Particle Track and Analysis (PTA) Version 1.2

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Contents

1 About this plugin

1.1 ATTENTION

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1.2 Acknowledgement

2-dimensional Gaussian fitting is done by Levenberg-Marquardt method through the cminpack library (cminpack.a, incorporated into the fit2DGauss library).

In addition, JFreeChart (and JCommon) packages are used for Graph visualization.

1.3 Abstract

The purpose of this plugin is "to track the particles and analyze them". This plugin works as a plugin of ImageJ. Thus, this plugin does not depend on the difference of platforms. It works as

- This plugin detects and tracks the particle by centroid and 2-D Gaussian distribution. The 2-D Gaussian fitting part is written in C, connected by JNI (Java Native Interface). Thus, fitting speed is relatively high compared to the JAVA based system.
- Simple and easy data management by table.
- X-Y trajectories are viewed as graphically by using JFreeChart.
- Analysis of mean square displacement and velocity change.
- Easy to make a coloring for individual particle's ROI.
- Export as text data.

2 Install

To use this plugin, several external packages are needed. And the place where files put on is different depending on the platforms.

2.1 Required files

- ImageJ (ver 1.43g or higher)
  - Download ImageJ from the site, http://rsbweb.nih.gov/ij/. Update ImageJ from its help menu.
  - In case of Winodws, choose the 32bit or 64bit ImageJ depending on your OS type.
- jfreechart.jar
  - Package for drawing the graph.
  - Download from this site... http://www.jfree.org/jfreechart/
- jcommon.jar (included jfreechart file)
- PTA_.jar
  - This is the heart of Particle Track and Analysis. ImageCapturing plugin is also included.
- libfit2DGauss.jnilib (for Mac), fit2DGauss.dll (for win)
  - JNI library for 2-D Gaussian fitting. The fitting algorithm is Levenberg-Marquardt method by using cminpack library (http://devernay.free.fr/hacks/cminpack.html)

2.2 Install to MacOSX

This plugin runs on MacOS10.5 or higher with Intel CPU.

- Install the ImageJ. Then, update the ImageJ via Help→Update ImageJ... command.
- Put the PTA_.jar file into the plugins folder (/Applications/ImageJ/plugins/)
- Put the libfit2DGauss.jnilib into the ImageJ folder (not plugins folder) (/Applications/ImageJ)
- Put the jcommonXXX.jar, jfreechartXXX.jar (XXX is version code) into the plugins folder (/Applications/ImageJ/plugins/)

2.3 Install to Windows

This plugin runs on 32bit or 64 bit versions of ImageJ. I confirmed XP, Vista and 7.

- Install the ImageJ (If your OS is vista or 7, you had better to install documents folder (not Program files folder). Then, update the ImageJ via Help→Update ImageJ... command.
- Put the PTA_.jar file into the plugins folder (∼/ImageJ/plugins/)
- Put the appropriate fit2DGauss.dll depending on your ImageJ bit into the ImageJ folder (not plugins folder)
- Put the jcommonXXX.jar, jfreechartXXX.jar files into the plugins folder (∼/ImageJ/plugins/)

After installation, start ImageJ and the PTA submenu will be found in plugins menu. Choose the PTA:Particle Track and Analysis. When you can see the threshold window and PTA Window, your installation was succeeded.

3 How to use this plugin

3.1 Tentative using: for still image

Use the cell colony image available through the ImageJ sample to resemble particles.

- Select File→Open Samples→Cell Colony (31k)
- Convert to 8bit grayscale Image by →Type→
- Invert gray level by Edit→Invert (This process may not be required)
- Start PTA plugin by Plugins→PTA→PTA:Particle Track and Analysis
- Check dark background by Threshold Window. Don't press the Apply button, or image will be banalized.
- Press [Preview] button on PTA window. Pre-detected Particles were viewed as square ROI. The function of [Preview] is only applied for viewed frame.
- Press [Track] button on PTA window. Particles were detected and Table window will appear.
- Select the rows what you want to watch the particle. Multiple selections is also functional.
- When you check the [All] check box in Appearance of PTA window AAll of detected ROI's will be viewed (Figure1)B

