Microsystems for Cultivation and Analysis of Cells

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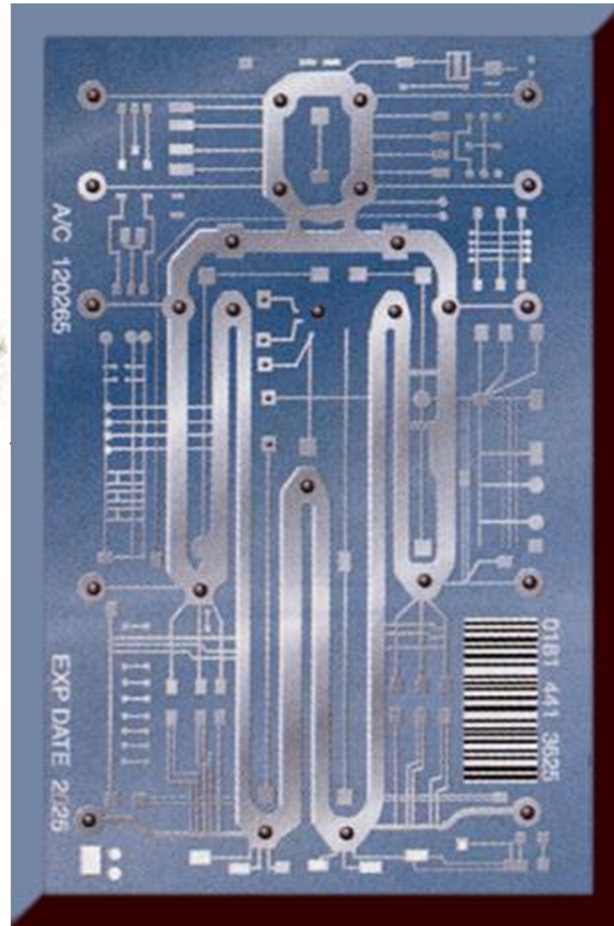
Cellular Microsystems and Biosensors Laboratory

Research Thrusts of the Lab:

- **Developing microphysiological systems or organs-on-chip for disease modeling and individualized medicine.**
- **Microsystems and biosensors for point of care testing.**

Why Microphysiological Systems?

Rationale: in vitro culture systems are simplistic. Animal models are complex, difficult to study cell signaling, may not be reflective of human physiology.



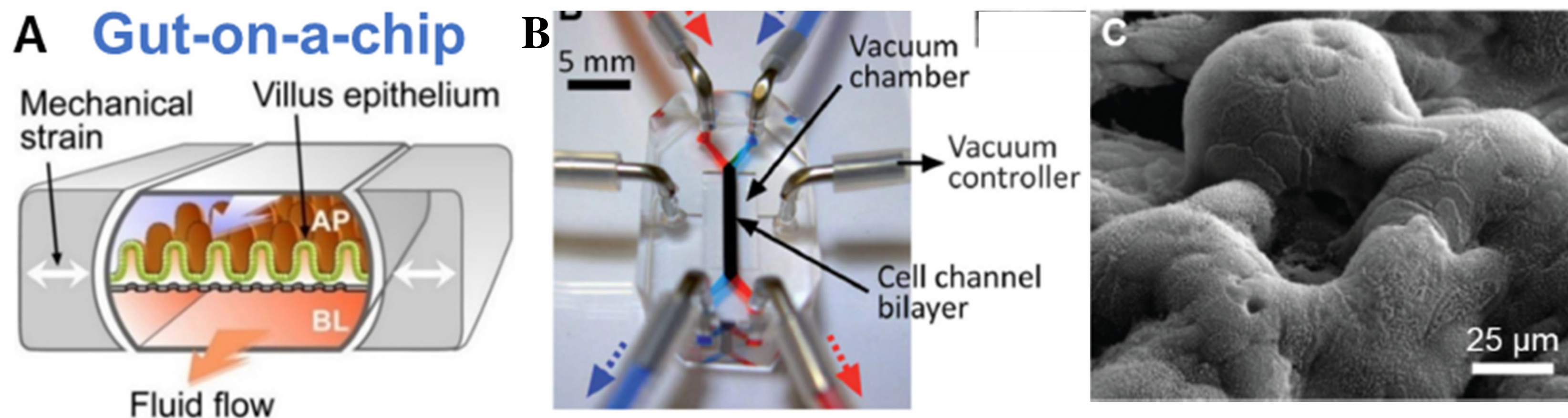
What is Needed for Microphysiological Systems

- **Microfabrication** – miniaturization of cell culture units using soft lithography and photolithography.
- **Microfluidics** – to connect cell culture units in a physiological manner via a set of miniature pumps and routers.
- **Biosensors and sampling units** – miniature devices used to convert biological events, release of signal or consumption of metabolite, into an electrical or optical signal.

Examples of Microphysiological Systems

Gut-on-chip device developed by Ingber lab showed that flow and cyclical strain were needed to achieve appropriate differentiation of gut epithelial cells.

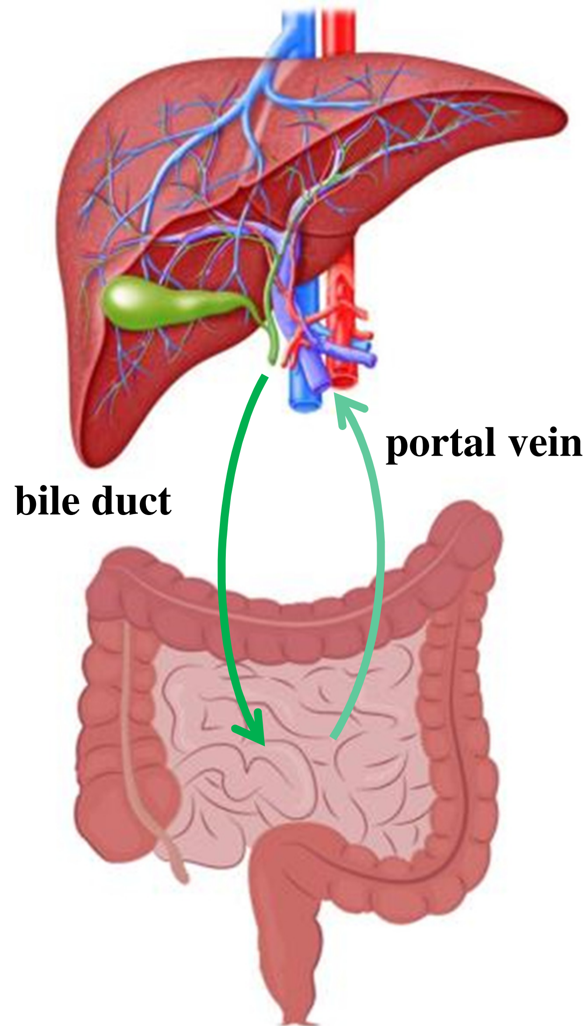
Oxygen gradients can be established to mimic lumen of the gut. Microbes and immune cells have been integrated.



Ingber lab, Wyss Institute/Harvard

Modeling Liver-Gut Crosstalk Using Microfluidics

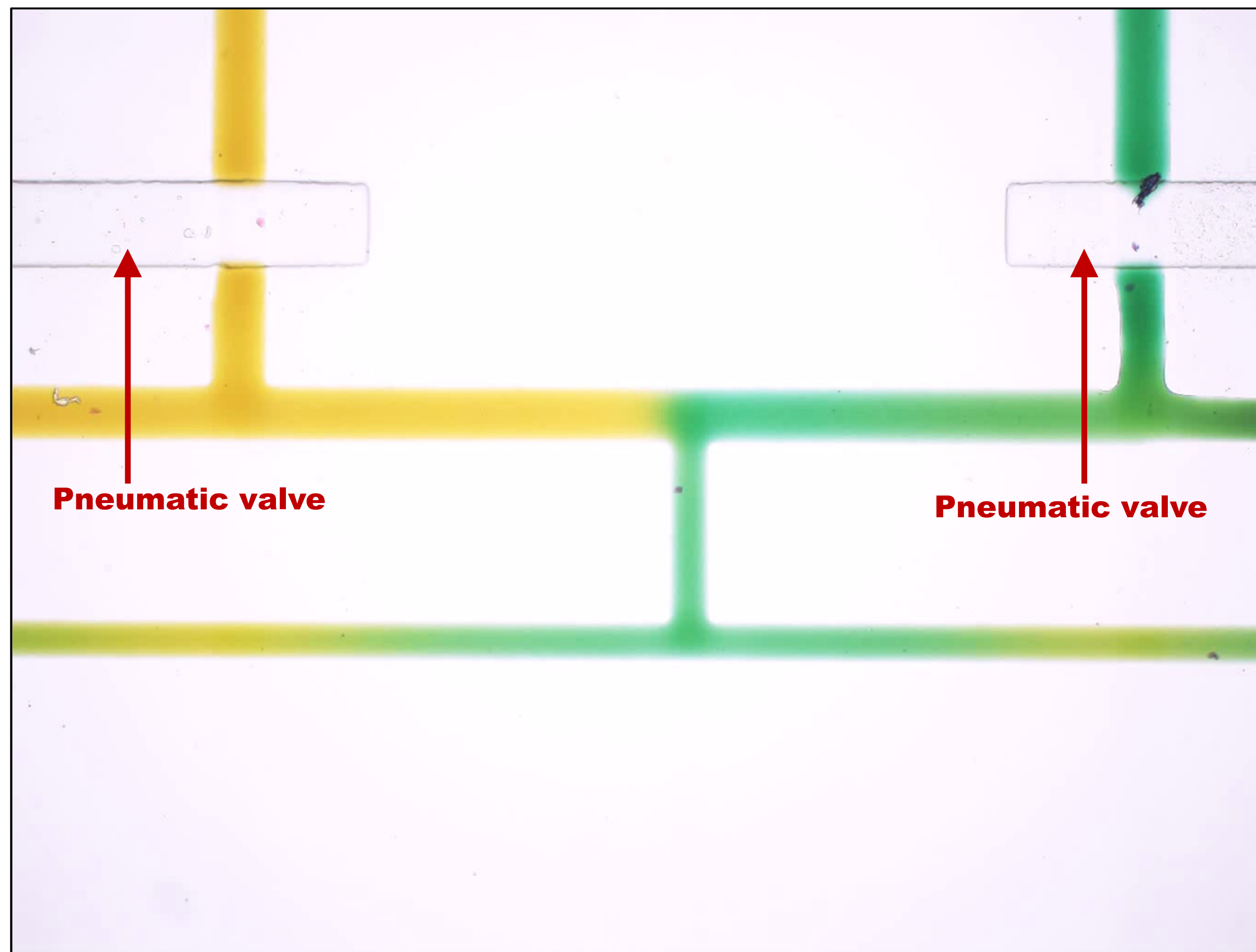
Entero-hepatic circulation



- Alcohol injury affects gut permeability, gut-derived products leach into the liver and cause inflammation/fibrosis. Difficult to untangle direct effects of alcohol injury to the liver and contribution of the gut.
- Bile acid homeostasis – feed forward and feed back communication between the gut and the liver. Difficult to model in vitro using standard approaches.

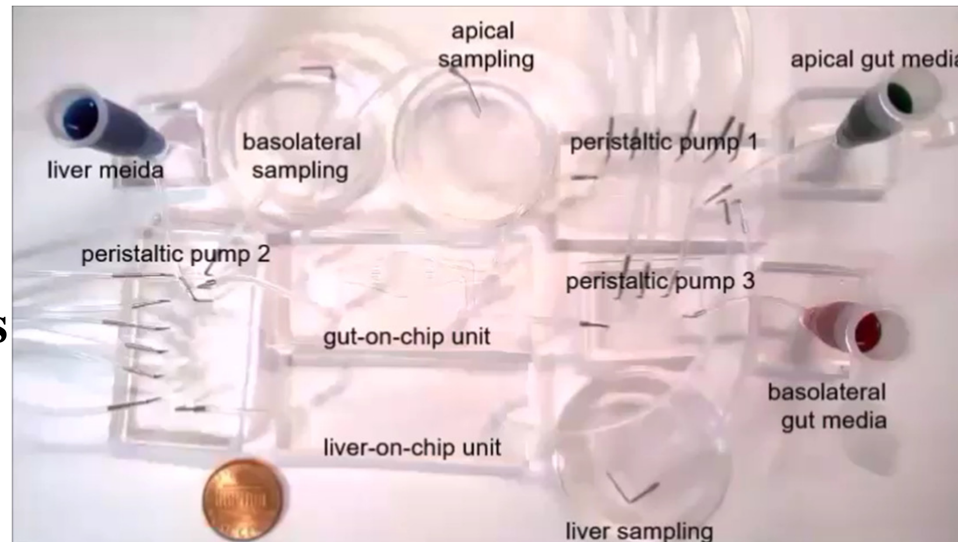
Microfluidic Devices with Built-in Microvalves

- First developed by Quake et al in ca 2000
- Useful for pumping, routing and sampling in microfluidic devices

