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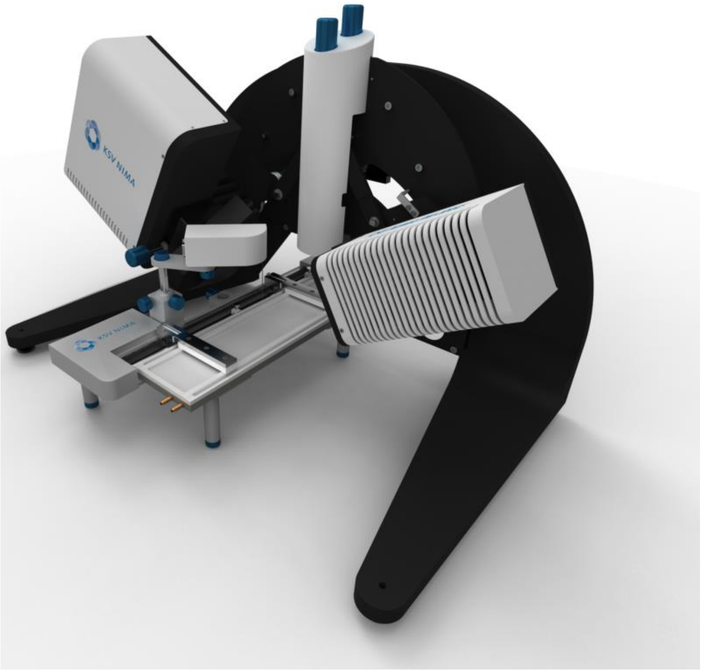
User manual



KSV NIMA PM-IRRAS

Polarization Modulation Infrared Reflection Absorption Spectrometer

Revision 1.3

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1. Introduction

The PM-IRRAS (Polarization Modulation Infrared Reflection Absorption Spectroscopy) technology allows the measuring of surface specific FT-IR spectra of materials because of the differences in the reflection of p- and s-polarized light from interfaces. PM-IRRAS contains a state-of-the-art compact Fourier Transform IR-spectrometer. The polarization modulation method eliminates background signals coming from environmental factors such as water vapor and CO_2 which removes the need for protective gasses used with other IR reflection instruments.

The spectrometer and polarization modulation units are mounted on one arm of the goniometer with a highly sensitive MCT-detector on the other arm. The spectrometer and PM-unit operate at different frequencies enabling separation of the two signals at the detector. A resolution of 8 cm^{-1} makes it possible to observe the processes of interest in the monolayer.

By positioning the spectrometer and detector on a goniometer above the surface the experimental setup is simplified and provides results within minutes. The open design also allows combined use of other complementary equipment like external UV light source and temperature heater. The instrument can be positioned above a fully equipped analytical KSV NIMA Langmuir Trough, facilitating accurate monolayer studies without restrictions.

This is the software manual for the PM-IRRAS infrared spectrometer. It features the basics of the PM-IRRAS theory, how to run the experiments, and also gives tips on how to analyze the data. If you are using the equipment together with a KSV NIMA Langmuir-Blodgett trough, please make sure to check the device-specific manual for details on Langmuir isotherms.

If any problem would appear for the product, then please contact KSV NIMA support department at www.biolinscientific.com/ksvnima/services or contact a local distributor. For further information and for the contact details of the nearest distributor, please visit our website at www.biolinscientific.com/ksvnima.

2. Theory

2.1 Overview

PM-IRRAS is an easy-to-use, highly surface specific FTIR (Fourier Transfer – Infrared Spectroscopy) method that is capable of detecting chemical compositions from interfacial films down to one molecule thick films. The PM-IRRAS technique allows enhanced detection on substrates and measurements from the air-water interface. Changes in the PM-IRRAS signal intensity and position can be used to infer for example:

- Molecular absorption/desorption behavior and kinetics
- Molecular packing and orientation
- Phase transitions
- Different surface reactions in a thin film

2.2 Background

2.3 IR Spectroscopy

IR spectroscopy is an optical measurement technique for characterization of thin films. The main benefits of the technique are that it is a non-invasive method and suitable for most interfaces. The structural information that can be obtained is based on molecules and molecular groups that have distinct characteristic spectra. [1]

In IR spectroscopy, photons of specific wavenumbers between 10^2 and 10^4 cm^{-1} induce a transition from a vibrational ground level of the molecule under study to an excited state. [2] The molecule in the excited level returns very fast to the ground state by thermal relaxation, seen as molecular vibrations. This means that when IR radiation passes through (transmittance) or reflects from a sample (solid, liquid or gas), certain frequencies of the radiation are absorbed by the molecules of the substance leading to molecular vibrations. Examination of the transmitted/reflected light reveals how much energy was absorbed at each wavelength. The amount and frequency that is absorbed can be correlated with the molecular structure of the sample, which means that each material has its own spectral “fingerprint”. Additionally, functional groups have their characteristic frequencies where they absorb.

The design of most interferometers today is based on the two-beam interferometer originally designed by Michelson in 1891 [3, 4]. In an interferometer a polychromatic beam of radiation is divided into two beams, which are recombined after a path length difference has been introduced. Thereby interference occurs between the beams. The intensity variations of the resulting beam are measured as a function of the path length difference.

The Michelson interferometer (Figure 1) consists of two mutually perpendicular mirrors, of which one can move (moving mirror). Between the stationary and moving mirror is a beam splitter, where the light of the IR source can be partially transmitted to the stationary mirror and partially reflected to the movable mirror. FTIR spectrometers operate mainly with two scan modes, either with the mirror moving continuously or only at each sampling point.

The resulting beam from the Michelson interferometer part is focused in a sample compartment, usually with the help of some mirrors. The light beam is focused on a detector after passing the sample compartment. The IR detector and sampling electronics record the irradiance changes.

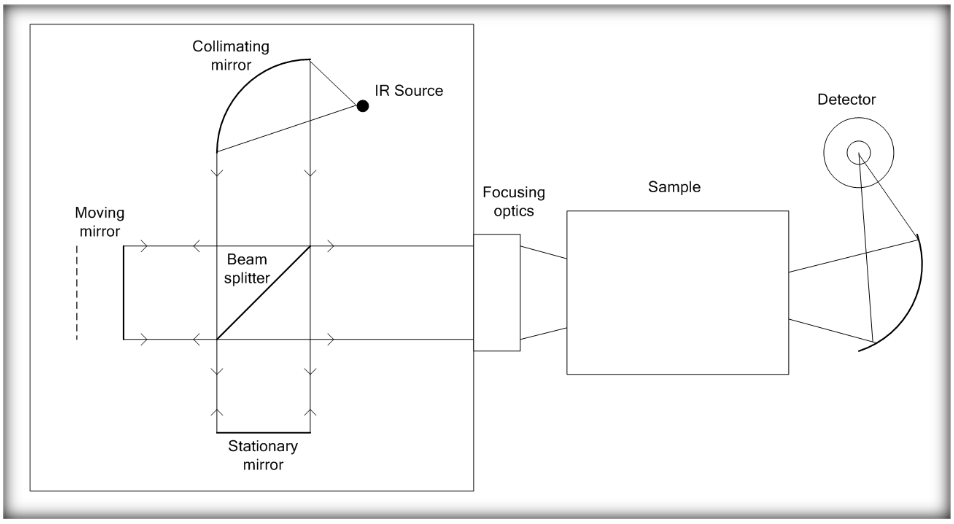


Figure 1. The principle of the Michelson two-beam interferometer.

The interferometer provides a time-domain graph showing how the interference signal changes over time. From this data, Fourier transform is used to obtain the FTIR spectrum. The spectra describes how much of the signal lies within each given frequency band over a range of frequencies. When measuring a sample, a reference spectrum (spectrum taken with a clear substrate) is used to resolve the response of the sample from the spectrum of the instrument.

2.4 Water in IR spectroscopy

One of the problems in FTIR spectroscopy is that water absorbs IR light, and shows strong absorption bands around 1700 and 3700 cm^{-1}. [2] Usually, the amount of water vapour in the atmosphere within the spectrometer is reduced by purging with dry nitrogen gas or by putting the optical part of the FTIR spectrometer in vacuum. Additionally, the path length of the IR light should be kept as small as possible when exposing the measured system to atmospheric conditions.

A FTIR spectrum can be obtained either in reflection or transmission mode. In transmission mode, the measurement requires IR -transparent substrates. In biological applications, water is a common subphase for thin films. Since water is a strong absorber in the infrared region, measurements on water surfaces generally require the use of a reflective measurement. The measurements at reflective mode enable molecular scale analysis of thin films at air-water, air-solid, and even liquid-solid interfaces.

Infrared Reflection Absorption Spectroscopy (IRRAS) is a known spectroscopy method for molecular characterization of thin films on water. However, the method necessitates running successive long-time measurements with a covered and uncovered water surface. This is because of the drift in the FTIR spectrometer signal and strong absorption of the water vapour that evaporates from the surface. As a result, the most interesting spectral region remains hidden. These effects can be minimized using a polarization modulation (PM) unit in combination with an IRRAS -measurement.

2.5 KSV NIMA PM-IRRAS

The KSV NIMA PM-IRRAS consists of an FT-IR spectrometer mounted on one arm of a goniometer and a polarization modulation unit mounted on the other arm (Figure 2). The angle and height of the interferometer and detector can be adjusted using the goniometer. The instrument can be positioned above a fully equipped analytical KSV NIMA Langmuir Trough for studies on water surface. Alternatively, a horizontally leveled solid sample can be used.

