SAND2015-4741PE

Sandia's Microfluidic Applications in Biosciences

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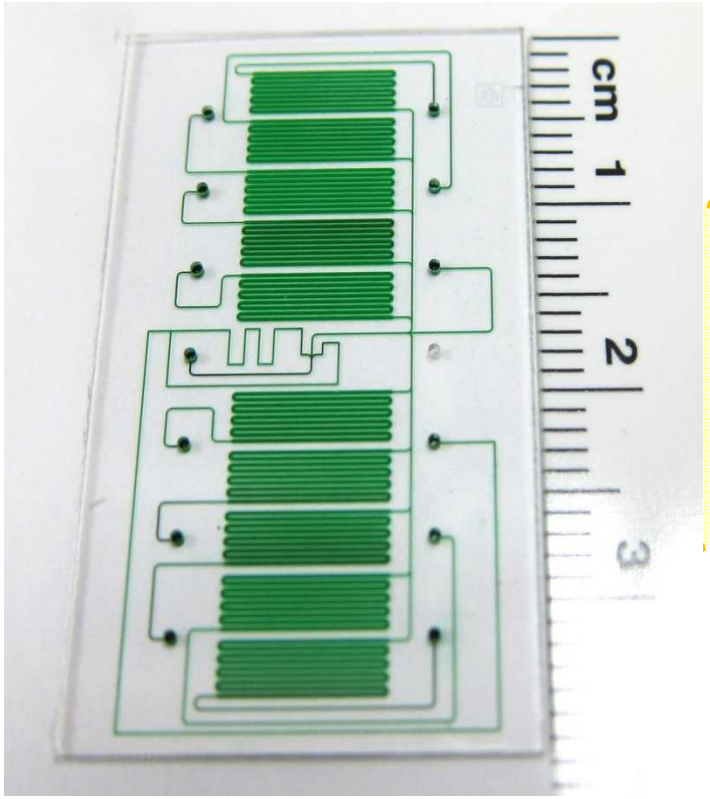
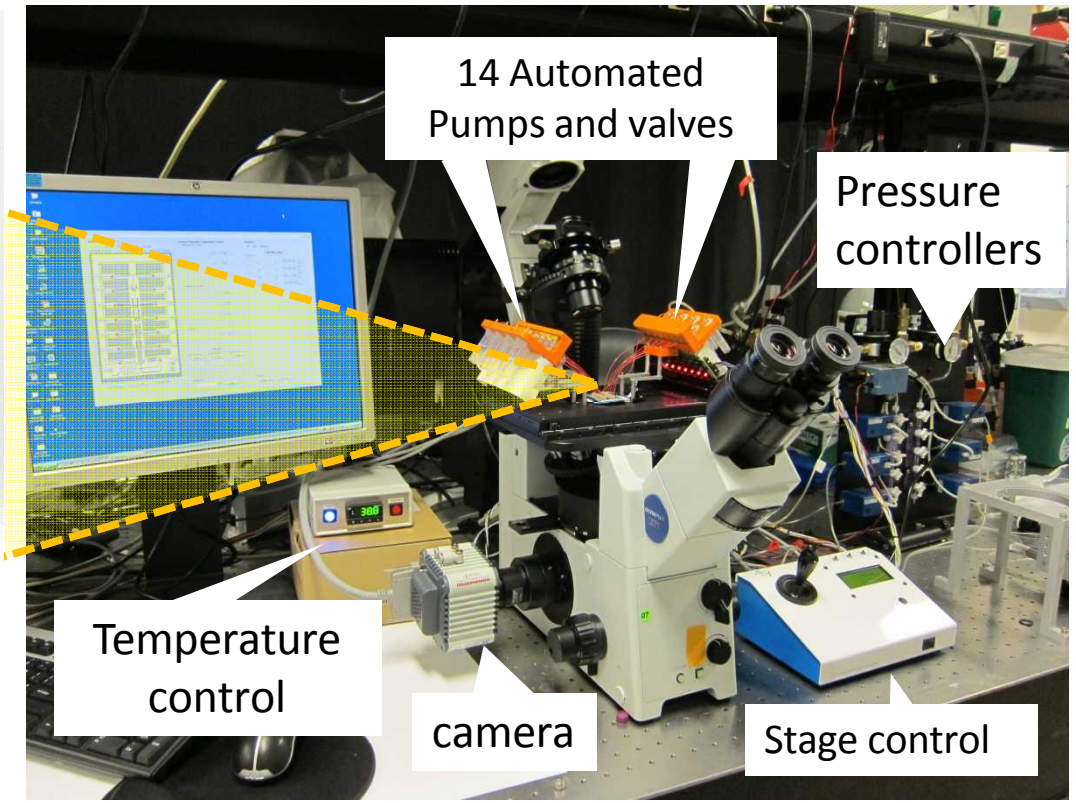
Integrated Microfluidic Platform for Single-cell Analysis

10 chamber microfluidic chip



Automated assay platform



In situ hybridization (miRNA, mRNA, DNA)
Immunostaining (Proteins)

