

## MACSima Imaging System Standard Operation Protocol



MACSima

Miltenyi Biotec

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.

	$ \square$ $\times$
	የያል ነ ጉ
	Redock Grid Layer Column Stack
Preferences	
Info Personal Machine Updates	
Account Settings 1.4.2	Username: Cindy CPOS Password Expiration: 2035-02-22 Role: esearcher Change Password R 1 4 3
Reset Password	
The password must be at le	ast 8 characters and contain at least one capital letter, one umber and one special character
Old Password	
New Password*	
Confirm Password*	
	* This field is required Cancel Save
	1.4.4

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.



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

New Sample	
Name:	Hypothalamus
Collection Date:	2022-11-11 11:11:11 Mitteleuropäische Zeit
Project Name:	
Sample Type:	Tissue
Cell Type:	Select 👻
Species:	Human
Organ:	Brain
Disease Type:	
Diagnosis Remarks:	
Experimental Condition:	
Fixation Method:	PFA 🗸
Comment:	
	Cancel

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.

Duplicate Sample			
Number of Copies:	4		•
Name:	Hypothalamus		
Collection Date:	2022-11-11 11:11:11 Mitteleuropäische Zeit		
Project Name:			
Sample Type:	Tissue		•
Cell Type:	Select		•
Species:	Human		•
Organ:	Brain		•
Disease Type:			
Diagnosis Remarks:			
Experimental Condition:			
Fixation Method:	PFA		•
		Cancel	Duplicate

- 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**.

issue Micro Array 🚺								
Harren Harren	man hi							
	5 U	Tonsil 16	2024-07-26 11:	Not In Use				
2.6.1 Set up sam	ples in the arr	ay and se	lectthem	Nath Use				
	111				Tissue	Mouse	Tonsil	FFPE
•••					Tissue	New Sample		
	100		2024-07-26 11:		Tissue	New Sample G	iroup	
1		Tonsil 10	2024-07-26 11:	Not In Use	Tissue	berne to c	omploy	oomolo
No.	and and	Tonsil 9.0.2	202407-26 11	Not in Use	Convert	. N CHITSpirt (Des)	complex	sample
					Tissue	Convert to Cor	mplex Sample (eg.	TMA)
	7 01				Tissue	Remove Samp	les	
					Tissue	Share		
					Tissue	NIGUSE	TOTION	
( 00 00 · ( 00 0	0							
particular part								

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

🖾 Edit Complex S	ample					
Name:		ТМА				
Comment:	[					
Treat as complex s	sample (eg. TMA)	<ul><li>✓</li></ul>		Clear Filter 🔘	Filter to Assignat	ble
		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:	Ν
Tonsil 10	2024-07-26 11:	Cindy	2024-07-26 11:	Cindy	2024-07-26 11:	Ν
Tonsil 11	2024-07-26 11:	Cindy	2024-07-26 11:	Cindy	2024-07-26 11:	Ν
Tonsil 12	2024-07-26 11:	Cindy	2024-07-26 11:	Cindy	2024-07-26 11:	Ν
Tonsil 13	2024-07-26 11:	Cindy	2024-07-26 11:	Cindy	2024-07-26 11:	Ν
Tonsil 14	2024-07-26 11:	Cindy	2024-07-26 11:	Cindy	2024-07-26 11:	Ν
Tonsil 15	2024-07-26 11:	Cindy	2024-07-26 11:	Cindy	2024-07-26 11:	Ν
Tonsil 16	2024-07-26 11:	Cindy	2024-07-26 11:	Cindy	2024-07-26 11:	Ν
Tonsil 17	2024-07-26 11:	Cindy	2024-07-26 11:	Cindy	2024-07-26 11:	Ν
Tonsil 18	2024-07-26 11:	Cindy	2024-07-26 11:	Cindy	2024-07-26 11:	N
				Ca	ncel Save	

### 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** 

Racks 🗙					~ G	T   + 🔪	🛍 i 🛛 🕯
Name	- <b>A</b>	Position	Туре	Prep Date	Scan Date	Owner	Creati
MACSwell1 Template							
MACSwell2 Template							
MACSwell24 Template		New Deals					
MACSwell4 Template		New Rack					
		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.

