MACSima™ Platform

Explore the full potential of spatial multiomics

Miltenyi Biotec
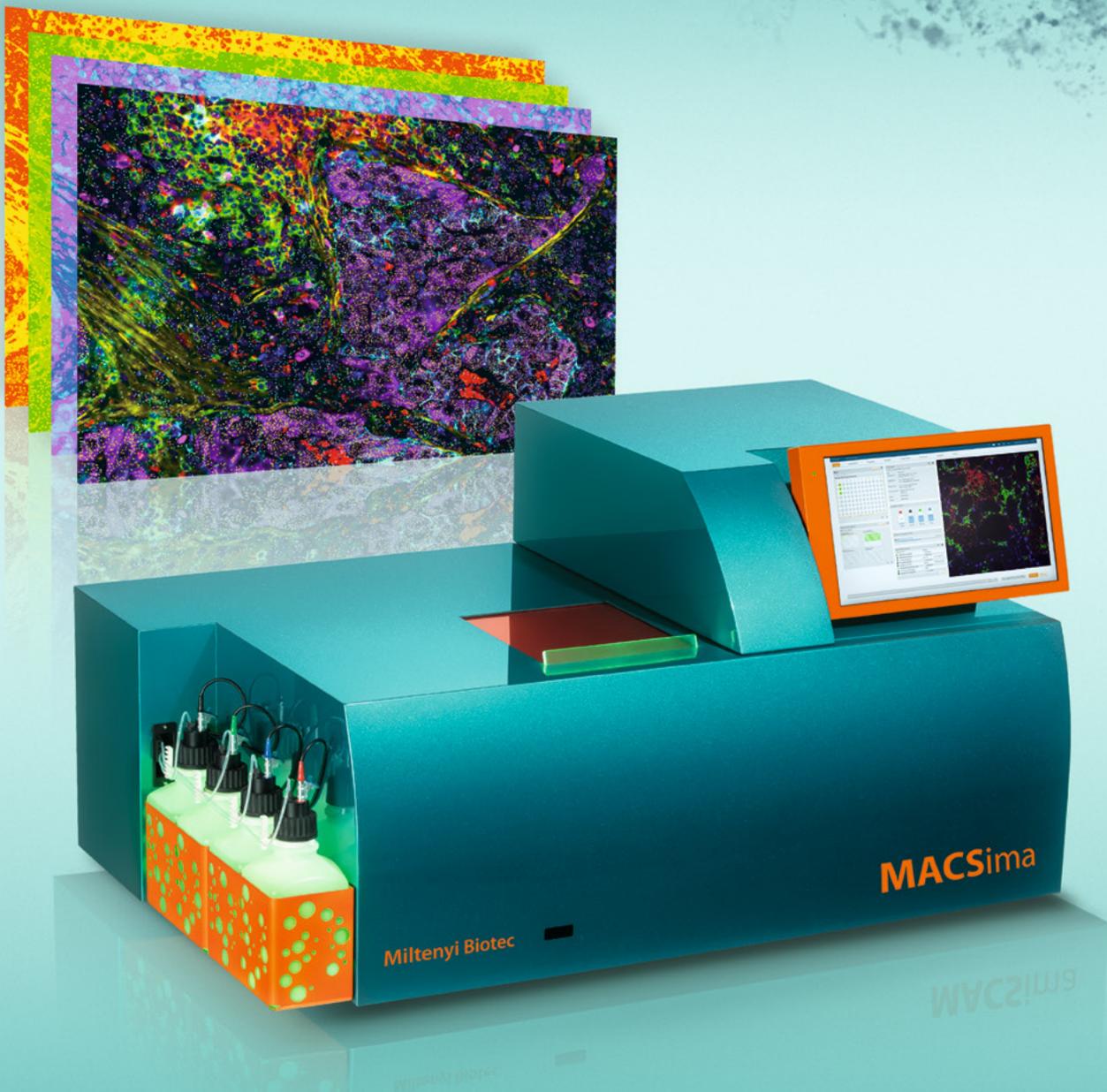
MACSima

The MACSima™ Platform

## Easily understand the complex interplay of cells in tissues

In our body's tissues, cells interact closely, affecting each other's functions and states. For example, in the tumor microenvironment, cancer cells can stop immune cells from working properly.

Those cellular complexities cannot be fully understood by analyzing individual cells or cell suspensions. We need to study them in their spatial context in their natural settings – the essence of spatial biology.

Enter the world of spatial biology with the high-plex proteomic approach of the MACSima Platform. Benefit from unparalleled flexibility: Analyze hundreds of protein markers across every cell in your tissue sample or focus on a more specific panel of around 20 markers for targeted or preliminary studies. Don't stop at proteins. Go deeper with spatial RNA detection, seamlessly integrated into the MACSima Platform for a unified multiomics workflow and analysis on the same tissue section.

Returning to the example of the tumor microenvironment, this can help us uncover why certain cells fail to do their job or why specific drugs aren't effective.

Content without context is just noise.
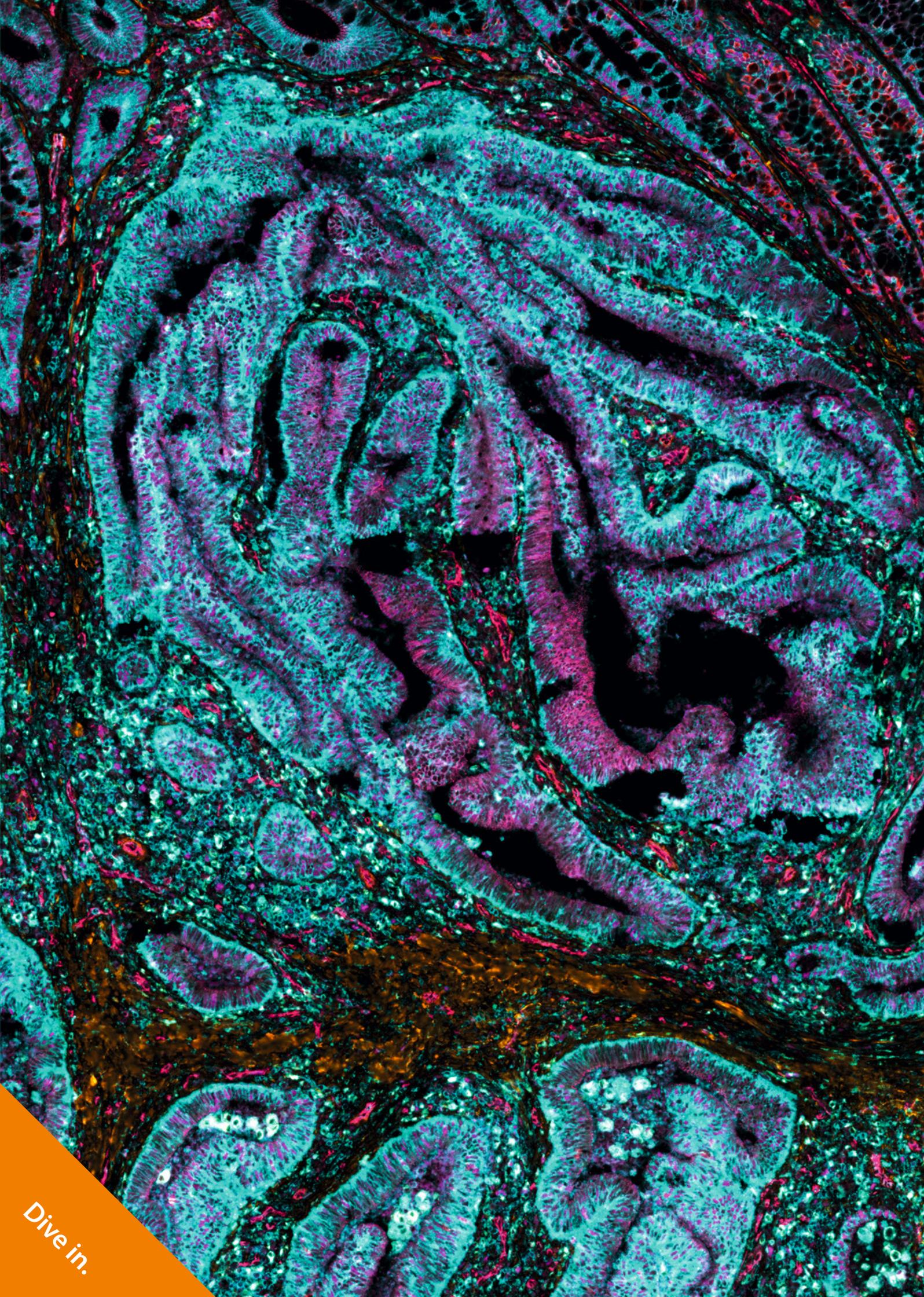
Dive in.

