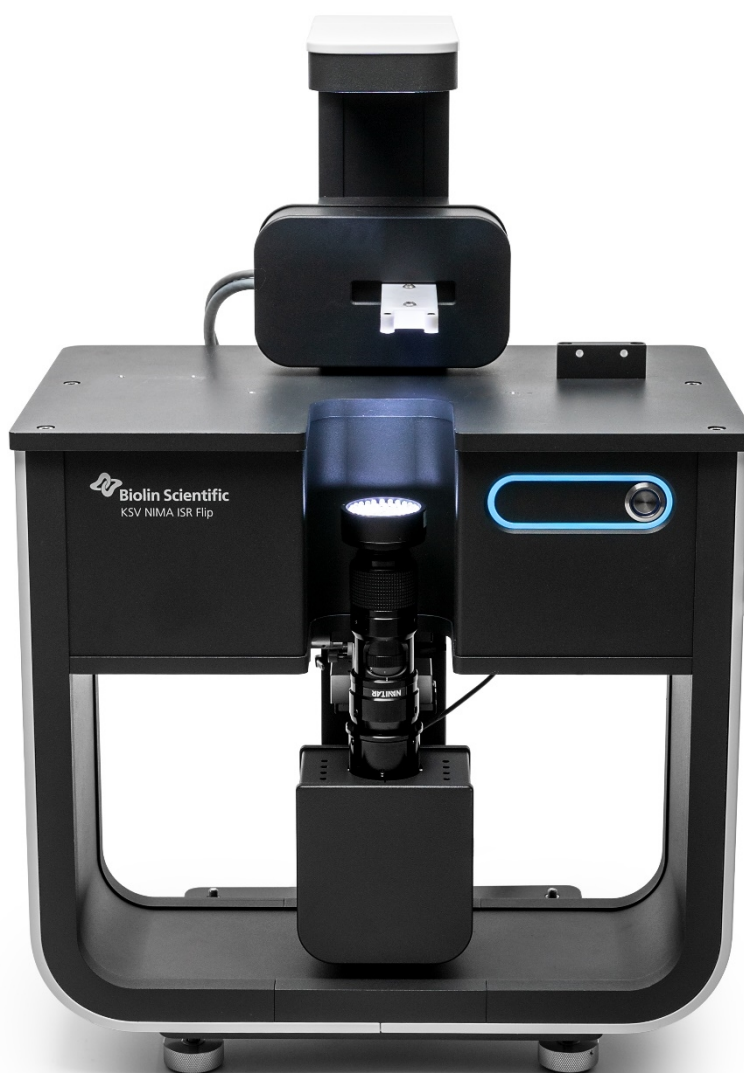


# KSV NIMA ISR Flip

## User Manual



MANUAL25362 – 3

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# 1 Introduction

The KSV NIMA ISR Flip provides an extremely sensitive method to measure the shear properties of films at fluid interfaces (gas/liquid and liquid/liquid). Viscoelastic properties and surface pressure can be measured simultaneously. The film packing density can also be controlled while measuring.

The KSV NIMA ISR Flip can be equipped with either a KSV NIMA High Compression ISR trough which can be used for both air-liquid and liquid-liquid measurement as well as for simultaneous control of the film packing density or a Low Volume Measurement cell to work with small interfacial areas and reduced subphase volumes.

Both systems enable surface pressure measurement thanks to the integrated highly sensitive Wilhelmy balance. Both troughs are divided into an upper and lower compartment which can be used to study film viscoelasticity at the liquid-air or liquid-liquid interface.

This is the user manual for KSV NIMA ISR Flip, featuring instructions on how to setup and, run the experiments, and how to analyze the data.

If any problem would appear with the product, then please contact KSV NIMA support department at <http://www.biolinscientific.com/ksvnima/services/> or contact a local distributor directly. For further information and for the contact details of the nearest distributor, please visit our website at [www.biolinscientific.com/ksvnima](http://www.biolinscientific.com/ksvnima).

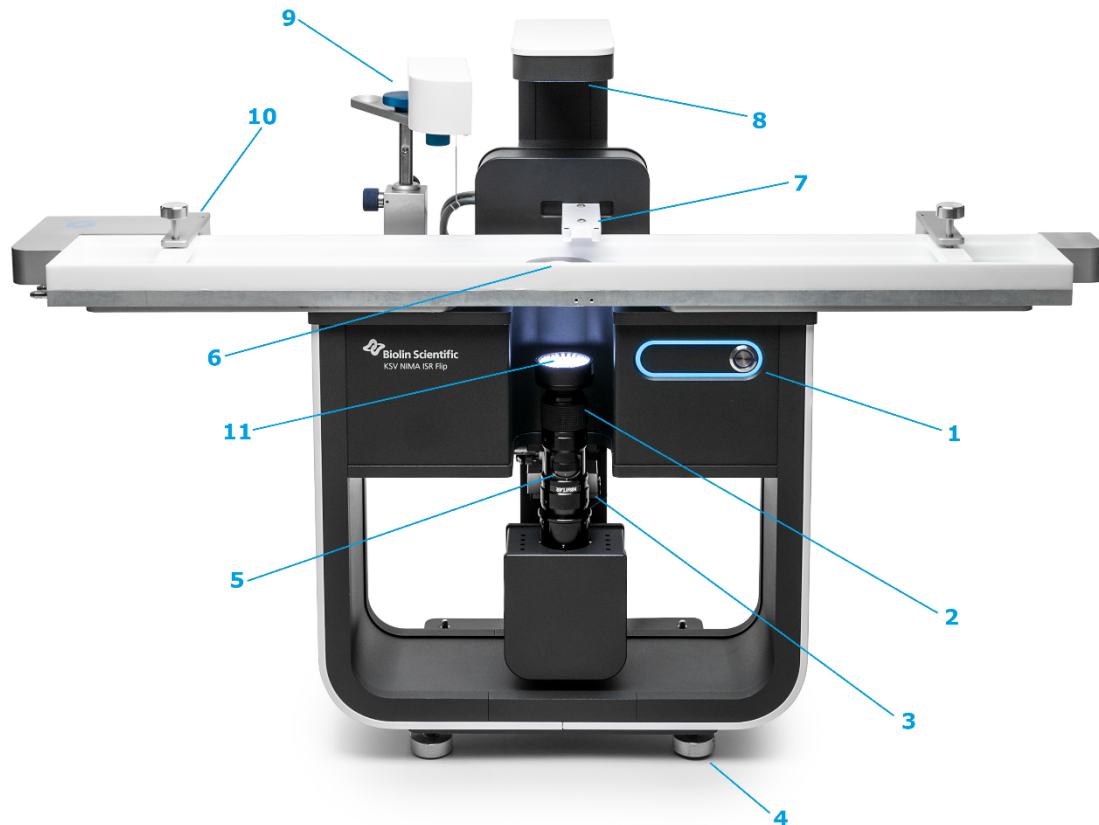
## 1.1 Purpose of this manual

This manual provides information on how to operate KSV NIMA ISR Flip along with some practical advice on the measurement techniques. To obtain the maximum performance from the instrument this manual should be read and kept available for reference. For more detailed instructions on how to use KSV NIMA LB software to control for example the balance and the barrier movement, user is referred to **KSV NIMA LB software manual**.

For information on the installation of ISR Flip and the software please see the separate **Installation Manual**.

## 1.2 Physical description

The figure below describes the basic hardware of ISR Flip.



### ISR Flip physical features.

- |  |                            |
|--|----------------------------|
| 1) Power button and status indicator LED | 7) Magnetic trap           |
| 2) Camera lens fine focus adjustment     | 8) Backplane light         |
| 3) Camera position adjustment screws     | 9) Balance                 |
| 4) Level adjustment feet                 | 10) Barrier                |
| 5) Camera lens zoom adjustment           | 11) Camera objective light |
| 6) Microscopy window                     |                            |

The instrument front panel contains the power button, and status indicator LED.

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

Electrical connections are available on the instrument rear panel. A power cord and USB3 cable need to be connected to operate the instrument. The rear panel also contains a USB port to connect the interface unit. When the instrument is on, do not touch the backside of the instrument. To disconnect the instrument, after turning the instrument off, remove the power cord plug from the electric outlet.

## 2 Safety



### **WARNING!**

The safety requirements listed in this manual must be followed 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 KSV Nima 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 the equipment is exposed to hazardous material.

The instrument is heavy (21 kg). Grab the ISR Flip on the bottom of the frame when lifting and moving it. Lift it with care and in a good lifting position.



### **WARNING!**

**BRIGHT LIGHT. AVOID EXPOSURE.** The camera objective light is very bright. Do not stare into light. The brightness of light may cause eye injury.



### **WARNING!**

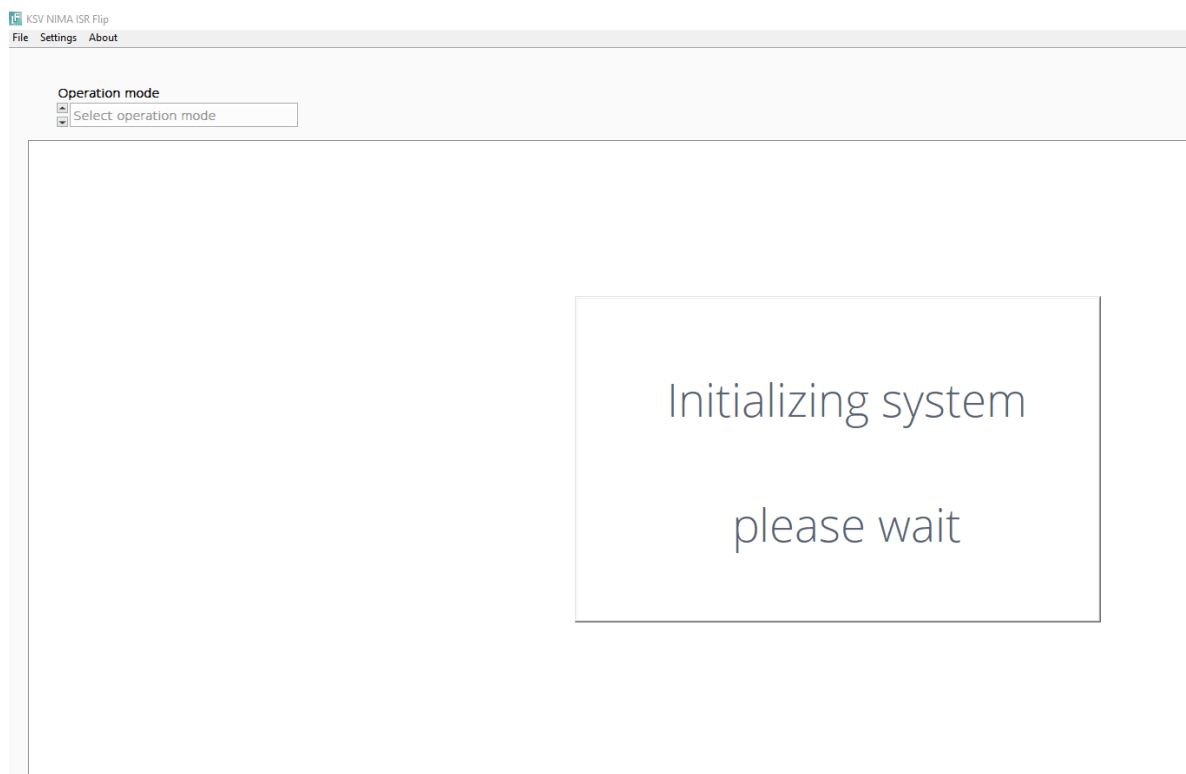
**RISK OF CRUSHING.** Locations, where crush hazard exists, are marked with crush hazard symbol.

### **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.

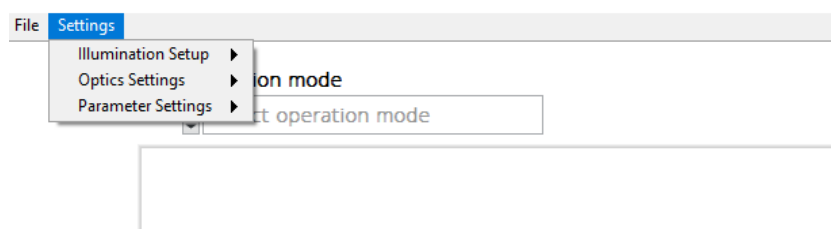
## 3 Operation of the instrument

The operation of the instrument is done through the KSV NIMA ISR Flip software, and if the compression is being used the KSV NIMA LB software. For detailed information about the KSV NIMA LB software, the instruction can be found in the KSV NIMA user manual. When the ISR Flip software is turned on, the system will be initialized by moving the magnetic trap.



