User Manual
Monolith® NT.115

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Safety Considerations

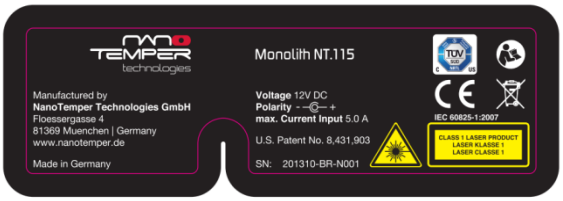
To ensure operation safety, this instrument must be operated correctly and maintained according to a regular schedule. Carefully read to fully understand all safety precautions in this manual before operating the instrument. Please take a moment to understand what the signal words **WARNING!**, **CAUTION** and **NOTE** mean in this manual.

Safety symbols

WARNING!	A **WARNING!** indicates a potentially hazardous situation which, if not avoided, could result in serious injury or even death.
CAUTION	A **CAUTION** indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury. It may also be used to alert against damaging the equipment or the instrument. Do not proceed beyond a **WARNING!** or **CAUTION** notice until you understand the hazardous conditions and have taken the appropriate steps.
NOTE	A **NOTE** provides additional information to help the operator achieve optimal instrument and assay performance.

Read manual label. This label indicates that you have to read the manual before using the instrument. This label is positioned at the back of the device.

Warning symbol. This symbol indicates laser radiation, it is put on products which have a laser built in. This warning label is positioned at the back of the device.



Identification label. This label is positioned at the back/ rear panel of the device.

WARNING! Only operate the Monolith NT.115 instrument with the supplied external power supply (SPU63-105, Sinpro Electronic Co Ltd or Ael60US12, XP Power LLC or GS90A12-P1M, MeanWell or GS60A12-P1J, MeanWell). Only use the supplied cables and plugs. If not doing so you risk electric shock and fire.

WARNING! Do not operate the Monolith NT.115 with substances and under conditions which cause a risk of explosion, implosion or release of gases. Only operate the Monolith NT.115 with aqueous solutions.

CAUTION Ensure that the power plug of the external power supply is easily accessible. The Monolith NT.115 instrument has to be installed in a way that it does not hinder the access to the external power supply and its power plug.

CAUTION The weight of the Monolith NT.115 instrument is approx. 24 kg, do not move the instrument alone (two persons required for transport). If you move the instrument alone it poses a risk of personal injury or damage to the instrument.

CAUTION The manual opening of the instrument is not allowed. Manual opening poses a risk of personal injury or damage to the instrument. Contact NanoTemper Technologies service personnel if you need to open the instrument.

CAUTION Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous invisible radiation exposure. The instrument contains an IR-laser module (invisible laser radiation Class 3B according to IEC 60825-1: 2007). Lasers or laser systems emit intense, coherent electromagnetic radiation that has the potential of causing irreparable damage to human skin and eyes. The main hazard of laser radiation is direct or indirect exposure of the eye to thermal radiation from the infrared B spectral regions (1,400 nm – 3,000 nm). Direct eye contact can cause corneal burns, retinal burns, or both, and possible blindness. Do not open the instrument while it is switched on. Manual opening of the instrument during operation poses a risk of personal injury or damage to the instrument. When the instrument is used as intended it is LASER CLASS 1.

CAUTION Mechanical moving parts within the instrument can pinch or injure your hands or fingers. Do not touch the instrument while parts are moving.

CAUTION The instrument contains a temperature regulator to control the sample temperatures. Some accessible parts of the instrument can reach temperatures up to 50°C. Avoid touching the temperature controlled parts of the instrument for a longer time when you have set the temperature controller to high temperatures.

CAUTION Only NanoTemper Technologies staff is allowed to service and open the instrument. Turn off the power switch and unplug the power cord before servicing the instrument, unless otherwise noted. Connect the equipment only to the delivered power source. Do not use extension cords. Have an electrician immediately replace any damaged cords, plugs, or cables. Not doing so poses a risk of personal injury or damage to the instrument.

CAUTION Only open the sample loading site when moving parts inside the instrument are at rest. Do not insert or remove a sample while linear actuator, laser or LED is at work. Moving parts in the instrument can be harmful.

CAUTION Do not use the instrument in a cold room.

CAUTION Turn off the main circuit breaker in the back of the chassis, when the instrument is not in use.

CAUTION Do not use ethanol or other types of organic solvents to clean the instrument as they may remove the instrument paint.

CAUTION Use the instrument only for biomolecule analytics with aqueous solutions and do not open the instrument on other site than for sample loading.

CAUTION Only use aqueous sample for analysis in the instrument.

CAUTION Do not use the instrument with hazardous substances or substances/materials which pose a risk of infections.

