



National Research Council  
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Canada

National Institute for Nanotechnology

# Tomography Alignment Software

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**User Manual**

Version 1.1

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UNIVERSITY  
OF ALBERTA



THE UNIVERSITY OF BRITISH COLUMBIA

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# **1. Introduction**

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Tomography Alignment Software was developed initially in MATLAB by Dr. Misa Hayashida with the National Research Council's National Institute for Nanotechnology at the University of Alberta. The software was translated to Python by students at the University of British Columbia.

## **1.1. Overview**

The following manual describes Tomography Alignment Software, also known as Alignment Software. The software is used to find and track particles in three dimensions as they spin around a holder that is rotated in space at set increments.

In the software, the user will be able to:

1. Load images.
2. Contrast adjustment across all images.
3. Filter, binning, bulk image shift and bulk image rotation.
4. Inter-image alignment via cross-correlation to maintain stability.
5. Particle detection.
6. Automatic particle tracking with manual adjustment.
7. Track optimization and tomography.
8. Save results.

## **1.2. User Groups**

National Research Council of Canada nanotechnology researchers at the University of Alberta.

## **2. Getting Started**

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### **2.1. Licensing & Cautions**

Note the following licences associated with this software:

National Research Council of Canada:

<https://nrc.canada.ca/en/research-development/intellectual-property-licensing>

Python:

<https://docs.python.org/3/license.html>

Matplotlib:

<https://matplotlib.org/stable/users/project/license.html>

NumPy/SciPy:

<https://numpy.org/doc/stable/license.html>

When using this software, ensure to follow the National Research Council of Canada's Intellectual Property policy.

### **2.2. Set-up Considerations**

Alignment Software uses tools that users need to download on their machine. If you want to download and run the software on your machine, you must verify that your machine has permission to download Python and batch files required. If you are unsure if your machine has permission, please contact your IT support service.

### 3. Installation Guide

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Use the following guides to install Alignment Software on your Windows machine.

#### 3.1. Windows Install

1. A compatible version of **Python** (Python >=3.9) must be installed.
  - a. See: <https://www.python.org/downloads/windows/>
2. Extract the full source code to a folder on your machine.
  - a. Go to the GitHub repository, and click the green button that says "Code" then **Download Zip**.
  - b. Extract the zip file. (Unzip the file)
  - c. See:  
<https://github.com/UBCO-COSC-499-Summer-2022/matlab-to-python-application-translation-project2-nrc.git>
3. Navigate into the **Alignment Software folder**.
4. Run the `install.py` file in the folder with Python. This installs the software.
  - a. You can do this by right clicking on the file and click **with Python**.
5. Run the `main.py` file in the folder with Python. This runs the software.
  - a. You can do this by right clicking on the file and click **with Python**.

## 3.2. Mac/Linux Install

1. A compatible version of Python (Python >=3.9) must be installed.
  - a. Using a package manager such as `brew` or `apt` is recommended.
    - i. `brew install python@3.9`
    - ii. `sudo apt install python3.9`
2. Install supporting libraries for Tcl/Tk.
  - a. `brew install python-tk`
  - b. `sudo apt install python3-tk`
3. Extract the full source code to a folder on your machine.
  - a. Go to the GitHub repository, and click the green button that says "Code," then click **Download Zip**.
  - b. Go to the GitHub repository, and click the green button that says "Code" then **Download Zip**.
  - c. Extract the zip file. (Unzip the file)
  - d. See:  
<https://github.com/UBCO-COSC-499-Summer-2022/matlab-to-python-application-translation-project2-nrc.git>
4. Navigate into the **Alignment Software folder**.
5. Run the `install.py` file in the folder with Python. This installs the software.
  - a. You can do this by right clicking on the file and click **with Python**.
6. Run its `main.py` file in the folder with Python. This runs the software.
  - a. You can do this by right clicking on the file and click **with Python**.

### **3.3. Executables**

The user can also run Alignment Software using an executable file (EXE file). Executables are easily run on Windows machines. Simply download the file onto your machine, double-click on the file in your directory, and the software will begin.

## 4. System Organization & Navigation

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**Alignment Software** has one **Main Window** with several pop-up windows for each step in the alignment process. On the main window, there are seven buttons labelled for each step on the left and an area for viewing the images on the right. On start up, the first step, **Open First Image in Set**, is the only button enabled.

**Prerequisite to Step 1:** Images that are to be uploaded to this software should be formatted on your machine as "AAAAXXX.dm3", where XXX is a number which starts at 001. Images must be square and the size of the image must be a power of two to make the software function properly.

### Step 1: Open First Image in Set

For the first step, click on the **Open First Image in Set** button. This button opens a popup window where users will select the first **.DM3** file in their set to upload. The software finds and uploads all associated images in the set. In the **Main Window**, the first image will be displayed. View the entire slideshow of images by dragging the **slider** in the scrollbar in the lower left corner of the **Main Window**. After successful upload, the **Contrast Adjustment** button is enabled.

### Step 2: Contrast Adjustment

For the second step, click on the **Contrast Adjustment** button. This button opens a popup window to perform contrast adjustment on the images. By modifying the contrast, particles can be found in a simpler manner during the Auto Detection step. The histogram displays the current contrast of the image. Choose the **Rejection percentile** for the contrast by typing into the box. Select **Adjust discretely** to apply smoother contrast adjustment across all the images. Click **Apply**. Alternatively, adjust the contrast manually using the min and max sliders under **Manual Adjustment**. The min slider adjusts the lightest areas in the image. The max slider adjusts the darkest areas in the image. As contrast is adjusted, the results are displayed in the **Main Window**. View how contrast was applied to all the images in the set using the scrollbar. The red box drawn on the histogram in the

Contrast Adjustment Window indicates the new contrast. Keep editing the contrast until satisfied. After finishing the contrast adjustment, close the window. The **Transform Image** button (step 3) and **Coarse Alignment** button (step 4) are now enabled on the Main Window.

### **Step 3: Transform Image**

The third step is optional. For the third step, click on the **Transform Image** button. This button opens a popup window where users can manually adjust the parameters of the images for the coarse alignment process. **Offset X** shifts all the images in the X or horizontal direction. **Offset Y** shifts all the images in the Y or vertical direction. **Angle** rotates all the images a set amount in degrees. **Binning** changes the resolution on the samples, with binning down the images allows them to be faster to process. **Sobel Filter** applies a filter to the images to emphasize edges within the image. Users can view changes as they are being made on the images displayed in the **Main Window**. Close the **Transform Window** when finished with transformations.

### **Step 4: Coarse Alignment**

For the fourth step, click on the **Coarse Alignment** button. This activates the coarse alignment and the user will see the images scrolling through the set of images on the **Main Window** as coarse alignment is being performed. Then, a popup window will open to signal that coarse alignment has been completed. Coarse alignment determines the X-Y shift required between each image to keep the particle holder centred and stable in the image, to allow for tracking in future steps. Click the **OK** button on the popup window and view the adjustments that have been made to the images in the main window. The image set will appear more stabilized as the user scrolls through. The **Auto Detection** and **Manual Detection** buttons are now enabled on the Main Window.

### **Step 5: Auto Detection**

For the fifth step, click on the **Auto Detection** button. This button opens the **Auto Detection** popup window. In this step, users can select particles from one image and the software will track the movement of the particle through the rest of the

images in the set. The user will interact between the **Auto Detection Window** and the **Main Window** to perform the auto detection.

Select particles to track:

1. On the **Auto Detection Window**, click on a numbered radio button, next to the numbers 1-13.
    - a. Each of these radio buttons will be associated with a selected particle. The row of values and buttons next to the radio button are associated with that particle. X, Y, IM1, IM2, Mark End, Reset, Track.
  2. Click on the desired particle for tracking on the first image on the **Main Window**.
    - a. Two boxes will be drawn around the selected location. The small box marks the radius of the particle. The large box marks the radius of the search area when tracking occurs.
  3. To redraw the placement of the boxes, simply click again on the desired particle.
  4. Repeat these steps until all desired particles are selected; users can track up to 13 particles.
- Reset a particle's selection by clicking the **Reset Button** next to the associated particle.
  - Select which particles to track by selecting/deselecting the **Track** selection box next to each associated particle.
  - Select the colour of the particles that are being tracked using the **Particle Colour** radio buttons.

Track Particles:

- When finished selecting the particles, click the **Track all selected** button to perform particle tracking. The user will see the images strolling through the set of images on the Main Window as particle tracking is being performed.
- When tracking is finished, the user can view where the software tracked each particle on each image by scrolling through the images on the Main Window.
- In the Auto Detection window, the values associated with each particle will be updated. The X and Y values display the value in pixels of the location of the

particles on the current image in pixels. The IM1 and IM2 values show the first and last image that the particle is tracked on.

- Note: If the image has both black and white particles: select all the black particles, indicate the colour using the particle colour radio button, then track, then select all the white particles, indicate the colour using the particle colour radio button, then track.

