Dianthus NT.23 system

User manual

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1. About this user manual

This user manual gives guidance on the correct use of the Dianthus NT.23, Dianthus NT.23Pico and Dianthus NT.23PicoDuo systems. It covers system specifications, safety considerations and installation as well as why and how to run experiments with Dianthus NT.23. Please read this manual carefully before starting and make sure the contents are fully understood. Keep this manual available near the system for future reference. In case of loss, please contact NanoTemper Technologies customer support (https://nanotempertech.com/support) for a replacement copy of this manual.

1.1. Directions for more detailed information

There are two sources for further, more detailed information on scientific principles and recommendations for assay development as well as software usage. One is the **NanoPedia** knowledge base that is integrated into the DI.Control and DI.Screening Analysis software. It contains a variety of articles about the biochemistry of interaction analysis, TRIC as a method and the operational functions of Dianthus. A Pdf version of the **NanoPedia** can be accessed through the NanoTemper Technologies **Explorer Community**.

The NanoTemper Technologies **Explorer Community** is an online community to obtain resources for NanoTemper products such as application-notes, tech-notes or protocols. It is also a place to exchange best practices directly with other Dianthus users. You can also pose questions and have them answered by NanoTemper support guides. Follow https://nanotempertech.com/be-an-explorer/ for access to the Explorer Community.

2. Safety information

To ensure operation safety, this system must be operated correctly. Carefully read this chapter to fully understand all necessary safety precautions before operating the system.

2.1. Symbols and descriptions

This section describes the safety symbols and descriptions used in this manual, as well as the labels on the system.

Please take a moment to understand what the signal words **WARNING!** **CAUTION** and **NOTE** mean in this manual.

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. **CAUTION** may also be used to alert against damaging the equipment or the system.

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 system and assay performance.

Read manual label. This label indicates that you must read the manual before using the system. This label is positioned on the back of the instrument.

Warning symbol. This symbol, when used on its own or in conjunction with any of the following icons indicates the need to consult the provided manual, because a potential risk exists if the operating instructions are not followed. This label is positioned on the back of the instrument.

Warning symbol. This symbol indicates moving parts that can crush and cut. This warning label is positioned on the instrument tray holding the microwell plate.

Warning symbol. This symbol indicates the presence of electric shock hazards. To avoid risk of injury from electric shock, do not open the enclosure. The enclosure should only be opened by NanoTemper authorized personnel. This label is positioned on the back of the device.

2.2. Use and misuse

Use the Dianthus NT.23 system only after having read and fully understood this user manual. Use the system only in perfect condition. If the system shows any signs of damage, stop operation and contact NanoTemper Technologies customer support.

Do not modify the system in any way. Do not use it for anything other than its intended purpose.

2.3. Safety instructions

WARNING! The door of the system can pinch or injure your hands or fingers. Keep fingers safe while opening and closing the door. Do not touch the instrument while parts are moving. Do not reach into the opening when the door is open.

WARNING! Connect the Dianthus NT.23 to the AC power supply using the supplied power cable. Since the instrument is assembled in line with the specifications for safety class IEC 61010-1:2010, it must only be connected to an outlet that has a ground contact.

WARNING! Do not attempt to change the Fuses. Consult NanoTemper Support (https://nanotempertech.com/support) if you think the fuse is broken.

WARNING! Danger of electric shock, fire and skin burns. Do not open the system (other than operating the door/loading hatch via the software). Do not reach into the door opening.

WARNING! Using hazardous or infectious substances in the system may pose a risk of explosion, implosion, release of gases or infection. Use only non-hazardous, non-infectious, aqueous samples. Dispose of used microwell plates according to the substances contained in them and according to locally applicable regulations concerning chemical waste.

CAUTION The instrument contains up to two IR-laser modules (invisible laser radiation class 3B according to IEC 60825-1: 2014) and two red laser modules (visible laser radiation class 3B according to IEC 60825-1: 2014). Lasers or laser systems emit intense, coherent electromagnetic radiation that has the potential of causing irreparable damage to human skin and eyes. Direct eye contact can cause corneal burns, retinal burns, or both, and possible blindness. Do not attempt to open the instrument as this poses a risk of personal injury or damage to the instrument. When the instrument is used as intended it emits laser radiation of LASER CLASS 1.

