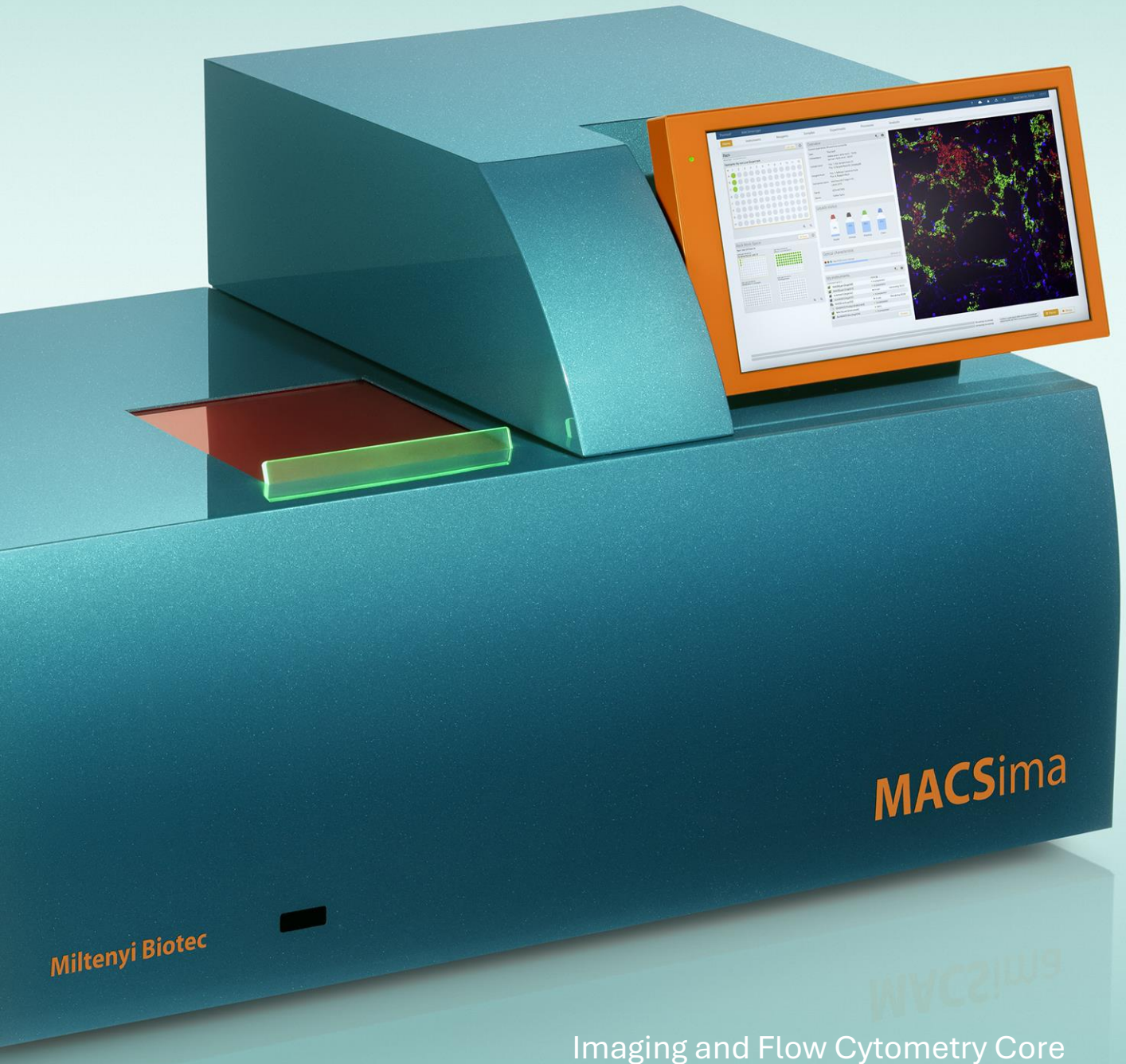
Miltenyi Biotec

MACSima Imaging System
Standard Operation Protocol



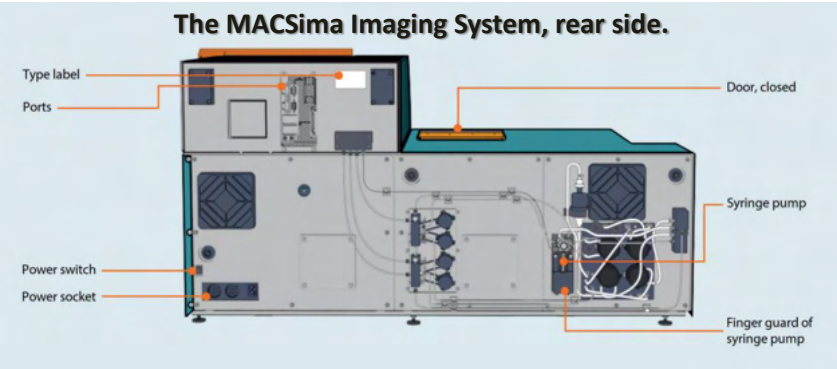
Imaging and Flow Cytometry Core
Center for PanorOmic Sciences

Contents

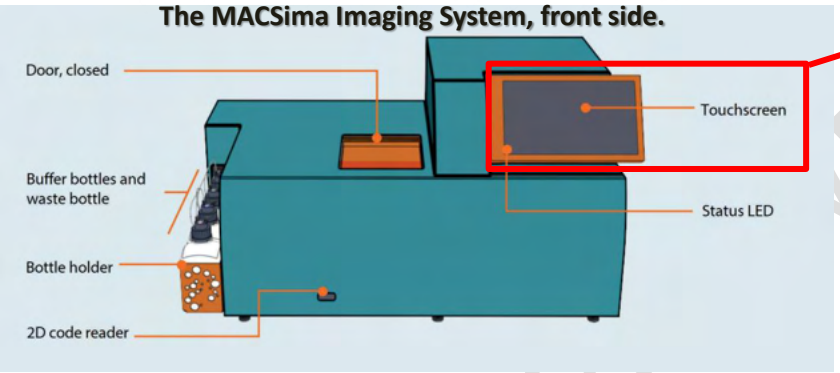
1 Startup of MACSima System

Switching on the Instrument

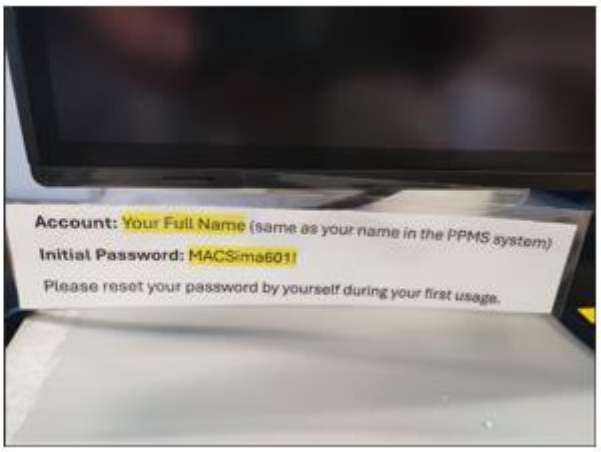
1. Switch on the instrument. The power switch is located on the rear side of the instrument.



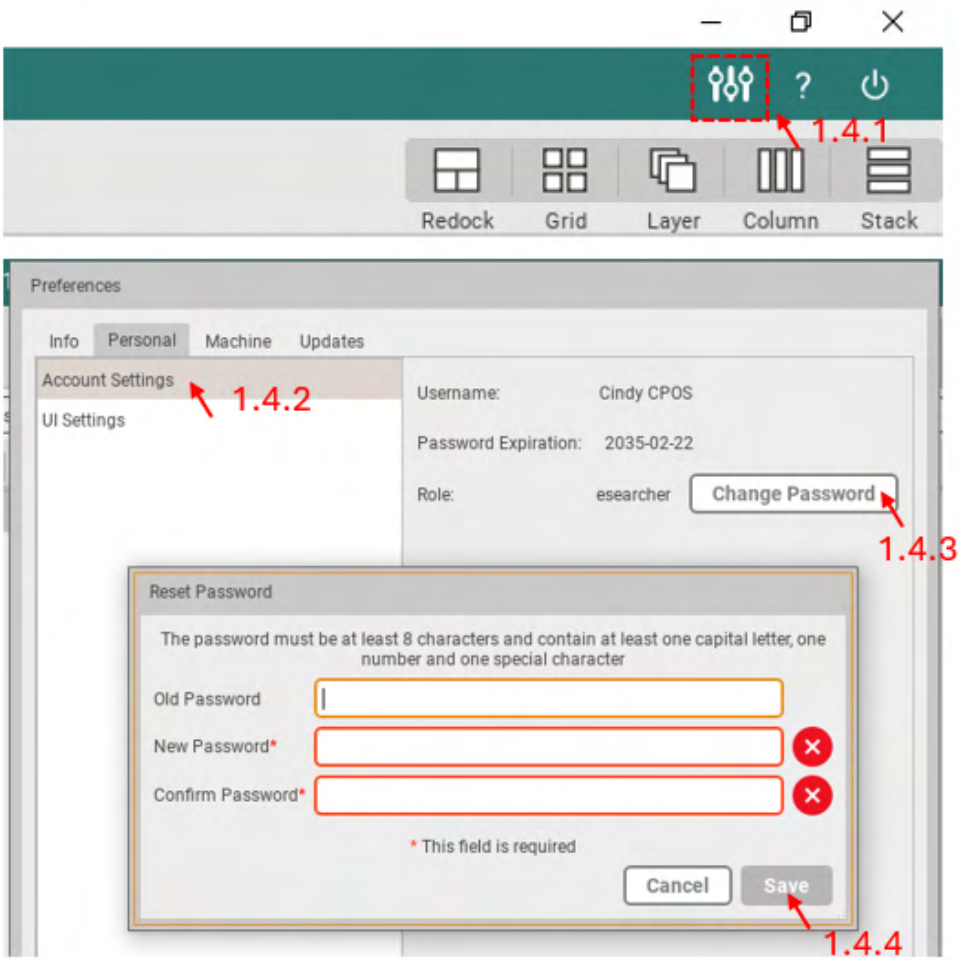
2. Tap the screen to boot the instrument and start the MACS iQ View Software Control Module.



