

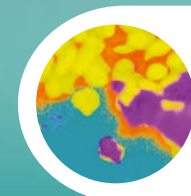
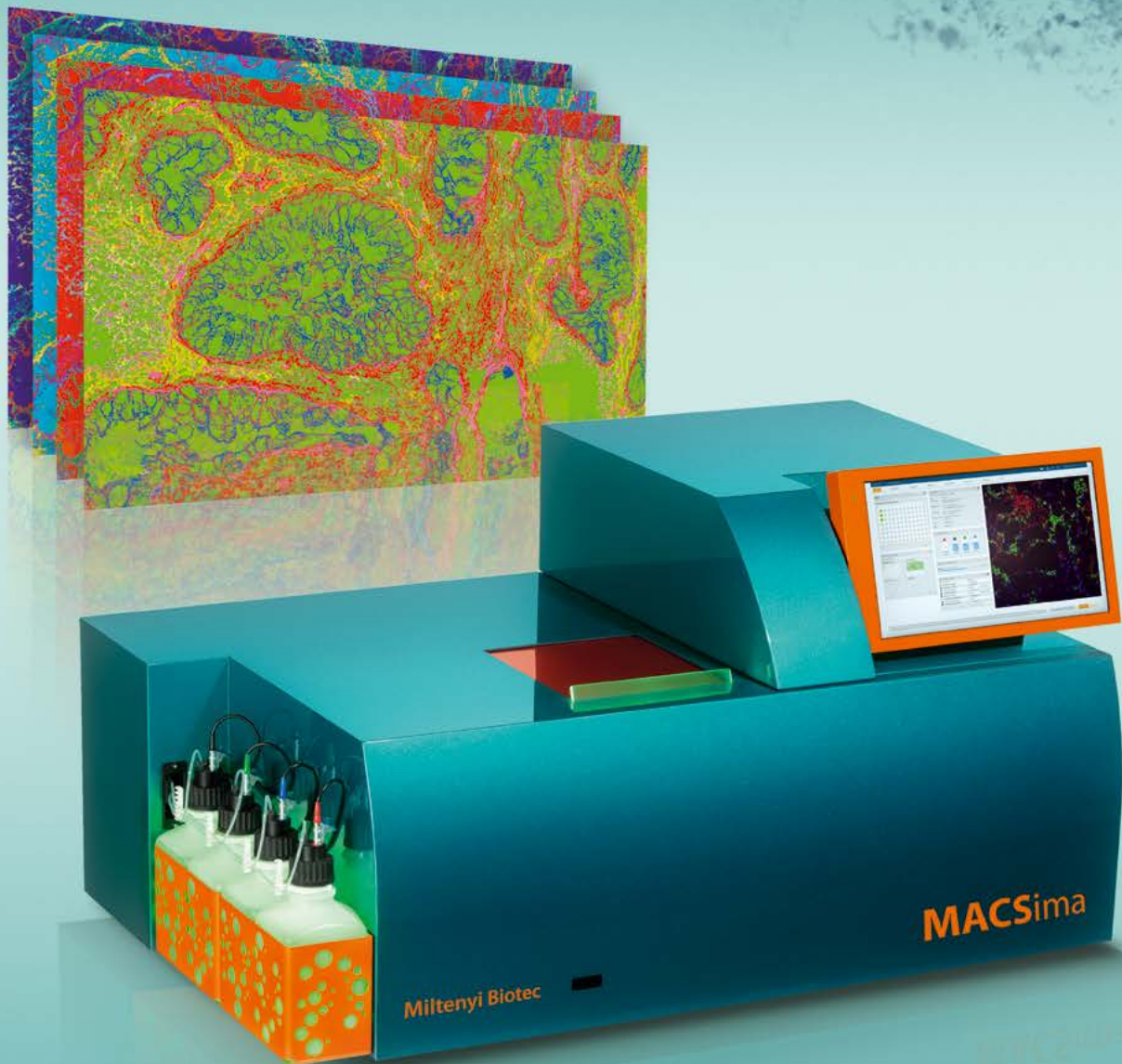


Miltenyi Biotec

CytoGenics   
life science Innovation

# MACSima™ Imaging Platform

The easy way to ultrahigh-content imaging



UNDERSTAND  
NATURE'S  
COMPLEXITY

# The MACSima™ Imaging Platform

Everything you need  
for fully automated,  
ultrahigh-content imaging

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 Imaging Platform, which enables fully automated high-content 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.

One sample,  
hundreds  
of markers

The picture shows an ovarian carcinoma sample stained and imaged with the MACSima Imaging System. MICS technology allowed labeling with 152 antibodies. Selected stainings displayed here:

- DAPI
- CD326 (EpCAM)
- CD105
- CD38
- CD73
- CD227
- Ki67

## Explore the unknown.

Learn how to generate feature-packed images like this one: Explore the details of our MACSima Imaging Platform and watch the video at

► [miltenyibiotec.com/MACSima](http://miltenyibiotec.com/MACSima)

