Switchable Antibodies for Increased Throughput in Iterative Immuno-Fluorescent Staining of Cancer Tissue Using ChipCytometry™

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Highlights

- Historically, spatial analysis of the tumor microenvironment has been limited by low plex methods (e.g., IHC).
- Iterative immuno-fluorescence staining is an emerging method for precise spatial multiplexing.
- Drawbacks of iterative methods are often slow throughput or harsh sample treatments for the inactivation/removal of fluorescence dyes.
- Our new switchable antibodies allow for the fast (5 min) deactivation of fluorescent signals on whole-slides under mild conditions.
- This allows for an increase in throughput while preserving the integrity of the sample.

Methods

- We present the analysis of human breast cancer carcinoma samples with an 8-plex protein panel (Tab. 1) using our new switchable antibodies on the ChipCytometry™ platform.
- Staining was performed in two cycles with 4 different antibodies each, followed by a DNA stain.
- A commercial antibody was included in the last cycle to show that our new switchable antibodies can be combined with off-the-shelf antibodies.
- After each cycle, the fluorescence signal of the switchable antibodies was removed by a short 5 min incubation with a fluorophore deactivation solution.

Tab. 1: Switchable Antibody Staining Plan

	Target	Clone	Dye	Type
Staining Cycle 1	CD45	HI30	Atto488	Switchable antibody
	CD3	UCHT1	Atto532	Switchable antibody
	CD45RA	HI100	Atto594	Switchable antibody
	CD326	9C4	Atto647N	Switchable antibody
Staining Cycle 2	CD4	RPA-.T4	Atto488	Switchable antibody
	panCK	C11	Atto532	Switchable antibody
	CD8a	RPA-T8	Atto594	Commercial antibody
	CD27	LG.3A10	Atto647N	Switchable antibody
DNA Stain	Draq5			

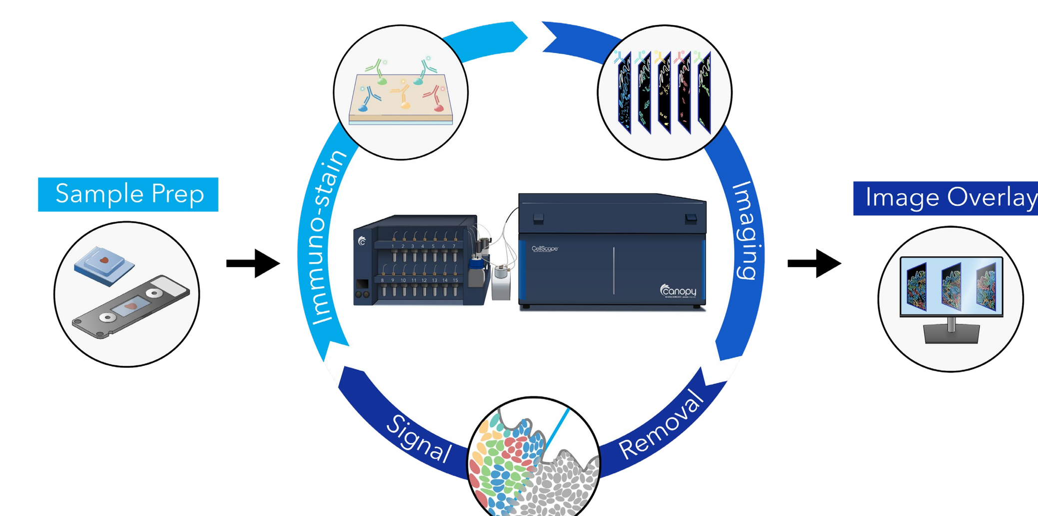

Figure 1: Step by step overview of sample preparation, image acquisition and data analysis.

Fluorophore Deactivation of Switchable Antibodies to Enable Fast Multiplexing

After the first staining cycle with four differently conjugated switchable antibodies (**Fig. 2A**), fluorescence signals of the antibodies were deactivated by incubation with a fluorophore deactivation solution (**Fig. 2B**). The fast and gentle fluorescence inactivation of our switchable antibodies showed to be effective in just 5 min for the whole slide. This allows for a higher throughput while preserving the integrity of the sample. Removal of the fluorescence stain allowed for the staining of the next four antibodies, bearing the same fluorophores as in staining cycle 1.

