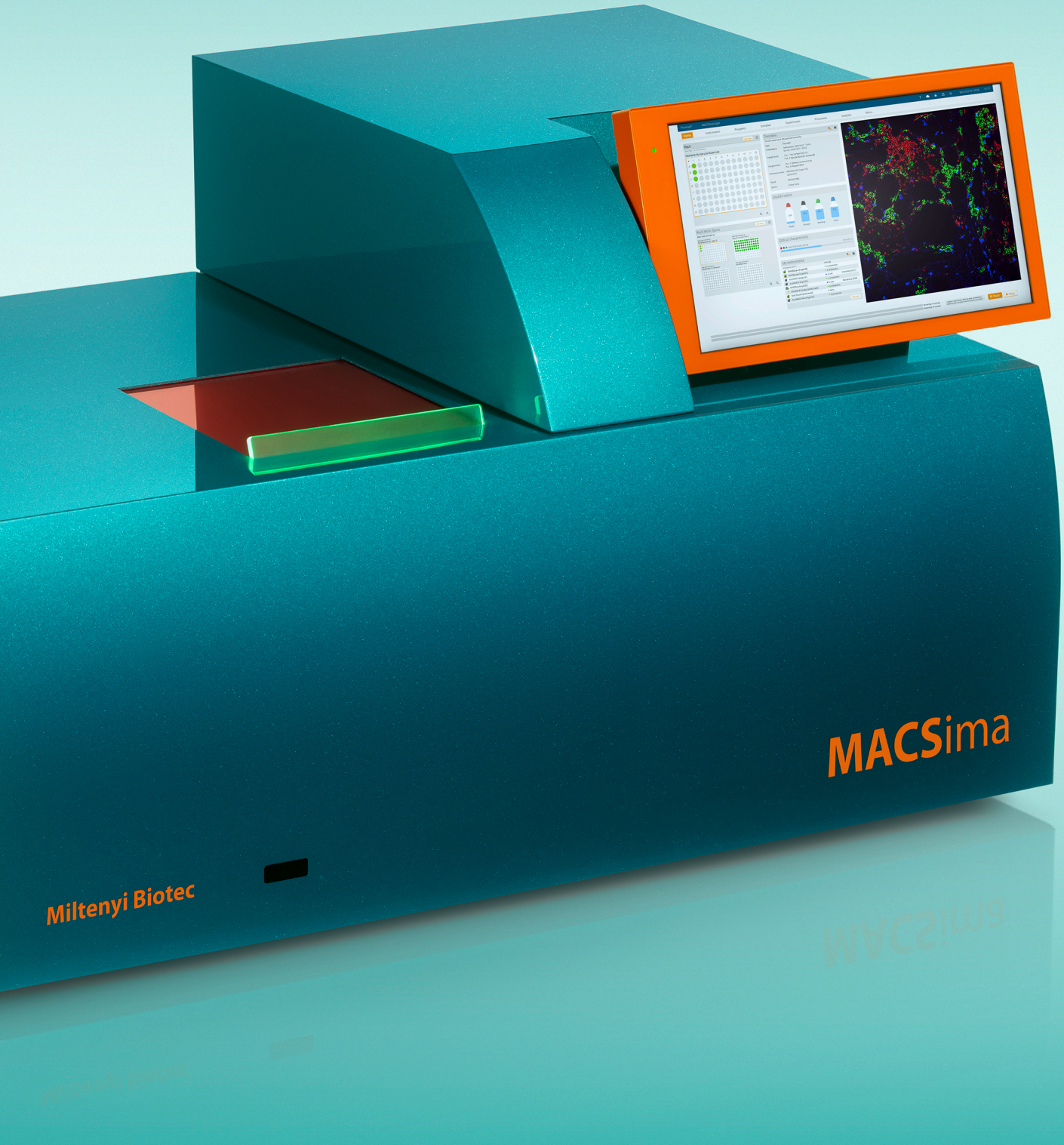
Miltenyi Biotec

# MACSima™ Imaging System

## Software user manual



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# MACSima™ Imaging System

## User manual

Original instructions

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# Read the chapter **Important safety information** and all other information in the hardware user manual before using the instrument

⚠WARNING Before using the MACSima Imaging System, read the chapter **Important safety information** in the MACSima Imaging System hardware user manual and all other information contained in this software user manual, including any safety and operating instructions. Pay special attention to all warnings displayed on the instrument. Failure to read and follow these guidelines could lead to improper or incorrect usage and result in damage to the instrument. Improper usage could also cause severe personal injury, death, unpredictable results, instrument malfunction, and premature wear to components shortening the lifetime of the instrument. Such actions may void your warranty. Keep the user manual and any other safety and operating instructions provided with the instrument in a safe place accessible to all users for future reference.

If you have a serious concern regarding the safe use of your instrument, contact your authorized Miltenyi Biotec service provider or call Miltenyi Biotec Technical Support.

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# 1 Basic functions



## 1.1 Switching on the instrument

1. Switch on the instrument. The power switch is located on the rear right side of the instrument.
2. Tap the screen to boot the instrument.
3. Start the MACSima software.

## 1.2 Switching off the instrument

Under normal working conditions, it is unnecessary to switch off the instrument. In case of an error, or if cleaning and maintenance is required, switch off the instrument as follows:

1. Quit the MACSima software.
2. Shut down the Windows system.
3. Switch off the instrument using the power switch on the rear side of the instrument. If for any reason it is necessary to disconnect the power supply, unplug the power cable from the electrical outlet.

## 1.3 Long-term storage

The instrument is automatically flushed with Storage Solution after each run. No special precautions have to be taken to store the instrument.