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.



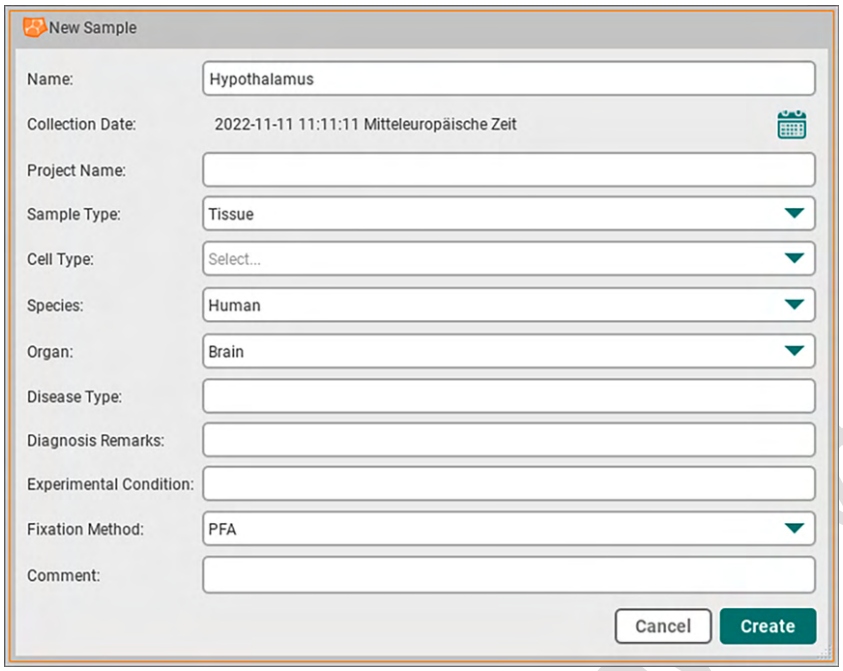
5. Wait for MACSima System warmup for 2 hours (If the system is not shut off by the previous user, you don't need to warm up it.)

2 Setup Experiment

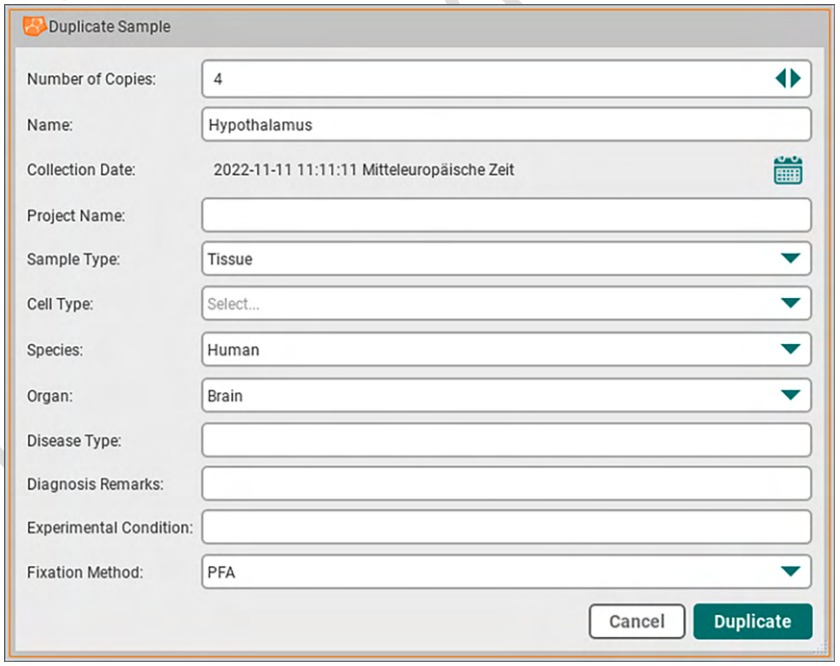
2.1 Create Sample

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.

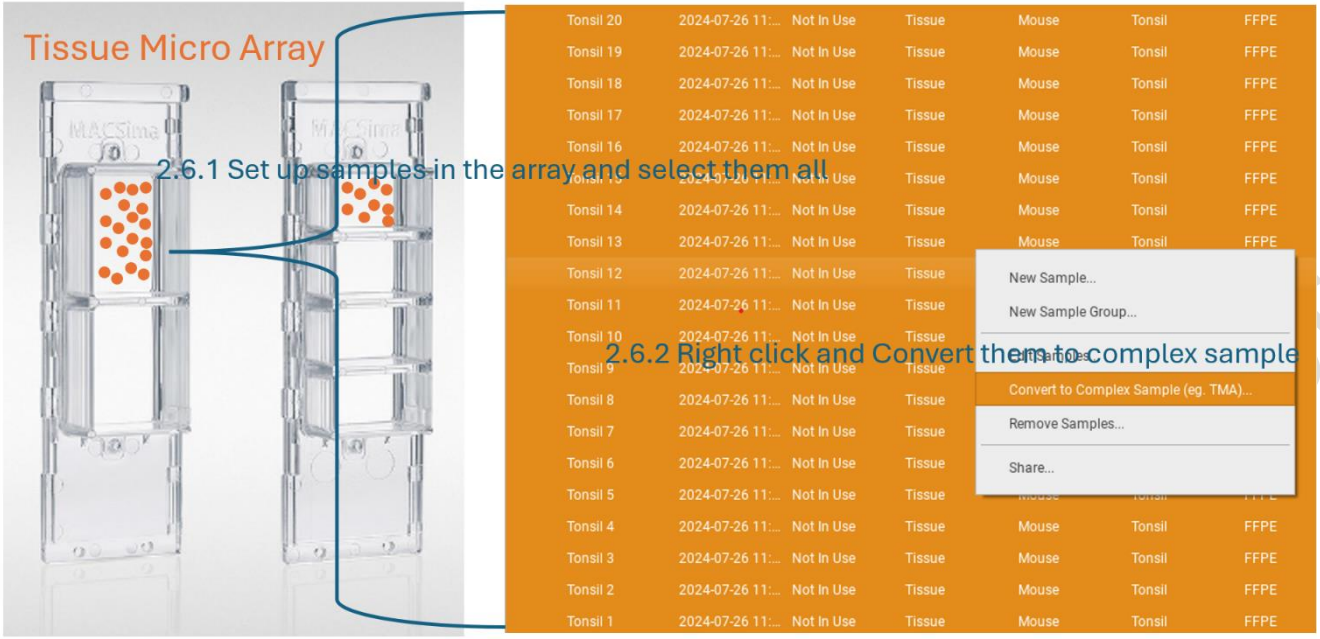


4. If all your samples are of the same type, you could duplicate the sample created with several copies, then the sample creation is finished.
Right-click the newly created sample and click **Duplicate Sample...** to open the **Duplicate Sample window**. Enter the Number of Copies and edit the other fields as needed. Click the **Duplicate** button to create the samples.

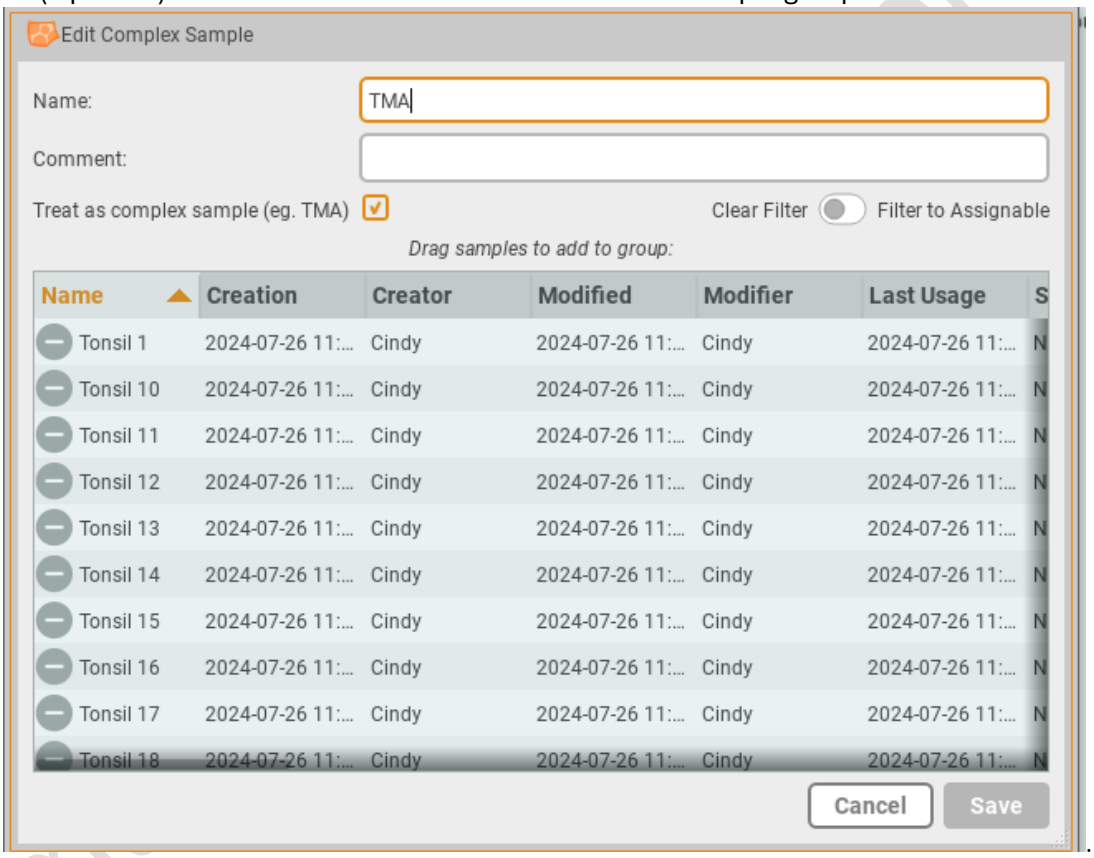


5. If all your samples are of different types, please create all the samples one by one until finished.
6. (Optional) If your sample is a **TMA** (tissue micro array) in the same chamber, please create all the samples in

