# Antibody Validation for ChipCytometry™

## Highlights

- ChipCytometry™ is an all-in-one high-plex imaging platform for spatially resolved single-cell phenotyping
- ChipCytometry antibodies leverage decades of histopathology tradition, using clones validated for flow cytometry and immunohistochemistry (IHC)
- Commercially available, fluorescently conjugated antibodies are part of a flexible and practical solution for spatial multiplexing
- ChipCytometry antibodies are fully validated in both single-marker and combination assays for specificity, sensitivity, and reproducibility

## Background

Immunofluorescence techniques depend on thoroughly validated antibodies to resolve antigens in the tissue (Du et al., 2019). Although they are some of the most commonly used research tools, there are no universally accepted guidelines for antibody validation (Bordeaux et al., 2010). Furthermore, many commercial platforms use proprietary reagents or do not provide transparent information on reagent validation. ChipCytometry™ addresses these challenges by employing fluorescently conjugated antibodies from any vendor (Figure 1).

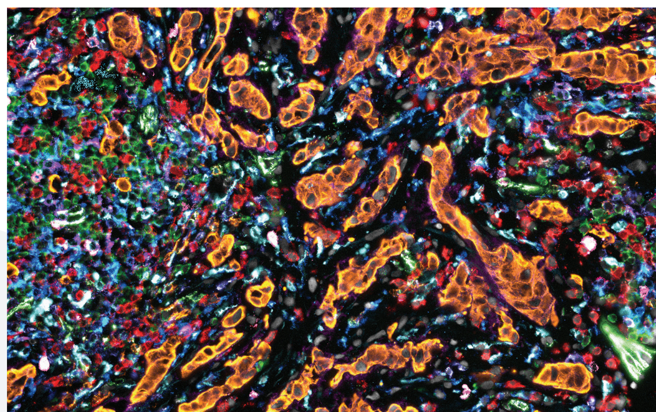

**Figure 1.** A human breast cancer FF tissue stained with 16 fluorescently conjugated antibodies in a ChipCytometry assay.

## Introduction to ChipCytometry

Deep profiling of the tissue microenvironment is essential for understanding the biology of disease and its progression. Investigation of therapeutically relevant cells in their native environment requires a spatially resolved high-plex imaging approach. ChipCytometry enables scientists to visualize and quantify cells in their native microenvironment. ChipCytometry is based on iterative immunofluorescence using the CellScape™ instrument, a fully integrated automated imaging system (Figure 2). High-resolution, high-dynamic range imaging achieves true single-cell resolution, enabling researchers to collect accurate data on a range of high- and low-expressing proteins.

**Figure 2.** CellScape is an all-in-one instrument for spatial multiplexing that combines iterative rounds of immunofluorescence staining with advanced image analysis software.

ChipCytometry enables spatially resolved multiplexing with features including:

- Ability to profile dozens of biomarkers
- Compatible with FFPE and fresh frozen tissue as well as cell suspensions
- HDR imaging for accurate quantification of high- and low-expressing proteins
- Single-cell quantification and spatial analysis with custom software
- Sample preservation for up to 2 years on proprietary ZellSafe™ chips
- Whole slide imaging or ROI selection

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## Types of ChipCytometry Antibodies

ChipCytometry offers the flexibility to mix and match pre-validated antibodies to create unique panels for immunology, oncology, and neurobiology applications. ChipCytometry antibodies have been fully validated for precise and consistent performance in cell suspensions and both FFPE and fresh frozen tissue sections. Antibodies are selected for their scientific application in relevant species, including human, non-human primate, and mouse samples (Figure 3).

**Figure 3.** ChipCytometry antibodies are for research use in: human, non-human primate, and mouse FFPE or fresh frozen tissue samples or cell suspensions.