# 2 Setting up an experiment

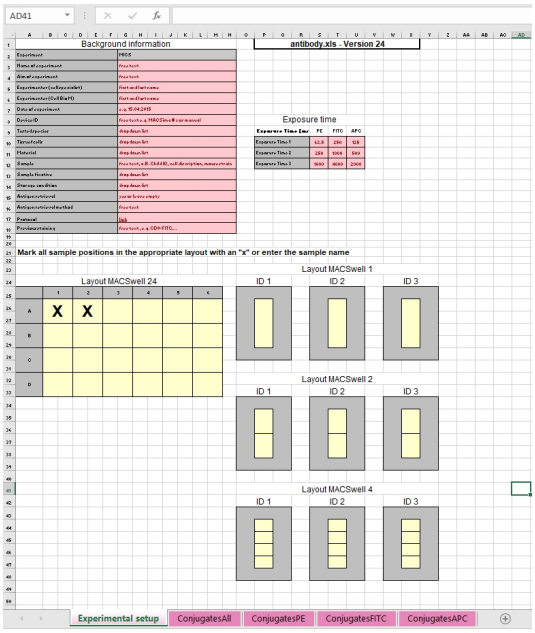


## 2.1 Enter your experiment data into the Excel sheet

### 2.1.1 Background information, exposure time and used slides

**1** Open the Microsoft Excel workbook antibody.xlsm on a personal computer. The file is located on the desktop of the MACSima Imaging System.

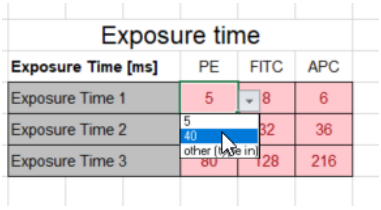
**2** Go to the worksheet **Background information**. In this worksheet, you can enter background information for your experiment (table Background information, optional), define the plate layout (mandatory), and enter the exposure time for the applicable fluorochromes (table Exposure time, mandatory).




**3** Enter the information associated with your experiment into the table **Background information**. Some values can be selected from a drop-down list, click into the cells to expand the drop-down menu.

| Background information | |
|---|---|
| Experiment | MICS |
| Name of experiment | free text |
| Aim of experiment | free text |
| Experimenter (cell specialist) | first and last name |
| Experimenter (Cell Bio M) | first and last name |
| Date of experiment | e.g. 15.04.2015 |
| Device ID | free text e.g. MACSima # x or manual |
| Tested species | dropdown list |
| Tissue/cells | dropdown list |
| Material | dropdown list |
| Sample | free text, z.B. Child ID, cell discription, mouse stra |
| Sample fixative | dropdown list |
| Storage condition | dropdown list |
| Antigen retrieval | yes or leave empty |
| Antigen retrieval method | free text |
| Protocol | link |
| Previous staining | free text , e.g. CD9-FITC.... |

4 Define the exposure time for all used fluorochromes. Click into the red-shaded cells of the table **Exposure time** and select the desired value from the drop-down menu. If your experiment requires a different time, select **other** and type in a value.

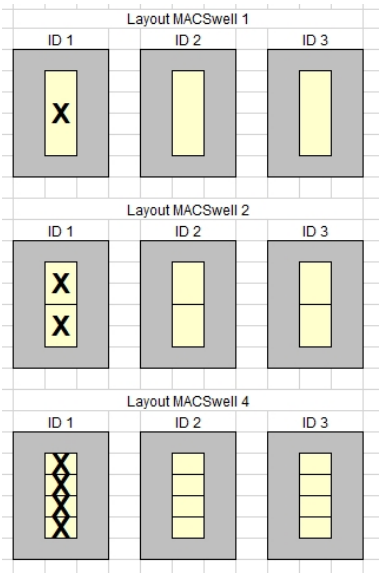



## 2.1.2 Sample frames and plates

Use either sample frames or plates for your samples. Refer to **Consumables in the MACSima Imaging System Hardware manual** for purchasing information.

### Imaging frames

Mark the location of your sample(s) on the frame with an "X" or the sample name. This layout is suitable for MACSwell Imaging Frames with up to four available positions.



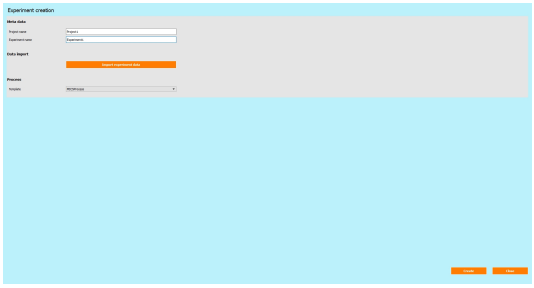

### Create an experiment file

Before you start an experiment, ensure that the waste bottle is empty and that the buffer bottles are full.

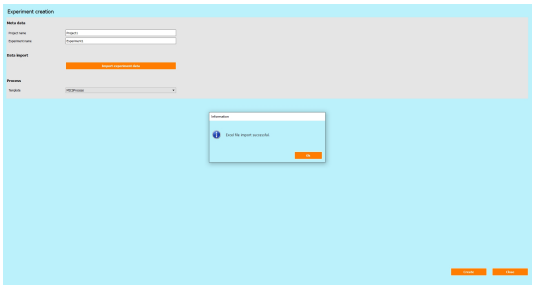
1 Click **Experiment creation**.



2 Enter a project name (max. 15 characters) and experiment name (max. 25 characters). Do not use special characters such as **/, ?**, etc.



3 Click **Data Import** and select the desired Excel workbook. If the Excel workbook loaded successfully, you will receive a confirmation message. Click **Ok**.



4 Click **Create** to proceed or **Cancel** to return to the previous window.



### Imaging Frames

Mark the location of your samples with an "X" or the sample name. In this example, the first two positions are occupied. This layout is suitable for MACSwell 24 Imaging Plates.

Layout MACSwell 24

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| A | X | X | | | | |
| B | | | | | | |
| C | | | | | | |
| D | | | | | | |

## 2.1.3 Fill out the conjugates worksheets

The worksheets for the respective conjugates can be filled out manually as described below. Alternatively, you can copy the data from the current antibody selection file provided by Miltenyi Biotec.

1 Select the respective conjugates worksheet (ConjugatesPE, ConjugatesFITC, ConjugatesAPC) in the workbook Antibody.xlsm. In this example, ConjugatesPE is shown.

