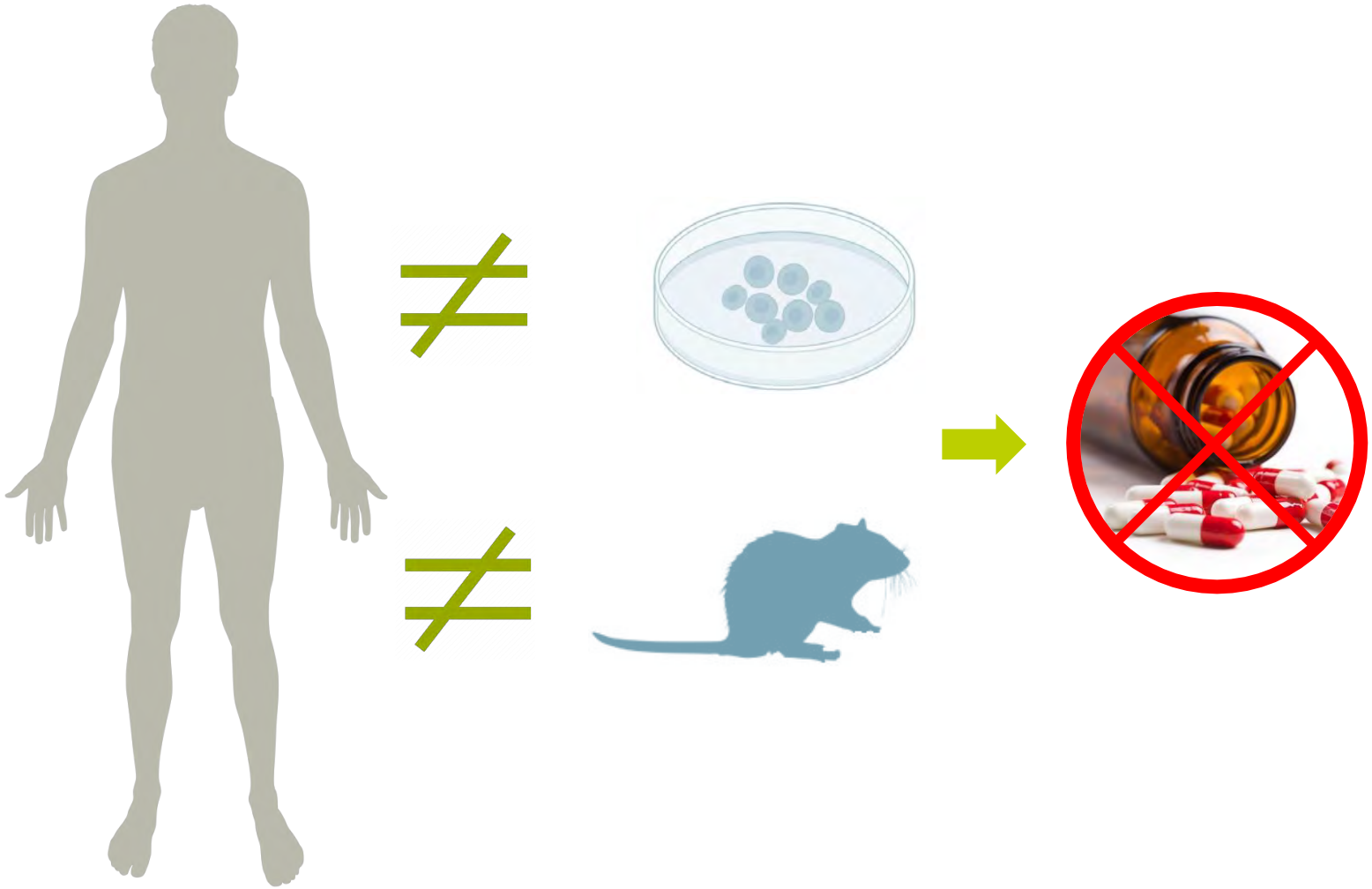
Coupling organ-on-chip and multimode plate readers: Real-time assessment of snake venom toxicity on a three-dimensional blood vessel model

Sebastiaan J. Trietsch

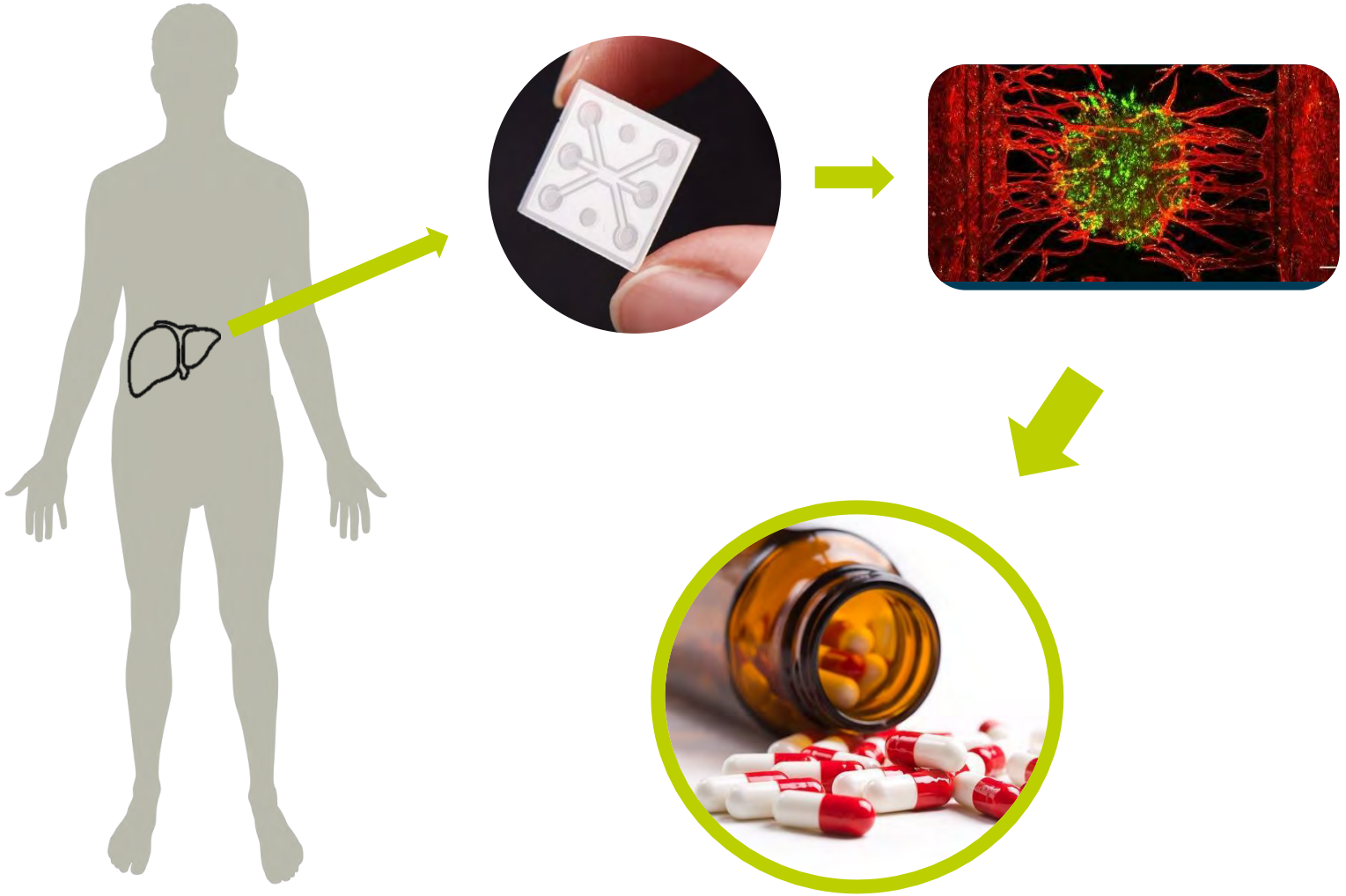
Grow. Learn. Discover.

