Application – Microfluidic Cell Analysis Devices (II)

Date: 2013/05/31

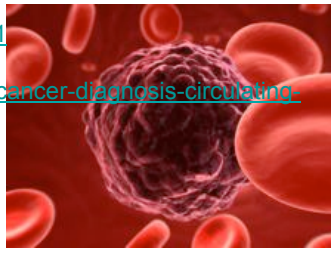
Dr. Yi-Chung Tung

Circulating Tumor Cells (CTCs)

A typical cancerous tumor contains millions or even billions of cells harboring genetic mutations driving them to grow, divide, and invade the local tissue in which they're embedded. However, as the cells proliferate, they don't all stay in the neighborhood. Some cells slough off the edges of a tumor and are swept away by the bloodstream or lymphatic system. These so-called circulating tumor cells (CTCs) can remain loose in circulation, cluster together as they travel, or lodge themselves in new tissues. Whatever their path, their common origin means that CTCs hold information about a tumor, information that researchers think could be key to cancer diagnosis or treatment.

http://www.pnas.org/content/110/13/4861.full#ref-1

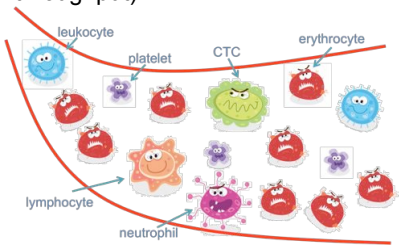
http://c0nc0rdance.com/2012/04/12/cutting-edge-cancer-diagnosis-circulating-tumor-cells/



Probing CTCs in Microfluidics

The difficulty in using CTCs lies in their extremely low concentrations in blood samples: a normal concentration in a human cancer patient is approximately 1–100 CTCs per mL of blood. In designing and evaluating CTC isolation systems, it is important to note the three design objectives of an ideal CTC isolation system:

1. Isolate all of the CTCs in the blood sample (high capture efficiency)
2. Isolate only the CTCs, with no other cells accidently isolated (high isolation purity)
3. Perform this isolation quickly (high system throughput)



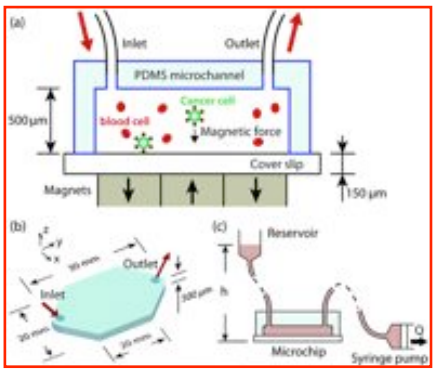
Probing CTCs in Microfluidics

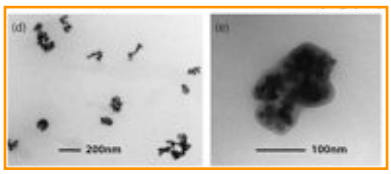
1. Magnetic-Based CTC Separation or Detection
2. Affinity Chromatography CTC Separation
3. Size- and/or Deformability-Based CTC Separation
4. Combination of Multiple CTC Separation Mechanisms
5. Others…

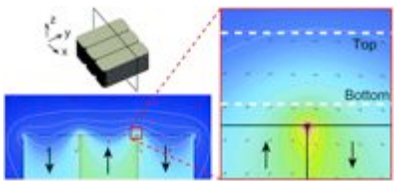
Magnetic-Based CTC Separation or Detection

Immunomagnetic Detection of CTCs

Microchip design for immunomagnetic detection of cancer cell . Schematic showing the principle of operation. CTCs in blood are labeled with EpCAM functionalized Fe_3O_4 magnetic nanoparticles , and captured by the magnetic field as the blood flows through the microchannel.