Name		Creation 🔻	Status	Sar	New Rack			
Ð	Liver	2024-07-26 11:	Not In Use	Tiss	Name:	MACSwell4 1		
Ð	TMA (20)	2024-07-26 11	Not In Use	Tiss		(@		
	Tonsil	2024-07-26 11:	Not In Use	Tiss	Type:	MACSwell Four		•
	Tonsil	2024-07-26 11:	Not In Use	Tiss	Owner:			
	Tonsil	2024-07-26 11:	Not In Use	Tiss	Lot:			
	Tonsil	2024-07-26 11:	Not In Use	Tiss	Serial:			
	Tonsil	2024-07-26 11:	Not In Use	Tiss	Ordor	[		
	Tonsil	2024-07-26 11:	Not In Use	Tiss	order.			
	Tonsil	2024-07-26 11:	Not In Use	Tiss	Preparation date:	2024-07-26 10:11:03	China Standard Time	
	Tonsil	2024-07-26 11:	Not In Use	Tiss				Clear Filter 🔘 Filter to Assignable
	Tonsil	2024-07-26 11:	Not In Use	Tiss			drag samples to wells	
	Tonsil	2024-07-26 11:	Not In Use	Tiss			A (	
	Tonsil	2024-07-26 11:	Not In Use	Tiss				
	Tonsil 9	2024-07-26 11:	Not In Use	Tiss				
	Tonsil 8	2024-07-26 11:	Not In Use	Tiss				
	Tonsil 7	2024-07-26 11:	Not In Use	Tiss	A			<b>i</b>
	Tonsil 6	2024-07-26 11:	Not In Use	Tiss				
	Tonsil 5	2024-07-26 11:	Not In Use	Tiss				
	Tonsil 4	2024-07-26 11:	Not In Use	Tiss				Cancel Create
	Tonsil 3	2024-07-26 11:	Not In Use	Tissa	ie iviouse	1011311	1112	1
	Tonsil 2	2024-07-26 11:	Not In Use	Tissu	e Mouse	Tonsil	FFPE	
	Tonsil 1	2024-07-26 11:	Not In Use	Tissu	e Mouse	Tonsil	FFPE	

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:	Hypothalamus Rack	
Туре:	I MACSwell Four	
Owner:		
Lot:		
Serial:		
Order:		
Preparation date:	2022-11-11 11:11:11 Mitteleuropäische Zeit	
	Clear Filter  Filter to Assignable drag samples to wells	
	<b>▲</b>	
Samples: <u>Hypoth</u>	nalamus 1	
Unassign San	nples Cancel Create	

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

	Sug.		
Ó	<u></u>		
(nor			

### 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.

🌱 Edit Reagent	
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 💌
	Cancel Save
ar5050 0TINCIS	

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.

Reagents X	00		1 0	Q CD3 C	Panels (	Ň	
Filters AND OR	Name	Antigen	Antibody	Fluorochron	n. Name		
pecies (any of)	🕀 🛛 🖯 CD	317 (B., CD317 (BS)	T2) CD317_R	A202 APC	$\Theta \rightarrow$	BioQC pla	ate, human, PFA,
Human 🗙	🕂 🔴 CD	305 (L., CD305 (LAI	R-1) CD305_R	A447 APC	0	REAscree	en MAX, mouse, i
AND	🕀 🔴 CD	3 Antib CD3	CD3_REAT	151 APC	0	REAscree	en, MAX, human,
luorochrome (any of)					$\Theta \rightarrow$	REAscree	en MAX, human,
APC ×					<b>( ( ) () ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( ) () ( ) ()()</b>	REAscree	en MAX, mouse, l
AND	1 New Rea	gent Group					iman,
endor (any of)	Name	20240726 Immuno	-oncology mouse	FEPE			Jman,
MB ×							p-onco
AND	Comment:						
ixation Method (any of)	Application						
FFPE ×	Category:						
AND		Drag rea	agents or panels to	add to panel:		00 00	• 1
untibody Type (any of)	Name	Owner	Antigon	Antibody	Eluorochrom	Vendor	
REA 🗙		D3 A	cos	CD3 REA1151	APC	MR	
AND			000	COS_ALATION	Aro	MC	1
earch String							
CD3 ×							
Actin (Smooth Muscle)							
AKT Pap (PKP)							
							ato
untibody Type					Car	Crea	are
U Buffer							
🔄 Hybridoma 🗹 REA							

