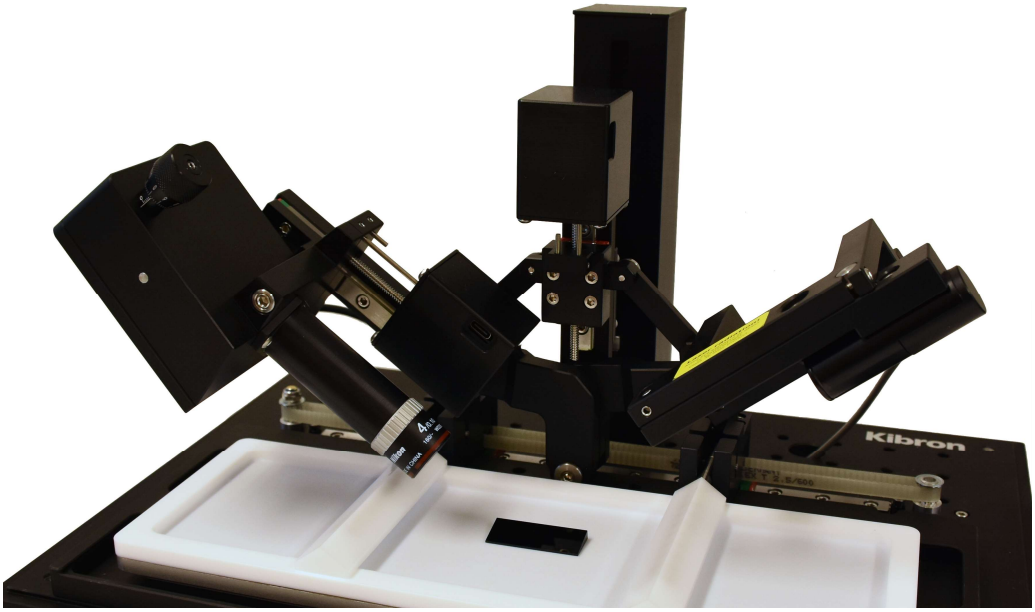
Kibron

G-BAM

User´s Guide



Kibron G-BAM
Brewster angle microscope
User´s Guide

October 3rd, 2022
Revision E

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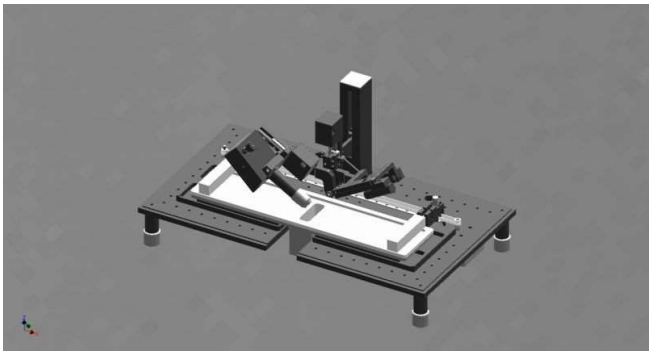
Preface

Congratulations for your purchase of Kibron's G-BAM. This manual will acquaint you with the basic features of the control software and hardware. Should you have any questions or other queries related to this product, please contact Kibron by phone or e-mail:



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Document Conventions

This manual uses the following typographic conventions:

Example	Description
	This icon alerts the user to the presence of important operating and maintenance (servicing) instructions
	This icon indicates a warning or caution.

Revision History

Revision	Date	Changes
A	09/2021	First release
B	12/2021	Update following software version BAMwareX 1.1
C	05/2022	Manual update for BAMwareX 1.2. The software update is fairly large and significantly improves fps count. This has consequences for many functionalities in the software.
D	09/2022	Manual update for BAMwareX 1.22. The software is update with "Dynamic flat field correction" background. The update function has been changed so that it only affects height tracking reference image.
E	10/2022	Update for BAMwareX 1.23. Number of background subtraction methods has been reduced.

CONTENTS

IMPORTANT WARNINGS!

It is important that you take good care of your Brewster angle microscope. The image in Brewster angle microscopy is based on very small changes in reflected light intensity. Any misalignment or deposits on the lenses will compromise image quality. The components of the microscope have been pre-aligned at Kibron's premises and any further adjustments should be considered carefully. Warranty does not cover misalignment, and we do charge a fee for realigning. The technique is extremely sensitive to the angle of incidence; an error of 0.2° in the alignment of the laser beam results in an increase in the background intensity comparable to a surfactant monolayer! Dust on the components in the optical path of the laser result in diffraction patterns and reduces the quality of your micrographs. Keep the instrument clean and store it in a dust free location.

Microscope placement and laser safety

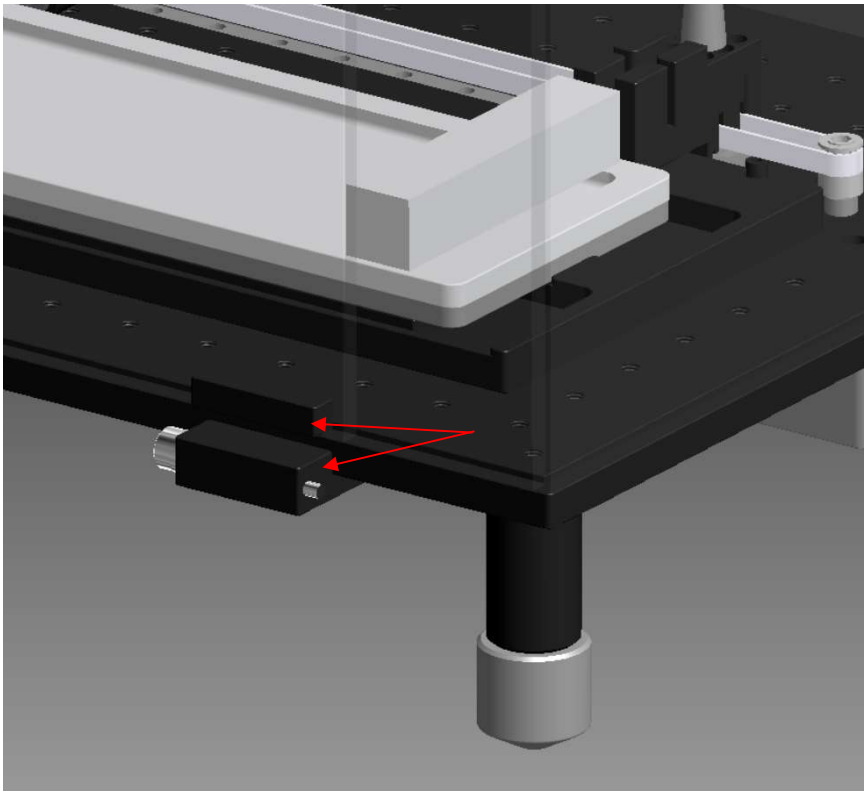


Figure. Interlock mounted on a MTX G2. The interlock consist of a proximity switch (lower part) and a magnet (upper part), which control the power to the laser.

The BAM contains a class 3B laser which can damage the eyes even after brief exposure. It is mandatory to place the microscope so that laser safety and regulations are guaranteed.

1. Use an enclosure for the microscope that prevents the beam from escaping in any direction. Use an interlock that cuts the power to the laser if the enclosure is opened. One interlock is provided with the microscope.

or

2. Use the dark dust cover provided with the Microtrough G baseplate. The baseplate must be equipped with an interlock to cut the power if the hatch is opened.

The microscope must be used so that so that the laser beam, including specular reflection, is confined at all times. Place the microscope in an enclosure with an interlock, for example the interlock supplied with the instrument.

If you need to setup your own interlock, the power to the laser should be cut between the laser in the instrument and the laser control unit (USB) using a simple circuit switch.

Always use a dark wedge to absorb the beam.

Integrated safety mechanisms

1. The microscope is delivered with an interlock. Always use it. Do not bypass it.

2. The laser is automatically shut down after 10 s, if the communication to the computer is broken, for example in the event of a disconnected cable or software failure.

 AVOID AND PREVENT DIRECT EYE EXPOSURE! The BAM must be placed in an enclosure equipped with an interlock!