Spatial protein analysis of
61 markers in colorectal
cancer tissue using the
MACSima Platform.
Selected stainings
displayed here:

- Collagen I (REAL 958)
- PCNA (REA858)
- CD107a (REAL653)
- CD31 (REA1312)
- Actin (REAL650)
- CD68 (REA1306)
- CD11c (REA1310)
- CD4 (REA1307)
- CD324 (REA1319)
- CD326 (REA1311)
- Calponin (REA1104)

Explore the unknown

Your tissue sample is full of secrets, just waiting
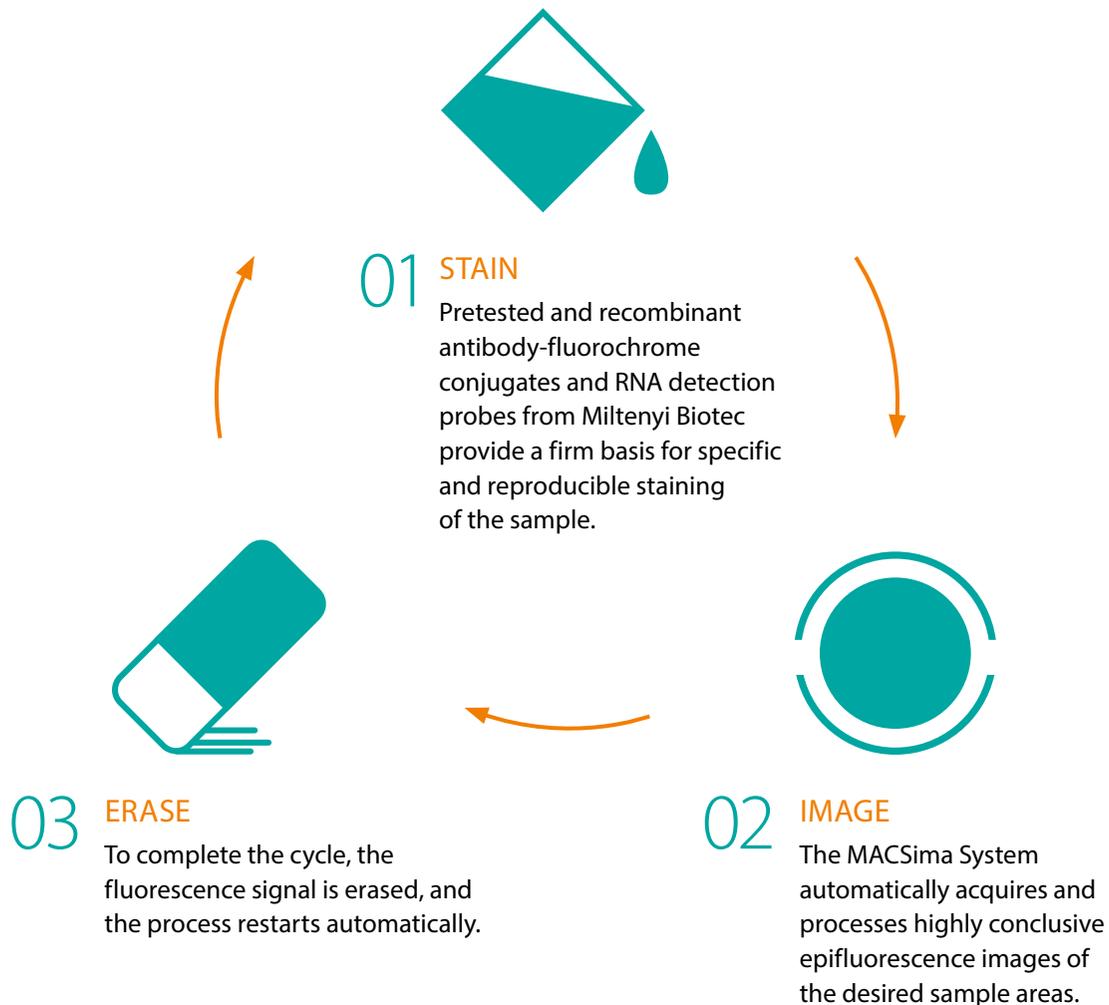to be unlocked. Start your journey of discovery at

▶ **miltenyibiotec.com/MACSima**

MICS technology – the basic principle

The MACSima Platform utilizes MICS (MACSima Imaging Cyclic Staining) technology. This approach captures microscopy data of hundreds of markers without compromising the sample for further downstream processing. Initially, the sample is stained with multiple primary fluorochrome-conjugated antibodies or RNA detection probes. In the subsequent step, an image is captured using the widefield microscope, and to conclude the cycle, the fluorescence signal is erased.

This cycle is automatically repeated as many times as necessary, enabling the user to label an unlimited number of protein markers and detect a comprehensive range of RNAs on the same sample. To investigate protein expression in tissue, utilize our pretested antibody portfolio or enjoy the flexibility to employ your own antibodies. For RNA detection, choose between predefined or custom RNA panels to investigate the targets of your choice.

01 STAIN
Pretested and recombinant antibody-fluorochrome conjugates and RNA detection probes from Miltenyi Biotec provide a firm basis for specific and reproducible staining of the sample.

02 IMAGE
The MACSima System automatically acquires and processes highly conclusive epifluorescence images of the desired sample areas.

