Software Manual

MO.Control 2

Contents

The MO.Control 2 software is dedicated to running and analyzing MicroScale Thermophoresis (MST) experiments. It guides the user step-by-step through planning, setup and execution of experiments and helps to evaluate and analyze measured data. It also recommends assay improvement strategies and quantifies binding parameters such as dissociation constants (K_d) or EC_{50} values.

This manual explains the main functions integrated in the MO.Control 2 software. Additional and more detailed information can be found within the software itself. Due to the intuitive and self-explanatory design, the user is able to set up and perform MST experiments using MO.Control 2 without previous training.

1. System Requirements

If the necessary licenses have been purchased, MO.Control 2 software can be installed on additional computers for convenient data analysis and experimental planning. Computers must meet the following requirements:

Operating system:	Windows 7/10 (x86 or x64)
CPU:	Intel Core i5, 6th generation or higher
RAM:	8 GB or higher
Hard disk:	20 GB available space, SSD recommended
Display resolution:	Full HD @ 125 %
Software:	Microsoft .NET Framework 4.5.1or higher (included in installer of MO.Control 2 software)
Operating system language:	English or German

An external computer mouse is necessary to access all software features.

2. Installation, License Activation, and Software Updates

The software can be installed on any computer and will automatically start in trial mode, when opened for the first time. The trial version offers full functionality and has to be activated with a license key purchased from NanoTemper Technologies after 30 days.

To activate the license, navigate to the main menu and select *Licensing Status*. Please follow the instructions provided. One software license is valid for one account per computer and licenses can be transferred between computers. To transfer a license, please deactivate it first via the *Licensing Status* dialogue and reactivate it on the new computer.

When a software update is available, a green button will appear next to button on the top right corner of the software window. This feature is accessible only if your device is connected to a network.

3. General Layout and Features

After starting MO.Control 2, the user is prompted to either "start a new session" or to "browse previous sessions". A new session will create a .moc2 file in the specified location. Each .moc2 file can contain multiple experiments. Existing .moc2 files can be opened using "browse previous sessions", and further experiments can be added.

Next, the user is asked to choose the excitation color filter for the upcoming experiment. This will trigger the adjustment of some experimental parameters and recommendations at later stages. The excitation color will be set automatically if the connected Monolith instrument contains only one excitation color filter. If the user chooses to browse a previous session, the session's excitation color setting will automatically be applied. The excitation color can be changed at the start of each new experiment (see red highlight in **Figure 1**).

Protein labeling assistant, designed to help find best labeling strategy and labeling kit for the target protein, can be accessed by scanning the QR code (Figure 1).

3.1. Experimental Modes

In this screen, the user is also asked to choose the mode for the upcoming experiment. The options are (see **Figure 1**):

- **Pretest**: The *Pretest* routine guides you through basic tests that are recommended before conducting a binding experiment. This includes e.g. checking fluorescence after labeling and in LabelFree experiments. The *Pretest* is recommended when starting on a new or newly labeled target and allows to compare up to six different buffer conditions in a single experiment.
- **Binding Check**: The *Binding Check* routine guides you step-by-step through a quick and easy MST assay development set-up and analyzes whether a binding event can be detected (yes/no answer). The *Binding Check* allows to compare up to six different buffer conditions in a single experiment.
- **Binding Affinity**: The *Binding Affinity* routine provides detailed instructions for performing full, quantitative affinity measurements (K_d answer).
- **Expert Mode**: The *Expert Mode* gives you the freedom to use unconventional experimental setups, but does not offer features for instruction or automatic evaluation. Recommended only for advanced users.
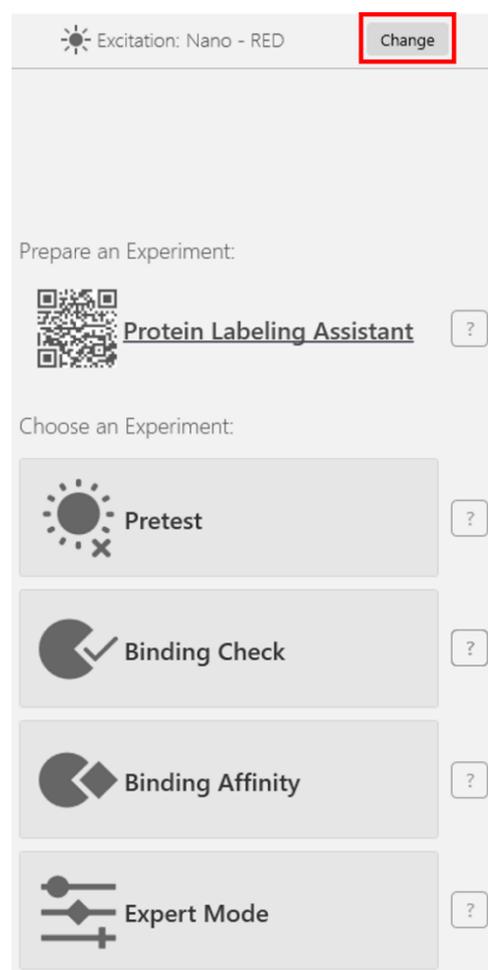

