

# **RT Microscope Accessory**

for PerkinElmer Lambda 650/750/850/950/1050

## User manual



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APPENDIX 2: CALIBRATION OF THE TRANSMITTANCE REFERENCE SAMPLESERROR! BOOKMARK NOT DEFINED

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### **RT Microscope Accessory Set**

#### Inventory

ltem	Description	Quantity
1	Reflectance/transmittance microscope accessory	1
2	Motorized XY stage with controller and joystick	1
3	Cables	6
4	Test report	1
5	USB stick	1
6	Manual	1
7	covers	6

Contact OMT Solutions BV if not all items are delivered or in case an item is damaged due to transport.

#### Hardware requirements

The use of the microscope accessory requires the PerkinElmer Lambda 650/750/850/950/1050 to be equipped with the standard detector unit.

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### Microscope basics

#### Description

The optical system is depicted in figure 1. The mirrors M5, M6 and M7 form the imaging part of the microscope. The remaining mirrors are flat and only used to fold the beam. M5 forms a reduced image of the aperture that is placed before mirror M1. This image is further reduced by the Schwarzschild combination M6 and M7. The result is an image of the aperture on the sample that is reduced by a factor 80.

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The optical paths of the various beams in figure 1 are:

Incident beam : sample chamber entrance port - Aperture - M1 - M2 - M3 - M4 - M5 - M6 - M7 - sample

Reflected beam : sample -M7 - M6 - M5 - M4' - M3' - M2' - M1' - sample chamber exit port Transmitted beam: sample <math>-M7'' - M6'' - M5'' - M4'' - M3'' - M2'' - M1' - sample chamber exit port





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The measurement mode (reflectance or transmittance) can be selected by the knob on the left side of the upper part of the microscope:



The knob on the right side controls the camera view and measurement mode M:



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During measurement its position is in the middle (M). In the upper position the camera image is taken through the reflection optics M7 - M6 (see figure 2a) and the height of the imaged sample view is approximately 0.2 mm. This position is used to obtain a focus on the sample. In the lower position the camera image is taken behind M1', directly in front of the sample chamber exit port (see figure 2b). This camera view is used to obtain the focus of the transmittance optics..





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#### **Spot size limitations**

From the user's point of view, the angle of incidence and the solid angle of the beam on the sample need to be as small as possible. On the other hand we have to take into account the optical geometry of the Spectrophotometer. A design for a reflection / transmission microscope has to deal with conflicting demands and the physical limitations of the optical geometry and detector sensitivity of the spectrophotometer's beam. In the following sections we will discuss some of the effects that limit the spot size.

#### Helmholt – Lagrange Invariant

In optical systems energy conservation is defined by the so-called Helmholt – Lagrange Invariant, which roughly states that image area multiplied by solid angle is constant throughout a system, considering only rays that can pass the system. Input for a microscope that fits into the sample compartment is the slit image of the Lambda 650/750/850/950/1050, having the approximate size of  $6 \times 12 \text{ mm}$  (width x height) and a beam solid angle of about 0.0062 steradians In case of our microscope accessory, the magnification is 80 times and the total solid angle of the beam at the sample is roughly 0.356 steradians. Since the beam area at the entrance of the microscope is 80x80=6400 times larger, the solid angle of the beam accepted at the entrance of the microscope is 6400x smaller, namely 0.0000556 steradians. The maximum energy throughput due to the Helmholt – Lagrange Invariant is in this case 0.0000556/0.0062 = 0.9%, with a spot size of 75 x 150 micrometer. A spot size of  $12 \times 24$  micrometer can be obtained by placing a 0.48 x 0.96 mm aperture at the entrance of the microscope (Lambda 650/750/850/950/1050 slit image) which reduces the energy by an additionally factor 39. The total throughput (not counting the reflection losses of the optics) is now only 0.9%/39 = 0.024%.

#### **Reflection losses**

Aluminum coated mirrors have reflection values in the range 85% - 97%, depending on the wavelength. The minimum reflection of 85% occurs around 830 nm and at 860 nm, the wavelength where the spectrophotometer has its lowest sensitivity, is the reflectance is  $\approx$  86%. The Lambda 650/750/850/950/1050 beam reflects 14 times on the mirrors in the microscope. As a result, the transmission of our microscope using Aluminum mirrors is  $\approx$  12% at 860 nm so we lose 88% of the beam energy due to reflection losses!

**Note**: An alternative is to equip the microscope with silver coated mirrors. This results in a much higher throughput (86% after passing 14 mirrors at 860 nm) but limits the lower end of the wavelength range to approximately 400 nm so that measurements in the UV become impossible.

#### **Beam geometry**

Figure 3 shows the limiting rays in the incident, reflected and transmitted beams at the sample position.