Parts and installation of the Brewster angle microscope

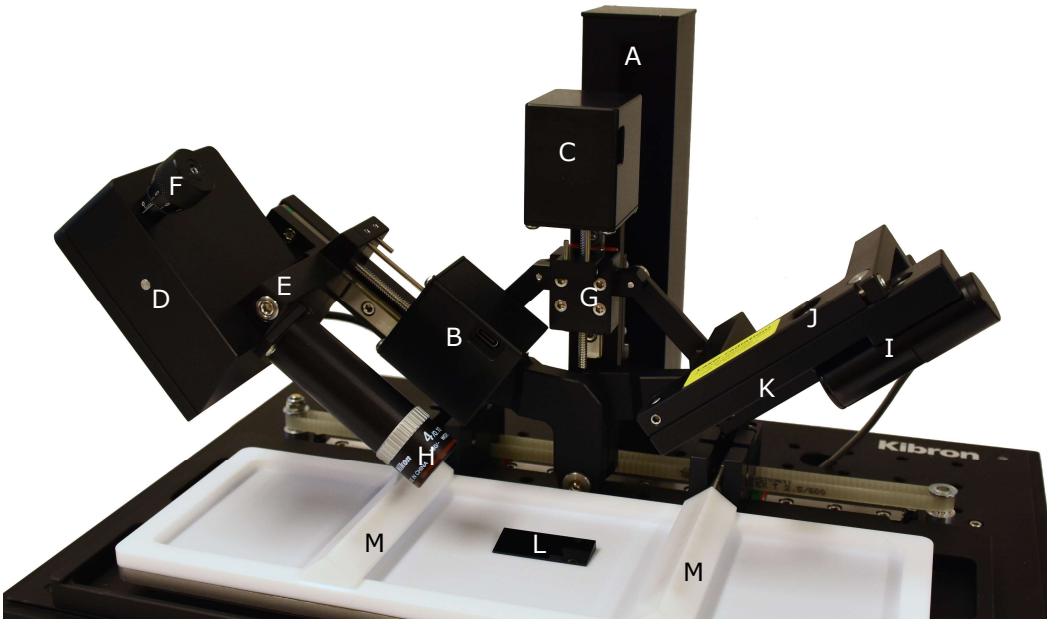


Figure. Most important parts of the brewster angle microscope.

A) Height motor and lift
B) Focus motor
C) Angle motor for goniometer
D) Camera
E) Polarizer, allen screws for turning and locking polarizer
F) Camera tilt knob
G) Goniometer, ca. 40-60 degrees.
H) Lens, default 4X finite conjugate, 0.10 NA, RMS thread.
I) Laser
J) Horisontal alignement screws for laser
K) Vertical alignment screws for laser
L) Dark glass wedge for beam absorption and deflection
M) Wedged barriers to allow for maximum compression without interfering with the beam.
N) (Not shown) Screw knob below the baseplate for attaching the microscope to the trough.

Installation

Firstly, less vibration and draft translates directly into image quality, so take care to choose a pieceful location and a very stable table. A heavy stone table or actively vibration damped table is ideal, but you can set up the BAM on an ordinary laboratory benchtop. If the table is shaking too much you will see it in the image. We strongly recommend that the instrument is on a separate table from any other work, including the keyboard and mouse of the computer.

Remove packing material around the microscope. Grab the microscope lift and position the microscope onto the baseplate. The microscope should be placed behind the barrier track of the Microtrough, as shown in in the figure below. On the G1, G2 or G4, make sure you place the microscope so that so that the barrier carriages can move freely behind the camera and laser. On the G1 and G2 the lens will be close to the midline of the trough. Alternatively, you can place the BAM at the end of the trough and work in one barrier mode (not available on the G1). Firmly fix the microscope with the screw knob.

You can also fix the microscope in other places on the baseplate, or on other supports, as long as the focus point is sufficiently far from the edge of the trough, so that any curvature of the subphase meniscus due to the edge is negligible and the liquid surface is horizontal. In practice, this corresponds to a distance of at least 10 mm.

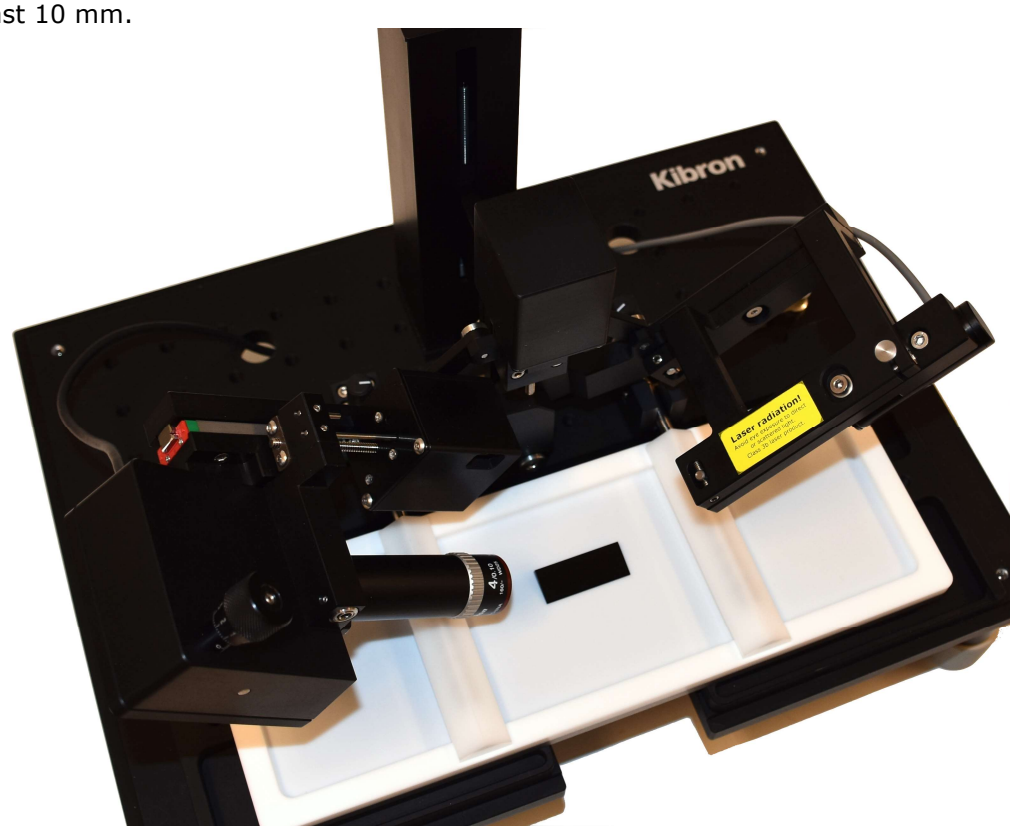


Figure. Placement of microscope on baseplate of a G2 Langmuir instrument.

Thread the short USB cables provided through the cable holes on the baseplate and connect to the respective motors and the USB hub using the short USB cables with 90 degree USB-C connectors. Connect the USB hub to the PC. In Windows Device Manger the peripherals should now appear under 'Ports' as 'USB Serial Device (COM *n*)'. Most modern computers can provide enough current

through USB3 ports to run all motors and the laser on an unpowered USB hub. If running all three motors at the same time fails, you may have to get a USB hub with a separate power supply. Make sure the power supply can provide at least 500 mA per port. This means at least 2000 mA in total.

 Make sure that the cables have a bit of slack so that, so that the motors can move freely. If the cables are too tight you will hear a knocking sound when the motor skips steps. If this occurs you must initialize the motors again to get an accurate position reading.

Thread the camera cable through one cable hole on the baseplate and connect it to the computer. There is a lot of data transfer from the camera to the PC, so we recommend connecting the camera to a separate USB3 port, rather than the USB hub. The software must be installed for the camera to appear in the device manager under Imaging devices, see Installing software. If you connect the camera to a hub with the laser and motors, the camera will operate in USB2 mode, and consequently the frame rate will suffer significantly.

 In case of problems, please make sure that the laser, 3 motors and camera are shown in the Device Manager. The camera is shown under imaging devices and requires the software to be installed first. Make sure the camera is indicated as a USB3 device.

