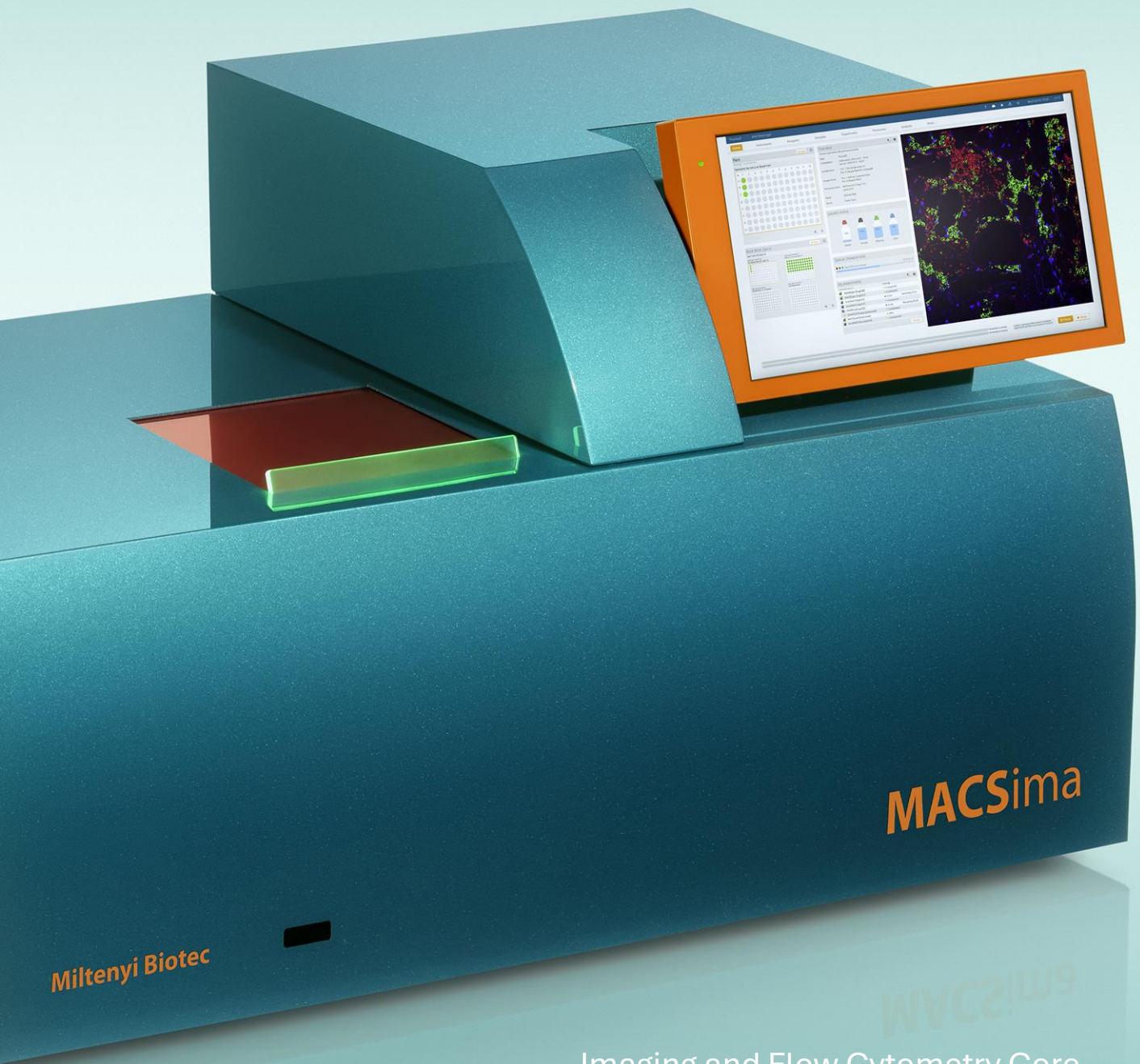




Miltenyi Biotec

MACSima Imaging System Standard Operation Protocol



Imaging and Flow Cytometry Core
Center for PanorOmic Sciences

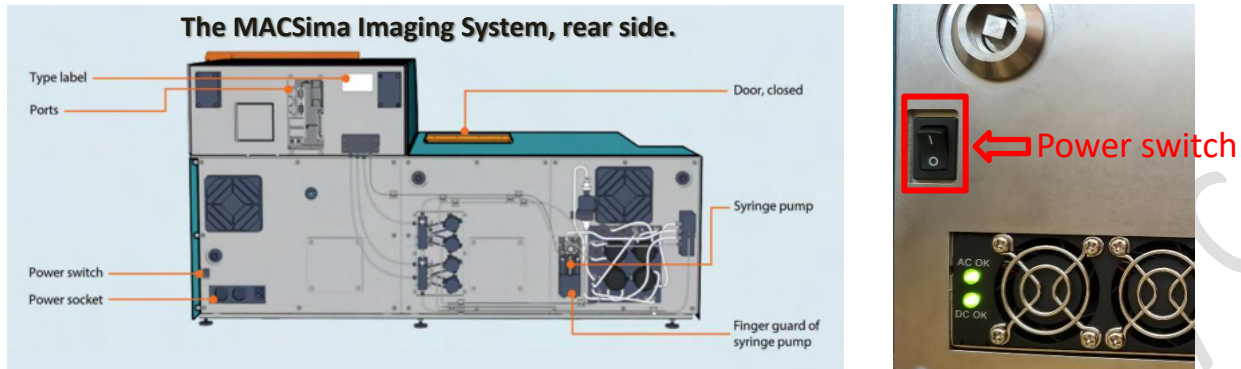
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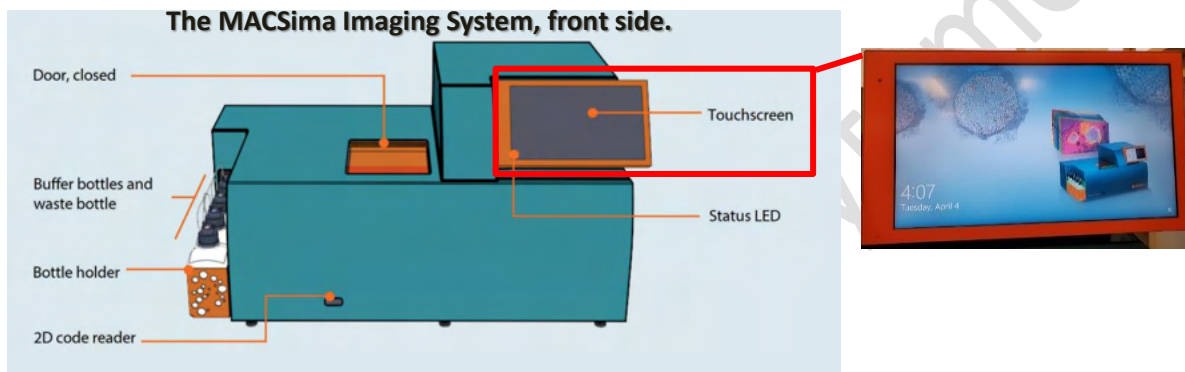
1 Startup of MACSima System

Switching on the Instrument

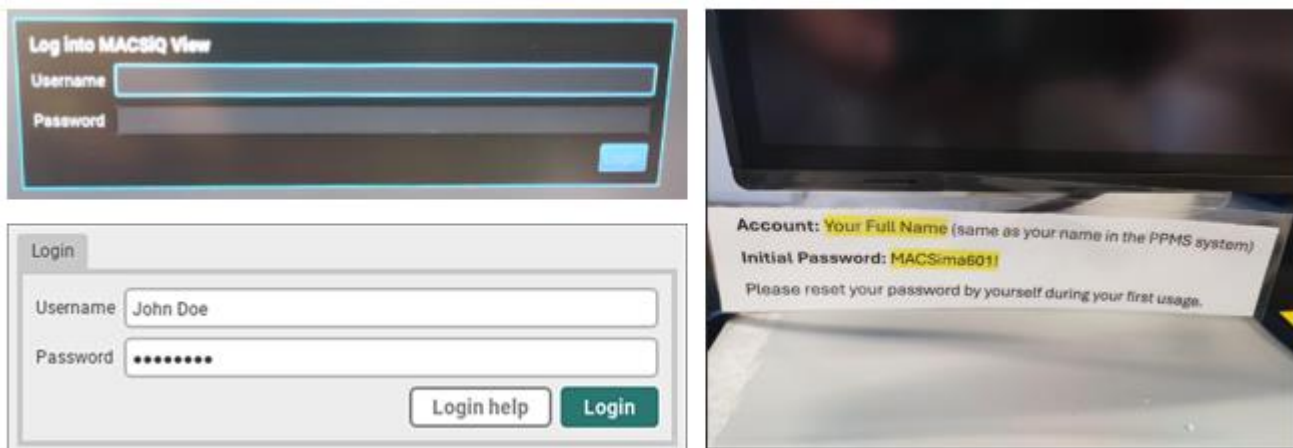
1. Switch on the instrument. The power switch is located on the rear side of the instrument.