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



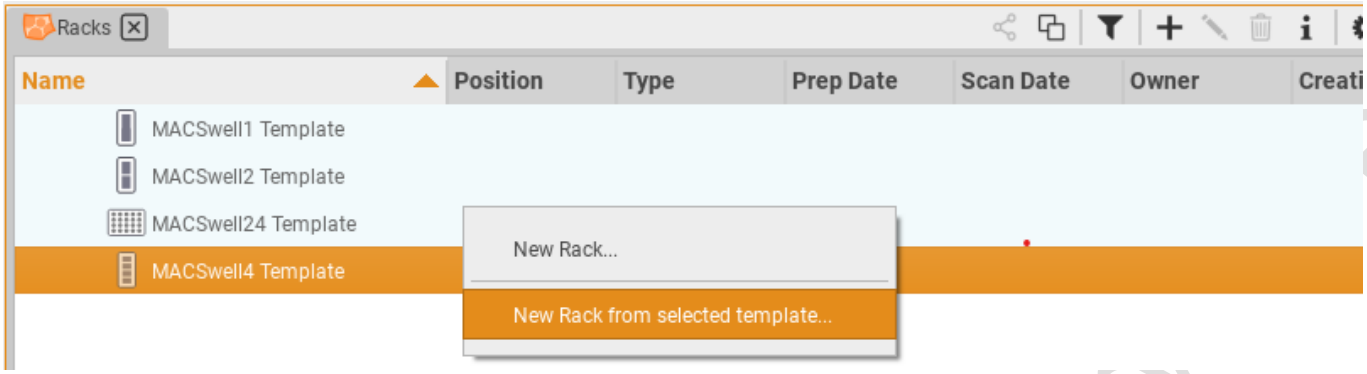
7. (Optional) Click the **SAVE** button to create the TMA sample group



2.2 Assign Sample to Racks

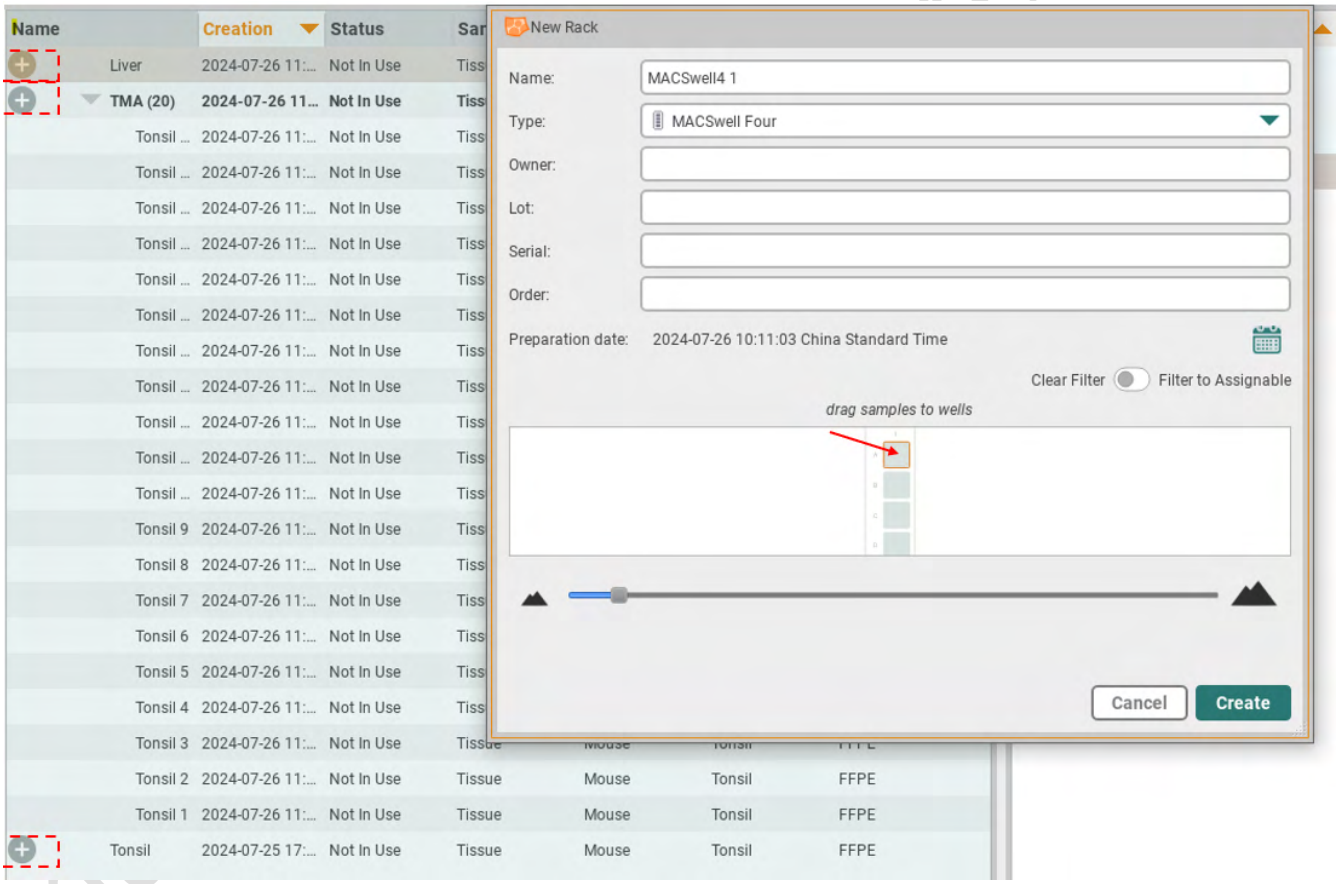
In this step, the racks are added to the experiment and the samples will be assigned to a well in the rack(s).

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**

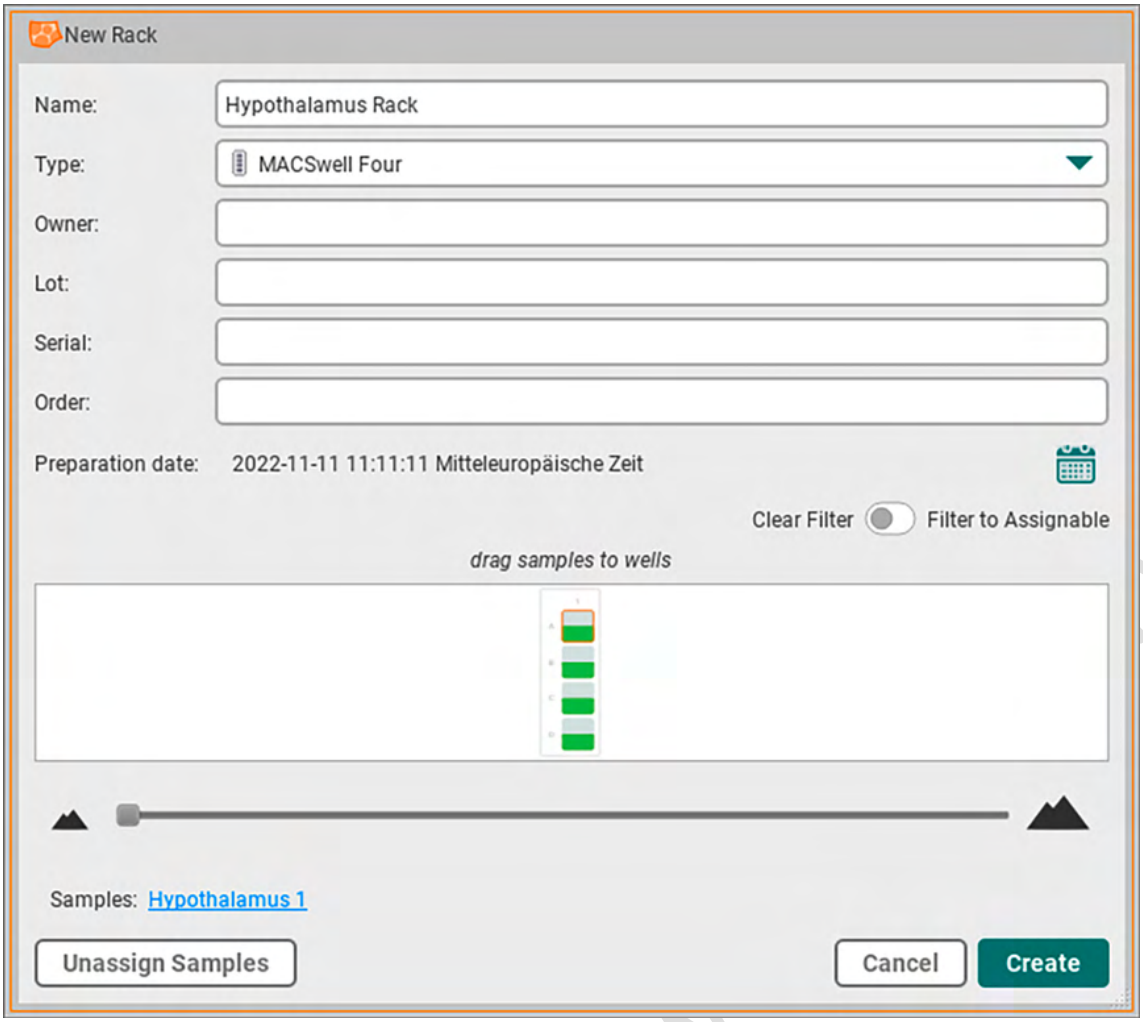


2. In the **New Rack** window, fill in all the required fields..

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.)



