ParticleStats

Open source software for the analysis of particle motility and cytoskeletal polarity

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User Guide

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Contents

ParticleStats:Compare

The dynamics of particle movement, such as in the case of motor driven transport, can be explored through the calculation of runs and paused in the movement. The ParticleStats:Compare module takes two sets of tracked data and makes a comparison between the sets of X,Y coordinates. Statistical comparisons can be made between different populations of coordinates such as in the case of a wild type Vs mutant.

Input Files

The minimal input for running the ParticleStats:Compare module is an Excel format file containing X and Y coordinates.

Excel File:

The excel format was chosen as several tracking programs output the coordinate data directly to Excel format. Although the Excel file format is a closed-format the files can be written using open source software such as OpenOffice.

Coordinate Data:
The coordinates should be supplied in the format outlined in the image below. The order of the columns is not important, however the spelling of the headers should be exact including the case of the letters. The columns are: "Image Name", "Image Plane", "Object #", "Frame #", "X", "Y", "Time Interval"

	A	B	C	D	E	F	G	H	I
1	Image Name	Image Plane	Object #	Frame #	X	Y	Distance	Velocity	Time Interval
2	C2	1	1	4	88	79	0.161	0.032	5
3	C2	2	1	5	88	77	0.322	0.064	5

Correction Data:
A set of coordinates can be supplied to correct for any movement or drift observed in the original image data. The sheet in the Excel file must be called “Correction Data”. The columns are: "Image Name", "Stack", "X", "Y"

	A	B	C	D
1	Image Name	Stack	X	Y
2	C2	1	96	82
3	C2	2	96	82

Axis Data:
Axis data should be provided to allow the orientation of each of the movies. This is appropriate if the cell or area being tracked has a polarity, like in the Drosophila oocyte (Dorsal, Ventral, Anterior, Posterior). The Excel sheet must be called “Axis Data”. The columns are: "Image Name", "Stack", "X", "Y"

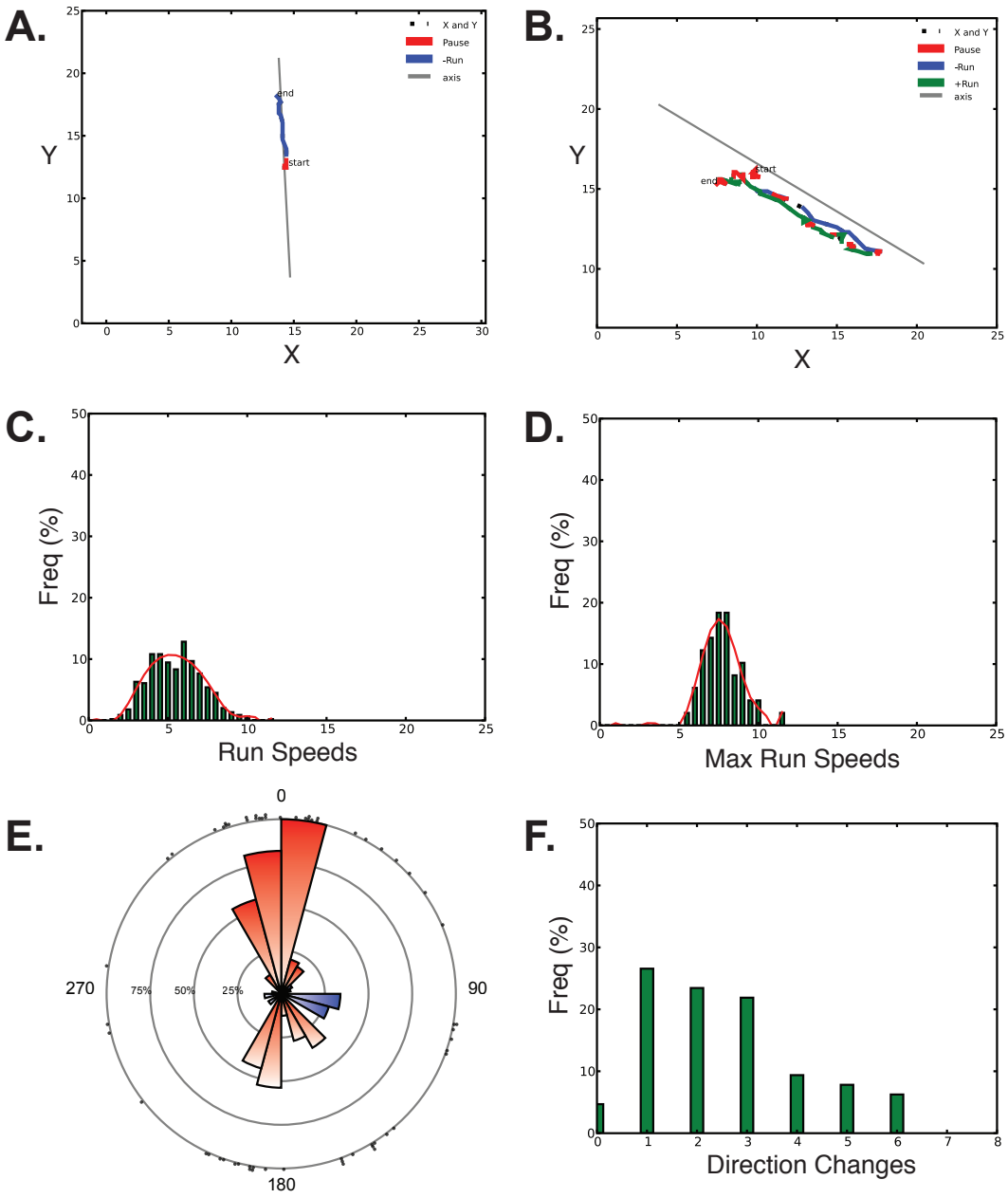
	A	B	C	D	E
1	Image Name	X1	Y1	X2	Y2
2	C2	98	25	92	141
3	C5	52	38	70	139

