3. Type in the name of the filter in the [Filter] field.

Example of filter configurations for U-FFWR and U-FFWO in the [Device Settings] dialog box

4. Add additional filters if required by selecting the appropriate position and selecting the name of the filter.

5. Click the [OK] button to save the changes.

6. Subsequently you have to configure an observation method in the [Device Customization] dialog box to be able to use the new filter.

21.5 Manual device configuration

This chapter describes the manual configuration of VS200 hardware devices.

21.5.1 ORCA monochrome camera

If your VS200 kit contains an ORCA-Flash 4.0, ORCA-Fusion or ORCA-Fusion BT camera you need to manually add it to the device list as those two cameras are not part of the default setup.

1. Open the [Device List] dialog box and go to the [Camera] tab.
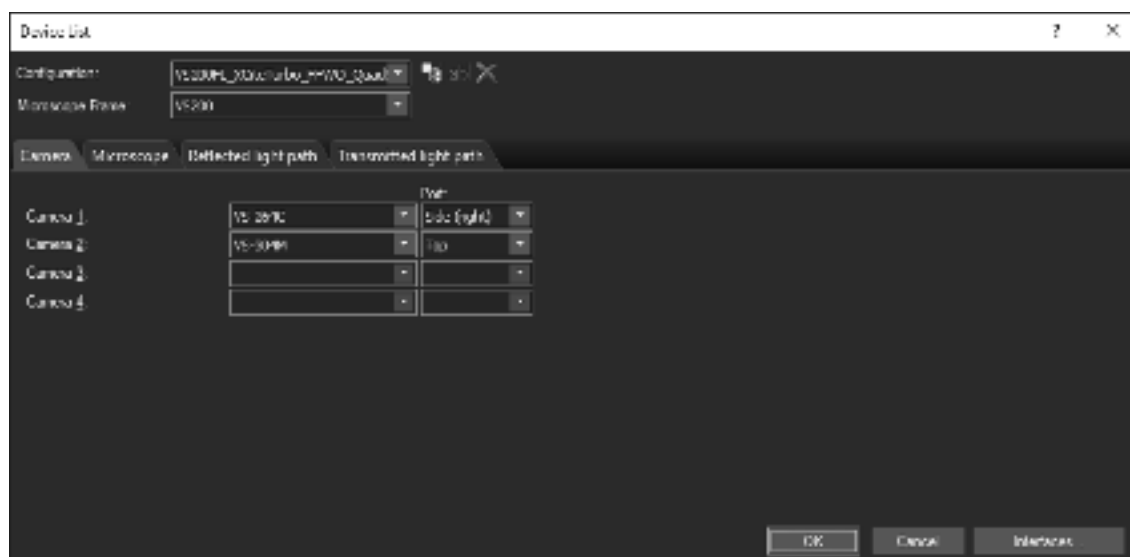
2. In the [Camera 2] list select either the ORCA-Flash 4.0, ORCA-Fusion or ORCA-Fusion BT.

3. Make sure that the correct camera adapter magnification (1.0x) is selected in the [Device Settings] dialog box. To open the [Device Settings] dialog box use the [Acquire] > [Devices] > [Device Settings] command.
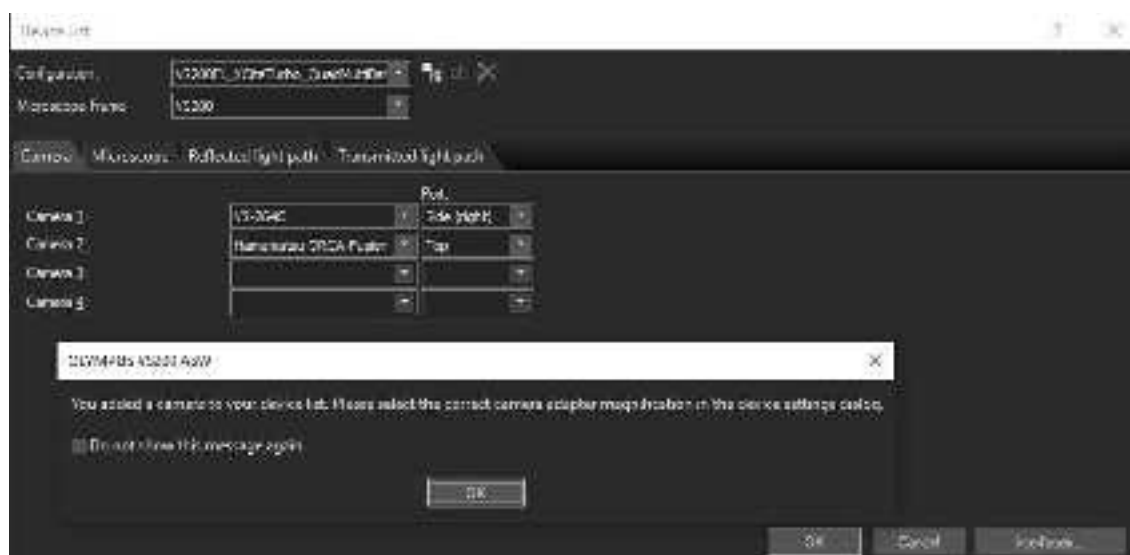
As none of the ORCA cameras are part of the default device configurations each observation method uses an ORCA camera needs to be adjusted manually. See <u>Device customization on page 121</u> for further information.

21.6   Device customization

In the [Device Customization] dialog box you can make changes to existing obser-
vations methods. For example, you can add a different monochrome camera or you
can add new observation methods.

21.6.1   ORCA camera adjustments

1. Select the [Acquire] > [Devices] > [Device Customization] command to
   open the [Device Customization] dialog box.

2. Select the [BFMono] observation method (OM).

3. Unlock the [BFMono] observation method by clicking on the lock icon.

4. In the [Status] picklist, set the ORCA camera to the status [Use].

5. In the [Image type] picklist, change the [Current] entry to the [16-bit grayscale] entry.

6. Do this for all other mono camera-related observation methods.

7. Set the correct VS200 LED lamp voltages for the individual objectives. Refer to chapter VS200 LED lamp voltages on page 131 for detailed information.

21.6.2 Hamamatsu ORCA-Flash 4.0 special settings

> Since the ORCA-Flash 4.0 camera does not support exposure times below 3.2 ms, additional settings must be made in order to guarantee proper functioning of the VS200 system.

1. Insert the IX3 filter cube plus ND6 (neutral density filter) into the IX3-RFACA.

   » Insert the filter cube into position 7 of the IX3-RFACA. How you can insert a filter is described in chapter U-FDICT filter cube on page 79.

2. After installation of the new filter cube make sure that you register it afterwards in the [Device Settings] dialog box. See Device settings - filter on page 118.

3. Configure the [BFMono] observation method to use the IX3 (ND6) filtercube. Refer to chapter ORCA camera adjustments on page 121 and select the IX3 Mirror Turret from the available components.

4. Set the VS200 LED lamp voltages for the [BFMono] observation method as listed in chapter ORCA-Flash 4.0 (monochrome camera) voltages (%) on page 134.

