PCD Detect Guide

Installing PCD Detect

PCD Detect has been designed to work on Microsoft Windows. The programs should be extracted into a folder on your hard drive called PCD-Detect. There are 64-bit and 32-bit version of the programs depending on your computer architecture. The 32-bit version does not support parallel processing (multiple core processing) in the Cilia Averaging program. For the programs to run a matlab runtime library needs to be installed. Install the runtime files (R2020a (9.8) for 64-bit and R2015b (9.0) for 32-bit version) that are included with the programs or can be download from the following weblink;
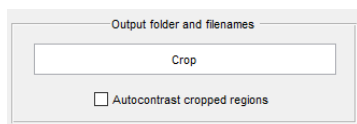https://uk.mathworks.com/products/compiler/matlab-runtime.html.

Cilia Crop Guide
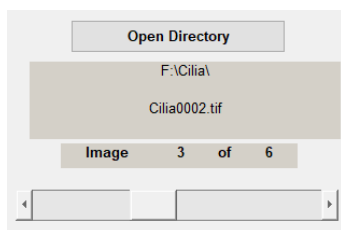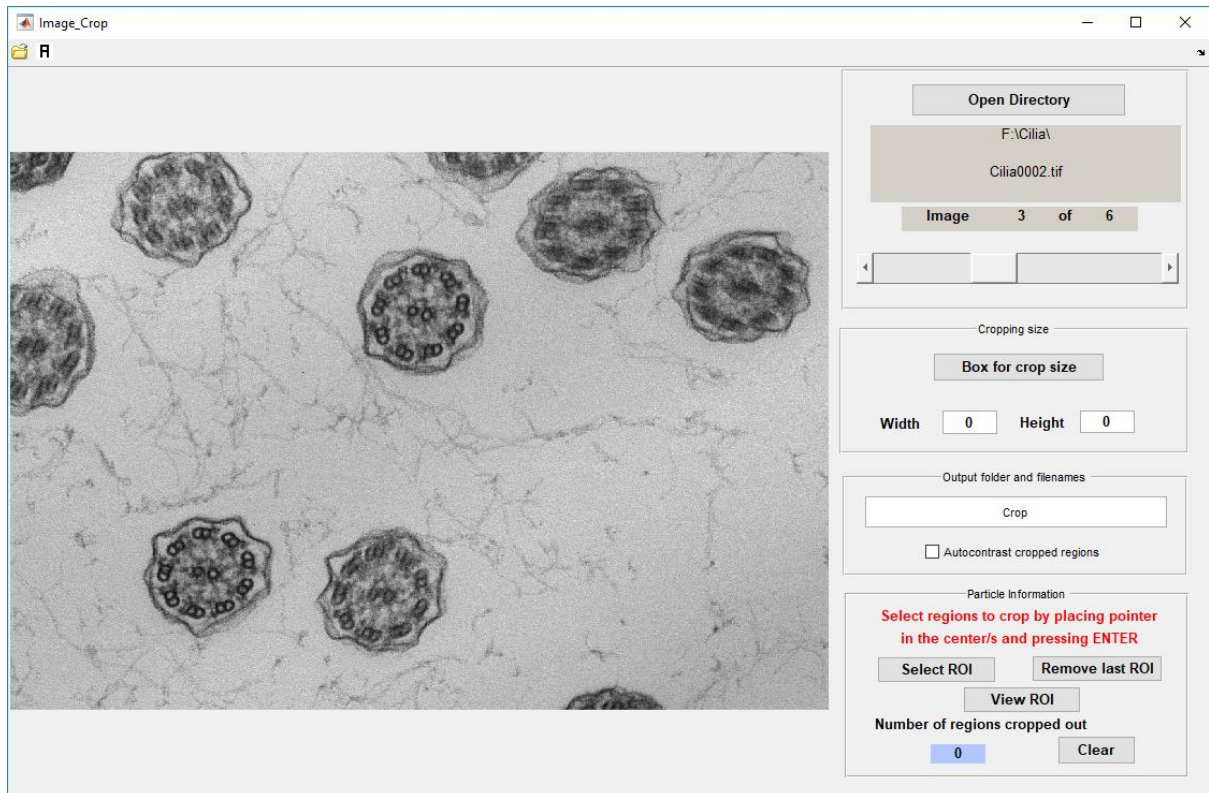
1.	Press 'Open Directory' to select directory containing the electron microscopy images and select the first image in the folder and press 'open'
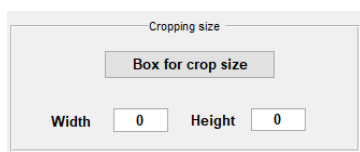
2.	A box can be drawn around a cilium by pressing 'Box for crop size' or the width and height of the box in pixels can be entered in manually.