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

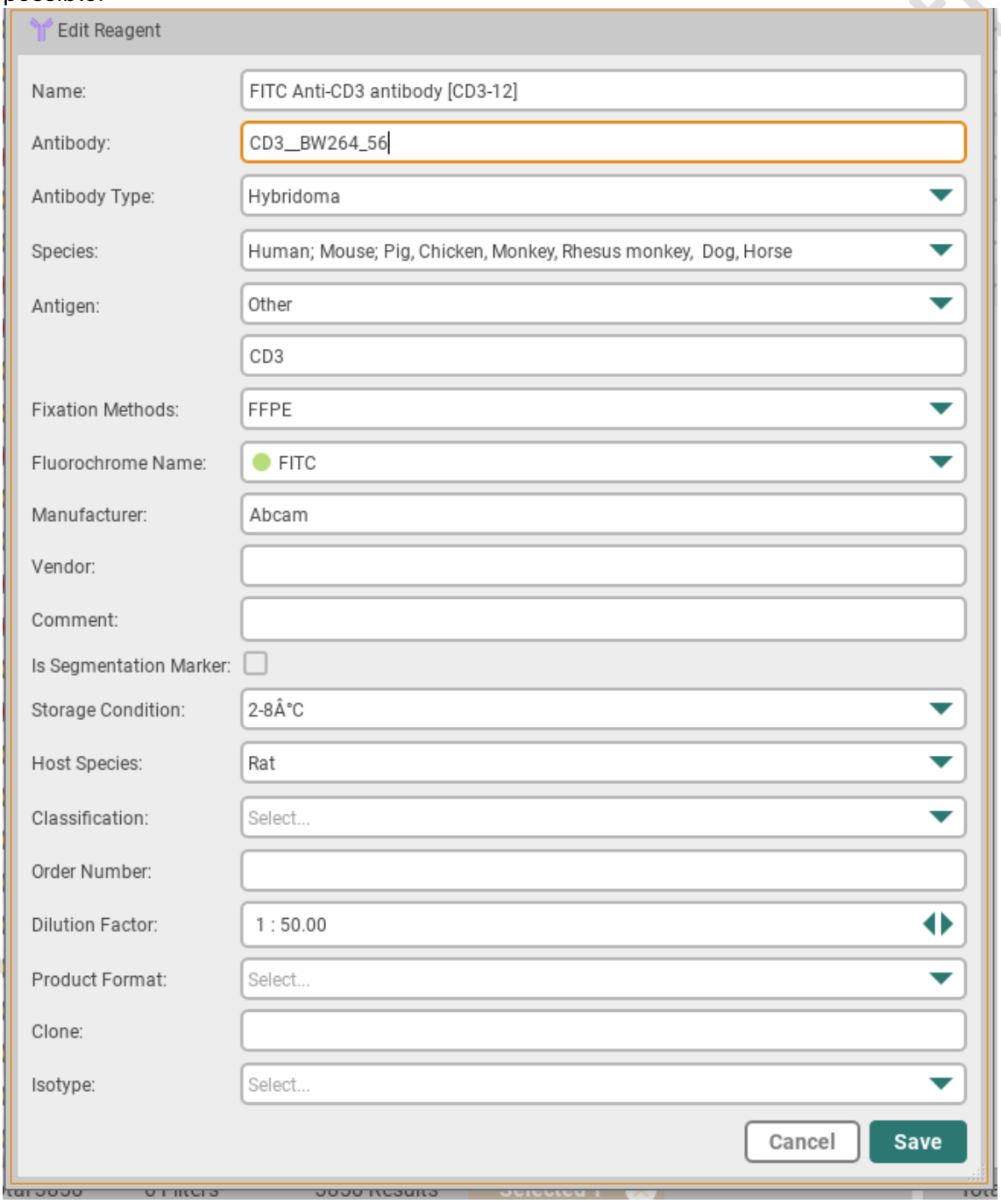
2.3 Managing the Reagents

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, skip2.2.1. 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

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.

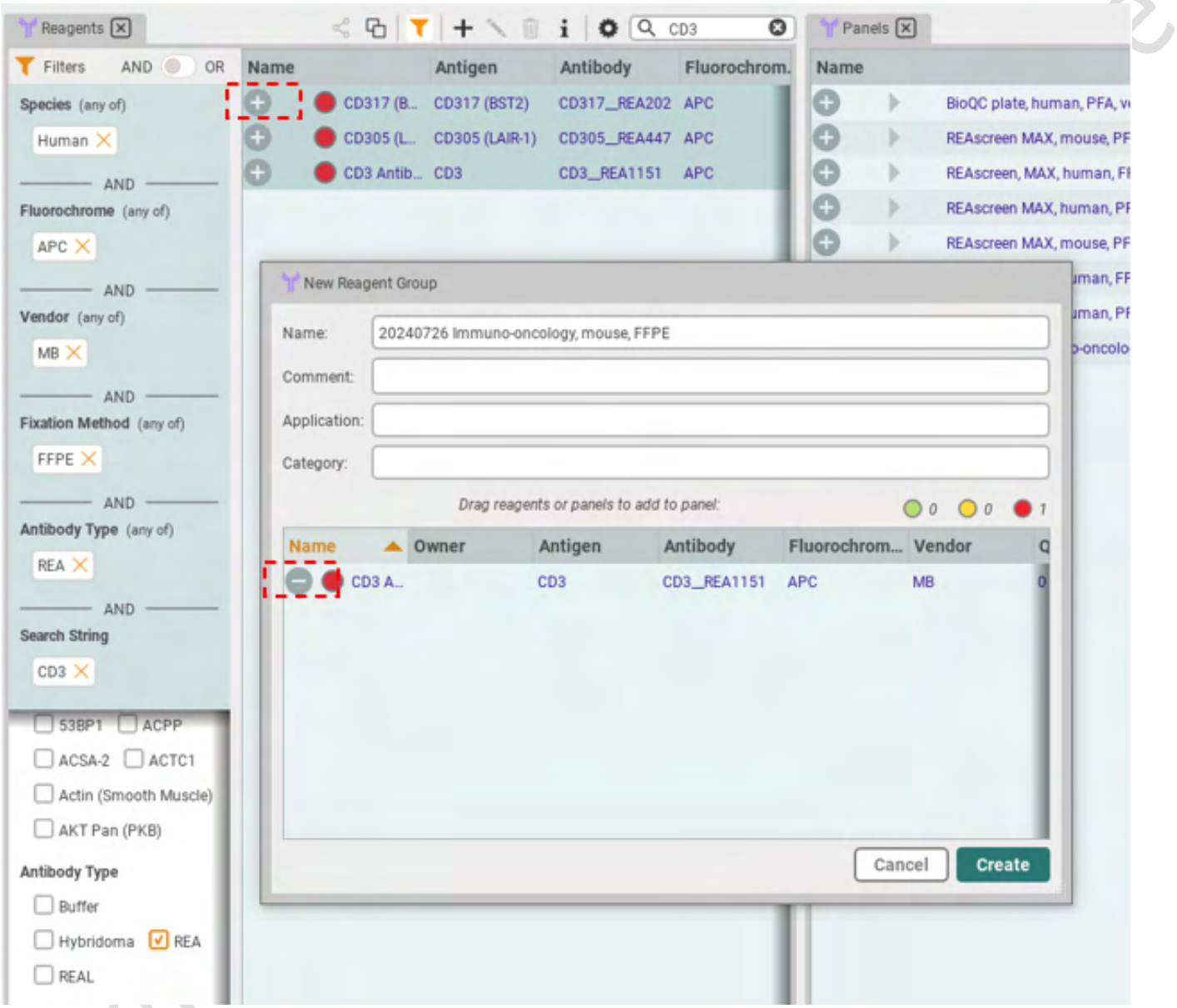


5. Click the **Save** button to add the reagent to the **Reagents** window.

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.

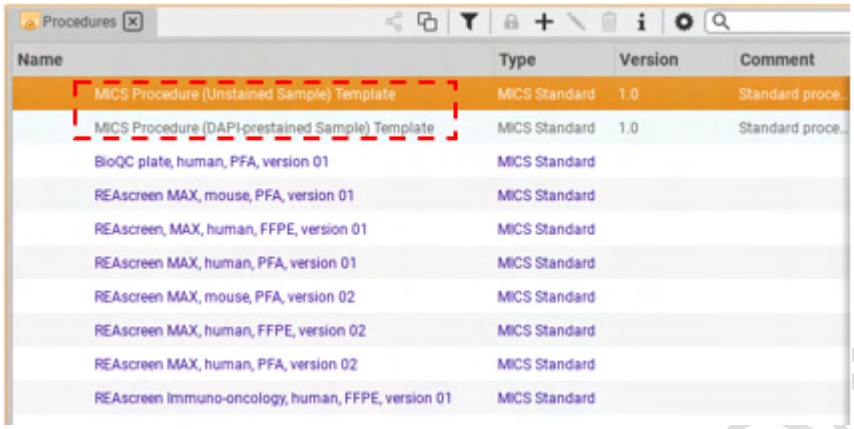


4. Until you filled the New Reagent Group with all the antibody you will use in your imaging. You could remove the wrong antibody that you added by clicking the “Minus” button. Click the **Create** button to create the panel.

