MACS
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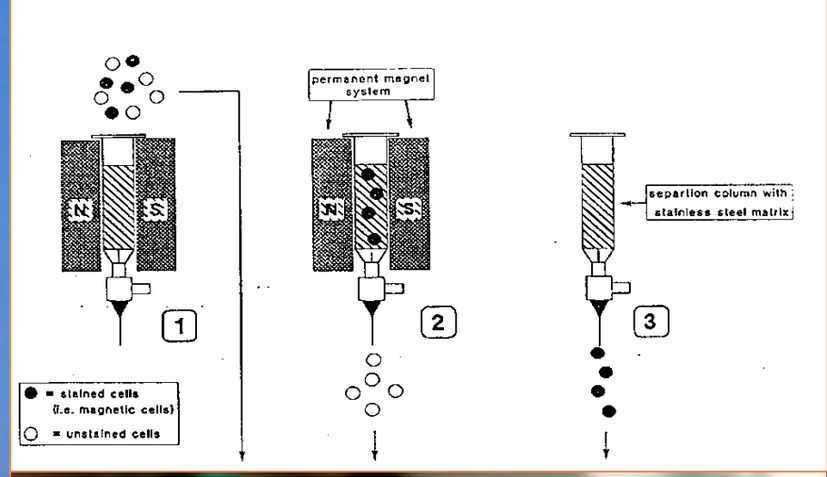
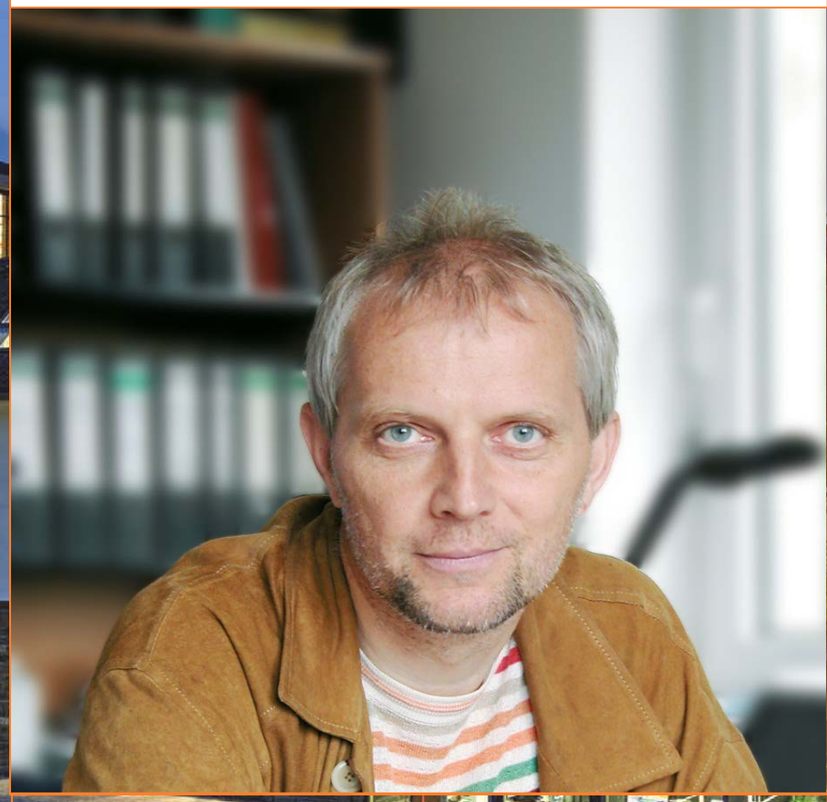
Innovation & Creativity





Research to bedside

Miltenyi Biotec fast facts

1989 Miltenyi Biotec was founded in Germany

14.000 products available worldwide

More than **55.000** cell therapy procedures with Miltenyi Biotec products

2.200 employees worldwide

44 nations represented within our staff

2.500 patients treated annually with cell products using Miltenyi Biotec technologies

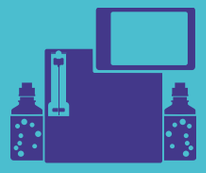
73 countries served through distributors

28 countries with direct sales

475 employees in R&D, engineering, and clinical development

Miltenyi Biotec Integrated Workflows

Pioneering solutions and integrated workflows



Devices

Reagents

Expertise

Consumables

Prerequisite for Single Cell Analysis

- Single cells suspension
- Excellent viability
- Debris & dead cells free

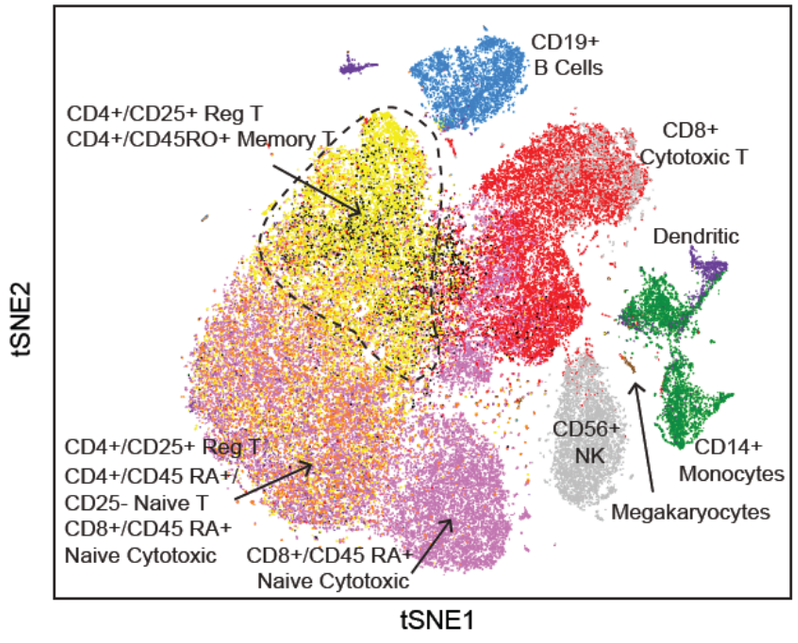
Risks

- Background noise in sequencing data
- Impact on efficiency of cell partitioning
- Impact on Cell Counting Accurancy

Sample Preparation from Tissues

Tools & Innovations

[Single Cell Suspensions]



MACS Tissue Storage Solution

Store Tissue up to 48 hours

No Background effect

Tested on many tissue

Cell Viability

Security

Flexibility

gentleMACS Octo Dissociator with Heaters

Fully automated dissociation

Pre-set protocols

Both cellular & molecular

Reproducible results & time saving

Security - Quality

Flexibility

Tissue Dissociation Kits

Pre-titrated enzymes

Highly purified enzymes

Limit unspecific tryptic activity

Reproducible results

No batch to batch variation

Epitopes preservation

Removal of large particules after dissociation: SmartStrainers

Improved ventilation	Fit with 15 ml & 50ml tubes	Stackable
No clogging	Flexibility	Save time

Removal of large agregates: SmartStrainers

Straining has no impact on Cell viability

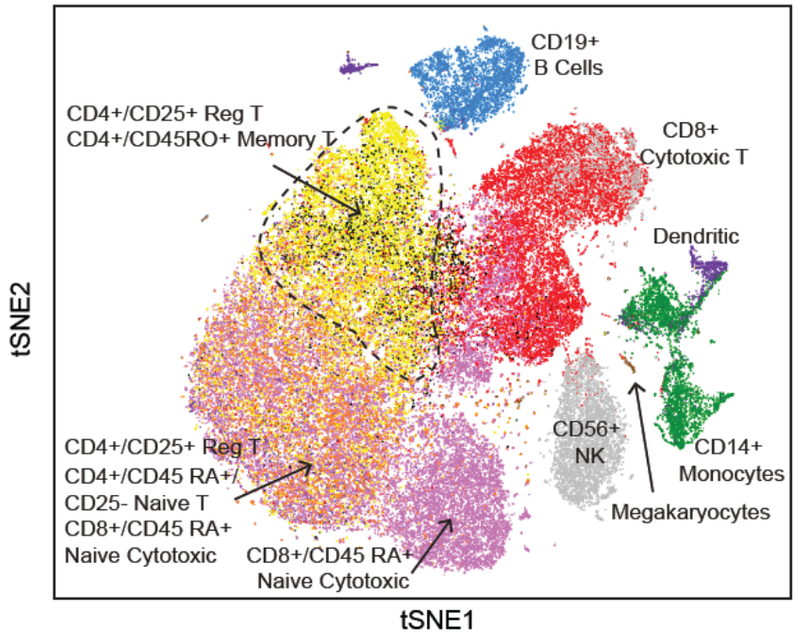
Recommended as SmartStrainers

cause minimal changes to the cell concentration

Sample Clearing

Tools & Innovations

[Single Cell Suspensions]



Removal of Dead Cells

10x GENOMICS®

TECHNICAL NOTE

Removal of Dead Cells from Single Cell Suspensions Improves Performance for 10x Genomics® Single Cell Applications

PBMC suspension #1 (Control):	Cells loaded immediately for partitioning after sample preparation
PBMC suspension #2 (24 h at RT):	Cells left at room temperature (RT) in PBS for 24 h
PBMC suspension #3 (Digitonin Low):	Cells treated with 5 ng/ml Digitonin. Cell suspension resulted in < 5% viable cells and was mixed with >90% viable PBMCs at a 1:1 ratio
PBMC suspension #4 (Digitonin High):	Cells treated with 5 ng/ml Digitonin. Cell suspension resulted in < 5% viable cells and was mixed with >90% viable PBMCs at a 5:1 ratio

Removal of Dead Cells

	Control	24 h at RT	Digitonin Low	Digitonin High
Pre Dead Cell Removal	85%	50%	50%	20%
Post Dead Cell Removal	85%	86%	85%	82%

Table 1. Cell viability for each PBMC suspension assessed with Trypan Blue and the Countess II Automated Cell Counter after sample treatment and removal of dead cells with the Dead Cell Removal Protocol.

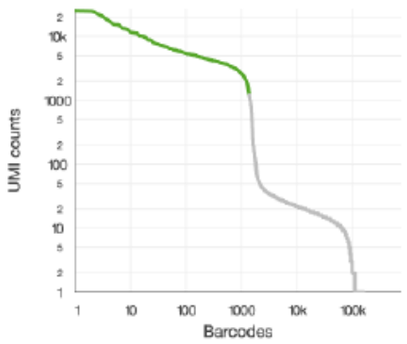
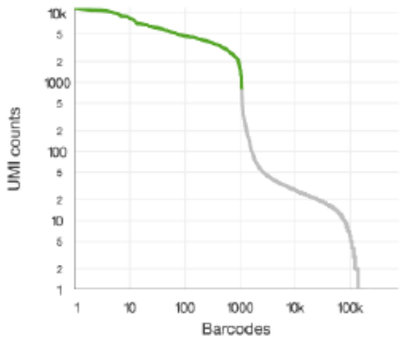
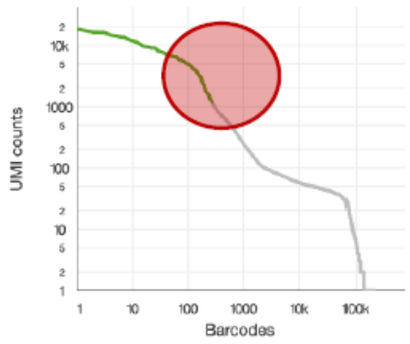
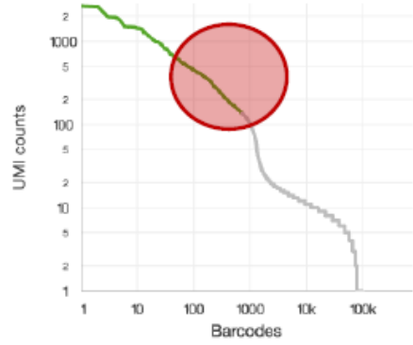
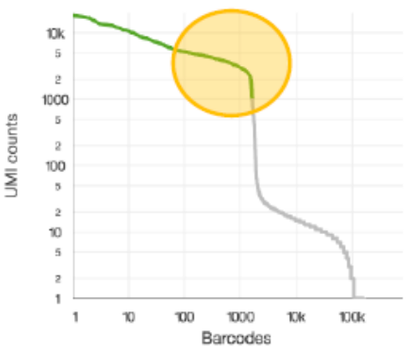
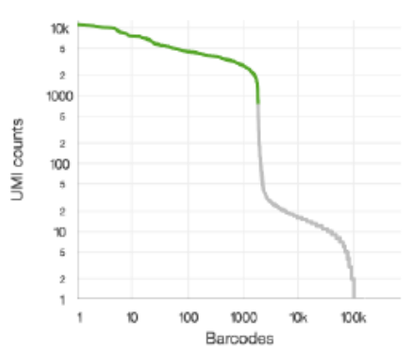
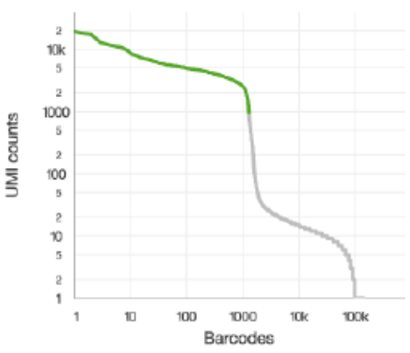
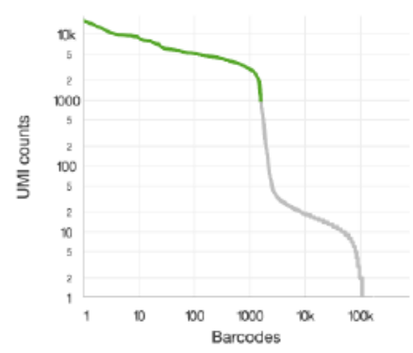
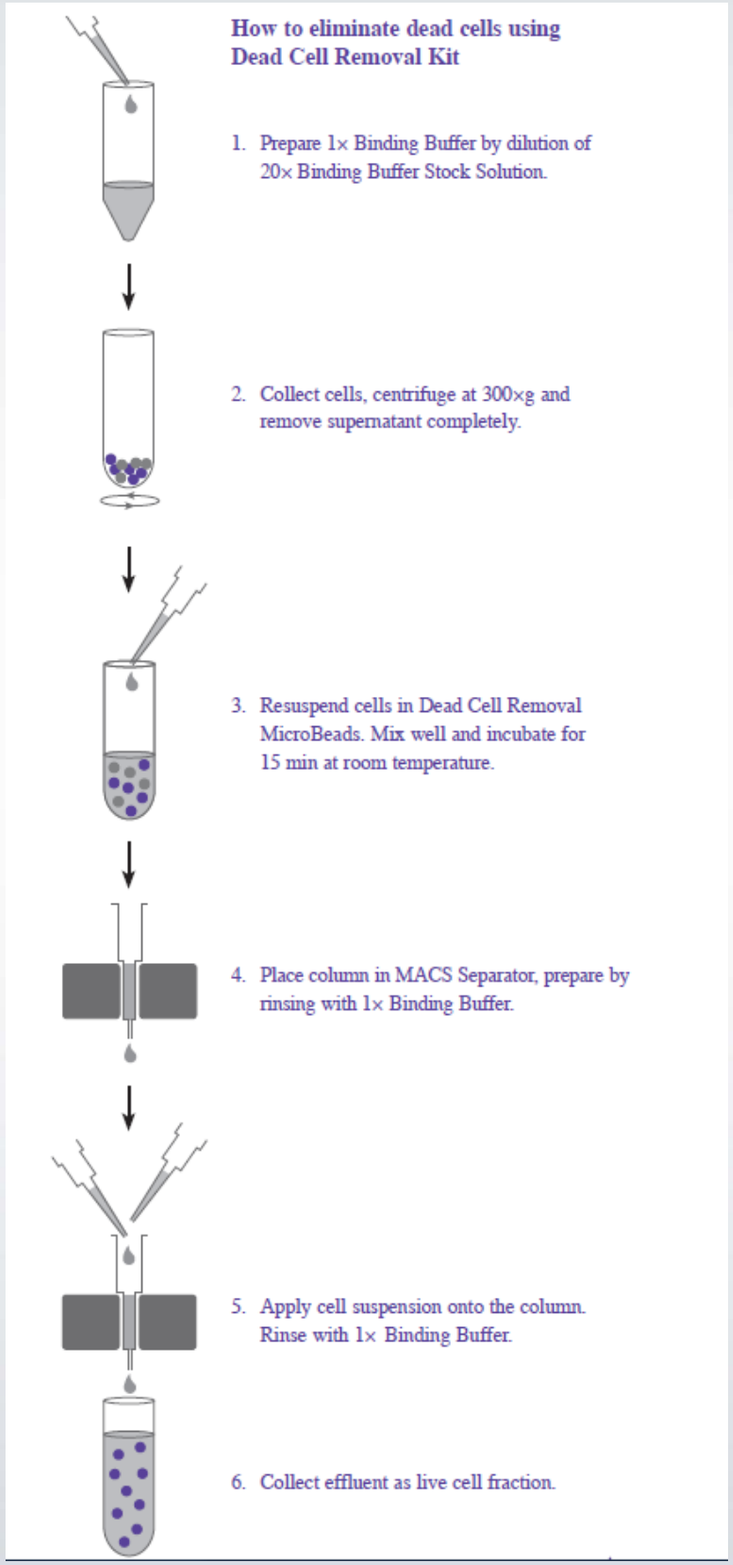
Treatment	Control	24 h at RT	Digitonin Low	Digitonin High
Pre-Dead Cell Removal				
Post-Dead Cell Removal				