Levelling the instrument when the BAM is attached on the baseplate

(This section does not apply to stand-alone BAM model). The baseplate of the MTX G*x* should be as close to horizontal as possible. We recommend using a high precision spirit level to adjust the baseplate feet. Place the spirit level directly on the baseplate for best accuracy. Adjust both horizontal directions.

Installing software

The software can be acquired in different ways:

1: On the Kibron memory stick provided with the microscope
- look for the 'Kibron BAM' directory and **BAMWareX_*ver*_inst.exe.**

2. Via a 'DropBox', 'WeTransfer' or similar
This is often distributed as a 'zip' file - just right-hand click on the zip file and use the Windows extractor to unzip it. Look for **BAMWareX_*ver*_inst.exe** in the unpacked directory.

Run **BAMWareX_*ver*_inst.exe** and follow the instructions on the screen. When prompted, also install the drivers for the camera.

You must be running FilmWareX 4.3 or later for communication from the trough, please see 'Connection to FilmWareX' later in this manual.

Storage of the microscope

Store the microscope in a dust free environment, for example mounted on the baseplate under the dust cover. It is very important that the microscope is kept clean!
For long term storage we recommend extra pre-cautions. You may use plastic bags or pieces of take to prevent dust from collecting on the lenses of the objective and laser, and then placing the microscope in a box.

Cleaning of the optics

The preferred method to remove dust from the optics is by using compressed air. Make sure that the air is oil free/solvent free. Cleaning with compressed air should be sufficient for almost all situations.

In case you need more thorough cleaning, acquaint yourself with cleaning of optics. You can study the guides on the websites of optics retailers (Edmund Optics, Thorlabs, Newport, etc.).

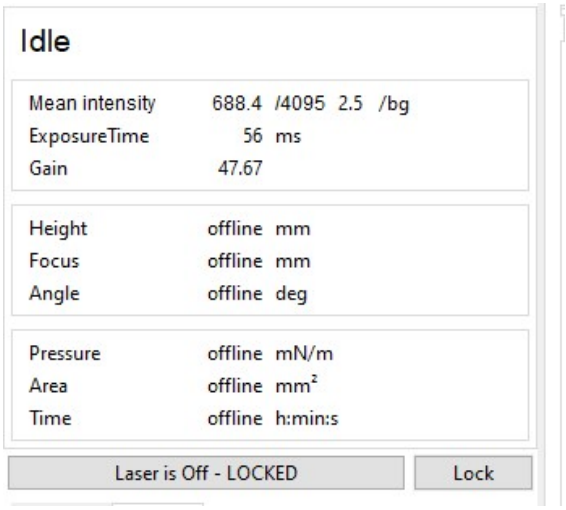
 Cleaning with detergents or solvents is not without risk and may leave permanent stains. You can also contact Kibron for a clean up / renewal of the optical components.

Structures and functions of the software

Here, we give a presentation of the functions available in the software. There is a step-by-step guide for measurements later on in the manual.

Default values for most fields can be restored by **double clicking on the field**. Remember to click 'Apply' to commit the value.

Status display



The 'Status display' is located at the upper left corner of the screen. It contains information on the current state of the microscope, with the exception of sampling and video recording, which are located at the status bar at the bottom of the screen.

The 'offline' text indicates that the peripheral is not present, communication is not setup, or a part is broken. Make sure all peripherals are connected, and then restart the software. You may also attempt the 'Connect peripherals' functionality found in the 'Tools'-menu. This searches for the camera and motors. If key components related to the microscope are offline, the software may become unstable. It is not mandatory to run the trough control software, FilmWareX, and the 'Pressure', 'Area' and 'Height' fields only affect sampling functions.

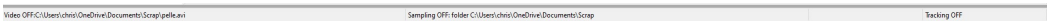
The 'Mean intensity'-field shows the mean intensity of the last frame both for the pixel ensemble on a scale 0-4095, and a second value referred to the light intensity of background frame. The first value is important when setting correct camera parameters, while the latter is a measure of the film reflectivity, and hence a helpful measure of the film density during an experiment.

'Exposure time' and 'Gain' show the current settings of the camera. Exposure time can be set between 10 ms and 6000 ms. The gain range is 1-50. Notice that the exposure time affects the frame rate of the camera. It should be set to provide enough light for the measurement, but as short as possible for high fps.

'Height', 'Focus' and 'Angle' show the current positions of the motors.

'Pressure', 'Area' and 'Time' show the main experimental parameters obtained from FilmWareX. Setting up of the connection is described later on in this manual. You may change between 'Pressure' and 'Tension', as well as 'Area' and 'Area/molecule' by clicking on the labels.

The status bar on the bottom of the screen contains information on video sampling. The ON/OFF text indicates whether the function is activated and is followed by the respective folder / file name.



The third field of the sdtatusbar shows an estimate of the optimal camera tilt for a fully focused image plane, calculated based on the lens data and goniometer angle. Whenever you change the goniometer angle, you should check and change the camera angle dial on the BAM accordingly. The camera tilt should be set so that the image is in focus throughout. The 'cam angle dial' will give a good guess for an overall focused image. If incorrectly set, the image may be in focus only along a horizontal slice, and blurry above and below the slice. When adjusting, you may have to fine tune height (or focus) as well. A good approach is to keep the mid-section of the image in focus and then tuning the knob.

The fourth field shows the state of the height tracking algorithm. When activated the field shows the current offset from the height where the background was recorded and the correlation strength. It is normal for the correlation to decrease with time due to evaporation and especially changes in film coverage. These processes affect the fine structure in the laser pattern used for tracking. When the signal strength drops to the 20-30% range, the tracking reference image (background) should be updated with 'Update' on the camera tab, if possible. Always ensure that the height is correctly set before looking either at either the laser pattern or the focus of the image.

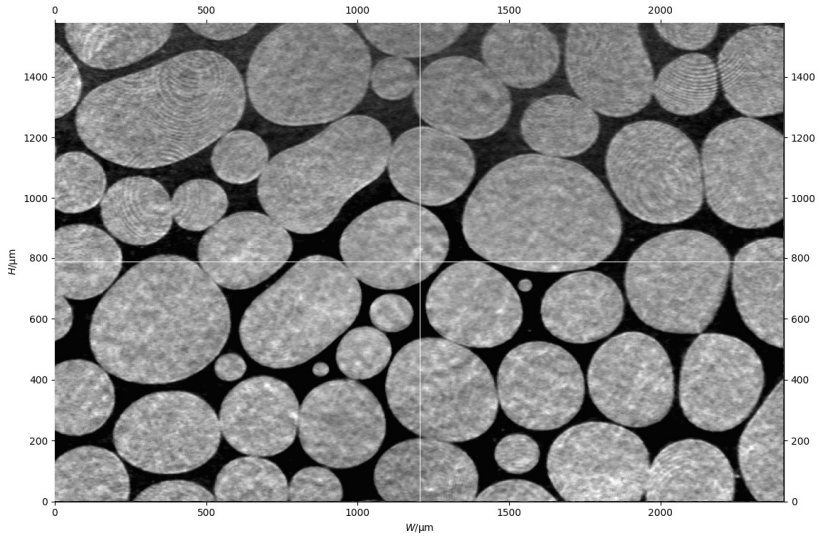
Laser on/off and lock

The laser on/off button is located just below the status panel. The laser is locked to prevent unauthorized and accidental activation of the laser. The pass key is 'R1332C'. The user must actively start the laser before performing any steps requiring the laser. The button is red when the laser is on, and grey otherwise. Turn off the laser after the experiment. Lock the laser whenever you work with the instrument and there is any risk of the laser causing injury.

 The laser is not turned on automatically to avoid any risks of injury! Make sure it is safe to turn on the laser. Also consider the reflected beam. Make sure the instrument placement ensures safe working in the lab throughout experiments. Aim the reflected beam towards an object in the vicinity, for example back wall of the instrument. You may for example attach a black background or paint the dust cover to absorb the reflected beam.

 The user must take care that local regulations are followed and necessary safety equipment is present. This may require you to use a darker dust cover. You may for example paint the original dust cover.

