

Combining Microfluidics and Nanosensors for Future Diagnostics

Megan Yi-Ping Ho 何亦平

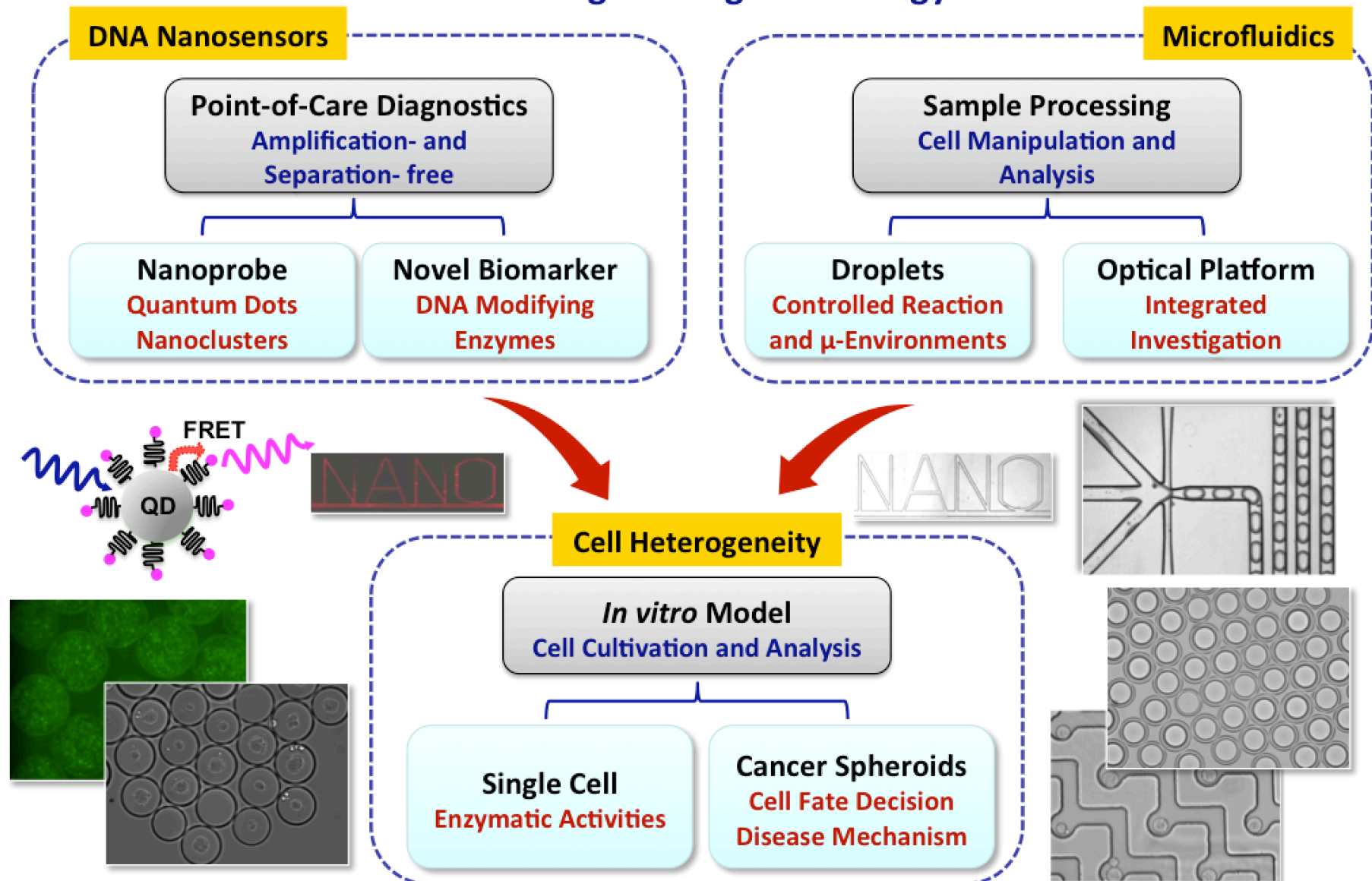
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PhD, Mechanical Engineering, The Johns Hopkins University, USA
MS, Mechanical Engineering, Tsing-Hua University, Taiwan
BS, Mechanical Engineering, Tsing-Hua University, Taiwan

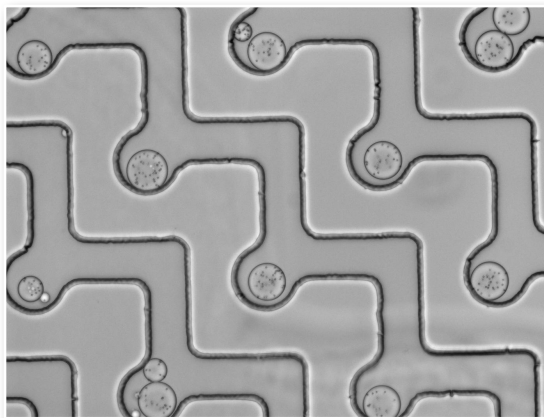
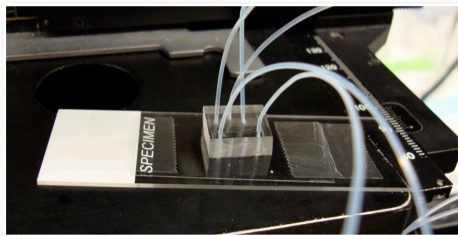
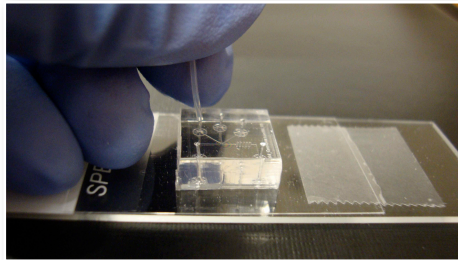
Where the Engineering and Biology Meet



