

Confocal Imaging Profiler

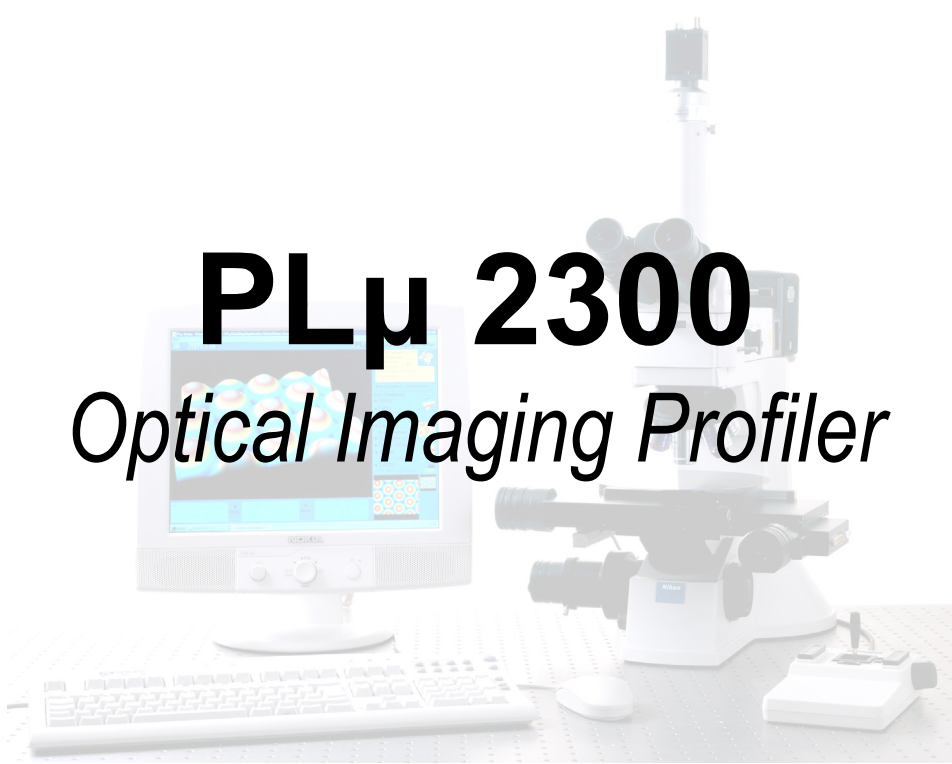
PL $\mu$

SENSOFAR<sup>®</sup>



# USER'S MANUAL





**PL $\mu$  2300**  
*Optical Imaging Profiler*

**USER'S MANUAL**

---

*PL $\mu$  2300 Software Version 2.3*

September, 2004

2001-2004, Sensofar-Tech, S.L.

This document contains proprietary and confidential information of Sensofar-Tech, S.L.

The contents of this document may not be copied nor duplicated in any form, in whole or in part, without prior written consent from Sensofar-Tech, S.L.

By purchasing this product, the Purchaser(s) and/or any subsequent legitimate owner(s) of the product, henceforth referred to as "the Purchaser," agree(s) to abide by the terms of this Agreement and read and recognize the following set of definitions appertaining to the intellectual-property items and trademark references as can be found throughout this User's Manual.

Sensofar-Tech, S.L. provides the information and data included in this document for the Purchaser's benefit, but it is not possible for Sensofar-Tech, S.L. to entirely verify and test all of this information in all circumstances, particularly information relating to non-Sensofar-Tech, S.L. manufactured products. Sensofar-Tech, S.L. makes no warranties or representations relating to the quality, content, or adequacy of this information. Every effort has been made to ensure the accuracy of this manual; however, Sensofar-Tech, S.L. assumes no responsibility for any errors or omissions in this document. Sensofar-Tech, S.L. shall not be held liable for any errors or for incidental or consequential damages in connection with the furnishing, performance, or use of this User's Manual or the examples herein.

Sensofar-Tech, S.L. assumes no responsibility for any damage or loss resulting from the use of this User's Manual, loss or claims by third parties which may arise through the use of this product, any damage or loss caused by deletion of data as a result of malfunction or repair, or any other damage related to the use of this product or associated documentation. The information in this document is subject to change without notice.

#### **Definitions of Intellectual Property and Trademark Attributions**

This Section is intended to ensure proper attribution and honoring of any and all trademarks and intellectual-property items in terms of attribution to their respective owners as mentioned in this User's Manual. The reader is encouraged to consult this Section whenever uncertainty presents itself as to the terms, their meaning within the User's Manual, and the trademarks and intellectual-property items they stand to identify, whether by themselves or in conjunction with other terms and items.

Sensofar® is a registered trademark of Sensofar-Tech, S.L.; Microsoft, DirectShow and Windows are either trademarks or registered trademarks of Microsoft Corporation in the United States and/or other countries; All other products, brand names, company names are trademarks or registered trademarks of their respective owners.

Registration or Update of this Manual, January 2004
---

PRINTED IN SPAIN



# Contents

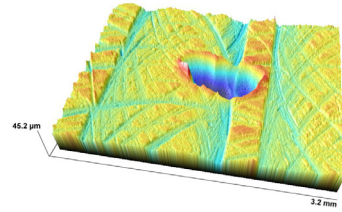
<b>Contents</b> .....	<b>i</b>
<b>Introduction</b> .....	<b>1</b>
1.1. What is a confocal microscope? .....	1
1.2. Differences between a confocal scanning laser microscope (CSLM) and the PL $\mu$ .....	2
1.3. System requirements and Windows configuration.....	3
<b>PL<math>\mu</math> Software Interface Organization</b> .....	<b>4</b>
2.1. The Acquisition Screen .....	4
2.2. The Display and Analysis Screen .....	5
2.3. The Configuration Screen .....	5
<b>Preparing for measurement</b> .....	<b>7</b>
3.1. Focusing the slit pattern .....	7
3.2. Selecting the acquisition parameters .....	8
3.3. Setting the Z scan range .....	9
<b>The Motors Control</b> .....	<b>11</b>
4.1. Stop Motion .....	11
4.2. Spider Motion .....	<b>¡Error! Marcador no definido.</b>
4.3. XY Motion.....	11
4.4. Z Motion .....	11
4.5. Relative and Absolute Motion .....	12
4.6. Home, Define Home and Center Table.....	12
<b>The Acquisition Screen</b> .....	<b>14</b>
5.1. Menu Toolbar .....	14
5.2. Configuration and Alignment.....	15
5.3. The Image View Option.....	15
5.4. The Autofocus Option .....	16
5.5. The Measurement Window .....	16
• What is a <i>Profile</i> ? .....	17
• What is an <i>Extended Profile</i> ? .....	18
• What is a <i>Multiple-Profile</i> ? .....	20
• What is a <i>Coordinate</i> ?.....	21
• What is <i>Topography</i> ? .....	21
• What is an <i>Image</i> ? .....	22
• What is <i>Extended Topography</i> ? .....	23
• What is a <i>Thickness</i> measurement? .....	25
• What is a <i>Custom Application</i> ? .....	26
5.6. The Objective Window .....	26

5.7. The Area & Length Window .....	27
5.8. The Z Scan Window .....	30
5.9. The Threshold Edit .....	35
<b>The Display and Analysis Screen .....</b>	<b>36</b>
6.1. Organization of the Display and Analysis Screen .....	36
6.2. File Menu .....	36
6.3. The Numerical Data Area .....	37
• Measure, Zoom and Cursor .....	37
6.4. The Analysis Area .....	39
• What does “Restore” mean? .....	39
• How to align with a “Term” (line, circle, plane & sphere).....	39
• Filter.....	42
6.5. The View Area .....	43
• Display .....	43
• What does “Invert” mean? .....	44
• 3D View .....	45
• Colour Scale .....	45
• Scale.....	46
• Zoom .....	47
6.6. The Measurement Report Area .....	47
• The Edit Comment button .....	47
• The New Acquisition button.....	47
6.7. The Print Report .....	48
<b>The Configuration Screen .....</b>	<b>50</b>
7.1 Options .....	50
7.2 Selecting the software language .....	52
7.3 Setting the “Lost pixels” value .....	52
7.4 Configuring the available hardware (objectives, PZT and XY table).....	53
7.5 Positioning and aligning the slit pattern.....	53
7.6 Calibrating the Objectives .....	54
7.7 Calibrating the Z Scale Factor.....	54
<b>Error Messages .....</b>	<b>55</b>
<b>Troubleshooting.....</b>	<b>58</b>

## Chapter 1

# Introduction

---



Thank you for acquiring the PLμ. The PLμ uses a user-friendly software application based on Windows 2000/XP. All commands are of the push-button type, and are operated by placing the mouse pointer on them and pressing the left button of the mouse.

The software of the PLμ controls all the electronic devices contained in the hardware. If any of the controllers is switched off, the software informs you that this electronic device is not available. Also, if one of the controllers fails during a running session, the software informs you about it.

### 1.1. What is a confocal microscope?

Confocal scanning optical microscopy has recently emerged as a significant new technique which exhibits several advantages in comparison with conventional systems based on classical microscopy. The most important of these advantages stems from the fact that out-of-focus blur is essentially absent from confocal images, giving the possibility of direct non-invasive serial optical sectioning of intact and even living specimens. This leads to the possibility of generating three-dimensional images of thick transparent objects such as biological cells and tissues. Similarly, it allows profiling of the surfaces of three-dimensional objects and multi-layer structures such as integrated circuits deposited on silicon, again by a non-contact, non-destructive method.

In conventional microscopy, the simultaneous illumination of the entire field of view of a specimen will excite fluorescent emissions or reflections throughout the whole depth of the specimen, rather than just at the focal plane. Much of the light collected by the objective lens to form the image will thus come from regions of the specimen above and below the selected focal plane, contributing to the out-of-focus blur of the final image and seriously degrading it by reducing contrast and sharpness. This explains why studies of biological specimens using conventional immunofluorescence microscopy are most informative on well-flattened cultured cells, and why the maximum usable thickness of frozen tissue sections for immunofluorescence observations is about 10 microns.

Confocal microscopy has other additional advantages over conventional optical microscopy, including a small but significant improvement in lateral resolution. It

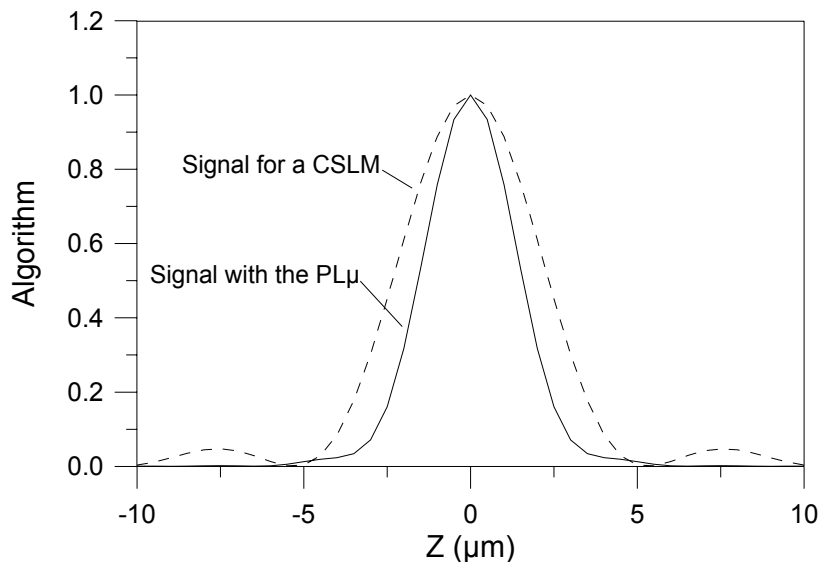
rejects stray light from the out-of-focus specimen planes as well as light scattered in the optical instrument, resulting in increased contrast and signal-to-noise ratio in the final image. Confocal microscopy is also compatible with computer image storage techniques, allowing generation of high-resolution digitized data sets of the 3D topography of a surface, suitable for subsequent data analysis.

## 1.2. Differences between a confocal scanning laser microscope (CSLM) and the PL $\mu$

A *Confocal Scanning Laser Microscope* (CSLM) uses laser light and a scanning device to achieve a confocal image. The laser beam illuminates a pinhole, which is imaged by the microscope objective on the sample. The light, which has been either reflected or scattered by the sample, passes again through the same objective and strikes a second pinhole called the confocal aperture. A photodetector is placed behind the confocal aperture. If the sample is exactly in focus, it will project the image of itself and the pinhole on the confocal aperture at the same time, and the signal in the photodetector will be high. In contrast, if the sample is out of focus, the image on the confocal aperture will be the unfocused sample and pinhole, and the signal will be low.

In the PL $\mu$  the pinhole has been replaced by a structured pattern consisting of a set of parallel slits, and the photodetector measuring the light intensity passing through the confocal aperture is replaced by a CCD. Instead of looking for the confocal information point by point, the PL $\mu$  looks for the confocal information in all the points contained in each line. This is done by using the digitized information in each pixel of the CCD, which functions as a pinhole and a photodiode at the same time. With this arrangement the PL $\mu$  is able to obtain confocal information from several thousands of points per second.

Moreover, the PL $\mu$  uses a novel “high-contrast” algorithm, which allows it to achieve sharper confocal images. With the aid of the “high-contrast” algorithm, the PL $\mu$  provides much better axial resolution (more than 30% more) than a standard CSLM system.



### 1.3. System requirements and Windows configuration

- System requirements.

PL $\mu$  hardware comes with its own compatible computer, which has been tested to run the PL $\mu$  software properly. The minimum requirements for the computer are a Pentium III 600MHz microprocessor, with 256 MB of RAM memory and 10GB of hard disk.

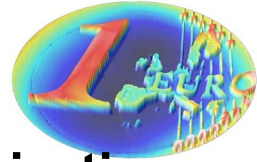
Matrox Frame Grabber hardware compatibility needs the Plug and Play option of the computer Bios to be disabled. If not, Windows will detect new hardware, and it will try to install its own controller files. To disable the plug and play, enter the Bios configuration menu during the start up of the computer, look for this option in the advanced hardware configuration and disable it.

- Operating system requirements.

PL $\mu$  software runs under both Windows 2000 and Windows XP.

Both operating systems need a desktop area configuration of 1024x768 pixels with 24-bit colour and small fonts. If the desktop area configuration is different, the software will tell you to change the resolution. Also, if you are using large fonts, the software will run, but many of the features of the software will be out of the desktop area.