CAUTION The system must be installed in a way that does not hinder access to the power switch and power plug.

CAUTION Broken glass can cut skin. Do not use if the front or back glass is broken.

CAUTION The weight of the Dianthus NT.23 instrument is approx. 70 kg, do not move the instrument alone (four persons required for transport). If you move the instrument alone, you risk personal injury or damage to the instrument.

CAUTION Only NanoTemper Technologies staff may service and open the instrument.

NOTE Insufficient air supply can cause overheating of the system. Assure enough air supply by not covering the back of the system. Leave at least 15 cm of space between system and any wall or other obstruction.

3. The Dianthus NT.23 system

3.1. General

3.1.1. Intended use

The Dianthus NT.23 system provides fast and highly sensitive detection and quantification of molecular interactions in microwell plates. The system is intended for research purposes only. It is not to be used for diagnostic purposes.

3.1.2. Conformity

The following safety and electromagnetic standards were considered:

- IEC 61010-1:2010 Safety requirements for electrical equipment for measurement, control and laboratory use.
- IEC 61326-1:2012 EMC, Electrical equipment for measurement, control and laboratory use – EMC requirements.
- IEC 60825-1:2014 Safety of laser products.

3.1.3. Identification

The identification labels (Figure 1) are positioned at the rear panel of the instrument. They include manufacturer information, system model name and serial number (SN), electrical requirements, and the CE conformity symbol.

Figure 1: Identification labels for Dianthus NT.23.

3.2. Technical information

3.2.1. Technical specifications

Electricity	
Input Voltage AC	AC 100-240 V -15 % +10 %
Voltage phase	Single phase
Mains frequency	50-60 Hz ± 6 %
Overvoltage category	CAT II
Input current AC	6 /3.2 A at 120 / 230 Vac
Pollution degree	2
Environmental	
Operating temperature	20–30 °C (indoor use only)
Storage temperature	-20–30 °C
Humidity	5–70 %, non-condensing
Operating altitude	max 2000 m
Dianthus NT.23 dimensions	
Width	61 cm (24.0”)
Height	42 cm (16.5”)
Depth	57 cm (22.4”), with open tray: 69 cm (27.2’’)
Weight	70 kg (154.3 lbs) net
Red Laser	
Wavelength	635 nm ± 3 nm
Power	40 mW max.
IR Laser	
Wavelength	1475 nm ± 15 nm
Power	120 mW max.
Dianthus NT.23 Laser classification	
The device is LASER PRODUCT CLASS 1	
Temperature control	
Temperature control range	20 °C–25 °C

Maximum difference to room temperature*	± 5 °C
Precision of temperature control	± 0.25 °C

*for best possible results we recommend operating Dianthus at a temperature setpoint equal to the ambient temperature of the room.