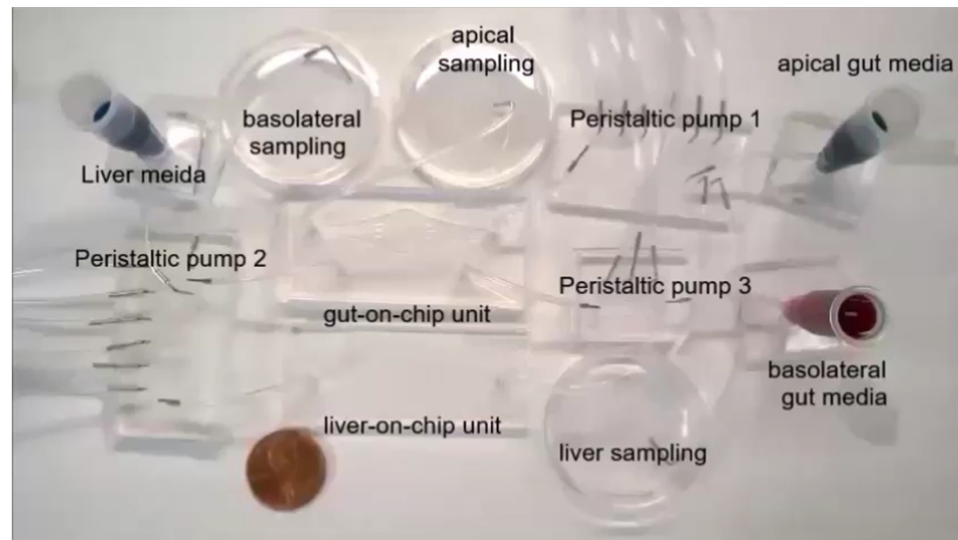


Microfluidic Cell Culture Circuits with Microvalves

Individual organ cultures

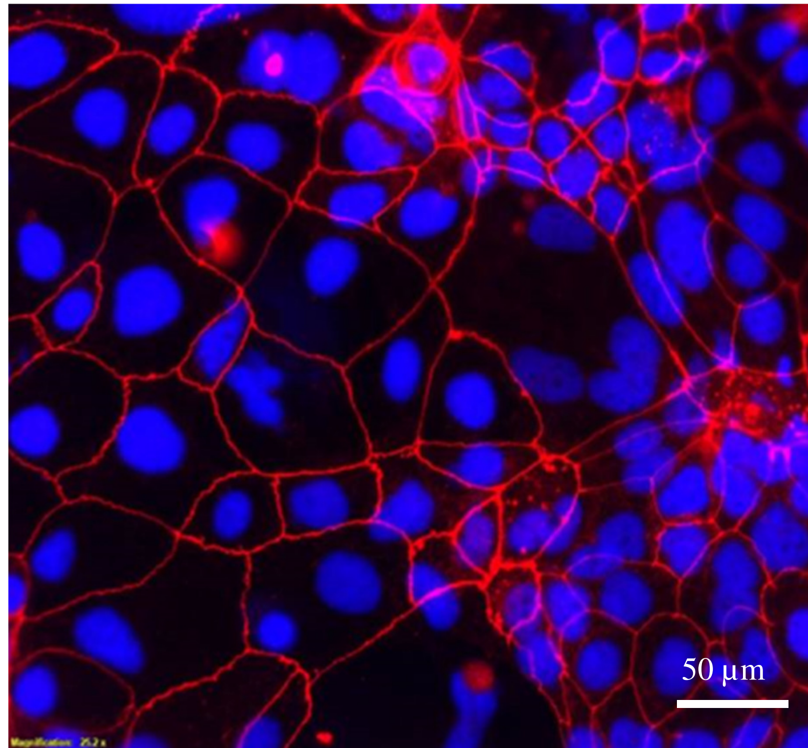


Enterohepatic circulation



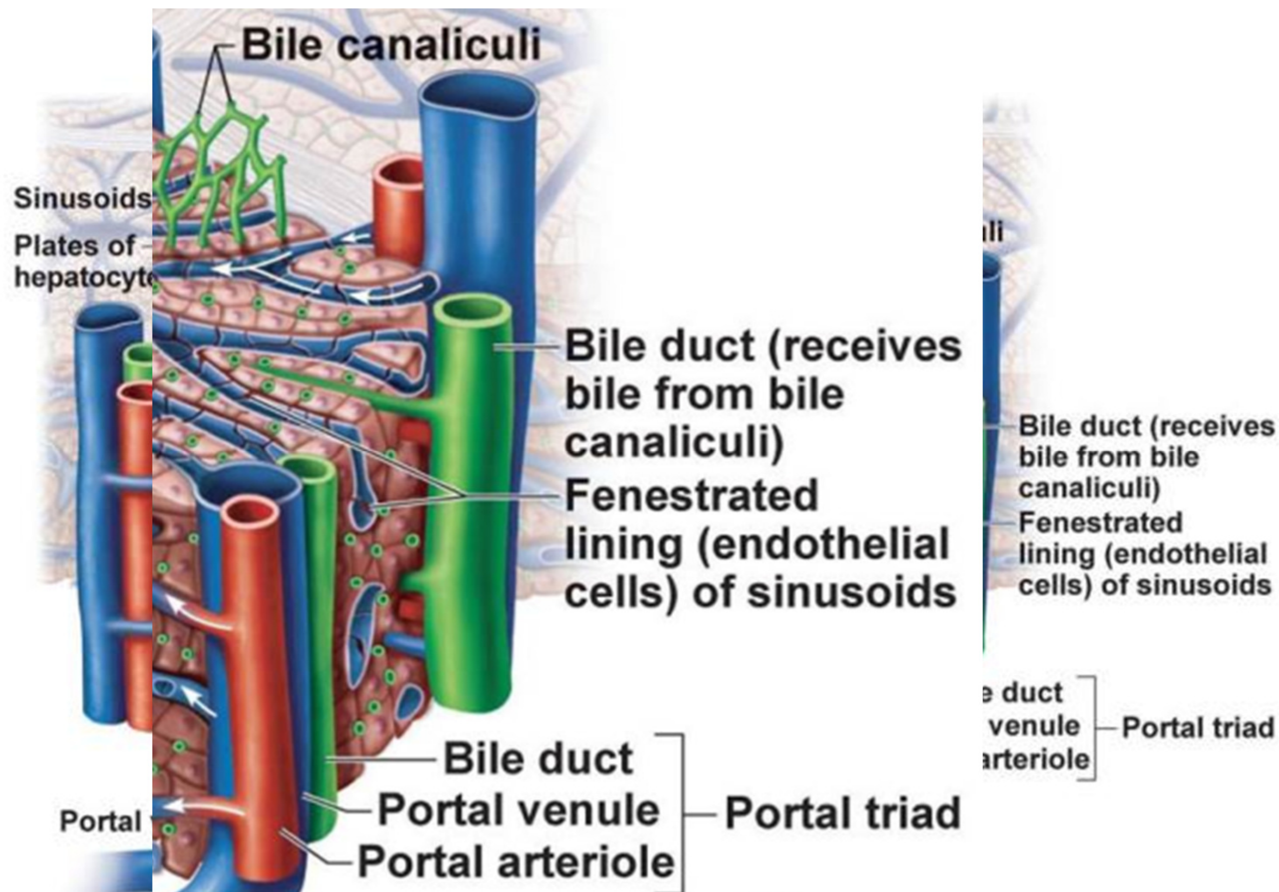
Function of Cells in Microfluidic Devices

- Need to ensure that microphysiological systems are physiological.
- Hepatocytes maintain function in microfluidic devices for several weeks in devices but what about polarization?



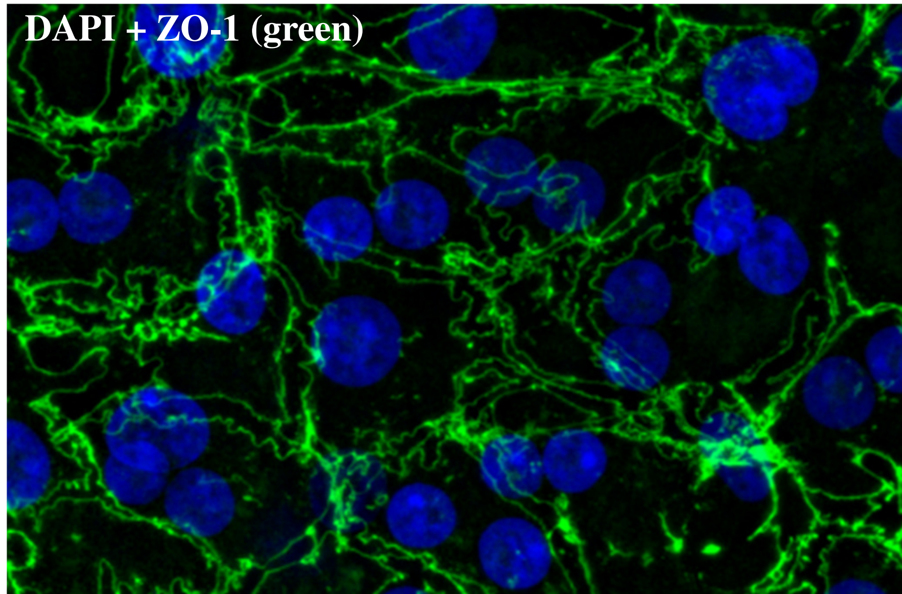
Function of Cells in Microfluidic Devices

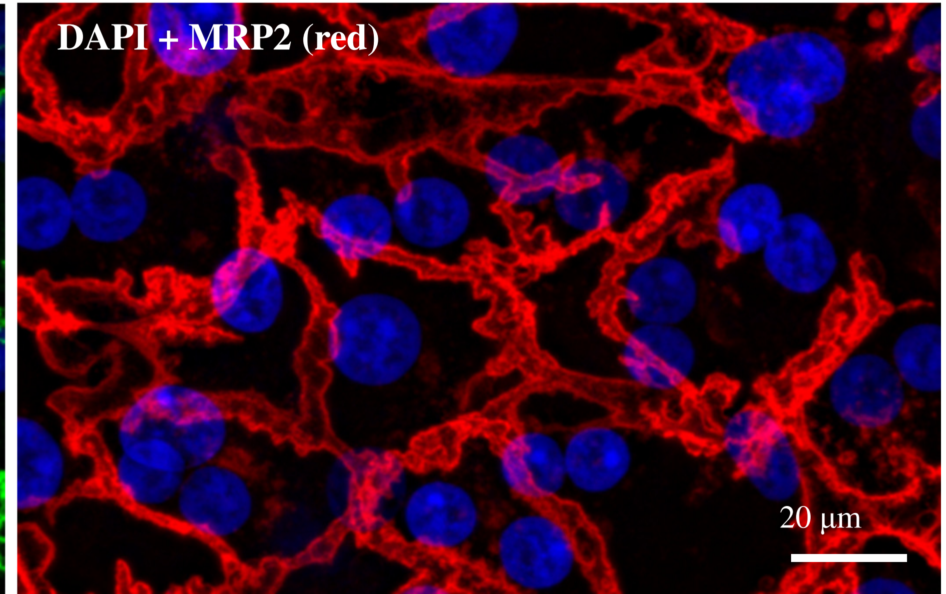
- Need to ensure that microphysiological systems are physiological.
- Hepatocytes maintain function in microfluidic devices for several weeks in devices but what about polarization?
  Formation of bile canalicular network needed.



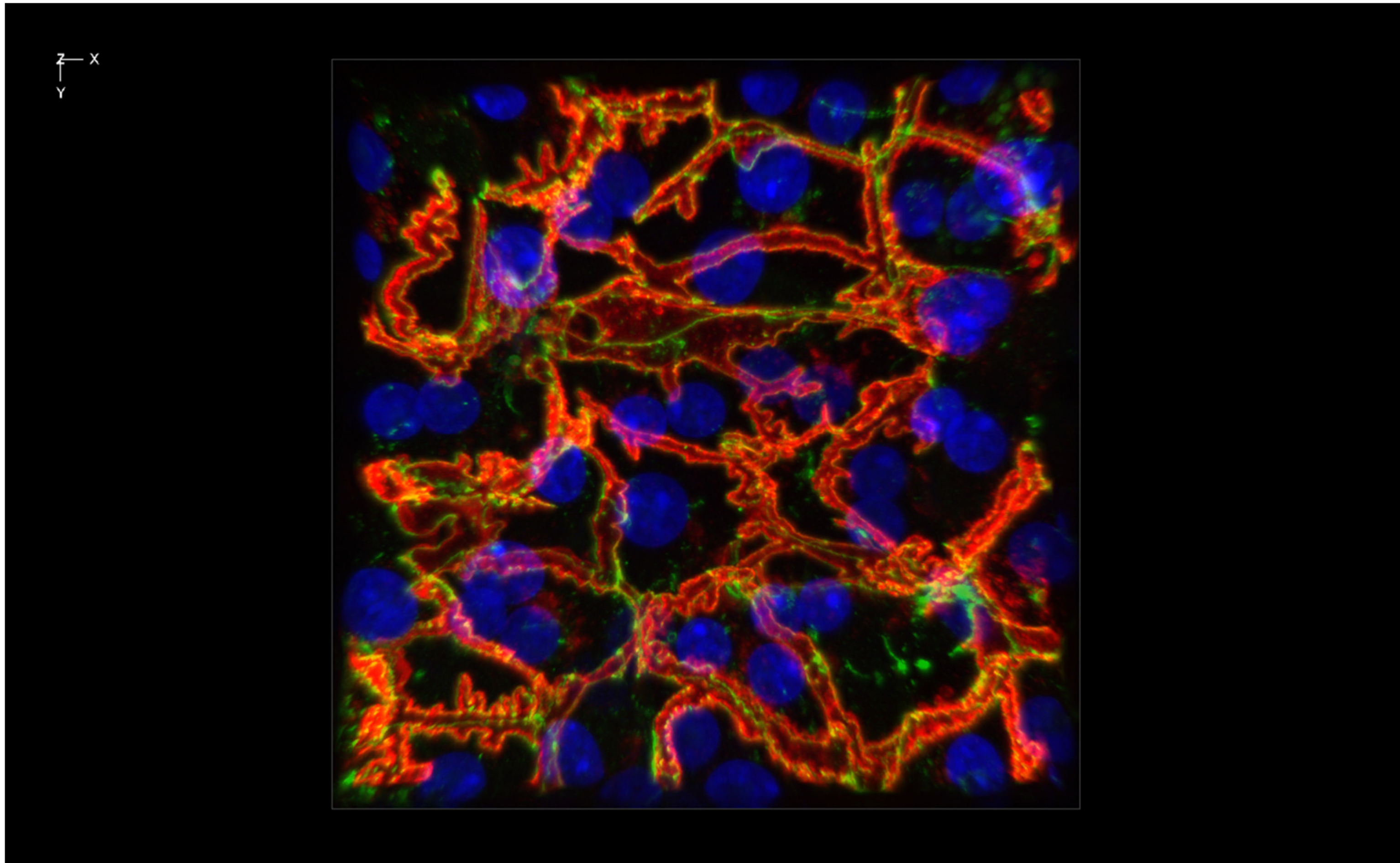
Function of Cells in Microfluidic Devices

- Optimized conditions to culture primary hepatocytes in microfluidic devices in collagen gel to ensure correct polarity.
- MRP2 – bile acid transporter. Extensive, branched in vivo-like network of canals observed in microfluidic devices.

