Development of microfluidic devices for the separation and characterization of CAR T cells samples

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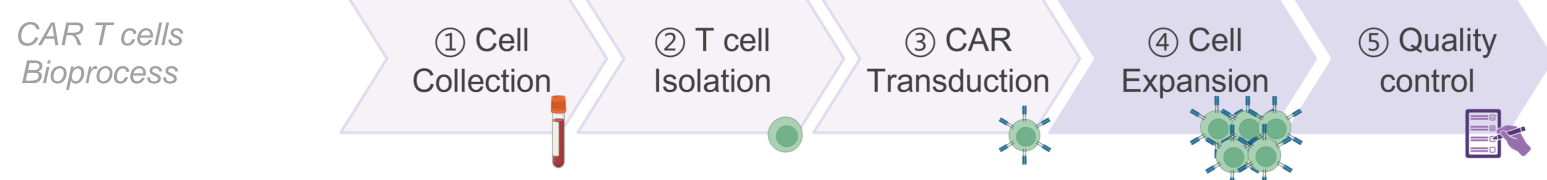
Introduction & Context

CAR T cells = Chimeric Antigen Receptor T cells → Immune cells modified to target specific proteins expressed by cancerous cells.
→ Engineered immunotherapies for cancer treatment produced from the people's own T cells
Cost: 300–500 k€ per patient [1]→ **Need for improved bioprocesses (BioImp project)**

New smart microsystems for in-line quality control during the production.
→ Specifically during the cell expansion step to identify the different cells present in the sample as well as the phenotypes of CAR T cells



Objectives

✓ **Identify and quantify the different immune cell** populations present in a CAR T cells production sample: B cells, T cells, monocytes and NK cells.

✓ Perform **rapid analysis** using the **smallest possible sample volume** (results obtained within half a day) and **minimal human intervention**.

✓ **Identify and quantify T-cell phenotypes**: naïve, central memory, effector memory, effector among CAR T cells.

