

APPLICATION CARD

Nikon BioImaging Lab

– Europe –



Hoechst, B2220, F4/80, CD8a, GR1, CD4, CD169

Mouse spleen



Deeper Spatial Insight from Tissue Sections Through Multiplex Staining and Imaging

Multiplex staining and imaging maximize insight from scarce tissue by extracting more data from each section, enabling richer spatial phenotyping for translational and drug-development programs. However, multiplexing is often technically challenging, time-consuming, and dependent on specialized imaging hardware for optimal spectral separation. Utilizing *Kromnigon's* multiplex chemistry and filter technology, NBIL offers a streamlined solution for fast, biologically meaningful visualization of multiple proteins while preserving precious epitopes.

CHALLENGES

Scaling multiplex panels beyond 3–4 antibodies is often slow and technically challenging.

Conventional methods rely on aggressive antibody-stripping cycles that can compromise tissue morphology, deplete precious epitopes, and reduce sensitivity for low-abundance targets. Spectral overlap and autofluorescence further limit usable channels, leading to lengthy panel optimization and restricting routine use in translational and drug-development pipelines.

Our solution eliminates antibody stripping by enabling the simultaneous staining of up to seven antibodies in a single tissue section, preserving epitope integrity while accelerating multiplex spatial analysis.

APPLICATIONS

- ✓ Multiplex 7-marker staining, imaging, and spatial phenotyping on delicate tissues
- ✓ Scalable, as-a-service multiplex workflows that minimize panel development time
- ✓ Integration with spectral imaging for robust unmixing and quantitative downstream analysis

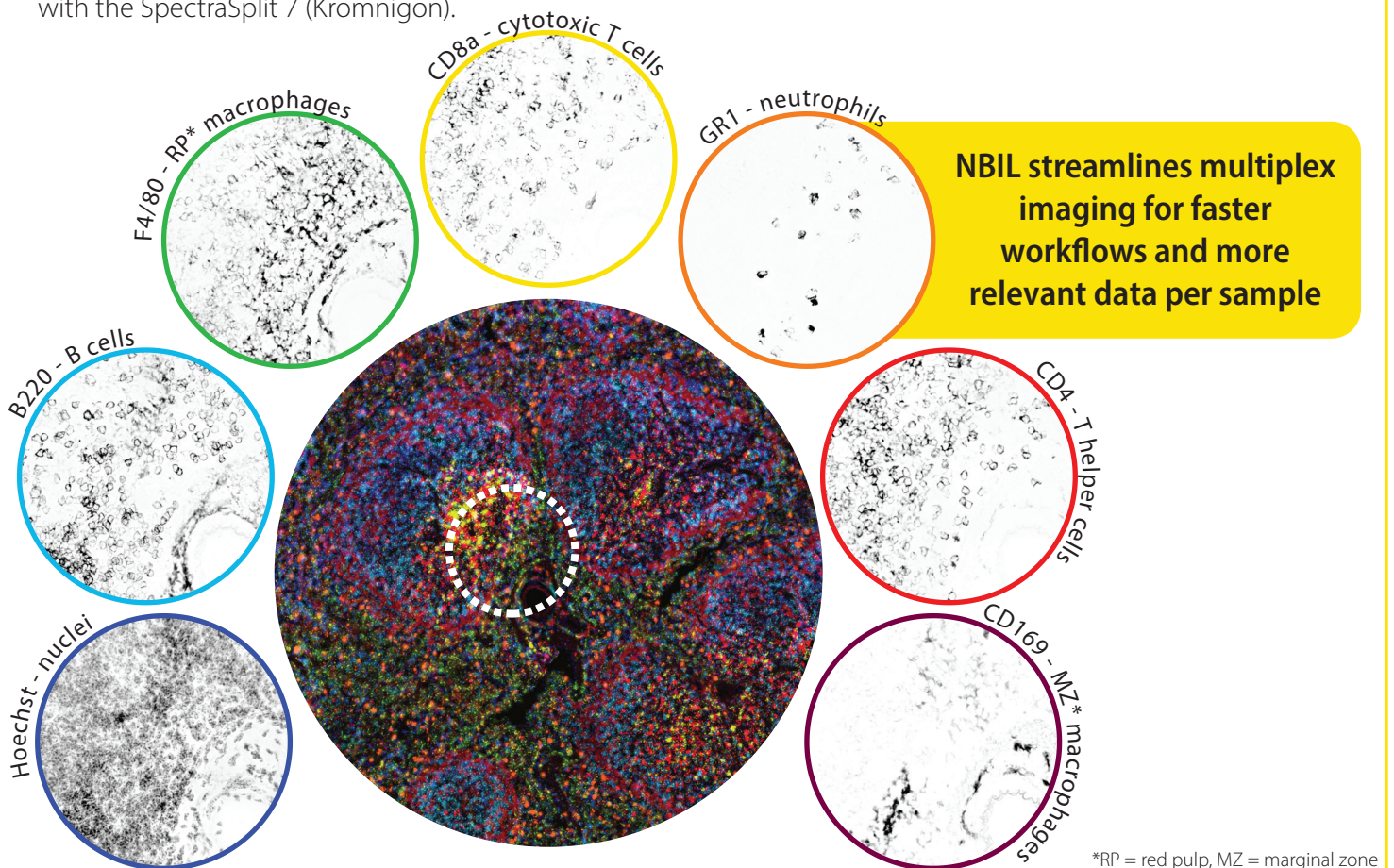
Talk to an expert

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Multiplex Staining and Imaging of Tissue Sections

In this assay, mouse spleens were snap-frozen, cryosectioned, and fixed in cold acetone. Staining was performed using the SpectraClick-Multiplex Immunofluorescence kit (Kromnigon). Sequential staining cycles targeted splenic cell markers (CD4, CD8a, CD169, B220, GR1, and F4/80) plus a Hoechst nuclear counterstain. Each cycle used a biotinylated primary antibody bound to StreptaClick HRP (monovalent streptavidin-HRP), where HRP activates tyramide-conjugated fluorophores in a Tyramide Signal Amplification (TSA) reaction, leading to fluorophore deposition on and around the antibody. Sections were imaged on a Nikon Ti2 microscope equipped with the SpectraSplit 7 (Kromnigon).



OUR APPROACH

We combine a gentle, enzyme-amplified staining chemistry with *Kromnigon's* patented filter technology to deliver high-content, tissue-preserving multiplexing.

- ✓ **Fast, reliable results from scarce samples:** a gentle, strip-free chemistry preserves tissue and epitopes, reducing repeat stains and panel optimization to extract more data per section with shorter turnaround.
- ✓ **Single-sample, multi-parameter readout:** the 7-plex panel provides richer biological insight into protein interactions and spatial organization within a single tissue section.
- ✓ **Integrated spectral imaging:** Nikon ECLIPSE Ti2 with Kromnigon SpectraSplit 7 enables faster plate throughput and richer high-content data per tissue, without added assay complexity, so imaging can scale with demand.

Altogether, this workflow delivers sensitive, reproducible 7-marker spatial phenotyping from precious samples, unlocking deeper biological insight and enabling more scientific questions to be answered from a single tissue section.

Nikon BioImaging Lab's services:

01 Assay development > 02 Imaging > 03 Analysis > 04 Reporting