• AnalLS

AnalLS analyses linescan data generated with ScanImage. These are usually present as image stacks, only that lines in an image represent subsequent recordings of the same line.

Note: Required include files are:
LoadScanImage.ipf

See also: LoadScanImage

AnalLS (*LSwave, start, stop*)
This operation extracts the average brightness of a one-dimensional ROI over time (if the data has been generated in ScanImage and loaded using the LoadScanImage function) or frame number. *Start* and *stop* are the left and right pixels (!) of the ROI. The resulting wave will be named nameofwave(LSwave)+"_LS" and contain the normalised fluorescence, ΔF/F.

CallAnalLS ()
This operation calls AnalLS and prompts the user for parameters. This function is useful to be placed in a menu for quick access.

• AutoRegistration

Use this operation to call RegisterStack on multiple files in a folder. This is useful for registering a large number of image stacks overnight or while away from the computer. Call AutoRegistration() and specify one or many .tiff files. These will be sequentially loaded, registered and saved as Igor binary files in the origin folder.

Note: Required include files are:
LoadScanImage.ipf
RegisterStack

• CenterOfMass

The functions in CenterOfMass are used for calculating centers of mass of regions of interest based on brightness, displaying them and measuring distances between curves (waves) and points.

CenterOfMass (*image,ROI*)
This operation calculates the center of mass for ROIs based on the intensity of *image*. The ROI wave *ROI* must be a MultiROI ROI wave. The result will be stored in the two-dimenasional wave CoM. CoM[][0] contains the scaled X data, CoM[][1] contains the scaled Y data. The precision of CoM is higher than that of pixels in *image*.

See also: MultiROI ROI wave

CoMDistances (*CoM, [index]*)

This operation calculates the (scaled) distances between all CoMs in wave *CoM* and returns the result as the matrix *DistanceMatrix*. The optional parameter *index* can be used to spexify the layer of a compound CoM wave (as returned by the operation ComByLayer.

DistanceMatrix2Colum (*DistanceMatrix, [index]*)

This operation puts all distances of the distancematrix *DistanceMatrix* in a single column of the wave *DM2C*, so that these date can be used for a histogram, clustering, etc. If the optional parameter *index* = 1, the output wave, *DM2C*, has three columns, the first one holds the distances between two traces, the second and the third hold the index of the traces that the distance was measured between.

See also: HiClu, HiClu2D, HiCluCP

GeometricCenter (*ROI*)

This operation calculates the geometric center (centroid) for ROIs in the MultiROI ROI wave *ROI*. The result will be stored in the two-dimenasional wave GeoC. GeoC[][0] contains the scaled X data, GeoC[][1] contains the scaled Y data. The precision of CoM is higher than that of pixels in *ROI*.

ShortestDistance (*func, relX, relY*)

This function returns the shortest distance between a function *func* (1D wave) and a point, defined by *relX* and *relY*. The wave has to be scaled, as have the points.

See also: ShortestDistance2

ShortestDistance2 (*xfunc,yfunc, relX, relY*)

This function returns the shortest distance between a curve, defined by *xfunc* and *yfunc* and a point, defined by *relX* and *relY*. Waves and points have to be scaled.

See also: ShortestDistance

DrawCoMNumbers (*CoMWave, bgimg, WindowName, [fontsize]*)

This function draws the ROI numbers as stored in *COMWave* at the position of the center of mass in the window *WindowName*. *bgimg* is needed to get the dimension of the background image, which should be displayed in *WindowName*. *Fontsize* is optional and useful for very large images, as fonts don't scale when the image size is changed.

Note: Numbers are drawn relative to the borders of the window, not the image. Therefore changing the axes won't affect the drawn numbers. Also note that the origin of the image is expected to be in the lower left. If the image is to be displayed with the origin in the upper left, the function has to be adjusted accordingly. A comment in the function points out where this has to be done.

Related Topics: ResultsByCoef

- **Crop**

The functions in crop allow GUI-style cropping of images and image stacks. More

advanced users may want to use the Duplicate command with the /R flag.

CropXYFromWindow ()

For this function to work, a marquee must be drawn on the top window. When this function is called, the top image or image stack will be cropped along the borders delimited by the marquee. For added educational value, the function also prints the appropriate Duplicate command in the command line. The cropped image will be saved as *w_Cropped*.

CropZFromWindow ()

When this function is called, a popup menu appears for the user to specify the first and last frame, either in frame numbers or scaled variables. The top image stack will be cropped accordingly. For added educational value, the function also prints the appropriate Duplicate command in the command line. The cropped image will be saved as *w_Cropped*.

• Customcolor

rainbowLUT (*levels, outputwave*)

This function generates a rainbow lookup table with a number of different colours equal to *levels*. *Outputwave* will be the name of the so generated lookup table. This function is called automatically in QuickAnal in order to generate as many colours as there are regions of interest.

Related Topics: ModifyImage

• Difference

The functions in Difference are used to calculate a "difference image", i.e. subtract a baseline image from a response image. Usually, frames over a specified time interval are averaged to generate the baseline and response images. Thes functions are called from the ImageAnalysis Control Panel, when "Response" is selected as the thresholding method.

RunDifference (*stack3D, startbaselineF, stopbaselineF, startresponseF, stopresponseF*)

This function calculates the difference image by averaging frames in *stack3D* from *startbaselineF* to *stopbaselineF* to calculate the baseline image and by averaging frames from *startresponseF* to *stopresponseF* to calculate the the response image. The resulting image is named NameOfWave(*stack3D*)+"_RES".

GetTimes (*stack3d, name*)

This function calls prompts to enter start and stop times and then calls RunDifference using these variables.

Note: Required include file:

z-project.ipf

- ## dif_image

The functions in dif_image are used to generate the 2nd derivative of grayscale images and threshold these.

dif_image (*image, [targetname]*)
This function calculates the 2nd derivative of *image* in the x and y axis and sums them. The optional parameter *targetname* is the name of the so calculated image. If left out, the name will be nameofwave(image)+"_dif".

dif_image3D (*image, [targetname]*)
This function calculates the 2nd derivative of the voluma data *image* in the x, y and z axis and sums them. The optional parameter *targetname* is the name of the so calculated image. If left out, the name will be nameofwave(image)+"_dif".

difloop (*imagestack, [targetname]*)
This function calculates the 2nd derivative of *imagestack* in the x and y axis and sums them for each frame. The optional parameter *targetname* is the name of the so calculated image. If left out, the name will be nameofwave(image)+"_dif".

mod_img (*image, i_limit, [targetname]*)
This function performs (negative) thresholding on *image*:
First, pixels with a value larger than -*i_limit* will be set 1 (outside ROI), then those with a value <= 0 will be set 0 (inside ROI.) The optional parameter *targetname* is the name of the so calculated image. If left out, the name will be nameofwave(image)+"_mod".

Note: The threshold, *i_limit*, is not normalized to *image* within this function. When mod_img is called from ManThresh, however, the input to *i_limit* is first multiplied by the standard devation of *image* so that similar levels will yield similar results independent of absolute values in *image*.

xydif_image (*image, [targetname]*)
This function calculates the 1st derivative of *image* in the x and y axis, and thus returns two images. The optional parameter *targetname* is the name of the so calculated images which "_x" and "_y" appended to their names. If left out, the names will be nameofwave(image)+"_difx" and nameofwave(image)+"_dify". This function is not called in automated analysis, but left for completeness.

- ## DistanceTransform

These functions calculate a distance transform of a 2D or 3D binary wave. The output can be Euclidean distances in pixels, Manhattan distances in pixels or scaled Euclidean distances.

Note: These functions use a *very* slow algorithm. Although the functions are multithreaded, processing large waves will take a very, very long time. Processing speed can be increased by calculating the distance transform of a pixelated wave and then resampling the result, using the ImageInterpolate function with the pixelate and

resample keywords, respectively.