3.2.2. Connections for input and output

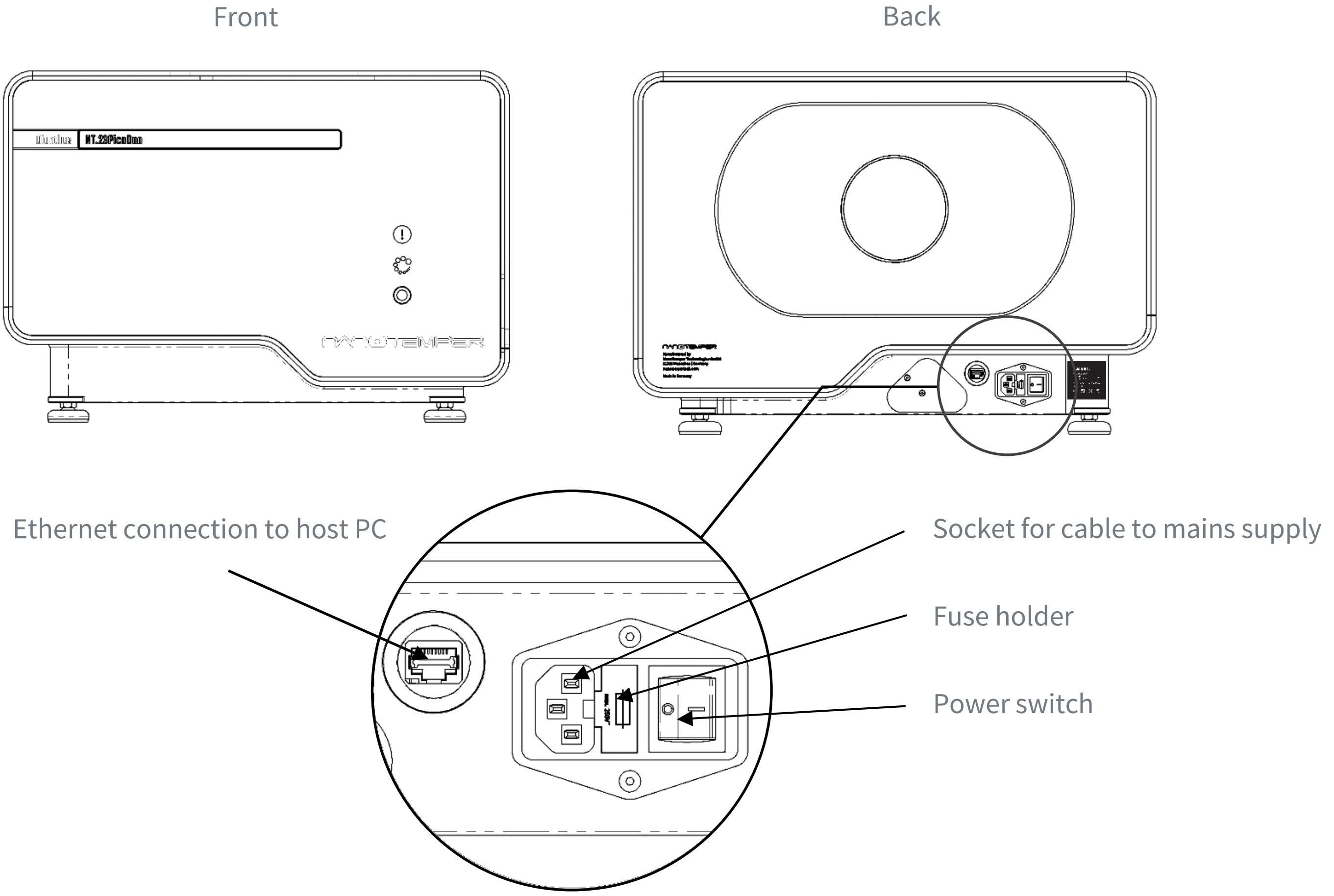


Figure 2: Connections on the Dianthus NT.23 device.

Type	Function	Position
Ethernet	To connect the system to the Control PC/LAN via Ethernet cable	Back panel
AC Power	To connect the system to electrical power	Back panel

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

3.2.3. LED panel and device status

Warning state: Appears when there is an error state, such as a failed initialization.

Measuring state: Indicates that the instrument is actively measuring and acquiring data. Even if connection to the control PC is lost or interrupted, measurement continues, and data is saved.

Initialization: Flashes yellow while initializing and while temperature setpoint is not yet reached. Blue when instrument is initialized and ready.

3.3. Legal

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 purposes 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 content of this manual is 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 controlling PC. In this case, take corrective action by uninstalling the conflicting product(s).
7. NanoTemper and Dianthus are registered trademarks of NanoTemper Technologies GmbH in the United States of America and other countries.
8. Unauthorized resale is not permitted.

