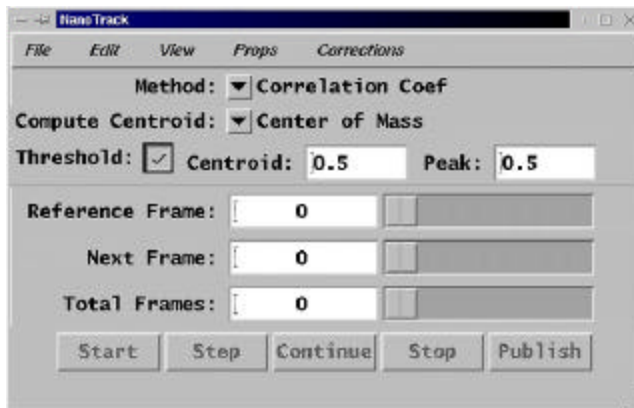




NanoTrack Cell and Particle Tracking Primer

The NanoTrack Pnode allows the user to track single cells and particles with nanometer precision at very fast tracking speeds. The speed of the tracking is dependent on the size of the image to be tracked, the number of display windows opened during the tracking sequence and the amount of RAM and processor in the computer. Nanotrack can track objects, cells, and particles in the form of images list and QuickTime movies.

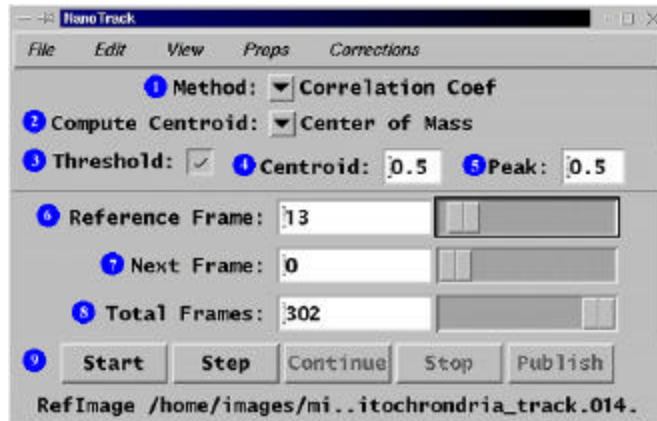


- | File | Edit | View | Props | Corrections |
|---|---|---|---|---|
| <ul style="list-style-type: none"> •Open Image List Reference Corr. •Open Movie File •Open Text for Results •Save Results As... •Save Image with Overlay •Save State as Results •Reset Defaults •Set Factory Defaults •Close | <ul style="list-style-type: none"> •Optics •Clear Track <ul style="list-style-type: none"> •Reset Reference for Movie Display •Reset Next to Movie •Reset Reference to First Image •Reset Next to Reference Image <ul style="list-style-type: none"> •Reset Next to First Image •New Reference •Save Reference | <ul style="list-style-type: none"> •Track Display •Results <ul style="list-style-type: none"> •Display Reference Image •Display Next Image •Filtered Reference •Filtered Source <ul style="list-style-type: none"> •Reference Window •X vs Y Plot •<r> vs T Plot •dX vs T Plot •dY vs T Plot •P vs T Plot •L vs T Plot •MSD vs T Plot •Correlation | <ul style="list-style-type: none"> •Calibration •Publish Format •Kernal •Auto-Threshold Control •Missing Track Option •Enhancement •Display Control •Source Control •Movie Control •SGI Movie Converter | <ul style="list-style-type: none"> •Save Last Track as •Reset Reference Track |

NanoTrack Cell and Particle Tracking Primer

1. NanoTrack Property Screen

From the Nanotrack Property Screen, the user can setup all the parameters required to track a particle or object, do statistical analysis of the graphical plots, and publish and save the text data in ASCII format.



