



# **Microfluidics for the Interrogation of Circulating Biomarkers in Cancer Patients**

Shannon Stott, PhD

Assistant Professor, Harvard Medical School

Massachusetts General Cancer Center

Arab-American Frontiers of Science, Engineering and Medicine  
November 3<sup>rd</sup>, 2017

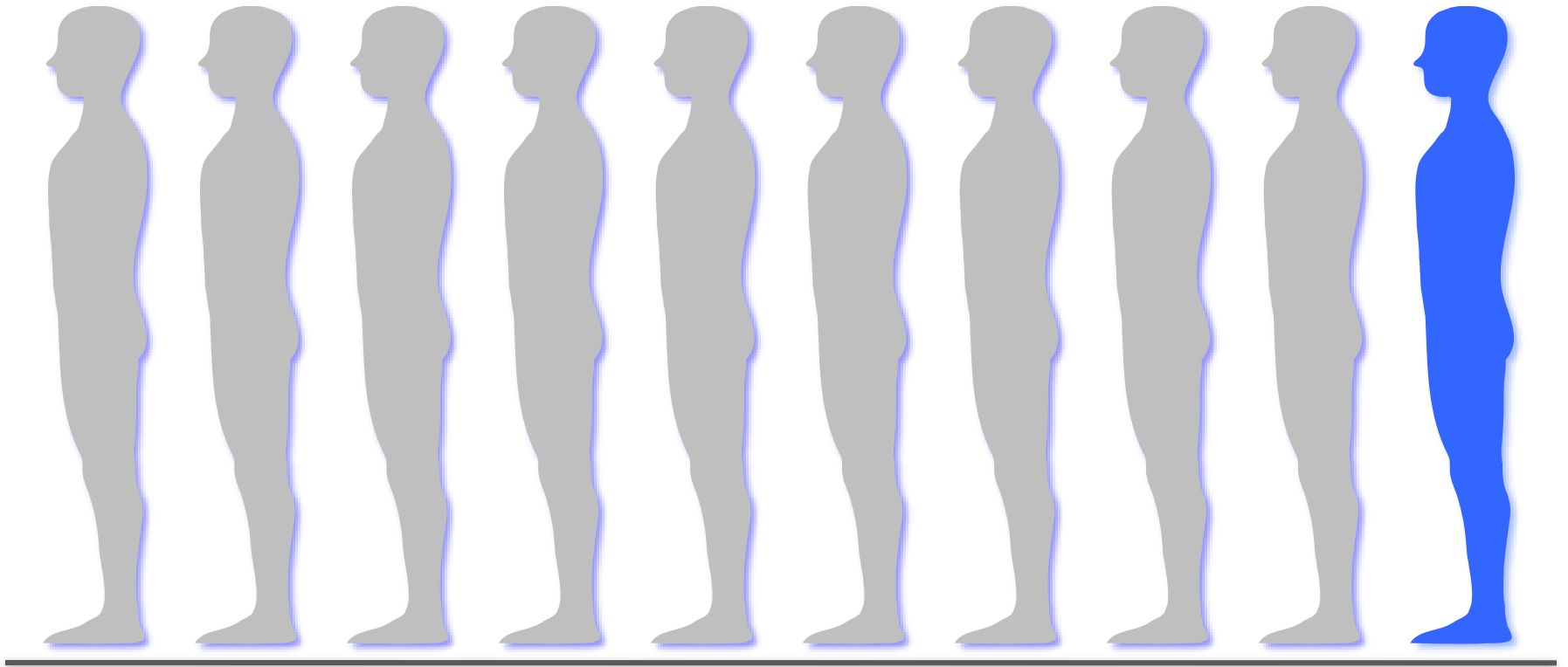
# Putting Cancer into Perspective

Number of deaths from cancer each **day** in the US:

1,600 deaths / day (2016)

Number of deaths from cancer each **day** in the world:

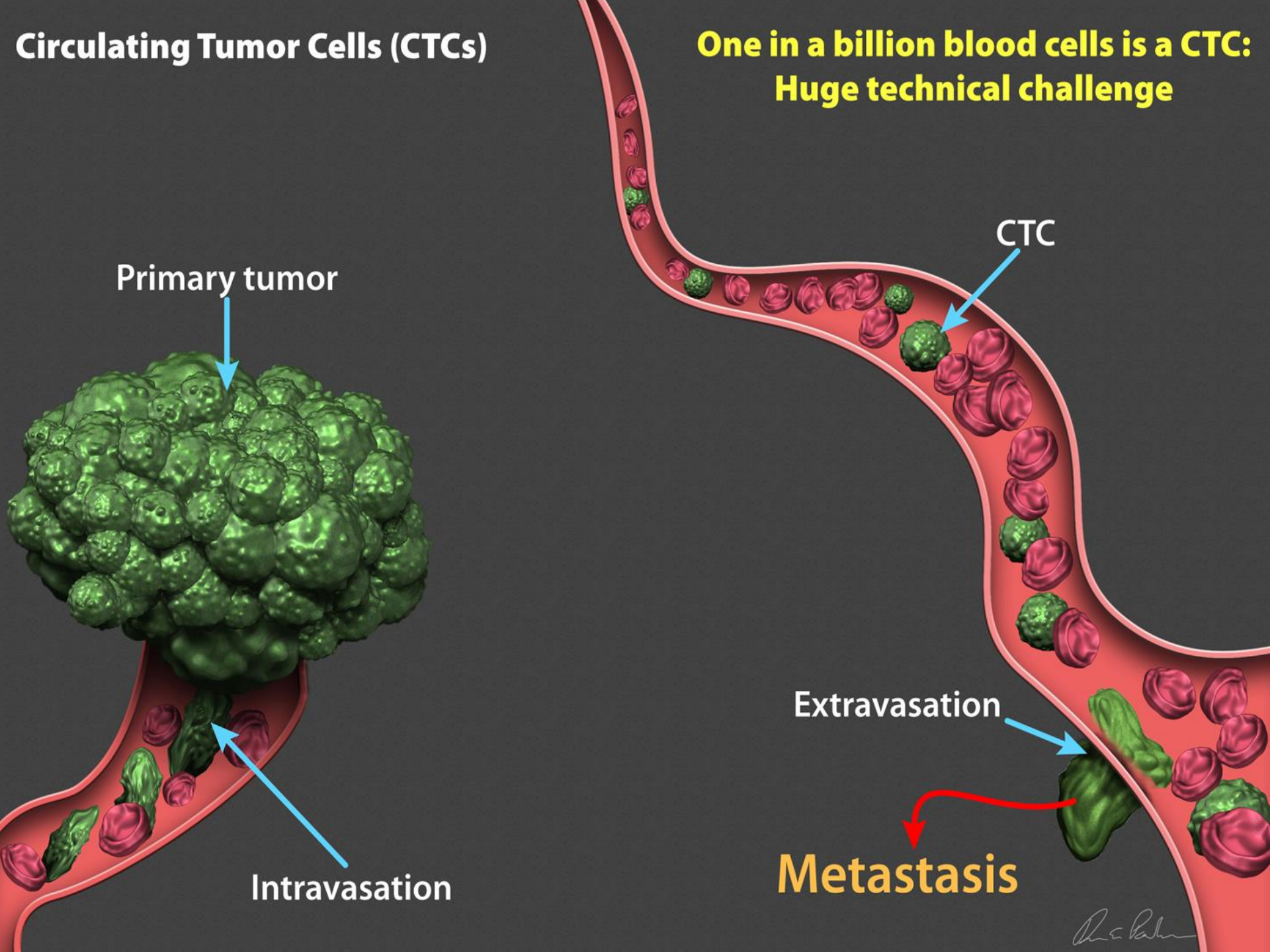
24,000 deaths / day (2016)



9 out of 10 deaths in cancer is due to  
**metastasis!**

**Circulating Tumor Cells (CTCs)**

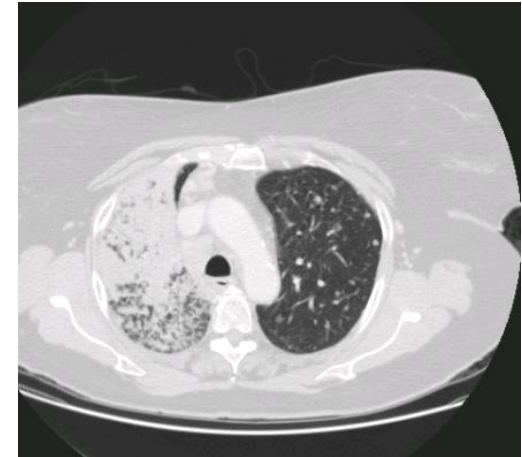
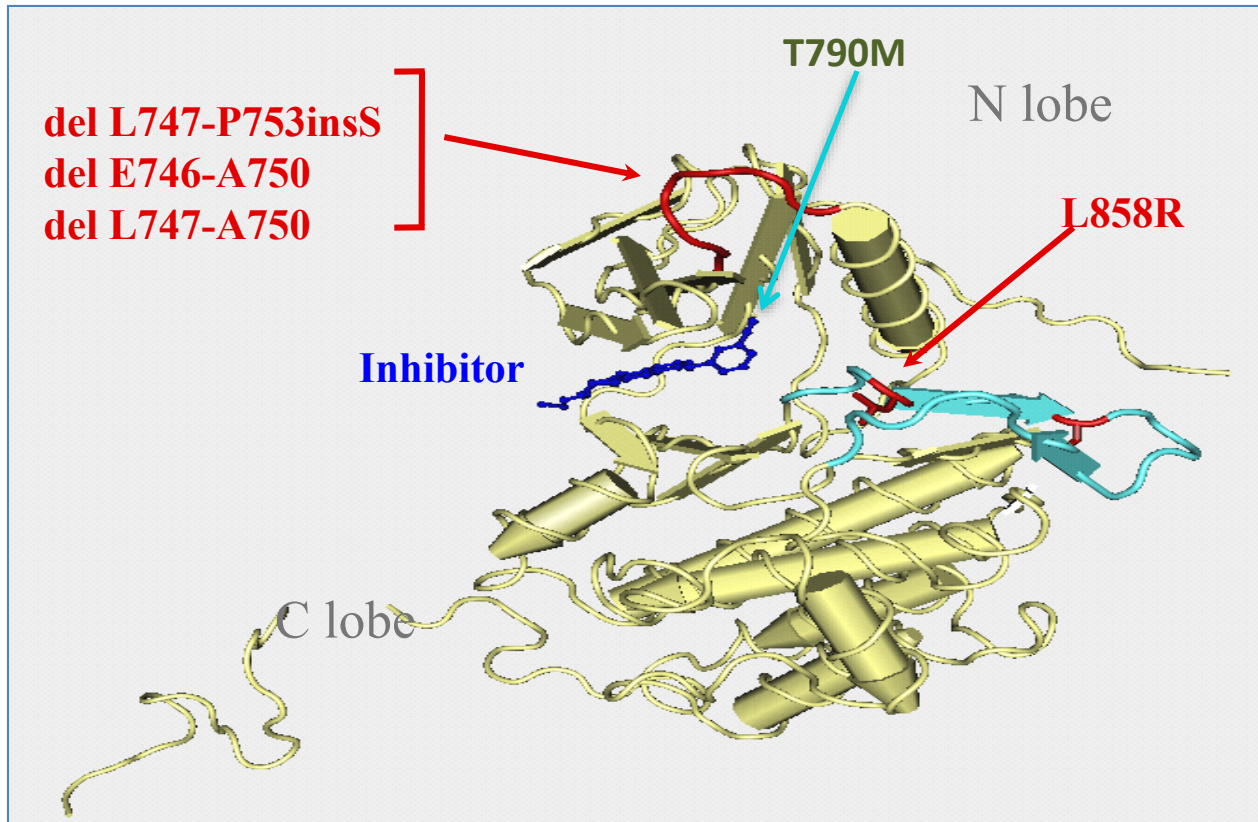
**One in a billion blood cells is a CTC:  
Huge technical challenge**