Edit for improved auto tracking:

- If there are errors in the tracking, there are a few actions that users can take to aid Automatic Detection before having to perform Manual Detection.
- The search areas can be edited in the **Shift Search Areas** frame. The search area is the area that the software uses to track the particles. Edit the **search area** box **width** and **height** to change the size of the search area box around the particles. Edit the **marker radius** to change the size of the box drawn around the particles to match the particles' sizes. Click the **Track all selected** button to perform the tracking again.
- To end the particle tracking of a certain particle on a particular slide in the set of images, select the radio button for that particle, scroll to the desired ending slide, and click the **Mark End** button to stop the tracking at this slide.
  - To start tracking again at another slide after the Mark End slide, scroll to the desired slide and select the particle on this slide. Click the **Track all selected** button to track again. The particle will be tracked until the 'Mark End' slide, then resume tracking where the second selected slide is.
  - The IM1 and IM2 values on the Automatic Detection window will be updated to display the slides affected by the latest tracking performed on the particles.
  - Scroll through the images to view the tracking on the particles.
- Note: tracking can also be edited during the Manual Detection step.

Interpolation:

- The user can also use the **Mark End** button in combination with the **Interpolation** button to re-track a poorly tracked particle.
- Mark the end of the tracking where the particle was tracked incorrectly on the slides using the **Mark End** button.

- Then, scroll to the next slide that the particle can be clearly selected and indicate the particle location here. Click the **Track all Selected** button to track the path of the particle starting from this location.
- When scrolling through the slides, the particle will be tracked up to the Mark End slide, and then resume tracking where the newly selected slide was. There will be a gap between the Mark End slide and the newly selected slide, where the particle is not tracked. Use Interpolation to fill the gap.
- Click **Interpolation**. Linear Interpolation will be performed on the particle location to estimate where the particle's location is in the gap between slides and re-track the particle. The particle will now be fully tracked.

## **Step 6: Manual Detection**

For the sixth step, click on the **Manual Detection** button. This button opens the **Manual Detection** popup window. Automatic particle tracking is normally an accurate process, but in the rare cases of particle overlap or occlusion, it is possible that particles are tracked incorrectly. The manual tracking adjustment window allows users to inspect the tracking quality and adjust tracked particle locations in the event of error. In order to complete optimization on all desired particles, ensure that tracking is complete for all desired particles.

Check each particle, chosen via the numbered radio buttons, by:

- Looking at each particle's **Y Position** and **X Position** plots, which should be smooth within detection error, and should not have any discontinuities.
- Examining the particles' tracked locations in the images as the images are scrolled through using the slider tool below the plots.
- Look at the indicators to see if the tracking is complete or not.

Tracking a new particle manually:

- Select the particle location on all of the images.
- Select a **particle number radio button**.
- Click on the proper location of the particle on the image in the **Main Window**.
- Use the **Location Adjustment Arrow buttons** to move the selected particle around without having to click on the image. The buttons will move the

particle in the direction of the arrow on the button by the amount of pixels in the Pixel Adjustment spinbox.

- Adjust the amount of pixels the arrow buttons will move the particle by editing the number in the **Pixel Adjustment spinbox** in the middle of the arrow buttons.
- Repeat until the particle location is selected on all of the images.

Tracking a particle using interpolation:

- Select a **particle number radio button**.
- Select the location of the particle on the image.
- Do this once on every few slides, every other 5-15 slides from the beginning to end.
- Click **interpolation**. The program will fill in the gaps and track the particle on every slide.

If a track found the wrong particle, it needs to be adjusted by the following means:

- Select the appropriate particle number via the radio buttons.
- Use the **Location Adjustment Arrow buttons** to move the selected particle around OR Click the proper location of the particle on the image.
- Repeat until the track looks correct.

The **Optimization** button is now enabled on the Main Window.

## Step 7: Optimization

For the seventh step, click on the **Optimization** button. This button opens the **Optimization** popup window, with the original images displayed. The process of optimization starts with the particles tracked through the image set after a translation has been applied, and computes a set of rotation and translation values to stabilize the entire image set even further.

Optimization Settings: Indicate how the images were acquired. **Either:**

- Check the **CSV File** radio button and having a CSV file with tilt angles in it in the same folder as the images
  - Uploads a CSV file with an array of tilt angles that do not have to have constant step values.
  - Tilt angles should be written in 'tilt\_angle.csv', placed in the same

folder as the tilt series of images.

- Select the **Constant Step** radio button and enter the **Start Angle** and **Angle Step** values properly.
  - **Constant Step** assumes a constant step change between tilt angles, entered in the entry boxes beside the radio button.
  - The **Start Angle** value is the first image's angle.
  - The **Angle Step** value is the incremental change in angle between images.

Now, select the option desired for optimization.

- The options include: fixed rotation and magnification, one rotation and fixed magnification, group magnifications and one rotation, group magnifications and group rotations, adjust azimuth angle amount, and group tilt angles.

Click the *Optimization* button to perform optimization, and watch as the images are displayed.

- The accuracy of the optimization is displayed as the images start to appear on screen.
- Once the *Alignment* button turns back to grey, the process is done and the software is finished with processing of images

### Saving results:

Results are saved on the user's machine throughout the alignment process. Find these results stored in a folder named **image\_output** where the initial PRZ images are stored on the device. The results that are saved include numbered **TIFF** image files for each of the images in the set, a CSV file named **marker\_data.csv**, and a CSV file named **transform.csv**.

The TIFF images are the images adjusted according to the coarse alignment.

The marker\_data.csv file saves information about where the particles are for tracking.

The transform.csv file stores information about contrast adjustment, transformations, coarse alignment, and optimization for each image in the set. The contrast\_min column stores the blackest values for each image. The contrast\_max

column stores the lightest values for that image. The transform\_x column stores the x offset values on a scale from -1 to 1, with -1 the image being completely off screen to the left and 1 the image being completely off screen to the right. The scale column stores the value for the scaling of the image during transformation. 1 is a normal 100% zoom, 2 is 200%, 0.5 is 50%, etc. The coarse\_x column stores the x changes during coarse alignment measured in pixels. The coarse\_y column stores the y changes during coarse alignment measured in pixels.

The **Restore Previous Session** button in the lower left corner on the Main Window can use the saved results to reopen the progress of a previous alignment session. To do this, open the first image in the set, and then click **Restore Previous Session**. The progress that was made the last time the software was used will be restored and the user can continue.

### **Tips for using Alignment Software:**

If a track found the wrong particle, it needs to be adjusted by the following means:

- Edit the box around the particle
- Mark End, track the rest, Interpolation
- Manual tracking

If there are particles that are both black and white and tracking is not working:

- Select the black particles, then track, then select the white particles, then track
- Do not track black and white particles at the same time

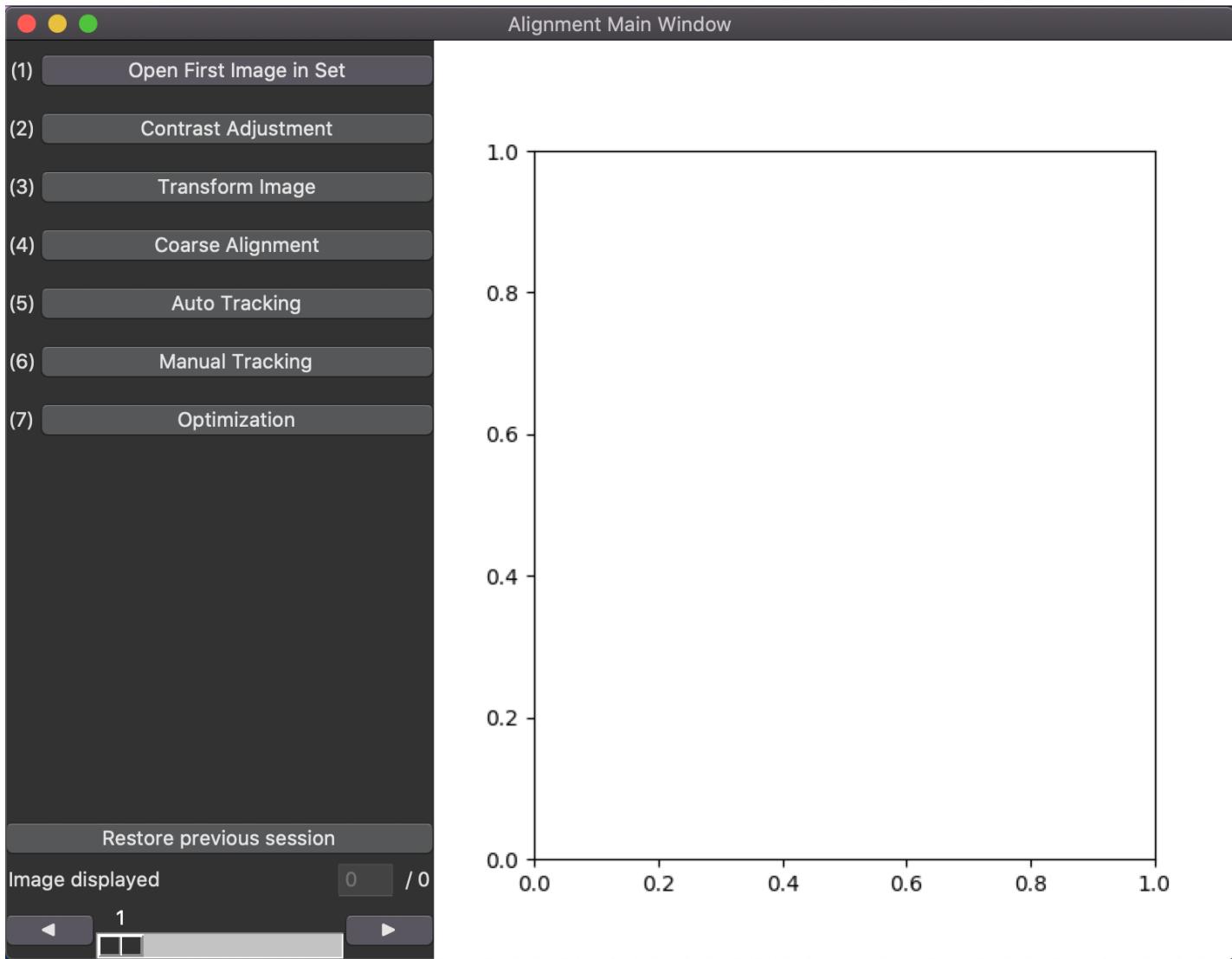
How to make the alignment process quicker:

- *Binning* down samples the images to make them faster to process.

