

Manual for AFS graphical user interface (GUI) for data analysis

This GUI was developed using the MATLAB® App Designer using version R2022b and compatibility was not tested with previous versions. The installation of the Communications Toolbox, Data Acquisition Toolbox, Optimization Toolbox and Statistics and Machine Learning Toolbox is required. For the best visualization of all GUI features, a monitor size of 24" or more is recommended. It is important that all functions and subfunctions for the GUI are included in the current folder in MATLAB®. Most buttons execute a single function and/or output plots. Each function is supplemented with a brief description of the syntax as well as comments embedded within the code.

The manual is written to be followed step by step to provide the user with a suggested workflow for data analysis. Some tasks may not work if previous tasks were not executed. For example, it is not possible to perform a force calibration or rupture force determination without determining the anchor point first.

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GUI layout and data structure

GUI layout description

Figure S1 shows a screenshot of the GUI after startup. The GUI consists of 3 main areas indicated by the colored boxes. The blue box marks the navigation panel, where beads can be loaded in or removed as well as individual traces selected for visualization or certain computations. In addition, the system settings for magnification and bead diameter are found here. The green box covers the visualization panel, which displays the x/y position and z/power over time for a selected trace, and features a status update field to inform the user of the successful execution of operations. The red box outlines the individual features organized in tabs for anchor point and drift correction, filtering out traces based on thresholds, performing the force calibration (manual and automated), determining the rupture force or generating the force factor heat map.

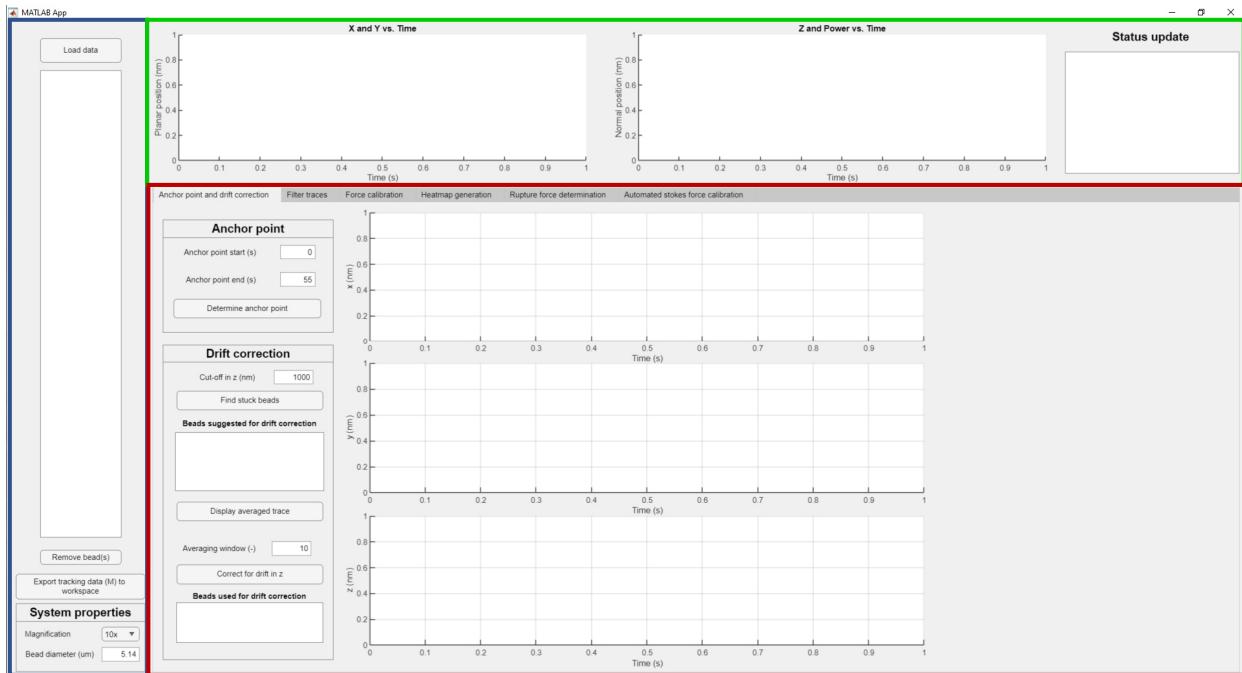


Figure S1: Layout of GUI upon startup. The blue framed box indicated the navigation panel as well as system properties. The green box contains the main trace plots and status updates. Individual tasks are organized in tabs indicated by the red frame.

Main data storage structure (M and N)

The MATLAB-based GUI operates mainly on data structs called M and N. The struct M carries the individual bead trace data along with outcomes of computations performed for each trace such as anchor point determination, stokes force calibration (SFC) or rupture force determination. Not all substructures of M are filled for each data set due to the nature of the experiment (stokes force calibration experiment vs. rupture force experiment). Figure S2 outlines the overarching structure of the data struct M. Fields shaded in green and yellow are only created and populated during SFC and rupture force experiments respectively. The struct N carries/collects the data from multiple SFC runs to create the final force factor heat map subsequently.

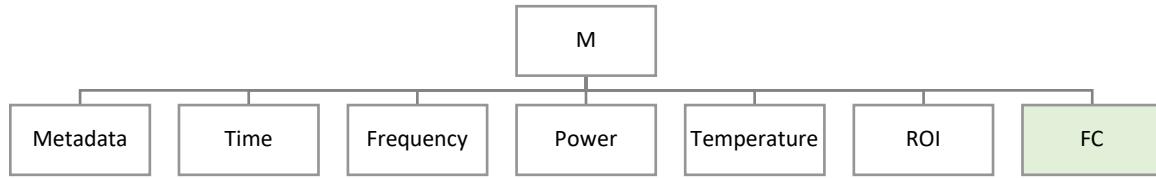


Figure S2. Structure of variable M. The green shaded field FC is only created if M is processed as part of the stokes force calibration analysis.

The M substructure Metadata contains details such as file name, information about anchor point or drift correction as well as the time when a force ramp was applied (ramp_start) as shown in Figure S3.

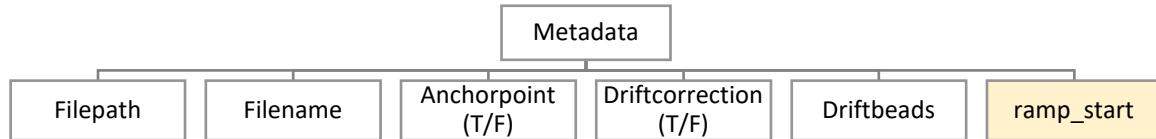


Figure S3: Substructure of the field Metadata as part of struct M. The fields Anchorpoint and Driftcorrection are True/False indicators. Ramp_start is only created when the data set is analyzed for rupture forces.

The substructure FC contains information necessary for the SFC such as the (relative) time array of the same size as the z-data during force application (Time_FC), the applied power value (Power_FC) as well as the (absolute) indices of the time where power was turned on (P_on_idx) and turned off (P_off_idx) as summarized in Figure S4.

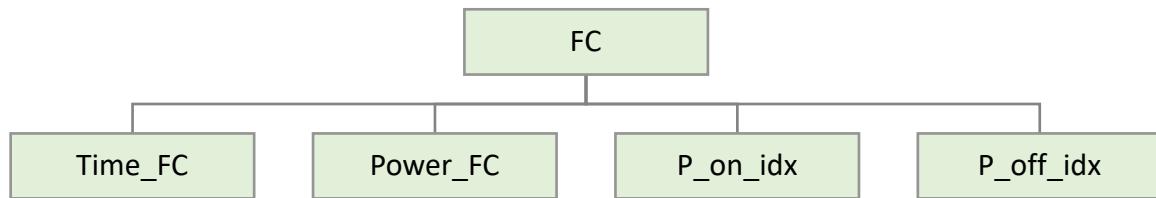


Figure S4: Substructure RF and FC as part of the main data struct M.

The field ROI contains information which is unique for each trace, such as the bead number (bead) and the tracking data in the x, y and z position. Furthermore it contains the anchor point position of the bead (Anchorpoint) and the root-mean-square (RMS) value (1) and standard deviation in z (StDz) during the same time frame as anchor point as shown in Figure S5.

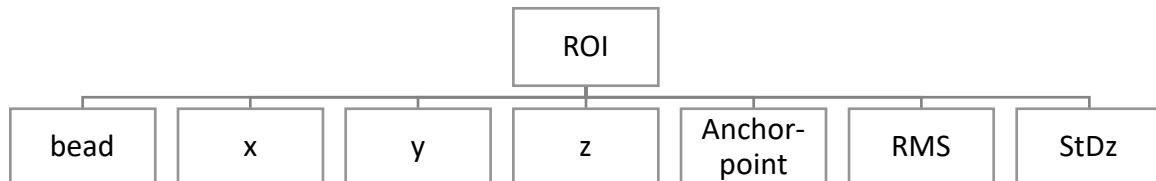


Figure S5: Part of substructure ROI, which will be created regardless of analysis mode (SFC or rupture force determination).