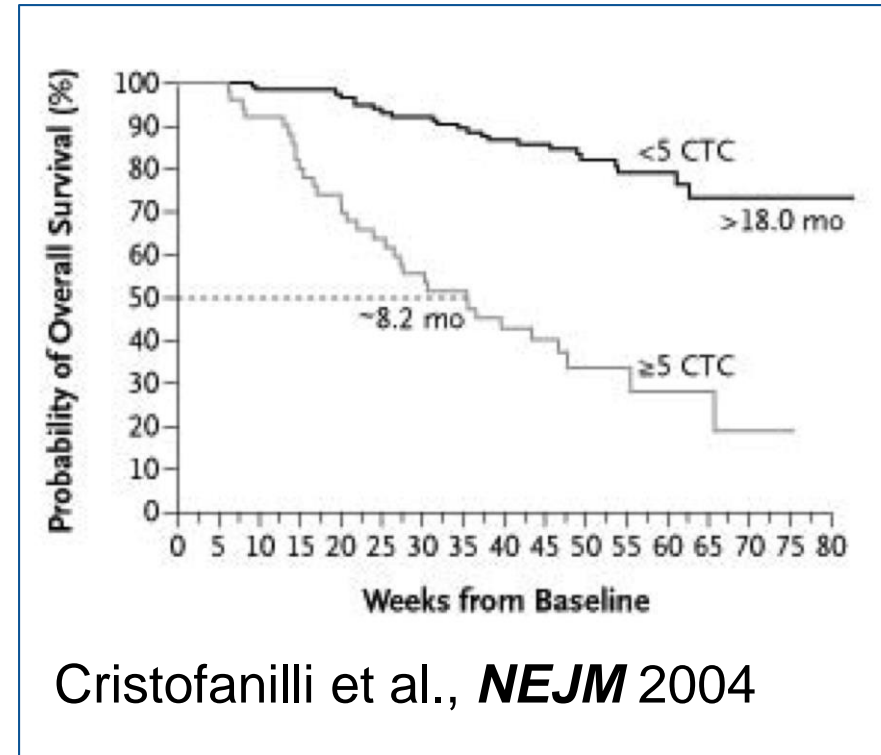
*Dr. Balu*

# “Liquid Biopsy” in Targeted Therapy

- Detection of sensitizing EGFR mutations
- Acquisition of drug resistance



# 2007: CTCs are found in ~20% of metastatic cancers using modified FACS and MACS, and filters



Cristofanilli et al., **NEJM** 2004

## CTCs useful mostly for prognosis



Why are the CTCs observed only in a small percentage of metastatic patients?

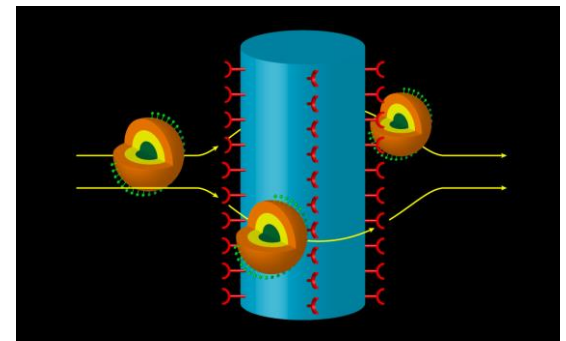
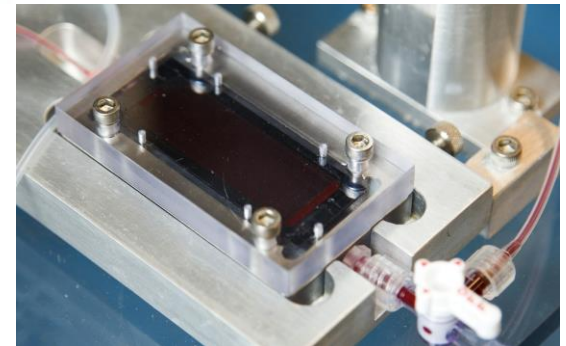
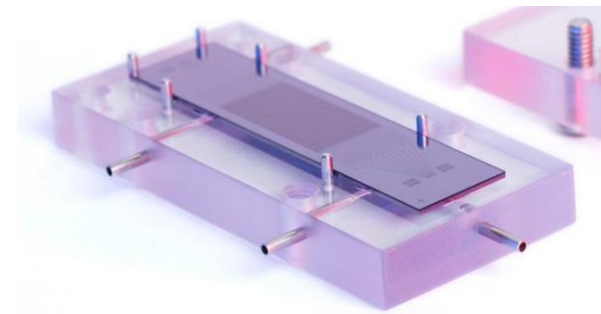
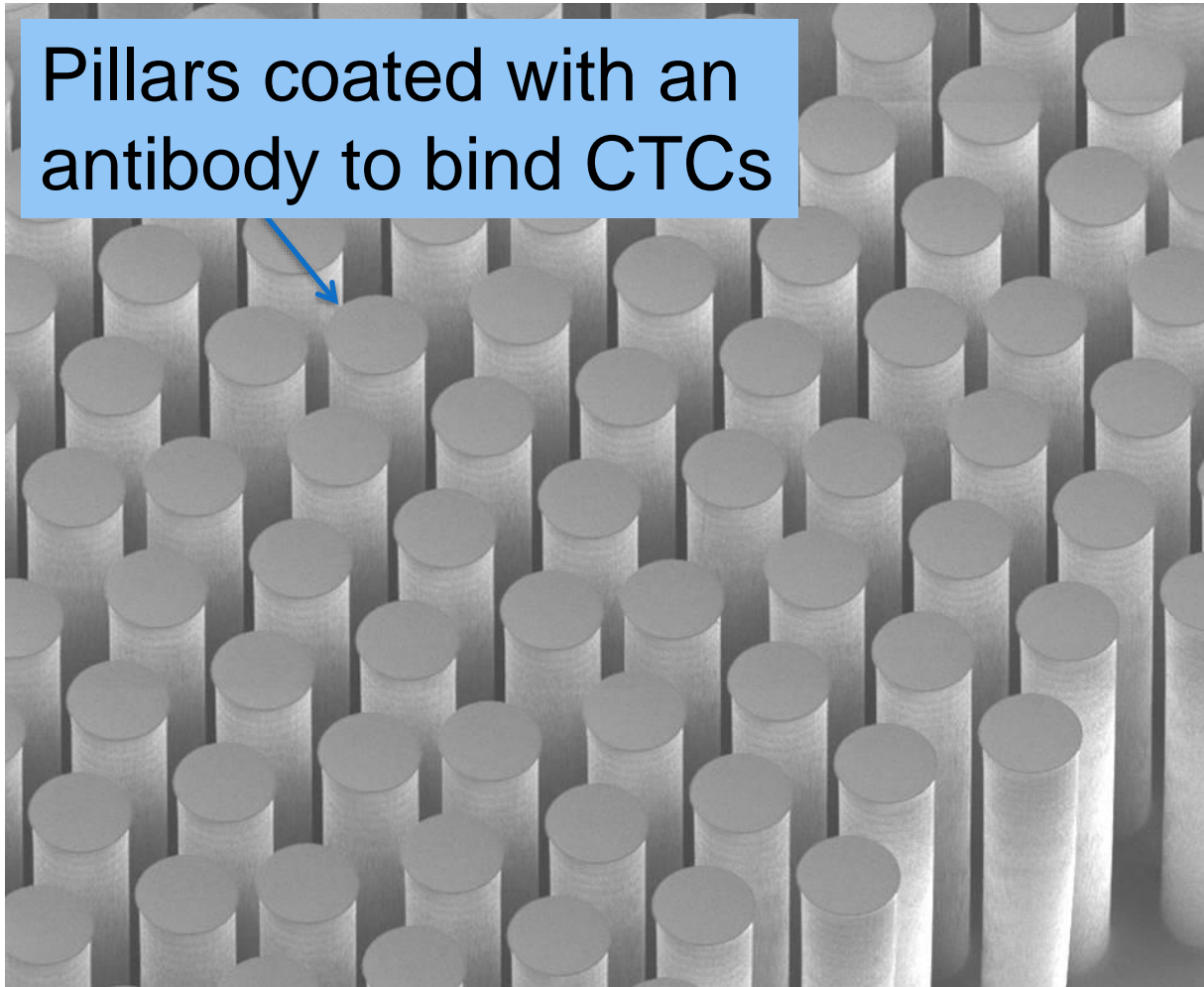
**Hypothesis:**

Biology vs. Technology

# 1<sup>st</sup> Generation Micropost CTC-Chip

## Validation Studies

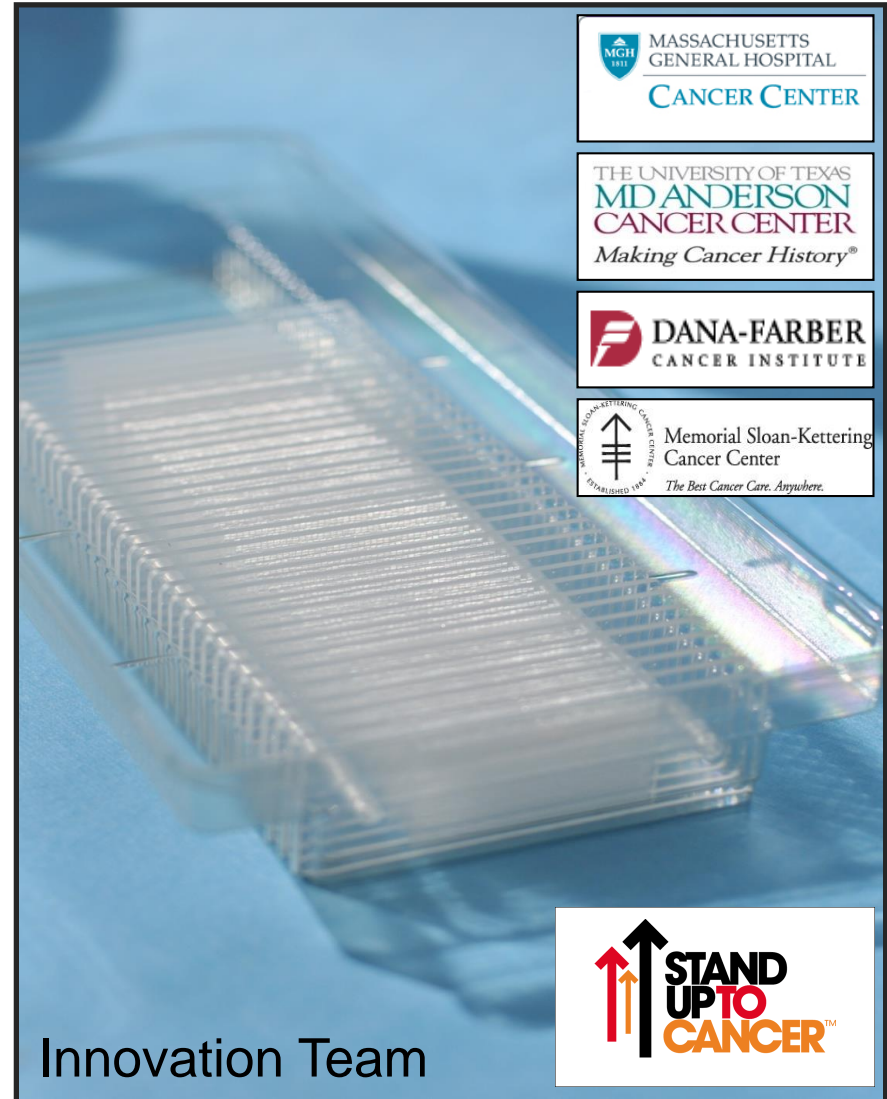
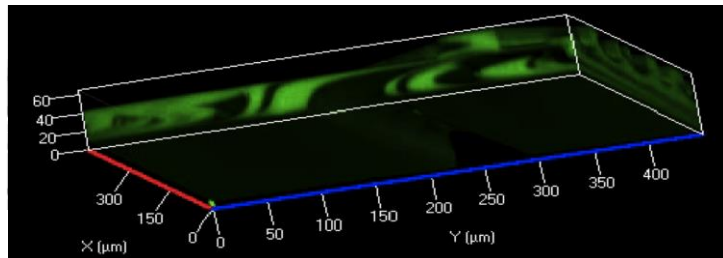
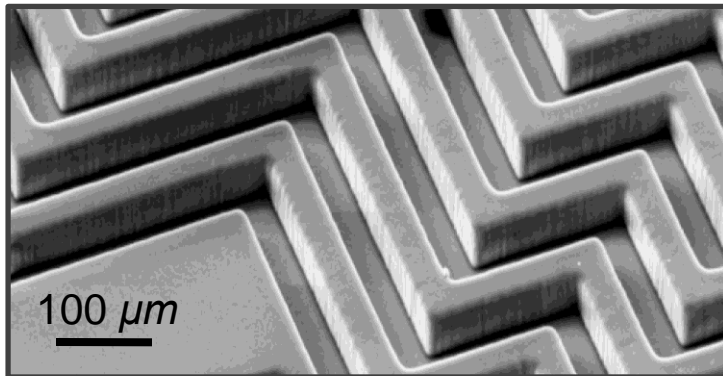
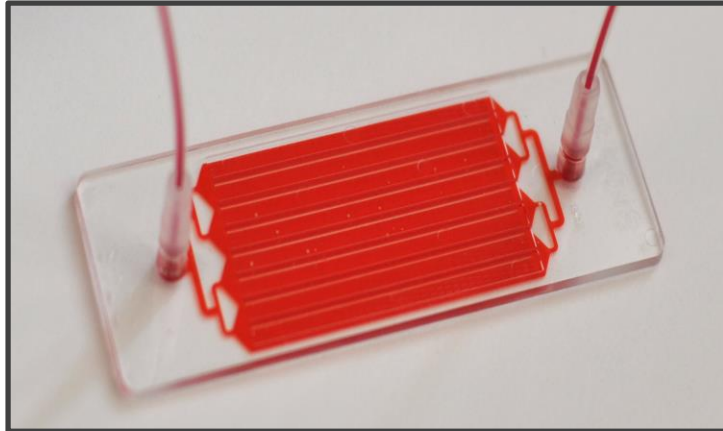
Pillars coated with an antibody to bind CTCs





