ImageJ Find Peaks Plugins

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Introduction

The Find Peaks ImageJ plugins allow the identification of peak intensity regions within 2D and 3D images. There are four different plugins that can be run from the ImageJ plugins menu:

1. Find Peaks
2. Find Peaks (Frame)
3. Find Peaks Optimiser
4. Find Peaks Optimiser (Frame)

The main algorithm is contained within the Find Peaks plugin. This is a standard ImageJ plugin filter that operates on an opened image. It shows a dialogue box to request parameters and then performs the image processing. Results are presented as a mask image, shown in tabular format and/or saved to file.

The Find Peaks (Frame) opens a permanent window frame within ImageJ. It has the same parameters as the Find Peaks plugin. It runs the Find Peaks plugin to perform the processing work. The advantage of the frame is that the user can quickly change different settings and repeatedly run the algorithm until they get the desired results.

The Find Peaks Optimiser allows a user to find the best parameters to use in the algorithm. There are many options for the peak finding algorithm and consequently thousands of combinations. The optimiser allows the user to mark maxima of interest on a representative image using the ImageJ ROI tools. The optimiser uses this image as a reference. It iterates through thousands of combinations and identifies the best parameters to find the desired peak regions. These parameters can then be easily applied to other images of the same type using the ImageJ scripting tools.

The Find Peaks Optimiser (Frame) opens a permanent window within ImageJ. It allows the user to select an image and run the Find Peaks Optimiser. This is a convenience plugin to allow the optimiser to be run repeatedly on one or many images.

In addition to the plugins that run the Find Peaks algorithms there are several plugins that provide useful functionality for peak analysis:

1. Find Peaks Helper
2. Match Calculator
3. Point Aligner

The Find Peaks Helper provides semi-automated assistance to aid identification of peaks in an image. The plugin uses the Find Peaks algorithm to identify all possible peaks in an image. Then when a user clicks on the image to label a peak using the ImageJ ROI tools the plugin updates the point, moving it to the nearest peak position if one is available. The plugin selects peaks within a search radius using either the nearest-neighbour or the highest peak. The plugin supports dragging points and removing false assignments. Results can be saved to a table.

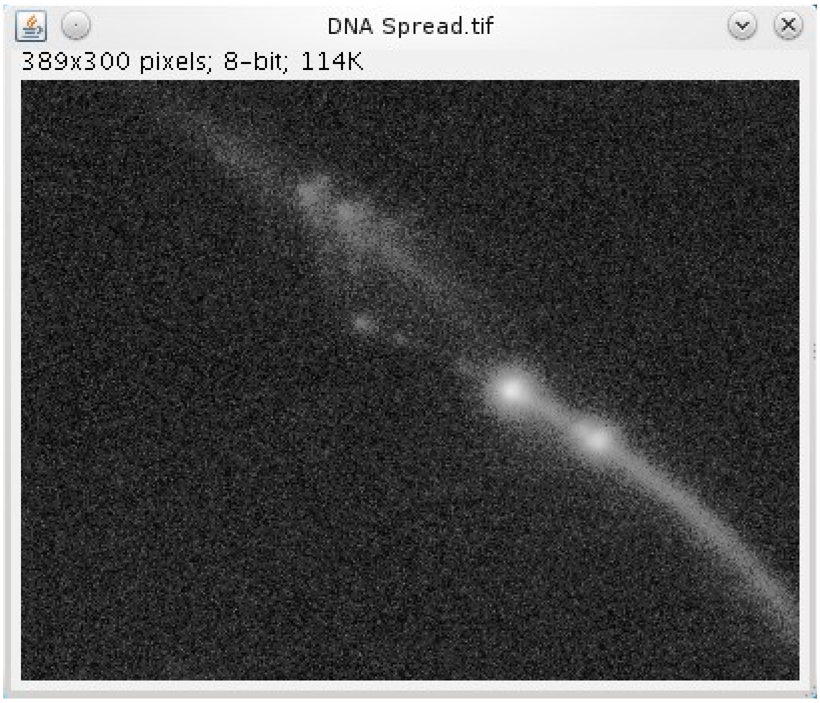
The Match Calculator compares the ROI points marked on two images and calculates the number of points that match. Points are aligned using a nearest-neighbour ranking within a specified distance threshold and the results are displayed as a statistics table. Using the image intensity for each point allows further analysis of the data including a scatter plot of intensity for matched points and analysis of matches within each quartile of the data. The matched and unmatched points can be shown as a colour-coded overlay on the input image.

The Point Aligner can move the current ROI points to align with the true image peaks found using the Find Peaks algorithm. Points are aligned using a nearest-neighbour ranking. Alignment results are output to a result table and can be used to update the ROI positions or shown as a colour-coded overlay on the image.

Find Peaks

ImageJ plugin that finds areas of maximum intensity in 2D and 3D images.

Example Input

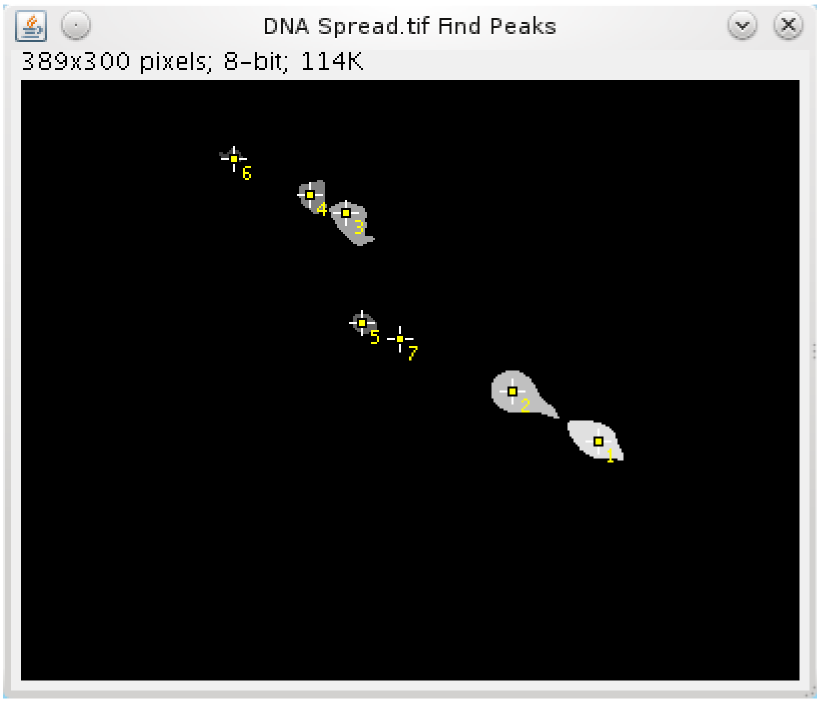


DNA Spread.tif

389x300 pixels; 8-bit; 114K

Input image with regions of high intensity for peak analysis.

Example Output



DNA Spread.tif Find Peaks

389x300 pixels; 8-bit; 114K

Output image showing the identified peak regions. Each peak centre is labelled with a point ROI marker.

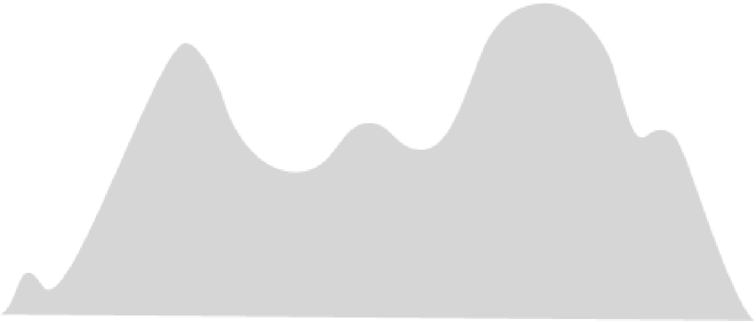
Features

- Processes 8-bit and 16-bit greyscale images
- Processes 2D and 3D images (i.e. image stacks)
- Allows hyperstacks but only processes the current channel and time frame
- Finds peak regions above a configurable background
- Merges sub-peaks into their parent using merging criteria
- Ranks peaks using configurable criteria and outputs the top N
- Outputs the peaks regions as a new image
- Generates a results table
- Marks peak centres using ImageJ point ROIs
- Saves results text table and images to a directory
- All functionality is scriptable
- Efficient two-stage algorithm is fast

Overview

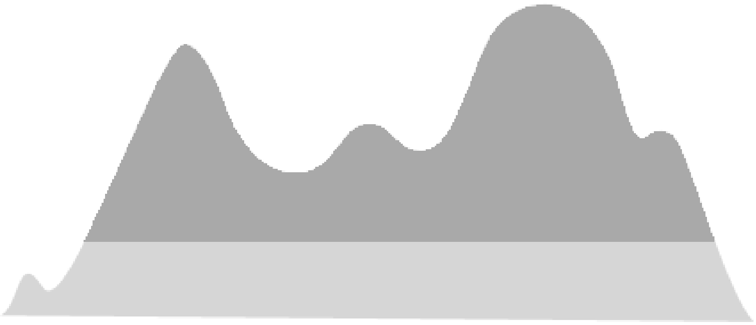
The Find Peaks plugin finds all the points of maximum intensity and expands them into regions. The initial regions are processed using merging criteria to produce a cleaner final result.

The following diagram shows an intensity profile to which the peak finding algorithm will be applied.



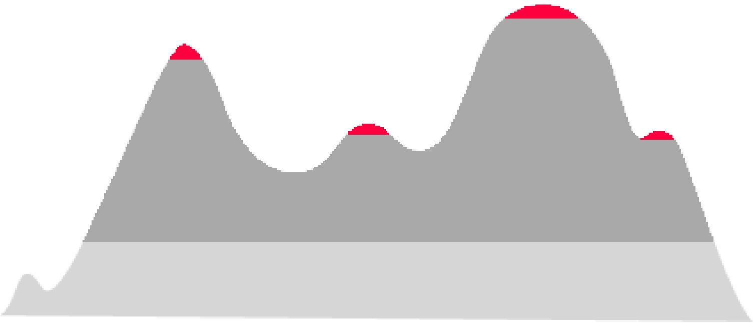
Stage 1: Assign Background

Assign the background level (all pixels below this level are ignored).



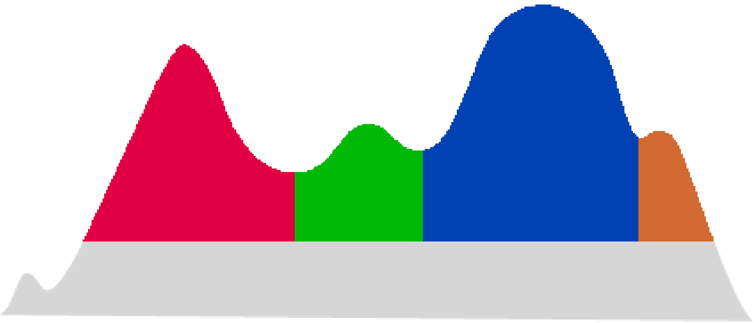
Stage 2: Assign Maxima

Find the points with an intensity higher than all the surrounding points. These local maxima are potential peaks.



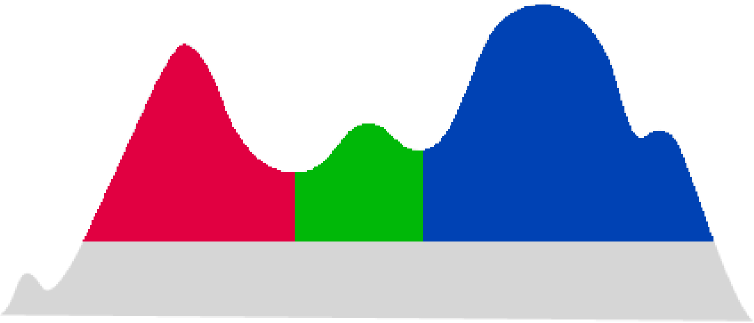
Stage 3: Expand Peaks

Expansion is then performed by progressively assigning points of lower intensity to the peak area above it. This allows all points to be processed in a single pass from the highest to the lowest. The result is an initial assignment of all the points above the background to a peak.



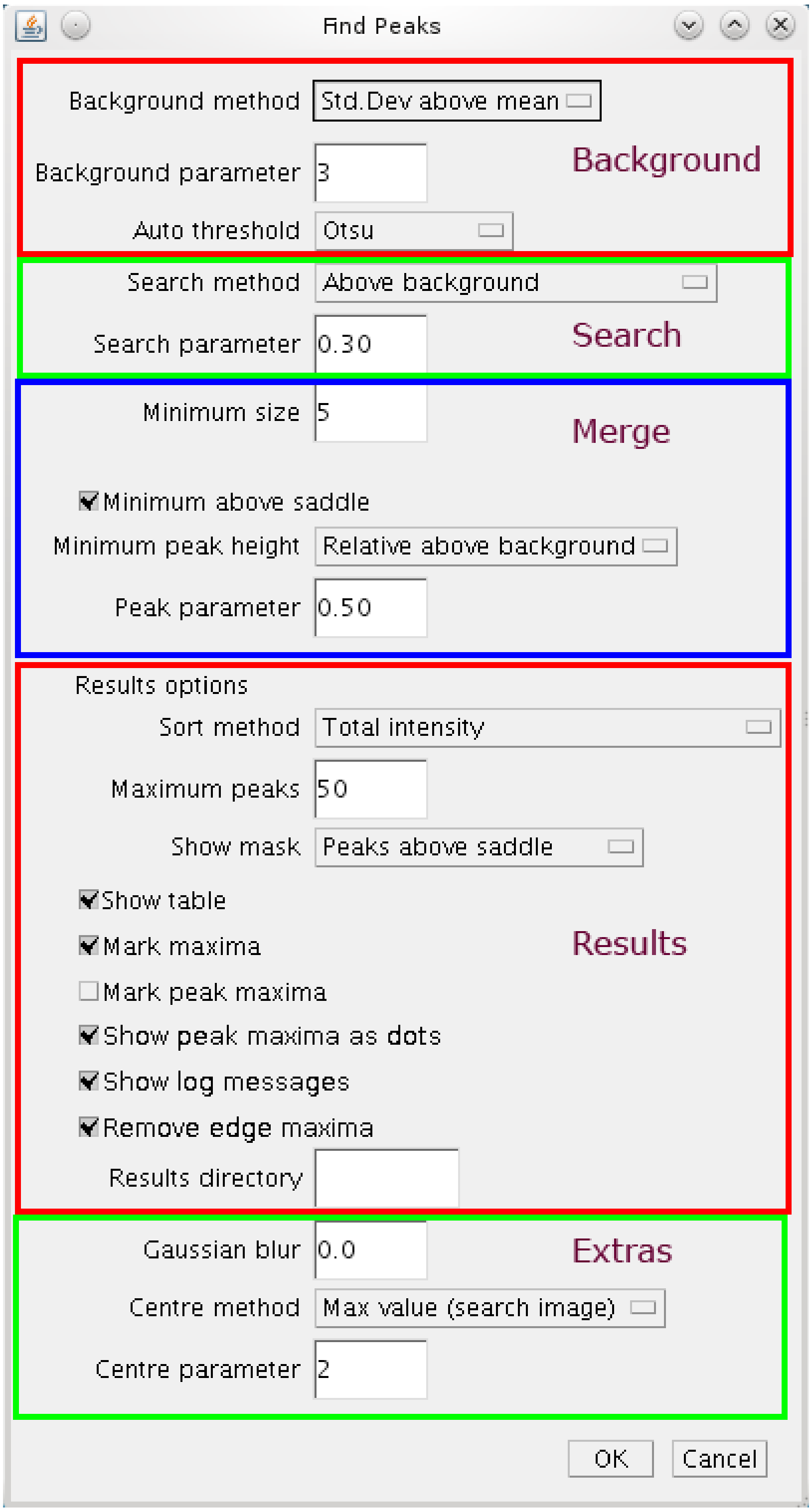
Stage 4: Merge Peaks

The final stage is to merge insignificant peaks into their parent peak. This can be done if the sub-peak is not high enough or covers insufficient area. This stage is optional but can improve results on noisy images.