★ *Method:* Function

- a. Allows the user to select the correlation method to be used
- b. Computes the correlation between imaging using:
 1. **Correlation Coefficient**
 - a. Default Setting
 - b. Yields a broader peak but is less sensitive to changes in background
 - c. $\{N * \text{Sum}(XY) - \text{SUM}(X)*\text{SUM}(Y)\} / \text{SQRT}(\{N*\text{SUM}(X*X)-\text{SUM}(X)*\text{SUM}(X)\} * \{N*\text{SUM}(Y*Y) - \text{SUM}(Y)*\text{SUM}(Y)\})$
 2. **Correlation Index**
 - a. Gives a more narrow peak but is very sensitive to changes in the object's shape
 - b. $1.0 - \text{SQRT}(\text{SUM}((Y-X) * (Y-X)) / \text{SUM}(X*X))$
 3. **Low Intensity**
 4. **High Intensity**

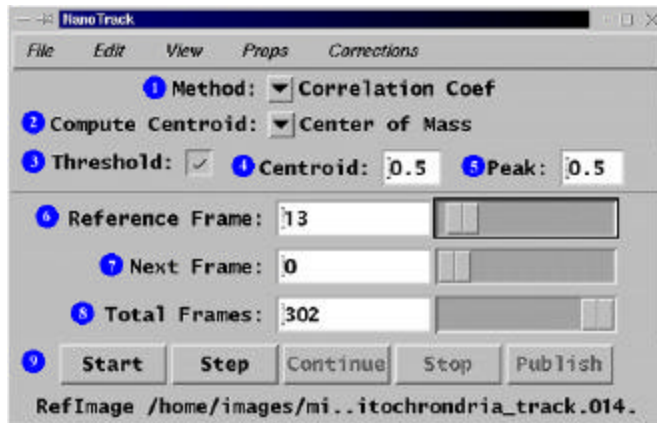
🕒 *Compute Centroid:* Function

- a. Allows the user to determine the method of computing the centroid
- b. Computes the centroid using
 1. **Correlation Peak**
 - a. Uses a simple peak search to give displacement
 - b. This method does not give subpixel positioning
 2. **Center of Mass**
 - a. Default setting
 - b.. Uses the values in the threshold correlation matrix's weights to compute the displacement between the source and the reference
 3. **Parabolic Surface Fit**

- a. Fits the correlation matrix to a 2-dimensional parabolic function to compute the displacement
- 4. **Adaptive Center of Mass**
 - a. Performs a feature extraction in the correlation matrix to remove any correlation with other objects in the scan area. It then computes the center of mass

