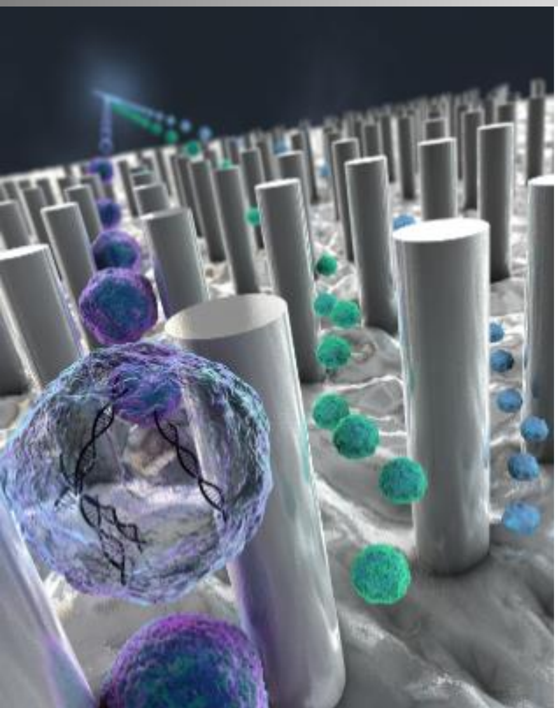
IBM Research



Identifying nanobiotechnology-based solutions for opportunities in personalized healthcare

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IBM T.J. Watson Research Center

Nanobiotechnology Group

November 15, 2018

NCI Southwest + NACK Network

Hosted by: ATE Central

Today's Presenters

IBM Research Staff Member

– Technology development
– Electrical engineering

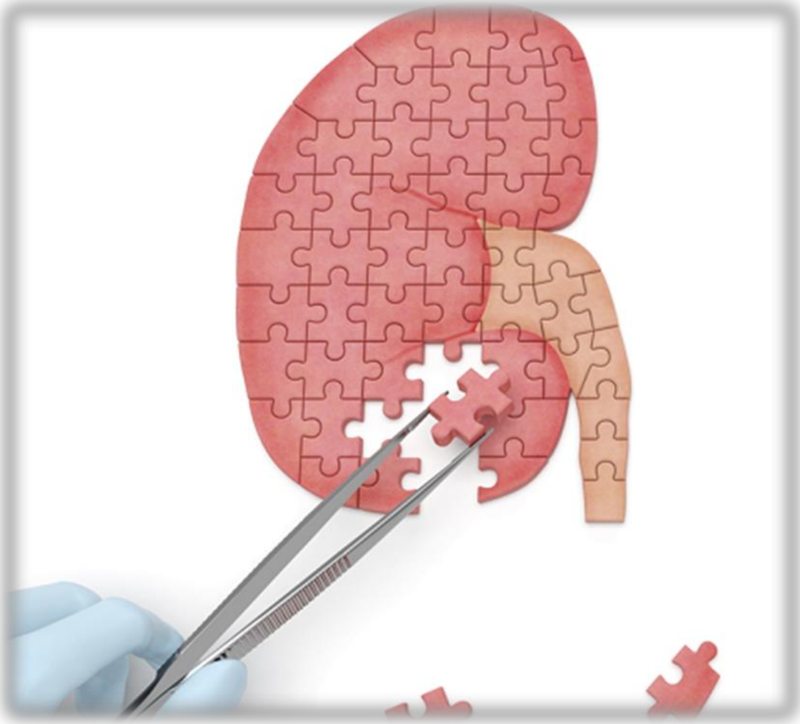
IBM Research Staff Member

– Biological applications
– Biochemistry

Challenges with the gold standard of tissue biopsy

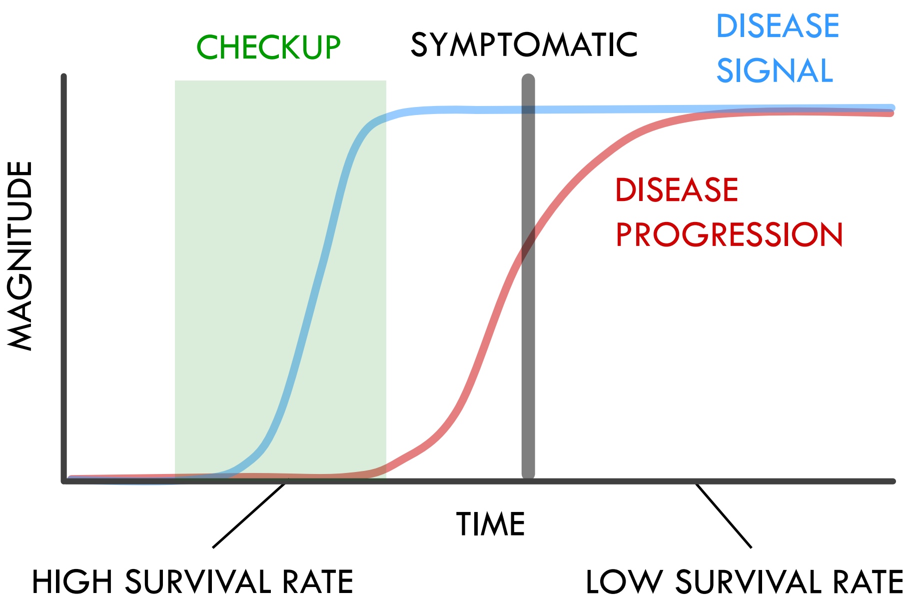
Tissue biopsy requires removal of tissue for histopathological analysis to determine malignancy

- Invasive
- Side effects include pain and infection
- Time-consuming
- Incomplete sampling of tumor tissue
- Limited sample size
- Incompatible with continuous monitoring and screening



Liquid biopsy offers advantages over tissue biopsy

Biomarker sampling from bodily fluids offers a non-invasive alternative to tissue biopsy

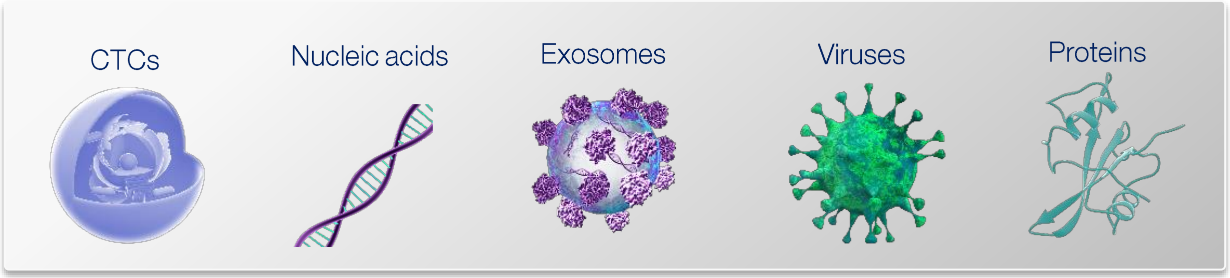


- Non-invasive or minimally invasive
- Low-risk and few side effects
- Early screening and detection
- Continuous monitoring
- Small volumes required
- More complete sampling of many tissues
- Diversity of biofluid sources
- Monitor health and disease beyond cancer

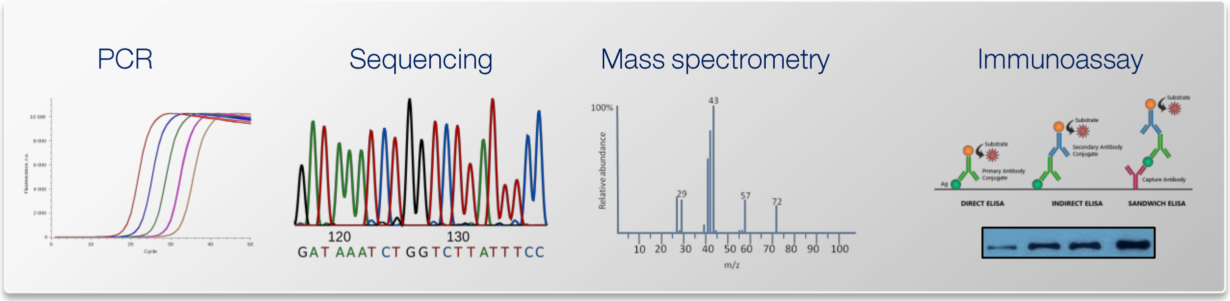
Diversity in liquid biopsy biomarkers and analysis

Biomarkers can be derived from nearly all biofluids including urine, blood, and saliva

Types of biomarkers

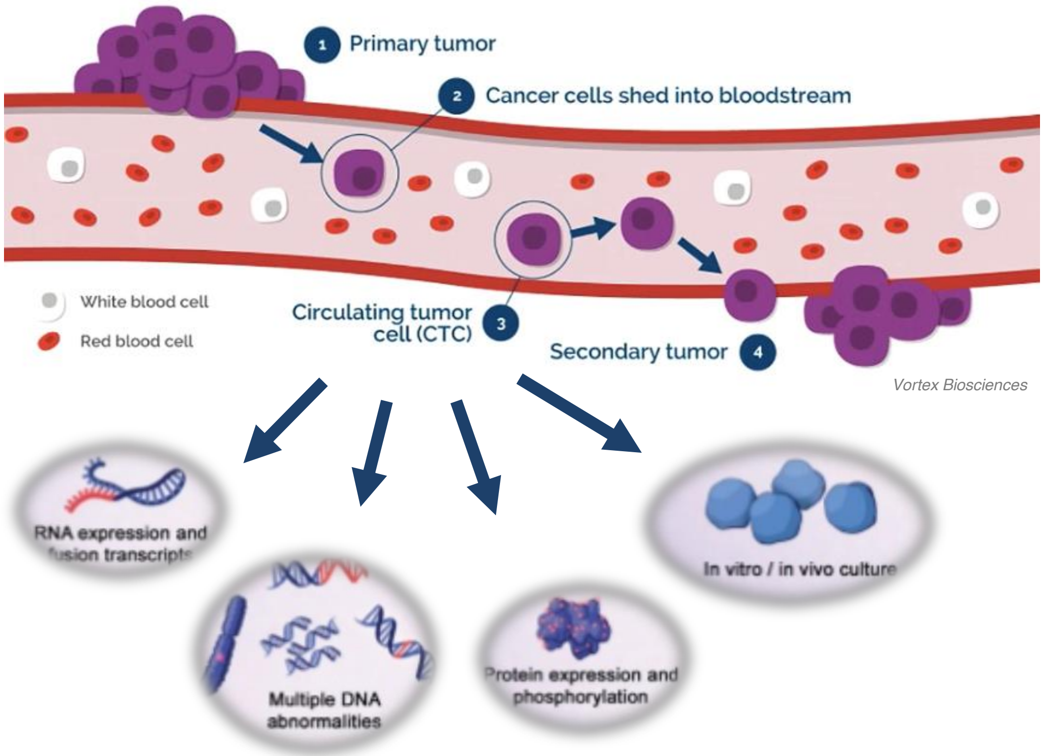


A subset of biomarker analysis methods



Circulating tumor cells in liquid biopsy

Cells migrating from the primary tumor to sites of metastasis carry molecular information to guide treatment