ConjugatesPE --> MICS-Cycle 0 is already filled with "autofluorescence", insert all PE-conjugates below, first column "dilution factor 1:x"

| No | MICS-Cycle | Dilution factor 1:x | Species | Antibody | Antigen | Clone | Short name | Order number | Fluorochrome | PlatePos | ABtype | RackID |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | | | autofluorescence_PE | | | | | PE | | | |
| 2 | 1 | 50 | human | CD180__REA956 | CD180 (RP105) | REA956 | CD180_2 | 130-115-848 | PE | 1 | REA | 1 |

2 The first row must always contain "autofluorescence" in column E Antibody.

> Do not delete the first row.

3 The columns **No.** and **MICS Cycle** will be filled automatically. Fill out the worksheet starting with column 3, **Dilution factor**. Do not use any special character such as **/, ?,** etc..

4 The column **Species** can contain "mouse“, "human“, or "rat“.

5 The column **Fluorochrome** can contain "FITC“, "APC“, "PE“, depending on the selected tab.

6 The column **PlatePos** can contain values from 1-96. The plate position is counted from the upper left corner of a plate, moving down and then right. E.g. for a plate with 4 rows (A-H) and 12 columns, the positions on the first column have the numbers 1-8, the samples on the second column have the numbers 9-16, and so forth. Once a plate is full, numbering starts at 1 again. Increment the Rack ID number by 1 for each new plate (see column **RackID**).

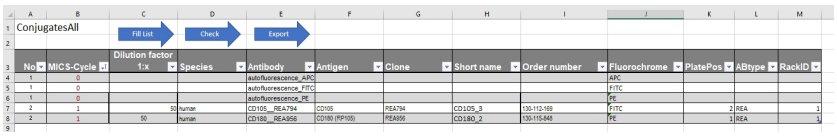
7 In the column **ABtype**, enter either "REA", "REAL", or "Hybridoma".

8 The column **RackID** must contain integers, beginning with one. Once a plate is full, increment the Rack ID number by 1 for each new plate (the first plate has RackID 1, the second plate has RackID 2, and so forth).

9 Fill out the worksheets for all used fluochromes. Note that all three conjugates worksheets must have the same number of rows. If one conjugates worksheet contains fewer antibodies, add blanks to match the length. To enter a blank, fill column **Antibody** with “empty”. If more than one blank row is required, fill the respective cells with “empty1”, “empty2”, and so forth.

### 2.1.4 Export your file

1 Go to the worksheet **ConjugatesAll**. Click the blue arrow **Fill list**. All information will be retrieved from the three conjugates worksheets and compiled into one table. Doublets will be highlighted in red. If you wish to make corrections, go back to the respective conjugates worksheet. After making your corrections, click the blue arrow **Fill list** again to update the **ConjugatesAll** list.




2 Click the blue arrow **Check**. If no sample positions were selected, an error message appears.

3 Click the blue arrow **Export** to export the data as an .xlsx file, which is required for running an experiment on the MACSima Imaging System. Enter a new name for the file and click **Save**.

## 2.2 Configure reagent and sample racks

1 Click **Rack configuration**.




2 Assign sample racks and reagent racks to the 4 available mounts.

## 2.2.1 Sample racks

REAscreen antibody panels are supported as of CUI 14.3 or later.

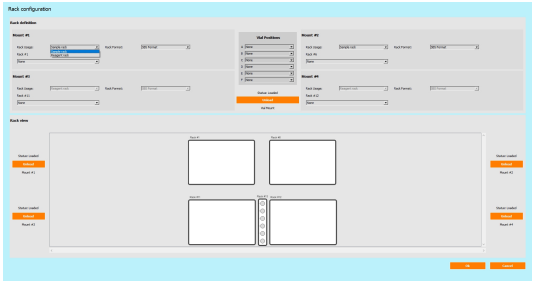
In the following example, Mount #1 and Mount #2 are used for samples. Mount #1 is used for plates, Mount #2 is used for sample slides. If Mount #1 and Mount #2 are occupied by sample plates or slides, Mount #3 and Mount #4 must be used for reagent plates. If only one Mount is required for samples, you can also use Mount #1 or Mount #2 as a reagent rack. Mount #3 and Mount #4 can only be used for reagents.

Only one mount can be used for 24-well plates. If using Mount #1 for a 24-well plate, then Mount #2 can only hold slides, and vice versa.

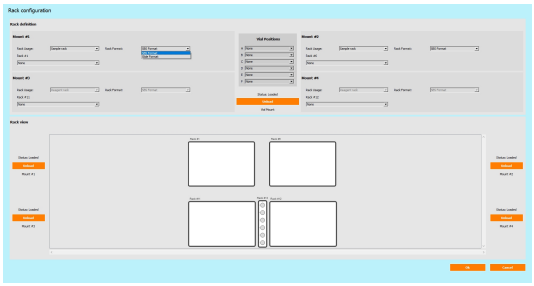
### Sample racks: SBS Format

1 Define the usage of **Mount #1** (upper left corner).

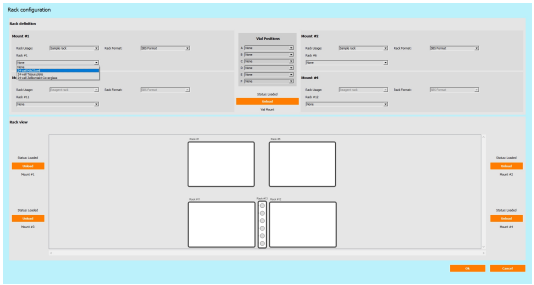
2 From the drop-down menu next to **Rack Usage**, select **Sample rack**.



3 From the drop-down menu next to **Rack Format**, select **SBS Format**.



4 From the drop-down menu under **Rack #1**, select either **24 well MACSwell** or **24 well Tissue plate**.



### Sample racks: Slide Format

1 Define the usage of **Mount #2** (upper right corner). To illustrate, Mount #1 is used for SBS plates, Mount #2 is used for slides in this example.

