Biolin Scientific
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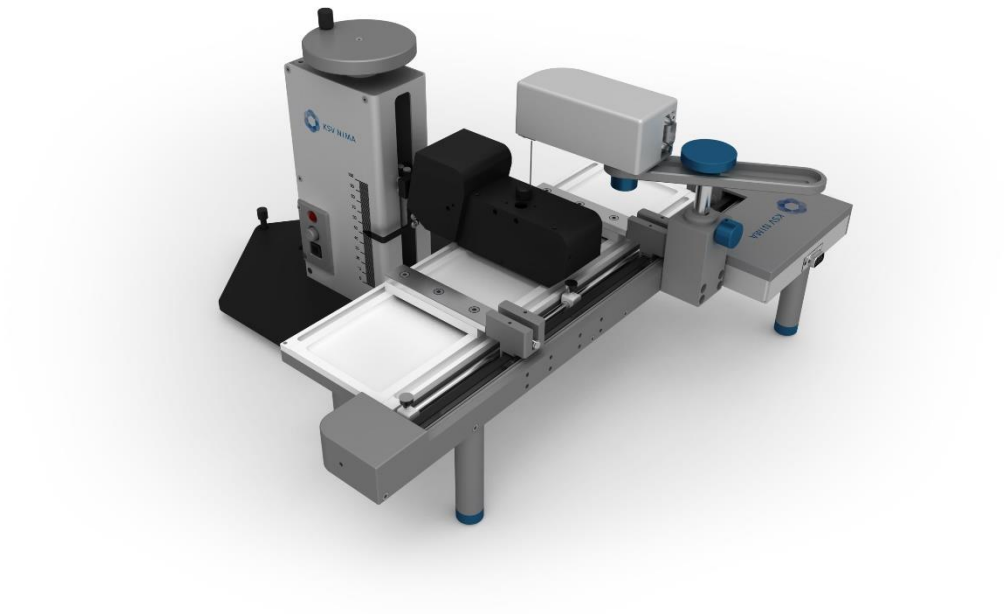
KSV NIMA MicroBAM Manual



Revision 1.7

Biolin Scientific
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Laser safety notes for the MicroBAM

Read these safety notes completely before you operate the Mi

The MicroBAM is a research instrument, to be handled by qualified personnel in a laboratory environment. It is intended to be mounted on a Langmuir trough. As such, its working principle requires a high-power, free air laser beam to illuminate an aqueous surface. Therefore, the laser beam might under certain circumstances impose a risk to your health. It is required that you read, understand and strictly adhere to the following laser safety rules with this product.

The MicroBAM emits visible laser radiation. Never look directly into the laser beam!

Class 3B (III-B) laser radiation is dangerous for the eye and potentially also for the skin.

Laser class:	3B according to DIN EN 60825-1:2001-11 (III-B according to 21 CFR 1040.10)
Max. Laser power:	50mW (at laser aperture)
Wavelength:	659 nm
Beam characteristics:	Collimated beam of approximately 6mm diameter.

Do not remove laser warning labels!

Strictly adhere to local safety regulations.

Ask your local laser safety representative about the regulations that are valid for your laboratory.

(For example in Germany: Unfallverhütungsvorschrift "Laserstrahlung" (BGV B2), §5 Anzeigen von Lasern der Klasse 3B, or in the USA: 21 CFR, Chapter J, Department of Health and Human Services)

Use laser safety goggles that protect from laser class 3B at minimum!

With laser safety goggles you are protected in particular against secular reflections of laser light, as may happen when reflecting objects interfere with the laser beam path, such as tweezers etc, or when the black glass (see manual) is not properly positioned underneath the instrument.

Safety features of the MicroBAM

Be careful when you set up the instrument to make sure that everything is properly placed according to the manual before you switch on the instrument.

The MicroBAM has the following built-in safety features:

- Key switch for the laser
- Laser warning light on interlock box that is ON if the laser is emitting.
- Interlock switch fitted for safe removal of the MicroBAM head from the stage
- Mechanical shutter

Operation of the MicroBAM

- Make sure that the supplied black glass plate is inserted into the trough and properly positioned to avoid diffuse reflections from the trough bottom.
- Avoid looking at diffuse reflections of the laser beam, for example from the trough bottom.
- Do not operate tweezers, scissors, pipettes or other tools in the vicinity of the laser beam.
- Avoid strong water waves in the trough, as this may cause secular reflection into unexpected directions. Switch of the laser or close the mechanical shutter before filling, adding or removing the water in the trough.
- Switch off and disconnect the instrument when you disassemble it from the trough.
- Do not remove covers from the instrument

Liability statement

BiolinScientific shall have no liability for any error or damage of any kind resulting from the use of this document. BiolinScientific shall also not be responsible for any damages or injuries caused by misuse of the KSV NIMA **MicroBAM** instrument.

Very important

The original mains cables of the instrument and the computer must always be used and connected to a grounded wall socket. Ungrounded wall sockets may cause dangerous voltages between the instrument, the computer and the real ground.

1. Introduction

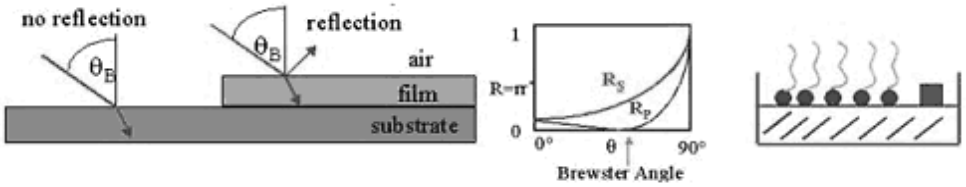
In the beginning of 19th century Sir David Brewster, a Scottish physicist, discovered the polarization phenomenon of light reflected at specific angles. In his studies on polarized light, Brewster discovered that when light strikes a reflective surface at a certain angle, the light reflected from that surface is polarized into a single plane i.e. plane-polarized. This angle is commonly referred to as **Brewster's angle**, and can be easily calculated utilizing the following equation for a beam of light traveling through air:

$$\mathbf{n = \sin(\theta_i)/\sin(\theta_r) = \sin(\theta_i)/\sin(\theta_{90-i}) = \tan(\theta_i)}$$

where **n** is the refractive index of the medium from which the light is reflected, θ_i is the angle of incidence, and θ_r is the angle of refraction.

The KSV NIMA MicroBAM is designed to the purpose i.e. for imaging thin films on water and/or rigid substrates. The principle behind the Brewster Angle Microscope (BAM) makes use of the zero reflectance of an air/water interface or dielectric substrate for vertically linearly polarized (p-polarized) light at the Brewster Angle of incidence. The Brewster angle is determined by the refractive indexes of the substrates involved for example for air/water (refractive index of 1.333), air/glass (refractive index of 1.515), and air/diamond (refractive index of 2.417) interfaces the critical (Brewster) angles are 53, 57, and 67.5, respectively.

When a condensed phase of a (mono)layer with different refractive index is spread or deposited on the interface of interest, a measurable change in reflectivity will occur. The reflected light can then be used to form a high contrast image of the lateral morphology of the spread or deposited layer. For example, a monolayer spread on an air/water interface is extremely thin, approximately 0.5 % of the wavelength of visible light. The relative effect it has on the electric field reflected from a water surface is therefore very small and the monolayer is under normal conditions quite invisible. However, if the water surface is illuminated with pure vertically linearly polarized light at the Brewster angle before spreading the monolayer at the air/water interface, there is no reflection from the water surface. The background is then completely dark and after spreading of the monolayer and compressing it the tiny effect of the monolayer can be visualized.



The user-friendly hardware and software tools make the set-up and alignment of the instrument easy and quick.

Inside this manual you will find information on how to install and use your KSV NIMA MicroBAM. In order to obtain the maximum performance from your instrument you should read this manual and keep it available for reference.

Maintenance: The hardware of your KSV NIMA MicroBAM is manufactured to the highest quality standards and when used properly should yield a lifetime of trouble-free use.

1.1. Why brewster angle microscopy - BAM

Optical techniques are used in a large variety of research fields of pure and applied science for investigating surface properties. The biggest advantage of optical techniques in surface characterization is that they are non-invasive (non-contact), which is important in circumstances were the surface structures are either very soft or must not be contaminated or scratched. Brewster Angle Microscopy is one optical technique that has been widely used for the past 10 years, especially in thin film research. The Brewster angle microscopy (BAM) technique was developed slightly more than 10 years ago mainly for characterizing one molecular thick monolayers at the air/water interface (e.g. a monolayer with typical thickness of ca 2 nm). Later it was also adapted for studies concerning interfaces such as glass, mica and SiO_2.

A large range of other surface characterization methods such as X-ray Photoelectron Spectroscopy (XPS or ESCA), Secondary Ion Mass Spectrometry (SIMS), Small Angle X-ray Scattering (SAXS), synchrotron X-ray diffraction, Ellipsometry, Imaging Ellipsometry, fluorescence microscopy, Transmission and Scanning Electron Microscopy (TEM and SEM), Scanning Tunnelling Microscopy (STM), Scanning Probe Microscopy (SPM), Raman and IR spectrometry also exists. Some of these techniques are non-imaging techniques and gives information about the chemical composition of the surfaces, and often the main interest lies in the information of the morphology or phase behaviour of thin films. In such cases one has to rely on the scanning microscopes (TEM, SEM, STM and SPM), SAXS, synchrotron X-ray diffraction, Imaging Ellipsometry, fluorescence microscopy techniques, Raman and IR spectrometry and BAM. However, the main drawbacks of most of these techniques are that they are very expensive and they are only suitable for characterizing films on solid substrates. Only fluorescence microscopy techniques and BAM can be considered to be cheap surface characterization techniques with the advantage that they can be used for characterizing both solid and liquid interfaces. Furthermore, in comparison to fluorescence microscopy techniques the main advantage of the BAM technique, apart from being non-invasive, is that it allows the direct observation of ultra-thin films on air/water interfaces or on dielectric substrates without using any fluorescent probes in the studied materials.

1.2. Applications of BAM

Thin organic films are the source of high expectations as being useful components in many practical and commercial applications such as sensors, detectors, displays and electronic circuit components. Various functionalities of supported thin films, spread monolayers, bilayer membranes, Langmuir-Blodgett (LB) or self-assembled films have been achieved by mixing organic or amphiphilic molecules with different active components. Thus, ordered multicomponent molecular assemblies with controlled concentrations of the functional units have become the subject of intensive research. This research aims for example at the construction of ultrathin optical and electronic devices, understanding of the structural arrangement and intermolecular interactions in model biological systems, and the characterization of recognition processes at a molecular level. Functioning of biomembranes, as well as the conducting and spectroscopic properties of thin films, depends on the spatial

distribution of their constituents. Therefore, the characterization of the miscibility or phase separation of the thin film components appears to be an essential step in the design and preparation of functionalized structures. BAM is a technique, which easily helps to increase this kind of knowledge. A partial list of the application areas of the BAM is shown below:

- Phase behaviour of monolayers i.e. domains and order phenomena
- Influence of subphase compositions on monolayer structures
- Phase separation in monolayers and thin films
- Real time monitoring of photochemical reactions
- Real time monitoring of polymerization reactions
- Detection of polymers and materials which cannot be detected with fluorescent probes
- Adsorption kinetics
- Gibbs adsorption layers
- Formation of multilayers
- Monitoring surface treatments
- Determining the quality and homogeneity of thin (organic) films and LB films
- LB-films on solid structures

2. Installation

This section of the manual describes how to install the KSV NIMA MicroBAM instrument and its software components. Your KSV NIMA MicroBAM is delivered in one crate. The crate should carefully be opened and checked to see that it contains the following components:

- MicroBAM laser unit
- MicroBAM stand
- Black glass plate
- CD-ROM with MicroBAM software, Camera drivers and KSV NIMA MicroBAM manual
- USB-cable
- Tool set

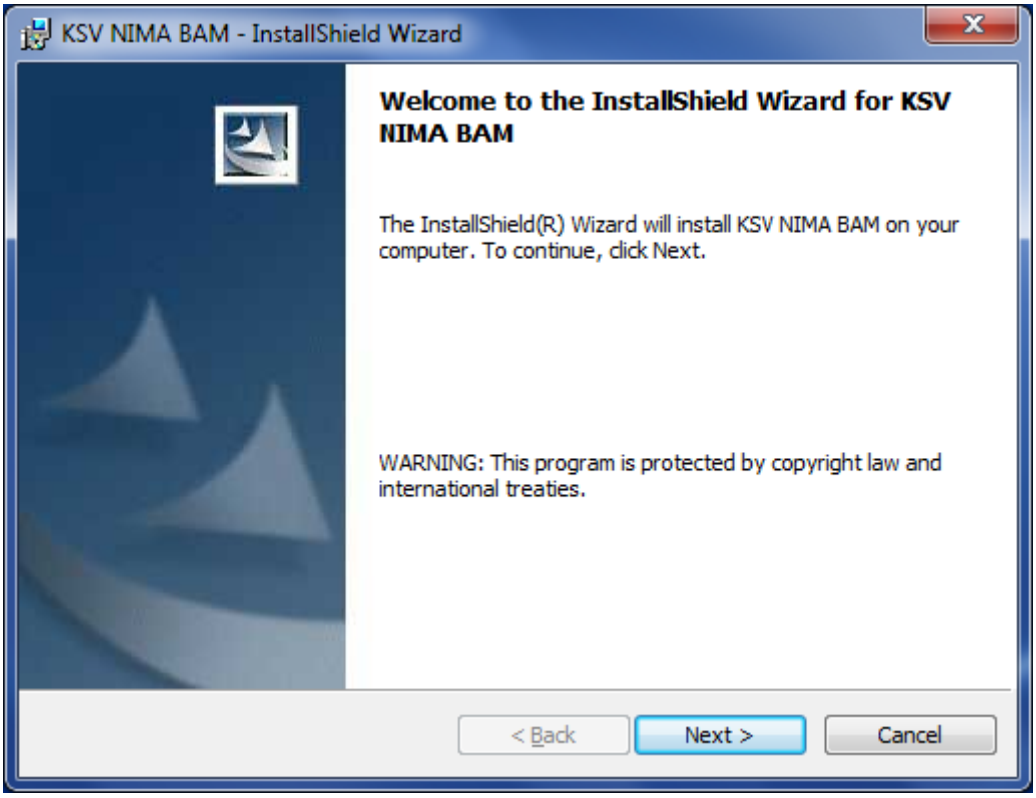
Optional items may include the following components:

- Active vibration isolation table
- KSV NIMA Langmuir-Blodgett trough equipment
- Dust protection cabinet
- Computer with pre-installed software

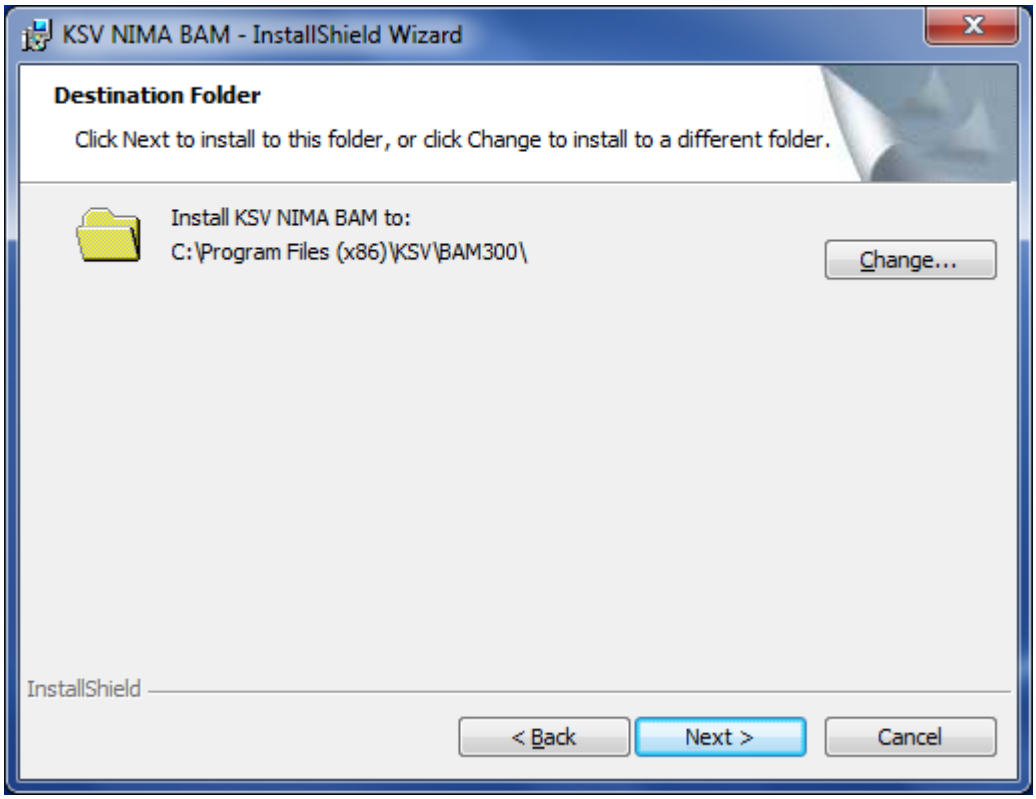
2.1. KSV NIMA MicroBAM software installation

The installation of the software for running the KSV NIMA MicroBAM instrument is done in two steps; first the Intel's imaging library which is used by the KSV NIMA MicroBAM software is installed, and then the KSV NIMA MicroBAM software itself is installed.

