

# TweezPal – Optical tweezers analysis and calibration software

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## Abstract

Optical tweezers, a powerful tool for optical trapping, micromanipulation and force transduction, have in recent years become a standard technique commonly used in many research laboratories and university courses. Knowledge about the optical force acting on a trapped object can be gained only after a calibration procedure which has to be performed (by an expert) for each type of trapped objects. In this paper we present TweezPal, a user-friendly, standalone Windows software tool for optical tweezers analysis and calibration. Using TweezPal, the procedure can be performed in a matter of minutes even by non-expert users. The calibration is based on the Brownian motion of a particle trapped in a stationary optical trap, which is being monitored using video or photodiode detection. The particle trajectory is imported into the software which instantly calculates position histogram, trapping potential, stiffness and anisotropy.

## PROGRAM SUMMARY

*Manuscript title:* TweezPal – Optical tweezers analysis and calibration software

*Authors:* Natan Osterman

*Program title:* TweezPal

*Licensing provisions:* CPC non-profit use licence agreement

*Programming language:* Borland Delphi

*Computer(s) for which the program has been designed:* Any PC running Microsoft Windows

*Operating system(s) for which the program has been designed:* Windows 95, 98, 2000, XP, Vista, 7

*RAM required to execute with typical data:* 12 Mbytes

*Has the code been vectorised or parallelized?:* no

*Number of processors used:* 1

*Supplementary material:* two sample data sets

*Keywords:* laser tweezers, optical trapping, calibration

*CPC Library Classification:* 3; 4.14; 18; 23;

*External routines/libraries used:-*

*CPC Program Library subprograms used:-*

*Nature of problem:* Quick, robust and user-friendly calibration and analysis of optical tweezers. The optical trap is calibrated from the trajectory of a trapped particle undergoing Brownian motion in a stationary optical trap (input data) using two

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methods.

*Solution method:* Elimination of the experimental drift in position data. Direct calculation of the trap stiffness from the positional variance. Calculation of 1D optical trapping potential from the positional distribution of data points. Trap stiffness calculation by fitting a parabola to the trapping potential. Presentation of X-Y positional density for close inspection of the 2D trapping potential. Calculation of the trap anisotropy.

*Restrictions:* None.

*Unusual features:* None.

*Additional comments:* None.

*Running time:* Seconds

*References:* The theoretical underpinnings for the trap calibration are found in [E. L. Florin, A. Pralle, E. H. K. Stelzer and J. K. H. Hörber, Appl. Phys. A **66** (1997), S75-S78]

## **Introduction**

Optical tweezers are a powerful tool for manipulating microscopic particles by exerting forces via a highly focused laser beam. The instrument is capable of precise manipulation and detection of sub-nanometer displacements of (sub)micrometer dielectric particles. Since its inception in 1986 [1] they have become an established technique in a wide range of scientific disciplines such as physics, biophysics, biology, chemistry, etc. The tweezers have been used to manipulate colloidal particles, viruses, bacteria, living cells, organelles and DNA molecules, and to measure various properties of these systems, such as elasticity, force, torsion, position, surface structure and interaction between particles.

Although one can purchase turnkey optical tweezers systems from companies such as Aresis, Arryx, Elliot Scientific, etc., traditionally the optical tweezers setups used in research laboratories and (under)graduate courses are still custom-built [2]. When an object is successfully trapped for the first time in a new optical tweezers the system is far from ready to be used in experiments. After a tedious beam alignment procedure to achieve the best trapping conditions the system should be prepared for simple everyday use by non-expert users. This includes development of the supporting software for data acquisition, trap control and calibration. In this paper a software tool for quick analysis and calibration of the optical trap is presented.

In practically all applications of the laser tweezers, it is desired to have a knowledge about the optical force acting on a trapped object. The optical trap has to be calibrated for each type of the trapped objects. Moreover, the tweezers setups, especially custom built, often get misaligned which results in

weaker trapping and loss of radial symmetry of the trapping potential well. A consequence of misalignment is also dependence of the trap stiffness and shape on the lateral position in the trapping plane.