Plugin Interface

The Find Peaks interface uses the standard ImageJ Generic Dialog. The plugin has many options but these can be divided into sections controlling parts of the algorithm. The different sections are shown in the following image and are described below.



Find Peaks

Background method: Std.Dev above mean

Background parameter: 3

Auto threshold: Otsu

Search method: Above background

Search parameter: 0.30

Minimum size: 5

☑ Minimum above saddle

Minimum peak height: Relative above background

Peak parameter: 0.50

Results options

Sort method: Total intensity

Maximum peaks: 50

Show mask: Peaks above saddle

☑ Show table

☑ Mark maxima

□ Mark peak maxima

☑ Show peak maxima as dots

☑ Show log messages

☑ Remove edge maxima

Results directory:

Gaussian blur: 0.0

Centre method: Max value (search image)

Centre parameter: 2

Background Parameters

The background parameters set the lowest intensity level used in the search for peak areas. Pixels that are below this level will not be included in the peak areas.

A simple approach would set the background to 0 (zero). However this means that all pixels in the image must be processed resulting in a slower runtime and the identification of many false peaks with insignificant heights. The plugin therefore provides different options for setting a background above zero.

The background parameters section contains three input fields: the main field (Background method) allows the selection of the background method and the other two fields pass parameters to the selected method if applicable.

Background Method	Description
Absolute	The background intensity is set using the input value in the Background parameter text field
Mean	The background intensity is set using the mean of the image
Std.Dev above mean	The background intensity is set using the mean of the image plus the Background parameter field multiplied by the standard deviation of the image
Auto threshold	The background intensity is set using the Auto-threshold method. The Auto threshold option uses the Auto Threshold plugin developed by Gabriel Landini (see http://pacific.mpi-cbg.de/wiki/index.php/Auto_Threshold). The plugin uses various methods to partition an image into foreground and background. Only the foreground pixels are then used in the peak finding algorithm. In most cases the Otsu method provides a fast and robust partitioning.
None	The background intensity is set as 0. Equivalent to using Absolute with a value of zero

Search Parameters

The search parameters control how far the algorithm expands local maxima into peak regions. This can be used to reduce the area of peaks on an image to only part of the peak above the background. In an image that has hundreds of peaks this can make the peaks easier to view distinctly in the output image.

The section contains two input fields: the main field (Search method) allows the selection of the search method and the other field (Search parameter) passes a parameter to the selected method if applicable.

Search Method	Description
Above background	A region is grown until the intensity drops below the background (default method)
Half peak value	A region is grown until the intensity drops to halfway between the value at the peak (the seed for the region) and the background level. This is equivalent to using the Fraction of peak - background option with the threshold value set to 0.5
Fraction of peak - background	A region is grown until the intensity drops to:

background + (parameter value) * (peak height)

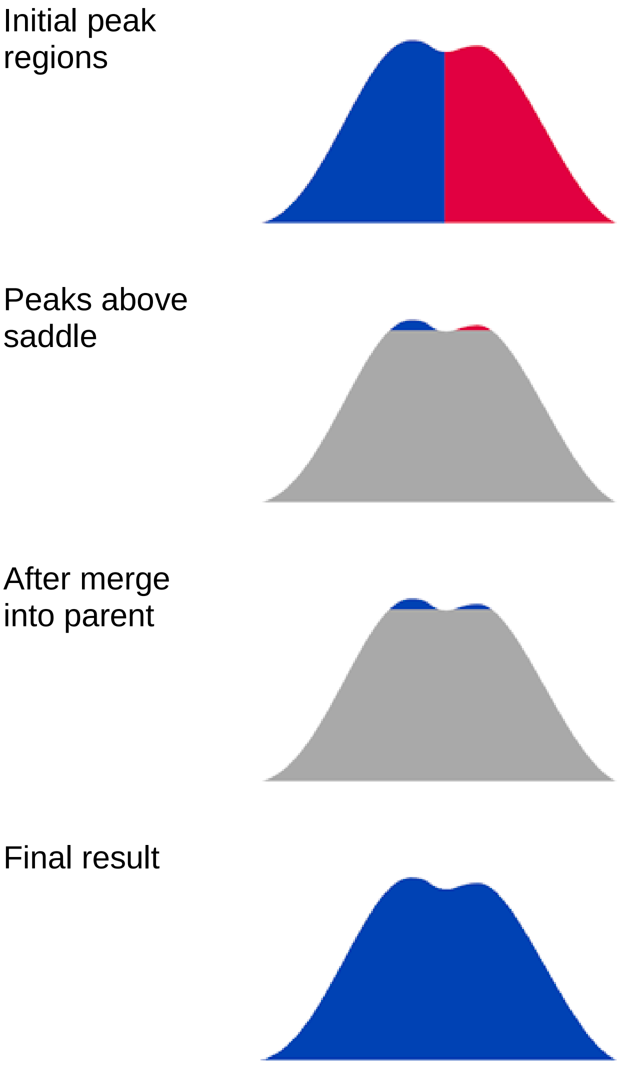
E.g. a Search parameter of 0.2 would grow the peak to 80% of its height above the background.

Peak Merging Parameters

The peak merging parameters are applied after the initial pass of the algorithm has identified all the peak regions. The parameters are used to control removal of insignificant peaks from the results.

Insignificant peaks are only removed if they have no neighbour peaks. If they have a neighbour then the peak's pixels are added to the highest neighbour peak (the parent peak). This is effectively merging a sub-peak into the larger peak.

The following diagram shows the merging process:



Initial peak regions

Peaks above saddle

After merge into parent

Final result

Peaks can be merged using two criteria: the peak size and the peak height.

Peak size

The Minimum size parameter sets the minimum size of a peak. If the peak is below this size then the peak is merged/removed. Set to zero to show all peaks including isolated local maxima.

The Minimum size above saddle optional restricts the peak size criteria to the pixels above its contact with a neighbour peak (the saddle point). This can be useful for eliminating sub-peaks which may only have a few pixels above the saddle to the parent peak but have been allocated a large number of pixels below the saddle height.

Peak Height

The peak height sets a minimum height that the peak must be above the highest saddle point, i.e. how far the peak stand out from any neighbours.

The peak height contains two input fields: the main field (Minimum peak height) allows the selection of the method and the other field (Peak parameter) passes a parameter to the selected method if applicable.

Minimum Peak Height	Description
Absolute Height	The peak must be an absolute height above the highest saddle point. The height is specified by the Peak parameter
Relative Height	The peak must be a relative height above the highest saddle point. The height is calculated as peak intensity * parameter value. E.g. a value of 0.2 indicates that the peak must contains at least 20% of its total height above the saddle point
Relative above background	The peak must be a relative height above the highest saddle point. The height is calculated as (peak intensity – background) * parameter value. E.g. a value of 0.2 indicates that the peak must contains at least 20% of its height over the background above the saddle point

Note that the if the peak has no neighbours, i.e. they have no saddle points, then the peak height limit is applied relative to the background.

Results Parameters

The results parameters control how the results will be displayed within ImageJ.

The Sort method options correspond to different columns of the results table (see the results table section). This allows the peaks to be sorted by any column in the table.

Maximum peaks specifies a limit (N) on the number of peaks that will be reported. Peaks are ranked using the specified sort method and then the top N are selected.

The Show mask parameter sets the type of output image to display. The image will be displayed in a image window with the original image title plus 'Find Peaks'. If this window exists (i.e. the plugin has already been run on the input image) then the plugin will update the existing window. Therefore you should save your result mask image if you do not wish it to be overwritten.

The mask is shown as a greyscale image with value 0 corresponding to no peak region. ImageJ's max display settings are adjusted so that the highest value is white. In all output mask images the peak maxima (X,Y,Z coordinates) are shown as white pixels.

The mask options are shown below:

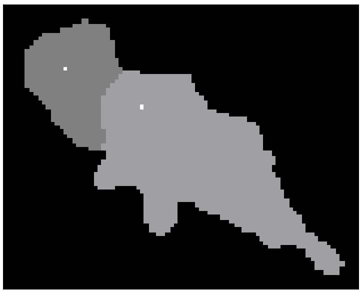
Result **Description**

None

No mask image is shown

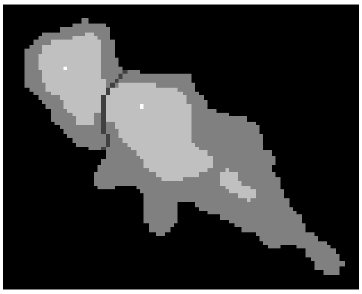
Peaks

The mask image contains all the pixels in the peak regions. Peak regions are assigned a value that corresponds to their ranking. The highest ranked peaks are shown as the lightest colours. The mask value for each peak is shown in the results table



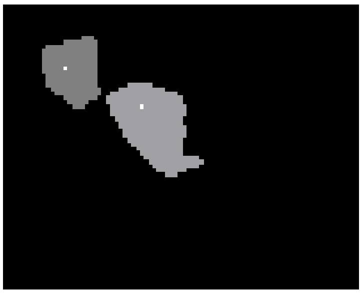
Threshold

The mask image contains all the pixels in the peak regions. Each peak region is thresholded using the auto-threshold method. This shows the high parts of the peak in a light shade and the low parts in a dark shade. The saddle pixels between peaks are shown in a dark grey



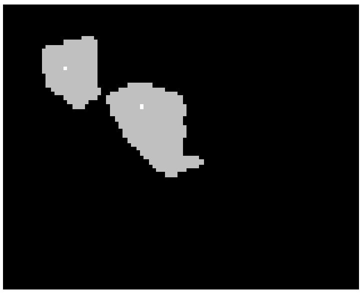
Peaks above saddle

The mask image contains all the pixels in the peak region above the highest saddle point. Peaks are coloured as the Peaks output above



Threshold above saddle

The mask image contains all the pixels in the peak region above the highest saddle point. Peaks are coloured as the Threshold output above



There are several flags for the results parameters as shown below:

Show table

Show the results table. This contains all of the information on the peak regions including height, centre, area and intensity. Note that if a results table is already present the results will be appended

Mark maxima

Label the maxima on the input image using ImageJ point ROIs. Existing ROIs will be deleted

Mark peak maxima

Label the maxima on the result mask image using ImageJ point ROIs

Show peak maxima as dots

If this option is selected then the pixel location at the centre of the maxima will be given the maximum value displayed in the mask (this can be used instead of Mark peak maxima). Disable this if the mask will be used for region labelling based on pixel value

Show log messages

Allow the plugin to write messages to the ImageJ log window. This provides additional information on the peak finding algorithm

Remove edge maxima

Removes any maxima from the results that contain pixels at the image border. This leave only peaks that are entirely within the image

The Results directory parameter allows the user to specify a directory to use to save the results. The directory must exist otherwise the plugin ignores the parameter. If the directory exists then the plugin will create 3 files named with the prefix FindPeaksYYYYMMDD_HHmmss (the datestamp ensures that files will not be over-written). The 3 files are:

1. .txt: A text file containing the results as written to the result table (the Show table option is not required)
2. .roi: An ImageJ ROI file. This uses ImageJ's ROI format. It can be loaded into ImageJ using the ROI manager
3. .params: A text file containing all the parameters used to run the plugin including the original input image filename

Extra Parameters

The Gaussian blur parameter is used to apply a Gaussian blur to the input image before running the peak finding algorithm. This is very useful for noisy images since it can eliminate peaks that are single pixel local maxima by smoothing the image content. This dramatically improves the algorithm speed due to the smaller number of merging steps required to eliminate all the sub-peak regions. Note that if a Gaussian blur is applied the final results are still calculated using the original image (e.g. the total intensity values).

The Centre method contains two input fields: the main field allows the selection of the method used to calculate the centre of the peak and the other field passes a parameter to the centre method if applicable.

Centre Method	**Description**
Max value (search image)	Define the peak centre using the highest pixel value of the search image (default). In the case of multiple highest value pixels, the closest pixel to the geometric mean of their coordinates is used
Max value (original image)	Re-map peak centre using the highest pixel value of the original image. If no blur is applied then this matches the result using the search image
Centre of mass	Re-map peak centre using the peak centre of mass (COM). The COM is computed within a given volume of the highest pixel value. Only pixels above the saddle height are used to compute the fit. The volume dimensions are specified using 2xN+1 where N is the Centre parameter
Gaussian	Re-map peak centre using a Gaussian fit. Only pixels above the saddle height are used to compute the fit. The fit is performed in 2D using a projection along the z-axis. If the centre parameter is 1 a maximum intensity projection is used; else an average intensity project is used. The z-coordinate is computed using the centre of mass along the projection axis located at the xy centre. Note: Gaussian fitting functionality is experimental. This method rarely produces better results than the simpler methods above since a large peak is required for a fit. In addition the current method does not fit the correlation between axes and so poorly models elliptical peaks. If no fit is possible then the peak is not remapped from the 'Max value (search image)' result

Results Table

The results table contains details about each peak region. The table contains the following information:

Result	**Description**
Peak #	The number of peak
Mask Value	The value used for the peak in the mask image (applies to the mask Peaks option)
X, Y, Z	The peak maxima location in X, Y and Z coordinates

Size	The number of pixels in the peak
Max	The maximum intensity value of the peak
Total	The total intensity of pixels in the peak
Saddle value	The intensity value of the highest saddle point
Saddle Id	The Id of the neighbour peak sharing the saddle
Abs.Height	The height of the peak above the highest saddle
Rel.Height (>Bg)	The relative height above the background: (absolute height) / (max value – background) i.e. The amount of the peak height that is above the saddle point
Size > saddle	The number of pixels above the highest saddle
Total > saddle	The total intensity above the highest saddle
Av	The average intensity of the peak
Total (>Bg)	The total intensity in the peak above the background level
Av (>Bg)	The average intensity in the peak above the background level
% Signal	The total intensity divided by the total image intensity
% Signal (>Bg)	The total intensity above background divided by the total image intensity above the background
Signal / Noise	The peak maximum value divided by the background

Note: Although not all the columns are listed in the Sort method parameter, some values are derived from others and it should be possible to order the results using any column.

Find Peaks Frame

ImageJ plugin that finds areas of maximum intensity in 2D and 3D images. The frame passes the selected parameters to the Find Peaks plugin for processing.

Features

- Permanent frame within ImageJ
- Allows different images to be selected
- Runs the Find Peaks algorithm to identify peak regions
- Supports the ImageJ macro recorder

Note that the the plugin supports the ImageJ macro recorder for the Find Peaks command. This means that a user can record their actions with the Find Peaks Frame within a macro and it will successfully record the execution of the Find Peaks plugin.

Plugin Interface

The Find Peaks Frame uses a Java frame within the ImageJ application. The plugin supports all the options of the Find Peaks plugin. Full details of the parameters can be found in the Find Peaks Plugin Interface section.

Since the interface is not limited by the layout of the standard ImageJ dialogue the design has been altered. The window is smaller and several less used options are accessed by clicking the 'Advanced options …' button. The smaller size makes the plugin a better option to use on monitors with a low resolution.