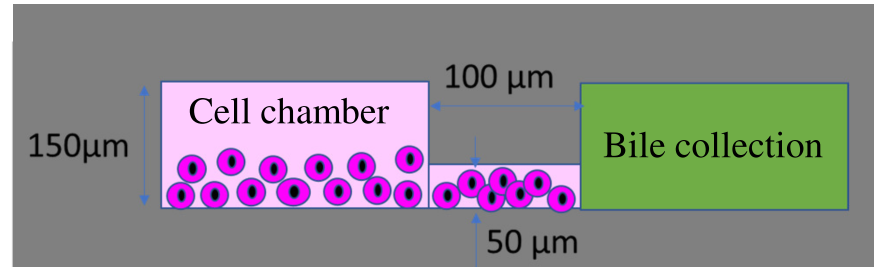


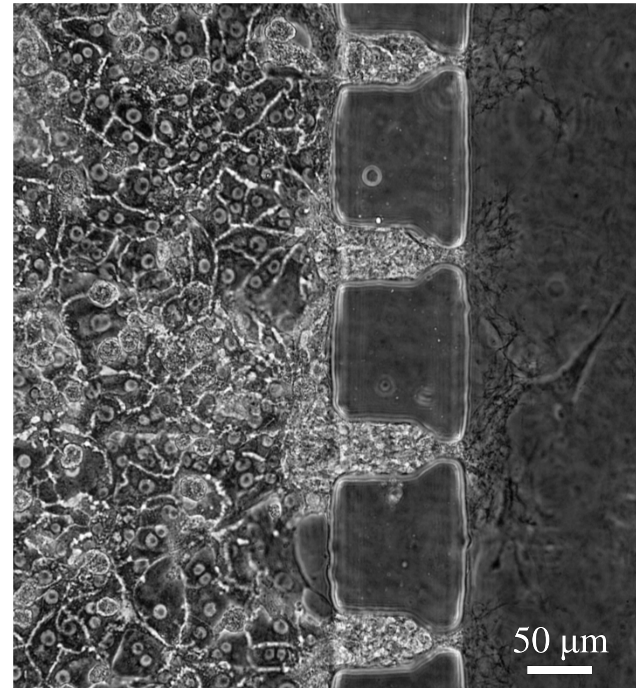


3D Bile Canalicular Network in a Microfluidic Device



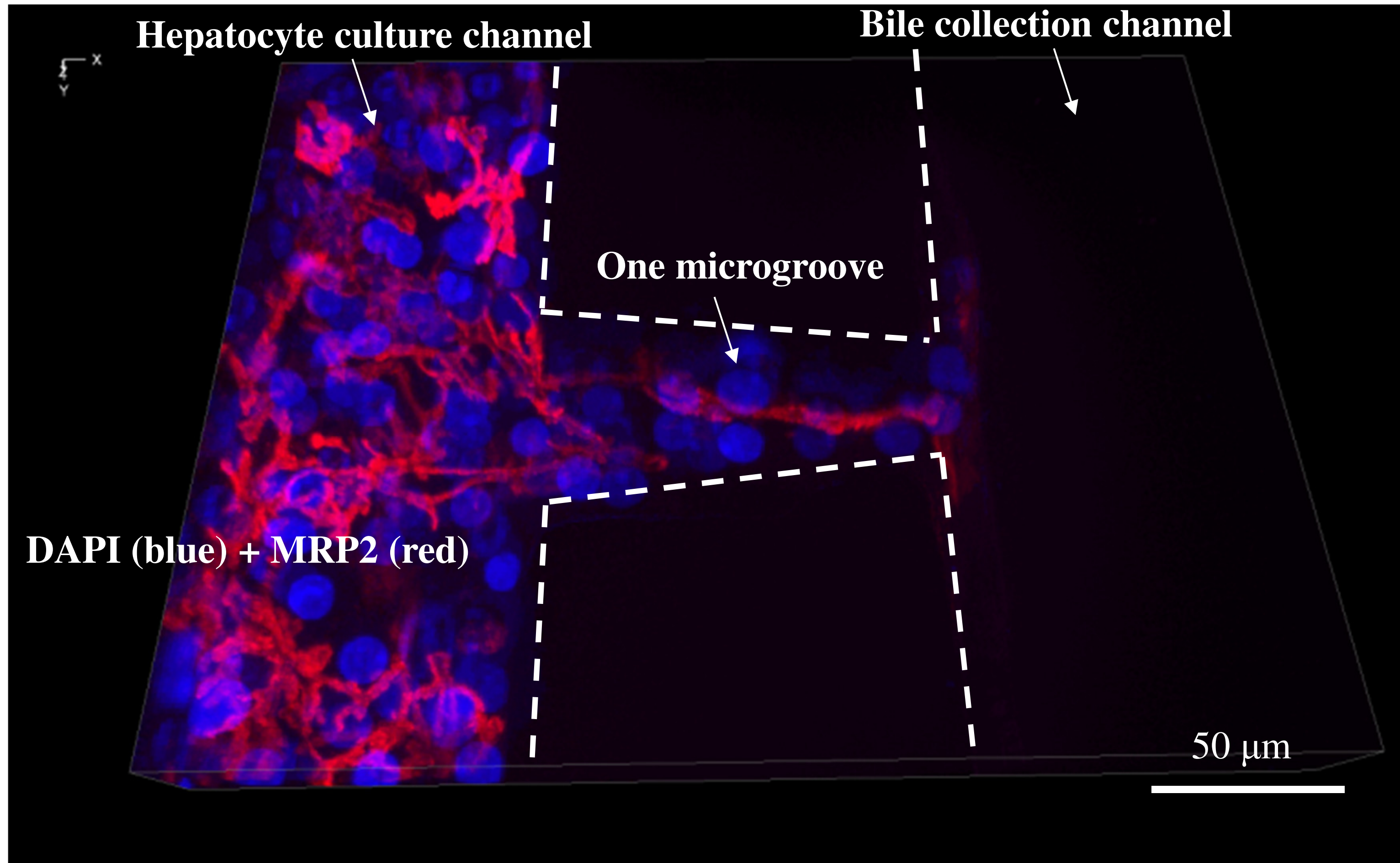
Microfluidic Device for Bile Collection





Hepatocytes at Day 4

Guidance of Bile Canals Into Microgrooves



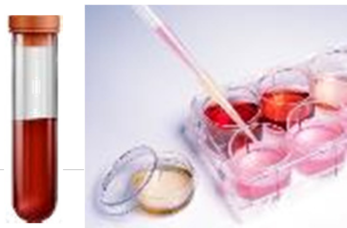
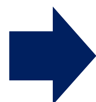
Summary I

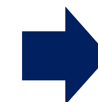
- Developed components of a microfluidic circuit: cell culture chambers, pumps, switches. Able to assemble these components in a physiological manner.
- Established long-term microfluidic cultures of hepatocytes.
- Polarizing hepatocytes and working to collect bile-enriched products using microfluidic devices. Important for enterohepatic circulation.
- Characterizing bile acid synthesis by mass spectrometry.

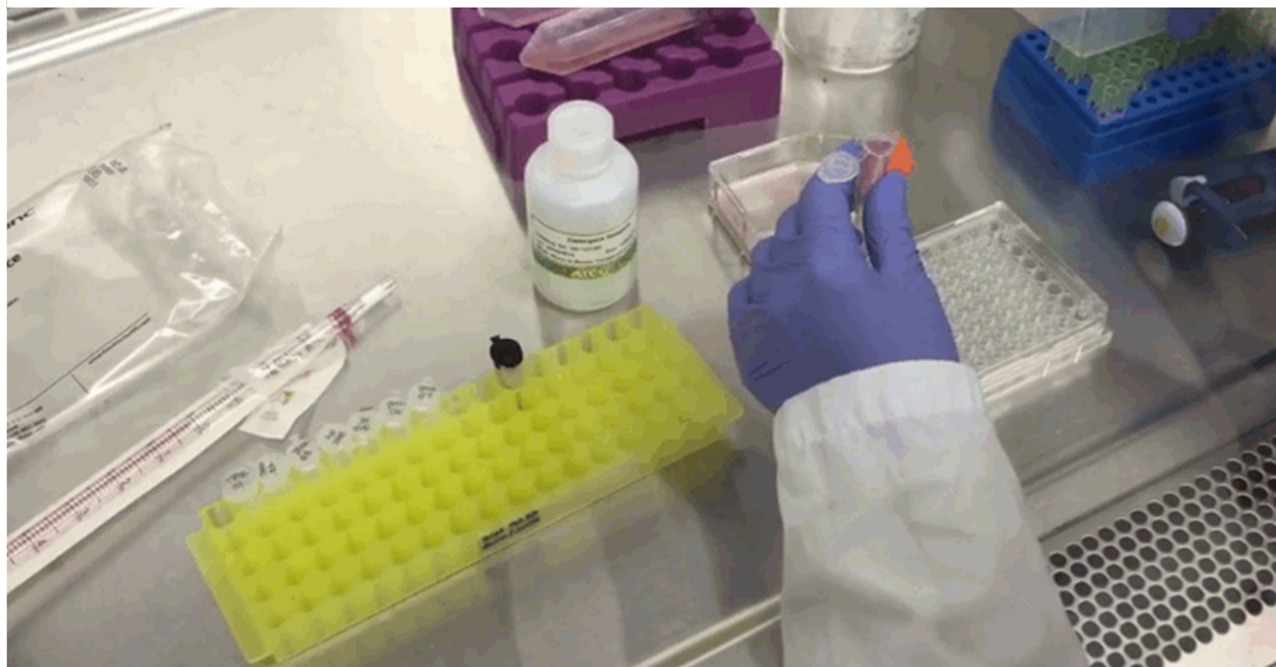
What about sampling and analysis of cell-conditioned media in microfluidic devices? Total volume ~ 50 μL.

Conventional Mix-and-Read Assays

Sampling | **Manual sample dilution / assay reagent handling** | **Spectrophotometry**





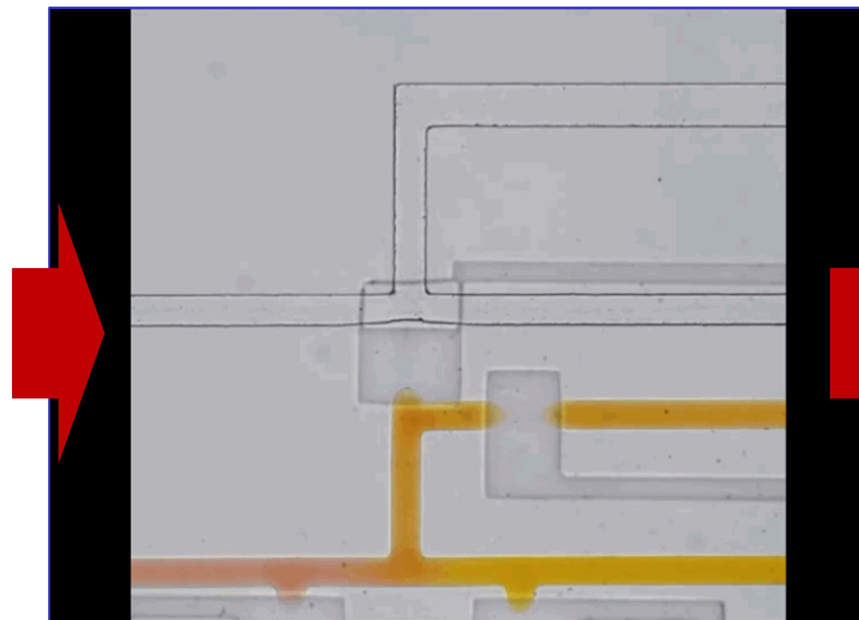
- ✓ **Repetitive**
- ✓ **Time-consuming**
- ✓ **Labor-intensive**
- ✓ **Requirement of large sample/reagent volume**

Microfluidic Mixing and In-Droplet BioAssays

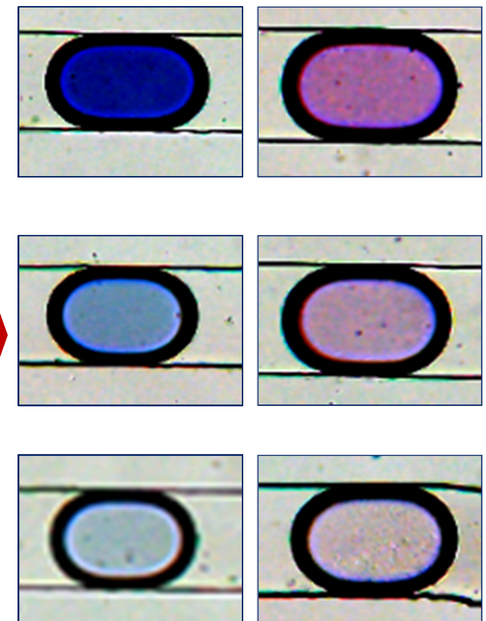
- Water-in-oil droplets generated at the crossflow junction of water and oil streams
- Microfluidic automation employs computer-controlled microvalves built into microfluidic devices.