Device 1 : Microfluidic Cytometer

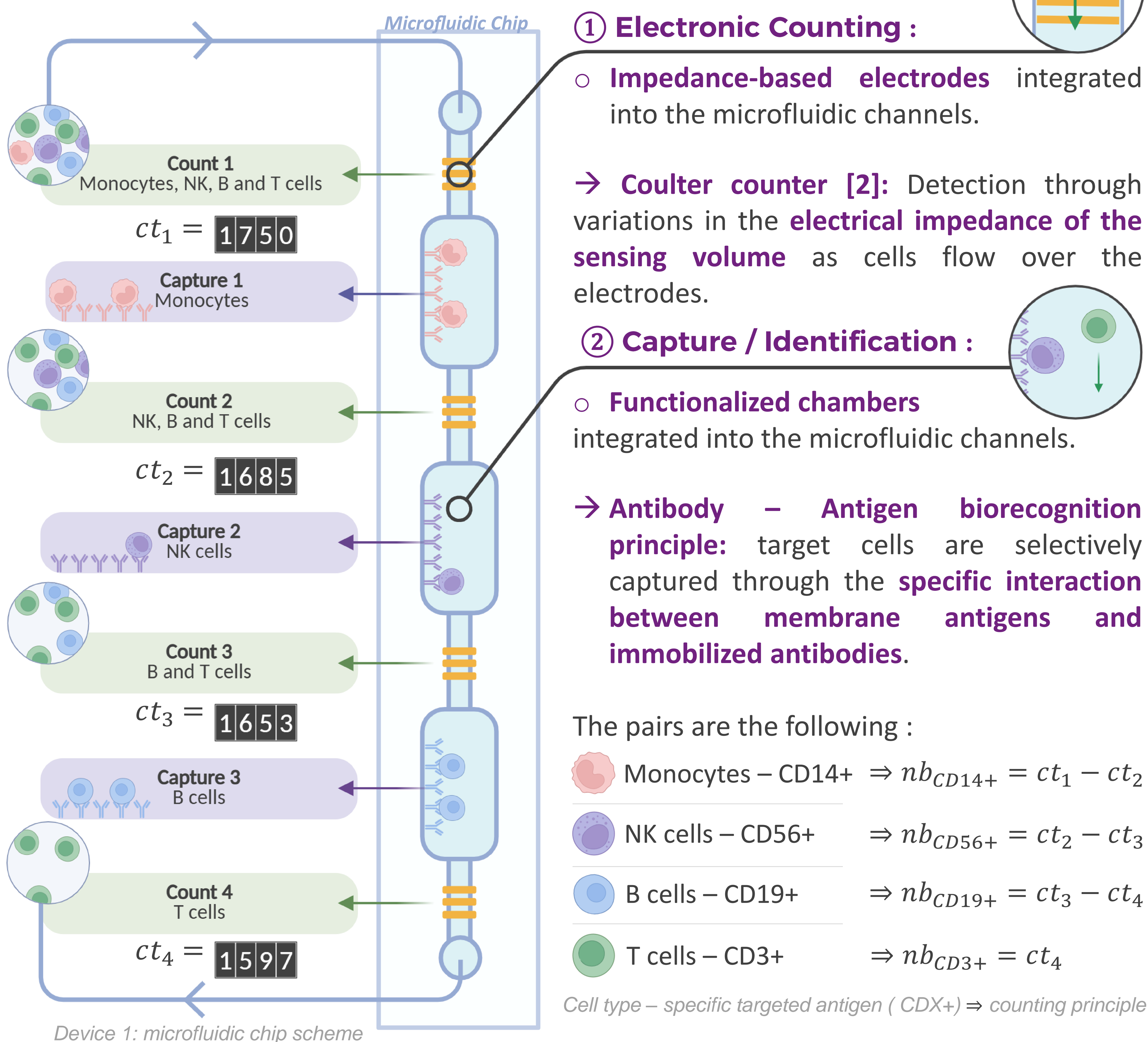
Device 2 : Phenotype Detector

→ **Multiplexed microfluidic biosensors for immune cells analysis**

Device 1 : Microfluidic Cytometer

Identification & Counting Immune Cells

Basic Principle :
Combining capture chambers to differentiate between the cells in the sample and electronic detection to effectively count them.



Device 1: microfluidic chip scheme

① Electronic Counting :

- **Impedance-based electrodes** integrated into the microfluidic channels.

→ **Coulter counter [2]:** Detection through variations in the **electrical impedance of the sensing volume** as cells flow over the electrodes.

② Capture / Identification :

- **Functionalized chambers** integrated into the microfluidic channels.

→ **Antibody – Antigen biorecognition principle:** target cells are selectively captured through the **specific interaction between membrane antigens and immobilized antibodies.**

The pairs are the following :

Monocytes – CD14+	$\Rightarrow nb_{CD14+} = ct_1 - ct_2$
NK cells – CD56+	$\Rightarrow nb_{CD56+} = ct_2 - ct_3$
B cells – CD19+	$\Rightarrow nb_{CD19+} = ct_3 - ct_4$
T cells – CD3+	$\Rightarrow nb_{CD3+} = ct_4$

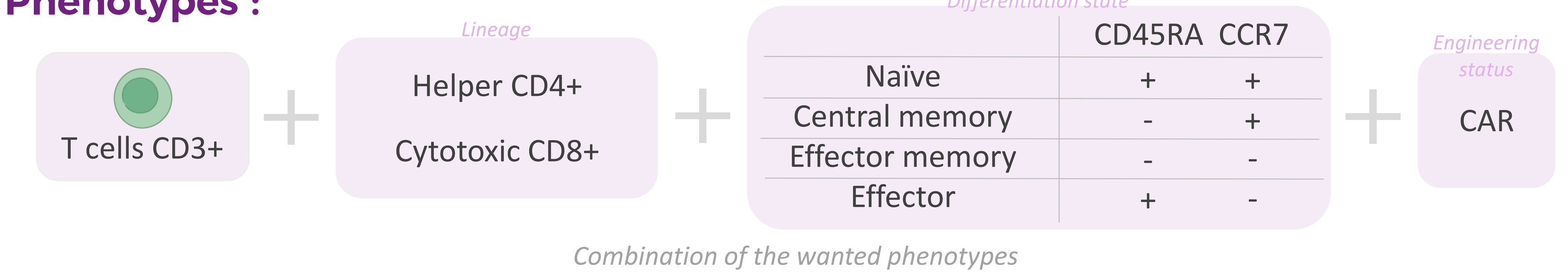
Cell type – specific targeted antigen (CDX+) ⇒ counting principle

Future work :
- Test the specificity and sensitivity of the antibodies
- Design the counting electrodes and capture chambers

Device 2 : Phenotype Detector

→ Identification & Counting T cells phenotypes

Phenotypes :



Combination of the wanted phenotypes

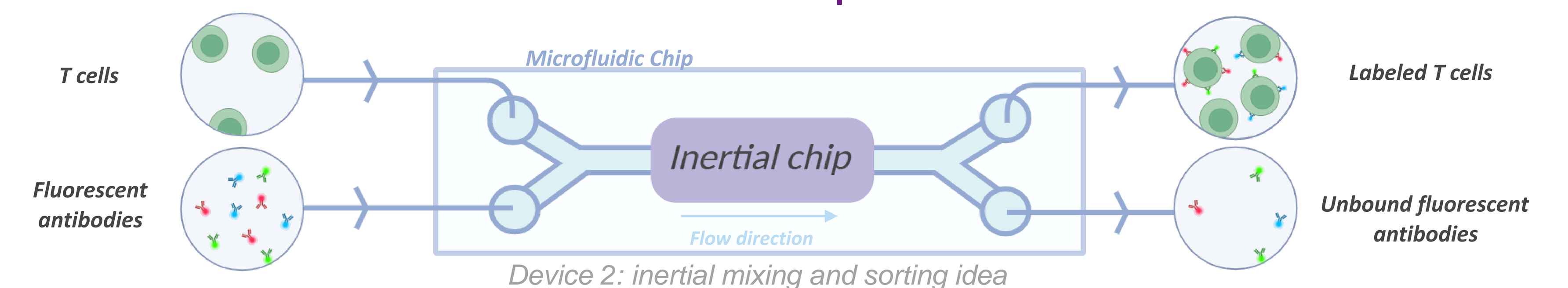
Challenge : Phenotypes are defined by combinatorial surface marker expression.
→ Solution: **Simultaneous detection of fluorescent antibodies** sharing a common excitation wavelength but exhibiting distinct emission spectra.