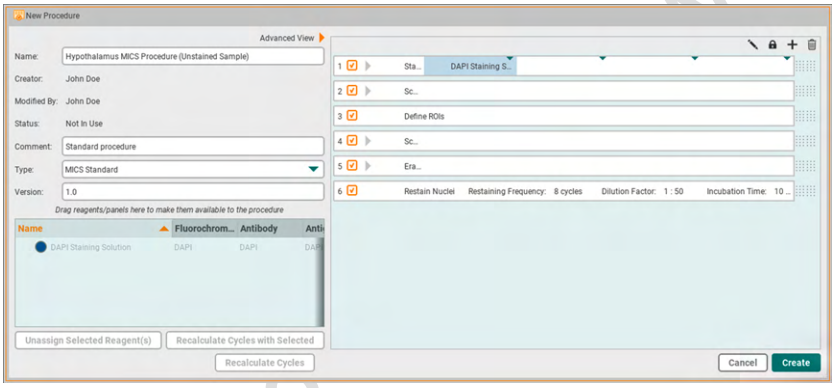
2.4 Creating a Procedure

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

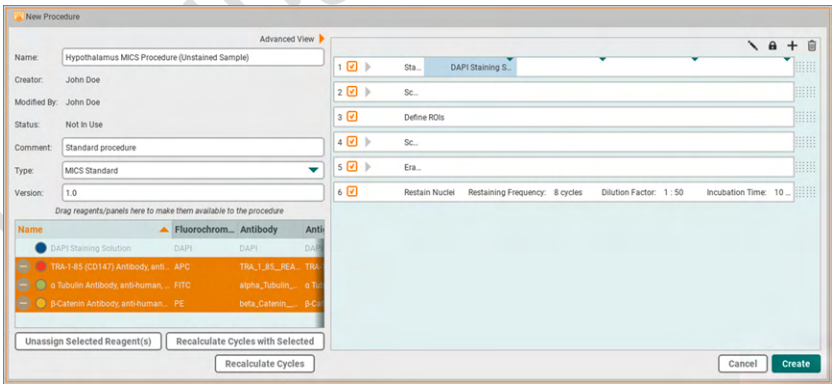
2. Select a template from the **Procedures** window.
3. The **Procedures** window already contains **templates for unstained and DAPI-prestained samples** as well procedures for all available REAscreen Antibody Panels. The unstained template still requires DAPI in a specific well, the DAPI-prestained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



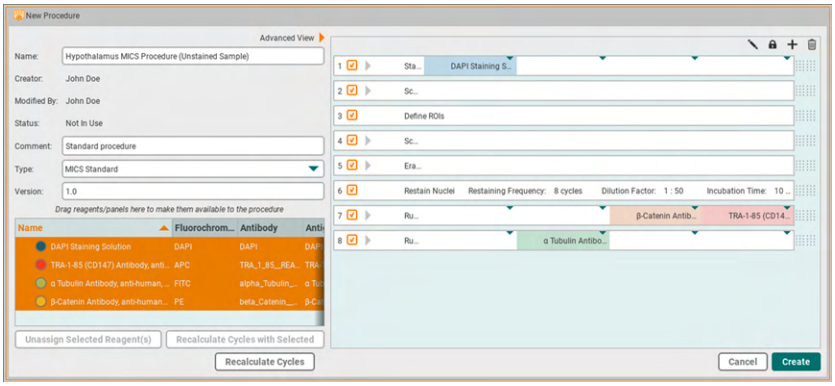
2. Click **Add** > **Create From Template...** in the Procedures table header.
3. Enter the name for the panel in the New Procedure window



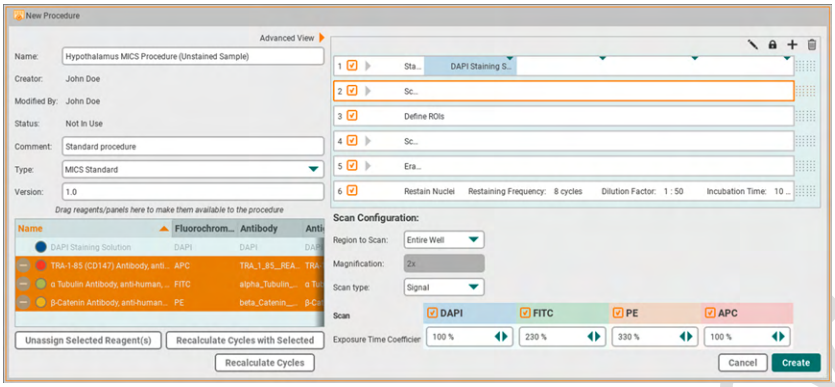
4. Drag panels and/or reagents from the **Inventory**, **Panels**, and/or **Reagents** window to the **New Procedure** window.



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.

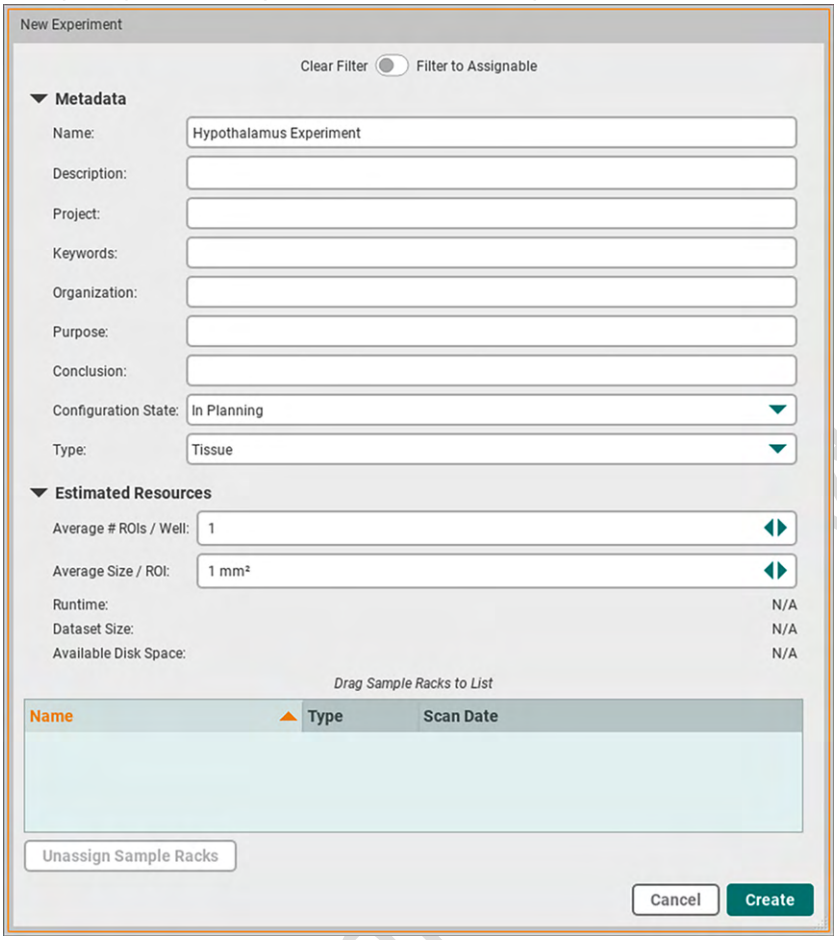
7. Click the **Create** button to save the procedure

2.5 Creating an Experiment

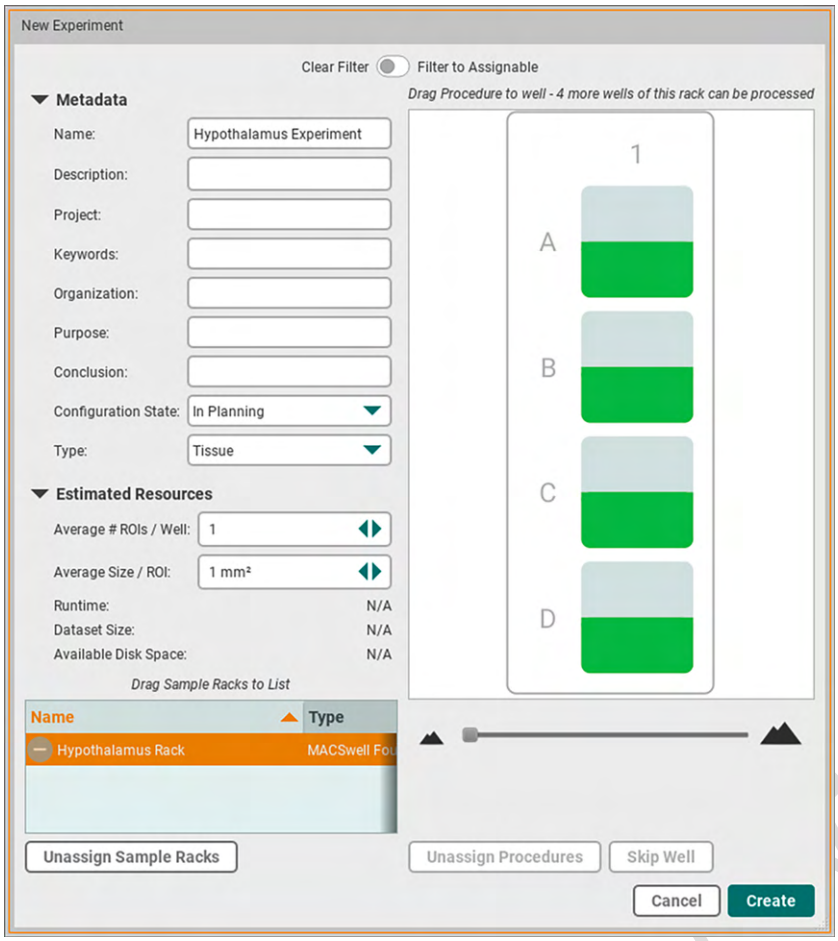
In this step, the previous inputs are combined into an experiment.