As part of the SFC, ROI as shown in Figure S6, is further filled with the z-trace data during the power application (z_FC), shifted such that $z_{(t=0)}=0$ (2), the fit parameters as an array (fitp), the R^2 value during fit (RSQ), the predicted z-data based on the optimal fit parameters (z_fit), the z-node based on the optimal fit parameters (znode) as well as the force at 1 μm (F) if the button **Visualize force distribution in FoV** is pressed.

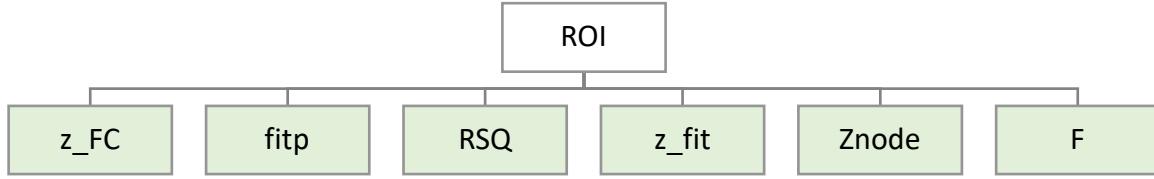


Figure S6: Substructure of ROI created during analysis of M for stokes force calibration (SFC).

During the analysis of traces of a rupture force experiment, the following fields of ROI as shown in Figure S7 are created. The time of bond rupture (RuptureTime), the power value at bond rupture (RupturePower), the rupture force based of the interpolated force calibration factor for a given anchor point position (RuptureForce) as well as the time until bond ruptured (BondLifeTime) and the loading rate (LoadingRate).

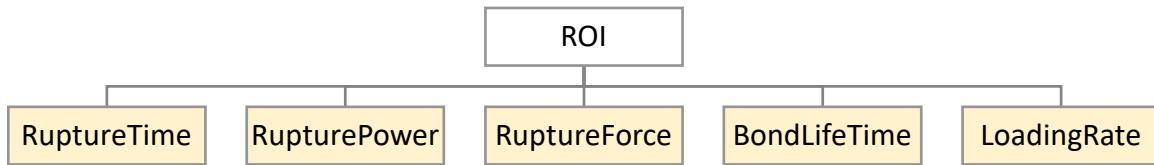


Figure S7: Substructure of ROI during analysis of traces of a rupture force experiment.

The structure of N shown in Figure S8 and contains only the necessary information to create a force factor heatmap. For each bead, which passes the filters described in the main text, the applied power value (Power), anchor point in x and y (APx and APy, respectively) and fit parameters (fo, kp, phip and R^2 , respectively) and the first z-node (znode) are stored.

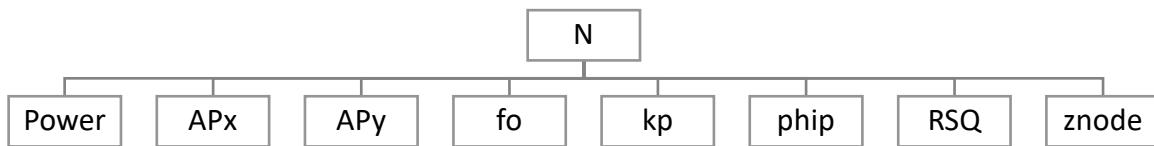


Figure S8: Structure of N, which collects data from individual SFC runs for the generation of the force factor heatmap.

Data analysis example

The following section describes the use of the GUI to analyze experimental data in the same order as a typical AFS experiment. The preparation of a bead sample for SFC or rupture force determination is described in the main text. An example data set for each operation is part of the supplementary material.

Finding a good field of view

The force field varies significantly across the channel of the AFS (2), hence it is imperative to find a field of view (FoV) where the force variation is minimal. This can be easily achieved with the developed GUI. After a set of beads (between 80-160 beads of 5.14 μm diameter in the FoV at 10x magnification) was subjected to the SFC protocol (described in the main text), the software returns a heatmap visualizing the force distribution as well as the z-node distribution. Based on that information, the user can iteratively find a more homogeneous FoV by moving the stage towards the area where forces appear to be more homogeneously distributed and repeat the SFC. With the user-friendly GUI developed, this process takes less than 30 minutes. The analysis steps are described below.

- 1) Load in the data set. Click on **Load Data**. A pop-up window opens up and the user can select the TDMS file *Find_FoV_1.tdms*. Once the import is completed, the list box in the navigation panel lists all beads and the Status update window shows “Data set Find_FoV_1.tdms successfully imported”. Upon clicking on one of the traces, the x, y, z and power data is visualized in the two plots as shown in Figure S9.

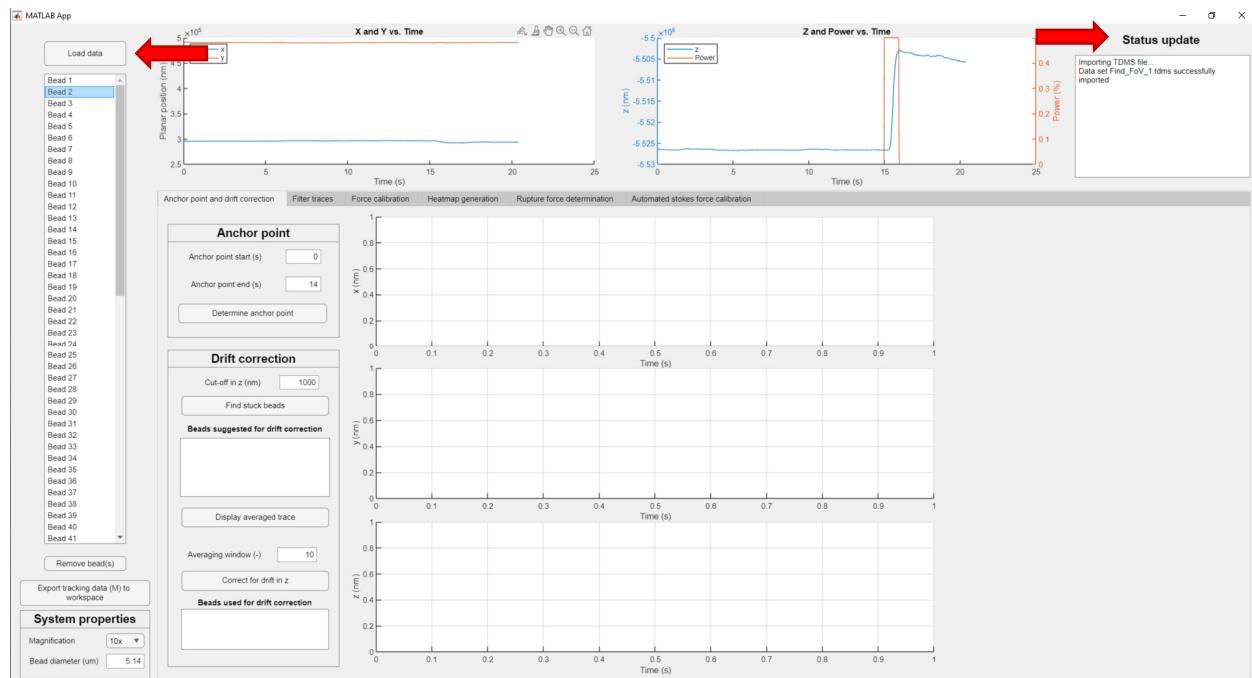


Figure S9: GUI with data set *Find_FoV_1* imported and visualization of raw tracking data. The load data button and status update fields are indicated by the red arrow.

- 2) To convert the absolute positional data to useful relative motion, the anchor point needs to be determined. Select the time frame where no force was applied by filling out the fields for *Anchor point start (s)* and *Anchor point end (s)* as 0 and 14 respectively. Next, click on **Determine anchor point**. Clicking on a bead from the list box updates the traces as shown in Figure S10. Beads which contain tracking error such that the anchor point falls outside the limits set by the magnification drop-down menu, will be automatically removed from the navigation panel.