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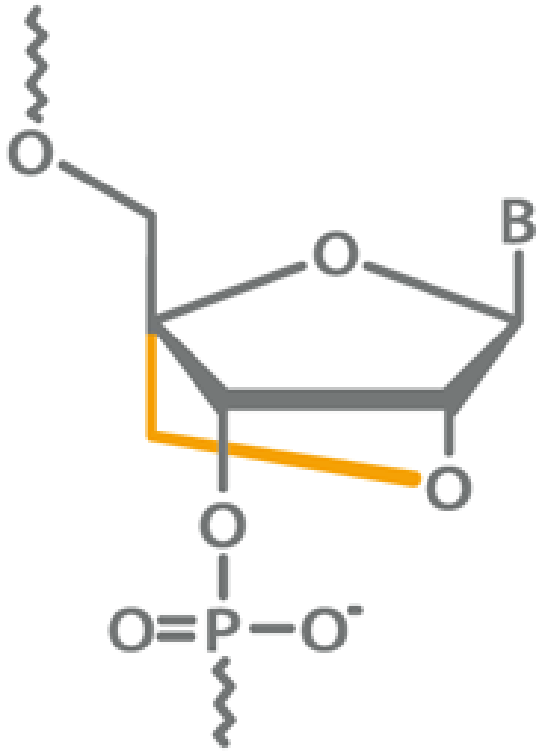
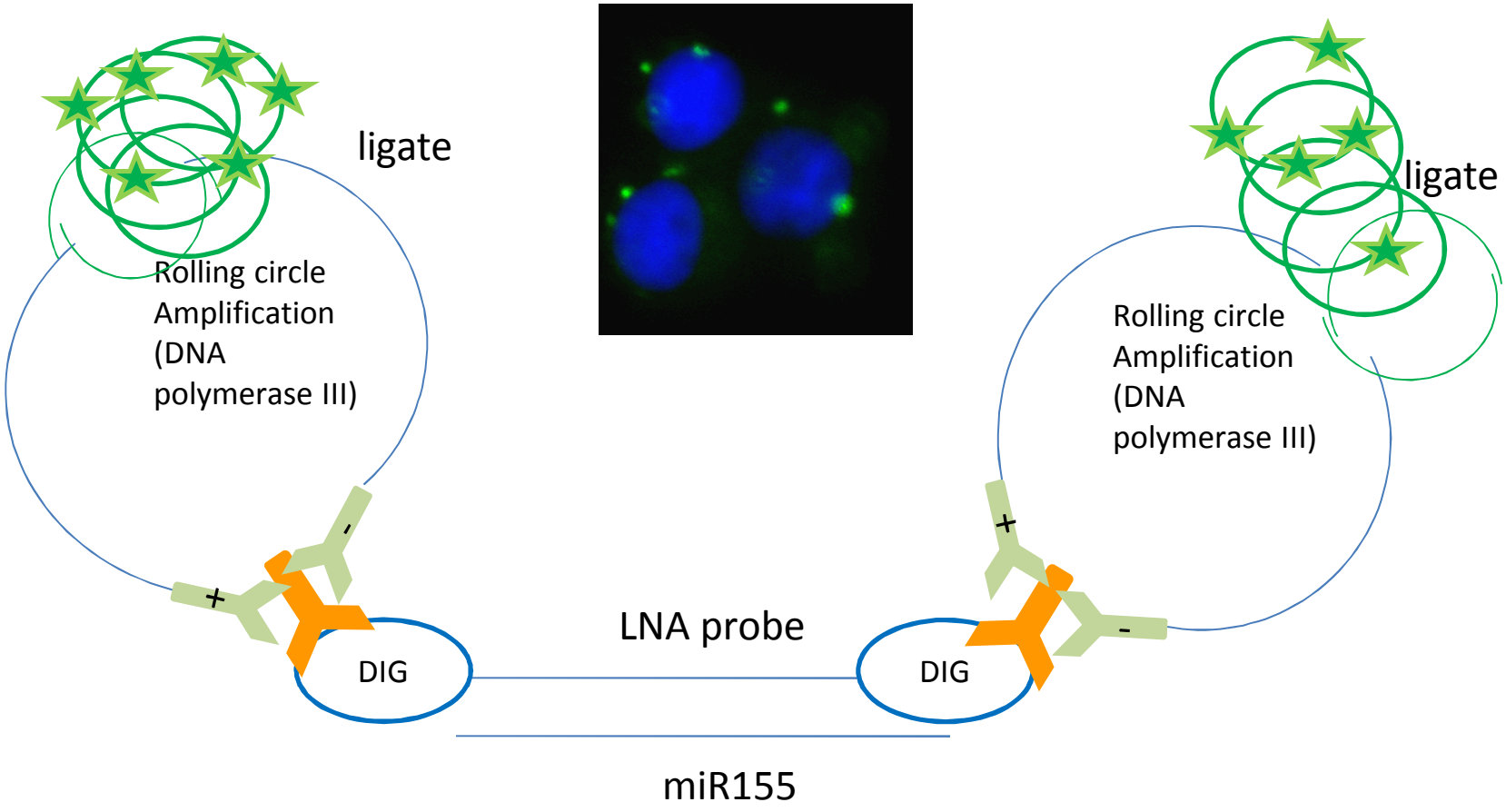
Use Locked Nucleic Acid probes to detect miRNA



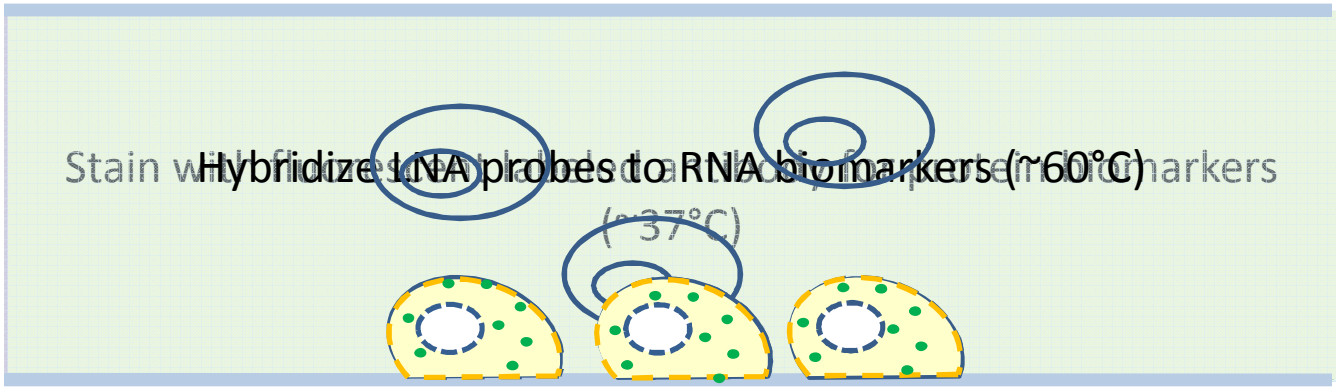
LNA - The ribose ring is connected by a methylene bridge (orange) between the 2’-O and 4’-C atoms, “locking” the ribose ring in the ideal conformation for Watson-Crick binding.