3.4. Limited warranty

Products sold by NanoTemper Technologies, unless otherwise specified, are warrantied to be free of defects in materials and workmanship for a period of one year from the date of shipment. If any defects in the product are identified 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 operation, maintenance, adjustment or calibration.
3. Unauthorized modification or misuse.
4. Use of unauthorized microwell plates and accessories.
5. Use of consumables, disposables and parts not supplied by an authorized NanoTemper Technologies distributor.
6. Corrosion due to the use of improper solvents, samples, or due to surrounding gases.
7. Accidents beyond NanoTemper Technologies' control, including natural disasters.

This warranty does not cover consumables like microwell plates, reagents, labeling kits and the like. It also does not cover normal wear-and-tear.

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 system (see 3.1.3).

4. Dianthus NT.23 setup

The Dianthus NT.23 should be installed by NanoTemper Technologies personnel to ensure safety measures are taken and to confirm proper functionality of the instrument.

4.1. Scope of delivery

Upon receiving the system, please check package contents for completeness. The Dianthus NT.23 system package contains the following items:

Item	Description
Dianthus NT.23 system	-
User manual	This user manual
Cables	Power cord for power supply, Network cable for connection to control PC
Control PC and Monitor	Desktop control PC with monitor for Dianthus NT.23 system

4.2. Unpacking

The Dianthus system should only be unpacked and installed by trained NanoTemper Technologies personnel to ensure proper functionality of the instrument upon delivery.

4.3. Startup

Connect the Dianthus NT.23 system to power by plugging in the power supply cable. Connect the Dianthus NT.23 system to the control PC using the ethernet connection at the back of the instrument. The system starts upon switching the power switch. The LED display shows the Connected state when ready.

4.4. Cleaning

The Dianthus NT.23 system does not need any regular maintenance.

To clean the outside surface of the system, unplug the power supply at the back. Wipe the surface, including the front display, with a cloth slightly dampened with water or ethanol.

4.5. Software updates

Software updates of the embedded system can only be performed by instructed NanoTemper Technologies personnel and is part of regular maintenance visits. Software updates of DI.Control and DI.Screening Analysis can be performed by the users within the respective software solution.

5. Using Dianthus NT.23

The Dianthus instruments analyze Temperature Related Intensity Change (TRIC) signals for fast and easy detection and quantification of binding events in industry standard microwell plates. TRIC is based on the generation of a rapid and highly precise temperature change in a sample well by infrared (IR) laser light. Changes in sample fluorescence upon activation of the IR laser are monitored to characterize interactions and derive affinity constants. The response of the sample fluorescence upon IR laser activation is based on distinct physical principles which are described in the following section.

5.1. Dianthus 384-well plates

NOTE Dianthus instruments can only be used with Dianthus 384-well plates which can be purchased from NanoTemper Technologies.

CAUTION Using any other plate with the instrument could result in damage or unreliable results.

The well geometry and the specific coating of Dianthus 384-well plates ensure highest reproducibility and data quality. The plate has a working volume of 18-25 µL. However, we strongly recommend a working volume of 20 µL. The plates are made of black polystyrene material, ensuring that samples are not subjected to sunlight and do not bleach easily. The bottom of the plate is made of transparent material as the Dianthus optical system measures the sample from the bottom. This has the added advantage that the top of the plate can be sealed to avoid sample oxidation and evaporation. The plates are barcoded with a unique barcode that is recognized by the Dianthus instrument. The barcode in addition contains information about the production date of the plates for tracking the shelf-life. The plates are coated with a special polymer to avoid protein adsorption to well walls and well bottom.

General tips for handling plates

- Do not leave fingerprints on the foil bottom. In case, wiping the bottom with a lint-free tissue can remove fingerprints.
- Avoid dust and scratches on the plate bottom. Scratches influence the measurement, while dust generally does not. Still, one should avoid taking too much dust into the instrument. In case, wiping the bottom with a lint-free tissue can remove dust.
- Use reverse pipetting where possible to avoid air bubbles. If you pipette manually in a 384-well plate it is advisable to use reverse pipetting in cases where it is possible (e.g. when dispensing the buffer for a dilution series). This does of course not work if mixing is necessary.
- In general, avoid air bubbles at the plate bottom. This can affect the measurement. If air bubbles appear, centrifuge the plate **open** (i.e. without seal).
- For few samples, first pipette in tubes and then transfer to the plate using reverse pipetting. In tubes it is easier to see while pipetting, therefore results are often more accurate.
- When pipetting directly and manually in the plate, avoid piercing the foil bottom with your pipette tip.