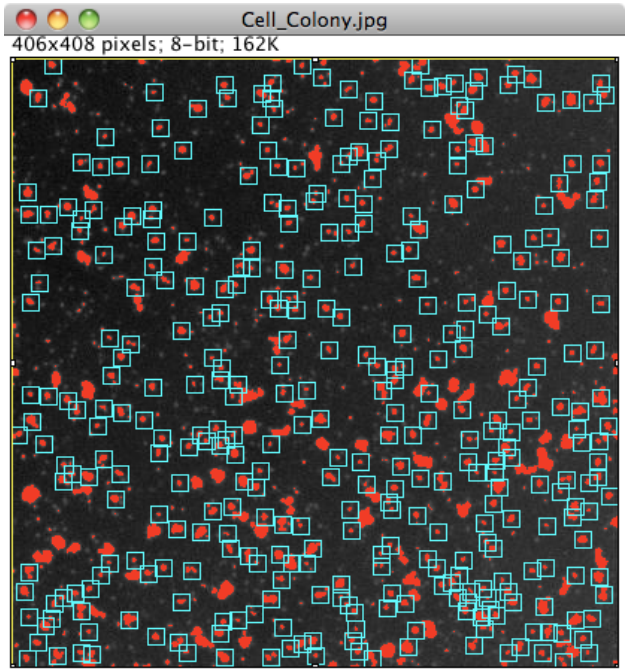


Figure 1: Detected particles

3.2 Tentative using: for stack images

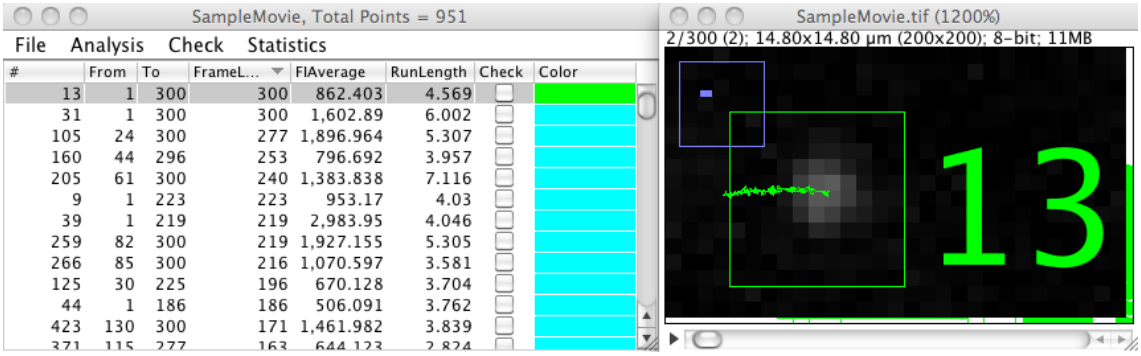
We use SampleMovie.avi as example.

- Open SampleMovie.avi by File→Open...
- Set the scale and frame interval by Image→Properties...
  1. Unit of Length: um (automatically translate to μm)
  2. Pixel Width: 0.074
  3. Pixel Height: 0.074 (may be changed automatically when you type on Pixel Width form)
  4. Frame Interval: 0.05 sec
- Check [Convert to Grayscale] in opening option window.
- Start PTA plugin by Plugins→PTA→PTA:Particle Track and Analysis. (Notice: PTA plugin can be started before opening imaging files)
- Set the Threshold value. Just check the Dark Background may be o.k.
- Press [Preview] button on PTA window. Pre-detected Particles were viewed as square ROI.
- Check [all Frames] check box.

- Press [Track] button on PTA window.
  - Detect particles for whole frames. When detection process is finished, the plugin link the each particles automatically.
- After finishing the detection and linking, all of detected trajectories will appear.

Now we can detect the moving particles. Let's choose the particle by Table. Trajectory window will appear will appear.

- Table can be sorted by columns. Sort by FrameLength as top-down.
- Select the top of row (#13) (Figure2)
- Roi and trajectory will appear on the movie (stack) window.
- Trajcetories Window(Figure3) will also appear. When the movie frame is changed, Roi on movie window and cross-cursors on the Trajectories Window will move.