## 5. Step-by-Step Guide

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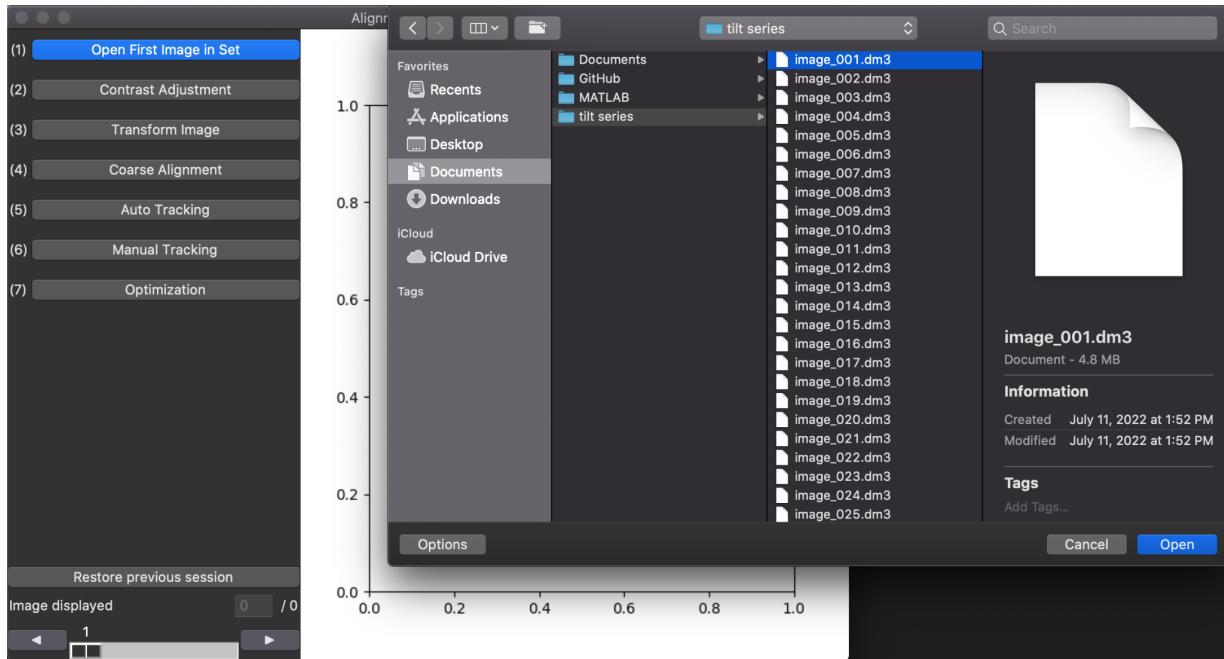
The Alignment Software main window:



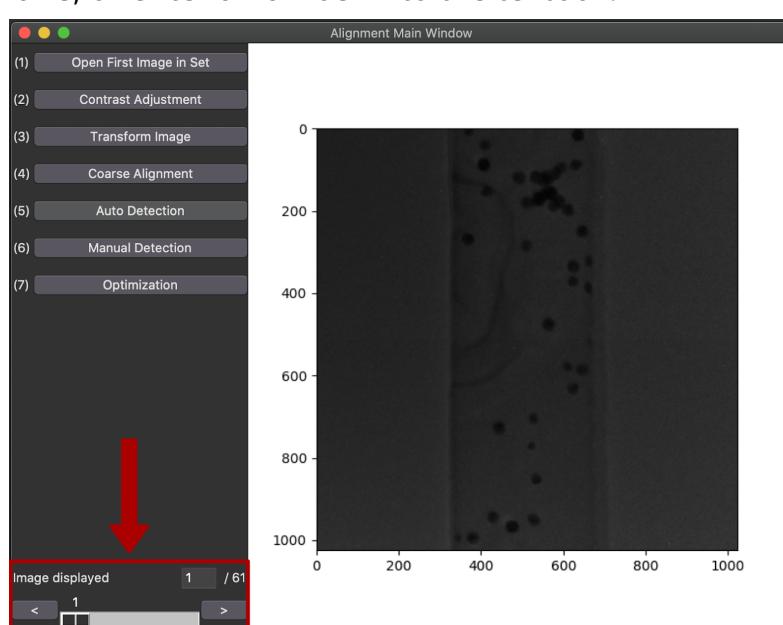
This is the Alignment Software Main Window, on the left are the buttons for the seven steps in the alignment process. On the right is where the images will be displayed. In the lower left corner, there is an image scrollbar where the user will be able to scroll through each image in the set, once uploaded. There is also the restore previous sessions button.

## Step 1: Open First Image in Set

1. Click on the Open First Image in Set button in the Main Window.

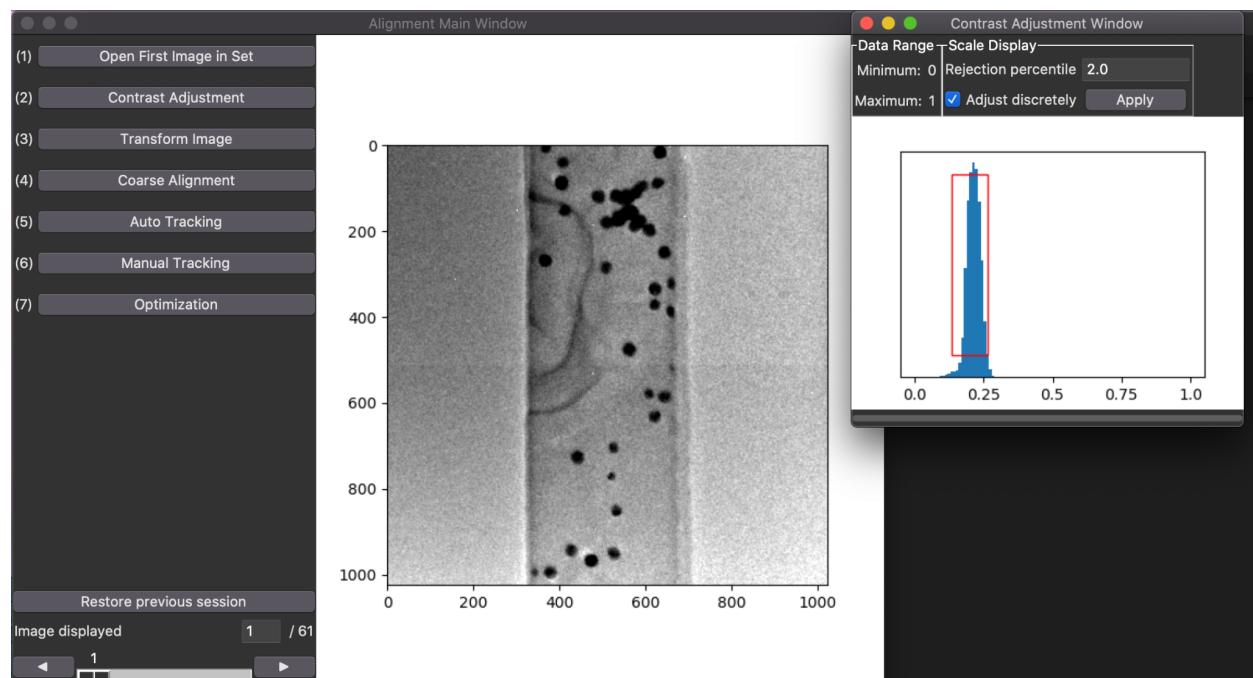
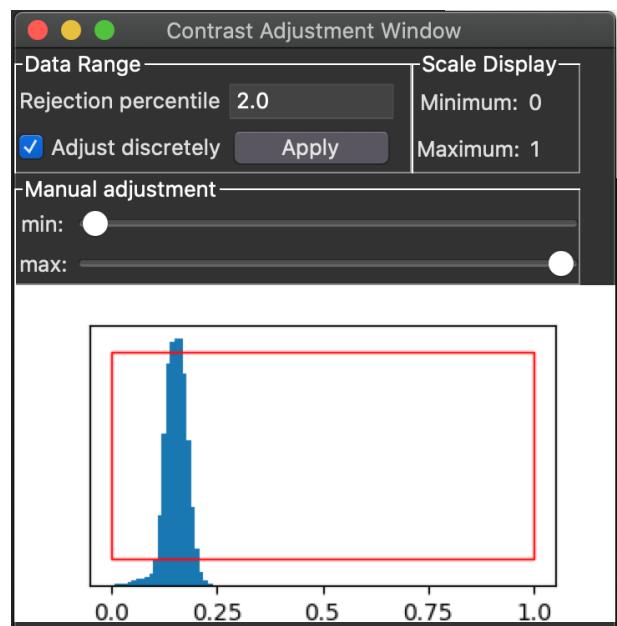


2. In the popup window, navigate to where the first image is stored on the device.
3. Click **Open**. The whole set of images will appear in the Main Window.
  - View each image using the image scrollbar: drag the slider, click the arrows, or enter a number into the textbox.