Figure 1: Initial setup of an experiment.

3.2. Session Overview and Guidance Panels

Throughout the software, the left and right panels will always remain visible. The *Session Overview* panel on the left lists all experiments in the .moc2 file and indicates their respective experimental modes. These experiments can be selected, renamed (by clicking the pen button) and removed (by clicking the *X* button). Use the *Save* button to save a file at any time. An asterisk in the software title bar indicates unsaved changes in the file; the user will be prompted to save changes when closing the software. New experiments with blank settings can be started from the *Session Overview*.

The right panel is called the *Guidance*. It is context-sensitive and always shows information that is relevant to the task performed in the main window. Clicking on the question mark buttons [?] will trigger item-specific information to be shown in the *Guidance*. Use this function if a term or a concept is unclear, or if you would simply like more information on a specific topic.

3.3. NanoPedia
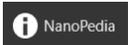
Clicking the [ⓘ NanoPedia] button in the top right corner of the software will open a separate window containing *NanoPedia*, an extensive knowledge base with background information concerning MicroScale Thermophoresis and further related topics (see **Figure 2**).

By default, the *NanoPedia* window is pinned, meaning it will always stay on top of the MO.Control 2 software window. Unpin it to trigger normal window behavior.

NanoPedia articles are sorted alphabetically. Use the magnifying glass button to search *NanoPedia* for a specific keyword.

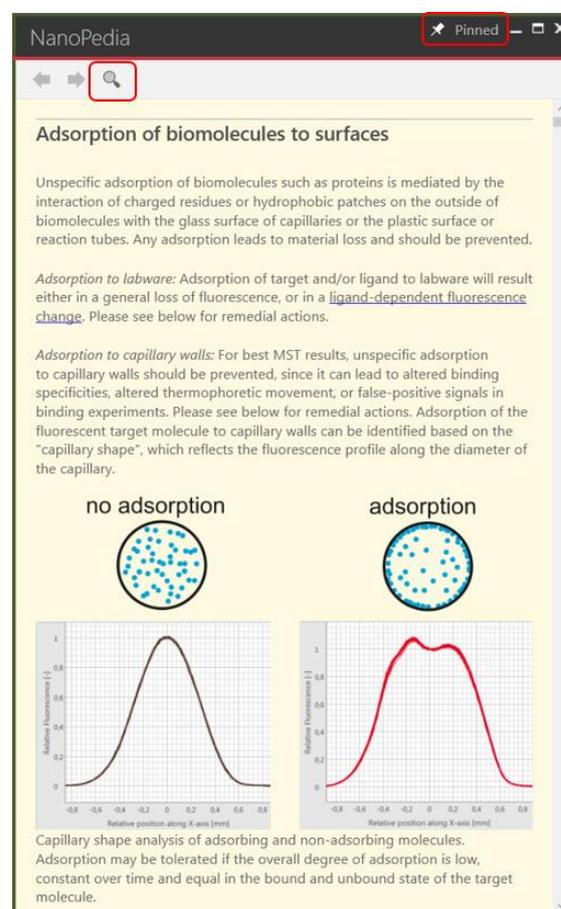Clicking on blue hyperlinks in the Guidance will open the corresponding *NanoPedia* entry.