ChipCytometry antibody validation is a multi-stage, iterative process (Figure 4) to evaluate antibodies for:

- **Specificity**: Antibodies bind their intended targets and do not demonstrate off-target effects
- **Sensitivity**: Antibodies detect low quantity antigens in order to differentiate positive cells from background fluorescence
- **Reproducibility**: Antibodies produce consistent results in similar or the same specimens over time

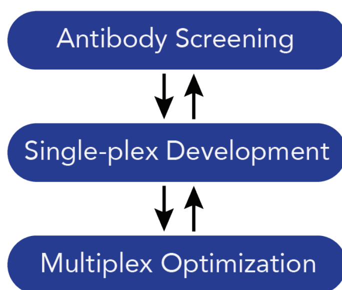


**Figure 4.** ChipCytometry antibodies are validated in a multi-stage and iterative process.

## Clone Testing

ChipCytometry antibodies leverage decades of histopathology experience, sourcing clones from a variety of vendors and previously validated for flow cytometry or IHC assays. New clones are selected based on the compatibility of conjugated fluorophore with ChipCytometry instrumentation. Up to three clones are tested in the first stain cycle in samples expected to show postitive staining. Figure 5 and Figure 6 show the results of clone screening for antibodies that target CD56. Each clone is evaluated to identify a strong and specific signal with minimal off-target staining.

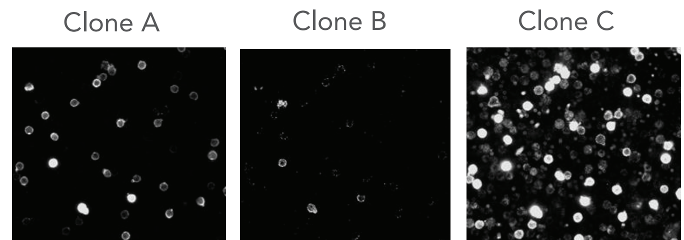


**Figure 5.** Human PBMCs stained with three different CD56 clones. Clone A passed validation tests for specificity, sensitivity, and reproducibility. Clone B failed due to weak, but specific, staining. Clone C failed due to nonspecific staining.

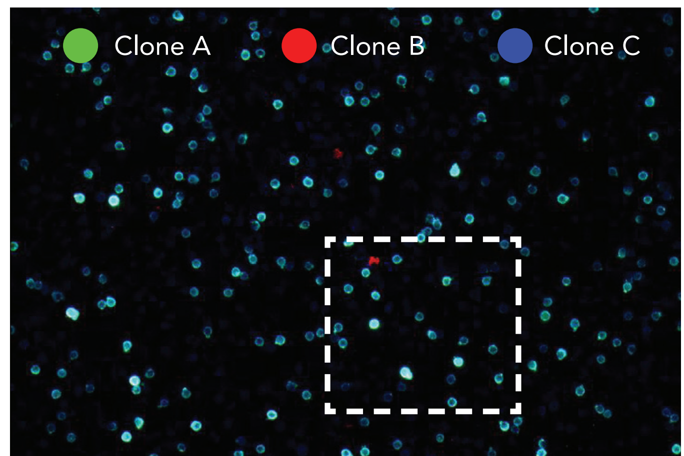


**Figure 6.** Image overlay of the three CD56 clones. Clone A (green) and Clone C (blue) both demonstrate sensitive and specific staining of NK cells, but Clone C additionally stains cells expected to be CD56 negative. Clone B gives nonspecific signal that neither Clone A or Clone C shows.

## Antibody Titration

Each antibody is titrated to select the correct concentration to optimally resolve cell populations. Too low of a dilution yields a weak signal, while too high of a dilution increases off-target binding effects and background fluorescence. Therefore, each antibody is titrated prior to experiment start to optimize the signal-to-noise ratio to ensure reliable analyses can be performed. Figure 7 and 8 show the results of titration experiments for Pan-CK, CD45RA, and CD8 antibodies.

