

Introduction

SimplePCI Motion Tracking and Analysis (MTA) expands **SimplePCI** to enable objects to be tracked across multiple time separated fields.

Fields are first collected and analyzed for size (Area), position (Center of Gravity), intensity and time (Field Time). The **MTA** module then rapidly tracks objects and reports distance moved with straight, curve line velocity, and all related statistics.

The module supports the following features:

- Fast Object Tracking
- Track Multiple Objects
- Adjustable Parameters
- Object Velocity
- Object Intensity
- Replay Objects with Track and intensity
- View Individual Object Trajectory
- Extensive Statistics
- Save Tracked Objects as AVI
- Straight and Curve Line Velocity
- Save Tracked Objects as AVI
- Export Data to Spreadsheet

Expand the functionality of **SimplePCI** and **AIC-AD** by adding the following optional modules:

- **AIC**, automated control and image acquisition
- **DIA**, dynamically measure intensity over time
- **IPA**, develops icon-driven work files for automatic image analysis and processing
- **QFA-FRET**, accurate FRET measurements and cross talk correction
- **VIS-MD**, provides rapid 3D visualization of multi-dimensional data sets
- **DNN**, Remove or Restore blur in images using fast algorithms
- **DNN-2D**, a Point Spread Function is derived and used in restoration

Getting Started

This **Quick Start Guide** contains examples of how to utilize **MTA**. For further assistance, refer to the online help, manual, or visit support at <http://www.cimaging.net>, for access to the latest **How to's** and frequently asked questions. Additional support is available at e-mail: support@cimaging.net, or Tel: 412-741-7920.

Example guides:

- ◆ Create a Workfile to Track Moving Objects 2
- ◆ Run a Workfile to Track Moving Objects 3
- ◆ Glossary of Tracking Parameters 4
- ◆ Glossary of MTA Statistics 5

Create a Workfile to Track Moving Objects

1. Go to the **File Menu** and click on **New** (fig.1).
2. Select **Workfile Document** and click **OK**.
3. Click the **Camera icon**, the **Capture window** appears (fig.2).
4. Select **Disk** and **Auto Depth** from the top-right drop-down list.
6. Click **Browse**, (fig.2) locate your data file and click **Open**. The image sequence appears in the **Image Display**.
7. Close the **Capture** window by clicking **OK** (fig.1).
8. Click the **Identify** icon (fig.3) to open the **Identify Objects** window. Set the Threshold of your image by adjusting the **Min.** and **Max.** slider until the cell or your object of interest is covered by a green binary overlay (fig.4). Click **OK**.
9. Click the **Modify** icon (fig.3) if you need to modify your binary image with a binary filter, such as **Erode**, **Dilate**, **Close**, **Open**, etc. In this example, a **Close** binary operation was applied.
10. Click the **Qualify** icon (fig.3) to reject unwanted objects from your binary overlay by adjusting the **Min** and **Max** slider.
11. Click the **Measure** icon (fig.3). Check **Area**, **COFG_X** and **COFG_Y**. Click **OK** (fig.5).
12. Save your workfile. Go to the **File Menu** and click **Save As**, assign a file path and name to your Workfile and click **Save** (fig.6). You can now run your new Workfile to track your sample, see following page.