#	From	To	FrameL...	FIAverage	RunLength	Check	Color
13	1	300	300	862.403	4.569		
31	1	300	300	1,602.89	6.002		
105	24	300	277	1,896.964	5.307		
160	44	296	253	796.692	3.957		
205	61	300	240	1,383.838	7.116		
9	1	223	223	953.17	4.03		
39	1	219	219	2,983.95	4.046		
259	82	300	219	1,927.155	5.305		
266	85	300	216	1,070.597	3.581		
125	30	225	196	670.128	3.704		
44	1	186	186	506.091	3.762		
423	130	300	171	1,461.982	3.839		

Figure 2: Table and correspond particle on movie window When you select the rows on table window, corresponding particles will appear. Selecting multiple rows lead to multiple ROI on movie window. In case of this figure, corresponding number is viewed by checking the [Number] on Appearance.

4 Functions

4.1 PTA Window

Let's learn about PTA window.

1. Preview: Perform detection for current image as a test.
2. Track: Start detecting particles

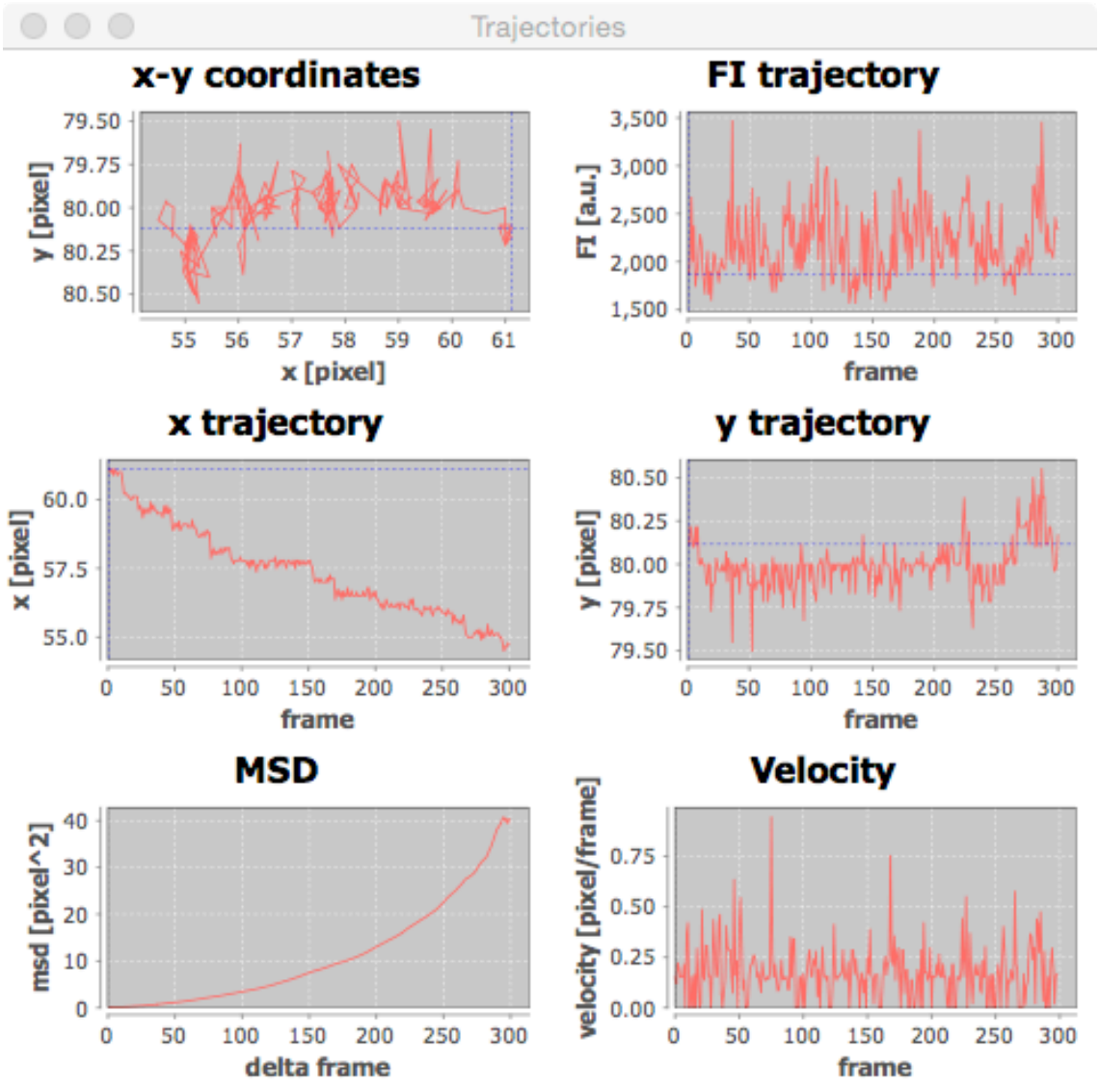


Figure 3: Trajectory data of selected particles

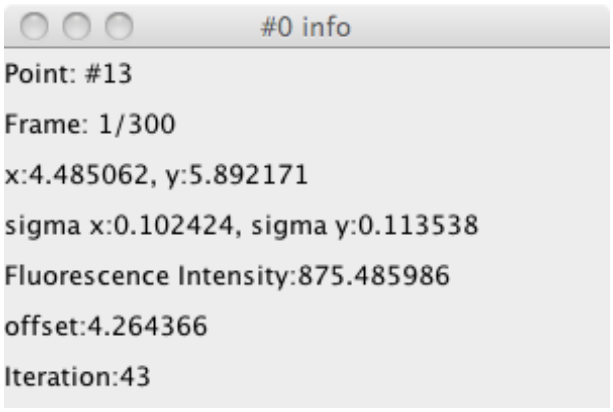


Figure 4: Information window

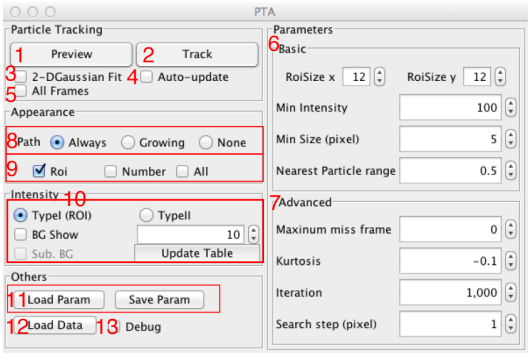


Figure 5: PTA Window Main windows

Tips: Scan area can be determined by square ROI.

3. 2-DGaussian Fit : Detect particles by 2D-Gaussian distribution fitting
4. Auto-update: Perform detection for current images as a test automatically following current Parameters and Threshold changing. This mode is convenient for detail parameter determination. WARNIG: Rapid changing parameter will be a cause of crash.
5. All Frames: For whole stacks detection.
6. Basic: Relatively basic parameters