## Step 2: Contrast Adjustment

1. Click on the **Contrast Adjustment** button on the Main Window.
2. Adjust contrast settings in the Contrast Adjustment Window:
  - The graph displays the current contrast of the image.
  - Choose the **Rejection percentile** by typing into the box.
  - Select **Adjust discretely** to apply smoother contrast adjustment across all the images.
  - Manually adjust using the min and max sliders.
3. Click **Apply**.
  - See changes made to images in the Main Window. Scroll through the images to see changes using the image scrollbar.
  - The red box drawn on the graph indicates the area where the new contrast is.
4. Keep adjusting until satisfied.
5. Close Contrast Adjustment Window.



### Step 3: Transform Image

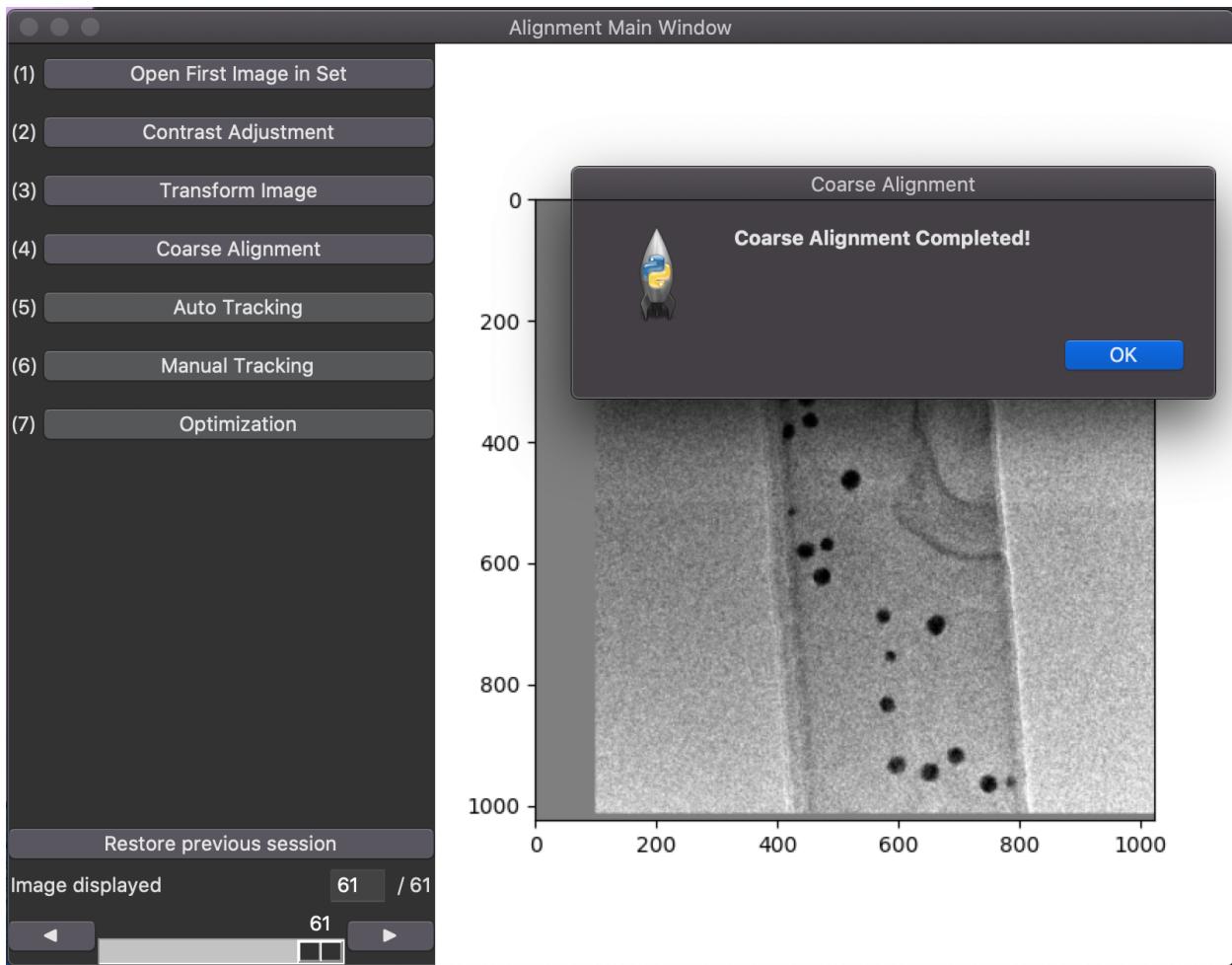
1. Click on the **Transform Image** button on the Main Window.
2. Transform the image in the **Image Transformation Window**:
  - **Offset X** - Drag the slider, enter a number into the textbox, and/or click on the up and down arrows to adjust. Positive numbers transform the image upwards, negative numbers transform the image downwards.
  - **Offset Y** - Drag the slider, enter a number into the textbox, and/or click on the up and down arrows to adjust. Positive numbers transform the image upwards, negative numbers transform the image downwards.
  - **Scale** - Drag the slider, enter a number into the textbox, and/or click on the up and down arrows to adjust zoom on image. Positive numbers make the image larger, negative numbers make the image smaller.
  - **Angle** - Drag the slider, enter a number into the textbox, and/or click on the up and down arrows to adjust degrees. This rotates the image.
  - **Binning** - Select a radio button to change binning.
  - **Sobel Filter** - Apply sobel filter by selecting the checkbox.



3. As adjustments are made, view them in the Main Window.
4. When finished with adjustments, close the Image Transformation Window.

## Step 4: Coarse Alignment

1. Click on the **Coarse Alignment** button on the Main Window.
  - a. The user will see the images scrolling through the set of images on the Main Window as coarse alignment is being performed.
2. A popup window opens to signal that coarse alignment has been completed



3. Close the popup window by clicking **OK** and view the adjustments made to the images using the image scrollbar in the Main Window.

## Step 5: Auto Detection

1. Click on the **Coarse Alignment** button on the Main Window.

**Automatic Detection Window**

1.	X	Y	2.	IM1	IM2	3.	4.	5.
<input checked="" type="radio"/> 1	-	-		1	61	<b>Mark End</b>	<b>Reset</b>	<input type="checkbox"/> Track
<input type="radio"/> 2	-	-		1	61	<b>Mark End</b>	<b>Reset</b>	<input type="checkbox"/> Track
<input type="radio"/> 3	-	-		1	61	<b>Mark End</b>	<b>Reset</b>	<input type="checkbox"/> Track
<input type="radio"/> 4	-	-		1	61	<b>Mark End</b>	<b>Reset</b>	<input type="checkbox"/> Track
<input type="radio"/> 5	-	-		1	61	<b>Mark End</b>	<b>Reset</b>	<input type="checkbox"/> Track
<input type="radio"/> 6	-	-		1	61	<b>Mark End</b>	<b>Reset</b>	<input type="checkbox"/> Track
<input type="radio"/> 7	-	-		1	61	<b>Mark End</b>	<b>Reset</b>	<input type="checkbox"/> Track
<input type="radio"/> 8	-	-		1	61	<b>Mark End</b>	<b>Reset</b>	<input type="checkbox"/> Track
<input type="radio"/> 9	-	-		1	61	<b>Mark End</b>	<b>Reset</b>	<input type="checkbox"/> Track
<input type="radio"/> 10	-	-		1	61	<b>Mark End</b>	<b>Reset</b>	<input type="checkbox"/> Track
<input type="radio"/> 11	-	-		1	61	<b>Mark End</b>	<b>Reset</b>	<input type="checkbox"/> Track
<input type="radio"/> 12	-	-		1	61	<b>Mark End</b>	<b>Reset</b>	<input type="checkbox"/> Track
<input type="radio"/> 13	-	-		1	61	<b>Mark End</b>	<b>Reset</b>	<input type="checkbox"/> Track