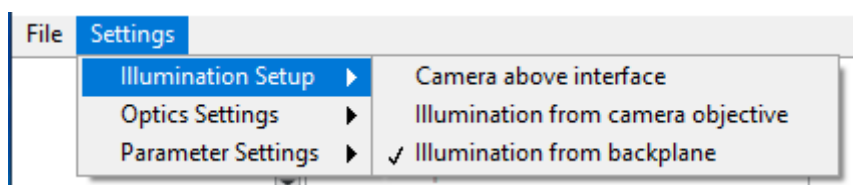
### 3.1 Instrument settings

Instrument settings can be adjusted from the Settings tab.

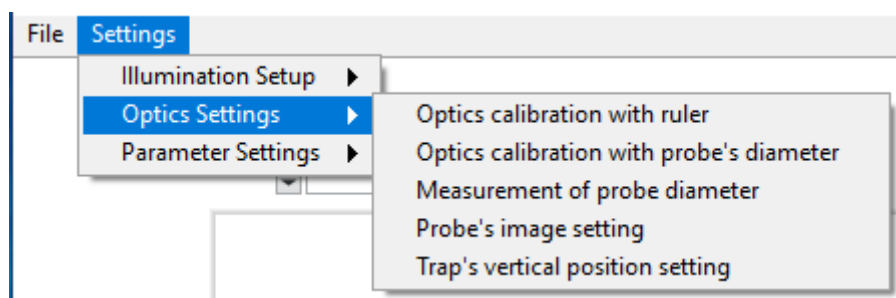


## Illumination setup

Illumination should always be checked when the software is opened. When the device is switched on, the last light settings used are applied. The default position for the camera is below the interface. When the camera is below the interface, Illumination from the backplane is selected. If the camera is installed above the interface select Camera above and Illumination from the camera objective.



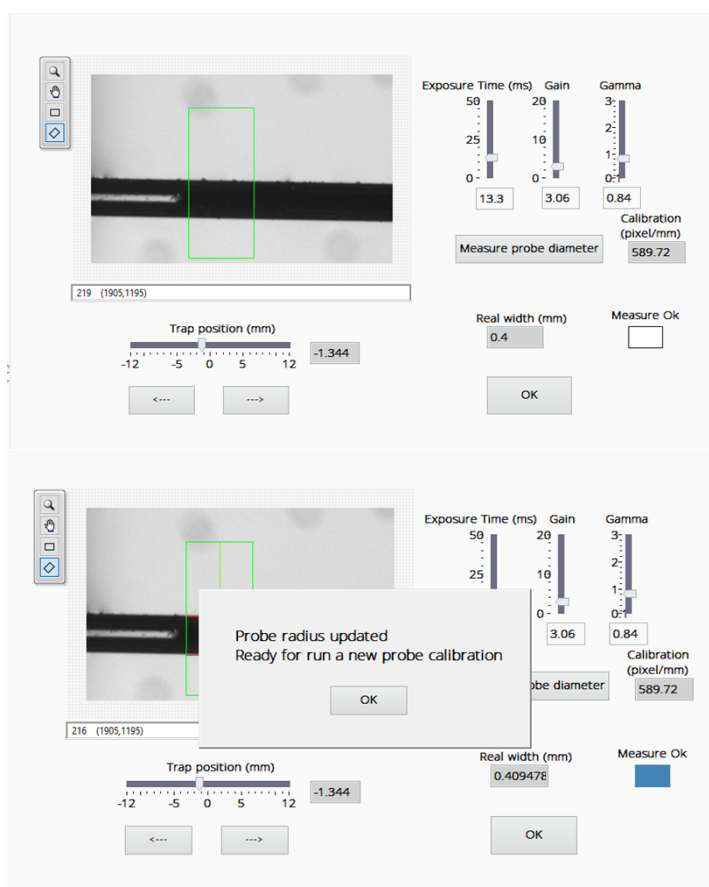
**Optics settings** contain five different functions. First, there are two ways to perform the optics calibration, and those are explained in section 3.2. The other three options are explained below.




**Measurement of probe diameter.** It is possible to determine the probe's diameter. This is useful as the probe radius is needed when the probe is calibrated. Also, the probe diameter can be used for optics calibration which is explained later in section 3.2.2. To measure the probe's diameter follow the below steps:

1. Move the camera from the three screws to find the needle. You can also change the trap position by using the left and right arrows under the image.
2. Adjust camera parameters for optimal visualization and check that the needle is in focus.
3. Select a RoI that covers both edges of the needle.
4. Click Measure probe diameter. Measure ok box turns Blue if the measurement was successful otherwise an error message appears. The calculated diameter will be shown in the Real width box.





**Trap's vertical position setting** can be used to move the trap up and down when the measurement is not ongoing. The current height is shown on the left. Insert target height on the right and click Go To h button. The Busy indicator light will switch on and the trap holder moves to the target position. The Busy light goes off when the desired height is reached. The current height should match the target height. **NOTE!** that the allowed height range is related to the loaded probe calibration file. If the probe calibration has not been done at a height lower than e.g. 5 mm the trap cannot be moved lower than that.

<b>Actual Trap Height (mm)</b>	<b>Target Trap Height (mm)</b>
13.987	10
<b>Go To h</b>	<b>Busy</b>
	
<b>Exit</b>	

**Parameter settings** adjustment is covered in the calibration and data analysis section.

## 3.2 Optics calibration

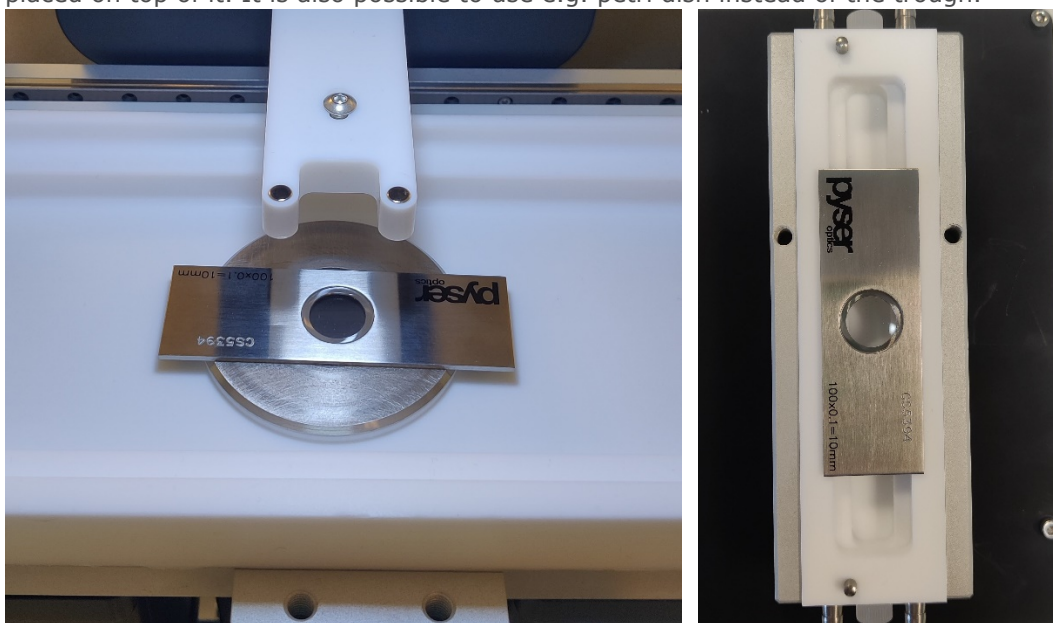
The accuracy of the rheological measurements relies directly on the accuracy of the measurements of the probe's tip position. Hence, accurate calibration of the camera/optics system is critical for the good performance of the instrument. Perform optics calibration every time magnification is changed.

**Note that after calibration has been done, you should only focus the camera by moving the camera itself, not from the focus ring.** There are two options for optics calibration; Calibration with the precision ruler and calibration with the probe diameter.

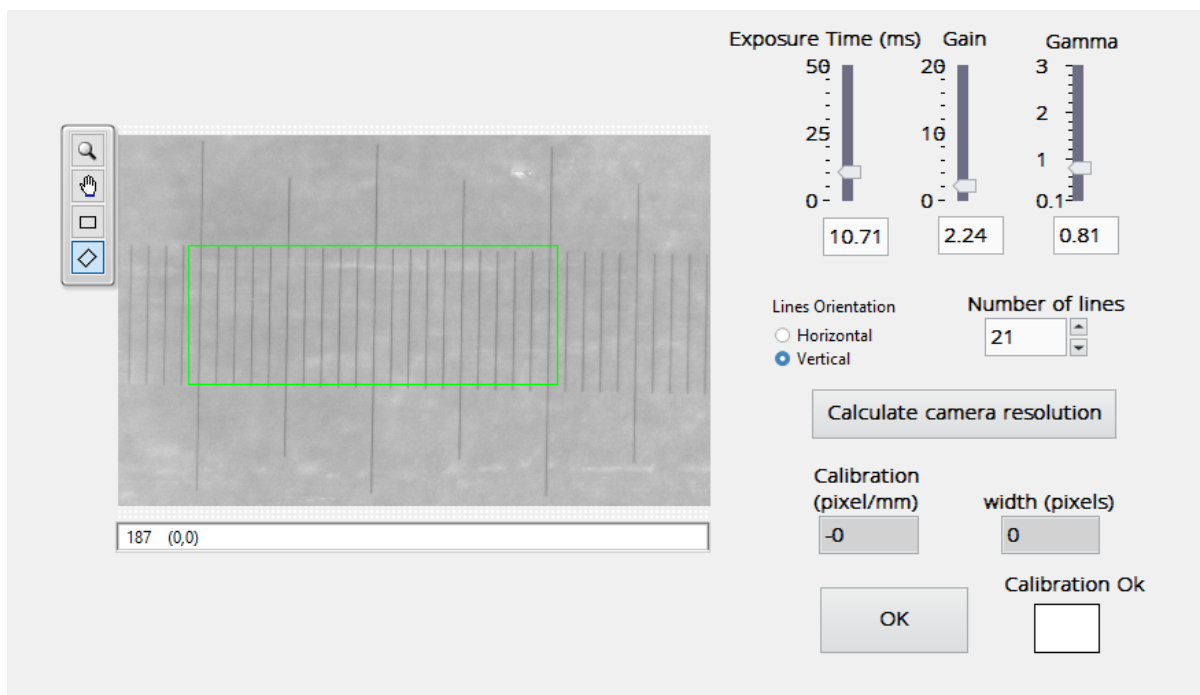
### 3.2.1 Optics calibration with a precision ruler (recommended)

This method works by measuring the number of pixels between some well-separated stripes of a high-precision ruler engraved in a transparent glass slide.

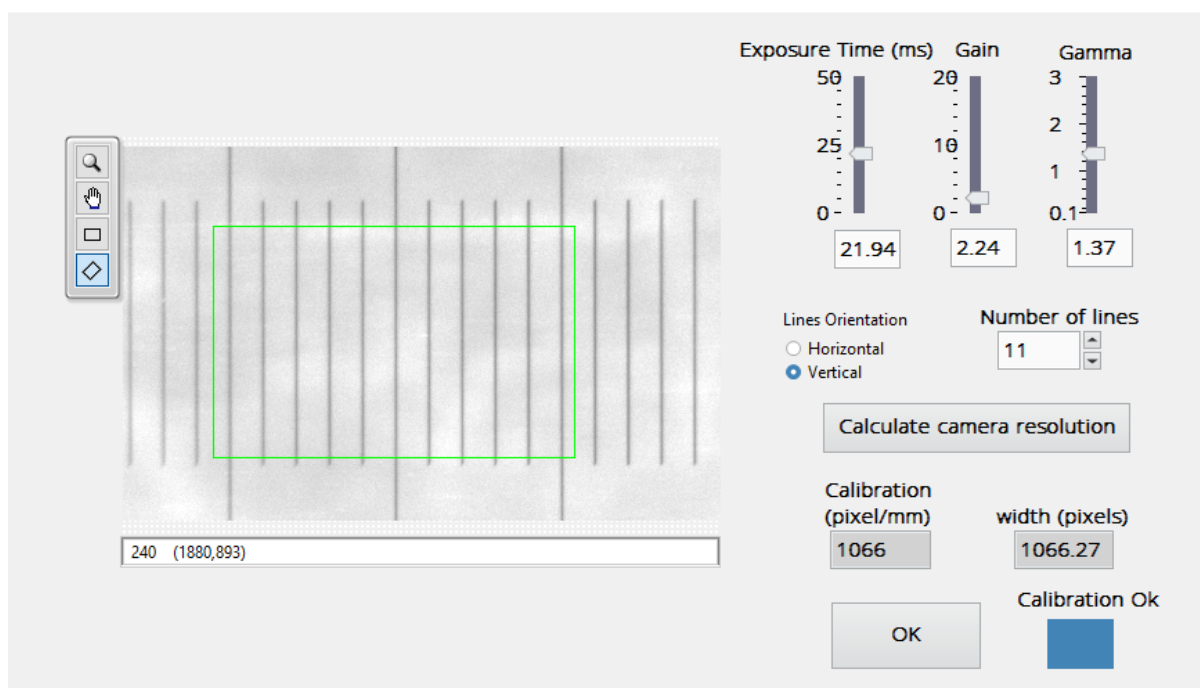
1. Open Optics settings → Optics calibration from ruler
2. Select the magnification used for measurement
3. Position the ruler on top of the trough window. If low volume cell is used, the ruler can be placed on top of it. It is also possible to use e.g. petri dish instead of the trough.



