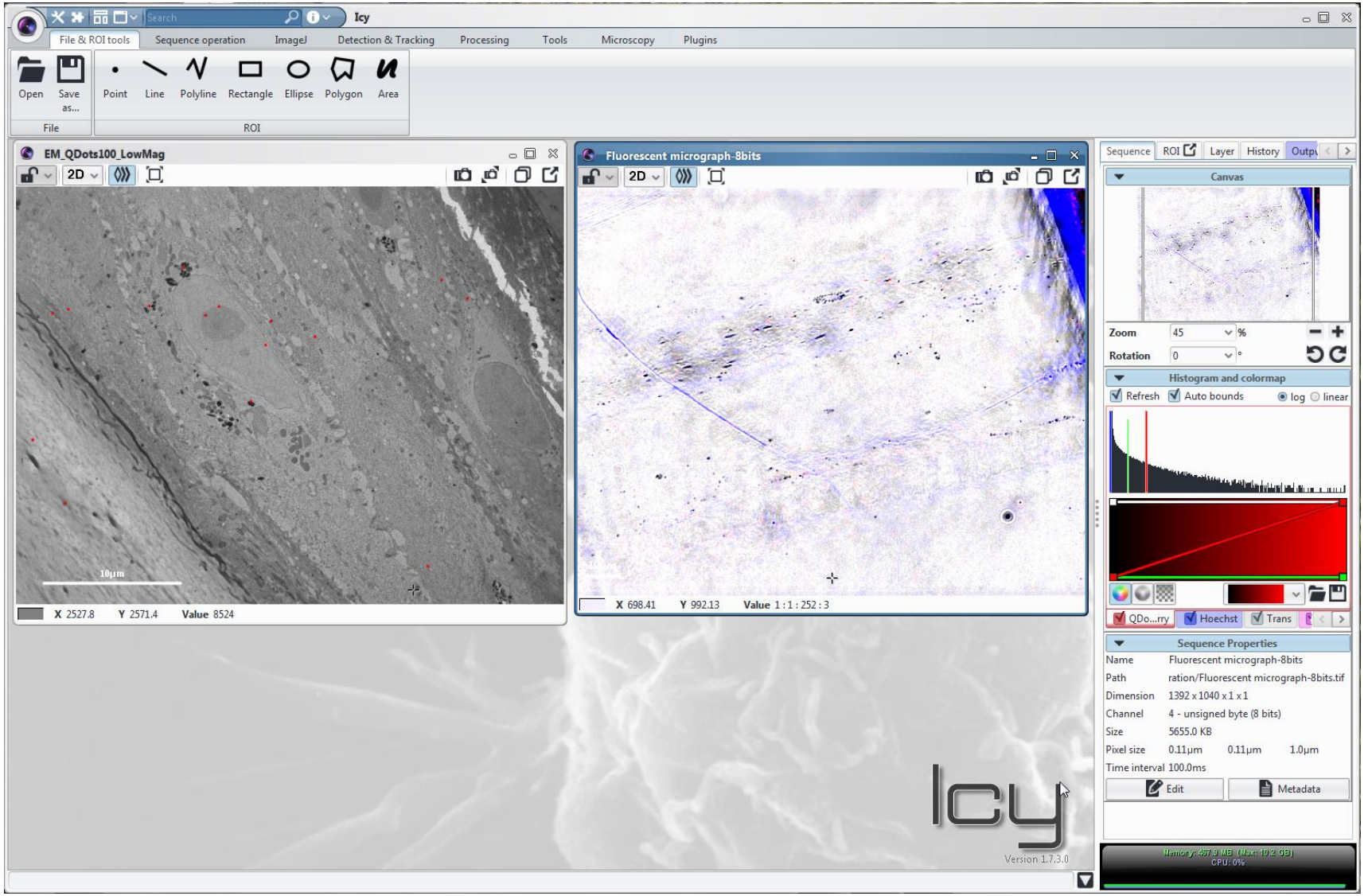
Step by step Guide for eC-CLEM

2D to 2D registration

Open both light and electron microscopy images

Drag and drop

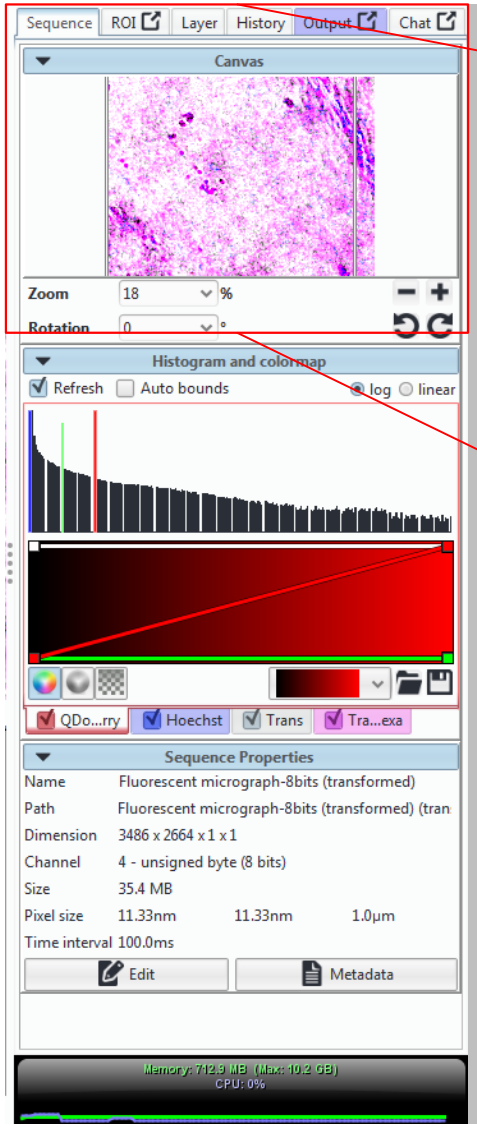


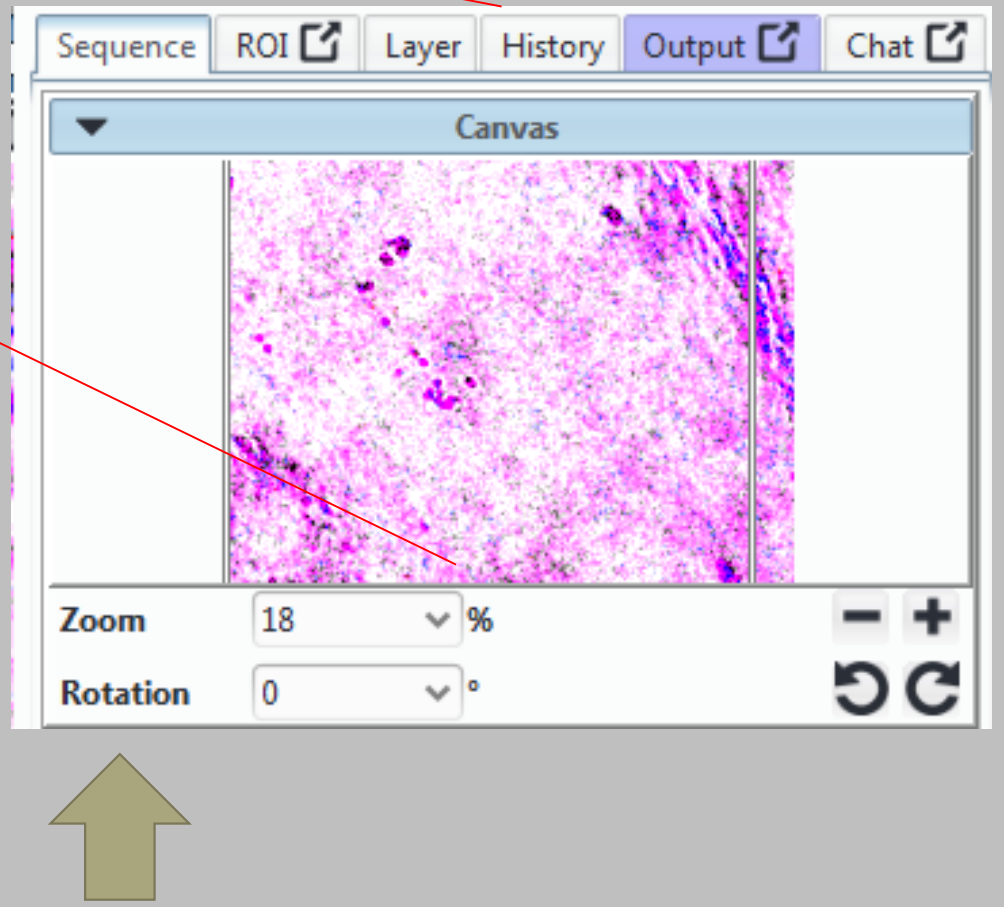
Display your images at your convenience

On the left panel, the top part has several tabs.

Sequence tab shows the navigator canvas with the display zoom and the rotation display.

Be careful, this rotation is only a graphical display, not a transformation calculated and applied to your image.