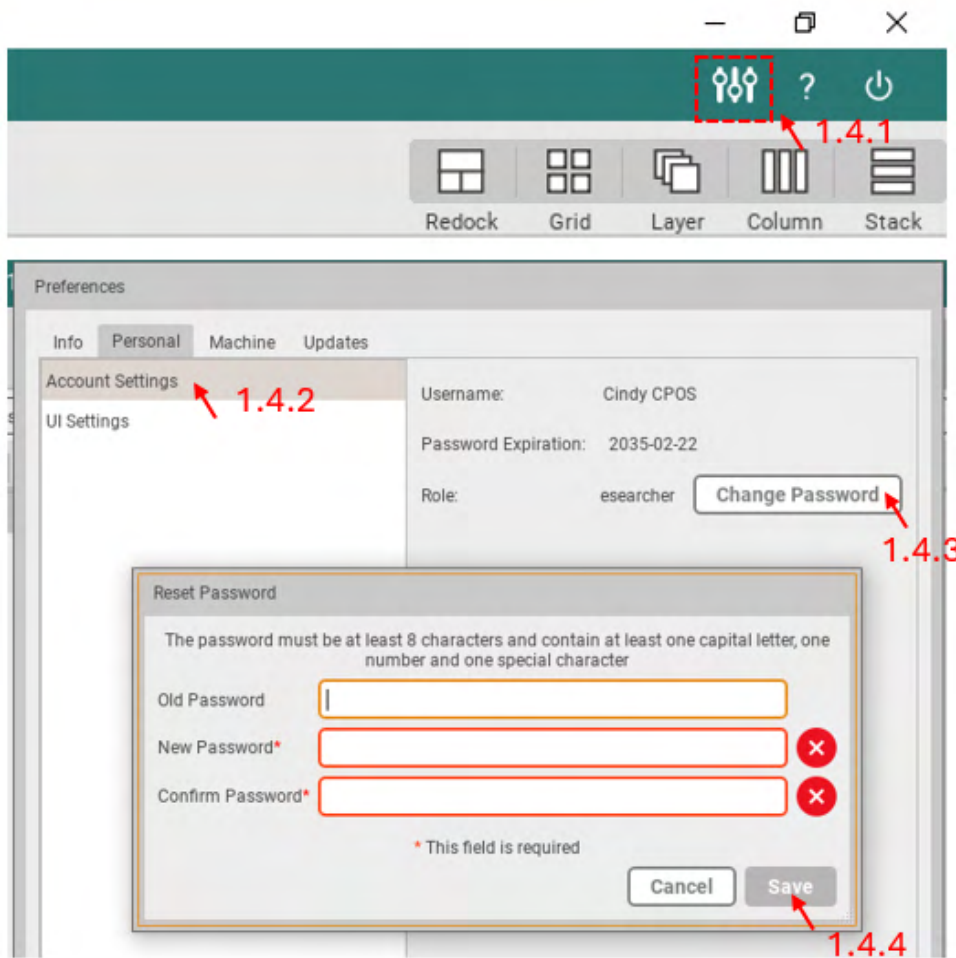
2. Tap the screen to boot the instrument and start the MACS iQ View Software Control Module.



3. Enter your user account with the initial password "MACSima601!" attached at the bottom of the screen.



4. (Optional) Please reset your password at your first time usage as below image.




5. Wait for MACSima System warmup for 2 hours (If the system is not shut off by the previous user, you don't need to warm up it.)

2 Setup Experiment

2.1 Create Sample

1. Click the **Sample Setup** tab.



2. Click **Add**  **> New Sample...** in the Samples table header to open the New Sample window.
3. Fill in all the required fields for the experiment. Click the **Create** button to save your sample.

A screenshot of the 'New Sample' window. It features a title bar with a plus icon and the text 'New Sample'. The form contains several fields: 'Name' (text input with 'Hypothalamus'), 'Collection Date' (calendar icon with '2022-11-11 11:11:11 Mitteleuropäische Zeit'), 'Project Name' (empty text input), 'Sample Type' (dropdown menu with 'Tissue'), 'Cell Type' (dropdown menu with 'Select...'), 'Species' (dropdown menu with 'Human'), 'Organ' (dropdown menu with 'Brain'), 'Disease Type' (empty text input), 'Diagnosis Remarks' (empty text input), 'Experimental Condition' (empty text input), 'Fixation Method' (dropdown menu with 'PFA'), and 'Comment' (empty text input). At the bottom right, there are 'Cancel' and 'Create' buttons.

4. If all your samples are of the same type, you could duplicate the sample created with several copies, then the sample creation is finished.

Right-click the newly created sample and click **Duplicate Sample...** to open the **Duplicate Sample window**. Enter the Number of Copies and edit the other fields as needed. Click the **Duplicate** button to create the samples.

A screenshot of the 'Duplicate Sample' window. It features a title bar with a plus icon and the text 'Duplicate Sample'. The form contains several fields: 'Number of Copies' (spin box with '4'), 'Name' (text input with 'Hypothalamus'), 'Collection Date' (calendar icon with '2022-11-11 11:11:11 Mitteleuropäische Zeit'), 'Project Name' (empty text input), 'Sample Type' (dropdown menu with 'Tissue'), 'Cell Type' (dropdown menu with 'Select...'), 'Species' (dropdown menu with 'Human'), 'Organ' (dropdown menu with 'Brain'), 'Disease Type' (empty text input), 'Diagnosis Remarks' (empty text input), 'Experimental Condition' (empty text input), 'Fixation Method' (dropdown menu with 'PFA'), and 'Comment' (empty text input). At the bottom right, there are 'Cancel' and 'Duplicate' buttons.

5. If all your samples are of different types, please create all the samples one by one until finished.
6. (Optional) If your sample is a **TMA** (tissue micro array) in the same chamber, please create all the samples in

the array and convert them to a **TMA** type. Select the samples and right click-to **Convert To Complex Sample**.

Tissue Micro Array

2.6.1 Set up samples in the array and select them all

2.6.2 Right click and Convert them to complex sample

Name	Creation	Status	Tissue	Mouse	Tonsil	FFPE
Tonsil 20	2024-07-26 11:...	Not In Use	Tissue	Mouse	Tonsil	FFPE
Tonsil 19	2024-07-26 11:...	Not In Use	Tissue	Mouse	Tonsil	FFPE
Tonsil 18	2024-07-26 11:...	Not In Use	Tissue	Mouse	Tonsil	FFPE
Tonsil 17	2024-07-26 11:...	Not In Use	Tissue	Mouse	Tonsil	FFPE
Tonsil 16	2024-07-26 11:...	Not In Use	Tissue	Mouse	Tonsil	FFPE
Tonsil 15	2024-07-26 11:...	Not In Use	Tissue	Mouse	Tonsil	FFPE
Tonsil 14	2024-07-26 11:...	Not In Use	Tissue	Mouse	Tonsil	FFPE
Tonsil 13	2024-07-26 11:...	Not In Use	Tissue	Mouse	Tonsil	FFPE
Tonsil 12	2024-07-26 11:...	Not In Use	Tissue	Mouse	Tonsil	FFPE
Tonsil 11	2024-07-26 11:...	Not In Use	Tissue	Mouse	Tonsil	FFPE
Tonsil 10	2024-07-26 11:...	Not In Use	Tissue	Mouse	Tonsil	FFPE
Tonsil 9	2024-07-26 11:...	Not In Use	Tissue	Mouse	Tonsil	FFPE
Tonsil 8	2024-07-26 11:...	Not In Use	Tissue	Mouse	Tonsil	FFPE
Tonsil 7	2024-07-26 11:...	Not In Use	Tissue	Mouse	Tonsil	FFPE
Tonsil 6	2024-07-26 11:...	Not In Use	Tissue	Mouse	Tonsil	FFPE
Tonsil 5	2024-07-26 11:...	Not In Use	Tissue	Mouse	Tonsil	FFPE
Tonsil 4	2024-07-26 11:...	Not In Use	Tissue	Mouse	Tonsil	FFPE
Tonsil 3	2024-07-26 11:...	Not In Use	Tissue	Mouse	Tonsil	FFPE
Tonsil 2	2024-07-26 11:...	Not In Use	Tissue	Mouse	Tonsil	FFPE
Tonsil 1	2024-07-26 11:...	Not In Use	Tissue	Mouse	Tonsil	FFPE

7. (Optional) Click the **SAVE** button to create the TMA sample group

Edit Complex Sample

Name:

Comment:

Treat as complex sample (eg. TMA) Clear Filter Filter to Assignable

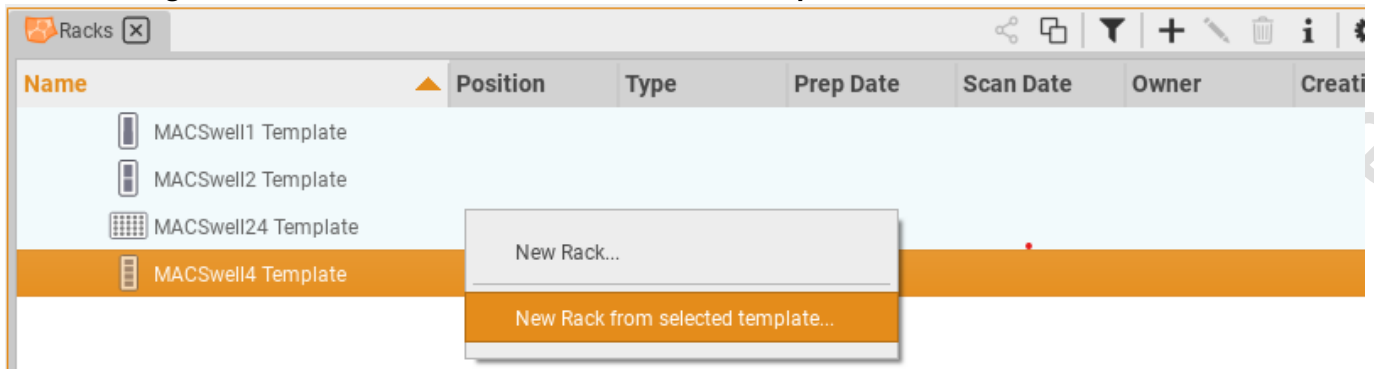
Drag samples to add to group:

Name	Creation	Creator	Modified	Modifier	Last Usage	S
Tonsil 1	2024-07-26 11:...	Cindy	2024-07-26 11:...	Cindy	2024-07-26 11:...	N
Tonsil 10	2024-07-26 11:...	Cindy	2024-07-26 11:...	Cindy	2024-07-26 11:...	N
Tonsil 11	2024-07-26 11:...	Cindy	2024-07-26 11:...	Cindy	2024-07-26 11:...	N
Tonsil 12	2024-07-26 11:...	Cindy	2024-07-26 11:...	Cindy	2024-07-26 11:...	N
Tonsil 13	2024-07-26 11:...	Cindy	2024-07-26 11:...	Cindy	2024-07-26 11:...	N
Tonsil 14	2024-07-26 11:...	Cindy	2024-07-26 11:...	Cindy	2024-07-26 11:...	N
Tonsil 15	2024-07-26 11:...	Cindy	2024-07-26 11:...	Cindy	2024-07-26 11:...	N
Tonsil 16	2024-07-26 11:...	Cindy	2024-07-26 11:...	Cindy	2024-07-26 11:...	N
Tonsil 17	2024-07-26 11:...	Cindy	2024-07-26 11:...	Cindy	2024-07-26 11:...	N
Tonsil 18	2024-07-26 11:...	Cindy	2024-07-26 11:...	Cindy	2024-07-26 11:...	N

2.2 Assign Sample to Racks

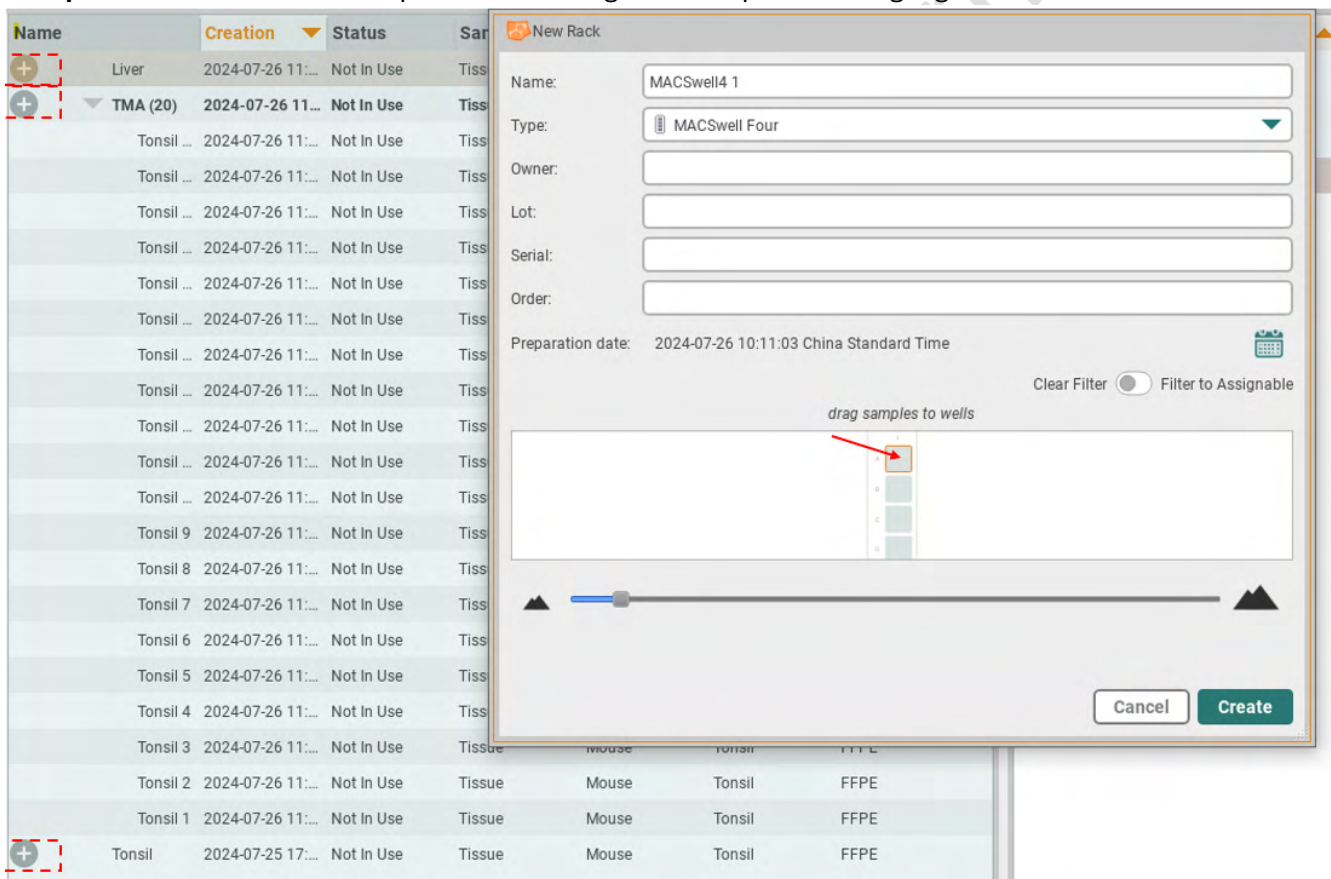
In this step, the racks are added to the experiment and the samples will be assigned to a well in the rack(s).

1. Click the Racks Tab, you could see four Racks Templates. Select the proper one based on your sample carrier and right click, to select **New Rack From Selected Template**



2. In the **New Rack** window, fill in all the required fields..

3. In the **New Rack** window, highlight the well located with sample (red arrow indicated). Select a sample in the **Samples** window and click the plus icon to assign the sample to the highlighted well.



4. Repeat this step until all samples used in your experiment have been assigned to the correct wells. (Not all wells need to be used if you have empty wells.)

New Rack

Name:


Type:

Owner:

Lot:


Serial:

Order:

Preparation date: 2022-11-11 11:11:11 Mitteleuropäische Zeit 

Clear Filter Filter to Assignable

drag samples to wells



▲ ▲

Samples: [Hypothalamus 1](#)

5. Click the **Create** button to create the new rack.

2.3 Managing the Reagents

The software is delivered with a complete list of all pre-tested Miltenyi antibodies. This list can be extended with non-Miltenyi antibodies. If all the reagents you used are from Miltenyi, skip 2.2.1. If you used Non-Miltenyi reagents, follow the guide below:

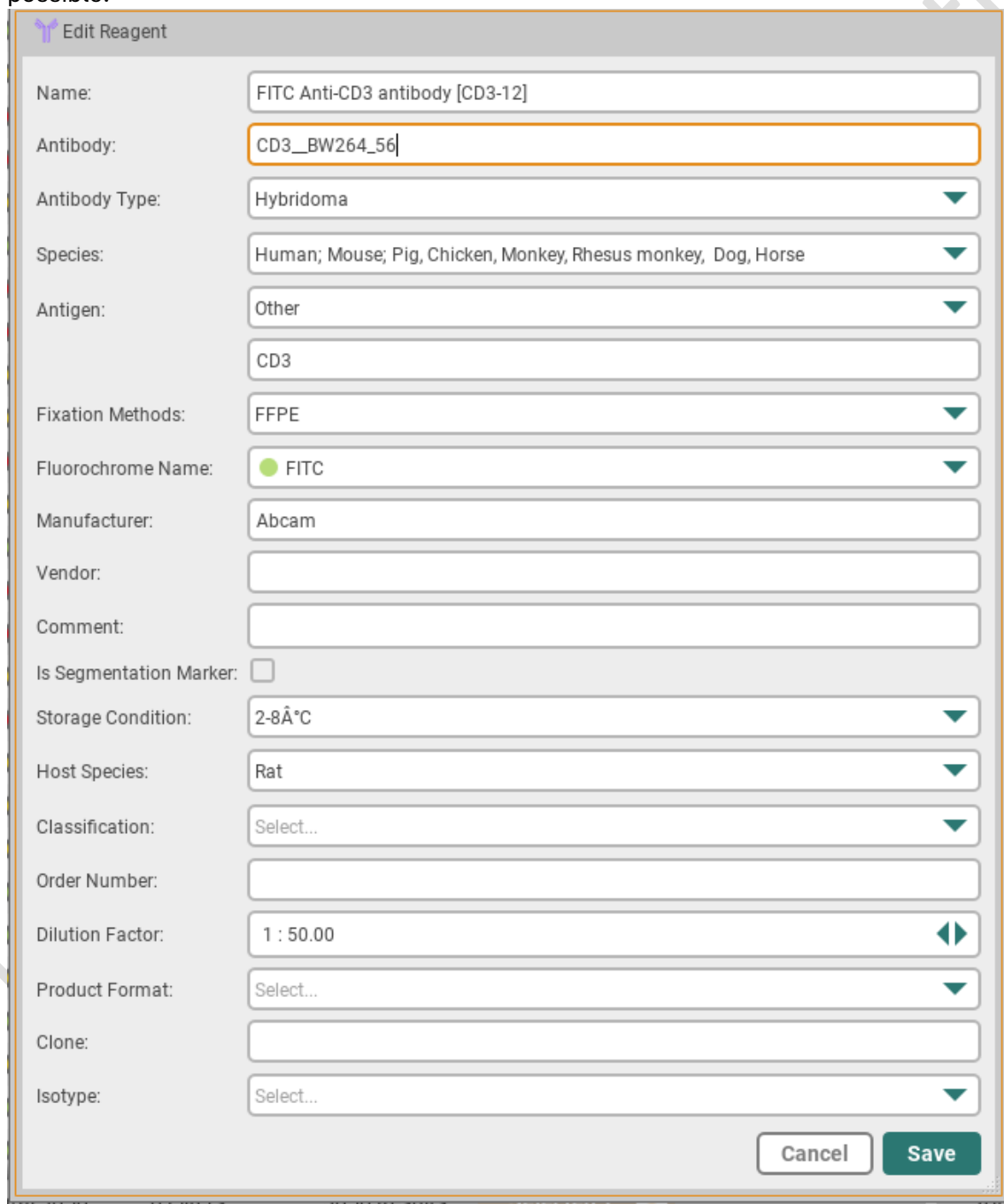
2.3.1 Register a Non-Miltenyi Biotec Reagent