4. Adjust the camera position using three screws and the focus tool to find ruler lines. Fine tune the camera parameters (Exposure time, Gain, and Gamma), to achieve a nice ruler image with good intensity contrast.
5. Select 'Line Orientation' either Horizontal or Vertical according to the correct orientation of the ruler stripes.
6. Select a rectangular RoI from the image by pressing the mouse left button at one angle drawing the area you want to use. The selected area appears as a green box. The more lines selected in the image, the better. Use at least five lines if possible. NOTE! The selected area must be parallel to the ruler lines. You can rotate the box orientation if needed.
7. Calculate the number of lines inside the green RoI. Insert that value to 'Number of lines'



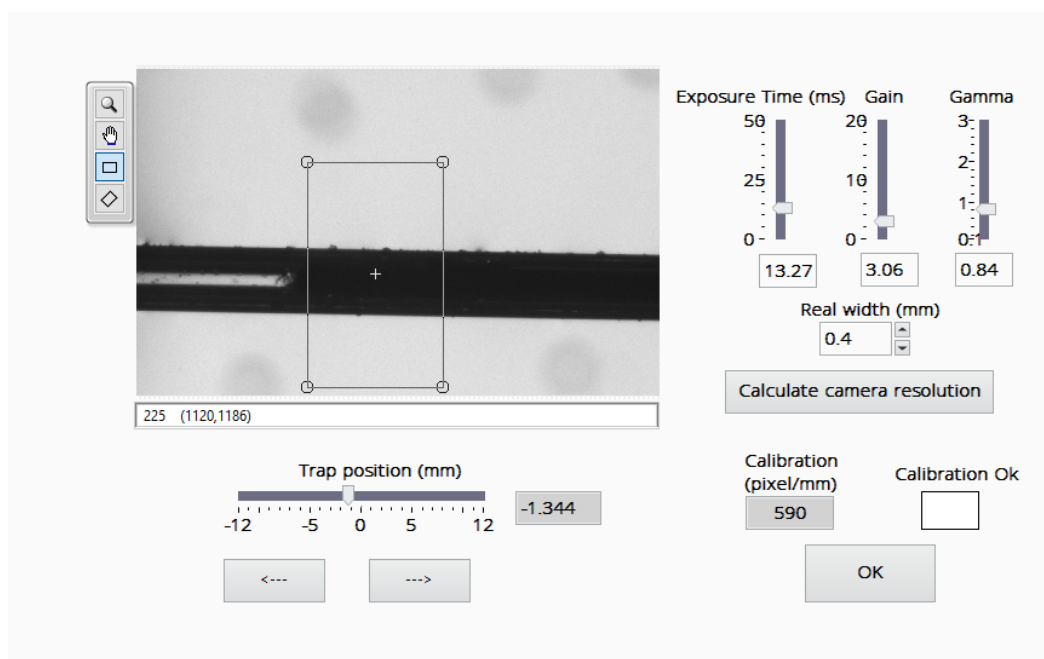
8. Click calculate camera resolution. If calibration is OK, Calibration OK box will turn blue and Calibration constant (pixel/mm) is shown in the box. Click OK to end calibration.
9. If calibration is not accepted, an error message will appear. Adjust camera parameters to achieve good intensity contrast. Make also sure that 'Number of Lines' value is correct.



### 3.2.2 Optics calibration with probe diameter

This method works by measuring the number of pixels between the optical borders of a KSV Nima standard capillary-based probes. To use this method the trough is filled with the subphase, e.g. water, and the probe is placed there. Although calibration with the ruler is recommended, the

possibility to calibrate with the probe is useful if the magnification needs to be changed during the measurement.



1. Open Optics settings → Optics calibration from probe diameter
2. Select the magnification used for measurement
3. Find the probe into the image by moving the camera
4. Adjust the camera position using three screws and focus on the probe. Fine-tune the camera parameters (Exposure time, Gain, and Gamma), to achieve a good image of the probe
5. Select RoI to cover the whole height of the probe.
6. Click Calculate camera resolution. If calibration is OK, calibration OK box will turn blue and Calibration constant (pixel/mm) is shown in the box. Click OK to end calibration.
7. If calibration is not accepted, an error message will appear. Adjust camera parameters to achieve good intensity contrast.

### 3.3 Probe calibration

A probe calibration must be performed when any of the following conditions are met:

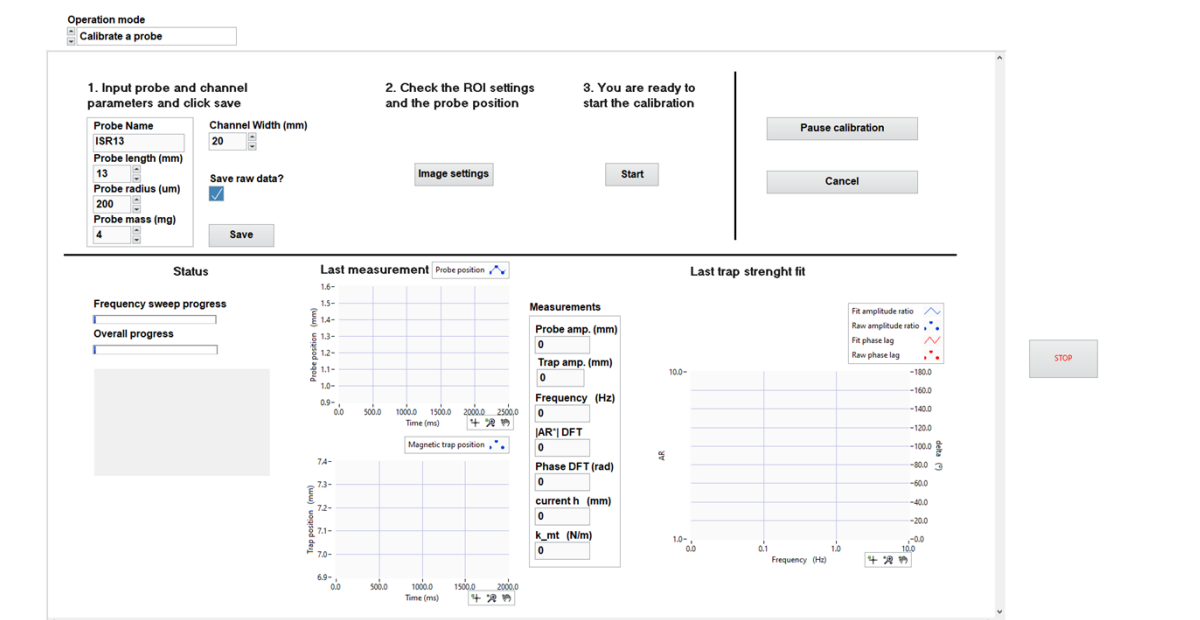
- A new probe is going to be used for the first time
- No probe calibration data file exists on the user's PC
- The magnets or the magnet support part have been removed and reinstalled or replaced.

The probe calibration is typically performed at the air-liquid interface but other subphases are also possible. The same calibration file can be used later, regardless of the subphase in which the calibration was performed. After the calibration has been performed, the same calibration file can be used for all the upcoming measurements as long as previously mentioned limitations are considered.

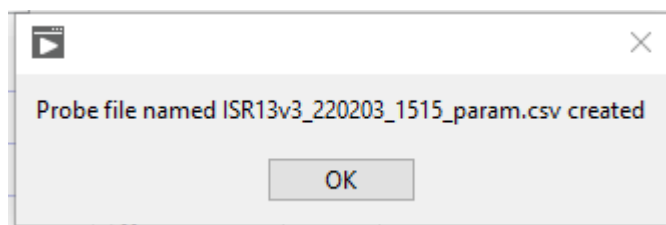
### 3.3.1 Performing the probe calibration

To calibrate the probe, select the probe calibration from the measurement menu. There are predefined probe calibration parameters set and those are explained in section 5.

1. Clean the Langmuir trough, barriers, the channel, and the probe with adequate solvent and rinse them thoroughly with Milli-Q water
  2. Place the trough in place and pour the subphase liquid. Place the channel and the channel holder in place. Put the probe at the interface underneath the trap.
  3. Fill in the windows on the screen in order. Start from no 1. Input probe and channel parameters:
- Probe's length
  - Probe's diameter
  - Channel width (20 mm for high compression channel, 10 mm for low volume cell)
  - Select save raw data