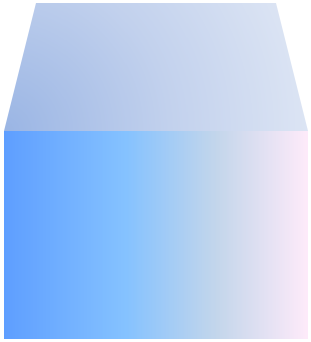
Oligonucleotide probes with LNA form much more stable complementary duplexes.

Use Rolling Circle Amplification (RCA) to Amplify miRNA signal in single cells



Typical automated microfluidic experiment

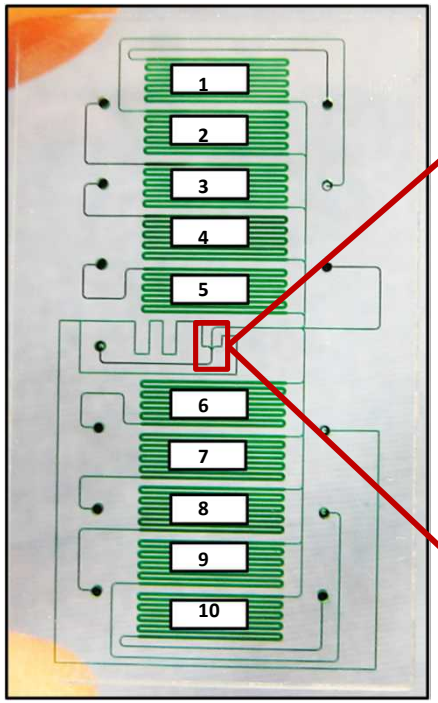


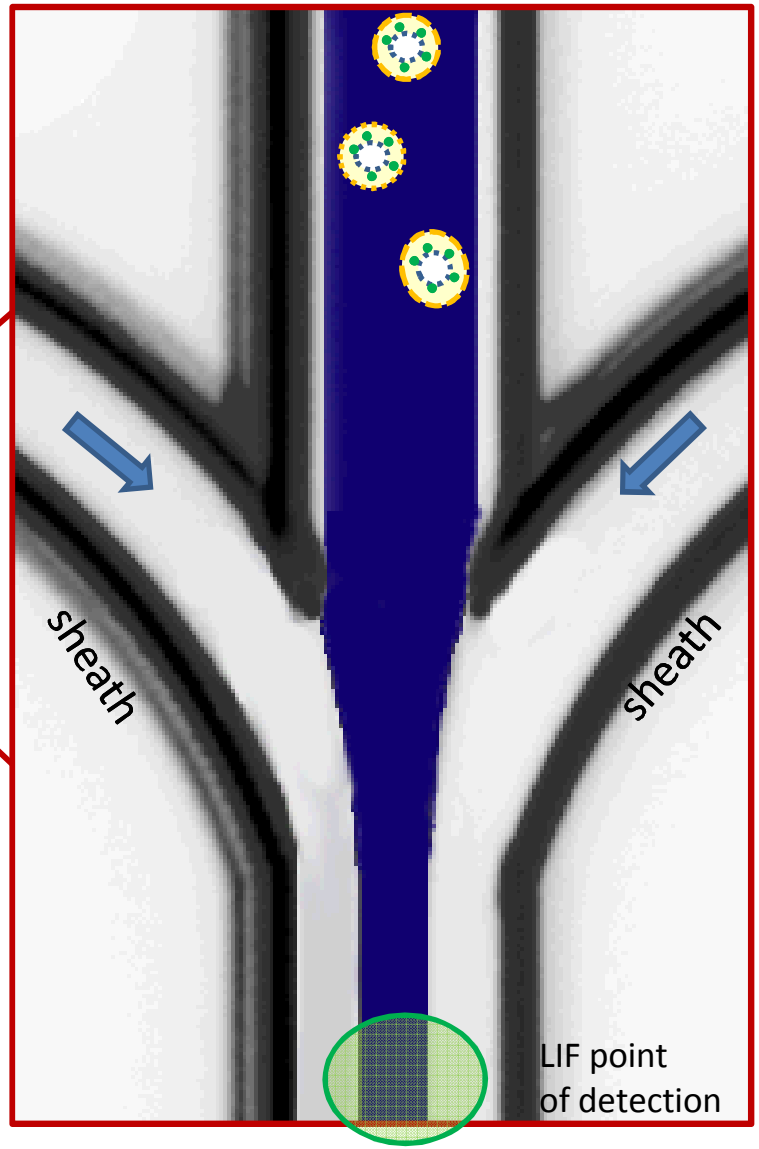


