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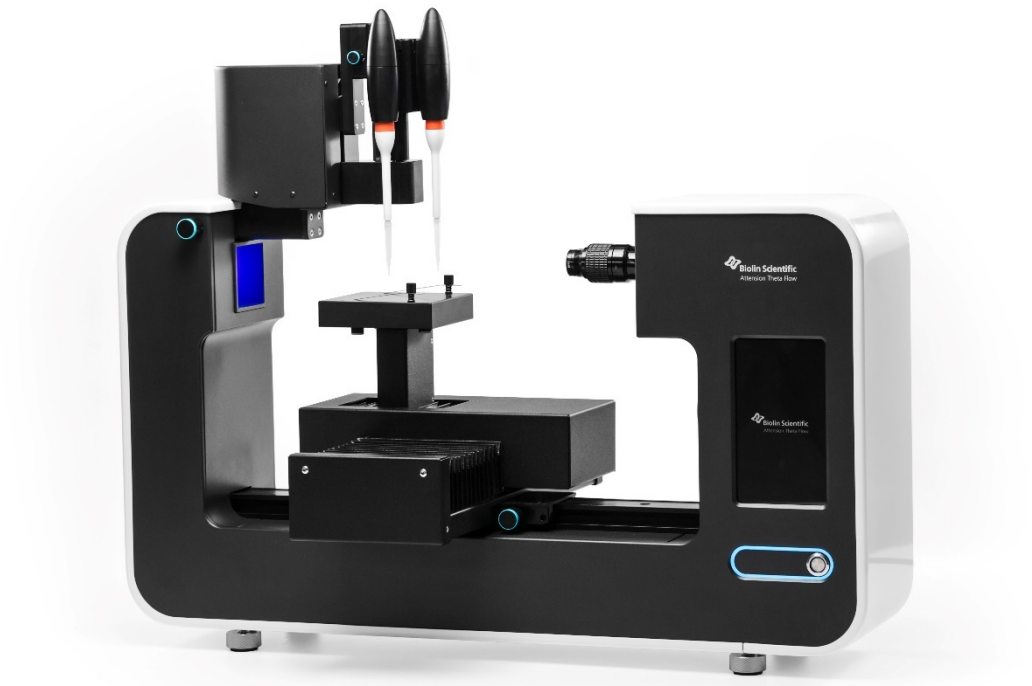
Theta Flow

User Manual – OneAttension

Original Instructions



Optical Tensiometer

MANUAL25100-8

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1 Introduction

Attension Theta Flow Optical Tensiometer is a computer controlled and user programmable video-based instrument designed for the measurement of:

- Surface and interfacial tension
- Static and dynamic contact angles
- Surface free energy of solids
- Dilatational interfacial rheology

Theta Flow includes a video camera, an adjustable sample stage and a LED light source. The open design and modular construction allow the instrument to be adapted to a wide variety of applications. In addition to the basic measuring unit, Theta Flow can be equipped with the following components. For a full list of the available accessories, please see our website at www.biolinscientific.com/attension.

- Automated dispensers
- Dispenser rotation module and Dual dispenser unit
- Picoliter dispenser
- Motorized sample movement mechanisms
- Environmental measuring chambers
- Interfacial measuring chambers
- Thermostatted measuring chambers
- Tilting cradle
- Pulsating drop module
- 3D Topography module
- High pressure chamber

The OneAttension software included with this instrument allows the easy operation of a variety of pre-programmed experiments which can be modified to any needs. The data is stored to the hard drive and can be retrieved and analyzed later. The data analyses can be done without instrument connection. Data files can be easily exported and imported if another data reduction software than the one provided is preferred.

For any questions or comments about Theta Flow or any Attension products please contact us at support@attension.com.

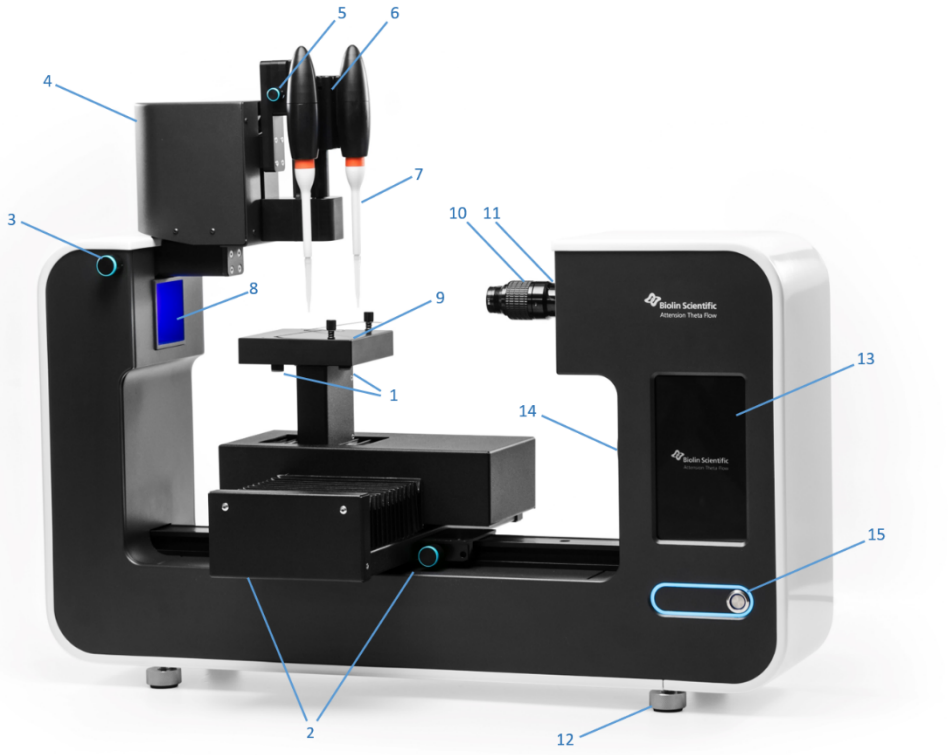
1.1 Purpose of this manual

This manual provides information on how to operate Theta Flow along with the major points of the theory involved in the measurements and some practical advice on the measurement techniques. In order to obtain the maximum performance from the instrument this manual should be read and kept available for reference.

For information on the installation of Theta Flow and OneAttension please see the separate **Installation Manual**.

1.2 Physical description

The figure below describes the basic hardware of Theta Flow.



1) Stage level adjustment
2) Stage rail locks
3) Syringe lateral adjustment
4) Liquid dispenser holder
5) Syringe attachment lock
6) Optional rotation module
7) Dispenser
8) LED light source
9) Sample stage with sample attachment clips
10) Camera lens focus adjustment
11) Camera lens zoom adjustment
12) Level adjustment feet
13) Touch display
14) Camera tilt adjustment
15) Power button and status indicator light

The instrument front panel contains the power button, status indicator (#15) and touch display (#13). The instrument status is indicated as follows:

- Indicator light is on when the instrument is ready to be used
- Indicator light spins during recording
- Indicator light blinks when the software asks for input from user

From the touch display it is possible to control automated devices including dispensers, dispenser holder and automated stages. The touch display also shows instrument levelness information, camera tilting angle, ambient temperature and probe temperature.

Electrical connections are available on the instrument rear panel. A power cord and USB3 cable need to be connected in order to operate the instrument. The remaining device ports are used for additional modules (see Chapter 5 for details). The rear panel also contains USB2 connector for external USB devices such as barcode scanner or Topography module.

The light source (#8) is based on monochromatic LEDs in order to assure a sharp image even for moving objects, with minimal sample heating.

The instrument uses a USB3 camera. The camera has the following image modes:

- 60 FPS @ 2592 x 2048
- 100 FPS @ 1984 x 1568
- 252 FPS @ 1216 x 961
- 1059 FPS @ 672 x 320
- 2266 FPS @ 672 x 107
- 3422 FPS @ 672 x 44

The presented frame rates are maximum values. Some functions such as using the live analysis may slow down the actual imaging speed.

The optics has a working distance of 142 – 187 mm depending on focus. Magnification of the image seen on the screen during measurement is changed with the camera lens zoom adjustment (#11). It is advisable to always have as magnified an image as possible to have as accurate results as possible. You can also adjust the angle of the camera optics from the knob (#14).

The instrument is equipped with thumbscrews for the easy adjustment of the position of the sample stage and the syringe. These screws are used for the positioning of the sample and the syringe so that their images appear at the appropriate positions when recording images.

The instrument is equipped with a system levelness sensor, an integrated temperature sensor that measures ambient temperature and an integrated humidity sensor. An external temperature sensor to monitor the measurement conditions can be connected to **TEMP** port on Theta Flow rear panel.

2 Safety

WARNING!
The safety requirements listed in this manual must be followed in order to avoid personal injury and damage to the instrument. If the equipment is used in a manner not listed in this manual, protection provided by the equipment may be impaired.

WARNING!
RISK OF ELECTRICAL SHOCK. Do not connect this instrument to electrical power if the enclosure is damaged or any of the covers or panels are removed. Make sure the voltage rating on the instrument matches the line voltage available. Make sure the power cord is not damaged and it is properly connected to the instrument and a power outlet with protective earthing. Make sure that the power cord is easily accessible after the equipment has been installed and set at its working position.

WARNING!
RISK OF ELECTRICAL SHOCK OR FIRE HAZARD. The instrument has been designed for indoor use only. Do not expose it to rain, snow or dust. During storage or transport the instrument should be kept dry. Temperatures below 0°C and above 70°C should be avoided. Do not operate at ambient temperatures below 15°C and above 30°C.

WARNING!
RISK OF INJURY. Do not configure the instrument with parts that are not supplied by Biolin Scientific and not intended to be used with Attension instruments. Do not install substitute parts that are not described in this manual. Do not perform any modifications to the product.

If dangerous liquids are used, adequate protection such as proper ventilation, safety glasses, etc., should be used: refer to the safety information from the supplier and general safety regulations in your country. Carry out appropriate decontamination if equipment is exposed to hazardous material.

WARNING!
RISK OF BURNS. Exercise caution when touching heated measurement chambers (optional). The chamber surface will reach dangerous temperatures when heated. The chambers are marked with hot surface warning symbol.

CAUTION!
Camera optics and instrument rear panel are sensitive to electrostatic discharges (ESD). Take appropriate ESD prevention precautions when handling these parts.

Make sure that the power is switched off when making any electrical connections (apart from the USB cables). Connecting cables with power on may damage instrument electronics. To disconnect the instrument, after turning the instrument off, remove the power cord plug from the electric outlet.

3 Brief instructions

This section contains the minimum necessary information about each stage of making measurements with Theta Flow. It is intended as a reminder for those who are already familiar with basic functioning of the unit. The rest of this manual has much additional detail on the installation and the operation of the unit. This information should be read with care to ensure the accurate collection and analysis of data.

3.1 Installation

Mechanical installation

It is advised to have two persons lifting the instrument.

Place Theta Flow on a vibration free table in an area where local air currents are at a minimum. The instrument features an integrated level indicator that can be used to level the instrument. Monitor the instrument levelness from the touch display from the levelling tab and rotate the feet until the bubble sits in the central marked circle. Note that this part of the setup is only possible when the software is installed. The touch display indicates constantly Yes/No depending whether the instrument is levelled or not.

Attach the sample stage and level it by using the three adjustment screws under the stage. Underneath the large samples stage top is a smaller sample stage top. To be able to attach different measuring chambers, it is necessary to remove the large sample stage top to reveal the smaller one. Loosen the screw on the side of the sample stage to remove the large sample stage top if needed.

Connect the cables. The device cables should be connected starting from the first **DEV** port without leaving any empty ports between. If the automated dispenser holder is used that should be connected to the first serial port. If motorized XYZ sample stage is used that should be the last one. Otherwise the order of connected accessories is not critical.

Software installation

Run the installation from the USB stick and follow the instructions. Please see more detailed installation instructions in the **OneAttension Theta Flow and Theta Flex Installation Manual**.

3.2 Calibration

Turn Theta Flow on. Wait for the computer to recognize the camera before you turn on OneAttension. Notice that when you open the measurement for the first time, the instrument will initialize all the automated functions (e.g. dispenser holder and X-stage). Care should be taken that there is enough space for all the movements to be completed.

Choose measurement method by clicking a measurement icon (sessile drop, batch sessile drop, pendant drop, meniscus, pulsating drop or automatic DCA). Click Adjust Camera Parameters and choose automated camera adjustment by clicking “Auto adjust”. You are able to adjust the camera settings also manually if needed.

Place a clean calibration ball and its magnetic holder on the sample stage underneath the dispenser. Lift or lower the sample stage to bring the ball into the image on-screen. Lift the dispenser away from the image. Adjust the zoom from the lens zoom adjustment (#11). A recommended default zoom setting is such that the calibration ball fills most of the image. Press “Autofocus” and enable it. You can also manually focus the ball if needed. To do this, disable the autofocus and then rotate the focus ring (#10) so that it is midway between its two extreme positions. Then move the camera from

the controls until the edges of the ball are sharp. Fine-focus the image with the focus adjustment ring. You can use the focus measurement tool by right clicking at the edge of the calibration ball and observing how sharply the light intensity appears to change at the edge of the calibration ball. After focusing, press "Calibration invalid" and accept the calibration ball diameter (standard 4 mm) to complete the calibration. After the calibration is done, do not change the zoom or focus from the focus ring otherwise the calibration has to be redone. If you need to change the focus after calibration, use "Autofocus" or do it by moving the whole optics (from the software) or the stage. Since calibration is fairly easy and simple, it is advisable to do it before every set of measurements. Calibration can also be conducted with a needle. In this case, choose "Use calibration needle" and give the outer diameter of the needle. Please see detailed instructions for calibration in Chapter 4.2.

3.3 Sessile drop contact angle experiments

Preliminaries

For sessile drop experiments it is recommended to adjust the camera angle to -2 degrees.

When a manual syringe is used, clean the syringe, fill it with the studied liquid and attach the syringe to the syringe clamp.

If an automatic single liquid dispenser is used, the tubings need to be filled. This can be done using the device control buttons or by manually pushing liquid through the tubings and the needle. Click **dispense** to push liquid from the tubing. The dispensing should be performed until there are no air bubbles left in the tubing.

When a disposable pipette dispenser is used, the OneAttension software will automatically recognize the dispenser. Please select which tip size is used from the recipe. (**Note!** The Automatic single liquid dispenser should not be connected at the same time). Fill the dispenser using the device control buttons. Please see detailed instructions for automated dispensers from Chapter 5.

When the multi-liquid dispenser is used, the dispenser must be enabled from main menu → global settings → Select multidispenser in Theta. The controls for multi-liquid dispenser will now be visible in the Controls tab (see more detailed instructions under **"using multi-liquid dispenser"**).

Prepare the solid sample and place it on the sample stage. From **Start tab**, choose Sessile drop experiment.

Image Recording

Lift or lower the sample stage until the surface of the solid sample is visible on the bottom part of the screen. Set the baseline to the sample surface. Lower or raise the syringe until it is just visible at the top of the screen. Fill in the relevant data on recipe tab. The OneAttension software has default recipes for each of the measurement types. These recipes are suitable for most applications. It might be necessary to add users, liquids or solids to the database manager found in the **main menu**.

In case the manual dispenser holder is used, alter the depth of the dispenser so that when the dispenser is pushed down the syringe does not hit the sample. This is essential because if the syringe hits the sample, it might damage the surface and alter the contact angle data obtained.

Systems with manual dispenser: Select Start from Trigger in the Saving options in the recipe if you wish. Lower an appropriate drop from the syringe. Place trigger below the droplet if used. Click Record in controls if no automated devices are present or Start if automated devices are connected. Press down and release on the dispenser (manual dispenser holder) or wait for the automated dispenser holder to lower the drop if present and wait for the images to be recorded.

Systems with automatic dispenser and dispenser holder: Fill in the experiment name and choose the appropriate drop size, usually 3 – 6 µl. If you want to use 3 µl droplets or smaller, decrease the drop rate to 0.5 – 1.0 µl/s. "Volume from image" function is on as a default, and thus the volume of the

droplet is calculated based on the image calibration, and not only by using dispenser step motor. "Volume from image" –function is able to provide always the same droplet volume despite possible air bubbles in the needle or the tubings. **Press start.** The measurement is now done automatically.

See analysis of the results from chapter 6.

3.4 Batch sessile drop experiments

Note! Batch sessile drop experiment is not intended to be used with Tilting Cradle.

A Batch sessile drop experiment is a set of sessile drop experiments for a batch of samples. Contact angles from a batch of samples are measured successively using the same measurement recipe, and the results can be further analyzed and compared together. This can be convenient for example for quality control of samples.

Preliminaries

For sessile drop experiments it is recommended to adjust the camera angle to -2 degrees.

Clean the syringe, place the studied liquid into it and attach it to the syringe clamp. If an automatic dispenser is used, the tubings should also be filled. This can be done using the device control. Click **dispense** to expel liquid from the tubing. The dispensing should be performed until there are no air bubbles left in the tubing.

When a disposable pipette dispenser is used, the OneAttension software will automatically recognize the dispenser. Please select which tip size is used from the recipe. (**Note!** The Automatic single liquid dispenser should not be connected at the same time).

When the multi-liquid dispenser is used, the dispenser has to be enabled from main menu → global settings → Select multidispenser in Theta. The controls for multi-liquid dispenser will now be visible in the measurement window (see more detailed instructions under **"using multi-liquid dispenser"**). Please see detailed instructions for automated dispensers from Chapter 5.

Prepare the solid samples and place the first sample on the sample stage. From **Start tab**, choose Batch sessile drop experiment.

Image Recording

Lift or lower the sample stage until the solid is visible on the bottom part of the screen. Set the baseline to the sample surface. Lower or raise the syringe until it is just visible at the top of the screen. Alter the depth of the dispenser so that when the dispenser is pushed down the syringe does not hit the sample. This is essential because if the syringe hits the sample, it might damage the surface and alter the contact angle data obtained.

Fill in the relevant data on recipe tab. If you want to measure several points per sample define the points in the recipe, or if you have an automatic sample stage set the sequencer accordingly. In batch sessile drop measurements, the image analysis is made from the live image at your chosen points of time. Use the Single point mapper to fill in the points of time you want to use for analysis.

To begin the batch measurement, **press start**. The instrument will measure the first sample, and let you know when it is ready to measure the next sample. Follow the given instructions to measure the rest of the samples. The same points of time and place will be used for the analysis of all samples. When all the samples in your batch have been measured, choose **End batch**.

3.5 Pendant drop experiments

A Pendant drop experiment is very similar to a Contact angle experiment in procedure. A note on terms: a surface tension measurement involves a liquid drop and a gas whereas an interfacial tension measurement involves two liquids.

Preliminaries

For pendant drop experiments it is recommended to adjust the camera angle to 0 degrees.

Clean the syringe, place the liquid into it and attach it to the syringe clamp. With a standard surface tension measurement, lower the sample stage so that it is out of the way.

With a standard interfacial tension measurement place the less dense liquid in the syringe, the denser liquid in a cuvette and the cuvette on the sample stage. Change the syringe needle to a hook needle and attach the filled syringe to the syringe clamp.

From the **Start tab** choose the Pendant drop experiment.

Image recording

Fill in the relevant data in the recipe tab. It might be necessary to add users, liquids or solids to the database manager found in main menu.

With a surface tension measurement simply lower an appropriate drop. With an interfacial tension measurement lift the sample stage until the cuvette is visible, lower the hook needle into it, lower an appropriate drop and select Flip Y. Press Start or Record from the controls and wait for the images to be recorded.

3.6 Curve fitting and data analysis

After recording, choose the **Analysis tab** and double click on your experiment. If you wish to analyze a single measurement, accept either the default fitting parameters or adjust them from the Drop analysis window. The software will then process all of the chosen images. The red line represents the baseline, and it should connect the widest points of the curve profile and its reflection to each other. Check this visually, and if unsatisfied unselect Automatic baseline and set it manually. In sessile drop contact angle experiments the baseline is set at the solid surface, whereas in pendant drop experiments it is adjusted at the end of the needle.

By right clicking on the results, you are able to perform further data analysis, for example by hiding unnecessary data points. Selected data points can be plotted to a graph or statistically analysed on the Graph and Statistics tabs, respectively. You will also find the option to export data by right clicking on the results. By right clicking on the drop image you are able to save the separate pictures or the video. Calculated results are shown in the right side of the image. Values used to calculate result value are marked blue in the table. Result report in .pdf-format can be also created from the Report tab.

The analysis of batch measurements is described in more detail in chapter 5.2.

3.7 Surface free energy experiments

Surface free energy is automatically calculated for each experiment, if enabled on the recipe. The setting can be changed on the recipe and results are shown on the lower right-hand corner after each experiment. Typically, contact angles measured with 2-5 different probe liquids are used in surface energy calculations. On analysis side, different experiments can be combined into an SFE group by choosing them on the analysis window, right-clicking and selecting "Calculate SFE". SFE is then automatically calculated in calculated results. The angles can be also chosen manually from the measurement data by right-clicking on the contact angle and choosing "mark as contact angle". Detailed instructions are explained in Chapter 6.4.

3.8 Surface tension components experiments

In a similar way as surface free energy, the surface tension of liquid can be divided into dispersive and polar parts. Surface tension components calculations can be calculated on the Analysis side. In surface tension components (STC) calculations, data from Sessile Drop and Pendant Drop experiments are utilized. To make STC calculations, measure first the contact angle with a liquid of interest against a known dispersive substrate, for example Teflon. Then measure surface tension of the same liquid using pendant drop. On analysis side, the two experiments can be combined by choosing them on the analysis window, right-clicking and selecting "Calculate STC". STC is then automatically calculated in calculated results. The angle and the surface tension can be also chosen manually from the measurement data by right-clicking on the value and choosing "mark as contact angle/surface tension". Detailed instructions are explained in Chapter 6.5.

4 General operation instructions

4.1 Touch display overview

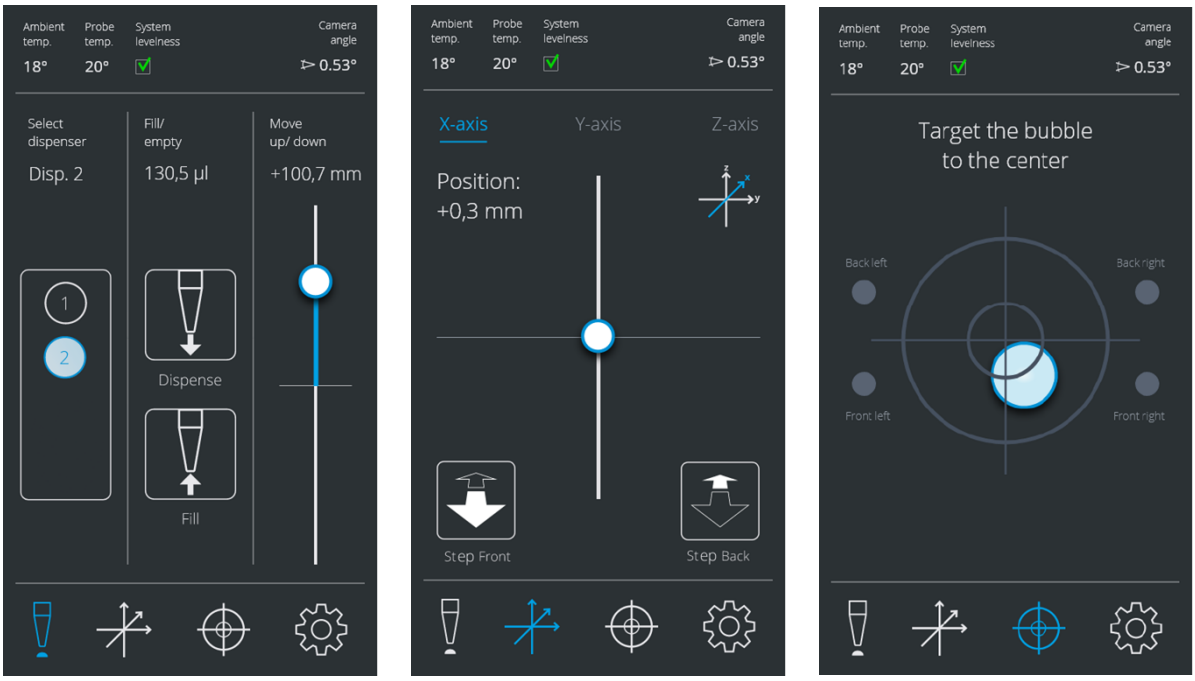
The integrated touch display can be used to operate the automated devices and to level the instrument. Note that OneAttension software needs to be turned on to operate the instrument from the touch display.

The upper part of the screen displays the ambient temperature of the integrated temperature sensor, status of the instrument levelness and the camera angle. It also displays the probe temperature if an external temperature probe is connected to the instrument. The middle part of the screen contains control buttons for the devices on separate tabs. From the bottom part of the screen, you can navigate through the different tabs.

The first tab contains controls for the dispenser and the dispenser holder. In case multiple dispensers are connected to the instrument, choose the active dispenser from the left side of the screen. Use the Dispense and Fill control buttons to fill and empty the dispenser. The dispenser holder is controlled from the vertical slider. Drag and hold the slider up to move the dispenser holder upwards, and vice versa for down. Release to stop the movement. The further away from the center you move the slider, the faster the dispenser holder moves.

The second tab contains controls for the automated stages (automatic X sample stage and automatic XYZ sample stage). The stage movements are controlled similarly to the dispenser holder movement. With the automatic XYZ sample, choose first to which direction you want to move the stage (X-axis, Y-axis, Z-axis). You can also move the stage by steps with "Step" buttons in all directions. The step length for the X-direction can be set in the recipe and is only valid when the measurement script is opened. Otherwise default step lengths are used.

The third tab shows the system levelness from the integrated level sensor. To level the instrument, rotate the instrument feet and target the bubble to the centre of the crosshair. The touch display will indicate when the system is levelled.



Touch display tabs.

The fourth tab displays the firmware version of the touch display and has controls to adjust the display brightness.

4.2 Software overview

The OneAttension software that runs Theta Flow has an easy and user-friendly interface. When starting the OneAttension program (located by default on the desktop and in the Start menu under Programs>OneAttension) first time, you are able to choose User Manager option (see figures below) In the User Manager option you are able to create different user levels in the setup window by clicking Create new user under the user settings. In addition to Administrator, who can manage all the users (i.e. create and remove users), Programmer and Operator user levels can be created. The Programmer can create recipes but cannot manage the other users. The Operator can run ready-made recipes. See below a complete list of what each user level can do in the software. The User Manager option allows setting preferred level of privacy: recipes and saved results can be chosen to be private only for the user.

Administrator:

- Creates new users including operators, programmers and administrators
- Adjusts permission levels and passwords of existing users
- Can switch privacy settings on specific experiments
- Modifies global settings, including path for saving data
- Edits experiment recipes
- Can delete experiments
- Can import experiments
- Can export experiments
- Can run experiments

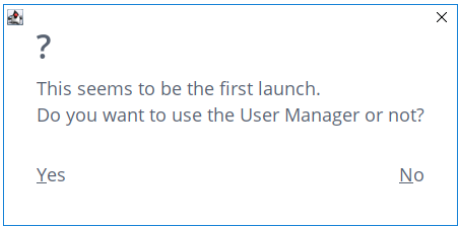
Programmer:

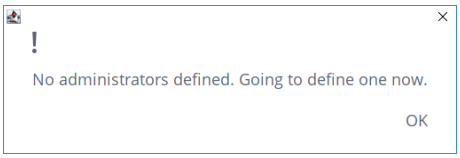
- Modifies global settings, including path for saving data
- Edits experiment recipes

- Can delete experiments
- Can import experiments
- Can export experiments
- Can run experiments

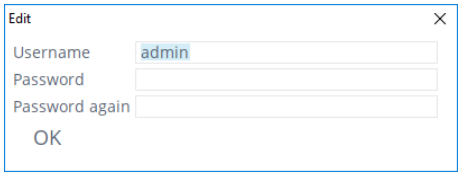
Operator:

- Can export experiments
- Can run experiments

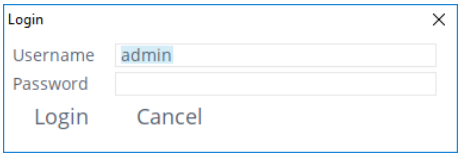




The first launch will give you an option to use User Manager. In case the User Manager option is chosen the administrator will be created.



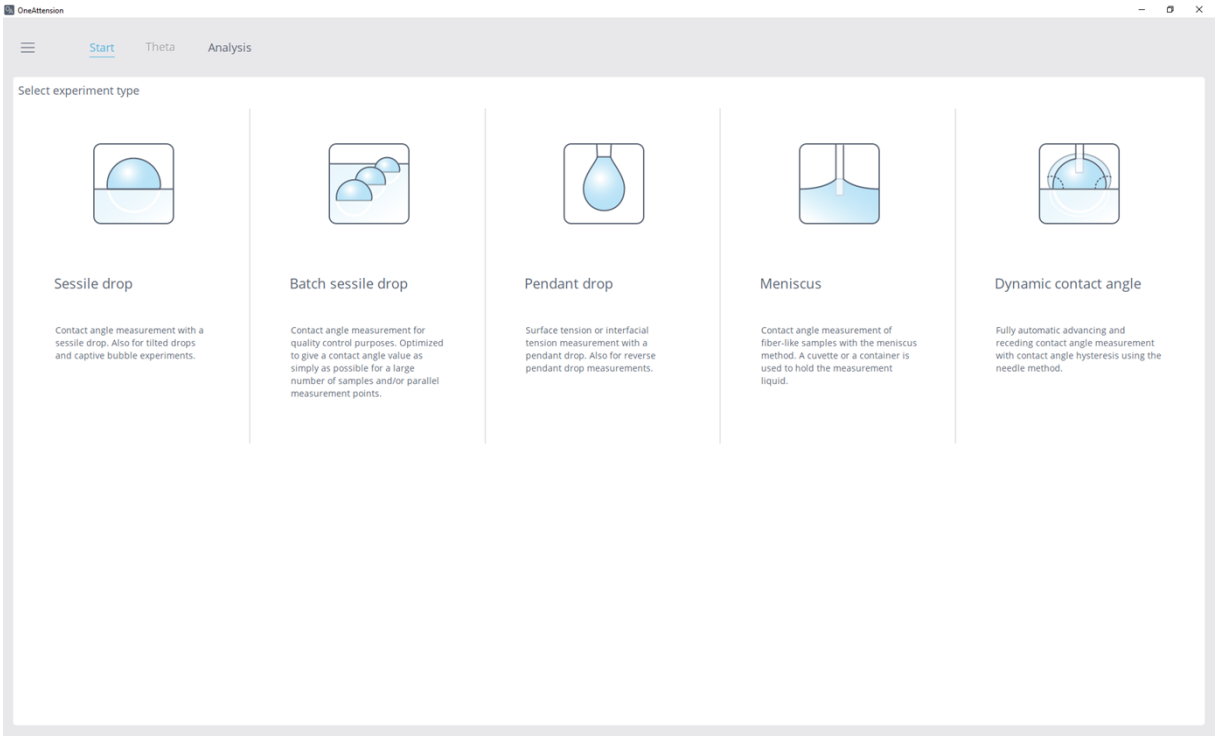
Create username and password for the administrator.



Login with the created username and password in the OneAttension software.

OneAttension is easy-to-use software with a clear and logical user interface. OneAttension includes the following parts:

Start tab: This is the default view after starting OneAttension software. The tab shows all experiment that can be performed with your instrument. Please note that if no instrument is connected to the computer, this tab is not visible. By clicking an icon, a new experiment is be started and the software will switch to **Theta tab**. The experiment choices with Theta Flow include Sessile drop, Batch sessile drop, Pendant drop, Meniscus, Pulsating drop and Automatic dynamic contact angle (auto-DCA). Please note that the Pulsating drop experiment is available only when Pulsating drop module is connected to the instrument. Automatic dynamic contact angle is available only when an automatic dispenser is connected to the instrument

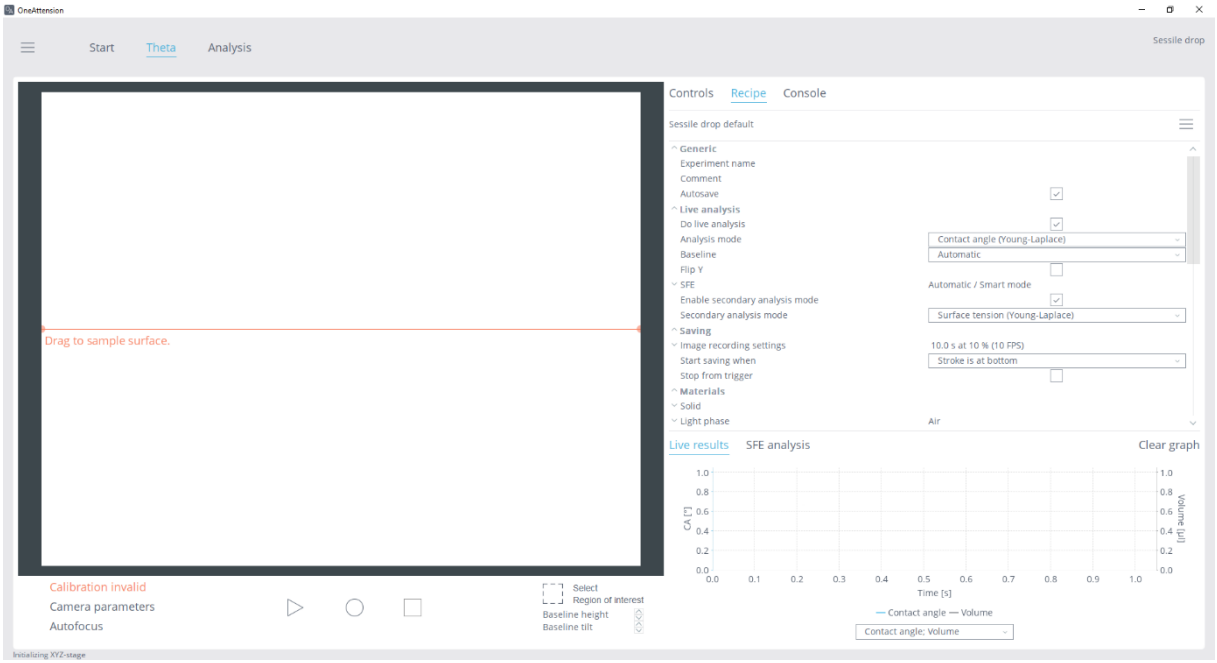


Start tab.

Recipes contain a set of experiment parameters. Default recipe is loaded automatically when an experiment is selected. A recipe is considered default if its name contains the word "default" (case-insensitive). If there's more than one candidate, the first one (or the first one owned by the current user) is selected. Users can create and save recipes, as well as browse and load them by clicking on the top right corner of Recipe tab. The measurement window will then automatically appear with the pre-defined values in the recipe.

You can also open a pre-defined experiment recipe by scanning a barcode that is saved to the barcode database. This can also be done when an experiment is opened. If a measurement is running, the software will ask you to abort it before opening the pre-defined experiment.

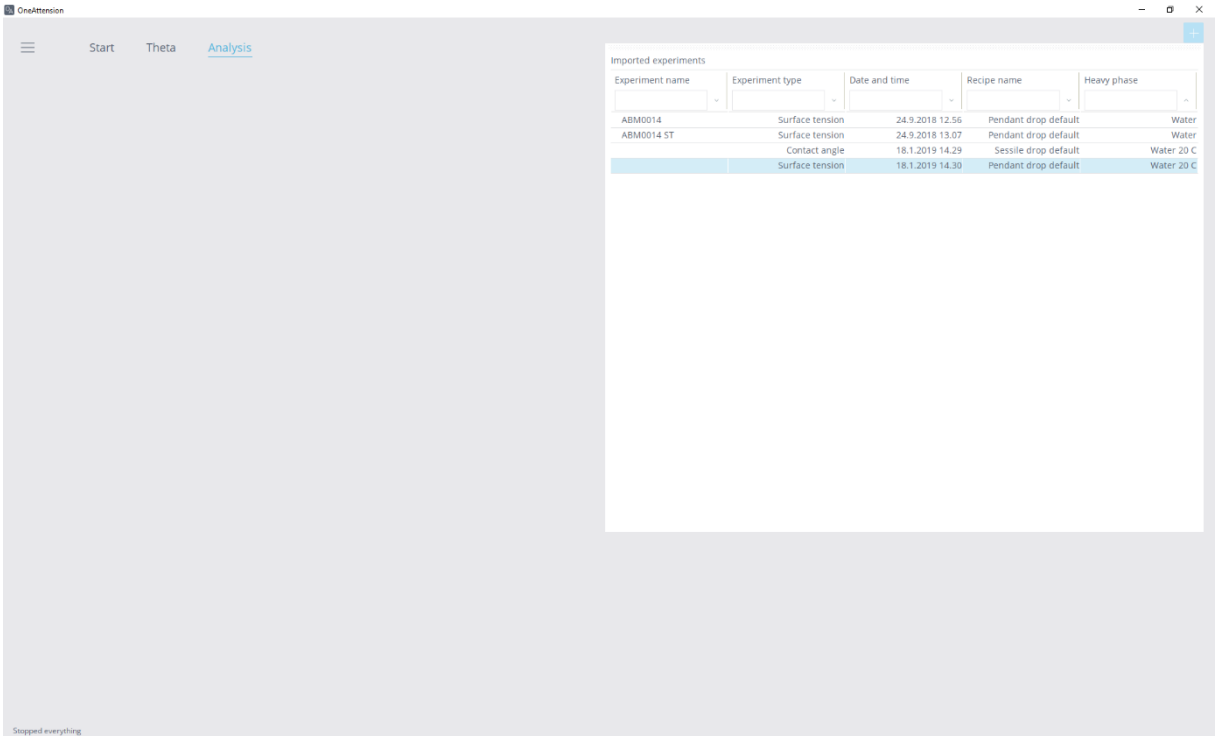
Once a pre-programmed experiment has been chosen by choosing an experiment icon in the **Start tab**, **Theta tab** opens and the computer must be informed with various parameters specific to the current experiment. The values for these parameters are entered in the Recipe tab on the right-hand side of the window. This also helps you to keep track in your performed measurements. You may click on the field and type in the required information or select from choices available in a particular Database. The actual performance of the experiment occurs after the Recipe has been filled out and the materials for the experiment are in place. Once all the experimental controls have been chosen the experiment can be started. The experiment will then continue unsupervised until completion.



Theta tab with Recipe tab visible.

Analysis tab: After an experiment has been performed you can return to the data for further analysis. Click the + sign on top right corner to open up the list of experiments and select the experiment you would like to analyze by double-clicking it. Now the data for that experiment will be displayed. Values used for the calculated results are marked as blue.

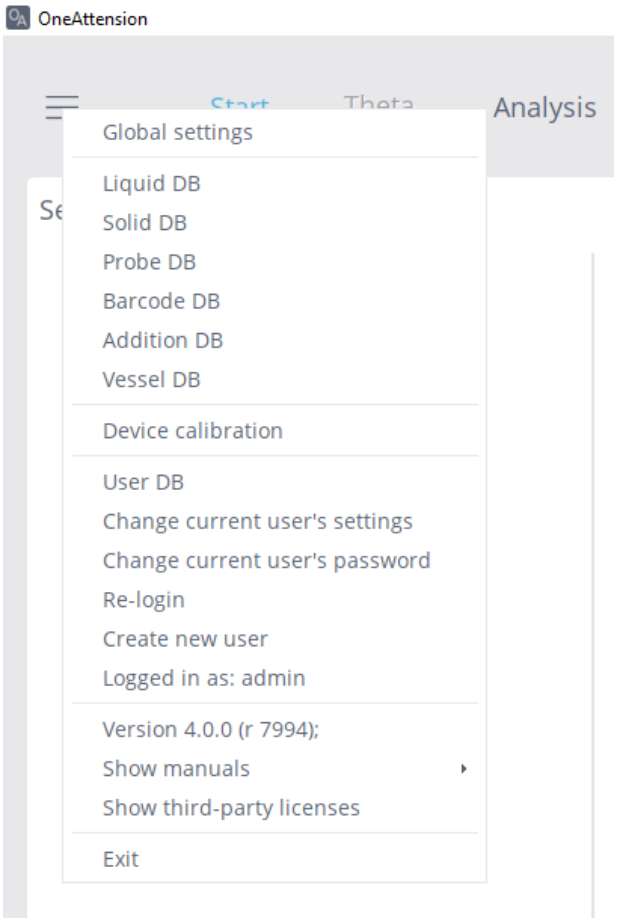
You then have the option of viewing and editing the recipe. This can be very helpful if you wish to recalculate the data produced based on new information about the materials involved. You can also look at a variety of graphs for your data. Calculation of additional results and export of data can be done. You can also print reports of experiment from the **Analysis Tab**. By right clicking on the experiment name on the list of experiments, you are able to Import or Export an experiment in OneAttension .bs-or Excel (.xls/xlsx) format, among other options. You can also select multiple experiments and export them simultaneously. If you scan a barcode when the cursor is either in Experiment name or Recipe name field, only those experiments/recipes that match with the barcode are shown.



Analysis tab.

Main menu: You can open the main menu from top left corner of the window. The menu provides access to all application settings, including liquid, solid, probe, barcode, user, addition and vessel databases as well as global settings and device calibration window. From the menu you can also change current user's settings and password and create new user accounts. In addition, by clicking "Show manuals" you are able to access your instrument manuals.

Adding information to the databases is simple. Just open the database and right click on top of it and choose add. Different information is asked depending on the database. It is also possible to import data from Excel (.xls/xlsx) or comma-separated values (.csv) files to the database.



Main menu.

Global settings: In this part of the software, you are able select the path where your experiments are saved, choose user language (English/Chinese), as well as choose the additional hardware you are using (see figure below).

Liquid database: Database for creating and editing probe liquid alternatives for recipes.

Solid database: Database for creating and editing solid material alternatives for recipes.

Probe database: Database for creating and editing Sigma force tensiometer probe alternatives for recipes. Not utilized with Theta optical tensiometers.

Barcode database: Database for creating and editing barcodes to quickly open experiment recipes with optional barcode scanner.

Addition database: Database for creating and editing addition alternatives for recipes. This section is mainly related to Sigma force tensiometer measurements.

Vessel database: Database for creating and editing vessel alternatives for recipes. This section is mainly related to Sigma force tensiometer measurements.

Systems diagnostics/ Device calibration: Function for analyzing the communication between devices and computer as well as for the calibration of devices. Can be activated by selecting **Device calibration** on the main menu. Automated single liquid dispenser can be calibrated from this section (see figure below).

User settings in the main menu will allow managing the user properties as follows:

User DB: Shows the list of all users. Administrator level users can edit other user accounts. It also possible to export and import user lists (.xls, txt or .csv file formats).

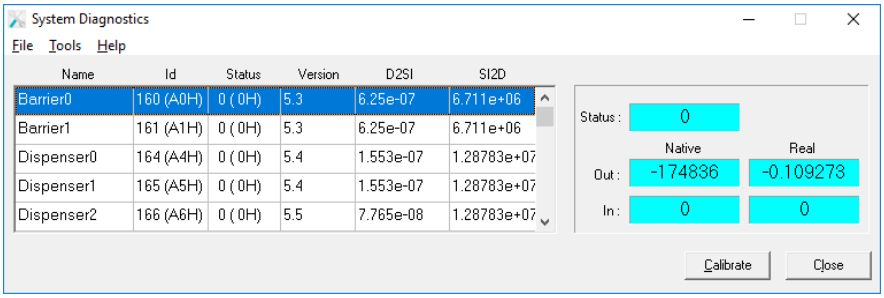
Change current user's settings: Enable to select the path for each user, where the measurements will be saved.