   (a) RoiSize (pixel): Roisize when detect particles. Roisize can be modified for each x and y length independently. Too big or Too small Roi does not work for detection. About 10% larger than particles size works well.
   (b) Min Intensity :Minimum intensity for particle detection. In case of centroid, the intensity is sum of the pixel value within threshold. In case of 2D-Gaussian fitting, intensity is a coefficient of Gaussian distribution.
   (c) Min Size (pixel): Minimum particle size for detection
   (d) Nearest Particle Range : If the detected particles are located within the range of this value x Roisize, this plugin identify these particles as a single particle. This value is used to verify the overlapped particles and link the particles among frames.
7. Advanced : Relatively advanced parameters
   (a) Maximum miss frame: Sometimes, particles are missing for a while (Blinking, and so on). This factor determine how many frames are concerned for missing particles.
   (b) Kurtosis: Particles which have higher kurtosis value than this value.
   (c) Iteration: Upper limit number of iteration number for 2-D Gaussian fitting
   (d) Search step (pixel): Scanning step size when searching and detecting particels. High number of this parameter lead to rising detection time, whereas the probability of detection failure rate may be raised. 1$\sim$2 is proper.
8. Path : Parameters how to draw the trajectory path
   - Always: Always drawing trajectory path
   - Growing: Drawing trajectory path depending on the frame change
   - None: Do not draw the trajectory path
9. Roi: Parameters how to draw the ROI
   - Roi: On and off drawing of ROI

