生物樣本前處理技術
Bio-sample Process Techniques

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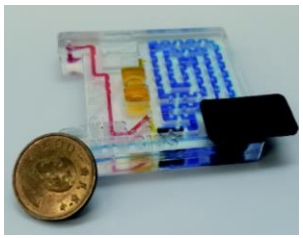
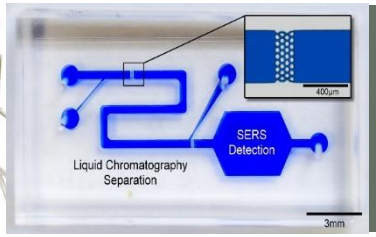
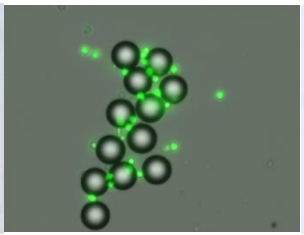
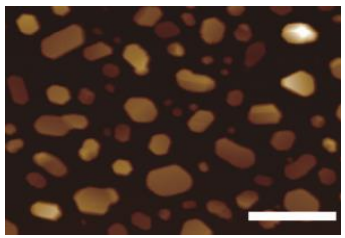
2018/10/26 Introduction to Lab on a Chip

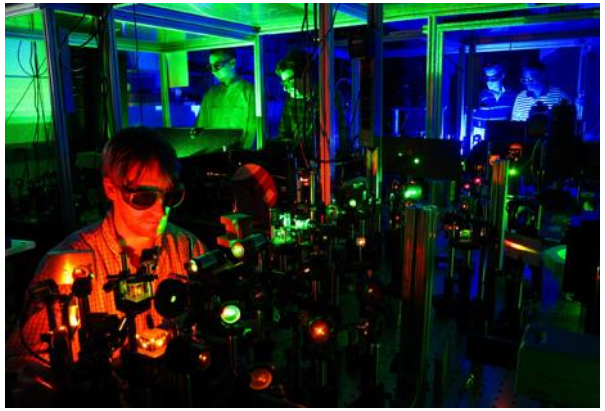








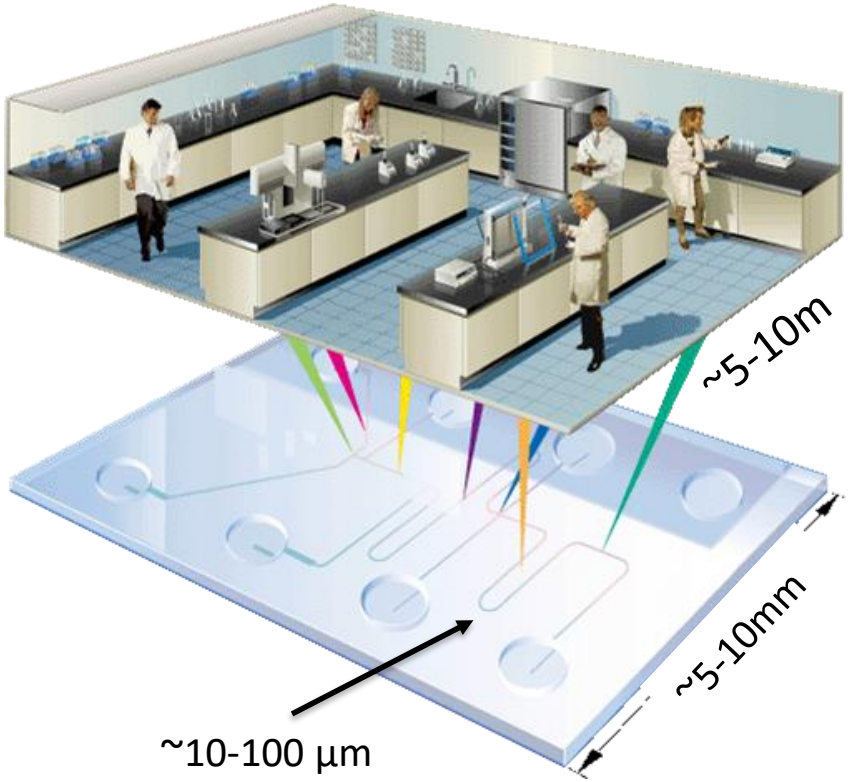
Laboratory…




THE BIGGEST BLIZZARD IN HISTORY
THE SECOND COMING
ZOMBIE OUTBREAK
LIFE IN GENERAL
THINGS THAT COULD BE HAPPENING OUTSIDE THAT YOU WOULDN'T KNOW ABOUT BECAUSE YOUR LAB/OFFICE HAS NO WINDOWS.

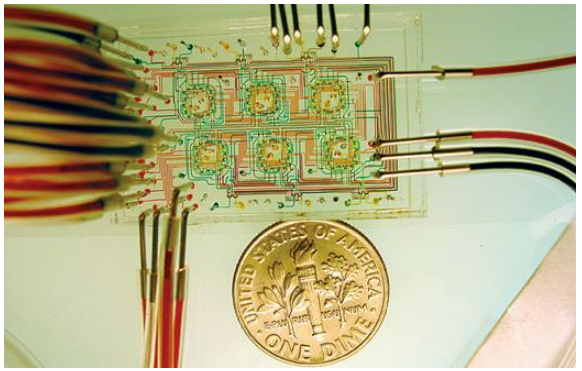
Lab-on-Chip

- Miniaturization and integration of laboratory sample preparation processes
- Consists of microfluidic channel, microsensors and microacutators
- Reduce cost and waste of bio-diagnostics
- Personalized medicine and healthcare

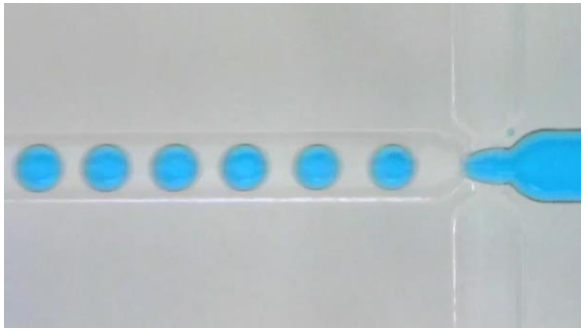


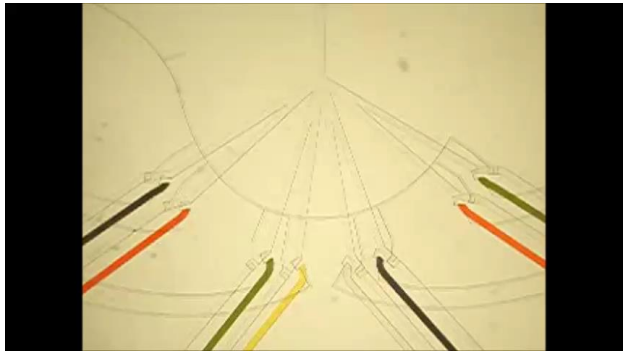
Microfluidics

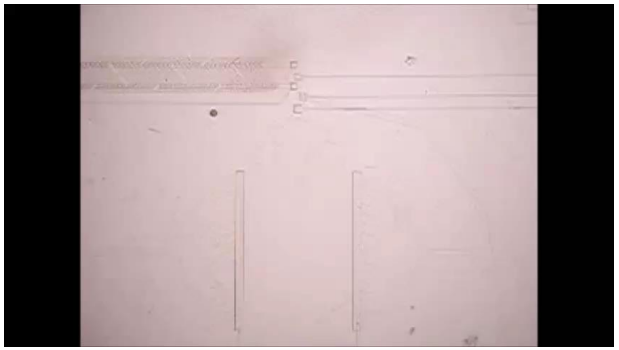
- The science and technology of manipulating and controlling fluids, between 10^{-6} to 10^{-12} L
- Networks of channels with dimensions from ~10-100 μm
- The research field origins in 1990s and grow dramatically due to development of analytical chemistry and microelectronic fabrication technologies.

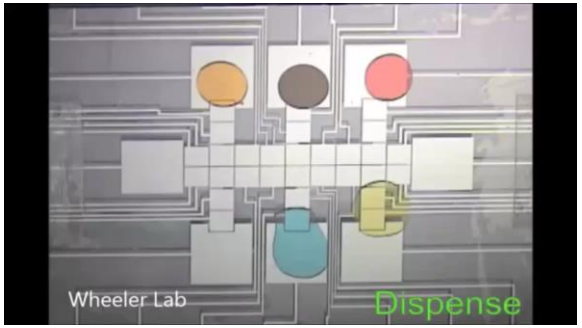


(Science, 309, 137-140, 2005)









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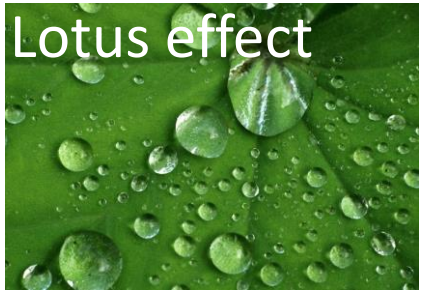
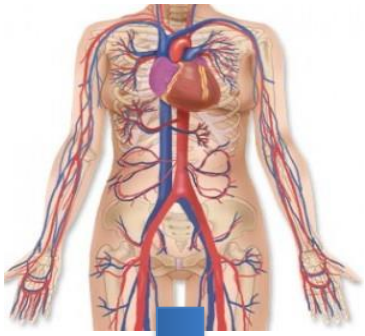
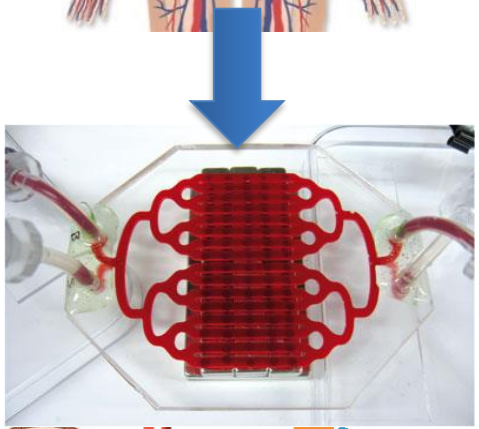
Microfluidics

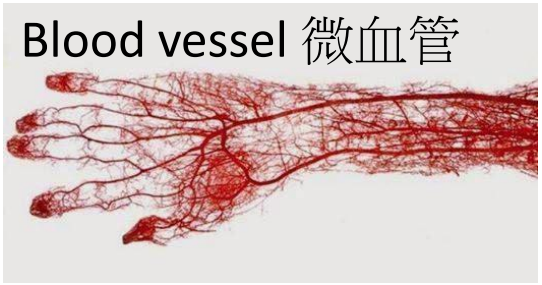
- Microfluidics in the environment?
- Microfluidics in the human body?
- Organs on Chip: design a whole body biomimetic device

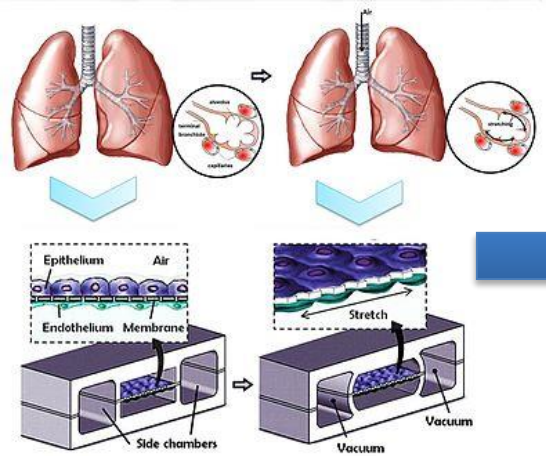


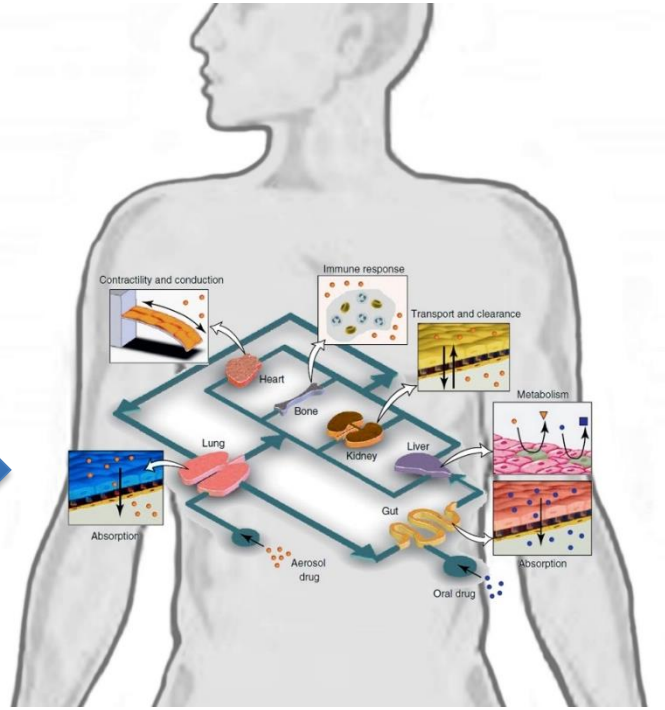




(Nature 471, 661–665)







http://wyss.harvard.edu/viewpage/240/

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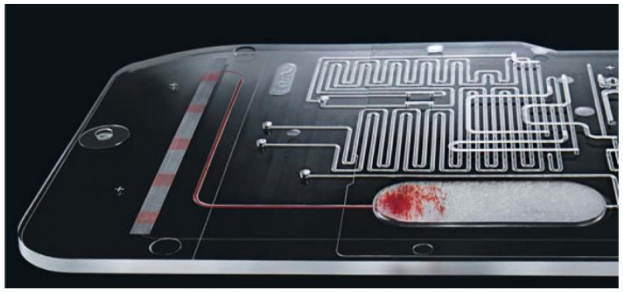
INNOVATE

Innovation: A Blood Test on a Chip

Claros Diagnostics has created the mChip, which can produce accurate test results from a single drop of blood in 10 minutes.

By Christine Lagorio-Chafkin *Senior writer, Inc.* @Lagorio

WRITE A COMMENT



CREDIT: Nigel Cox

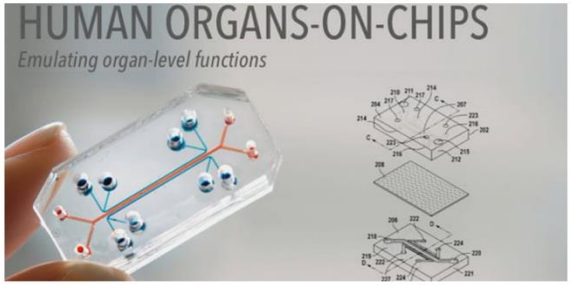
A lab on a chip

Many laboratory blood tests take several days to process. A group of Harvard University researchers has developed a device, the mChip, that produces accurate test results from a single drop of human blood in about 10 minutes. After blood is injected into the credit-card-size cartridge, it interacts with antibodies housed in hairline channels. The cartridge is then placed in a portable device that analyzes the results and displays them on a digital screen. One mChip can test for up to 10 disease biomarkers, including those for hepatitis C and HIV. Claros Diagnostics, co-founded by Vincent Linder and Samuel Sia, two of the mChip's inventors, has received approval to market a version of the device for prostate cancer screening in Europe. Next year, Claros, based in Woburn, Massachusetts, plans to apply

Organs-on-chips: Will They Lead to the End of Animal Testing?

Adriana Kyvik on 07/12/2015 FROM THE BENCH

1 SHARES



Miniature human organ models made using microchips...really? Is that even possible and could this be the end of animal testing?

emulate | insphero Biomimetic Microtissue Technology | MIMETAS the organ-on-a-chip company

These organs-on-chips are considered "organs" because they contain perfused chambers of living cells. These are arranged so that they simulate living tissues and function at the organ level in their physiology. So far, organs which have been replicated to the 3D microchip level include the heart, liver, lungs, gut and also bone marrow, all made into microfluidic cell culture-devices. Bionic!

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April 10, 2017

Quick and accurate measurement of circulating tumor cells (CTCs) in the bloodstream is a popular way to learn if tumors are metastasizing in the body. Current CTC isolation techniques are mostly based on immunomagnetic and microfluidic enrichment methods, both of which present accuracy issues with low yields of CTCs.