03 ERASE
To complete the cycle, the fluorescence signal is erased, and the process restarts automatically.

All you need for your spatial biology journey

The MACSima Platform streamlines your spatial biology research from A to Z, reducing the experimental workload and generating impactful data in no time. As you step into the realm of spatial biology using the MACSima Platform, you receive more than just an instrument – you gain access to a comprehensive spatial biology solution, encompassing various sample carriers, an extensive antibody portfolio, supportive reagents, and an intuitive analysis software. And that's not all: We provide support throughout the entire workflow, from panel design to data analysis, addressing both technical and biological aspects. As we use the MACSima Platform in our own research, rest assured we understand scientists' needs.
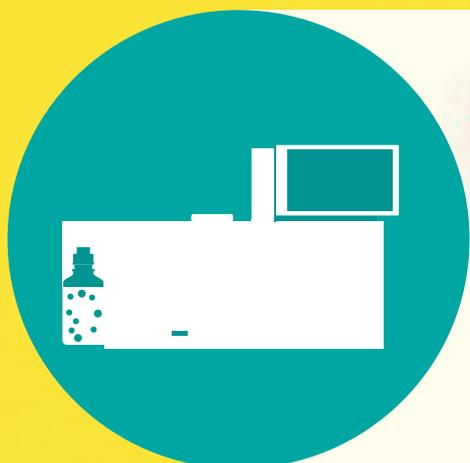
MACSima System

At the core of the platform is the MACSima System, an instrument that executes the iterative staining cycle and data acquisition in a fully automated manner.

## Protein and RNA detection panels for spatial multiomics

Save time using pretested and ready-to-use reagents for your multiomic experiments from our extensive portfolio.

MAC
Platform

Sima

MACSwell™
Sample Carriers

Specially designed sample carriers from Miltenyi Biotec ensure a failure-free, automated MICS process and offer the flexibility to use various sample types.
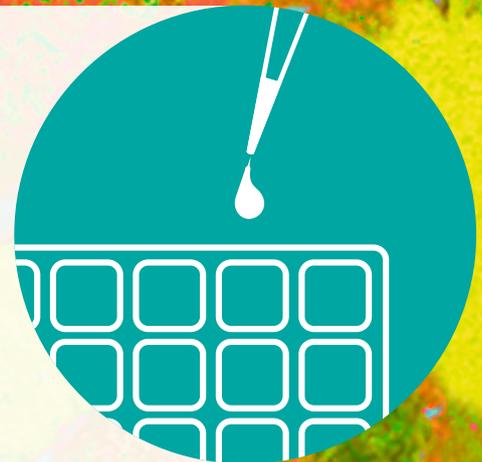
MACS® iQ View
Analysis Software

Specifically developed to analyze the large and valuable data stacks obtained by the MACSima System, this software is the perfect tool to dissect the multidimensional datasets and to extract the underlying information in an easy and comprehensive manner.

Ready, set, explore!
Your shortcut to spatial data.

1 PANEL DESIGN

Accelerated and simplified preexperimental work with Miltenyi Biotec's portfolio of pretested recombinant antibodies and plug-and-play antibody and RNA detection panels.

2 AUTOMATED MICS EXPERIMENT

Efficiency through automation of the MACSima System, saving time for tackling other challenges in your project.
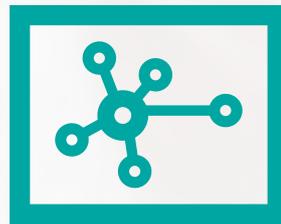
3 SPATIAL DATA ANALYSIS

Rapid generation of conclusive and publication-ready results with MACS iQ View – Spatial Biology, an intuitive software that makes complex spatial data analysis accessible to users of all backgrounds.

MACSima

MACSima System

Fully automated sample handling and data acquisition

Excellent optics, a state-of-the-art sCMOS camera, accurate liquid handling, and high computer capacity are the hallmarks of the MACSima System.

Experience hands-free MICS experiments with our system components that operate in perfect harmony.

Computers

Two computers with high storage capacity allow for detailed data analysis while the image stack continues to grow cycle after cycle.
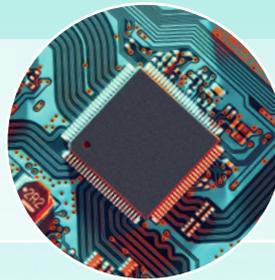
Liquid handling system

The robotic needle arm eliminates pipetting errors and saves valuable time as it can handle hundreds of antibodies and all other necessary liquids accurately and fully automatically. Automated washing processes prevent carryover of reagents to maintain fluorescent signal specificity across all images.


Ultraprecise stage

Hosting the sample and all reagents, the ultraprecise stage serves as the MACSima System's work bench. Accurate positioning of all components throughout the MICS experiment ensures reliable execution of the iterative process and exact maintenance of the field of view.

State-of-the-art microscope and camera

The widefield microscope includes one objective for a fast overview scan. With two 20× objectives, our system provides flexibility for both long working distance and short working distance imaging, enabling you to unlock subcellular details regardless of your sample format (slides or 24-well plates). The sCMOS camera provides excellent image quality.