- Number: Draw the particle number on right side
- All: Drawing all of ROI

10. Intensity Panel
    - Roi, Gaussian Int: Choose which type of intensity representation
        - o Type I(ROI): The sum of the intensity
        - o Type II: With 2D Gaussian fitting; coefficient of Gaussian distribution Without 2D Gaussian fitting: The sum of the intensity higher than threshold value (same as previous version)
    - BG Show: As a background signals indicated in the number of right box, the Roi intensities at the last frame's position were appended on the trajectory graph.
    - Sub. Bg: Subtracting the background signals from intensity.
    - Update Table: Apply with or without background subtraction in the average intensity on table.
11. Load Param, Save Param: Load and Save Parameters
12. Load Data: Load the table data from the [Save Table] menu of table. When you load the table data,
13. debug : Debug check box for developer

4.2 Data table

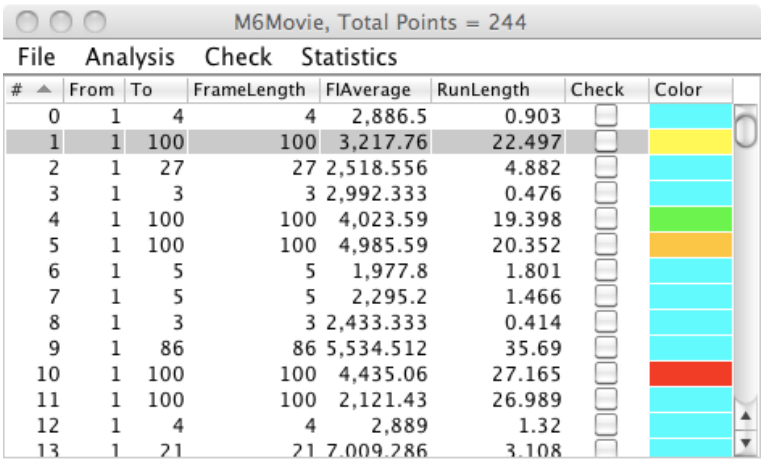
All of particle datas are stored in the Data table.

Image name and number of detected particles are shown on title bar. When you select the rows on table window, corresponding particles will appear. Selecting multiple rows lead to multiple ROI on movie window. The trajectory data and information are viewed on the another window.

4.2.1 Role of each columns

From left

1. # : Number of detected individual particles
2. From : First frame
3. To : Last frame



#	From	To	FrameLength	FIAverage	RunLength	Check	Color
0	1	4	4	2,886.5	0.903		
1	1	100	100	3,217.76	22.497		
2	1	27	27	2,518.556	4.882		
3	1	3	3	2,992.333	0.476		
4	1	100	100	4,023.59	19.398		
5	1	100	100	4,985.59	20.352		
6	1	5	5	1,977.8	1.801		
7	1	5	5	2,295.2	1.466		
8	1	3	3	2,433.333	0.414		
9	1	86	86	5,534.512	35.69		
10	1	100	100	4,435.06	27.165		
11	1	100	100	2,121.43	26.989		
12	1	4	4	2,889	1.32		
13	1	21	21	7,009.286	3.108		

Figure 6: Data table Same as Fig2

4. Frame Length: Frame length
5. FIAverage : Average intensity
6. RunLength: Total running length
7. Check : Checkbox for the any kind of purpose (for example, determine the good or bad particle)
8. Color : Color of ROI and Trajectories

Tips : Each columns can be sorted by clicking the each header.Sorting key is up to 2. For example, when you sort the FIAverage and then Check columns, the checked datas are sorted the order of FIAverage.

4.2.2 Menus of Table

1. File : Saving the data as various ways
   - Save all: Export all of particles data as text
   - Save selected : Export selected particles data as text
   - Save checked : Export checked particles data as text
   - Save Table as Text Data : Export table data as text
   - Save Table : Serialize and export all of particles data as .dat files. The saved data can be l oaded by [load Table] button on PTA window. Original movie should be opened in advance, and do not change the movie filename, otherwise particles data cannot be loaded

2. Analysis : Perform several analysis.

   - calc MSD : Calculate mean square displacement (MSD) for selected data (multiple selection is available). The MSD data can be fitted by linear or polynomial automatically.

