

The MACSima™ **Platform**

Everything you need for fully automated, spatial analysis

Biological systems and cellular processes are inherently complex due to the interaction of thousands of proteins involved in proper functioning of the entire organism. Thus, an in-depth analysis of biological systems requires the examination of a plethora of parameters in order to decipher the underlying principles. Currently available techniques can provide only a very limited perspective on the complexity of biological systems.

Miltenyi Biotec's MICS (MACSima Imaging Cyclic Staining) technology impressively overcomes these limits as it makes innovative use of trusted fluorescence microscopy techniques to allow the microscopic analysis of an unprecedented number of proteins or other antigens on a single sample.

Based on this technology, Miltenyi Biotec developed the MACSima Platform, which enables fully automated high-plex imaging. The possibility of evaluating localization, expression, and potential interaction of a multitude of different proteins allows scientists to tap the full potential of spatial biology.

D117⁻CD138+CD141⁻CD142+CD155+CD171-CD309⁻CD318-CDCP1+Anti-SSE D156c-ADAM10+- CD184-CXCR4+ CD195+ CD223-LAG3- CD240DCE+- CD27 105⁻CD206⁻ CD317-BST2⁻ Anti-HLA-ABC+- IgD⁻ TSPAN-8+CD3⁻ CD4⁻ CD8 - CD279-PD-1- CD298- CD38-CD46+ CD47+- CD183+- CD27- CD31- CD49f+ :de⁻ CD66c⁻ CD44+ ICAM-1-CD54+- CD59+- HLA-DR+- HLA-DQ- CD45- C[⁻ CD19⁻ CD28⁻ CD49B⁺ CD51⁺ CD56⁺ CD80⁺ CD94⁺ CD95L-CD178-FasL⁺ CDCP1+ Anti-SSEA-4+- IgG- Oct-4- CD23- CD24+ CD58+ CD34+- CD73+- CI DCE+- CD273-PDL-2- CD278-ICOS- CD326+ Anti-CLA+ Podoplanin- CD49c-CD3⁻ CD4⁻ CD8⁻ CD18⁻ CD29+ CD45RO⁻ CD49e+- CD71+- CD146+- CD147+ C CD31⁻ CD49f⁺⁻ CD107a⁺⁻ CD162⁻ CD274-PD-L1⁺⁻ CD163⁻ IgA⁺⁻ CD276⁺⁻ CD² -DQ- CD45- CD20+ CTLA4-CD152- CD86- GITR-CD357- ki-67+- 0X40-CD13 CD73+- CD90+ CD95-Fas+- CD133+ CD156c-ADAM10+- CD184-CXCR4+ CD19 n⁻ CD49c-ITGA3+ CD55-DAF+ CD68- CD105-CD206- CD317-BST2- Anti-HL +- CD147+ CD166+ CD204- CD227+ CD239- CD279-PD-1- CD298- CD38-CD :D276+- CD45RA-Anti-HLA-A2-A28+ CD66acde- CD66c- CD44+ ICAM-1-C X40-CD134⁻ SSEA-1+- Vimentin⁻ CD11c⁻ CD14⁻ CD19⁻ CD28⁻ CD49B+ CD51⁺ D142+ CD155+ CD171- CD309- CD318-CDCP1+ Anti-SSEA-4+- lgG- Oct-4- C 184-CXCR4+ CD195+ CD223-LAG3⁻ CD240DCE+- CD273-PDL-2⁻ CD278-ICC :D298⁻ CD38⁻CD46+ CD47-⁻ CD183+- CD27- CD31- CD49f+- CD107a+- CD162 028⁻ CD49B+ CD51+ CD56+ CD80+ CD94+ CD95L-CD178-FasL+- CD104-Inte lgG⁻ Oct-4⁻ CD23⁻ CD24⁺ CD58⁺ CD34⁺⁻ CD73⁺⁻ CD90⁺ CD95-Fas⁺⁻ CD133 DL-2⁻ CD278-ICOS⁻ CD326+ Anti-CLA+ Podoplanin⁻ CD49c-ITGA3+ CD55-

- CD162 A4-CD15 D104-In CD95-Fa GA3+CI 5+ CD204 -HLA-A2 Viment CD318-CD240D N-8+CD3 CD31⁻ CE -DQ⁻CD

CD178-F

CD90+0

rga3+ ci

5+ CD204

One sample, hundreds of markers

> 40-CD13 D155+ CD 195+ CD BC+- IgD⁻ 7⁺⁻ CD18:

