

Image registration

What is image registration?

- An image of an MS imaged sample taken by a different device is called as a “reference image”
 - e.g. Stained photo, fluorescent photo
- The reference image can be used in analysis
 - ROI settings
 - Similar image extraction
- Before using the image in analysis, it is necessary to align the images
 - This is referred to as “image registration”

Steps

1. Read in the image file that will become a reference image
2. Align the images (carry out image registration)
 1. Rough image registration (linear deformation)
 2. Detailed image registration (non-linear deformation)

1. Read in reference images

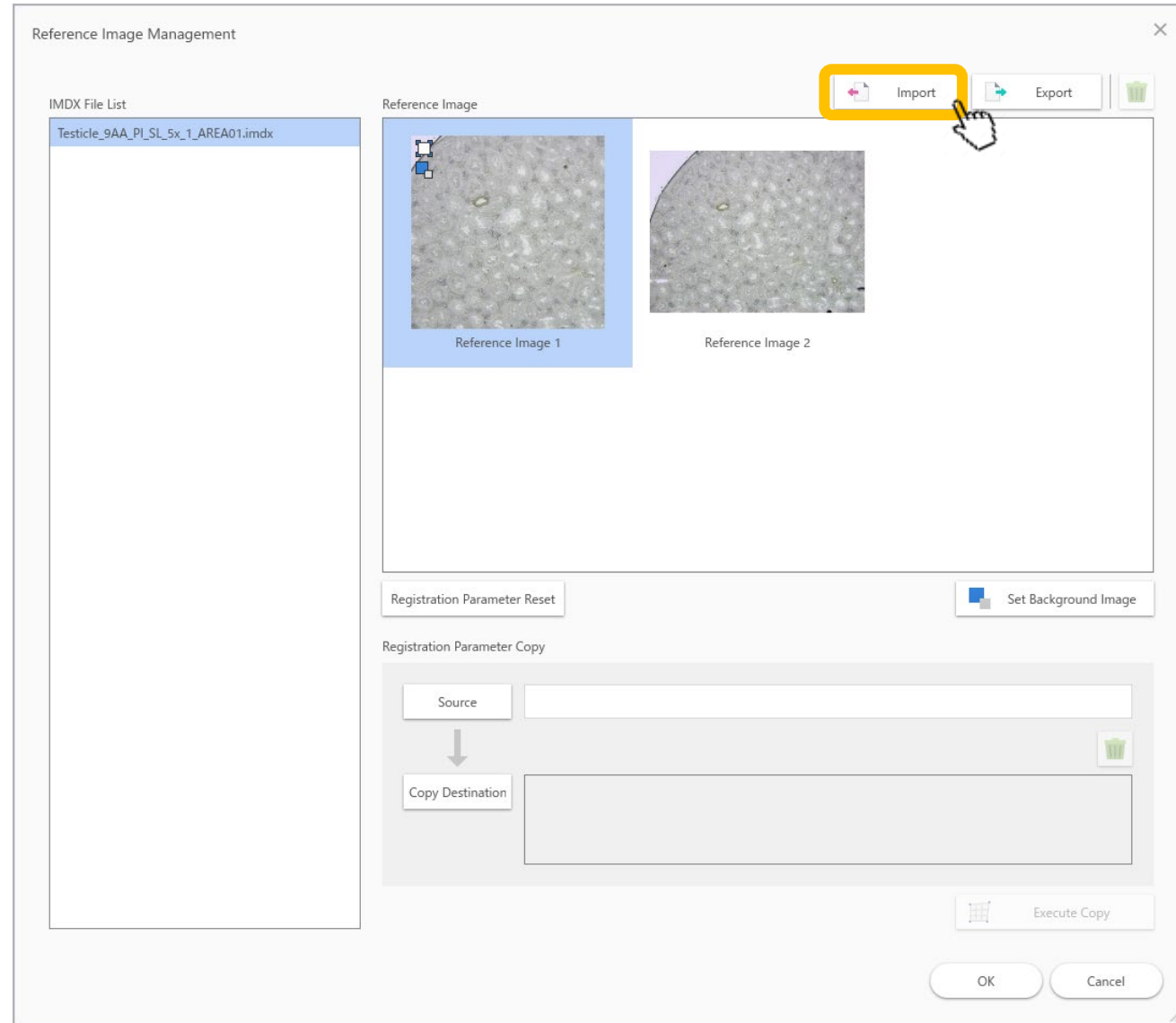
Load the image file to be used as a reference image.
Select from the menu bar, “File” → Reference Image Management .