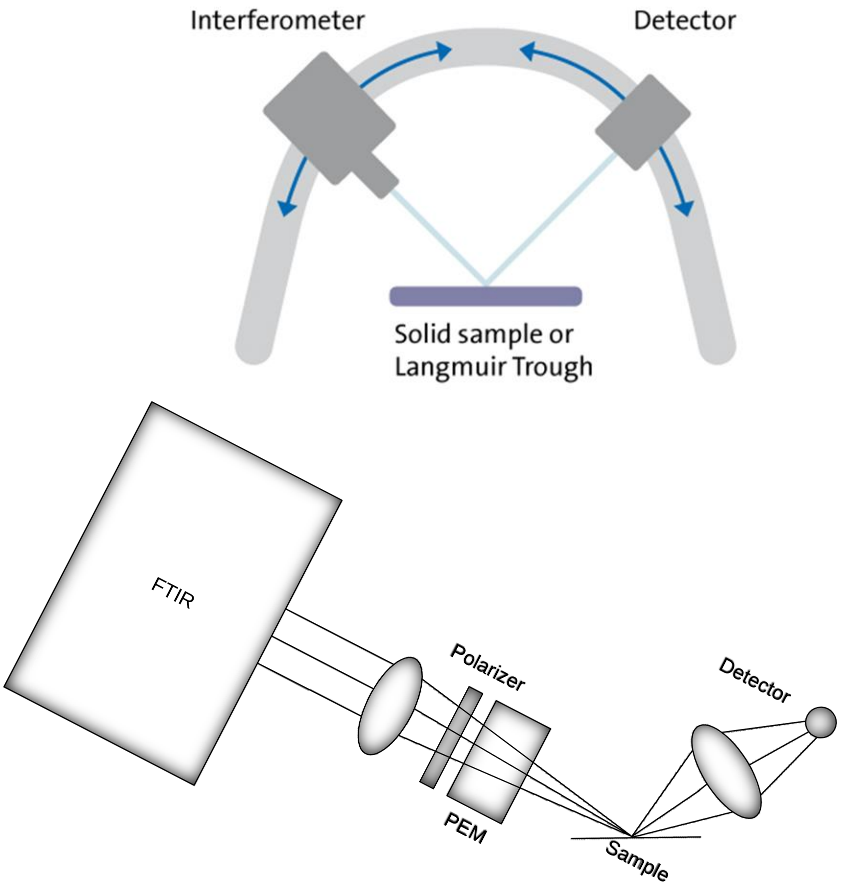


Figure 2.The set-up of KSV NIMA PM-IRRAS.

The operation principle of the KSV NIMA PM-IRRAS is based on a reflective IR measurement in combination with a PM -unit. In order to interpret the PM-IRRAS data, it is essential to understand the principle of the PEM (photo-elastic modulator), the angle of incidence and the demodulation process at the detector. This chapter introduces the basic principle of operation. For deeper understanding, detailed reference material can be found under ‘References and further reading’.

2.5.1. Polarization Modulation

When light is reflected from a surface, the Fresnel equations describe the amount of reflected (and transmitted) light. [5] The incoming light can be split into a polarized component parallel to the plane of reflection and a component perpendicular to this plane (Figure 3). By knowing that light with different polarisations interacts differently with the interface it is reflected from, we can use polarisation modulation to reduce the noise of reflective FTIR -measurements and to compensate for the water vapour absorption bands.

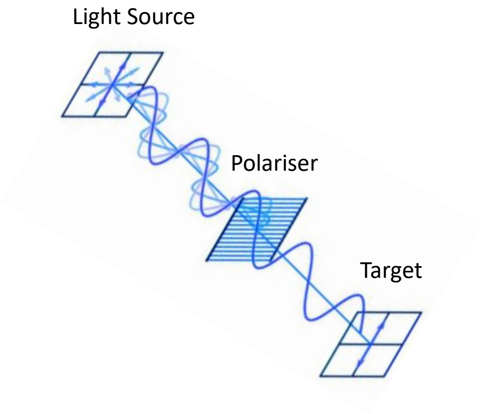


Figure 3. Polarization of light from a monochromatic light source.

Polarisation modulation can be realized using a mechanically rotated polarizer, but these polarizers are generally slow and require a mechanical connection. In KSV NIMA PM-IRRAS, a photo-elastic modulator from Hinds Instruments is used to modulate the polarization of the light in addition to the intensity modulation of the spectrometer (Figure 4). The frequencies of these modulations differ so that the demodulation of the signals can be separated.

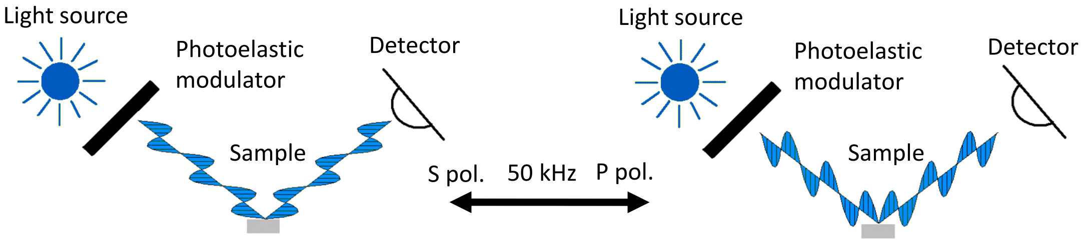


Figure 4. The principle of the photo-elastic modulator.

In the photo-elastic modulator, the birefringence of an IR transparent crystal is controlled by applying a variable stress by electric transducers. This causes a variable optical retardation in one optical axis. When PEM is oriented at 45 degrees from the plane of incidence of the beam and operated at half-wave retardation, s-polarized light is produced at the retardation maxima, while p-polarized light is produced at zero PEM retardation. The modulation depth follows the shape of the Bessel function, see Technology Primer from Hinds Instruments [6] for a more in-depth description. Both, s- and p- polarized light are collected at the detector.

2.5.2. Demodulation

The measured signal at the detector consists of two parts; a high frequency component from the difference Δ in light intensities between the p- and s-polarized light in the photo-elastic modulator and a low frequency component from the sum Σ of these signals from the FTIR unit. These signals contain different information and are processed in two different ways. The demodulation is performed using a Lock-In Amplifier (LIA), where an arbitrary, adjustable phase angle is used. After Fourier transform, the final FTIR spectrum is obtained. The normalized differential reflectivity spectrum S is calculated from the collected difference (ΔR) and sum spectra (ΣR) of the detected intensities of the p- and s- polarized light as;

$$S = \frac{\Delta R}{\Sigma R} = \frac{(R_s - R_p)}{(R_s + R_p)} \quad \text{(Eq.1)}$$

The PM-IRRAS spectrum obeys the second order Bessel function. The shape can be seen as a wavenumber dependent efficiency function for the polarization modulation. The PEM should be adjusted so that a maximum value of the second order Bessel function is obtained in the wavenumber region of interest. For biological layers, the most interesting wavenumber regions are the fingerprint region around 1500 cm^{-1} and the CH absorption region around 3000 cm^{-1}. To measure both of these regions, the PEM should be tuned to approximately 1500 cm^{-1}. [2]

In order to detect the chemical information about the layer, the spectrum should be corrected. The Bessel function can be corrected for example using a baseline or a mathematical second order Bessel function. In practice, it is much easier to determine the spectrum also for a bare substrate and take a ratio of the two spectra. Essentially, this gives the normalized and background corrected spectra where the peaks can be easily analysed from.

2.5.3. Angle of incidence

The angle of incidence has an effect on the intensity of the detected PM-IRRAS signal.

On a dielectric surface, such as a thin phospholipid or fatty acid monolayer on water, electric fields both perpendicular and parallel to the substrate surface are present. Now, the field can interact with both transition dipole moments on the surface. The magnitude for the absorbance of a thin film parallel and perpendicular to the surface can be calculated based on the refractive indices of the monolayer and the substrate, and these results can be presented as a function of the angle of incidence. For a thin film on water surface, the optimum angle of incidence is 76° [7].

The basic interpretation of the PM-IRRAS band directions is illustrated in Figure 5. At an incident angle of 76°, an upward oriented band indicates a transition moment preferentially parallel to the surface of the substrate, whereas a downward oriented band indicates an orientation preferentially perpendicular to the substrate surface. [8]

For a metal surface, the electric field is maximized around 80°. On a metal surface s-polarized light (parallel to the metal surface) shows no IR absorbance, and the electric field is oriented according to the p-polarized component (perpendicular to the surface).

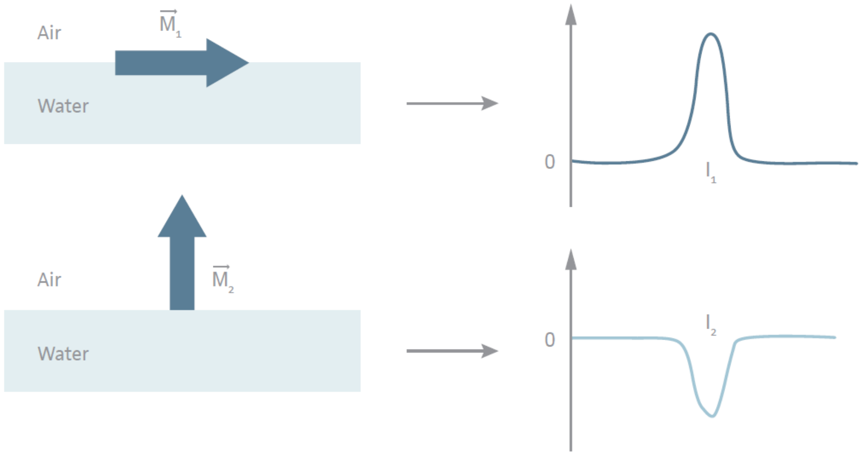


Figure 5. Schematical presentation of the linking between the transition moment orientation and the sign and intensity of the PM-IRRAS absorption band at the air-water interface at an angle of incidence of 76°. Adapted with permission [8]. Copyright 1996 American Chemical Society.

2.5.4. Transmission experiments

Polarization modulation experiments can be done in reflection or transmission, and for different angles of incidence. In transmission geometry the measured noise of the IR light is increased because the projection of the effective sample surface on the detector becomes smaller than the detector area. Reflection experiments are more suited for a high angle of incidence because the sample surface area is used more efficiently when compared to transmission experiments.

2.5.5. Advantages

In traditional FTIR, the amount of light measured on the detector is very high and only a small part of the total light that is absorbed by the thin layer is measured. With the signal coming from the polarization modulated light, the intensities of the p- and s-polarized light are equal and only the absorbance of the thin layer at the substrate surface causes differences in the light intensity. In this case, the measured signal is caused by the difference in absorption between p- and s-polarized light instead of the absorbance of the thin film. Because this signal can be amplified before passing to the AD-converter, the signal-to-noise ratio is better than in a regular IRRAS -measurement.