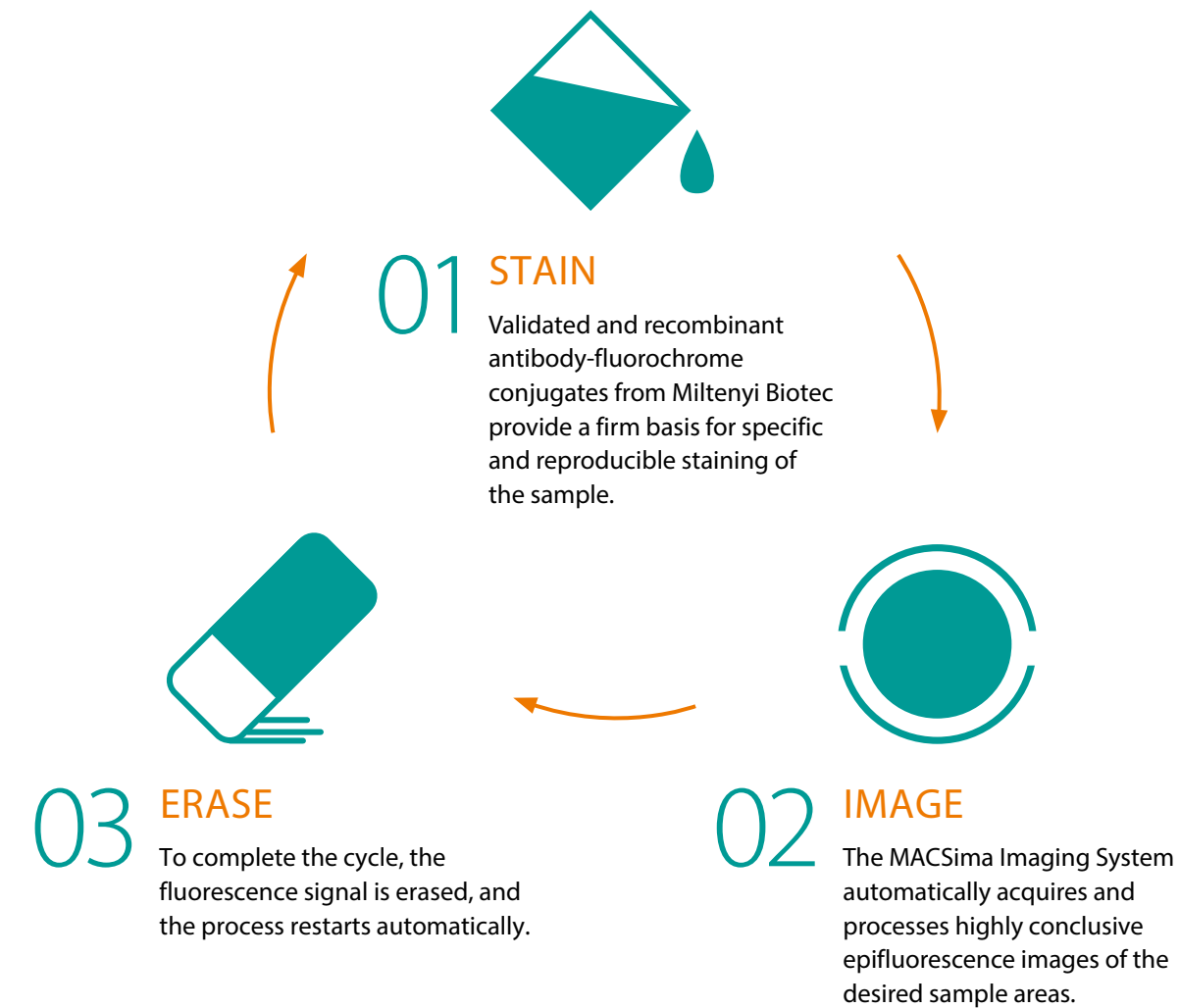


Dive in.

## 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 fluorochrome-conjugated 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 erasure of the fluorescence signal, all of which are conducted by the MACSima Imaging 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.



## Complete platform solution

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

### Hundreds of markers on one sample

Obtain high-content imaging 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 Imaging System.

### Broad portfolio of validated antibodies

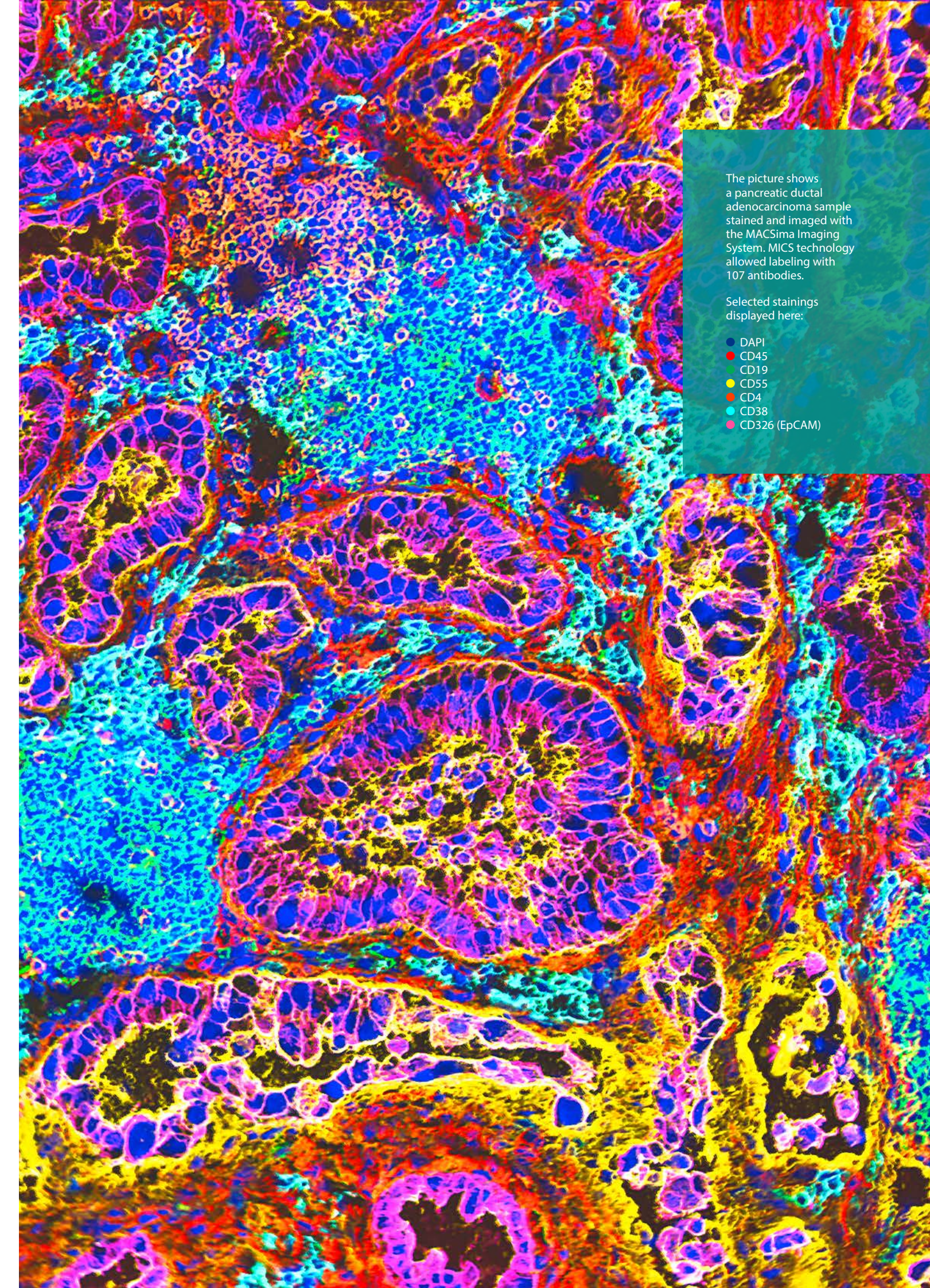
Analyze hundreds of markers on your sample based on a plethora of recombinantly engineered, fluorochrome-conjugated antibodies, validated specifically for MICS.

