AKADEMIE FÜR IMMUNOLOGIE

SPRING SCHOOL ON IMMUNOLOGY

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Cell separation as a tool in immunology research

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Notes

Materials from companies used in this presentation are for illustration of scientific and technical aspects, and not a recommendation to use their products.

Some of the texts in this presentation have been derived from chats like Google Bard, Microsoft Bing, and ChatGPT openAI.

Abundant instructions on using cytometry including cell separation is available in the publication authored by more than 200 experts in the field:
Cossarizza, Andrea, et al. "Guidelines for the use of flow cytometry and cell sorting in immunological studies." *European journal of immunology* 49.10 (2019): 1457-1973.
In this presentation it is referred to as “Guidelines” with page numbers.

Cell Separation in Immunology

Cell separations are important functions in immunology research, allowing scientists to isolate specific populations of immune cells from a complex mixture. This purified population can then be studied in detail to understand their function, role in disease, and potential for therapeutic development.

Isolation and analysis of single cells improve the understanding of the complexity of immune systems and allow for the development of cell cultures from individual cells e.g for monoclonal antibody production.

Large scale isolation of specific cell subsets improves the efficiency and safety of cell therapy e.g. CD34 positive hematopoietic stem cell purification.

(from ChatGPT, Bard, MS Bing feedback for “How is cell sorting used in immunology research?”)

Cell Selection Applications and Requirements

Single Cells

- Creation of single clone hybridomas for antibody production
- Single Cell Genomics
- Single Cell Proteomics

Few cells

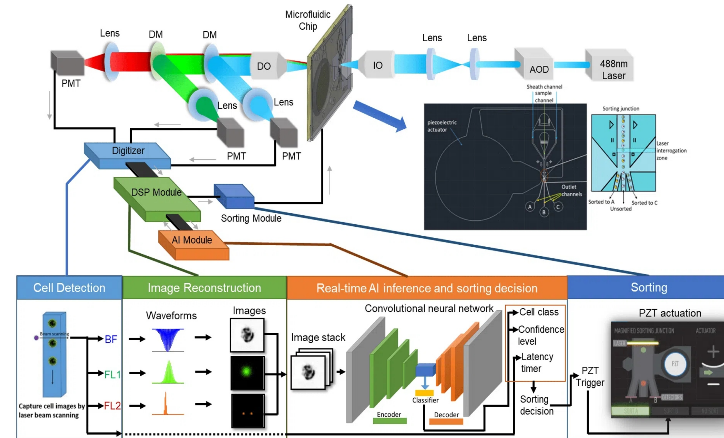
- Studying immunoglobulin class switch (A Radbruch)
- Weissman identifying root hematopoietic stem cell (I Weissmann)
- Immune response profiles

Many Cells

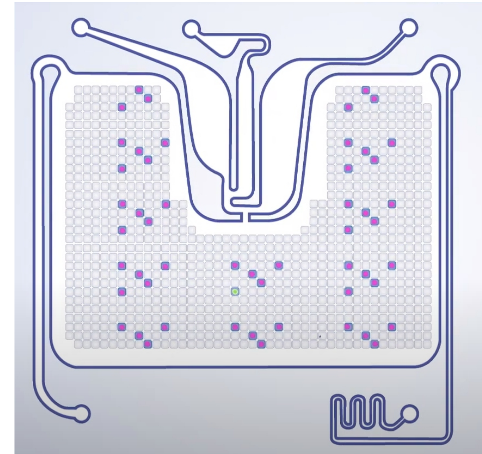
- Metabolomics
- Cell Therapy e.g. 200 million cells need sorting to obtain 2 million CD34 cells
  (sequential 5.5hr at 10000/s, parallel about 1 hr)

Sequential Single Cell Selection Technologies

- Limiting Dilution
- Droplet Sorting (spectral analysis, imaging, morphology fingerprint and AI,...)
- Fluid Channel MEMS walve
- Fluid Channel stream switching
- Dielectric movement with microscopic observation
- Optical tweezers



https://nanocellect.com/image-guided-cell-sorting/



siliconbiosystems.com/en-us/DEPArray-PLUS

Parallel cell separation technologies

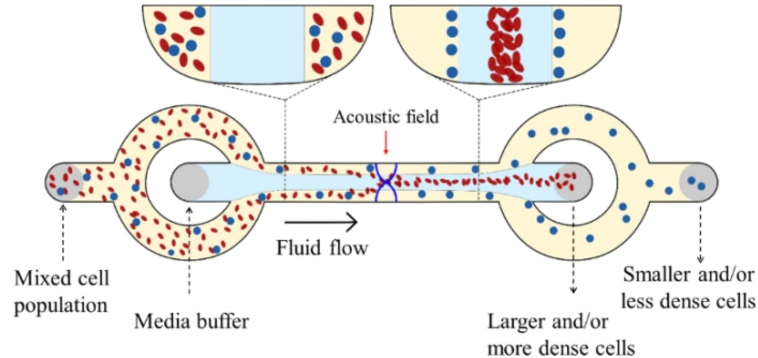
Older methods

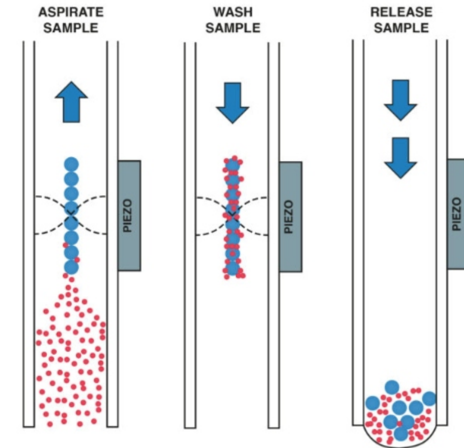
Magnetic (application need based MACS development)