Wrong model means wrong conclusions



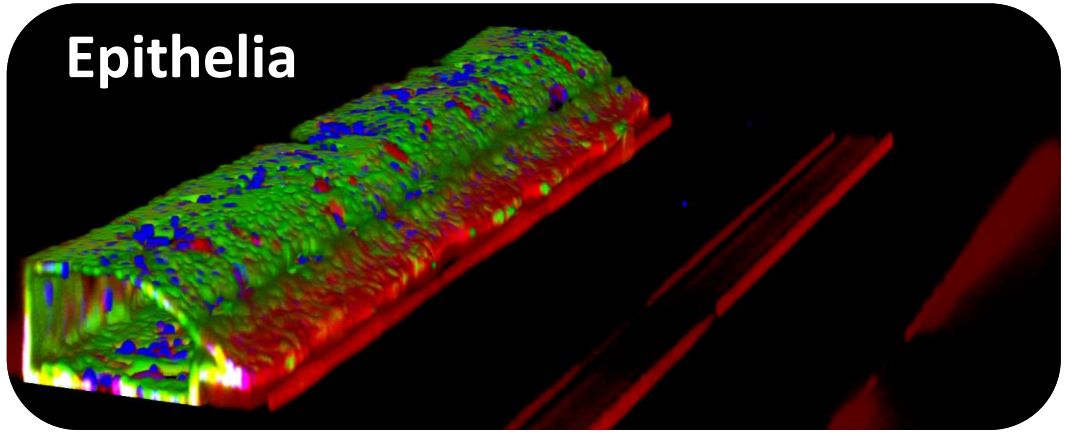
Simple cell cultures & animal models fail to capture the complexity of human disease

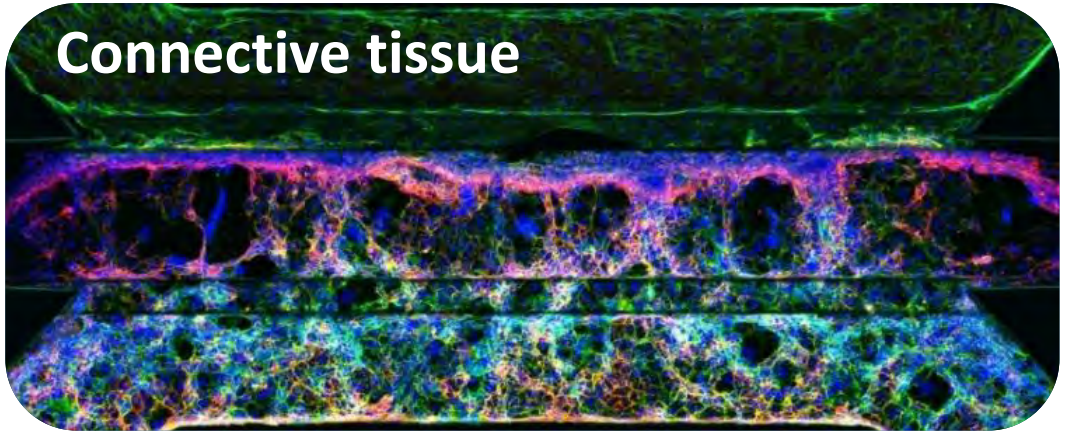
MIMETAS provides the right model

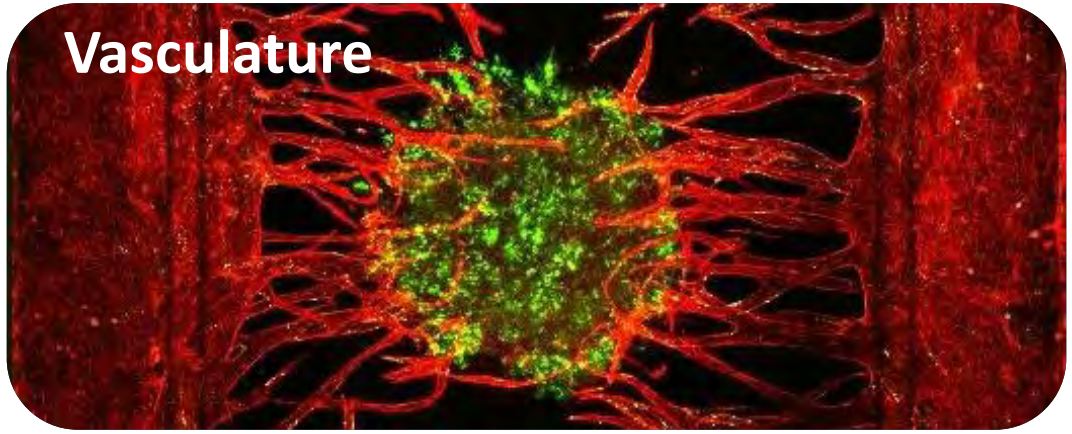


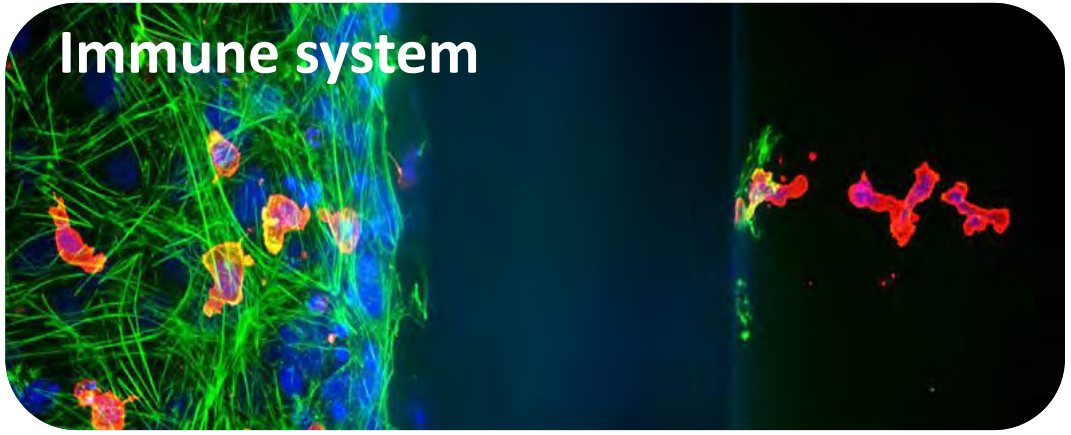
Human Organ-on-a-Chip technology recapitulates the human organ niche and captures the complexity of disease