1. Click the Reagent Definition tab



Reagent Definition

2. The non-Miltenyi antibodies must first be added to the Reagents list.
3. Click **Add > Create Non-MB Reagent...** in the Reagents table header to open the New Reagent window.
4. Specify all the required fields for your reagent. The fields Antibody, Antibody Type, Species, Antigen, Fixation Methods, and Fluorochrome Name are mandatory. It is recommended to collect as much information as possible.



The screenshot shows the 'Edit Reagent' window with the following fields and values:

- Name: FITC Anti-CD3 antibody [CD3-12]
- Antibody: CD3_BW264_56
- Antibody Type: Hybridoma
- Species: Human; Mouse; Pig, Chicken, Monkey, Rhesus monkey, Dog, Horse
- Antigen: Other
- CD3
- Fixation Methods: FFPE
- Fluorochrome Name: FITC
- Manufacturer: Abcam
- Vendor:
- Comment:
- Is Segmentation Marker:
- Storage Condition: 2-8°C
- Host Species: Rat
- Classification: Select...
- Order Number:
- Dilution Factor: 1 : 50.00
- Product Format: Select...
- Clone:
- Isotype: Select...

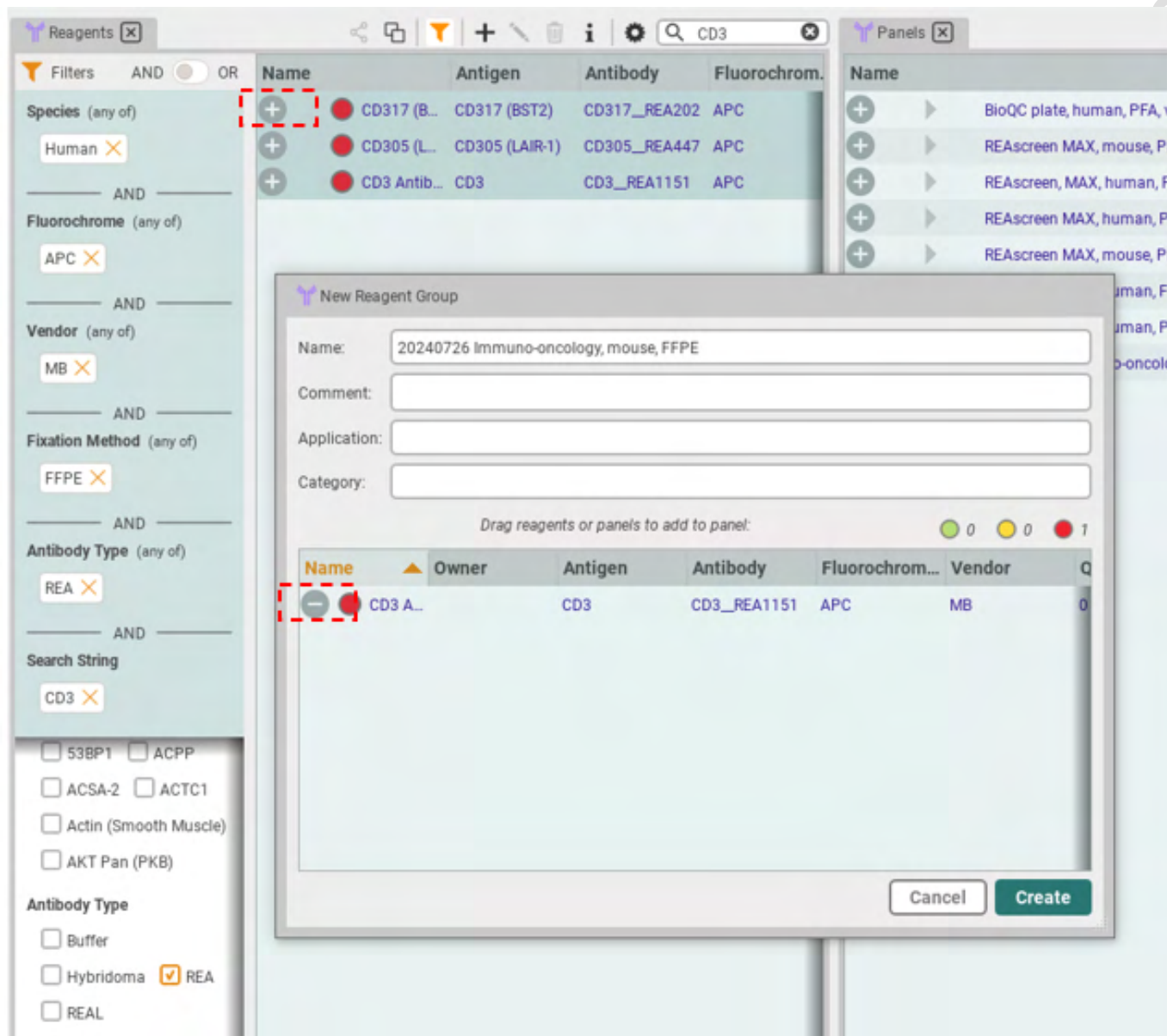
Buttons: Cancel, Save

5. Click the **Save** button to add the reagent to the **Reagents** window.

2.3.2 Creating a Reagent Panel

Creating a panel is optional but recommended if an established group of antibodies is used regularly.

1. In the Panels Window, click **Add icon > New Panel...** in the **Panels** table header
2. Specify all the required fields for the panel in the **New Reagent Group** window.
3. Sort the antibody in the **Reagents** Window, select the antibody that you used in your experiment, and click the add button to add them to the **New Reagent Group** Window.



4. Until you filled the New Reagent Group with all the antibody you will use in your imaging. You could remove the wrong antibody that you added by clicking the "Minus" button. Click the **Create** button to create the panel.

2.4 Creating a Procedure

1. Click the **Procedure Creation** tab.



2. Select a template from the **Procedures** window.

3. The **Procedures** window already contains **templates for unstained and DAPI-prestained samples** as well as procedures for all available REAscreen Antibody Panels. The unstained template still requires DAPI in a specific well, the DAPI-prestained template can be used directly.

The screenshot shows the 'Procedures' window with a table of procedures. The first two rows are highlighted with red dashed boxes, indicating they are selected. The table has columns for Name, Type, Version, and Comment.

Name	Type	Version	Comment
MICS Procedure (Unstained Sample) Template	MICS Standard	1.0	Standard proce...
MICS Procedure (DAPI-prestained Sample) Template	MICS Standard	1.0	Standard proce...
BioQC plate, human, PFA, version 01	MICS Standard		
REAscreen MAX, mouse, PFA, version 01	MICS Standard		
REAscreen, MAX, human, FFPE, version 01	MICS Standard		
REAscreen MAX, human, PFA, version 01	MICS Standard		
REAscreen MAX, mouse, PFA, version 02	MICS Standard		
REAscreen MAX, human, FFPE, version 02	MICS Standard		
REAscreen MAX, human, PFA, version 02	MICS Standard		
REAscreen Immuno-oncology, human, FFPE, version 01	MICS Standard		

2. Click **Add > Create From Template...** in the Procedures table header.

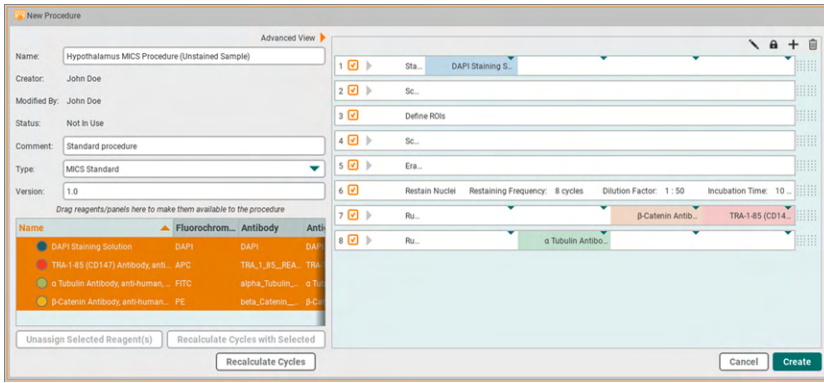
3. Enter the name for the panel in the New Procedure window

The screenshot shows the 'New Procedure' window. The 'Name' field is filled with 'Hypothalamus MICS Procedure (Unstained Sample)'. The 'Type' is set to 'MICS Standard' and the 'Version' is '1.0'. The 'Advanced View' tab is active, showing a list of steps: 1. DAPI Staining S..., 2. Sc..., 3. Define ROIs, 4. Sc..., 5. Era..., 6. Restain Nuclei (Restaining Frequency: 8 cycles, Dilution Factor: 1:50, Incubation Time: 10...). The 'Reagents/Panels' section is empty, with a prompt to 'Drag reagents/panels here to make them available to the procedure'.