3.	Set the output file and folder name (a subfolder with this name will be created containing the cropped cilia).

Particle Information
Select regions to crop by placing pointer in the center/s and pressing ENTER

Select ROI | Remove last ROI

View ROI

Number of regions cropped out

0 | Clear

4. 'Select ROI' (region of interest) allows a point to be placed in the centre of one or multiple cilia and by pressing the ENTER key on the keyboard the cilia will be cropped out.

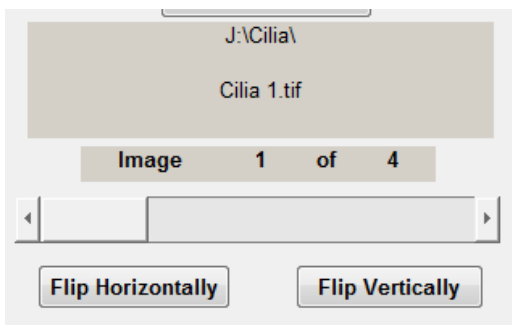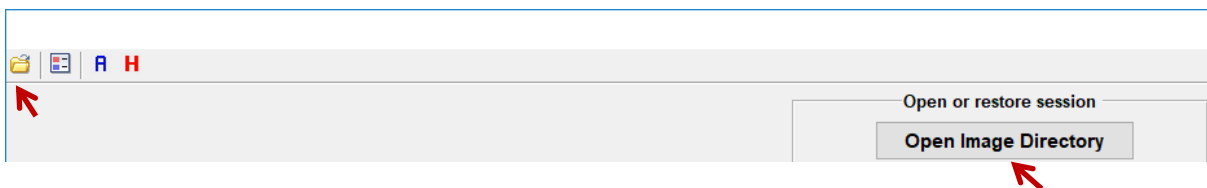5. View cilia that have been cropped by pressing 'View ROI'.