Text Output

The calculations of the runs and pauses for the movement each of the tracked objects are presented in text form, an example is shown below.

```
Runs for particle   11 sheet  3  (Excel Row= 1883  File= Mad2TEV.xls )
 No Coords = 102  No +ve Runs =  3  No -ve Runs =  2  No Pauses =  10
 Event       Start End Dir   Dist  SDist  RDist  Angle  Speed SSpeed RSpeed   Time
 Run     1 [     1   5  -1    1.4    1.1    1.2  324.3   3.32   2.62   2.82     25
 Pause   2 [     5   7  +0    0.5    0.2    0.0    0.0   1.94   0.60   0.00     15
 Pause   3 [    11  13  +0    0.5    0.0    0.0    0.0   2.05   0.00   0.00     15
 Pause   4 [    18  20  +0    0.7    0.3    0.4    0.0   2.79   1.20   1.70     15
 Run     5 [    22  27  -1    2.4    0.3    0.3   54.9   4.71   0.67   0.68     30
 Pause   6 [    27  31  +0    1.1    0.0    0.0    0.0   2.75   0.00   0.00     25
 Pause   7 [    35  43  +0    1.9    0.5    0.4    0.0   2.54   0.63   0.58     45
 Pause   8 [    47  55  +0    1.7    0.2    0.3    0.0   2.21   0.28   0.35     45
 Pause   9 [    59  63  +0    0.1    0.1    0.2    0.0   0.36   0.36   0.36     25
 Run    10 [    65  68  +1    1.8    1.1    1.1  238.0   5.30   3.43   3.42     20
 Pause  11 [    68  70  +0    0.5    0.3    0.3    0.0   1.94   1.34   1.34     15
 Run    12 [    72  78  +1    3.0    2.2    2.3  236.7   5.07   3.82   3.95     35
 Pause  13 [    78  81  +0    0.8    0.5    0.6    0.0   2.32   1.62   1.85     20
 Pause  14 [    85  88  +0    0.4    0.2    0.2    0.0   1.09   0.64   0.64     20
 Run    15 [    90  97  +1    2.2    1.9    1.9  246.5   3.27   2.78   2.79     40
 Average Speed       = [+ve    4.54][-ve    4.02][all    2.78]
 Average Run Length  = [+ve    1.77] [-ve  189.63]
 Standard Deviations = Speed [+ve 1.11 ] [-ve 0.98 ]
                       Dist  [+ve 0.59 ] [-ve 0.59 ]
 Standard Errors     = Speed [+ve 0.37 ] [-ve 0.49 ]
                       Dist  [+ve 0.20 ] [-ve 0.29 ]
```

Graphical Output



ParticleStats:Compare. (A-B). Two examples of plots of kinetochore separation. The movements are divided up into runs (+ green, - blue) and pauses (red) with a user supplied orientation line (grey). **(C)** A frequency distribution for run speeds, where the runs have been split into overlapping three frame windows. **(D)** The maximum three frame speed is plotted for each run. **(E)** Rose diagram with individual run angles plotted on the circumference, and the petals showing run angle frequencies. **(F)** A frequency distribution of the changes in direction for runs in a set of kinetochore separation examples.

Command Line: Full Options

To view the full list of options for ParticleStats:Compare use the -h (help) flag as shown below:

```
python ParticleStats_Compare.py -h
```

There is an extensive list of user customisable options, however most have default values which should be suitable for a first run approach. The options can then be configured as required. The minimal requirements for running ParticleStats:Compare are the excel files containing the coordinates (See Input Files below).

```
Usage: ParticleStats_Compare.py [--a=ExcelFile1] [--b=ExcelFile2]

Options:
  --version             show program's version number and exit
  -h, --help            show this help message and exit
  -a EXCELFILE1, --xls1=EXCELFILE1
                        Name of first Excel File
  -b EXCELFILE2, --xls2=EXCELFILE2
                        Name of second Excel File
  -o OUTPUTTYPE         print text ot html style output: DEFAULT=text
  --outdir=OUTPUTDIR    Specify a directory for the output files
  --outhtml=OUTPUTHTML  Specify a web location for the HTML output
  --trackingtype=TrackingType
                        Source of tracked coords: DEFAULT=metamorph
  -g, --graphs          print graphs
  -t, --trails          print trails
  -r, --regression      Run linear regression analysis
  -d, --debug           print full debug output
  --timestart=TIMESTART
                        Provide a time point start point for movement
                        calculations
  --timeend=TIMEEND     Provide a time point end point for movement
                        calculations
  --pausedefinition=PAUSEDEF
                        Pause definition: speed or distance DEFAULT=distance
  --rundistance=RUNDISTANCE
                        Run Distance in nm: DEFAULT=1.1
  --runframes=RUNFRAMES
                        Run Frames: DEFAULT=0
  --pausedistance=PAUSEDISTANCE
                        Pause Distance in nm: DEFAULT=10
  --pauseduration=PAUSEDURATION
                        Pause Duration in miliseconds: DEFAULT=2000
  --pausespeed=PAUSESPEED
                        Pause Speed: DEFAULT=0.25
  --pauseframes=PAUSEFRAMES
                        Pause Frames: DEFAULT=3
  --reverseframes=REVERSEFRAMES
                        Reverse Frames: DEFAULT=2
  --flipY               Changes the default orientation for the Y axis.
                        Default y=0 is at the top of the image
  --imagesize=IMAGESIZE
                        Image size to define the range of the coordinates
                        DEFAULT=512
  --pixelratio=PIXELRATIO
                        Pixel Ratio (nm per pixel): DEFAULT=1.00
  --pixelratiomethod=PIXELRATIOMETHOD
                        Pixel Ratio calculation method <multiply/divide>:
                        DEFAULT=multiply
  --dimensions=DIMENSIONS
                        Number of dimensions (1DX, 1DY, 2D): DEFAULT=2D
```