Miltenyi Biotec
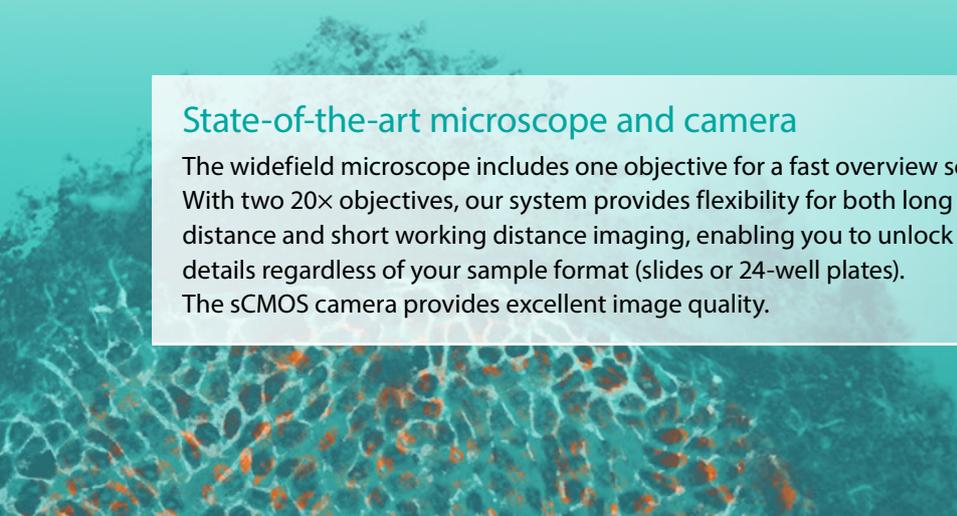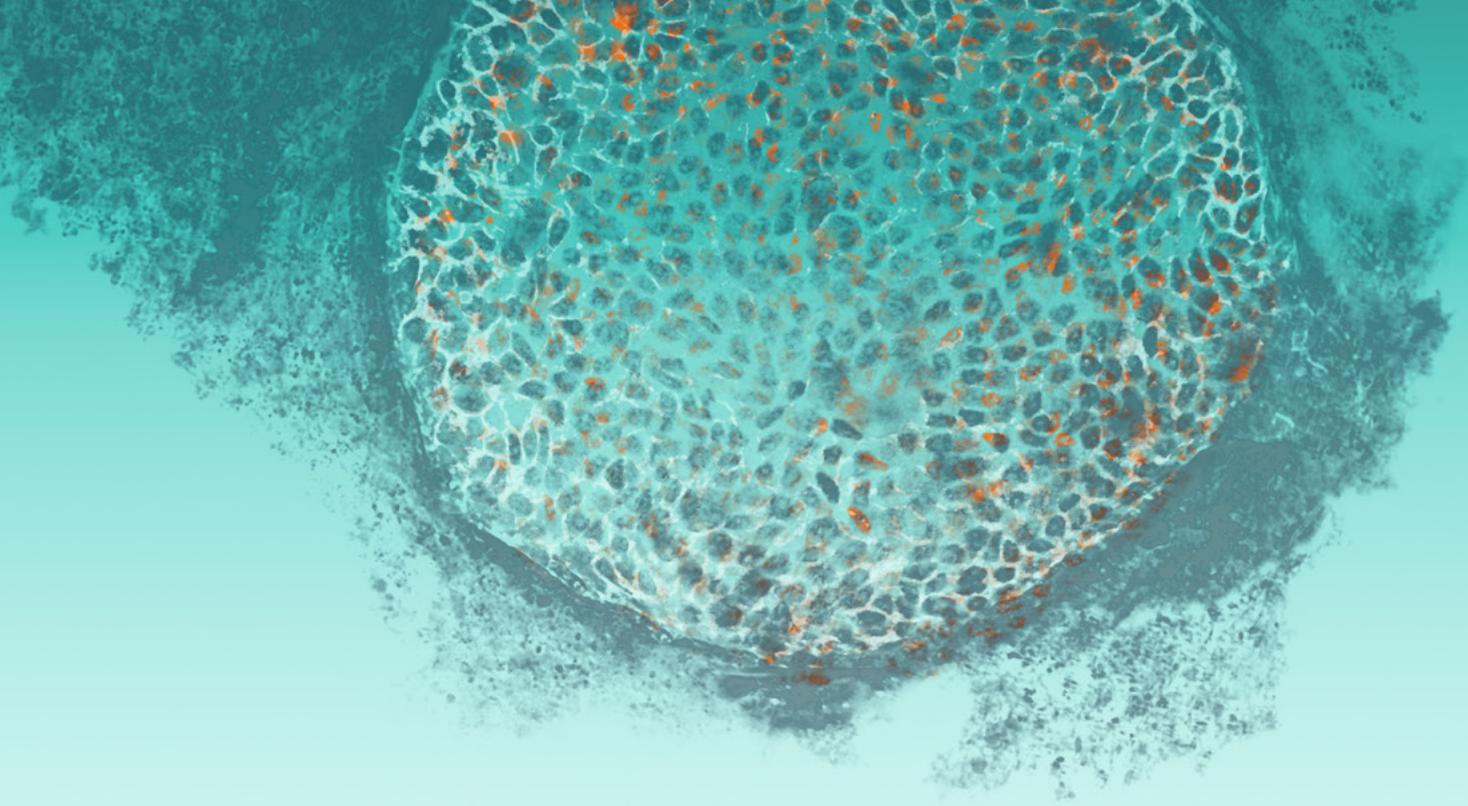
MACSima

MACSwell Sample Carriers

Analyze any kind of fixed sample

To answer complex scientific questions, you shouldn't be held back by technical constraints. That's why we developed MACSwell Sample Carriers for the MACSima System, providing you with total flexibility in analyzing fixed samples.

These carriers are designed to support various types of samples, including tissues and adherent cells. Each carrier features a precisely designed reaction cavity, ensuring confidence and trouble-free MICS experiments.

TISSUE
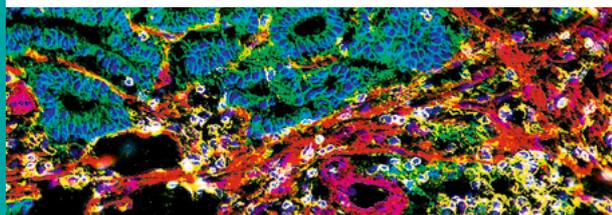
MACSwell Imaging Frames

MACSwell Imaging Frames can be attached to any standard microscope slide and provide the reaction cavity needed for a MICS experiment. These frames come in different sizes, ensuring a perfect fit for your tissue sample. They are fully compatible with our MACSwell HighRes Slides, allowing protein and RNA detection on the same sample.
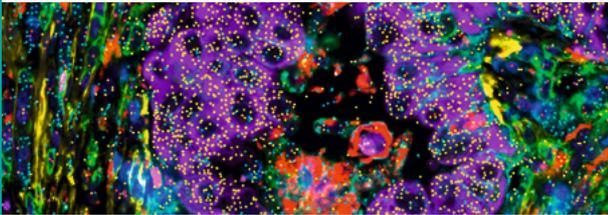
ADHERENT CELLS
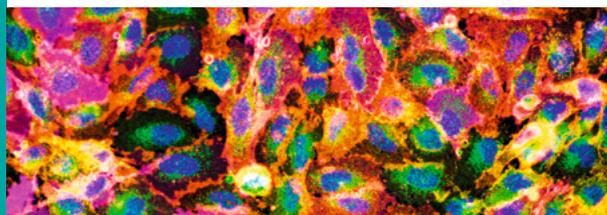
MACSwell 24 Imaging Plates

MACSwell 24 Imaging Plates come with 24 rectangular wells and a clear, 170 µm thick glass bottom. Just pipette your cell suspension onto the plate, culture it as usual, and then follow your standard protocol to fix the sample directly on the plate.

SPATIAL MULTIOMICS

MACSwell HighRes Slides

To achieve even higher resolution, use our MACSwell HighRes Slides, featuring 170 μm thick glass. They allow for exceptional detail capture and execution of spatial multiomics experiments with detection of proteins and RNA on the same sample.

Pretested antibodies for MICS

Specific staining – conclusive results

To achieve reliable and error-free analyses, rely on the world's largest portfolio of fluorochrome-conjugated primary antibodies for spatial biology:

- Pretested for MICS technology on the MACSima Platform, to be compatible with FFPE or frozen (PFA or acetone-fixed) samples of human or mouse origin
- Recombinantly engineered for high specificity
- Lot-to-lot consistent, ensuring reproducible results over time

The two mechanisms for signal erasure

After staining with fluorochrome-conjugated antibodies and image acquisition of the stained sample, the fluorescent signal can be erased by photobleaching or fluorochrome release (fig. 1).

Erase by photobleaching

The fluorescent signal of samples that were stained with fluorochrome-conjugated antibodies, such as our recombinant REAfinity® Antibodies or third-party antibodies, coupled to non-photostable FITC, PE, or APC fluorochromes, can be erased via photobleaching.