* Computer flow controller

* Droplet-based biochemical assay



* Assay results



Device operation: Droplet generation

4. New droplets
3. Incubation and
solution exchange
Reagents

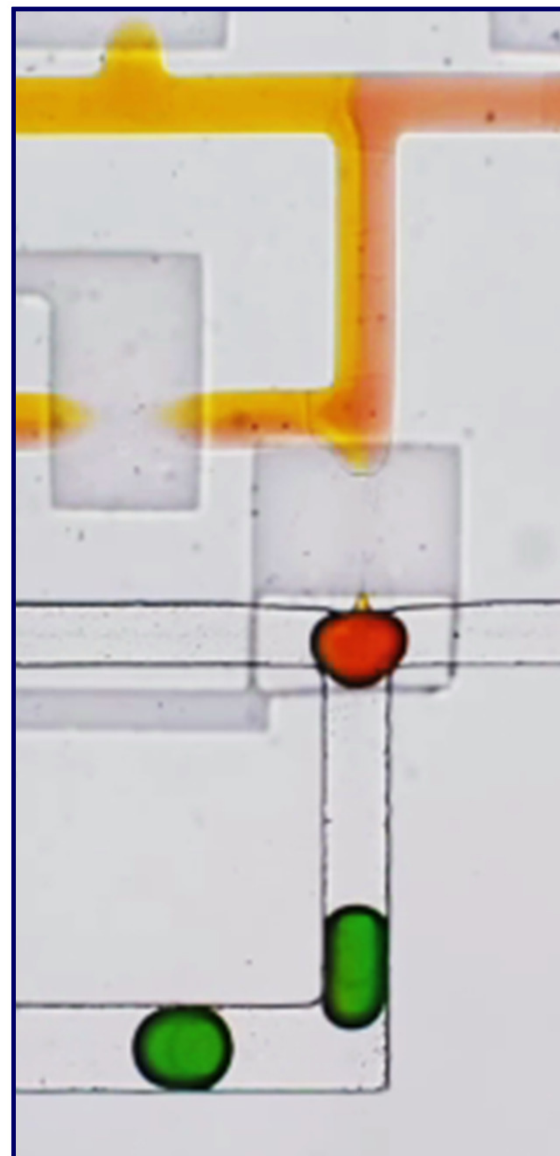
Aqueous stream

Oil stream

Closed valve

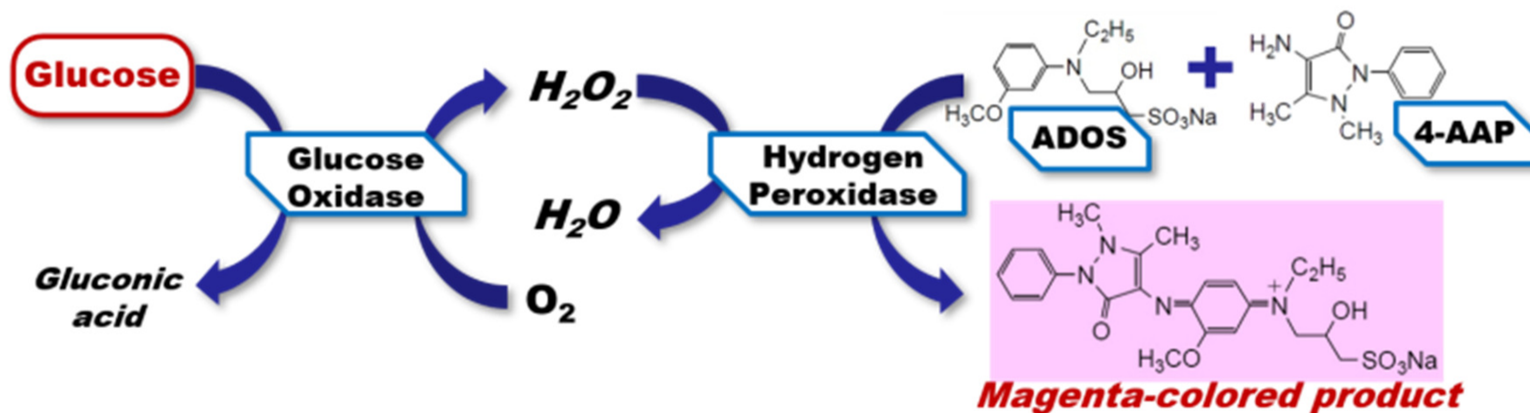
Opened valve

Outlet

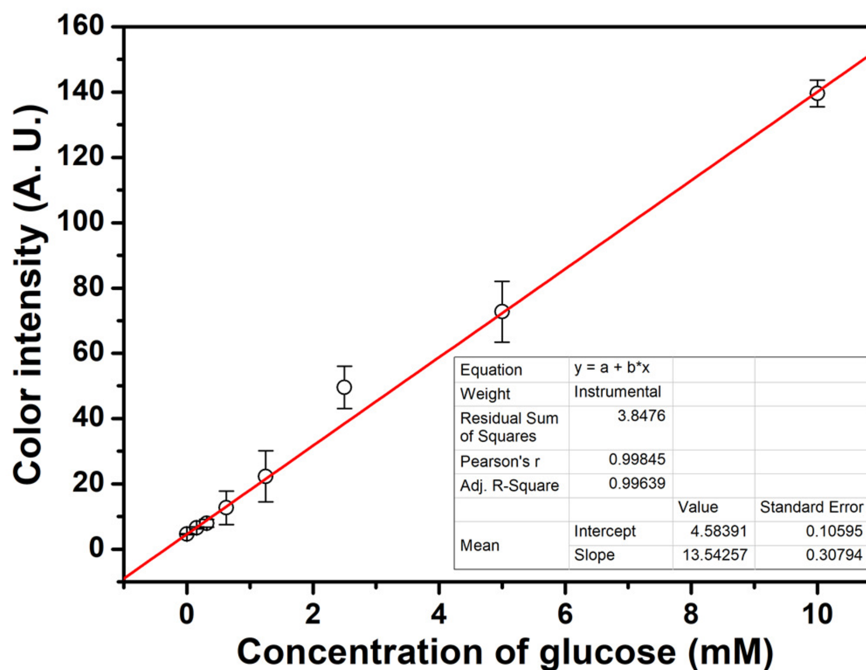


Cedillo, Han … Revzin, *Analytical Chemistry*, **2019**

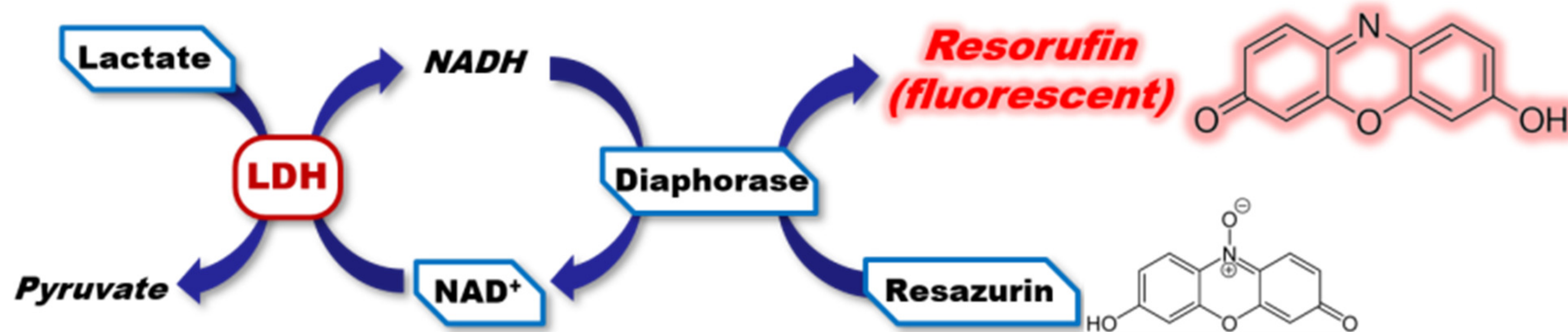
Colorimetric Glucose Assay



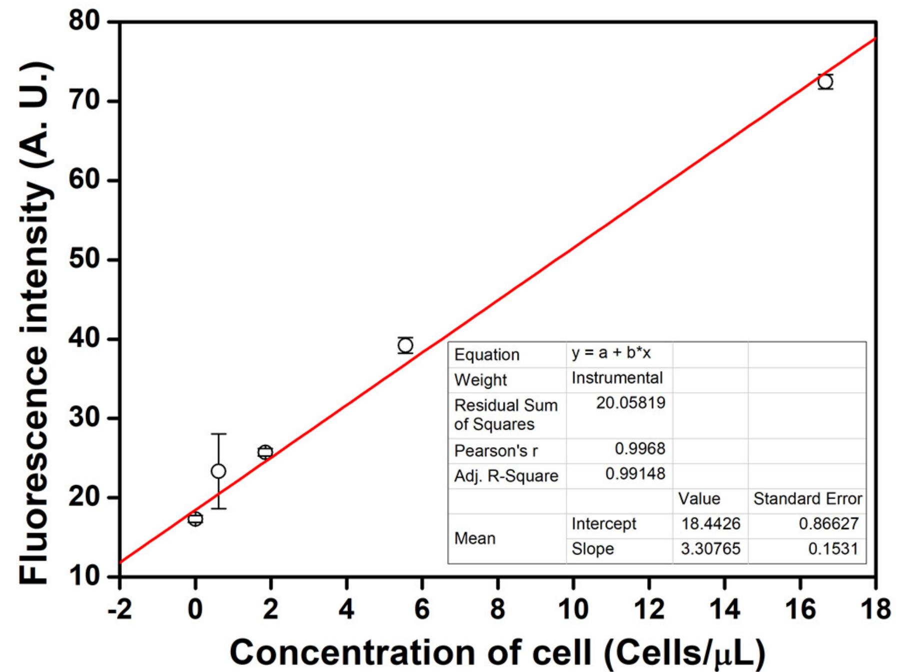
0 mM	0.3 mM	1.3 mM
5 mM	**25 mM**	**Limit of detection**
		0.04 mM



Fluorescence LDH Assay

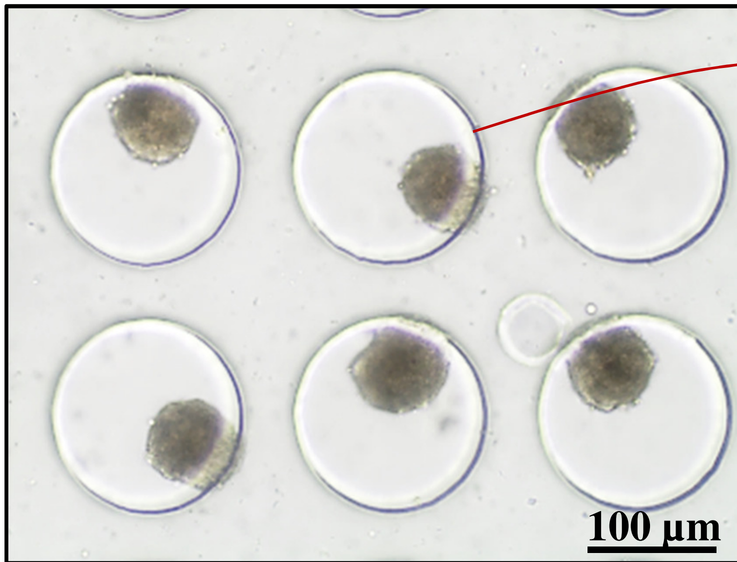


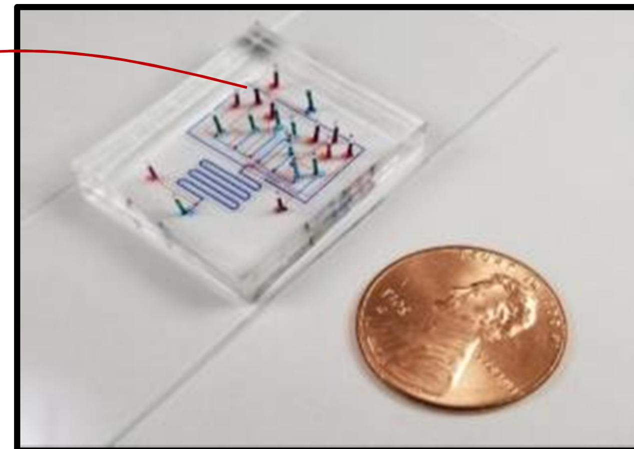
0 Cells/μL	0.6 Cells/μL	1.8 Cells/μL
5.5 Cells/μL	**16.6 Cells/μL**	**Limit of detection**
		50 Cells/mL



Connecting Microfluidic Culture and Analysis Modules

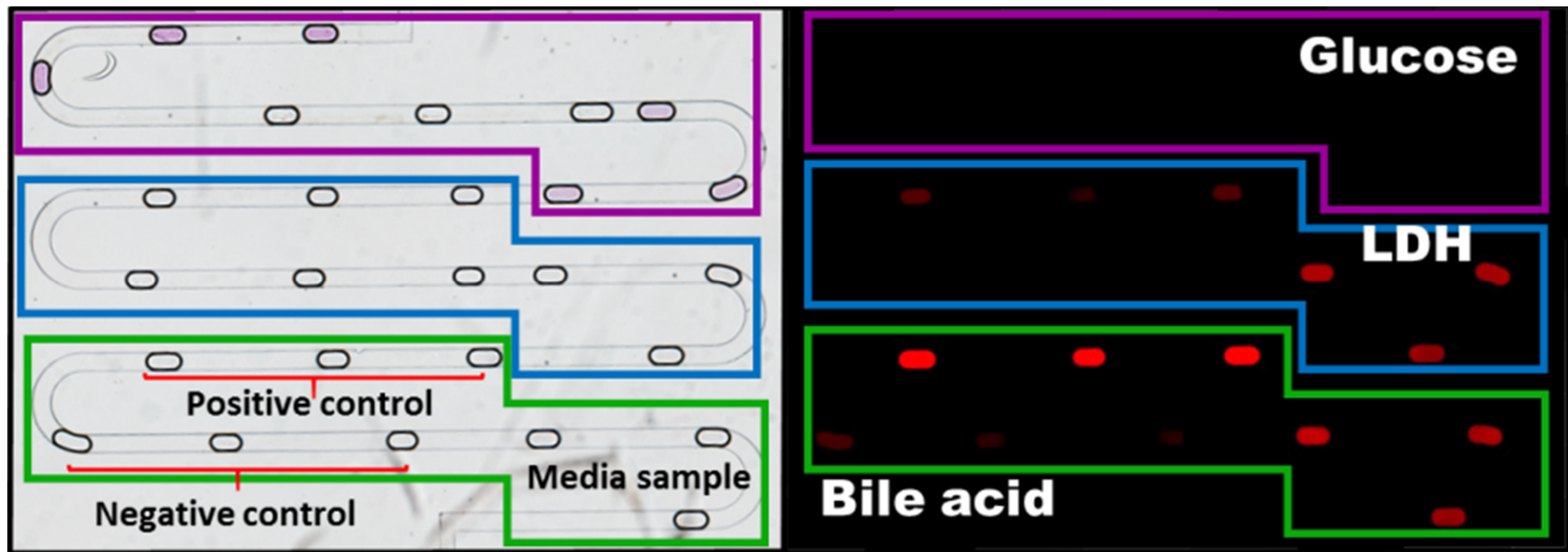
- Culture hepatocyte spheroids in the presence of palmitate (lipotoxic agent) for 4 days.
- Measure LDH (cytotoxicity), glucose and bile acid synthesis (hepatic function) daily using microfluidic analysis module.