The screenshot displays the software's main interface. The 'File' menu is open, and 'Reference Image Management...' is highlighted with a yellow box. A hand cursor is pointing at this option. The background shows a mass spectrum graph titled 'Testicle_9AA_PL_SL_5x_1_AREA01.imdx Whole_Ave.' with the x-axis labeled 'm/z' (ranging from 700 to 900) and the y-axis labeled 'Intensity' (ranging from 0E+00 to 2E+06). Several peaks are labeled with their m/z values: 721.48186, 767.49182, 795.52084, 796.52363, 797.52374, 798.52545, 837.53900, and 885.53782. The right panel shows a color-coded image of the testicle with a 250 µm scale bar. The bottom right panel shows the 'MS Image List' with 'Testicle_9AA_PL...' selected.

Analysis Parameters

No.	Name	Value
Normalization Not Calculated		

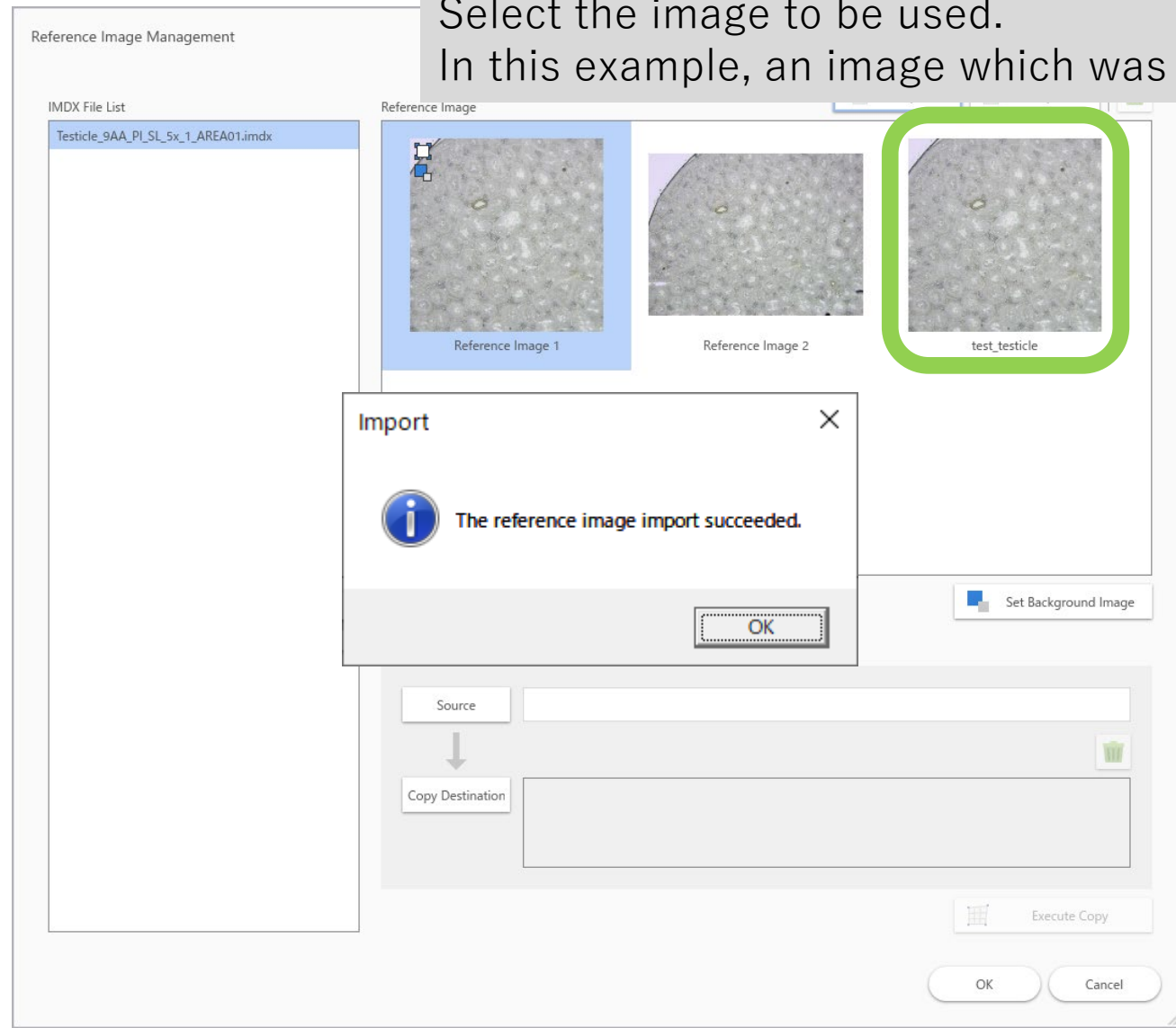
1.1 The “Reference Image Management” screen opens