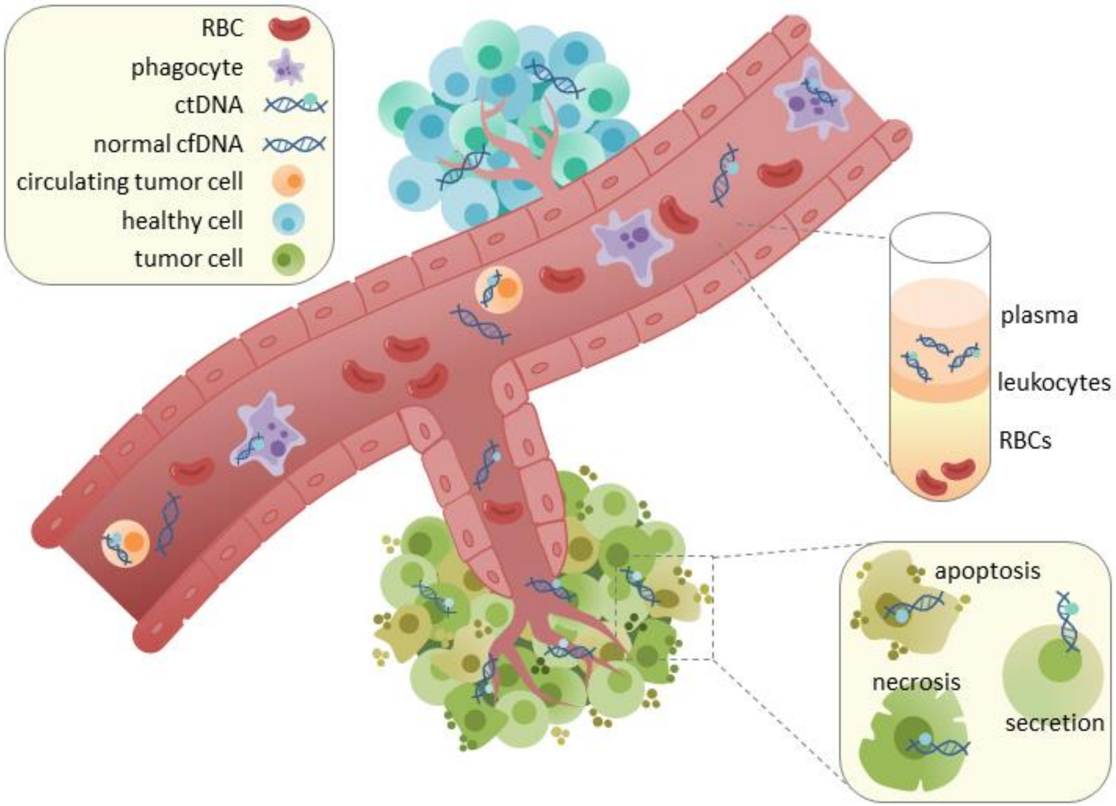
CELLSEARCH® is the only CTC-based liquid biopsy test

EpCAM-positive circulating tumor cells offer prognostic value for epithelial cancers

- Counts EpCam^{+} and CD45^{-} epithelial CTCs
- Sensitive to 1 cell per 7.5 mL whole blood
- CTCs in peripheral blood associated with decreased survival in metastatic breast, colorectal, and prostate cancer patients
- Limited predictive power in drug response and resistance
- Dropped from Medicare coverage in 2017 citing lack of impact on patient outcomes

Circulating tumor DNA and cell-free DNA as biomarkers

ctDNA and cfDNA are derived primarily from blood and can serve as biomarkers for diseases



Many cfDNA and ctDNA tests available

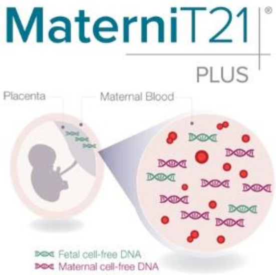
IVDs offer test from companion diagnostics to prenatal screening, but not all are FDA-approved

cobas EGFR Mutation Test v2
Non-small cell lung cancer
Companion diagnostic

Guardant360
Non-small cell lung cancer
Comprehensive mutation analysis

Abbot RealTi*me* IDH2
Acute myeloid leukemia
Companion diagnostic

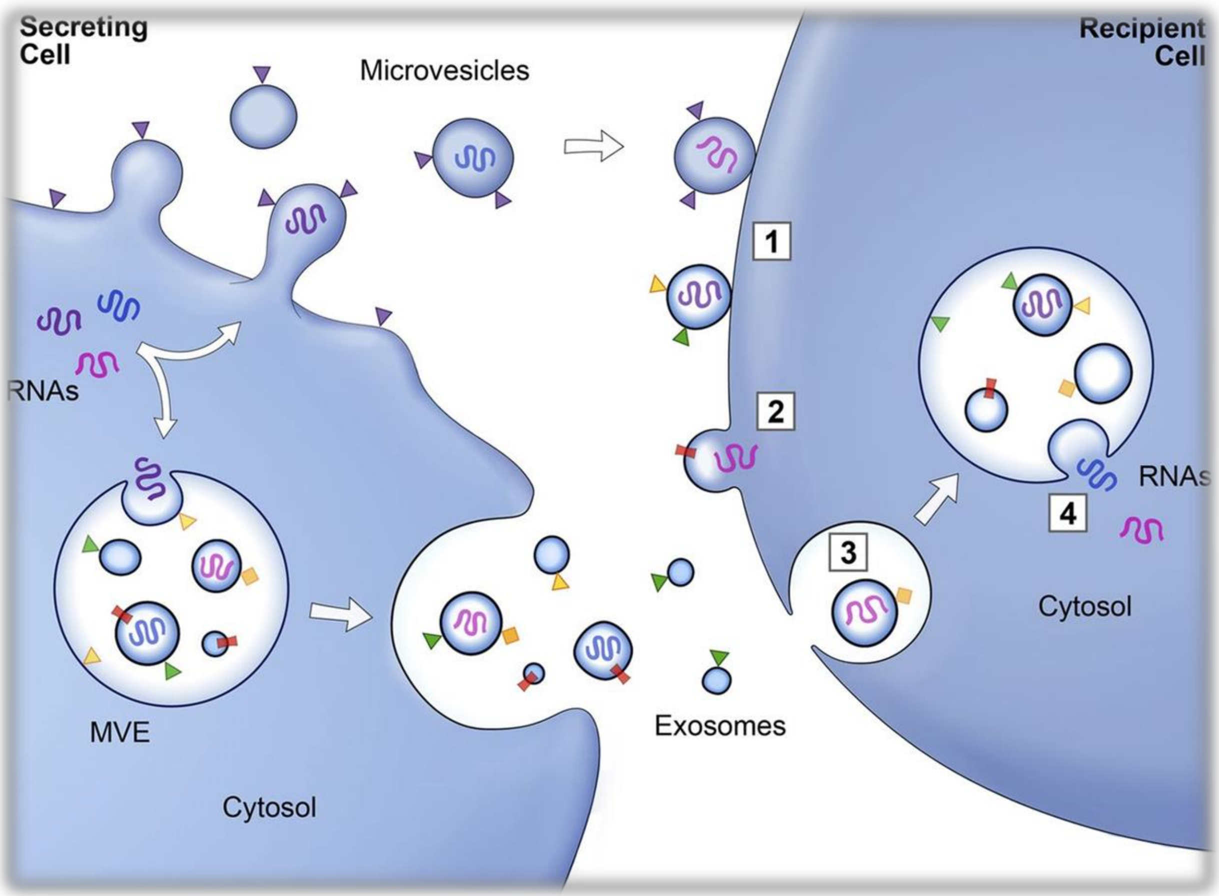
Natera panorama
Prenatal screening
Aneuplodies and common mutations



MaterniT21 Plus
Prenatal screening
Trisomy screening

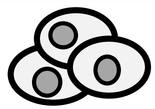
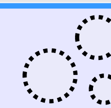
Extracellular vesicles as biomarkers for disease

EVs offer a diverse array of biomarkers for many diseases from nearly all biological fluids