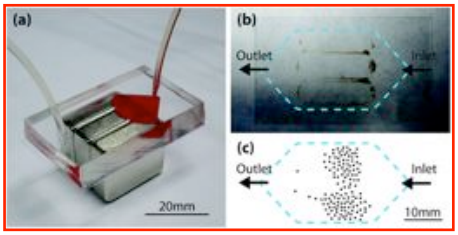


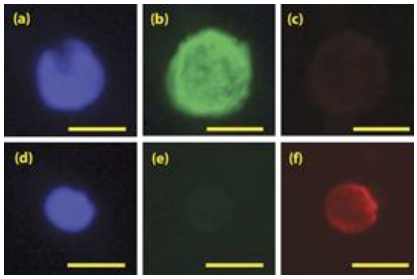




Immunomagnetic Detection of CTCs

Blood screening experiment. (a) Photograph of the experimental setup. Blood sample is being introduced into the microchannel. (b) Bottom glass slide removed from the channel after screening. (c) Example of COLO205 cell distribution on the glass slide. The picture is a trace of a manually drawn sketch, approximating the coordinates of the cells .





Examples of (a) DAPI (blue), (b) CK (green) and (c) CD45 (red) fluorescence images of captured COLO205 cell . (d) DAPI, (e) CK and (f) CD45 images of a white blood cell , which shows clear CK negative and CD45 positive images, are also shown. Bars = 10 μm.

Immunomagnetic Detection of CTCs

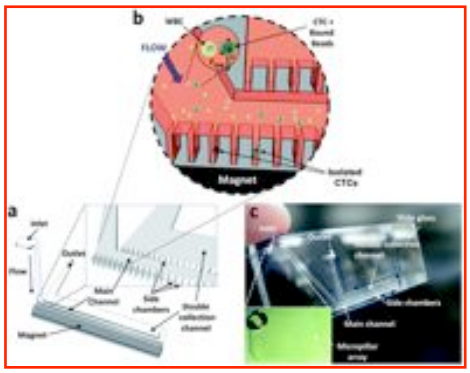
Recovery Rate of Spiked Blood Experiment.

Ctrl1	14	52	81	164	968	1477
Ctrl2	12	45	84	150	893	1496
Ctrl average	13.0	48.5	82.5	157.0	930.5	1486.5
Cells found	7	37	58	160	625	1129
Capture rate	54%	76%	70%	102%	62%	76%

Cell	Tube	Ferrofluid/blood/μL mL^{-1}	Flow rate/mL h^{-1}	Ctrl 1	Ctrl 2	Ctrl average (A)	Cells found (C)	Capture rate (C/A)	Average capture rate
COLO-205	EDTA	30	2.5	75	46	60.5	61	108%	79%
	EDTA	30	2.5	115	114	114.5	92	80%	
	EDTA	30	2.5	139	130	134.5	92	68%	
	CellSave	30	2.5	75	46	60.5	40	66%	65%
	CellSave	30	2.5	139	130	134.5	70	52%	
	CellSave	30	2.5	48	53	50.5	49	96%	
	CellSave	7.5	2.5	109	126	117.5	125	106%	53%
	CellSave	7.5	2.5	180	193	186.5	83	28%	
	CellSave	7.5	2.5	180	193	186.5	53	44%	
	CellSave	7.5	10	252	275	263.5	239	91%	90%
	CellSave	7.5	10	252	275	263.5	257	98%	
	CellSave	7.5	10	252	275	263.5	267	101%	
	CellSave	7.5	10	252	275	263.5	228	87%	
	CellSave	7.5	10	252	275	263.5	240	91%	
	CellSave	7.5	10	252	275	263.5	203	77%	
SKBR3	CellSave	7.5	10	711	927	819.0	713	87%	86%
	CellSave	7.5	10	711	927	819.0	704	86%	

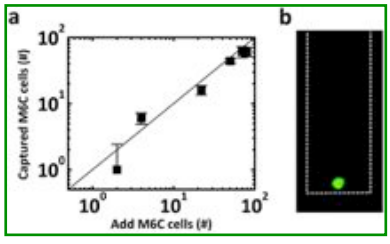
Micromagnetic-Microfluidic Device for CTC Detection and Culture

