Cell counting and identification through microfluidic impedance counting and capture chambers

Céleste Baquet[1] , Himanshi Kharkwal[1], Jean-Francis Berry[2] ,Elodie Bôle-Richard[2,3], Thérèse Leblois[1], Vincent Humblot[1]

[1] Université Marie et Louis Pasteur, CNRS, Institut FEMTO-ST, F-25000 Besançon, France
[2] Université Marie et Louis Pasteur, INSERM, EFS BFC, UMR1098, F-25000 Besançon, France
[3] Franche-Comte' Innov, Bionoveo, F-25000 Besançon, France

Despite advances in conventional therapies such as chemotherapy and stem cell transplantation, hematological cancers remain a significant clinical challenge due to treatment resistance and toxicity [1]. Over the past decade, **chimeric antigen receptor (CAR) T-cell therapy has emerged as a promising immunotherapy for refractory hematological malignancies [2]**. However, **autologous CAR T-cell manufacturing is complex and costly**. It involves isolating T cells from the patient's blood, transducing them to express the CAR, expanding them to a therapeutic dose, and performing quality control before reinfusion. **New technologies, particularly microfluidics, are being developed to improve manufacturing efficiency and access to treatment. These new solutions could particularly be applied to improve and automate the monitoring of the expansion phase.**

→ **Challenge : Automating cell analysis during the expansion phase of CAR T-cell bioproduction**

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

Solution

Microfluidic Cytometer

Microfluidic device able to count and capture the main immune cell populations for identification

Primary interest of this poster

Cell capture on functionalized surfaces enabling separation and identification

Microfluidic cytometer chip scheme

Capture / Identification

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

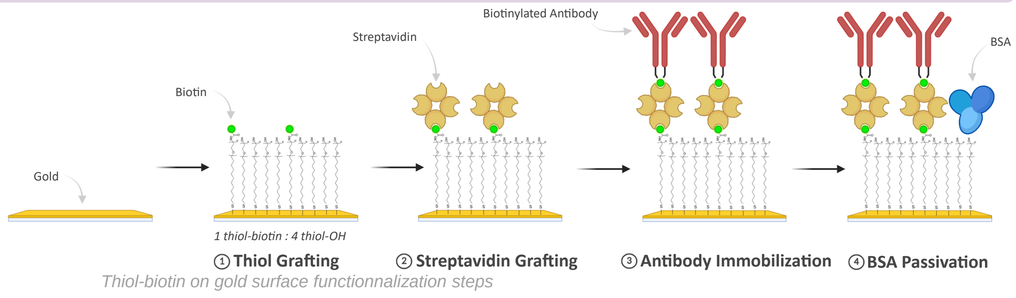
→ **Antibody – Antigen biorecognition principle:** target cells are selectively captured through the **specific interaction between cell antigens and immobilized antibodies** (see table on the right).

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+	$\Rightarrow nb_{CD3+} = ct_4$

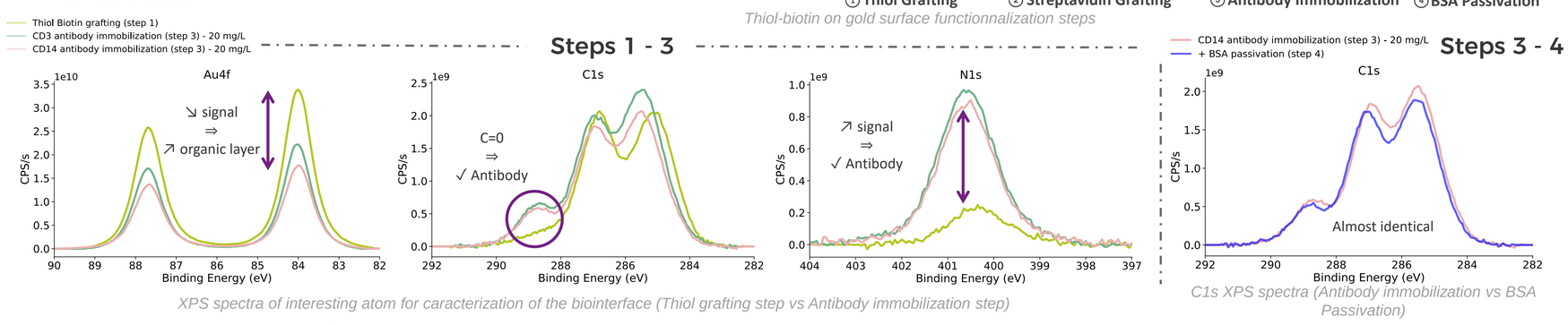
Cell type – specific targeted antigen (CDX+) ⇒ counting principle

① Biointerface → Thiol chemistry strategy : 4 steps

- Characterization of the biointerface is needed to **validate good grafting of the antibodies on the gold surface.**
- Use of **Streptavidin and Biotin specific interaction** to allow better orientation of the antibodies.



Thiol-biotin on gold surface functionnalization steps



XPS spectra of interesting atom for caracterization of the biointerface (Thiol grafting step vs Antibody immobilization step)

C1s XPS spectra (Antibody immobilization vs BSA Passivation)

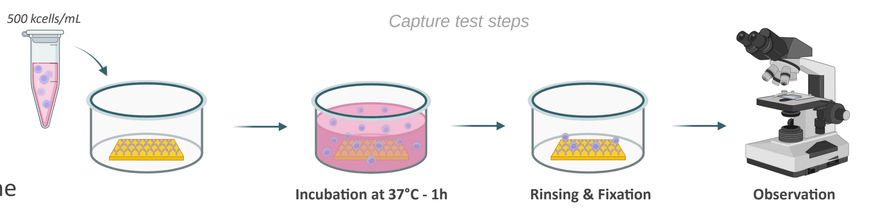
→ ✓ **Biotinylated antibodies (CD3 and CD14)** were **correctly immobilized** on the gold surface.
✓ **BSA Passivation** step seemed to have **no impact** on the organic layer.