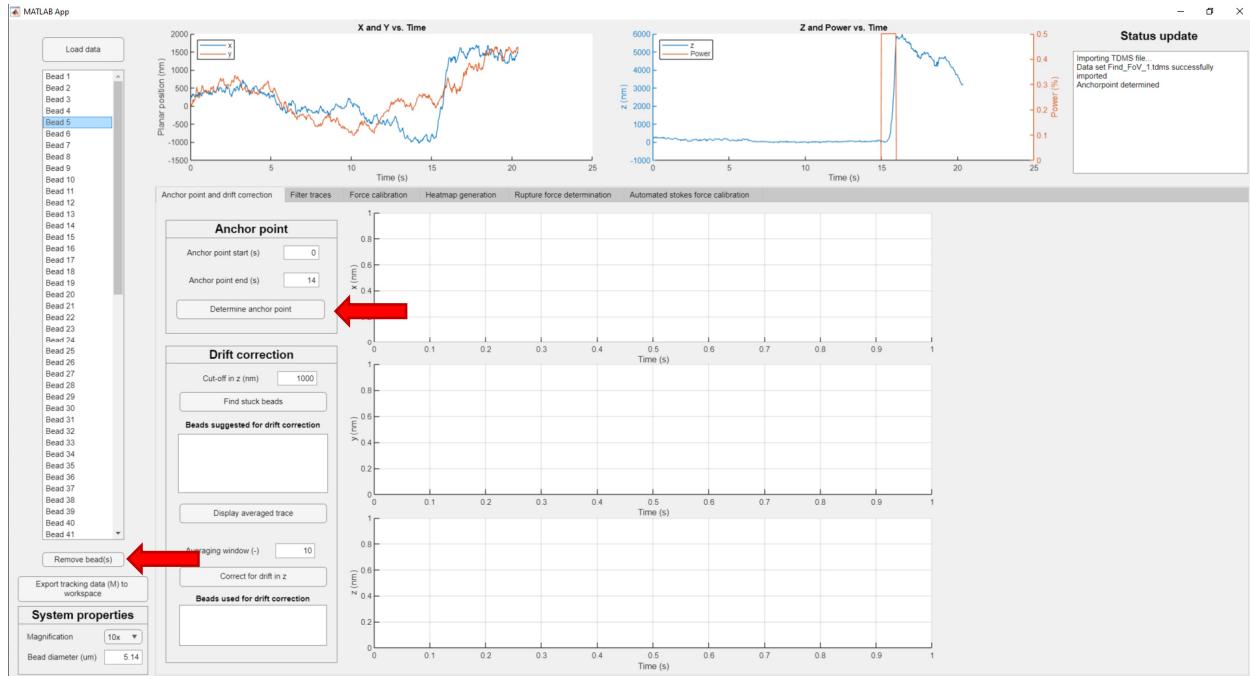


Figure S10: GUI after determining the anchor point. The Determine anchor point button and remove beads button are indicated by the red arrow.

- 3) The user can manually browse and inspect individual traces by clicking on their name in the navigation panel. The trace data will automatically be updated. For example, bead 14 shows fluctuations in the x, y and z. This bead can be removed by selecting it in the navigation panel and clicking the button **Remove bead(s)**. Multiple traces can be removed at once by holding down the ctrl-key while clicking on a bead.
- 4) Alternatively, beads with tracking errors can be removed by switching to the *Filter traces* tab and create a histogram of RMS and standard deviation in z during the time frame for anchor point determination by clicking the button **Plot RMS and Std. Dev. (z) histograms**. Beads outside user specified bounds in *RMS Lower cut (nm)* and *RMS Upper cut (nm)* as well as *Std. Dev. (z) cutoff (nm)* (in our example 5, 1000 and 1000 nm respectively) will be removed by clicking the button **Remove RMS and Std. Dev. (z) outliers**. Figure S11 shows the histograms before (left) and after (right) applying these filters.
- 5) To visualize the force distribution in the current FoV, the SFC needs to be performed. Although the implemented SFC fitting algorithm is robust, it may be helpful to filter out traces with tracking errors or otherwise unsuitable traces prior to SFC. Move to the tab *Force Calibration* tab and define the time range for SFC in the fields *Force calibration start (s)* and *Force calibration end (s)* as 14 and 17, respectively. It is important to define the time range such that the force application is fully captured. Only the approximate time range is needed, since the algorithm will identify the exact start and end time as indices (*P_on_idx* and *P_off_idx* in M/Metadata, respectively). Depending on the chip specification and position of the z-node, the cut-off for z can be defined in the field *z cut-off (um)*, for example 10 μm . Once those parameters are set, pressing the button **Find beads** returns a plot of beads suitable for force calibration as well as a list of all beads which may not be suitable for SFC as shown in Figure S12. The feature is not 100% accurate, and a manual verification of suggested beads is still necessary.

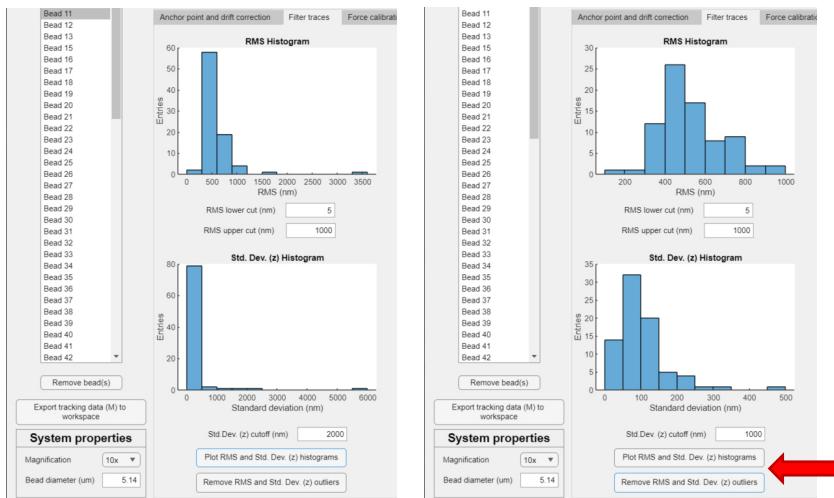


Figure S11: Histograms of RMS before (left) and after (right) applying RMS and standard deviation in z filters. The buttons Plot RMS and Std. Dev histograms as well as remove RMS and Std. Dev. outliers are indicated by the red arrow.

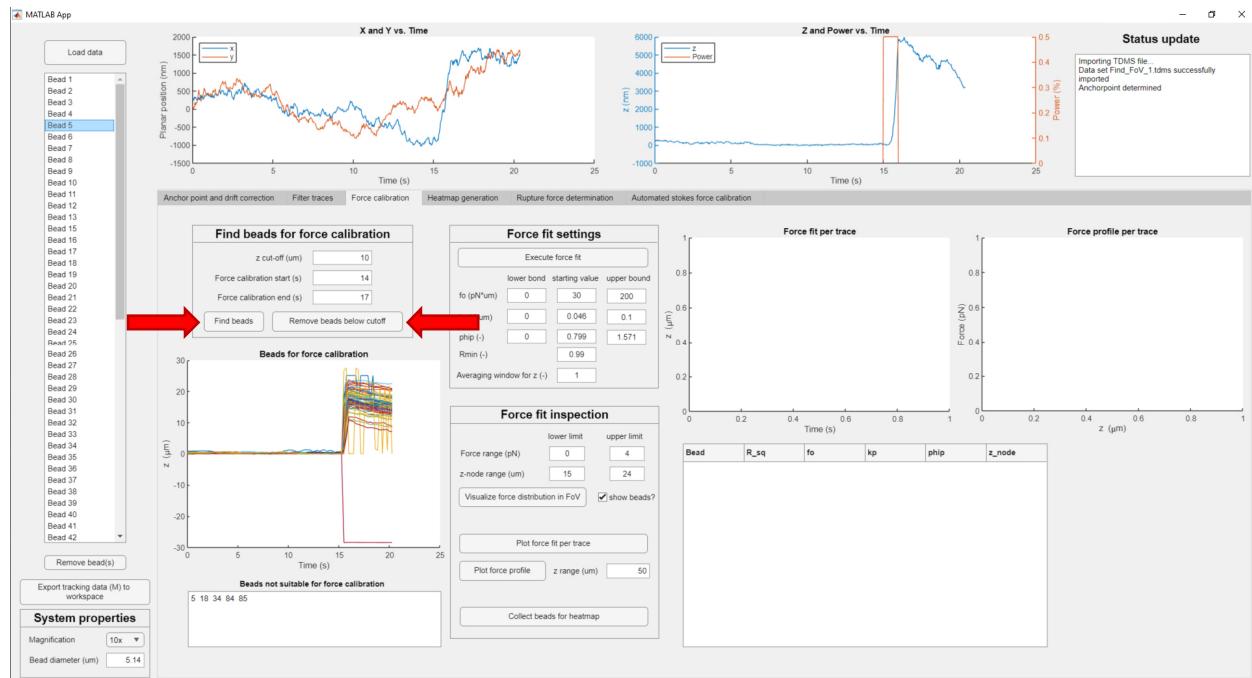


Figure S12: Visualization of beads suitable for force calibration and listing beads identified not to be suitable for SFC. For example, Bead 5 does not contain any tracking errors (see x/y and z/p plots), but the threshold for z of 10 μm was not met (bead lifted up only to about 6 μm as seen in the z/p plot on the top right). The find beads and Remove beads below cutoff buttons are indicated by the red arrow.

- 6) The button **Remove beads below cutoff** removes all beads listed in the text field at the bottom of Figure S12.
- 7) The SFC algorithm needs starting parameters and boundary conditions, which can be defined as shown in Figure S13 (red box). The R^2 value of the force fit can be specified in the field *Rmin*. The default value is 0.99 and the algorithm will change the step size and function tolerance of the fitting function to reach the specified R^2 value. In addition, the trace can be smoothed by applying a moving average. By default, the averaging window field *Averaging window for z (-)* is set to 1, which means no averaging is applied. The button **Execute force fit** starts the SFC. The SFC completion is indicated in the *Status update* field as shown in Figure S13.