The right model looks like this…





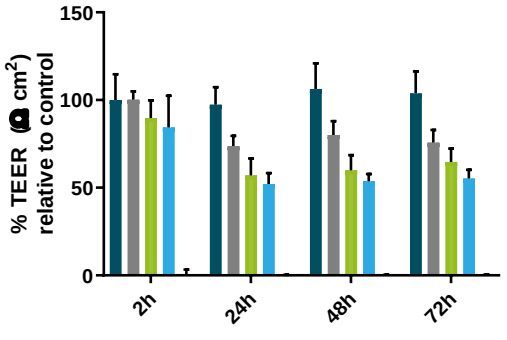




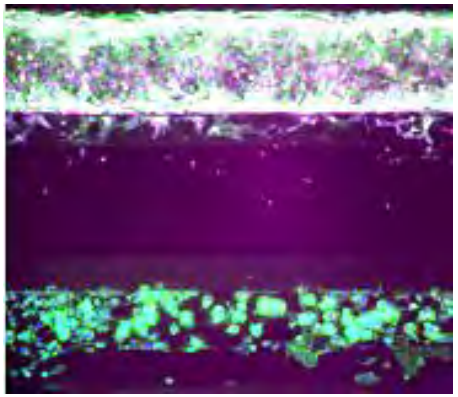
… yields robust and quantitative data

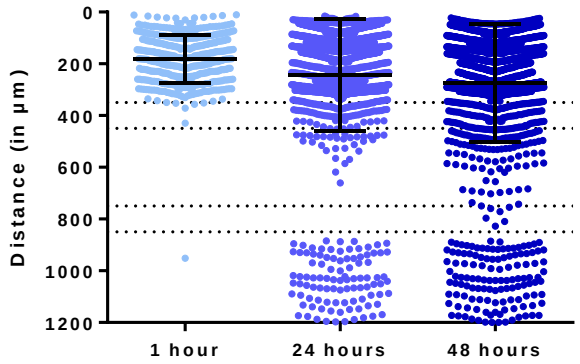
TEER measurement

2CC Apical exposure

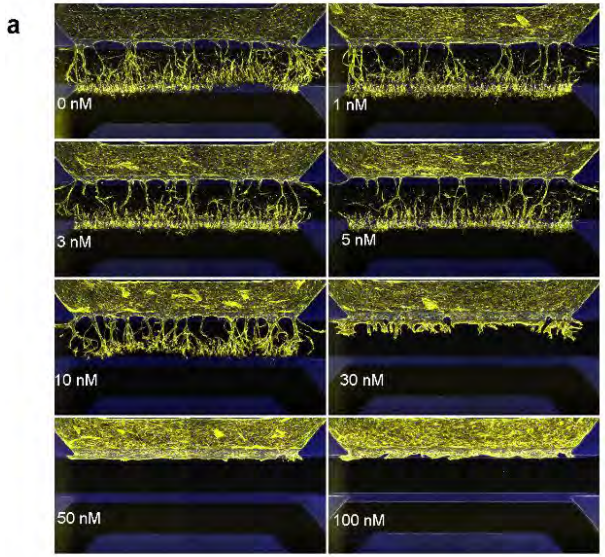


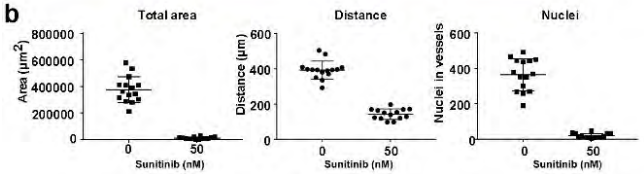
Immune cell Migration





Angiogenesis





… and can be repeated thousands of times

Automated workflow

Screenable platform

High content screening systems and multimode readers

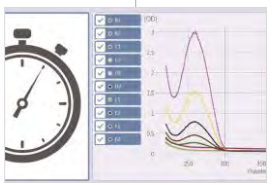
Thousands of chips

Spark® Cyto – live cell multimode plate reader with real-time image cytometry

A POWERFUL TOOL FOR MULTIPLEXING

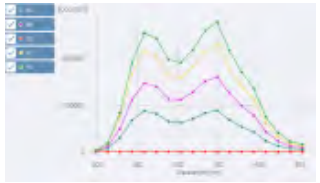
TECAN

Absorbance with high-speed **monochromators**

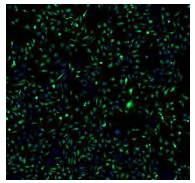


Fluorescence with **FUSION Optics**

Luminescence with BRET & NanoBRET™

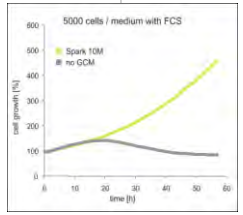


Brightfield and **Fluorescence Imaging**

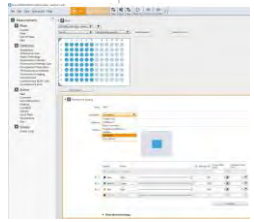


Automate your workflow with **Lid Lifter™**

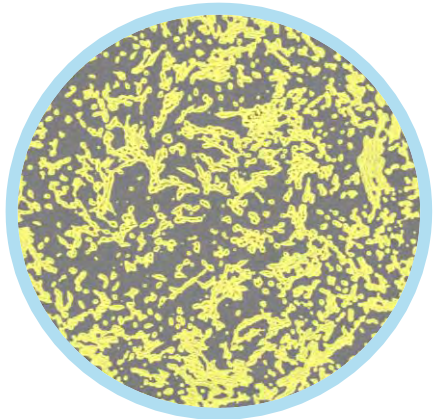
Cell incubation with temperature/ gas control and humidity cassette



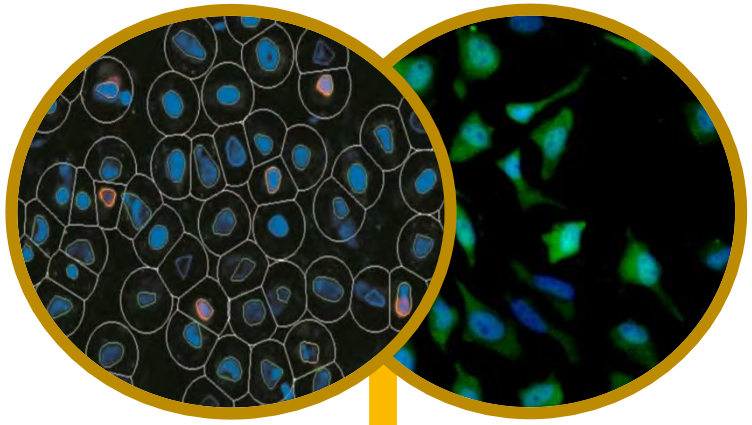
Intuitive and **user-friendly** SparkControl™ **software**