- One should always work with a volume of 20 µL per well. In this range small volume errors will not affect the measurement. 25 µL is the maximum volume a well can hold.
- If plates are kept in a fridge or in an incubator with significantly different temperature than the device temperature, it is advisable to incubate the plates 30 min before measuring at device temperature.
- Mixing the sample well before measurement is pivotal for reproducibility and high data quality in Dianthus measurements. When using liquid handling solutions with pipette tips we recommend at least 15 mixing cycles with at least 80% of the final volume. When using contact-less liquid handling we recommend plate shaking with at least 10 G for 15 seconds and a subsequent 30 min incubation time.

5.2. Dianthus software products

Two software products are needed to make most efficient use of Dianthus' capabilities. The **DI.Control** software is needed to access all of Dianthus' functionalities. It controls the instrument and is used for assay development as well as screening data acquisition. The **DI.Screening Analysis** software provides a convenient and easy to use solution for the in-depth analysis of large datasets. It can automatically identify hit compounds in single-dose screens and fit dose-response data with different fit models.

5.3. Underlying physical principles

Temperature Related Intensity Change 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**. TRIC constitutes an effect where the fluorescence intensity of a fluorophore is temperature dependent (Figure 3).

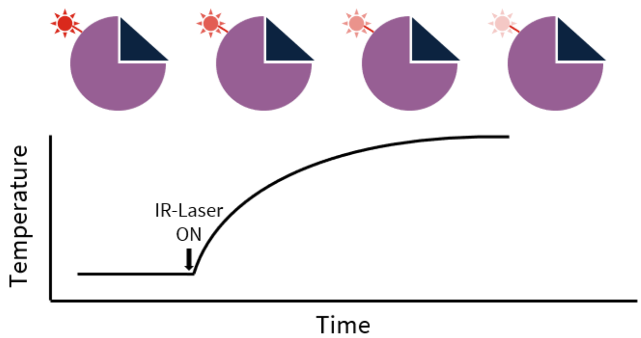


Figure 3: Illustration of a temperature related intensity change for a fluorescent probe (red) attached to a target molecule (purple) with an IR-laser induced change in temperature.

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. Chemical environment changes for the fluorophore through ligand binding can occur by 2 distinct mechanisms. Either by binding of a ligand in close proximity to the position of the fluorophore or by inducing a conformational change in the target molecule (Figure 4).

Figure 4: Illustration of chemical environment changes for fluorescent probes upon target-ligand complex formation.

5.4. TRIC measurement and data analysis

A TRIC measurement in a Dianthus instrument starts with a well-scan in three dimensions to first carefully position the optical focus in the well center, close to the transparent plate bottom (see next chapter for details). This process completes within less than one second. After optics positioning, steady-state fluorescence at ambient temperature is recorded for a period of 1 second, followed by IR-laser activation for a defined *on*-time (Figure 5.1) after which the IR laser is switched off and the next well is scanned. Such a TRIC measurement is done for each sample and results in the typical TRIC-trace as a measured signal. Usually one would compare TRIC for a target in absence and presence of ligand (Figure 5.2), corresponding to two different samples in two different wells. Due to pipetting errors, small variations in absolute fluorescence can be observed (Figure 5.2). These pipetting errors are eliminated by data normalization (Figure 5.3) of all TRIC-traces to relative values starting at 1. After normalization one can usually see clear differences for TRIC-traces obtained for the target in absence and presence of ligand. The two states differ in their TRIC response which results in a quantifiable *Signal Amplitude* (Figure 5.3). To further process the data and correlate it with ligand concentrations one calculates F_{norm} values (Figure 5.4), which correspond to the ratio between the fluorescence F_1, obtained for a selected on-time (note that this selected *on*-time can be shorter than the measured *on*-time) and the fluorescence F_0 obtained at ambient temperature (also *Initial Fluorescence*). The resulting values are multiplied with 1000 and then correspond to values in ‰. The obtained F_{norm} values for different concentrations of added ligand can then be used to quantify the interaction between target and ligand.