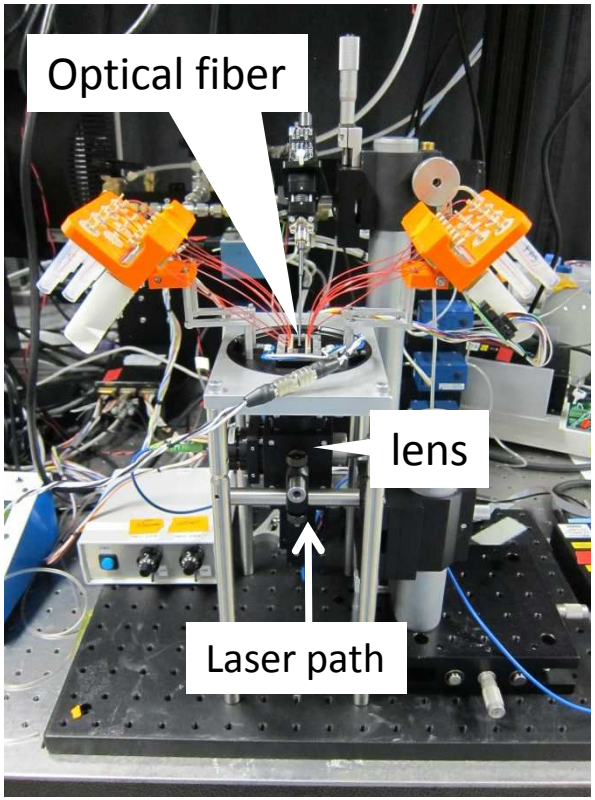
Take photographs of cells in the chip

Objective

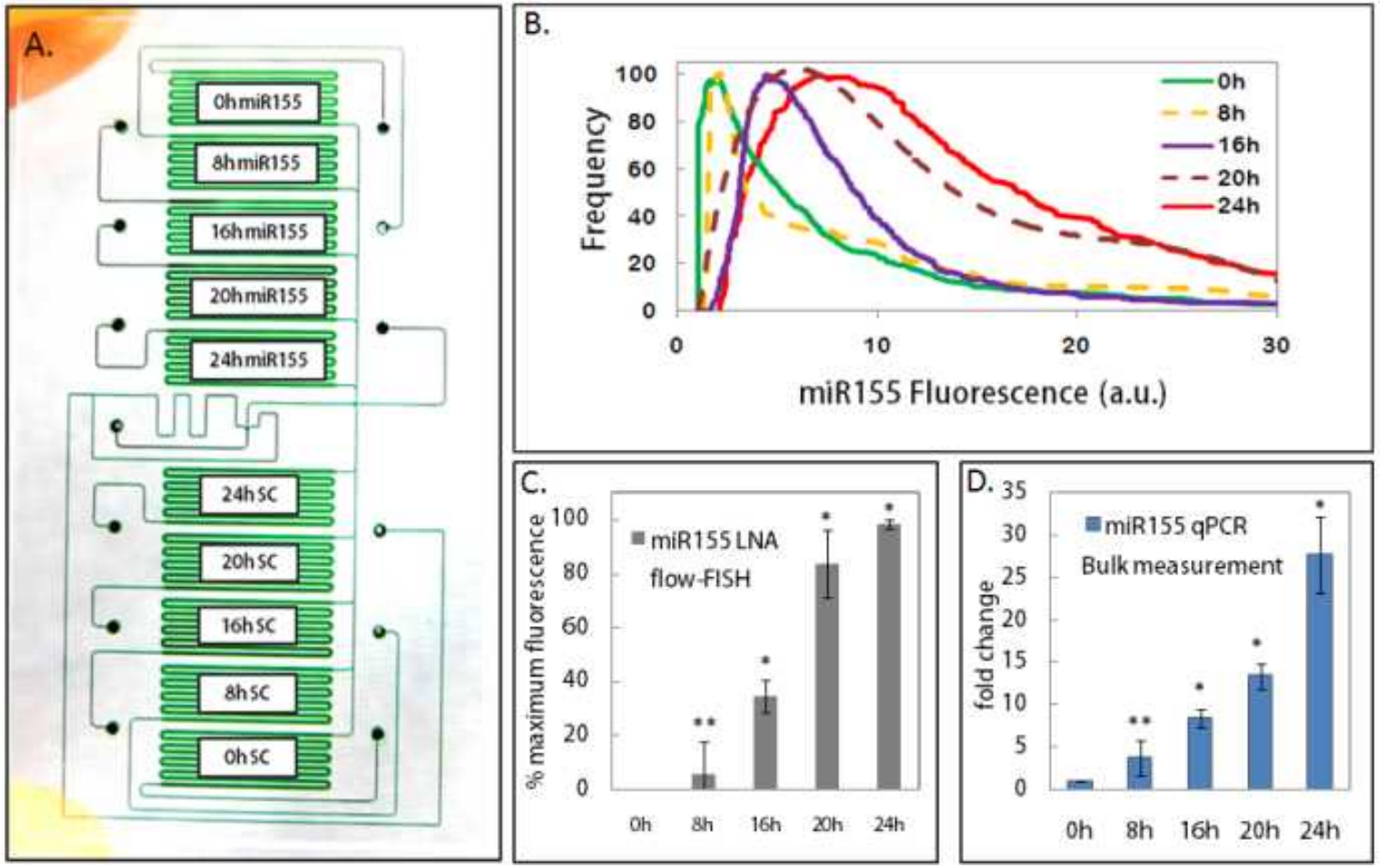
On-chip microflow cytometry







LNA flow-FISH profiling of miR155 expression



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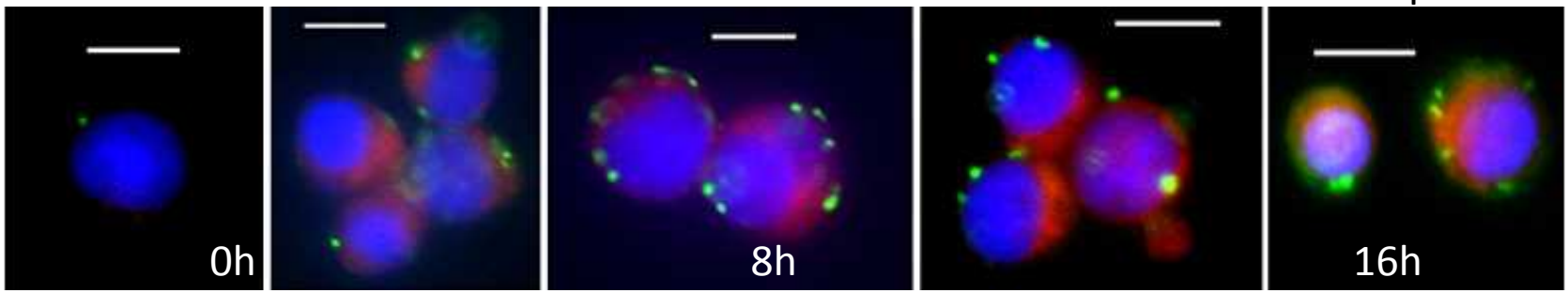
