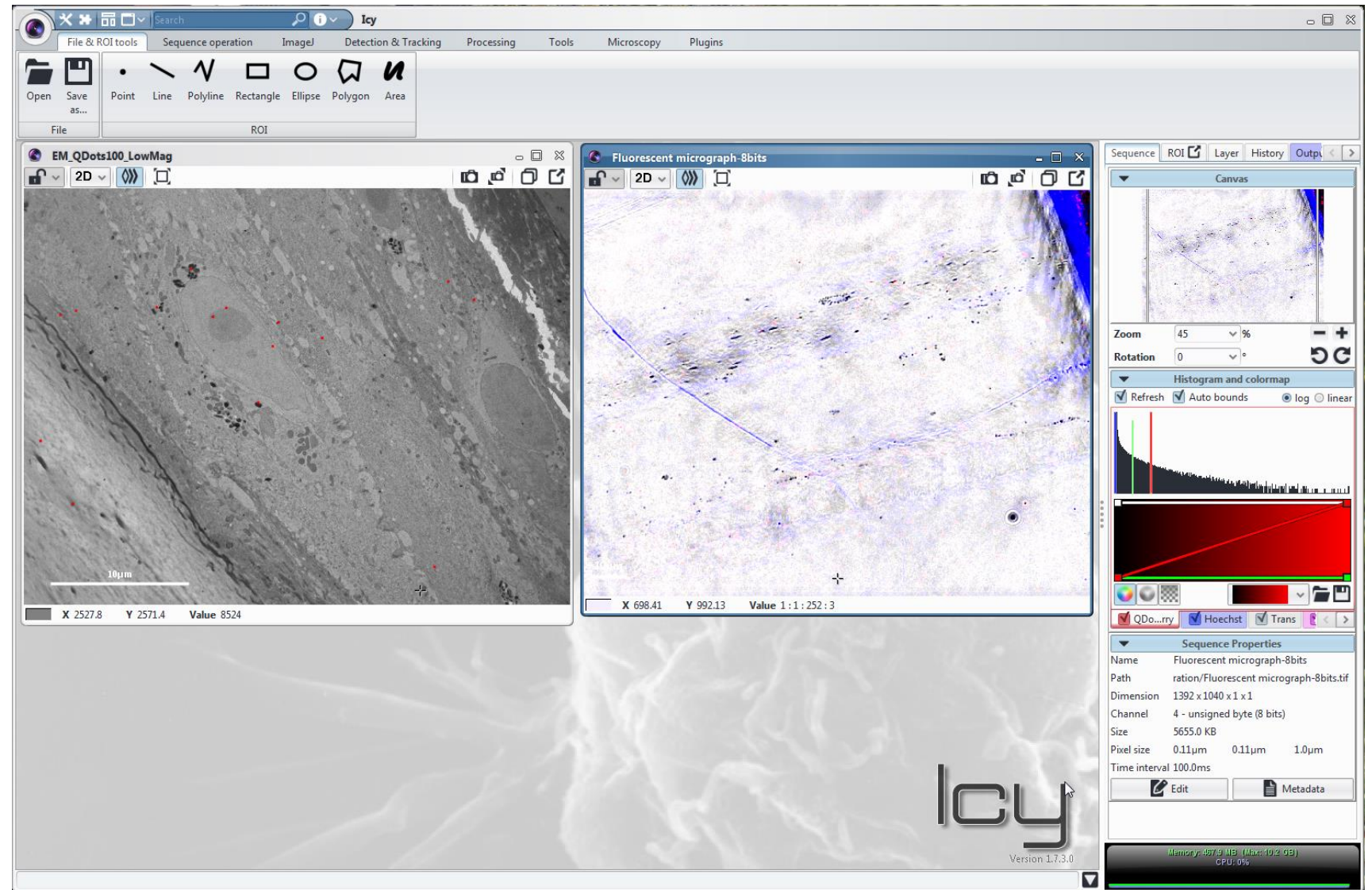


# Step by step Guide for eC-CLEM

2D to 2D registration

# Open both light and electron microscopy images

Drag and drop

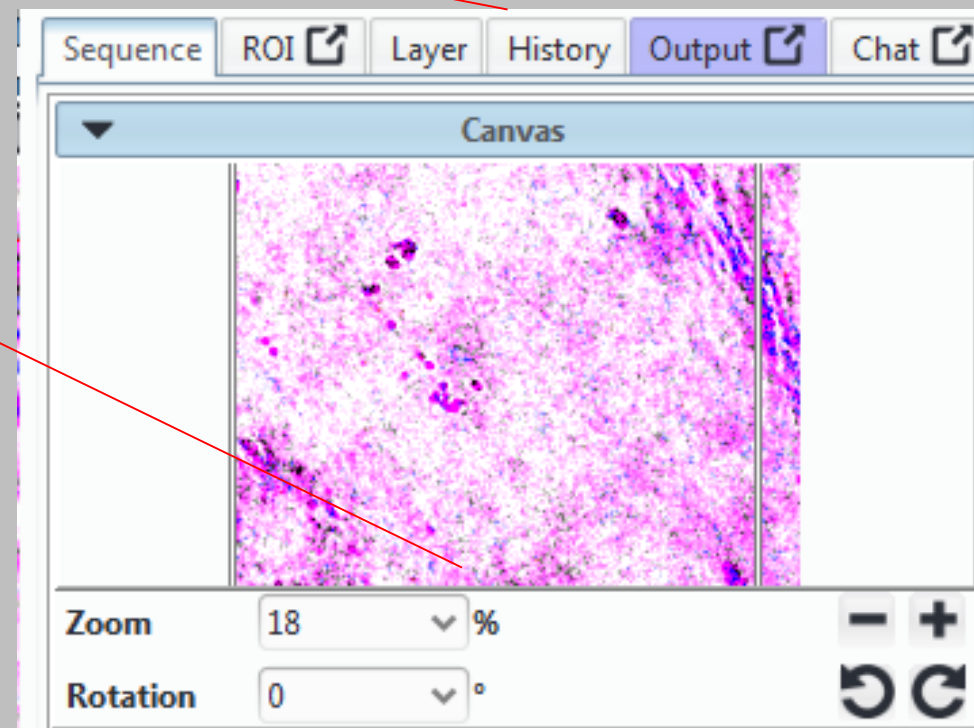
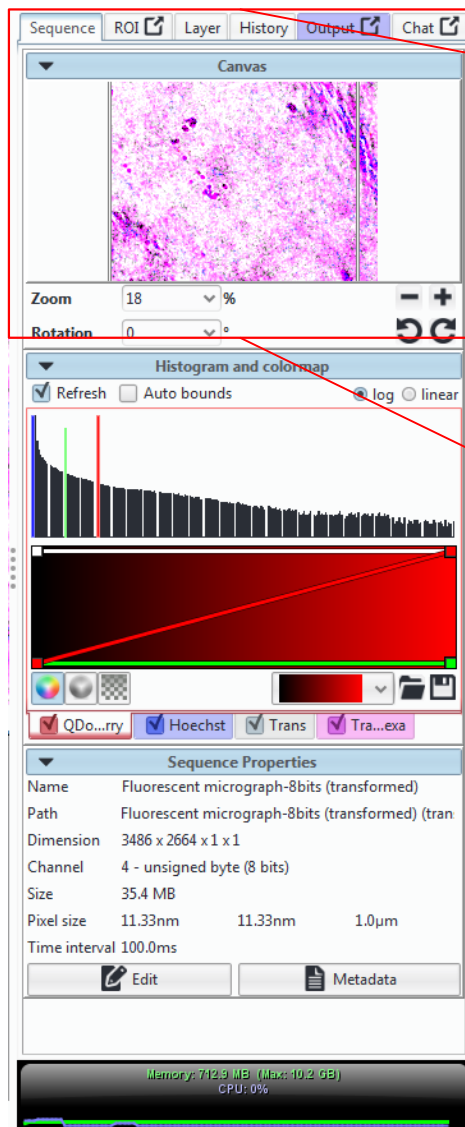


# Display your images at your convenience

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

The screenshot displays a software interface with the following components:

- Canvas:** Shows a histology image with a zoom of 18% and a rotation of 0 degrees.
- Histogram and colormap:** Features a histogram with a red vertical line and a color calibration tool. A red arrow points from the histogram to the color calibration tool.
- Sequence Properties:** A table containing the following data:

Sequence Properties			
Name	Fluorescent micrograph-8bits (transformed)		
Path	Fluorescent micrograph-8bits (transformed) (tran		
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		

Three numbered arrows indicate key features: arrow 1 points to the color calibration tool, arrow 2 points to the histogram, and arrow 3 points to the 'Edit' button in the 'Sequence Properties' panel.

Type « eC-CLEM » in the search bar

Click on the plugin to launch it

The screenshot displays the Icy software interface with the search bar at the top containing the text « eC-CLEM ». The search results show the eC-CLEM plugin, which is highlighted. A green arrow points to the search bar. The main window shows a grayscale electron micrograph (EM\_QDots100\_LowM) with several red dots. A scale bar indicates 10 μm. The status bar at the bottom shows X: 3236.0, Y: 5.4479, and Value: 4977. The right sidebar contains a description of the eC-CLEM plugin, a diagram of the plugin's interface, and a list of sequence properties.