2 From the drop-down menu next to **Rack Usage**, select **Sample rack**.

3 From the drop-down menu next to **Rack Format**, select **Slide Format**.

4 Select either **MACSwell one**, **MACSwell two**, and /or **MACSwell four racks**. The ID refers to the specific rack definition in the Excel file.



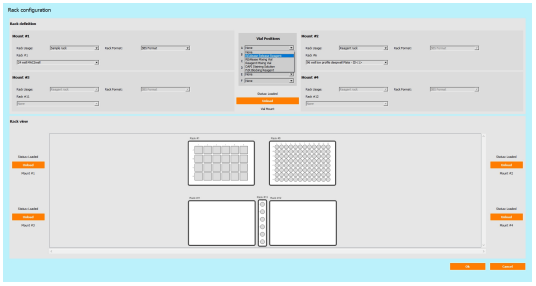
### 2.2.2 Configure reagent racks

1 Alternatively, Mount #1 or Mount #2 can also be used for reagent plates. In this case, select **Reagent rack** from the drop-down menu next to **Rack Usage**.

2 From the drop-down menu next to **Rack Format**, select **96 well low profile deepwell Plate - ID<1>**.



3 If using reagent vials, select a vial position and choose a reagent type from the drop-down menu.



4 If both Mount #1 and Mount #2 are occupied by sample frames or plates, you can use Mount #3 and Mount #4 for reagent plates.

Mount #3 and Mount #4 cannot be used for samples.

### 2.2.3 Reagent and sample rack: Configuration error

If sample or reagent plates are defined in the Excel workbook, but are not configured during experiment setup, the user is informed about the error.

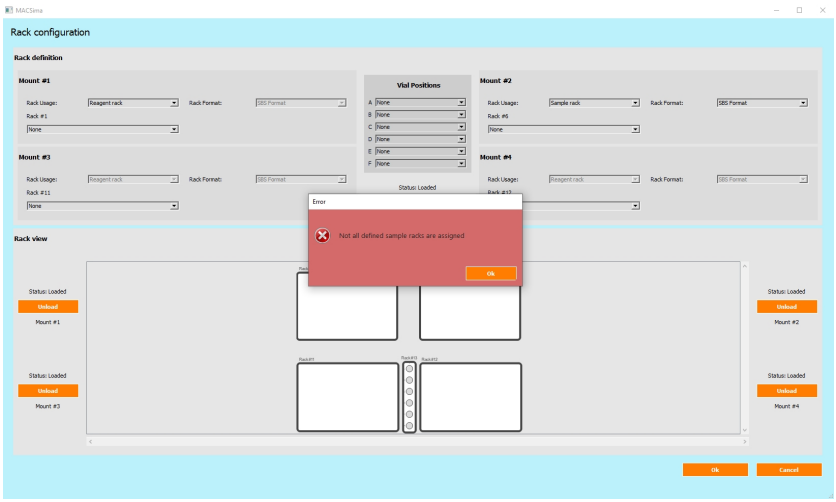



**Figure 2.1:** Configuration error: Sample rack is not defined.

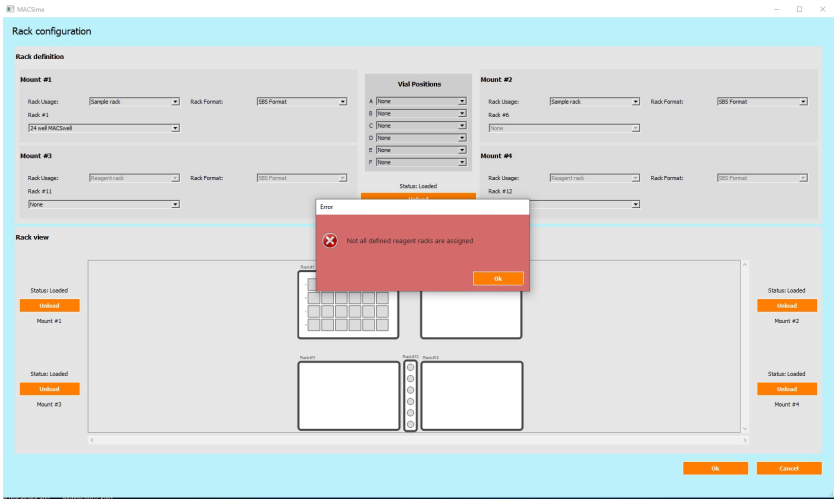


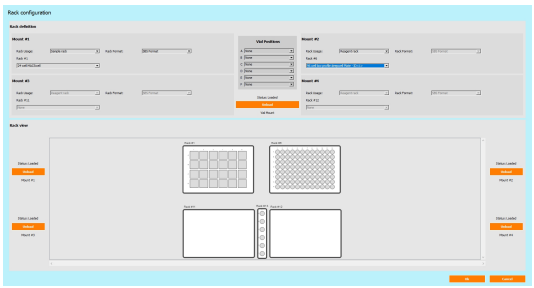

**Figure 2.2:** Configuration error: Reagent rack is not defined.

## 2.3 Load reagent and sample racks

BIOLOGICAL HAZARD

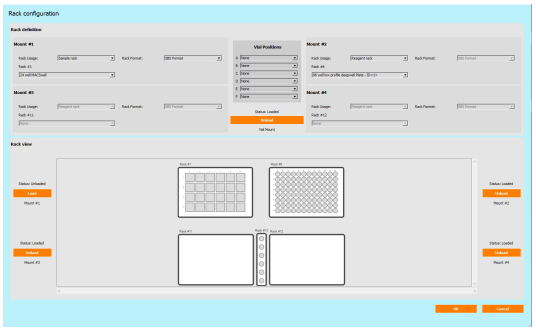
**WARNING** **Contamination or infection may lead to death or serious injury depending on the material used.**
Take care when handling samples and reagents. To avoid spilling of liquids, remove all caps and lids from any sample or reagent container before you place them into the instrument. As an exception, do not remove the aluminum sealing foils on antibody plates. Follow the instructions in the data sheet for each disposable.