### Effortless experiment preparation

Benefit from ready-to-use 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 ultrahigh-content imaging 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.



The picture shows a pancreatic ductal adenocarcinoma sample stained and imaged with the MACSima Imaging System. MICS technology allowed labeling with 107 antibodies.

Selected stainings displayed here:

- DAPI
- CD45
- CD19
- CD55
- CD4
- CD38
- CD326 (EpCAM)

## Ultrahigh-content imaging made easy



- 1 Set up experiment**  
Prepare your sample and antibody panel, taking your preferred fixation method into account.



- 2 Start experiment**  
Place your sample and reagent plates in the MACSima Imaging System and define your preferred imaging area. Start the experiment and leave the iterative MICS technology-based process to the MACSima Imaging System.



- 3 On-the-fly data analysis**  
Start analyzing your acquired data, even if the experiment is still running.

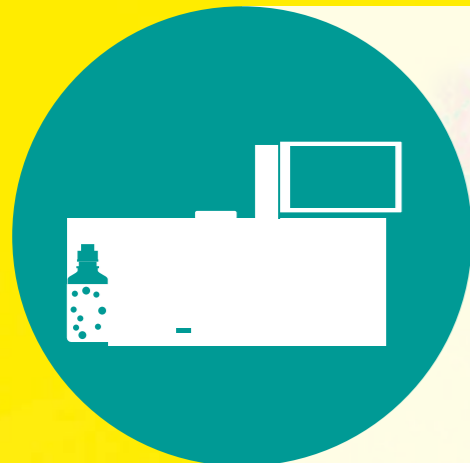
MACSima

# 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 Imaging Platform relies on four essential pillars that are combined seamlessly to ensure the easy generation of conclusive high-content imaging data.

## MACSima Imaging Platform



### MACSima IMAGING SYSTEM

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



### VALIDATED ANTIBODIES FOR MICS

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

### 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 enormous data stacks obtained by the MACSima Imaging System, this software is the perfect tool to dissect the multidimensional datasets and to extract the underlying information in an easy and comprehensive manner.



# MACSima Imaging System

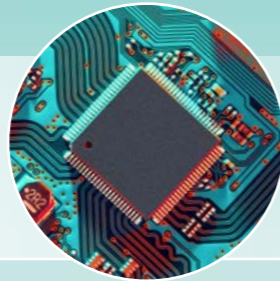
Fully automated sample handling and data acquisition

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

All system components are perfectly geared to each other, allowing truly automated and conclusive ultrahigh-content imaging 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.