6. If a cilia that has been cropped out needs to be deleted press 'Remove last ROI'.

7. To remove all cilia that have been cropped and to start again press 'Clear'.
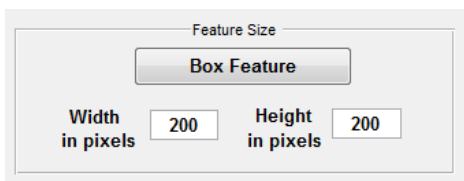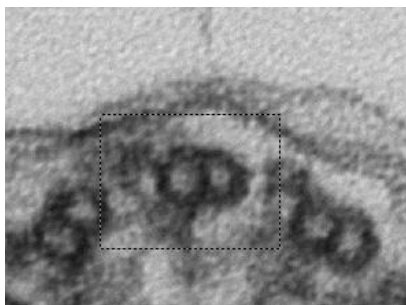
Cilia Averaging Guide
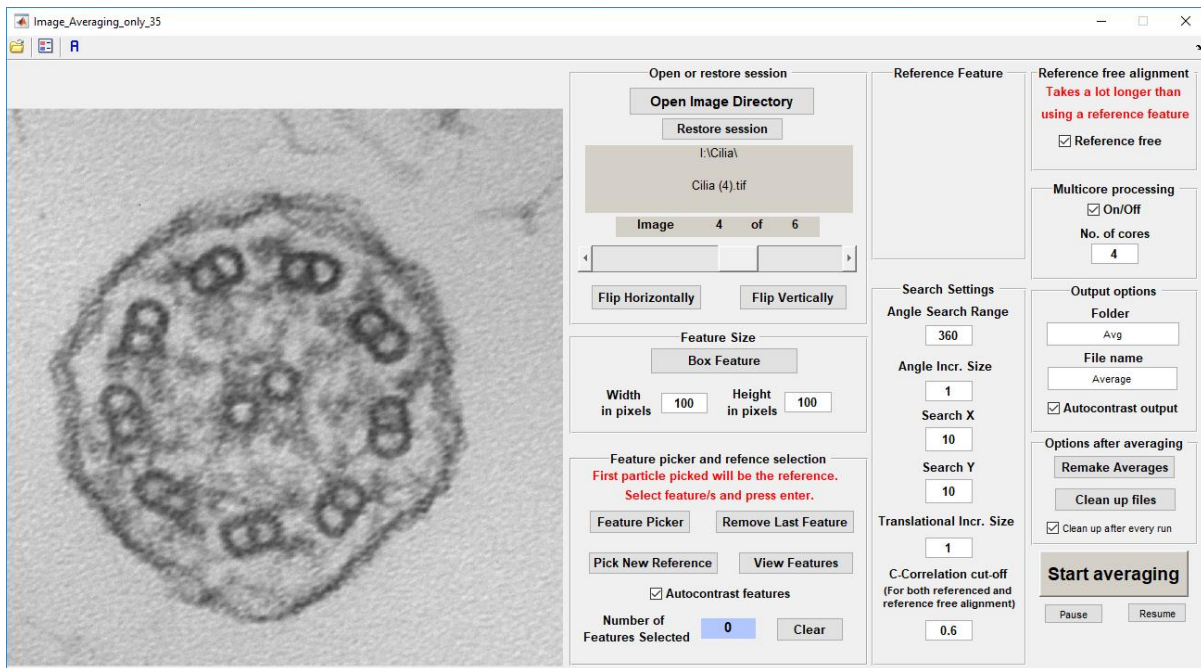
1. Open Image Directory by pressing on either of the following and selecting the first image in the folder before pressing 'Open'
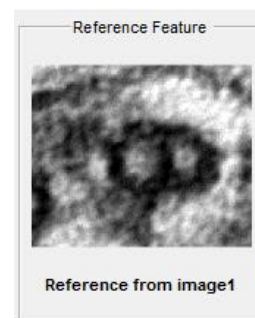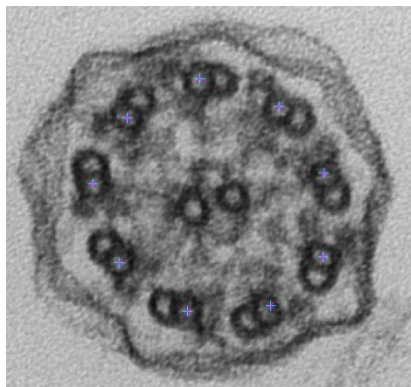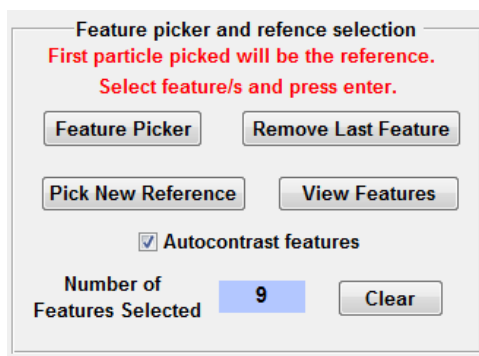

2. Make sure all images have the same orientation by flipping them horizontally or vertically


3. Press 'Box Feature' and draw box around feature or alternatively input desired width and height of feature.

4. Press 'Feature Picker' and in each image select feature/s and press Enter on the keyboard. The first feature automatically becomes the reference feature.


To remove an unwanted or mistakenly selected feature press 'Remove Last Feature'. If a new reference feature needs to be picked press on 'Pick New Reference'. All features can be seen as a montage image by pressing 'View Features'. When 'Autocontrast feature' is on, the contrast of all features used will be automatically contrasted.
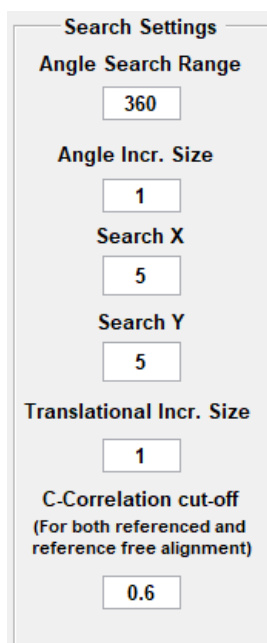
5. Set the search angle and translational (Search X/Y) range (the program will rotate and move a feature in X and Y to attempt to get the best match to another feature) as well as the search angle (degrees) and translational (pixels) increments size (Incr. Size) (the size of each step for the rotational and translational search).