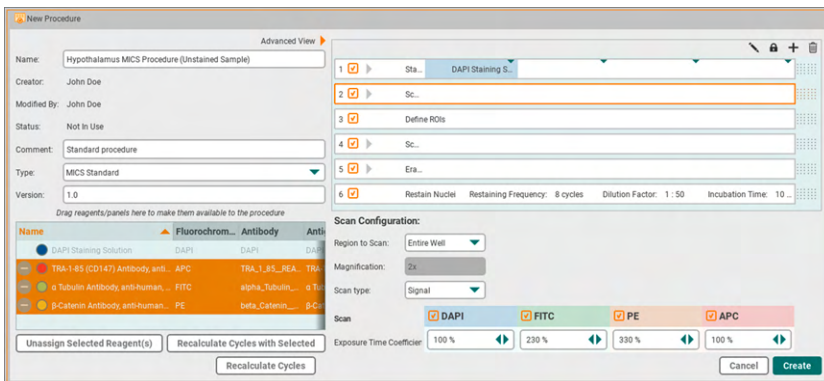
4. Drag panels and/or reagents from the **Inventory, Panels, and/or Reagents** window to the **New Procedure** window.

The screenshot shows the 'New Procedure' window with the 'Reagents/Panels' section populated. The 'Name' field is 'Hypothalamus MICS Procedure (Unstained Sample)'. The 'Advanced View' tab is active, showing the same list of steps as the previous screenshot. The 'Reagents/Panels' section now contains several items, including 'DAPI Staining Solution', 'Fluorochrom...', 'Antibody', and 'Anti...'. The 'Unassign Selected Reagent(s)' and 'Recalculate Cycles with Selected' buttons are visible at the bottom.

5. If you added or removed antibodies in **New Procedures** Window, you could click **Recalculate Cycles** to calculate a procedure.



6. Optional: Adjust the numerical values (Dilution Factor, Incubation Time, Exposure Time Coefficient, Erase Method, Bleach Energy, and Wash Dilution) in each step of the procedure to suit your needs.



Any deviation from the recommended values is the responsibility of the user.

7. Click the **Create** button to save the procedure

2.5 Creating an Experiment

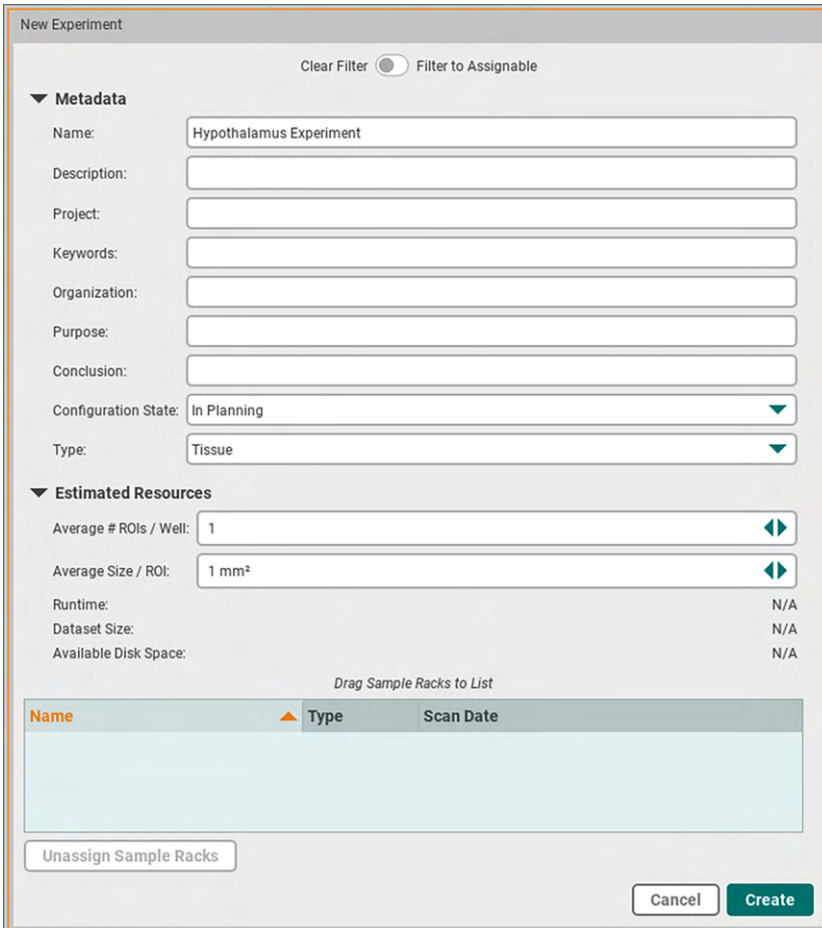
In this step, the previous inputs are combined into an experiment.

1. Click the Experiment Planning tab.



Experiment Planning

2. Click **Add > New Experiment...** in the Experiments table header
3. Specify all the required fields for the experiment. It is recommended to collect as much information as



New Experiment

Clear Filter Filter to Assignable

▼ **Metadata**

Name: Hypothalamus Experiment

Description:

Project:

Keywords:

Organization:

Purpose:

Conclusion:

Configuration State: In Planning

Type: Tissue

▼ **Estimated Resources**

Average # ROIs / Well: 1

Average Size / ROI: 1 mm²

Runtime: N/A

Dataset Size: N/A

Available Disk Space: N/A

Drag Sample Racks to List

Name	Type	Scan Date
------	------	-----------

Unassign Sample Racks

Cancel Create

4. Drag the rack(s) from the **Racks** window to the **New Experiment** window. Only racks with assigned samples can be added.

New Experiment

Clear Filter Filter to Assignable

Drag Procedure to well - 4 more wells of this rack can be processed

▼ Metadata

Name:

Description:

Project:

Keywords:

Organization:

Purpose:

Conclusion:

Configuration State:

Type:

▼ Estimated Resources

Average # ROIs / Well:

Average Size / ROI:

Runtime: N/A

Dataset Size: N/A

Available Disk Space: N/A

Drag Sample Racks to List

Name	Type
Hypothalamus Rack	MACSwell Four

Unassign Sample Racks

Unassign Procedures

Skip Well

Cancel

Create

5. Drag the procedure(s) from the **Procedures** window to the wells used in the **New Experiment** window. Procedures can be assigned to multiple wells with different samples. Samples that are not used do not need to be assigned a procedure.

New Experiment

Clear Filter Filter to Assignable

No more wells of this rack can be processed

▼ Metadata

Name:

Description:

Project:

Keywords:

Organization:

Purpose:

Conclusion:

Configuration State:

Type:

▼ Estimated Resources

Average # ROIs / Well:

Average Size / ROI:

Runtime: 6 m 1 s

Dataset Size: 5,20 GB

Available Disk Space: N/A

Drag Sample Racks to List

Name	Type	Scan Date
Hypothalamus Rack	MACSwell Four	

Unassign Sample Racks

Unassign Procedures

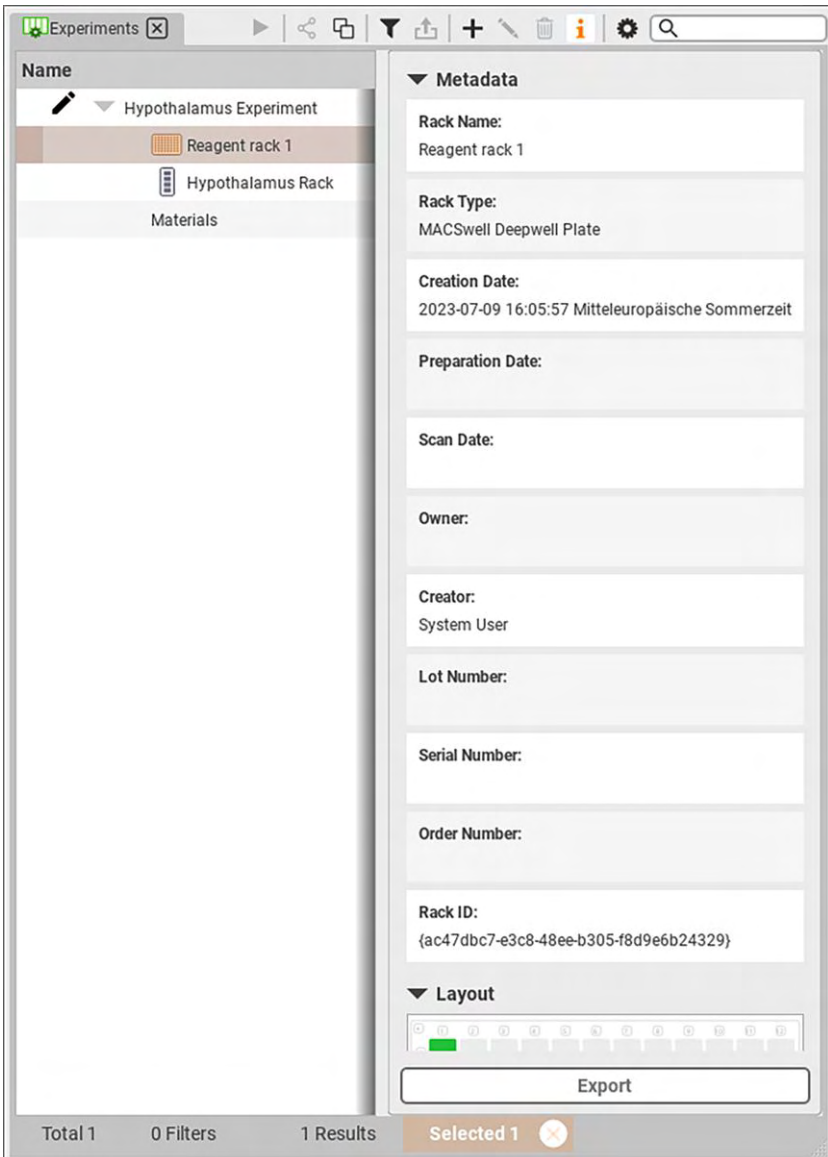
Skip Well

Cancel

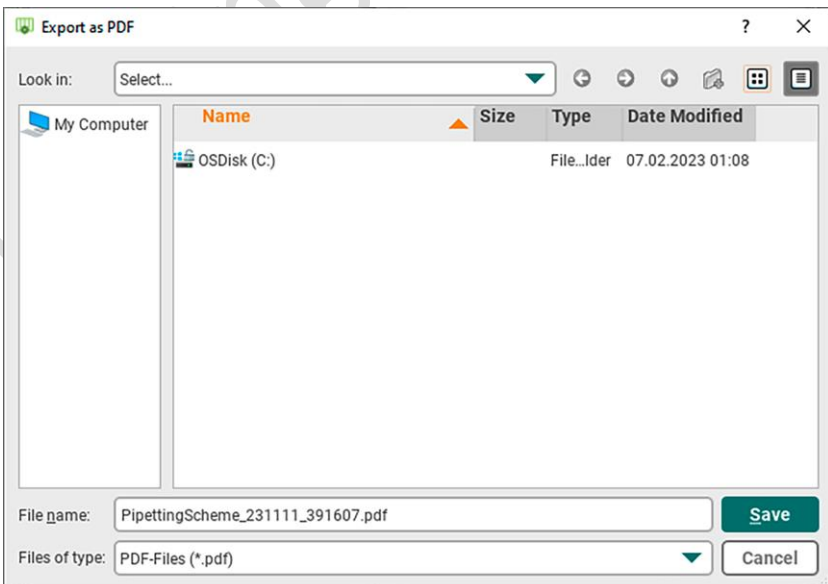
Create

3

6. Optional: Click the well(s) to see the assigned procedure and sample.
7. Optional: Specify the Average # ROIs / Well and the Average Size / ROI to get a Runtime and Dataset Size estimate.
8. Click the **Create** button to save the experiment.
9. Click the Info button in the **Experiments** table header.



10. Click the **Export** button to save the pipetting scheme as PDF.



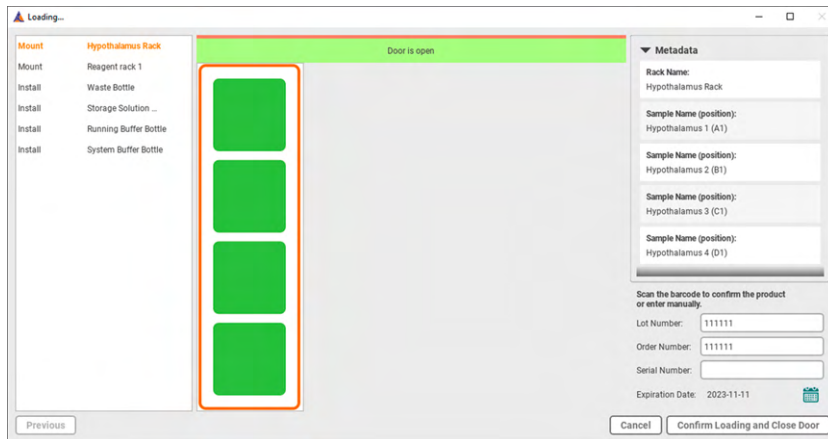
2.6 Running an Experiment

1. Click the **Experiment Planning** tab.

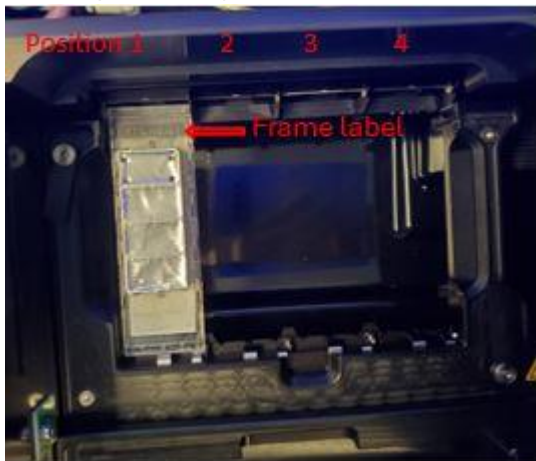


Experiment Planning

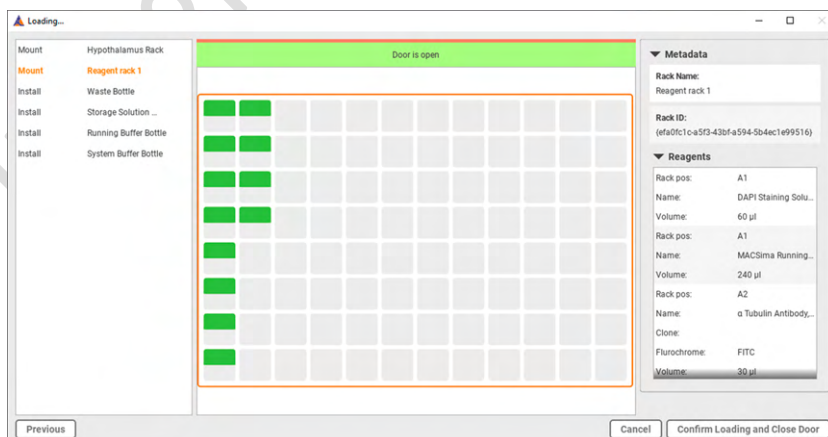
2. Right-click the experiment and click **Run Experiment...** to open the **Loading...** window.
3. Hold the rack(s) in front of the 2D barcode reader. Make sure the 2D barcode is clearly visible.
4. Optional: Enter Lot Number and Order Number for your rack(s).



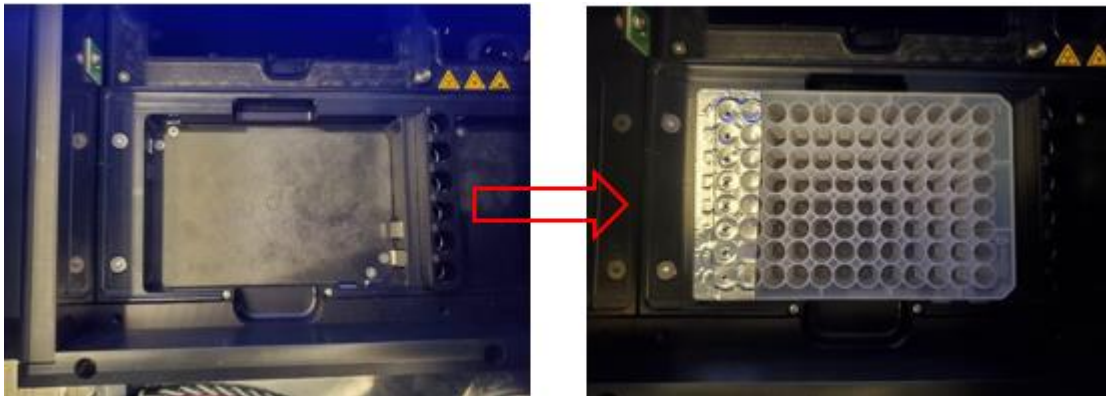
5. Place the sample racks(s) on the indicated imaging stage rack position(s). The stage is moved into the correct position by the instrument.



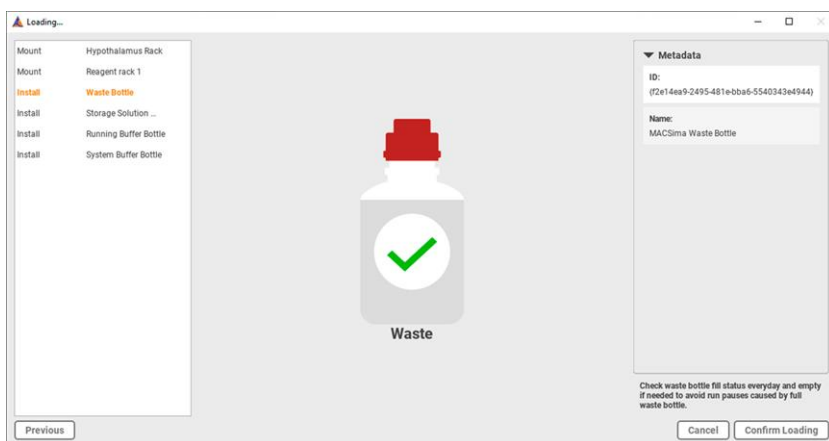
6. Click the **Confirm Loading and Close Door** button. The door on the instrument closes and opens again as indicated by the animation on-screen.