The optical tweezers software usually consists of several scripts that have to be run within a higher programming language such as Matlab, IDL, LabView, etc. The scripts are generally written by an expert and meant to be used by one. New users of tweezers therefore must be first taught the basics of the specific programming language and then of the analysis and calibration scripts. The problem arises when these custom-written scripts either give unexpected results or stop working, especially if their author is not present. Therefore a user-friendly software that implements a simple, robust and quick procedure to check and calibrate the tweezers is always needed. A stand-alone application, TweezPal, that runs in the Windows operating system has been developed for this purpose. The software has a graphical user interface, is user-friendly, intuitive, simple to use and yet powerful enough to perform optical trap calibration and analysis of the trapping potential.

## **Calibration**

The easiest way of tweezers calibration is to analyze the Brownian motion of a trapped particle, such as spherical bead. The bead is held in a stationary trap for a certain amount of time (typically, a few minutes) and its position fluctuations are recorded. If an application needs a high bandwidth the acquisition of bead positions is accomplished using a quadrant photo-diode. The detector response has to be measured [3] beforehand in order to convert the detector signal to the displacement of the trapped bead. A simpler and more common option is to use a CMOS or CCD camera to record a video of the bead and subsequently extract its positions using a dedicated software employing image recognition algorithms [4] to track the bead. The result is a data series of bead positions given in pixel coordinates. To convert to physical units (micrometers are used in TweezPal) one has to multiply the extracted values by a conversion factor which can be determined by using a ruled microscope slide or precisely known movement of an object fixed on a calibrated microscope stage. The position resolution when tracking a micrometer-sized bead using even simple image segmentation algorithms can be well under 10 nm.

Once the bead positions are obtained there are two methods to calibrate the trap. The more complex one is a calibration based on the power spectrum of fluctuations [5, 6], where the bead radius and the

viscosity of the liquid have to be known, whereas the simpler method reveals trap stiffness directly from the position fluctuations using a statistical approach [7]. This method does not rely on specific information about the medium or the trapped particle. It is independent of viscous drag, particle size and shape, and needs only the absolute sample temperature  $T$  as a parameter.

### Calibration using equipartition theorem

Assuming that the optical trapping potential is harmonic the trap can be calibrated using the equipartition theorem, which states that in equilibrium each degree of freedom contributes  $k_B T/2$  to the average energy, where  $k_B$  is the Boltzmann constant. The trap stiffness  $k_x$  can be directly calculated from the averaged square of the displacement of the bead from the trap center,

$$\sigma_x^2 = \langle (x - x_{\text{mean}})^2 \rangle, \quad (1)$$

using

$$k_x = k_B T / \sigma_x^2. \quad (2)$$

However, the results are not accurate for particle positions far from the center of the trap where the harmonic model fails.

### Calibration using Boltzmann statistics

The optical potential reconstruction using Boltzmann statistics can be used to determine any continuous trapping landscape in the region accessible by thermal agitation. In equilibrium the probability density  $\rho(x)$  of the 1D particle position is given by

$$\rho(x) = C \exp(-U(x)/k_B T), \quad (3)$$

where  $C$  is a normalization constant and  $U(x)$  is the trap potential. The shape of  $U(x)$  can be obtained from the normalized histogram of the trapped particle positions as

$$U(x) = -\ln(\rho(x)). \quad (4)$$

In the case of the commonly used TEM<sub>00</sub> Gaussian trapping beam, which results in a harmonic trapping potential, one can fit a parabola  $y = ax^2 + b$  to the data in the central region of the potential to extract the trap stiffness and check for possible deviations from the perfect harmonic shape. The stiffness

coefficient  $k=2a/k_B T$  obtained in such manner is more accurate than (2). Another advantage of such calibration is that it also gives the information about the potential in the region away from the trap center where the optical potential is non-harmonic.

## Program usage

### File specification and sample data

The default format of a time series of N data points is a text file holding one bead position  $(x_i, y_i, z_i)$  at a specific time  $t_i$  in each line:

```
t1    x1    y1    z1
t2    x2    y2    z2
...
tN    xN    yN    zN
```

Time column can be omitted. The calibration is performed for all coordinates that are included in the data file, typically x and y, since simple video particle tracking normally only provides 2D particle position information.