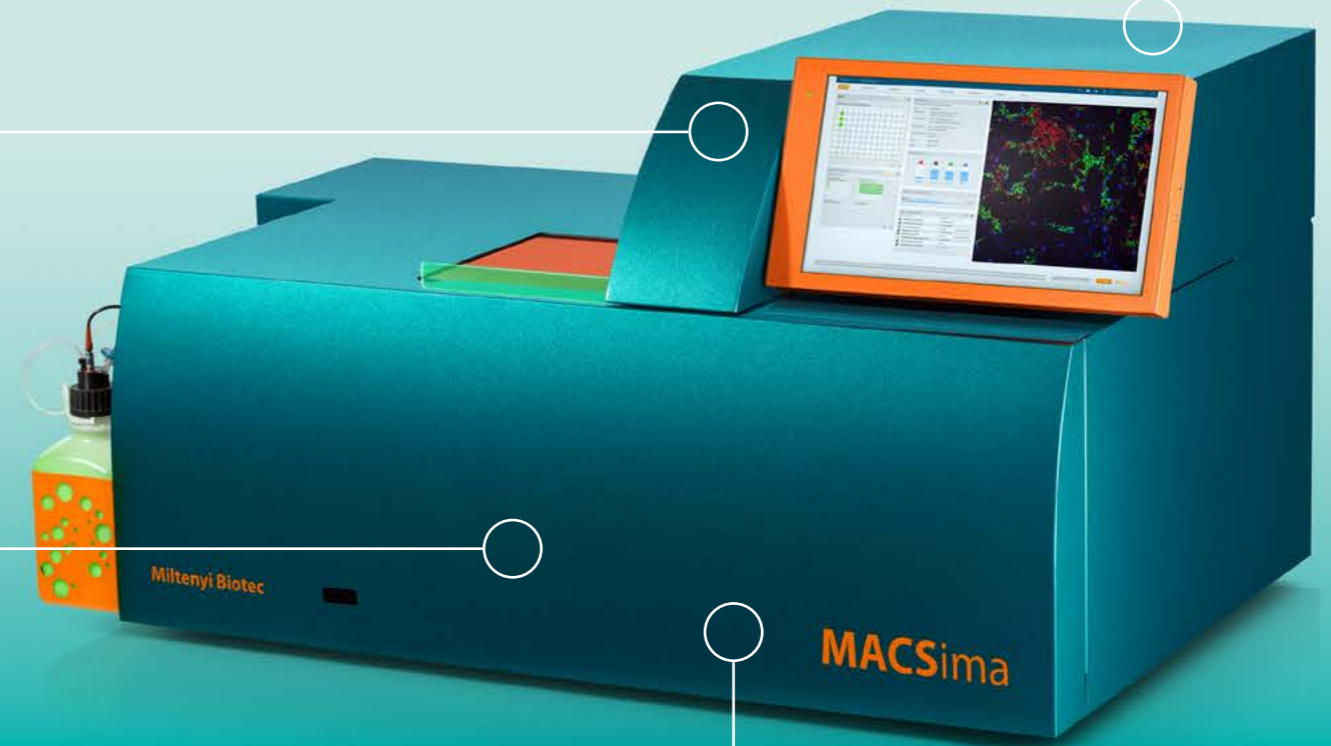
## Ultraprecise stage

Hosting the sample and all reagents, the ultraprecise stage serves as the MACSima Imaging 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 20x 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.