5. Perform the shading correction for the [BFMono] observation method.

21.6.3   Setup phase contrast (PH) observation method

If you want to add a phase contrast observation method do the following:

1. Select the [Acquire] > [Devices] > [Device Customization] command to open the [Device Customization] dialog box.

2. Select the [BFMono] observation method.

3. Use the [Copy Observation Method] ⬛ button to make a copy of the observation method.

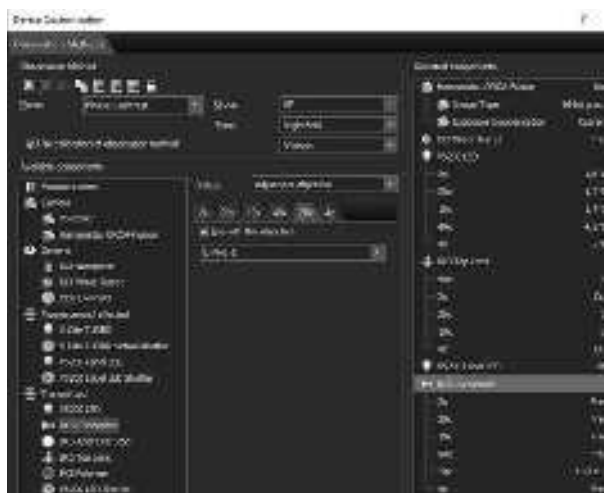4. Type in a name, e.g. "Phase Contrast".

5. In the [Type] picklist select [Phase Contrast].

6. Select the [BX3 Condenser] entry from the available components and choose the [Adjust per objective] entry in the [Status] pick list.

7. Select the e.g. 20x phase contrast objective and select the [Use with this objective] check box.

Refer to the following table for the correct assignment of the different phase contrast inserts to the correct magnification:

| Objective magnification	BX3 condenser PH insert
10x	U-PH1-S
20x	U-PH1-S
40x	U-PH2-S
60x	U-PH3-S
100x	U-PH3-S

8. Select the [BX3 Top Lens] entry from the [Available components] and select the [Top Lens] > [In] option for the 20x PH objective.

Refer to chapter for the correct lamp voltages for phase contrast acquisition.

21.6.4   Setup polarization (Pol) observation method

If you want to add a polarization observation method do the following:

1. Select the [Acquire] > [Devices] > [Device Customization] command to open the [Device Customization] dialog box.

2. In the [Device Customization] dialog box select the [BF] observation method.

3. Use the [Copy Observation Method] button ![icon] to make a copy of the observation method.

4. Type in a name, e.g. Polarization.

5. In the [Type] picklist select [Polarized].

6. Select the [IX3 Mirror Turret] entry from the available components and choose the [Adjust] entry in the [Status] pick list (this step is not needed in case the manual analyzer is used).

7. In the [All objectives] tab select the [U-FDICT] analyzer for all objectives (this step is not needed in case the manual analyzer is used).

8. Select the [BX3 Polarizer] and set the [Status] field to [Use].

9. Select the [Polarization Angle Changer] entry from the available compon-
ents and choose the [Adjust] entry in the [Status] pick list.
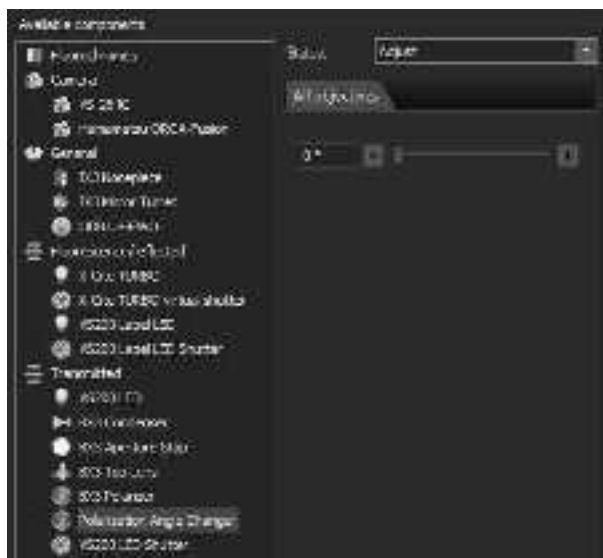
10. Use the slide control to rotate the motorized polarizer or enter a defined
angle value in the field.

21.6.5   Create or adjust an observation method

The easiest way to create a new observation method (OM) e.g. for fluorescence
image acquisition is to copy an existing one and adjust the settings according to
your needs in the [Device Customization] dialog box.

Open the [Device Customization] dialog box

1. Use the [Additional layouts] ⇄ button to go to a different layout. You can
find the [Additional layouts] button at the top right in the navigation bar
on the VS200 ASW software's start page.

2. At the top right, on the menu bar click the [Manual Control] button.

3. Select the [Acquire] > [Devices] > [Device Customization] command to
open the [Device Customization] dialog box.

Configuring an observation method

1. Select an observation method (e.g. CY3) in the [Name] list. This list con-
tains all of the observation methods that have already been defined.

2. Click the [Copy Observation Method] button to create a copy of the observation method.

3. Enter a name for the observation method in the [Enter a New Observation Method Name] dialog box.

4. In the [Available Components] list select the [Fluorochromes] entry.

5. Select the [Use] entry from the [Status] list.

6. In the [Fluorochrome] list select the fluorochrome that fits to your new filter combination.
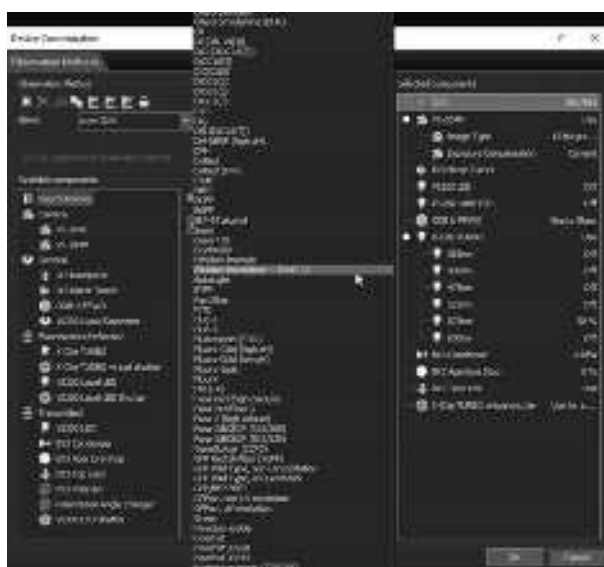
7. In the [Available Components] list select the [ODB U-FFWO] entry.

8. Select the new filter you have added.

9. Do the same if you e.g. added a new excitation filter for the ODB U-FFWR or a new IX3 filter cube in the IX3 Mirror Turret.

10. Click [OK] to save the changes.

11. As you created a new observation method you now have to carry out the shading correction. See Shading correction for fluorescence observation methods on page 209.