Command Line: Basic Usage

```
python ParticleStats_Compare.py -a EXCELFILE1 -b EXCELFILE2
```

Where EXCELFILE1 and EXCELFILE2 are Excel files to be compared from the tracking software such as Metamorph.

Command Line: Published Usage Examples

Vendra, G., Hamilton, R.S. & Davis, I. (2007) Dynactin suppresses the retrograde movement of apically localized mRNA in Drosophila blastoderm embryos. *RNA*, **13**, 1-8. [DOI]

```
python ParticleStats_Compare.py -o text -g -t -d --runframes=3 --pausespeed=0.25
--pauseframes=3 --pixelratio=216 -a ParticleStats_Vendra_wild.xls -b
ParticleStats_Vendra_glued.xls
```

Oliveira, R.A., Hamilton, R.S., Pauli, A., Davis, I., Nasmyth, K. (2009) Cohesin cleavage and Cdk inhibition trigger formation of daughter nuclei. *Nature Cell Biology,* **12**, 185-192 [DOI]

```
python ParticleStats_Compare.py -o text -g -r --runframes=3 --pausespeed=0.25 --
pauseframes=3 --pixelratio=0.15 -a control.xls -b Mad2TEV.xls
```

ParticleStats:Directionality

Summary

The directionality of a set of tracked particles is determined using directional statistics. The windmaps are a novel way of visualizing bias in the travel direction of particles. Further evidence is provided with rose diagrams and radial histograms

Input Files

The minimal input for running the ParticleStats:Directionality module is an Excel format file containing X and Y coordinates and a dingle image file.

Images:

The images can be in several formats (TIFF, PNG, JPG, GIF) and can be any frame from a time series or Z-stack. Ideally the image will be one to best represent the entire movie of images used for the tracking. The results including the trails and windmaps are then plotted using this image as a background.

Excel File:

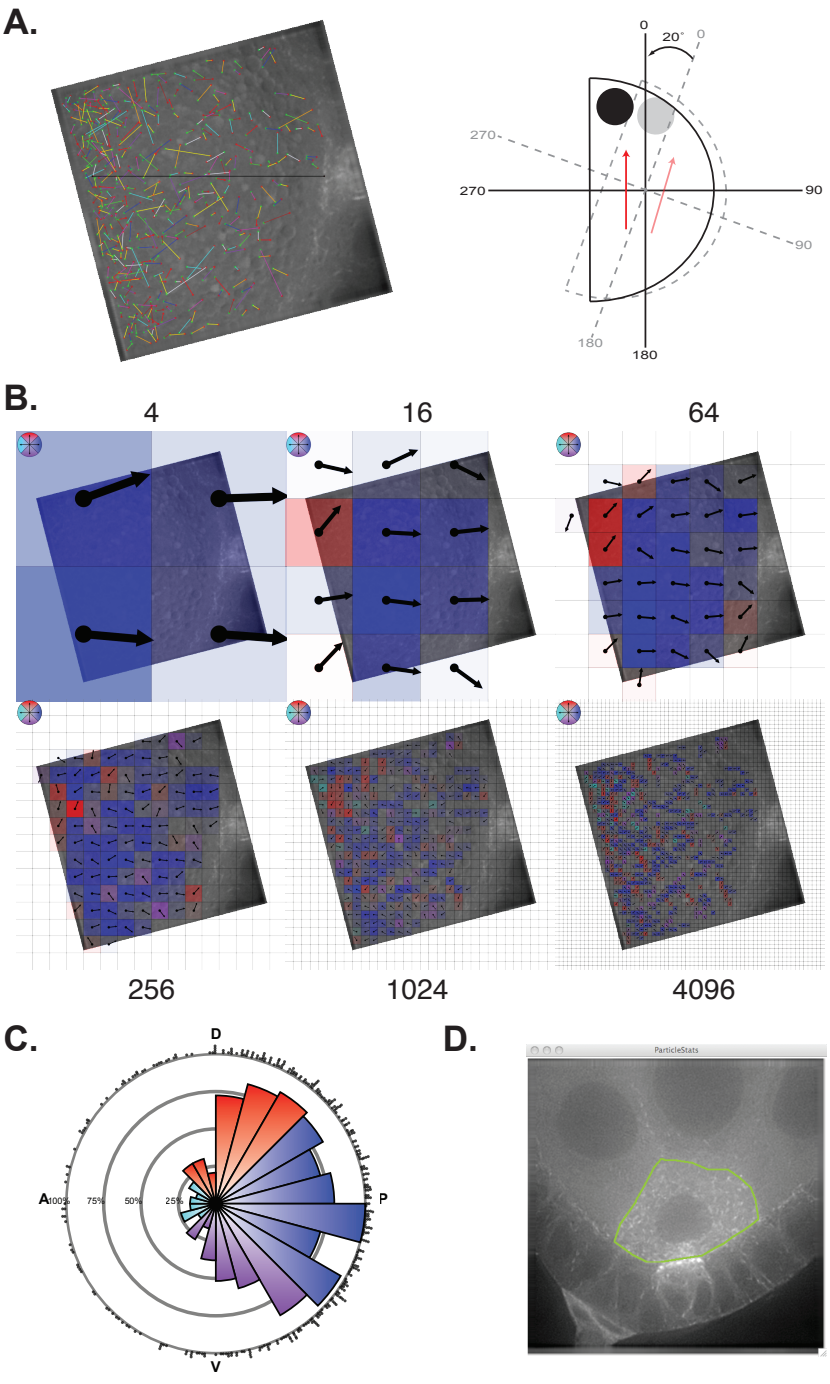
The excel format was chosen as several tracking programs output the coordinate data directly to Excel format. Although the Excel file format is a closed-format the files can be written using open source software such as OpenOffice.

