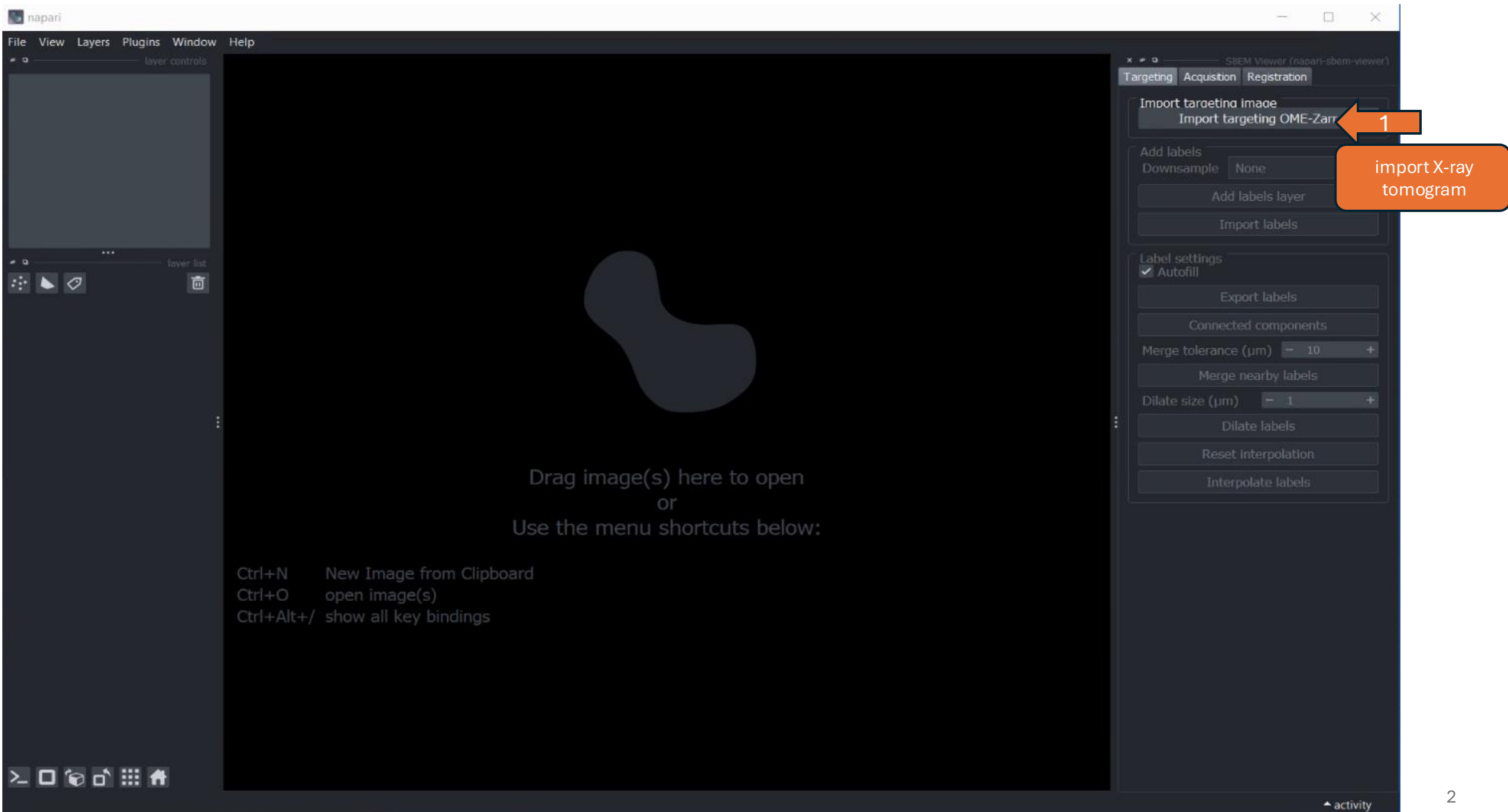
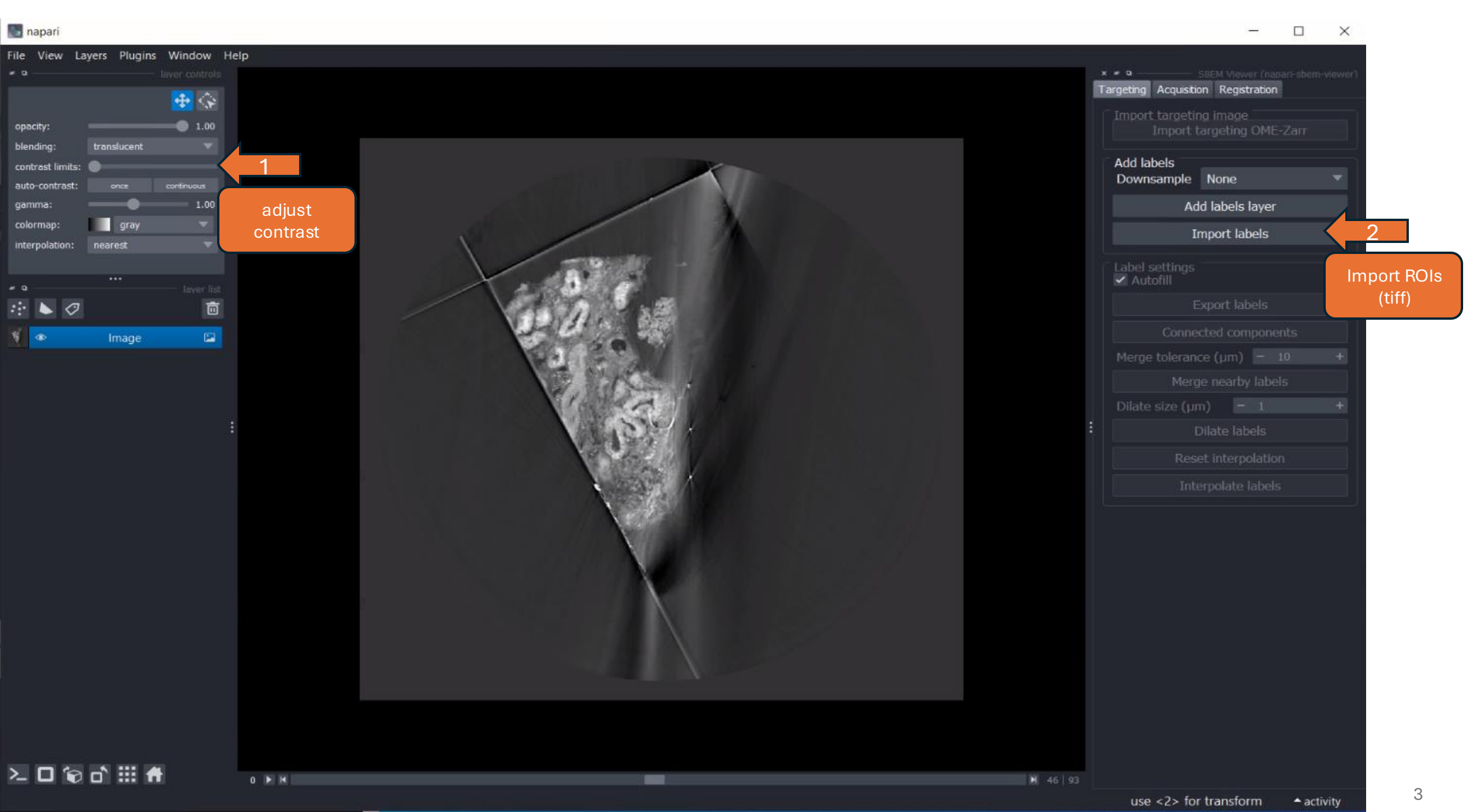
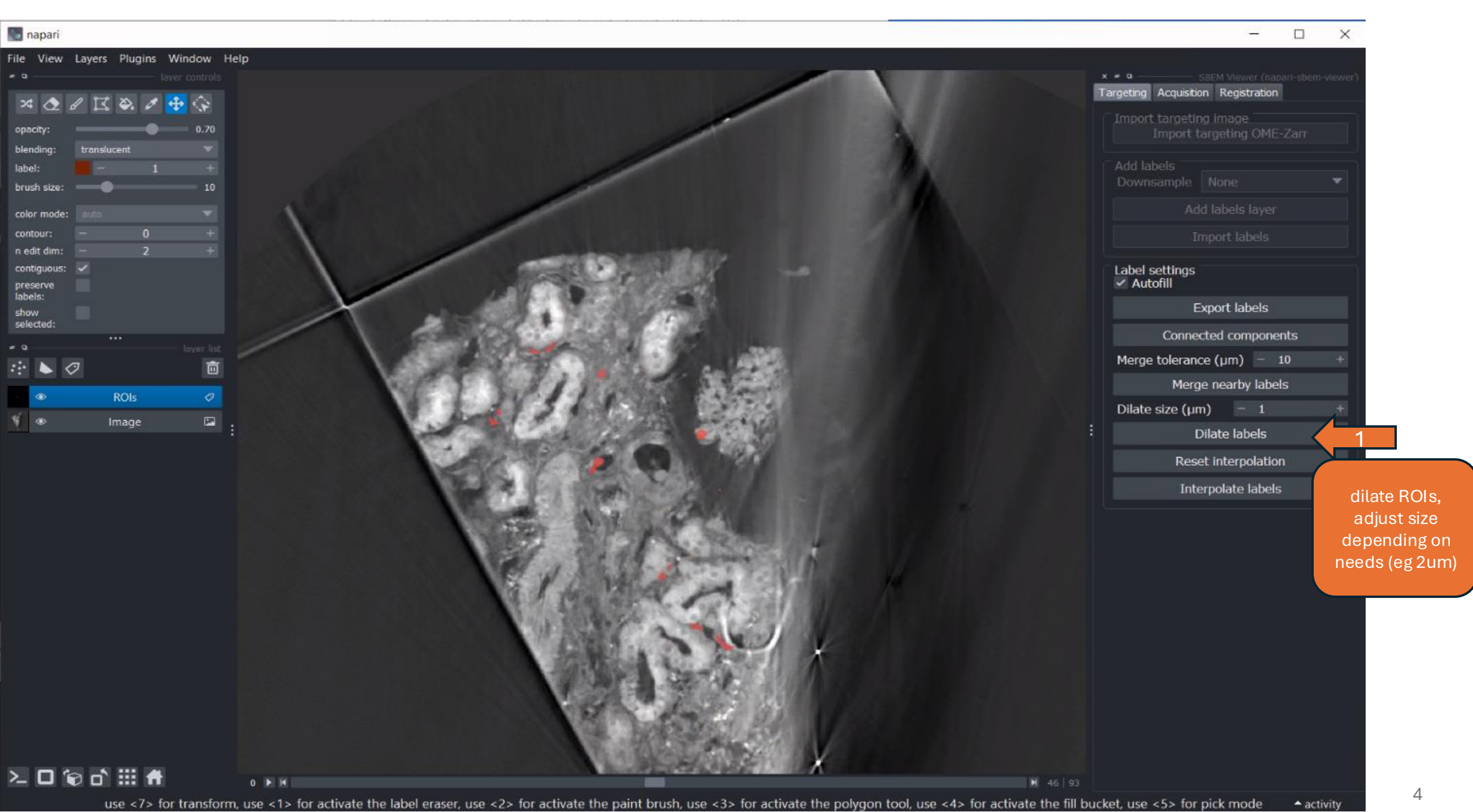


