DIANTHUS

PRODUCT MANUAL

What is included in this product manual

DIANTHUS SYSTEM

User Manual

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1. About this user manual

This user manual gives guidance on the correct use of the Dianthus system. It covers system specifications, safety considerations and installation as well as why and how to run experiments with Dianthus. 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 visit the NanoTemper Technologies Support Center (**https://support.nanotempertech.com/hc/en-us**) for a replacement copy of this manual.

1.1. Directions for more detailed information

For further, more detailed information on scientific principles and recommendations for assay development as well as software usage visit our Support Center (**https://support.nanotempertech.com/hc/en-us**).

The NanoTemper Technologies **Support Center** is an online community to obtain resources for NanoTemper products such as application-notes, tech-notes or protocols. You can also pose questions and have them answered by NanoTemper support guides.

Do not miss out on our online-tool “Degree of labeling calculator” that helps with calculation of the degree-of-labeling (DOL): **https://nanotempertech.com/user-tools/dol-calculator/**

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 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 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! 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 an IR-laser module (invisible laser radiation class 3B according to IEC 60825-1: 2014) and a green laser module (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 protects the user from dangerous laser radiation and is classified as a **class 1 Laser Product.**

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

CAUTION Do not replace the detachable power cord with an inadequately rated cord.

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

CAUTION The weight of the Dianthus 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.

CAUTION Disconnect the mains supply before changing the fuses.

CAUTION This equipment is not intended for use in residential environments and may not provide adequate protection to radio reception in such environments. According to IEC 61326-1, in connection with CISPR 11, this device is: Group 1, Class A.

Group 1: Equipment has intentionally generated and/or uses conductively-coupled radio frequency energy that is necessary for the internal function of the equipment itself.

Class A: Equipment is suitable for use in all establishments other than domestic and those directly connected to a low-voltage power supply network that supplies buildings used for domestic purposes. There may be potential difficulties in ensuring electromagnetic compatibility in other environments due to conducted and radiated disturbances.

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 system

3.1. General

3.1.1. Intended use

The Dianthus 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/AMD1:2016, Safety requirements for electrical equipment for measurement, control and laboratory use. Part 1 General Requirements.
- IEC 61326-1:2021 EMC, Electrical equipment for measurement, control and laboratory use – EMC requirements.
- IEC 60825-1:2014, Safety of laser products.
- Complies with 21 CFR 1040.10 and 1040.11 except for conformance with IEC 60825-1 Ed. 3., as described in Laser Notice No. 56, dated May 8, 2019

3.1.3. Identification

The identification label (Figure 1) is positioned at the rear panel of the instrument. It includes manufacturer information, system model name and serial number (SN), electrical requirements, and the CE conformity symbol

Figure 1: Identification labels for Dianthus.

3.2. Technical information

3.2.1. Technical specifications

Electricity

Input Voltage AC	AC 100-240 V -10 % +10 %
Voltage phase	Single phase
Mains frequency	50/60 Hz
Overvoltage category	CAT II
Input current AC	6 - 3.2 A
Pollution degree	2
Fuse	Fuse link 5 x 20 mm, 10 A, 250 V, time-lag T (2x)

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 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

Green Laser

Wavelength	520 nm ± 10 nm
Power	10 mW max.

IR Laser

Wavelength	1475 nm ± 15 nm
Power	120 mW max.