The PM-IRRAS sum spectrum (denominator in Eq. 1) is used to compensate for the water vapour in the signal. The absorbance of all random oriented absorbents, such as CO_2 and water vapour cancels out when the sum spectrum is measured simultaneously with the delta spectrum. The simultaneous measurement also allows longer measurement times, because changes in the light source intensities or water surface height changes do not affect the final spectrum. In normal IRRAS -measurements the reference and measurement signals have to be taken separately, which increases the chance for errors.

2.5.6. Example PM-IRRAS spectra

For most molecules the IR spectrum will show several absorption bands. In most cases these bands are caused by fundamental vibrations. Fundamental vibrations can be either stretching, bending or torsion vibrations. Generally, these happen at different wavenumber regions. The vibrations can also be classified by symmetry, where symmetrical vibrations retain the symmetry of the group and asymmetrical vibrations disturb the symmetry elements of the molecule.

Many structural groups have vibrational wavenumbers that are nearly independent of the rest of the molecule. Characteristic wavenumbers of vibrations for most groups of interest are in the 800-4000 cm^{-1} region. Generally, two interesting regions can be distinguished; the absorption bands of the CH stretching vibrations are visible around 3000 cm^{-1} and many other vibrations are visible in the fingerprint region from 800 to 1800 cm^{-1}.

Figure 6 presents an example PM-IRRAS spectra of Stearic acid (SA) deposited using a Langmuir-Blodgett trough with a subphase containing 0,1 mM of $CdCl_2$. Salts are generally used in the subphase to improve the stability of the monolayer. The spectra show the CH_3 symmetric stretch (2960 cm^{-1}) and CH_2 symmetric (2850 cm^{-1}) and asymmetric (2918 cm^{-1}) stretches. In the pH 2.5 spectrum, one can observe an upward oriented C=O band at 1710 cm^{-1}. In the pH 7 spectrum, there is a downward oriented band at 1650 cm^{-1}, which indicates strong water absorption [7]. Also, there is a clear peak at 1540 cm^{-1} for the COO^- asymmetric stretch. This shows that at a lower pH, the stearic acid molecule remains protonated, i.e. the carboxyl group -COOH is in its native form. At a higher (neutral) pH, the -COOH group is able to dissociate and react with the salt in the subphase, resulting in a deprotonated COO^- -group and strong water absorption.

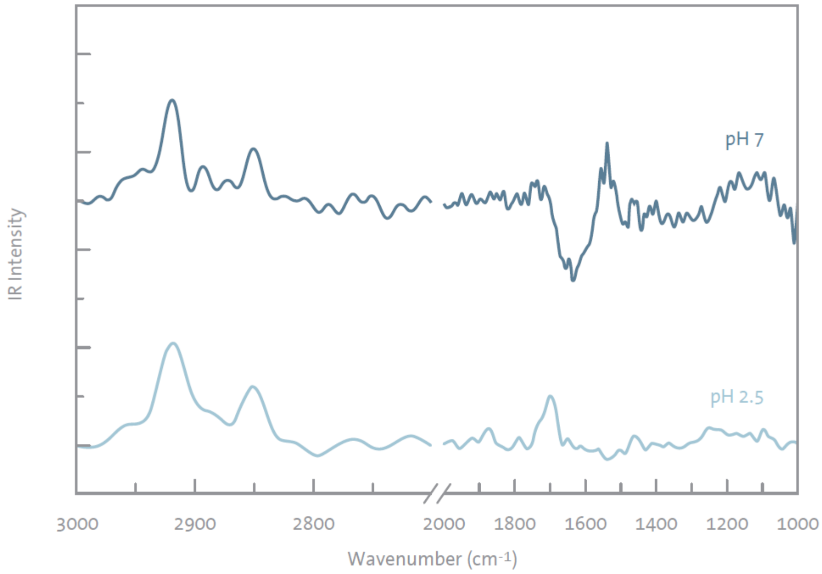


Figure 6. PM-IRRAS spectra of Stearic acid Langmuir films at pH 2.5 and pH 7.

Figure 7 represents the spectra for layers of Stearic acid deposited on a gold subsbrate at different subphase acidities, pH 2 and pH 6. From the PM-IRRAS spectra, it is possible to distinguish the CH_3 symmetric stretch (2960 cm^{-1}) and CH_2 symmetric (2850 cm^{-1}) and asymmetric (2918 cm^{-1}) stretches.

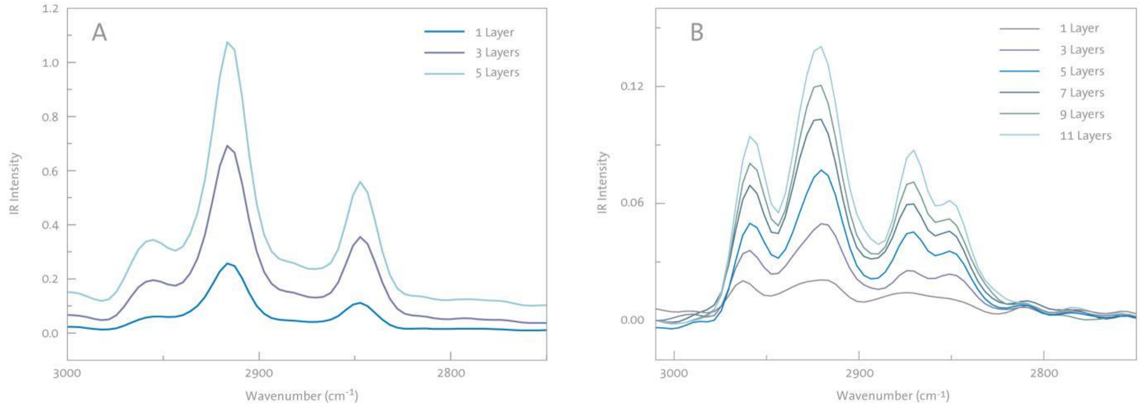


Figure 7. PM-IRRAS spectra of SA Langmuir-Blodgett films of increasing thickness on gold at (A) pH 2 and (B) pH 6 in the C-H stretch region

The fingerprint region for the films is presented in Figure 8. For the films made at pH 2, the C=O stretch (1710 cm^{-1}), COO^- symmetric stretch (1430 cm^{-1}) and CH_2 wagging and twisting (1200-1350 cm^{-1}) were identified. For pH 6 films there was no C=O stretch, but the COO^- symmetric stretch was much more pronounced and also COO^- asymmetric stretch (1540 cm^{-1}) was identified. This clearly shows the effect of the dissociated carboxyl groups at pH 6.

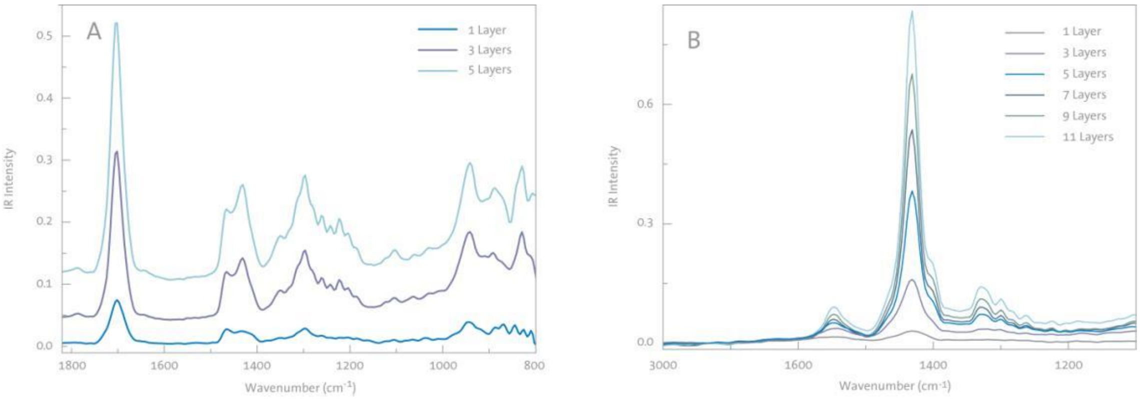


Figure 8. PM-IRRAS spectra of SA Langmuir-Blodgett films of increasing thickness on gold at (A) pH 2 and (B) pH 6 between 1800 and 800 cm^{-1}.

2.6 References and further reading

1) Griffiths, P. R.; de Haseth, J. A., Fourier Transform Infrared Spectrometry, John Wiley & Sons, INC., USA 1986.

2) Kosters, P., FT-IR Spectroscopy of thin biological layers, University of Twente, 2000.

3) Michelson, A.A., Phil. Mag. 1891, 31 (5), 256.

4) Michelson, A.A., Light Waves and Their Uses, University of Chicago Press, Chicago 1902

5) Reitz J.R.; Milford F.J.; Christy R.W., Foundations of electromagnetic theory, 3rd Ed., Addison-Wesley, Amsterdam 1979.

6) Photoelastic Modulator Technology Primer, Hinds Instruments, http://www.hindsinstruments.com/knowlege-center/technology-primer/

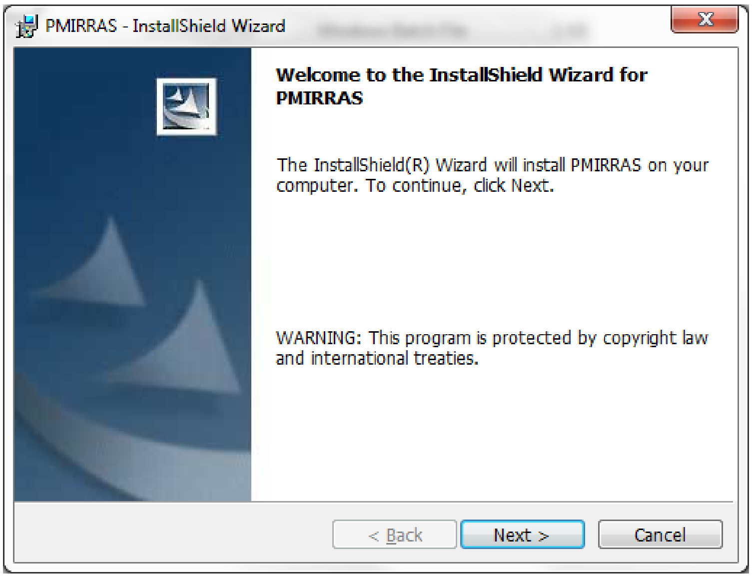
7) Blaudez D.; Buffeteau T; Cornut J.C.; Desbat B.; Escafre N.;Pezolet M.; Turlet J.M., Appl. Spectrosc. 1993, 47 (7), 869.