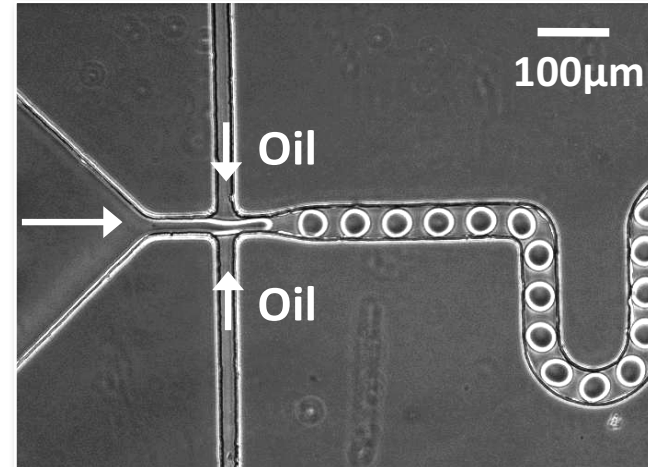
Lab-on-a-Chip: Making the Small

■ Development and Fabrication of Microfluidics

Overview of the Chip

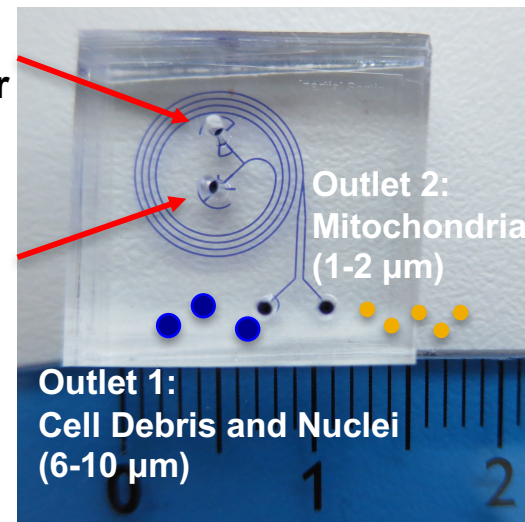


Aqueous
Phases



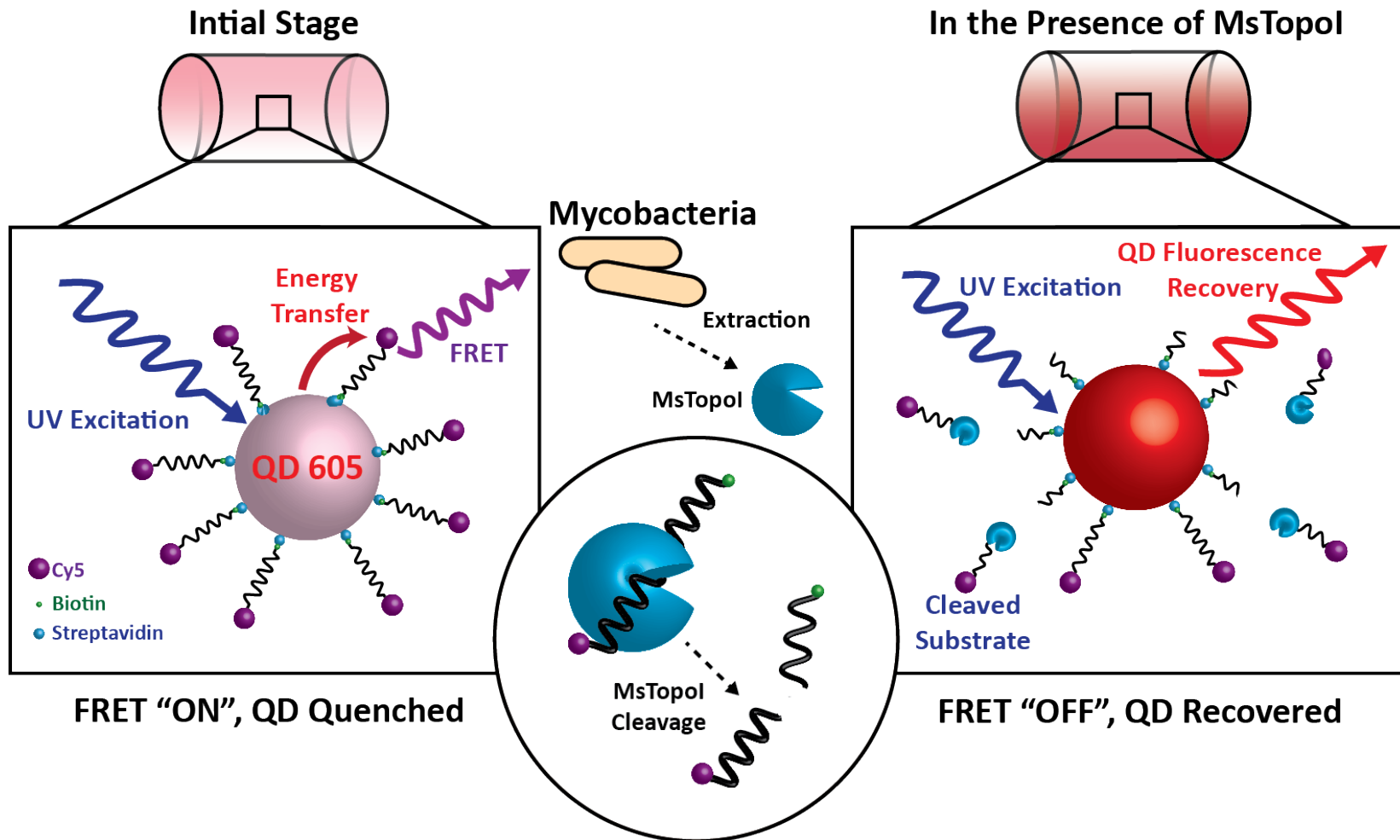
Inlet 1:
Sheath Buffer

Inlet 2:
Cell Lysate



Molecular Imaging: Reading from the Small

■ Biosensing *via* Nanosensors



M. L. Jepsen, C. Harmsen, A. Godbole, V. Nagaraja, B. R. Knudsen, Y. P. Ho*, *Nanoscale*, 8(1): 358-364, 2016

Introduction

Biomarkers

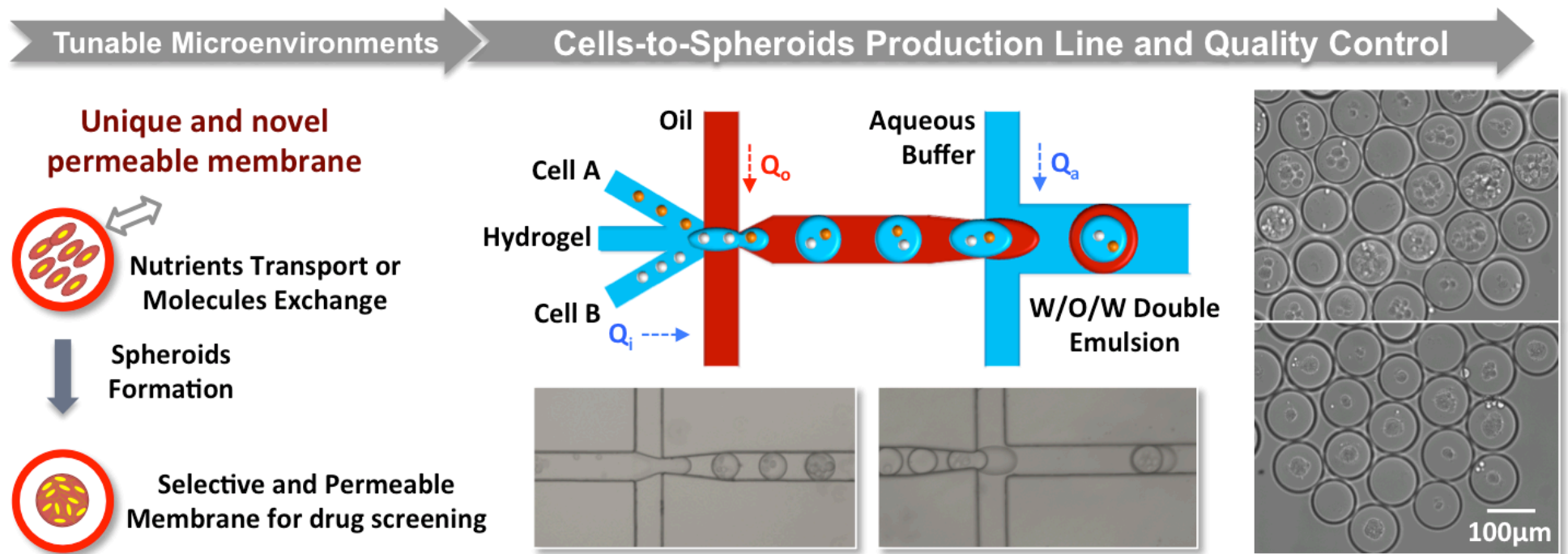
Organelles

3D Culture

Conclusion

Cell/Organelles Manipulation and Analysis: Learning from the Small

- Microfluidics based double-emulsion as a new cultivation vessel



Towards Future Disease Diagnostics

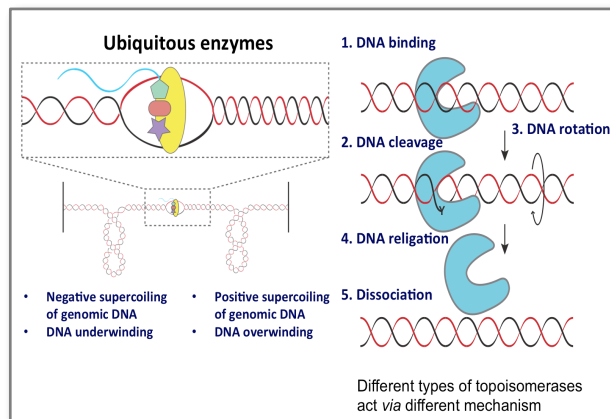
➤ Combining Microfluidics and DNA Nanosensors