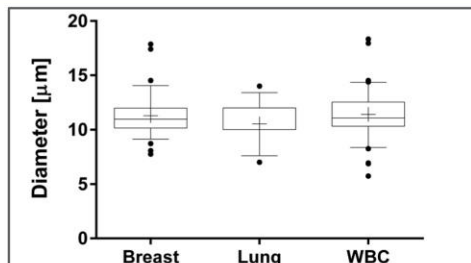
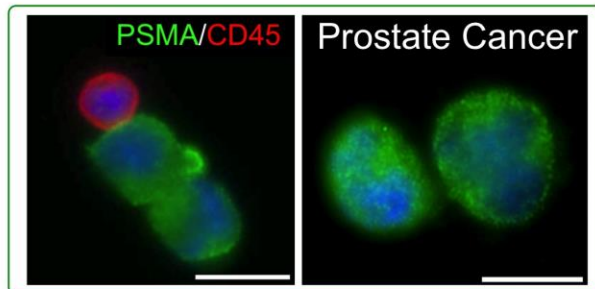
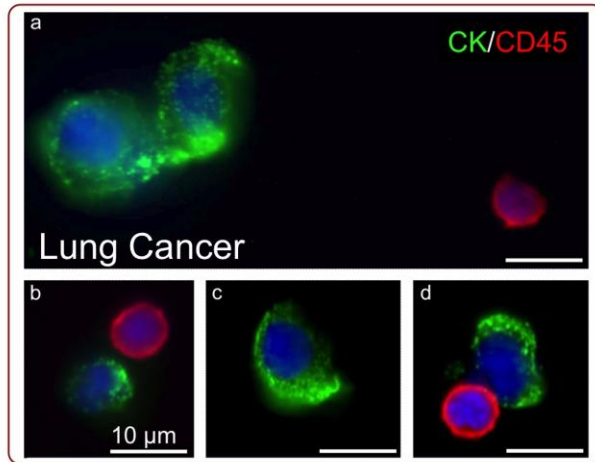
# 2<sup>nd</sup> Generation Herringbone CTC-Chip

## Scale-up and Dissemination

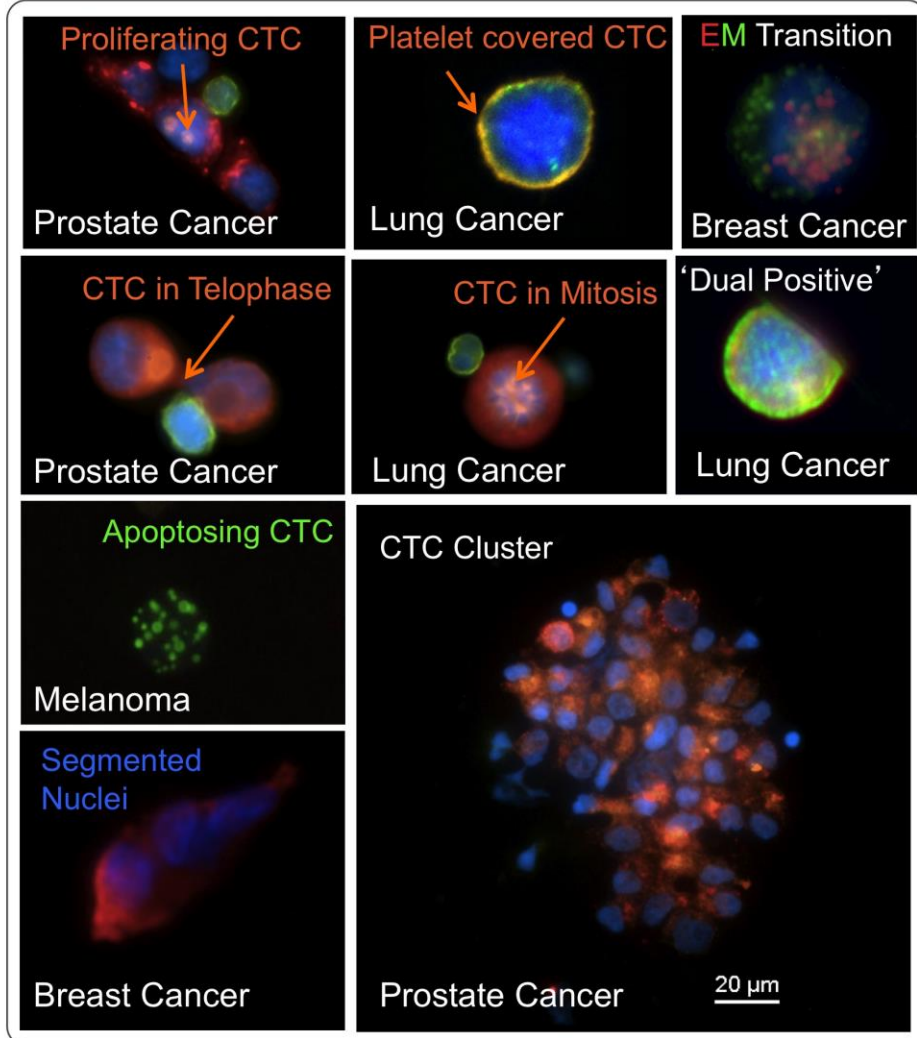


# Patient CTCs come in different size, shape & phenotype

## Size & Shape of CTCs



## Phenotype of CTCs



# All cancers and all CTCs

***Avoid bias*** in CTC isolation and ***improve yield***, especially in non-epithelial cancers, or cancers undergoing EMT.



## **Negative depletion:**

Removing the hay to uncover the needle...

HIGH  
THROUGHPUT



EPITOPE  
INDEPENDENT  
SELECTION



ENHANCED  
CTC ANALYSIS



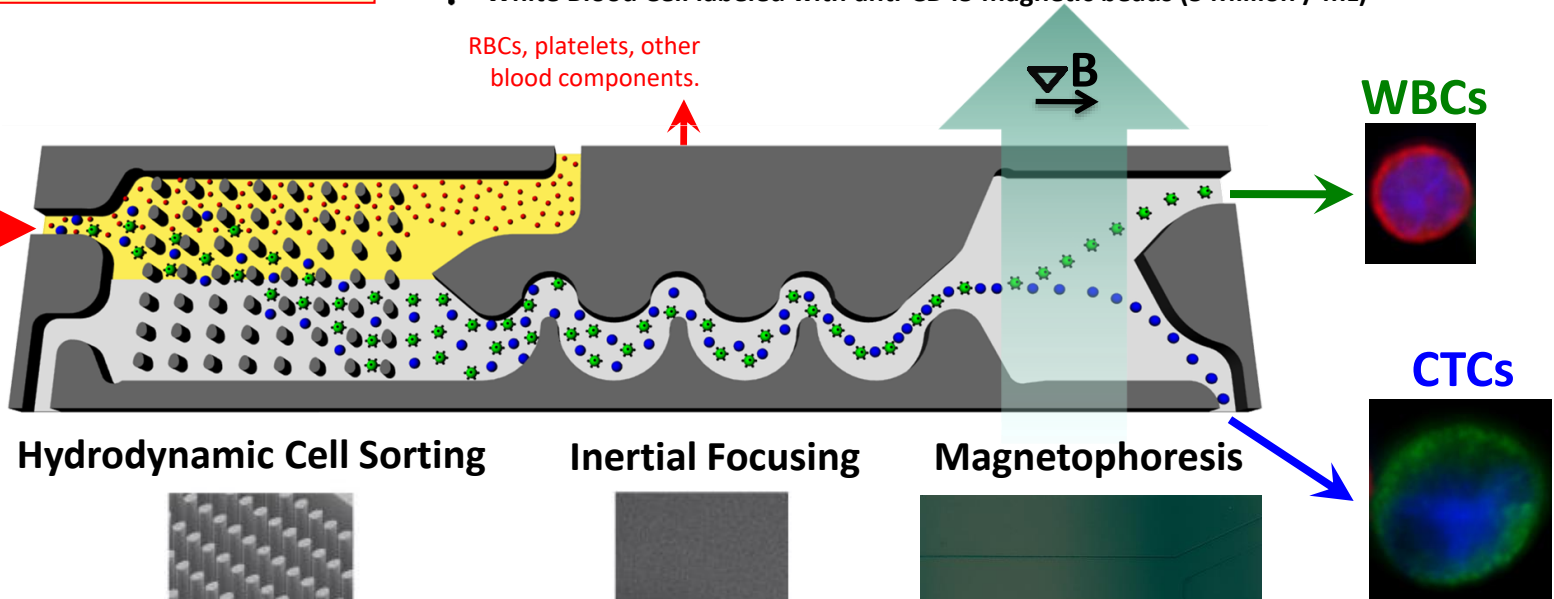
# CTC-iChip: *Negative Selection*

Magnetic bead-tagged  
WBCs

- Red Blood Cell (8 Billion / mL)
- CTCs (1-100 / mL)
- White Blood Cell labeled with anti-CD45 magnetic beads (5 Million / mL)

RBCs, platelets, other  
blood components.