Dianthus 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

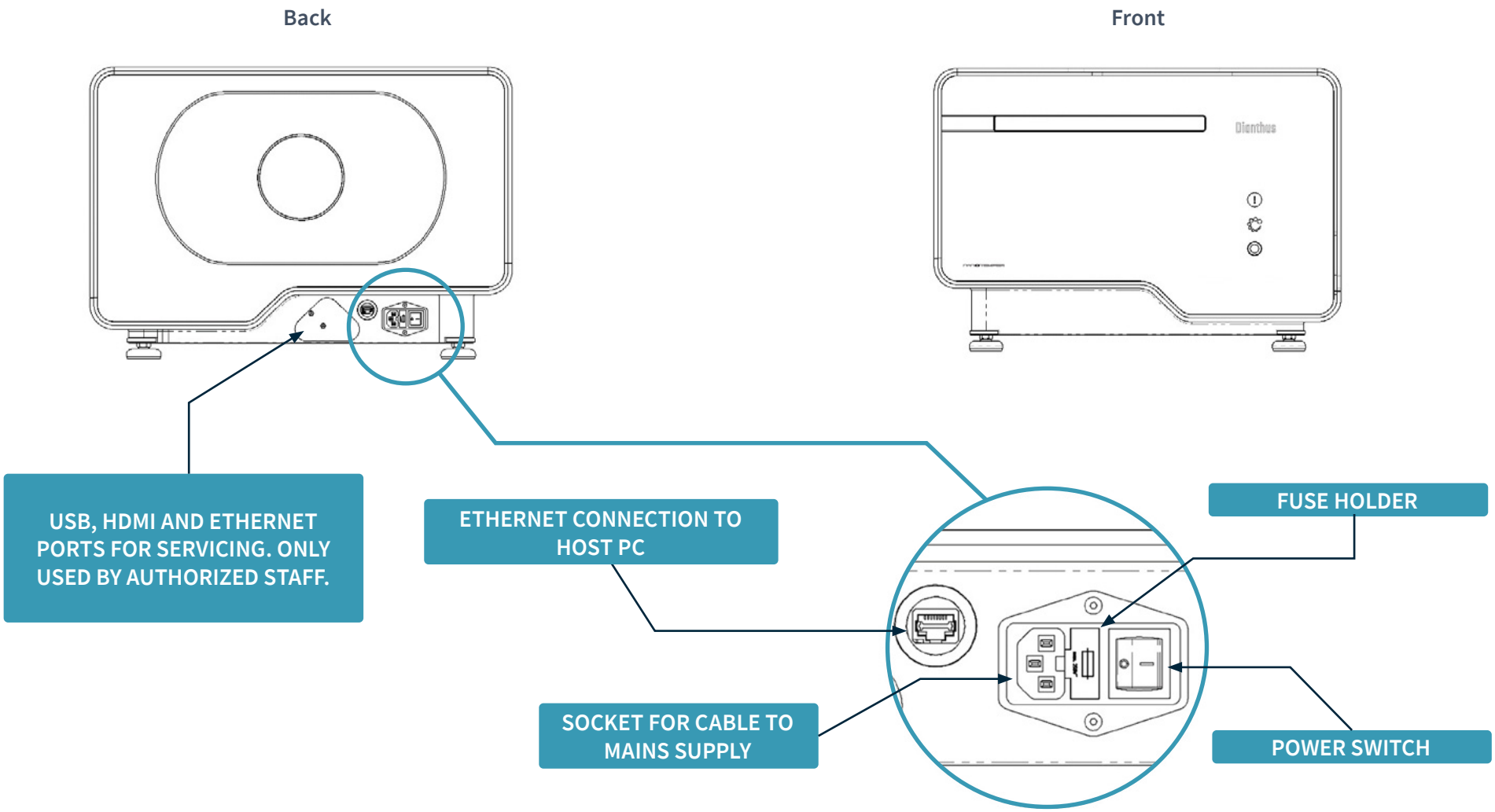

Figure 2: Connections on the Dianthus device.

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 of the instrument.

3.2.3. Replacement of fuses

We recommend not to change the fuses on your own. Please consult NanoTemper Support (**https://nanotempertech.com/support**) if you think the fuse is broken. To replace the fuses, disconnect the mains supply and open the fuse holder (see Figure 2). Replace the two fuses with new ones of type fuse link 5 x 20 mm, 10 A, 250 V, time-lag T (available, e.g. from ESKA, art. no. 522727).

3.2.4. LED panel and device status

Warning state: Appears when there is an error state, such as a failed initialization. Open DI.Control to receive more detailed information about the device status. Contact support if issue cannot be resolved by power-cycling the instrument.

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.

3.2.5. Stacking of instruments

To save space in your lab, it is possible to stack a maximum number of two instruments on top of each other.

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 setup

The Dianthus 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 system package contains the following items:

Item	Description
Dianthus 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 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.

Note: After transport it is possible that the instrument is cool and needs some time to equilibrate to room temperature. If the instrument is turned on while the instrument temperature is significantly lower than the set temperature the measurement accuracy may be reduced.

4.3. Startup

Connect the Dianthus system to power by plugging in the power supply cable. Connect the Dianthus 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 (initialization LED is blue).

The instrument is controlled from the DI.Control Software, including opening and closing of the plate-tray.

4.4. Cleaning

We recommend regular maintenance of the instrument by trained NanoTemper Technologies personnel to ensure internal components are functional. 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 users.

5. Using Dianthus

The Dianthus instruments assess two distinct photophysical properties of fluorophores for fast and easy detection and quantification of binding events. These two properties are changes in the fluorescence emission spectrum (Spectral Shift) and changes in the fluorophore's temperature dependence (TRIC).