## Chapter 2



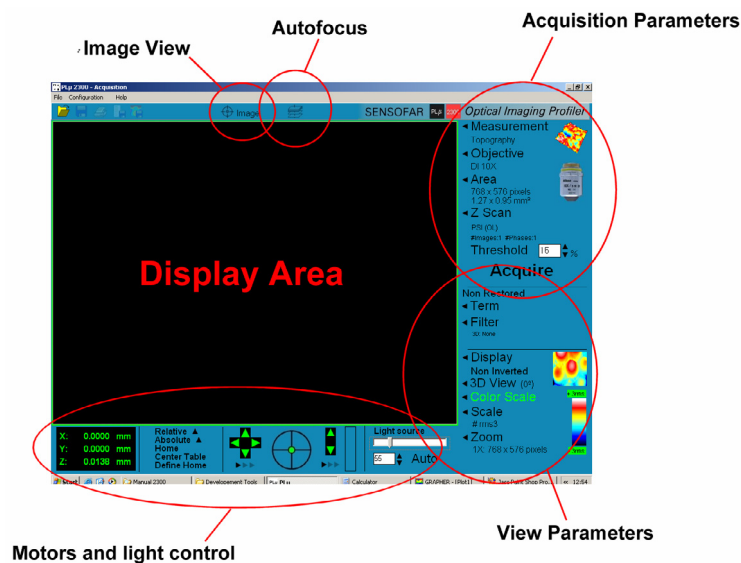
# PLμ 2300 Software Interface Organization

The software of the PLμ 2300 is organized in three different windows: the Acquisition screen, the Display and Analysis screen and the Configuration screen.

Once the program is started, the display always shows the Acquisition screen. The Display and Analysis screen is displayed after acquiring a new measurement or loading a previous one. The Configuration screen is displayed when the “Configuration and Alignment” option in the Acquisition screen is clicked.

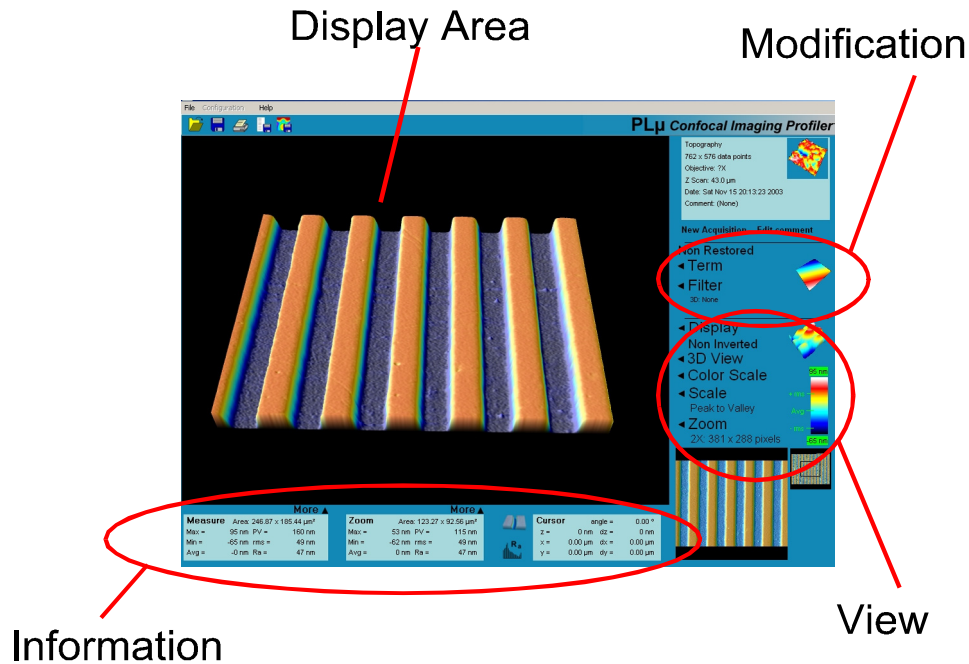
## 2.1. The Acquisition Screen

You will use the acquisition screen to (i) place the sample in the right position under the microscope objective and (ii) to select the acquisition and view parameters before starting the measurement. Depending on the option of profiler you have purchased you will also use this screen to control the motorized stages of the microscope. The Display Area shows the live image seen by the CCD camera. You will use this live image to focus the slit pattern on the sample.



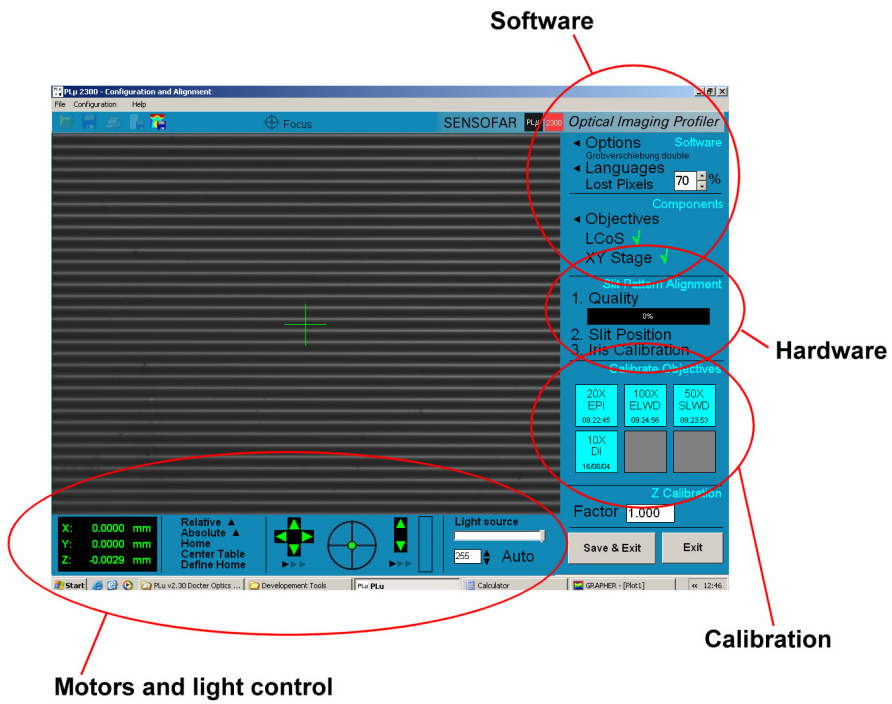
## 2.2. The Display and Analysis Screen

Once a measurement has been acquired or a previous one has been loaded, the PLμ 2300 software switches automatically to the Display and Analysis screen. In this window you can look at the results in two or three dimensions, with different colours, zooms and scales. You can also invert, remove a term to align the measurement or apply a filter in order to obtain roughness and waviness parameters according to different surface texture standards.



## 2.3. The Configuration Screen

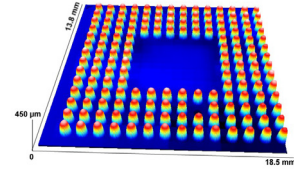
You will use the Configuration screen to (i) select software options and hardware components (ii) align the slit pattern and (iii) calibrate the objectives. Alignment and calibration procedures are performed with a high-quality flat mirror as a sample (see Section 7 of this manual). You will use the display area and the motor control to focus the slit pattern onto the surface of the mirror.





## Chapter 3

# Preparing for measurement



Before acquiring a measurement follow the procedure stated below. First of all, check for the right calibration of the objectives that you want to use (see Section 7). If very precise measurements are to be acquired, a warming-up time of roughly ten minutes before calibrating the objectives is always recommended.

### 3.1. Focusing the sample

#### 3.1.1 Focusing a confocal objective

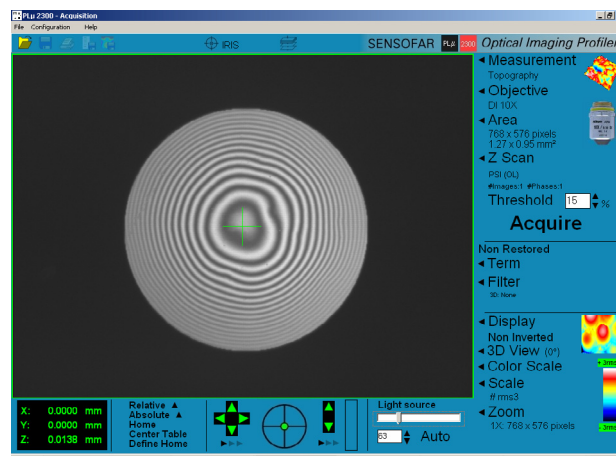
Place the sample on the holder and choose the microscope objective that you want to use (preferably a 20XEPI). In order to get a fast focus of the sample (especially for smooth surfaces), press the “Image View” button at the top of the acquisition screen. This button switches between a conventional microscope image to a parallel slit pattern projected onto the surface.

Use the coarse control buttons of the joystick to place the surface close to the working distance of the objective (left button moves the sample downwards and right button moves the sample upwards). You will see the unfocused image of the sample in the display area. Then move slowly until the image begins to appear bright. If the sample has low reflectivity, increase the amount of light by using the adjustment control of the microscope. In these conditions you can look for the region of the sample to be measured. Use the manual XY control knobs or the joystick to move the stage until this region is contained within the field of view. If this region is very difficult to find, we recommend you use a lower magnification objective.

Once the surface is close to focus use the fine movement of the stage on the joystick until the slit pattern structure appears in the display area. If you have a flat, smooth sample you will be able to see almost all the slits focused at the same time. But for rough or tilted samples, you will see the slit pattern only in those regions of the surface which are in perfect focus.

#### 3.1.2 Focusing an interferometrical objective

Place the sample on the holder and choose the microscope objective you want to use. Generally interferential fringes appear in a very narrow plane centered on the best focus position of the sample. If the sample is rough, then focusing is achieved by focusing the sample itself. Nevertheless, if the sample is smooth focusing is very difficult. To aid focusing very smooth samples, press the “Image View” button in the top of the acquisition screen. This button switches between a conventional microscope image and a circular iris centered on the screen. When the sample is out of focus, one iris can be seen. This corresponds to the reflection on the reference mirror of the interferential objective, and the focus of that does not change with the position of the sample. When the sample gets closer to the focus, a second iris reflection appears, and coincides with the previous one when the sample is just placed on the focus of the objective.



With the sample in focus you have to adjust the intensity of light. If some pixels of the CCD camera are saturated because of excessive intensity, the software shows these regions in red. For flat samples increase intensity to just below saturation. For rough, tilted or structured samples check for different focus positions to avoid saturation.



To use the coarse control buttons of the joystick, the right focusing stop ring of the microscope stage must be always released. Moving the focus with the focusing stop ring clamped may cause irreversible damage to the mechanical stage.

### 3.2. Selecting the acquisition parameters

There are six parameters in the Acquisition Parameters zone, which must be selected before pressing the acquisition button (see Section 5 for more details). You will have to choose the kind of measurement that you want to obtain, the objective, the measurement area, the direction of the scanning of the sample along the z-axis, the length of the scan along the z-axis, and the allowed measurement

error. If the sample has a marked lay direction, and you want to measure the structure perpendicular to the lay, you probably need a profile or a multiple-profile measurement. Alternatively, if you want to measure the roughness or waviness of a sample you have to choose the extended profile measurement and enter the desired length. Finally, if you have a 3D structure, you can inspect it by acquiring a confocal image, or measure it with the Topography option.

In any case it is very important to make the right selection of the objective that will be used for measurement. If the selection does not coincide with the objective, the result will be completely useless. It is also important to ensure the direction and the total scanning length along the z-axis.

### 3.3. Setting the Z scan range

Once the sample is focused and the acquisition parameters have been selected, it is important to ensure enough range for Z scan displacement. If the range is too broad, acquisition will waste a lot of time and sometimes you may even obtain a measurement with lower resolution. If the range is too narrow, there will be zones in the measurement without confocal or interferential information, and the measurement will show “non-measured” points.

To avoid problems with the Z scan range setting, the software checks that the stated value is contained within an interval which is dependent on the objective and the kind of measurement that has been previously selected. If, for instance, the stated value for Z range is close to the working distance of the objective the software will automatically reduce the setting to the maximum value of the allowed interval. In contrast, if the setting is too low it will be automatically increased to the minimum value of the allowed interval in order to ensure enough information when the sample is swept through the confocal depth of focus.

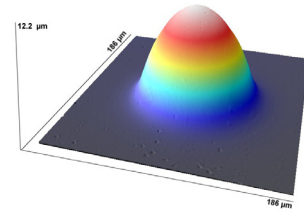
Any value contained within the allowed interval will be accepted. Nevertheless, it is important to be as close as possible to the optimum value for each measurement. You can estimate this optimum value by simply moving the sample along the z-axis (using the joystick or the virtual joystick contained in the motor control zone of the acquisition screen). Move the stage downwards to such a point that you are no longer able to see neither, the slit pattern or the interferential fringes focused somewhere on the sample. Take the reference of this z position (using the scale of the manual fine knob of the microscope stroke or the display of the virtual joystick). Then move the stage upwards to such a point that you are no longer able to see the pattern focused somewhere on the sample. Take the reference of this new z position. Measure the difference between the two z positions, increase this value by roughly 20% and use the result in the Z scan edit box.

As stated earlier, if the range is too narrow, there will be zones in the measurement without confocal information, and the results will show “non-measured” points at the top or the bottom of the profile or the topography. In this case you must check that

both the direction and the starting point of z scanning were correct (see Section 5.8). Otherwise you must increase the Z scan value.

## Chapter 4

# The Motors Control



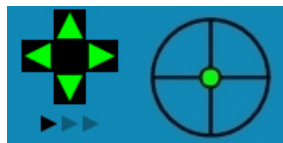
There is a zone for the control of the motorized stages of the microscope in both the acquisition and the configuration screens. In this zone you can move the sample in three dimensions, in the same way as you can with the joystick.

### 4.1. Stop Motion

This is a safety control which is always enabled. To stop any motor motion, press the ESC key of the keyboard.

### 4.2. XY Motion

This control acts as a virtual joystick and allows the sample to move in XY directions in discrete steps. The size of each step depends on the objective previously selected.

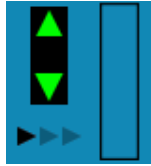


Objective Magnification	10X	20X	50X	100X
XY velocity ( $\mu\text{m/s}$ )	30	15	6	3

With the green arrows the XY stage is moved continuously. To increase or reduce the speed use the black arrows. With the use of the green dot inside the circle, the XY stage is moved in any direction.

### 4.3. Z Motion

This control acts as a virtual joystick and allows the sample to move in Z direction in continuous movement. To move the Z motor up or down press the green arrows. To increase or reduce the speed use the black arrows. To fine adjust the focus, click and drag the vertical box.

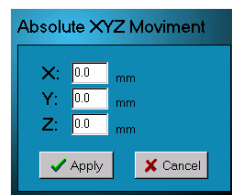
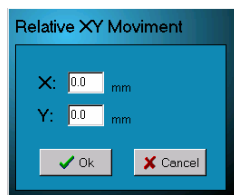


## 4.4. Relative and Absolute Motion

These controls allow the execution of a relative or an absolute movement of the sample. The relative movement is executed from the current position to the new one, and is only available for the XY directions. Alternatively, the absolute movement is executed from the defined home to the new position and is available in all three dimensions. Nevertheless, the Z movement is limited from the home position to half of the working distance of the previous selected objective.



If the selected objective is not the current objective in the microscope, the software lets you enter a large Z scan value that may cause the objective to crash with the sample.