Two sample data files, `weaktrap.dat` and `strongdrift.dat`, are included with the program. Each consists of 20000 subsequent positions of a 1.6-micrometer silica bead trapped in a weak optical trap. The first set is a trajectory of a “perfectly” trapped bead whereas the trajectory in the second file includes a strong drift.

## Application

Upon start-up of the program the „Main“ panel is displayed (Figure 1). To analyze a data file the user should proceed as follows:

1. Click on the “Open” button. Browse for and open the data file to be analyzed. A new window displays a first few lines of the data file (Figure 2). Each column can be designated (time, x, y, z or discard) by a left-mouse click. If the coordinates in the data file are not given in micrometers

enter the conversion factor into the appropriate field (e.g. enter 1000 if positions are in *mm* or 0.001 if positions are in *nm* or some other conversion factor if data is given in pixels or other units). The time column can be omitted. Click “OK” and wait a few moments until all data points are imported.

2. The Information Box (Figure 1C) shows the name of the data file and the number of imported points. A long term drift (slope of a linear function fitted to all data points) for each imported coordinate column is calculated and displayed. Particle trajectories are shown in the upper left chart (Figure 1A). Trap stiffnesses determined directly from the variance of positions according to (3) are displayed under the chart.
3. Raw data points (trapped particle positions) usually include a fixed offset. For the analysis it is useful if a mean value of each coordinate is subtracted from each data point. This can be achieved by selecting “Subtract mean” option in the “Raw data manipulation” box (Figure 1B). If a particle trajectory also contains a drift as a consequence of experimental conditions, the positional variance increases, which results in spurious trap stiffness. To correct for the drift the program includes a drift compensation option, which can be enabled by selecting “Subtract drift” option in the “Raw data manipulation” box. The drift is modeled by a 3<sup>rd</sup> degree polynomial [8] (obtained by fitting), which is then subtracted from the data.
4. All data points are taken into account for further calculations by default. If you want to time-limit the input data just click and hold left mouse button on the left side of the particle trajectories chart (Figure 1A). A red line which represent the lower boundary of the input data appears. Keep holding the left mouse button to drag the line and change the input data region. Similarly you can click and hold the left mouse button on the right side of the chart to move a green line, representing the upper boundary. The data points between the red and green lines are used for further calculations which can be executed by clicking the “Recalculate” button.
5. Histogram of particle positions is shown in the upper right chart (Figure 1D). The number of histogram bins can be changed in the input box below the chart. Click “Recalculate” button to calculate distributions using the new settings.
6. Trapping potential (in the units of  $k_B T$ ) is displayed in the lower right chart (Figure 1E). In the case of harmonic trapping potential data points can be fitted with a parabola to directly extract the trap stiffness. To select the upper and lower fitting boundaries follow the same procedure as

described in step 4. Once the fitting region is selected click the “Fit Potential” button to perform the fitting. Fitted curves (solid lines) appear on the chart with their equations displayed underneath. The trap stiffnesses obtained from the fitted values according to (3) are displayed in bold together with a relative statistical error value.

### **Other panels**

Apart from the “Main” panel the program includes the following special panels: “Trap Anisotropy”, “XY Chart”, “Trajectory”, “Position distribution” and “Trap potential”. Each panel hosts one large chart with zooming option for close inspection of the data. To zoom-in click and hold the left mouse button, which selects the upper left corner of the region of interest. Drag the mouse holding the left button to select the lower right corner of the zooming region. Release the button to zoom-in. To zoom out repeat the action but select the second point of the zooming rectangle above the first point.

The charts in the panels can be exported either as images (use the “Save Image” button to save them into a JPG file) or as raw data (use the “Save data” button). Chart data points are stored into text files, one chart series per text file.

The “Trap Anisotropy” panel displays a 2D probability density chart. The whole trapping region is divided into  $N \times N$  square bins which are colored according to probability that the trapped particle is in them. The number of bins in one dimension can be entered in the “Bins” field.

This panel is especially useful for the analysis of the optical trap. Trapping potentials are often anisotropic due to low quality of the laser source, artifacts in the trapping beam, inhomogeneity of the trapped particle, heating etc. The long white line in the chart represents the direction of the minimum trap stiffness, whereas the short line represents the direction of the maximum stiffness. The information box displays trap anisotropy (defined as the ratio between the maximum and the minimum stiffness), the angle of the minimum stiffness with respect to x-axis and the minimum and the maximum stiffnesses.