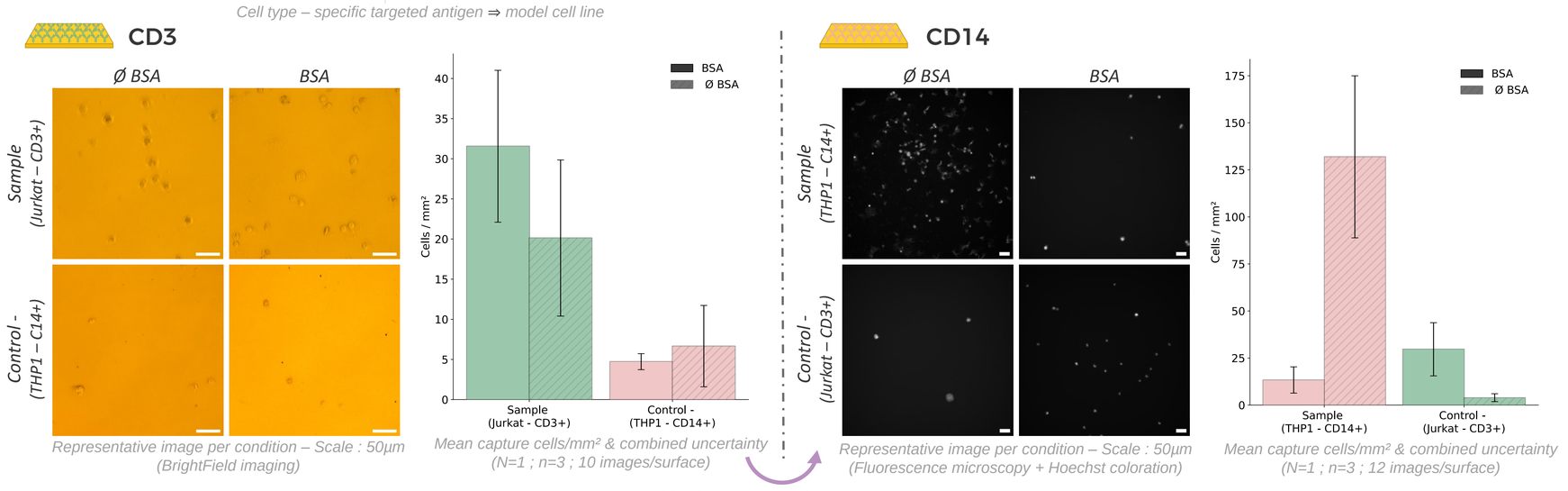
② Specificity Validation → Static capture on the surfaces

- **Validation of the specificity** of our biointerfaces. We performed **static capture assays on functionalized surfaces** using **model immortalized cell lines** that mimic specific immune cells presenting target antigens.

Jurkat cell line ⇐ CD3+ – T cells | Monocytes – CD14+ ⇒ THP1 cell line

Cell type – specific targeted antigen ⇒ model cell line





Representative image per condition – Scale : 50µm (BrightField imaging)

Mean capture cells/mm² & combined uncertainty (N=1 ; n=3 ; 10 images/surface)

Representative image per condition – Scale : 50µm (Fluorescence microscopy + Hoechst coloration)

Mean capture cells/mm² & combined uncertainty (N=1 ; n=3 ; 12 images/surface)

BF to Fluorescence for improved contrast

→ ✓ **Target-specific cell capture was achieved** using CD3- and CD14-functionalized gold surfaces.
✓ **Fluorescence labeling enables clear visualization and automated quantification** of captured cells compared to Brightfield imaging.
→ **BSA seemed counter effective** as it decreased specificity especially on the CD14 biointerface.

Electronic Counting

- Detection through variations in the **electrical impedance of the sensing volume** as cells flow over the electrodes. Allows single cell counting [3] as shown in the figures bellow.

→ **Microfabrication of the electrodes in gold and the microfluidic channel are ongoing, as well as flow simulations.**

Counting chip design

Counting principle

Conclusion & Perspectives

→ **Ongoing validation of the immobilized antibodies biointerfaces** through surface characterization and specific cell-capture assays. **Primary studies (N = 1) show promising results** before transferring to dynamic cell capture in microfluidic chambers.

→ **Counting chips are on their way to microfabrication** and are currently being complemented with fluidic simulations.

[1] Cancer Treat. Res. Commun. 2026, 46, 101074, DOI: 10.1016/j.ctarc.2025.101074.
[2] Front. Immunol. 2022, 13, 927153, DOI: 10.3389/fimmu.2022.927153.
[3] Microfluid. Nanofluid. 2025, 29, 10, DOI: 10.1007/s10404-024-02782-4.

This work was supported by the BioImp project and the European Union. Ref: BioIMP BFC000802
This work was partly supported by the French RENATECH network and its FEMTO-ST technological facility.

Contact
Céleste BAQUET
E-mail : celeste.baquet@femto-st.fr
FEMTO-ST Institute, UMR CNRS 6174, UMLP
15B Avenue des Montboucons, 25000 Besançon, F
www.femto-st.fr