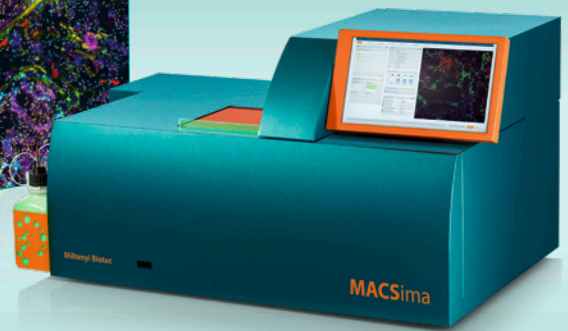
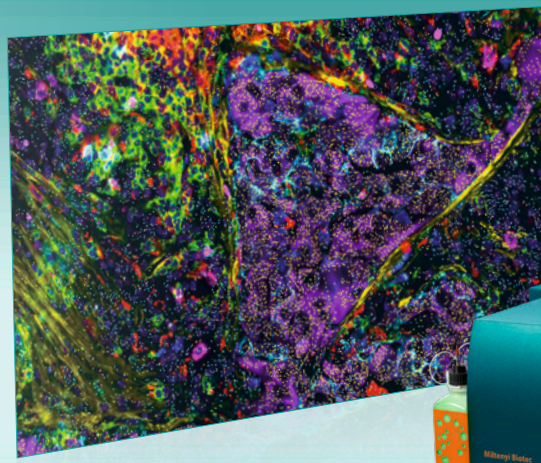




Miltenyi Biotec



From tissue sample to publication-ready results in record time

MACSima™ Platform's streamlined same-section multiomics workflow for spatial biology

No barriers, no limits

The MACSima Platform empowers researchers to explore the intricate world of spatial biology with a workflow solution, that guides researchers from sample preparation to data analysis. The MACSima Platform not only streamlines high-plex proteomics analysis but also enables the seamless integration of RNA detection, all conducted on a single tissue section.

The MACSima Platform integrates all crucial components into a single-source solution (Fig. 1). This eliminates the need to navigate multiple vendors and software packages, saving researchers valuable time and effort.

The workflow unfolds in four key steps:

1. Sample preparation
2. Defining an antibody and RNA panel
3. Fully automated staining and imaging of RNA targets and protein markers, ranging from 20 to hundreds.
4. Spatial data analysis

Each step in the workflow is thoroughly designed to expedite research, streamlining complex tasks and propelling you towards publication-ready data with effortless ease, all while maintaining flexibility to pursue your unique research vision.

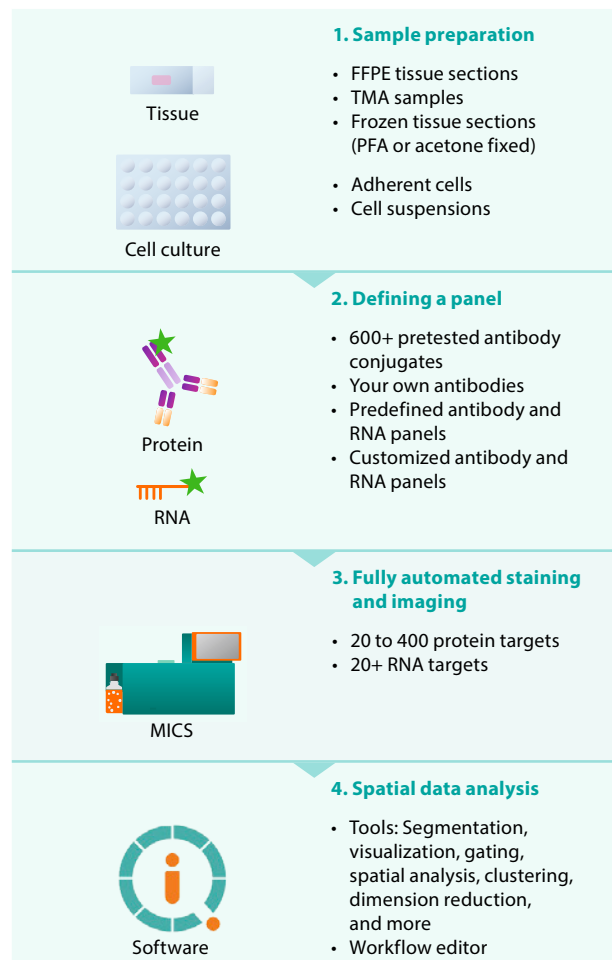


Figure 1: Overview of the MACSima Platform's same-section multiomics workflow and its components

Background

What is Spatial Biology?