Image panel



The image panel contains the current view of the microscope and some controls. There is currently an extra tab which in the next release will have a short user guide. Select the **Live video** tab.

The image can be zoomed by holding the right mouse button and dragging the mouse or sliding two fingers in opposite directions on a mouse pad.

Hold the left mouse button and drag in the desired direction to pan.

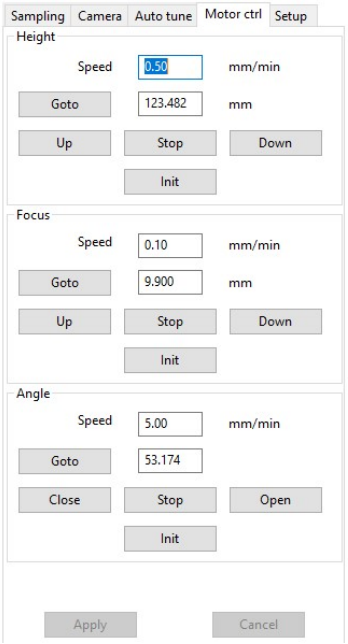
Left double click restores the default zoom level and pan position.

A right double click on the image shows or hides the crosshair located at the middle of the camera sensor. The crosshair is a convenient tool for adjusting the height of the microscope. It is also convenient when performing alignments.
If the cross is shown it also appears in saved images and video, so remember to hide the cross when it is not needed for aligning.

Motor ctrl tab

The motor control tab provides functions to move the motors individually. The 'Init' functions run the respective motor to its datum position. This is an important step to ensure that the positions of the motors are correct. You should use 'Init' if you suspect that the positions are incorrect or if you notice that a motor has skipped steps, for example when an object is preventing motion. Otherwise, the positions are stored in the onboard memory and do not need resetting (There may be minute errors in the saved positions due to mismatch between the real position and magnetic equilibrium positions, if a motor is disconnected).

Remove the dust cover when initiating the height motor!



The most common operations on the setup tab is adjustment of height and focus. Use decreasing speeds to target in on the correct position, and slow speeds, <<1 mm/min, to fine tune.

Please be aware that the speed in the angle motor is in the unit of mm/min of the carriage on the linear track, and not angular speed. Constant angular speeds are computationally more demanding, and we rather use the resources for image processing.

Autotune tab

The autotune tab contains functions for detecting the surface and setting the height, as well as, finding Brewster's angle. These functions are intended as tools for automatically setting up the BAM for a measurement. They are intended to help you get started quickly and easily.

There is also a possibility to save a position, so that you can access it quickly if you need it later.

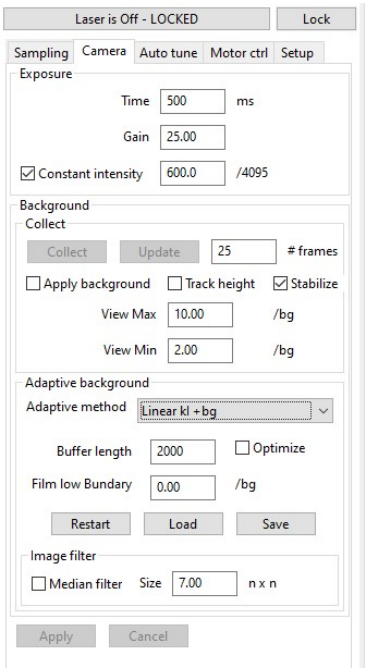
Firstly, 'Goto ideal focus' will move the camera to the correct focus position for the selected lens. This is a preset value, which should give a focused image when the height has been set correctly. We recommend that the focus is kept in this position at all times, and if there is a need to focus, height adjustments should be considered first.

Before searching for the Brewster angle you must align the height of the microscope with the liquid level, so that the center of the laser pattern aligns with the horizontal line of the cross-hair, see Motor ctrl tab. Check the angular search range and click on 'Find Angle'. Large scan ranges take a good while, so we recommend keeping the range small. If your microscope is well tuned and has not been moved, a search over ±0.5 degrees off the anticipated Brewster angle of the subphase should be sufficient, but you may want to decrease the range to ±0.15 degrees if you know the angle well. The software will scan through the search range while adjusting gain and exposure time. Wait for the 'Idle' status to return. The gain and exposure time found during the search will be set after the search has completed.

If everything is perfectly in tune, the sample should now be in focus. Otherwise, you may need to fine tune. We recommend primarily using the height adjustment for aligning the center of the laser pattern with the middle of the image. Use the crosshair for this (right double click on the image). Then if the image is not in focus, fine tune with the focus motor, and possibly with the camera angle tilt knob to obtain an overall focused motor. Be aware that in order to verify the focus you need some features on the surface to focus on. You cannot focus on the laser pattern – it is only a reflection.

The obtained Brewster angler depends on the tilt of the baseplate. You can calibrate the goniometer angle on the setup tab, provided that you know the Brewster angle of your sample. Do not do this, unless you are sure that everything is well aligned and that the subphase you use is clean.

The Camera tab



The 'gain and exposure time' control the amount of light collected before readout of the camera cell. They must be adjusted so that the light intensity is sufficient, while avoiding saturation of the pixels of the camera (bright white). For a clean subphase surface, it is good to obtain an intensity of 200-600 on a scale from 0-4095.

There are two parameters to adjust the intensity, namely 'Gain' and exposure 'Time'. You can find them on the 'Camera'-tab on. The effect of each parameter is nearly linear, so the intensity remains constant when doubling exposure time while halving the gain. There are trade-offs between both parameters. Large gain increases noise. On the other hand, if the objects on the surface are moving, extended exposure times cannot capture a good image. We recommend keeping the gain high (50) and controlling intensity primarily by setting the exposure time.

It may be necessary to change the Gain and Exposure time settings during an experiment as a film develops. This is especially true for optically dense, i.e. highly reflective films.

The 'Constant intensity' function adjusts the intensity whenever it deviates by more than 50 units from the setpoint. We recommend using the constant intensity function in experiments, as it reduces the risk that pixels in the image are saturated when the film density increases. A good default value is 300-600. The 'Constant intensity' option is automatically turned off when searching for height and Brewster's angle, so you do not need to remember this. However, turn it off for any operations where you need to follow the follow the light intensity. A pragmatic approach is to keep the constant intensity setting in the 300-600 range all the time.

Background

In the beginning of an experiment a background frame of the pure subphase should be recorded, before applying a surface film. The background frame is used to compensate for spatial variations in the laser beam intensity. It is also used to set a reference image for the height tracking algorithm.

'Collect' collects several frames and computes the median image, and stores this as the background image. It sets the background intensity level, to give a reference for quantitative analysis of a film and should be used in the beginning of an experiment on clean subphase surfaces only. If you use 'Collect' after applying the film, you will lose the background intensity reference for the clean surface. Use Gain and Exposure time or 'Constant intensity' to set the mean intensity. We recommend an intensity value of ca. 300-1500 for recording the background frame. Ideally, there should not be completely white (4095) or black (0) pixels.
Click 'Collect' to record the background. Once the background has been collected mark the 'Apply background' checkbox. The image should now be rather smooth with an intensity distributed close to unity.

The 'Update function collects and computes the median of a number of frames. It then stores a reference image for height tracking algorithms only. The purpose of the 'Update' function is to aid height tracking algorithm when the coverage has changed. Make sure that the microscope is at the right height when you do this, by verifying that the laser pattern is aligned with the horizontal line of the crosshair or that the image is in focus.

 Both 'Collect' and 'Update' are based on a collecting a median image. If there are any contaminations or film patches present they must be moving so that their effect is averaged in the median image. In other words, every pixel in the median image should be representative of the long term median.
Neither method is suited for stagnant films, and should be used only on clean subphase surfaces, or on moving films where time averages and medians are applicable.