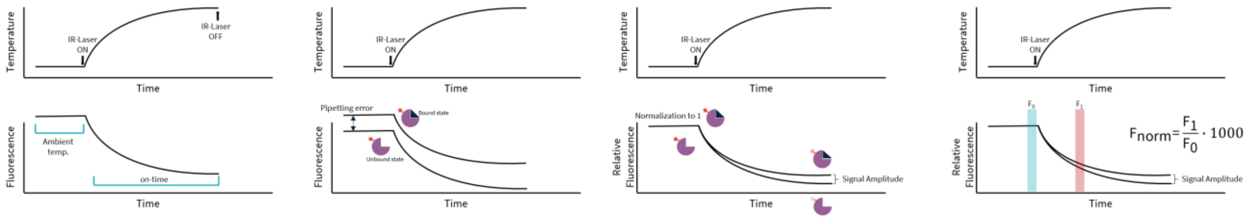


Figure 5 from left to right. 1: Illustration of a TRIC measurement. The lower panel shows the obtained fluorescence over time while the upper panel illustrates the change in temperature occurring through IR-laser activation. 2: TRIC-traces for an unbound and bound target molecule, resulting in traces that can be shifted in their absolute fluorescence values by pipetting errors. 3: Normalization of TRIC-traces results in observable differences for bound and unbound state of the target. The result of normalization is a quantifiable signal amplitude as a function of the *on*-time. 4: The user can select a different on-time that can be shorter than the measured on-time. F_{norm} values are then calculated as shown and used for further quantification of the binding event.

5.5. Using F_{norm} values for qualitative and quantitative description of binding events

The observed change in fluorescence after IR laser activation results from a change in fluorescence due to TRIC. Measured values in TRIC-traces are displayed as relative fluorescence, where the fluorescence obtained at ambient temperature is normalized to one (see previous section for details). Further data processing is done with normalized fluorescence values (F_{norm}) which describe the ratio between fluorescence values (F_1) after and fluorescence values (F_0) prior to IR laser activation and are typically given in ‰:

$$F_{norm} = \frac{F_1}{F_0} \cdot 1000$$

As described, an interaction between a fluorescent target molecule and a ligand molecule would result in an alteration of the observed TRIC signal. Bound and unbound (complexed and free) target molecules contribute differently to the overall TRIC signal i.e. the resulting F_{norm} signal from these two samples would be different. Differences in TRIC signal comparing samples with ligand (added at one fixed high concentration) and without ligand (e.g. a DMSO reference sample) can be used to qualitatively analyze binding events and pre-screen compound libraries for hits that bind to the target molecule of interest (single dose screening). In such a case one would simply look for ligands that induce a significantly different F_{norm} signal as the target alone would have (Hits) or for those that don't (Non-binders). Instead, analyzing a series of samples with an increasing ligand concentration can be used to quantitatively describe any type of interaction with a derived affinity constant. To extract affinity constants from titration data one needs to analyze F_{norm} signals as a function of ligand concentration (Figure 6).