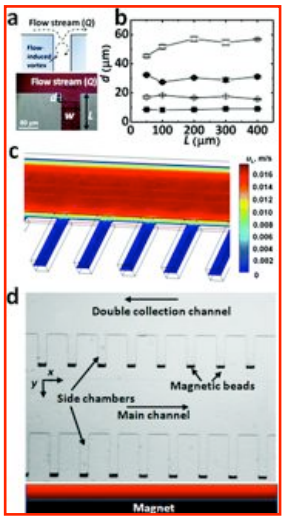
The rare circulating cell isolation microfluidic device contains an angled inlet channel that connects to a main channel followed by a double collection channel before reaching the outlet. The main channel and double collection channel are lined by rows of dead-end side chambers where magnetic bead-bound cells are collected when a permanent magnet is placed directly beneath the lower row of side chambers.



Micromagnetic-Microfluidic Device for CTC Detection and Culture

Schematic view of an intersection between a main flow channel and a side collection chamber showing how recirculation and shear stress are induced by flow.

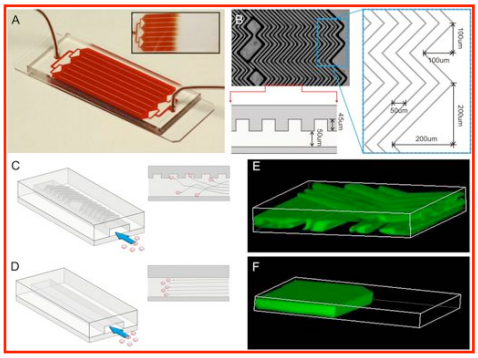




Affinity Chromatography CTC Separation

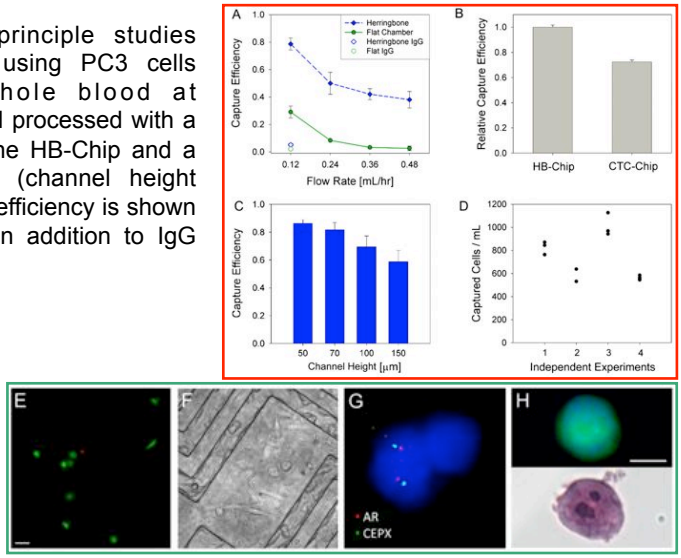
Isolation of CTCs Using a Microvortex-Generating Herringbone-Chip

This work reports a high-throughput microvortex mixing device that ensures effective contacts of cells with antibody-coated surfaces, while designed with simple geometry that is amenable for large-scale manufacturing. The Herringbone (HB)-Chip effectively captured CTCs from patients with metastatic prostate cancer, and its low shear flow properties revealed the presence of previously unappreciated microclusters of CTCs.



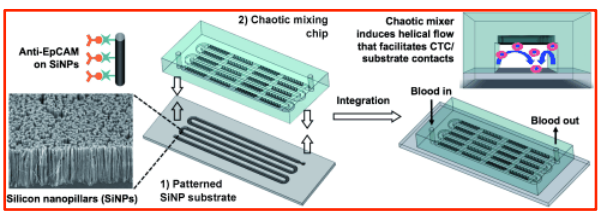
Isolation of CTCs Using a Microvortex-Generating Herringbone-Chip

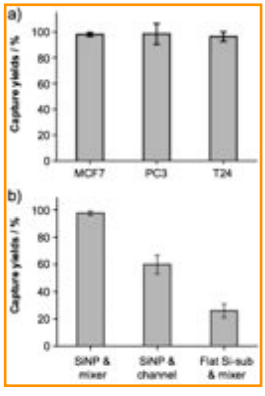
Device proof-of-principle studies were conducted using PC3 cells spiked into whole blood at 1,000 cells/mL and processed with a small version of the HB-Chip and a flat-walled device (channel height 100 µm). Capture efficiency is shown for both devices in addition to IgG controls.