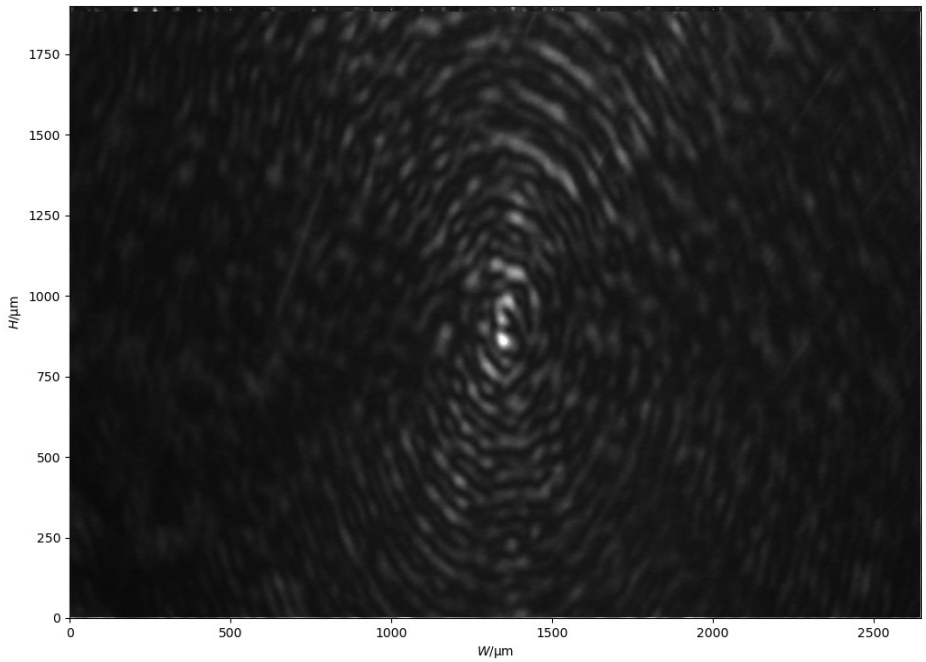


Figure. Image of the background with the birefringence ring center in the middle of the image.

The 'Track height' checkbox activates the height controller. This is reacting slowly to changes in height by running the height motor in the corresponding direction. The 'Stabilize' checkbox activates a digital stabilization algorithm, which reacts to changes in height on a shorter timescale, by offsetting the image digitally. An important difference is that 'Track height' moves the microscope so that the focus distance is maintained, while 'Stabilize' does not prevent the sample from moving out of focus.

The background images are automatically stored in the configuration file. In case the software crashes or you must restart for some reason, the last recorded background frame is loaded. You can also save and load background and fitting data for each background correction method.

Once the background has been set by checking 'Apply background', the image values are expressed relative to the background intensity. You can use 'View max' and 'View min' to set these. A value of 1 corresponds to the background intensity level. It may be convenient to set 'View Min' to 1 or 1.5 to hide non-idealities in the background.

Selecting background type

There are two different options for background correction. These are both flat field corrections, but differ in the how frames for the corrections are estimated. automatically optimizing the background. If 'Optimize' is checked, the image stream will be analyzed and the background and linearity of each pixel adjusted. All background optimization types are based on the assumption that the time average or running median over an interval should be equal for all pixels. Thus, these methods are only applicable to moving films. The averaged pixel values are then used to separate foreground and background from the image stream images.

The state of the correction depends on history, much like a diffusion process. If the movement of the film stops or is too slow, bright areas will gradually fade while dark areas will become brighter.

 The background method and optimization options affect the achievable framerate. For higher framerates, turn off optimization.

The **'Dynamic flat field correction'** is the preferred method for most cases and the most stable algorithm. It corrects the image by

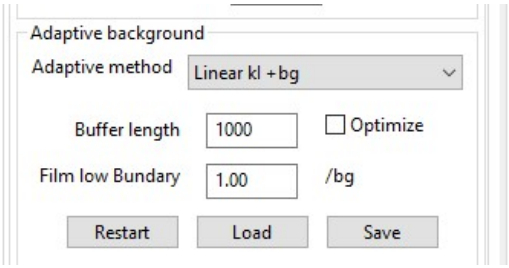
$$C = \frac{R - D}{F - D} \times m$$

Where *C* is the corrected image, *R* is the raw image, *D* is the background (darkframe), *F* is the flat field (white field) image accounting for different sensitivity at each pixel, and *m* is a magnification factor scaling the image intensity to the background intensity. If 'Optimize' is checked, *F* and *D* are continuously estimated from the image stream. The buffer length corresponds to a time constant relating to the update rates of *F* and *D.*
The high and low percentile affect the fractions used to estimate *D* and *F* are estimated from the image stream. Ideally, the ratios should correspond to film coverage, but the algorithm is fairly insensitive to changes in the values, so crude estimates can be used.
The 'Use low percentile as background' uses the dark frame *D* to estimate the background intensity of the bare subphase surface. This is convenient if the background has not been recorded from a clean surface. Quantitative analysis of intensity should be done with caution, as the intensity of *D* may not correspond to the bare subphase.
If the film layer becomes stagnant you need to turn off the optimization or increase the buffer length time constant. Otherwise, the background image will incorrectly approach the flat field image.

'Linear *k*I +bg*' – This method corrects the frame by:

$$C = \left[\frac{R - D}{k} + D\right] \times m$$

Where *D* is the background (dark frame), *k* is a pixel specific intensity response slope and *m* is a scaling factor relating the mean intensity to the background intensity. Only *k* is optimized when checked. Since *D* is not optimized, the method is sensitive to impurities during recording of the background, as well as changes in the height relative to the subphase surface. It should be used with the 'Stabilization' and 'Track height' activated, or in conditions where the surface level is very stable. 'Film low Boundary' is a threshold that must be reached for a given pixel to be used for optimization of *k*.



'None' – The corrected image is calculated simply through:

$$C = R/D$$

No adaptive method is applied, so *D* always corresponds to the recorded background.

Each method has an **'Optimize'** checkbox. If checked the algorithms strive to optimize the image correction of each pixel. All these methods use the 'Buffer length' as an argument for controlling the influence of one image frame on the ensemble. It is important that this parameter is set correctly. A too large number makes the filters slow or unresponsive. For slowly moving films, the buffer length needs to be large, otherwise the contrast in the image will gradually fade, and "shadows" created behind a moving film edge. The user must set the parameter during measurement, and switch between methods as the experiment progresses.

The **'Restart'** button first sets the buffer length to a short value and then gradually increases it back to the value set by the user. This provides a means to temporarily increase the responsiveness of the adaptive method. This is useful if an obvious fallacy has developed in the background frame or if the user wants to increase the speed of adaptation.

Backgrounds can also be **saved** and **loaded**. The files are specific to the background type.

'Image filter' – Applies a spatial median filter on the image with the given kernel size. A spatial median filter is suited for removing "salt and pepper" noise, while preserving edges.

Setting up sampling scheme

On the **'Sampling tab'** you can find various ways of storing your image data. Please be aware that the images take a lot of space on your computer. You must always make sure that you have sufficient disk space for your images.
Images can be saved with or without the axes, while video is always recorded without to reduce the frame size.

Choose the folder to save the files in using **'Save to'**. The image file names are automatically generated from the area, pressure and time data. Take care to save in as correctly named folder so that you can keep track of your data.

The image scale can be reduced to save disk space with the **'Scale'** choice menu.

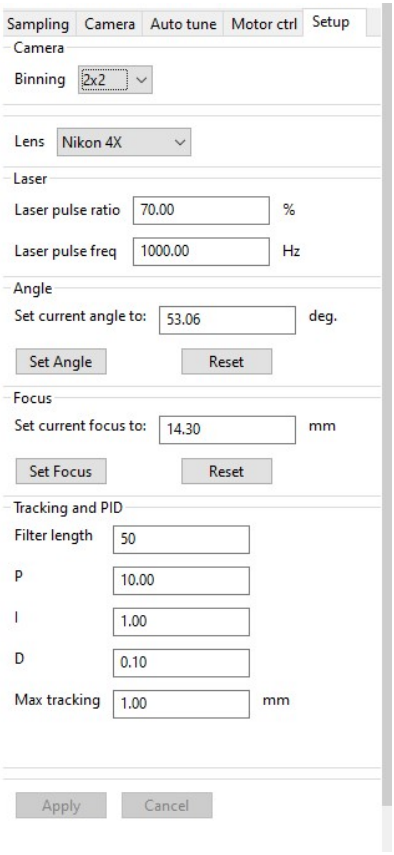
Image saving can be manually triggered at any time by clicking on the **'Snap'** button, independently of other sampling regimes. You can also setup an automatic saving protocol. Select the data type(s) and interval(s) you want to use for triggering of automatic saving. It is good to select these already at the start of the experiment. Then click **'Start'** to engage the saving protocol.