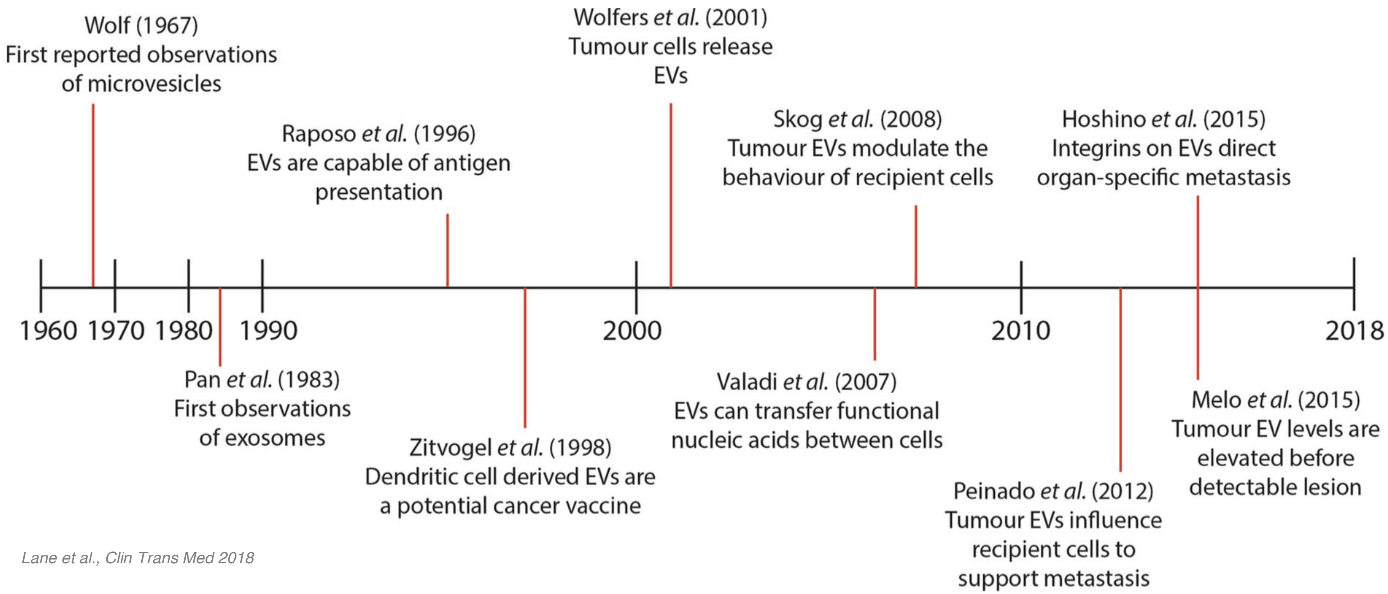
Raposo and Stoorvogel, JCB 2013

Circulating biomarkers in disease

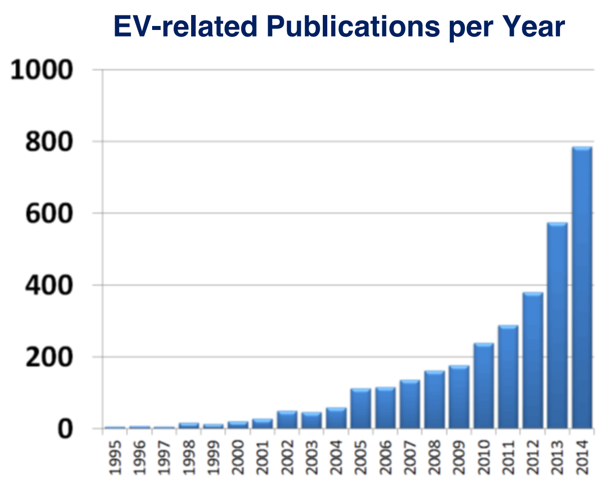
Applications	Metastatic cancer	Tumor, heart, brain, prenatal, autoimmune, infectious	
Biomarker	 CTCs	 ccfDNA	 Exosomes
Concentration	1-10 per mL	10-1,500 ng/mL	10^9-10^{12} per mL
Size	10 µm	100-10,000 bp (R_G = 5-250 nm)	30-150 nm
Challenges	Rare	High background	Small

- Abundantly available
- Broad application to disease
- Accessible in most bodily fluids
- Contain RNA, DNA, and proteins
- Protected from degradation

Timeline of extracellular vesicle discovery



Lane et al., Clin Trans Med 2018



exosome-rna.com

Extracellular vesicles in the clinic

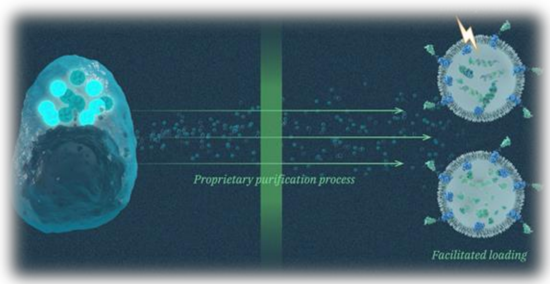
A promising new modality for diagnostics and therapeutics

- Exosome Diagnostics released exosomal RNA-based diagnostic tools for prostate cancer diagnosis and lung cancer treatment guidance
- Prostate(IntelliScore) reimbursable by Medicare
- Codiak Biosciences developing exosome-based therapeutics using engEx™ system of custom engineered exosomes with specific targeting and drug delivery

ExoDx® Prostate(IntelliScore)

ExoDx® Lung(ALK)

CODIAK

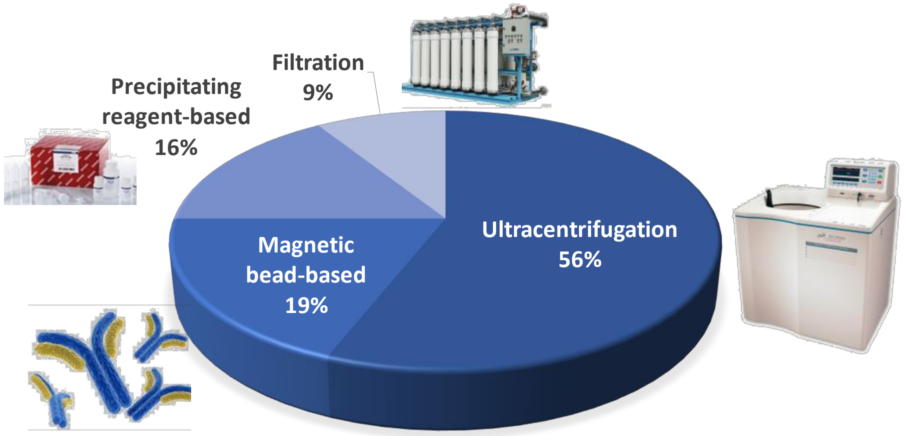


EV isolation techniques and challenges

Difficulty in isolation has hindered progress in EV applications

- Sub-fractionation of exosomes by size and chemistry is extremely limited
- Contamination high and poorly characterized
- Lacks reproducibility and standardization
- Methods are non-automated
- Methods require sophisticated lab equipment and long run time

Exosome isolation

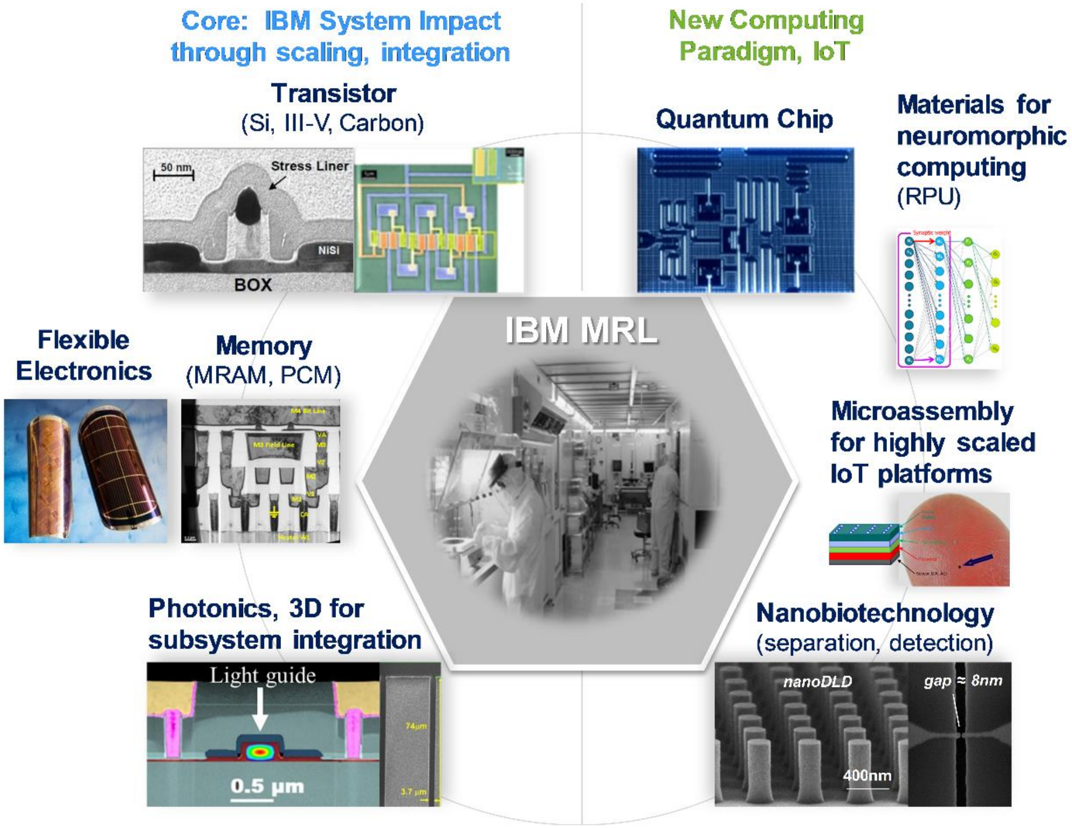


IBM Research nanofabrication strategy in MRL

Microelectronic Research Laboratory provides a foundation for core/new technology development

- State-of-the-art design, fabrication and packaging facility to rapidly prototype and integrate novel materials and structures for devices, sensors, and systems

- 40,000 sq.ft. class 100 CR, 200 mm wafer line w/ advanced CMOS and packaging capabilities



Redefining healthcare at the nanoscale

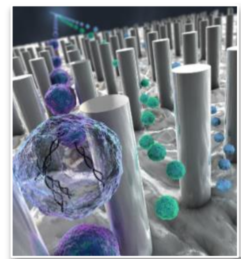
Early-stage disease detection and overall health wellness requires a new set of tools

Today

Future

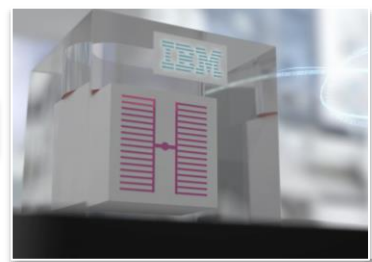
Goals

Research



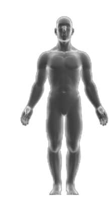
- High fidelity biomarker isolation on-chip
- Automated liquid-biopsy processing solutions

Clinical



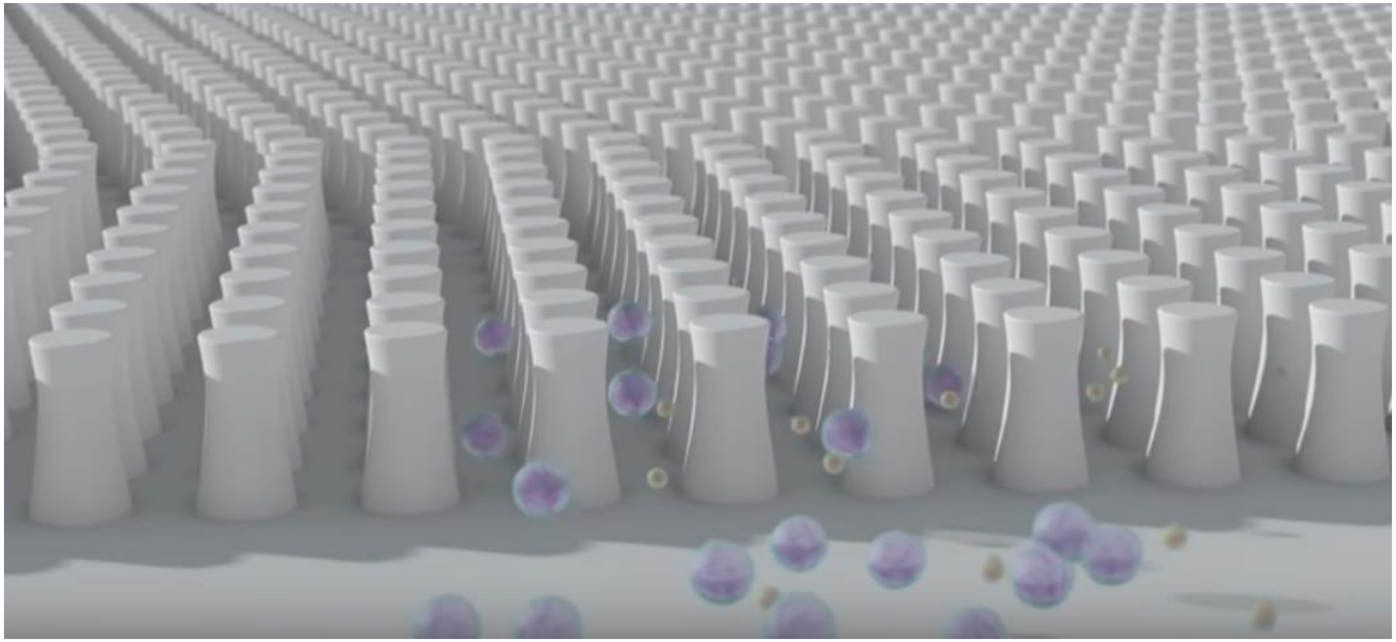
- POC diagnostic results for patients at the clinic
- Collect molecular data for IVD development