To improve this situation, Balaji Panchapakesan, an associate professor of mechanical engineering and director of the Small Systems Laboratory at Worcester Polytechnic Institute (WPI), has developed a liquid biopsy chip

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Streamlining Next-Generation Sequencing Experiments with NeoPrep Digital Microfluidics

by
Lisa C. Watson and Gary P. Schroth, Illumina Applications Group | Feb 27, 2015

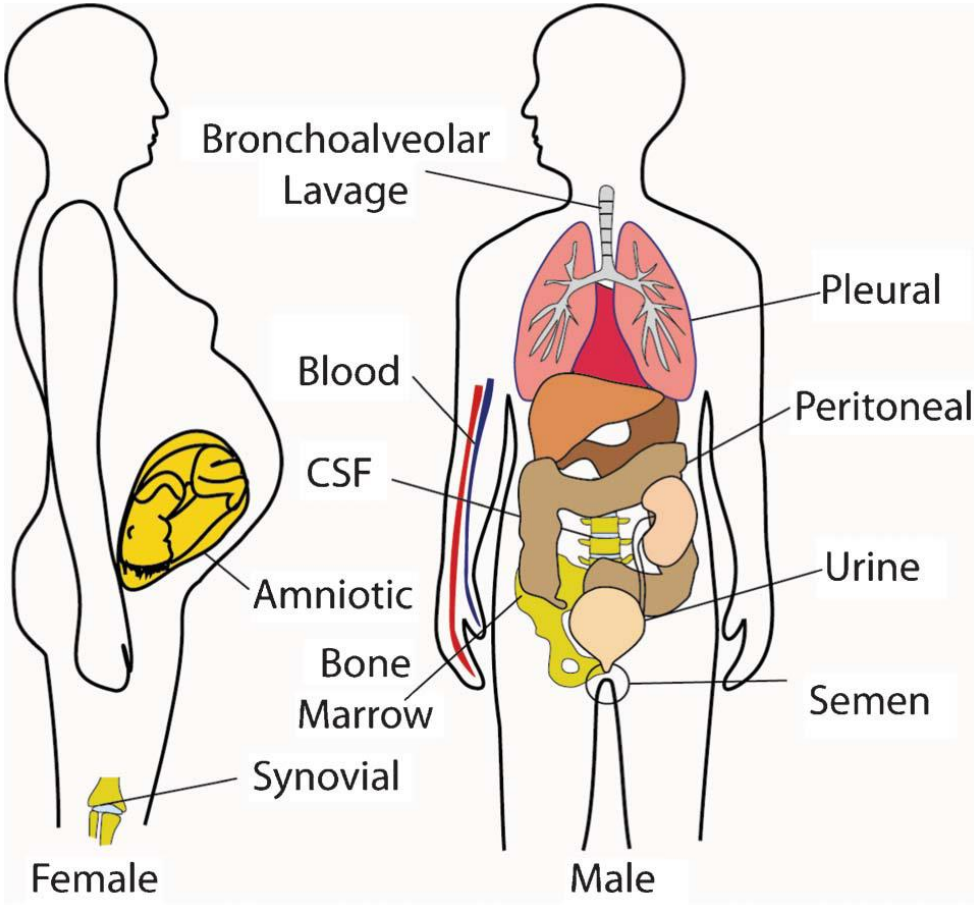
This week, Illumina launched the NeoPrep System – the first Illumina digital microfluidics platform for automated preparation of sequencing libraries. Digital microfluidics uses electrical voltage to manipulate nanoliter volume droplets through standard library prep chemistry, to ultimately transform sheared DNA or total RNA into ready-to-sequence libraries. This revolutionary technology enables 16 libraries to be prepared in parallel, with only 30 minutes of initial hands-on time, freeing up your schedule for other projects or planning your next

Bio-sample preparation process using microfluidics

- What is Bio-sample preparation process?
- Biofluid in Human System
- Blood
  - Composition
  - Non-Newtonian fluid
- Blood separation methods
  - Blood cells
  - Blood plasma
- Microfluidics for Whole Blood Processing
- Point-of-care (POC) biochips for sample process

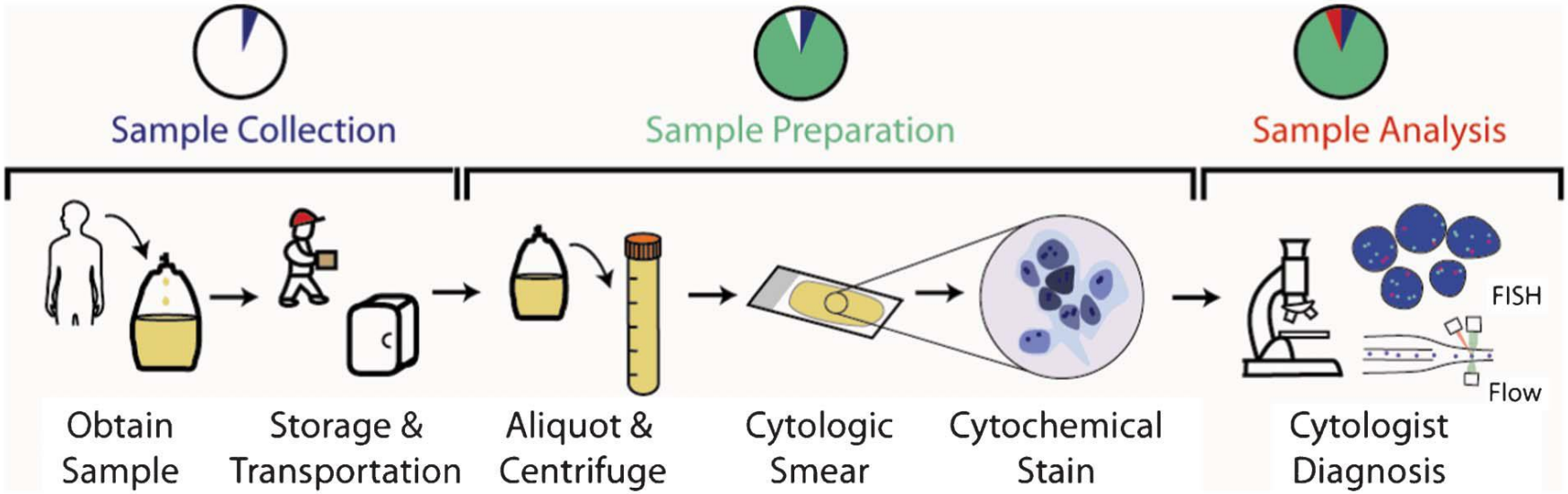
Biofluid in Human System

- Blood (血液)
- Pleural and peritoneal fluid (胸膜和胸腹水)
- Amniotic fluid(羊水)
- Urine(尿液)
- Bone marrow aspiration(骨髓切片檢查)
- Bronchoalveolar fluid (支氣管肺泡灌洗液)
- Synovial fluid (關節液)



Current Bio-Sample Process

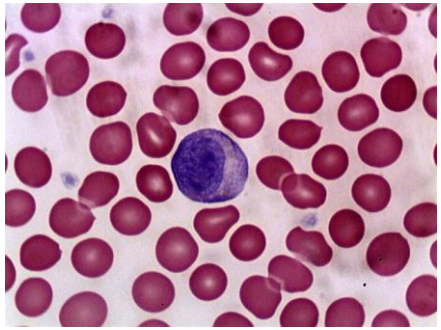
- Sample process: multiple centrifugation, cell fixing, washing and cytochemical staining
- Sample preparation process require most time

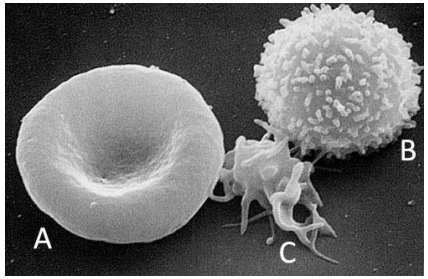


We should develop a platform to efficiently perform sample preparation!!!

Whole Blood

- Whole blood: 54.3% plasma, 45% red blood cells, 0.7% white blood cells
- Blood cell types
  - WBC: 4-10 x 10^6 cells/mL,12 ~ 20um
  - RBC: 4-6 x 10^9 cells/mL, 6 ~ 8um
  - Platelet: 4-10 x 10^6 cells/mL, 2 ~ 3um
- 4.7-5L in a health adult (7% of weight)



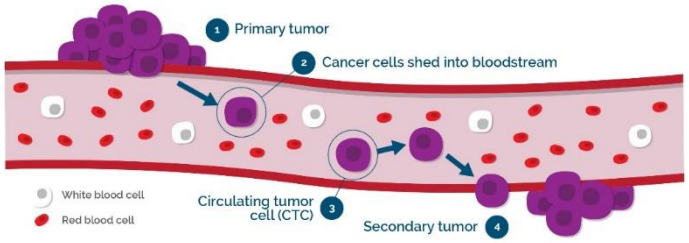


- One of the most important bio-sample
  - Can be used for screening pathogenic bacteria infection, metabolic diseases or diagnosis of cancer

Bacteria trapping

Diabetes diagnosis

CTC trapping



Blood Plasma Composition

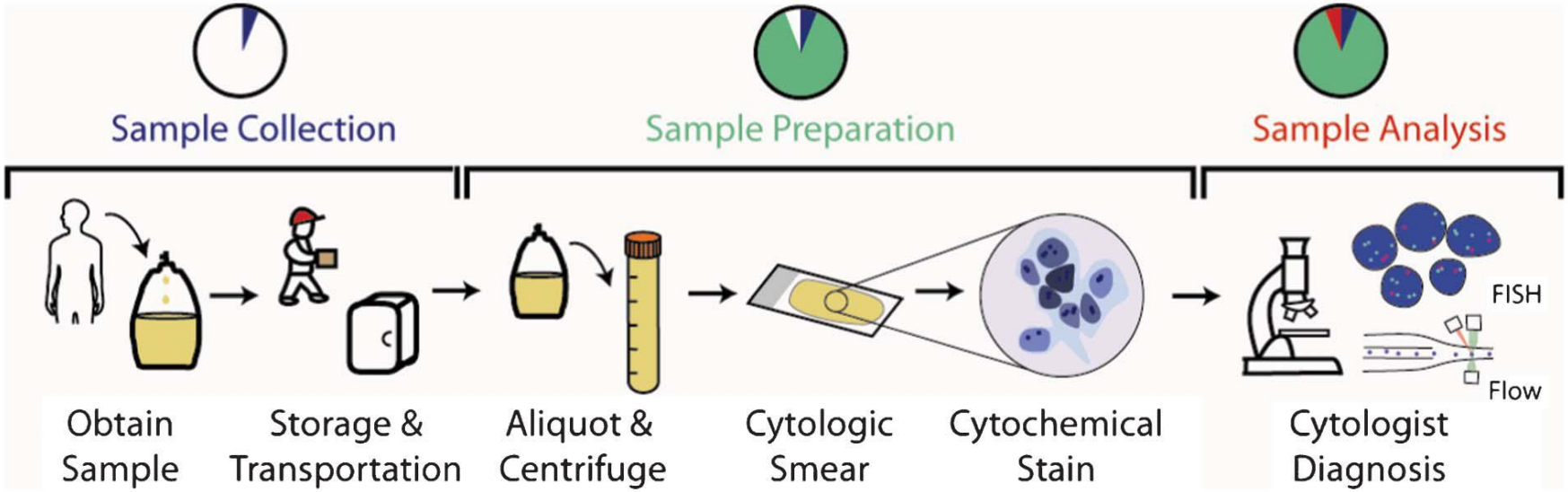
- Blood Plasma
  - Water (~91%)
  - Protein(~7%)
  - Bacteria, Fungi and Micro-organisms(traces)
  - Metabolites(traces)
    - glucose, total cholesterol(總膽固醇), melanin(黑色素)
    - urea(尿素), Hormones(賀爾蒙激素)
  - Circulating Nucleic Acids(traces)
    - Health patients: DNA (~1.8-35ng/mL), RNA(~2.5ng/mL)
    - Depending on conditions
      - Tumor DNA (cancer), Viral DNA(infection), Fetal DNA(pregnacy), Donor DNA(transplantation)

Disease Diagnosis from Blood Cells

- Blood Cells
  - Complete blood count (CBC)
    - Leukemia: abnormal increase of immature white blood cells
    - HIV: count CD4+ T-cells
    - Malaria: the level of parasitemia (volume of blood occupied by malaria parasite)
  - Rare cell counting
    - Circulating tumor cells (CTC): cancer relapse or mutation states
    - Fetal cells: determine the health of developing fetus
  - Long term cell culture
    - Monitor immune cell conditions
    - Search for bacteria, virus

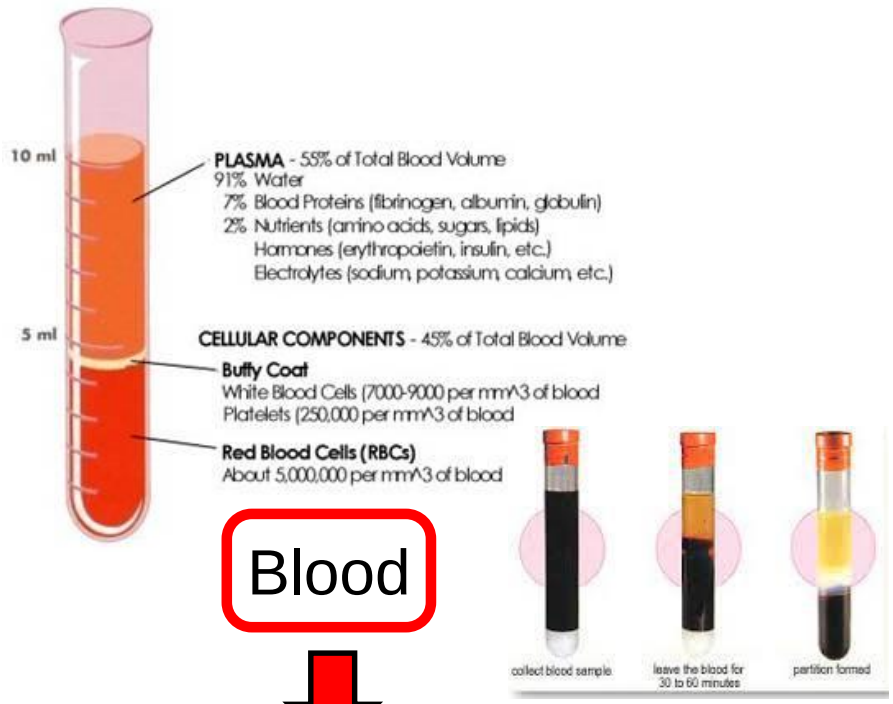
Current Bio-Sample Process Problem

- Sample preparation: centrifugation, cell fixing, washing and cytochemical staining
- The quality of biomarker detection will be affected by sample preparation



A platform to efficiently perform sample preparation and in-situ analyte detection!!!