- 8) The fit for one or multiple traces can be inspected by selecting one or more traces from the navigation panel and then clicking on the button ***Plot force fit per trace***. The data points and fit are shown in the graph *Force fit per trace* and fit parameters listed in the table below. Further, the force profile can be plotted by defining a limit in *z range (um)*, for example 50 μm . The button ***Plot force profile*** displays the force profile for the selected trace(s) as shown in the Figure S13.

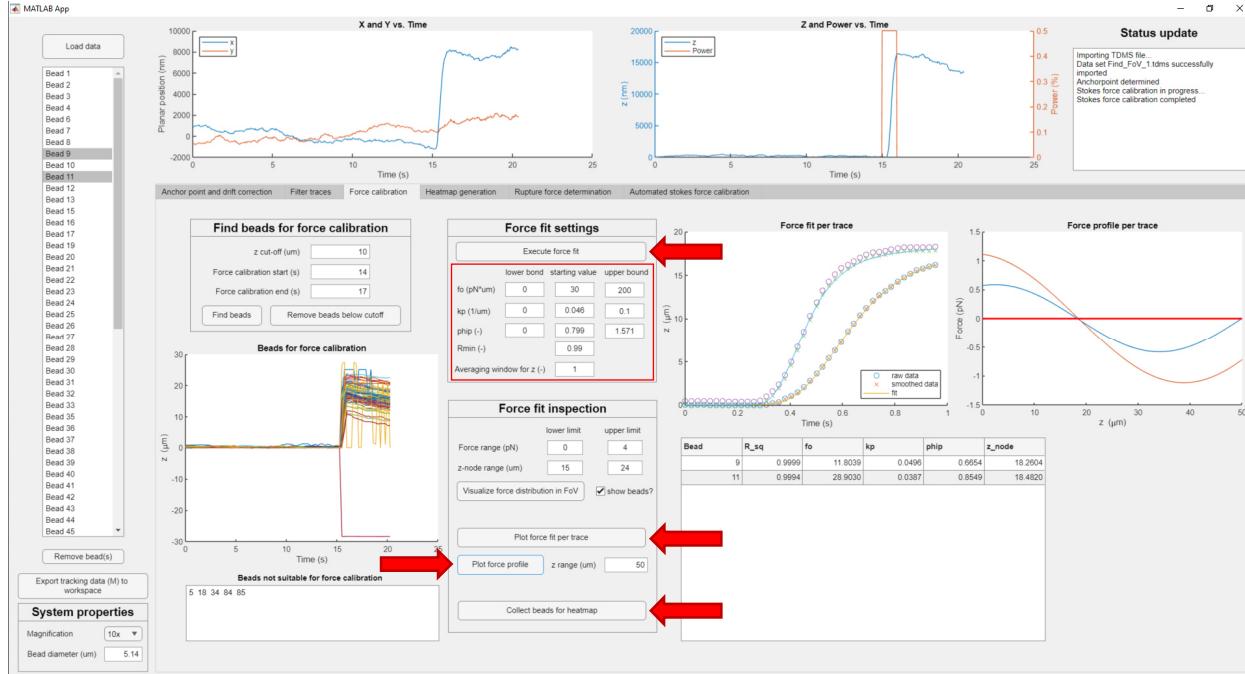


Figure S13: After completion of SFC, individual traces can be inspected. For example, the individual force fits as well as the associated parameters and the force profile in z can be readily accessed with the functions implemented in the GUI. The force fit parameters are highlighted by the red box. The buttons *Execute force fit*, *Plot force fit per trace*, *Plot force profile* and *Collect beads for heatmap* are indicated by red arrows.

- 9) The fit parameters can be visualized as distributions in the tab *Filter traces*. The button ***Plot force fit histograms*** updates the plots for f_0 , k_p , ϕ_p , R^2 and z -node as shown in Figure S14 (left). Outliers can be removed by specifying the cutoffs for each parameter in the input fields beneath each plot. The button ***Remove force fit outliers*** will remove all beads from the struct which are outside of the defined cut-offs Figure S14 (right).

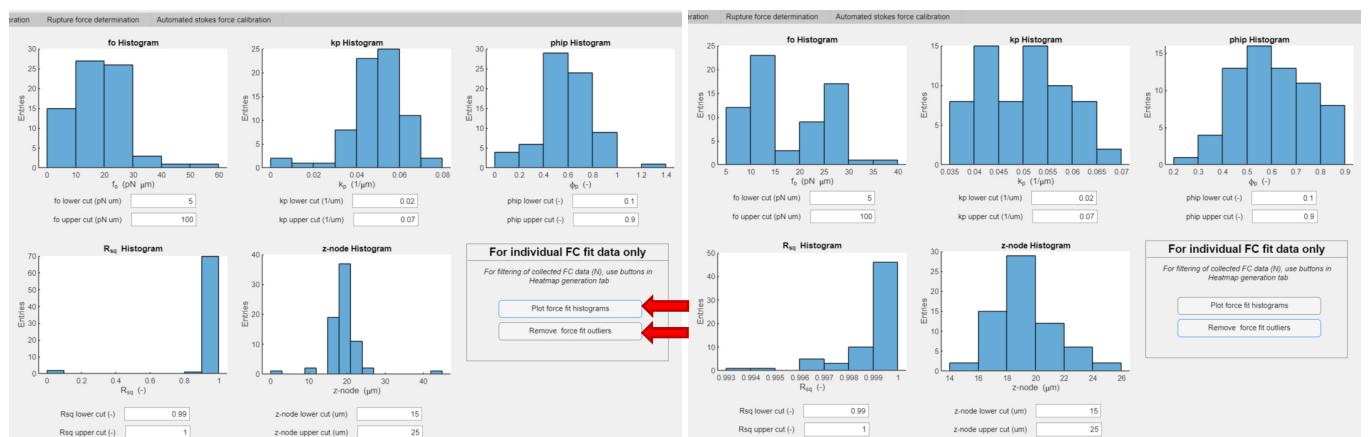


Figure S14: Histograms of force fit parameters before (left) and after (right) applying filters. The buttons *Plot force fit histograms* and *Remove force fit outliers* are indicated by the red arrows.

- 10) To get a quick estimate of the force and z-node distribution in the current FoV, the button **Visualize force distribution in FoV** in the *Force calibration* tab can be pressed. A pop-up window will appear, which displays a heatmap for the force at $z=1\text{ }\mu\text{m}$ as well as the z-node distribution. Furthermore, the histograms of force and z-node are shown right next to the heatmaps. As seen in Figure S15 (left), for the data set *Find_FoV_1* most forces are less than 1 pN.
- 11) If the force field appears inhomogeneous or low, the stage of the AFS chip can be moved towards the area which appears to exert higher forces and the analysis can be repeated. The pop-up window for the force and z-node heatmap can be kept open so that multiple runs for different FoVs can be compared to identify the best FoV for an experiment. For this demonstration, the process described above is repeated for the file *Find_FoV_2.tdms* (FoV moved $\sim 400\text{ }\mu\text{m}$ to the right relative to *Find_FoV_1.tdms*) and the heatmap comparison is shown in Figure S15.