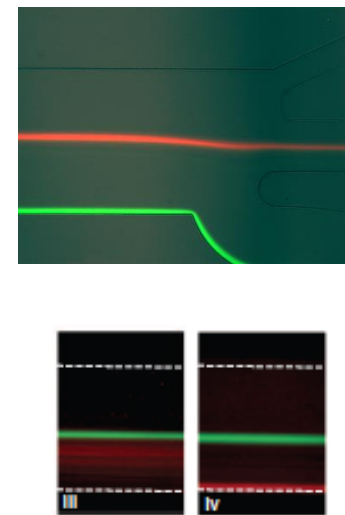
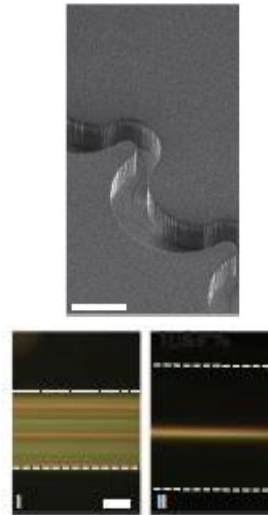
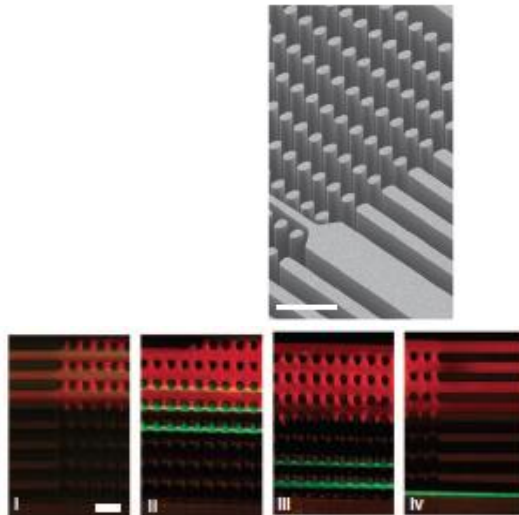
Blood