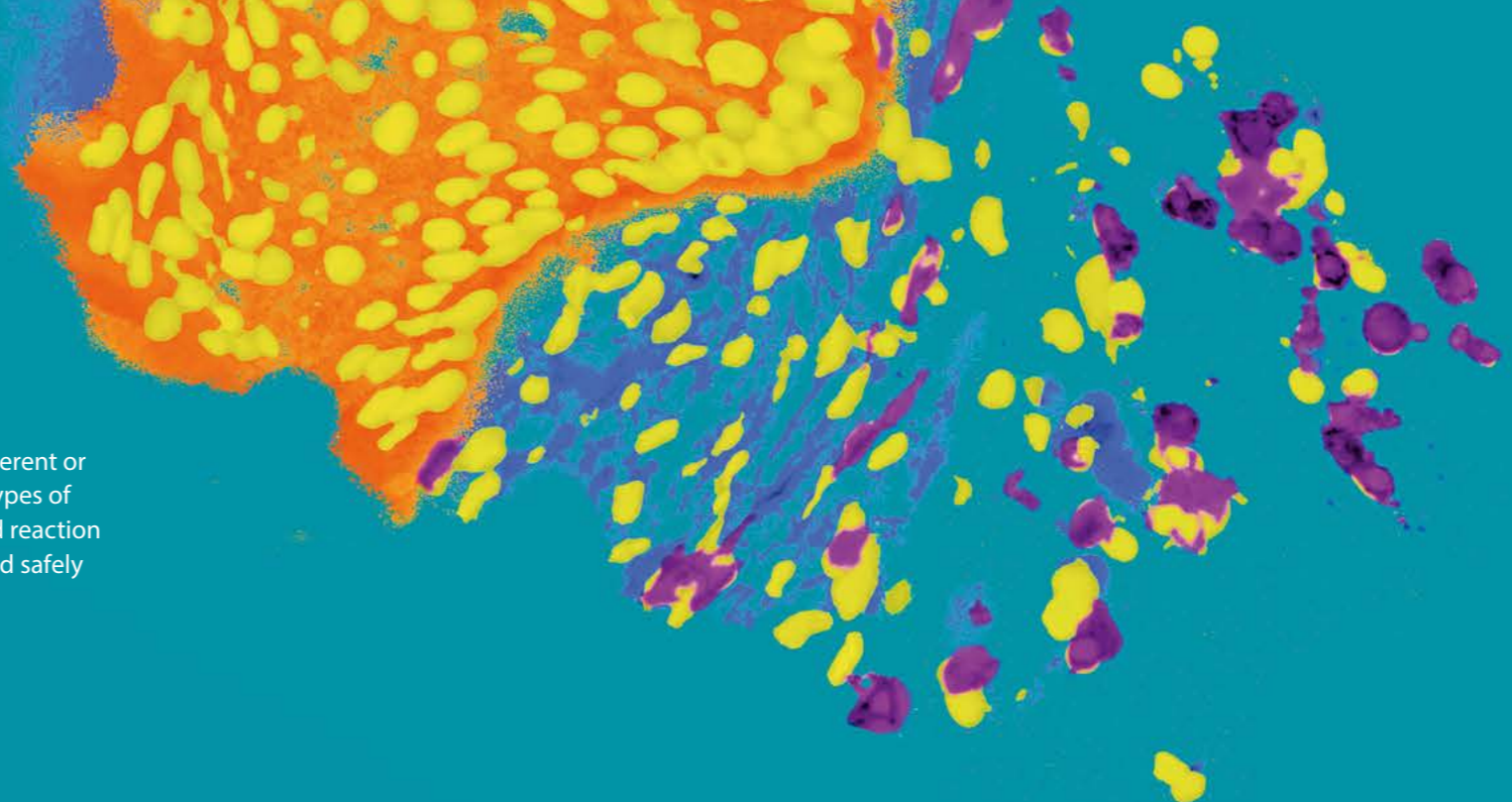


# MACSwell™ Sample Carriers

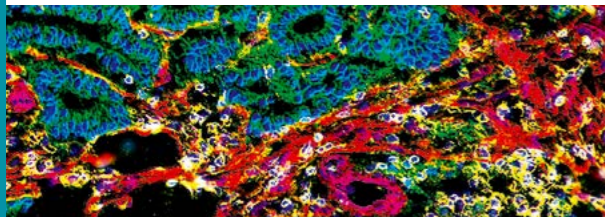
Analyze any kind of fixed sample

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 Imaging 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.



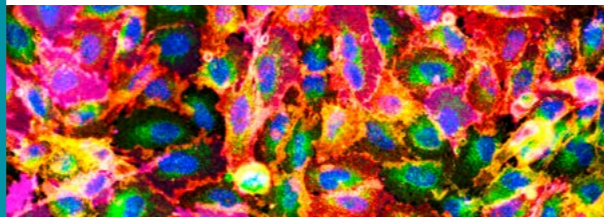
## TISSUE



### 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.

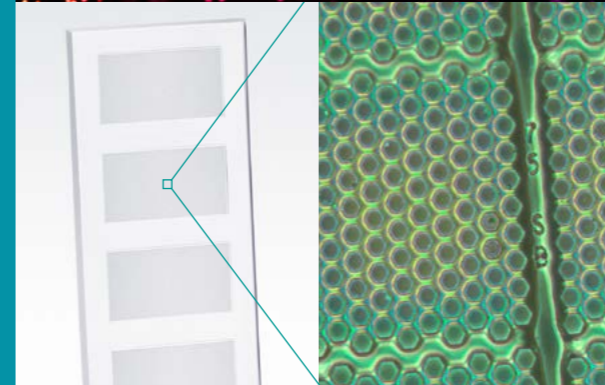
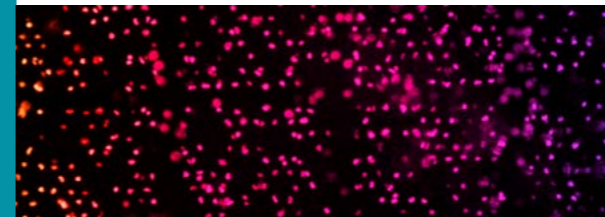
## ADHERENT CELLS



### MACSwell 24 Micro 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

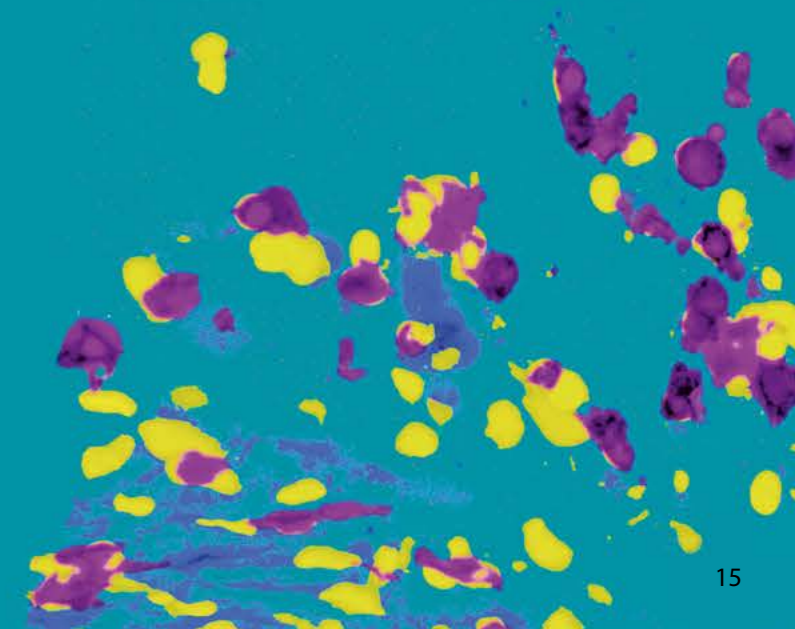


### MACSwell Sample Carriers with microcavities

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



# Validated antibodies for MICS

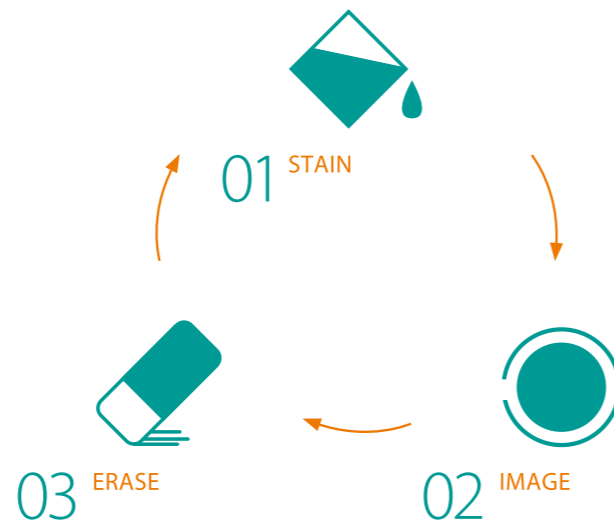
## Specific staining – conclusive results

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

- World's largest portfolio of antibodies for ultrahigh-content imaging
- Recombinant antibodies specifically validated 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

### The two mechanisms for signal erasure

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

Explore   
**500+**  
**antibodies**  
 ▶ [miltenyibiotec.com/MICS-antibodies](https://miltenyibiotec.com/MICS-antibodies)



**Antibody conjugates for MICS**  
 Choose from a huge portfolio of MICS-validated antibodies and a variety of fluorochromes to design your panel flexibly.