### **Acknowledgement**

The author would like to thank Blaž Kavčič for careful reading of the manuscript and helpful comments, Igor Poberaj for stimulating discussions, and Jean Debord for DMATH mathematical library which is used for data fitting. This work was supported by Slovenian Research Agency (Grants No. J1-2200 and No. J1-2209).

## Bibliography

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- [3] A. Pralle, M. Prummer, E.-L. Florin, E.H.K. Stelzer, J.K.H. Hörber, Three-dimensional high-resolution particle tracking for optical tweezers by forward scattered light, *Microscopy Research and Technique* **44** (1999), 378-386
- [4] J. C. Crocker, D. G. Grier, Methods of digital video microscopy for colloidal studies, *J. Colloid Interface Sci.* **179** (1996) 298-310. Scripts using particle tracking algorithms can be downloaded from <http://www.physics.emory.edu/~weeks/idl/>. Commercial software for video based object tracking is also available, e.g. Able Particle Tracker, DiaTrack, etc.
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- [7] E. L. Florin, A. Pralle, E. H. K. Stelzer, J. K. H. Hörber, Photonic force microscope calibration by thermal noise analysis, *Appl. Phys. A* **66** (1997) S75-S78.
- [8] The main cause of the drift usually lies in slow temperature fluctuations of the experimental setup which include many different time-scales. A possible model of the drift would be running average of data points with a time window that is much longer than the characteristic relaxation time of a trapped object. In order to keep the program simple and to minimize the number of parameters we decided to model the drift by a third degree polynomial function, which (according to our experience) performs well.