The beam interacting with the sample is well defined and has an average angle of incidence of 25.00° and the average degree of polarisation of zero. The minimum and maximum angle of incidence on the sample are respectively 17.3° and 32.7°.

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Figure 3 Schematic drawing of the optical rays in the reflectance/transmittance microscope. (red = incident and transmitted beam, blue = reflected beam)

#### Implementation of a sample cryostat

It is possible to implement a sample cryostat. The available space between the upper and lower Schwarzschild optics is  $\approx$  45 mm. For a cryostat with a height of 40 mm and the sample position half way at 20 mm, the cryostat windows should have a clear aperture of 28 mm to pass the beam.

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### Installation

#### Handling the microscope

The microscope accessory is quite heavy. This weight is necessary for stability. There are different ways to lift the microscope accessory. You can choose which way suits you the best. In order to carry the microscope, the microscope is equipped with a handle underneath the rectangular lid on the top:



The microscope can also be handled at its base plate:



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To lift the microscope into the Lambda 650/750/850/950/1050 the recommended position to handle it is shown below with the green arrow (at both sides one hand). There are no optical components at this position at the inside so the microscope can be lifted here with one hand at the left side and one at the right side.



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#### Setting up the microscope

- 1. Remove the top and front cover of the sample compartment of the Lambda 750/950/1050
- 2. Remove the (standard) compartment base plate of the Lambda 750/950/1050
- 3. Remove the black steel sample compartment frame of the Lambda 750/950/1050
- 4. Lift the microscope body (without the microscope sample compartment covers) into the spectrophotometer's sample compartment. Make sure that the base plate of the accessory stays horizontal when it is lowered into position. Ask the help of a colleague if necessary. The installation engineer has marked the position where the microscope is placed with black lines, see green arrows in the picture below.
- 5. Make sure that the microscope is placed correctly onto the 4 guidance pins, see red arrows. If placed correctly the microscope will have (almost) no play and is leveled with the spectrophotometer's baseplate



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6. Install the 2 side covers with the 4 M4 screws (blue arrows) at the sides and the 3 M3 screws (yellow arrows) at the front to connect the two side covers to each other.



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 At the back of the microscope, connect the USB cable of the camera to an USB port of the PC with the EO uEye software (see software section) and the power supply cable to one of the Accessory connectors of the Lambda 650/750/850/950/1050



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8. Connect the XY-stage as follows:



The XY-stage can either be controlled with the joy-stick or with the delivered software (USB connection to the PC).

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9. If the XY-stage is powered the stage can be moved in order to lock the 2 M4 screws to attach it to the microscope.

#### Software

#### **Camera software**

On the USB stick open the Software/EO software directory. Depending on your PC choose the 32 or 64 bit to unzip:

uEye\_BatchInstall\_4.50\_32\_WHQL uEye\_BatchInstall\_4.50\_64\_WHQL

After unzipping the directory double-click uEyeBatchInstall file and follow the instructions:



#### XY-stage software

On the USB stick open the Software/Marzhauser software directory:



Open the Switchboard directory:

Documentation BSwitchBoard\_169\_Setup

Double-click the SwitchBoard\_169\_Setup file and follow the instructions. In the Documentation directory you can find the User Manual for further instructions.

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#### **UVWinlab Settings**

For Reflectance measurements (%R) to following standard settings can be used. The integration times may be changed to optimize scan duration and desired noise level.



For Transmittance measurements the Ordinate mode (at Data collection) must be set to '%T ':

Ordinate I	Mode	
%T	~	

The other settings can remain the same as with the ' %R ' measurement.

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#### At Corrections the followings settings are recommended:

Open Out	Copy Paste Report 665.38 nm - C	Send To DPV Start 0.0019 A S 2	Stop Set λ Autoze	ro Algn	
Corrections Folder List Folder List Folder List Folder Collection Forgram Forgram Forgram Forgram Forgram Results Coutput Forgram Coutput	Baseline Corrections         Baseline Determination         Always at task start         V 100%T / 0A Baseline (Autozen         V 0%T / Blocked Beam Baseline         V Use internal attenuator         Do not invalidate baselines with	o)	ngs		Expire Corrections O Never In 8  Hours
	Reflection Corrections Correction Type None	×			
	Light Spectral Reference	Spectralon		2	
	Attenuator Corrections Correction Determination Measure Do not invalidate attenuator of	orrections			

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#### Setting the focus

The procedure for focusing the microscope for **reflectance** measurements is as follows:

Step 1: Set the monochromator of the Lambda 650/750/850/950/1050 at 0 nm to produce a white light beam (select "<u>Application</u>" in the menu bar of UV WinLab and click on "<u>G</u>oto WI..." to get the Goto Wavelength dialog, or use the shortcut function F5)

Step 2: Place the reflectance sample at the measurement position



Step 3: Turn the camera image knob to the following position:



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Step 4: At the front panel push the green button to turn on the led light to illuminate the sample area:



Note: The red indicator shown above will light up if the sample stage is at the lowest or highest position. In this case do not move further!