The interface has a drop-down menu that is used to select the image. This allows the user to quickly try the same parameters on different images that are open. The image list is populated with the currently open 8-bit and 16-bit greyscale images. Any images with the title ending in 'Find Peaks' (i.e. previous results) are also ignored.

The interface has been designed to only allow the user to set the options that are relevant. For example it is only possible to set the Threshold method when the Background method is set to Auto threshold. This makes it easier for a user to set the parameters for the algorithm.

The following image shows the interface for the Find Peaks Frame:

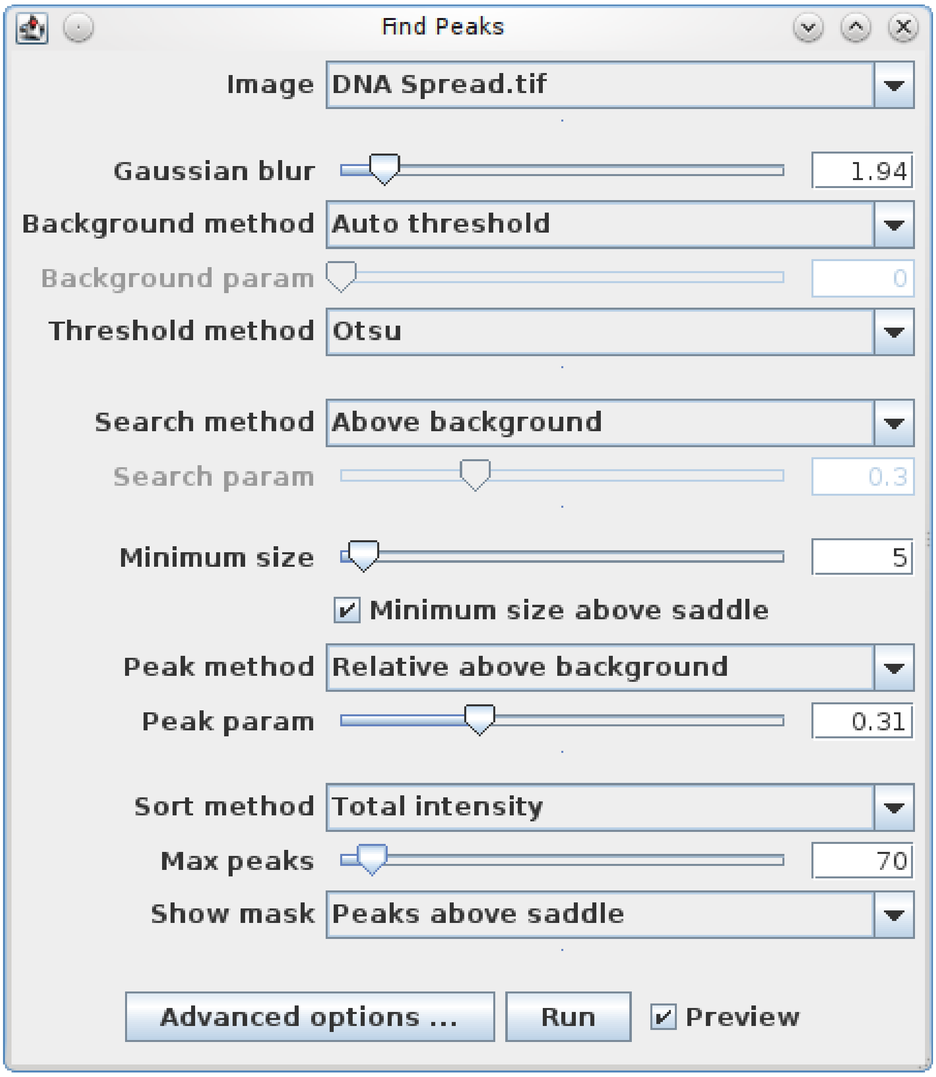
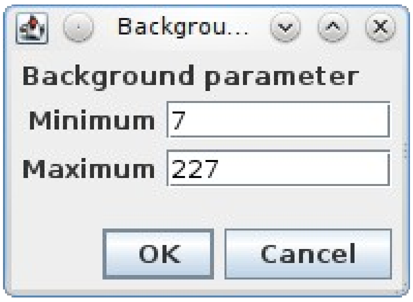


Image: DNA Spread.tif
Gaussian blur: 1.94
Background method: Auto threshold
Background param: 0
Threshold method: Otsu
Search method: Above background
Search param: 0.3
Minimum size: 5
☑ Minimum size above saddle
Peak method: Relative above background
Peak param: 0.31
Sort method: Total intensity
Max peaks: 70
Show mask: Peaks above saddle
☑ Preview

The interface has sliders to adjust the numeric parameters. The parameter can also be entered using the input text box. The range for the sliders are set to a default scale depending on the parameter. For example the background parameter is limited to the minimum and maximum values found in the image. The range for the slider can be updated by double-clicking the slider. This will display a dialog where the range can be updated:



Background parameter
Minimum: 7
Maximum: 227

Preview

The Find Peaks Frame interface has a preview option. If this option is checked then the plugin will automatically update the results based on any changes to the parameters. The plugin stores intermediate results in memory. When a parameter changes the plugin identifies the stages of the calculation that must be re-run and updates the results.

The preview provides the ability to change the parameters and quickly see the effects on the output mask and results table. However since the full Find Peaks plugin is not run there is no output to the ImageJ log window and the command is not recorded to the ImageJ macro recorder. Only the RUN button supports these options.

The preview option requires a large amount of memory to store the intermediate results. It may not be suitable for large stack images depending on the configuration of your system. The memory used by the preview is released when the preview option is unchecked. This method can also be used to reinitialise the preview in the event that it has stopped responding to parameter changes.

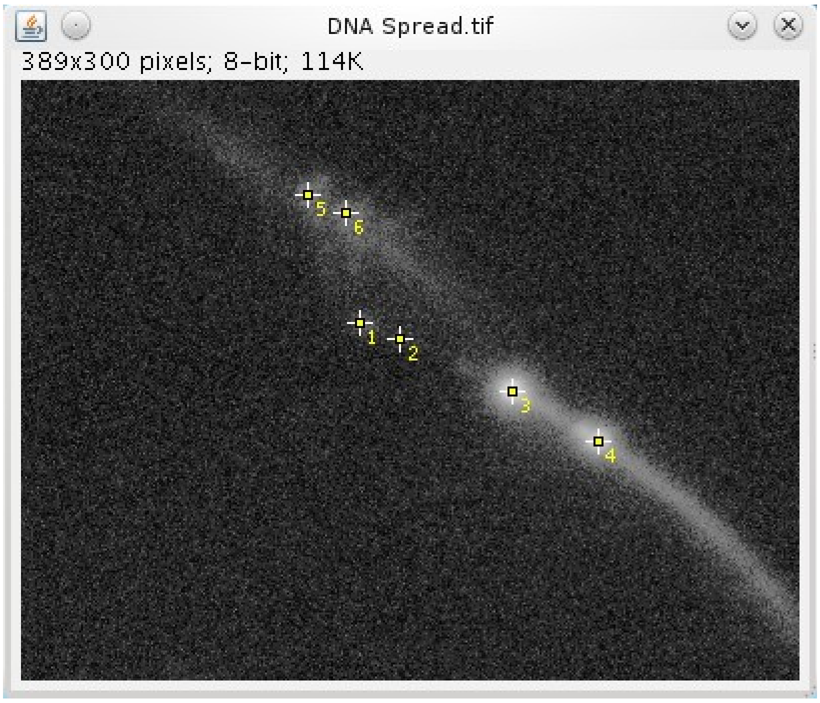
Java Requirements

The Find Peaks Optimiser Frame uses the BeansBinding Java framework. More details can be found in Appendix 1: BeansBinding Java framework.

Find Peaks Optimiser

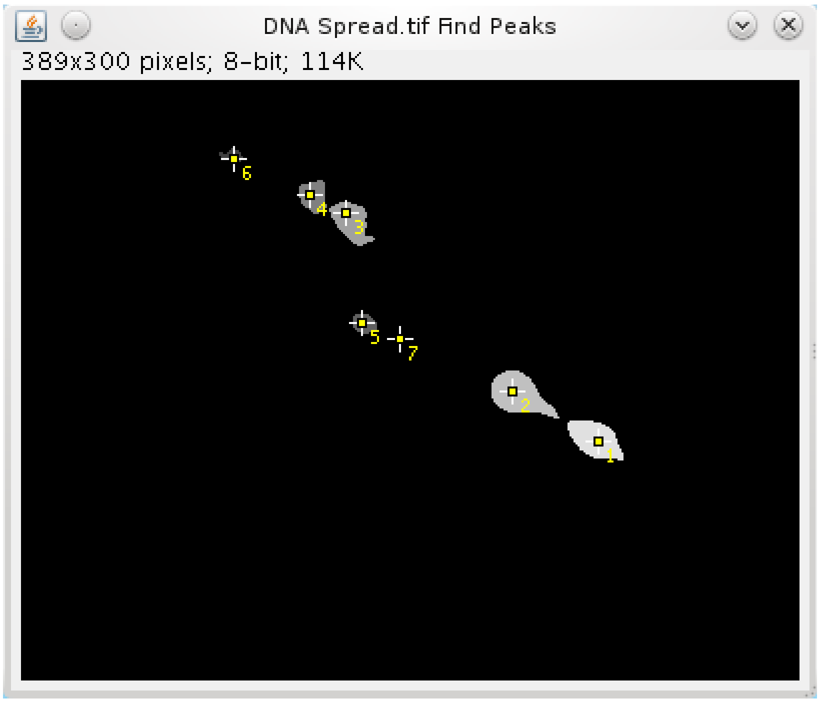
ImageJ plugin that iterates through combinations of parameters for the Find Peaks plugin. Results are compared to an optimal reference image and the best parameters to match the reference result are identified.

Example Input

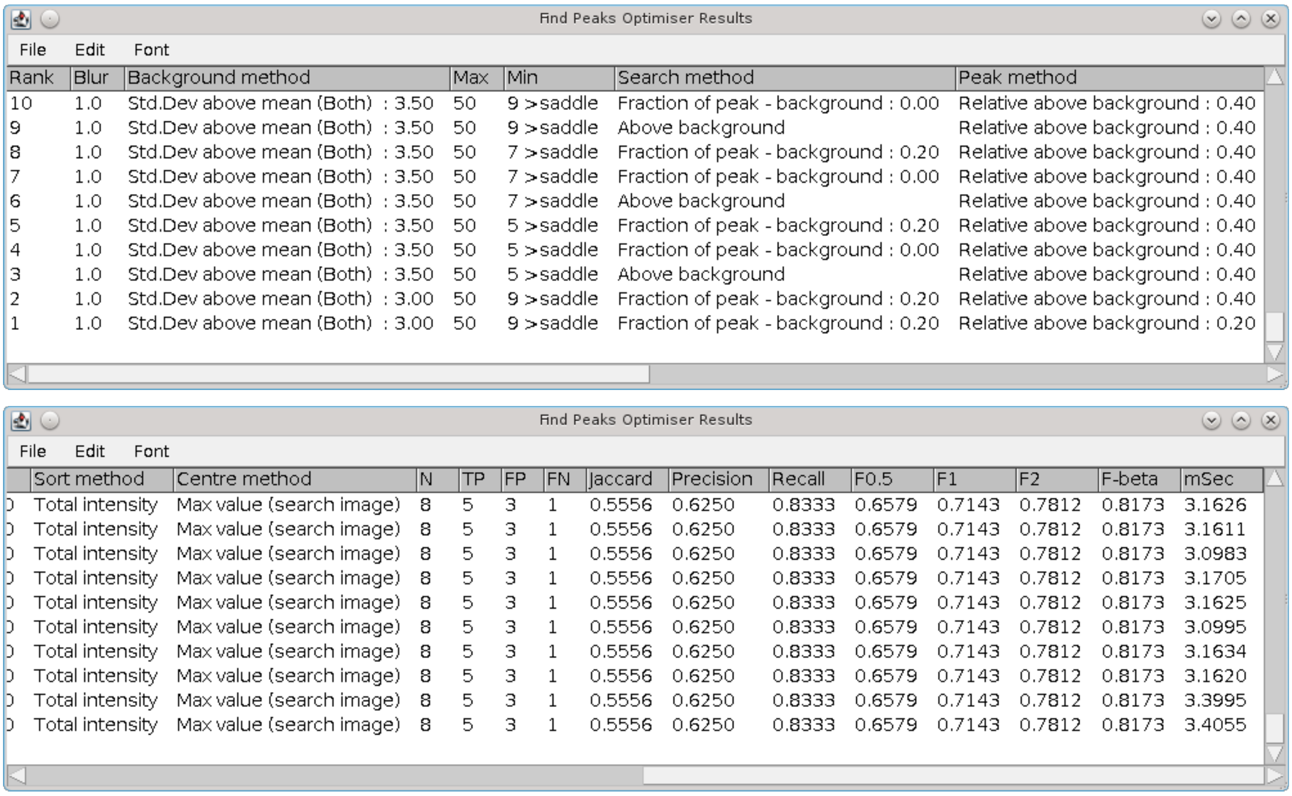


Input image with manually identified peaks. These are used a reference points for optimisation of the Find Peaks algorithm.

Example Output



Output image showing the peaks identified with the best match to the input image.



Rank	Blur	Background method	Max	Min	Search method	Peak method
10	1.0	Std.Dev above mean (Both) : 3.50	50	9 >saddle	Fraction of peak - background : 0.00	Relative above background : 0.40
9	1.0	Std.Dev above mean (Both) : 3.50	50	9 >saddle	Above background	Relative above background : 0.40
8	1.0	Std.Dev above mean (Both) : 3.50	50	7 >saddle	Fraction of peak - background : 0.20	Relative above background : 0.40
7	1.0	Std.Dev above mean (Both) : 3.50	50	7 >saddle	Fraction of peak - background : 0.00	Relative above background : 0.40
6	1.0	Std.Dev above mean (Both) : 3.50	50	7 >saddle	Above background	Relative above background : 0.40
5	1.0	Std.Dev above mean (Both) : 3.50	50	5 >saddle	Fraction of peak - background : 0.20	Relative above background : 0.40
4	1.0	Std.Dev above mean (Both) : 3.50	50	5 >saddle	Fraction of peak - background : 0.00	Relative above background : 0.40
3	1.0	Std.Dev above mean (Both) : 3.50	50	5 >saddle	Above background	Relative above background : 0.40
2	1.0	Std.Dev above mean (Both) : 3.00	50	9 >saddle	Fraction of peak - background : 0.20	Relative above background : 0.40
1	1.0	Std.Dev above mean (Both) : 3.00	50	9 >saddle	Fraction of peak - background : 0.20	Relative above background : 0.20

Sort method	Centre method	N	TP	FP	FN	Jaccard	Precision	Recall	F0.5	F1	F2	F-beta	mSec
Total intensity	Max value (search image)	8	5	3	1	0.5556	0.6250	0.8333	0.6579	0.7143	0.7812	0.8173	3.1626
Total intensity	Max value (search image)	8	5	3	1	0.5556	0.6250	0.8333	0.6579	0.7143	0.7812	0.8173	3.1611
Total intensity	Max value (search image)	8	5	3	1	0.5556	0.6250	0.8333	0.6579	0.7143	0.7812	0.8173	3.0983
Total intensity	Max value (search image)	8	5	3	1	0.5556	0.6250	0.8333	0.6579	0.7143	0.7812	0.8173	3.1705
Total intensity	Max value (search image)	8	5	3	1	0.5556	0.6250	0.8333	0.6579	0.7143	0.7812	0.8173	3.1625
Total intensity	Max value (search image)	8	5	3	1	0.5556	0.6250	0.8333	0.6579	0.7143	0.7812	0.8173	3.0995
Total intensity	Max value (search image)	8	5	3	1	0.5556	0.6250	0.8333	0.6579	0.7143	0.7812	0.8173	3.1634
Total intensity	Max value (search image)	8	5	3	1	0.5556	0.6250	0.8333	0.6579	0.7143	0.7812	0.8173	3.1620
Total intensity	Max value (search image)	8	5	3	1	0.5556	0.6250	0.8333	0.6579	0.7143	0.7812	0.8173	3.3995
Total intensity	Max value (search image)	8	5	3	1	0.5556	0.6250	0.8333	0.6579	0.7143	0.7812	0.8173	3.4055

Result table showing the match scoring statistics comparing the identified peaks to the reference image for each set of parameters.