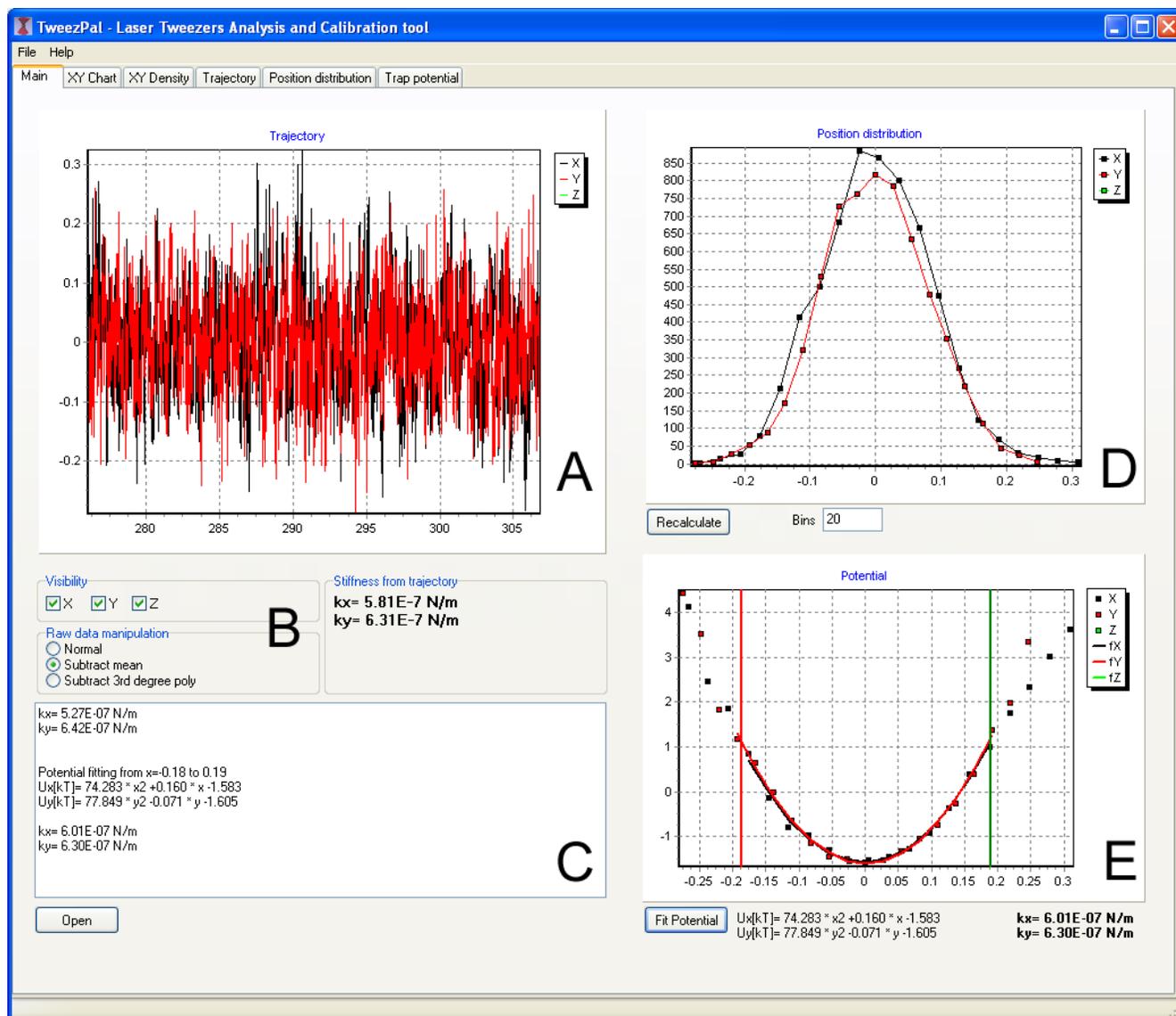


Figure 1: The main program window comprises a chart showing the particle trajectory (A), panel for data source, visibility and manipulation selection (B), information box (C), position histogram (D) and trapping potential with fitting region selector (E). The “Open” button is used to load data points from a trajectory file, the “Recalculate” button initiates recalculation of all charts and trap coefficients, and the “Fit Potential” button fits a parabola to the potential in the selected fitting region.

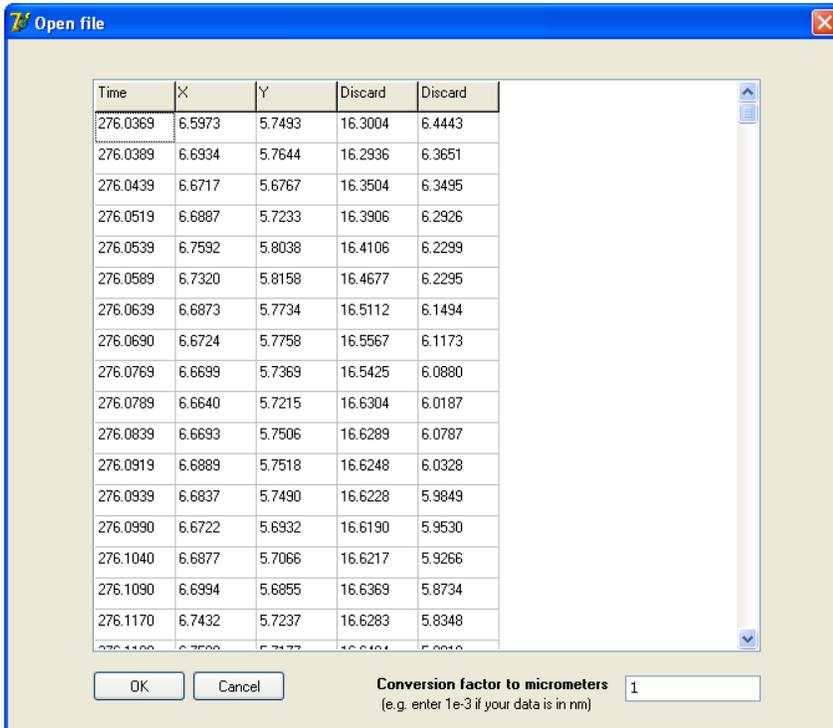


Figure 2: The import window displays the contents of the data file. Each column can be designated (Time, X, Y, Z or Discard) by a left mouse click on the column. Conversion factor between units used in the data file and micrometers should be entered in the appropriate field.

