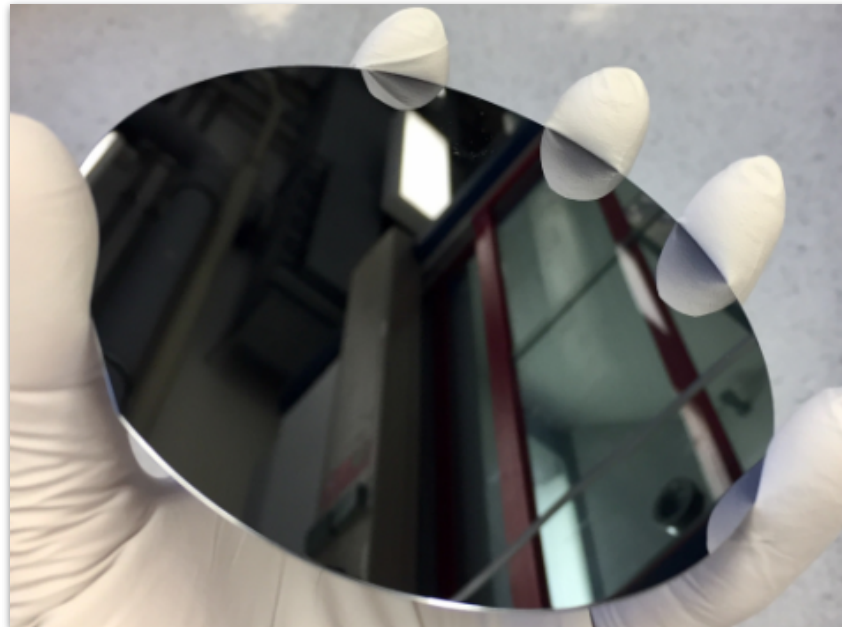
- ▶ Hot embossing
- ▶ Injection molding
- ▶ Casting techniques

➡ Materials: Silicon, plastics, glass, ceramics, metals, etc.

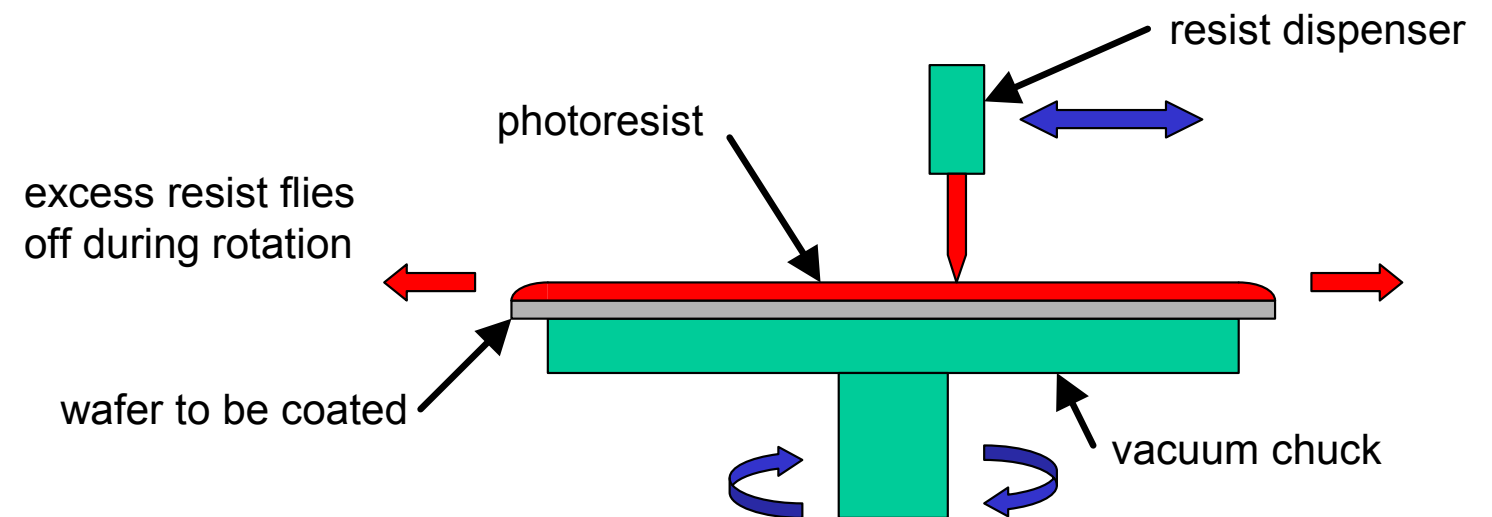
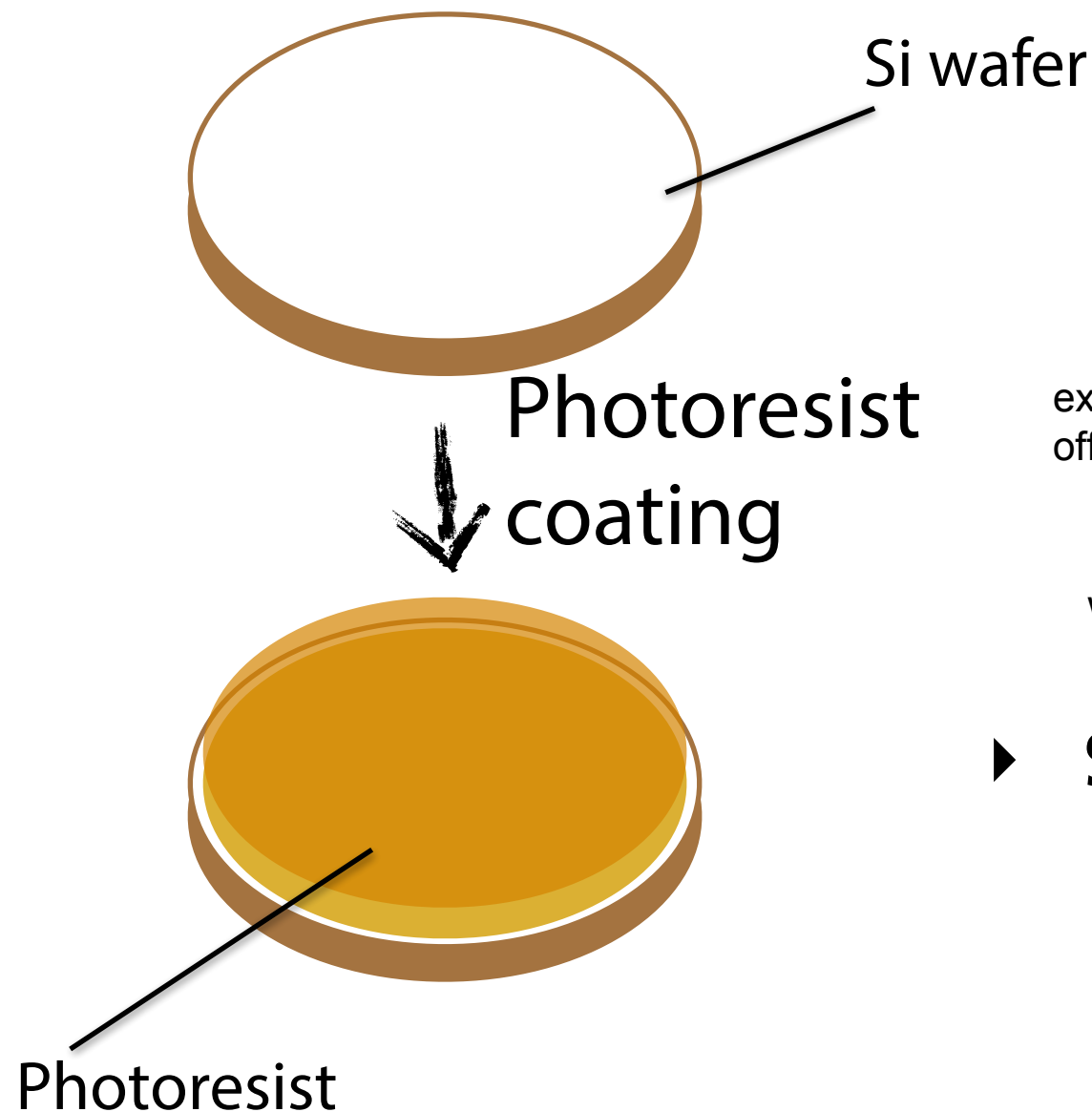
➡ Photolithography



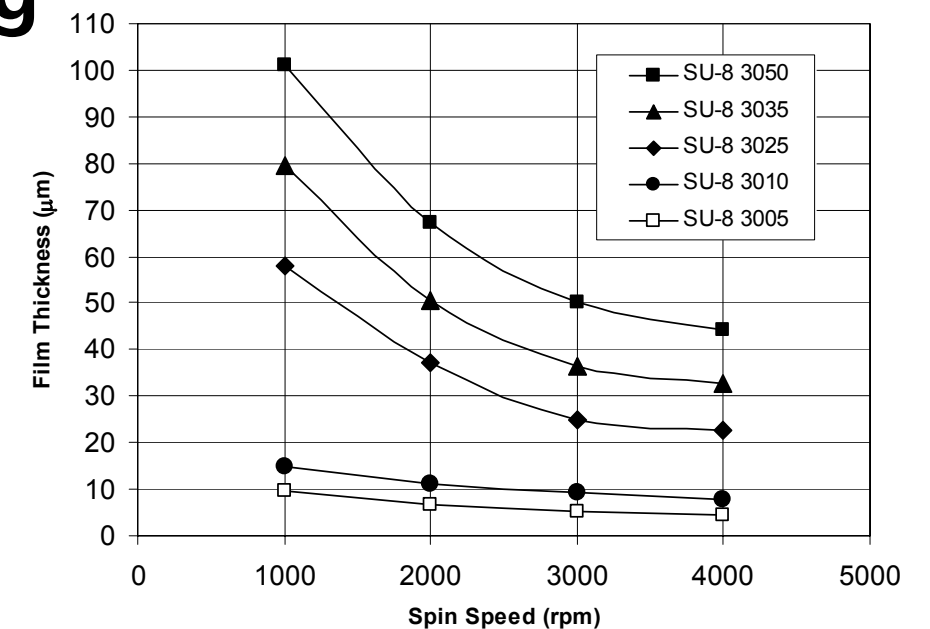
- ▶ **sub-nm roughness**
- ▶ **extremely clean**



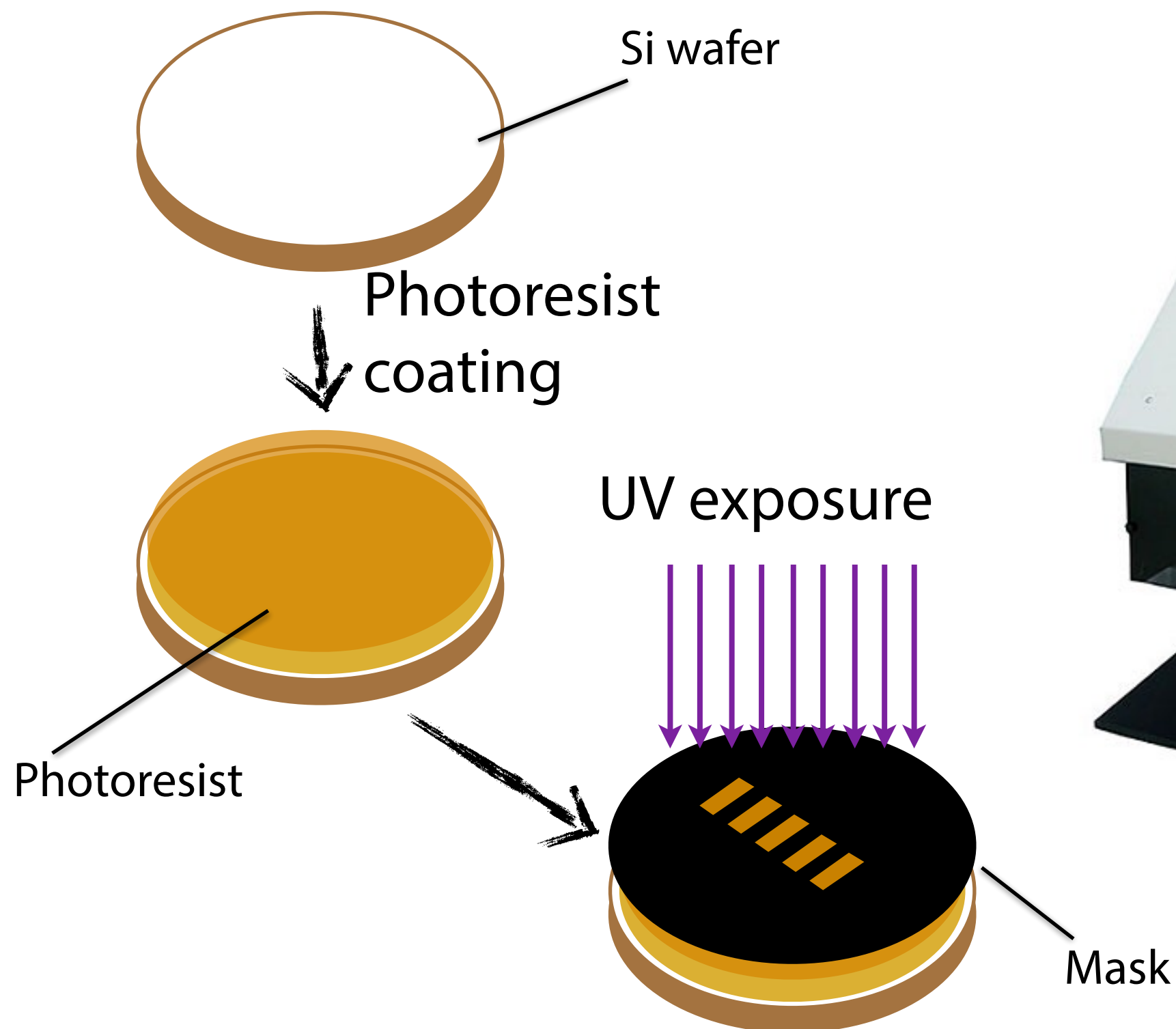
➔ Photolithography



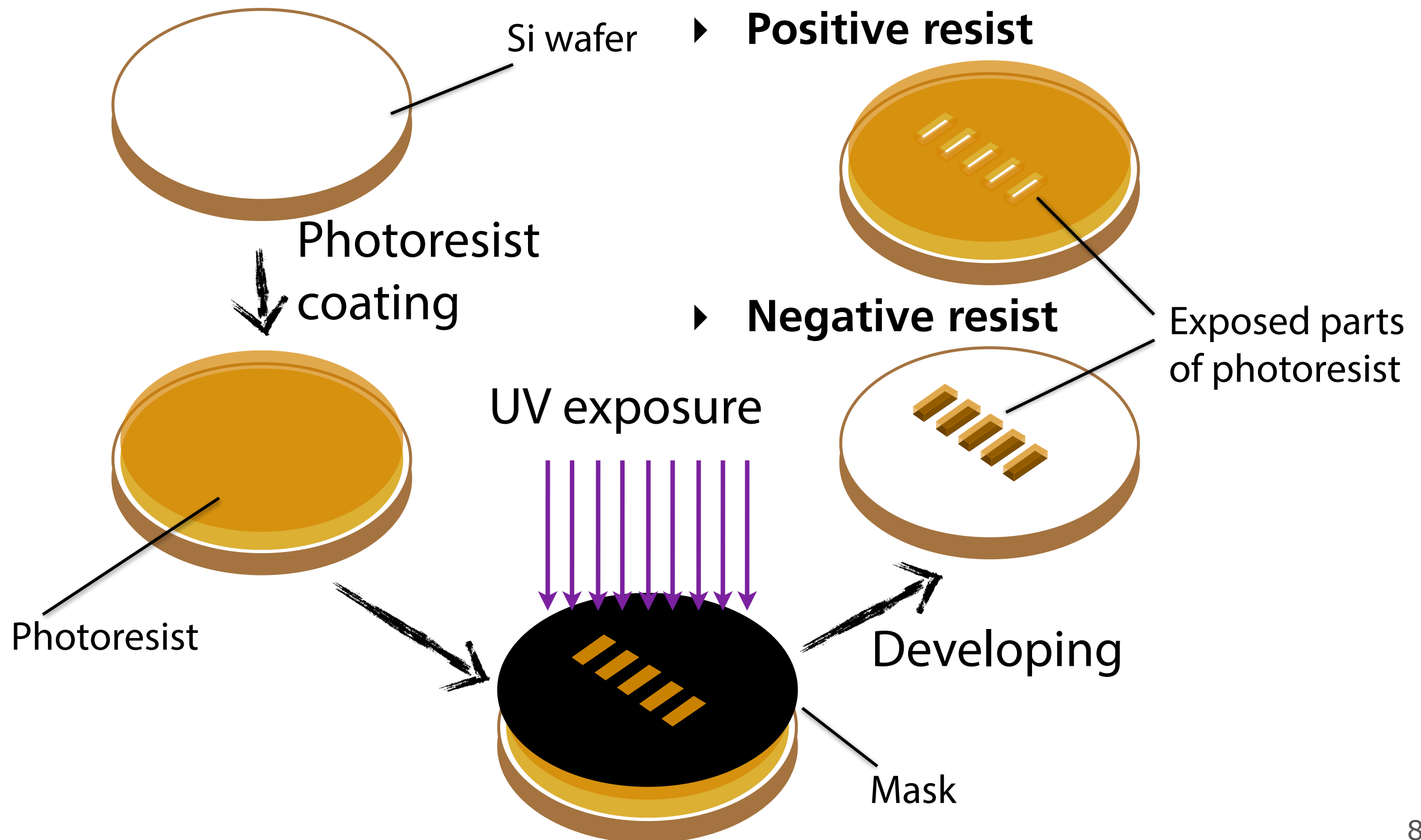
► Spin coating



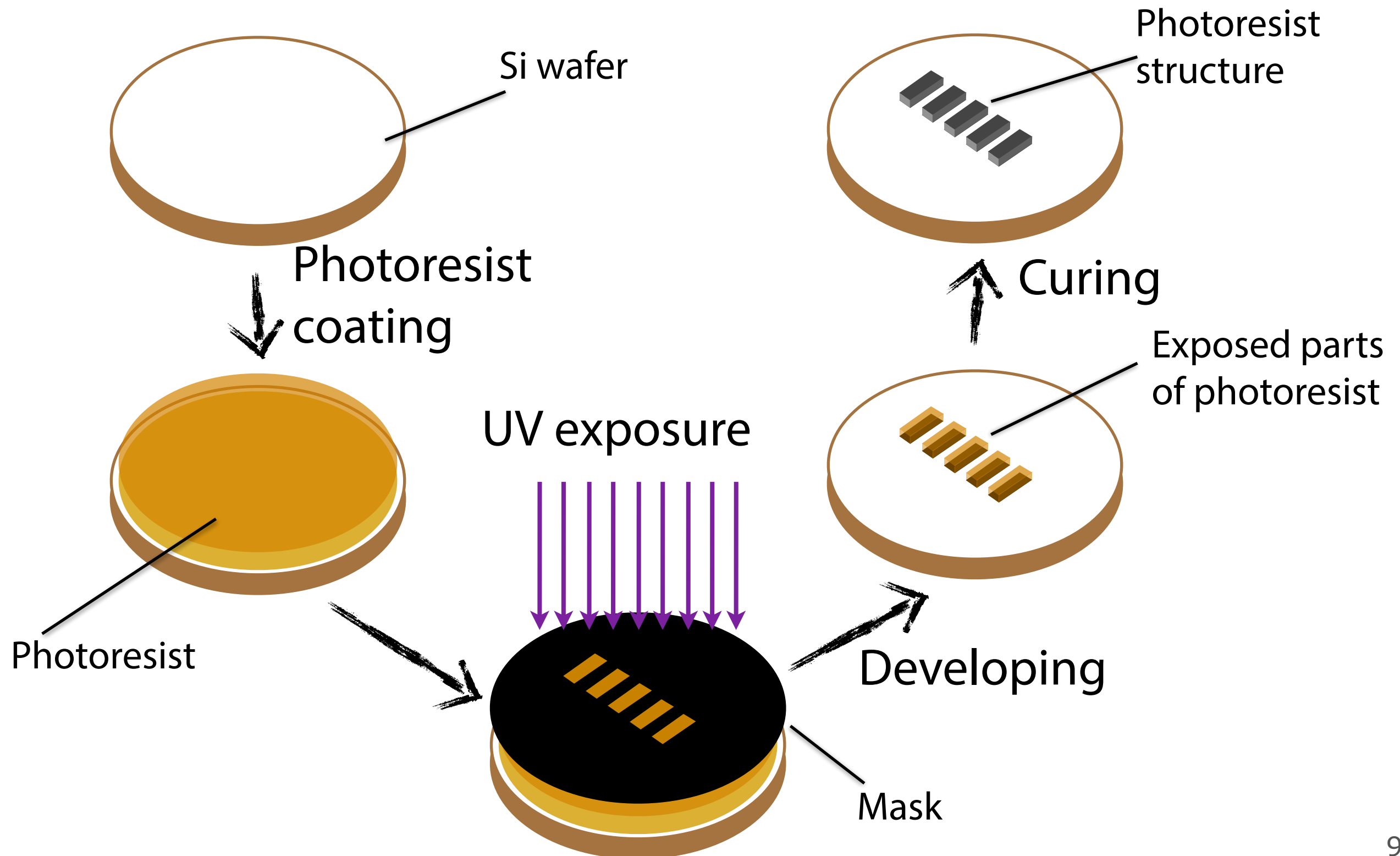
➔ Photolithography



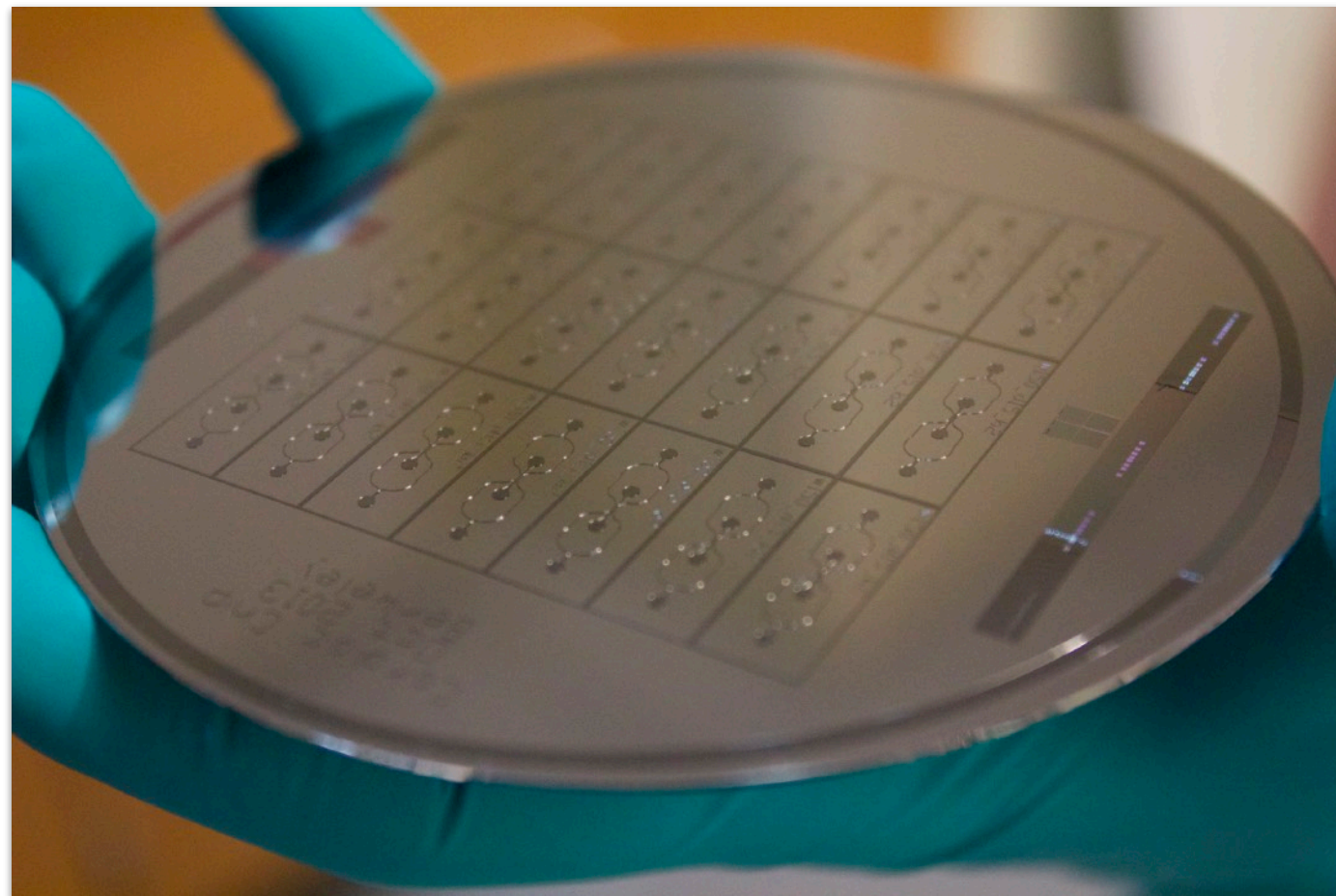
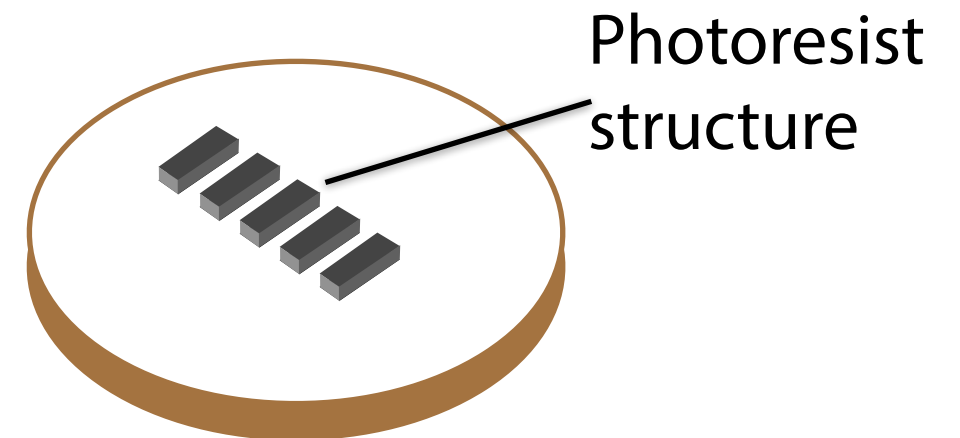
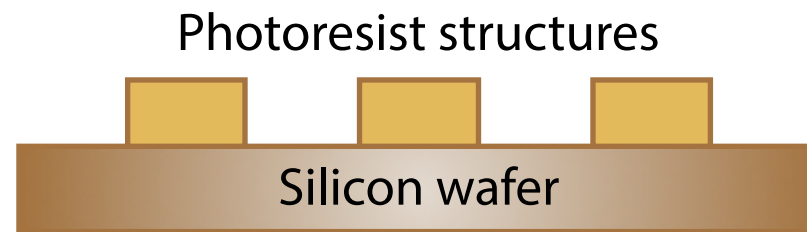
➔ Photolithography



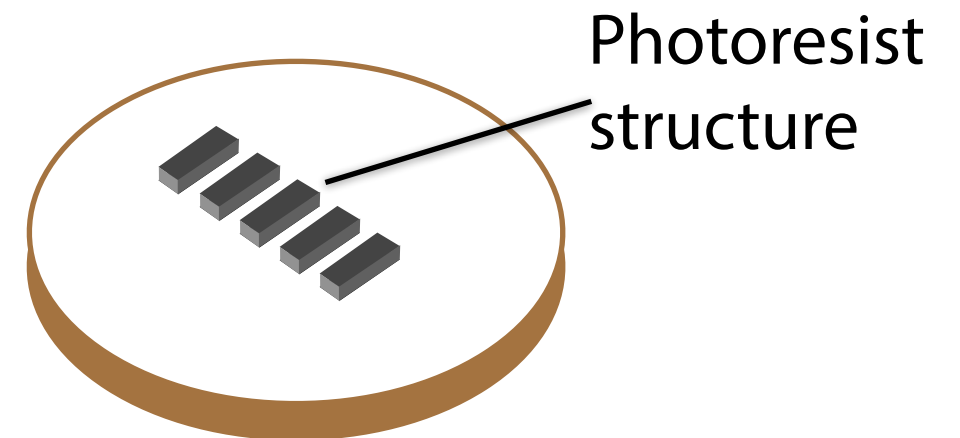
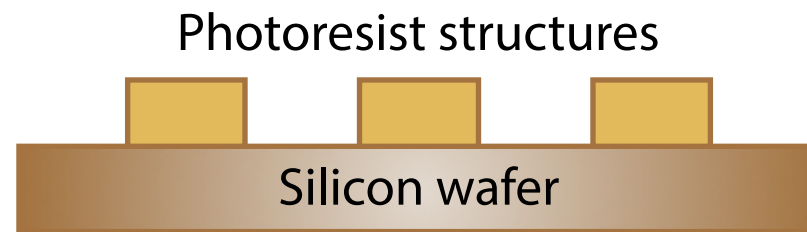
➔ Photolithography