Change current user's password: The User can change her/his password.

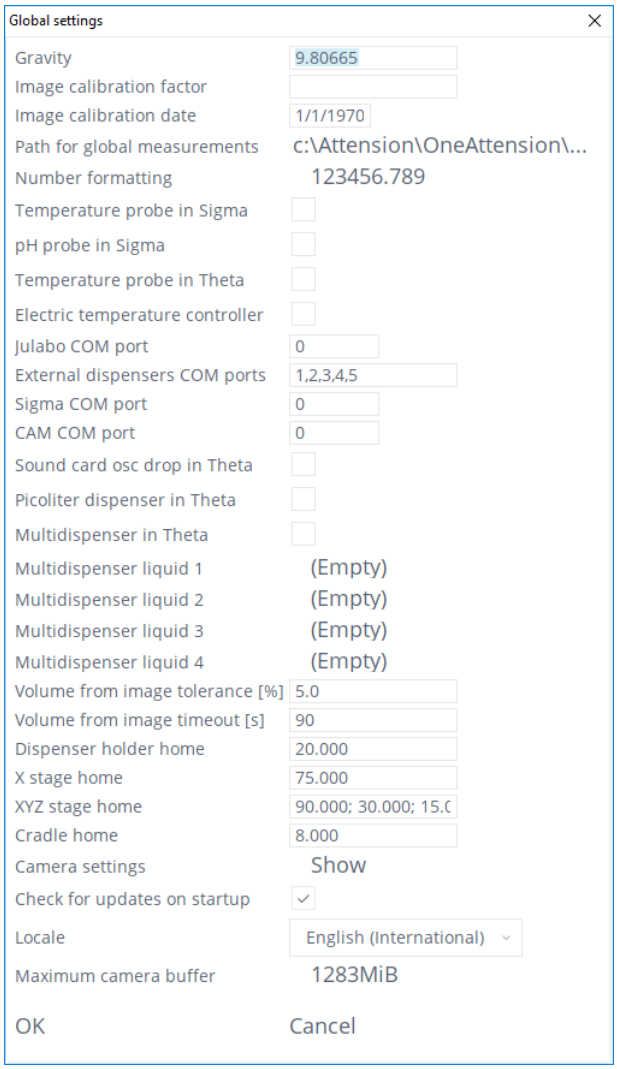
Re-login: The other user can re-login in the system without shutting the program down.

Create new user: Administrator level users can create new users.

Logged in as: Will show the name of the current user.



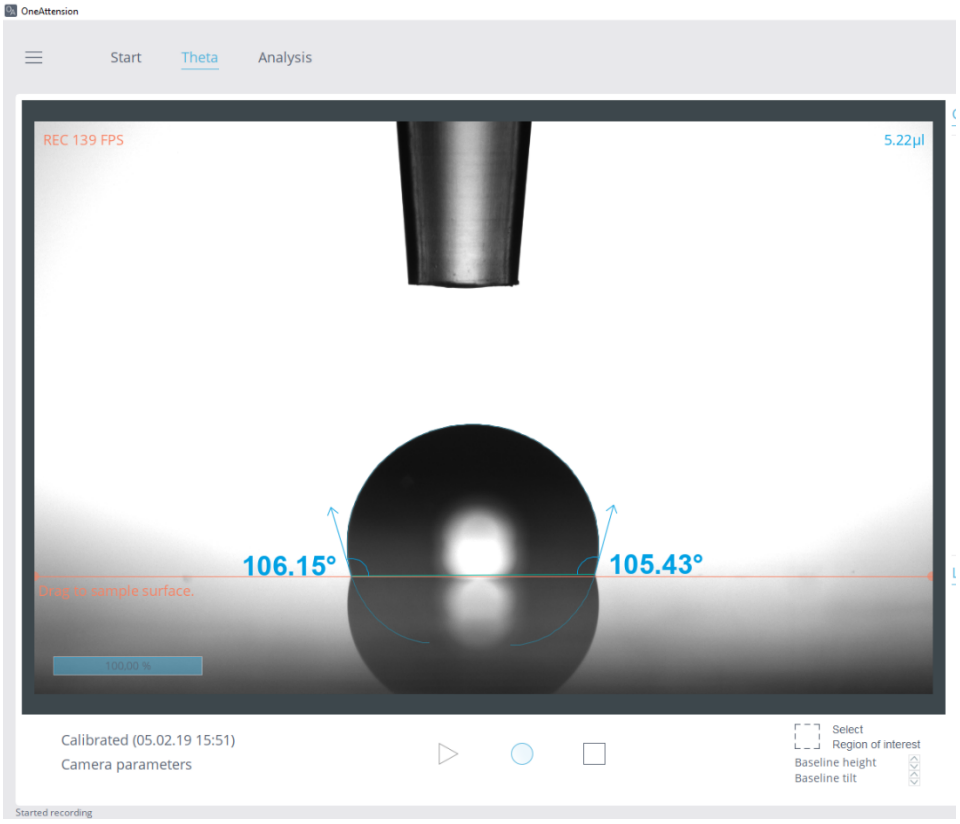
System Diagnostics window.



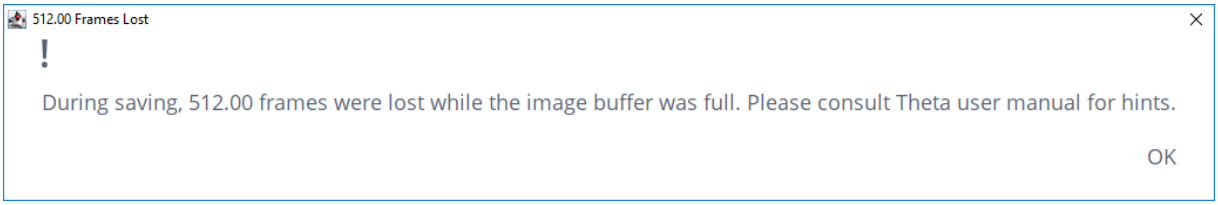
Global settings window.

One of the parameters in the Global Settings is called **Maximum camera buffer**. The cameras have their own memory buffer which they use to store images during measurement. From the buffer, the pictures are saved to the hard disk of the PC. During a measurement, at the same time data is being transferred to the camera buffer and from there to system hard disk.

Especially with the USB3 camera, it is possible to obtain so much data that the camera buffer will get full during a measurement due to the high resolution of the pictures and high framerates. If the buffer is full, it is no longer possible to save more images until some of the data is moved into system hard disk. For user, this will appear as some frames not being saved during a measurement. If this happens, OneAttension will indicate that the buffer may get full during measurement and after measurement it will show how many pictures were lost due to buffer being full.



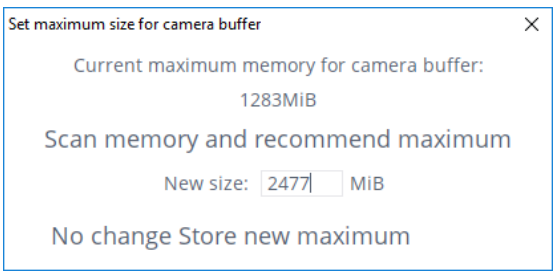
Camera buffer indicator at the bottom left corner of the image will appear if it looks like the image buffer might get full. When it's at 100%, the buffer is full and some images are being lost.



Post-measurement message indicating that some frames were lost during saving.

<u>Tips for how to avoid losing images during high speed and high-resolution measurements:</u>

- **Adjust the camera buffer**. The buffer has a default value that depends on your computer RAM. If the buffer starts getting full, the buffer size can be adjusted in Global Settings → click on the maximum camera buffer. OneAttension can scan your computer's RAM to recommend a new maximum value. After scanning, choose "Store new maximum" to save the scan result.



- In order for the images to transfer quickly to your PC hard disk, the disk needs to be fast in data transfer. For optimal results, an **SSD hard disk with > 500 Mb/s transfer speed** is required. Please consult your IT department on the possibility to get an SSD hard disk installed. After installing the disk, update the path where the results will be saved in Global Settings → Path for global measurements to point at the new SSD disk.

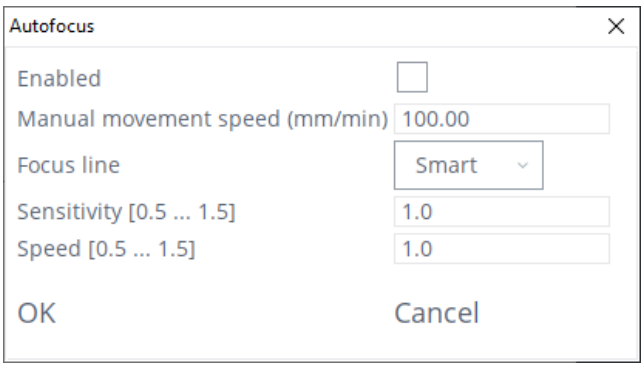
- **Close any heavy processes** that might be running at the background of the PC. Do not for example do any copy-pasting or downloading while the image capturing is in progress.

Only save the required frames. Most phenomena requiring high speed imaging, such as adsorption, happen usually within a few seconds. Optimize the measurement time and use the trigger function to start the measurement to only take the frames needed. This will also save hard disk space on your PC.

4.3 Autofocus

Theta Flow features an autofocus function that ensures the measurement is done with optimal focus automatically. Press **Autofocus** button at the bottom left of **Theta tab** to activate it.

There are three different autofocus modes. **Smart** mode prioritizes the focusing on the droplet. In case there is no droplet in the camera view, smart mode will focus on the needle. **Custom** mode features a focus line that can be dragged to the position desired to be focused on the image. **Middle** mode will always focus into the middle of the view. If you are using dual dispenser unit, rotate the camera lens focus ring (#10) all the way to look close to give enough space for the camera to move in autofocusing.



Autofocus window.

Sensitivity (scale from 0.5 to 1.5) determines how easily the autofocus starts looking for a new focus point. High sensitivity means the autofocus will re-focus when there is even a slight change in the focus. Low sensitivity means the change in focus needs to be larger for the refocusing to occur.

Speed (scale from 0.5 to 1.5) is the movement speed.

The autofocus performance relies on the camera calibration factor. If the calibration factor isn't correct, the autofocus may not work well.

It is possible to also focus the image manually. Disable the autofocus function from the Autofocus window and focus the image using the camera lens focus ring (#10). Depending on your configuration you might need to move the camera on its rail to get the image focused. You can move the whole camera stage on its rail from the controls. The speed for the manual camera movement can be set from the 'Autofocus' window.

4.4 Calibration

Calibration with ball

A calibration must be made when you use the instrument for the first time and every time the magnification (zoom) has been altered. If Autofocus is not used and you need to change the focus after calibration, do it by moving the whole optics (from the software) or the stage, not from the focus ring. This is especially important when Pendant drop mode is used. If change focus from the focus ring during Pendant drop measurement it is also good to recalibrate the system. After logging in to the OneAttension for the first time, select **Calibration invalid** at bottom left of **Theta tab** to perform calibration. After the system has been calibrated, the latest calibration time is shown in this button.

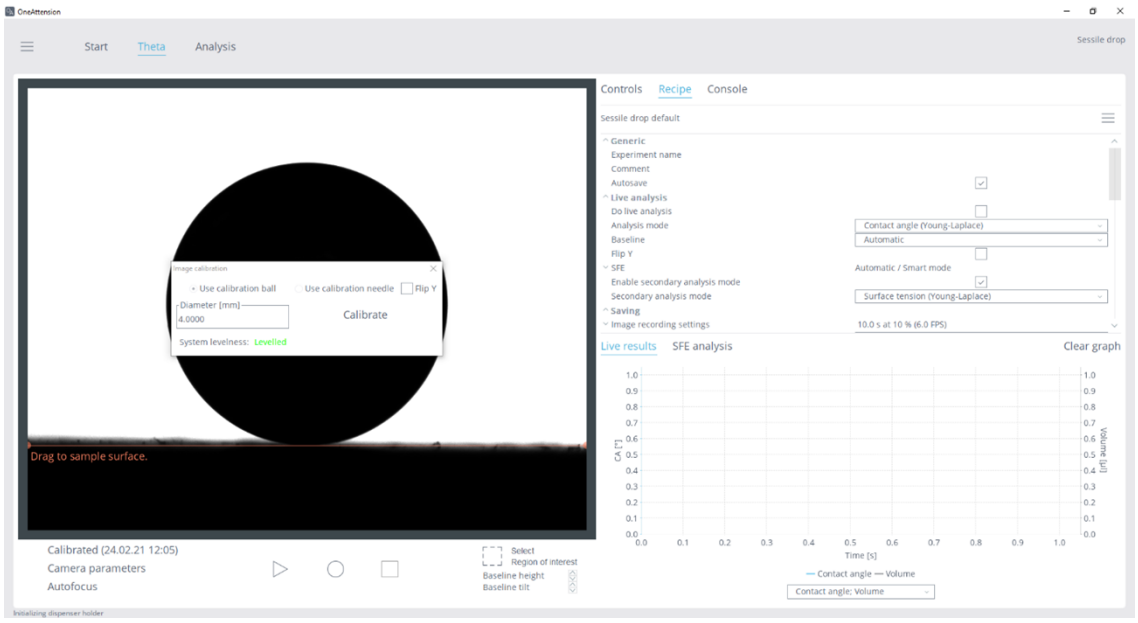
The calibration is valid for all camera modes even though it has been performed on one mode only if the magnification isn't changed.

Place a calibration ball and its magnetic holder on the sample stage. Bring the ball into the image on-screen and zoom it appropriately by altering the lens zoom adjustment (#11). The larger the drop on screen, the greater the accuracy. Therefore, always try to have the most zoomed image possible whilst still seeing the entire image. If the zoom is changed, you must recalibrate! While doing the calibration, you can also adjust the angle at which the camera looks at the sample stage. This is done by turning the camera tilt adjustment knob (#14).

Camera tilt adjustment knob.

You can either use the autofocus function of the instrument or manually focus the image. Enable the autofocus by pressing **Autofocus** underneath the camera view. To manually focus the image, first disable the autofocus. Then turn the camera lens focus adjustment (#10) until the image is focused. If necessary, you can move the camera from the controls either away or towards the sample stage. Set the speed for the camera movement from the Autofocus window. In manual focusing, the focus tool is a useful tool: right-click on the image on the left or right edge of the calibration ball and click 'Focus tool'. A window pops up, showing the horizontal intensity and its gradient around the focus point. The focus point can be re-selected by clicking on the image again. Now, adjust camera focus so that the peak of the intensity gradient, corresponding the edge of the ball, becomes as high and sharp as possible. The optimal contrast is found when the difference between black and white is high enough, but the image is still not burned. This can be used together with 'Adjust camera parameters' dialog to optimize the image quality.

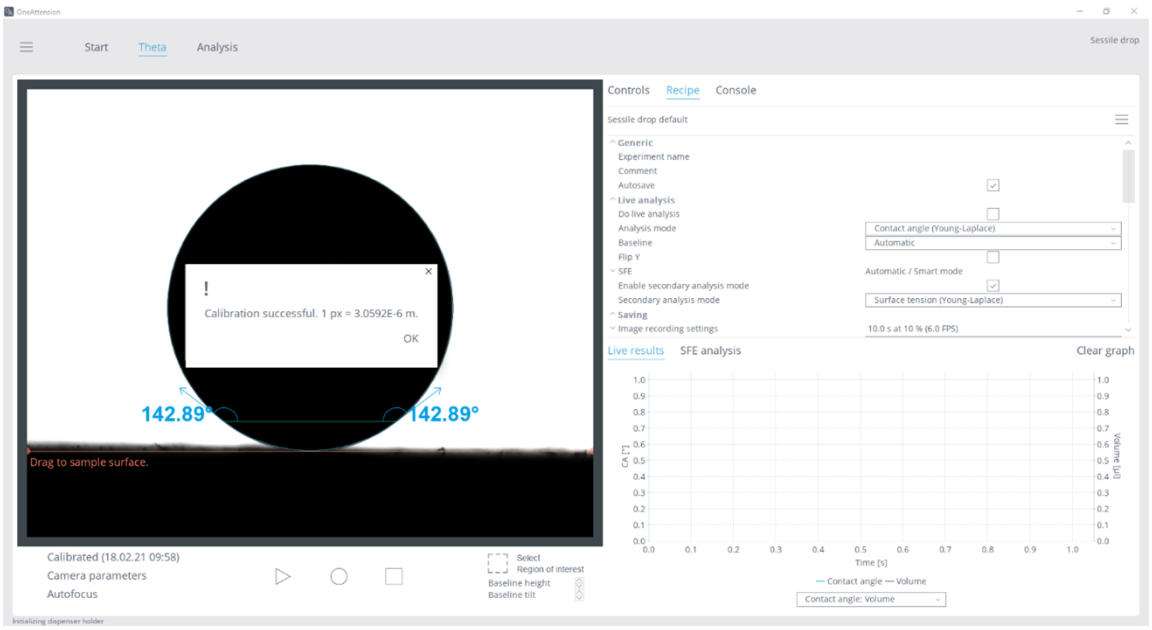
Once the calibration ball is in the center and focused (see figure below) press Calibration invalid. A screen will appear prompting for the diameter of the calibration ball. The nominal diameter is 4 mm, however the actual diameter of each calibration ball can be checked from the certificate that is delivered with the ball. The actual diameter should be used for calibration.



Choose the calibration method and accept the dimensions of the calibration tool.

There should now be a green line around the image of the calibration ball showing the curve that was fitted on it and screen showing the calibration factor. If the green line is not fitted closely around the ball image, the ball should be cleaned and image parameters and focus should be re-checked, and the calibration should be performed again.

Theta Flow is now calibrated. Press OK to finish.



Calibration performed successfully.

Calibration with needle

Calibration can be done using a needle. It is useful option e.g., when using picoliter dispenser (see Chapter 5).

This requires a needle or an equivalent object, such as calibration tool of the picoliter dispenser, with a precisely known width. The calibration is performed exactly in similar way than with the calibration ball, excluding that the calibration needle is chosen for a calibration method. You are able to fill any needle width as a dimension.

Needle diameter is commonly announced in gauges (G) which can be converted to mm. Table below shows the diameters of the needles provided by Biolin Scientific.

Needle gauge	Diameter (mm)
14 G	2.108
22 G	0.718
30 G	0.311

4.5 Setting up an experiment

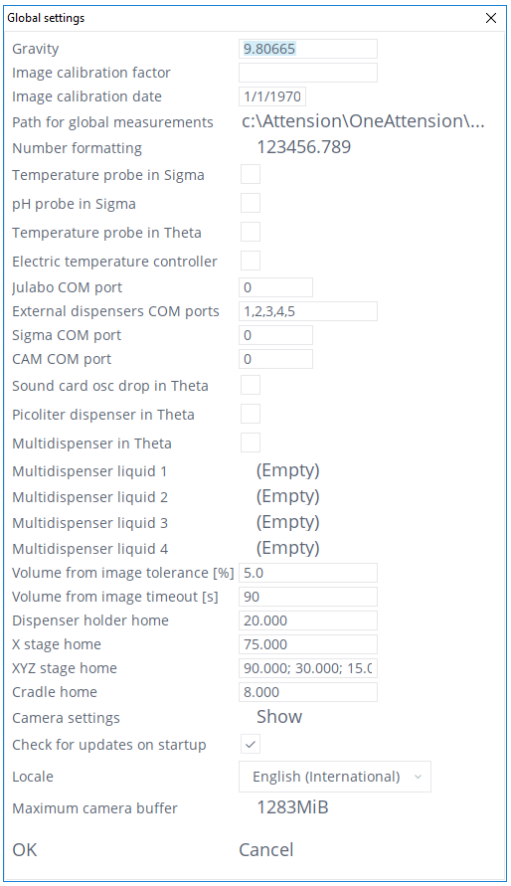
A. Preliminaries

Theta Flow instrument has six experiment types: Sessile drop, Batch sessile drop, Pendant drop, Meniscus, Pulsating drop and Auto-DCA. From the data obtained through Sessile drop experiments a further type of analysis, Surface free energy, is also available. From the data obtained through Sessile drop and Pendant drop experiments a further type of analysis, Surface tension components, is also available.

The procedures of the Sessile drop, Batch Sessile drop, Pendant drop Meniscus and Auto-DCA experiments are very similar and thus the main parts of each step will be explained together with further details per experiment type as necessary. Pulsating drop experiment operations will be explained in Chapter 5.

Before starting an experiment, you are able to modify databases (e.g. liquid or user databases) in the main menu. In Global settings window you are able to see the instrument parameters and also adjust them if needed. You are also able to set default liquids for each multi-liquid syringes. Following equipment need to be also enabled from here before the experiments:

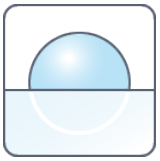
- Temperature probe
- pH probe
- Temperature controller
- External liquid bath (Julabo)
- Picoliter dispenser
- Multi-liquid dispenser
- Theta COM port: COM port number for older RS-232 connected Thetas. Use 0 unless you have such.
- Sound card osc drop in Theta: Check this if, and only if, you're using the old, sound card based pulsating drop device.



Global settings window

Start the experiment

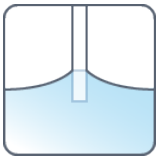
Choose the preferred experiment type by clicking the experiment icon. After this, automated devices will automatically initialize their locations. You can also open a pre-defined experiment recipe by scanning a barcode that is saved to the barcode database. This can also be done when an experiment is opened. If a measurement is running, the software will ask you to abort it before opening the pre-defined experiment.



Sessile drop experiment

Batch sessile drop

Pendant drop experiment

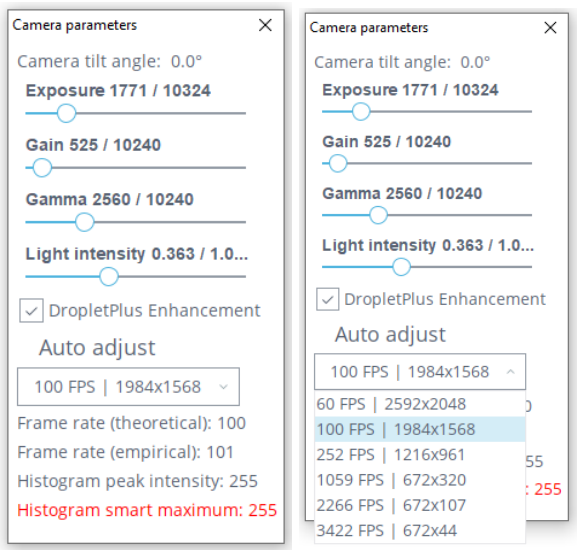


Meniscus experiment

Pulsating drop experiment (requires pulsating drop module)

Automatic dynamic contact angle (Auto-DCA) experiment (requires an automatic dispenser)

After the experiment is selected, it is possible to adjust camera parameters by clicking **Adjust camera parameters** at bottom left of **Theta tab**.



Camera parameters.

Select auto adjust to let OneAttension to optimize the camera parameters. You can also fine tune the parameters manually. The camera parameters window also displays the camera angle.

Some cameras have more than one camera mode that can be used. You can choose which mode to use in the drop-down menu. Each mode has its own camera parameters, so you need to change the parameters for each mode separately.

The presented frame rates are maximum values. Some functions such as using the live analysis may slow down the actual imaging speed.

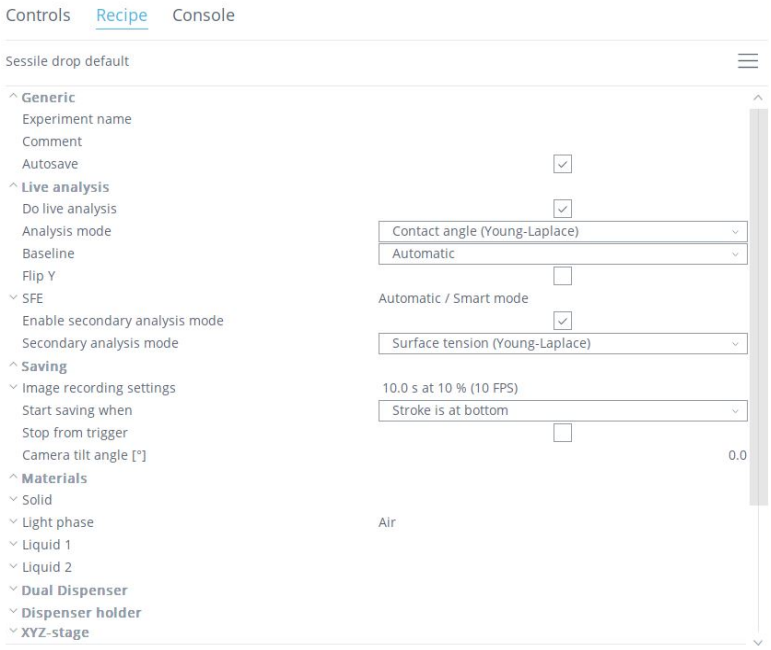
Theta Flow features a Droplet Plus technology that uses image enhancement algorithms to optimize the image quality. It enhances the droplet sharpness and baseline visibility. You can activate it from the camera parameters by enabling **DropletPlus Enhancement**.

Make sure that all the equipment to be used with the experiment are cleaned, usually performed by rinsing with ethanol and distilled water. Bring the dispenser into the image and then bring the solid sample stage to the appropriate position so that for

- Sessile drop and Auto-DCA experiments the solid surface is visible and level.
- Pendant drop experiments the sample stage can be removed from the image.
- Interfacial tension experiments the liquid inside a cuvette, or another vessel completely fills the screen.
- Meniscus experiments the liquid surface is visible and level.

Check the quality of the image. Do not change the zoom without a recalibration! Enable the autofocus by pressing **Autofocus** button at the bottom left of **Theta tab** and choose one of the autofocus methods. Autofocus feature ensures the measurement is done with optimal focus automatically. In case manual focusing is preferred, disable the autofocus function. Focus the image using the camera by primarily moving the camera from the controls, use focus ring only if needed. After calibration change the focus only by moving the camera from the controls. The speed of the camera movement can be set from the 'Autofocus' window. An image focused on the needle is generally also focused on a drop from that needle.

When starting a measurement, the first thing will always be to fill in the experimental parameters in the recipe tab (see figure below). After the parameters have been filled once, you are able to save the recipe and load it next time by clicking on the top right corner of the recipe tab



Recipe tab. Created recipes can be loaded or saved by clicking the top right corner.

The Recipe parameters are as follows (please notice not all parameters are visible at once – they depend on the measurement type and hardware configuration):

Generic

Experiment name: a name needs to be given for your experiment. This also makes the recognition of the experiment easier later on, when browsing experiments for further analysis.

Comment: Additional comments can be added if necessary.

Autosave: Software automatically saves your experiment. If not chosen, the software asks after each experiment, do you want to save the results.

Private to admin: Only the current user can see the results. If not chosen, results are available for all users. Option is only available if user manager is enabled.

Live analysis

Do live analysis: Enables real-time result analysis. Results are shown in the Live analysis screen in the lower part of the tab.

Analysis mode: Drop-down menu enabling to choose preferred analysis (i.e., fitting) mode.

Baseline: Use manual, automatic, or circular (automatic) baseline in the live analysis.

Flip Y: Needs to be chosen if a hooked needle is used. The analysis software will then flip the image vertically to be able to perform the analysis. Image will not be flipped on the screen.

Evaporation compensation: If enabled, the algorithm uses the dispenser to automatically fine-tune the drop size so that it stays constant in the long run. For the evaporation compensation algorithm to work, live analysis must be running, and the dispenser must be enabled. The evaporation compensation features a feedback loop mechanism to control the droplet volume to stay constant during the measurement. Based on the difference between the current and the desired droplet

volume, the instrument uses PID controller to apply correction to enhance or reduce the dispensing rate. PID controller consists of proportional, integral, and derivative terms.

PID Drop-down menu to set the PID controller's coefficients used in evaporation compensation. Coefficient P is a weighting factor for the current error, i.e., the error between current and target droplet volumes. The greater the weighting factor is, the stronger the system tries to correct the error. Too great value will result in an overshoot of the droplet volume, while with a too small value the droplet will never reach the desired volume. The integrator term is used to compensate possible overshoot error by integrating the error over time, and is weighted by coefficient I. The derivative term, weighted by coefficient D, further improves the dynamic system controlling by altering dispensing rate based on the error's current rate of change. It might be necessary to adjust the weighting factors as the same values might not work with different sized droplets, in different temperatures or humidities, and different applications might demand more accurate droplet volume than others. In these cases, it is advised to understand the principle of a PID controller before changing the values. The fine-tuning can be started by setting the I and D terms to be zero and then increasing the P term until system reaches oscillating behaviour around the set droplet size. Then increase the I term to dampen the oscillation and D term to get faster system response.

Enable secondary mode analysis: If enabled, OneAttension can automatically switch between two analyze modes to fit whichever works better with the image onscreen. Can be used for example for determining hanging drop volume in sessile measurements with manual dispenser when surface tension has been chosen as the secondary analysis mode.

Secondary analysis mode: Drop-down menu enabling to choose preferred secondary analysis (i.e., fitting) mode for live analysis.

SFE: Shows surface free energy analysis mode and contact angle selection.

SFE Enabled: Enables SFE calculation on live side.

SFE Contact angle: Drop-down menu for choosing contact angle to calculate surface free energy on live side. Smart mode is used as default, in which data is partitioned into subsections, analyzed and ranked in order to favor less noisy subsections, and finally the subsections are combined, and average CA is calculated. Additionally, last analysed frame or N seconds after first analysed frame can be used.

SFE Analysis model: Drop-down menu for choosing surface free energy analysis model on live side. Automatic mode uses Equation of State for single-liquid measurements. For multi-liquid measurements, OWRK is used.

N seconds: Defines N seconds when using “N seconds after first analyzed frame” in “SFE Contact angle”.

Surface tension: Shows automatic surface tension result selection mode.

Enabled: Enables the automatic surface tension result selection.

Surface tension: Drop-down menu for automatic surface tension result selection mode. Smart mode is used as default, in which data is partitioned into subsections, analyzed and ranked in order to favor less noisy subsections, and finally the subsections are combined, and average ST is calculated. Additionally, last analysed frame or N seconds after first analysed frame can be used.

N seconds: Defines N seconds when using “N seconds after first analyzed frame in automatic surface tension result.

Saving (options depend on the configuration)

Image recording settings: Summary of chosen image recording settings. By clicking this button, different camera parameter combinations can be saved.

Time points: The only image recording parameter in a Batch sessile drop measurement. Create a list of points of time when the contact angle is analyzed from the live image.

Mode: *Basic* mode is chosen as a default mode, which enables to image one phase frame per second (FPS) measurements. In case two phase imaging (initial + final) is preferred, choose the *Advanced* mode. *Live images only* mode records only live images, in which the frame rate cannot be adjusted.

Total duration [s]: Total recording time including both initial and final recoding times.

Initial pause [s]: Pause before starting recording. Typically used in contact angle experiments, when the most stable contact angle value is recorded after a certain time period. You can also set a negative value as the Initial pause. In that case, results will be saved already before the recording is set to start. For example, if you are using a trigger to start the recording and set the Initial pause to -1, frames from one second before the trigger fires will be added to the beginning of the measurement.

Initial FPS: In case Advanced Image recording mode is used, the initial frame rate can be set here.

Initial duration [s]: In case Advanced Image recording mode is used, the initial recording time is set here. Software automatically calculates the initial duration, after the total and final duration has been set on the screen.

Final FPS: In case of Advanced mode is used, the frame rate of the final phase is set here. In Basic mode, the frame rate is set here as well.

Final duration [s]: In case of Advanced mode is used, the final frame rate can be set here.

Start saving when (available when there is an automated dispenser): Saving options for measurement. Options include for example start from trigger and start when the drop is out.

Start from trigger (available when there is no automated dispenser): Create a trigger on screen. Recording starts when the pixel changes either from white to black or from black to white.

Stop from trigger: Create a trigger on screen. Recording stops when the pixel changes either from white to black or from black to white

Camera tilt angle: Specify here what tilting angle you want to use in the measurement. Then set the tilting angle from the camera tilt adjustment wheel. If the specified tilting angle differs more than ±0.1° from the actual angle, the software will warn you before starting the measurement. Recommended values are 0° for pendant drop measurements and 0°...-2° for sessile drop measurements.

Materials

Solid: You can either choose a solid from the readily created solid database or add a new sample of your choice. This can be done the same way as the addition of the probe and the vessel. This time the "Editor" window will ask you a name for your sample, surface tension or surface energy of your sample (γ), dispersive component of surface tension (γ_d), acid component of surface tension (γ^+), base component of surface tension (γ^-) as well as the density (ρ).

Light phase: Can be chosen from the readily created database or be added as above. Additional parameters are the viscosity (η), temperature, and molecular weight of the phase.

Heavy phase: Can be chosen from the readily created database or be added as above.

Sdr roughness [%]: The Sdr roughness value of the specimen surface can be inserted here. The value can be known if the surface has been measured using a surface profiler. If the Sdr roughness has been written, the calculated roughness-corrected contact angles can be examined. For the definition of Sdr and information on the effect of roughness to contact angle values, please see the Topography section in the Theory section in this manual.

After *Materials* the recipe sheet will include separate parameters for all the automated devices, which are connected in the system. These parameters as well as device control options for automated devices will be explained in the Chapter 5.

When the recipe sheet has been filled, the experiment can be started from the buttons at bottom of **Theta tab**.

Start button starts the experiment according to all experimental parameters filled in the recipe sheet. Used only for experiments with automated functions. Not visible if no automated devices can be found.

Stops image recording and retains already recorded pictures.

Only record button will start recording according to Image recording parameters defined in the recipe sheet. Used for recording the results of manual experiments.

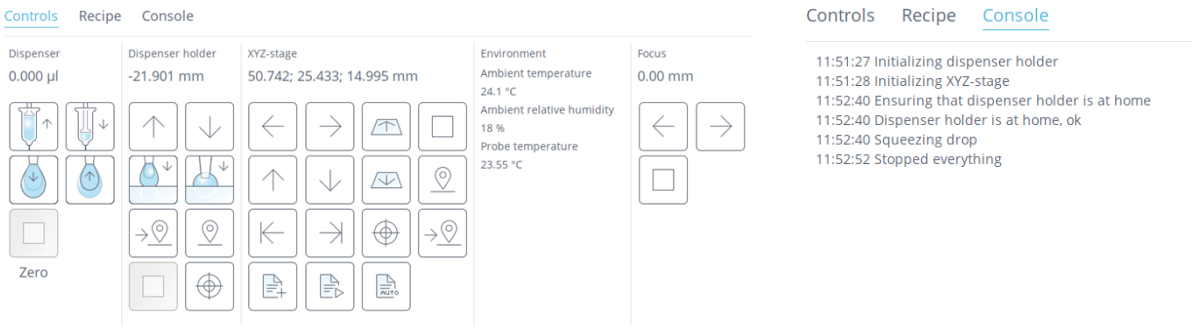
Back button goes back one sample in a Batch sessile drop measurement.

Forward button goes forward one sample in a Batch sessile drop measurement.

Real-time operating functions can be followed on the Console tab as shown in the figure below. The Console tab records all the actions. This might be useful especially with long lasting automated measurements when the user is not following the measurements in real-time. The current action can be seen also in the lower left corner of the whole screen. When it states 'Stopped recording', the results can be viewed in the **Analysis tab**.

Automated devices and manual camera movement can be controlled from the Controls tab. Controls tab displays ambient temperature and relative humidity measured by the instrument's integrated

sensors. It also shows probe temperature if an external temperature probe is connected and enabled from the Global settings.

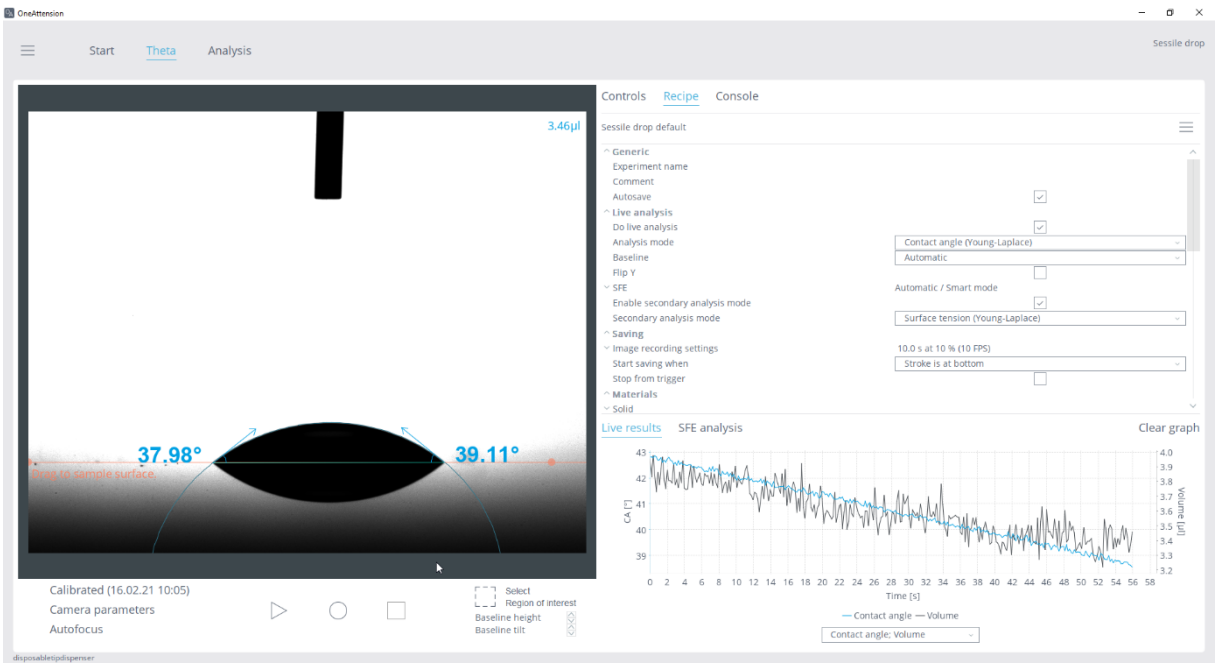


Controls and Console tabs.

Note! Some options in main menu are not available after the experiment type has been chosen. Click on the Start tab to quit the current experiment to enable all options in main menu.

B. Sessile drop experiments

Set the desired parameters in the recipe before starting the measurement. Then place "Drag to sample surface" line to the sample surface. Press start to begin the measurement. With automated devices, the dispenser will automatically create the droplet and place it on the surface.



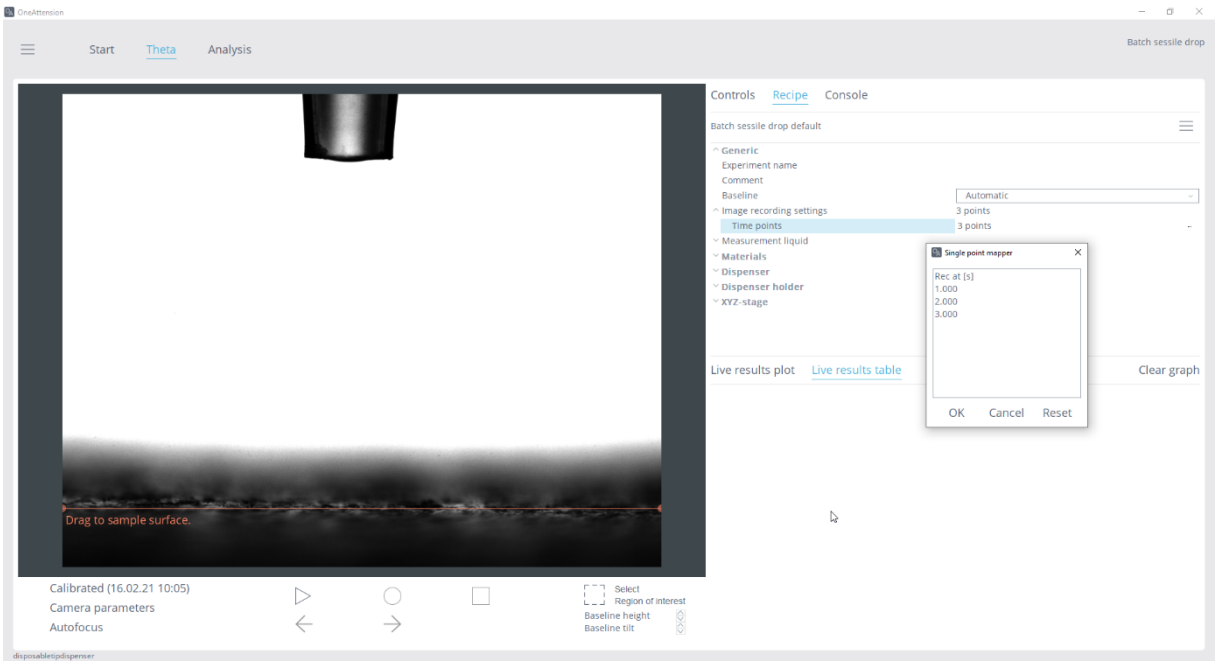
Live contact angle results.

C. Batch sessile drop experiments

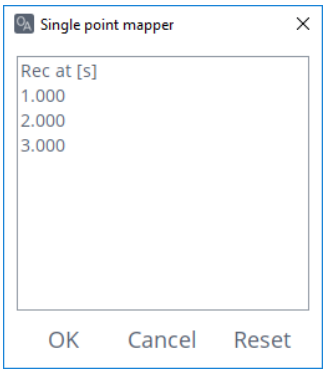
Note! Batch sessile drop experiment is not intended to be used with Tilting Cradle.

A Batch sessile drop measurement is a set of sessile drop contact angle measurements on several samples. The preliminaries and recipe parameters for a batch measurement are the same as for a single sessile drop measurement apart from the Image recording settings. In a Batch sessile drop

measurement, the contact angle analysis is done from the live image. Select the points of time at which you want to get a value for the contact angle by selecting Time points in the recipe sheet (see the image below). A single point mapper will appear. Fill in the desired points of time one below each other.



Creating a Batch sessile drop measurement recipe.

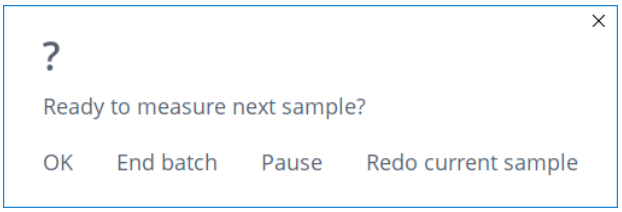


Single point mapper used in a Batch sessile drop measurement.

If you want to measure several points on one sample, set the sequencer accordingly. See instructions about the use of automatic sample stage movement and other automatic modules in Chapter 5.