21.7   Adjust the lamp intensities
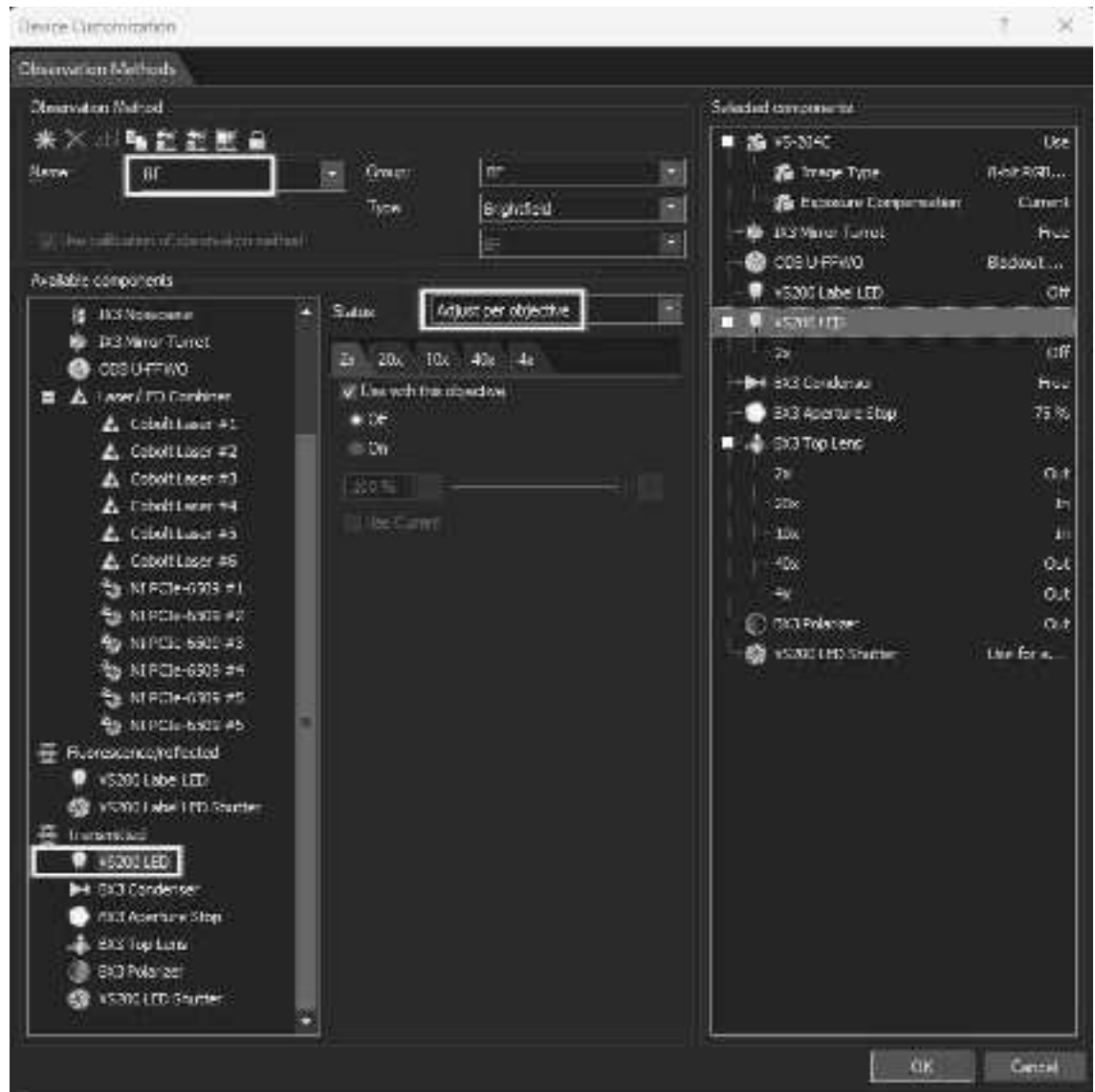
21.7.1   General information

These steps describe the procedure for adjusting the intensities of the VS200 LED lamps for the [BF] and [BFMono] observation modes in the VS200 ASW software. This adjustment is necessary with every fresh software installation or when the beam-splitter has been replaced.

Follow these step-by-step instructions to avoid any unexpected difficulties.

> The units described below must be assembled and adjusted by Evident. If these units are assembled or adjusted by the customer, the operations are not ensured.

21.7.2   Adjust the BF lamp intensities to reach 500 μs

1. Load the calibration slide on the stage.
2. In the VS200 ASW software, go to the [Manual control] layout.
3. Use the [BF] observation method and start the live mode.
4. In the [Camera Control] tool window, select the [Exposure] > [Automatic] option.
5. Focus on the calibration slide pattern.
6. Open the [Device Customization] dialog box. To do so, click the [Device Customization] button in the [Microscope Control] tool window's toolbar.
7. In the [Name] list, select the [BF] observation method.
8. Click the lock button to unlock the observation method.
9. In the [Available components] list, select the [VS200 LED] entry. Select the [Adjust per objective] entry in the [Status] list.

10. Choose the first objective, for example 2x, and make sure it is actually the objective placed in the light path.

11. Adjust the lamp voltage of the VS200 LED for the 2x objective to reach an exposure time of 500 µs.

    You can check the exposure time in the [Camera Control] tool window **(1)**.

12. Repeat the last 2 steps for all other objectives.

13. Close the [Device Customization] dialog box and confirm the message with [OK].

14. Stop the live mode.

21.7.3 Adjust the BFMono lamp intensities to reach 500 µs +/- 50 µs (VS304M, ORCA Fusion, ORCA Fusion BT, not for ORCA Flash 4.0 v3)

1. Load the calibration slide on the stage.

2. In the VS200 ASW software, go to the [Manual control] layout.

3. Use the [BFMono] observation method and start the live mode.

4. In the [Camera Control] tool window, select the [Exposure] > [Automatic] option.

5. Focus on the calibration slide pattern.

6. Open the [Device Customization] dialog box. To do so, click the [Device Customization] button in the [Microscope Control] tool window's toolbar.

7. In the [Name] list, select the [BFMono] observation method.

8. Click the lock button to unlock the observation method.

9. In the [Available components] list, select the [VS200 LED] entry.

10. Select the [Adjust per objective] entry in the [Status] list.

11. Choose the first objective, for example 2x, and make sure it is actually the objective placed in the light path.

12. Adjust the lamp voltage of the VS200 LED for the 2x objective to reach an exposure time of 500 µs (+/- 50 µs).

13. Repeat the last 2 steps for all other objectives.

14. Close the [Device Customization] dialog box and confirm the message with [OK].

15. Stop the live mode.

21.7.4 Adjust BFMono lamp intensities to reach 15 ms (+/- 1 ms) (for ORCA Flash 4.0 v3 only)

1. Follow the instructions to use a ND6 filter for the [BFMono] method. See Device customization on page 121.

2. Load the calibration slide on the stage.

3. In the VS200 ASW software, go to the [Manual control] layout.

4. Use the [BFMono] observation method and start the live mode.

5. In the [Camera Control] tool window, select the [Exposure] > [Automatic] option.

6. Focus on the calibration slide pattern.

7. Open the [Device Customization] dialog box. To do so, click the [Device Customization] button in the [Microscope Control] tool window's toolbar.