Fig.1. Barcode Rank Plot for 8 samples pre and post Dead Cell Removal treatment. Plotted is the distribution of barcode counts (x-axis) and the corresponding total UMI (Unique Molecular Identifier) counts (y-axis). Cell-containing partitions (barcodes on x-axis) are shown in green. Background partitions are shown in grey. Red circle indicates lack of steep drop-off. Yellow circle indicates one example of clear steep drop-off.

Removal of Dead Cells



How to eliminate dead cells using Dead Cell Removal Kit

1. Prepare 1× Binding Buffer by dilution of 20× Binding Buffer Stock Solution.
2. Collect cells, centrifuge at 300×g and remove supernatant completely.
3. Resuspend cells in Dead Cell Removal MicroBeads. Mix well and incubate for 15 min at room temperature.
4. Place column in MACS Separator, prepare by rinsing with 1× Binding Buffer.
5. Apply cell suspension onto the column. Rinse with 1× Binding Buffer.
6. Collect effluent as live cell fraction.

Removal of Dead Cells

Less cell loss than density gradient

Recovery

Fast

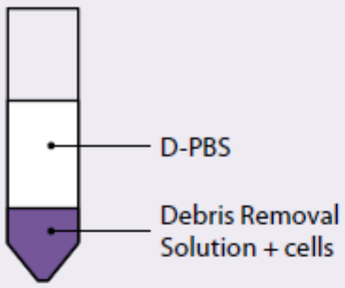
(20-25 min)

Save time

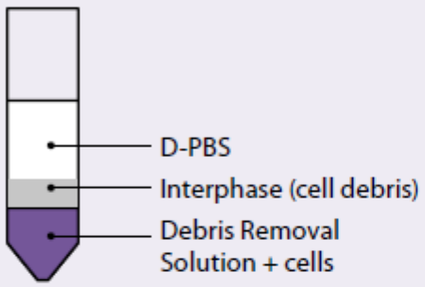
Highly viable cell sample

Suitable for Single Cell Analysis

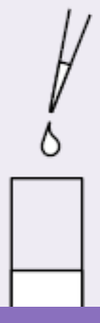
Debris Removal kit & Red Blood Lysis Solution



- **Overlay with 4 mL D-PBS**
- **Centrifugation**
  10 min, 3000×g, 4 °C



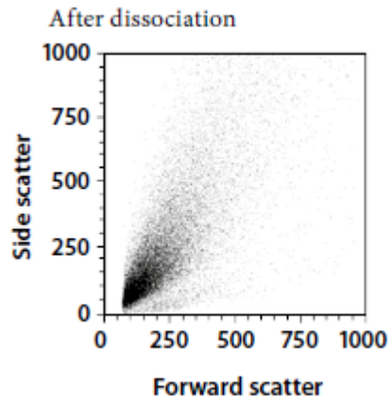
- **Remove D-PBS**
- **Remove interphase**

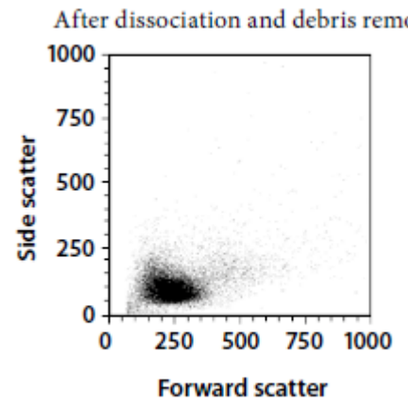


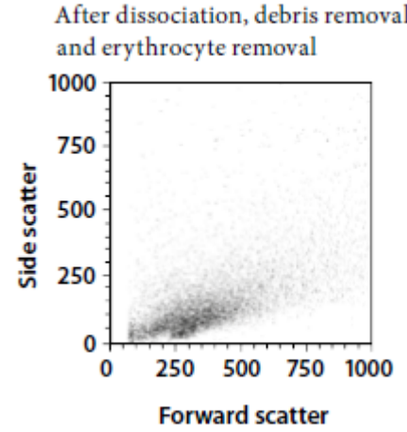
- **Add D-PBS to a final volume of 15 mL, invert gently**
- **Centrifugation**
  10 min, 1000×g, 4 °C

ernatant
cell pellet

Example of debris removal after adult rat heart dissociation using the Debris Removal Solution







Developped for efficient debris removal

Quality

Standardized protocole

Reproducibility

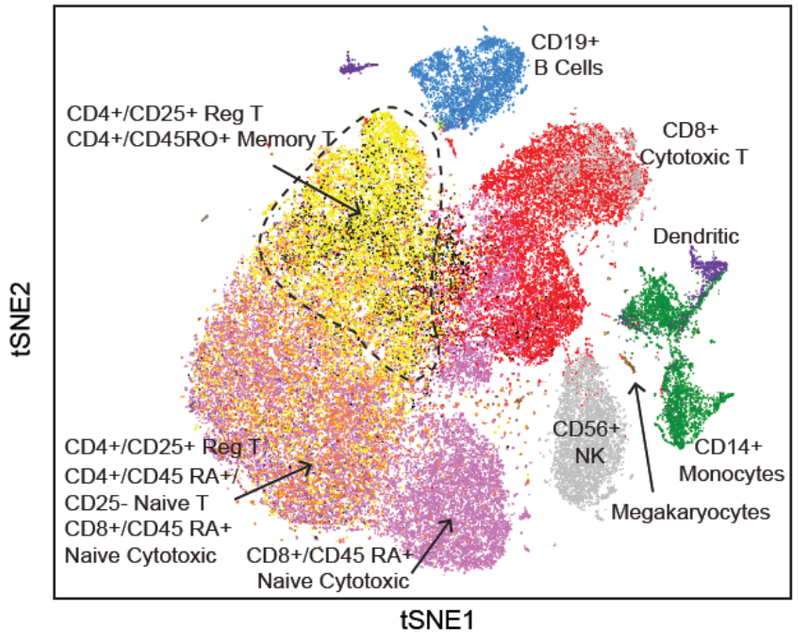
Full acceleration & Full brake

Save time

Cell Separation

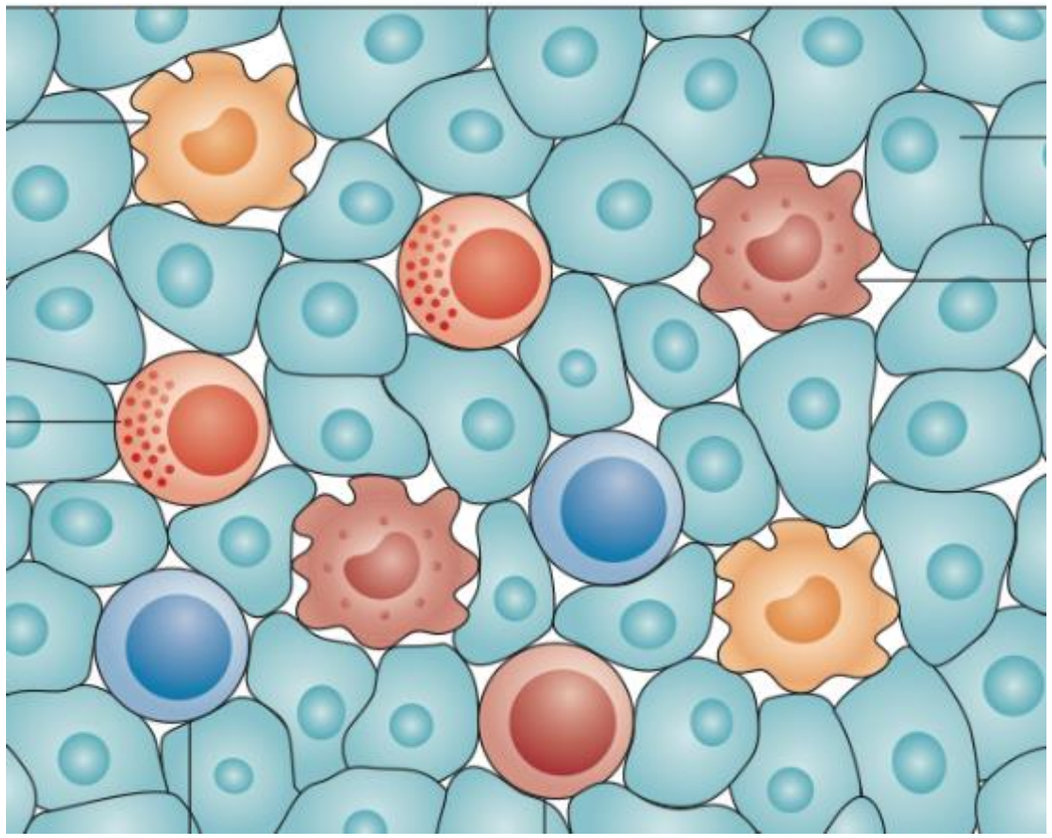
Tools & Innovation

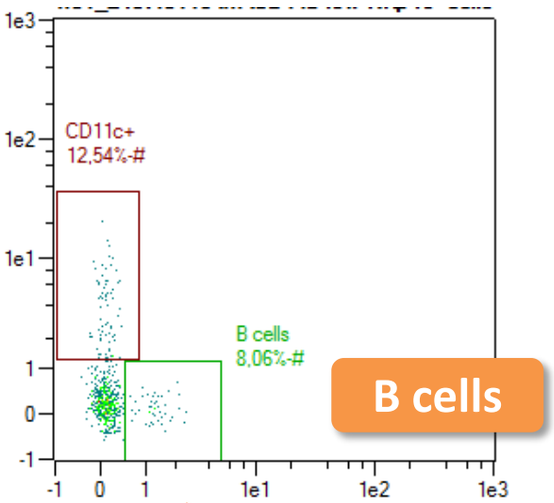
[Single Cell Suspensions]