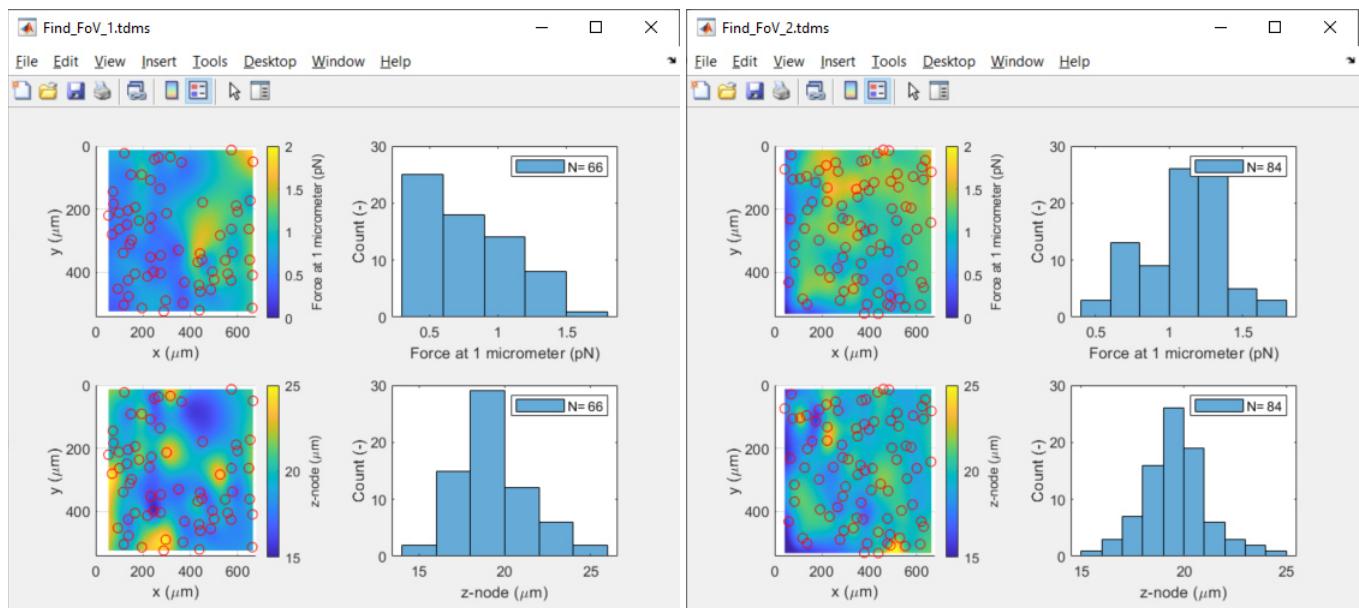


Figure S15: With the GUI, the user can quickly analyze data and obtain a qualitative comparison between different FoVs. Comparison of the force and z-node distribution of two different field of views (FoV). For the data set *Find_FoV_1* (left), most forces are $< 1\text{ pN}$, whereas data set *Find_FoV_2* shows most forces $> 1\text{ pN}$. The pop-up window is created by pressing the button **Visualize force distribution in FoV**.

Stokes Force Calibration (SFC)

Once an acceptable FoV has been found, the heatmap of the force calibration factors can be created. The process is very similar to the steps described above for finding a FoV. Typically, with the same set of beads in the AFS chip, 3-5 SFC runs can be recorded. As mentioned in the main text, the power values applied should be in a range that yield enough data points per trace for the time range where the beads was rising towards the z-node. The examples below describes the process of manually creating the heatmap as well as the automated generation. The data set used for the automated SFC can be used for manual SFC as well.

Manual Stokes Force Calibration

- 1) Load in the data set *Automated SFC example_1.tdms*. The status field should display “Importing TMDS file...” and “Data set Automated SFC example_1.tdms successfully imported”.
- 2) Determine the anchor point between 0 and 14 seconds. The status field should display “Anchor point determined”.
- 3) Filter RMS between 5-1000 nm and standard deviation in z < 1000 nm.
- 4) Define the time range for force calibration between 14 and 17 seconds.
- 5) Find beads for SFC with a z cut-off of 10 μm between 14 and 17 seconds.
- 6) Remove all outliers.
- 7) Set the fit parameters to the values shown in Figure S13 and run the force fit. The status field should display “Stokes force calibration completed”.
- 8) Move to the *Filter traces* tab and remove beads with fit parameters outside the defined specifications shown in Figure S14.
- 9) Up to this point, the steps were identical to the steps described in finding a good FoV. The GUI uses 2 types of data structures, *M* for individual experiments and *N* for collecting SFC data from multiple SFC runs. Once the data set has been inspected manually (for example inspecting the force fit or force profiles of selected beads), and deemed useful, the beads can be moved to the collection-struct *N* by pressing the button **Collect beads for heatmap** in the *Force calibration* tab. As shown in Figure S8, only the anchor point data, applied power value and force fit parameters are transferred from *M* to *N*.
- 10) Repeat the process with the data sets *Automated SFC example_2.tdms* and *Automated SFC example_3.tdms* and append *N* struct after each analysis by pressing the button **Collect beads for heatmap**. Pressing the button twice would append the data set twice to *N*, so attention must be paid during the manual force calibration. The status update on the top right corner of the GUI provides information about the collection process by displaying “Current data set stored for heatmap”.
- 11) The progress of collecting data for the heatmap can be visualized in the tab *Heatmap generation*. The table in *N struct content* below the bead visualization graph summarizes the number of beads for each applied power value that are currently stored in *N*. Their distribution in the FoV can be visualized by selecting a power value (such as 0.3 for this particular example) and pressing **Visualize bead collection** as shown in Figure S16.
- 12) Once sufficient data points (recommended > 1000 per unique power value) have been collected, the force factor heatmap can be generated. Since forces vary in z, the z-position for the heatmap needs to be defined in *z for force estimation (um)* such as 1 μm . The heatmap algorithm creates a mesh defined as per user input and pools beads according to their anchor point location for all power values. Each mesh point is only populated if more than 2 beads (either from one or more power values) are present within the mesh bounds. For the described example, the mesh size can be set in *Mesh merging size (um)* to 30 μm , since there are only a few beads in the FoV. The

heatmap is generated by pressing the button **Create heatmap**, which also creates a histogram of the force factors. The fit of force factors to a normal distribution is summarized below the histogram and the values in square brackets denote the 95% confidence interval of the parameter estimation as shown in Figure S16.

- 13) The heatmap can be saved by pressing the button **Save Heatmap**.

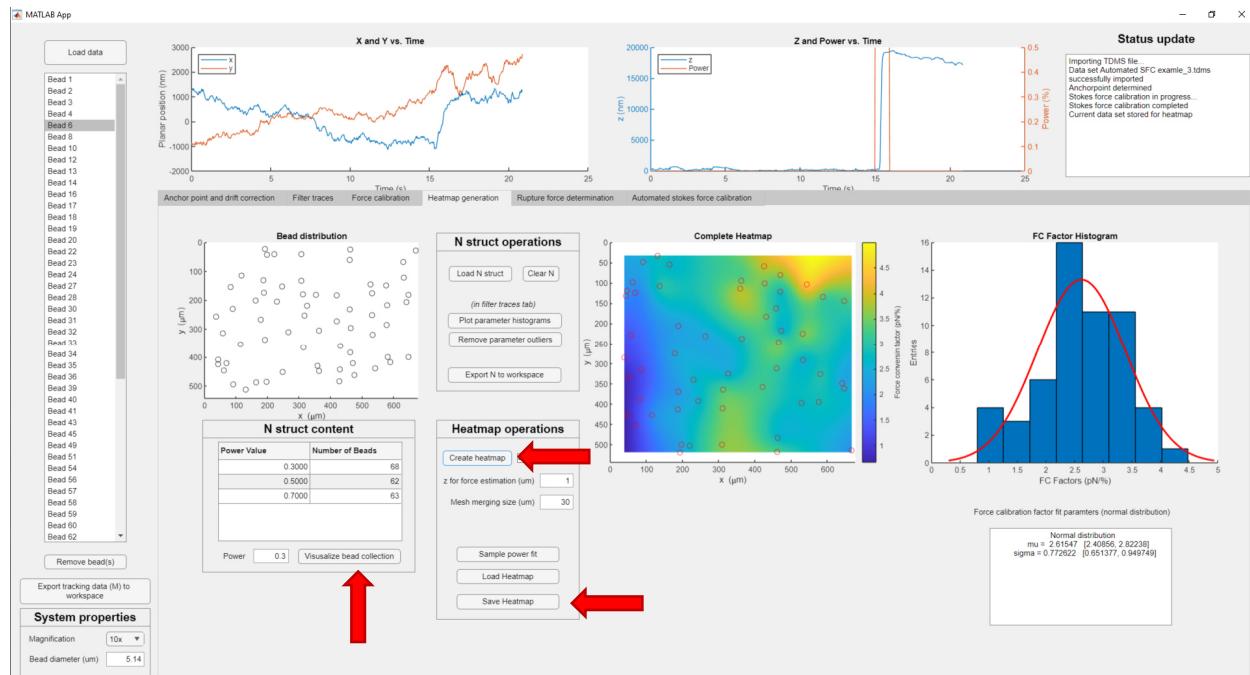


Figure S16: Manual stokes force calibration and force factor heatmap generation using the GUI. The GUI displays the heatmap and force factor distribution and provides a summary of the normal fit below the histogram. The buttons Visualize bead collection, Create heatmap and Save Heatmap are indicated by red arrows.

Automated Stokes Force Calibration

Since 40-60 data sets need to be analyzed to create a sufficient force factor heatmap, the execution of the steps described above are automated within the GUI. The automated SFC protocol replicates the manual force calibration steps as it calls the same functions **in the same order** as the user would during manual force calibration. This means, that the same input fields in the *Anchor point* tab and *Force calibration* tab need to be defined correctly. **The GUI recognizes a folder and will read in all TDMS files contained within that folder.** Thus, it is important that only data relevant for SFC is present in that folder. The automated script works best if all data sets have the same time signal, e.g., no force for 15 seconds followed by 1 second of power on, followed by 5 seconds power off. If traces are not identical, it is necessary to define the time range for anchor point and force calibration such that all individual data sets fall within the specified range. The bead tracking software provided by LUMICKS can execute protocols automatically, thus ensuring that all data sets display the same time range for anchor point and force application. It is important to define and test the fit parameters for SFC described in the manual SFC section above, before starting the automated SFC protocol to ensure error-free execution of functions. It is not necessary to define the range for the fit parameters in *Filter traces* tab, since outliers will be removed based on the z-score > 3 automatically and the user can filter the data further after automated SFC is completed as demonstrated in the example below.