## 4.5. Home, Define Home and Center Table

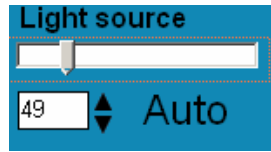
If you click “Home” the program executes a command that moves the XY table and the Z motor to the previously defined home.

If you click “Define home”, the home is defined in the current position.

If you click “Center table” the program executes a movement in the X and Y direction to search the middle point of the table and moves there. Then, it defines home in this position.

## 4.6 Light control.

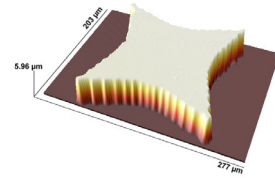
PLμ 2300 has an LED as a light source. The light intensity of the LED is controlled by 8 bit microcontroller PWM signal. The maximum light intensity is achieved with a 255 value, while the minimum is 1.



## Chapter 5

# The Acquisition Screen

---



The acquisition screen is the main window that always appears after starting the PLμ 2300 software. In this window you can get ready to acquire a new measurement or you can load a previous measurement from the hard disk. You can also enter the Configuration screen and move the motorized stages as explained earlier in Section 4.

### 5.1. Menu Toolbar

- **Load**

Loads a previous measurement from the hard disk. All measurements are saved as “\*.plu” files and the software recognizes the kind of measurement. Once a user has loaded a file, the software automatically enters the Display and Analysis screen and applies all the processing information taken in the last saved session.

- **Save acquisition macro**

Saves the current parameters selected for a measurement, including the kind of Measurement, the Objective, the Area, the Scan, the Term, the Filter, the Display, the Colour, the Scale and the Zoom.

This function is useful for automating processes, where the user needs to take a large number of measurements under the same conditions.

- **Load Acquisition macro**

Loads a saved macro.

- **Print Setup**

Enters the printer setup window.



- **Print Screen**

Prints the whole desktop area.

- **Exit**

Exits the software.

## 5.2. Configuration and Alignment

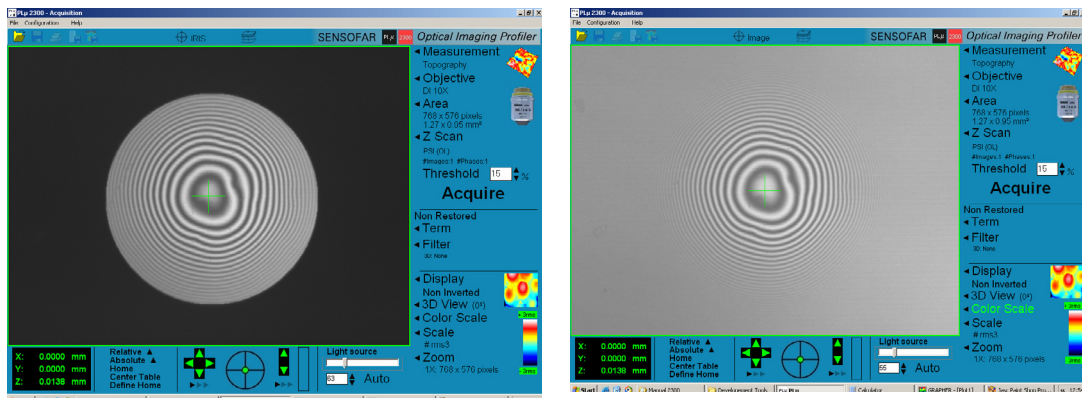
The configuration and alignment button enters the Configuration screen. This window must be entered by trained people to avoid any improper configuration of the instrument.

## 5.3. The Image View Option



When the “Focus” label appears in the “Image View” of the acquisition screen, the software shows a conventional image. The way it is achieved is by showing a white image in the microdisplay, doing a wide illumination of the sample.

When the “Image” label appears in the “Image View” of the acquisition screen, the software shows an aiding tool to get focus. If a confocal objective is selected, the Image view projects a slit pattern on the surface. The focus of the sample is achieved by focusing the slits. If an interferential filter is selected, the “Image View” projects a circular iris. The focus of the sample is achieved when two equal iris overlaps (see section 3.1).



## 5.4. The Autofocus Option

The autofocus command is used to fine focus the sample in order to optimize the starting z coordinate for the acquisition.

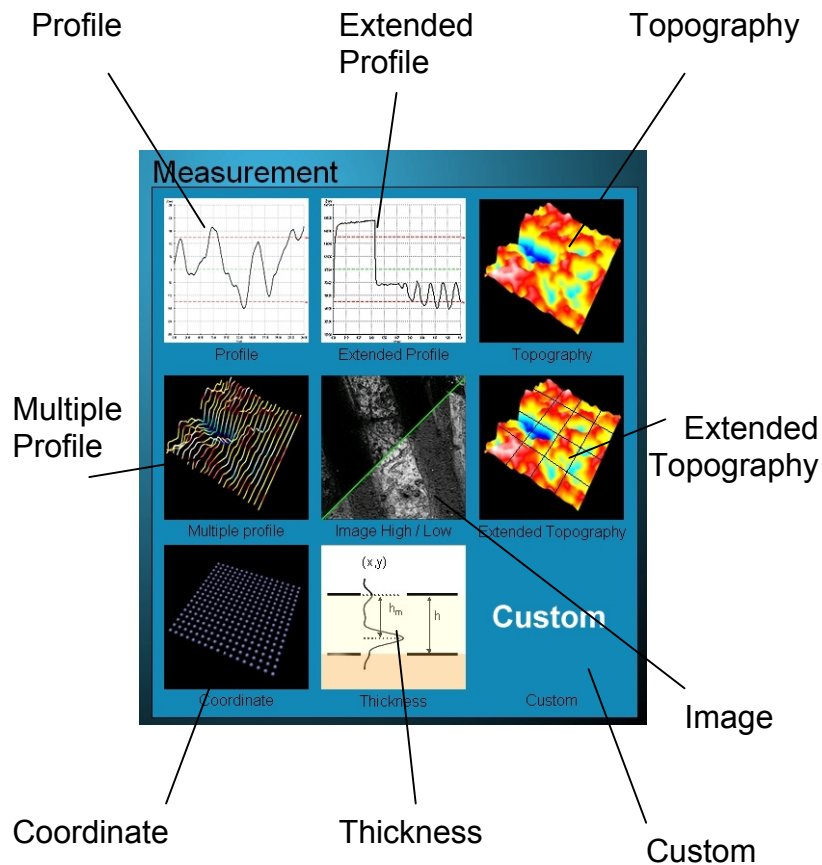
If a confocal objective is selected, the autofocus performs a scanning of a vertical predefined value range for each objective and looks for the best confocal position of the overall image. Then it moves to this position and carries out a second scanning with a half range. This procedure is repeated four times. The values of the initial Z scan range are shown below.

Objective	10X	20X	50X	100X
Initial Z scan range ( $\mu\text{m}$ )	200	100	20	20

If a interferential objective is selected, the autofocus performs a scanning of a 40  $\mu\text{m}$  vertical range and looks for the highest contrast of the fringe pattern of the overall image. Then it moves to this position and carries out a second scanning with the half range. This procedure is repeated four times.

## 5.5. The Measurement Window

You will use the Measurement window to select the kind of measurement that you want to acquire. There are several measurements, namely *Profiles*, *Multiple-Profiles*, *Extended Profiles*, *Coordinate*, *Topographies*, *Extended Topographies*, *Conventional image*, *Confocal Images* and *Thicknesses*. There is also a free slot for a *Custom Application*. Each kind of measurement has its own properties, both in software and hardware configuration and the user should be familiar with them.



- **What is a *Profile*?**

A *Profile* is a 2D measurement of the heights of the points situated along the central slit of the pattern imaged on the sample. The result of a *Profile* is similar to that obtained with a mechanical contact instrument. In this case the length that can be measured is limited by the field of view of the selected objective. The *Profile* is, by far, the fastest measurement taken by the PLμ 2300 (about 3 s), and is very useful for checking acquisition parameters such as the Z Scan range and the Threshold.



Single profiles can only be acquired using confocal objectives

The *Profile* is also the measurement recommended for those samples with a marked lay direction and for the evaluation of roughness and waviness with short evaluation lengths.

- **What is an *Extended Profile*?**

An *Extended Profile* is a profile with a total measurement length larger than that obtained with a single *Profile*. It is obtained by measuring and stitching together many different single contiguous profiles.



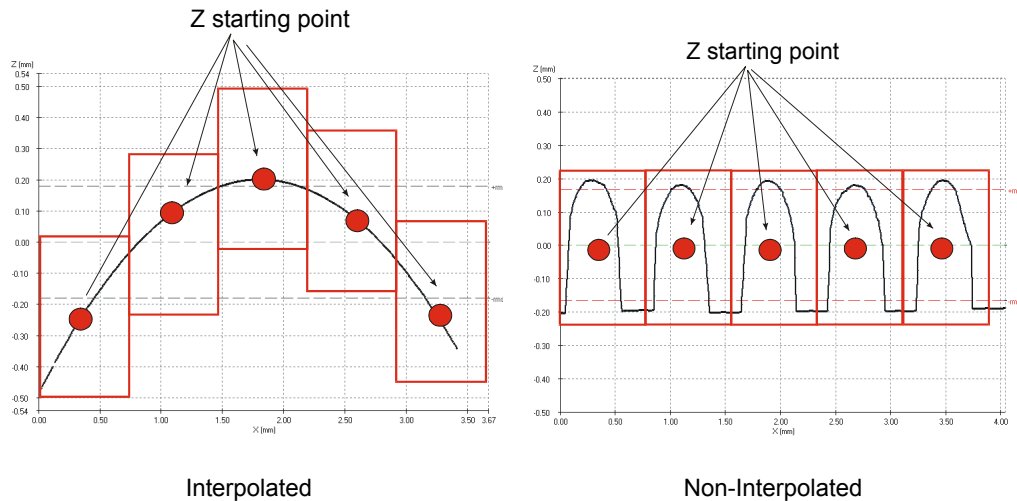
The *Extended Profile* option is only available for that configuration of the PLμ containing the XY motorized stage.

The *Extended Profile* option can only be used with confocal objectives.

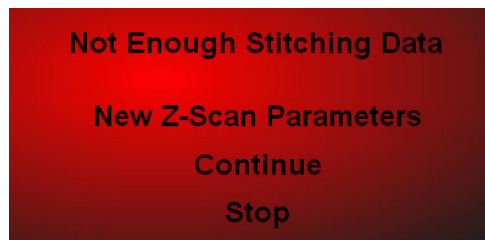
With *Extended Profile* measurement you will be able to obtain profiles as large as 100 mm. The user has to select the objective and enter the desired length in the Length edit. The PLμ 2300 software calculates how many single profiles are needed to obtain the requested measurement length. When the acquisition is running, the software drives the XY motorized stage to measure the profiles. In each single measurement, the software calculates stitching information and local slope in order to follow the shape of the sample.

To start an *Extended Profile* measurement place the sample under the microscope with the starting point in the left. Also, the *Extended Profile* is always symmetrical in the Z scan window, which means that the upward and downward options are not available.

Users can choose between *Interpolated* and *Non-interpolated* options in the Z Scan window. The *Non-interpolated* option simply measures each profile from the same Z starting point, and is only useful for flat surfaces. With the *Interpolated* option the software fits a second order polynomial to each single profile measured in order to evaluate the curvature of the sample. In the next profile, the software changes the Z starting point to the position given by the polynomial. The *Interpolated* option is very useful for samples with curved shapes. However, the measurement will be aborted when the calculated Z starting point is too close to the objective.

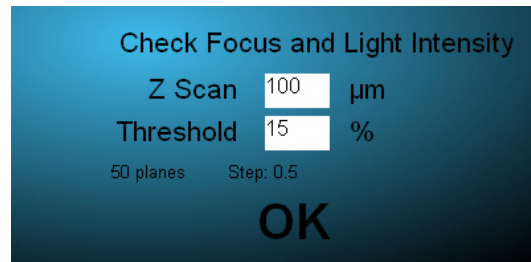


Sometimes, there is not enough information to stitch together two contiguous profiles. This problem arises when there are not enough measured points at the end of a single profile or at the beginning of the next one. In this situation the software shows a *Lost Profile* dialog window to help the user to solve the problem. There are three different possibilities: *Continue*, *Stop* and *New Z-Scan Parameters*.



Lost Profile dialog window

With the *Continue* option, the software continues to the acquisition of the next profile, but stitching of the current one will be wrong. With the *Stop* option, the software stops measuring and shows the result of the measurements taken up to that moment. With the *New Z-Scan Parameters* option, the user can refocus the sample (using the manual focus knob of the microscope stand), increase or decrease the amount of light, and enter new *Z-Scan* and *Threshold* parameters in order to perform a new scan in the same region of the sample. The new parameters will be only used in the current profile acquisition.



New Z-scan parameters

The reasons why it is not possible to measure enough points at the end of a single profile or at the beginning of the next one may be twofold:

- (i) The Z range is out-of-focus and/or too narrow. In this case you can try refocusing the sample and/or increasing the *Z Scan*.
- (ii) The slope in these regions is too steep. In this case you can try lowering the *Threshold* and/or increasing the light intensity. If the problem persists you will have to obtain a new measurement with a higher numerical aperture objective.

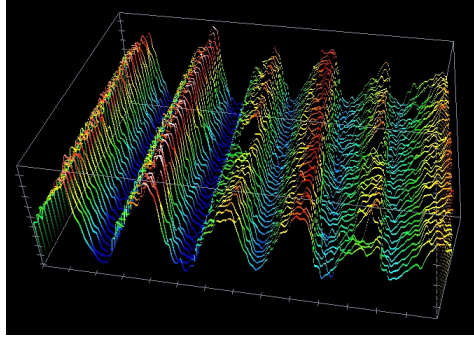
- **What is a *Multiple-Profile*?**

A *Multiple-Profile* is a profile measurement which is taken simultaneously at all the slits of the pattern imaged on the sample. The measurement principle is the same as in a *Profile*, but the difference is that the *Multiple-Profile* measures the metrological information contained not only in the central slit but in all the slits of the image.



The *Multiple-Profile* option can only be used with confocal objectives.

The *Multiple-Profile* result looks like a Topography. This is a very fast measurement and is very useful for samples with a marked lay direction in which it is not important to measure very fine details.



Example of a Multiple-Profile

- **What is a *Coordinate*?**

A *Coordinate* is a measurement of a large sample point by point, up to 100 x 72 mm<sup>2</sup>.



The *Coordinate* option is only available for the configuration of the PLμ containing the XY motorized stage.

The Multiple-Profile option can only be used with confocal objectives.

To obtain a *Coordinate* measurement the user has to write a file into the hard disk. The file layout contains the X, Y and Z positions of the points to be measured arranged in three different columns. The X and Y positions are the coordinates of the point that has to be measured, and Z is a rough estimation of the height of that point.