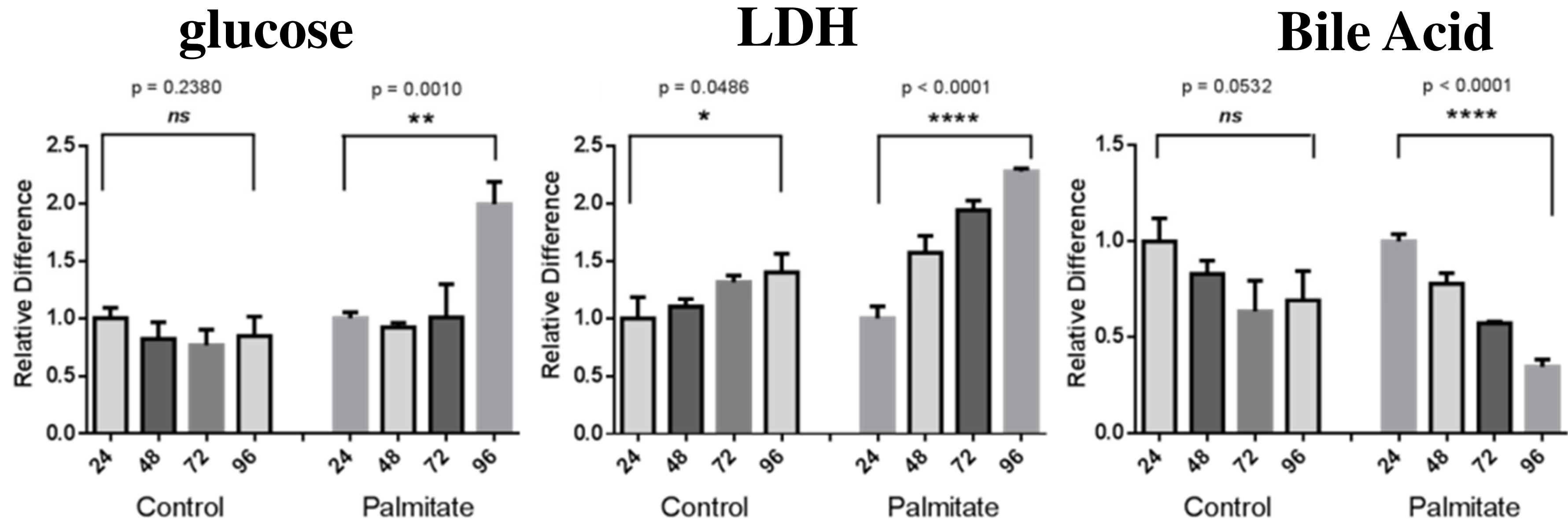
Analysis of Liver Cells in Microfluidic Devices

Each sensing session includes 3 types of assay of cells-conditioned media, positive and negative controls. Everything done in triplicate. ~100 nL of sample volume used.



Results of Hepatocyte Injury

- Cytotoxicity increase, reduction in bile acid synthesis, decrease in glucose consumption after 4 days of injury.



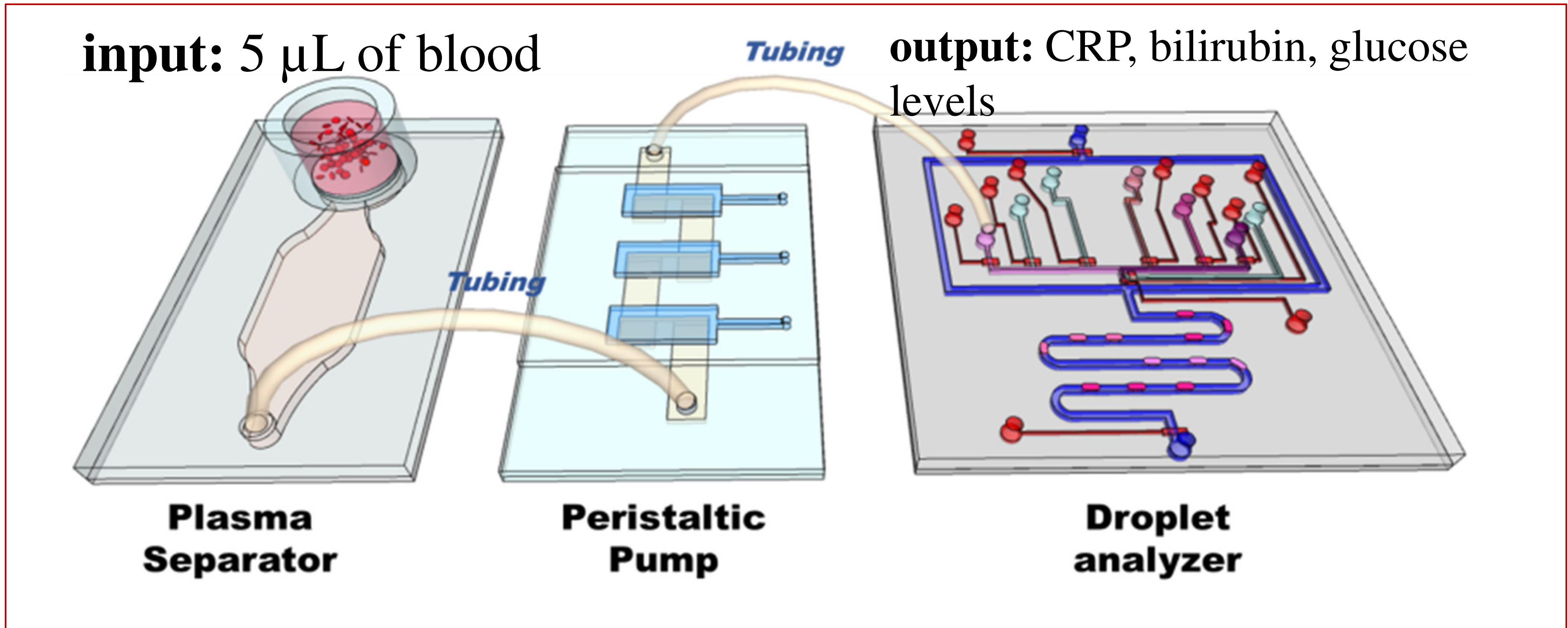
Summary II

- Microfluidic automation can be used to analyze small volumes of cell-conditioned media or physiological fluid.
- Multiple assays with built-in repeats, positive and negative control can be run based on ~2-5 μl of sample.
- Platform technology can be used with most commercial mix-and-read assay.
- Fluorescence and absorbance measurements can be made.
- 5-plex assay possible with current design. Multiplexing capabilities may be increased further.

Clinical Application – Neonatology

- Premature babies weigh as little as 1500g with total blood volume of 50 mL.
- Each blood draw requires 0.5 mL of blood, multiple blood draws can result in 20% blood loss. Anemia is a problem.
- C-reactive protein (CRP) and bilirubin are some of the most common biomarkers monitored in neonates. Commercial assays for both analytes are available.
- Teaming up with Dr. William Carey (neonatologist) to detect CRP and bilirubin in 5 μL of blood. 100x volume reduction.

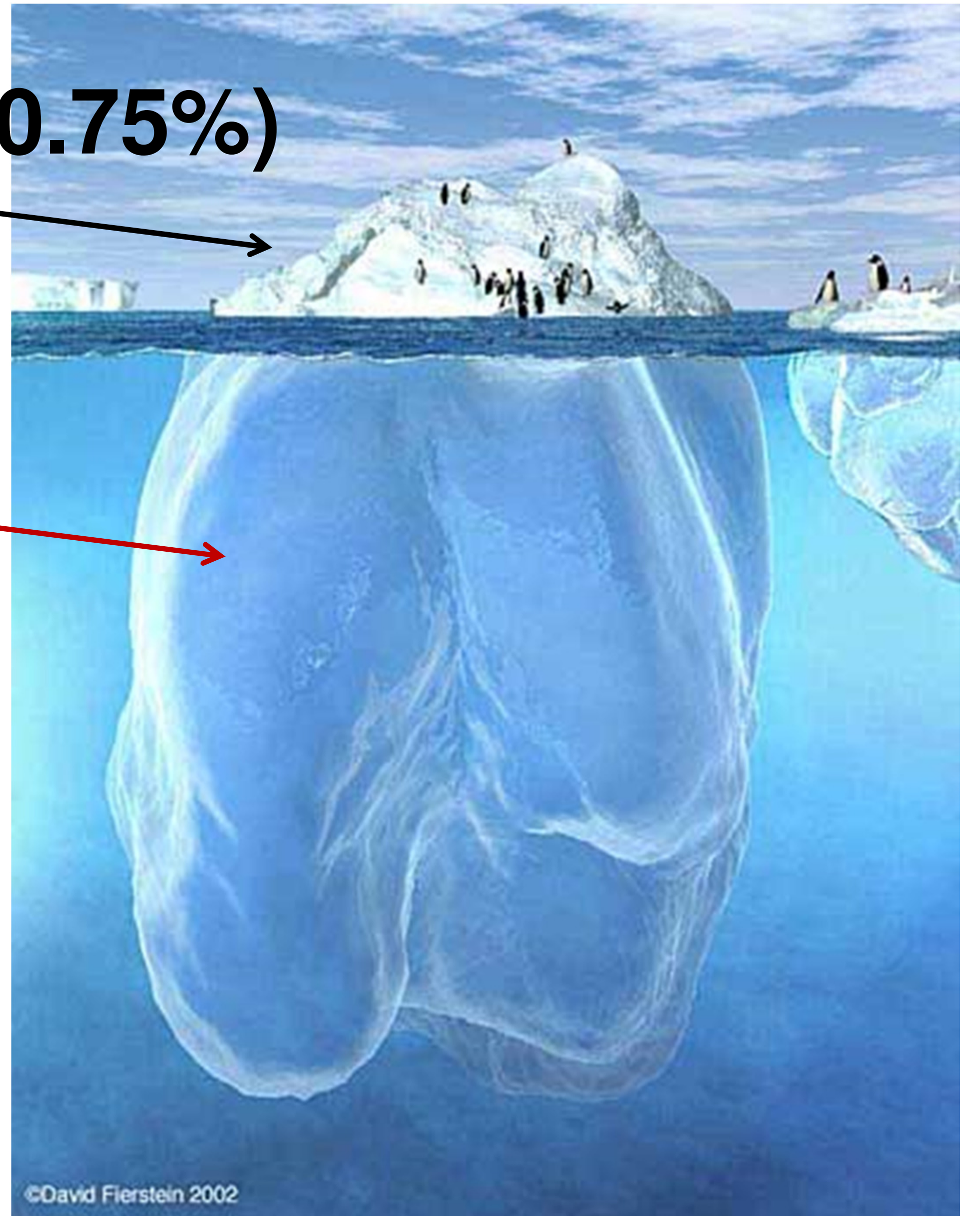
Blood Analysis in Neonates



Latent TB infections (LTBI)

15M active TB cases **(0.75%)**

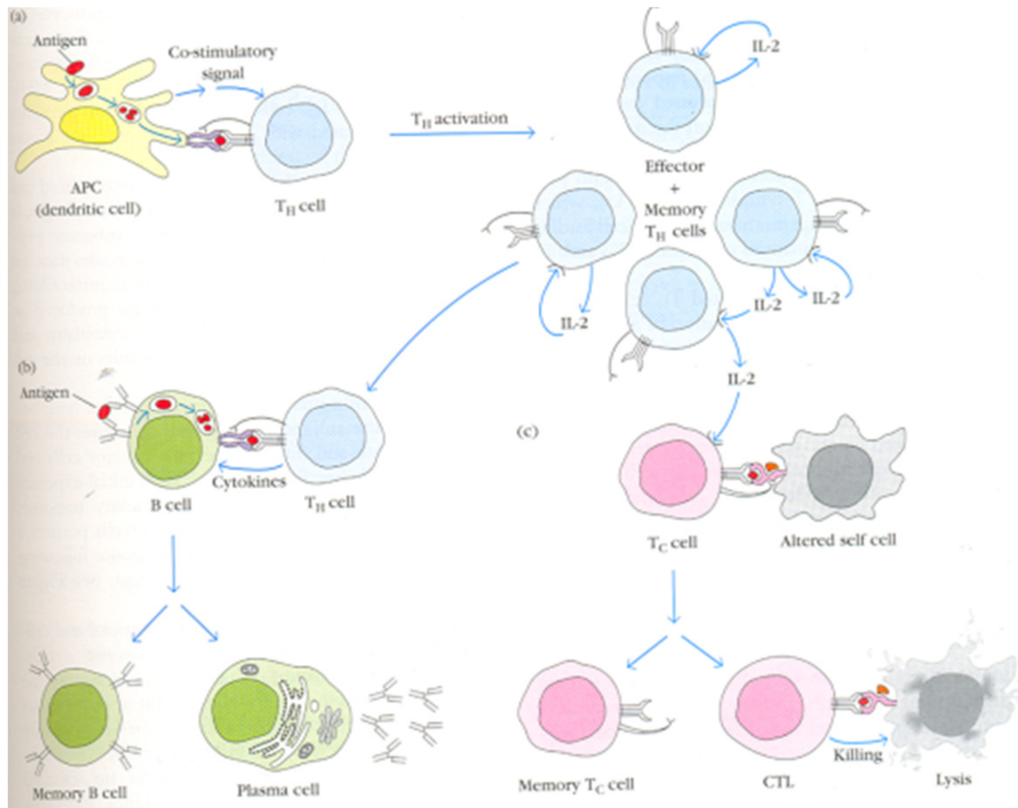
2.2B latently infected individuals

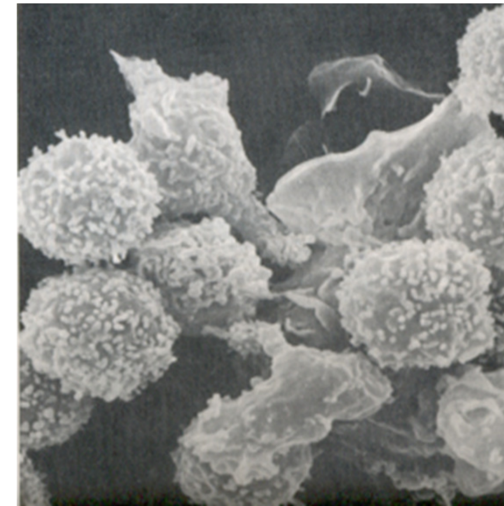


Why care about latent TB?
Anything that compromises immune system can trigger latent to active transition.

Cytokine Detection – IFN-γ Release Assay

- T-cell are part of adaptive immune system and release IFN-γ to stimulate other immune cells and mount immune response.
- **T-cells remember.** Once infection passes, T-cells specific to this infection are kept on file and can be recalled when infection reappears.
- **IFN-γ release assay (IGRA)** – stimulating immune cells with Tuberculosis antigens to detect latent TB



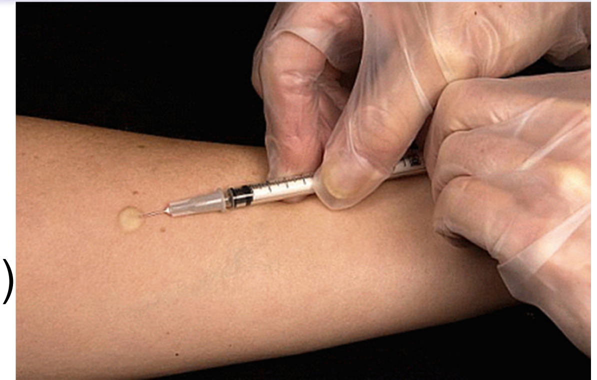


Macrophage surrounded by T-cells

Kuby Immunology, 4th edition

Current TB diagnosis

- Tuberculin skin test (TST)
  - — Qualitative bump – introduced in 1890
  - — Reduces risk of active disease by 60% (Pai *et al.*, 2004)
  - — Requires 48 – 72 h



- T-cell based interferon gamma release assays (IGRAs)
  - — Elispot (T SPOT-TB, Oxford Immunotec, UK)
  - — QuantiFERON-TB-Gold (QFT TB, 2001, Cellestis, Australia)
  - — Requires 16 – 24 h

- Nucleic acid amplification test (NAAT)
  - — GeneXpert (Cepheid, US)
  - — Sputum-based, requires 2 h. Active TB test.

