NUVISAN
The Science CRO

Zoom In and Out: A Comprehensive Immunologic Evaluation of Human, Murine and Rat Samples

Christos Nikolaou, Martin Lange, Alexandra Eichten, Oliver von Ahsen , Barbara Nicke, Charlotte Kopitz, Krzysztof Brzezinka

NUVISAN ICB GmbH | Muellerstr. 178 | 13353 Berlin | Germany

Introduction

Fluorochrome-conjugated antibodies applied in conventional FACS are widely used but have limited utility for high-parameter studies. Spectral cytometry overcomes those limitations since the emission spectrum of every fluorescence molecule is detected across a defined wavelength range. Employing spectrum cytometry for immunophenotyping at NUVISAN we can zoom in and out of the immune system of :

1. **Human**
2. **Mouse**
3. **Rat**

with less sample material needed to extract complex information vital for immunological studies. Immune responses to cancer are highly influenced by the tumor microenvironment, where the delicate balance between suppressor and effector cells steers the prognosis after therapy. Proper establishment of multiparameter panels require accurate assessment of isolation and preservation protocols as practical considerations on epitope stability of immune cells vital for downstream assays and immunophenotyping.

We use spectral cytometry to map immune cell subsets and correlate them with tumor progression in human, mice and rats. Using unsupervised dimensionality reduction tools (e.g viSNE) we identify major immune subsets as well as analyze their expression of stimulatory and inhibitory molecules in tissues and periphery.

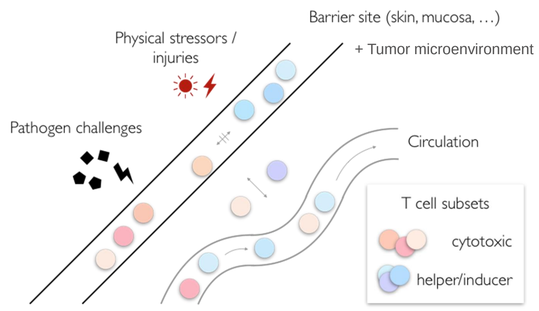
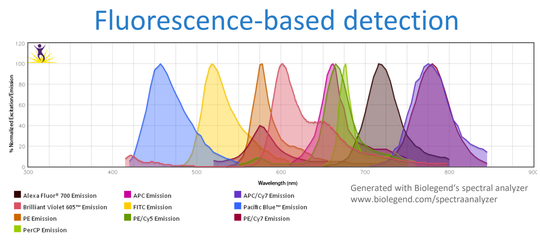


Fig. 1: Cytotoxic and helper-type circulating and tissue resident T cells at barrier sites as well as tumor microenvironment.



+ Utilising full emission spectrum signatures
+ >30 markers simultaneously
+ Autofluorescence extraction
+ Flow rate 20.000 cells/second
+ Sorting of live cells possible
+ Inexpensive

Employing Spectral Cytometry for Immunophenotyping

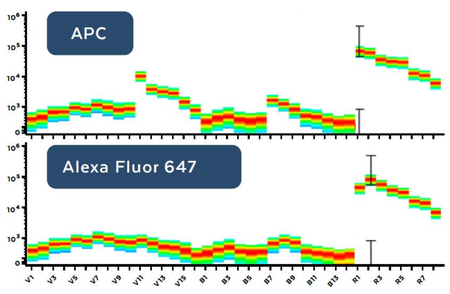


Fig. 2: Full spectrum plots from a 3-laser CYTEK Aurora show distinct signatures for APC and Alexa Fluor 647.

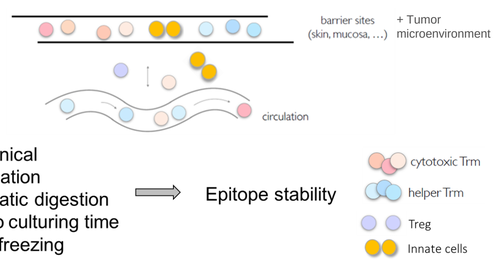


Fig. 3: Establishment of multiparameter panels require proper assessment of isolation and preservation protocols as practical considerations on epitope stability of immune cells vital for downstream assays and immunophenotyping.

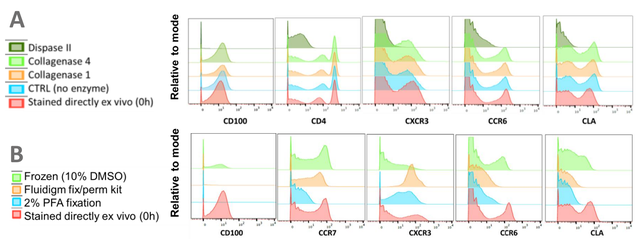
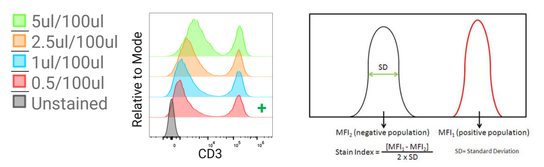


*Fig. 4: **A)** Representative examples of effect of collagenase 1, 4 and Dispase II after 6 hours of digestion, 37 °C onto epitope recovery. **B)** Representative examples of effect of fixation and freezing onto epitope recovery. Human PBMCs, gated on live single CD45+ cells.*



Proper Titration ensures minimal spectral overlap and better resolution of distinct cell populations.