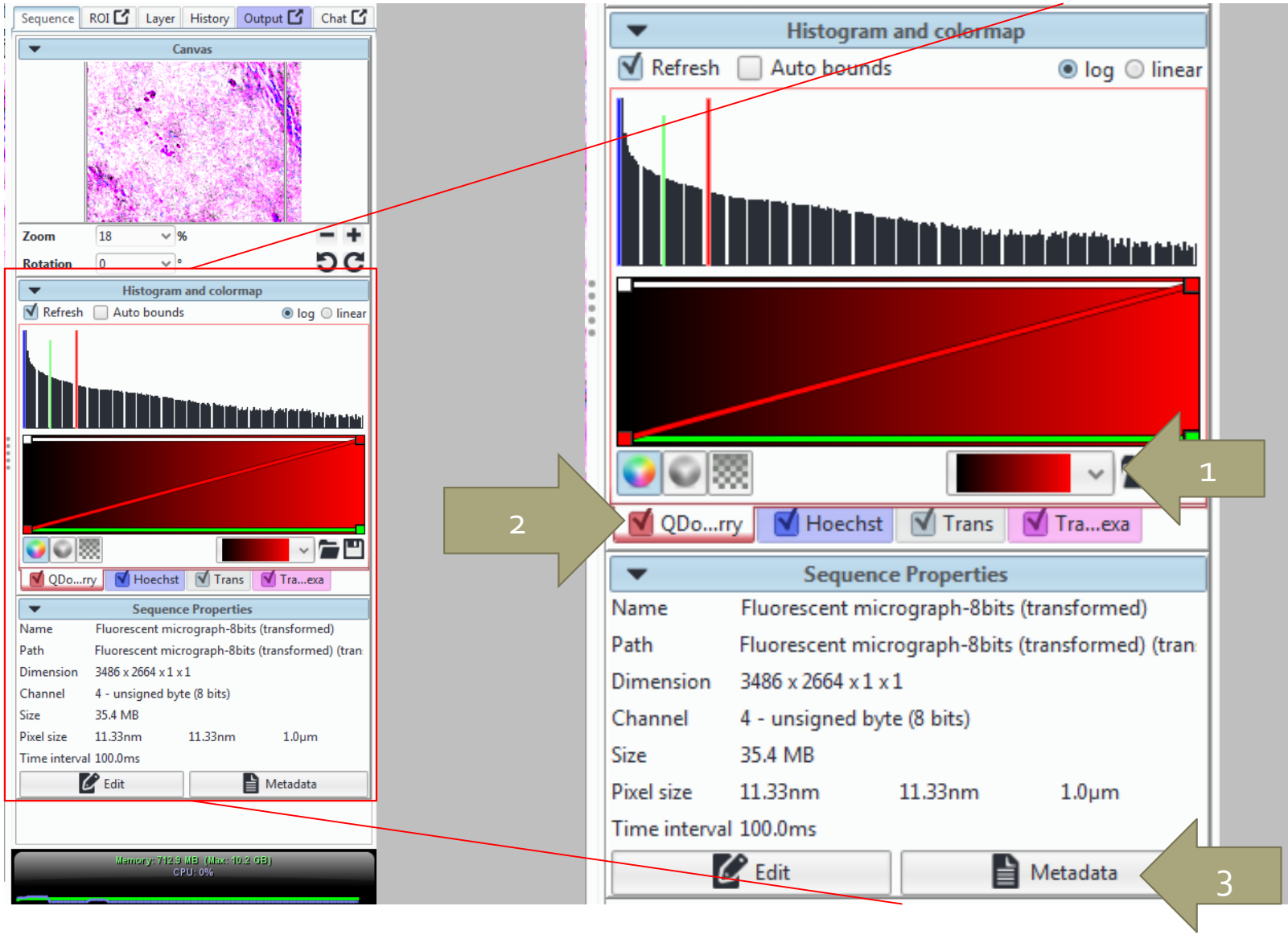


Display your images at your convenience

On the left panel, the top part has several tabs.

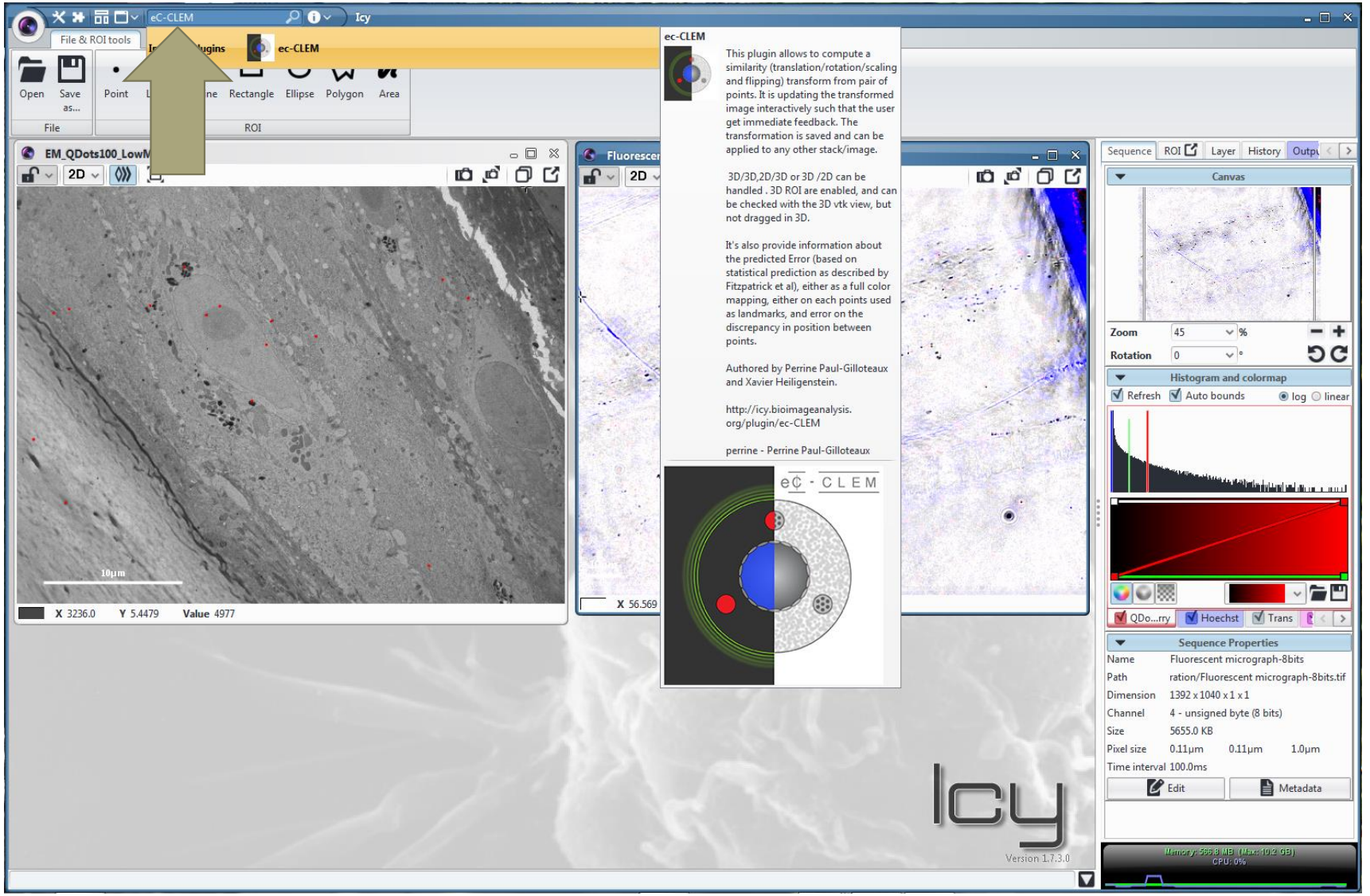
Below, the color display is tunable (1) channel by channel (2)

Check the metadata and edit them if necessary (3). Pay attention to the pixel size as it defines the error calculation estimation



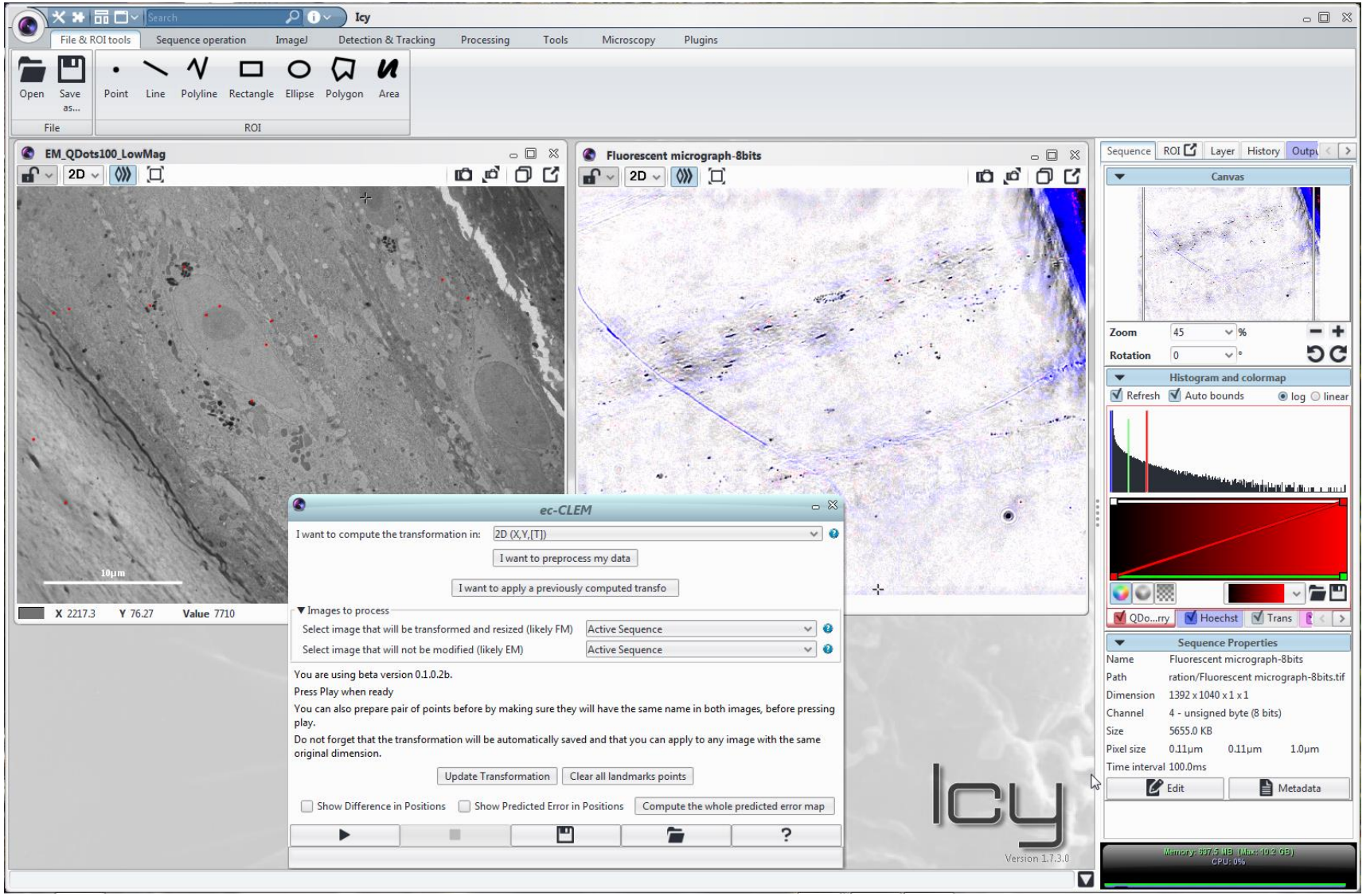
Type « eC-CLEM » in the search bar

Click on the plugin to launch it



eC-CLEM starts

If the plugin is not already installed, it will automatically download and install it. No Icy restart is required after installation