Acoustic (deflection, trapping)

Hydrodynamic

Optical



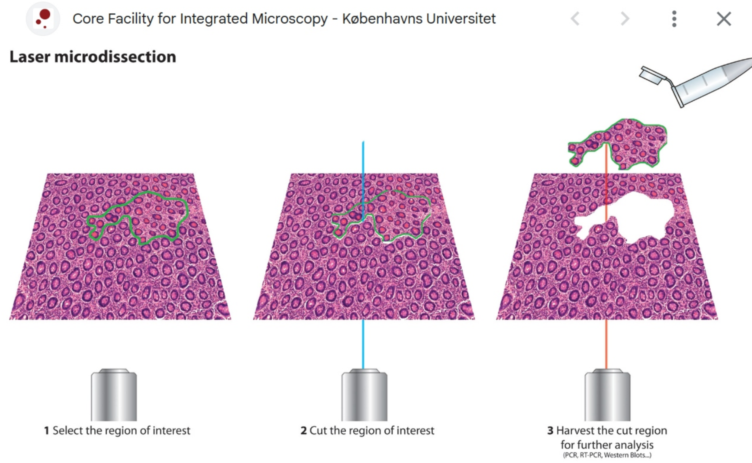


https://acousort.com/technology/

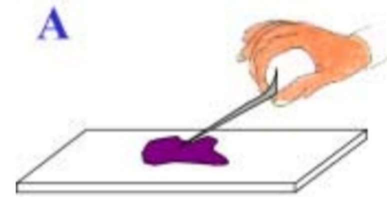
Tissue Dissection

Mechanical dissection under microscopic observation

Laser Capture Microdissection

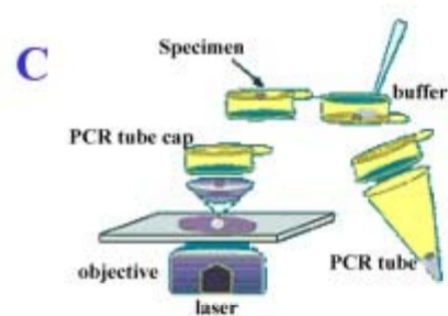


E. Heinmöller et al. / Microdissection and molecular analysis of single cells or small cell clusters 127

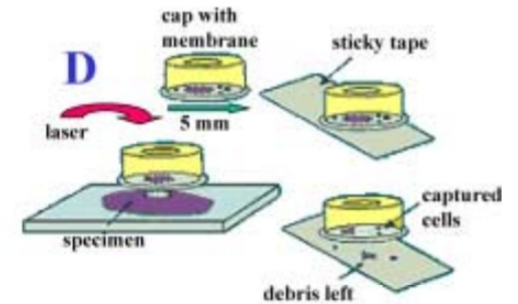


Manual microdissection

Micromanipulator assisted microdissection



Laser Pressure Catapulting



Laser Capture Microdissection

Historical Parallel Selection Methods

I. Separation by physical parameters

- Density e.g. Ficoll, Percoll
- Lysis e.g. erythrocyte removal
- Adhesion e.g. nylon wool

II. Cell separation by immunological parameters

- Complement mediated specific lysis of Ab-coated cells
- Specific adherence of cells to Ab- coated plastics
- Rosetting
- Avidin columns
- Change of buoyancy by cell-cell contact across surface molecules