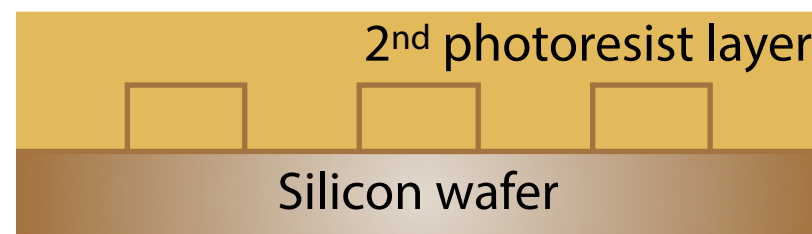
➔ Secondary processing



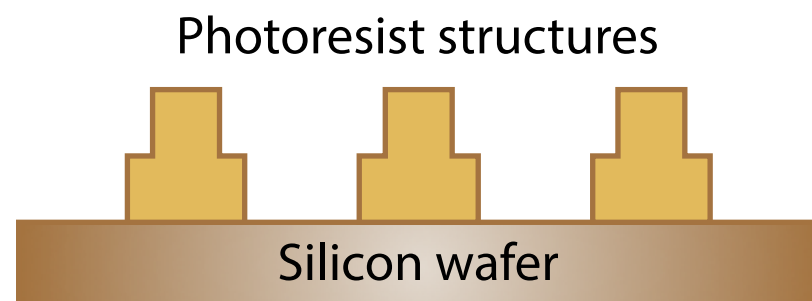
➔ Secondary processing



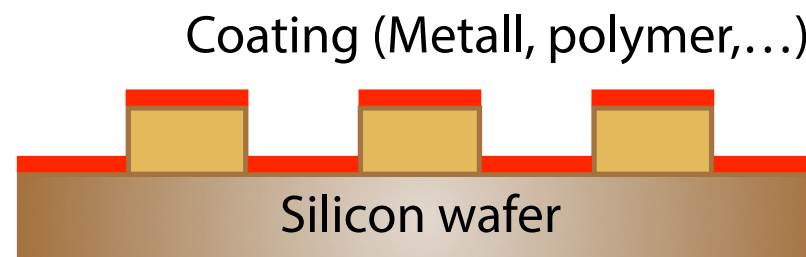
► Two step lithography



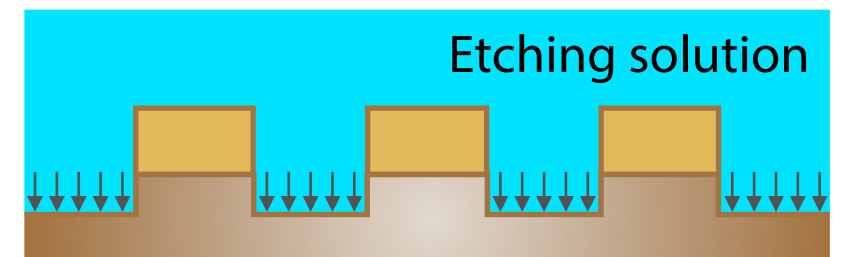
Photolithography



► Deposition



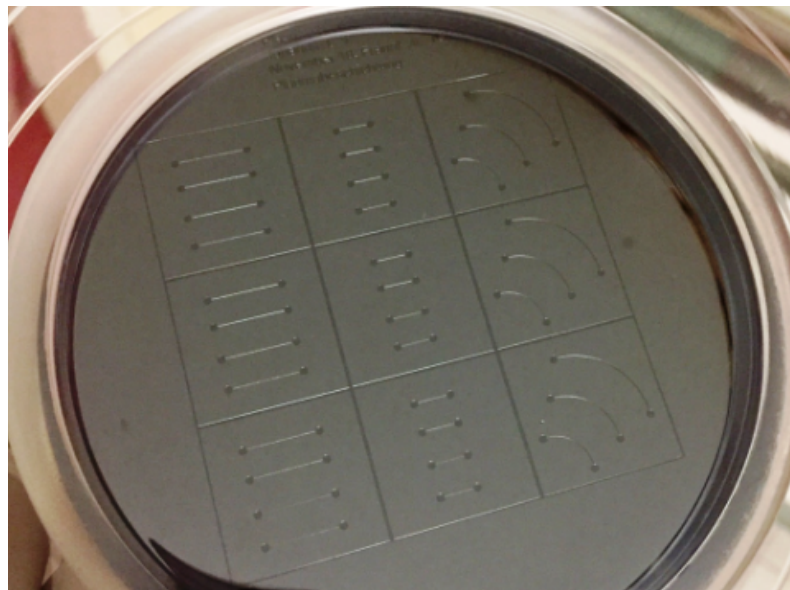
► Etching (Wet, dry)



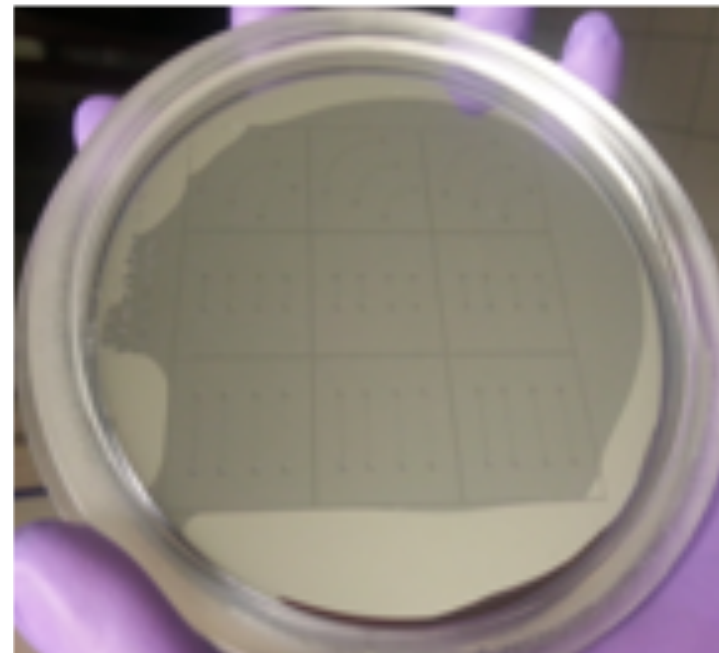
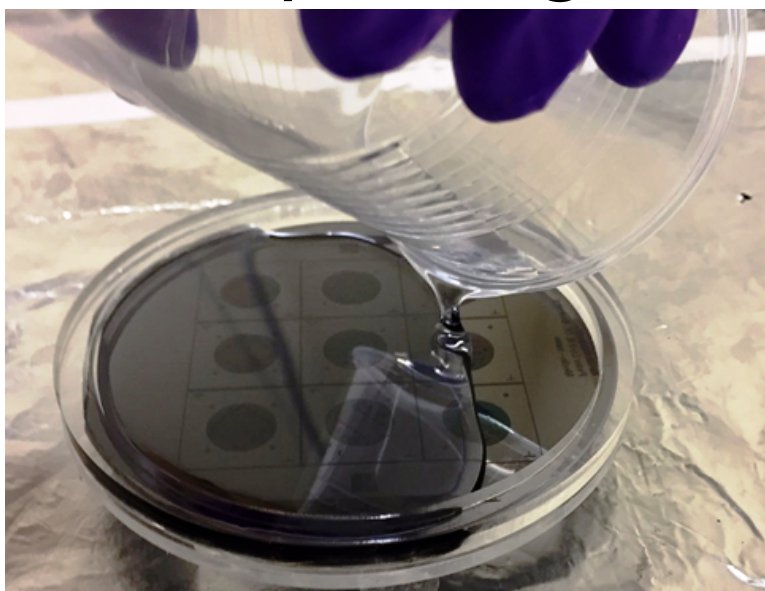
Silicon wafer

➔ Replica molding

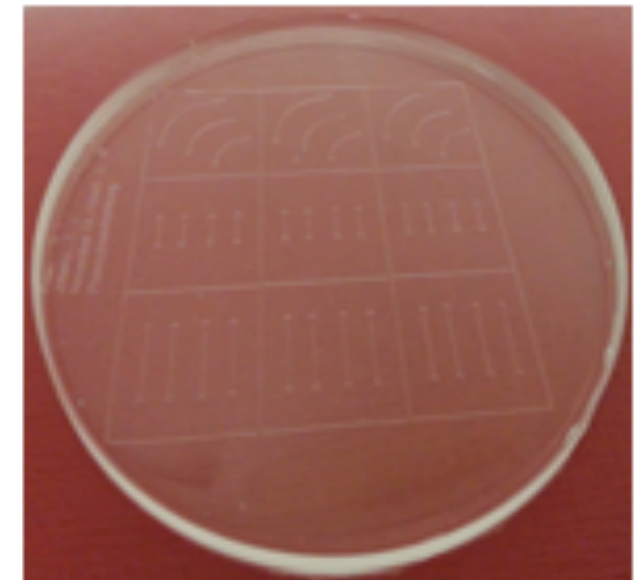
- ▶ Molding of polydimethylsiloxan (PDMS) against microfabricated master



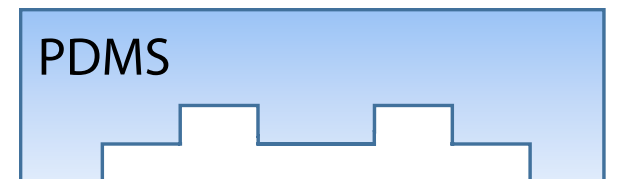
PDMS pouring



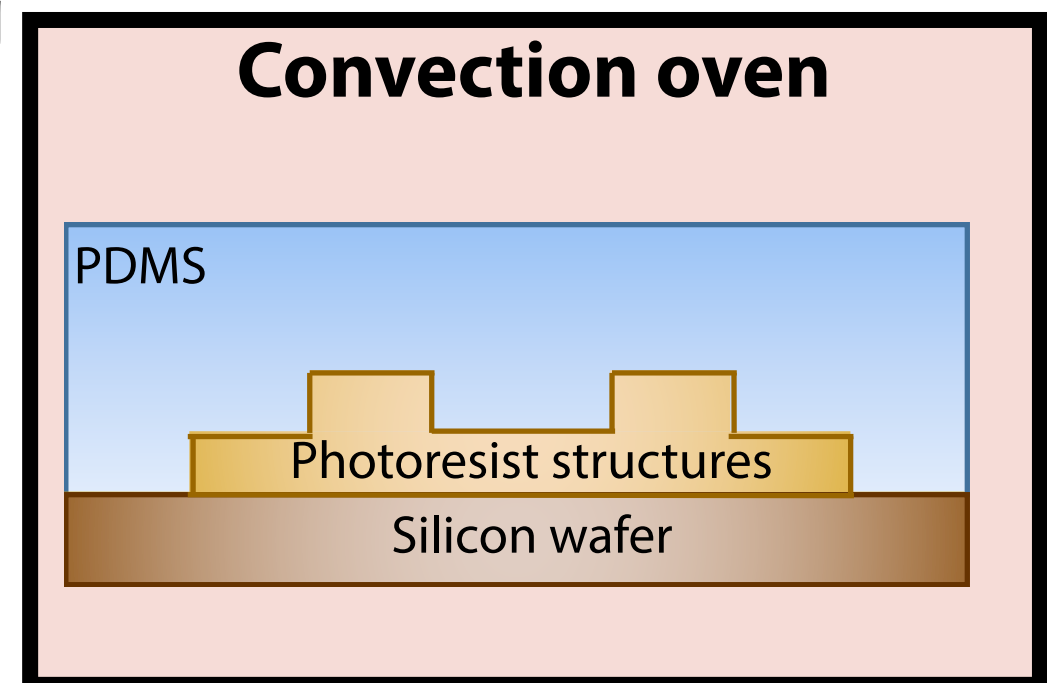
Cooling



Peel off



Curing

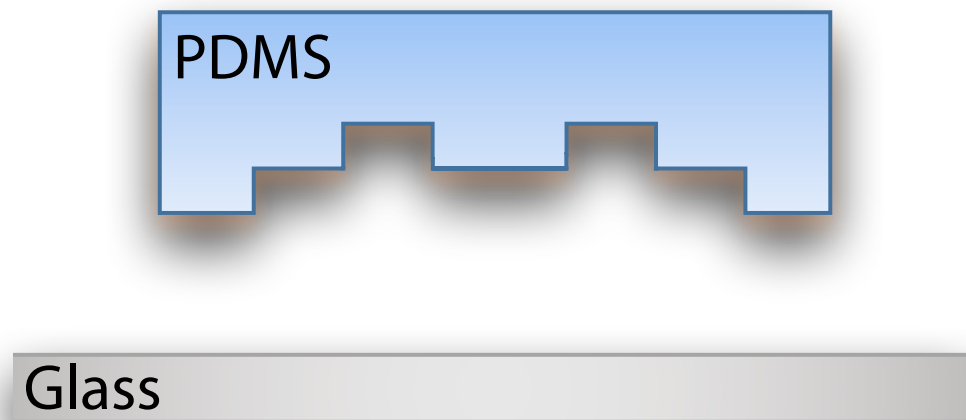


➡ Replica molding

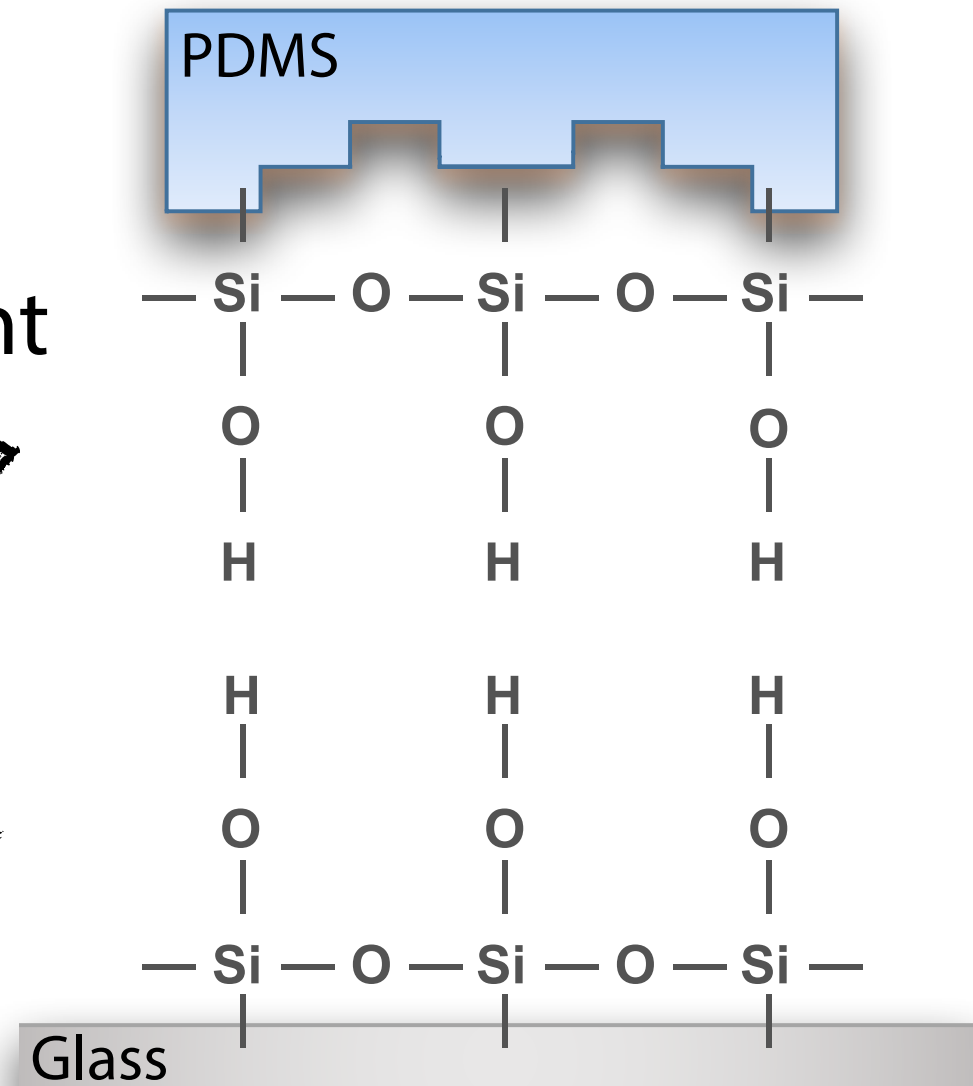
- ▶ Molding of polydimethylsiloxan (PDMS) against microfabricated master
- ▶ Fast and cheap process
- ▶ Reusable Master



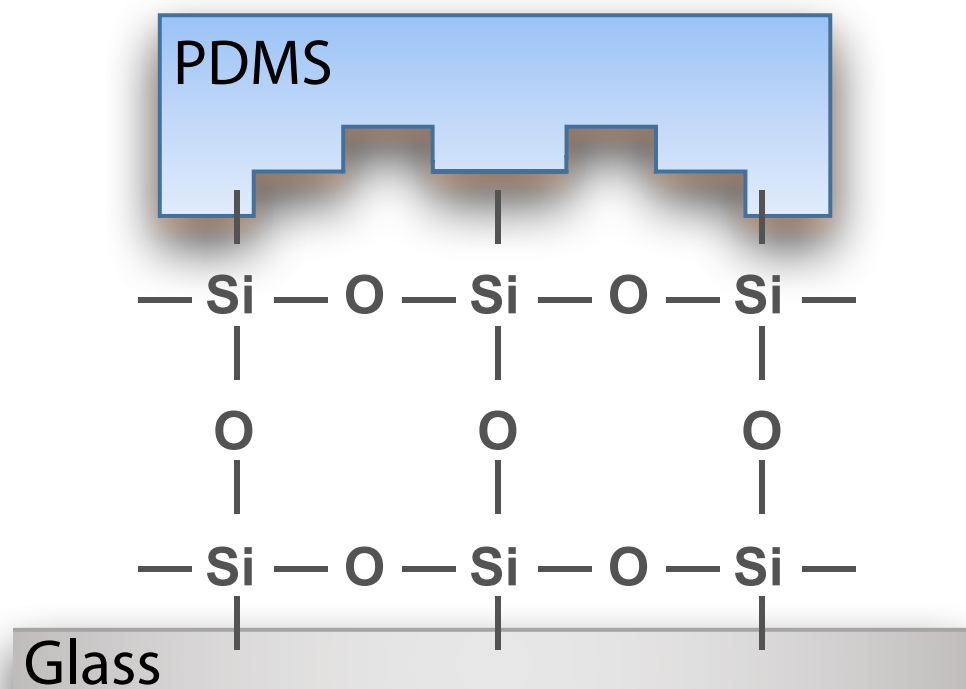
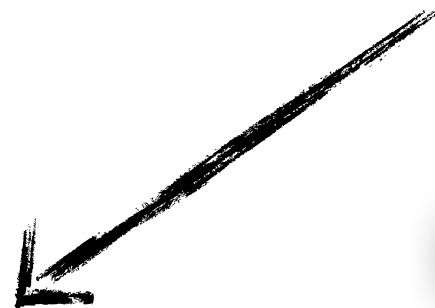
→ PDMS Bonding



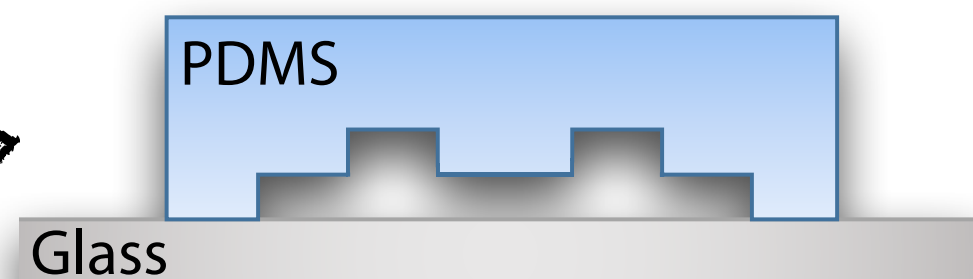
Plasma
treatment



Bonding

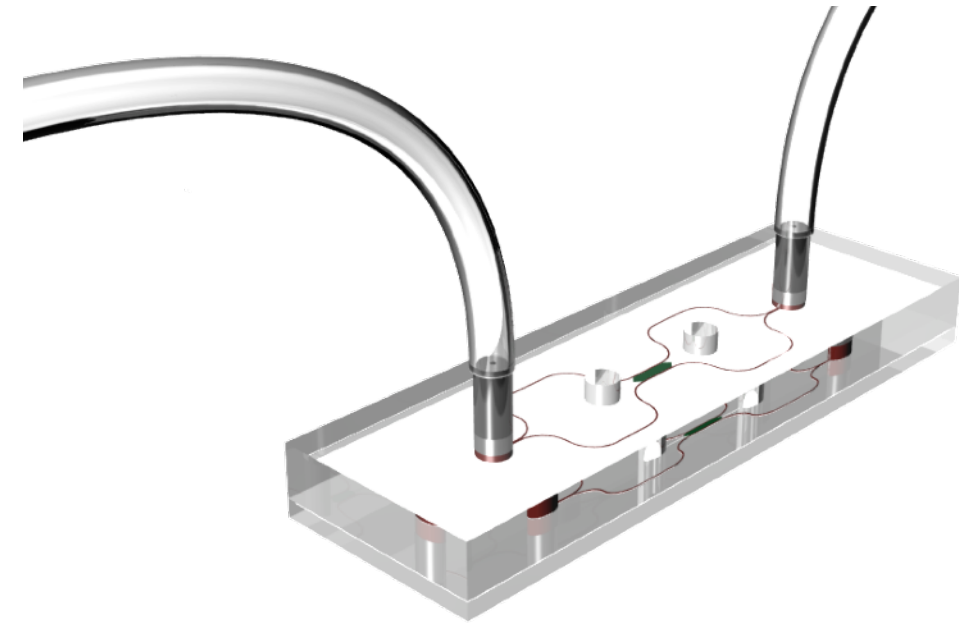


Curing



➔ Replica molding

- ▶ Molding of polydimethylsiloxan (PDMS) against microfabricated master
- ▶ Fast and cheap process
- ▶ Reusable Master



Chip
assembly

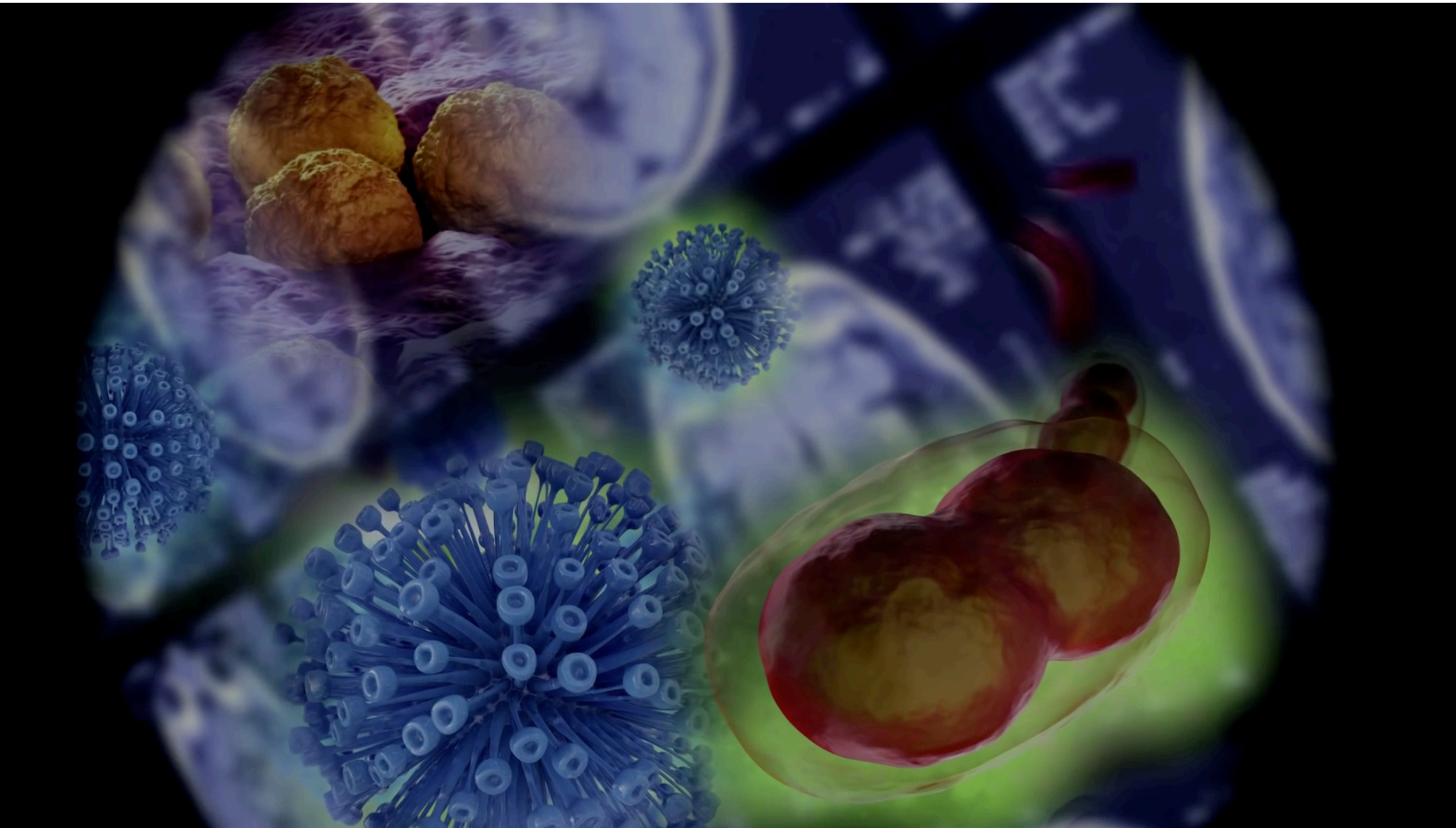


... implement operations that used to require an entire lab in a single micro-sized device:

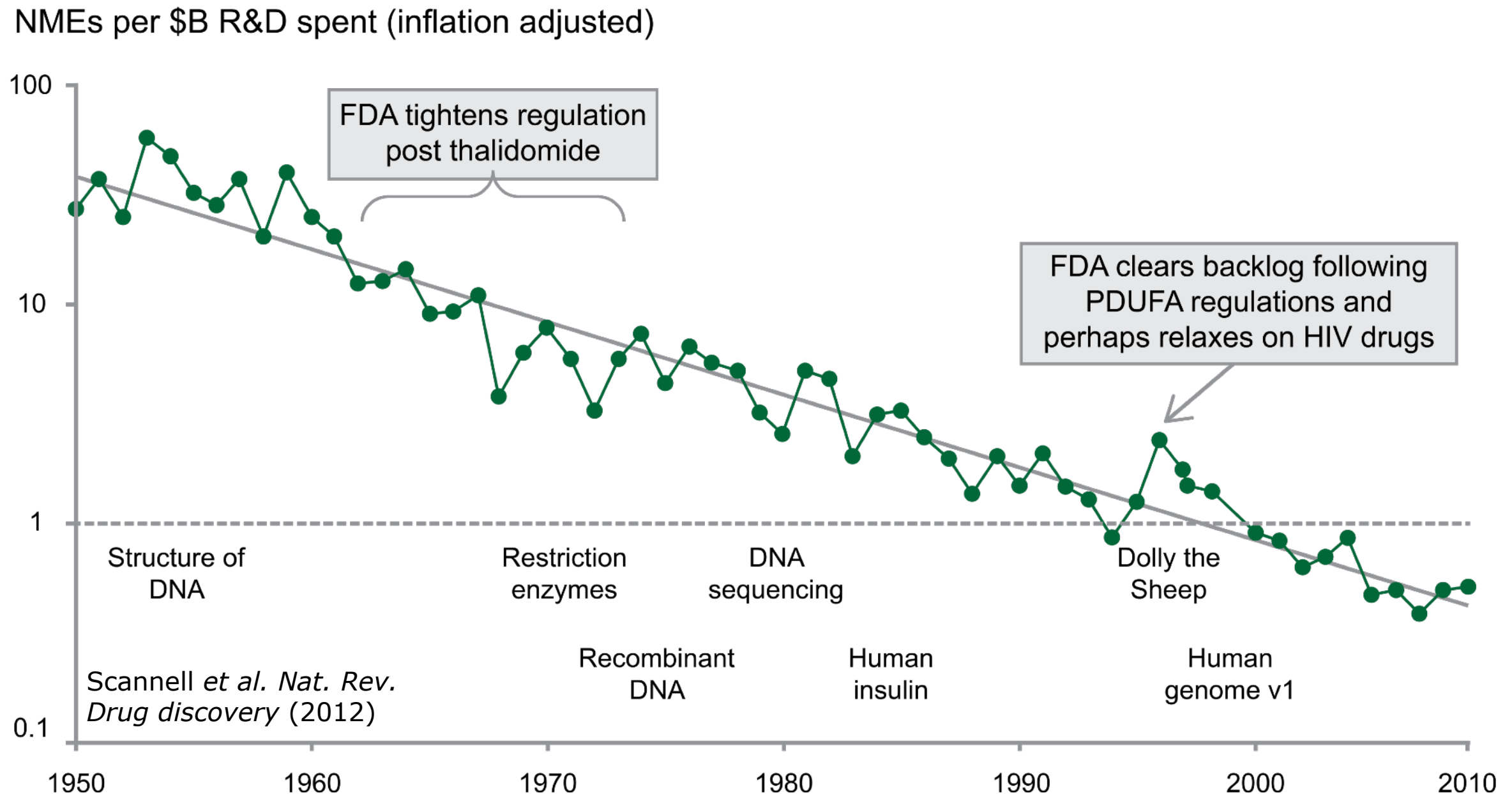
- ▶ Chemical synthesis
- ▶ Cell analysis & sorting
- ▶ Diagnostic tools (Point-of-care)
- ▶ Organs-on-a-chip