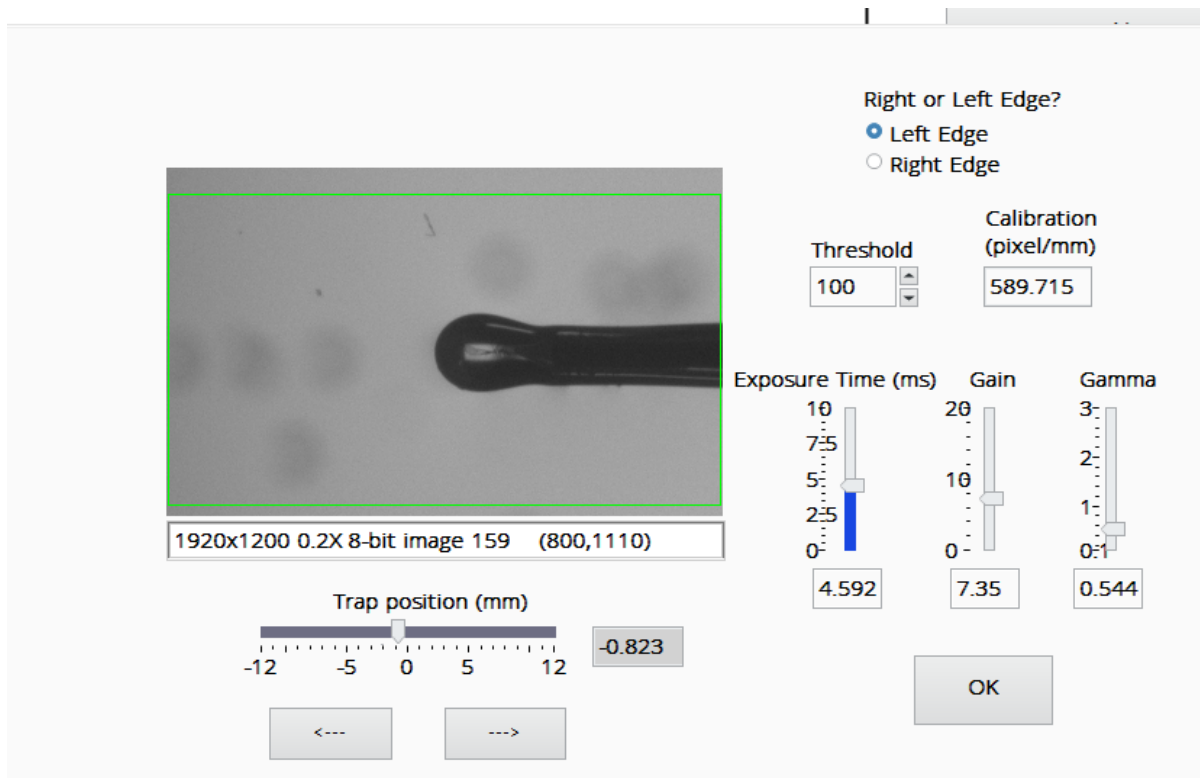


4. Click Save. The software informs that a calibration file has been created

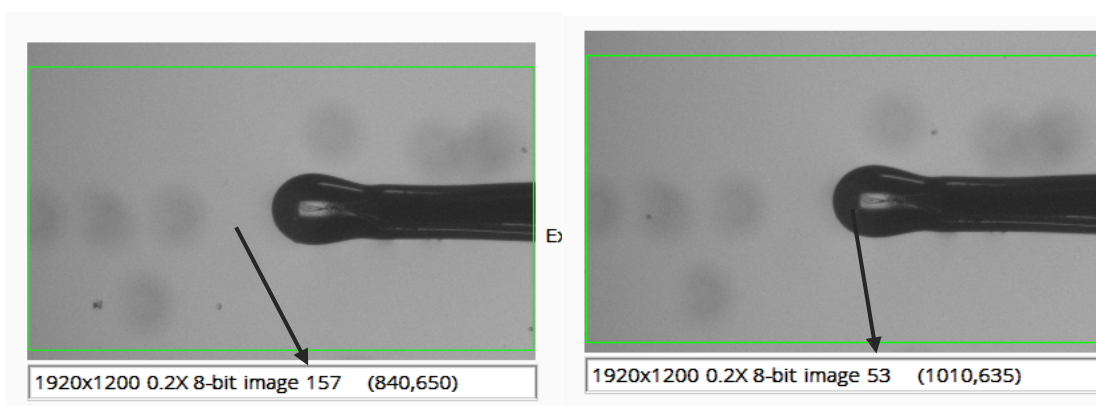


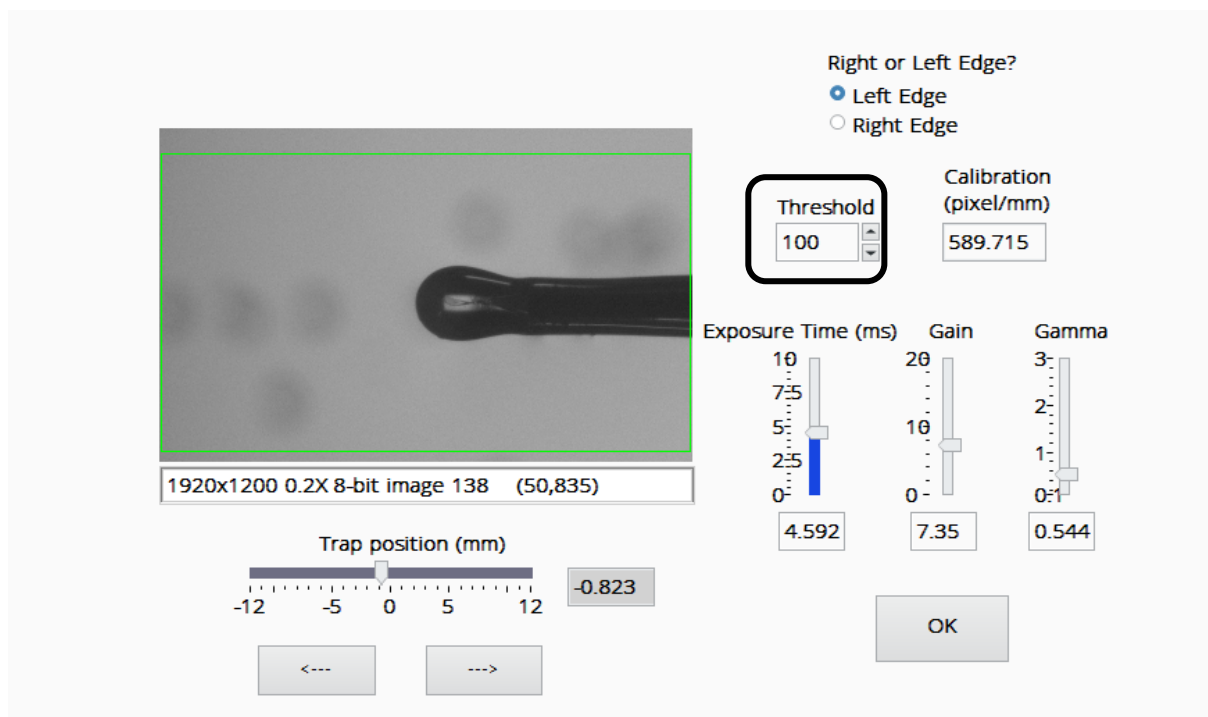
5. Open Image settings. Adjust camera position, focus, and lightning to be able to have a nice image of either end of the needle. The magnetic trap can be moved to the left/right using the arrows shown below the trap position scale.

6. Select either Left Edge or Right Edge depending on which is shown in the camera view.



- 7.
8. Set the intensity threshold value for the detection of the probe's edges. At the line below the image, the numbers between parenthesis indicate the coordinates of the cursor in the image (with the origin at the top left corner). The number preceding the left parenthesis is the intensity at the cursor position. To select the intensity threshold:
- Move the cursor around the bright zones of the image and notice the typical intensity value.
  - Move the cursor around the darkest zones of the probe's image and notice the typical intensity value.
  - Set the threshold value between the brightest and darkest intensity values.





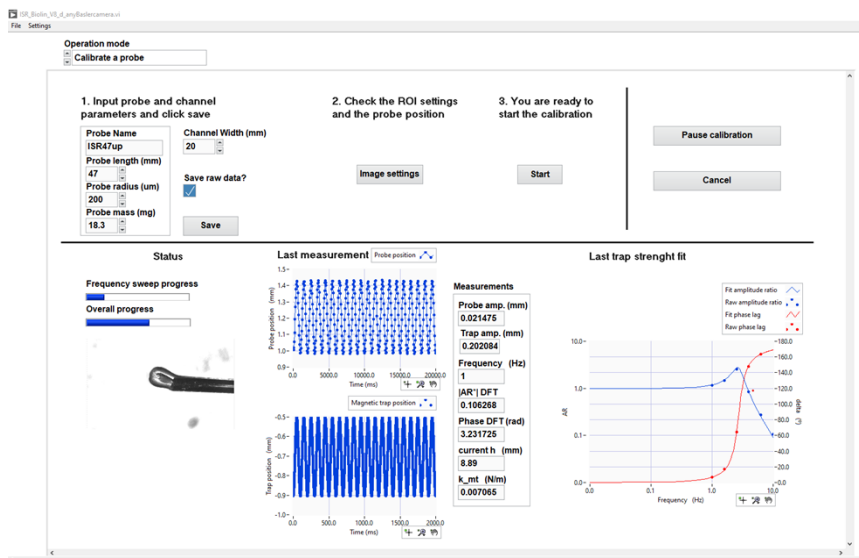
9. Click Start button to start calibration.

Bars at the Status panel will inform the progress of the ongoing tasks. A live image of the probe is shown below that.

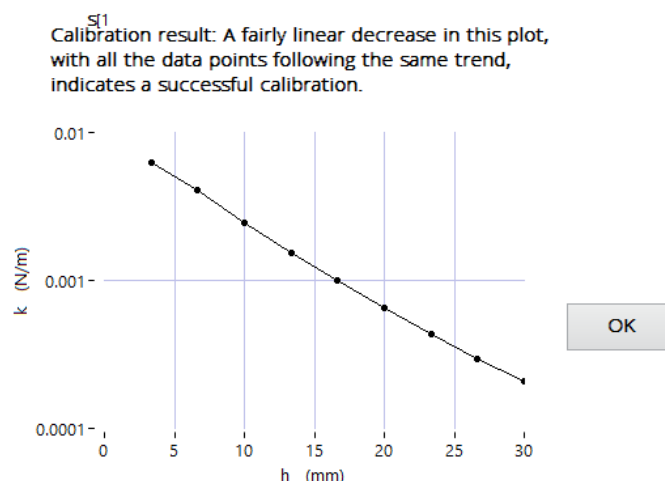
The two central chart graphs will display the evolution of the trap and probe tip position after the image analysis is finished. The numerical displays values from analyzed measurement:

- The amplitude of the probe's oscillation in mm.
- The amplitude of the trap's oscillation in mm.
- The oscillation frequency in Hz.
- The modulus of the complex amplitude ratio obtained numerically from the Discrete Fourier Transforms (DFT hereafter) of the trap and probe oscillation signals.
- The argument of complex amplitude ratio obtained from the DFTs of the trap and probe oscillation signals.
- The current trap height in mm.
- The trap constant corresponding to the current trap height (in N/m).

The chart graph at the right shows the amplitude and phase experimental data (symbols) and the corresponding fit (continuous lines) for each frequency sweep



10. Clicking the Pause calibration button will pause the procedure when the current frequency sweep ends. A pop-up window is shown, and it allows to change manually the trap height, the optical settings, to resume the calibration or abort it.
11. It may happen that during the measurements the amplitude of the oscillation drives the probe's tip out of the RoI, then the pop-up window will show up to inform the user. Then, Click the OK button and the Pause menu will appear. Open ROI Settings. Adjust the camera position if the probe is disappeared. Adjust lighting if needed and select new RoI. Click ok and continue the calibration by clicking Resume Calibration.
12. When calibration is finished Calibration result window will pop up. Calibration is ok if all the data points are following the same trend.





### 3.3.2 Probe calibration at a single height

Probe calibration at a single height is useful when the measurement is run on a trough or other container like a Petri dish and the height of the interface is not the same as with the troughs typically used. Note that to use the single height calibration, the full calibration file of the probe needs to be available as the single height calibration is used to correct that.

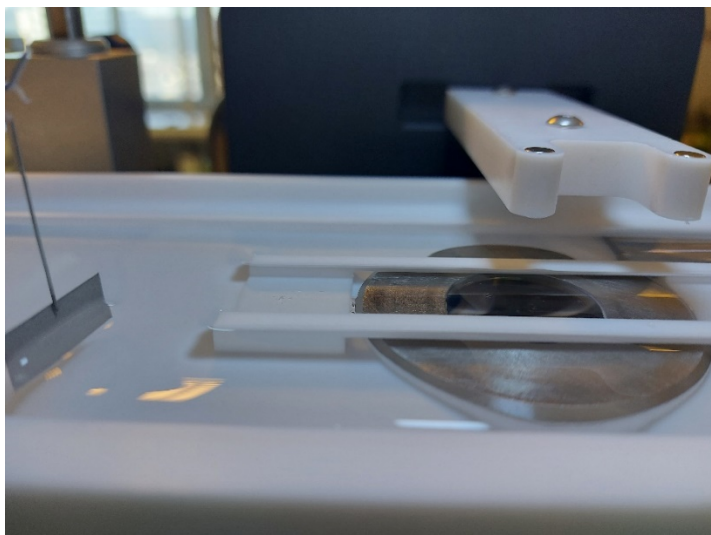
A quick single height calibration process allows the user to obtain the trap constant at the actual interface height that can, subsequently, be used to obtain the actual interface height by interpolation in the  $k_{mt}(h)$  curve corresponding to the trap-probe combination used. The software then uses the actual interface height to correct the difference between the two reference heights corresponding to the actual Langmuir trough and the one used for the full calibration of the probe. In that way, the probe calibration curve can be automatically used if the trap vertical position is changed during the later measurements.

**IMPORTANT: When using this Operation Mode, the channel width should be set at the same value that was used in the original probe calibration for the correct values of the drags to be used.**

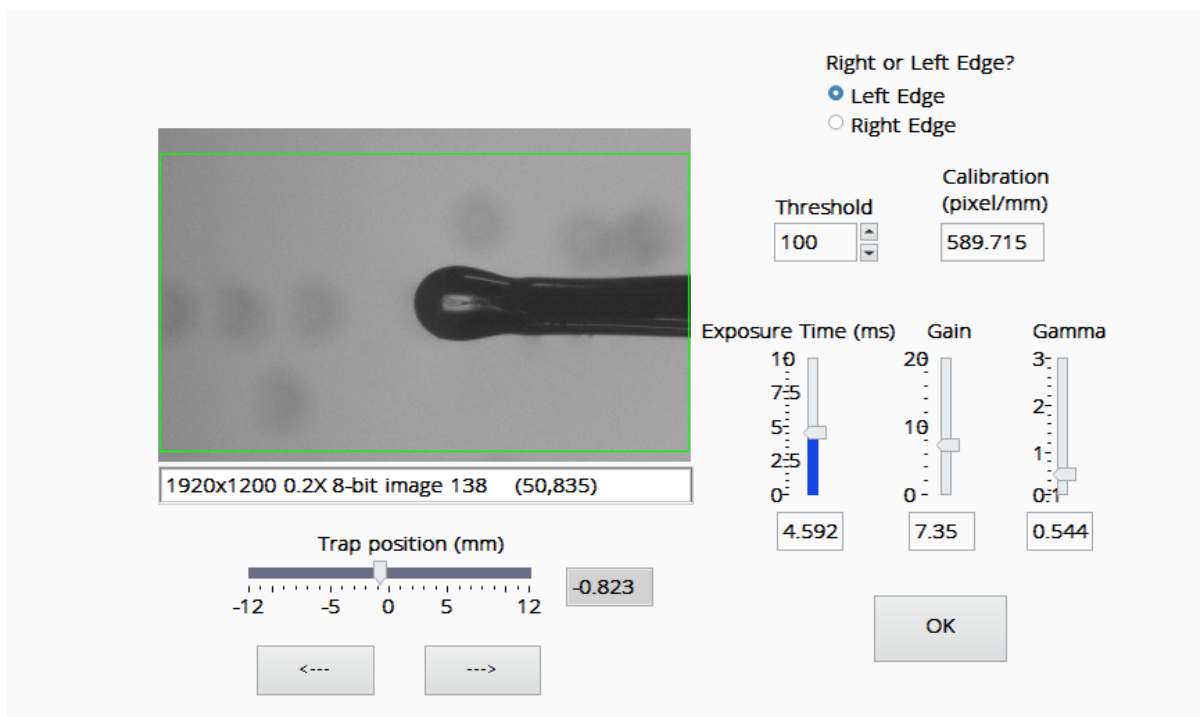
## 3.4 Setting up the measurement

Before the measurement can be started, it is important to make sure that all the parts in contact with the subphase and monolayer are cleaned. This includes the trough, barriers, the channel and the channel holder, probe, and the Wilhelmy plate or the rod.