The force fit algorithm is sensitive to the starting parameters and it is advised to test the SFC analysis protocol for multiple starting parameters to assess which starting conditions yield more normal distributed fit parameters.

- 1) In the *Automated stokes force calibration* tab, define a file name for the N struct, which collects relevant SFC data. After the automated SFC, the N struct will be saved using the name specified in the *File name for N struct* field.
- 2) Define time frame for anchor point and force calibration, standard deviation in z during anchor point, z-position cut-off and starting parameters for the SFC fit as for the manual SFC example above.
- 3) The analysis is executed by clicking the button **Select file path and run automated force calibration**. It opens a pop-up window to specify the folder, in which all TMDS files will be analyzed. It is important to note that only traces designated for automated SFC analysis should be contained in the specified folder. A status update field specific to the automated SFC displays the progress of data analysis as shown in Figure S17. The general status update window on the top right of the corner of the GUI will update as well as shown in Figure S17. The automated SFC protocol follows the same steps as the manual execution described above. Upon completion, the N struct is saved as a MATLAB variable (*.mat file format) in the folder where the TDMS files are located. In this example the N struct is saved as *automated_SFC_demo.mat* and supplied as part of the supplementary information for reference.

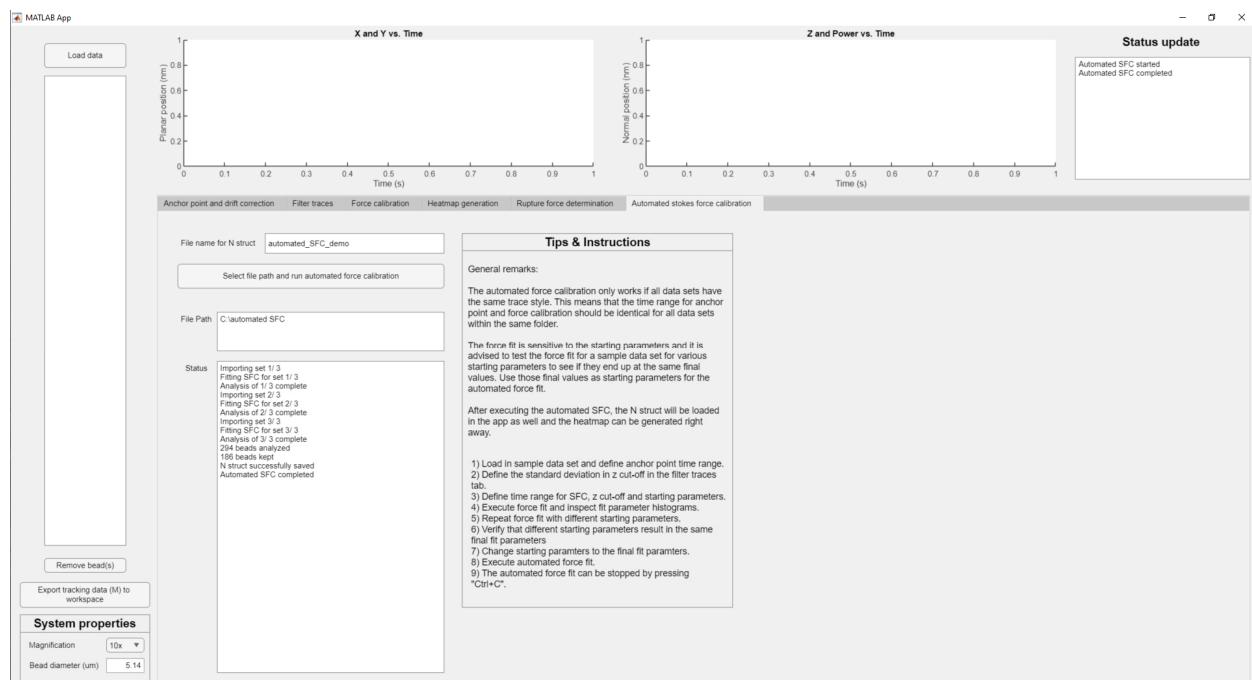


Figure S17: Automated stokes force calibration tab shows the status of data analysis.

- 4) After completion, the collected beads for each applied power value can be visualized by pressing the button **Visualize bead collection** in the *Heatmap generation* tab as mentioned above. If needed, additional SFC data can be generated and added to the N struct via manual SFC after the automated analysis by following the steps outlined above. It is not possible to automatically analyze more data and add to the existing N struct. If more data is needed, then the automated SFC needs to be repeated.
- 5) If the GUI has been closed after SFC, the N struct can be loaded in again. Load in *N_example.mat* by pressing the **Load N struct** button in the *Heatmap generation* tab. This file contains the fit parameters of 70 individual data sets (> 6000 individual beads analyzed) to generate a full force factor heatmap.
- 6) Before creating the heatmap, it is advised to check the values for individual fit parameters.

- 7) All fit parameters can be visualized by pressing **Plot parameter histograms** in the *Heatmap generation* tab. The fit parameters are displayed in the *Filter traces* tab as shown in Figure S18 (left).
- 8) Set the filters for all fit parameters as shown in Figure S18 in the *Filter traces* tab, move to the *Heatmap generation* tab and press **Remove parameter outliers** to remove outliers and update histograms. Moving back to the *Filter traces* tab, the histograms should appear as shown in Figure S18 (right).

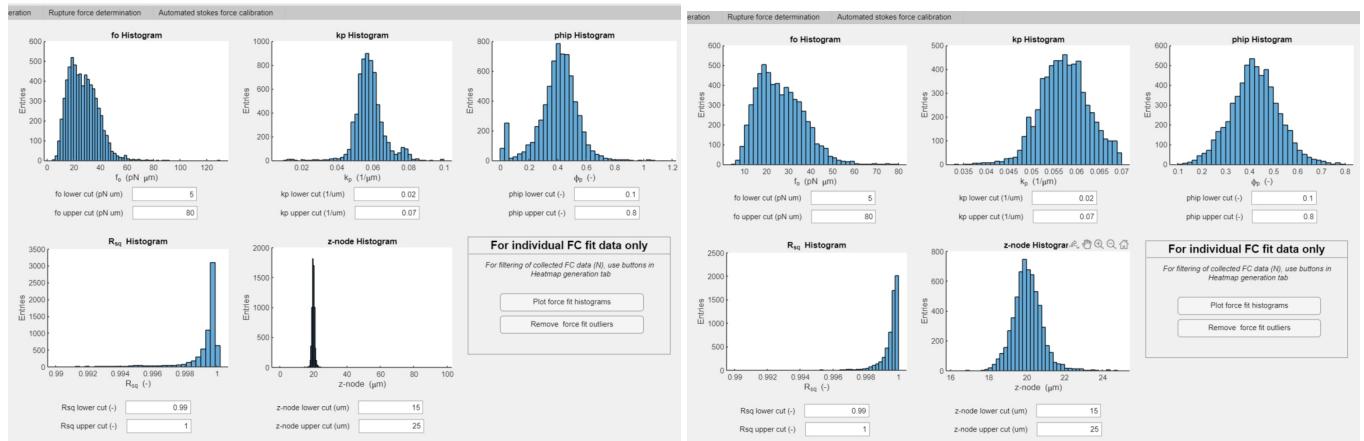


Figure S18: Fit parameter histograms of N containing > 6000 individual bead force calibration points before (left) and after (right) applying the filter criteria.

- 9) The force factor heatmap is created in the *Heatmap Generation* tab. Set *z for force estimation* as $1 \mu\text{m}$ and the *mesh size for merging* to $10 \mu\text{m}$. The heatmap and force factor histogram as shown in Figure S19 is created by pressing **Create heatmap**. If *show beads?* is unchecked, then the force factor heatmap is shown without the merged bead locations (shown as red circles in heatmap).