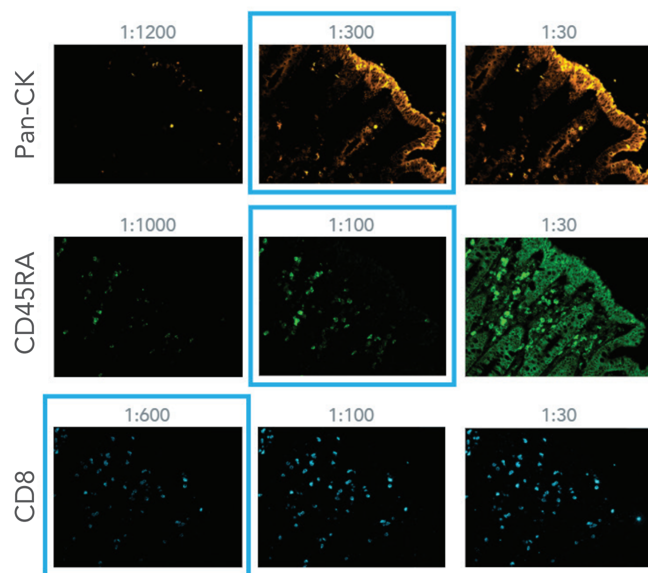


**Figure 7.** A human colorectal cancer FFPE tissue stained with Pan-CK, CD45RA, and CD8 antibodies at low, medium, and high concentrations.

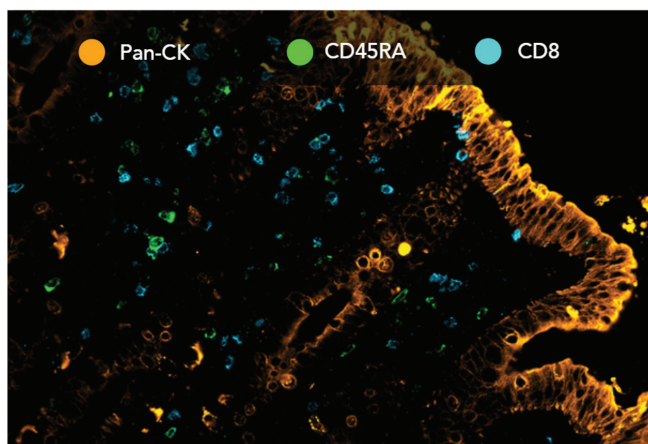


**Figure 8.** Composite image of optimal dilutions for Pan-CK (orange), CD45RA (green), and CD8 (cyan) antibodies.

## Localization Check

ChipCytometry antibodies stain their intended tissue structures (e.g., epithelial tissue, stromal region, lymphoid follicles) and localize to the expected subcellular region. Figure 9 shows examples of surface, intracellular, and nuclear markers in tissue samples and cell suspensions. Canopy scientists review current resources to verify that the staining pattern is expected in the specific cell and tissue types being tested.

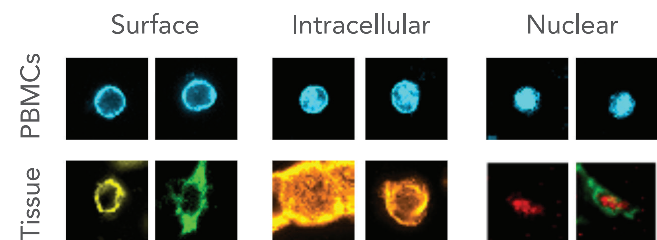


**Figure 9.** ChipCytometry antibodies are evaluated for expected surface, intracellular, or nuclear staining.

Table 1 shows some common surface, intracellular, and nuclear markers for ChipCytometry assays. Some ChipCytometry antibodies, including cluster of differentiation (CD) molecules, stain the cell surface, while other targets are found in the cell (e.g. CASP-3) or within the nucleus (e.g. FoxP3).

| Surface | | Intracellular |
|---|---|---|
| CD3 | PD-1 | CASP-3 |
| CD4 | PD-L1 | Granzyme B |
| CD8 | EpCAM | IL-10 |
| CD11 | Pan-CK | IL-12 |
| CD20 | SMA | IL-17A |
| CD56 | Vimentin | INFy |

| Nuclear | | |
|---|---|---|
| FoxP3 | p-Histone H3 | p-Stat1 |
| Ki-67 | T-bet | p-Stat2 |