Figure 2: NanoPedia knowledge base. See highlights for pinning the NanoPedia window or searching for specific key words.

3.4. Starting a New Experiment

There are several ways to start a new experiment. The first one is to start a new session (see section 3). The second one is to use the *New Experiment* button in the *Session Overview* panel (see section 3.2). In both cases a blank experiment not containing any user-supplied information will be started. The third option is available after an experiment is finished. On the bottom right of the *Results* overview page (see section 4.3), the software will suggest a suitable follow-up experiment (see **Figure 3**). Use the arrow button to expand and access different experimental mode options. An experiment started from here will always contain previous user-supplied information and settings.
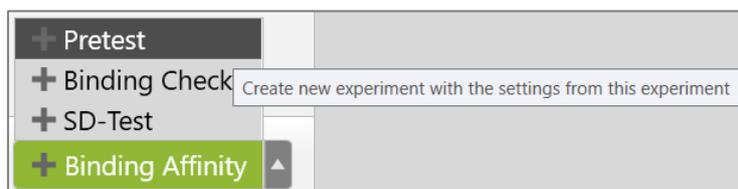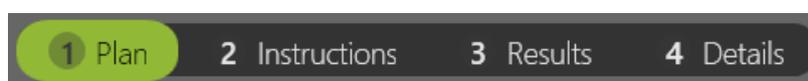

Figure 3: After an experiment finishes, start a new one directly from the *Results* page.

4. Running MST Experiments

4.1. Planning

Setting up a new experiment starts with the *Plan* page. While the exact setup of the *Plan* page is specific to the experimental mode, the general purpose is the same: to plan the current experiment. Information on interacting molecules, experimental conditions, affinities and concentrations can be entered. Green fields are mandatory, since their information is used to calculate pipetting steps on the *Instructions* page. Other information is conveniently stored with the experiment, making it easy to keep track of assay optimization steps and experimental conditions (see **Figure 4**). Some fields already contain recommendations. For example, depending on the type of detector used, a typical target concentration is suggested.

Click the question mark buttons to show explanatory information on individual items in the *Guidance* panel.

Figure 4: Information stored with a typical experiment. The information ribbon at the top of the window shows measurement meta-data and information on experimental conditions. From top left to right: experiment number, experimental mode, target concentration, highest ligand concentration in the assay, buffer, capillary type, detector type and excitation power, MST power, comments, type of instrument, experimental status and date.

The information on the *Plan* page can be altered even after an experiment is finished. To do this, click the *Alter Data* button. Concentration and K_d calculations on later pages will be updated accordingly.

Temperature Control

This top ribbon also contains the temperature controls if supported by the Monolith instrument (see **Figure 5**). By default, measurements on Monolith instruments are carried out at 25 °C. If a different temperature is needed, click the thermometer icon to open the temperature control, then choose the desired temperature using the arrow buttons. Click *Set* to start the temperature control.


Figure 5: Temperature controls. Left panel: click the thermometer icon to open the temperature control. Right panel: choose the desired temperature using the arrow buttons, then click *Set* to start the temperature control.

4.2. Pipetting
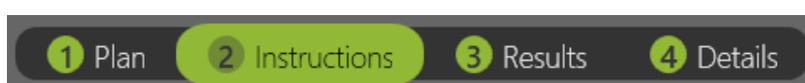
The *Instructions* page uses the information previously entered on the *Plan* page to give specific, step-by-step pipetting instructions using experiment-specific molecule names and concentrations. Of course, the instructions are adapted to the chosen experimental mode. After preparation is complete and samples are loaded into the instrument, start the measurement.

4.3. Results and Details
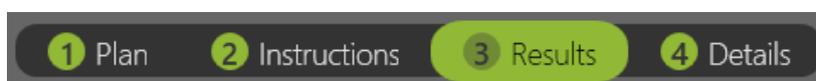
Data acquisition can be followed on the *Results* page while experiments are running. After the measurement is finished, the *Results* section summarizes the findings of an experiment. The measurement is assessed, and the data and conclusion are displayed here, along with recommendations for improving the assay. Depending on the experimental mode, the three panels on the *Results* page show different parameters. By clicking the *Review* buttons of the individual parameter panels, details concerning the respective parameter can be accessed (see sections 4.3.2 through 4.3.5).

