PCD Detect Guide

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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

Mage_Crop	- 0 ×
ä A	
	Open Directory
	F:\Cilia\
	Cilia0002.tif
	Image 3 of 6
and the second	
	Cropping size
	Box for crop size
13. MAT AR De June Sug	Width 0 Height 0
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	Autocontrast cropped regions
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the second se	Select regions to crop by placing pointer in the center/s and pressing ENTER
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and the lite of the	View ROI
	Number of regions cropped out
	0 Clear

	Оре	en Direc	tory		
		F:\Cilia	a/		
	(Cilia000	2.tif		
	Image	3	of	6	
4					Þ

1. Press 'Open Directory' to select directory containing the electron microscopy images and select the first image in the folder and press 'open'

	Cropping size		
	Box for crop size		
Width	0 Height 0		

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.

Output folder and filenames
Сгор
Autocontrast cropped regions

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

Select regions to cr	nformation op by placing pointer nd pressing ENTER
Select ROI	Remove last ROI
Vie	w ROI
Number of regions of	ropped 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'.

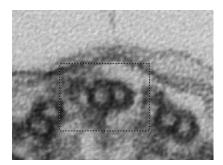
Cilia Averaging Guide

Image_Averaging_only_35			- D >
E A			
	Open or restore session	Reference Feature	Reference free alignment
	Open Image Directory		Takes a lot longer than using a reference feature
	Restore session		Reference free
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	Cilia (4).tif		Multicore processing
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A SHALL SHE	Flip Horizontally Flip Vertically	Search Settings	Output options
BORRAR	The forzontary The ventury	Angle Search Range	Folder
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· 是是在中心这些思想。""你们的"	in pixels 100 in pixels 100	Search X	Autocontrast output
A MALE FOR THE LET BE		10	Options after averaging
The set of	Feature picker and refence selection First particle picked will be the reference.	Search Y	Remake Averages
Store a a for a	Select feature/s and press enter.	10	Clean up files
V Method F	Feature Picker Remove Last Feature	Translational Incr. Size	Clean up after every run
Man and a start of	Pick New Reference View Features	1 C Correlation aut aff	Start averaging
the state of the s	Autocontrast features	C-Correlation cut-off (For both referenced and	Start averaging
and the second s	Number of O Clear	reference free alignment) 0.6	Pause Resume

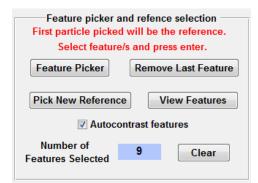
1. Open Image Directory by pressing on either of the following and selecting the first image in the folder before pressing 'Open'

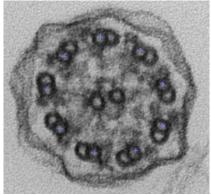
🗃 🗉 R H	
ĸ	Open or restore session
	Open Image Directory
	7
J:\Cilia\	
Cilia 1.tif	2. Make sure all images have the same orientation
Image 1 of 4	by flipping them horizontally or vertically
4	
Flip Horizontally Flip Vertically	
Feature Size	3. Press 'Box Feature' and draw box around
Box Feature	3. Press 'Box Feature' and draw box around
	feature or alternatively input desired width and height
in pixels in pixels	of feature.

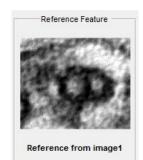
of feature.



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:

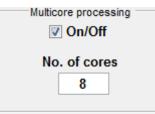
Reference free alignment Takes a lot longer than using a reference feature

Reference free

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'

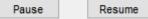
Additional features

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

Restore session

Remake Averages

2. Sessions can be paused and resumed later on, or resumed after restoring a session



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.



Classification12_include_align		- 🗆 X
R		لا
	Open completed ave	eraging session
	Ready to generate o	class averages
Averaging	g session information	Reference Feature
Working directory	Cilia\	
Working file name MT	ס	
Average Feature size	x 220 Y 220	
Feature search range	4 10 Y 10	
Number of feartures 11	17 Reference from 1 image 1	Fitered reference
Method of alignment	Reference-free	
PCA and K-mean numbe	k-mean classification	
PCA coefficients to use to cal between 1 to 6 and use -		
Cross-correlation cut-off(each clas		
Do not include features be	low cross- correlation threshold 0.3	Hierarchy classification
	h feature for PCA and K-mean	Alternative classification system that can be used
	eference free alignment) trast enhance output	after performing reference free alignment
	to assist PCA classification	Class size cutoff (>%) 10
∠ Lowband pass filter		Cross-correlation 0.6
PCA plots K-mean	clustering Generate classes	Contrast enhance output

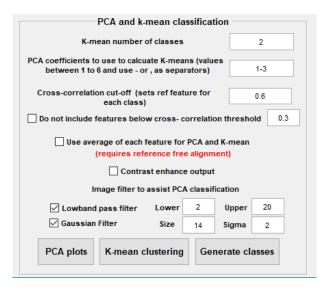
Cilia Classification Guide

Open completed averaging session	
Ready to generate class averages	

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

'Open completed averaging session'. If there

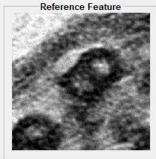
Averaging session information					
Working directory	I:\Cilia\				
Working file name	MTD				
Average Feature size	x	220	Y	220	
Feature search range	x	10	Y	10	
Number of feartures	117	Ref	erence from image	1	
Method of alignment	Reference-free				

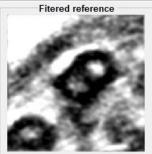


 Once the session file has been open, information regarding the averaging that was done is displayed here.