Once the user has clicked the *Acquire* button, the software moves the sample point by point and measures the fine position for the Z coordinate. The fine Z position is calculated as the average of the heights measured with a *Single-Profile* measurement within the most central points of the field of view of the objective that is being used.

When the *Coordinate* measurement is finished, the software tells the user the name of the file that contains the calculated fine Z positions for each point.

- **What is *Topography*?**

*Topography* is a 3D measurement of the whole volume of the sample, within the field of view of the objective.

To measure *Topography*, just place the sample under the microscope and focus. Then select the *Area*, the *Range* and the *Threshold* and click the *Acquire* button.

The PLμ software automatically calculates the number of planes to be acquired and the required Z scan movement depending on the acquisition mode. It then drives the Z motor to obtain a confocal image in each plane. After acquiring all the confocal information, the software calculates the metrological data and switches to the Display and Analysis screen to show the result.

Because each confocal image requires a large amount of RAM memory, the number of planes to be acquired is limited depending on the selected resolution and the Z *range*. For instance, if you want to measure an *Area* of 768x576 pixels, the maximum number of planes will be limited to 100, and 170Mb of RAM memory will be allocated.

- **What is an *Image*?**

An *image* is the result of acquiring the intensities of all the pixels contained in the camera.

If a confocal objective is used, confocal and conventional images can be acquired. A confocal image is somewhat different to a conventional image. As explained in Sections 1.1 and 1.2, a confocal image has bright pixels in zones where the sample is close to the focus, and dark pixels for the zones that are out of focus. The PLμ 2300 software acquires the confocal image according to the confocal algorithm selected in the options window of the Configuration and alignment screen. The conventional image is directly the broadband illumination image seen by the CCD camera.

If an interferential objective is used, a conventional image can be acquired.

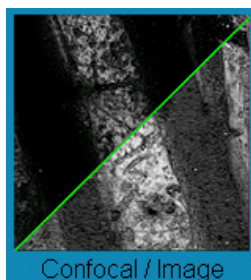


Image option using confocal objectives

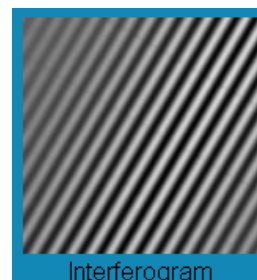


Image option using interferential objectives



To acquire an Image just choose the Area and click the Acquire button. The software drives the microdisplay and shows the result in the Display and Analysis screen.



Example of an *image low*



Example of an *image high*

- **What is *Extended Topography*?**

Extended Topography is a 3D measurement of the whole volume of the sample, with a field of view that can be greater than that of the objective.



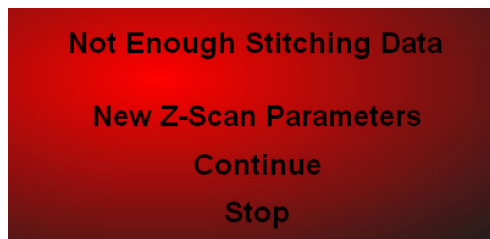
The Extended Topography is only available for the configuration of the PLμ 2300 containing the XY table and the PZT device.

To measure Extended Topography just place the sample under the microscope and focus. Then select the Area, the Range and the Threshold and click the Acquire button.

The software of the PLμ automatically calculates how many single Topographies are needed in order to fill the desired area. Once the user has clicked Acquire, the software goes to the first Topography and measures it with the Z Scan and Threshold values selected. Then it moves to the contiguous Topography by just the full field of view of the objective minus a selectable amount of overlapping area. Once all the Topographies have been measured, the software calculates stitching parameters in order to avoid slippage of the sample and the mechanics, and copies all the data to a final result. Then the software switches to the Display and Analysis screen, where the stitched Topography is treated as a single Topography.

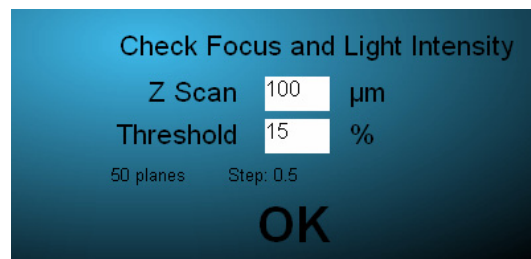
To know how to choose the Area and Scan parameters, please refer to the Area and Z scan section.

Sometimes, there is not enough information to stitch together two contiguous Topographies. This problem arises when there are not enough measured points at the edge of a single Topography or at the beginning of the next one. In this situation the software shows a *Lost Topography* dialog window to help the user to solve the problem. There are three different possibilities: *Continue*, *Stop* and *New Z-Scan Parameters*.



Lost Topography dialog window

With the Continue option, the software continues to the acquisition of the next Topography, but stitching of the current one will be wrong. With the Stop option, the software stops measuring and shows the result of the measurements taken up to that moment. With the New Z-Scan Parameters option, the user can refocus the sample (using the manual focus knob of the microscope stand), increase or decrease the amount of light, and enter new Z-Scan and Threshold parameters in order to perform a new scan in the same region of the sample. The new parameters will be only used in the current Topography acquisition.



New Z-scan parameters

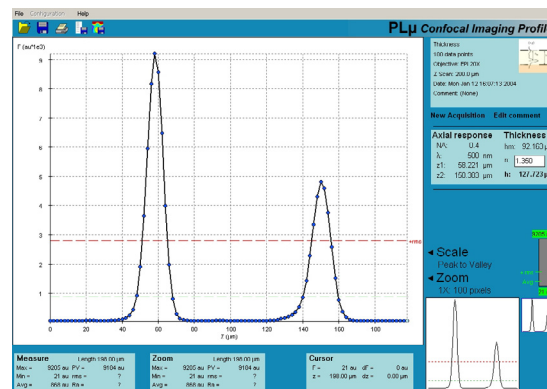
The reasons why it is not possible to measure enough points at the edge of a single Topography or at the beginning of the next one may be twofold:

- (i) The Z range is out-of-focus and/or too narrow. In this case you can try refocusing the sample and/or increasing the Z scan.

- (ii) The slope in these regions is too steep. In this case you can try lowering the *Threshold* and/or increasing the light intensity. If the problem persists you will have to obtain a new measurement with a higher numerical aperture objective.
- **What is a *Thickness* measurement?**

This is a measurement that gives as a result the thickness of a transparent layer or a transparent foil in a single point within the field of view. The PLμ will supply accurate results whenever the measured layers or foils have thicknesses larger than a few microns. The thinner the layer, the higher the NA of the objective needed for the measurement. The PLμ is also able to measure thin films (under 1 μm) on structured surfaces containing dissimilar materials, but this is a special application not included in the standard PLμ software. If you need more information, please contact the manufacturer (info@sensofar.com).

To measure Thickness, just place the sample under the microscope, focus and click on the position that you want to measure within the acquisition screen. Then select the Range, type the Index of refraction *n* of the transparent material and click the Acquire button.



Example of Thickness Measurement

The result of the Thickness measurement shows the confocal axial response with the peaks corresponding to the two interfaces of the layer or the foil. The information related to this measurement appears on the right area of the Display screen:

- NA is the numerical aperture of the selected objective.
- $\lambda$  is the wavelength used by the PLμ.
- $Z_1$  and  $Z_2$  are the height coordinates of the two peaks of the axial response.
- $h_m$  is the difference in height between  $Z_1$  and  $Z_2$ .

- $n$  is the index of refraction. The user can update this figure in the display screen.
- $h$  is the real thickness of the layer or the foil.

The thickness result  $h$  is obtained automatically by the software from  $h_m$  and the correction factor to compensate for the depth distortion effect.

- **What is a *Custom Application*?**

A *Custom Application* is a user-defined measurement specially programmed for different samples and customers.

To develop a *Custom Application*, please contact your distributor.

## 5.6. The Objective Window

The *Objective* window allows you to select the Objective that will be used for the measurement.



The Objective Window

The PLμ 2300 has available twelve different confocal objectives and five interferential. Six of the confocal are Super Large Working Distance (SLWD) and Extra Large Working Distance (ELWD) objectives (20X, 50X and 100X), and six more of them are EPI Plan objectives (5X, 10X, 20X, 50X, 100X, and 150X). There are three DI interferential objectives (Mirau type) and two TI interferential (Michelson type).

To select the *Objective*, just click on it.

If the Objective is not available, the software shows a dimmed image. Also, if the objective is available but not calibrated, the software shows a red message saying that the objective is not available. In both cases the user cannot use this objective.



It is very important to select the right objective before acquiring. If the objective selected does not coincide with the objective being used, the result will be completely useless.

## 5.7. The Area & Length Window

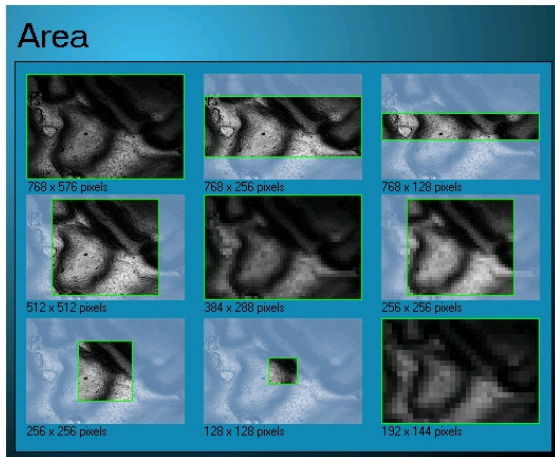
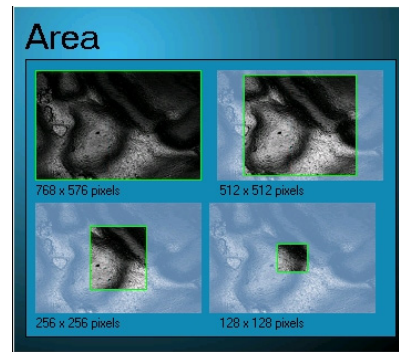
The *Area* and *Length* window is used to select the size of the area (for *Multiple-Profile*, *Topography*, *Extended Topography* and *Image* measurements) or the size of the length (for *Profile* and *Extended Profile* measurements).

To select the *Area*, just click on the image. The PLμ 2300 software automatically calculates the real area seen by the objective and shows the result just below the *Area* label.

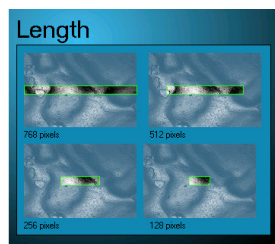
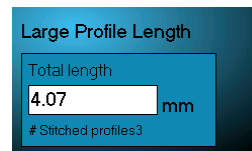
There are five different windows of the *Area* and *Length*, depending on the measurement selected.

For a *Topography* the *Area* window shows nine selections: full area with high-resolution (768x576), medium-resolution (384x288) and low-resolution (192x144); a large square area of high-resolution (512x512) and a large square area with medium-resolution (256x256); two more square areas of high-resolution, (256x256) and (128x128); and finally two rectangular areas with high-resolution, (768x256) and (768x128).

In the same way, for an *Image* and *Multiple-Profile* measurement, the *Area* window shows four selections for different areas, in all cases with high-resolution.

Area window for a *Topography*Area window for an *Image and Multiple-Profile*

The *Length* window for a *Profile* shows four different lengths, all of them with high resolution. The window for *Extended Profile* shows an entry field, where the user has to introduce the desired total measurement length.

Length window for a *Profile*Length window for an *Extended Profile*

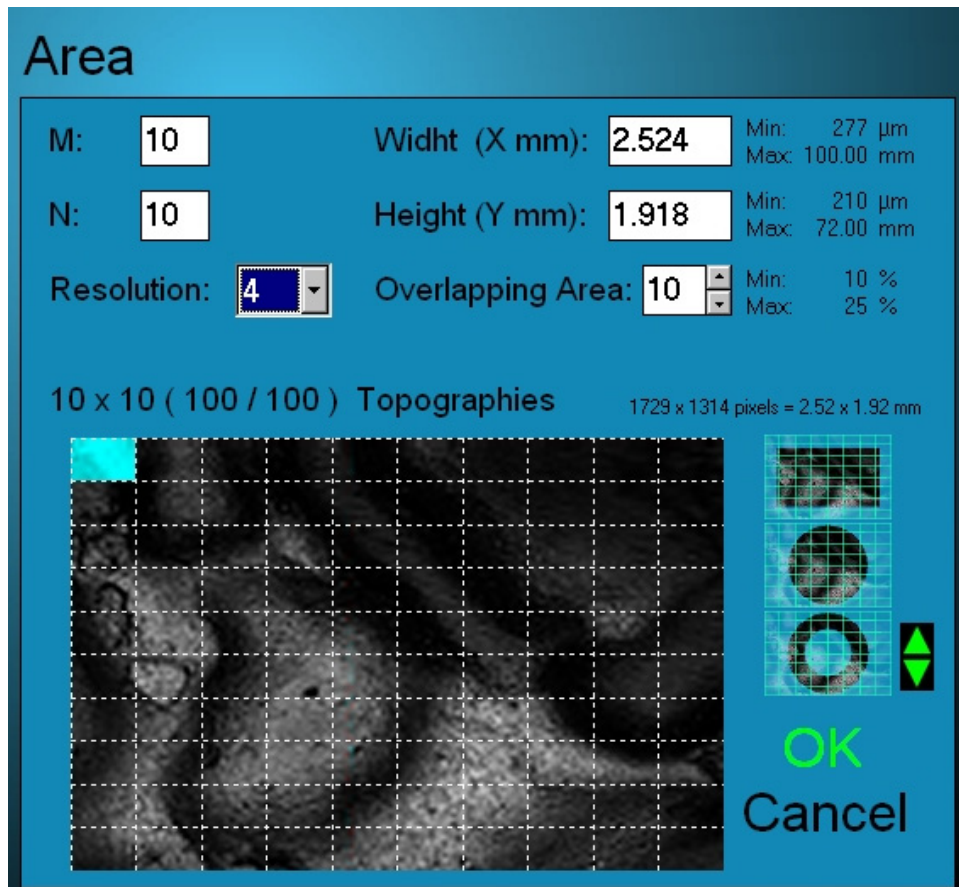
For an *Extended Topography* the Area Window shows an interactive way in order to choose the desired acquisition parameters.

There are several options:

- N and M. These figures are used to select the number of single Topographies (N rows and M columns) to measure. The software calculates the Width and Height of the area and updates it.
- Resolution. This figure stands for the resolution of a single Topography. The higher the resolution number, the lower the number of measured pixels. The selectable resolutions are 1, 2, 4, and 12. For resolution 12, and confocal objectives, the PLμ 2300 software doesn't need to scan the

slit pattern with the microdisplay, and the single multiple-profile measurement is taken very quickly.