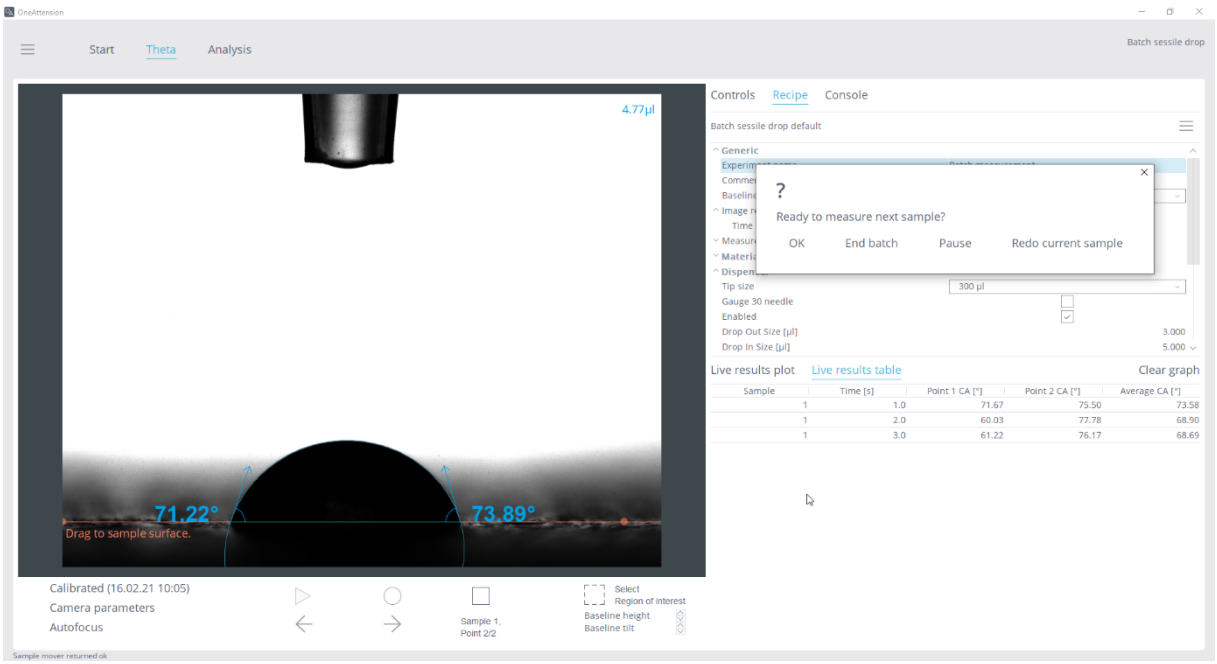
During the batch measurement, OneAttension software will give you instructions on what to do next. Depending on the configuration of your Theta Flow, you may be asked to dispense a droplet on the sample stage, move the sample on the sample stage, or place the next sample of the batch on the sample stage. Follow the instructions given by the software.

After measuring each sample, you are asked if you are ready to move to the next sample in the batch. If you are, place the next sample on the sample stage and press OK. You can also choose to redo the current sample, pause the measurement, or end the batch measurement.



After measuring one sample you are asked if you want to move to the next sample. If you want to move to measuring the next sample, place the next sample on the sample stage and press OK.

You are also able to keep an eye on the live results table or plot. If you want to measure some of the samples again you can use the Back and Forward buttons next to the Start and Stop buttons to move back and forward in the batch.

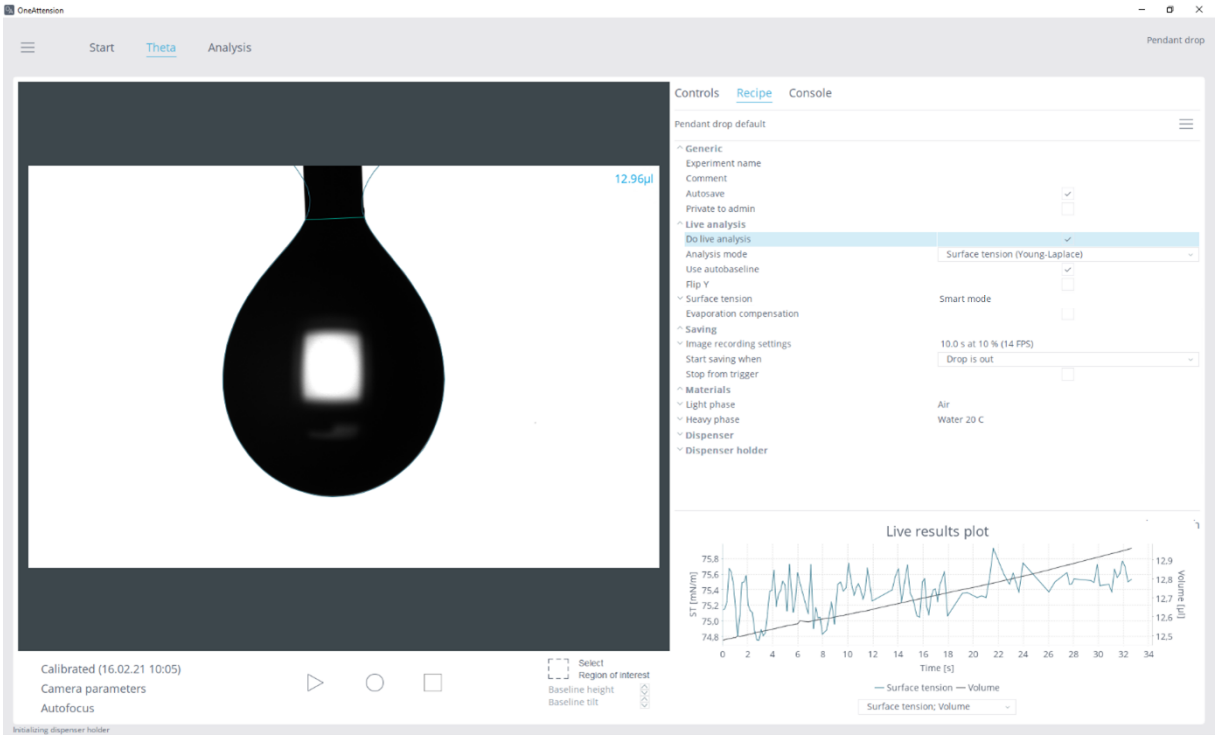


Live results.

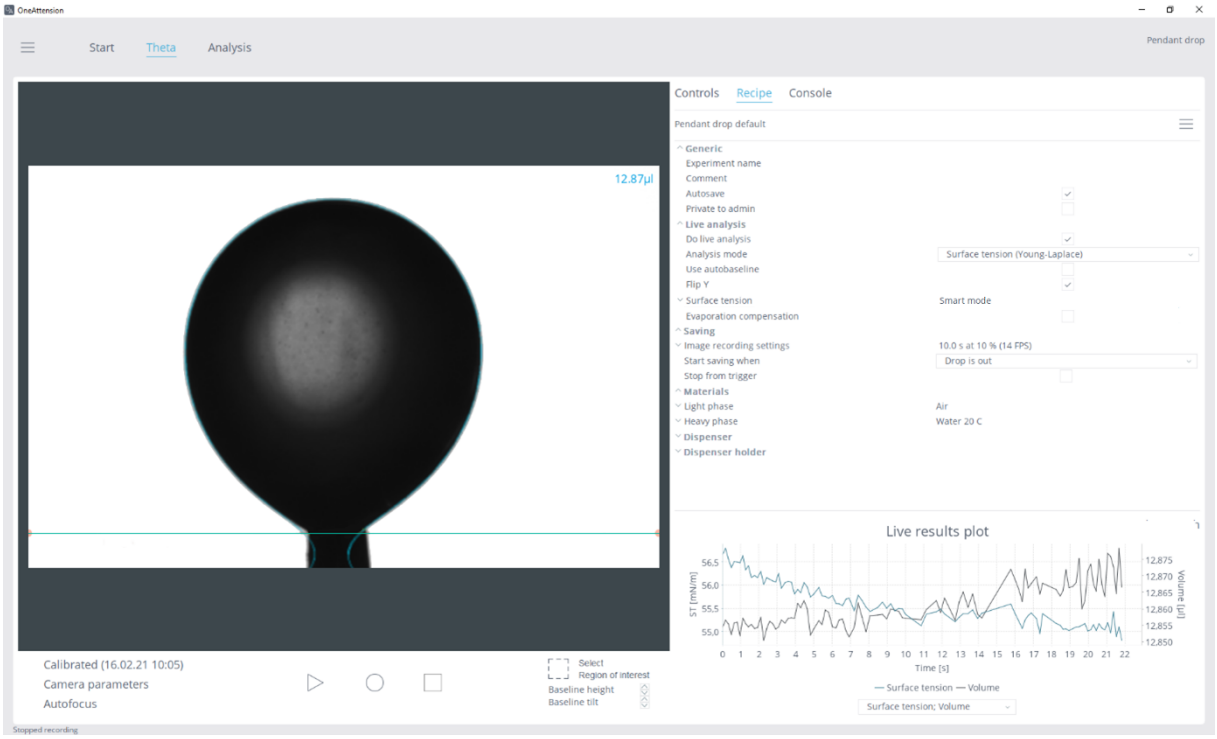
When you have measured all the samples in your batch, select **End batch**. See the analysis of batch measurement results in Chapter 6.

D. Pendant drop experiments

In pendant drop experiments adjust the drop size as large as possible and then record the image(s). If you need to manually adjust the focus after calibration, do it by moving the camera from the controls. A pendant drop experiment can also be done with one liquid within another if the two are immiscible. This kind of interfacial tension measurement is done with a hooked needle with the denser liquid in a cuvette around it. A drop is pushed up from the tip of the hook and measurement is done in the same way as a normal Pendant drop measurement except that a hooked needle is used. Flip Y must be selected from the recipe sheet if Live analysis is used. Notice that the denser liquid around the needle is now the Heavy phase.



Air-liquid surface tension measurement by pendant drop method.



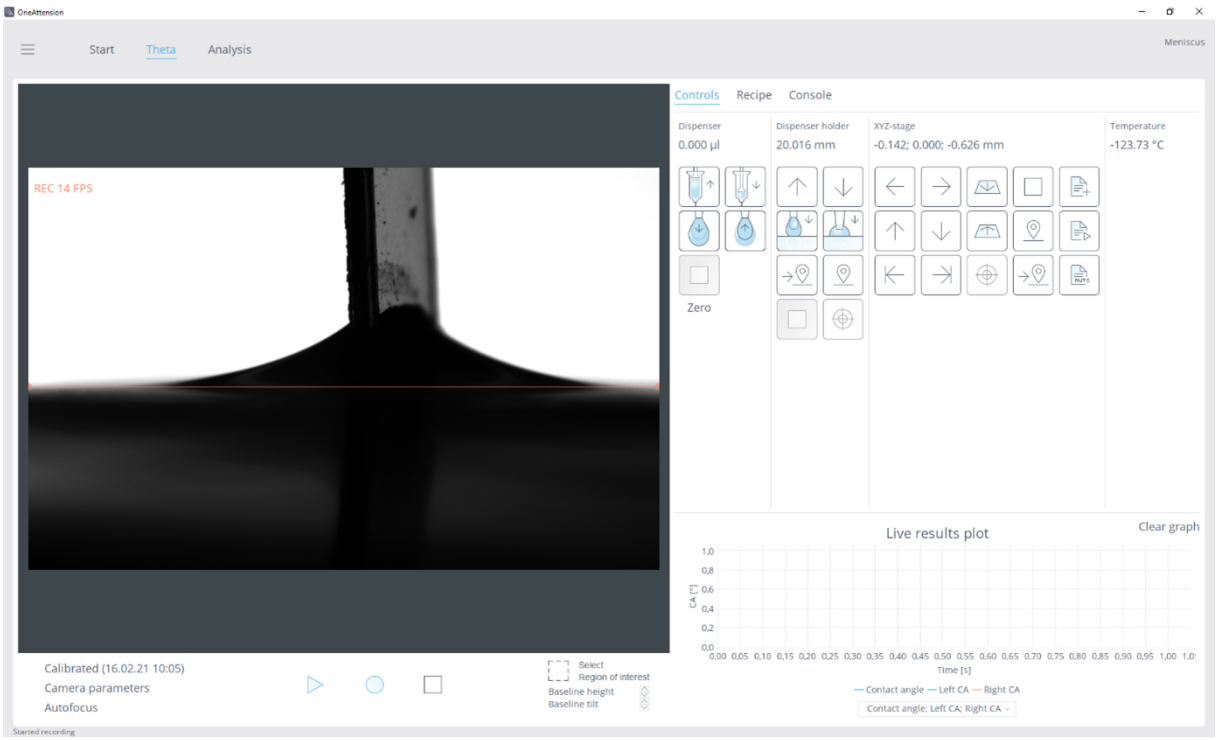
Liquid-liquid interfacial tension measurement by pendant drop method.

E. Meniscus experiments

The fibers commonly used with Meniscus experiments are very thin, and often require the use of a zoom lens and additional lighting for the zoom lens. Before beginning the measurement, clean the solid appropriately and attach it to the syringe clamp.

Theta instrument can be used for studying cylindrical shapes such as rods or fibers as well as shapes that are not symmetrical about their axis such as plates. For convenience, the solid sample will be referred as a rod hereafter throughout this section.

Adjust the position of the rod until it is visible on the image. Lift the liquid or lower the rod until the two surfaces intersect and a meniscus is formed. If necessary, use a trigger. In most cases it should be sufficient to manually press Start/ Record once the meniscus is visible.

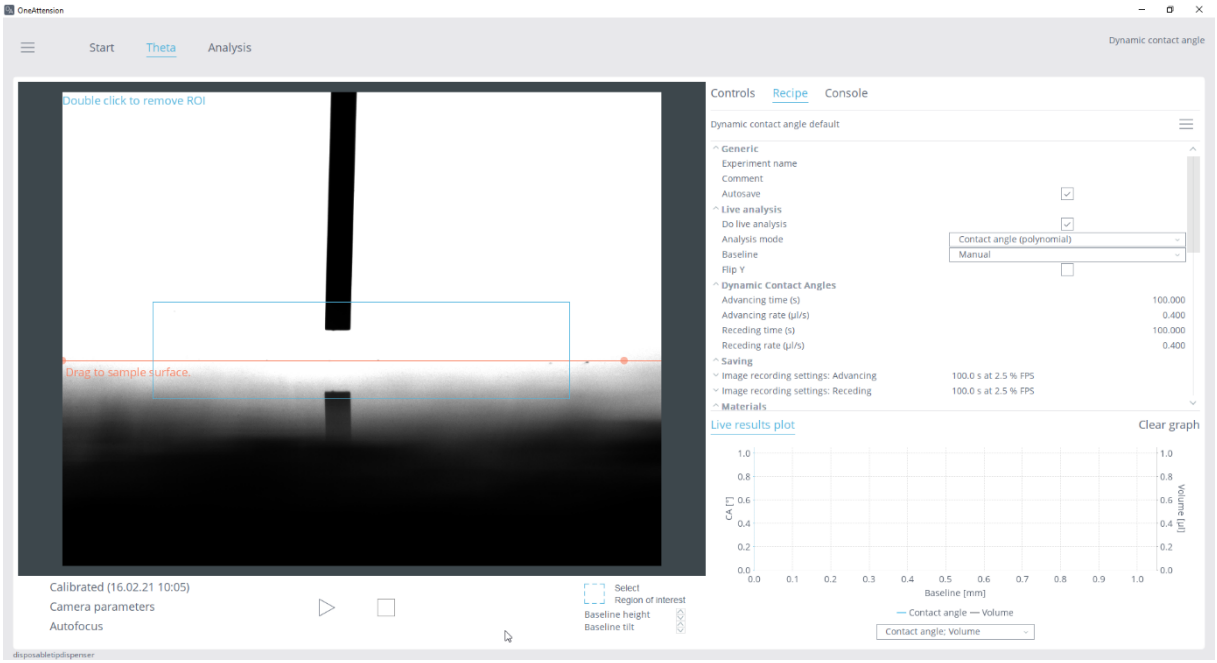


Recording the images of the meniscus experiment.

F. Automatic dynamic contact angle (Auto-DCA) experiments

Dynamic contact angle measurements define the contact angle hysteresis (advancing and receding angles). The measurement can be performed either with the Auto-DCA measurement mode or manually using the sessile drop measurement mode or a tilting cradle/tilting stage (Chapter 5). The measurement can be carried out either by using the C201 dispenser with a 30-gauge needle or automatic C311 dispensers with 30-gauge needle and an adapter. The adapter is simply mounted to the dispenser, and the needle is connected to the adapter.

To measure dynamic contact angles with the Auto-DCA mode, bring the tip of the needle close to the solid surface. It is also possible to start the measurement with a small drop so that the drop touches the surface. Choose a region of interest before starting the measurement, as shown below.



Needle close to the surface and ROI selected in auto-DCA measurement mode.

Recording possibilities are set in the recipe in Dynamic Contact Angles:

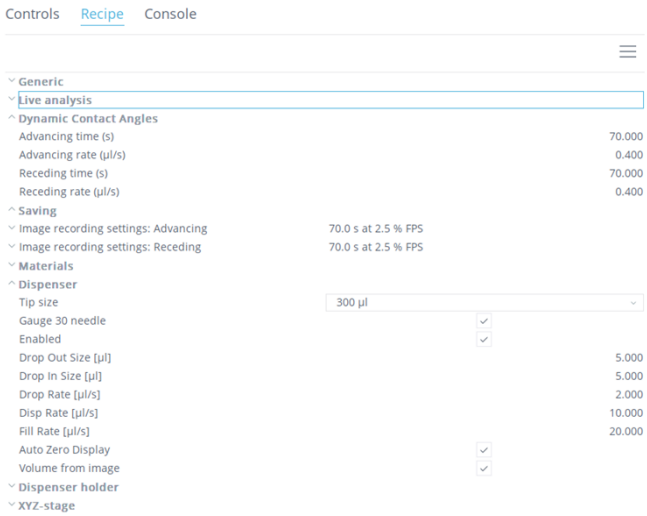
Advancing time: The time to measure advancing angle

Advancing rate: Dispensing rate during advancing angle measurement

Receding time: The time to measure receding angle

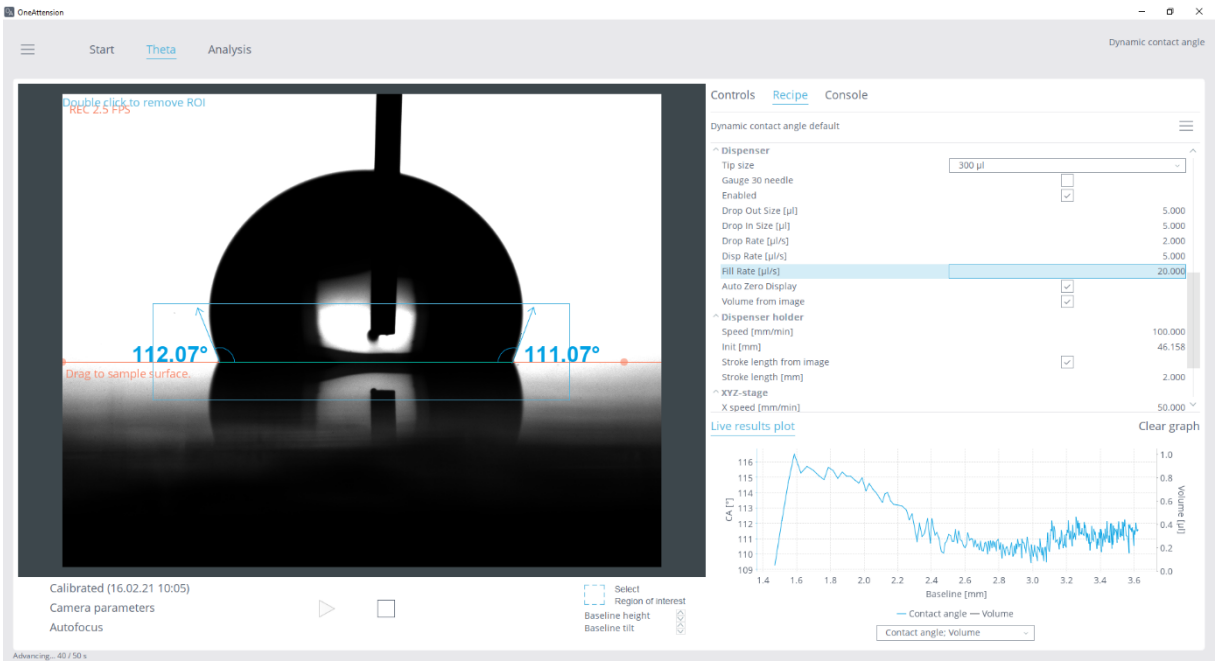
Receding rate: Dispensing rate during receding angle measurement

Image recording settings: can be adjusted for advancing and receding angles separately.



Recipe settings for Auto-DCA measurements. Remember to check the Gauge 30 needle for measurements.

Press start and the measurement will be carried out automatically according to the recipe settings. During the measurement the drop may fall out of focus of the camera, thus it is recommended to enable the autofocus function in dynamic contact angle measurements. Otherwise, the focus needs to be manually adjusted.



Auto-DCA measurement during advancing angle.

Manual DCA measurements

To measure advancing angles with sessile drop method manually, lower the syringe tip near the solid so that the tip remains attached to the drop after contact with the solid. Create the advanced angle by expanding the drop with the embedded syringe tip. Use the smallest, cleanest tip available and move the execution area (area enclosed by the blue rectangle) to neglect the section of the drop distorted by contact with the tip. To produce receded angles, use the embedded syringe tip method described above but remove fluid from the drop as opposed to adding fluid to it. Suggestion for experimental parameters can be found in application note #15.

4.6 Useful tips

High speed camera modes: The USB3 camera contains high speed modes that enable imaging even up to 3422 fps. The trade-off with these modes is that the field of view is limited especially vertically. In order to perform a contact angle measurement, the droplet may need to be stroked from outside the picture. With manual dispenser holder, check that the deposition height is correct. With automated dispenser holder, follow these steps:

1) Lower the dispenser holder so that the dispenser is almost touching the sample surface. Mark this as the home position of the dispenser holder.
2) Raise the dispenser holder up approximately 3 mm. Check the position of the dispenser holder on the Monitor tab. In the recipe under the Dispenser Holder, disable "Stroke length from image". Update the "Stroke length" to be the value in the monitor tab subtracted by 0.3 mm (for example Monitor tab gives 3.00 mm as the dispenser holder position, mark 2.70 into recipe).
3) Mark the current position as the new home position for the dispenser holder.

4) If you are using an automated dispenser, disable "Volume from image" on the recipe.
5) Start a measurement normally. The droplet will be created and stroked to surface from outside of the picture. As the droplet volume cannot be seen while creating the droplet, the method is best suited for automated dispensers that use the distance moved by the dispenser motor to calculate dispensed volume.

Please notice that large droplets and/or droplets with high contact angles may not fit completely in the image when using the high-speed camera modes. If the droplet top is only slightly outside the picture, the software may still give reasonable contact angle results. If the droplet extends considerably outside the picture, either use a smaller droplet volume or another camera mode.

The high-speed modes are application-wise important mainly in the contact angle measurement due to adsorption or similar effects. They are typically not used in surface/interfacial tension measurements as there the imaging speed isn't typically an essential factor.

Cleanliness: as in all surface chemistry applications cleanliness is essential for reproducible results. All liquids should be pure and uncontaminated. Any plastic or glassware that comes into contact with liquids should be scrupulously rinsed to remove any traces of surfactants used in cleaning. In order for the results to be reproducible, the solids that are tested must have a consistent history of events or exposures which might affect the surface.

Lighting: The lighting system for Theta Flow provides ideal illumination for sharp image capture. The brightness of the image may be adjusted with the Adjust camera parameters. Note that the image doesn't need to be bright to get good results. In most bright lighting situations, the highlights present on the drop may detract from the ability to model the curve profile accurately.

Magnification: The quality of the image profile and curve fitting is enhanced if the image of the drop fills a larger part of the screen. Whenever possible set the magnification so that the image of the drop is as large as possible.

Size of drops: Ideal drop size varies with different applications. Line tension effects may cause small drops to exhibit higher contact angles, whereas the gravity has larger influence on larger drops. Drops of 1 - 10 μl are reasonable; the most important factor is the consistent use of the same volume.

Assignment of baseline: One of the major factors limiting the reproducibility of contact angle measurements is the accurate assignment of a baseline for the image analyzed. Two methods are commonly employed. One is to make the surface of the solid horizontal with respect to the camera. The solid thus appears as a sharp-edged, flat object with no three-dimensional aspect. The image does not show the surface of the solid receding above the front edge of the sample. Place the drop near the front edge of the sample and use the sharp front edge on the image as the baseline.

The other approach is to adjust the sample stage angle so that the edge facing the camera is tilted downwards slightly (less than 5°). The more distant surface of the sample is seen receding above the front of the sample. In this case the reflection of the drop onto the solid surface may be observed. The baseline is assigned at the point where the curve of the drop and its reflection meet. Alternatively, adjusting the camera angle downwards will produce the same effect.

Analysis of Porous Solids: When a solid has a porous structure (paper, packed powder, etc.) the liquid may imbibe into the solid causing the contact angle to decrease over time. Researchers generally report graphs of contact angle, drop volume, baseline length or height of droplet as a function of time. These graphs are used to characterize the wetting behavior. The use of Theta on-screen trigger and fast recording mode are recommended in situations where liquid adsorption is rapid.

Adjusting camera angle: When adjustment of either sample stage or camera angle is required for baseline assignment, it is preferable to adjust the camera angle.

The camera angle should be zero when performing pendant drop experiments, because tilting the camera can affect the results.

4.7 Cleaning

The exterior of the instrument can be cleaned with a damp cloth. Ensure that the surfaces are dried immediately after cleaning. If liquid is accidentally spilled on the instrument, wipe the instrument using a dry, clean cloth.

4.8 Maintenance

Only persons authorized by Biolin Scientific are allowed to perform maintenance and repairs on the instrument. In case repair is needed, contact Biolin Scientific or an authorized representative directly.

5 Operation of additional modules

Theta Flow can be upgraded with various hardware modules for increased automation and functionality. All the modules operate with plug-and-play installation, therefore also the plug-in instructions are included in the user manual in addition to operation instructions.

A connection cable is supplied with each hardware module which must be connected to the **DEV** or **Ext I/0** inputs on the Theta Flow. Connect the first module to **DEV1**, second module to **DEV2** and so on. If only one additional module is used, it must be connected to **DEV1**.

CAUTION!
Make sure that the power is switched off when making any electrical connections (apart from the USB cables). Connecting cables with power on may damage instrument electronics.

5.1 Automatic movement

A. Motorized dispenser holder

T301 Motorized dispenser holder for single dispenser is used for automatic movement of the liquid dispensing unit vertically. This greatly facilitates automatic drop placement on the solid sample surface.

Motorized dispenser holder.

If your Theta Flow was purchased with T301 it will arrive with the module already attached. Connect the supplied device cable from the 15-pin connector (upper) on the module to Theta Flow rear panel.

The first time after the experiment tab will be opened, the stage will initialize itself by driving to the top end to properly establish its position. After this, the stage will return to the zero position.

Note! Be sure that nothing is on the sample stage and preferably lower the sample stage to ensure that no accidents occur during homing.

In OneAttension, module settings are available on the **Theta tab** under Recipe tab (see figure below). Module settings can be adjusted as follows:

Speed [mm/min]: The speed of dispenser holder movement.

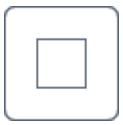
Init [mm]: The position where the dispenser holder is driven when initialized.

Stroke length from image: When checked, the software will determine the stroke length from the live image. It is important to set the baseline correctly so that proper stroke length can be determined and the needle does not hit the sample surface.

Stroke length [mm]: If stroke length from image is not selected, the stroke length can be set manually.

Module settings for the Motorized dispenser holder.

Module controls are available on the **Theta tab** under Controls tab.



Stop: Stops all movement

Up: Moves the dispenser holder upwards

Down: Moves the dispenser holder downwards

Stroke: The needle tip is moved down from the current position to just above the surface and back. This is used to place a drop on the surface

Place drop: Dispenses a drop and then executes a stroke

Set Home: Sets home position, which is defined by the user. The home position is also automatically set to current position when a measurement is started

Go Home: Moves the dispenser holder to home position

An integral part of using the automated dispenser movement is the ability to automatically dispense a drop onto the surface and then to lift the needle away from the drop. This requires that the software knows how far to move the stage, which is indicated by the baseline. First, move the dispenser holder so that the tip is at the top of the screen. Now move the baseline to the level of the surface. You can adjust the baseline either by dragging or by using the BL height and BL tilt buttons. BL height changes the baseline height whereas BL tilt tilts the baseline.

With a manual syringe, draw out an appropriate size drop and press Stroke. This lowers the needle almost to the level of the surface and then back up, leaving the drop behind. Alternatively with an

automatic dispenser simply press Place drop and the dispenser will first dispense a drop and then lower and lift the stage as is done by the stroke command.

The only way for the software to know the distance between the needle tip and surface is with appropriate use of the baseline.

Note! As the distance for moving the stage is obtained from the distance on screen, it highly depends on the calibration. Check that the calibration is accurate before using the Stroke or Place drop functions!

B. Automatic X sample stage

The T320 Automatic X sample stage is used for motorized movement of the solid sample to different positions relative to the dispensing point. When combined with an automatic dispenser holder movement and a dispenser the Sequencer utility allows multiple drops on a sample to be measured automatically.

Automatic X sample stage.

Connect the supplied device cable from the 15-pin connector on the module to Theta Flow rear panel.

The first time after the experiment tab is opened, the sample stage will initialize itself, after which the stage will return to the zero position. **Note!** Be sure that no measurement chambers or temperature modules are attached to the sample stage during homing to avoid accidents.

In OneAttension, module settings are available on the **Theta tab** under Recipe tab (see figure below). Module settings can be adjusted as follows:

Speed [mm/min]: The speed of automatic X sample stage movement.

Step [mm]: Step size.

Center [mm]: Sets home position.

Point mapping: Opens Single point mapper window.

X-stage		
Speed [mm/min]		100.000
Step [mm]		5.000
Center [mm]		75.000
Point mapping	No points	

Module settings for Automatic X sample stage

Module controls are available on the **Theta tab** under Controls tab.

Stop: Stops all movement

Left: Moves the sample stage to the left

Right: Moves the sample stage to the right

Step Left: Moves the stage one step to the left

Step Right: Moves the stage one step to the right

Set Home: Sets home position, which is defined by the user. The home position is also automatically set to current position when a measurement is started

Add to List: Add current location to sequencer list

Drive Through: Drives through the locations set in the sequencer list by Add to list function

Sequencer: Open Sequencer generator window, which enables to set amount of measurements points with defined distance in X direction, when automatic dispenser holder, dispenser and X sample stage are used.

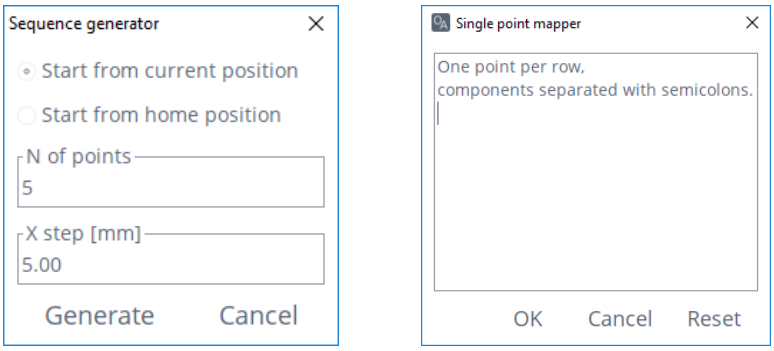
Go Home: Moves the sample stage to home position

Init: Initializes the module

A sequence can be created in two ways: 1) by defining the dimensions between the droplets or 2) by marking the preferred measurement points in the software by driving to the preferred positions.

A point mapping option will open a Single point mapper window to see the coordinates, which have been set in case the sequencer is used. Points can be added by Add to list button from the device

controls by running the sample stage in the preferred position and then pressing the Add to list in control device.



Sequencer windows. The point mapping option in the recipe sheet will open the Single point mapper, where the points added by Add to list function are shown in the list. Sequence generator will appear by pressing the Automatic sequencer from the device controls.

C. Automatic XYZ sample stage

The T330 Automatic sample stage is an additional module that enables motorized substrate movement in all three dimensions. The stage consists of three individual motorized axes. The stage is an alternative to the manual sample stage or the automatic X sample stage. The stage attaches to the optical rail. Automatic XYZ sample stage is required for the 3D Topography module. **Note!** Remove the sample clips if the stage is used with the 3D Topography module.

Automatic XYZ sample stage.

Connect the supplied device cable from the 15-pin connector on the module to Theta Flow rear panel.

The first time after the experiment tab is opened, the sample stage will initialize itself. After that the stage will return to the zero position. **Note!** Be sure that no measurement chambers or temperature modules are attached to the sample stage during homing to avoid accidents.

In OneAttension, module settings are available on the **Theta tab** under Recipe tab (see figure below). Module settings can be adjusted as follows:

X/Y/ZSpeed [mm/min]: The speed of automatic X/Y/Z movement.

X Step [mm]: Step size in X direction.

Center [mm]:Sets home position.

Point mapping: Opens Single point mapper window.

XYZ-stage		
X speed [mm/min]		50.000
Y speed [mm/min]		50.000
Z speed [mm/min]		25.000
X Step [mm]		5.000
Center [mm]	90.000; 30.000; 15.000	
Point mapping	No points	

Module settings for the Automatic XYZ sample stage

Module controls are available on the **Theta tab** under Controls tab. The control buttons are similar to automatic X sample stage (see section B above), excluding the additional direction buttons (Up, Forward, Down and Backward).

A point mapping option will open a Single point mapper window to see the coordinates, which have been set in case the sequencer is used. Points can be added by Add to list button from the device controls by running the sample stage in the preferred position and then pressing the Add to list in control device. Automatic sequencer from the device controls provides also the possibility to adjust measurements points in all XYZ directions.

D. Dispenser rotation module and Dual dispenser unit

Dispenser rotation module and dual dispenser unit (DDU) allow simultaneous use of two samples with C311 automatic single liquid dispensers. There is an automatic as well as manual dispenser rotation module available. Manual dispenser rotation module can be used with manual one-touch dispenser (OTD). Automatic dispenser rotation module is configured with motorized vertical dispenser movement.

Manual dispenser rotation module is fastened to OTD with a thumbscrew. DDU is mounted to rotation module with a set screw, and C311 dispensers are placed in their slots on the DDU. When only one dispenser is used, the un-used slot must be protected with the included cover block.

Manual dispenser rotation module and dual dispenser unit.

Automatic dispenser rotation module is placed on the slot on the motorized dispenser holder.

Automatic dispenser rotation module and dual dispenser unit.

Dispenser rotation module has two positions. In each position one of the automatic dispensers is positioned on the line from camera to LED light source. Manual version of the module is simply turned by hand to change between the positions. In automatic version this is done automatically when user selects which dispenser position is active.

Connect the supplied device cable from the DDU module to Theta Flow rear panel.

In OneAttension, module settings are available on the **Theta tab** under Recipe tab (see figure below). Automatic dispensing needs to be enabled before using it. Module settings can be adjusted as follows:

Tip size: Volume of the disposable pipette tip used.

Gauge 30 needle: Will optimize volume from image functionality for gauge 30 thin needles. Select if you are using the gauge 30 needle and volume from image functionality

Enabled: Select to enable automatic dispensing.

Use liquid 1 (or 2): Select to enable automatic dispensers 1 or 2.

Drop Out Size: Volume of drop dispensed when Drop Out is pressed.

Drop In Size: Volume of drop retracted when Drop In is pressed.

Drop Rate: Rate of Drop Out or Drop In dispensing.

Disp Rate: Rate of dispensing when Dispense is pressed.

Fill Rate: Rate of filling when Fill is pressed.

Auto Zero Display: When checked the current volume reading is zeroed whenever Drop Out or Drop In is pressed.

Volume from image: Software is able to define the drop volume from the image due to calibration.

Check ST prior to measurement: Only for sessile and batch sessile measurements. If checked, the software will check the surface tension of the liquid before contact angle measurement.

ST check tolerance [%]: Surface tension check tolerance compared to the value reported in the database

ST check volume [ul]: Volume of the drop for surface tension check

Dual Dispenser	
Tip size	300 µl
Gauge 30 needle	☐
Enabled	☑
Use liquid 1	☑
Drop Out Size [µl]	5.000
Drop In Size [µl]	5.000
Drop Rate [µl/s]	2.000
Disp Rate [µl/s]	20.000
Fill Rate [µl/s]	20.000
Auto Zero Display	☑
Volume from image	☐
Use liquid 2	☑
Drop Out Size [µl]	5.000
Drop In Size [µl]	5.000
Drop Rate [µl/s]	2.000
Disp Rate [µl/s]	20.000
Fill Rate [µl/s]	20.000
Auto Zero Display	☑
Volume from image	☐
Check ST prior to measurement	☐
ST check tolerance [%]	5.000
ST check volume [µl]	6.000
Check ST prior to measurement	☐
ST check tolerance [%]	5.000
ST check volume [µl]	6.000

Module settings for Dual Dispenser.

Module controls are available on the **Theta tab** under Controls tab.

Stop: Stops all movement

Fill: Retracts liquid into the syringe at the rate defined in the Fill rate field.

Dispense: Dispenses liquid at the rate defined in the Disp rate field.

Drop Out: Dispenses the volume defined in the Drop Out Size field at the rate defined in the Drop rate field.

Drop In: Retracts the volume defined in the Drop In Size field at the rate defined in the Drop rate field.

Init: This button is available with automatic dispenser rotation module only. It can be used to move the rotation module to its home position, which is dispenser position 1. Please note that initialization of the rotation module produces noise as the shaft is rotated against a mechanical limit for a short time.

Zero

Zero: Resets the current volume reading.

1 2

1 and 2: Select which one of the dispenser positions is active. With automatic rotation module the selection also moves the selected dispenser into the image.

5.2 Automatic dispensers

There are four different automatic dispenser types that can be used with Theta Flow. C311 Automatic single liquid dispenser is the default option. C311, C201 Automatic single liquid dispenser and T315A Picoliter dispenser can be used with either manual or motorized dispenser holders.

T314 Multi liquid dispenser requires the motorized dispenser holder.

A. Automatic single liquid dispenser

The C201 Automatic single liquid dispenser is used to automatically dispense a precise volume of liquid, often a drop. When combined with an automatic sample stage and dispenser holder the Sequencer utility allows multiple drops on a sample to be measured automatically.

Automatic single liquid dispenser with Theta Flow.

Place the plastic syringe piston inside the Hamilton 1 ml syringe. Place the syringe in the groove and turn the plastic cover clockwise to the horizontal, 'closed,' position. Pull the piston of the syringe down to the arm of the dispenser and screw on the supplied thumbscrew. With the needle and tubing connected to the mounted syringe, mount the needle in the manual one-touch or automated dispenser holder.

Connect the supplied device cable from the 15-pin connector on the module to Theta Flow rear panel.

In the **Theta tab** the software will automatically provide the device controls. In the recipe sheet dispenser needs to be enabled before using it. In case manual dispenser is used instead of automatic

one, disabling the dispenser from the recipe sheet allows the user to leave the cable connections plugged in. Adjustable recipe sheet parameters are as follows:

Drop Out Size: Volume of drop dispensed when Drop Out is pressed.

Drop In Size: Volume of drop retracted when Drop Out is pressed.

Drop Rate: Rate of Drop Out or Drop In dispensing.

Disp Rate: Rate of dispensing when Dispense is pressed.

Fill Rate: Rate of filling when Fill is pressed.

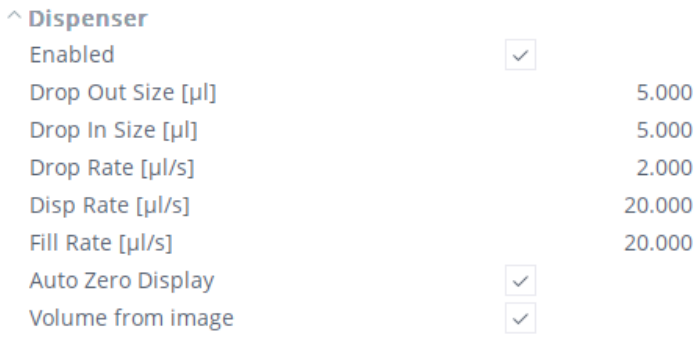
Auto Zero Display: When checked the current volume reading is zeroed whenever Drop Out or Drop In is pressed.

Volume from image: Software is able to define the drop volume from the image due to calibration.

Check ST prior to measurement: Only for sessile and batch sessile measurements. If checked, the software will check the surface tension of the liquid before contact angle measurement.

ST check tolerance [%]: Surface tension check tolerance compared to the value reported in the database

ST check volume [ul]: Volume of the drop for surface tension check



Module settings for automatic single liquid dispenser.

Module controls are available on the **Theta tab** under Controls tab.

Stop: Stops all movement

Fill: Retracts liquid into the syringe at the rate defined in the Fill rate field.

Dispense: Dispenses liquid at the rate defined in the Disp rate field.

Drop Out: Dispenses the volume defined in the Drop Out Size field at the rate defined in the Drop rate field.

Drop In: Retracts the volume defined in the Drop In Size field at the rate defined in the Drop rate field.

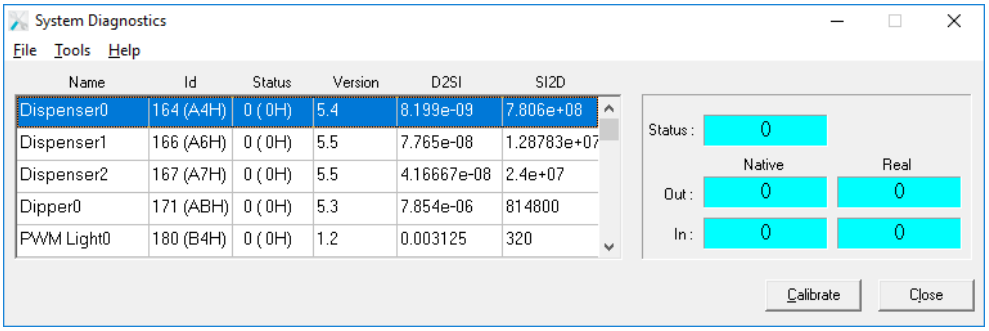
Init: This button is available with automatic dispenser rotation module only. It can be used to move the rotation module to its home position, which is dispenser position 1. Please note that initialization of the rotation module produces noise as the shaft is rotated against a mechanical limit for a short time.

Zero: Resets the current volume reading.

Calibrating the dispenser

The automatic single liquid dispenser has been factory calibrated and generally doesn't have to be calibrated prior to use. If it for some reason needs to be recalibrated, please follow these instructions.

Click Device calibration in the main menu and the System Diagnostics window will appear. Select Dispenser0 and click Calibrate. Fill the dispenser syringe with a liquid. Weigh an empty container and dispense the contents of the syringe into it by pressing the Start button. Allow the syringe to empty and weigh the container again. Subtract to calculate the weight of the liquid and divide by its density calculate the volume dispensed. Enter this information into the Volume field and press Next to complete the calibration of the dispenser.



System diagnostic window will appear by clicking the Device calibration in the Setup tab.

B. Automatic single liquid dispenser using disposable pipette tips

The C311 Automatic single liquid dispenser using disposable pipette tips is used to automatically dispense a precise volume of liquid, often a drop. When combined with an automatic sample stage and dispenser holder the Sequencer utility allows multiple drops on a sample to be measured automatically.

Automatic single liquid dispenser with disposable pipette tips.