**1** Click **Unload** next to the depicted frame or plate.



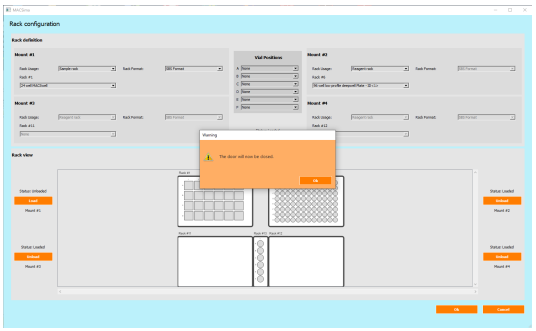
2 Load the corresponding racks with your samples and reagents into the instrument.

3 Click **Load** to lock the rack.




4 Click **Ok**.

5 If one mount is still in **Unload** position, the user is informed that the door is closing. Confirm with **Ok**.

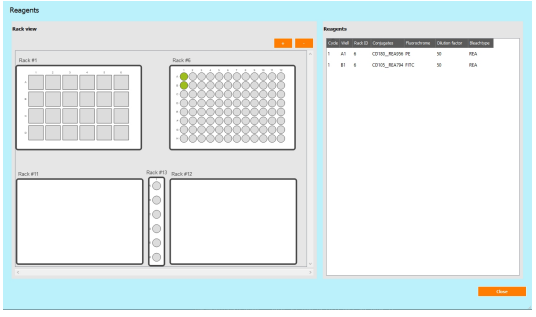



## 2.4 Review reagent plate configurations

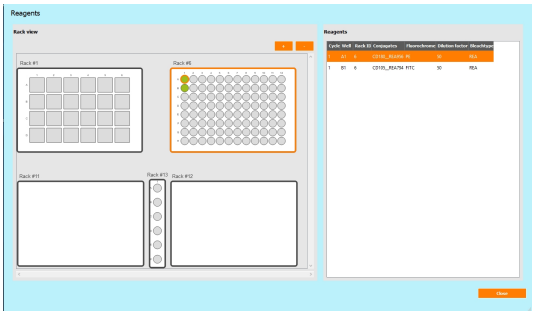
1 Click **Reagents** to review reagent plate configurations.




2 Click a reagent rack position to highlight it in the table on the right. Alternatively, select a reagent on the right to show its position on the rack.



3 Corresponding reagents and their positions are marked in orange.



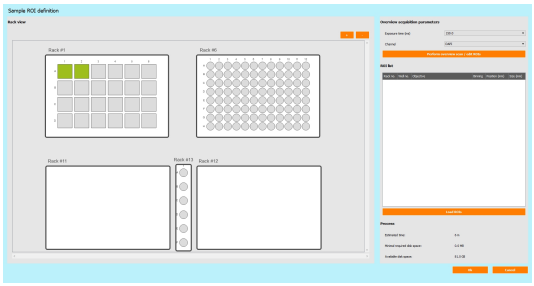
4 Click **Close** to return to the **Main** screen.

## 2.5 Sample ROI (region of interest) definition

1 Click **Sample ROI definition**.

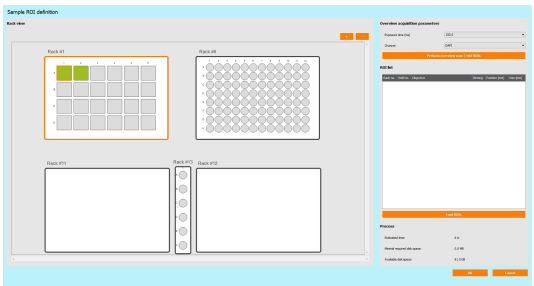


2 Select a sample position on the left. Positions containing a sample according to the Excel workbook are depicted in green.

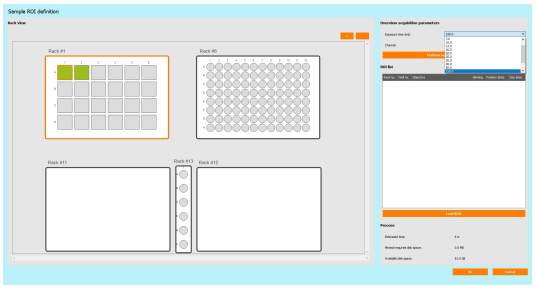


Ensure that the sample position on the plate correspond to the correct sample position in the Excel workbook.

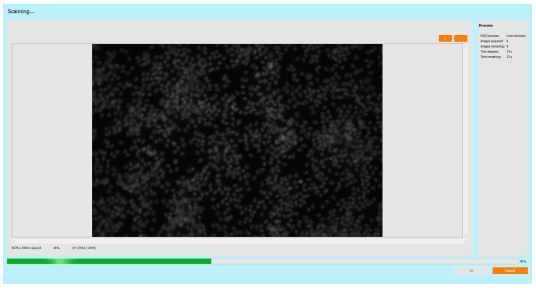
3 Click a sample to select it.



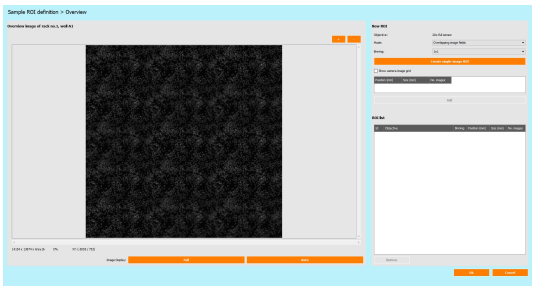
**4** Select an exposure time from the drop-down menu.