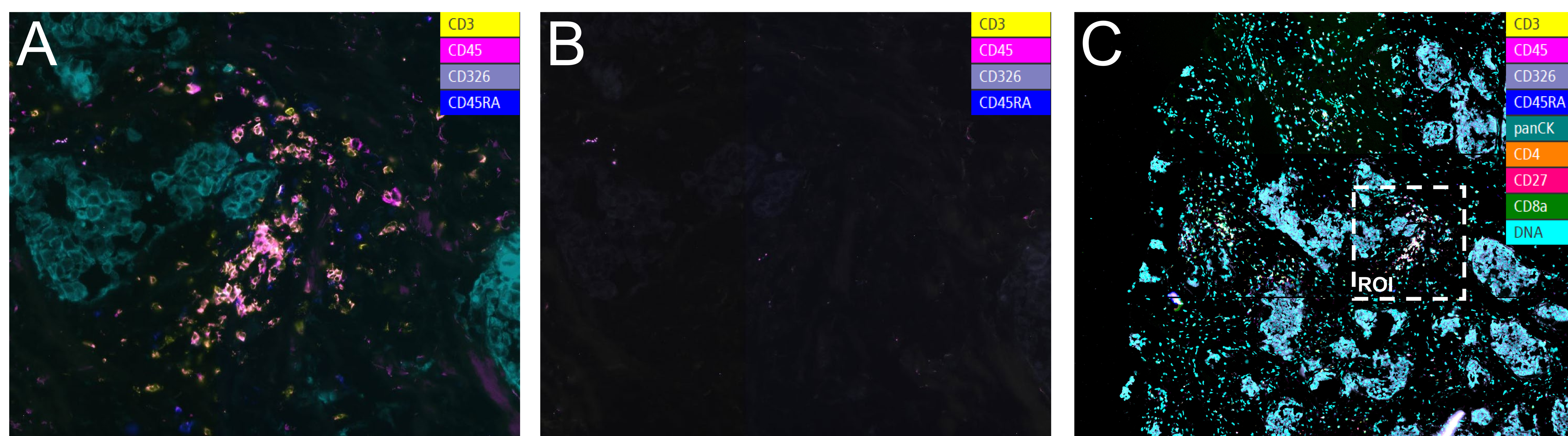

Fig 2: Overview image of a breast cancer carcinoma fresh-frozen tissue section stained with our switchable antibodies before and after incubation with a fluorophore deactivation solution. (A) Raw fluorescence picture of 9 FOVs (3x3) after the first staining cycle in **Table 1**. **(B)** Raw fluorescence picture of the same 9 FOVs after a 5 min incubation with the fluorophore deactivation solution. The specific stain could be completely removed. This allows for the staining with the next set of four antibodies in staining cycle 2. **(C)** Pseudo-colored composite images of the marker fluorescence signals for CD45, CD3, CD4, CD8a, CD27, CD45RA, CD326 (EpCAM), and panCK. The ROI is magnified in **Fig.3**. Note: Images **A** and **B** show the raw fluorescence images while **C** is background subtracted.

Using Switchable Antibodies to Detect T Cell Subtypes in Human Breast Cancer Carcinoma

The results show precise expression levels for each of the eight markers in the assay in each individual cell. Different subtypes of T cells could be detected as well as tumor cells. This shows that our switchable antibodies not only increase throughput but allow for a precise and specific detection of several markers on the ChipCytometry™ platform. Furthermore, staining with a commercial antibody (CD8a) and the DNA stain was not impaired.