Blood Separation Methods



Blood

- Plasma Separation
- Cell Separation

Label-free

No external field

- Micro-scale filters
- Hydrodynamic filtration
- Deterministic lateral displacement
- Inertial
- Gravity and sedimentation
- Aqueous two-phase systems

With external field

- Acoustophoresis
- Magnetophoresis
- Optical
- Dielectrophoresis

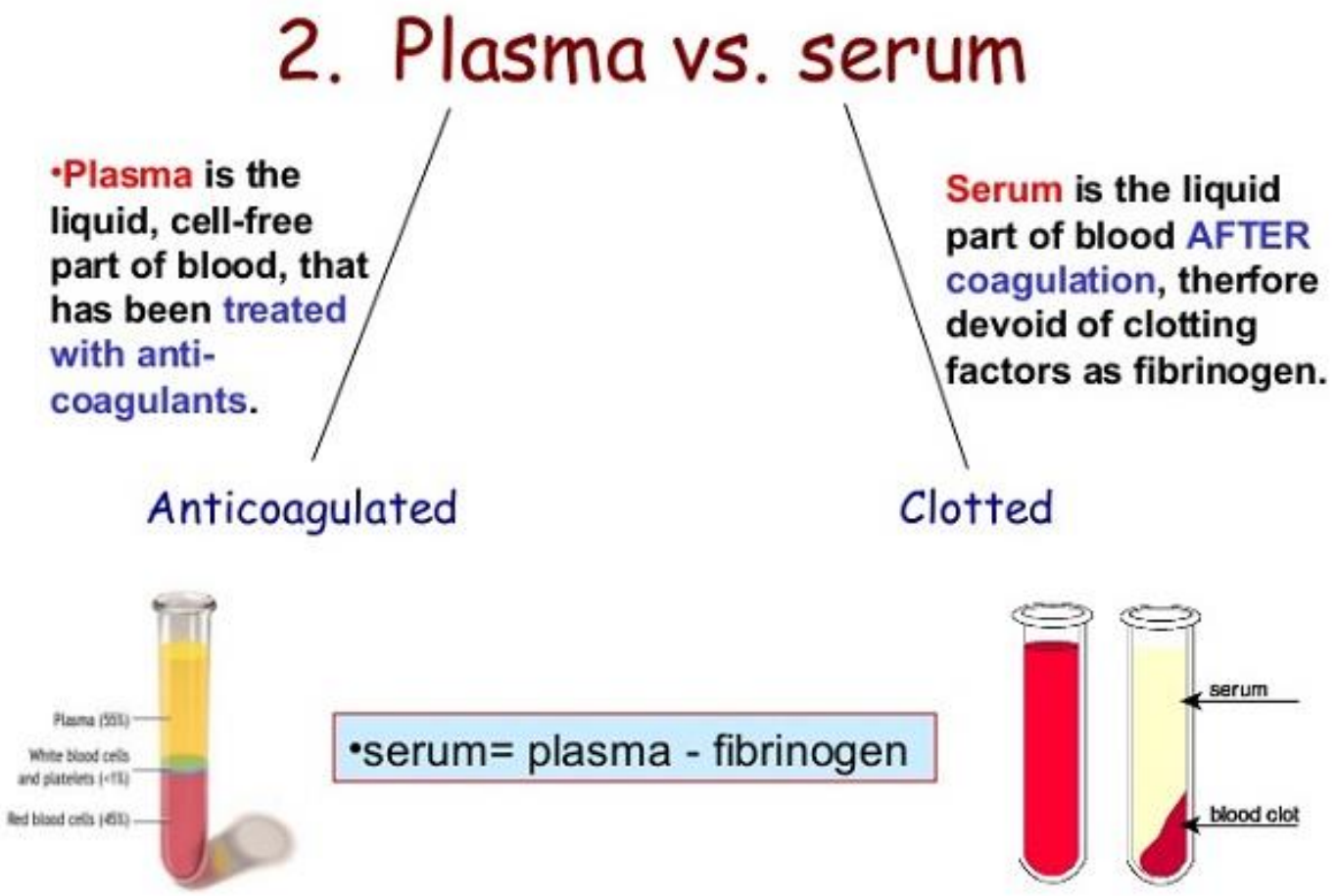
Other format

- CD
- Paper

Labeled

- Fluorescence-activated cell sorting
- Magnetic-activated cell sorting

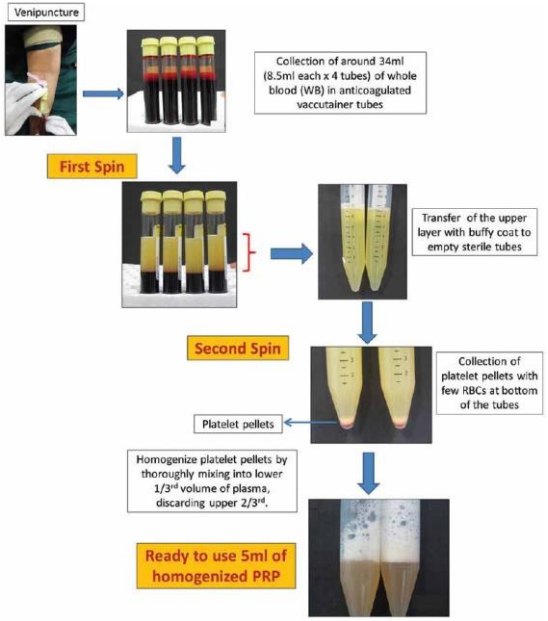
Difference between Plasma and Serum



http://www.microbiologynotes.com/differences-between-serum-and-plasma/

Blood Separation Method

- Centrifuge



- Platelet-Rich Plasma (PRP) treatment

PRP刺激細胞修復 Kobe 伍茲都說讚

2016年05月12日 傳送 讚 99 G+

Kobe曾接受PRP治療膝傷。資料照片

【王翊亘／綜合報導】PRP治療全名為platelet-rich-plasma，高濃度血小板血漿治療，許多國外運動名將都曾使用過此方式治療患處，包括高爾夫前球王老虎伍茲(Tiger Woods)、男網球星納達爾(Rafael Nadal)以及今年剛從NBA退休的Kobe(Kobe Bryant)。

PRP治療是透過使用患者自身的血液，用離心機把血小板分離出來，再把血小板注射到受傷部位，刺激細胞修復，手術過程1小時完成。技術原理為將血漿濃縮，讓血液中的血小板破裂，釋出生長因子，促進軟組織修復和再生。

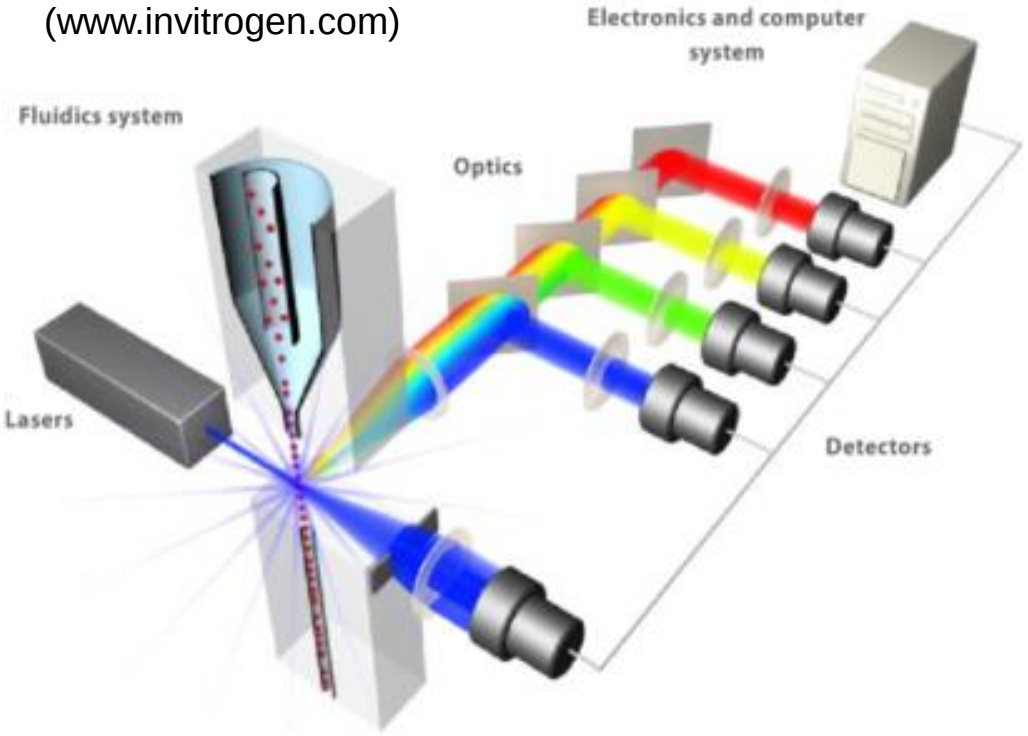
這種治療方式一開始是使用在醫學美容，後來改應用在關節、韌帶的治療修復，在歐美已存在許久，Kobe在2011年時，就曾到德國進行PRP治療。在台灣若進行PRP治療，價格約在1萬至2萬元間，配合手術使用效果最佳，林智勝2013年進行左膝手術時，也曾在縫合的韌帶上施打PRP，幫助加速癒合。

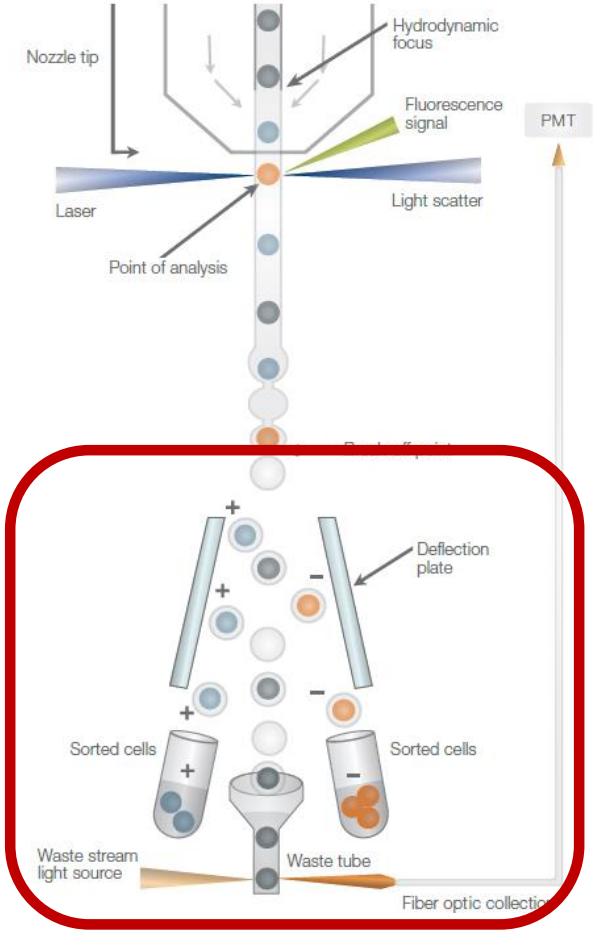
台灣蘋果日報 TW 說這專頁讚 370 萬 按讚次數

Labeled Cell Separation Methods

- Fluorescence-activated cell sorting(FACS)

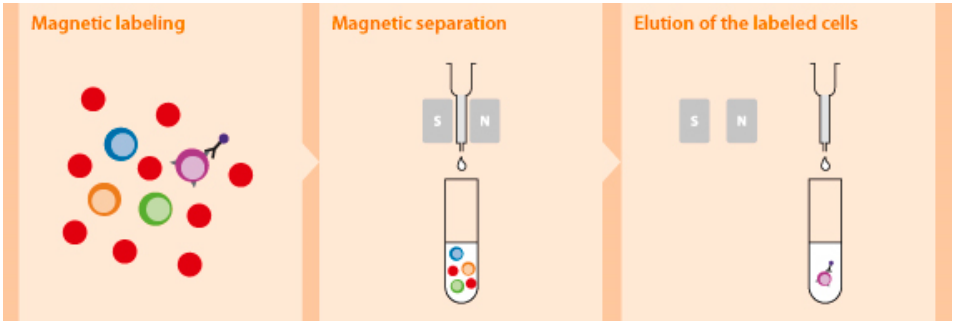
(www.invitrogen.com)

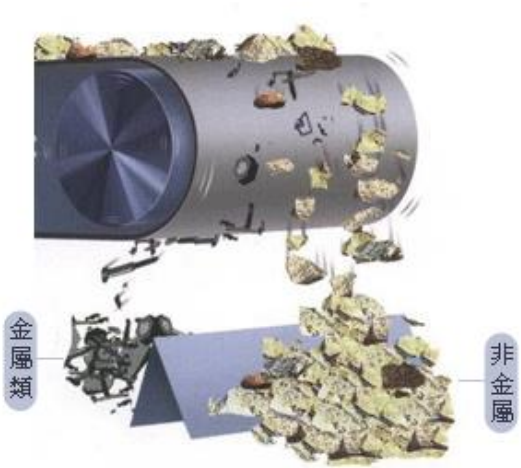




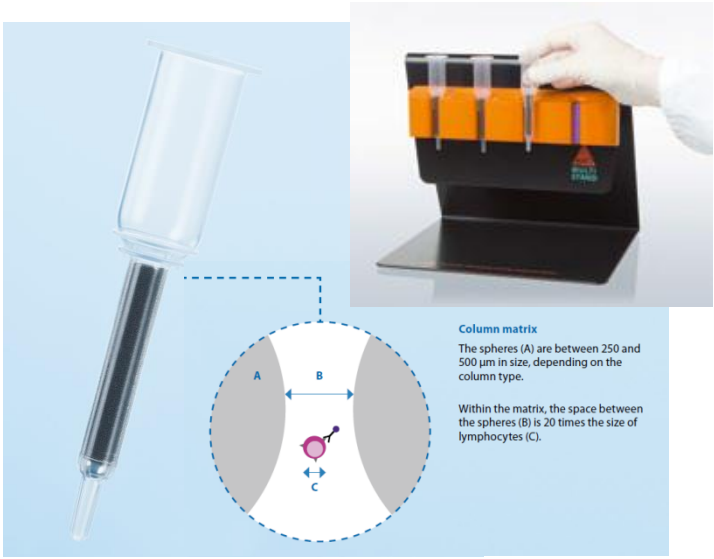
Labeled Cell Separation Methods

- Magnetic-activated cell sorting(MACS)
  - Magnetic bead labeling
  - Magnetic column
  - Cell analyzer





http://www.proten.com.tw/products-3.htm



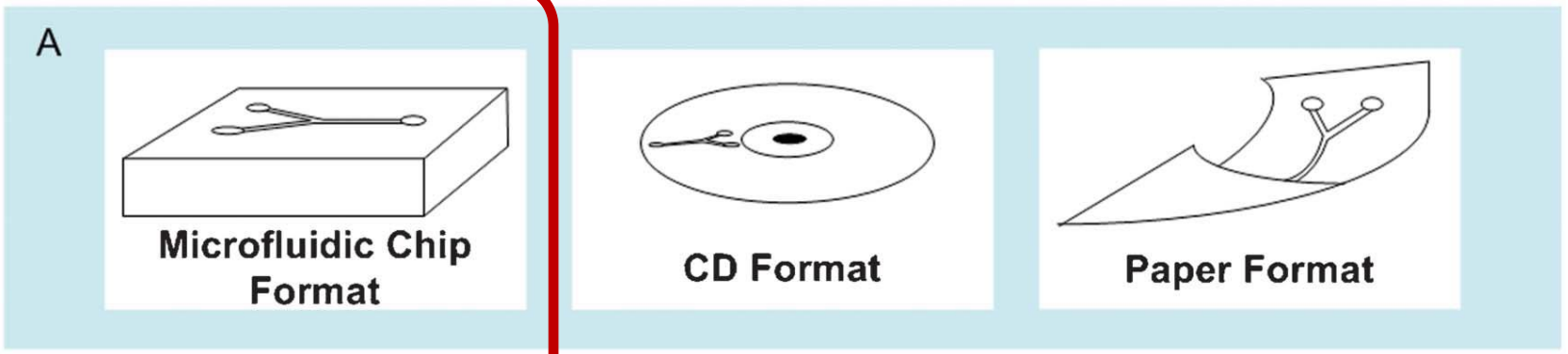
https://www.miltenyibiotec.com/

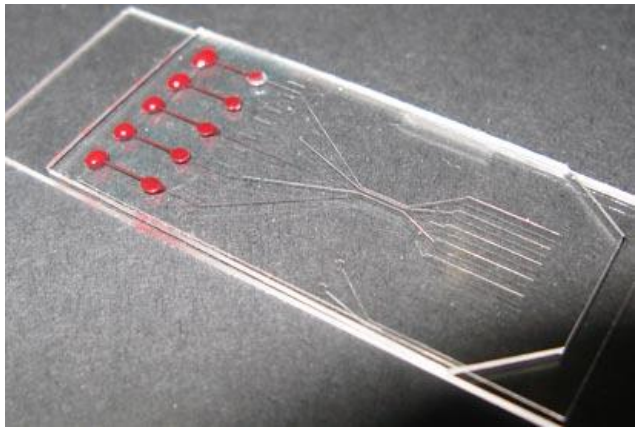
Challenges of Whole Blood Process

- High cellularity of samples
- Cell components aggregation
  - EDTA: prevent platelet activation
  - Dilution factors
  - Red blood cell lysis: remove 99% of cellular contents
- Large blood volume process
- Long sample culture time

Can we do a simpler whole blood process in a resource limited environment?