**ec-CLEM**

This plugin allows to compute a similarity (translation/rotation/scaling and flipping) transform from pair of points. It is updating the transformed image interactively such that the user get immediate feedback. The transformation is saved and can be applied to any other stack/image.

3D/3D,2D/3D or 3D/2D can be handled. 3D ROI are enabled, and can be checked with the 3D vtk view, but not dragged in 3D.

It's also provide information about the predicted Error (based on statistical prediction as described by Fitzpatrick et al), either as a full color mapping, either on each points used as landmarks, and error on the discrepancy in position between points.

Authored by Perrine Paul-Gilloteaux and Xavier Heiligenstein.

<http://icy.bioimageanalysis.org/plugin/ec-CLEM>

perrine - Perrine Paul-Gilloteaux

**eC - CLEM**

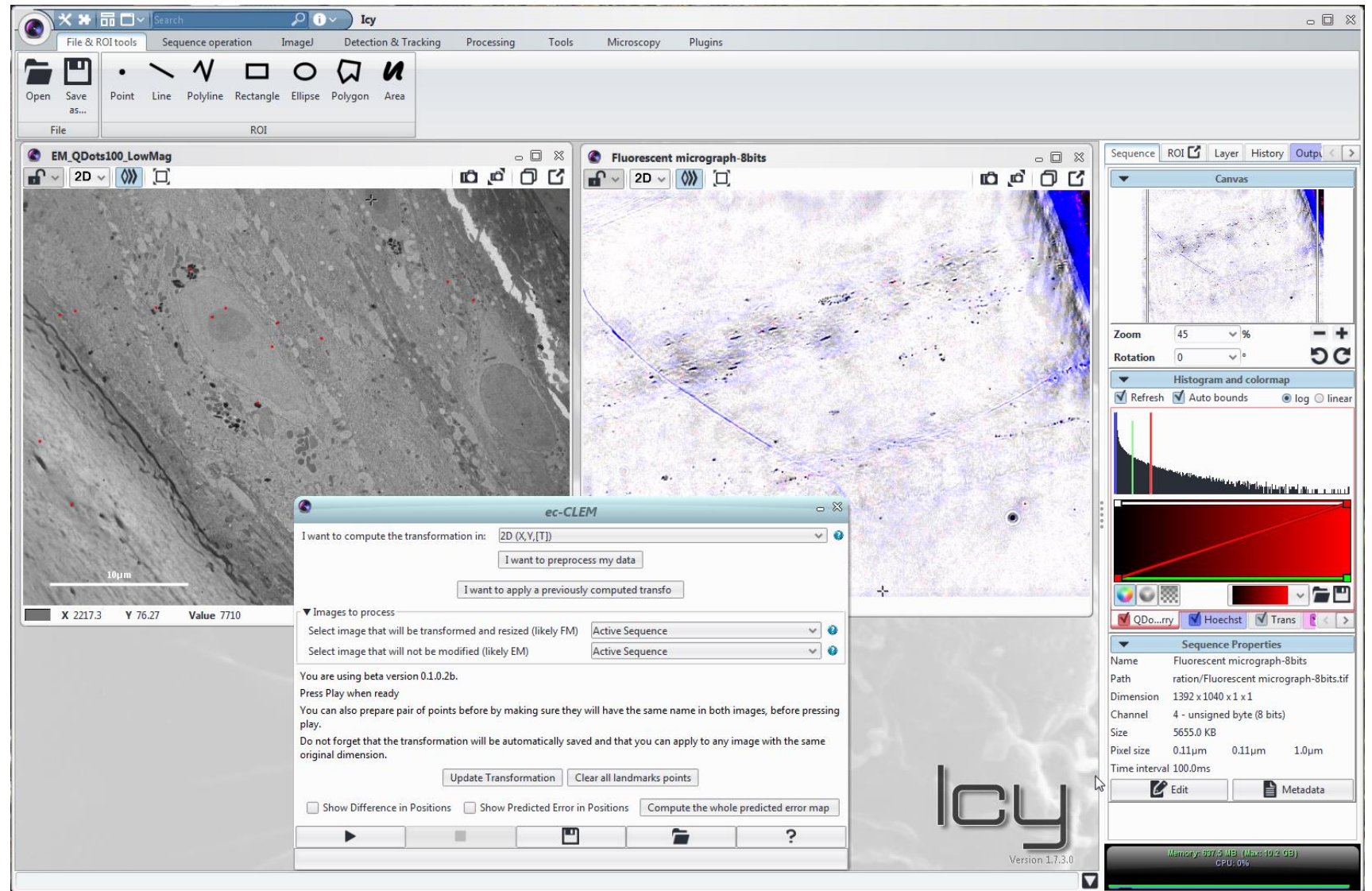
**Sequence Properties**

Name	Fluorescent micrograph-8bits
Path	ration/Fluorescent micrograph-8bits.tif
Dimension	1392 x 1040 x 1 x 1
Channel	4 - unsigned byte (8 bits)
Size	5655.0 KB
Pixel size	0.11 μm 0.11 μm 1.0 μm
Time interval	100.0ms