Erase by fluorochrome relase

Staining of samples with REAdye_lease™ and REAlease® Fluorochrome-Conjugated Antibody complexes allows for fast and gentle signal erasure via a controlled release of fluorochromes.
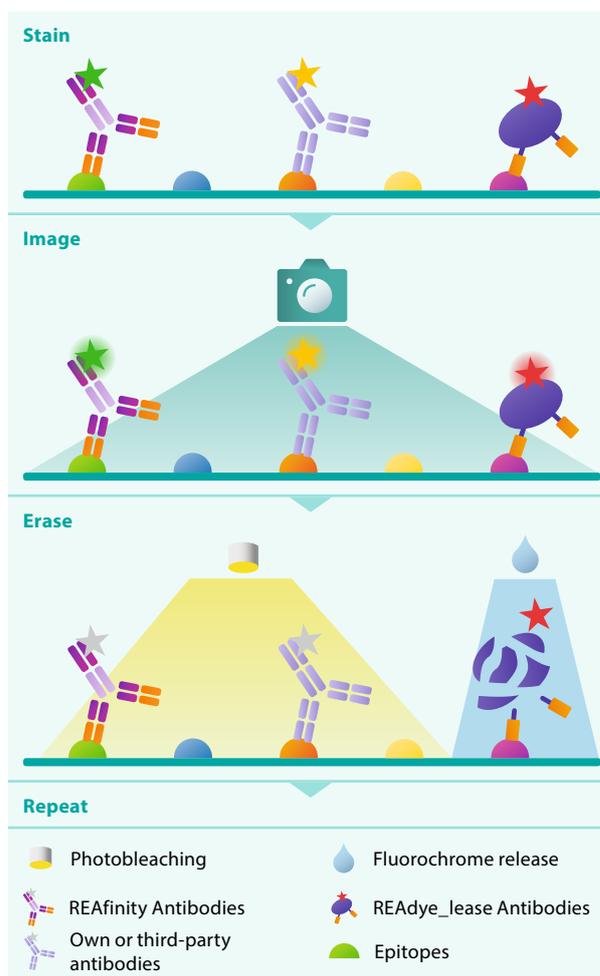
Figure 1: MICS cycles are performed automatically by the MACSima System. Fluorescence signal erasure occurs by photobleaching or fluorochrome release.
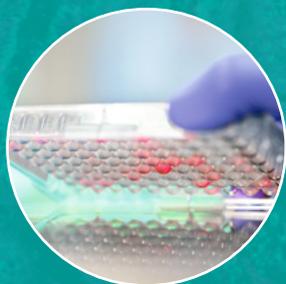
Convenient antibody panels – pretested for MICS

Predefined antibody panels

REAplex Panels

- Up to 20 markers, curated to visualize key tissue features.
- Full guidance from experiment setup to data analysis.
- Flexibly adaptable and expandable.

REAscreen™ Panels

- Skip the panel design and pipetting by using comprehensive, predefined panels.
- Convenient, standardized reagent format with dried antibodies.
- Freely expandable with liquid antibodies.

Custom antibody panels
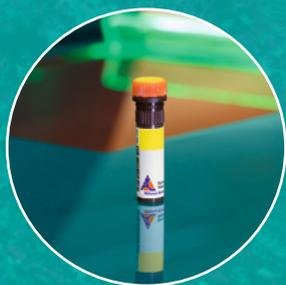
Individual antibody conjugates

- Huge catalog of fluorochrome-conjugated antibodies.
- Pretested for MICS on FFPE and frozen tissue sections.
- Build your panel with full flexibility.
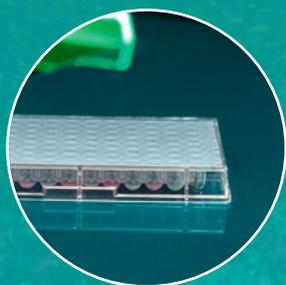
REAscreen Design custom panels

- Our highly standardized REAscreen Format – tailored to your panel.
- Define your time-saving and scalable panel with Miltenyi Biotec antibodies.
- Ideal for studies with large sample numbers.
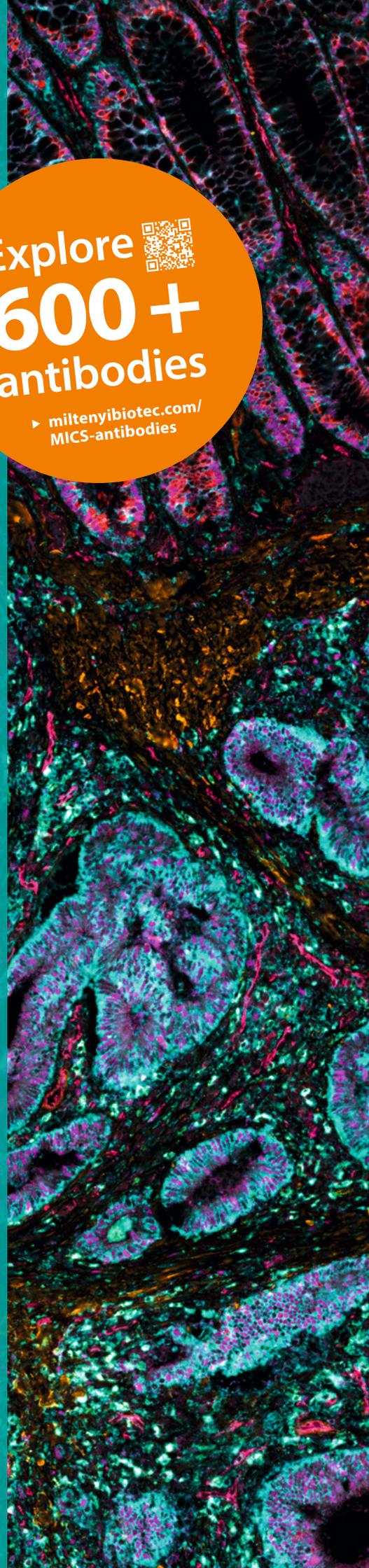
Same-section spatial multiomic analysis of non-small cell lung cancer tissue using the MACSima Platform. In this experiment, MICS technology was used for the simultaneous detection of 40 protein markers and quantification of 27 RNAs on a single tissue section.

Selected protein targets:
- CD4 (REA1307)
- Ki67 (REAL1047)
- CD68 (REA1306)
- Mast Cell Tryptase (REAL798)
- CD57 (REA769)
- CD11b (REA3121)
- Cytokeratin (REA831)
- CD326 (REA1311)
- Actin (REAL650)
- CD20 Cytoplasmic (REA1087)

Selected RNA targets:
- CD3D
- Tumor markers (EGFR, EPCAM, ERBB2)
- CD68
- MKI67
- MS4A1

RNAsky
Detection Probes

Same-section spatial multiomics

Our RNAsky® Technology empowers you to complement your ultrahigh-plex spatial proteomics experiment with RNA transcript detection – all within the same tissue section. Leveraging Miltenyi Biotec's MICS technology, gene expression of selected RNA targets can now be seamlessly incorporated into the comprehensive MACSima Workflow.

▶ **miltenyibiotec.com/spatial-multiomics**

Download our flyer on same-section spatial RNA and protein analysis.