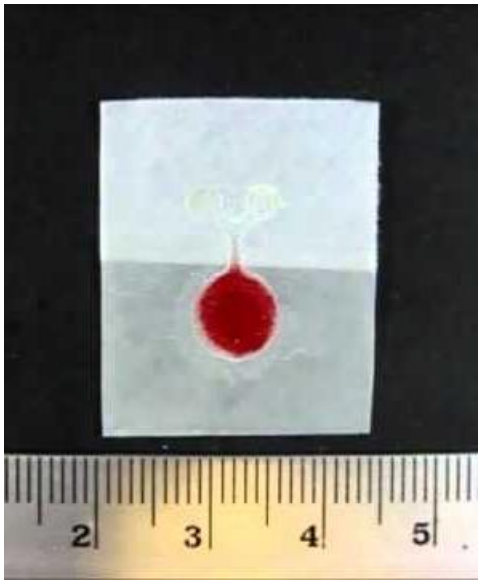
Three formats for Whole Blood Process





Multifunctional
Flow rate control

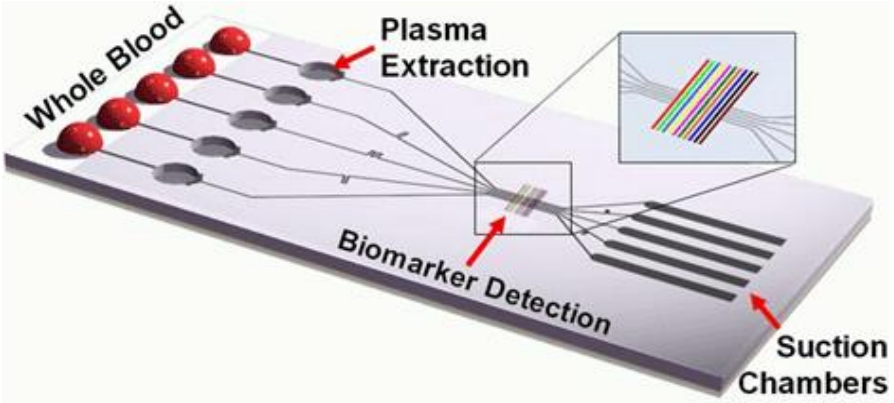
Fast response
Easy of integration



Power-free
Easy to discard

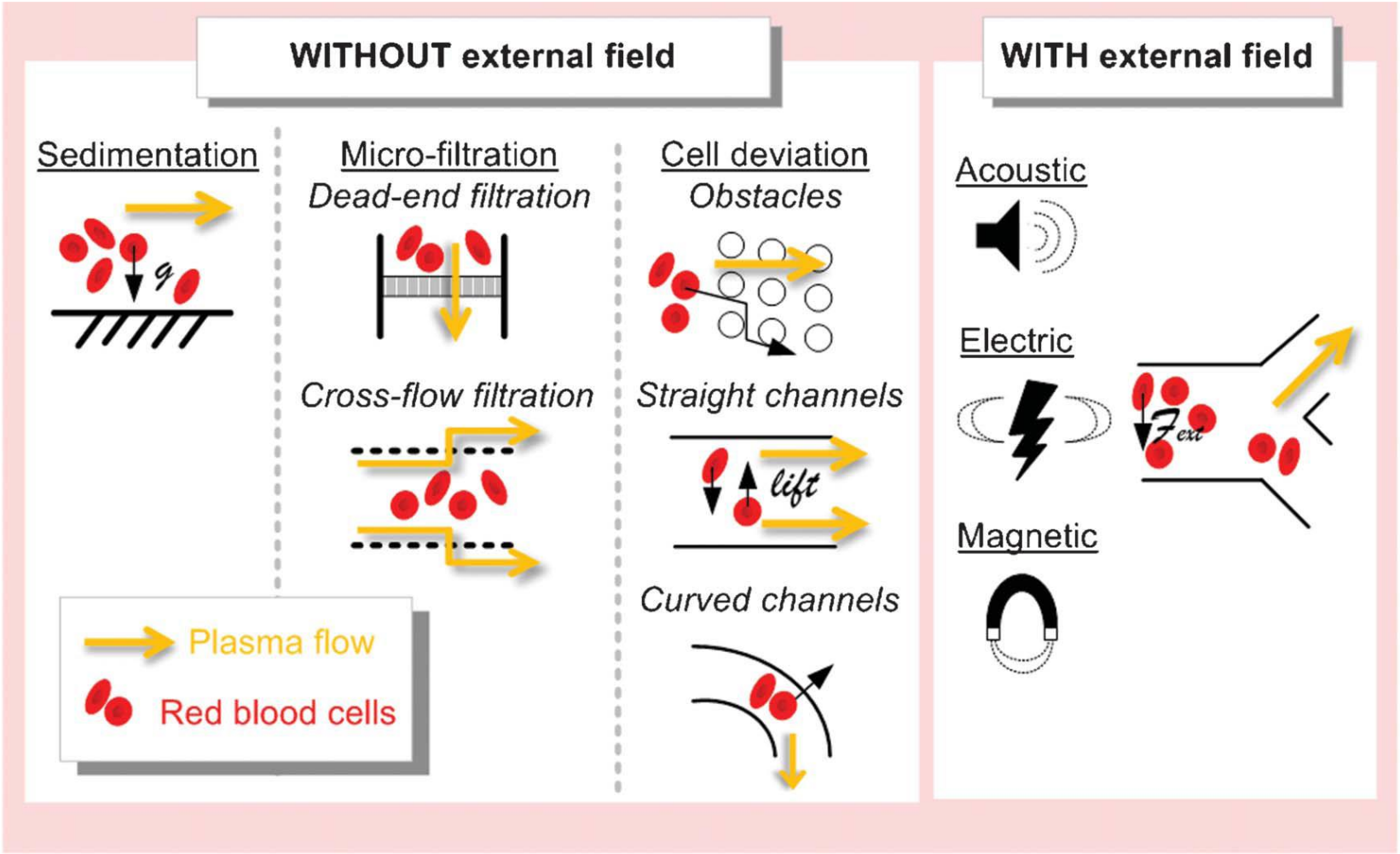
Microfluidics for Whole Blood Process

- Why microfluidics for whole blood process?
  - Cost-effective, portable, disposable
  - Low sample volume
  - Fast response
  - Multi-functional
- Four important parameters:
  - Dilution ratio
  - Throughput
  - Purity
  - Yield



L. Lee Group at UC Berkeley

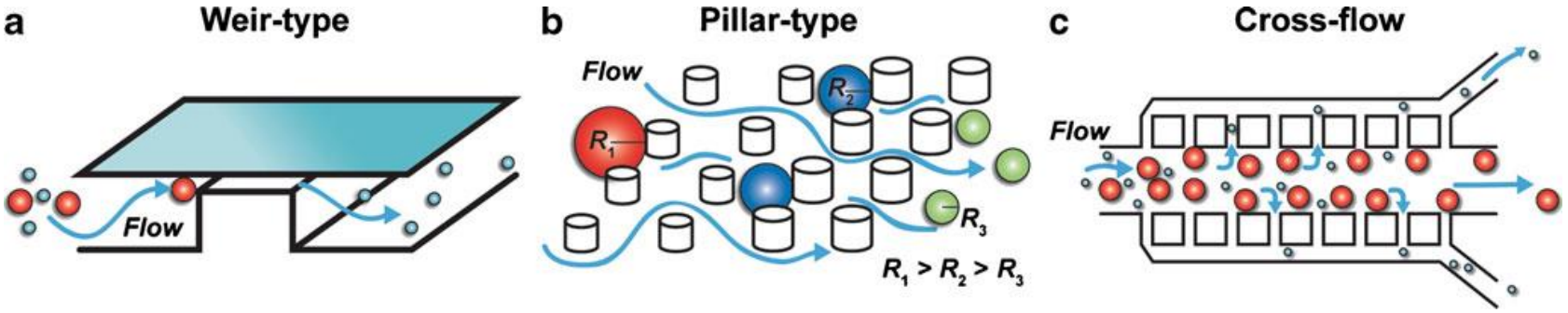
Label-free Blood Separation Methods



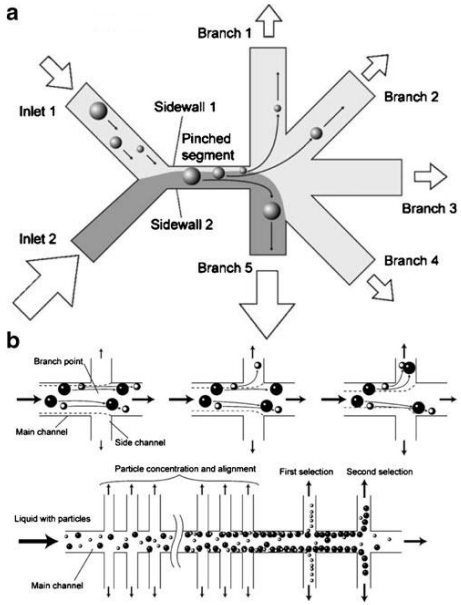
Do we want blood plasma or blood cells?

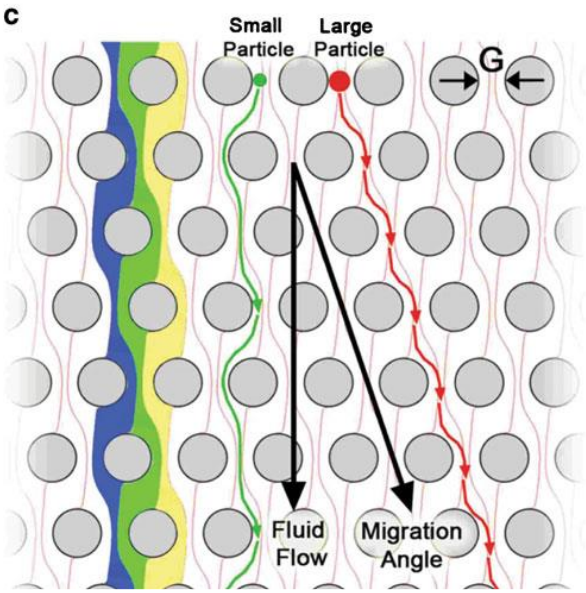
Blood Cell Separation Methods

- Micro-scale filters (Size, deformability)

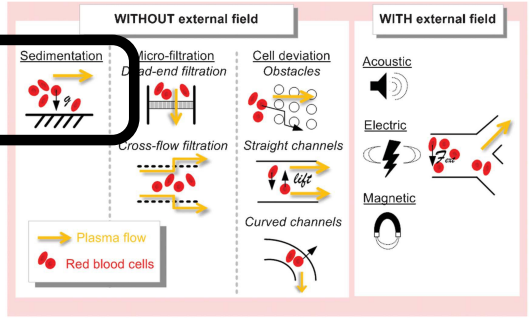


- Hydrodynamic filtration (size, shape)
- Deterministic lateral displacement (size)

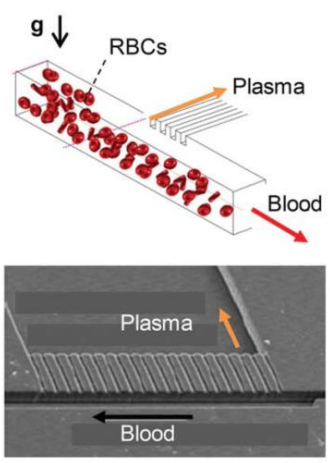
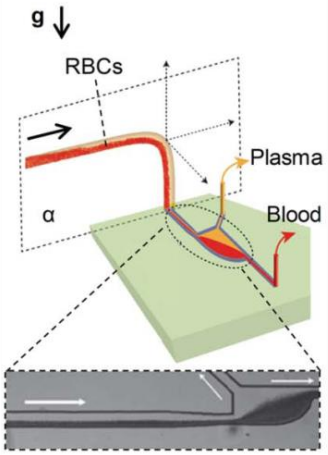
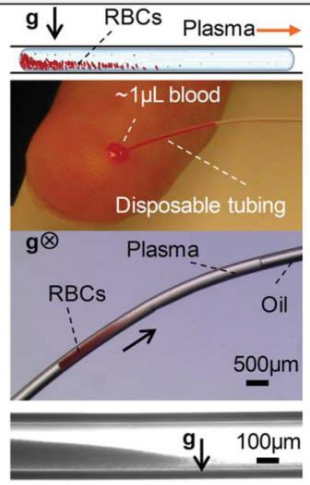
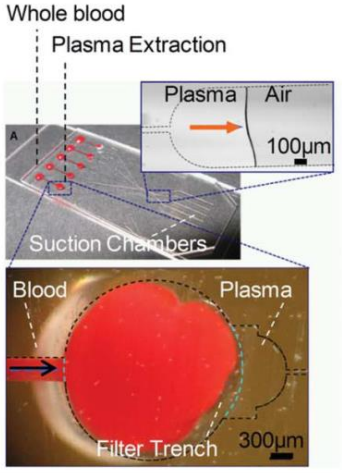




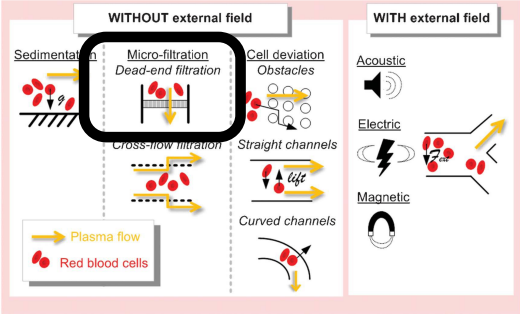
Sedimentation Method



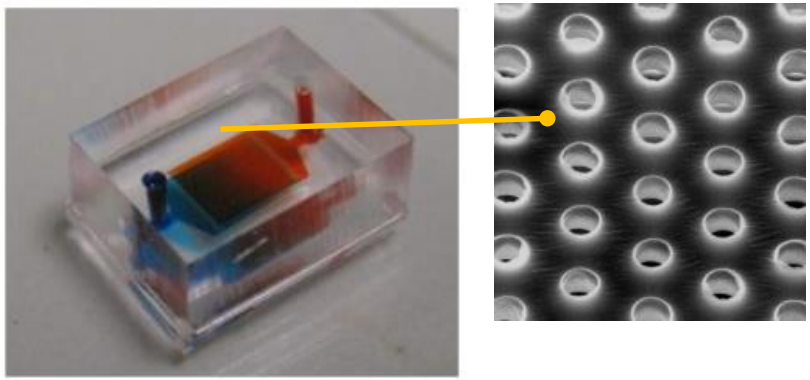
- Based on gravity
- Blood cell separation
  - May not be suitable
- Blood plasma separation
  - The easiest method

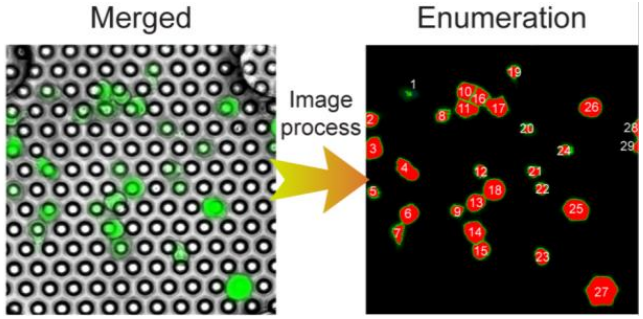
A - Sedimentation with Cross-Flow Filtration	B - Sedimentation with Back-Facing Step	C - Sedimentation in a Plug	D- Sedimentation in Trenches
			
Dilution ratio: None Flow rate: 0.1 μL/min Purity: N/A Yield: 20%	Dilution ratio: 1:5 Flow rate: 15 μL/min Purity: 99% (Hematocytometer) Yield: 66%	Dilution ratio: 1:5 Flow rate: 0.5 μL/min Purity: 100% (Image Analysis) Yield: 60%	Dilution ratio: None Flow rate: 0.83 μL/min Purity: 100% (Image Analysis) Yield: N/A