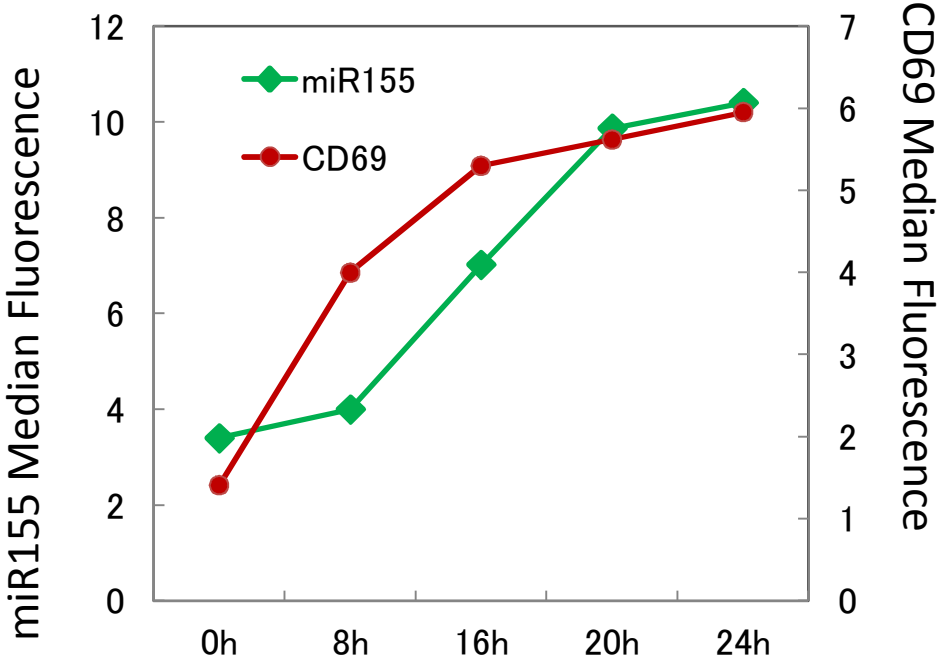
Multiplexing Results

All scale bars = 10 µm



miR155 (green), nucleus(blue), CD69 early T cell activation marker (red)



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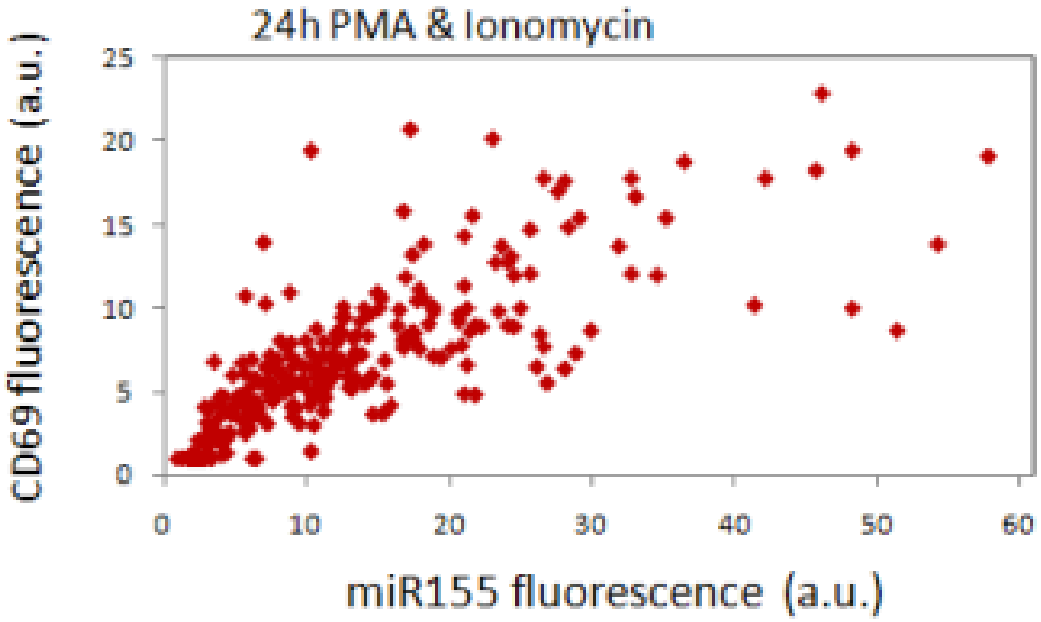
miRNA detection at single cell resolution shows heterogeneity in the population