1. Click the Experiment Planning tab.

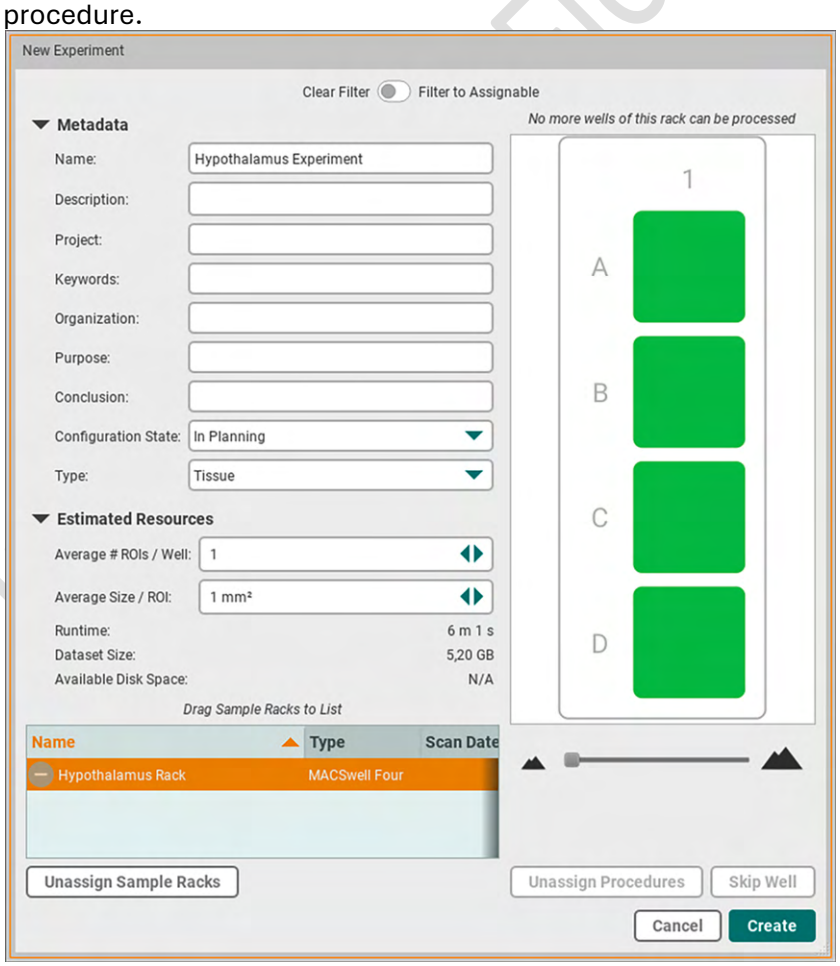
2. Click **Add** > **New Experiment**... in the Experiments table header
3. Specify all the required fields for the experiment. It is recommended to collect as much information as



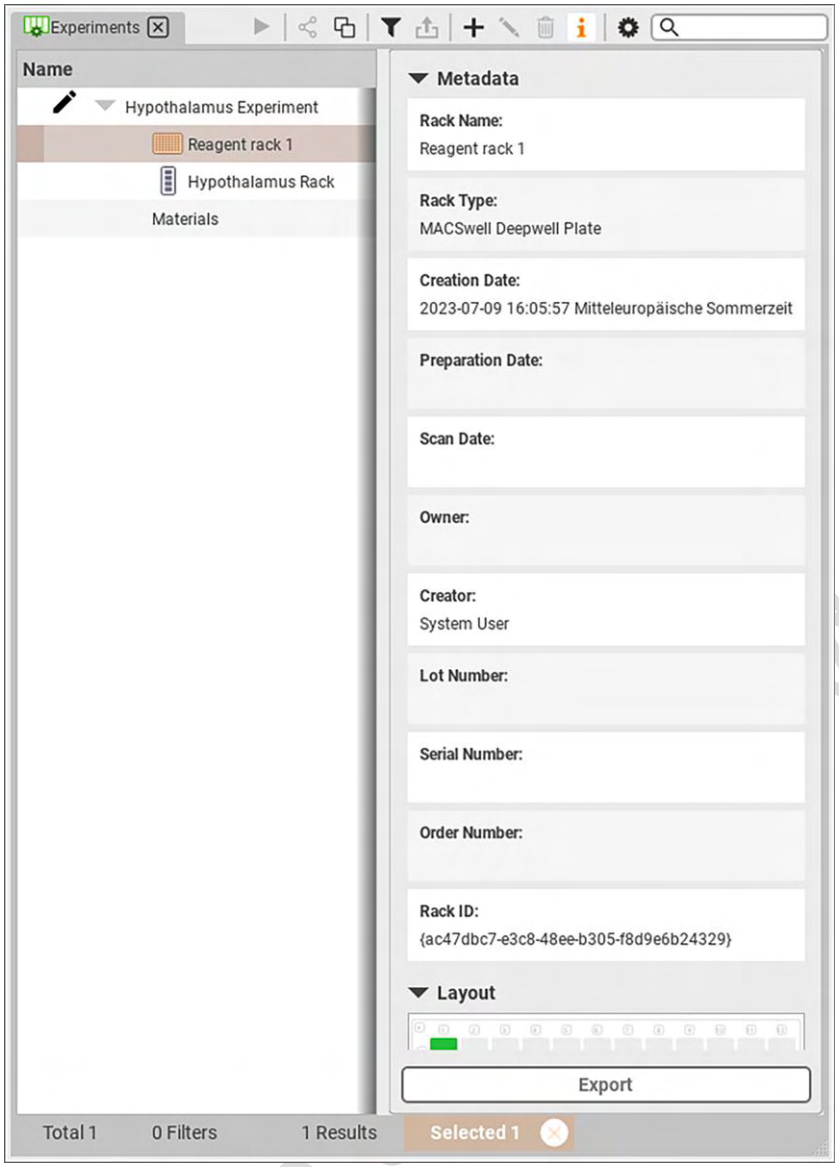
4. Drag the rack(s) from the **Racks** window to the **New Experiment** window. Only racks with assigned samples can be added.



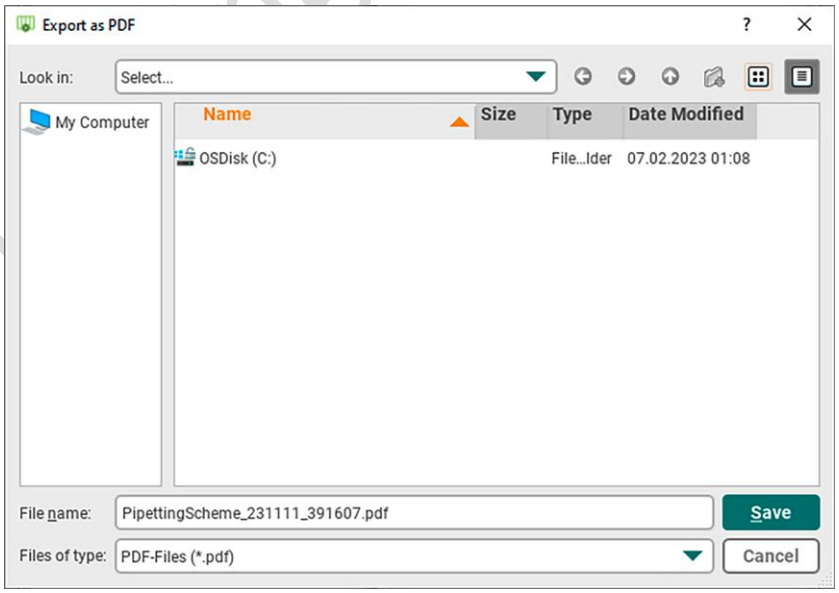
5. Drag the procedure(s) from the **Procedures** window to the wells used in the **New Experiment** window. Procedures can be assigned to multiple wells with different samples. Samples that are not used do not need to be assigned a procedure.



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.



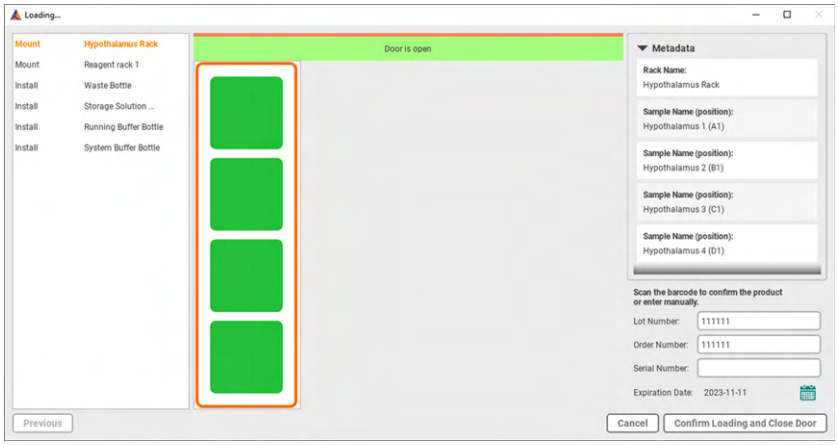
10. Click the **Export** button to save the pipetting scheme as PDF.



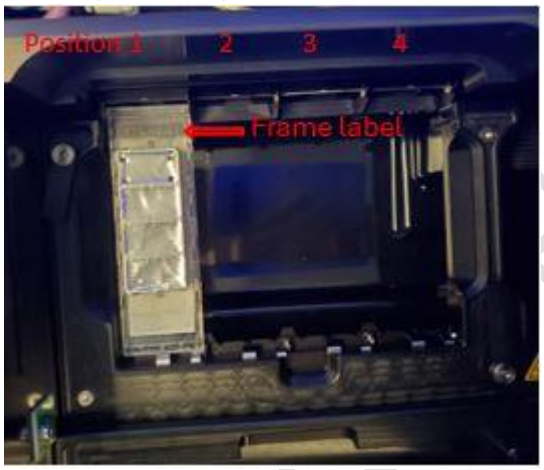
2.6 Running an Experiment

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

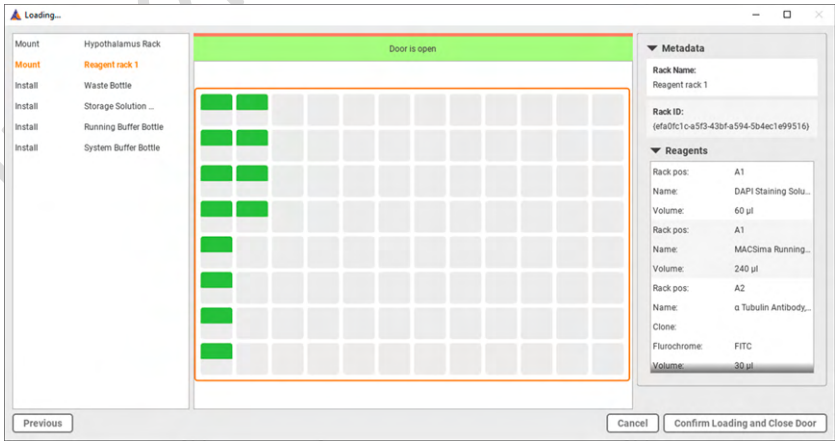
2. Right-click the experiment and click **Run Experiment**... to open the **Loading...** window.
3. Hold the rack(s) in front of the 2D barcode reader. Make sure the 2D barcode is clearly visible.
4. Optional: Enter Lot Number and Order Number for your rack(s).