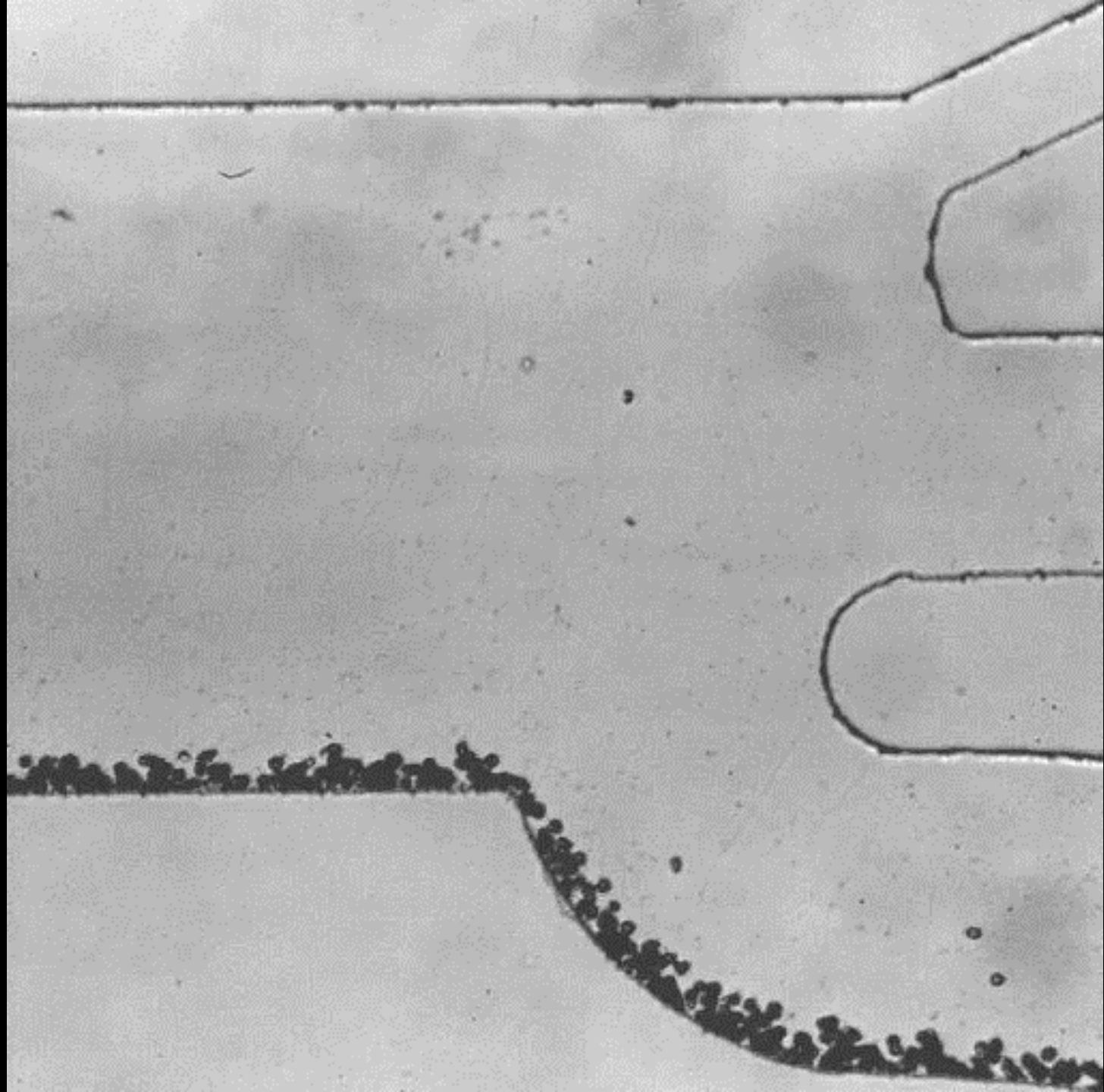


Hydrodynamic Cell Sorting

Inertial Focusing

Magnetophoresis





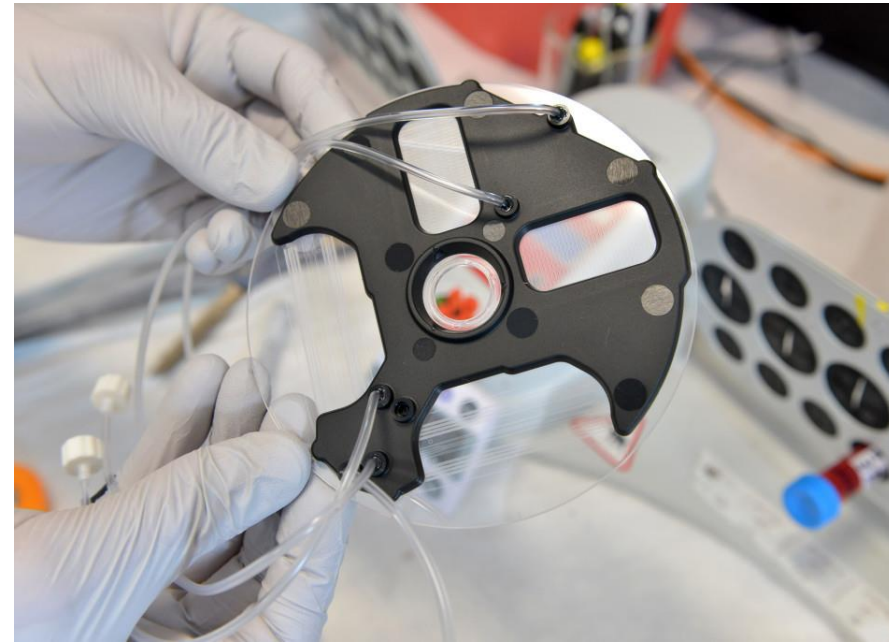
# “World-to-Chip” Coupling

Processor



**CATAPULT!**  
product development

CTC-iChip



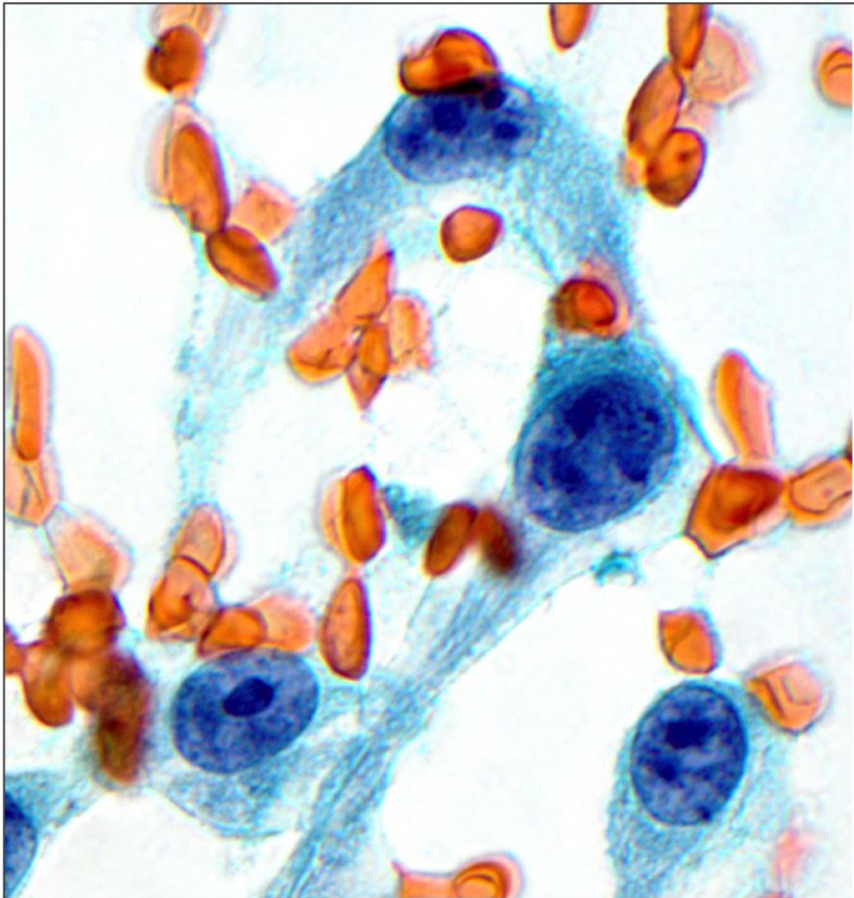
**strattec** ● ●  
biomedical

**Automated sample processing**  
Flowrate: 10-20 mL whole blood/hour  
20-30 million cells/second

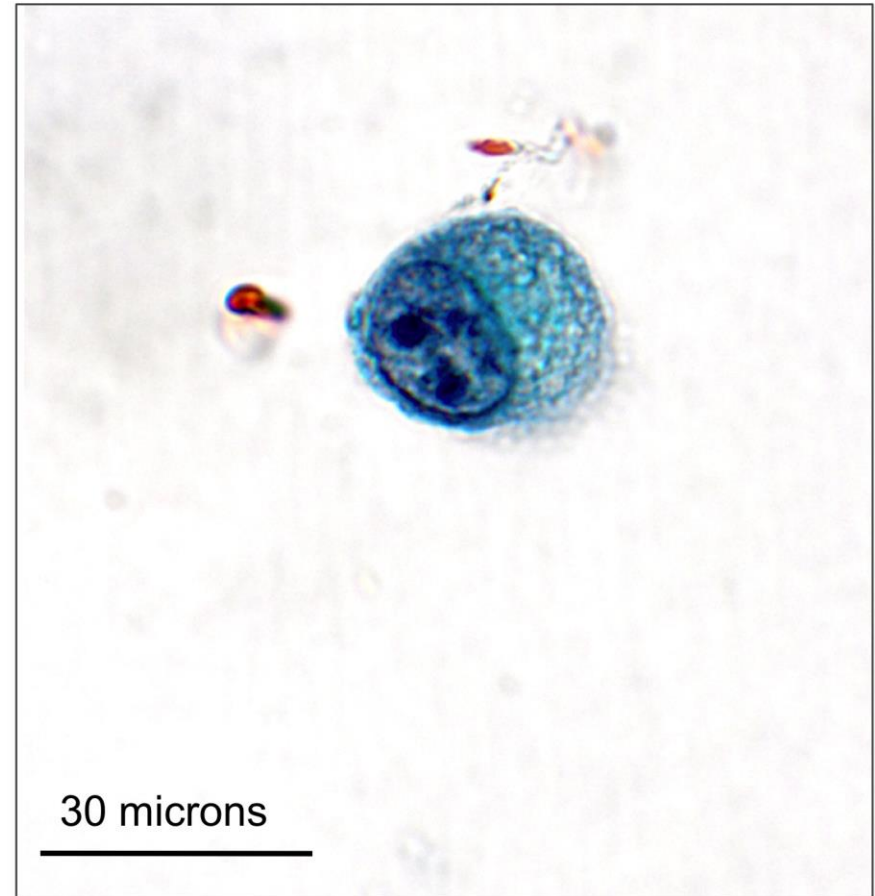


# CTC-iChip enables light microscopy

FNA

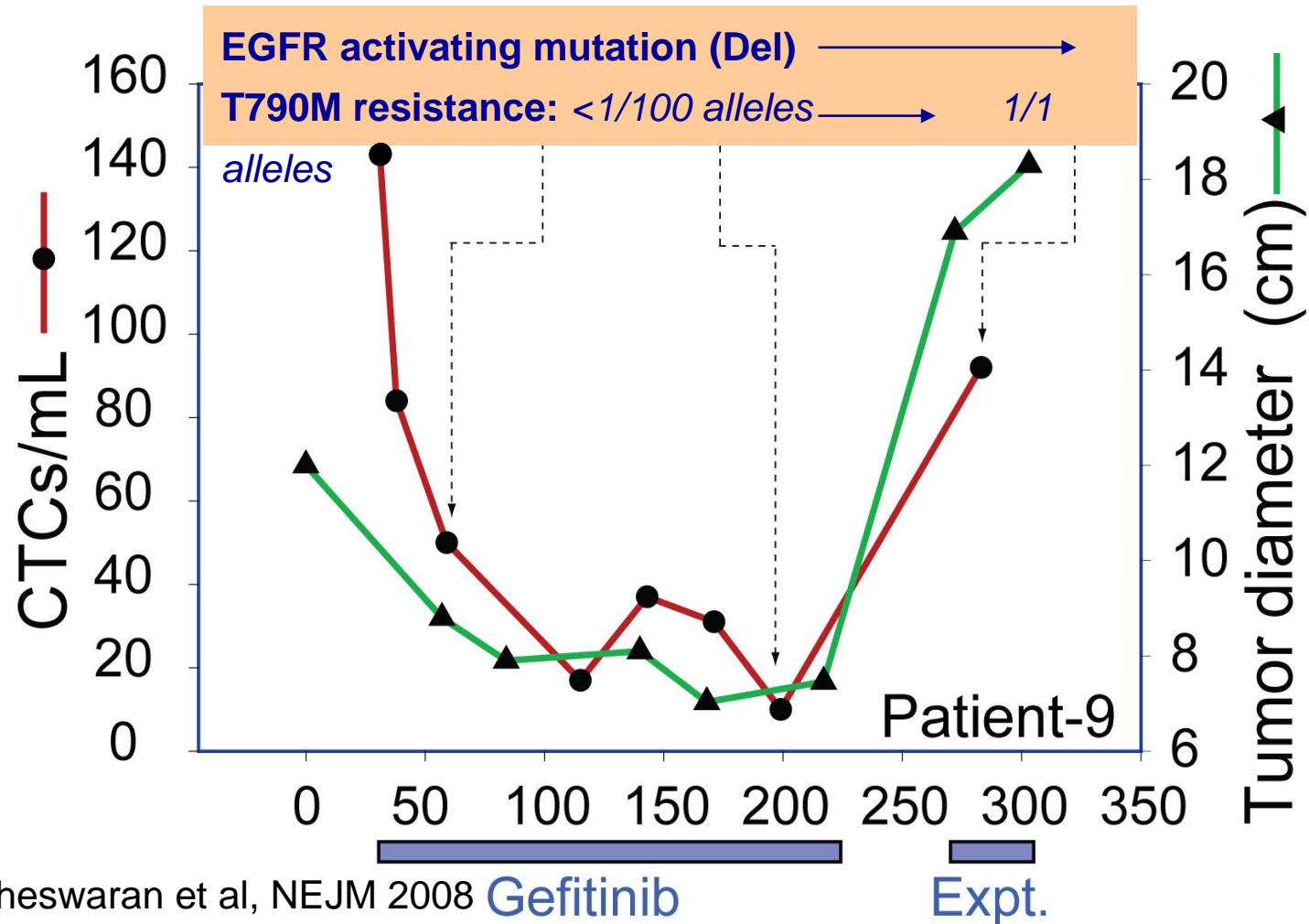


CTC



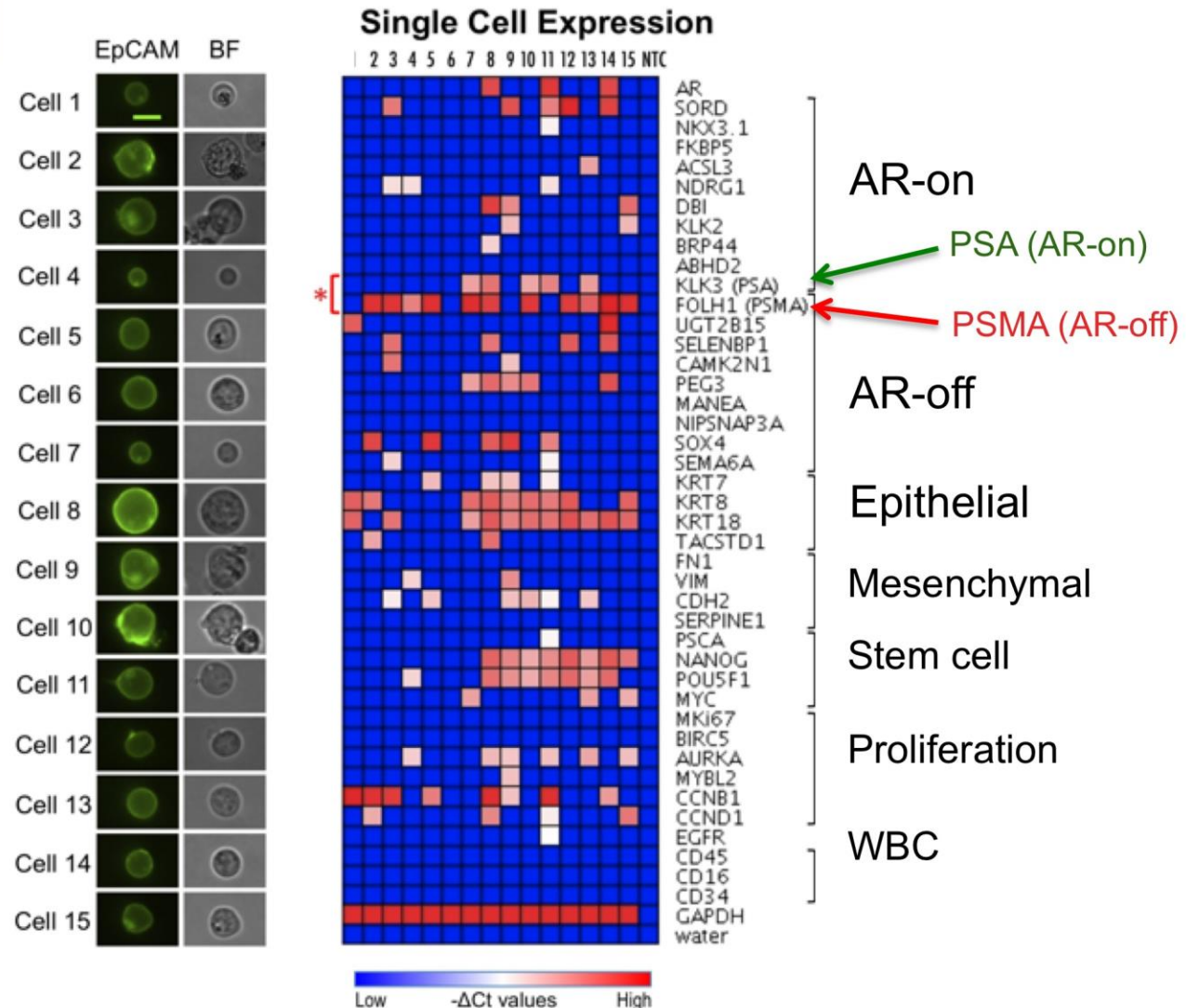
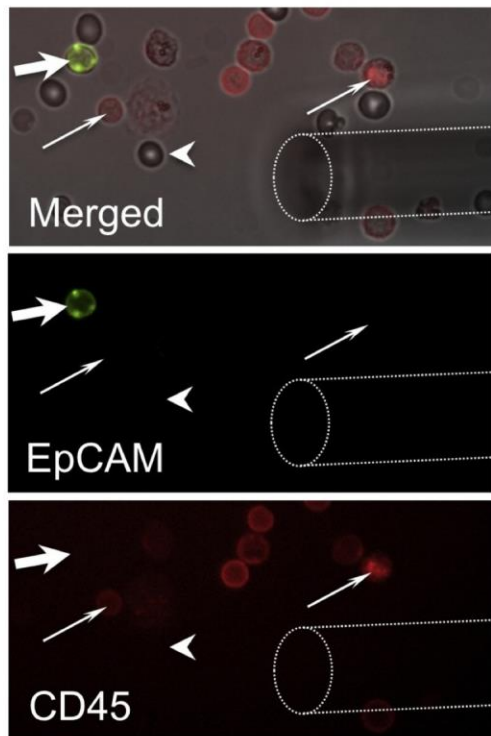
Papanicolaou staining of melanoma CTCs

# Lung Cancer: Serial Monitoring of EGFR Genotypes



# Measuring androgen signaling pathways in prostate cancer CTCs

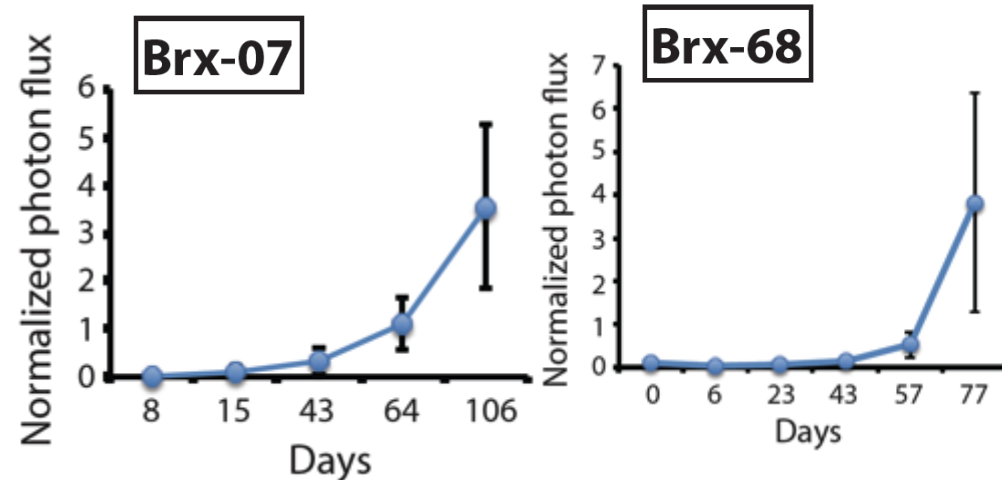
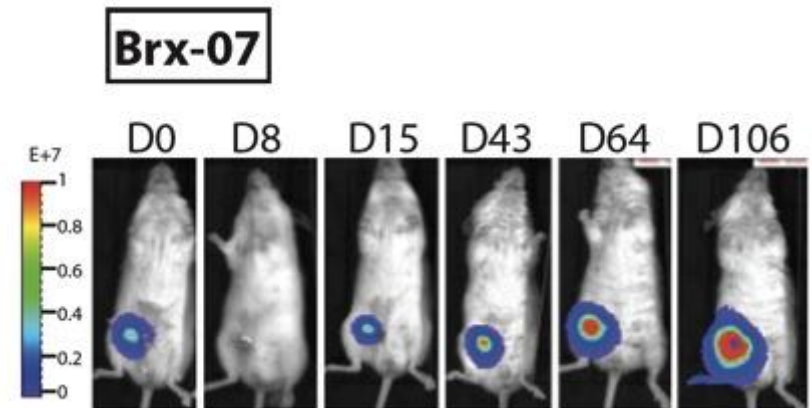
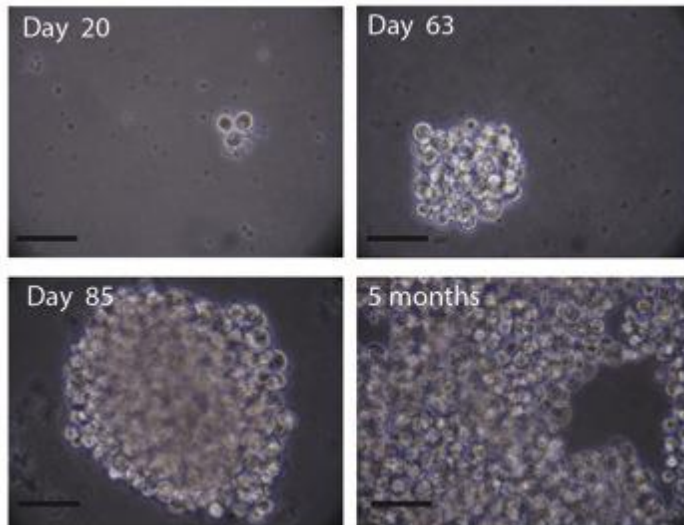
## qRT-PCR (Fluidigm)