▶ **miltenyibiotec.com/RNAsky-flyer**

Unlock spatial gene expression insights with RNAsky® Technology

Spatial multiomic analysis at its finest

RNAsky Technology enhances the MACSima Imaging Platform, enabling *in situ* RNA detection alongside protein analysis in the same tissue section. This multiomic approach preserves spatial context while providing deeper biological insights.

Why choose RNAsky Technology?

- Epitope preservation – protease-free protocol ensures protein integrity.
- High specificity – proprietary probe design algorithm minimizes off-target binding.
- Robust performance and sensitivity – multiprobe approach enhances detection.

RNAsky Design: custom RNA panels for your research

Custom RNAsky Panels allow researchers to target virtually any gene with high specificity. Available in two formats, 12-plex and 24-plex, these panels provide flexibility for different research needs. The standard design service covers well-annotated human, mouse, and rat transcriptomes, while the advanced service extends to non-standard organisms, viral and bacterial targets, and novel sequences.

How does RNAsky Design work?

01 **PANEL REQUEST**
- Fill out our online form to connect with an expert.
- Discuss your specific experimental needs.
- Choose from our 12-plex and 24-plex panel formats.

02 **DESIGN REVIEW**
- We review your panel request and refine it together.
- You'll receive a quote and confirm the order.

03 **PANEL DESIGN AND PRODUCTION**
- We design your custom probes using our proprietary probe design algorithm optimized to yield highly specific probes.
- After precise design, we produce your panel.

04 **DELIVERY**
- We deliver your custom RNA panel.
- It's ready to use, so you can start your experiments immediately!

Figure 2: Workflow for RNAsky Design – from request to delivery.

LEARN MORE

Get more information on our RNAsky Design panels.
▶ **miltenyibiotec.com/RNAsky-Design**

MACS iQ View – Spatial Biology
Sophisticated yet simple analysis software

The MACS iQ View – Spatial Biology Software Package was specifically designed to manage and analyze the vast amount of valuable data generated by the MACSima Platform. Our primary goal in developing this software was to make spatial data analysis accessible to users from all backgrounds.

With MACS iQ View, handling and analyzing hundreds of images couldn't be simpler. You can easily display individual experimental markers or any combination you require. Efficiently organize your data using various display options and save your preferences to increase the consistency and reproducibility of your analyses.

Interactive and dynamic data display

The software's user interface is highly intuitive and easy to use. Interactive and dynamic gating or clustering enables you to view the results immediately in a variety of graphs, tables, and a vast array of plots.

LEARN MORE

Visit our website to learn more about MACS iQ View – Spatial Biology.

▶ **miltenyibiotec.com/MACS-iQ-View**

Figure 3: Graphical user interface of the MACS iQ View – Spatial Biology Software.

Easy segmentation

Defining the cell and nucleus boundaries is an effortless task with MACS iQ View – Spatial Biology. You can begin your analysis quickly with simple definition of parameters and fast processing. Choose between various predefined segmentation options or conveniently import your own trusted segmentation mask.
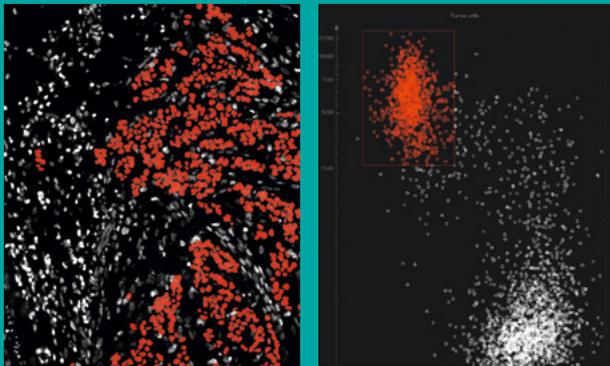
Multifaceted gating and clustering enable deep phenotyping

Deep phenotyping can be achieved through highly interactive, dynamic manual gating both on the graph and the image. A workflow editor lets you keep track of all selection steps. You can also use the integrated clustering options according to the features of your interest. All this allows you not only to phenotype the cells, but to also view their position on the image and analyze spatial relationships and more.
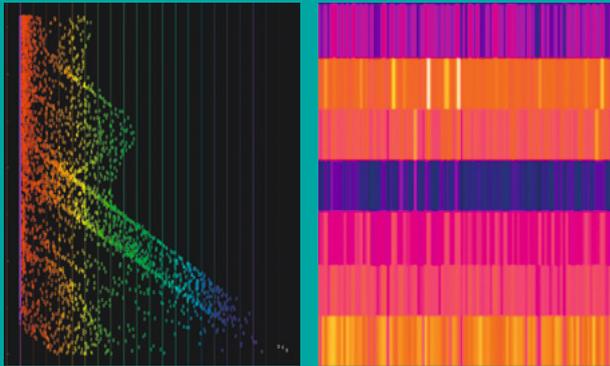
Cutting-edge analysis and plotting options for clear data display

MICS data enable you to examine tissue samples and cells in great detail. To display the high-dimensional data on the cells' complex phenotypes and spatial organization in an easy-to-grasp way, the software offers sophisticated analysis tools. Dimension reduction plots (t-SNE and UMAP), distance mapping, and a range of additional display options, such as heatmaps, let you visualize your intricate data.
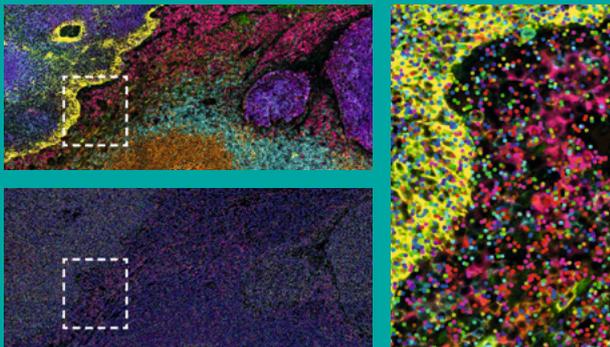
Same-section multiomics analysis