NanoTrack Cell and Particle Tracking Primer

1. NanoTrack Property Screen

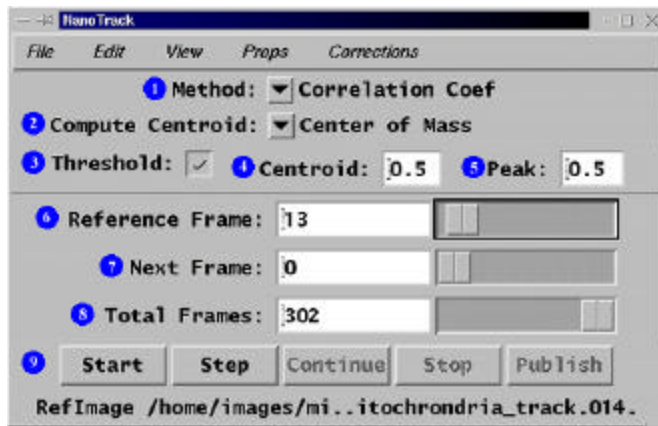


- ⌚ *Threshold:* Function
 - a. Default is enabling Thresholding
 - b. When the Threshold function is checked , each time a correlation matrix is computed, the corresponding value given in the Correlation property is used as a threshold to isolate the peak for computing the centroid.
 - c. Only values between 0.0 and 1.0000 may be entered for the Correlation threshold.
- ⌚ *Centroid:* Function
 - a. Displays the centroid value of the peak
- ⌚ *Peak:* Function
 - a. Displays the peak value of the correlation matrix
- ⌚ *Reference Frame Slider* Function
 - a. Allows the user to select the reference frame either by manual input or using the interactive slider bar
 - b. The Reference Frame is the first frame in the tracking sequence and will be the base line for all measurements.
 - c. The Reference Frame Slider Bar is interactive with NanoTrack Display Window.
- ⌚ *Next Frame Slider* Function
 - a. Displays the next frame in the tracking sequence
 - b. Allows the user to manually input the next frame or use the interactive slider bar
- ⌚ *Total Frames Slider* Function

- a. Displays the total number of frames in the tracking sequence
- b. Allows the user to interactively set the total number of frames to be tracked either by manual input or using the slider bar.

NanoTrack Cell and Particle Tracking Primer

1. NanoTrack Property Screen



🕒 Action Bar Function

- a. Allows the user to start and stop the tracking sequence and publish the results of the tracking sequence
- b. **Start** Function
 1. Starts the tracking sequence
 2. When the tracking sequence is started, the Stop Function becomes active.
- c. **Step** Function
 1. Allows the user to step through the tracking sequence one frame at a time
- d. **Continue** Function
 1. The Continue function restart the tracking sequence if the tracking sequence is stopped prior to reaching the last frame in the sequence.
 2. The tracking sequence is restarted at the frame displayed in the *Next Frame* 🕒 function.
- e. **Stop** Function
 1. Stops the tracking sequence
 2. This function becomes active when the tracking sequence is started.
- f. **Publish** Function
 1. Allows the user to send the numerical results of the tracking sequence to the Text Journal

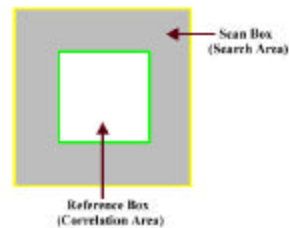
2. This function becomes active once the track sequence has been either stopped or is completed.

NanoTrack Cell and Particle Tracking Primer

1. NanoTrack Display Screen

- a. The NanoTrack Display Screen is used designate the object to be track using the Tracking ROI and displays the tracking sequence in real time.
- b. When the tracking sequence is started, the Tracking ROI will interactively move with the object from frame to frame. The Reference Image (start point) of the tracking sequence will be designated with a red box..
- c. As the tracking sequence is started, the region in each image's green box is correlated with the reference image's red box over the range of the yellow box . From this a correlation matrix is created. A correlation Threshold is then applied to isolate a peak matrix and the centroid of the peak determines the displacement of the object.
- d. The ROI Tracking Frame is an interactive ROI that can be positioned and sized by the user, it consists of three rectangular boxes: red, green and yellow. Initially, only the green and yellow boxes are visible to the user as the red box lies under the green box.

1. The Green Box is used to determine the correlation.
2. The Yellow Box is the search area.
3. The Red Box is the reference image .



NanoTrack Cell and Particle Tracking Primer

1. NanoTrack Display Screen (continued)

- e. The accuracy and speed of the tracking sequence is dependent upon a number of factors.
 - 1. Empirically, the best results have been achieved using the "Correlation Coefficient" Method and the "Center of Mass" Centroid.
 - 2. Reduce the size of the **Reference Box** of the Tracking ROI to the smallest area which includes the target to be tracked.
 - 3. The **Scan Box** should be large enough to include the maximum displacement between successive frames.
 - a. As the size of the **Scan Box** increases, the processing time increases.

- f. To Position the ROI Tracking Frame over the object to be tracked:
 - 1. Place the mouse pointer over the ROI Tracking Frame:
 - 2. Hold down the right mouse button.
 - 3. Drag the ROI Tracking Frame over the object, positioning the green frame over the object.