TIL subpopulations in 4T1 tumor – bulk tumor

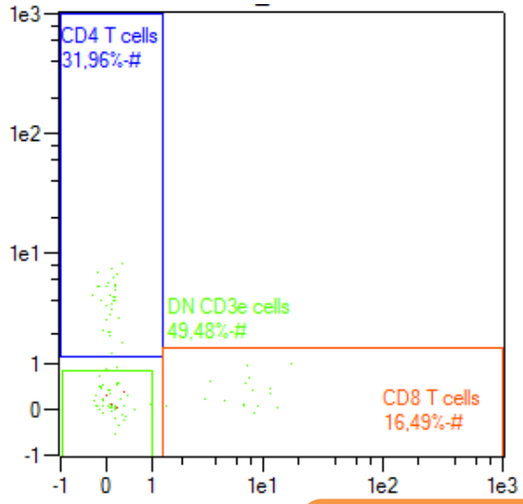
Tumor





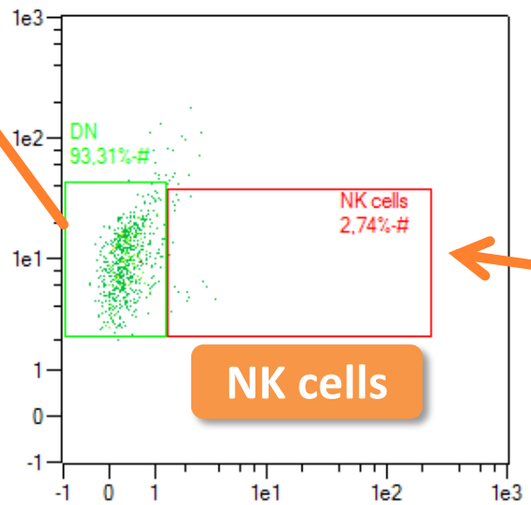
B cells

T helper cells



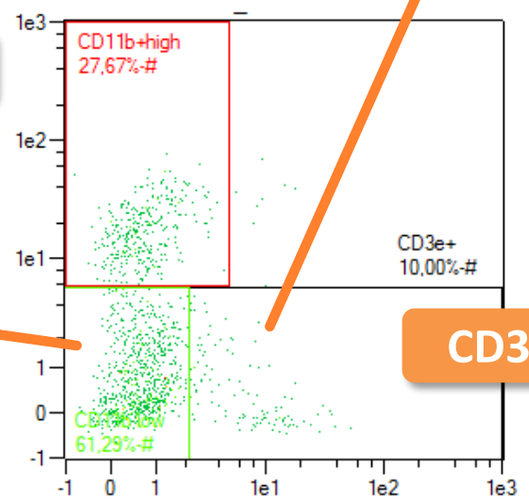
Cytotoxic T cells

CD11b



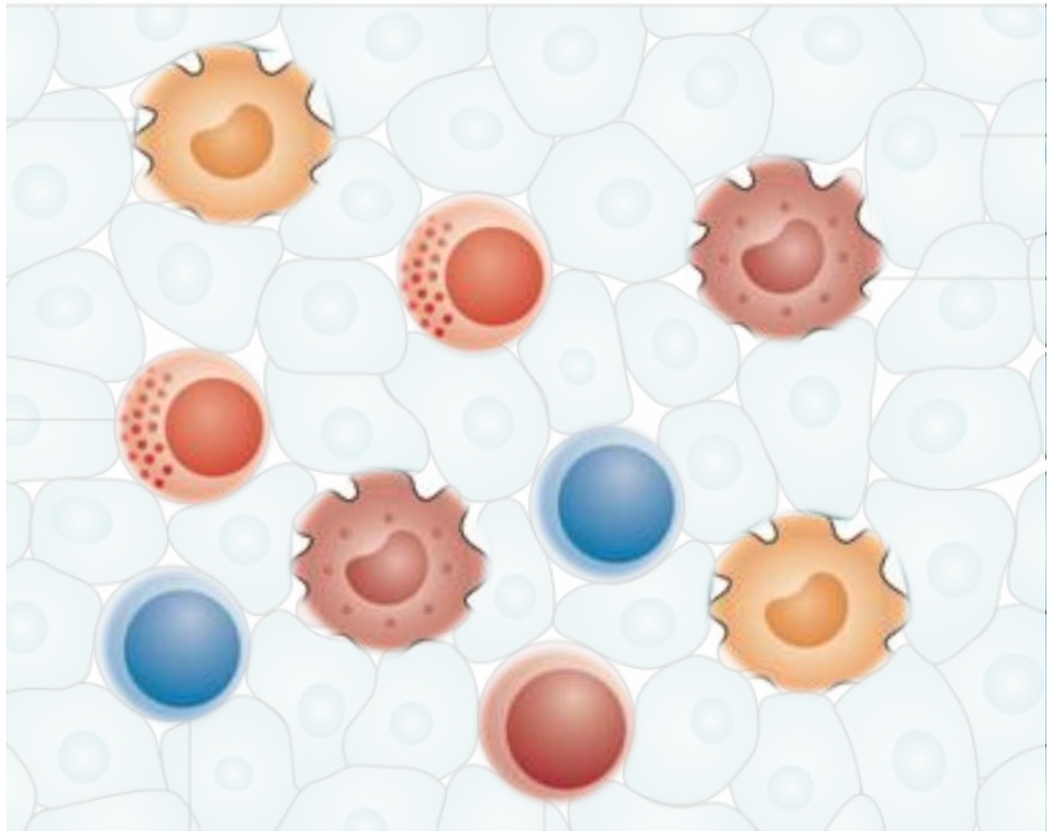
NK cells

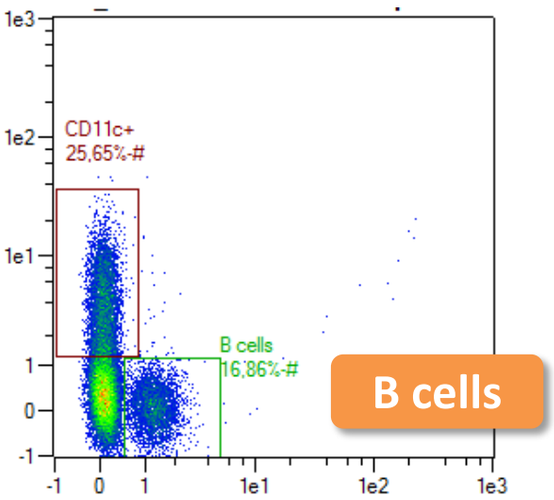
CD3



TIL subpopulations in 4T1 tumor – enriched CD45+ cells

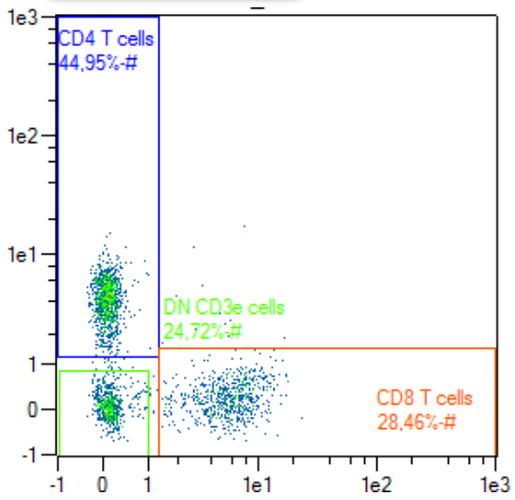
Tumor



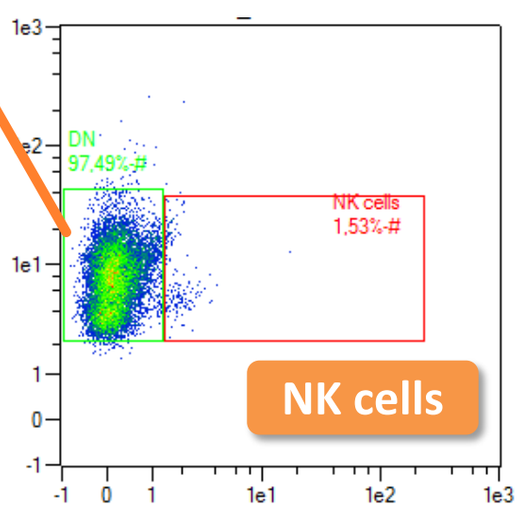


B cells

T helper cells

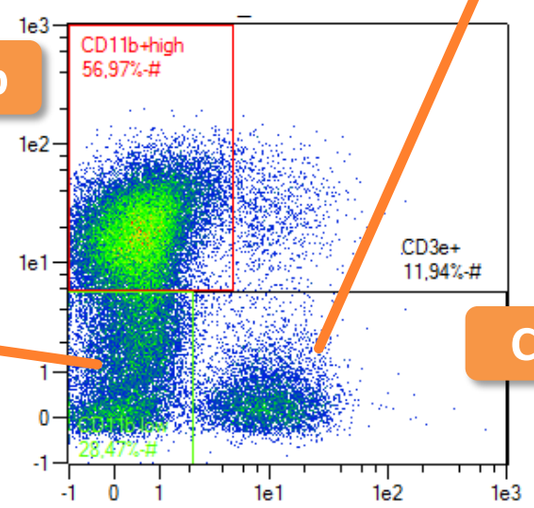


Cytotoxic T cells



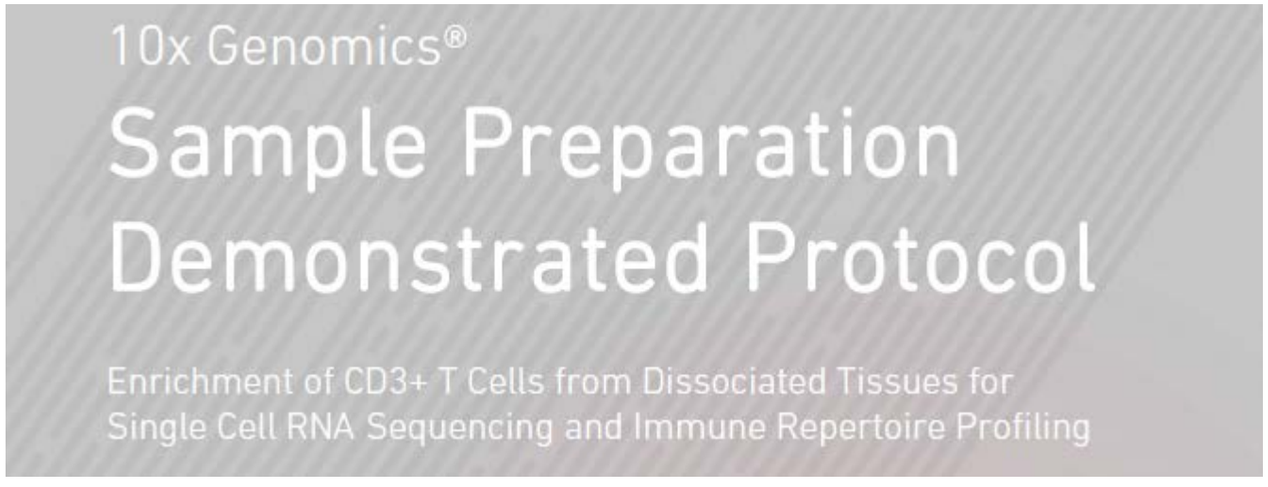
NK cells

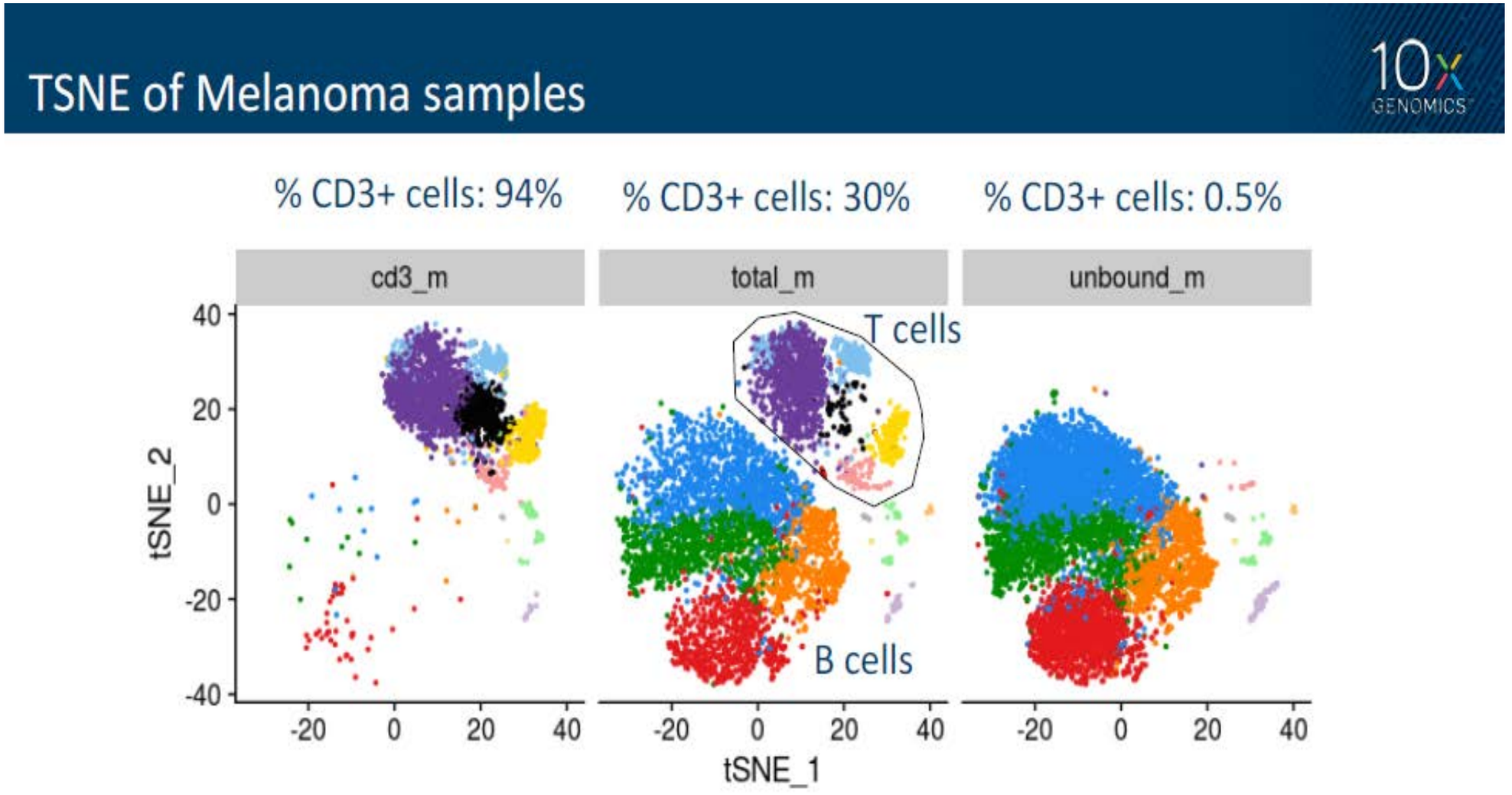
CD11b



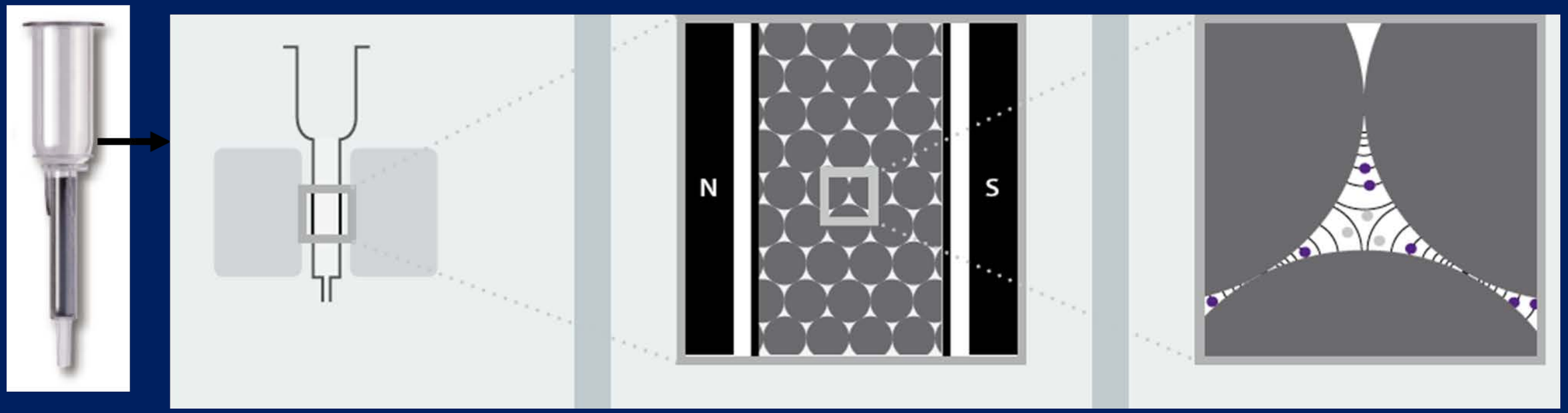
CD3

Impact of cell separation on single cells analysis





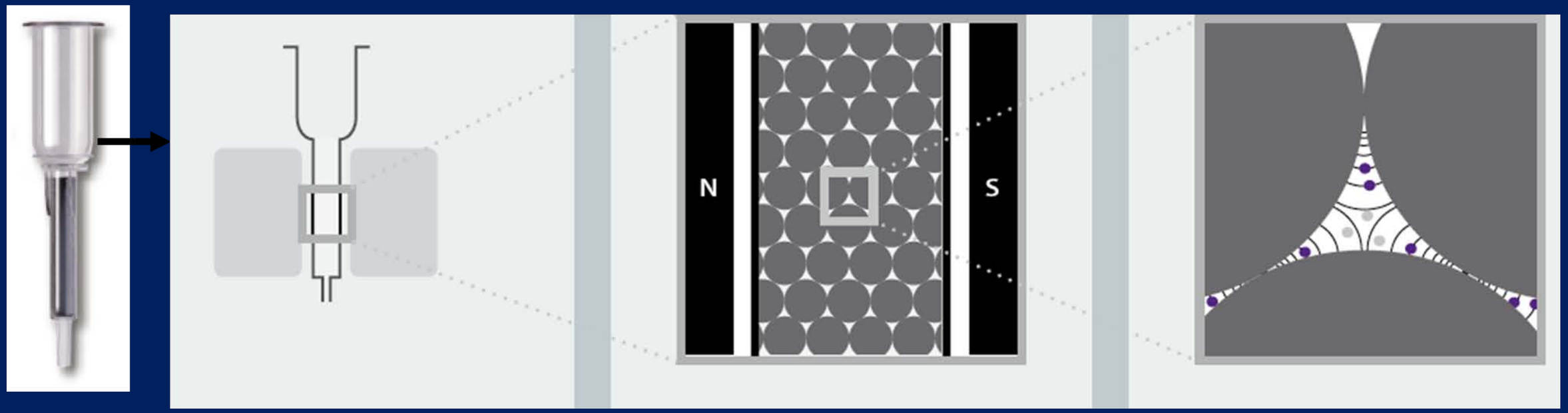
MACS Cell Separation Reagents



Minimal labelling

Gentle for cells

MACS Cell Separation Reagents



Minimal labelling

Fast Enrichment

Gentle for cells

Save time for downstream analysis

MACS Cell Separation Reagents

Starting Materials