A conclusion (e.g. a K_d or a definitive qualitative answer on binding) is given in the green box on the bottom left, if available. Recommendations for improving the assay are given on the bottom right. These recommendations are prioritized by relevance and should be considered one at a time. Use the *Guidance* panel and *NanoPedia* to get more information on assay optimization strategies by clicking question mark buttons and hyperlinks.

At the bottom of the *Results* page, click *Create Report* to create a PDF document containing all relevant information on the measurement for easy filing. The green button on the bottom right allows to start a new experiment. The software recommends an experimental mode; for example, if a *Binding Affinity* experiment was measured and needs improving, another *Binding Affinity* experiment will be suggested. By clicking on the button, a new experiment will be created and settings from the current experiment will be transferred. This is different from the *New Experiment* button in the Session Overview, which will open a new, blank experiment (see 3.2).

4.3.1. Quality Checks

The conclusion and consequential recommendations on the *Results* page are based on quality checks. These are computed automatically but can be overridden. Quality checks are used throughout the *Results* pages to visualize the results of checks performed on the measured data. Next to all quality checks is a question mark button providing additional information on the assessed parameter. Quality checks can have three different outcomes: positive, negative and inconclusive (see **Figure 6**). A negative quality check can be overridden if you disagree or if you think the detected issue is negligible. Override by clicking on the quality check button. A positive quality check can of course also be overridden. Note the small arrow indicating a user-made decision to override a result. This information will be stored in the file. An inconclusive quality check means that user input is needed because additional data is required. In these cases, the software will provide information on the necessary input and how to get it (see section 55).

Figure 6: Quality Checks. Left panel: possible results. Center panel: overriding a negative quality check. Right panel: Overriding a positive quality check. Note the small arrow indicating an overridden quality check.

A conclusion (e.g. a K_d or a definitive qualitative answer on binding) will only be given if all quality checks are positive. The final decision on all quality checks is left up to the user.

4.3.2. Capillary Scans

The *Capillary Scans* panel shows the fluorescence peaks recorded for all individual capillaries. The peak shape indicates whether the fluorescent molecule is adsorbing to the inside capillary wall, while the peak height indicates fluorescence intensity. Both parameters are important for a successful MST experiment and are assessed by the software automatically.

Capillary scans are recorded before and after the MST measurement and can be displayed either together or separately. Capillary peak overlays enable easy visual assessment of adsorption, for example of target alone compared to target in complex. In *Pretest* and *Binding Check* experiments, this overlay is done automatically.

Individual capillaries can be selected as outliers by clicking on the respectively numbered box below the image. Quality checks and conclusions will be updated accordingly. Mouse over a capillary peak to display its number.

In this section, the software automatically assesses the following parameters and display the results as quality check symbols (see 4.3.1):

- Initial fluorescence intensity of each capillary (peak height)
- Fluorescence homogeneity (whether capillaries containing identical target concentration show identical fluorescence intensities)
- Adsorption (peak shape)
- Changes in initial fluorescence induced by the addition of ligand

4.3.3. MST Traces

The *MST Traces* panel shows all traces recorded for the individual capillaries. The shape of the MST traces gives information on homogeneity and photostability of the sample. Both parameters are important for a successful MST experiment and are assessed by the software automatically.

In this section, the software automatically assesses the following parameters and displays the results as quality check symbols (see 4.3.1):

- Aggregation in the sample (shape of MST trace)
- Photobleaching behavior of the sample and whether it is changed by the addition of ligand (slope of the first seconds of the MST trace)

Individual capillaries can be selected as outliers by clicking on the respectively numbered box below the image. Quality checks and conclusions will be updated accordingly. Mouse over an MST trace to display its capillary number.