DistanceTransform (*image,metric*)

Calculates the distance transform of the 2D or 3D wave *image*, which must be binary, i.e. contain only 0 and 1. Metric can be 0 (Euclidean distances in pixels), 1 (Manhattan distances in pixels) or 2 (scaled Euclidean distances). Scaling does work, even if pixels are not quadratic (or cubic).

Related Topics: ImageInterpolate

• EqualizeScaling

These functions quickly transfer scaling or size from one wave to another, even if the numbers of dimensions mismatch.

CopyScaling (*source, destination*)

This function applies point-by-point scaling from *source* in all 4 dimensions plus the data full scale to *destination* and copies the wavenote. Source and destination don't need to have the same number of dimensions. If *source*, for instance, has 3 dimsensions, but *destination* only 2, then those 2 will be appropriately scaled.

Note: Dimension labels are not transferred.

CopySize (*source, destination*)

This function applies the length of the respecive axes from *source* to *destination*, regardless of the number of points. It also copies the data full scale and the wave note. This is useful, for instance, after resampling or pixelating an image.

Related Topics: SetScale, ImageInterpolate

• EventDetection_MT

These functions detect events in time-series data and fit single exponential time-courses to rise and decay. The fitting is done multi-threaded, therefore a multi-core processor is required to run them.

EventDetection (*trace, bin, durationr, durationd, direction, thr,[show, verbose]*)

This function detects events using the 2nd derivative to find peaks. It returns the resulting wave, *Events* (see below).
Input:

trace: 1D wave to be analysed
bin: number of points to be averaged - reduces the number of points; set to 1 to analyse non-smoothed trace.
durationr, *durationd*: times for rise and decay - The baseline is measured between -*durationd* and *durationr*, decacays are fitted from -durationr to peak and from peak to *durationd*.
direction: 1 for positive going peaks, -1 for negative going ones.
thr: threshold on the 2nd derivative (normalised by SD) to detect peaks.
show=1 displays results
verbose: prints errors and keeps intermediate results for

debugging/demonstration

Results are stored in the wave events with 5 columns and nevents rows.
Column 0 (tpeak): time of peak (scaled).
Column 1 (baseline): local baseline (treat with care).
Column 2 (amplitude): relative amplitude, i.e. abspeak - basline (treat with care)
Column 3 (abspeak): absolute amplitude
Column 4 (risetime): tau of single exp. fit (check for fitting errors)
Column 5 (decaytime): : tau of single exp. fit (check for fitting errors)

EventStats (*events*)

This function calculates average, SD, SEM, Median, Q25, Q75 and nEvents for inter-event-intervals (IEI), local baseline, local amplitude, absolute amplitude rise- and decay times of the wave *events*, which is the output of EventDetection. Returns the wave *EventsStats*.

• ExpDataBase2

These functions combine PopulationWaves and associated results into a database and allow extraction of data from a database. Version 1 operated by an entirely different principle and was never adopted by the community.

Databases can be built by launching the control panel, EDB2_CP. Information can be extracted using custom functions. A template is given in ExpDB2_Extraction.

The PopWaveBrowser3 allows browsing of databases and viewing/changing associated information.

Data is stored by adding a "footer" to the end of a PopulationWave that stores associated information as well as scaling, name of origin, etc., making use of Igor's Dimension Labels. Waves with different numbers of points can be combined, the resulting database will leave the appropriate fields of waves with less points empty (i.e. contain NaN). This allows analysis of the associated information in respect to the original traces, or other associated information. For instance, one can compare traces obtained at diiferent ages, or check whether there is correlation of age with size (see figure).

RO Label		Time		
Row	c0214e003_DB	c0214e003_DB	c0214e003_DB	c0214e003_DB
	x \ ROIN			
889	Time	219.977	120.569	240.061
890	Time	206.995	127.984	229.343
891	Time	202.937	117.602	218.018
892	Time	186.78	117.388	233.849
893	Time	207.924	143.731	230.923
894	Time	218.286	116.973	219.175
895	Time	216.663	134.218	240.887
896	Time	234.666	112.807	230.147
897	Time	210.676	144.348	229.679
898	Time	179.172	144.242	249.072
899	Time	181.246	124.26	238.945
900	ROINr	0	1	2
901	Age	8	8	8
902	Position	39	69	31
903	Size	3.02848e-11	1.0816e-11	2.1632e-11
904	ONOFF	2	0	0
905	TSus			
906	Stim			
907	BaseLine	199.144	136.43	212.611
908	Experimenter	6	6	6
909	AnalysisBitMask	13	13	13
910	nPoints	900	900	900
911	XDelta	0.2	0.2	0.2
912	XOffSet	0	0	0
913	XUnit	-1	-1	-1
914	OriginID	6.46762e+24	6.46762e+24	6.46762e+24
915				

Storage model of the database

Global and local constants

The following global constants are defined at the top of the procedure file. Some will need adjustment for different users:

k_LabelList is a list of labels that are stored with the database. The defaults list is (beware the linebreaks):

```
k_LabelList="ROINr;Age;Position;Size;ONOFF;TSus;Stim;BaseLine;Experimenter;Analys
isBitMask;nPoints;XDelta;XOffSet;XUnit;OriginID"
```

k_NaturalUnits is a list of SI units that the wave scaling can assume. More can be added at the end, as they are index-coded. However, Igor might not scale them normally. For instance, if the unit is inches ("in"), then thousands of them will become kiloinches ("kin"). Why do you think did we come up with SI units in the first

place?

k_Experimenter is a list of people analysing experiments. This helps keeping track of who analyzed an experiment. If this is changed after some databases have been built, add names at the end, as the names are index-coded.

k_Template is the template for names of PopulationWaves that will be added to the database using the GrepString operation. Associated information is contained in waves that have specific suffixes to their names (see next point).

k_Suffixes specifies the name suffix of waves containing a certain type of information that will be added to the database.

k_Autodetect is a list of labels that will be detected automatically, based on whether waves with the appropriate name+suffix are present

See also: ExpDB2_Extraction, PopulationWave, GrepString and Regular Expressions

BuildDataBaseFromSubFolders (*[Template]*)

This function screens all subfolder for waves whose name matches *Template* (default: see Global and local constants) and compiles them into a new database.

CombineDataBases (*ListWave*)

This function combines (proto-)database waves referenced in the reference wave *ListWave* into a single database, combining the labels of all of them, if they are different. See the example of how to make a reference wave.

Example:

```
make/wave ListWave
ListWave = {DataBase1, DataBase2, DataBase3} //no duoble quotes
CombineDataBases(ListWave)
```

See also: Wave reference waves

DBFooter (*PopWave, [LabelList,ResultName]*)

This function appends a footer to *PopWave* and thus converts it to a proto-database. The following fields are automatically filled in: ROINr, nPoints, XDelta, XOffSet, XUnit, OriginID. The optional parameter *LabelList* specifies the labels that are added (default is the constant *k_LabelList*). The optional parameter *ResultName* specifies the name of the resulting wave (default: "F0_"+NameOfWave(*PopWave*)). The function returns the resulting wave reference.

EDB2_CP ()

This function launches the control panel to specify a populationwave and waves containing associated information to be compiled into a database. If an existing database is selected, the new information will be appended, otherwise a new proto-database will be created.

TraceFromDB (*DataBase, index, [ResultName]*)

This function extracts a single trace from *DataBase* at index *index* and returns it as *ResultName* (the default is the name of the original wave+"_"+Num2Str(index).

See also: PopFromDB

MakeDataBase (w*List,[LabelList]*)

This function makes proto-databases out of the PopulationWaves referenced in the reference wave *wList* and combines them into a single database. It also checks if waves specified by the local constants *k_Suffixes* and *k_AutoDetect* are present and adds their data in the respective fields.
The optional parameter *LabelList* specifies the labels that are added (default is the constant *k_LabelList*). The resulting database wave is returned by the function. See the example of how to make a reference wave.