Memory: 289.8 MB (Max: 10.2 GB)  
CPU: 0%

# eC-CLEM starts

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



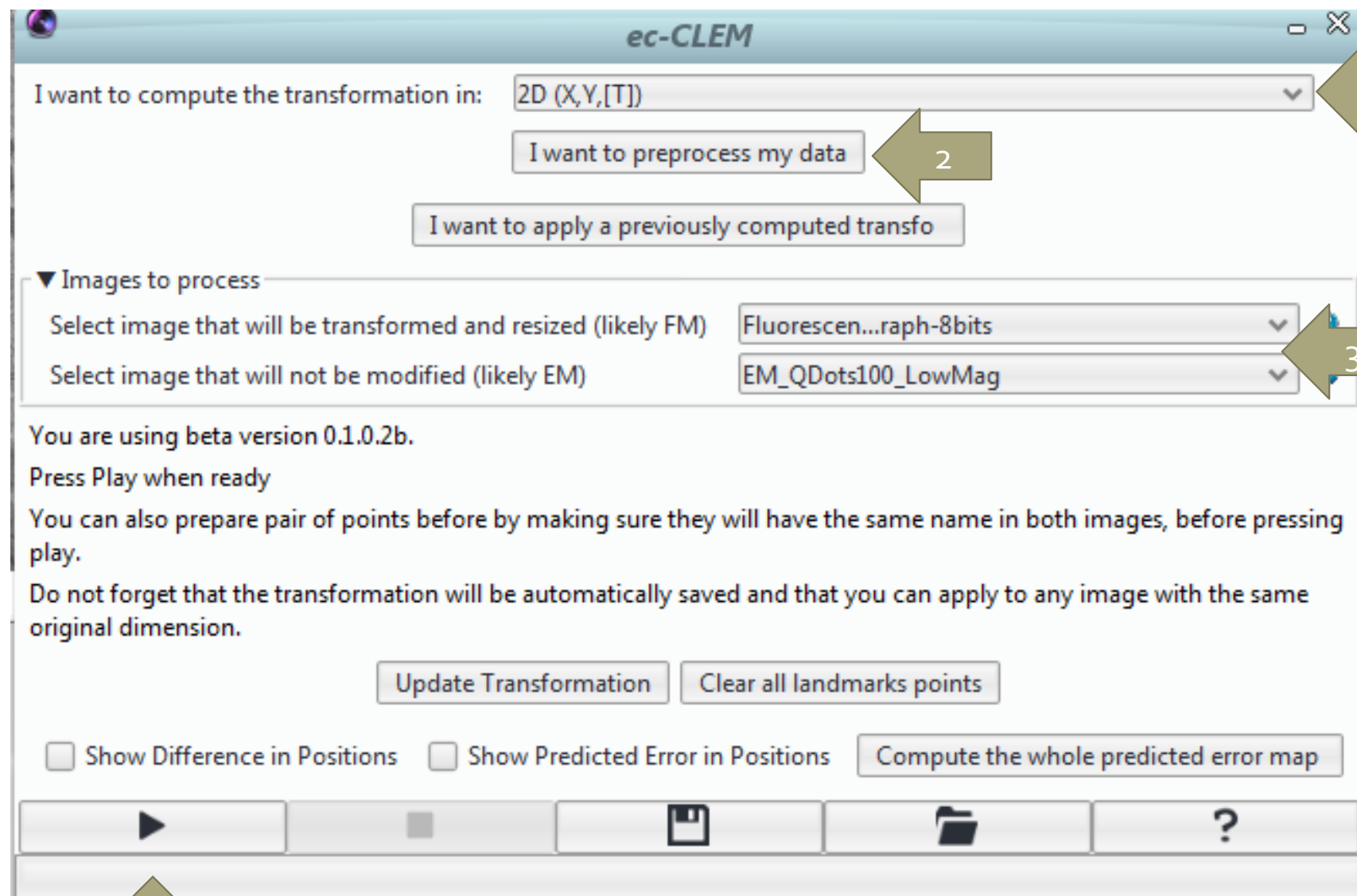
# The eC-CLEM interface is kept simple

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

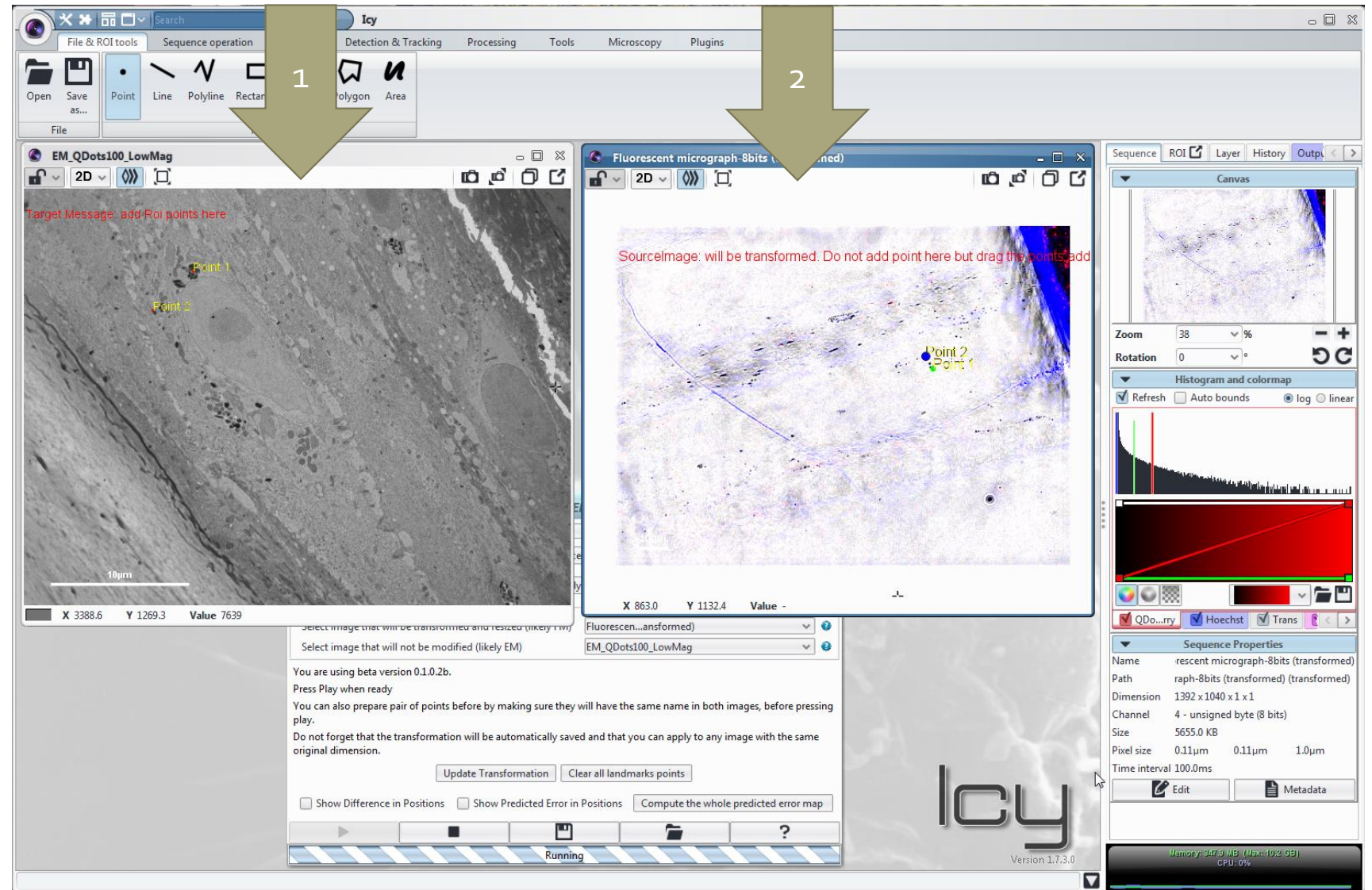
When starting from a z-stack of fluorescence from a section, a « beginners » preprocessing step can be used (2). See article for more details.

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

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



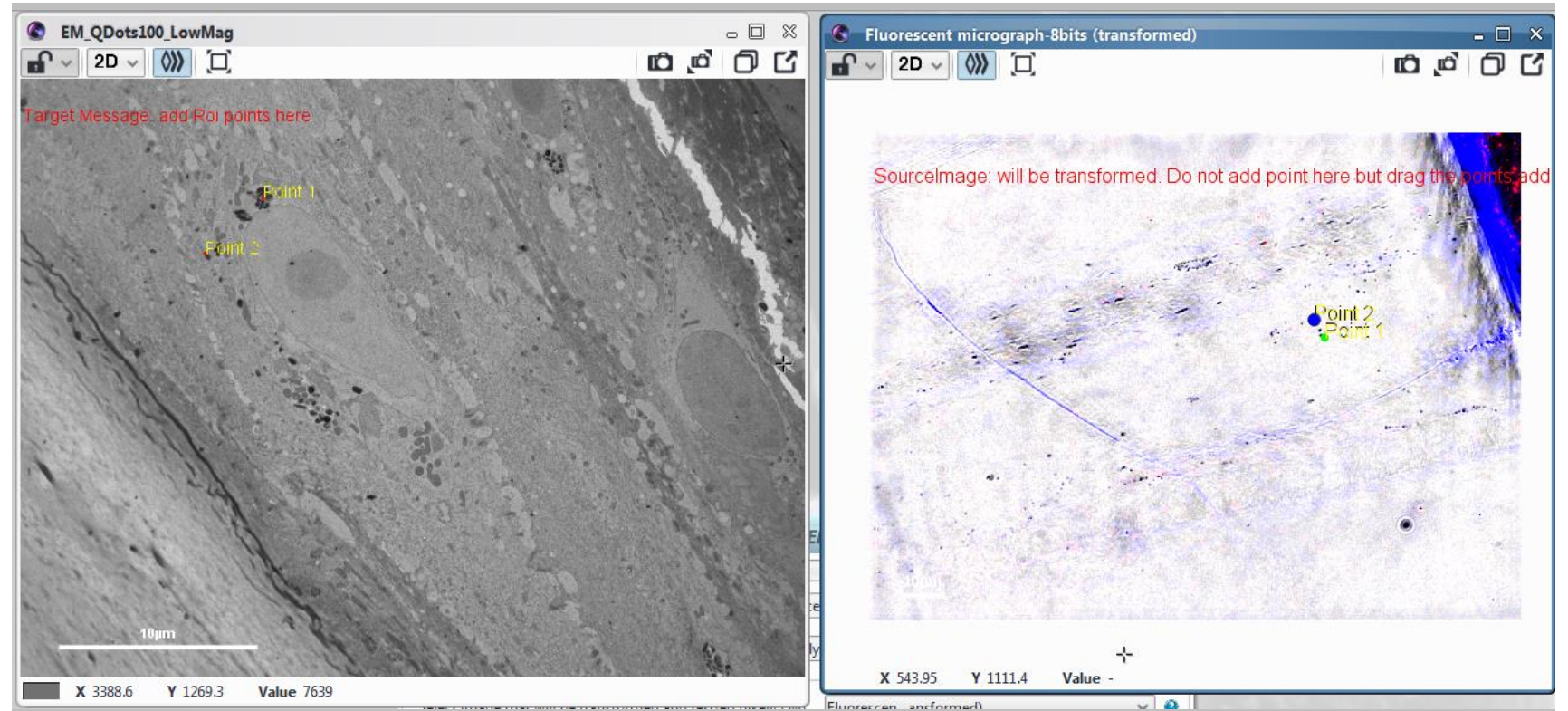
Seed the point on the target image (1), adjust it on the source (2)