The disposable pipette tip dispenser is to be mounted on to the motorized dispenser holder or to dual dispenser unit. If you are upgrading from a manual precision syringe, loosen the thumbscrew on the vertical arm of the motorized dispenser movement, lift the syringe holder assembly up out of the motorized vertical arm of the device and slide the pipette dispenser down into the same slot. Ensure that the **Theta Flow is turned off for this procedure**. After assembly re-tighten the thumb screw.

When a disposable pipette dispenser is used, the OneAttension software will automatically recognize the dispenser.

In OneAttension, module settings are available on the **Theta tab** under Recipe tab. They are the same as those for the automatic single liquid dispenser. Please select which tip size is used from the recipe. **Note!** The Automatic single liquid dispenser should not be connected at the same time). The C311 dispenser does not need to be calibrated. Module controls are available on the **Theta tab** under Controls tab.

The disposable tip is filled by placing a cup of liquid on the sample stage. The disposable tip is immersed into the liquid and **fill** is pressed. You may have to decrease the filling rate to e.g. 5 ul/s, in order to get proper filling. Note that there is a maximum position where the piston of the dispenser can go. When this position is reached, there will be no further filling of the tip, even though the Controls tab shows otherwise. If you are not able to get the tip filled completely, you have to first dispense the air out from the tip (drive to piston of the dispenser to the down position).

The tips used are disposable and easy to change. Attension offers disposable tips made from polypropylene, as well as PFA and parylene coated tips.

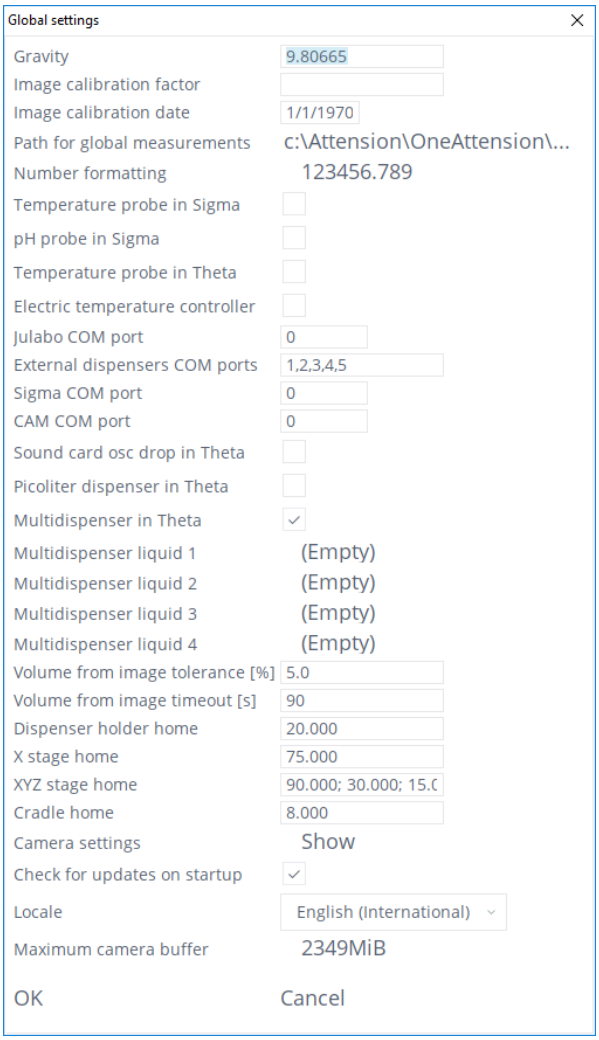
C. Multi liquid dispenser

The T314 Automatic Multi Liquid Dispenser consists of four glass syringes that are connected by three-way valves to either a liquid container or to a needle. The syringes are moved in unison, with normally only one valve open to the needle. The four needles are combined into a needle array.

Multi liquid dispenser with Theta Flow.

Connect the syringe pump module to the mains. The tubes from black couplings go to the liquid containers and the tubes from white couplings go to the needles. This is important, as the syringes will be emptied when Theta Flow is turned on. Connect the pump module to the dispenser holder lower port with the supplied cable. **Turn the dispenser on first, and Theta Flow on only afterwards.**

The multi liquid dispenser has to be enabled from main menu → global settings → Select 'Multidispenser in Theta'. Liquids can also be set in the Global settings window but it is not mandatory since it can be done in the recipe as well.



Global settings window.

When the multi liquid dispenser is recognized, the initialization of the needle shift is done when the measurement is started. The first time after the experiment tab is opened the motorized actuators (needle array, dispenser holder and sample stage) are initialized by moving to a limit switch. This is why it is important to have no measurement chambers/attachments in place since this might lead to a mechanical collision.

First, the needle number one has to be set; Select number 1 from the dispenser device controls. Move the needle number one to the center of the image by turning the screw that moves dispenser holder. The correct operation of the system can be checked by pressing e.g. number 3 and the needle shift should now move so that the needle number three is in the center of the image.

The module controls and settings in the recipe tab are the same than with the single liquid dispenser, excluding the following additional functions:

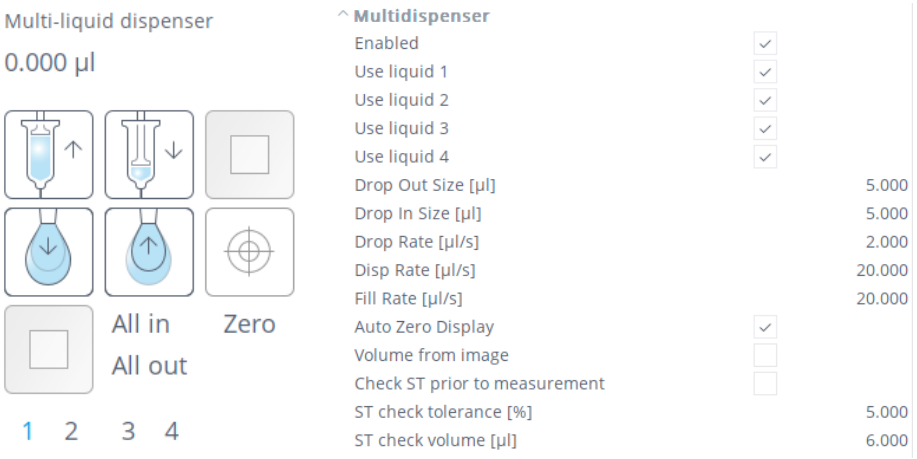
Use Liquid 1-4 (recipe)/ 1-4 (buttons in controls): Enabling corresponding channel translates corresponding needle into the view and enables its valve for output.

All In: all channels are connected to the containers. Use this for flushing or filling the syringes.

All Out: all channels are connected to the needles. Use this for e.g. flushing the needles.

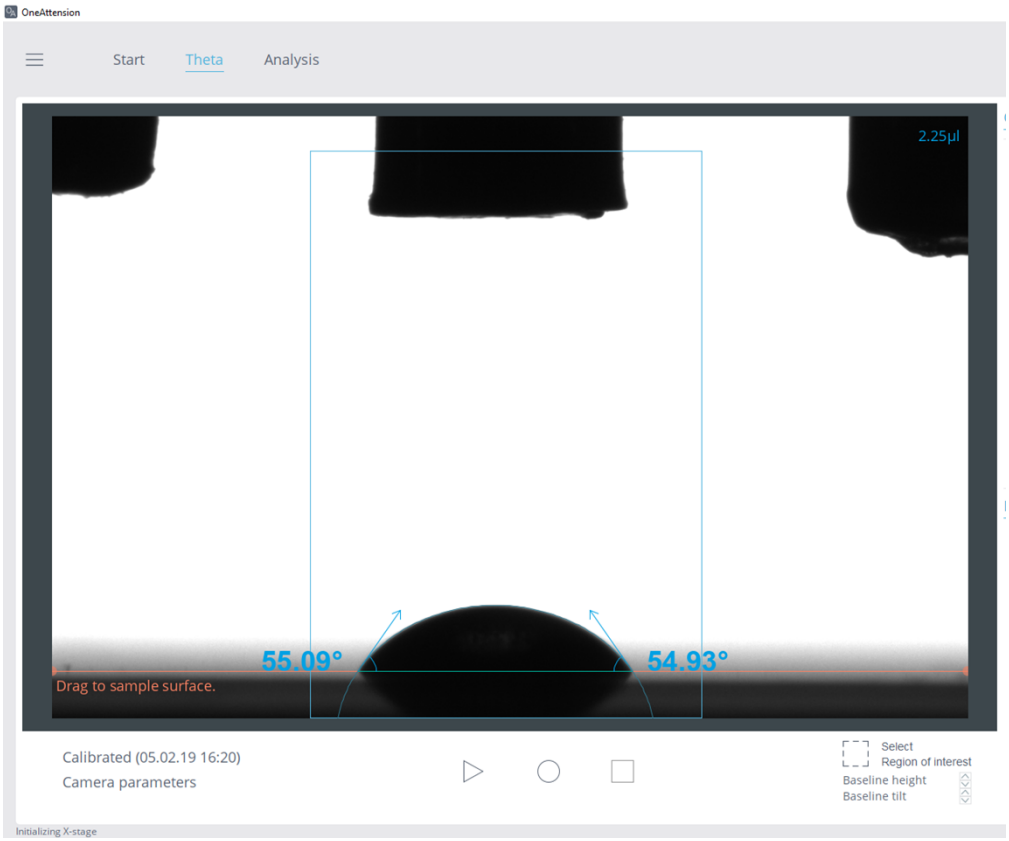
Note that when the measurement is done, only the needle shift is moved. If the stage is not manually moved, the next liquid will be placed on the same spot as the previous one. The automatic X sample

stage or XYZ sample stage can also be programmed to work together with the multi-liquid dispenser, so that no manual stage movement is needed and sequencer can be used instead: The sequencer will automatically change to the next enabled liquid when placing drops onto to sample surface. You can choose to use 1 to 4 liquids during the same experiment.



Module controls and recipe sheet parameters for multi liquid dispenser.

When images are analyzed, it is important to use **ROI** (Region Of Interest) function to exclude parts of the image that may disturb successful fitting of the droplet. **ROI** is explained in detail in chapter 6.1.



ROI function.

Technical specifications

Input voltage range	100 – 240 Vac
Frequency	50 – 60 Hz
Input current	110 – 130 mA
Mains fuses	T3.15A 5x20 mm

D. Picoliter dispenser

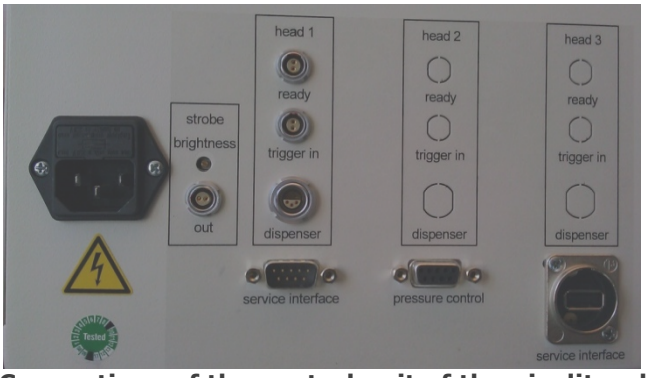
The T315A picoliter dispenser is an additional module used to enable the dispensing of picoliter sized drops. The picoliter dispenser consists of a liquid vessel, dispenser head with chosen nozzle size, calibration tool, additional optics and a control unit. The dispenser head is connected with an additional part to the manual or automatic dispenser holder. Automatic dispenser holder is recommended with the system.

Picoliter dispenser.

The picoliter dispenser is handled by the control unit. The additional zoom optics is required to see the small drops that can be observed. The liquid vessel holds the liquid sample. The dispenser head launches the liquid onto the substrate.

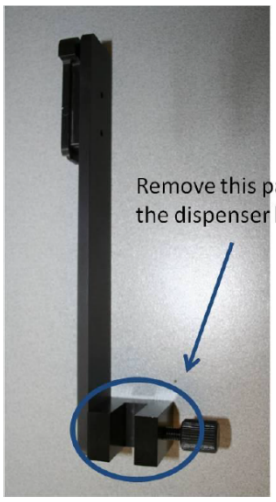
Installation

Plug in the serial cable between Theta Flow (**Ext I/0**) and control unit (**trigger in**). Connect also the white cable coming from the **dispenser** to the dispenser port of the control unit. Plug in also the power cable of the control unit.



Connections of the control unit of the picoliter dispenser.

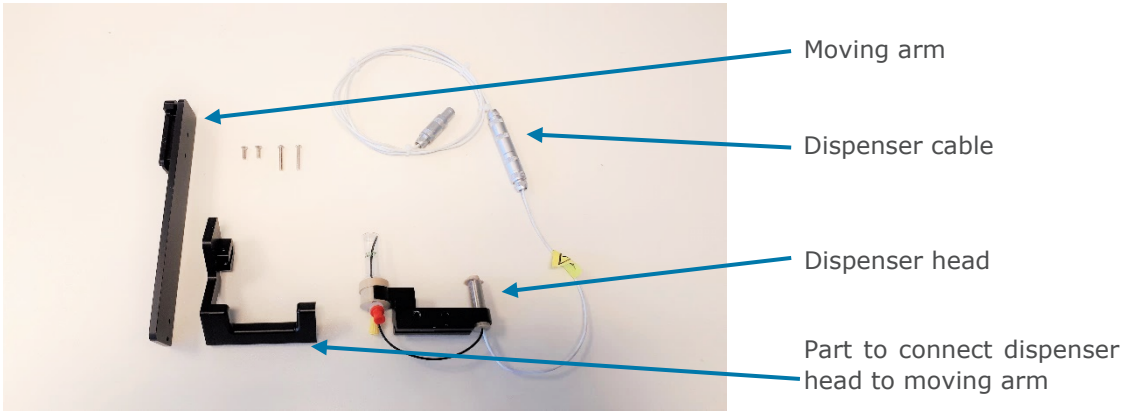
Attach the dispenser head to dispenser holder according to the instructions given below:



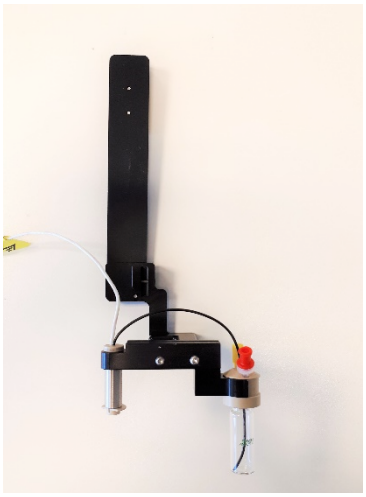
Automated dispenser holder.

Remove the holder part from the automated dispenser holder (in figure above) or in the one-touch manual dispenser holder.

Attach the additional part, which connects the dispenser to the dispenser holder. In case of automatic dispenser, the additional part is connected to the moving arm, whereas with manual dispenser holder thicker additional part is connected to the one-touch manual dispenser holder.



Picoliter dispenser parts.

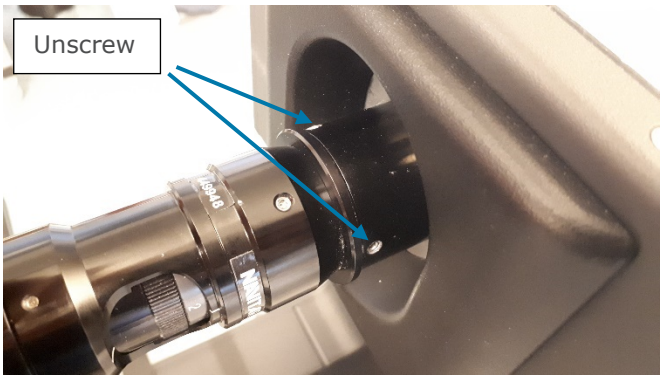


Complete assembly with automated dispenser holder.

Due to the small size of the droplet, additional optics are needed for higher magnifications. Attach the additional optics according to instructions given below:

Magnification lens is attached to zoom lens with adapter.

Replace the standard camera optics with additional picoliter optics. First remove the camera linear adjustment lock screw from the instrument rear panel and slide the camera stage all the way towards the light source to make the screws accessible. Unscrew the screws (3 pcs) shown in figure below to change the optics.

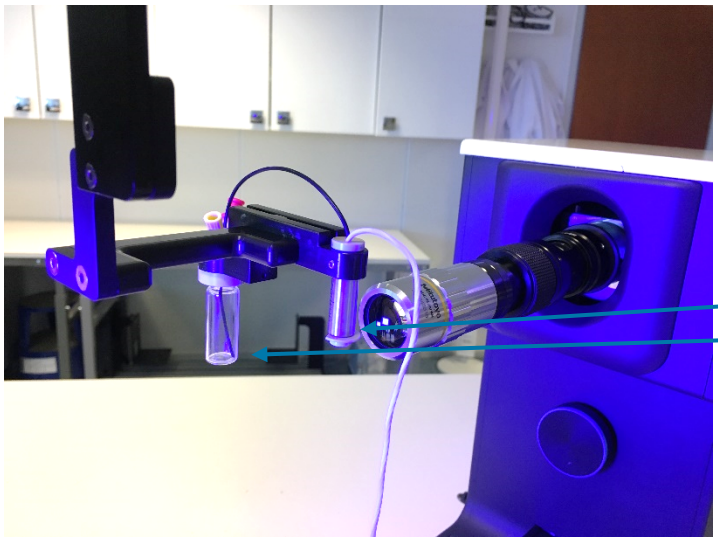


Camera optics.

Mount the magnification lens to zoom lens with the supplied adapter. Then mount the zoom lens to the optics tube.

Please note that you might need to adjust linear position of the camera to get enough room for the longer additional optics. Adjust the position, so that the optics is as close to the sample stage as possible. Also remember to mount back the linear adjustment lock screw and lock the camera stage in place.

Care has to be taken especially when using the motorized XYZ sample stage together with the picoliter dispenser. During the initialization of the stage, the table part may hit the optics or the liquid container. Press stop button at the XYZ controls, if this happens. **Never let the instrument to initialize without monitoring!**



Complete system with additional optics.

Using the picoliter dispenser

Control unit

Turn on the control unit. Wait until a Start buttons appears at the bottom right of the screen. Press Start.

If the third button from the top says Cont. then press it once to change it to Burst.

If the fourth button from the top says Intern then press it once to change it to Extern. After changing Intern to Extern, you need to press still once on/off on the touch screen to open the connection to OneAttension software. The on/off button is then dark.

Optimized control parameters are set according to separate information sheet.

The picoliter dispenser is now set to deposit one drop of your sample liquid when instructed to do so. This drop will have a volume between 20 pl and 380 pl depending on the inner nozzle size of the dispenser, control settings (voltage and pulse width) and liquid properties (e.g. viscosity and surface tension). Control unit parameters are optimized for each nozzle and they are unique for each liquid as well. The optimal parameters are delivered with each system.

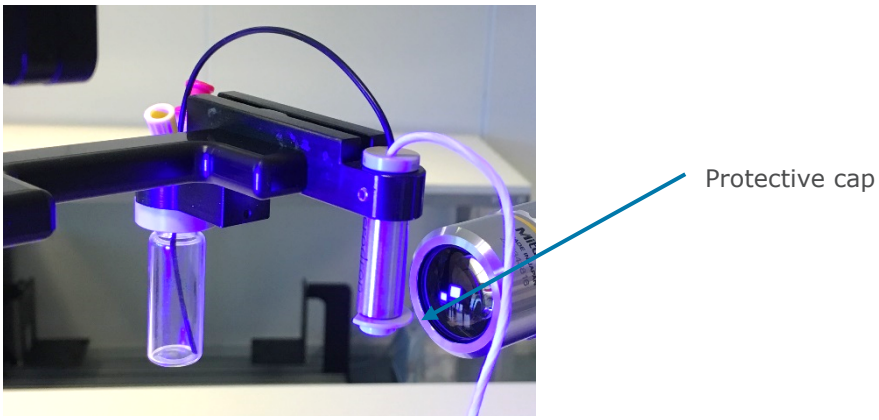
For manual use set the fourth button to Intern. In this case nozzle will produce one additional droplet, after which the system is ready to deposit droplets by pressing On/Off from control unit.

Conducting a measurement and calibration

Take the sample vessel off the system, and place the used liquid to the sample vessel though the filter by using the syringe as shown in the figure below. The filter pore size should be 5 µm or less. **Never place liquid without using the filter! Use only the liquid(s) for which the parameters are optimized, otherwise the system may get jammed.**

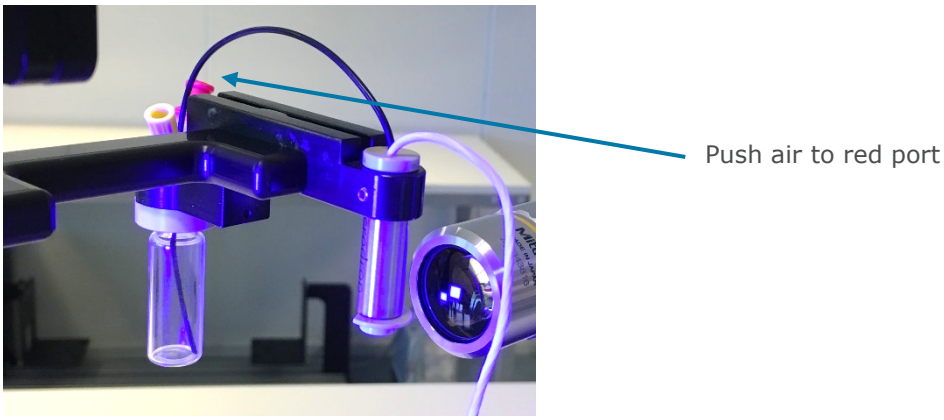
Syringe with filter.

Remove the plastic cap at the end of the capillary.



Protective cap.

Use a dry disposable syringe to press air into the liquid vessel to push the sample liquid out from the dispenser as shown in the figure below. This is to ensure that the tubing is full of liquid and system is pressurized.



Push air to liquid vessel.

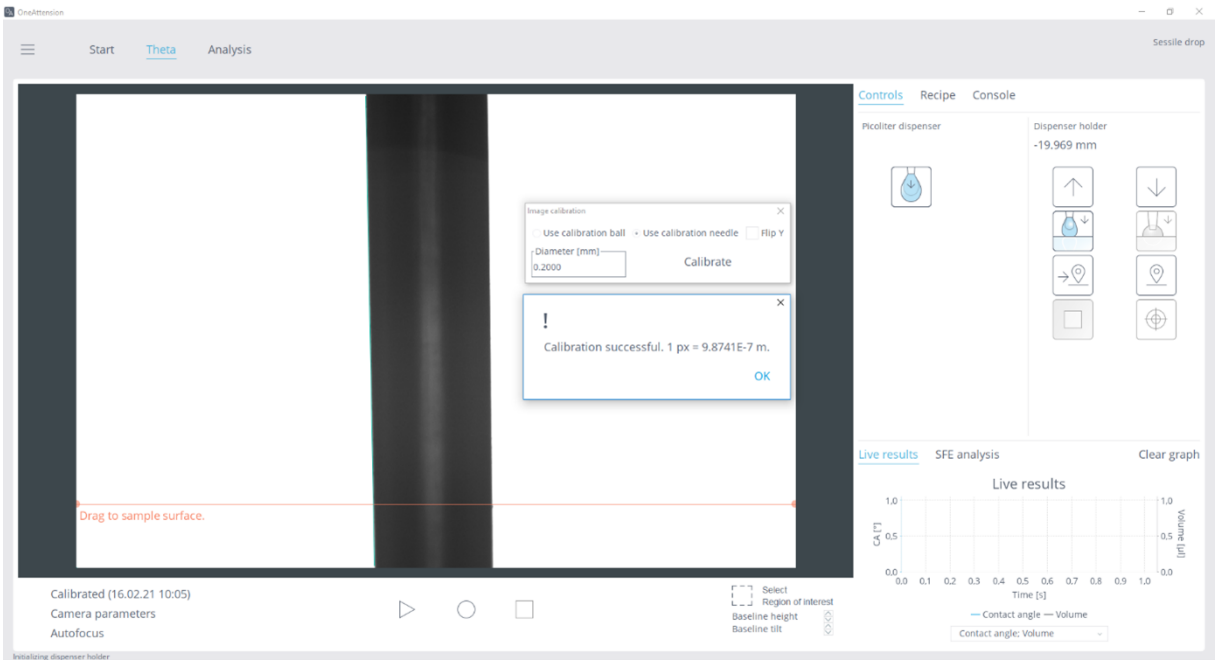
Start OneAttension software and enable the picoliter dispenser in main menu -> Global settings. In case other dispensers are used, the cables can be connected during the picoliter dispenser use. When the standard dispenser is preferred, just disable the picoliter dispenser from the global settings.

Begin a sessile drop experiment. Press Adjust camera parameters and increase the light intensity if needed. In the recipe tab the picoliter dispenser needs to be enabled.

If you want to measure droplet volume, the picoliter dispenser requires a needle calibration, which is performed with the special needle calibration tool coming along with the instrument. However, the zoom level should be adjusted before the calibration in the right level. This is done by seeking the dispenser head in the upper part of the image and e.g. sample stage in the lower part of the image. Then focus the image and deposit a few droplets from picoliter device controls. According to the droplets, adjust the zoom and check the focus approximately (move the camera rail position if needed). After this, perform the needle calibration. Do not change significantly the zoom or the place of the camera stage.

Place the calibration tool under the dispenser. Adjust the thin needle of the calibration tool in the image. Focus the image and then press calibrate, after which the Image calibration window opens as shown in the figure below. Select the needle calibration, which gives the right dimension (0.2 mm)

as default setting. Press Calibrate and then press OK. A new calibration is demanded if zoom or camera stage position is changed.

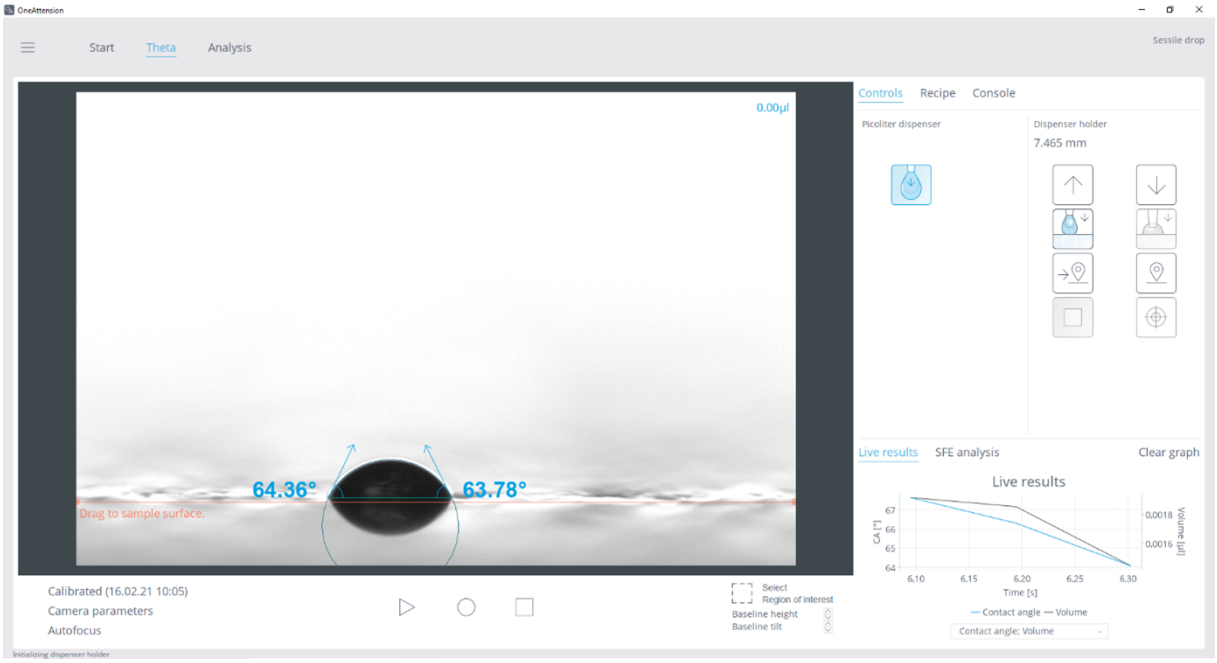


Calibration of the picoliter dispenser.

After the calibration, adjust the dispenser head in the preferred position and deposit a drop from the picoliter dispenser's device controls. It might be necessary to adjust the focus by using test droplets. Test droplets can be deposited from the picodispenser device controls.

When pressing the start from the Controls, the dispenser will deposit the drop and start to record at the same time.

Cleaning and other maintenance of the picoliter dispenser need to be performed according to separate picoliter dispenser manual, which is delivered together with the product. It is highlighted that the system needs to be cleaned thoroughly after each measurement, if some other than pure solvents are used.



Contact angle measurement performed by picoliter dispenser. Picodispenser device control includes the test drop button. The picoliter dispenser needs to be enabled from the recipe sheet.

5.3 Electrically heated temperature control unit

A. Safety

WARNING!

RISK OF BURNS. Exercise caution when touching heated measurement chambers (optional). The chamber surface will reach dangerous temperatures when heated. The chambers are marked with hot surface warning symbol.

B. Instructions

The computer-controlled Temperature Control Unit (TCU) for use with Theta Flow consists of an electronics control box, a sample compartment surrounded by a ceramic insulator and a temperature probe. Plug in the serial cable between the control unit and the Theta Flow. Connect the serial cable to the port of the control unit and then to the **Ext I/O** port of Theta Flow rear panel with the cable supplied. The chamber is connected on the back of the temperature control unit. Connect the temperature probe to **TEMP** port on Theta Flow rear panel.

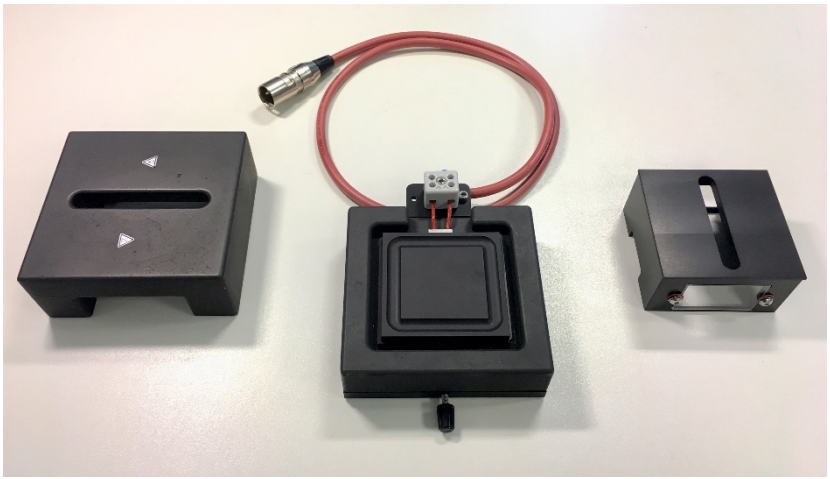
Connection ports on the back of the temperature control unit.

Technical specifications

Dimensions of control electronics box	130 x 230 x 185 mm^3 (h x w x d)
Dimensions of sample compartment	40 x 82 x 82 mm^3 (h x w x d)
Dimensions of sample compartment surrounded with ceramic insulator	90 x 115 x 115 mm^3 (h x w x d)
Heating plate power	150 W
Temperature range	from room temperature to 250 °C (C203E)
Accuracy	+/- 1 °C
Input voltage range	100 – 240 Vac
Frequency	50 – 60 Hz
Input current	450 – 1100 mA
Mains fuses	T3.15A 5x20 mm
Heater fuse	T3.15A 5x20 mm

The sample compartment consists of the following items:

- An insulating ceramic base plate with the heating plate and a cable for connecting to the heater port. The bottom of the ceramic base plate is made so that it fits perfectly on the sample stage of Theta Flow.
- Environmental chamber (made of aluminum) with glass windows and a lid with a hole for the needle. There is also one hole for the temperature sensor near one of the windows.
- An insulating ceramic lid with a corresponding hole for the needle as in the environmental chamber, and jackets for the windows.



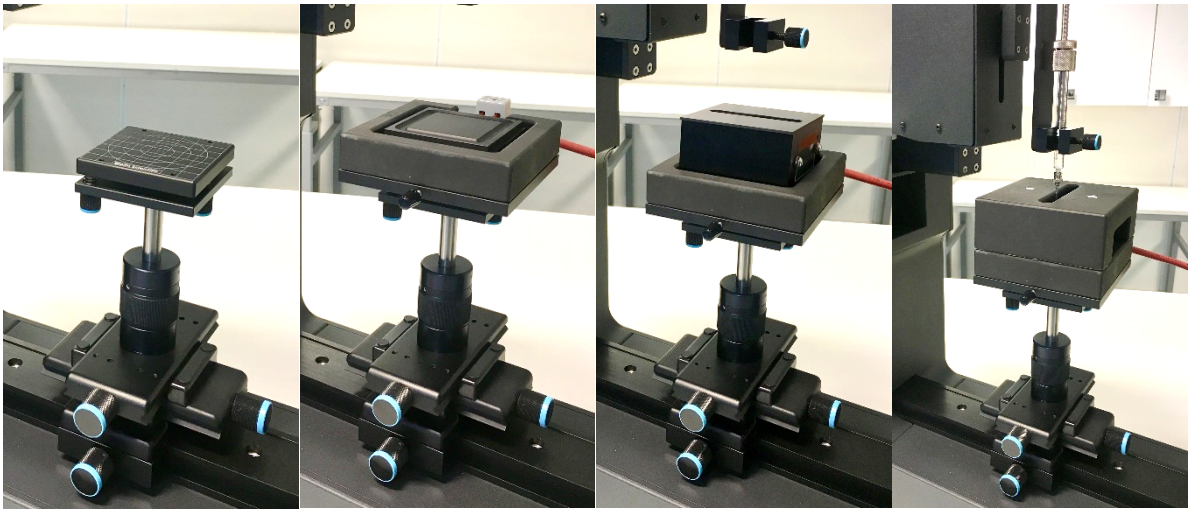
The insulating ceramic lid (left), ceramic base plate with heating plate and environmental chamber.

Use of the Temperature Control Unit (TCU)

After connecting the cables, the TCU is ready to be used with Theta Flow. Turn on both Theta Flow and the TCU.

Note! When using TCU with Automatic XYZ sample stage, remove the top plate from sample stage before mounting TCU.

Remove the large sample stage top by first loosening the screw on the side of the sample stage to reveal the smaller sample stage top. Place the ceramic base plate on the smaller sample stage so that the thick red cable is located behind the instrument. Insert the environmental chamber on the ceramic base plate in the slot reserved for it (fits only one way), then place the ceramic lid on top of the environmental chamber so that the windows are visible and so that the needle fits through the hole at the top into the sample compartment. For individual steps see pictures below from left to right.



Setting up the temperature control.

Start OneAttension software and enable the temperature control in main menu -> Global settings. After this, the target temperature of the temperature control unit can be adjusted from the recipe tab. In addition, the ambient temperature can be set, which has an influence on the heating power. Temperature control unit needs to be enabled from the recipe tab. In the Controls tab, monitor sheet will show the real time temperature if the temperature probe is used.

5.4 Tilting cradle

A. Safety

WARNING!

RISK OF INJURY. Tilting cradle is equipped with emergency stop button. Movement is stopped when the button is pressed. Tilting cradle must be positioned so that the emergency stop button is always accessible.

Grab the tilting cradle on the bottom frame ends when lifting and moving it. Do not lift the cradle when Theta is attached to it. Pay attention to the free-hanging support bracket when lifting or moving the cradle.

WARNING!
RISK OF CRUSHING. Locations where crush hazard exists are marked with crush hazard symbol. Stay clear of the instrument during tilting.

CAUTION!
Make sure no objects are positioned so that they obstruct movement of the cradle.

To disconnect the cradle, after turning the instrument off, remove the power cord plug from the electric outlet.

B. Instructions

The automated tilting cradle is an additional module that enables the investigation of dynamic contact angle i.e. advancing and receding contact angles.

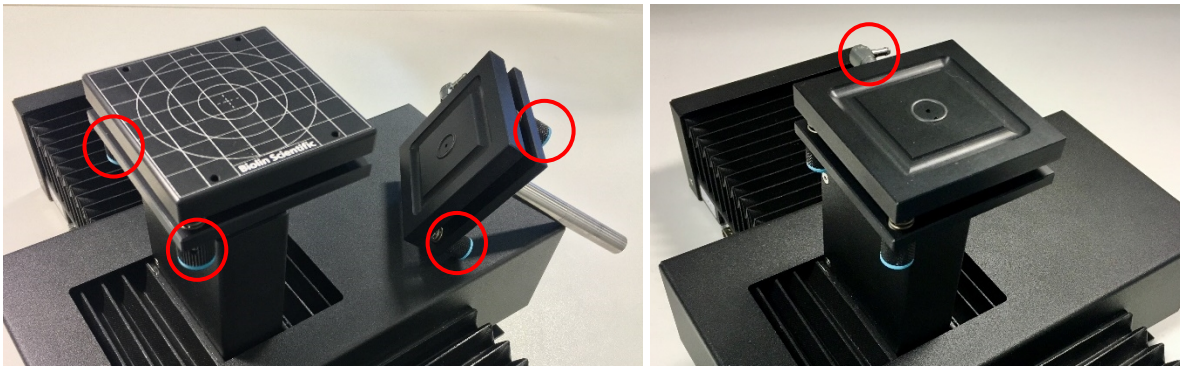
The automated tilting cradle consists of a frame that supports Theta Flow on an axle turned by a motor on one end and a pivot on the other. The motor is driven by the OneAttension software to turn Theta Flow at precise speeds to defined angles. As the stage tilts, so tilts the camera and thus the image onscreen is similar to what would be seen with a standard contact angle experiment.

Remove the instrument feet and lift the instrument on top of the tilting cradle. Note that it is advised to have two persons to lift the instrument. Attach the instrument to the tilting cradle with additional screws that come with the tilting cradle, do not use the instrument feet to attach the instrument.

Plug in the device cable between the tilting cradle and Theta Flow rear panel (**DEV X**). Connect the tilting cradle power cord to mains outlet with protective earthing. Connect Theta Flow power cord from tilting cradle to Theta Flow rear panel.

Please note that other accessories such as the 3D Topography module or temperature control chambers need to be removed prior to using the tilting cradle. Failing to do so may lead to injuries or instrument damage. The support leg of the 3D Topography module may be fixed during tilting as long as it's being made sure not to hit anything while tilting.

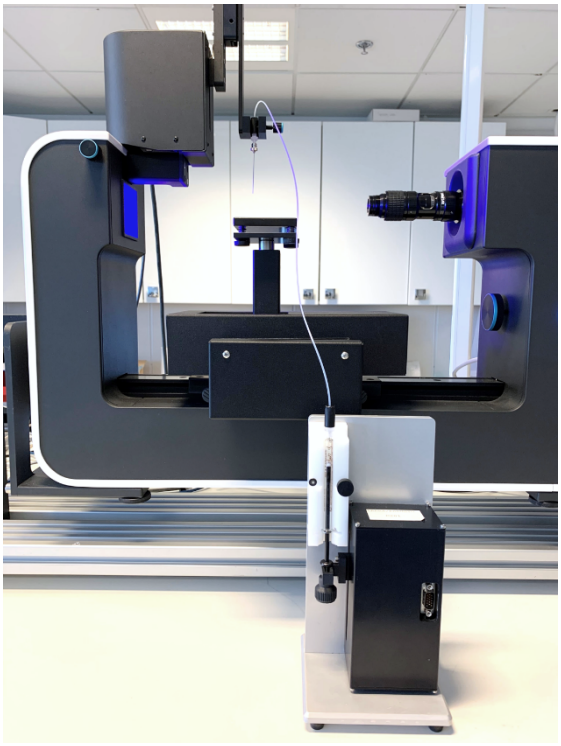
If you have also acquired the C204B Vacuum stage top, it needs to be inserted to your sample stage. If you have T310 Manual sample stage, T320 Automatic X sample stage, or T3X0L Large sample stage top the vacuum stage top is simply inserted to replace the current stage top you have by loosening the thumb screw fixing the stage top and inserting the new stage top. If you have T330 Automatic XYZ sample stage, the change is done by only switching the upper plate of the vacuum stage top into to the T330. Please see the pictures below for instructions.



Installing the vacuum stage top to T330 Automatic XYZ sample stage. Start by opening the thumb screws keeping the upper stage plate attached to the lower plate, both on the T330 and on the vacuum stage top (left image, a total of 6 screws). Be careful not to lose the springs in between the plates. Then attach the upper plate of the vacuum stage top to T330 with the same thumb screws that were holding the plate earlier. The springs go also between the plates as they originally were. After this, you have the T330 with the

vacuum stage ready to be used (right image). The vacuum pump tubing is connected to the adapter marked in the image. Please see a separate sheet on how to use the vacuum pump.

If you are using the C201 Automatic single liquid dispenser, place the dispenser in front of the instrument so that the instrument and its modules do not hit the dispenser when tilting. Observe especially the dispenser holder and the stage. If you are using the T330 Automatic XYZ sample stage, place the C201 Automatic single liquid dispenser on the right side of the stage's extension or in front of it so that dispenser is slightly more on the right side, to avoid collision.



Place the C201 Automatic single liquid dispenser either on the right side of the stage's extension or in front of it so that the dispenser is slightly more on the right side like shown in picture.

The first time after the sessile drop experiment tab is opened, the cradle will initialize itself. When initializing, the tilting cradle is driven to its limit switch position which is 8 degrees on the side. With the help of this limit switch position, zero position is defined and the cradle will move to the zero position. **Note!** Be sure that nothing is on the sample stage during homing.

In OneAttension, module settings are available on the **Theta tab** under Recipe tab**.** Speed of the movement, target angle and initialization angle can be adjusted. The function of the tilting cradle can be also disable.

Module settings for the tilting cradle.

Module controls are available on the **Theta tab** under Controls tab.

Stop: Stops all movement

Set Home: Sets home position, which is defined by the user. The home position is also automatically set to current position when a measurement is started

Go Home: Moves the sample stage to home position

Init: Initializes the module

Tilt Right: Tilt instrument clockwise from the point of view of the tilting motor.

Tilt Left: Tilt instrument anti-clockwise

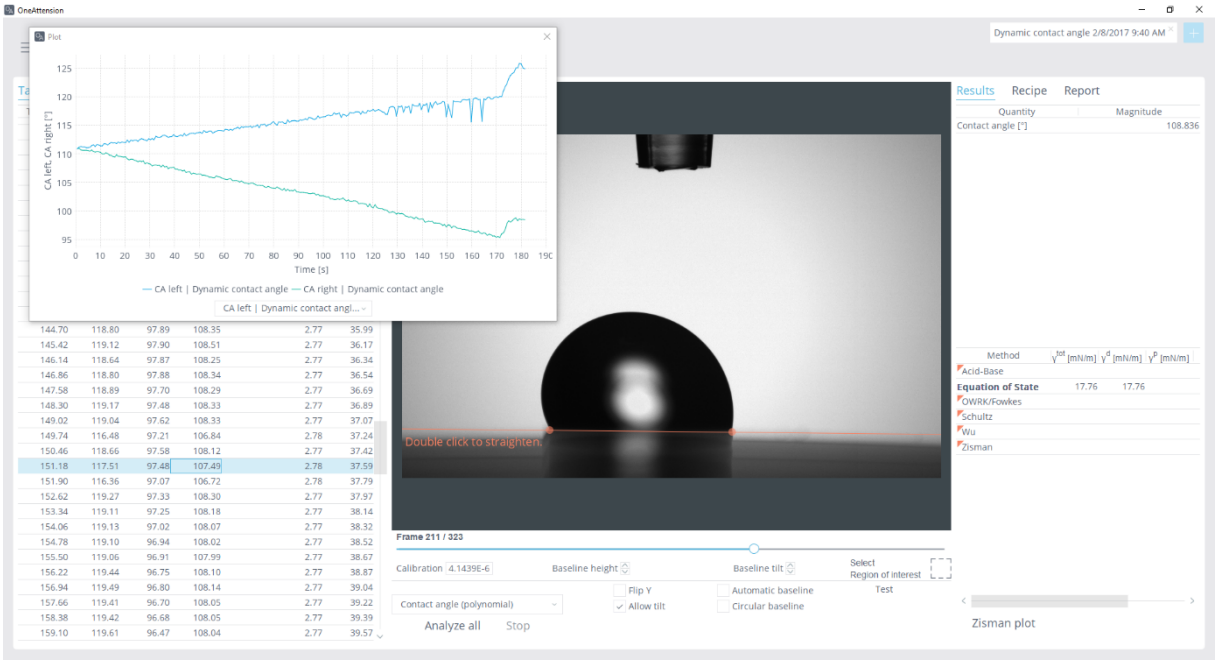
Set target to the tilting angle.