Coordinate Data:
The coordinates should be supplied in the format outlined in the image below. The order of the columns is not important, however the spelling of the headers should be exact including the case of the letters. The columns are: "Track #", "X", "Y", "Z" - not fully implemented, so can take any integer value, "Image Name"

	A	B	C	D	E
1	Track #	X	Y	Z	Image Name
2					
3	1	477	339	1	DirectionalityExample
4	1	68	234	1	DirectionalityExample
5					
6	2	187	342	1	DirectionalityExample
7	2	165	353	9	DirectionalityExample
8					
9	3	149	345	6	DirectionalityExample
10	3	123	335	13	DirectionalityExample
11					
12	4	107	360	1	DirectionalityExample
13	4	73	335	12	DirectionalityExample
14					
15	5	117	403	15	DirectionalityExample
16	5	95	395	23	DirectionalityExample

The excel file can contain multiple sheets of coordinate data. Each sheet is treated as a "set" of data.

Output Files



ParticleStats:Directionality. (**A)** The tracked particles are plotted and rotated according to a user defined axis (red arrow). **(B)** Windmaps visually display trends in the directionality of tracked particles. The windmaps are created for a range of square resolutions (4 up to 4096) and are coloured according to the user defined axis. e.g. dorsal (red), posterior (blue), ventral (purple), anterior (cyan). **(C)** A rose diagram showing the angle of each track around the circumference of the plot. The petals of the rose show angles for tracks in defined angle ranges. **(D)** A screenshot of the simple graphical tool used to generate coordinates for a regions of interest (ROI).

Windmaps are generated at different resolutions, set by the number of squares (4, 16, 64, 256, 1024, 4096). For each square tracks are converted to vectors for the part of the track contributing to the square. The resultant vector direction is then plotted as an arrow for that square. The opacity of the square is proportional to either the number of tracks or the magnitude of the resultant vector.

Text Output

Summaries of the orientation biases are given for the input data set. The table below shows these summaries divided up into different ranges of angles. If a Region of Interest (ROI) is supplied then an additional table displays the orientation summaries for ROI as well as for the full image.

0 to 45	16.19% (57/352)
45 to 90	22.16% (78/352)
90 to 135	19.32% (68/352)
135 to 180	19.89% (70/352)
180 to 225	6.82% (24/352)
225 to 270	4.26% (15/352)
270 to 315	4.55% (16/352)
315 to 360	6.82% (24/352)
0 to 90	38.35% (135/352)
90 to 180	39.20% (138/352)
180 to 270	11.08% (39/352)
270 to 360	11.36% (40/352)
0 to 180	77.56% (273/352)
180 to 360	22.44% (79/352)
315 to 45	23.01% (81/352)
45 to 135	41.48% (146/352)
135 to 225	26.70% (94/352)
225 to 315	8.81% (32/352)

Region of Interest

If a region of interest is given only coordinates within this region are considered in the analysis.