- Spectral Shift is based on the detection of emission wavelength changes of a fluorescent probe. Changes in fluorescence emission are detected using a ratiometric dual-wavelength approach. Spectral Shift can be used to characterize interactions and derive affinity constants.

- 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.

Spectral Shift and the response of the sample fluorescence upon IR laser activation are based on distinct physical principles which are described in section 5.3.

Measurements in Dianthus instruments occur in industry standard microwell plates.

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. Importantly, 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).

- 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 that are pipetted manually, 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 at least 30 min before measuring at device temperature. At best, pre-incubation is done inside the instrument.

- 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 180 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

Spectral Shift

Spectral Shift detection is a biophysical technique that measures the strength of the interaction between two molecules by detecting a variation in the fluorescence emission profile of a fluorescently labeled or intrinsically fluorescent target when a binding event occurs.

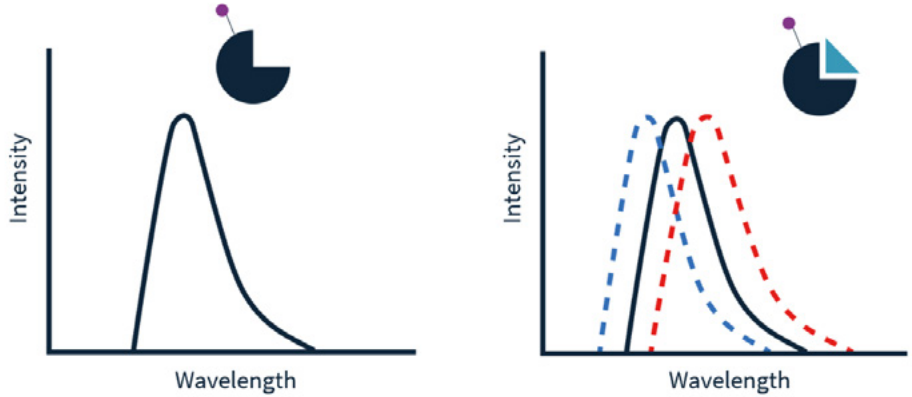

Figure 3: Illustration of a binding-induced change in the fluorescence emission spectrum. Spectrum can shift either to longer wavelengths (red-shift), or to shorter wavelengths (blue-shift).

Temperature Related Intensity Change

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.**

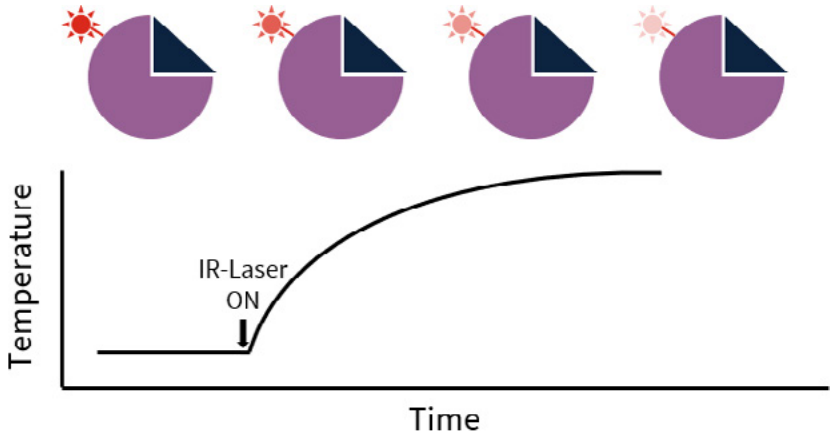

Figure 4: Illustration of a temperature related intensity change for a fluorescent probe (red) attached to a target molecule (purple) with an IR-laser induced change in temperature.

The emission wavelength as well as the extent of the temperature dependence of 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 5).