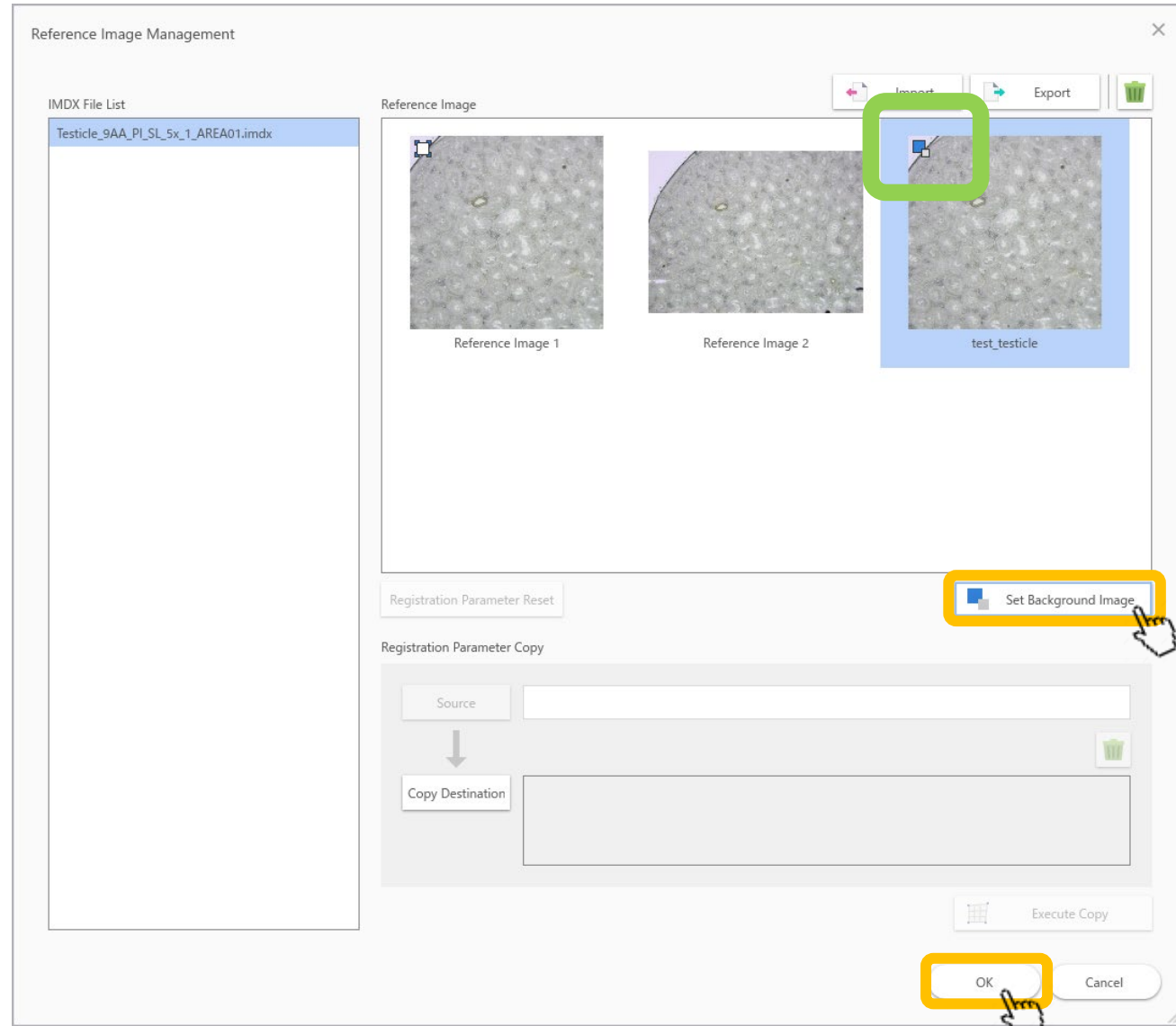
1.2 Import an image to use

Select the image to be used.

In this example, an image which was already read in is imported.