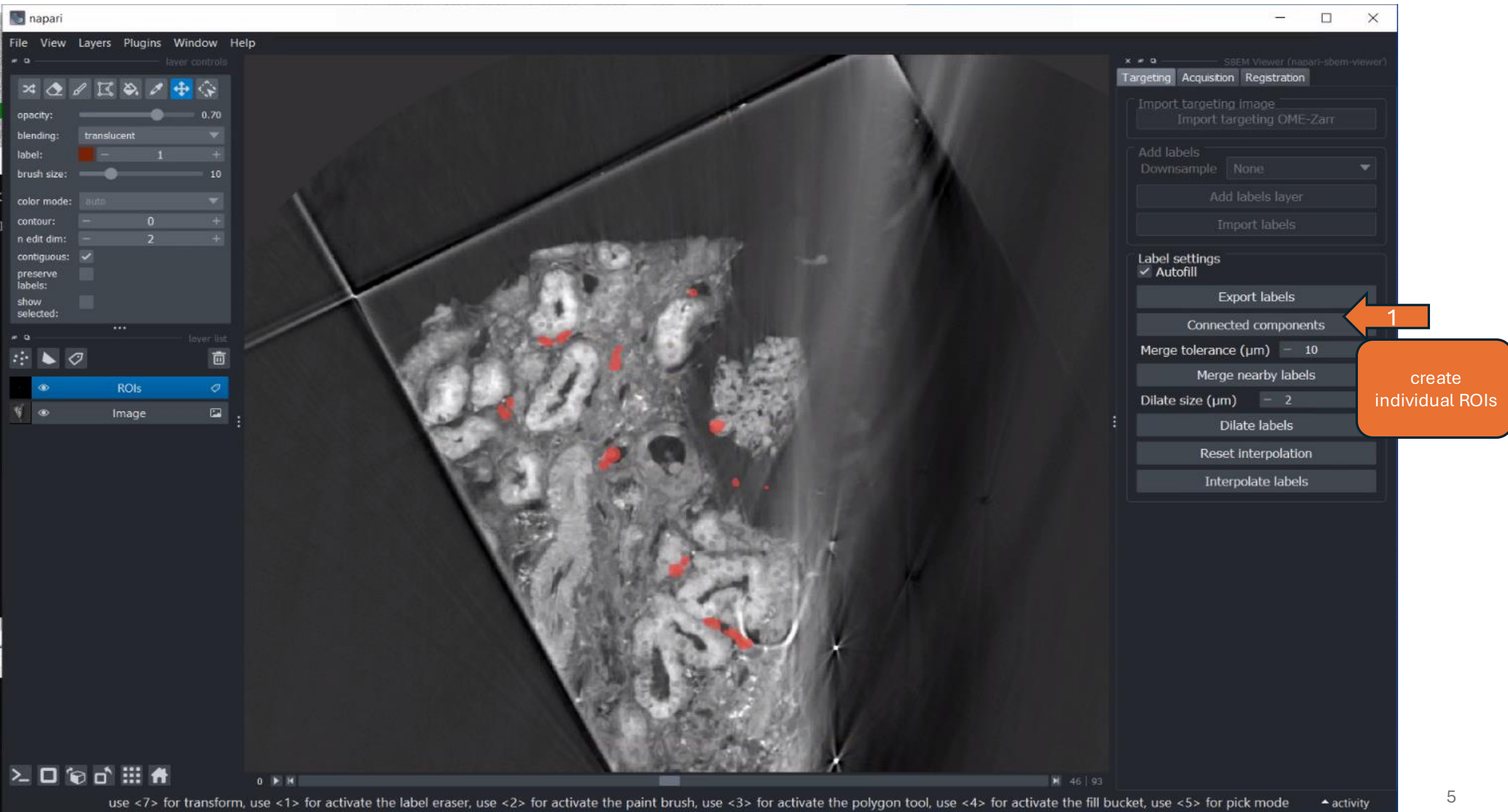
Napari iSBEM

Users Guide









napari

File View Layers Plugins Window Help

layer controls

opacity: 0.70

blending: translucent

label: 1

brush size: 10

color mode: auto

contour: 0

n edit dim: 2

contiguous: ☒

preserve labels: ☐

show selected: ☐

layer list

ROIs

Image

SBEM Viewer (napari-sbem-viewer)

Targeting Acquisition Registration

Import targeting image

Import targeting OME-Zarr

Add labels

Downsample: None

Add labels layer

Import labels

Label settings

☒ Autofill

Export labels

Connected components

Merge tolerance (μm) - 10 +

Merge nearby labels

Dilate size (μm) - 2 +

Dilate labels

Reset interpolation

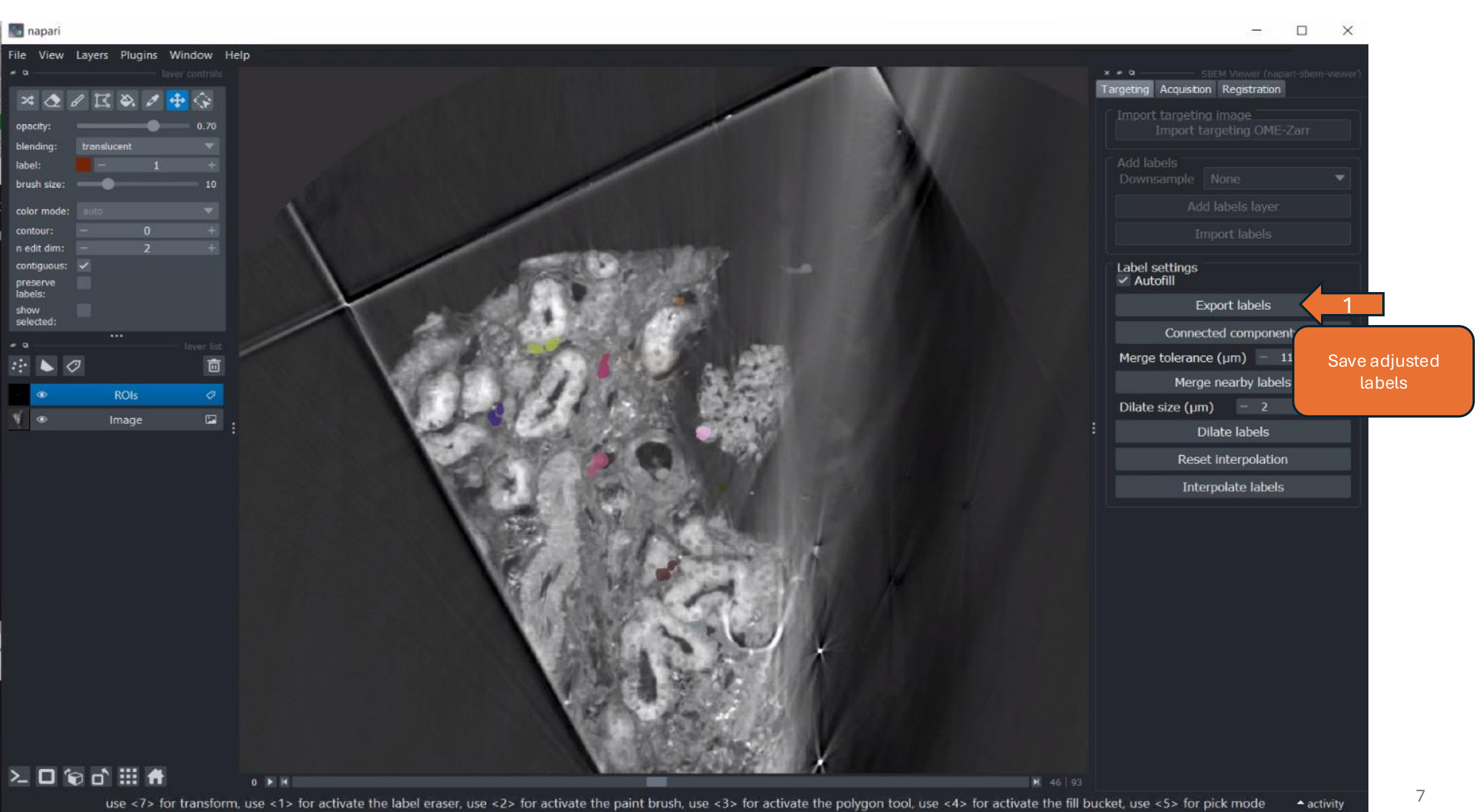
Interpolate labels

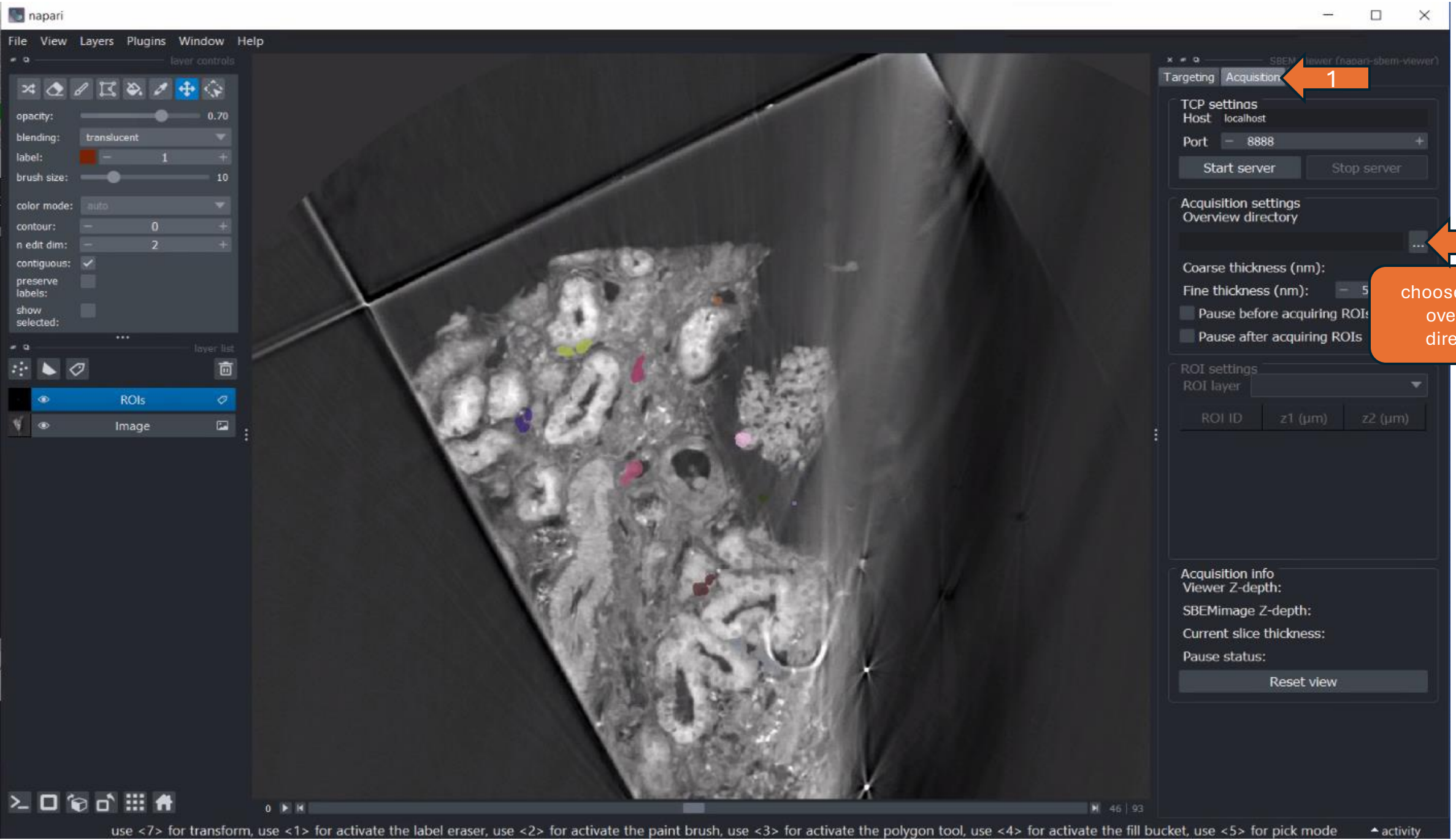
1

merge nearby labels (to prevent double scanning), adjust tolerance based on tile size (eg 11um)