Regulatory Statement

The following safety and electromagnetic standards were considered:

- IEC 61010-1:2001 Safety requirements for electrical equipment for measurement, control and laboratory use. Part 1 General Requirements
- IEC 61010-2-010:2005 Safety requirements for electrical equipment for measurement, control and laboratory use. Part 2-010: Particular requirements for laboratory equipment for the heating of materials.
- IEC 61010-2-081:2001 Safety requirements for electrical equipment for measurement, control and laboratory use. Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes.
- IEC 60825 Laser safety.
- Complies with 21 CFR 1040.10 and 1040.11 except for deviations pursuant to Laser Notice No. 50, dated June 24, 2007
- IEC 61326-1:2006 EMC, Electrical equipment for measurement, control and laboratory use – EMC requirements.
- IEC 61000-3-2:2006 EMC, Limits for harmonic current emissions (equipment input current up to and including 16A per phase).
- IEC 61000-3-3:2008 EMC, Limits

Specifications

Input external power supply: 90–264 VAC ± 10% 47–63 Hz, 230 VA max
Output external power supply: 12 VDC, 5.0 A max, 2.4 A typical

Electrical input Monolith NT.115 instrument: 12 VDC, 5A

Environmental
Operating temperature 15 – 30°C (Indoor use only)
Storage temperature -20 – 30°C
Humidity 0–80%, noncondensing
Operating altitude max 2000m

Monolith NT.115 size
Width 33 cm (13”)
Height 45 cm (17.5”)
Depth 51 cm (20”)
Weight 24 kg (51 lbs) net

Power supply size
Width 10.6 cm (4.2”)
Height 3 cm (1.2”)
Depth 6.5 cm (2.6”)
Power supply weight 0.23 kg (0.51 lbs) net max

IR laser
Wavelength: 1475 nm +/- 15 nm
Power: 120 mW max.

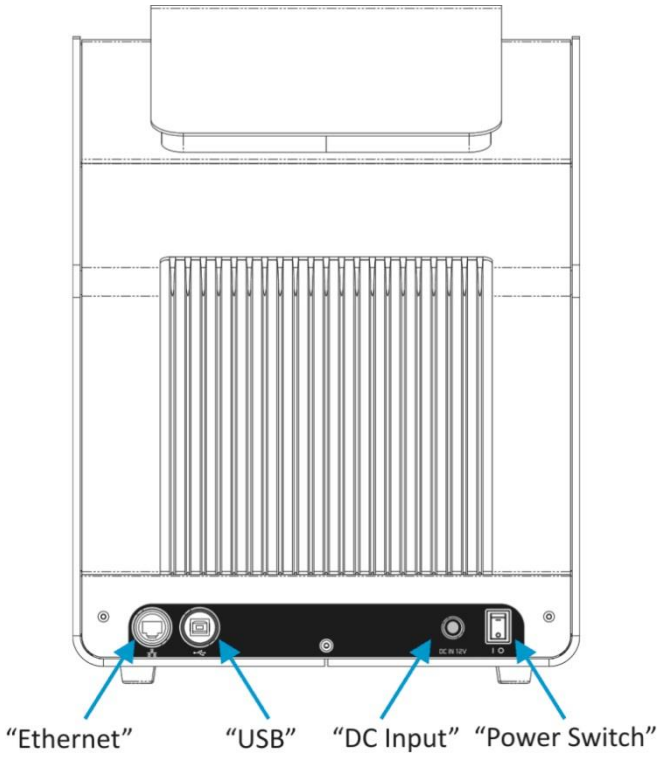
Monolith NT.115 laser classification
Device is LASER PRODUCT CLASS 1

Temperature control
Range: 22°C – 45°C (at Room Temperature 25°C)
Accuracy: +/- 0.3°C

Noise level of Monolith NT.115 instrument: max. 64 dB(A)

Connections

All ingoing and outgoing connections can be found at the rear panel of the instrument.



Name	Function
Ethernet	Connector for connecting the Ethernet cable to the PC/notebook.
USB	Connector for connecting the USB cable to the PC/notebook. Only used for service by NanoTemper Technologies service.
Power Switch	When the switch is turned on (position "I"), the instrument is switched on.
DC Input	The connector to the external power supply. Connect with the external power supply.

Preface

This manual is your guide for using the Monolith NT.115 and doing MicroScale Thermophoresis (MST) measurements. It instructs first-time users on how to use the instrument, and serves as a reference for experienced users.

Before using the Monolith NT.115 instrument, please read this instruction manual carefully, and make sure that the contents are fully understood. This manual should be easily accessible to the operator at all times during instrument operation. When not using the instrument, keep this manual in a safe place. If this manual is lost, order a replacement from NanoTemper Technologies GmbH, by contacting your local NanoTemper Technologies representative.

Notices

1. NanoTemper Technologies shall not be held liable, either directly or indirectly, for any consequential damage incurred as a result of product use.
2. Prohibitions on the use of NanoTemper Technologies software
    - Copying software for other than backup
    - Transfer or licensing of the right to use software to a third party
    - Disclosure of confidential information regarding software
    - Modification of software
    - Use of software on multiple workstations, network terminals, or by other methods
3. The contents of this manual are subject to change without notice for product improvement.
4. This manual is considered complete and accurate at publication.
5. This manual does not guarantee the validity of any patent rights or other rights.
6. If a NanoTemper Technologies software program has failed causing an error or improper operation, this may be caused by a conflict from another program operating on the notebook (PC). In this case, take corrective action by uninstalling the conflict product(s).
7. NanoTemper Technologies is a registered trademark of NanoTemper Technologies GmbH in Germany and other countries.