Spatial biology is a rapidly developing field with the goal of understanding biological processes within their spatial context. It takes a holistic approach by looking at how molecules like proteins or RNA are organized and interact within tissues. This provides deep insights into complex disease mechanisms, drug responses, and the development of personalized medicine. To efficiently navigate the intricate world of spatial biology, researchers need strategies and technologies that streamline their workload while ensuring the integrity of their data.

The power of spatial proteomics

Recent years have seen a breakthrough in spatial biology, merging classic methods like immunohistochemistry with high-throughput techniques like transcriptomics and proteomics. While RNA analysis is well-established, high-plex proteomics is emerging quickly. This is exciting, as proteins drive biological processes and offer tissue architecture insights missing in RNA data. Examples include:

- Understanding how cancer cells interact with their surrounding environment.
- Studying how the immune system functions in different tissues.
- Developing new drugs that target specific cell types and interactions.

High-plex proteomics meets RNA detection

Until recently, spatial biology relied on single-omics approaches, like transcriptomics or high-plex proteomics. New platforms allow researchers to combine omics data, like high-plex transcriptomics with complementary protein information or high-plex proteomics with complementary RNA information. These advancements have the potential to revolutionize both scientific research and clinical practice.

Stepping through the MACSima Platform's workflow

1. Sample Preparation

The MACSima Platform allows researchers to analyze several sample formats. Miltenyi Biotec provides protocols for preparing FFPE (formalin-fixed, paraffin-embedded) or frozen tissue sections, as well as cell culture samples. Additionally, they offer a protocol for RNA sample preparation to achieve same-section multiomics. However, you can still customize these protocols based on your specific experimental needs.

1.1 Tissue samples

The platform accepts a variety of tissue sample formats, including FFPE tissue sections, tissue microarray (TMA) samples, frozen tissue sections (PFA or acetone fixed). These can be presented on standard slides, or for high-resolution imaging, MACSwell™ HighRes Slides with their 170 µm thin glass window. Specifically designed MACSwell Imaging Frames (MACSwell One, Two, Four, or One Small Imaging Frames) can be easily assembled around these microscope slides, providing the necessary reaction cavity for the antibody solution.

1.2 Cell culture

For adherent cell culture, simply utilize preassembled MACSwell 24 Imaging Plates. For single suspension cells, opt for the MACSwell 24 Micro Imaging Plates, featuring hundreds of thousands of hexagonal microcavities, each housing a single cell. Just pipette your cells and insert the plates into the MACSima System.

2. Defining a panel

Users of the MACSima Platform benefit from a wide range of panel design options. The MACSima Software provides convenient ways to create, manage and run sophisticated panels and experiments.

2.1 For protein analysis

Single antibodies: Choose from over 600 pretested recombinant antibodies for either FFPE or frozen (human or mouse) samples. For a quick start, simply select one of our 2000+ pre-conjugated antibodies. You can add your own or third-party antibodies for ultimate flexibility.

Predefined antibody panels (liquid, REAplex): Address specific research questions with modular panel kits containing a selection of ready-to-use antibody conjugates (e.g. 20-plex).

Predefined antibody panels (dried, REAscreen™): Maximize insights from your valuable samples with our ultra-high-plex, plug-and-play panels, perfect for deep phenotyping (100s of markers). Offered in a convenient 96-well format, this approach saves precious research time by eliminating laborious panel design, while still offering the flexibility to add your own or third-party antibodies.

Customized antibody panels (dried, REAscreen Design): For researchers who already have specific research questions, our custom antibody panels are an option. These panels can be individually tailored to specific applications in a plug-and-play 96-well format.