8) Payan, S.; Desbat, B.; Destrade, C.; Nguyen, H.T., Langmuir 1996, 12, 6627.

3. Software installation

In order to install the PM-IRRAS software, select INSTALL.bat in the software installation folder. This batch file will install all the necessary PM-IRRAS software components. Alternatively, you can install both ippsetup.exe and setup.exe separately. If your operating system asks to give permissions for the installer to modify computer's hard drive, always agree.

PM-IRRAS InstallShield Wizard opens. Select Next.



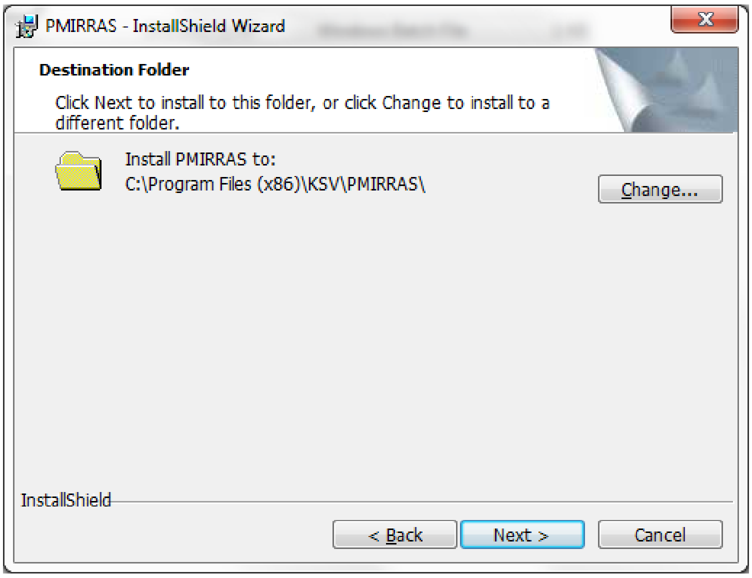
PMIRRAS - InstallShield Wizard

Welcome to the InstallShield Wizard for PMIRRAS

The InstallShield(R) Wizard will install PMIRRAS on your computer. To continue, click Next.

WARNING: This program is protected by copyright law and international treaties.

Select the destination folder for PM-IRRAS installation. Select Next.



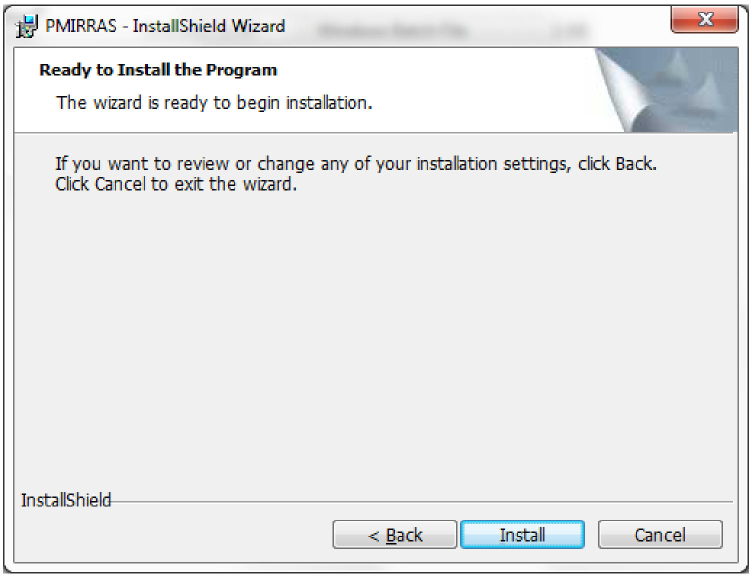
PMIRRAS - InstallShield Wizard

Destination Folder

Click Next to install to this folder, or click Change to install to a different folder.

Install PMIRRAS to:
C:\Program Files (x86)\KSV\PMIRRAS\

Select Install.

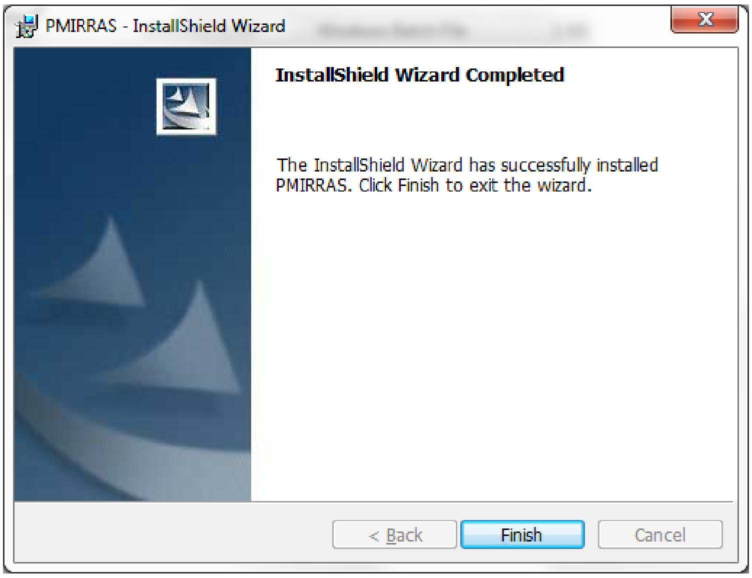


Ready to Install the Program

The wizard is ready to begin installation.

If you want to review or change any of your installation settings, click Back. Click Cancel to exit the wizard.

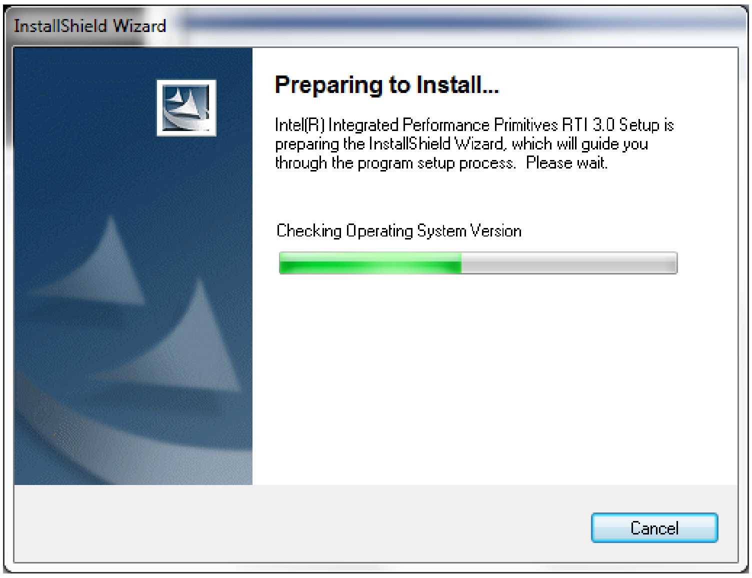
Select Finish.



InstallShield Wizard Completed

The InstallShield Wizard has successfully installed PMIRRAS. Click Finish to exit the wizard.

Next, the installer will automatically install a programming library. The installer will open and automatically close when the installation is complete. When also the black command prompt window closes, the installation is complete.



Intel(R) Integrated Performance Primitives RTI 3.0 Setup is preparing the InstallShield Wizard, which will guide you through the program setup process. Please wait.

Checking Operating System Version

The software can be uninstalled in Windows using the “Programs and Features” functionality in Windows Control Panel. Both PMIRRAS and Intel Integrated Performance Primitives need to be removed.

3.1 Setting the amplitude and the offset

The interferometer of KSV NIMA PM-IRRAS has two settings that ensure maximum signal strength: amplitude and offset. The optimal values are determined by KSV NIMA on factory site and they can be found on the delivery document that arrives with the instrument.

Note! With newer PM-IRRAS instruments the amplitude and offset settings are saved to instrument hardware. If your instrument serial number is ABH55023 or higher, setting the amplitude and offset on your computer is not required.

The parameters are set on the computer that the PM-IRRAS is used on during the PM-IRRAS installation by KSV NIMA specialist. If for some reason the PC needs to be changed after the installation, follow these instructions to update the amplitude and offset values:

1) Locate the pmirras.ini file at your PC after PM-IRRAS software installation. The place of the file depends on the operating system, for example in Windows 7 it can be typically found in C:\Users\username\AppData\Local\VirtualStore\Program Files (x86)\KSV\PMIRRAS\. Please notice “AppData” may be a hidden folder but will open after you type the path into the explorer.

2) Locate the amplitude and offset values specific for your instrument in your delivery document.

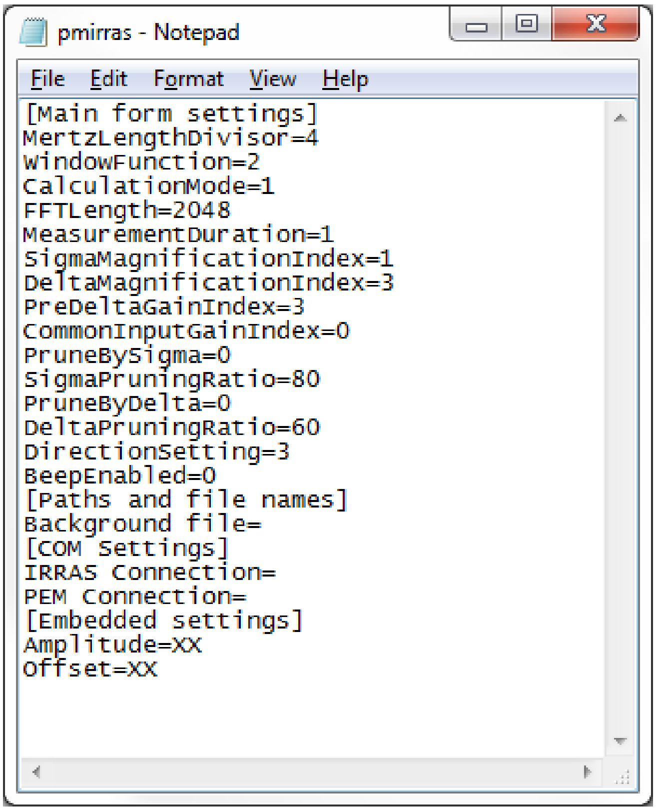
3) Open the pmirras.ini using for example notepad. To the end of the file, write

[Embedded settings]

Amplitude=xx

Offset=yy

where xx and yy are the amplitude and offset values for your system.