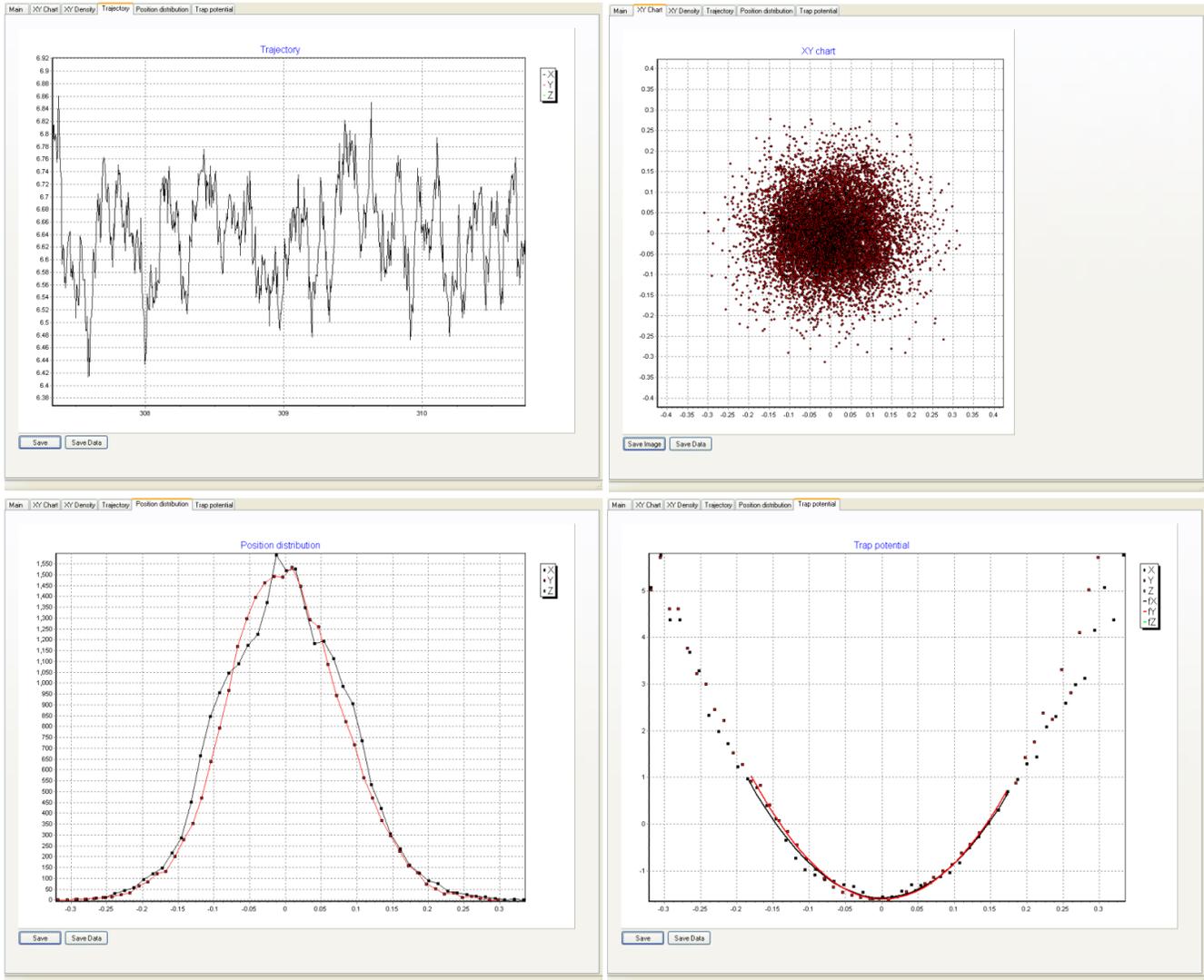


Figure 3: Other program panels host larger versions of the following: Trajectory, XY chart, Position distribution histogram and Trapping potential. Each chart can be exported as an image file (“Save Image” button) or as a set of raw data points (“Save data” button). Zooming-in for close inspection of the charts is achieved using the left mouse button.

