nanotemper

AN.Control

USER MANUAL

Table of contents

1.	Technical Information	2
1.1.	System Requirements	2
1.2.	Backward Compatibility	2
2.	Home Screen and General Usage	3
3.	Choose Excitation Color	3
4.	Discovery Scan	4
5.	Melting Scan	5
6.	Annotations and Results	7
7.	Data Export	8
7.1.	Discovery Scan	8
7.2.	Melting Scan	8
7.3.	Annotations and Results	8

NanoTemper® Technologies' Andromeda series instruments detect changes in the fluorescence of GFP fusion and labelled His-tagged proteins over a wide range of temperatures. The instruments can be used to induce thermal unfolding of proteins and to determine thermal unfolding transition temperatures.

The AN.Control software is dedicated to running and analyzing thermal unfolding experiments on Andromeda and Andromeda Plex instruments.

1. Technical Information

1.1. System Requirements

If the necessary licenses have been purchased, AN.Control software can be installed on additional computers for convenient data analysis. The computers have to meet the following requirements:

Operating system:	Windows 7 64 Bit or higher
CPU:	Intel Core i5 or better
RAM:	8 GB or more
Hard disk:	20 GB or more free disk space available
Display resolution:	1600 x 900 or better
Software:	Microsoft .NET 4.6.2 framework (included in installer of AN.Control software)
Operating system language:	English or German

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

1.2. Backward Compatibility

Measurements collected with all previous versions of AN.Control software are compatible with this version.

NanoTemper Technologies provides support for all current and previous versions of AN.Control.

2. Home Screen and General Usage

To perform a new measurement, start the AN.Control software, which will show the *Project* home screen. Click *Create New Project* to enter a file name and location. The file will be saved in .anc format. Optionally, enter relevant information into the *Project Name* and *Comments* fields.

Alternatively, previous project files can be loaded by clicking *Browse to analyze* previous experiments or to add additional measurements. Recently loaded files are listed chronologically on the right.

Use the *Save Changes* button at any time to save modifications of the file. An asterisk in the software title bar indicates unsaved changes in the open file. Closing the software will trigger a dialogue box asking whether you want to save the changes.

After an initial setup step (Choose Excitation Color), there are three tabs that guide the user through running and analyzing Andromeda measurements:

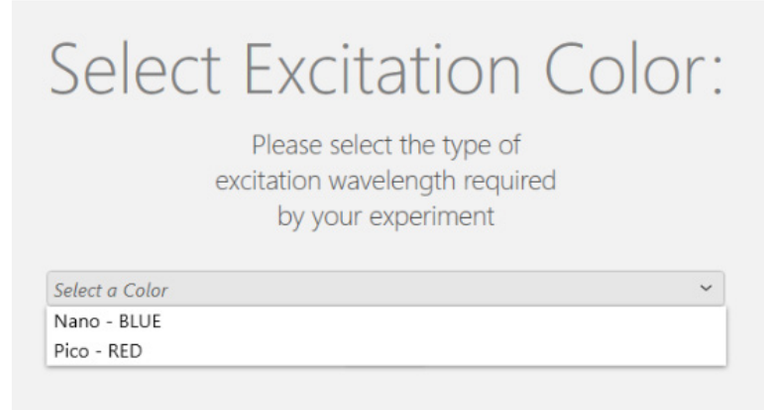
1. Discovery Scan
2. Melting Scan
3. Annotations and Results

Proceed from 1 to 3 to set up, run and analyze an Andromeda measurement. As soon as the measurement is started, navigate between all three tabs freely to re-analyze, modify or annotate. More details on each tab follow below.

The keyboard shortcut ctrl + z will undo any action, while ctrl + y will redo.

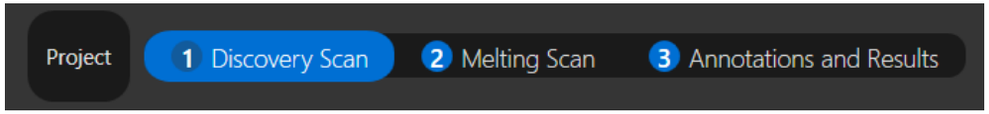
Any graph displayed in the software can be exported by clicking the Export button. Options are to copy the graph to the clipboard, to save it as an image (.png or .svg file formats), or to export the raw data needed to recreate the graph in third-party software (.xlsx file format).

3. Choose Excitation Color

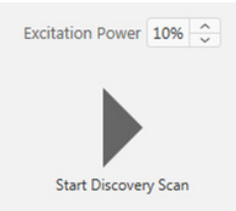


After creating a new project or loading a previous project file, first select the appropriate Excitation Color based on your sample type. For His-tagged proteins, choose the RED fluorescence channel to perform thermal unfolding after labeling your samples with the Andromeda His-Tag Labeling Kit (Cat. No. AN-030002). For GFP fusion proteins, choose the BLUE channel to perform thermal unfolding without labeling your samples.