**REAscreen™ Standardized Antibody Panels**  
 Ready-to-use panels of dried MICS-validated 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



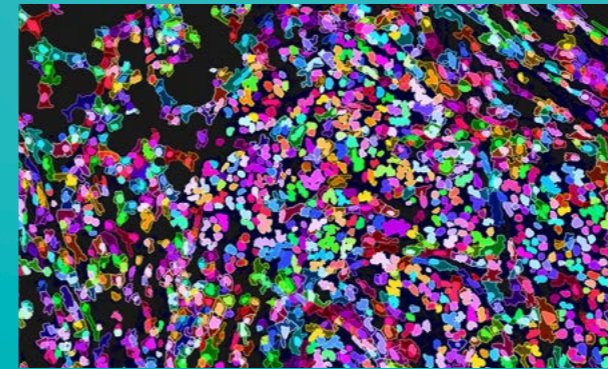
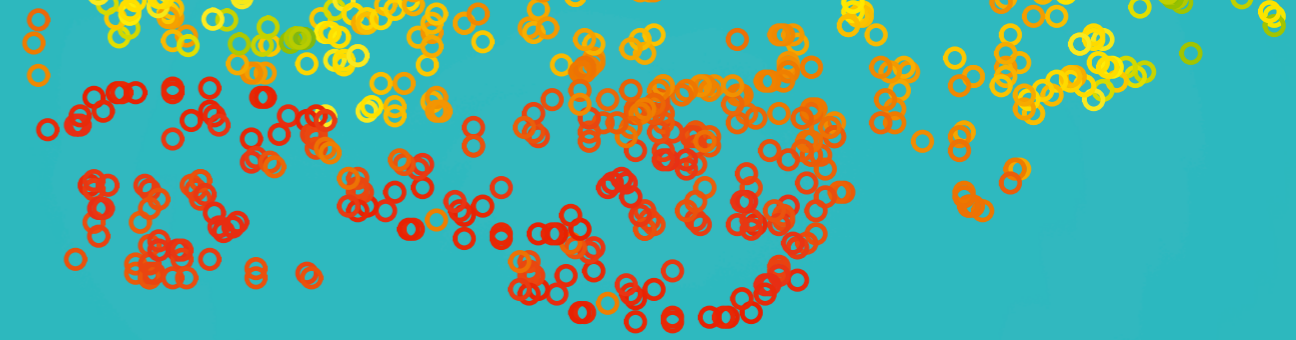
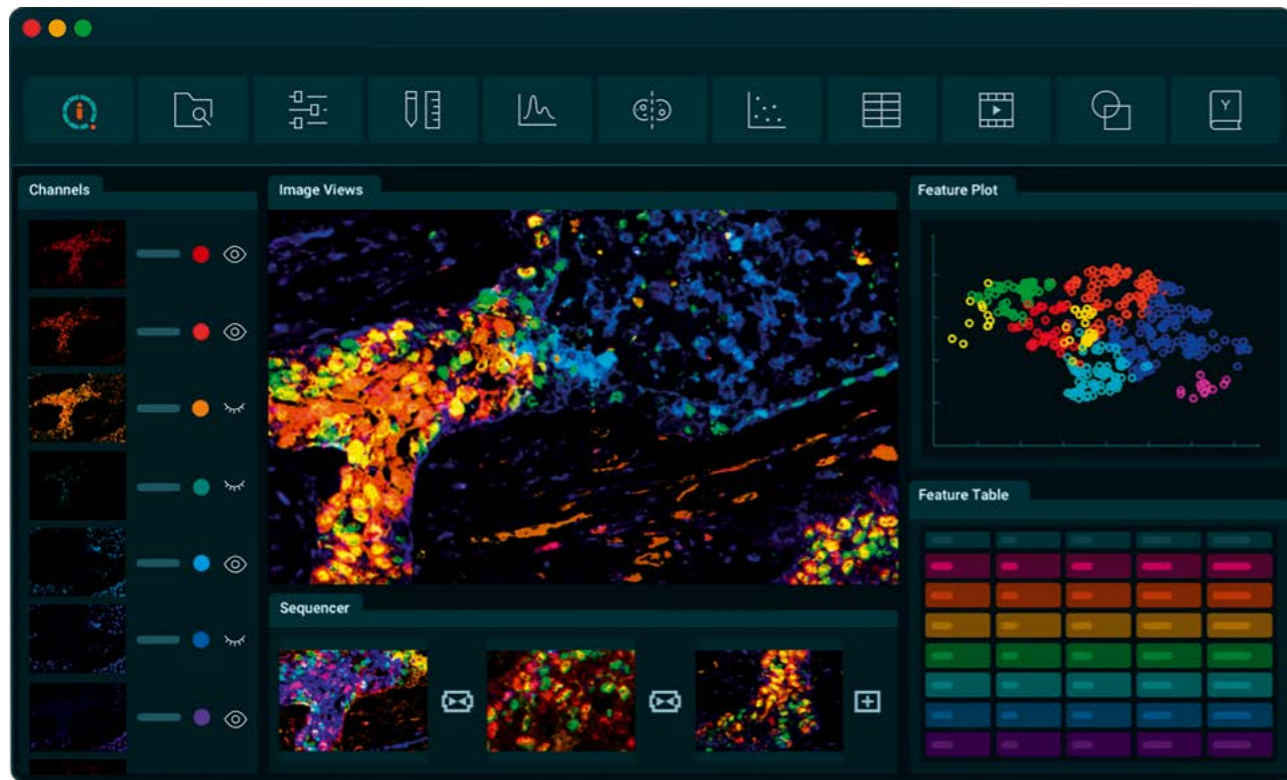
MACS iQ is a family of easy-to-use, powerful software solutions that unlock the full capabilities of Miltenyi Biotec instruments. As the first family member, MACS iQ View was specifically developed to analyze the unprecedented amount of data created by the MACSima Imaging System. MACS iQ View makes handling 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.