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.





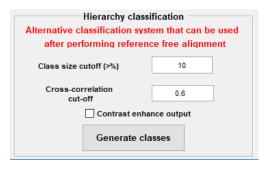
5. To use averaged versions of each feature to calculate the PCA rather than single images 'Use average of each feature for PCA and k-mean' can be pressed. This is only available if a previous reference free alignment was done. To determine the averages the 'Cross-correlation cut-off' needs to be set 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 Kmeans'

- 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:

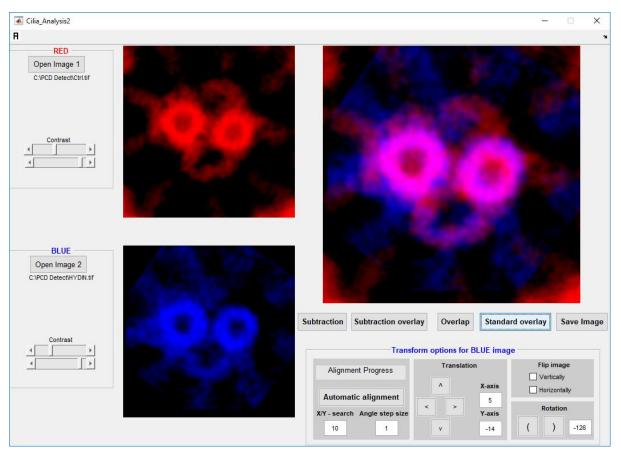


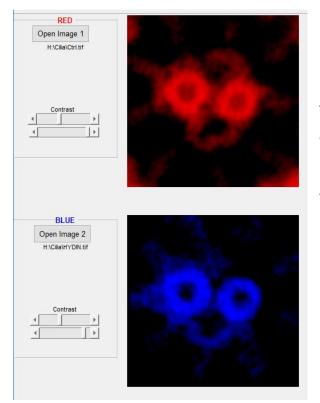
1. An alternative method to generate class averages is the Hierarchy classification that can be used if a previous reference free alignment was done. This uses the features that has most other similar other features based on cross correlation and find those that are most dissimilar to generate

class averages.

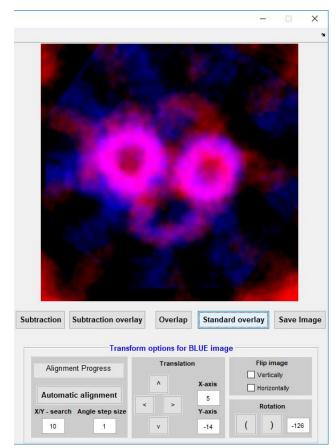
- If a class average is to be made up of less than the 'class size cutoff' (% of the total number of features input into the averaging scheme) no class average will be generated
- 'Cross-correlation cut-off' determines the cross-correlation (feature similarity) required to be group features together. When comparing two features if the crosscorrelation calculated is below the cut-off value they will be grouped together.
- 4. Press 'Generate classes' to generate hierarchy class averages.

Image Analysis Guide

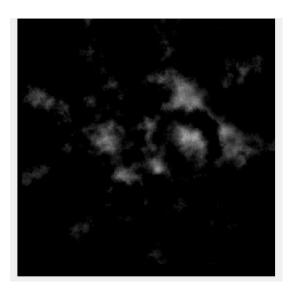




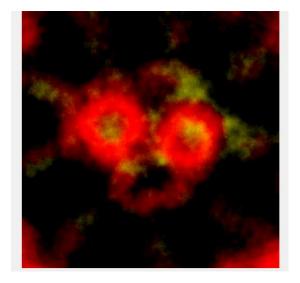
1. Open two averaged images of ciliary that are to be compared structures (in the example shown is the central pair) by press 'Open Image 1' and 'Open Image 2'. These will appear Red and Blue in colour respectively and the contrast can be adjusted using the sliders.



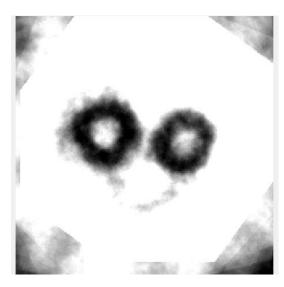
The two images can be aligned
by transforming the blue image by
moving/rotating manually or by using the
automatic alignment.



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'.