







Soluzioni tecnologiche per l'analisi cellulare e immunologica: CTL ImmunoSpot e CytoFLEX System

Giuseppe Stefanetti, Ilaria Conti, Davide Torre







## Why Cellular and Immunological analysis matter



### **Critical for Diagnostics and Research**

Cellular and immunological analysis is essential for monitoring cell function, proliferation and responses and allow a better understanding disease mechanisms



### Study Cellular Functionality

Analyze cytokines, growth factors, and protein secretion to evaluate cellular responses in health and disease.



### **Detect rare antigen-specific cells**

Techniques like ELISpot allow identification of rare antigen-specific cells—key for vaccine efficacy, disease diagnostics and immune response studies.



### **Characterize Cell Populations**

Flow cytometry supports detailed analysis of cell subsets to uncover insights into infections, cancer, and autoimmune disorders.







## **ELISpot and Flow Cytometry techniques**

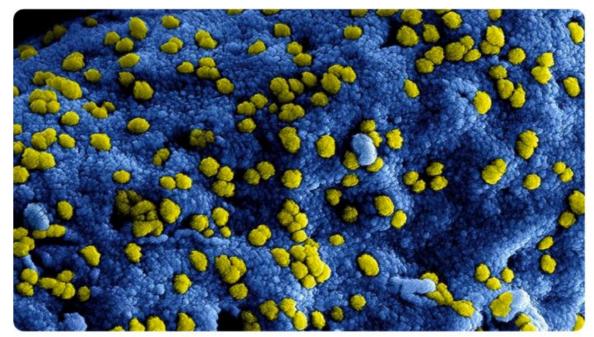
Advanced Tools for Cellular and Immunological Analysis

**ELISpot**: *single-cell sensitivity*. Detect cytokine-secreting cells at the single-cell level—ideal for antigen-specific immune responses

Flow Cytometry: *multiparametric analysis*. Rapid, simultaneous analysis of multiple cell markers for detailed immune cell characterization

**Complementary Technologies**: ELISpot measures functional immune responses; Flow Cytometry provides detailed phenotypic analysis

**Automation and High Throughput:** Both techniques support automation, enhancing efficiency and scalability for large studies









## **High-Impact Applications of ELISpot and Flow Cytometry**

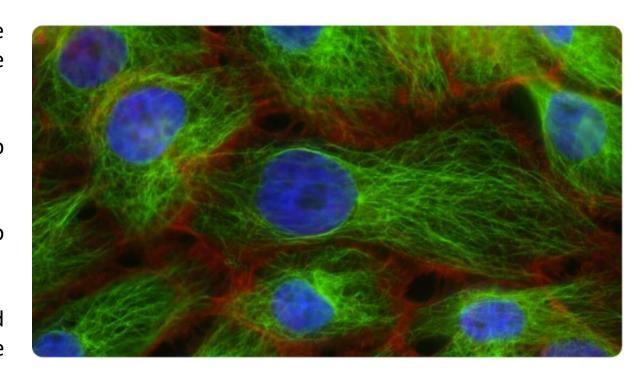
Transforming vaccine development, immunotherapy and research

**Vaccine Development:** Evaluate antigen-specific immune responses in preclinical and clinical trials to determine vaccine efficacy

**Immunotherapy Monitoring:** Analyze T and B cell responses to therapies for cancer and autoimmune diseases

**Infectious Disease Research:** Understand immune responses to infections for better treatments and diagnostics

**Autoimmune Disease Studies:** Characterize dysregulated immune cells to uncover mechanisms driving autoimmune conditions.









«ELISPOT represents the most sensitive technology for the detection of immune cells secreting signature proteins, such as cytokines.

It represents the *state-of-the-art* for the detection, measurements and functional analysis of immune cells»

## **Strenghts of ELISpot**

- Visualization of individual antigen-specific T or B cells
- Detection at single-cell level





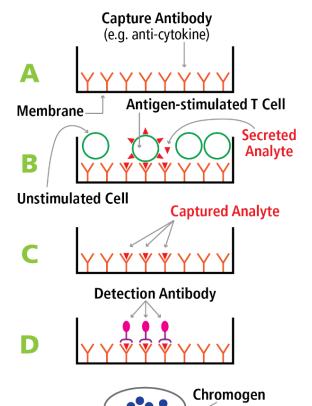
Ilaria Conti, PhD 17/12/2024







How it works



- Each spot represents an individual cytokine-secreting cell
- Software for automated spot recognition, gating and count in each well (up to 8-color analysis)









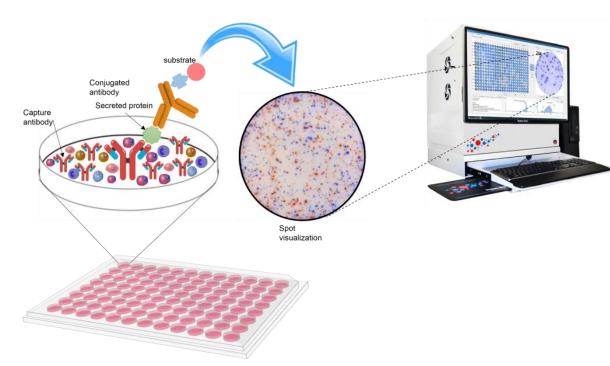
In ELISPOT assays, analytes are captured around secreting cells and visualized. For T cell assays, antigen-induced production of cytokines, such as IFN-γ, IL-2, IL-4, IL17, or granzyme B, perforin and TRAIL can be measured. The assay permits the detection of the rare antigen-specific T cells within the PBMC plated. Spot numbers delineate the frequency of the analyte-producing cells. Spot morphologies provide insight into productivity and secretion kinetics of each analyte. ImmunoSpot® analyzers automatically recognize spots of different morphologies and apply statistics-based gating, thereby providing scientifically validated and user-independent results.