4.3.4. Signal-to-Noise Ratio (Binding Check)

For a qualitative answer on whether an interaction is taking place in the investigated sample, the MST signal of target is compared to the signal of complex (target + ligand). An interaction can only be assumed if the difference in the respective MST signals is large enough. The signal-to-noise ratio is used to evaluate the quality of the binding data. It is defined as the response amplitude divided by the noise of the measurement. A signal-to-noise ratio of more than 5 is desirable, while more than 12 reflects an excellent assay. The noise is defined as the average standard deviation of the mean of all points of target and complex, respectively, while the amplitude is the difference between the average complex and target signals.

Individual capillaries can be selected as outliers by clicking on the respectively numbered box below the image. Quality checks and conclusions will be updated accordingly. Mouse over a point to display its capillary number.

By default, the software will evaluate the measurement automatically. This means that the evaluation mode is chosen based on experimental parameters and to yield the best signal-to-noise ratio. To change the evaluation mode, deselect *Automatic Mode*, then choose an evaluation mode from the drop-down menu.

In this section, the software automatically assesses the following parameters and displays the results as quality check symbols (see 4.3.1):

- Response amplitude
- Signal-to-noise ratio

4.3.5. Dose Response (Binding Affinity)

The *Dose Response* analysis displays concentration dependent changes in the measurement parameter (usually the MST signal), which are used to calculate K_d or EC_{50} values. The signal of each capillary is plotted against the ligand concentration in the capillary.

In this section, the software automatically assesses the following parameters and displays the results as quality check symbols (see 4.3.1):

- Response amplitude
- Signal-to-noise ratio

The signal-to-noise ratio is used to evaluate the quality of the binding data. It is defined as the response amplitude divided by the noise of the measurement. A signal-to-noise ratio of more than 5 is desirable, while more than 12 reflects an excellent assay. The noise is defined as the average standard deviation of all points from the fitted curve, while the amplitude is the difference between the unbound state and the bound state, or the two plateaus of the fitted curve. Only if both parameters can be determined, and if the signal-to-noise ratio is deemed sufficient, a binding curve will be fitted and a K_d or EC_{50} will be derived.

Individual capillaries can be selected as outliers by clicking on the respectively numbered box below the image. Quality checks and conclusions will be updated accordingly. Mouse over a point to display its capillary number.

By default, the software will evaluate the measurement automatically. This means that the evaluation mode is chosen based on experimental parameters and to yield the best signal-to-noise ratio. To change the evaluation mode, deselect *Automatic Mode*, then choose an evaluation mode from the drop-down menu. The default fit model is the K_d model, which can also be changed here.

5. Specificity Tests

When a ligand-induced fluorescence change is detected in an experiment, an additional specificity test is required to enable unambiguous interpretation of MST data and to distinguish between specific and non-specific effects. For samples labeled via the His-tag and RED-tris-NTA, it is recommended to perform an ECP-Test (EDTA/Control Peptide Test). For other samples, please perform an SD-Test (SDS denaturation test).

The automatic assessment performed by the MO.Control 2 software will identify these cases. It will suggest the appropriate specificity test and provide step-by-step instructions.

6. Data Visualization and Export

At the bottom of the *Results* page, click *Create Report* to create a PDF document containing all relevant information on the measurement for easy filing. This PDF report contains experimental settings, concentrations, user-supplied information and comments, and experimental results (capillary scans, MST traces and dose response/signal-to-noise ratio plots).

On each *Results Details* page, the image can be exported as a .png or .svg file by clicking on the *Export* button above the image.

To access the raw measurement data, open the measurement file in MO.Affinity Analysis 3 and export from there. Please refer to the MO.Affinity Analysis 3 user manual for more information.