- ➡ Microfabrication techniques
- ➡ Organ-on-a-chip technology

The Problem



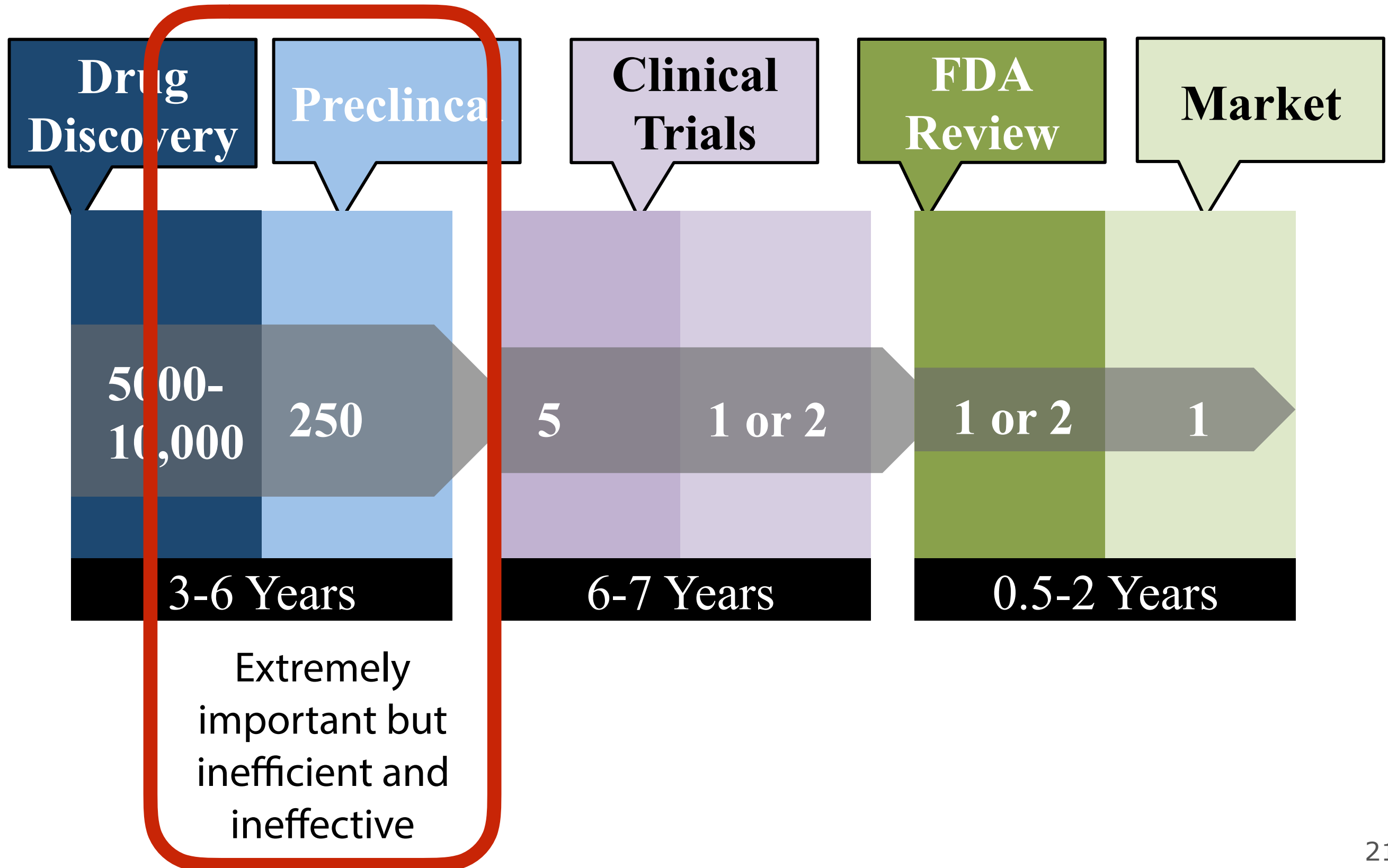
EROOM's law

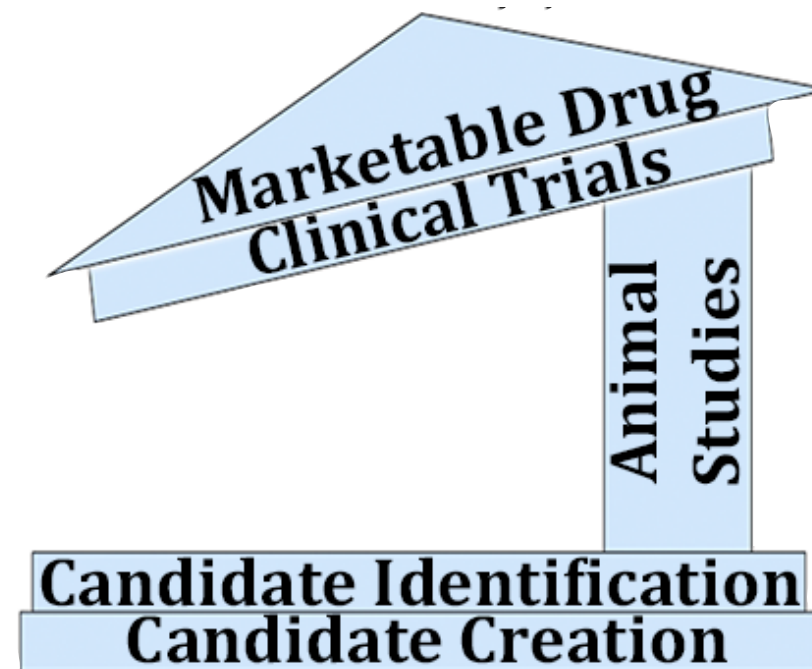


The cost of creating a new drug ~ \$5 Billion

Company	Number New Drugs	10 Year R&D Spending (\$Mil)	R&D Per Drug (\$Mil)
Abbott	1	13.183	13.183
Sanofi	6	60.760	10.128
AstraZeneca	4	38.245	9.561
Hoffmann-La Roche	8	70.928	8.866
Pfizer	10	77.786	7.779
Wyeth	3	22.702	7.567
Eil Lily	4	26.710	6.678
Bayer	5	33.118	6.624
Schering-Plough	3	18.845	6.282
Novartis	10	60.727	6.073

Drug development

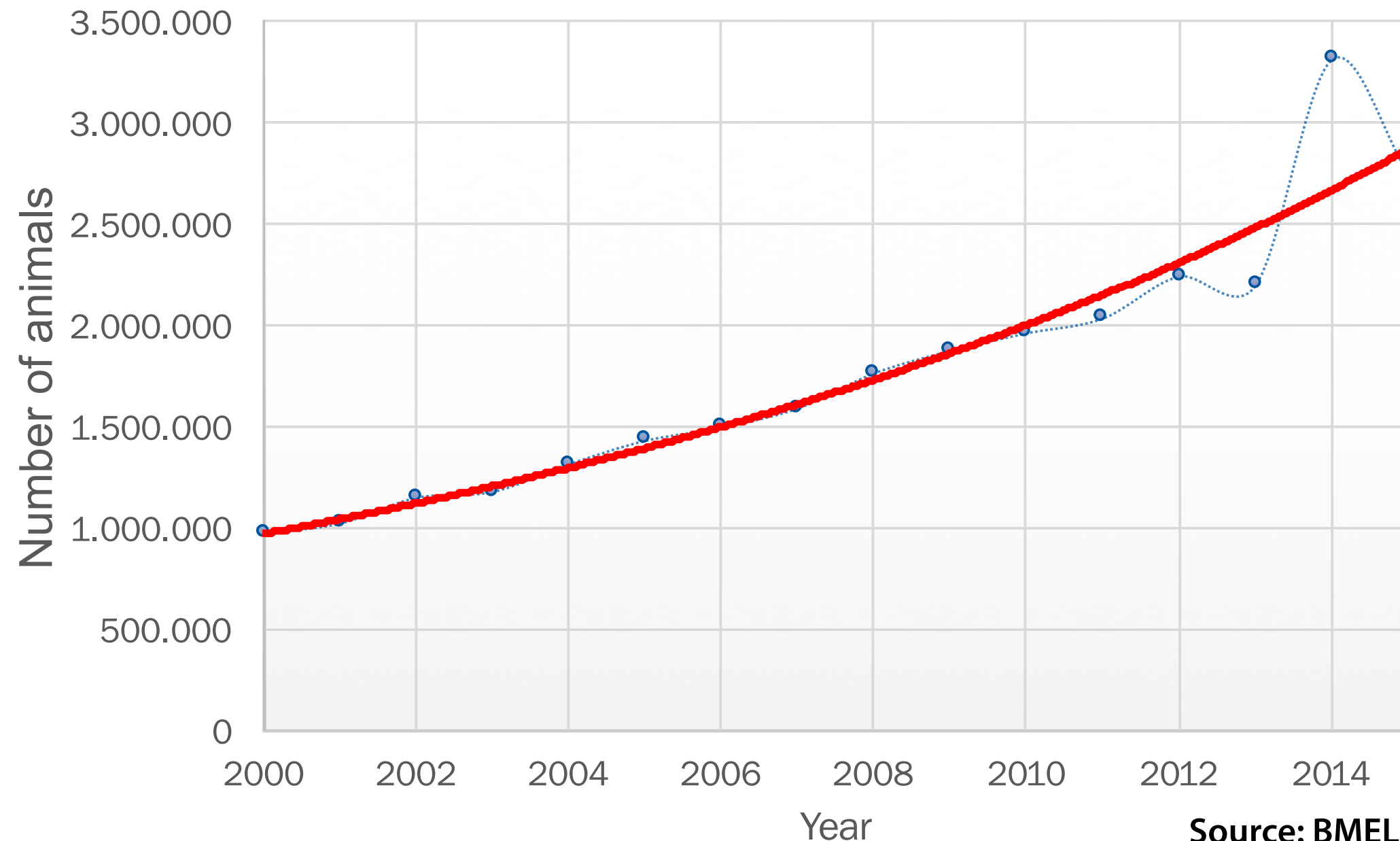




↳ Relies on animal models

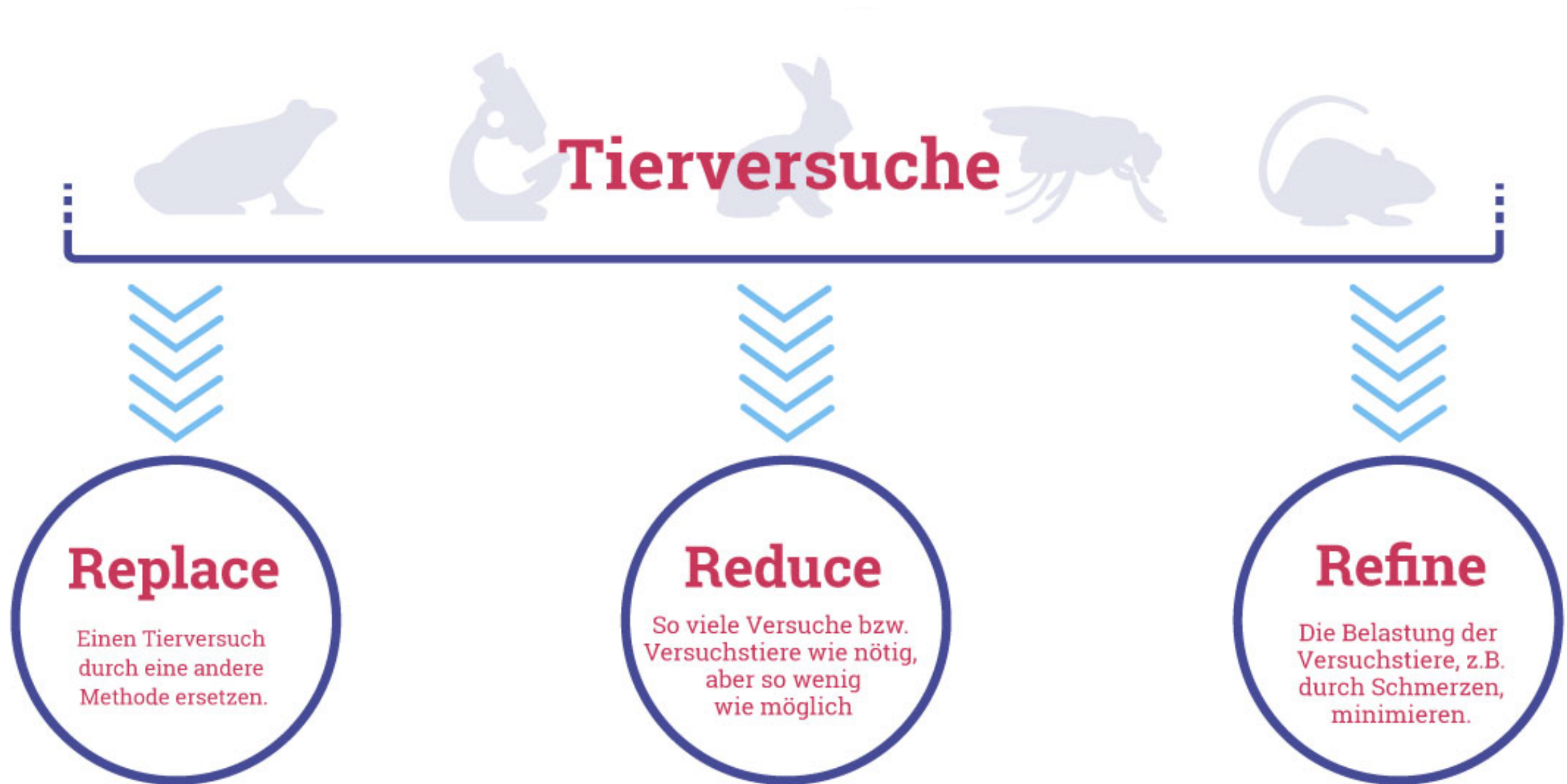
- ▶ fail to resemble human physiology
- ▶ impossible to create humanized disease systems
- ▶ ethical concerns

Use of animal models in Germany



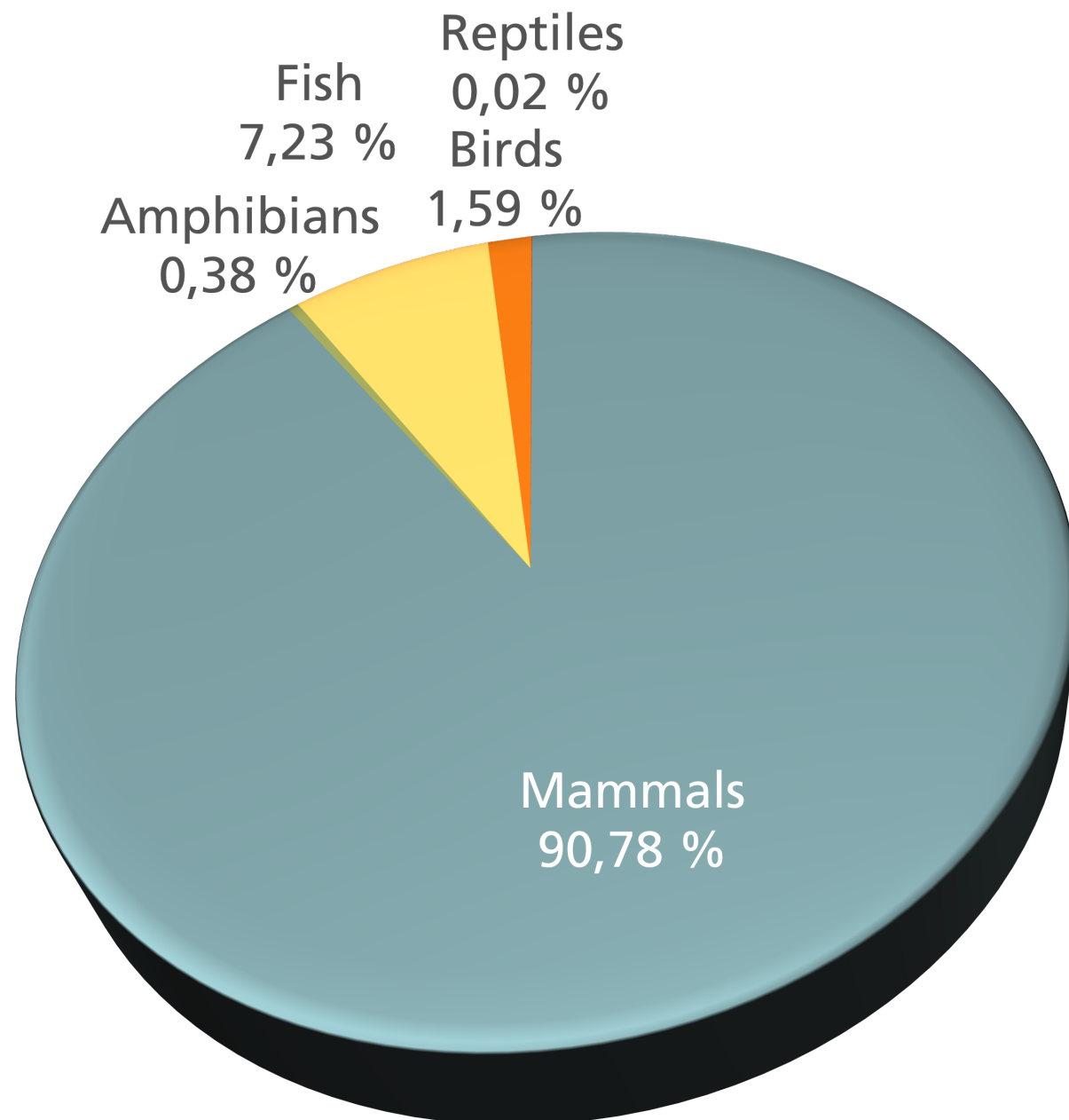
Source: BMEL 2017

3R principle



Quelle: Deutsches Zentrum zum Schutz von Versuchstieren (Bf3R), 2015

Types of animal models



Mammals	Number
Rodents	2.396.475
Rabbits	111.718
Carnivores	6.526
Equines (Horses...)	1.525
Even-toed ungulates	20.643
Primates	3.141
Further mammals	1.590
Total	2.541.618

Source: BMEL 2017

Rodent models

- ▶ 95% of genes identical to human
- ▶ Similar organ structure



Advantages

- ▶ Small: Advantageous in terms of housing and facility size
- ▶ Fast breeding

Disadvantages

- ▶ Specific environment and diet
- ▶ Strain-specific results due to breeding
- ▶ Compared to human:
 - ▶ Different physiology (heart rate 500–700 bpm)
 - ▶ Large differences in immune system

Rodent models

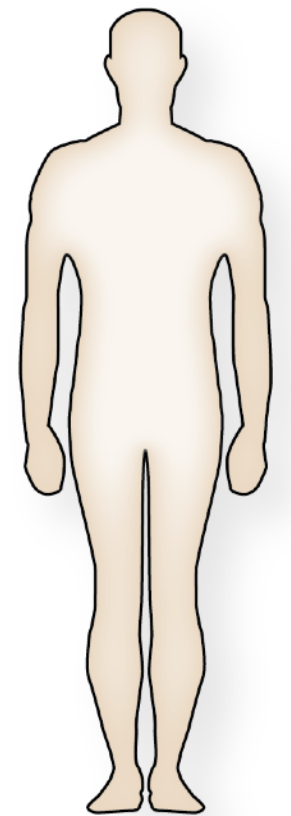
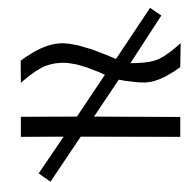
The New York Times

SCIENCE

Mice Fall Short as Test Subjects for Some of Humans' Deadly Ills

By GINA KOLATA FEB. 11, 2013

"But now, researchers report evidence that the mouse model has been totally misleading for at least three major killers—sepsis, burns, and trauma. As a result, **years and billions of dollars have been wasted** following false leads, they say".

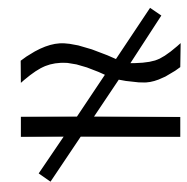


Rodent models

PNAS 2013

Genomic responses in mouse models poorly mimic human inflammatory diseases

Junhee Seok^{a,1}, H. Shaw Warren^{b,1}, Alex G. Cuenca^{c,1}, Michael N. Mindrinos^a, Henry V. Baker^c, Weihong Xu^a, Daniel R. Richards^d, Grace P. McDonald-Smith^e, Hong Gao^a, Laura Hennessy^f, Celeste C. Finnerty^g, Cecilia M. López^c, Shari Honari^f, Ernest E. Moore^h, Joseph P. Mineiⁱ, Joseph Cuschieri^j, Paul E. Bankey^k, Jeffrey L. Johnson^h, Jason Sperry^l, Avery B. Nathens^m, Timothy R. Billiar^l, Michael A. Westⁿ, Marc G. Jeschke^o, Matthew B. Klein^j, Richard L. Gamelli^p, Nicole S. Gibran^j, Bernard H. Brownstein^q, Carol Miller-Graziano^k, Steve E. Calvano^r, Philip H. Mason^e, J. Perren Cobb^s, Laurence G. Rahme^t, Stephen F. Lowry^{r,2}, Ronald V. Maier^j, Lyle L. Moldawer^c, David N. Herndon^g, Ronald W. Davis^{a,3}, Wenzhong Xiao^{a,t,3}, Ronald G. Tompkins^{t,3}, and the Inflammation and Host Response to Injury, Large Scale Collaborative Research Program⁴



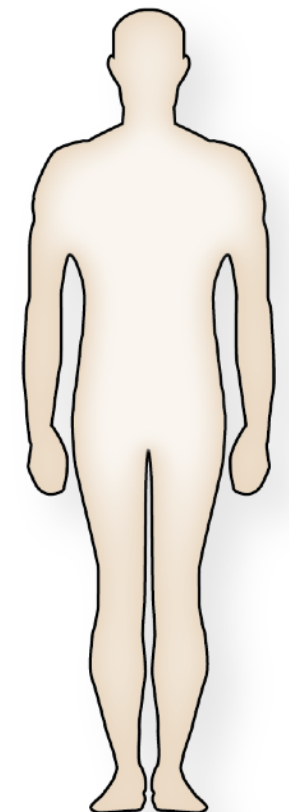
PNAS 2014

Genomic responses in mouse models greatly mimic human inflammatory diseases

Keizo Takao^{a,b} and Tsuyoshi Miyakawa^{a,b,c,1}

^aSection of Behavior Patterns, Center for Genetic Analysis of Behavior, National Institute for Physiological Sciences, Okazaki, Aichi 444-8585, Japan; ^bCore Research for Evolutional Science and Technology, Japan Science and Technology Agency, Kawaguchi, Saitama 332-0012, Japan; and ^cDivision of Systems Medical Science, Institute for Comprehensive Medical Science, Fujita Health University, Toyoake, Aichi 470-1192, Japan

Edited by Ruslan Medzhitov, Yale University School of Medicine, New Haven, CT, and approved June 11, 2014 (received for review January 31, 2014)



„It is generally well known and accepted among researchers using animal models that mouse models **mimic only partial aspects of human disorders.**“

Rodent models

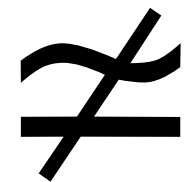


© psdesign1 - Fotolia

Couzin-Frankel Science (2013)



When Mice Mislead



New stroke therapies

➡ 603 drugs tested in animals

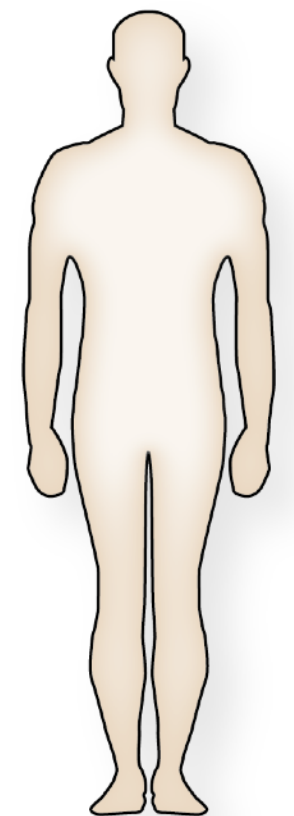
- ▶ 374 showed positive healing effects
- ▶ 97 tried in humans

➡ 1 worked

"...wasn't tested because of animal data at all, but because it had already benefitted patients with heart attacks."

"The way we do our research with our animals is stone-age."