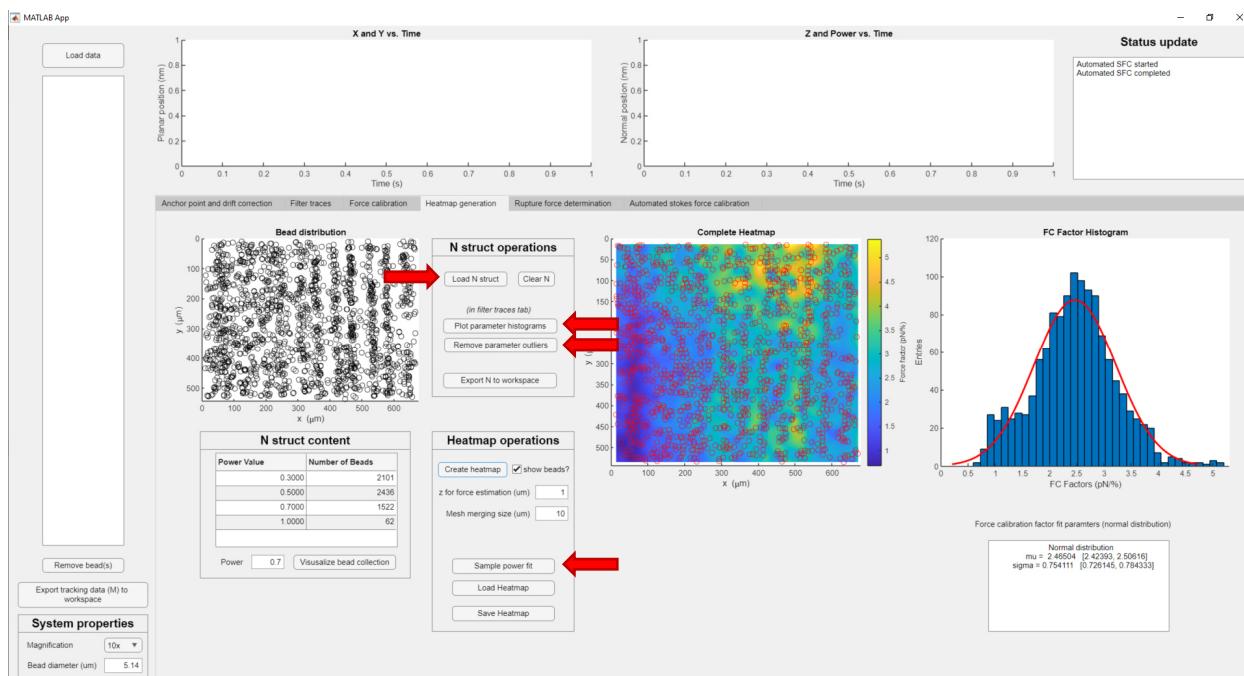


Figure S19: Full heatmap example automatically created with the GUI after loading in N struct from an automated SFC analysis. The buttons *Load N struct*, *Plot parameter histograms*, *Remove parameter outliers* and *Sample power fit* are indicated by red arrows.

10) The force-power fit can be qualitatively assessed by pressing the button **Sample power fit**. This creates a pop-up window displaying the fit of 9 randomly selected mesh grid points along with their location in the FoV as shown in Figure S20.

11) The final heatmap can be saved with the button **Save Heatmap** for subsequent use in rupture force experiments. For reference, the final heatmap displayed here is provided as part of the supplemental material in *Heatmap_example.mat*.

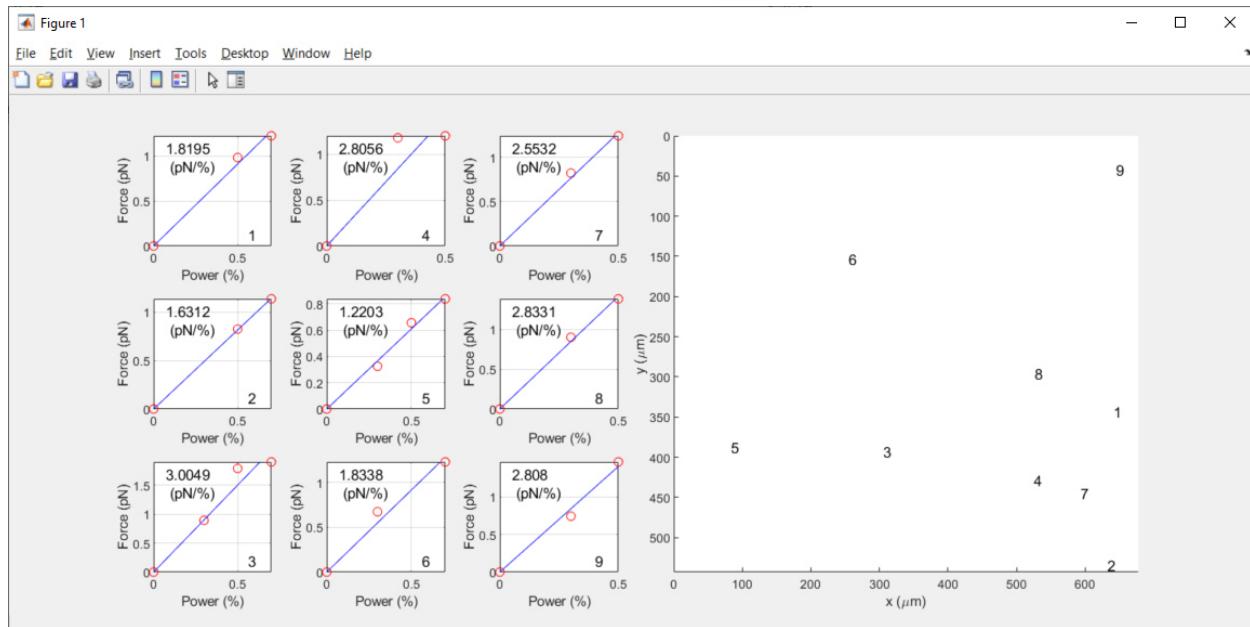


Figure S20: Sample of force power fits (left) and their location within the FoV (right). The red circles represent the force-power data for one mesh grid point and the blue line represents the linear power fit to correlate the force with the applied power. The inset in each figure at the top left shows the force calibration factor and the mesh grid point is indicated at the bottom right for the respective mesh grid point.

Rupture force determination

In addition to finding the force factor heatmap, the GUI can be used to analyze bead rupture force data. The experimental setup is described in the main text and the steps in analysis are outlined below:

- 1) Load in the data set *Rupture force example.tdms*.
- 2) Determine the anchor point between 0 and 55 seconds.
- 3) Optional drift correction. If the acoustic force was applied over a long time (such as minutes or hours), the fluid will warm up and traces show signs of drift. This can be corrected by subtracting the relative motion of a bead stuck to the surface. Such beads are found by setting the *Cut-off in z (nm)* to 1000 and pressing the button **Find stuck beads**.
- 4) Each stuck bead can be manually inspected by clicking on the bead in the navigation panel, which updates the main plots. One or more beads suited for drift correction (such as Beads 41, 44 and 69), can be selected from the navigation panel and a preview of the average trace is displayed by pressing the button **Display averaged trace** as shown in Figure S21.

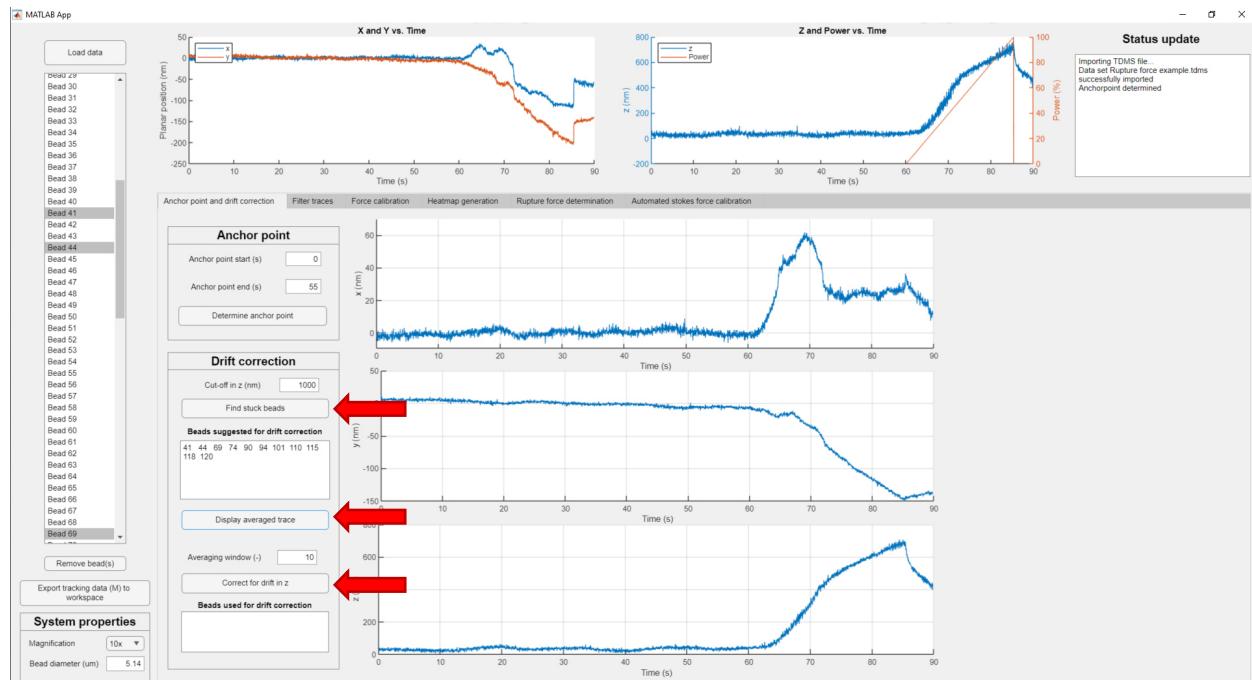


Figure S21: Preview of an averaged trace from Bead 41, 44 and 69 show that those 3 beads are suitable to correct the entire data set for drift. Traces containing tracking errors (such as sudden changes in x, y or z) should not be used for drift correction. The buttons **Find stuck beads**, **Display averaged trace** and **Correct for drift in z** are indicated by red arrows.