A-A2-A28

D309⁻ CD

0223-LA

ABC+- IgE

4- CD183

HLA-DI

0+ CD94

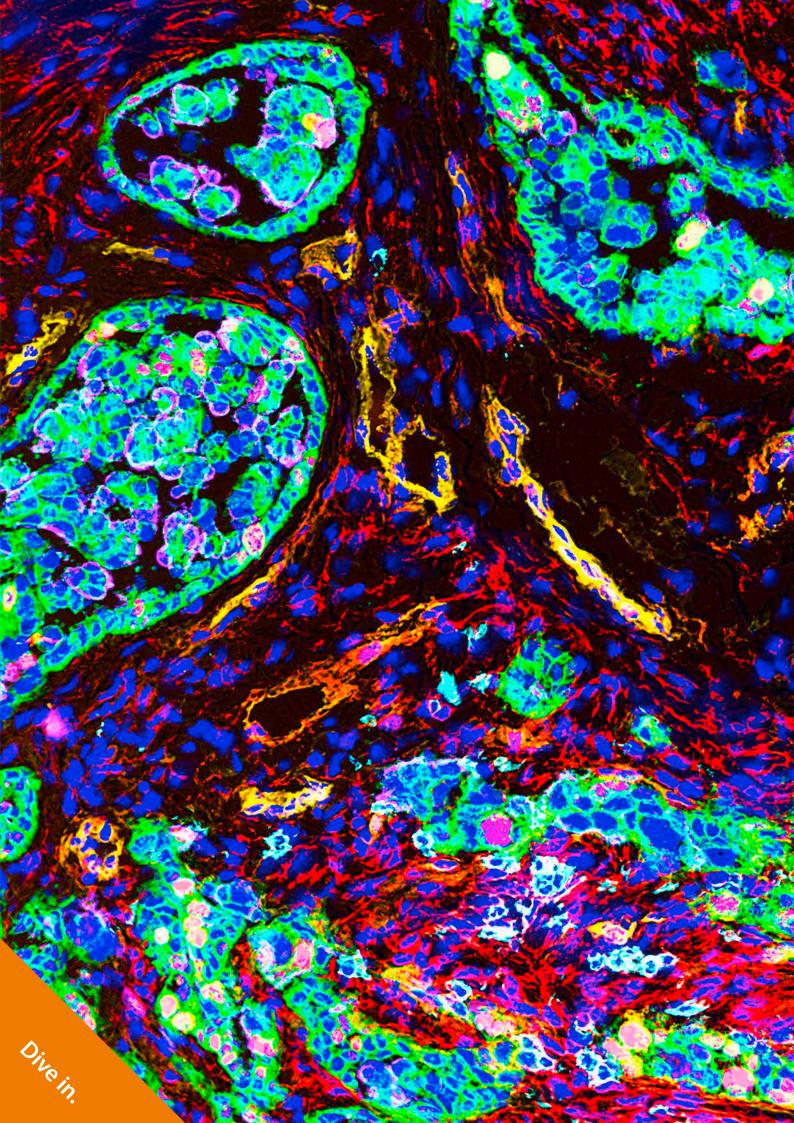
D34⁺⁻ CD

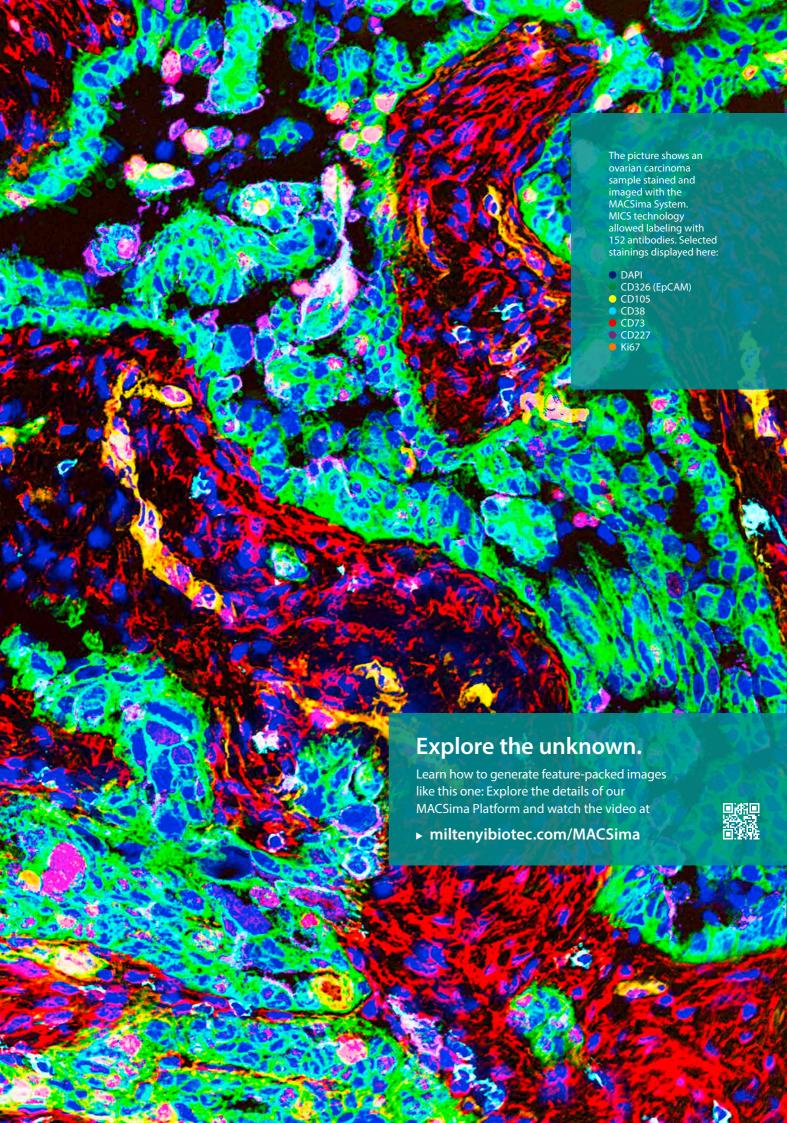
oplanin⁻

46+- CD1

′6+- CD45

Vimentin- CD11c- CD14- CD19- CD28- CD49B+ CD51+ CD56+ CD80+ CD94 CD318-CDCP1+ Anti-SSEA-4+- lgG- Oct-4- CD23- CD24+ CD58+ CD34+- CE CD240DCE+- CD273-PDL-2- CD278-ICOS- CD326+ Anti-CLA+ Podoplanin-N-8+CD3-CD4-CD8-CD18-CD29+CD45RO-CD49e+-CD71+-CD146+-CD CD31- CD49f+- CD107a+- CD162- CD274-PD-L1+- CD163- IgA+- CD276+- CD2 4-DQ- CD45- CD20+ CTLA4-CD152⁻ CD86- GITR-CD357- ki-67+- OX40-CD13 CD90+CD95-Fas+-CD133+CD156c-ADAM10+-CD184-CXCR4+CD195+CD n⁻ CD49c-ITGA3+ CD55-DAF+ CD68⁻ CD105⁻CD206⁻ CD317-BST2⁻ Anti-HL*F* 5+- CD147+ CD166+ CD204⁻ CD227+ CD239⁻ CD279-PD-1- CD298⁻ CD38⁻CD CD276+- CD45RA-Anti-HLA-A2-A28+CD66acde- CD66c- CD44+ ICAM-1-C X40-CD134⁻ SSEA-1⁺⁻ Vimentin⁻ CD11c⁻ CD14⁻ CD19⁻ CD28⁻ CD49B⁺ CD51⁺ CD142+ CD155+ CD171- CD309- CD318-CDCP1+ Anti-SSEA-4+- lgG- Oct-4- CI -BST2-Anti-HLA-ABC+- IgD-TSPAN-8+CD3-CD4-CD8-CD18-CD29+CD45 .D298⁻ CD38⁻CD46⁺ CD47⁺⁻ CD183⁺⁻ CD27⁻ CD31⁻ CD49f⁺⁻ CD107a⁺⁻ CD162⁻ D28- CD49B+ CD51+ CD56+ CD80+ CD94+ CD95L-CD178-FasL+- CD104-Int lgG⁻ Oct-4⁻ CD23⁻ CD24⁺ CD58⁺ CD34⁺⁻ CD73⁺⁻ CD90⁺ CD95-Fas⁺⁻ CD133⁻ DL-2-CD278-ICOS-CD326+ Anti-CLA+ Podoplanin-CD49c-ITGA3+CD55-CD317-BST2-Anti-HLA-ABC+- IgD-TSPAN-8+CD3-CD4-CD8-CD18-CD2 CD298- CD38-CD46+ CD47+- CD183+- CD27- CD31- CD49f+- CD107a+- CD16 D28- CD49B+ CD51+ CD56+ CD80+ CD94+ CD95L-CD178-FasL+- CD104-Into | lgG⁻ Oct-4⁻ CD23⁻ CD24⁺ CD58⁺ CD34⁺⁻ CD73⁺⁻ CD90⁺ CD95-Fas⁺⁻ CD13





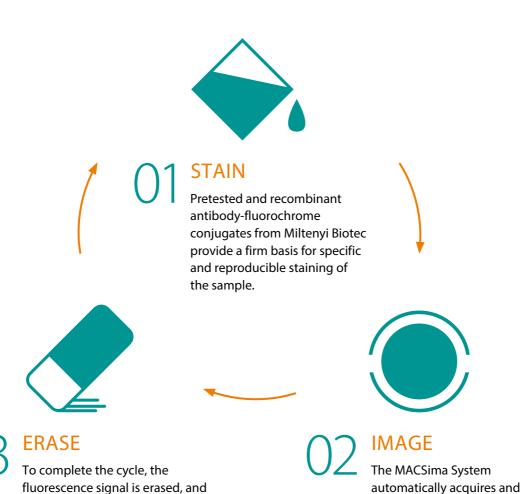
MICS technology – the basic principle

MICS technology enables the simultaneous analysis of hundreds of markers on a single sample based on fluorescence microscopy. It uses the principle of iterative staining with different fluorochromeconjugated antibodies to acquire microscopy data for a multitude of parameters without harming the sample. The iterative process comprises three main steps: fluorescent staining, image acquisition, and

the process restarts automatically.