Tilts to the target angle.

Note! The emergency stop button stops the movement of both tilting cradle and Theta Flow.

Use a levelling tool to locate the position in which Theta Flow is horizontal, this should occur at roughly eight degrees from the initialization position. When Theta Flow is perfectly horizontal (adjust the legs of the frame as necessary), press Set home from the device controls. Now conduct the experiment as normal, either by recording a series of images at certain angles or by using the trigger to observe the moment the drop leaves the surface.

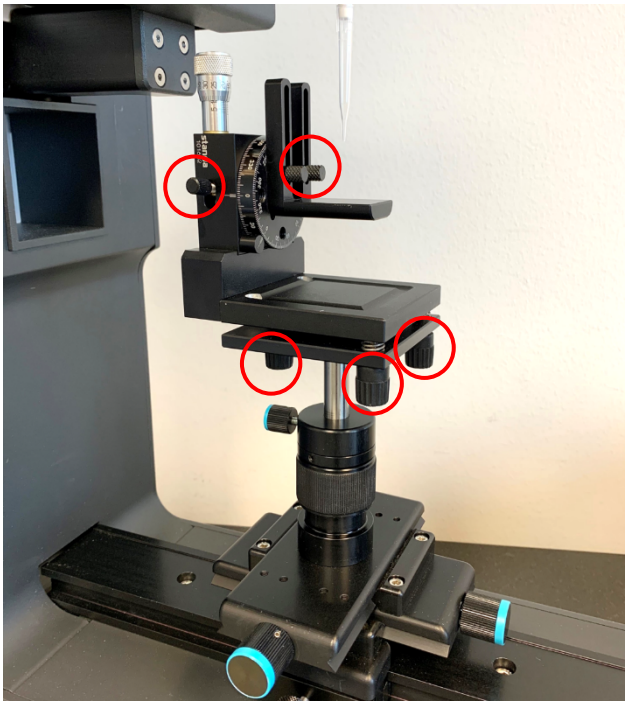
When the images are ready, image analysis is the same as with a standard contact angle experiment (see Chapter 6), except that the left contact angle will now show an advancing contact angle and the right contact angle will show a receding contact angle.



Example of result analysis of the dynamic contact angle measurement performed with tilting angle.

5.5 Manual tilting stage

Manual tilting stage enables the study of dynamic contact angle i.e. advancing and receding contact angles. Manual tilting stage is attached to Theta Flow similarly as standard sample stage. Open the thumbscrew under the sample stage, replace the sample stage with tilting stage and then lock the thumbscrew.

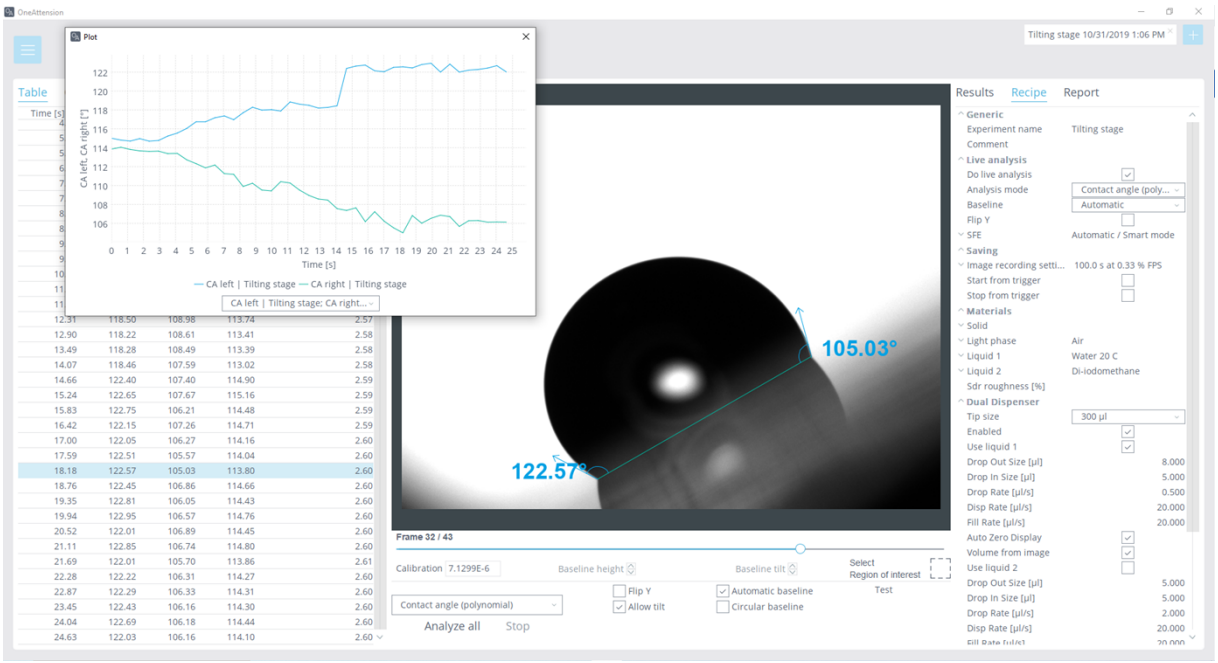


Manual tilting stage. Upper left thumb screw to lock the wheel movement. Upper right thumb screws to move the stage. Lower three thumb screws to level the base plate.

First the base plate must be levelled from the three thumbscrews, using a levelling tool. Initially the scale must be set to zero on the stage wheel and then locked with the small thumbscrew. Secondly, the tilting stage must be levelled by using a levelling tool and then locking the two thumbscrews. At this point you can turn the instrument on and look at the image to find a correct height for the stage to match your sample.

For a successful measurement it is essential that the sample and sample stage are positioned so that the top surface of the sample is located on the center of rotation of the tilting stage. If that is not the case, the droplet will move away from the image when the stage is tilted. It also helps to dispense the droplet to the middle point of the sample stage and position it in the middle of the image.

To conduct a measurement with the tilting stage, first place a droplet on your sample. The larger the droplet, the easier it is to get it rolling-off the surface. Lift the stage so that the droplet is somewhat in the middle of the screen. You may need to lift the dispenser up so that it does not hit the droplet. Use the automatic baseline as the baseline will tilt when the sample is rotated. Choose the polynomial fitting as the analysis mode. Start recording with a low frame rate and tilt the stage from the wheel until you find a position where the droplet is about to start moving along the sample. Then lock the wheel in position with the lock screw. Adjust the fine-tuning screw until the droplet starts to move. End the recording and analyze your measurement data. Find the frame where the droplet starts moving and determine the advancing and receding angles and the contact angle hysteresis from that frame. The left contact angle will show the advancing contact angle and the right contact angle will show the receding contact angle. Contact angle hysteresis is the difference between these two. If needed the baseline can be adjusted for individual frames manually in the analysis side.



Example of result analysis of the dynamic contact angle measurement performed with tilting stage.

5.6 Pulsating drop module

Overview

The Attension PD200 is a computer controlled module that enables a controlled perturbation (such as sinusoidal oscillation, triangular and square perturbation) for pendant drops or bubbles, and thus study of dilatational interfacial rheology. The perturbation is achieved with a piezo-pump enclosed in a chamber. The piezo-pump is driven through a pulse modulating electronic units.

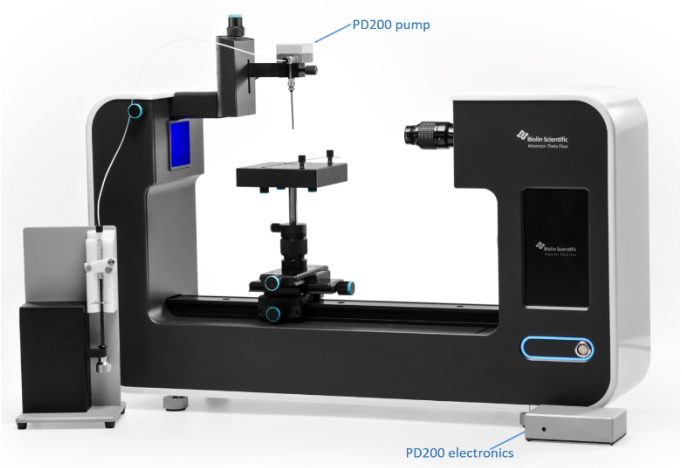
Technical specifications of piezo module

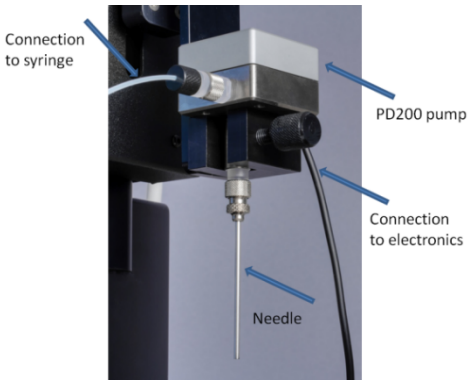
Perturbation principle	Piezo-electric membrane enclosed in a chamber
Frequency range	0-30 Hz (the most practical range 0.01 – 10 Hz)
Frequency resolution	0.001 Hz
Maximum volume amplitude	~0.8 µl
Sample liquid volume needed	5 µl
Material	PEEK, PTFE, FPM, titanium

Connections

The pump chamber contains the piezo pump that oscillates the drop at the end of the needle. A syringe is used as the liquid reservoir. Both automated (C201) and manual dispensers can be used with the PD200, however only automated dispenser enables compensation of evaporation with "volume from image"-function. A Teflon tube is used to connect the syringe to the pump or the pump to the needle.

The pump is controlled by the electronics unit. Connect the serial cable to the port of the control unit of the PD200 and then to a **DEV** port on Theta Flow rear panel with the cable supplied. Then connect the pump to the control unit of the PD200, after which PD200 is ready to use (see Figure below).





Theta Flow with PD200 and automated C201 dispenser.

Using the PD200

Start OneAttension software and begin a Pulsating drop experiment. PD200 device controls and adjustable parameters in the recipe tab will appear automatically.

Note! Use only chemicals, which are tolerated by the pump materials (see pump materials above). Isopropanol and water are recommended for cleaning.

Pulsating drop device controls:

Start: Starts the oscillation according to the experimental parameters in the recipe sheet (not according to set points). Measurement will not be recorded. This function is very useful for pre-testing the system and making sure that the pulsation is stabile (typically this indicates if there is air in the pump).

Stop: Stops the oscillation.

Recipe tab:

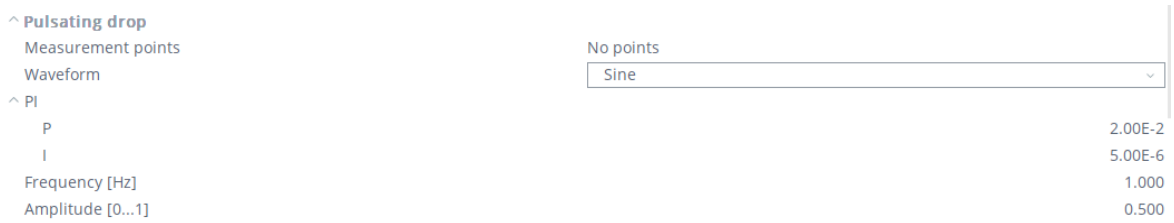
Measurement points: Several frequencies can be measured automatically during one recording. From the Measurement points the Triple point mapper will appear. Adjustable parameters consist of the *Wait time* (s) before the first frequency and between the different frequencies, *Frequencies* (Hz) for each measurement point and *number of cycles.*

Waveform: Select an appropriate Waveform, the choices are Sine, Square, Triangle and Constant. The Sine wave is the most typical form for interfacial rheology experiments.

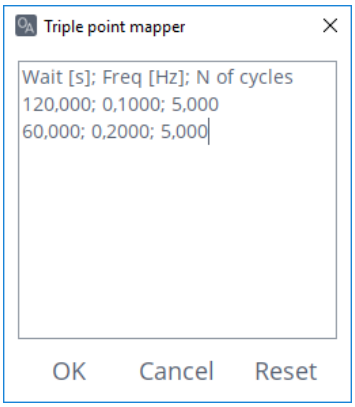
PI Drop-down menu to set the PI controller's coefficients used in evaporation compensation. Coefficient P is a weighting factor for the current error, i.e., the error between current and target droplet volumes. The greater the weighting factor is, the stronger the system tries to correct the error. Too great value will result in an overshoot of the droplet volume, while with too small value the droplet will never reach the desired volume. The integrator term is used to compensate possible overshoot error by integrating the error over time, and is weighted by coefficient I. In case it is needed to fine-tune the weighting factors, it is advised to understand the principle of a PI controller before changing the values. The fine-tuning can be started by setting the I term to be zero and then increasing the P term until system reaches oscillating behaviour around the set droplet size. Then increase the I term to dampen the oscillation.

Frequency [Hz]: Adjust the frequency, which will be used in the oscillation started from the Oscdrop device controls.

Amplitude[-1…1]: Set a suitable amplitude.



Module settings of PD200.



Triple point mapper to set several frequencies for the pulsating drop measurement.

Filling the system

There are several ways to make the measurement; either by filling the system with the liquid to be studied, or by using cuvette and hooked needle. To fill the system with liquid, start by filling with water. Then suck a small amount of the liquid to be studied. Filling the system with water will make changing the studied liquid easier, since there is no need to extensively wash the pump after measurements. This method will be explained in more detail below. Using the hooked needle is explained separately. By using any of these methods, it is also possible to measure liquid-liquid interfaces. When a straight needle is used the heavier liquid must be in the needle, but for hooked needle measurements the heavier liquid must be in the cuvette.

Using a straight needle

1. Fill the syringe with water, attach the syringe to the Teflon tube and push liquid manually into the pump. You may have to repeat this a few times (2-5) to make sure there is no air in the piezo pump since that will cause problems with the operation of the pump. If you are filling the system with the studied liquid the system is now ready to be used.
2. Place the beaker containing the liquid of interest under the needle and suck a small amount of that into the needle (~20 µl) by using "droplet in" function.
   - Volume of the straight needle and the connection (14 gauge) is about 150 µl

   Usually only a small amount of liquid is consumed during the measurement. Now the system is ready to be used.
3. Set oscillating amplitude to 1 and oscillating frequency to 0.5 Hz. Test that the oscillation behaves correctly by squeezing out the droplet and pressing the start button from the Controls tab. Follow the live results. The volume change should be at least 0.8 microliters and the curve sinusoidal. Note however that if the computing power of the computer is low, it might be that the blue fitting line is not able to follow the edge of the droplet which will make the live analysis curve look rough. If you record the oscillation and do the analysis in the analysis part, the curve probably looks smoother. In case the pulsation is not stable, the most common reason is having air in the pump.

Using a hooked needle

Using a hooked needle has some advantages over the pendant drop. The stability of the measurement is higher since the system is not affected by the air flow so easily. Also the evaporation losses are negligible.

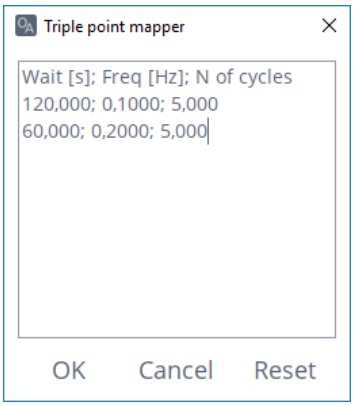
1. Fill the syringe with water, attach the syringe to the Teflon tube and push liquid manually into the pump. You may have to repeat this a few times to make sure there is no air in the piezo pump since that will cause problems with the operation of the pump.
2. Put the liquid that you want to study in the cuvette.

3. In case you are studying air-liquid interface, suck a small amount of air into the needle (~15 µl). **Be sure not to suck too much causing the air plug to reach the pump.**
4. In case you are studying liquid-liquid interface, add the less dense liquid in the needle by using fill option (follow volume from monitor).
    - Volume of the hooked needle and the connection (22 g) is about 24 µl
5. Immerse the needle into the liquid.
6. Set oscillating amplitude to 1 and oscillating frequency to 0.5 Hz. Test that the oscillation behaves correctly by squeezing out the droplet and pressing the start button from the Controls tab. Follow live results. The volume change should be at least 0.8 microliters and the curve should be nicely sinusoidal. Note however that if the computing power of the computer is really low, it might be that the blue fitting line is not able to follow the edge of the droplet which will make the live analysis curve look rough. If you record the oscillation and do the analysis in the analysis part, the curve probably looks smoother.

The recipe and recording

Fill in the recipe.

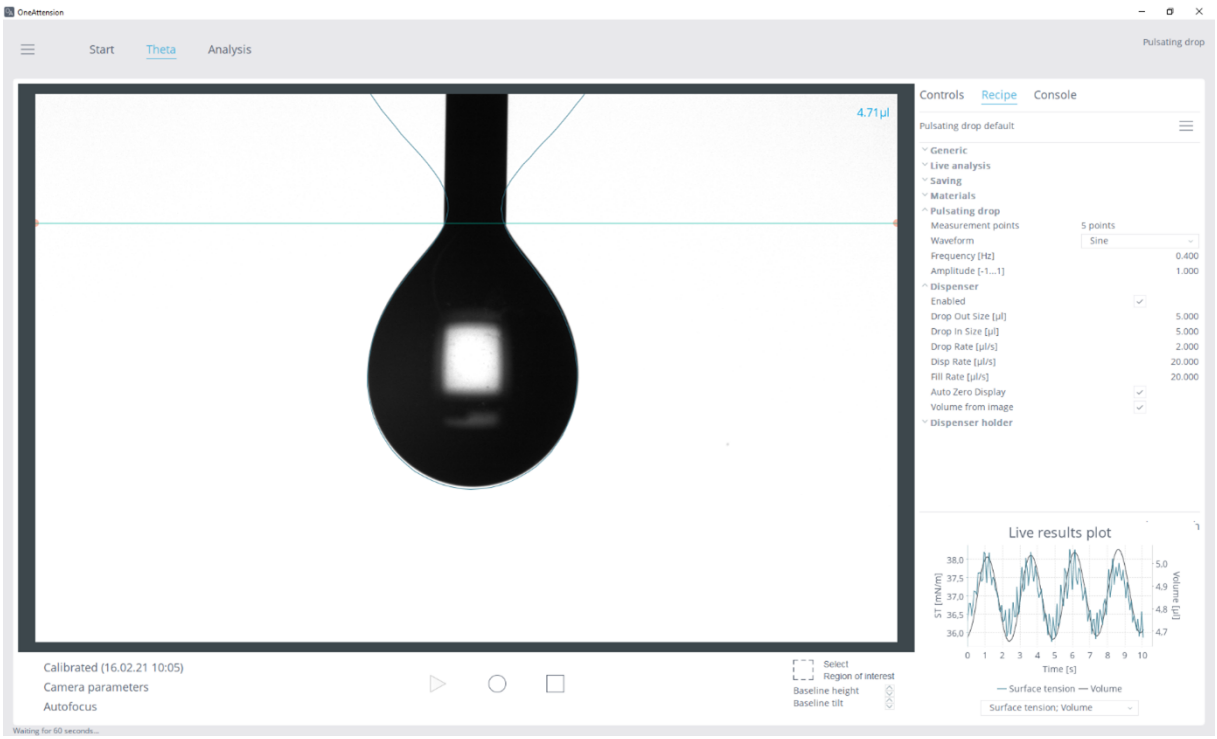
1. The image recording time has to be long enough for a sufficient number of complete oscillations to occur. This will depend on the oscillation frequency being requested. The recording time is number of cycles/frequency. For example, if 10 cycles with 0.1 Hz is used, the recording time is 100 s. You do not have to calculate the recording time, just mark long enough time and the software will calculate the suitable time for each frequency by itself.
2. The light and heavy phase has to be selected since a density difference value is needed for the surface tension measurement.
3. Under Pulsating drop; measurement points are written. Wait time defines how long the system is allowed to stabilize after the frequency is changed and before the recording is started. Depending on the type of surfactants studied, the time can be very long (up to several hundred seconds). A typical measurement script is presented below. Waveform is typically sine and the amplitude is set to one.



4. Drop out size depends on the sample. Relatively large drops should always be used. Air condition (air flow) and table vibration can cause problems especially when large droplets are used (see how measurements can be done with hooked needle). If the automatic single liquid dispenser and volume from image functions are used, the volume of the droplet will stay constant throughout the whole measurement. If a manual syringe is used, the decrease in volume can be detected.
5. Press **Start** to perform measurement according to recipe settings.

Pulsating drop experiments feature an evaporation compensation algorithm. The algorithm uses the dispenser to automatically fine-tune the drop size so that it stays constant in the long run. For the evaporation compensation algorithm to work, live analysis must be running and the dispenser must

be enabled. To disable the evaporation compensation algorithm, disable live analysis or dispenser (or both). The evaporation compensation features a feedback loop mechanism to control the droplet volume to stay constant during the measurement. Based on the difference between the current and the desired droplet volume, the instrument uses PI controller in the pulsating drop module measurement mode to apply correction to enhance or reduce the dispensing rate. PI controller consists of proportional and integral terms, and their weighting factors can be changed in the recipe.

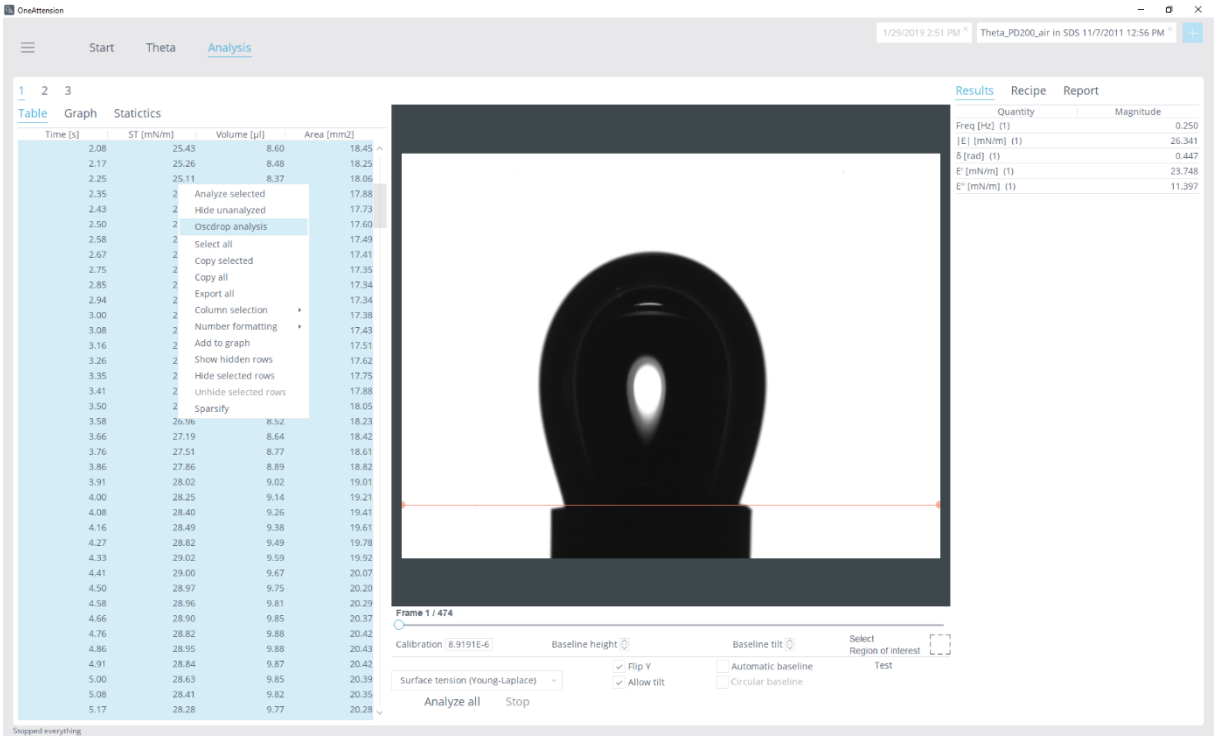


Pulsating drop experiment tab during the measurement and stable pulsation.

Curve fitting and data analysis

Curve fitting and basic data analysis is done in the same way as with a normal Pendant drop experiment (see Chapter 6).

The PD200 complements surface / interfacial data with viscoelastic characteristics of surface / interfacial layers. Choose all the data rows to be analysed, right click the analyzed results and choose Oscdrop analysis. The calculated results will then appear in the **Analysis tab** giving the viscoelastic parameters of the surface / interfacial layers. The analysis needs to be done separately for each measured frequency. By right clicking the calculated results and choosing the Graph Oscdrop, the Graph from the calculated results is drawn as a function of used frequencies. The steps for result analysis are illustrated in the figures below.

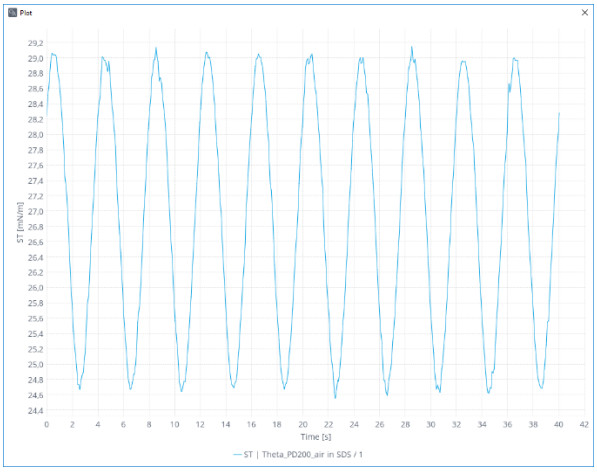


Analysis tab of the PD200 experiment. Right click and choose Oscdrop analysis.

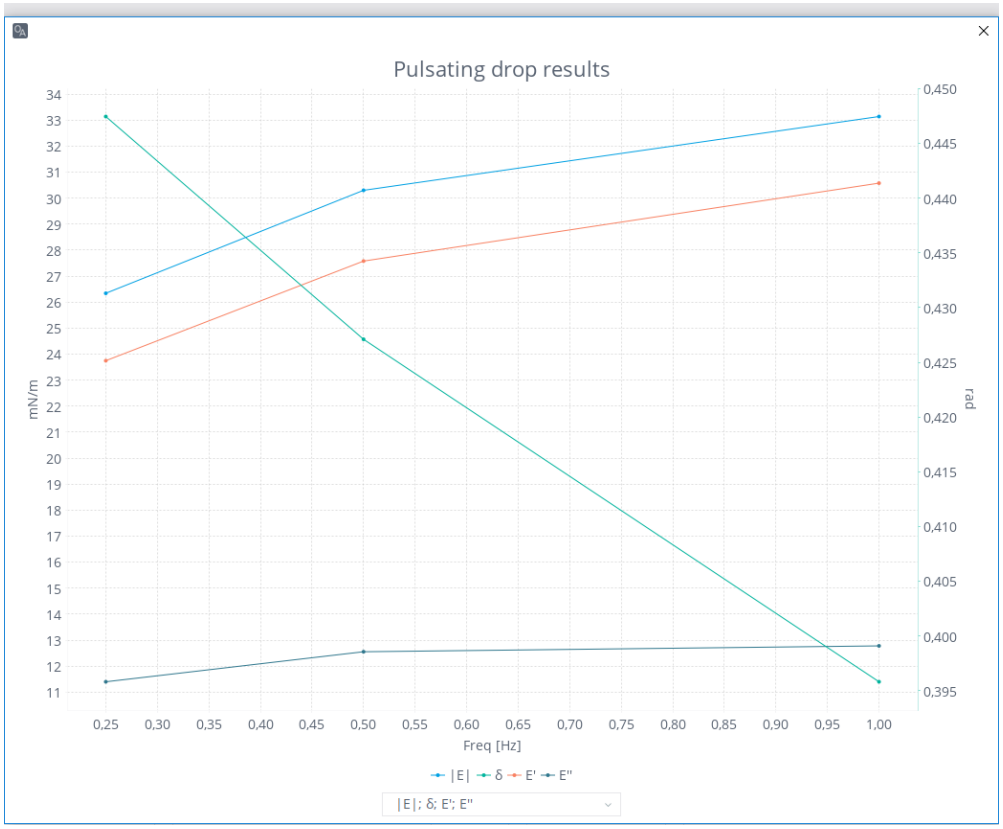
Calculated results and a graph of the analyzed surface tension and drop area as a function of time.

Results Recipe Report

Quantity	Magnitude
Freq [Hz] (1)	0.250
|E| [mN/m] (1)	26.341
δ [rad] (1)	0.447
E' [mN/m] (1)	23.748
E'' [mN/m] (1)	11.397



Graph Oscdrop results function to draw a graph of the oscillation results as a function of the frequencies.



Oscdrop result graph as a function of the used frequencies.

5.7 3D Topography module

A. Overview

In real life, wettability and contact angles are dependent not only on the surface chemistry of the studied material but also on the topography. High surface roughness increases contact angle with hydrophobic materials (CA > 90°) and decreases contact angle with hydrophilic materials (CA < 90°). The effect of the surface roughness to contact angle and wetting properties can be studied by measuring surface topography and applying the results through Wenzel equation. For more detailed theory on the influence of surface roughness on contact angle and wettability, please refer to section 7.4.

Optional 3D topography module for Theta measures surface roughness optically with a non-invasive procedure. Topography measurement results include quantitative two- (2D) and three-dimensional (3D) surface roughness parameters, optical image of the surface as well as both 2D and 3D topography graphs. Quantitative surface roughness values can be used in further calculations to combine with traditional contact angle measurement results, resulting in roughness corrected contact angle and surface free energy (SFE). The topography and contact angle measurements can be programmed to be performed automatically at the same sample area.

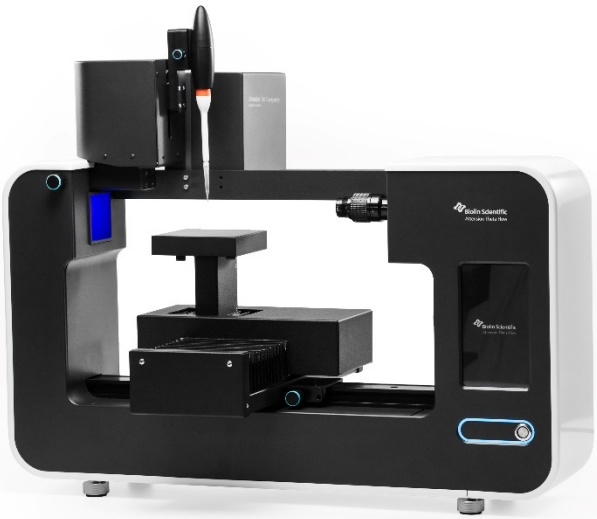
The measurement is based on the principle of fringe projection phase shifting in which a structured illumination pattern of sinusoidal intensity is projected on a surface. The fringe images are recorded on a digital camera. 3D contours of the surface modulate the phase of the fringe pattern seen from the camera's point of view. By calculating the phase shift caused by this modulation the 3D shape of the surface can be determined. The method in general form can be expressed by the following formula:

$$\varphi = \arctan\left(\frac{\sum bI}{\sum aI}\right), \text{ where}$$

$$a = \sin(\omega n)$$

$$b = \cos(\omega n)$$

I is the intensity in recorded image. If three phase shifts are used, ωn = [0, π/3, 2π/3]. The principle is described in more detail in: G.-S. Han, S.-W. Kim, "Numerical correction of reference phases in phaseshifting interferometry by iterative least-squares fitting", Applied optics 33, 7321-7325 (1994).



3D Topography module with Theta Flow.

Technical specifications and requirements

Dimensions:	17 cm x 16.5 cm x 11.5 cm (+ support arms 35 x 8 x 25 cm)
Weight:	2.6 kg (+ support arms 2.3 kg)
Maximum sample size:	Unlimited x 18 cm x 2.2 cm (XYZ)
Power supply input:	100…240 VAC. 50…60 Hz, 350 mA
Topography module input:	12 VDC, 350 mA
Method:	Fringe projection phase-shifting
XY pixel size:	1.1 µm x 1.1 µm
Measured range in Z direction:	1 µm – 60 µm
Lateral sampling (XY):	1.41 mm x 1.06 mm (Stitching option up to 4.2mm x 4.2 mm)
Working distance:	18 mm
Imaging options:	Optical image, 2D and 3D roughness graphs
Measurement speed:	5-30 s (1280 x 960 measurement points)
Sample requirements:	Diffuse reflecting surface required

Note! Topography module is based on the idea of measuring first the surface roughness of a sample area and subsequently measuring the contact angle at this exact same area. Therefore, an automatic

XYZ sample stage is required for topography module to function. Also, a motorized dispenser holder and an automated dispenser are required for sequencing measurements.

B. Installation

CAUTION!
Remove the sample attachment clips from the sample stage when using 3D Topography module.

1. Mount the supplied square bar to Theta Flow with supplied M6x16 screws (2 pcs).

2. Fasten the Topography module to the square bar with the supplied M4x16 screws (2 pcs). Note that there are two mounting positions.
   a. Use the position closer to the camera when either manual or automatic dispenser rotation module is attached.
   b. Use the position closer to the light source with other dispenser configurations.

3. Attach the motorized XYZ-stage as described earlier in the "Motorized XYZ-stage" chapter.

4. Connect the supplied USB2 cable from topography module to **EXT USB** connector on Theta Flow rear panel, or directly to your PC. Next, plug in the power cord and turn on the module from the on/off button. Topography light source should initiate when the module is turned on. Turn on your Theta Flow.

5. Install the latest OneAttension software provided on USB drive with your 3D topography module. For specific installation guidance, see the OneAttension Theta Flow installation manual.

The 3D topography module is now installed. When OneAttension is opened, it shows Topography tab next to **Theta tab**.

CAUTION!
When you open the measurement for the first time, Theta will initialize all the automated functions (e.g. dispenser holder and XYZ-stage). Care should be taken that there is enough space for all the movements to be completed.

C. Calibration

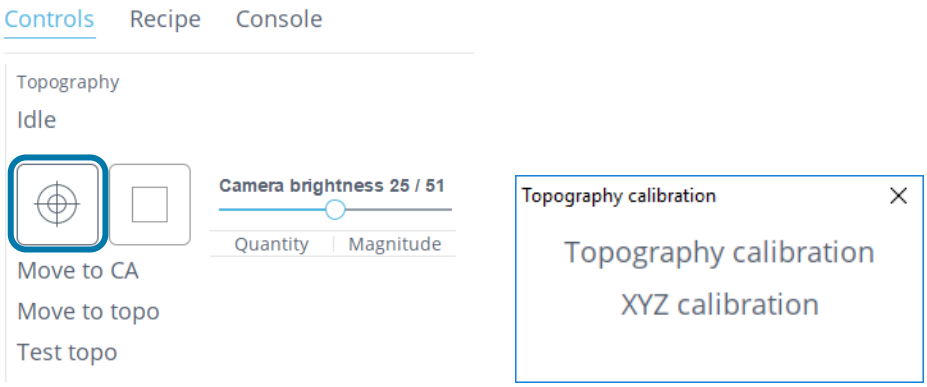
The topography module calibration consists of two parts: topography calibration and XYZ calibration. The purpose of the topography calibration is to calibrate topography movements. This value is used, when the surface roughness is measured. The purpose of the XYZ calibration is to tell OneAttension what is the distance between topography and contact angle measurement positions. This information is used when both topography and contact angle measurements are wanted to be done at the exact same place.

Before performing these topography calibrations, calibrate Theta Flow camera according to the instructions in section 3.2.

XYZ calibration

If the XYZ calibration has not been done, OneAttension will use standard values stored in its memory. These values may be inaccurate for your system. Therefore, it is strongly suggested to perform the calibration for your system whenever the system configuration has been changed (after topography installation or after the instrument has been moved). **Note!** If the XYZ calibration has not been performed accurately, topography and contact angle measurements cannot be performed exactly at the same area.

The XYZ calibration is done by selecting Topography → Controls → Calibration icon -> XYZ calibration.

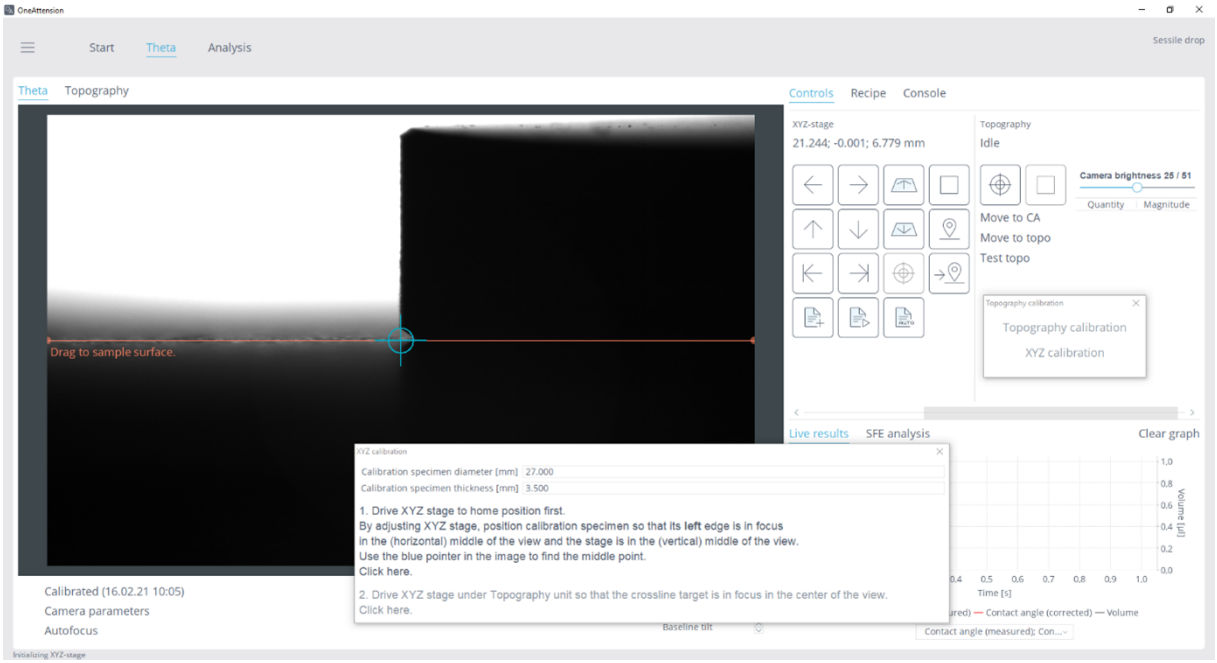


XYZ calibration.

First, move the stage to the home position. Move the dispenser to the image and adjust focus so that the dispenser is focused. **Do not adjust the focus from the optics at this point or afterwards, and disable the autofocus function at this point.** Then place the calibration specimen on the sample stage so that its middle point is below the tip of dispenser pipette. After this, move the dispenser out of the image.

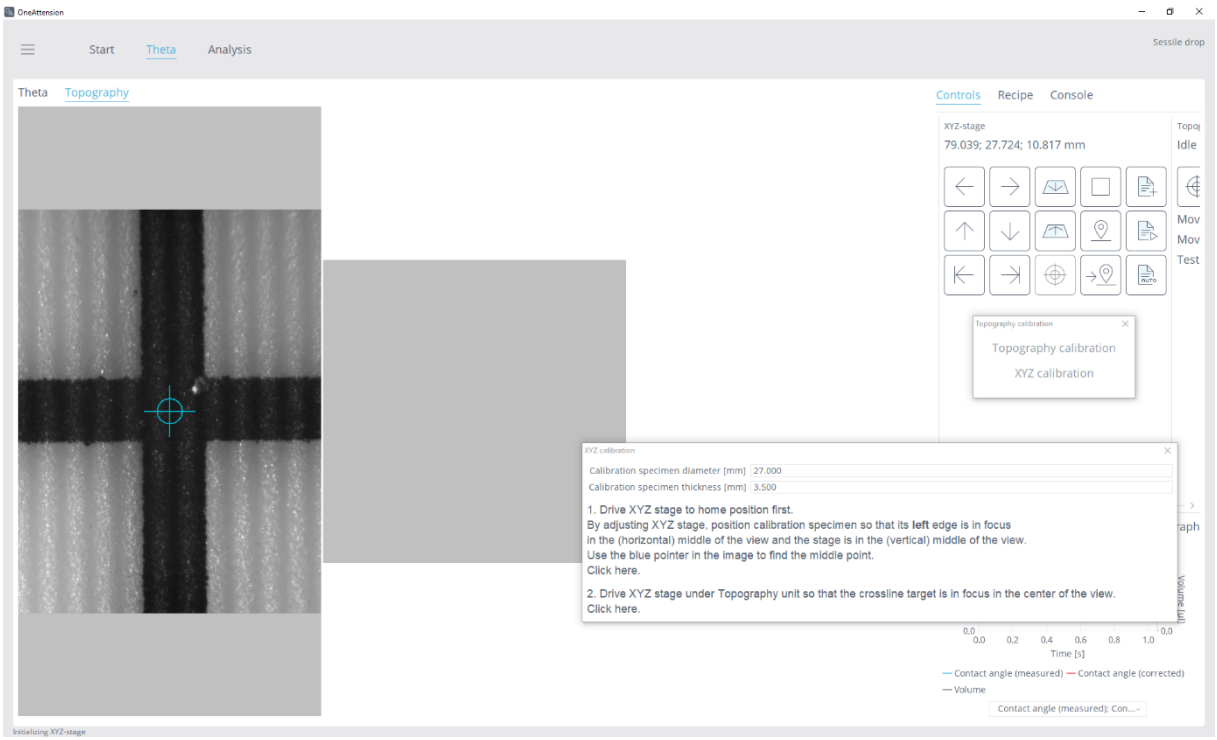
Then, move the stage so that the left side of the calibration specimen is visible in the middle of the screen and it is focused. Move the stage so that the vertical line of the calibration target crosshair is in line with the vertical side of the calibration specimen.

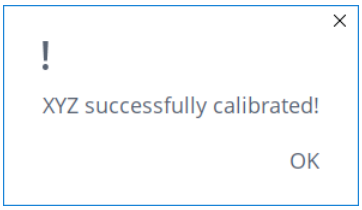
Then, move the stage so that also the horizontal line of the calibration crosshair is in line with interface between the calibration specimen and the stage. Do this by using the controls that are found under the XYZ-stage tab (you may have to adjust XYZ speeds in order to find the correct spot accurately. **Do not move the position of the red line.** This is the position where your contact angle measurements will be done. Insert the calibration specimen diameter. Click the first button at the calibration screen.