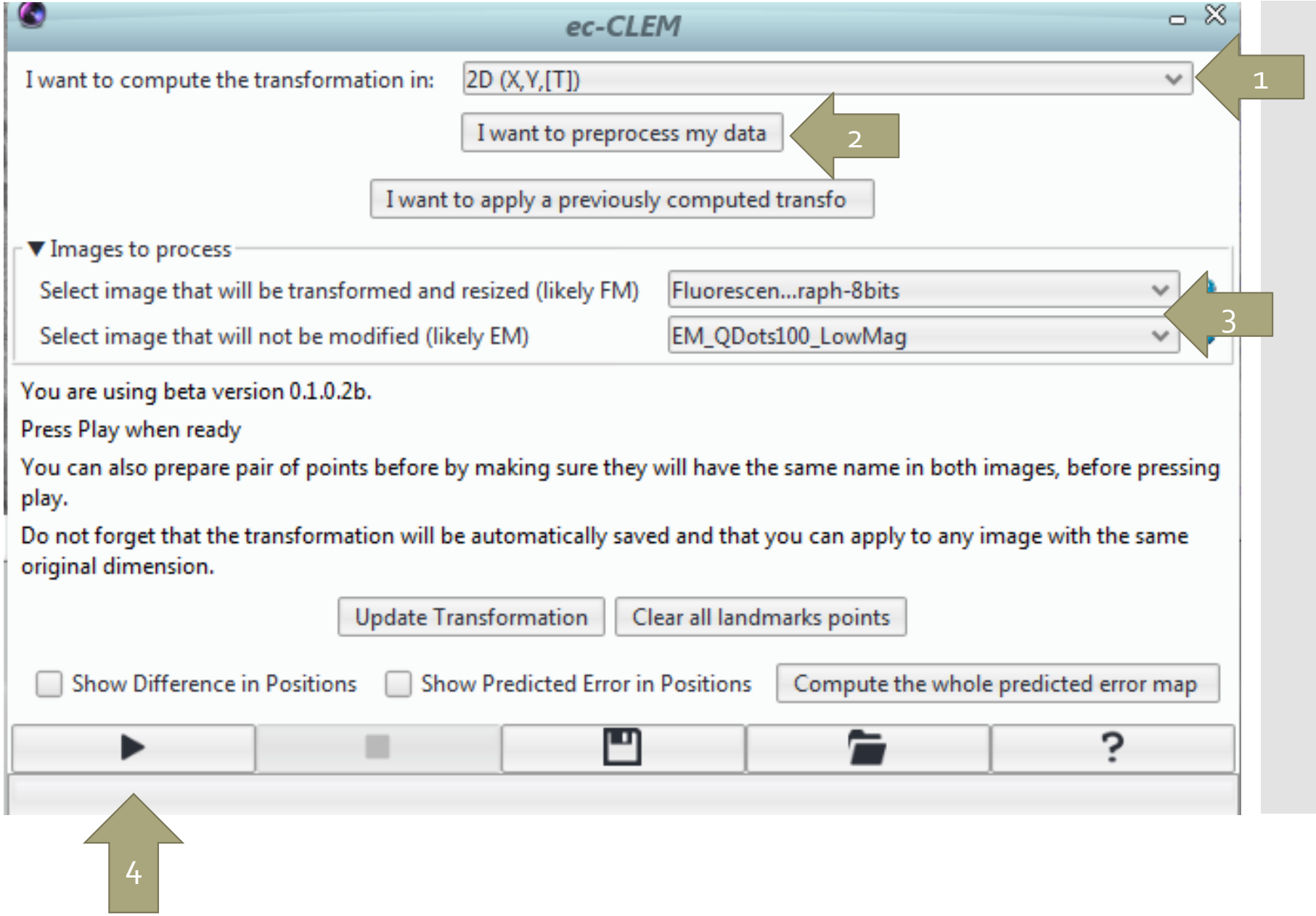
The eC-CLEM interface is kept simple

The first step is to decide whether the final overlay will be 2D or 3D (1).

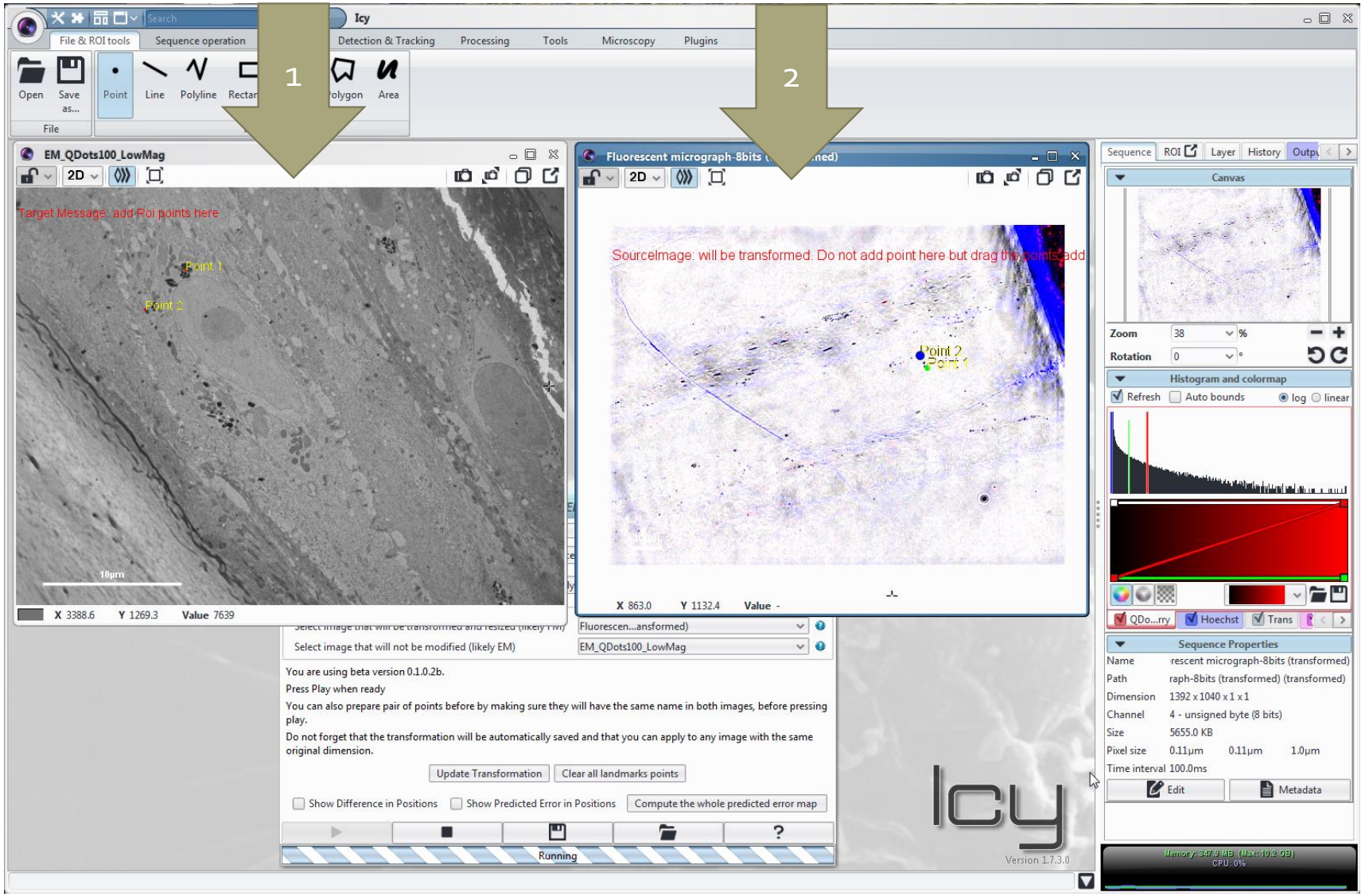
When starting from a z-stack of fluorescence from a section, a « beginners » preprocessing step can be used (2). See article for more details.

Select the source image to transform and the target image (3)

Launch the plugin for live seed and transform process (4)

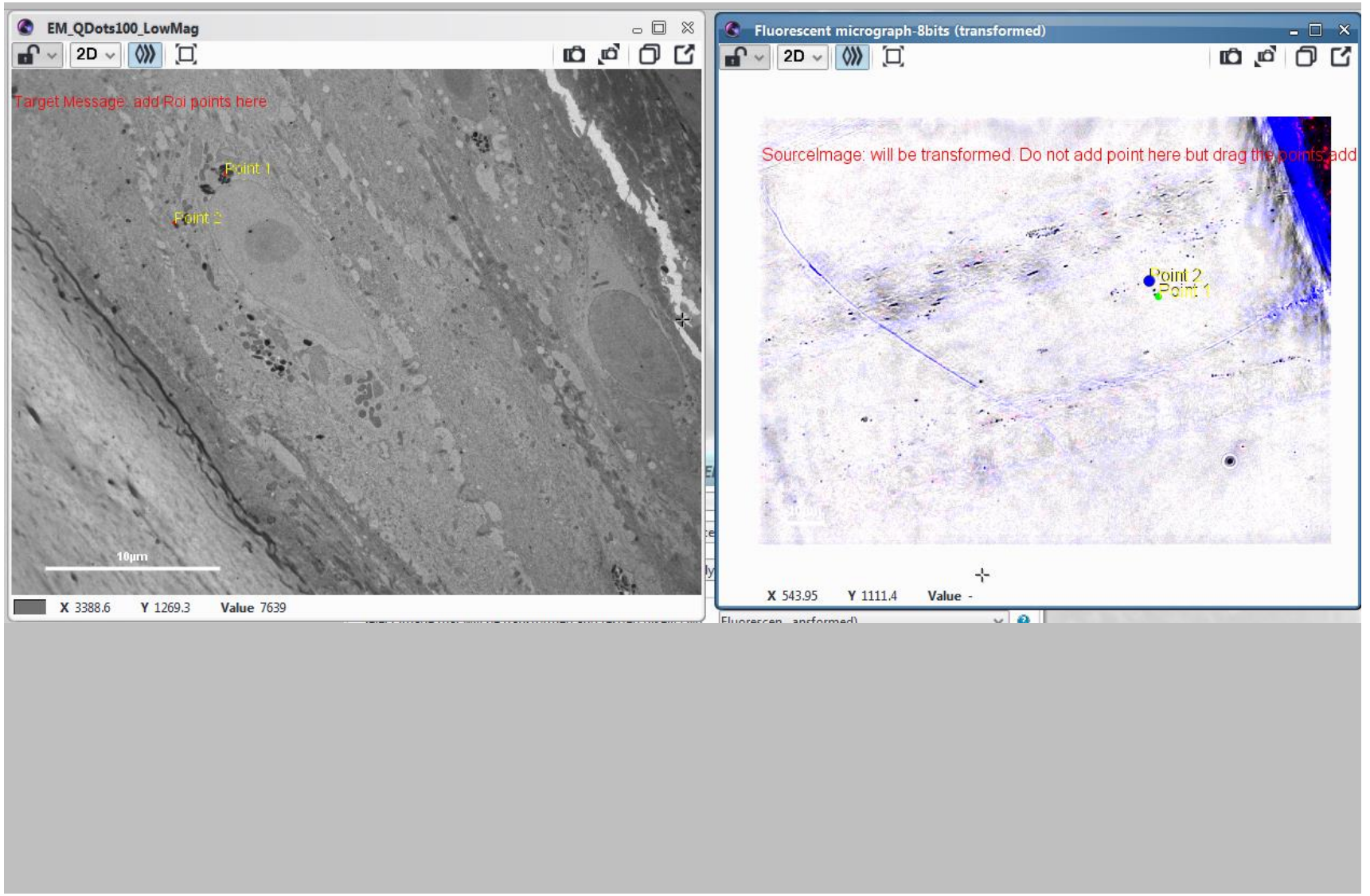


Seed the point on the target image (1), adjust it on the source (2)



In 2D registration, 3 initial points are necessary to compute the initial transformation