erasure of the fluorescence signal, all of which are conducted by the MACSima System in a fully automated manner. The resulting stack of potentially hundreds of marker images provides an unprecedented insight into the physiological or pathological characteristics of the sample. Due to on-the-fly processing, data analysis can start at any time, even when the iterative process is still running.

processes highly conclusive epifluorescence images of the desired sample areas.



Complete platform solution

With its automated processes and optimized components, the MACSima Platform reduces the effort required for the generation of complex spatial biology data to the basic essentials. The benefits of this complete, harmonized solution speak for themselves.

Hundreds of markers on one sample

Obtain high-plex spatial data to analyze hundreds of proteins and other antigens on a single sample.

Fully automated instrument

Plan your experiment and leave the execution to the MACSima System.

Broad portfolio of pretested antibodies

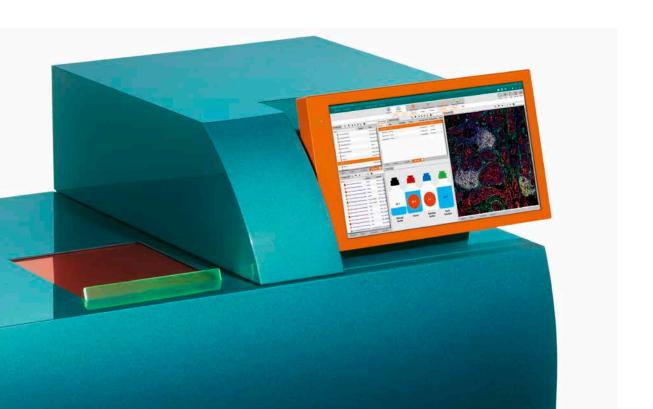
Analyze hundreds of markers on your sample based on a plethora of recombinantly engineered, fluorochrome-conjugated antibodies, tested specifically for MICS.