Command Line: Full Options

To view the full list of options for PS_Compare use the -h (help) flag as shown below:

```
python ParticleStats_Directionality.py -h

Usage: ParticleStats_Directionality.py [-x Excel [-t tif] [-s squares]

Options:
  --version             show program's version number and exit
  -h, --help            show this help message and exit
  -o OUTPUTTYPE, --outputtype=OUTPUTTYPE
                        print text ot html style output: DEFAULT=text
  --outdir=OUTPUTDIR    Specify a directory for the output files
  --outhtml=OUTPUTHTML  Specify a web location for the HTML output
  -x EXCELFILE, --xls=EXCELFILE
                        Name of Excel File
  -i IMAGEFILE, --image=IMAGEFILE
                        Name of image file: Tif(8bit)/PNG/GIF/JPG
  -s SQUARES, --squares=SQUARES
                        Number of squares (1,4,16,64,256,1024,4096): DEFAULT=4
  -a, --axis            Axis Angle included as first coordinate points?
  -p POLYGON, --polygon=POLYGON
                        Name of file containing polygon region coordinates
  --pixelratio=PIXELRATIO
                        Pixel Ratio (nm per pixel): DEFAULT=1.00
  --pixelratiomethod=PIXELRATIOMETHOD
                        Pixel Ratio calculation method <multiply/divide>:
                        DEFAULT=multiply
  --flipY               Changes the default orientation for the Y axis.
                        Default y=0 is at the top of the image
  -g, --grid            Toggle on / off the display of the grid
  -r, --rectangles      Toggle on / off the display of the coloured rectangles
  -c, --arrows          Toggle on / off the display of the direction arrows
  --ArrowColour=COLOUR  Colour specification for the arrows: DEFAULT=white
  --ROIColour=COLOUR    Colour specification for the ROI: DEFAULT=white
  --scalerose           Scale the Rose Diagrams so axis scales to the data,
                        not just to 100%
  --AxisLabels=AXISLABELS
                        Specification direction axis labels e.g.
                        North/South/West/East: DEFAULT=NSWE
```

Command Line: Basic Usage

```
python ParticleStats_Directionality.py -x EXCELFILE1 -t TIFFILE
```

The minimum required input is an excel file (see below for format) and a representative tiff image (see below for details)

Command Line: Published Usage Examples

Parton, R.M., Hamilton, R.S., Cullen, F., Ohkura, H. & Davis, I. (2009) Novel mapping of microtubule polarity and dynamics in the Drosophila oocyte provides an explanation for biased random RNA transport. *In preparation*

ParticleStats:Kymographs

Summary

ParticleStats:Kymographs was developed to analyse kymographs depicting chromosome separation during cell division . During mitosis, individual sister chromatids move apart to opposite poles of the cell, to allow efficient separation of the DNA into two genetically identical sets. Instead of tracking individual centromeres, kymographs show the averaged movement of all centromeres. Each kymograph was treated as two halves for the left and right kinetochores, and the pixel intensity values were extracted for each time point.

Noise Estimation

Each kymograph was treated as two halves for the left and right kinetochores, and the pixel intensity values were extracted for each time point. A weighted average calculation was used to determine the average separation distance for each of the halves of the image (Figure 3A,B). The noise levels were estimated using the edge pixel values with an additional customisable threshold of 95% of the maximum intensity above noise. We implemented a further noise estimation method, a diagonal edge-based pixel measure, where pixels are taken from a diagonal line from the top centre of the image to the bottom right. This second method improves noise estimation in the kinetochore separation kymographs, as the intensities are also mainly on a diagonal. The weighted average distances and weighed standard deviations are plotted on the kymographs for visual inspection, then a user defined time range is used to calculate the speeds of the kinetochore separation.

Input Files

The minimal data required for running PartileStats:Kymographs are a set of kymograph images

Images:

The kymograph images should ideally be in 8-bit format. As ParticleStats:Kymographs reads each of the pixel values from the input images is is best to use a format which does not use compression. There is support for inputting PNG, JPG, GIF and TIFF image formats. To upload the images to the ParticleStats web page, they must be archived together into one archive file. The supported formats for the archive are zip (.zip) and compressed tar (.tar.gz)

Excel File:

The excel format was chosen as several tracking programs output the coordinate data directly to Excel format. Although the Excel file format is a closed-format the files can be written using open source software such as OpenOffice. The excel file must contain the headers as in the example below. The order of the columns is not important, however the spelling of the headers should be exact including the case of the letters. The columns are: "Image Name" (must be the exact name of the kymograph image), "Time Interval", "Pixel Size". The excel file can contain multiple sheets of kymograph data. Each sheet is treated as a "set" of data.

