BioTechniques
The International Journal of Life Science Methods

In Focus

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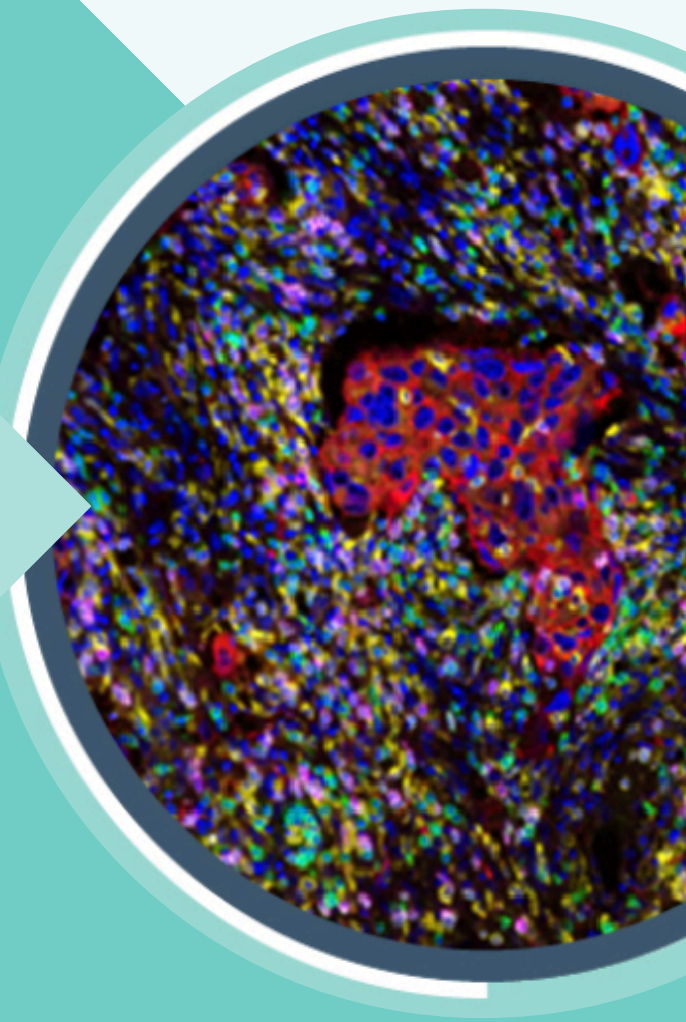