Using dried REAscreen Antibody Panels saves you valuable time while eliminating pipetting errors and reducing handling mistakes, leading to more reliable and reproducible results.

2.2 For combined RNA detection

For same-section multiomics analysis, seamlessly add RNA panels to your experiment. This reveals valuable details like cytokine expression alongside protein data, enriching the understanding of your sample. Our RNAsky® Detection Probes are designed to target virtually any RNA of interest with high sensitivity and specificity.

Design your custom RNAsky panels or use our 24-plex RNAsky IO Explore Panel, featuring immuno-oncology markers.

3. Fully automated staining and imaging

After sample preparation and panel design, the MACSima System takes over for an automated, high-precision experiment. Simply load your samples and antibody panels, define your region of interest (ROI), and press start. The system handles the rest, freeing you to focus elsewhere. An initial overview scan optimizes your ROI selection, pinpointing the most intriguing areas for in-depth characterization.

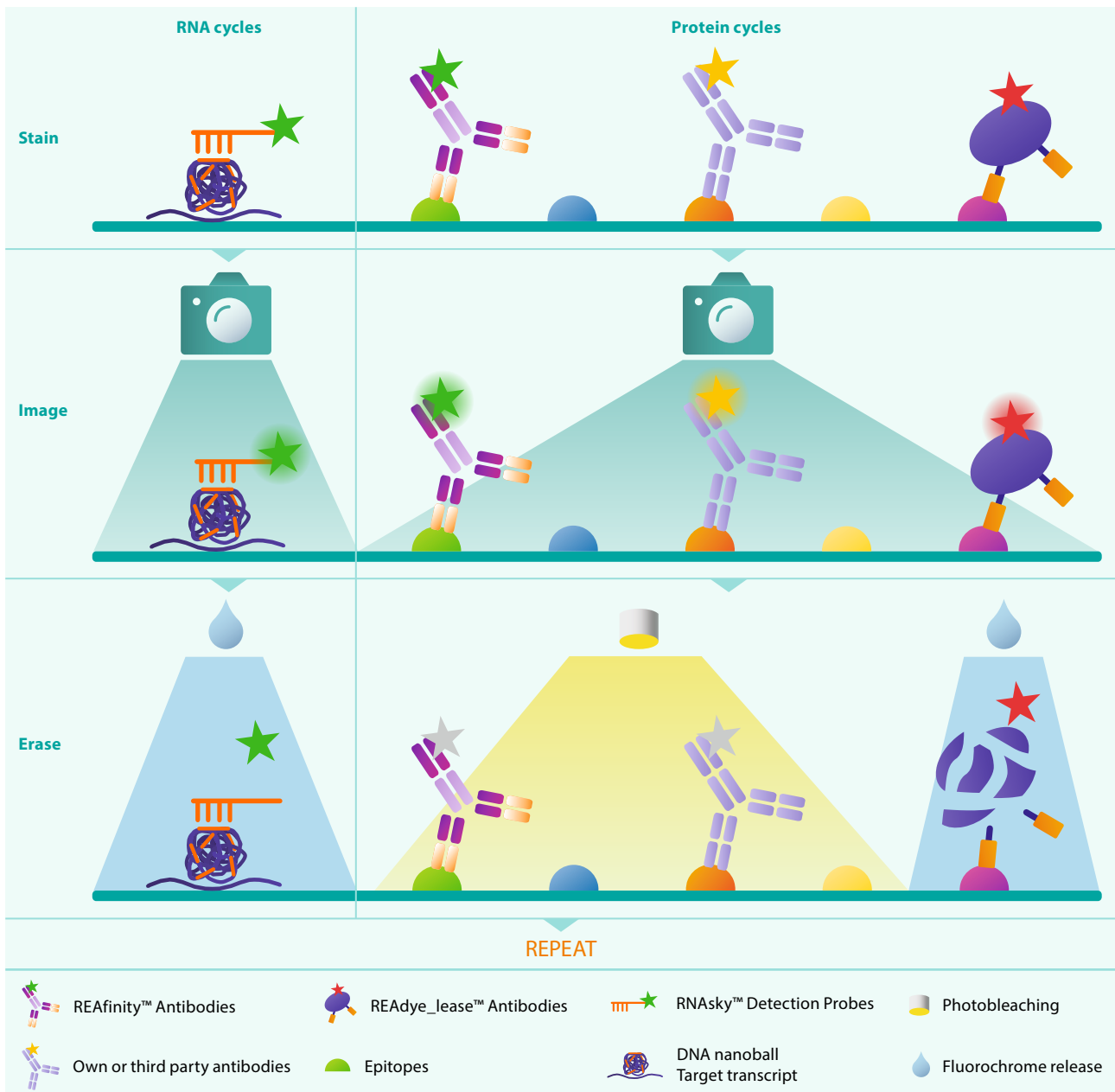


Figure 2: MICS cycles automated by the MACSima System. Both RNA and protein targets are analyzed on the same tissue section, with RNA cycles performed first, followed by protein cycles.

The MACSima Platform utilizes MICS (MACSima Imaging Cyclic Staining) technology, employing iterative staining cycles with either primary, fluorochrome-conjugated antibodies (protein) or RNAsky Detection Probes (RNA) to capture microscopy data of hundreds of markers