Nanostructured Silicon Substrates for CTC Capture

Schematic representation of the configuration and operational mechanism of an integrated device for capturing circulating tumor cells (CTCs). The device is composed of two functional components, a patterned silicon nanopillar (SiNP) substrate (1) with anti-EpCAM-coating exhibiting vastly enhanced CTC-capture affinity, and an overlaid microfluidic chaotic mixing chip (2) capable of promoting cell–substrate contact frequency. See text for details.





Micro-Post Array for CTC Capture

Vol 450|20/27 December 2007|doi:10.1038/nature06385 nature

LETTERS

Isolation of rare circulating tumour cells in cancer patients by microchip technology

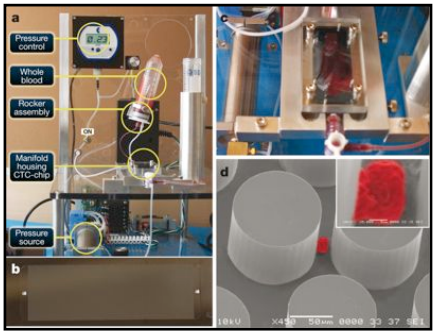
Sunitha Nagrath[1]*, Lecia V. Sequist[2]*, Shyamala Maheswaran[2], Daphne W. Bell[2]†, Daniel Irimia[1], Lindsey Ulkus[2], Matthew R. Smith[2], Eunice L. Kwak[2], Subba Digumarthy[2], Alona Muzikansky[2], Paula Ryan[2], Ulysses J. Balis[1]†, Ronald G. Tompkins[1], Daniel A. Haber[2] & Mehmet Toner[1]

Viable tumour-derived epithelial cells (circulating tumour cells or CTCs) have been identified in peripheral blood from cancer patients and are probably the origin of intractable metastatic disease[1–4]. Although extremely rare, CTCs represent a potential alternative to invasive biopsies as a source of tumour tissue for the detection, characterization and monitoring of non-haematologic cancers[1–4]. The ability to identify, isolate, propagate and molecularly characterize CTC subpopulations could further the discovery of cancer stem

Here we describe the development and application of a microfluidic device (the 'CTC-chip') that can efficiently and reproducibly isolate CTCs from the blood of patients with common epithelial tumours (Fig. 1, and Supplementary Fig. 1). The CTC-chip (Fig. 1b) consists of an array of microposts (Supplementary Fig. 1c) that are made chemically functional with anti-epithelial-cell-adhesion-molecule (EpCAM, also known as TACSTD1) antibodies. Anti-EpCAM provides the specificity for CTC capture from unfrac-