III. Separation using biological characteristics

- Fe-Phagocytosis

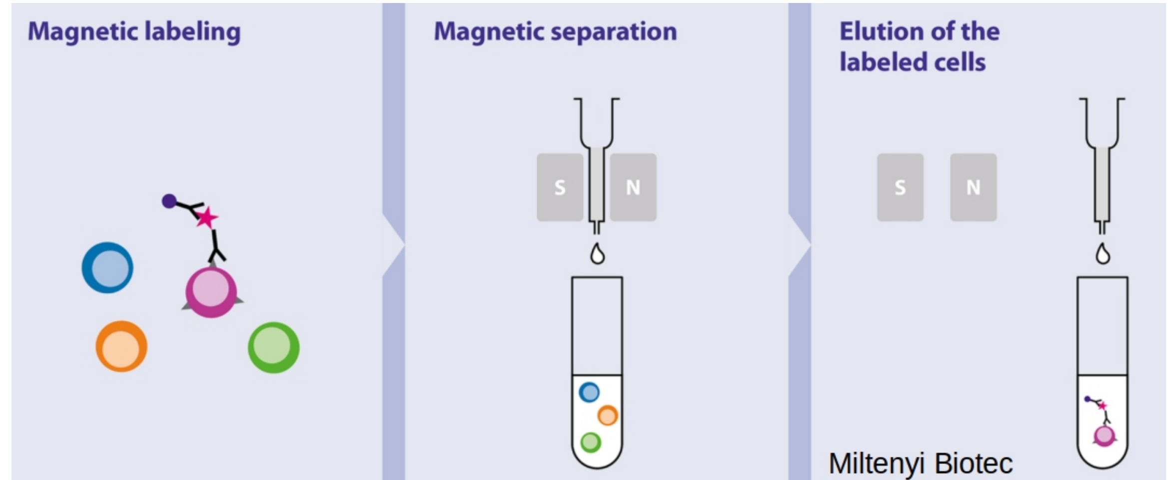
IV. Separation by biochemical characteristics

- L-leucine methyl ester (microglia, macrophages)
- Antibiotic resistance
- Selection of gene-targeted cells

From: "Historical and Useful Methods of Preselection and Preparative Scale Sorting: Charlotte Esser
I. BACKGROUND." Cell Separation Methods and Applications. CRC Press, 1997. 21-34; Table 9

Parallel Magnetic Cell Separation

1. Single-cell suspension of cells in an appropriate buffer
2. Avoid prolonged incubation (cell death, non-specific interactions)
3. Keep cell suspensions at 4°C
4. Use aseptic techniques
5. Avoid air bubbles, harsh vortexing
6. Use optimized parameters
7. Get cell counts of all fractions

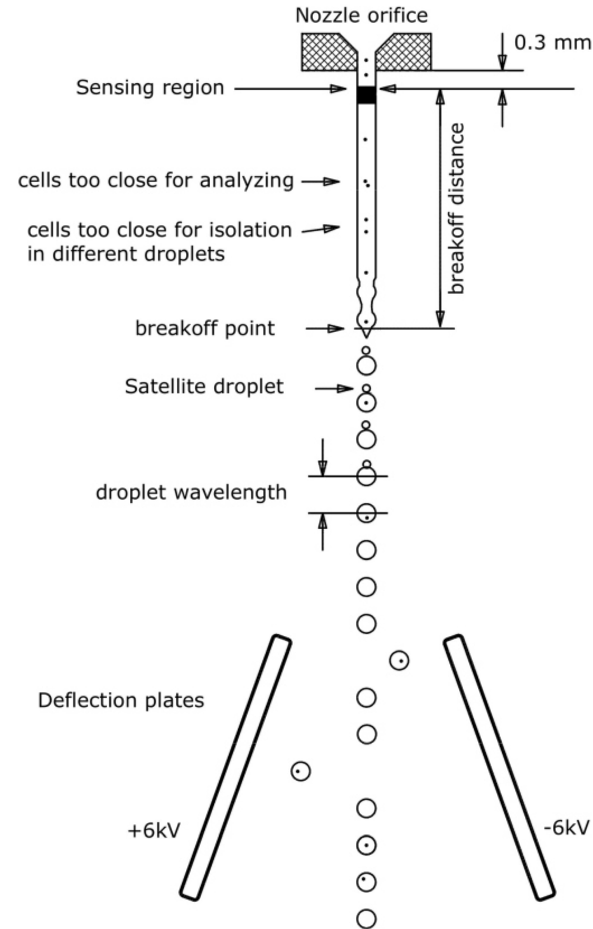


(from ChatGPT, Bard, MS Bing feedback for "What are sample preparation and handling considerations for magnetic cell sorting?")

Sequential Single Cell Sorting

1 Ensure a single-cell suspension
2 Use a cell-friendly sorting buffer
3 Keep samples cold
4 Optimize sample concentration
5 Sort promptly
6 Use appropriate instrument setup
7 Choose the appropriate sort mode
8 Protect cells from intense light exposure
9 Use appropriate collection
10 Verify sorted populations

(from ChatGPT, Bard, MS Bing feedback for "What are sample preparation and handling considerations for FACS cell sorting?")