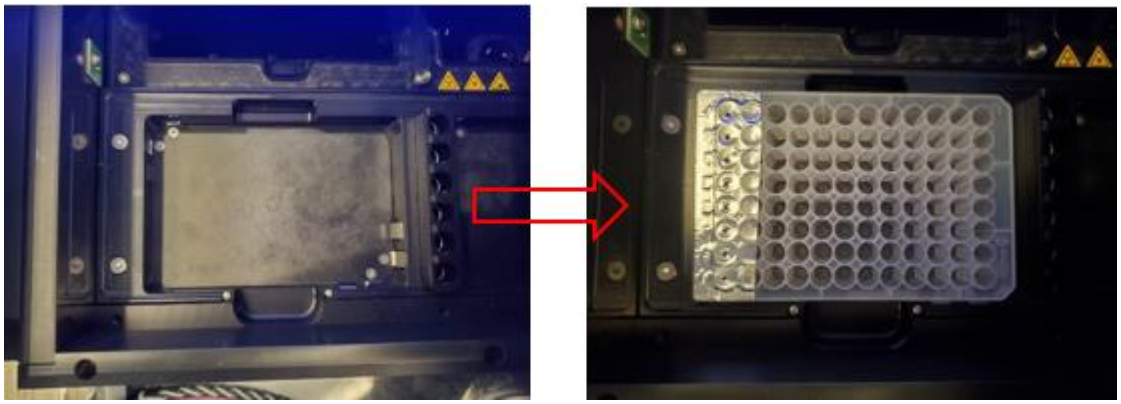
5. Place the sample racks(s) on the indicated imaging stage rack position(s). The stage is moved into the correct position by the instrument.



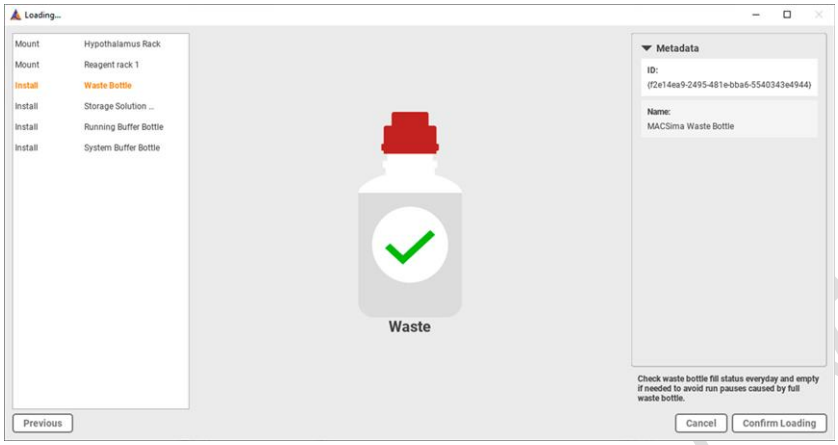
6. Click the **Confirm Loading and Close Door** button. The door on the instrument closes and opens again as indicated by the animation on-screen.



7. Place the reagent rack.



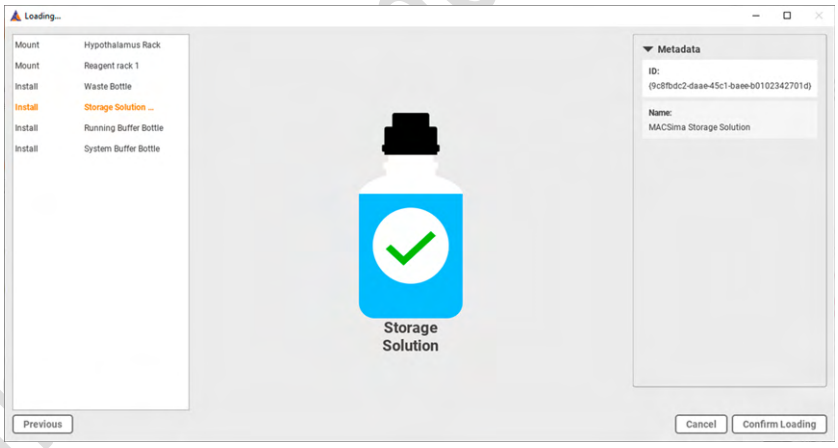
8. Click the **Confirm Loading and Close Door** button. The door on the instrument closes and opens again as indicated by the animation on-screen.



9. Empty the waste bottle.

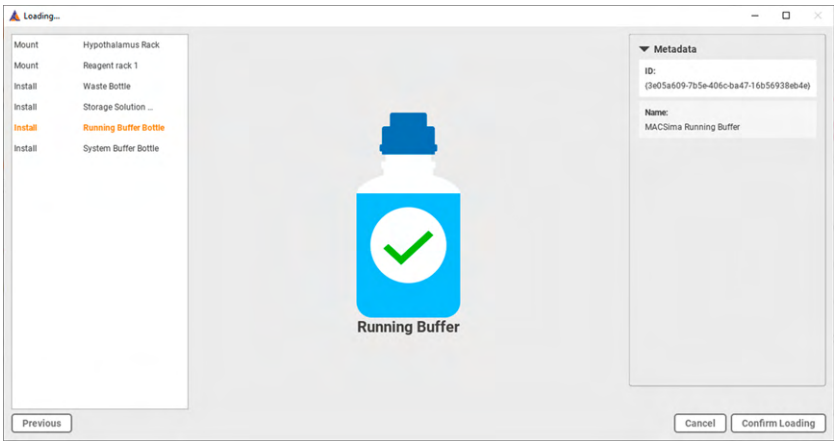
Check the fill status of the waste bottle daily. Ask Technical staff to empty if necessary to avoid run pauses caused by a full waste bottle.

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



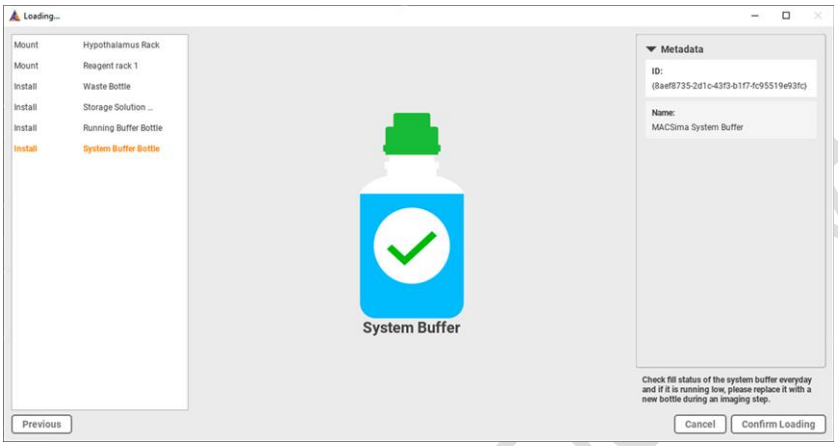
Check fill status of the storage solution. Ask Technical staff to replace the bottle if necessary.

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.

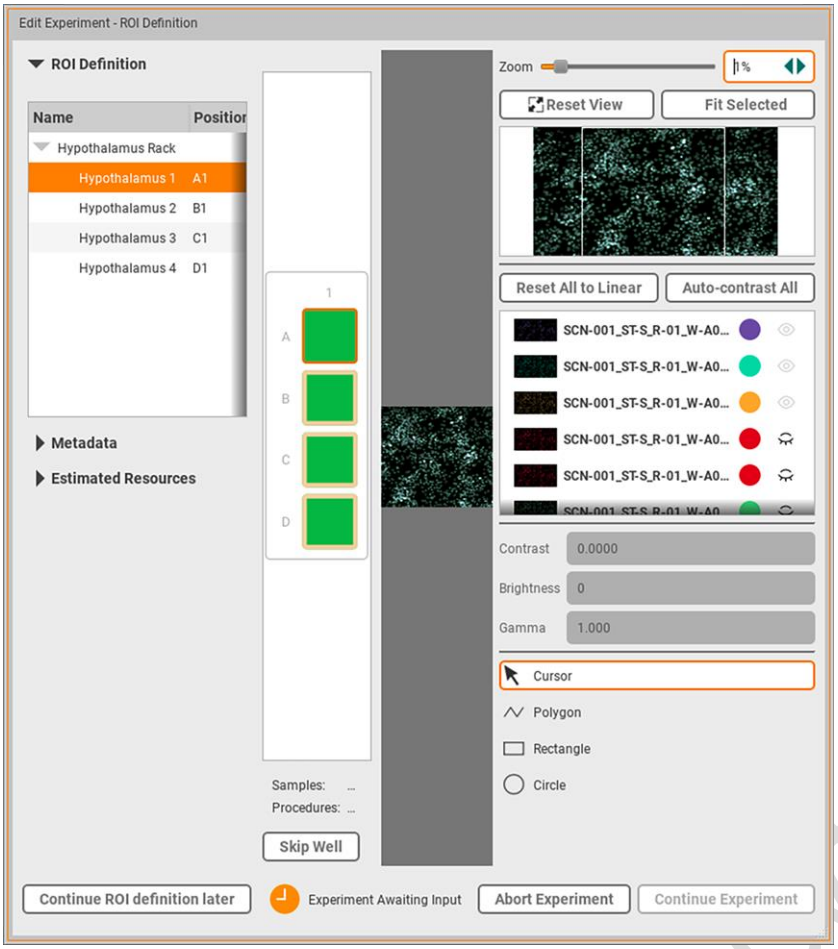
12. Click the **Confirm Loading** button. Check the system buffer