**8. Track all selected**

**9. Interpolate selected**

Particle Color	Shift Search Areas
<input checked="" type="radio"/> Black <input type="radio"/> White	Marker radius (pixel) <input type="text" value="20"/>
6.	Search width (pixel) <input type="text" value="80"/>
	Search height (pixel) <input type="text" value="80"/>

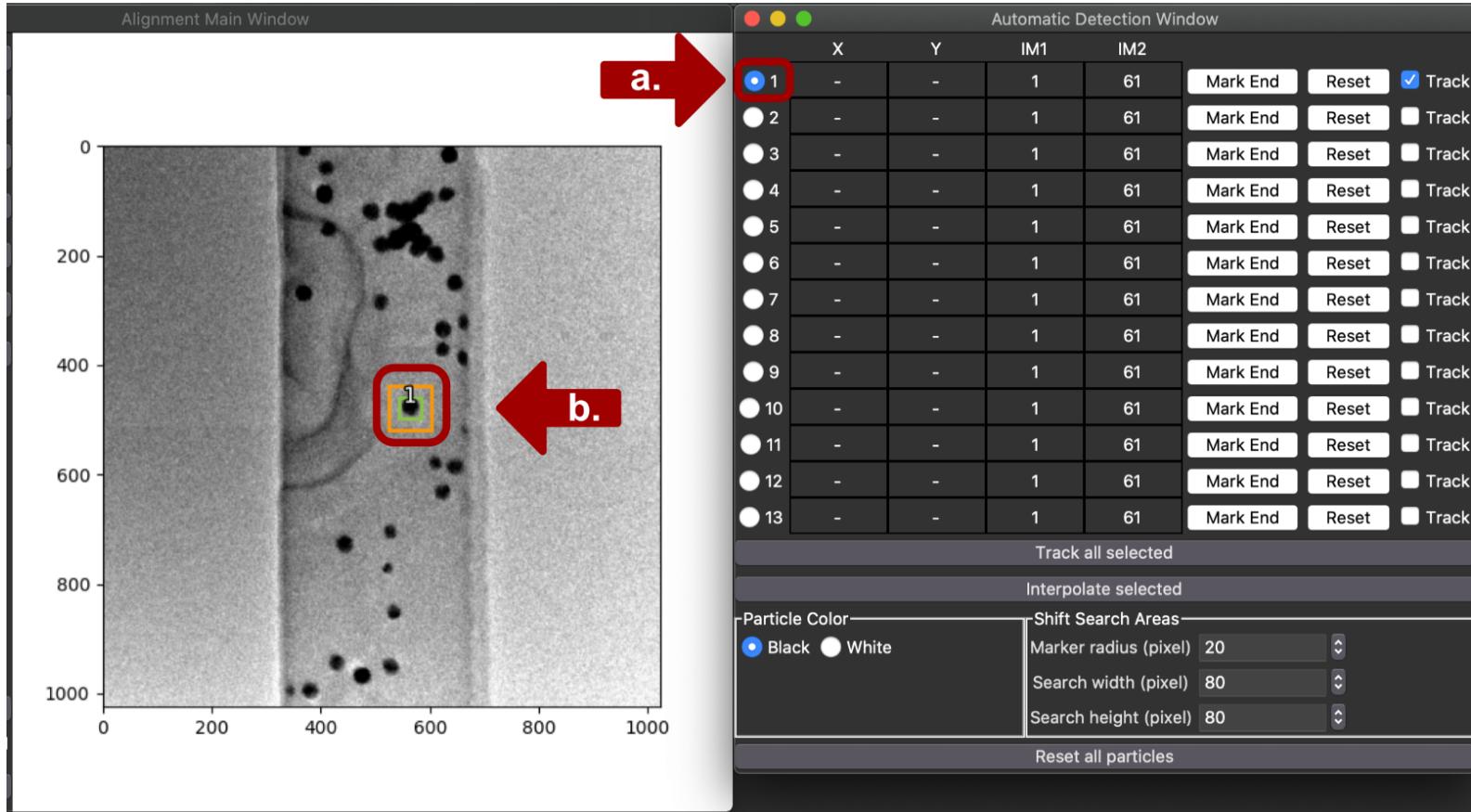
**7.**

**10. Reset all particles**

#	<u>Feature</u>	<u>Description</u>
1	Particle Numbers	<ul style="list-style-type: none"> <li>• This set of radio buttons represents individual particles that can be found and tracked, up to 13.</li> <li>• Selecting a radio button allows the user to adjust the search location of the particle.</li> </ul>
2	Particle Locations	<ul style="list-style-type: none"> <li>• These display boxes show the locations for each particle on the currently displayed image.</li> <li>• If the displays show all zeros, then that particle has not been tracked yet.</li> <li>• The range of images that has been tracked is shown in <i>/M1</i> and <i>/M2</i>.</li> </ul>
3	Mark End	<ul style="list-style-type: none"> <li>• This checkbox ends the tracking of a certain particle at a selected image, in case the particle is occluded partway through the set of images.</li> <li>• This is an optional setting.</li> </ul>
4	Reset button	<ul style="list-style-type: none"> <li>• This button resets the selected particle search area to default values.</li> </ul>
5	Track Checkbox	<ul style="list-style-type: none"> <li>• This checkbox specifies that the particle is to be tracked.</li> <li>• If unchecked, the particle on that row is not to be tracked.</li> </ul>
6	Particle Colour Selection	<ul style="list-style-type: none"> <li>• This area specifies what colour the particles are relative to the background.</li> <li>• Choose <i>Black</i> or <i>White</i> to match how the image appears.</li> </ul>
7	Search Area Width and Height Parameters	<ul style="list-style-type: none"> <li>• These numeric entry boxes specify the width and height of the search areas, as shown in the image.</li> </ul>
8	Track all button	<ul style="list-style-type: none"> <li>• This button resets the selected particle search area to default values.</li> </ul>
9	Interpolation	<ul style="list-style-type: none"> <li>• Track the gap if tracking was not fully successful</li> </ul>
10	Reset All button	<ul style="list-style-type: none"> <li>• This button resets all search areas for particles to default values.</li> </ul>

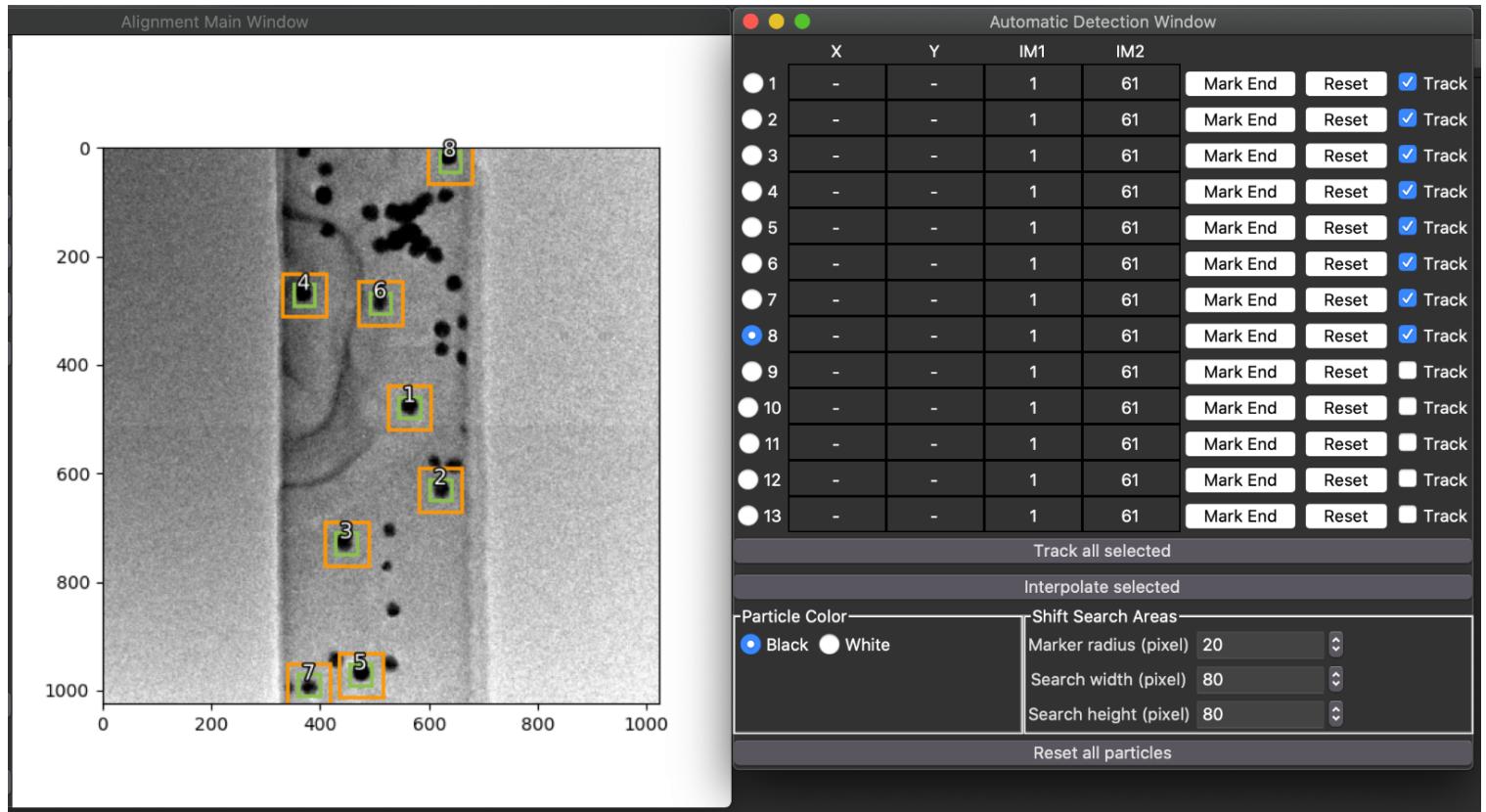
2. First select particles to track in the image:

- a. In the **Auto Detection Window**, click on Particle Number radio button 1.
- b. Click on the desired particle for tracking on the first image on the **Main Window**.
  - i. Two boxes will be drawn around the selected location.
  - ii. To redraw the placement of the boxes, click again on the image.