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.

Proc	edures 🗶 🗠 🤘 🕇		i O	Q
Name		Туре	Version	Comment
	MICS Procedure (Unstained Sample) Template			
	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

New Proc	cedure														
	Advanced View 🕨													A .	+ =
Name:	Hypothalamus MICS Procedure (Unstained Sample)		ה		C14 1	ADI Ctainie									
Creator:	John Doe		2	-	518	AFTOLEIIIII	y o								
Modified By:	: John Doe	2	2	▶.	Sc										
Status:	Not in Use	3 🖸	2		Define ROIs										
Comment:	Standard procedure	4 6	2	Þ	Sc										
Type:	MICS Standard	5 💽	2	Þ.	Era										
Version:	1.0	6 🖸	7		Restain Nucle	Restain	ing Freque	ency: 8 cycl	es Dil	ution Factor	1:50	Incubation	Time:	10.	
1	Drag reagents/panels here to make them available to the procedure														
Name	<ul> <li>Fluorochrom Antibody Anti-</li> </ul>														
• DJ	API Staming Solution DAPI DAPI DAP														
Unassig	gn Selected Reagent(s) Recalculate Cycles with Selected														
	Recalculate Cycles											Car	icel	C	reate

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.

New Pros	cedure													
		Advanced	View 🕨											+
Name:	Hypothalamus MICS Procedure (U	Instained Sample)								•				-
Creator:	John Doe			10	P	Sta	DAPI	I Staining S						
Modified By:	John Doe			2 🗹	▶	Sc								
Ctatue	Not in Line			3 🗸		Define	ROIs							
Status:	Not in Use		_		h	60								
Comment:	Standard procedure				-	06								
Type:	MICS Standard		•	5 🗹	▶.	Era								
Version:	1.0			6 🗹		Resta	in Nuclei	Restaining Free	quency: 8 cycles	Dilution Fa	actor: 1:50	Incubation	Time:	10
1	Drag reagents/panels here to make the	em available to the procedure		7 🔽	•	Ru.		*		•	B-Catenin Antib.	TRA-1	-85 (CD	14
Name	🔺 Flu	uorochrom Antibody	Anti					•		*	•			-
<b>O</b>	API Staining Solution DA		DAPI	8 🗹	▶	Ru			a Tubulin Antib	0				
<b>О</b> ТГ			TRA											
			a Tub											
			<b>β-Cat</b>											
Unassig	n Selected Reagent(s) Re	ecalculate Cycles with Selec	ted											
		Recalculate Cycle	s									Cano	el	Creat

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.