Example:

```
make/wave ListWave
ListWave = {PopWave1, PopWave2, PopWave3}     //no duoble quotes
CombineDataBases(ListWave)
```

See also: Wave reference waves

PopFromDB (*DataBase, [ResultName]*)

This function removes the footer from *DataBase* and applies the wave scaling, thus making it into a PopulationWave. However, this works only if all the traces in *DataBase* have the same number of points and scaling in the x dimension. The result is returned as *ResultName* (default: NameOfWave(*DataBase*)+"_Pop")

See also: TraceFromDB

• ExpDB2_Extraction

This function provides a template to programatically extract information from an ExpDataBase2 database.

Related Topics: ExpDataBase2, Bitwise and Logical Operators

• GammaCorrCP

This control panel allows the change of the gamma value for a grayscale image. It actually creates a lookup table, rather than changing the image values. Note that changing the gamma value of an image constitutes a non-linear adjustment which has to be disclosed in most scientific journals.

• HClu

The functions in HClu are used for the implementation of hierarchical clustering algorithms of time-series data (in the form of a PopulationWave).

NormalizeTraces (*PopWave, options*)

This function normalizes traces in the PopulationWave *PopWave* from 0 to 1 (*options* bit 0 set) and then divides by its standard deviation (*options* bit 1 set)

See also: Setting Bit Parameters

BinPop (*PopWave, ResultName, nBins, [smth]*)

This function changes the y data from *PopWave* into *nBins* bins and returns the result as $*ResultName*. The optional parameter *smth* is the number of smoothing operations to be applied for binomial smoothing (default: 0, ie no smoothing).

See also: Smooth

PopDistances (*PopWave, options*)

This function calculates a distance matrix of all traces in *PopWave*. The metering method is specified by *options*:

options = 0 Euclidean
options = 1 Chebychev
options = 2 Hamming (for categorical data)
options = 3 Chebychev after binning with 5 bins, smoothing 5
options = 4 normalized Euclidean
options = 5 Pearson distance (Pearson distance = 1 - Pearson's R)
options = 6 Manhattan

HiClu (*Sorted, cutoff*)

This function calculates the membership of traces in *sorted* according to *cutoff*. *Sorted* is a wave with three columns, the first one holds the distances between two traces, the second and the third hold the index of the traces that the distance was measured between. *Sorted* is ususally the output of the function DistanceMatrix2Column, with the optional parameter *index* = 1 that has been sorted by the function SortByFirst. The resulting wave, DM2C, holds the number of the cluster respective traces, specified by the row (i.e. [p]), belong to. Traces are added by single linkage (a.k.a. nearest neighbor clustering) to clusters, i.e. if the distance to any trace in a cluster is less than *cutoff*.

See also: HCluCP

HiClu2D (*Sorted*)

This function calculates the nodes where subclusters of traces in *sorted* converge with higher clusters in a dendrogram. *Sorted* is a wave with three columns, the first one holds the distances between two traces, the second and the third hold the index of the traces that the distance was measured between. *Sorted* is ususally the output of the function DistanceMatrix2Column, with the optional parameter *index* = 1 that has been sorted by the function SortByFirst. The resulting wave, Clusters2D holds the number of the cluster respective traces, specified by the row (i.e. [p]), belong to. Each column represents one iteration, so that in the first column each trace is a cluster on its own, and in the last column all traces belong to the same cluster. The wave Cluster_Dist holds the absolute distances for each column.

See also: HCluCP

SortByFirst (*DM2C, rev*)

This function sorts the three-column wave *DM2C* by the entries in the first colum. If *rev* > 0, sorting is done in ascending order, else in descending order. This function returns a wave named NameofWave(*DM2C*)+"_s".

Renumber1D (*wv*)

This function renumbers the integer numbers in wv so that all copnsecutive numbers are present and none are left out.

- **HiCluCP**

This function launches the control panel for hierarchical clustering. Functions are called in the following order:

(optional) NormaliseTraces
PopDistances
DistanceMatrix2Column
SortByFirst
HiClu

- **IPLPosCP**

This function launches a control panel to access functions to determine the position of regions of interest in a layered structure.

Overview

Following steps will be executed:

1. Calculating centers of mass of the regions of interest.
2. Determining the borders of the structure, either by thresholding or free-hand drawing.
3. Interpolating the borders to have 8 times the number of points than the X dimension has pixels and smoothing by applying the robust form of the Loess algorithm.
4. Calculating percentiles between the two borders.
5. Determinig the percentile closest to each center of mass.

See also: CenterOfMass, Interpolate2, IPLPosition, Loess

The Panel

First, a grayscale image of the desired structure and an image specifying the regions of interest have to be specified in the appropriate pull-down menus. The latter has to be a MultiROI ROI wave. Then, a method for thresholding the borders has to be selected. *Iterative* works well, if a bright structure is well separated from the background and no other bright structures are present. If this fails, *Manual Treshold* may still work. Borders can always be specified by *Manual Drawing*, where the user has to draw points along the borders.

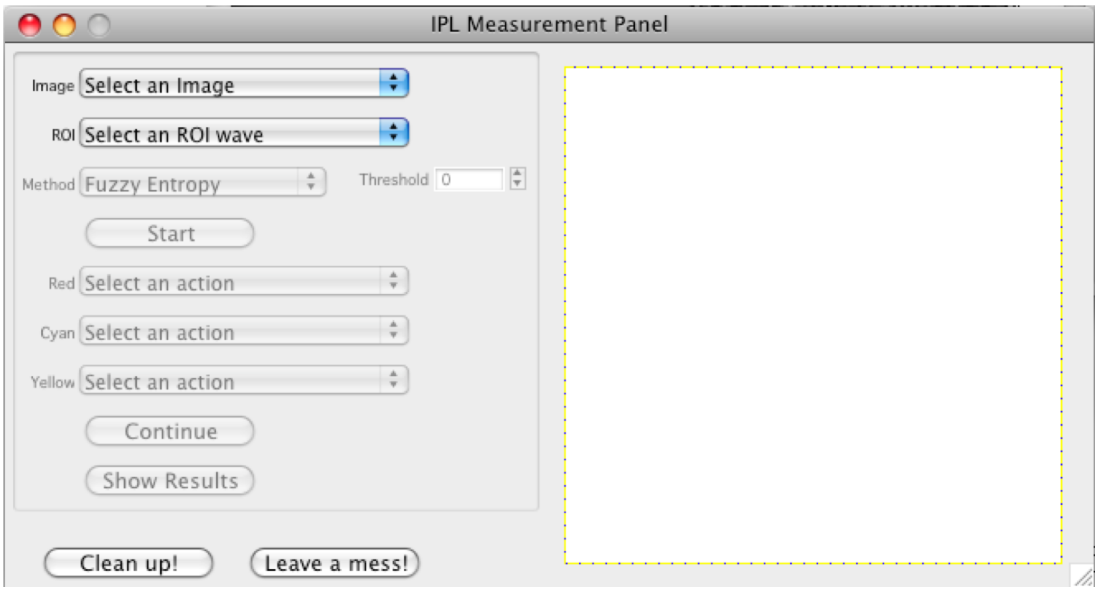
When *Start* is clicked, centers of mass for the regions of interest will be calculated and the thresholding applied. If *Manual Drawing* has been selected, then the user will be prompted to draw the borders (see below). The results are then displayed:
Three coloured lines: red, cyan and yellow, as well as the centers of mass.

The user has to specify, which of these lines is at the top, i.e. 100% (by definition, this is the photoreceptor side in the inner plexiform layer) and which at the bottom, i.e. 0% (ganglion cell side in the inner plexiform layer). Since automated thresholding sometimes breaks a border, two can be specified for either top or

bottom.