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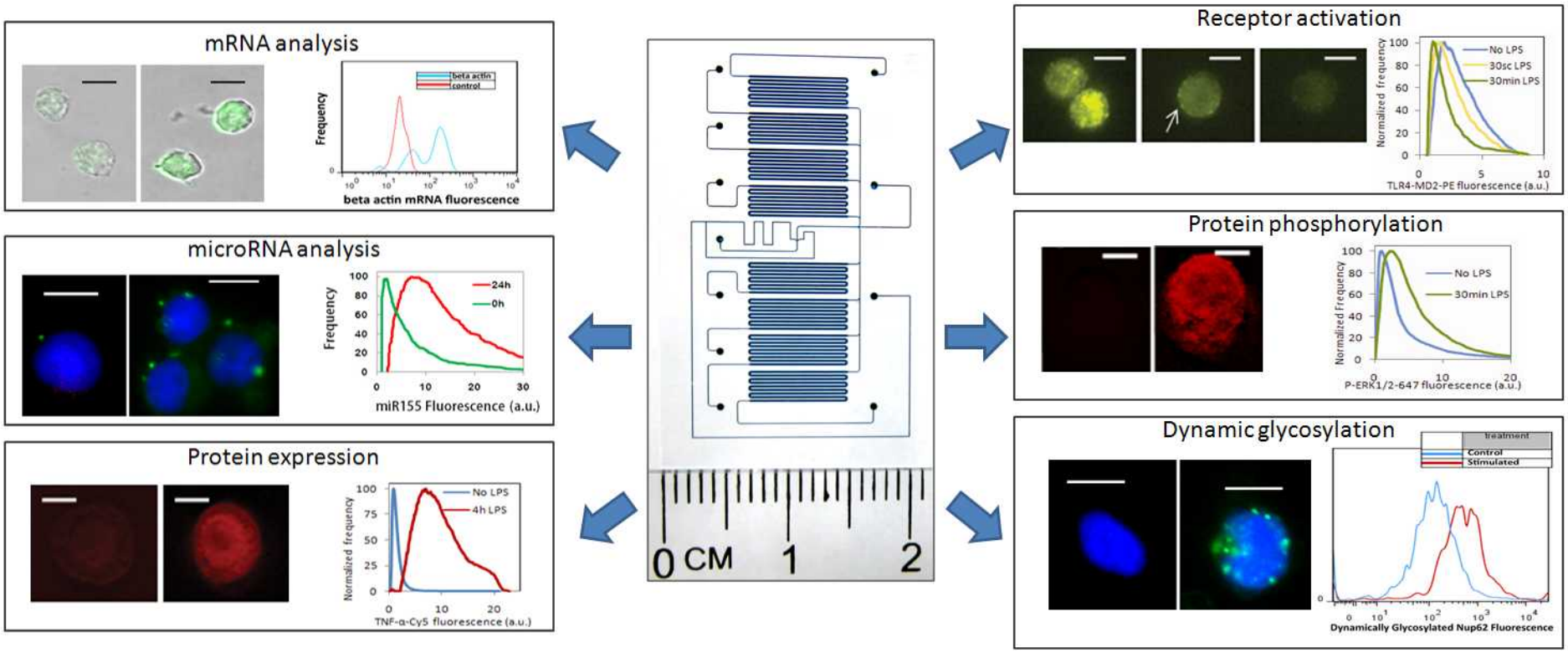
Portfolio of molecular assays developed for the microfluidic platform



All molecular assays are compatible for multiplexing

Sandia's Microfluidic platform addresses technology gap

- Single-cell resolution: preserve cellular context
- Imaging and flow cytometry: "where" and "how much" biomarkers in the cell
- Automated microfluidic sample preparation
- Cost-effective: decrease reagent cost by over 95%
- Multiplexing: detect multiple categories of biomarkers in the same cell

Related Publications and Patents

Wu M, Singh AK. Single Cell Cytokine Analysis, Methods in Molecular Biology Chapter (In preparation)

Wu M, Singh AK. Microfluidic molecular assay platform for the detection of miRNAs, mRNAs, proteins, and posttranslational modifications at single-cell resolution. Journal of laboratory automation. 2014 Dec;19(6):587-92. PubMed PMID: 25027027.

Wu M, Piccini ME, Singh AK. MiRNA detection at single-cell resolution using microfluidic LNA flow-FISH. Methods in molecular biology. 2014;1211:245-60. PubMed PMID: 25218391.

Wu M, Piccini M, Koh CY, Lam KS, Singh AK. Single cell microRNA analysis using microfluidic flow cytometry. PloS one. 2013;8(1):e55044. PubMed PMID: 23383050. Pubmed Central PMCID: 3559333.

Wu M, Perroud TD, Srivastava N, Branda CS, Sale KL, Carson BD, et al. Microfluidically-unified cell culture, sample preparation, imaging and flow cytometry for measurement of cell signaling pathways with single cell resolution. Lab on a chip. 2012 Aug 21;12(16):2823-31. PubMed PMID: 22777012.

Wu M, Singh AK. Single-cell protein analysis. Current opinion in biotechnology. 2012 Feb;23(1):83-8. PubMed PMID: 22189001. Pubmed Central PMCID: 3283030.