For averaging using a reference feature, an additional average is generated consisting only features that match with a cross-correlation above a given threshold value can be set. When performing reference free averaging this value is used to determine the best feature to average other feature to it.

**For reference free aligned and averaging setup the following:**
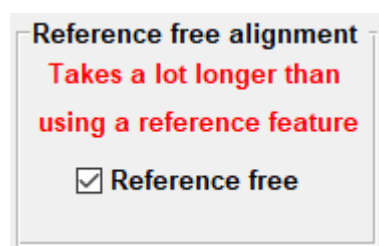
1. For reference free averaging follow all the previous steps.

2. Turn on 'Reference free'. When this is on, the reference feature will not be used (this takes a lot longer than when using a reference).

Final setup for both referenced and reference free alignment and averaging are as follow:
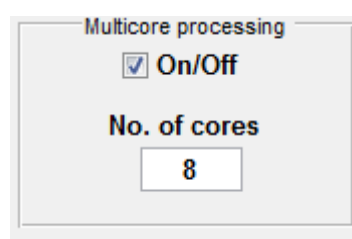
1. Multicore processing can be set on/off and the number of cores to be used can be set. (This is recommended for reference free alignment as it can take a long time to run).
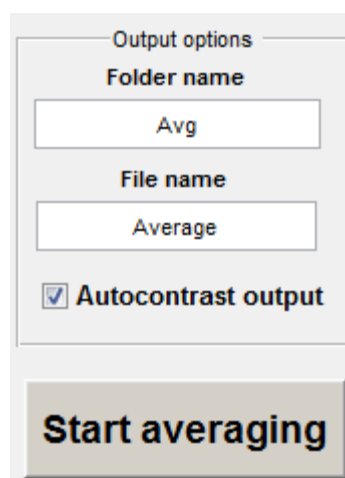
Input the output folder and file name as well as if the output should be auto-contrasted before pressing 'Start averaging'

Additional features

1. After running an averaging processes, a session file is automatically generated. When opening a session, instead of having to run the alignment and averaging again remake average can be used to quickly generate new averaged images
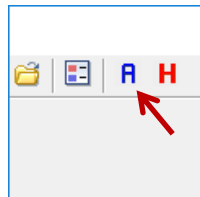
2. Sessions can be paused and resumed later on, or resumed after restoring a session

[Pause]   [Resume]
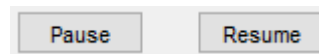
3. The default setting can be change by pressing the following button on the Toolbar.
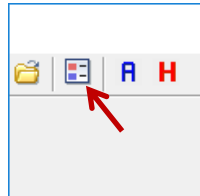
4. Press the A icon if you wish to email and leave feedback.

Cilia Classification Guide

1. Open session file averaging after cilia averaging has been completed by pressing 'Open completed averaging session'. If there

Averaging session information

Working directory	I:\Cilia\			
Working file name	MTD			
Average Feature size	X	220	Y	220
Feature search range	X	10	Y	10
Number of feartures	117	Reference from image	1	
Method of alignment	Reference-free			

PCA and k-mean classification

K-mean number of classes	2
PCA coefficients to use to calcuate K-means (values between 1 to 6 and use - or , as separators)	1-3
Cross-correlation cut-off (sets ref feature for each class)	0.6
☐ Do not include features below cross- correlation threshold	0.3

☐ Use average of each feature for PCA and K-mean
(requires reference free alignment)
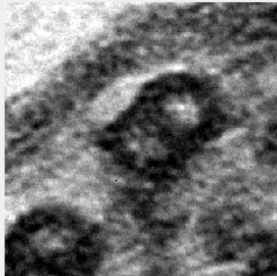
☐ Contrast enhance output

Image filter to assist PCA classification

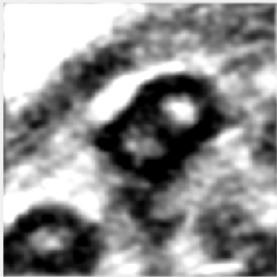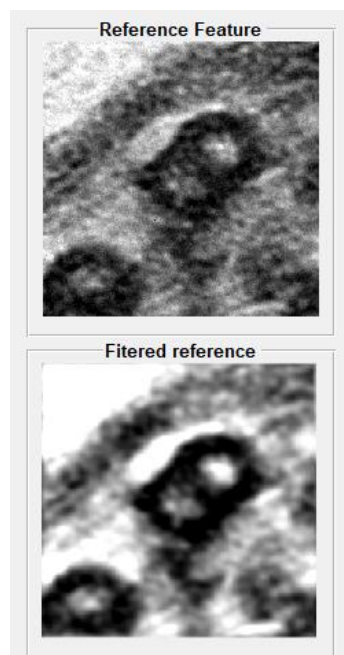|				
☑ Lowband pass filter	Lower	2	Upper	20
☑ Gaussian Filter	Size	14	Sigma	2