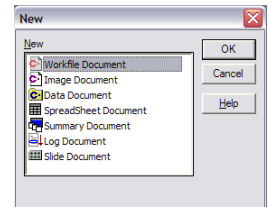


fig.1

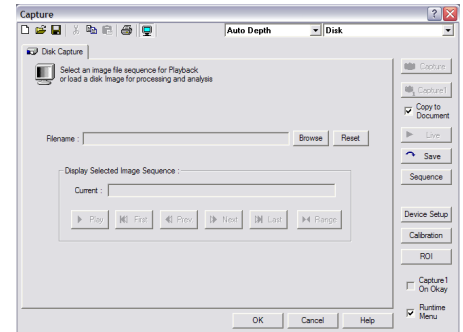


fig.2

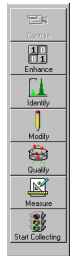


fig.3

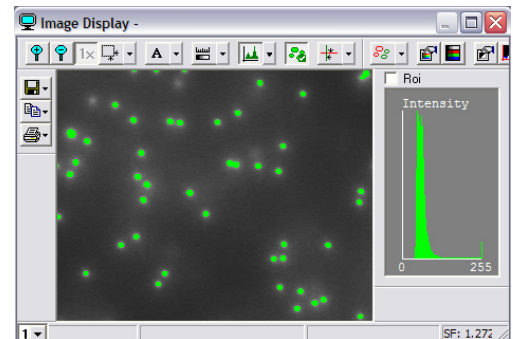


fig.4

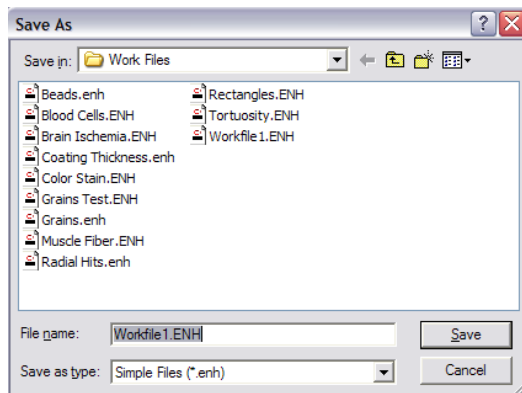


fig.6

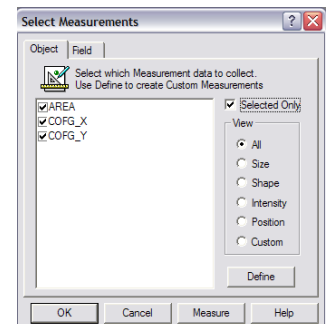


fig.5

Run a Workfile to Track Moving Objects

1. Open your **Workfile** if it's not open. **File Menu > Open > Workfile Document > OK > Open.**
2. Open your **Data Document**. **File Menu>Open>Data Document>OK>Open.**
3. Click the **Start Collecting** icon (**fig.7**) in the Data Document toolbar, a dialog window appears (**fig.8**). Click **Replace** if you want to replace existing data. Click **New** if you want to create a new data file, click **Cancel** to cancel the process.
4. Check all the data components you wish to have in your data file and click **OK** (**fig.9**).
5. Click the **Start** icon in the toolbar (**fig.10**). **SimplePCI** starts loading and analyzing each image into a new cxd.
6. Close the original cxd if a new cxd has been created, and activate the new cxd by clicking on it. Expand **Field 1** and click on **Original Image** to activate its toolbar. Click the **Tracking Properties** icon (**fig.11 & 12**). Enter the **Absolute (micron/sec)** and **Relative (diam/sec)** in the **Maximum Speed** box. Click **Apply** to get a preview. **Note:** if you don't see any result, change the **Absolute** and **Relative** values until you get a satisfactory result. Also, ensure you have loaded the correct calibration file, and that the timing between field is correct. Click **OK** once you are pleased with the result.
7. Click the **Track Image Sequence** icon (**fig.11**).
8. Click the **Play** button (**fig.11**) to play the image sequence. Notice the green line which indicates the object's track.
9. Click on the track to get its statistical values. (**fig.11, & 13**).
10. Click on **Summary Track Info** (**fig.11**) to view a spreadsheet with all the tracks in the cxd.
11. To create an AVI file from the tracked images, Right-Click on an image and select **Export Display Image + All Layers** image from the **Export Image Sequence** sub-menu. **Select Movie File(*.avi)** from the drop-down list in the Export Image window.