1.3 Set the imported image as a background image



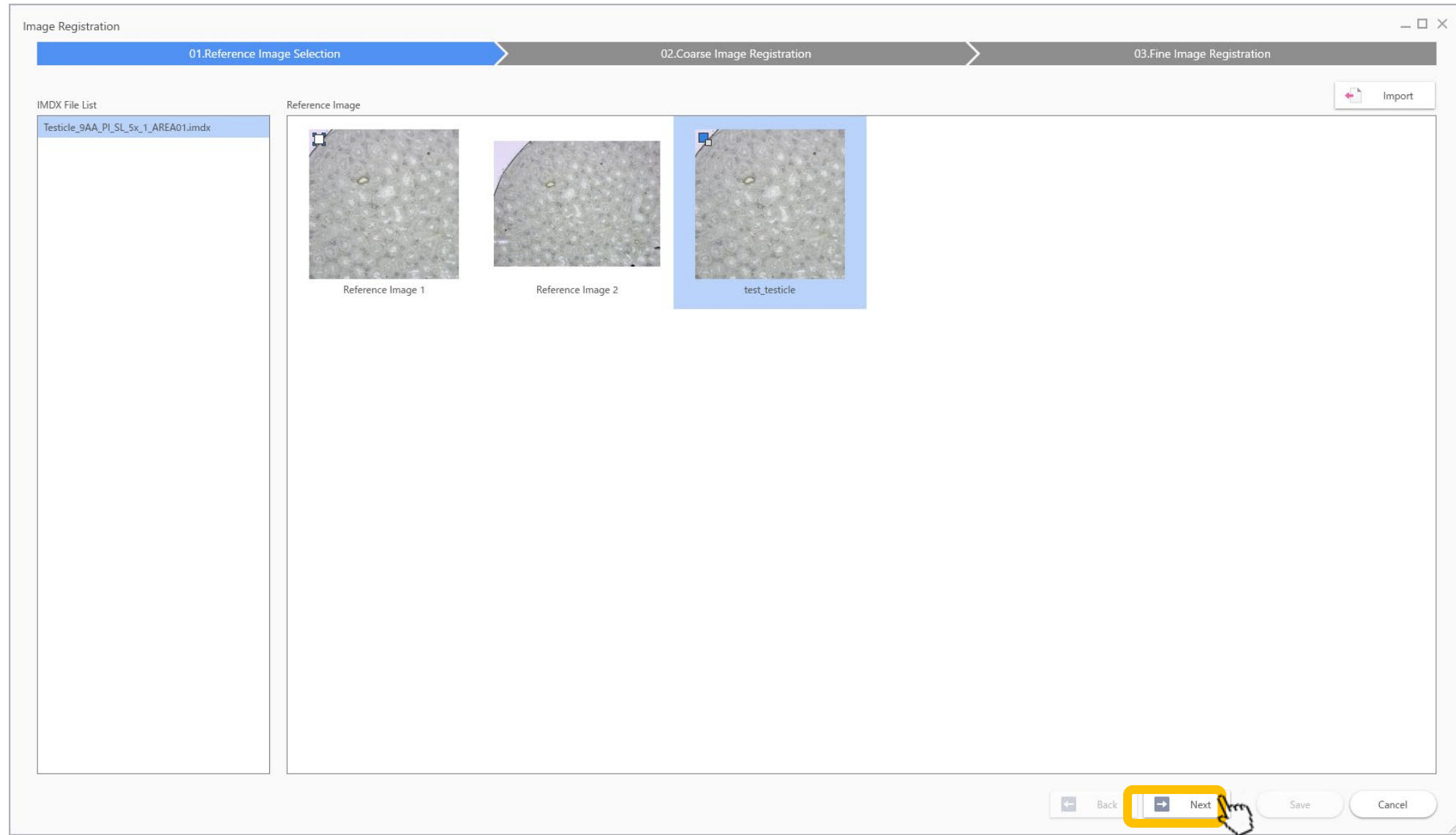
2. Image registration

Select "Image registration"

The screenshot displays the software interface with the following components:

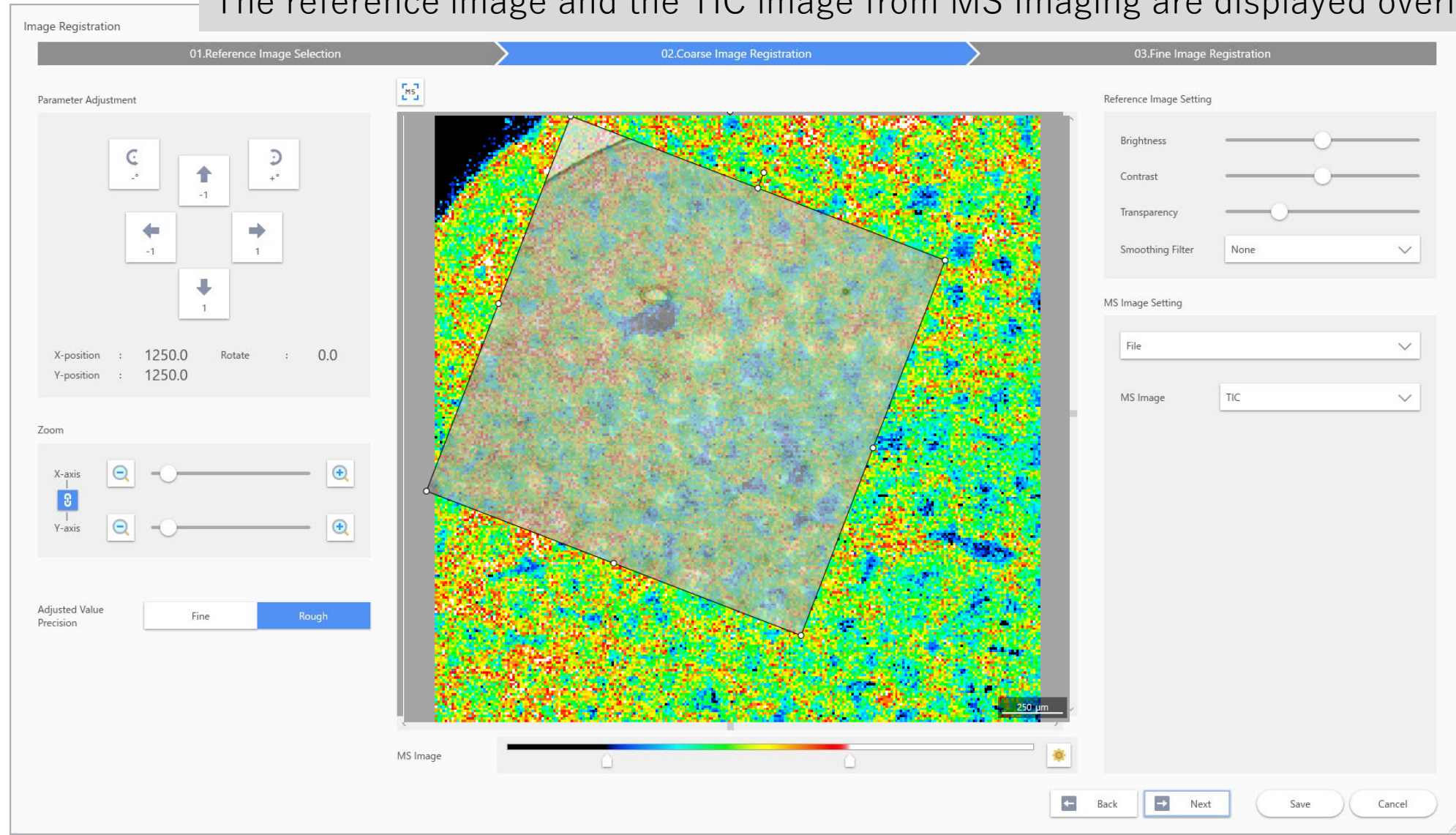
- Left Sidebar:** Contains various tool icons. The 'Image Registration' icon is highlighted with a yellow box and a mouse cursor.
- ROI List:** A table with columns: No., Use, File Name, ROI Name, Data Points. It contains one entry: 1, [checked], Testicle_9AA..., All, 62500.
- Data Matrix Table:** A table with columns: No., Use, Tag, Label, m/z, Formula, Adduct Ion, Matrix, Polarity. It is currently empty.
- Graph:** A mass spectrum plot titled 'Testicle_9AA_Pi_Sl_5x_1_Area01.i.mdx Whole_Ave.' showing Intensity vs. m/z. The x-axis ranges from 700 to 900, and the y-axis ranges from 0E+00 to 2E+06. Several peaks are labeled with their m/z values: 721.48186, 767.49182, 795.52084, 796.52363, 797.52374, 798.52545, 837.53900, and 885.53782.
- MS Image List:** A panel on the right showing a list of images. The first entry is 'Testicle_9AA_Pi_Sl_5x_1_Area01.i.mdx' with a thumbnail image.