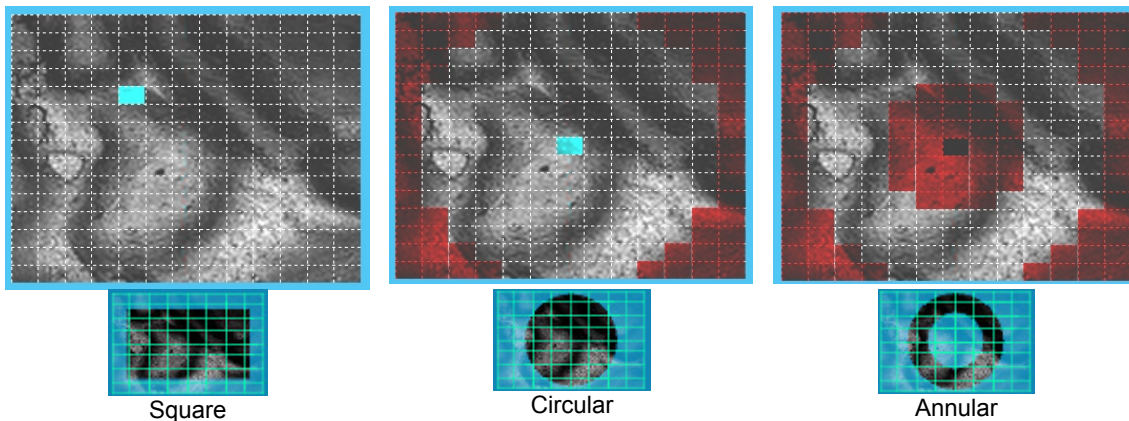
- Width and Height. These numbers are used to select the measurable area in mm. The software calculates the N and M and updates it.
- Overlapping Area. This is the amount of overlapping area between contiguous Topographies. The available overlapping areas are 10%, 15%, 20% and 25%. The greater the overlapping, the greater the number of single Topographies required to reach the desired measuring area. The default amount is 10%.



Once the acquisition parameters have been selected, the software displays a mesh of NxM single Topographies. The user can interact with this mesh in two different ways:



- Select the current position of the sample. By clicking the left button of the mouse on the mesh, the user can select the single Topography corresponding to the field of view of the current position of the sample. The result is shown as a blue Topography. The measurement is always taken line by line from left to right. For example, if a symmetrical sample has to be measured, place the sample under the microscope in the middle and choose the current position also in the middle of the mesh.
- Avoid measuring some Topographies. By clicking the right button on the mesh, the user can uncheck a single Topography, and the software will not measure it. The result is shown as a red Topography. The user can also select three different cropping geometries: square, circular and annular.



## 5.8. The Z Scan Window

There are two different Z Scan windows depending on the selected objective.

### 5.8.1 The Z Scan Window for confocal objectives.

The *Z Scan* window and the *Z Scan* edit are used to select the direction, the length and the speed of the scanning of the sample along the z-axis. There are two kinds of Z Scan: Single and Dual Z Scan.

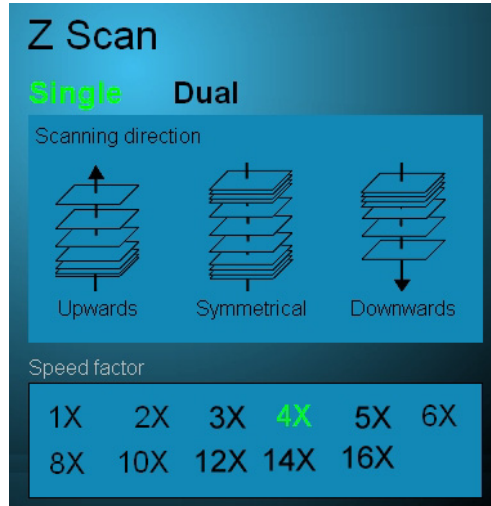
- Single Z Scan:

For the Single Z Scan, the most useful scanning option is the *Symmetrical*. To use it, you have to focus the sample right in the middle point between the most out-of-focus part and the most in-focus part of the sample. In the *Thickness* measurement you have to focus in the middle point between the two interfaces of the layer or the foil in the selected position within the field of view. Alternatively, if you want to avoid



obtaining confocal information on some parts of the sample, you can use the *Upwards* or *Downwards* scanning direction.

To use the *Upwards* direction turn the fine movement of the Z-axis of the joystick counter-clockwise, to the desired Z start position. To use the *Downwards* direction turns the fine movement clockwise.



Single Z Scan

The Z Scan edit is used to introduce the desired Z scan range. Refer to Section 3.3 to evaluate the optimum Z range value for each measurement. Once the user has entered the range, the software calculates the number of planes needed to carry out the measurement and shows the result below the Z Scan label.



The calculation is made with internal values of the optimum step for each objective. For example: if the user has entered a 20-μm range with the 20X EPI objective, the software will acquire 40 planes with steps of 0.5 microns. But if the objective is the 100X EPI, the software will acquire 100 planes with steps of 0.2 microns.

Objective Type	Z Scan step	Objective Magnification			
		10X	20X	50X	100X
CFI Plan EPI	Optimum step (μm)	2	0.5	0.2	0.2
	Maximum step (μm)	32	8	3.2	3.2
CFI Plan ELWD and SLWD	Optimum step (μm)	-	1	0.5	0.2
	Maximum step (μm)	-	16	8	3.2

Nevertheless, the Z Scan range has some limitations. As explained earlier in Section 3.3, when the range is too great, acquisition will waste a lot of time and sometimes you may even obtain a measurement with lower resolution. If the range is too narrow, there will be zones in the measurement without confocal information, and the measurement will show “non-measured” points.

To avoid these problems with the Z scan range, the software automatically checks either the range or the total number of planes. If the Z range is not enough for the measurement, the software changes it in order to reach a minimum number of planes. Alternatively, if the number of planes calculated with the desired range, the optimum step and the resolution is greater than the maximum allowed by the available memory, then the software changes the range value to the maximum allowed.

	Minimum number of planes	Maximum number of planes
<i>Profile</i>	50	Memory dependent
<i>Multiple-Profile</i>	50	
<i>Extended Profile</i>	50	
<i>Coordinate</i>	50	
<i>Topography</i>	20	
<i>Extended Topography</i>	20	

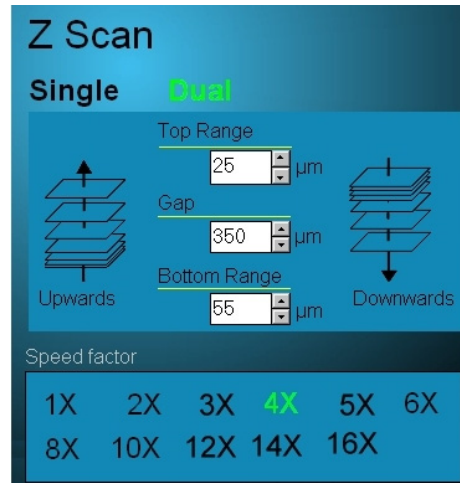
The *speed factor* can be used to reduce the acquisition time. A speed factor of 1X means that the software will do the acquisition using the optimum step between planes. With a 2X value the software will acquire using a double step size and half number of planes (a maximum of 16X is allowed). While increasing the speed factor, the acquisition time is reduced but also the profiling performances of the system get worse. The optimum speed factor depends on the sample roughness and the user has to check which one is the maximum acceptable for its application.



Speed factors above 4X can lead to inaccurate results for smooth surfaces. Check the correct speed factor for your sample to keep the needed accuracy. See the Troubleshooting section for more information.

- Dual Z Scan.

The *Dual Z Scan* is used to measure samples with a high step, in which it makes no sense to scan the step section. To do a *Dual Z Scan*, just click on *Dual* in the Z Scan window.



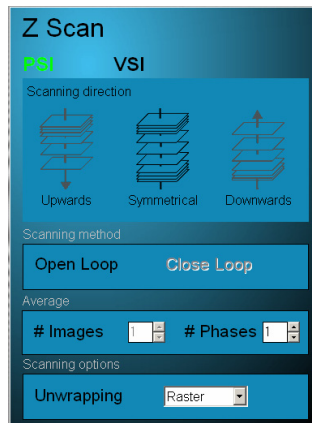
Dual Z Scan

Focus the sample at either the top or the bottom and choose the upwards or downwards scanning direction. Enter the desired scanning amount in the *Top Range* and *Bottom Range*, and the *Gap*. Select the *Speed Factor*.

Once the user has clicked on *Acquire*, the software scans the first region, then moves the amount of *Gap* without scanning, and scans the second region. The result is treated as a regular measurement. The effect of the speed factor on the number of planes scanned along the top and bottom ranges is the same as in a single z scan.

### 5.8.2 The Z Scan Window for Interferential objectives

There are two different Techniques for interferential measurements: the Phase Shifting Interferometry (PSI) and the Vertical Scanning Interferometry (VSI). To choose between these two kinds of techniques, click on the desired one.

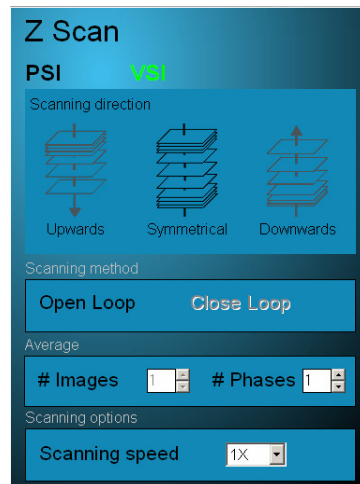


If the PSI technique is selected, the “Z Scan” edit box is disabled. After clicking the “Acquire” button, the PLμ software will scan 6 μm in the Z direction in order to acquire the phase map. There are three different selections that can be done using the PSI technique:

- Scanning method. Open loop and Close loop. These options are applied to the scanning device available in the hardware. If the system does not contain a close loop device, this option is disabled.
- Average. There are two possibilities: to average images and phases. Averaging images is only available in those systems that contain a close loop device. In that mode the Z plane is maintained for every Z scan step and a given number of images of the ccd camera are there averaged. Averaging images is available all the scanning devices. In that mode a given number of phase maps are acquired and then averaged. The use of Phase and Image averaging helps to reduce the noise in the final measurement.
- Scanning Options. This is the unwrapping algorithm available for PSI measurements. There are three different algorithms: Raster, Quality and Goldstein.

Raster algorithm is a point by point unwrapping algorithm, very fast and suitable for very smooth surfaces. Quality algorithm is a modified raster looking for different regions. Goldstein algorithm is very slow but usable for rough surfaces of surfaces that contains discontinuities.

If the VSI technique is selected the “Z Scan” edit box is enabled. The available options are the same that for PSI except for the Scanning Options. In that you can select the scan speed of 1X or 3X. Using 1X scanning speed the sample is scanned along the Z axis at 3 μm/s, while using the 3X scanning speed is moved at 9 μm/s.



## 5.9. The Threshold Edit

The *Threshold* edit is used to ignore pixels with low measurement reliability.

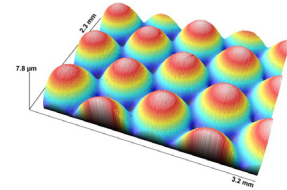


The default value for the *Threshold* is 15%. If the measured result shows large number of lost pixels, try reducing the *Threshold*.

Reducing the *Threshold* to a value below 10% on a rough surface may cause spikes to appear. Avoid using low values.

## Chapter 6

# The Display and Analysis Screen



## 6.1. Organization of the Display and Analysis Screen

The Display and Analysis screen is organized in five different zones: the File menu, the Numerical Data area, the Analysis area, the View area and the Measurement Report area. The Numerical Data area supplies statistical information about the measurement, while the Analysis and View parts allows the user to modify the data either by applying a filter or modifying the display conditions.

## 6.2. File Menu

You can use the File menu to save a measurement, save a bitmap of the display area, export the data, save a graphic file, subtract two measured files or print a report containing the information of the measurement.

The saved file contains not only the raw information of the measurement, but also the modifications introduced by the user. If the user has applied a filter when the file is loaded from the acquisition window, the software applies that filter and shows the result. Of course, the user can remove the filter and retrieve the raw data.

The Save as BMP option allows the user to save a Bitmap file containing the information of the display area.

The Export option allows the user to export the measurement data in two formats: a DAT file or a RAW file. With the DAT format, the data is saved as an ASCII two or three columns. If the measurement is 2D, the file contains two columns; the first one is the X-axis coordinate and the second is the Z-axis data. In contrast, if the measurement is in three dimensions, like a Topography or an Image, the file contains three columns; the first and the second are the X and Y coordinates respectively, and the third one is the Z-axis data. In contrast, the RAW format saves the data in binary, placing only the values of the z coordinate only for a Topography or an Image.



The *Export* option saves the data displayed on the screen.

The Save Graphic option allows the user to save a graphic file containing the information of the display area. The user can select a Windows Bitmap (\*.BMP) or a Joint Photographic Experts Group (\*.JPEG) format.

The Load and Subtract option allows the user to load a file from the hard disk and subtract it from the current measurement. The two measurements have to have been acquired in the same conditions regarding objective, field of view and resolution. The user can undo the subtract action by clicking again in the same command.

### 6.3. The Numerical Data Area

In this area either numerical data about the measurement or volume calculations are displayed, depending on the selected icon.

- **Measure, Zoom and Cursor**

Inside each blue box contained in the Numerical Data area there is statistical information about the measurement.

The *Measure* zone shows numerical data related to the whole measurement area or length including the restored pixels. The *Zoom* zone shows numerical data related to the zoomed area. Displayed information contains the point with the maximum height (Max.), the point with the minimum height (Min.), the average (Avg.), the peak to valley (PV), the root mean square (rms.) and the Ra.

The *Cursor* area shows the position x, y and z of the mouse while it sweeps over a contour or a profile display. The dx, dy, dz and slope (angle) values appear when the mouse is clicked and dragged over the measurement.

Measure <span>More ▲</span>		Zoom <span>More ▲</span>		Cursor	
Area:	292.98 x 221.38 $\mu\text{m}^2$	Area:	292.98 x 221.38 $\mu\text{m}^2$	z =	0 nm
Max =	1.24 $\mu\text{m}$ PV = 2.440 $\mu\text{m}$	Max =	1.24 $\mu\text{m}$ PV = 2.440 $\mu\text{m}$	x =	0.00 $\mu\text{m}$
Min =	-1.20 $\mu\text{m}$ rms = 492 nm	Min =	-1.20 $\mu\text{m}$ rms = 492 nm	y =	0.00 $\mu\text{m}$
Avg =	-0 nm Ra = 407 nm	Avg =	-0 nm Ra = 407 nm	angle =	0.00 °

Note: the values appearing in “measure” or “zoom” can change with the use of the restored option.

The “more” window shows statistical information related to the measurement taking into account the filter that has been applied and excluding the restored pixels. Refer to the ASME B46.1 or DIN 4768 standards for roughness and waviness parameters.