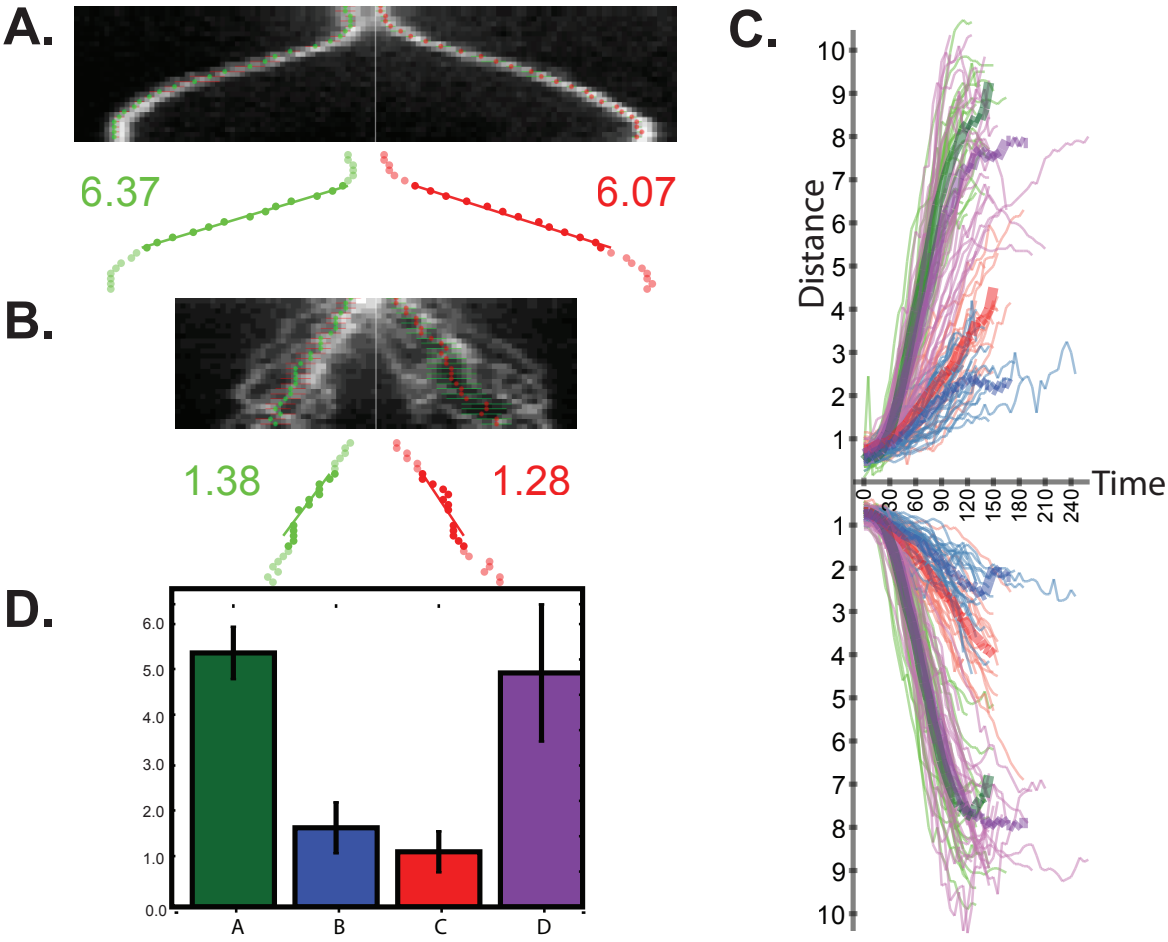
	A	B	C
1	Image Name	Time Interval	Pixel Size
2	KymographExample_b1.tif	5	0.1612
3	KymographExample_b2.tif	5	0.1612
4	KymographExample_b3.tif	5	0.1612
5	KymographExample_b4.tif	5	0.1612
6	KymographExample_b5.tif	5	0.1612
7	KymographExample_b6.tif	5	0.1612
8	KymographExample_b7.tif	5	0.1612
9	KymographExample_b8.tif	5	0.1612
10	KymographExample_b9.tif	5	0.1612
11	KymographExample_b10.tif	5	0.1612
12	KymographExample_b11.tif	5	0.1612
13	KymographExample_b12.tif	5	0.1612
14	KymographExample_b13.tif	5	0.1612
15	KymographExample_b14.tif	5	0.1612
16	KymographExample_b15.tif	5	0.1612
17	KymographExample_b16.tif	5	0.1612
18	KymographExample_b17.tif	5	0.1612
19	KymographExample_b18.tif	5	0.1612
20	KymographExample_b19.tif	5	0.1612
21	KymographExample_b20.tif	5	0.1612
22	KymographExample_b21.tif	5	0.1612
23	KymographExample_b22.tif	5	0.1612
24	KymographExample_b23.tif	5	0.1612
25	KymographExample_b24.tif	5	0.1612
26	KymographExample_b25.tif	5	0.1612
27	KymographExample_b26.tif	5	0.1612
28	KymographExample_b27.tif	5	0.1612
29	KymographExample_b28.tif	5	0.1612
30	KymographExample_b29.tif	5	0.1612

Text Output

The text output shows the results of the analysis of the kymograph images. The features of the kymograph are extracted using a weighted mean calculation (intensity and distance). These means are then used to calculate the speed of the movement over specified time ranges from the kymograph.

```
 + Image Name            = kymo1.tif
 + Image Size            = (110, 22)
 + Pixel Ratio           = 0.1612
 + Time Interval         = 5
 + Data Mid Point        = 55
    + Time Point 1  (t=0  ) Weighted Mean 0.75 0.63 Threshold 8.24 7.76 Noise 33.73 36.08
    + Time Point 2  (t=5  ) Weighted Mean 0.81 0.71 Threshold 8.59 8.12 Noise 31.37 33.73
    + Time Point 3  (t=10 ) Weighted Mean 0.85 0.79 Threshold 8.24 7.41 Noise 28.24 31.37
    + Time Point 4  (t=15 ) Weighted Mean 0.96 1.09 Threshold 8.29 6.94 Noise 23.92 25.10
    + Time Point 5  (t=20 ) Weighted Mean 1.01 1.30 Threshold 7.12 6.47 Noise 23.14 21.96
    + Time Point 6  (t=25 ) Weighted Mean 1.35 1.59 Threshold 7.53 6.94 Noise 17.65 20.39
    + Time Point 7  (t=30 ) Weighted Mean 1.61 2.10 Threshold 8.35 6.12 Noise 15.69 18.43
    + Time Point 8  (t=35 ) Weighted Mean 1.95 2.53 Threshold 7.65 5.59 Noise 12.94 15.69
    + Time Point 9  (t=40 ) Weighted Mean 2.34 2.84 Threshold 6.59 5.06 Noise 12.16 12.16
    + Time Point 10 (t=45 ) Weighted Mean 2.81 3.14 Threshold 6.00 4.94 Noise 11.37 13.73
+ Kymo Ave Speed = L 5.38 R 3.79 Ave 4.5
```