Crossline target.

Next, move the stage under the topography camera so that the crossline target is in the middle of the topography camera screen. Choose **Move to Topo** on Controls tab in order to move under topography quickly and then fine-adjust the correct position. This is the position where the topography measurements will be done. Click the second button in the calibration window. Choose Yes. A confirmation for calibration will appear.





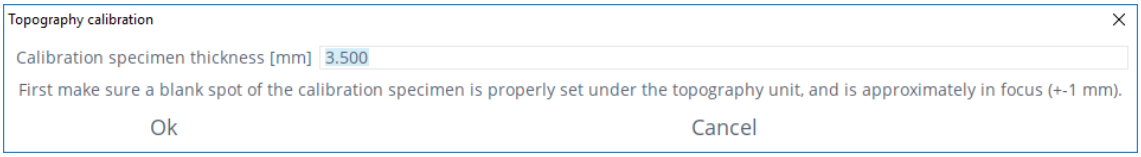
Succesful XYZ calibration.

Topography calibration

Click Calibration icon on the Controls tab and select Topography Calibration

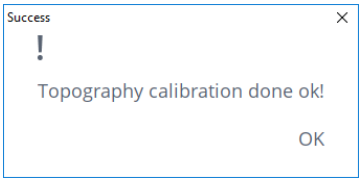
Place the calibration sample provided with the topography module on the Theta sample platform. Move the sample stage under the topography. After the stage has moved, find an approximate focus for the camera by using the controls under the XYZ-stage tab. Use a white area of the calibration sample (not area with black cross) for calibration.

OneAttension gives note that the calibration specimen must be properly set and that it should be in approximate focus (± 1 mm). Insert the calibration specimen thickness and select Ok. The calibration sample thickness is written on the sample box.



Once Ok has been selected, the topography module will calibrate the height automatically. **Note!** It is crucial that there are no vibrations around the instrument during calibration, otherwise the calibration will not be successful.

After the calibration is done, OneAttension will announce "Topography calibration done ok!".

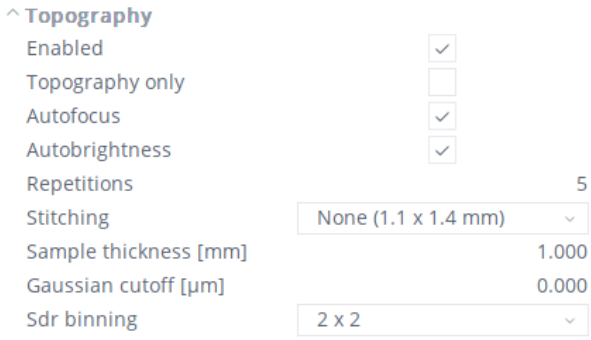


Topography measurements can't be performed until a valid z height calibration has been verified. The calibration should be done always after moving the instrument or if there is a reason to believe the calibration is no longer valid. Generally, the calibration is very stable and needs to be redone seldom. The calibration is computer-specific, meaning it needs to be performed always if the measuring computer is changed.

D. Measuring with the topography module

To combine contact angle and surface roughness measurements, choose sessile drop experiment. After the automated modules have initialized and you have performed the calibrations depicted above, the equipment is ready for measurements.

If Topography module is used with dual-dispenser unit software rotates the dual dispenser to position 2 when measurement is started. This prevents a crash between the stage and the dispenser 2.



Topography options and controls

The recipe features some topography-specific parameters when the topography module has been connected.

Enabled Yes/No: Is the module enabled. Always tick when module is in use.

Topography only Yes/No: Do you want to use only topography (not contact angle measurement as well).

Autofocus Yes/No: Does the topography module find the focus automatically or if the focus is set manually by moving the XYZ stage to the correct position. If not ticked, the topography measurement will be performed with the exact same focus you have set prior to starting a measurement.

Autobrightness Yes/No: Does the topography module find the brightness level automatically or if the brightness is set by the user in the Topography tab. If not ticked, the topography measurement will be performed with the exact same brightness you have set prior to starting a measurement.

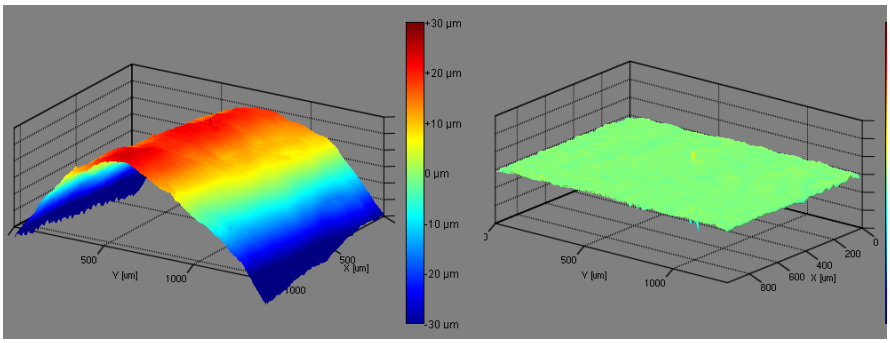
Repetitions: How many measurements will OneAttension perform in a single run. The results shown are averages of all measurements if repetitions > 1. Large number of repetitions will lower random result variance, but the measurements will take slightly more time. Default value is 5.

Stitching: Option for enlarging the topography measurement area by performing multiple measurements from adjacent areas. These individual measurements are then automatically combined for one larger analysis. The options are "None (1.1 x 1.4 mm)" for a standard measurement, "2 x 1 (2.1 x 1.4 mm)" for a stitching of 2 measurements, "3 x 2 (3.2 x 2.8 mm)" for a stitching of 6 measurements, and "4 x 3 (4.2 x 4.2 mm)" for a stitching of 12 measurements. The first number tells the amount of adjacent horizontal measurements, the second number tells the amount of adjacent vertical measurements, and the numbers in brackets show the total measurement area. Please read more on the stitching option from "Performing a stitched measurement" below.

Sample thickness (mm): The thickness of sample, should be accurate to ± 0.5 mm. Must be written prior to experiments and changed always if sample thickness changes.

Gaussian cutoff (µm): Software parameter for removing certain wavelengths in the topography. Topography of a sample may consist of multiple different topography components. The topography can be divided into multiple sinusoidal waves with different wavelengths. The Gaussian cutoff analyses the topography and filters away all the sinusoidal forms that have a greater wavelength than the value of the Gaussian cutoff. However, the value of 0 µm signifies that the filter is not in use.

The Gaussian cutoff is especially useful when the examined surface has also other curviness in addition to "true" surface roughness. For example, if the topography measurement is done on a rod, the curviness of the rod itself can be removed. This leaves only the roughness of the surface in the analysis. If used incorrectly, Gaussian cutoff may remove sinusoidal forms that are essential for a surface topography.



The same measurement with 0 µm Gaussian cutoff (left) and 50 µm Gaussian cutoff (right). The macroscopic surface shapes with long wavelengths (> 50 µm) are filtered, and only small-scale surface roughness remains.

Default value is 0 µm. As a default, no wavelengths are filtered and the image is shown as measured. When reporting topography results, always denote if cutoff filtering has been used on the data.

This parameter only affects data analysis, not the measurement itself. Therefore, this parameter can also be changed after the measurement at data analysis stage.

Sdr binning: Software parameter for averaging results. Sdr binning combines pixels for each area of defined size in the images. Based on these values, a new S_{dr} value is calculated (the averaging is not shown in the images).

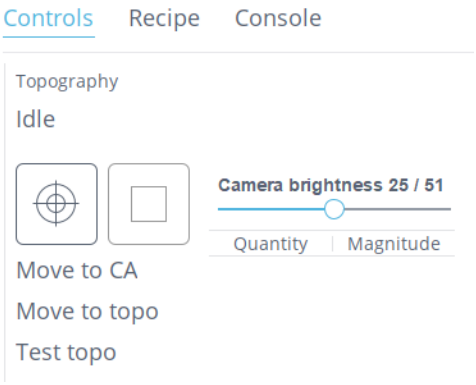
Sdr binning is used for reducing the effect of single incorrect data point values. The more noise your measurements have, the more you should use Sdr binning. However, the resolution of the analysis will be reduced significantly if Sdr binning is used. For example, 2x2 binning reduces the resolution to 1/4 of the original, and 3x3 binning reduces the resolution to 1/9 of the original. This will make smaller topography details undetectable. Also, fewer pixels will inherently lead to smaller S_{dr} values altogether.

Default value is 2 x 2. The 2x2 value will do some averaging and therefore, the effect of single pixels is reduced. Also, the resolution is not as reduced as with larger binning values. This value is suitable for most measurements. When reporting topography results, always denote if binning has been used on the data.

This parameter only affects data analysis, not the measurement itself. Therefore, this parameter can also be changed after the measurement at data analysis stage.

Controls tab shows XYZ position of the autostage and the status of topography unit. Console shows all messages, now including also topography-related messages. Also, the stage controls at the XYZ-stage tab are the same as without topography (see 4.1, motorized XYZ sample stage).

Topography controls are available on the Controls tab**:**

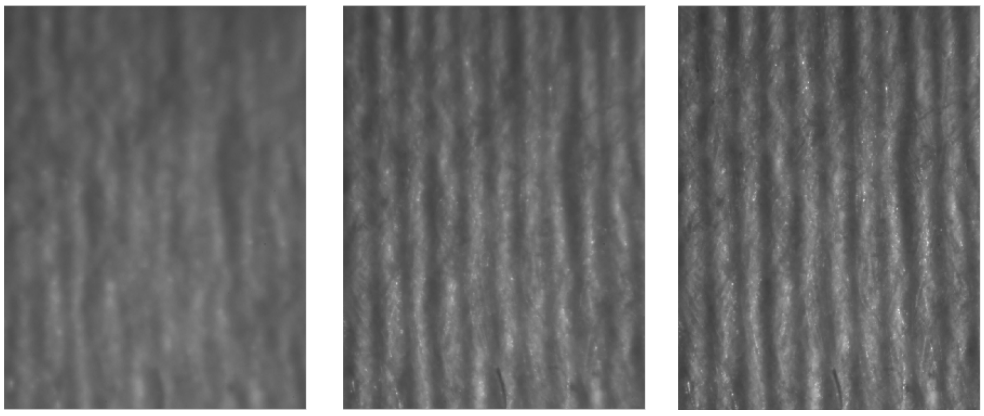


- **Move to CA**: Moves the autostage to the contact angle measuring point (in front of Theta Flow camera). The movement is based on the XYZ calibration results. The movement rate is hard-coded and cannot be changed.
- **Move to topography**: Moves the autostage to the topography measuring point (under topography camera). The movement is based on the XYZ calibration results. The movement rate is hard-coded and cannot be changed.
- **Test topography**: Performs a topography measurement without using sample thickness and without saving the results. Shows results at the lower right corner of the screen. If you wish to save these results, you must copy-paste them to another workbook. Please note that the sample must be in approximate focus prior to test topography.
- **Calibration**: For performing calibrations, see "Calibration" chapter above.
- **Camera brightness**: For setting the topography camera brightness. If Autobrightness is not chosen in the recipe, this brightness value will be used in the measurements.
- **Stop**: Stops a run

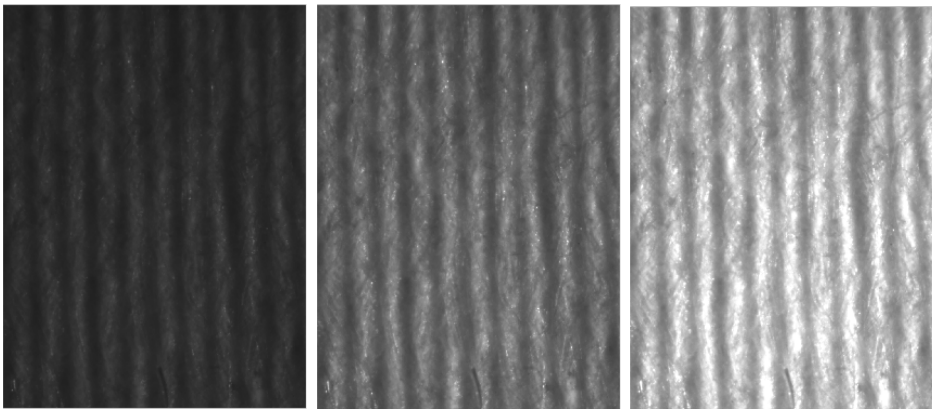
Choosing the correct focus and brightness

In most cases, autofocus and autobrightness functions are able to define optimal level of stage height and brightness for each sample. However, sometimes samples require special conditions for the best results. The focus and the brightness can then be set manually.

Focus level can be set manually by moving the XYZ stage up or down when topography module's autofocus is disabled in the recipe. For more accurate setting, it's a good procedure to lower XYZ stage Z speed to for example 10 mm/min. Good focus is achieved when surface features look the sharpest. Examples for inadequate (left), close (middle), and good (right) focus levels are shown below.

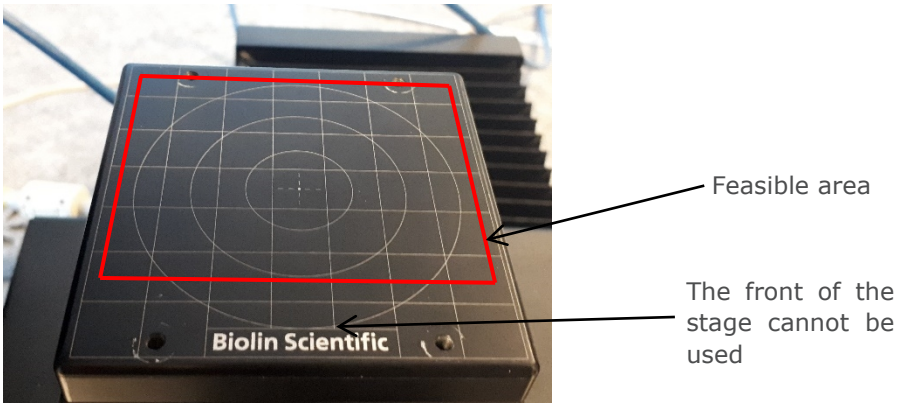


Brightness level can be set in the Topography tab. For best results, the brightness should be as high as possible without overexposing too big friction of the image. Autobrightness aims for this by overexposing a small proportion of all pixels. Examples of too dark (left) and feasible (middle and right) images are presented below.



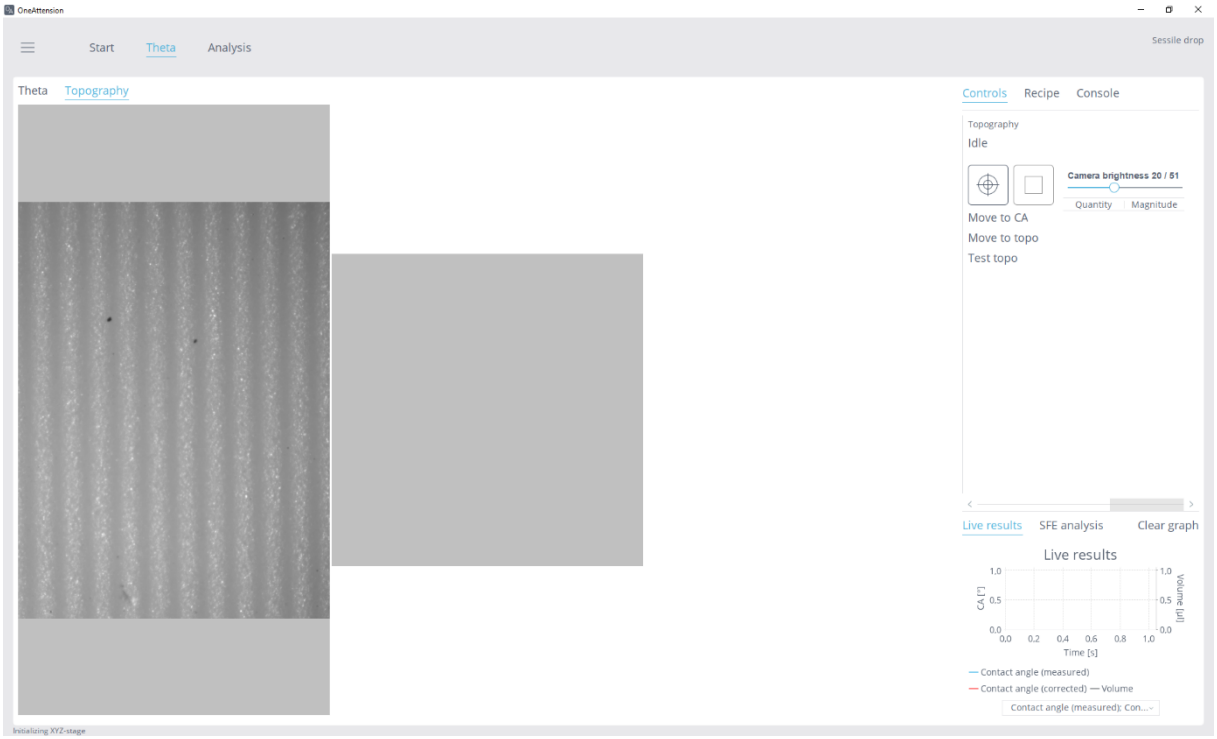
Feasible sample stage areas

The measured sample area must be placed on the sample stage in an area that can be seen in the Topography camera. The exact feasible sample stage area is dependent on each system, but the approximate feasible area is depicted below.

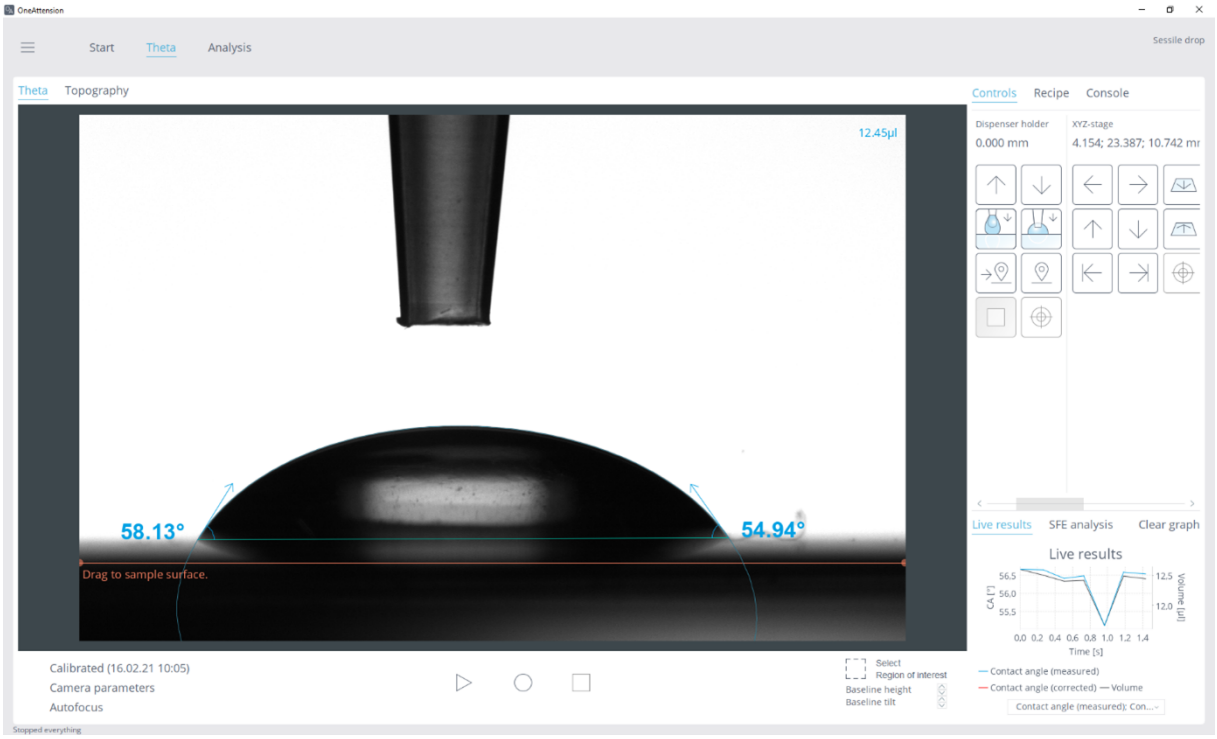


Performing a single measurement

- If the "Topography only" is not selected, both topography measurement and contact angle measurement will be performed. If Topography only is selected, only topography measurement will be performed.
- Place your sample on the sample stage. You can now choose the sample area in which the measurements will be performed. Move the sample stage either below the topography camera or the Theta Flow camera depending on which you prefer by using the "Move to CA" and "Move to topography" controls under the Topography tab. For example, if you wish to make the topography measurement in a certain area in the sample, you can place the sample below the topography camera so that this exact spot is visible in the topography camera. When you start the measurement, this exact area will be used in topography measurement and also in the contact angle measurement if the XYZ calibration has been performed accurately.



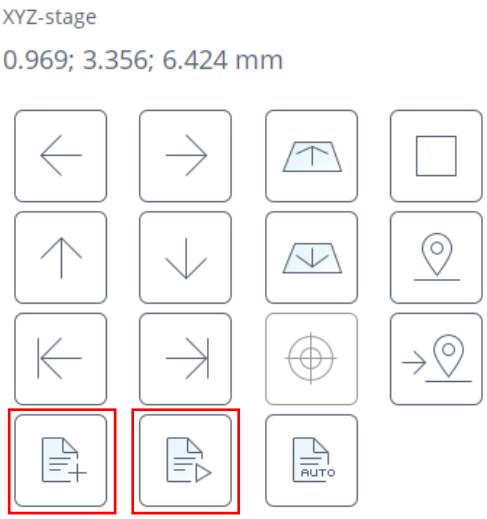
- If you wish to make the contact angle measurement on a certain sample area, you can look for the correct sample area with the Theta Flow camera. When the stage is under Theta Flow camera when the measurement is started, this exact point will be used in the contact angle measurement and also in the topography measurement if the XYZ calibration has been performed accurately. In case your sample has variation in z direction, it is recommended to start the measurement under the Theta Flow camera. If you are using an automatic dispenser, please make sure that when starting a measurement the tip of the dispenser is visible in the Theta camera the same way it is visible in standard Theta Flow measurements.
- Check that the sample thickness has been set correctly in the recipe. Choose Start below the image to start a measurement. The autostage will move itself to the positions defined earlier in calibration automatically. The topography measurement will be performed first, followed by the contact angle measurement. The contact angle measurement will be performed at the exactly same manner as usually. If you have an automated dispenser, it will dispense a droplet automatically. If you have a manual syringe, you must create the droplet yourself.



Performing a measurement sequence

Note! A motorized dispenser holder and an automatic dispenser are required for a full automatic measurement sequence.

- If the Topography only is not selected, both topography measurement and contact angle measurement will be performed. If Topography only is selected, only topography measurement will be performed.
- With Attension Theta Flow and the topography module, you can perform multiple measurements in a single run. You can measure the topography of multiple areas in your sample, and then automatically do the contact angle measurements on the exact same areas.
- In order to create a measurement sequence, choose either Sequencer under the XYZ-stage tab or map the desired points manually with "Add to list" selection. Please see the detailed information on the XYZ stage automatic point mapping functions under the chapter "XYZ stage". If you are using an automatic dispenser, please make sure that when starting a measurement the tip of the dispenser is visible in the Theta camera the same way it is visible in standard Theta Flow measurements.



The sequencer in the XYZ stage controls tab.

- Check that the sample thickness has been set correctly in the recipe. Once the desired sample areas have been mapped, select Start to start the measurement. The topography measurements will be performed first, followed by the contact angle measurements. The contact angle measurements will be performed at the exactly same manner as usually. If you have an automated dispenser, it will dispense droplets automatically. If you have a manual syringe, you must create the droplets yourself.

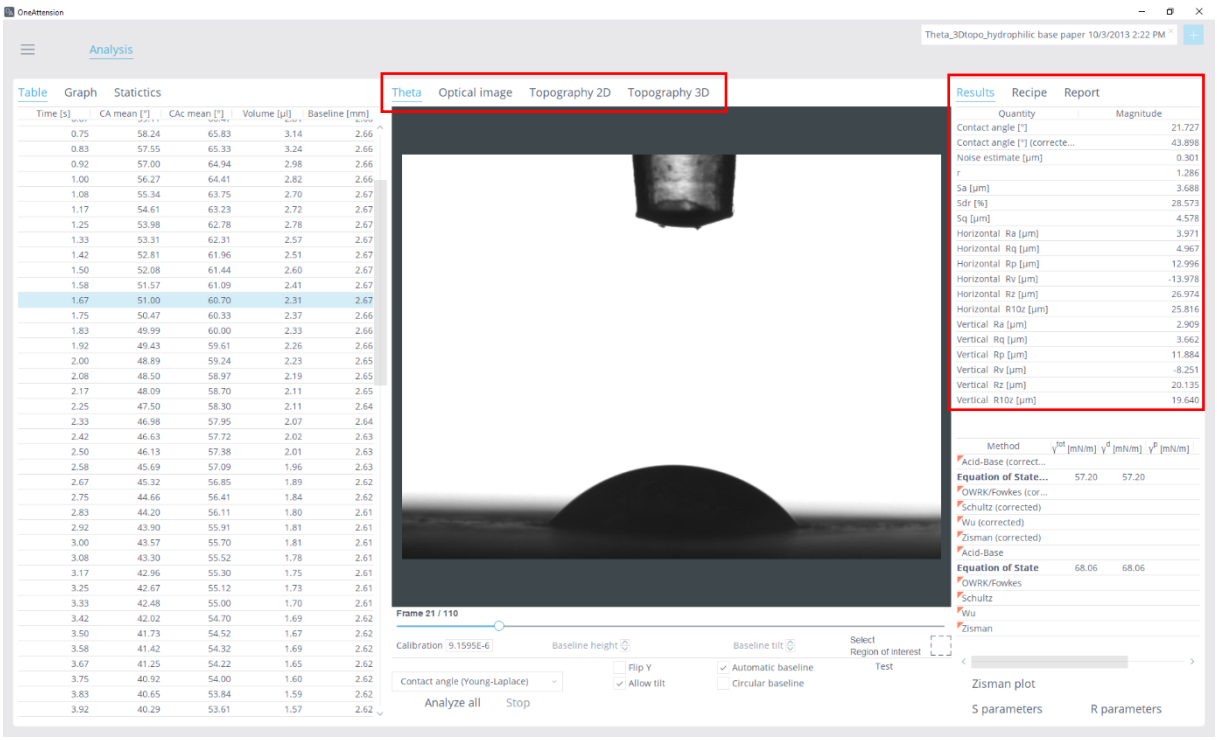
Performing a stitched measurement

- Attension topography module enables also stitching operation. In stitching, multiple measurements are made from adjacent areas in the sample. The individual measurement results including the images are then automatically combined into one larger result file. Normal analysis can be then performed from the results.
- With stitching, a larger sample area can be topographically mapped. Therefore, your liquid drop in contact angle measurements can be larger than the single measurement area of topography camera and still fit the topographically mapped area. You may also use stitching to study topographical surface homogeneity from a larger sample area.
- If the Topography only is not selected, both topography measurement and contact angle measurement will be performed. If Topography only is selected, only topography measurement will be performed.
- In order to perform a stitched measurement, choose a suitable stitching option from the recipe depending on your preferences, please see the more detailed description of the choices above in the recipe parameters. If the measurement is started under the topography camera, the visible area will become the upper left hand side area of the stitched area, i.e. the entire stitched area will span to the right and downwards from the starting point. If you'll start the measurement from below the Theta Flow camera, the area visible in the Theta Flow camera will become the center for the stitching operations. If you are using an automatic dispenser, please make sure that when starting a measurement, the tip of the dispenser is visible in the Theta Flow camera the same way it is visible in standard measurements.
- Optimal focus and brightness are defined separately for each single image. Therefore, the brightness and/or focus levels may differ between the images. This is normal and also important for the best results possible. Effectively, you may notice small borders between the single images after the images have been combined.

- Please note that Gaussian filter with stitched images will be applied to each original area separately if in use. The filter affects the numerical results but not the images. With standard measurements, Gaussian filter results are also shown in images.

E. Data analysis

Once measurements have been performed, the results can be seen in the **Analysis tab**. Double-clicking a measurement in the measurement list opens the results of that particulate measurement. Contact angle results are shown similar to other measurements (see section 6 for more information). Values used for calculated results are shown blue. However, the view with droplet images at the center of the screen has now four different tabs: Theta, Optical image, Topography 2D, and Topography 3D. In addition, right part of the screen has room for calculated topography results. All the results are analyzed similarly independent of whether they are standard measurements, measurement sequences, or stitched measurements.

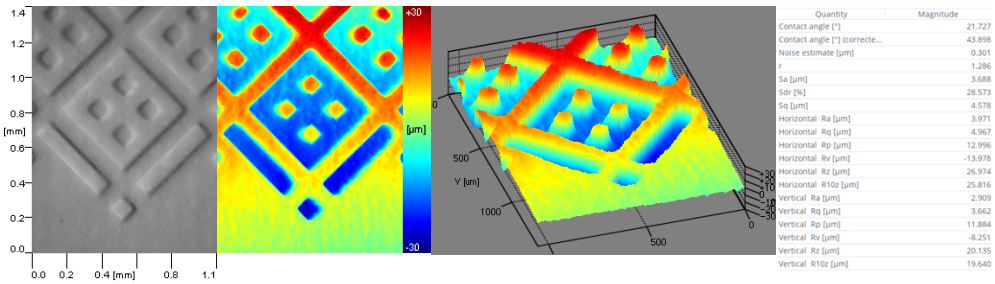


The analysis of a combined topography / contact angle measurement. Different result tabs and calculated results highlighted.

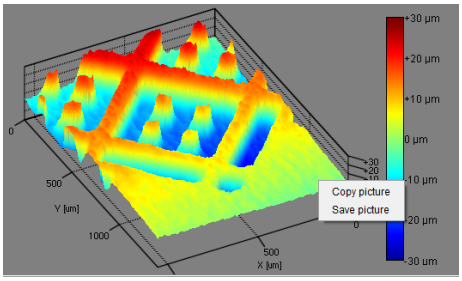
Theta tab above the droplet image includes the contact angle measurement results. These are otherwise similar to standard contact angle results, but now the roughness-corrected contact angle (CAc mean) is also shown in the calculated results. The roughness-corrected contact angles are seen in surface free energy (SFE) calculations similarly to standard contact angles. Please see "Surface free energy calculations" section in the Analysis section of this manual.

The other tabs include the actual topography information. **Optical image** tab shows the optical image taken by the topography camera. This picture is used by the software for topography calculations. **Topography 2D** tab has a digital 2D surface representation of the sample based on the optical image. The same topography information is presented three-dimensionally in the **Topography 3D** tab. Finally, the quantified topography parameters are shown in the **calculated results**.

All the images can be saved or copied by right-clicking the images and selecting the appropriate action.

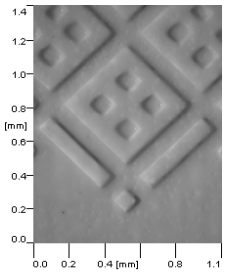


Example topography results. From left to right: optical image, 2D representation, 3D representation, and quantified roughness parameters.



Right-click any image in order to copy or save them.

The roughness data consists of 17 parameters. Please see the descriptions for each parameter in the theory section (chapter 7.4) of this manual. The parameters appear automatically when a measurement is opened. If they are deleted, they can be recalculated by choosing "S parameters" and/or "R parameters" below the calculated results. A report of the results in pdf form can be saved by selecting the "Generate report".

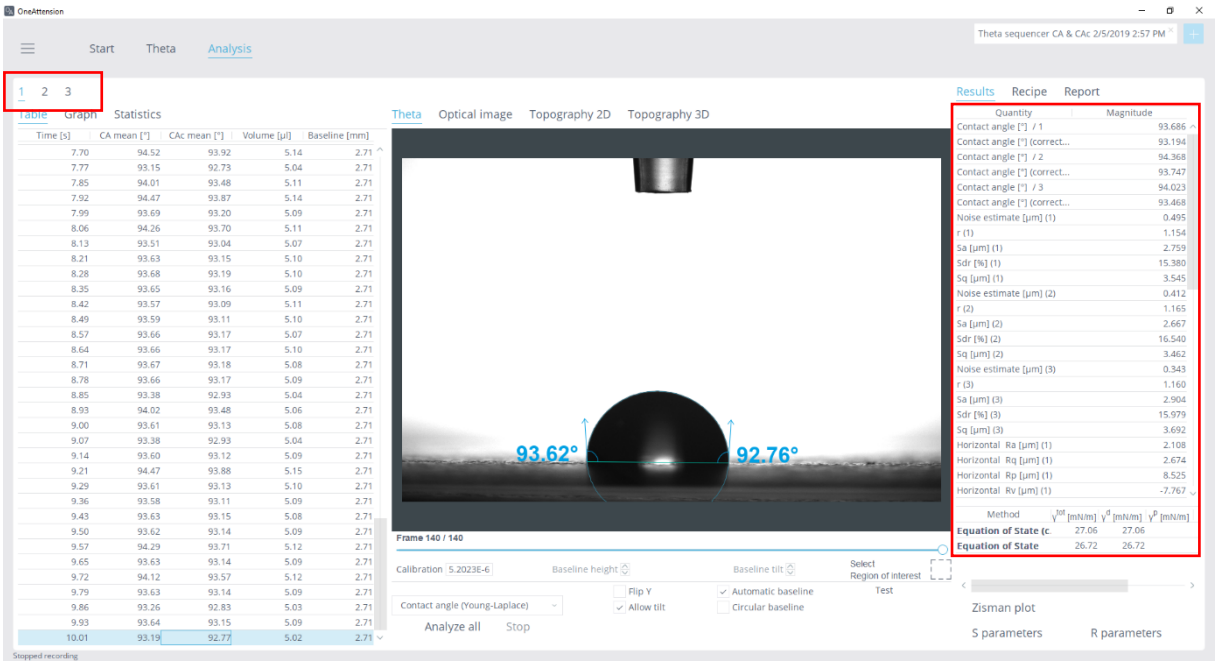


S and R parameters can be recalculated using the "S parameters" and "R parameters" buttons. Generate report creates automatically a pdf report of the results.

The noise estimate parameter depicts the noisiness of the analysis. When the number of repetitions is greater than 1, multiple analyzes are performed at the sample. There is always some variation between the results of these repetitions. The noise estimate is the standard error of the mean for each pixel height. Higher relative noise estimate compared to Sq value indicates lower repeatability of the measurement.

Note! If the topography camera settings such as brightness have not been adequate or the studied surface has been too diffuse, the noise level in the images may have raised substantially. If the noise estimate is more than 50% of the S_q value in a measurement, a warning is presented in the roughness data section. In this case, be aware your results may include substantial error.

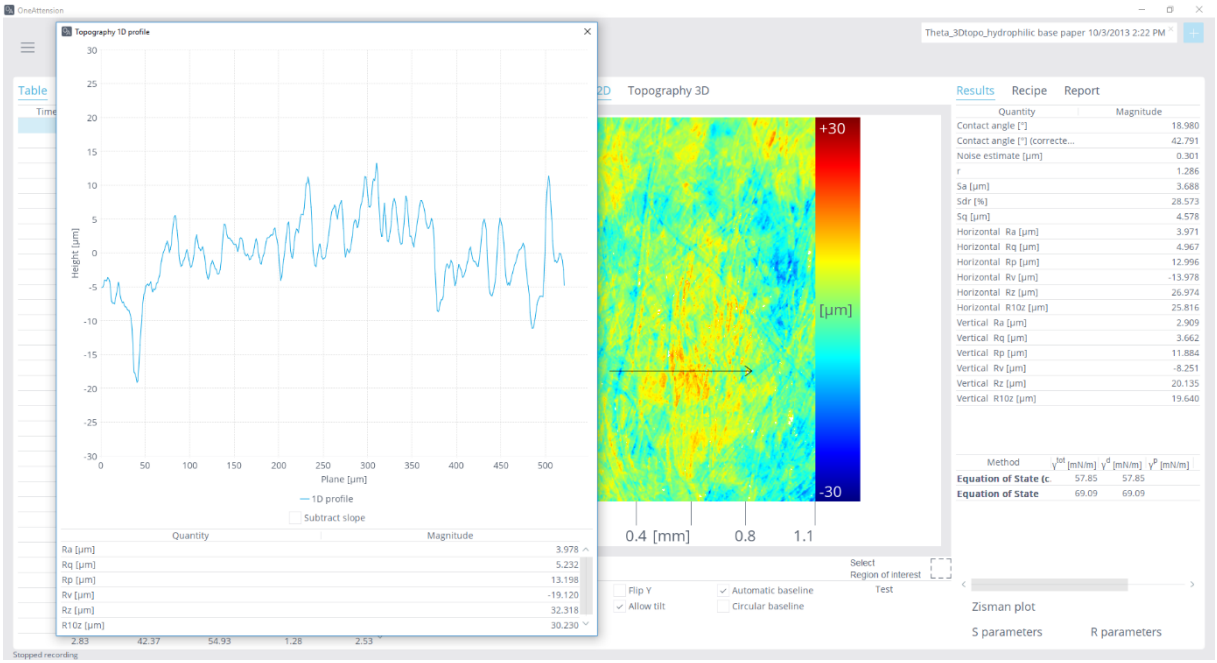
When a **measurement sequence** has been performed, the results for different measurements are presented with tabs on the left side of the window. Also, the quantified topography results are marked with the number of the measurement.



The results for a sequenced measurement of 3 points.

Whenever a stitched measurement is analyzed, each measured subarea is calculated separately. In this case, the calculated results are averages of these results. For each parameter, also the standard deviations are presented. The results are shown as average ± standard deviation. If for example 3 x 2 stitching measurement has been performed, each of these 6 subareas have been calculated their own topography values. The averages and standard deviations have been calculated based on these values. If Region of Interest (ROI) function has been used, the values and the standard deviations are calculated based on only the subareas in the selected ROI. Please see below for more information on the Region of Interest function.

The roughness profile of any one-dimensional line in the surface profile can also be analyzed. In the Topography 2D tab, an arrow can be freely drawn from any point to another by selecting a spot in the picture with left mouse click and dragging the mouse to another spot. OneAttension will automatically analyze this profile and open a new result window. The new window includes a plotted surface profile as well as some quantified roughness parameters (R_a, R_q, R_p, R_v, R_z and $R10_z$).



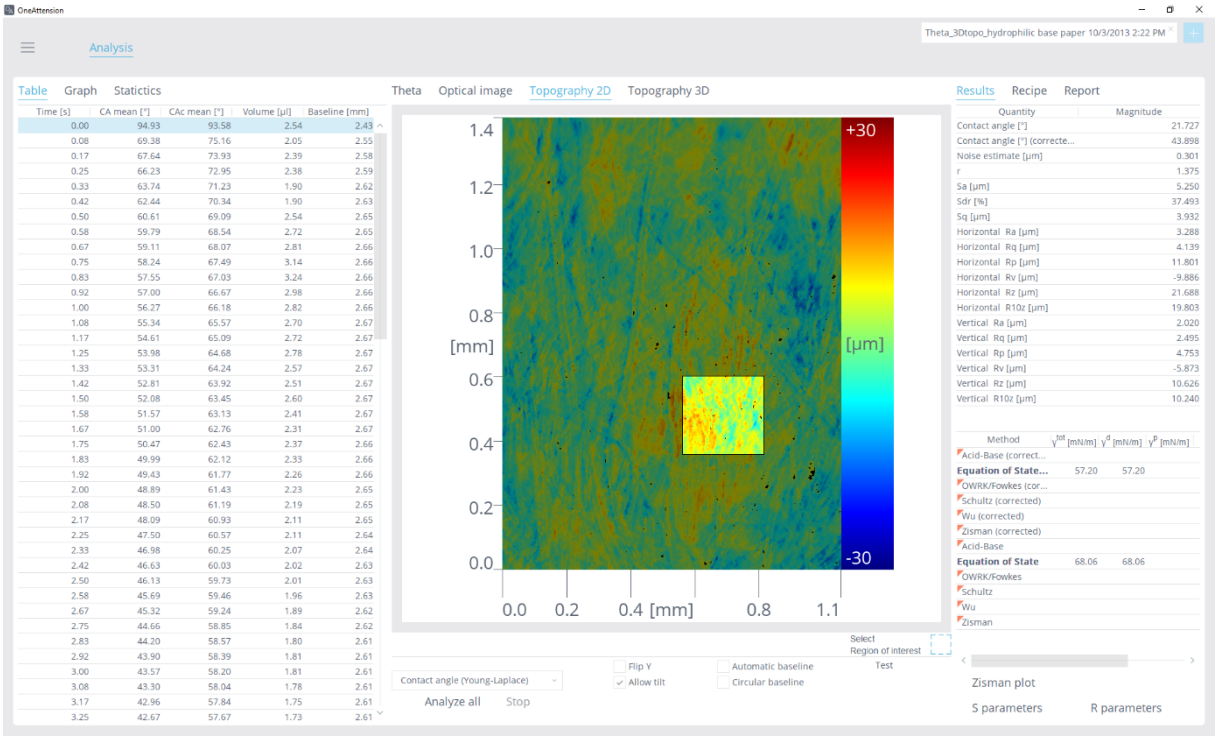
OneAttension showing surface profile information for a custom line drawn to the Topography 2D representation.

Options for picture properties can be opened by right-clicking the image. The options are similar to those of standard results graph. Please see Analysis section of this manual for more information.

Subtract slope option is available when viewing 2D roughness profile. With each 3D topography profile, a zero topography level is calculated automatically in order to subtract the effect of sample position and stage tilting. The zero level is then automatically subtracted from the results. Subtract slope performs the same calculations for custom 2D roughness profiles. When the option has been ticked, the curve and the R values are presented with the subtraction on. If the option has not been ticked, the curve and the values are presented exactly as measured with no correction. Generally, steep slopes shouldn't be subtracted as it distorts the profile. Use the subtract slope option only if you are certain you understand its meaning and its effects to the results.

ROI (Region Of Interest) can be used for selecting only a part of the entire image for the quantitative analysis. ROI is especially useful when part of your image contains data that you want to exclude from analysis.

In the "Topography 2D" tab, select ROI. You can now draw custom rectangles into the 2D surface representation. When a selection is performed, the calculated results chance accordingly. The calculated area must be at least 100 px x 100 px.



Using ROI function

The area that isn't in the ROI selection is plotted more dark than the area selected. In order to remove ROI selection, double click on the 2D image when the ROI button is active.

Topography results can also be **post-processed** using software-based numerical methods. You can select a measurement from the analysis section and change either the "**Sdr binning**" or the "**Gaussian cutoff"** values in the recipe. The results will automatically change accordingly. See the recipe parameters description above for further details on these parameters. Please note that changes in Sdr binning values are not depicted in the figures, only the parameters. The images are always presented without any binning. Also, the effects of Gaussian filters are not presented in the stitched images. The Gaussian filters are applied to each subimage separately in stitched measurements due to possible brightness or focus differences in subimages. In these cases, only the numerical parameters are affected.

If you notice some areas **depicted as white** in your 2D Topography image, the same areas are also missing from the 3D Topography image. These areas are either overexposed, underexposed or out of focus. As a default, the autobrightness aims for overexposing a small amount of all pixels. This has been found to produce the most reliable topography parameters. The white areas are always excluded from topography calculations and hence, do not interfere with the results.