- c. Repeat these steps with the next radio button number, until all desired particles are selected; users can track up to 13 particles.
  - Reset a particle's selection by clicking the **Reset** button for that particle.
  - Select which particles to track by selecting/deselecting the **Track** selection box.
  - Select the colour of the particles using the **Particle Colour** radio buttons.

The following image shows an example of eight particles selected for tracking:



### 3. Track Particles

- Click the **Track all selected** button.
  - The user will see the images strolling through the set of images on the Main Window as particle tracking is being performed.
- When tracking is finished, view where the software tracked each particle on each image by scrolling through the images on the Main Window using the scrollbar.

### 4. Edit for improved tracking:

- Reset a particle's tracking by clicking the **Reset** button for that particle. Click the **Track all selected** button to re-track.
- Shift Search Areas** frame.
  - Edit the marker radius to change the size of the box drawn around the particle.
  - Edit the search area box width and height to change the size of the search area box around the particle.
  - Click the **Track all selected** button to re-track.

In the photos below, the tracking jumps to the wrong particle because it got confused when two particles got really close to each other. There are a few things that the user can do to fix this:

Image 20: Correct particle

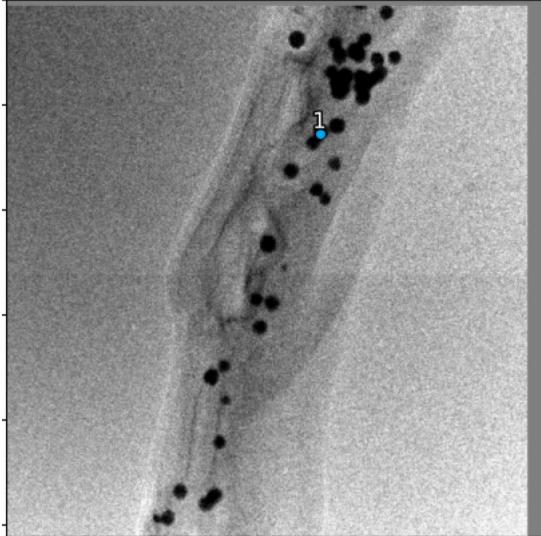
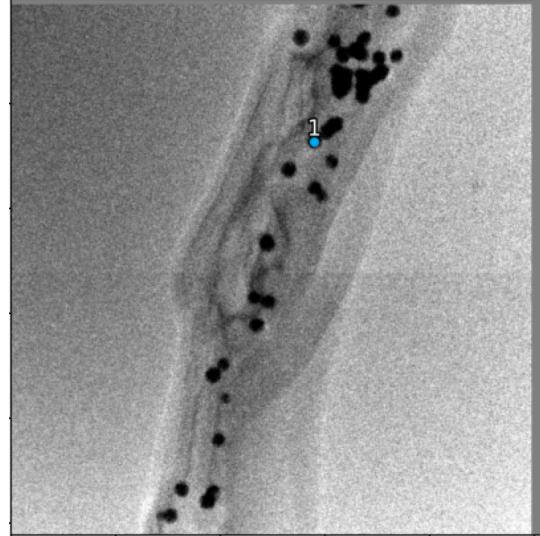


Image 21: Incorrect particle



- **Mark end:** end particle tracking at a particular image:
  - Scroll to the last image that the particle was tracked correctly on and click the **Mark End** button.
  - The IM2 value will update for that particle.

Image 20:

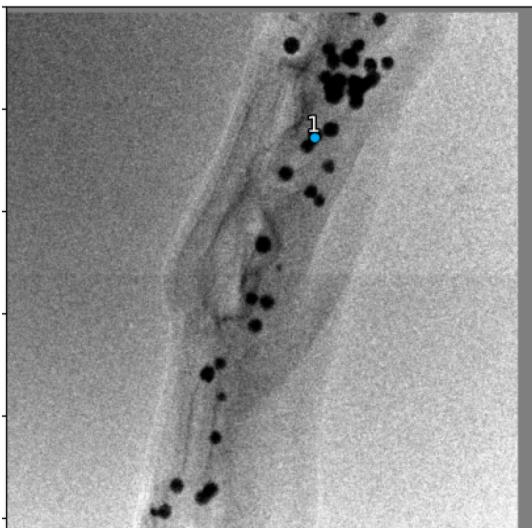
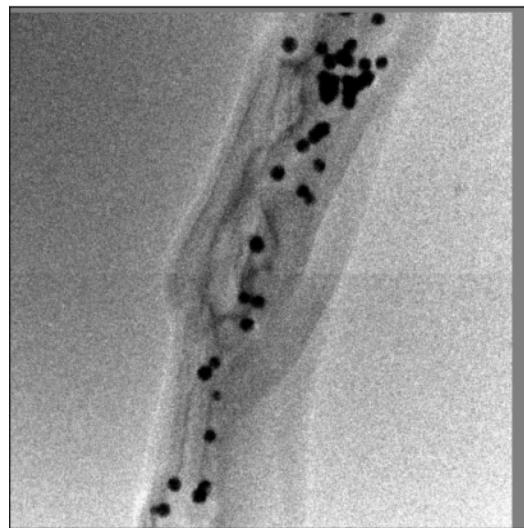
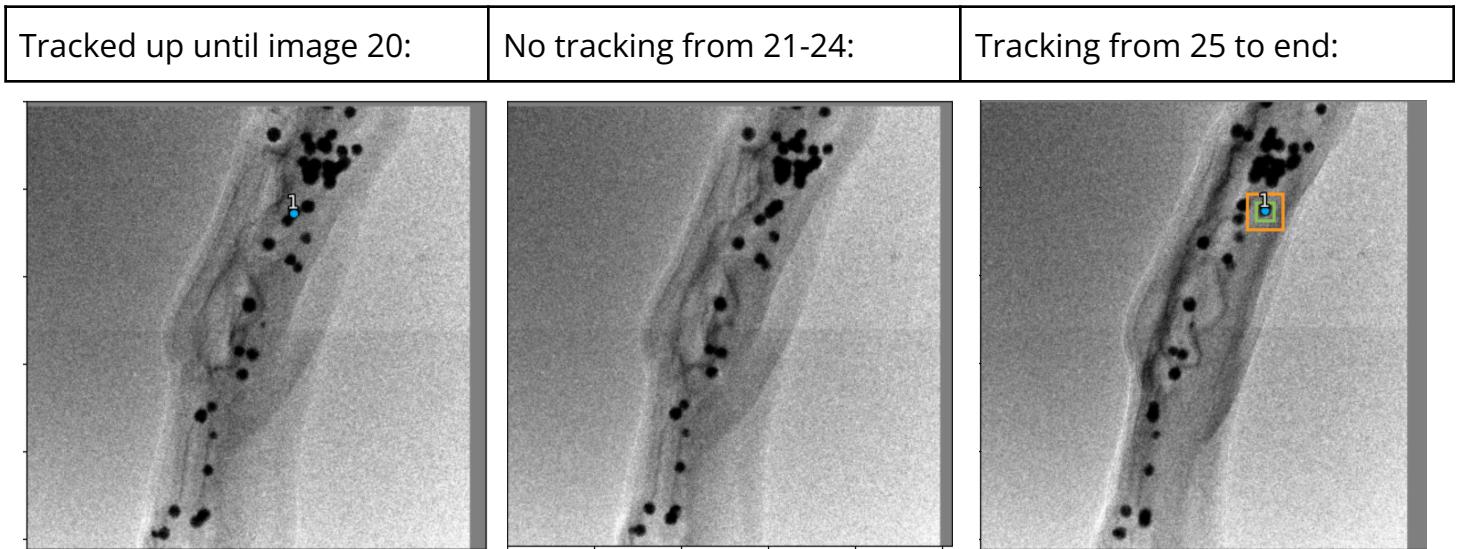


Image 21:



- To track the particle again after marking the end, go to the next slide where the particle is clearly visible and select here. Then click the **Track all Selected** button to re-track.
  - The IM1 and IM2 values will be updated based on this latest tracking, but the tracking on this particle saved from previous trackings will still be saved in the images - check the tracking using the scrollbar.