without compromising the sample. The process comprises three steps (stain, image, and erase), all performed fully automated by the MACSima System. Both RNA and protein targets are analyzed on the same tissue section, with RNA cycles performed first, followed by protein cycles. Initially, the sample is stained with RNAsky Detection Probes or fluorochrome-conjugated antibodies, respectively (Fig. 2, Stain). In the subsequent step, an image is captured using the widefield fluorescent microscope (Fig. 2, Image). Then, the fluorescence signal is erased without harming the sample (Fig. 2, Erase). Thanks to the mild erasure method, the cycles can be repeated as often as needed, allowing users to label an unlimited number of markers.

For signal erasure, the MICS technology supports two different mechanisms:

- Photobleaching (REAbye_lease™, REAlease®, and REAfinity™): Fluorescent signals are erased from any antibodies conjugated to photosensitive fluorochromes such as FITC, PE, or APC.
- Fluorochrome release: Fluorochromes are detached from antibody complexes (REAbye_lease, REAlease) and RNAsky Detection Probes in a controlled and specific manner upon incubation with a certain reagent.

Both signal erasure mechanisms are sample-friendly, preserving epitope integrity while efficiently clearing signals for subsequent cycles. This enables highly flexible panel design and cycle assignment, a key advantage of MICS. Individual antibody conjugates can be incorporated into early or late staining cycles, freely combined with other relevant antibodies, all within the same experiment.