**Table 1.** Common markers in ChipCytometry assays.

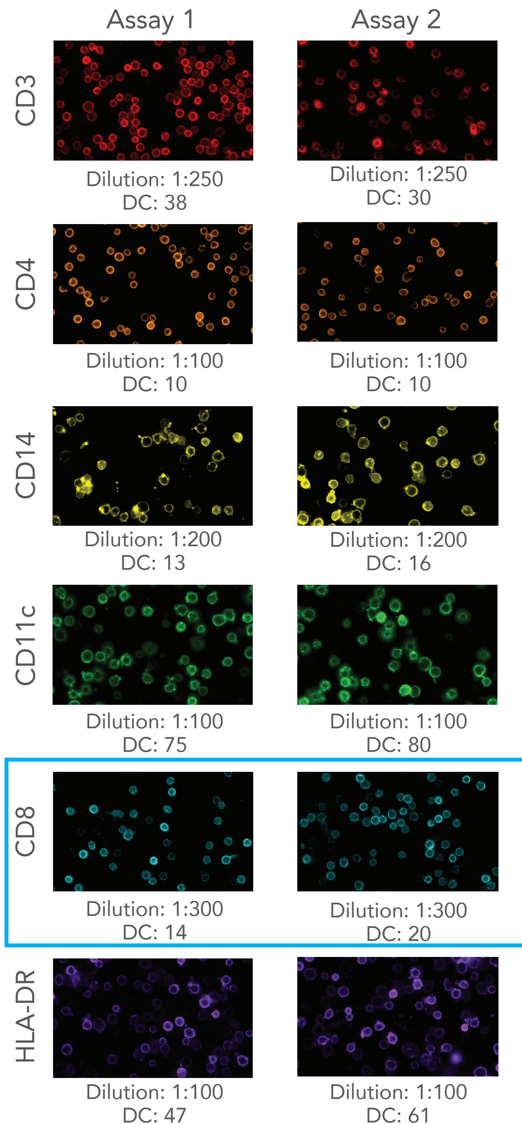


**Figure 10.** Antibody staining in PBMCs in early (Assay 1) or later cycles (Assay 2). DC is dynamic compression.

## Steric Hinderance Assessment

Two experiments, where the antibody was either added in an early cycle (Assay 1) or late cycle (Assay 2) were designed to assess steric hinderance and cross-reactivity. Qualitatively, signals were similar regardless of cycle number (Figure 10). Observations were confirmed quantitatively with hierarchical gating to identify positive cell populations. Figure 11 shows the results for a CD8 antibody with a high degree of concordance for positive cells, suggesting staining order has minimal effect on resolving cell populations.

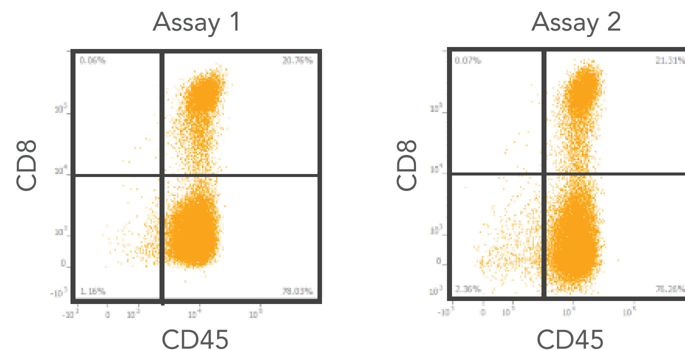


**Figure 11.** Quantitative results of CD8 antibody staining in early cycle (Assay 1) and later cycle (Assay 2). Each dot represents a single cell.

## Conclusion

Spatial biology requires highly validated reagents to accurately resolve proteins in the tissue microenvironment. ChipCytometry employs commercially available antibodies as part of a practical solution for spatial biology research. Here, we present how ChipCytometry antibodies are fully validated through specificity, sensitivity, and reproducibility tests for use in spatial multiplexing assays.

### References


1. Bordeaux et al. (2010). Antibody validation. BioTechniques, 48(3), 197–209.
2. Du et al. (2019). Qualifying antibodies for image-based immune profiling and multiplexed tissue imaging. Nature Protocols, 14(10), 2900–2930.


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