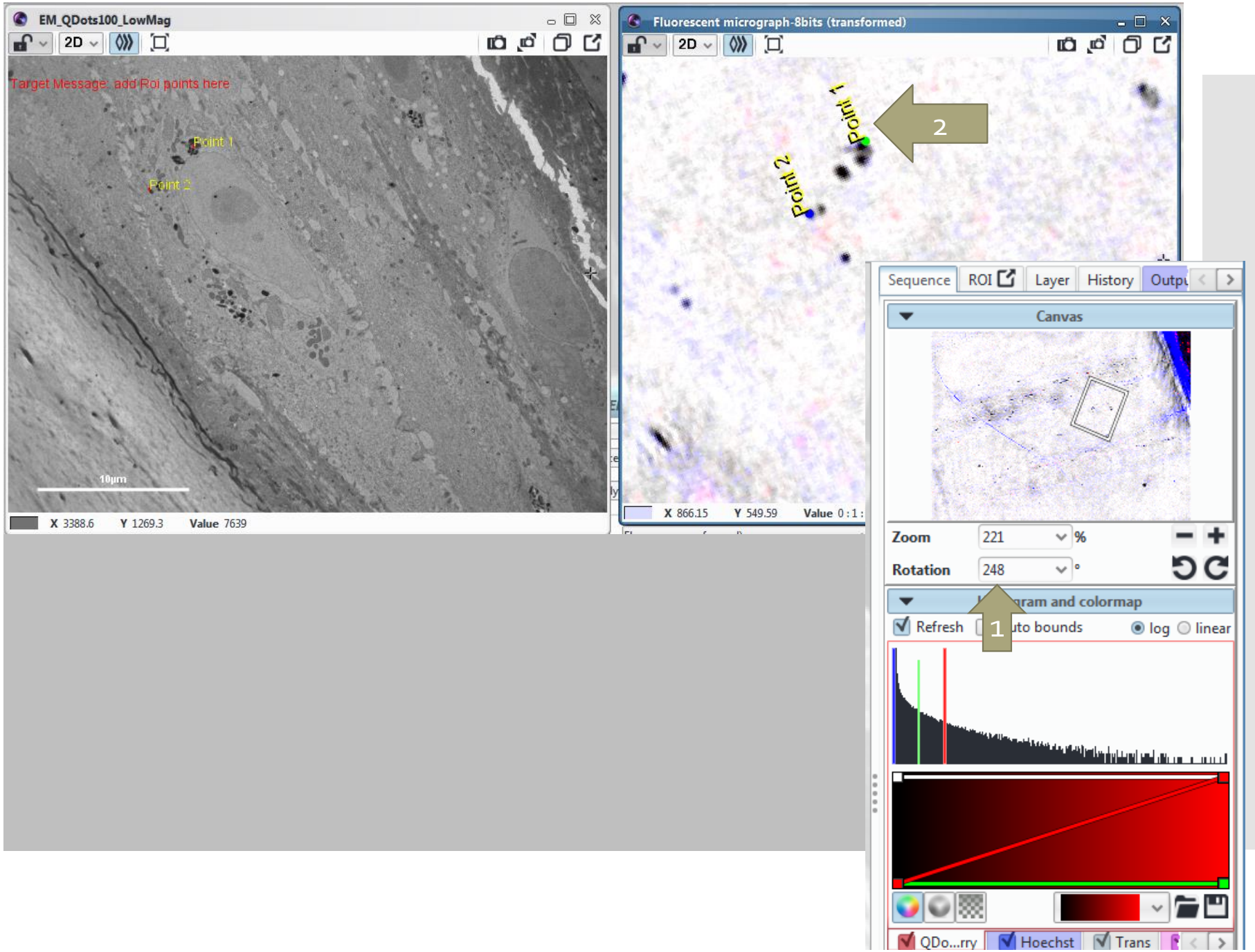
A prior knowledge of at least 3 homology points is necessary.



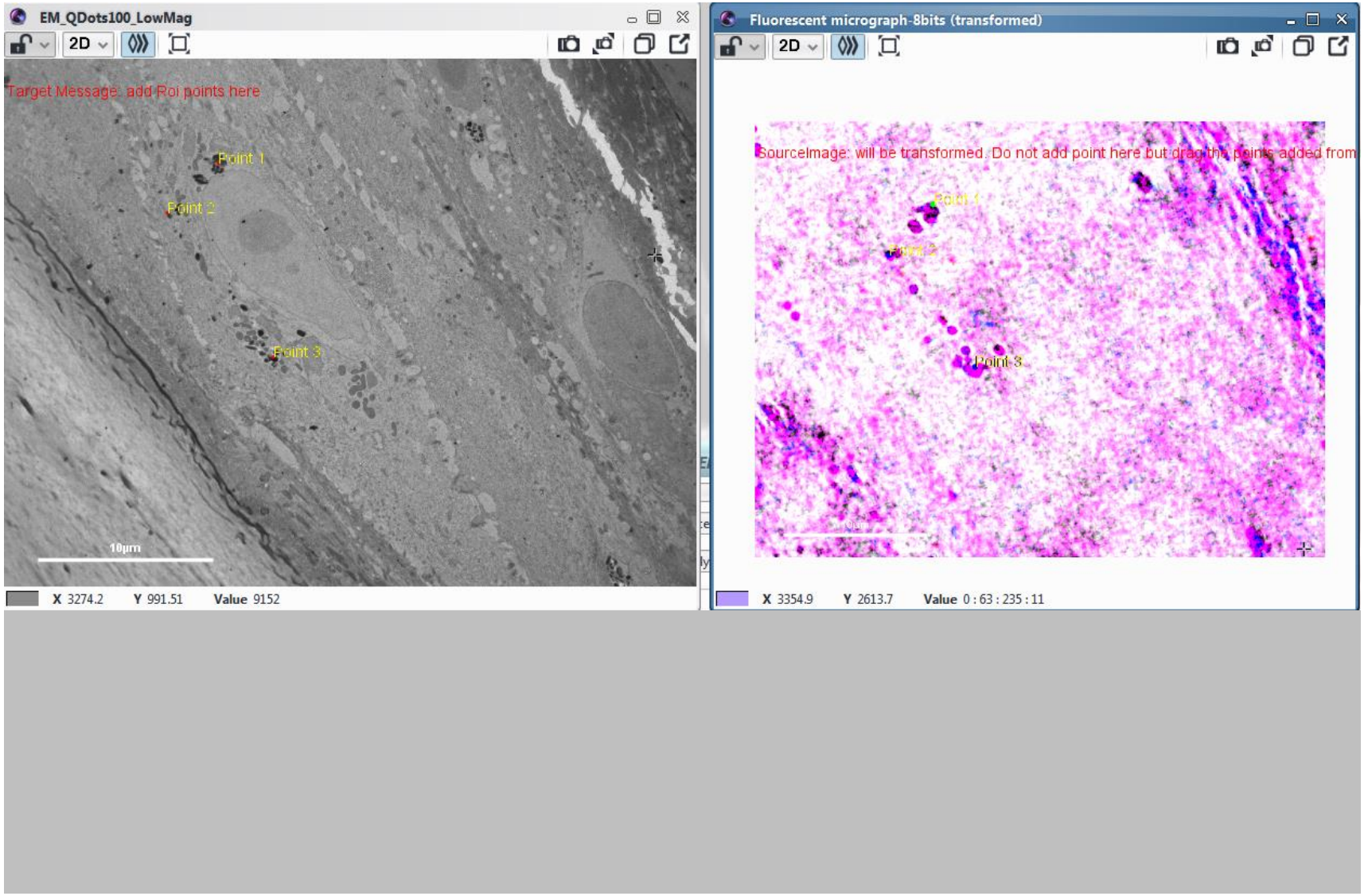
Right click and shift allows manual « display rotation » (1)

No transformation is computed, but it might help to seed the first 3 points.

Note that the display only is affected : labels are rotated together with the image (2).