Consumer



- Regular self-monitoring of molecular profiles
- Cognitive healthcare assistant for personalized wellness

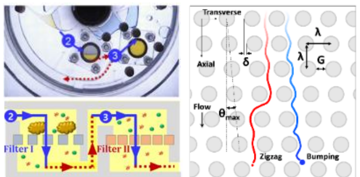
IBM nanoDLD for accelerating early detection of diseases



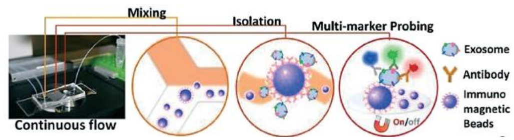
https://www.youtube.com/watch?v=FBJ02gheVFM

LOC implementations for EV isolation

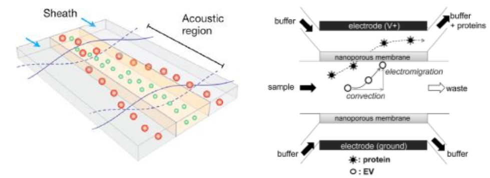
Size-based



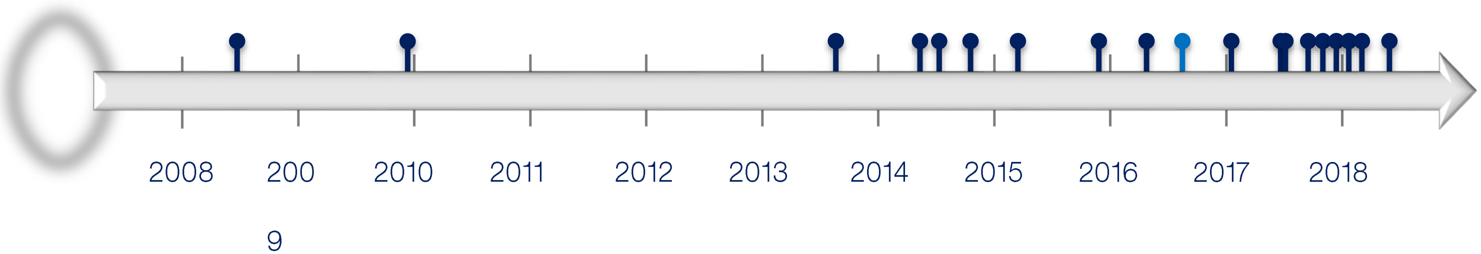
Immunoaffinity



Dynamic forces



Publication chronology on microfluidic-based exosome isolation techniques

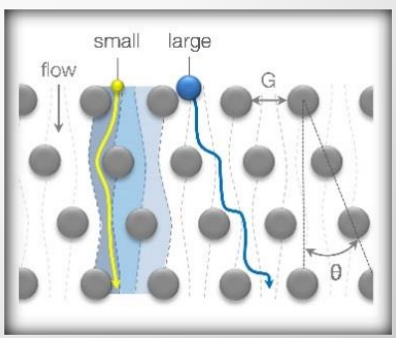


1. **Flow field-flow fractionation**
University of Medicine and Science, Korea
D. Kang et al., *J. Proteome Res.*, 7, p. 3475 (2008)
2. **Immunoaffinity-based microfluidics**
MGH, Harvard Medical School
C. Chen et al., *Lab Chip*, 10, p. 505 (2010)
3. **Ciliated micropillars**
University of Texas at Austin
Z. Wang et al., *Lab Chip*, 13, p. 2879 (2013)
4. **ExoChip (immunoaffinity assay)**
University of Michigan
S. S. Kanwar et al., *Lab Chip*, 14, p. 1891 (2014)
5. **Integrated immunomagnetic isolation**
University of Kansas
M. He et al., *Lab Chip*, 14, p. 3773 (2014)
6. **Deterministic lateral displacement**
Cornell University
S. M. Santana et al., *Biomed Microdevices*, 16, p. 869 (2014)
7. **Acoustic nanofilter (SSAW)**
MGH, Harvard
K. Lee et al., *ACS Nano*, 9, p. 2321 (2015)
8. **ExoSearch chip (immunomagnetic bead capture)**
University of Kansas
Z. Zhao et al., *Lab Chip*, 16, p. 489 (2016)
9. **Electrophoretic migration through a dialysis membrane**
Pohang University of Science and Technology, Korea
S. Cho et al., *Sensors and Actuators B*, 233, p. 289 (2016)
10. **Nanoscale DLD (nanoDLD)**
IBM Research
B. Wunsch et al., *Nature Nanotech*, 11, p. 936 (2016)
11. **Exodisc isolation of exosomes (nanofilter)**
UNIST, Korea
H.-K. Woo et al., *ACS Nano*, 11 (2), p. 1360 (2017)
12. **Alternating current electrokinetic microarray**
University of California San Diego
S. D. Ibsen et al., *ACS Nano*, 11 (7), p. 6641 (2017)
13. **Viscoelastic flows**
Chinese Academy of Sciences, China
C. Liu et al., *ACS Nano*, 11 (7), pp. 6968 (2017)
14. **Acoustofluidics (taSSAW)**
Duke University
M. Wu et al., *PNAS*, 114 (40), p. 10584 (2017)
15. **Exosome total isolation chip (ExoTIC) filtration**
Stanford University
F. Liu et al., *ACS Nano*, 11 (11), p. 10,712 (2017)
16. **Nanowires**
Nagoya University, Japan
T. Yasui et al., *Sci. Adv.*, 3, p. e1701133 (2017)
17. **HB-Chip**
MGH, Harvard Medical School
E. Reátegui et al., *Nat. Commun.*, 9, p. 175 (2018)
18. **Asymmetric flow field-flow fractionation**
Drukier Institute for Children's Health
H. Zhang et al., *Nat. Cell Biology*, 20, p. 332 (2018)
19. **ICP electrokinetic concentrator**
New York Univ., Adu Dhabi
L. S. Cheung et al., *Micromachines*, 9, p. 306 (2018)

Deterministic lateral displacement (DLD) technology

An adaptable technology offering concentration and purification of analyte in continuous flow

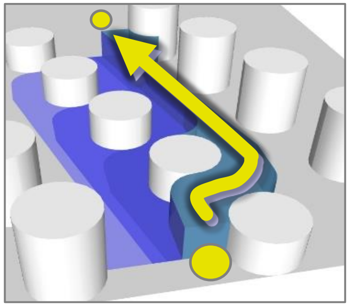
Principle of Operation



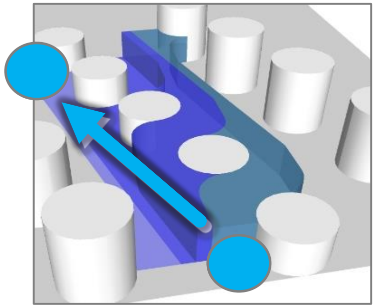
$\theta = \tan^{-1}(\delta/\lambda) = \tan^{-1}(\varepsilon)$

$D_C \approx 1.2G\varepsilon^{0.5} \approx 0.38\ G$

$D_P \leq D_C$

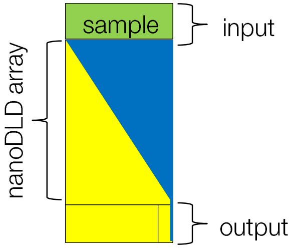


$D_P > D_C$

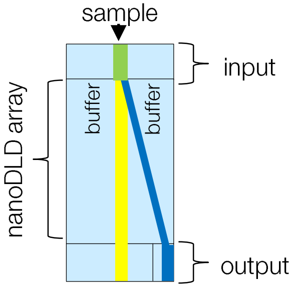


Operational Modalities

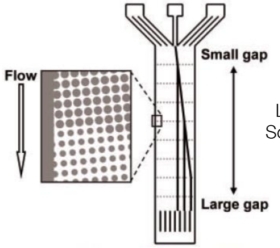
Enrichment and concentration



Purification and isolation



Staged separation

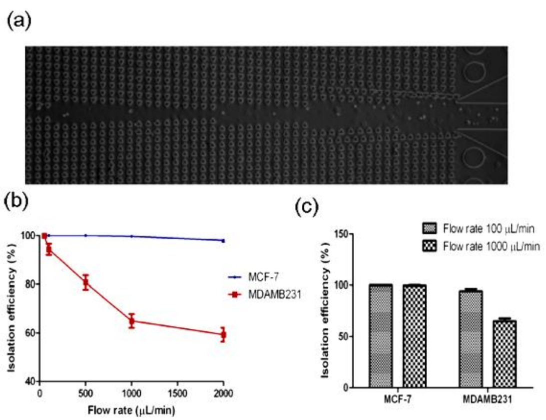


L. R. Huang, *et al.*, Science, 304, p. 987 (2004)

Deterministic lateral displacement (DLD) technology

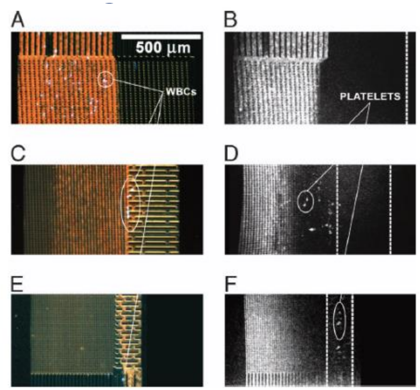
A diverse set of applications in biology have been demonstrated at the micron scale

CTC Isolation



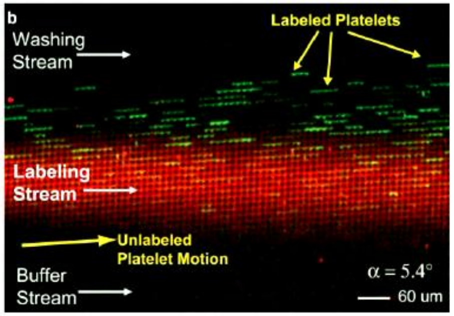
Z. Liu, *et al.*, Biomicrofluidics, 7, p. 011801 (2013)