Video recording is started by first selecting the video file name, and then clicking 'Record'. There is no checking if the file exists when you click record, so take care not to overwrite previous recording which you want to store. You can also **'Annotate'** the videos with the surface pressure, area and/or time. These data are integrated into the image by changing the pixel color. Saving videos takes a lot of space, so make sure that you remove unnecessary videos. The videos can be recorded at constant fps, or at variable fps only if a new frame is available with the 'Only updated' option. For the latter the fps setting serves as an upper limit and the video does not reproduce real time when played back.

The **ON/Off** indicators on the bottom status bar show the state of the sampling.

The Setup tab

The setup tab provides the user with settings and parameters which affect the performance of the instrument. Most of the time you do not need to change these settings – the default settings should work well.

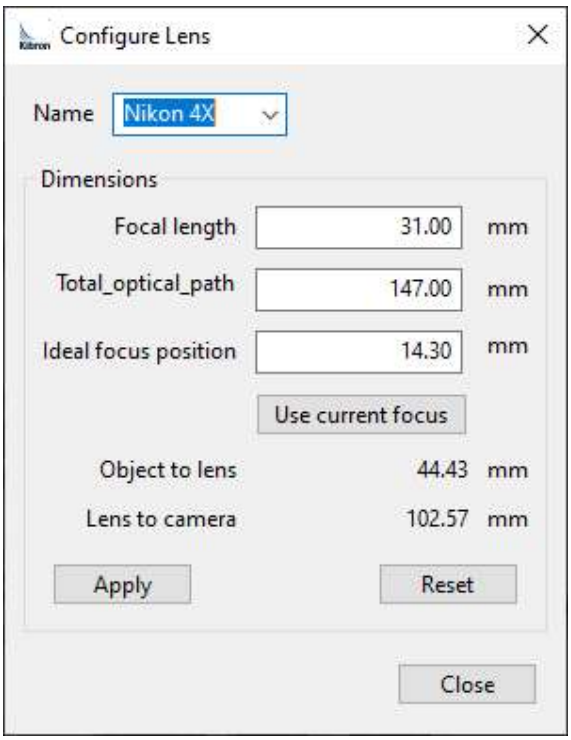


With the **'Binning'** option it is possible to choose 1x1 binning or 2x2 binning. The latter provides less resolution, but increased frame rate and smaller file sizes since there is less data handled.

The default 'Lens' delivered with the microscope is a Nikon 4X finite objective with a numerical aperture of 0.10. If you use another objective, you have to select it from the drop down menu. Please refer to 'Editing lens information' for setting your own parameters.

One important setting is the 'Set Angle' functionality. It can be used to calibrate the goniometer angle. For example, once you have fine tuned the microscope exactly at the Brewster angle, you can tare the angle so that it matches the theoretical Brewster angle of the liquid you have just measured.

Editing lens information



You can edit lens information under the Tools menu. While we at this stage provide only the Nikon 4X objective with the BAM, this functionality is here to be able to configure the microscope with other objectives. Choose the lens you want to view or edit from the drop down menu. We recommend that you do not alter the pre-existing lenses, but rather edit new ones. These have names starting with an '_' (underscore). Edit the name and remove the '_'. Otherwise, the new lens is not shown on the setup tab. Enter the focal length for the objective. You can usually obtain this from the vendor or manufacturer.

The total optical path is the distance between the camera cell and the sample surface measured along the optical axis. You can use the 'Use current focus' to calculate the optical path, if the axis of the goniometer, i.e. height is on the sample plane, and the sample is in focus. The ideal focus position is used as a guide to help adjusting the BAM when starting an experiment. It should correspond to a focused image when the sample height is correctly set. The parameters here are also to calculate the axes scales and **ideal camera tilt angle** estimate. You may need to fine tune the values to account for the finite thickness of the objective lens.

There are some practical limits on the objectives that can be used. These result from the angular range of the camera and goniometer. Typically, focal lengths should be in the 15 to 40 mm range (4X-10X objectives) and should have an RMS thread. Shorter focal lengths cannot be used without the objective touching the liquid surface. Higher magnification (shorter focal length) also require higher tilt of the camera cell, resulting in a loss of light intensity.

Connection to FilmWareX

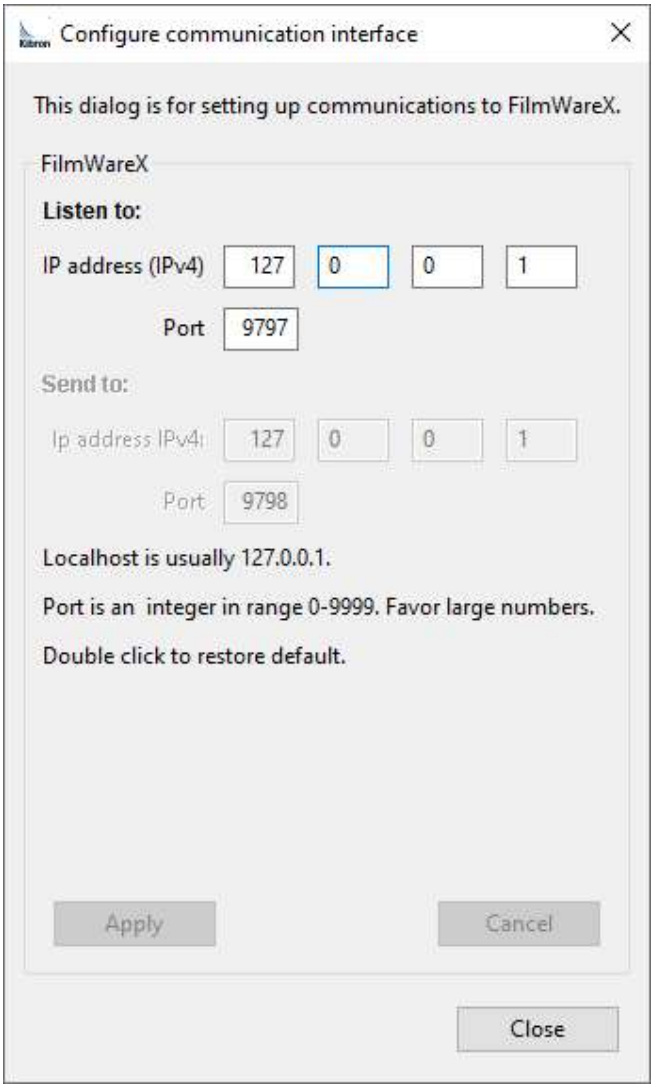


Fig. Setup of connection to to FilmWareX in BAMWareX.

The BAM software can be setup to receive data from the trough control software, FilmWareX 4.3 or later. This is a simple data transfer of current area, experiment time, and pressure. The data exchange between the trough FilmWareX, and BAM software is handled through a UDP/IP connection. You can thus run both software on the same computer, or on different computers connected to the network.

When both software run on the same computer, you can use the localhost address '127.0.0.1' for the connection. You may also have to select different ports if the suggested ports are in use already. It is good practice to choose large port numbers, e.g. between 9000 and 10000. In the BAM software you only need to specify the port to which the software listens, while in FilmWareX you need to specify the port to which data is sent in Tools => Connection to BAM:

Fig. Setup of connection to BAM in FilmWareX.

When running on the same local area network, you need to setup your firewall correctly to allow for incoming/outgoing traffic, for example by allowing inbound and outbound traffic for FilmWareX*nn*.exe and BAMWareX*nn*.exe in 'Windows Defender Firewall with Advanced Security'.
Furthermore, you need to set the address and port to which the BAM software is listening. There are various tools to find your local area IP address, you can for example run ipconfig from the Windows command prompt. Then look for the IPv4 address.
In principle, the softwares can communicate when running on separate local area networks, however, it is more complicated to configure as you must also set your router firewall and port forwarding. Please contact your IT department for help.