**Parameters**

**Height**

Pa: 1.855 μm	Pt: 25.13 μm	PzISO: ? μm
Pq: 2.379 μm	Ppi: ▶	PmaxISO: ? μm
Pp: 10.90 μm	Ppm: ? μm	PzDIN: ? μm
Pv: -14.83 μm	Pti: ▶	PmaxDIN: ? μm

**Spacing**

Sm: ▶	Del: 1.000 mrad	Psk: 0.166 μm
Sr: ? μm	Delq: 1.596 mrad	Pku: 3.530 μm
Pc: ? peaks/mm		

**Hybrid**





**Shape**

▼ Close

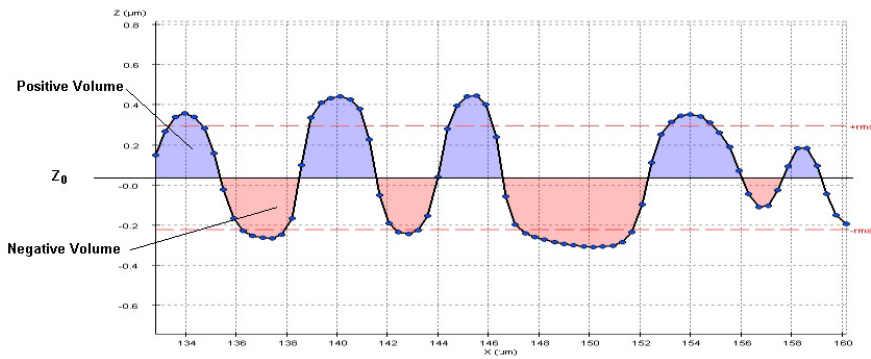
Note: The values appearing in the “Measure” and “zoom” don’t will coincide with the values appearing in “More” for a restored measurement. The values in More don’t change with the use of the restored option.

• **Volume**

To calculate the volume of any part of the sample click on the volume icon. The following area will appear:

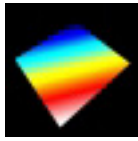
<b>Volume Assessment</b>	<b>Mask</b>					<b>Cursor</b>	angle = 0.00 °
V(+)= 13234 μm <sup>3</sup>	Full					z = 0 nm	dz = 0 nm
V(-)= -13219 μm <sup>3</sup>	Area = 0.065 mm <sup>2</sup>					x = 0.00 μm	dx = 0.00 μm
z0 = -0.000 μm						y = 0.00 μm	dy = 0.00 μm

V(+) and V(-) are the positive and the negative volumes calculated from the Z<sub>0</sub> reference height. If any term is removed, then Z<sub>0</sub> is the average of the surface, being set to the zero height. If a term is removed, then the Z<sub>0</sub> value is set to the average of the points selected by the user.



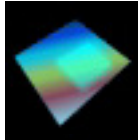


In the Mask region the area used to calculate the volume is displayed:



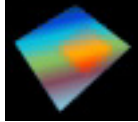
**Full:**

The volume is calculated taking into account all the points of the sample.



**Inclusive:**

The user can select which region of the sample will be used to calculate the volume. Just click and drag over the measurement.



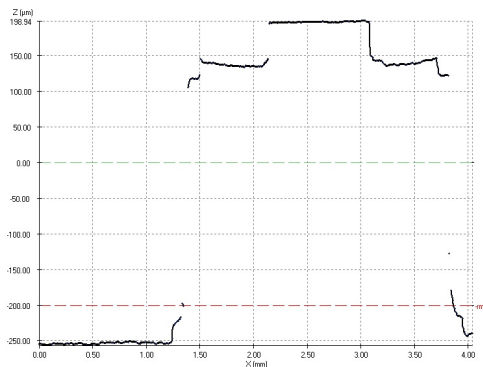
**Exclusive:**

The user can exclude one region of the sample from the calculation. Just click and drag over the measurement.

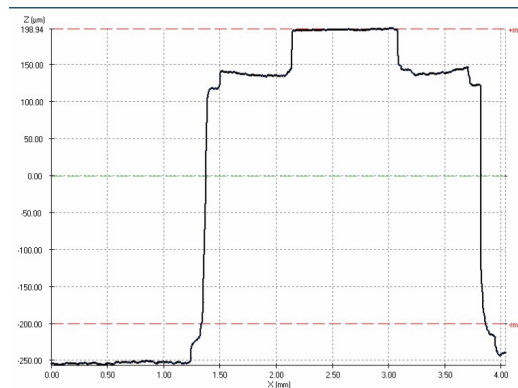
## 6.4. The Analysis Area

- **What does “Restore” mean?**

The Restore option interpolates and fills the points of the measurement that were lost during the acquisition procedure. By pressing the Restore label, the software interpolates with a spline curve and changes the message to “Restored”. To remove the interpolation, just click again on the label. Profile displays show the restored points in red.



Non-restored profile

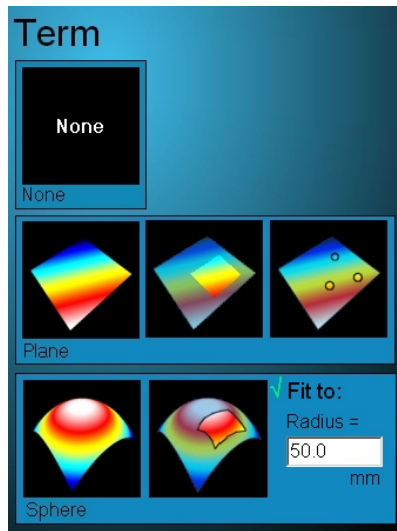


Restored profile

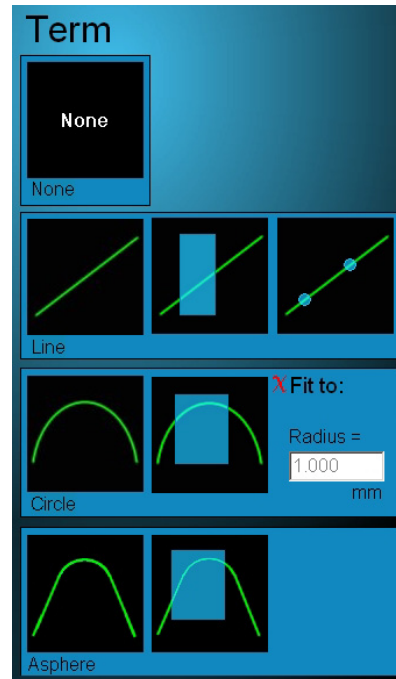
- **How to align with a “Term” (line, circle, plane, sphere and Asphere)**

“Term” is used for samples that need to be aligned, because of a tilt, an inherent spherical or aspherical component.

There are three kinds of terms: the plane term, the spherical and the aspherical term.



Term in 3D



Term in 2D

With a Plane or a Line term the PLμ software fits the best linear mathematical function and removes it from the measurement. This option is useful for those samples that have been measured with a significant tilt. It is also possible to remove a plane/line from the whole sample, a selected area of the sample, or user-given points.

With a Sphere or Circumference term the PLμ software fits a non-linear mathematical function, removes it and shows the curvature radius. It is also possible to remove this term from a selected area.

If the *Fit to:* label is selected, then the radius of curvature has to be set by the user. The software removes the sphere with the set radius that fits better to the measured results.

If an aspheric term has to be removed then it is needed to click on the aspheric image that shows the whole profile or a portion of a profile. After selecting the portion of the profile a user configurable fitting window appears.

**Aspheric Term Fitting**

Original Data and fit Graph

Aspheric Equation

$$Z(x) = \frac{X^2 / R}{1 + \sqrt{1 - (K+1) \frac{X^2}{R^2}}} + A_4 X^4 + A_6 X^6 + A_8 X^8 + A_{10} X^{10}$$

Fitting Parameters

Radius (R)	10	mm	✗
Conic Constant (K)	-1.5		✗
Aspheric Term 1 (A4)	0	mm <sup>3</sup>	✓
Aspheric Term 2 (A6)	0	mm <sup>5</sup>	✓
Aspheric Term 3 (A8)	0.1234E-8	mm <sup>7</sup>	✗
Aspheric Term 4 (A10)	0.1234E-12	mm <sup>9</sup>	✓

Regression coefficient (ρ): 0.99999 X Center: 100.000

**FIT** Save

OK CANCEL

User configurable fitting window for an aspheric term.

The aspheric term is fitted according to the following formula:

$$Z(x) = \frac{X^2 / R}{1 + \sqrt{1 - (K+1) \frac{X^2}{R^2}}} + A_4 X^4 + A_6 X^6 + A_8 X^8 + A_{10} X^{10}$$

Where R is the radius of curvature, K the conic constant, A<sub>4</sub>, A<sub>6</sub>, A<sub>8</sub> and A<sub>10</sub> the non aspheric terms.

In the upper-left part of the windows appear the original selected data in black and the best fitting in red. In the lowest-left part appears the residuals between the original and the best fit data. In the right part there is a window where the user can select what parameters are free for the fitting and what are fixed. To change the state of a parameter just click on the icon on the right. A red cross means that the parameter is fixed and the user can enter and change its value. A green check means that the parameter is free for fitting. For example, if a pure conic has to be fitted, then select the R and K with a green check and, A<sub>4</sub>, A<sub>6</sub>, A<sub>8</sub> and A<sub>10</sub> with a red cross and a 0 value.

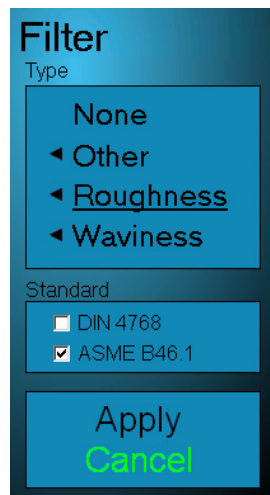
To see the effect of the fitting click the *FIT* label. The result of the quality of the fitting is shown as a regression coefficient (ρ) and the centering in the X direction. To save the fitting parameters click the *Save* button.

The *OK* label applies the fitting and shows as a result the residuals on the main screen of the software. The *Cancel* label cancels all the operation.

- **Filter**

The Filter window is used to modify the raw measurement data in order to get statistical information. In this way, you can filter to obtain roughness or waviness parameters according to surface texture standards, or simply apply median and enhancement filters.

To remove all filters, just click None.



In the “Other” option there are three different available filters: the Kernel filters, the Fast Fourier Transform (FFT) filters, and the cut filter.

The Kernel filters are applied in a neighbourhood of 3x3 pixels. There are the Median, Harsh, Sobel and Prewitt filters. The Median filter is used to smooth the measurement, while the Harsh filter is used to sharpen it. In contrast, the Sobel and Prewitt filters are used to show the slopes in two different directions.



Example of a *Harsh* filter

Example of a *Sobel* filter

Example of a *Prewitt* filter

To apply an FFT filter, just choose the frequency and select the filter (Low pass and High pass). The frequency is defined as a percentage of the maximum, and values between 1% and 100% are accepted.

The Cut filter is used to remove parts of the measurement. To restore all the cuts made, click on UnCut.

To apply a Roughness or a Waviness filter, just choose the Long Wavelength Cut-off, the filter (Gaussian or 2RC) and the number of cut-offs. Then press the Apply button.

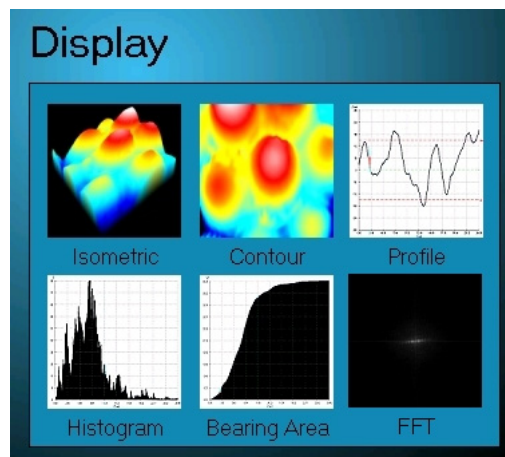
## 6.5. The View Area

The View area is used to select how the measurement results will be displayed. In this way, you can choose between different view angles, use the inversion option, modify the colours and the scale, and apply the zoom.

- **Display**

The Display window is used to select how the results will be displayed. You can choose between 3D Isometric, Contour mode, 2D Profile, Histogram, Bearing Area and Fast Fourier Transform.

Isometric and Contour views are not available for Profile and Extended Profile measurements.



If the acquired measurement is Topography or an Image, the profile view will show a 2D cut of the 3D measurement. On the right side of the window the user can select the position and the direction of the profile (X, Y or skew). To make a cut, just click and drag on the window. For the X and Y cuts, the length of the profiles is the length of the zoomed are. For a skew cut, the total length is selected by the user. The skew cut can also be moved using the right button of the mouse.

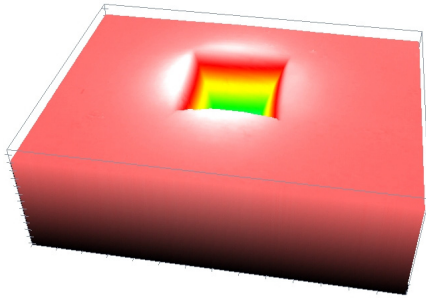
In a Contour view Topography or an Image is seen from the top. To know the dx, dy and dz values, just click and drag on the view. A blue line will appear, showing the initial and final positions.

In a Profile view just click and drag to know the dx and dz values. Two vertical lines will appear showing the initial and final positions.

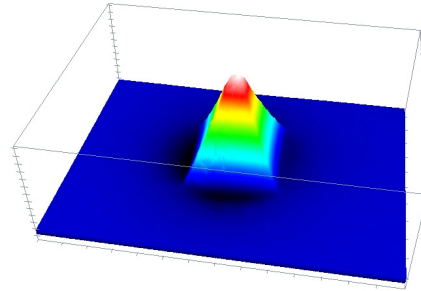
The Fast Fourier Transform (FFT) display is available for Profiles, Images and Topographies without lost pixels. If necessary, the user has to *Restore* the measurement to fill the missed pixels. To obtain the FFT, the software processes an amount of pixels that has to be a power of two. Therefore, the software will expand the measurement to the requested format just above the dimensions of the original one. Then it calculates the FFT and displays the result.  $f_x$  and  $f_y$  are the frequencies (in  $\text{mm}^{-1}$  units) of the measurement, while  $I$  is the weight of a given frequency. Because of periodic features of the samples use o have low frequencies, the maximum displayed frequency is 25% of the highest allowable. To increase the contrast of the FFT, the colour scale and the scale options can be modified.

- **What does “Invert” mean?**

The Invert option is used to invert the values of the measurement. To invert, just click on the label. When the measurement is inverted, the software shows the message “Inverted” in red. To remove the inversion just click again on the label.