Micro-Filtration Method



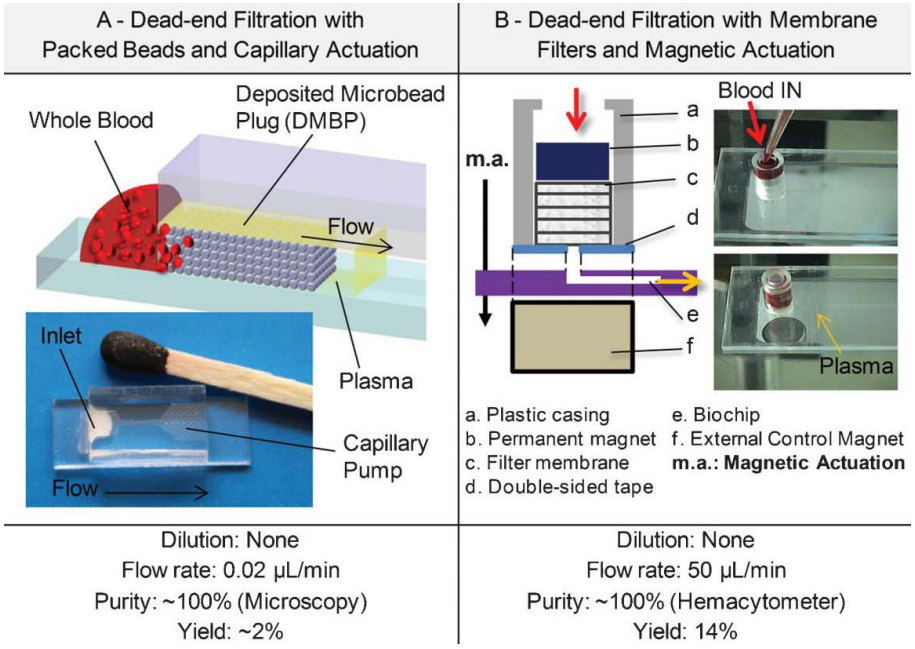
- Based on cell size
- White blood cell separation



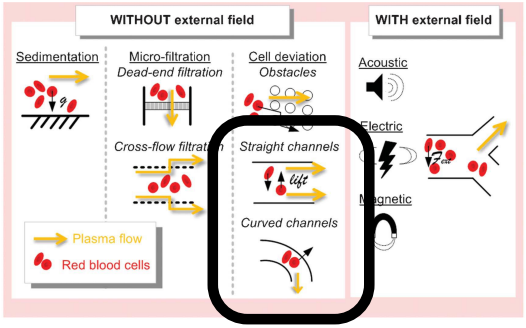


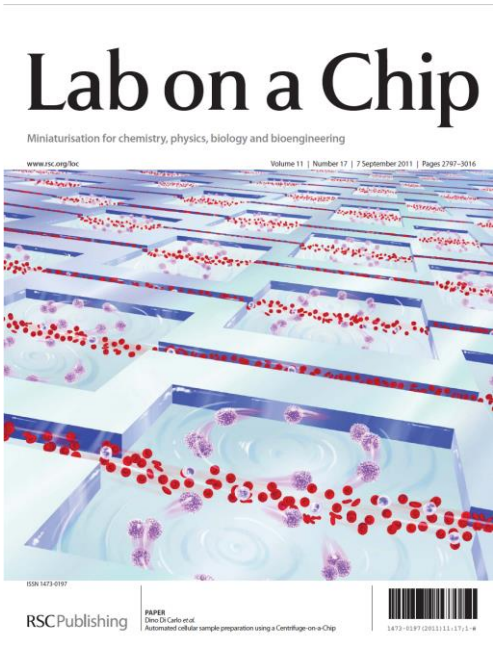
K. Kurabayashi Group, U Michigan

- Blood plasma separation

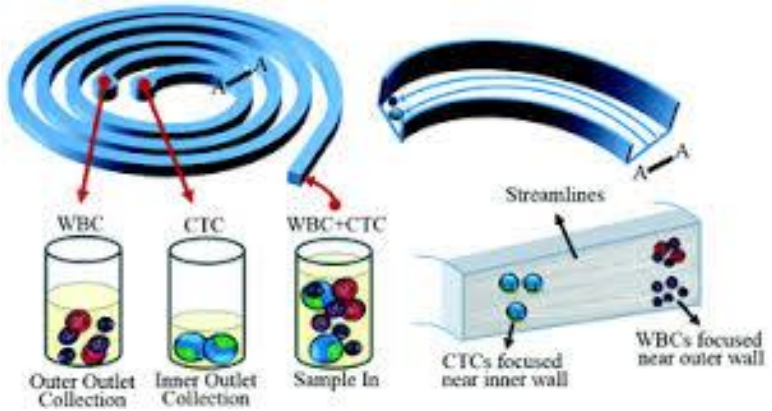


Cell Deviation Method

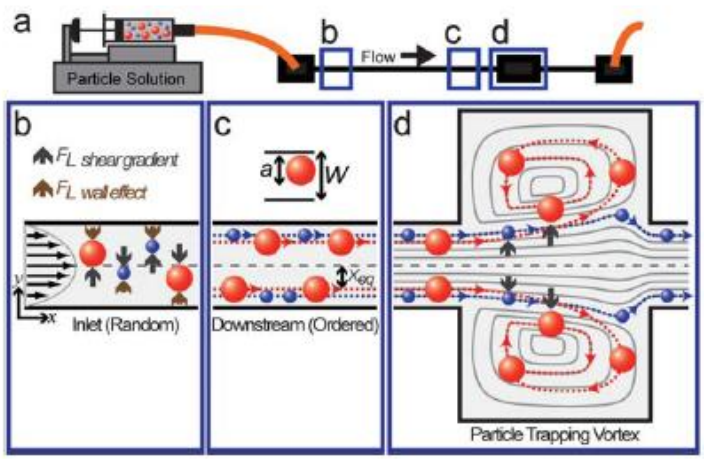




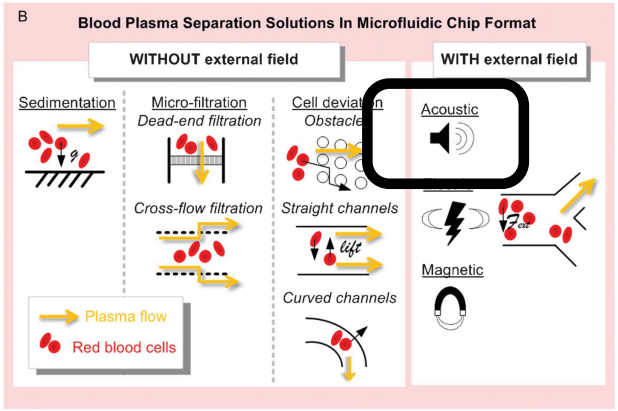
- Blood Cell Separation



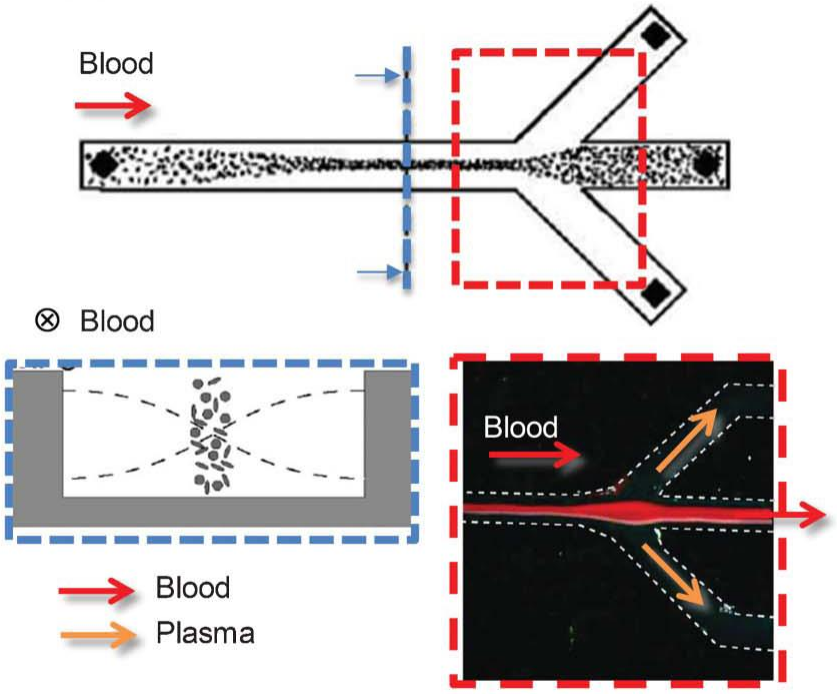
- The balance of shear gradient and wall effect life force



Acoustic Field Separation



- Blood plasma separation

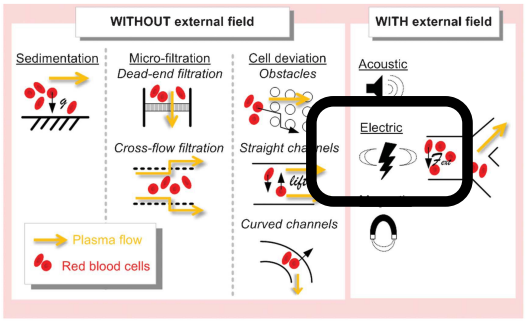


- Based on cell size, density and compressibility
- Can be used as acoustic flow cytometry: https://www.youtube.com/watch?v=b2ilHEnugE0

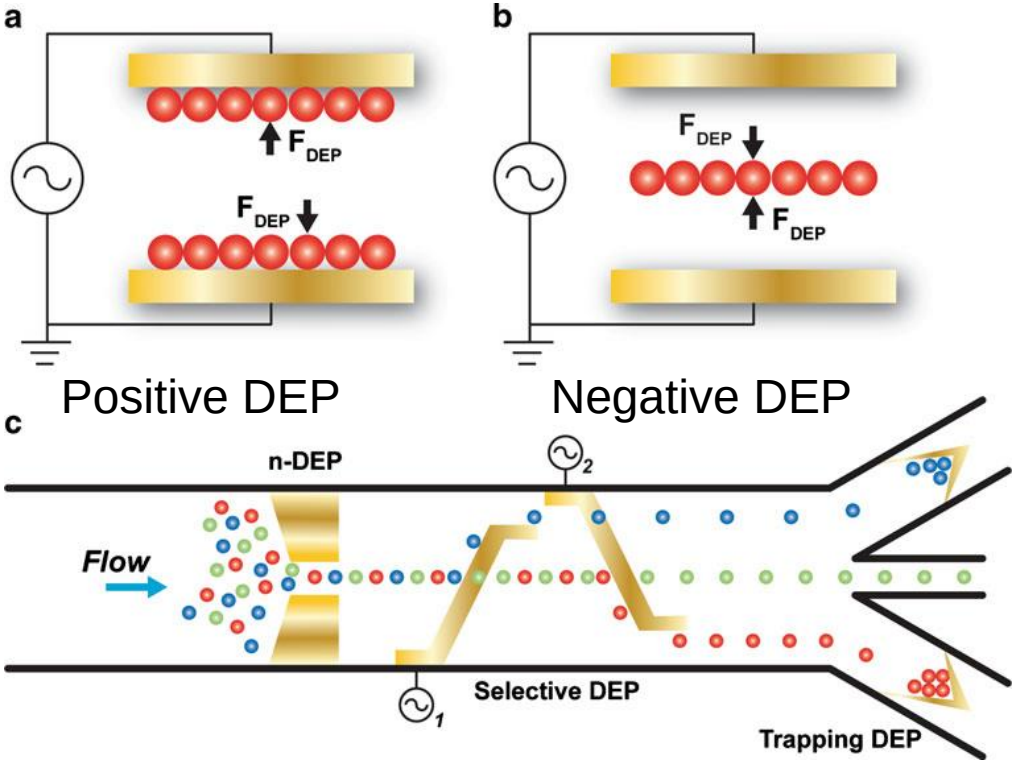
A. Lenshof, A. Ahmad-Tajudin, K. Jaras, A.-M. Sward-Nilsson, L. Aberg, G. r. Marko-Varga, J. Malm, H. Lilja and T. Laurell, Anal. Chem., 2009, 81, 6030–6037.

Electrical Field Separation

- Blood Cells Separation



- Dielectrophoresis (DEP)
  - The force applied to the dielectric particle when subject to an non-uniform electrical field
  - Particles did not have to be charged
  - The DEP force depends on particle size, shape, freq. of E filed

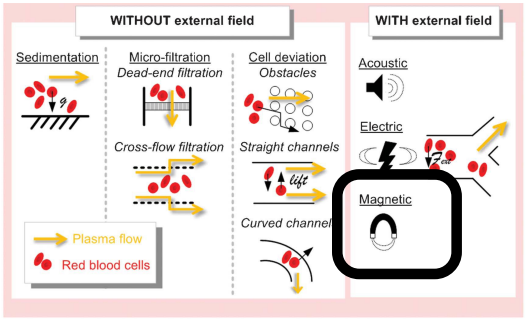


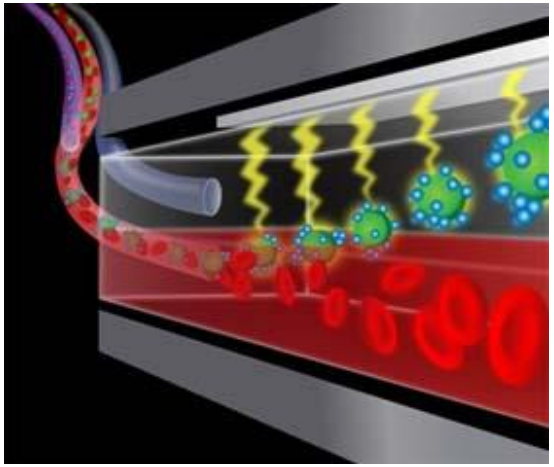
$$\langle F_{\mathrm{DEP}} \rangle = 2\pi r^3 \varepsilon_m \mathrm{Re}\left\{ \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \right\} \nabla \left| \vec{E}_{rms} \right|^2$$

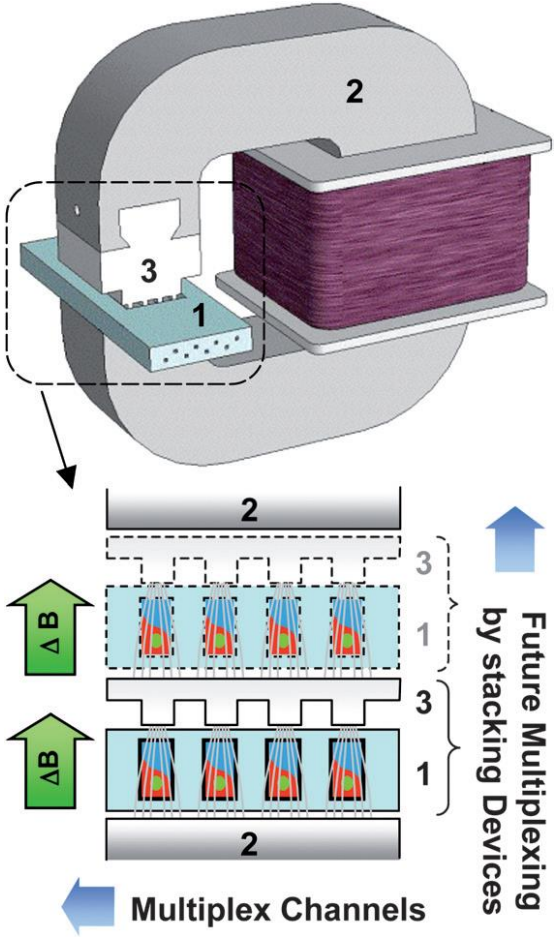
https://www.youtube.com/watch?v=Hf0sen7bJ6A

Magnetic Field Separation

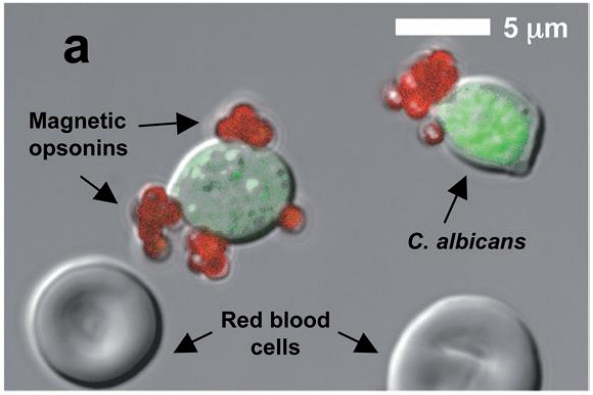
- Blood Cells Separation

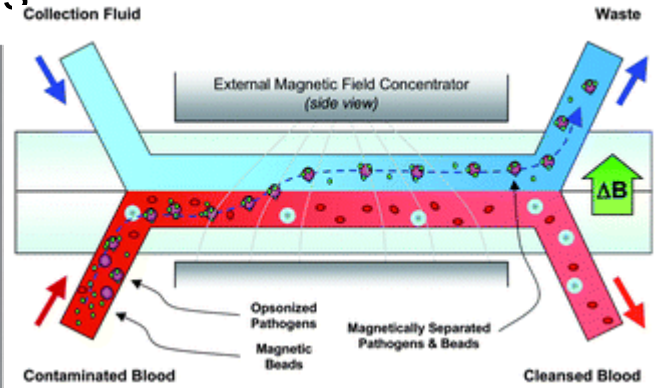






- C.albicans fungi:
  - a leading cause of sepsis-related deaths





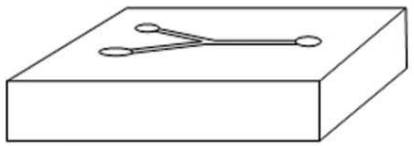
Micromagnetic–microfluidic blood cleansing device ***Lab Chip***, 2009,**9**, 1171-1177