Guide to a measurement on liquid surfaces

The microscope and software forms a quite detailed entity which takes time to learn. Here, we try to give a stepwise guide on how to do a measurement. Please refer to previous sections for detailed information on the available functions

Many of these steps can be skipped if already set!

1. **Setting up the Langmuir experiment**
   Clean the trough thoroughly and place it on the baseplate. The correct cleaning procedure depends on the chemicals you use. If you are not sure, we recommend rinsing the trough thoroughly with water, let the water flow off, fill the trough with ethanol and let it soak for a few minutes. Then rinse the trough with water. Let the water flow off and put the trough on the baseplate. Remove any residual droplets with an aspirator equipped with a clean tip.

   Fill the trough with subphase. You can reduce the amount of dust in the compression area by first compressing the barriers fully, then fill the subphase on the outside of the barriers to normal level, let the trough sit for a while and relax the barriers fully. You may need to use an aspirator to remove residues if your subphase is impure.

2. **Place the wedge in the trough**
   Place the beam reflector-absorber wedge at the bottom of the trough using a clean tweezer. The wedge should be placed with the beam incident on the glossy side. The laser beam should be deflected/absorbed so that light reflected or diffracted from the bottom does not enter the objective and compromise image quality.
   The slight heating of the wedge at the laser spot causes a liquid flow. In shallow troughs this may translate into a significant surface flow. While it is difficult prevent, you can change the direction of the flow by turning the wedge around the vertical axis.

3. Then turn on the laser
   The password for the laser is ‘R1332C’.

 Make sure the beam is safely reflected and confined. An open laser beam can cause eye injury. Turn off the laser before performing any work on the trough.

4. **Aligning the height of the BAM**
   Adjust the height using the controls on the Motor Ctrl tab so that the center spot of the laser pattern is as close to the middle of the image as possible. Use the crosshair (right double click on the graph) for aiming and aligning the center spot with the horizontal line of the crosshair.

5. **Setting the angle**
   If you have already the correct angle for your subphase you can skip this step. If it is the first time you setup, or you have moved your instrument, make sure the Brewster angle is within the search range on the

'**Autotune'** tab. Click on **'Find angle'** and wait for the 'Idle' text to return. When the search has finished, check, and readjust the camera tilt if necessary. If you always measure on subphases with similar composition, and the baseplate is at the same position, there readjusting the goniometer angle should not be necessary.

Even small deviations below 0.1 degrees affect the image quality negatively. It is not recommended to rely solely on setting the angle to the theoretical Brewster angle using the motor controls. Likewise, if you have moved your instrument it is good to check that the goniometer is a at an optimal angle.

6. **Focus on the surface**

   If you have already used your microscope it may be unnecessary to tune the focus again.
   Your microscope has been setup so that when the center of the laser pattern is in the middle of the screen the correct focus position should be close to the preset value. Move the lens to the correct distance by clicking on 'Goto ideal focus' on the Autotune tab. You may have to fine tune using the height or focus motors.
   Focusing on the surface is a somewhat ambiguous task. If the surface is perfectly clean without any dust or visible film, then there is nothing to focus on. In this case you may have to set the position of the focus motor as close as possible and continue with the next step.
   However, if you can spot small dust particles moving on the surface. Move the focus position slowly and try to get the targets as small as possible without diffraction rings.
   You may also have to adjust the camera tilt angle. It should be set so that the complete image is in focus. If it is incorrectly set the only a narrow slab will be in focus.

7. **Collect background**
   Adjust the 'Gain' and 'Exposure time' or 'use the Constant intensity' on the 'Camera' tab so that the mean intensity is ca. 300 to 600. Then click on 'Collect'. Finally, click on 'Apply background' You may want to uncheck the constant intensity option once you have found good parameters and an interesting state of your film.

8. **Select the background correction method and check the 'Optimize' checkbox**
   We recommend the dynamic flat field correction.

9. **Set the 'Track height' and 'Stabilize' checkboxes.**
   It may be necessary to update the background after spreading the film using the **'Update'** option, or as the density of the film changes with compression. Make sure that the image is in focus and height set correctly before updating!

10. **Spread the film**
    You may now spread the film on the sample surface. If it is your first experiment with the BAM we recommend testing for example cholesterol (1 mg/ml in chloroform). Cholesterol tends to form both continuous film with holes and islands, so there is a lot to view.
    Stearic acid (1 mg/ml in chloroform) is also a good option. Stearic acid

forms large, dense domains and is thus very well visible. a very good option for your first experiments. Add 10-20 µl to a G1 or G2 trough. Another suitable system is a 0.5 mg/ml cholesterol + 0.5 mg/ml palmitic acid in chloroform.

11. **Set up the sampling scheme**
    Once the film is spread, set up your sampling scheme.

12. **Start the experiment in FilmWareX**
    Then you may start your experiment in FilmWareX, for example a compression isotherm. We recommend slow compression speeds (<20 mm/min).

13. **Update the tracking reference image**
    It may be necessary to update the reference background image for the tracking algorithm with increasing coverage. We recommend doing this if the tracking correlation falls below 30 %. Make sure the image is in focus and then click 'Update' on the camera tab.

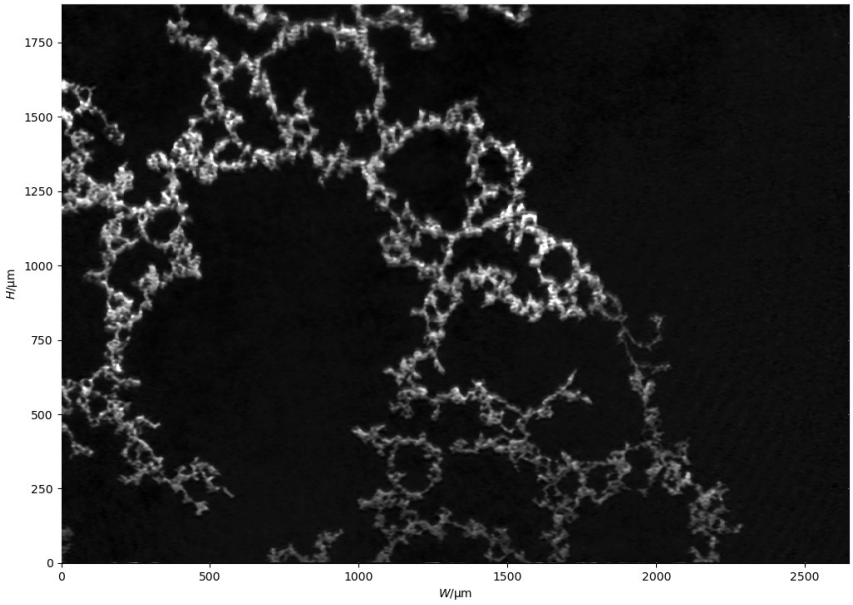


Figure. Stearic acid on water subphase.

Imaging of solid samples

The Brewster angle microscope has been developed for aqueous subphases. However, it can also be used for imaging of flat and smooth solid samples with their Brewster angles withing the goniometer range. Imaging of solid samples is more challenging, since, especially for samples with parallel front and back surfaces, there is a strong interference pattern from the reflections of the laser light.

Due to the strong interference pattern from reflection on the bottom surface, you need to have a means of adsorbing/preventing the reflection from the back surface. You can attempt to paint the back surface black.

Another alternative is to attach a prism or wedge to the back surface, using a liquid with high boiling point and a refractive index matched to the substrate, for example, glycerol (n=1.47) is well matched to PMMA (n=1.49). Place a small drop on the prism and press it against the back surface.

Additionally, the background collection and adaptation methods are based on a moving background, so you need to move the sample in the horizontal plane. You can for this purpose place the sample on the barriers and control the barrier movement from FilmWareX. If you use both barriers, you can lock the barriers to move in the same direction and place them far enough apart so that the goniometer does not prevent them from moving.

First, set the height of the microscope manually following the same principles as for liquid surfaces.