—ULRICH DIRNAGL, CHARITÉ UNIVERSITY MEDICINE BERLIN



Conventional cell culture

“Biologists have constructed an entire, self-consistent biology using monolayer monocultures on plastic”

(John Wikswo, Professor of Biomedical Engineering, Molecular Physiology & Biophysics, and Physics at Vanderbilt University)

Advantages

- ▶ Simple handling & logistics
- ▶ Use of cell lines (unlimited availability)
- ▶ Reproducible & standardisable
- ▶ Cheap
- ▶ Various ways of analysis

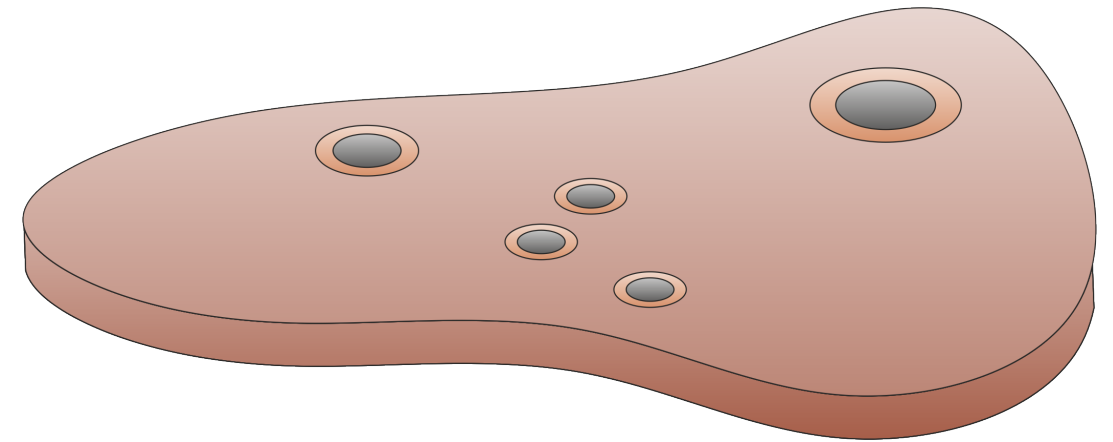
Disadvantages

- ▶ 2D cell monolayers
- ▶ Cell / media ratio magnitudes too low
- ▶ Typically mono-cultures
- ▶ No perfusion
- ▶ No mechanical forces
- ▶ On (hard) plastic surfaces

Conventional cell culture

➔ Tissue slices

- ▶ Vascularised tissue
- ▶ includes all cell types
- ▶ embedded in native ECM



Precision Cut Liver Slices

Advantages

- ▶ Physiological model of human tissue

Disadvantages

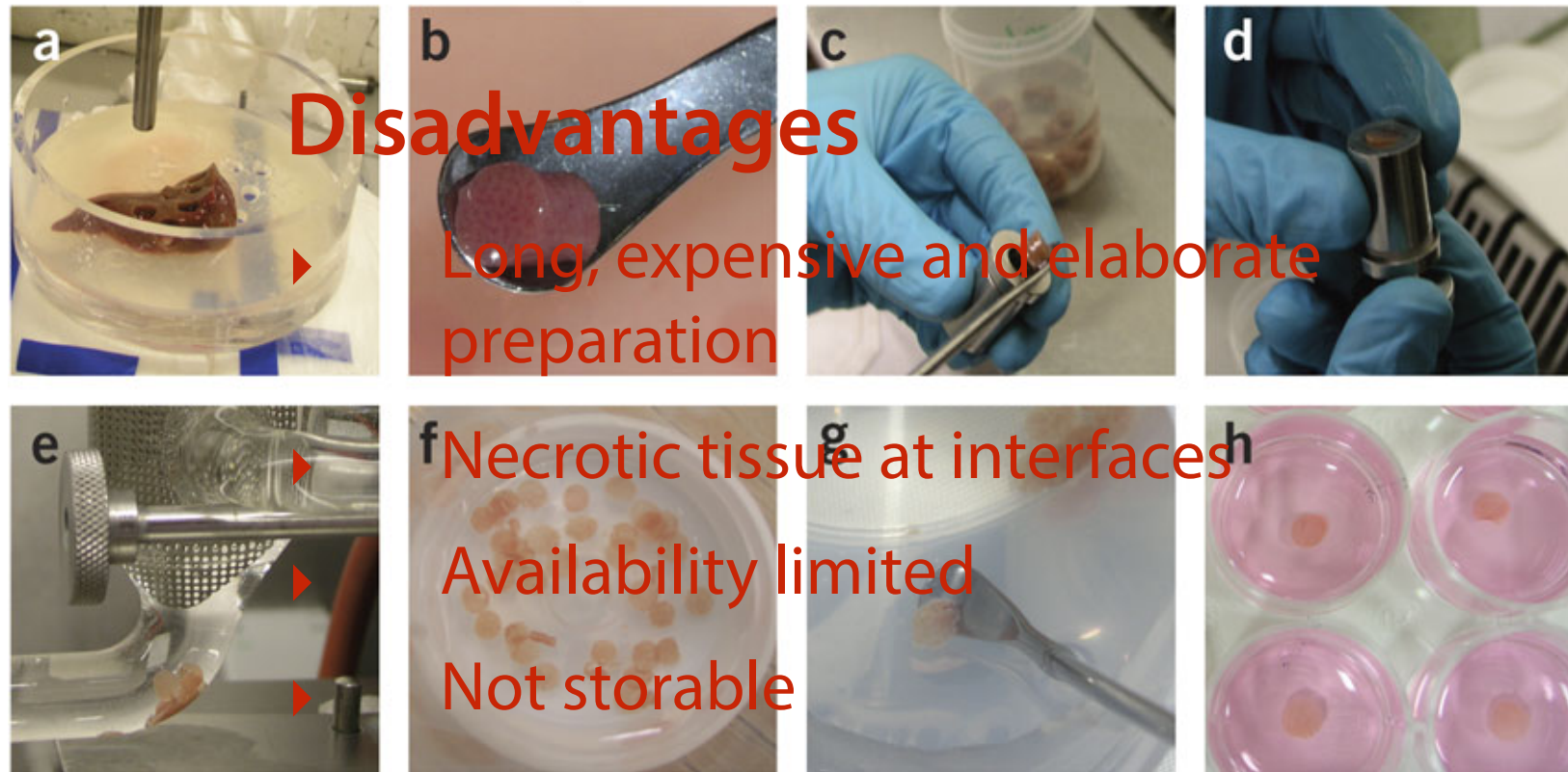
Long, expensive and elaborate preparation

Necrotic tissue at interfaces

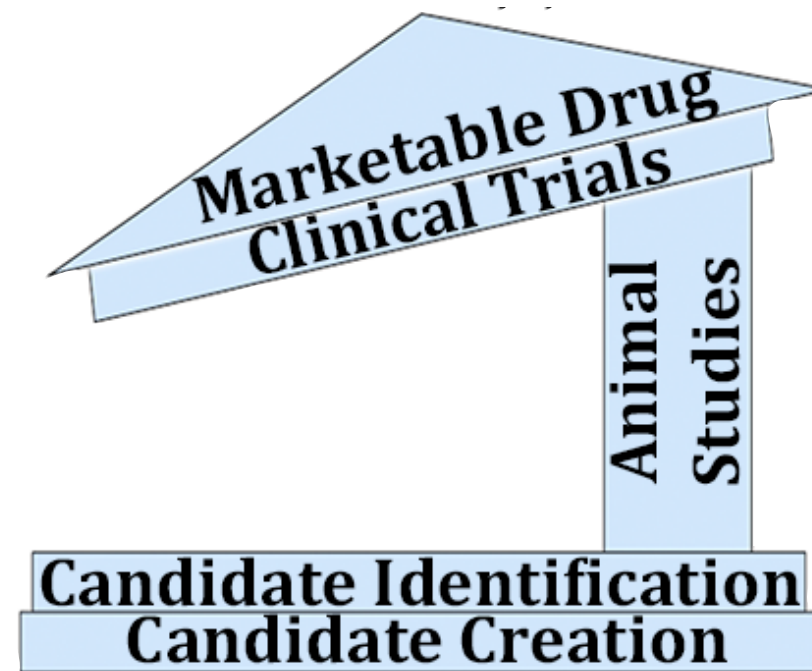
Availability limited

Not storable

Large variability due to patient heterogeneity



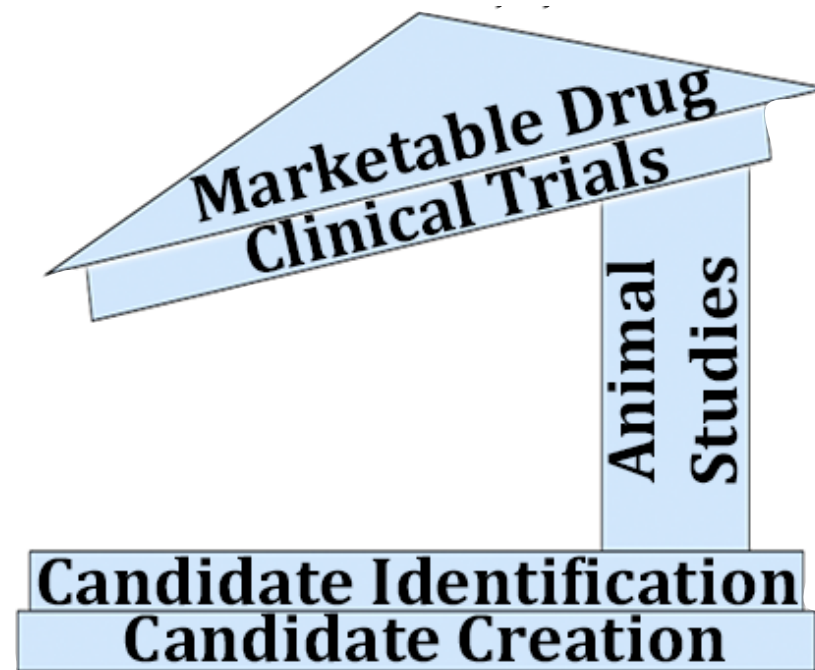
de Graaf et al. Nature Protocols (2010)



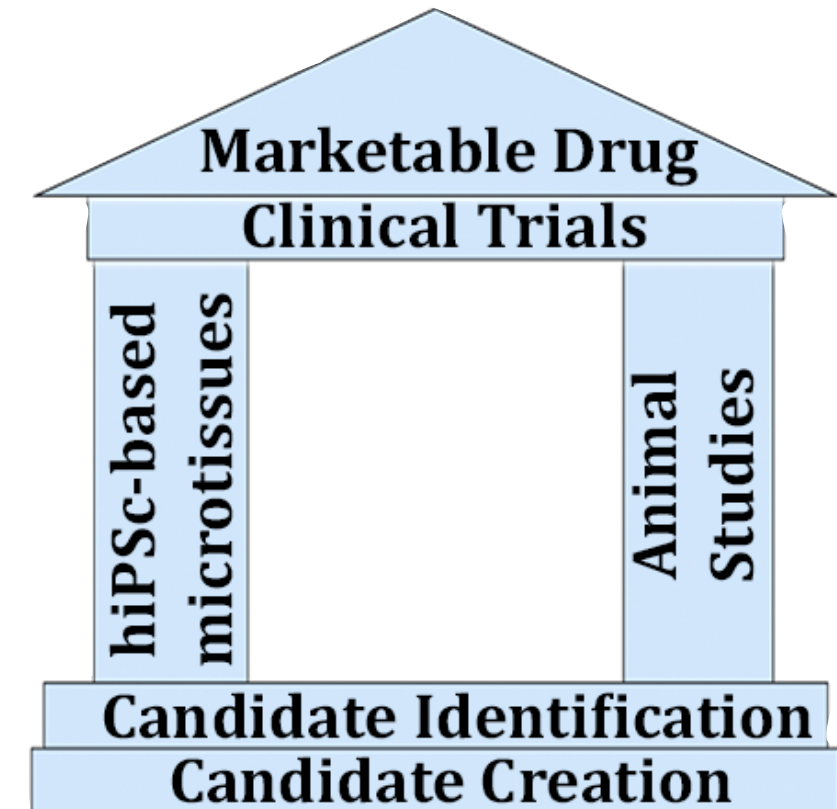
↳ Relies on animal models

- ▶ fail to resemble human physiology
- ▶ impossible to create humanized disease systems
- ▶ ethical concerns

Classical approach



Next generation approach



➡ Relies on animal models

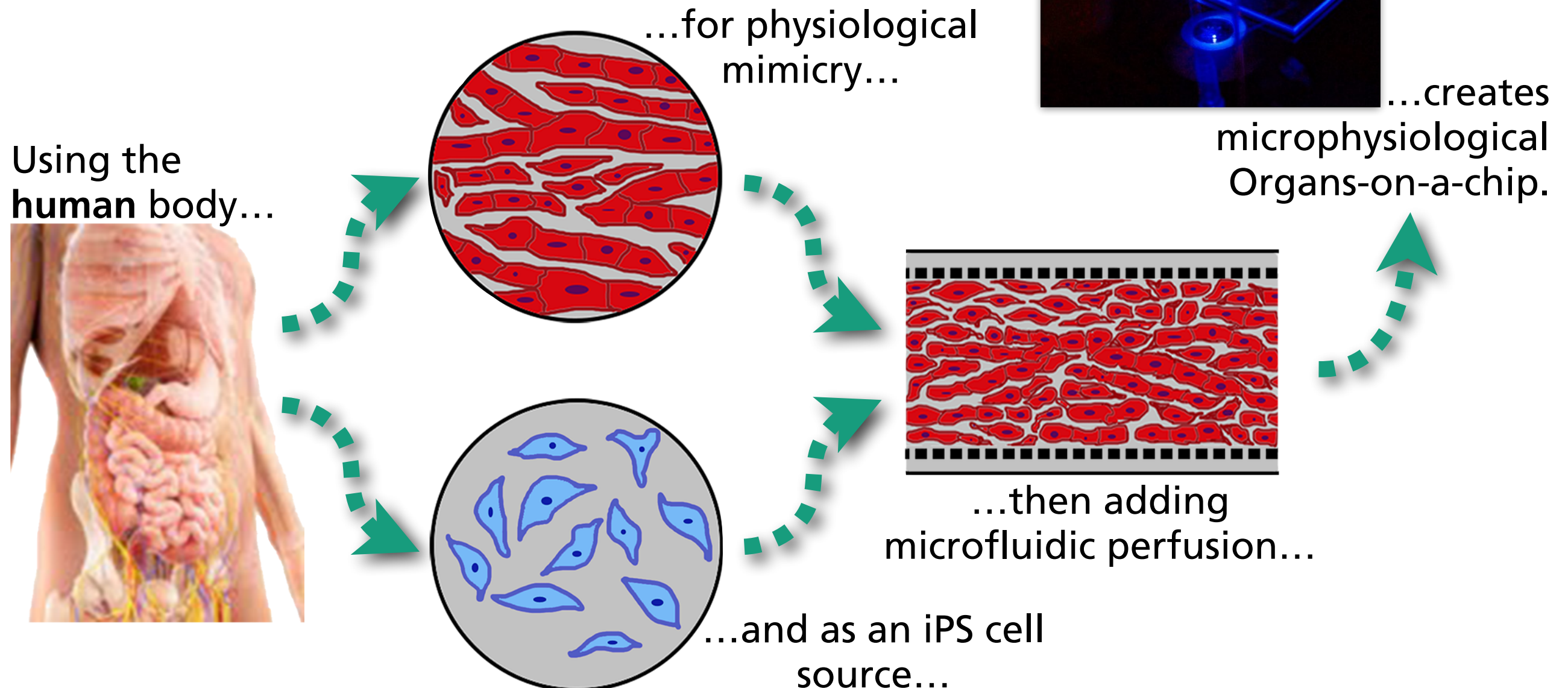
- ▶ fail to resemble human physiology
- ▶ impossible to create humanized disease systems
- ▶ ethical concerns

➡ Adds organs-on-a-chip

- ▶ resemble human physiology
- ▶ patient and disease specific
- ▶ no ethical concerns

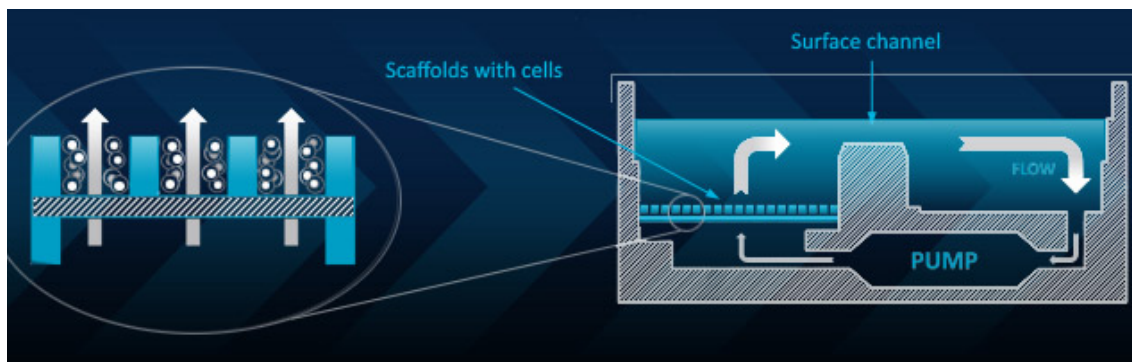
➔ How do we define organs-on-a-chip?

- Human tissue
- Physiological structure & function
- Microfluidic perfusion



Perfused Trans-Well Systems

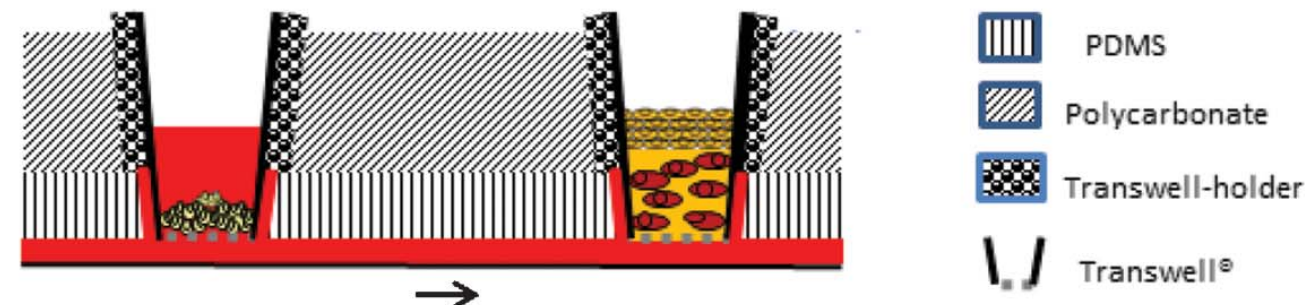
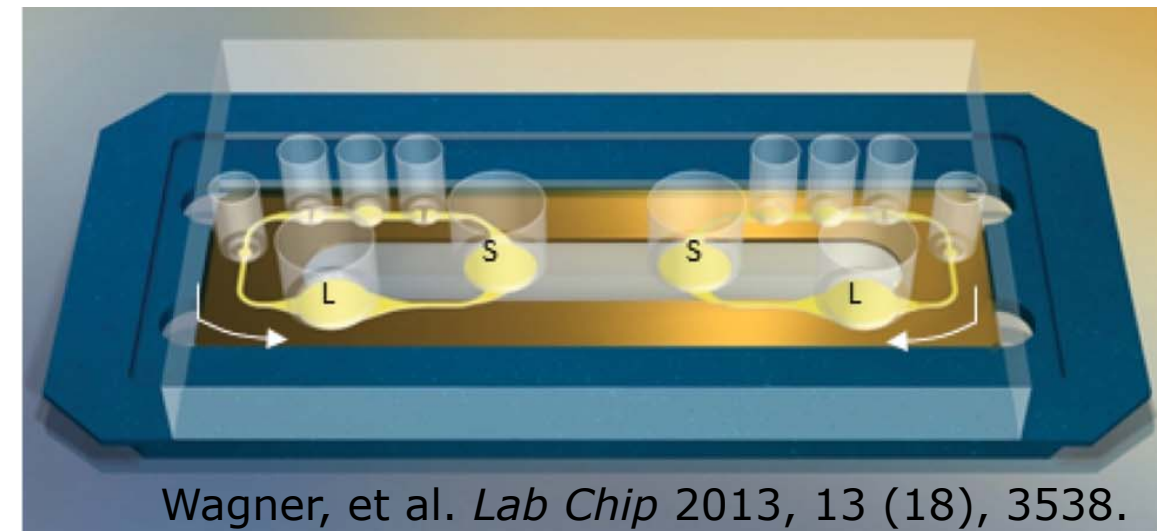
Liver Module - Linda Griffith (MIT)



Advantages

- ▶ Commercially established
- ▶ Multi-organ-versions

Multi-Transwell module - Uwe Marx (Berlin)



Disadvantages

- ▶ Large perfusion volumes
- ▶ Difficult to perform in situ imaging
- ▶ Organs not sustained off platform
- ▶ Tethered to pneumatic controller

The next step:

Microphysiological Systems (MPS) /
Microfluidic Organs-on-a-chip /
Tissue Chips

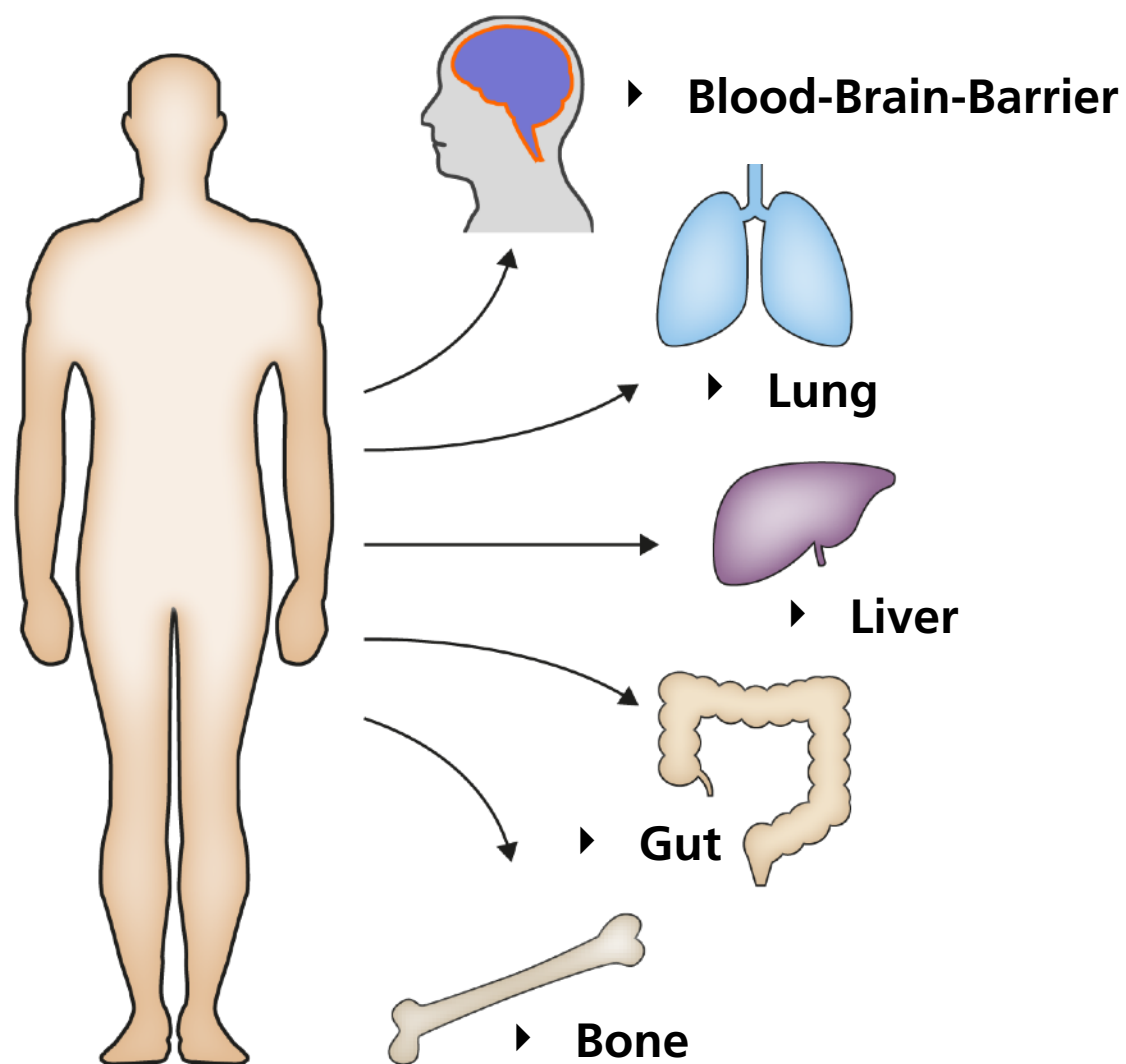
➡ **Creating microscopic “minimal functional units” of organs in a microfluidic environment**

Microfluidic Organs-on-a-chip

„... a microfluidic cell culture device created with microchip manufacturing methods that contains continuously perfused chambers inhabited by living cells arranged to simulate tissue- and organ-level physiology. “

Bhatia SN, Ingber DE. Nature Biotechnology 2014;32:760-772

➔ **Simulate tissue-/organ-level functionality**

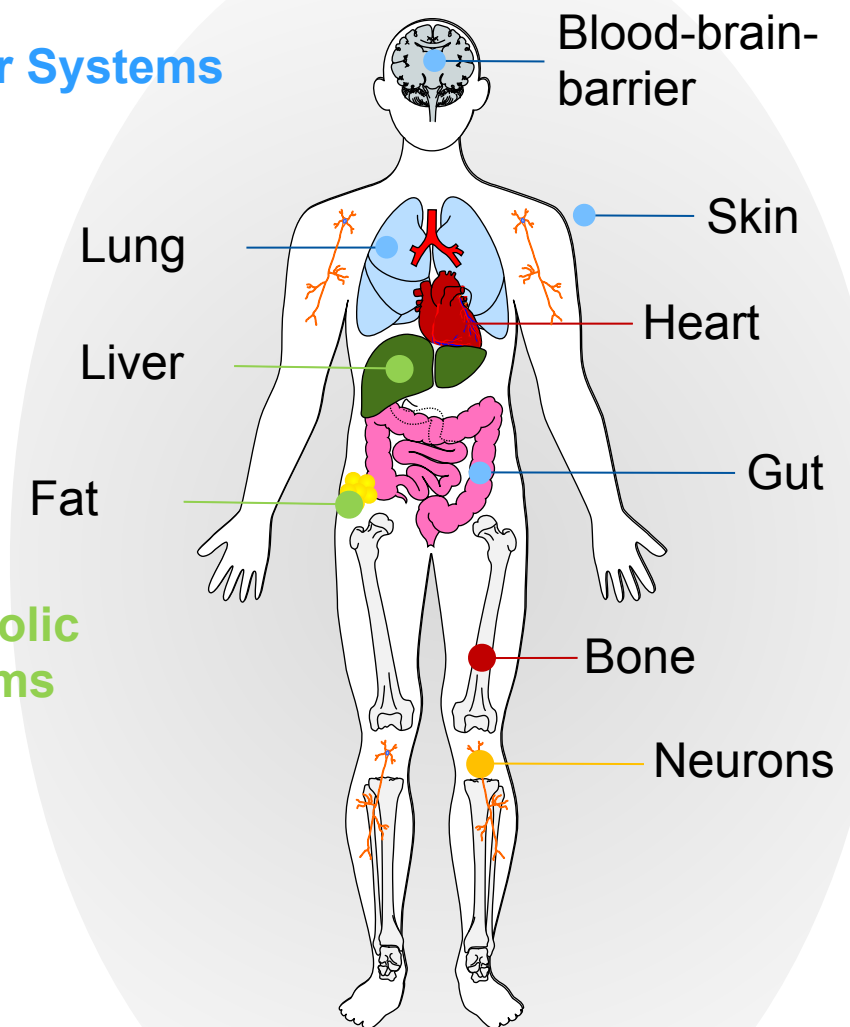


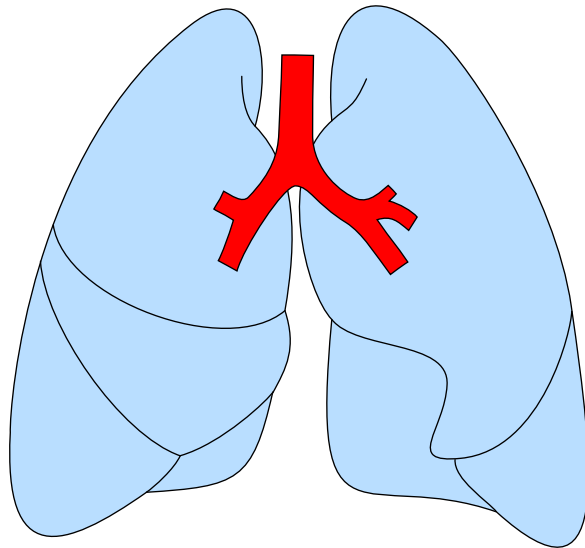
Barrier Systems

Metabolic Systems

Mechanical Systems

Neuronal Systems





Lung on a chip - Don Ingber (Wyss Institute / Harvard)

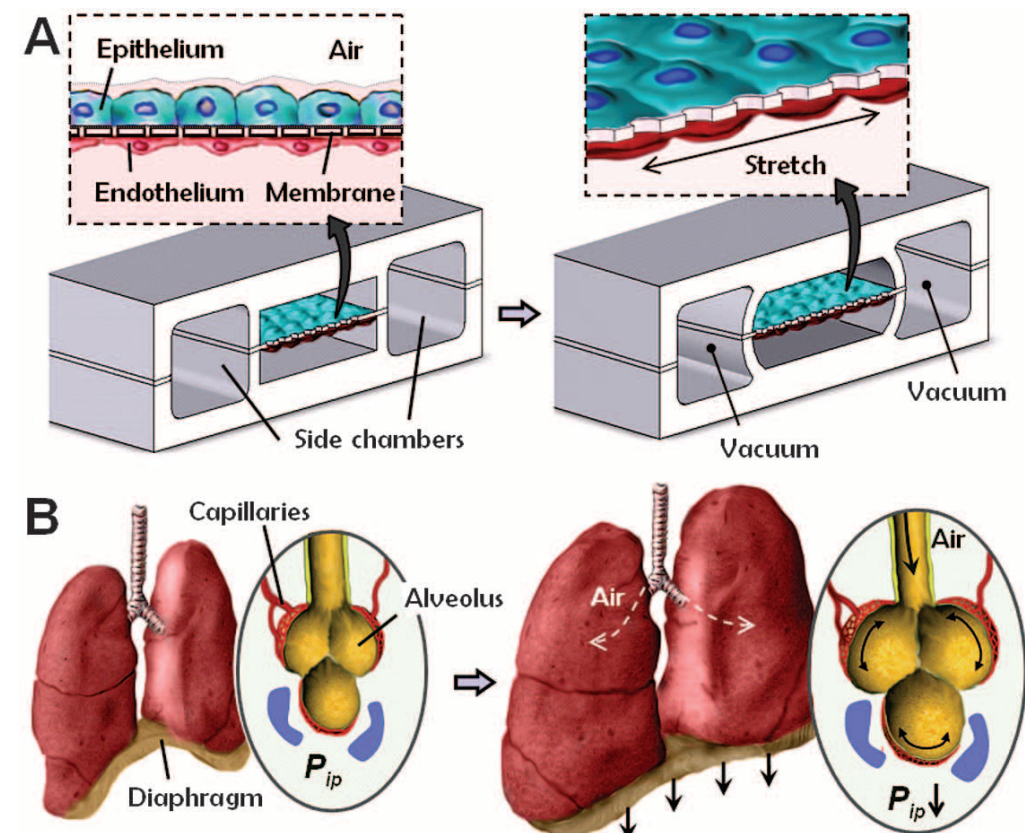
Reconstituting Organ-Level Lung Functions on a Chip

Dongeun Huh,^{1,2} Benjamin D. Matthews,^{2,3} Akiko Mammoto,² Martín Montoya-Zavala,^{1,2}
Hong Yuan Hsin,² Donald E. Ingber^{1,2,4*}