The white areas are most common with non-diffuse materials such as many metals. Also, large topographical variance (> 100 µm) might cause more over- or underexposure.

The number of over- and underexposed areas can be minimized by finding the suitable brightness and focus levels manually, if the autofunctions don't bring optimal results. Also, another area in the same sample might produce a higher quality images.

Focus can be found manually quite easily be ascending and descending the sample stage. The Z moving speed could be set to relatively slow in order to get the focus more easily.

Manual brightness can be determined most easily by trying a few different brightness levels. For the best images, set the brightness to a level where the over- or underexposed area is minimized. For

the most accurate calculated parameters, set the brightness as high as possible without overexposing a significant part of the area.

6 Analysis

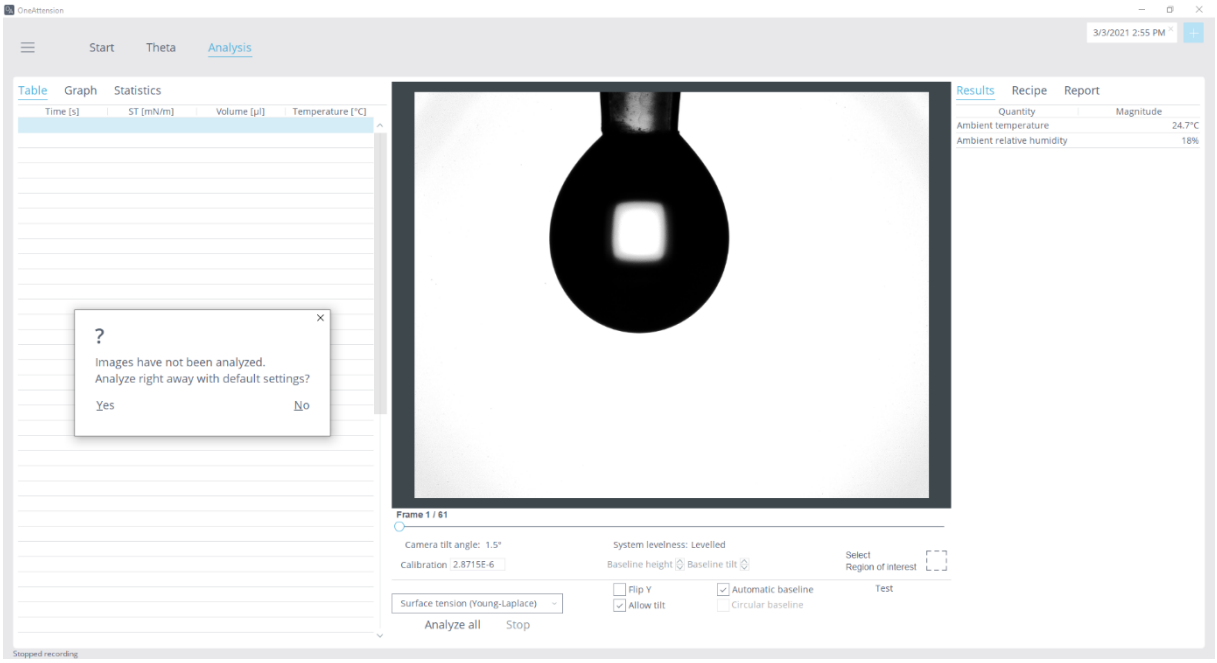
6.1 Curve fitting and data analysis

The curve fitting and data analysis is done in **Analysis tab** after the images have been recorded in the **Theta tab**. In curve fitting the profile of the drop is converted into a curve from which surface tension and/or contact angle can be calculated.

After recording the images in the **Theta tab**, move to the **Analysis tab** by clicking the Analysis button in top left corner.

Choose your experiment results by double-clicking the preferred experiment row. Different kinds of filter options are located in the upper part of the **Analysis tab**, such as experiment name and type, date and time, and user name. These may help to find the preferred experiment. If you scan a barcode when the cursor is either in Experiment name or Recipe name field, only those experiments/recipes that match with the barcode are shown.

When the experiment results are opened for the first time, the software states: "Images have not been analyzed. Analyze right away with default settings?". By clicking Yes, the software will automatically curve fit and analyze the results according to the default settings shown in Drop Analysis window. The settings will be copied from Live analysis settings if Live analysis was used.



OneAttension software enables automated curve fitting and data analysis if preferred.

If the default settings are not preferred for your sample, the settings can be adjusted from the Data Analysis window below the drop image. Curve fitting method can be chosen from the Data Analysis drop down menu.

The Young-Laplace has been chosen to be the default setting and it can be used for all experiments, Bashforth / Adams is just for Pendant drop experiments and Circle and Polynomial are just for Contact Angle experiments.

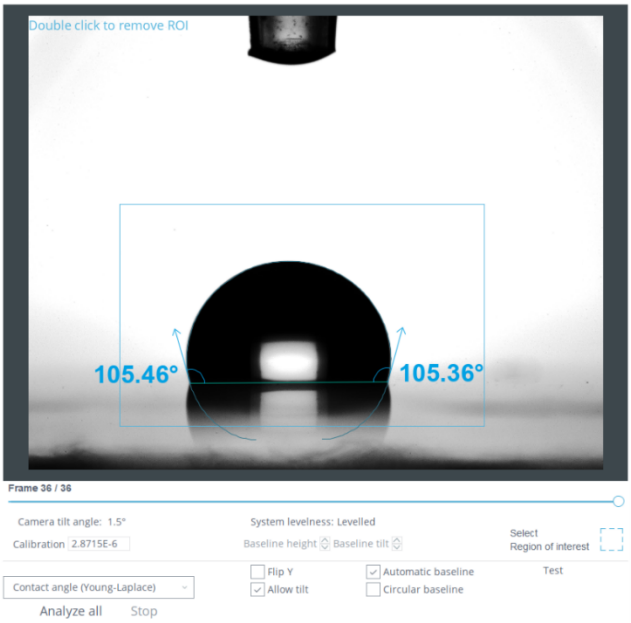
Flip Y function needs to be enabled when the hooked needle has been used to flip the image in data analysis upside down. However, the image will not be flipped visually in the **Analysis tab**.

In case the sample surface is curved, the Use circular baseline may need to be enabled.

The red line shown in the image is the baseline. It denotes the level of the solid or liquid surface for Contact angle, Auto-DCA and Meniscus experiments and the tip of the needle for Pendant drop experiments. Automatic baseline has been chosen to be the default setting. However, some surfaces (e.g. very rough surfaces) may demand a manually set baseline. Then the "Use automatic baseline" needs to be disabled from the Data Analysis. The Test autobaseline button displays where the auto baseline will place itself. Use this to verify that the baseline is set correctly. Allow tilt enables tilting of angle fitting. If not selected, the fitting is done symmetrically on both sides. The baseline can be straightened by double clicking the baseline.

In addition to dragging the baseline, it can be set manually also by using the BL height and BL tilt buttons. BL height changes the baseline height and BL tilt changes the tilt angle of the baseline.

If you want to exclude a part of the image from analysis, **ROI** (Region Of Interest) can be used. This means creating an area of interest around the droplet. To select the region of interest, click on the ROI button below the image. Then draw a rectangle of your choice to surround the area you wish to be analyzed. To remove the ROI double click on the image. For Auto-DCA analysis, ROI should always be used.

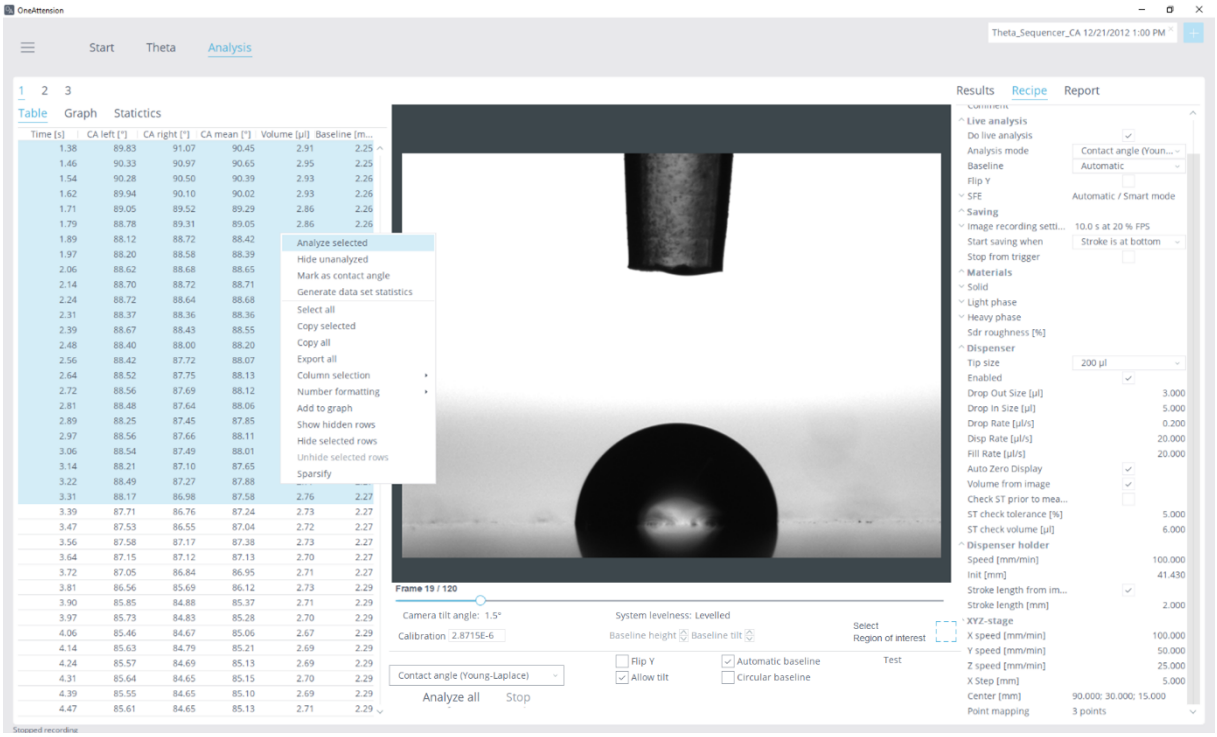


ROI can be used to select a region of interest for the analysis.

If all the data points are analyzed with the same settings, the Analyze all option can be used from the Data Analysis window. Any group of data points can be also chosen for the analysis by clicking on a row which highlights the selected data point(s). In this case, select the experiments which you prefer to analyze. Then choose the preferred Data Analysis settings, after which you can select Analyze selected. Undesirable data points can be hidden by right clicking the mouse and choosing Hide selected rows. The hidden data points can be returned from Show hidden rows.

Fitting can be stopped at any time by clicking Stop from the Data analysis window.

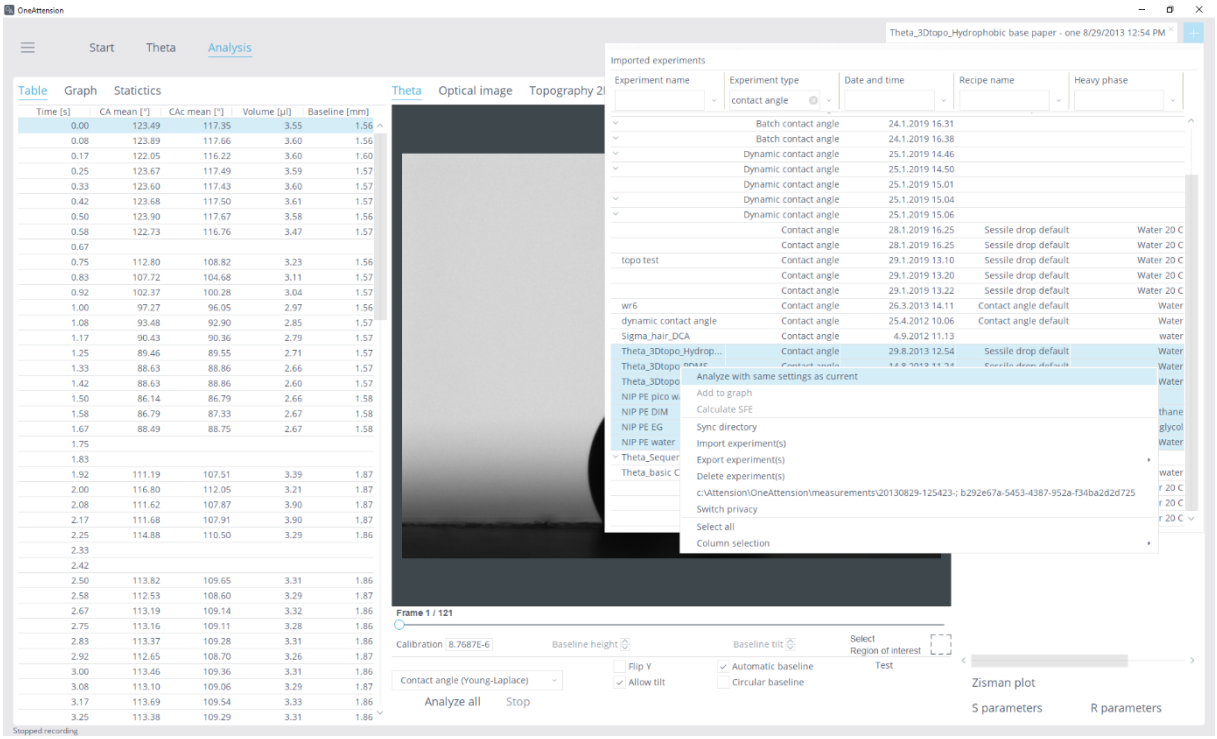
The quality of the curve fitting performed may be inspected visually during the fitting. A turquoise line is superimposed on the image and the calculated values will be shown in the image. The turquoise line represents the fitted curve and the green line the fitted baseline. If this line does not display an acceptable convergence with the actual profile it is possible to change the fitting parameters and repeat the fitting.



Curve fitting and data analysis options can be set also manually from the Data Analysis window for chosen data points.

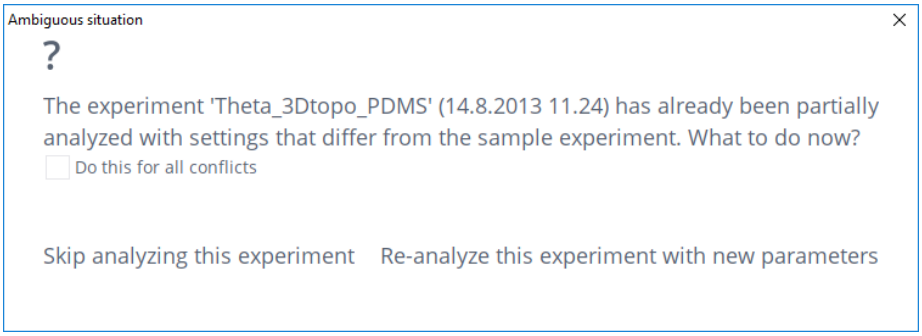
Group analysis

If you have a large number of measurements that you want to analyse similarly, it is possible to do group analysis of the measurements. First, choose one of the measurements that you wish to analyze and analyze it with the desired settings. Then select all the measurements that you want to analyse, right-click on them, and choose “Analyze with same settings as current” (see the image below).



Group analysis.

If some of the measurements have been analyzed before with different settings, you will be asked if you want to reanalyze the measurement.



Conflicting analysis.

6.2 Data handling and storage

After data has been analyzed it might be useful to review the data, print reports, view and manipulate graphs, make further calculations or export the data to another application. Calculated results are shown in the result tab on the right. Values used for the calculations are highlighted in blue. Surface free energy calculation is advised in the chapter 6.4 and surface tension component calculations in the chapter 6.5.

By highlighting the result row(s) on Table tab and right clicking with the mouse, a menu including options for data handling will appear:

Analyze selected: Enables recalculating your data according to chosen Data Analysis settings.

Hide unanalyzed: Hides all the rows that are not analyzed.

Mark as contact angle: You can mark any data point or group of the data points to be used in surface free energy calculations. Selected data point, or average of the selected group of data points will be shown in Results tab.

Mark as corrected contact angle: Similarly, you can mark a data point or group of the data points to be used as the corrected contact angle in surface free energy calculations.

Generate data set statistics: Generates statistics for a set of measurements. You are able to choose the statistics parameters and points of time that you want to examine.

Mark as surface tension: You can mark any data point or group of the data points to mark as surface tension result for the measurement. Selected data point, or average of the selected group of data points will be shown in Results tab.

Select all: Enables highlighting all the data points.

Copy selected: Copy selected data points.

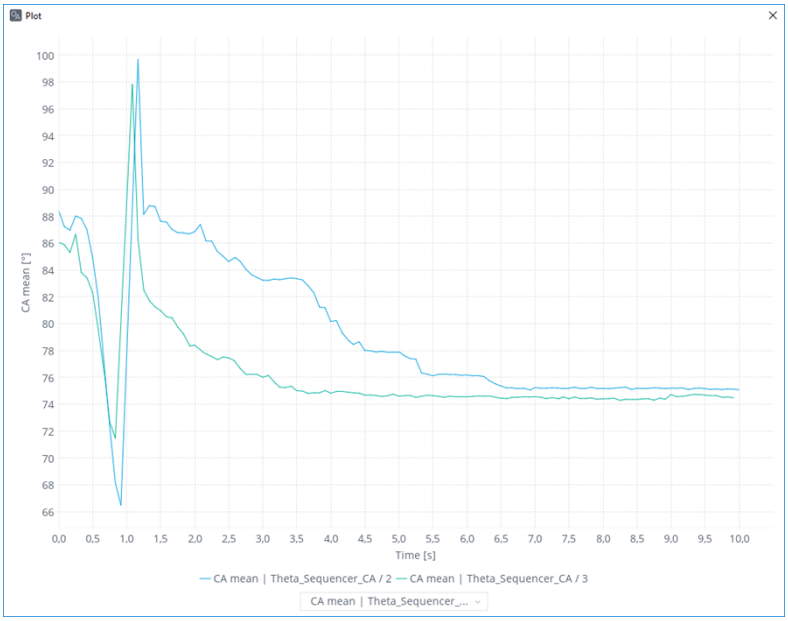
Copy all: Copy all the data points.

Export all: Export all the data points. The exported file format can be chosen (.xls, txt or .csv). The Excel has been set to be as a default option.

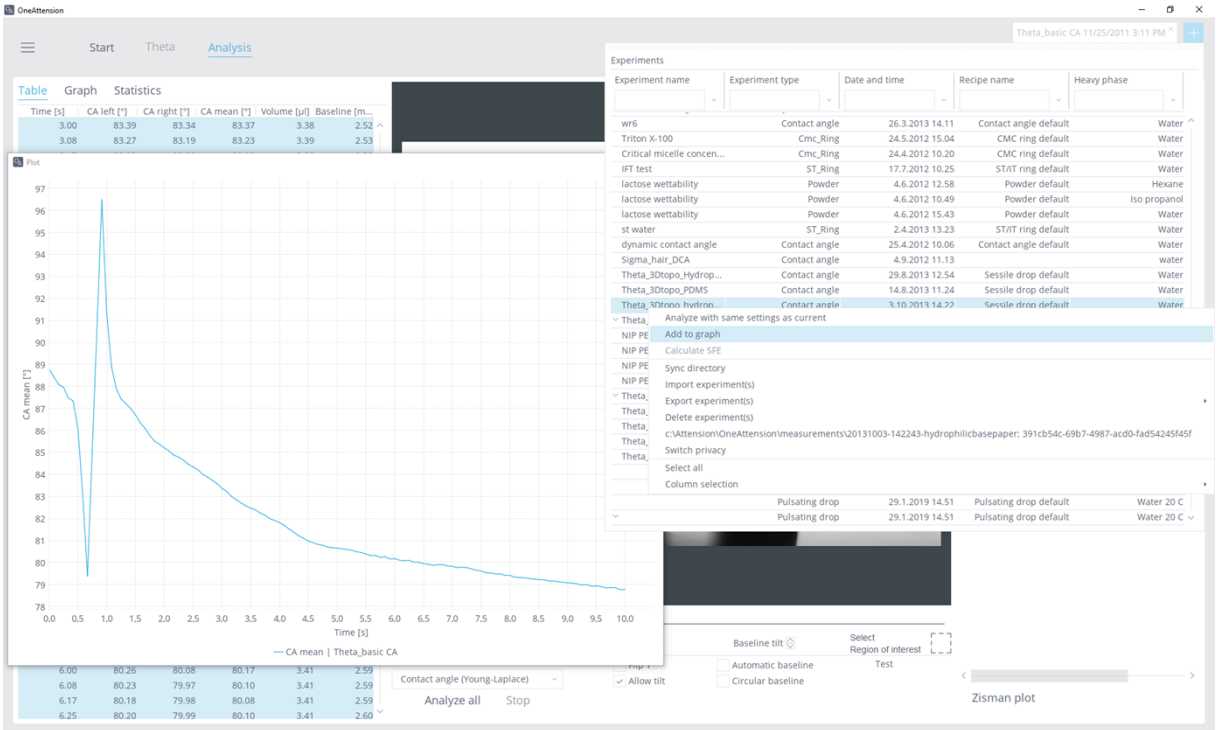
Column selection drop down menu: Enables to choose the columns to be visible in the **Analysis tab**. The result column options are clarified below.

Number formatting drop down menu: For selected result columns the number formatting can be selected here. Default settings might be necessary to change e.g. in case of using the high speed camera (time) or picoliter dispenser (volume).

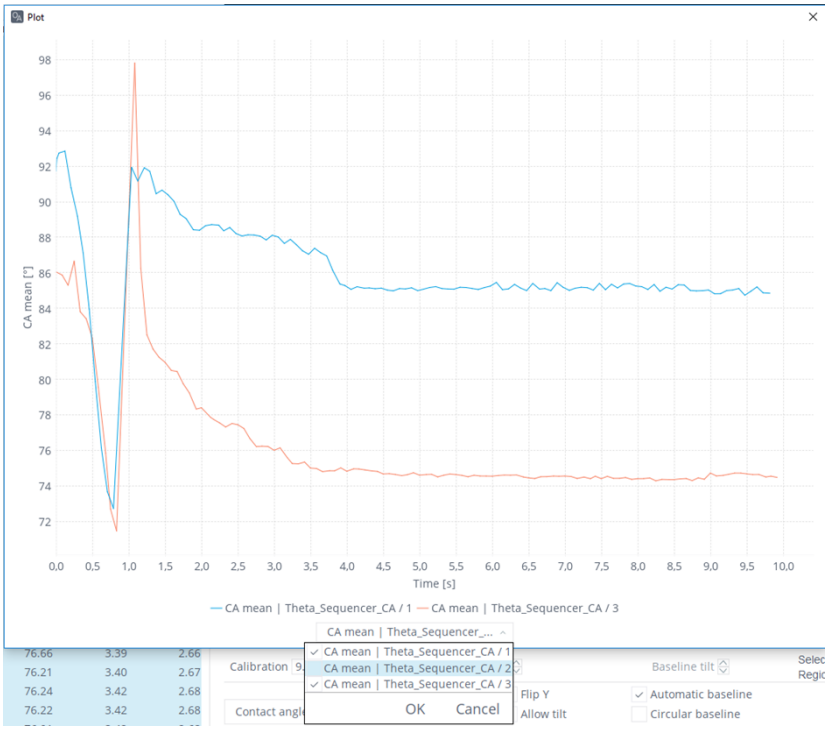
Graph: To create a graph, there are several graphing options. To select the values of the axis, right click the analyzed results and then select the axis from the Graph wizard window. You can either select to draw the graph from whole range of data points or just the selected range by selecting the option from the Graph wizard window. In addition, logarithmic scales are available to be chosen in the Graph wizard window. To display more than one experiment at a time on the same graph, create the first graph as normal and then right-click on the experiments to add and select Add to Graph. You can then select / deselect the added experiments in the graph window using the drop-down menu at the bottom of the screen. By right clicking the created graph you are able to adjust the graph properties, save, copy and print the graph, and adjust the axis properties (Zoom in/out and Auto range).



The graph window. Added datasets can be selected and deselected at the bottom. Right-click shows options for graph properties.



How to plot several measurements in the same graph.



Select which measurements are shown in the graph.

Hide selected rows: Enable to hide the selected row(s). The hidden rows will not be included in the further analyses or graphs.

Show hidden rows: Enables to return the hidden rows.

Sparsify: In case the number of data points need to be reduced the Sparsifier window will able to choose every Nth data point to be hidden or kept.

On Statistics Tab you can create a statistic report. The preferred result columns can be chosen from the Column selection drop down menu and are as follows:

Time [s]: Time at which image was captured in seconds

FPS: Frames per second

CA (Left): Left contact angle

CA (Right): Right contact angle

CA (Mean): Mean contact angle

CAc (Left): Left roughness-corrected contact angle

CAc (Right): Right roughness-corrected contact angle

CAc (Mean): Mean roughness-corrected contact angle

ST (mN/m): Surface tension of the liquid used

Height (mm): Height of drop

Area (mm^2): Area of the drop

Volume (µl): Drop volume

Baseline (mm): drop diameter

Tilt: Tilt of baseline

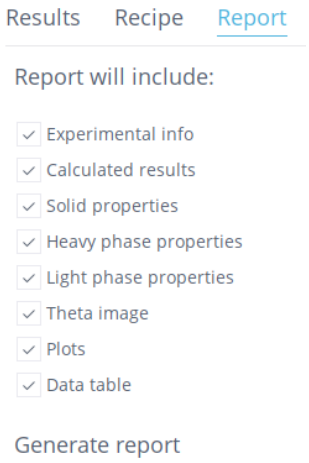
Cradle angle [°]: The cradle angle on that data point

Y-L β: The shape factor of the Young-Laplace fitting

Y-L r (mm): Radius of drop curvature at apex in Young-Laplace fitting

WA (J/m^2): Work of adhesion

Generate report function on the right side of the window will generate a pdf-report from the results. Choose the report content you want by ticking the wanted boxes. If you have SFE analysis and/or a graph open, they will be added to the report as well.



Choosing the PDF report content.

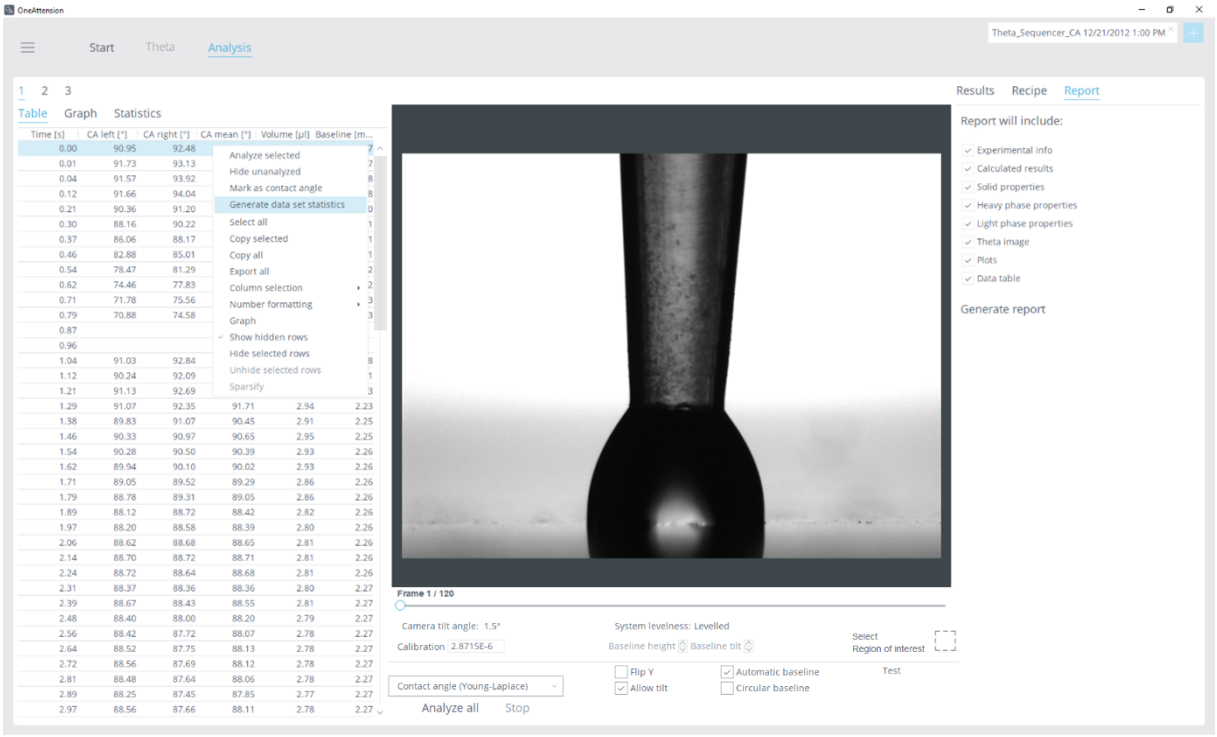
Ambient temperature and ambient relative humidity information is stored in the **Results** tab. Camera tilt angle and information about the system levelness are shown below the drop image.

In addition, the images can be saved either as separate pictures or as video by right clicking the image. You can 'Copy picture with overlays' or 'Copy picture in native size' to copy it to clipboard, 'Save picture with overlays' or 'Save picture in native size' to save it to a file, 'Save video' to save the whole experiment as a video clip, or 'Save all frames' to save each frame as a separate picture. The available video encoders depend on what is installed on the computer.

By right clicking the image in the Analysis tab, the recorded images can be saved either as separate picture, which can be selected by the frame selector, or as video. Camera tilt angle and information about the system levelness are shown below the drop image.

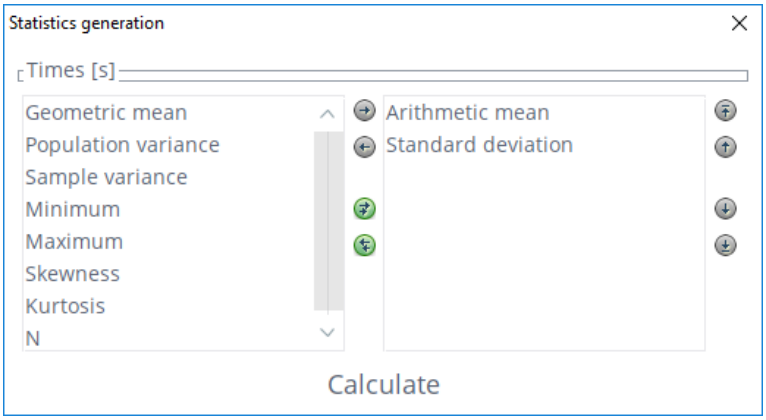
6.3 Data set statistics

Data set statistics can be created when an automatic sample stage and sequencer have been used to measure contact angles at several points on a sample. Choose the experiment you want to create statistics for by double-clicking it in the experiment row. Tabs will open for the sequenced points (see the image below). After analyzing the images, open one of the measurement tabs and choose all the columns that you are interested in. Right-click on any of the measurement rows and select "Generate data set statistics". This will create statistics for the values in the columns you have selected.



Generate data set statistics after analyzing your measurements.

The following window will open:



Statistics generation.

Select the time points that you want to appear in the statistics. Select also the parameters that you want to appear in the statistics by moving them into the box on the right using the left and right arrows. You can change the order of which the statistics are displayed by moving them up and down in the box on the right using the up and down arrows. The statistics parameters to choose from are:

Geometric mean: A geometric mean of the values at a time point

Aritmethic mean: An aritmethic mean of the values at a time point

Standard deviation: Standard deviation of the values at a time point

Sample variance: Sample variance of the values at a time point

Minimum: The minimum value at a time point

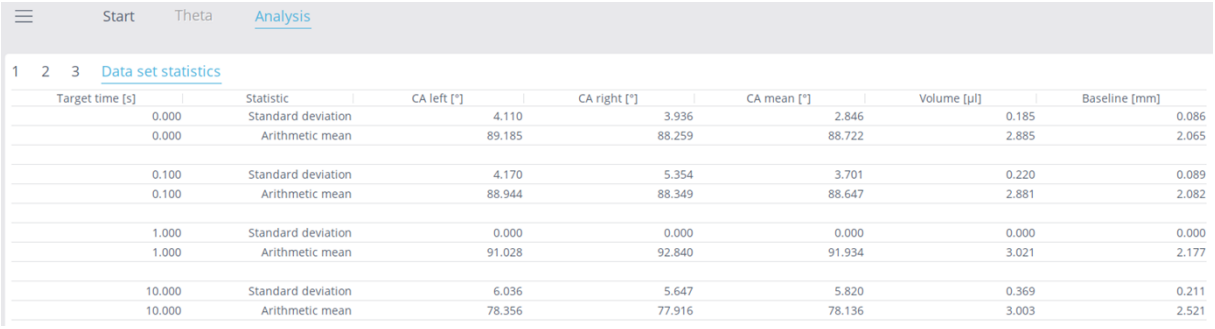
Maximum: The maximum value at a time point

Skewness: The asymmetry of the results distribution at a time point

Kurtosis: The degree of "peakedness" of the results distribution

N: The number of sequenced points used

When you have chosen the desired time points and statistics parameters, **press Calculate**. A data set statistics tab will appear with statistics for all the desired measurement parameters.



Target time [s]	Statistic	CA left [°]	CA right [°]	CA mean [°]	Volume [µl]	Baseline [mm]
0.000	Standard deviation	4.110	3.936	2.846	0.185	0.086
0.000	Arithmetic mean	89.185	88.259	88.722	2.885	2.065
0.100	Standard deviation	4.170	5.354	3.701	0.220	0.089
0.100	Arithmetic mean	88.944	88.349	88.647	2.881	2.082
1.000	Standard deviation	0.000	0.000	0.000	0.000	0.000
1.000	Arithmetic mean	91.028	92.840	91.934	3.021	2.177
10.000	Standard deviation	6.036	5.647	5.820	0.369	0.211
10.000	Arithmetic mean	78.356	77.916	78.136	3.003	2.521

Data set statistics.

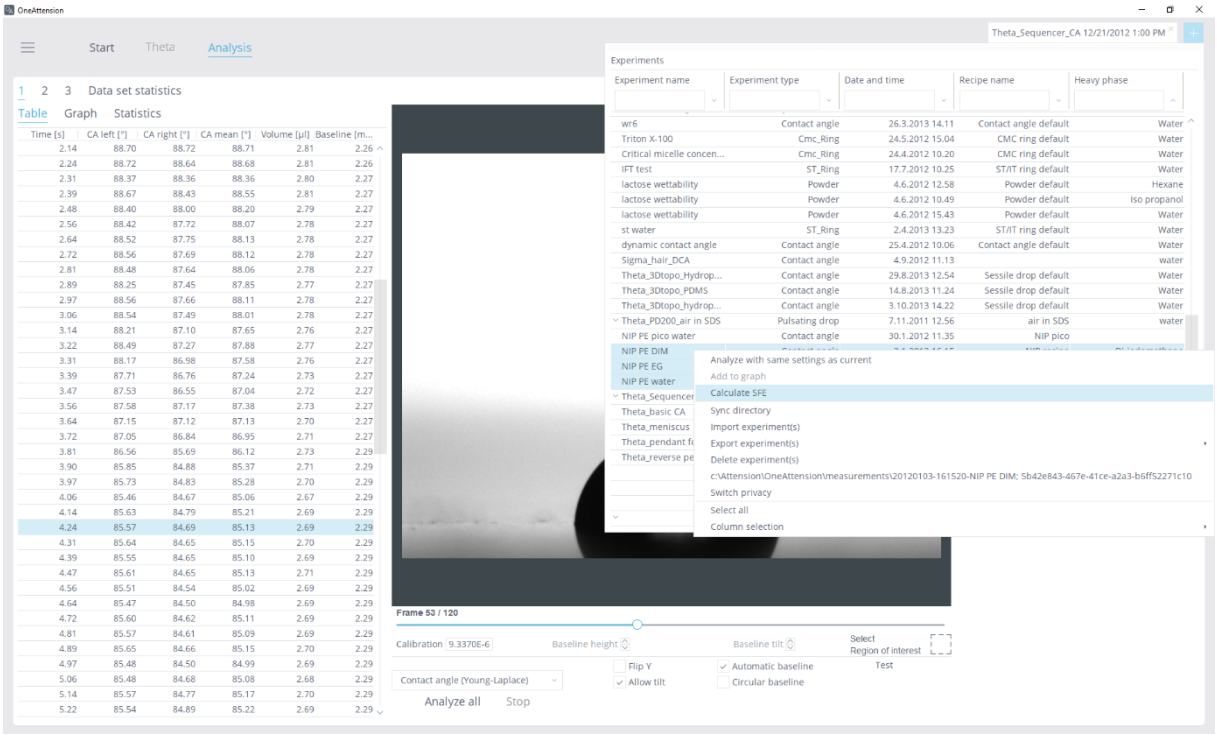
If you later want to change the displayed columns or points of time, select "Calculate statistics" from the bottom of the results page.

6.4 Surface free energy calculations

In surface free energy (SFE) calculations, data from Sessile Drop experiments is utilized to calculate the surface free energy of the solid. Please see the Chapter 7 to go through the theory of the surface free energy.

The SFE calculations appear in the Results tab automatically per each measurement. For individual or multi-point measurements, the same analysis modes and contact angles are used as in the measurement tab. The used angles and corresponding SFE values are displayed in Results tab. If the contact angle has been roughness-corrected either by using the 3D Topography module or by inserting Sdr value manually, also the corrected values are seen in the Results tab. SFE results calculated from roughness-corrected contact angles are postscripted with _c. In the automatic mode, Equation of State is highlighted for single-liquid measurements and OWRK for multi-liquid measurements.

SFE calculation can be performed by combining multiple liquids to same measurement. This is done by right-clicking on a set of measurements and clicking Calculate SFE. If these measurements have different modes for choosing contact angle, the original angles are shown in Results tab. In the recipe, the contact angle mode is shown as Manual, and the angles can be manually changed (see below).



Mark the preferred measurements for SFE calculations. Right-click and choose Calculate SFE to create a new measurement with the SFE calculations.

The SFE contact angles can be defined also manually. In the **Analysis tab**, select the chosen angle by highlighting the row and then right clicking the Mark as contact angle. If applicable, the roughness corrected values will be sent to Results tab.

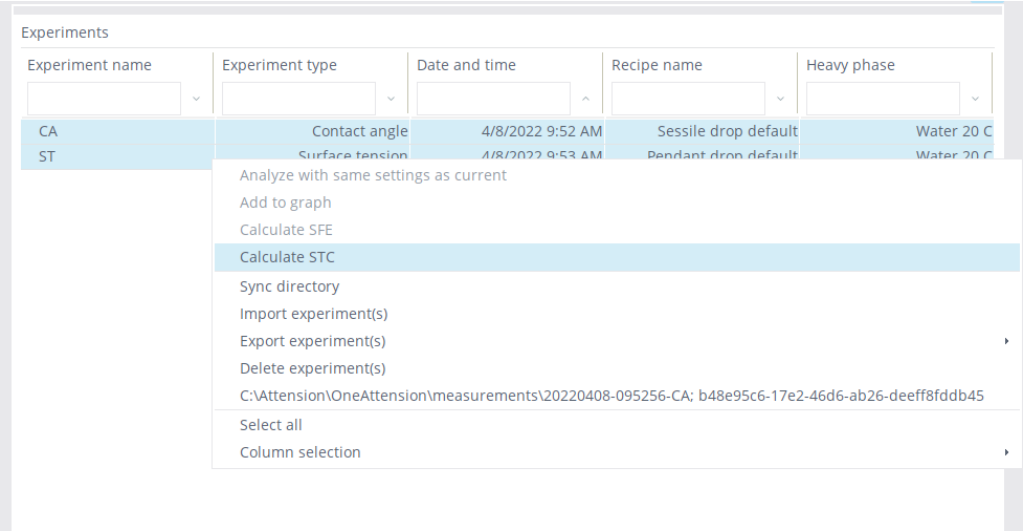
The available SFE models include: Zisman, OWRK / Extended Fowkes, Wu, Acid Base, Equation of State, Simple Fowkes, Schultz 1 and Schultz 2. Graph of the Zisman plot is available from Zisman plot button.

If the contact angle data doesn't fit some of the SFE models, the SFE values are not calculated and an error is shown next to the method name.

6.5 Surface tension components calculations

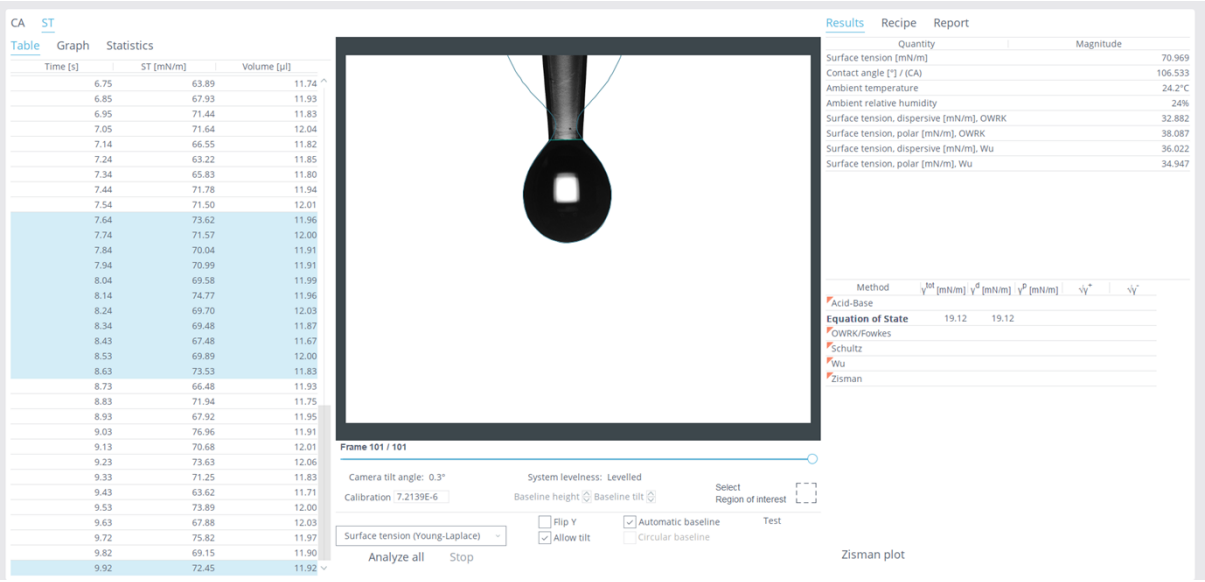
In a similar way as surface free energy, the surface tension of liquid can be divided into dispersive and polar parts. In surface tension components (STC) calculations, data from Sessile Drop and Pendant Drop experiments are utilized. To make STC calculations, measure contact angle with a known dispersive substrate, for example Teflon. Then measure surface tension of the same liquid using pendant drop.

STC calculations are done on the Analysis side. Select both contact angle and surface tension measurements on the Analysis tab and right click and select Calculate STC. New STC experiment will be created. If the measurements have not been analysed, they are first analysed.