Graphical Output



ParticleStats:Kymograph (A) Kymograph of eight aligned kinetochore pairs. Weighted averages of the intensities are used to pick out the kinetochore paths (upper panel) and linear regression is used to determine the speed of the kinetochore separation (lower panel). **(B)** A kymograph showing less synchronous separation of kinetochores. **(C)** A plot of the kinetochore paths for approximately thirty examples of four variants of kinetochore separation experiments. Each of the four variants has an averaged line for the weighted averages. **(D)** A plot of the average speeds for the four variant kinetochore separations with error bars showing weighted standard deviations.

Command Line: Full Options

To view the full list of options use the -h (help) flag as shown below:

```
python ParticleStats_v2_b001_Kymographs.py -h

Usage: ParticleStats_Kymographs.py -xls1 <Excel>

Options:
  --version             show program's version number and exit
  -h, --help            show this help message and exit
  -o OUTPUTTYPE, --outputtype=OUTPUTTYPE
                        print text ot html style output: DEFAULT=text
  -n NOISE, --noise=NOISE
                        Correct for noise <None/segmented/edge/segmented_diag>
  -t THRESHOLD, --threshold=THRESHOLD
                        Provide a cut off for the segmented threshold 0.10 =
                        top 90% intensity above noise
  --speed_start=SPEED_START
                        Provide a time point start point for speed
                        calculations
  --speed_end=SPEED_END
                        Provide a time point end point for speed calculations
  -x EXCELFILE, --xls=EXCELFILE
                        Name of Excel File
  --pixelratio=PIXELRATIO
                        Pixel Ratio (nm per pixel): Default=0.15
  --TimeInterval=TIMEINTERVAL
                        Time Interval between data collection point in
                        kymograph: Default=5secs
  --tiffdir=TIFFDIR     Specify a directory containing the kymograph tiff
                        files
  --outdir=OUTPUTDIR    Specify a directory for the output files
  --outhtml=OUTPUTHTML  Specify a web location for the HTML output
```

Command Line: Basic Usage

```
python ParticleStats_v2_b001_Kymographs.py -x EXCELFILE1
```

The kymograph image files must be in the same directory as the input EXCELFILE1

Command Line: Published Usage Examples

Oliveira, R.A., Hamilton, R.S., Pauli, A., Davis, I., Nasmyth, K. (2010) Cohesin cleavage and Cdk inhibition trigger formation of daughter nuclei. *Nature Cell Biology,* **12**, 185-192 [DOI]

```
python ParticleStats_v2_b001_Kymographs.py -x kymo.xls -n segmented_diag -t 0.15
--speed_start=6 --speed_end=18
```

ParticleStats:ROI

Summary

The ParticleStats:ROI module is currently a stand alone application for generating region of interest (ROI) coordinate pairs for a user drawn area of interest. In future versions of ParticleStats the ROI tool will be implemented on the main ParticleStats server. The ROI coordinates can be generated by several methods, most image software will show the coordinates for particlulat pixels in an image. However we have provided a simple too to make generating ROI coordinates more straight-forward.

Input Files

The only input for the ROI tool is an example image for the data set being analysed. This image can be in several common image formats (PNG, GIF, JPG, TIFF)

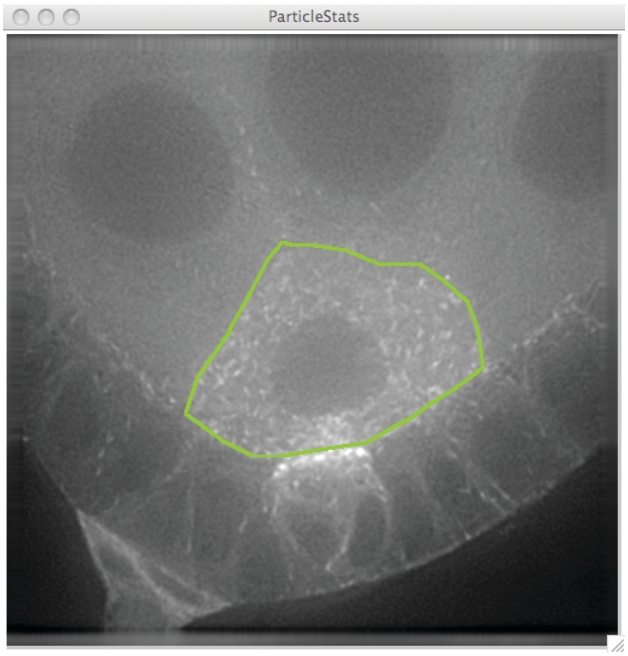
Usage

To view the full list of options use the -h (help) flag as shown below:

```
python ParticleStats_ROI.py -h
```

To start the ROI tool use the command below:

```
ParticleStats_ROI.py --img XXX.png --out YYY.text
```



Output

The ROI coordinates are printed to the screen, and to the specified output file. The format for the coordinates are:

```
0 [368.0, 208.0]
1 [361.0, 213.0]
2 [346.0, 229.0]
3 [339.0, 247.0]
```

Trouble Shooting Guide

Excel Files

Each of the ParticleStats modules requires that the coordinate data or image information be presented in an Excel file format.