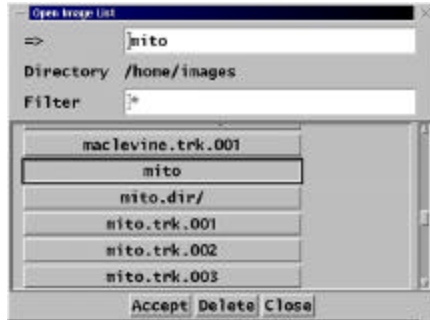
- g. To Size the **Reference Box** of the ROI Tracking Frame:
 - 1. Place the mouse pointer inside the **Reference Box** of the ROI Tracking Frame.
 - 2. Press and hold down the " SHIFT " key.
 - 3. Drag the mouse pointer to grow or shrink the **Reference Box**.

- h. To Size the **Scan Box** of the ROI Tracking Frame:
 - 1. Place the mouse pointer inside the **Scan Box** of the ROI Tracking Frame.
 - 2. Press and hold down the " Control " key.
 - 3. Drag the mouse pointer to grow or shrink the **Scan Box**

NanoTrack Cell and Particle Tracking Primer

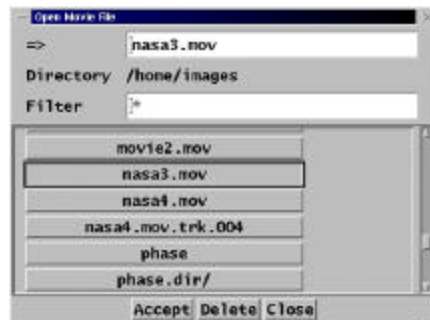
File Menu

1. *Open Image List* Function
 - a. Opens the Image List Menu
 - b. Allows the user to select the image list to be tracked



1. Click on the Image List desired.
2. Click on the Accept button.

2. *Open Movie File* Function
 - a. Opens the Movie File Menu
 - b. Allows the user to select movies in QuickTime format to be tracked
 - c. Movies in QuickTime format have the extension .mov



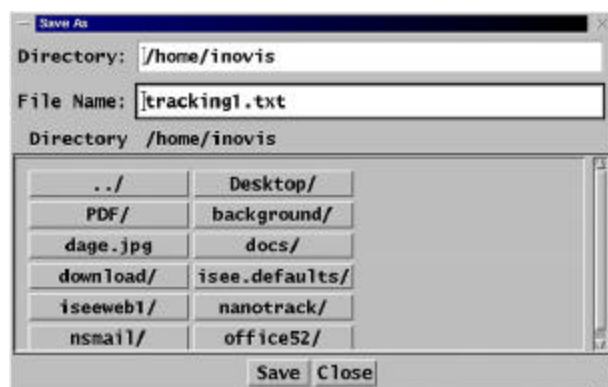
3. *Open Text for Results* Function
 - a. Opens the Text Journal that will display the numerical results of the track when the Publish button is pressed.



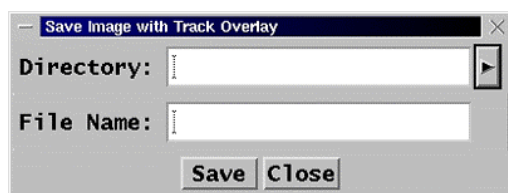
NanoTrack Cell and Particle Tracking Primer

File Menu

4. *Save Results As...* Function
 - a. Allows the user to input the file name to save the text journal



5. *Save Image with Track Overlay* Function
 - a. Allows the user to save the current image in the Display Screen with the track overlay