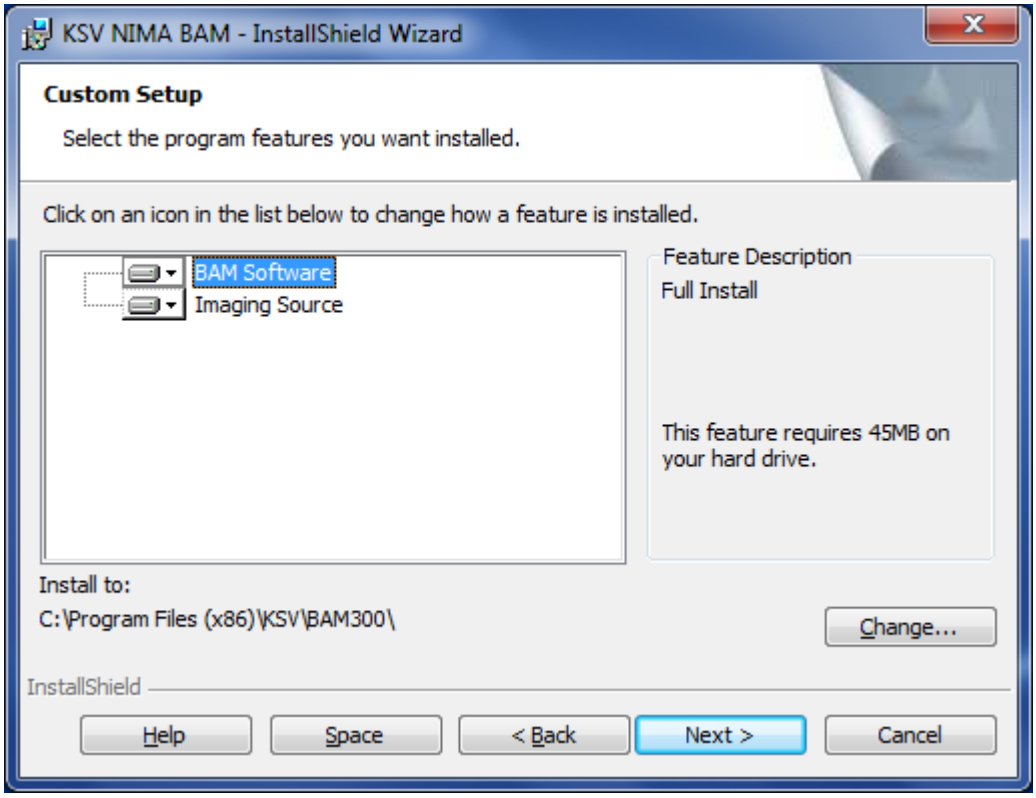
Insert the KSV NIMA MicroBAM software installation CD in your computers CD-ROM drive, open Windows explorer and locate the setup.exe file from the root of the CD and run it by double-clicking on it. The software Install Shield Wizard screen will then appear.



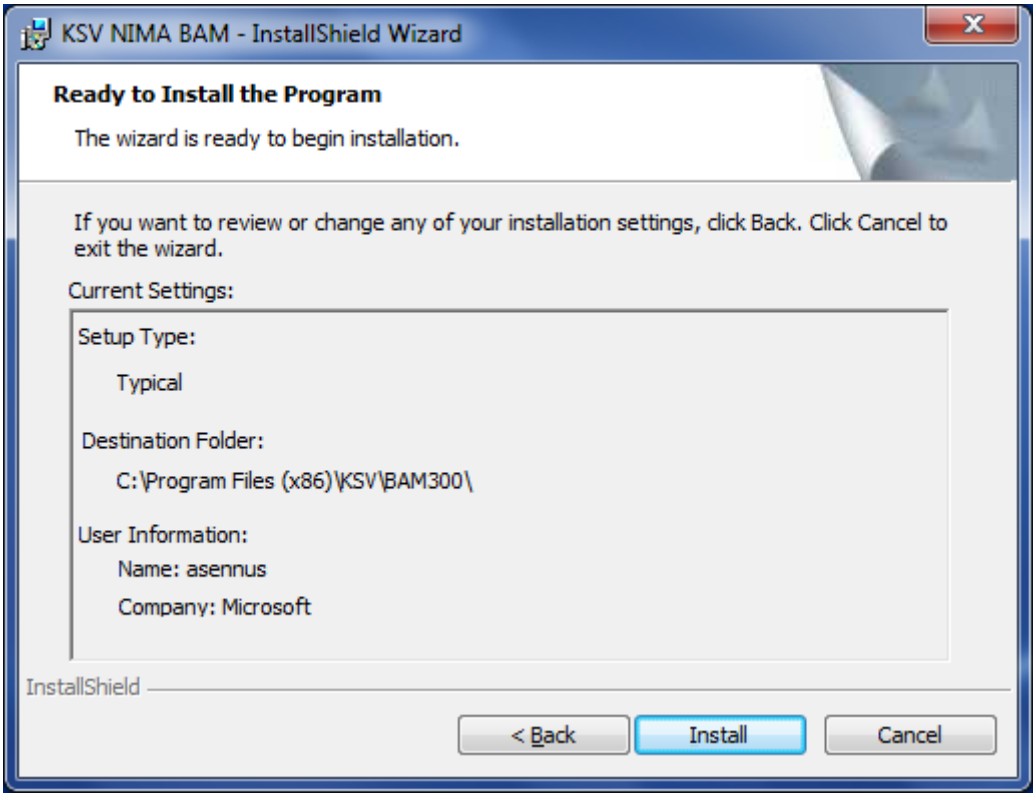
To continue with the installation press Next.



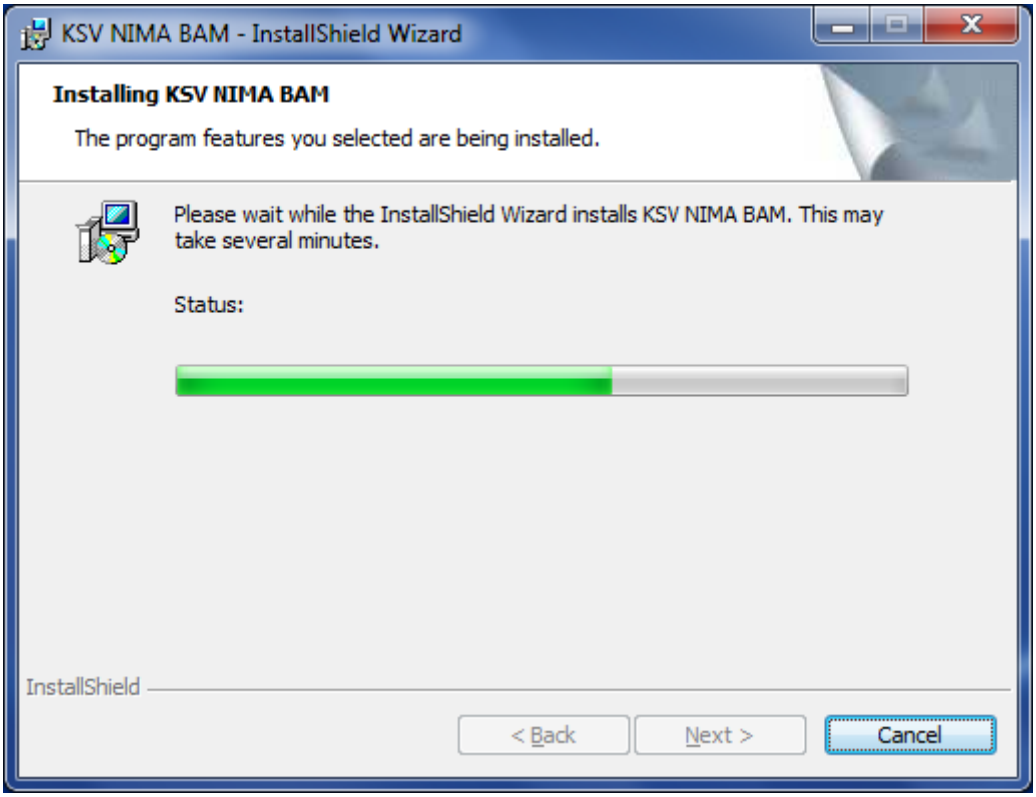
Use Change button to select different installation destination if needed. Otherwise continue with the installation by pressing Next.



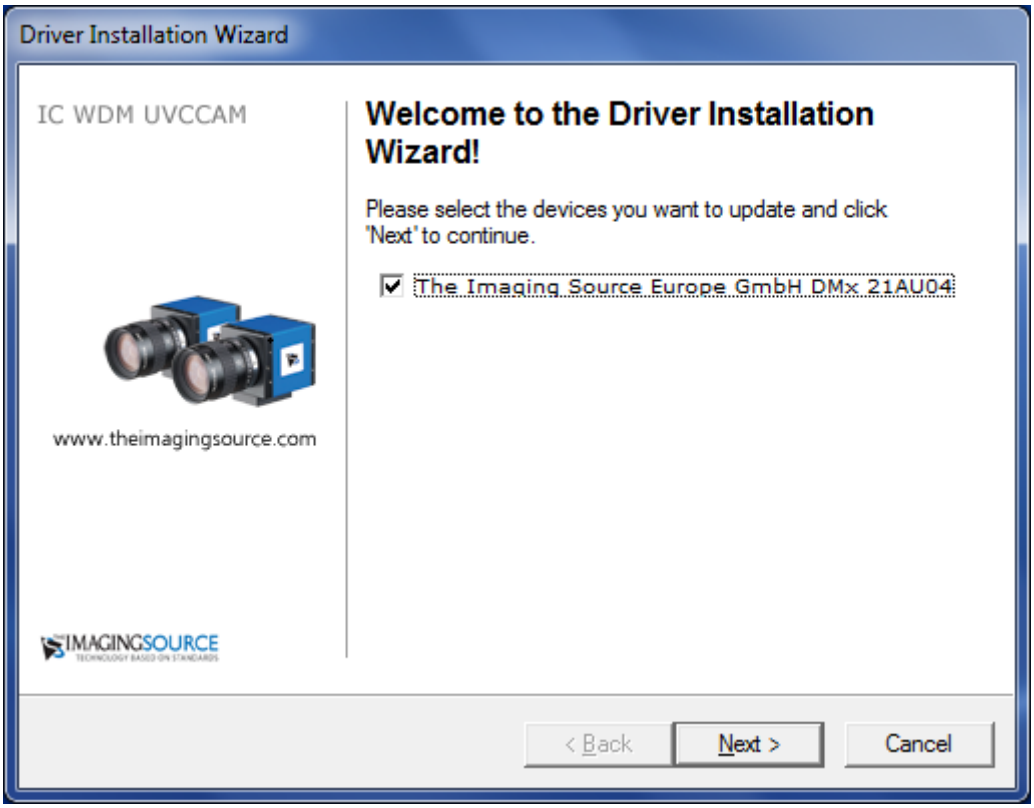
For first time installation both BAM software and Imaging Source packages are required. Continue installation by pressing Next.



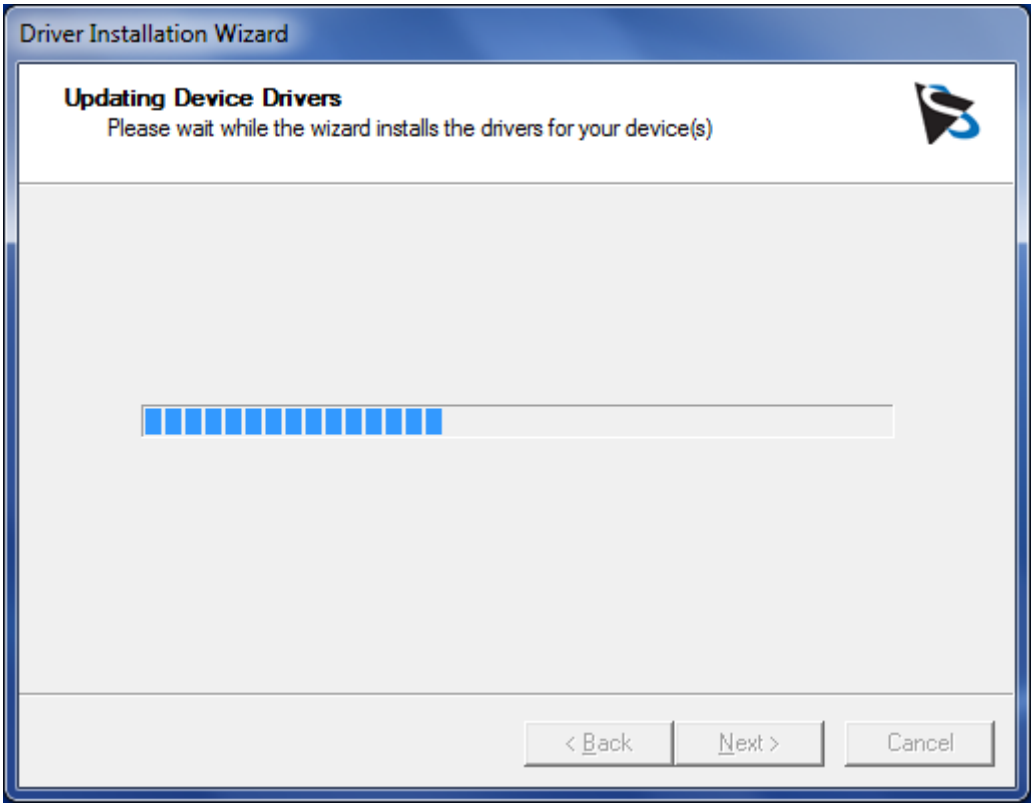
Confirm that destination folder is correct and press Install. The installation will start.



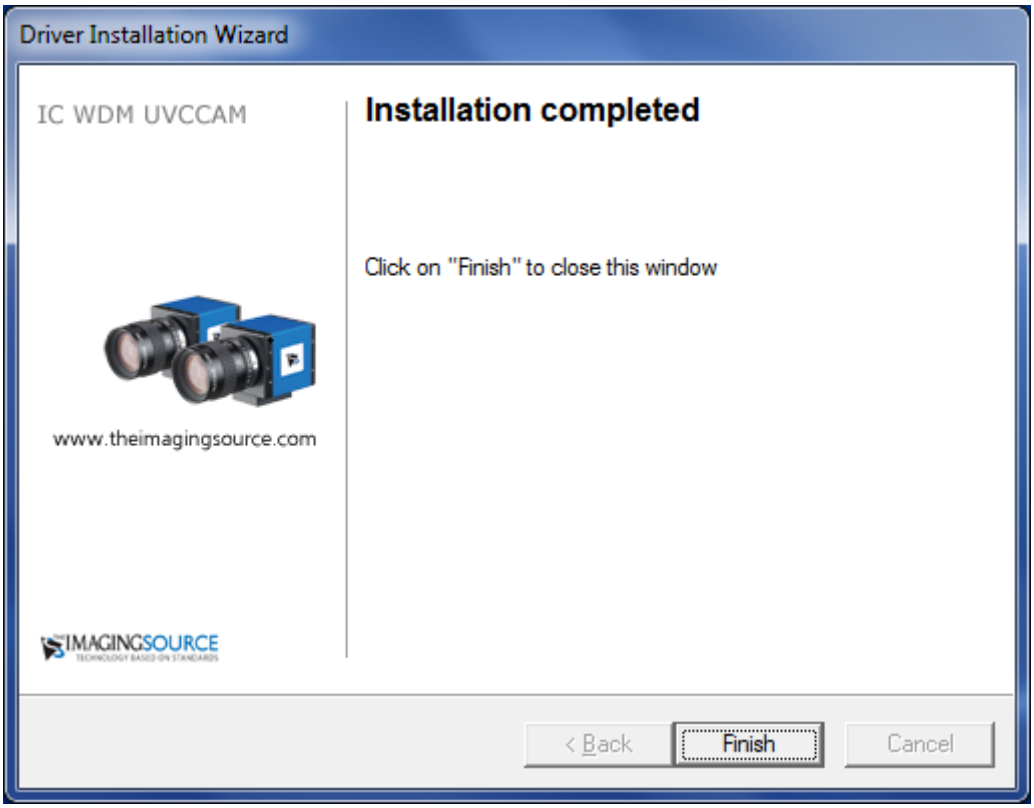
The BAM software is installed first and after it is finished the camera installations opens a new window. Connect the MicroBAM camera by plugging in the USB connector. After a short period of time the installation software should recognize the camera as shown in the following screen.



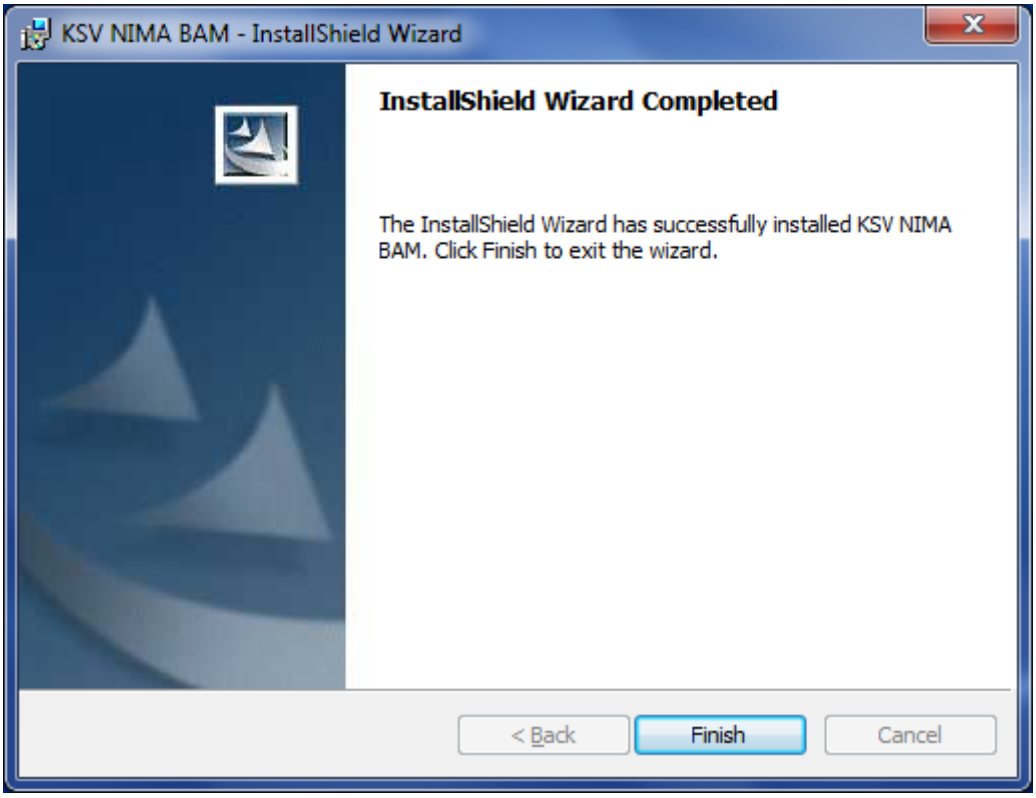
Continue installation by pressing Next.



With successful driver installation the blue progress bar should be visible for a moment.



Press Finish after the camera drivers are installed and the windows should disappear.



Press the Finish button to end the installation of the KSV NIMA MicroBAM software. Proceed to the next section if you use the KSV NIMA MicroBAM instrument in combination with a KSV NIMA Langmuir instrument.