Work Flow for IGRAs

- IGRAs are:
  - —more specific than TST
  - —cannot tell between latent/active TB
  - —cannot resolve latent TB spectrum for treatment
- Complicated lab based test. There remains a lack of a point-of-care test

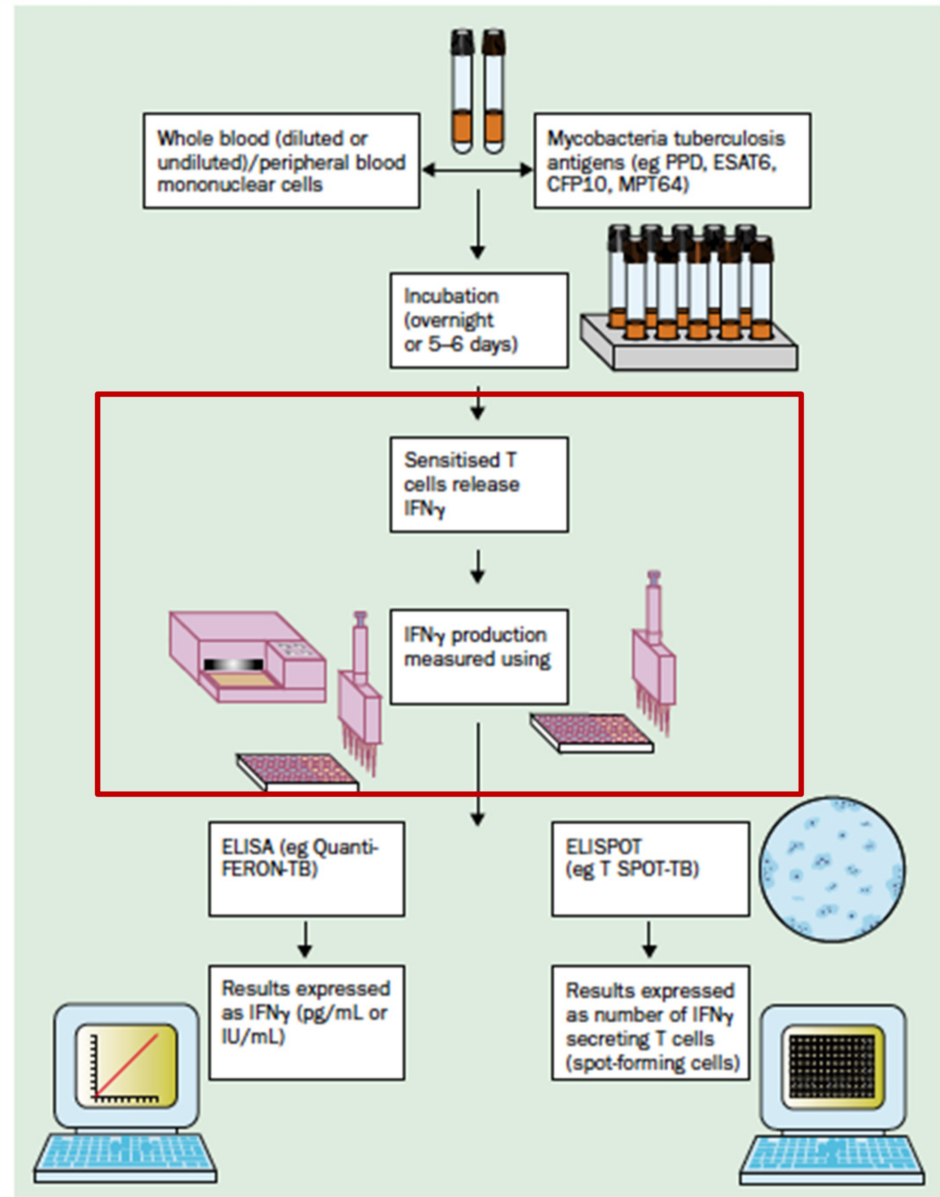


Figure 2. Overview of the interferon-γ (IFNγ) assay technology.

Pai *et al.*, 2004

IGRAs are Complex

8. Mix the conjugate and plasma samples/standards thoroughly using a microplate shaker for 1 minute.

11. Tap plates face down on absorbent towel to remove residual wash buffer. Add 100μL of Enzyme Substrate Solution to each well and mix thoroughly using a microplate shaker.

12. Cover each plate with a lid and incubate at room temperature (22°C ± 5°C) for 30 minutes.

    - Plates should not be exposed to direct sunlight during incubation.

13. Following the 30 minute incubation, add 50μL of Enzyme Stopping Solution to each well and mix.

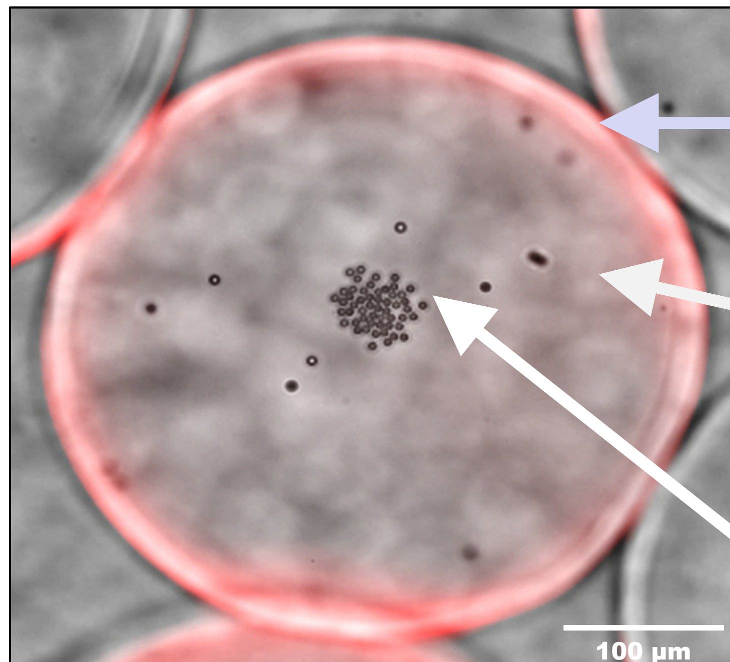
    - Enzyme Stopping Solution should be added to wells in the same order and at approximately the same speed as the substrate in step 11.

14. Measure the Optical Density (OD) of each well within 5 minutes of stopping the reaction using a microplate reader fitted with a 450nm filter and with a 620nm to 650nm reference filter. OD values are used to calculate results.

from Quantiferon Gold technical instructions

Microencapsulated Immunoassays

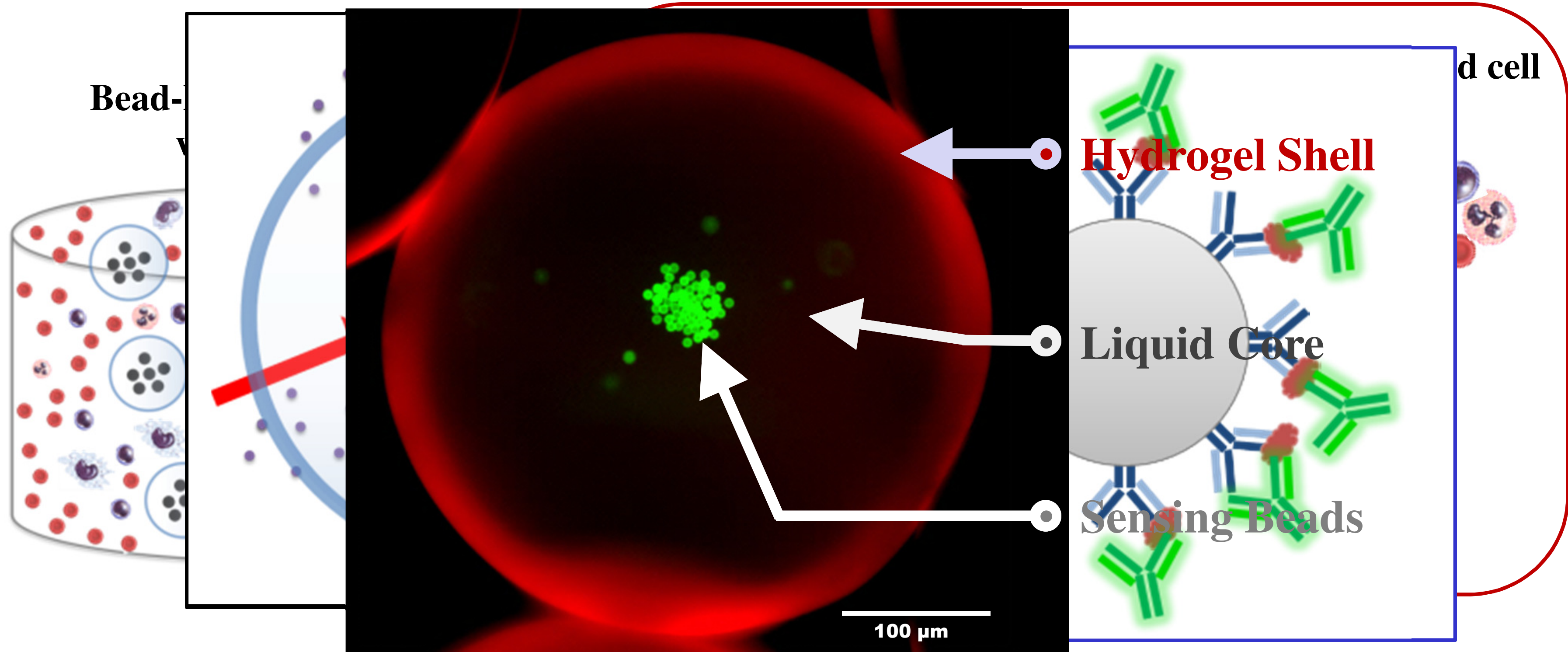
- Encapsulating antibody-functionalized beads to enable cytokine sampling in whole blood.
- No blood processing steps are required, beads are protected from blood cells by non-fouling hydrogel capsule.



- **Hydrogel Shell**
- **Liquid Core**
- **Sensing Beads**

Workflow of Microencapsulated Immunoassay

Advantage: capsules reside in whole blood, sample cytokines. No need for plasma separation.

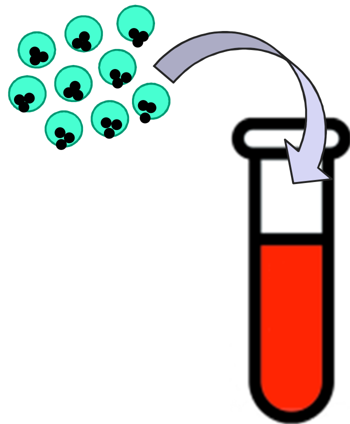


Rahimian … Revzin, ACS Sensors **2019**

Workflow of Microencapsulated Immunoassay

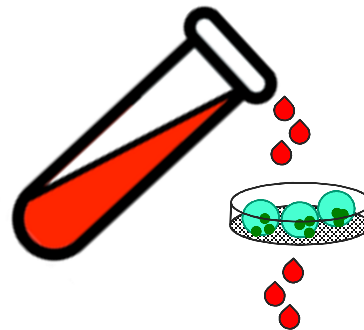
- ✓ No sample processing is required
- ✓ Works directly with whole blood specimen