**5** Click **Perform overview scan / edit ROIs**.



**6** On the left, an image of the entire well will appear.

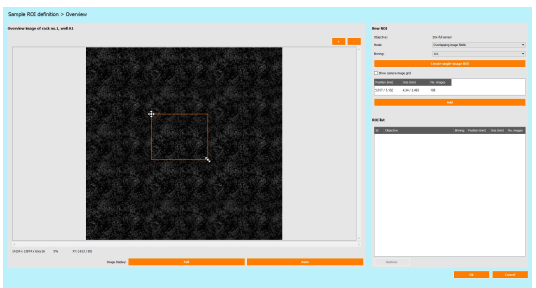


**7** Select an image display option:

- **Full**: Displays the entire dynamic range of the image. Details may not resolve well for weakly stained samples.
- **Auto**: The dynamic range is adjusted for an optimal contrast in the live image display.

If no cells are depicted, check whether the designated sample positions in the Excel workbook correspond to the plate.

**8** Click into the image and drag a ROI around the region of interest. Release if finished.

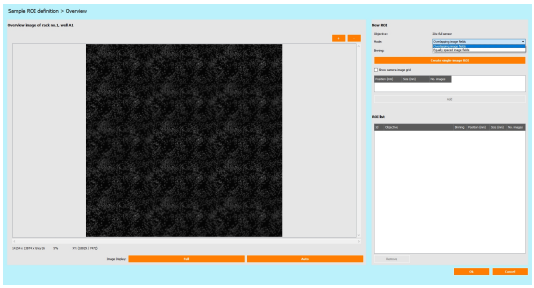


**9** Click the handle at the lower right corner of the rectangle to modify the size as needed.

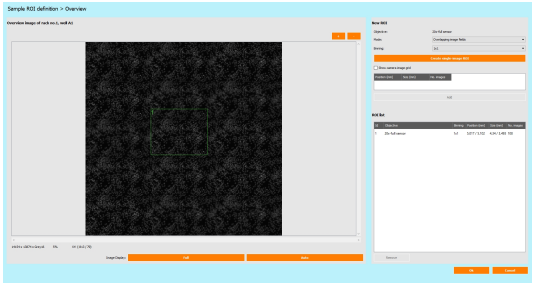
**10** Click the handle at the upper left corner of the rectangle to move the rectangle as needed.

**11** From the **Mode** drop-down menu, select one of the following imaging modes:

- **Overlapping image fields**: The acquired images have a defined overlap.
- **Equally spaced image fields**: There is a defined gap between adjacent images.

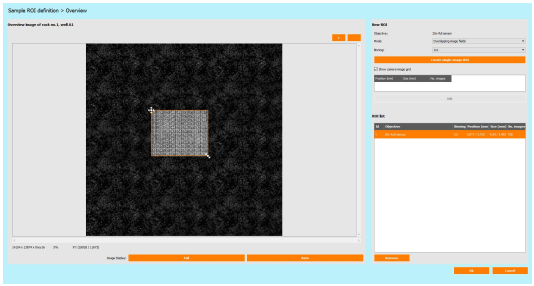


**12** Click **Add**. The ROI will now be displayed with a green rim. The ROIs will be displayed in the ROI list on the right.



**13** Add more ROIs as needed.

**14** Click **Show camera image grid** to display the camera grid in the selected ROI.



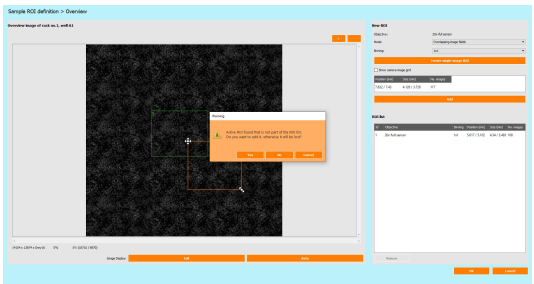
**15** To remove a ROI, select it in the ROI list and click **Remove**.

**16** Click **Ok** to proceed.

## 2.6 Sample ROIs not added to the ROI list

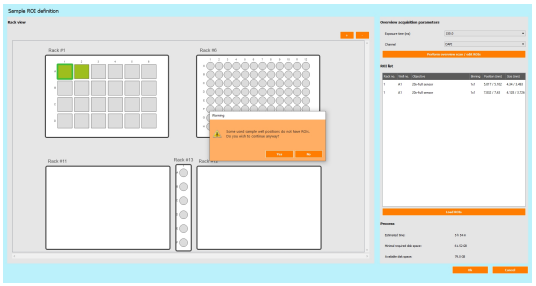
**1** If a ROI is not added to the list before leaving the sample ROI definition screen, the user is informed.

**2** Click **Ok** to add the ROI to the list, click **No** to return to the previous screen and discard the scan, or **Cancel** to modify the ROI further.



## 2.7 Incomplete sample ROIs

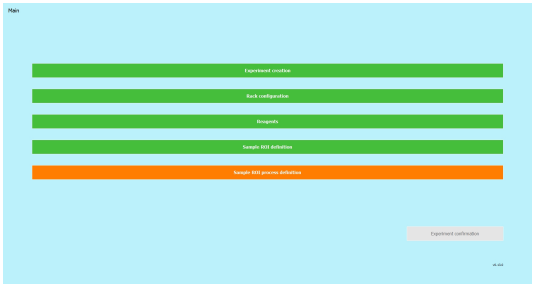
**1** If one or more samples are missing a ROI, the user is informed.



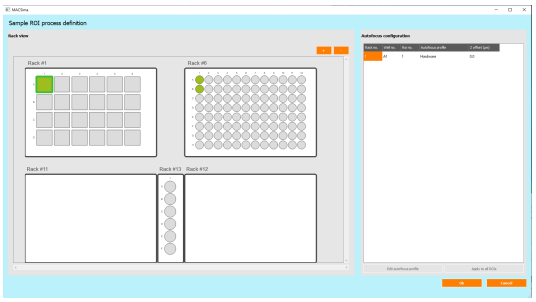
Sample positions without ROIs will be omitted.

## 2.8 Sample ROI process definition

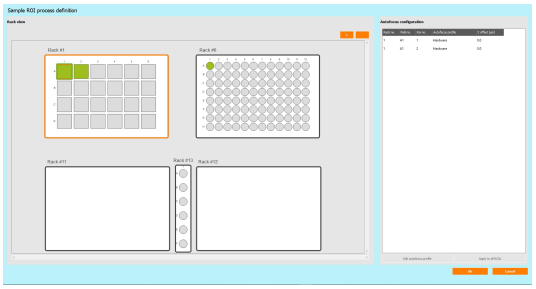
**1** Click **Define Sample ROI process definition**.