Basic Principle :
Combining on-chip cell labeling and fluorescence-based detection to reproduce the operating principle of a miniaturized flow cytometer.

① On-chip Cell Labeling :

- **Inertial mixing** is used to mix efficiently cells and fluorescent antibodies as well as separating unbound antibodies.

→ **Inertial mixing and separation:** Dean vortices generated within the microfluidic channels **promote antibody–cell interactions** while enabling **size-based separation**.



Device 2: inertial mixing and sorting idea

② Fluorescence Detection :

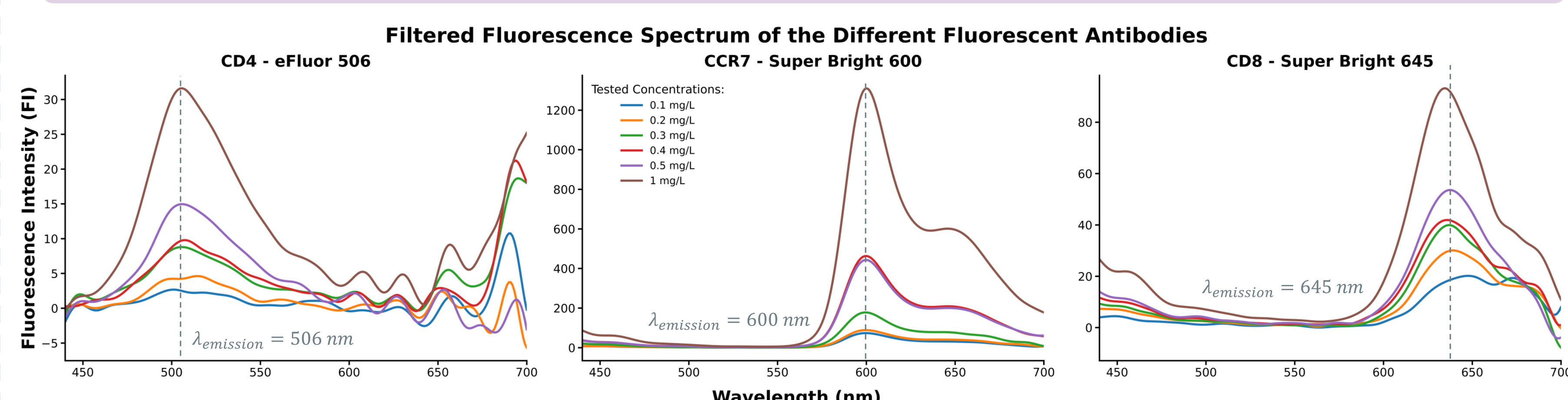
- Different cell phenotypes are identified through **multiplex fluorescent labeling**, since individual cells can express several target surface antigens simultaneously.

→ **Optical detection :** As cells flow individually through the laser beam, **fluorescently labeled antibodies are excited and emit fluorescence** at their specific emission wavelengths. The selected fluorophores share a common excitation wavelength while **exhibiting distinct emission spectra, enabling their differentiation.**

Antibody – Fluorescent couple	$\lambda_{excitation}$	$\lambda_{emission_max}$
CD4 – eFluor 506	405 nm	506 nm
CD8 – Super Bright 645	405 nm	645 nm
CCR7 – Super Bright 600	405 nm	600 nm

Table of the fluorescent antibodies characteristics

Preliminary Results



- **Distinct emission spectra** for the different fluorescent antibodies
- **Strong potential for multiplexing applications**, provided that spectral overlap is properly addressed

Future work :
- Combine fluorescent to develop a signal deconvolution approach
- Design of the inertial sorting and mixing microfluidic chip

Conclusion & Prospects

2 microfluidic devices developed to allow quality control of CAR T cell production samples

→ One device for **cell identification and counting** → Antigen – antibody couples are still to be investigated

→ One device for **immunophenotyping of T cells** → Fluorescent spectra obtained while mixing samples are to be investigated for a deconvolutional analysis

Acknowledgments

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[1] Gérard de Pouvourville. Car-T cells: Price, cost-efficiency and sustainability in France. 2024.
[2] Cole RH et al. Development of a multi frequency impedance measurement system for use in MEMS flow cytometers. 2017

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