Limited Warranty

Products sold by NanoTemper Technologies, unless otherwise specified, are warrantied for a period of one year from the date of shipment to be free of defects in materials and workmanship. If any defects in the product are found during this warranty period, NanoTemper Technologies will repair or replace the defective part(s) or product free of charge.

THIS WARRANTY DOES NOT APPLY TO DEFECTS RESULTING FROM THE FOLLOWING:

1. IMPROPER OR INADEQUATE INSTALLATION.
2. IMPROPER OR INADEQUATE TRANSPORT.
3. IMPROPER OR INADEQUATE OPERATION, MAINTENANCE, ADJUSTMENT OR CALIBRATION.
4. UNAUTHORIZED MODIFICATION OR MISUSE.
5. USE OF UNAUTHORIZED CAPILLARIES AND CAPILLARY TRAYS.
6. USE OF CONSUMABLES, DISPOSABLES AND PARTS NOT SUPPLIED BY AN AUTHORIZED NANOTEMPER TECHNOLOGIES DISTRIBUTOR.
7. CORROSION DUE TO THE USE OF IMPROPER SOLVENTS, SAMPLES, OR DUE TO SURROUNDING GASES.
8. ACCIDENTS BEYOND NANOTEMPER TECHNOLOGIES'S CONTROL, INCLUDING NATURAL DISASTERS.

This warranty does not cover consumables like capillaries, reagents, labeling kits, and the like.

The warranty for all parts supplied and repairs provided under this warranty expires on the warranty expiration date of the original product. For inquiries concerning repair service, contact NanoTemper Technologies after confirming the model name and serial number of your NanoTemper Technologies instrument.

Installation Requirements

To ensure operation safety, observe the following conditions:

1. Only operate the Monolith NT.115 instrument with the supplied external power supply (SPU63-105, Sinpro Electronic Co Ltd or Ael60US12, XP Power LLC or GS90A12-P1M, MeanWell or GS60A12-P1J, MeanWell).
2. Only connect the external power supply of Monolith NT.115 to an electrical socket containing a protective conductor terminal.
3. Ensure that the power plug of the external power supply is easily accessible. The Monolith NT.115 instrument has to be installed in a way that it does not hinder the access to the external power supply and its power plug.
4. Only operate the instrument with the delivered PC (notebook).
5. Only operate the instrument with original Monolith NT.115 capillary trays.
6. The maximum noise level of the instrument is 64 dB(A). Only operate the instrument in an environment where this noise level is appropriate.
7. Operate the instrument in a temperature range of 15 – 30°C.
8. Operate the instrument in a humidity range of 0 – 80 % (RH). If ambient humidity exceeds 80 % (RH), condensation may deteriorate optical components.
9. Operate the instrument in an atmospheric pressure range of 800 – 1060 hPa.
10. Do not operate the Monolith NT.115 under conditions which pose a risk of explosion, implosion or the release of gases.
11. Avoid strong magnetic fields and sources of high frequency. The instrument may not function properly when near a strong magnetic field or high frequency source.
12. Avoid vibrations from vacuum pumps, centrifuges, electric motors, processing equipment and machine tools.
13. Avoid dust and corrosive gas. Do not install the instrument where it may by exposed to dust, especially in locations exposed to outside air or ventilation outlets.
14. To clean the instrument, only use water.
15. Do not install the instrument in a location where it may be exposed to direct sunlight.
16. Install the instrument in a horizontal and stable position. (This includes a table, bench or desk upon which the instrument is installed).
17. Ensure that no air conditioner blows air directly onto the instrument. This may prevent stable measurements.
18. Install the instrument in a location that allows easy access for maintenance.

Note: *The above conditions do not guarantee optimal performance of this instrument.*

Installation and Connecting Cables

Preparation

Prepare a table which can bear a weight of about 50 kg and has a free area of 80 cm (width) x 50 cm (depth).

Put the Monolith NT.115 instrument and the PC/notebook on this free area.

CAUTION The weight of the Monolith NT.115 instrument is approx. 24 kg, do not move the instrument alone (two persons required for transport/movement). If you move the instrument alone it poses a risk of personal injury or damage to the instrument.

Connecting the power supply and the Monolith NT.115

Confirm that the power switch of the Monolith NT.115 instrument is off (power switch is at the back, left of the instrument).

WARNING! Only operate the Monolith NT.115 instrument with the external power supply provided (SPU63-105, Sinpro Electronic Co Ltd or Ael60US12, XP Power LLC or GS90A12-P1M, MeanWell or GS60A12-P1J, MeanWell). Only use the supplied cables and plugs. If not doing so, you risk electric shock and fire.

Connect the external power supply to the electrical socket. Then connect the power supply to the Monolith NT.115 instrument.