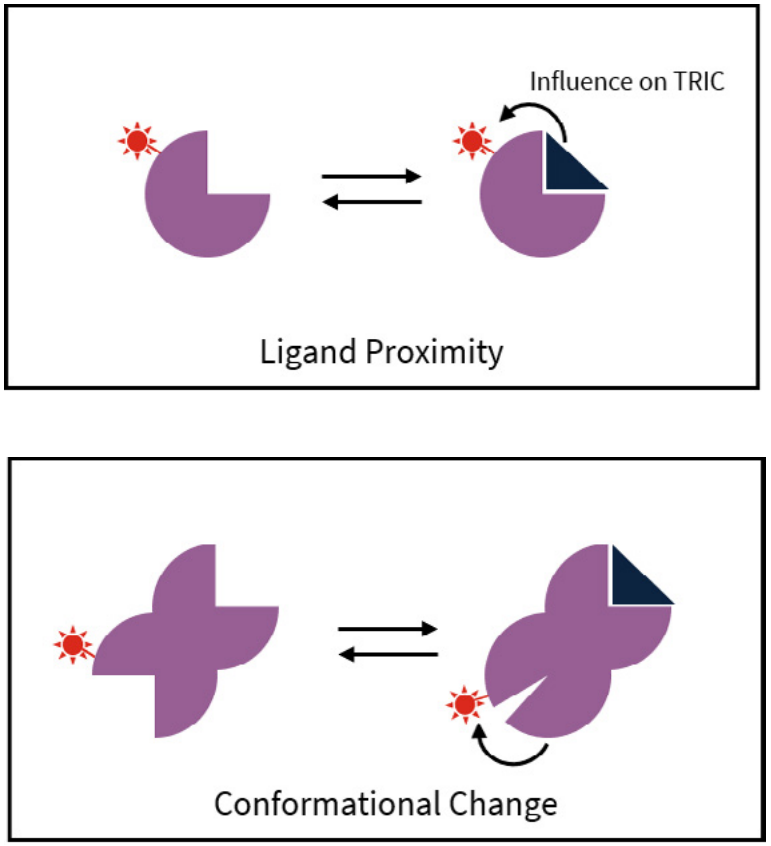

Figure 5: Illustration of chemical environment changes for fluorescent probes upon target-ligand complex formation.

5.4. How does a Dianthus measurement look like?

The Dianthus instrument series uses 384- microwell plates as a sample vessel. The measurement time for one well depends on the user settings. Users can choose to measure spectral shift only (35 min for one 384-well plate) or spectral shift and a subsequent TRIC experiment (70 min for one 384-well plate).

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. For further information on automation please contact our support.

5.5. Sequence of positioning and data acquisition for one well

Before one well can be measured, the optical system needs to be carefully positioned.

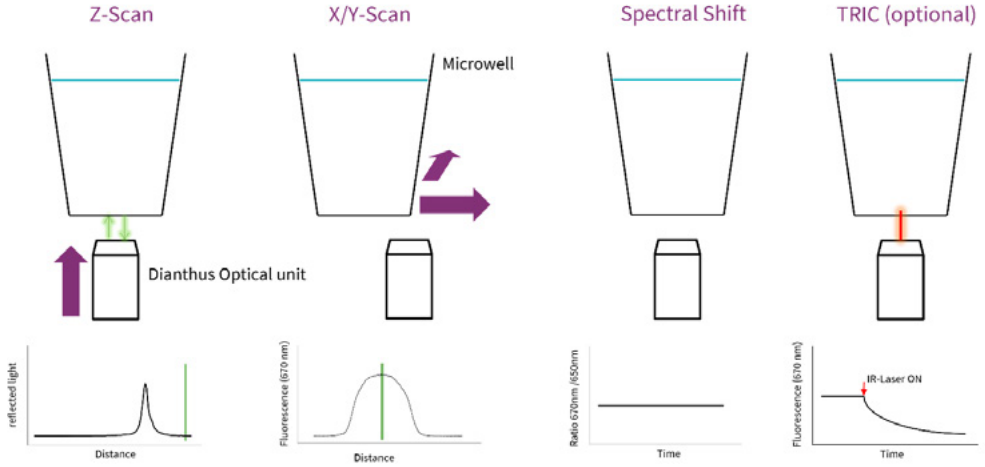

Figure 6: Illustration of the serial steps of establishing the spatial dimensions of one specific well. Z-Scan, X-Scan and Y-Scan and ensure high precision positioning of the optical system before a spectral shift measurement and (optionally) a TRIC-trace is recorded for that well.

As a first step of optics positioning, Dianthus 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 (Figure 6.1). This careful and highly precise positioning of the optical system is pivotal, especially for reproducibility of the temperature increase in TRIC measurements.

The Z-scan is followed by a sample scan to measure sample fluorescence while the well moves in x- and y-direction above the optics (Figure 6.2). From the resulting well profile, Dianthus determines the well center in x and y, here indicated as a green line. Following optics positioning,

Dianthus now performs the spectral shift measurement (Figure 6.3), followed by an optional TRIC-scan (Figure 6.4). Note that the Z-position for the spectral shift measurement is different from the Z-position for a TRIC measurement. While spectral shift is measured at a distance of 1.5 mm from the well-bottom, the TRIC measurement occurs right at the well bottom. Hence the absolute fluorescence signals can be different for Spectral Shift and TRIC data.

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

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

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NanoTemper cares for the future of our planet, therefore we want to do our best to protect it and reduce the environmental footprint of our products.

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