8. In the [Name] list, select the [BFMono] observation method.

9. Click the lock button to unlock the observation method.

10. In the [Available components] list, select the [VS200 LED] entry. Select the [Adjust per objective] entry in the [Status] list.

11. Choose the first objective, for example 2x, and make sure it is actually the objective placed in the light path.

12. Adjust the lamp voltage of the VS200 LED for the 2x objective to reach an exposure time of 15 ms (+/- 1 ms).

13. Repeat the last 2 steps for all other objectives.

14. Close the [Device Customization] dialog box and confirm the message with [OK].

21.8 VS200 LED lamp voltages

21.8.1 VS-264C (color camera) voltages (%)

Objective	Observation method			
	BF (AS 75%) initial value*	DF (AS max)	POL (AS 75%)	PH
2x PLN	60	not supported	100	not supported
4x UPLFLN	100	not supported	100	not supported
10x UPlanXApo	65	100	100	not supported
20x UPLXAPO	64	not supported	100	not supported

40x UPLXAPO	100	not supported	100	not supported
40x UPlanXApoS	100	not supported	100	not supported
60x UPLXAPO	100	not supported	100	not supported
100x UPLXAPO	100	not supported	100	not supported
10x UPLFLNPH	65	100	100	100
20x UPLFLNPH	64	100	100	100
40x UPLFLNPH	100	100	100	100
60x UPLFLNPHO	100	100	100	100
100x UPLFLNPHO	100	not supported	100	100

* These values must be adjusted for every fresh software installation or after beam splitter substitution. To adjust the lamp voltage, see Adjust the BF lamp intensities to reach 500 µs on page 128.

21.8.2 VS-304M (monochrome camera) voltages (%)

Objective	Observation method		
	BFMono (AS 75%) initial value*	DFMono (AS max)	PHMono (AS 75%)
2x PLN	1.6	not supported	not supported
4x UPLFLN	2.0	40	not supported
10x UPlanXApo	1.7	40	not supported
20x UPlanXApo	1.7	not supported	not supported
40x UPlanXApo	4.2	not supported	not supported
40xUplanXApoS	4	not supported	not supported
60x UPlanXApoO	5	not supported	not supported
100x UPlanXApoO	7	not supported	not supported
10x UPLFLNPH	1.7	70	100
20x UPLFLNPH	1.7	100	100
40x UPLFLNPH	4.2	100	100
60x UPLFLNPHO	5	100	100
100x UPLFLNPHO	7	not supported	100

* These values must be adjusted for every fresh software installation or after beam splitter substitution. To adjust the lamp voltage, see Adjust the BF lamp intensities to reach 500 µs on page 128.

21.8.3   ORCA-Flash 4.0 (monochrome camera) voltages (%)

Objective	Observation method		
	BFMono (AS 75%) initial value*	DFMono (AS max)	PHMono (AS 75%)
2x PLN	1.3	not supported	not supported
4x UPLFLN	1.2	40	not supported
10x UPlanXApo	1.4	40	not supported
20x UPlanXApo	1.5	not supported	not supported
40x UPlanXApo	4.0	not supported	not supported
40xUplanXApoS	4.0	not supported	not supported
60x UPlanXApoO	4.4	not supported	not supported
100x UPlanXApoO	5.0	not supported	not supported
10x UPLFLNPH	1.4	70	10
20x UPLFLNPH	1.5	100	30
40x UPLFLNPH	4.0	100	18
60x UPLFLNPHO	4.4	100	20
100x UPLFLNPHO	5.0	not supported	25

* These values must be adjusted for every fresh software installation or after beam splitter substitution. To adjust the lamp voltage, see Adjust the BF lamp intensities to reach 500 μs on page 128.

21.8.4   ORCA-Fusion / Fusion BT (monochrome camera) voltages (%)

	Observation method		
Objective	BFMono (AS 75%) initial value*	DFMono (AS max)	PHMono (AS 75%)
2x PLN	1.8	not supported	not supported
4x UPLFLN	1.2	40	not supported
10x UPlanXApo	1.8	40	not supported
20x UPlanXApo	2.1	not supported	not supported
40x UPlanXApo	5.7	not supported	not supported
40xUplanXApoS	5.7	not supported	not supported
60x UPlanXApoO	6.8	not supported	not supported
100x UPlanXApoO	2.3	not supported	not supported
10x UPLFLNPH	1.8	70	100
20x UPLFLNPH	2.1	100	100
40x UPLFLNPH	5.7	100	100
60x UPLFLNPHO	6.8	100	100
100x UPLFLNPHO	2.3	not supported	100

* These values must be adjusted for every fresh software installation or after beam splitter substitution. To adjust the lamp voltage, see Adjust the BF lamp intensities to reach 500 µs on page 128.

22   How to insert a slide into a tray

1. To insert a slide into a tray push the button on the tray away from the slide pocket to open.
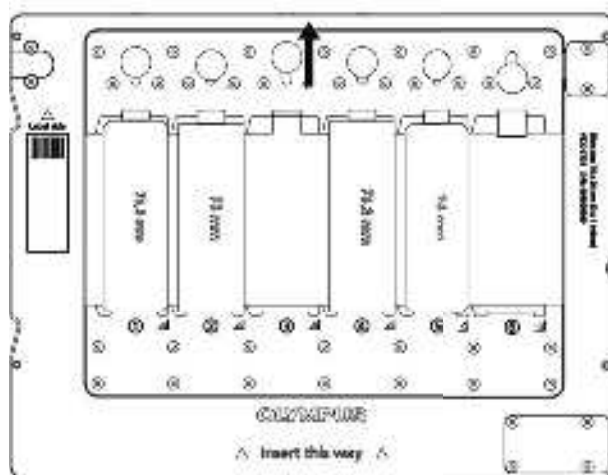
2. Hold the button in place and put a slide into the slide pocket with the label facing the top as indicated on the tray (see imprint [Label side]).

3. Push the slide to right side and release the button.

> If you place a 76x26 mm (3x1 inch) slide into the slide pocket of the tray, the slide will not always be positioned exactly the same. The maximum tolerance for the XY-positioning of the slide in the slide pocket is +/- 150 µm.

22.1   How to insert a tray into the VS200 scanner

CAUTION
Pinching hazard when inserting the tray into the VS200 scanner
The motorized drive of the door flap in the scanner poses a pinching hazard. Your hands and fingers risk being pinched when you insert a tray.
▸ Make sure that the VS200 ASW software isn't performing any functions while you are inserting the tray.

ATTENTION
Risk of damage to device due to improperly inserted tray
If a tray is inserted into the VS200 scanner improperly, the top lens can be damaged.
▸ When inserting a tray, refer to the [Insert this way] lettering and the orientation of the triangles on the tray.