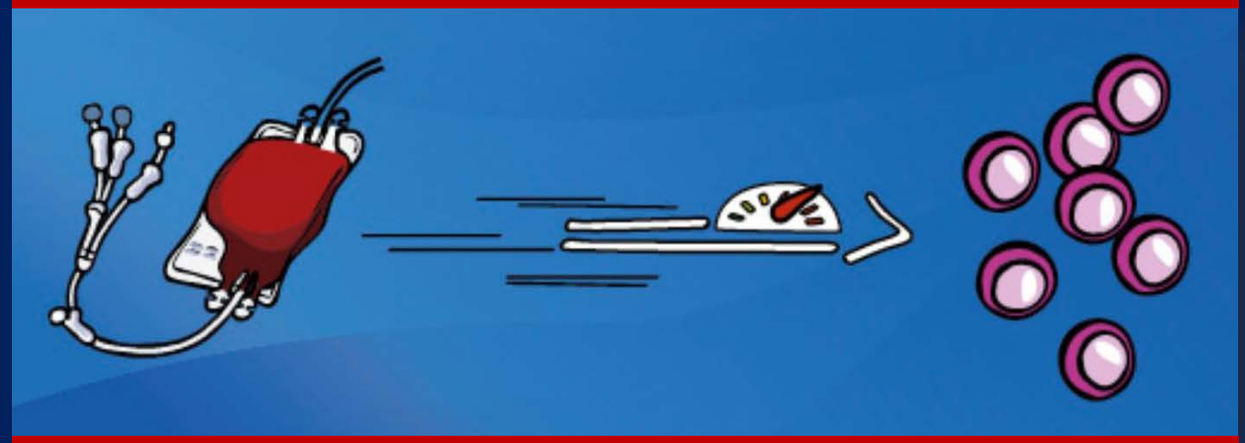
Single cell suspension

PBMC, dissociated tissues

MicroBeads & Isolation Kits

Blood products

Whole blood, Buffy coat, Apheresis products



StraightFrom Technology

Minimal labelling

Gentle for cells

Fast Enrichment

Save time for downstream analysis

Automation & Highthroughput

Reproducibility & time saving

MACS Cell Separation Reagents

Upscale your cell separation

Manual	Automated	High throughput	Automated high throughput

Minimal labelling	Fast Enrichment	Automation possible
Gentle for cells	Save time for downstream analysis	Reproducibility

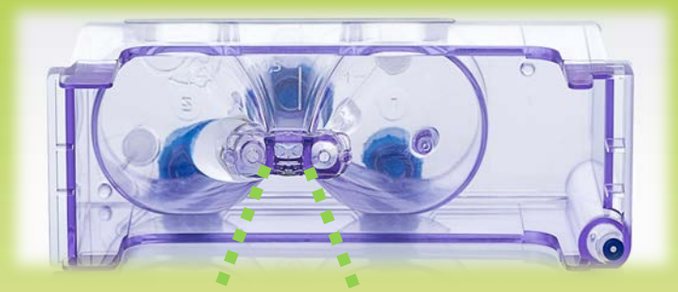
MACSQuant Tyto

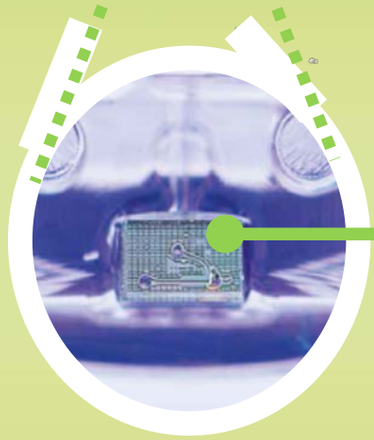
Miltenyi Biotec

Reliable

Closed system

Translational





Microchip

MACSQuant Tyto

Low pressure

No decompression

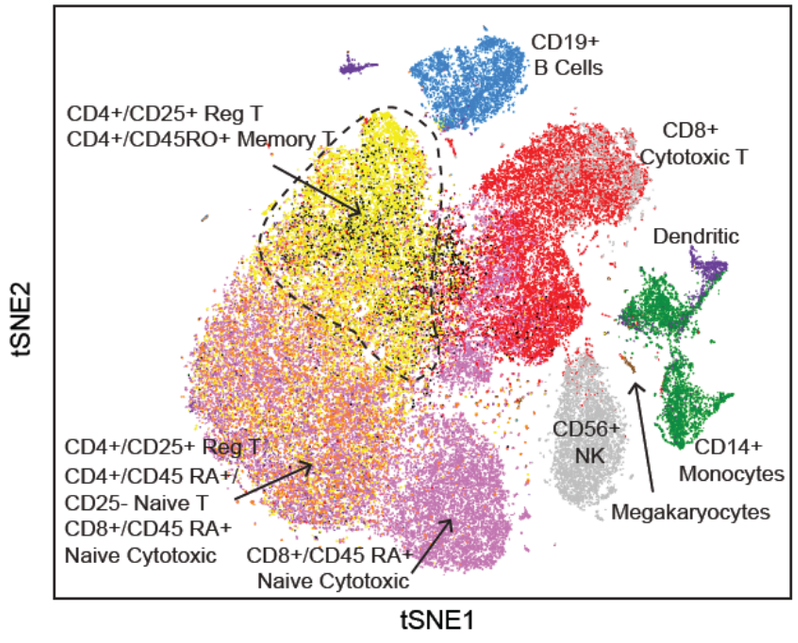
Enables re-sorting of functionnal cells

Full Cell Viability & Functionnality

Additionnal Technologies to improve your SCG Results

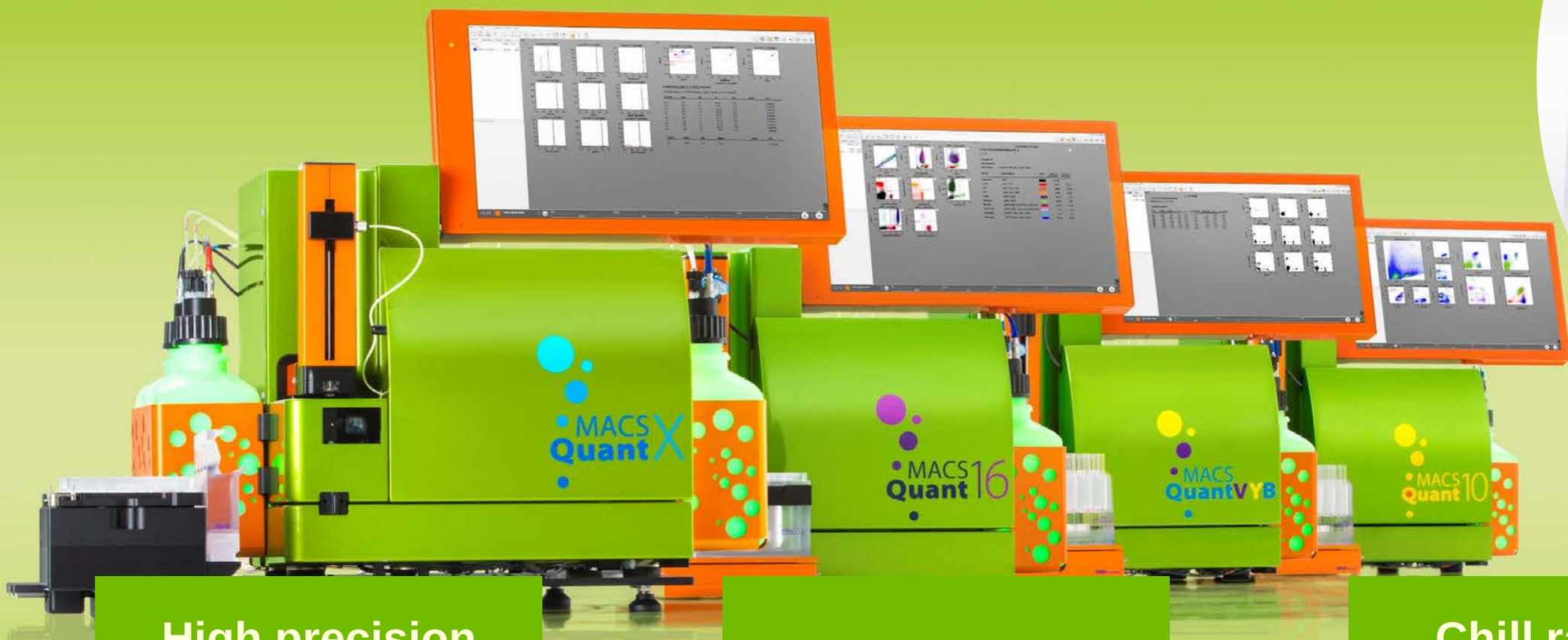
Tools & Innovations

[Single Cell Suspensions]