FACS™ Guidelines pg 1598

Sequential Single Cell Sorting

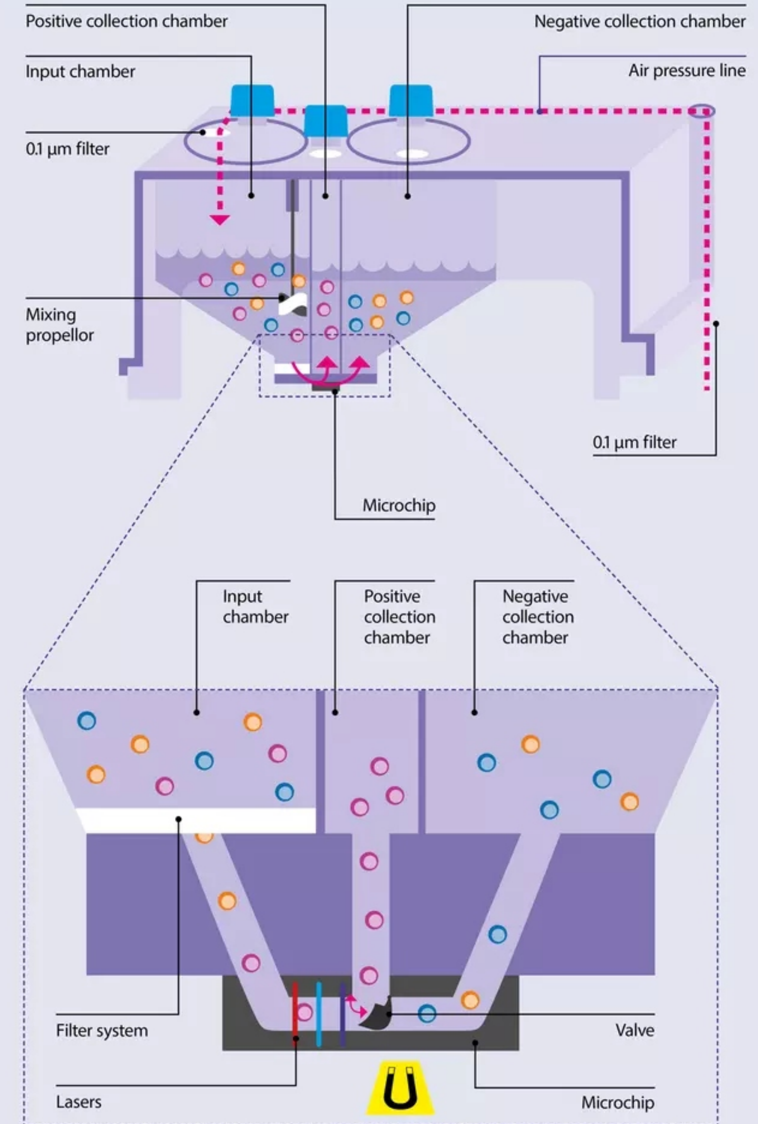
Table 2. Expected purities, yields, and processing times for different starting cell concentrations

Total cells/mL	10^6	10^7	10^8
Purity in Yield Sort [%]	96	69	18
Yield in Purity Sort [%]	96	64	11
Time to process cells	309	31	3:05

Microfluidic sequential sorting

Flow rate	Pulse duration	Cell conc	Fraction target	Volume per pulse	Target cells per displaced volume	Non-target cells per displaced volume	Purity	Yield
mL/s	usec	1/mL	%/100	mL				
1.00E-03	50	1.0E+06	1.00E-03	5E-08	0.00005	0.04995	0.952	0.9512
1.00E-03	50	1.0E+06	1.00E-01	5E-08	0.005	0.045	0.957	0.9512
1.00E-03	50	1.0E+05	1.00E-01	5E-08	0.0005	0.0045	0.996	0.995

Guidelines pg 1617



Benefit of parallel pre-enrichment before sequential sorting

Andrea Cossarizza et al. Eur. J. Immunol. 2017. 47: 1584–1797

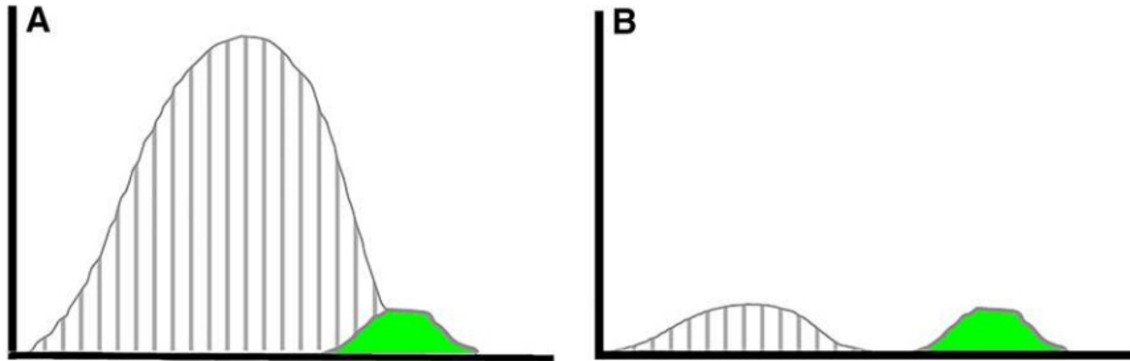


Figure 11. Improvement of population discrimination after pre-enrichment. Cytometer histograms of unwanted (gray lines) and wanted (solid green) populations. (A) A large excess of an unwanted population may create substantial overlap with the target population, making it impossible to achieve a good single-cell sort. (B) After a pre-enrichment bulk sort, which removes most of the unwanted population a good discrimination between the two populations can be achieved.