CAUTION Ensure that the power plug of the external power supply is well accessible. The Monolith NT.115 instrument has to be installed in a way that it does not hinder the access to the external power supply and its power plug.

Connect the Monolith NT.115 instrument to the notebook by using the supplied network cable.

Switch on the Monolith NT.115 and the notebook.

Maintenance and Operation

Pay attention to the instrument operating environment and always keep it clean so that the instrument can be used in a stable condition over a long period. Do not place anything on top of the instrument.

Cleaning the Monolith NT.115 instrument

Switch off the instrument and remove the power plug of the external power supply from the electrical socket. Only use a dry cloth or a cloth dampened with water for cleaning the instrument.

CAUTION Do not use ethanol or other types of organic solvents to clean the instrument as they may remove the instrument paint.

Transporting the Monolith NT.115 instrument

Switch off the instrument and remove the power plug of the external power supply from the electrical socket. Do not carry the instrument alone, two people are needed for transportation.

CAUTION The weight of the Monolith NT.115 instrument is approx. 24 kg, do not move the instrument alone, two persons required for transport/movement. If you move the instrument alone it poses a risk of personal injury or damage to the instrument.

Malfunction

In case of a malfunction switch off the Monolith NT.115 instrument and wait for five minutes, then switch on the instrument again. If the malfunction persists switch off the device, unplug the power cable and contact the NanoTemper Technologies customer support.

Repairing the Monolith NT.115 instrument

Do not try to repair the instrument. Contact the NanoTemper Technologies customer support for instrument repairs.

CAUTION The manual opening of the instrument is not permitted. Manual opening poses a risk of personal injury or damage to the instrument. Contact NanoTemper Technologies customer support staff if you need to open the instrument.

Waste Treatment

Waste treatment is your own responsibility. You must hand the instrument to a company specialized in waste recovery. Do not dispose the instrument in a litter bin or at a public waste disposable site. For detailed information please contact the NanoTemper Technologies service.

1. Introduction to MicroScale Thermophoresis (MST)

MicroScale Thermophoresis™ (MST) provides an easy, fast and precise approach to quantify biomolecular interactions. It allows for the detection of a wide range of interaction types, ranging from ion and fragment binding up to interactions of macromolecular complexes such as liposomes or ribosomes [1-3]. The main key feature of MST is the capability to work under close-to-native conditions: Measurements are performed immobilization-free and in any buffer, or even in complex bioliquids such as serum and cell lysate. In addition, the method combines straight forward sample preparation, low sample consumption of less than 4 µl per sample at nM concentration, and a large dynamic range with dissociation constants from the sub-nM to mM range. The NanoTemper Technologies Monolith® instruments are maintenance-free, have low running costs and use an intuitive software user interface with provides guided assay development and automated data analysis, making MST a one-of-a-kind, low-hurdle biophysical technology for every lab.

1.1. Method

The Monolith instruments analyze MST signals for rapid and easy determination of affinity constants. MST is based on the generation of a locally restricted and highly precise temperature change in a sample by infrared (IR) laser light, which cannot be achieved by conventional heating procedures using e.g. thermal elements. Changes in sample fluorescence upon activation of the IR laser are monitored to characterize and quantify binding events. The response of the sample fluorescence upon IR laser activation is based on distinct physical principles which are described in the following section.

1.1.1. Underlying physical principles

MicroScale Thermophoresis (MST) is a biophysical technique that measures the strength of the interaction between two molecules by detecting a variation in the fluorescence signal of a fluorescently labeled or intrinsically fluorescent target as a result of an **IR-laser induced temperature change**. The range of the variation in the fluorescence signal correlates with the binding of a ligand to the fluorescent target.

Two major factors contribute to the variation in the fluorescence signal:

1- TRIC (**Temperature Related Intensity Change**) - An effect where the fluorescence intensity of a fluorophore is temperature dependent. The extent of the temperature dependence is strongly related to the chemical environment of the fluorophore, which can be changed by the binding of a ligand to the target.

2- **Thermophoresis** -An effect where the movement of fluorescent molecules along temperature gradients results in a quantifiable change in their local concentration and therefore of the observed fluorescence. The extent of the concentration change depends on the molecule's overall properties like size, charge and conformation.

Both TRIC and thermophoresis are influenced by binding events and therefore contribute to the overall recorded MST signal.

In some cases, the fluorescence of the target molecule changes upon ligand binding even in the absence of an IR-induced temperature gradient. These cases can be analyzed using the **Fluorescence Analysis Mode**. The phenomenon is referred to as ligand-induced fluorescence quenching or enhancement and is typically caused by changes in target conformation or binding of the ligand in proximity to the target's fluorophore(s). Since the fluorescence detection in all Monolith® instruments is highly precise, the change in target fluorescence upon ligand binding can be directly used to calculate binding affinities without application of the IR laser.