Spark Cyto imaging - key applications at a glance

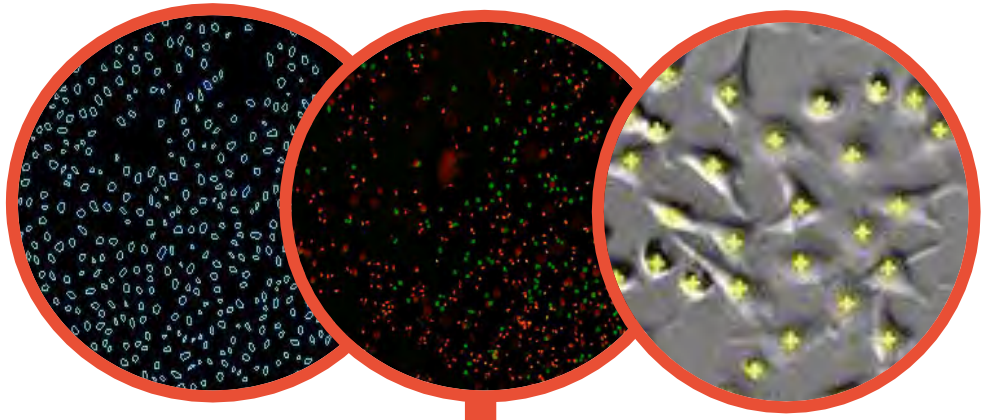


Confluence



- Apoptosis
- Transfection efficiency

Multi-Color applications



- Nuclei counting
- Viability
- Label-free cell counting

Counting

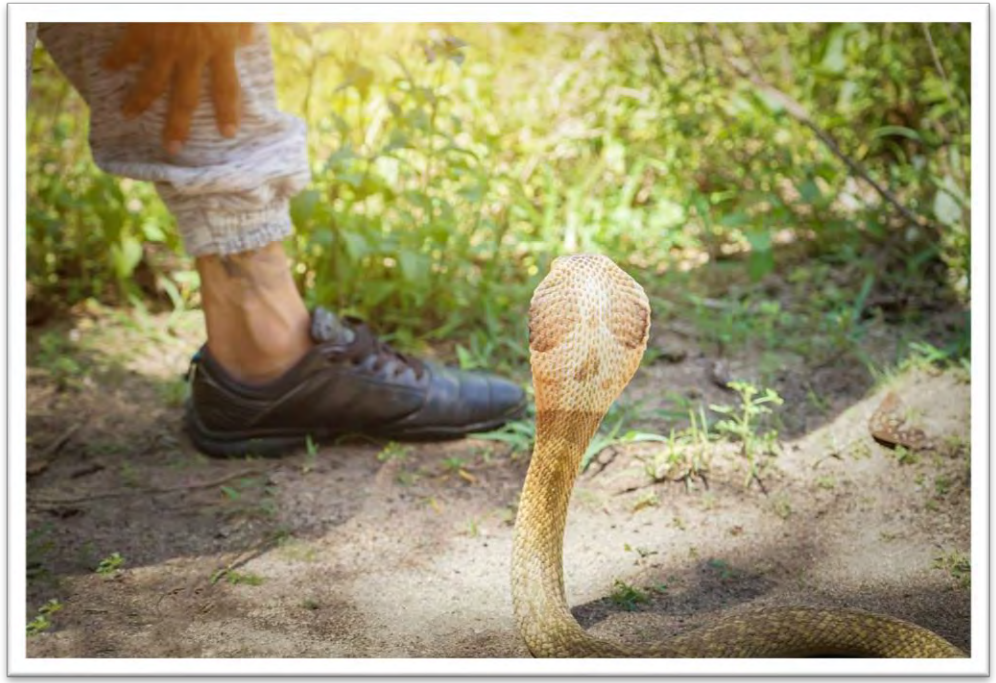
Synergy between OrganoPlate and Tecan Spark Cyto

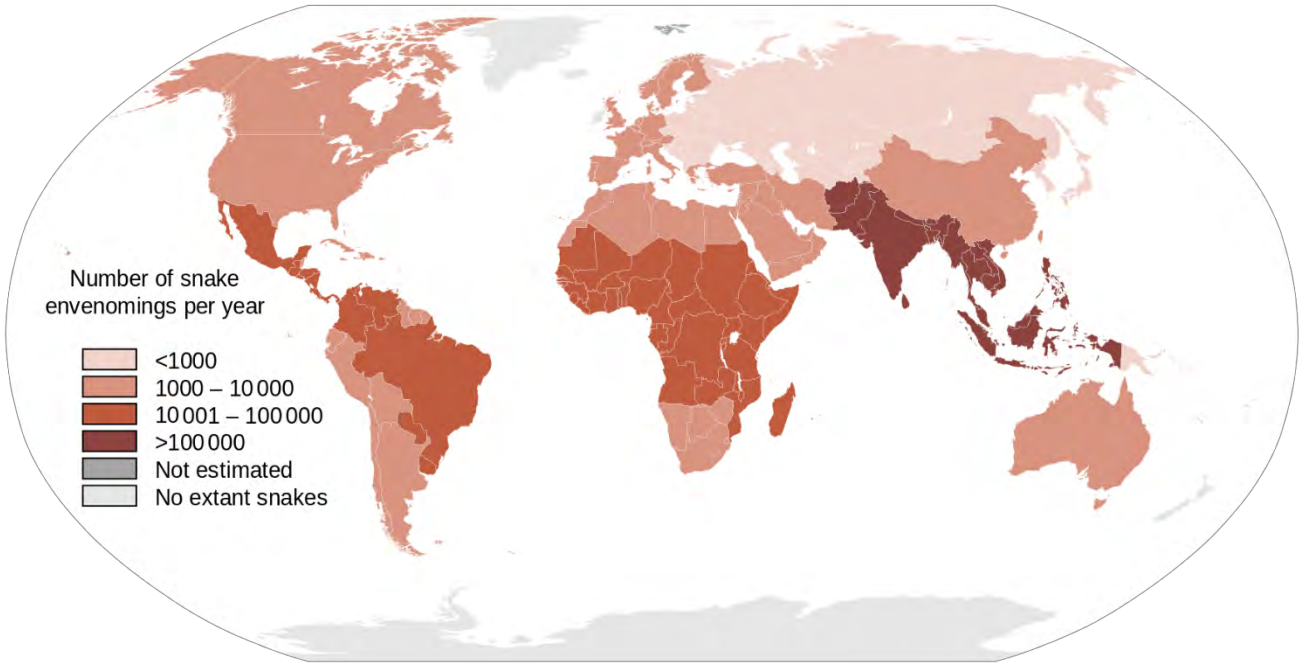
OrganoPlate

Tecan Spark Cyto

✓	Ease of use	✓
✓	Scalable and throughput capable	✓
✓	Imaging capable	✓

Venomous snake bites affects millions





Snakebite seriously injure up **to 2.7 million people** and claim some **125,000 lives**.
(World Health Organization)