2.1 The background image is selected



2.2 The image registration window opens

The reference image and the TIC image from MS Imaging are displayed overlaid.



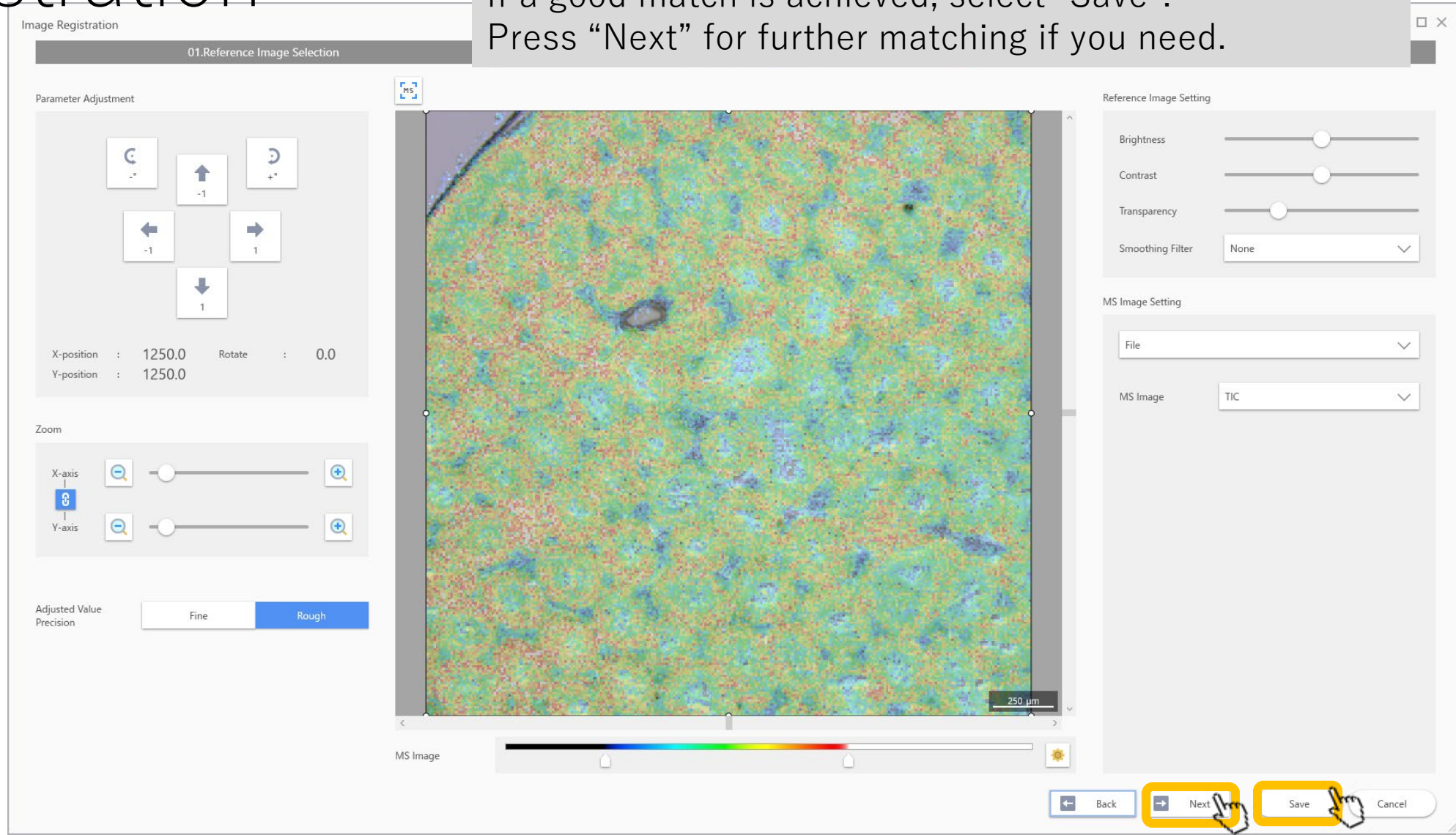
2.3 Coarse image registration

Use the parameter adjustment buttons and the zoom tool on the left-hand side of the window to get a good match between the reference image and the TIC image.