MACSQuant Analyzers : Absolute counting & co

Miltenyi Biotec



High precision syringe

Autolabelling

Chill racks & gentle mixing

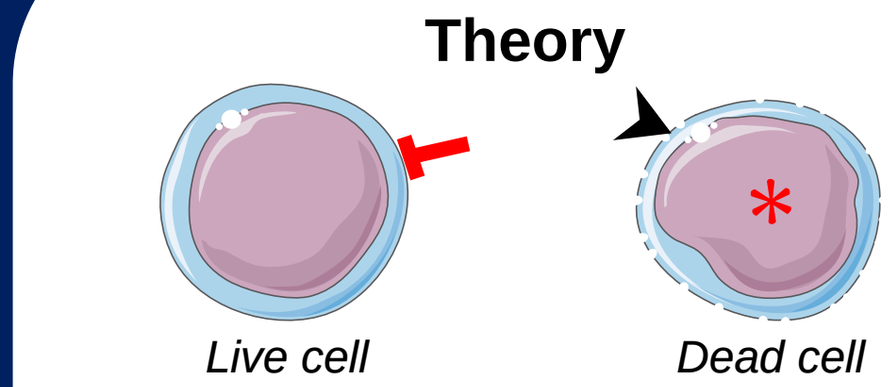
Absolute counting

Ideal for Viability check

Viability Accuracy

Viability Check by Flow Cytometry

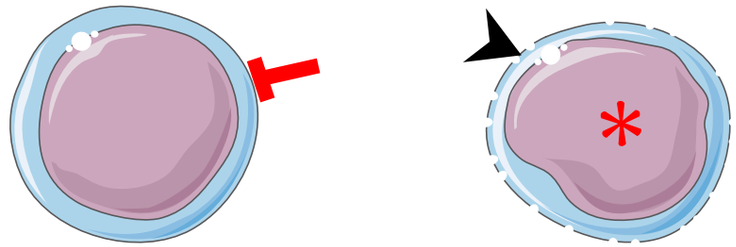
7-AAD
DAPI
IP



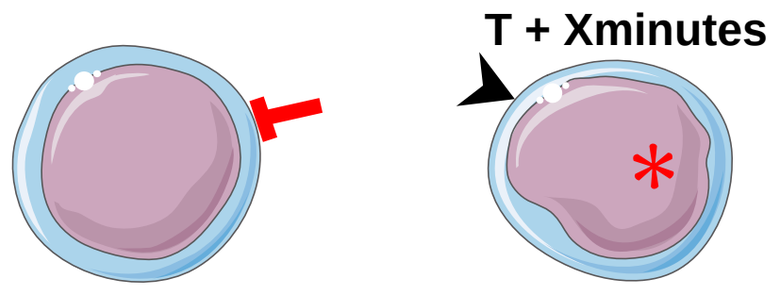
Viability Check by Flow Cytometry

7-AAD
DAPI
IP

Theory



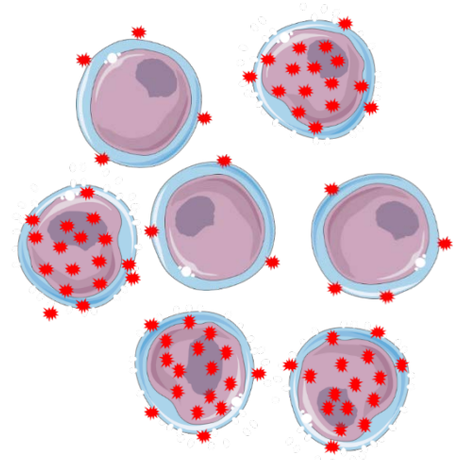
Practical



New

Viobility™ Fixable dyes

Amine groups binding



*Cells stained with **Viobility Dye***

New

Viobility™ Fixable dyes

Blue Laser

Viobility™ 488/520 Fixable Dye

Equivalent fluorochromes	Capacity	Reference	Price
FITC / VioBright FITC /Alexa488	100 tests	130-109-814	60 €
	500 tests	130-110-206	240 €

Violet Laser

Viobility™ 405/452 Fixable Dye

Equivalent fluorochromes	Capacity	Reference	Price
VioBlue / Pacific Blue / V450 / BV421	100 tests	130-109-816	60 €
	500 tests	130-110-205	240 €

Viobility

Equivalent fluorochromes
VioGreen / V500 / BV510

Non toxic for cells

No passive diffusion

High SI

Viability

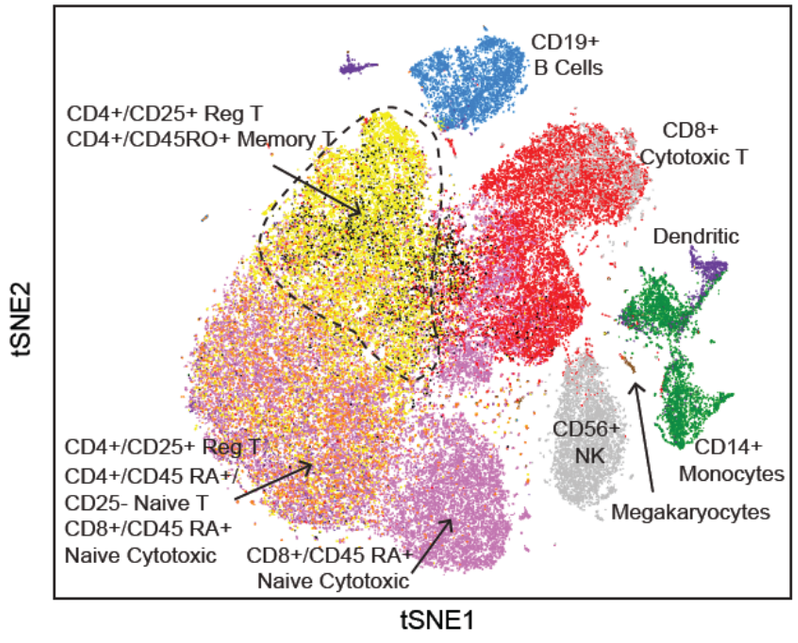
Viability Accurancy

Better discrimination

Studies from Tumors

Tools & Innovations

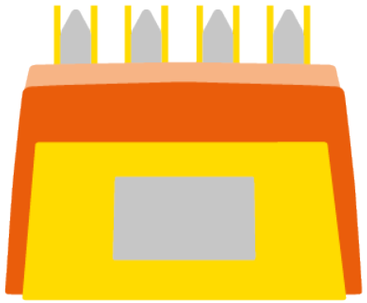
[Single Cell Suspensions]



Cell preparation from tumor tissue - workflow

MACS Miltenyi Biotec

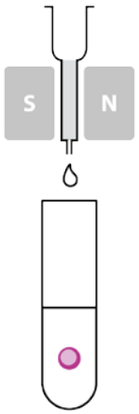
Tissue dissociation



Filtration

RBC lysis

Dead cell removal



Study design

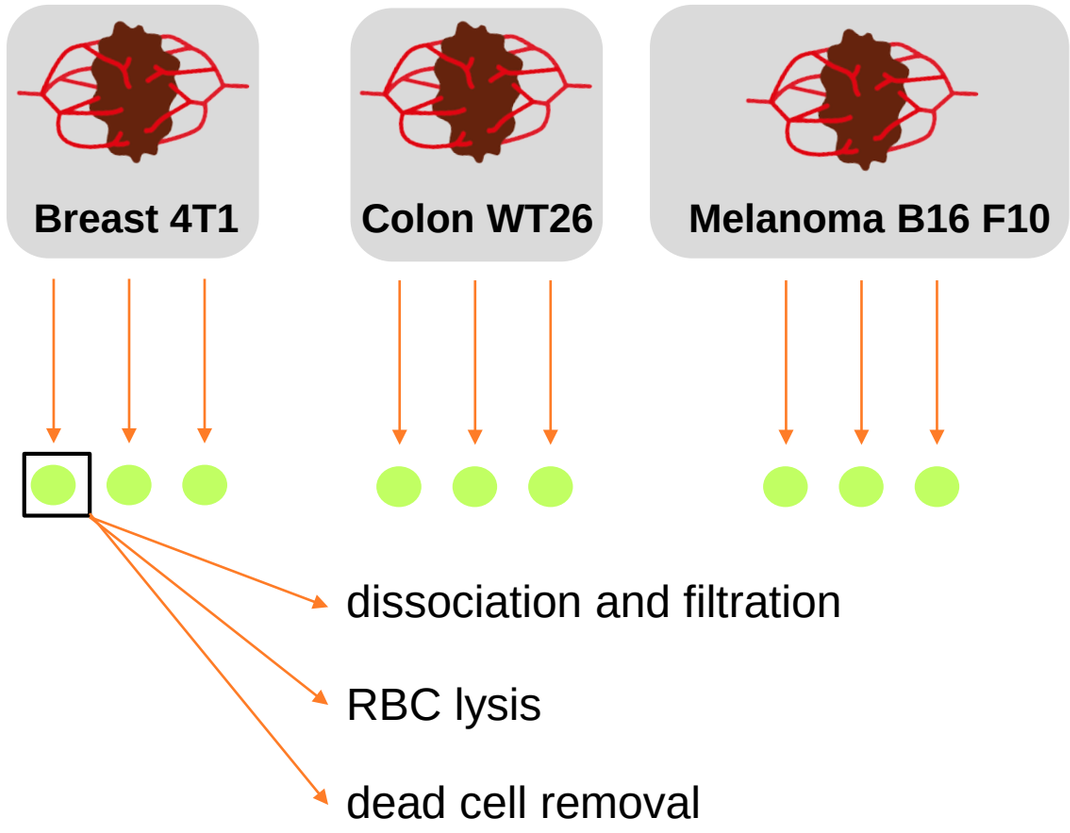
Syngeneic Mouse Tumor Model

Tumor originated in an inbred strain

Tumor cell line grown *in vitro*

Tumor cells implanted in mice of the same inbred strain

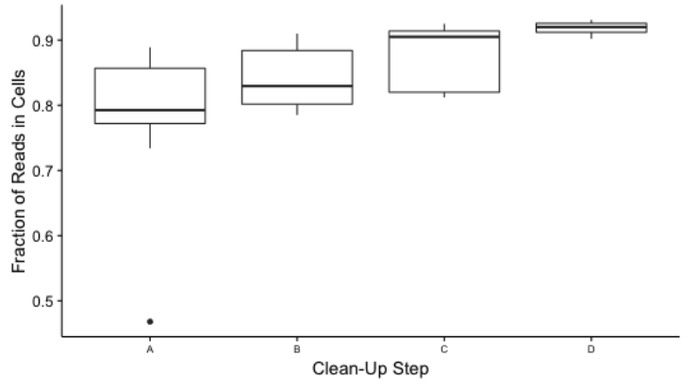
Experimental setup



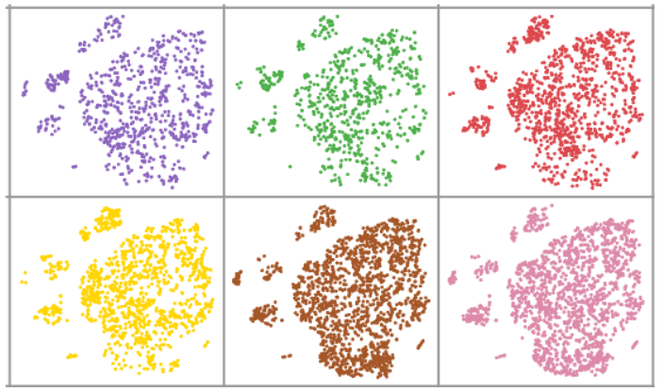
9 Tumors: 3 tissues, 3 reps
47 sequencing libraries
84,042 single cells sequenced