Features

- Enumerates combinations of parameters for the Find Peaks plugin
- Compares the results to a reference image
- Reports the best parameters to identify the reference peaks
- Save the results table to file
- Staged processing allows fast enumeration of parameters

Overview

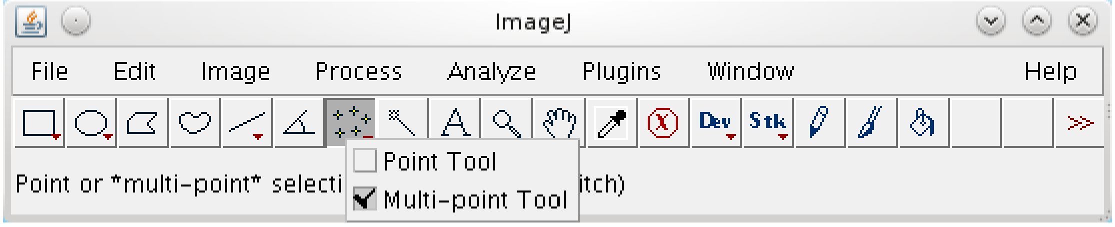
The Find Peaks Optimiser provides the ability to set multiple options for each parameter of the Find Peaks plugin. In the case of selection inputs and flags it is possible to specify more than one option for each parameter. In the case of numeric inputs it is possible to select a lower limit, upper limit and increment to use, e.g. 0 to 10 in 2 step intervals. The optimiser then runs the Find Peaks plugin for all the combinations of parameters.

Since the Find Peaks algorithm can be broken down into stages (background thresholding, maxima identification, peak growing and peak merging) it is possible to runs only the parts of the algorithm that differ when changing a subset of the parameters. This allows the optimiser to efficiently run thousands of combinations of parameters.

Each run of the Find Peaks plugin is compared to a reference image with marked maxima and scored using various metrics. The final results are ranked and the best parameters identified.

Stage 1: Mark Reference Image

The optimiser requires a reference image where peaks have been marked using the ImageJ ROI tool. Select the Multi-point tool by right-clicking on the Point Tool option in the toolbar and selecting the Multi-point Tool option.



Point or *multi-point* selecti itch)

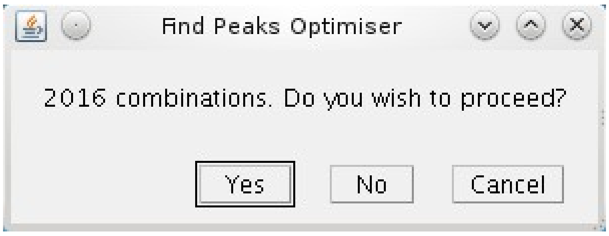
Open a reference image. Click the image to mark a maxima. Points can be moved by hovering over an existing point and dragging them. Points can be deleted by holding down the ALT key and clicking them.

Once the image maxima have been labelled you can save the image as a TIFF. ImageJ will save the ROI points to the image and they will be shown when next the image is next opened.

Stage 2: Run the Optimiser

Select the different parameters for the optimiser to enumerate.

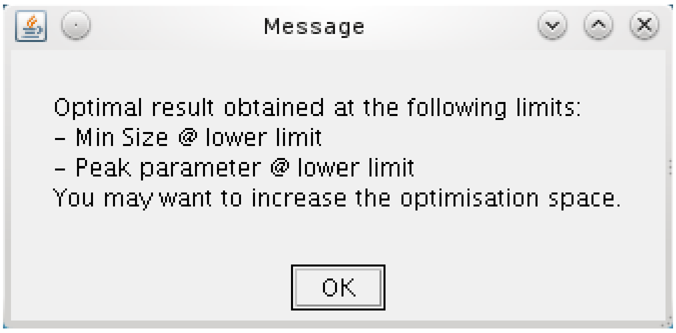
Click OK and the optimiser will show a dialogue containing the number of combinations. You can then select to continue or cancel to stop the optimiser and change the parameters.



2016 combinations. Do you wish to proceed?

If you run the optimiser a progress bar will show in the ImageJ window that tracks the number of combinations currently completed. When the optimiser is finished a results window will appear showing the top N results. The number of results can be configured in the optimiser dialogue.

If the best result is obtained using a numeric parameter that lies on an extreme of the test range then the plugin will show a warning message:



Optimal result obtained at the following limits:
- Min Size @ lower limit
- Peak parameter @ lower limit
You may want to increase the optimisation space.

This allows the user to increase the range to ensure that the optimal parameters are identified.

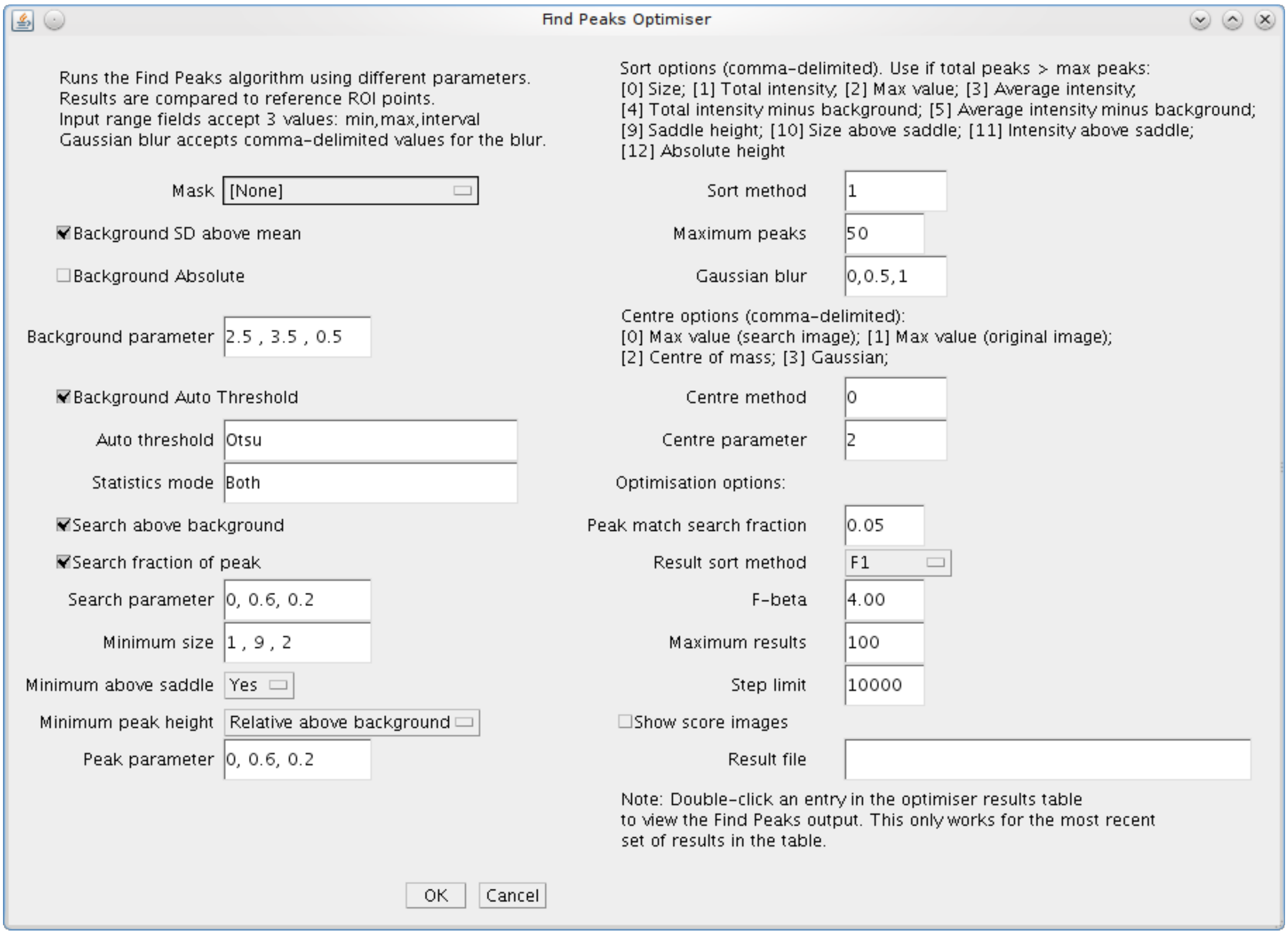
Result Assessment

The optimiser scores the results of the Find Peaks plugin by comparing the locations of the identified maxima to the reference image. Maxima are deemed to be correct if they are within a set distance of a reference point. The cut-off distance is configured in the plugin options (default is 5% of the image width).

The maxima assignment process is iterative. The maxima closest to any reference point is assigned as a match and the pair removed from the search. This is iterated until no more reference points exist, or all maxima are too far from a reference point to be a match. The results are then scored using the metrics detailed in Appendix 2: Binary Scoring Statistics.

Plugin Interface

The Find Peaks Optimiser interface uses the standard ImageJ Generic Dialog. The plugin has many options but these can be divided into sections controlling parts of the algorithm. The different sections are shown in the following image and are described below. Full details for each of the parameters can be found in the Find Peaks Plugin Interface section.



Find Peaks Optimiser

Runs the Find Peaks algorithm using different parameters.
Results are compared to reference ROI points.
Input range fields accept 3 values: min,max,interval
Gaussian blur accepts comma-delimited values for the blur.

Mask [None]

☑ Background SD above mean

□ Background Absolute

Background parameter 2.5 , 3.5 , 0.5

☑ Background Auto Threshold

Auto threshold Otsu

Statistics mode Both

☑ Search above background

☑ Search fraction of peak

Search parameter 0, 0.6, 0.2

Minimum size 1 , 9 , 2

Minimum above saddle Yes

Minimum peak height Relative above background

Peak parameter 0, 0.6, 0.2

Sort options (comma-delimited). Use if total peaks > max peaks:
[0] Size; [1] Total intensity; [2] Max value; [3] Average intensity;
[4] Total intensity minus background; [5] Average intensity minus background;
[9] Saddle height; [10] Size above saddle; [11] Intensity above saddle;
[12] Absolute height

Sort method 1

Maximum peaks 50

Gaussian blur 0,0.5,1

Centre options (comma-delimited):
[0] Max value (search image); [1] Max value (original image);
[2] Centre of mass; [3] Gaussian;

Centre method 0

Centre parameter 2

Optimisation options:

Peak match search fraction 0.05

Result sort method F1

F-beta 4.00

Maximum results 100

Step limit 10000

□ Show score images

Result file

Note: Double-click an entry in the optimiser results table
to view the Find Peaks output. This only works for the most recent
set of results in the table.

Initialisation Parameters

The Find Peaks algorithm must be initialised with the image pixels that are to be processed. The pixels are then used to calculate image statistics and a background level for the peak finding algorithm. The optimiser will run the Find Peaks plugin for each background option that is enabled. There are addition fields that allow parameters to be passed to the background method and control the pixels used for the statistics.

Initialisation Option	Description
Mask	Optionally select a mask to define the pixels to process. Only images with the same dimensions as the input image are listed. Any non-zero pixel is included in the analysis
Background SD above mean	Use the SD above Mean background method. It is not allowed to use this at the same time as the Absolute method
Background Absolute	Use the Absolute background method.

	It is not allowed to use this at the same time as the SD above Mean method
Background parameter	Set the lower limit, upper limit and increment for enumerating the background parameter passed to the background method. This applies to either SD above mean or Absolute
Background Auto Threshold	Use the Auto Threshold background method
Auto threshold	The method for performing the Auto Threshold calculation. The text field supports a comma-delimited list for multiple options
Statistics mode	When a mask image is used it is possible to set the region used to calculate the image statistics: Inside – Use the pixels inside the image mask Outside – Use the pixels outside the image mask Both – Use all the pixels in the image When no mask image is used this option is ignored

Search Parameters

The optimiser will run the Find Peaks plugin for each search option that is enabled. There is an addition field that allows a parameter to be passed to the search method.

Search Option	Description
Search above background	Use the Search above background method
Search fraction of peak	Use the Fraction of peak – background method
Search parameter	Set the lower limit, upper limit and increment for enumerating the search parameter passed to the fraction of peak method

Peak Merging Parameters

The optimiser will run the Find Peaks plugin for each combination of the following peak merging options.

Peak Merge Option	Description
Minimum size	Set the lower limit, upper limit and increment for enumerating the minimum size parameter passed to the peak merge method
Minimum above saddle	Set the option for limiting the peak size to above the saddle. Select Yes; No; or Both
Minimum peak height	Set the method for setting the minimum peak height
Peak parameter	Set the lower limit, upper limit and increment for enumerating the parameter passed to the minimum peak height method

Results Parameters

Option	Description
Sort Method	Set the method used to sort the peaks before extracting the top N. Multiple options can be specified using a comma-delimited list
Maximum peaks	Set the maximum number of peak results

Extra Parameters

Control other options available within the Find Peaks plugin.

Option	Description
Gaussian blur	Set the size of the blur to apply before running the Find Peak algorithm. Multiple options can be specified using a comma-delimited list
Centre method	Set the method used to determine the centre of the peak. Multiple options can be specified using a comma-delimited list
Centre parameter	Set the lower limit, upper limit and increment for enumerating the parameter passed to the centre method

Optimisation Parameters

The Find Peaks Optimiser plugin has several options for controlling how the Find Peaks results are calculated and ranked.

Option	Description
Peak match search fraction	Set the distance limit used to determine if a peak maxima and a reference point are a match. This value is specified as a fraction of the image short edge, e.g. 0.05 for a 600x400 pixel image would be 20 pixels (400 x 0.05)
Result sort method	Specify the metric used to sort the results
F-beta	Specify the beta parameter used to calculate the custom F-score
Maximum results	Specify the maximum number of results to include in the result table
Step limit	The optimiser will not run if the total number of combinations (steps) is above the Step limit. This parameter can be used to increase the potential optimisation space

Additional Parameters

The Find Peaks Optimiser plugin has several options for controlling how the Find Peaks results are calculated and ranked.

Option	Description
Show score images	If enabled the optimiser will show three duplicates of the input image with ROI points marking the True Positives, False Positives and the False Negatives
Result file	Provide the prefix for the results files. The prefix should be a full path to a valid directory and include a filename prefix, e.g. /tmp/FindPeaks Results files will have a suffix added corresponding to the result: .results.txt – Contains the optimiser results .points.csv – Contains the peak coordinates from the best result

Range Limit Fields

The plugin has several text fields that accept a lower limit, upper limit and interval. The numbers must be comma-delimited. If three numbers are present then the plugin will assume that they are lower, upper and interval. Otherwise only the upper limit is set using the first number in the text field. This is equivalent to using a single value and not a range of values.