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

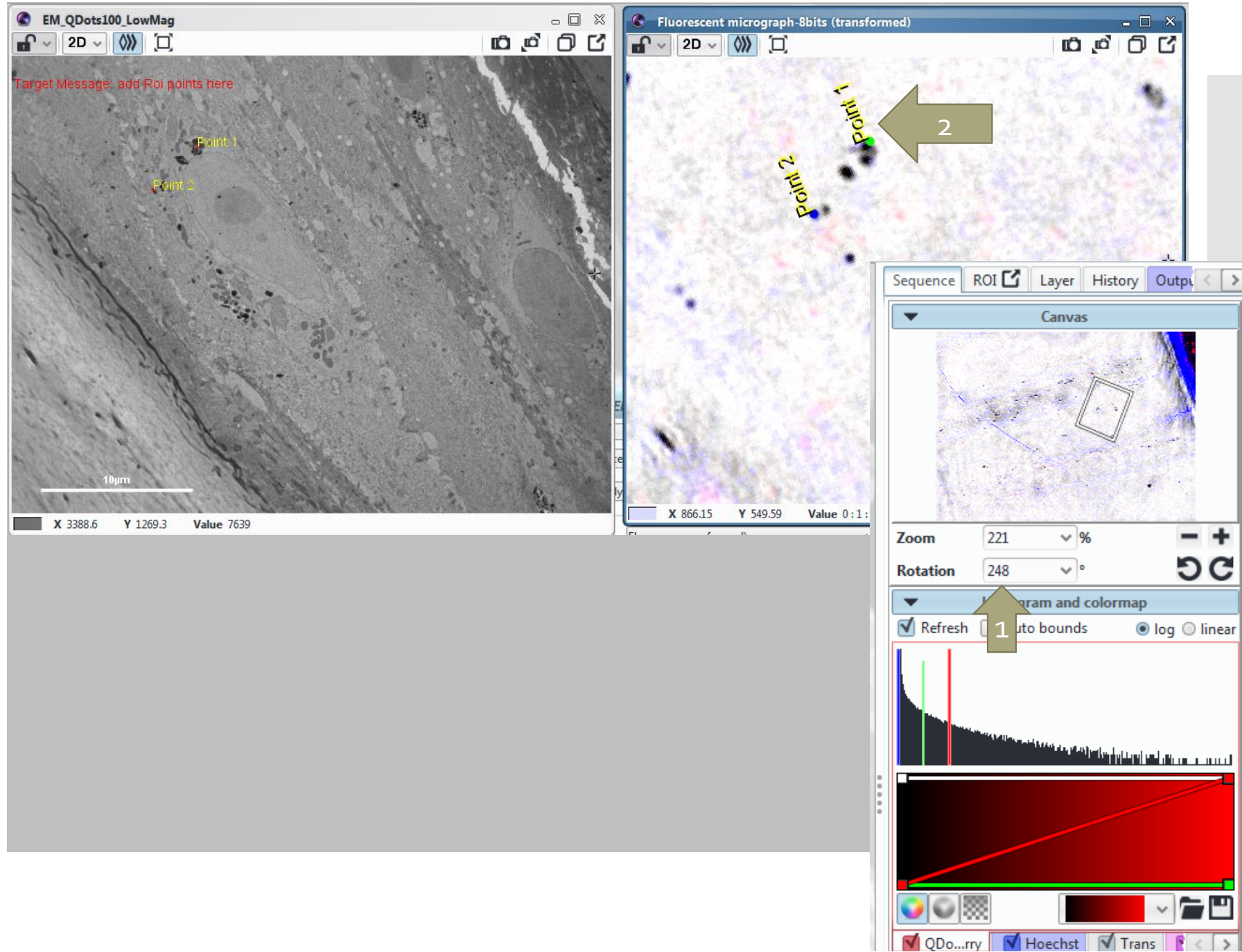
A prior knowledge of at least 3  
homology points is necessary.



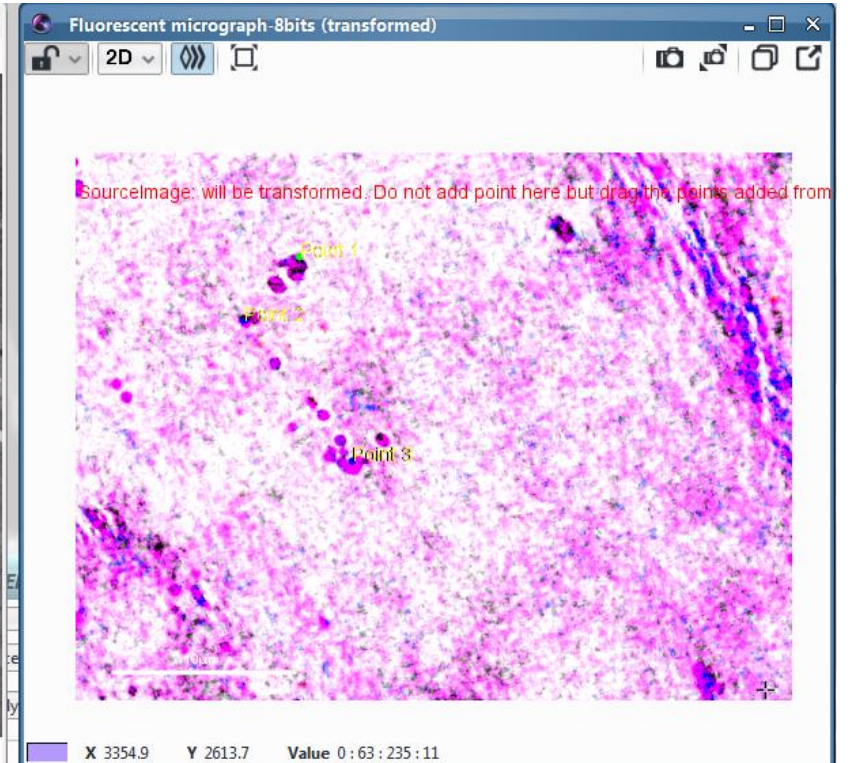
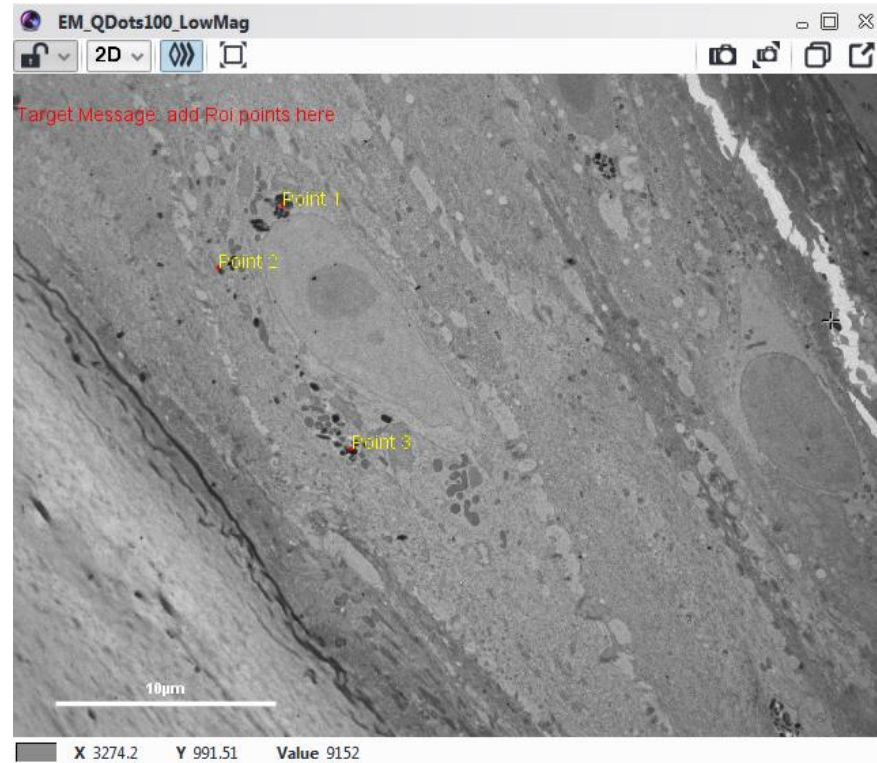
# Right click and shift allows manual « display rotation » (1)

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).