All graphs can be zoomed and adjusted for optimal visualization. Check the *Auto Zoom* checkbox to adjust all data in the chart to the chart size. Zooming in-and-out of the chart is performed by scrolling the mouse wheel. Horizontal or vertical zooming can be performed by pressing shift or control on the keyboard while scrolling, respectively. Click and hold the mouse wheel and move the mouse to drag the chart (see **Figure 7**).
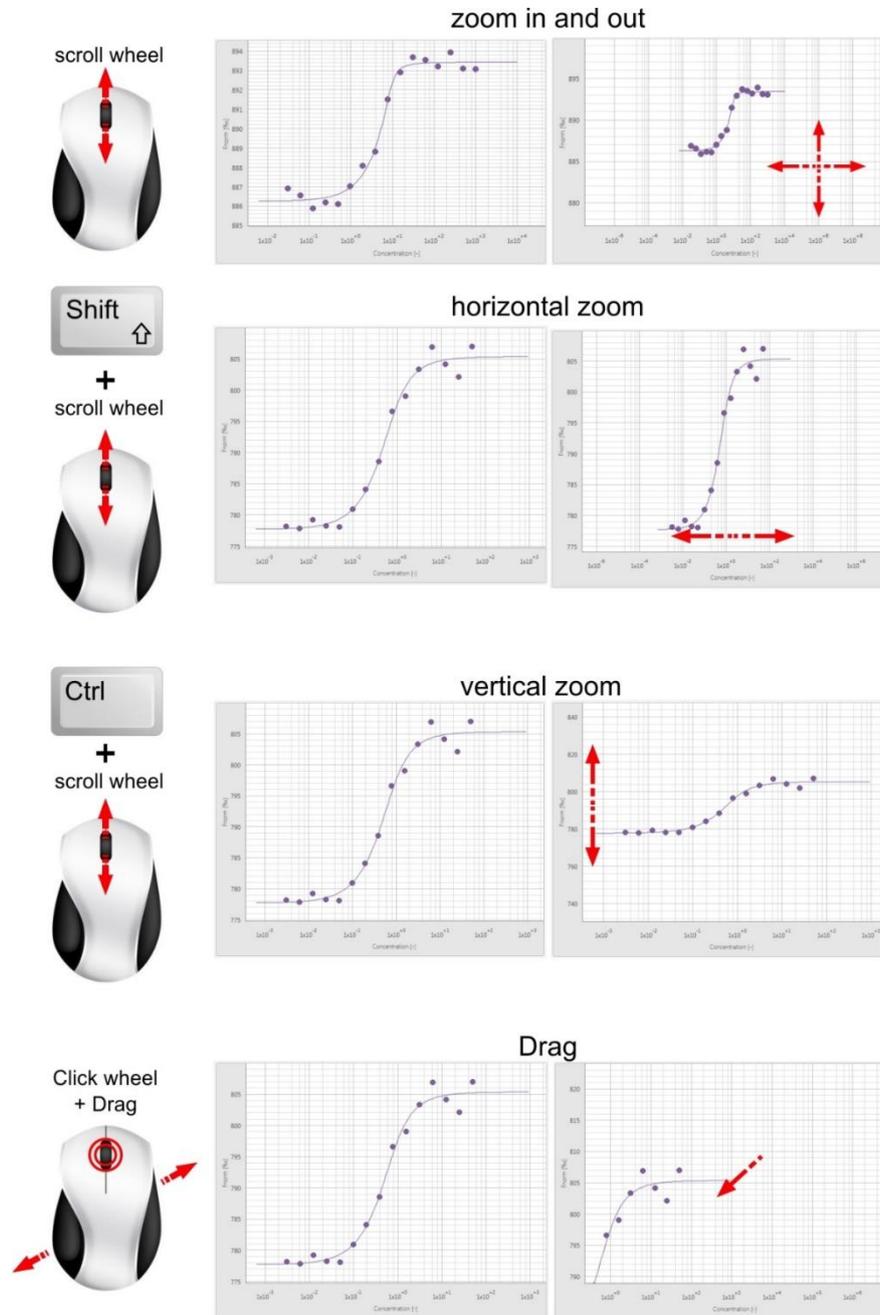

Figure 7: Mouse control of chart visualization.

7.Patents and intellectual property

Monolith, MST and TRIC technology are patent protected, especially by the following patents, US8431903B2, US8853650B2, US10345312B2, US8741570B2 including their application and registration in different other countries.

Contact

NanoTemper Technologies GmbH

Global headquarters :
Floessergasse 4
81369 Munich
Germany

Phone: +49 (0) 89 4522895 0
Fax: +49 (0) 89 4522895 60

info@nanotempertech.com
nanotempertech.com

V01_2012-10-15