Study goals

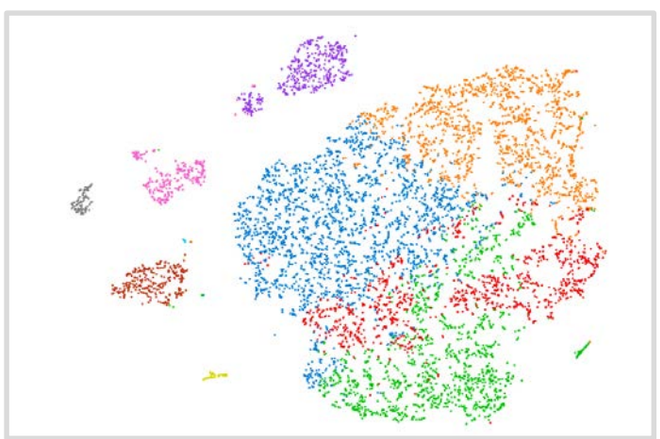
- **Performance**



- **Reproducibility**



- **Tumor heterogenity**

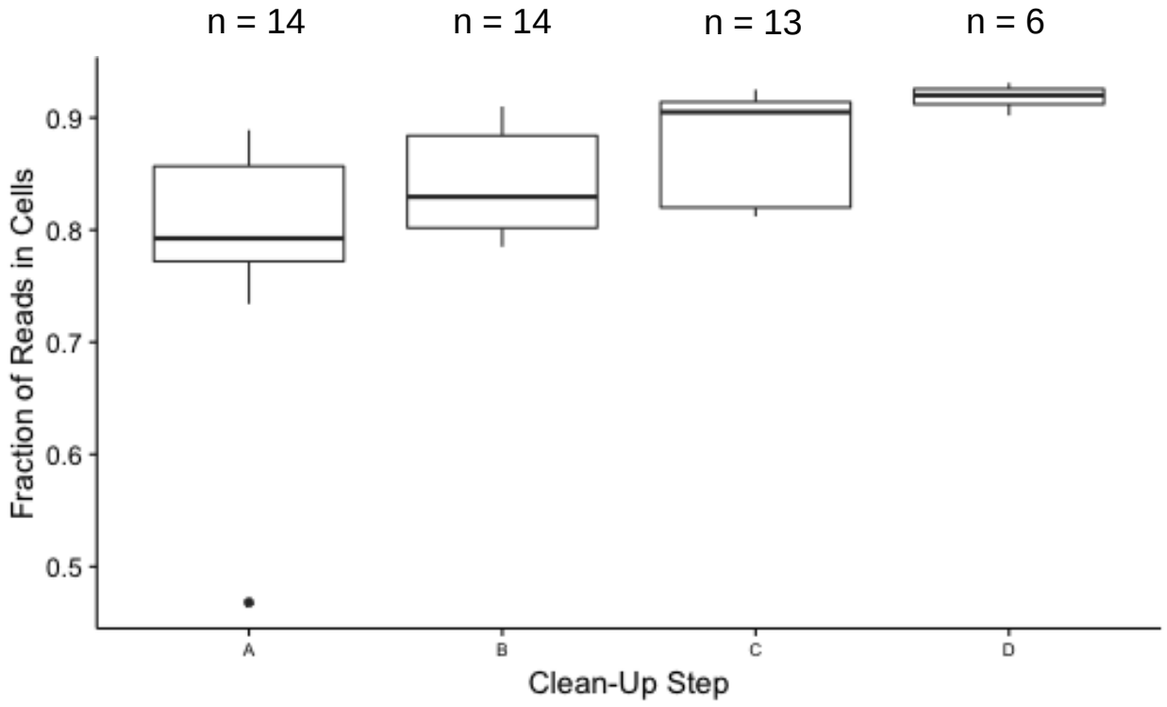


Performance

Increase in Cleanliness and Complexity

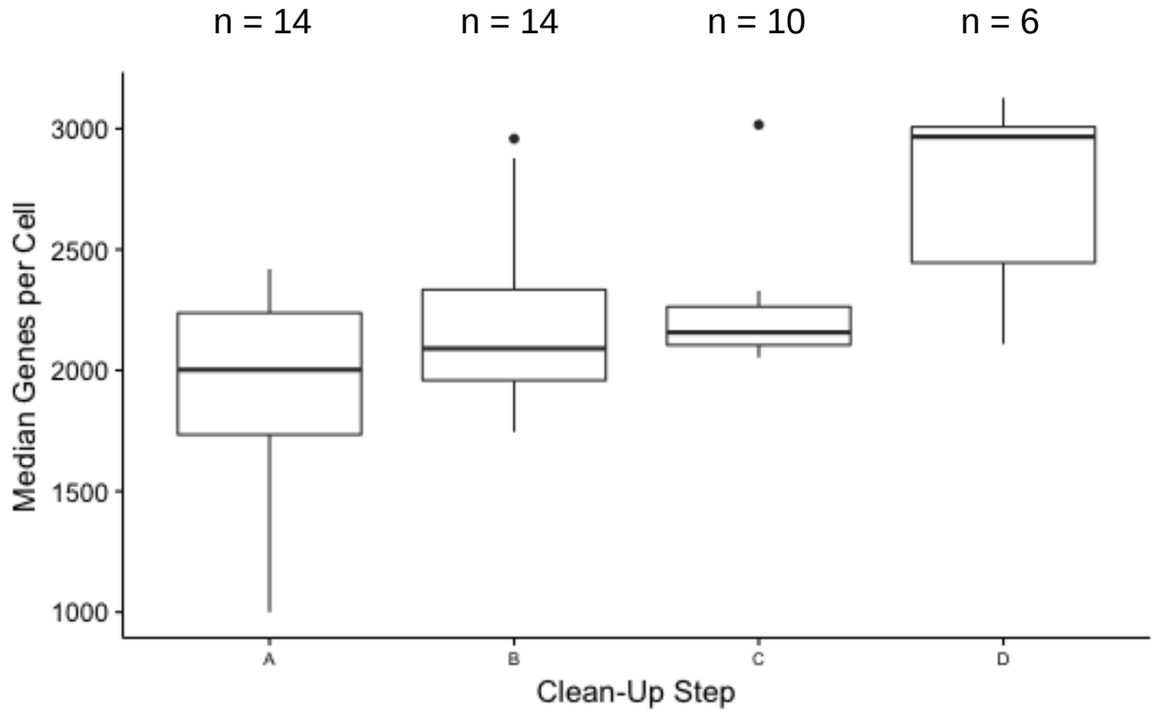
Cell Ranger™ Software Pipeline

Library Cleanliness



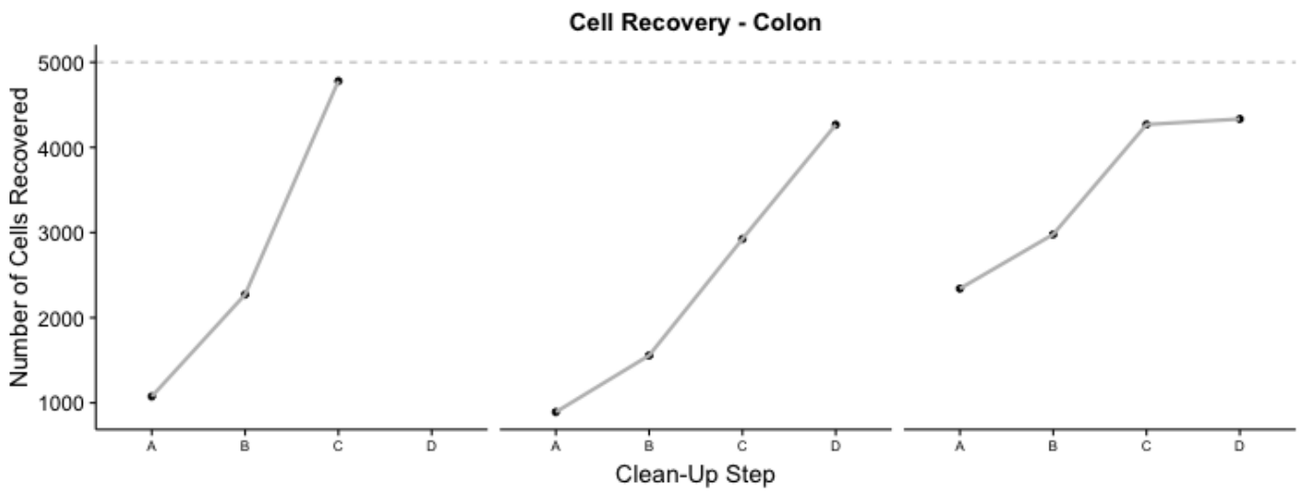
A = Post Filtration | B = Post RBC Lysis | C, (D) = Post Dead Cell Removal(s)

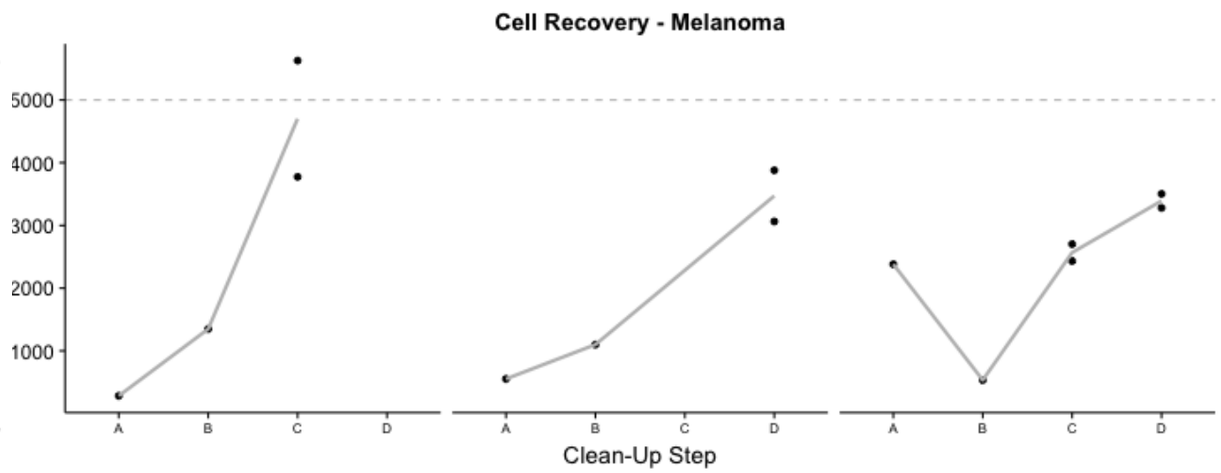
Library Complexity

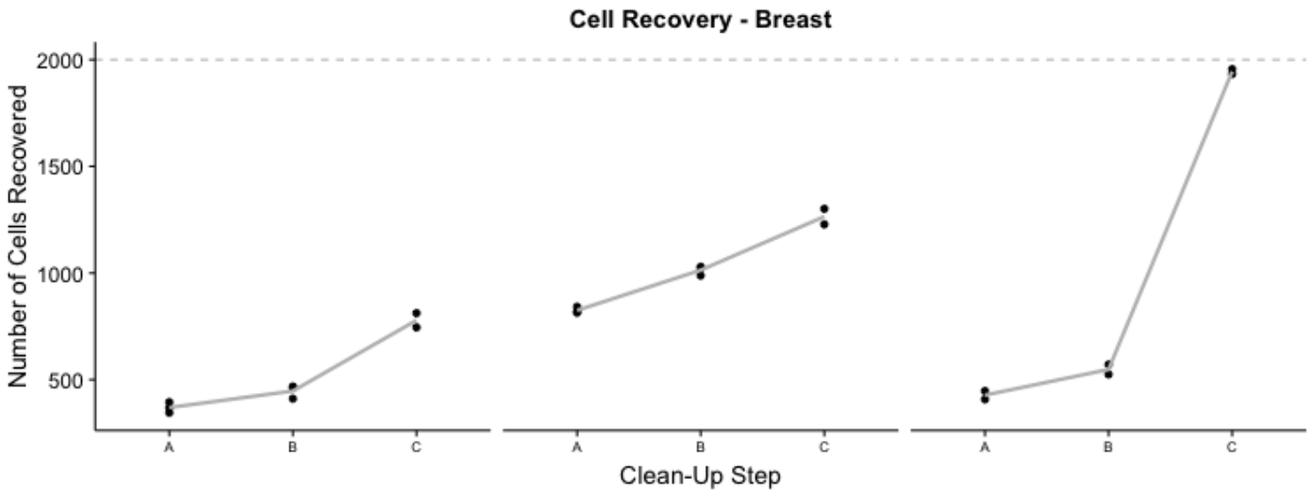


20,000 reads per cell (low seq. depth)

Substantial Increase in Cell Recovery







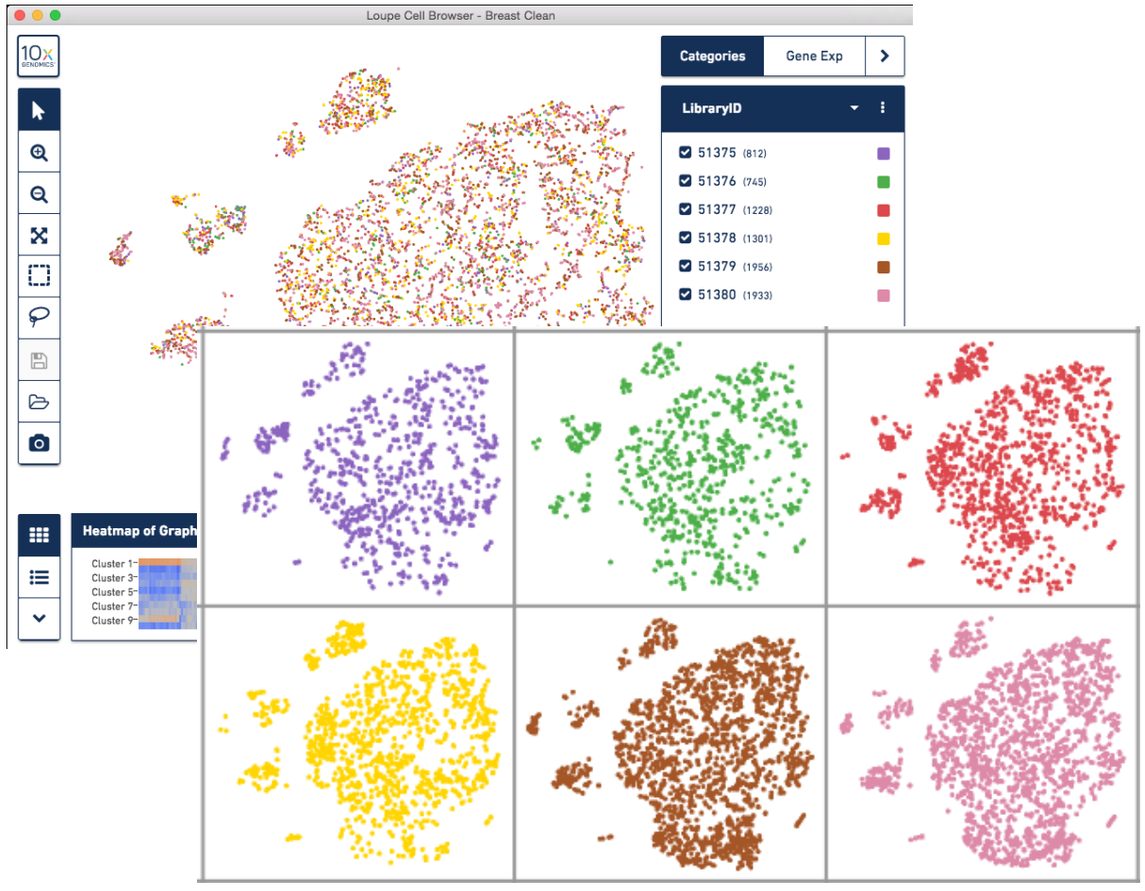
A = Post Filtration | B = Post RBC Lysis | C, (D) = Post Dead Cell Removal(s)