Challenges of Microfluidics for Whole Blood Process

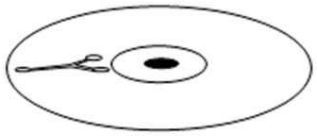
- Easy-of-use: automating multi-step sample preparation
- Yield: Preparing samples with high cellularity
- Purity: achieving high purity cell populations
- Throughput: concentrating rare cells from large volumes
- Multiplexity: preparing small volume sample for multiple assays

Three formats for Whole Blood Process

A

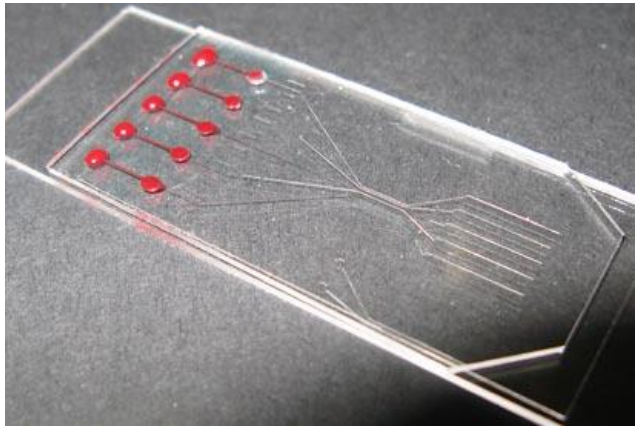


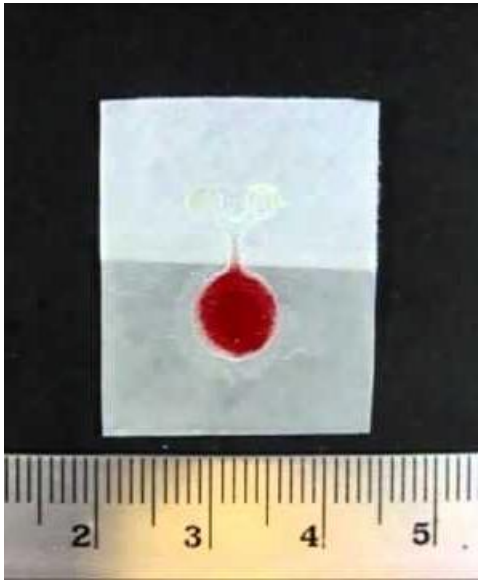
Microfluidic Chip Format



CD Format

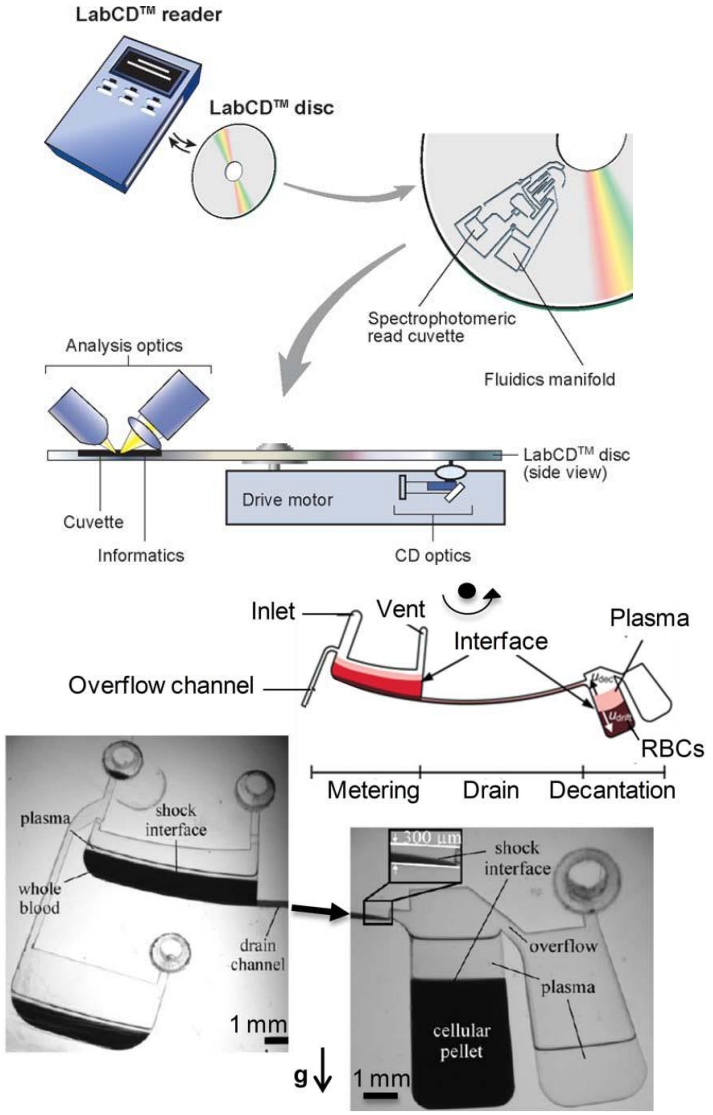
Paper Format





Blood Plasma Separation by CD

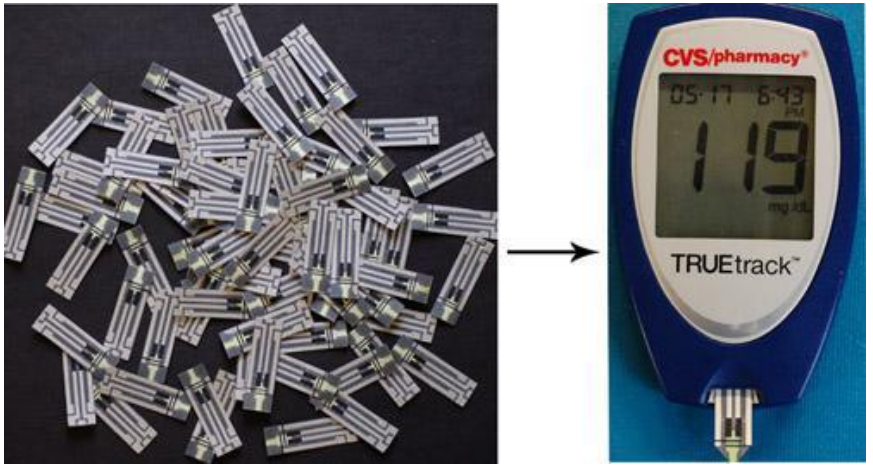
- Use centrifugal force
- Advantages:
  - Cost-effective
  - High throughput
  - Fast response
- Problems:
  - Not easy to adjust flow rate
  - Require valves
  - Tubing is difficult

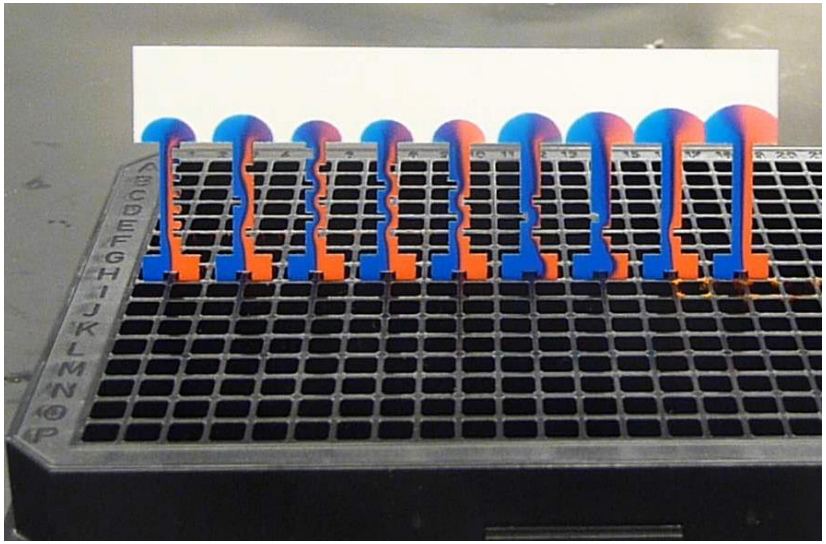


https://www.youtube.com/watch?v=iXUtVtpP6Q8

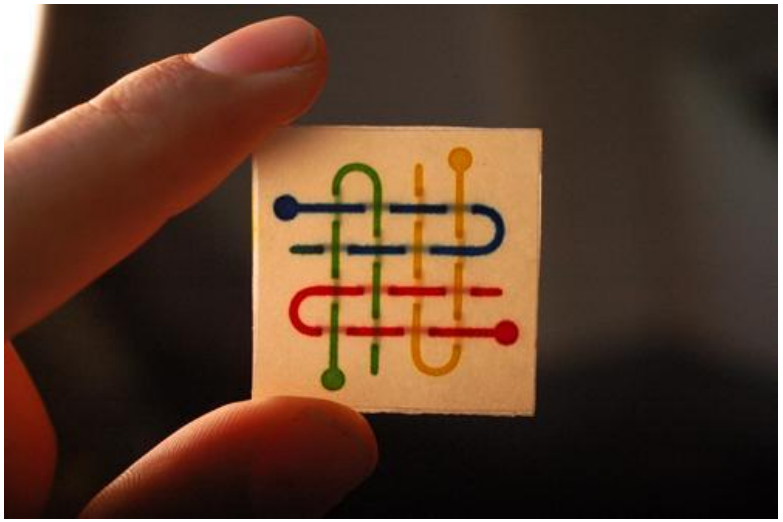
Paper-based Microfluidics

- Low cost
- Easy of fabrication
- Long term storage





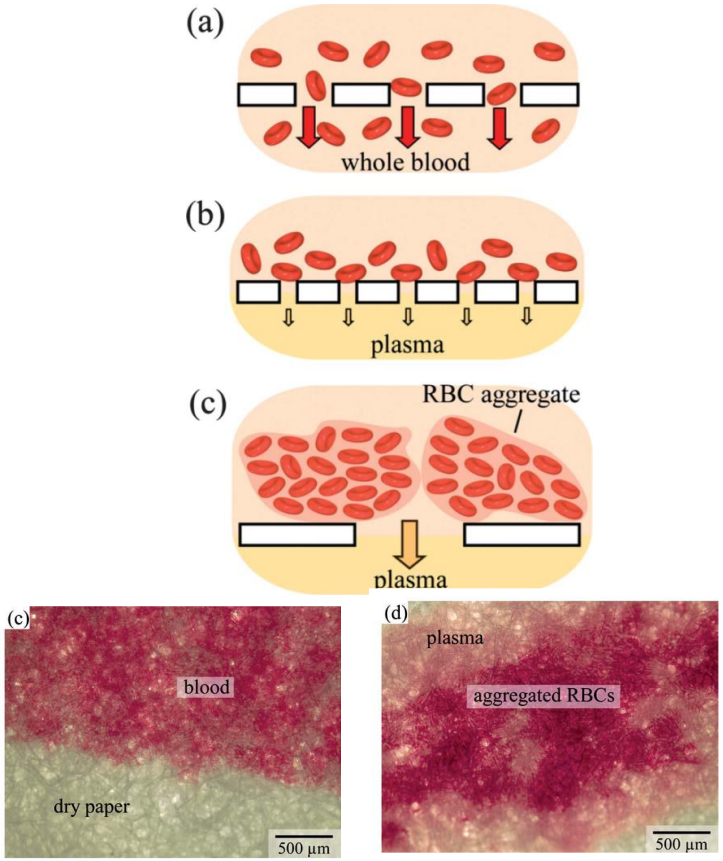
P. Yager Group, U Washington

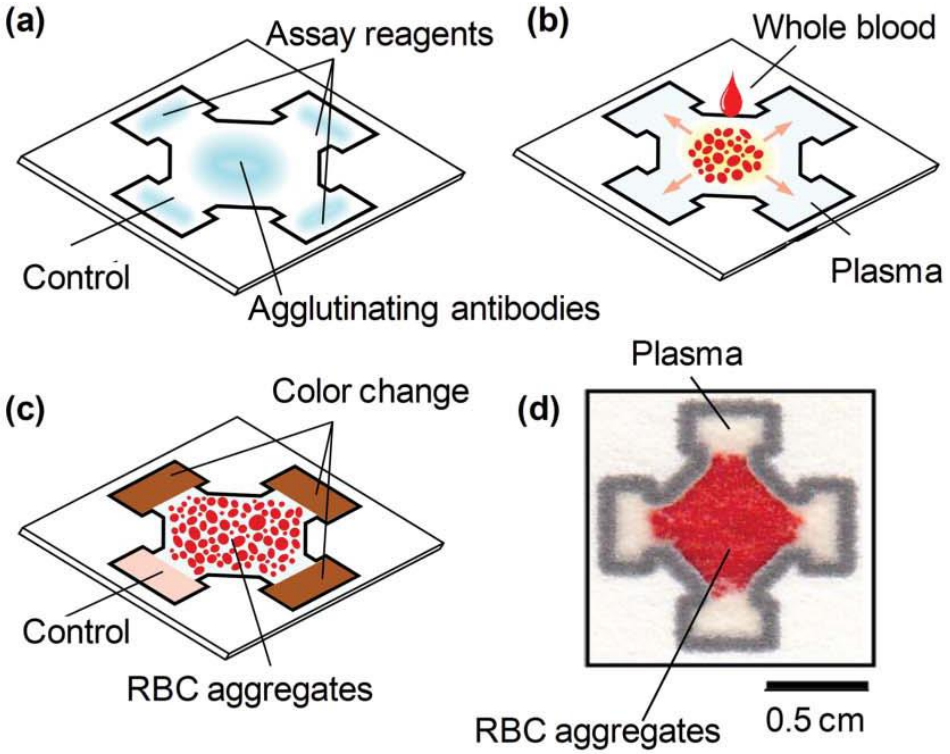


G. Whiteside Group, Harvard

Blood Plasma Separation by Paper

- Use capillary force to draw liquid
  - RBC aggregation helps plasma separation

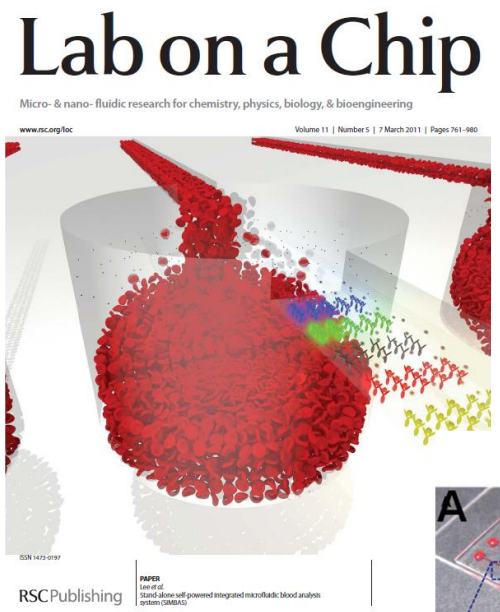


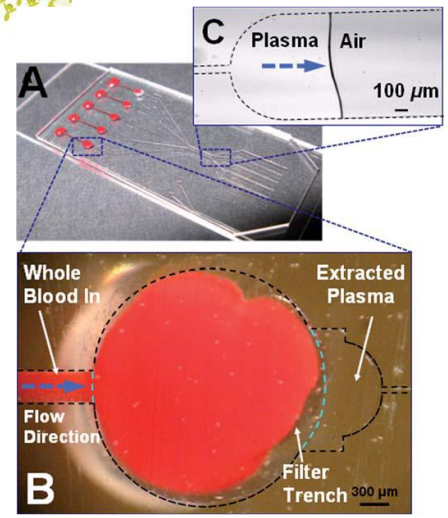


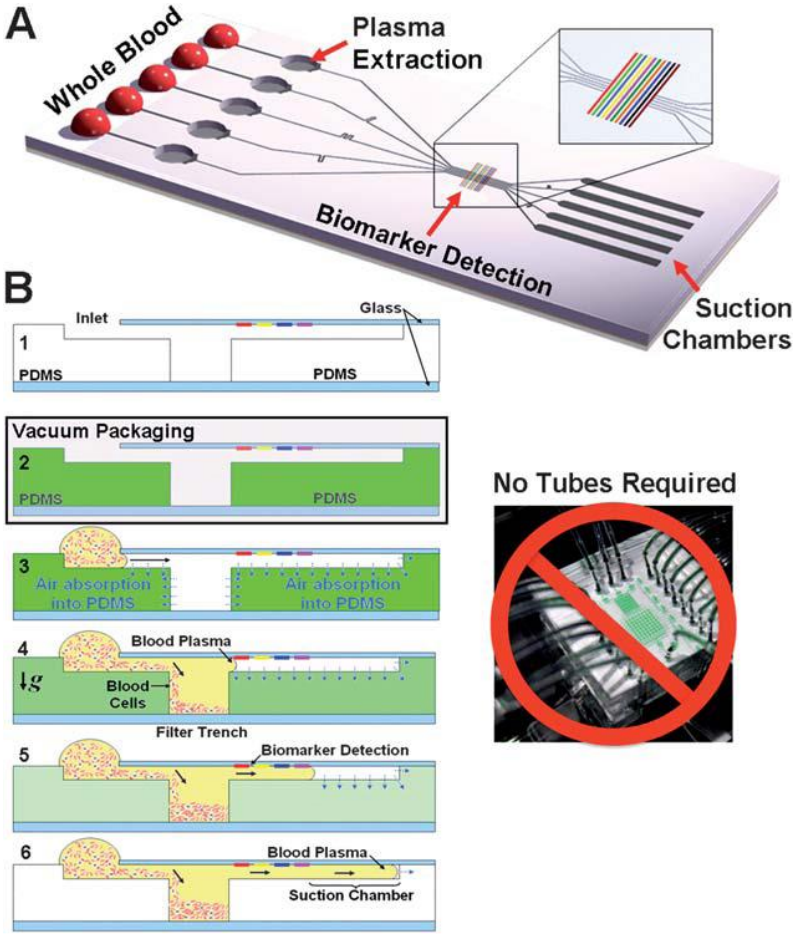
X. Yang, O. Forouzan, T. P. Brown and S. S. Shevkoplyas, Lab Chip, 2012, 12, 274–280