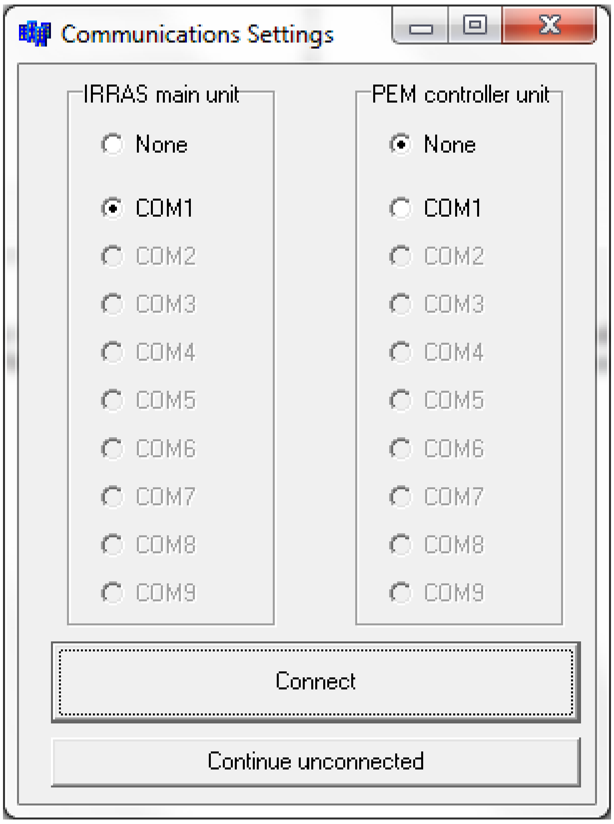


```
[Main form settings]
MertzLengthDivisor=4
WindowFunction=2
CalculationMode=1
FFTLength=2048
MeasurementDuration=1
SigmaMagnificationIndex=1
DeltaMagnificationIndex=3
PreDeltaGainIndex=3
CommonInputGainIndex=0
PruneBySigma=0
SigmaPruningRatio=80
PruneByDelta=0
DeltaPruningRatio=60
DirectionSetting=3
BeepEnabled=0
[Paths and file names]
Background file=
[COM settings]
IRRAS Connection=
PEM Connection=
[Embedded settings]
Amplitude=XX
Offset=XX
```

4) Save the changes and close the file.

4. Software components

The PM-IRRAS software is used for both performing measurements and accessing old measurement results. When the software is opened, a Communications Settings window opens. In this window, you must select the correct COM ports for both IRRAS main unit and PEM controller unit according to your setup. Once the correct COM ports have been chosen, select “Connect” and a connection is established to the devices. If you only wish to browse old measurements, you can also select “Continue unconnected”.



Communications Settings

IRRAS main unit
○ None
● COM1
○ COM2
○ COM3
○ COM4
○ COM5
○ COM6
○ COM7
○ COM8
○ COM9

PEM controller unit
● None
○ COM1
○ COM2
○ COM3
○ COM4
○ COM5
○ COM6
○ COM7
○ COM8
○ COM9

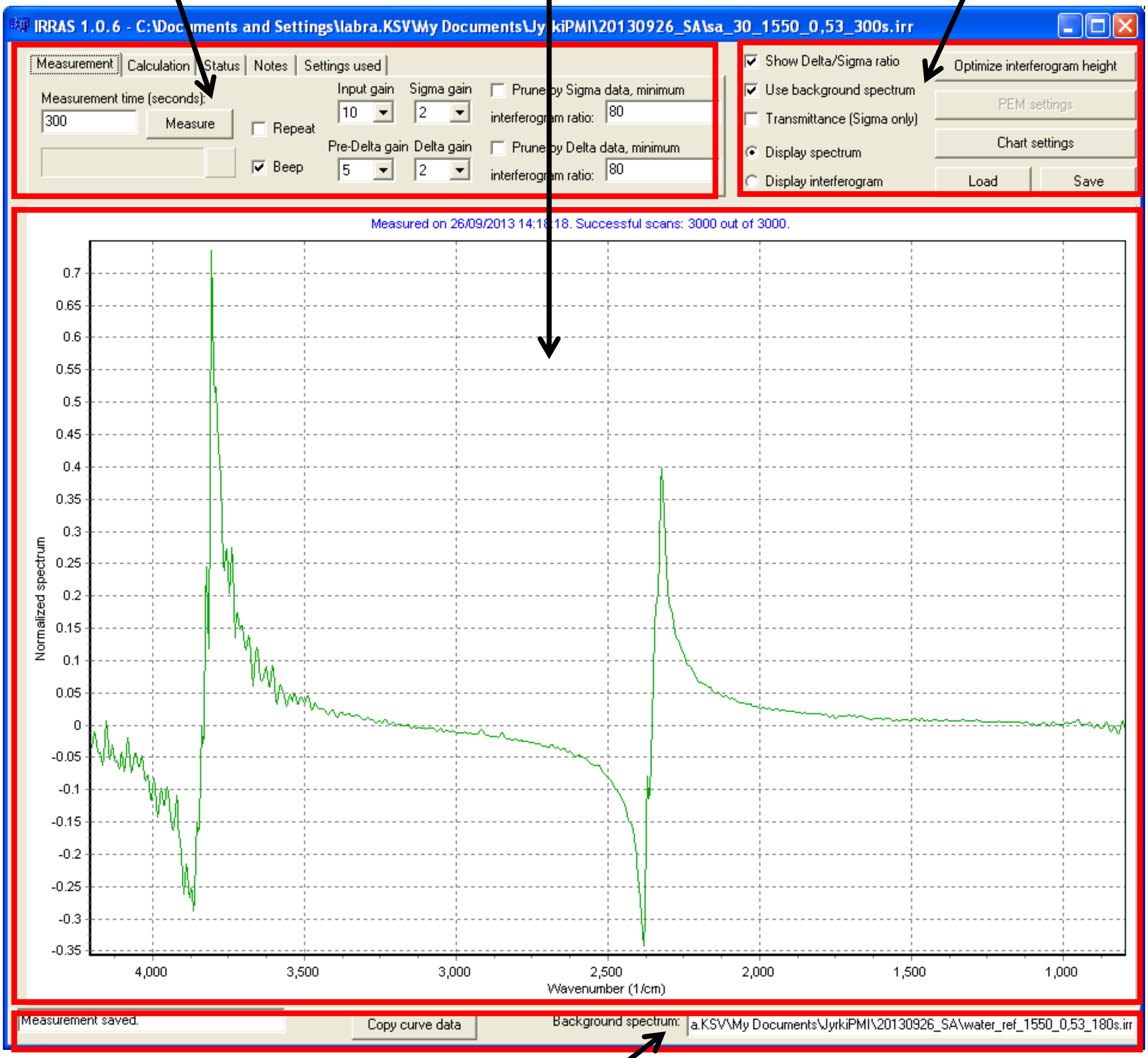
The main PM-IRRAS software window consists of:

- Tab selection bar in the upper left corner with tab-specific controls
- Display mode controls and miscellaneous buttons in the upper right corner
- Result graph in the middle
- Bottom bar below the graph

Tab selection bar with tab-specific controls

Result graph

Display mode controls and miscellaneous buttons



IRRAS 1.0.6 - C:\Documents and Settings\labra.KSV\My Documents\JyrkiPMI\20130926_SA\sa_30_1550_0,53_300s.irr

Measurement time (seconds): 300

☐ Repeat

☑ Beep

Input gain: 10

Sigma gain: 2

Pre-Delta gain: 5

Delta gain: 2

☐ Prune by Sigma data, minimum interferogram ratio: 80

☐ Prune by Delta data, minimum interferogram ratio: 80

☑ Show Delta/Sigma ratio

☑ Use background spectrum

☐ Transmittance (Sigma only)

● Display spectrum

○ Display interferogram

Measured on 26/09/2013 14:18:18. Successful scans: 3000 out of 3000.

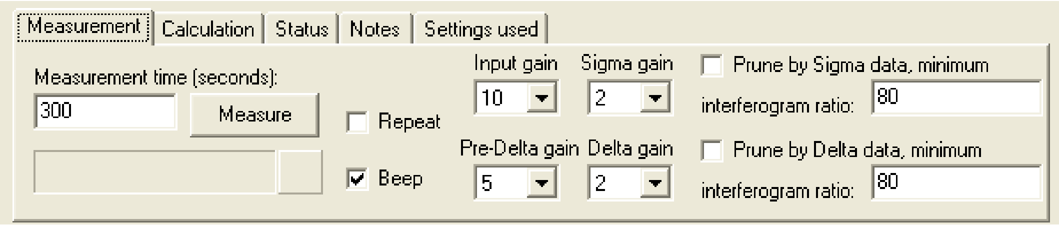
Measurement saved.

Background spectrum: a.KSV\My Documents\JyrkiPMI\20130926_SA\water_ref_1550_0,53_180s.irr

Bottom bar

4.1 Measurement tab

Measurement settings can be adjusted and measurement started in the measurement tab.



Measurement time (seconds): 300

☐ Repeat

☑ Beep

Input gain: 10

Sigma gain: 2

Pre-Delta gain: 5

Delta gain: 2

☐ Prune by Sigma data, minimum interferogram ratio: 80

☐ Prune by Delta data, minimum interferogram ratio: 80

Measurement time (seconds): Specifies the length of the measurement. The measurement time does not grow longer even if some of the scans fail. The IRRAS unit performs 10 scans per second, 5 to both directions. For example, 10 second measurement includes 100 scans.

Measure/Connect: For initiating a measurement. This button shows "Measure" when the program is connected to the IRRAS main unit and "Connect" when not connected.