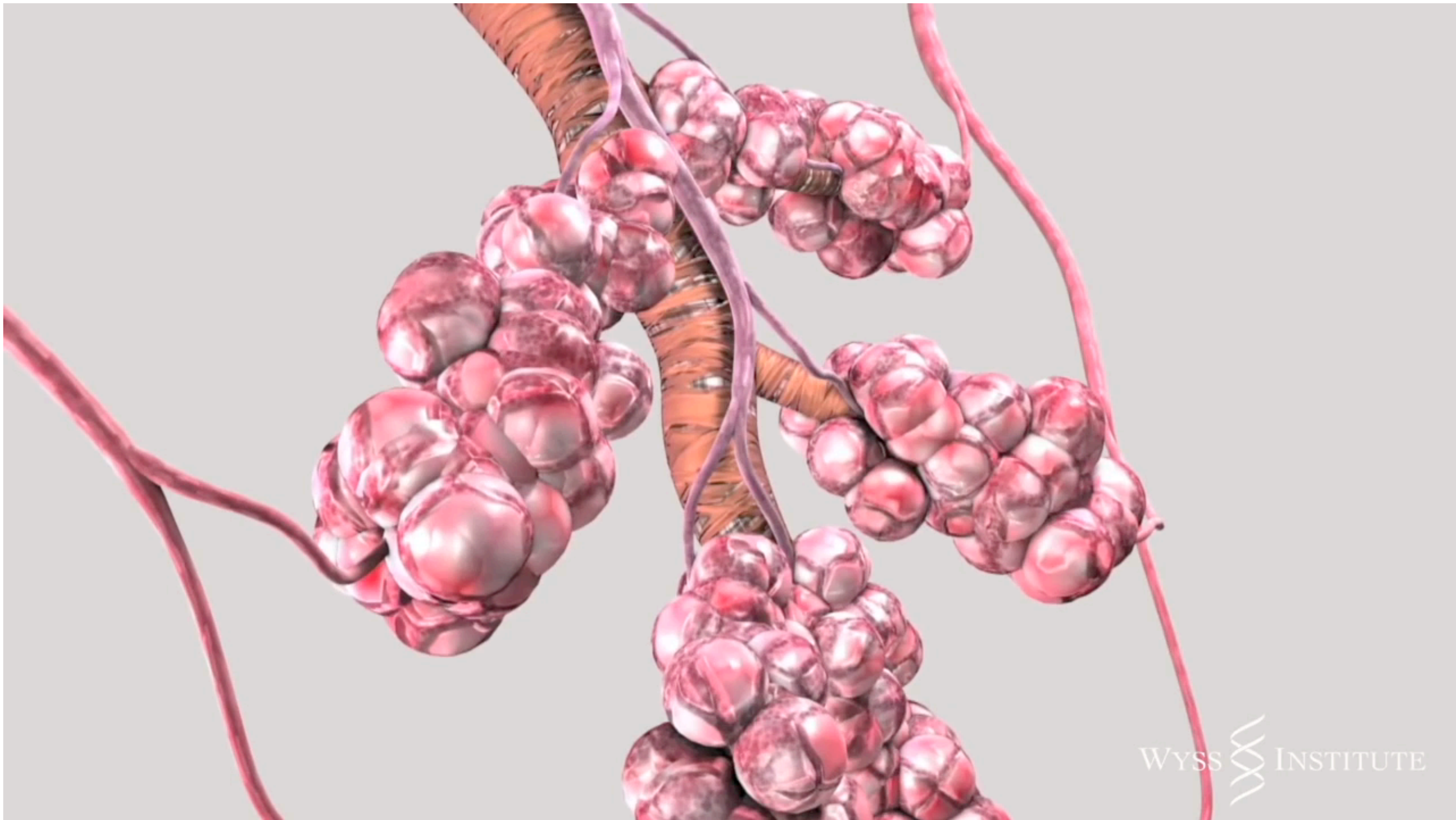
Science, 2010

Lung characteristics

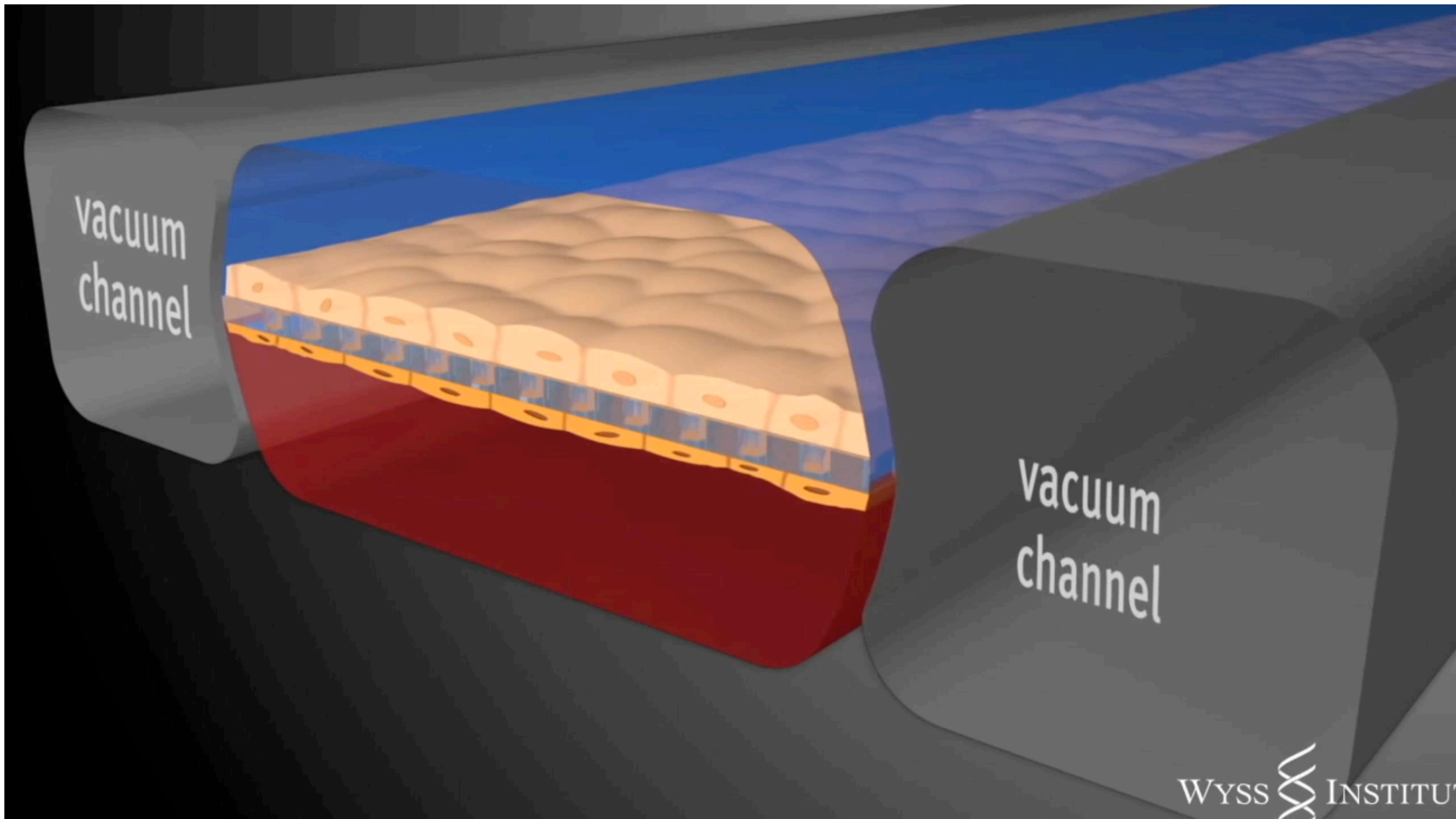
- ➡ Breathing motion:
Mechanical stimulation
- ➡ Air-Blood interface
- ➡ Immune competent



Lung on a chip - Don Ingber (Wyss Institute / Harvard)

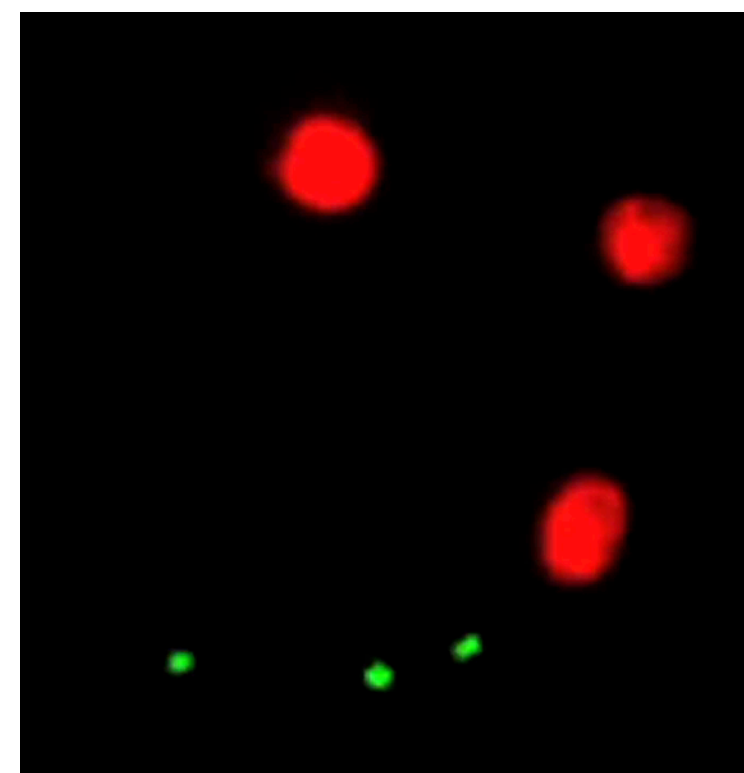
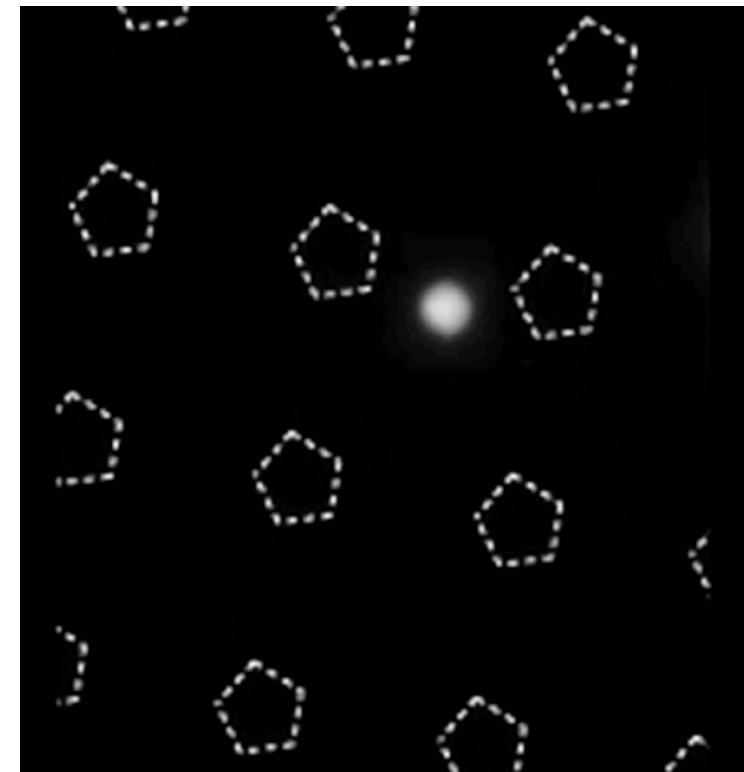
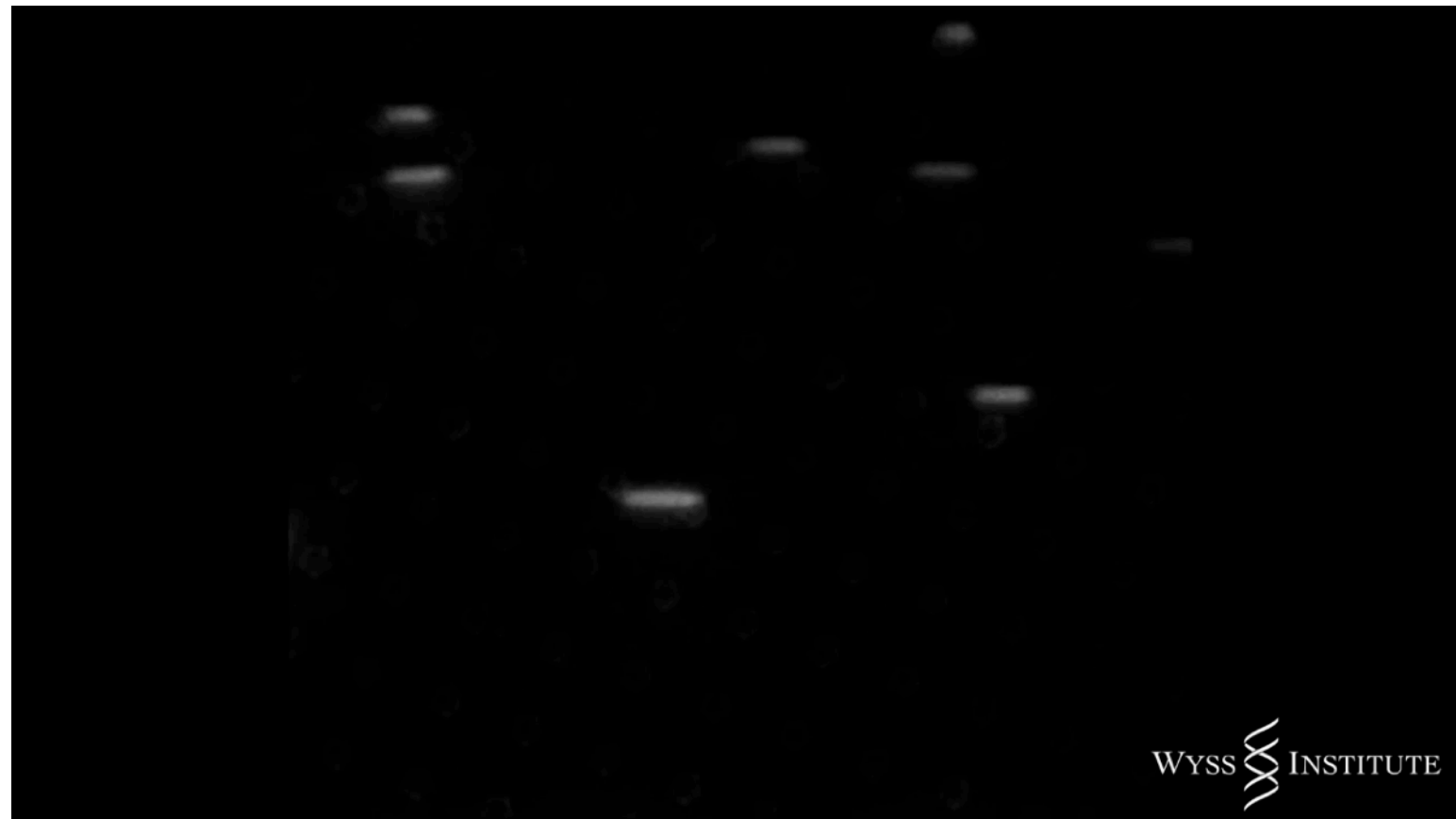


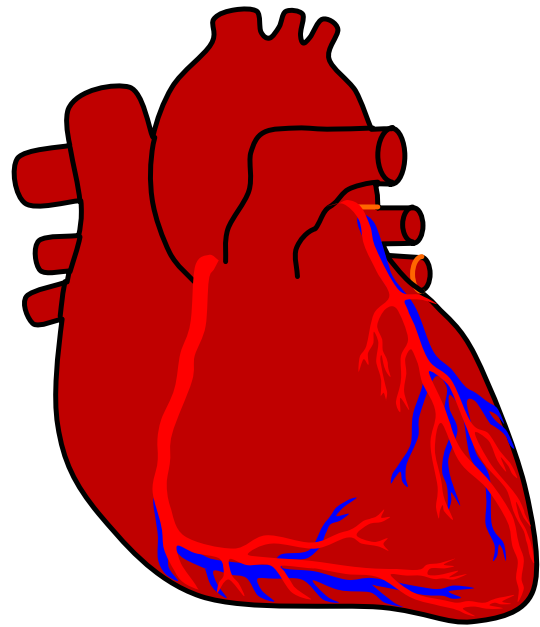
Lung on a chip - Don Ingber (Wyss Institute / Harvard)



Lung on a chip - Don Ingber (Wyss Institute / Harvard)

- ➔ Immune competent
 - ▶ Injection of white blood cells

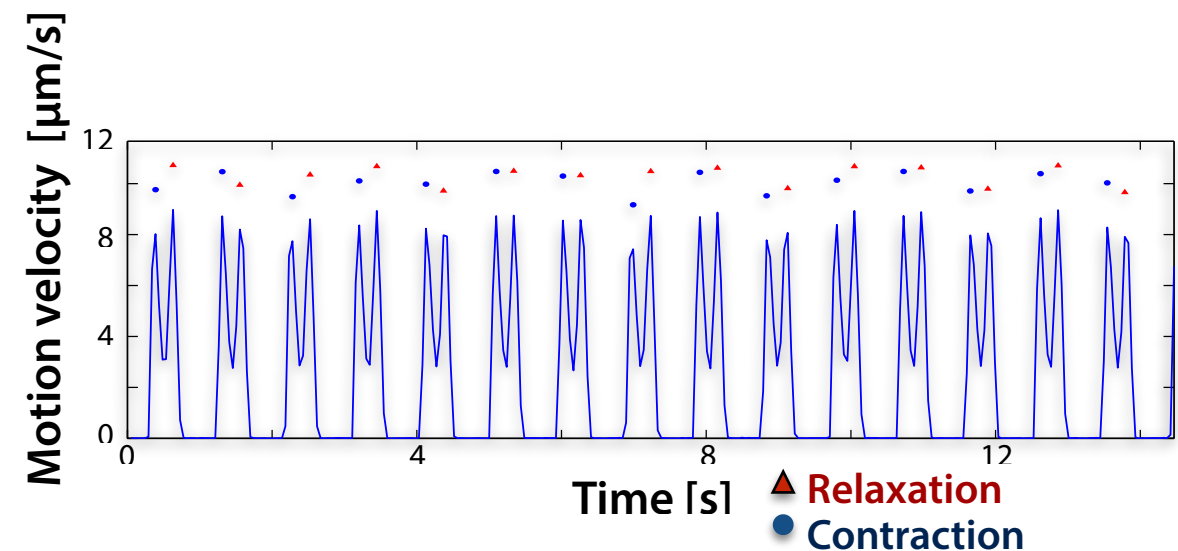
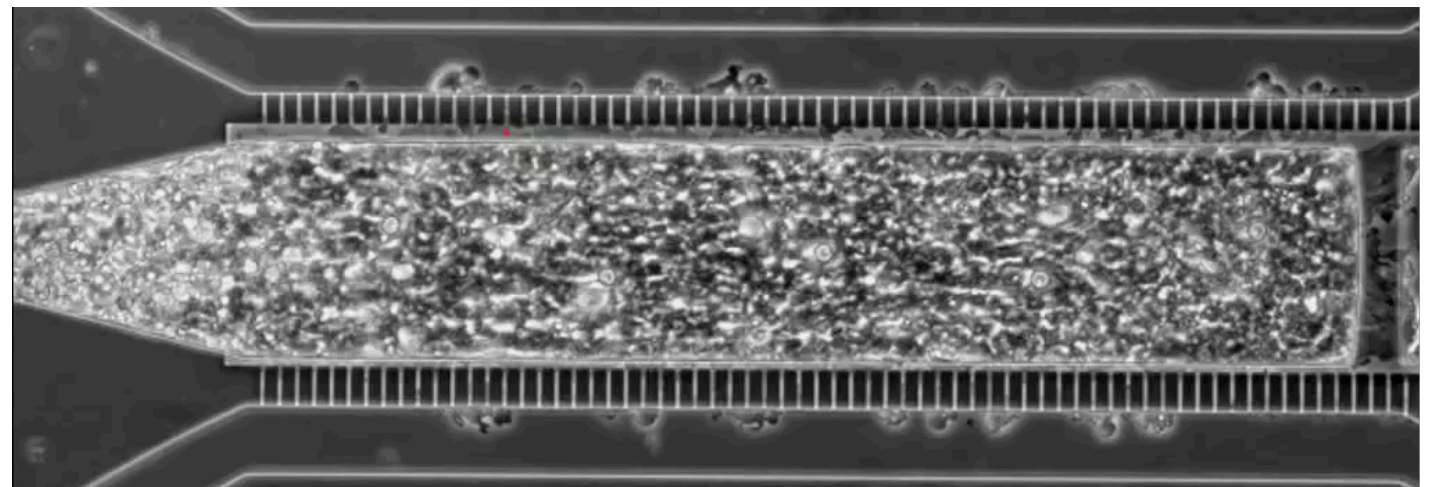




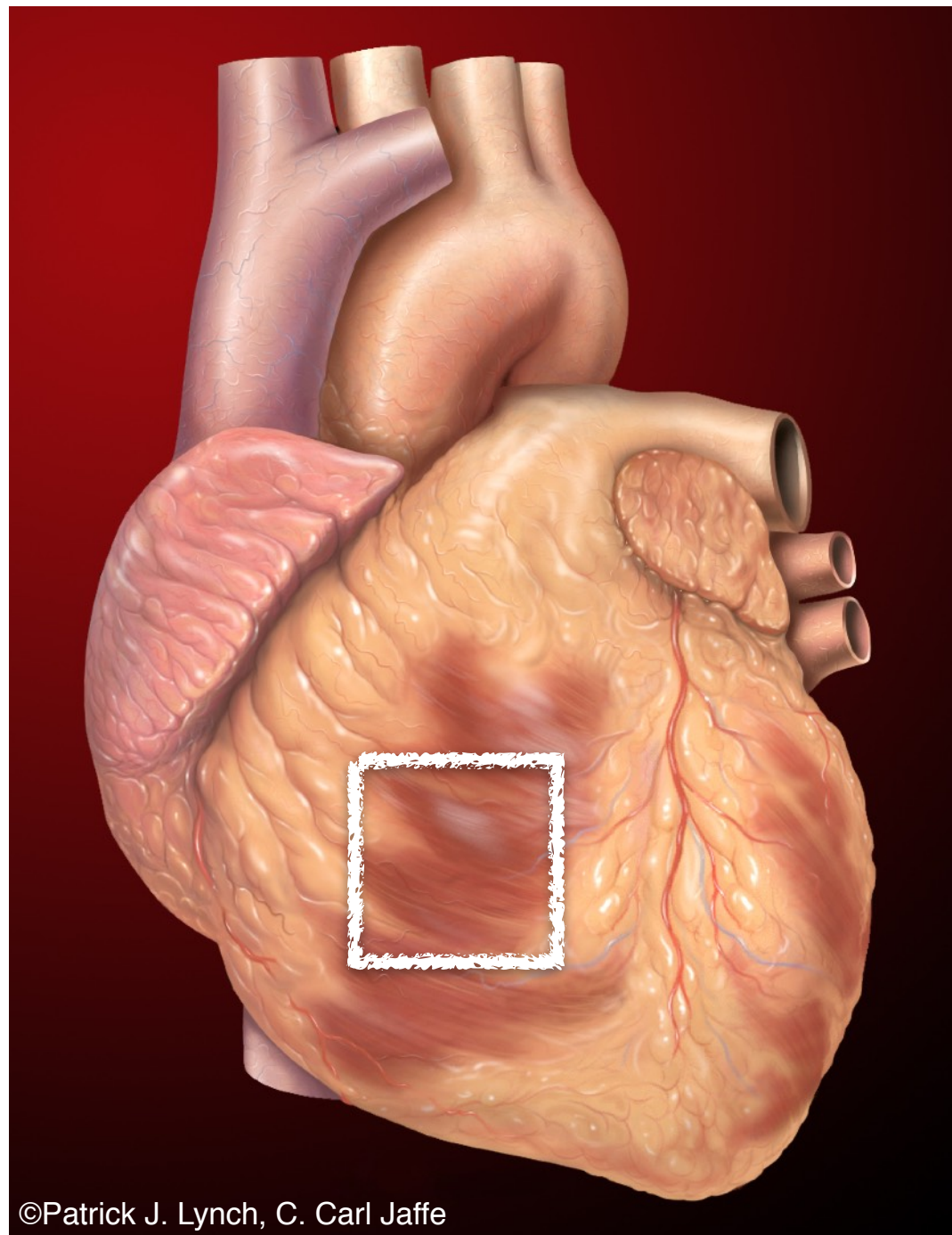
Heart characteristics

- ➡ Spontaneous beating
- ➡ High mechanical stresses
- ➡ Physiological drug response

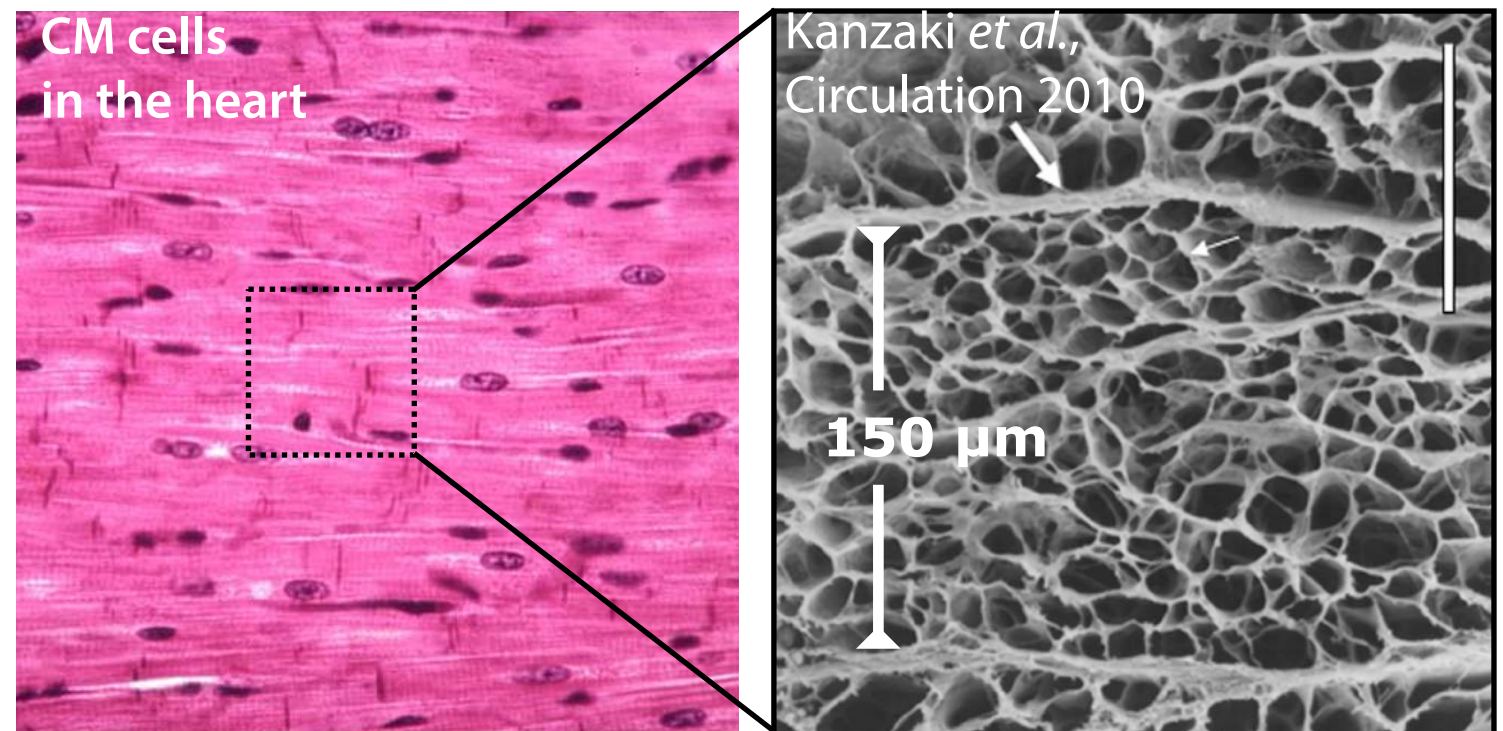
Heart on a chip - Peter Loskill (Fraunhofer IGB / UC Berkeley)



Heart on a chip - Peter Loskill (Fraunhofer IGB / UC Berkeley)