The screenshot displays the coarse image registration software interface. On the left, the 'Parameter Adjustment' panel includes directional buttons (up, down, left, right, and a central '1' button) and a 'Rotate' button. Below these, the 'Zoom' panel features sliders for the X-axis and Y-axis, each with a magnifying glass icon. At the bottom left, the 'Adjusted Value Precision' panel has 'Fine' and 'Rough' tabs, with 'Rough' currently selected. The central area shows a large, noisy 'MS Image' with a semi-transparent reference image overlaid. A yellow circular arrow icon is positioned at the top of the reference image, and three large yellow arrows point towards the corners of the reference image, indicating registration adjustments. A scale bar in the bottom right corner of the image area indicates '250 μm'. On the right, the 'Reference Image Setting' panel contains sliders for 'Brightness', 'Contrast', and 'Transparency', along with a 'Smoothing Filter' dropdown menu set to 'None'. Below this, the 'MS Image Setting' panel includes a 'File' dropdown menu and an 'MS Image' dropdown menu set to 'TIC'. At the bottom right, there are 'Back', 'Next', 'Save', and 'Cancel' buttons.

2.4 If you need, go to next “Fine image registration”

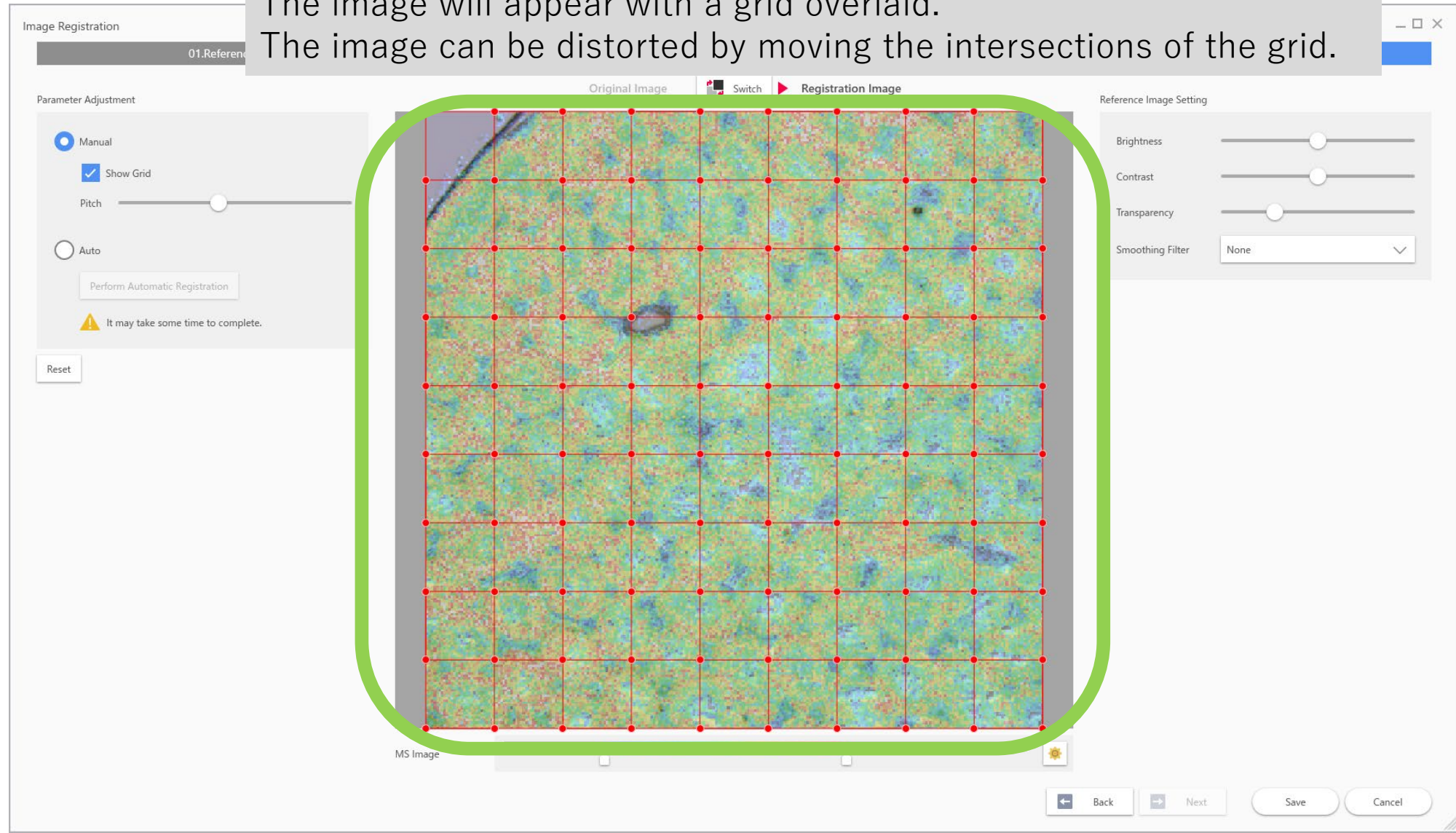
If a good match is achieved, select "Save".
Press “Next” for further matching if you need.