Biomarkers

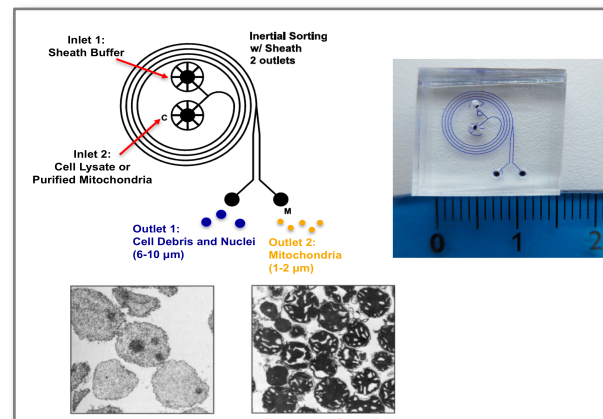
Sub-Cellular
Organelles

3D Culture

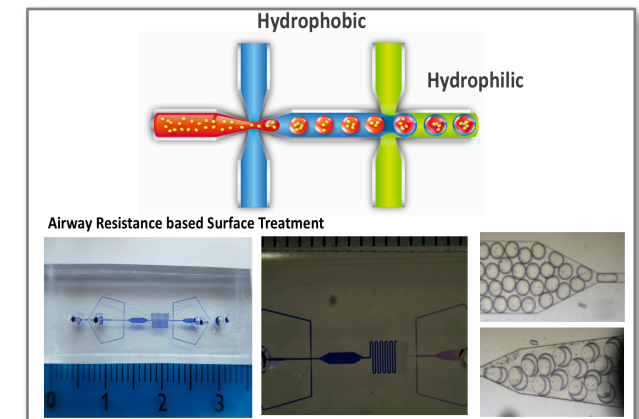
DNA Nanosensors



Extraction and Isolation of Mitochondria



Multicellular Spheroids in Double Emulsions



Introduction

Biomarkers

Organelles

3D Culture

Conclusion

Diagnosics of Infectious Diseases



■ A New Class of Biomarker

Pathogens

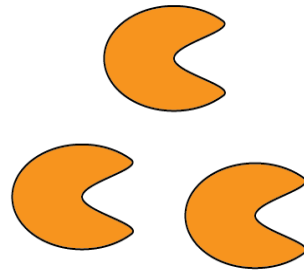


Pathogens specific essential enzymes



Only viable pathogens are detected

DNA
Modifying
Enzymes

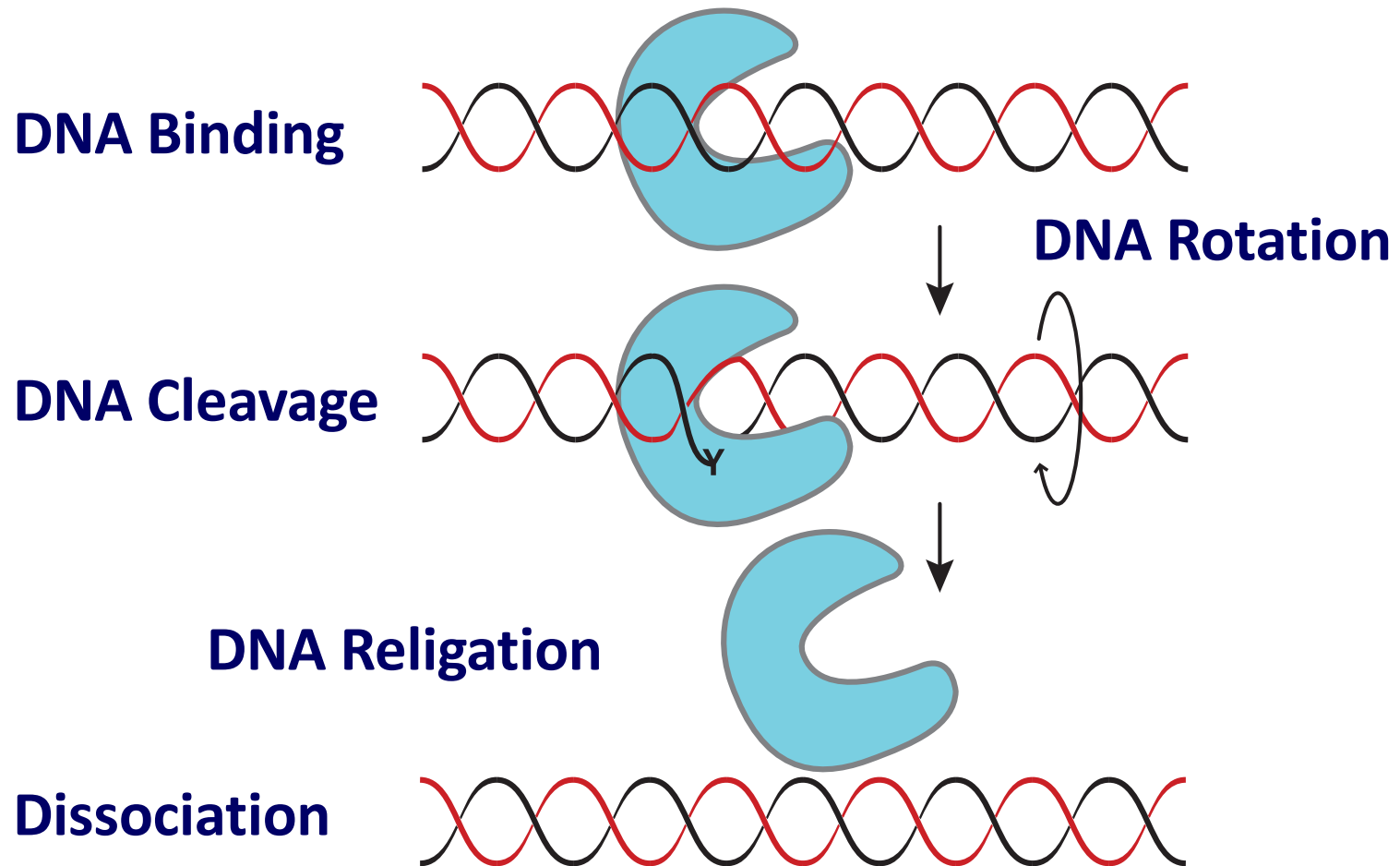


Direct measurement of enzymatic activities in crude samples

Novelty: Intrinsic Amplification, Improved Detection Sensitivity, Live-Pathogen Detection

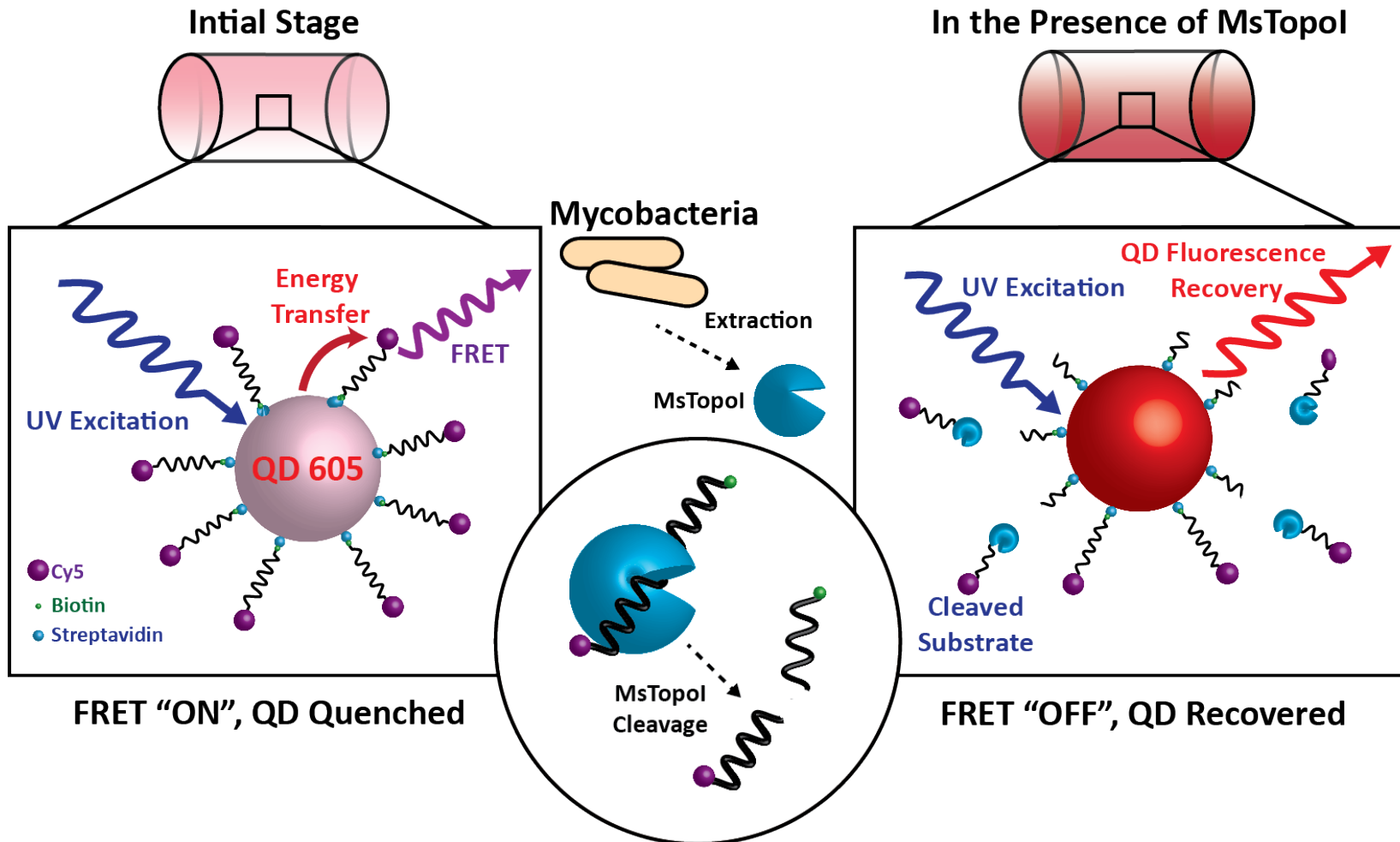
Why Activity of DNA Modifying Enzymes?

- Essential for the Organisms
- Intrinsic Amplification



QD-Nanosensor for Rapid Detection of TB

■ Detection *via* the cleavage activity



M. L. Jepsen, C. Harmsen, A. Godbole, V. Nagaraja, B. R Knudsen, Y. P. Ho*, *Nanoscale*, 8(1): 358-364, 2016

Introduction

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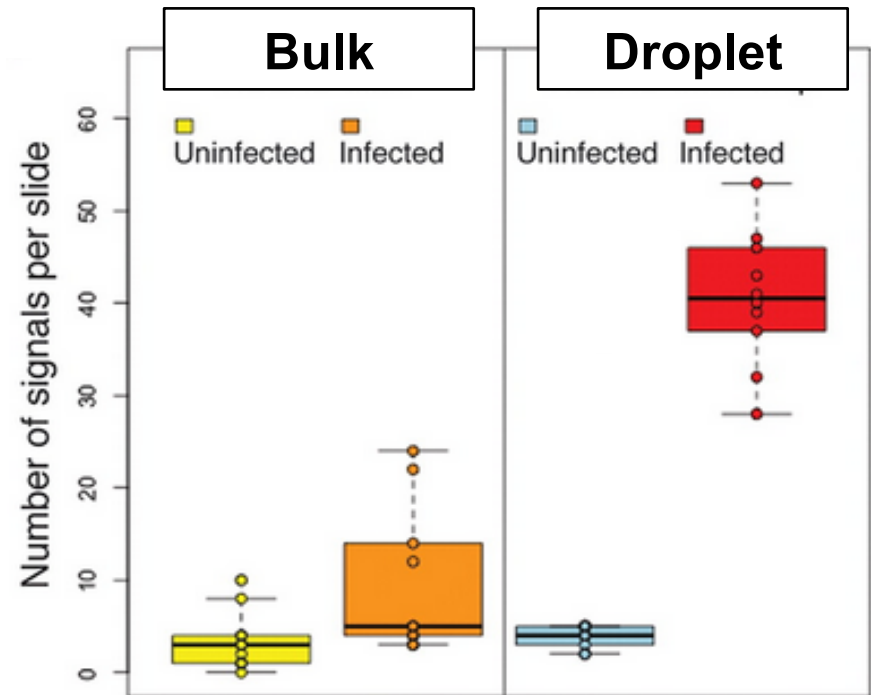
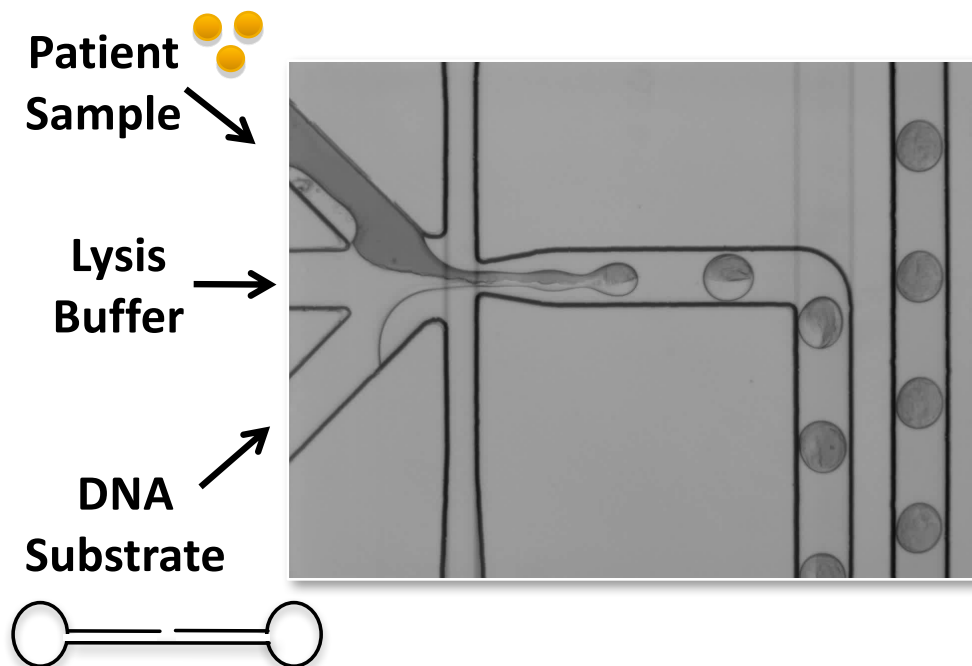
Organelles