Clicking *Continue* will then calculate the percentage for each center of mass and store them in the wave *positions*.

The panel can be closed by clicking the button *Clean Up* or *Leave a Mess!*. The former will keep only the wave positions and kill all intermediates, while the latter will keep all the waves generated in the procedure and only remove gloval variables associated with the panel.



The IPL Measurement Panel

Manual Drawing

If *Manual Drawing* has been selected, then clicking *Start* will open a new window in which the background image is shown. By clicking in this window the user can specify points, between which a line will be drawn. When finished, double-click in the window and close it. This procedure is then repeated for the second border. After the second window has been dismissed, the results will be shown in the panel. Note that the lines specified by the user will be interpolated and smoothed.

Related Topics: CenterOfMass, IPLPosition, MultiROI ROI wave

• IPLPosition

These functions are used for determining the positions of regions of interest within a layered structure. They were written to analyse data from the inner plexiform layer of the retina, but may work for other layered structures as well. These functions are best accessed by the function IPLPosCP which is in the file IPLPosCP.ipf.

Note: Required include files are:
CenterOfMass.ipf
NaNBust.ipf

DrawCoMPositions(*CoMWave, positions, bgimg, WindowName, [fontsize, TextRot]*)

This function draws the Positions as stored in *positions* at the position of the center of mass, which are stored in *CoMWave*, in the window *WindowName*. *Bgimg* is needed to get the dimensions of the background image, which should be displayed in *WindowNam*e. *Fontsize* is optional and useful for very large images, as fonts don't scale when the image size is changed. The optional parameter *TextRot* sets the rotation of the labels in degrees.

Horizontal(*Xfunc, Yfunc*)

This function returns 1, if *Yfunc* vs *Xfunc* is roughly horizontal, and 0 if it is more vertical.
Horizontality is determined by first dividing the waves by their maximum value, then differentiating them. Then the maxima (maxX and maxY) and minima (minX and minY) of the derivatives are calculated. If the product of the maxX * minX is larger or equal to the product of maxY * minY, then 1 returned, else 0.

InterpAndLoess (*XWave,YWave,interpNumber, [SmoothNumber]*)

This function interpolates the curve *YWave* vs *XWave* linearly to *interpNumber* of points and then smoothes *YWave* vs *XWave* using locally-weighted regression smoothing with a factor of *SmoothNumber* (0<SmoothNumber<1; default = 0.5).

See Also: Interpolate2, Loess

MeasureDistances (*CoM, BottomX, BottomY, TopX, TopY*)

This function calculates the relative distance of centers of mass (stored in *CoM*) from the borders *BottomY* vs *BottomX* and *TopY* vs *TopX*. The top border is by definition 100%, the bottom 0%. The results are stored in the wave Positions.

This is achieved by first calculating percentiles between the two borders and then determining which of the percentile lies closest to each of the points. CoM is a two-dimensional wave, where Y coordinates are stored in CoM[][1] and X coordinates in CoM[][0].

Note: Points outside the borders will not be calculated precisely, but are assigned values of -1 or 101. You might want to remove them before doing any statistics.

See Also: CenterOfMass

MeasureDistancesFromPercentiles (*CoM, Percentiles*)

This function calculates the relative distance of centers of mass (stored in *CoM*) from the *percentiles* which have been calculated beforehand using the function MeasureDistances. The top border is by definition 100%, the bottom 0%. The results are stored in the wave Positions.

This is achieved by first calculating percentiles between the two borders and then determining which of the percentile lies closest to each of the points. CoM is a two-dimensional wave, where Y coordinates are stored in CoM[][1] and X coordinates in CoM[][0].

Note: Points outside the borders will not be calculated precisely, but are assigned values of -1 or 101. You might want to remove them before doing any statistics.

See Also: CenterOfMass

QuickPA (*image, size, method, [level]*)

This function applies particle analysis using a predetermined set of parameters. *Image* is the wave containing the image to be analysed, *size* is the minimum size in pixels for particles found, *method* specifies the thresholding method (see ImageThreshold for more details) and the optional parameter *level* is used if manual thresholding (method=0) is selected.

After removing extreme values (i.e. those coinciding with the borders of the image), the three longest contingent borders are returned as the waves
TopY vs TopX,
BottomY vs BottomX, and
ThirdY vs ThirdX

Note: While pairs of point match in all of these resulting Y vs X waves, they might not be ordered in a left-to-right fashion. This depends on the actual symmetry of the object to be detected. The function reorder has to be used to return them into an ascending order.

The core function of the code is the following:

```
ImageThreshold/I/M=(method)/Q image
ImageAnalyzeParticles /f/E/W/Q/M=0/A=(size) stats, M_ImageThresh
  //M_ImageThresh is the result of the ImageThreshold operation
```

See Also: ImageThreshold, ImageAnalyzeParticles

Reorder(*XWave,YWave*)

This operation reorders pairs of points in *YWave* vs *XWave* in ascending order of *XWave*.

Thickness(*Percentiles*)

This operation calculates and prints statistics on the distances between the 0th and 100th percentile in wave *percentiles*. Percentiles are a result of the MeasureDistances operation. Since the results are calculated using the WaveStats operation, its automatically created variables can be used.

See also: WaveStats

• LoadScanImage

The functions in LoadScanImage load gif images, stacks and movies recorded with the ScanImage plugin for Matlab (Pologruto et al. 2003, Journal Biomed Eng Online) and extract the information stored in the header. Files saved with other programs will be loaded, but since the header info is not available, or in a different format, it can't be used to set wave scaling, splitting channels, etc.

Note: Two constants are set at the top of the procedure file, which may need to be adjusted between different setups:
Z_factor is the factor by which distances in the z dimension have to be multiplied in

order to return actual distances in μm. This is important only for the correct scaling of image stacks, but not for single images or movies.
ImageLength is the side length in μm of an image taken at zoom 1. This will, among other factors, depend on the objective used. This is important to get the correct X and Y scaling of images.
For time series, the internal clock of the recording computer is assumed to be correct, so no adjustment is being made.

LoadScanImage ( )

This function loads an image or a stack of images and puts them in a 2D or 3D wave that has the same name as the the image file (without the extension). The header will be copied nto the wave note. This functions returns the name of the so generated wave as a string.

Note: On a Macintosh OSX computer, this operation seems to stall the Igor Pro application with the spinning wait cursor (a.k.a. spinning pizza of death) appearing. This behaviour is normal (sort of), with the duration of that effect being roughly proportional to the size of the image file being loaded. Other applications work normally in that time.

See also: ImageLoad, Note

LoadMovie ( )

This function loads multiple images into one 3D wave that has the same name as the the first image file (without the extension). The header will be copied into the wave note. This functions returns the name of the so generated wave as a string. This is usefulto load a timelapse movie that has been acquired in ScanImage.

The user is prompted to specify one file. This file is the first in the sequence to be loaded. The last three characters of the filename (not the extension) have to be numbers. This operation will then load all consecutive images in that folder into one 3D wave.

Note: On a Macintosh OSX computer, this operation seems to stall the Igor Pro application with the spinning wait cursor (a.k.a. spinning pizza of death) appearing. This behaviour is normal (sort of), with the duration of that effect being roughly proportional to the size of the image file being loaded. Other applications work normally in that time.

See also: ImageLoad, Note

ZoomFromHeader (*PicWave*)

This function returns the zoom factor from the wave note of *PicWave*.

FramesFromHeader (*PicWave*)

This function returns the number of frames from the wave note of *PicWave*.

XRelFromHeader (*PicWave*)

This function returns the relative X position of the stage from the wave note of *PicWave*.

YRelFromHeader (*PicWave*)

This function returns the relative Y position of the stage from the wave note of *PicWave*.