If you are using the KSV NIMA stand-alone MicroBAM instrument without a KSV NIMA Langmuir system, the installation of the instrument is finished. In order to learn more about using the KSV NIMA MicroBAM instrument, proceed to the sections **SOFTWARE OVERVIEW and ADJUSTMENT OF THE KSV NIMA MicroBAM.**

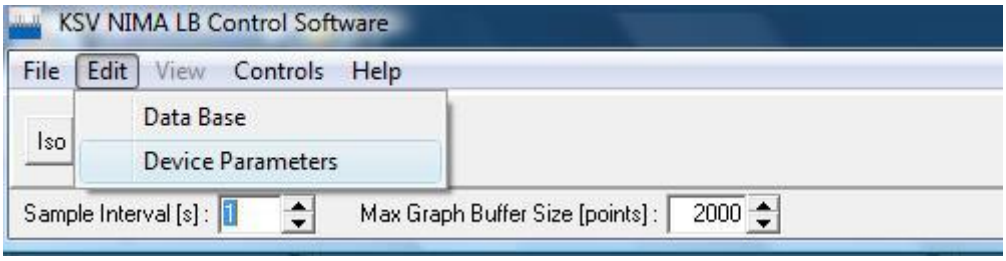
2.2. KSV NIMA LB software installation

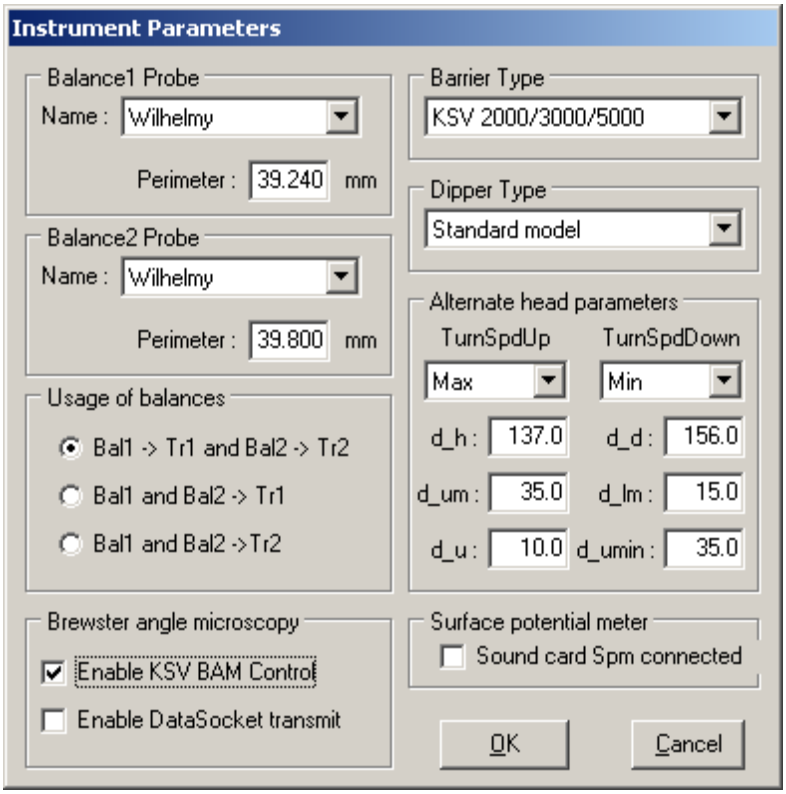
When the KSV NIMA MicroBAM instrument is used in combination with a KSV NIMA Langmuir instrument, the installation of the KSV NIMA LB software will be necessary in order to be able to control the BAM measurements through the KSV NIMA LB software. If you have WinLB software installation package, please contact your local distributor for an upgrade.

PLEASE NOTE! These upgrades require that you have at least some earlier KSV NIMA LB software or KSV NIMA WinLB software already installed on your computer. In some cases the KSV NIMA WinLB software needs to be upgraded before the KSV NIMA LB software can be installed. If no previous version of the KSV NIMA LayerBuilder or WinLB software have been installed the upgrade packages might not work.

Here it is assumed that the user is familiar with the KSV NIMA LB software. If more precise descriptions about the KSV NIMA LB software are needed the user is referred to the KSV NIMA LB software manuals.

The integration of the KSV NIMA LayerBuilder and the KSV NIMA MicroBAM software enables the user to store data such as molecular area, surface pressure, temperature, pH etc. to the captured images at certain time or surface pressure intervals. To activate the integration of the KSV NIMA MicroBAM software to the KSV NIMA LB software the connection between the two programs has to be activated from the KSV NIMA LB software. This is done by choosing Edit -> Device Parameters in the KSV NIMA LayerBuilder Control Software window and checking the box Enable KSV NIMA BAM Control in the Instrument Parameters window. Hereafter, press the OK button, close all the KSV NIMA LB software windows and restart the KSV NIMA LB software.





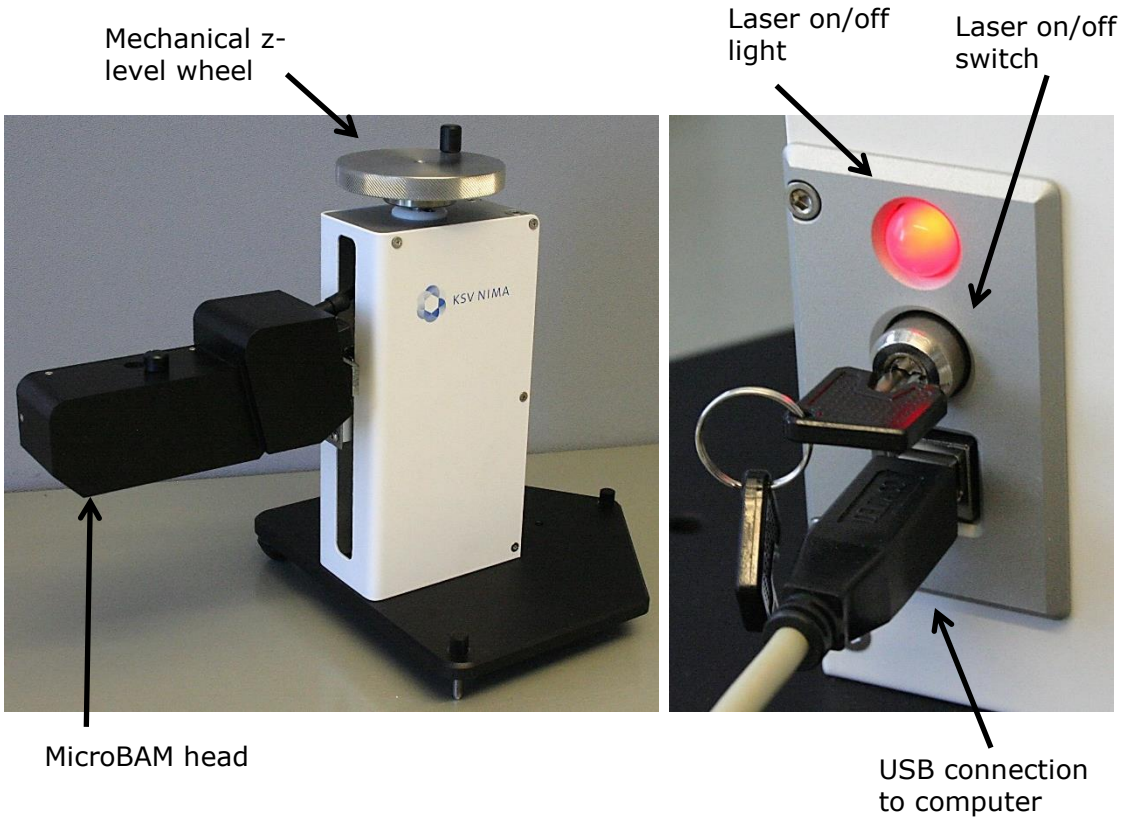
After this activation step, the KSV NIMA MicroBAM software will automatically start when the KSV NIMA LB software is started.

To deactivate the auto start of the KSV NIMA MicroBAM software when starting the KSV NIMA LB software just uncheck the box Enable KSV NIMA BAM Control in the Instrument Parameters window, press the OK button, close all the KSV NIMA LB software windows and restart the KSV NIMA LB software.

Now the installation of the instrument is finished. In order to learn more about using the KSV NIMA MicroBAM instrument, proceed to the sections SOFTWARE OVERVIEW and ADJUSTMENT OF THE KSV NIMA MicroBAM.

3. Description of the instrument

3.1. Physical description



3.2. Prepare the Langmuir trough and the MicroBAM for integration

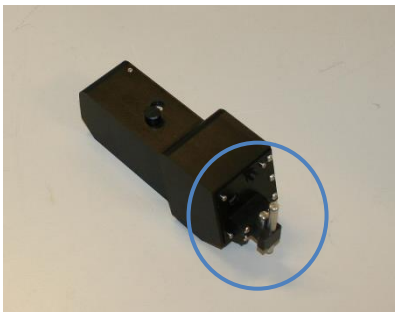
1. Ensure that you have a clean, spacious work area before you begin assembling the microscope. When microscope is not in use, always cover it to protect it from dust. For best results, operate MicroBAM on a laboratory bench that is free of vibrations and air turbulence. An undisturbed water surface makes laser alignment easier and prevents flickering of the live video feed from the CCD camera. Enclosing the trough/microscope setup in a Plexiglas box will eliminate air turbulence and gives a stable, more accurate surface pressure reading.

2. Clean the Langmuir trough with standard cleaning procedure explained in Monolayer Kit manual section 5.1. Preliminaries.

3. Verify that the MicroBAM stand slides easily underneath the trough.

3.3. Attaching the MicroBAM head to the stand

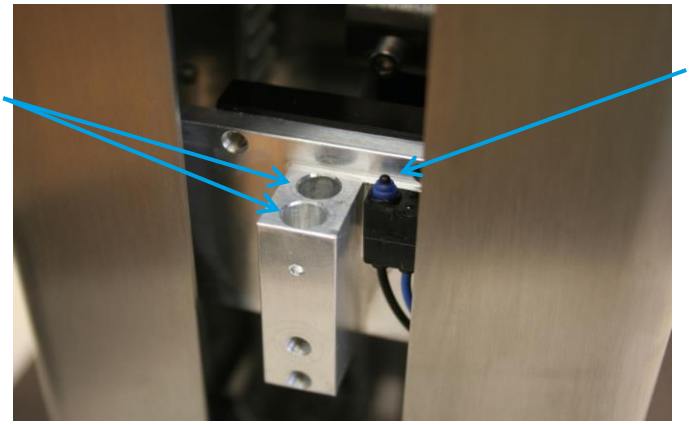
1. The MicroBAM arrives with separate measuring head and stand.



2. Attach the USB cable and MicroBAM interlock cables to the back of the camera.

3. Insert camera mounting studs into mounting slots and ensure that the camera is lowered down fully, so as to compress the safety interlock microswitch.

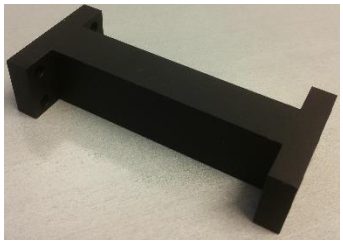
Insert camera head into the two mounting holes



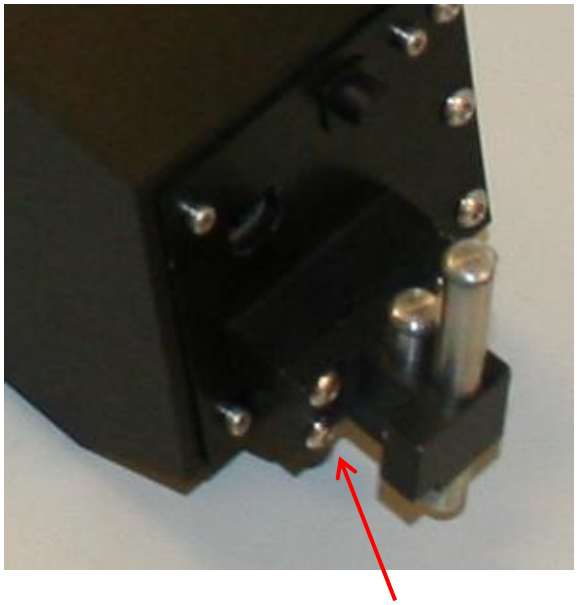
Ensure microswitch is fully compressed

Please also note that an extension arm has been supplied with your instrument for cases when you need extended reach for the system. This is the case for example

when using the MicroBAM with the Alternate in order to study the backtrough section.



The extension arm can be fixed between the MicroBAM head and the mounting studs. Open the screws that connect the studs to the head. Using the screws provided, fix the extension arm to the MicroBAM head on the other side and to the mounting studs on the other side.



Open these 4 screws in order to separate the head and the studs. Put the extension arm between the head and the studs. Fix the arm with the screws on

3.4. Positioning of the MicroBAM

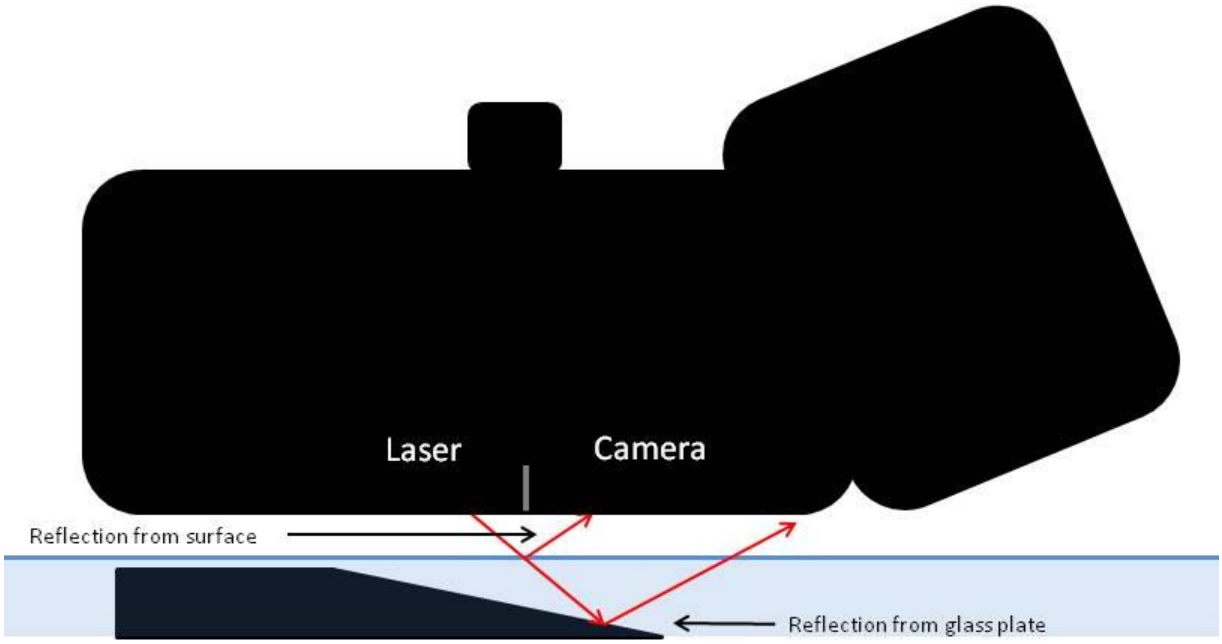
1. Position the MicroBAM head above the area of the trough that you wish to observe. Verify that the MicroBAM head sits approximately 1 cm above the water surface and that the opening for the laser beam path reaches over the trough surface.

2. You can use the scale in the MicroBAM stand to place the MicroBAM to the approximately right level if you have set the MicroBAM level before to the system. The final height adjustment must always be made by observing MicroBAM image.

3.5. Adjusting the KSV NIMA MicroBAM

POSITION THE BLACK GLASS PLATE

1. Place the clean black glass plate underneath the vertical mark on the MicroBAM head so that the wedge-shaped end will sit directly underneath the laser beam path. The black glass absorbs the refracted beam (which contains >99% of the incident energy) from the laser. The glass plate prevents this light from reaching the detector and also acts as a safety device to prevent random diffraction of the laser beam. Align the edge of the black plate with the edge of the MicroBAM head that contains the vertical mark. This mark indicates the opening through which the laser light passes.



2. Fill the LB trough with aqueous subphase to the appropriate level as indicated in the KSV NIMA Monolayer manual. Make sure the glass plate is submerged in the

water. Do not aspirate the water surface at this time; you will do this in a later step.

ADJUST THE 'Z' POSITION OF THE MICROBAM

1. Fill trough with water

2. Position MicroBAM above trough

3. Place black glass under laser

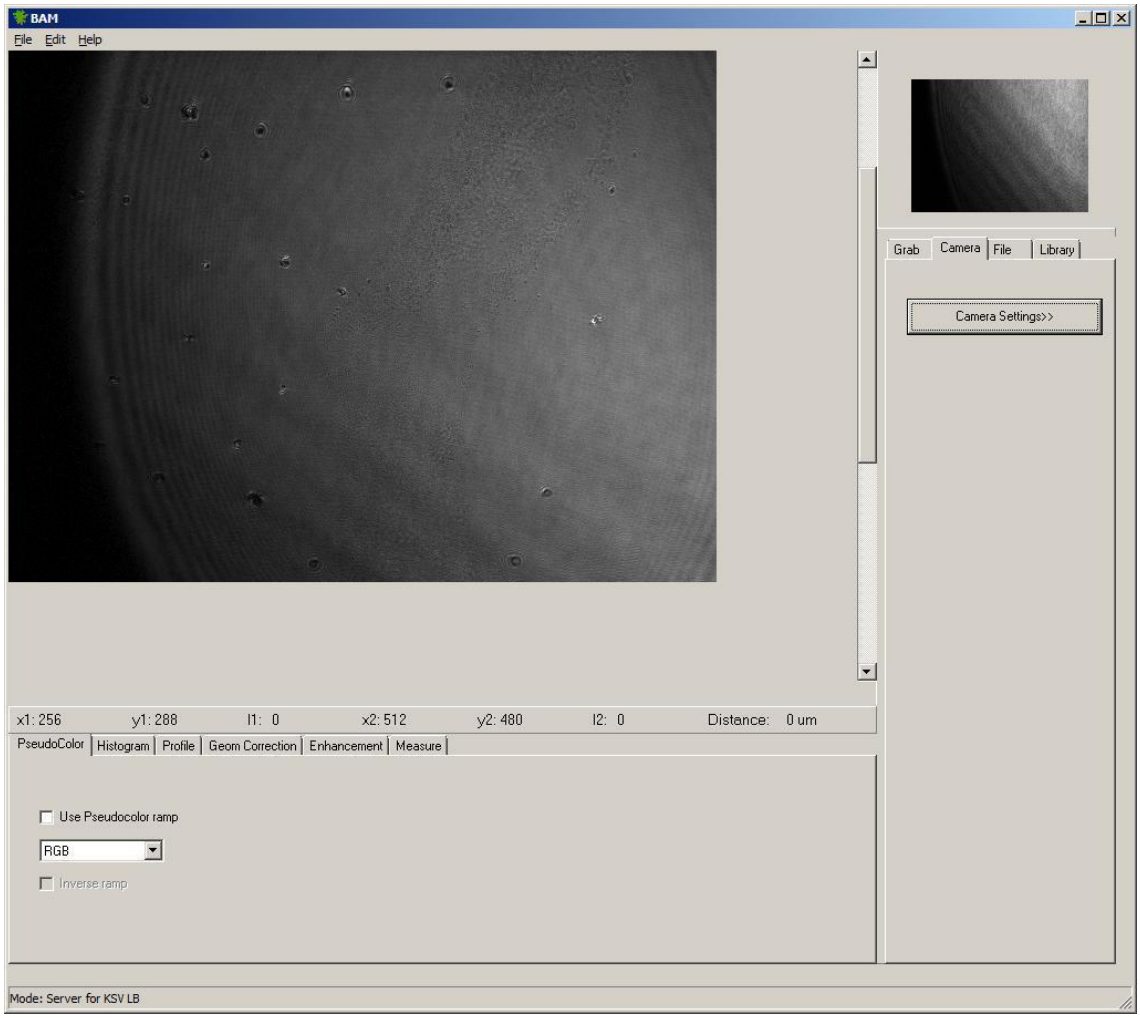
4. Switch on laser and ensure indicator lamp is illuminated, to indicate that laser is active.