1. Clean the Langmuir trough, barriers, the channel, and the probe with adequate solvent and rinse them thoroughly with Milli-Q water
2. Place the trough on and put the channel inside. Use channel holder if wanted. Pour the subphase liquid. The liquid is filled all the way to the edge so that the liquid is leveled with the channel surface. If the water surface is too high, the surface becomes convex.
3. Check the cleanliness of the subphase using LB software. Zero the balance and start to close the barriers. The balance reading should not increase more than 0.2 mN/m. If needed clean the subphase surface with e.g. vacuum suction pump and repeat the test.



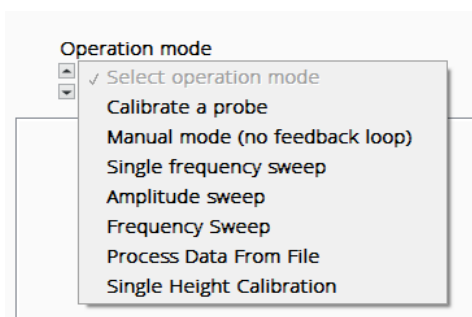
4. Put the probe at the interface underneath the trap.
5. Test that the probe stays in the middle of the channel when the trap is moved to the highest position. By doing this, you can make sure that the probe will be trapped throughout the measurement independent of the trap position. This can be done by selecting settings → optics setting → trap vertical height. If the probe does not stay in the middle, you should either add or remove liquid from the trough and repeat the test.
6. If you are performing liquid-liquid measurements, pour the lighter phase on top. This should be done extremely slowly to make sure that phases will stay in the preferred order. It is advised not to pour the liquid on top of the window area or the channel holder as that easily leads to wetting of the solid with light phase.
7. The monolayer material is then added. This is done as instructed in the KSV NIMA LB manual. Note that you need to set up the experiment first in the KSV LB software if you are going to compress the monolayer. In the case of liquid-liquid measurements, the monolayer material should be carefully added at the liquid-liquid interface.
8. After adding the monolayer material, the system is ready for the measurement.
9. Next, select the measurement mode you want to use (see different measurement modes in the next section). In all modes fill the following information:
  - a. Input channel width (for high compression trough 20 mm, for low volume 10 mm)
  - b. Click "Load probe calibration". Select the folder with the probe calibration files and click select.
  - c. Select the working directory where the measurement results should be saved.
  - d. Give a name for the experiment "Filename".
  - e. Select if you want to save the raw data.
10. Make the image settings by clicking "image setting".
  - a. Place the probe end at the center of the image. If focusing is needed, move the whole camera up and down (do not change the focus in the optics as this might affect the calibration).
  - b. Select ROI around the probe by drawing a rectangle around the probe.
  - c. Adjust camera parameters for good visualization of the probe.
  - d. Set intensity threshold value. This is set between the intensity value of the black and the white area. Place the cursor on the black area of the tip, and check the intensity value at the bottom of the image. Do the same outside the probe and set the threshold between those numbers.



11. Change measurement mode specific parameters if needed. Different modes are explained below.

## 3.5 Measurement modes

There are four measurement modes in ISR Flip; single frequency sweep, amplitude sweep, frequency sweep, and manual mode.



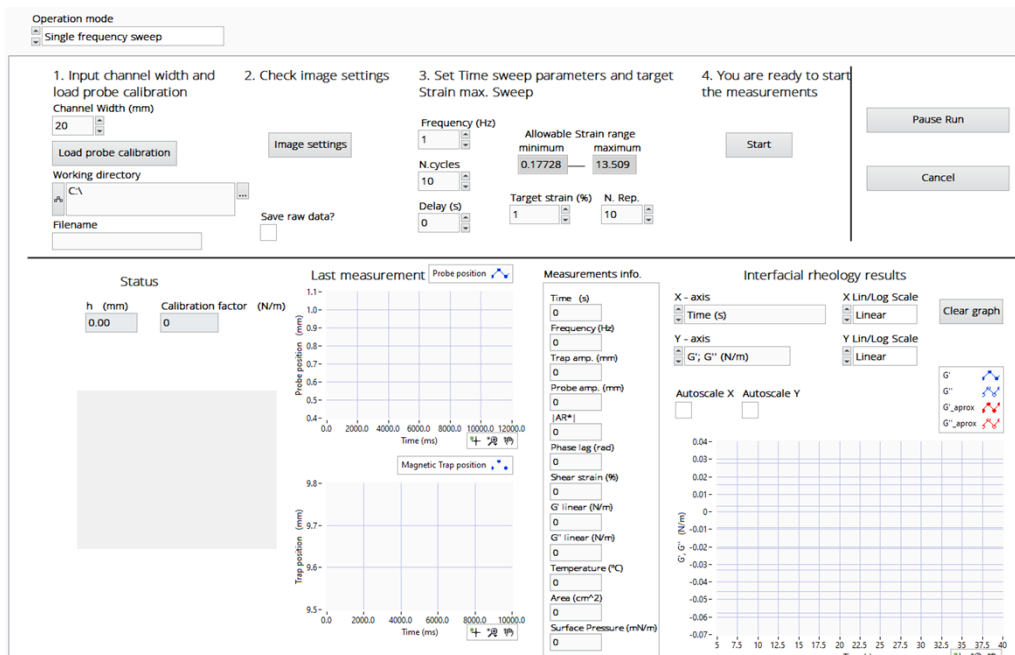
### 3.5.1 Single frequency sweep

Using this mode, the instrument will perform the measurement at single frequency while keeping the constant nominal interfacial strain. Single frequency measurement can be used to define the time dependency of viscoelastic properties. When combined with the ISR Flip high compression trough, the viscoelastic changes as a function of surface pressure can be defined.

In this operation mode, the height of the trap and the trap oscillation amplitude are automatically adjusted by the software to keep the amplitude of the probe oscillation constant within 5% of the selected target strain value. Long-time measurements may be performed by setting a constant nominal strain value and many repetitions.

Single frequency measurement is typically done either so that you compress the monolayer to the desired surface pressure and run the measurement, or you slowly compress the monolayer while measuring at the single frequency. The later one will give you a viscoelastic response as a function of surface pressure in a single measurement file. The former one requires you to construct the same graph from the individual measurement files after all the measurement points have been completed. Which one should be used, depends on the expected behavior of the monolayer.

## 1. Set the measurement parameters



The screenshot shows the Biolin Scientific software interface. At the top, the 'Operation mode' is set to 'Single frequency sweep'. Below this, there are four numbered steps: 1. Input channel width and load probe calibration (Channel Width: 20 mm, Load probe calibration button, Working directory: C:\, Filename: ), 2. Check image settings (Image settings button, Save raw data? checkbox), 3. Set Time sweep parameters and target Strain max. Sweep (Frequency: 1 Hz, N cycles: 10, Delay: 0 s, Allowable Strain range: 0.17728 to 13.509, Target strain: 1%, N. Rep.: 10), and 4. You are ready to start the measurements (Start button). To the right of these steps are 'Pause Run' and 'Cancel' buttons. Below the steps, there are three main sections: 'Status' (h: 0.00 mm, Calibration factor: 0 N/m), 'Last measurement' (Probe position: 0.9 mm, Time: 0.0 to 12000.0 ms, Magnetic Trap position: 9.5 to 9.8 mm), and 'Measurements info.' (Time: 0 s, Frequency: 1 Hz, Trap amp: 0 mm, Probe amp: 0 mm, [AR\*]: 0, Phase lag: 0 rad, Shear strain: 0%, G' linear: 0 N/m, G'' linear: 0 N/m, Temperature: 0 °C, Area: 0 cm², Surface Pressure: 0 mN/m). To the right of these sections is the 'Interfacial rheology results' section, which includes 'X-axis' (Time: 0 s, X Lin/Log Scale: Linear), 'Y-axis' (G', G'' (N/m), Y Lin/Log Scale: Linear), 'Autoscale X' and 'Autoscale Y' checkboxes, and a 'Clear graph' button. The main graph area shows a plot of G' and G'' (N/m) versus Time (s), with a legend indicating G' (blue line with circles), G'' (red line with circles), and G' approx (blue line with triangles), G'' approx (red line with triangles).

- **Frequency (Hz):** The frequency used
  - **N cycles:** Number of trap oscillation cycles that form the driving waveform
  - **Delay (s):** Delay between the ending oscillation train and the beginning of the next
  - **Allowable Strain range (%):** Minimum and maximum values of nominal strain that can be used
  - **Target strain (%):** Target nominal strain
  - **N. Rep.:** Number of repetitions of the constant nominal strain measurement. If you want to measure a long measurement, this should be set to a high value (max. 9999).
2. Click Start button. If the used filename already exists, the software will give you the option to overwrite it or give a new filename. Otherwise, the measurement is now started.
  3. Do the first measurement at the initial surface pressure. If you are going to compress as you measure the rheology, wait for the instrument to take rheology measurement before starting to compress. This way, you will get rheology data also at the initial conditions. Then start compressing.
  4. Clicking the Pause Run button will pause the procedure when the current frequency sweep ends. A pop-up window is shown, and it allows to change manually the trap height, the optical settings, to resume the calibration or abort it. Cancel button ends the whole experiment.
  5. It may happen that during the measurements the amplitude of the oscillation drives the probe's tip out of the RoI, then the pop-up window will show up to inform the user. Then, Click the OK button and the Pause menu will appear. Open ROI Settings. Adjust the camera

position if the probe is disappeared. Adjust lighting if needed and select new RoI. Click ok and continue the calibration by clicking Resume Calibration.

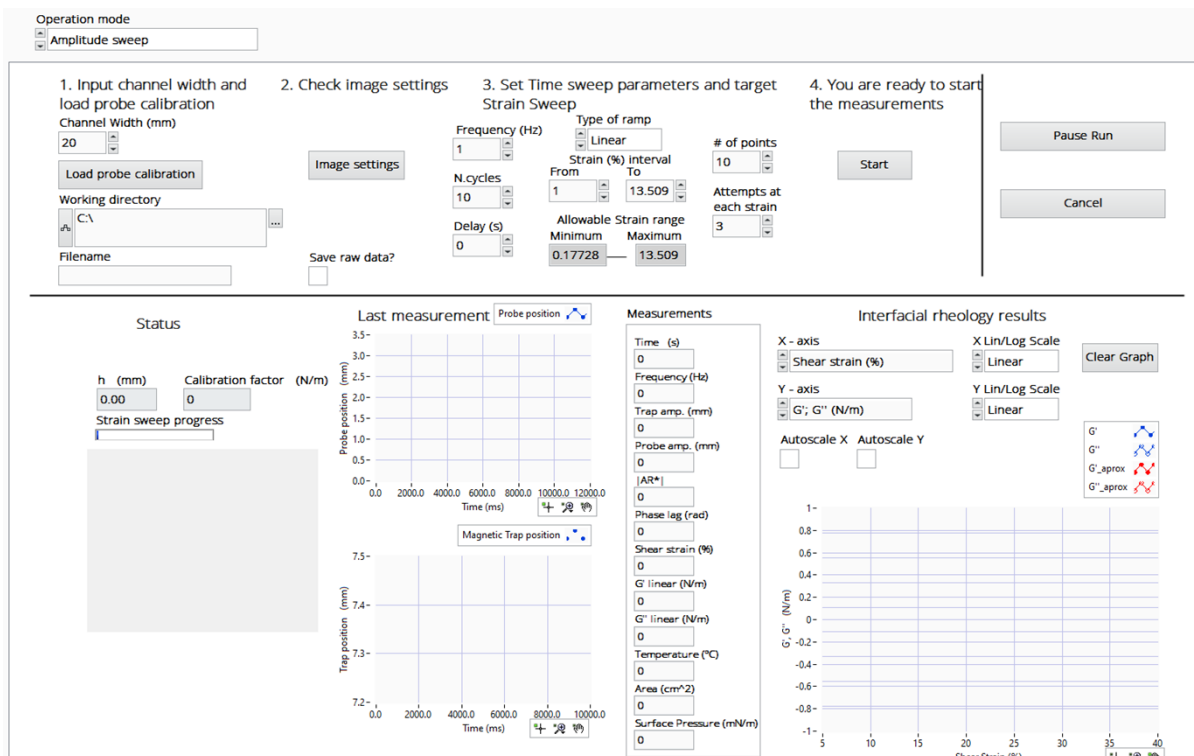
- Once the experiment is finished Experiment has finished window will appear

### 3.5.2 Amplitude sweep

Using this mode, the instrument can perform automatic nominal strain sweeps at constant oscillation frequency. The actual interfacial shear strain is equal to the nominal strain when the interfacial velocity profile is linear, i.e., when the Boussinesq number is  $Bq \gg 1$ . The nominal strain  $s$  is just the ratio between the probe oscillation amplitude,  $A_p$  and the half channel width,  $d/2$  i.e.  $\gamma = 2A/d$ .

This mode is typically used to find the linear regime for the viscoelasticity for example before running the constant frequency measurement at a constant nominal strain.