Reproducibility

Breast tumors – reproducible results

Filtered Only

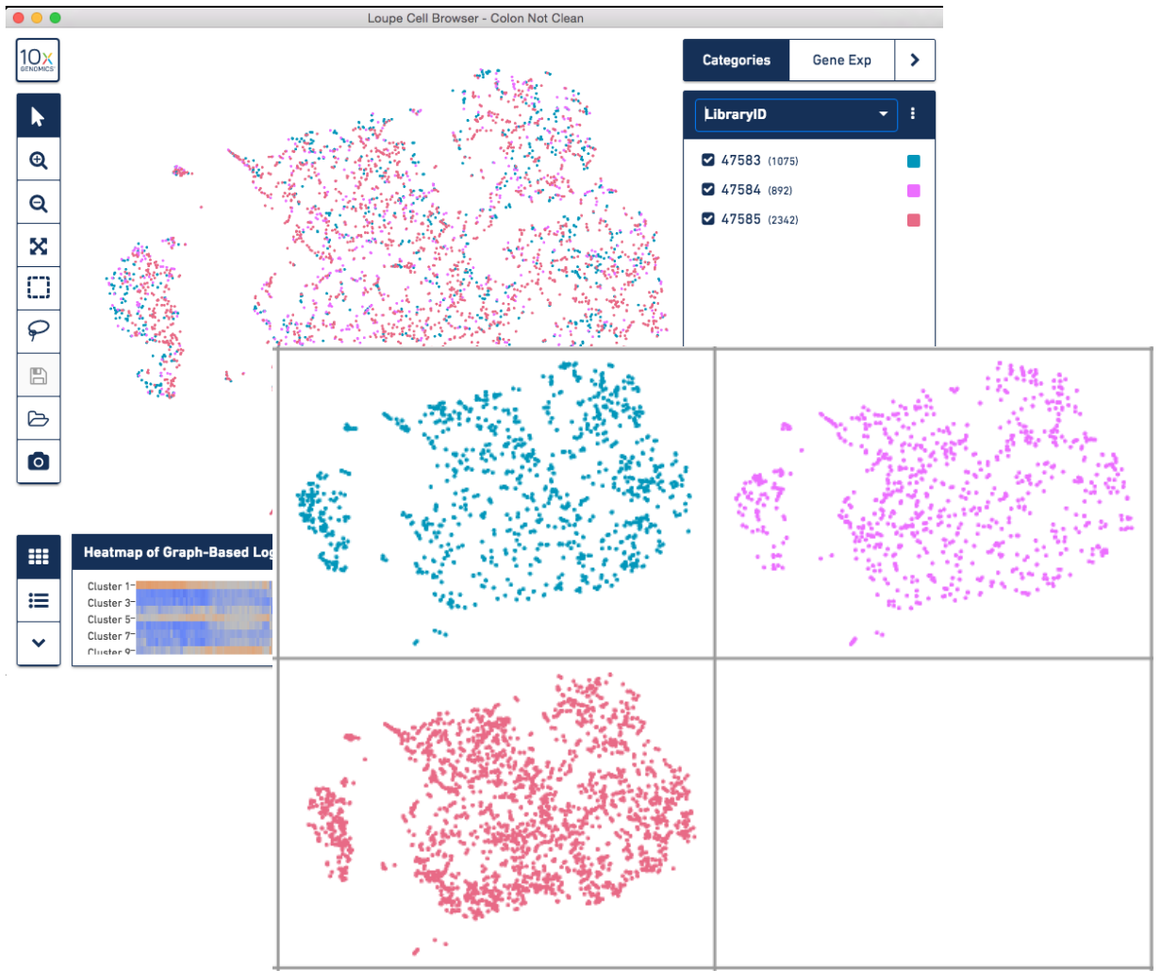
Plus RBC and Dead Cell Removal



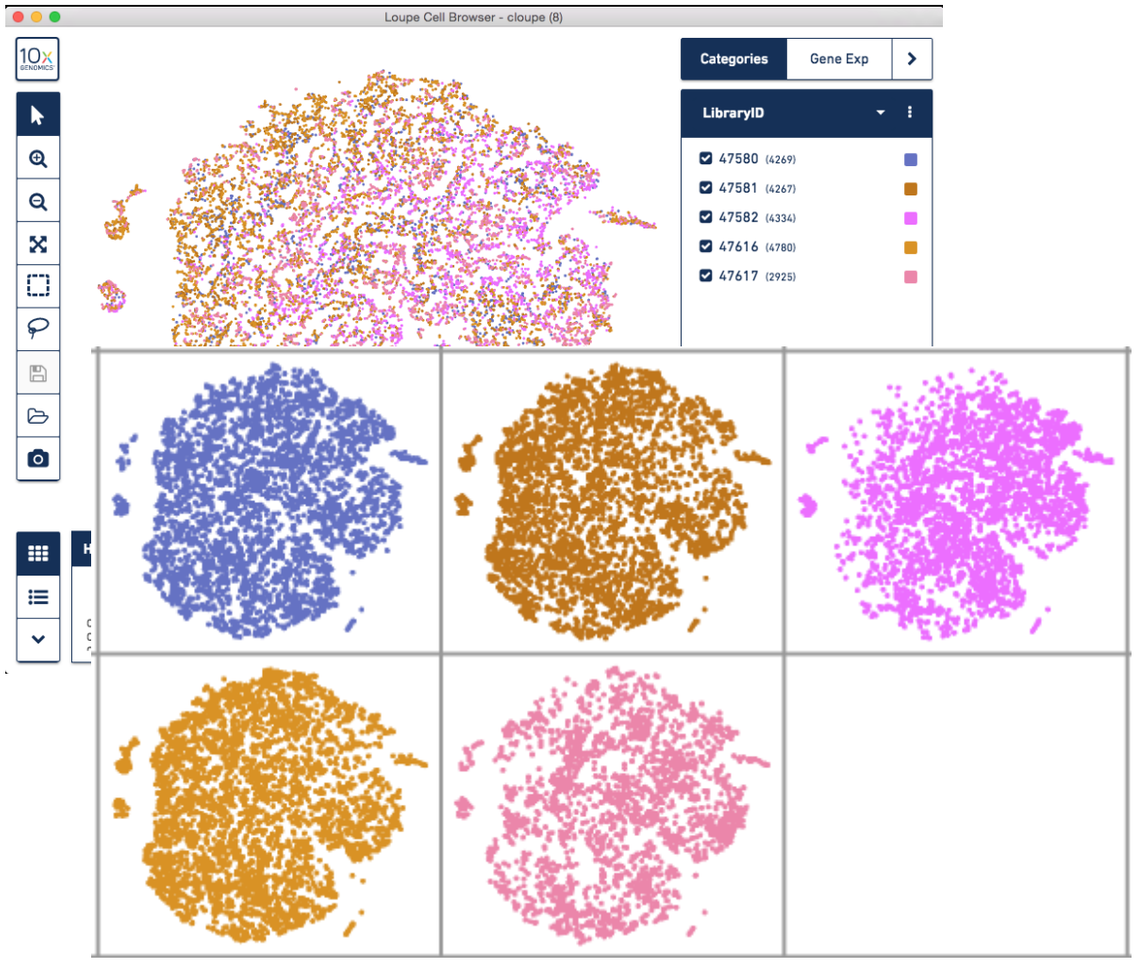
Loupe Cell Browser™ Software

Colon tumors – reproducible results

Filtered Only



Plus RBC and Dead Cell Removal

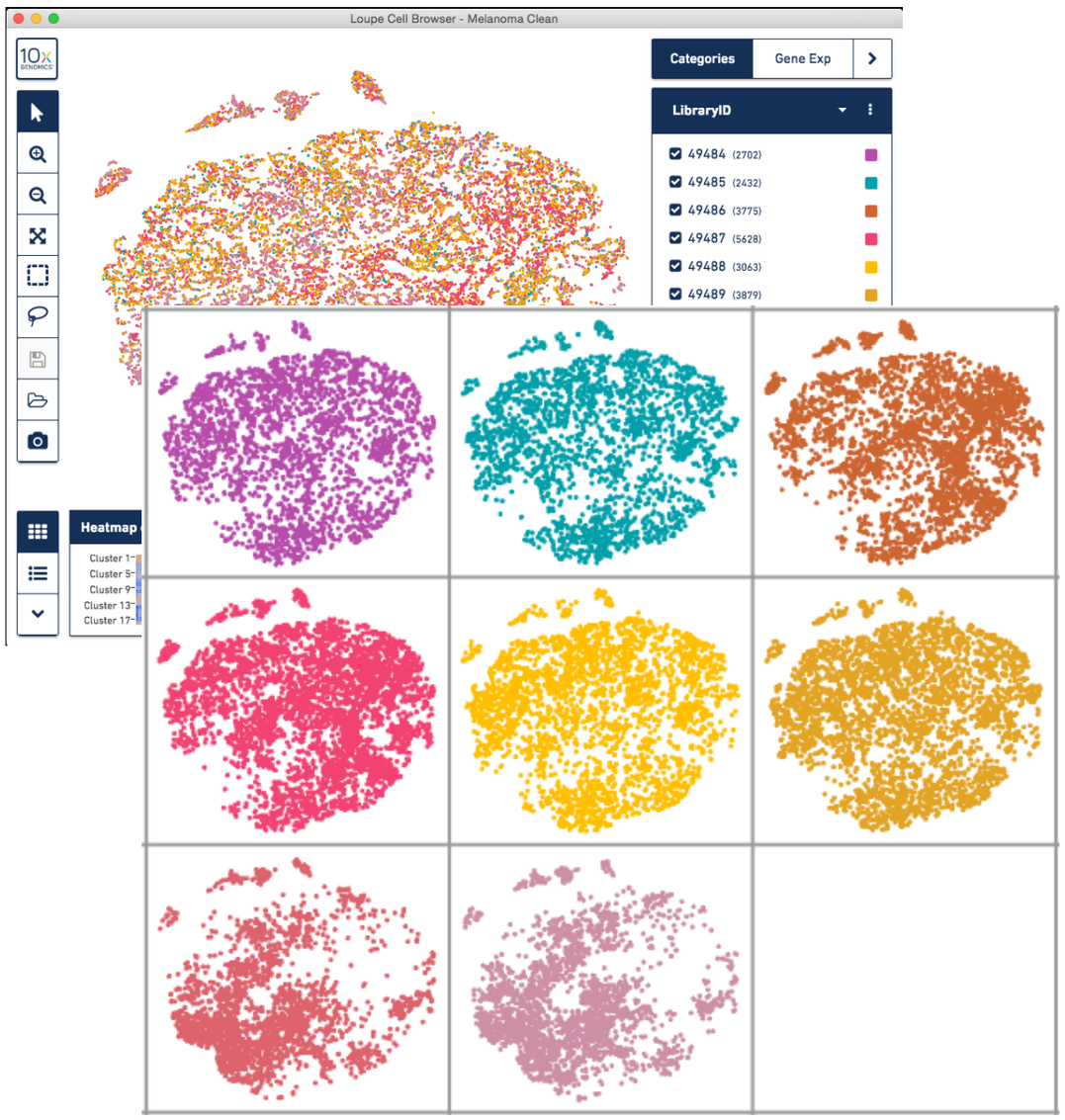


Melanoma – additional clean-ups increase the reproducibiliy of results

Filtered Only

poor reproducibility

Plus RBC and Dead Cell Removal

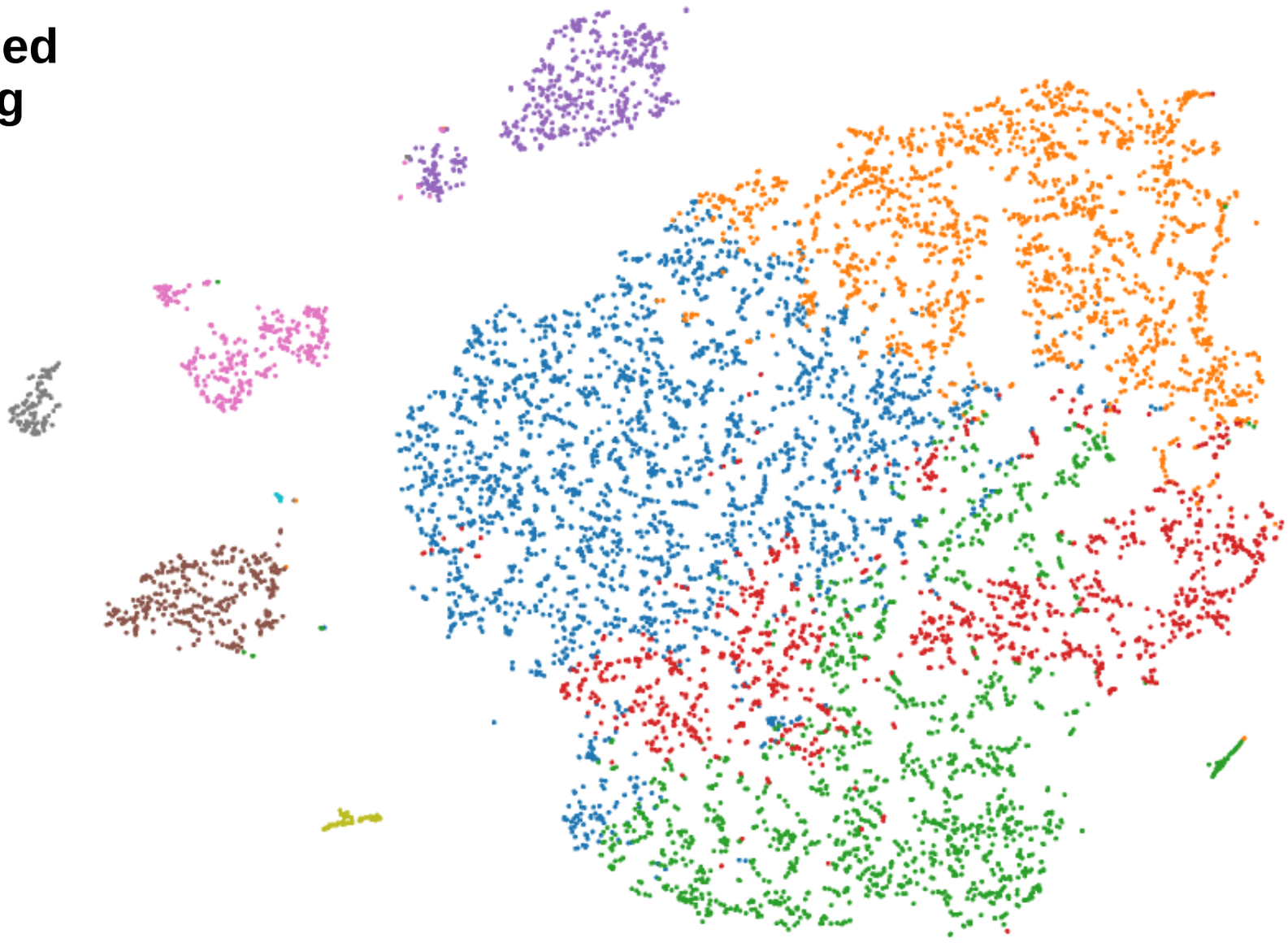


Tumor heterogenity

Tumor heterogenity – analysis of cell types

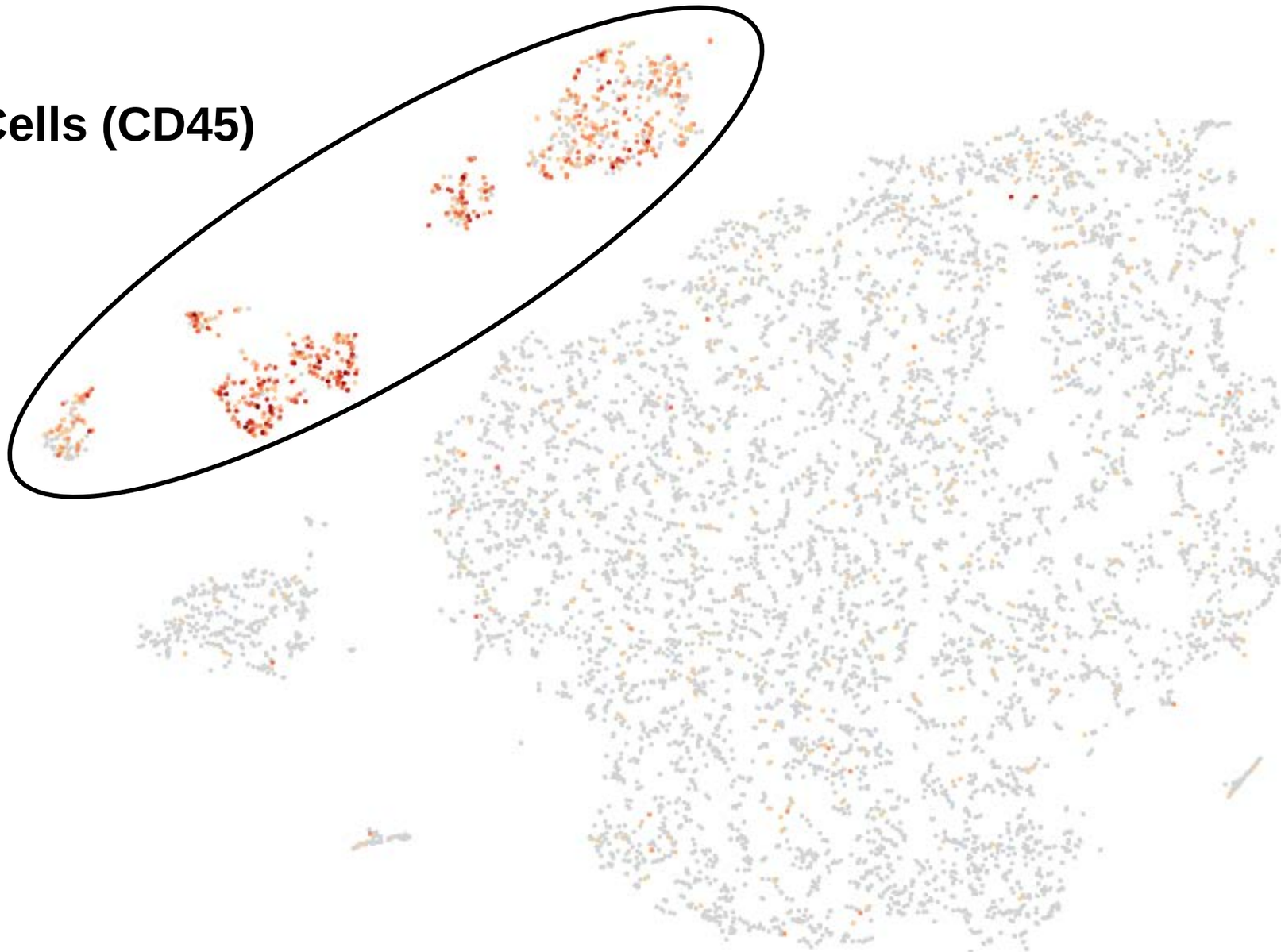
Breast Tumor: 6335 Cells

Graph-based clustering

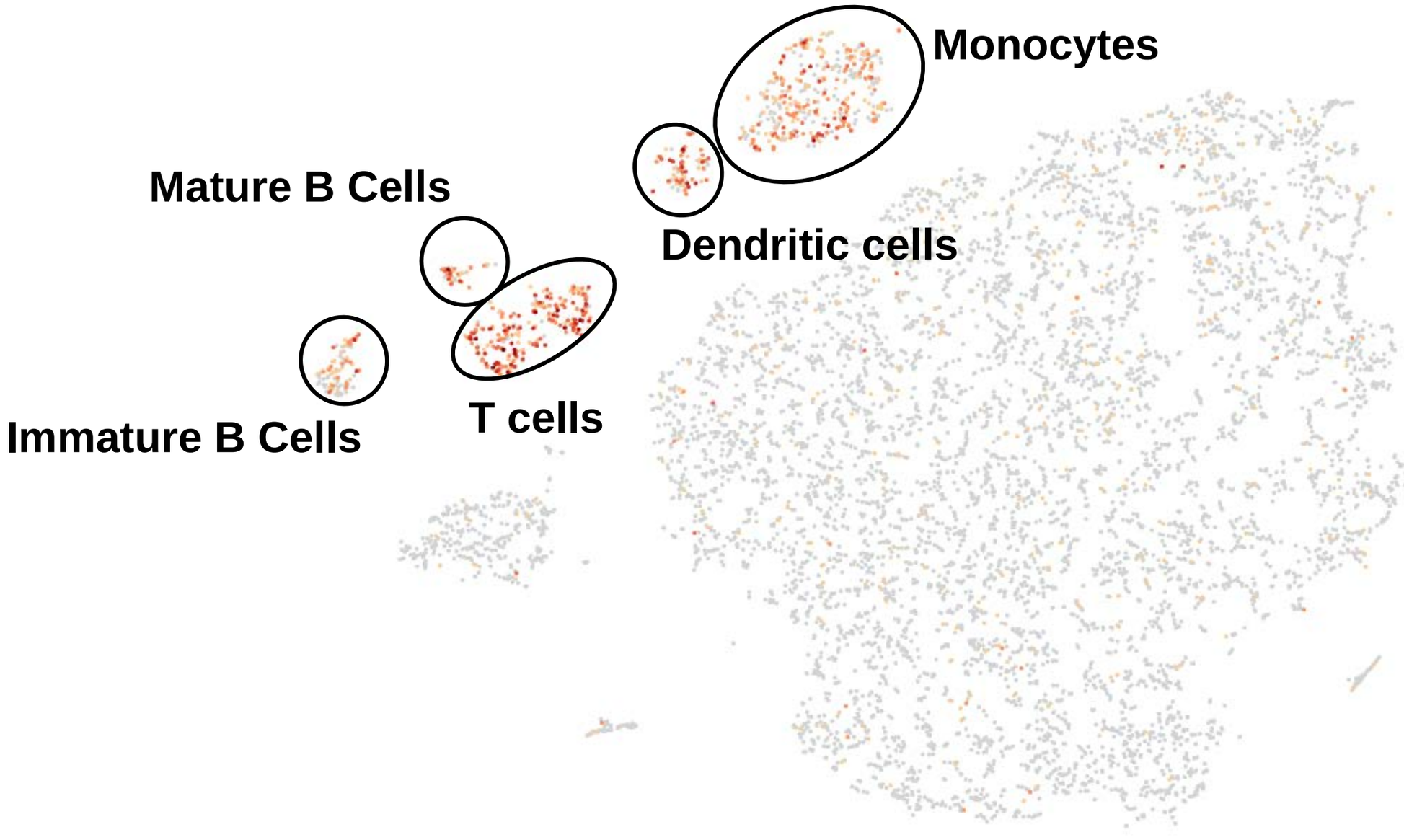


Tumor heterogenity – analysis of cell types

Immune Cells (CD45)



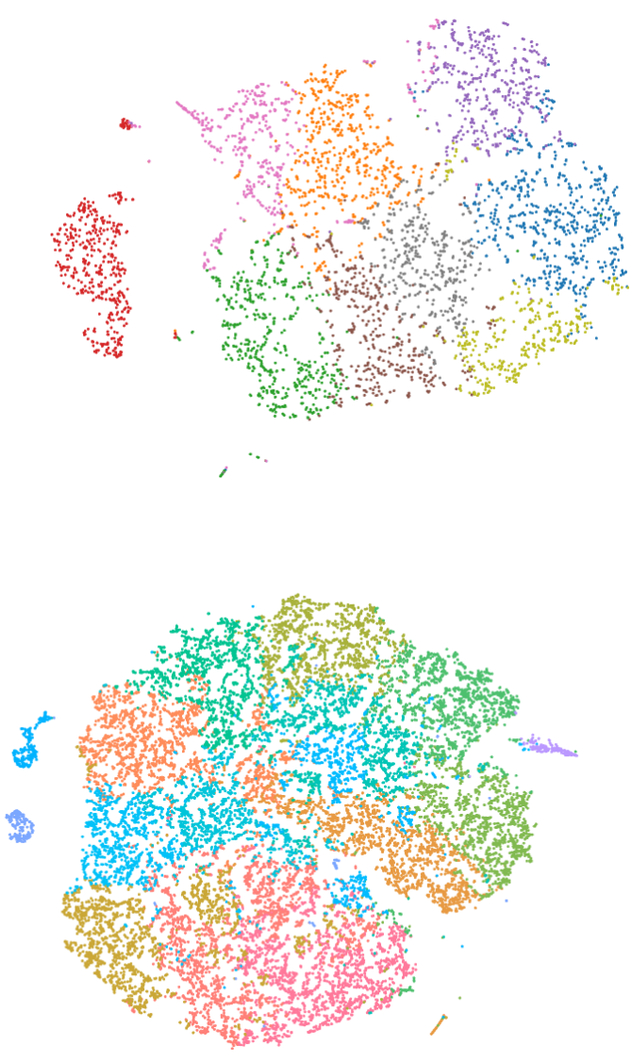
Tumor heterogenity – analysis of cell types



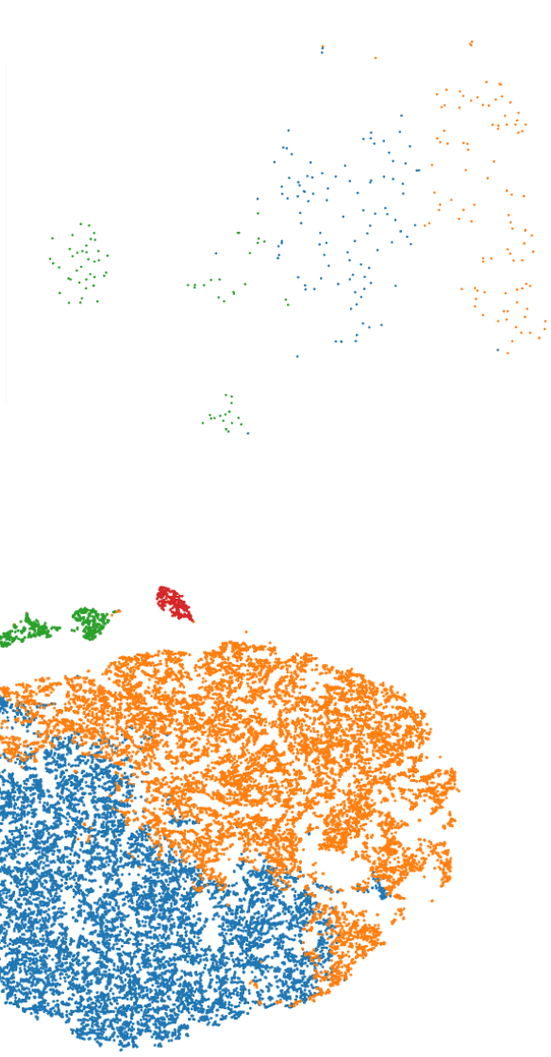
Tumor heterogenity – analysis of cell types

Breast

Colon