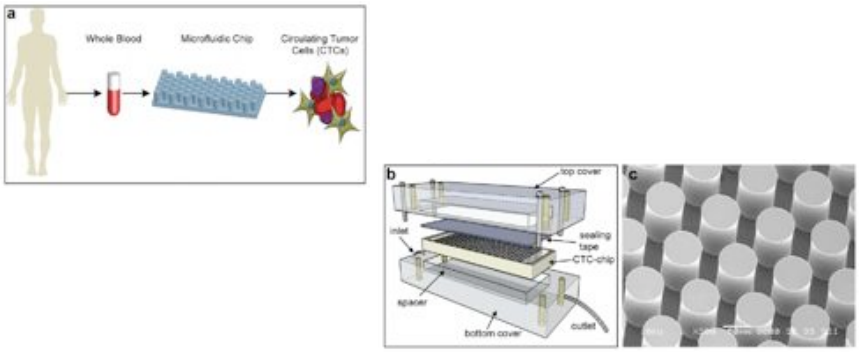
Micro-Post Array for CTC Capture

(a) The workstation setup for CTC separation. The sample is continually mixed on a rocker, and pumped through the chip using a pneumatic-pressure-regulated pump. (b) The CTC-chip with microposts etched in silicon. (c) Whole blood flowing through the microfluidic device. (d) Scanning electron microscope image of a captured NCI-H1650 lung cancer cell spiked into blood (pseudo coloured red). The inset shows a high magnification view of the cell.



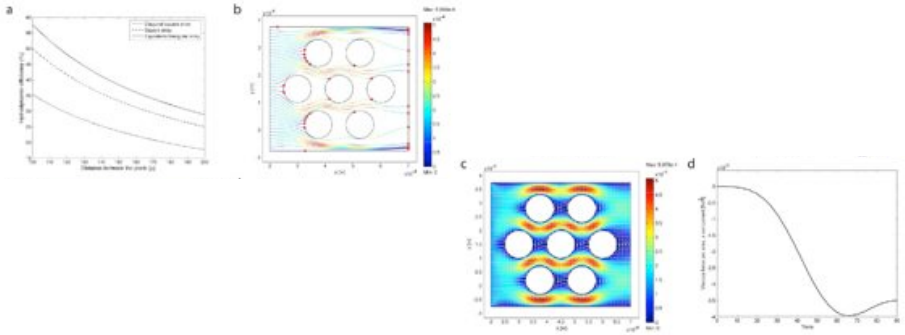
Micro-Post Array for CTC Capture

Microfluidic approach to isolate circulating tumor cells.(a) One-step process for point-of-care isolation of CTCs from peripheral blood. (b) Schematic of the manifold assembly. The microfluidic chip is sealed from above with a biological grade adhesive tape and placed in the manifold. (c) Scanning electron micrograph (SEM) image of the microposts array.



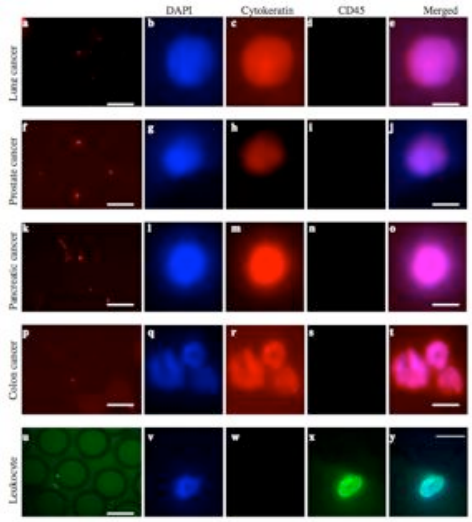
Micro-Post Array for CTC Capture

Design criteria and computational an alysis of hydrodynamics in the microfluidic chip. (a) Comparison of the hydrodynamic efficiency of different post array arrangements by distance between the posts using square, diagonal square, and equilateral triangular arrays. (b) Computational analysis of the micropost array. Cell trajectories (solid lines) are based on particle tracings and the end positions of the cells are indicated by red dots. (c) Flow profile. (d) Shear stress, x-component along the surface of the post.



Micro-Post Array for CTC Capture

Captured cell purity check using immunofluorescence staining.



Micro-Post Array for CTC Capture

Table S1. Quantification of circulating tumor cells per mL of blood in healthy subjects.

None of the samples from healthy subjects had any detectable CTCs.