0 46 | 93

use <7> for transform, use <1> for activate the label eraser, use <2> for activate the paint brush, use <3> for activate the polygon tool, use <4> for activate the fill bucket, use <5> for pick mode





napari

File View Layers Plugins Window Help

layer controls

opacity: 1.00

blending: translucent

contrast limits:

auto-contrast: once continuous

gamma: 1.00

colormap: gray

interpolation: nearest

layer list

EM overview

ROIs

Image

SBEM Viewer (napari-sbem-viewer)

Targeting Acquisition Registration

TCP settings

Host: localhost

Port: 8888

Start server Stop server

Acquisition settings

Overview directory

E:/Paolo/EM04652_2_slice18/overviews/ov000

Coarse thickness (nm): 100

Fine thickness (nm): 50

Pause before acquiring ROIs

Pause after acquiring ROIs

ROI settings

ROI layer

| ROI ID | z1 (μm) | z2 (μm) |
|--------|---------|---------|
|--------|---------|---------|

Acquisition info

Viewer Z-depth: 57.41 μm

SBEMImage Z-depth:

Current slice thickness:

Pause status:

Reset view

use <2> for transform activity

Added directory and overview. Stack is uploaded but most probably does not show because it is on a different "height".

napari

File View Layers Plugins Window Help

layer controls

opacity: 1.00

blending: translucent

contrast limits: [slider]

auto-contrast: once continuous

gamma: 1.00

colormap: gray

interpolation: nearest

layer list

- EM overview
- ROIs
- Image

SBEM Viewer (napari-sbem-viewer)

Targeting Acquisition Registration

TCP settings

Host: localhost

Port: 8888

Start server Stop server

Acquisition settings

Overview directory

E:/Paolo/EM04652_2_slice18/overviews/ov000

Coarse thickness (nm): 100

Fine thickness (nm): 50

Pause before acquiring ROIs

Pause after acquiring ROIs

ROI settings

ROI layer

| ROI ID | z1 (μm) | z2 (μm) |
|--------|---------|---------|
|--------|---------|---------|

Acquisition info

Viewer Z-depth: 41.71μm

SBEMimage Z-depth:

Current slice thickness:

Pause status:

Reset view

use <2> for transform activity

move slider to the left to see EM overviews

Rotation Transformation

The screenshot displays the napari SBEM Viewer interface. The main view area shows a 3D volume (X-ray) and a 2D slice (EM overview). The left panel contains layer controls and a layer list. The right panel has tabs for Targeting, Acquisition, and Registration. The Registration tab is active, showing options for saving/loading transforms, 3D rotation, Z alignment, and 2D alignment. An orange callout box points to the 'Show rotation' button in the 3D rotation section.

1. Click on "show rotation". A window pops up with Xray volume in cyan

2. Click on "show rotation". A window pops up with Xray volume in cyan

napari

File View Layers Plugins Window Help

layer controls

opacity: 1.00

blending: translucent

contrast limits:

auto-contrast: once continuous

gamma: 1.00

colormap: gray

interpolation: nearest

layer list

EM overview

ROIs

Image

SBEM Viewer (napari-sbem-viewer)