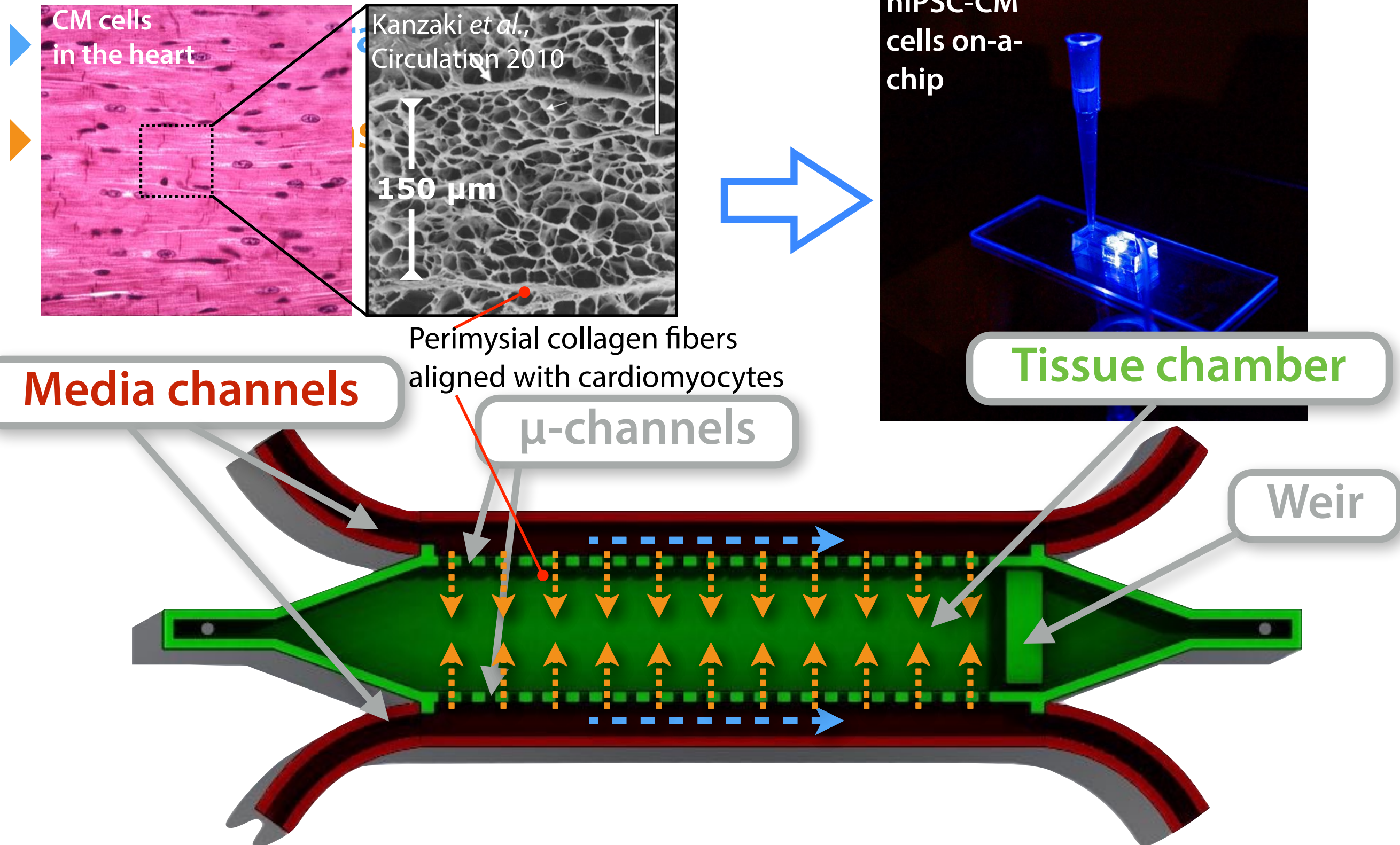


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- ➡ Highly aligned Cardiomyocytes
- ➡ 3D architecture

Microfluidic Organs-on-a-chip



Microfluidic Organs-on-a-chip

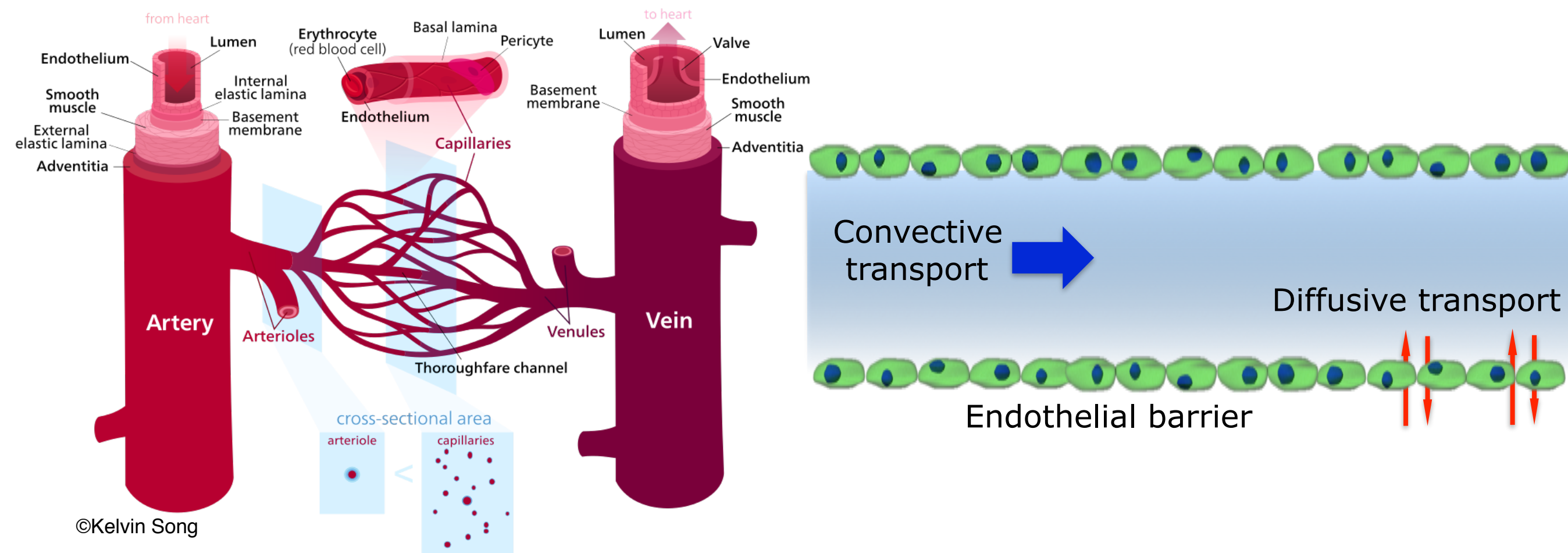
► **Convective Transport**

➡ Mimicking **vasculature**

► **Diffusive transport**

➡ Mimicking shear force protection of **endothelial barrier**

Human vasculature



► **Convective Transport**

➡ Mimicking **vasculature**

► **Diffusive transport**

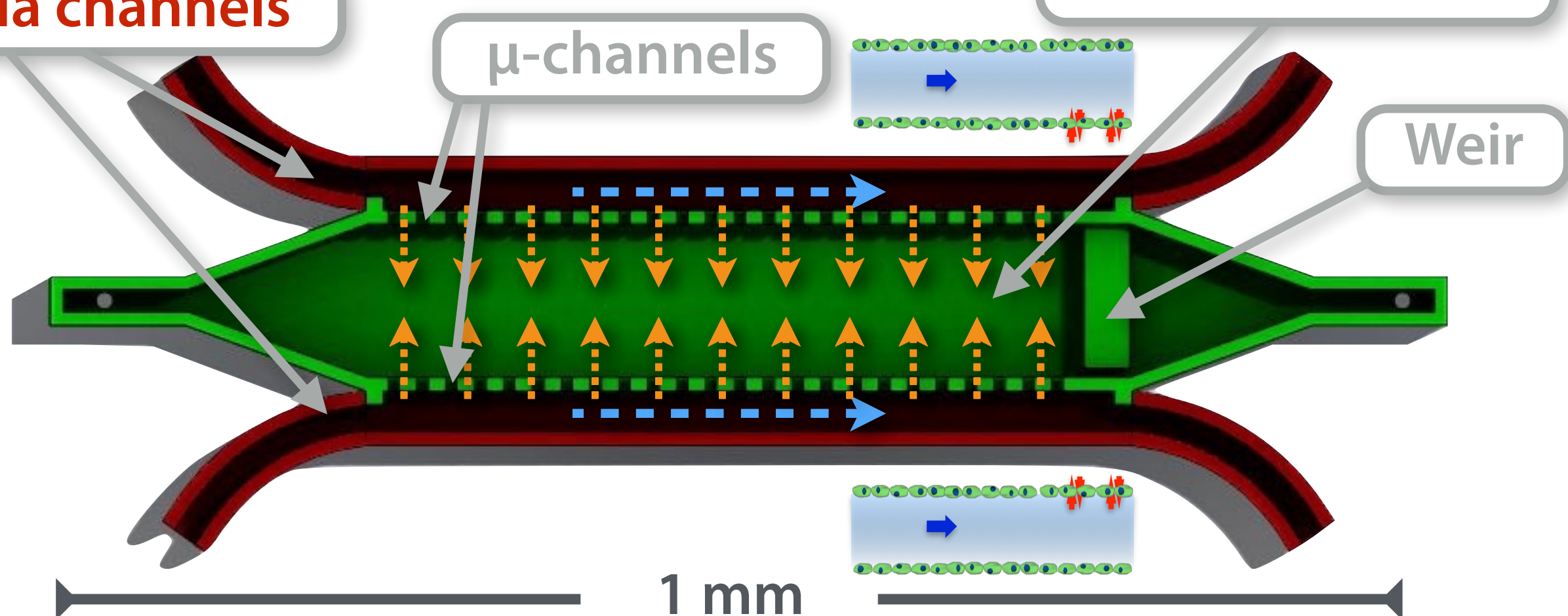
➡ Mimicking shear force protection of **endothelial barrier**

Media channels

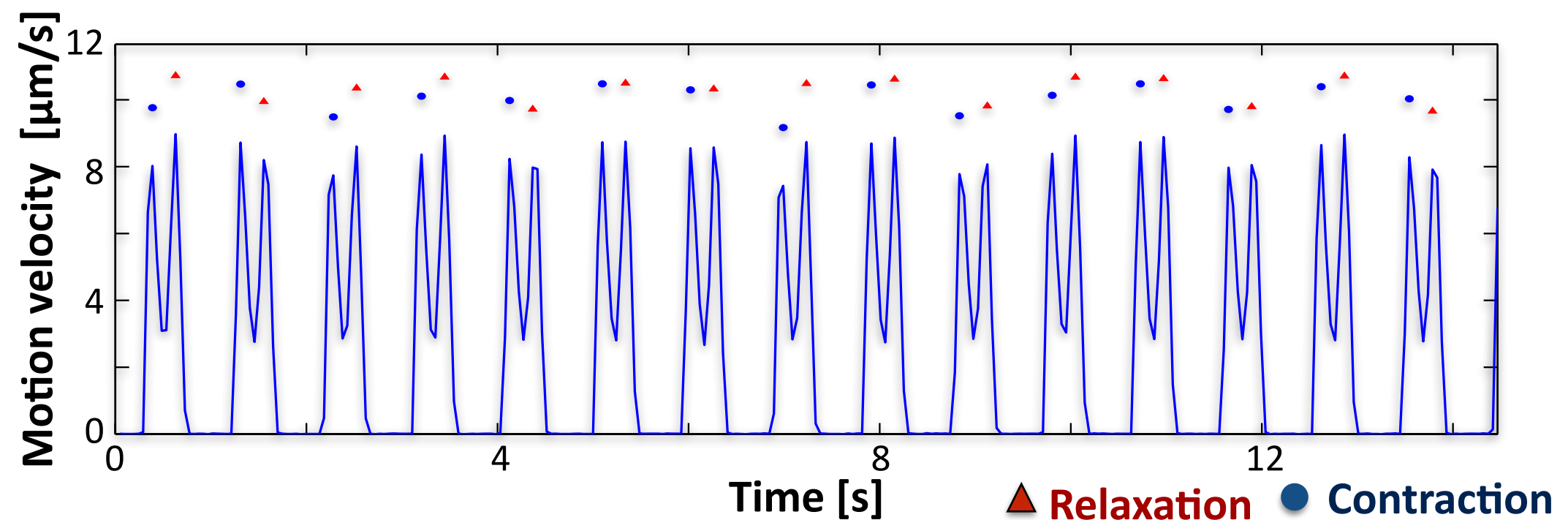
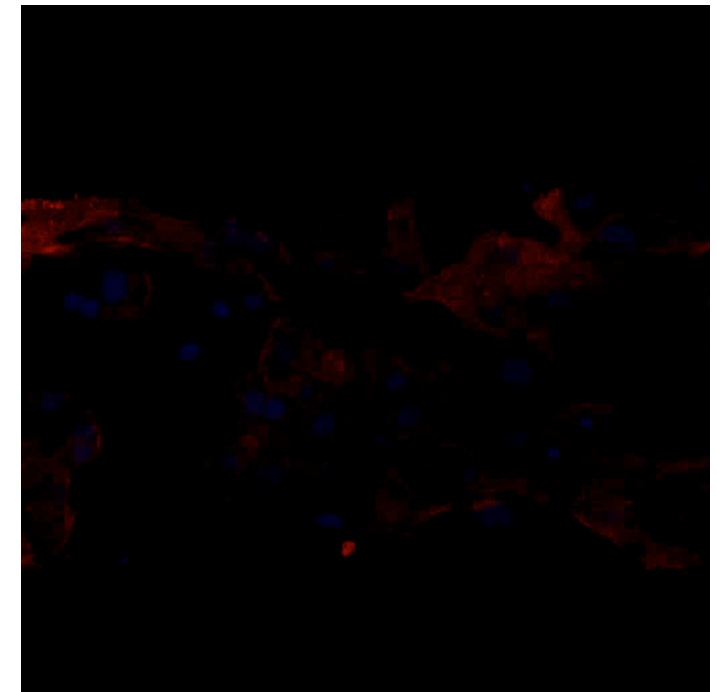
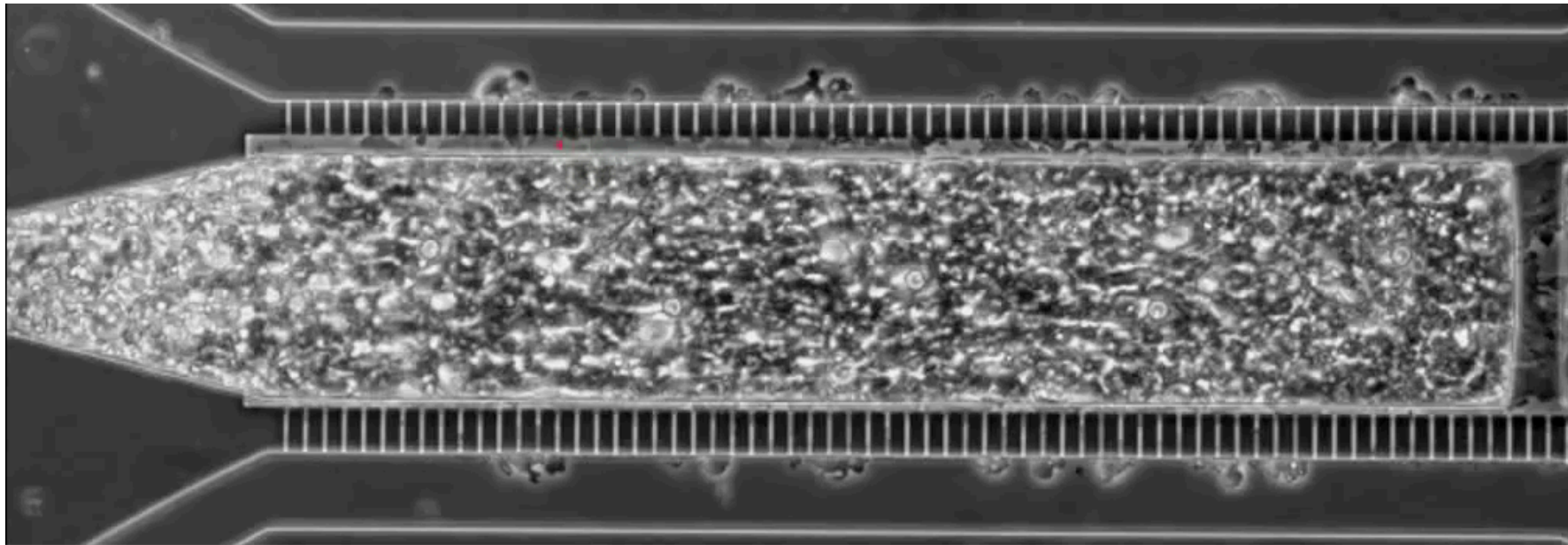
μ -channels

Tissue chamber

Weir

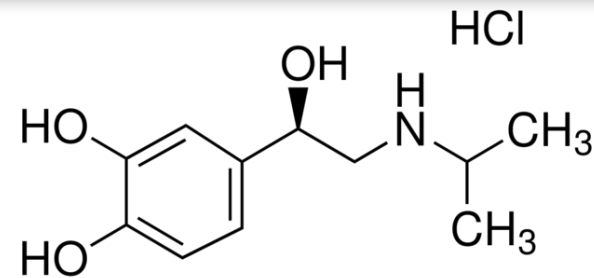


Microfluidic Organs-on-a-chip



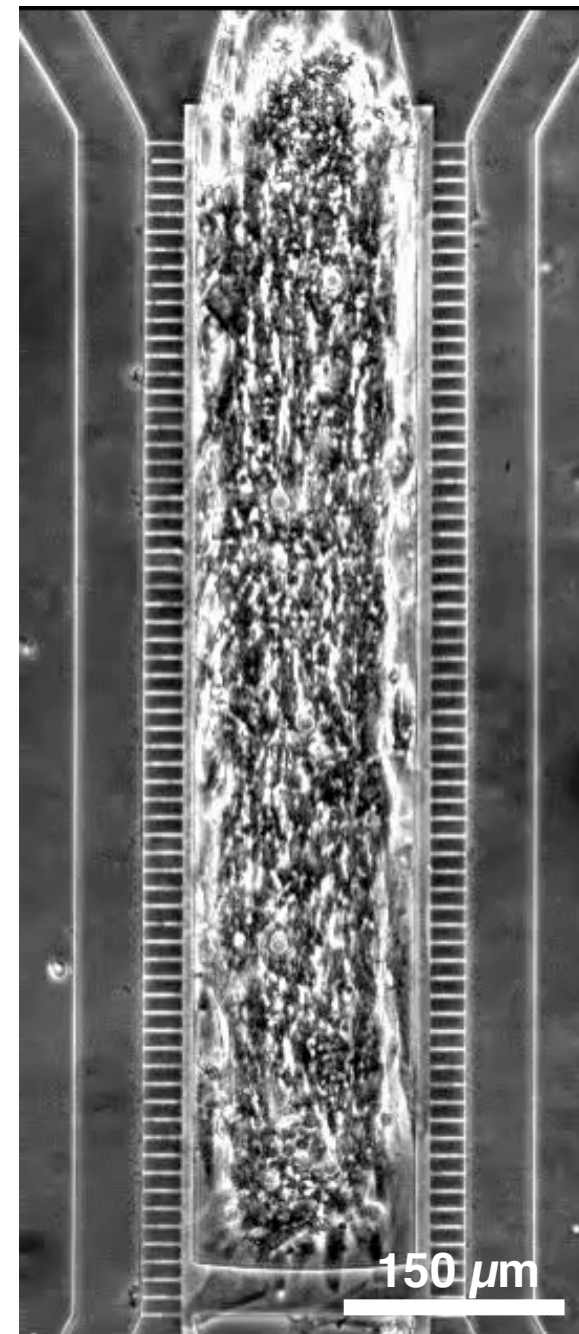
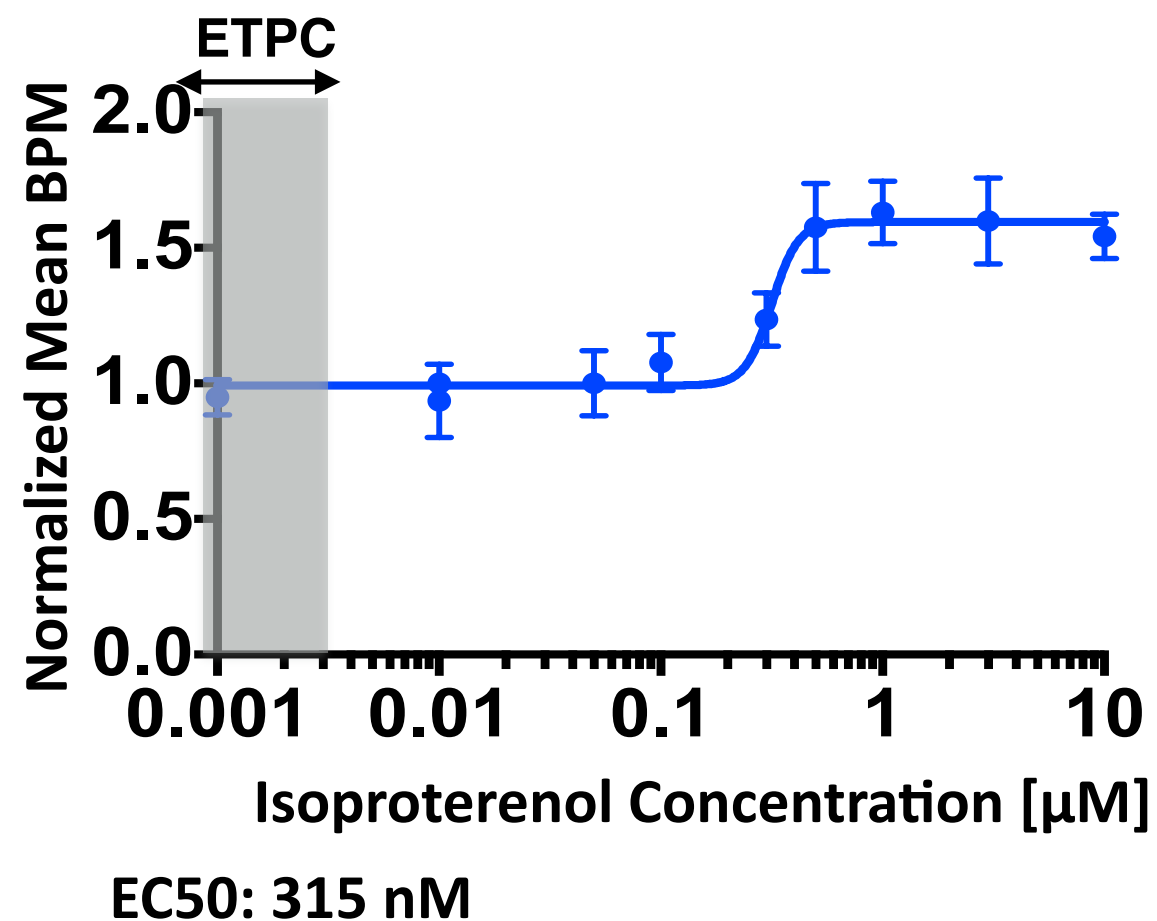
Microfluidic Organs-on-a-chip