The following fields allow range limits:

- Background parameter
- Search parameter
- Minimum size
- Peak Parameter
- Centre parameter

The plugin has several text fields that accept multiple values in a comma-delimited list. The following fields allow a comma-delimited list:

- Auto-threshold
- Statistics mode
- Sort method
- Centre method
- Gaussian blur*
- The use of a list for Gaussian blur is due to the fact that changes in the blur radius are most noticeable at increasing increments, e.g. 1, 2, 4, 8, 16. In this case a range field with a linear increment is unwanted.

Output

The Find Peaks Optimiser records the parameters and results of each run of the Find Peaks algorithm. The best results are shown in a result table and the complete results can be saved to file. The plugin also runs the Find Peaks plugin using the optimal parameters on the input image to show the result peaks. Further details are outlined below.

Result Table

The result table shows the top scoring results from all the parameter combinations. The results are sorted using the Result sort method parameter and limited to the number specified by the Maximum results parameter. Results are presented in descending order so that the best result from the most recent analysis is shown at the bottom of the table.

In the event that two sets of parameters have an equal score then the parameters that have the most conservative settings are ranked first. The following logic is used:

- Lowest blur
- Lowest background threshold level
- Background method: None < Auto-threshold < Std.Dev above mean < Mean < Absolute
- Lowest minimum size
- Search method: Above background < Fraction of peak - background < Half peak value
- Lowest search parameter
- Peak method: Relative above background < Relative height < Absolute height
- Lowest peak parameter
- Lowest run time

The result table shows the result rank and then columns containing the parameters. The table then shows the following scoring metrics:

Metric	**Description**
N	The number of maxima in the result
TP (True Positives)	The number of maxima in the result that match a reference point
FP (False Positives)	The number of maxima in the result that do not match a reference point
FN (False Negatives)	The number of reference points that were not identified as maxima
Jaccard	Measure of the overlap similarity between the reference and the maxima
Precision	Measure of how many identified maxima are correct
Recall	Measure of how many reference points were identified
F-score	Combined score that provides a weighted measure of the prediction accuracy: β is a weighting factor between the Precision and Recall. The F-score is calculated using β of 0.5, 1, 2 and the provided input F-beta parameter.
mSec	The number of milliseconds taken to perform the calculation. If the metrics are identical between results then the fastest calculation is scored higher

Note that the metrics Jaccard, Precision, Recall and F-score all range from 0 to 1. Further details of the metrics can be found in Appendix 2: Binary Scoring Statistics.

A blank line is inserted at the end of the result table to allow easy visual separation of the results from multiple runs of the plugin.

Showing a result

By default the Find Peaks Optimiser shows the result of running the Find Peaks algorithm with the best result. However it is possible to show any result by double-clicking a row in the result table. The Find Peaks algorithm will then be re-run with the parameters from that row.

Note that it is only possible to view the results for the latest run of the optimiser. Parameters for prior runs are discarded.

Find Peak Images

Upon completion the optimiser runs the Find Peaks algorithm on the input image to display the peaks. To preserve the input image ROI points a copy is made of the original. This is named with the suffix ' clone'. Find Peaks is then run on the clone image to mark the peak ROI points. A mask image showing the peaks above the saddle points is also created with the suffix ' clone Find Peaks'.

Score Images

If the Show score images parameter is enabled then the input image is duplicated three times. These images are then marked with the ROI points for either the True Positives (TP), False Positives (FP) or False Negatives (FN).

If the optimiser is re-run on the same input image then the output result images will be detected (using the image title) and updated. This feature allows the images to be updated with the results of using the specified parameters when double-clicking on the result table (see section Showing a result).

Find Peaks Optimiser Frame

The plugin provides an ImageJ window frame that runs Find Peaks Optimiser on the selected image.

Features

- Permanent frame within ImageJ
- Allows different images to be selected
- Runs the Find Peaks Optimiser algorithm to identify peak regions
- Supports the ImageJ macro recorder

Note that the the plugin supports the ImageJ macro recorder for the Find Peaks Optimiser command. This means that a user can record their actions with the Find Peaks Optimiser Frame within a macro and it will successfully record the execution of the Find Peaks Optimiser plugin.

Plugin Interface

The Find Peaks Optimiser Frame uses a Java frame within the ImageJ application. The plugin provides a selection box of the currently open images that can be processed by the optimiser.

The following image shows the interface for the Find Peaks Optimiser Frame:

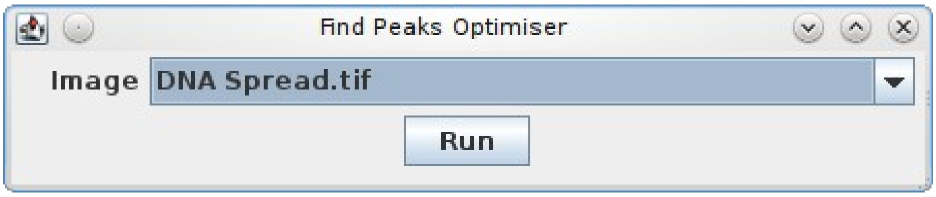


Image: DNA Spread.tif

Clicking the Run button launches the Find Peaks Optimiser on the selected image. Full details of the parameters can be found in the Find Peaks Optimiser Plugin Interface section.

The interface has a drop-down menu that is used to select the image. This allows the user to quickly try the same parameters on different images that are open. The image list is populated with the currently open 8-bit and 16-bit single channel images that have an ImageJ Point ROI. Any images with the title ending in 'Find Peaks' (i.e. previous results) are also ignored.

Java Requirements

The Find Peaks Optimiser Frame uses the BeansBinding Java framework. More details can be found in Appendix 1: BeansBinding Java framework.

Find Peaks Helper

An ImageJ plugin that aligns the manually added point Regions of Interest (ROIs) with the peaks found by the Find Peaks algorithm.

Features

- Processes 8- or 16-bit images
- Extracts a single channel and time frame for processing N-dimensional images
- Computes the image peaks using the Find Peaks algorithm
- Intercepts user clicks with the multi-point ROI tool and assigns the marked points to the true peaks
- Supports dragging points
- Outputs assignments to the ImageJ log window
- Optional output of a results table of point positions with intensity

Overview

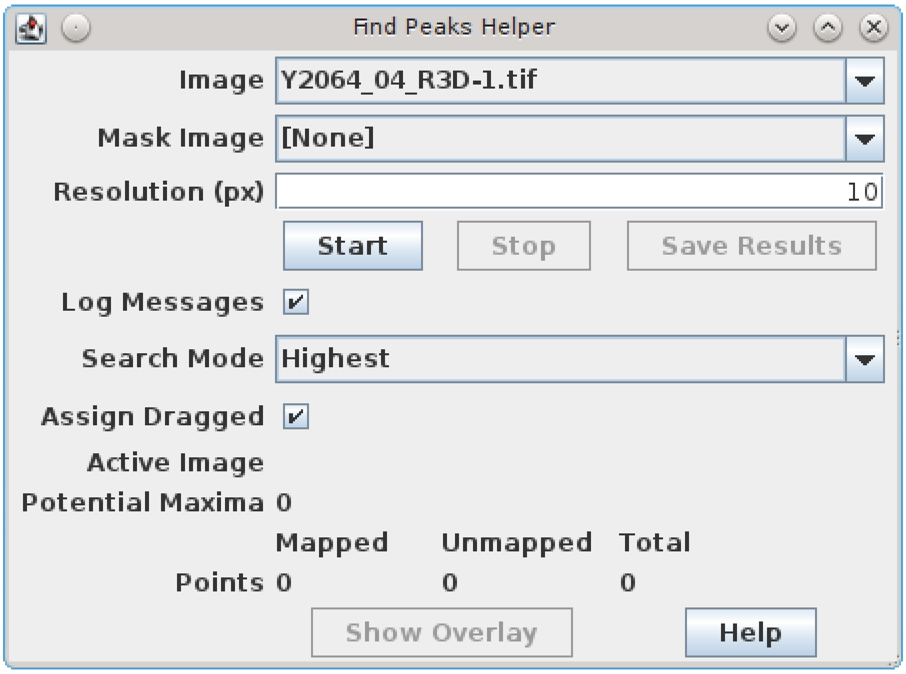
The Find Peaks Helper provides a semi-automated tool to assist in manually counting the peaks in an image. The tool is designed to work in conjunction with the ImageJ multi-point ROI tool.

The plugin must be attached to an image and initialised. During initialisation all the potential peaks in an image are calculated using the Find Peaks algorithm.

The plugin then listens for mouse-click events from the ImageJ multi-point ROI tool. If a new ROI point is added then the plugin attempts to align the point to an available peak within a set distance. The alignment is performed using the highest or the closest peak. If an existing point is moved then the plugin will detect the drag and attempt to align the point when it is dropped.

Plugin Interface

The Find Peaks Helper uses a Java frame within the ImageJ application. The following image shows the plugin window.



Find Peaks Helper

Image Y2064_04_R3D-1.tif

Mask Image [None]

Resolution (px) 10

Start Stop Save Results

Log Messages ☑

Search Mode Highest

Assign Dragged ☑

Active Image

Potential Maxima 0

	Mapped	Unmapped	Total
Points	0	0	0

Show Overlay Help

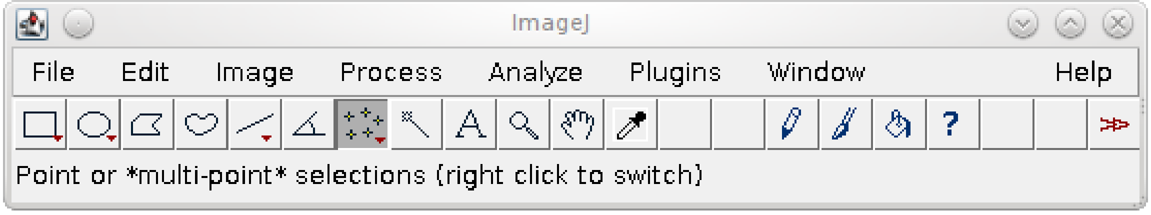
Parameters

Parameters	Description
Image	The target image
Mask Image	Optional mask image defining the region where points will be aligned
Resolution (px)	Defines the search radius for aligning a point (in pixels)
Log Messages	If selected record details of the point alignments to the ImageJ log window
Search Mode	Define the search mode for aligning points to the available maxima: Highest or Closest
Assign Dragged	If selected the plugin will re-align dragged points when they are dropped

Workflow

Multi-point ROI Mode

The ImageJ multi-point ROI tool must be activated. This can be found on the ImageJ toolbar as shown below:



Point or *multi-point* selections (right click to switch)

If the single-point ROI mode button is shown then the multi-point mode can be enabled by right-clicking the button and toggling the mode.

The multi-point ROI mode allows the user to manually mark multiple points on an image. Each point is represented by a cross and shows a numbered label (if more than 1 point is selected).

Initialisation

The Find Peaks Helper shows a list of the available images that can be processed. This will be all the 8- and 16-bit images. Select the image and the list of possible masks images will be updated. Any non-zero pixel in the mask defines the region of the image where potential maxima will be detected. The mask image is optional.

Next configure the resolution for point assignment. The resolution defines the search distance around the point that can be used to locate a maxima. The resolution is fixed once the helper is initialised.

Click Start to initialise the helper. The plugin supports images with multiple channels or time frames but will only calculate the peaks on a single channel and time point. If a multi-dimensional image is selected then the plugin will show a dialogue requesting the user to select the channel and/or frame to be processed.

The plugin will calculate all the potential maxima above the mean of the image using the Find Peaks algorithm. The mean is used as a reasonable threshold to avoid identifying false peaks.

If the image contains an existing multi-point ROI then the plugin will attempt to assign each point. If the Highest Search mode is enabled the plugin will process the points in descending height order. If the Closest Search mode is enabled the points will be processed using the closest pairing of point and maxima, iterating until no more assignments can be made.

The number of potential maxima along with the count of mapped and unmapped points will be displayed in the plugin window as shown below:

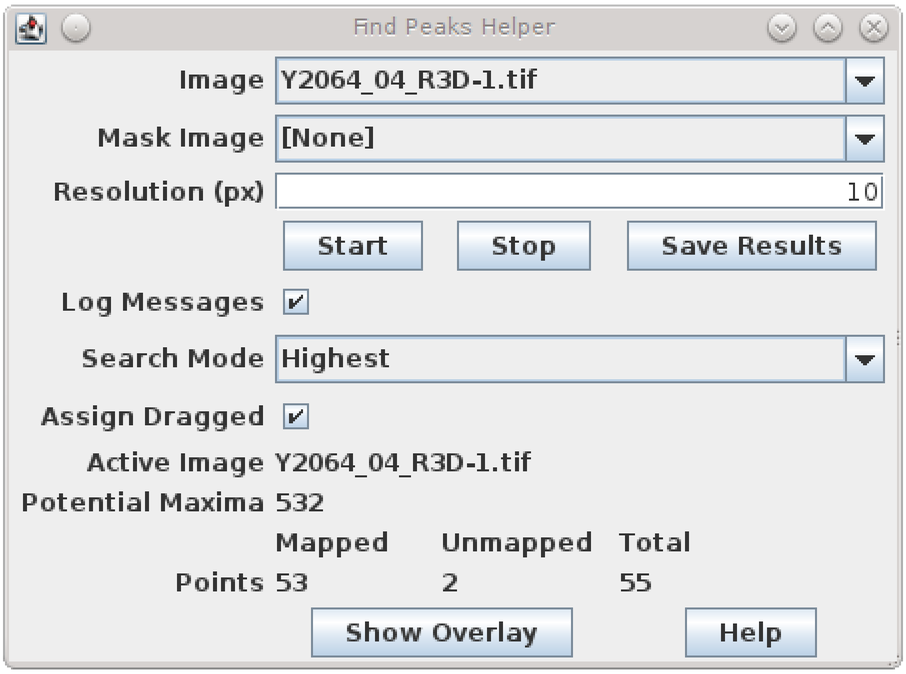


Image: Y2064_04_R3D-1.tif

Mask Image: [None]

Resolution (px): 10

Log Messages: ☑

Search Mode: Highest

Assign Dragged: ☑

Active Image: Y2064_04_R3D-1.tif

Potential Maxima: 532

	Mapped	Unmapped	Total
Points	53	2	55

Marking Peaks

Mark a new peak by clicking on the image. The plugin will search for the closest or highest unassigned maxima within the search resolution.

If a maxima is found the point will be moved to the maxima. The mapped count on the plugin interface will increment.

If no maxima is found then the point will be unchanged. The unmapped count on the plugin interface will increment.

Note that if the Search mode is set to highest then a check is made for the highest peak within the search radius. If present then the search resolution is updated to the distance to the highest assigned peak. This means that the plugin requires the user to click closer to a second unassigned peak than to an existing assigned high peak in close proximity. This prevents the plugin moving the clicked point past an already assigned peak to reach an unassigned peak. If the plugin does not assign the peak as expected it can be moved by dragging as described below.

Moving Peaks

If the mouse cursor is hovered over an existing point it will change to a finger. The point can then be moved by holding down the mouse button and moving the mouse to drag the point.

The plugin will identify the point that has been dragged. If it is an aligned point the mapped count on the interface will decrement, otherwise the unmapped count will decrement.

When the mouse button is released the plugin will detect the point has been dropped. If the Assign Dragged option is enabled the plugin will attempt to align the point. Otherwise the point will be unchanged and the unmapped count will increment.

Note that is is possible to disable and re-enable the Assign Dragged option during the course of marking an image. This allows points to be moved without alignment or using alignment.

It is possible that the plugin fails to correctly detect that the peak was assigned before dragging. This will cause the mapped and unmapped counts to show errors. This can be solved by deleting a point which forces all existing points to be remapped without movement. The point can then be added back.