Toxicity of snake venoms

Snake venom

Circulatory system

Damage to blood or blood vessels
Internal bleeding and organ dysfunction

Neuromuscular system

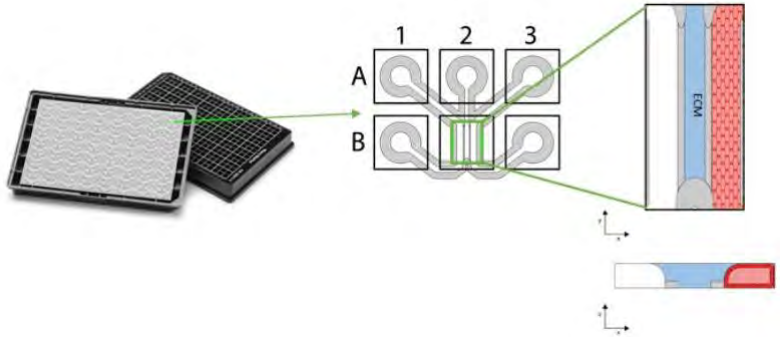
Damage to neurons and muscles
Paralysis, respiratory failure, cardiac arrest

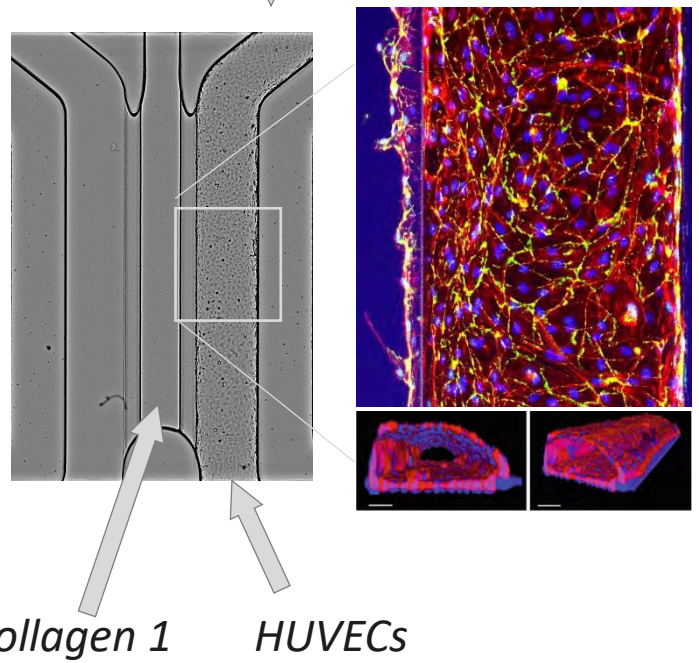
Snake venoms are comprised of a complex cocktail of bioactive substances. They can have multiple effects, including affecting the circulatory or neuromuscular system.
The effect of many venoms on the human body is unknown

Venom-induced vascular toxicity model

Four different venomous snake species

Snake venom





Collagen 1

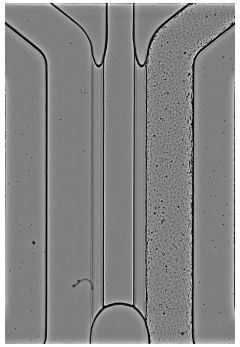
HUVECs

Vascular leakage
Morphology
Viability
Toxicity

Integrated multiplex workflow

- One integrated workflow protocol
- Multiplexed fluorescence intensity measurements and imaging
- Temperature controlled environment

Snake venom



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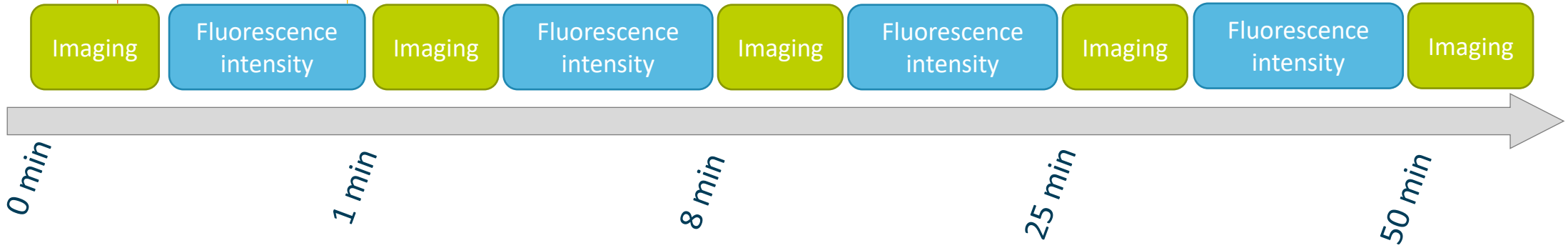
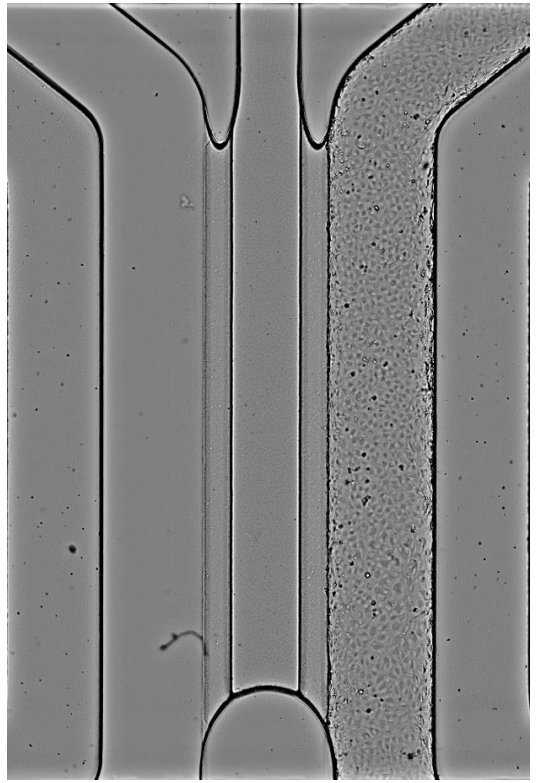