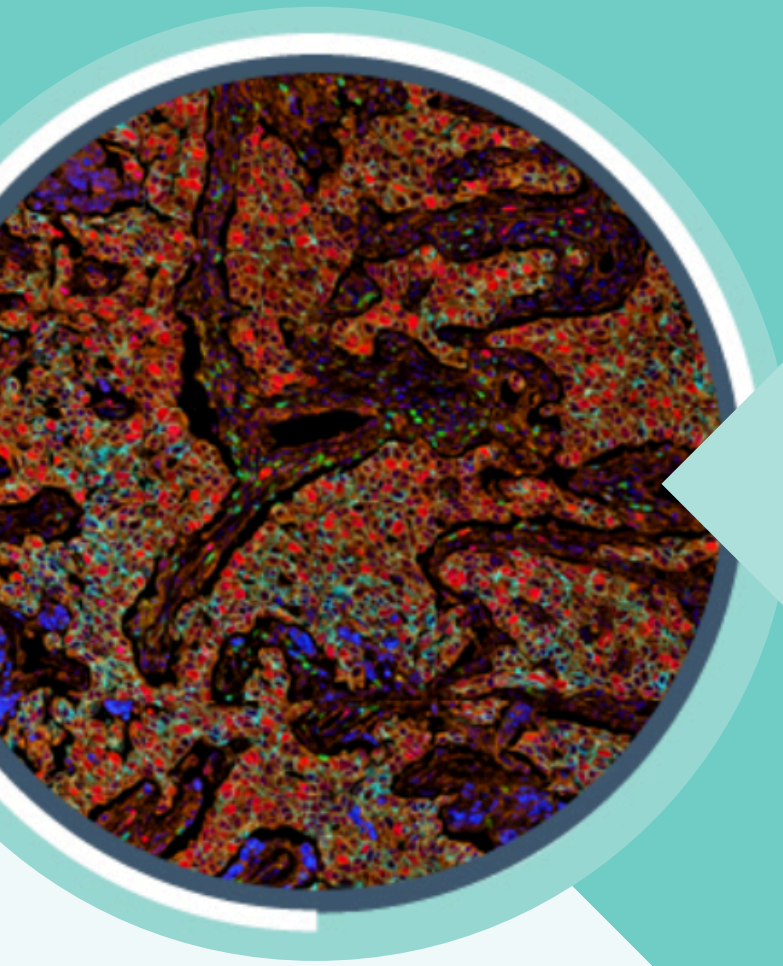

Multiplex immunofluorescence for cancer research

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Taylor & Francis

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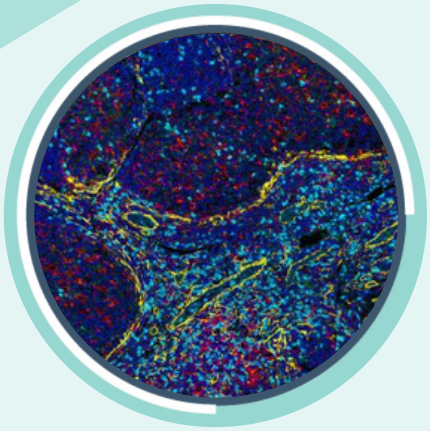

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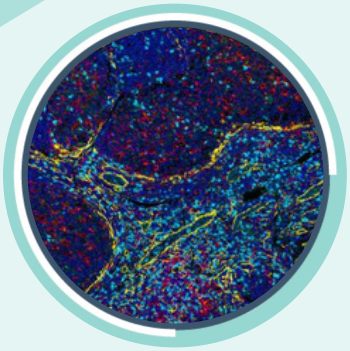
Introduction



As our attempts to develop more targeted and effective drugs continue, the need to better understand the protective and confounding effect of the tumor microenvironment grows. The study of such a complex environment, composed of many cell types with varying, often-corrupted, functions, requires tools that can examine and characterize numerous factors simultaneously and with spatial context.

By deploying technologies such as multiplex immunofluorescence effectively, we can design therapies that are more targeted to the tumor and its microenvironment, while remaining resistant to its protective immunosuppressive effects.

This In Focus will provide insight into tools and how they can be applied in the study of the tumor microenvironment.

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The video

Multiplex analysis of the tumor microenvironment

Introducing our In Focus, this video provides an introduction to the tumor microenvironment, the complex interplay of the cells within and how multiplex immunohistochemistry can be used to investigate this space.

Animation by James Harvie.

The podcast

Mitochondria, the immune system and cancer: discovering new insights with spatial technologies

In this episode, supported by Fortis Life Sciences, we delve into the relationship between mitochondria, inflammation and cancer, discussing the new techniques that are bearing fruit in this field, such as spatial analysis.

Our expert insight for this episode comes from Phillip West, Principal Investigator of the West lab at Texas A&M Medicine (TX, USA). Philip explains the role mitochondria can play in cancer and heart disease, reveals some of his most exciting discoveries of late and provides technical tips for investigating this field.

Listen on to discover how his use of spatial techniques has helped uncover mechanisms linking mitochondrial damage to the stifling of the immune system in the tumor microenvironment and the latest breakthroughs at the intersection of mitochondria and cancer.

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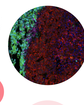
Multiplex immunofluorescence techniques explained

Multiplex immunohistochemistry-immunofluorescence (mIF) techniques enable the simultaneous detection of multiple proteins of interest in a single sample. This provides numerous benefits in the examination of different tissues, such as a tumor biopsy.



Top 5 benefits of mIF approaches:

1. Make the most of limited samples by visualizing multiple targets within a single tissue section.
2. Preserve tissue architecture. Provide contextual, spatial data regarding colocalization and orientation of cells and proteins.
3. Capture microenvironment data.
4. Enable relative quantitation of target through assessment of fluorescent signal intensity.
5.

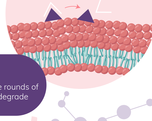
Key techniques

Tyramide signal amplification

Working principle:

Deparaffinize sample in xylene and hydrate with ethanol and distilled water.