Isoprenaline/ Isoproterenol

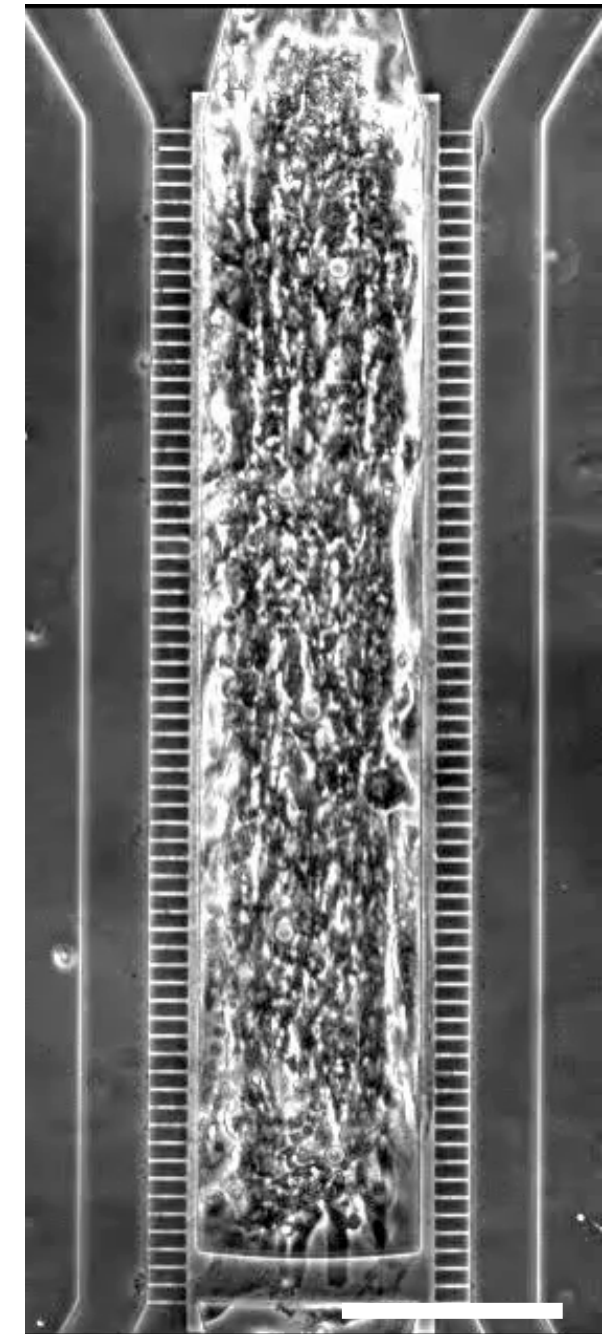


- ➔ β adrenergic agonist
- ➔ Used as cardiac stimulator

➔ Time: 30 min



Baseline - No Drug
55 bpm

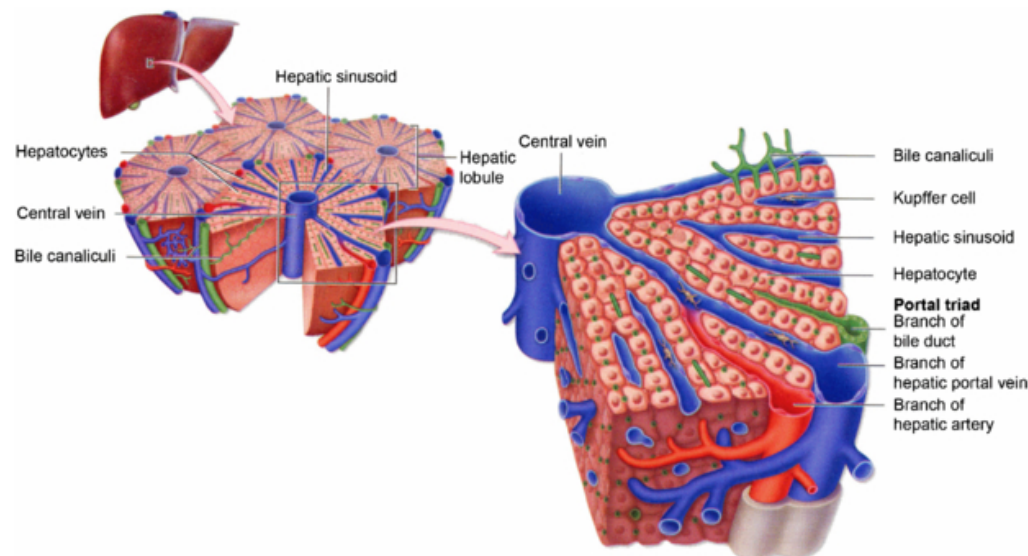


30 min on drug
124 bpm

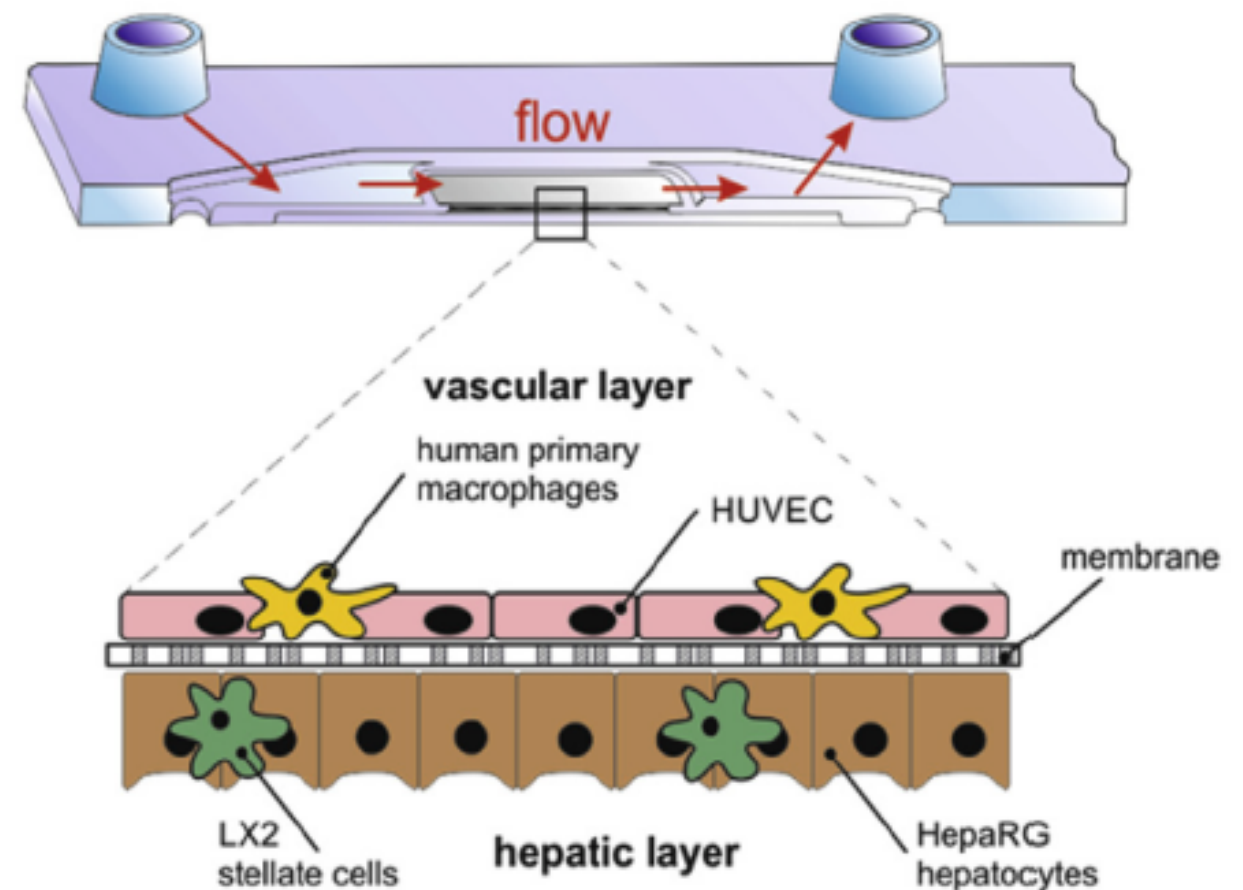


Liver characteristics

- ➔ Multi-layer structure
- ➔ Metabolism
- ➔ Non parenchymal cells

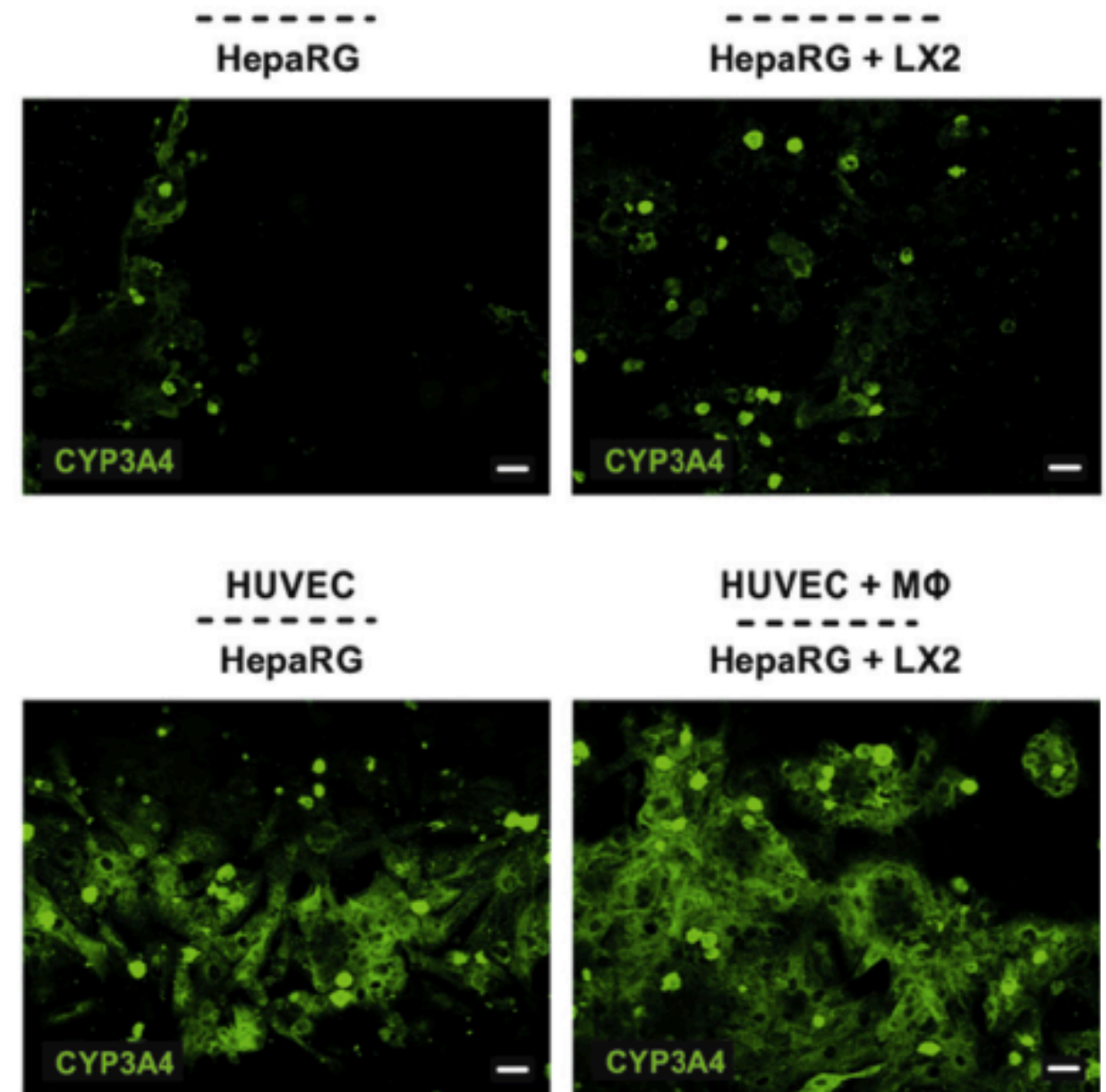
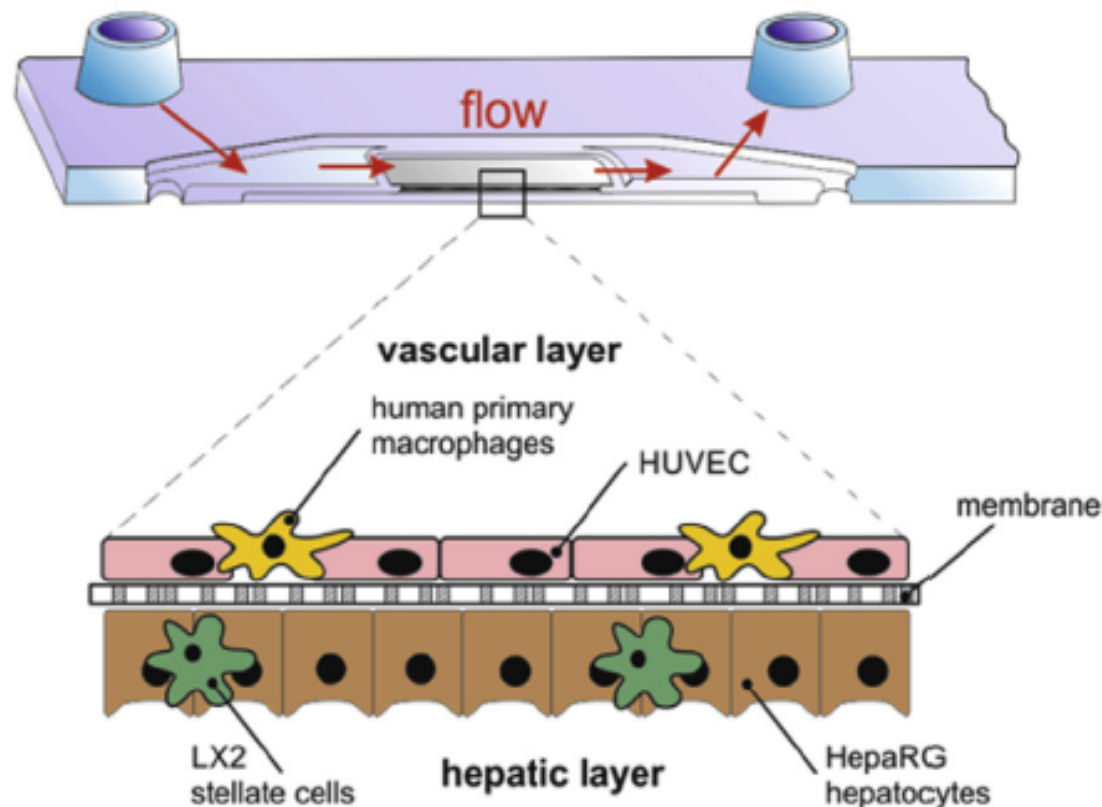


Liver on a chip - Alexander Mosig (Jena University Hospital)

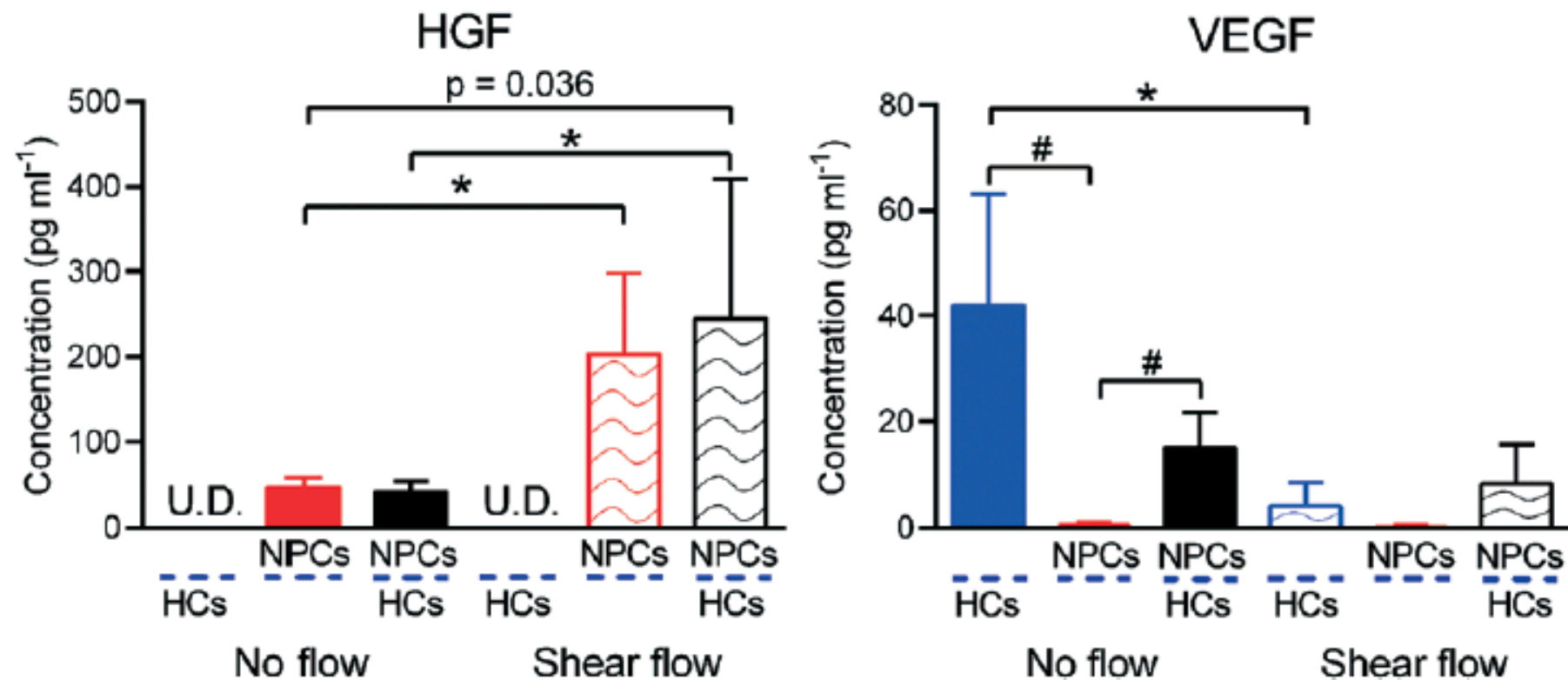


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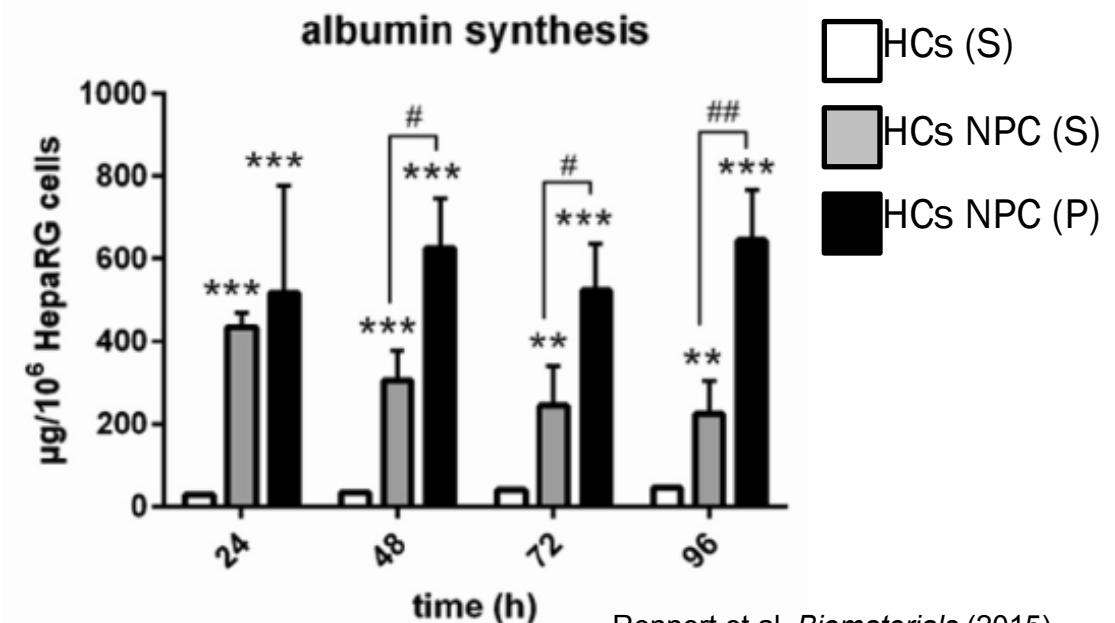
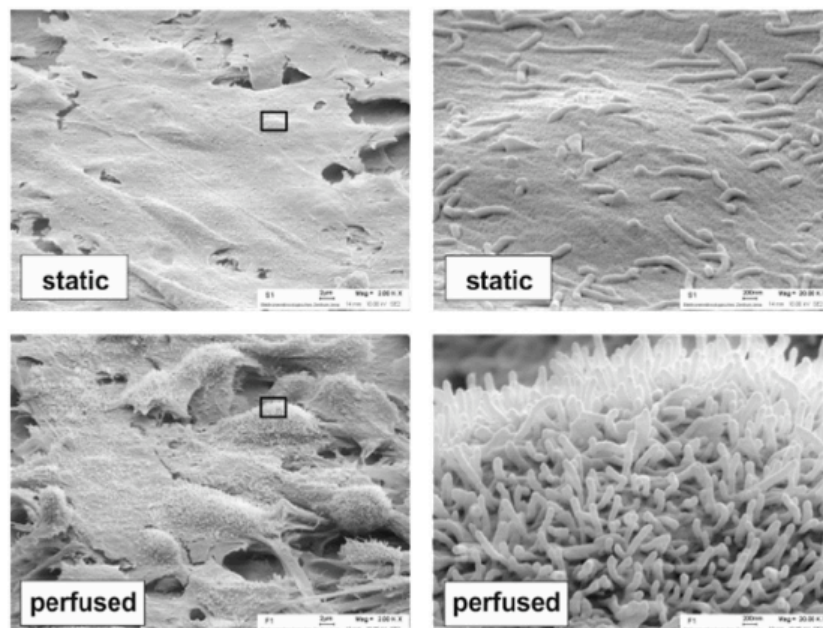
Intercellular signalling
stabilises cell differentiation



Liver on a chip - Alexander Mosig (Jena University Hospital)



Du et al. *Lab Chip* (2017)

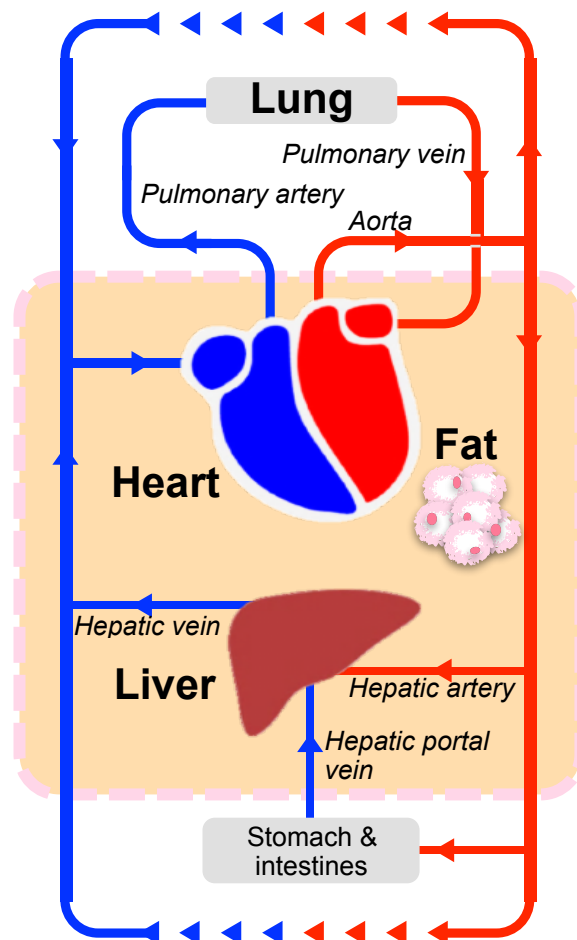


Rennert et al. *Biomaterials* (2015)

The next level: Multi-Organ-chips

Integration of multiple organs

- ➡ capture of metabolite and off-target toxicity
- ➡ studies on organ-organ interactions

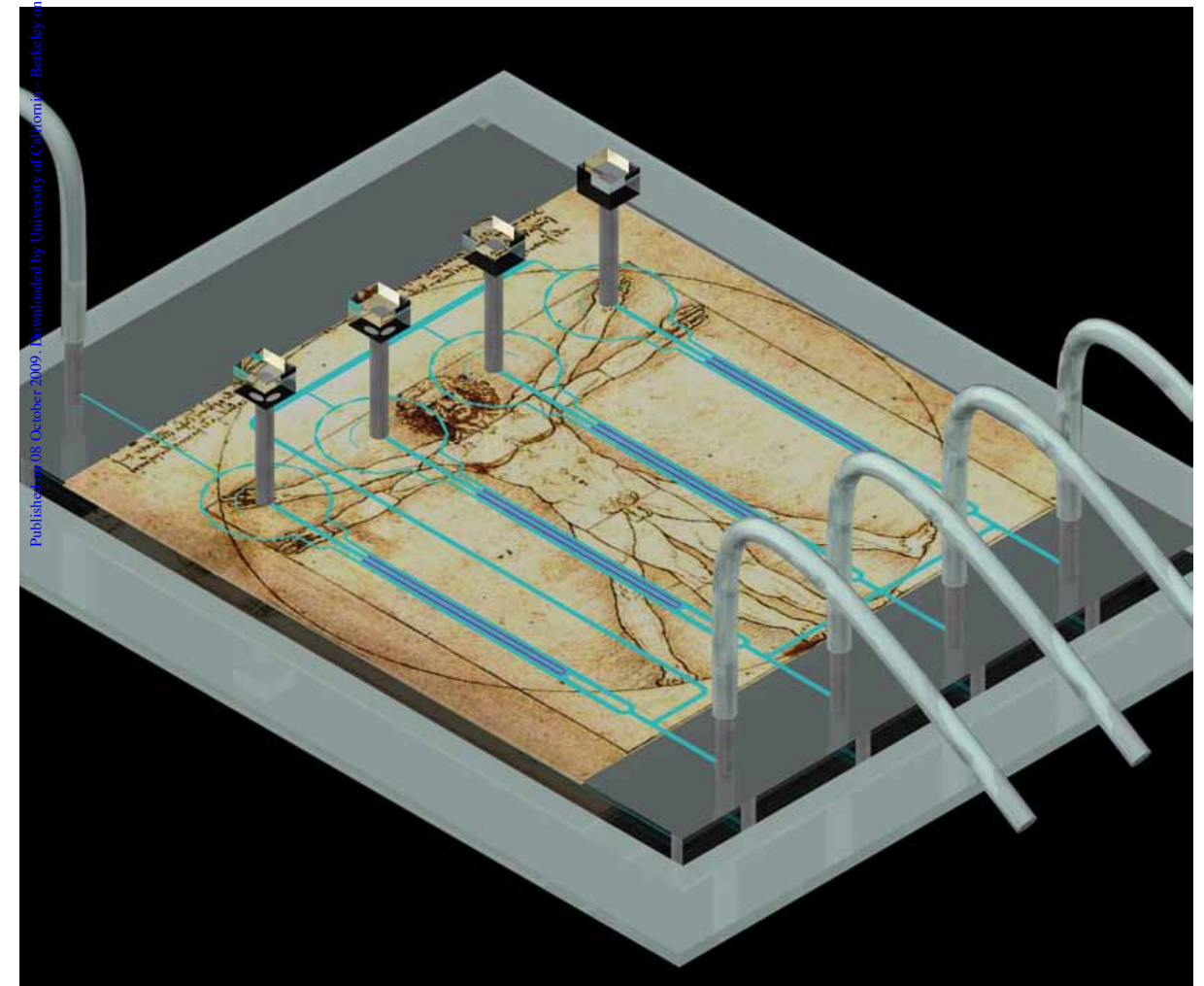


Lab on a Chip

Miniaturisation for chemistry, physics, biology, & bioengineering

www.rsc.org/loc

Volume 9 | Number 22 | 21 November 2009 | Pages 3165–3312



ISSN 1473-0197

RSC Publishing

Yu
Human on a chip
Selvaganapathy
Precise microinjection

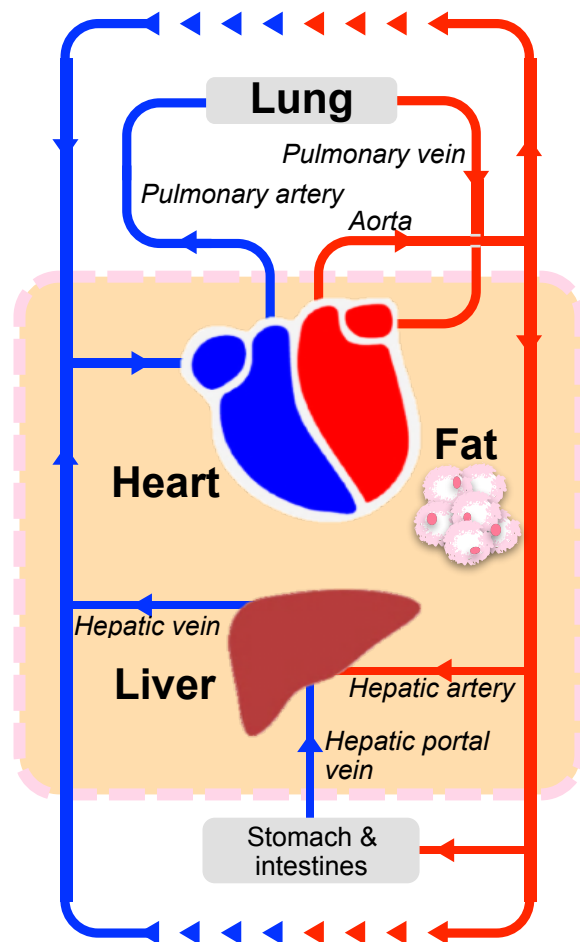
Chou
Enhanced DNA hybridization
Chiou
Light actuated droplets

Zhang, C. *et al.* Lab Chip 2009

The next level: Multi-Organ-chips

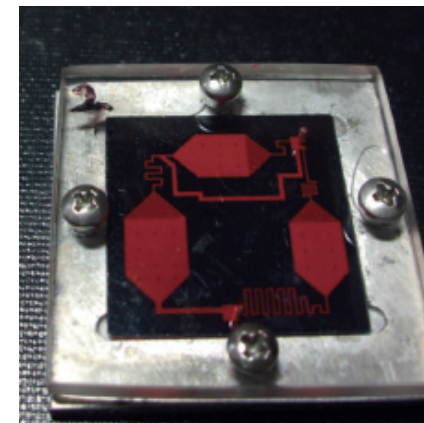
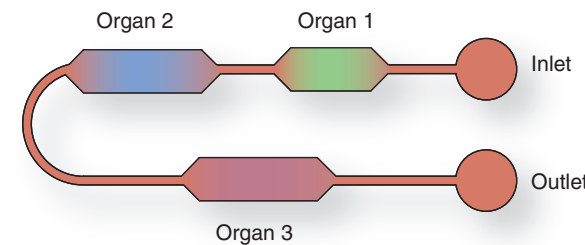
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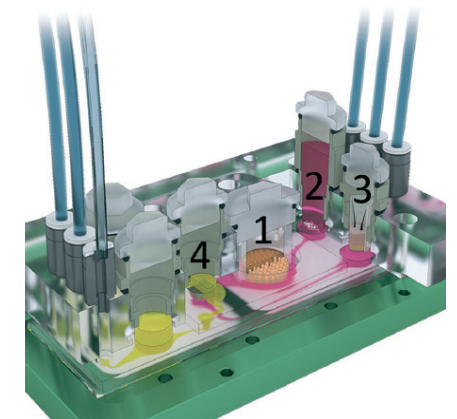
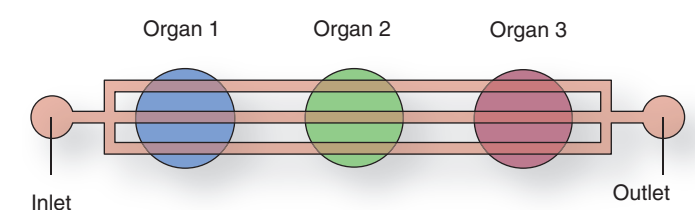


Concepts for multi-organ-chips

- Static systems
- Semistatic systems



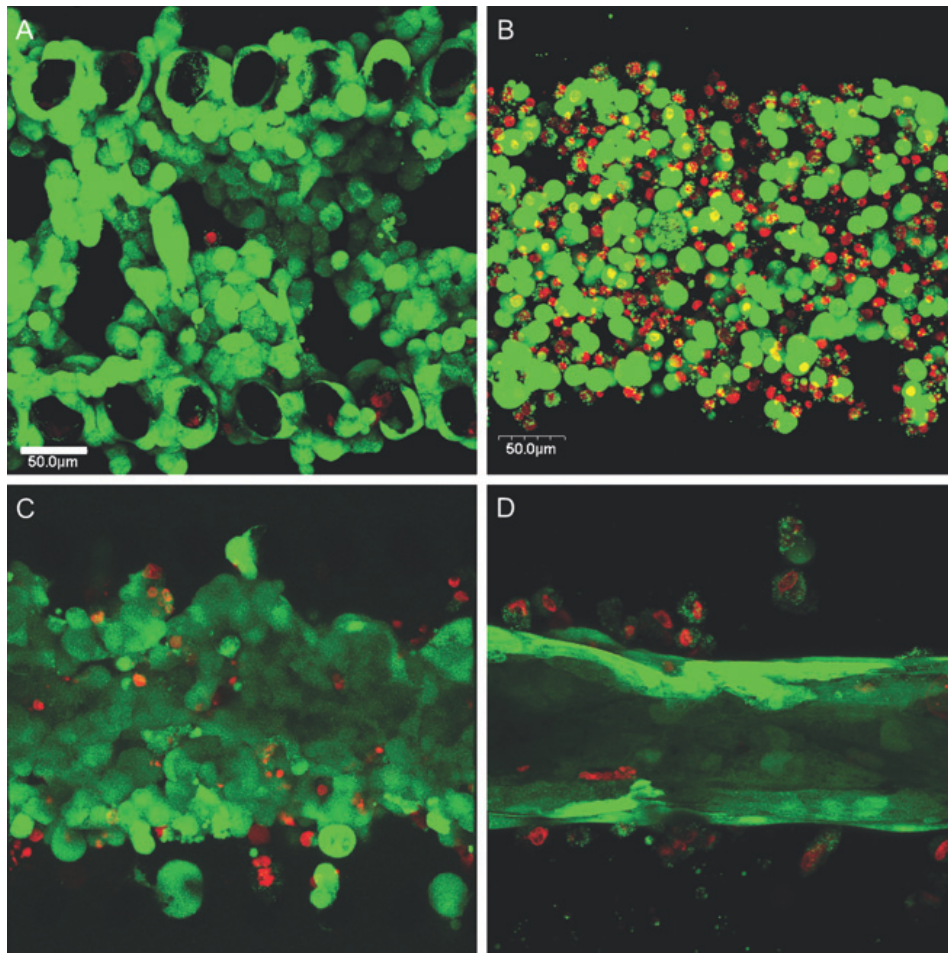
J.H. Sung et al. *Lab Chip* (2009)