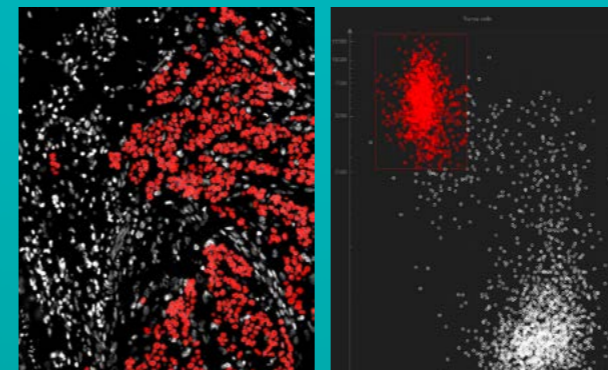
Visit our webpage to learn more about the MACS iQ View Software.

► [miltenyibiotec.com/MACS-iQ-View](https://miltenyibiotec.com/MACS-iQ-View)



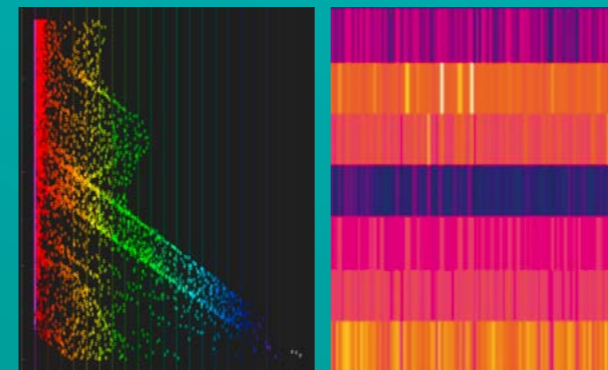
## Easy segmentation

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.



## 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 easily illustrate your intricate data.





The picture shows a sample of lung adenocarcinoma that was stained with more than 125 antibodies to characterize the tumor microenvironment in detail. Identification of more than 20 known cell populations based on more than 40 established markers enabled the dissection of intricate marker combinations for a deep phenotyping of the sample. However, the option to use such a large number of antibodies on a single sample also allows the discovery of as yet unknown cell populations.

- CD4<sup>+</sup> T cells
- CD8<sup>+</sup> 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

## The true meaning of ultrahigh-content imaging

From sample handling to the conclusive visualization of hundreds of markers on a single sample, the MACSima Imaging Platform covers the complete workflow. Experience what ultrahigh-content imaging based on MICS technology really means, and imagine the new possibilities this opens up for you to push the boundaries of spatial biology.

► [miltenyibiotec.com/MACSima](https://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](https://miltenyibiotec.com/MACSima-lung-TME-appnote)



# One experiment, myriads of findings



## Deep phenotyping

Characterization of hundreds of markers on all cells of a tissue sample enables you to identify a multitude of different cell types and explore their spatial relationships. These data provide you with an immense resource for deep phenotyping studies.



## Biomarker research and discovery

Understanding how the different markers correlate in your sample is crucial for the discovery of biomarker signatures. Ultimately, these findings can help you to develop patient stratification strategies and novel therapies for many diseases.



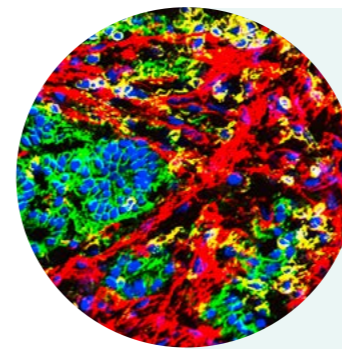
## Drug target discovery and validation

Ultrahigh-content analysis of this huge number of markers also allows you to identify potential drug targets. Resulting candidates can then be further analyzed for their effectiveness and validated in the spatial context of the full microenvironment in preclinical research studies using the MACSima Imaging Platform.

# Ultrahigh-content imaging in action

The MACSima Imaging Platform revolutionizes the world of high-content 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-content imaging in spatial biology. Here are some examples.