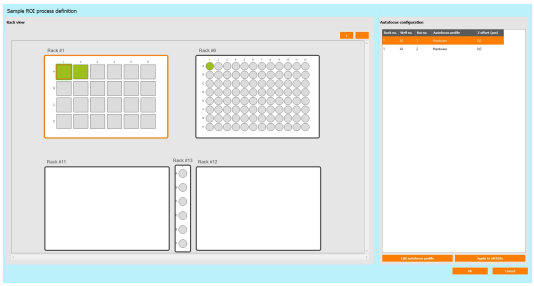
**2** Sample positions with an assigned sample are depicted in green.



**3** Select a sample position on the left to display associated ROIs and their autofocus profiles on the right.

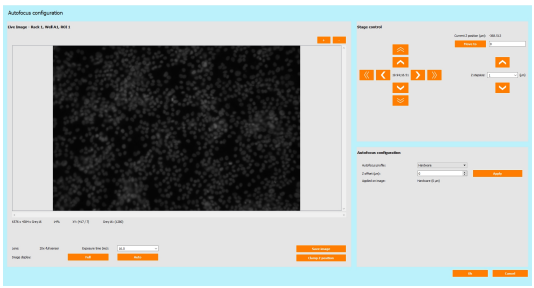


4 Select a ROI in the list on the right.



5 Click **Edit autofocus profile** to edit.

6 On the left, a live image appears. It displays the center of the selected ROI.



7 Check whether the image is in focus. Use the navigation cross in the **Stage control** section to move in X-Y-direction to another area within the selected ROI.

8 If the image is not in focus, the user can choose and define another autofocus profile. Select one of the following options from the drop-down menu in the **Autofocus configuration** section:

- Hardware based
- Image based
- Combination of Hardware based and image based.

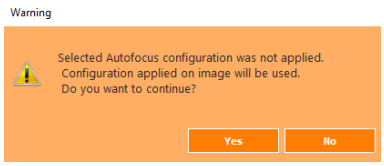
Refer to **Autofocus profiles on the next page** for detailed information on autofocus adjustments.

9 The last applied autofocus profile (results shown in the image on the left) is displayed under **Applied on image**.

10 Click **Apply** to apply the selected settings to the area displayed in the live image. The live image will be refreshed.

11 Click **Ok** to confirm the selected profile.

12 If the selected autofocus profile differs from the last applied one, the following warning will be shown:




13 Do this either for all ROIs, or, optionally, Click **Apply to all ROIs** to use the defined autofocus profile for all ROIs. Check for each ROI whether the selected settings yield good results.

14 Click **Ok** to return to the **Main** screen.

## 2.9 Adjust the autofocus settings

### 2.9.1 Autofocus profiles

| Option | Description |
|---|---|
| Hardware based | The software attempts to identify the interface between glass slide and sample (=bottom of the sample). |
| Image based | The software creates several images (z-stack) and automatically selects the best image. The live image area is refreshed automatically. |
| Combination of Hardware based and on image based profile | First, the hardware-based profile is applied to identify the transition zone between glass slide and sample. Second, the image-based profile is applied based on the identified transition zone. |

**Table 2.1:** Autofocus configuration options.

### 2.9.2 Hardware based autofocus

When adjusting the hardware based autofocus, the software attempts to identify the transition zone between glass slide and sample (=bottom of the sample). To determine in which distance to the glass slide the image is to be taken:

- Enter a value in the field **Z offset (µm)**. Click **Apply** to apply the entered value. After sample focusing, the live image will refresh automatically.
- Alternatively, you can use the up/down buttons on the right side of the **Stage control** section. The image will refresh automatically. You can select a step size from the drop-down field between the arrows. Click **Clamp Z position** to transfer the selected value to the **Z offset** field.

The Z offset field is only available in combination with the Hardware based profile.

### 2.9.3 Autofocus adjustment options

| Option | Description |
|---|---|
| **Save Image** button | Saves the current image as a tiff file. |
| **Clamp z-position** button | Transfers the adjusted z offset to the **Z offset** field. |
| **Exposure time** drop-down menu | In case of overexposure or weak staining, the exposure time can be de- or increased, respectively. |
| **Full** button | Displays the entire dynamic range of the image. Details may not resolve well for weakly stained samples. |
| **Auto** button | The dynamic range is adjusted for an optimal contrast in the live image display. |

**Table 2.2:** Options to adjust the autofocus.

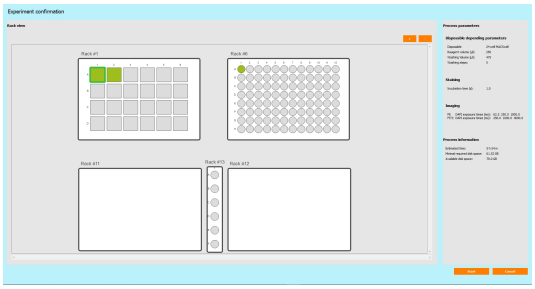
## 2.10 Start your experiment

Ensure that the fluid bottles are full and that the waste bottle is empty. You can exchange the bottles before a run, or if the software prompts you to do so. Do not change the bottles while the instrument is running. Refer to **Exchanging fluid bottles** and **Empty the waste container** in the MACSima Imaging System hardware manual for details on how to change a bottle.

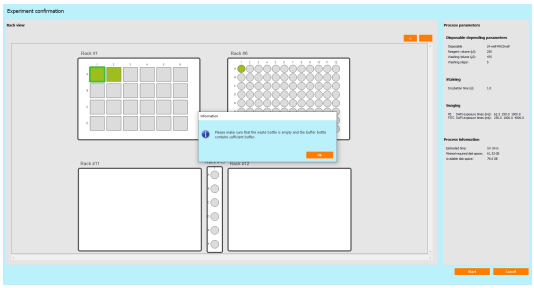
1 Click **Experiment confirmation**.