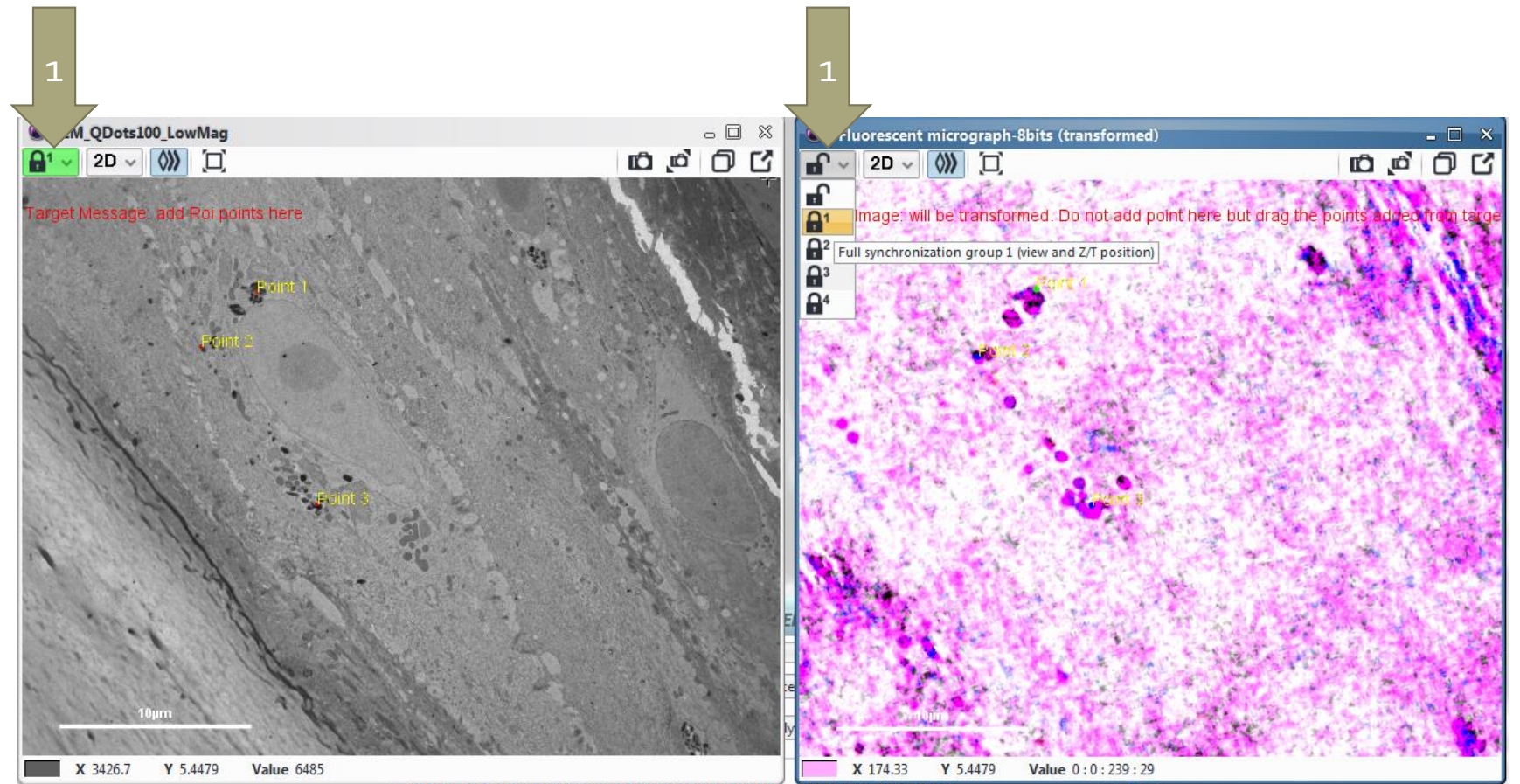


After 3 points, the transformation is applied and displayed



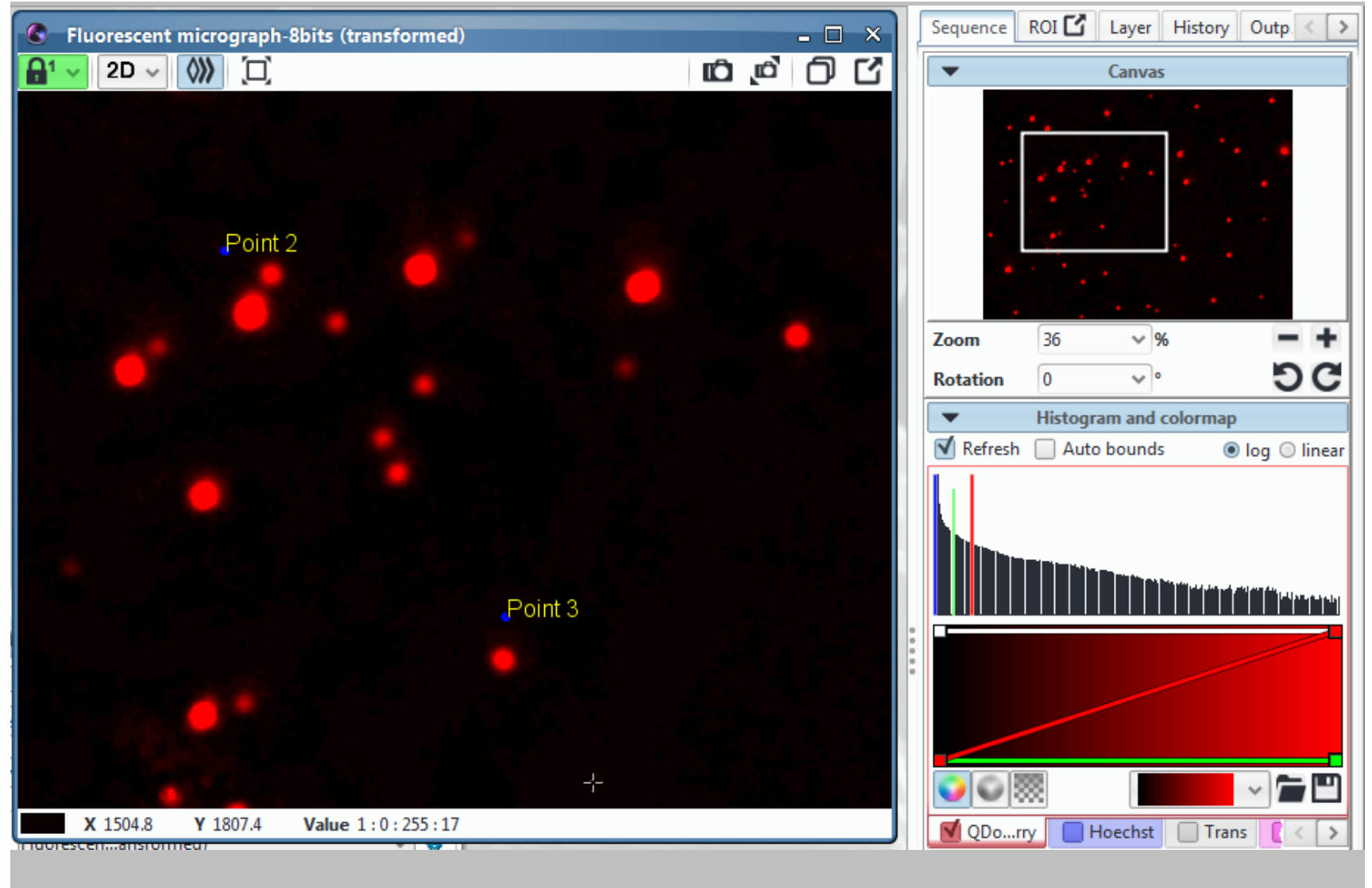
# Lock both images together

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



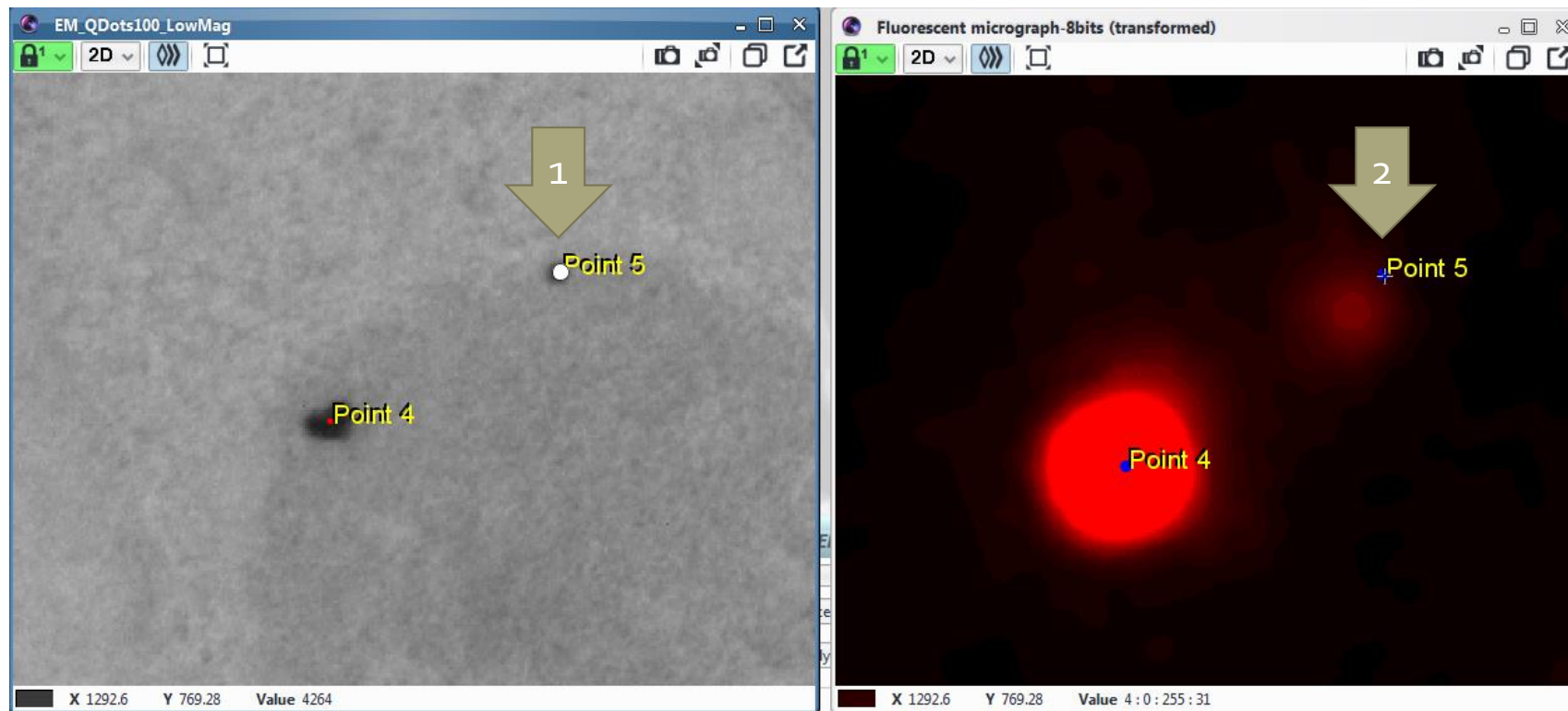
# Select and adjust the contrast from the channel containing the registration landmarks