- Enhanced Sensitivity
- Specificity Improvement
- Cost-Efficiency
- Reproducible & Saves Time
- Minimizes Non-Specific Binding
- Reduces Sample Variability
- Maintains Cell Viability
- Facilitates Multicolor Panel Design

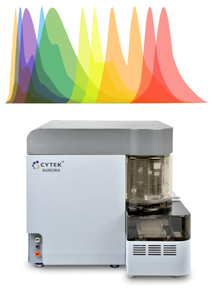
Fig. 5: Representative example of CD3 titration for the identification of the best stain index value .

Establishments & optimisations of FACS panels are crucial steps in flow cytometry experiments, as they optimize sensitivity, specificity, and reproducibility, leading to improved data quality and reliability. Find out more at https://www.nuvisan.com/home.html to see how we are committed to supporting cutting-edge research endeavours. As part of our comprehensive suite of services, we take great pride in offering specialized assistance as well as expertise in immunology and Fluorescence-Activated Cell Sorting (FACS) experiments.
SCAN here →

NUVISAN

Off the shelf NUVISAN panels available

Cytek Aurora (VBR lasers)



Human Immunophenotyping Panel (≥30 parameters) of B, T, NK, DC, ILC and monocyte subsets in PBMCs.

Murine Immunophenotyping Panel (≥ 28 parameters) of B, T, NK, DC, and monocyte subsets in Thymus, blood, spleen, BM, tumors.

Rat Immunophenotyping Panel (≥ 13 parameters) of B, T, NK, DC, and monocyte subsets in Thymus, blood, spleen, BM, tumors.

human	mouse	rat
CD11c	CD4	CD4
CD45RA	CD19	CD45RA
CD3	Ly6G	CD8
CD25	CD69	CD3
IgD	CD45	CD62L
CD95	CD44	Gr
CD11b	CD11b	Ki67
CD38	CD62L	CD161a
CD57	NKp46	CD45RC
CD27	CD137/41bb	Live/dead
CD123	CD8	CD28
CD127	CD45R/B220	IgM
HLADR	CTLA4	CD38
CCR7	F4/80	CD45
CD19	CD3	
CD16	Ly6c	
TCRgd	TCRgd	
CD14	CD11c	
CD8	PD1	
CD1c	CD25	
PD1	SLAMF7	
CD56	CD206	
CD45RA	Live/dead	
CD28	MHCII(IA/IE)	
SLAMF7	CD38	
CXCR3		
CCR6		
cKIT		
IgM		
Live dead		

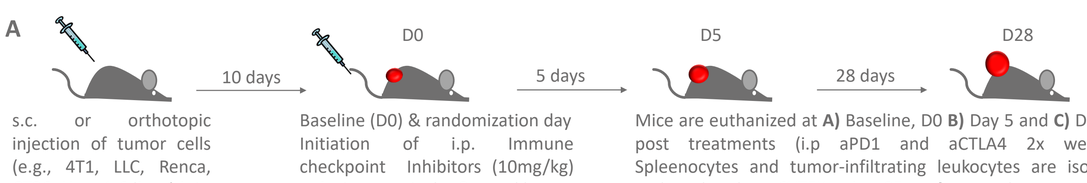
Advantage of Spectral flow cytometry:
Less sample material needed to extract more complex information!

Human immunophenotyping

*Fig. 6: **A)** Identification of differentially distributed cellular phenotypes by opt-SNE Probability Binning. Representative example of opt-SNE from freshly isolated PBMCs from 1 healthy donor showing CD3 intensity among cell subsets. **B & C)** CD45+ populations in PBMCs identified and visualised by FlowSOM. **C)** . The heatmap shows median marker intensities within each immune population (red represents high and blue represents low intensity). **D & F)** Representative histograms of CD4+ and CD8+ T cells depict the expression of the indicated surface molecules on T cells freshly isolated ex vivo (0h), stimulated with aCD3,aCD28,IL2 & ex vivo cultured (2 weeks), or unstimulated CTRL only cultured with IL2 (2 weeks). **E)** Representative plots showing CD8+ and CD4+ T cells respectively with or without stimulation with aCD3,aCD28, IL2 ex vivo cultured(2 weeks)*

Murine immunophenotyping

Experimental design 4 groups (aPD1 vs isotype control, aCTLA4 vs isotype control)



s.c. or orthotopic injection of tumor cells (e.g., 4T1, LLC, Renca, CT26, Hepa1-6 lines) into WT mice

Baseline (D0) & randomization day Initiation of i.p. Immune checkpoint Inhibitors (10mg/kg) vs isotype controls, 2x weekly

Mice are euthanized at **A)** Baseline, D0 **B)** Day 5 and **C)** Day 28 post treatments (i.p aPD1 and aCTLA4 2x weekly). Spleenocytes and tumor-infiltrating leukocytes are isolated and analyzed on CYTEK using 25 surface markers as well as using IHC.