- 5) If the averaged trace does not show any sudden changes or noise in z such as the one displayed in Figure S21, then the user can proceed with drift correction by pressing the button **Correct for drift in z**. This corrects all traces for drift in z based on the average of the selected traces.
- 6) Filter traces with RMS outside 5-200 nm and a standard deviation in z more than 500 nm in the *Filter traces* tab by pressing **Remove RMS and Std. Dev. (z) outliers**.
- 7) In the *Rupture force determination* tab, the time range for determining the rupture force is set by the input fields *Rupture force start (s)* and *Rupture force end (s)*. Specify the time range as 55 and 86 seconds, respectively. The GUI determines the rupture time as the time point where the (averaged) z position is above the user defined threshold specified in *Cut-off for z (nm)*. In this example, *Cut-off for z (nm)* and *Averaging window for z(-)* are set as 2000 nm and 120, respectively.

- 8) Pressing the button ***Find Rupture Force*** determines the rupture time and associated power value for all beads. Beads, with an inconclusive rupture event, are listed in the field *Traces for manual rupture force determination* as shown in Figure S22. By default, such beads are assigned 0 for rupture time and rupture power until the user specifies otherwise.
- 9) Beads can be inspected manually, by selecting the bead in the navigation panel and pressing the button ***Plot rupture-bead trace***. This will display the selected bead's z trace (black) and averaged z trace (green) and the suggested rupture time as a red dot. The suggested rupture time is also displayed in *Suggested rupture time (s)* field as shown in Figure S22.
- 10) In case of Bead 20, the GUI- suggested rupture time seems to indicate the real rupture event and the rupture time can be assigned to this bead by pressing the button ***Assign rupture time***. By default, the field *Rupture time input (s)* displays -1, which means that the value displayed in *Suggested rupture time (s)* will be assigned to the selected bead. The user can, however, input any rupture time (within the time range of the trace) in the field *Rupture time input*. Bead 109, for example shows a suggested rupture time of 69.4 seconds, however it appears to be a stuck bead. In that case, either the *Rupture time input* field can be specified as 0 before the button ***Assign rupture time*** is pressed (Figure S22) or the bead can be removed by clicking ***Remove bead(s)*** in the navigation panel. Once all rupture times have been automatically or manually determined, the rupture force for each bead can be assigned based on the location within the FoV. Note that pressing the button ***Find Rupture Force*** again would overwrite all manual rupture time data.
- 11) If the GUI has been closed after the heatmap generation, the heatmap can be loaded in by switching to the *Heatmap generation* tab and clicking the button ***Load Heatmap***. Use *Heatmap_example.mat* for this example.
- 12) Switching back to the *Rupture force determination* tab, the rupture power is converted to the rupture force by clicking the button ***Assign rupture force and plot rupture force and loading rate histogram***. This will output a rupture force and loading rate histogram as shown in Figure S22. Note that beads with zero rupture force are removed from the histogram plots for clarity but are still stored in the M struct.
- 13) Both rupture force and loading rate histograms can be fitted to either a normal and lognormal distribution by selecting the option from the drop-down menu and pressing ***Fit loading rate and rupture force*** as shown in Figure S22.
- 14) The rupture force and loading rate data along with bead data can be saved as a *.csv file. Specify the file name and file path in *File name* and *File path*, respectively and press the button ***Export rupture force data***.
- 15) Tracking data (position and power trace over time) is not exported but can be accessed by clicking the button ***Export tracking data (M) to workspace*** in the navigation panel. This will save the *M* struct to the MATLAB workspace as shown in Figure S23.

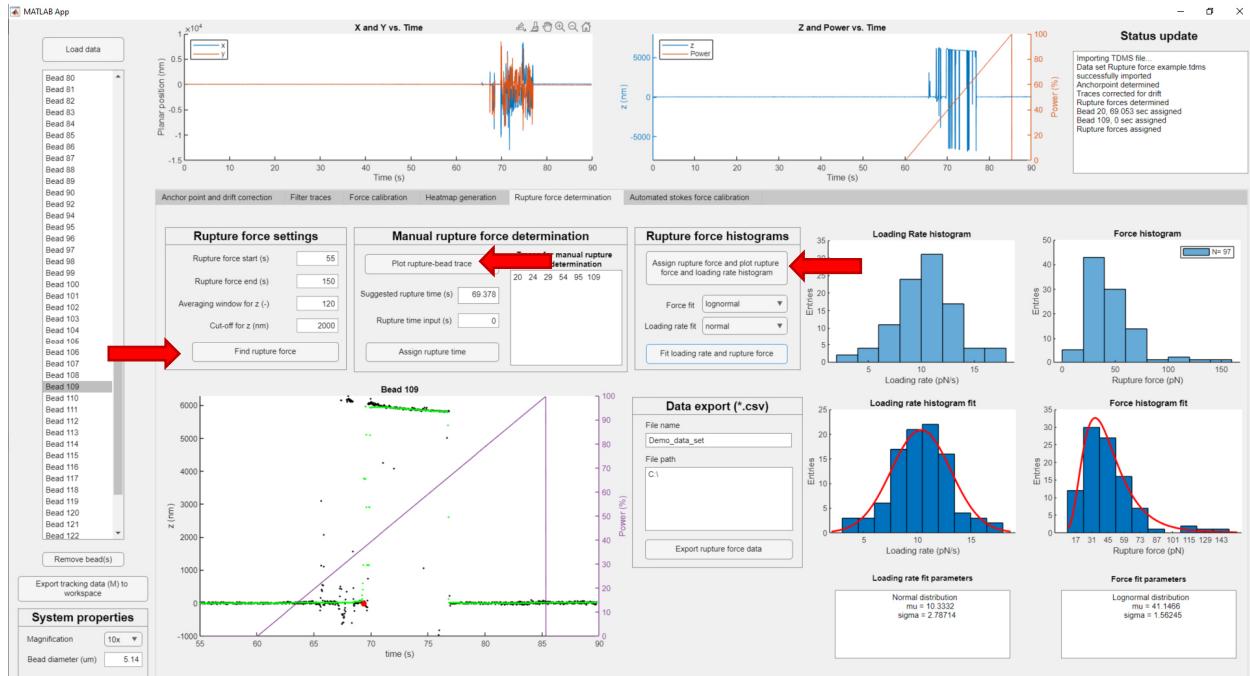


Figure S22: Rupture force determination tab of the developed GUI. The feature to manually assign rupture forces allows the user to collect more data per experiment while the plotting feature on the right side provides a quick overview of the results. After analysis, the rupture force data can be exported in csv format for subsequent analysis. The buttons Find rupture force, Plot rupture-bead trace and assigning rupture force and plotting histograms are indicated by red arrows.

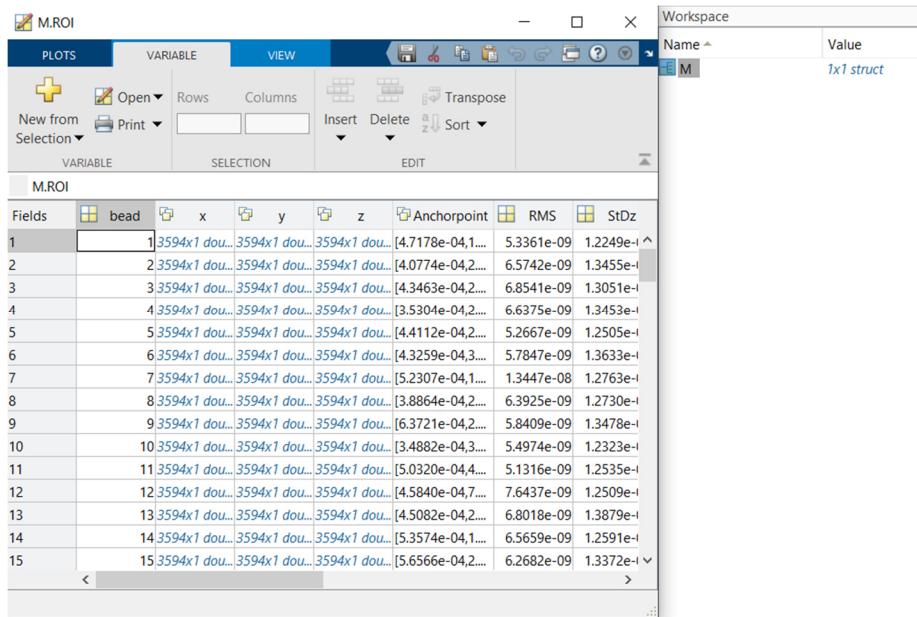


Figure S23: Screenshot of a data set (M struct) exported to MATLAB® workspace. The tracking data can be accessed under M.ROI.

References

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