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Measuring Perfomance

Calculation of yield of “negative” subsets (all numbers in fractions [%/100]):

$$Purity = \frac{(posFraction \cdot posYield)}{(posFraction \cdot posYield + negFraction \cdot negYield)}$$

$$negYield = \frac{(posYield \cdot posFraction \cdot (1 - Purity))}{(Purity \cdot (1 - posFraction))}$$

negYield measures how poorly the system removes cells which should not be selected.
posYield measures how well the system captures the desired cells.
These yield and the original fraction of desired cells (**posFraction**) determine the sort **Purity**.

Sorting Performance (literaure):

System	Purity %	Yield %	Starting %	Y-	-log (Y-)
Aria CD19	99.23	*90*	14.84	1.2E-3	2.9
Aria CD3	94.61	*80*	0.1	4.6E-5	4.3
CliniMACS	85	79	0.09	1.3E-4	3.9
CYTOMX sorter	97.8	70	20	3.9E-3	2.4
Easysep CD34	95	75	0.08	3.2E-5	4.5
EasySep CD4	96	55	28	8.9E-3	2.1
Ebio T-cells	97.4	95	53.8	3.0E-2	1.5
FACS CD4	98.7	*90*	21.2	3.2E-3	2.5
Imag CD4	89.3	55	20.3	1.7E-2	1.8
MoFloXDP CD19	99.91	*90*	14.84	1.4E-4	3.8
MoFloXDP CD3	99.48	*80*	0.1	4.2E-6	5.4

Note: The examples above reflect the results from specific experimental conditions, and not necessarily the capabilities of a specific system.

Negatives Yield Calculator:

	A	B	C	D	E	F
1	Purity	pos Yield	pos Fraction	neg Yield	-log(Y-)	P_calc check
2	0.987	0.92	0.17	2.5E-03	2.6	0.987

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Large Scale Rare Cell Isolation Example

Table 2 Therapeutic Scale Isolation of CD34 Progenitor Cells from Leukapheresis Harvest of Filgrastim-Stimulated Patients

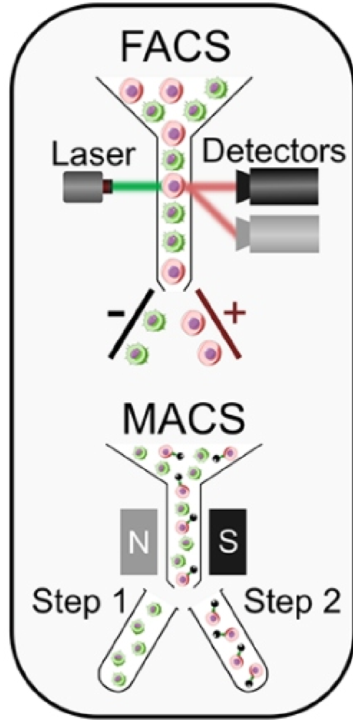
	CD34 cells[a]			Log Depletion		
	Original	Purity	Yield	CD3 T cells	CD14 monocytes	CD20 B cells
Average	0.8%	95%	92%	4.5	3.9	4.1
SD	0.3	4	5	0.2	0.2	0.3
Range	0.4–1.3%	88–99%	84–99%	4.1–4.7	3.4–4.4	3.6–4.7

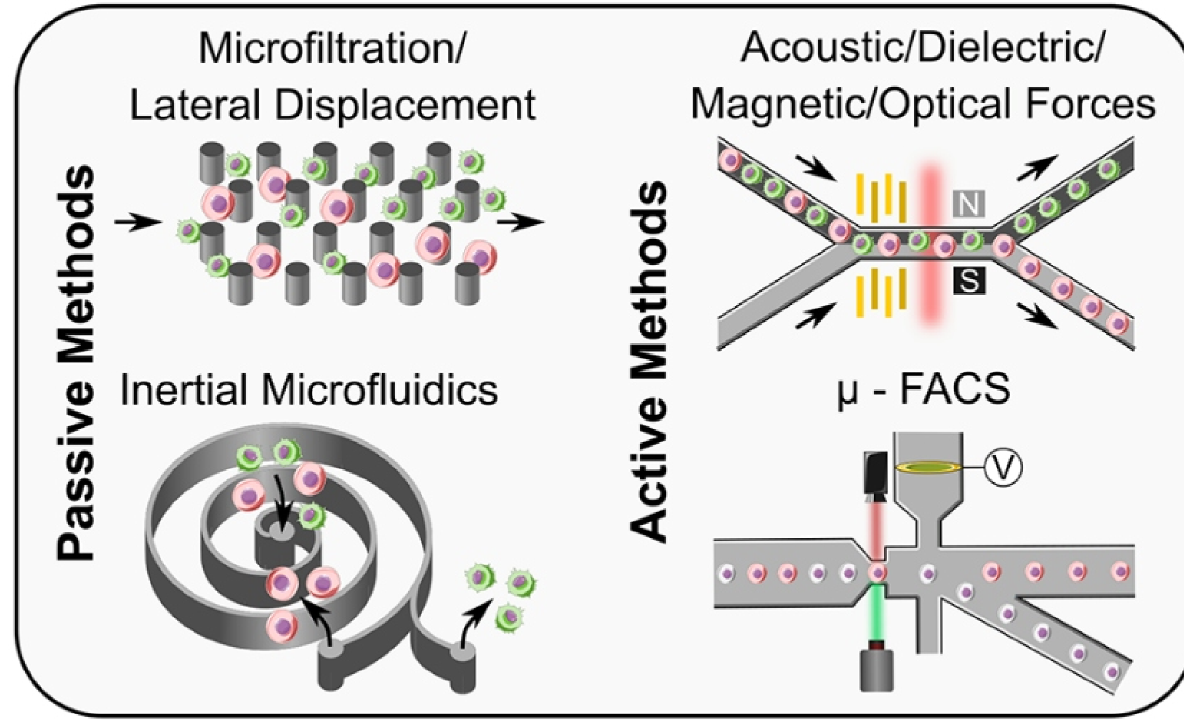
[a] $n = 10$ for CD34 cells data and 5 for log depletion data.