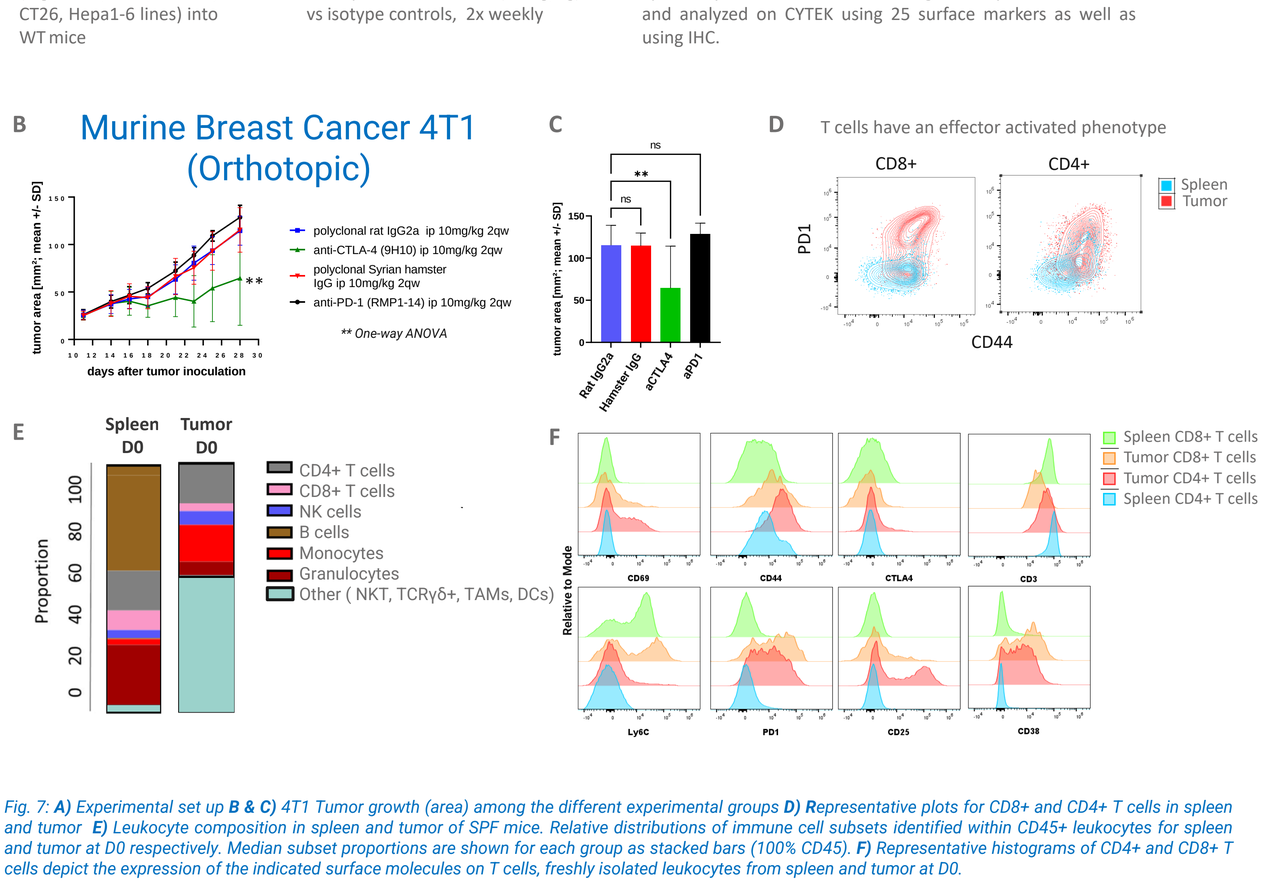


*Fig. 7: **A)** Experimental set up **B & C)** 4T1 Tumor growth (area) among the different experimental groups **D) R**epresentative plots for CD8+ and CD4+ T cells in spleen and tumor **E)** Leukocyte composition in spleen and tumor of SPF mice. Relative distributions of immune cell subsets identified within CD45+ leukocytes for spleen and tumor at D0 respectively. Median subset proportions are shown for each group as stacked bars (100% CD45). **F)** Representative histograms of CD4+ and CD8+ T cells depict the expression of the indicated surface molecules on T cells, freshly isolated leukocytes from spleen and tumor at D0.*