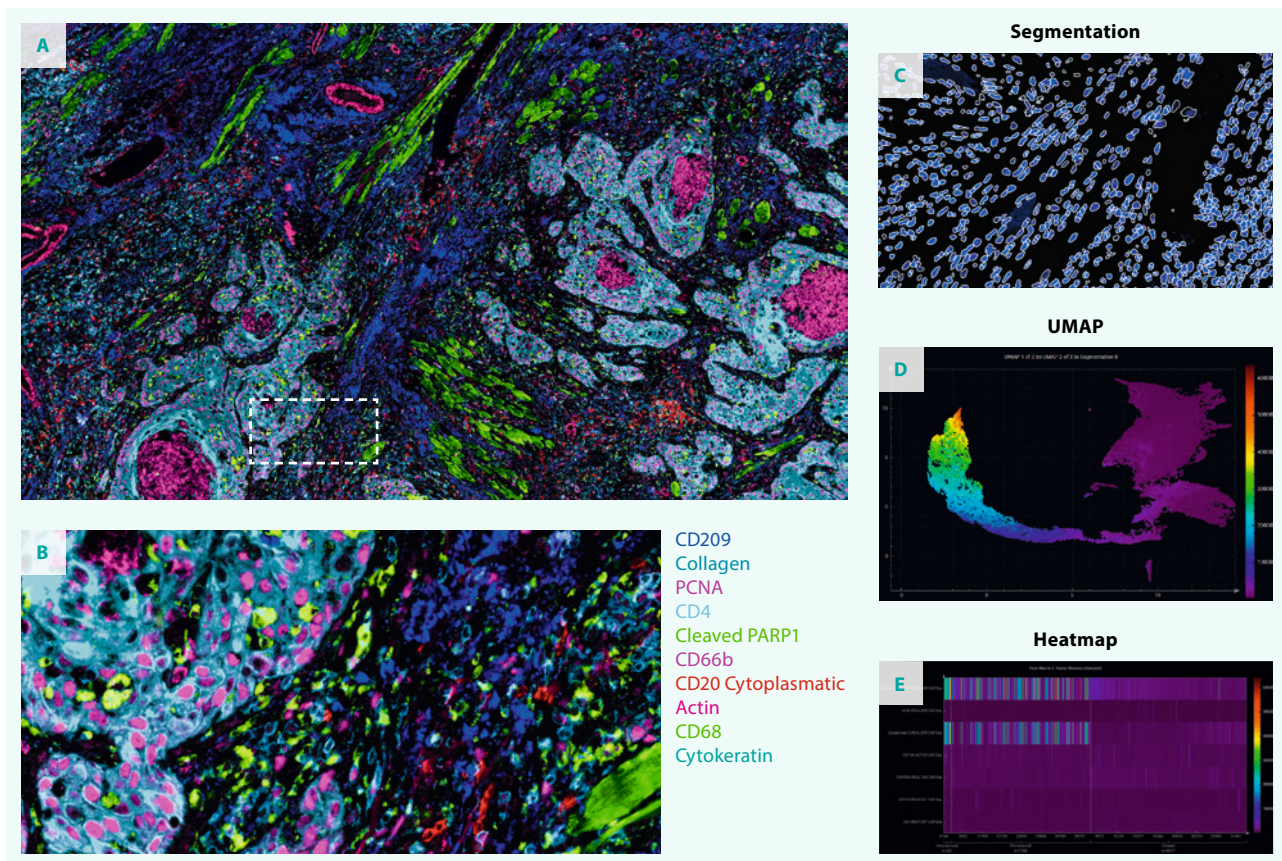


Figure 3: Head and neck squamous-cell carcinoma tissue stained with 61 markers. Overview showing 10 markers (A), zoom-in (B) and exemplary data analysis plots (C–E).

4. Spatial data analysis

MACS® iQ View – Spatial Biology is a software package specifically designed for spatial data analysis. The software is easy to learn and use, even for researchers without extensive experience in data analysis. The MACS iQ View – Spatial Biology Software offers a wide range of tools to visualize and analyze spatial data:

- **Visualization and navigation:** Effectively visualize and navigate through your data with the Image Viewer, using histogram tools, pixel-based correlations, and line intensity profiles.
- **Segmentation:** The segmentation algorithm enables both classic cell morphology (based on nuclei detection) and superpixel (image-based) segmentation methods. You can also import segmentation masks from external sources.
- **Gating:** Use interactive gating to analyze and sort cells based on specific markers. The software's dynamic data display enables you to gate cell populations or even select single cells on your plot and immediately see where they are located in the image or data table and vice versa.
- **Spatial analysis:** The standard image analysis tools include correlation matrices, along with data extraction. Benefit from advanced spatial analysis capabilities, particularly the precision in relative distance measurements, crucial for accurate tumor analysis, and relative density measurements, essential for biomarker discovery.
- **Clustering and dimension reduction:** For a more unbiased and qualitative data analysis, use clustering and dimension reduction tools, such as UMAPs and t-SNE plots.

The MACS iQ View – Spatial Biology Software is flexible as it allows you to import external data, customize workflows, and perform advanced analysis using Python. As one of the highlights, you can design, visualize, and save your workflows with the workflow editor. For enhanced automation, use this tool to duplicate entire workflows for use with different datasets. Perform cross-dataset analysis to compare and analyze data from multiple experiments.

Finally, turn your data into publications with ease. Easily export your results in publication-ready CSV and TIFF formats, eliminating tedious formatting and streamlining your publishing process.