PLEASE NOTE: LASER GOGGLES SHOULD BE WORN TO PROTECT DAMAGE TO YOUR EYES

5. Ensure safety window is open

6. Level the BAM head so that the spirit level is in the centre. Use the two adjustable legs on the frame to control the levelling.

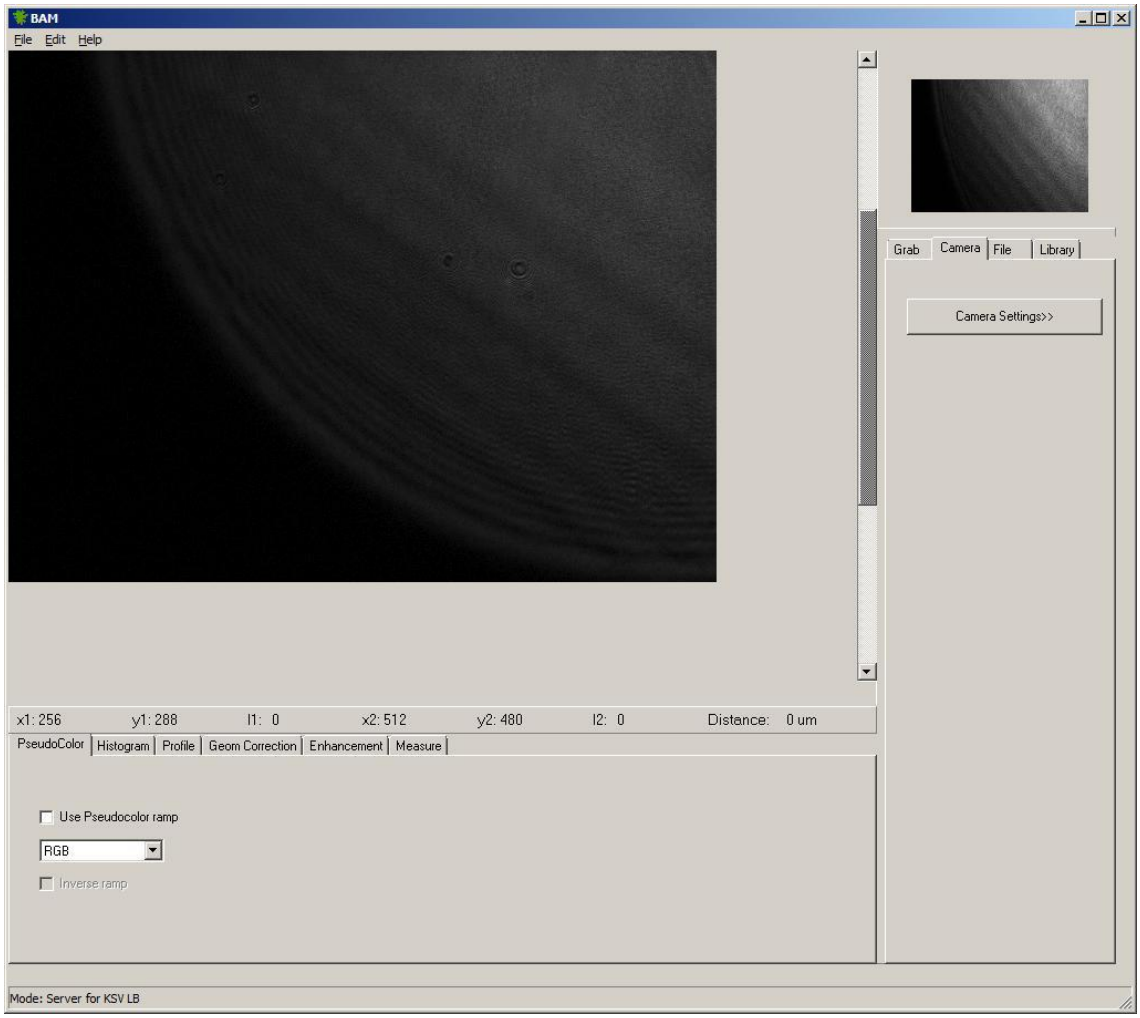
7. Run KSV NIMA BAM software



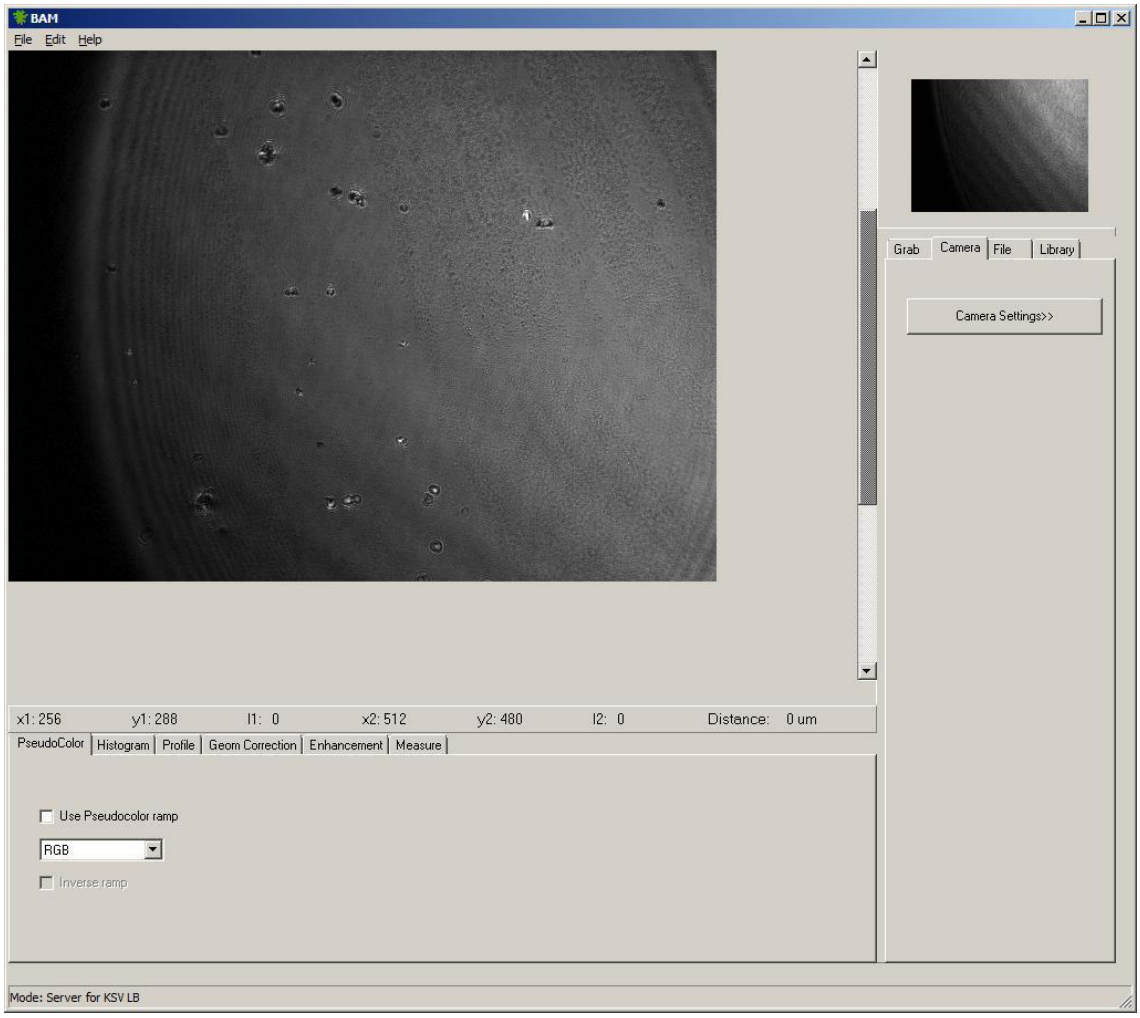
8. Ensure that the Live video is set to "Left" window

9. Lower the MicroBAM head using the mechanical turning wheel on top of the machine.

If the image is all grey, or shows a semi-circular display at top of screen then the MicroBAM is too far away from the water surface:



If the image shows a semi-circle on bottom of image, then the MicroBAM is too close to water surface:



When adjusted correctly, the image will fill the screen.

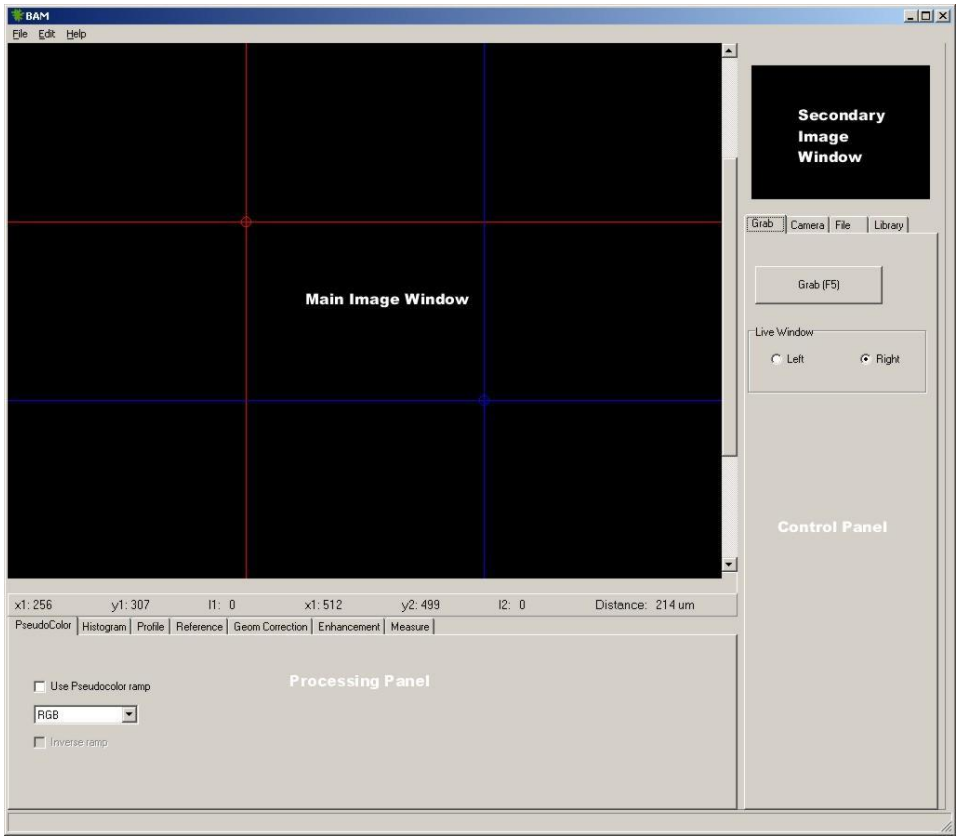
4. KSV NIMA MicroBAM software overview

This section describes the basic functions and main components of the KSV NIMA MicroBAM software.

The KSV NIMA MicroBAM software is started by choosing the Windows Start button → Programs → BAM → BAM or by double-clicking on the 

icon on the desktop.

When the KSV NIMA MicroBAM software is started the BAM main window of the software will appear. The BAM main window can be divided in 4 major sections: Main Image Window, Secondary Image Window, Control Panel and Processing Panel.



MAIN IMAGE WINDOW

The Main Image Window is primarily used for showing the live video image during the measurements and still images during the post-processing and analysis. The Live Window selection in the Control Panel's Grab page is used to select the live video image destination between Main Image Window and Secondary Image Window.

SECONDARY IMAGE WINDOW

The Secondary Image Window is used for still images during measurements and for live video frame during post-processing. See Main Image Window for details how to select live video image output window.

CONTROL PANEL

The Control Panel lets you do the following things:
Grab pictures
Set Camera settings
Define where to store images during measurements
Browse existing image libraries

GRAB PAGE

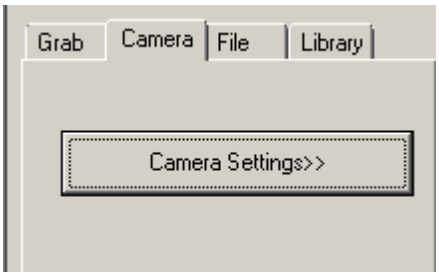
Grab

By pressing the Grab button the current live video frame is grabbed and shown in the still image window (Main Image Window or Secondary Image Window depending on the Live Window setting). Pressing function key F5 also initiates a grab. **PLEASE NOTE**! If Enable Autosave is not activated in the File tab of the control panel, the grabbed image has to be saved manually by pressing the Save button.

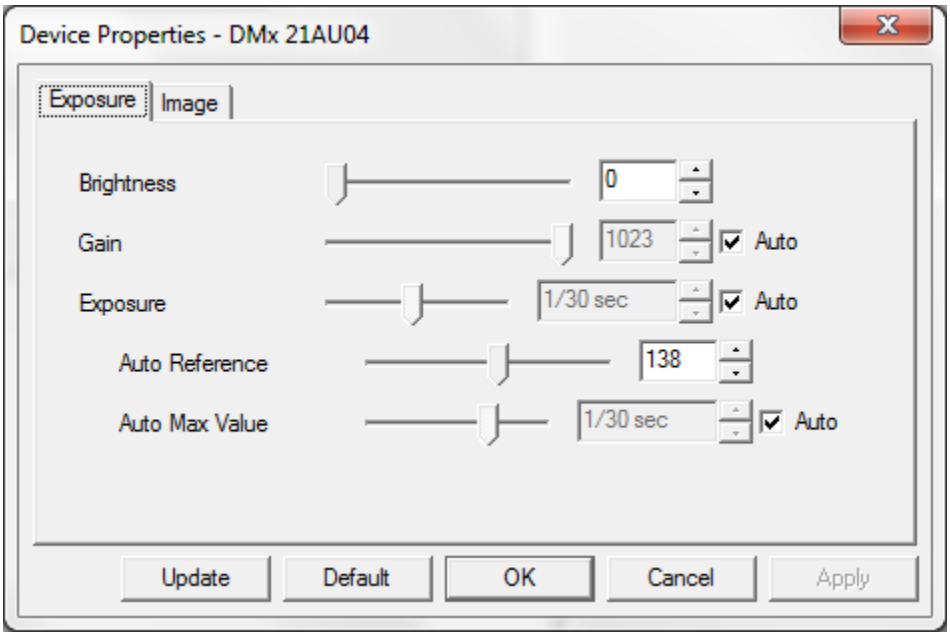
Live Window

Selects the destination window for live video image feed. Left corresponds to the Main Image Window and Right to the Secondary Image Window.

CAMERA PAGE



This page lets you to adjust the digitising settings of the camera. By selecting Camera Settings>>, a new window opens.



Brightness

Adjusts the brightness of the image from the camera

Gain

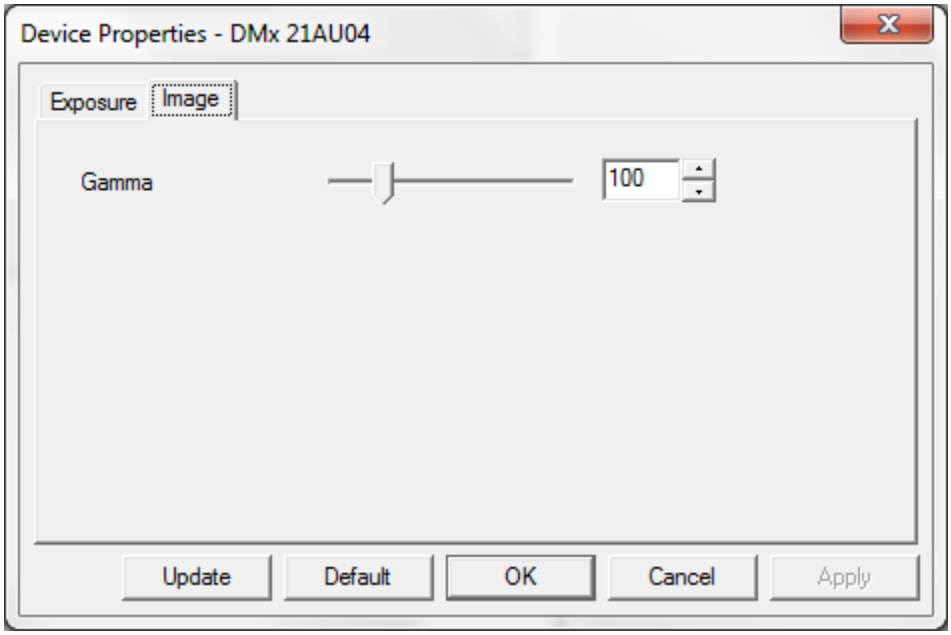
Adjusts the gain of the image from the camera

Exposure

Adjusts the exposure of the image from the camera

Default

Restores the factory settings



Gamma

Adjusts the gamma correction of the image from the camera

FILE PAGE

Save
Saves the current raw image to a file on the computer's hard disk

Load
Loads a raw image from a file for further processing

Format
Selects a picture file format (JPEG or BMP) for the saved image files. Applies also when Enable Autosave is activated.