Bead laden Microcapsules

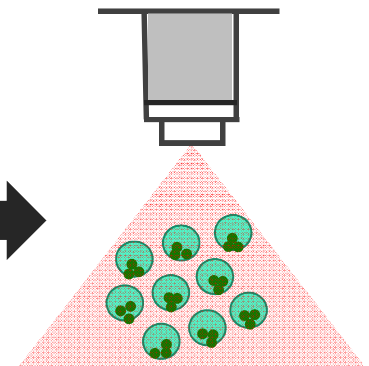


Blood sample

Incubation

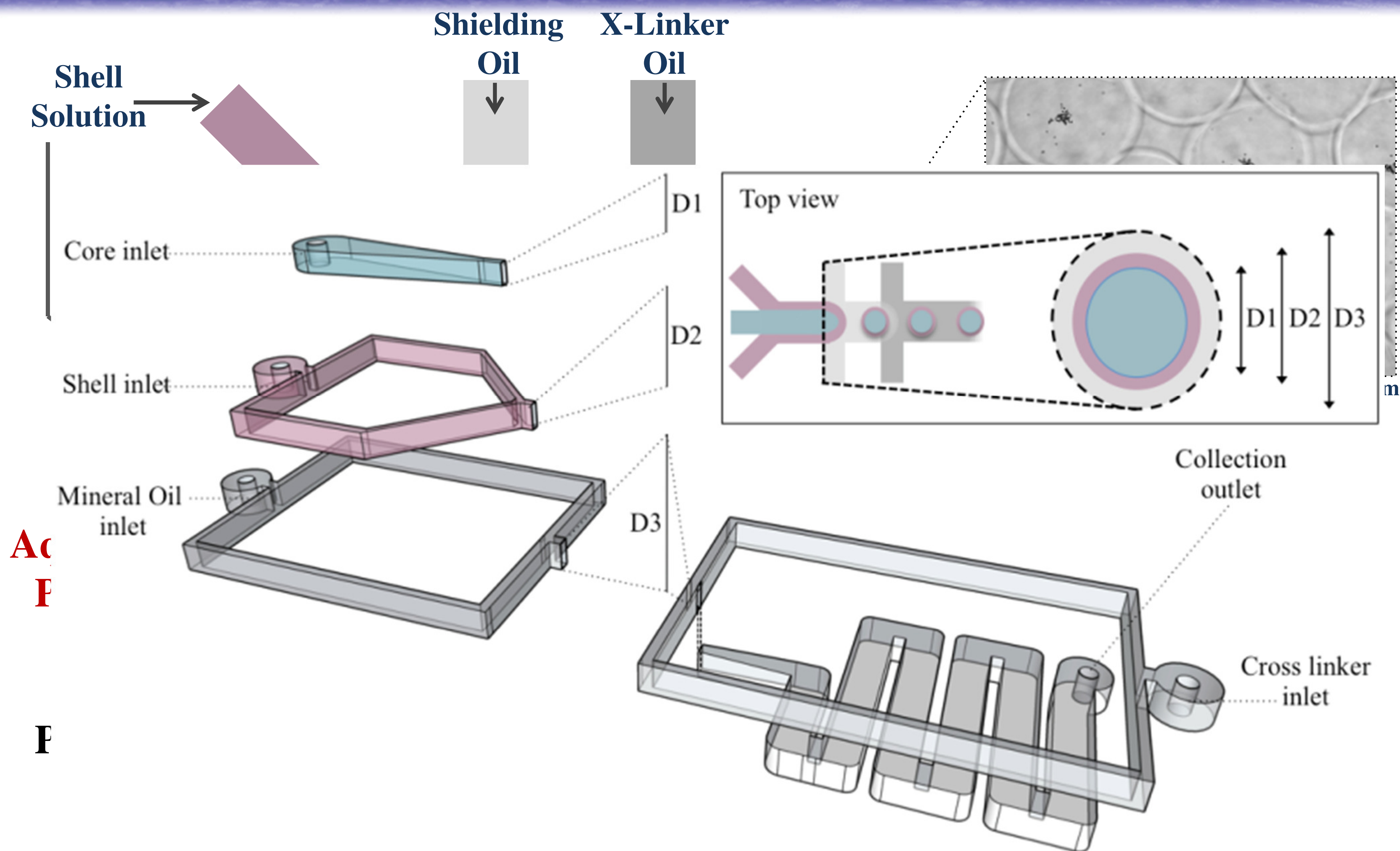


Separation & Fluorescent Labeling



Fluorescent Detection

Microfluidic Fabrication of Capsules



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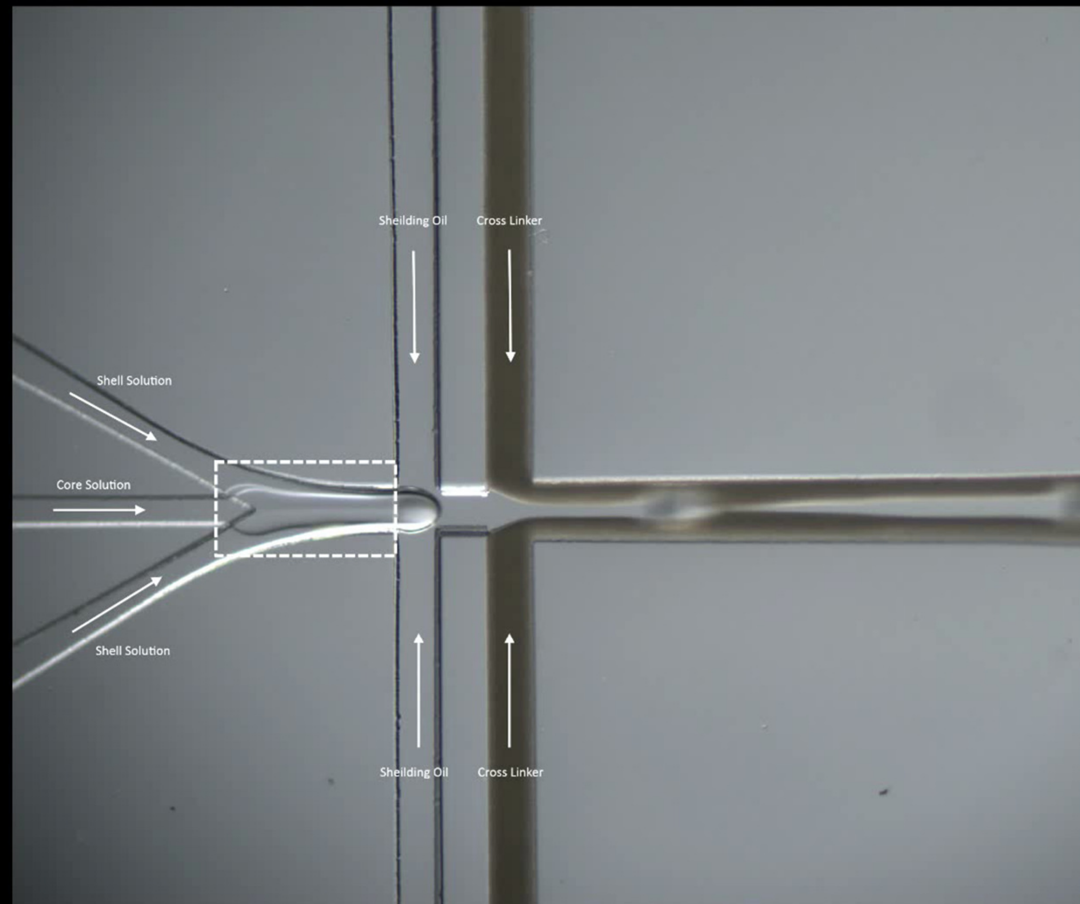
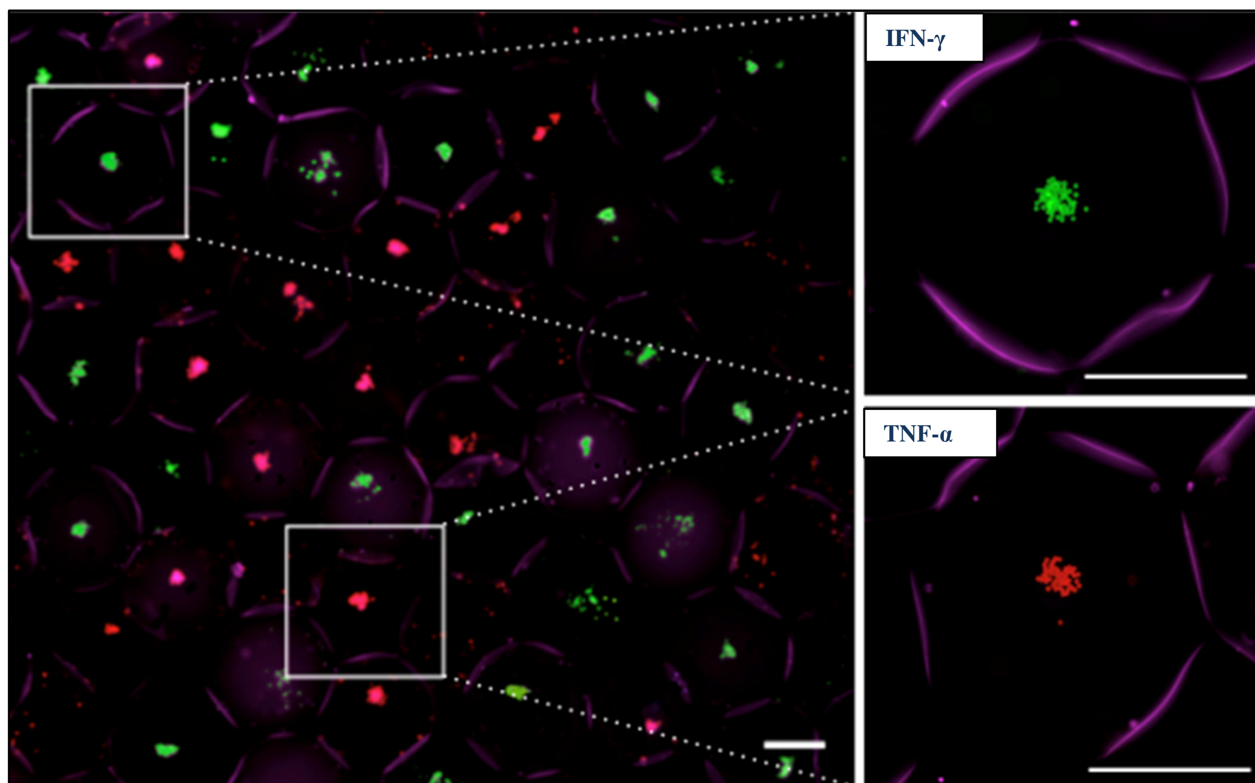
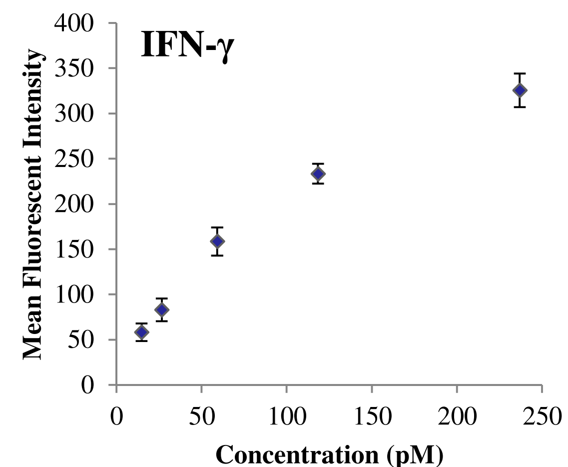
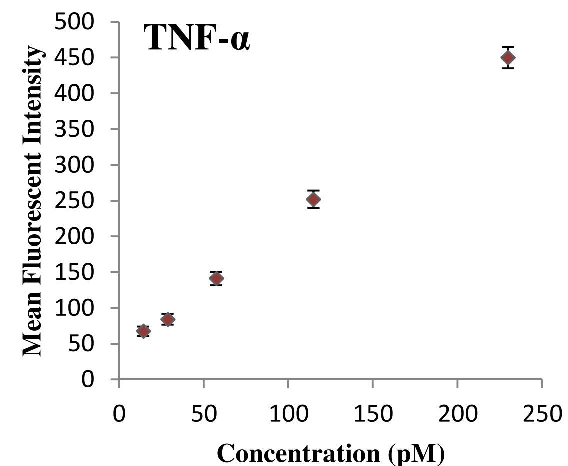
Microfluidic Fabrication of Capsules



Multiplexed Detection of TNF-α and IFN-γ







- The microcapsules were challenged with different concentrations of recombinant IFN-γ and TNF-α
- LOD for both targets: ≈ 14 pM

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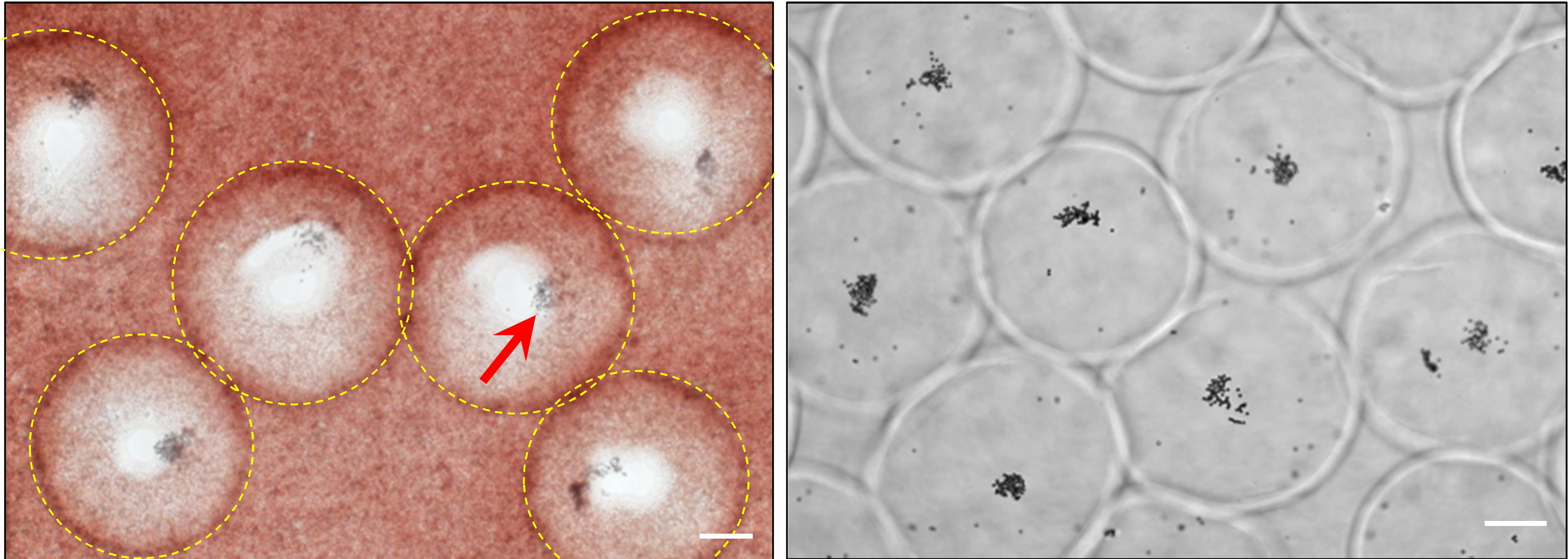
Microcapsules Are Not Fouled in Blood

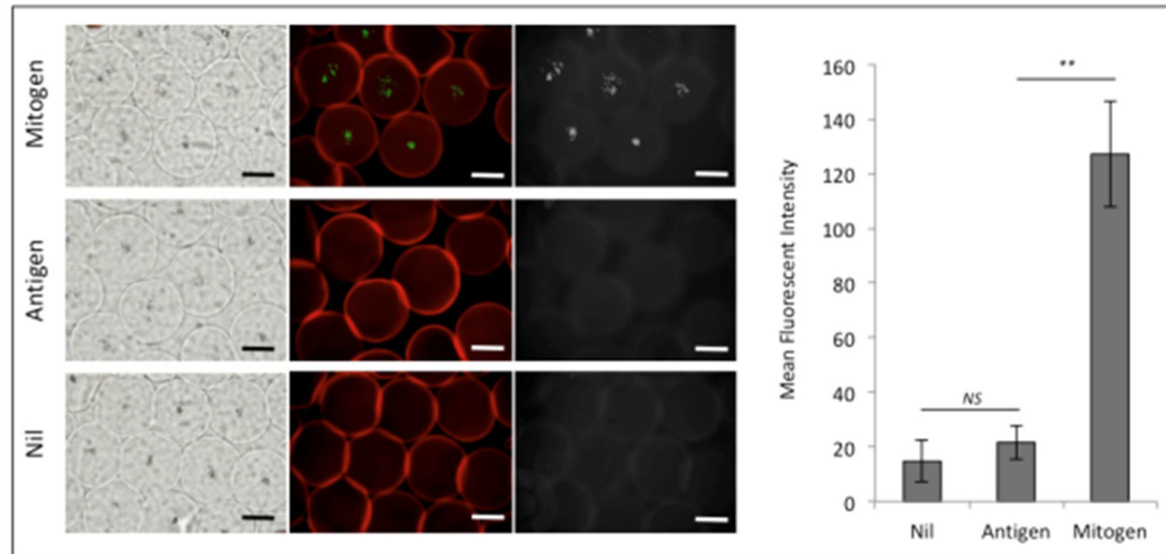


- The capsules show no cell attachment after <u>48 hours</u> incubation in human blood sample – Red arrow points to the encapsulated sensing beads