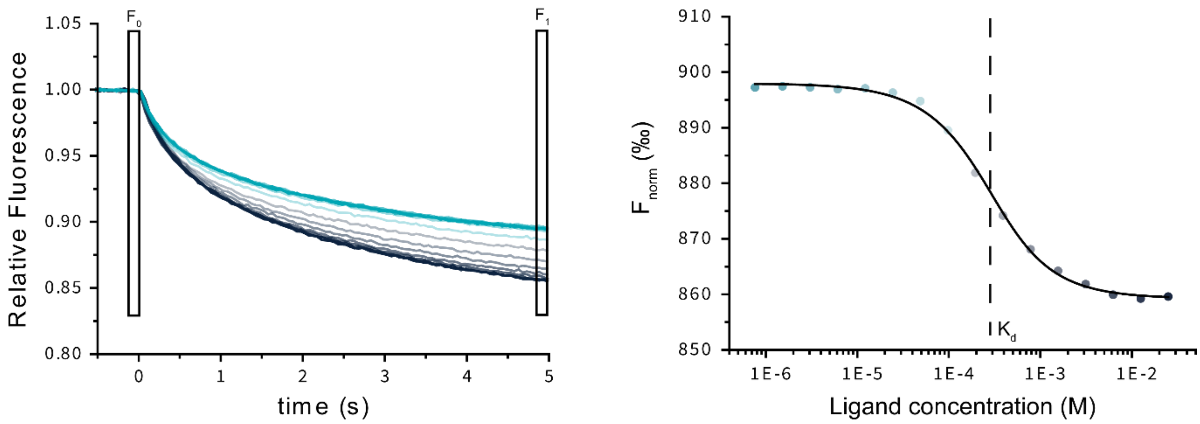
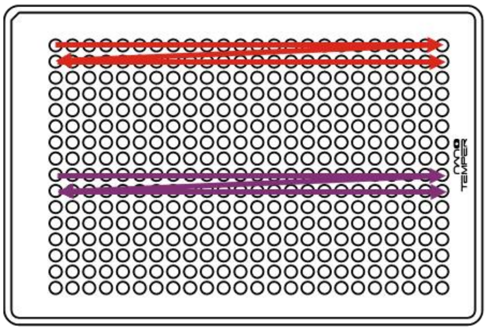


Figure 6: Left: TRIC traces for a fluorescent target in presence of a varying amount of added ligand molecule. Right: Fnorm response obtained for the traces left plotted against the concentration of added ligand in M. The data is fitted with a dose-response curve that describes the law of mass action for this interaction with the K_d as one fit parameter.

5.6. How does a Dianthus measurement look like?



The Dianthus NT.23 instrument series uses 384- microwell plates as a sample vessel. To measure one well (i.e. one datapoint) requires approximately seven seconds and one full plate takes 30min to measure provided both optical elements are used for the measurement. Depending on instrument variant two optical system can work in parallel, making the system twice as fast. While optic *top* measures well A1, optic *bottom* can measure well I1 at the same time. To increase the throughput and make the technology applicable for compound or fragment library screening the instrument can be fully integrated into lab automation instruments using google remote procedure call.

5.7. Optical positioning prior to TRIC measurements

Before one well can be measured for TRIC the optical system with the infra-red laser needs to be carefully positioned.