1. To insert a tray into the VS200 scanner use the [Exchange Trays] function of the VS200 ASW software to open the door flap and to extend the microscope stage.

2. Insert the tray horizontally (following the orientation which is printed on the tray [Insert this way]) into the rails of the microscope stage and push it inside (about 6cm) until you cannot push it any further.
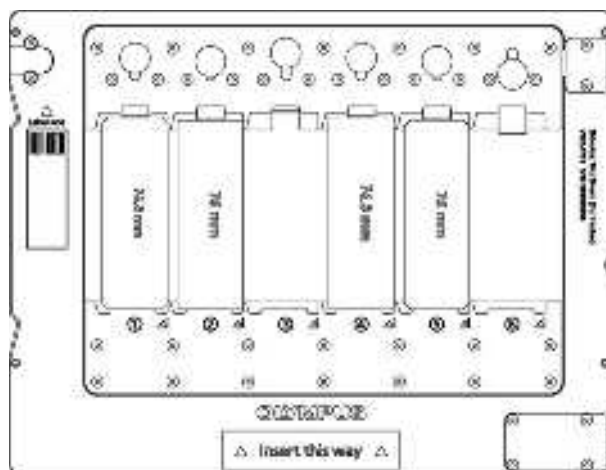
3. Click the [Close] button to close the door flap.

22.2   Insert a tray into the VS200 loader

1. To insert a tray into the VS200 loader use the [Exchange Trays] function of the VS200 ASW software to drive the VS200 loader into the loading/unloading position. This unlocks the door of the loader. Click the [Exchange Trays] button on the start page of the VS200 ASW software.

2. Open the VS200 loader door and insert the tray horizontally following the orientation which is printed on the tray ([Insert this way]) into an empty position.
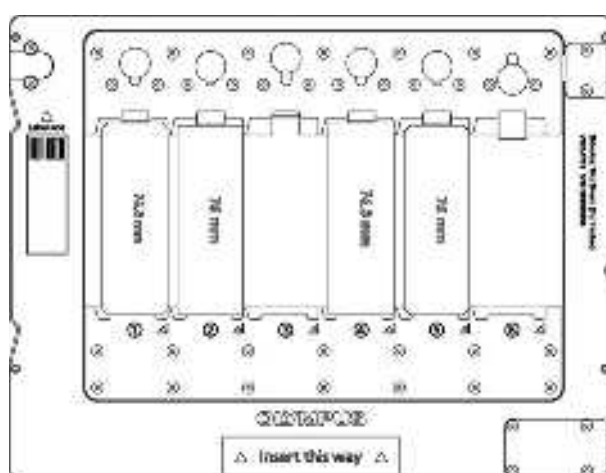
3. Push the tray to the left-most side and all the way in until the tray indicator LED on the left side is green.

4. Close the VS200 loader door and click the [Lock Door] button in the VS200 ASW software.

5. Either use [Select Slide for Calibration] on the start page of the VS200 ASW software to select a slide and tray for calibration or select any scan mode to select and load a tray into the VS200 scanner.

23   Calibrate VS200 using the VS Calibration Slide

This chapter describes how to calibrate a VS200 system.

The VS200 ASW software offers a dedicated calibration wizard for all necessary calibrations.
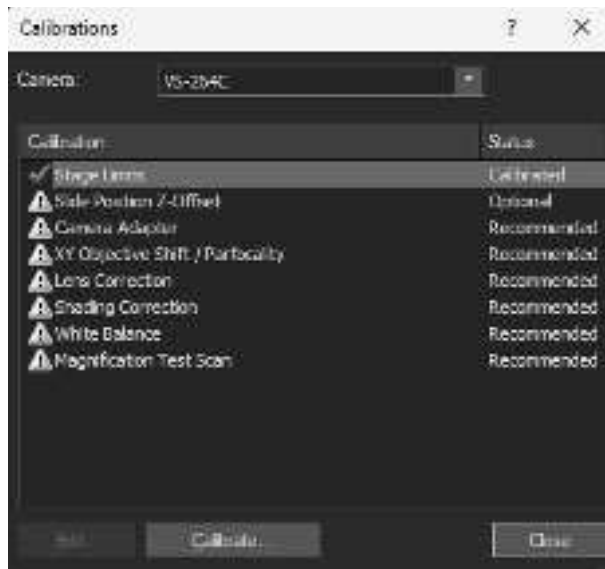
The image shows the [Calibrations] dialog box.

In order to get optimal results, we recommend using the VS Calibration Slide v2.0 to calibrate the system.

Layout of the Calibration Slide (Version 2.0)

The image shows the layout of the VS Calibration Slide v2.0. Always insert it with the label-area (left side) towards the defined label-area of the slide tray.

1. Insert the VS-calibration slide into position 3 of the slide tray.

2. Click the [Exchange Trays] button on the start page of the VS200 ASW software to insert the tray.

3. Click the [Select slide for calibration] button.

4. Load the tray (either manually or using the VS200 loader) by clicking the [Load Slide and Calibrate] button.

» The software switches to the [Manual Control] layout.
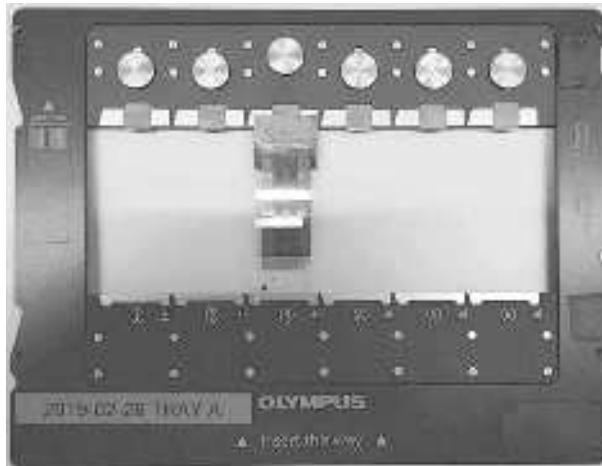
5. In the [Manual Control] layout select the [Acquire] > [Calibrations] command.

23.1 Stage Limits - Z Axis

> 💡 The X and Y axes do not have to be calibrated again. However, the Z axis calibration is dependent on the glass slide thickness. Therefore it is necessary to calibrate the Z axis.