Blood Cell



J. A. Davis, *et al.*, PNAS, 103, p. 14779 (2006)

Lysis and Labeling

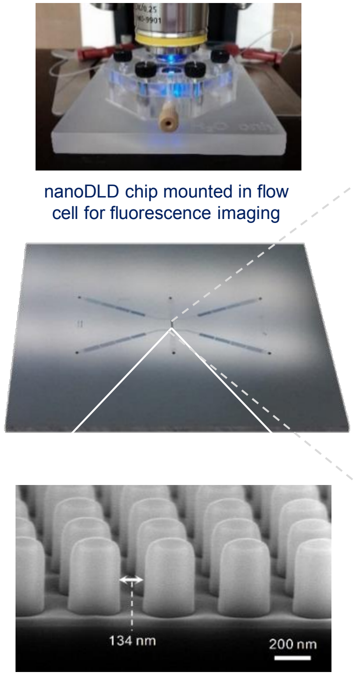


K. J. Morton, *et al.*, Lab Chip, 8, p. 1448 (2008)

nanoDLD separation of nanoscale colloids

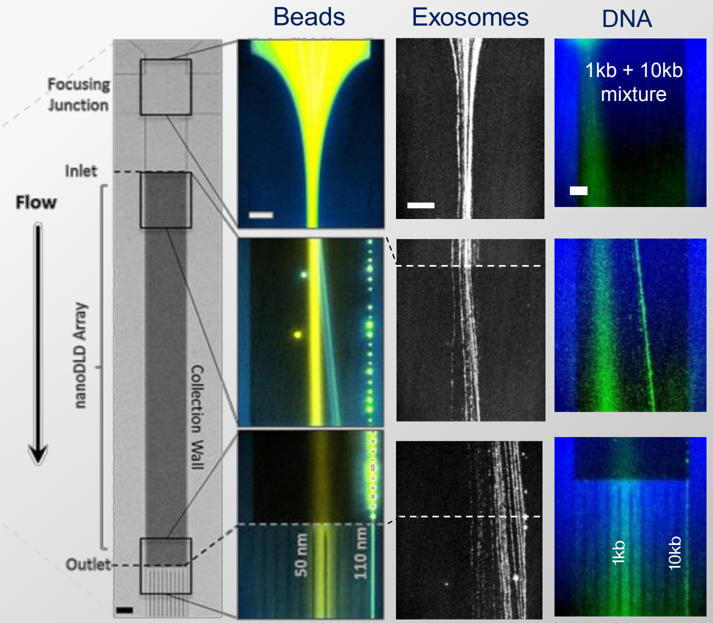
nanoDLD-based separation is applicable to a variety of nanoscale materials

Test Platform



nanoDLD chip mounted in flow cell for fluorescence imaging

Separation down to 20 nm with nanoscale resolution

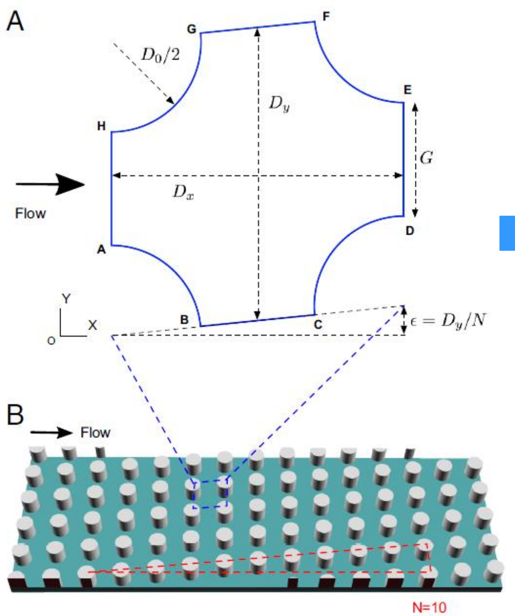


B. Wunsch *et al.*, Nature Nanotech, 11, pp. 936 (2016)

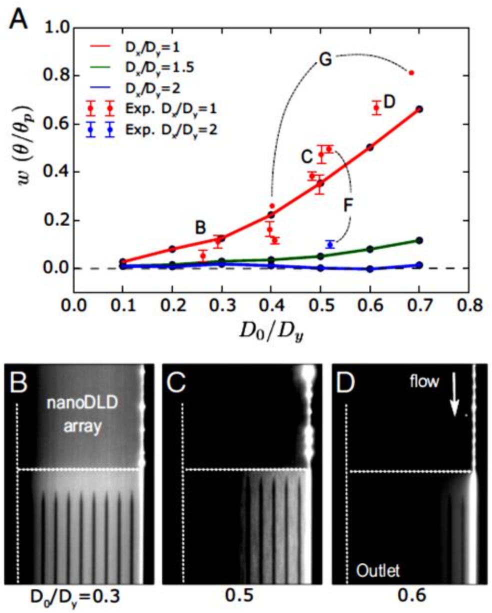
Understanding and utilizing the physics of nanoDLD

Partial displacement modes between zigzag and bump conditions can be modelled

Simulation model



Experimental verification



S.-C. Kim *et al., PNAS,* 114, pp. E5034 (2017)

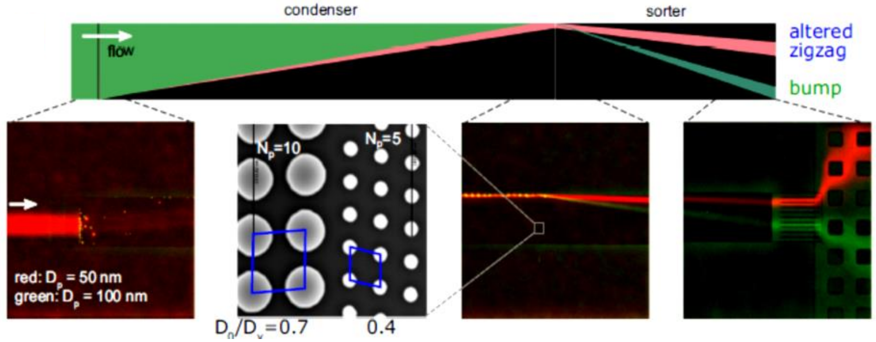
- Unified theoretical framework describes multi-modal trajectories
- D_0 / D_y ratio provides a mechanism for tuning displacement behavior

Understanding and utilizing the physics of nanoDLD

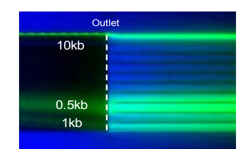
Partial displacement behavior can be exploited to offer devices with new functionality

- Altered trajectories provide a route toward dilution-free processing
- Multi-mode separation of DNA achieved in a multi-staged array series

Full-width separation with no dilution

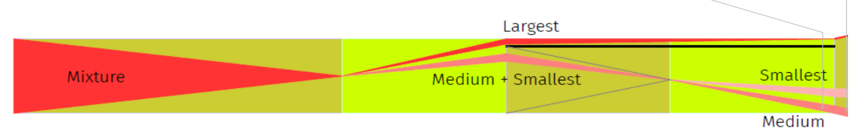


S.-C. Kim *et al.*, *PNAS*, 114, pp. E5034 (2017)



Multi-stage separation

full-width injection



Advancing nanoDLD as a sample preparation technology

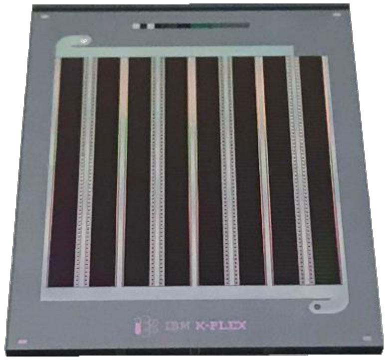
Parallel array processing of sample fluids greatly enhances throughput volumes

Prototyping

- Observational layout
- Single array prototyping
- Throughput ~ 0.2μL/hr @ 10 bar

Large-Scale Integrated Designs

lightly scaled



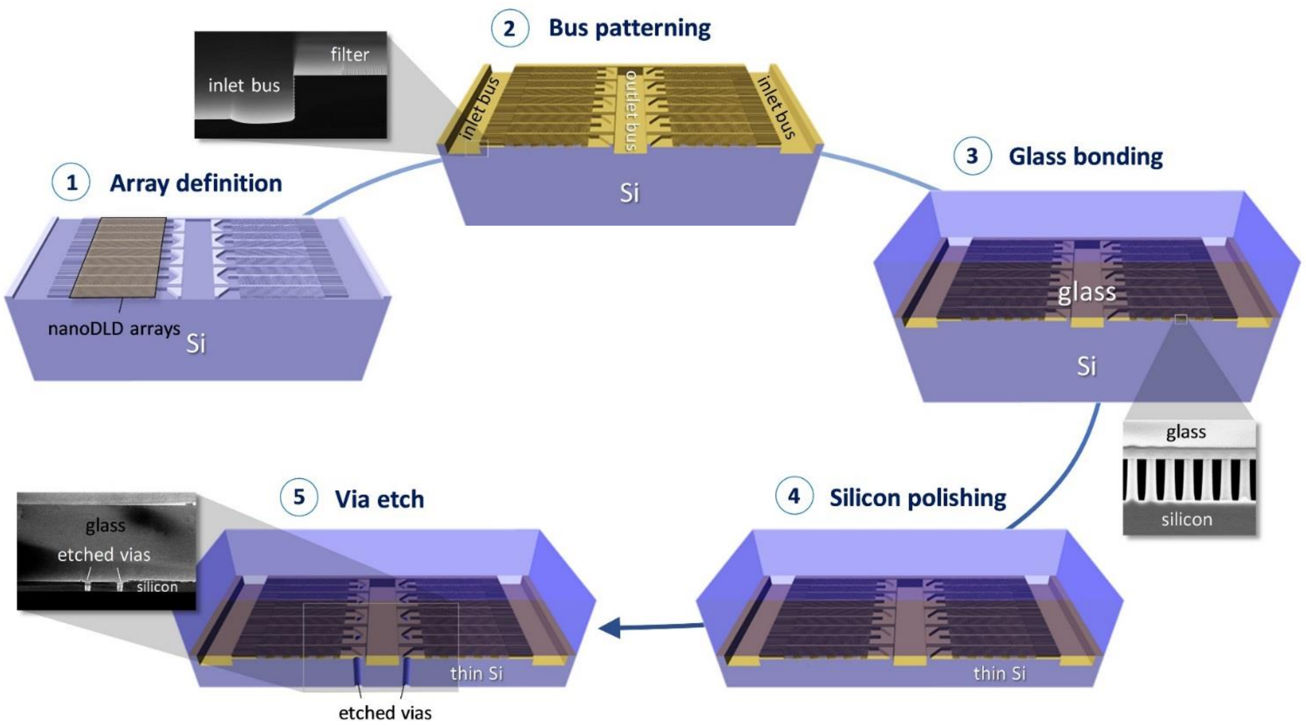
- 1,024 arrays in parallel
- Fluid rates: ~ 140μL/hr @ 2 bar
  ~ 900μL/hr @ 10 bar