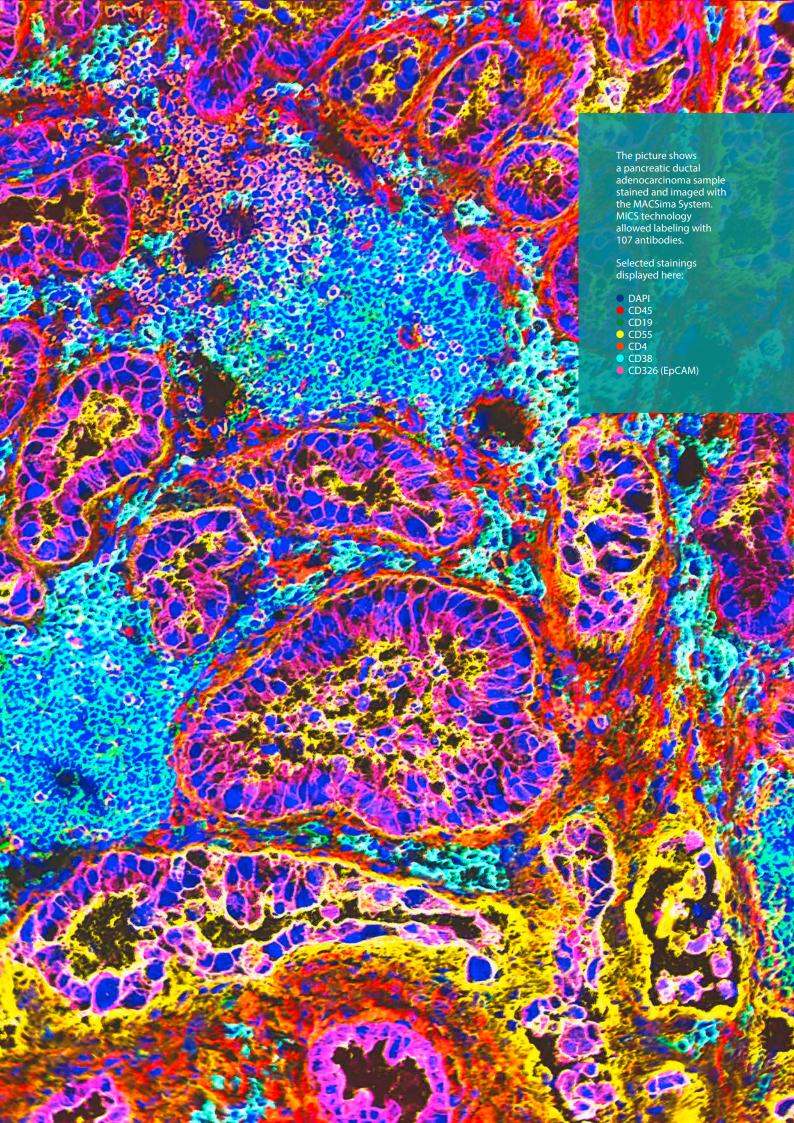
Effortless experiment preparation

Benefit from plug-and-play REAscreen™ Antibody Panels containing pre-defined sets of antibodies from Miltenyi Biotec for an effortless yet comprehensive analysis. Analyze any kind of fixed sample with our MACSwell™ Sample Carriers and benefit from various formats of antibodies for full flexibility.

Sophisticated yet simple analysis software

Analyze your high-plex spatial data easily and comprehensively using MACS® iQ View. Its versatility and intuitive user interface make this image analysis software the perfect companion for your spatial biology experiments.



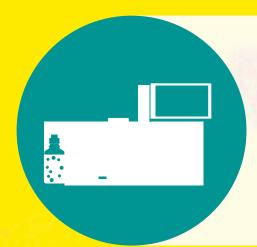






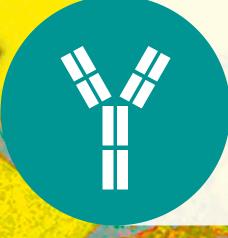
Integrated system allows for effortless and reliable processing

MICS technology is based on the well-established and straightforward technique of immunofluorescence staining. However, to translate this technique into comprehensive and effortless multiparameter imaging, a functional, well-orchestrated system is a prerequisite. The MACSima Platform relies on four essential pillars that are combined seamlessly to ensure the easy generation of conclusive spatial data.



MACSima SYSTEM

At the heart of the platform is the MACSima System, an instrument that performs the iterative staining cycle and data processing in a fully automated fashion.



PRETESTED ANTIBODIES FOR MICS

An extensive and continuously growing portfolio of pretested antibodies for MICS, including recombinantly engineered antibodies, ensures specific staining and reliable analysis of hundreds of markers.





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.

All system components are perfectly geared to each other, allowing truly automated and conclusive spatial analysis based on MICS technology.

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 required liquids accurately and fully automatically. Automated washing processes prevent carryover of reagents to maintain fluorescence signal specificity across all images.



Ultraprecise stage

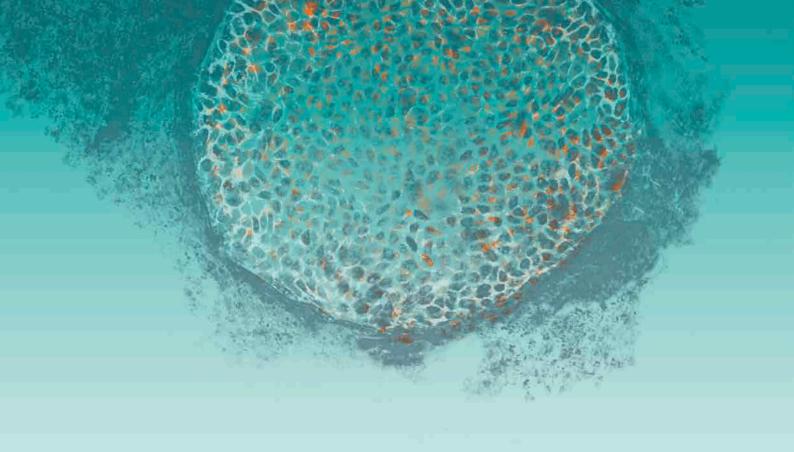
Hosting the sample and all reagents, the ultraprecise stage serves as the MACSima System's work bench. Accurate positioning of all components throughout the MICS experiment ensures reliable execution of the iterative process and exact maintenance of the field of view.



State-of-the-art microscope and camera

The widefield microscope includes one objective for a fast overview scan. Two 20× objectives enable optimal imaging of subcellular details, regardless of the sample carrier used – be it long working distance with microscope slides and MACSwell Imaging Frames or short distance with MACSwell 24 Imaging Plates. The sCMOS camera provides excellent image quality.









Analyze any kind of fixed sample

To answer complex scientific questions you can't afford to be restricted by technical limitations. To give you complete flexibility in the type of fixed sample you want to analyze with the MACSima System, we have developed the MACSwell

Sample Carriers. To support either tissue, adherent or suspension cells, we have designed various types of devices. Each of them contains a well-defined reaction cavity to perform MICS experiments easily and safely and assure you failure-free experimentation.

TISSUE



MACSwell Imaging Frames

can be mounted around any standard microscope slide and provide the reaction cavity needed for a MICS experiment.