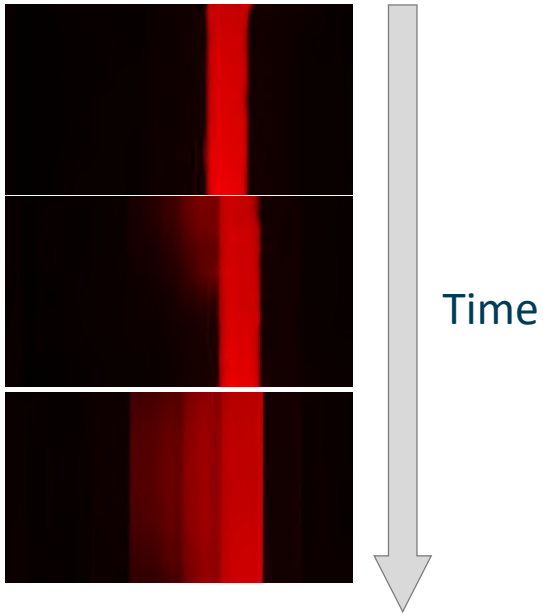
Vascular leakage assessment

TRITC-Dextran

Snake venom

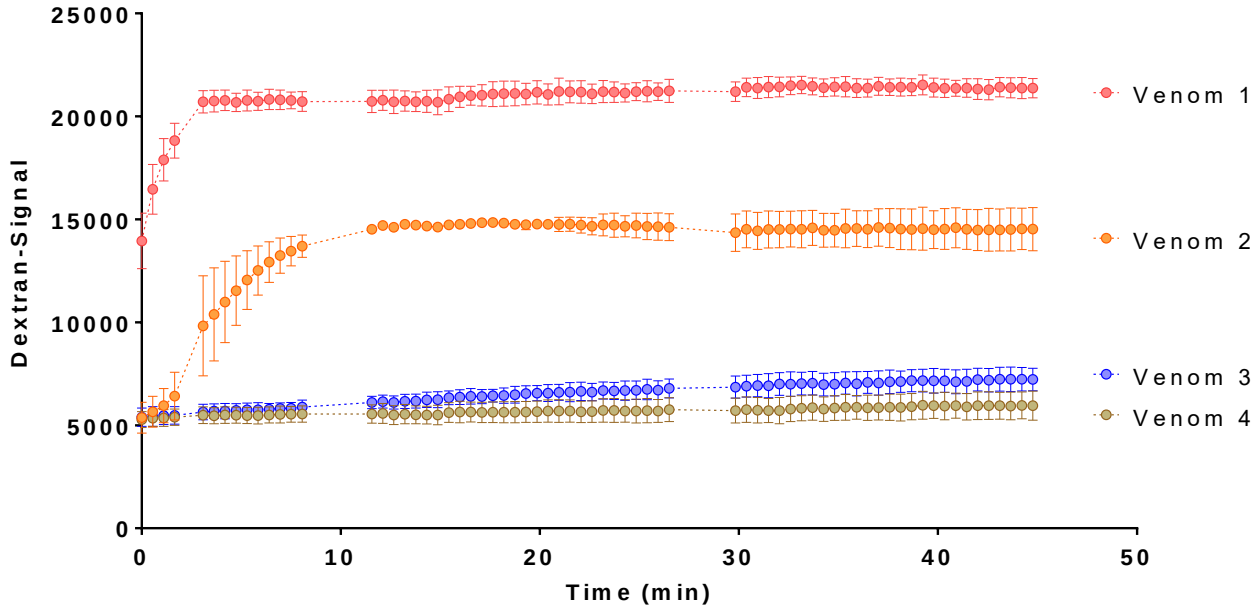


Dextran is retained in the blood vessel lumen but permeates in the adjacent channel if the barrier is disrupted



Fluorescence intensity measurement

Vascular leakage



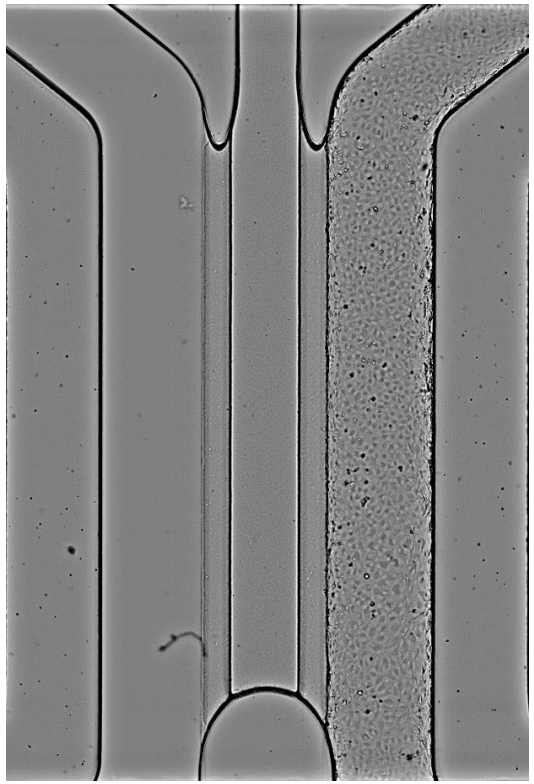
Venom 1 and Venom 2 appear to induce massive leakage seconds or minutes after addition. Venom 3 shows slight leakage over time while Venom 4 did not induce vascular leakage in this timeframe.

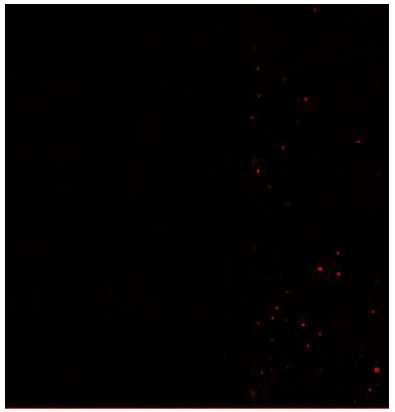
Dead nuclei count

TRITC-Dextran

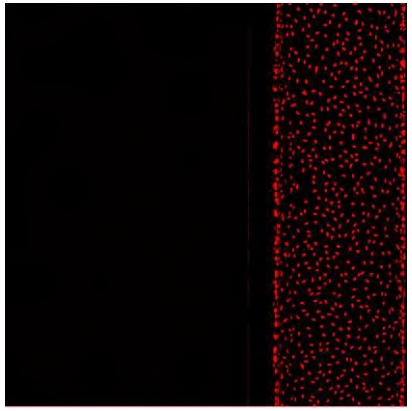
Snake venom

DRAQ7



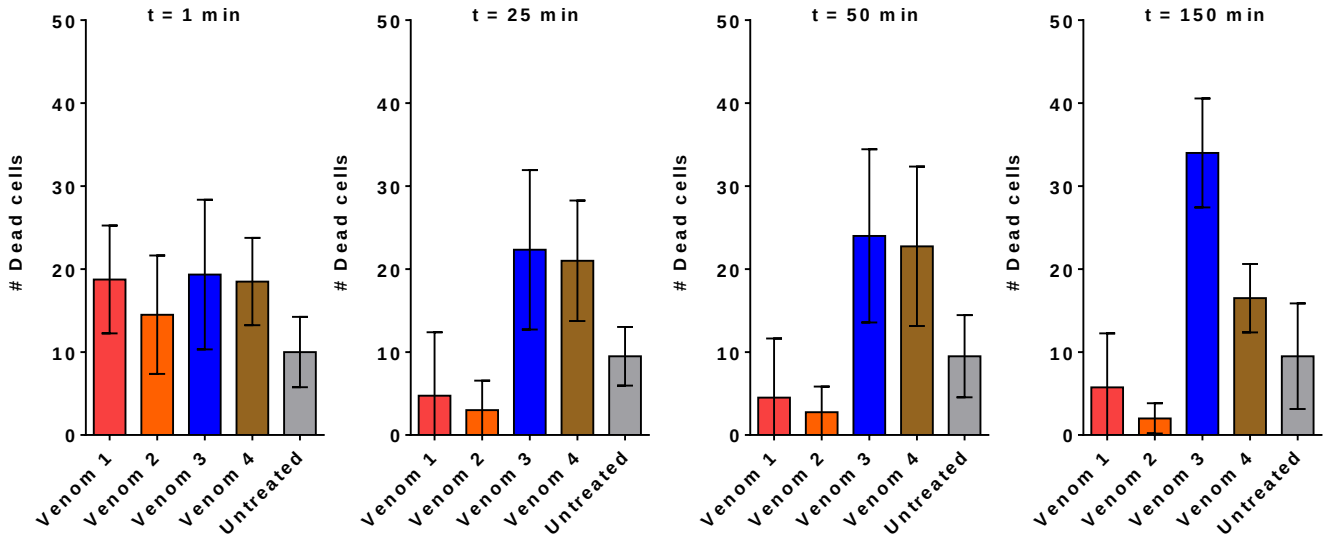


1% Triton control (100% dead cells)