The most common source of errors in reading the data in the Excel files are the headers of the columns. The headers must be spelled exactly as in the examples shown in this document and this includes the case of the headers i.e. capital and lower case lettering.

Another source of error is the inclusion of empty sheets in the excel files. By default Excel includes three sheets. Deleting these empty sheets can cure import problems

Image Formats (ParticleStats:Kymographs)

Each pixel value from the kymograph images are read to determine their intensity values. In order to ensure that these intensities are accurate it is best to use images in an uncompressed format. This will ensure that the original pixel intensity values are maintained.

Installation Instructions

ParticleStats is a command line program written in the Python programming language so therefore requires the Python interpreter to be installed on the computer intended to run it. Python is platform independent so should run on most major operating systems. ParticleStats is still undergoing active development so new releases are made available on a regular basis.

Please check www.ParticleStats.com for the latest version.

Any questions or comments can be sent to Russell Hamilton (Russell.Hamilton-@-bioch.ox.ac.uk)

Platforms

ParticleStats was written as platform independent open source software. It will therefore run on *nix style platforms (Linux, UNIX, Mac OSX) as well as on Windows XP. However ParticleStats was developed and tested on Linux and OSX so is more likely to work on these platforms rather than Windows XP.

Prerequisites

Programming Languages

- **Python** for Linux/MacOSX
- **ActiveState** for Microsoft Windows
- *Description:*
- Python Programming Language
- *Version:*
- 2.6.2 tested
- **R**
- *Description:*
- R Statistical Computing
- *Version:*
- 2.9.1 tested

Python Modules

In order to run ParticleStats several python modules must be installed. Details are provided below of which modules are needed and where to download them. Instructions for their installation can be found on their respective web pages. Each python module is platform independent so should work on all the main operating systems.

- **xlrd**
- *Description:*
- Library for extracting data from Microsoft Excel spreadsheets
- *Version:*
- 0.6.1 tested
- **matplotlib**
- *Description:*
- A python 2D plotting library
- *Version:*
- 0.90.0 tested
- **RPY2**
- *Description:*
- Python interface to the R Programming Language
- *Version:*
- 2.03 tested
- **PIL**
- *Description:*

- Python Image Library
- *Version:*
- 1.1.6 tested
- **SciPy**
- *Description:*
- Scientific Python
- *Version:*
- 0.5.2 tested
-

Installation

From python distribution:

1. Download ParticleStats
   ParticleStats comes as a tar archive file and as a zip archive.
   Download the appropriate version from www.ParticleStats.com
   (tar.gz for Linux/Unix/OSX; .zip for Windows XP).

2. Unpack
   ```
   tar -zxvf ParticleStats_XXX.tar.gz
   ```
3. Install
   ```
   python setup.py install
   ```
4. Test
   ```
   python ParticleStats_Compare.py -h
   python ParticleStats_Directionality.py -h
   python ParticleStats_Kymographs.py -h
   ```

From source code:

1. Create Directories
   Create a directory to put all the ParticleStats source code.
   e.g. in your home directory
   ```
   mkdir ParticleStats
   ```
   Create directories for the results generated by ParticleStats.
   ParticleStats generates a large number of png graphs for tracked particles,
   so it best to hae them create in a separate directory.
   ```
   mkdir GraphOutput
   ```
2. Download ParticleStats
   ParticleStats comes as a tar archive file and as a zip archive.
   Download the appropriate version from www.ParticleStats.com
   (tar.gz for Linux/Unix/OSX; .zip for Windows XP) and save it in
   the directory you created in step 1.
3. Unpack
   ```
   tar -zxvf ParticleStats-0.1.tar.gz
   ```
4. Test
   ```
   python ParticleStats_Compare.py -h
   python ParticleStats_Directionality.py -h
   python ParticleStats_Kymographs.py -h
   ```

Modifying ParticleStats

ParticleStats is open source and intended to be utilised in the analysis of and X and Y based coordinate results - typically particle tracking. We have added a wide range of analyses, however there may be others required. These can either be added by submitting a request to Russell.Hamilton@bioch.ox.ac.uk or by making modifications to the code. If you do make additions to the code we would love to hear about them and include them in future releases of ParticleStats.

Python is a scripting language that is fairly easy to pick up for anyone with some programming experience. Modifying ParticleStats should be possible to make without the requirement of a computer science degree!

Adding/Modifying the R statistical comparisons ParticleStats makes use of the R statistical language to perform statistical comparisons. The R language features all the common statistical functions, and can be added into ParticleStats. The code can be altered by following the examples already in the ParticleStats code.

FileList

Main programs:

- ParticleStats_Compare.py
- ParticleStats_Directionality.py
- ParticleStats_Kymographs.py
- ParticleStats_ROI.py ParticleStats.pl

Function modules:

- ParticleStats_Inputs.py
- ParticleStats_Maths.py
- ParticleStats_Outputs.py
- ParticleStats_Plots.py

Data Files:

- ParticleStats_Vendra_wild.xls
- ParticleStats_Vendra_glued.xls

Other Files:

- ParticleStats_LICENSE.txt
- ParticleStats_README.txt

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Version 3, 29 June 2007

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