3D Culture

Conclusion

Small Droplet, Substantial Improvement

Sample Processing by Droplet Microfluidics

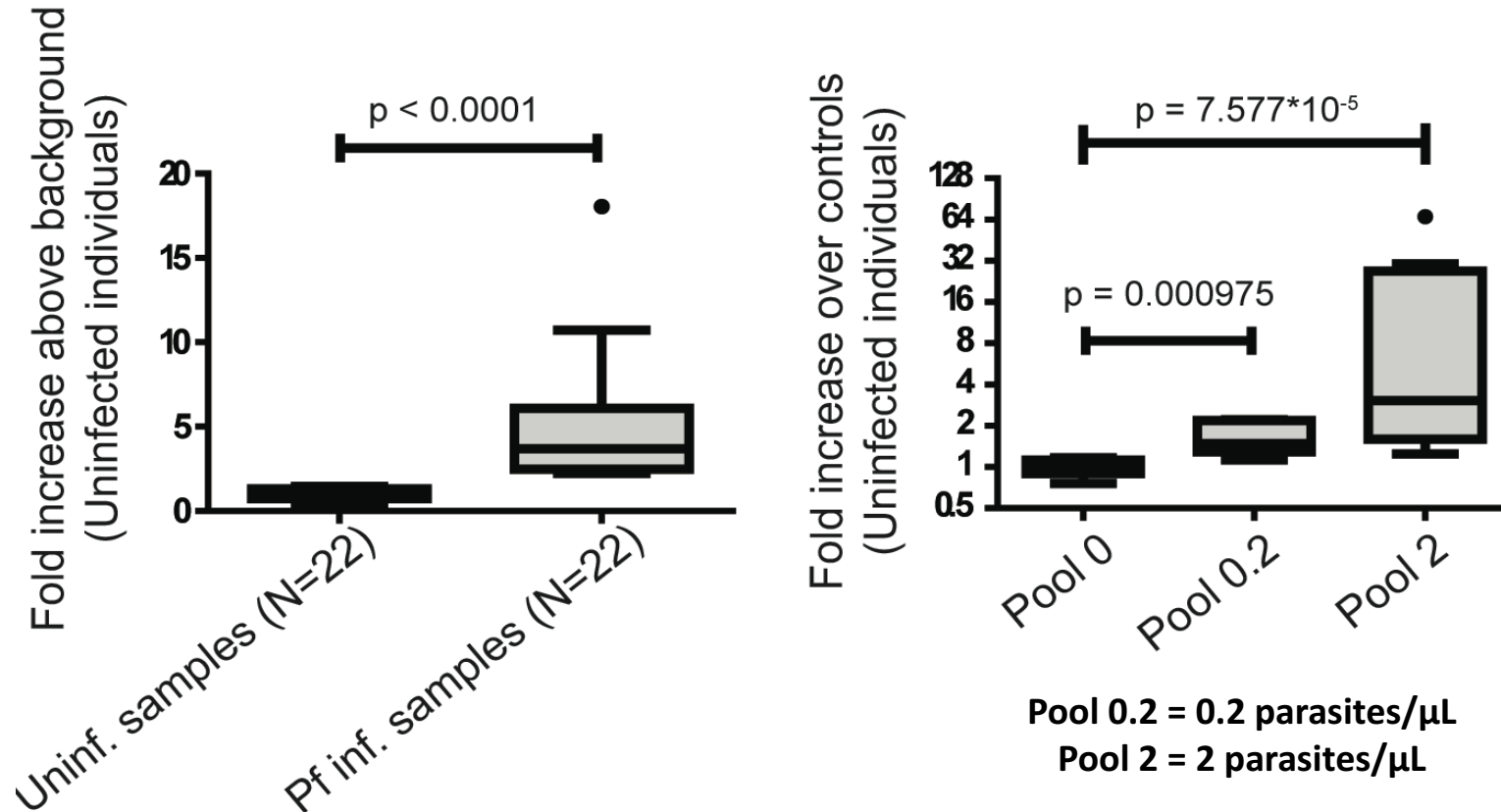


S. Juul, *et al.*, *ACS Nano*, 6 (12): 10676-10683, 2012

- 20 μL blood or saliva patient sample
- Detection Sensitivity: 0.06 parasite/ μL

Improved Detection Sensitivity Enables Malaria Detection in Noninvasive Saliva Samples

Detecting Malaria in Saliva for POC



- Manual Operation of Droplet Generation and Extraction
- Colorimetric Readout (HRP)

Marianne S. Hede et. al., Scientific Reports 8 (1), 4122, 2018
(collaboration with Zymonostics and Medical Research Center in Gabon)

Promotion to the Clinic

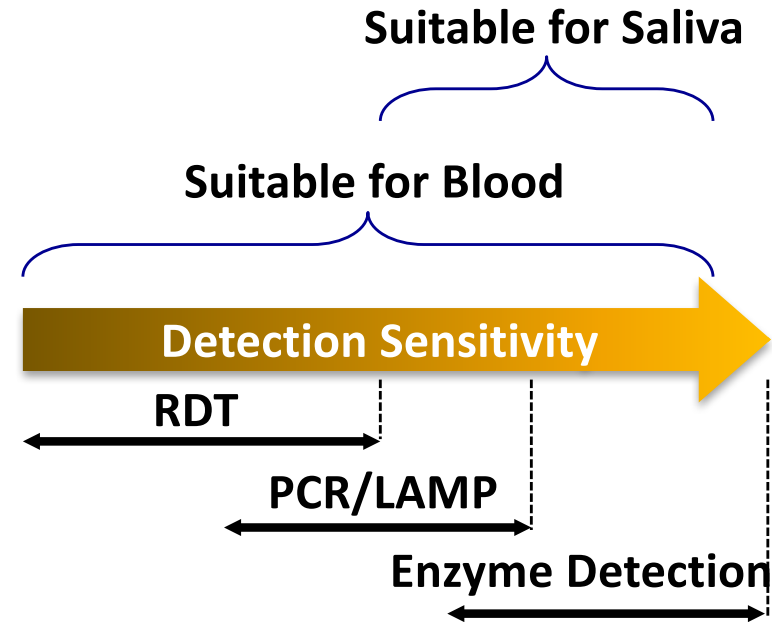
Zymonostics®



- Start-up 2013-2017
- Point-of-Care (POC) Diagnostics for Malaria



- Start-up 2018-
- Rapid Diagnostics for Food Pathogens (*E. Coli* and *Salmonella*)



Feature	vPCR	Traditional method	Rapid method (PCR)
Price per test	€2	€3	€6
Hours to result	8	120	24-30
Targeting	Live bacteria	Live bacteria	Live or dead bacteria

Introduction

Biomarkers

Organelles

3D Culture

Conclusion

Towards Future Disease Diagnostics

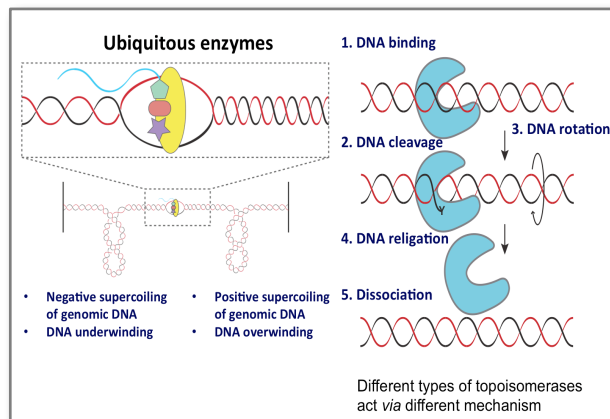
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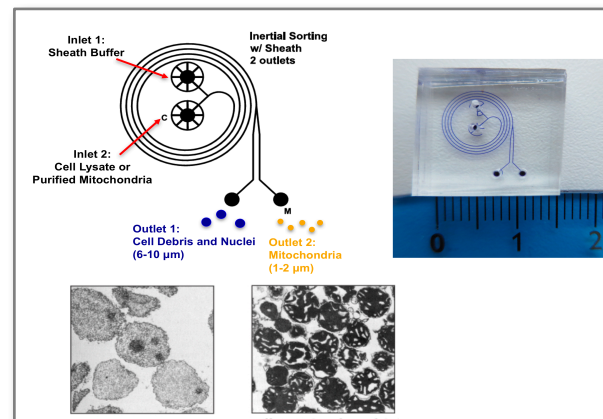
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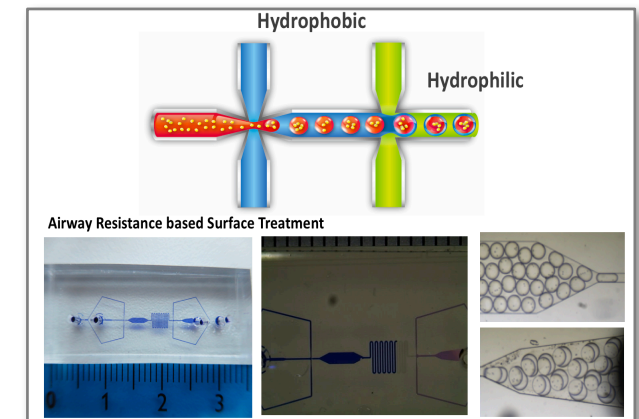
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Extraction and Isolation of Mitochondria