7. Place the reagent rack.



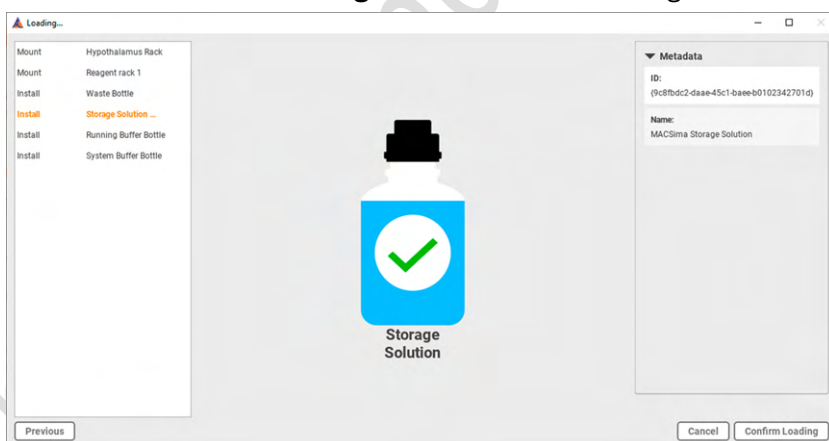
8. Click the **Confirm Loading and Close Door** button. The door on the instrument closes and opens again as indicated by the animation on-screen.



9. Empty the waste bottle.

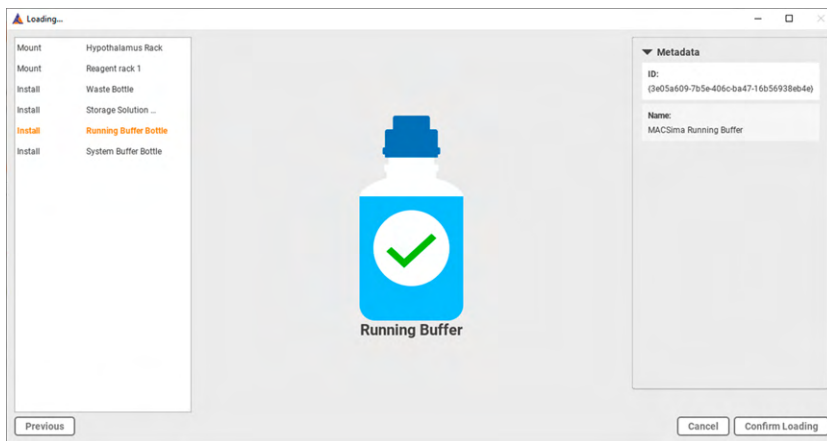
Check the fill status of the waste bottle daily. Ask Technical staff to empty if necessary to avoid run pauses caused by a full waste bottle.

10. Click the **Confirm Loading** button. Check the storage solution



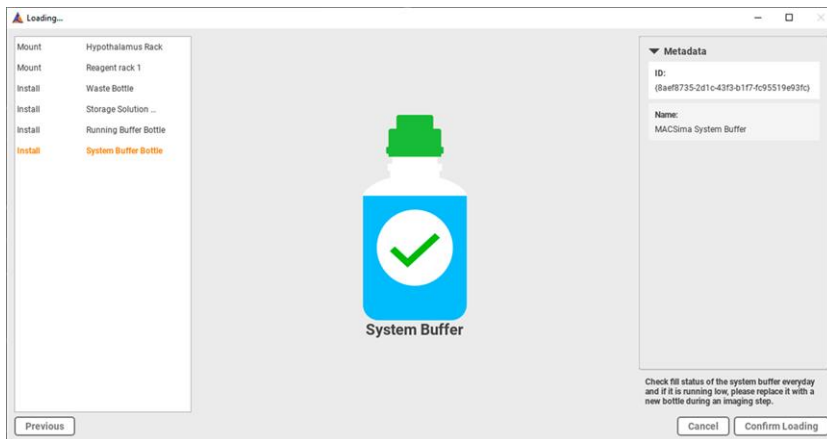
Check fill status of the storage solution. Ask Technical staff to replace the bottle if necessary.

11. Click **Confirm Loading**. Check the running buffer



Check the fill status of the running buffer. Ask Technical Staff to replace the bottle if necessary.

12. Click the **Confirm Loading** button. Check the system buffer

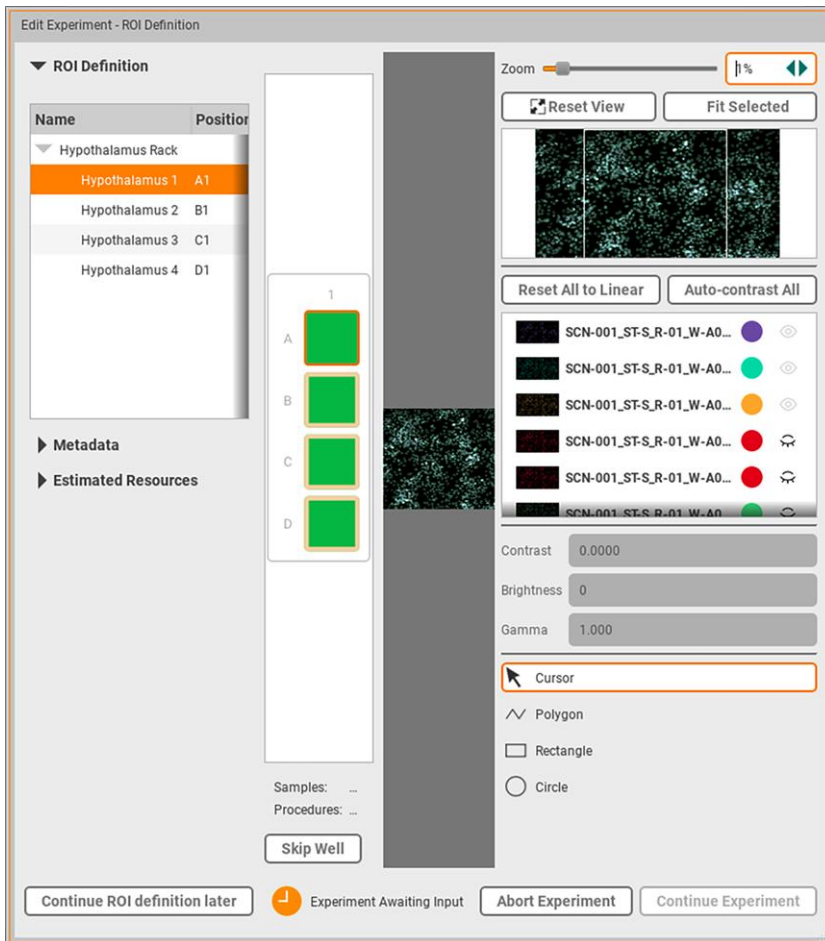


Check the fill status of the system buffer. Ask Technical Staff to replace the bottle during the imaging step if necessary.

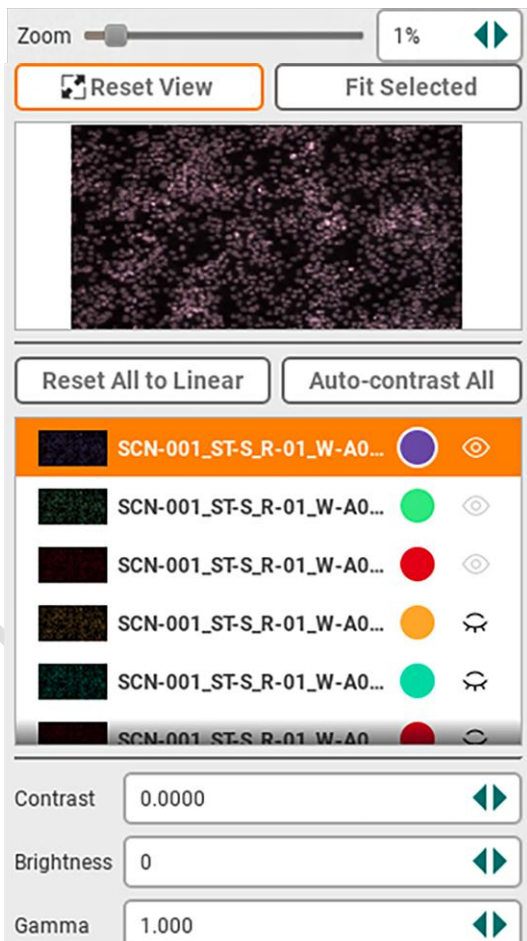
13. Click the **Confirm Loading** button. Click the **Start Experiment** button.



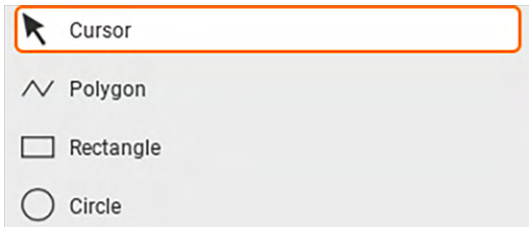
14. Wait until the preview scan has finished. The **Edit Experiment - ROI Definition** window opens.