			Advanced 1	View 🕨										\ A	+ 0
ime:	Hypothalamus MICS Proceed	ure (Unstained Sam	iple)			01.	0.000							1 8	
reator:	John Doe				10 1	Sta	DAPT S	aining s							
odified By	John Doe				2 🗸 🕨	Sc									
atus	Not in Lise				3 🗸	Define	ROIs								
mment	Standard procedure			_	4 🗸 🕨	Sc									
Armingare.	Standard procedure			=	5 🕢 🕨	Fra									
/pe:	MICS Standard			_		0.0.0									
rsion:	1.0				6 🗹	Restain	Nuclei Re	staining Fi	equency: 8 cy	cles D	lution Factor:	1:50	Incubation 1	Time: 1	0
	Drag reagents/panels here to ma	ke them available to	the procedure	_	Scan Configura	ation:									
ime	Drag reagents/panels here to ma	Fluorochrom	Antibody	Anti	Scan Configura	ation:									
ime	Drag reagents/panels here to ma	Fluorochrom DAPI	Antibody DAPI	Anti-	Scan Configura Region to Scan:	Entire	Well								
lame	Drag reagents/panels here to ma API Staining Solution RA-1-85 (CD147) Antibody, anti.	Fluorochrom DAPI APC	the procedure Antibody DAPI TRA_1_85REA_	Anti DAP TRA	Scan Configura Region to Scan: Magnification:	Entire	Well								
Name	API Staining Solution API Staining Solution RA-1-85 (CD147) Antibody, anti- Tubulin Antibody, anti-human,	Ke them available to Fluorochrom DAPI APC FITC	the procedure Antibody DAPI TRA_1_85REA alpha_Tubulin	Anti DAP TRA- a Tub	Scan Configura Region to Scan: Magnification: Scan type:	Entire 2x Signal	Well								
Name	Drag reagents/panels here to ma API Staining Solution RA-1-85 (CD147) Antibody, anti. Tubulin Antibody, anti-human, Catenin Antibody, anti-human.	ke them available to Fluorochrom DAPI APC FITC PE	the procedure Antibody DAPI TRA_1_85_REA. alpha_Tubulin beta_Catenin	Anti DAP TRA- a Tub B-Cat	Scan Configura Region to Scan: Magnification: Scan type:	Entire 2x Signal	Well		FITC				PAPC		
	prag reagents/panels here to ma API Staining Solution RA-1-85 (CD147) Antibody, anti. Tubulin Antibody, anti-human Catenin Antibody, anti-human	ke them available to Fluorochrom DAPI APC FITC PE	the procedure Antibody DAPI TRA_1_85REA. alpha_Tubulin beta_Catenin	Anti DAP TRA- a Tub B-Cat	Scan Configura Region to Scan: Magnification: Scan type: Scan	Entire 2x Signal	Well		₹ FITC		V PE		Z APC		
lame 0 D 0 T 0 G 0 G 0 G 0 G 0 G 0 G 0 G 0 G	Drag reagents/panels here to ma API Staining Solution 8A-1-85 (CD147) Antibody, anti- Tubulin Antibody, anti-human. Caternin Antibody, anti-human in Selected Reagent(s)	ke them available to Fluorochrom DAP1 APC PE Recalculate C	the procedure Antibody DAPI TRA_1_85REA_ atpha_Tubulin beta_Catenin ycles with Select	Anti; DAP TRA: a Tub B-Cat	Scan Configura Region to Scan: Magnification: Scan type: Scan Exposure Time Co	ation: Entire 2x Signal ( perficier	Well DAPI 100 %		<b>FITC</b> 230 %	•	<b>V PE</b>	•	<b>APC</b>	•	

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			
Туре:	Tissue		▼	κŲ
▼ Estimated Resource	es			
Average # ROIs / Well:	1		•	
Average Size / ROI:	1 mm²		•	
Runtime:			N/A	
Dataset Size: Available Disk Space:			N/A N/A	
	Drag Sa	mple Racks to List		
Name	🔺 Туре	Scan Date		
Unassign Sample Ra	cks			
			Cancel Create	
				4

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 t	o Assignable	
▼ Metadata Drag Pro	cedure to well - 4 more wells of this rack can be processed	
Name: Hypothalamus Experiment		
Description:	1	
Project:		
Keywords:	A	
Organization:		
Purpose:		
Conclusion:	В	
Configuration State: In Planning		
Type: Tissue		
▼ Estimated Resources	C	
Average # ROIs / Well: 1		
Average Size / ROI: 1 mm <sup>2</sup>		
Runtime: N/A	D	
Available Disk Space: N/A	_	
Drag Sample Racks to List		
Name Type	-	
Hypothalamus Rack MACSwell Fou		
		KV
Unassign Sample Racks Una	ssign Procedures Skip Well	
	Cancel	