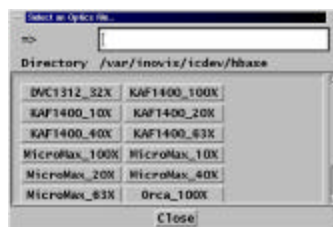
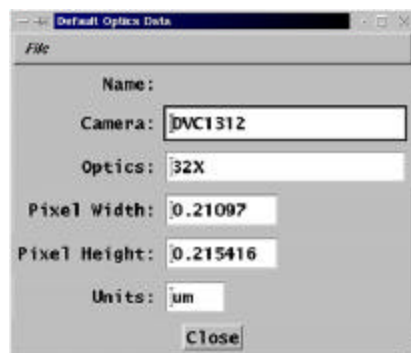
6. *Save State as Defaults* Function
 - a. Saves the current settings as the default values
7. *Reset Defaults* Function
 - a. Resets the NanoTrack Properties Screen to the saved defaults values
8. *Set Factory Defaults* Function
 - a. Resets the NanoTrack Properties Screen to the factory default values
9. *Close* Function
 - a. Closes the NanoTrack Pnode

NanoTrack Cell and Particle Tracking Primer

Edit Menu

1. Optics Function

- a. Allows the user to input objective magnification used to acquire the image sequence, the camera used in the acquisition, the pixel width and height of the imaging CCD and the unit of measure.
- b. Pixel spacing and units of measure are required for the calibration measurement.
- c. This function is related to the *Calibration Function* under the **Props** Menu.
- d. The values can be manually input or recalled from a stored file.



2. Clear Track Function

- a. Clears all the tracking data stored in the RAM memory

3. Reset Reference from Movie Display Function

- a. When an image list or movie file is being processed, selecting this function resets the reference to the image currently in the NanoTrack Display Window.

4. Reset Next from Movie Display Function

- a. When an image list of movie file is being processed, selecting this function sets the **Next Frame** property to the image currently being displayed.

5. Reset Reference to First Image Function

- a. Resets the Reference imaging to the first image in the image list of movie file

6. Reset Next to Reference Image Function

- a. Resets the next image in the sequence to the Reference Image

7. Reset Next to First Image Function

- a. Resets the next image in sequence to the first image in the image list or movie file

8. New Reference Function

a. Clears the current reference image, and if not set, automatically sets it from the next image in the sequence.

9. *Save Reference Function*

a. Creates a new copy of the reference image and uses it. In cases where the image input to NanoTrack are from static buffer which is continuously being rewritten, this insures the reference image is not overwritten.

NanoTrack Cell and Particle Tracking Primer

View Menu

1. *Track Display Function*

a. Displays the NanoTrack Display Screen
b. This function is used when the Display Screen is close during the tracking sequence.

2. *Results Function*

a. Opens a non-editable screen that displays the centroid (XY) of the last image tracked, the displacement from the reference, (XY Offset), and the peak correlation index.



3. *Display Reference Image Function*

a. Displays the Reference Image in the NanoTrack Display Screen

4. *Display Next Image Function*

a. Displays the next image in the sequence in the NanoTrack Display Screen

5. *Filtered Reference Function*

a. When the Enhancement Function, from the Props Menu is enabled, the post processed reference image is displayed in a separate window.

6. *Filter Source Function*

a. When the Enhancement Function, from the Props Menu is enabled, the post-processed source or next image is displayed in a separate window.

NanoTrack Cell and Particle Tracking Primer

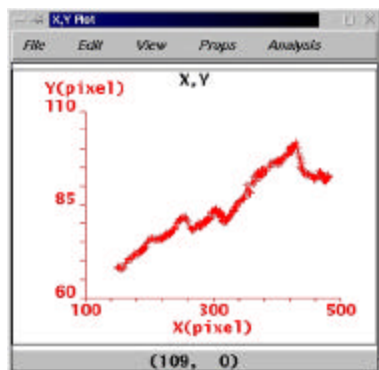
View Menu

7. *Reference Window Function*

a. Displays the reference image in a separate window

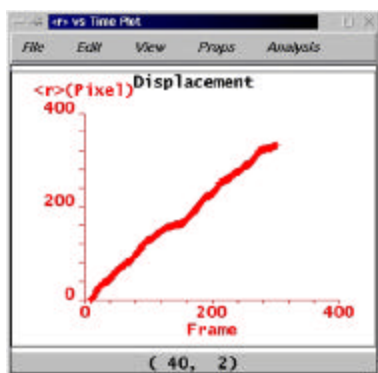
8. *XY Plot*

- a. Displays and graphs the calibrated X, Y position of the object being tracked



9. *<r> Plot*

- a. Displays and graphs the displacement of the object being tracked.