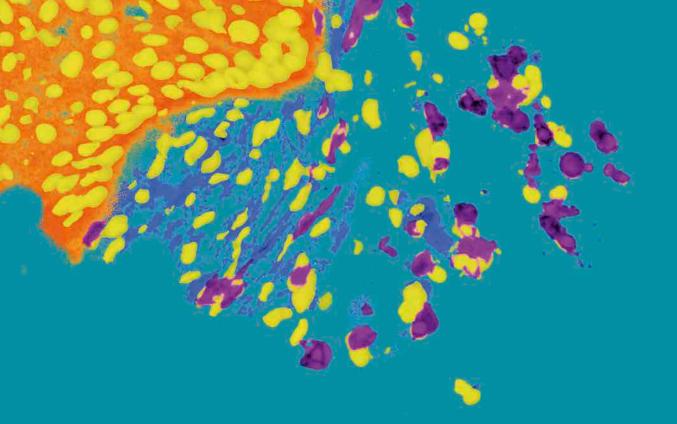
MACSwell Imaging Frames are provided with various different sizes of reaction cavities to perfectly fit the size of your tissue sample.

ADHERENT CELLS

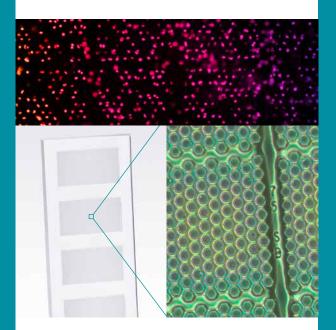


MACSwell 24 Imaging Plates

are pre-assembled and contain 24 rectangular wells with a clear 170 μm thick cover glass bottom. Simply pipet your cell suspension onto the plate, culture as usual, and fix the sample according to your standard protocol directly on the plate.



SUSPENSION CELLS

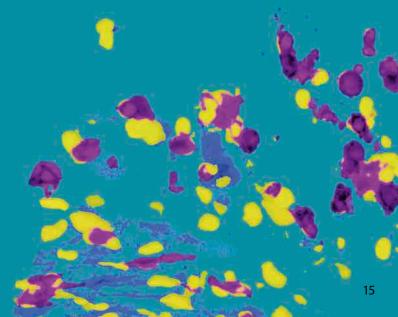


MACSwell Sample Carriers with microcavities

MACSwell Micro Slides contain over 1.5 million hexagonal microcavities, which are perfectly sized to fit exactly one cell. Simply mount your choice of MACSwell Imaging Frame around the slide and pipet your suspension cells in the resulting reaction cavity. For even greater flexibility, MACSwell 24 Micro Imaging Plates complete the portfolio of sample carriers featuring microcavities.

VALIDATED FIXATION METHODS

- ✓ Formalin-fixed paraffin-embedded (FFPE)
- ✓ Paraformaldehyde (PFA)
- ✓ Acetone



Pretested antibodies for MICS

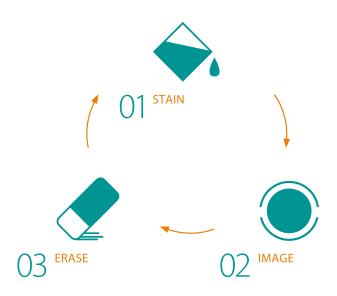
Specific staining – conclusive results

If you want to achieve high reproducibility and error-free analysis, rely on recombinant antibodies.

- World's largest portfolio of antibodies for spatial analysis
- Recombinant antibodies specifically tested for MICS technology
- Tested for compatibility with FFPE-, PFA-, or acetone-fixed samples of human or mouse origin
- Lot-consistent and reproducible results due to sophisticated recombinant antibody technologies



After staining with fluorochrome-conjugated antibodies (01) and image acquisition of the stained sample (02), the fluorescent signal can be erased by either of the two mechanisms shown below.





The fluorescence signal of samples that were stained with fluorochrome-conjugated antibodies, such as our recombinant REAfinity™ Antibodies coupled to non-photostable fluorochromes, can be erased via photobleaching.



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.





Convenient antibody formats



Antibody conjugates for MICS

Choose from a huge portfolio of MICS-pretested antibodies and a variety of fluorochromes to design your panel flexibly.



miltenyibiotec.com/ MICS-antibodies

REAscreen™ Standardized Antibody Panels

Plug-and-play panels of dried MICS-pretested antibody conjugates are designed for maximum convenience and reproducibility. Whether with panels developed by Miltenyi Biotec for specific applications or with customer-designed panels (REAscreen Design), these plates save you time and effort as they eliminate the need for tedious and error-prone manual pipetting.

MACS® iQ View



Analysis software that is sophisticated yet simple

MACS iQ is a family of easy-to-use, powerful software solutions that unlock the full capabilities of Miltenyi Biotec instruments. As the first family member, MACS iQ View was specifically developed to analyze the unprecedented amount of data created by the MACSima Platform. MACS iQ View makes handling and analysis of hundreds of images as easy as it gets: Conveniently display all experimental markers individually or in any combination you need. Organize your data efficiently based on a variety of display options. 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.