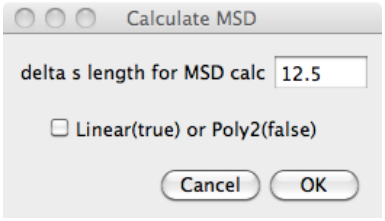


Figure 7: Calculate MSD dialog window

     – delta x length for MSD calc : Determine how length of time (frame, sec etc.) series is used for linear or polynomial fitting

     – Linear (true) or Poly2(false) : How fit the MSD data, linear (for freely diffusing particles) or polynomial (for directional moving particles)

     Export MSD data as text files (you are asked the filename). Fitted data will be appear on result window.

   - calc Velocity :Calculate velocity trajectory data for selected data (multiple selection is available)

   - Re-fit by 2D Gauss: Perform 2-D Gaussian fitting for selected data (multiple selection is available). (Figure8)
     Tips: 2-D Gaussian fitting takes a little time. Thus, I recommend you to detect the particle by centroid first, then choose the valuable data and perform Re-fit by 2D Gauss.

   - Extract point along LineRoi : Extract and check the particles which is on the line or segmented ROI.

     – This method can be useful when you want to extract the particles which on the specific line.

3. Check : Changing the selected particles check status.

   - Uncheck : Uncheck the selected particles
   - Check : Check the selected particles
   - Reverse : Reverse the selected particles