moderately scaled

- 7,600 arrays in parallel
- Fluid rate: ~ 940μL/hr @ 2 bar

Technology designed for large-scale manufacturing

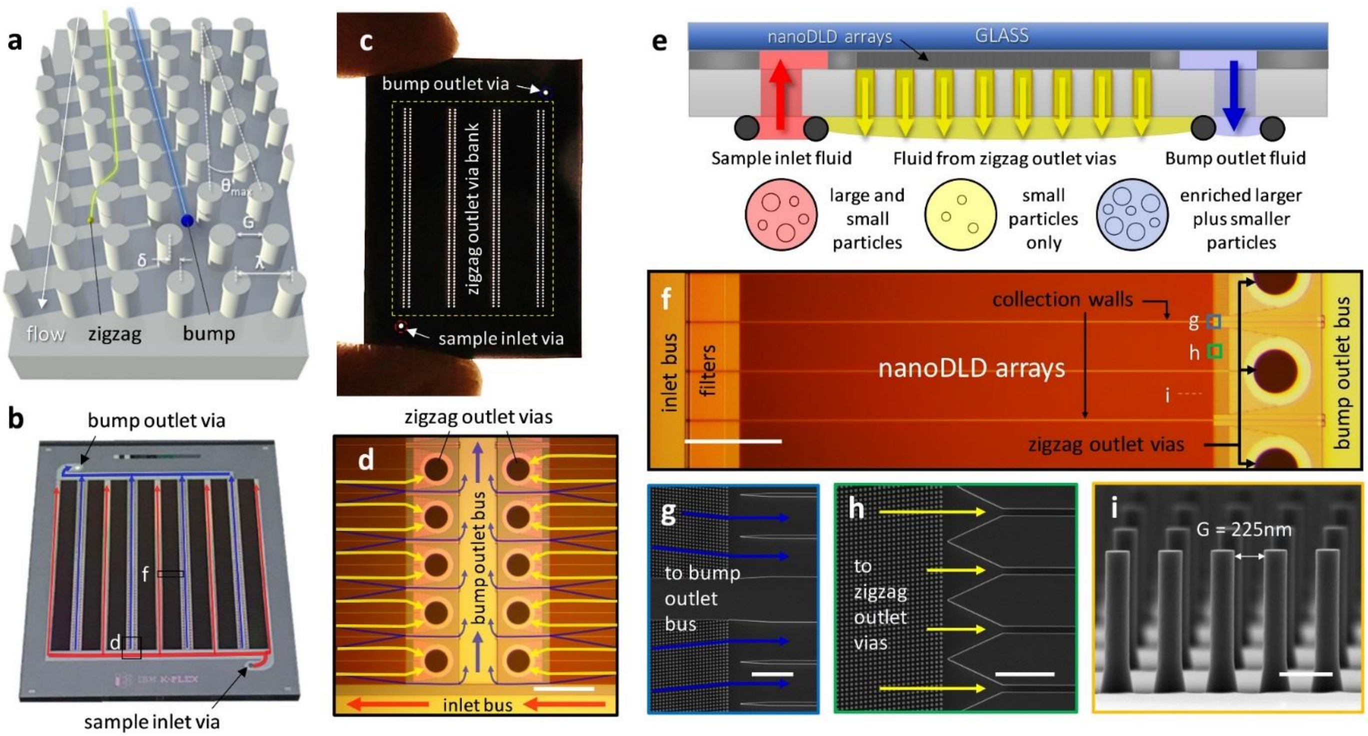
Integrated nanoDLD chip process flow designed for scalability



J. T. Smith *et al.*, Lab Chip, accepted

Multiplexed array chip architecture and operation

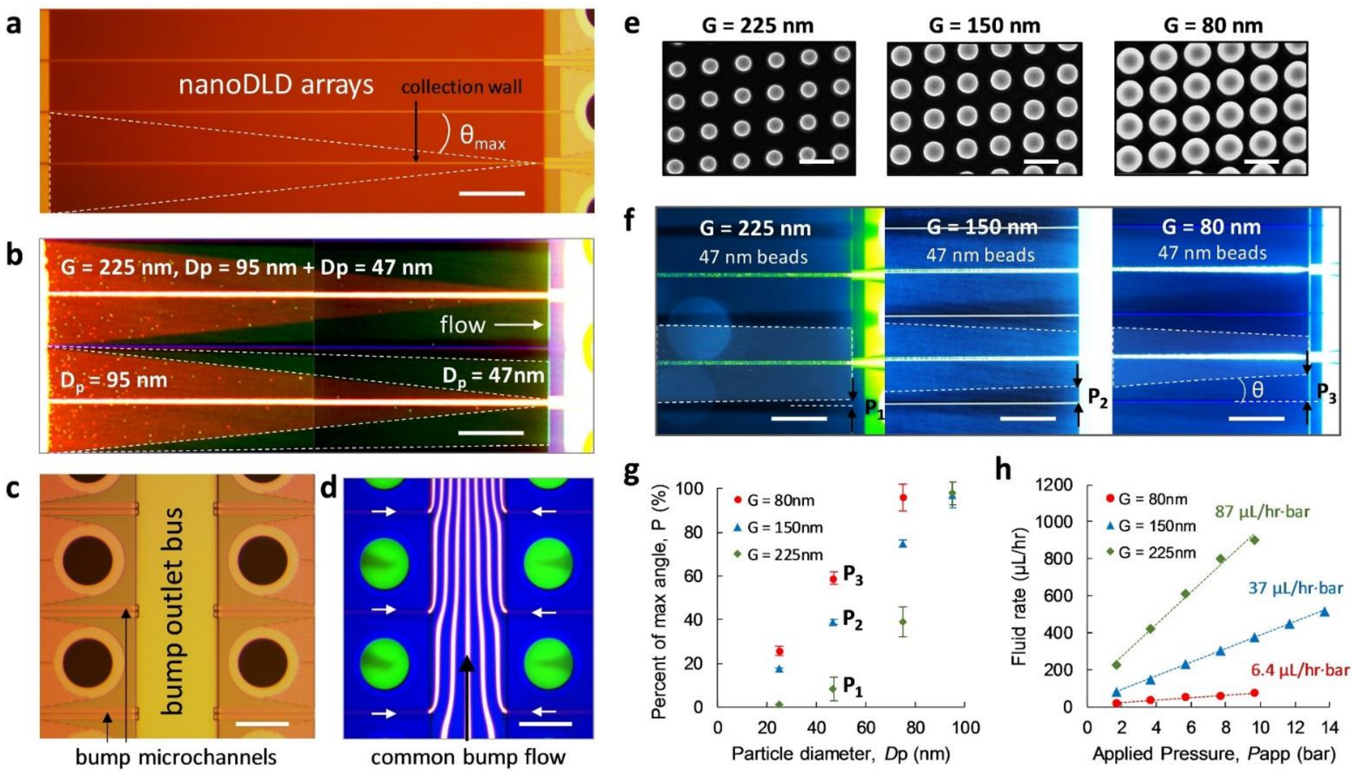
Common collection reservoirs permit efficient extraction of separated material



J. T. Smith *et al.*, Lab Chip, accepted

In situ operation and calibration of integrated arrays

Parallel array systems preserve displacement behavior observed in single array chip designs



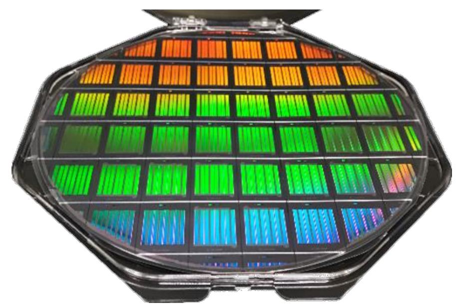
J. T. Smith *et al.*, Lab Chip, accepted