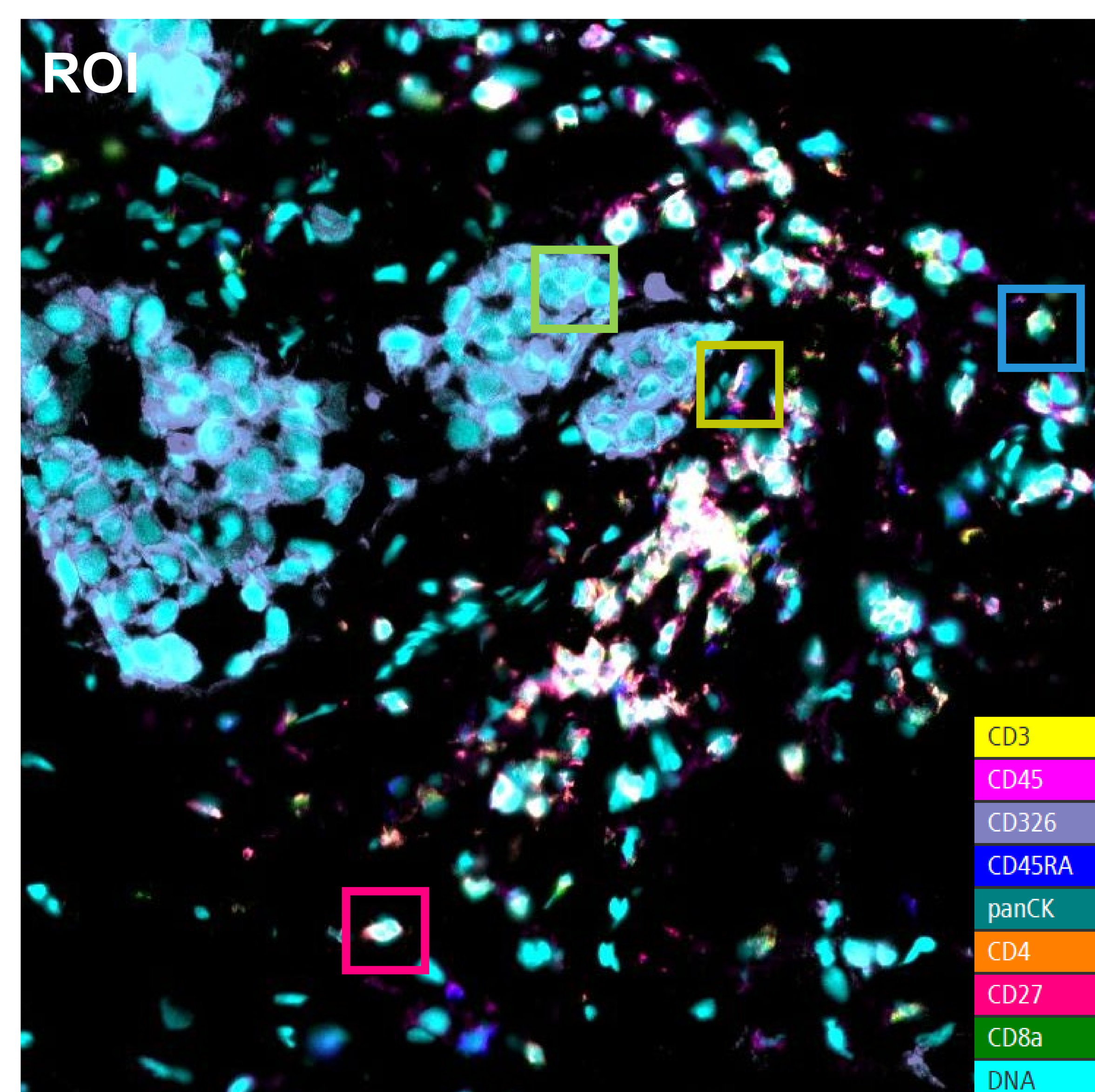

Fig 3. Zoomed in view of the ROI. CD3 illustrates T cell distribution. CD326 marks tumor cells.