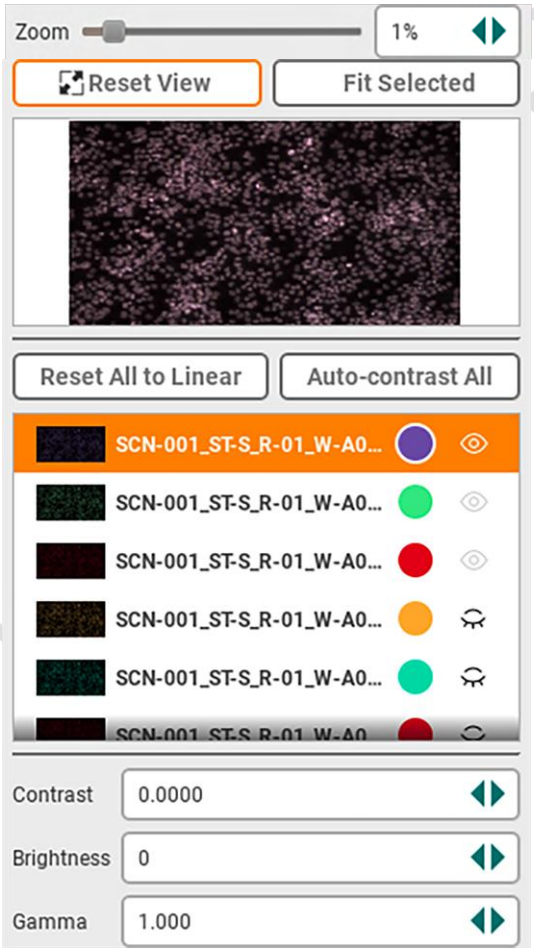
Check the fill status of the system buffer. Ask Technical Staff to replace the bottle during the imaging step if necessary.

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

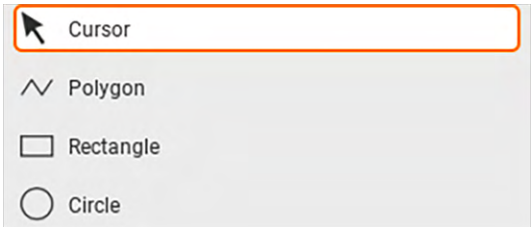
14. Wait until the preview scan has finished. The **Edit Experiment - ROI Definition** window opens.



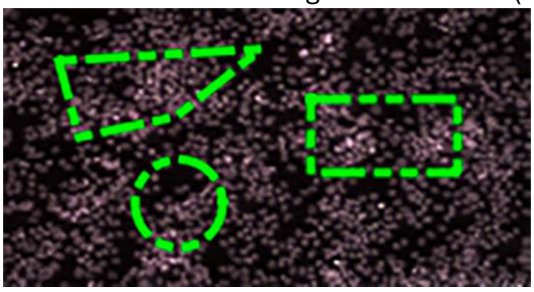
15. Zoom into the preview picture of the first well by using the Zoom slider or your mouse wheel. Adjust the contrast, brightness, and gamma values



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

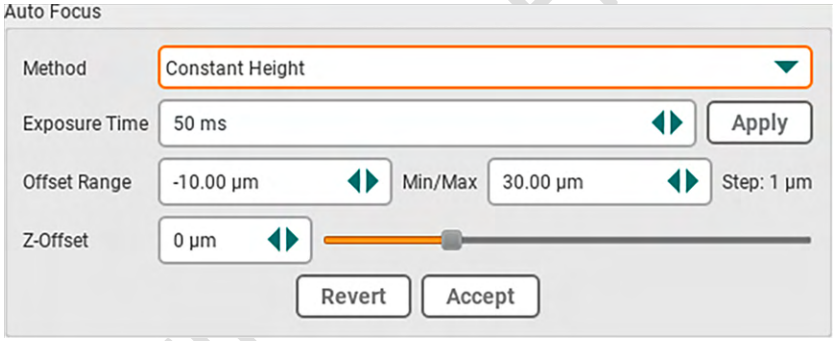


17. Draw one or more regions of interest (ROI).



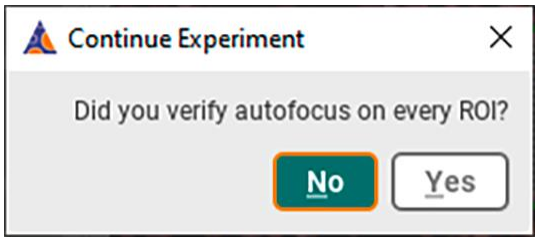
18. Check autofocus for all ROIs in the first well.

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.

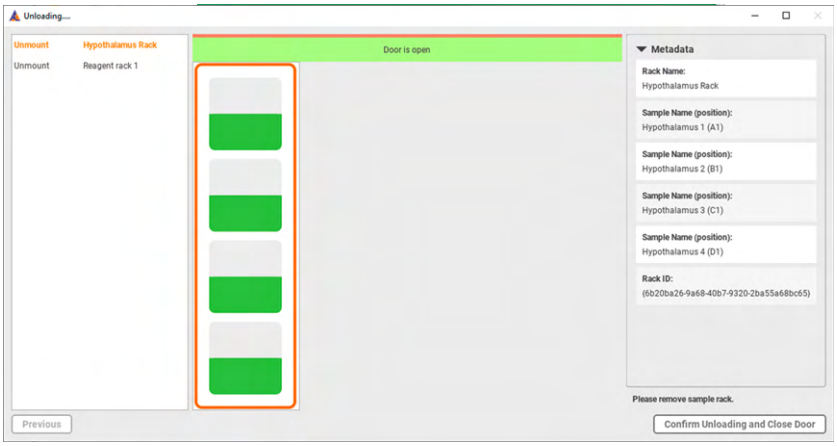
20. Click the Accept button to use the auto focus settings.
21. Repeat steps 18– 20 for all wells in the experiment.
22. Click the Continue Experiment button to start the measurement. A message reminds to set the auto focus for all ROIs. If you confirm the autofocus for each ROI is ok, click "**Yes**" to continue experiment.



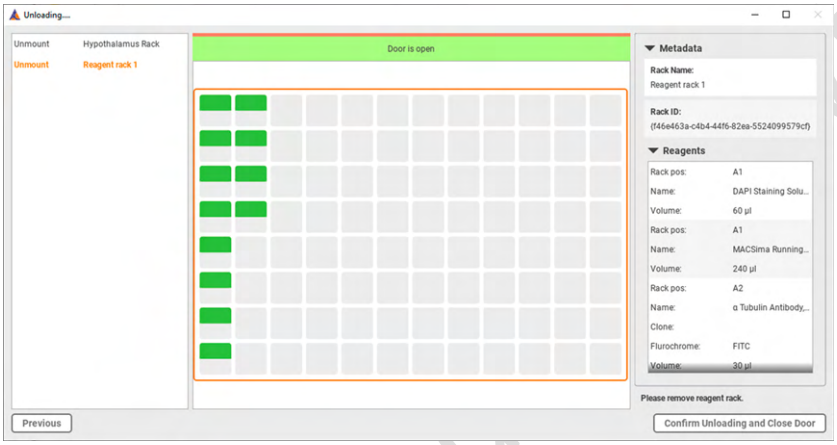
23. You could leave now, and the system will do automatic cycling staining and imaging for you. If it is a long-term imaging (e.g., 3-7 days), please remind Technician to refill the imaging buffer during office hours in case experiment suspension due to insufficient buffer.

3 Backup Data and Shut Down System

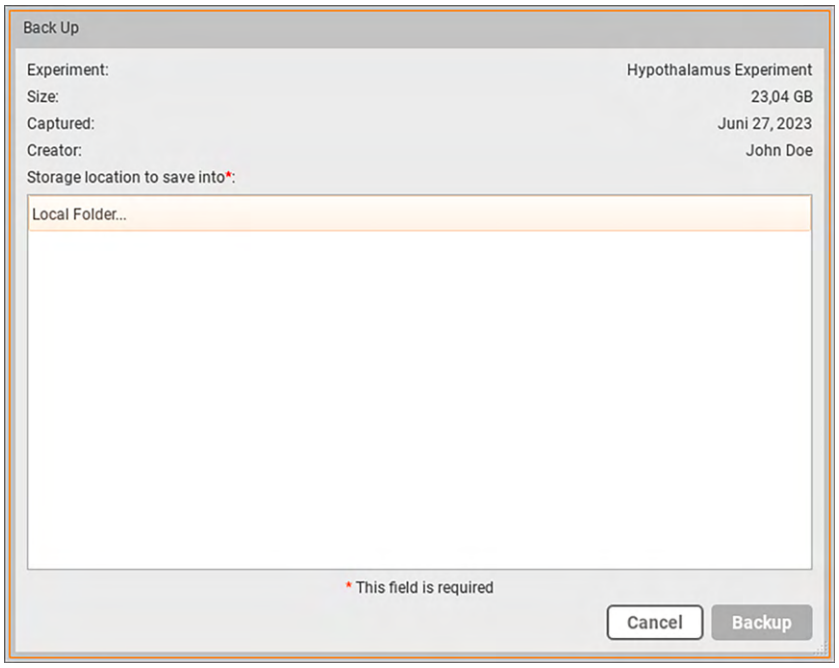
1. Wait until the experiment is finished.
2. Click the **Begin unload** button to open the **Unloading…** window.



3. Remove the sample racks(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.