Automated liquid biopsy processing

Integrated array architectures to push-button processing

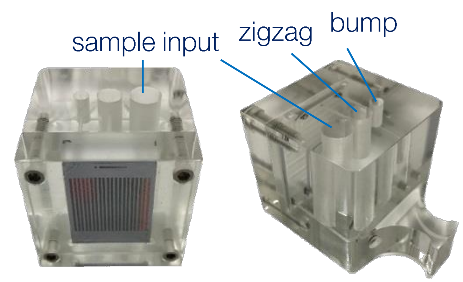
Integrated array designs

Wafer-level processing

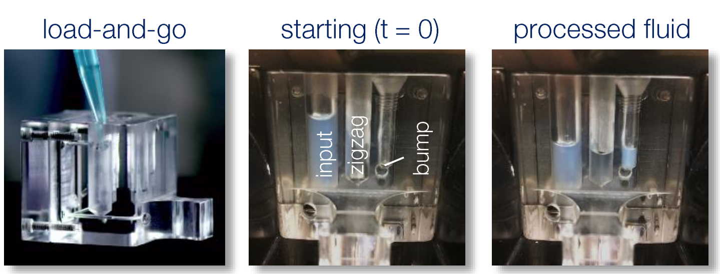


Processed 200 mm wafer

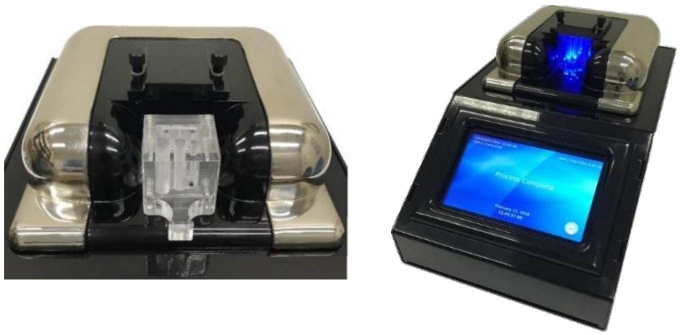
Cartridge-based sample loading



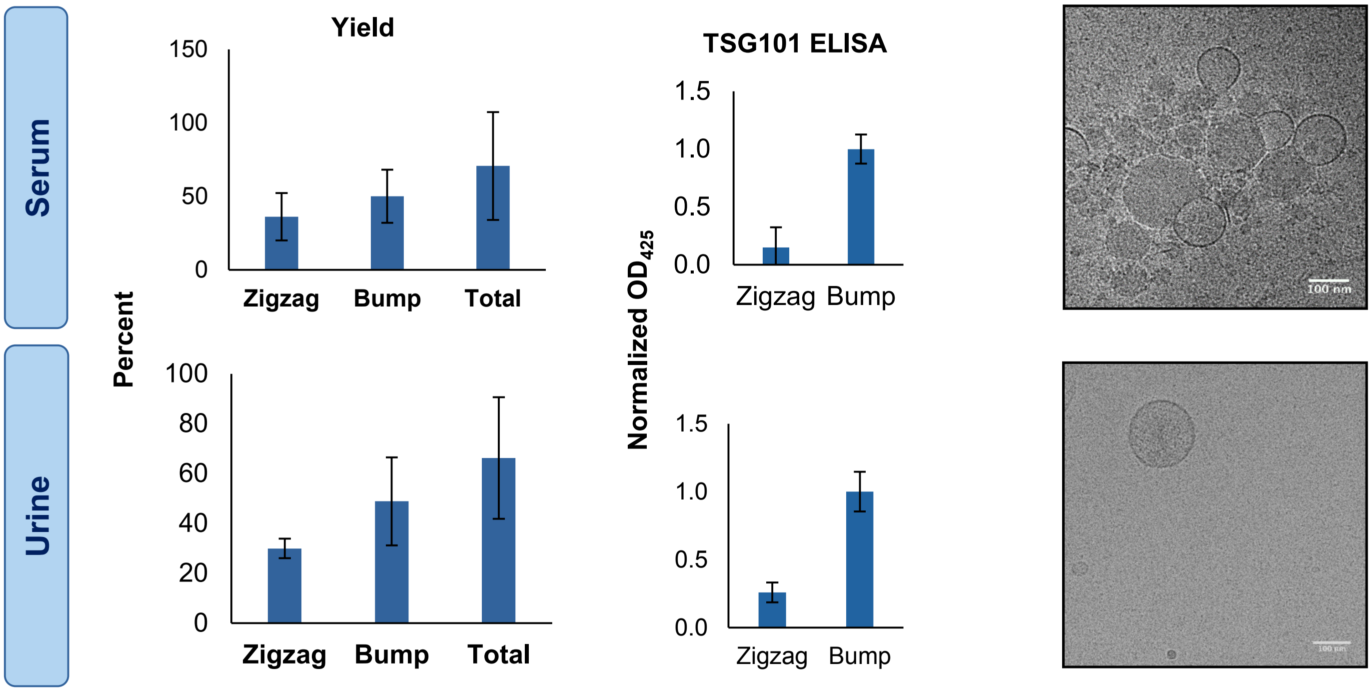
Demonstration of sample processing



Benchtop ARES prototype system



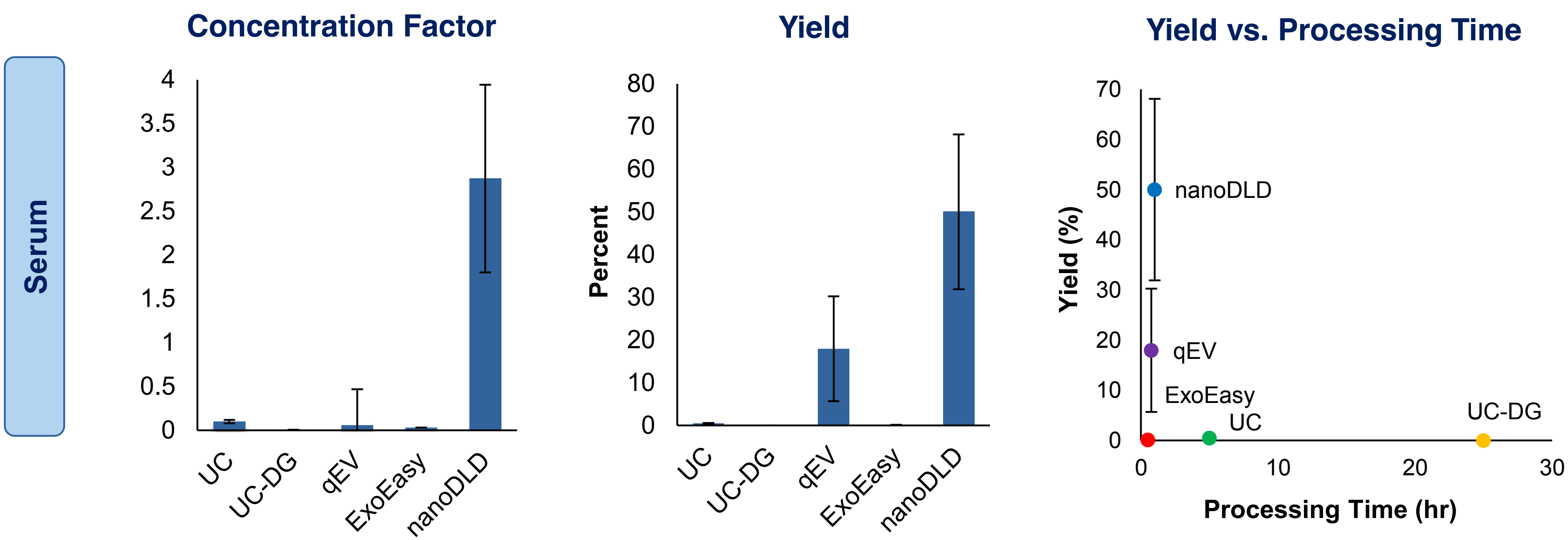
nanoDLD isolation of urine and serum EVs for off-chip analysis



J. T. Smith *et al.*, Lab Chip, accepted

Benchmarking of nanoDLD exosome isolation

Improving yield and processing time with smaller volumes



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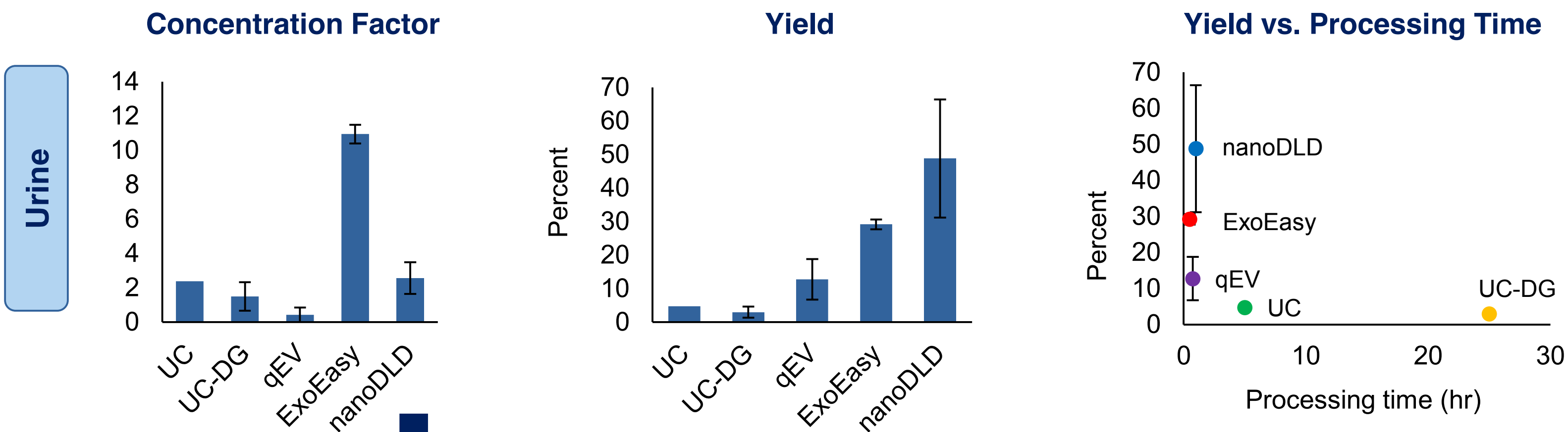
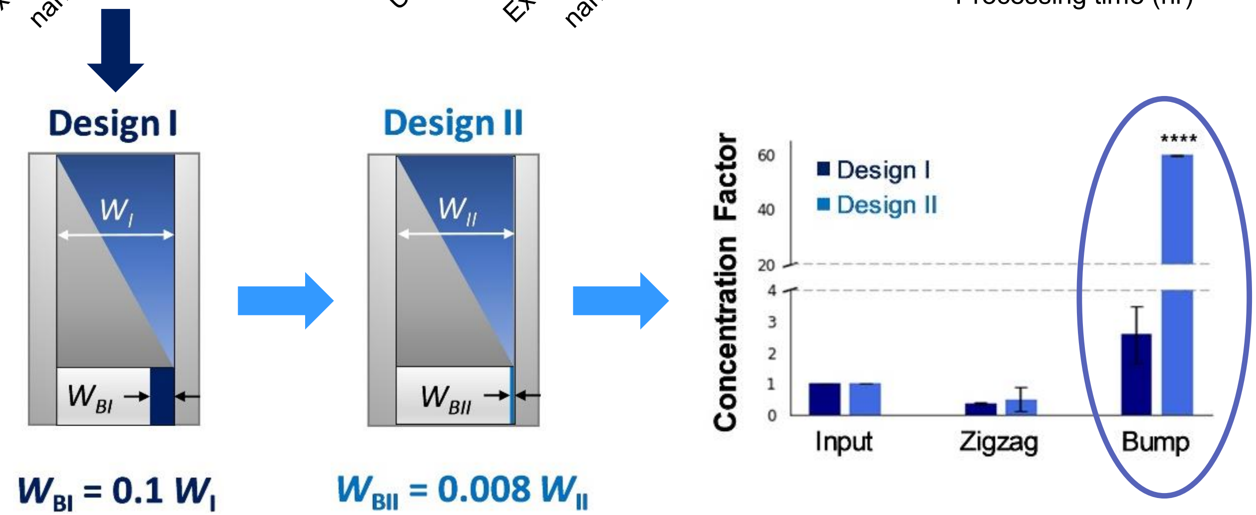
Increasing concentration through chip engineering