Examples

Figure 3 illustrates a representative example of high-plex proteomics analysis using the MACSima Platform workflow on a head and neck squamous-cell carcinoma tissue sample. The REAscreen Immuno-oncology Panel, consisting of 61 protein markers, was used to stain the tissue. Ten of these markers are shown (Fig. 3A), along with a zoomed-in view demonstrating single-cell resolution (Fig. 3B). In spatial analysis, the initial step is segmentation (Fig. 3C), which defines an identity for each cell and enables further analysis with tools like UMAP (Fig. 3D). Heatmaps are a valuable tool to visualize the expression levels of specific markers depending on their distance from the tumor (Fig. 3E).

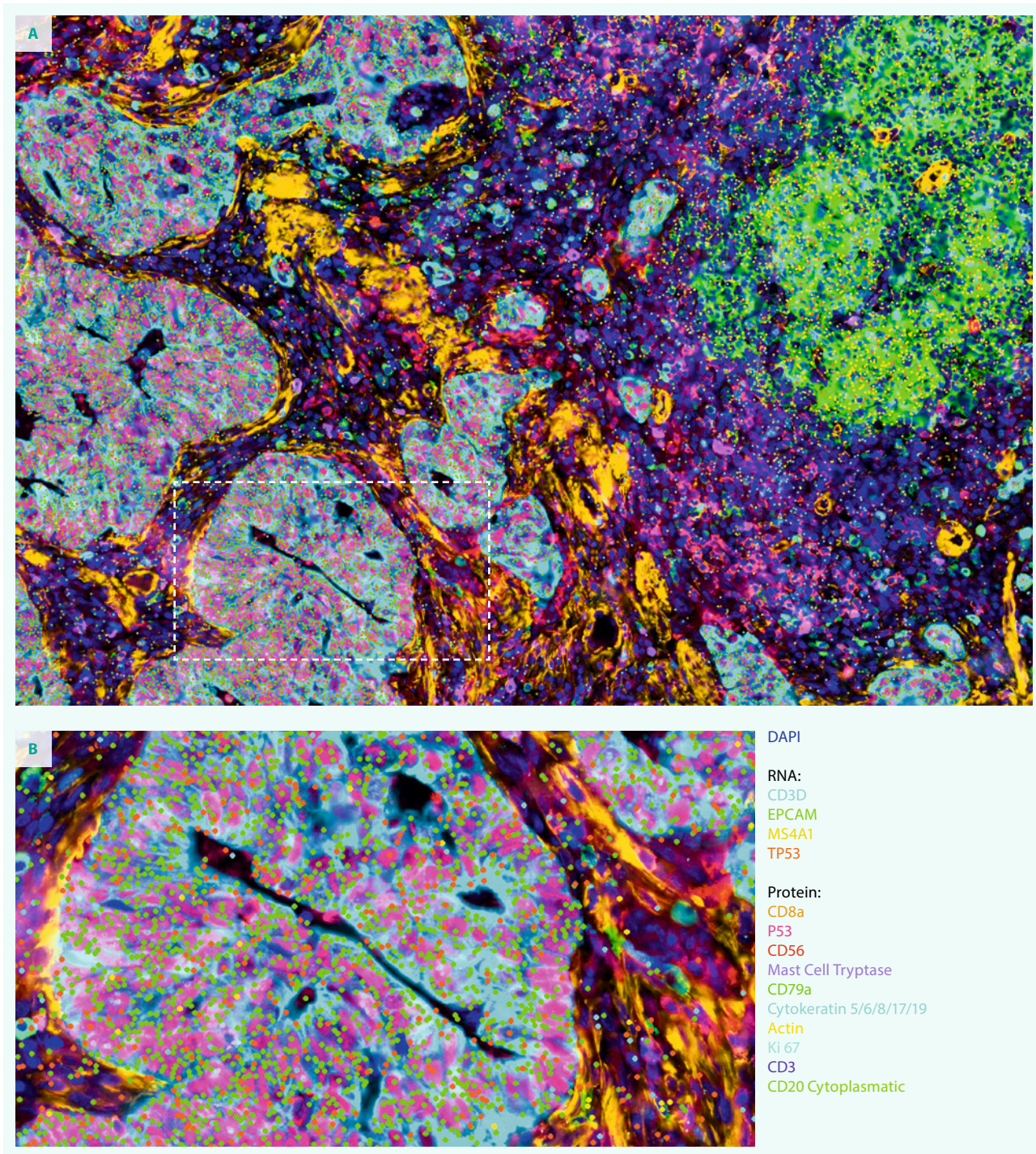


Figure 4: Colorectal cancer tissue stained with 40 protein markers and 27 RNAs. Overview showing 10 protein markers and 4 RNA targets (A), and zoom-in (B).