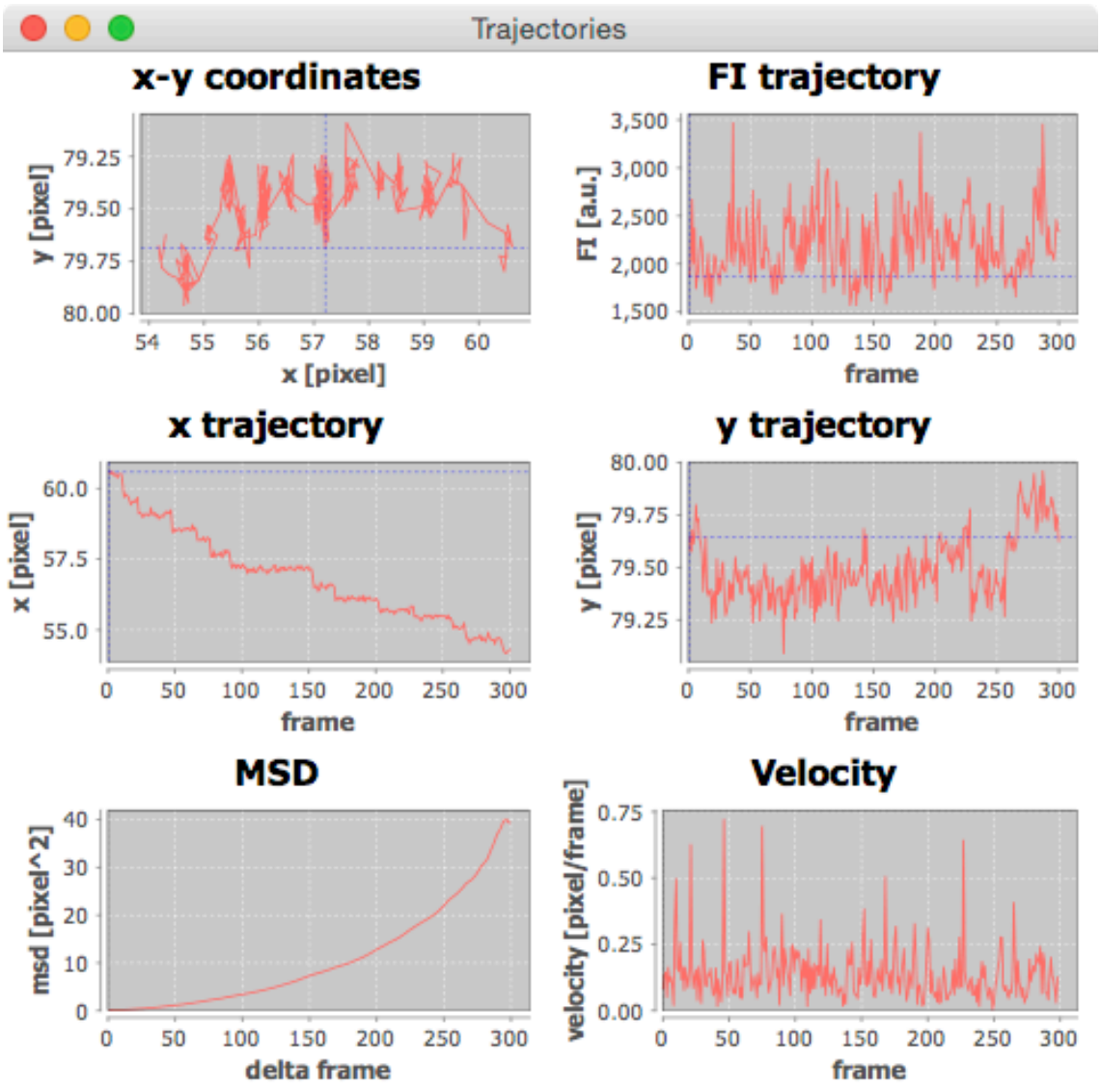


Figure 8: This trajectory is a result of re-fitted by 2D Gaussian for Figure3 data. You can find the quality of the data is little bit improved.

4. Statistics : Showing the histograms of averaged intensity and its s.d., averaged offset intensity and its s.d. duration time, run length, averaged value of X and Y and their s.d. (Figure 9). The bin width is determined by Sturges' formula.
    - All points of statistics : Show all particles histograms
    - Checked points of statistics : Show checked particles histograms
    - show Statics: Export selected particles data statics as text.

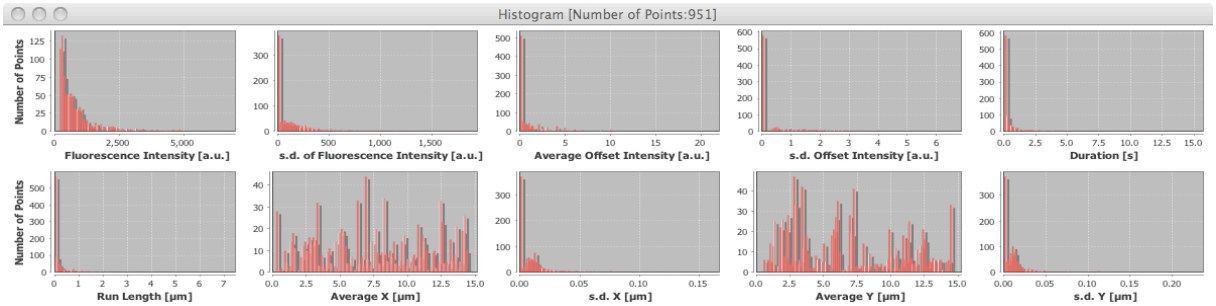


Figure 9: Histograms for each statistic data

	A	B	C	D	E	F
1	#Point:1	--	--	--	--	--
2	#Frame	x	y	sx	sy	F.I.(I)
3	1	100.955889	187.737031	1.820366	1.876283	637
4	2	100.89025	187.691647	1.871149	1.987383	685
5	3	100.881946	187.826788	1.861555	1.914374	672
6	4	100.915884	187.771094	1.824906	1.869139	643
7	5	100.874451	187.708521	1.774636	1.951668	639
8	6	100.911471	187.739191	1.845544	1.873213	642

Figure 10: Exported particles data as text

- #Point:[number] Particle number
- #Frame x y sx sy F.I.(I) F.I.(II) offset Iteration: frame, x position, y position, σx, σy,Intensity, offset (background) intensity, number of iteration value when 2-D Gaussian fitting is performed. F.I.(I) and F.I.(II) are corresponds to the Type I

(ROI Intensity) and Type II (coefficient of Gaussian distribution or the sum of the intensity higher than threshold), respectively.

- Each particles frame data is extracted as text in one line.

Next particle data will be start from #Point line
Space separated values.

6 CaptImage

This plugin can make the movie with ROI and trajectory data. As shown in the name, this plugin just capturing the movie window. This plugin may be useful for presentation.

How to use This plugin capture the movie in "the state as it is". Thus, size of window, zoomed state is also exported (What you see is what you want). When you choose the [Growing] on PTA window, you can obtain the movie which showing the growing trajectory depending on the particle movement.

- Enter the first and end frame
- Press OK button

WARNING: Again, this plugin just capture the movie. Do not overlay the any other window on the movie window and hide.