Imaging

Dead nuclei count

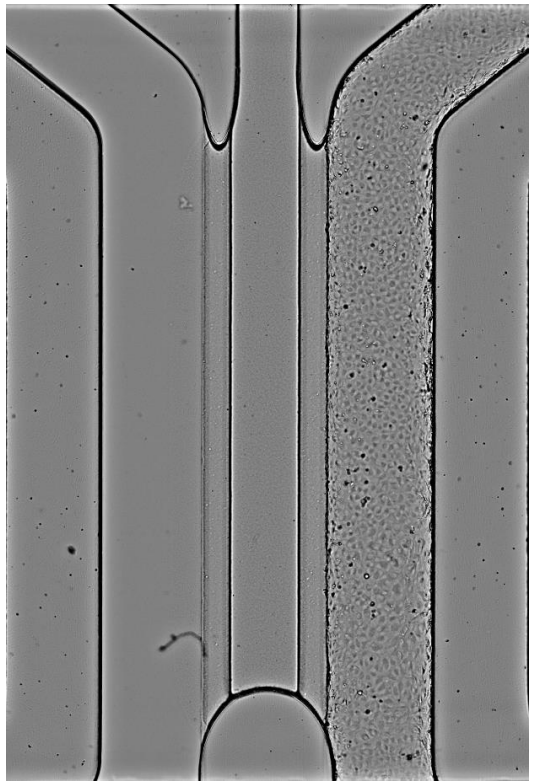


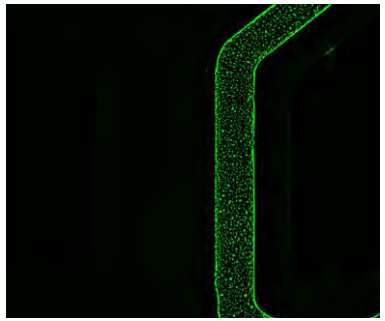
Although Venom 1 and Venom 2 induce massive leakage, no increase in dead cells was observed. Venom 3 and (to a lesser extent) Venom 4 appear to be causing cell death over time

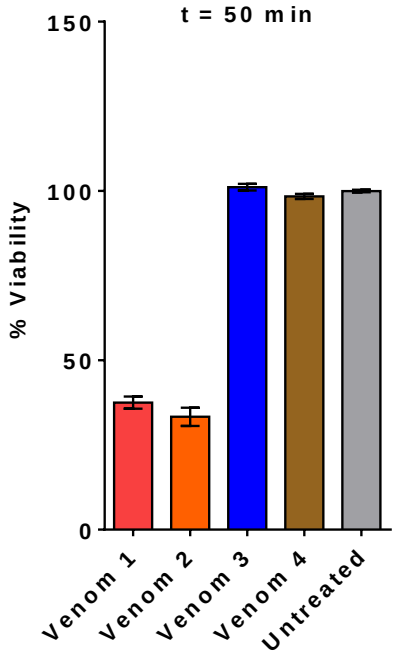
Viability assessment

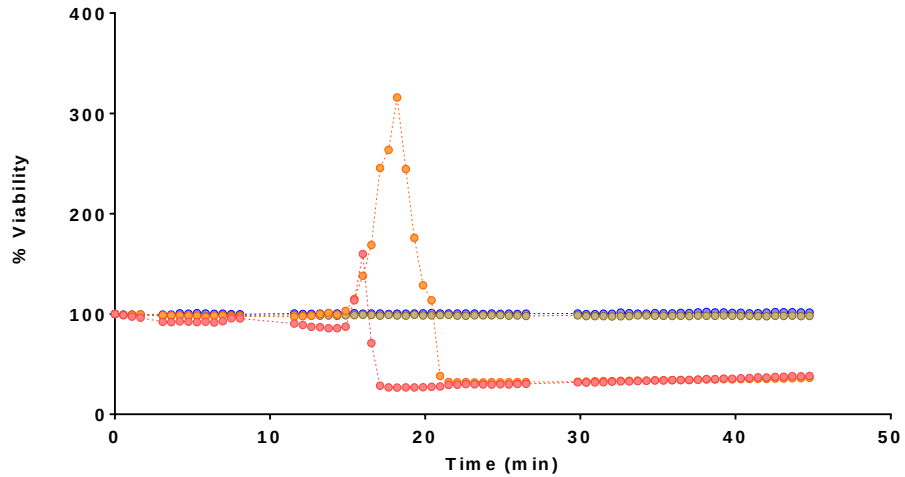
Fluorescence intensity measurement

Viability / morphology







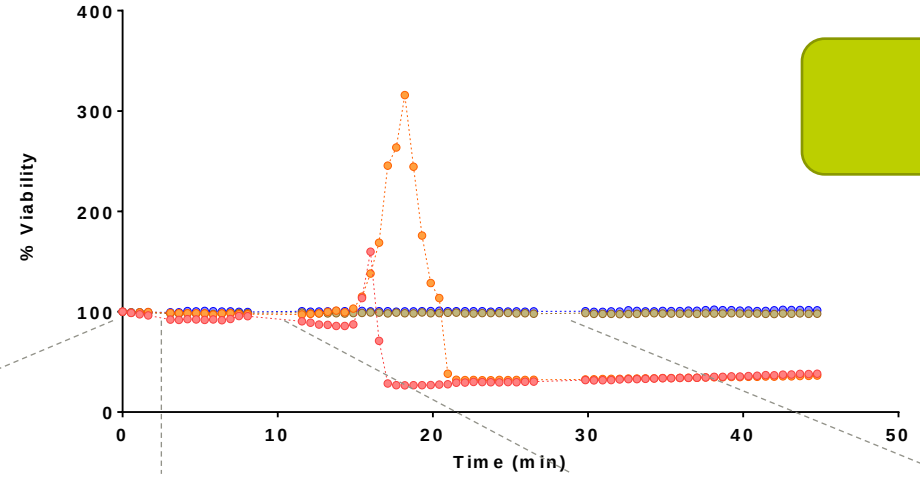


After 50 min incubation, Calcein-AM signal was significantly reduced for Venom 1 and Venom 2. Over-time assessment indicates the emergence of distinct "spikes" followed by a substantial drop in signal. Venom 3 and 4 do not seem to significantly affect viability.