A new set of tools allows researchers to extract and quantify RNA expression data obtained by using RNAsky Detection Probes. With these tools, a true same-section multiomic experiment can now deliver both multiplex protein and RNA expression data for comprehensive analysis. Spatially resolved cellular transcript counts can now be used for distance and density studies, representing a new dimension for the spatial biology analysis workflow.
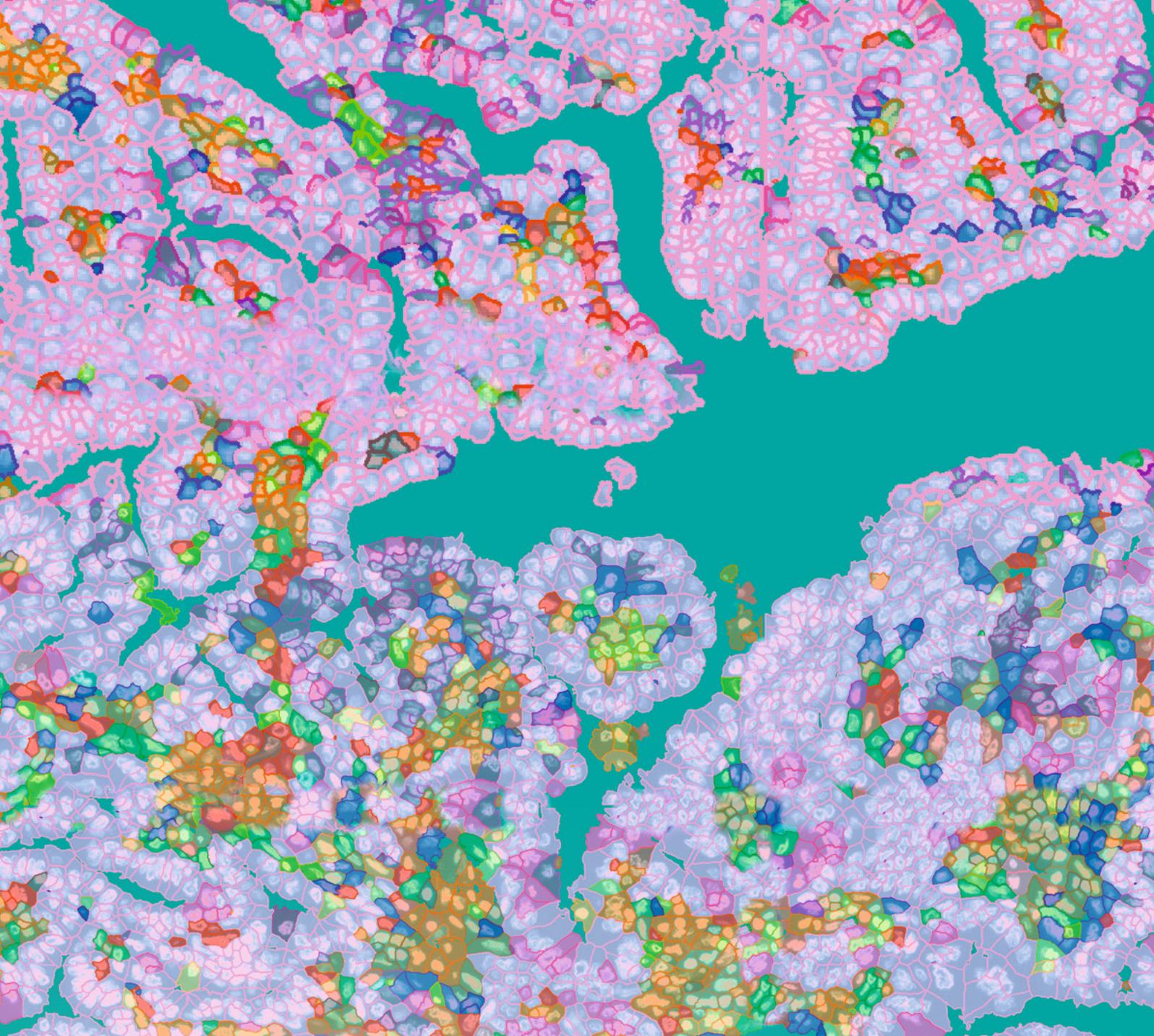
Turn spatial data into discoveries

Experience what highly multiplexed imaging based on MICS technology can bring to your research. The possibilities with spatial biology are endless and you can bring new discoveries to light that could revolutionize the study of disease characterization and the development of new therapies.

▶ **miltenyibiotec.com/MACSima**

Download our application note to learn more about the analysis of the tumor microenvironment in lung adenocarcinoma.

▶ **miltenyibiotec.com/MACSima-lung-TME-appnote**
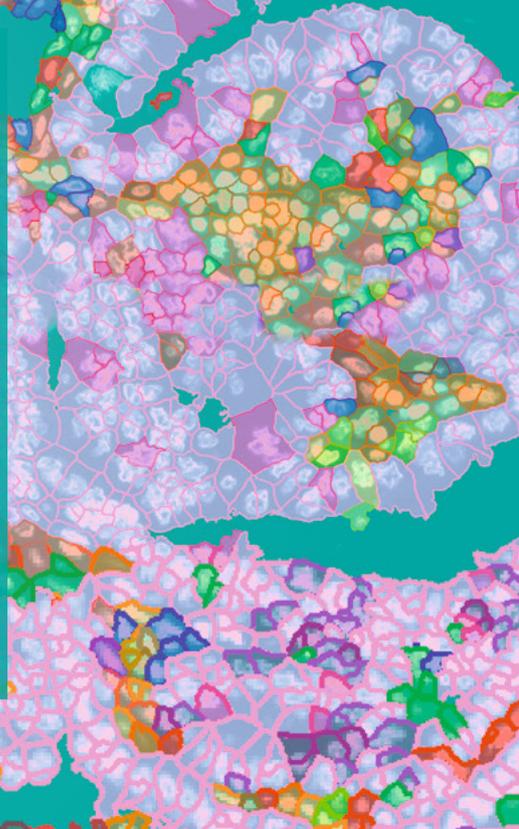
The picture represents a sample of lung adenocarcinoma stained with over 125 antibodies to characterize the tumor microenvironment. Utilizing 40 established markers enabled the identification of over 20 known cell populations, enhancing the discovery of unique marker combinations for deep phenotyping. Staining with such a high number of antibodies also provides the opportunity to discover previously unknown cell populations.

- CD4+ T cells
- CD8+ T cells
- Dendritic cells
- NK cells
- Monocytes
- Myeloid cells
- Neutrophils
- Granulocytes
- Macrophages
- M1 macrophages
- M2 macrophages
- Cancer stem cells
- Tumor cells
- Blood vessels
- Antigen-presenting cells
- Anti-apoptotic cells
- Activated T cells
- Plasma cells
- B cells
- Proliferating cells
- Fibroblasts
- Activated fibroblasts

One experiment, endless discoveries

Deep phenotyping

By examining hundreds of protein markers in their spatial context, researchers can uncover intricate details about cell heterogeneity, microenvironment interactions, and disease-specific patterns, ultimately advancing our knowledge of complex biological systems.
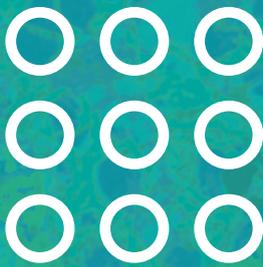
Biomarker research and discovery

The correlation between various markers in your sample is vital for uncovering biomarker signatures. This understanding plays a pivotal role in developing patient stratification strategies and innovative therapies tailored to individual needs, revolutionizing the treatment of numerous diseases.

Drug target discovery and validation

By conducting spatial analysis with a wide range of markers, you can identify potential drug development targets. Using the MACSima Platform, these candidates can be further assessed and validated in preclinical research studies, considering the comprehensive microenvironment. This approach can enhance the accuracy and depth of drug effectiveness analysis.

Where every marker tells a story

Thoroughly analyzing a wide range of markers not only boosts your current research but also creates a valuable archive for addressing future questions, even those you haven't thought of yet. You can revisit datasets from your MICS experiments anytime to uncover new insights. Your spatial data holds unlimited potential for discoveries. Explore how other researchers used the MACSima Platform in their groundbreaking research in the examples below.
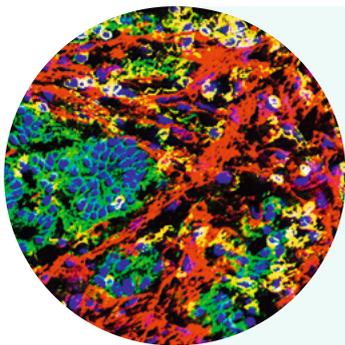
Spatial single-cell atlas of the liver

MICS technology helped researchers to generate a cellular atlas of the entire human and murine liver.