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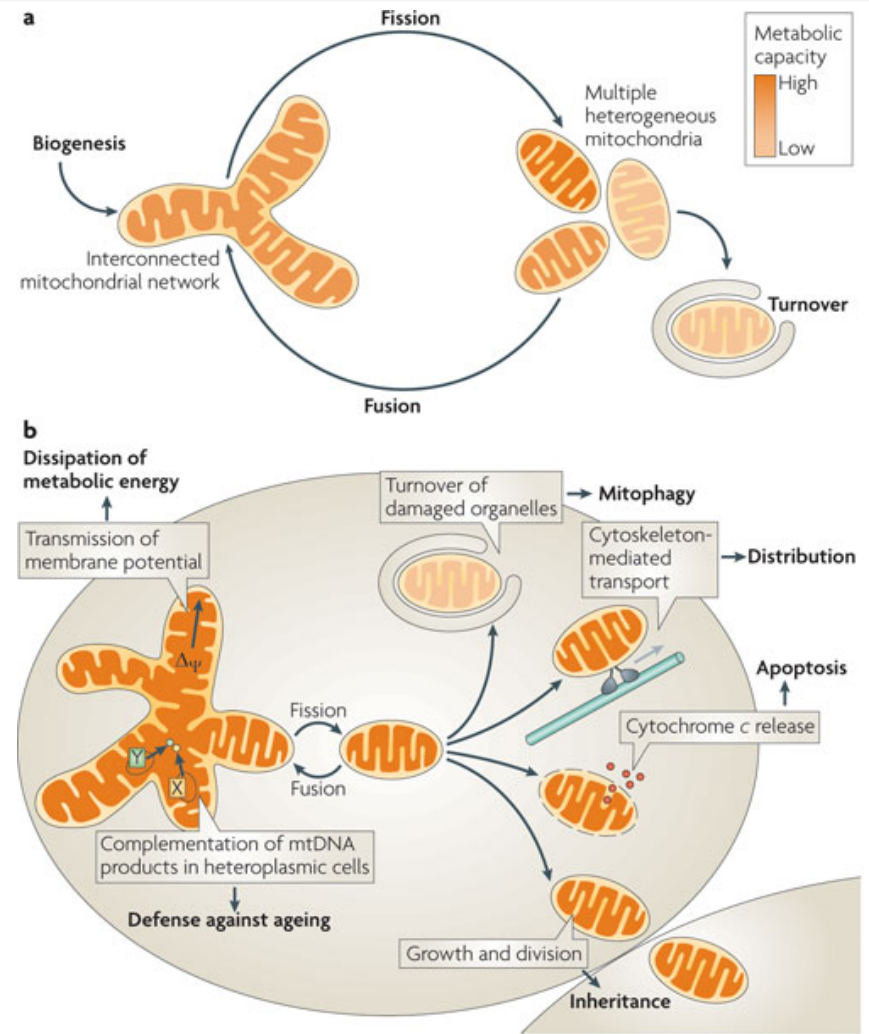
Organelles

3D Culture

Conclusion

Importance of Mitochondria

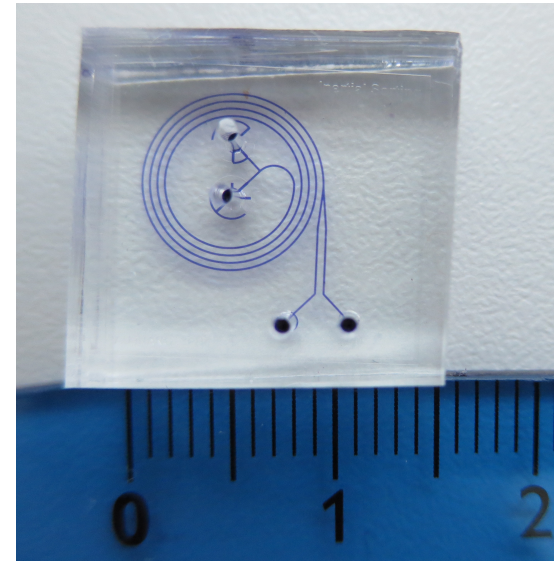
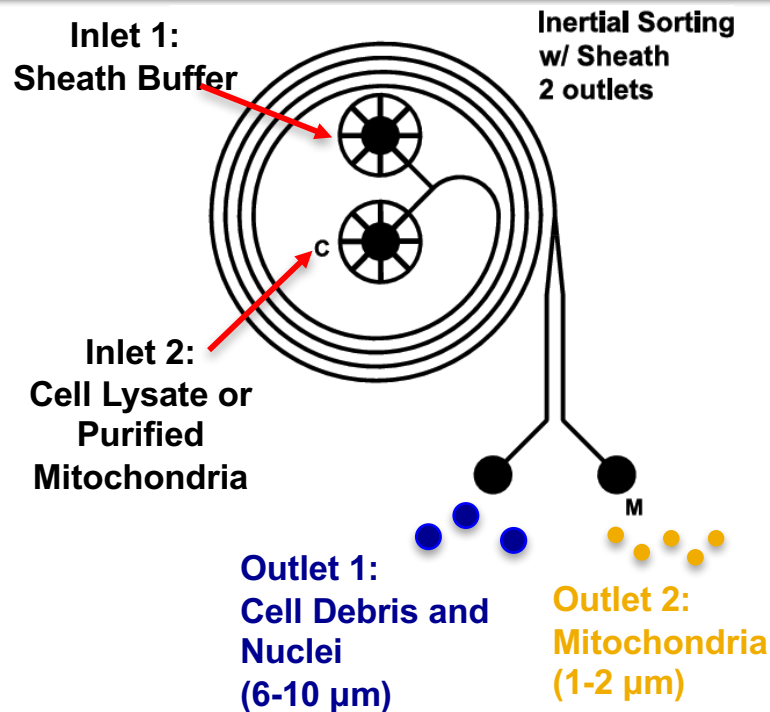
- Mitochondria are the powerhouses of the cells
- Mitochondria make decisions of cell fates:
 - Response to stress
 - ROS signaling
 - Apoptosis/necrosis
- Defective mitochondria have been linked to:
 - Cancers
 - Premature aging syndromes
 - Diabetes
 - Neurodegenerative disorders



Nature Reviews | [Molecular Cell Biology](#)

Ref: Westermann, B. (2010). *Nature Reviews Molecular Cell Biology*, 11(12), 872–884.

Isolating Mitochondria based on Inertia



Tesaro et al., RSC Advances 7 (38), 23735-23741, 2017

- Completely new concept
- The benefits of microscale sorting:
 - When to-be-sorted organelles are fragile (as opposed to centrifugation)
 - Small amount of precious/clinical samples (ex: cancer stem cells)
- The benefits of sorting by inertia: (v.s. IEF or antibodies-based microchip):
 - No pre-functionalization (ex: antibodies)
 - No external electric field (as required for the IEF)
 - Easy and robust operation (a pump and the chip)

Introduction

Biomarkers

Organelles

3D Culture

Conclusion

Innovations and Practical Significance

- Effective approach to isolate mitochondria from small scale (< 200 μ L) of biological crude sample (i.e. patient sample from the clinics)
- Applicable for other sub-cellular organelles
- Comparison with existing mitochondria isolation kits:

	This Study	Thermofisher	Abcam	Qiagen
Working Principle	Inertial Microfluidics	Differential Centrifugation	Differential Centrifugation	Density Gradient
Assay Time (Post-Lysis)	10 min	40 min	>30 min	>45 min
Required Cells (Total)	$10^2 - 10^4$	2×10^7	4×10^7	5×10^6
Bench Top Availability	Yes	Yes	Yes	Yes
Purity	High	High	High	High
Required Reagent Exchange	No	Yes	Yes	Yes
Price Per Isolation (HKD)	20	45	97	296

Introduction

Biomarkers

Organelles

3D Culture

Conclusion

Towards Future Disease Diagnostics

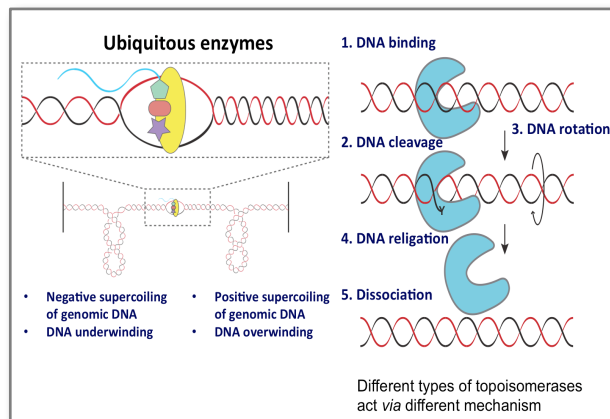
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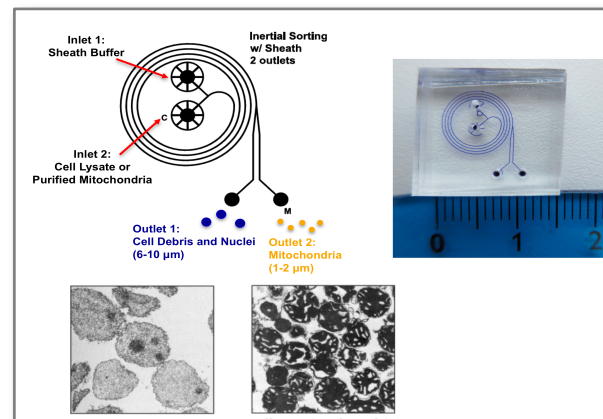
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Organelles

3D Culture

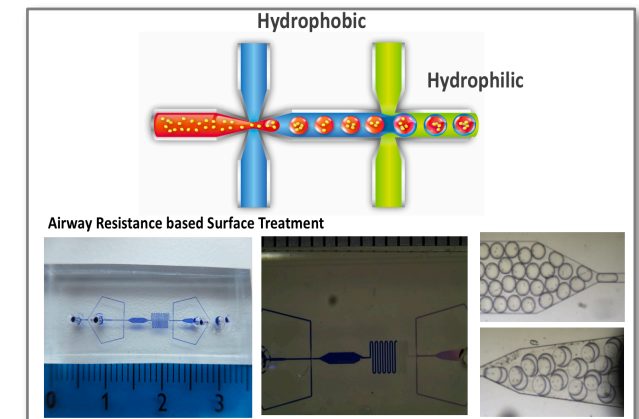
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Introduction

Biomarkers

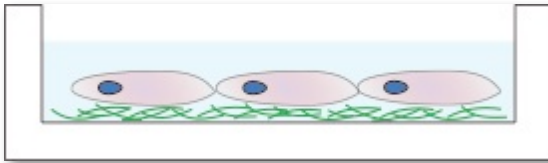
Organelles

3D Culture

Conclusion

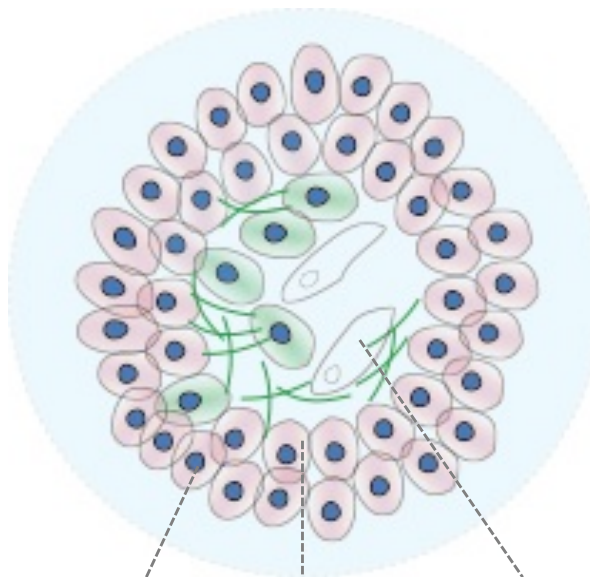
Bridging *In Vitro* and *In Vivo*

2D Culture in Petri Dish



- + Standard Assay
- 2D Cell Signaling
- 2D Drug Response

3D Multicellular Spheroids



- Proliferating Zone
- Quiescent Zone
- Necrotic Zone

Animal Model



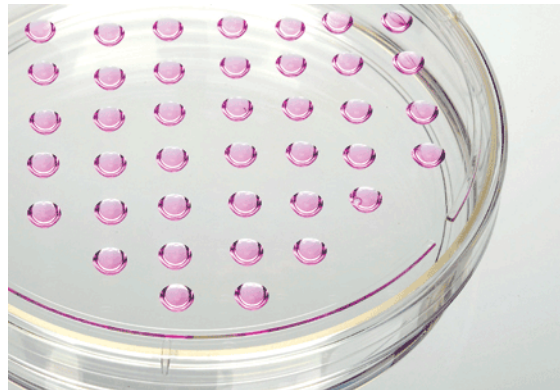
- + 3D Cell Signaling
- + 3D Response
- Expensive