Decreasing bump volume increases EV concentration





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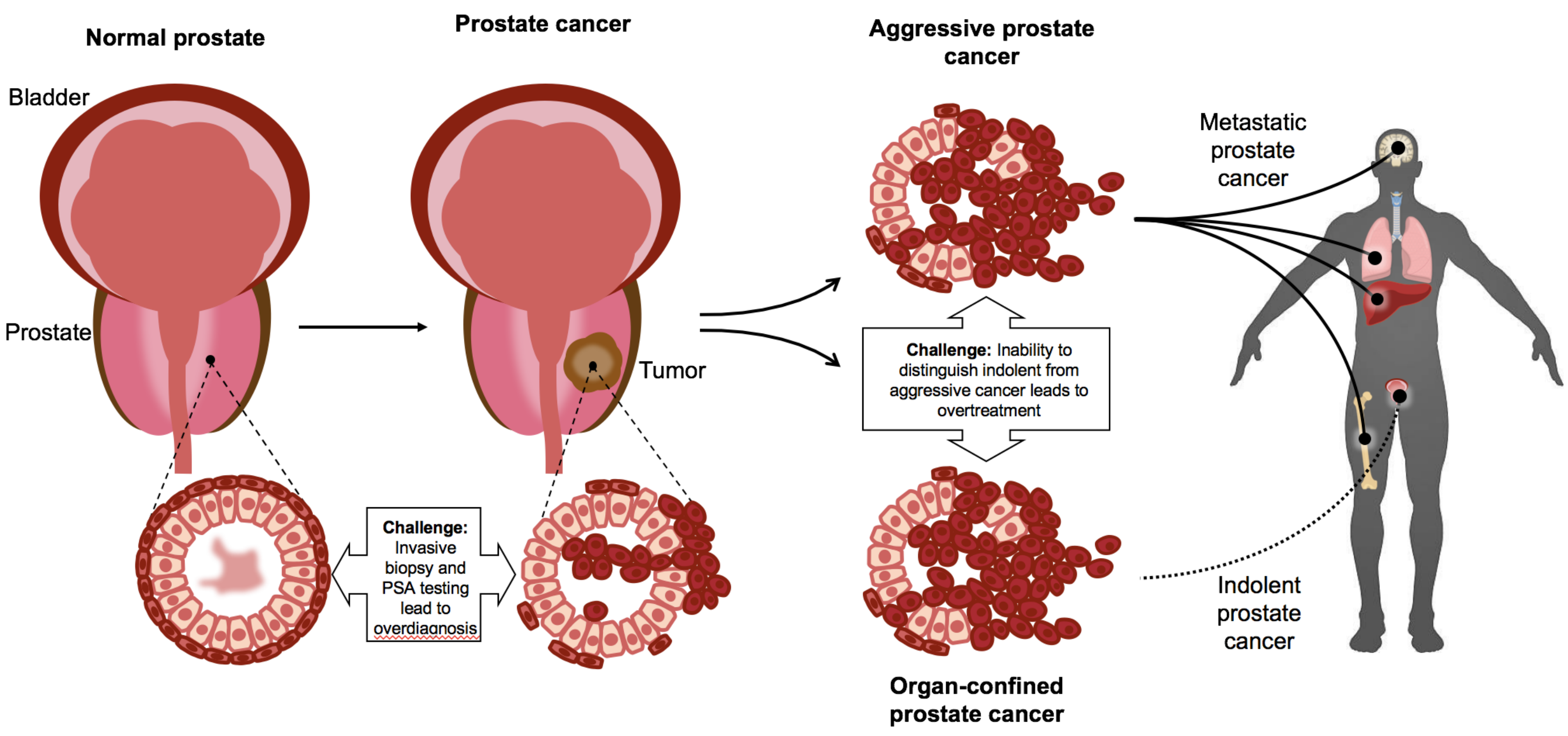
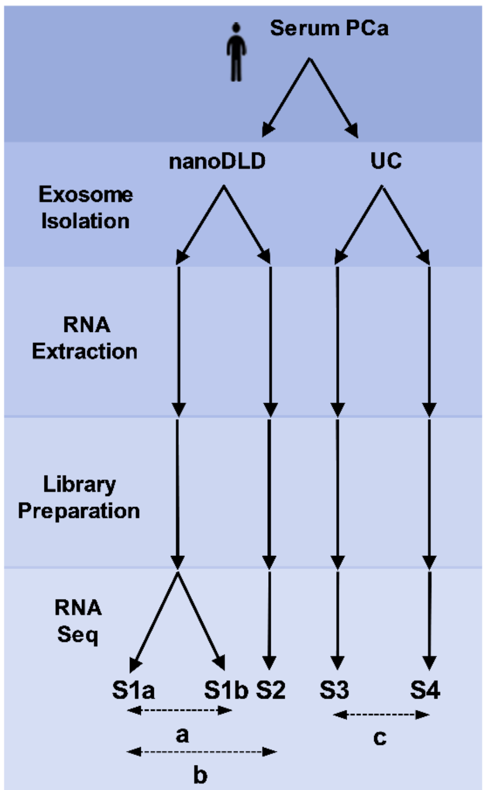
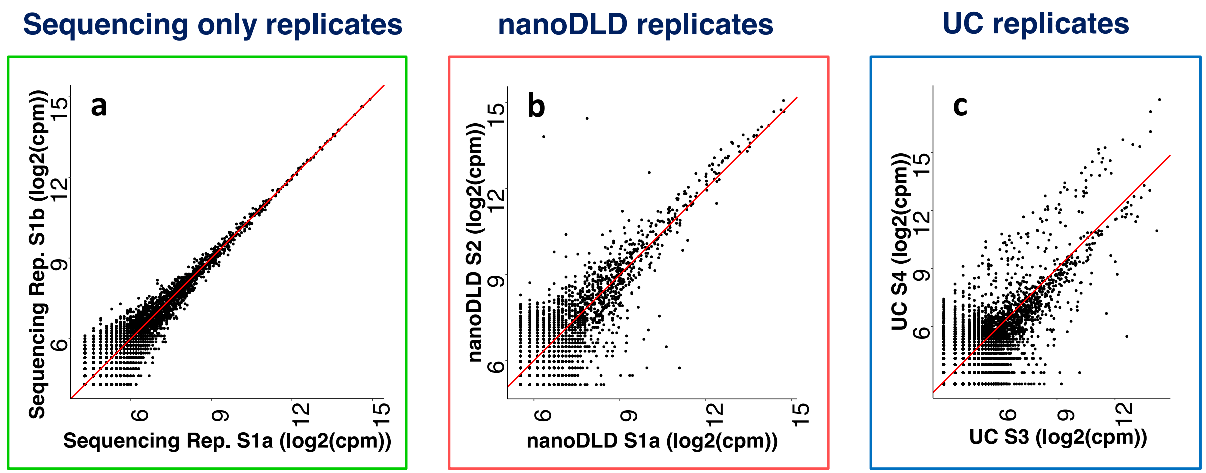
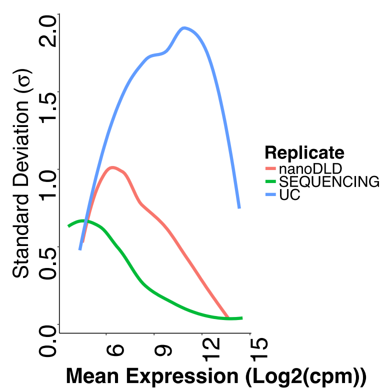
Applying nanoDLD to prostate cancer prognosis and treatment



nanoDLD-isolated RNA shows greater sequencing reproducibility







- Sequencing contributes minimal sequencing variability
- nanoDLD shows reduced variability compared to UC

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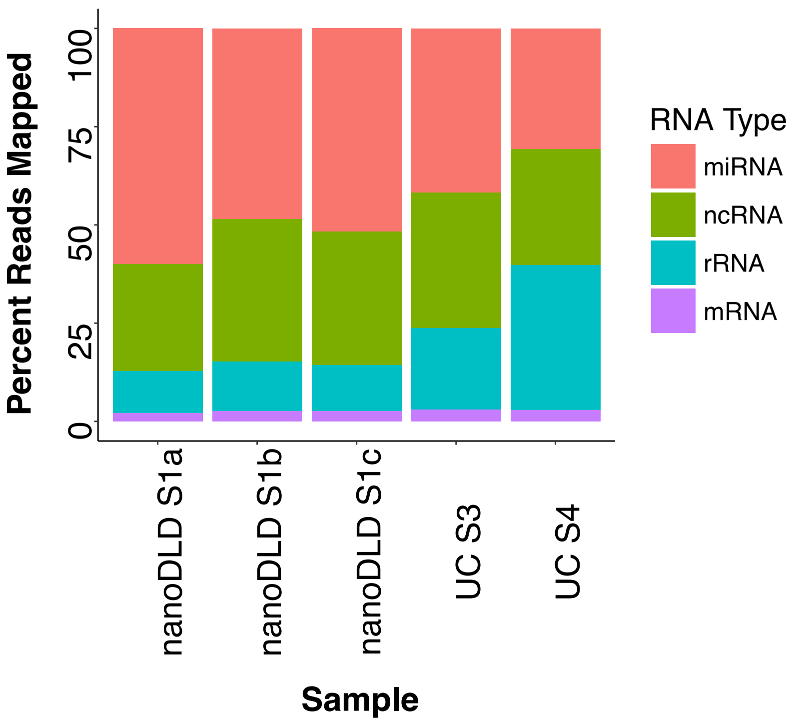
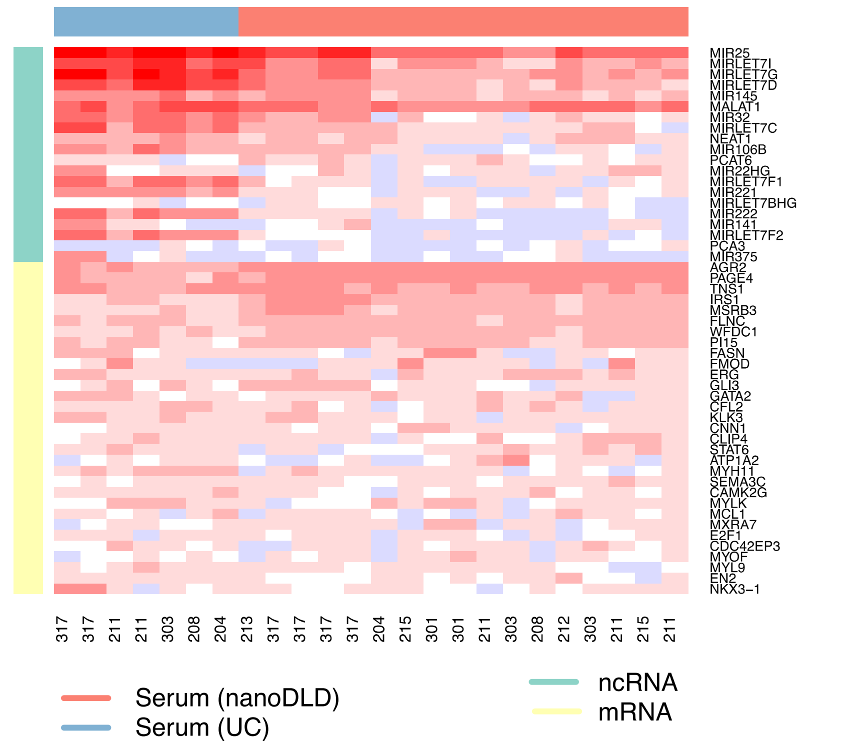
Serum exosomes as prostate cancer (PCa) biomarkers

Candidate PCa markers are enriched in nanoDLD-isolated serum exosomes of PCa patients





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Conclusions

- Extracellular vesicles offer a diverse array of biomarkers for disease
- EV isolation presents the greatest challenge in clinical applications
- nanoDLD offers improved concentration, yield, and processing time over existing EV isolation methods
- nanoDLD isolates EV RNA with greater reproducibility than UC and isolates known prostate cancer RNA biomarkers

Future work

- Develop prostate cancer RNA biomarker panel to differentiate indolent and aggressive disease
- Engineer nanoDLD chips to increase purity
- Partner with new collaborators for EV applications and beyond