Conduct heat-induced epitope retrieval to facilitate antibody binding.



Tip

mIF is optimized for formalin-fixed paraffin-embedded tissues. Multiple rounds of heat-induced epitope retrieval can degrade other sample types.

Incubate with IHC-validated primary antibody.

Wash and incubate with horseradish-peroxidase (HRP) conjugated secondary antibody.

Wash and apply fluorophore-conjugated tyramide.

When tyramide interacts with HRP it forms covalent bonds with the tyrosine residues in or near the target protein.

Tip

Fluorophore pairings should be carefully considered for targets in the same cell type and especially in the same subcellular location. In these cases, use fluorophores with spectra that don't overlap.

Repeat these steps up to 8 times for different protein targets.

Counterstain with DAPI.

Tip

Pair high-intensity fluorophores with antibodies targeted to a low-abundance protein.

Image the panel and analyze the results.

Tip

The order of staining and imaging is important! For certain antibody–antigen pairs, stain intensity can vary based on its position in the workflow. Optimize your workflow to accommodate antibody–antigen pairs that are impacted by this.

Key Benefits

Compatible with any immunohistochemistry-validated antibodies.

1. Can detect low-abundance proteins.
2.

Cyclic Immunofluorescence

Working principle:

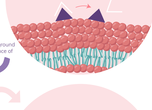
Deparaffinize sample with xylene and rehydrate with water.

Conduct heat-induced epitope retrieval to facilitate antibody binding.

Capture background autofluorescence of the sample

Stain with fluorophore-conjugated primary antibodies for first two targets.

Acquire immunofluorescence.



Inactivate the fluorophore with:

Stain with fluorophore-conjugated antibodies against two more targets.

Acquire new immunofluorescence.

Repeat for up to 60 targets.

Once you have completed your rounds of Cyclic IF, conduct Hematoxylin and Eosin staining to allow for a conventional histopathology examination.

Compile image stacks for analysis.

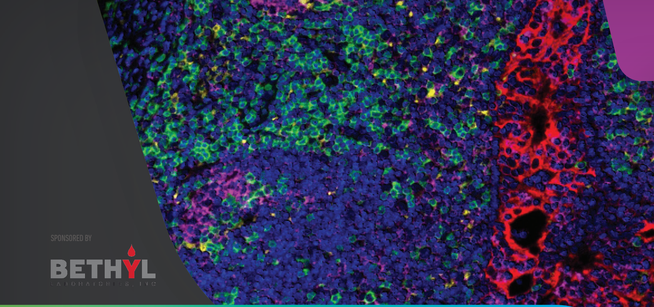
Tip

Include a nuclear stain in each round of imaging to use for the alignment of image stacks.

Key Benefits

Can identify up to 60 targets.

1. Uses the same fluorophore in multiple rounds of staining, minimizing the need to use of uncommon fluorophores.
2.



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Multiplex IHC: Making Discoveries Multicolor

Complexity of the Tumor Microenvironment

The tumor microenvironment (TME) is a complex mass of malignant and nonmalignant cells, signaling molecules, extracellular matrix, and blood vessels. Immunomodulation of the T-cell response within the TME, via inhibition of immune checkpoints and co-inhibitory molecules such as CTLA-4 and PD-1, is a promising cancer therapy. Multiplex immunohistochemistry (mIHC) enables the tracking of multiple markers within the TME, predicting therapeutic response and highlighting new therapeutic targets.

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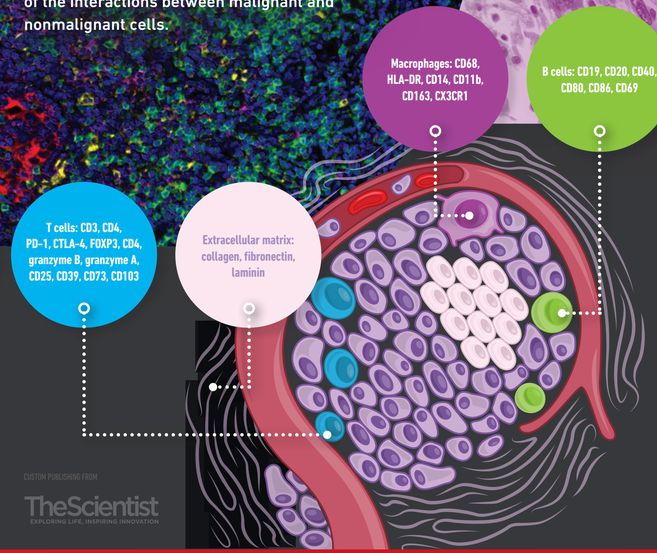
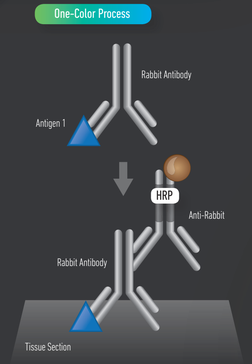
TME Expression Profiles