Enable Autosave
When this box is checked every grabbed image (Grab button or F5) is automatically saved on the computer's hard disk. You have to specify a File Body in order to specify specific file names for the captured images. The Session Folder defines the destination location for the captured images on the computer's hard disk. Format setting defines images' file format.

File Body
In Enable Autosave-mode an increasing serial number is added to the file body specified, e.g. picture0001.jpg, picture0002.jpg and so forth.

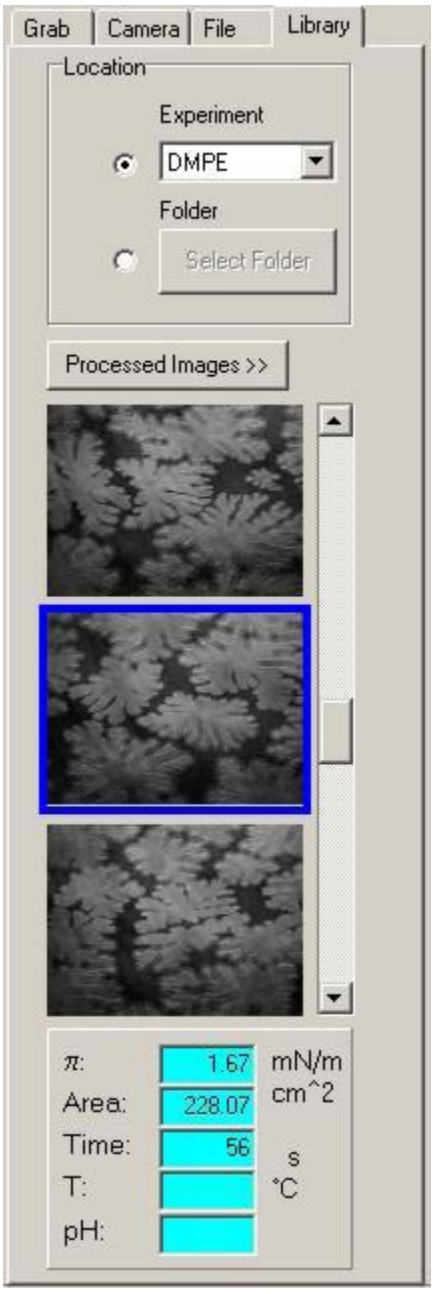
Session Folder
Defines the location (destination folder) on the computer's hard disk where the captured images will be stored.

Browse
You can browse for the destination folder by pressing the Browse –button.

NOTE ! When used in co-operation with KSV NIMA LB software the KSV NIMA LB software handles the file naming.

LIBRARY PAGE

Library is used for browsing your measurements.



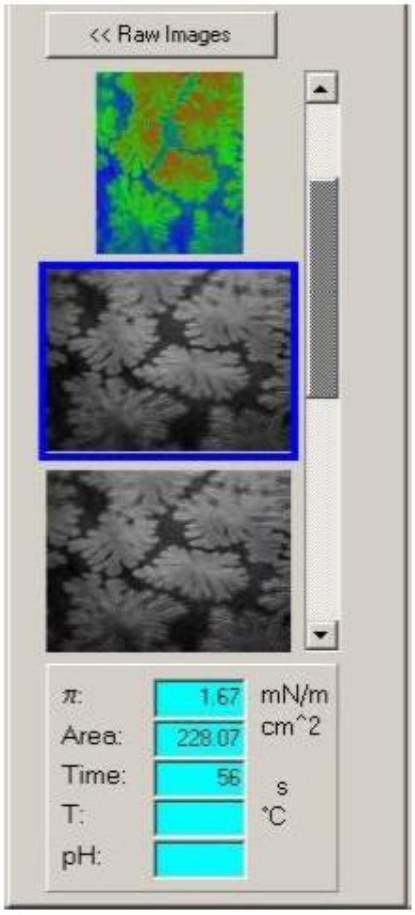
Location
Defines the library location. When used with KSV NIMA LB experiments, select the experiment name from the Experiment drop-down menu.

If used without a KSV NIMA Langmuir system, use Select Folder button to select the library location on the computer's hard disk.

Thumbnail view in Raw Mode
This view shows you three consecutive raw pictures from the experiment. You can scroll through the library with the scroll bar or use the mouse wheel.

Under thumbnails you can see LB experiment quantities stored by the KSV NIMA LB software. If the MicroBAM is used without a KSV NIMA Langmuir system, these figures will not be seen.

Thumbnail view in Processed Mode



This mode is only active for BAM images captured during an LB measurement. The raw images captured during an LB measurement are stored on the computer's hard disk in a folder under the KSV NIMA LB software DATA folder. However, also the processed images of the LB measurement can be stored and viewed in the same folder separately from the raw images. After processing, images you can browse through processed images attached to each raw file by pressing the Processed Images button. Further, by pressing the Raw Images button the view goes back to the raw image mode.

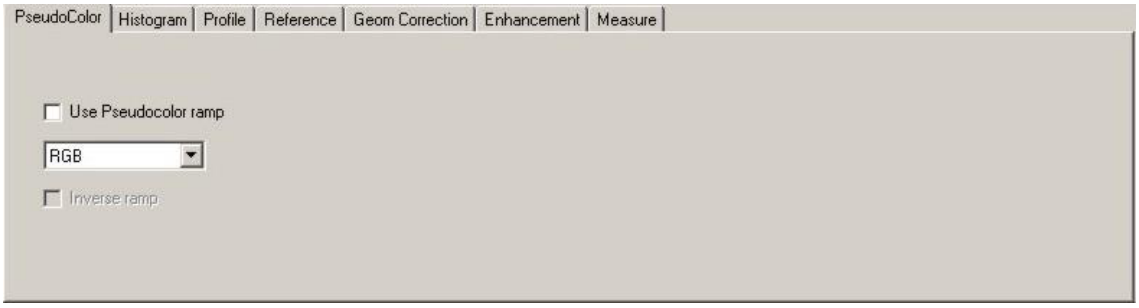
You can open raw images into the BAM software just by double-clicking on the thumbnail. You can also copy an image to the Windows clipboard by right-clicking on the thumbnail and selecting Copy to Clipboard.

PROCESSING PANEL

Processing panel mainly controls how the software processes raw images, and also facilitates simple analysis.

PSEUDO-COLOR

Pseudo-coloring or "false coloring" is a method where monochrome (grey-scale) images are artificially processed to have color information. This is accomplished by doing a mapping from grey-scale values to RGB values. Pseudo-coloring can often help to get more visual details out of scientific images.



Use Pseudo-color ramp

When checked, Pseudo-color ramp is applied to the raw image. Select the ramp type from the drop-down menu. There are currently four ramps available:

RGB: "rainbow" mapping, dark areas have shades of red, mid-tones are shades of green and bright areas are shades of red

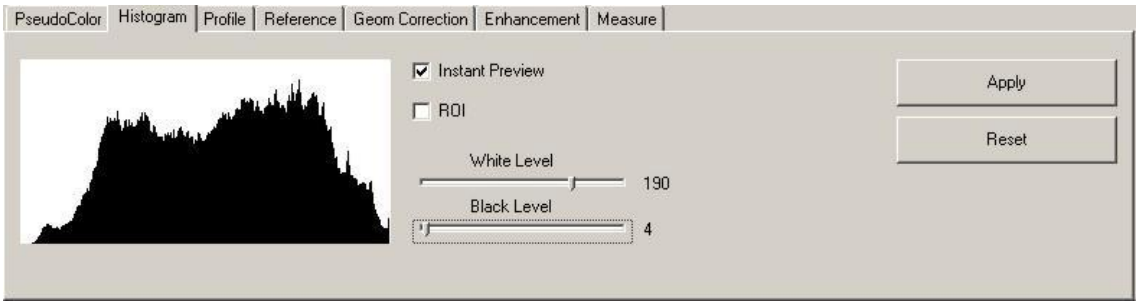
Red, green and blue ramps: linear one-to-one mapping from grey-scale value to the corresponding color channel, e.g. grey-scale value of 53 is mapped (0,0,53) in the blue ramp mode.

Inverse ramp

Inverts the ramp, grey-scale values are first mapped from [0,255] to [255,0] and pseudo-coloring is then applied as above depending on the setting.

HISTOGRAM PAGE

Here you can set the "levels" of the picture with the help of an intensity histogram or just simply look at the intensity distribution of the image.



Instant Preview

When this option is set, the changes of the slider positions are applied immediately. If not checked, pressing the Apply button applies the changes.

ROI (Region of Interest)

When this button is checked the histogram is calculated only from the ROI (area bound by the cursors (if both are enabled)).

White Level

Sets the White Level. This level and brighter levels are mapped to be white (255).

Black Level

Sets the Black Level. This level and darker levels are mapped to be black (0).

Reset Button

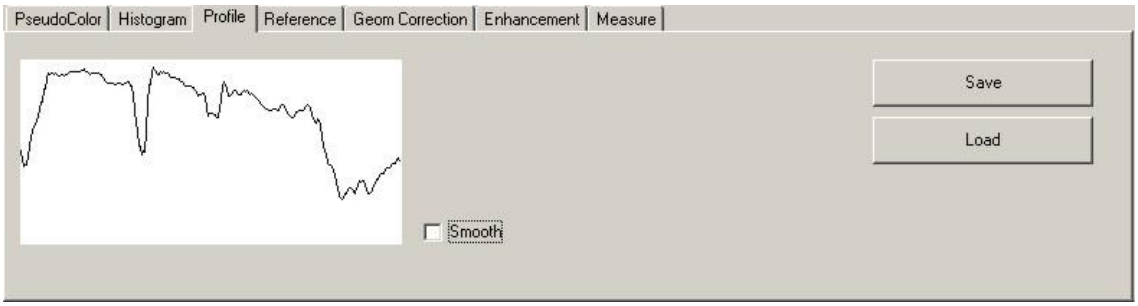
Resets the Level sliders

Apply

Applies the current settings. Inapplicable if Instant Preview is checked.

PROFILE PAGE

Profile lets you see intensity distribution in the picture through one line segment. The line segment's end points are defined by the cursor locations. Cursor 1 (Red) is the start point of the segment and Cursor 2 (Blue) is the end point of the segment.

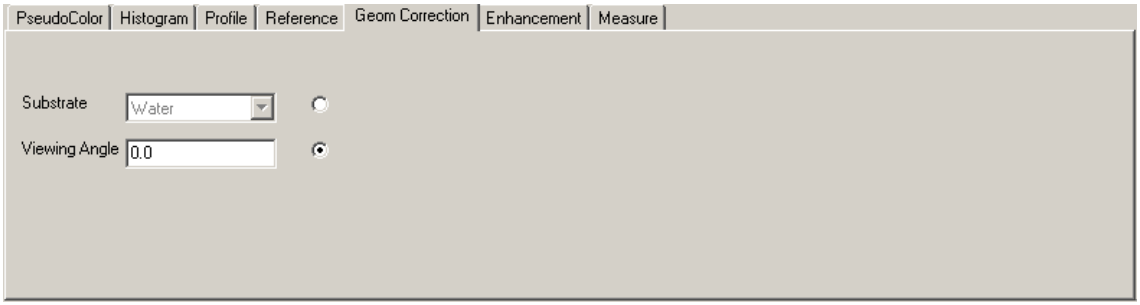


Smooth

Checking Smooth will smooth the curvewhich removes some noise..

GEOMETRIC CORRECTION

BAM looks at the specimen plane in an oblique angle. Due to the high magnification the longitudinal magnification of the image is different from the transverse magnification thus shortening the picture in the longitudinal direction. You can specify the viewing angle so the picture can be stretched back to an isotropic magnification by defining the viewing angle here.



Substrate

There are four pre-defined substrates for which the viewing angles are pre-calculated:

- Water: 53.0
- Si/SiO_2: 73.0
- Glass: 57.0
- Diamond: 67.5

Select from the list your substrate or click the radio-button on the right side of "Viewing Angle" in order to specify a custom angle. See below how to define the viewing angle.

Viewing Angle

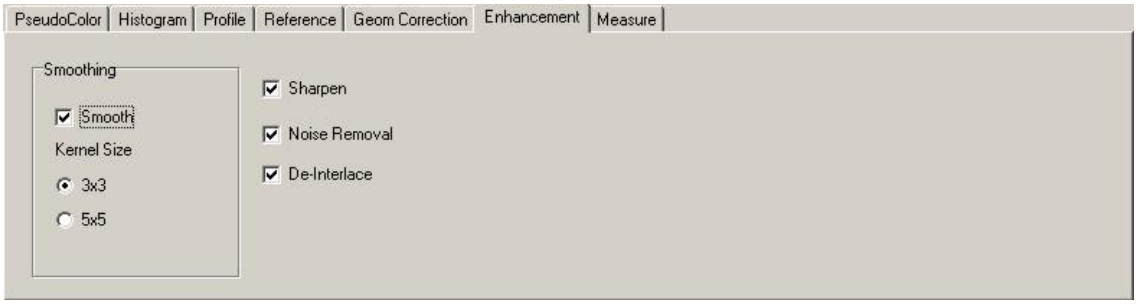
Enter the viewing angle here in degrees. Please do note that the viewing angle is the Brewster angle for your current sample. This can easily be calculated from the Brewster's law:

$\tan \beta = n_2 / n_1$,

where β is the Brewster angle, n_2 is the refractive index of the denser media (in most cases water) and n_1 is the refractive index of the lighter media (in most cases air).

ENHANCEMENT PAGE

This page contains some helpful standard image processing functions to enhance the image quality.



Smoothing

Enables Gaussian smoothing.

Kernel Size

Selects the Kernel Size of the Gaussian Filter. 5x5 gives smoother results than the 3x3 filter.

Sharpen

Applies a 3x3-sharpening filter to the image.

Noise Removal

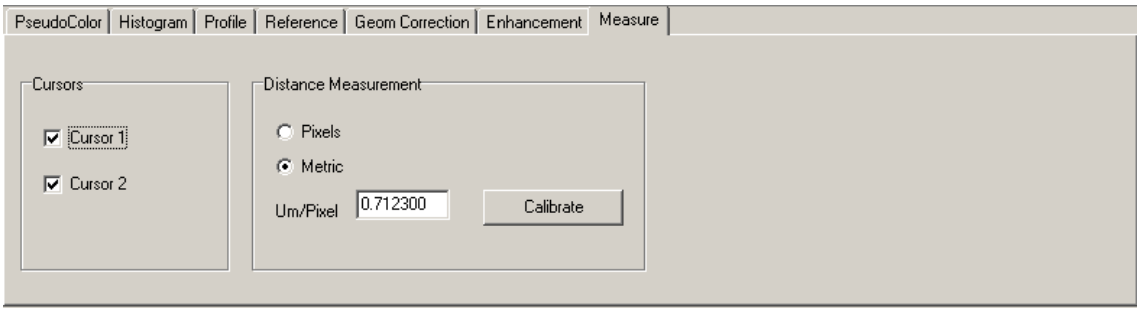
When checked suppresses noise in the picture.

De-Interlace

When recording moving objects (monolayers on water etc.) interlaced video cameras causes disturbing wiggles in the pictures if the domains are moving quickly. Enabling De-Interlacing removes this problem.

MEASURE PAGE

This page controls the cursors on the Main Image Window



Cursors

You can enable the cursors by checking the boxes in the Cursors window. Cursor 1 is Red and Cursor 2 is Blue. Both cursors are needed in the Profile view and in the case when you want to see the location and the distance data on the Measurement panel:

X1 is the x co-ordinate of Cursor 1

Y1 is the y co-ordinate of Cursor 2

I1 is the intensity level under Cursor 1

X2 is the x co-ordinate of Cursor 2

Y2 is the y co-ordinate of Cursor 2

I2 is the intensity level under Cursor 2

Distance is distance between cursors in micrometers or pixels

Distance Measurement

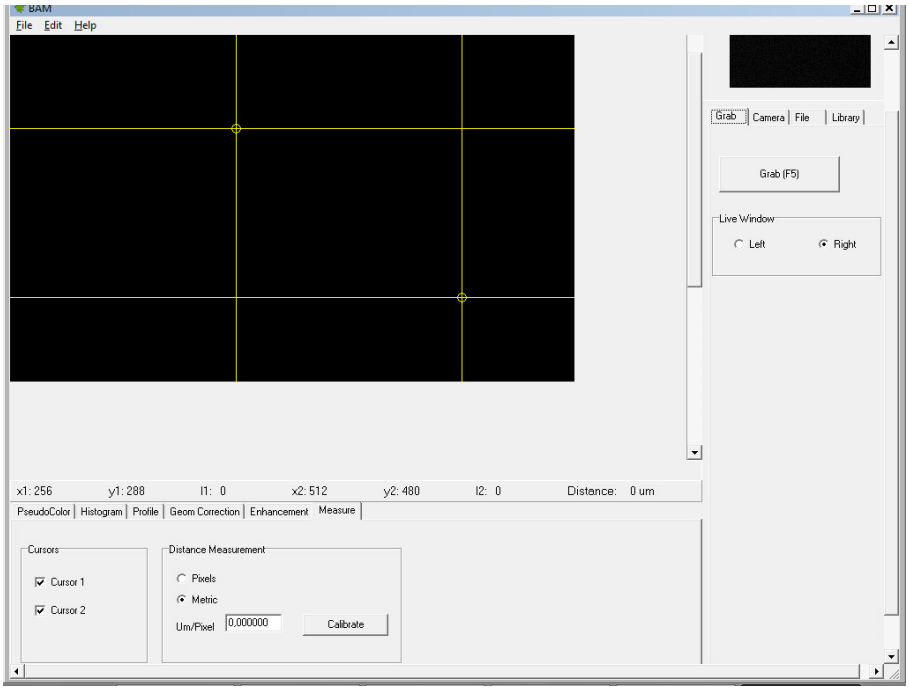
In this window you can select if you want to see the Distance in pixels or micrometers.

Um/Pixel field

This is the "calibration" factor, which converts pixel distances to metric distances. This conversion is only approximate. You can estimate this "calibration" factor by dividing the horizontal field of view by the number of horizontal pixels in the imaging element of the camera as explained below in the "Camera distance measurement calibration".