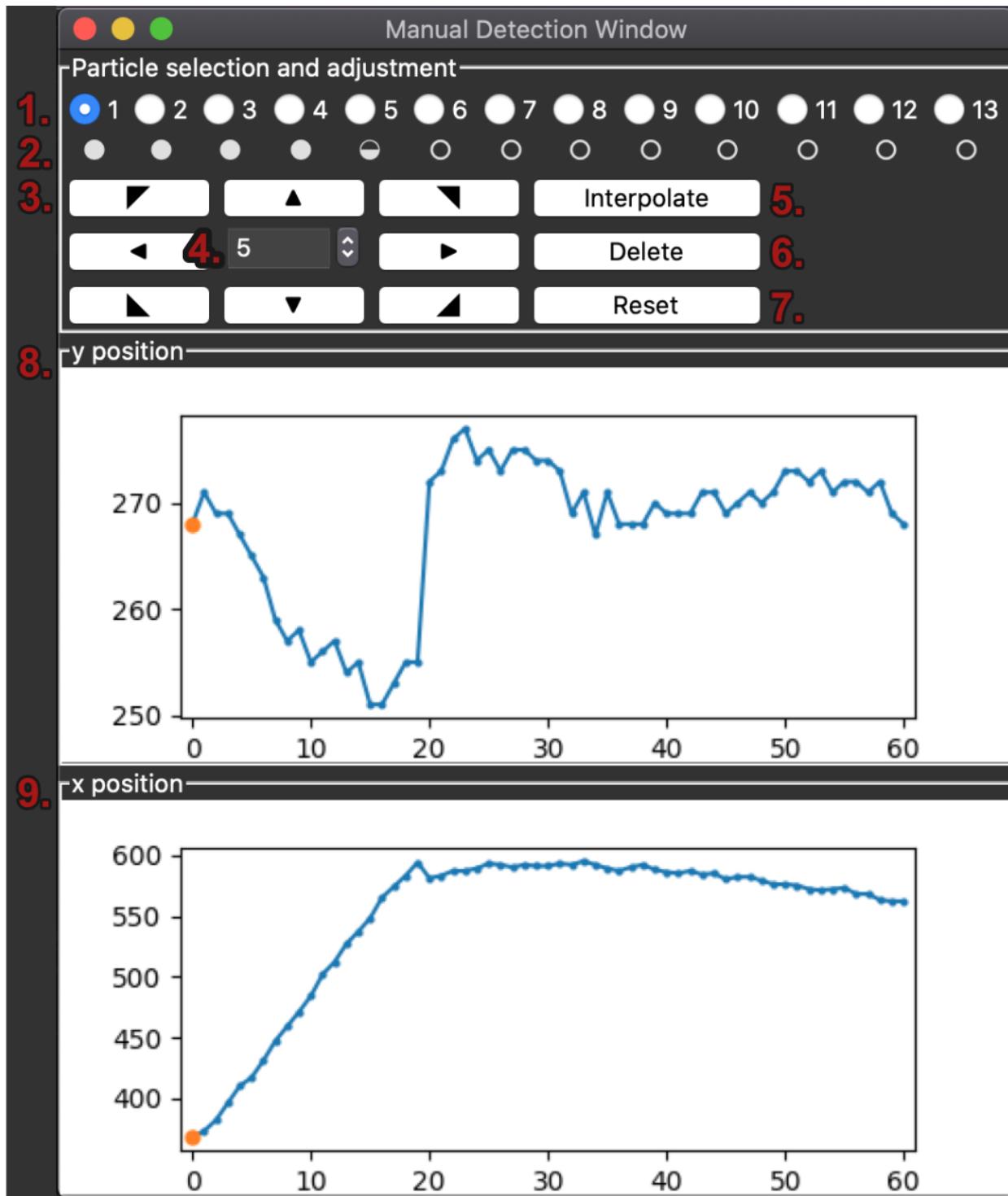
- When scrolling through the slides, the particle will be tracked up to the Mark End slide, and then resume tracking where the newly selected slide was.
- Notice that there is a gap between the Mark End slide and the newly selected slide, where the particle is not tracked on a few slides. Use Interpolation to fill the gap.

- **Interpolation:**

- **Prerequisite** - perform Mark End on the particle and re-track where available. The particle should be mostly tracked with a few slides with a gap in the middle where it could not be tracked. Follow the Mark End step above to do this.
- Click **Interpolation**.
- The particle will now be fully tracked where there was previously a gap.

## Step 6: Manual Detection

1. Click on the **Coarse Alignment** button on the Main Window.



#	<u>Feature</u>	<u>Description</u>
1	Particle Numbers	<ul style="list-style-type: none"> <li>• This set of radio buttons represents individual particles that can be found and tracked, up to 13.</li> <li>• Selecting a radio button allows the user to adjust the search location of the particle.</li> </ul>
2	Particle Completion Status	<ul style="list-style-type: none"> <li>• These indicators will show the completion status of each particle's tracking</li> <li>• Empty circle - no tracking</li> <li>• Half circle - partial tracking</li> <li>• Full circle - complete tracking across all images</li> </ul>
3	Location Adjustment Arrow buttons	<ul style="list-style-type: none"> <li>• Adjust the location of the particle on the images using the 8 arrows, without having to re-click on the image</li> </ul>
4	Pixel Adjustment	<ul style="list-style-type: none"> <li>• The entry box shows how many pixels the location adjustment arrows shift the particle by</li> </ul>
5	Interpolate	<ul style="list-style-type: none"> <li>• The interpolate button fills gaps in the tracking.</li> <li>• Use interpolation after selecting the particle's location on every few slides from the beginning to end.</li> </ul>
6	Delete	<ul style="list-style-type: none"> <li>• Deletes the particle tracking on the current image.</li> </ul>
7	Reset	<ul style="list-style-type: none"> <li>• Reset the tracking for the current selected particle.</li> </ul>
8	y position	<ul style="list-style-type: none"> <li>• This plot shows the selected particle's Y-position (vertical location) plotted against image number.</li> <li>• Ideally, in a good tracking scenario with smooth motion, there should be no discontinuities and smooth transitions on this plot, within error of location noise.</li> </ul>
9	x position	<ul style="list-style-type: none"> <li>• This plot shows the selected particle's X-position (horizontal location) plotted against image number.</li> <li>• Ideally, in a good tracking scenario with smooth motion, there should be no discontinuities and smooth transitions on this plot, within error of location noise.</li> </ul>

Adjust a particle's location:

- Use the **Location Adjustment Arrow buttons** to move the selected particle around without having to click on the image. The buttons will move the particle in the direction of the arrow on the button by the amount of pixels in the Pixel Adjustment spinbox.
- Adjust the amount of pixels the arrow buttons will move by editing the number in the **Pixel Adjustment spinbox** in the middle of the arrow buttons.

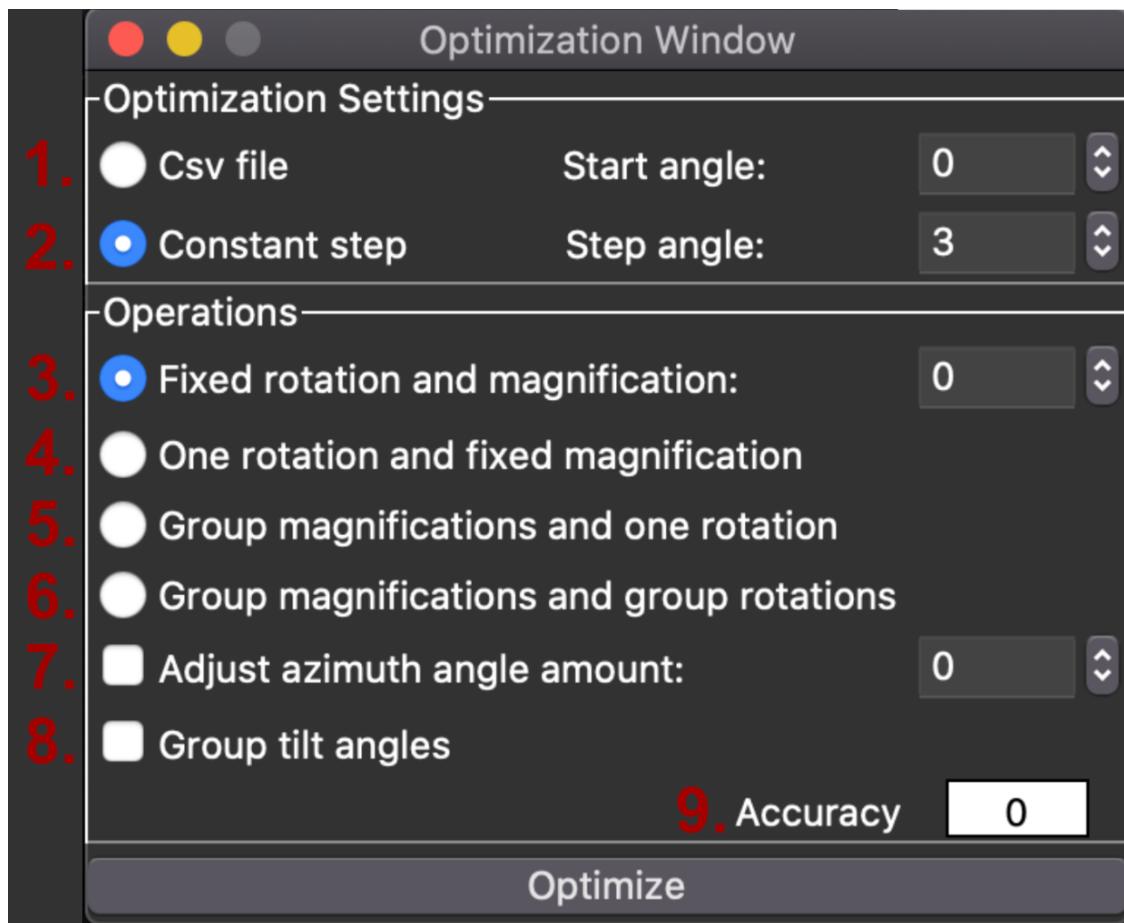
Track a new particle manually:

- Select a **particle number radio button**.
- Select its location by clicking on the image. Adjust.
- Repeat until the particle location is selected on all of the images.