Healthy Subject Sample No.	Gender	Age	Volume Processed (mL)	CTCs/mL
1	F	30	3.4	0
2	F	26	3.2	0
3	M	42	2.5	0
4	M	27	3.0	0
5	F	25	2.7	0
6	M	45	2.9	0
7	F	30	3.4	0
8	F	30	3.5	0
9	F	52	3.2	0
10	M	41	2.4	0
11	M	41	4.1	0
12	F	26	3.0	0
13	F	24	2.8	0
14	F	24	3.1	0
15	M	33	2.4	0
16	M	26	2.1	0
17	M	29	3.4	0
18	F	32	2.8	0
19	M	29	3.1	0
20	M	29	3.0	0

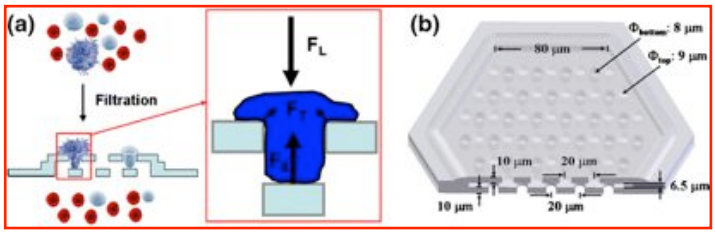
Table S2. Quantification of circulating tumor cells per mL of blood among 116 samples from patients with epithelial cancers including NSCLC (n=55), prostate cancer (n=26), pancreatic cancer (n=15), breast cancer (N=10) and colorectal cancer (n=10).

Sample	Cancer Type	Gender	Age	ml	CTC/mL	%Purity
1	NSCLC	M	58	3.7	156	52
2	NSCLC	M	58	4.3	8	16.7
3	NSCLC	M	58	2.1	6	20.0
4	NSCLC	F	61	5.1	73	48.5
5	NSCLC	M	70	1.4	279	52.4
6	NSCLC	M	55	2.2	57	41.9
7	NSCLC	M	55	3.0	113	33
8	NSCLC	M	59	0.9	771	62.2
9	NSCLC	F	66	4.6	24	42.1
10	NSCLC	F	66	0.9	1281	50.7
11	NSCLC	F	74	1.4	740	93.2
12	NSCLC	M	64	3.1	196	65.5
13	NSCLC	F	65	2.0	538	72.1
14	NSCLC	M	62	4.1	42	25.0

Size- and/or Deformability-Based CTC Separation

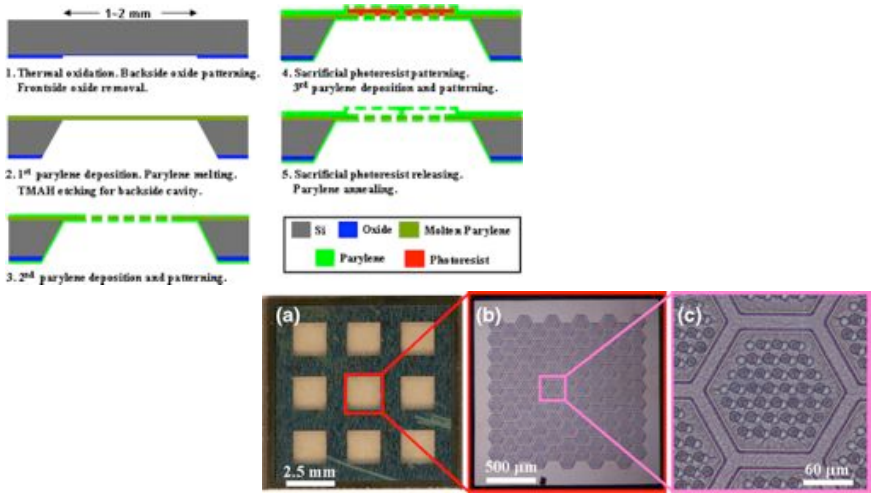
3D microfilter device for viable CTC enrichment from blood

This device consists of two layers of parylene membrane with pores and gap precisely defined with photolithography. The positions of the pores are shifted between the top and bottom membranes. The bottom membrane supports captured cells and minimize the stress concentration on cell membrane and sustain cell viability during filtration. Viable cell capture on device was investigated with scanning electron microscopy, confocal microscopy, and immunofluorescent staining using model systems of cultured tumor cells spiked in blood or saline.