ZRelFromHeader (*PicWave*)

This function returns the relative Z position of the stage from the wave note of *PicWave*.

FilePathFromHeader (*PicWave*)

This function returns the file path where*PicWave* was loaded from as a string.

FileNameFromHeader (*PicWave*)

This function returns the file name where*PicWave* was loaded from as a string.

msPerLineFromHeader (*PicWave*)

This function returns the acquisition time for one line in milliseconds from the wave note of *PicWave*.

sPerLineFromHeader (*PicWave*)

This function returns the acquisition time for one line in seconds from the wave note of *PicWave*.

ExpDateFromHeader (*PicWave*)

This function returns the date of the experiment (i.e. when the image was acquired) from the wave note of *PicWave* as a string.

ZSlicesFromHeader (*PicWave*)

This function returns the number of Z slices from the wave note of *PicWave*. It will be 0, if a movie, rather than a Z stack had been acquired.

ZStepSizeFromHeader (*PicWave*)

This function returns the Z step size of slices from the wave note of *PicWave*. This is the number stored in the header, rather than a distance. This number multiplied by the constant Z_factor returns the actual distance (in μm).

nChannelsFromHeader (*PicWave*)

This function returns the number of channels that *PicWave* was acquired in (currently 1 - 3).

SplitChannels (*PicWave, nChannels*)

This function splits *PicWave* into *nChannels* stacks, each corresponding to the recording of a separate channel.

ApplyHeaderInfo (W*ave3D*)

This function applies X, Y and Z scaling from stored in the wave note to *Wave3D*. If *Wave3D* is a Z stack, then the Z scaling will be a unit of length, if it is a movie, it will be a unit of time, and if it is a single frame, then only X and Y scaling will be set.

InvertImage (*Image*)

This function inverts image by calculating v_max - *image* and returning the result as NameOfWave(*image*)+"_inv".

LoadMovie ()

This function lets the user specify a single file on the hard drive. All sequentially numbered .tiff files with the same base name are loaded into an image stack. The images must have the same number of pixels in the x and y dimension. Note that the numbering is taken from the last 3 characters of the filename, e.g., MyFile013.tif would return 13 as the file's number and attempt to load MyFile014.tif next. The function automatically stops if no matching file is found in the same directory. Use this function to load time-lapse data.

See Also: SetScale

• ManThresh

The functions in ManThresh create a panel to threshold a grayscale image. The resulting MultiROI ROI wave will be saved as MTROIWave.

The thresholding will be performed on the sum of the 2nd derivatives in the X and Y dimension. Optionally, regions of interest smaller than a given size can be excluded.

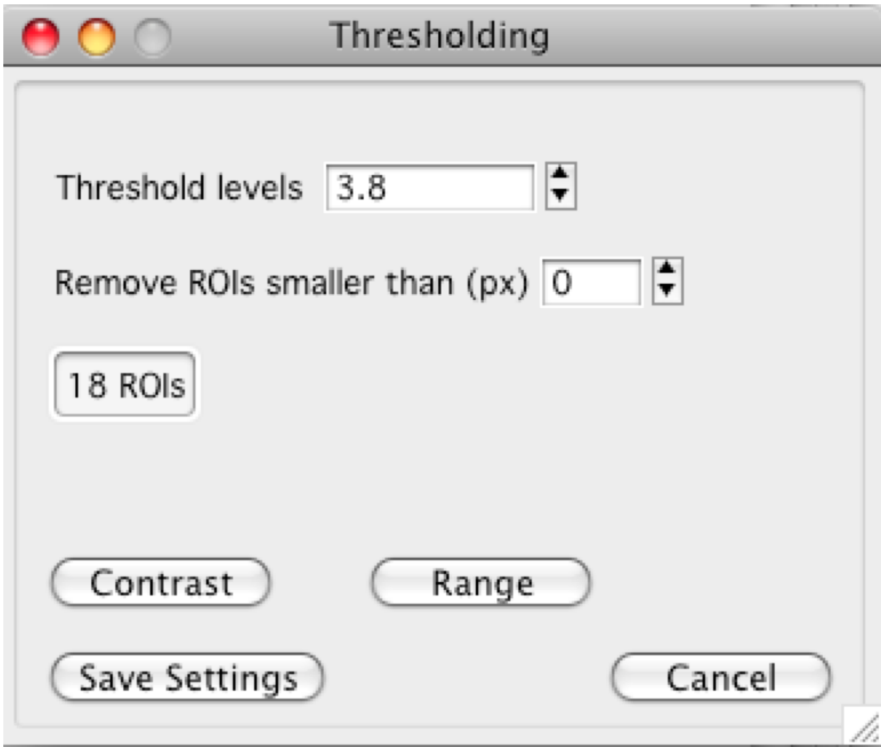
See Also: dif_image, MultiROI ROI wave

ManThresh (*picwave, startlevels*)

This function opens the thresholding panel. *Picwave* is the grayscale image to be thresholded, *startlevels* is the threshold level to be started with. This number is multiplied by the standard deviation of the 2nd derivative image (see above). Empirically, 3 is a good number to start.

Calling ManThresh opens two new windows: The control panel and a preview window. The user changes the threshold level until thei are happy with the preview. By clicking *Save Settings*, the result will be saved as MTROIWave. *Cancel* aborts the operation and saves no wave. *Contrast* and *Range* can be used to change the way *picwave* is displayed in the preview window. A Textbox will show how many regions of interest (ROIs) have been found.

Note: The algorithm to remove ROIs smaller than a given size is very slow with resolutions over a few thousand pixels. Using the arrows will make Igor calculate all intermediate steps, so it is better to enter numbers directly.



The ManThresh control panel

- **MultiROI ROI wave**

The multiROI ROI wave is a concept to encode different regions of interest (ROIs) in a single mask. Inbuilt functions in Igor use a binary ROI in which pixels with a value of 1 lie outside the ROI, those with a value of 0 inside. MultiROI ROI waves encode multiple ROIs using increasing negative numbers (-1 based).The function MultiROIZStack in Z-Project needs a MultiROI ROI wave passed as a parameter.

MultiROI (*sourcewave, targetwave*)

This operation modifies a binary ROI mask to become a MultiROI ROI wave. *sourcewave* is a binary ROI mask in which pixels with a value of 1 lie outside the ROI, those with a value of 0 inside. The function MultiROI assigns all connected pixels, i.e. pixels that lie in one of the eight surrounding pixels of any pixel with a value of 0, a negative number. It scans first line-by-line, increasing the rows at the end of each line, starting from the origin.
The string *targetwave* will be the name of the so generated MultiROI ROI mask.

Note: If two pixels are "connected" only on an edge, they will still be assigned the same ROI number. This behaviour can be changed by commenting out the four appropriate conditional assignements in the code (i.e. where neither x2 nor y2 is 0).

MultiROIByLayer (*sourcewave, targetwave*)

This operation calls MultiROI on each layer of the 3D wave *sourcewave*. The string *targetwave* will be the name of the so generated MultiROI ROI mask.

MultiROIStats (*image, ROI,[m]*)

This operation calculates statistics of the ROIs defined by *ROImask* on *image*. Set *m* to 2 in order to calculate the higher statistical moments; The default is 1. The results are stored in the wave ROIStats. Each row corresponds to an ROI, the different statistics are stored in columns as follows:

ROIStats[][0] = v_avg	Average of pixel values.
ROIStats[][1] = v_min	Minimum pixel value.
ROIStats[][2] = v_max	Maximum pixel value.
ROIStats[][3] = V_npnts	Number of points in the ROI.

(*m*=2 only):

ROIStats[][4] = v_sdev	Standard deviation of pixel values.
ROIStats[][5] = v_rms	Root mean squared of pixel values
ROIStats[][6] = v_skew	Skewness of pixel values.
ROIStats[][7] = v_kurt	Kurtosis of pixel values.
ROIStats[][8] = v_adev	Average deviation of pixel values.