Figure 4 illustrates an example of same-section multiomics analysis using an FFPE colorectal cancer tissue. In this sample, 40 proteins and 27 RNAs were analyzed simultaneously within the same tissue section. The tissue section was stained using Miltenyi Biotec antibodies and RNAsky Detection Probes. Figure 4A shows a selection of 10 protein markers and 4 RNA markers, while figure 4B provides a zoomed-in view demonstrating the spatial correlation of Cytokeratin and p53 markers with high gene expression of the tumor markers EPCAM and TP53.

Discussion

Navigating the complex spatial biology landscape can be time-consuming and challenging, frustratingly slowing down the path to publication. The MACSima Platform simplifies and accelerates this process, empowering researchers to achieve publication-ready results faster.

Unlike workflows requiring multiple tools, MACSima offers an all-in-one solution. It streamlines every step, from sample preparation, automated staining and imaging, to advanced data analysis, including both high-plex protein and RNA analysis on a single slide. This reduces time spent managing different vendors, devices, and software, allowing researchers to focus on groundbreaking discoveries.

From setup to publication-ready results in 2–9 weeks

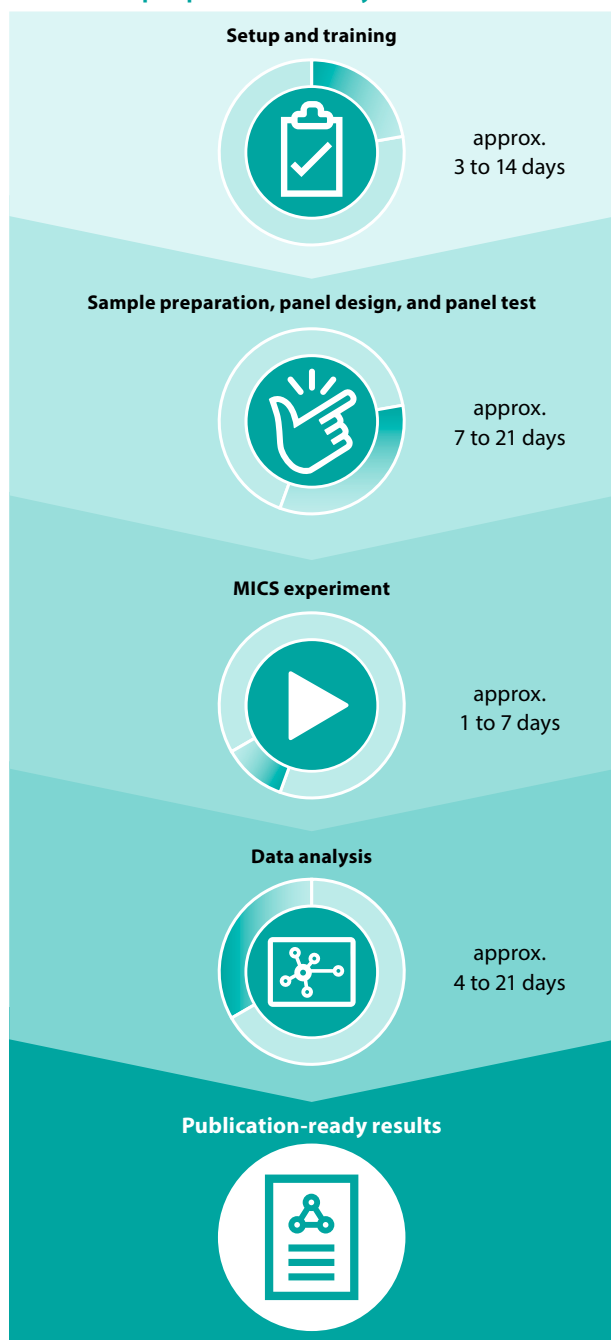


Figure 5: Anticipated time spans for each step in the spatial biology workflow using the MACSima Platform

Figure 5 illustrates the anticipated time spans for each step in the spatial biology workflow using the MACSima Platform, from device setup to obtaining publication-ready results.