1.1.2. MST Data Analysis

MST experiments can be carried out at different MST intensities, which are defined by the power of the IR-laser and thus the extent of the temperature change. The higher the MST intensity, the faster and larger is the temperature increase. During an MST experiment, the sample fluorescence is recorded, starting with a 3 sec period at ambient temperature to monitor steady-state fluorescence, followed by IR-laser activation for a defined *MST-on* time. For data analysis, the change in sample fluorescence upon IR-laser activation is monitored by calculating the ratio between the fluorescence after a given *MST-on* time (F_1) and the fluorescence prior to IR laser activation (F_0).

1.1.3. Calculation of normalized fluorescence and binding affinities

The observed change in fluorescence after IR laser activation stems from a change in fluorescence due to TRIC and a change in the concentration of fluorophores due to MST. The overall change in fluorescence after IR laser activation can thus be expressed as

$$\frac{\partial}{\partial T}(cF) = F\frac{\partial c}{\partial T} + c\frac{\partial F}{\partial T}$$

Where $c\frac{\partial F}{\partial T}$ describes changes in fluorescence due to TRIC. The thermophoretic change in concentration is described by $F\frac{\partial c}{\partial T} = -S_T c$. The Soret coefficient, S_T, describes the percentage of the concentration change per Kelvin [5].

Measured fluorescence values are displayed as normalized fluorescence (F_{norm}) which relates to the fluorescence values prior (F_0) to and after (F_1) IR laser activation:

$$F_{norm}=F_1/F_0$$

$$\mathrm{F_{norm}} = (1\text{-x})\mathrm{F_{norm}^{[A]}} + \mathrm{xF_{norm}^{[AT]}}$$

The latter equation describes the contribution of the bound and unbound state of a fluorescent target molecule (A) to the F_{norm} signal. $F(A)_{norm}$ is the contribution of the unbound fluorescent molecule A, $F(AL)_{norm}$ is the contribution of the complex of the fluorescent molecule A and its interacting ligand L, and x is the fraction of fluorescent molecules that formed the complex. Upon binding of a ligand to the target molecule, changes in the MST signal are quantified based on the altered MST signal of the target-ligand complex. By analyzing a series of samples with an increasing ligand concentration at constant target concentration, this change in MST signal, expressed as ΔF_{norm}, can be used to calculate changes in the fraction of bound target molecule to derive binding affinities.

The dissociation constant, K_d, is obtained by fitting a dose-response curve to a plot of F_{norm} vs ligand concentration. The K_d is calculated from the law of mass action, which is defined as

$$K_d=[A]*[L]/[AL]$$

where [A] is the concentration of free fluorescent molecule, [L] the concentration of free ligand and [AL] is the concentration of the complex of A and L. The free concentrations of A and L are $[A]=[A_0]-[AL]$ and $[L]=[L_0]-[AL]$, respectively. $[A_0]$ is the known concentration of the fluorescent molecule and $[L_0]$ is the known concentration of added ligand. This leads to a quadratic fitting function for [AL]:

$$[AL]=1/2*(([A_0]+[L_0]+K_d)-(([A_0]+[L_0]+K_d)^2-4*[A_0]*[L_0])^{1/2})$$

The concentration of fluorescent molecule $[A_0]$ is kept constant during the experiments and the concentration of ligand $[L_0]$ is varied in a dilution series. The signal obtained in the measurement directly corresponds to the fraction of fluorescent molecules that formed the complex $x=[AL]/[A_0]$, which can be easily fitted with the derived equation to obtain K_d.