Targeting Acquisition Registration

Save / load transforms

Upload transform Save transform

Reset transform

3D rotation

Show rotation

Rotate Z-Y 0.0

Rotate Z-X 0.0

Position 0.48

Apply rotation

Z alignment

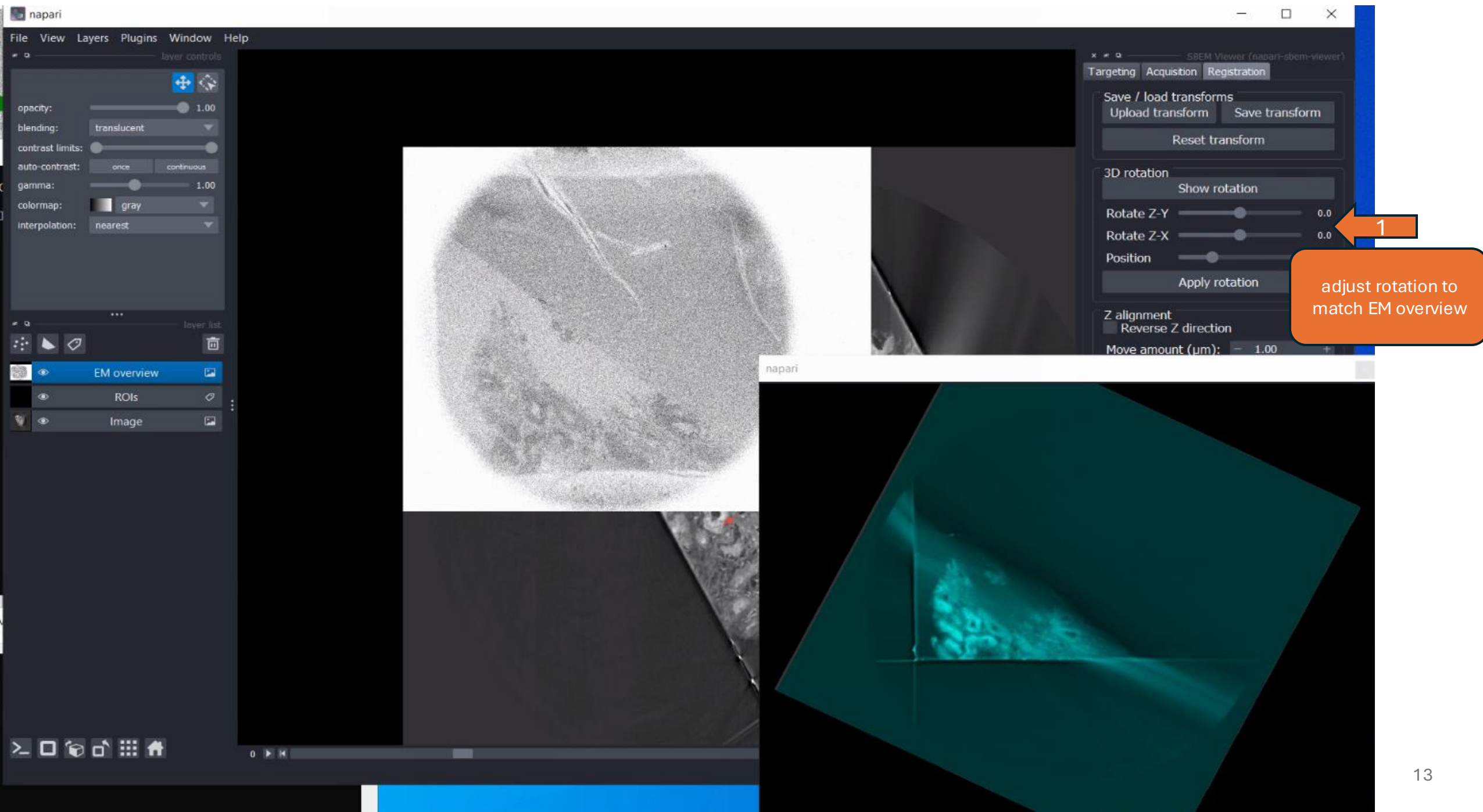
Reverse Z direction

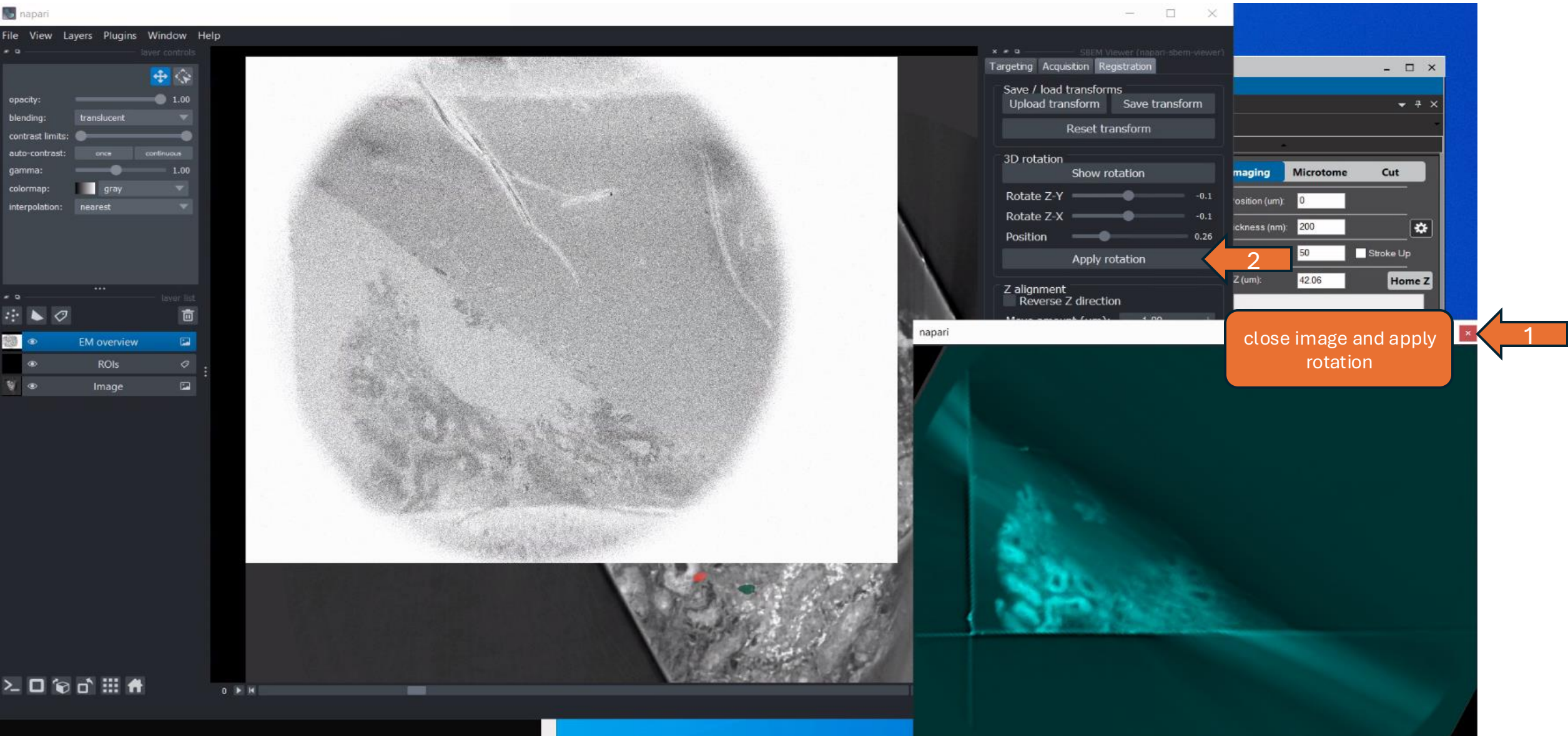
1

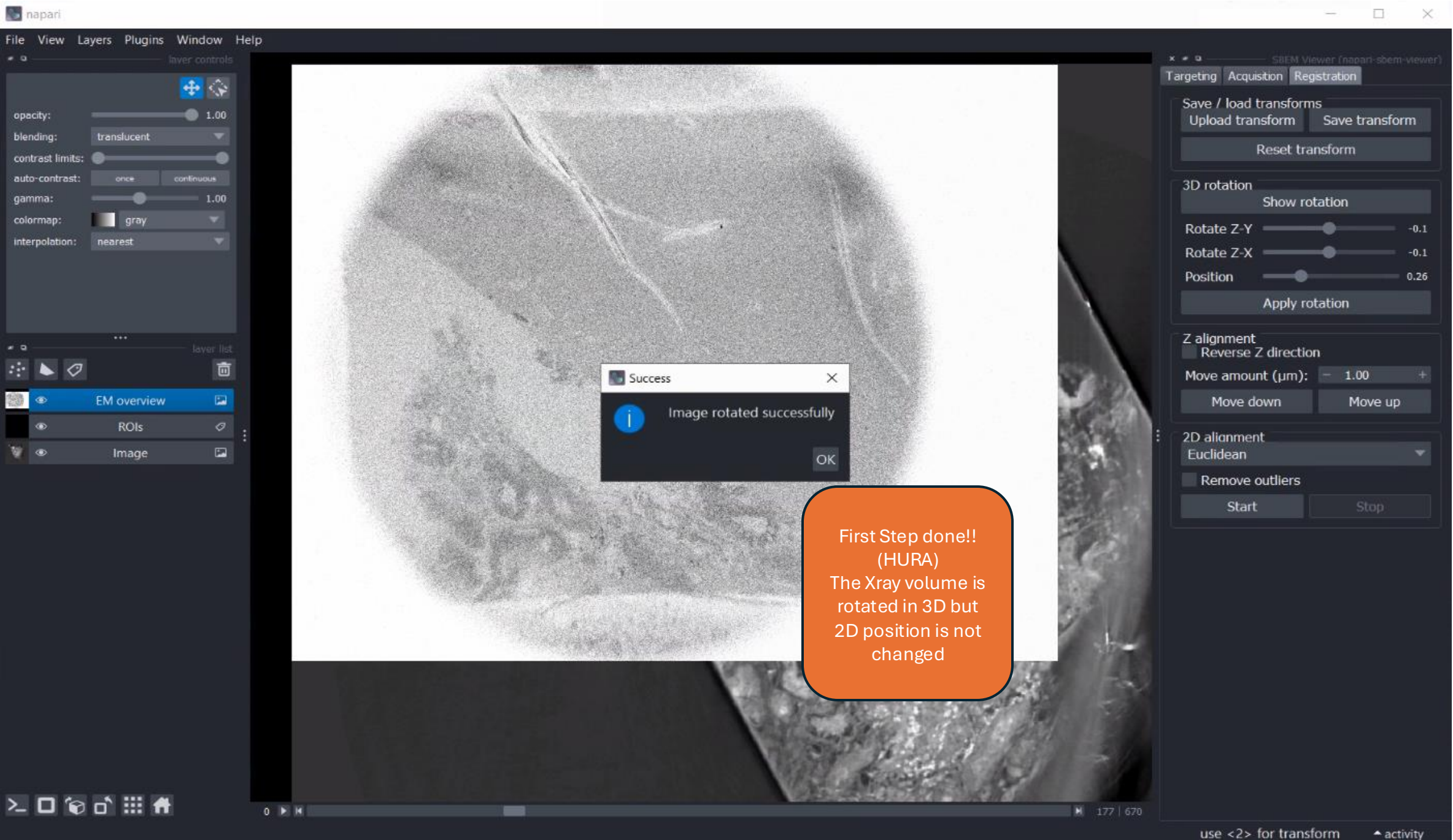
Grab the image and rotate to roughly match the OV orientation

2

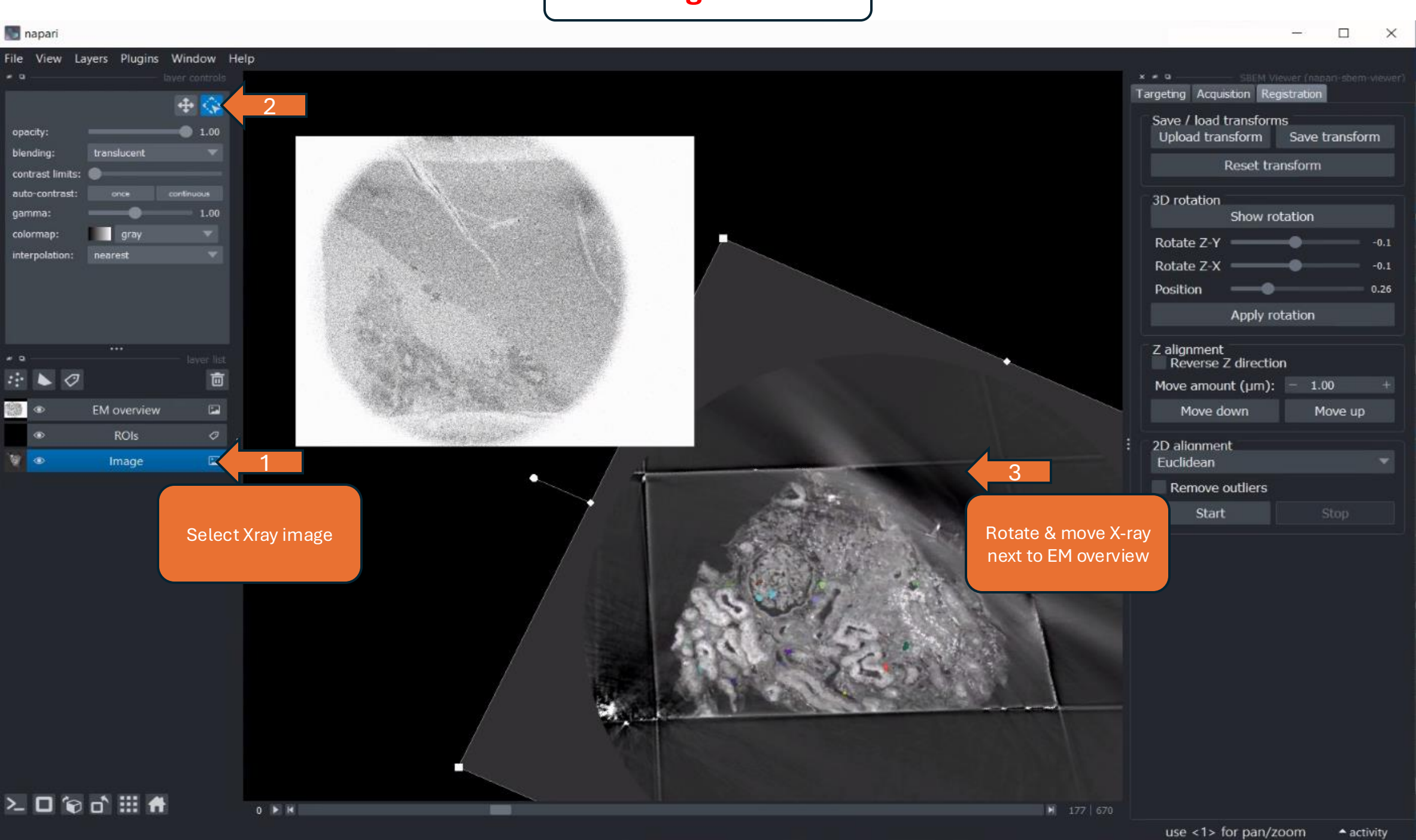
Adjust position to match EM overview

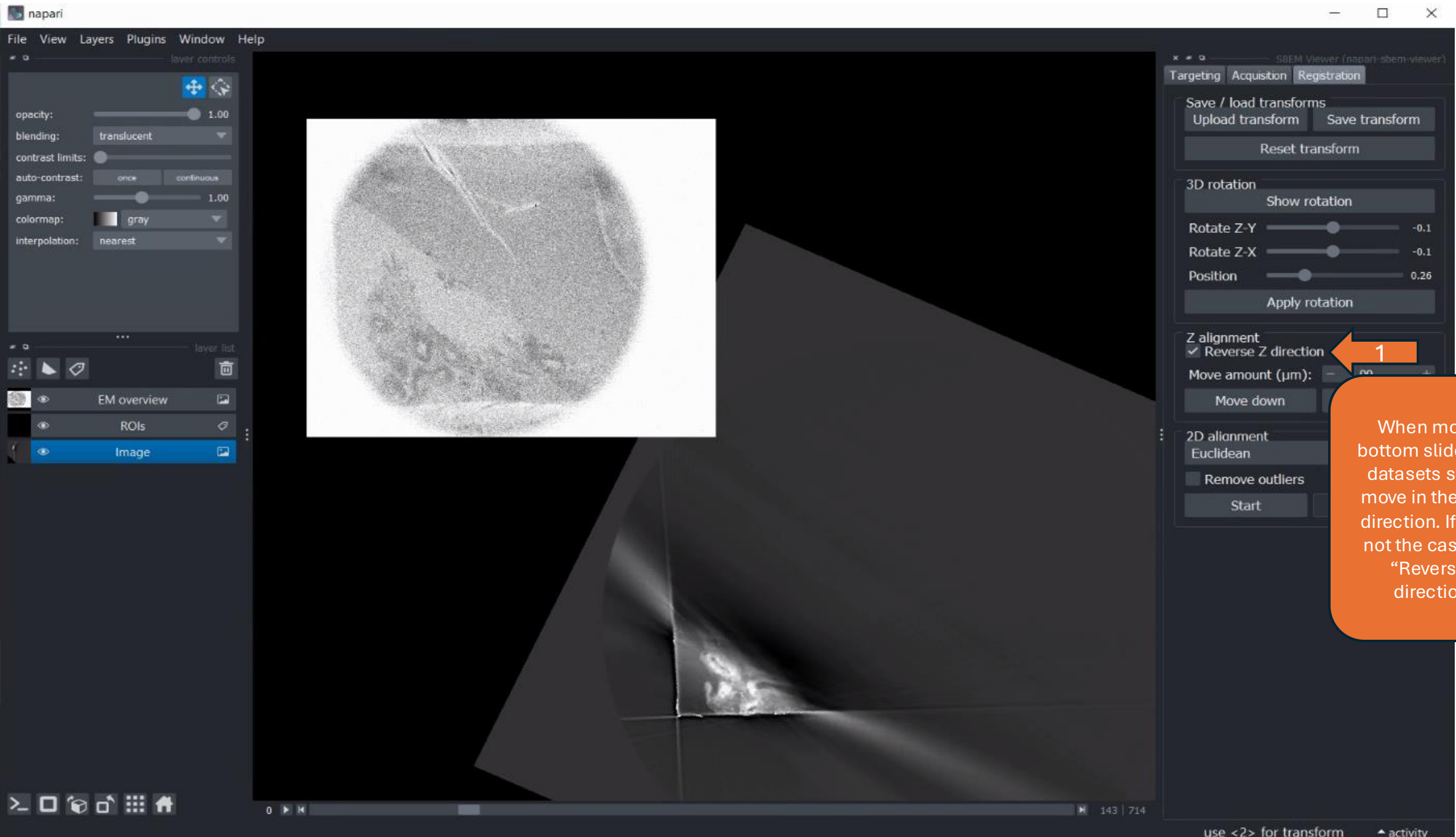






First Step done!!
(HURA)
The Xray volume is
rotated in 3D but
2D position is not
changed