- Detection of rare antigen-specific cells
- Different colors indicate different cytokines
- Spot morphology reflects cytokine kinetics:
  - Rapid secretion rate: Large and fuzzy spots
  - Slow and steady secretion: Smaller and denser spots









## **Advantages**

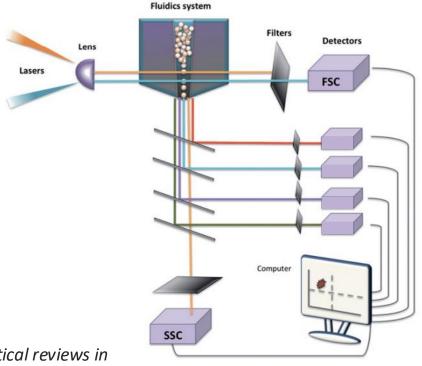
- **Exceptional Sensitivity:** Detection of 1 in 1 million antigenspecific cells (0.0001%; detection limit of flow-cytometry 0.1%)
- Efficient cell utilization: Requires minimal cell numbers;
  cells are often reusable
- **Automated analysis:** Streamlines data processing for consistent results
- **High-throughput capability:** Designed for large-scale studies with enhanced productivity







## How it works



- A flow cytometer is an instrument used to analyze individual cells or particles suspended in a liquid by combining lasers, optics, and detectors
- It measures physical and chemical properties of cells, such as size, internal complexity, and the presence of labeled molecules

Adan, Aysun et al. *Critical reviews in biotechnology* vol. 37,2 (2017): 163-176. doi:10.3109/07388551.2015.1128876

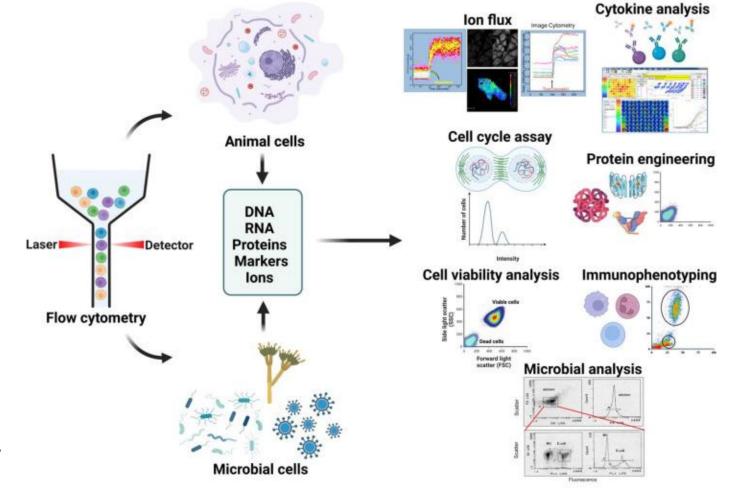








CytoFLEX Application



Robinson, J Paul et al. Cells vol. 12,14 1875. 17 Jul. 2023, doi:10.3390/cells12141875







#### **Exquisite Sensitivity**

- Unique collection of innovative technologies provide exceptional detection capabilities
- Large dynamic range to resolve dim and bright populations in the same sample

#### **Low Maintenance**

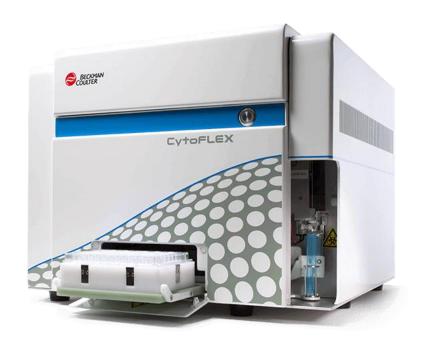
- Lasers, steering optics and flow cell are all mounted onto a vibration isolated table, maintaining optical alignment
- Low pressure fluidic system
- Preventive Maintenance can be completed by the user, no service visit required

#### **Platform Versatility**

- 9 detector for multiparametric analysis
- Implementable number of parameters
- Includes full complement of repositionable bandpass filters

#### **Automation Enabled**

 96-well plate sample loader module







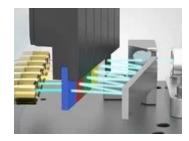


## CytoFLEX technology

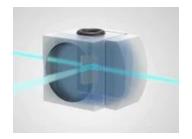
The platform's exquisite sensitivity is achieved through advanced technologies like the Wavelength Division Multiplexer (WDM). Using fiber optics and band-pass filters, the WDM separates light wavelengths without needing multiple dichroic filters. This simplifies fluorescence channel configuration and enhances light efficiency by minimizing refraction losses.

The WDM utilizes <u>Avalanche Photodiode</u> <u>detectors (APD)</u>, versus Photomultiplier tubes (PMT). One hallmark of the photodiode is the high quantum efficiency in excess of 80%, especially for wavelengths greater than 800 nm.

The CytoFLEX improves efficiency with integrated optics that focus light directly onto the flow cell, ensuring optimal excitation and emission of fluorochrometagged cells for superior performance.













**Grazie per l'attenzione**