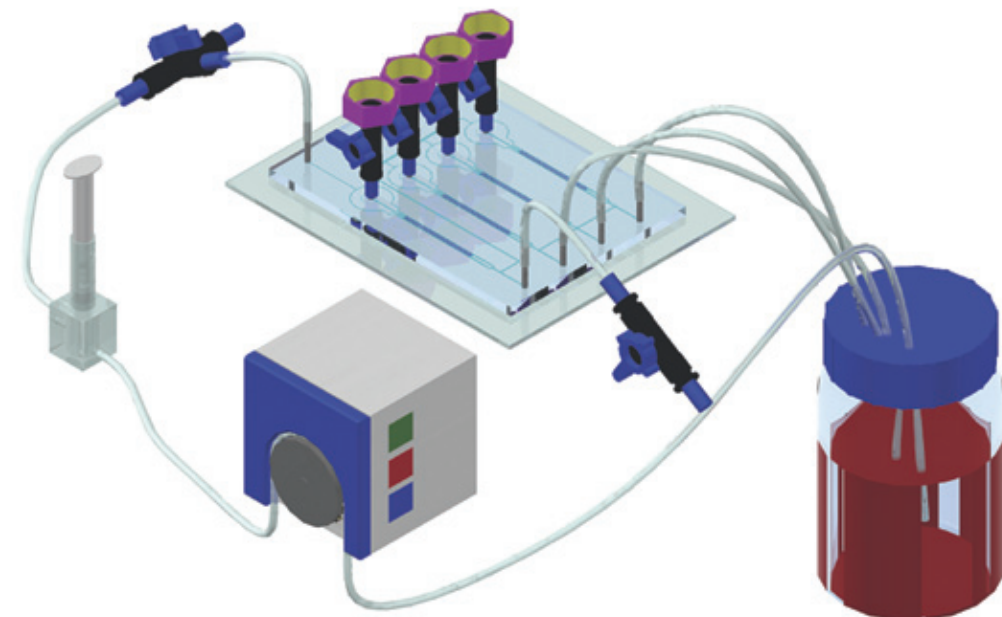
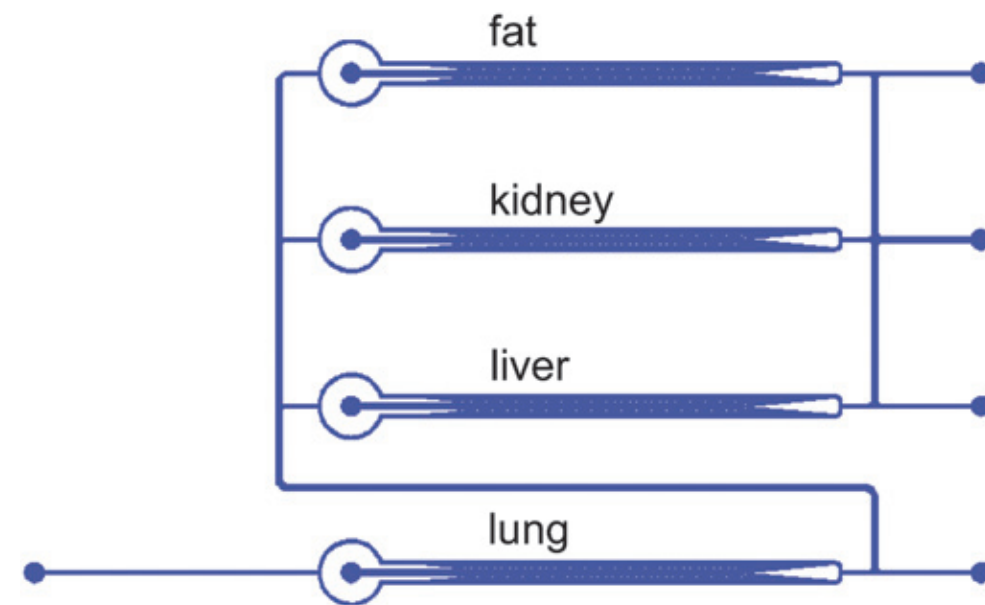
I. Maschmeyer et al. *Lab Chip* (2015)

The next level: Multi-Organ-chips

Parallel Cell Culture Chambers - Hanry Yu (Singapore)

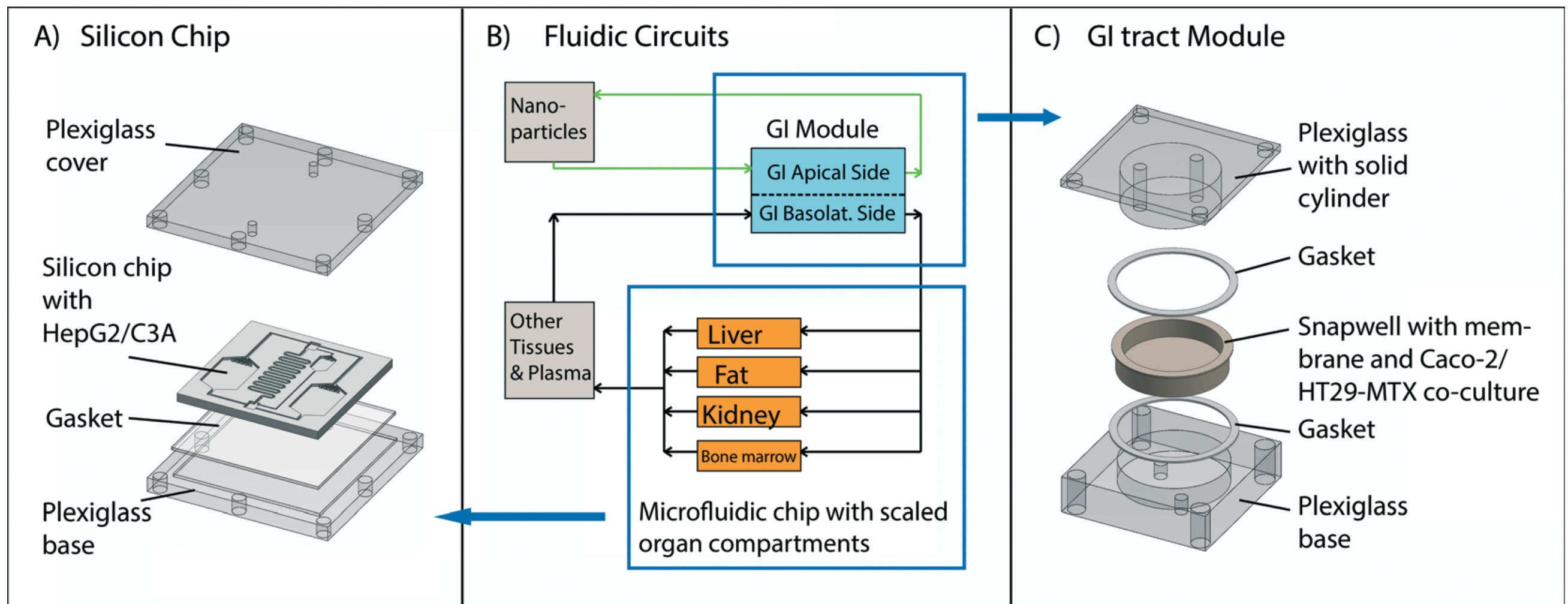


Zhang, C. *et al.* Lab Chip 2009



The next level: Multi-Organ-chips

Combined Silicon Chip - Michael Shuler (Cornell University)

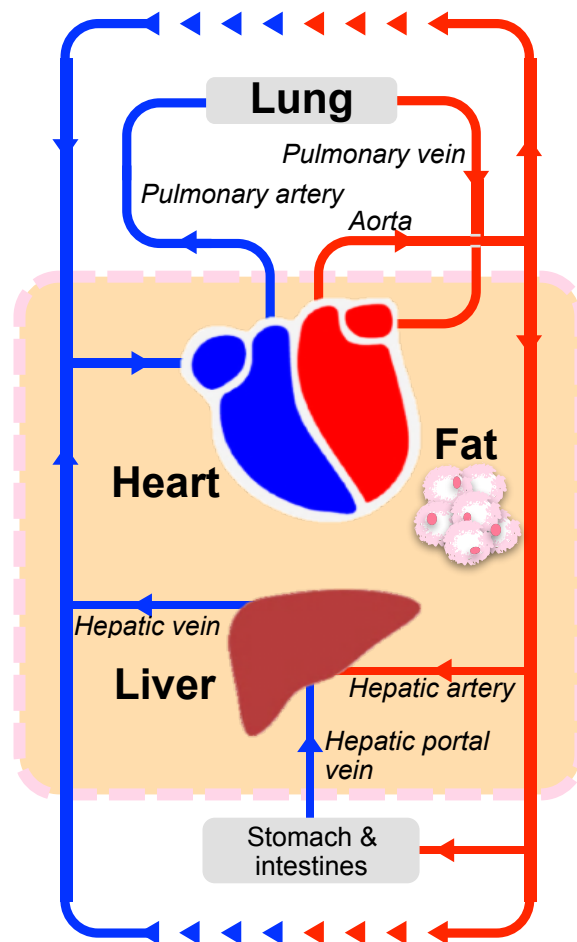


Esch, M. B *et al.* Lab Chip 2014

The next level: Multi-Organ-chips

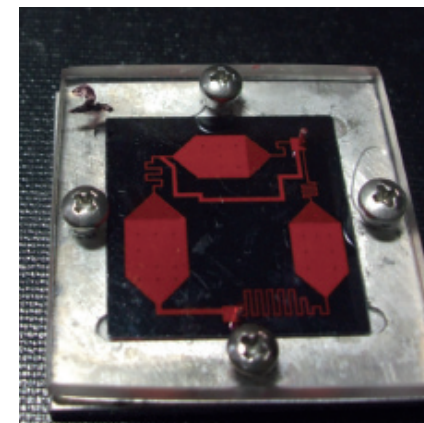
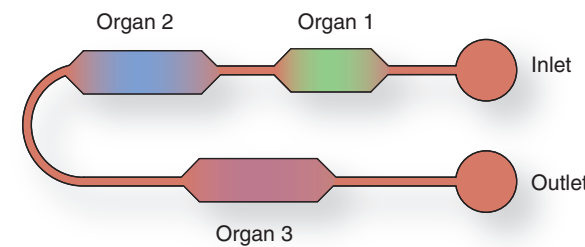
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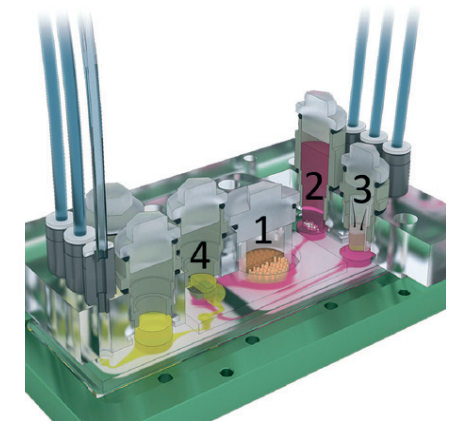
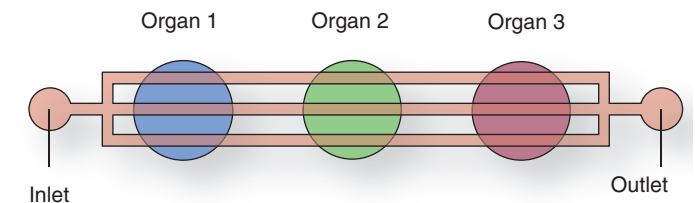


Concepts for multi-organ-chips

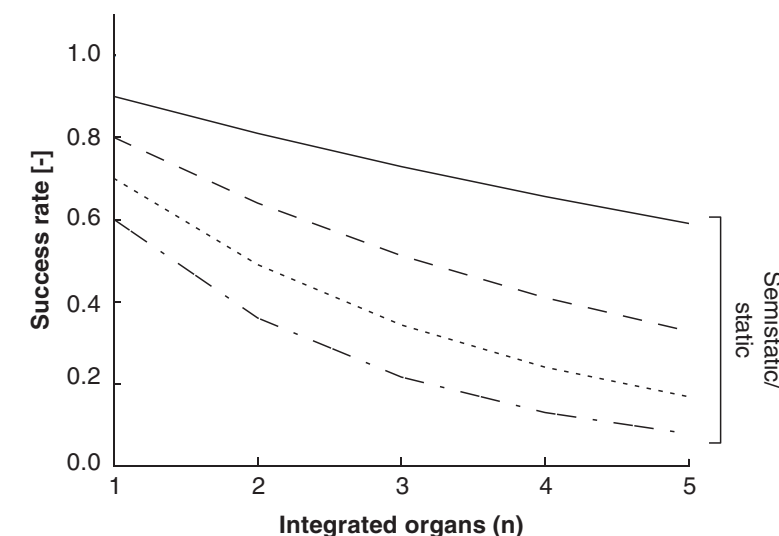
- Static systems
- Semistatic systems



J.H. Sung et al. *Lab Chip* (2009)



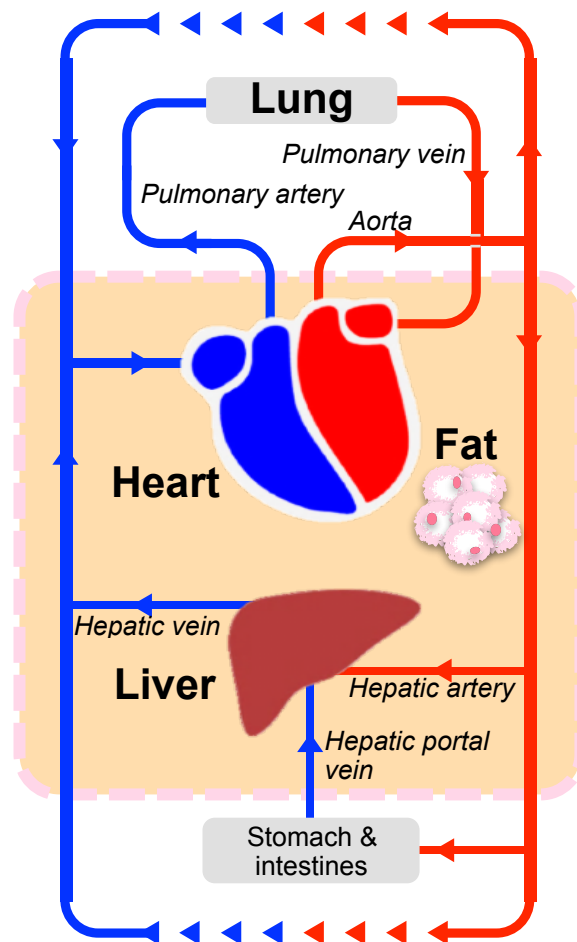
I. Maschmeyer et al. *Lab Chip* (2015)



The next level: Multi-Organ-chips

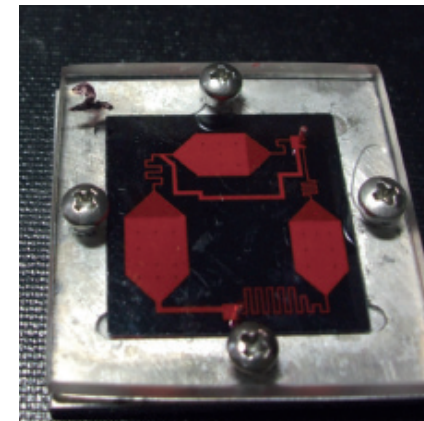
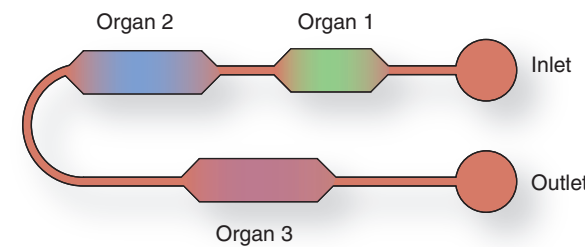
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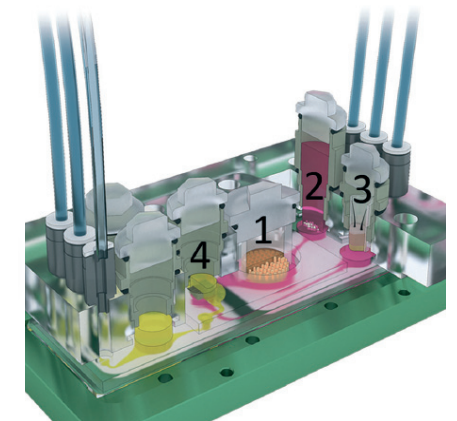
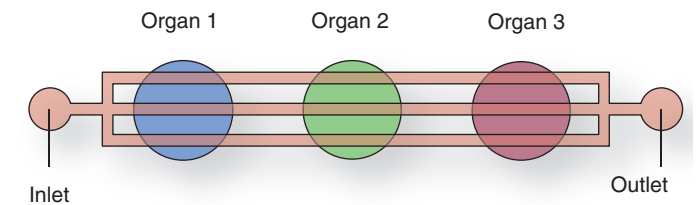


Concepts for multi-organ-chips

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- Semistatic systems

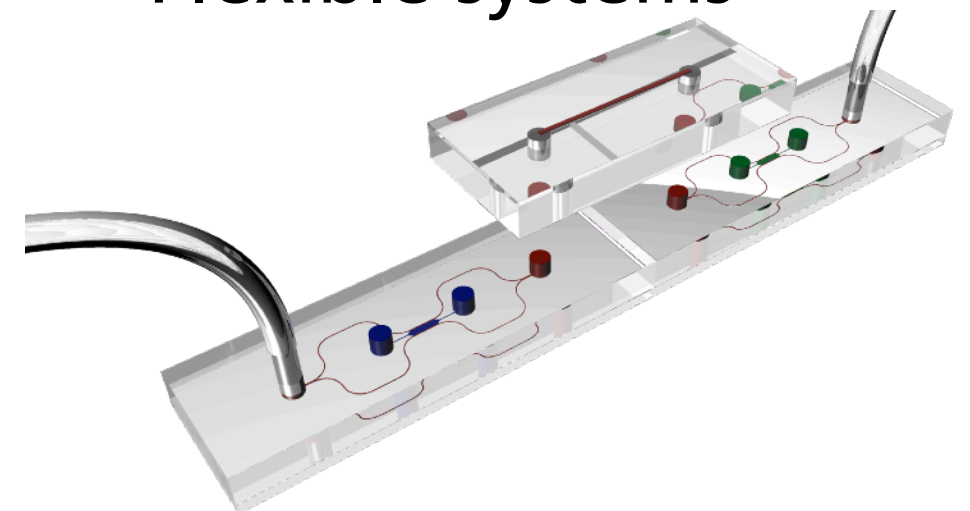


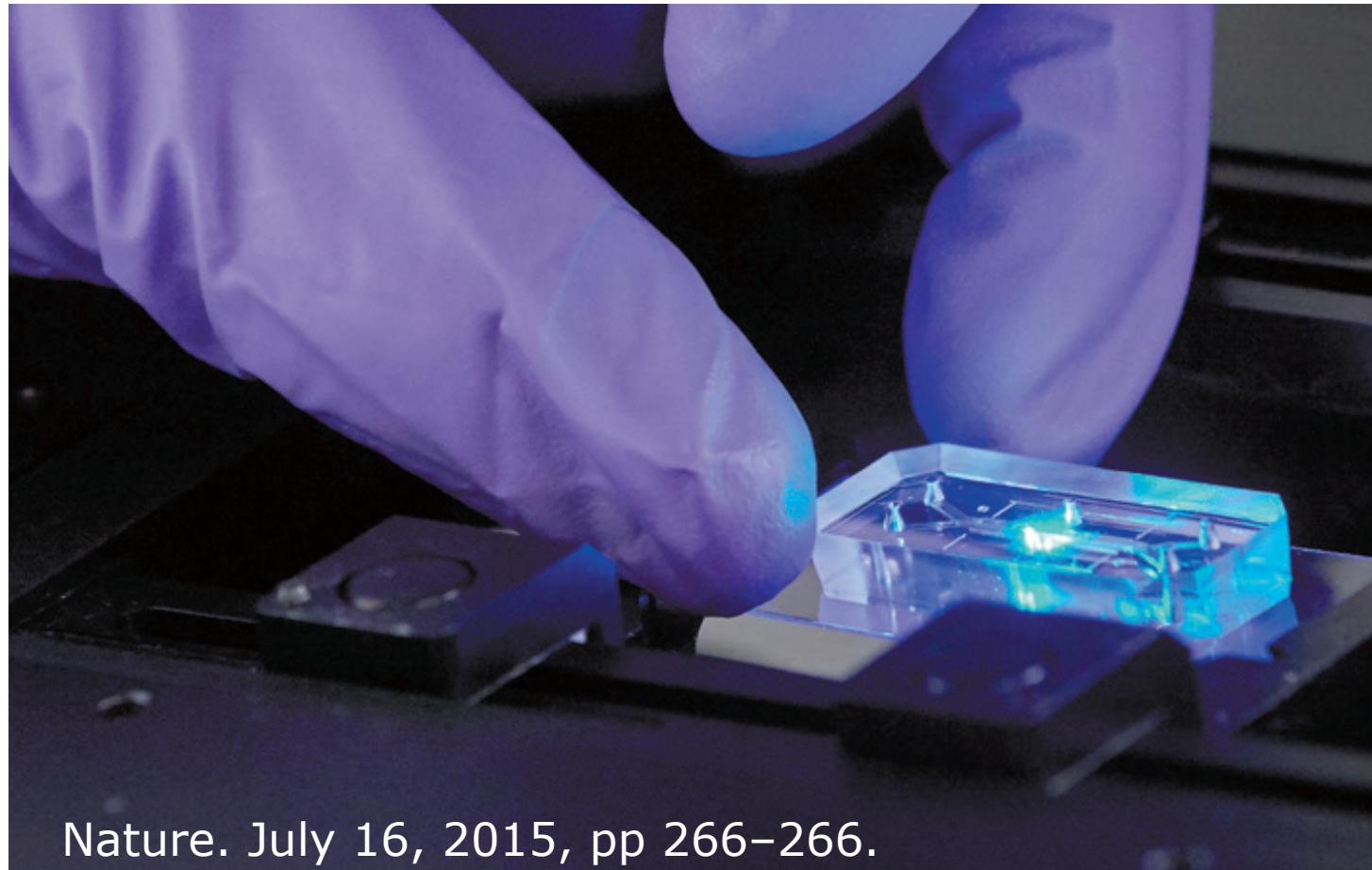
J.H. Sung et al. *Lab Chip* (2009)



I. Maschmeyer et al. *Lab Chip* (2015)

- Flexible systems





Nature. July 16, 2015, pp 266–266.

Miniature devices that mimic human organs could help to replace animals used in drug testing.

BIOTECHNOLOGY

‘Organs-on-chips’ go mainstream

Drug companies put in vitro systems through their paces.

www.rsc.org/loc

CRITICAL REVIEW

Engineers are from PDMS-land, Biologists are from Polystyrenia

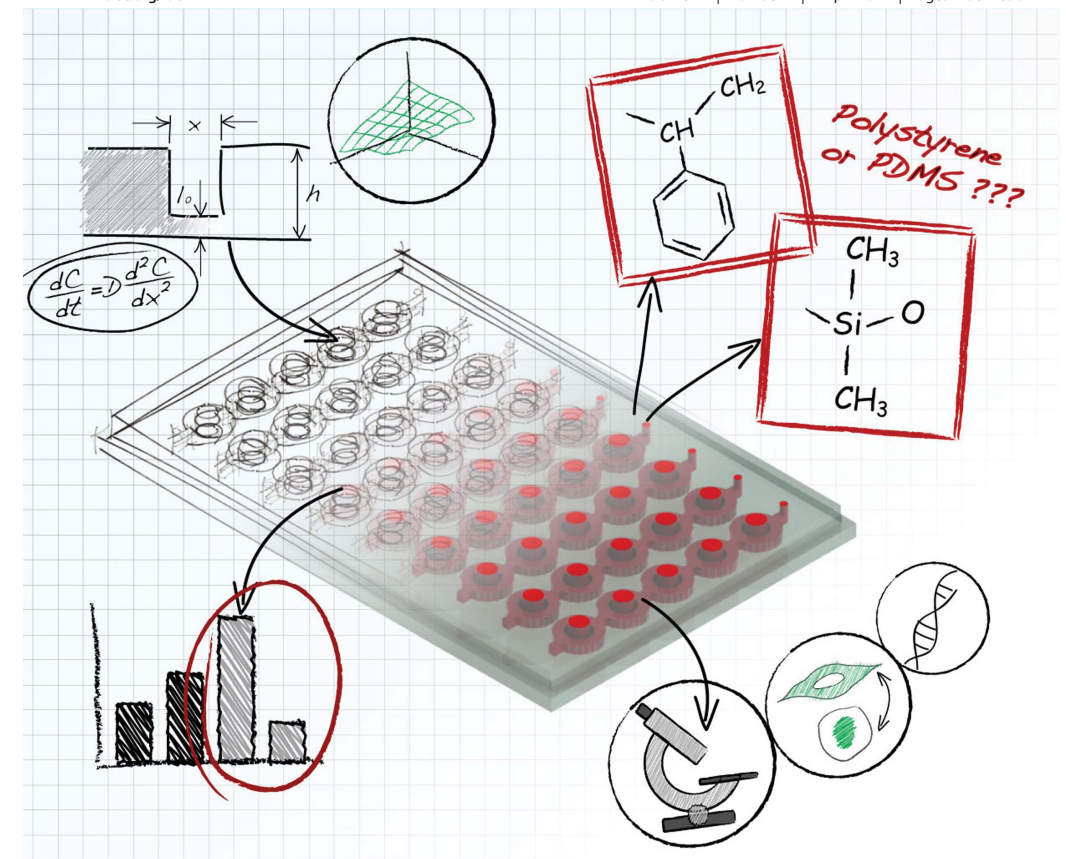
Erwin Berthier,^{†a} Edmond W. K. Young^{†b} and David Beebe^{*b}

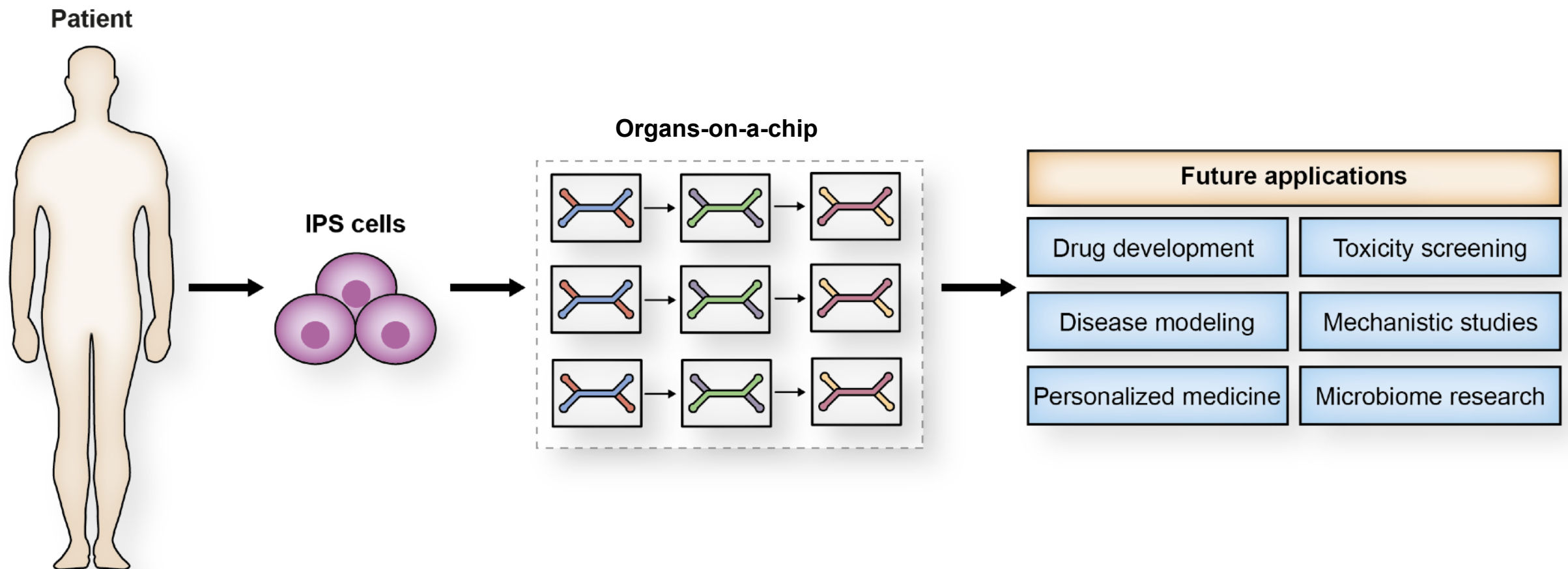
Lab on a Chip

Miniaturisation for chemistry, physics, biology, materials science and bioengineering

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Volume 12 | Number 7 | 7 April 2012 | Pages 1193–1396





► More than just drug screening tools!