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



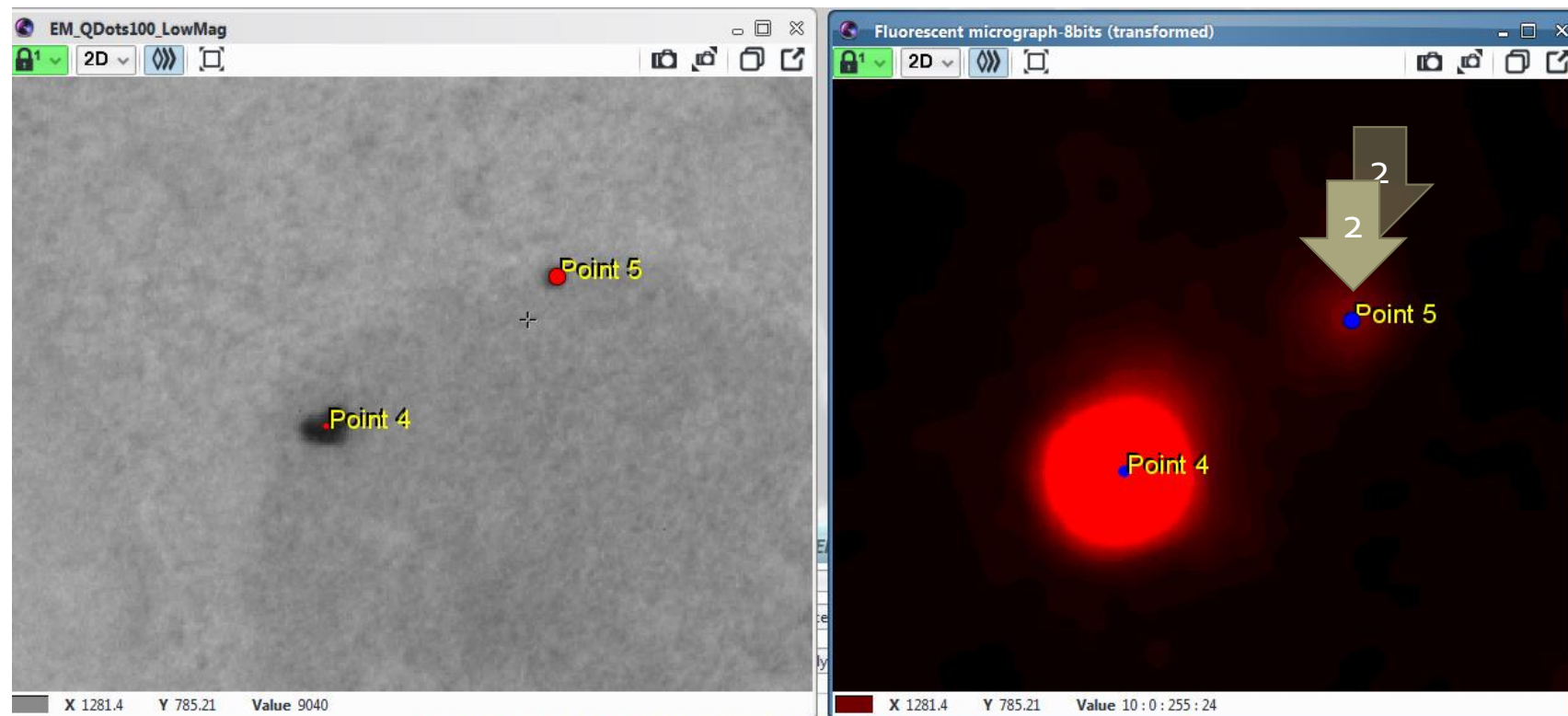
Seed a point on the target image (1).  
Its homologous point is automatically placed on the source image (2)

The localization is calculated according to the previous seed points



# Adjust the seed point on the source image (2)

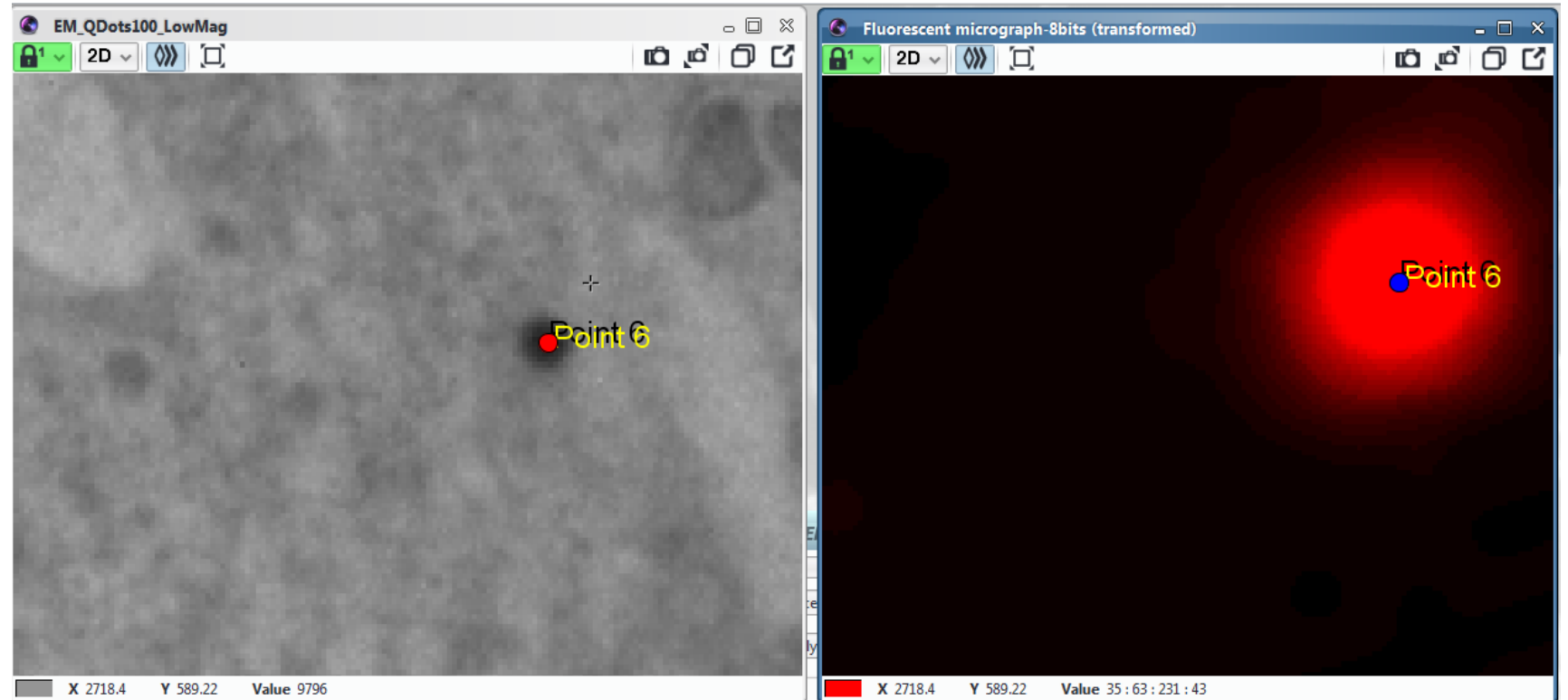
Drag and drop the point at sub-pixel precision