1. In the [Calibrations] dialog box select the [Stage Limits] entry and click the [Calibrate] button.
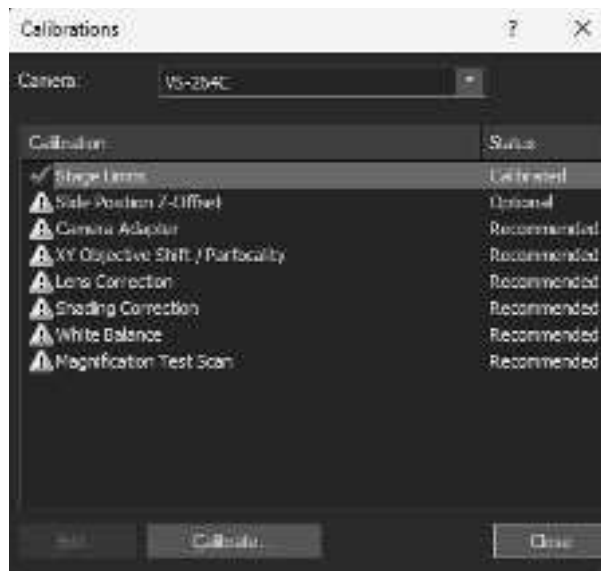
2. Proceed with [Next] and follow the instructions of the wizard.
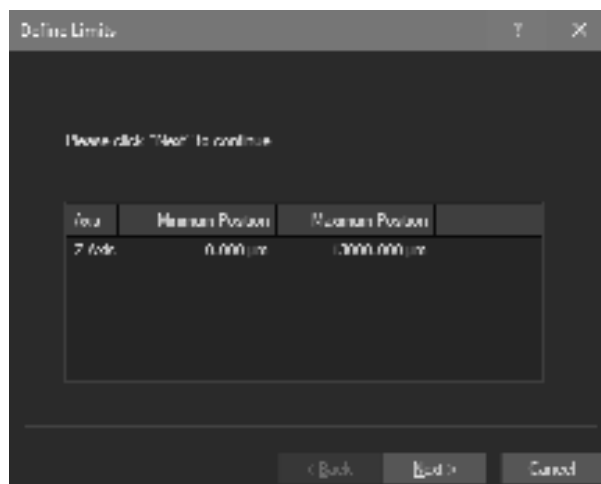
3. Select the [Z Axis] check box and continue with [Next].

4. At the top right in the menu bar click the [Manual Control] entry to switch the layout.

5. In the [Manual Control] layout, change to the 20x objective.

6. In the [Camera Control] tool window, click the [Live] button to switch to live mode.

7. Use the stage navigator or left click on the center of the concentric circles while holding down the [Ctrl] key to move to the center of the slide.

8. Use the [Ctrl] + mouse wheel combination to focus the sample. If you have trouble getting the image into focus, use a lower magnification first.

9. Once the image is in focus click the [Set Focus] button.

» Do not change the value for the Z-limit. The value should be 200.

10. Click the [Finish] button to finalize the calibration process.
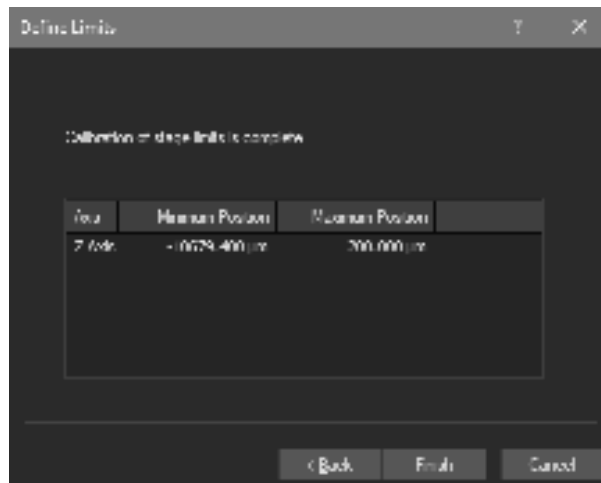
23.2   Slide Position Z-Offset

This calibration corrects Z variations between different trays and slide pockets. This calibration is mainly for a VS200 loader system.

Preconditions

✓ A 4x objective is used for this calibration. It has to be mounted on nosepiece position 6 and configured in the [Device Settings] dialog box.

✓ Use the [Clean Objectives] button on the start page of the VS200 ASW software and unmount the objective from position 5 (if there is one) to avoid collisions.

✓ Use the [Exchange Trays] button on the start page of the VS200 ASW software to remove the calibration slide from the current tray. This step can also be performed from the [Calibrate Slide Position Z-Offset] dialog box when clicking on the [Exchange Trays] button.

✓ Put all empty trays into the VS200 loader.

During this calibration, the objective will focus on the supporting plate of every slide pocket (two positions on the top plate and one position on the bottom plate), average them, and then save this value as the Z offset. The calibration will take around 2 minutes per tray (standard tray, 6 slide pockets), including the loading time for the tray.

1. In the [Calibrations] dialog box, select the [Slide Position Z-Offset] entry and click the [Calibrate] button.
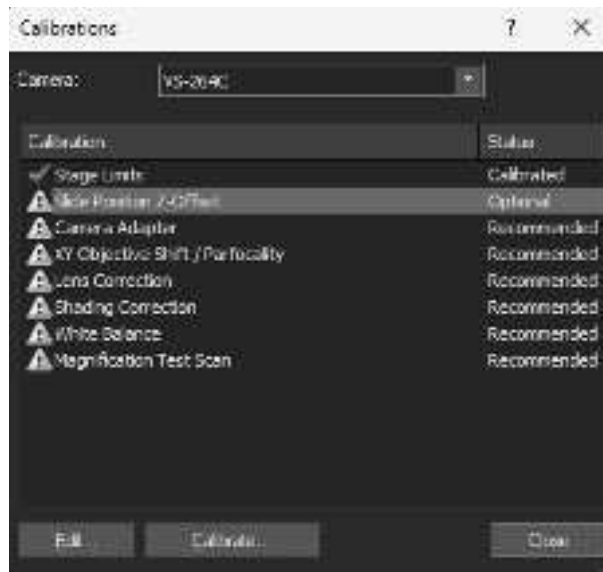
2. The stage moves automatically to the metal area of the supporting tray. Focus on the area and proceed with [Next]. All trays inside the loader will be calibrated.
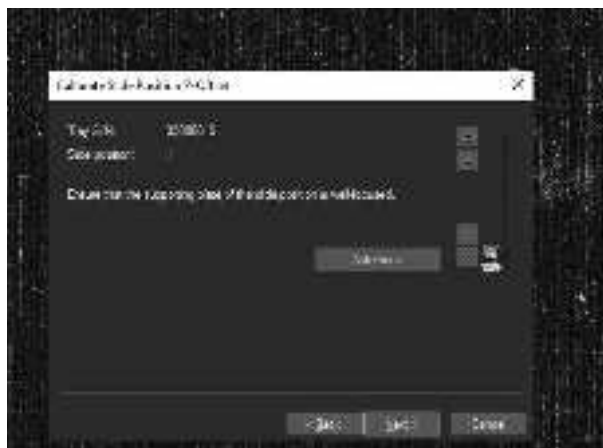
3. Results will be displayed in a table after the calibration is done. Click [Back] to calibrate more trays or to repeat the calibration. Otherwise proceed with [Next] and [Finish].
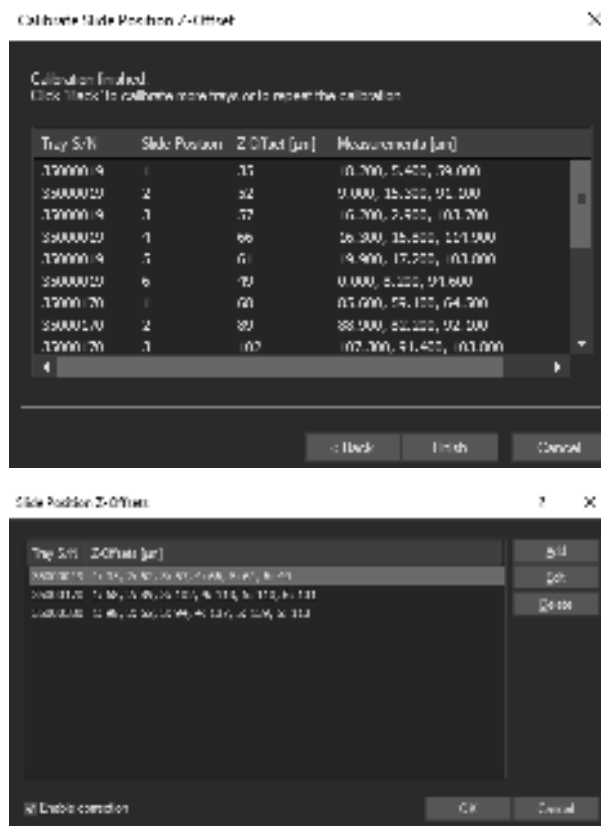
4. You can now mount the objective back on Position 5 (if there was one). Remove the 4x objective if it is a service objective that does not belong to the customer. Remember to remove it in the [Device Settings] dialog box as well.