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.

ew Experiment				
- Matadata	Clear Filter	Filter to Assigna	able No more wells of this ra	ck can be processed
<ul> <li>Metadata</li> </ul>			(	
Name:	Hypothalamus Experiment			1
Description:				·
Project:				
Keywords:			A	
Organization:				
Purpose:				
Conclusion:			В	
Configuration State:	In Planning	•		
Туре:	Tissue	•		
<ul> <li>Estimated Resource</li> </ul>	ces		С	
Average # ROIs / Well:	1	•		
Average Size / ROI:	1 mm²	•		
Runtime:		6 m 1 s	D	
Dataset Size:		5,20 GB		
Available Disk Space:	rag Sample Racks to List	N/A		
lame	🔺 Туре	Scan Date		
Hypothalamus Rack	MACSwell For	ar 👘	A -	
	_			
Unassign Sample Ra	acks	(	Unassign Procedure	s Skip Well
			[ Ca	ncel Create
			Ca	create

- 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.

ne	▼ Metadata	
<ul> <li>Hypothalamus Experiment</li> <li>Reagent rack 1</li> </ul>	Rack Name: Reagent rack 1	
Hypothalamus Rack Materials	Rack Type: MACSwell Deepwell Plate	c C
	Creation Date: 2023-07-09 16:05:57 Mitteleuropäische Sommerzeit	
	Preparation Date:	$P_{\mathbf{x}}$
	Scan Date:	
	Owner:	
	Creator: System User	×O`
	Lot Number:	
	Serial Number:	
	Order Number:	
	Rack ID: {ac47dbc7-e3c8-48ee-b305-f8d9e6b24329}	
	▼ Layout	
	Export	

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

Export as	PDF					?	
Look in:	Select.		8	• 0	000	2 🙂	
S My Com	puter	Name	▲ Size	Туре	Date Modi	fied	
		11을 OSDisk (C:)		FileIder	07.02.2023	01:08	
File <u>n</u> ame:	Pipetti	ngScheme_231111_391607.	pdf			<u>S</u> a	114
							_

### 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).

ount	Hypothalamus Rack	Door is open	▼ Metadata
ount	Reagent rack 1		Rack Name:
tall	Waste Bottle		Hypothalamus Rack
all	Storage Solution		Sample Name (position):
all	Running Buffer Bottle		Hypothalamus 1 (A1)
all	System Buffer Bottle		Sample Name (position):
			Hypothalamus 2 (B1)
			Sample Name (position):
			Hypothalamus 3 (C1)
			Sample Name (position):
			Hypotnalamus 4 (01)
			Scan the barcode to confirm the product
			Lot Number: 111111
			Order Number: 111111
			Serial Number:
			Expiration Date: 2023-11-11

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.

Loading			- D ×
Mount	Hypothalamus Rack		▼ Metadata
Mount	Reagent rack 1		ID:
Install	Waste Bottle		(f2e14ea9-2495-481e-bba6-5540343e4944)
Install	Storage Solution		Name:
Install	Running Buffer Bottle		MACSima Waste Bottle
Install	System Buffer Bottle		
		· · · · · · · · · · · · · · · · · · ·	
		Waste	
			Check waste bottle fill status everyday and empty if needed to avoid run pauses caused by full waste bottle.
Previous	5		Cancel Confirm Loading

### 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

🛦 Loading	•		- 0
Mount	Hypothalamus Rack		▼ Metadata
Mount	Reagent rack 1		ID:
Install	Waste Bottle		(9c8fbdc2-daae-45c1-baee-b0102342701
Install	Storage Solution		Name:
Install	Running Buffer Bottle		MACSima Storage Solution
Install	System Buffer Bottle		
		Storage	
		Solution	
		00.000	

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

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

Loading			- 0
Mount	Hypothalamus Rack		▼ Metadata
Mount	Reagent rack 1		ID:
Install	Waste Bottle		(3e05a609-7b5e-406c-ba47-16b56938eb4e
nstall	Storage Solution		Name:
nstall	Running Buffer Bottle		MACSima Running Buffer
Install	System Buffer Bottle		
		Running Buffer	
Desident			
Previous			Cancel Confirm Loadin

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

# Image: Image:

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.