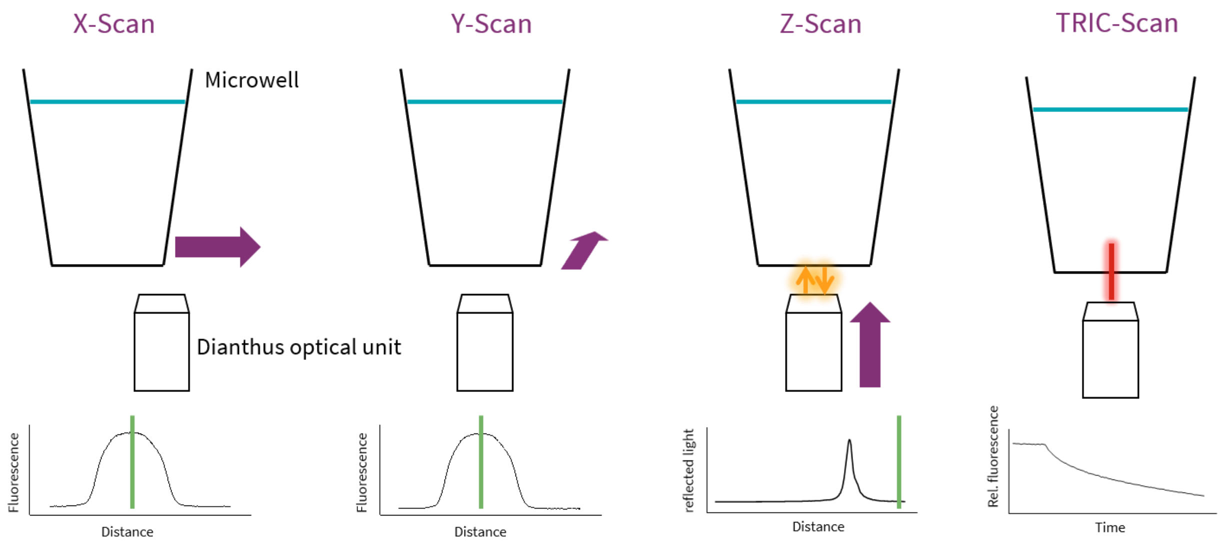


Figure 7: Illustration of the serial steps of establishing the spatial dimensions of one specific well. X-Scan, Y-Scan and Z-Scan ensure high precision positioning of the optical system before the final step (TRIC-Scan) where the TRIC-trace is recorded for that well.

As a first step of optics positioning, Dianthus uses sample scan to measure sample fluorescence while the well moves in x-direction above the optics (Figure 7.1). From the resulting well profile, Dianthus determines the well center in X, here indicated as a green line. It then does the same in Y (Figure 5.2).

After these two steps the well center is well defined for the resulting Z-Scan (Figure 5.3). Here Dianthus now moves the optical setup in Z-direction and uses reflected laser light to determine the accurate position of the interface between air and plate bottom, as well as plate-bottom and sample. Dianthus then positions the z-focus at a micrometer distance above the well bottom. This careful and highly precise positioning of the optical system in all three dimensions is pivotal for reproducibility of the temperature increase. Despite the high precision all three scans require less than one second of accumulated measurement time. Following optical positioning the TRIC-Scan is started (for details see previous chapter), followed then by positioning for the next well.

6. Troubleshooting

6.1. Wrong insertion of Dianthus 384-well plates

In case a plate is inserted in a wrong way and blocks the instrument, the drawer cannot close, the instrument will stop and go into a warning state. In such a case please switch off the instrument and contact the NanoTemper Technologies support (www.nanotempertech.com/support).

6.2. Disconnect from control PC

In case of a disconnect between instrument and control PC the instrument would still complete the ongoing measurement. This is indicated by the LED panel. Please do not switch off the instrument in this state. Wait until the measurement is complete and reestablish the connection. The DI.Control software will then obtain the measured data from the internal instrument PC.

6.3. Restarting Dianthus NT.23

In case the system freezes, wait one minute. If it does not un-freeze, use the switch at the back of the system to switch off the instrument. Wait 30 seconds for complete shutdown, then restart the instrument. The system will start up again automatically.

6.4. Customer support

In case of any issues not described in this user manual, please don't hesitate to contact NanoTemper Technologies customer support at www.nanotempertech.com/support.

7. Patents and intellectual property

Dianthus and TRIC technology are patent protected, especially by the following patents, US8431903B2, US8853650B2, US9459211B2, US10345312B2, US8741570B2 including their application and registration in different other countries.

8. Transport and disposal

8.1. Repackaging for transport

The Dianthus instrument should be repacked only by trained NanoTemper Technologies personnel to ensure safety and stability during transport. Please store the instrument box for that purpose. If the instrument box was discarded NanoTemper Technologies can provide replacement at the cost of packaging material and shipment.

8.2. Waste disposal

Please dispose of used microwell plates according to the substances contained in them and according to locally applicable regulations concerning chemical and glass waste.

8.3. System disposal

The system may need to be decontaminated before disposal. Please contact NanoTemper Technologies for more information.

This symbol indicates that this system may not be disposed of as unsorted municipal waste and must be collected separately. It must be disposed of according to locally applicable regulations regarding electrical and electronic equipment. The symbol is positioned at the back of the instrument.

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

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