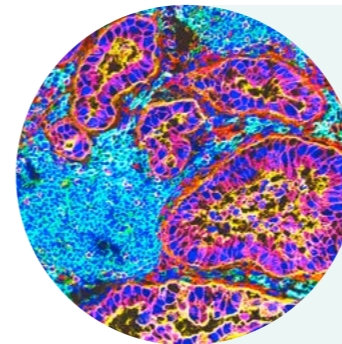


## Spatial single-cell atlas of the liver

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](https://cell.com/cell/fulltext/S0092-8674(21)01481-1)

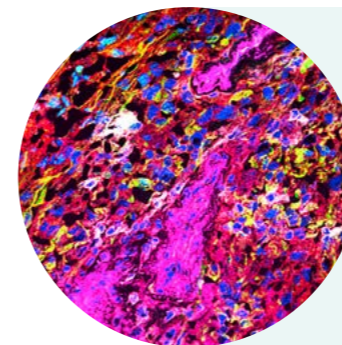


## Identification of target candidates for CAR T cell-based immunotherapy of pancreatic adenocarcinoma

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](https://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](https://nature.com/articles/s41598-022-05841-4)



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

► [miltenyibiotec.com/MACSima-resources](https://miltenyibiotec.com/MACSima-resources)



# All you need for your ultrahigh-content experiment

MACSima Imaging System specifications	
<b>Microscope</b>	
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	<ul style="list-style-type: none"> <li>• 2× objective to generate overview images; NA 0.1</li> <li>• 20× long working distance objective (designed for 1 mm thick slides); NA 0.45</li> <li>• 20× objective with high numerical aperture (designed for 170 µm thick cover glass); NA 0.75</li> </ul>
Bleaching unit	<ul style="list-style-type: none"> <li>• Separate bleaching unit for optimized signal erasure</li> <li>• Illuminated area: 3 mm × 3 mm</li> <li>• Light intensity: 2 W</li> </ul>
<b>Liquid handling system</b>	
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
<b>Sample and reagent stage</b>	
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
<b>Technical data</b>	
Computer	Two integrated computers for experiment planning/execution and image analysis
Monitors	<ul style="list-style-type: none"> <li>• Tiltable integrated touch display (Full HD) for PC1</li> <li>• External 4K monitor for PC2</li> </ul>
Storage/ports	<ul style="list-style-type: none"> <li>• 25 TB integrated storage to store data of several experiments</li> <li>• 2× USB 3.0</li> <li>• HDMI port</li> </ul>
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
<b>MACS iQ View Software</b>	
Control software	<ul style="list-style-type: none"> <li>• Comprehensive sample and reagent management system</li> <li>• Easy planning and design of both simple and complex experiments</li> <li>• User-friendly execution and monitoring of experiments</li> </ul>
Analysis software	<ul style="list-style-type: none"> <li>• Easy display of hundreds of markers from high-dimensional datasets</li> <li>• Fast and flexible segmentation</li> <li>• Interactive gating</li> <li>• Unbiased data analysis (k-means clustering, UMAP and t-SNE calculation)</li> <li>• Multiple plotting options (histogram, scatter plot, strip and violin plots, heatmaps)</li> <li>• Workflow editor</li> <li>• Distance analyses</li> </ul>
<b>Size and weight</b>	
Dimensions	<ul style="list-style-type: none"> <li>• Instrument dimension: 1,220 mm × 780 mm × 650 mm (w × d × h); footprint: 1,210 mm × 750 mm (w × d)</li> <li>• MACSima Table<sup>1</sup>: 1,250 mm × 765 mm × 813 mm (w × d × h)</li> </ul>
Weight	<ul style="list-style-type: none"> <li>• Instrument: 160 kg</li> <li>• MACSima Table<sup>1</sup>: 120 kg</li> </ul>

Product type	Order no.
<b>Instrument</b>	
MACSima Imaging System	130-121-164
MACSima Table	150-001-781
MACSima 4K monitor	150-001-775
<b>Software</b>	
<b>MACS iQ View Analysis Software Licenses<sup>2</sup></b>	
<ul style="list-style-type: none"> <li>• Annual license</li> <li>• Permanent license</li> </ul>	
<b>Antibodies and reagents</b>	
<b>MICS-validated antibody conjugates<sup>3</sup></b>	
<ul style="list-style-type: none"> <li>• REAfinity Recombinant Antibodies</li> <li>• REA_dye_lease Releasable Fluorochromes</li> <li>• REAlease Releasable Antibodies</li> </ul>	see website
<b>Pre-defined Antibody Panels for MICS<sup>3</sup></b>	
<ul style="list-style-type: none"> <li>• REAscreen MAX Kit, human, FFPE</li> <li>• REAscreen MAX Kit, human, PFA</li> <li>• REAscreen MAX Kit, mouse, PFA</li> </ul>	see website
<b>Support Reagents for MICS</b>	
<ul style="list-style-type: none"> <li>• MACSima Stain Support Kit, human</li> <li>• MACSima Stain Support Kit, mouse</li> </ul>	130-127-574 130-127-575
<b>Sample carriers<sup>4</sup></b>	
<ul style="list-style-type: none"> <li>• MACSwell One Imaging Frames</li> <li>• MACSwell One Small Imaging Frames</li> <li>• MACSwell Two Imaging Frames</li> <li>• MACSwell Four Imaging Frames</li> <li>• MACSwell 24 Imaging Plates</li> </ul>	see website
<b>Buffers and accessories</b>	
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 <sup>®</sup> /MACSima Storage Solution 6×1.5 L	130-092-748
MACSwell Deepwell Plates	130-126-865
MACSwell Sealing Foils	130-126-866

<sup>1</sup> The MACSima Imaging System is delivered with a trolley table specifically designed to support the system. It has anti-vibration properties and provides a closed storage compartment.

<sup>2</sup> For detailed information about the different license options, visit [miltenyibiotec.com/MACS-iQ-View](https://miltenyibiotec.com/MACS-iQ-View)

<sup>3</sup> For details on the wide range of Miltenyi Biotec antibodies for MICS, visit [miltenyibiotec.com/MICS-antibodies](https://miltenyibiotec.com/MICS-antibodies)

<sup>4</sup> For more information about the latest sample carriers from Miltenyi Biotec, visit [miltenyibiotec.com/MICS-sample-carriers](https://miltenyibiotec.com/MICS-sample-carriers)



[www.cytogenics.com.br](http://www.cytogenics.com.br)



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