ROI Definition				Zoom	[h%]
				Reset View	Fit Selected
Name	Position			P]Reserview	The Science
Hypothalamus Rad	ж				
Hypothalamus	1 A1				and the second
Hypothalamus	2 B1			- 32 - 35	
Hypothalamus	3 C1				
Hypothalamus	4 D1			Reset All to Linear	Auto-contrast All
				Reservanto Eniedi	
		A		SCN-001_ST-S_F	R-01_W-A0 🔵 💿
	- 1			SCN-001_ST-S_F	R-01_W-A0 🔵 💿
		в		SCN-001_ST-S_F	R-01_W-A0 😑 💿
Metadata			Constrainty .	SCN-001_ST-S_F	R-01_W-A0 🔴 🛱
Estimated Resources	rces	С		SCN-001_ST-S_F	R-01_W-A0 🔴 😪
p Lotinated Reood				SCN.001 STS	2-01 W-40
		D			
				Contrast 0.0000	
				Brightness 0	
				Gamma 1.000	
				Cursor	
				✓ Polygon	
				Rectangle	
		Samples:		O Circle	
		Procedures:			
		Skip Well			
		-		-	
Continue ROI defin	ition later	Experiment	Awaiting Input	Abort Experiment	Continue Experiment

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

Zoom 🗕	)		1%		
Res	set View	Fit	Select	ed	
Reset A	ll to Linear	Auto-c	ontras	t All	
	6CN-001_ST-S_R	-01_W-A0	. ()	۲	
5	SCN-001_ST-S_R	-01_W-A0	. 🔴	$\odot$	
\$	SCN-001_ST-S_R	-01_W-A0	. 🔴	$\odot$	
5	SCN-001_ST-S_R	-01_W-A0	. 🔴	Ŷ	
5	SCN-001_ST-S_R	-01_W-A0	. ●	Ŷ	
SCN-001 ST-S R-01 W-40					
Contrast	0.0000				
Brightness	0				
Gamma	1.000				

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

K	Cursor
$\sim$	Polygon
	Rectangle
0	Circle

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



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

Auto Focus				
Refine AF				
Method	Constant Height			
Exposure Time	50 ms			
Offset Range	-10.00 µm	Min/Max	30.00 µm	Step: 1 µm
Z-Offset	0 µm -			
	Арг	ply to all ROIs in	Well	

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

Method	Constant Height				
Exposure Time	50 ms	Apply			
Offset Range	-10.00 µm	Step: 1 µm			
Z-Offset	0 µm 🜗 ———				
	Revert Accept				

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.

🛕 Continue Experiment	×
Did you verify autofocus on ever	y Roi?
<u>N</u> o <u>Y</u>	es

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.

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### 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.

noun	Hypothalamus Rack	Door is open	▼ Metadata	
Unmount	Reagent rack 1		Rack Name: Reagent rack 1	
			Rack ID: (f46e463a-c4b4-44f6-82ea-552409	9579cf)
			▼ Reagents	
			Rack pos: A1 Name: DAPI Stainin Volume: 60 µl Rack pos: A1 Name: MACSima R	Solu
			Volume: 240 µl	
			Rack pos: A2 Name: a Tubulin An Clone:	ibody,
			Flurochrome: FITC Volume: 30 µl	_
			Please remove reagent rack.	

- 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.)

Back Up		
Experiment:		Hypothalamus Experiment
Size:		23,04 GB
Captured:		Juni 27, 2023
Creator:		John Doe
Storage location to save into*:		
Local Folder		
	* This field is required	
		Cancel Backup

- 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.

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