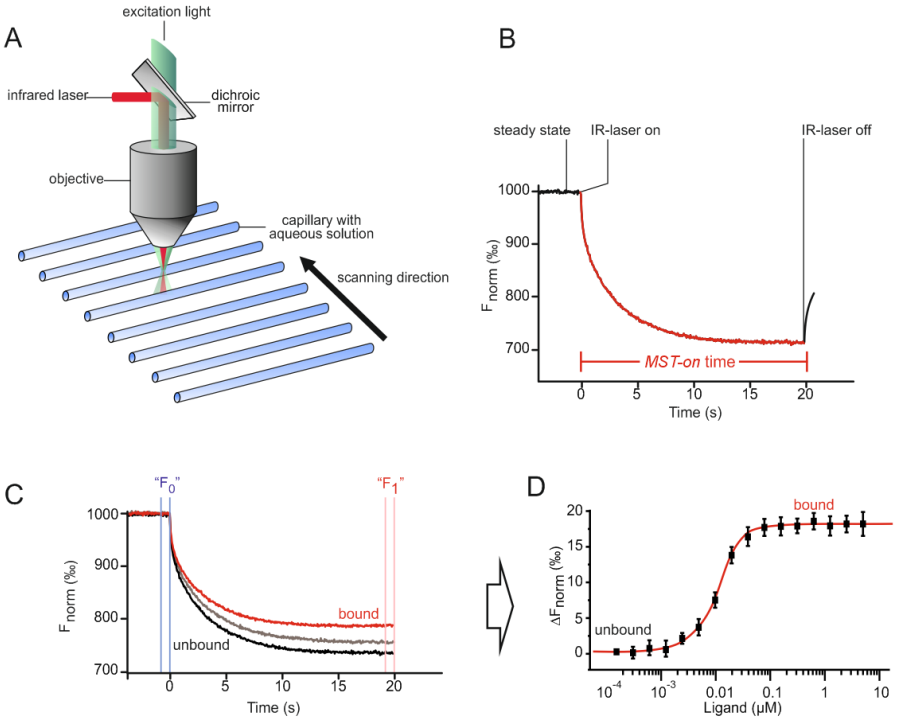


Figure 1: MST setup and experiments. (A) Schematic representation of MST optics. MST is measured in capillaries with a total volume of down to 4 µl. The fluorescence within the capillary is excited and detected through the same objective. A focused IR laser is used to locally heat a defined sample volume. The MST response of fluorescent molecules within the temperature gradient are detected. (B) Typical signal of an MST trace. Initially, the molecules are homogeneously distributed and a constant ‘‘initial fluorescence’’ is detected. After activation of the IR laser, a drop in fluorescence is observed, which corresponds to TRIC triggered by the fast temperature change and thermophoretic movement of the fluorescent molecules out of the heated sample volume. After deactivation of the IR laser, a fluorescence increase occurs, caused by the ‘backdiffusion’ of molecules and a decrease in sample temperature. MST, MicroScale Thermophoresis; IR, infrared. (C) Typical binding experiment. The MST signal of a fluorescent molecule (black trace; ‘‘unbound’’) changes upon binding to a non-fluorescent ligand (grey trace, intermediate state; red trace, ‘‘bound’’), resulting in different traces. (D) For analysis, the change in MST signal is expressed as the change in the normalized fluorescence (ΔF_{norm}), which is defined as F_1/F_0. Titration of the non-fluorescent ligand results in a gradual change in MST, which is plotted as ΔF_{norm} against the ligand concentration to yield a dose-response curve, which can be fitted to derive binding constants.

1.1.4. Software

MST experiments are analyzed using the dedicated **MO.Control** and **MO.Affinity Analysis Software**, using the above equations. Prior to determining binding affinities, the MO.Control software performs several **automated quality checks** of the samples to detect protein adsorption, aggregation, or other irregularities. Based on the results, a **guided assay development algorithm** provides detailed instructions on how to efficiently improve assay quality (if necessary). Moreover, the analysis algorithms automatically determine optimal analysis settings, with the aim of minimizing exposure times of biomolecules to the IR laser and to limit the temperature increase to < 10 °C. The optimal binding signal is derived based on the identification of the best signal-to-noise ratio at the shortest possible MST-on times.

1.2. Literature

1. Jerabek-Willemsen, M., et al., MicroScale Thermophoresis: Interaction analysis and beyond. Journal of Molecular Structure, 2014(0).
2. Seidel, S.A.I., et al., Label-Free Microscale Thermophoresis Discriminates Sites and Affinity of Protein–Ligand Binding. Angewandte Chemie International Edition, 2012. 51(42): p. 10656-10659.
3. Wienken, C.J., et al., Protein-binding assays in biological liquids using microscale thermophoresis. Nat Commun, 2010. 1: p. 100.
4. Duhr, S. and D. Braun, Why molecules move along a temperature gradient. Proc Natl Acad Sci U S A, 2006. 103(52): p. 19678-82.
5. Baaske, P., et al., Optical thermophoresis for quantifying the buffer dependence of aptamer binding. Angew Chem Int Ed Engl, 2010. 49(12): p. 2238-41.

2. Monolith® NT.115 Instrument

2.1. Models and Most Common Fluorophores

Fluorophore	Excitation [nm]		NT.115 Blue/Green	NT.115 Blue/Red	NT.115 Green/Red	NT.115 Pico	Emission [nm]
BCECF	480						525
GFP	488						507
NT-495 (BLUE)	493						521
Fluorescein (FITC)	495						519
Alexa488	495						519
YFP	514						527
Alexa532	530						555
TAMRA	546						579
Cy3	550						570
RFP	555						584
NT-547 (GREEN)	557						574
Alexa546	560						572
Cy5	649						670
NT-647 (RED)	650						670
Alexa647	652						668

Note: *Excitation and emission wavelengths can vary depending on the environment (buffer, pH). Colors are only a very rough approximation. The minimum concentration for use in NT.115 series instruments can vary.*

2.2. Application Range

The Monolith NT.115 allows measurement of the binding of virtually all biomolecules over a large range of binding affinities (K_d = 1 nM - 500 mM).

2.3. Sensitivity

The Monolith NT.115 can measure as little as the binding of single ions (40 Da) or small molecules (300 Da) to a target as well as the binding of large complexes such as ribosomes (2.5 MDa).

2.4. Sample Consumption

The Monolith NT.115 requires minimal sample material: typically 1 -10 nM of the labeled interaction partner and a dilution series of the unlabeled ligand starting about 20-fold above the expected dissociation constant. For standard applications, 4 µl of sample material is loaded in the capillary.

2.5. Capillary Format

The capillary format used for the Monolith NT.115 is inexpensive, easy to handle and offers maximum flexibility in the experiment scale. The standard sample tray format allows automatic processing of up to 16 capillaries (e.g. for a detailed K_d analysis).