Melanoma



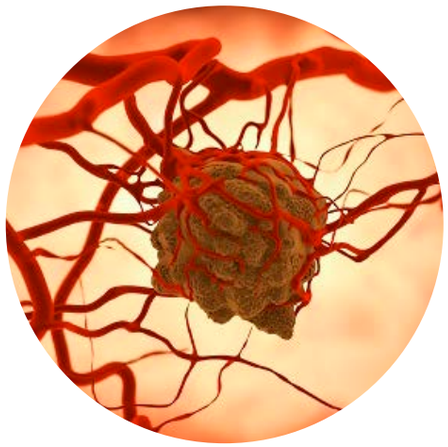
Filtered Only quality

Plus RBC and Dead Cell Removal

Summary of the results

Performence Parameter	Measurement	Filtered Only	With additionnal clean-ups: RBC Lysis & DCR
Library Complexity	Median genes per cell	2112	2967
Library Cleanliness	Fraction of reads in cells	79%	92%
Cell Recovered Melanoma & Colon	Estimated number of cells (Target=5000)	984	3691
Cell Recovered Breast	Estimated number of cells (Target=2000)	427	1264
Reproducibility	Loupe Cell Browser	2/3	3/3
Heterogeneity	Loupe Cell Browser	3/3	3/3

- Samples yielded good performance before(!) and after additional clean-up steps
- Cell Recovery is significantly improved with additional clean-up steps
- Optimization may be required to maximize % of non-tumor cells

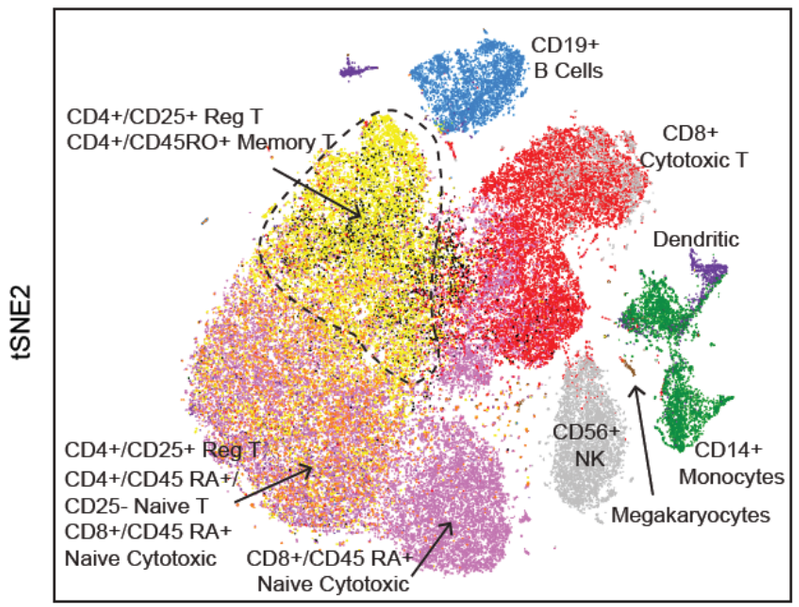


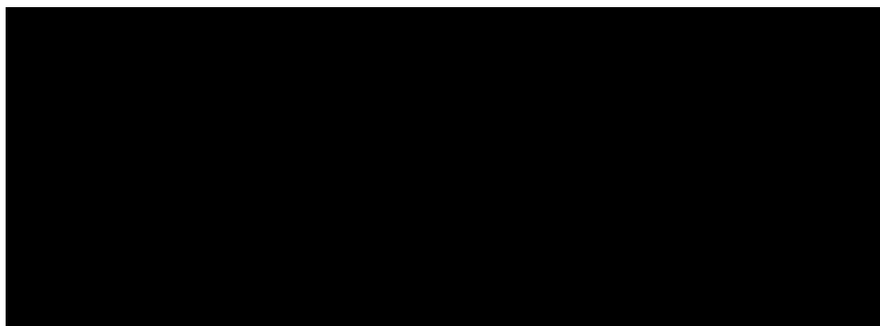
Tissue Storage

Sample Preparation

Cell Separation

Flow Cytometry





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