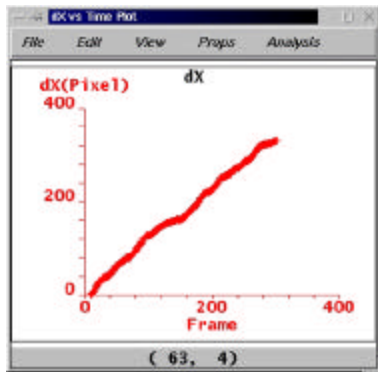


NanoTrack Cell and Particle Tracking Primer

View Menu

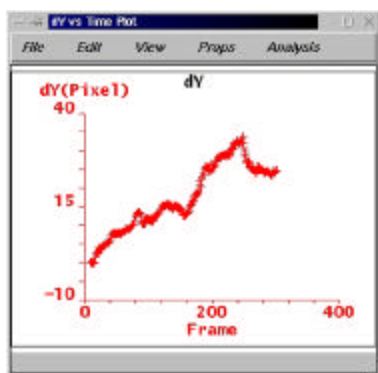
10. *dX vs T Plot*

- a. Displays and graphs the change in the X position versus time.



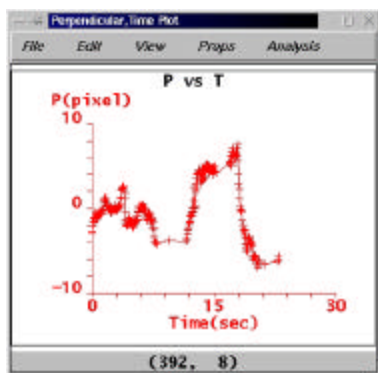
11. dY vs T Plot

- a. Displays and graphs the change in the Y position versus time.



12. P vs T Plot

- a. Displays and graphs the Perpendicular fit versus time
 b. Used in conjunction with the Publish Function, in the Props Menu, and the Publish button



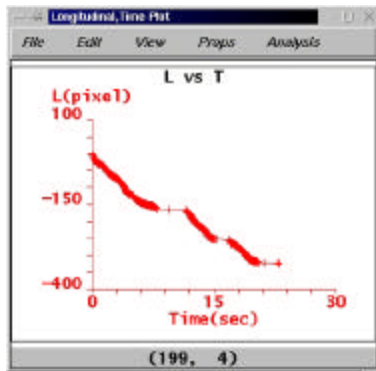
1. After the tracking sequence has been completed, open the *Publish Format* Function under the **Props Menu**.
2. Select one of the formulas under the *Fit Data To:* property.
3. Open the P vs T Plot .
4. Click the **Publish** button on the NanoTrack Property Screen.

NanoTrack Cell and Particle Tracking Primer

View Menu

13. *L vs T Plot*

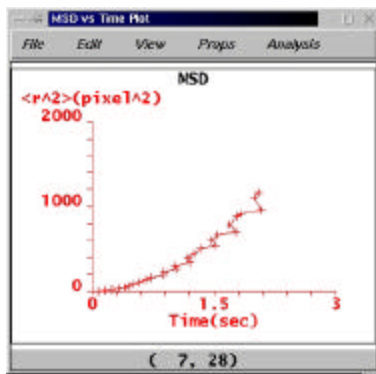
- a. Displays and graphs the Longitudinal fit versus time.
- b. Uses in conjunction with the Publish Function in the Props Menu and the Publish button



1. After the tracking sequence has been completed, open the *Publish Format* Function under the **Props Menu**.
2. Select one of the formulas under the *Fit Data To:* property.
3. Open the *P vs T Plot*.
4. Click the **Publish** button on the NanoTrack Property Screen.