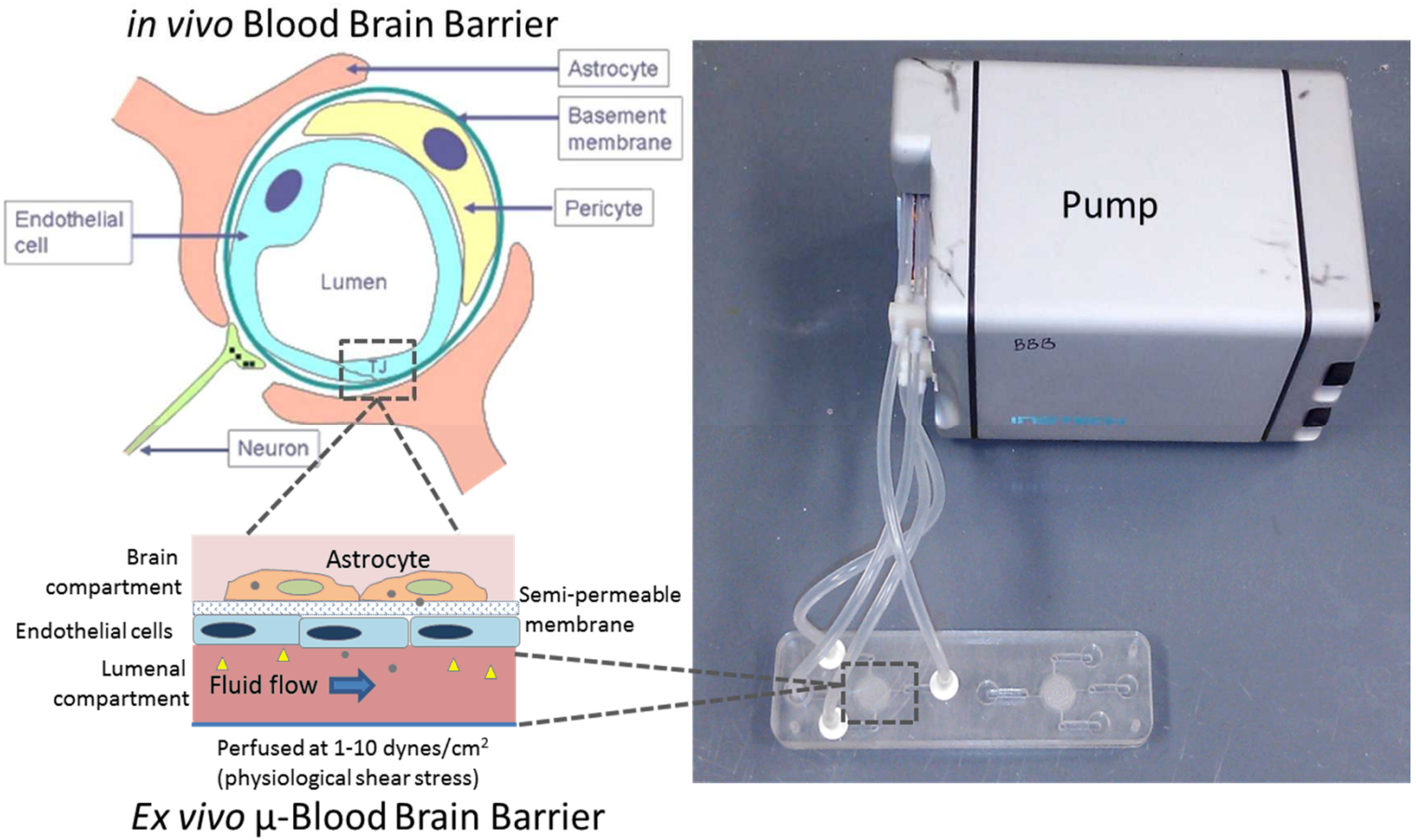
Srivastava N, Brennan JS, Renzi RF, **Wu M**, Branda SS, Singh AK, et al. Fully integrated microfluidic platform enabling automated phosphoprofiling of macrophage response. Analytical chemistry. 2009 May 1;81(9):3261-9. PubMed PMID: 19323537.

Perroud TD, Meagher RJ, Kanouff MP, Renzi RF, **Wu M**, Singh AK, et al. Isotropically etched radial micropore for cell concentration, immobilization, and picodroplet generation. Lab on a chip. 2009 Feb 21;9(4):507-15. PubMed PMID: 19190785.

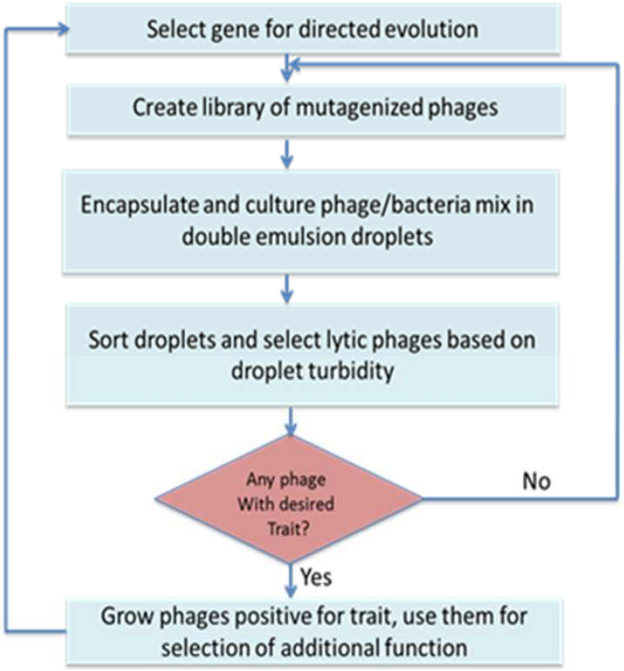
Wu M, Singh AK; MICROFLUIDIC PLATFORM FOR MULTIPLEXED DETECTION IN SINGLE CELLS AND METHODS THEREOF (filed 12/19/2014, 14/575,886).