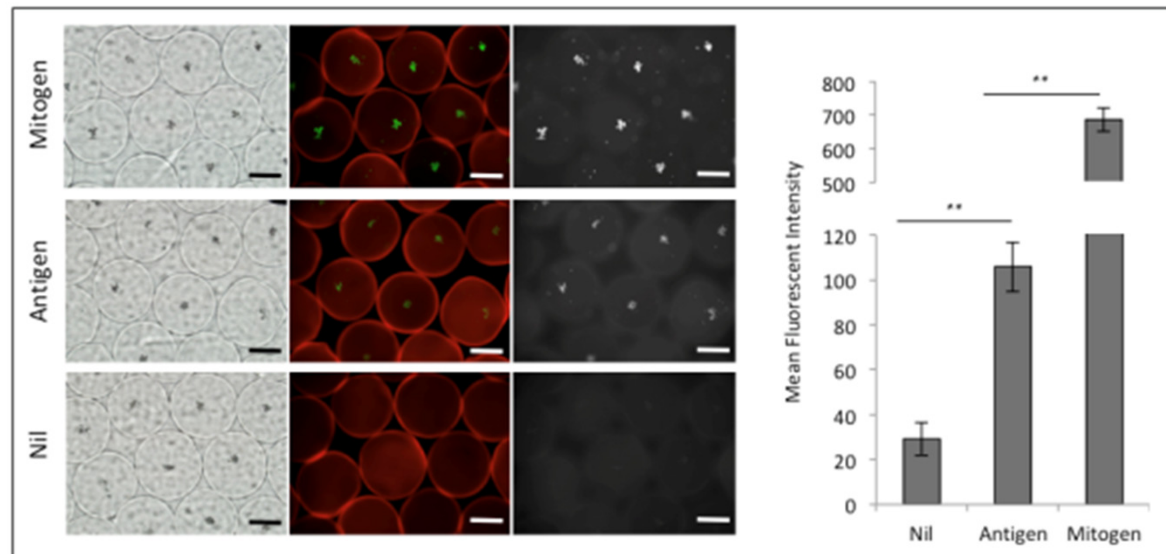
Detection of LTBI in Patient Samples

In collaboration with Dr. Patricio Escalante, infectious diseases / pulmonology

An Example of a Negative Test Result

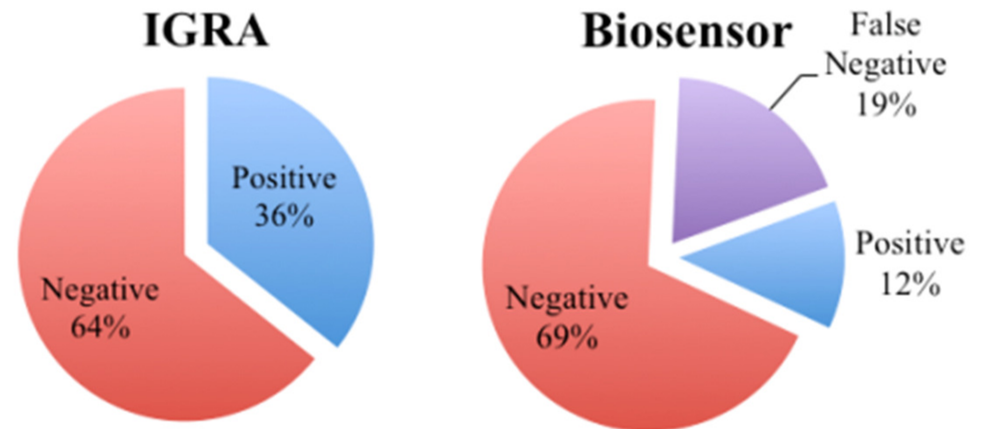


An Example of a Positive Test Result



QuantiFeron vs. Microcapsule Immunoassay

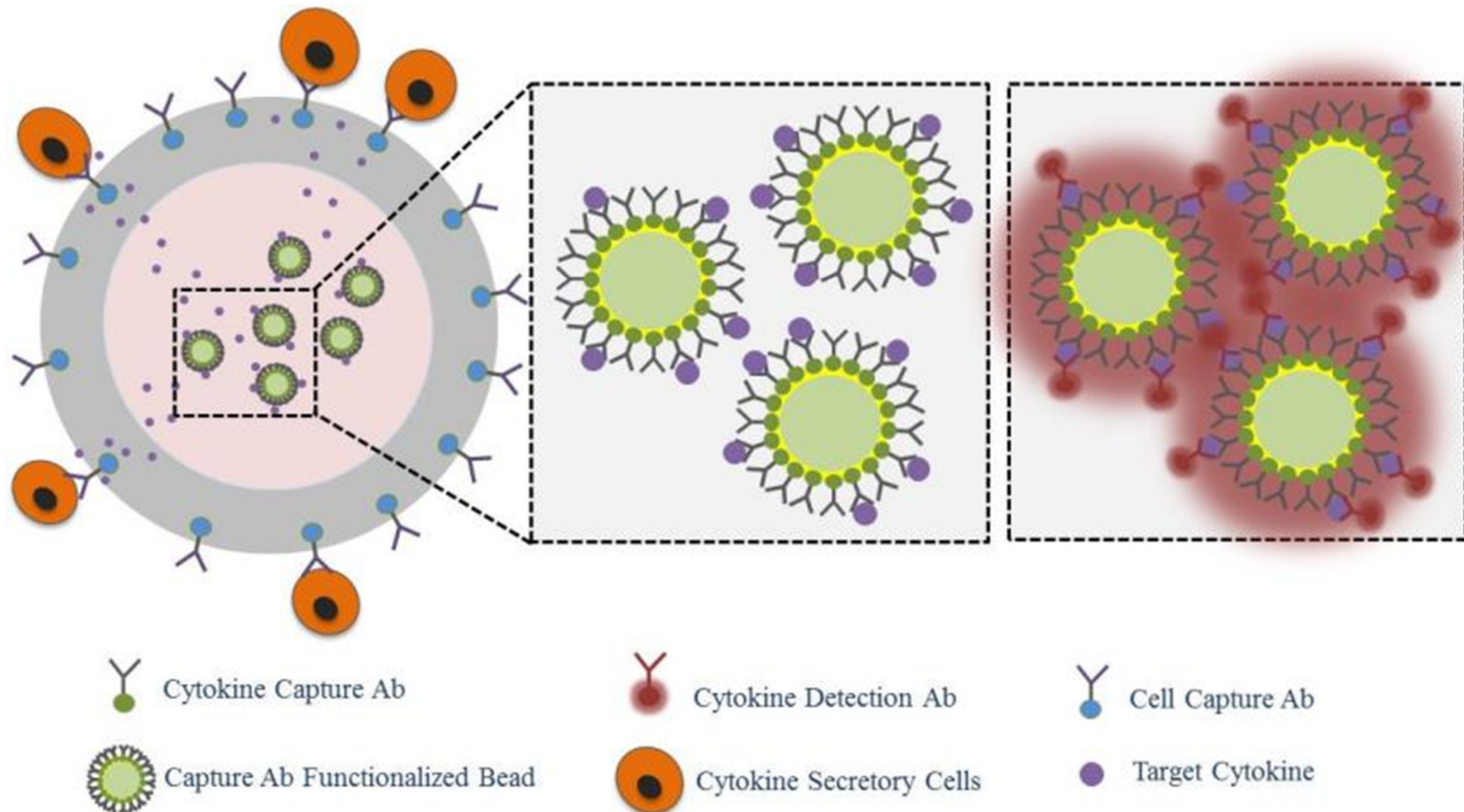
Sample ID	IGRA	Biosensor
1-TB253	Negative	Negative
2-TB254	Positive	Negative
3-TB255	Negative	Negative
4-TB256	Positive	Positive
5-TB257	Negative	Negative
6-TB258	Negative	Negative
7-TB259	Negative	Negative
8-TB260	Negative	Negative
9-TB261	Negative	Negative
10-TB262	Positive	Negative
11-TB264	Negative	Negative
12-TB265	Negative	Negative
13-TB266	Positive	Intermediate
14-TB267	Positive	Positive



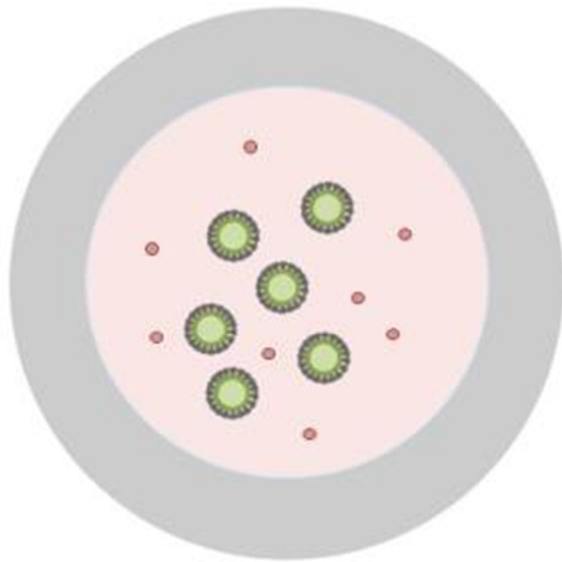
- Tested 14 patient samples. 80% agreement with IGRA.
- **Problem:** insufficient sensitivity of the capsule assay resulted in false negatives. 17 pg/ml threshold for positive IGRA.
- **Solution:** decrease the number of capsules from 100 to 20 capsules per mL. Microcapsule limit of detection decreased to 15 pg/ml.

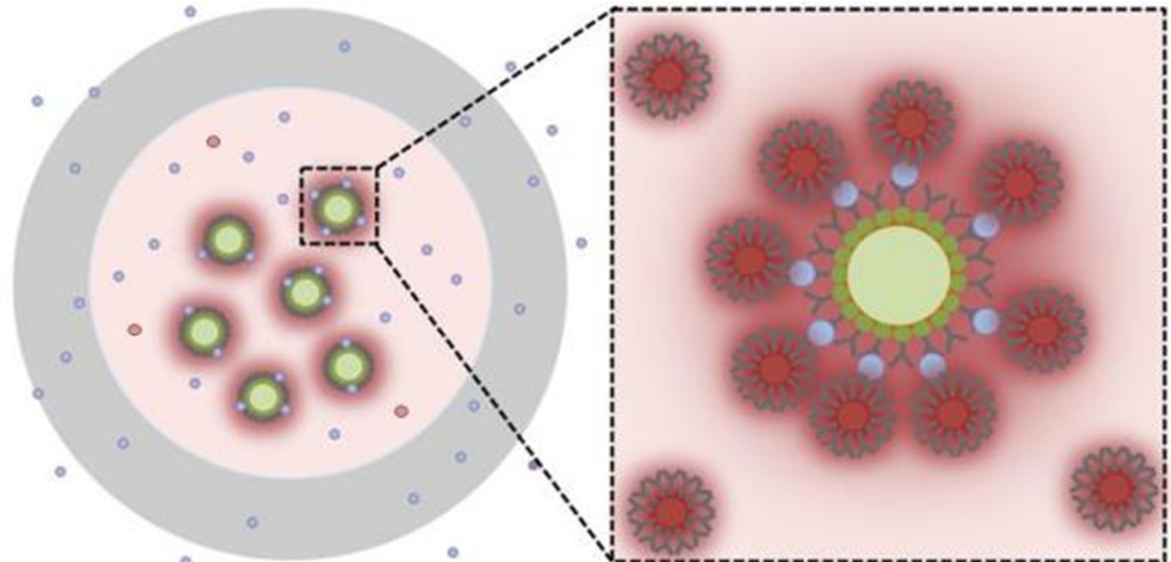
Evolution of Microcapsule Immunoassays

Concepts that we are interested in implementing going forward–
leukocyte capture and cytokine detection with the same capsule.
Increasing sensitivity of the assay by placing cells close to sensors.



One-Step Immunoassay – Eliminating Labeling Steps





ABSENCE
of the *Target Analyte*

PRESENCE
of the *Target Analyte*

Target Analyte

Detection Bead

Capture Bead

Summary III

- Developed encapsulated immunoassay for detection of IFN-γ and TNF-α. Easy to expand to other cytokines.
- Immunosensors remain functional after 48h incubation in whole blood.
- Demonstrated proof-of-concept detection of IFN-γ from TB patients.
- Looking for ways achieve more facile, single-step in-capsule detection of cytokines.

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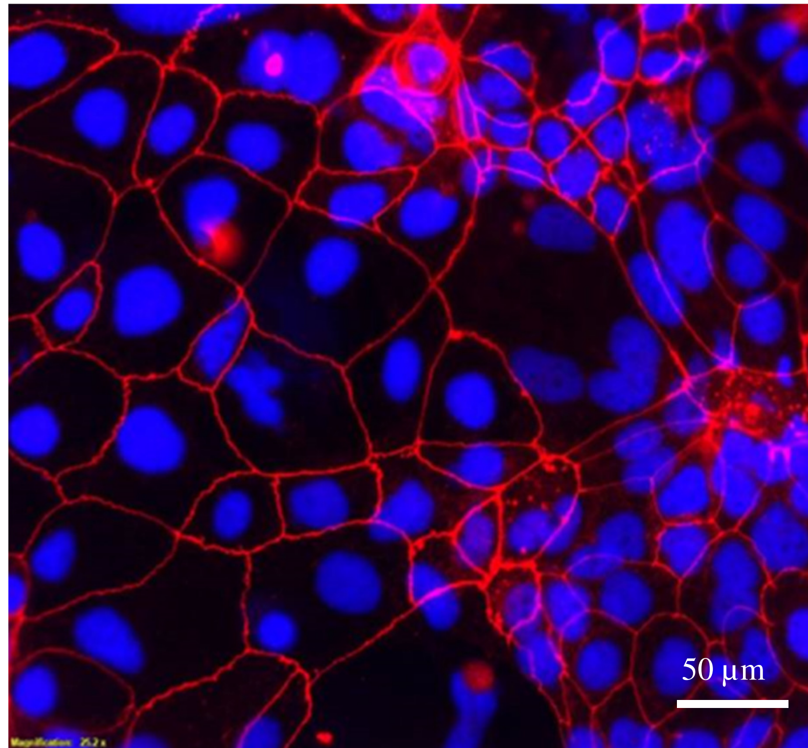
Function of Cells in Microfluidic Devices

- Need to ensure that microphysiological systems are physiological.
- Hepatocytes maintain function in microfluidic devices for several weeks in devices but what about polarization?



Microfluidic Devices with Built-in Microvalves