14. *MSD vs T Plot*

- a. Displays and graphs the Mean Square Displacement fit versus time
- b. Uses in conjunction with the Publish Function in the Props Menu and the Publish button.



1. After the tracking sequence has been completed, open the *Publish Format* Function under the **Props Menu**.
2. Under the *Fit to Data:* property, select:
Compute Mean Square Displacement
3. Open the *MSD vs T Plot*
4. Click the **Publish** button on the NanoTrack Property Screen.

15. *Correlation Function*

- a. Displays the correlation matrix as an image
- b. This window automatically opens when a new reference ROI is selected and is closed when the tracking sequence is started. This is the default setting found in the *Display* Function in the **Props Menu**

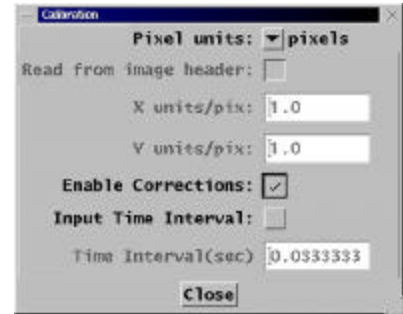


NanoTrack Cell and Particle Tracking Primer

Props Menu

1. Calibration Function

- Allows the user to select properties for controlling the calibration
- Default value is in pixels
- Measurement formats include:
 - Microns
 - Nanometers
- When microns or nanometers are selected, the user can either input the values directly or they can be automatically taken from the header file.



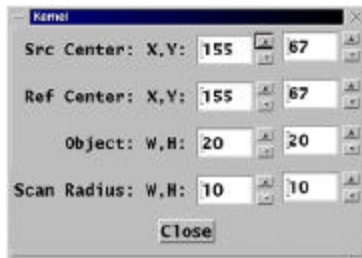
2. Publish Format Function

- Allows the user to select the values to be published in the Journal Text Screen and to delineate the formulas for the L vs T, P vs T, and MSD vs T plots



3. Kernal Function

- Allows the user to manually change the kernel and the ROI Tracking Frame



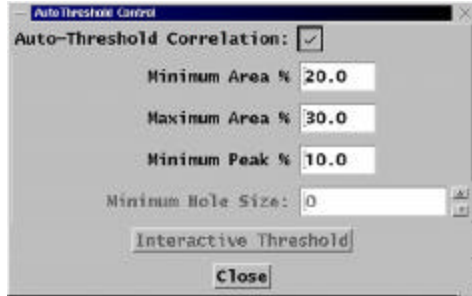
- Src Center: X, Y
 - Displays the center position of the source ROI in pixels
 - This is automatically updated during processing
- Ref Center: X, Y
 - Displays the center position of the reference ROI in pixels
 - This is automatically updated during processing.
- Object: W, H
 - Displays the width and height of the area used for the correlation
- Scan Radius: W, H
 - When the area over the which the Reference ROI is correlated with the Source ROI, this width and height less the Object W,H determines the size of the correlation matrix.

NanoTrack Cell and Particle Tracking Primer

Props Menu

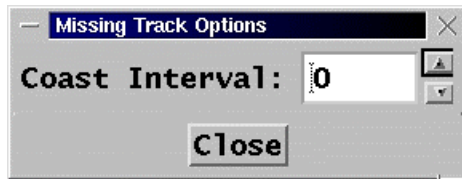
4. Auto-Thresholding Function

- a. When the Auto-Thresholding is enabled , the threshold for the correlation matrix is automatically computed.
- b. The Minimum % Area and the Maximum % Area properties allow the user to determine the lower and upper limits of the number of pixels in the peak as a percentage of the matrix size.
- c. If the Auto-Thresholding is disabled, the user can interactively set up the thresholding.