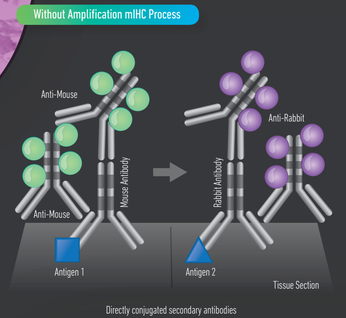
TME expression profiles guide understanding of the interactions between malignant and nonmalignant cells.



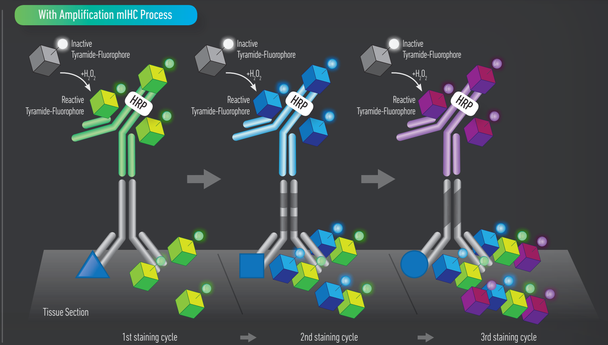
Traditional One-Color IHC | Traditional mIHC

Traditional, one-color IHC cannot differentiate between multiple cell types and targets in tumors. To immunostain more than two targets, serial sections or serial staining procedures must be used.





Tyramide Signal Amplification mIHC | SEEING LIFE IN MULTICOLOR



mIHC allows multiple targets to be labeled. Traditional mIHC uses primary antibodies from different species. Tyramide signal amplification mIHC uses multiple rounds of staining and antibody removal.

Multiplex IHC for Immuno-oncology: Seeing Life in Multicolor

Sequential staining of different antigens with:

- Primary antibody
- HRP-conjugated secondary antibody
- Tyramide-conjugated fluorochromes

Tyramide labels adjacent to the antigens and binds covalently.

Primary and secondary antibodies are removed by microwaving after each round of staining.

ADVANTAGES OF MULTIPLEX IHC

1. Multiplex IHC allows for visualization of multiple targets within a single tissue section, critical for limited samples.

2. Tissue architecture is preserved allowing for observation of spatial information and co-expression within the TME, unlike alternative multiplex approaches such as NGS, PCR, mass spectrometry, etc.

3. Fluorophore detection systems offer major advantages over chromogenic detection:

- Fluorophores have a wider dynamic range and larger linear range than chromogenic substrates, tyramide-based multiplexing enhances fluorescence signal enabling detection of low-level binding sites.
- DAPI (DNA/nuclear counterstain) is superior to hematoxylin, which can be obscured by other targets with chromogenic staining.
- Fluorescence signals can be overlaid and seen as single or multi-channel, allowing for intensity measurements for each target.

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The webinar

An end-to-end workflow for exploring spatial biology through multiplex IHC

Analysis methods enhance our understanding of spatial biology, providing deeper insights into cellular interactions and tissue architecture.

In this webinar, we explore the intricacies of multiplexed immunohistochemistry (IHC), a cutting-edge technique that allows for the simultaneous detection of multiple biomarkers in a single tissue section. We will also focus on how spatial analysis methods enhance our understanding of spatial biology, providing deeper insights into cellular interactions and tissue architecture. First, we will review an open-source workflow for pathologist-in-the-loop segmentation and classification. Then, we will demonstrate the practical applications of this approach across several peer-reviewed publications.

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