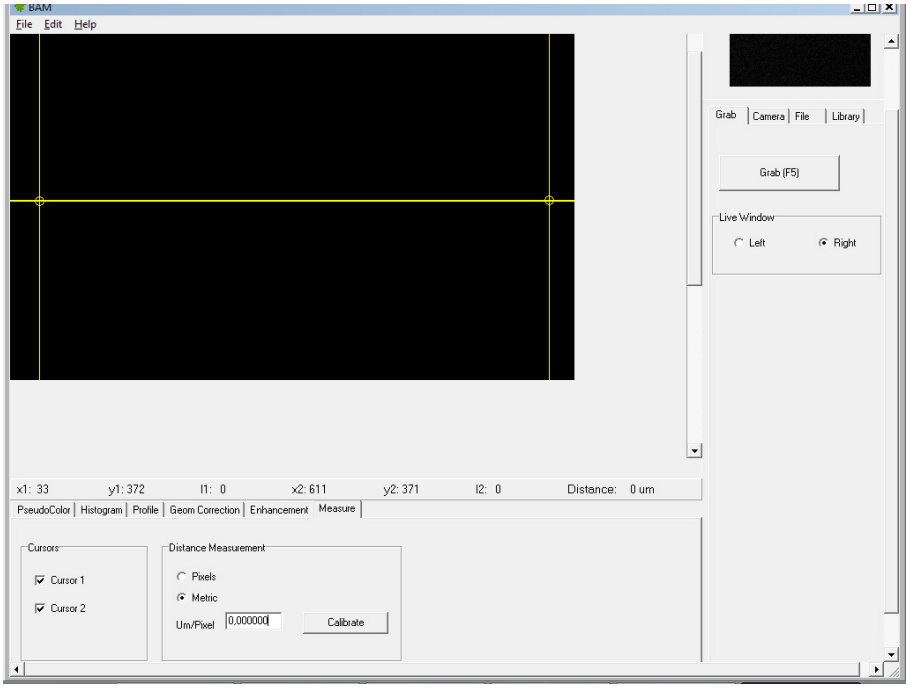
Camera distance measurement calibration without calibration image.

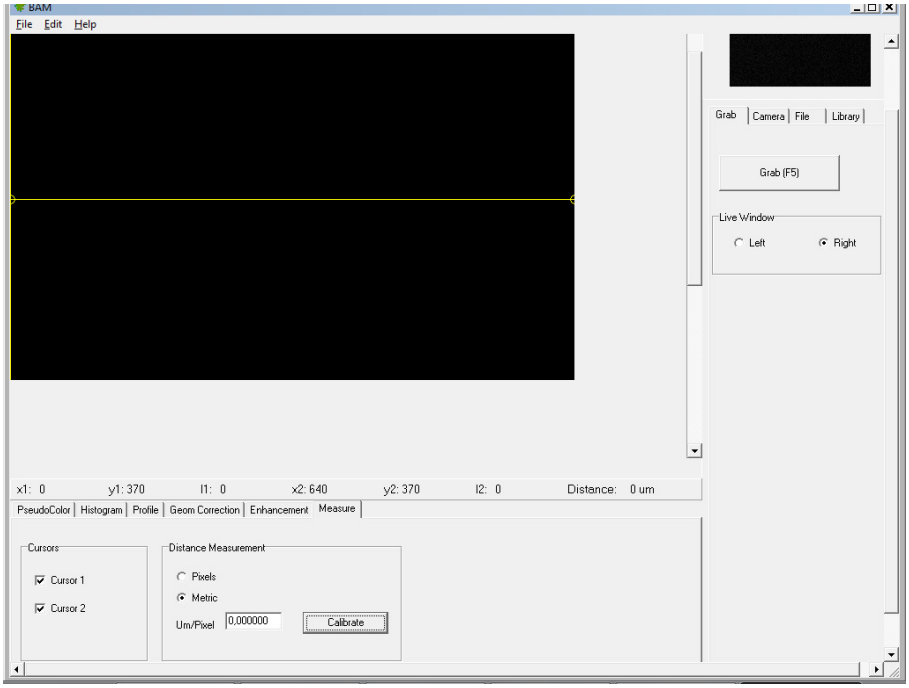
The pixel size of the camera is 5.6 µm. You can manually type this in to the "Um/Pixel" field. If needed, you can also calibrate the camera:



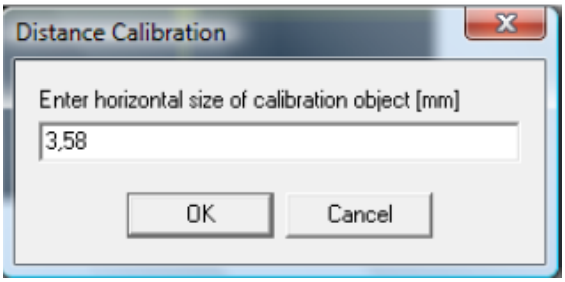
The easiest is to use the known width of the image size (3580 µm):

First carefully move the cursors horizontally to the edges of the figure.

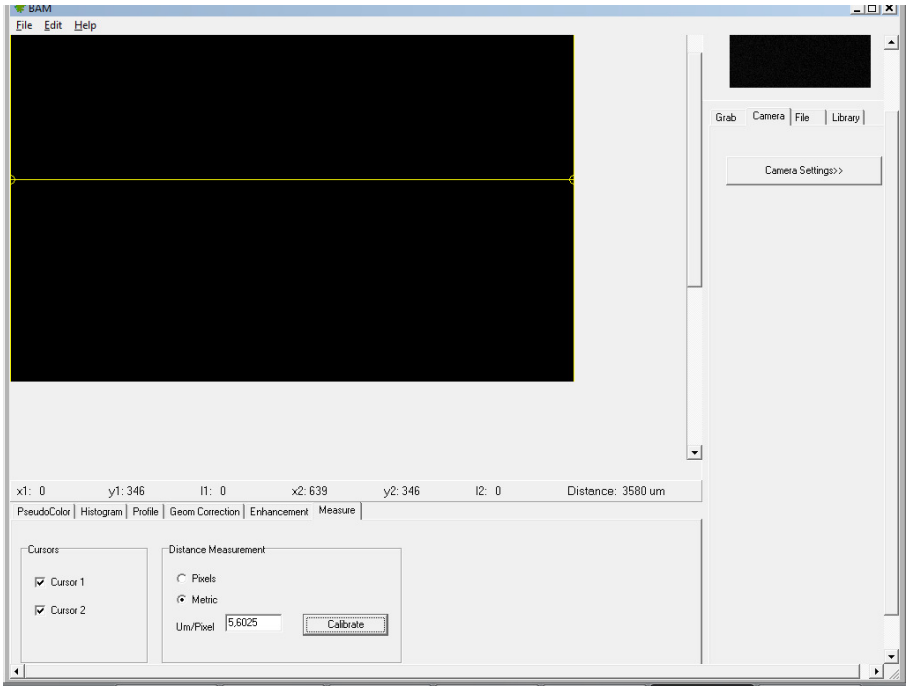




Then click on Calibrate, type in 3,58 and click on OK.



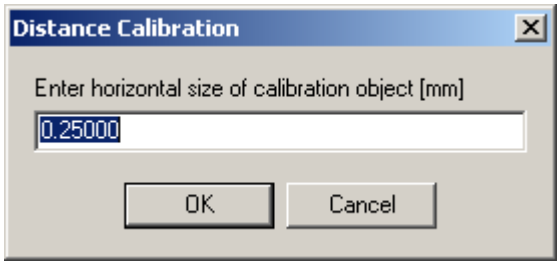
The µm/pixel field reads 5,6 and your camera is calibrated.



Camera distance measurement calibration

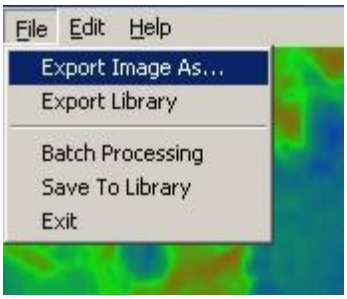
There is an approximate method to calibrate the distance. The procedure is as follows:

- Place calibration target into the middle of the field of view of the camera
- Set the blue cursor to the left edge of the calibration object
- Set the red cursor to the right edge of the calibration object
- Press “Calibrate”. The following dialog will pop up:



- Enter the width of the calibration in mm.
- Press Ok

FILE MENU



Export Image As

Saves processed images in jpeg or bmp format. Define the file name and format in the Save As dialog.

Export Library

Exports pictures from the folder where the images from a LB measurement are stored to another folder (can also be the same as for the LB measurement).

When choosing the Export Library the following Dialog window will appear.

Source Library

Select source library from the drop-down menu.

Destination Folder

Select destination folder by clicking "…" button.

File Body

Define a file name for the picture files. Files are numbered filename0001, filename0002, etc.

Raw Pictures

If checked, Raw Pictures are exported

Processed Pictures

If checked, processed pictures are exported

#Pictures

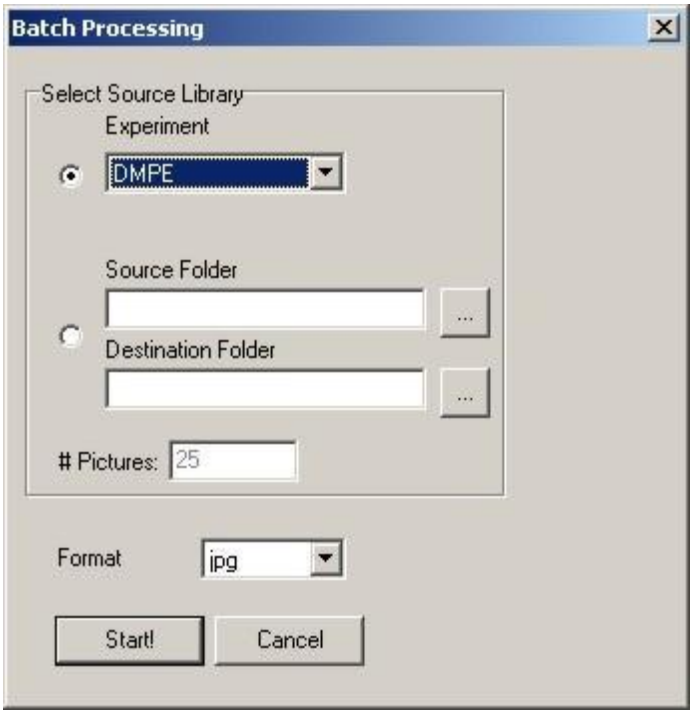
Shows how many pictures are selected (Raw and/or Processed) for exporting

Start Export

Starts the export process.

BATCH PROCESSING

Batch processing is a convenient way to apply image processing to a large library of images. Images will be processed by the current settings set in the Processing Panel



Select Source Library
Select KSV NIMA LayerBuilder Experiment from the drop-down menu. Processed images will be stored in the corresponding library and marked as processed images.

Source/Destination Folder
Use this setting if used without KSV NIMA LB software. Enter here the Source and Destination Folders for the images.

#Pictures
Indicates how many candidates for processing exists in the selected library/folder.

Format

Selects the format (jpeg or bmp) for the processed images.

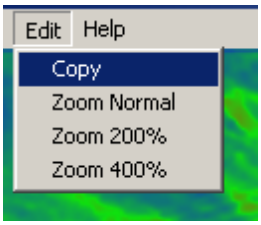
Start!

Starts processing the images. Depending on the number of images and amount of processing functions applied this operation may take some time.

SAVE TO LIBRARY

When used with the KSV NIMA LB software this command saves the current processed images to the current library. Use Control Panel's library page to switch between libraries.

EDIT MENU



COPY
Copies the current image in the Main Image Window to the Windows clipboard.

Zoom Normal
Changes the zoom back to normal

Zoom 200% and Zoom 400%
Zooms the current view to 200% or 400% correspondingly.

HELP MENU

BAM HELP

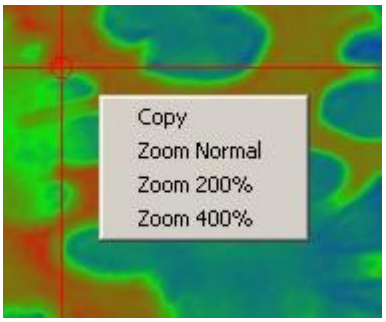
Shows the Help for the KSV NIMA MicroBAM software.

ABOUT

Shows i.e. software version number.

CONTEXT MENU

When you right-click the mouse in the Main Image Window the following menu pops up.



COPY
Copies the current image in the Main Image Window to the Windows clipboard.

ZOOM NORMAL
Changes the zoom back to normal.

ZOOM 200% AND ZOOM 400%
Zooms the current view to 200% or 400% correspondingly.

5. Performing a measurement with KSV NIMA MicroBAM

After installing the software and making the final adjustments to the KSV NIMA MicroBAM, making a measurement is quite straightforward.

If you are using a KSV NIMA LB instrument in combination with the KSV NIMA MicroBAM, the measurement is controlled directly from the LB software. Also, the images are captured, stored and named (same as the filename for LB measurement) automatically to the hard disk. This will be described in more detail below.

If you are using the KSV NIMA MicroBAM without a KSV NIMA LB system, the images have to be captured, stored and named manually. The basic use of the KSV NIMA MicroBAM software is described in the SOFTWARE OVERVIEW section. The performing of a measurement without a KSV NIMA LB instrument will briefly be outlined here.

MicroBAM with KSV NIMA LB trough

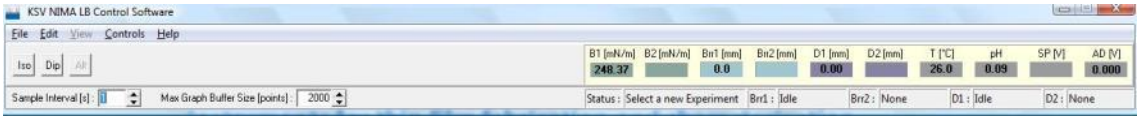
Before starting any measurements, please familiarize yourself with the procedure of making a normal Compression isotherm by reading carefully the LB manual that is delivered with your LB instrument. This should be useful as the most measurements made with the KSV NIMA MicroBAM will probably include a performance of a compression isotherm of a floating monolayer while at the same time grabbing images of the formation and changes in the monolayer domains. From the LB manual you can find hints and more detailed information about the use of the LB software for this purpose.

After cleaning the trough and barriers, pouring the subphase in the trough, cleaning the subphase surface and finalizing the adjustments of the KSV NIMA MicroBAM instrument, please make sure that the subphase surface in the trough is clean enough for making a measurement. This can be confirmed by looking on the Main Image Window (assumed this has been chosen to the Live Window) and see if it contains a lot of small bright spots (dust particles) or floating bright islands (organic contaminants). If the surface is not clean enough rinse it with an aspirator, and adjust the height of the Goniometer accordingly to obtain a dark screen when the subphase level decreases. If the screen looks OK, then proceed with the measurement according to the following procedure.

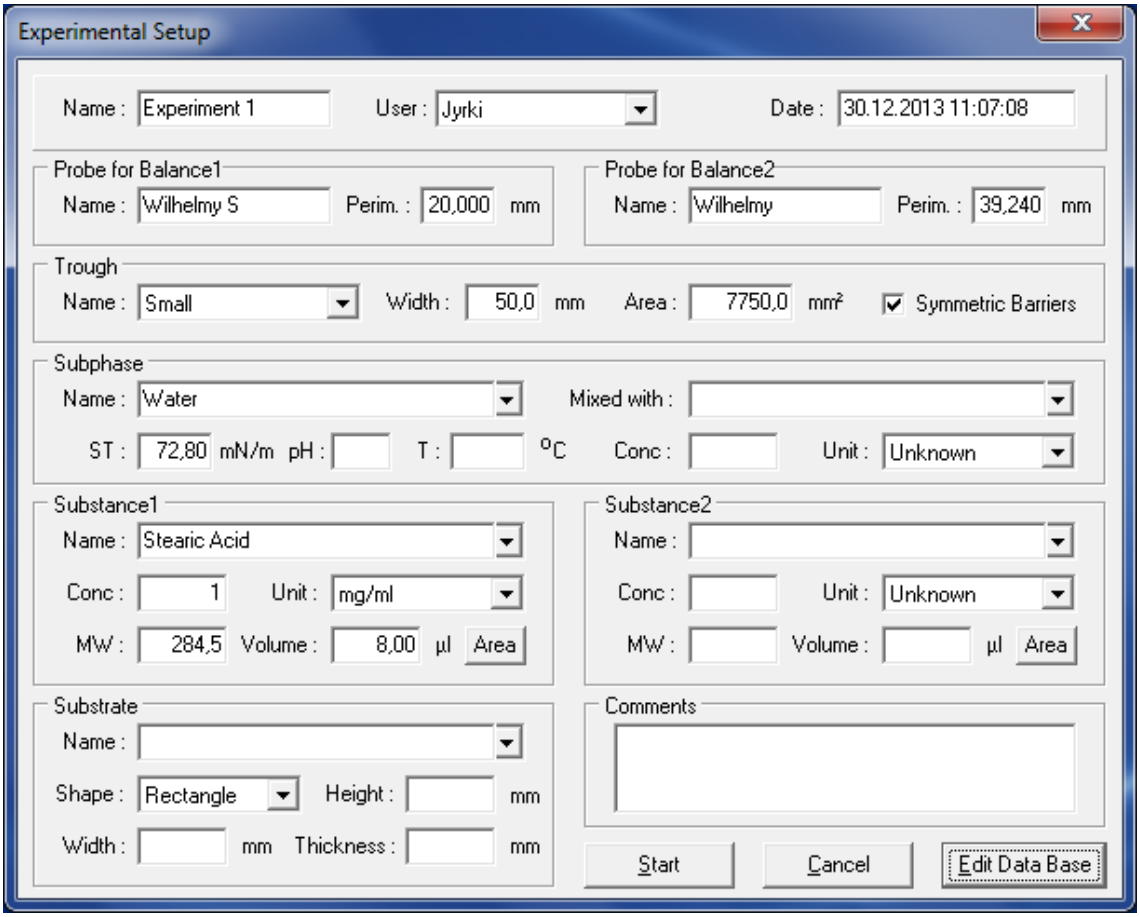
1. Choose as the Live Window the bigger screen i.e. the left Main Image Window from the Grab page. Clean the subphase surface and drive the barriers to their outmost position, zero the balance and position of the barriers from the KSV NIMA LB software Control Panel.