Existing 3D Culture Models

- **Scaffold-Based**

- Hydrogels
- Inert matrix

- **Scaffold-free**



<http://www.nature.com>



www.mdpi.com

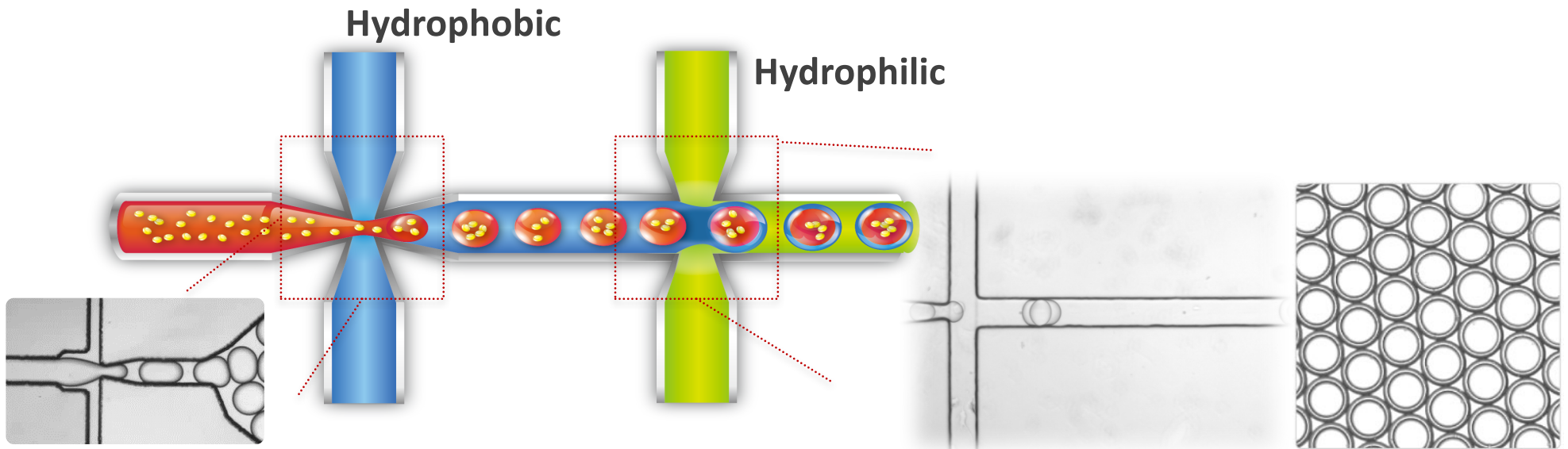
Advantage(s)

Disadvantage(s)

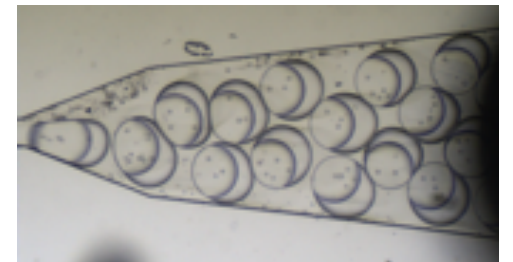
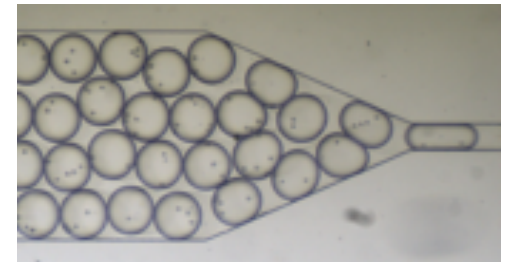
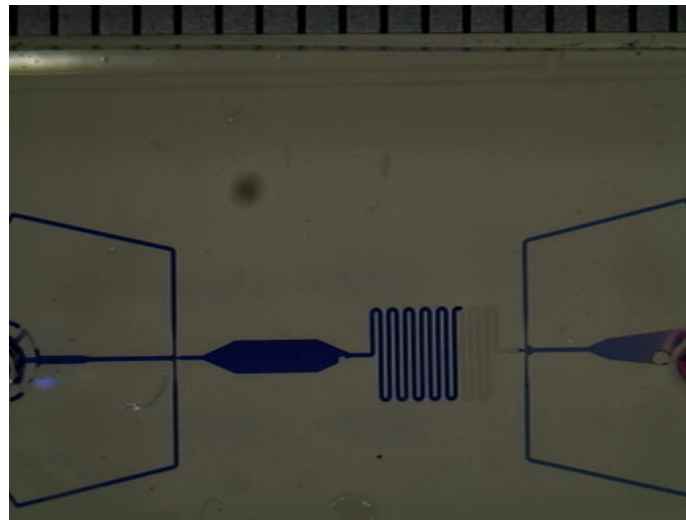
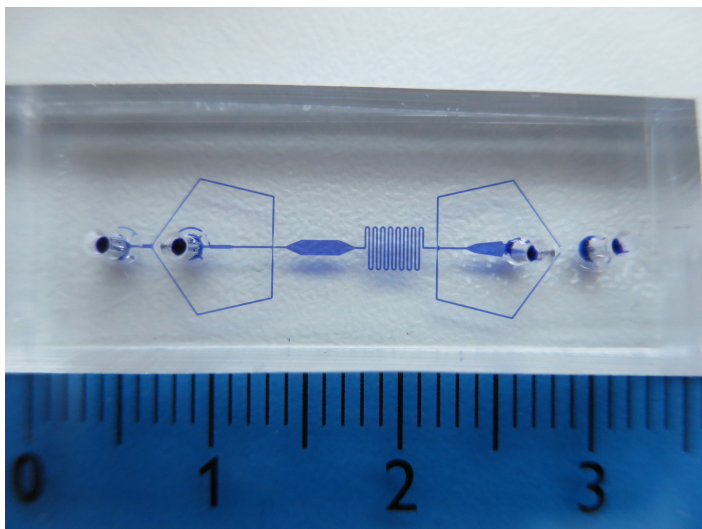
Hanging Drop	<ul style="list-style-type: none"> • Simple • Uniform spheroid size 	<ul style="list-style-type: none"> • Labor-intensive • Difficult to change medium
Spinner flask/ suspension	<ul style="list-style-type: none"> • Simple • Easy to scale up 	<ul style="list-style-type: none"> • Heterogeneous in spheroid size
Micromolded plate	<ul style="list-style-type: none"> • Uniform spheroid size • High-throughput 	<ul style="list-style-type: none"> • Delicate skills required for spheroid culture

***none of the techniques allows facile addition of exogenous ECM cues**

Challenges on Surface Treatment



Airway Resistance based Surface Treatment



Ma *et al.*, *J. of Micromechanics and Microengineering*, v. 27 (9): 095014, 2017