See Also: ImageStats

MorphROI (*ROI, type*)

This function applies a morphological operator to the ROIMask *ROI*, which is specified by *type* (0=Erosion, 1=Dilation, 2=Closing, 3=Opening).

Related Topics: ImageGenerateROIMask, MultiROI, Z-Project

• MultiROIBeams

The functions in MultiROIBeams are used to extract time-course information of pixels in an image stack based on a binary ROI mask or a MultiROI mask.

MultiROIBeams (*wv, ROIMask*)

This operation will Extracts the beams (z-dimension data) from the iage stack *wv*, based on the ROI mask *ROIMask*, which can either be a binary ROI mask (ROIs have a value of 0), or a MultiROI ROI wave.
The function produces 2 waves: NameOfWave(wv)+"_ROIBeams", which is the populationwave containing all the beams, and NameOfWave(wv)+"_index" which contains the ROInumbers that the traces belong to.

SortPopBtIndex (*pop, index, [rev]*)

Sorts the populationwave *pop* so that entries with the same index in *index* are adjacent. If the optional parameter *rev* is TRUE, the sorting will be reversed. This function is called by MultiROIBeams.

ROIBeams2Traces (*ROIBeams, index, ResultName*)

Averages all traces in *ROIBeams* with the same index, provided by *index*, and stores the results in a populationwave named $*Resultname*.

MultiROIBeams_prompt ()

Calls a prompt window to select the inputs for MultiROIBeams.

- ## NaNBust

The functions in NaNBust replace numbers or NaN entries in waves. They are slightly more comfortable to use than inbuilt functions.

NaNBust (*wv, [newnum]*)
This operation will replace NaN values in the wave *wv* with 0 or the value of the optional parameter *newnum*. The heart of this function is the MatrixOP function:

```
MatrixOP/o/free NaN_Busted = ReplaceNaNs(wv, newnum)
```

Replace (*wv, findVal, replacementVal*)
This operation will replace values equal to *replace* in the wave *wv* with the value of *with*. The heart of this function is the MatrixOP function:

```
MatrixOP/o/free w_Replaced = Replace(wv, findVal, replacementVal)
```

Note: *replace* can be any number, but not NaN or ±inf. The function NaNBust can be used in the former case.

Related Topics: MatrixOP, NumType, SelectNumber

- ## Normalize

The functions in Normalize normalize waves and images.

IACPNormalize (*sourcewave, targetwave*)
This operation normalizes *sourcewave* by dividing it by the average of all points and then substracting 1. The string *targetwave* is the name of the so calculated wave. Hence, the average of $*targetwave* will be 0.

Note: This function normalizes to the average of all values in *sourcewave*. Often it is better to normalize to a baseline. This function is useful, however, if a baseline can not be automatically determined.

Normalise (*wv, from, to, [name]*)
This operation normalizes all values in the wave wv so that the values will be spread from *from* to *to*. The optional parameter name is the name of the so calculated wave, the default being nameofwave(*wv*)+"_nor".

This operation first substracts the smallest value (min) of *wv* from *wv* and then adds *from*. Then, *wv* will be multiplied by *to* and divided by (max - min). Max is the largest value in *wv*.

This is essential when a double precision wave is to be saved as an image, as images can only assume limited values, e.g. from 0 to 255 for 8 bit grayscale, 0 to 65535 for 16 bit grayscale, 0 to 255 in three channels for 24 bit colour, etc.

NormalizePop (*sourcewave, targetwave*)
This operation normalizes all rows in the populationwave *sourcewave* by dividing each

column by its average and then substracting 1. The string *targetwave* is the name of the so calculated populationwave.

Note: Confusingly, columns of a wave will be plotted against the X axis, while rows will be plotted against the Y axis in an image.

Note: This function normalizes to the average of a whole column in *sourcewave*. Often it is better to normalize to a baseline. This function is useful, however, if a baseline can not be automatically determined.

See Also: Populationwave

NormalizePopByInterval (*pop, start, stop*)
This operation normalizes all rows in the populationwave *pop* by the average of all values between the scaled timepoints*star*t and *stop* (F_0), so that

$F_{norm} = (F(x)-F_0)/F_0$

i.e., this function calculates $\Delta F/F_0$ with F_0 being the average of values between timepoints *start* and *stop*.

Note: The timepoints*star*t and *stop* are scaled (x), and not points (p).

Rectify (*sourcewave, targetwave, [direction]*)
This operation sets all negative values in *sourcewave* to zero. If the optional parameter *direction* is set to a value smaller than zero, all positive values in *sourcewave* will be set to zero instead. The string *targetwave* is the name of the so calculated wave. The heart of this function is the SelectNumber function:

```
wv_calc = SelectNumber((wv_calc[p][q][r][s])*direction<0,wv_calc[p][q][r][s],0)
```

See Also: SelectNumber

• OneClickSmooth

The functions in OneClickSmooth provide quick access to Igor's inbuilt image filtering algorithms, as well as filtering 3D data by principal component analysis (PCA).

FIltering by PCA lets the user choose the number of principal components to keep. Therefore, a higher number means less filtering.

OCS ()
opens a user interface from which the following parameters can be specified:
Image - The top wave in the top graph will be automatically chosen, but others can be specified from the pull-down menu.
Method - See the ImageFilter, MatrixFilter and PCA operations for a detailed description.
Filter Size - In pixels. Odd numbers should be preferred, as these preserve the filter symmetry. This won't have an effect if the method Hybridmedian has been selected.

If PCA is selected, this number will specify the numbers of principal components that are not rejected (i.e. kept).

The filtered image will have the same name as the original with "_fil" appended to its name.

Note: For three dimensional waves (i.e. image stacks or movies) the appropriate 3D (*n* x*n* x*n*) filters will be used, while 2D (*n* x*n*) filters will be used for two dimensional data. The method Hybridmedian exists only as a 3D filter, for 2D data the method FindEdges will be used instead. PCA works only on 3D data.

Filter2 (*image*)

The Filter2 operation works similar to OCS, only that the image to be filtered is passed as the parameter *image*. A popup will prompt the user for Method and Filter Size.

Related Topics: ImageFilter, MatrixFilter, PCA

• Populationwave

A populationwave is a 2D wave that contains multiple 1D waves with the same scaling and number of points. The X axis scaling/units of a populationwave is the same as the X axis/units scaling of the 1D waves, while the Y dimension is the index. The data full scale of the populationwave contains the units of the 1D waves.

The functions in Populationwave generate and separate populationwaves.

AverageWavesFromWindow ()

This operation first stores all traces from the top graph in a populationwave called *WinPop* and then calculates the average, SD and SEM of these traces. The results are stored in the waves *W_PopAvg*, *W_PopSD* and *W_PopSEM*, respectively. Furthermore, the average trace will be displayed as a bold black trace in the top window, and the average trace plus error bars (SD) will be displayed in a new graph.

PopCorrelate (*pop, [auto]*)

This function calculates the normalized cross-covariance of all traces in the populationwave *pop* at the x (time) shift where it is maximal. This differs from the Pearson correlation, as the latter calculates the correlation without a shift in the x-Axis. The results are stored in the waves *MaxCorr* and *PopCorrelogram*. *MaxCorr* is a 3D distance matrix, which contains the maximum normalized cross-covariance between each pair of traces in layer 0 and the scaled x location (time) of the maximum in layer 1. *PopCorrelogram* is a 3D matrix, which contains the normalized cross-covariance of all pairs of traces. The traces are arranged in x/y and the time in z. If the optional parameter *auto* is set to 1, then the auto cross-covariances are stored in the resulting waves, otherwise a NaN will be placed in their positions (default).