# i-Chip: Culture of patient CTCs

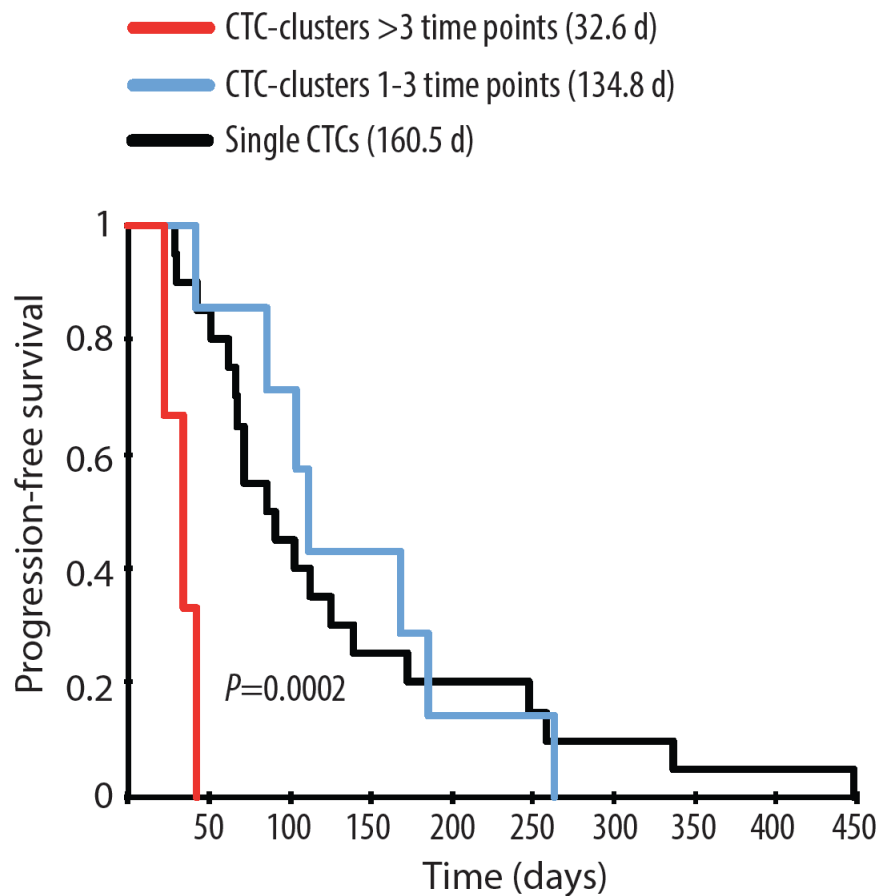
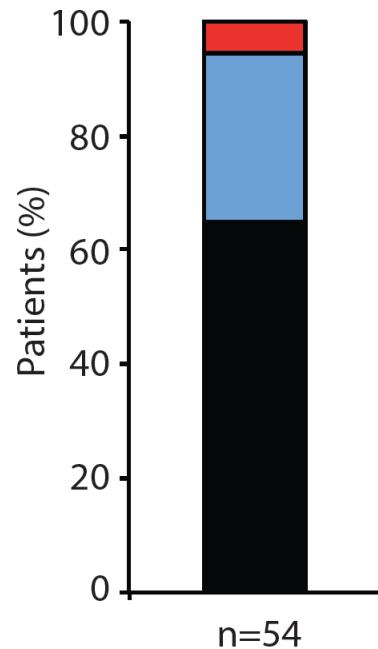
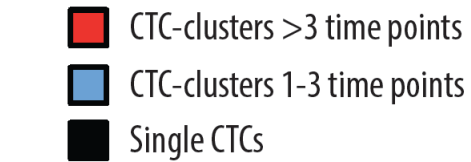
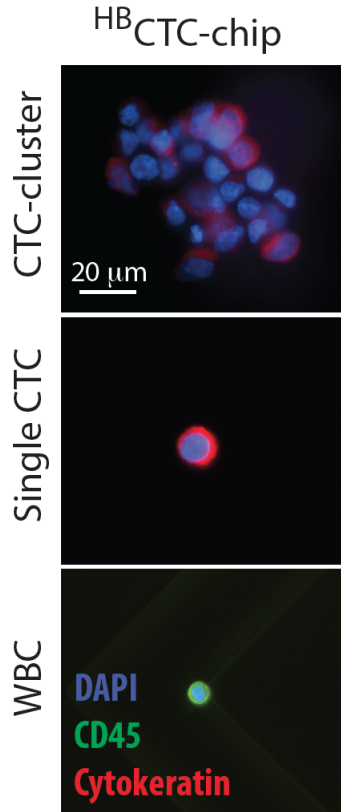
## Tumorigenesis in mouse models

*In vitro* expansion of CTCs  
from patients with  
hormone-positive breast  
cancer



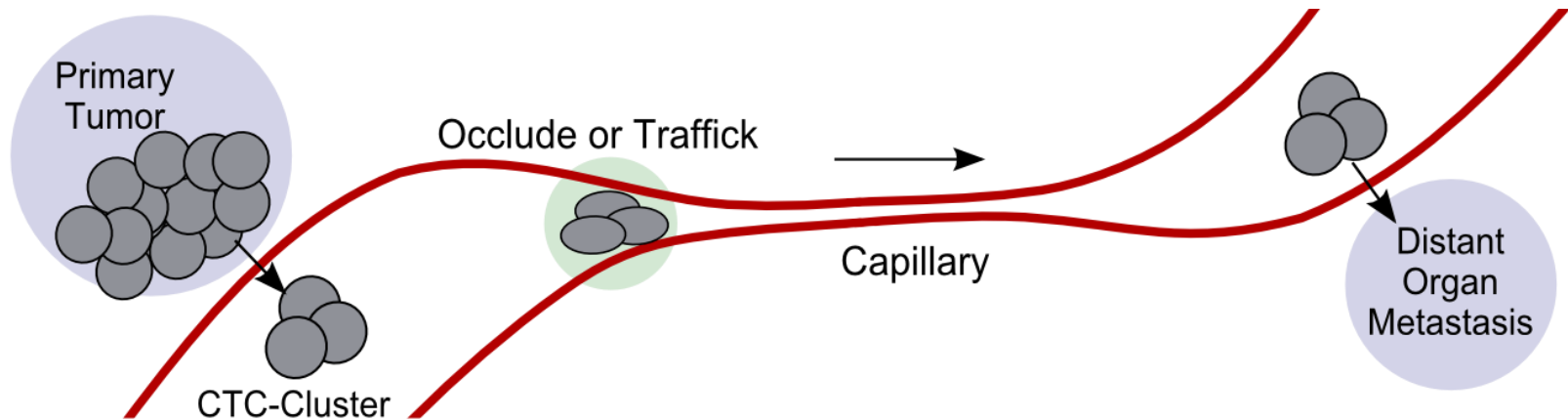


# Clinical response and CTC clusters

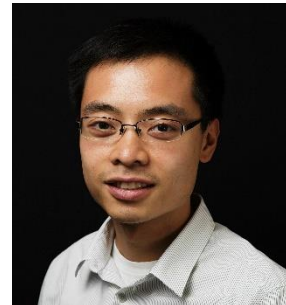


# CTC-Cluster Transit

- CTC-Clusters (2-100+ cells) believed to occlude 5-10  $\mu\text{m}$  capillaries  $\rightarrow$  cannot contribute to dissemination of tumors to distant organs

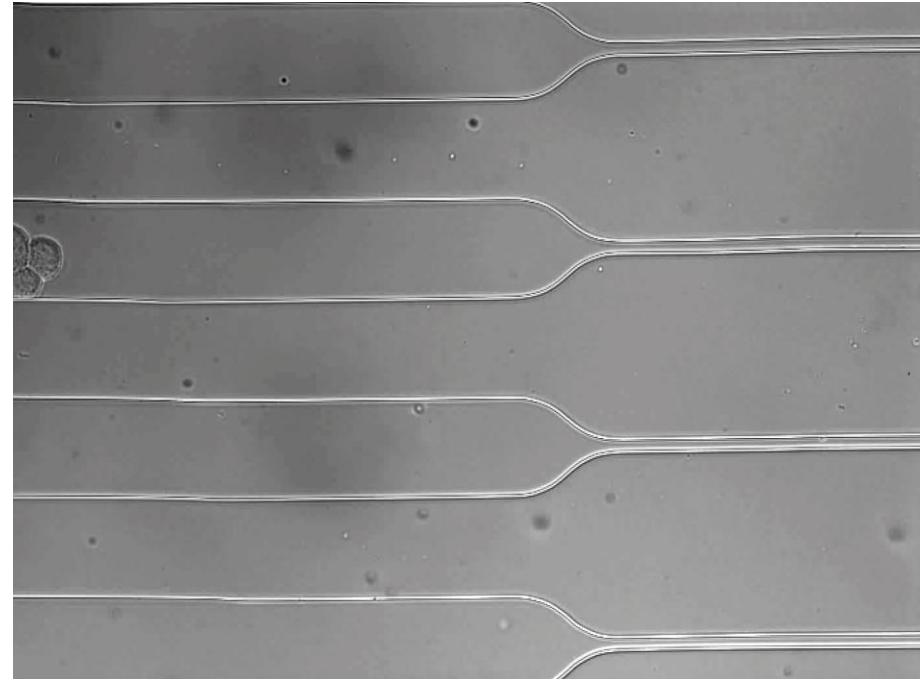
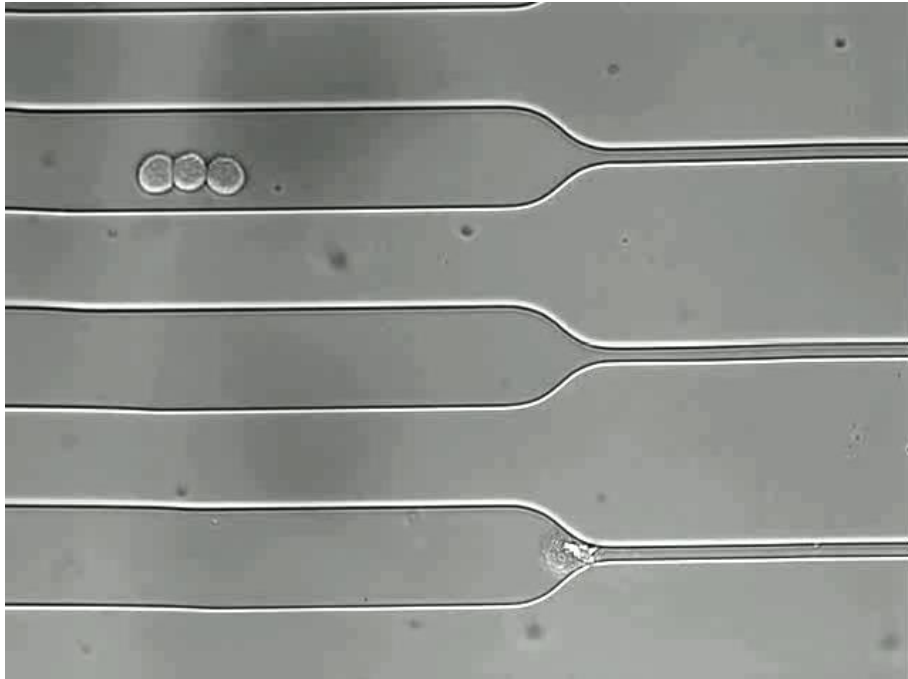
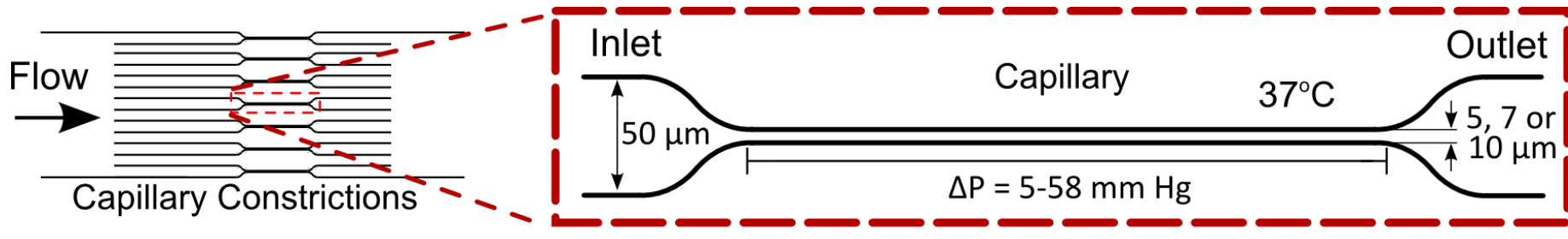