Introduction

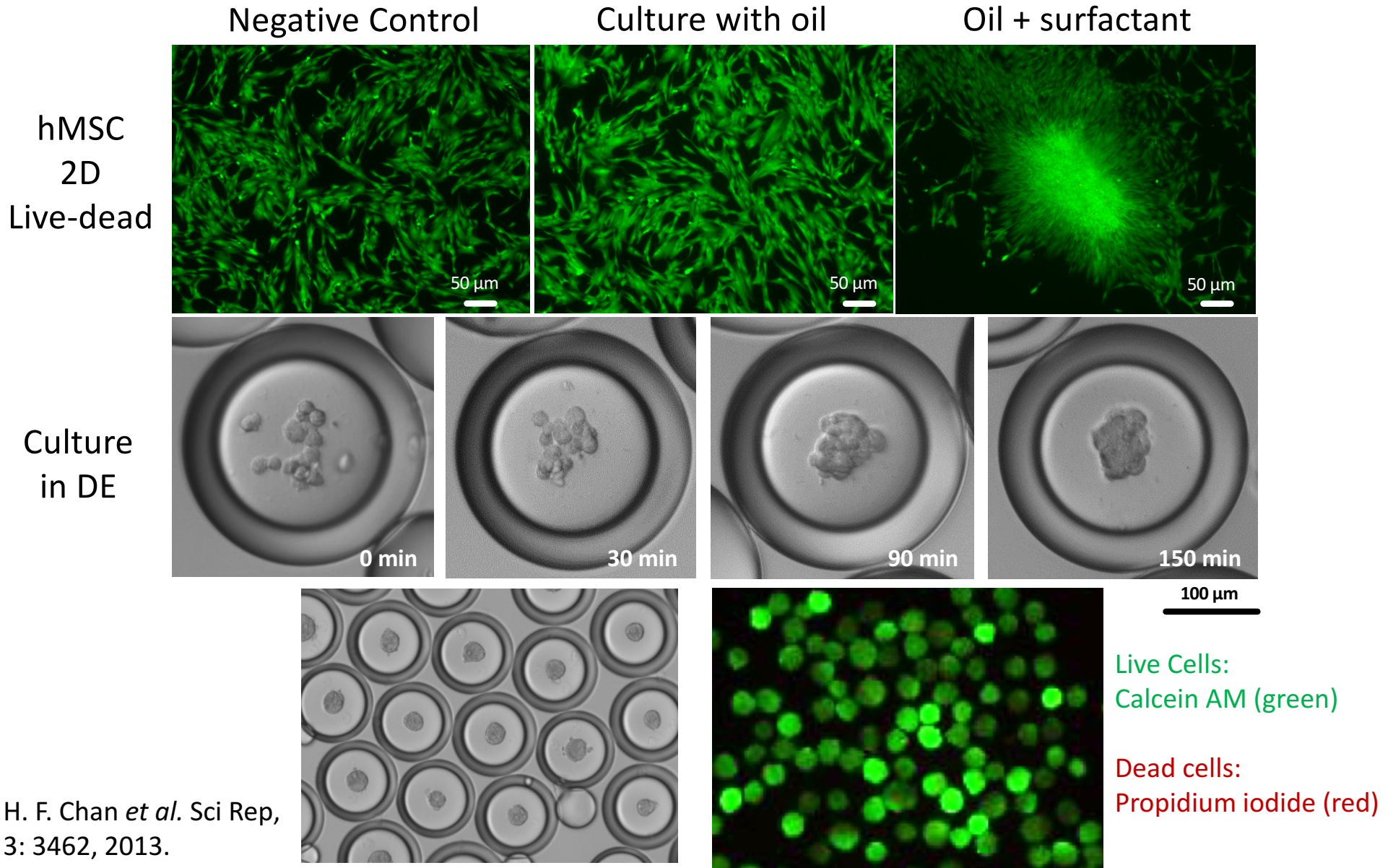
Biomarkers

Organelles

3D Culture

Conclusion

Surfactant Induced Spheroid Formation



Introduction

Biomarkers

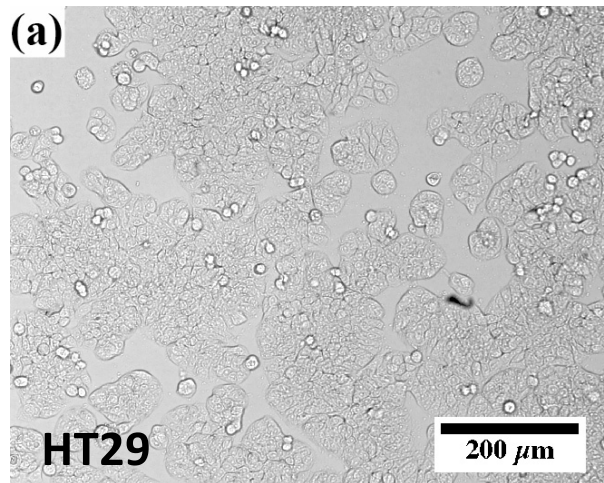
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3D Culture

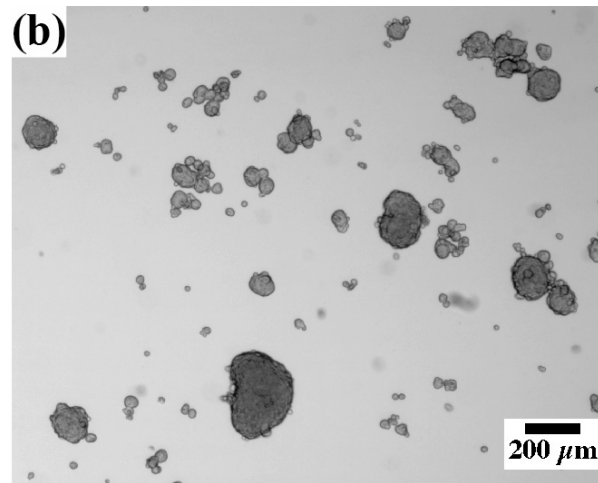
Conclusion

Comparison with Existing Strategies

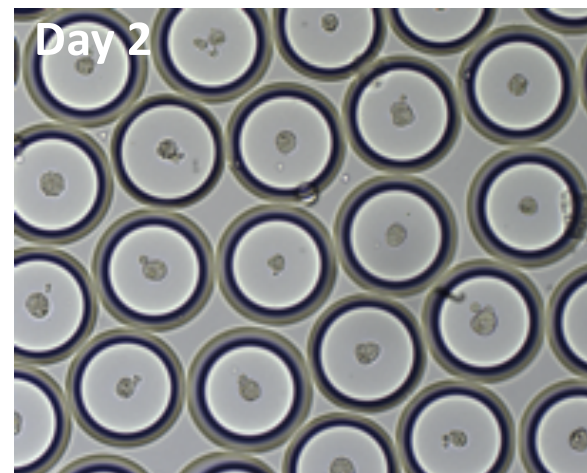
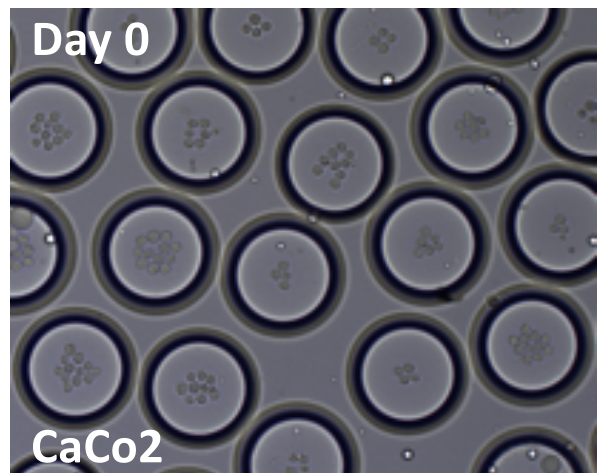
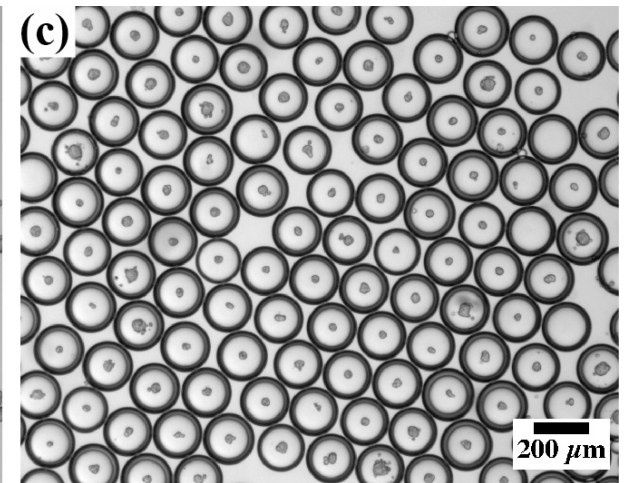
2D Culture



Ultra Low Attachment Plate



Double Emulsions



Ma *et al.*, J. of Micromechanics and Microengineering, v. 27 (9): 095014, 2017

Introduction

Biomarkers

Organelles

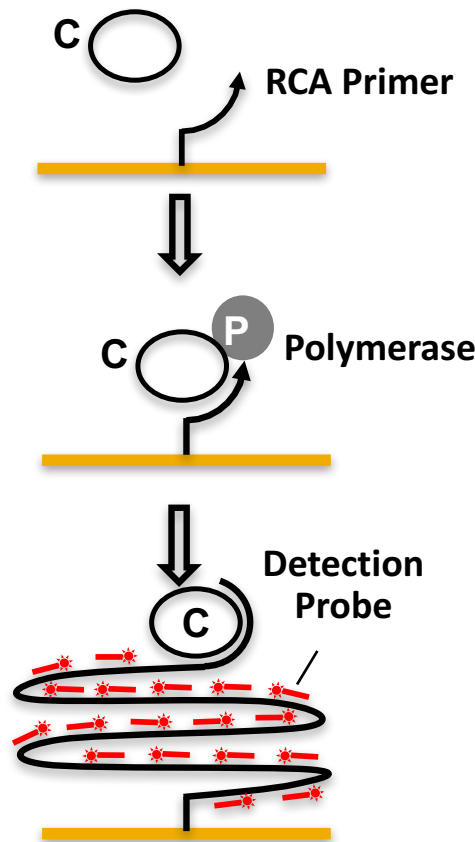
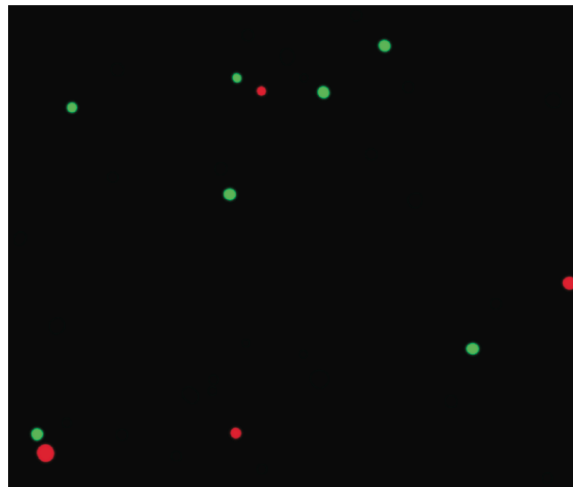
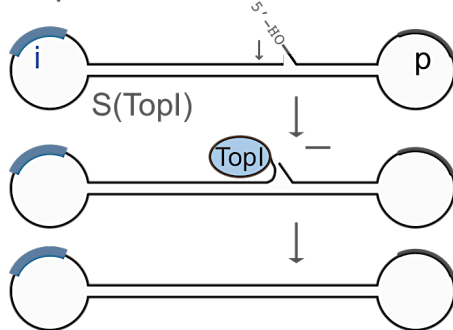
3D Culture

Conclusion

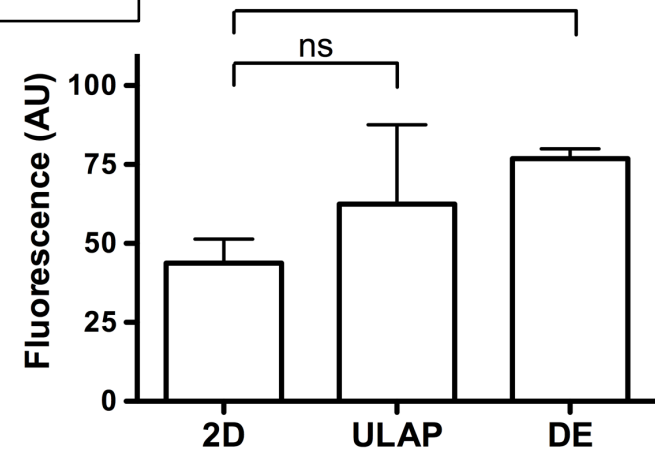
Reliable and Consistent Analysis as Each Droplet Serves as an Individual Incubator