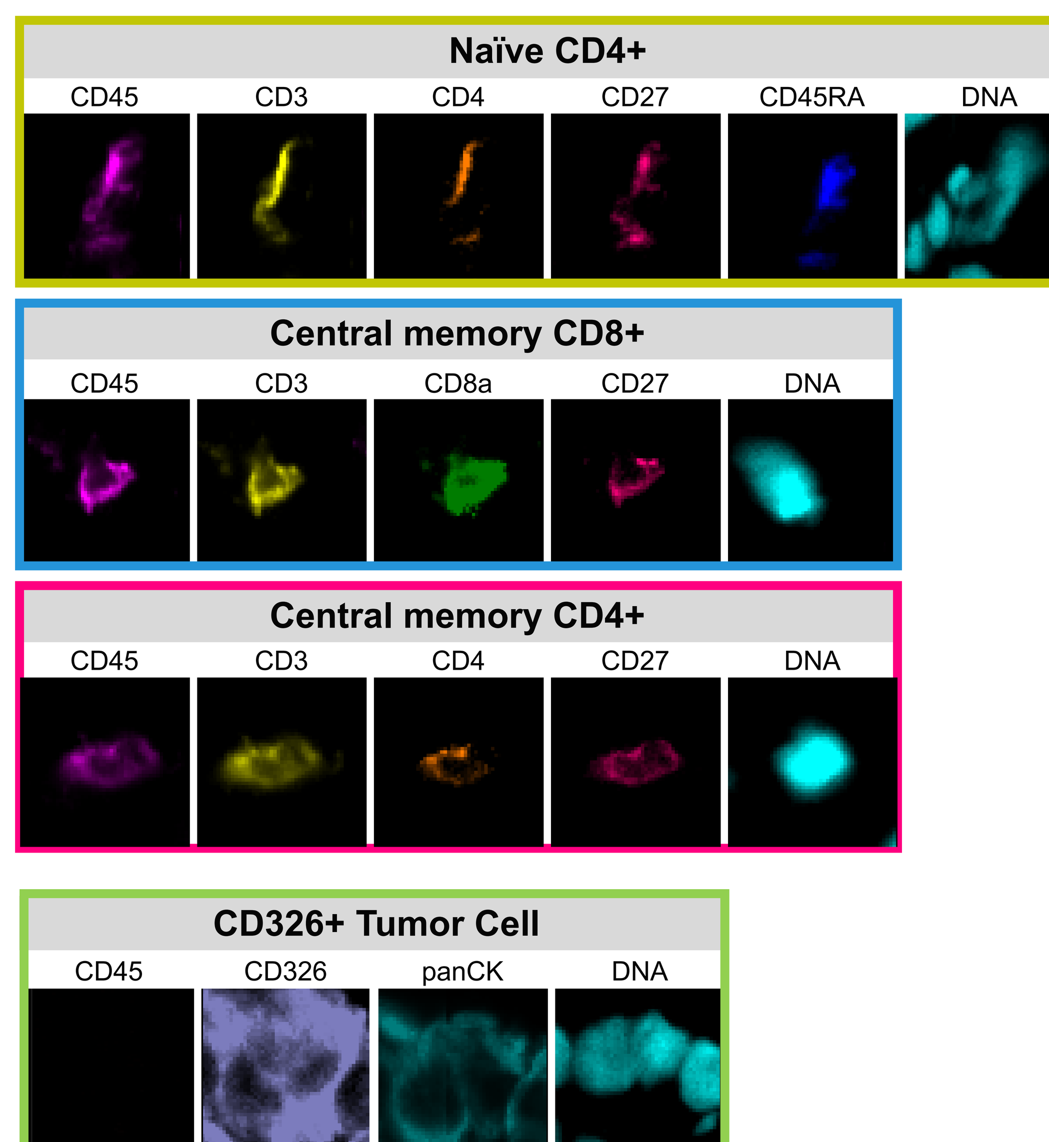

Fig. 4: Expression profile of individual cells from the ROI. These markers strips show the channels used to identify exemplar T cell subtypes including naïve CD4+, central memory CD4+ as well as central memory CD8+ cells.

Conclusions

- ChipCytometry™ in combination with our novel switchable antibodies allows for the simultaneous detection of multiple protein markers on a single tissue section for deep immune cell profiling with increased throughput in comparison to commercially available antibodies.
- Signal removal, which is essential for multiplexing, was fast (5 min) and gentle for our switchable antibodies.
- While switchable antibodies allow for an increased throughput in comparison to commercially available antibodies, a combination of both types of antibodies is possible and expands the capabilities of ChipCytometry™, giving researchers the highest flexibility in choosing their marker panel.

Acknowledgement

The development of switchable antibodies is supported in part by a grant from the BMBF (Photiomics KFZ 13N15767)

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Selected Publications

1. Jarosch, S. *et al*. Multiplexed imaging and automated signal quantification in formalin-fixed paraffin-embedded tissues by ChipCytometry. Cell Rep. Methods 1, 100104 (2021).
2. Jarosch, S. *et al.* ChipCytometry for multiplexed detection of protein and mRNA markers on human FFPE tissue samples. STAR Protoc. 3, 101374 (2022).
3. Hagel, J. P. *et al*. Defining T Cell Subsets in Human Tonsils Using ChipCytometry. J. Immunol. Baltim. Md 1950 206, 3073–3082 (2021).

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