Power-free Blood Separation Microfluidics

- Fluid is driven by vacuumed PDMS

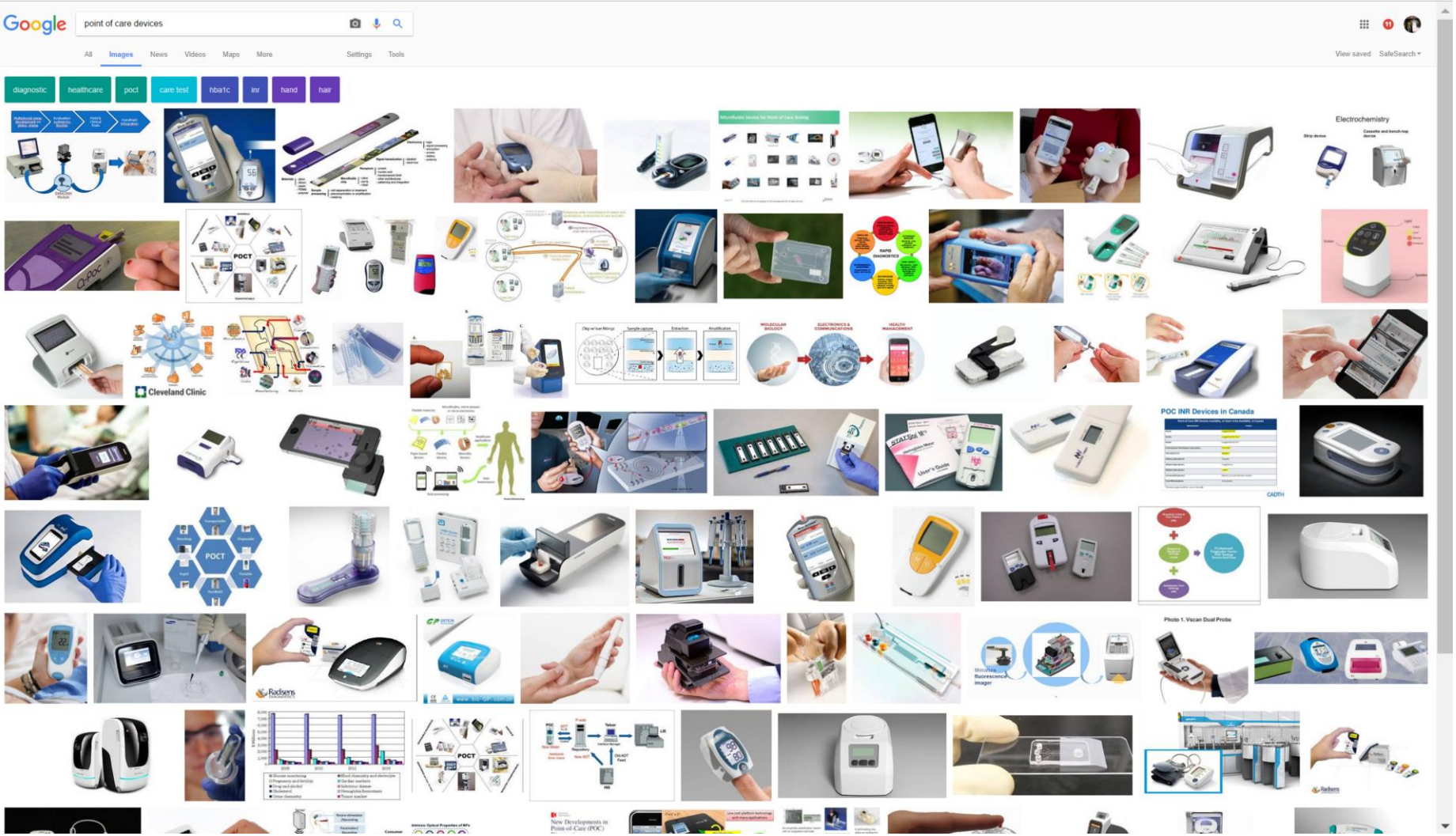






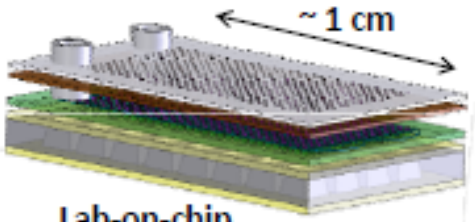
L. Lee Group at
UC Berkeley

Point-of-care (POC) System



Point-of-care (POC) System

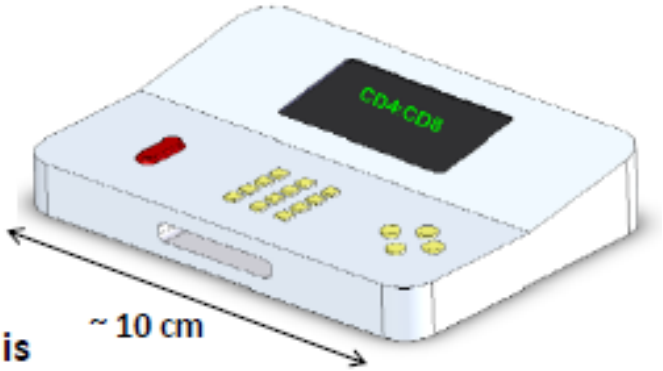
1. Blood is obtained from the patient's finger



Lab-on-chip System

2. The sample is being processed by the biochip

3. The biochip is placed inside the data analysis device for blood analysis.



Discussion:

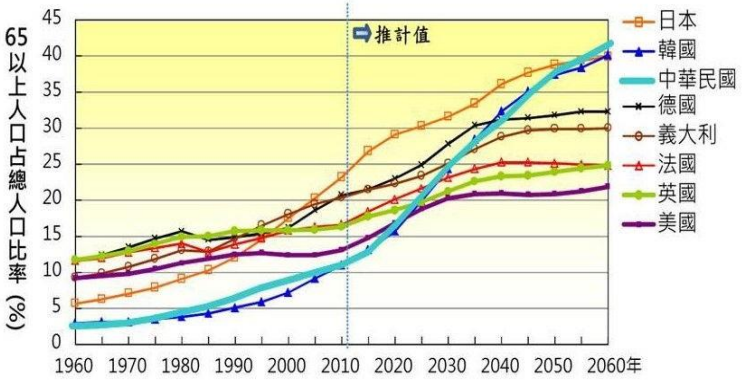
Why point-of-care systems? Where and when do we need it? advantages and challenges?

Guidelines of POC system

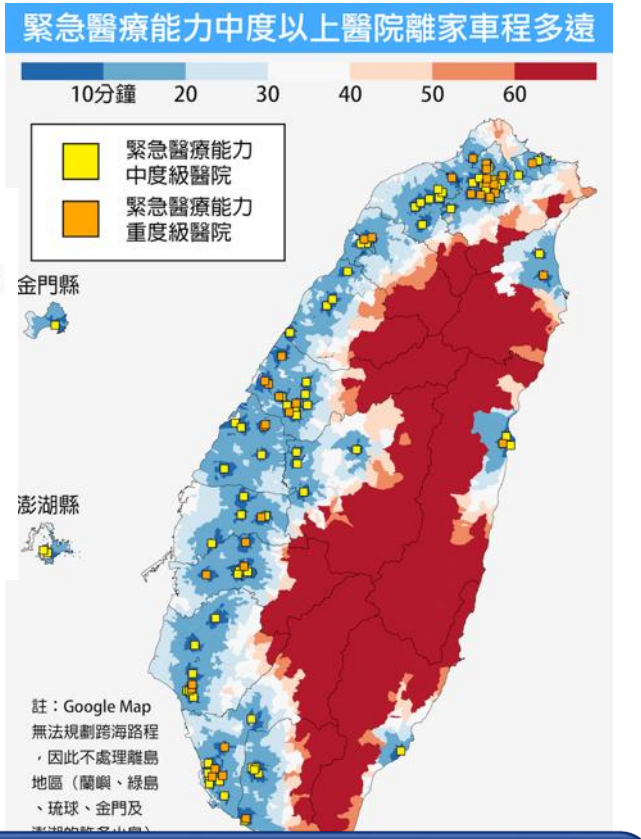
- World Health Organization (WHO)'s ASSURED challenge
  - **A**ffordable by those at risk of infection
  - **S**ensitive (few false-negatives)
  - **S**pecific (few false-positives)
  - **U**ser-friendly (simple to perform and require minimal training)
  - **R**apid treatment at first visit, and Robust, with no need for special storage
  - **E**quipment-free
  - **D**elivered to those who need it

Current medical problems in Taiwan

- Aging society
- Uneven medical resources distribution



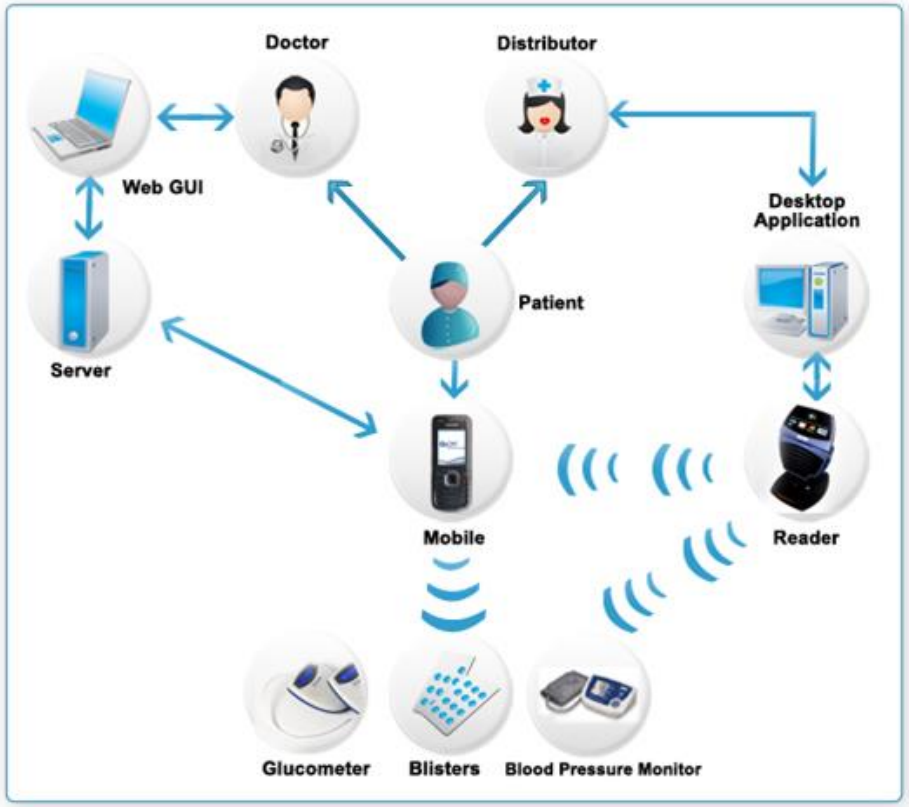
Ref: 國發會



Personalized healthcare system for point-of-care testing

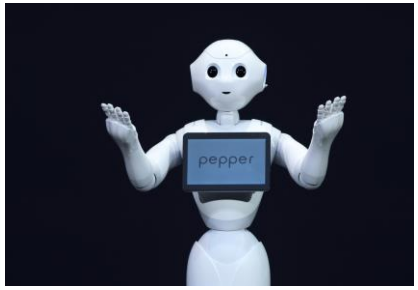
A Personalized Healthcare System

- The core of the personalized healthcare system is “patients” not “doctors” or “hospitals”
- Portable medical devices market worth $20 Billion by 2018



http://www.themobileknowledge.com/content/healthcare

Pepper Robot

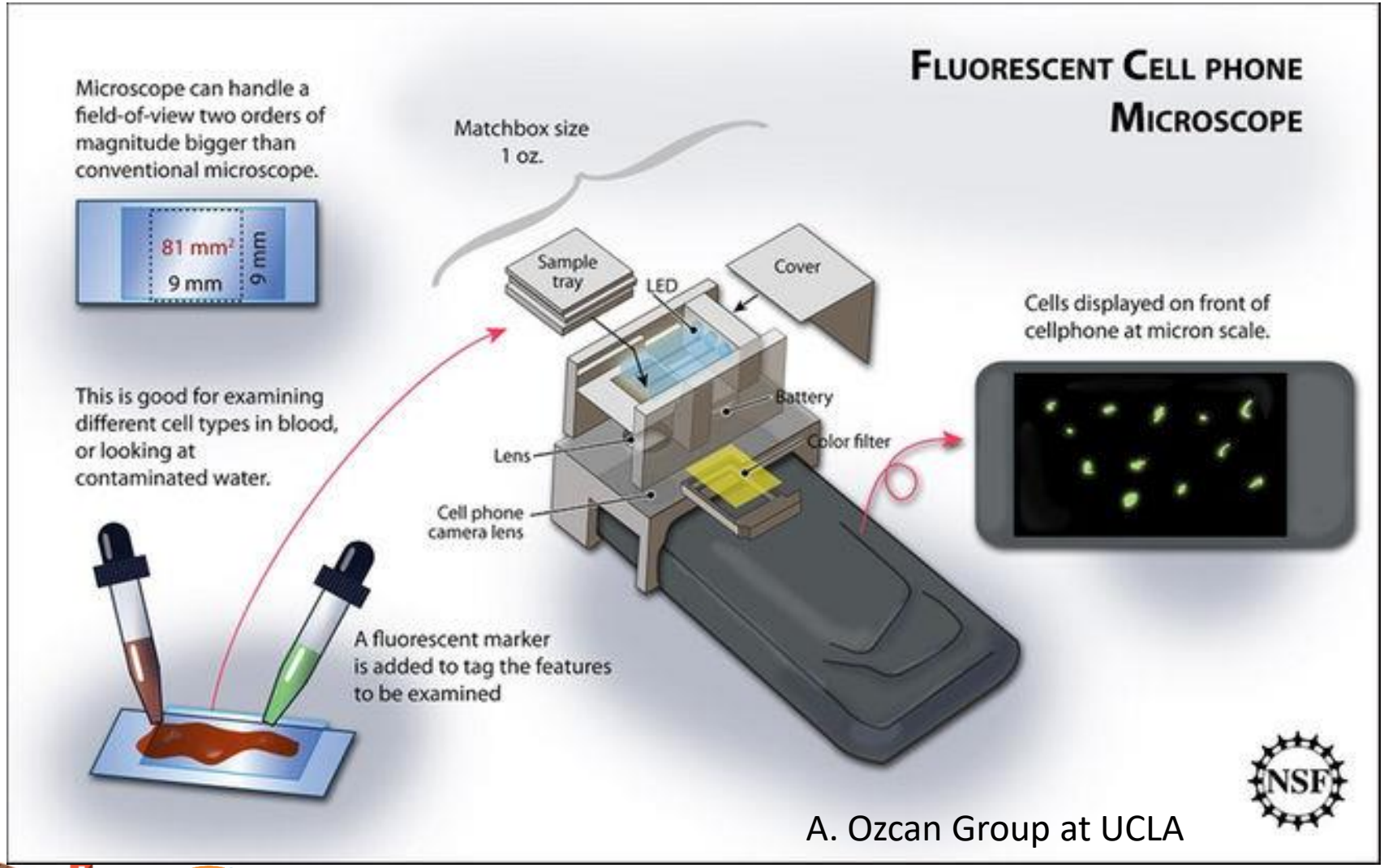


Baymax Robot

“I am Baymax, your personal healthcare companion”