Currently, the angular autotune function has been optimized for liquid subphases, and is slow for searching for Brewster's angle at other substrates. If you know the refractive index of your material, you can calculate a good estimate and then fine tune the angle using the motor controls. You may also have to adjust the laser intensity on the 'Setup' tab.

Adjust the focus on a small detail on the surface.

Record the background from an uncoated part of the substrate or a clean substrate surface. For best results, the substrate should move slowly (<10 mm/min) in the horizontal plane so that the any artefacts can be removed from the background image.

Switch to your coated sample and continue with imaging following the same principles.

Known bugs:

1. The software does not fully close when exited, with the last message being 'Except'. Just close the terminal window.

2. The color indicator for the Track offset field in the bottom status bar, spreads out to all fields. This happens particularly when switching between BAMwareX and other software.

3. The software crashes when using the 'Dynamic flat field background'. The reason is unknown for the moment, but it is linked to updating gain and or time. We believe that turning the 'Constant intensity' option off may help preventing the crash.

Appendix I. Aligning the laser

The instrument is prealigned when manufactured. There should not be a need to realign without a known cause. This procedure should be done only if the alignment has been lost. This procedure should be done only by persons with sufficient safety training! Take care that all steps are performed meticulously.

1. Make sure the MTX baseplate (or microscope stand) is horizontal. For this purpose you can use a spirit level and the liquid in the trough.
2. Place a solid sample with well-defined feature under the microscope. The sample can for example be a glass slide with a calibration patterns.
3. Illuminate the sample with a bright light. Do not turn on the laser yet.
4. Run the focus motor to the ideal focus position.
5. Run the microscope to the correct height corresponding to exactly the axis of the goniometer and focus at the feature. Close and open the goniometer and make sure the feature stays in the same spot. The ends of the range may be unreliable, so use a range between 47 and 57 degrees. Use the crosshair to your advantage (double right click on the image). The location is relative to the image size – NOT the grid lines or tick marks – look only at a feature at the mid horizontal line. If the feature is moving the height is incorrect. If the sample moves **down** when **closing** the goniometer, move the microscope **down**. Usually increments around 0.2 mm are good to start with, and then fine tune.
6. Once the microscope has been set at the correct height and the feature is well focused, you need to adjust the focus motor position. For the Nikon 4X finite objective, the focus position should be 14.3 mm. Start by resetting the focus motor offset on the Setup tab.
   If the deviation is large (> 0.5 mm) tune using the trigger bar on the focus motor. Move bar down if value is too small and up if it is too large. Then do init and focus again.
   If the deviation is small, you can set the position on the Setup tab to 14.3 mm. This value is written in the EEPROM of the motor.
7. Next you need to aim the laser beam at the feature so that the feature is in the middle of the laser beam.
8. Set the laser intensity to 2 %. Turn on the laser
9. Loosen the locking screw a little bit of the vertical adjustment first. There should be a bit of friction to maintain accuracy in the adjustment.
10. Use the adjustment screws to bring the center of the beam on the level with the feature. This is done by looking at the beam on the sample – not with the microscope.
11. The adjustment screws should clamp the arm from both sides.
12. Repeat for the horizontal adjustment.
13. Finally, fine tune the alignment with water in so that the laser diffraction pattern center is in the middle of the image, when the focus is at the ideal focus position (adjust using height motor). You must be able to see some monolayer or contaminants in the trough for this step.
14. Tighten the locking screws and make sure the adjustment does not move.
15. Set the laser intensity back to 100 %.

The laser should now be aligned. You may adjust 'Ideal focus position' of the lens to correspond to the new alignment. Proceed to test the beam on a clean subphase surface. There should not be speckle spots from the laser hitting "non-optical" surfaces like metal.

Appendix II. Calibrating the goniometer angle and polarizer

This procedure is usually done at manufacture and should not be altered without careful consideration. This procedure should be done only by persons with sufficient safety training!

Coarse tuning:

1. Get an exact angle support, for example a ruler with a 30 or 45 degree angle. An angle of 53 degrees is ideal, as both 45 and 30 (60 degrees from the normal) degree angles are close to the extreme positions of the goniometer.
2. Run the goniometer to the corresponding angle
3. Place the ruler under laser arm of the goniometer. Make sure that you place it under the arm and not the laser support which may be at a different angle. Make sure that the angle is well aligned with the arm.
4. Adjust the angle of the goniometer using the open and close buttons until it matches the support angle.
5. On the setup tab, set the angle (measured from the norma) so that it corresponds to the angle used for the adjustments.

Fine tuning:

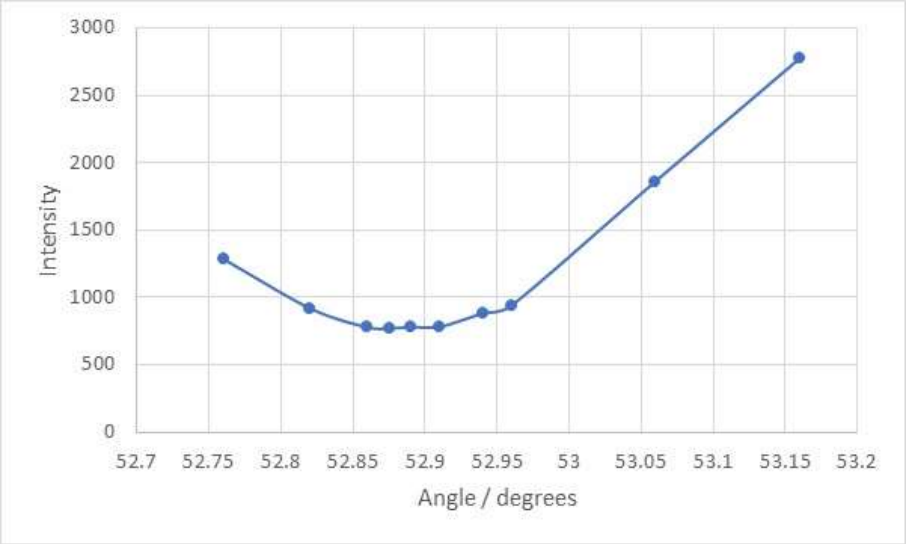


Figure. This fine tuning procedure was started at 53.06 degrees. Then the angle was adjusted until the minimum was found at 52.875 degrees.

6. Prepare a clean trough with a clean water surface. Make sure everything is clean, as even small amounts of impurities can drastically affect your results.
7. Run the goniometer to 53.06.
8. Fine tune height so that the center of the laser is exactly on the horizontal cross hair. You will need to check the height after every test angle below.
9. Loosen the polarizer lock screw.

10. Set camera gain and exposure time so that the intensity is around 2000. You can use "Constant intensity" for this, but turn it off once the intensity has been reached.
11. Slowly rotate the polarizer and find the minimum intensity position. Record the goniometer angle and the intensity.
12. Change angle by 0.1 degrees, and find the minimum intensity. Record the angle and intensity.
13. The next angle should be chosen with the goal of finding the minimum.
14. Repeat the procedure until you have found the minimum with reasonable accuracy, for example 0.02 degrees would be good.
15. Lock the polarizator.
16. Run an Find Brewster angle scan.
17. Set the angle to 53.06 on the setup tab.

The polarizer should now be oriented correctly, and the angle calibrated.

Appendix III. Raw data recordings

It is also possible to use a '.mkv' ending for the video file. This option saves the image data from the camera in an unpacked 8+8+8 bit format. Do not apply a background or any annotations while recording .mkv files. The 16-bit camera data is split so that the highest 8-bits are stored in the first layer, and the lowest 8-bits in the second. Thus, the raw image can be reconstructed by 256*L1+L2. The third layer contains the gain in positions [0,0,2], [1,0,2] and [2,0,2], and exposure time [0,1,2], [1,1,2] and [2,1,2]. These can be reconstructed by 4096*[0,x,x]+256*[1,x,x]+[2,x,x]. The first frame in the video contains the background frame.
The raw camera video recordings are HUGE, and can reach 100's of Gb!

Raw data recordings can be played back, by disconnecting the camera and then selecting **'Load raw video file'.** The video data replaces the camera input. You can thus apply all backgrounds etc in retrospect. Currently, the playback does not reproduce real time.