5. Use the [Exchange Trays] button on the start page of the VS200 ASW software to put back the calibration slide and load it into the base unit by clicking the [Select Slide for Calibration] button.

6. Proceed with the rest of the calibrations.

23.3   Camera adapter calibration for iDS (VS-264C) camera

» 3 mm hex key with angle

» 3 mm hex key

» The iDS color camera is mounted and calibrated ex-works.
The parfocality might change due to temperature changes. Therefore do not check this calibration with a "cold" system (e.g. directly after assembling the system). Wait at least 30 minutes with the system switched on to heat it up.

» If you use objectives with a coverslip correction collar it is recommended to adjust it prior to all following calibrations.

» The calibration process [Camera Adapter] also works with the 20x objective in case a 40x objective is not part of the system configuration.

» The acceptable tolerance for the focus distance is +/- 20µm.

Preconditions

✓ If your VS200 kit contains an objective with a coverslip correction collar ask the customer which coverslips they are using and note the thickness.

✓ Before you start with the camera adapter calibration make sure that the coverslip correction ring of the objective, for example 40x UPlanXApo, is set correctly. In most cases it should be in-between 0.15 – 0.17.

Follow the instructions below to align the camera adapter.

1. In the [Calibrations] dialog box select the [Camera Adapter] entry and click the [Calibrate] button.
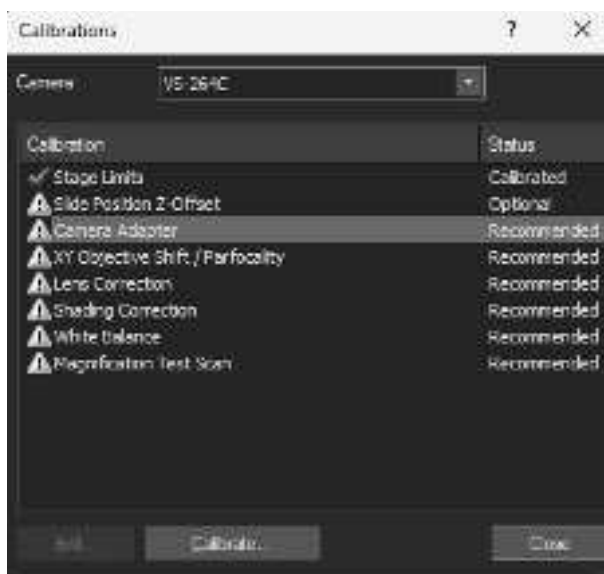
2. The system will use the objective with the highest magnification and switch to live mode. If the image is not in focus click the [Autofocus] button to perform an autofocus.
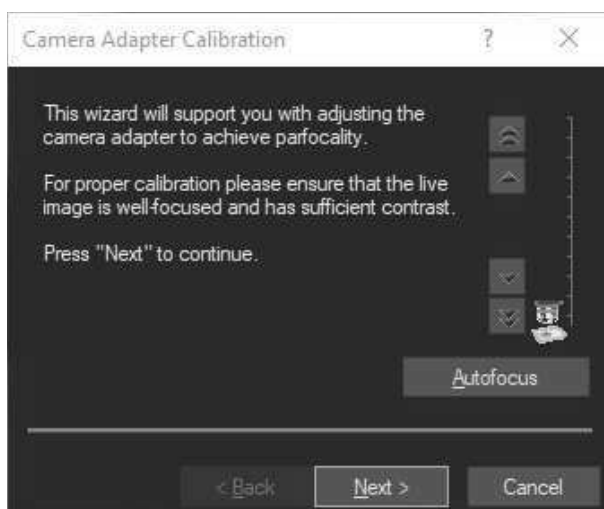
3. Select an area on the calibration slide were it is possible to focus with both the 40x and 2x objectives as shown in the screenshot below.

4. Click the [Next] button to continue.

» If the focus distance between the objectives differs too much, the wizard tells you to either shorten or stretch the camera adapter.
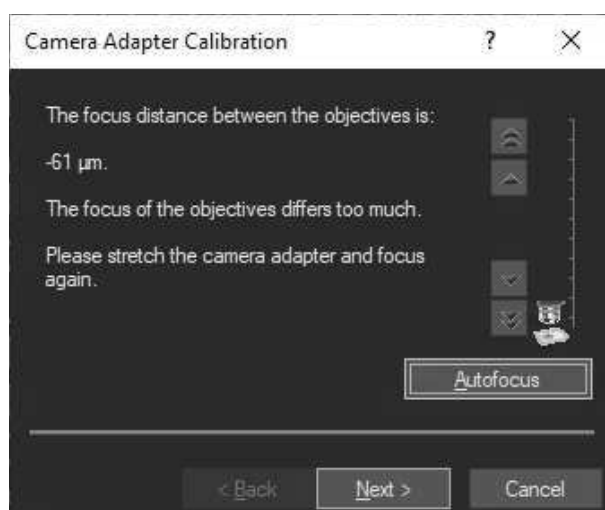
5. To adjust the distance, loosen the [LOCK] hex socket screw slightly and use the [FOCUS] hex socket screw to stretch or shorten the camera adapter. To turn the [FOCUS] screw use the angled hex key.
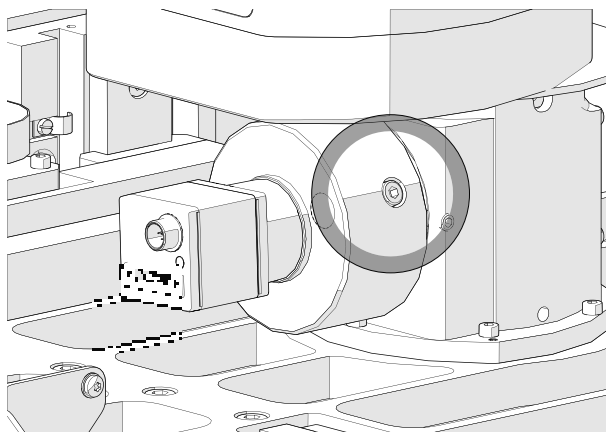
» If the value is positive, turn slightly clockwise.