1

When moving bottom slider both datasets should move in the same direction. If this is not the case, tick "Reverse Z direction"

napari

File View Layers Plugins Window Help

layer controls

opacity: 1.00

blending: translucent

contrast limits:

auto-contrast: once continuous

gamma: 1.00

colormap: gray

interpolation: nearest

layer list

EM overview

ROIs

Image

SBEM Viewer (napari-sbem-viewer)

Targeting Acquisition Registration

Save / load transforms

Upload transform Save transform

Reset transform

3D rotation

Show rotation

Rotate Z-Y -0.1

Rotate Z-X -0.1

Position 0.26

Apply rotation

Z alignment

☒ Reverse Z direction

Move amount (μm): 1.00

Move down Move up

2D align

Euclidean

Rem

click "move down" or "move up" to match X-ray to EM as accurately as possible

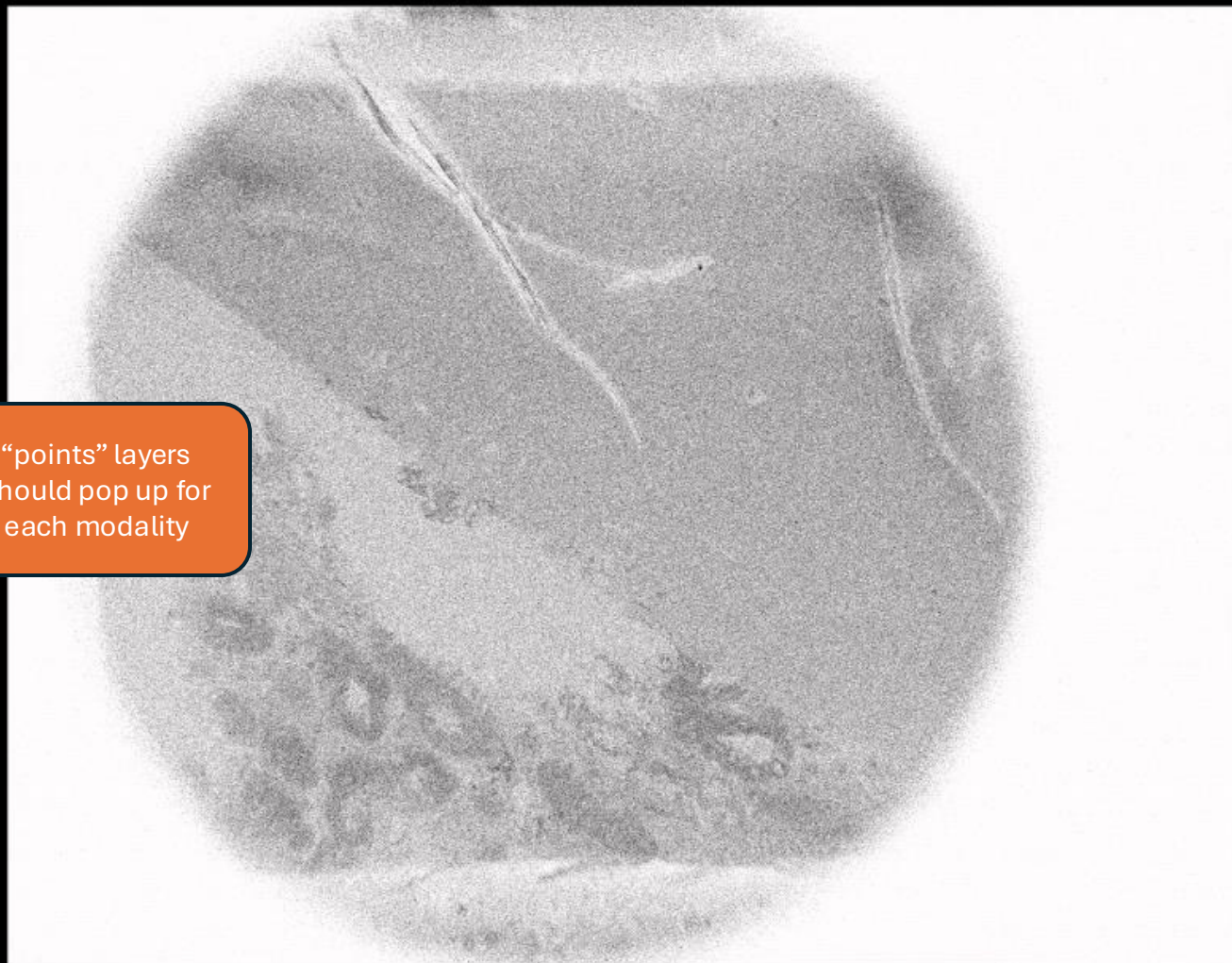
0 248 670

use <2> for transform ^ activity

opacity: 1.00
blending: translucent
point size: 10
symbol: disc
face color: #1f77b4ff
border color: dimgrey
display text: ☒
out of slice: ☐

EM overview_pts
EM overview
Image_pts
Image
ROIs

"points" layers
should pop up for
each modality



Save / load transforms

Upload transform

Save transform

Reset transform

3D rotation

Show rotation

Rotate Z-Y -0.1

Rotate Z-X -0.1

Position 0.26

Apply rotation

Z alignment

☒ Reverse Z direction

Move amount (μm): - 1.00 +

Move down

Move up

2D alignment

Euclidean

☐ Remove outliers

Start

1

Stop

Start 2D alignment
(here rigid registration selected)

napari

File View Layers Plugins Window Help

layer controls

opacity: 0.50
blending: translucent
contrast limits:
auto-contrast: once continuous
gamma: 1.00
colormap: gray
interpolation: nearest

layer list

- EM overview_pts
- EM overview
- Image_pts
- Image
- ROIs

select points for registration on EM and X-ray sequentially
(images change automatically)

SBEM Viewer (napari-sbem-viewer)

Targeting Acquisition Registration

Save / load transforms
Upload transform Save transform
Reset transform

3D rotation
Show rotation
Rotate Z-Y -0.1
Rotate Z-X -0.1
Position 0.26
Apply rotation

Z alignment
☒ Reverse Z direction
Move amount (μm): 1.00
Move down Move up

2D alignment
Euclidean
☐ Remove outliers
Start Stop

0 248 | 670

use <2> for transform ^ activity

napari

File View Layers Plugins Window Help

layer controls

opacity: 0.50

blending: translucent

contrast limits:

auto-contrast: once continuous

gamma: 1.00

colormap: gray

interpolation: nearest

layer list

- EM overview_pts
- EM overview
- Image_pts
- Image
- ROIs

SBEM Viewer (napari-sbem-viewer)