2.6. Close-to-Native Conditions

The Monolith NT.115 can monitor the binding affinity between all kinds of biomolecules under close-to-native conditions:

- immobilization-free
- in a solution of choice, ranging from standard buffers to complex bioliquids including blood serum, cell lysates, etc.

2.7. Dedicated MO.Control Software and MO.Affinity Analysis Software

The Monolith NT.115 is supported by software for instrument control and data analysis. For more detailed information please refer to the corresponding software manuals

3. Preparation of MST Experiments

3.1. Control Kits - Performing Standard Experiments

Control Kits containing standardized sample material are recommended

- when using the Monolith NT.115 instrument for the first time
- for monitoring the correct performance of the Monolith NT.115
- for training of new laboratory members

Optimized Control Kits for use in MicroScale Thermophoresis are available either with red- or green-fluorescently labeled biomolecules.

The kits contain a fluorescently labeled DNA molecule, unlabeled binding partner and buffer.

Cat #	Monolith Control Kits	Reactions
MO-C030	Monolith™ NT Control Kit RED Molecular Interaction Control Kit for Monolith™ NT.115 BLUE/RED or Monolith™ NT.115 GREEN/RED	5
MO-C031	Monolith™ NT Control Kit GREEN Molecular Interaction Control Kit for Monolith™ NT.115 BLUE/GREEN or Monolith™ NT.115 GREEN/RED	5

3.2. Selecting the Right Monolith Capillary

NanoTemper Technologies offers coated and uncoated special capillaries with high-precision inner diameter, best glass quality and verified functionality. Our capillaries are especially developed for usage with NanoTemper Technologies Monolith devices. It is highly recommended to use NanoTemper Technologies capillaries for high-quality and reproducible measurements. NanoTemper Technologies Monolith warranty is only valid if instrument is used with NanoTemper Technologies capillaries.

Cat #	Monolith Capillaries	Content
MO-D001	Monolith™ NT.115 Capillary Selection Kit	25 Monolith NT.115 Standard Treated Capillaries 25 Monolith NT.115 Hydrophobic Capillaries 25 Monolith NT.115 MST Premium Coated Capillaries
MO-K002	Monolith™ NT.115 Standard Treated Capillaries	1000 Capillaries
MO-K003	Monolith™ NT.115 Hydrophobic Capillaries	200 Capillaries
MO-K005	Monolith™ NT.115 MST Premium Coated Capillaries	200 Capillaries

3.3. Fluorescence Labeling

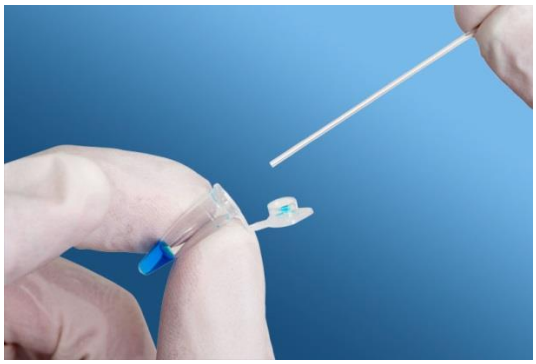
The following kits offer optimized fluorescence labeling and purification protocols for use in MicroScale Thermophoresis. The dyes have been widely tested with MST and the protocols are optimized to remove free, non-reacted dye:

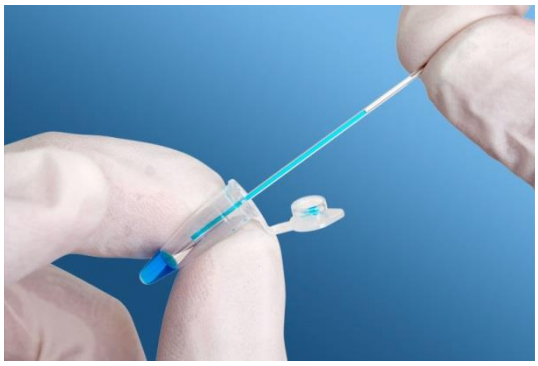
Cat #	Monolith Protein Labeling Kits	Reactions
MO-L001	Monolith™ Protein Labeling Kit RED-NHS (Amine Reactive)	4x 100 µg protein
MO-L002	Monolith™ Protein Labeling Kit GREEN-NHS (Amine Reactive)	4x 100 µg protein
MO-L003	Monolith™ Protein Labeling Kit BLUE-NHS (Amine Reactive)	4x 100 µg protein
MO-L004	Monolith™ Protein Labeling Kit RED-MALEIMIDE (Cysteine Reactive)	4x 100 µg protein
MO-L005	Monolith™ Protein Labeling Kit GREEN-MALEIMIDE (Cysteine Reactive)	4x 100 µg protein
MO-L006	Monolith™ Protein Labeling Kit BLUE-MALEIMIDE (Cysteine Reactive)	4x 100 µg protein
MO-L007	Monolith™ Antibody Labeling Kit RED-NHS (Amine Reactive)	4x 10 µg protein

3.4. Sample Loading

Be careful when using the glass capillaries. Broken capillaries are harmful to skin and eyes. Discard capillaries only in waste boxes that are intended for glass waste. Wear gloves, safety glasses and lab coat.