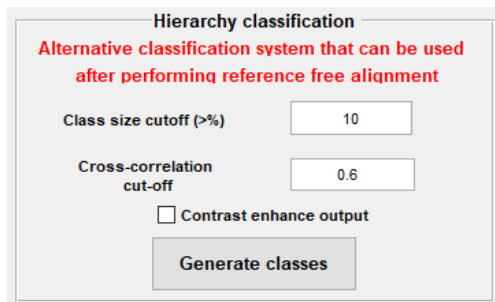| PCA plots	K-mean clustering	Generate classes

2. Once the session file has been open, information regarding the averaging that was done is displayed here.

3. These are the parameters for the principal component analysis classification. 'k-mean number of classes' defines the number of expected classes (e.g. 2 classes, if expecting some microtubule doublets with and other without outer dynein arms).

4. To assist the PCA classification and reduce background noise a lowband pass and Gaussian filter can be applied. When adjusting these parameters the filtered reference image will change accordingly.

Reference Feature

Fitered reference

5.   To use averaged versions of each feature to calculate the PCA rather than single images 'Use average of each feature for PCA and k-mean' can be pressed. This is only available if a previous reference free alignment was done. To determine the averages the 'Cross-correlation cut-off' needs to be set and an optional setting to exclude features that are dissimilar is available – 'Do not include features below cross-correlation threshold'

6.   Press 'PCA plots' and from the graphs given if there is multiple peaks in some of the graphs these can be used for the next stage by selecting the graph numbers in 'PCA coefficients to use to calculate K-means'

7. Pressing 'K-mean clustering' gives a plot shows how the different features are classed based on the PCA into different classes.

8. Press 'Generate classes' to get generate the class averages.

Hierarchy classification method:

1. An alternative method to generate class averages is the Hierarchy classification that can be used if a previous reference free alignment was done. This uses the features that has most other similar other features based on cross correlation and find those that are most dissimilar to generate class averages.

2. If a class average is to be made up of less than the 'class size cutoff' (% of the total number of features input into the averaging scheme) no class average will be generated

3. 'Cross-correlation cut-off' determines the cross-correlation (feature similarity) required to be group features together. When comparing two features if the cross-correlation calculated is below the cut-off value they will be grouped together.