2 Click **Start**.



3 A pop-up window asks you to empty the waste bottle. Also, check that you have enough buffer and solutions. Click **Ok** when done.

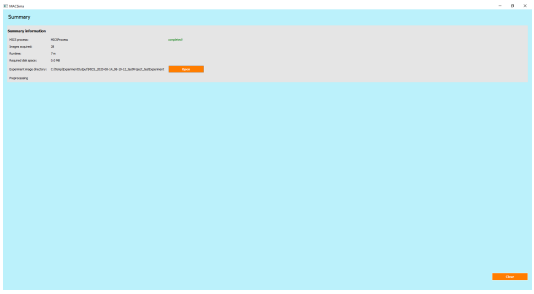


4 A green status bar indicates the progress. Click **Cancel** to abort the process.

After a process has been aborted, the instrument may keep on running for a while before it stops.



5 After completion, a summary screen appears. Click **Open** to retrieve the data.




6 Click **Close** to end the experiment.

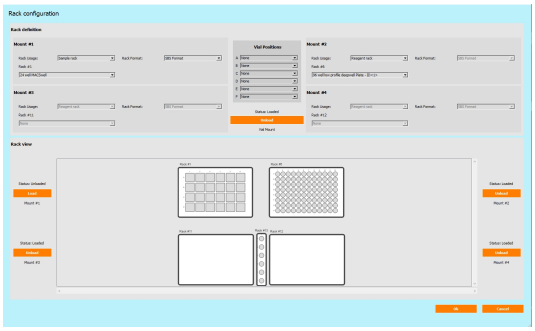
## 2.11 Discard used plates

1 After you closed the summary screen, you will be redirected to the main dialog screen.




2 Click **Rack Configuration** to retrieve your used plates.

3 Click **Unload** next to the respective plate to retrieve it.



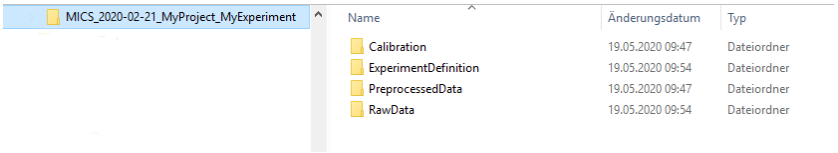
# 3
# Output data



## 3.1 Output data

After creating the experiment, an experiment data folder is created. The folder has the following name structure:

MICS_<Date>_<Project>_<Experiment>




| Folder | Description |
|---|---|
| Calibration | Contains calibration data for flatfield or darkframe correction, if existing |
| ExperimentDefinition | Contains all experiment configuration data both predefined parameters as well as user-made configurations |
| RawData | Contains raw image data as well as associated system-relevant files |

**Table 3.1:** The output data folder.

### 3.1.1 Calibration data

| File | Description |
|---|---|
| Darkframe_<binning>_<exposureTime [ms]>.tif | Darkframe correction, image file |
| Darkframe_<binning>_<exposureTime [ms]>.ready | Darkframe correction, system file required for synchronization with the Data Preprocessing Tool |
| Flatfield_<Fluorochrome>_<Lens>_ <binning>.tif | Flatfield correction, image file |
| Flatfield_<Fluorochrome>_<Lens>_ <binning>.ready | Flatfield correction, system file required for synchronization with the Data Preprocessing Tool |

**Table 3.2:** Calibration data files.

### 3.1.2 Experiment definitions

The subfolder **ExperimentDefinitions** has two subfolders, **MicsProcess** and **RoiDefinitions**.

#### Mics Process data

The folder **MicsProcess** contains the following data:

| File | Description |
|---|---|
| antibody.xlsx | Contains information about the marker: order in which they are applied, sample rack definitions, etc. |
| processConfig.json | Configuration file that contains information about the process parameter |

**Table 3.3:** Mics process files.

### Roi Definitions

The folder **RoiDefinitions** contains all information about ROIs used in this experiment.

| File | Description |
|---|---|
| standard.roi | Contains ROI definitions that can be loaded when setting up another experiment |
| OverviewImage_2x_<WellPosition>_<WellName>.tif | Overview image of the respective well |
| OverviewImage_2x_<WellPosition>_<WellName>_ RoiOverlay.tif | Overview image of the respective well with an overlayed image containing the ROIs |
| OverviewImage_2x_ <WellPosition><WellName>.tif.metaData. | Contains metadata of the overview image |

**Table 3.4:** ROI definition files.

## 3.1.3 RawData

This folder contains acquired images (.tif) with the following name structure:

<CycleId>_<CycleType>

<CycleId> designates the ID of the MICS cycle. <CycleType> designates whether the images were create after bleaching (<BleachCycle>) or after staining (<AntigenCycle>).

The folder also contains system files (.ready) required for synchronization with the Data Preprocessing Tool.

| Subfolder name | Description |
|---|---|
| <CycleId>_<CycleType>_<Antigen>_<Dilution>_<Fluorochrome>_<BitDepth>_M-<Lens>_ B-<Binning>_R-<RackId>_W-<WellId>_G-<RoiId>_F-<FieldID>_E-<ExposureTime[ms]>.tif | Images of the 1st marker cycle |
| <CycleId>_<CycleType>_<Antigen>_<Dilution>_<Fluorochrome>_<BitDepth>_W-<WellId>_R-<RoiId>_F-<FieldId>_E-<ExposureTime>_X-<StagePosX[mm]>_ Y-<StagePosY [mm]>_Z-<ObjectivePosZ[mm]>.tif | Images of the 1st bleach cycle |

**Table 3.5:** Acquired images.

# 4 Technical support



For technical support, contact your local Miltenyi Biotec representative or Miltenyi Biotec Technical Support at Miltenyi Biotec headquarters:

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Visit **www.miltenyibiotec.com** for local Miltenyi Biotec Technical Support contact information.

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