- Set the measurement parameters:



- Frequency (Hz):** The frequency used
- N cycles:** Number of trap oscillation cycles that form the driving waveform
- Delay (s):** Delay between the ending oscillation train and the beginning of the next
- Type of ramp:** Specify if the strain ramp is linear or logarithmic
- Strain interval (%):** Upper and lower values of the nominal strain that will define the strain ramp
- Allowable Strain range (%):** Two indicators display minimum and maximum values for nominal strain
- Target strain (%):** Target nominal strain

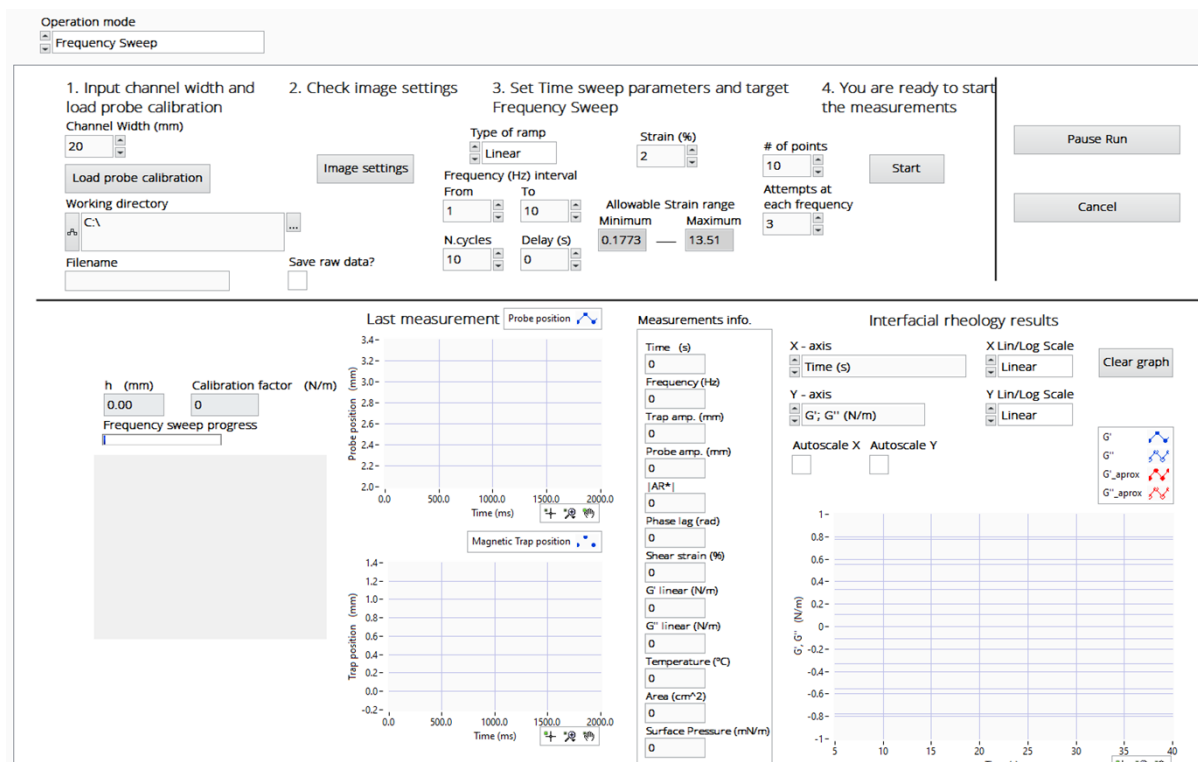
- **Number of points:** Number of different values of strain to be used in the ramp. If a linear ramp is selected, the measurement points are selected from a linear scale for example 10 points between the strain range specified. If a logarithmic scale is used, the points are selected at an even interval on a logarithmic scale.
  - **Attempts at each strain:** Maximum number of allowable attempts at each strain to fulfill the condition that the actual strain is within a 5 % of the target strain
2. Click Start button. If the used filename already exists, the software will give you the option to overwrite it or give a new filename. Otherwise, the measurement is now started.
  3. Do the first measurement at the initial surface pressure. If you are going to compress as you measure the rheology, wait for the instrument to take rheology measurement before starting to compress. This way, you will get rheology data also at the initial conditions. Then start compressing.
  4. Clicking the Pause Run button will pause the procedure when the current frequency sweep ends. A pop-up window is shown, and it allows to change manually the trap height, the optical settings, to resume the calibration or abort it. Cancel button ends the whole experiment.
  5. It may happen that during the measurements the amplitude of the oscillation drives the probe's tip out of the RoI, then the pop-up window will show up to inform the user. Then, Click the OK button and the Pause menu will appear. Open ROI Settings. Adjust the camera position if the probe is disappeared. Adjust lighting if needed and select new RoI. Click ok and continue the calibration by clicking Resume Calibration.
  6. Once the experiment is finished Experiment has finished window will appear

### 3.5.3 Frequency sweep

Using this mode the instrument can perform automatic frequency sweeps at constant nominal interfacial strain.

In this operation mode, the height of the trap and the trap oscillation amplitude are automatically adjusted by the instrument's software to keep constant the amplitude of the probe's oscillation within a 5% of the selected target strain value.

#### 1. Set the measurement parameters



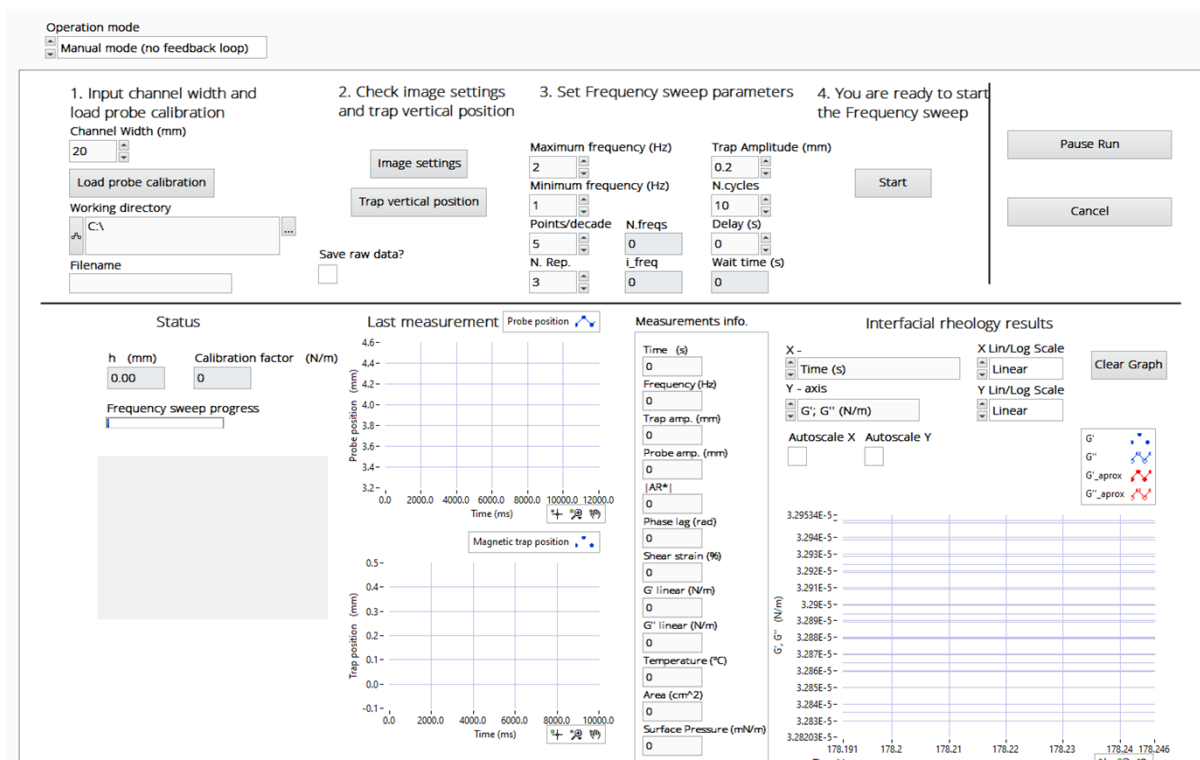
- **Type of ramp:** Specify if the strain ramp is linear or logarithmic
  - **Frequency interval (Hz):** Upper and lower values of the frequency that will define the frequency ramp
  - **Delay (s):** Delay between the ending oscillation train and the beginning of the next
  - **Strain (%):** Value of the nominal strain
  - **Allowable Strain range (%):** Two indicators display minimum and maximum values for nominal strain
  - **Target strain (%):** Target nominal strain
  - **Number of points:** Number of different values of strain to be used in the ramp
  - **Attempts at each strain:** Maximum number of allowable attempts at each strain to fulfil the condition that the actual strain is within a 5 % of the target strain
2. Click Start button. If the used filename already exists, the software will give you the option to overwrite it or give a new filename. Otherwise, the measurement is now started.
  3. Do the first measurement at the initial surface pressure. If you are going to compress as you measure the rheology, wait for the instrument to take rheology measurement before starting to compress. This way, you will get rheology data also at the initial conditions. Then start compressing.

- Clicking the Pause Run button will pause the procedure when the current frequency sweep ends. A pop-up window is shown, and it allows to change manually the trap height, the optical settings, to resume the calibration or abort it. Cancel button ends the whole experiment.
- It may happen that during the measurements the amplitude of the oscillation drives the probe's tip out of the RoI, then the pop-up window will show up to inform the user. Then, Click the OK button and the Pause menu will appear. Open ROI Settings. Adjust the camera position if the probe is disappeared. Adjust lighting if needed and select new RoI. Click ok and continue the calibration by clicking Resume Calibration.
- Once the experiment is finished Experiment has finished window will appear

### 3.5.4 Manual mode (no feedback loop)

Using this mode, the instrument can perform automatic frequency sweeps at a constant amplitude of the trap oscillation. In this mode height of the trap is not adjusted so the probe's amplitude might change over time. The height of the trap and the optical settings can be changed at any time after pausing the execution. A long-time constant frequency measurement can be performed by setting the same value at the high and low frequency limits and a large number of repetitions.

- Set the measurement parameters

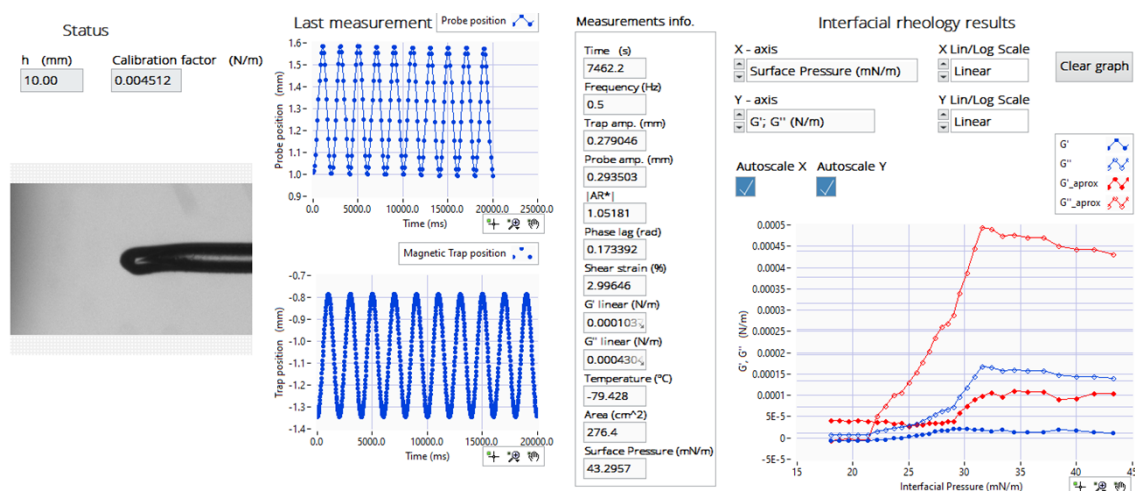




- **Maximum frequency (Hz):** The highest frequency used
  - **Minimum frequency (Hz):** The lowest frequency used
  - **Points/decade:** Number of different frequencies per each decade covered. Together with max and min frequencies determines the total number of different frequencies that will compose the sweep
  - **N. Rep.:** Number of repetitions of the N period oscillation train at each frequency. A long time constant frequency measurement can be performed by setting the same value at the high and low frequency limits and a large number of repetitions.
  - **N. freqs.:** Number of different frequencies that compose the sweep. Calculated from the max and min frequencies and the Points/decade selection.
  - **I\_freq:** Ordinal number of the current frequency
  - **Trap motion amplitude (mm):** Sets the amplitude of the Trap motion
  - **N cycles:** Sets the number of trap oscillation cycles that form the driving signal train
  - **Delay (s):** Delay between the ending oscillation train and the beginning of the next
  - **Wait time (s):** Total time span of the programmed measurements
2. Click Start button. If the used filename already exists, the software will give you the option to overwrite it or give a new filename. Otherwise, the measurement is now started.
  3. Do the first measurement at the initial surface pressure. If you are going to compress as you measure the rheology, wait for the instrument to take rheology measurement before starting to compress. This way, you will get rheology data also at the initial conditions. Then start compressing.
  4. Clicking the Pause Run button will pause the procedure when the current frequency sweep ends. A pop-up window is shown, and it allows to change manually the trap height, the optical settings, to resume the calibration or abort it. Cancel button ends the whole experiment.
  5. It may happen that during the measurements the amplitude of the oscillation drives the probe's tip out of the RoI, then the pop-up window will show up to inform the user. Then, Click the OK button and the Pause menu will appear. Open ROI Settings. Adjust the camera position if the probe is disappeared. Adjust lighting if needed and select new RoI. Click ok and continue the calibration by clicking Resume Calibration.
  6. Once the experiment is finished Experiment has finished window will appear

## 3.6 Real-time results and actions during measurements

During measurements, real-time results are drawn on the bottom part of the window. From the Status section, it is possible to see the current trap height and the corresponding trap constant. Below is a live image of the probe. The two central chart graphs at the Last measurement panel will display the evolution of the trap and probe tip position after the image analysis is finished.