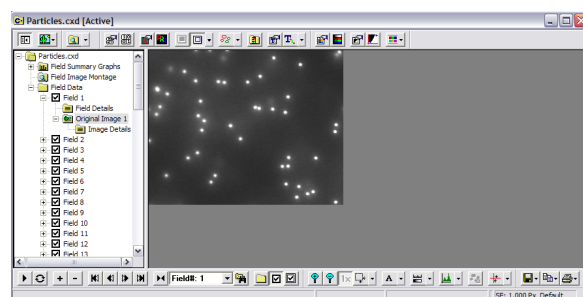


fig.7

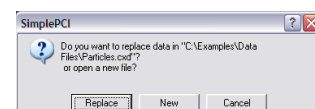


fig.8

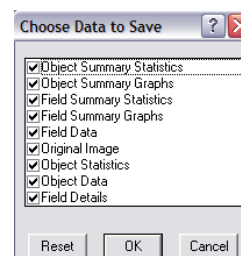


fig.9



fig.10

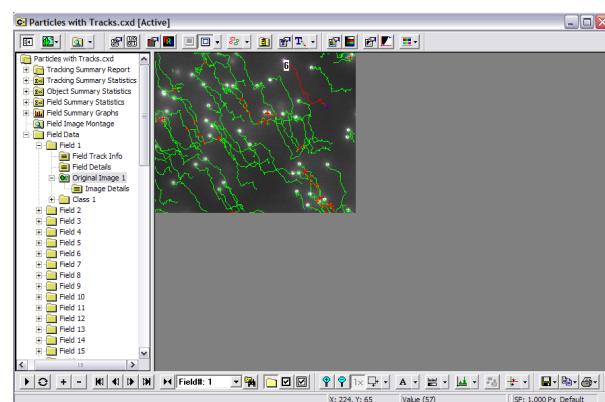


fig.11

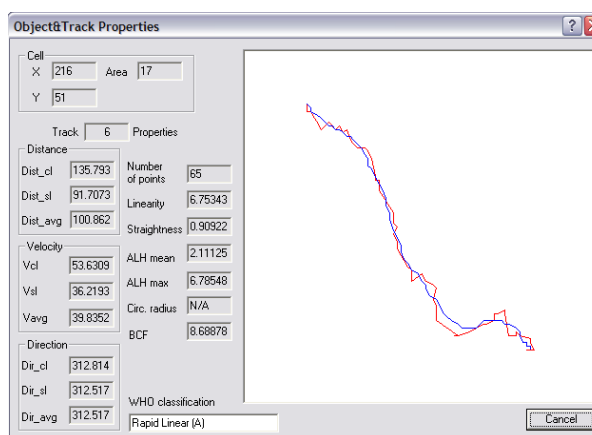


fig.13

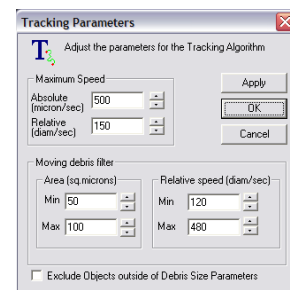


fig.12

Glossary of Tracking Parameters

Absolute Maximum speed - derives the Upper Distance Limit that objects in Field[n] will be tracked to objects in Field[n+1]

AbsMaxSpeed = Area (largest object) / time (between frames)

Relative Maximum speed - derives the Upper Distance Limit that objects in Field[n] will be tracked to objects in Field[n+1] with respect to object size

RelMaxSpeed = AbsMaxSpeed / sqrt(Area)

It's recommended to start with smallest area and watch the effects on the track as reduce RelMaxSpeed OR start with largest area and watch the effects on the track as increase RelMaxSpeed. Keep in mind that Decreasing these values too much will result in breaks in the tracks.

Moving Debris Filter Values:

Minimum Area - Objects below this number will have more stringent requirements when determining whether a matching object is found. (Two objects will need to be closer in size for a track to occur)

Maximum Area - Objects above this number will most likely be mapped to each other.

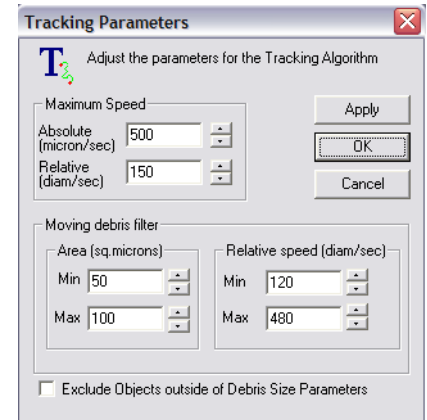
Minimum Relative Area - The smaller this number, the closer in size that two objects would need to be in order for a track to occur.

MinDebrisArea = Area of smallest object

MaxDebrisArea = Area of largest object.

MinDebrisRelSpeed = AbsMaxSpeed / sqrt(MaxDebrisArea)

MaxDebrisRelSpeed = AbsMaxSpeed / sqrt(MinDebrisArea)



Glossary of MTA Statistics

Cell position: X, Y coordinate **Cell area:** number of pixels

In the Object and Track Properties dialog there are two lines drawn to represent the track. The red line connects the position of the object at each point in the track. Measurements based on this path are called **_cl** or Curvilinear measurements.

The blue line represents a curve fitted through each point on the track to generate a mean path for the track. Measurements based on this path are called **_avg** or mean path measurements.

The start and end point of the track are used to generate a straight line representing the final distance traveled for the object. This line is not drawn. Measurements based on this path are called **_sl** or Straight Line measurements.

Distance - the length of the line in calibrated units (e.g. Microns).

Velocity - the average speed along the track (distance/time taken)

Direction - the angle measured from the 0-360 degrees

Number of points - number of consecutive images containing the same object used to make up the track

Linearity - $(\text{Dist_sl}/\text{Dist_cl}) \times 10$. A factor indicating how close the track is to a straight line. The maximum value is 10 for a perfectly straight line.

Straightness - $(\text{Dist_sl}/\text{Dist_avg})$ the fractional ratio between the straight line path and the mean path. The Maximum value is 1.0 for a perfectly straight line.

ALH Mean - Mean Amplitude of the transitions either side of the mean path. This calculation is useful for subjects having a regular beating motion (e.g. sperm). A higher value indicates the subject move vigorously away from the center line

ALH Max - Maximum Amplitude of the transitions either side of the mean path.

Circ Radius - The computed radius constraining the circular motion of a subject. Note this value cannot be computed for straight tracks as it tends to infinity. Also the value may not be useful when the direction of curvature of the mean path changes for the object.

BCF - The Beat Cross Frequency is calculated from the number of times the track crosses the mean path. It gives another indication of subject motility when applied to samples with a regular beating motion.

WHO Classification- World Health Organization classification scheme

Rapid Linear (A) - subject makes significant direct forward motion. High Vsl and High Straightness.

Slow Non-Linear (B) - subject makes motion, but moderate Vsl. High Vsl and low straightness.

Non-Progressive Motile (C) - subject makes some motion, but no progress low Vsl.

Non-Motile (D) - subject makes insignificant motion low Vavg.

Unclassified (U) - insufficient information.