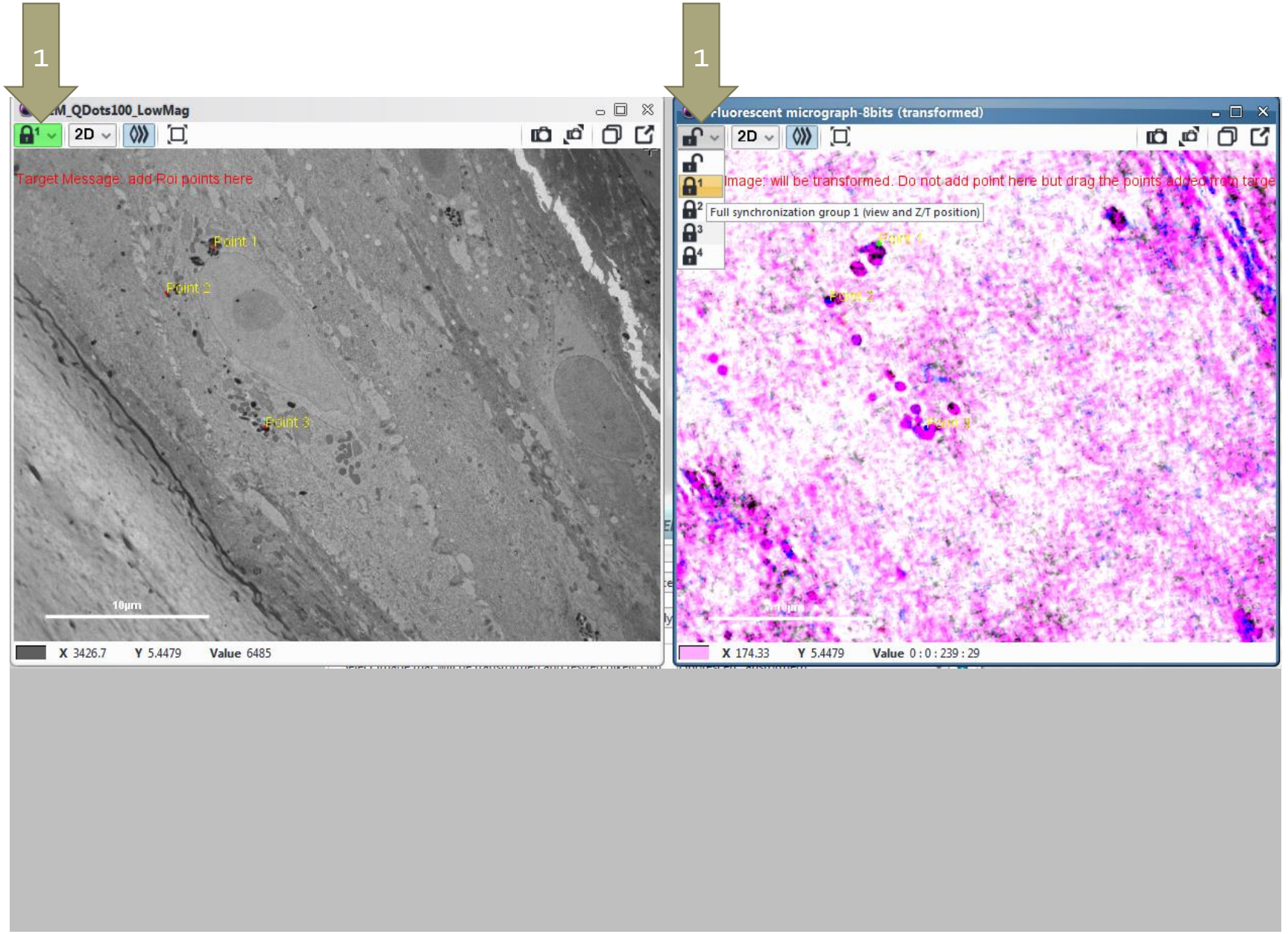


After 3 points, the transformation is applied and displayed



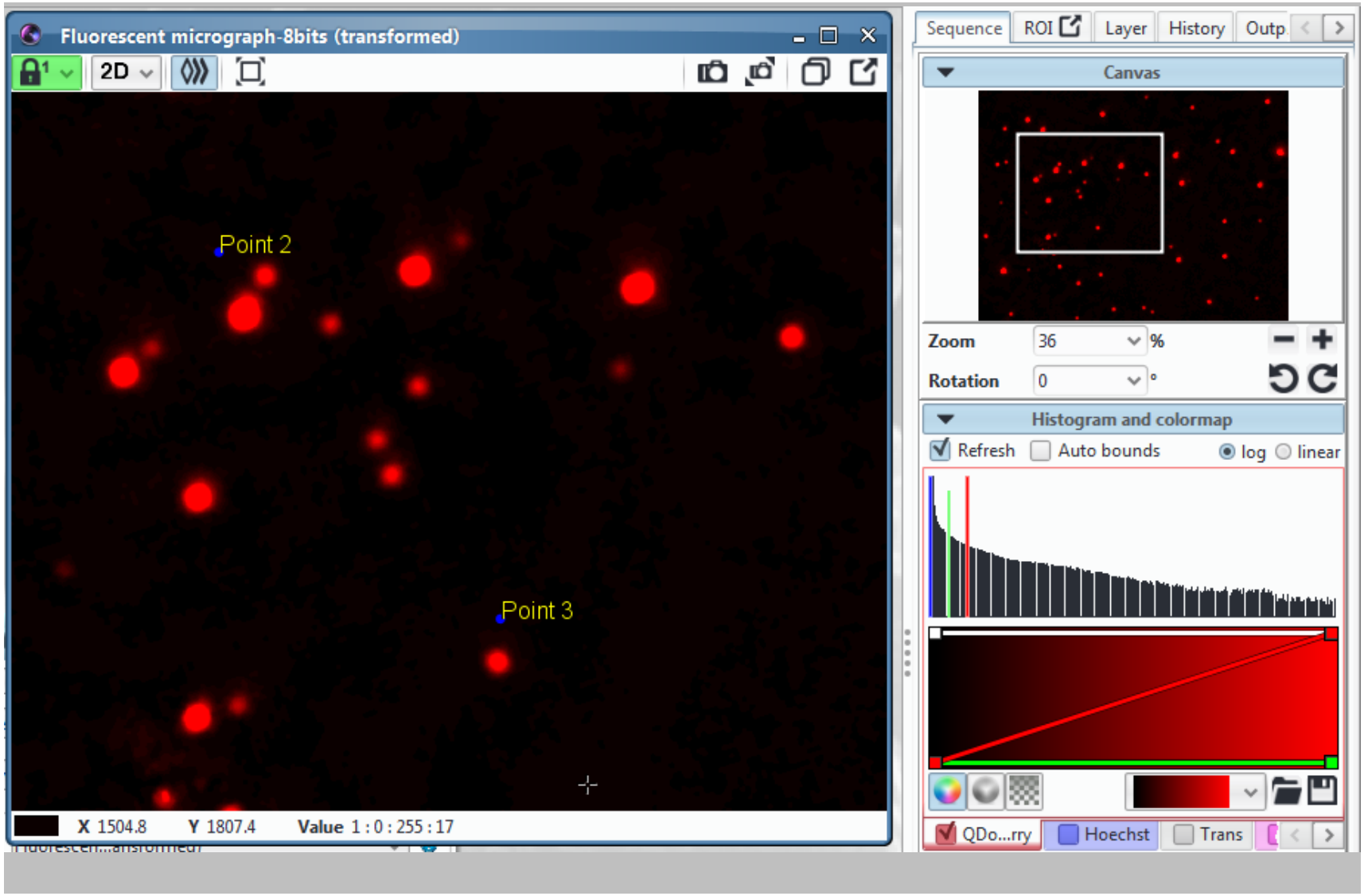
Lock both images together

A functionality of Icy allows to lock both images together, synchronizing the navigation while zooming in and out of the images.



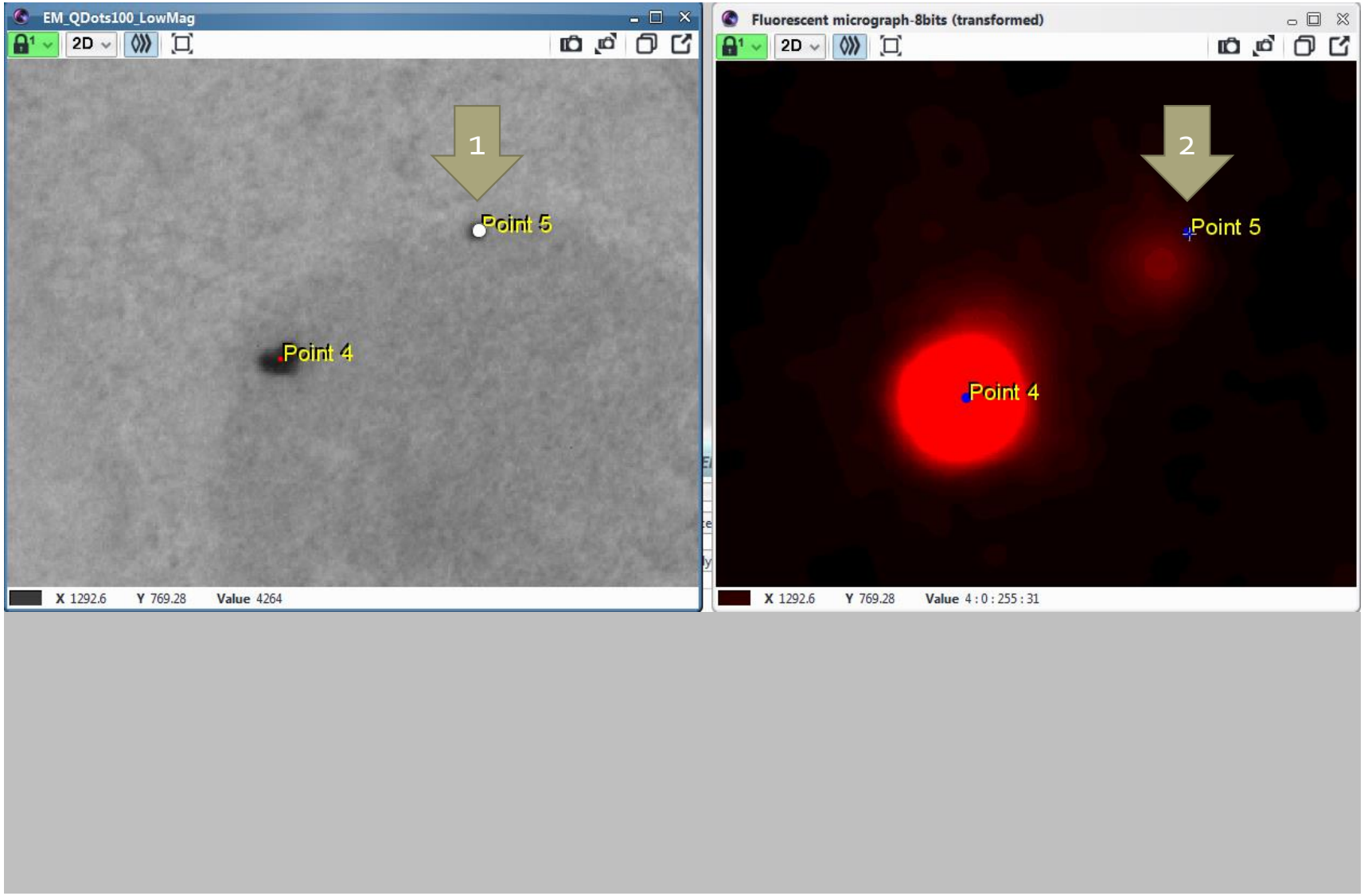
Select and adjust the contrast from the channel containing the registration landmarks

In our example, Qdots aggregates, visible both in EM and in mCherry channel are used to register the images and estimate the alignment accuracy.



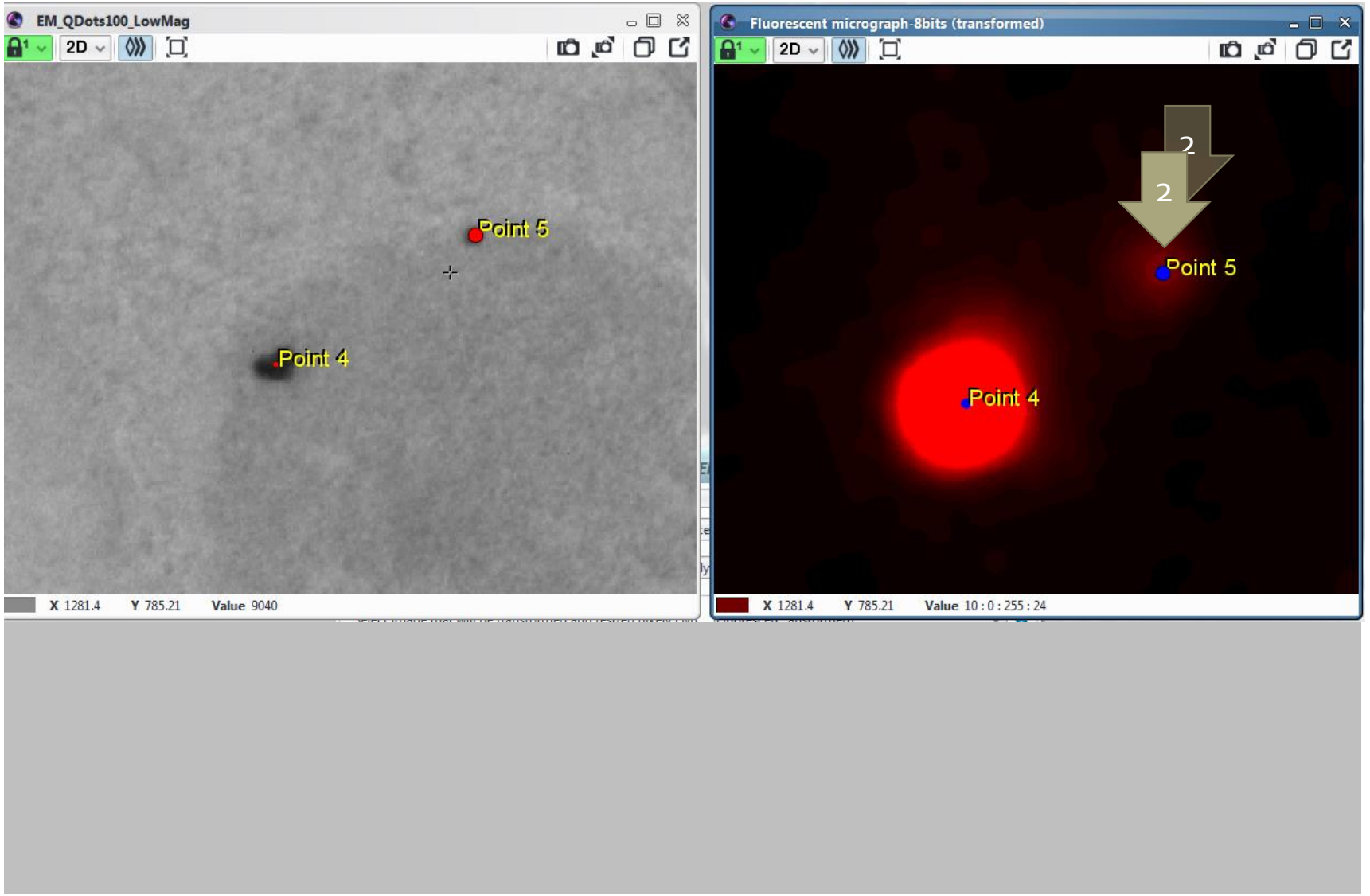
Seed a point on the target image (1). Its homologous point is automatically placed on the source image (2)