Tracking a particle using interpolation:

- Select a **particle number radio button**.
- Select the location of the particle on the image.
- Do this on every few images, every 5-15 slides from the beginning to end.
- Click **Interpolation**.

## Step 7: Optimization



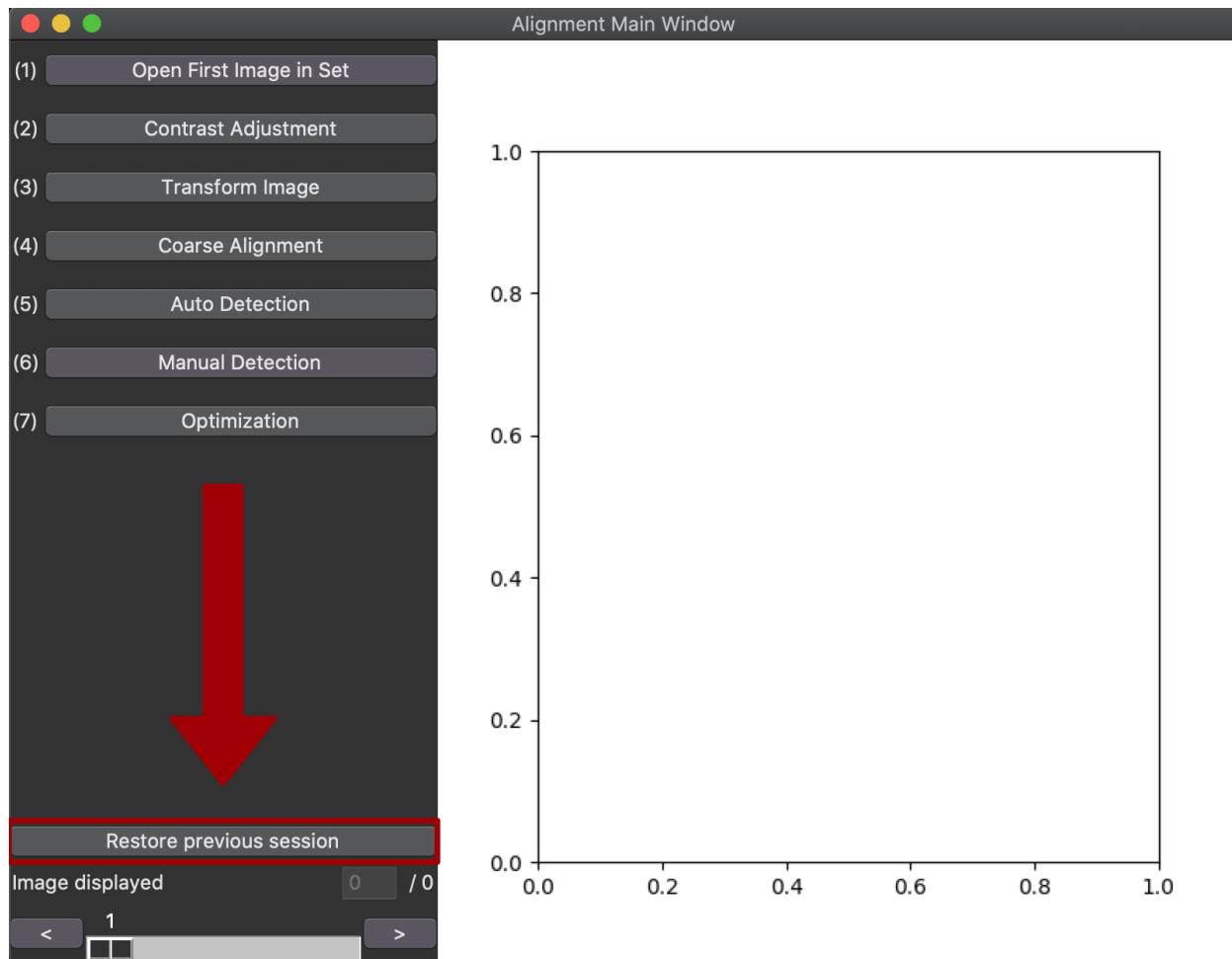
#	<u>Feature</u>	<u>Description</u>
1	Csv file	<ul style="list-style-type: none"> <li>• Uploads a CSV file with an array of tilt angles that do not have to have constant step values.</li> <li>• Tilt angles should be written in 'tilt_angle.csv', placed in the same folder as the tilt series of images.</li> </ul>
2	Constant step	<ul style="list-style-type: none"> <li>• Assumes a constant step change between tilt angles, entered in the entry boxes beside the radio button.</li> <li>• The start angle entry box is used as the first image's angle in degrees.</li> <li>• The step angle entry box is used as the step angle between consecutive images in degrees.</li> </ul>
3	Fixed rotation and magnification	<ul style="list-style-type: none"> <li>• Fixes rotation at a given value, typed into the spinbox, and fixes magnification at 1X.</li> </ul>
4	One rotation and fixed magnification	<ul style="list-style-type: none"> <li>• Has one rotation value shared by all frames and fixes magnification at 1X.</li> </ul>
5	Group magnifications and one rotation	<ul style="list-style-type: none"> <li>• Has one rotation value shared by all frames and a separate magnification value for each frame.</li> </ul>
6	Group magnifications and group rotations	<ul style="list-style-type: none"> <li>• A separate rotation value for each frame and a separate magnification value for each frame.</li> </ul>
7	Adjust azimuth angle amount	<ul style="list-style-type: none"> <li>• Allow for the optimization to adjust the 3rd axis or rotation (tilt, rotation, and azimuth) Adjust the amount using the spinbox.</li> </ul>
8	Group tilt angles	<ul style="list-style-type: none"> <li>• After the main optimization is done, comput a new 'corrected' tilt angle for each frame</li> </ul>
9	Accuracy	<ul style="list-style-type: none"> <li>• Displays the accuracy of the optimization.</li> </ul>

- Select the desired settings for optimization and operations to be performed then click the **Optimize** button.
- Results will appear in the Main Window.

## Restore Previous Session

### Restore Previous Session:

1. Open the software.
2. Open the first image in the set that needs to be restored.
3. Click **Restore Previous Session**. The progress that was made the last time the software was used will be restored and the user can continue.



## **6. Troubleshooting and Support**

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### **6.1. Error Messages**

- Incorrect Python version - Ensure that you have the correct Python version. There might have been an old version of Python already installed on your machine. In your Command Prompt, run:
  - `python --version`
- Python not installed in PATH - Ensure that Python is installed in the proper location on your machine. If the following runs and displays the correct version of Python, you have correctly installed it on your PATH variable. In your Command Prompt, run:
  - `python --version`
- Batch not running - Download the file on your machine and double-click on the file to start. If using Command Prompt, ensure that you are navigated to your batch file.

### **6.2. Support**

If you are using Alignment Software and notice there are errors, inconsistencies, or need other additional assistance, please contact Dr. Misa Hayashida at the National Institute for Nanotechnology with the National Research Council of Canada at the University of Alberta.

## **Appendix A - Record of Changes**

Record of Changes:

Version	Date DD/MM/YYYY	Author	Description of Changes
1.0	14/07/2022	Jasmine Mishra	Creation of User Manual.
1.1	16/08/2022	Jasmine Mishra	Finished adding all requirements

## **Appendix B - Notes to the Author**

This document is a User Manual for Alignment Software. The final document should be delivered in an electronically searchable format. The manual should stand on its own, with all elements explained for readers.

When modifying this document, the author should note that:

- When significant changes are made to the software, this document needs updating.
  - Note modifications made to this document in Appendix A.
- This document uses Open Sans font for accessibility and readability.
- Headings are left aligned, bolded, and size 18 font (except appendixes).
- There is a 12 font soft space between paragraphs and headings, except on the Step-by-Step Guide.
- Keep consistent styling.