2. Start a new LB measurement File → New Isotherm or press the **Iso** button, 

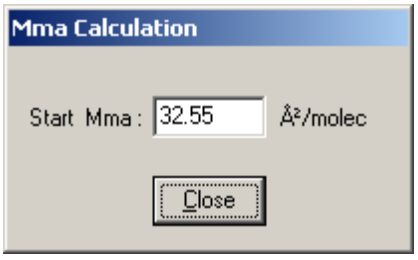
, in the LB Control Software window.



The following Experimental Setup window will appear.

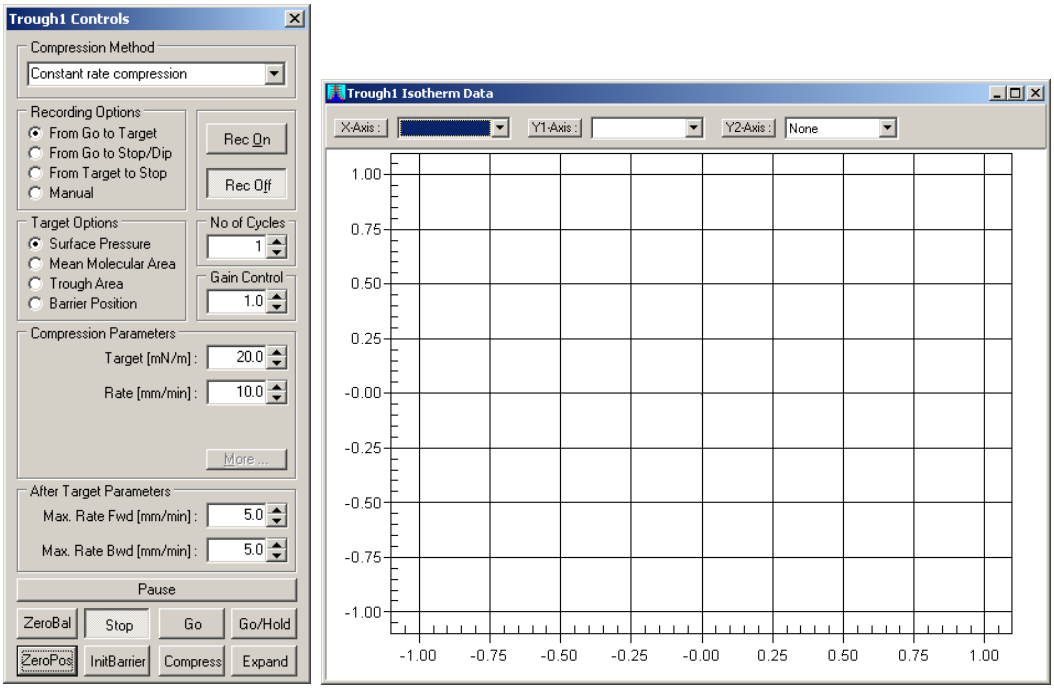


Fill in the Experimental Setup. If the concentration and molecular weight of the substance is known, and if the volume to be spread on the subphase is known the starting molecular area for the monolayer can be seen by pressing the Area button in the Substance# window.



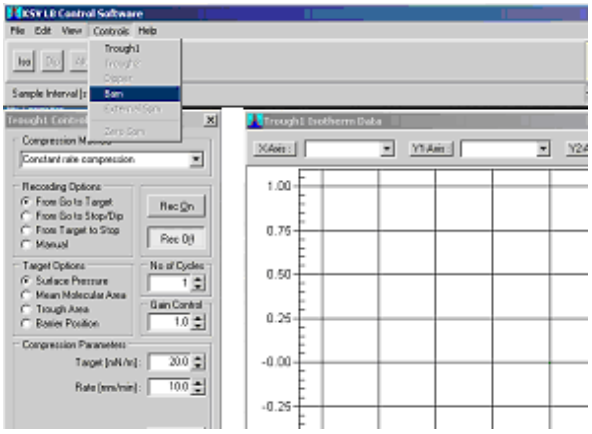
After filling in all the needed parameters in the Experimental Setup, press the Start button.

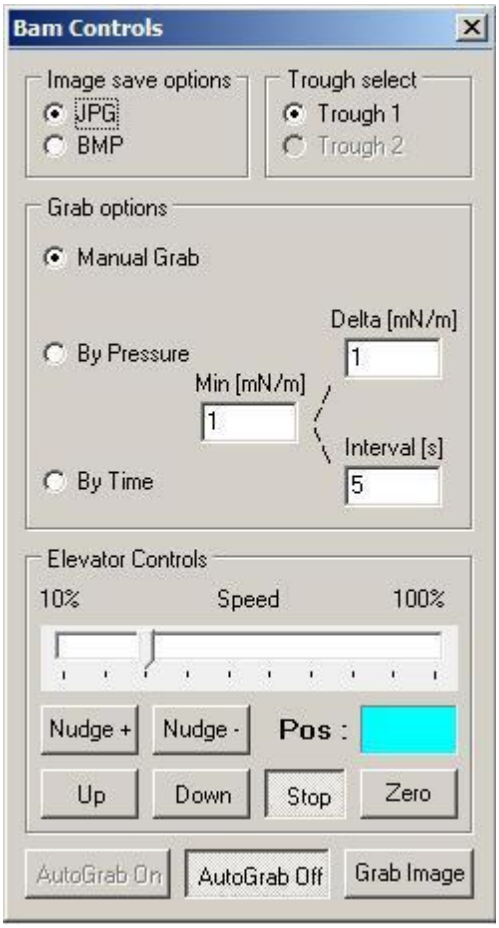
1. The Trough Control and Trough Isotherm Data windows will appear as below.



Set the parameters you like to use during the experiment in the Trough Control fields and choose the compression method. If you know that your experiment will take a long time, then increase the Sample interval value in the LB control software window.

4. Before spreading the substance on the subphase surface or starting the experiment you would need to define the parameters for capturing the images during the measurement. These parameters are set in the BAM controls window, which can be activated by choosing the Controls → BAM in the KSV NIMA LB Control Software window.





Define the parameters for your BAM measurement in this window by checking appropriate boxes and choosing suitable values for the capture intervals. The function and meaning of the boxes and options are the following:

Image save options

Saves the captured images in the chosen format. Options are JPEG and BMP format.

Trough select

Selects the trough in which the monolayer compression is performed. Trough 2 option only available for KSV NIMA Alternate LB system.

Grab options

Manual grab: Enables manual capture of images by pressing the Grab button. Manual capture of images requires that the AutoGrab Off button is activated before starting the LB measurement.

By Pressure: Captures images with a constant surface pressure interval defined in the Delta field. The capture of images is started after the value of the minimum surface pressure defined in the Min field is reached. Automatic capture of images requires that the AutoGrab On button is activated before stating the LB measurement.

By Time: Captures images with a constant time interval defined in the Interval field. The capture of images is started after the value of the minimum surface pressure defined in the Min field is reached. Automatic capture of images requires that the AutoGrab On button is activated before starting the LB measurement.

5. If a reference image is to be stored, it is useful to do this manually at this point before spreading the substance on the subphase.

6. After setting the parameters in the BAM controls window and capturing the possible reference image you can spread the substance on the subphase, wait for the solvent to evaporate and then start the measurement by pressing the Go button in the Trough Controls window.

7. After pressing the Go button the isotherm is recorded and simultaneously shown in the Trough Isotherm Data window until the surface pressure value reaches the defined Target Parameters, the barriers reaches the stoppers or alternatively the experiment is stopped by the user.

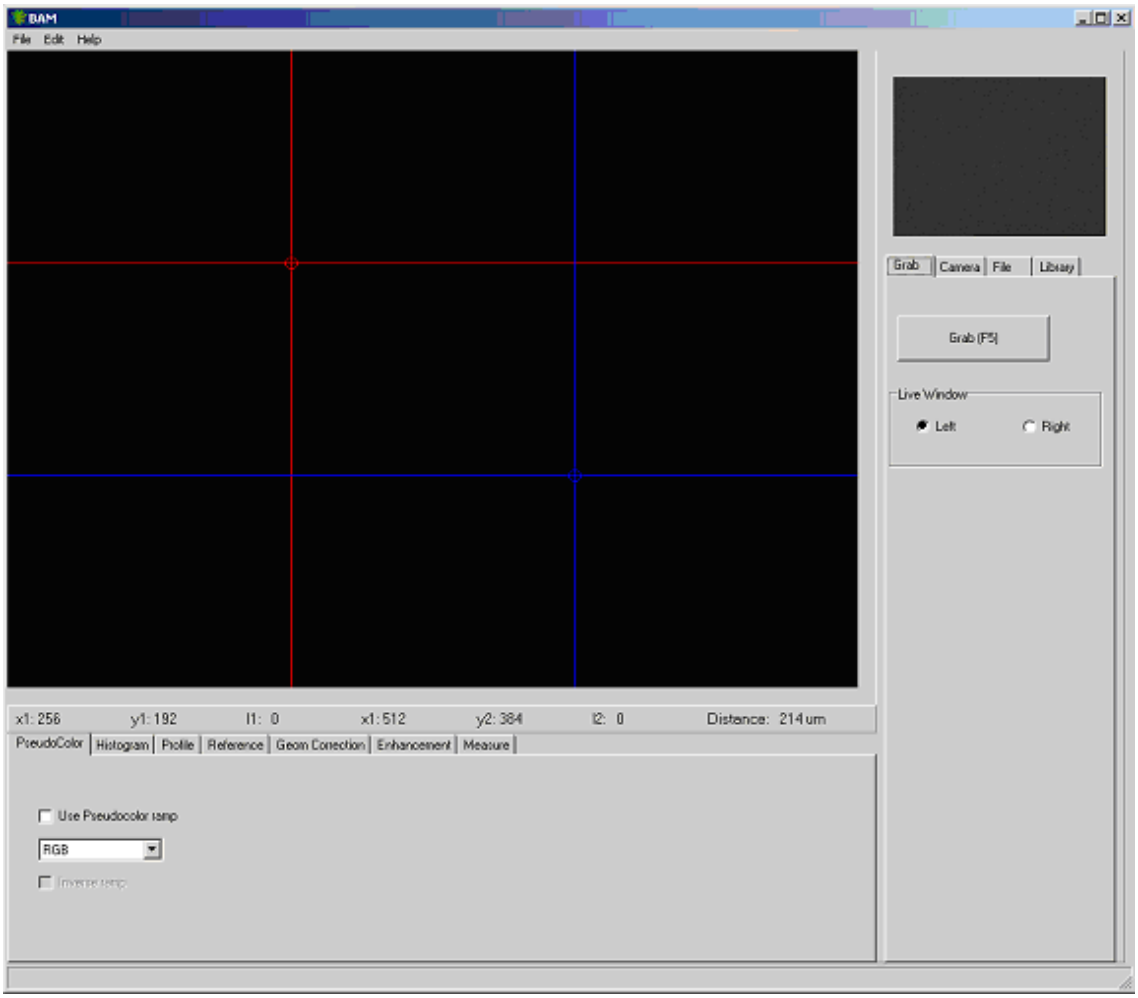
At the same time the KSV NIMA LB software controls the KSV NIMA MicroBAM software and initiates the capture of images at the values defined in the BAM controls window. The captured images are stored as separate files and the LB quantities are written into these from the KSV NIMA LB software database when the images are captured. The capture of images is stopped when the experiment is stopped by the user. Hereafter, you can past-process the images with the functions described in the SOFTWARE OVERVIEW section.

PLEASE NOTE! The image is in focus only in a narrow part of the screen due to the fact that we look on the surface in an oblique angle. This problem can be overcome by using a scanning option. However, this option is often not so useful for measurements done on a liquid surface as the domains are moving fast on the surface and the scanning option would have difficulties in keeping track of the moving domains. Usually the area in focus with the standard KSV NIMA MicroBAM setup is sufficient for most cases.

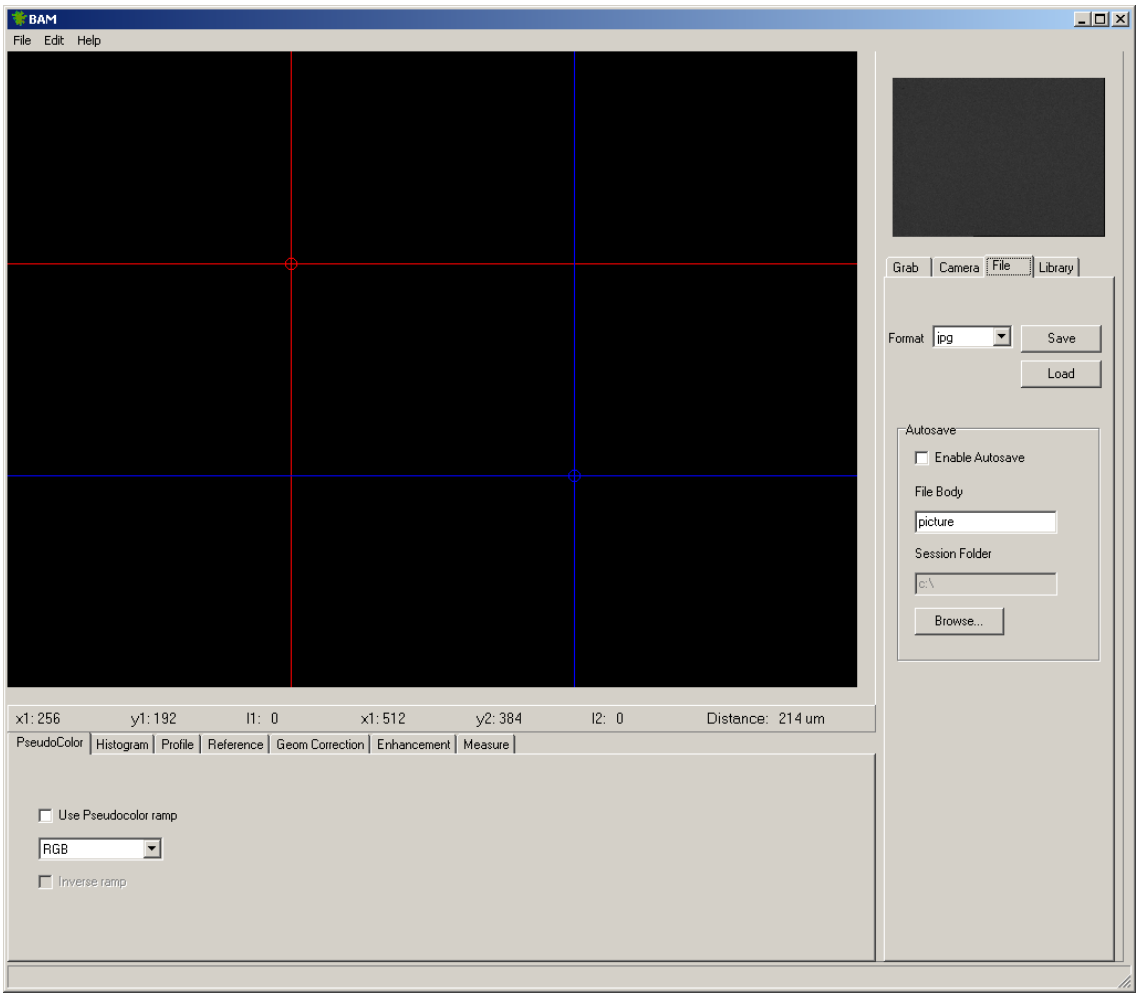
MicroBAM without KSV NIMA LB trough

After finalizing the adjustments of the KSV NIMA MicroBAM instrument, make sure that the subphase surface is still clean enough for making a measurement. This can be confirmed by looking on the Main Image Window (assumed this has been chosen to the Live Window) and see if it contains a lot of small bright spots (dust particles) or floating bright islands (organic contaminants). If the surface is not clean enough rinse it with an aspirator, and adjust the height of the Goniometer accordingly to obtain a dark screen when the subphase level decreases. If the screen looks OK, then proceed with the measurement according to the following procedure.

1. Choose as the Live Window the bigger screen i.e. the left Main Image Window from the Grab page.

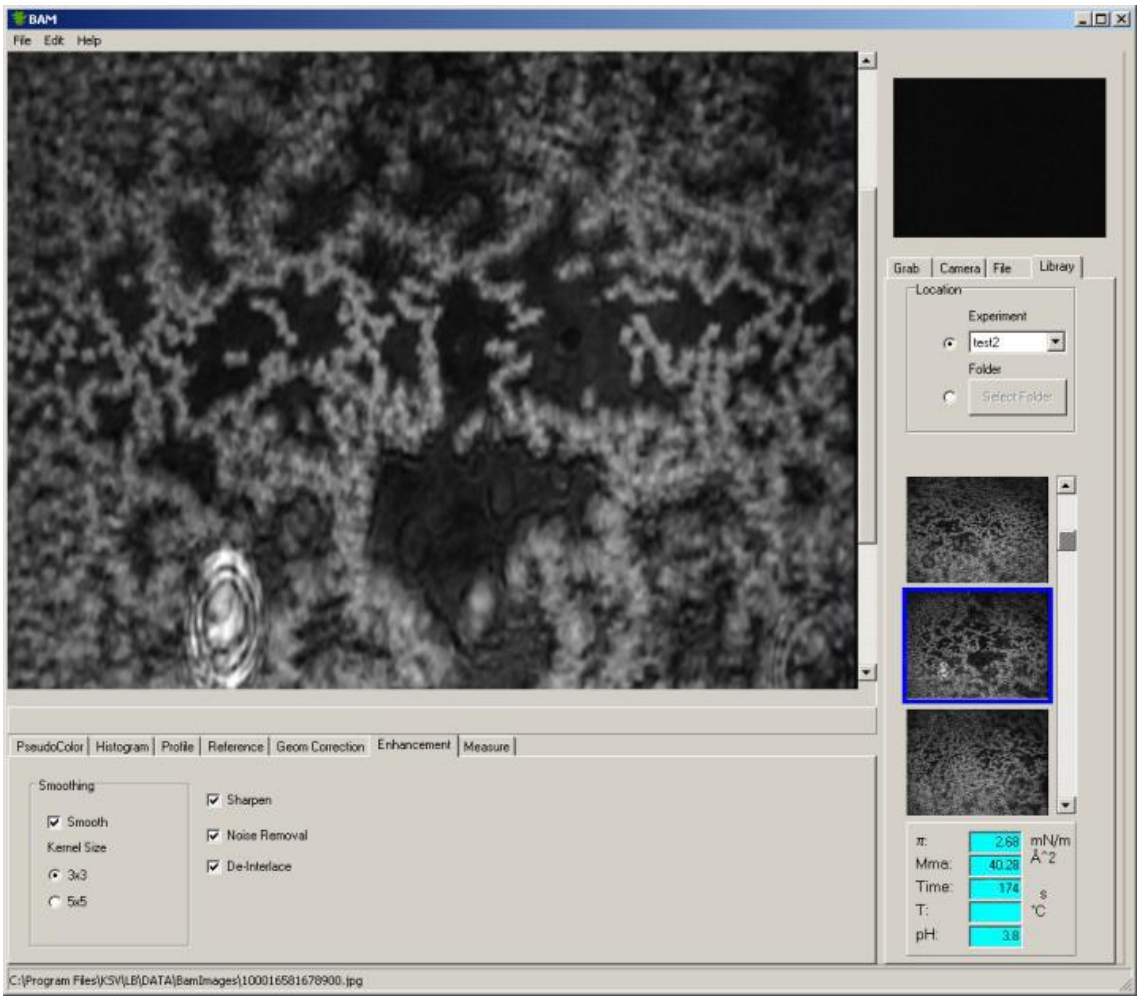


2. Define the format of the images to be captured from the Format drop down menu (alternatives; JPEG or BMP) in the File page. Here, also define the location i.e. Session Folder on your computer's hard disk where to store the captured images, and give the measurement a file name in the File Body field.