Sam Au et al. (PNAS, 2016)



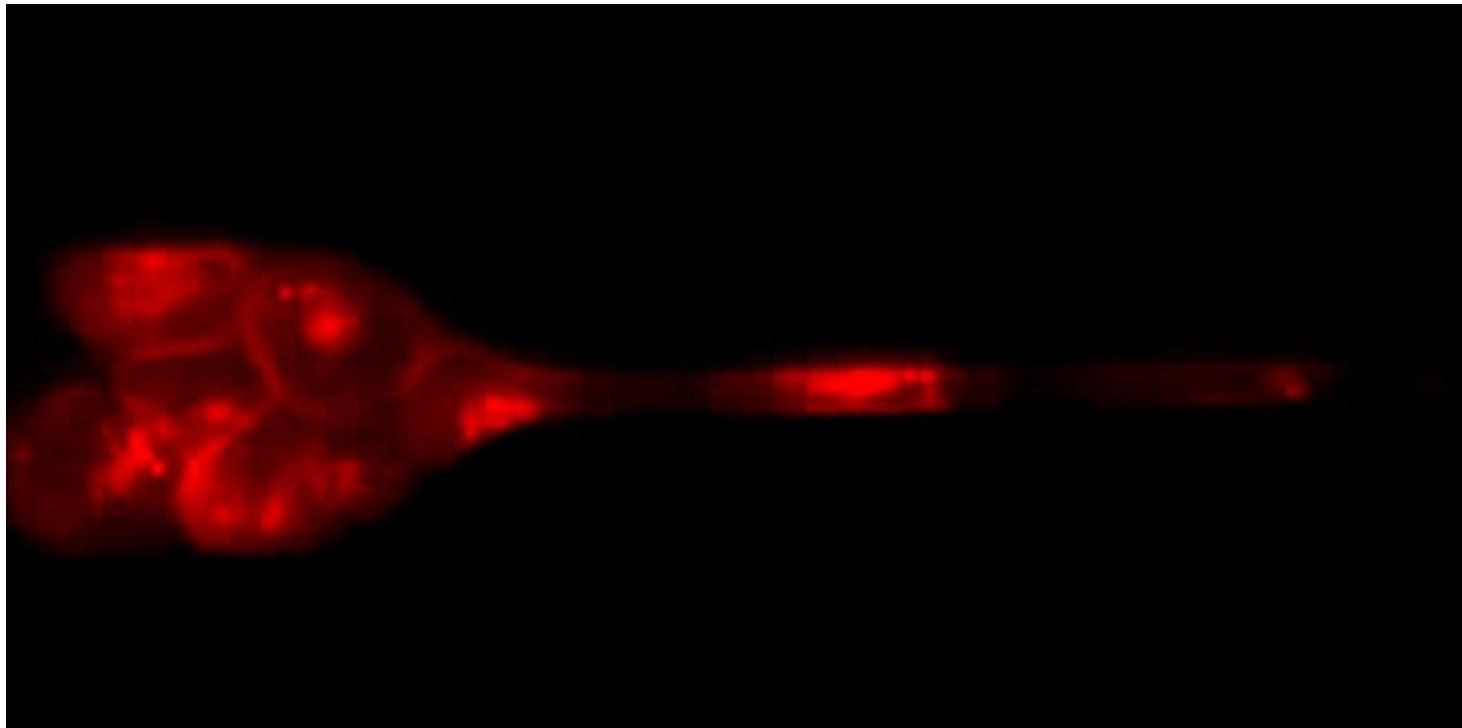


# Microfluidic Capillary Constrictions

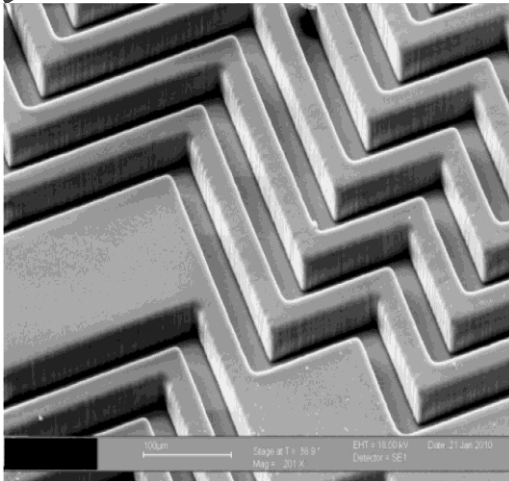
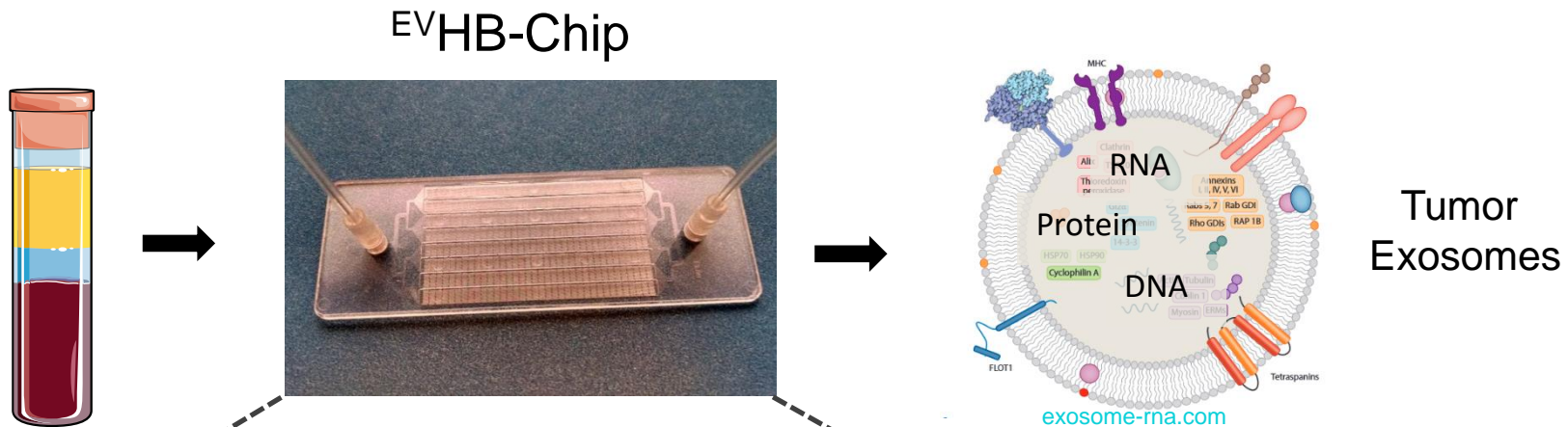


# Reorganization of Clusters at Entrance

- Cell membrane stain
- Complex organization dynamics



# Microfluidic Extracellular Vesicle Capture



## Downstream analysis:

- Digital Droplet PCR
- RNASeq
- Proteomics

## Functional Studies

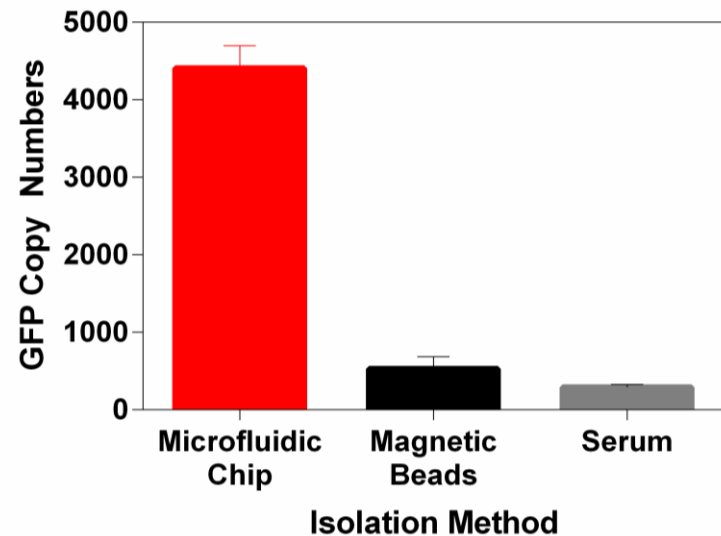
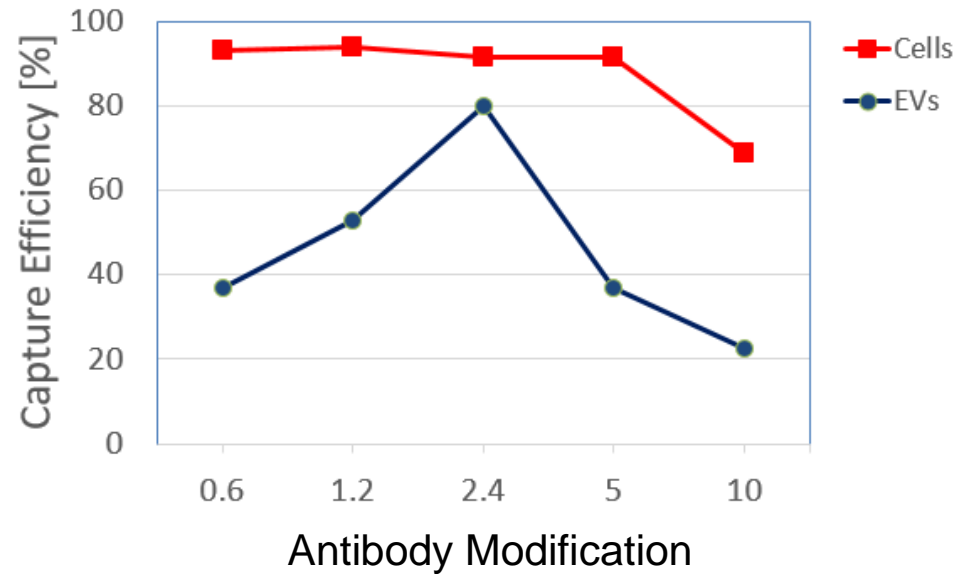
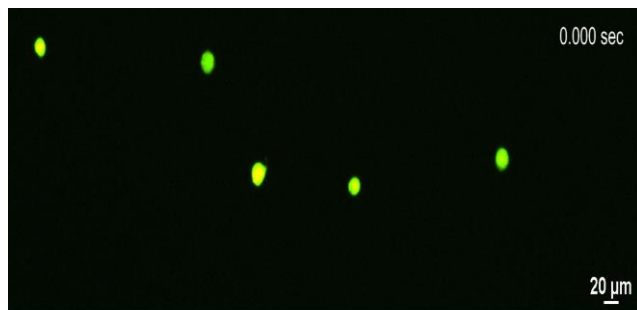
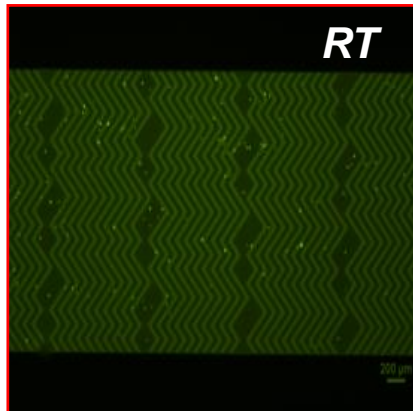
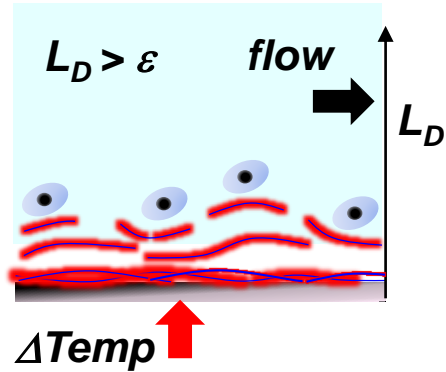
- Co-Culture