Morphological assessment

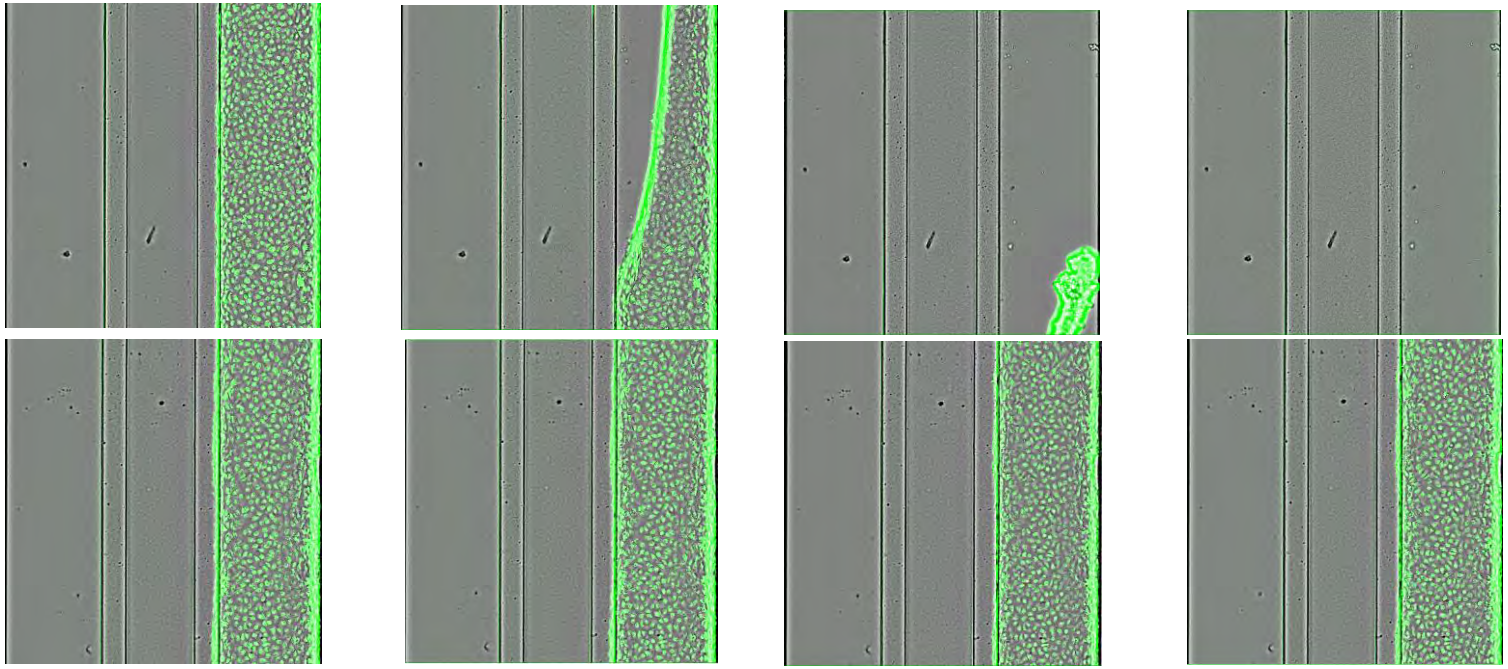
Imaging

Morphology



Venom 2

Untreated



The action of the venom does not cause the cells to die but rather to contract, hence an increase in signal is measured. Subsequently, the integrity of the whole structure is compromised, the tubule is lost and probably "flushed" out of the channel, leading to the loss of signal.

Summary

Circulatory system

Venom 1

Venom 2

No apparent cell necrosis but massive blood vessel structure damage and high blood vessel barrier disruption

Venom 3

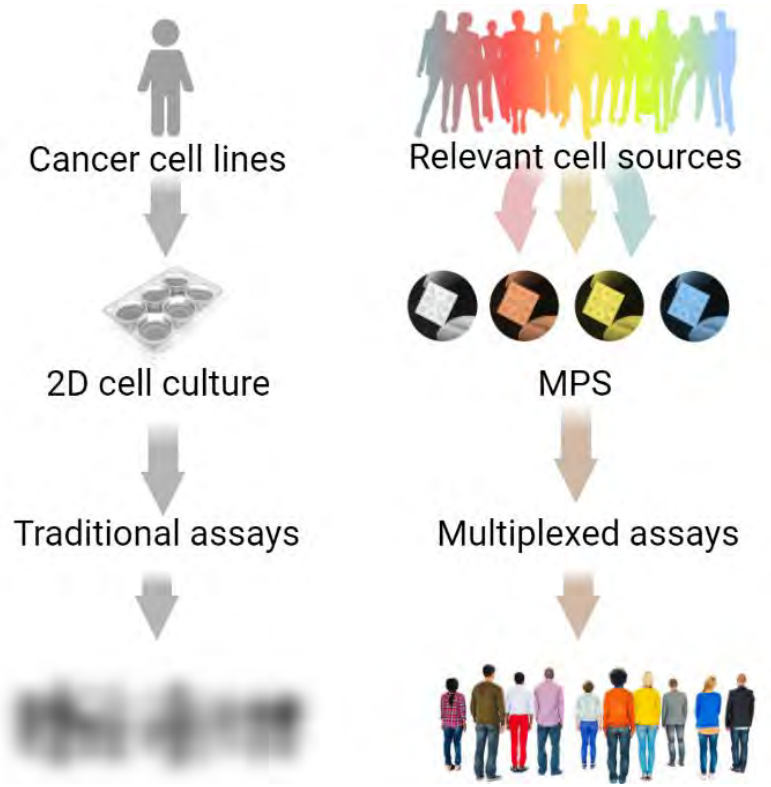
Indication of endothelial cell necrosis and elevated blood vessel leakage

Venom 4

Slight indication of endothelial cell necrosis but no clear sign of blood vessel leakage

Conclusion

- Increasing importance of *in vitro* pre-clinical test platforms
- Complex organ-on-chip models are used to perform functional assessment of bioactive substances, such as snake venoms
- This requires appropriate, automation capable and relevant 3D organ-on-chip models
- This also requires appropriate, multi-mode data acquisition systems
  - Inclusion of imaging expands the understanding of biological processes
  - Multiplexing with imaging allows for high sensitivity and temporal resolution while allowing morphological assessment and signal localization



Contributors

Flavio Bonanini[1], Mátyás Bittenbinder[2,3,4], Jeroen Kool[3,4], Freek J. Vonk[2,3,4], Sebastiaan J. Trietsch[1], Dorota Kurek[1]

1 Mimetas, Leiden, The Netherlands

2 Naturalis Biodiversity Center, 2333 CR Leiden, The Netherlands

3 Amsterdam Institute of Molecular and Life Sciences, Division of Bioanalytical Chemistry, Department of Chemistry and Pharmaceutical Sciences, Faculty of Science, Vrije Universiteit Amsterdam, De Boelelaan 1085, 1081HV Amsterdam, The Netherlands

4 Centre for Analytical Sciences Amsterdam (CASA), 1098 XH Amsterdam, The Netherlands

TECAN.

MIMETAS