Kantor, A. B., Gibbons, I., Miltenyi, S., & Schmitz, J. (1998). Magnetic cell sorting with colloidal superparamagnetic particles. In Recktenwald D, Radbruch A edts,Cell separation methods and applications, 153-173.

Selected New Technologies

Technologies for Cell Sorting





Mukherjee P et al (2022) doi.org/10.1021/acsnano.2c05494

Single Cell Isolation

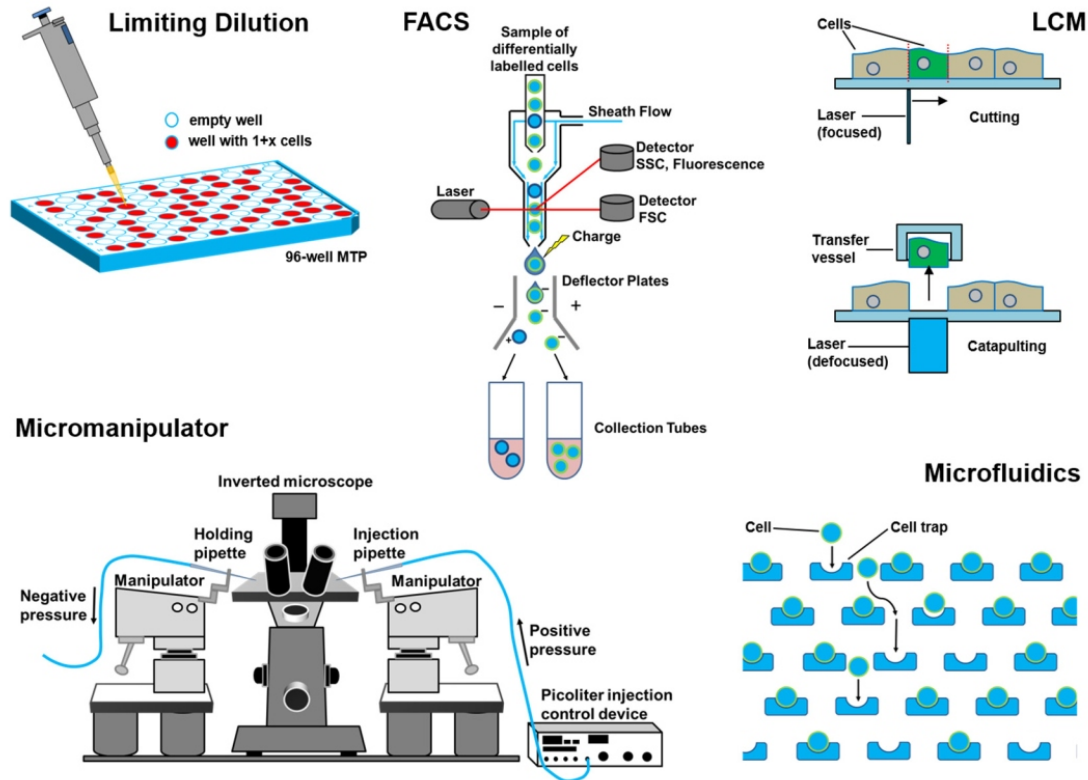


Figure 3. Schematic overview of single-cell separation technologies discussed in the following. The five technologies were identified through market studies as the most commonly used technologies for the handling of single cells (*cf.* (compare to) Figure 1).

Schoendube J et al (2015)
DOI: 10.3390/ijms160816897

High Speed Parallel Cell Sorter Chip

Figure 2. Important details of the chip design are shown. (**A**): individual sorter comprising serpentine inertial focuser, VACS device and output channels. (**B**): 4 × 4 array of VACS devices. (**C**): 4 × 4 array of microresistor actuators. (**D**): inlet manifold. (**E**): outlet manifolds. (**F**): photograph of the complete chip.