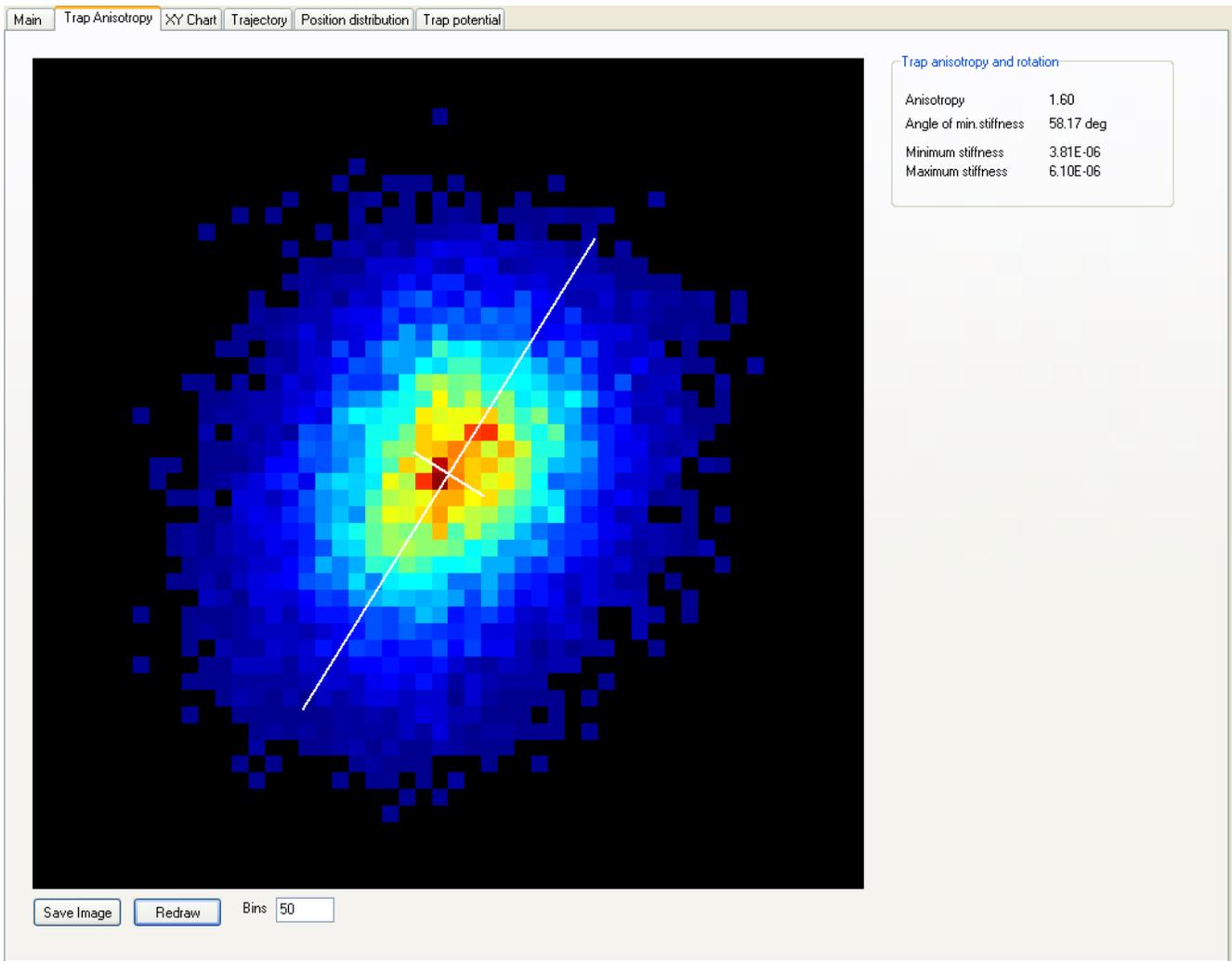


Figure 4: “Trap Anisotropy” panel hosts XY probability density chart. The whole trapping region is divided into  $N \times N$  square segments which are colored according to the probability that the trapped particle can be found in them. Red part of the color spectrum indicates higher 2D position density whereas darker shades represent areas which are rarely visited by the trapped particle. The white line represent the direction of the minimum trap stiffness. The information box displays trap anisotropy (defined as a ratio between the max. and min. stiffness), the angle of the minimum stiffness with respect to x-axis and the minimum and the maximum stiffnesses