Measurement progress bar: Shows the progress of the measurement.

Flash/Stop: Flashes when measurement is ongoing. Measurement can be aborted by clicking on the panel during measurement, after a confirmation dialog. All data from an aborted measurement is lost.

Repeat: When checked, a new measurement is started automatically right after a measurement is completed. This way the user can easily get an idea of how much variance there is in subsequent (short) measurements.

Beep: When checked, the program gives a sound signal when a measurement ends.

Input gain: Specifies the main input gain for both Sigma and Delta signals.

Sigma gain: Specifies the Sigma gain after Sigma/Delta signal differentiation.

Pre-Delta gain: Specifies the Delta gain after Sigma/Delta signal differentiation.

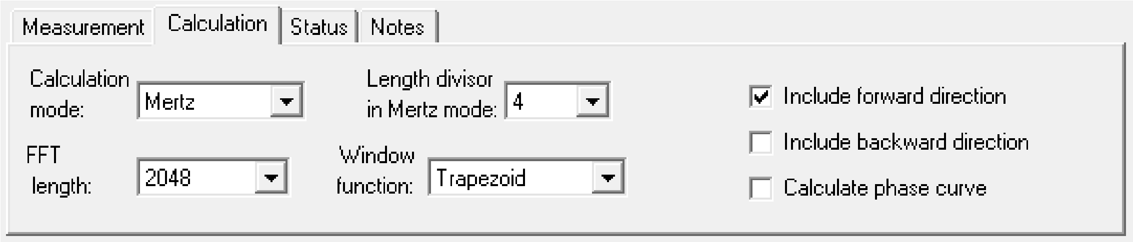
Delta gain: Specifies the Delta gain after Delta signal filtering.

Prune by Sigma data: Not in use.

Prune by Delta data: Not in use.

4.2 Calculation tab

Result calculation settings (Fourier transform settings) can be adjusted in the calculation tab.



Calculation mode: Mertz

Length divisor in Mertz mode: 4

FFT length: 2048

Window function: Trapezoid

☑ Include forward direction

☐ Include backward direction

☐ Calculate phase curve

Calculation mode: Specifies one of two calculation modes: Traditional or Mertz. “Traditional” refers to spectral calculation without the Mertz phase correction. This also influences how phase curves are calculated.

FFT length: Specifies the Fast Fourier Transform length. The smallest value for FFT length is the sample size, which is 2048, with the interferogram peak in the middle. The FFT length can be increased by zero-padding up to 65536 units. The FFT lengths available are 2048, 4096, 8192, 16384, 32768 and 65536. The higher the FFT length, the smoother the spectrum curve. Note, however, that phase curve calculation works properly only if FFT length is set to 2048.

Length divisor in Mertz mode: Specifies the fraction of data around the interferogram peak that is used for Mertz correction. If the value is 4, for example, the amount of data is 2048 / 4 = 512 samples. The larger the length divisor, the more influential the correction. The length divisor has no effect if “Calculation mode” is set to “Traditional”.

Window function: Selects the window function to be used in the Fourier transform.

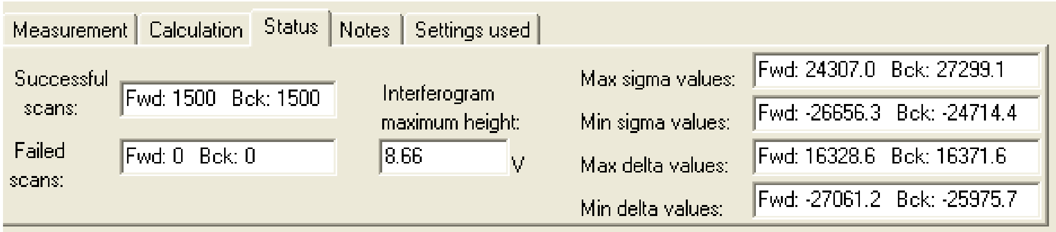
Include forward direction: When checked, scans to forward direction are included in calculation (except the failed ones). If both forward and backward scans are used, the spectrum displayed is the average of forward and backward spectra.

Include backward direction: When checked, scans to backward direction are included in calculation (except the failed ones). If both forward and backward scans are used, the spectrum displayed is the average of forward and backward spectra.

Calculate phase curve: When checked, an additional window is displayed, showing the phase curves for sigma and delta signals. The choice of forward and/or backward direction affects the phase curve.

4.3 Status tab

Measurement status information can be read in the status tab.



Successful scans: Fwd: 1500 Bck: 1500

Failed scans: Fwd: 0 Bck: 0

Interferogram maximum height: 8.66 V

Max sigma values: Fwd: 24307.0 Bck: 27299.1

Min sigma values: Fwd: -26656.3 Bck: -24714.4

Max delta values: Fwd: 16328.6 Bck: 16371.6

Min delta values: Fwd: -27061.2 Bck: -25975.7

Successful scans: The number of successful forward and backward scans.

Failed scans: The number of failed forward and backward scans. A scan may fail if the interferogram peak cannot be found in a suitable location in the scanning data.

Interferogram maximum height: The maximum level that the analog Sigma interferogram signal has reached during the scans of the measurement. The higher the value, the better the dynamic range. If the value gets close to 10 Volts, the signal may get clipped because of saturation. Please note that the voltage level is measured for Sigma signal only.

Max sigma values: Indicates the maximum values for the Sigma interferogram as sampling units. Higher values increase measurement sensitivity. Values over 30000 may cause signal saturation.

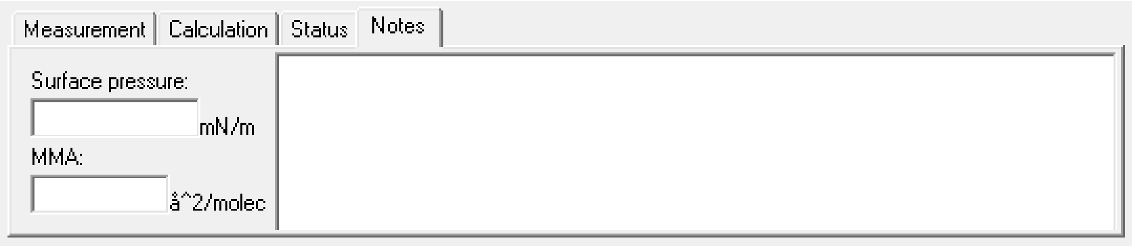
Min sigma values: Indicates the minimum values for the Sigma interferogram as sampling units. Lower values increase measurement sensitivity. Values under -30000 may cause signal saturation.

Max delta values: Indicates the maximum values for the Delta interferogram as sampling units. Higher values increase measurement sensitivity. Values over 30000 may cause signal saturation.

Min delta values: Indicates the minimum values for the Delta interferogram as sampling units. Lower values increase measurement sensitivity. Values under -30000 may cause signal saturation.

4.4 Notes tab

Measurement notes can be added to the notes tab.



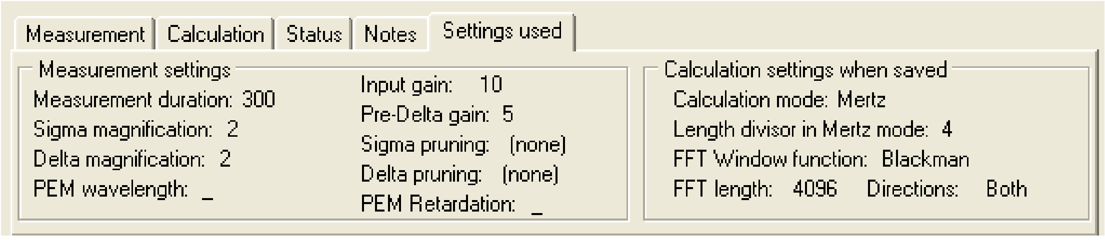
Surface pressure: You can write the surface pressure from your Langmuir experiment here. PM-IRRAS results depend on the surface pressure in which the monolayer is while being measured.

MMA: You can write the mean molecular area from your Langmuir experiment here.

Note section: You can freely add any measurement notes here.

4.5 Settings used tab (only displayed when viewing a saved measurement)

Measurement settings from past experiments can be seen in the settings used tab when a past experiment is open. The settings include both measurement and calculation settings.

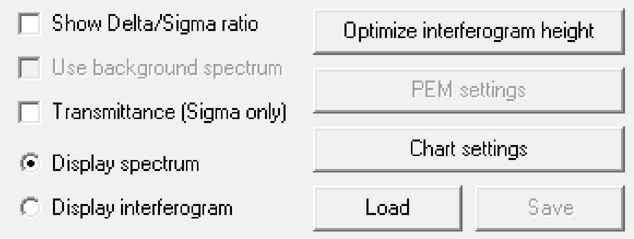


Measurement settings
Measurement duration: 300
Sigma magnification: 2
Delta magnification: 2
PEM wavelength: _
Input gain: 10
Pre-Delta gain: 5
Sigma pruning: (none)
Delta pruning: (none)
PEM Retardation: _

Calculation settings when saved
Calculation mode: Mertz
Length divisor in Mertz mode: 4
FFT Window function: Blackman
FFT length: 4096 Directions: Both

4.6 Display mode controls and miscellaneous buttons

Display mode controls set how the data is plotted in the graph. Miscellaneous buttons include more important measurement settings.



☐ Show Delta/Sigma ratio
☐ Use background spectrum
☐ Transmittance (Sigma only)
● Display spectrum
○ Display interferogram

Show Delta/Sigma ratio: If checked, purple Delta/Sigma curve is plotted.

Use background spectrum: If checked, green background corrected Delta/Sigma curve is plotted.

Transmittance (Sigma only): If checked, Sigma transmittance is plotted.

Display spectrum: If chosen, graph shows curves for the spectra calculated from the average interferograms of Sigma and Delta channels.

Display interferogram: If chosen, graph shows the average curves of Sigma and Delta interferograms to both directions.

Optimize interferogram height: Function for optimizing the interferogram height. See the instructions in “Performing a measurement” below.

PEM settings: Function for defining photoelastic modulator settings. See the instructions in “Performing a measurement” below.