The localization is calculated according to the previous seed points



Adjust the seed point on the source image (2)

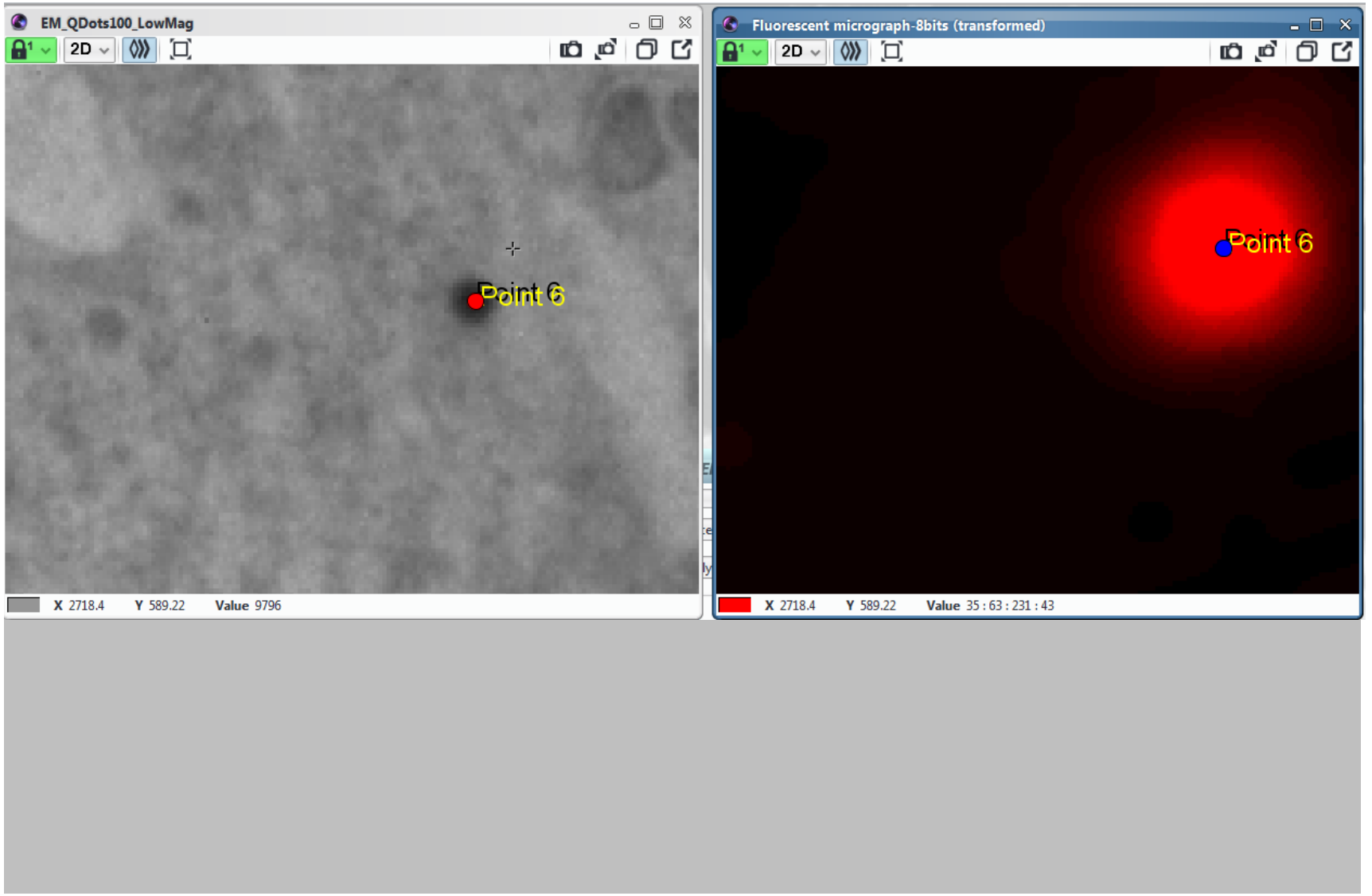
Drag and drop the point at sub-pixel precision



Select as many registration seeding points as required

eC-CLEM is not limited in the number of seed points and calculates the final transformation from the average transformations of all points.

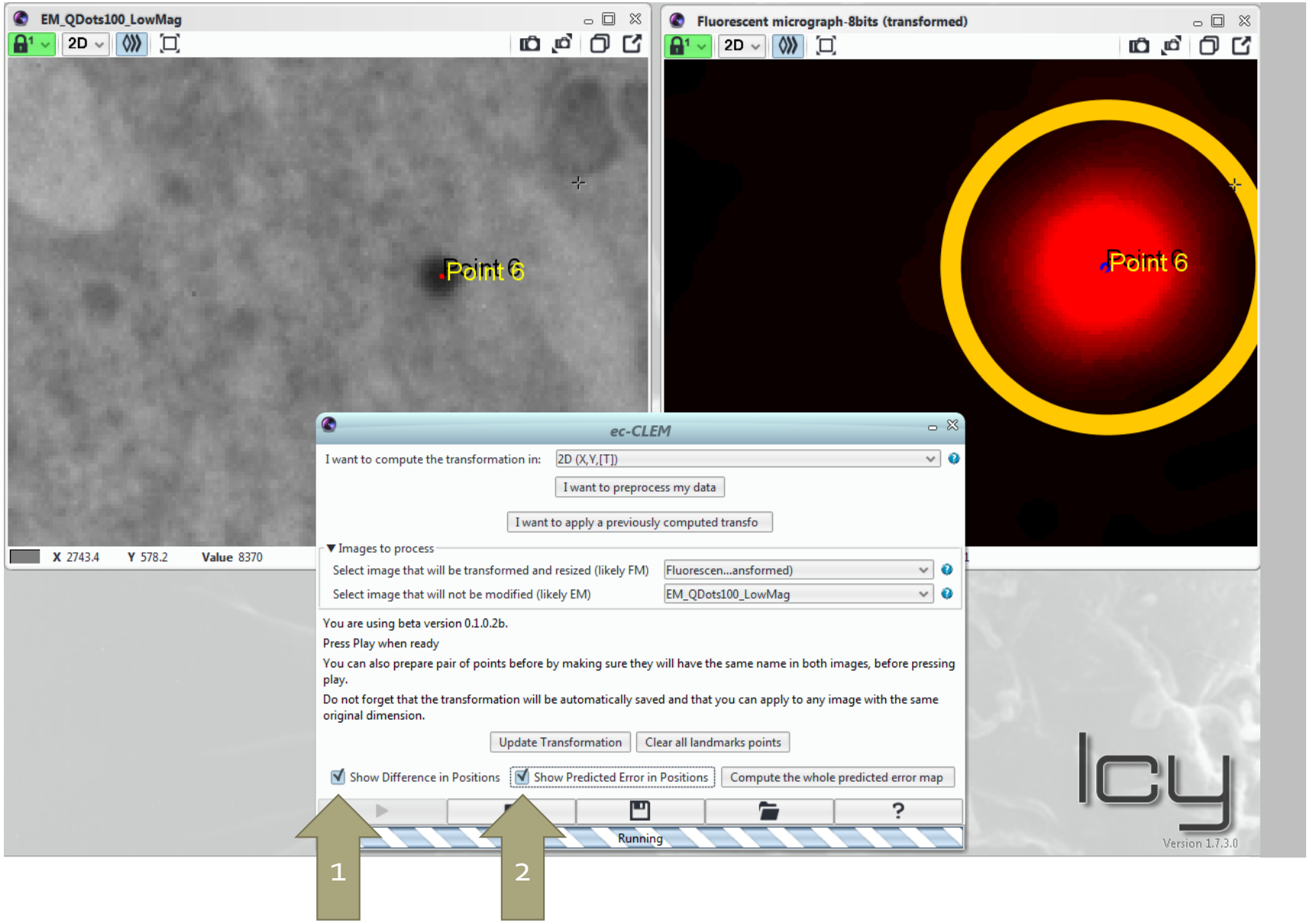
The seed points can be selected from different color channels if required. In this example, transmitted light channel was used for the first 3 points, then mCherry channel for the following points.



Display the difference in position (1) and the predicted error in position (2)