4. Press 'Generate classes' to generate hierarchy class averages.

Image Analysis Guide
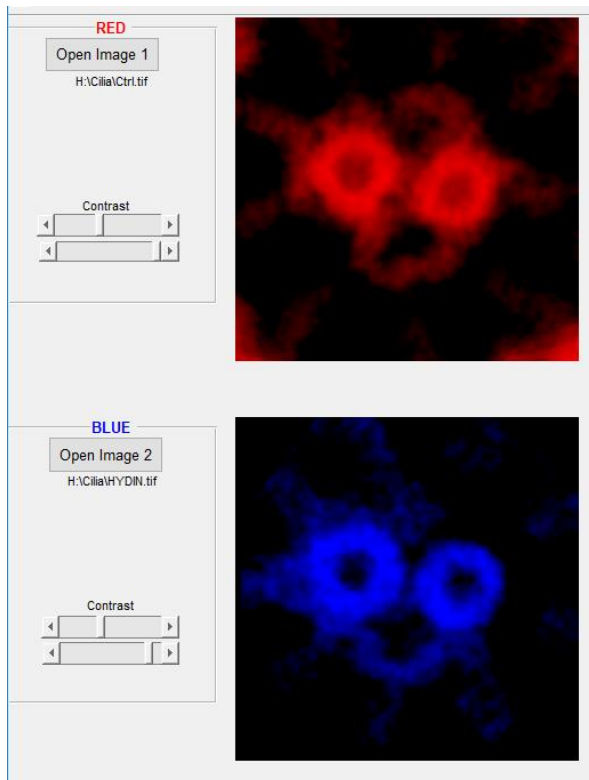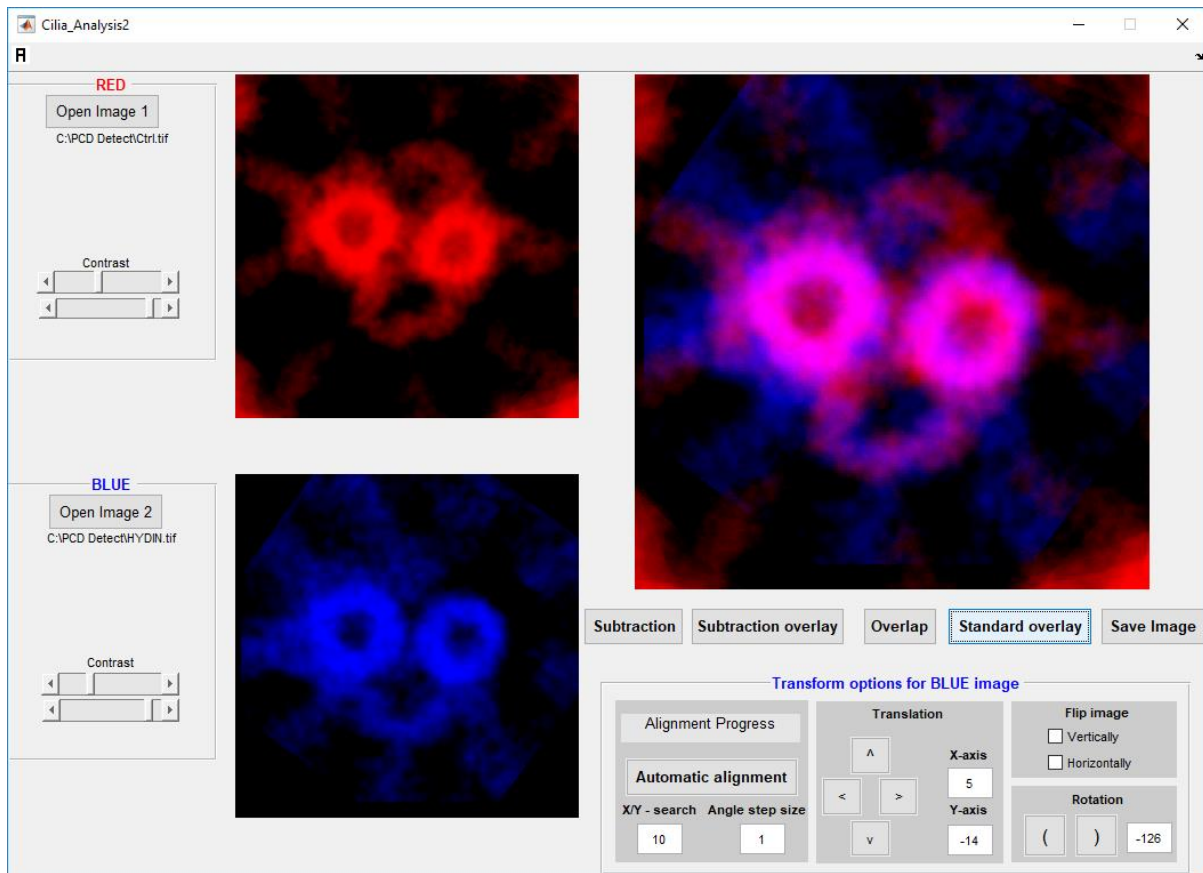
1.   Open two averaged images of ciliary that are to be compared structures (in the example shown is the central pair) by press 'Open Image 1' and 'Open Image 2'. These will appear Red and Blue in colour respectively and the contrast can be adjusted using the sliders.
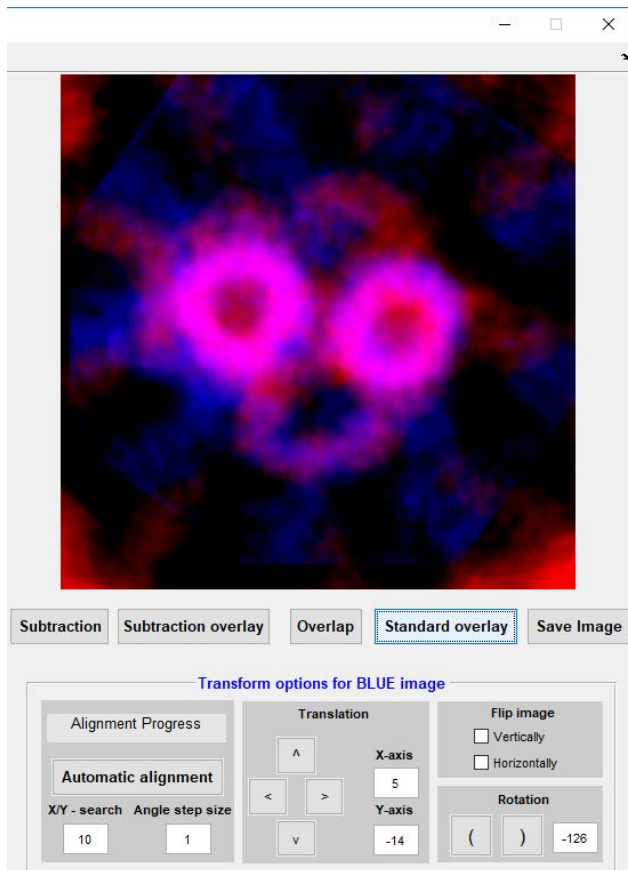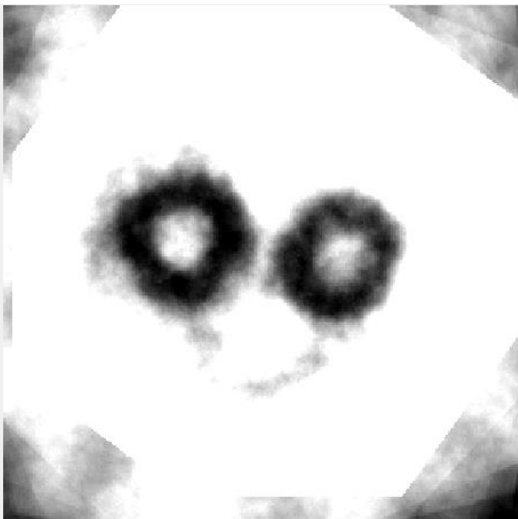
2.   The two images can be aligned by transforming the blue image by moving/rotating manually or by using the automatic alignment.
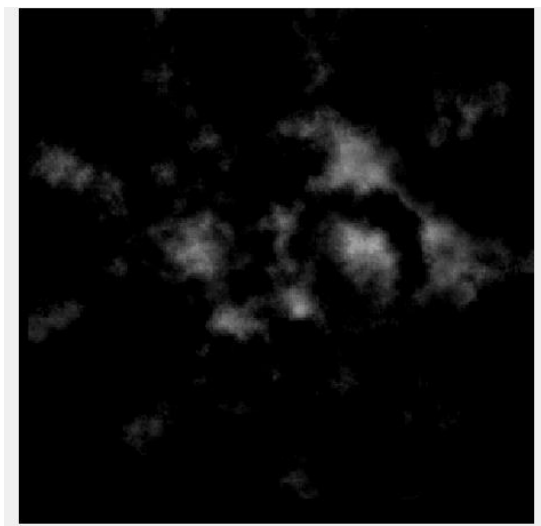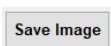