Visit our webpage to learn more about the MACS iQ View Software.

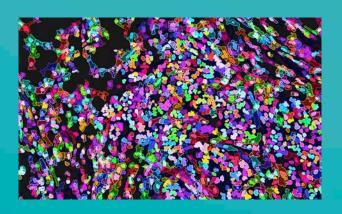


► miltenyibiotec.com/MACS-iQ-View



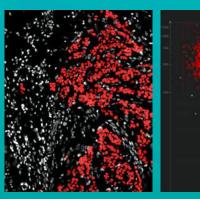


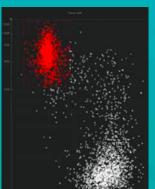




Easy segmentation

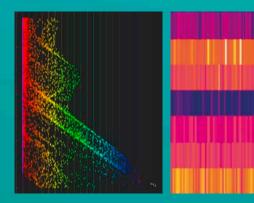
Defining the cell and nucleus boundaries is an easy task with the MACS iQ View Software. Simple definition of parameters and fast processing let you start with your analysis at once. Choose between various pre-defined segmentation options or conveniently import your own trusted segmentation mask.

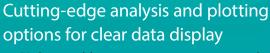




Multifaceted gating and clustering enable deep phenotyping

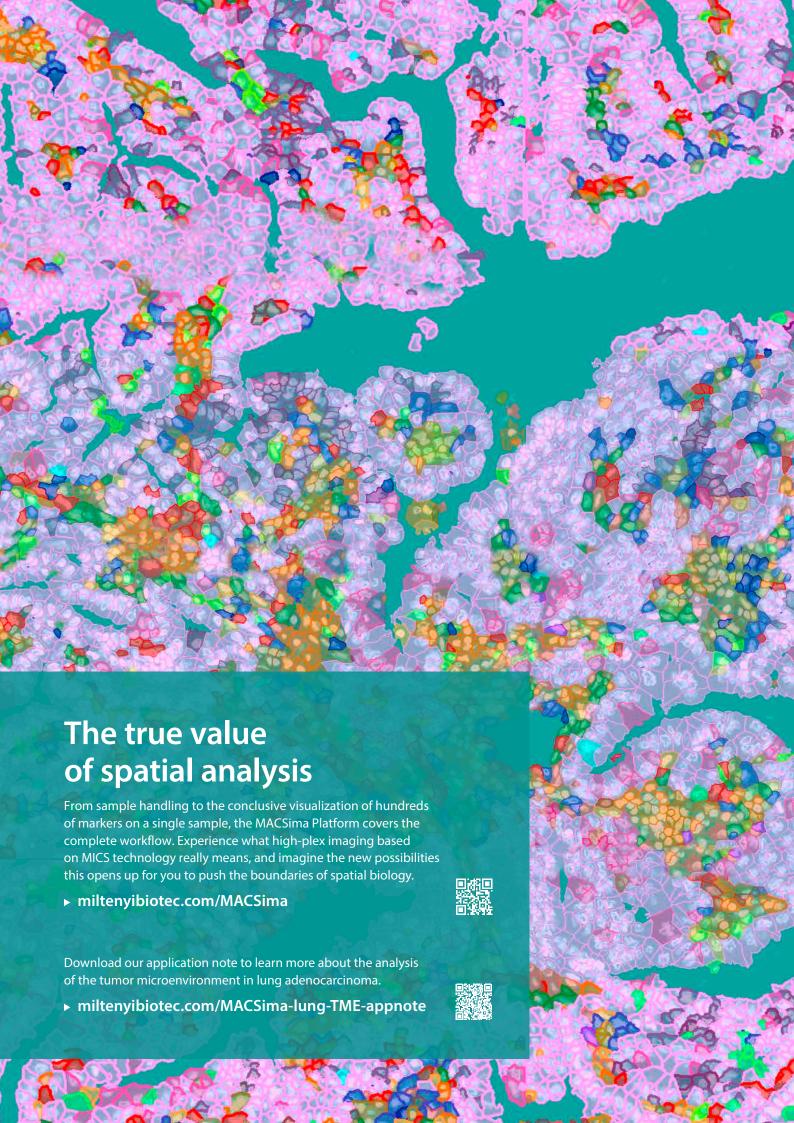
Deep phenotyping can be achieved through highly interactive, dynamic manual gating both on the graph and the image. A workflow editor lets you keep track of all selection steps. Alternatively, you can use the integrated clustering options according to the features of your interest. All this allows you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





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 easily illustrate your intricate data.