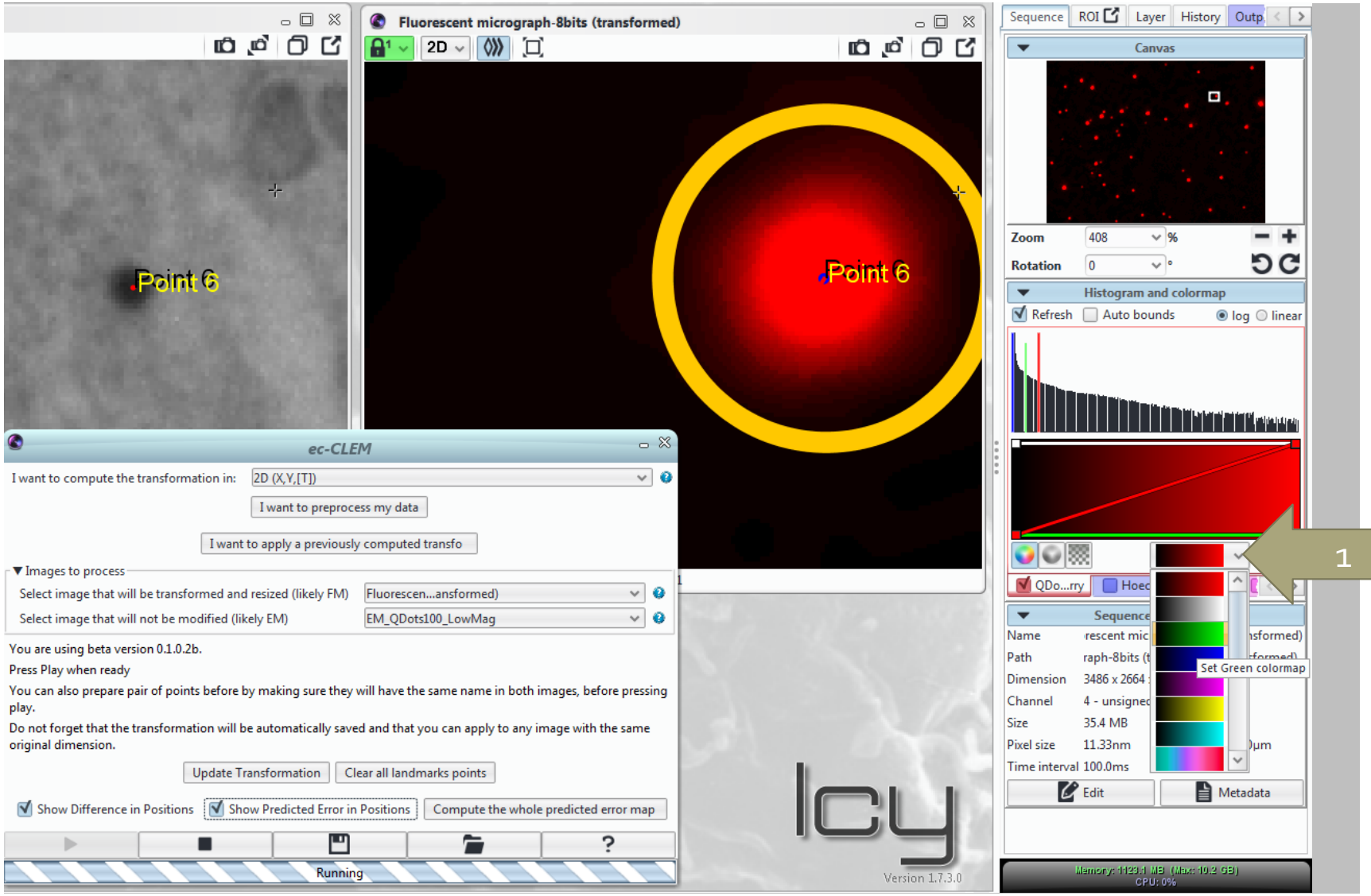
The difference in position is displayed as a red vector

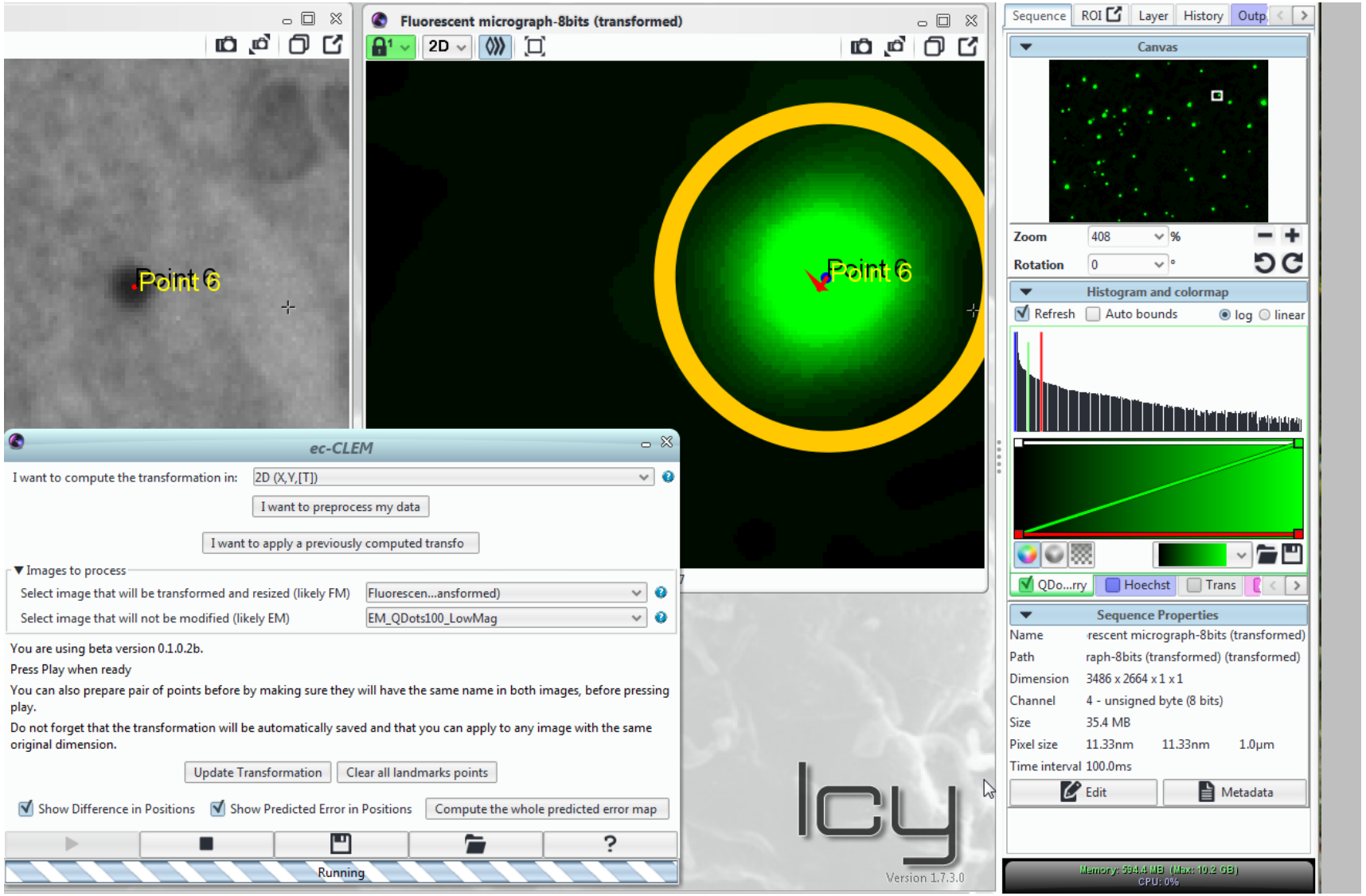
The predicted error in position is displayed as a orange circle



Change the display channel color to visualize the difference in position vector (1)

If the registration landmarks are in the red channel, arbitrarily change the display color to green (1).

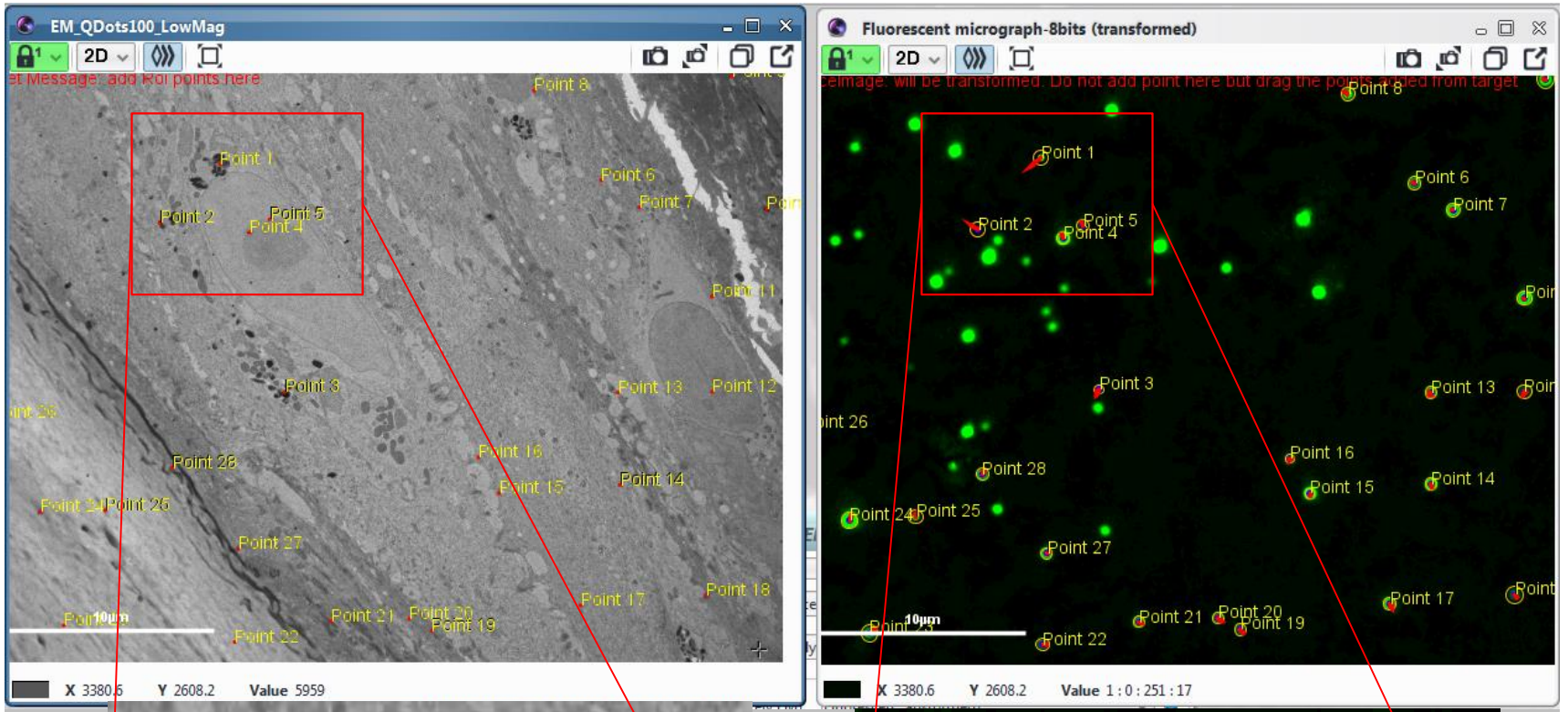


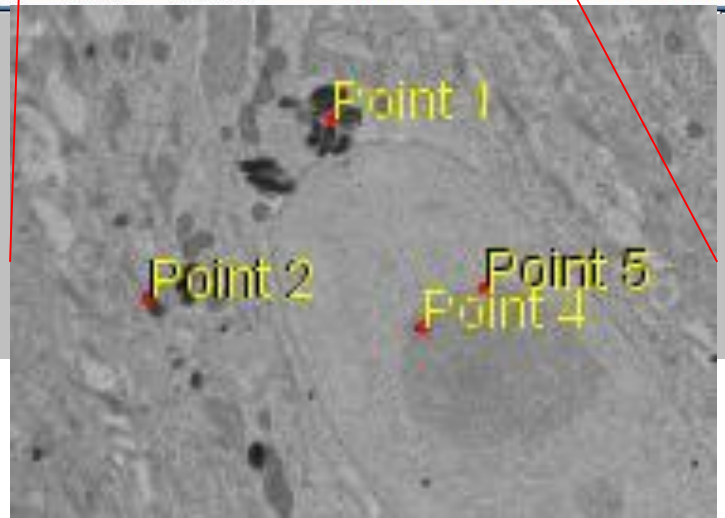

Point 6
Fluorescent micrograph-8bits (transformed)
2D
Point 6
ec-CLEM
I want to compute the transformation in:
2D (X,Y,[T])
I want to preprocess my data
I want to apply a previously computed transfo
Images to process
Select image that will be transformed and resized (likely FM)
Fluorescen...ansformed)
Select image that will not be modified (likely EM)
EM_QDots100_LowMag
You are using beta version 0.1.0.2b.
Press Play when ready
You can also prepare pair of points before by making sure they will have the same name in both images, before pressing play.
Do not forget that the transformation will be automatically saved and that you can apply to any image with the same original dimension.
Update Transformation
Clear all landmarks points
Show Difference in Positions
Show Predicted Error in Positions
Compute the whole predicted error map
Running
Version 1.7.3.0
Sequence
ROI
Layer
History
Canvas
Zoom
408
%
Rotation
0
Histogram and colormap
Refresh
Auto bounds
log
linear
QDo...rry
Hoechst
Trans
Sequence Properties
Name
rescent micrograph-8bits (transformed)
Path
raph-8bits (transformed) (transformed)
Dimension
3486 x 2664 x 1 x 1
Channel
4 - unsigned byte (8 bits)
Size
35.4 MB
Pixel size
11.33nm
11.33nm
1.0µm
Time interval
100.0ms
Edit
Metadata
Memory: 594.4 MB (Max: 10.2 GB)
CPU: 0%