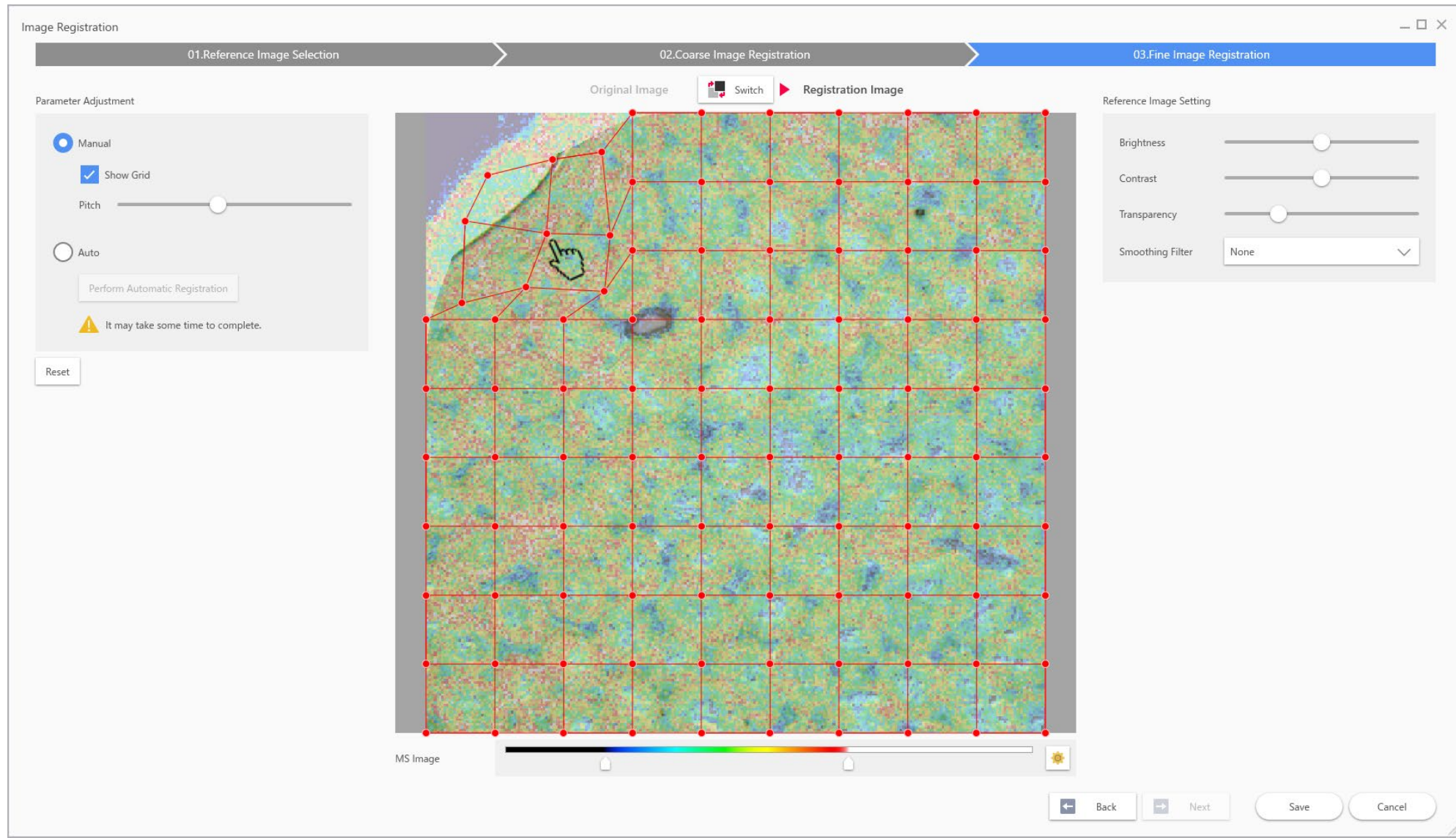
2.5 Fine image registration

The image will appear with a grid overlaid.

The image can be distorted by moving the intersections of the grid.



2.6 Example of a manipulated image



2.7 Alignment is complete, click “save”