PopDisplayAll (*popwave*)

This function displays all traces of *popwave* in a graph. It also serves as a programming example of how to implement a basic loop in a function.

PopulationWave (*basename, outputname,number,[offset]*)

This function generates a apopulationwave of *number* 1D waves. All 1D waves must have the same basename *basename* and end with numbers. The optional parameter *offset* specifies the number of the first 1D wave, the default being zero. The string *outputname* is the name of the so generated populationwave.

Examples

```
PopulationWave("MyWave_", "MyPopWave", 10)
//Generates a wave named MyPopWave out of the waves MyWave_0, MyWave_1, ...,
MyWave_9

PopulationWave("MyWave_", "AnotherPopWave", 5, offset=10)
//Generates a wave named AnotherPopWave out of the waves MyWave_10, MyWave_11,
..., MyWave_14.
```

PopStats(*PopWave*)

This operation calculates the average, SD and SEM of all lines in the populationwave *PopWave*. The results are stored in the waves *W_PopAvg*, *W_PopSD* and *W_PopSEM*, respectively.

PopWaveFromWindow ()

This operation generates a populationwave out of all waves displayed in the top window. The resulting populationwave will be named *WinPop*. This operation works if lines out of a populationwave are displayed in the top window.

Note: The number of points and the X and data scaling will be taken from the top wave, as they are assumed to be the same in all waves.

PopX2Traces (*PopWave, basename*)

This operation reverses the operation PopulationWave: It generates a 1D wave out of each column of the populationwave *PopWave*. The string *basename* will be the basename of the so generated waves.

PopY2Traces (*PopWave, basename*)

This operation is similar to PopX2Traces, only that it generates a 1D wave out of each row of the populationwave *PopWave*. The string *basename* will be the basename of the so generated waves. This is equal to calling PopX2Traces after TransposeXY.

TransposeXY (*basename, outputname*)

This function swaps rows and columns (and the respecitve scaling) of the wave *basename*. The string *outputname* is the name of the so generated populationwave. Some inbuilt functions in Igor operate along rows, rather than columns.

• RegisterStack

The functions in RegisterStack call the inbuilt operation ImageRegistration with a predefined set of parameters on a stack of images (3D wave).

Note: ImageRegistration for stacks has been implemented in Igor Pro version 6.1. For earlier versions, a workaround is implemented that is slower, and probably less efficient.

See also: ImageRegistration

RegisterStack (*picwave, [target]*)
This operation registers the imagestack *picwave*. The optional string *target* is the name of the registered stack (default: Nameofwave(*picwave*)+"_reg").

The core of this operation (for Igor Pro 6.1 or later) is:

```
imageregistration /q /stck /csnr=0 /refm=0 /tstm=0 testwave=regcalcwave,
  refwave=ref
```

Regcalcwave is a duplicate of *picwave*, ref is the first frame of *picwave*.

See also: ImageRegistration

Reg2 (*picwave*)
Works similar to RegisterStack, only that picwave is overwritten.

QuickReg ()
Calls Reg2 on the top wave of the top window.

• ResultsByCoef

The operations in ResultsByCoef are used to retrieve a subset of data from a wave. They are not called in any automated analysis.

Definition: A coeffitients wave as used by operations in this file consists of the elements one is interested in. For instance, if one was interested in retrieving the data from traces #3,4, 7 and 8 then one would make a coefficients wave like this:

```
make /o coef={3,4,7,8}
```

Coef2Cat (*coef, n, val*)
This operation generates a category wave out of *coef* and assigns *val* to all entries in coef. *n* is the number of ROIs in the category wave. The results are stored in the wave *C2C*.

Example:

```
make /o coef={0,4,5,7}
CoefByCat(coef, 10, 1)          //generates C2C
print C2C                       //prints C2C[0]= {1,0,0,0,1,1,0,1,0,0}
```

CoMByCoef (*CoM, coef*)
This operation retrieves paired data from the (2D) wave *CoM* stored at the rows

specified in the wave *coef* and stores them in the wave *CbC*. ComByCoef is generally used to retrieve centers of mass but may be applied to other 2D waves that store paired information.

See also: CenterOfMass

CoMByLayer (*Positions, CoM, Layers*)

This operation retrieves paired data from the (2D) wave *CoM* and stores it into the 3D *CoMPoP* wave with the 3rd dimension reflecting the respective layers. The layers are separated by the information in the wave *layers* which stores the center and width (=2*sigma^2) information in the fashion Layers={center(0), width(0), center(1), width(1),..., center(n),width(n)} which is compared to the position of each center of mass, as stored in *Positions*. A center of mass is supposed to lie in a particular layer if it is within center±width/2.

See also: IPLPosition, IPLPosCP

CoMByROI (*CoM, ROI*)

This function calculates the centres of mass, as specified by *CoM*, which coincide with a region of interest, as specified by *ROI*. The result is stored in *w_CoMbyROI*, where the row indicates the index of the centre of mass and the entry at that row specifies the ROI on which it lies, or NaN if it is not on any ROI. These results can then be processed by making a histogram with the same number of bins as there are ROIs in *ROI*. This histogram will then whow the number of centres of mass for each ROI.

CoMByROICP ()

Calls a control panel to to preprocess data and call the cfunction CoMbyROI. The ROImask will be dilated the number of times entered in the appropriate field in order to detect centres of mass on the border of a ROI.

ResultsByCat (*data,catWave,category*)

his operation retrieves data from the (1D) wave *data* if the variable *category* matches the entry of the wave *catWave* at that index.

ResultsByCoef (*data,coef*)

This operation retrieves data from the (1D) wave *data* stored in the points specified in the wave *coef* and stores them in the wave *results*.

Example:

```
make /o data={2,1,3,5,6,8,11,4,7}
make /o coef={0,4,5,7}
resultsbycoef(data,coef)
print results                    // prints Results[0]= {2,6,8,4}
```

ROI2Cat (*ROIWave,CatWave,[Bkgr]*)

This function transforms a MultiROI ROI wave so that the index of the ROI is replaced by the respective category, as specified by *CatWave*. The results are stored in the wave *ROI2C*.

ROIbyCoef (*ROIWave,Coef*)

This function extracts the ROIs specified by *Coef* from the MultiROI ROI wave *ROIWave* and stores them in the ROI wave *ROIbCoef*.

Related Topics: CenterOfMass, PopulationWave

• ROISize

The operations in ROISize determine the sizes of regions of interest (ROIs) in a multiROI ROI wave, remove ROIs below a certain size and renumber them.

ClearROIMarquee ()

To use this operation, first display a multiROI ROI wave, then drag a marquee over an area where the ROIs are to be removed. ClearROIMarquee then removes all ROIs within a selected marquee and renumbers them.
For quick access, this function should be placed in a menu.

RemoveROI (*ROIwave, threshold*)

This operation removes all ROIs in the multiROI ROI wave *ROIwave* whose size is smaller than or equal to *threshold*. The result is stored in the wave ROI_edit.

RenumberROI (*ROIwave*)

This operation renumbers all ROIs in the multiROI ROI wave *ROIwave* in order to account for numbers that have been removed.

ROISize (*ROIwave*)

This operation determines the sizes in pixels of regions of interest (ROIs) in the multiROI ROI wave *ROIwave* and stores them in the wave Size. More statistics can be calculated using MultiROIStats.

• RotateFunction

The operations in RotateFunction rotate functions (i.e. scaled 1D waves or two 1D waves specifying x and y coordinates waves), images, image stacks or 2-column waves specifying x and y coorinates (such as the output of CenterOfMass) in the x/y plane.