Eduardo Reátegui (Nat. Comm, Accepted)

In collaboration with the  
Breafield Laboratory

# Microfluidic Biomaterial Coating

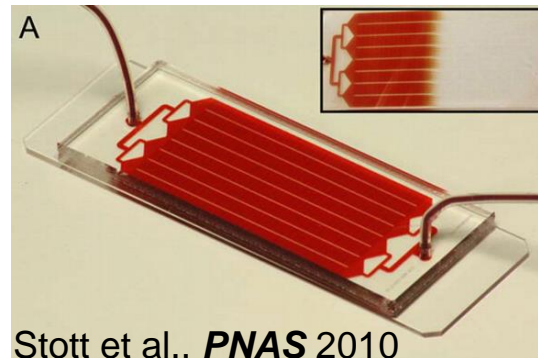


# CTCs and EVs in Precision Medicine

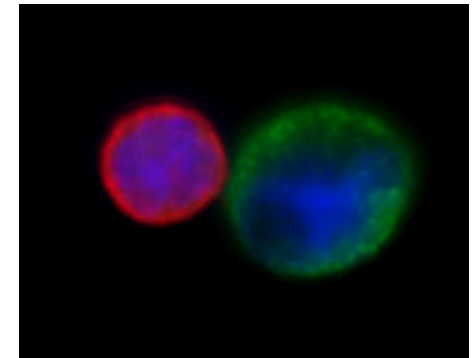
Blood cells  $\sim 10^{10}$   
CTCs 0~100



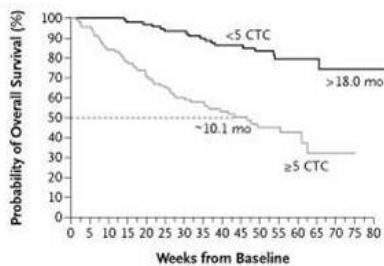
## Rare-Cell Isolation



## CTC

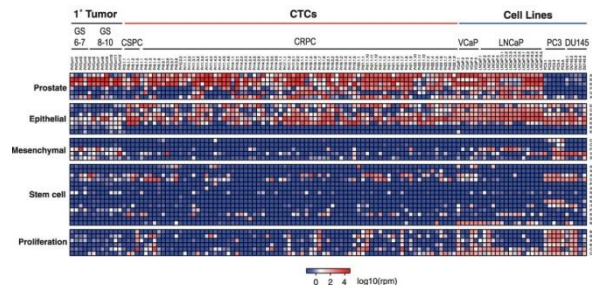


## prognosis

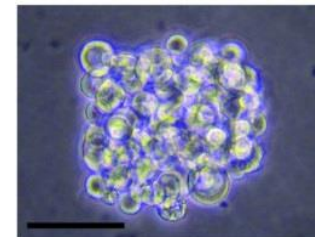


CTC count stable  
in fixed blood

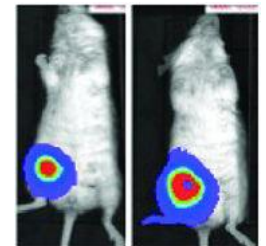
## expression profiling



## ex vivo culture



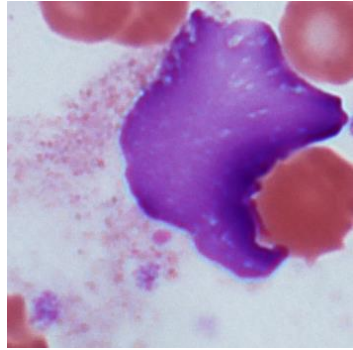
## personalized drug testing



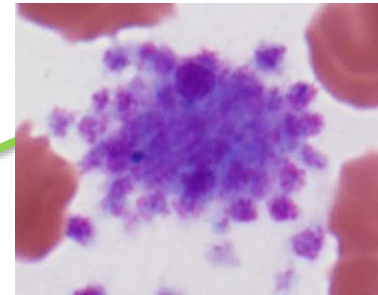
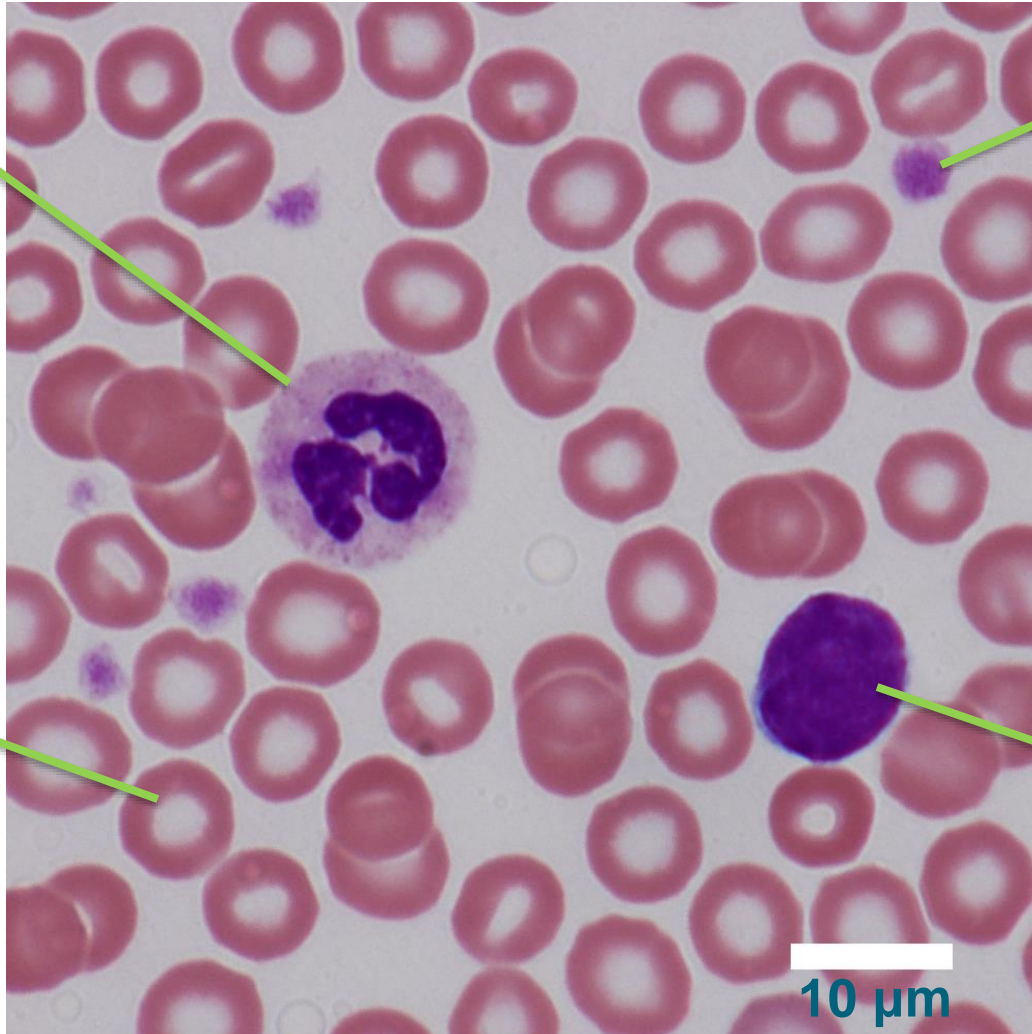
**Require live cells!**  
<4 hours after blood draw



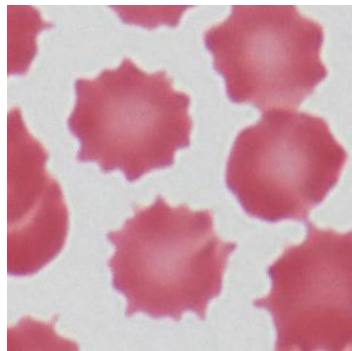
# Blood degradation damages target cells & cripples precise microfluidic technologies



**Oxidative bursts  
Extracellular traps**



**Aggregation  
Clotting**



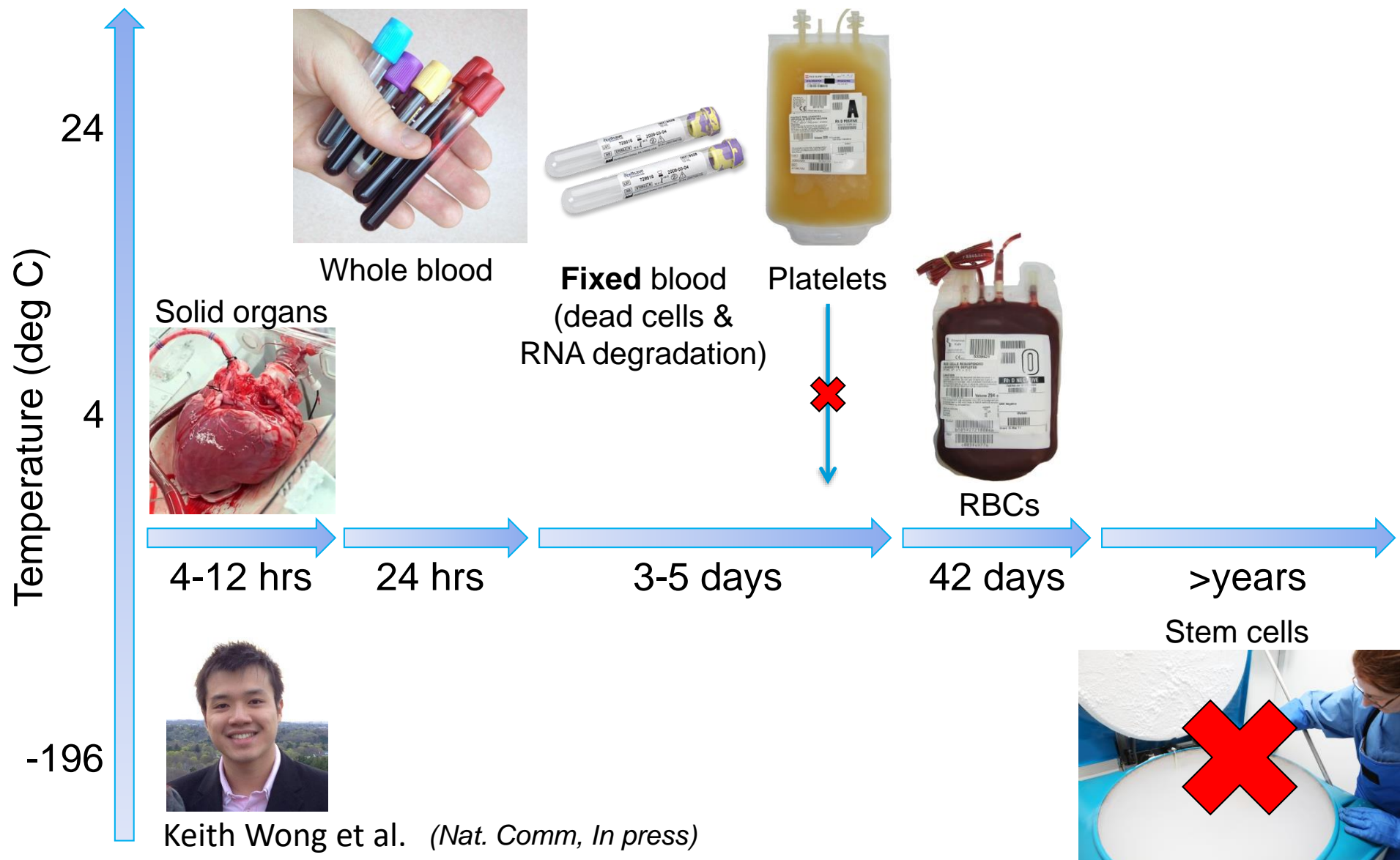
**Hemolysis**

**Activation  
Cytokines**

10 μm



# Challenges of *whole* blood preservation



# Microfluidics for Liquid Biopsy: An Enabling Technology for Cancer Care