Measurement info contains the following parameters:

- **Time (s):** Duration of the measurement
- **Frequency (Hz):** Value of the oscillation frequency
- **Trap amp. (mm):** Trap oscillation amplitude
- **Probe amp. (mm):** Amplitude of the probe oscillation
- **AR:** Modulus of the complex amplitude ratio
- **Phase (rad):** The argument of the complex amplitude ratio
- **Shear strain (%):** The nominal shear strain, considering a linear interfacial velocity gradient
- **G' linear (N/m):** Interfacial storage modulus at current frequency
- **G'' linear (N/m):** Interfacial loss modulus at the current frequency
- **Temperature (°C):** Temperature measured from the Langmuir trough
- **Area (cm<sup>2</sup>):** Trough area
- **Surface Pressure (mN/m):** Current surface pressure value

Results are drawn on the right side of the window. It is possible to change variables used in the axis and scale from the drop-down menu. The graph can be zoomed, and the user can change e.g. the color or fitting of the values by clicking the symbol presenting the variable.

G' and G'' are calculated using the nonlinear approximation and G'\_aprox and G''\_aprox calculated using the linear approximation

## 3.7 Cleaning

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

## 3.8 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.

## 4 Analysis of the data

There are different ways to perform the data analysis. All the data is stored as a .csv file and can be directly manipulated in Excel. The files that the instrument generates can be divided into three classes:

- Configuration files
- Calibration files
- Measurement data files

### 4.1 Data manipulation in Excel

#### 4.1.1 Configuration files

Configuration files are automatically placed by the instrument software in the following path:

Configuration files contain information about the device mounted in the system and some important physical parameters. These files should not be changed by the user.

ISR configuration file: contains the values of several global variables that are needed for system functioning. The name of the file is: ISR\_configuration.csv

The file contains several variables. These variables can be changed by the instrument's software during parameter adjustments using the Settings pull-down menu and sub-menus or during the execution of some Operation modes. Do not change the variable by directly editing the file.

An initial file of this kind will be supplied with the instrument. After a measurement/calibration session the instrument will update the values of the variable to those that were valid at the end of the measurement/calibration session. A typical ISR configuration file contains the following parameters, all the values are last valid values:

- **Exposure time (ms):** Exposure time of the camera
- **Gain:** Camera gain
- **Gamma:** Camera gamma parameter
- **Optics scale (pixels/mm):** Calibration constant of the optics.
- **RoI\_topleft\_X\_coord (pixel):** RoI's origin (at the top left corner) horizontal coordinate
- **RoI\_topleft\_Y\_coord (pixel):** RoI's origin (at the top left corner) vertical coordinate
- **RoI\_botleft\_X\_coord (pixel):** RoI's origin (at the bottom left corner) horizontal coordinate
- **RoI\_botleft\_Y\_coord (pixel):** RoI's origin (at the bottom left corner) vertical coordinate
- **Intensity Threshold value:** Intensity threshold used in probe's tip position measurement
- **Right/left probe's tip:** The parameter that selects which probe tip is being used for the position measurement.
- **Probe name:** Name of the probe
- **Probe length (mm):** Length of the probe
- **Probe radius (um):** Radius of the probe
- **Probe mass (mg):** Mass of the probe
- **Channel width (mm):** Total width of the channel
- **h (mm):** Trap height at which the last measurement/calibration was performed
- **k (N/m):** Trap constant at which the last measurement/calibration was performed
- **Maximum trap height (mm):** Maximum trap height admissible for the last probe used. This is the high height limit of the operation window of the last probe used.
- **Minimum trap height (mm):** Minimum trap height admissible for the last probe used.
- **Camera configuration:** Camera configuration 0 – below the trough 1 – above the trough
- **Fit coefficient:** Value of the logarithmic slope of the trap constant calibration curve for the trap/probe ensemble
- **Trap offset (mm):** The trap offset

- **At\_min (mm):** Lowest admissible trap oscillation amplitude; it is used in the constant strain operation modes.
- **At\_max (mm):** Highest admissible trap oscillation amplitude; it is used in the constant strain operation modes.
- **Directory for experiment files:**
- **Calibration path:**

Horizontal motion controller ID file: this file contains the ID number of the PI controller of the trap's horizontal motion. This number will never change unless the controller fails and must be replaced. The ID number should never be changed by direct editing of the file. The name of the file is: ISR\_PI\_SN.csv

#### 4.1.2 Calibration files

The corresponding calibration files are automatically placed by the instrument software in that folder. The calibration files contain information about the trap/probe ensembles that have been calibrated with the instrument. ATTENTION: Thifiles should be changed only by experienced users.

1. Probe parameters file: contains the physical parameters of the probe. The file name has a standard structure: "probe's name"\_"YYMMDD"\_"HHMM"\_params.csv where

A typical content of the file can be seen below.

2. Calculated drags file: contains the calculated values of the drags used to fit the resonance curve to the forced harmonic oscillator one. The file name has the standard structure:

"probe's name"\_"YYMMDD"\_"HHMM"\_drags.csv

The five columns contain, respectively, the real and imaginary parts of the subphase and superphase drags, and the frequency.

Frequency sweep files: a file containing the results of each frequency sweep performed at the calibration process. The structure of the file names is:

"probe's name"\_"YYMMDD"\_"HHMM"\_h"%p1f"\_"f"%p2f"\_"#"experiment number"\_cal.csv

where the terms between quotes have the usual meanings and the field following the h will indicate the height at which the frequency sweep was made. A typical content of the file can be seen in

The three columns contain, respectively, the values of the frequency, and the modulus and argument of the complex amplitude ratio obtained at that frequency.

Raw data files: if the Save raw data button has been selected, two files will appear for each frequency at each height. The file names will have the following structure:

"probe's name"\_"YYMMDD"\_"HHMM"\_h"%p1f"\_"f"%p2f"\_"#"experiment number"\_zprobe.csv

"probe's name"\_"YYMMDD"\_"HHMM"\_h"%p1f"\_"f"%p2f"\_"#"experiment number"\_ztrap.csv

where the terms between quotes have the usual meanings and the field following the f will indicate the frequency and the numbers following the # symbol will indicate the order in which the files were created. The files with extensions zprobe and ztrap contain, respectively, the data corresponding to the oscillations

5. A file containing the final result of the calibration process, i.e., two columns containing, respectively, the trap height and the trap constant at the corresponding height. The file name will have the following structure:

"probe's name"\_"YYMMDD"\_"HHMM"\_trap\_constant.csv

Fit coefficient file: contains the logarithmic slope of the trap constant calibration curve, which is needed for the constant strain Operation modes. The structure of the file name is:

"probe's name"\_"YYMMDD"\_"HHMM"\_fit\_coefficient.csv

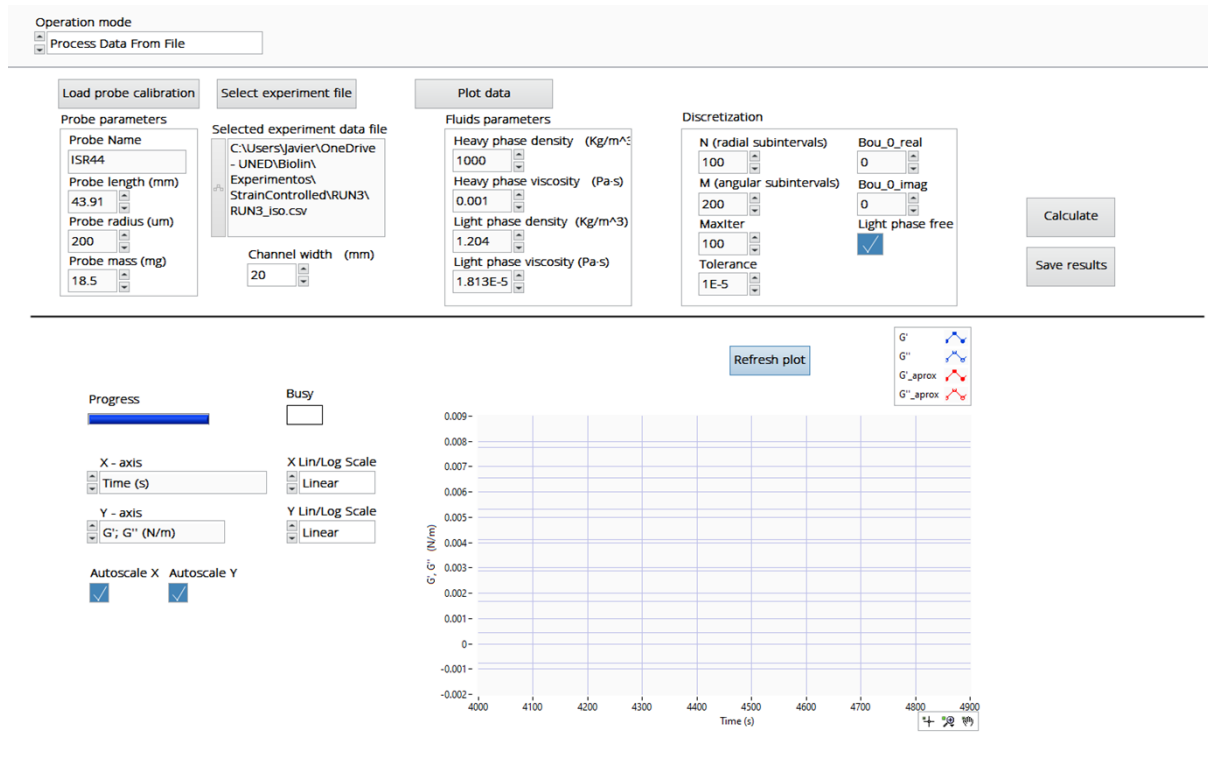
#### 4.1.3 Measurement data files

Measurement data is saved into the location defined in the measurements. A folder is created for each measurement. From these files it is possible and easy to draw e.g. graphs using Excel. If raw data is saved, it can be found from the RAW folder. Three different files are saved from each measurement:

1. "Experiment name".csv  
Contains data directly measured or calculated from the direct measured quantities. File contains the following information
  - **Time (s):**
  - **f (Hz):** Frequency of oscillation
  - **AR:** Modulus of the complex amplitude ratio
  - **Delta (rad):** Argument of the complex amplitude
  - **Shear strain (%):** Nominal shear strain calculated from the probe's motion amplitude
  - **G' linear (N/m):** Storage modulus according to the linear approximation
  - **G'' linear (N/m):** Loss modulus according to the linear approximation
  - **A (cm<sup>2</sup>):** Interfacial area calculated from data from the Langmuir trough
  - **P (mN/m):** Surface pressure
  - **T (°C)** Temperature, if temperature probe is used
  - **Probe amplitude (mm):** Probe's oscillation amplitude measured
  - **ktm (N/m):** Trap constant corresponding to the actual trap height
2. "ExperimentName\_Processed data files".csv
  - **Time (s):**
  - **f (Hz):** Frequency of oscillation
  - **G' (N/m):** Storage modulus according to the approximation
  - **G'' (N/m):** Loss modulus according to the approximation
  - **Delta (rad):** Argument of the complex amplitude
  - **A (cm<sup>2</sup>):** Interfacial area calculated from data from the Langmuir trough
  - **P (mN/m):** Surface pressure
  - **T (°C)** Temperature, if temperature probe is used
  - **Probe amplitude (mm):** Probe's oscillation amplitude measured
  - **ktm (N/m):** Trap constant corresponding to the actual trap height

## 4.2 Data analysis in KSV NIMA ISR Flip software

Process data from file operation mode.