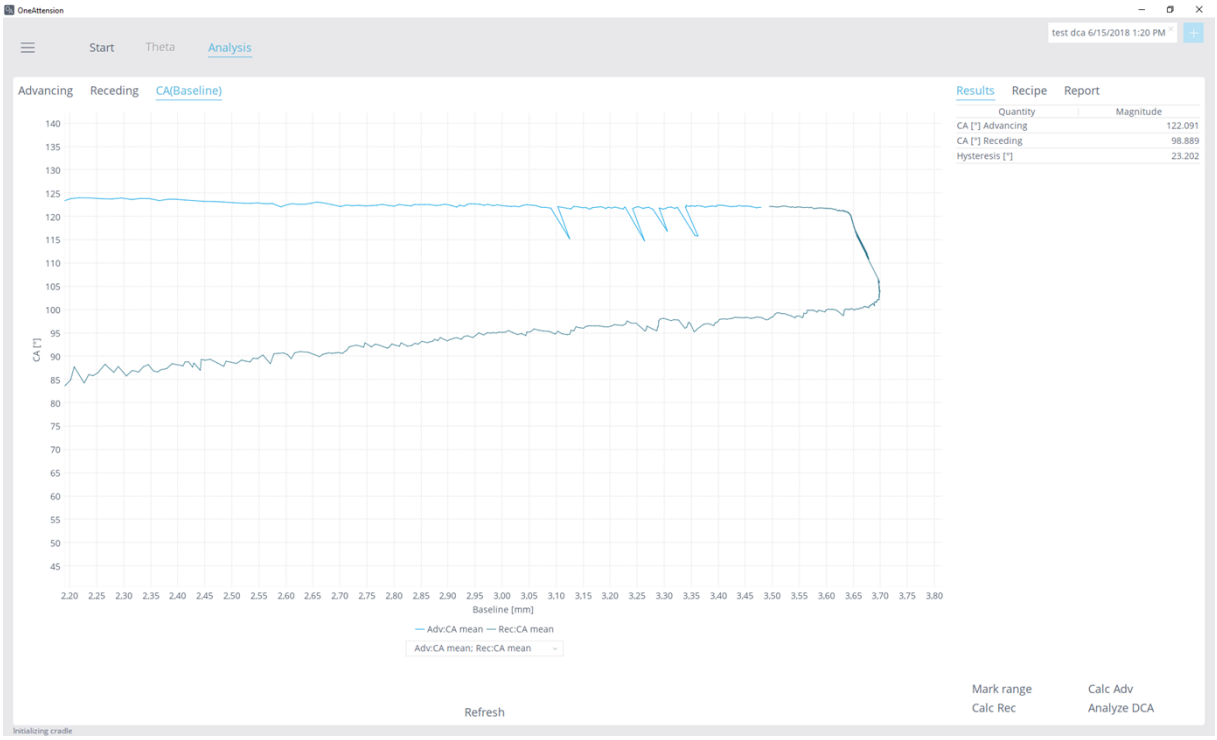
Mark the preferred measurements for STC calculations. Right-click and choose Calculate STC to create a new measurement with the STC calculations.

STC experiment includes contact angle and surface tension measurements. Results for STC are shown in the Results tab. CA and ST values used for the calculations are also shown in the table. Values used for CA/ST result are marked blue in the table. STC components, polar and dispersive part, calculated using OWRK or Wu approach are presented.



6.6 Automatic dynamic contact angle (auto-DCA) measurements

For auto-DCA measurements, the software automatically draws graphs and calculates the advancing and receding angles. Analyse the measurements first. The results are displayed on the CA(baseline) -tab.



Results of the auto-DCA measurement.

The calculated advancing angle is the average of contact angles where the baseline length is 96-99% of its maximum. Receding angle is the average of contact angles where the baseline length is 94-97% of its maximum (when the length is reducing). It is also possible to choose the ranges manually by using the 'mark range' option.

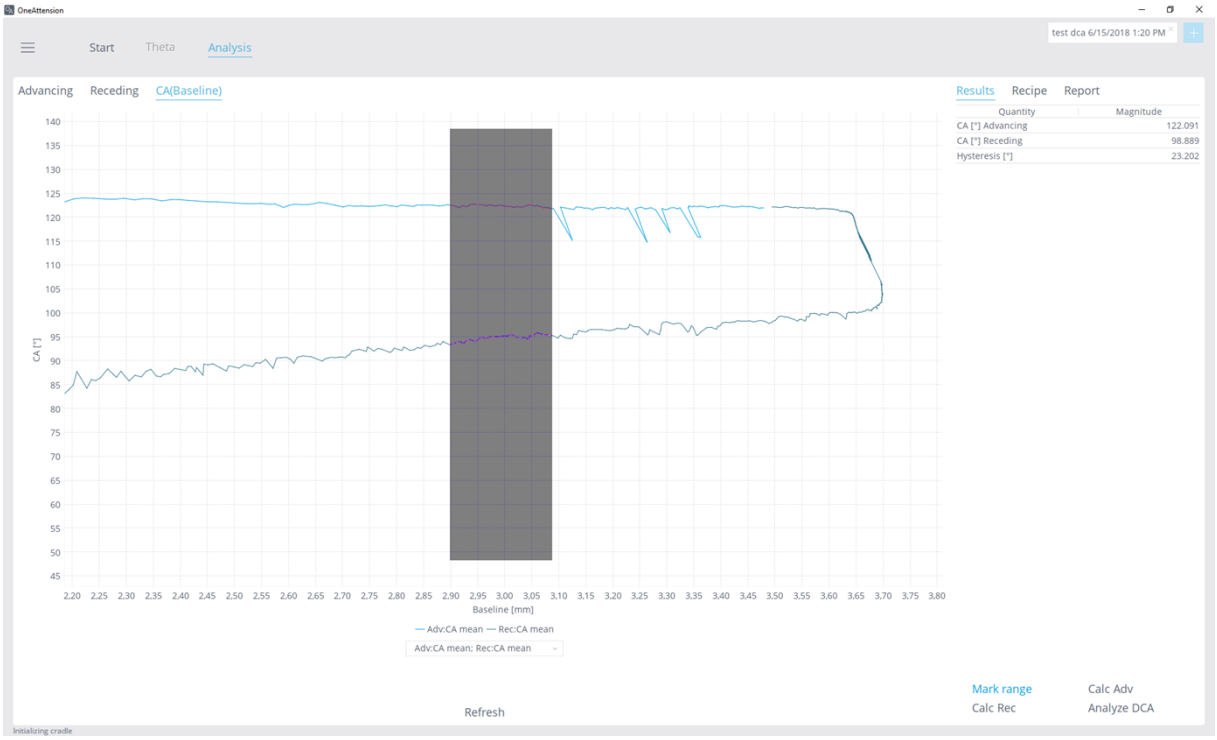
The buttons available for data analysis in calculated results.

Mark range: Enables the marking of the range in the graph for further calculations. Press this button and click the graph with your mouse first at point where you want to range to start and then click on the point where you want the range to end.

Calc Adv: Calculates advancing contact angle based on the marked range

Calc Rec: Calculates receding contact angle based on the marked range

Analyze DCA: Returns automatic DCA analysis



Range selection in Auto-DCA

6.7 Experiment browser functions

Right-clicking the experiment browser shows the following functions:

Export experiments: Export the selected experiment(s) as a .bs file, to be e.g. archived or transferred to another computer, or export the selected experiment(s) as a .xls file to be analyzed e.g. in Excel. When several measurements are exported to .xls format, keep one measurement open. The exported columns are determined by this open measurement.

Import experiments: Import .bs files that have been created via the Export experiments function.

Switch privacy: This only appears when the User Manager is in use. It switches previously private experiments public (visible to other users), or vice versa, if the current user's privileges allow it.

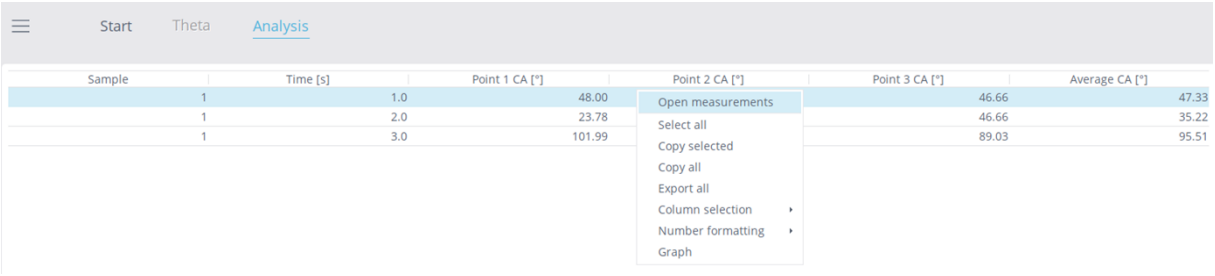
Sync directory: Brings up a 'Sync directory wizard', which can be used to synchronize the experiment browser database with the actual content in the disk, should they become out of sync. This may happen, for example, when using a network disk to save the experiments, and using a different computer to access (create, analyze, etc.) them.

Within the 'Sync directory wizard', following functionality is available:

- The path selection button (e.g. 'C:\Attension\OneAttension\measurements'), together with the 'Import new' button, can be used to import new experiments that have appeared to the specified path (e.g. when computer A has created experiments to a network disk, and they are wanted to appear in computer B's experiment browser).
- The 'Delete missing' button goes through the whole database and checks that all the entries actually exist in the disk. If something is missing (e.g. has been manually deleted from the disk), OneAttension suggests deleting the entries from the experiment browser database too.
- The 'Sync existing' button goes through the whole database and ensures that the metadata (e.g. experiment name, liquids, etc.) are in sync with the actual experiments in the disk. The metadata can become out of sync if computer B is used to modify an experiment (on a network disk), which is also referenced in computer A's experiment browser database.

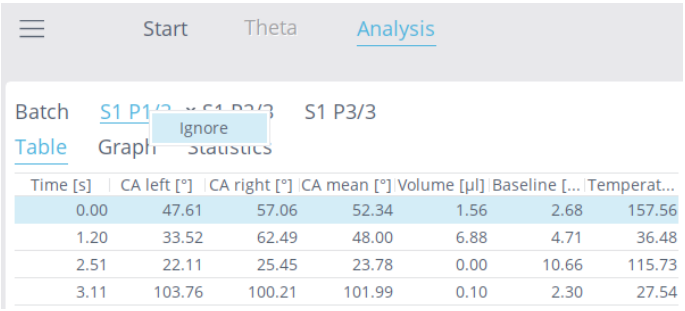
6.8 Analysis of batch sessile drop measurements

Results from batch sessile drop measurements are shown as an overview table. When you first open a batch measurement, a Batch tab opens up with an overview of the results. If you want to view individual measurements inside the batch, right-click on them and choose Open measurements.

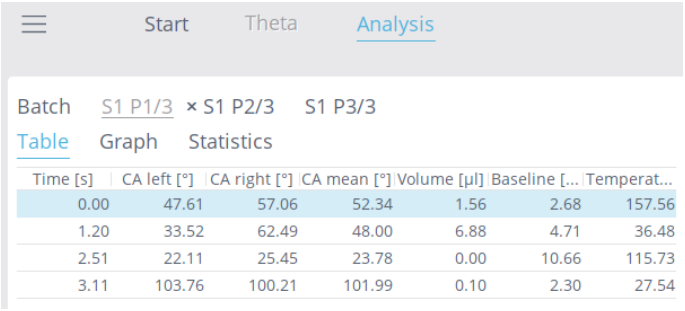


Batch sessile drop measurement results.

If for some reason you want to exclude one or more of the measurements from analysis, right-click on the desired tab and choose Ignore. The overview of the results will now be made without the ignored measurement.



Ignore a measurement from the batch results.



The ignored measurement tab is displayed in dark grey.

The leftmost column of the overview table displays the order number of the sample in the batch. The next column depicts the points of time when the analysis has been made. The points of time are displayed one below each other, each on its own row. The subsequent columns display the measured contact angles at each point of time. The number of the columns depends on how many points per sample have been measured. The last column in the table has the average contact angle in case several points have been measured on one sample.

At the bottom of the results window, you have the option to update the results table. By clicking the "Update data", the results table will be updated. If you have ignored or reanalyzed some of the individual measurements, the Batch results will now be updated according to the latest available data.

7 Theory

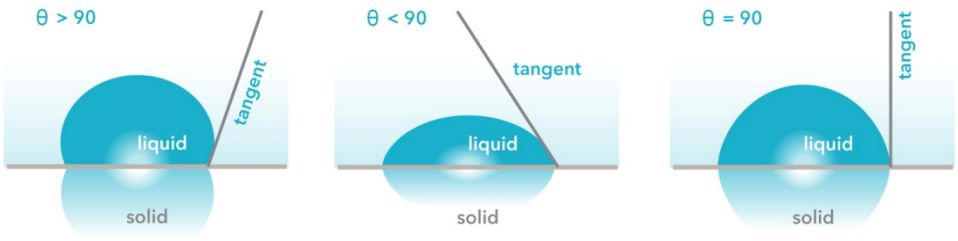
7.1 Contact angle

A. Definition of contact angle

Contact angle, θ, is a quantitative measure of the wetting of a solid by a liquid. It is defined geometrically as the angle formed by a liquid at the three phase boundary where a liquid, gas, and solid intersect. The well-known Young equation describes the balance at the three phase contact of solid-liquid and gas.

$$\gamma_{sv} = \gamma_{sl} + \gamma_{lv} cos\theta_Y$$

The interfacial tensions, γ_{sv}, γ_{sl} and γ_{lv}, form the equilibrium contact angle of wetting, many times referred to as Young contact angle θ_Y.



Different contact angles on a surface.

From the figure above, it can be seen that low contact angle values indicate that the liquid spreads on the surface while high contact angle values show poor spreading. If the contact angle is less than 90°, it is said that the liquid wets the surface, zero contact angle representing complete wetting. If contact angle is greater than 90°, the surface is said to be non-wetting with that liquid. Contact angles can be divided into static and dynamic angles. Static contact angles are measured when droplet is standing on the surface and the three phase boundary is not moving. Static contact angles are utilized in quality control and in research and product development. Contact angle measurements are used in fields ranging from printing to oil recovery and coatings to implants.

B. Goniometry

Goniometry is the analysis of the shape of a drop of test liquid placed on a solid. The basic elements of a goniometer include a light source, sample stage, lens, and image capturing device. Contact angle can be assessed directly by measuring the angle formed between the solid and the tangent to the drop surface.

Determining the tangent line which will define the contact angle is a factor that can limit the reproducibility of contact angle measurements. Conventional goniometry relies on the consistency of the operator in the assignment of the tangent line. This can lead to significant error and especially to subjective error between users.

Attension Theta Flow removes this problem by using computer analysis of the drop profile to generate consistent contact angle data. The software can fit the Young-Laplace equation to the shape of the drop accurately by using all of the points on the drop profile. The tangent is assigned as the gradient of the Young-Laplace equation where it intersects the baseline.

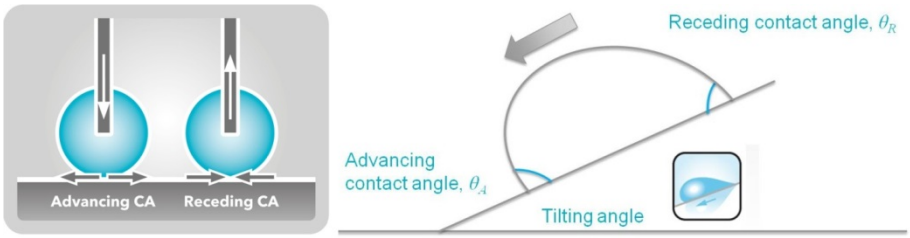
Many researchers report a single static contact angle without mentioning whether it is an advanced or a receded angle, though these may generally be assumed to be advanced angles. The literature regarding surface free energy calculations involves advanced angles almost exclusively.

C. Contact angle hysteresis

When the three phase boundary is moving, dynamic contact angles can be measured. These angles are referred to as advancing and receding angles. Contact angle hysteresis is the difference between the advancing and receding contact angles. Contact angle hysteresis arises from the chemical and topographical heterogeneity of the surface, solution impurities absorbing on the surface, or swelling, rearrangement or alteration of the surface by the solvent. Advancing and receding contact angles give the maximum and minimum values the static contact angle can have on the surface. Difference between advancing and receding angles can be as high as 50°. Dynamic contact angles and contact angle hysteresis has become a popular topic because of the recent interest in superhydrophobic and self-cleaning surfaces. Hysteresis is also important in other situations such as intrusion of water into porous media, coating, and adsorption at liquid/solid interface.

Both static and dynamic contact angles can be measured by using Theta Flow optical tensiometer. In practice, a droplet is placed on the solid surface and the image of the drop is recorded. Static contact angle is then defined by fitting Young-Laplace equation around the droplet, although other fitting methods such as circle and polynomial can also be used.

Dynamic contact angles can be measured by using two different approaches; changing the volume of the droplet or by using tilting cradle or tilting stage to tilt the droplet. The figure (a) below shows the principle of the volume changing method. In short, a small droplet is first formed and placed on the surface. The needle is then brought close to the surface and the volume of the droplet is gradually increased while recording at the same time. This will give the advancing contact angle. The receding angle is measured the same way but this time, the volume of the droplet is gradually decreased. In figure (b), the principle of the tilting cradle/tilting stage method is shown. The droplet is placed on the substrate which is then gradually tilted. The advancing angle is measured at the front of the droplet just before the droplet starts to move. The receding contact angle is measured at the back of the droplet, at same time point.



Schematics of dynamic contact angle measurements by using (a) volume changing method (b) tilting cradle/tilt stage.

D. Further reading

J. C. Berg, Wettability, Marcel Dekker, N.Y. (1993)

M. E. Schrader, G. Loeb, Modern Approach to Wettability, Plenum Press, N.Y. (1992)

W. Adamson, Physical Chemistry of Surfaces, Wiley & Sons, (1976)

S. Wu, Polymer Interface & Adhesion, Marcel Dekker, N.Y. (1982)

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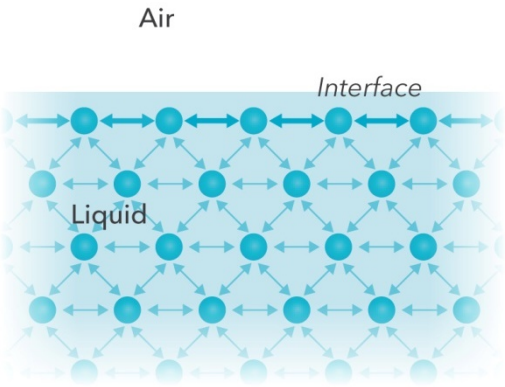
R. L. Bendure, J. Colloid Interface Sci., 42(1), 137-144(1973)

7.2 Surface tension

A. Definition of surface tension

The cohesive forces between liquid molecules are responsible for the phenomenon known as surface tension (ST). The molecules at the surface do not have the similar neighbouring molecules on all sides and thus they cohere more strongly to those directly associated with them on the surface. This forms a surface "film" which makes it more difficult to move an object through the surface than move it when it is completely immersed. The same situation applies also at the interface of the two liquids that do not mix together. In this case, the term interfacial tension (IFT) is used. There are several different units for surface and interfacial tension; typically mN/m (which is equivalent to dynes/cm) is used.



Interactions between molecules in the bulk rule out each other whereas on the surface the interaction between neighboring molecules is stronger.

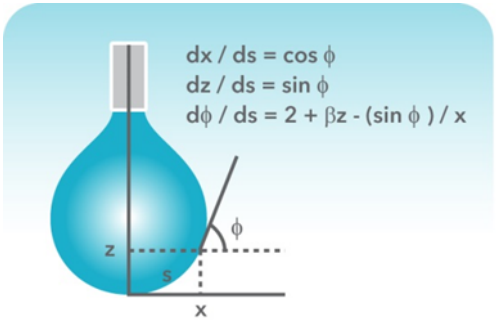
B. Pendant drop shape analysis

Surface and interfacial tension measurements can be done with optical tensiometer by so-called pendant drop shape analysis (or reversed pendant drop). The shape of the drop of liquid hanging on the needle is determined from the balance of forces which include the surface tension of that liquid. The surface or interfacial tension can be related to the drop shape by the equation

$$\gamma = \Delta\rho g\frac{R_0^2}{\beta},$$

where γ is surface tension, Δρ is density difference between fluids, g is gravitational constant, R_0 is radius of drop curvature at apex, and β is a shape factor.

β, the shape factor, can be defined through the Young-Laplace equation expressed as 3 dimensionless first order equations as shown in the figure below.



Pendant drop.

Modern computational methods using iterative approximations allow solution of the Young-Laplace equation for β to be performed. Thus, for any two fluids in contact which densities are known, the surface or interfacial tension may be measured based on the Young-Laplace equation. This method has the advantage of being able to use very small volumes of liquid and that it is possible to measure very low interfacial tension values. In practice, interfacial tension values below 0,01 mN/m are extremely difficult to measure. What has to be considered when measuring surface and interfacial tension is the size of the droplet used. The droplet should have the suitable pendant shape to achieve reliable results. When measuring surface tension, the density difference between liquid and gas (usually air) is so big that the droplet size from 5 to 20 ul is usually sufficient depending on the surface tension of the liquid. When measuring interfacial tensions, both the density difference and the interfacial tension have an effect on the required droplet size. As a rule of thumb, smaller the density difference, bigger the droplet has to be.

C. Further reading

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7.3 Surface free energy

A. Definition of surface free energy

Precise characterization of solid material surfaces plays a vital role in research and product development in many industrial and academic areas. Wettability of the surface is important in processes like painting and printing and has been utilized in the study of biomaterial – cell interactions to name a few application areas. Wettability can be studied by measuring contact angle of the substrate with given liquid. The well-known Young equation describes the balance at the three phase contact of solid-liquid and gas.

$$\gamma_{sv} = \gamma_{sl} + \gamma_{lv} cos\theta_Y$$

The interfacial tensions, γ_{sv}, γ_{sl} and γ_{lv}, form the equilibrium contact angle of wetting, many times referred as Young contact angle θ_Y. The Young equation assumes that the surface is chemically homogenous and topographically smooth. One of the important applications of the contact angle measurement is the assessment of the surface free energy (SFE) of the solid. Surface free energy of the solid is equivalent to surface tension of the liquid and the unit is the same mN/m (= dynes/cm).

Although contact angle itself also gives indications on the wetting properties of the surface, contact angle always depends also on the liquid used for the measurements.

In the Young equation, the surface free energy of the solid is described by using the contact angle θ_Y, surface tension of the liquid γ_{lv} and interfacial tension between solid and liquid γ_{sl}. The first two are easily measured but the problem is the unknown γ_{sl} which cannot be measured directly. To be able to solve the equation, more assumptions of the relationship between γ_{sv}, γ_{sl} and γ_{lv} has to be made.

To be able to understand the different methods, term "work of adhesion" has to be explained. Thermodynamic adhesion is the work required to separate surfaces into two new surfaces (see the figure (a) below). The equation for work of adhesion can be written as

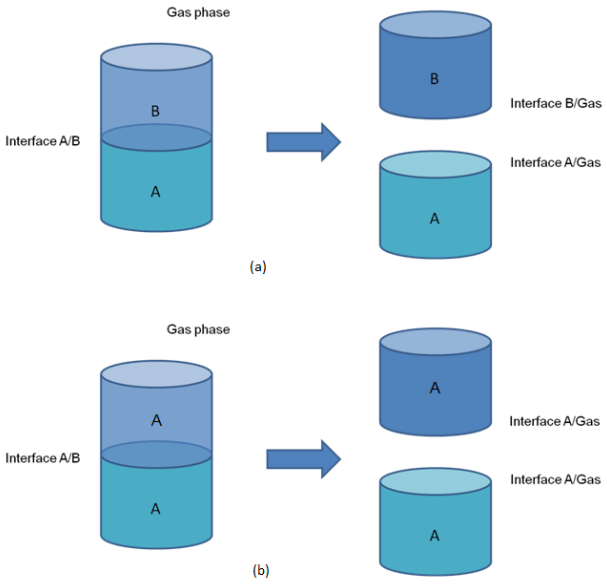
$$W_A = \gamma_A + \gamma_B - \gamma_{AB}$$

, where γ_{AB} is the interfacial tension between two phases, γ_A is the surface tension of phase A and γ_B is the surface tension of phase B. Now, if the other phase is solid and other liquid the equation can be written as

$$W_{sl} = \gamma_{lv} + \gamma_{sv} - \gamma_{sl}$$

From here, the Young-Dupré equation can be obtained:

$$W_{sl} = \gamma_{lv}(1 + cos\theta_Y)$$



Work of (a) adhesion, (b) work of cohesion.

In a similar way, the work of cohesion can be defined as shown in the figure (b) above.

$$W_C = \gamma_A + \gamma_A - 0 = 2\gamma_A$$

Berthelot initiated the direction to surface free energy calculations at the end of 19th century. He assumed that the work of adhesion (WA) between the solid and liquid is equal to the geometric mean of the cohesion work of a solid and the cohesion work of the measuring liquid.

$$W_{sl} = \sqrt{(W_{ss}W_{ll})}$$

And then combining with the equations the last equations:

$$W_{sl} = \sqrt{2\gamma_{lv}2\gamma_{sv}} = 2\sqrt{\gamma_{lv}\gamma_{sv}} = \gamma_{lv}(1 + cos\theta_Y)$$

This last equation is the basis for the following SFE theories.

B. Surface free energy theories

Equation of state

Although there are few different formulas based on Equation of state (EQS), the best known is one described by Neumann:

$$cos\theta_Y = -1 + 2\sqrt{\frac{\gamma_{sv}}{\gamma_{lv}}}e^{-\beta(\gamma_{lv}-\gamma_{sv})^2}$$

Here the coefficient β = 0.0001247 is determined experimentally. In principle, the equation of state requires the measurement to be done only by using one liquid. Regardless which liquid is chosen, the surface free energy result should be the same. There is a lot of criticism against this theory. The controversy deals with the question whether the constant β is universal constant of the materials or just quantity obtained as a result if the iterative procedures applied. Equation of state theory also does not divide the surface tension into different components as the other theories.

OWRK/Fowkes

The idea of dividing the SFE into individual components includes the assumption that γ_{sl} is determined by various interfacial interactions that depend on the properties of both the measured substrate and the measurement liquid. Fowkes assumed that surface free energy of a solid (and surface tension of a liquid) is a sum of independent components, associated with specific interactions:

$$\gamma_{sv} = \gamma_{sv}^d + \gamma_{sv}^p + \gamma_{sv}^h + \gamma_{sv}^i + \gamma_{sv}^{ab} + \gamma_s^o,$$

where γ_{sv}^d, γ_{sv}^p, γ_{sv}^h, γ_{sv}^i and γ_{sv}^{ab} are the dispersion, polar, hydrogen, induction and acid-base components, respectively. γ_{sv}^o refers to all remaining interactions. Fowkes investigated mainly systems containing substance (solid or liquid) in which only the dispersion interactions appear. According to Fowkes, dispersion interactions are connected with London interactions, arising from the electron dipole fluctuations. Owen and Wendt continued the Fowkes idea stating that all to components in the right side of the equation above, except γ_{sv}^d, can be considered polar (γ_{sv}^p). This will lead to equation:

$$\gamma_{sl} = \gamma_{sv} + \gamma_{lv} - 2\sqrt{\gamma_{sv}^d\gamma_{lv}^d} - 2\sqrt{\gamma_{sv}^p\gamma_{lv}^p}$$

And if combined with Young equation, the equation called OWRK can be written as

$$\sqrt{\gamma_{sv}^d\gamma_{lv}^d} + \sqrt{\gamma_{sv}^p\gamma_{lv}^p} = 0.5\,\gamma_{lv}(1 + cos\theta_Y)$$

Because there are two unknowns, γ_{sv}^d and γ_{sv}^p, in the equation, two liquids with the known dispersive and polar components are needed to solve it. The liquid with the dominant polar component should be chosen as one measuring liquid and a dispersive liquid as other one. Water, glycerol and formamide can be used as polar liquids and diiodomethane and α-bromonaphtalene as dispersive. Water and diiodomethane are the most often utilized. OWRK is one of the most common methods for SFE calculations.

Wu

Wu accepted to idea of Owen and Wendt to divide the SFE into polar and dispersive components. Instead of using the geometric mean, he used a harmonic one:

$$\gamma_{sl} = \gamma_{sv} + \gamma_{lv} - 4\left[\frac{\gamma_{sv}^{d}\gamma_{lv}^{d}}{(\gamma_{sv}^{d} + \gamma_{lv}^{d})} + \frac{\gamma_{sv}^{p}\gamma_{lv}^{p}}{(\gamma_{sv}^{p} + \gamma_{lv}^{p})}\right]$$

And if combined with Young equation, the Wu equation can be written as

$$\left[\frac{\gamma_{sv}^{d}\gamma_{lv}^{d}}{(\gamma_{sv}^{d} + \gamma_{lv}^{d})} + \frac{\gamma_{sv}^{p}\gamma_{lv}^{p}}{(\gamma_{sv}^{p} + \gamma_{lv}^{p})}\right] = 0.25\gamma_{lv}(1 + cos\theta_{Y})$$

As in the OWRK method, the Wu method requires the use of at least two liquids, one mainly polar and one dispersive. Water and diiodomethane are again often used. From a theoretical point of view, the geometric mean is more accurate than the harmonic one.

Acid-base

By using the acid-base approach, sometimes called Van Oss-Chaudhury-Good method, the polar component is further divided into acid and base components and the equation can be written as

$$\sqrt{\gamma_{sv}^{d}\gamma_{lv}^{d}} + \sqrt{\gamma_{sv}^{acid}\gamma_{lv}^{base}} + \sqrt{\gamma_{sv}^{base}\gamma_{lv}^{acid}} = 0.5\,\gamma_{lv}(1 + cos\theta_{Y})$$

Since there are three unknown, γ_{sv}^{d}, γ_{sv}^{acid} and γ_{sv}^{base}, at least three liquids with known properties are needed to solve the equation. One dispersive (e.g. diiodomethane) and two polar (e.g. water, glycerol) should be used. Acid-base method is one of the most recent developments in the field of SFE calculations. It has a potential to give more in depth information about surface properties of the solid but has been criticized by its sensitivity to even small variations in the contact angle measurements or properties of liquids used.

Zisman plot

Zisman plot is used for defining so-called critical surface tension which is the surface tension of the liquid needed to completely wet the solid (contact angle between the solid and liquid is zero). This critical surface tension value differs from the surface free energy of the solid, and is not divided into dispersive and polar components. In practice, critical surface tension is defined by measuring the contact angle between several different probe liquids and the studied surface. The results are then plot by having cos θ in y-axis and the surface tension of the liquid in x-axis. Straight line is then fitted to this measurement points and extrapolated to point cos θ=1 which will then give the critical surface tension value for the surface. In theory, only two measurement points would be needed but in practice, using just few different liquids will lead to incorrect results. Even negative values are often seen. At present, this method is not commonly used due to insufficient theoretical justification and laborious investigation procedure.

C. Further reading

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D. Li and A. W. Neumann, J.Colloid Interface Sci 148, 1, pp.190–200 (1991).

7.4 Topography

A. Influence of surface roughness on contact angle and wettability

Both chemical and topographical properties of the surface are important parameters in many different applications and processes, where wetting and adhesion behavior needs to be optimized. Wettability

can be studied by measuring contact angle of the substrate with given liquid. The well-known Young equation describes the balance at the three phase contact of solid-liquid and gas.

$$\gamma_{sv} = \gamma_{sl} + \gamma_{lv} cos\theta_Y$$

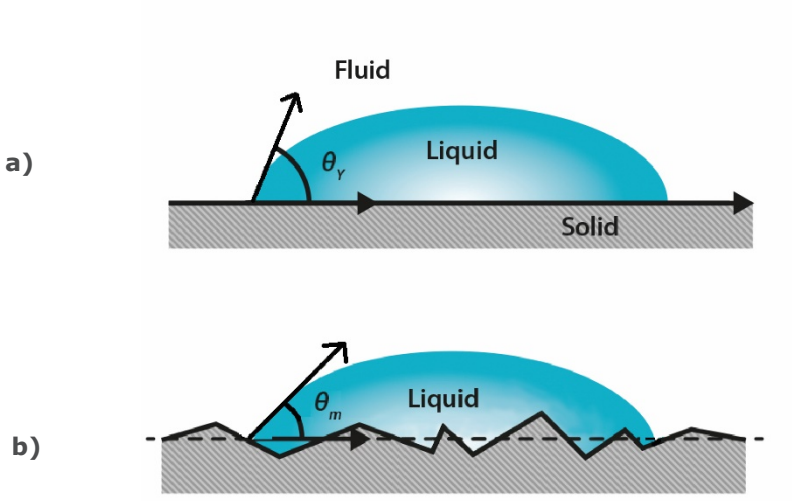
The interfacial tensions, γ_{sv}, γ_{sl} and γ_{lv}, form the equilibrium contact angle of wetting, many times referred as Young contact angle θ_Y. The Young equation assumes that the surface is chemically homogenous and topographically smooth. This is, however, not true in a case of real surfaces, which instead of having one equilibrium contact angle value exhibit range of contact angles between advancing and receding ones.

The figure below presents a droplet on ideal and real surface. On ideal surface, the Young equation applies and the measured contact angle is equal to Young contact angle (figure (a)). On a real surface, the actual contact angle is the angle between the tangent to the liquid-fluid interface and the actual, local surface of the solid (figure (b)). However, the measured (apparent) contact angle is the angle between the tangent to the liquid-fluid interface and the line that represents the apparent solid surface, as seen macroscopically. Actual and apparent contact angle values can deviate substantially from each other. To calculate the real surface free energies of the solid, the actual contact angles should be used.

The relationship between roughness and wettability was defined already in 1936 by Wenzel who stated that adding surface roughness will enhance the wettability caused by the chemistry of the surface [1]. For example, if the surface is chemically hydrophobic, it will become even more hydrophobic when surface roughness is added. This is also the way how superhydrophobic surfaces (contact angle over 150° with water) can be produced. Wenzel statement can be described with the equation

$$cos\theta_m = r cos\theta_Y$$

where θ_m is the measured contact angle, θ_Y is the Young contact angle and r is the roughness ratio. Roughness ratio is defined as the ratio between the actual and projected solid surface area (r=1 for a smooth surface and > 1 for a rough one). It is important to notice that the Wenzel equation is based on the assumption that the liquid completely penetrates into the roughness grooves (as in figure (b)). Wenzel equation is an approximation that becomes better as the drop becomes larger compared to the scale of roughness. It seems that if the drop is larger than the roughness scale by two to three orders of magnitude, the Wenzel equation applies [2].



Definition of different type of contact angles; (a) Contact angle on ideal surface is called Young contact angle (b) Apparent or measured contact angle on a real surface.

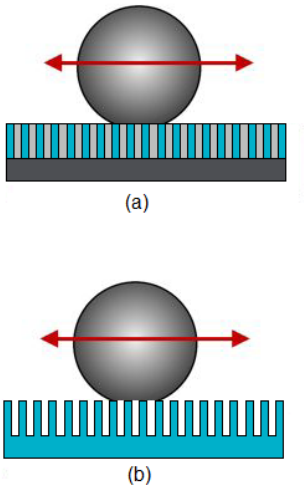
In cases where the liquid does not penetrate completely into the grooves, the Wenzel equation does not apply. In this case, the Cassie equation is used instead. The Cassie equation was first developed to describe chemically heterogeneous surfaces, with two different chemistries [3].

$$cos\theta_m = x_1 cos\theta_{Y1} + x_2 cos\theta_{Y2}$$

In the equation, the x is the area fraction characterized by the given chemistry and subscripts 1 and 2 indicate two different surface chemistries (figure (a) below). If instead of having different chemistries the second area is air like shown figure (b) below, the equation can be written as

$$cos\theta_m = x_1(cos\theta_Y + 1) - 1$$

since contact angle against liquid and air can be considered to be 180 ° ($cos\theta_{Y2}$ is -1) and the area fraction $x_2 = 1 - x_1$. This equation was developed by Cassie and Baxter and is thus often called Cassie-Baxter equation. It has been found that for the droplet to achieve the real Cassie-Baxter stage (no penetration of the liquid inside the grooves), the geometry of the roughness has to be carefully designed [4].



(a) Droplet on a chemically heterogeneous surface (b) Droplet standing on a pillar surface

The most stable contact angle is the one associated with the absolute minimum of the Gibbs energy curve which can be connected to Young contact angle. The contact angles calculated from the Wenzel and Cassie-Baxter equations has been found to be good approximations of the most stable contact angles [5].

B. Topography parameters

Surface roughness cannot be accurately characterized by using a single roughness parameter. Instead, a set of surface roughness parameters are defined. Parameters that characterize surface profiles are called 2-D parameters, and are mark with the letter "R". These parameters are widely utilized in different applications but are not really able to provide the full information on the three dimensional surfaces. Parameters to characterize surface topography are called 3-D parameters, and marked with letter "S". Some of the 3-D parameters have their 2-D counterparts; others are specifically developed for 3-D surfaces [6]. Here is a list of all topography parameters given by OneAttension.

Symbol	Name	Equations	Description
R_a, S_a	Arithmetic average	$S_a = \frac{1}{MN}\sum_{j=1}^{N}\sum_{i=1}^{M}\left\lvert\eta\left(x_i, y_j\right)\right\rvert$	Average of |z|
R_q, S_q	Root mean square (RMS) roughness	$S_q = \sqrt{\frac{1}{MN}\sum_{j=1}^{N}\sum_{i=1}^{M}\eta^2\left(x_i, y_j\right)}$	Standard deviation of z
R_p, S_p	Maximum height of peaks	$S_p = MAX\left(\eta_p\right)$	Max z
R_v, S_v	Maximum depth of valleys	$S_v = MIN\left(\eta_v\right)$	Min z
R_z, S_z	Maximum height of the surface	$S_z = \left(\lvert S_p\rvert + \lvert S_v\rvert\right)$	Max z- Min z
R_{10z}, S_{10z}	Ten point height	$S_z = \frac{\sum_{i=1}^{5}\lvert\eta_{pi}\rvert + \sum_{i=1}^{5}\lvert\eta_{vi}\rvert}{5}$	Average of five highest local maxima and five deepest local minima.
S_{dr}	Area factor	$S_{dr} = \frac{(Textured\ surface\ area) - (Cross\ sectional\ area)}{Cross\ sectional\ area} * 100\% = \frac{\sum_{j=1}^{N-1}\sum_{i=1}^{M-1} A_{i,j} - (M-1)(N-1)\Delta x\Delta y}{(M-1)(N-1)\Delta x\Delta y} * 100\%$	Ratio between the interfacial and projected areas

C. References

[1] R.N. Wenzel, "Resistance of solid surfaces to wetting by water", Industrial and engineering chemisty 28 (1936) 988

[2] A. Marmur, "Soft contact: measurement and interpretation of contact angles", Soft Matter 2 (2006) 12

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[4] A. Tuteja, W. Choi, M. Ma, J.M. Mabry, S.A. Mazzella, G.C. Rutledge, G.H. McKinley and R.H. Cohen, "Designing superoleophobic surfaces", Science 318 (2007) 1618

[5] A. Marmur, "Solid-Surface characterization by wetting", Annual review of materials research 39 (2009) 473

[6] K.J. Stout, P.J. Sullivan, W.P. Dong, E. Mainsah, N. Luo, T. Mathia, H. Zahouani, "The development of methods for the characterization of roughness in three dimensions", 1993

7.5 Interfacial rheology

A. Principle

Dilatational interfacial rheology enables the study of surfactant kinetics. Viscoelastic properties of an adsorbed interfacial layer have been shown to correlate with emulsion and foam stability.

Viscoelastic properties of an interface are measured by inducing a controlled oscillation to a pendant drop (or a bubble) and simultaneously recording the change in the interfacial tension γ and the drop area A.

From this data, viscoelastic properties of adsorbed molecular layer are calculated according to well established theories [1,2] with parameters and equations below:

|E|, complex surface dilatational modulus
δ , phase angle difference between the surface tension and drop area results
E’ , elastic (storage) modulus
E’’ , viscous (loss) modulus

$$|E| = \frac{\mathrm{d}\gamma}{\mathrm{d}\ln A} = A\frac{\mathrm{d}\gamma}{\mathrm{d}A}$$

$$E' = |E|\cos\delta, \qquad E'' = |E|\sin\delta$$

B. References

[1] Miller, R. Liggieri, L. (Eds), Progress in colloid and interface science series: Interfacial rheology (2009) Vol 1., Brill, Leiden

[2] Miller, R., Ferri, J.K., Javadi, A., Krägel, J., Mucic, N. and Wüstneck, R., Rheology of interfacial layers, Colloid. Polym. Sci. (2010) 288:937-350

8 Technical specifications

Available measurements

Experiments available	Static contact angle, advancing and receding contact angles, batch contact angle, meniscus contact angle, surface / interfacial tension by pendant drop method, topography profiles, surface free energy and dilatational interfacial rheology by oscillating drop method.
Surface free energy methods	Zisman Plot, OWRK/Extended Fowkes, van Oss Acid-Base, Wu, Neumann's Eq. of State, Schultz 1 and 2

Software and hardware

Measuring range (°, mN/m)	0…180, 0.01…2000
Accuracy (°, mN/m)	± 0.1, ± 0.01
Maximum sample size (mm)	Unlimited x 100 x 320 (w. stage).
Integrated sample holders	Yes
Maximum resolution (pixels)	2592 x 2048 (5MP)
Maximum measuring speed (fps)	3422
Camera	CMOS 1'' USB 3.0 digital camera with zoom.
Image focusing	Software-controlled autofocus, manual fine focus in optics
Image quality	Enhanced with DropletPlus technology
Camera protection	Inside instrument covers
Camera view angle (°)	-4…2.5, with digital scale
Light source and size	High power monochromatic LED, 62 x 62 mm.
Field of view (diagonal in mm)	1.44…45.3
Measurement indicator LED	Yes
Integrated touch display	Yes
Environment monitoring	Integrated digital ambient temperature, relative humidity and system levelness sensors
Disposable tip dispensing	Yes
Software	OneAttension, includes all measurement modes
Dimensions – Basic frame (LxWxH, mm)	765 x 230 x 435
Weight – Basic frame (kg)	29

Input voltage (Vac)	100…240
Frequency (Hz)	50…60
Input current (mA)	330…650
Fuses	T1.0A 5x20 mm

System requirements

Recommended system requirements for Theta Flow	2 GHz processor, 2 GB RAM, 120 GB hard disk drive*, 1920x1080 resolution, 1 USB 3.0 port *SDD hard disk (min. 500MB/s) needed for high speed recording with high resolution.
Operating system requirements	Windows 10 (32 or 64 bit).

Environmental conditions

Ambient temperature (°C)	15…30
Ambient pressure (hPa)	700…1060
Ambient humidity (%)	20-80 (non-condensing)

9 Contact information

If any problems arise please feel free to contact your local distributor or Biolin Scientific directly.

https://www.biolinscientific.com/contact-us

info@biolinscientific.com

10 EC Declaration of Conformity

Biolin Scientific
[Progress Together]

EU DECLARATION OF CONFORMITY

We,

Biolin Scientific Oy, Tietäjäntie 2, Espoo, Finland

as the manufacturer declare under our sole responsibility that the following products

Attension TFL400
Attension TFL400-Auto1
Attension TFL400-Auto2
Attension TFL400-Auto3
Attension TFL400-Auto4
Attension TFL400-Auto5
Attension TFL400-3D

are in conformity with the following European Directives

Low Voltage Directive 2014/35/EU

EMC Directive 2014/30/EU

RoHS Directive 2011/65/EU

The following harmonised European standards have been applied

EN 61010-1:2010

EN 61326-1:2013

Espoo, Finland
18.02.2021

Sten Brandt
Supply Chain Director
Biolin Scientific Oy

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