Zhukov AA et al (2021)
doi.org/10.3390/mi12040389

Acknowledments

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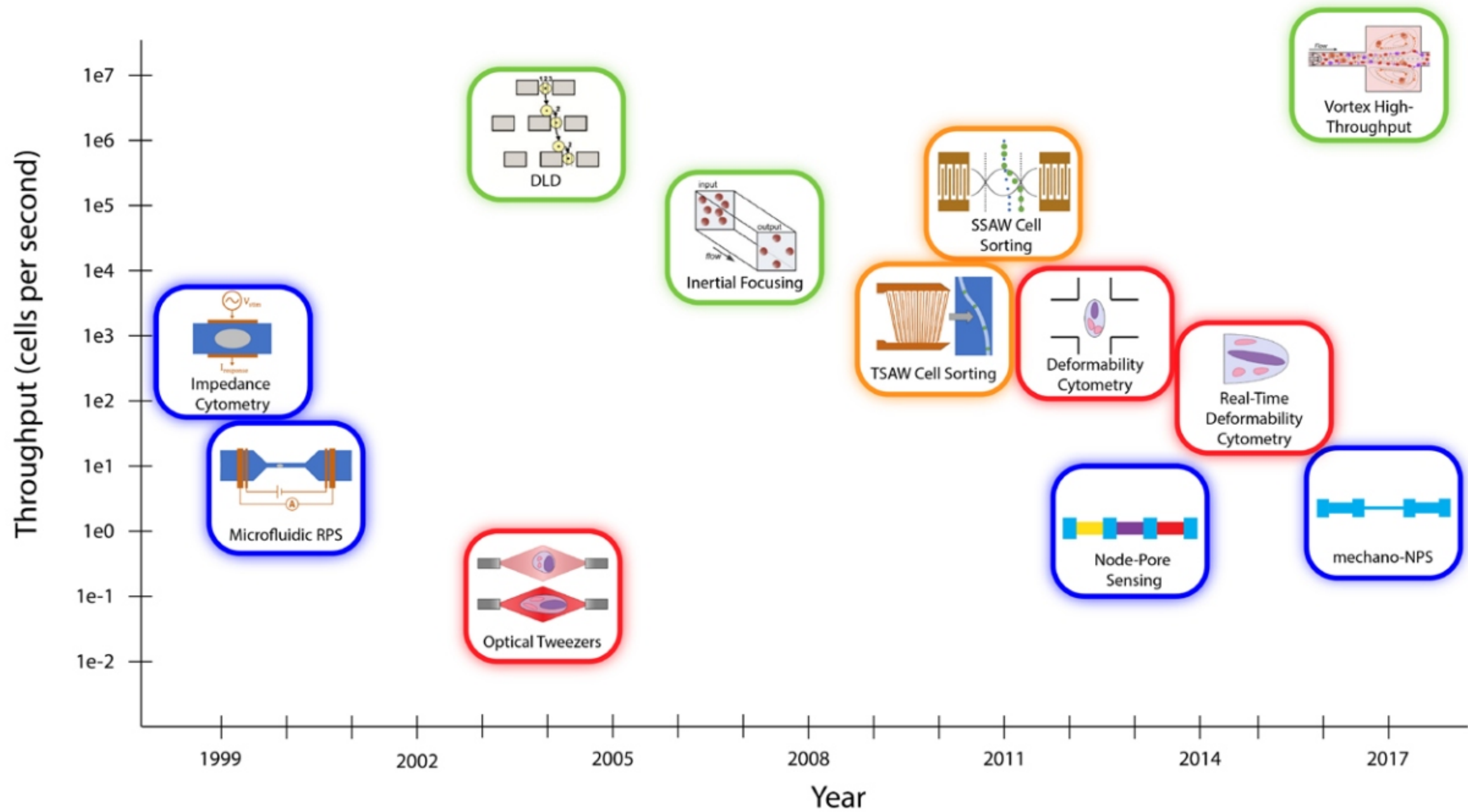
Additional Info



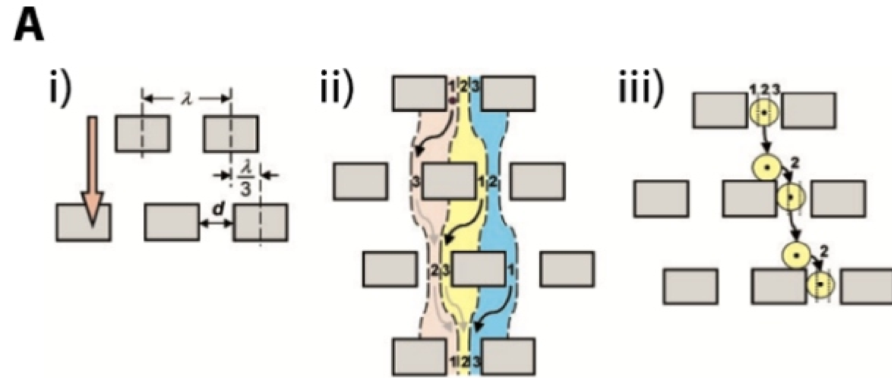
Many new tools that utilize microfluidic technologies for the label-free characterization and sorting of single cells have been developed in the last two decades. These methods can be broadly categorized as electrical (blue), optical (red), hydrodynamic (green), and acoustic (orange).