Setup and training

The first operational training occurs immediately upon installation, allowing experienced spatial biology users to begin their experiments right away. Newcomers to the field will be prepared in a dedicated user training. Miltenyi Biotec offers individualized, application-specific biological support throughout the entire experimental setup, encompassing everything from panel design to data analysis. This comprehensive support is built upon Miltenyi Biotec's extensive experience of over 30 years in research and development, including the use of its own products.

Sample preparation, panel design, and panel test

Complete the entire sample preparation, panel design, and panel testing process within just 7 to 21 days.

The easy-to-use MACSwell Sample Carriers allow you to directly use standard microscope slides, simply placing them around your sample. This eliminates the need to transfer your sample to a coverslip or other holder, saving time and minimizing the risk of sample damage.

With Miltenyi Biotec's pretested antibodies, you can skip the conjugation and validation processes that can take up to 4–7 months with other solutions. Jump right into your experiments with the readily available, pretested antibodies. Design your panel within hours, then test and optimize it for your specific research question and sample. Our Field Application Support team will partner with you to design the perfect panel for your needs. You can be ready to generate publishable data within just one week of testing.

For first-pass valuable insights, use our plug-and-play pre-defined panels. These panels eliminate the need for panel design and are rigorously tested by the platform developers, ensuring consistent performance and reliable results. This reduces the risk of errors and wasted time due to suboptimal antibody combinations or staining protocols. Set up your first experiment within hours.

The MACSima Platform's open architecture empowers you to leverage your existing expertise alongside the platform's capabilities. This allows you to seamlessly integrate your own antibodies or choose from the largest selection of pretested antibodies specifically designed for spatial biology.

MICS experiment

By automating many tedious tasks like staining cycles and data acquisition, the MACSima Platform enhances both efficiency and accuracy. This saves time while minimizing manual errors, ultimately leading to more reliable and reproducible results.

MICS technology's sample-friendly signal erasure mechanisms preserve epitope integrity while efficiently clearing signals for subsequent cycles, enabling highly flexible panel design and cycle assignment. Unlike multiplexing technologies using harsh antibody elution, MICS avoids the need for extensive validation due to minimal epitope damage, offering greater flexibility.

Data analysis

Despite the vast potential of spatial biology data, its analysis remains a challenge. Spatial biology experiments generate massive datasets – high-resolution images with hundreds of markers measured across thousands of cells. Spatial information like distances and densities adds complexity and combining different omics data further amplifies this issue. Consequently, sophisticated tools and workflows are crucial for comprehensive analysis.

The user-friendly yet sophisticated MACS iQ View – Spatial Biology Software empowers researchers to analyze spatial biology data without writing custom scripts for months or juggling multiple vendors and software packages. MACS iQ View – Spatial Biology was specifically designed for your experiments and needs, saving valuable time and effort.

Publication-ready results

The MACSima Platform offers convenient export options in publication-ready formats, eliminating the need for time-consuming formatting and facilitating a smooth transition to publication.

Conclusion

Spatial biology unveils the intricate world of biological processes in their true context, offering immense potential to revolutionize research and therapy. As a result, spatial biology will soon become an indispensable tool for (immuno-)oncology researchers, pathologists, and other professionals.

The MACSima Platform breaks down barriers by providing a seamless workflow and user-friendly solutions for each step. Unlike restrictive solutions, the MACSima Platform embraces flexibility, allowing the integration of your existing resources and expertise. This unique balance empowers researchers of all experience levels to achieve publishable results faster, unlocking the full potential of spatial biology and paving the way for groundbreaking discoveries.



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