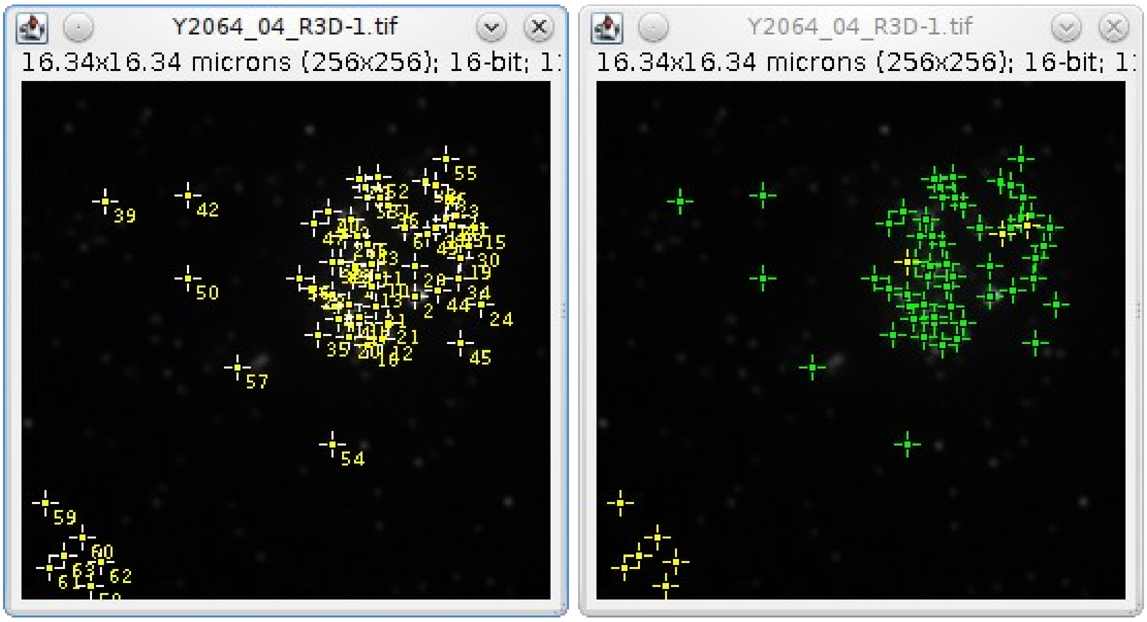
Deleting Peaks

If a the mouse cursor is hovered over an existing point it will change to a finger. It is possible to delete the point by clicking on it whilst holding down the Alt key on the keyboard.

The plugin will identify a point has been deleted. To maintain the ordering of points by ImageJ and the log message window the plugin will process all the remaining points and output the assignments using the new ImageJ point numbers.

Point Overlay

The current mapped and unmapped points can be displayed as an image overlay by clicking the Show Overlay button. This action saves the current ROI points and removes them from the image display. The points are then added back to the image as an overlay using green for the mapped points and yellow for the unmapped points. An example is show below:


Y2064_04_R3D-1.tif
16.34x16.34 microns (256x256); 16-bit; 1:
Y2064_04_R3D-1.tif
16.34x16.34 microns (256x256); 16-bit; 1:

The Show Overlay button is a toggle button. It will return to the off state under the following conditions:

Condition	Result
Show Overlay clicked	Removes the overlay and restores the ROI points
Image clicked	Removes the overlay and: • If using the multi-point ROI tool then the new point will be added to the ROI points • If a different ROI tool is used, for example the Rectangle tool, then the ROI points will be lost • If any other tool is used, for example to move or zoom the image, then the previous ROI is restored
Stop button clicked	Removes the overlay and restores the ROI points

Saving Results

The current point coordinates can be saved by clicking the Save Results button. The table contains the following information:

Result	Description
Id	The point identifier. This matches the ImageJ point ROI label
X	The point X coordinate
Y	The point Y coordinate
Height	The image intensity. Since only XY coordinates are used this is for the first plane of a multi-plane Z-stack
Assigned	True if the point is located on a Find Peaks maxima. False entries indicate points that could not be aligned

Stopping Point Alignment

Clicking the Stop button will halt the Find Peaks Helper. It will no longer intercept any mouse interactions with the image. All the working data used by the plugin is discarded and consequently the Save Results option will be disabled.

Note that clicking the Start button will reinitialise the Find Peaks Helper. This will set a new resolution if it has been changed and as part of the initialisation will perform alignment of the existing points to maxima. This can potentially move any points that have been dragged with the Assign Dragged option disabled, i.e. points that were previously unmapped.

Alignment Log

If the Log messages option is enabled then the alignment result of each point will be recorded to the ImageJ log.

Each entry is preceded by the point ID. The numbering matches the Ids assigned by ImageJ on the image. Following the ID is the keyword Mapped or Unmapped, depending on the alignment status, and then the XY coordinates of the point that was clicked. If a mapped point was moved to align to the true peak then the new coordinates will be shown along with the distance moved in pixels. Examples are shown below:

```
45: Mapped (166,80) => (167,80) (1.0px)
46: Mapped (201,51)
46: Unmapped (196,75)
```

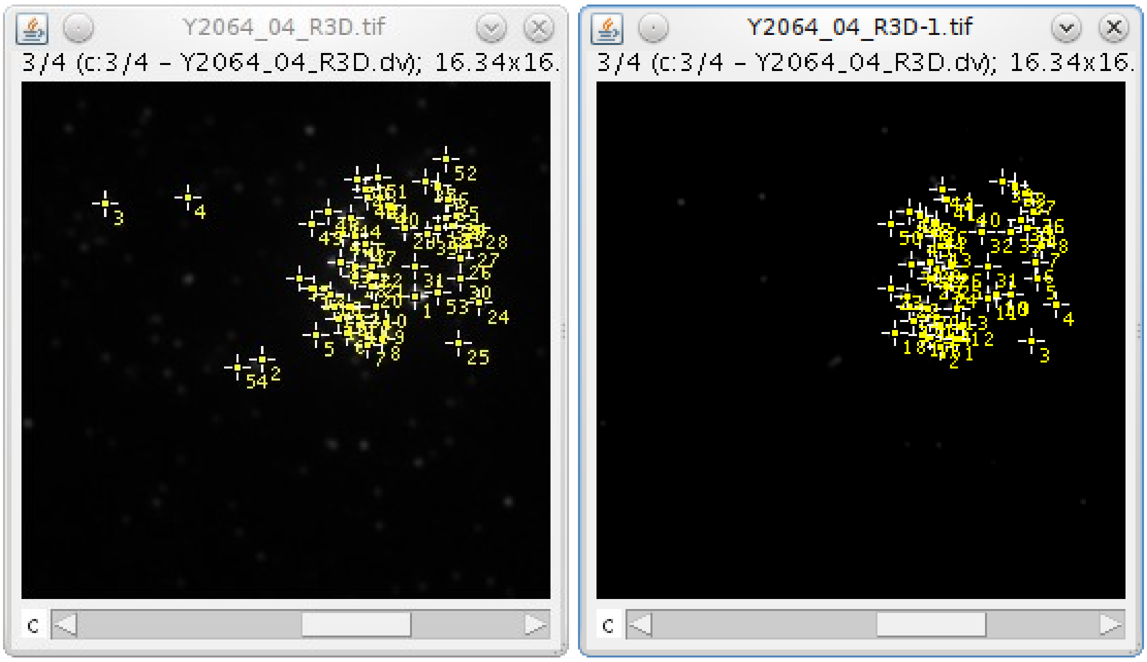
Java Requirements

The Find Peaks Optimiser Frame uses the BeansBinding Java framework. More details can be found in Appendix 1: BeansBinding Java framework.

Match Calculator

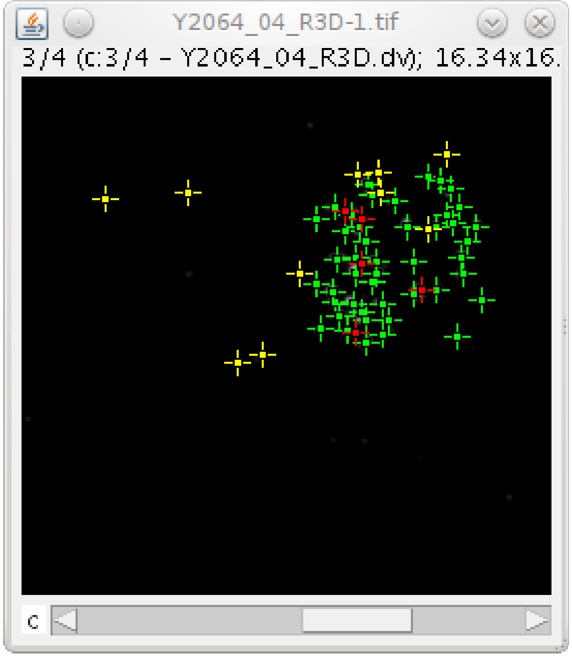
ImageJ plugin that compares the multi-point Regions of Interest (ROIs) between two images.

Example Input


Y2064_04_R3D.tif
3/4 (c:3/4 - Y2064_04_R3D.dv); 16.34x16.
Y2064_04_R3D-1.tif
3/4 (c:3/4 - Y2064_04_R3D.dv); 16.34x16.

Input images 1 and 2 containing multi-point ROI

Example Output



Output image overlay showing matched points in green, unmatched points from image 1 in yellow and unmatched points from image 2 in red.

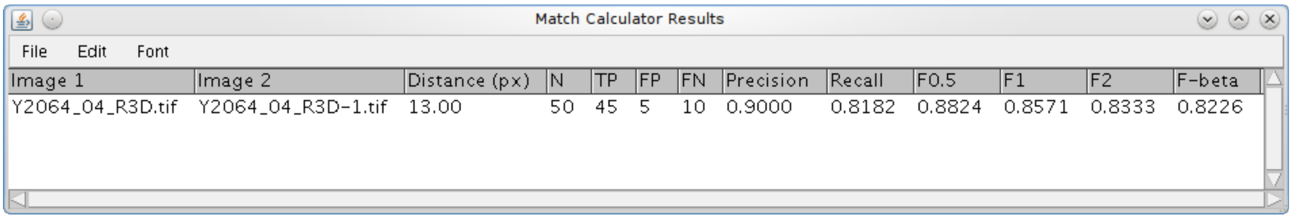


Image 1	Image 2	Distance (px)	N	TP	FP	FN	Precision	Recall	F0.5	F1	F2	F-beta
Y2064_04_R3D.tif	Y2064_04_R3D-1.tif	13.00	50	45	5	10	0.9000	0.8182	0.8824	0.8571	0.8333	0.8226

Results table showing the match statistics

Features

- Processes any images with point ROI
- Compares the ROI points and assigns matches using a nearest-neighbour method within a configurable distance threshold
- Generates a results table of match statistics
- Optional overlay of the match results onto the image
- Allows selection of channel and frame for multi-dimensional images when extracting heights
- Optional match statistics for each quartile of the data points when ranked by height
- Optional scatter plot of the height of matched and unmatched points
- Optional height analysis of the unmatched points

Overview

The Match Calculator plugin compares the ROI points on two images and computes the number of points that match. The matches can be used to generate match statistics as detailed in Appendix 2: Binary Scoring Statistics.

The plugin was developed to provide comparisons between the manual peak identification performed by different experimenters on the same image. The plugin can rapidly show differences between the peak labelling techniques used by two people.

Match Algorithm

Matches are calculated using a nearest-neighbour algorithm:

1. The coordinates of the ROI points from each image are used to build two sets, A and B
2. An all-vs-all distance matrix is computed for the two sets of points, i.e. the distance for any point in set A to any point in set B
3. The closest pair of points is identified
4. If the closest pair is below a distance threshold they are marked as a pair and removed from the sets. Step 3 is repeated
5. If the closest pair is above the distance threshold the search ends

The distance threshold can be set using an absolute number of pixels or can be set relative to the image size, e.g. 5% of the longest edge.

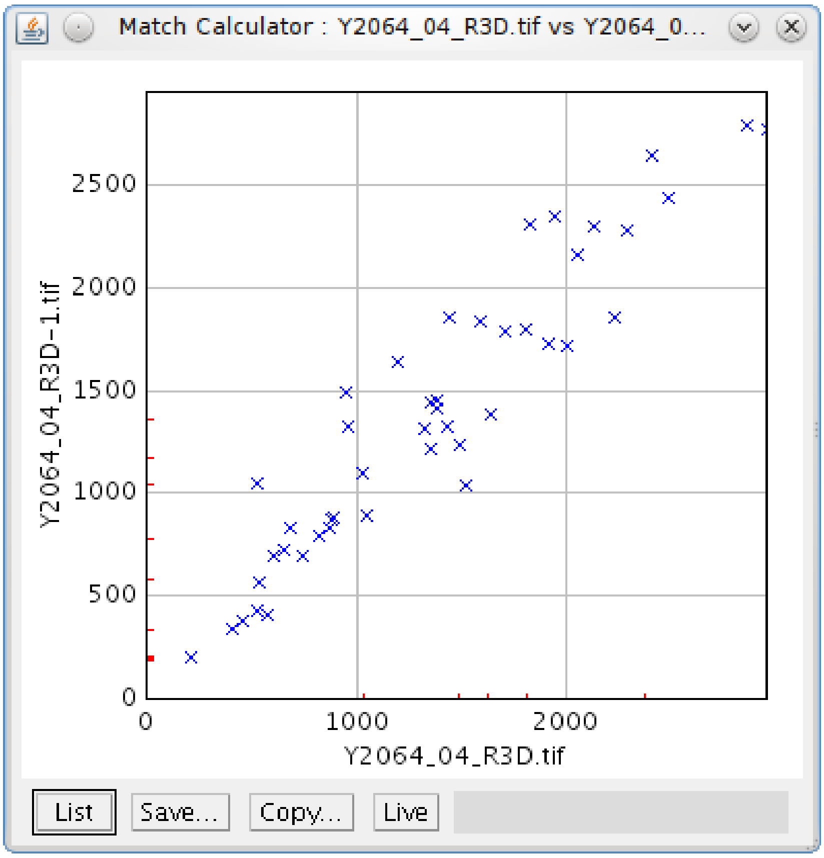
Height Analysis

The plugin can produce an analysis of the points using their heights (image intensity values). Since ImageJ point ROIs do not readily support z-stacks the plugin uses a maximum intensity projection for height analysis. It does allow selection of the channel/frame that is used for the heights for multi-dimensional images.

The heights for all the points can be used to divide the data into quartiles. All the heights from both sets are combined into a single dataset and used to set quartile boundaries. Each set of points is compared to the other using only the points that fall within each quartile boundary. This analysis will show if the higher points (greater intensity) have an increased number of matches. Due to subdivision of the data the analysis is less relevant when the number of points is small (e.g. less than 50).