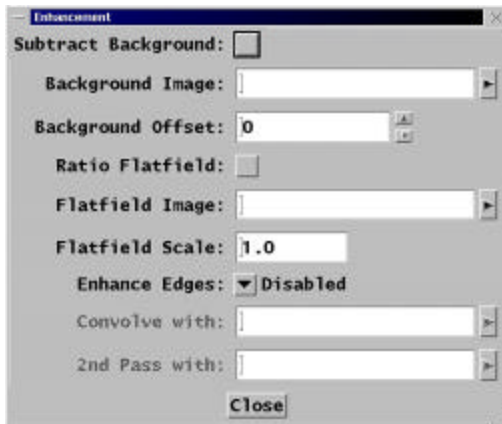
5. Missing Track Option Function

- a. The Coast Interval property allows the user to select the number of frames after a correlation search fails to continue before aborting the process.



6. Enhancement Function

- a. The Enhancement function allows the user to process images prior to computing the correlation matrix.



Edge Enhancement Filters include

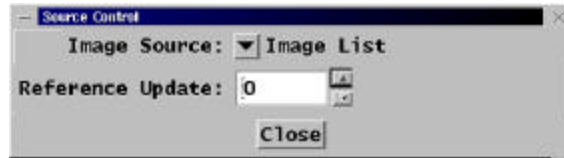
1. Sobel
2. Prewitt
3. Roberts
4. Laplacian
5. User Defined Kernels
6. Two Kernels

NanoTrack Cell and Particle Tracking Primer

Props Menu

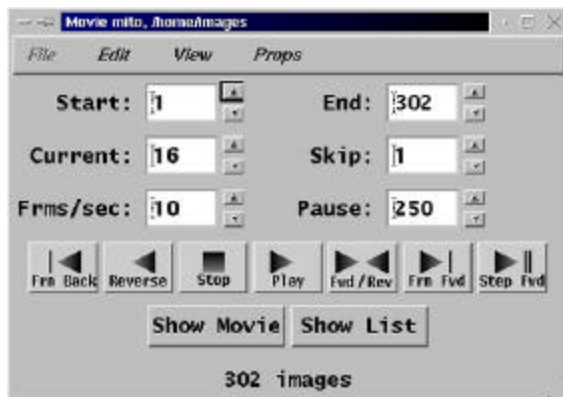
7. Source Control Function

- a. Allows the user to select the type of input format for the image sequence to be tracked.
- b. Input Formats available include;
 1. Node Input
 2. Input (Playback Enabled)
 3. LML Movie
 4. Image List



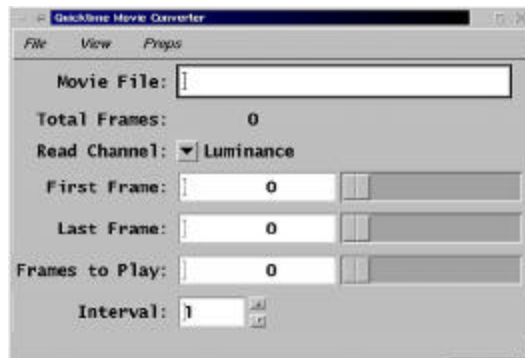
8. Movie Control Function

- a. Allows the user to view the tracking sequence as a movie with forward and reverse controls



9. Movie Converter Function

- a. Allows the user to review movie files and controls which channel is used to track (luminance, red, green, blue)
- b. Allows enables setting a ROI in a movie file.



NanoTrack Cell and Particle Tracking Primer

Corrections Menu

1. *Save Last Track as Reference for Correction* Function

- a. When selected, the last track processed is saved for correcting the subsequent tracks.
- b. In some cases, there may be movements which are due to vibration or other external factors. To correct, select and ROI in the image which is known not to move, and track it. After tracking this region, select this property to save this track. Now, any subsequent tracks will automatically be corrected by this reference data to remove any artifactual motion.

2. *Reset Reference Track* Function

- a. Deletes the reference track data.