**Operation mode**  
Process Data From File

**Load probe calibration**

**Probe parameters**

Probe Name: ISR44

Probe length (mm): 43.91

Probe radius (um): 200

Probe mass (mg): 18.5

**Select experiment file**

Selected experiment data file: C:\Users\Javier\OneDrive - UNED\Biolin\Experimentos\StrainControlled\RUN3\RUN3\_Iso.csv

Channel width (mm): 20

**Plot data**

**Fluids parameters**

Heavy phase density (Kg/m<sup>3</sup>): 1000

Heavy phase viscosity (Pa-s): 0.001

Light phase density (Kg/m<sup>3</sup>): 1.204

Light phase viscosity (Pa-s): 1.813E-5

**Discretization**

N (radial subintervals): 100

M (angular subintervals): 200

Maxiter: 100

Tolerance: 1E-5

Bou\_0\_real: 0

Bou\_0\_imag: 0

Light phase free: ☒

**Calculate**

**Save results**

**Refresh plot**

**Progress**

**Busy**

**X - axis**  
Time (s)

**Y - axis**  
G'; G'' (N/m)

**Autoscale X** ☒ **Autoscale Y** ☒

**X Lin/Log Scale**  
Linear

**Y Lin/Log Scale**  
Linear

**Plot area:** A graph showing G' and G'' (N/m) versus Time (s). The Y-axis ranges from -0.002 to 0.009, and the X-axis ranges from 4000 to 4900. The plot area is currently empty.

Measured data can be reanalyzed inside the software if needed. Reanalysis may be interesting in the following cases for example:

- Reanalyzing data that were processed with the wrong parameter setting
- Reanalyzing data with higher resolution meshes and/or lower tolerances for increased precision
- Reanalyzing data in case of the future improvements of the data analysis routines

In this operation mode, the instrument will automatically analyze, represent, and save the results of the new analysis.

1. Load the probe calibration for the probe used in the experiment.
2. Click select experiment file and select the measurement file that contains only the experiment name, (not for example the processed file)
3. Change fluid parameters and discretization parameters if needed.
4. Click Calculate data. Follow the progress from the progress bar.
5. Save the new processed results if you want.
6. BY right-clicking the image it is possible to e.g. copy it or export data.

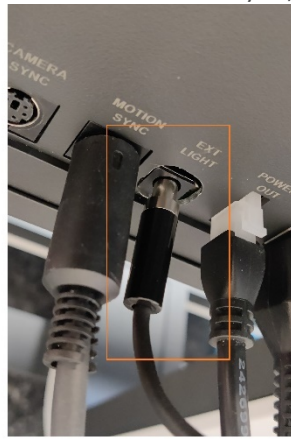
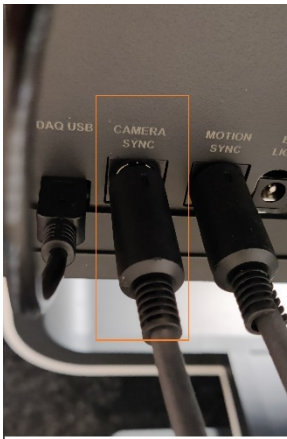
## 5 Changing the camera position

The ISR Flip is shipped mostly used with the camera at the bottom. However, if low volume cell is used, the camera needs to be moved to an upward position.

Instruction on how to change the camera from the below position to the above position is presented in this section.

The camera is adjusted in place with four screws. To move the camera, a screwdriver size 3 is needed.

1. Turn the instrument off. Disconnect the power cable.
2. Take off the camera and LED cables: camera sync, exit limit, one USB.

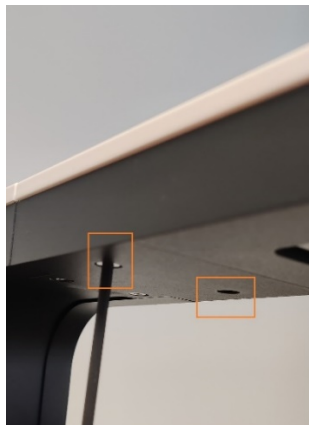


3. Open four screws, two on each side, that hold the camera in place.

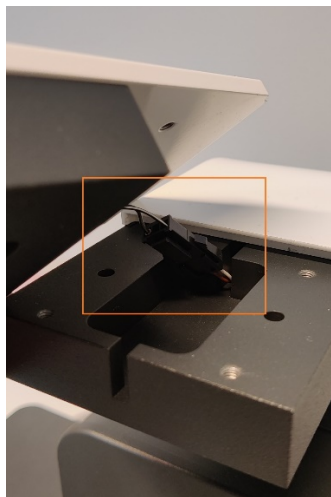




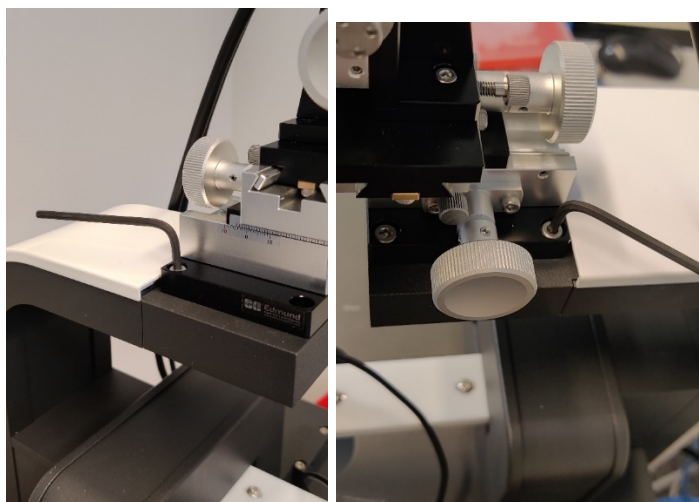
4. Open two screws underneath the light source



5. Detach the light cable.



6. Attach the camera with four screws to the point where the light was removed.



7. Attach cables and turn the instrument on.



## 6 Advanced settings

### 6.1 Probe calibration settings

#### 6.1.1 Probe calibration execution parameters

At the Settings pull-down menu select Probe calibration settings and Probe Calibration parameters. The pop-up menu window will appear. This window allows the user to Check/Setup the proper values for the parameters needed for the probe calibration execution. Remember that the probe calibration process involves measuring the probe's response by means of frequency sweeps at different trap heights.

Depending on used probe, maximum height it is important to set so that the needle such that the probe remains under the influence of the magnet. For example, with a small probe, a good maximum is about 20 mm. When choosing the altitude, it is good to note that after calibration, the trap can only be used at the range at which the calibration was performed.

**Calibration Parameters**

Height points to calibrate  
10

Maximum frequency (Hz)  
10

Minimum frequency (Hz)  
1

Frequency points per decade  
5

Repetitions  
1

Trap amplitude (mm)  
0.2

Number of periods  
20

Wait after motion (s)  
0

maximum height (mm)  
30

Close

The following parameters can be adjusted:

- **Height points to calibrate:** Number of different trap heights used for calibration measurements (must be  $\geq 2$ ).
- **Maximum frequency (Hz):** Value of the maximum frequency to be used (max value: 10 Hz).
- **Minimum frequency (Hz):** Value of the minimum frequency to be (min value Hz).
- **Frequency points per decade:** Number of different frequencies (per decade of frequency span)
- **Repetitions:** Number of times each frequency sweep must be repeated for averaging purposes
- **Trap amplitude (mm):** Trap movement amplitude. Value is kept constant during the calibration

- **Number of periods:** Number of periods composing the oscillatory motion of the trap
- **Wait after motion (s):** Delay time between consecutive oscillatory motions in second.
- **Maximum height (mm):** Maximum at which to perform a calibration (max value 30 mm).

### 6.1.2 Probe calibration FFBDA Parameters

This window allows the user to Check/set up the parameter values for the Flow Field-Based data analysis (FFBDA hereafter) numerical procedures that are used during the probe calibration measurements to transform the measured variables into values of the interfacial dynamic moduli or the complex interfacial viscosity. The pop-up window displays two separate tables. The upper table contains the physical parameters (density and viscosity) of the two bulk fluid phases that are in contact with the interface. The default values correspond to an air/water interface at 25 °C. The lower table comprises variables that are used in the flow field-based data analysis of the probe calibration measurements:

**Fluids parameters**

Heavy phase density (Kg/m <sup>3</sup> )	1000
Heavy phase viscosity (Pa·s)	0.001
Light phase density (Kg/m <sup>3</sup> )	1.204
Light phase viscosity (Pa·s)	1.813E-5

**Discretization**

# of points in freq.	50
N (radial subintervals)	60
M (angular subintervals)	120
Light phase free	<input checked="" type="checkbox"/>

Close

- **Number of points in frequency:** During the FFBDA of the measured data, some drag values can be calculated in advance and just once. Such procedure requires to define a number of points in a given frequency range, that are subsequently used to extract a functional  $\eta_t$  to the drag response that is later used in the conversion of the measured data to rheological variable values. (Default value: 50).
- **Number of radial subintervals:** Number of subintervals in the radial coordinate of the mesh used to numerically solve Navier-Stokes equations; increasing the value increases the precision but makes calculation last longer. (Default value: 100).

- **Number of angular subintervals:** Number of subintervals in the angular coordinate of the mesh used to solve Navier-Stokes equations; increasing the value increases the precision but makes calculation last longer. (Default value: 50).
- **Light phase free:** Selects if the upper end of the lighter bulk phase is open to air or closed by an upper rigid wall. This affects the boundary condition at the upper end of the bulk phase placed above the interface (default value: checked). Uncheck the indicator only if a rigid wall is in contact with the upper end of the bulk phase placed above the interface.

## 6.2 Measurement FFBDA parameters

At the Settings pull-down menu select Probe calibration settings and Measurements FFBDA parameters. The pop-up window will appear. This window allows the user to Check/setup the parameter values for the Flow Field-Based data analysis (FFBDA hereafter) numerical procedures that are used during the interfacial rheology measurements to transform the measured variables into values of the interfacial dynamic moduli or the complex interfacial viscosity.

The pop-up window displays two separate tables. The upper table contains the physical parameters (density and viscosity) of the two bulk fluid phases that are in contact with the interface. The default values correspond to air/water interfaces at 25 C. The lower table comprises variables that are used in the flow field-based data analysis of the probe calibration measurements. Here a detailed definition of all of the variables and some subtleties regarding how to fill in the tables are provided:

1. Read the liquid subphase temperature from the measurement of the Langmuir trough electronics.
2. Find the values of the density (in kg/m<sup>3</sup>) and the viscosity (in Pa\*s) of the two bulk fluid phases at the experiment's temperature.
3. Input the corresponding values for the upper and lower bulk fluid properties at that temperature. (Default values correspond to an air/water interface at T = 25 C).
4. ONLY FOR EXPERT USERS: Check/Input the parameters for the numerical calculations. The list of parameters, with their suggested/default values, is:
  - Number of points in the Radial coordinate: Number of points in the mesh used to numerically solve Navier-Stokes equations; increasing the value increases the precision but makes calculation last longer. (Default value:).
  - Number of points in the azimuthal coordinate: Number of points in the mesh used to solve Navier-Stokes equations; increasing the value increases the precision but makes calculation last longer. (Default value:).
  - Tolerance: Maximal discrepancy accepted between the experimental and calculated values of the amplitude ratio; decreasing the value increases the precision but makes calculation last longer. (De-fault value: ).
  - Maximal number of iterations: Value at which the iterative scheme is stopped if no convergence is found; increasing the value might make the calculation last longer. (Default value:).
5. Click the Close button

## 7 Technical specifications

<b>Frequency range (Hz)</b>	0.01-10
<b>Camera</b>	USB3 digital camera with zoom.
<b>Recommended system requirements</b>	Windows 10 (32 or 64 bit). One free USB3 port. 2,4 GHz processor, 8 GB RAM, 512 GB hard disk drive 1920x1080 screen resolution.
<b>Experiments available</b>	single frequency sweep, frequency sweep, amplitude sweep, and manual mode
<b>Dynamic moduli range</b>	$2 \cdot 10^{-8}$ -1
<b>Strain range (%)</b>	0.04-20*
	* The strain range is dependent on the channel width. For low volume the range is 0.08 - 40
<b>Instrument dimensions (LxWxH,mm)</b>	400 x 280 x 600
<b>Weight (kg)</b>	21
<b>Voltage and current</b>	
- <b>Input voltage range</b>	100 – 240 Vac
- <b>Frequency</b>	50 – 60 Hz
- <b>Input current</b>	210 – 380 mA
- <b>Fuses</b>	T1.0A 5x20 mm
<b>Environmental conditions</b>	
- <b>Ambient temperature</b>	15-30 °C
- <b>Ambient pressure</b>	700 to 1060 hPa
- <b>Ambient humidity</b>	20-80% (non-condensing)

## 8 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](mailto:info@biolinscientific.com)**

## 9 EC Declaration of Conformity

### EU DECLARATION OF CONFORMITY

We,

**Biolin Scientific Oy, Tietäjäsentie 2, Espoo, Finland**

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

**KSV NIMA ISR Flip**

**KSV NIMA ISR Flip High Compression**

**KSV NIMA ISR Flip Low Volume**

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/A1:2019**

**EN 61326-1:2013**

Espoo, Finland

16.05.2022



Sten Brandt

Supply Chain Director

Biolin Scientific Oy