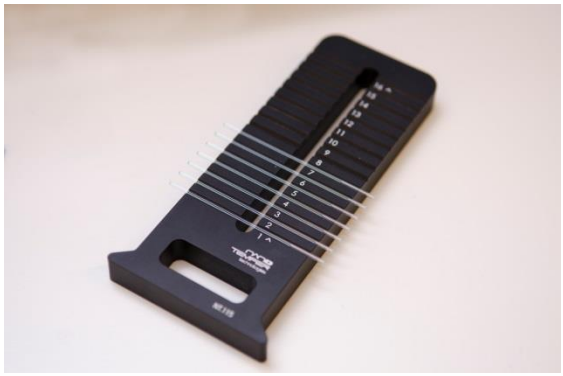
Place the capillary horizontally into the reaction tube to aspirate the sample. Don’t touch the capillary in the middle section where the optical measurement will be performed.





Position the liquid in the center of the Monolith Capillary by holding it vertically and shaking it after aspirating the sample.

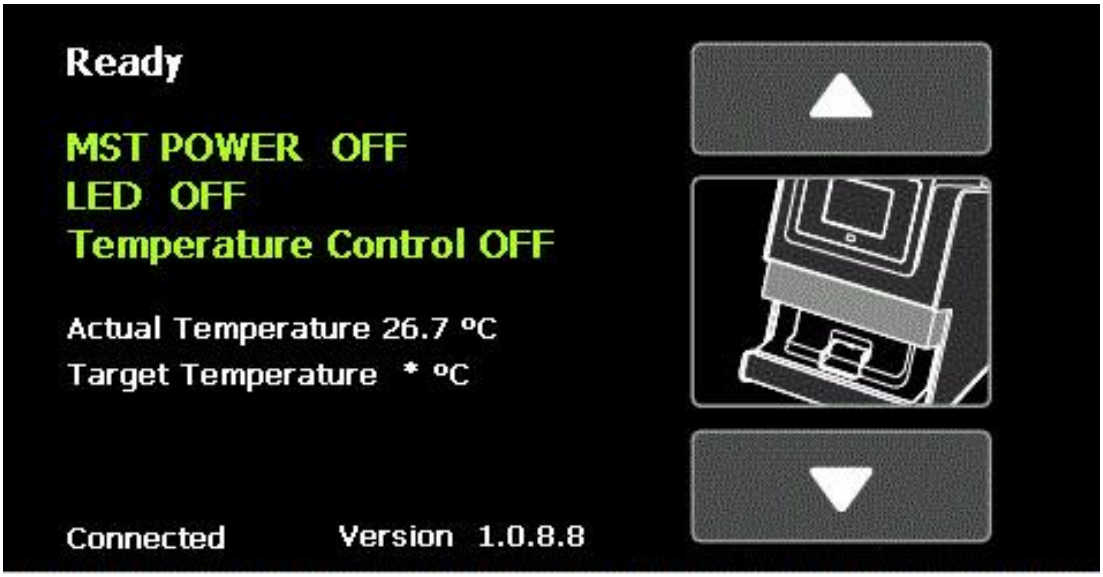
Put the capillaries in the slots on the sample tray. Note the order of the capillaries. The highest concentration is placed in the front of the tray. This position is denoted as “1” on the sample tray and in the control software.



Place the sample tray in the instrument by pushing it into the tray slot of the instrument as far as possible. The Monolith NT.115 instrument scans the tray and automatically determines the position of the capillaries on the tray.

4. Using the Monolith® NT.115 Instrument

Turn on the Monolith NT.115 instrument using the power toggle switch on the back of the instrument and start the control notebook. The display of the Monolith NT.115 instrument will show the following screen:



The words “Ready” in the first line and “Connected” in the last line indicate the successful connection between the Monolith NT.115 instrument and the control notebook.

The arrows on the right hand side (up and down) are used to open and to close the front door of the instrument in order to load/remove the sample tray.

The activities of the instrument are shown in green letters “MST POWER OFF” and “LED OFF”. When the experiment starts, these letters will turn red and indicate “MST POWER ON” and “LED ON”, respectively. The status of the LED informs you if fluorescence detection is running and the status of the MST power shows if the IR laser is working and generating temperature gradients in the samples.

In the middle of the screen you find information on the “Temperature Control”. First, you can see if the “Temperature Control” is activated (“OFF” or “ON”) – this is set with the control software. Below this, the “Actual Temperature” and the “Target Temperature” of the instrument is displayed. Please note that this is not the laser induced temperature gradient used for MST measurements.

For most measurements room temperature is appropriate, so there is no adjustment necessary.

IMPORTANT: Before running measurements, insert the tray containing capillaries filled with samples and wait for the “Target Temperature” to be reached!

To start a measurement, start the MO.Control Software. Please refer to the MO.Control software manual for details on performing measurements.

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