Targeting Acquisition Registration

Save / load transforms

Upload transform Save transform

Reset transform

3D rotation

Show rotation

Rotate Z-Y -0.1

Rotate Z-X -0.1

Position 0.26

Apply rotation

Z alignment

☒ Reverse Z direction

Move amount (μm): 1.00

Move down Move up

2D alignment

Similarity

Euclidean

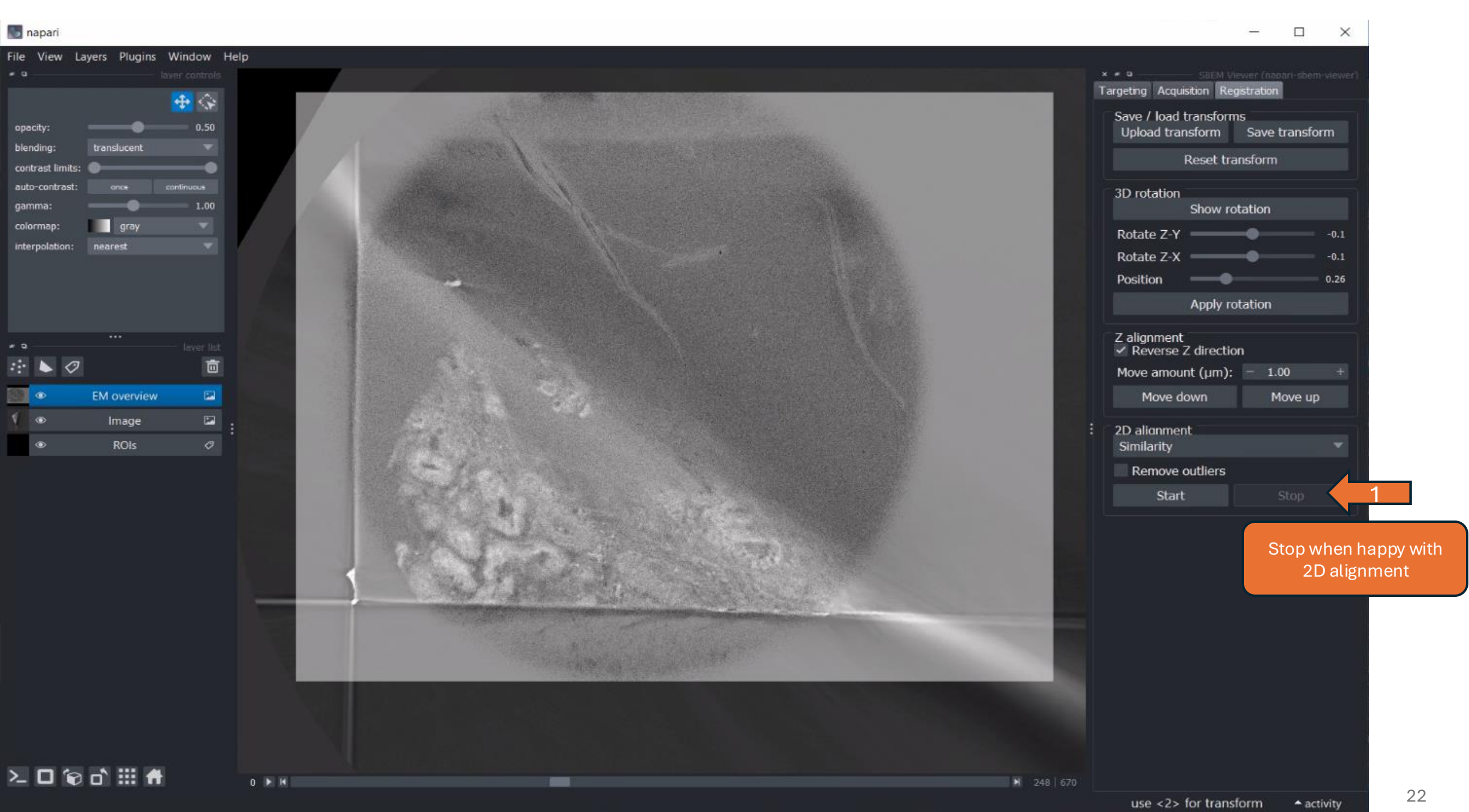
Similarity

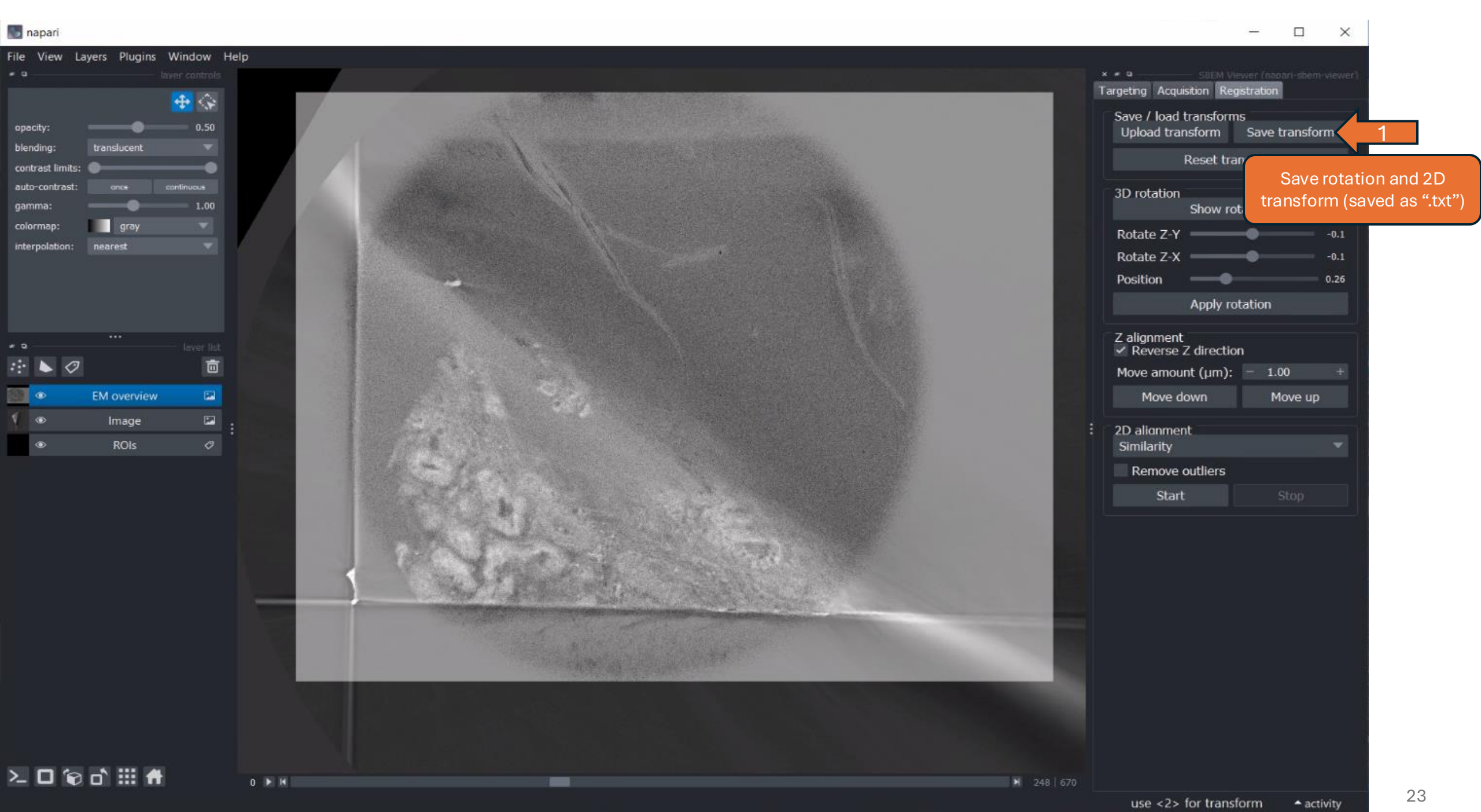
Affine

can select different transformation methods

Output is automatically updated in the viewer

use <2> for transform activity





napari

FileViewLayersPluginsWindowHelp

layer controls

opacity: 0.70

blending: translucent

label: 1

brush size: 10

color mode: auto

contour: 0

n edit dim: 2

contiguous: ☒

preserve labels: ☐

show selected: ☐

layer list

ROIs

EM overview

Image

0248670

use <7> for transform, use <1> for activate the label eraser, use <2> for activate the paint brush, use <3> for activate the polygon tool, use <4> for activate the fill bucket, use <5> for pick mode

activity

SBEM viewer (napari-sbem-viewer)

TargetingAcquisition

TCP settings

Hostlocalhost

Port8888

Start server

Acquisition settings

Overview directory

E:/Paolo/EM04652_2_slice18/overviews/ov000

Coarse thickness (nm): 100

Fine thickness (nm): 50

☐ Pause before acquiring ROIs

☐ Pause after acquiring ROIs

ROI settings

ROI layerROIs

| ROI ID | ROIs |
|--------|------------|
| 24 | 27.5055.50 |
| 26 | 33.5050.50 |
| 21 | 38.5059.50 |
| 22 | 38.5057.50 |

Acquisition info

Viewer Z-depth: 41.80µm

SBEMImage Z-depth:

Current slice thickness:

Pause status:

Reset view

select ROIs

27

napari

File View Layers Plugins Window Help

layer controls

opacity: 0.70

blending: translucent

label: 1

brush size: 10

color mode: auto

contour: 0

n edit dim: 2

contiguous: ☒

preserve labels: ☐

show selected: ☐

layer list

ROIs

EM overview

Image

ROIs [14 -20 76]: 0

SBEM Viewer (napari-sbem-viewer)

Targeting Acquisition Registration

TCP settings

Host: localhost

Port: 8888

Start server Stop server

Acquisition settings

Overview directory

E:/Paolo/EM04652_2_slice18/overviews/ov000

Coarse thickness (nm): 100

Fine thickness (nm): 50

Pause before acquiring ROIs

Pause after z

ROI settings

ROI layer

ROI ID

Acquisition info

Viewer Z-depth: 41.80µm

SBEMImage Z-depth:

Current slice thickness:

Pause status:

Reset view

Can adjust cutting thickness

Coarse thickness (nm) = cutting thickness for low resolution imaging, same as selected in SBEMImage for initial overview before registration

activity

napari

File View Layers Plugins Window Help

layer controls

opacity: 0.70

blending: translucent

label: 1

brush size: 10

color mode: auto

contour: 0

n edit dim: 2

contiguous: ☒

preserve labels: ☐

show selected: ☐

layer list

ROIs

EM overview

Image

ROIs [14 -20 76]: 0

SBEM Viewer (napari-sbem-viewer)

Targeting Acquisition Registration

TCP settings

Host: localhost

Port: 8888

Start server Stop server

Acquisition settings

Overview directory

E:/Paolo/EM04652_2_slice18/overviews/ov000

Coarse thickness (nm): 100

Fine thickness (nm): 50

☐ Pause before acquiring ROIs

☐ Pause after acquiring ROIs

ROI settings

ROI layer

ROI ID z1

Acquisition info

Viewer Z-depth: 41.80µm

SBEMImage Z-depth:

Current slice thickness:

Pause status:

Reset view

activity

Use case (e.g.) ROI deep in the specimen -> pauses before ROI -> can refocus

napari

File View Layers Plugins Window Help

layer controls

opacity: 0.70

blending: translucent

label: 1

brush size: 10

color mode: auto

contour: 0

n edit dim: 2

contiguous: ☒

preserve labels: ☐

show selected: ☐

layer list

ROIs

EM overview

Image

SBEM Viewer (napari-sbem-viewer)

Targeting Acquisition Registration

TCP settings

Host: localhost

Port: 8888

Start server Stop server

Acquisition settings

Overview directory

E:/Paolo/EM04652_2_slice18/overviews/ov000

Coarse thickness (nm): 100

Fine thickness (nm): 50

☐ Pause before acquiring ROIs

☐ Pause after acquiring ROIs

ROI settings

ROI layer

| ROI ID | z1 |
|--------|----|
|--------|----|

Acquisition info

Viewer Z-depth: 41.80µm

SBEMImage Z-depth:

Current slice thickness:

Pause status:

Reset view

Use case (e.g.) confirm that the complete region of interest was acquired

ROIs [14 -20 76]: 0

248 | 670

activity

Output in SBEMImage

SBEMImage 2025.3.25 dev - Main Controls - DEVELOPMENT VERSION

File Settings Configuration Calibration Import Export Update Help

Main controls Focus tool Notes Array MultiSEM Tests

SEM
ZEISS GeminiSEM
Beam 1.40 keV / 300 pA

Microtome/Stage
Gatan 3View
Last confirmed stage position:
X: -79.431 Y: -226.726
Z: 42.063

Overviews
OV 0
OV size: 4096 × 3072
Magnification 122.0
Dwell time 0.8 µs
Debris detection area:
[0, 0, 4096, 3072]
Location:
X: 94.324, Y: -279.714

Tile grids
GRID 0
Grid size: 18 × 15
Tile size: 4096 × 3072
Active tiles 0
Pixel size 10.0 nm
Dwell time 1.6 µs
Location of tile 0:
X: 0.913, Y: -265.697

Manual commands
Approach
Sweep
Grab frame
Screenshot
VP FCC
EHT ON/OFF

Stack acquisition
E:/Paolo/EM04652_2_slice18
Target Z depth (µm): 20.0 Slice thickness 50 nm

☒ E-mail monitor ☐ Disk mirror ☐ Image monitor ☐ Focus track ☐ Plasma clean ☒ Use TCP

Electron dose (min .. max) 0.03 .. 30.0 electrons per nm²
Tile acquisition area: Z depth: Data volume
15099.5 µm² 20.00 µm 65.4 GB
Estimated total duration (imaging / stage moves / cleanup) 1 d 14 h 44 min (79.9% / 15.9% / 4.2%)
Estimated date and time of completion 2025-05-16 at 07:17:13 (0 d 10 h 33 min remaining)
Current Z depth 14.550 µm (slice no. = 27) 72%