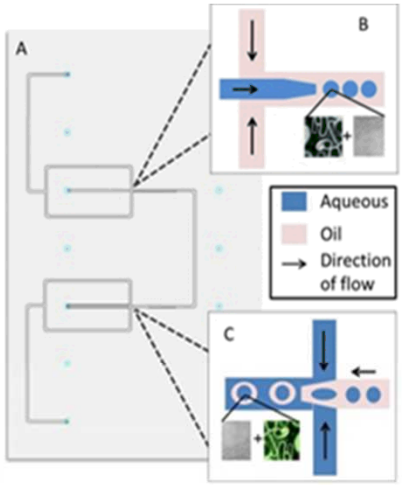
Other projects

μ-Blood Brain Barrier

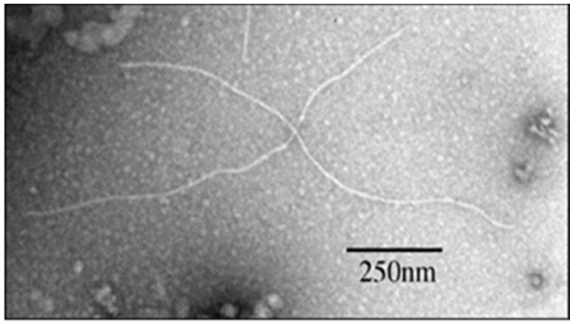


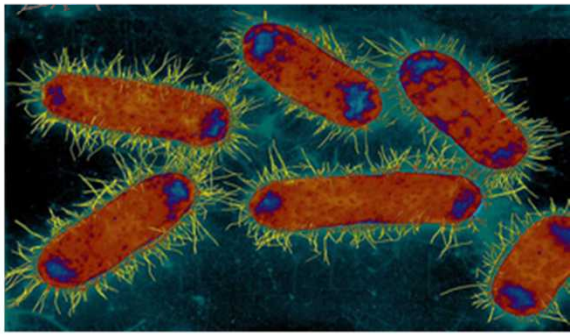
Directed Evolution of Bacteriophages as Novel Evolvable Antibiotics



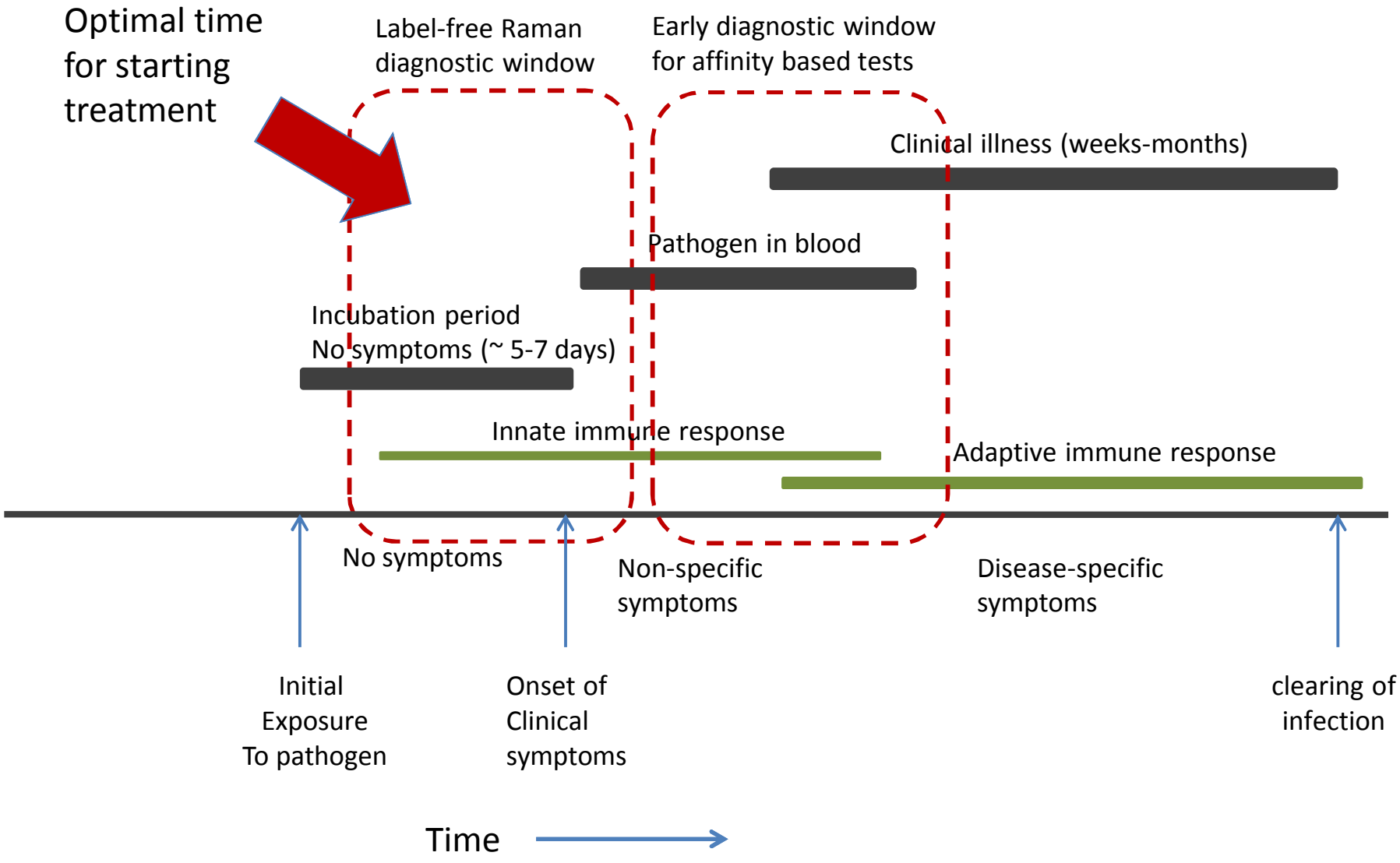


Double emulsion droplet Chip for ultra high throughput culture and screening





Early Disease Diagnostic System



Acknowledgements

Sandia National Laboratory

Anup Singh, PhD
Anson Hatch, PhD
Aarthi Chandrasakaran, PhD
Chung-yan Koh, PhD
Ryan Davis, PhD
Jim Brennen
Ron Renzi

UC Davis

Kit Lam, MD/PhD
Chuck Bevins, MD/PhD
Judy Kjelstrom, PhD

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