The intensity of the white led illumination can be varied with the knob on the back side of the microscope:



Step 5: In order to get a focused image of the reflected sample beam turn the right knob on the front panel (changes the sample height):



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The resulting image looks something like this in case of a glass sample (4%R):



Or like this in case of a mirror (90%R):



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The procedure for focusing the microscope for transmittance measurements is as follows:

Step 1: Set the monochromator of the Lambda 650/750/850/950/1050 at 0 nm to produce a white light beam (select "<u>Application</u>" in the menu bar of UV WinLab and click on "<u>G</u>oto WI..." to get the Goto Wavelength dialog, or use the shortcut function F5)

Step 2: Place the (transparent!) sample at the measurement position



Step 3: Follow the Steps 3 to 5 of the Reflectance procedure (described above) to focus the beam at the top side of the sample.



Step 4: Turn the camera image knob to the following position:

Step 5: In order to focus the transmittance optics turn the left knob on the front panel (this changes the height of the transmittance optics):



When focus is obtained the resulting image looks something like this (in case of a glass sample):



#### Adjusting the spot size

To adjust the spot size turn the following knob:



The reduction of the spot size can be seen with one of the two camera positions (for reflectance or transmittance)

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#### **Measurement Procedures**

#### **Reflectance measurement**

The following procedure is recommended for reflection measurements:

Step 1: Set the microscope in reflection mode and place the reference mirror on the sample stage.

Step 2: Select the UVWinLab settings for the microscope and set the monochromator to 0 nm to produce a white light beam.

Step 3: Focus the sample (procedure on page 18)

Step 4: perform an autozero to normalize the ordinate scale

Step 5: perform the first measurement scan on the reference mirror (MREF.1)

Step 6: perform a 0% measurement (reference mirror removed, no sample) (M<sub>0%,1</sub>)

Step 6: put a sample on the sample stage and focus the reflectance spot

Step 7: perform the measurement scan on the sample (M<sub>S</sub>)

Step 8: repeat steps 6 and 7 for additional samples (M<sub>S</sub>)

Step 9: after 2 or 3 samples, get a second scan on the reference mirror (MREF.2)

Step 10: as a last scan perform the second 0% measurement (M<sub>0%,2</sub>)

The reflectance values of the samples (R<sub>S</sub>) are determined by

$$R_{S} = R_{REF} \frac{2 M_{S} - M_{0\%,1} - M_{0\%,2}}{M_{REF,1} + M_{REF,2} - M_{0\%,1} - M_{0\%,2}}$$

in which  $R_{REF}$  is the calibrated reflectance of the reference mirror at 25° angle of incidence (random polarization).

Note : make sure that the sample illumination is switched off during measurement.

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#### **Transmittance measurement**

The following procedure is recommended for transmittance measurements:

Step 1: Set the microscope in transmission mode and place a blank reference substrate on the sample stage. This blank substrate which is used as calibration reference must have the same thickness as the samples!.

Step 2: Select the UVWinLab settings for the microscope and set the monochromator to 0 nm to produce a white light beam.

Step 3: Focus the sample (procedure on page 18)

Step 4: perform an autozero on the transmittance reference sample to normalize the ordinate scale.

Step 5: perform the first measurement on the transmittance reference substrate to obtain a first reference scan ( $M_{REF,1}$ )

Step 6: perform a 0% measurement with the sample beam blocked  $(M_{0\%,1})$ 

Step 7: put a sample on the sample stage and focus the reflectance spot and the transmittance optics

Step 8: perform the measurement scan on the sample (M<sub>s</sub>)

Step 9: repeat steps 7 and 8 for additional samples(Ms)

Step 10: after 2 or 3 samples, get a second scan on the transmittance reference substrate (MREF.2)

Step 11: as a last scan perform the second 0% measurement with the sample beam blocked  $(M_{0\%,2})$ 

The transmittance values of the samples  $(T_s)$  are determined by

$$T_{S} = T_{REF} \frac{2 M_{S} - M_{0\%,1} - M_{0\%,2}}{M_{REF,1} + M_{REF,2} - M_{0\%,1} - M_{0\%,2}}$$

in which  $T_{\text{REF}}$  is the transmittance of the reference substrate at 25° angle of incidence (random polarization).

Note : make sure that the sample illumination is switched off during measurement.