RotateFunction1 (*func, angle,CenterX,CenterY*)

This operation rotates *func* angle degrees around the coordinates specified by *CenterX* and *CenterY*. The x scaling is taken from the scaling of *func*. The output are two waves, *rot_x* and *rot_y*, which can be displayed using the following command:

```
Display rot_y vs rot_x
```

RotateFunction2 (*func, angle,CenterX,CenterY*)

This operation rotates *Yfunc* vs *XFunc* angle degrees around the coordinates specified by *CenterX* and *CenterY*. The output are two waves, *rot_x* and *rot_y*, which can be displayed using the following command:

```
Display rot_y vs rot_x
```

RotateImage (*image, angle*)

This operation rotates the image or image stack *image angle* degrees (specified if the origin is in the lower left) around its center. This function preserves the pixel values and looks for the closest matching pixel to put them in, thus no interpolation is performed, which is the main difference to ImageRotate. The output is the wave W_RotatedImage.

RotateImage (*CoM, image, angle*)

This operation rotates *CoM* around the center of *image* by *angle* degrees. *CoM* is a 2-column wave specifying x and y coorinates (such as the output of CenterOfMass). The output is *CoM_rot*.

• RotateGUI

RotateGUI() calls a panel to specify parameters for rotating an image or image stack in the x/y plane. Matrix Rotation calls the custom-written function RotateImage, which preserves pixel values, i.e. does not interpolate. Image Rotation, which calls ImageRotate, however, is much faster.

• SWT

The functions in SWT.ipf perform thresholding based on the à trous wavelet transform (or stationary wavelet transform) of 2D or 3D waves. Reference: Olivo-Marin 2002, Pattern Recognition 35:1989-1996

SWT2D (*image, [filter]*)

Calculates the à trous (or stationary) wavelet transform of the 2D wave *image*. The optional parameter *filter* specifies a threshold, below which wavelet coefficients are ignored. *Filter* is scaled by the median average deviation of the wavelet coeffitients.

SWT3D (*image, [filter]*)

Calculates the à trous (or stationary) wavelet transform of the 3D wave *image*. The optional parameter *filter* specifies a threshold, below which wavelet coefficients are ignored. *Filter* is scaled by the median average deviation of the wavelet coeffitients.

makeSWTkernel (*iter*)

Returns the *iter* th iteration of the kernel {1/16,1/4,3/8,1/4,1/16}, by adding 2^(*iter*-1)-1 zeroes between two taps.

BigPi2D (*imstack, [stop]*)

Returns the layer-by-layer product (∏) of all or *stop* layers of *imstack*.

BigPi3D (*imstack, [stop]*)

Returns the chunk-by-chunk product (∏) of all or *stop* chunks of *imstack*.

filterW2D (*wavelet, k*)

Filters the 2D wavelet representation *wavelet* by setting all values smaller than

*k**statsMAD(coefficient) to 0.

filterW3D (*wavelet, k*)
Filters the 3D wavelet representation *wavelet* by setting all values smaller than *k**statsMAD(coefficient) to 0.

statsMAD(*wv*)
Returns the Median Absolute Deviation of the wave *wv*.

• SaveTiff

This operation saves a 2D or 3D wave as a tiff image.

Note: Normalize.ipf is a required include file.

SaveTiff (*wv, [depth]*)
This operation saves the image or image stack *wv* as a tiff file. The optional parameter *depth* specifies the bit depth of the tiff file (default is 16). Only following bit depths are allowed: 1, 8, 16, 24, 32, 40.

The user will be prompted for a location and a file name.

Related Topics: Normalize

• Z-Project

The operations in Z-Project analyse or modify stacks of grayscale images, i.e. movies or volume data.

Definitions:
ROI...region of interest
Image stack...a stack of grayscale images, 4 dimensions (x,y,z, intensity). Stored in a 3D (!) wave
Image...a grayscale image, 3 dimensions (x, y, intensity). Stored in a 2D (!) wave.
Trace...a trace, 2 dimensions (x, y), stored in a 1D (!) wave.
Populationwave...a 2D wave that stores several traces. The trace number is encoded in the y dimension, while the y value of the traces is stored as the intensity.

Note: Required include files are:
MultiRoi.ipf
EqualizeScaling.ipf

See also: EqualizeScaling, PopulationWave, MultiROI ROI wave

AvgZ (*picwave, outputwave*)
This operation averages all layers of the image stack *picwave* using the inbuilt function:

```
imagetransform averageimage picwave
```

The string *outputwave* will be the name of the so generated image M_AveImage. The new image will have the same x and y scaling as *picwave*.

See also: Imagetransform

StdevZ (*picwave, outputwave*)

This operation averages all layers of the image stack *picwave* and returns the image showing the standard deviation using the inbuilt function:

```
imagetransform averageimage picwave
```

The string *outputwave* will be the name of the so generated image M_StdvImage. The new image will have the same x and y scaling as *picwave*. The string *outputwave* will be the name of the so generated image stack.

MaxZ(*picwave, outputwave*)

MaxZ generates a single image out of the stack *picwave* whose pixel intensities represent the maximum values of all layers of *picwave*. The string *outputwave* will be the name of the so generated image. The new image will have the same x and y scaling as *picwave*.

MinZ (*picwave, outputwave*)

MinZ generates a single image out of the stack *picwave* whose pixel intensities represent the minimum values of all layers of *picwave*. The string *outputwave* will be the name of the so generated image. The new image will have the same x and y scaling as *picwave*.

RangeZ (*picwave, outputwave*)

RangeZ generates a single image out of the stack *picwave* whose pixel intensities represent the maximum minus minimum values (range) of all layers of *picwave*. The string *outputwave* will be the name of the so generated image. The new image will have the same x and y scaling as *picwave*.

zStack (*picwave, outputwave, roiwave*)

zStack returns a Z-stack of all layers in the image stack *picwave*, averaging all pixels in *picwave* whose value in *roiwave* is 0. Obviously, *picwave* and *roiwave* must have the same number of pixels in the x and y dimension. *roiwave* is two-dimensional. The string *outputwave* will be the name of the so generated trace, whose x scaling will be the same as the z scaling of *picwave*, while its y scaling will not be set.

MultiROIZstack (*picwave, outputwave, roiwave*)

MultiROIZstack returns multiple Z-stacks of all layers in the image stack *picwave*, averaging all pixels in *picwave* that have the same negative value in *roiwave*. The string *outputwave* will be the name of the so generated 2D wave (a populationwave), whose x scaling will be the same as the z scaling of *picwave*. *roiwave* must be a MultiROI ROI wave, in which increasing negative numbers (-1 based) encode different ROIs.

SubstBG (*picwave, outputwave, roiwave*)

SubstBG returns an image stack with the same scaling as *picwave* that has the average

intensity of all pixels with a value of 0 in *roiwave* substracted from *picwave* on a layer-by-layer basis. Obviously, *picwave* and *roiwave* must have the same number of pixels in the x and y dimension. *roiwave* is two-dimensional. The string *outputwave* will be the name of the so generated image stack.

NormBG (*picwave, outputwave, roiwave*)

NormBG returns an image stack with the same scaling as *picwave.* The result is *picwave* divided by the average intensity of all pixels with a value of 0 in *roiwave* minus 1, on a layer-by-layer basis. Obviously, *picwave* and *roiwave* must have the same number of pixels in the x and y dimension. *roiwave* is two-dimensional. The string *outputwave* will be the name of the so generated image stack.

See also: PopulationWave, MultiROI ROI wave

SubstBGPoly (*picwave, outputwave, roiwave, order*)

This operation calls the function

```
imageremovebackground /r=roiwave /p=(order) frame
```

which removes a general background level, described by a polynomial of a specified order *order*, from the image in *picwave* layer by layer, which is copied into the wave *frame*. The string *outputwave* will be the name of the so generated image stack.

Note: This function is experimental and is not called by any automated image analysis procedures.

See also: ImageRemoveBackground

Related Topics: EqualizeScaling