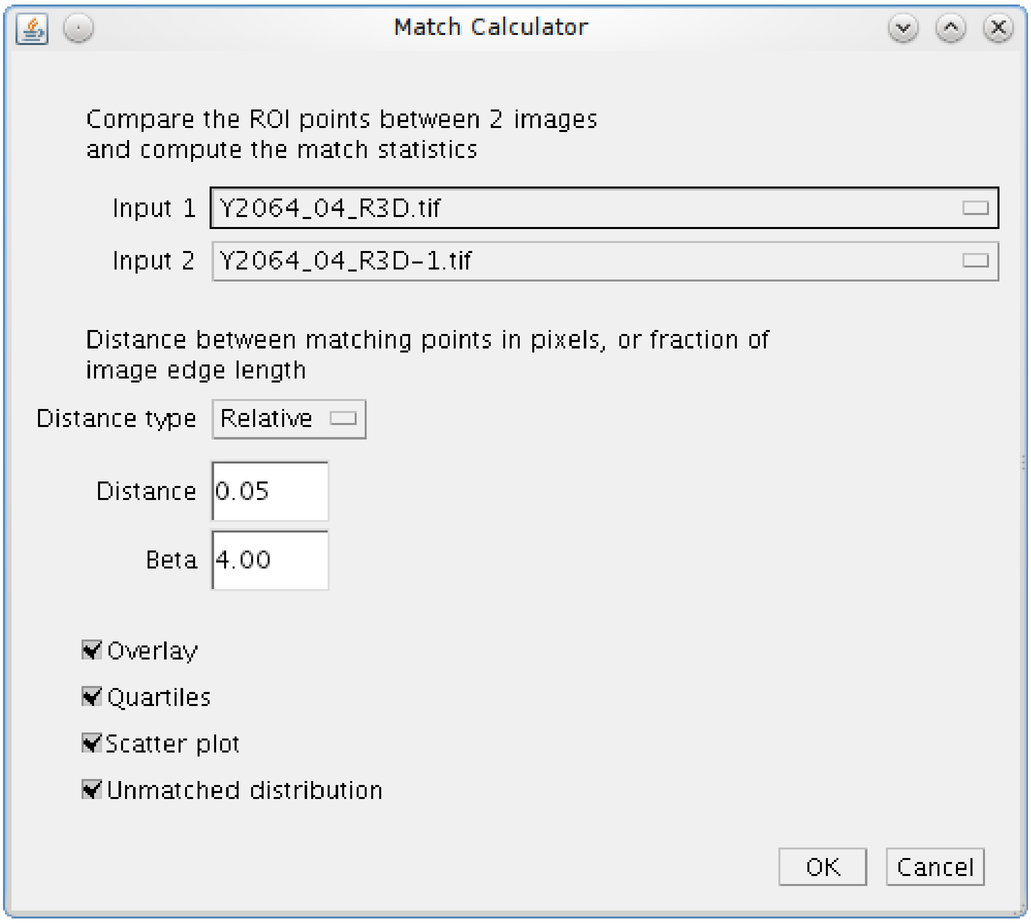
In addition the heights for the paired points can be used to divide the data. In this case the height is taken as the average height of the pair. The height distribution is used to set quartile boundaries. The unmatched points in a set can be counted for each of the height classifications defined using the boundary limits. This analysis shows where the unmatched points occur relative to points that have been matched.

A visual display of the heights for the matched and unmatched points uses a scatter plot of the point heights. The X-axis is the height of the point from set A and the Y-axis uses set B. If the points have been matched they will have both X and Y values. Otherwise they will be plotted on the axis. An example is shown below where matched points are shown in blue and unmatched points in red:



Plugin Interface

The Find Peaks interface uses the standard ImageJ Generic Dialog. The input parameters are shown in the following image and are described below.



Match Calculator

Compare the ROI points between 2 images
and compute the match statistics

Input 1 Y2064_04_R3D.tif

Input 2 Y2064_04_R3D-1.tif

Distance between matching points in pixels, or fraction of
image edge length

Distance type Relative

Distance 0.05

Beta 4.00

☑ Overlay

☑ Quartiles

☑ Scatter plot

☑ Unmatched distribution

Parameters

Parameter	Description
Input 1	The first input image
Input 2	The second input image
Distance type	Define the type for the distance parameter: Relative – A fraction of the longest image edge Absolute – A number of pixels
Distance	Specify the maximum distance allowed for a matched pair of points
Beta	The beta parameter for the F_β-score. This allows a customised weighting between the Precision and Recall scores.
Overlay	Show the matched and unmatched points using a coloured overlay on image 2 (see Example Output)
Quartiles	Compute the match statistics for the points divided into 4 sets

	using their heights to define quartiles
Scatter plot	Output a scatter plot of the heights of set A verses set B, matched points a plotted as height pairs
Unmatched distribution	Output a result table of the counts of unmatched points within the limits defined by the quartile boundaries of the matched points

Results Table

The results table contains details about the match statistics. The table contains the following information:

Result	**Description**
Image 1	The first input image
Image 2	The second input image
Distance (px)	The distance limit for macthed pairs. Note this is always specified in pixels even when using a relative distance type
N	The number of points in the first image
TP	The True Positives, i.e. the number of points that match
FP	The False Positives, i.e. the number of points in Image 2 that do not match Image 1
FN	The False Negatives, i.e. the number of points in Image 1 that do not match Image 2
Precision	The proportion of points in Image 1 that appear in Image 2
Recall	The proportion of points in Image 2 that appear in Image 1
F0.5	The $F_{0.5}$-score, a biased weighting of Precision over Recall
F1	The F1-score, a balanced weighting of Precision and Recall
F2	The F_{2}-score, a biased weighting of Recall over Precision
F-beta	The $F_{ß}$-score, a biased weighting of Precision and Recall using the Beta parameter for the weight

If the Quartiles parameter is selected then an additional set of columns will be appended for each quartile Q1 through to Q4. There will be a column for N, TP, FP, FN, Precision, Recall and F1 for each quartile.

Note: It will be necessary to close the result table if it has been previously generated without the additional columns.

Unmatched Results Table

If the Unmatched distribution parameter is selected then a second result table will be generated containing the height distribution of the unmatched points from each image relative to the matched points.

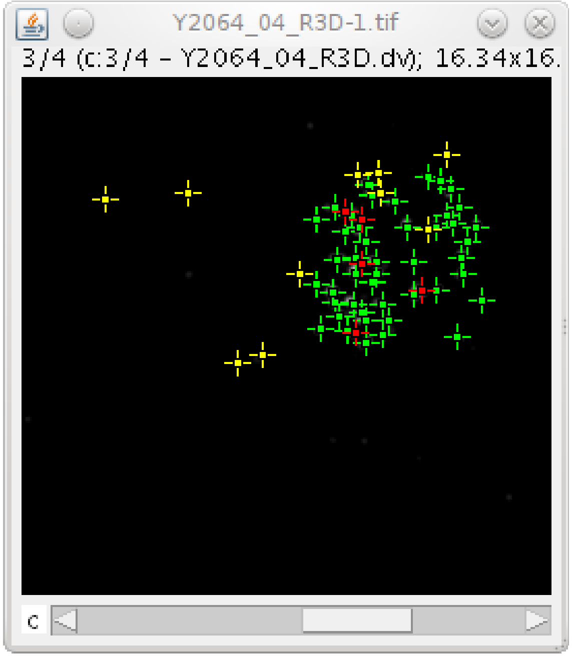
Result	**Description**
Image 1/2	The first/second input image title
N	The number of unmatched points
% <Q1	The percentage of points below the height of the lowest matched point
% Q1	The percentage of points within the first quartile of matched points
% Q2	The percentage of points within the second quartile of matched points
% Q3	The percentage of points within the third quartile of matched points
% Q4	The percentage of points within the forth quartile of matched points
% >Q4	The percentage of points above the height of the highest matched point

Overlay

The results of the match can be shown as an overlay on the original image 2 using the Overlay parameter. The following key is used for the overlay:

Classification	**Colour**
Matched	Green
Unmatched image 1	Yellow
Unmatched image 2	Red

The following image shows an example of an overlay applied to the image:



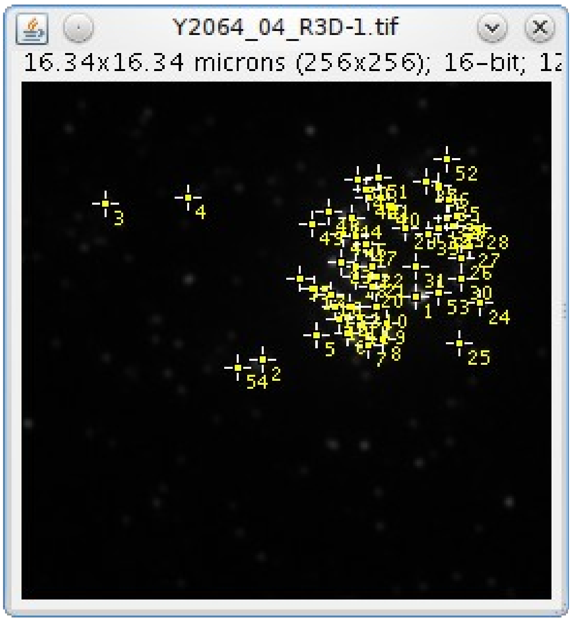
The overlay can be removed using Image > Overlay > Remove Overlay.

Note that the point ROI has been removed from the image. This can be restored using the ImageJ menu option Edit > Selection > Restore Selection (Ctrl+Shift+E). The point ROI are not removed if the overlay option is disabled.

Point Aligner

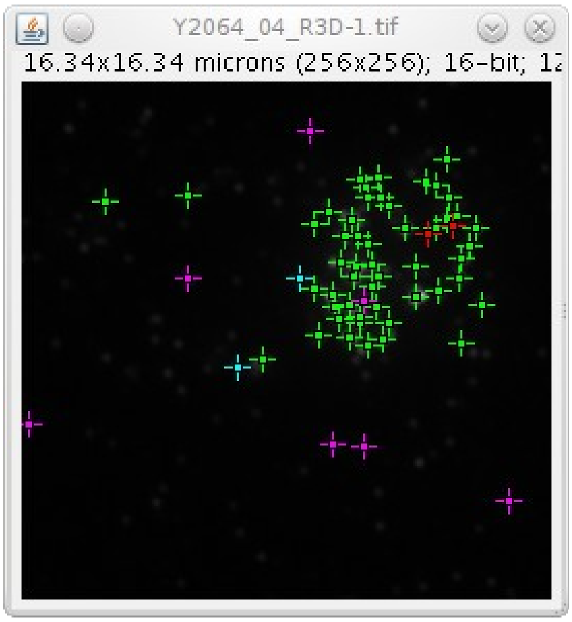
ImageJ plugin that aligns the marked multi-point Regions of Interest (ROIs) of an image with the peaks found by the Find Peaks algorithm.

Example Input

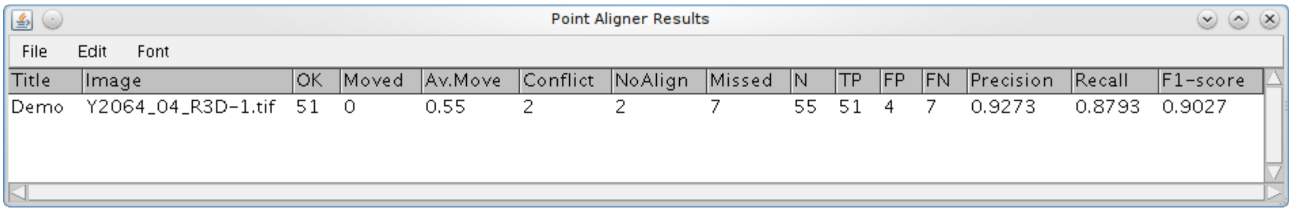


Input image containing multi-point ROI

Example Output



Output overlay showing aligned points in green, conflict points in red, unaligned points in cyan and missed points in magenta



Title	Image	OK	Moved	Av.Move	Conflict	NoAlign	Missed	N	TP	FP	FN	Precision	Recall	F1-score
Demo	Y2064_04_R3D-1.tif	51	0	0.55	2	2	7	55	51	4	7	0.9273	0.8793	0.9027

Results table showing the match statistics

Features

- Processes any single channel image with point ROI
- Computes the image peaks using the Find Peaks algorithm
- Assigns the marked points to the true peaks
- Generates a results table of alignment statistics
- Identifies any peaks that have been missed by the marked points
- Optional overlay of the alignment results onto the image
- Optional output of marked points that cannot by aligned (conflict regions) as images

Overview

The Point Aligner plugin aligns the ROI points on an image to the peaks found using the Find Peaks algorithm. Comparison of the original points and the aligned points can be used to generate match statistics as detailed in Appendix 2: Binary Scoring Statistics.

The plugin was developed to re-align the manual peak identification performed by an experimenter to the correct peak maxima location.

Alignment Algorithm

Alignments are calculated using the following algorithm:

1. The image ROI points are extracted into an XY coordinate set
2. The height of each point is calculated using a maximum intensity projection of the image (the projection is used due to the lack of a Z-coordinate for each point)
3. A background level is set using the lowest point height minus the standard deviation of the maximum intensity projection
4. The Find Peaks algorithm is used to find maxima above the background level. No merging is performed thus the output peak map contains all potential maxima
5. Points are processed in height order, highest first. Each point is aligned using the peak map (the pixels allocated to each maxima) effectively aligning the point by only proceeding up an intensity gradient to a peak. The maxima is then excluded from future alignments
6. If the point aligns to a previously assigned maxima a search is conducted within the distance to the excluded maxima for an alternative. The highest maxima within the radius is selected
7. Following alignment the heights of all the assigned maxima are analysed. The heights are used to set a limit for the minimum peak height that can be used for an alignment. Any point that aligns to a peak below the minimum peak height is reset to unaligned

Minimum Peak Height Limit

The minimum peak height is used to avoid points aligning to insignificant peaks. Note that the Find Peaks algorithm will identify any pixel higher than its neighbours and extend the peak using a downhill gradient. This can produce false peaks at a low intensity that are not above the image background. These are available to the alignment algorithm and so should be filtered at the end of the process.