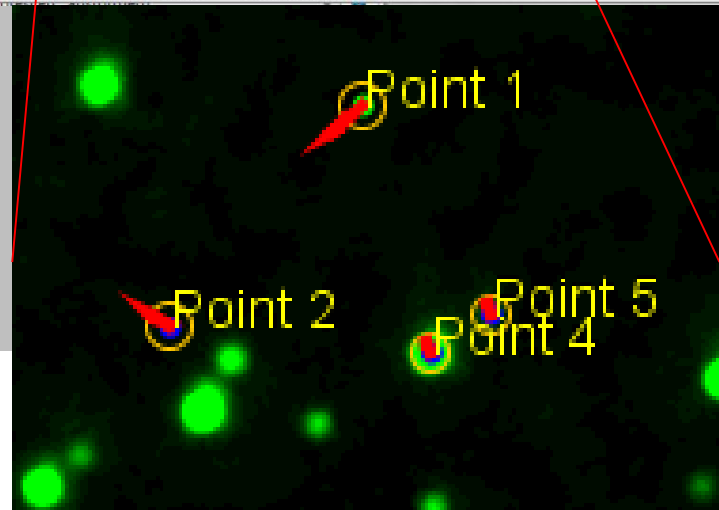
Direct display of the registration error estimation

The calculation of registration error is done on the fly. The display can be used as a guide to estimate the registration precision and the final alignment quality (see article).

Points 1 and 2 are slightly off targeted. Points 4 and 5 are accurately assigned according to all the other landmarks of the image.

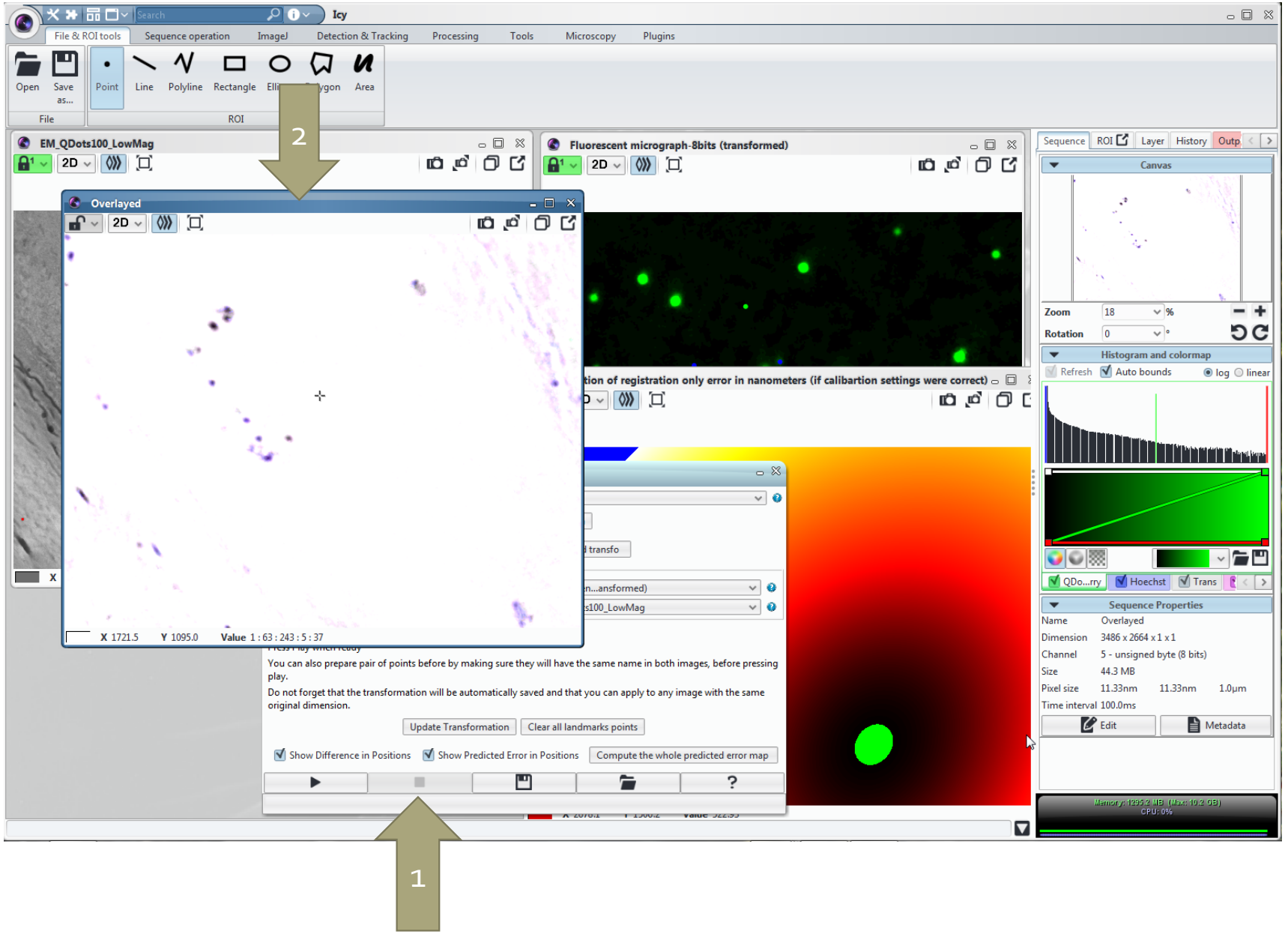






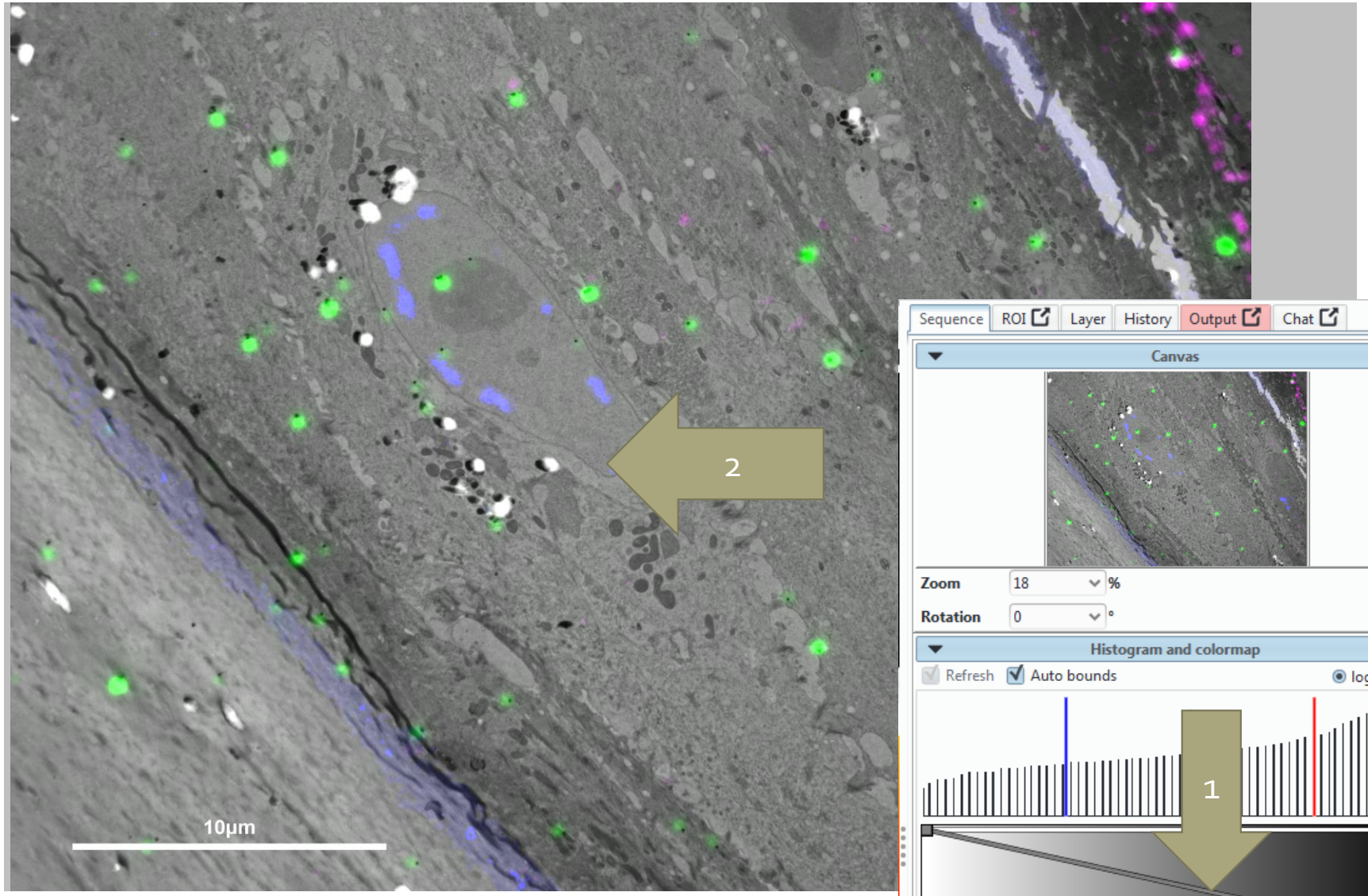
Automatic overlay production (2)

Once all registration landmarks have been seeded, stopping the plugin (1) creates instantly the multichannel overlay (2)



Adjust channels display to visualize the fluorescent signal onto the electron micrograph

The transmitted light channel is inverted (1) to appear white on the micrograph



A transformation file is automatically created in the source file folder (1)

This transformation can be applied to any other image (2) of the same size.

For example a time series from live cell imaging is a large dataset implying heavy processing load.

Using only the last frame for registration, the processing load remains reasonable. The final transformation can be later applied to the entire time series.