■ Detection of human Topoisomerase I (hTopI) activity

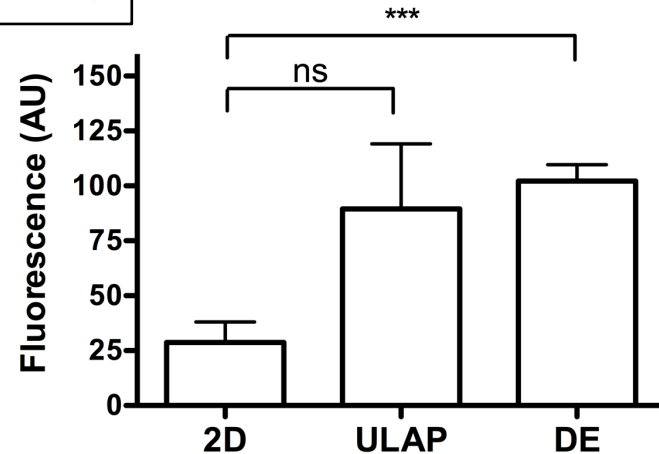
TopI reaction



CaCo2



HT-29



Unpaired t-tests: * $p < 0.02$, *** $p < 0.001$

Stougaard *et al.*, *ACS Nano*, 3(12): 4043-4054, 2009

Ma *et al.*, *J. of Micromechanics and Microengineering*, v. 27 (9): 095014, 2017

Introduction

Biomarkers

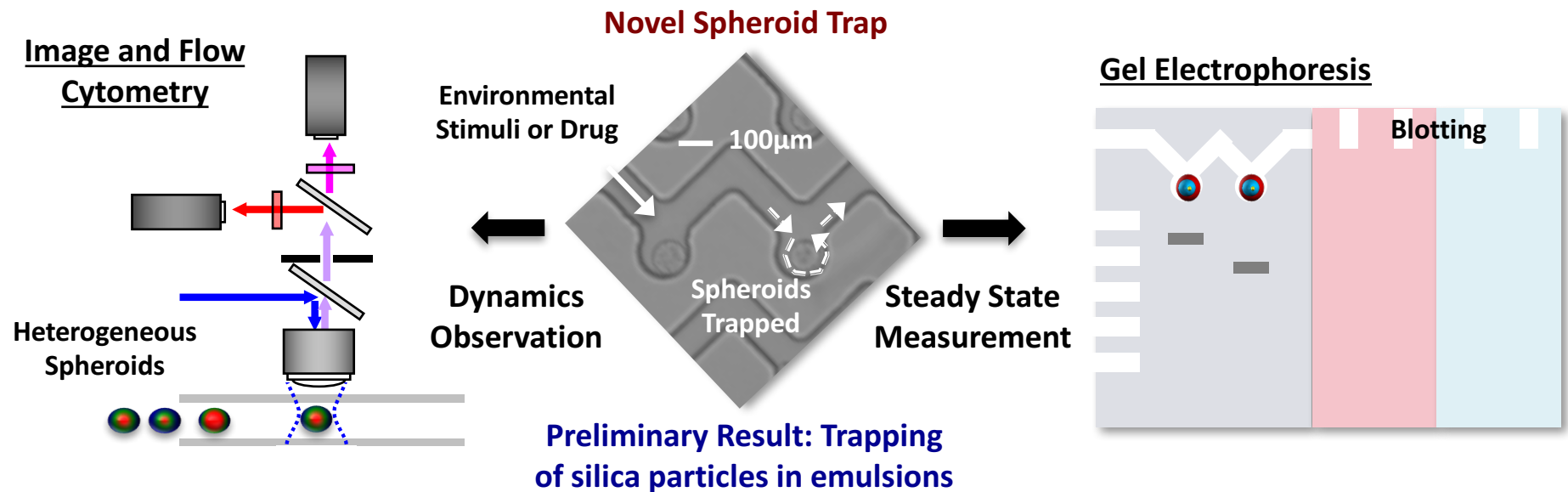
Organelles

3D Culture

Conclusion

Multifunctional Analytical Platform

Integrated Multifunctional Analytical Platform



■ Major Contributions:

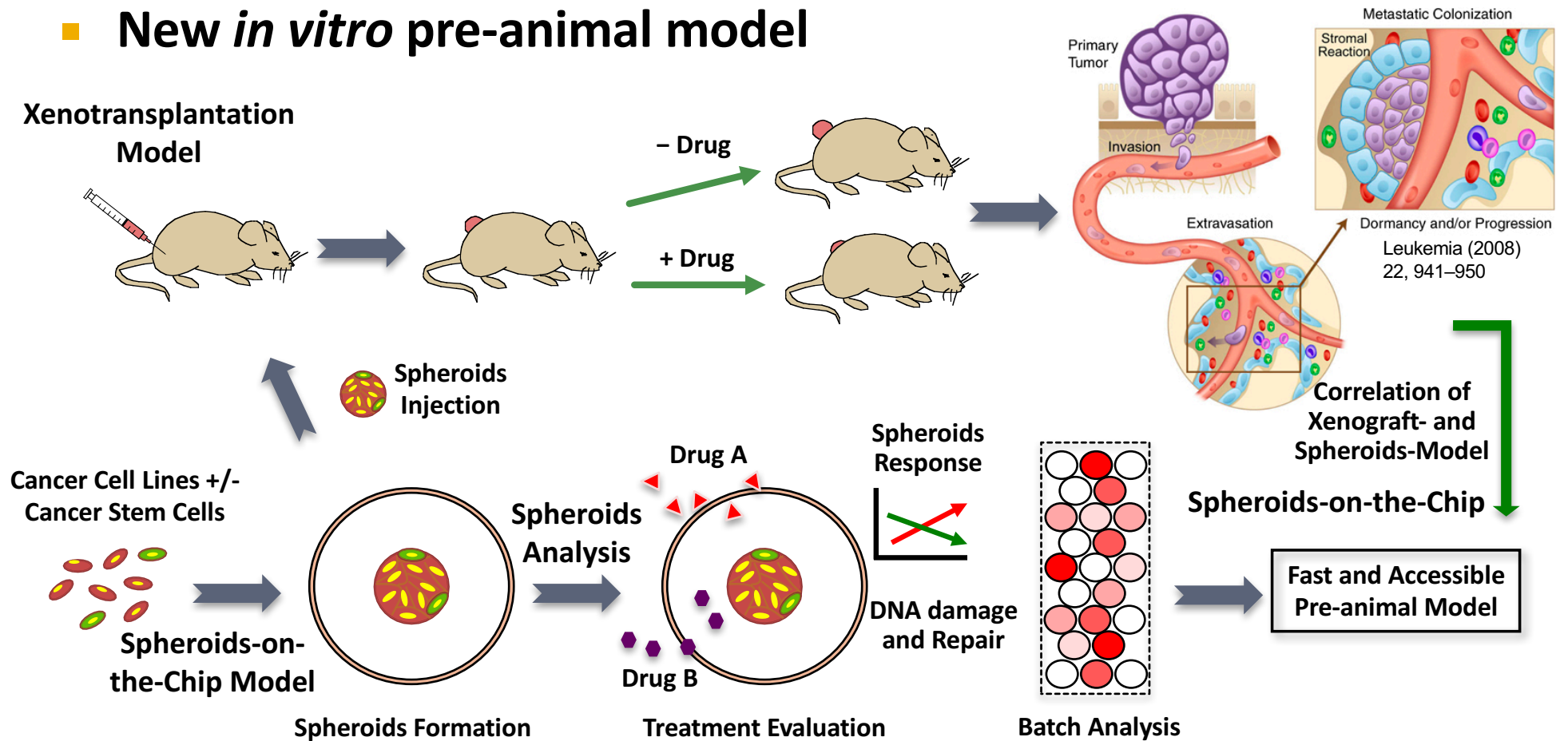
- New multifunctional platform for spheroid analysis
- Full package analysis and correlation between spheroid formation capability and molecular biological properties

■ Major Challenges:

- Performance of spheroid traps
- Integration of microfluidics, optics and gel electrophoresis

In vitro Cluster vs. In vivo Tumor?

■ New *in vitro* pre-animal model



■ Biology Endeavor

- Contribution of cancer stem cells to the spheroid formation?
- Effect of drug against stem cell-rich spheroids?

Introduction

Biomarkers

Organelles

3D Culture

Conclusion

Organoids as a Drug Screening Model

- Spheroids is referred to as organoids, when tissue-like phenotype is observed;
- Organoids recapitulate tissue architectures and functions *in vitro*
→ understand the biology and evaluate the drug efficacy



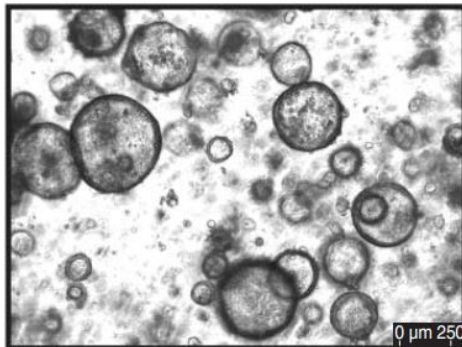
Access provided by The Chinese University of Hong Kong



Editorial

Method of the Year 2017: Organoids

The ability to prod stem cells into three-dimensional tissue models makes for a powerful way to study human biology. But these exciting tools are still works in progress.



RESEARCH

ORGANOIDS

Patient-derived organoids model treatment response of metastatic gastrointestinal cancers

- 100% sensitivity;
- 93% specificity;
- 88% positive predictive;
- 100% negative predictive value in forecasting response to targeted agents or chemotherapy in patients

G. Vlachogiannis *et al.*, Science, 2018, 359: 920

Introduction

Biomarkers

Organelles

3D Culture

Conclusion

Interested in Joining Us

- Students with strong background in **engineering, material science, medical or biological science** are welcome.

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Phone: 3943-4340

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WeChat: ypho13

Website: www.bme.cuhk.edu.hk/ypho/

See you at the Lab Tour (G20)