Read the paper in Cell.

▶ **cell.com/cell/fulltext/S0092-8674(21)01481-1**
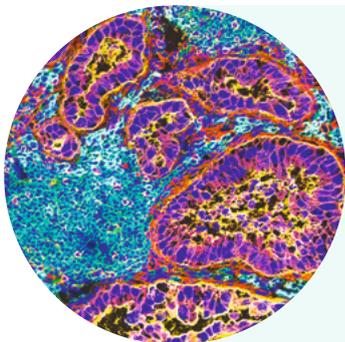
Deciphering functional tumor-immune crosstalk

Multiplexed data from the MACSima System was combined with single-cell laser microdissection and sensitive mass spectrometry to spatially profile tumor cells in human cancer samples.

Read the publication in Molecular Cell.

▶ **cell.com/molecular-cell/fulltext/S1097-2765(24)01041-4**
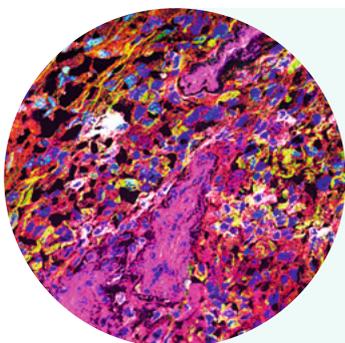
Deterministic neutrophil reprogramming in tumors

Data from the MACSima System were combined with RNA- and ATAC-sequencing to unravel distinct neutrophil states within tumors originating from different tissues.

Read the full story in Science.

▶ **science.org/doi/10.1126/science.adf6493**

LEARN MORE

Delve into cutting-edge spatial biology research with the MACSima Platform.

▶ **miltenyibiotec.com/MACSima-publications**

All you need for spatial biology excellence

MACSima Platform specifications	
Microscope	
Instrument type	Widefield epifluorescence microscope
Camera	Equipped with a latest-generation sCMOS camera with high resolution and thermoelectrically cooled sensor (15 megapixel, large 25 mm diagonal field of view)
Excitation	Six high-power excitation LEDs (filters: 386/23 nm, 420/10 nm, 470/40 nm, 531/46 nm, 628/32 nm, 725/40 nm)
Emission	Five emission filters (470/40 nm, 530/43 nm, 580/25 nm, 698/70 nm, 809/81 nm)
Autofocus	Dual approach of hardware- and image-based autofocus mechanisms
Objectives	• 2× objective to generate overview images; NA 0.1 • 20× long working distance objective (designed for 1 mm thick slides); NA 0.45 • 20× objective with high numerical aperture (designed for 170 µm thick cover glass); NA 0.75
Bleaching unit	• Separate bleaching unit for optimized signal erasure • Illuminated area: 3 mm × 3 mm • Light intensity: 2 W
Liquid handling system	
Needle arm	Robotic needle arm allowing fully automated liquid transfer
Steel needle	Washable stainless steel needle with minimized spillover
Syringe pump	Syringe pump drive for accurate volumetric staining of samples
Fluid containers	1.5 L fluid containers for automatic operation over several days without the need to exchange containers
Sample and reagent stage	
Automated stage	Stage with sub-micron positioning accuracy of 100 nm (automated image registration for different cycles)
Automated sample and reagent carrier positioning	Convenient loading and clamping of all supported sample carriers and reagents
Technical data	
Computer	Two integrated computers for experiment planning/execution and image analysis
Monitors	• Tiltable integrated touch display (Full HD) for PC1 • External 4K monitor for PC2
Storage/ports	• 25 TB integrated storage to store data of several experiments • 2× USB 3.0 • HDMI port
Network	1× 10 GbE 1× RJ45 GbE/10GbE
Power requirement and consumption	100–240 V, 50/60 Hz, max. 7/13A, max. 1,300 W
MACS iQ View Software	
Control software	• Comprehensive sample and reagent management system • Easy planning and design of both simple and complex experiments • User-friendly execution and monitoring of experiments
Analysis software (Spatial Biology Package)	• Easy display of hundreds of markers from high-dimensional datasets • Fast and flexible segmentation • Interactive gating • Unbiased data analysis (k-means clustering, UMAP and t-SNE calculation) • Multiple plotting options (histogram, scatter plot, strip and violin plots, heatmaps) • Workflow editor • Distance analyses
Size and weight	
Dimensions	• Instrument dimension: 1,220 mm × 780 mm × 650 mm (w × d × h); footprint: 1,210 mm × 750 mm (w × d) • MACSima Table[1]: 1,250 mm × 765 mm × 813 mm (w × d × h)
Weight	• Instrument: 170 kg • MACSima Table[1]: 120 kg

Product type

Instrument

MACSima System

MACSima Table

MACSima 4K monitor

Software

Licences for MACS iQ View – Spatial Biology[2]
- Annual license
- Permanent license

RNAsky Reagents
- RNAsky IO Explore Panel, human
- RNAsky Support Kit
- RNAsky Sample Preparation Kit
- RNAsky Design 12- and 24-Plex

Antibodies and reagents

MICS-pretested antibody conjugates[3]
- REAfinity Recombinant Antibodies
- REAdye_lease Releasable Fluorochromes
- REAlease Releasable Antibodies

Pre-defined Antibody Panels for MICS[3]
- REAscreen Immuno-oncology Kit, human, FFPE, version 01
- REAscreen MAX Kit, human, FFPE, version 02
- REAscreen MAX Kit, human, PFA, version 02
- REAscreen MAX Kit, mouse, PFA, version 02
- REAplex Immuno-oncology Core, human, FFPE

Support Reagents for MICS
- MACSima Stain Support Kit, human
- MACSima Stain Support Kit, mouse

Sample carriers[4]
- MACSwell One Imaging Frames
- MACSwell One Small Imaging Frames
- MACSwell Two Imaging Frames
- MACSwell Four Imaging Frames
- MACSwell 24 Imaging Plates
- MACSwell HighRes Slides

Buffers and accessories

MACSima Buffer Starting Kit

MACSima Running Buffer 6×1.5 L

MACSima System Buffer 6×1.5 L

MACSQuant®/MACSima Storage Solution 6×1.5 L

MACSwell Deepwell Plates

MACSwell Sealing Foils

[1] The MACSima System is delivered with a trolley table specifically designed to support the system.
It has anti-vibration properties and provides a closed storage compartment.
[2] For detailed information about the different license options, visit **miltenyibiotec.com/MACS-iQ-View**
[3] For details on the wide range of Miltenyi Biotec antibodies for MICS, visit **miltenyibiotec.com/MICS-antibodies**
[4] For more information about the latest sample carriers from Miltenyi Biotec, visit **miltenyibiotec.com/MICS-sample-carriers**

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