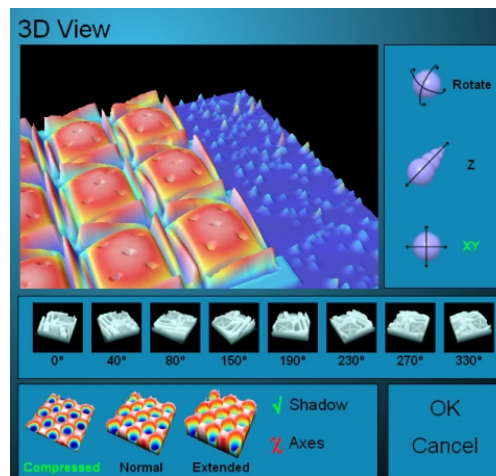
Non-Inverted Topography



Inverted Topography

- **3D View**

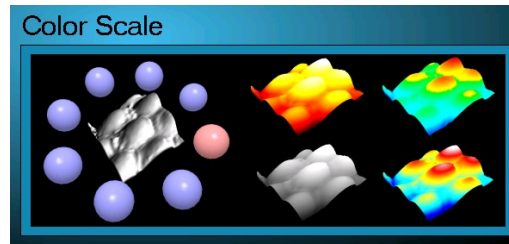
The view display lets the user to choose the isometric view layout. The surface can be rotated, translated or scaled just clicking and dragging on the screen. There are also predefined views and three different Z scaling: compressed, normal and extended. There is also the possibility to show a shadowing effect of the surface and the metrological axes.



- **Colour Scale**

The colour scale allows the user to choose between four different Z colours and eight slope colours. The Z colours assign the same colour to all the points with the same Z position. In contrast, the slope colour assigns a grey value to all the points with the same slope.

To choose a Z colour just click on the image. To choose a slope colour, first choose the slope direction by clicking on one of the eight blue balls, and then click on the Topography icon, which is in the middle of them.



- **Scale**

The Scale is used to define which part of the Z axis will be displayed, either in 2D or 3D measurements. The user can choose between Peak to Valley, rms and user-selected options.

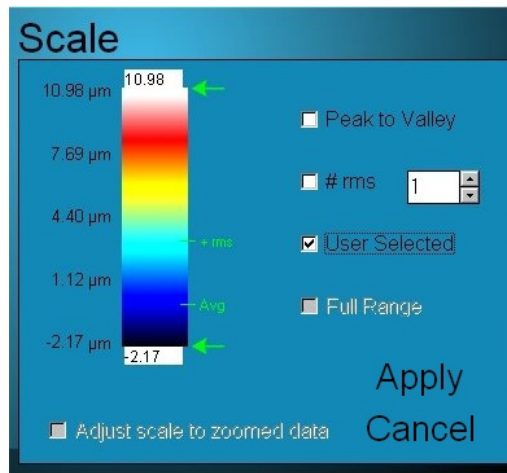
The Peak to Valley option displays the Z interval between Max and Min measured values and assigns the highest colour of the selected colour scale to the highest point of the measurement (Max), and the lowest colour to the lowest point (Min).

The rms option displays the Z interval between the average point and plus-minus the rms value selected. The highest and lowest colours of the colour scale are assigned to the extremes of this interval.

The user-selected option is a free option, which allows the user to select both the highest and the lowest point of the measurement and the maximum and minimum of the scale. To use the *User Selected* option just click and drag the green arrow to the desired position. While the arrow is being dragged, a green label appears with the current Z position. The initial User Selected option is limited to the Peak to Valley range, but the maximum and minimum values can be changed.

For the Image measurement, the Scale window shows a fourth option called "Full Range". This option shows the image from 0 to 255 grey levels.





The Scale is independent for each kind of display. The user can select different Scale options for every display of the same measurement. Changes introduced in the Scale for one kind of display will not affect the options previously selected for the other displays.

- **Zoom**

The Zoom option displays a smaller part of the measurement length or area. To use Zoom, click on the Zoom label and choose the magnification that you want to apply. The full measurement with a green box showing the zoomed area appears in the right side of the Display window. To change the zoomed area, just drag the green box on the display.

It is also possible to choose between rectangular and square zoom. To do this, just click on the image at the bottom of the Zoom window.

## 6.6. The Measurement Report Area

- **The Edit Comment button**

The Edit Comment button lets you write a comment of up to two lines in the comment area.

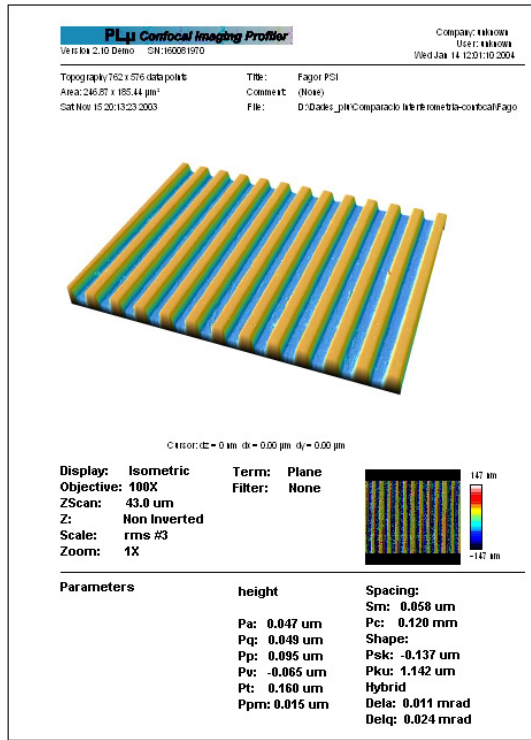
- **The New Acquisition button**

The New Acquisition button exits the Display and Analysis screen and enters the Acquisition screen. The user is prompted to save or delete the previous measurement.

## 6.7. The Print Report

To print a report of the measurement, press the print button on the tool bar, or the print command in the File menu.

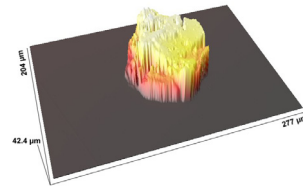
The software shows a print report as it will appear when printed.



The user, the measurement title and the measurement comment can be changed by clicking on it

## Chapter 7

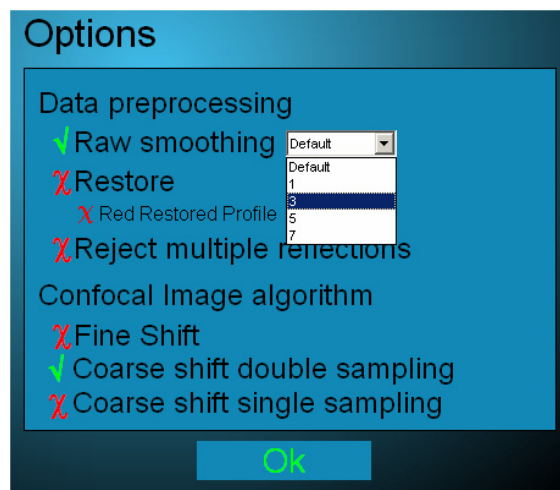
# The Configuration Screen



The *Configuration* screen is used to configure both the software and the hardware of the PLμ.

## 7.1 Options

The acquisition and display software of the PLμ 2300 can be configured using the *Options* commands.



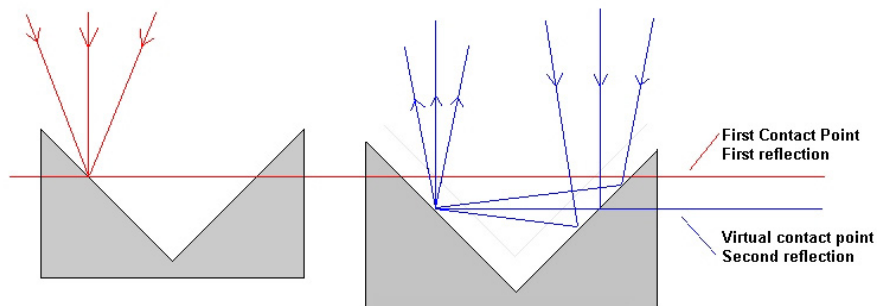
- Raw smoothing:

This option is used to smooth the confocal images while a topography is being acquired. *Default*, *1*, *3*, *5* and *7* are the allowed values, and stronger effect is achieved with higher values. The goal of the raw smoothing is to get lower measurement noise while keeping the maximum lateral resolution.



It is recommended to keep the *Raw Smoothing* option enabled as a default.

- **Restore:**  
If the restore option is enabled, then all the measurements are restored by default after the acquisition process. As a result, the *Restore* label in the Display and Analysis screen disappears, and the user is not able to remove the restored parts from the measurement.
- **Red restore profile:**  
If this option is enabled, then the restored pixels of a profile are displayed in red colour. If this option is disabled, then the restored pixels are displayed in black colour.
- **Reject Multiple Reflections:**  
This option can be used for those samples that can produce multiple reflections. This effect comes up in samples having, for instance, transparent coatings, multilayer structures or retro-reflecting geometries. For example, in a transparent layer or in a foil there may be more than one reflection, corresponding to the different interfaces. Another example is a smooth surface with a triangular 45 degree shape.



If this option is enabled, the software looks at each point of the surface for the location of the first peak in the axial response. Then, only the first surface will be measured, even in those samples where the reflection coming from deeper surfaces is stronger than that of the first one.



*Reject Multiple Reflections* has to be used only if there are multiple reflections on the surface. Otherwise, the result of the measurement may contain many lost pixels and non reliable values.

- **Confocal Image Algorithm.**

There are three possibilities: Fine Shift, Coarse Shift Double sampling and Coarse Shift Single Sampling.

**Fine Shift:**

Using the Fine Shift algorithm, the PL $\mu$  2300 projects parallel slits on the microdisplay spaced 12 pixels. The slits are also parallel to the X direction of the CCD camera. To get one confocal image 12 different images of the CCD camera for 12 different projected positions of the slits are needed. Fine Shift is a good algorithm for very rough surfaces.

**Coarse Shift Single Sampling.**

Using Coarse Shift is the same of Fine shift but projecting parallel slits with a spacemen of 4 pixels. The result is a faster acquisition rate, but high cross-talk. This is a good algorithm for smooth or moderately rough surfaces with low magnifications or all kind of surfaces with high numerical aperture and high magnification objectives.

**Coarse Shift Double Sampling.**

Using Coarse Shift Double Sampling is the same as Single Sampling but projecting parallel lines in both directions, X and Y. The parallel slits are spaced 4 pixels, and to get a confocal image 4 images parallel to the X direction and 4 to the Y direction are acquired. Coarse Shift Double Sampling is a good algorithm for almost all kind of surfaces, from very smooth to moderately rough for all magnifications.

## 7.2 Selecting the software language

The software of the PL $\mu$  is available in different languages. To choose the language, click on the languages label.

## 7.3 Setting the “Lost pixels” value

“Lost pixels” is a safety parameter, which is used during acquisition. This value is between 0% and 100%, and refers to the percentage of points that have been measured with sufficiently reliable confocal information.

If the measurement has a percentage of “non-measured” points below the stated “Lost pixels” figure, the software shows a message saying that there are too many lost pixels, and remains in the Acquisition screen without showing any result.

## 7.4 Configuring the available hardware (objectives and XY table)

This option is used to set up the PLμ 2300. If your hardware configuration has the XY table available, the related label has to be active. You can check the available objectives just clicking on the image appearing in the Objectives option.

If the XY table is unchecked, the Acquisition screen will not allow the user to acquire Extended Profiles or Coordinates.

## 7.5 Positioning and aligning the slit pattern



Hardware calibration of the PLμ has to be done by specialized people. Improper slit rotation and translation may lead to incorrect results.

To understand the meaning of this calibration, refer to the Installation Manual supplied with this document.

The slit pattern projected by the microdisplay inside the epi-illuminator of the microscope has to be aligned with the CCD the XY. The first step is the XY table rotation. This procedure is explained clearly in the Installation Manual.

The second step is to calibrate the CCD rotation. To do this, focus on the flat mirror supplied with the PLμ 2300. Ensure that the mirror is well focused. In the event of having some tilt or tip of the mirror, recalibrate the XY table position until the mirror appears focused on the whole image. Then click on the Quality label. A blue progress bar showing the amount of alignment between the slit pattern and the CCD will appear for 30s. To get a better alignment, unscrew the 0.5X CCTV adapter and rotate it until the progress bar shows a result better than 80%. Once you get this value, tighten the screw on the 0.5X.

The next step is to click on Slit Position. If the line position is correct, this option will draw the detected lines in red. If the slits are in the wrong position, the software will identify the problem. If the slits are too high, they have to be moved upwards, and if they are too low, they have to be moved downwards.

You can use the Slit Screw Calibration on both sides of the epi-illuminator to move the slits (see Installation Manual).

## 7.6 Calibrating the IRIS position.

This option is used to calibrate the center coordinates of the circular iris that is showed for interferential measurements. To calibrate that positions focus the slit pattern with a confocal objective. Then press the “Iris calibration” button. The software will calibrate that position.

## 7.7 Calibrating the Objectives

If the Slit Position has failed, the objective calibration is not available.



Objective calibration has to be done by specialized people. Improper calibration may cause the results to have a strong spherical aberration.

To calibrate a Confocal Objective, first focus on the flat mirror. Find a very clean area, without any scratches or dust particles. Then press the button of the objective to be calibrated. Calibration procedure will run automatically.

To calibrate an Interferential objective just click on the button. The software recognises that the user wants to enable that objective. The real calibration of an interferential objective is carried out during the fabrication process of the PLμ 2300 and the information regarding of all the calibration parameters are stored in the hardware configuration file.

## 7.8 Calibrating the Z Scale Factor



An incorrect value for the Z Scale Factor may result in larger or smaller data. If you doubt the accuracy of the value, reset to 1.000.

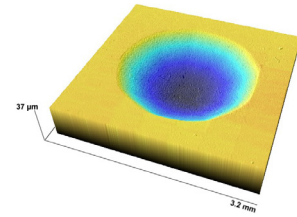
The Z Scale Factor is used to calibrate the non-linearity of the microscope's Z stage. This requires a calibration sample with known height parameters. First measure the sample with a Z scale factor 1.000 and look for the result. Then correct the result using the proper multiplication factor.



## Chapter 8

# Error Messages

---



- *The Motors control is not responding.*

This message means that something has occurred either in the motors controller or in the computer communication cable. First of all check the initialization procedure to be sure that the motors controller is switched on after booting into Windows. If not, exit the software, reboot the motors controller and reload the software. If the error message persists, check the serial communication cable between the computer and the controller and ensure that the cable is plugged into the computer in Serial Port number 2.

- *Cannot Grab MIL.*

This message appears because of a malfunction of the CCD-Frame Grabber system. Usually, the system fails because the CCD camera is not well connected to the Frame Grabber or is switched off. To solve the problem, exit the software and check if the CCD camera is switched on. If the problem persists, check the coaxial cable that connects the CCD camera to the computer.

- *Error in MdigGrab(): Error from Meteor II low level function.*

This message appears when the CCD camera causes an error during the normal operation of the PLμ system. This error can be due to a momentary disconnection of the CCD power supply or the signal cable that connects it with the computer. The Frame Grabber loses the synchronization and shows this error message. To avoid this problem, exit the software and reload it.