The height limit is set using the distribution of the assigned peak heights. The purpose is to identify any outlier low peaks that do not fit the distribution, similar to plotting outlier points on a box plot. The following methods are available, each method requires an input parameter *f*:

Method	**Description**
None	No height limit
Q1 - *f* * IQR	The limit is set using a factor of the Q1-Q2 interquartile range (IQR) subtracted from the Q1-Q2 boundary. A typical factor is 1.5
Mean - *f* * SD	The limit is set using a factor of the standard deviation subtracted from the mean. A factor of 3 will cover 99% of all the data assuming a standard normal distribution
nth Percentile	The limit is set using the *f*-th percentile of the heights. Using zero will not exclude any peaks. 100 will exclude all peaks. Note: This method is poor since it will not target outliers but penalises any low points
% Missed < *f*	The number of unassigned (missed) and assigned peaks above the lowest assigned peak are counted. The percentage missed peaks is calculated: Percentage missed = missed / (missed + assigned) If the percentage missed is above the specified factor then the lowest assigned peak is removed and the process repeated. Finally the limit is set using the lowest assigned peak height. Note: This method assumes that the purpose of the initial ROI point marking was to label the highest intensity values. If there are a large number of missed peaks above the highest assigned peak the method will fail. This can be the case if points were only labelled in a certain region of the image. This can be overcome using a mask image parameter

Alignment Results

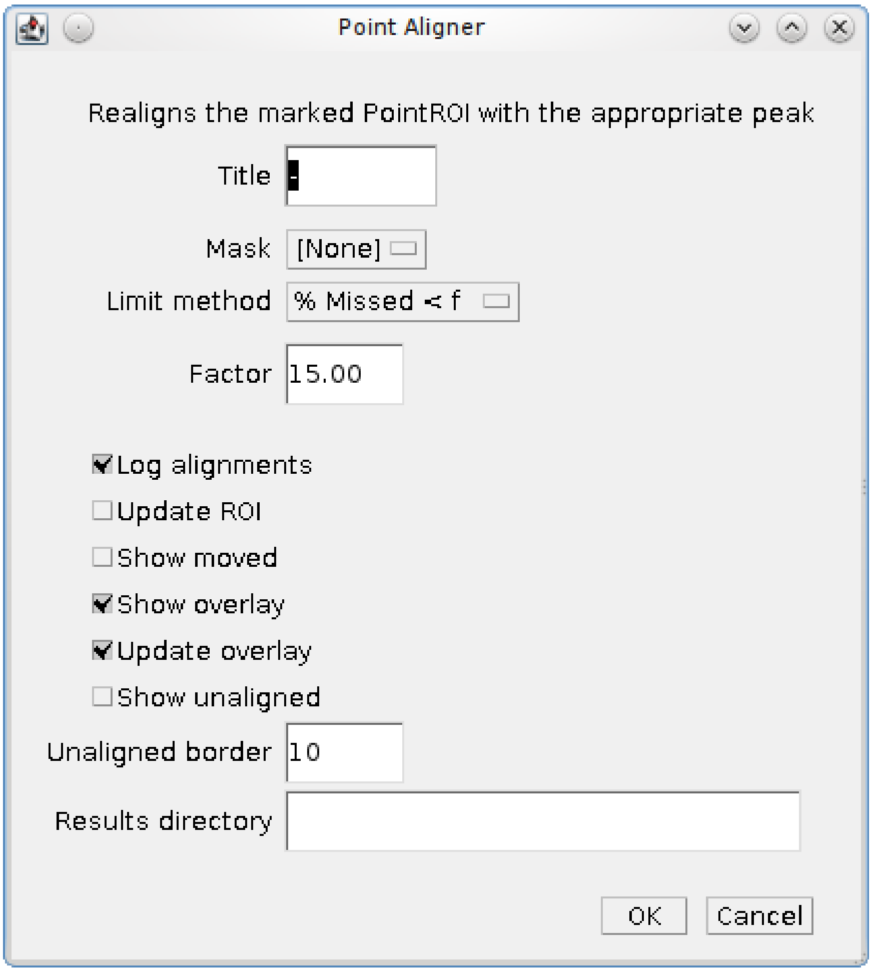
Each point in the original image is given an alignment result. In addition it is possible to analyse maxima found by the Find Peaks algorithm. The following alignment results are provided:

Result	**Description**
OK	The point aligns with a maxima and is in the correct location

Moved	The point aligns with a maxima but must be moved
Conflict	The point aligns with a maxima that has been assigned to a higher point, i.e. it conflicts with another aligned point
NoAlign	The point does not align with a maxima
Missed	The unassigned maxima is higher than the lowest maxima that has been assigned, i.e. it was missed in the original input points

Plugin Interface

The Point Aligner interface uses the standard ImageJ Generic Dialog. The input parameters are shown in the following image and are described below.



Point Aligner

Realigns the marked PointROI with the appropriate peak

Title

Mask [None]

Limit method % Missed < f

Factor 15.00

☑ Log alignments

□ Update ROI

□ Show moved

☑ Show overlay

☑ Update overlay

□ Show unaligned

Unaligned border 10

Results directory

Parameters

Parameters	**Description**
Title	A text title to be inserted into the results table. For example this can be used within automated scripts to identify the experimenter who scored the image
Mask	Allows selection of an image mask. Only images with the same dimensions are listed. The mask can be used to restrict the region of peaks provided by the Find Peaks algorithm; any peak

	located in a zero-value pixel will be excluded
Limit Method	The method used to set the minimum height for assigned peaks
Factor	The factor parameter used by the Limit method
Log alignments	Output the alignments to the ImageJ log window
Update ROI	If selected the point ROI on the image will be moved to the position of their assigned peaks
Show moved	Show the number of points that have been moved separately in the result table. The default is to show the OK and Moved counts together
Show overlay	Produce a coloured overlay on the image showing the points that were OK, moved, conflicting, missed or unaligned
Update overlay	If selected the overlay will show the moved points relocated to the correct positions. The default is to use the original location
Show unaligned	Extract an image containing each unaligned point. A sub-image is created containing the unaligned point plus a border of extra pixels. This allows examination of any points that cannot be aligned. If the point is unaligned due to a conflict then the previously assigned maxima is included in the image. An overlay is added showing the point location (and the maxima if available)
Unaligned border	The number of border pixels to use for an unaligned point image
Results directory	If provided the unaligned images will be recorded to this directory. The directory path must exist. Note: The Show unaligned option does not have to be enabled

Results Table

The results table contains details about the alignment results. The success of the alignment is assessed using binary scoring statistics. The original ROI points are compared against the potential maxima found by the Find Peaks algorithm. Only maxima above the lowest assigned maxima are included. Further details of the statistics can be found in Appendix 2: Binary Scoring Statistics.

The table contains the following information:

Result	**Description**
Title	The Title parameter
Image	The input image
OK	The number of points that were aligned without no move
Moved	The number of points that were aligned with a move. This is only

	shown if Show moved is enabled
Av.Move	The average distance that an aligned point is moved in pixels. If Show moved is disabled the average includes the points that are not moved and will be lower
Conflict	The number of points that conflict with another aligned point
NoAlign	The number of points that cannot be aligned
Missed	The number of maxima that are above the lowest assigned maxima
N	The number ROI points in the image
TP	The True Positives, i.e. OK + Moved
FP	The False Positives, i.e. Conflict + NoAlign
FN	The False Negatives, i.e. Missed
Precision	The proportion of points in the image that are aligned
Recall	The proportion of identified peaks in the image that have been assigned
F1	The F1-score, a balanced weighting of Precision and Recall. This provides an overall measure of the alignment success

Alignment Log

If the Log alignments option is enabled then the alignment result of each point will be recorded to the ImageJ log. The following text shows examples of each situation:

OK / Moved

If the peak can be aligned then the new coordinates and the pixel distance for the move are provided. The records are of the format:

```
Point [ID] <height> @ X,Y => <height> @ Xnew,Ynew (distance)
```

E.g.

```
Point [49] 793 @ 140,70 => 831 @ 141,70 (1.00)
Point [50] 784 @ 173,57 => 784 @ 173,57 (0.00)
```

Conflict

If the peak is in conflict then the ID of the other peak is provided. The coordinates of both peaks should be in close proximity. E.g.

```
Point [24] 1350 @ 205,67 => 1367 @ 205,68 (1.00)
Point [52] 717 @ 208,71 conflicts for assigned point [24]
```

NoAlign

If the peak is below the height threshold or cannot be aligned this is recorded.

```
Point [55] 479 @ 104,141 ~> 479 @ 104,141 (0.00) below height threshold (< 666)
Point [56] 186 @ 11,208 cannot be aligned
```

Unaligned Images

If a point cannot be aligned then the pixels surrounding the point can be extracted into a sub-image. A point can be unaligned for 3 reasons:

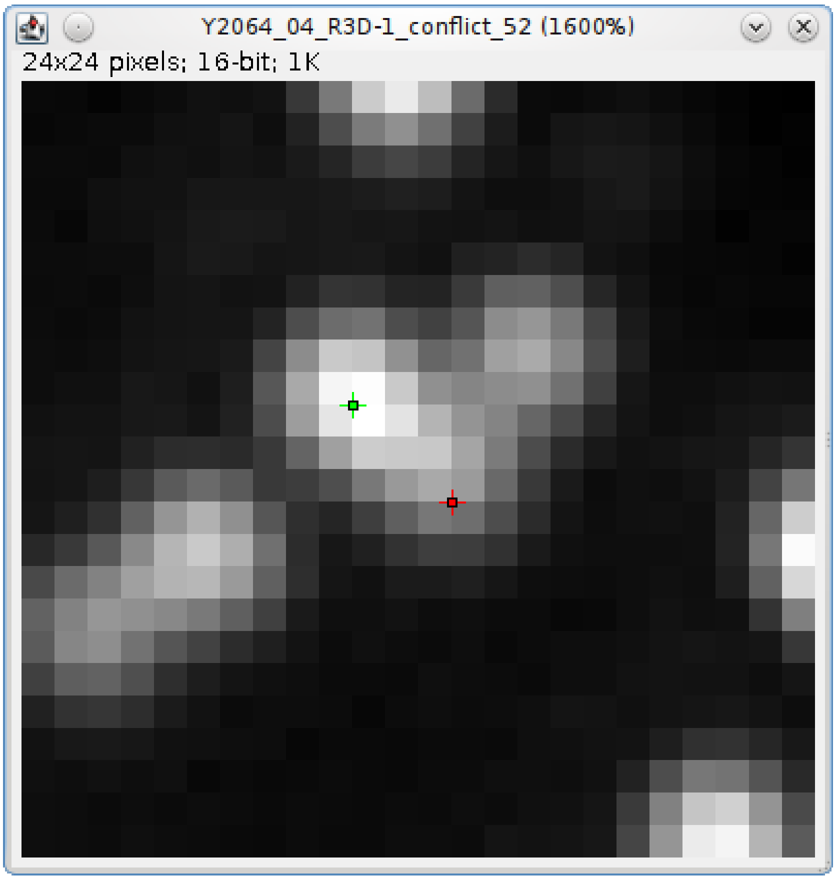
1. There is no suitable maxima [noalign]
2. It is below the height limit threshold [below_threshold]
3. It conflicts with another aligned point [conflict]

The sub-image is named using the Input image name, the classification and the point ID:

<Input image>_<classification>_<ID>

If the image shows a conflict point or a below_threshold point then the potential maxima is included in the image. An overlay is added to mark the unaligned point and the potential maxima (if available). The images can be displayed using the Show unaligned option and/or saved to the Results directory.

The following is an example of unaligned point sub-image.



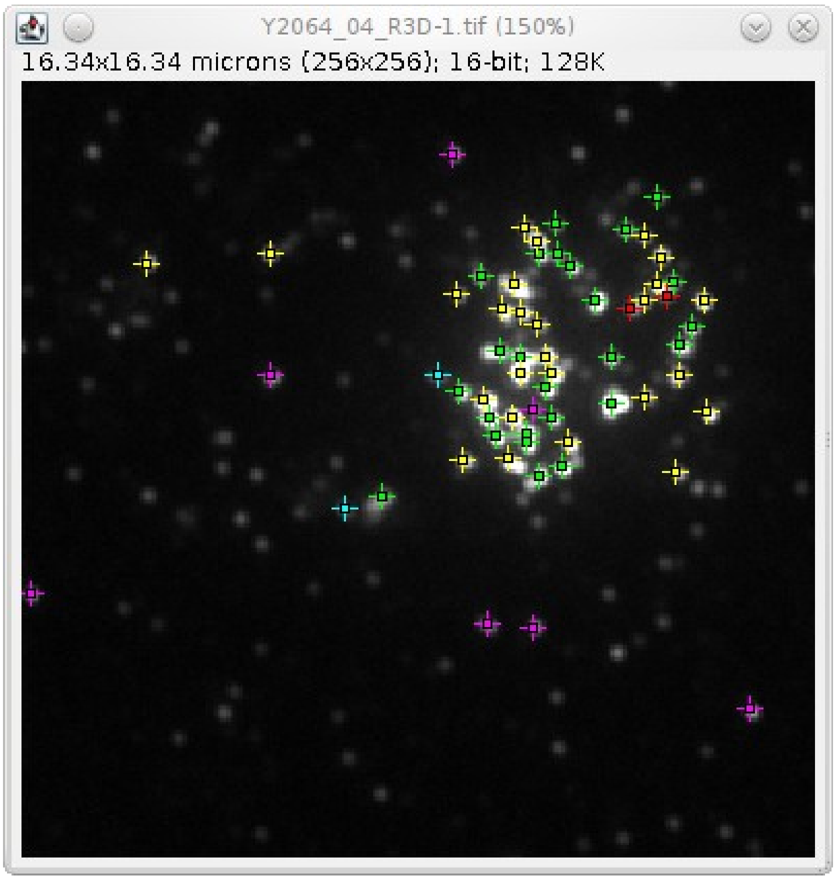
Note that in this case the conflict point (red) has been manually placed where there appears to be intensity from a second spot. However the Find Peaks algorithm does not identify this as a point since it uses a downhill gradient to define peaks from local maxima. This example demonstrates how the conflict images can be used to review where the Find Peaks algorithm differs from a manual peak assignment. Knowledge of the Find Peaks behaviour will allow better use of the semi-automated peak labelling tool provided by the Find Peaks Helper plugin (see section Find Peaks Helper).

Overlay

The results of the alignment can be shown as an overlay on the original image using the Show overlay parameter. The following key is used for the overlay:

Classification	Colour
OK	Green
Moved	Yellow
Conflict	Red
NoAlign	Cyan
Missed	Magenta

The following image shows an example of an overlay applied to the image:



Y2064_04_R3D-1.tif (150%)
16.34x16.34 microns (256x256); 16-bit; 128K

The overlay can be removed using Image > Overlay > Remove Overlay.

Note that the point ROI has been removed from the image. This is done before the overlay is added. The point ROI can be restored using the ImageJ menu option Edit > Selection > Restore Selection (Ctrl+Shift+E). The point ROI are not removed if the overlay option is disabled.

Appendix 1: BeansBinding Java framework

The default GDSC plugins package contains the BeansBinding framework. However if you are using a repackaged Jar file it may not contain the required library. The file can be obtained from:

http://www.sussex.ac.uk/gdsc/intranet/files/beansbinding-1.2.1.jar

This file can be placed in the ImageJ plugins directory.

If the BeansBinding jar cannot be found any dependant plugin will show an error message in the ImageJ log window when initialising. The error message should provide useful information for fixing the problem.

The following plugins use the BeansBinding framework:

- Find Peaks Frame
- Find Peaks Optimiser Frame
- Find Peaks Helper

Appendix 2: Binary Scoring Statistics

Several plugins within the GDSC package compute matches between points. One set of points can be labelled as the actual result, the second can be labelled as the predicted results. When comparing actual and predicted points the following combinations are possible:

Actual Point	Predicted Point	Classification	Label
Present	Present	True Positive	*tp*
Present	Absent	False Negative	*fn*
Absent	Present	False Positive	*fp*

The classification counts can be used to compute binary scoring statistics as described below.

Jaccard

The Jaccard measures the similarity between two sets and is defined as the size of the intersection divided by the size of the union:

$$jaccard = \frac{A \cap B}{A \cup B} = \frac{tp}{tp + fp + fn}$$

A score of 1 indicates that the overlap is perfect. Zero indicates no overlap.

Recall

Recall measures the number of actual points that are correctly predicted. It is also known as the True Positive Rate or sensitivity.

$$recall = \frac{tp}{tp + fn}$$

A score of 1 indicates that all the points were predicted, lower scores indicate that some points were missed.

Recall can be interpreted probabilistically as the chance that a randomly selected actual point will be predicted.

Precision

Precision measures the confidence of the predicted points. It is also known as the Positive Predicted Value.

$$precision = \frac{tp}{tp + fp}$$

A score of 1 indicates that all the predicted points were correct, lower scores indicate that some points are not correct.

Precision can be interpreted probabilistically as the chance that a randomly selected prediction is correct.

F-score

The precision and recall can be combined in a weighted score, the $F_ß$-measure or F-score.

$$F_B = (1+B^2) \cdot \frac{precision \cdot recall}{B^2 \cdot precision + recall}$$

A weight of 1 produces the balanced F-score where precision and recall are weighted equally. $F_{0.5}$ puts more emphasis on precision and F_2 puts more emphasis on recall. However the weight ß can be any non-negative real value. The score was derived so that it measures the effectiveness of retrieval with respect to a user who attaches ß times as much importance to recall as precision.

Defining the Actual or Predicted Points

Note that the categorisation of the actual and predicted points can be arbitrary. If the categorisation is reversed then the precision and recall scores will be reversed. However the F_1-score will remain identical because it is the harmonic mean of the two scores. The F_1-score is consequently a good measure of the similarity of two sets of points that have been aligned for matches.