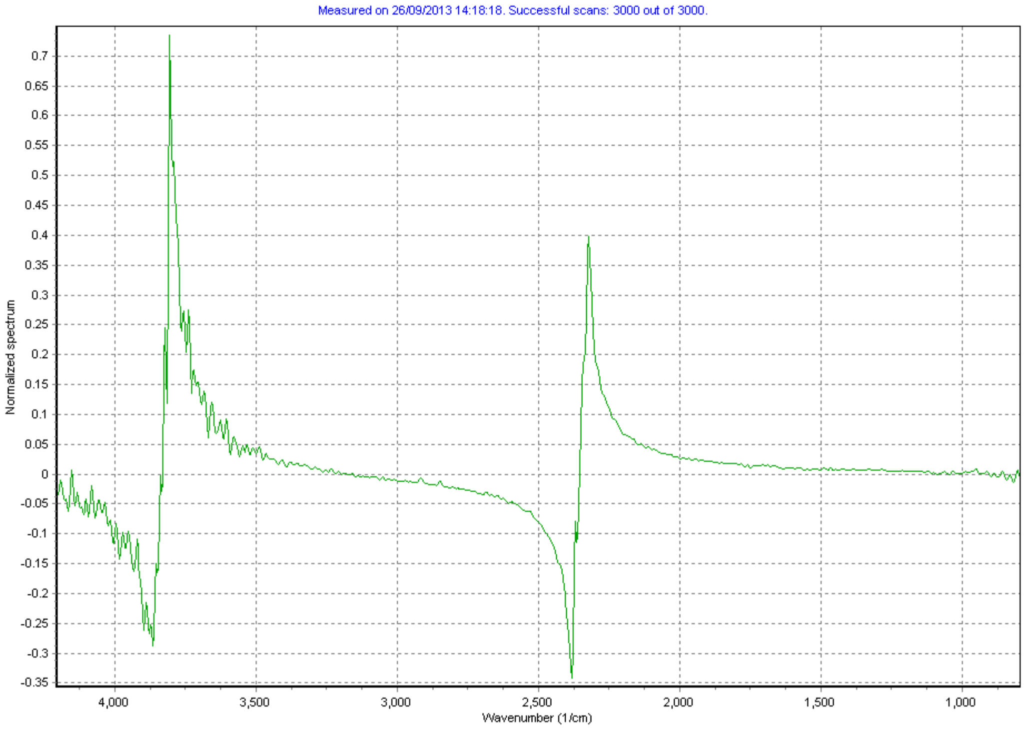
Chart settings: Function for defining chart plotting settings. See the instructions in “Measurement results” below.

Load: Loads a past experiment results.

Save: Saves the results for current experiment. **Results always need to be saved using this button, they are not saved automatically.**

4.7 Result graph

Results are presented graphically in results graph.



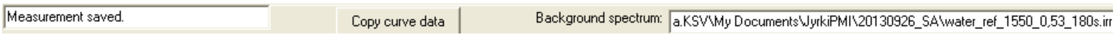
Measured on 26/09/2013 14:18:18. Successful scans: 3000 out of 3000.

The time of measurement as well as the number of successful scans are presented in the upper part of the graph.

The graph can be zoomed in by dragging mouse up-right (↗) or down-right (↘).Drag left (←) in order to reset the zoom.

4.8 Bottom bar

Additional functions are presented in the bottom bar.



Measurement saved.

Background spectrum: a.KSV\My Documents\JyrkiPMI\20130926_SA\water_ref_1550_0,53_180s.irr

Message box: Displays short measurement status messages that stay until replaced by the next message.

Copy curve data: Copies the results shown in graph to clipboard in ASCII format. Only the results shown in screen are copied. Therefore, only zoomed range of values is copied.

Background spectrum: Background spectrum is chosen here. Double-click on the edit box to browse directories.

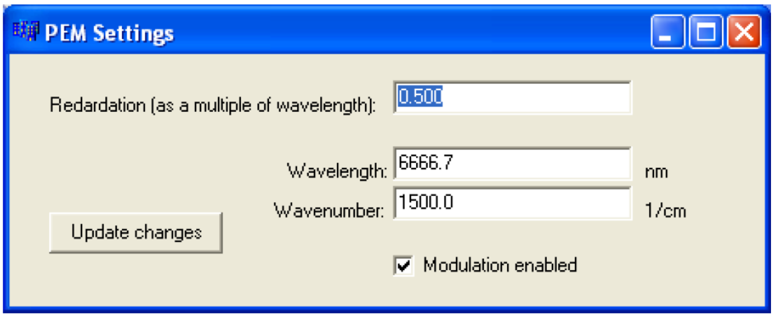
5. Performing a measurement

Usually a measurement involves first measuring the background spectrum and subsequently the sample spectrum. This allows the separation of background data (for example water, carbon dioxide) from the actual sample data.

Standard IRRAS measurements can also be done by disabling the PEM module. In this case, follow the steps below but turn off the PEM unit.

Perform the following steps in order to complete a measurement:

1) Go to ”PEM settings” in the main window. The PEM Settings Window provides the controls needed to set the magnitude and frequency of polarization modulation. Please note the PM-module can also be used in standalone mode in which case these settings can be adjusted in the PM-unit control box directly.



PEM Settings

Redardation (as a multiple of wavelength): 0.500

Wavelength: 6666.7 nm

Wavenumber: 1500.0 1/cm

☑ Modulation enabled

Retardation (as a multiple of wavelength): How much the light gets retarded when the oscillation of the modulator is at its most retarding orientation. The amount of retardation is given relative to the selected retardation wavelength.

Wavelength: The wavelength of retardation. If changed, the wavenumber changes accordingly.

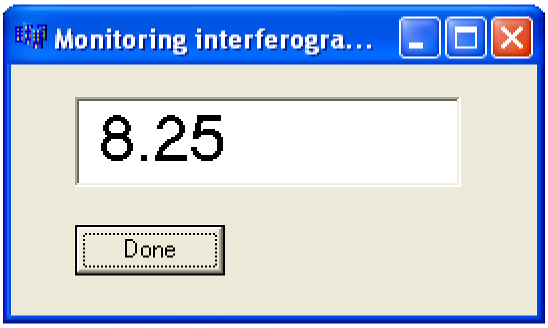
Wavenumber: The wavenumber of retardation. If changed, the wavelength changes accordingly.

Update changes: Updates the above changes to the PEM.

Modulation enabled: Is the module in use.

Here you can set the required photoelastic modulator settings prior to the measurement. Set the retardation wavelength/wavenumber setting according your research aims. The wavelength/wavenumber chosen is where photoelastic modulation works the most ideally—for which a retardation value of 0.5 implies a retardation of precisely half a wavelength. The retardation value (as a multiple of wavelength) is usually set to 0.5. Finally, click on “Update changes” to transfer the values to the photoelastic modulator unit, whose display should change accordingly.

2) Once the PEM settings have been set, choose "Optimize interferogram height" in the main window. The following screen will open.



8.25

Before adjusting the height setting of the transmitter-receiver system, place a sample in the approximate position where it will reflect the IR ray from the transmitter to the receiver. There are two settings on top of the goniometer: the height and the angle. The angle should be chosen based on theoretical considerations. If you are just trying to find the correct position where the signal gets reflected from the transmitter to the receiver, it's usually easier from a grazing angle such as 80°. Adjust the height according to the instructions below. However, when measuring a liquid surface from low angles, check that the edges of the vessel do not hinder signal transmission. When adjusting height, use the interferogram height monitoring mode that is started by pressing the button labeled "Optimize interferogram height" to find the maximum transmission from the transmitter to the receiver.

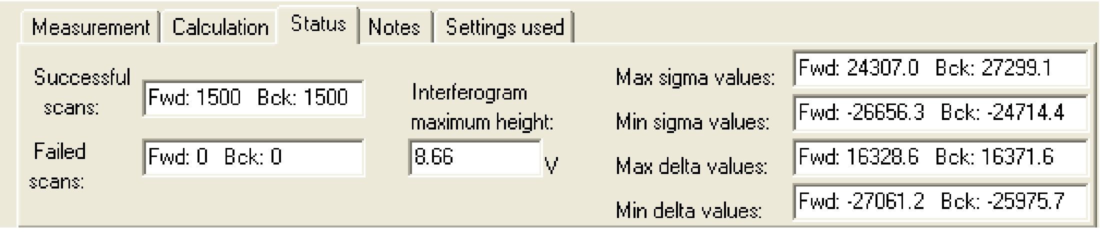
Alter the interferogram height until the value in the window is at its maximum. If the interferogram height values get over 9 V, close the interferogram height monitoring, set Sigma gain to a lower value (on Measurement tab) and restart interferogram height monitoring, and optimize the interferogram height further until it is at its maximum.

Higher interferogram height gives more sensitivity, but values over 9 V create a risk of signal saturation.

Please note that if you're performing a long experiment, some liquid may evaporate during the measurement. In this case, it might be beneficial to set the height of the interferogram close to the lower end of the optimal area in order to compensate for possible subphase level changes.

3) When the Sigma interferogram height has been adjusted to optimal, check that the level of Delta interferogram is within a suitable range. This is best done by short test measurements (e.g. 10 seconds). Adjust the measurement time to 10 seconds and choose "Measure".

To check the height of the Delta interferogram, check the values on the "Status tab". The maximum values should be as big as possible, but always below 30000. The minimum values should be as small as possible, but always above -30000.



Successful scans: Fwd: 1500 Bck: 1500
Failed scans: Fwd: 0 Bck: 0
Interferogram maximum height: 8.66 V
Max sigma values: Fwd: 24307.0 Bck: 27299.1
Min sigma values: Fwd: -26656.3 Bck: -24714.4
Max delta values: Fwd: 16328.6 Bck: 16371.6
Min delta values: Fwd: -27061.2 Bck: -25975.7

If the values are outside the limits, the gain values must be reduced. If the values are a lot smaller than 30000, the gain values must be increased. Please note that Sigma gain only affects Sigma values, Pre-Delta gain and Delta-gain only affect Delta gain, and Input gain affects both Delta and Sigma gain. Also the Sigma values must be between the limits.

After you have set new gain values, redo the 10 second measurement and check the values again. Re-iterate until the values are optimal. Higher values give more sensitivity, but values over 30000 (or under -30000) create a risk of signal saturation.