# 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.

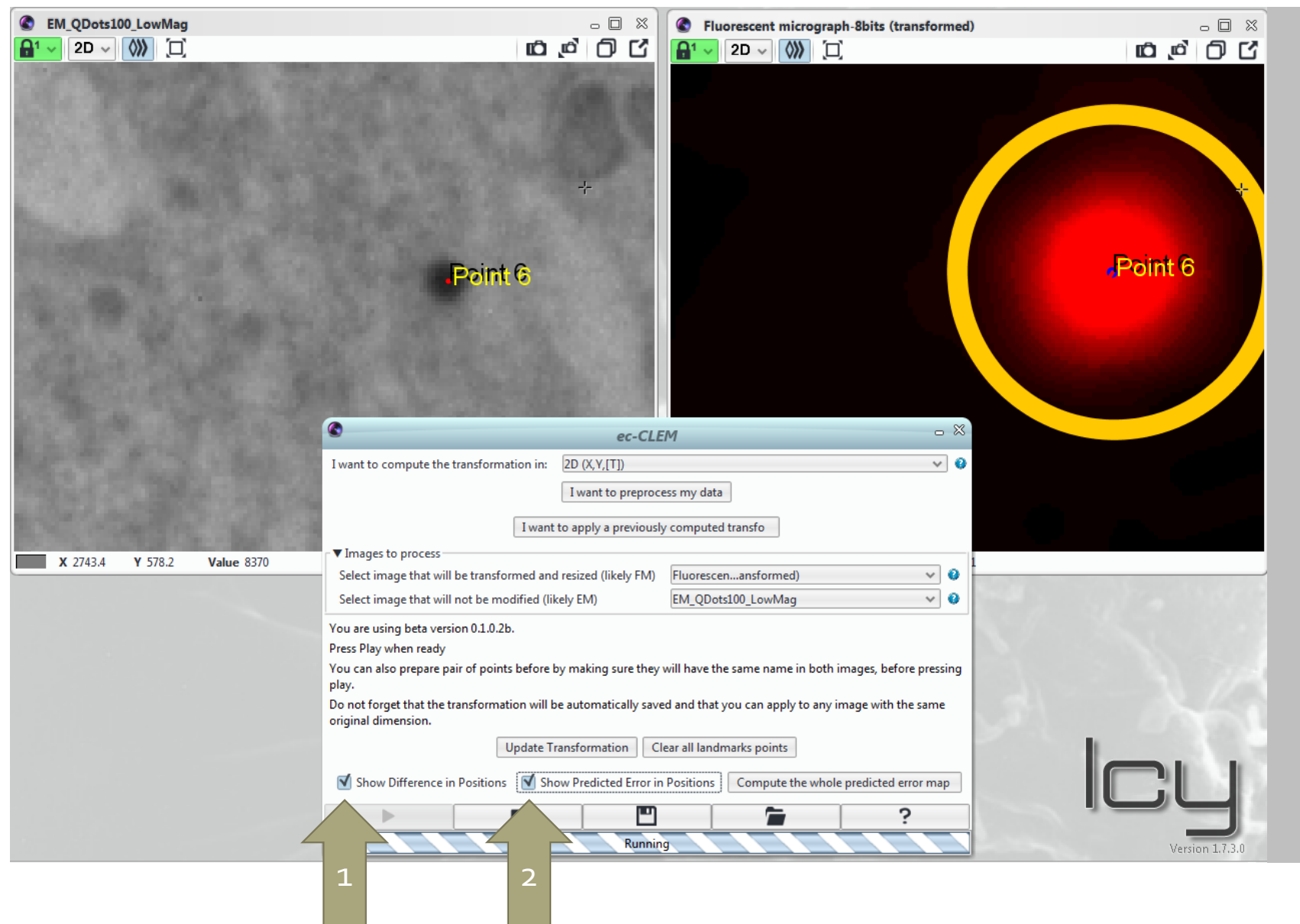




# Display the difference in position (1) and the predicted error in position (2)

The difference in position is displayed as a red vector

The predicted error in position is displayed as an orange circle

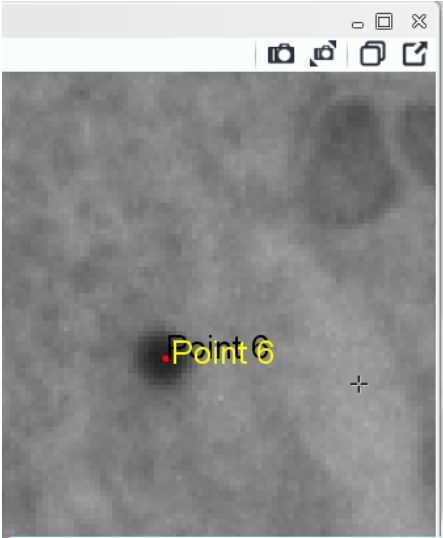


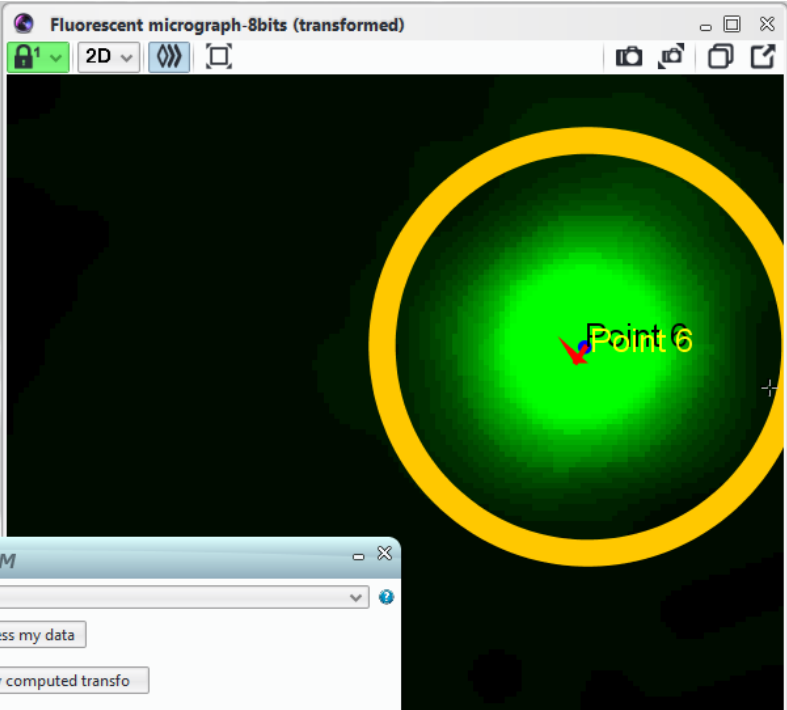
# Change the display channel color to visualize the difference in position vector (1)

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

The screenshot displays the Icy software interface with several windows open:

- Fluorescent micrograph-8bits (transformed)**: Shows a red circular region with a yellow border and a registration landmark labeled "Point 6".
- ec-CLEM**: A dialog box for computing transformations. It shows "2D (X,Y,[T])" as the transformation type. Under "Images to process", "Fluorescen...ansformed" is selected for transformation and "EM\_QDots100\_LowMag" is selected as the reference image. The "Update Transformation" button is visible.
- Canvas**: Shows a zoomed-in view of the registration landmarks.
- Histogram and colormap**: Shows a histogram and a color map. A green arrow labeled "1" points to the "Set Green colormap" button.
- Sequence**: Shows a list of images with columns for Name, Path, Dimension, Channel, Size, Pixel size, and Time interval.
- Footer**: Displays the Icy logo, version 1.7.3.0, and system information: Memory: 11231 MB (Max: 10.2 GB), CPU: 0%.





**ec-CLEM**

I want to compute the transformation in: 2D (X,Y,[T])

▼ 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.

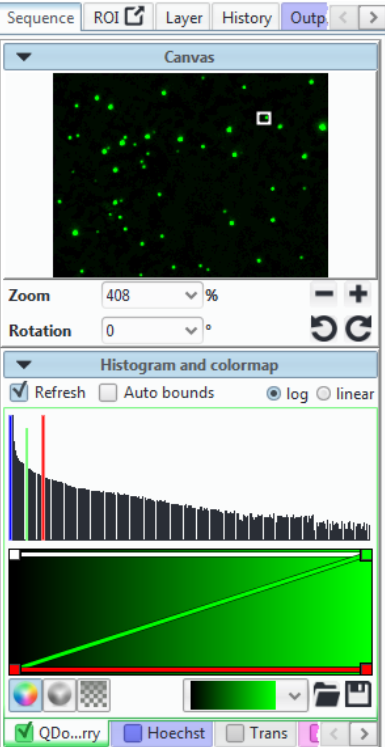
Show Difference in Positions  Show Predicted Error in Positions