3.   By pressing 'Subtraction' the difference between the two images will be given in grey scale (difference in grey/white).
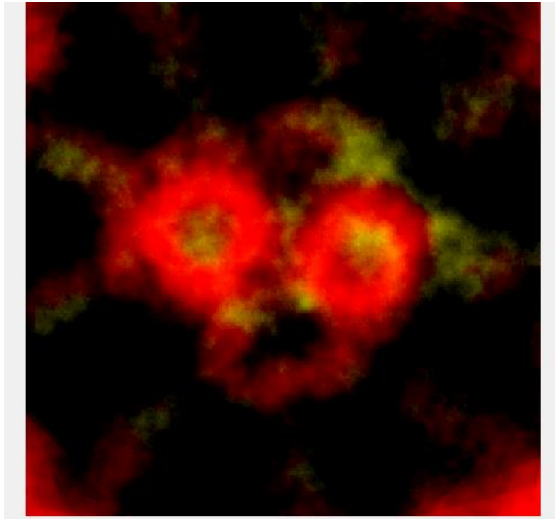
4.   Pressing 'Subtraction overlay' will show the difference between the images overlaid onto the red image.

5.   'Overlap' will show the overlapping information in the two images (this is shown in grey/black).

    6.   To save the any of the images or overlays press 'Save Image'.