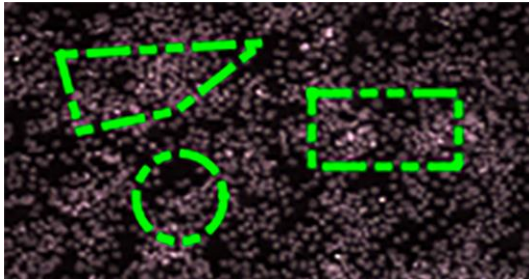
15. Zoom into the preview picture of the first well by using the Zoom slider or your mouse wheel. Adjust the contrast, brightness, and gamma values



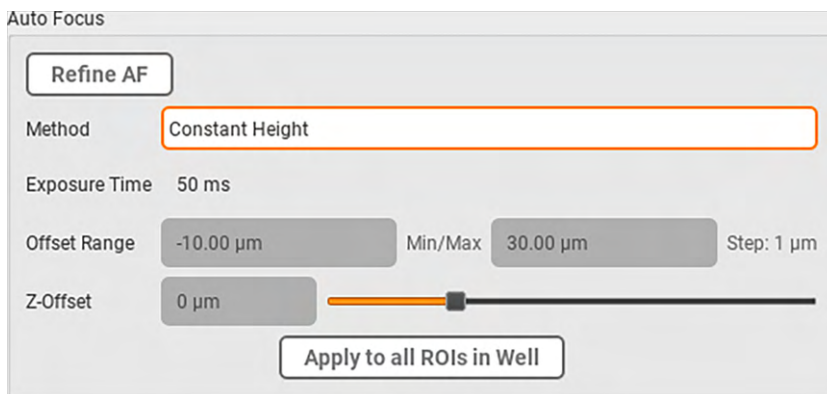
16. Select a drawing tool (**Polygon**, **Rectangle**, or **Circle**).



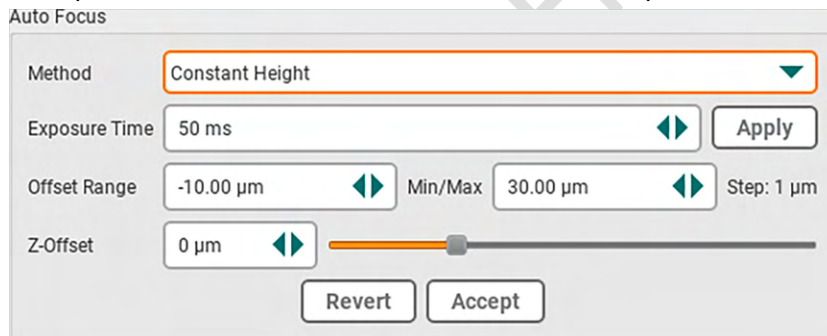
17. Draw one or more regions of interest (ROI).



18. Check autofocus for all ROIs in the first well.



19. Optional: Click **Refine AF** to set the Method, Exposure Time, Offset Range, and Z-Offset. Click “**Apply**” to

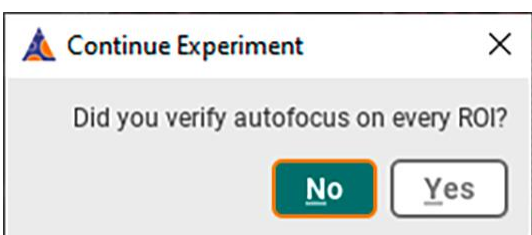


check the live view of current focus. Change the **Z-Offset** to change autofocus. Change **Exposure Time** if the signal in live view is too bright or dim.

20. Click the Accept button to use the auto focus settings.

21. Repeat steps 18– 20 for all wells in the experiment.

22. Click the Continue Experiment button to start the measurement. A message reminds to set the auto focus for all ROIs. If you confirm the autofocus for each ROI is ok, click “**Yes**” to continue experiment.

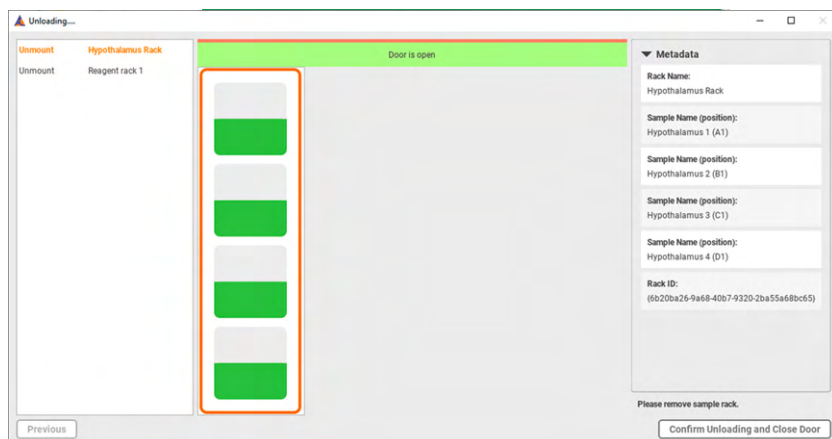


23. You could leave now, and the system will do automatic cycling staining and imaging for you. If it is a long-term imaging (e.g., 3-7 days), please remind Technician to refill the imaging buffer during office hours in case experiment suspension due to insufficient buffer.

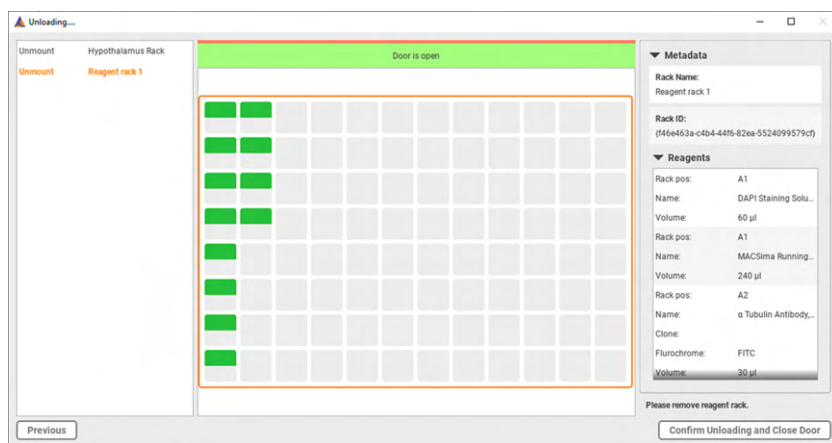
Imaging and Flow Cytometry Core

3 Backup Data and Shut Down System

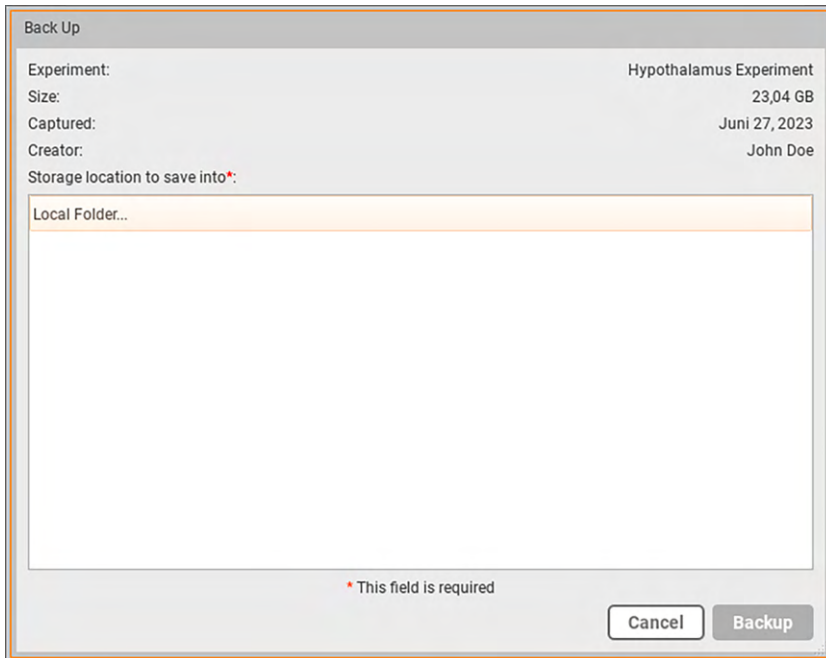
1. Wait until the experiment is finished.
2. Click the **Begin unload** button to open the **Unloading...** window.



3. Remove the sample rack(s) on the indicated imaging stage rack position(s). The stage is moved into the correct position by the instrument.
4. Click the **Confirm Unloading and Close Door** button. The door on the instrument closes and opens again as indicated by the animation on-screen.



5. Remove reagent rack.
6. Click the **Confirm Unloading and Close Door** button. The door on the instrument closes and the instrument is now in standby.
7. Right-click the executed experiment and select **Back-up...** to create a backup of the raw and processed images in **SSD borrowed from CPOS** or **your own formatted SSD**. (If you will use the SSD borrowed from CPOS, after the backup to your own laptop, please format the borrowed SSD before returning.)



8. Notice: Ensure that a backup has been created before deleting an experiment. Deleting an experiment deletes all measurement data.
9. Exit the software
10. Shut down the hardware. The power switch is located on the rear side of the instrument
11. Record logbook with your experiment real execution start and end time.