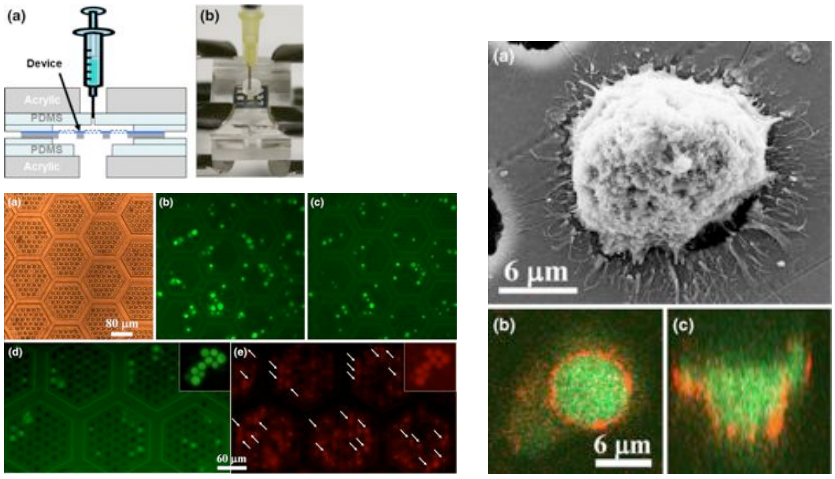
3D microfilter device for viable CTC enrichment from blood

Device Fabrication



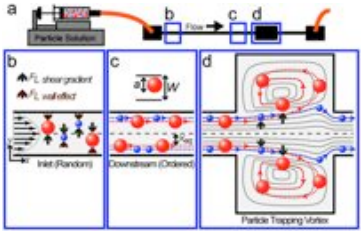
3D microfilter device for viable CTC enrichment from blood

Device Operation and Results (Great Cell Viability).

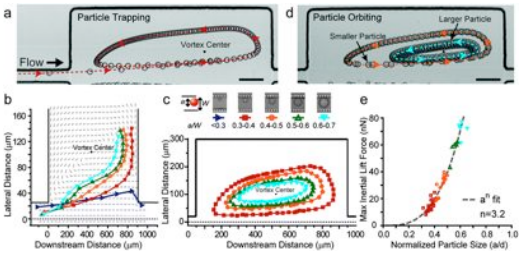


Centrifuge on a Chip

The Centrifuge-on-a-Chip device employs microscale fluid vortices to ‘trap’ and ‘release’ particles and cells in suspension.

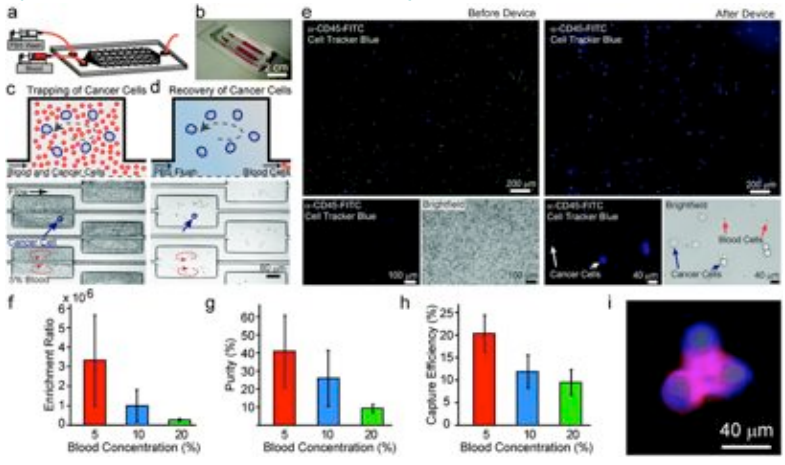


Size-based entry and maintenance of particles in microvortices.



Centrifuge on a Chip

High purity rare cell enrichment from whole human blood.
http://www.youtube.com/watch?v=OsbWOCkSejE



Circulating Tumor Cells (CTCs)

http://pubs.rsc.org/en/content/articlehtml/2013/lc/c2lc90148j