Host localhost
Port 8888
Sync data
OK

2025-05-15 19:00:00 and: ACTIVATE ARRAY GRID
2025-05-15 19:00:00 and: UPDATE GRID TILES WITH MASK
2025-05-15 19:00:00 TCP command: ADD ARRAY GRID
2025-05-15 19:00:00 TCP command: ACTIVATE ARRAY GRID
2025-05-15 19:00:00 TCP command: UPDATE GRID TILES WITH MASK
2025-05-15 19:00:00 EXCEPTION occurred for details.
2025-05-15 20:41:32 CTRL : Performing user-requested stage move.
2025-05-15 20:41:44 CTRL : User-requested stage move completed.
2025-05-15 20:43:23 CTRL : Stack started.
2025-05-15 20:43:23 CTRL : Settings saved to disk.
2025-05-15 20:43:24 SEM : Current WD/STIG_XY: 5.613945, -2.593627, 0.508597
2025-05-15 20:43:26 CTRL : Checking for TCP remote commands.
2025-05-15 20:43:27 CTRL : Processing TCP command: ADD ARRAY GRID
2025-05-15 20:43:27 CTRL : Processing TCP command: ACTIVATE ARRAY GRID
2025-05-15 20:43:27 CTRL : Processing TCP command: UPDATE GRID TILES WITH MASK
2025-05-15 20:43:27 CTRL : Processing TCP command: ADD ARRAY GRID
2025-05-15 20:43:27 CTRL : Processing TCP command: ACTIVATE ARRAY GRID
2025-05-15 20:43:27 CTRL : Processing TCP command: UPDATE GRID TILES WITH MASK
2025-05-15 20:43:27 CTRL : Processing TCP command: ADD ARRAY GRID
2025-05-15 20:43:27 CTRL : Processing TCP command: ACTIVATE ARRAY GRID
2025-05-15 20:43:27 CTRL : Processing TCP command: UPDATE GRID TILES WITH MASK
2025-05-15 20:43:27 CTRL : Processing TCP command: ADD ARRAY GRID

Ready. Active configuration: PR_MRC-MM_EM04652_2_slice18.ini / EMBL_Gemini_3View.cfg

check if ROI positions are correct

ROI 13 (inactive) ROI 25 ROI 2 (inactive) ROI 6 (inactive) ROI 3 (inactive) ROI 15 (inactive) ROI 7 (inactive) ROI 8 (inactive) ROI 23 ROI 17 (inactive) ROI 21 ROI 20 ROI 4 (inactive) ROI 5 (inactive) ROI 10 (inactive) ROI 16 (inactive) ROI 14 ROI 18 (inactive) ROI 26 ROI 19 (inactive)

GRID 0 (inactive)

Controls
All grid: Show tile previews All OV(s) Refresh OV(s) Image stub Show imported Show stub OV Show axes Zoom: Show labels Show stage position FOV: 522.2 µm × 417.7 µm

napari

FileViewLayersPluginsWindowHelp

layer controls

opacity: 0.70

blending: translucent

label: 1

brush size: 10

color mode: auto

contour: 0

n edit dim: 2

contiguous: ☒

preserve labels: ☐

show selected: ☐

layer list

ROIs

EM overview

Image

ROIs [14 -11 219]: 0

Congrats you've done it!

SBEM Viewer (napari-sbem-viewer)

TargetingAcquisitionRegistration

TCP settings

Host localhost

Port 8888

Start serverStop server

Acquisition settings

Overview directory

E:/Paolo/EM04652_2_slice18/overviews/ov000

Coarse thickness (nm): 100

Fine thickness (nm): 50

☐ Pause before acquiring ROIs

☐ Pause after acquiring ROIs

ROI settings

ROI layer ROIs

| ROI ID | z1 (μm) | z2 (μm) |
|--------|---------|---------|
| 24 | 27.50 | 55.50 |
| 26 | 33.50 | 50.50 |
| 21 | 38.50 | 59.50 |
| 22 | 38.50 | 57.50 |

Acquisition info

Viewer Z-depth: 41.80μm

SBEMImage Z-depth:

Current slice thickness:

Pause status:

Reset view

activity

Resuming from saved transformation

**Import X-ray, labels and EM
Overview as previously described**

The screenshot shows the napari SBEM Viewer interface. The main window displays a dark gray area with a faint, irregular shape. Below this shape, text reads: "Drag image(s) here to open or Use the menu shortcuts below:". Below the text, three shortcuts are listed: "Ctrl+N New Image from Clipboard", "Ctrl+O open image(s)", and "Ctrl+Alt+/ show all key bindings". On the right side, there is a panel titled "SBEM Viewer (napari-sbem-viewer)" with three tabs: "Targeting", "Acquisition", and "Registration". The "Targeting" tab is active. It contains several sections: "Import targeting image" with a button "Import targeting OME-Zarr" (highlighted by an orange arrow labeled "1" and a callout box "import X-ray tomogram"), "Add labels" with a "Downsample" dropdown set to "None" and an "Add labels layer" button, and "Import labels" button. Below these are "Label settings" with a checked "Autofill" checkbox, and buttons for "Export labels", "Connected components", "Merge tolerance (μm)" (set to 10), "Merge nearby labels", "Dilate size (μm)" (set to 1), "Dilate labels", "Reset interpolation", and "Interpolate labels". The bottom right corner shows an "activity" indicator.

1

import X-ray tomogram

Drag image(s) here to open
or
Use the menu shortcuts below:

Ctrl+N New Image from Clipboard
Ctrl+O open image(s)
Ctrl+Alt+/ show all key bindings

SBEM Viewer (napari-sbem-viewer)

Targeting Acquisition Registration

Import targeting image
Import targeting OME-Zarr

Add labels
Downsample None
Add labels layer
Import labels

Label settings
☒ Autofill
Export labels
Connected components
Merge tolerance (μm) 10
Merge nearby labels
Dilate size (μm) 1
Dilate labels
Reset interpolation
Interpolate labels

activity

napari

File View Layers Plugins Window Help

layer controls

opacity: 1.00

blending: translucent

contrast limits: **1**

auto-contrast: once continuous

gamma: 1.00

colormap: gray

interpolation: nearest

adjust contrast

layer list

Image

SBEM Viewer (napari-sbem-viewer)

Targeting Acquisition Registration

Import targeting image

Import targeting OME-Zarr

Add labels

Downsample: None

Add labels layer

2 Import labels

Label settings

☒ Autofill

Export labels

Connected components

Merge tolerance (μm): 10

Merge nearby labels

Dilate size (μm): 1

Dilate labels

Reset interpolation

Interpolate labels

Import ROIs (tiff)

Saved labels should be dilated and merged

use <2> for transform activity

32

napari

File View Layers Plugins Window Help

layer controls

opacity: 1.00

blending: translucent

contrast limits:

auto-contrast: once continuous

gamma: 1.00

colormap: gray

interpolation: nearest

layer list

EM overview

ROIs

Image

SBEM Viewer (napari-sbem-viewer)

Targeting Acquisition Registration

TCP settings

Host: localhost

Port: 8888

Start server Stop server

Acquisition settings

Overview directory

E:/Paolo/EM04652_2_slice18/overviews/ov000

Coarse thickness (nm): 100

Fine thickness (nm): 50

Pause before acquiring ROIs

Pause after acquiring ROIs

ROI settings

ROI layer

| ROI ID | z1 (μm) | z2 (μm) |
|--------|---------|---------|
|--------|---------|---------|

Acquisition info

Viewer Z-depth: 57.41 μm

SBEMImage Z-depth:

Current slice thickness:

Pause status:

Reset view

use <2> for transform activity

Added directory and overview. Stack is uploaded but most probably does not show because it is on a different "height".

napari

File View Layers Plugins Window Help

layer controls

opacity: 1.00

blending: translucent

contrast limits:

auto-contrast: once continuous

gamma: 1.00

colormap: gray

interpolation: nearest

layer list

EM overview

ROIs

Image

SBEM Viewer (napari-sbem-viewer)

Targeting Acquisition Registration

TCP settings

Host: localhost

Port: 8888

Start server Stop server

Acquisition settings

Overview directory

E:/Paolo/EM04652_2_slice18/overviews/ov000

Coarse thickness (nm): 100

Fine thickness (nm): 50

Pause before acquiring ROIs

Pause after acquiring ROIs

ROI settings

ROI layer

| ROI ID | z1 (μm) | z2 (μm) |
|--------|---------|---------|
|--------|---------|---------|

Acquisition info

Viewer Z-depth: 41.71μm

SBEMimage Z-depth:

Current slice thickness:

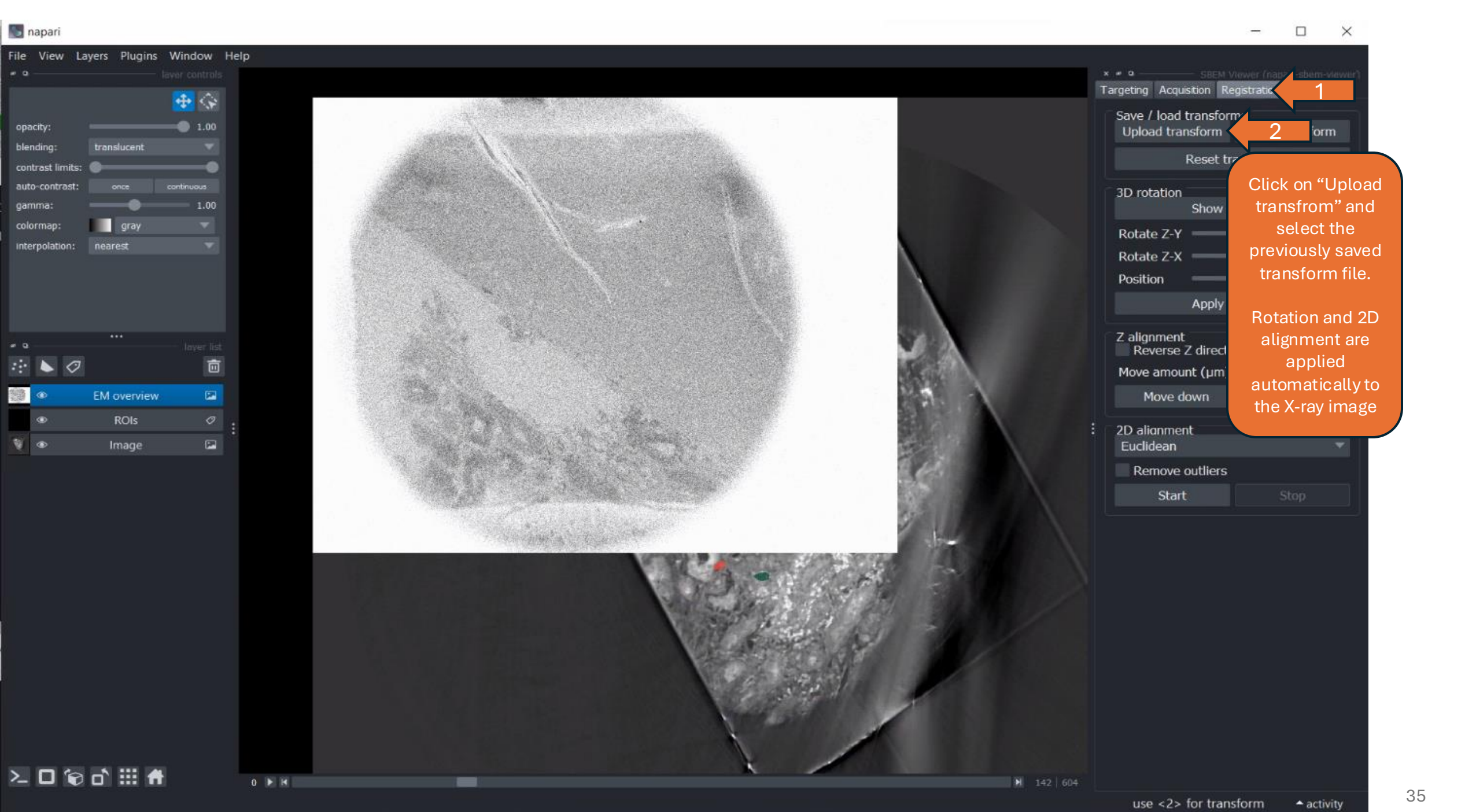
Pause status:

Reset view

use <2> for transform activity

0 142 | 604

move slider to the left to see EM overviews



resume with steps as previously described

The screenshot shows the napari SBEM viewer interface. The central panel displays a grayscale electron microscopy image with several colored regions of interest (ROIs) marked. The left panel shows layer controls for the 'ROIs' layer, including opacity, blending, label, brush size, color mode, contour, n edit dim, contiguous, preserve labels, show, and selected. The right panel shows acquisition settings, including TCP settings, Acquisition settings, ROI settings, and Acquisition info. An orange callout box labeled 'select ROIs' points to the ROI settings table.

1. Targeting Acquisition

2. ROI layer ROIs

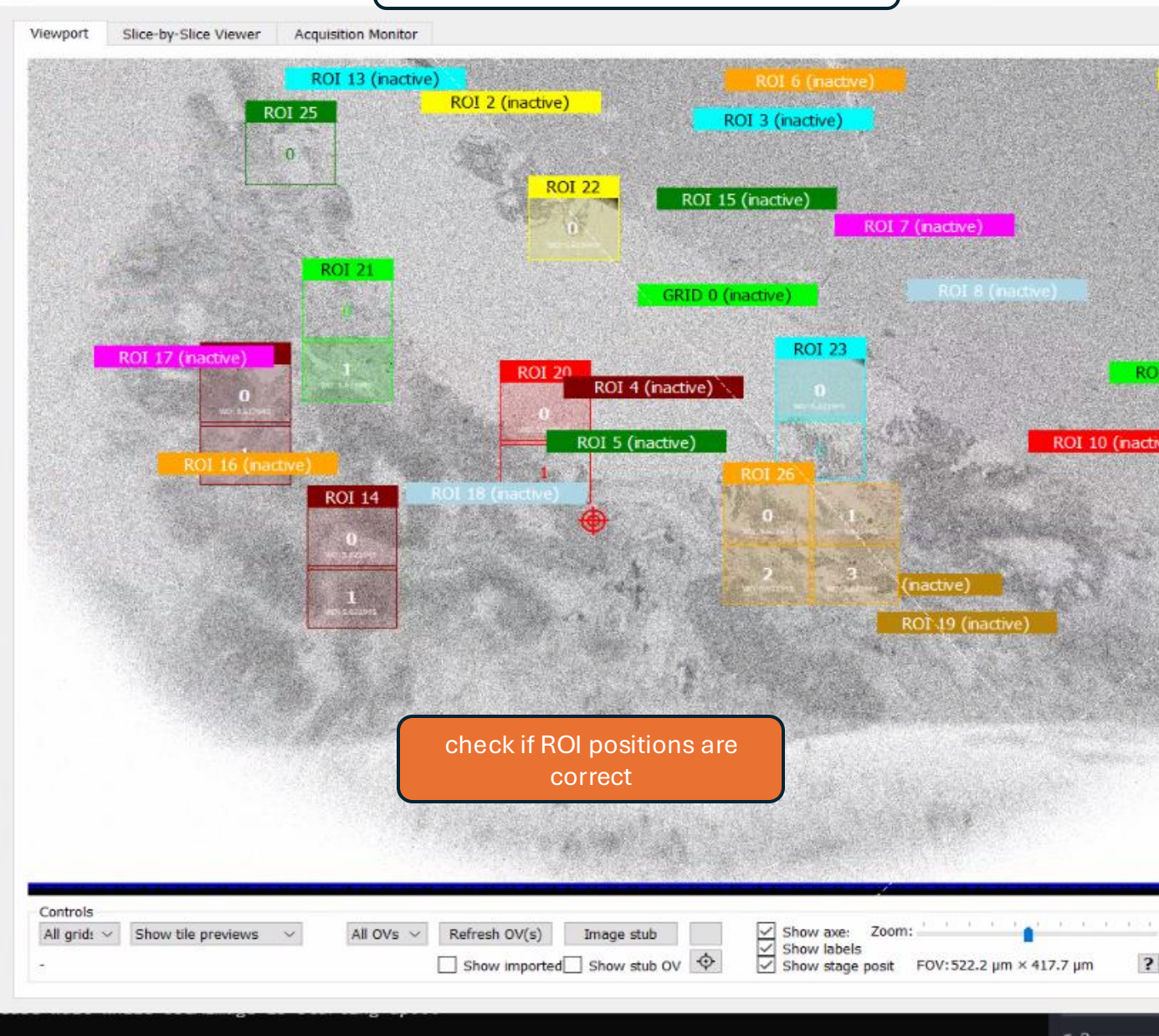
3. Start server

select ROIs

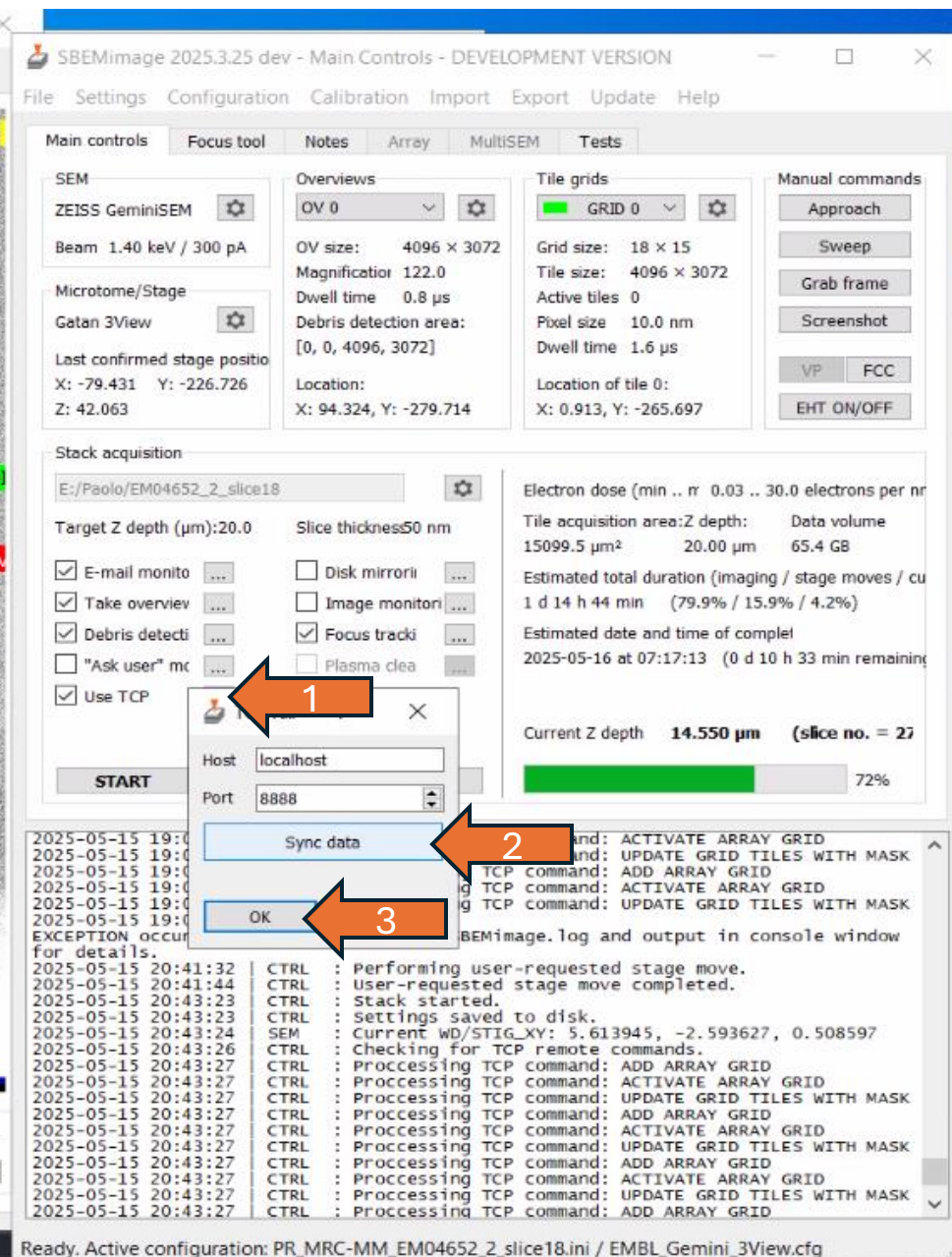
| ROI ID | ROIs |
|--------|-------------|
| 24 | 27.50 55.50 |
| 26 | 33.50 50.50 |
| 21 | 38.50 59.50 |
| 22 | 38.50 57.50 |

use <7> for transform, use <1> for activate the label eraser, use <2> for activate the paint brush, use <3> for activate the polygon tool, use <4> for activate the fill bucket, use <5> for pick mode

Output in SBEMImage



check if ROI positions are correct



napari

File View Layers Plugins Window Help

layer controls

opacity: 0.70

blending: translucent

label: 1

brush size: 10

color mode: auto

contour: 0

n edit dim: 2

contiguous: ☒

preserve labels: ☐

show selected: ☐

layer list

ROIs

EM overview

Image

Congrats you've done it!

SBEM Viewer (napari-sbem-viewer)

Targeting Acquisition Registration

TCP settings

Host: localhost

Port: 8888

Start server Stop server

Acquisition settings

Overview directory

E:/Paolo/EM04652_2_slice18/overviews/ov000

Coarse thickness (nm): 100

Fine thickness (nm): 50

☐ Pause before acquiring ROIs

☐ Pause after acquiring ROIs

ROI settings

ROI layer ROIs

| ROI ID | z1 (μm) | z2 (μm) |
|--------|---------|---------|
| 24 | 27.50 | 55.50 |
| 26 | 33.50 | 50.50 |
| 21 | 38.50 | 59.50 |
| 22 | 38.50 | 57.50 |

Acquisition info

Viewer Z-depth: 41.80μm

SBEMimage Z-depth:

Current slice thickness:

Pause status:

Reset view

ROIs [14 -11 219]: 0

activity