4. Discovery Scan



Once a fluorescence channel has been selected, perform a *Discovery Scan* (1) to determine optimal settings for the unfolding experiment. You can vary the excitation power between 1 % and 100 %. Re-scan with different excitation power settings if necessary.



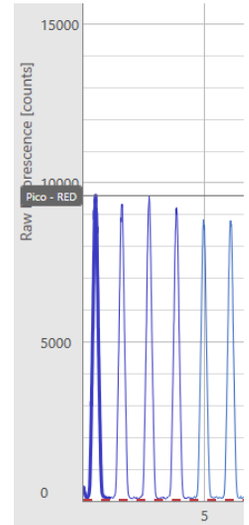
The discovery scan is used to detect the fluorescence intensity and position of each capillary along the entire length of the capillary tray. Each capillary will be visible as a peak, which represent the fluorescence intensity 670 nm in the RED channel or 510 nm in the BLUE channel, respectively. (Figure 1).

The upper and lower limits of detection are highlighted by dotted red lines in the discovery scan profile. The upper limit is 20,000 fluorescence counts in the RED channel and 2,000 fluorescence counts in the BLUE channel, respectively (Peak Fluorescence, meaning the height of the capillary peak). Please note that the lower detection limit is dynamically adjusted to the excitation power settings and thus may vary between experiments with different settings.

Zoom into the graph using the scrolling function of the mouse wheel. Holding down shift or ctrl while scrolling will zoom horizontally or vertically, respectively. Holding the mouse wheel also allows to move the graph. To reset the view, click *Zoom Extent*.

Note: *Try to aim for a fluorescence signal of approximately 10,000 counts in the RED channel, and of approximately 1,500 counts in the BLUE channel. With a final 1 nM concentration of RED-tris-NTA 2nd Generation dye in the sample, 10,000 counts should be obtained at 50 – 70 % excitation power in the RED channel.*

Note: *Photobleaching effects are negligible even at high excitation powers due to the rapid on-the-fly measurement mode.*



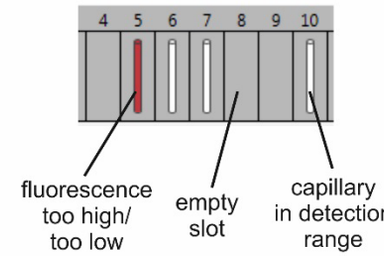
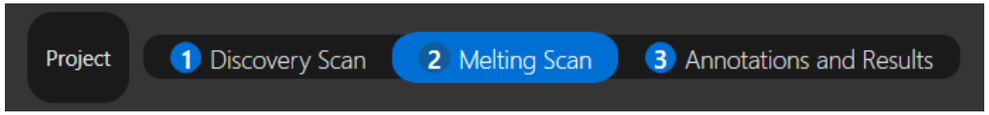


Figure 1: Discovery Scan. The discovery scan provides information about the fluorescence intensities of the samples, and is used to determine optimal measurement settings and the positions of occupied capillary slots. (Left) The fluorescence intensities of the 670 nm or 510 nm fluorescence channels are displayed as single peaks for each capillary. Clicking on a specific peak or capillary will highlight this capillary's fluorescence at the chosen wavelength, and also display its values for Peak Fluorescence and Integrated Fluorescence on the right of the screen. Each peaks should be between the upper and lower detection limits (dotted red lines). Capillaries with fluorescence intensities within the dynamic detection range are colored white; capillaries with too high or too low fluorescence intensities are colored red.

5. Melting Scan

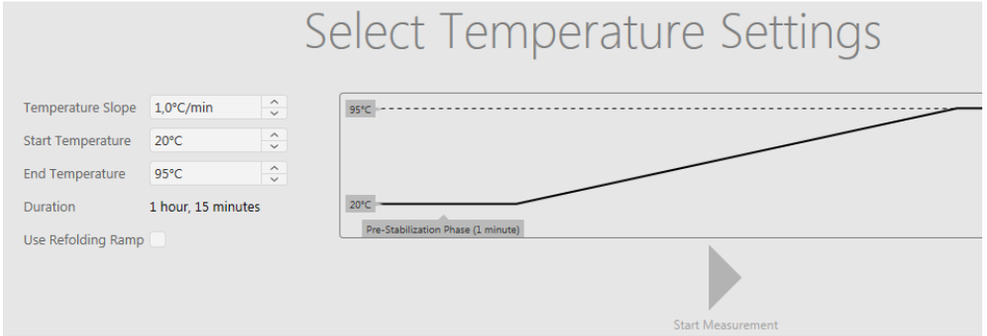


Once the optimal excitation power settings are determined, continue with the *Melting Scan* (2). The AN.Control software automatically identifies the capillaries for unfolding analysis from the discovery scan. By default, only capillaries that lie in the dynamic detection range are pre-selected for the unfolding experiment. However, you can also add capillaries that are marked in red.