- *Acquisition error: too many bad data points.*

This error message appears after a measurement containing too many badly measured points. To avoid this, try measuring the sample with a higher Z Scan value, or with a lower Threshold value. Also, check that the sample is focused in the right position according to the Z Scan direction. If the message persists, enter the *Configuration and Alignment* menu and increase the *Lost Pixel* value. Increasing this value lets the measurement have more erroneous pixels.

- *Cannot fit data.*

This message appears when the user is trying to remove a *Term* from a measurement and it cannot be done. The causes of the error can be due to there being too few points to adjust, or to a meaningless procedure. To avoid this problem, try selecting a larger or different area for the *Term*.

- *Cannot read PLμ calibration.*

This message appears when one of the setup files of the PLμ has been corrupted from the original one, or has been moved to another folder. The message appears during the PLμ software initialization, and warns the user that the system has lost the calibration.

- *Cannot open motors control serial port.*

This message appears if other software is using the same port to control the motors controller, or if a copy of the PLμ software is running. To avoid the problem, close all the programs and load the PLμ software.

- *Incorrect number of slits.*

This message appears during the *Slit Position* procedure of the system calibration and tells the user that the number of the slits appearing on the screen is not correct. Refer to the *System Installation Manual* for guidance in calibrating the system.

- *Invalid slit calibration.*

This message appears if the numbers of slits is incorrect or if the calibration image for the *Slit Position* procedure is saturated.

- *Non-suitable Z-axis movement.*

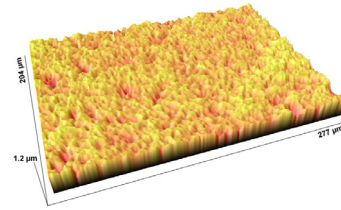
This message appears when the Z Scan range and direction are incompatible with the physical characteristics of the objective. Either the direction or the size of Z Scan range may cause the sample to push the objective, damaging or even crashing the optics. To avoid this message, check the values entered and try again.

- Z-axis motor not present, no measurement or calibration allowed.

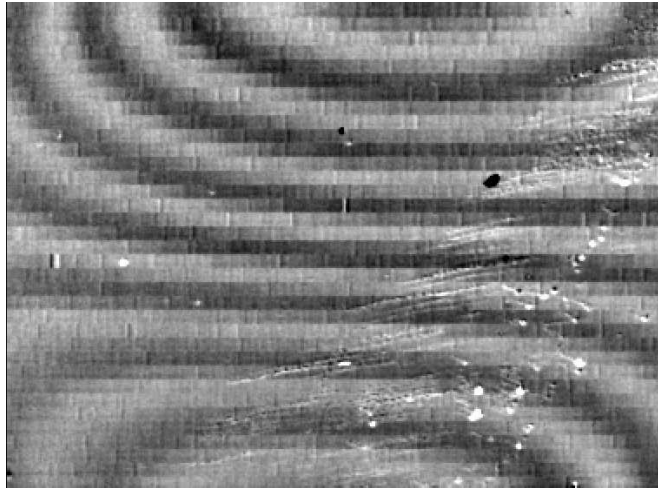
This message appears when the system has the Motors control present but it is switched off. Because all measurements need at least the Z-axis active, the software disables all the measurement and calibration options.

## Chapter 9

# Troubleshooting



- *Displacement of axis*

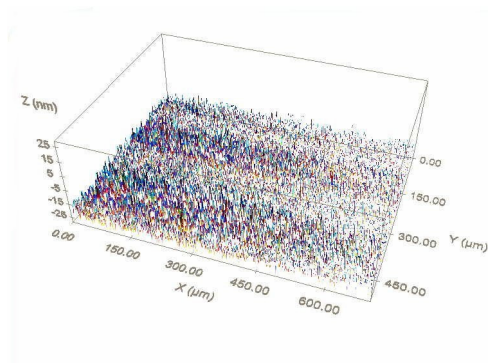


**Cause:** Mechanical displacement of the PLμ 2300. The CCD camera and the microdisplay have mechanically moved to a position where all the information about slit rotation and alignment is lost.

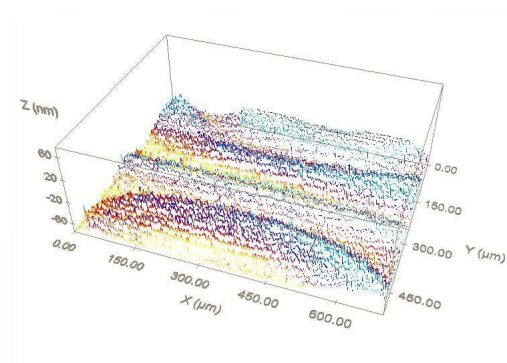
**Consequence:** Depending on the objective, a moiré pattern with amplitude between 2 and 50 nm appears in a Topography measurement.

**Solution:** Calibrate the PLμ 2300. Calibrate the slit position, rotation and all the objectives. Refer to the installation manual for details.

- *Undulations in Multiple-Profile.*



Fine Z Scan

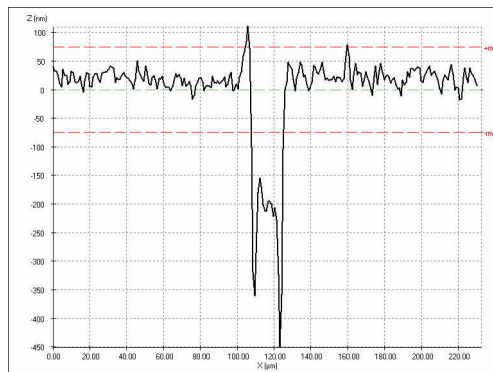


Coarse Z Scan

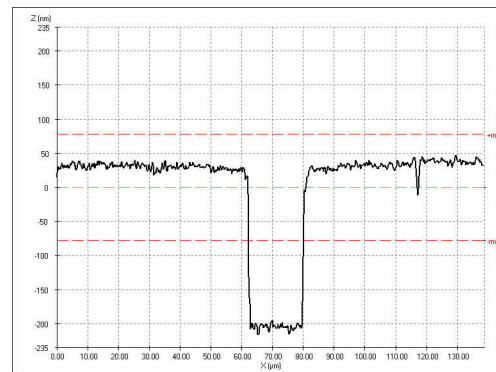
**Cause:** Use of *coarse* scan instead of *fine*. Although the *coarse* option in the Z Scan window makes the measurement faster, the fact that the sample is moving during the image acquisition makes undulations appear in the measurement.

**Solution:** Use a Z *fine* scan.

- *Peak structure*



Peak structure with 20X



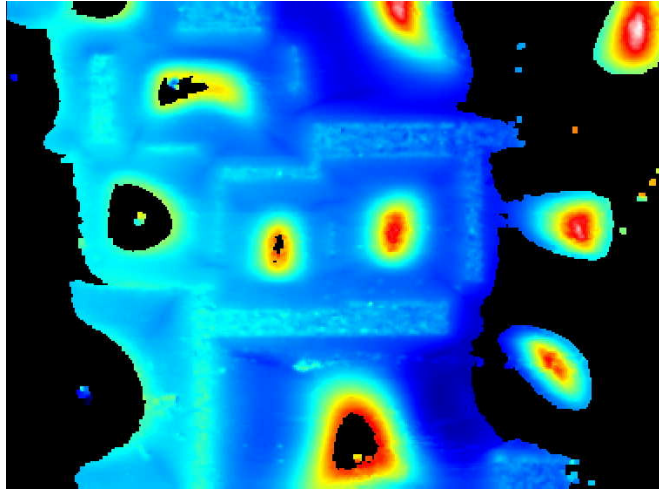
No peak structure with 100X

**Causes:** A peak structure appears in the corners of a step because of the light reflections from the walls. These peaks can be larger or shorter, depending on the objective, the reflectivity of the sample, and the different materials at the top and the bottom of the sample.

**Consequence:** Wrong measurement in the corners.

**Solution:** Try increasing the threshold value until the peaks disappear. If the peaks persist, use a higher magnification objective.

- *Narrow measuring range*

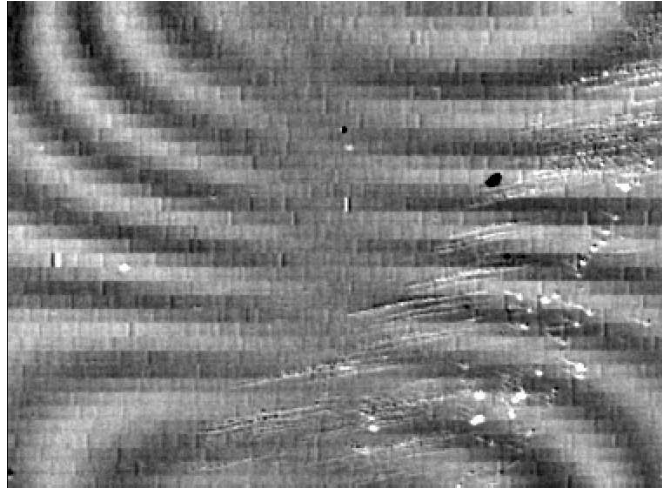


**Cause:** The Z Scanning value is very low and the scan has not reached the entire sample. The parts of the sample without confocal information cannot be measured, and are displayed as lost pixels.

**Consequence:** Many parts of the sample are not measured.

**Solution:** Increase the Z Scan range and measure again.

- *Rotation of axes*

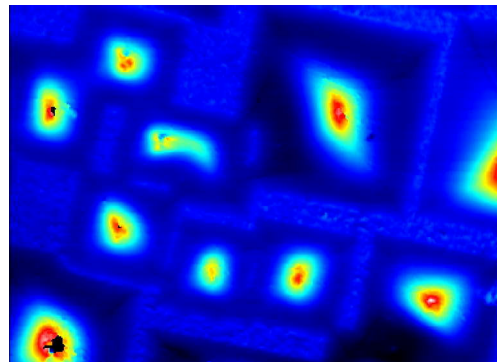
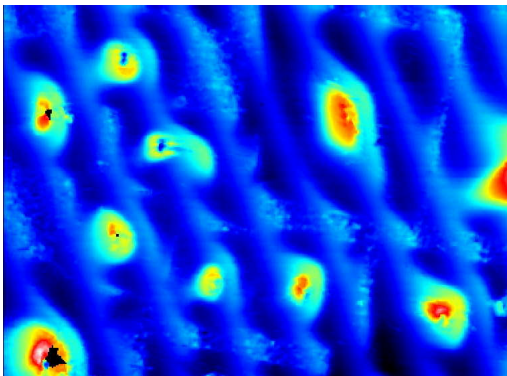


**Cause:** The CCD camera has been rotated with respect to the slit pattern.

**Consequence:** Depending on the objective, a rotated moiré pattern appears in a Topography measurement. This pattern has the center of rotation at the center of the image and the height amplitude varies from 2 to 50 nm.

**Solution:** Calibrate the PLμ. Calibrate the slit position, rotation and all the objectives. Refer to the installation manual for details.

- *Step height too high*

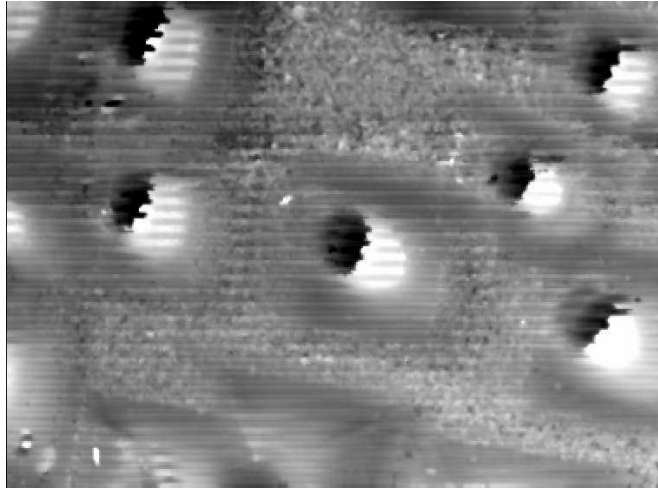


**Cause:** Step height too high. There are optimized values of the Z Step height for each objective. Using the *Coarse* option for the scanning, the software changes the optimized value in order to keep the number of measuring planes under 100. With the 20X, for example, the optimized value is 1 micron. This value is used while the scanning range is under 100 microns. If the user selects 400 microns with the *coarse* option, the step height is set at 4 microns.

**Consequence:** When the step height is too high, there are too few planes contained within the confocal depth of focus, even in rough surfaces, making the measurement to appear with periodic structures.

**Solution:** Try decreasing the Z Scan range close to the optimum value. If a large Scan is needed and the problem persists, try using the *fine* option. This option will use a value closer to the optimum, but it will need more planes and it will take more time to take the measurement.

- *Vibration during the acquisition*



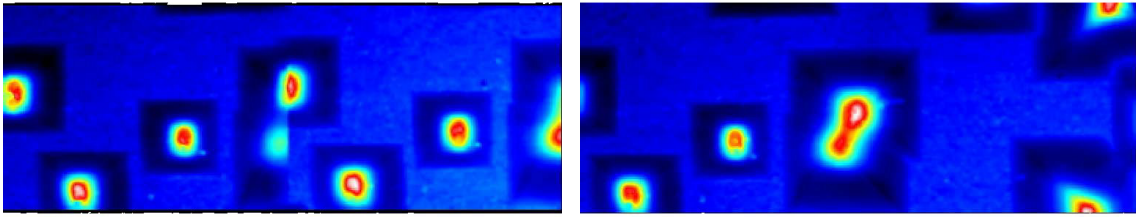
**Cause:** Vibration during the acquisition.

**Consequence:** Periodic structure parallel to the slits with random height values.

**Solution:** Repeat the measurement under lower vibration conditions. If the problem persists, check the vibration isolation of the table. Also, check if there is a vibration source that might affect the PLμ permanently, and at worst consider moving the profiler to a better measuring environment.

- *Wrong stitching*





**Cause:** The main cause of a wrong Profile or Topography stitching is slippage of the sample. If the sample is flat and polished on the lower side, the high acceleration of the XY table can cause the sample to slip.

**Consequence:** Wrong stitched result.

**Solution:** Fix the sample to the XY table.

- *Frequently lost Profiles/Topographies during Extended Profile/Topography acquisition*

**Cause:** The scan doesn't pass very often through the entire sample for different profiles/topographies.

**Consequence:** The *Lost Profile or Topography* dialog window frequently appears to help the user to solve the problem.

**Solution:** Stop the acquisition and start it again with a broader scanning range and/or lower threshold value. If the problem persists, try using non-interpolated scanning instead of interpolated. This is a usual problem for samples with steps.

- *Wrong vertical stitching*

**Cause:** Sometimes the lateral stitching is correct, but the vertical is not.

**Consequence:** Incorrect step in the stitching area.

**Solution:** This is a common problem when the sample is measured with a step height value that is very far from the optimized one. To solve it, try decreasing the Scan range as far as possible in order to use a step height value as close to the optimized as possible. If this is not possible, try using the *fine* option in the Z scan instead of the *coarse*.