4) Once the optimal gain values have been found, you are ready to start a measurement. Set the measurement time according to your measurement. The number of scans will be 10 scans/second. Choose “Measure”.

5) Once the measurement has been completed, save the results by selecting “Save”. **The results are not automatically saved!**

6) If this was your background measurement, you can now insert the monolayer and measure its spectrum. If you have checked “Repeat” in the measurement tab, two subsequent measurements will be performed automatically. **Please note!** Do not change any PM-IRRAS settings such as interferogram height or gain values between measurements you wish to compare directly.

6. Measurement results

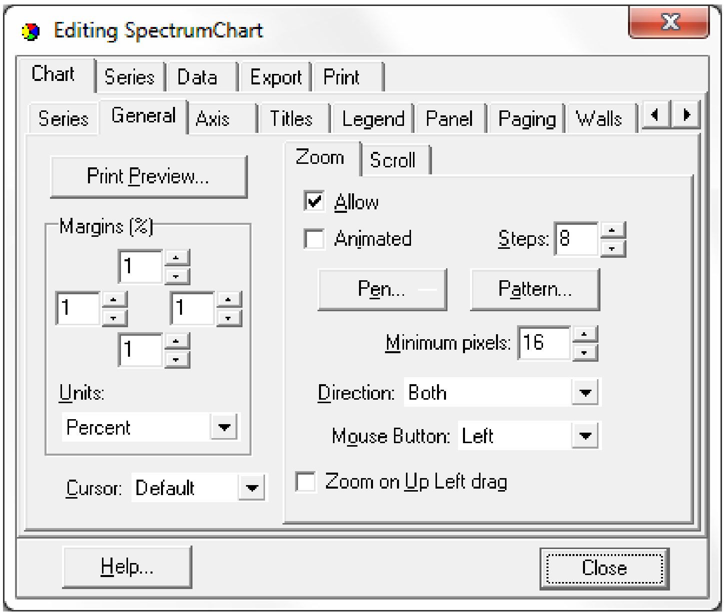
Always remember to save your results!

Once the measurement s have been completed and saved, they can be opened using the “Load” button. The results can be plotted using the display mode controls described before.

In order to subtract the background from the actual measurement, load the actual measurement to the graph screen and select the background measurement by double-clicking the “Background spectrum” box at the bottom of the screen.

The data can be copied to clipboard using the “Copy curve data” button at the bottom of the screen. This function always copies only the data that is visible in the graph. You can zoom in to include only a part of the data spectrum.

If you wish to edit the chart style, you can do this in “Chart settings”.



Margins (%): 1, 1, 1, 1

Units: Percent

Cursor: Default

☑ Allow

☐ Animated

Steps: 8

Minimum pixels: 16

Direction: Both

Mouse Button: Left

☐ Zoom on Up Left drag

Here you can find different style and visual settings such as legends and data series. Once you have added these, you either save the image using the “Export” tab in the settings or print the image using the “Print” tab.

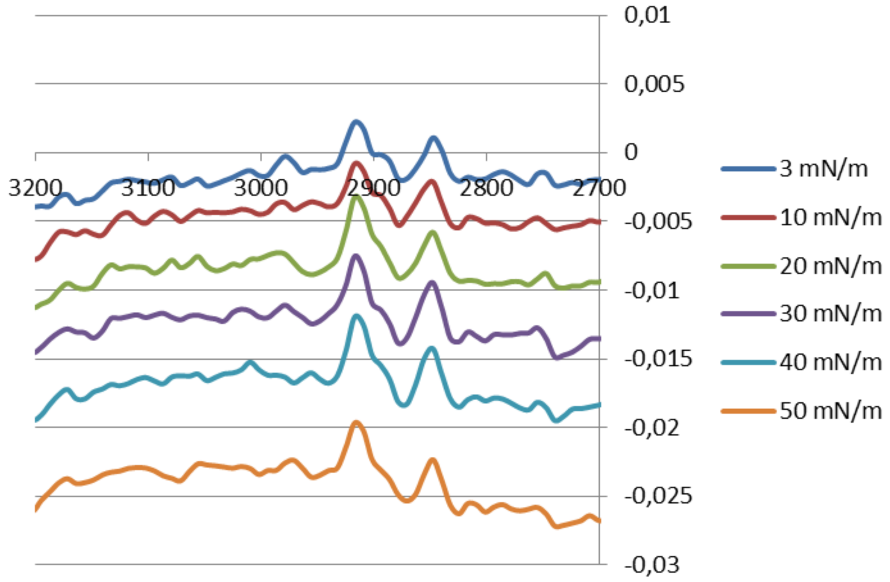
7. Example measurement - DPPC

A typical phospholipid used in Langmuir experiments, DPPC (dipalmitoylphosphatidylcholine), was used for PM-IRRAS experiment. The measurement was conducted in several surface pressures with the following parameters:

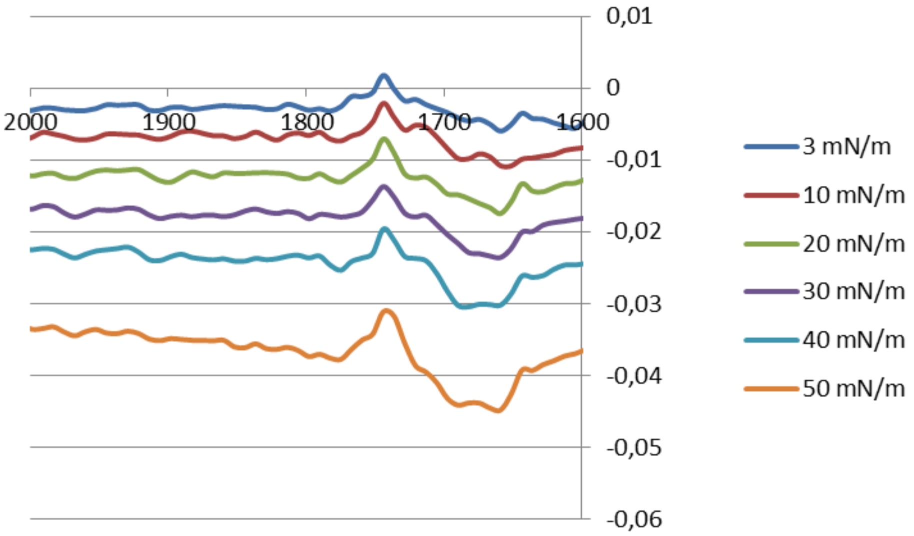
DPPC concentration:	1 mg/ml (in chloroform)
Evaporation time:	10 minutes
Spreading volume:	30 µl (with medium-sized trough top)
Compression speed:	20 mm/min
Subphase:	DI-grade water
Measurement time:	300 s (total of 3000 scans)
Window mode:	Hamming
Gains and interferometer height:	Optimized according to standard procedures
PM-module wavelength:	3000 cm^{-1}
PM-module retardation:	0.5
Environment:	Vibrations and air currents minimized
Background:	DI-grade water

After reducing the background from the results in PM-IRRAS software, the data was exported to MS Excel for further plotting. All surface pressures were plotted on the same graph for comparison.

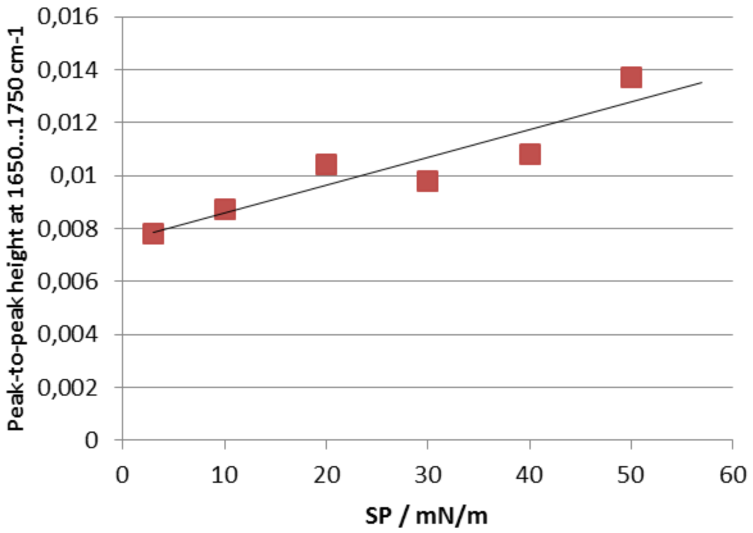
In the hydrocarbon area in 2800…3000 cm^{-1}, both typical CH$_2$ symmetric (2850 cm^{-1}) and asymmetric (2918 cm^{-1}) stretches were visible in all surface pressures. The peak height wasn't strongly dependent on the surface pressure.



In the carbonyl area, both an upward peak and a downward were visible. The downward dip (1650…1700 cm^{-1}) was very broad whereas the upward peak at ~1750 cm^{-1} was relatively sharp.



Moreover, there could be seen a peak size dependency based on the surface pressure. If the peak-to-peak height is measured and plotted as a function of the surface pressure, there is a linearly growing trend.



Troubleshooting

Issue	Possible solution
The noise level is high.	PM-IRRAS is very sensitive to vibration. Use as firm table as possible or active antivibration table (recommended). The use of cabinet for reducing air flow is also recommended. During measurement, do not work in the immediate proximity to the instrument.
The baseline is tilted.	The baseline may tilt over time due to subphase evaporation. You can reduce the evaporation by saturating the air with water vapour prior to measurement. Another way is to lower the goniometer a little bit after each measurement to compensate for the evaporation. Using 3000 cm^{-1} as the PM-module wavelength may also stabilize the baseline.
The system gives no signal.	Check all cable connections. Make sure there is nothing in the way of the signal. Adjust height of the goniometer to make sure you are at the correct level. If you have recently changed PC, make sure the amplitude and offset have been set for the new PC.
The signal shape is unexpected / different than usual.	Make sure the IR beam isn't hitting any part of the instrument such as the barriers, Wilhelmy plate or the limit switch. Also make sure the signal isn't saturated. Use smaller gains in the beginning of the signal amplifying (especially Input gain) and larger at the end of amplifying (especially Delta gain) rather than vice versa.

8. 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