Note: *You can multi-select capillaries using shift or ctrl + left click.*

Next, define the "Start" and "End" temperature for the thermal unfolding experiment, and set the "Temperature Ramp" to a heating rate of 7.0 °C/min.

Start the thermal unfolding experiment by pressing "*Start Measurement*". After a pre-melting phase in which the start-temperature is reached and the system is equilibrated, continuous scanning of the selected capillaries proceeds while a thermal ramp is applied. The thermal unfolding of proteins in each capillary can be followed in real time.



Once the final temperature is reached, unfolding transition midpoints will be automatically calculated by the software and can be displayed in the melting scan window (see Figure 2). The window will also display the unfolding curves and, if selected, the respective first derivative of the curves. In the first derivative view, each local maximum or minimum corresponds to an inflection temperature (T^i, also known as Inflection Point, IP). Inflection temperatures can be manually added or removed by clicking the *Add / Remove Marker* buttons. Colored buttons below the capillaries can be used to manually change the color of each curve.

The calculated unfolding transition temperatures as well as manually annotated markers will be added to the annotations table. Raw fluorescence and processed data (raw fluorescence and first derivative values) as well as the corresponding calculated unfolding transition midpoints and annotations can be exported in Excel™ format using the *Export button.* Images can be exported as .svg or .png files.

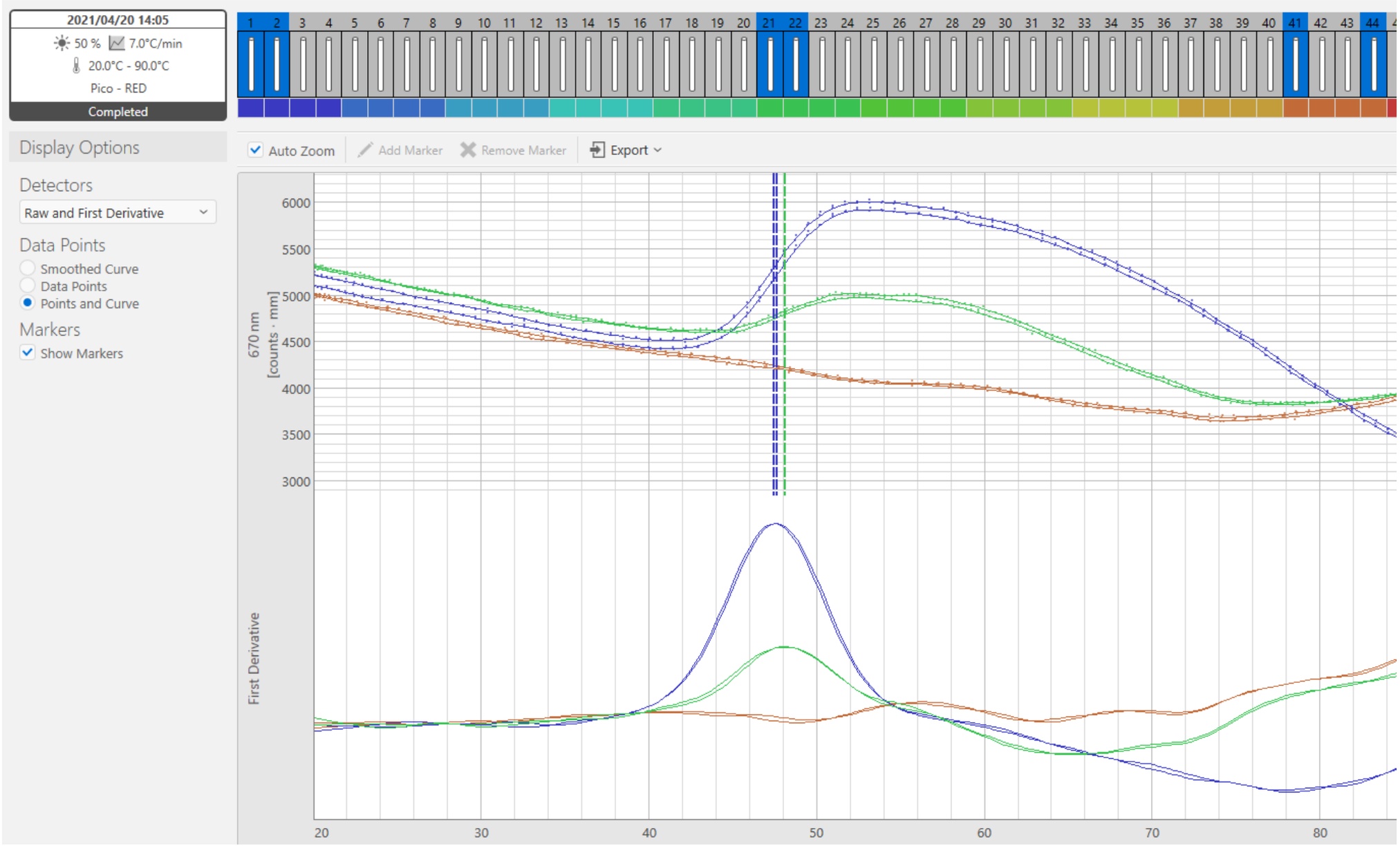
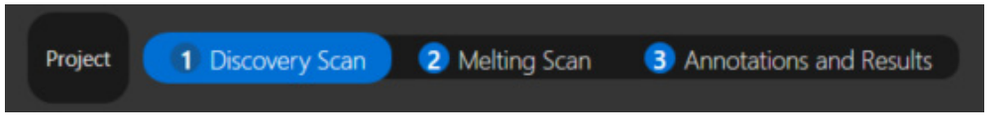