▶

Running

**Fluorescent micrograph-8bits (transformed)**

2D



**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		

Memory: 594.4 MB (Max: 10.2 GB)  
CPU: 0%

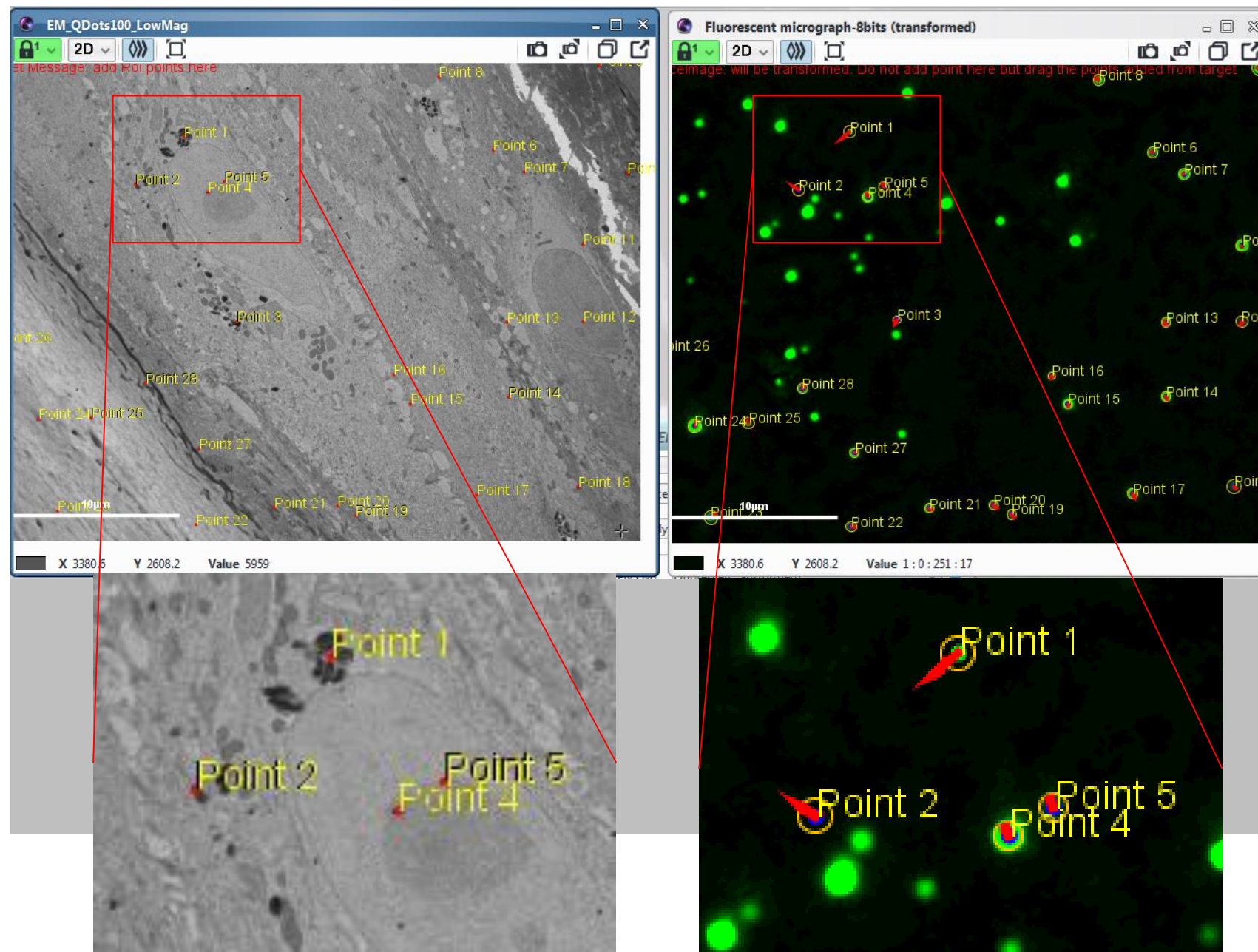
icy

Version 1.7.3.0

# Direct display of the registration error estimation

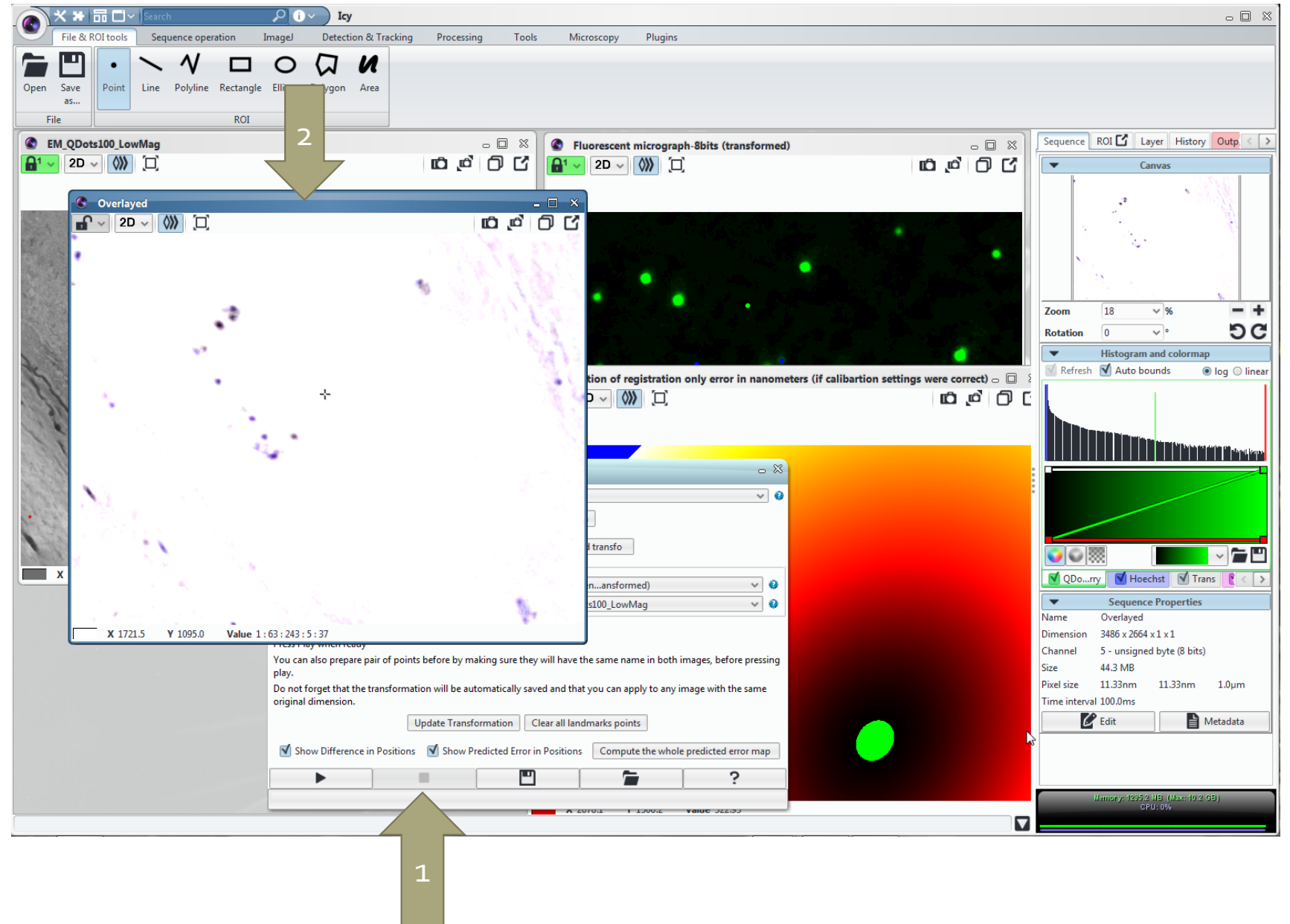
The calculation of registration error is done on the fly. The display can be used as a guide to estimate the registration precision and the final alignment quality (see article).

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



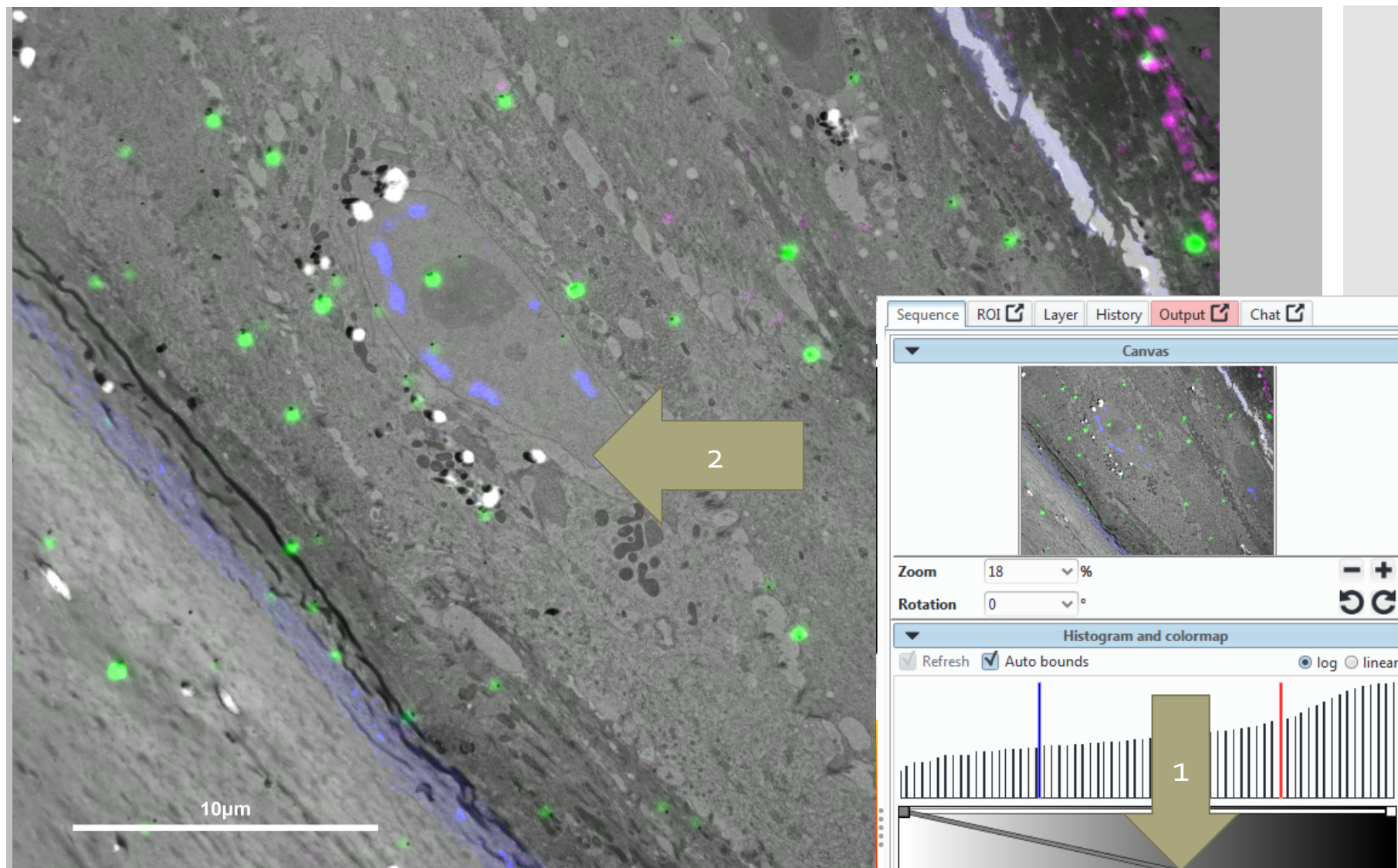
# Automatic overlay production (2)

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.

Name	Date modified	Type	Size
 EM_QDots100_LowMag.tif	10/11/2015 15:37	TIF File	21 351 KB
 EM_QDots100_LowMag.xml	28/01/2016 13:22	XML Document	8 KB
 EM_QDots100-HighMag.tif	31/10/2014 18:02	TIF File	23 986 KB
 EM_QDots100-HighMag.xml	26/01/2016 23:48	XML Document	4 KB
 Fluorescent micrograph-8bits.tif	10/11/2015 15:32	TIF File	1 857 KB
 Fluorescent micrograph-8bits.tif_transfo.xml	28/01/2016 13:50	XML Document	12 KB
 Fluorescent micrograph-8bits.xml	28/01/2016 13:22	XML Document	5 KB

