Ultivue

mIF vs IHC

Immunohistochemistry (IHC) has been the standard for research- and clinical-based tissue analysis for years.

And in the age of Immuno-Oncology (IO), IHC has been used as an effective biomarker screening tool. Over the last 20 years, though, an increasingly complex interplay between tumor cells and the tumor immune microenvironment has been revealed, indicating that single data points provided by IHC are not sufficient to provide the data needed not only for discovery but for patient stratification for targeted therapies and insight into clinical responses.

Multiplex immunofluorescence (mIF) builds on the foundations of IHC to provide unparalleled insight into the tumor microenvironment and overall effectiveness of targeted therapies, all while getting more data out of precious tumor samples. The information below compares IHC to mIF and demonstrates the impact that mIF can have on immunotherapeutic discovery.

Detection methods

While both IHC and mIF use antibodies to target individual proteins, IHC employs reporter enzymes that produce a chromogenic signal to indicate the presence of target proteins, while mIF uses fluorophores (figure 1, 2). Multiple fluorophores can be used to detect individual proteins, which enables the multiplex capabilities of mIF.

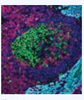

Figure 1
mIF

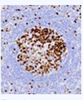

Figure 2
IHC using chromogenic reporter

targets per slide

The number of targets that can be detected simultaneously on a slide directly correlates with the number of different reporters that can be viewed simultaneously. IHC generally employs the commonly used chromagens, horseradish peroxidase (HRP) or alkaline phosphatase (AP) to produce a brown and red stain, respectively. This means that at most, IHC can detect two targets simultaneously. Fluorophores used for mIF instead exibit tight excitation and emission spectra that enable multiple fluorophores to be used simultaneously. For mIF, 5-8 targets can typically be detected simultaneously, although up to 50 or 60 can be possible with adaptations to the technology (figure 3, 4).

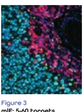

Figure 3
mIF: 5-60 targets

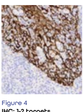

Figure 4
IHC: 1-2 targets

Dynamic range

Protein expression can change by up to 4 logs, and the ability to detect both high- and low-abundant proteins is critical for understanding protein expression and cell-cell interaction dynamics. The chromagens used for IHC do not conform to a linear relationship between the amount of chromagen and signal intensity and thus have a limited dynamic range of ~1 to 2 logs. Conversely, mIF technologies can exhibit linearity that provides a high dynamic range of ~5 logs for sensitive detection of proteins across a wide range of concentrations (figure 5, 6).

Figure 5
~5 logs

Figure 6
~1-2 logs

Capture area

The ability to analyze an entire slide means that no stone is left unturned during image analysis. While other commercially available mIF technologies must rely on analyzing just the regions of interest (ROI) rather than whole slide capture when working with more than 8 targets. With the use of Ultivue chemistry, you can enable whole slide capture of up to 16 targets (figure 7).

Figure 7

Spatial biology

The tumor microenvironment is complex, and only recently have we started to really understand the interaction between tumor, immune and stromal cells involved in cancer biology. mIF can be used to track individual cell populations and reveal their spatial relationship with other cell types, unlike IHC, due to its limited multiplexing and detection sensitivity (figure 8, 9).

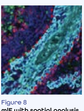

Figure 8
mIF with spatial analysis

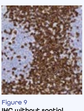

Figure 9
IHC without spatial analysis

Automatable

Both IHC and mIF rely on discrete processing steps, which makes both methods amenable to automation (figure 10).

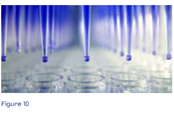

Figure 10

Digital image analysis

Both IHC and mIF are amenable to image analysis, although the characteristics of mIF outlined above make detailed image analysis more powerful for mIF than for IHC (figure 11).

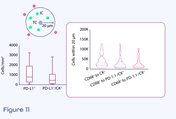

Figure 11

mIF provides unparalleled insight into the tumor microenvironment compared to IHC. Ultivue's mIF technology was designed with translational researchers and pathologists in mind to reveal actionable biological insights with low turnround-time for immuno-oncology applications.

Learn more at ultivue.com

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