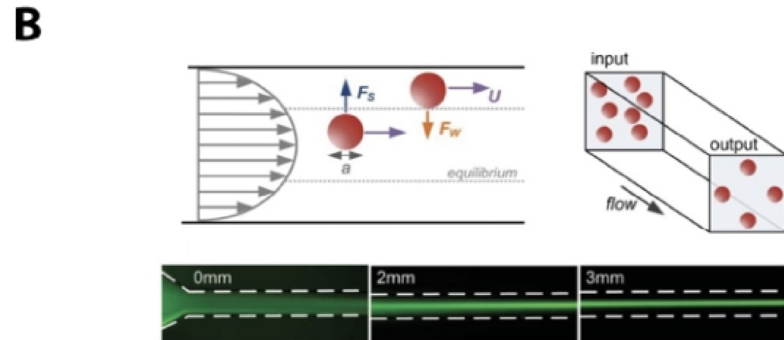
Carey TR 2019 doi: 10.1002/wnan.1529

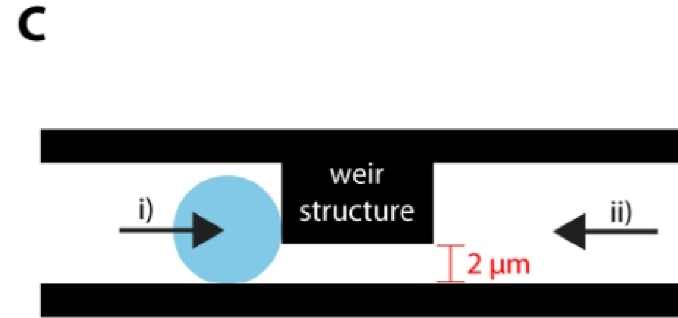
Hydrodynamic Cell Separation

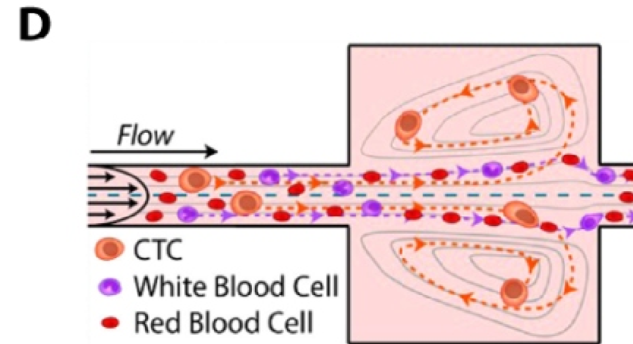
Continuous-flow hydrodynamic separation

Trapping-based hydrodynamic separation









Carey TR 2019 doi: 10.1002/wnan.1529

Node-Pore Sensing

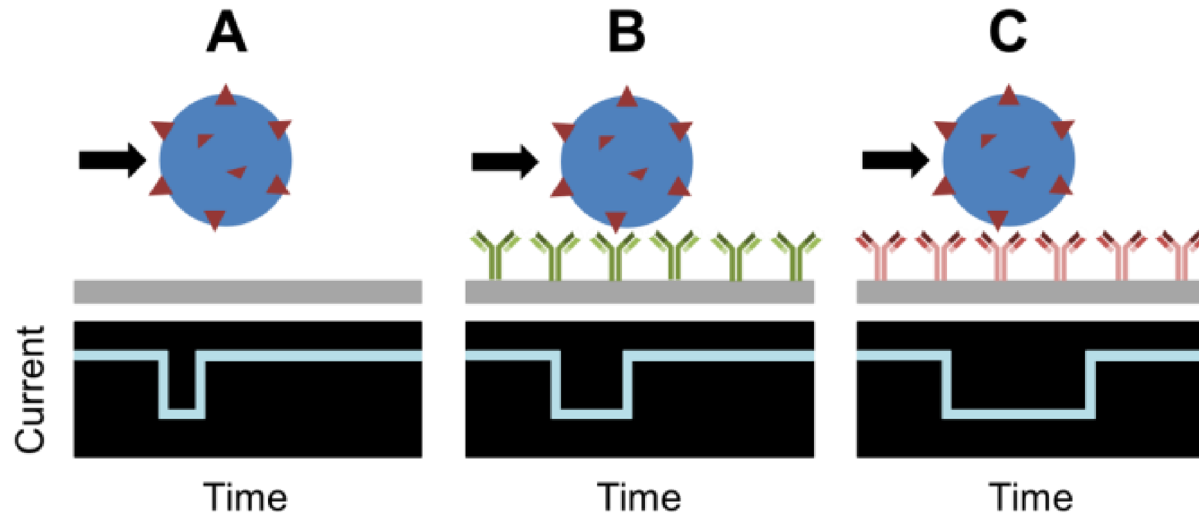


Fig. 2.6: Cell interactions in a pore. (A) A cell travelling through a blank pore does not experience significant retarding forces. (B) A pore functionalized with antibodies that exhibit nonspecific interactions with cell-surface receptors leads to a slightly longer transit time. (C) A pore functionalized with antibodies that exhibit specific interactions with cell-surface receptors leads to a significantly longer transit time.

Balakrishnan KR (2014) thesis https://escholarship.org/uc/item/3mg0n8p8