If the images should be automatically stored on your hard disk when pressing the Grab or F5 button then check the Autosave box in this section. If the Autosave box is not checked you need to save each captured image separately directly after grabbing it by pressing the Save button. If not pressing the Save button after grabbing an image the next image grabbed will overwrite the previous one in the Secondary Image window and the previous image will be lost.

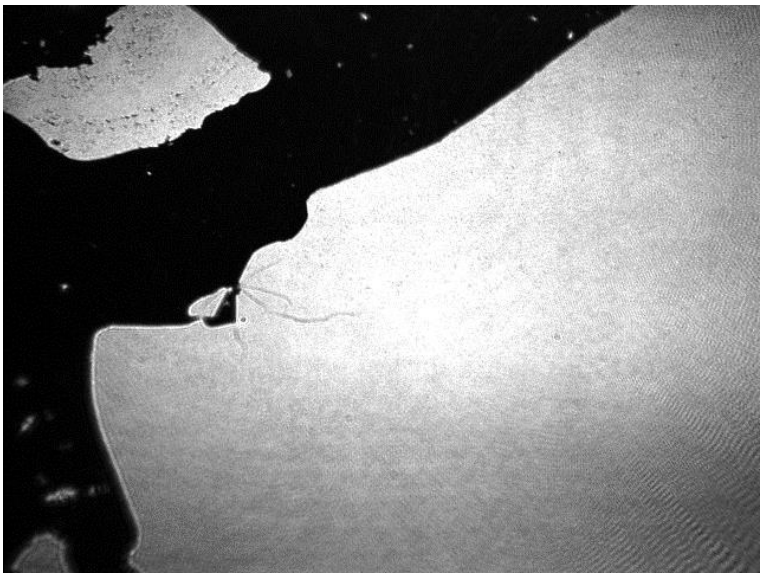
3. Spread your substance on the subphase until a suitable amount is added or until you see some domains form in the Live Window. After this, Grab and Save images whenever you want by first pressing the Grab button and then the Save button.

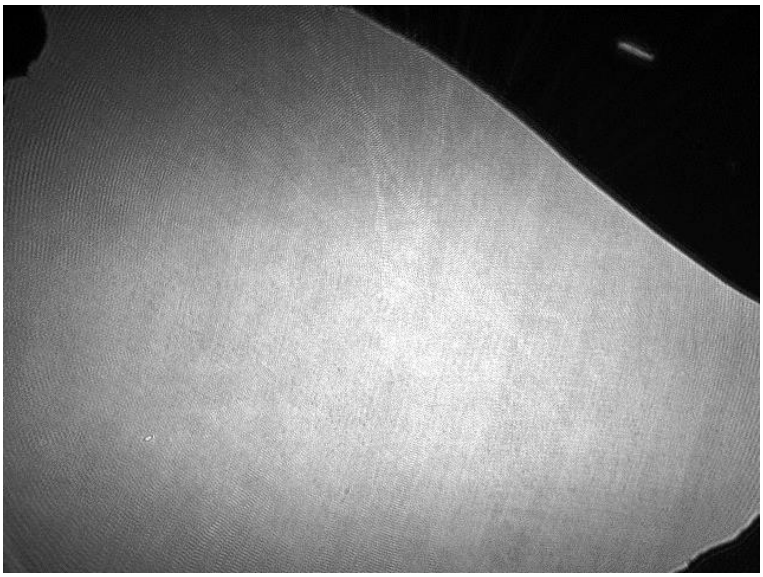


4. The measurement can be quit at any time by not taking any pictures. The pictures are then located as separate image files in the folder defined in the beginning of the measurement. Hereafter, you can postprocess the images with the functions described in the SOFTWARE OVERVIEW section.

6. Reference images

Examples of good quality MicroBAM images are presented below for your reference.

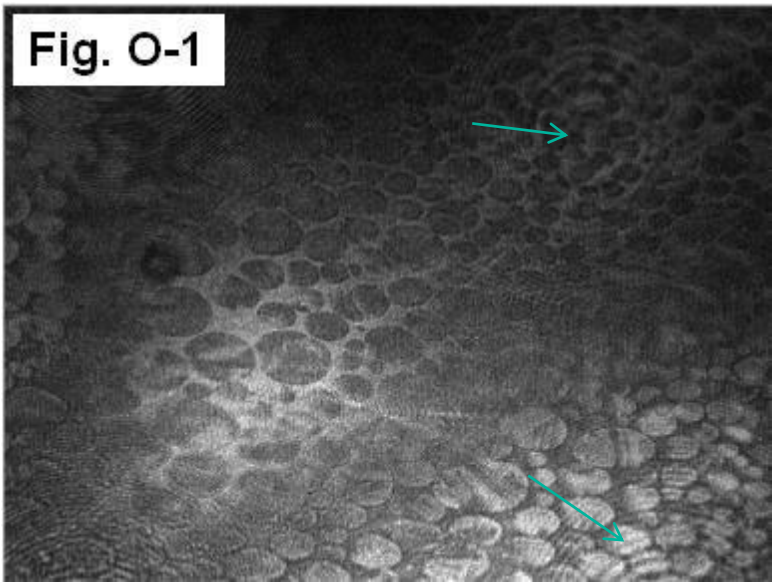




7. Image troubleshooting

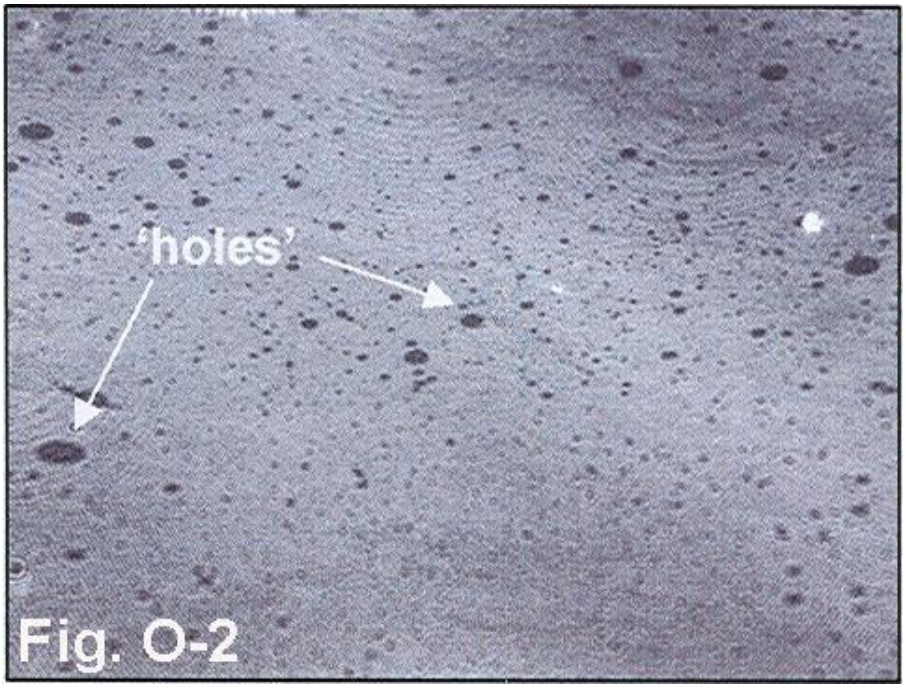
Please Note! Dust particles in the MicroBAM optics cause circular deviations to MicroBAM images (see Fig. O-1). The optics of MicroBAM is very sensitive and requires special handling. The MicroBAM should never be opened for cleaning by the users themselves. If dust particle deviations interfere with measurements, the MicroBAM should be sent to Biolin Scientific for special cleaning procedure. A "Warranty void if broken" sticker has also been placed in the microBAM to show the equipment cannot be self-serviced.

1. Ordered domains in the monolayer should appear as bright shapes on a relatively dark background.

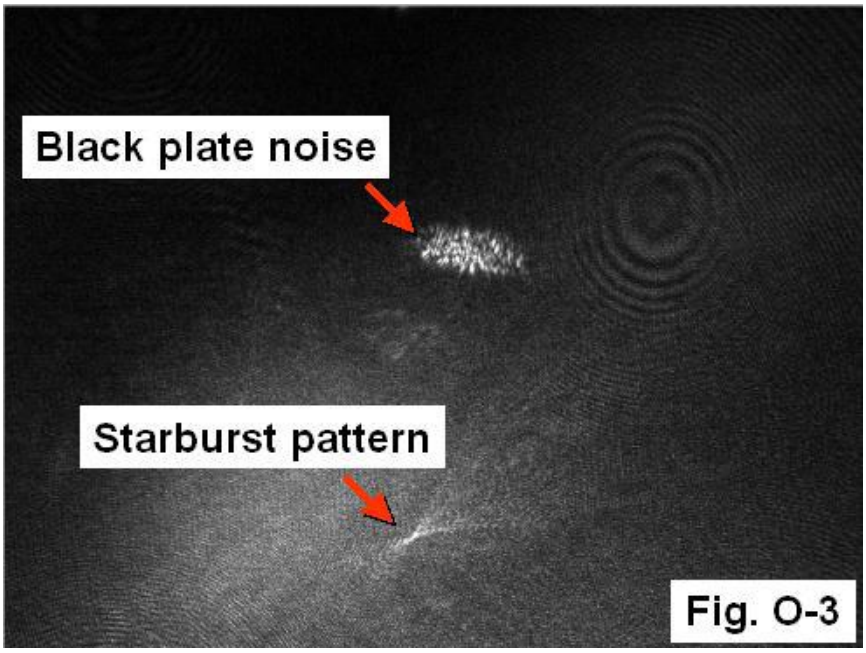


Deviations caused by dust particles shown with arrows.

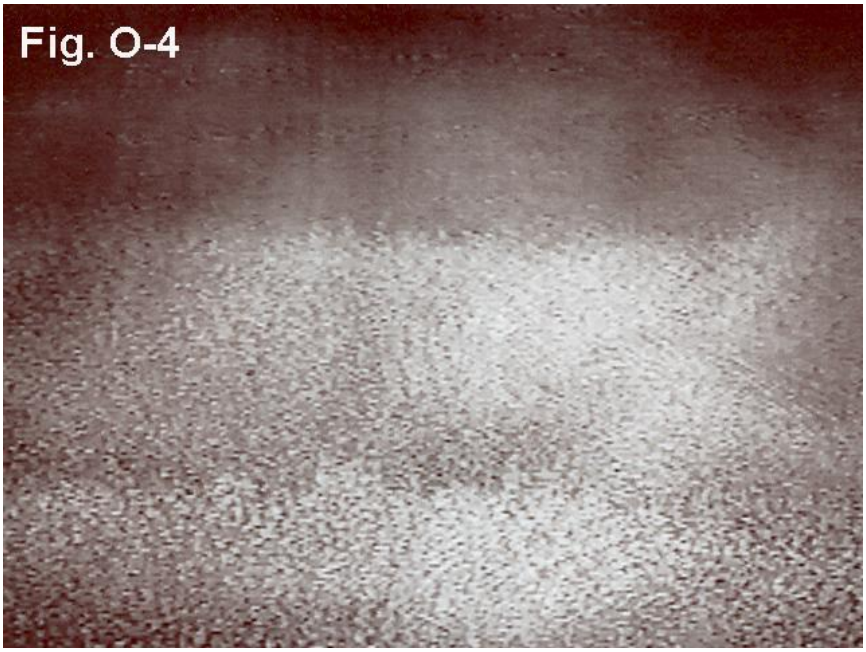
2. 'Holes' in the monolayer look like dark circular regions on a brighter background (fig. O-2). These 'holes' become bigger upon barrier expansion. Surface contaminants cause these 'holes', which are disordered regions of the monolayer. These contaminants may arise from an impure subphase surface or from an impure amphiphile sample.



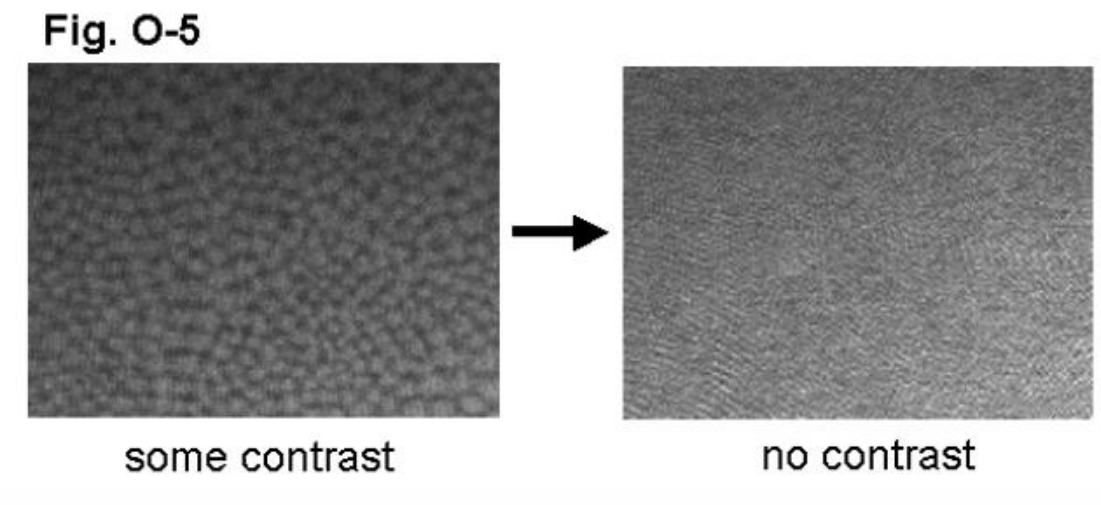
3. If you see objects on the screen that indicates dirt on the plate surface (fig. O-3).
    a. The glass plate must be cleaned to remove these objects.
    b. Starburst pattern can appear due to dirt or too high exposure and gain levels.



4. Strong shading effects across the image indicates that the hydrophobic chains of the molecules are arranged in different orientations (fig. O-4). Known as internal optical anisotropy, this condition signifies long-range orientational order of the hydrocarbon chains.



5. If you lose image contrast as you compress the monolayer, your molecules are orienting themselves into an upright position, perpendicular to the aqueous surface (fig. O-5).



6. Compress the trough barriers until you reach a surface pressure of 5-6 mN/m then stop trough barrier compression. Ordered monolayer domains form at this surface pressure which you will be able to visualize with the MicroBAM.

7. Once you see the domains of pentadecanoic acid clearly and have good contrast, you have achieved proper alignment of the MicroBAM. After the initial alignment of the microscope, adjustments you make in future MicroBAM experiments should be minor.

8. It is recommended to practice capturing video, images, saving images and editing/finishing the images.

8. Specifications

SOFTWARE	User friendly user interface, with built in controls for the KSV NIMA LB troughs.
COMPUTER REQUIREMENTS	
Recommended system requirements	2GHz processor, 2GB MB RAM, 100 GB hard disk drive (40GB free), 1280x1024 resolution, 1 USB Port
Minimum system requirements	1GHz processor, 512 MB RAM, 40 GB hard disk drive (20GB free), 1024x768 resolution
Operating system requirements	Windows 7 (32 bit and 64 bit), Windows 8 (32 bit and 64 bit), Windows 10 (32 bit and 64 bit)* *Windows 10 Anniversary Update software (version 1607) is not compatible with MicroBAM.
MECHANICS	
Goniometer	Fixed to 53 degrees for water
Vertical lift	High accuracy mechanical vertical adjustment of the MicroBAM, easy adjustment.
Mounting bridge	Flexible, customizable mounting bridge with levelling screws. Easy to install together with KSV NIMA LB troughs.
IMAGING	
Camera	Computer controlled, USB CCD camera with 640 x 480 pixels
Image processing	Continuous real time monitoring of moving objects Various dedicated image processing functions •Re-sizing •Profile •Background compensation •Geometric correction •Histogram analysis •Smoothing •Pseudo-coloring •Sharpening •De-interlacing •Image storage on hard disk
OPTICS	Laser diode, 659 nm, >20 mW, max 30 mV optical power aperture of the instrument. Laser class 3B according to DIN EN 60825-1:2001-11 (III-B according to 21 CFR 1040.10).
Field of View	3.6 mm x 4mm
Spatial Resolution	12 (horizontal image direction, centre) according to Rayleigh's criterion per pixel
Angle of Incidence	Fixed at 53°
DIMENSIONS, POWER & WEIGHT	
Measuring Head:	H: 72 mm, L: 57 mm, W: 162 mm
Stand dimensions:	H: 278 mm, L: 220 mm, W: 277 mm
Power supply:	100-240 V, 50/80 Hz (for KSV NIMA LB Interface Unit)

	USB-powered
Shipping weight:	10 kg
Shipping dimensions:	H: 350 mm, W: 560 mm, D: 440 mm
OPTIONS	•High performance PC Computer (please inquire for current specifications) •LB Troughs, several sizes and versions •Vibration isolation table

9. Contact information

If any problems arise please feel free to contact a local distributor or KSV NIMA directly.

KSV NIMA can be contacted from this address:

Biolin Scientific, KSV NIMA

Tietäjäntie 2

FIN-02600 Espoo

Finland

Tel. +358-(0)9-5497 3300

Fax +358-(0)9-5497 3333

info@biolinscientific.com for sales

support@ksvnima.com for service or technical questions

http://www.biolinscientific.com/ksvnima

Local distributors are listed at our website,
http://www.biolinscientific.com/ksvnima