Examples of Personalized Healthcare Device

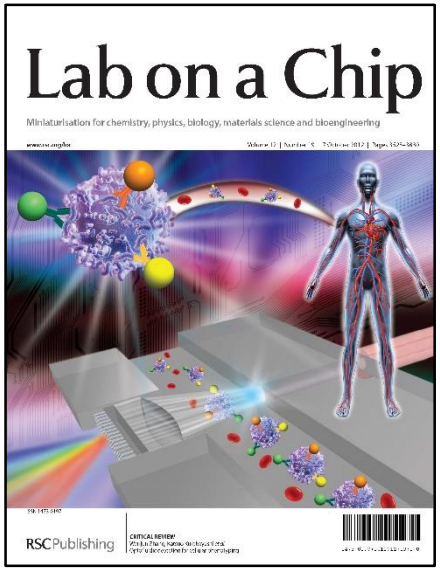
Cell-phone based Microscope

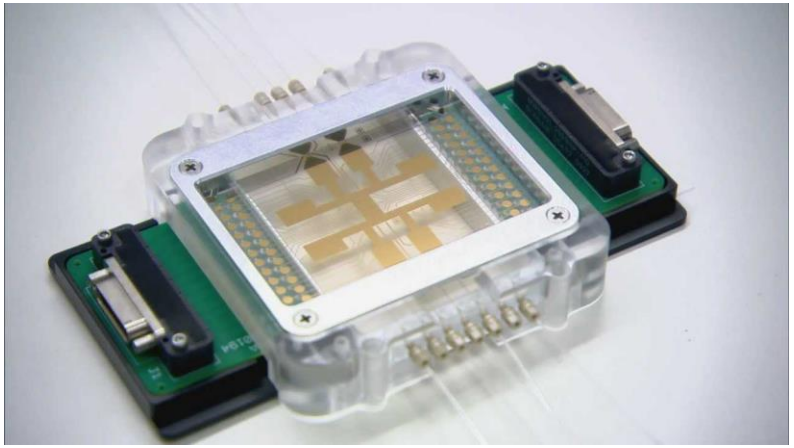


A. Ozcan Group at UCLA

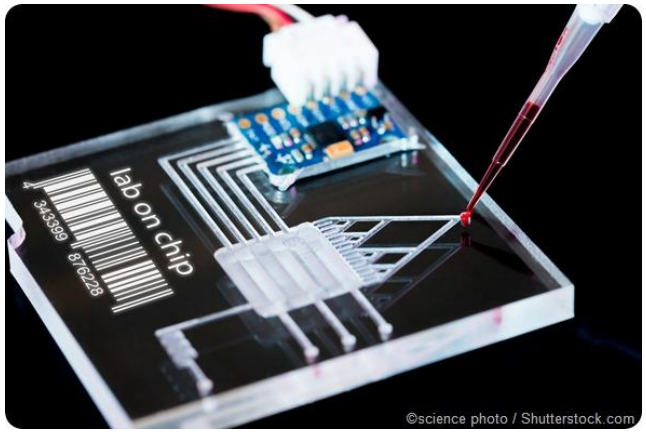
When Microfluidics meet Electronics or Optics…

- Problems to solve…
  - Bonding of different materials
  - Buffer conditions
  - Leakages
  - Packaging
  - Optical alignment
  - Standard fabrication protocols





(Ref: Sandia lab)



How to move research toward to clinical applications

- Hardware development
  - Develop new devices
  - Expand the functionalities of existing devices, such as smart phone, smart watch
- Software development
  - Personalized App
  - Big data analysis

Discussions:

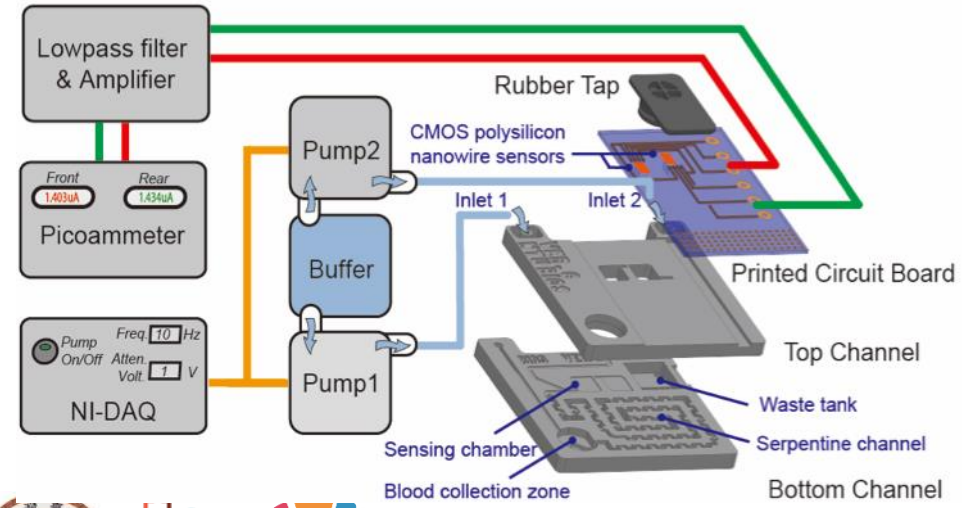
1. What is the current limitations of biomedical researches?

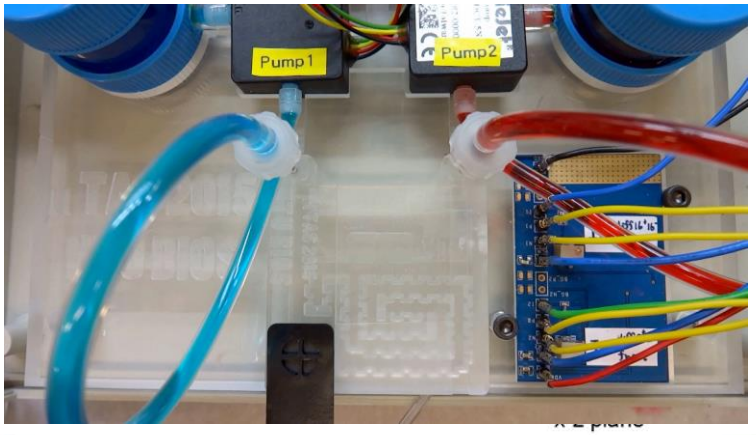
2. What are potential disease applications?

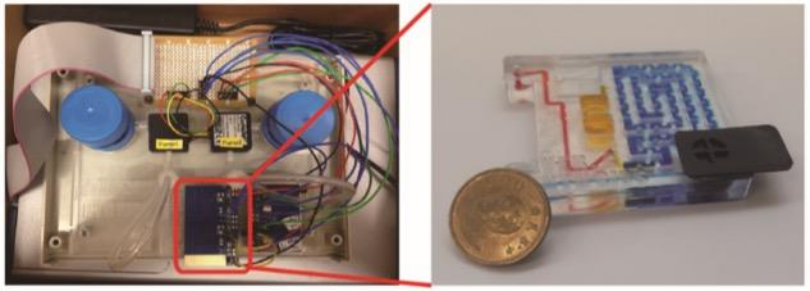
https://www.youtube.com/watch?v=VHHBKuNRtkk
https://www.youtube.com/watch?v=lYC6riNxmis

Microfluidics Integrating Nanowire Sensors

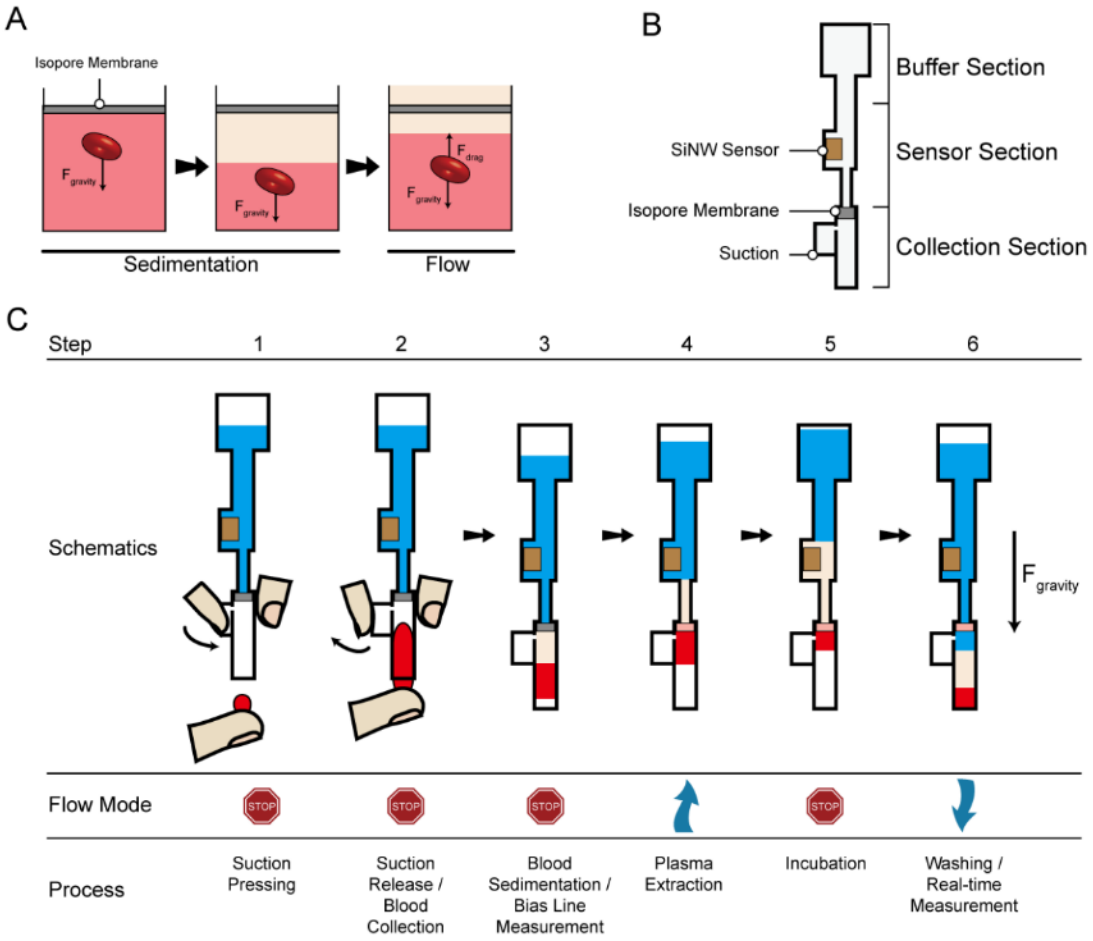
- Microfluidics + nanowires: on-chip whole blood processing and analytes detection
- Three-dimensional microchannel: blood cells trapping and plasma dilution
- Programmable piezoelectric pumps: automatic fluidic control
- CMOS nanowire sensors: label-free and dynamic detection of analytes
- Total assay time: <30 minutes
- Required blood volume: 5 μL

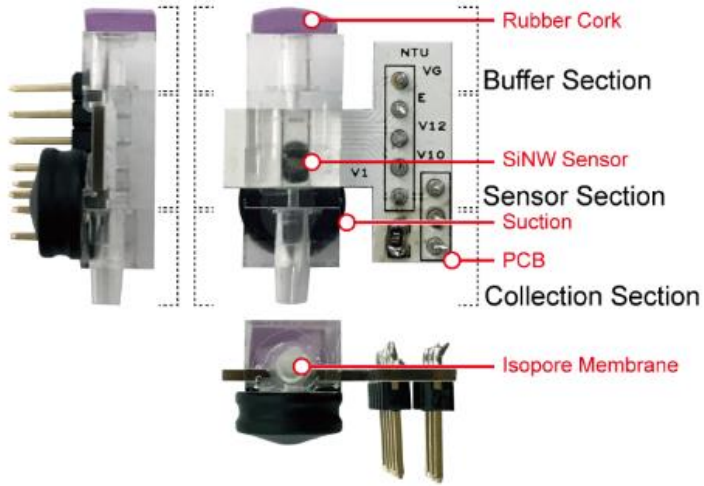






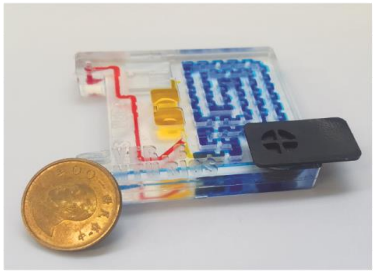
Microfluidic platform for Heart Failure Diagnosis

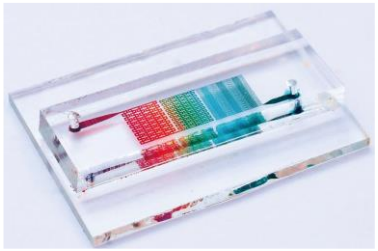




Microfluidic platform for Heart Failure Diagnosis

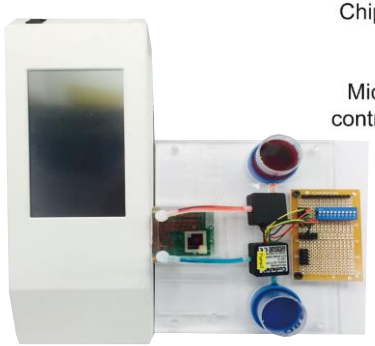
Disease	Diabetes (糖尿病)	Periodontal dialysis (腹膜透析)	Leukemia (白血病)	Bacterial Infection (細菌感染)	LQTS symptom (心律不整)	Blood Counting (全血分析)
Markers	Glycated Hemoglobin (HbA1C)	White Blood Cells (WBC)	White Blood Cells (WBC)	Bacteria	Mutated DNA sequence	WBC, RBC, Plasma

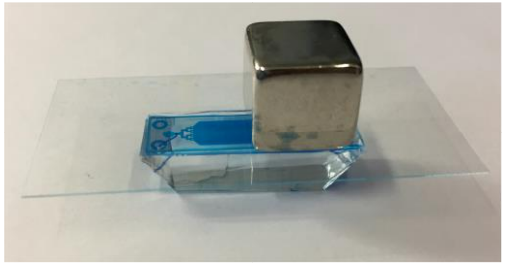




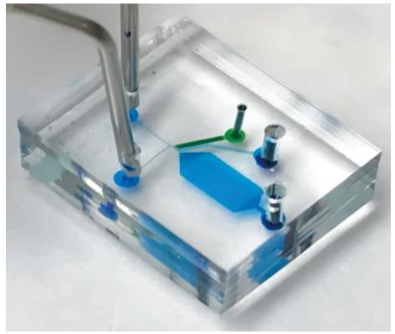
(Kuan et. al., Lab Chip, 2016)

(Huang et. al., Analyst, 2018)





(Huang et. al., Microfluid. and Nanofluid., 2018)



(Kuan et. al., Scientific Report, 2018)

Conclusions

- Bio-sample preparation is one of the most important steps to achieve successful bioassay analysis
- Blood is a complex body fluid since its compositions, but contain most valuable information in human body
- Microfluidics can potentially provide a multi-functional, cost-effective and disposable platform for point-of-care applications with low sample consumption and fast reaction time
- Developing lab-on-chip devices for point-of-care applications is still challenging