Figure 2: The Melting Scan window after an experiment has been completed.

6. Annotations and Results



It is not required to enter sample annotations prior to the experiment. Annotations for each capillary can be entered at any time while the melting scan is running or after it is finished. Annotations can either be entered by simple copy-and-paste from a spreadsheet software like Excel™ or manually. Columns can be added or removed by using the *Create New Column* and *Remove Column* buttons on the right side of the screen.

Annotations can be entered into multiple fields simultaneously after multi-selection, and subsequently sorted in ascending or descending manner by left-clicking on the column header.

Note: *Multi-selections of capillaries applied in the* Melting Scan *or* Annotations and Results *tabs persist after switching tabs.*

Capillaries can be selected and a single color can be assigned by clicking on the rectangle next to "*Single Color*".

Furthermore, capillaries can be colored by categories or gradients after selecting the cells that are to be used for categorizing.

The colors will directly translate into the melting curves in the *Melting Scan* window. Color assignment will only apply to selected fields. Multi-selection by using shift / ctrl + left click is possible.

The annotations table includes separate columns for unfolding transition midpoints (inflection points, IP also called T^i) and manually annotated points (M).

The thermal stability of a given protein is typically described by the thermal unfolding transition midpoint, also called inflection point (IP) or T^i (inflection temperature) [°C]. It is determined automatically by the AN.Control software via the derivative of the curve. This method circumvents the subjective determination of baseline levels and also allows for the determination of multiple unfolding transition midpoints, e.g. for more complex multi-domain proteins.

Annotation tables can be exported in Excel™ format (.xlsx).

7. Data Export

Each measurement and analysis performed can be exported to be used in third-party software. Throughout this software manual, different export options are mentioned. This section aims to give an overview regarding the file format and the content of each export.

7.1. Discovery Scan

The Discovery Scan tab contains three export options.

Clicking on *Export Raw Data* will create an Excel™ file (.xlsx) containing two sheets. The first sheet gives the major information for each peak, whereas the second one contains the information to reproduce the graph.

The *Copy Chart to Clipboard* button will copy the graph displayed so you can paste it into another software.

The *Export Graph* button will create an image file (.png or .svg) of the zoom extended view displayed by the software.

7.2. Melting Scan

The export button in this tab will display four options.

The *Copy Chart to Clipboard* button will copy the graph exactly how it is displayed in the software (for example only showing selected capillaries, or showing both raw data and first derivative) so you can paste it into another software.

The *Export Graph* button will create an image file (.png or .svg) of the graph as described above.

Clicking on *Export Raw Data* will create an Excel™ file containing four sheets. The first sheet gives an overview of all capillaries contained in the run, including unfolding transition midpoints, unfolding onsets and manually set markers. The other sheets contain the fluorescence at 670 nm or 510 nm as a function of temperature for all capillaries, respectively.

Using *Export Processed Data* will create the exact same Excel™ file but including also the data of the first derivative for each fluorescence channel as separate sheets.

7.3. Annotations and Results

The export option is located at the bottom right of the screen and it will create an Excel™ file containing the table displayed in this tab.

Andromeda™ is a trademark, registered with the U.S. Federal Trademark registration.

NanoTemper® is a registered trademark and registered in the U.S. Patent and Trademark Office.

V01_2021-06-16

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

NanoTemper® and **Andromeda™** are registered trademarks.