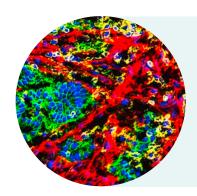




High-plex imaging in action

The MACSima Platform revolutionizes the world of high-plex imaging. Comprehensive analysis of a vast number of markers not only allows you to enhance your present research; the data will also provide you with a tremendous archive to answer future questions that you haven't even thought

about at the time of your experiment. You can go back to the complete datasets from your MICS experiment at any time and look at them from a new perspective. The sky is the limit when it comes to ultrahigh-plex imaging in spatial biology. Here are some examples.



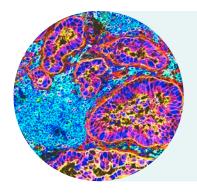
Spatial single-cell atlas of the liver

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





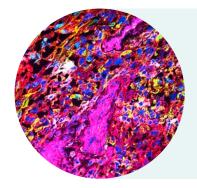
Identification of target candidates for CAR T cell-based immunotherapy of pancreatic adenocarcinoma

Pancreatic ductal adenocarcinoma samples were fixed with acetone and screened based on 107 markers.

Read the publication in Nature Communications.

▶ nature.com/articles/s41467-021-21774-4





New potential target pair for CAR T cell treatment of solid tumors

In this publication, more than 300 antibodies were applied to analyze a single PFA-fixed tissue sample.

Read the full story.

▶ nature.com/articles/s41598-022-05841-4



Take a look at our scientific content featuring the MACSima Platform.

▶ miltenyibiotec.com/MACSima-resources



All you need for your spatial biology experiment

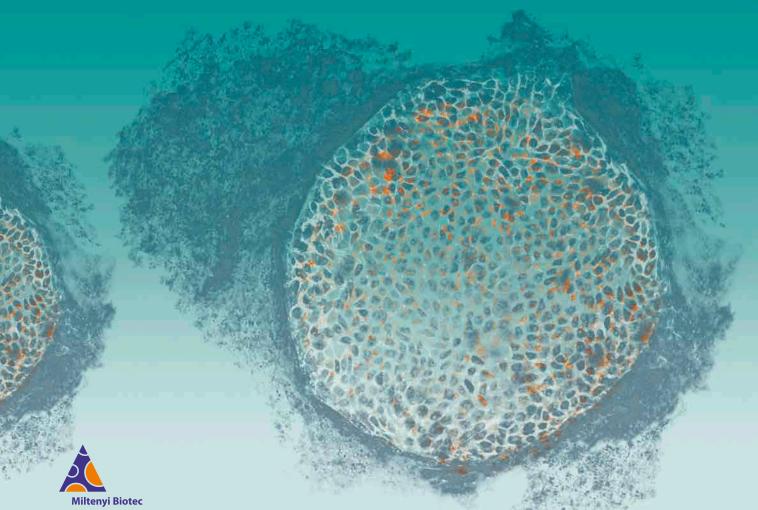
MACSima System specifications		
Microscope		
Instrument type	Widefield epifluorescence microscope	
Camera	Equipped with a latest-generation sCMOS camera with high resolution and thermoelectrically cooled sensor (15 megapixel, large 25 mm diagonal field of view)	
Excitation	Six high-power excitation LEDs (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 \times 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 PC1External 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	 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 \times 780 mm \times 650 mm (w \times d \times h); footprint: 1,210 mm \times 750 mm (w \times d) • MACSima Table¹: 1,250 mm \times 765 mm \times 813 mm (w \times d \times h)	
Weight	• Instrument: 170 kg • MACSima Table¹: 120 kg	

Product type	Order no.	
Instrument		
MACSima System		
MACSima Table	150-001-781	
MACSima 4K monitor		
Software		
MACS iQ View Analysis Software Licenses ²		
• Annual license		
• Permanent license		
Antibodies and reagents		
MICS-pretested antibody conjugates ³		
• REAfinity Recombinant Antibodies	see website	
REAdye_lease Releasable Fluorochromes	see website	
• REAlease Releasable Antibodies		
Pre-defined Antibody Panels for MICS ³		
• REAscreen MAX Kit, human, FFPE	see website	
• REAscreen MAX Kit, human, PFA	see website	
• REAscreen MAX Kit, mouse, PFA		
Support Reagents for MICS		
MACSima Stain Support Kit, human	130-127-574	
MACSima Stain Support Kit, mouse	130-127-575	
Sample carriers ⁴		
MACSwell One Imaging Frames		
MACSwell One Small Imaging Frames		
MACSwell Two Imaging Frames	see website	
MACSwell Four Imaging Frames		
MACSwell 24 Imaging Plates		
Buffers and accessories		
MACSima Buffer Starting Kit	130-125-753	
MACSima Running Buffer 6×1.5 L	130-121-565	
MACSima System Buffer 6×1.5 L	130-125-315	
MACSQuant®/MACSima Storage Solution 6×1.5 L		
MACSwell Deepwell Plates	130-126-865	
MACSwell Sealing Foils	130-126-866	

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**

miltenyibiotec.com/MACSima



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