» If the value is negative, turn slightly counter clockwise.

6. Tighten the [LOCK] screw.

7. Click the [Next] button in the [Camera Adapter Calibration] dialog box.

8. Repeat until the value meets the acceptable tolerance (+/- 20µm or better).
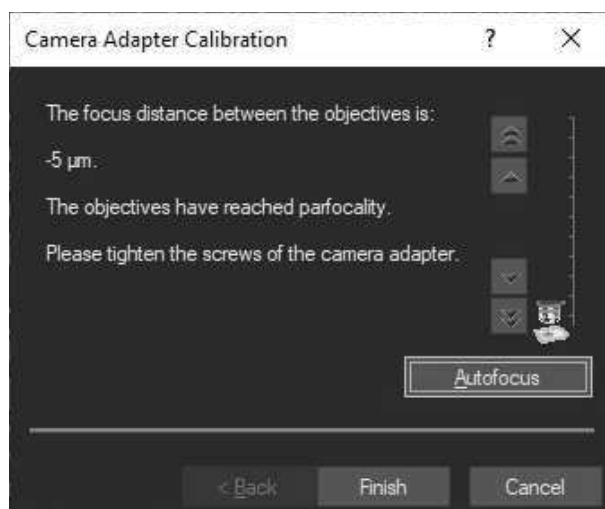
9. Click the [Finish] button to finalize the calibration process.

23.4   Check Koehler illumination

> ☒ The condenser is centered ex works in XYZ direction and fitted with an adjustment lock. Therefore, a recalibration on site is probably not necessary. The following check of the Koehler illumination must be performed by Evident before the initial start-up of the VS200 system.

» Hex key (size 3 mm with ball end)

1. Click the [Additional layouts] ⇄ button to go to a different layout.
2. Select the 10x or 20x objective.
3. Select the BF observation method and switch on live. Focus on a specimen.
4. Open the lock hex screw (size 3 mm hex key with ball end).
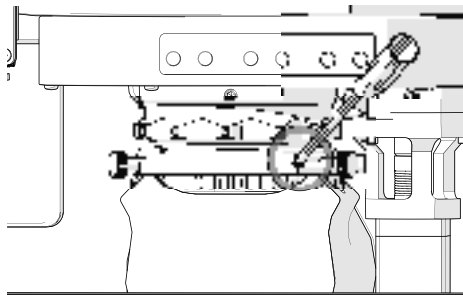
5. Close the fieldstop at the bottom of the condenser.
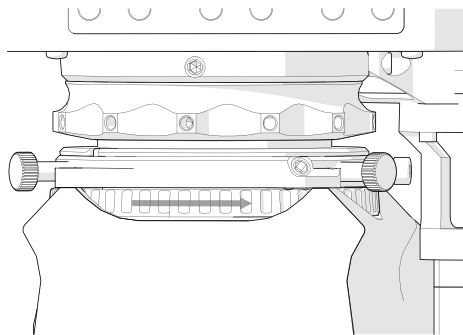
» If the circle you see in the live image is not in the center of the field of view refer to the troubleshoot chapter Setting the Koehler illumination on page 263 to learn how to adjust the Koehler position.

6. If the circle is centered open the field stop completely and close the lock screw again.

> 💡 In case the field stop is misaligned, the phase contrast rings needs to be adjusted. Contact an Evident service technician.

23.5   Check the rotation of the color camera iDS (VS-264C)

> 💡 The color camera is adjusted ex works and locked on place with a locker. Therefore, a recalibration on site is probably not necessary. The following check of the color camera rotation must be performed by Evident before the initial start-up of the VS200 system.

1. Load the calibration slide on the stage.
2. In the VS200 ASW software, go to the [Manual control] layout.
3. Use the [BF] observation method and start the live mode.
4. Check that the camera has the correct orientation by assuring that the letter matrix from the calibration slide appears up-side down.
    » If the letters appear in a different orientation, refer to the troubleshoot chapter. See Color camera rotation adjustment on page 267.
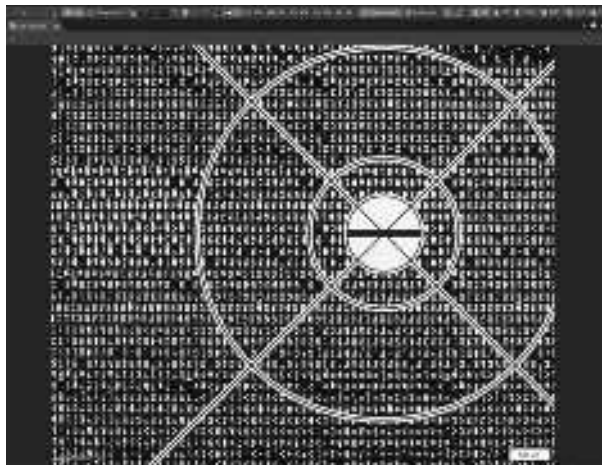
5. Activate the tool window [Digital Reticle]. To do so, click on [View] in the menu and select the [Digital Reticle] entry. You can change the format of the digital reticle and the color using the commands in the tool window.
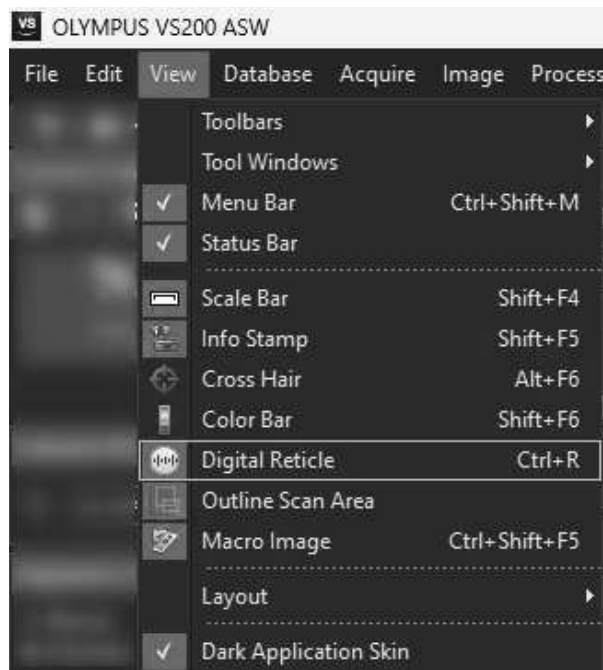
6. Navigate the slide in the Y direction, so the upper border of the letter matrix align to the central area of the field of view. The border should roughly align to the horizontal reference of the digital reticle. The VS200 system allows a deviation of 0,05° which will be calculated and corrected during the scan.
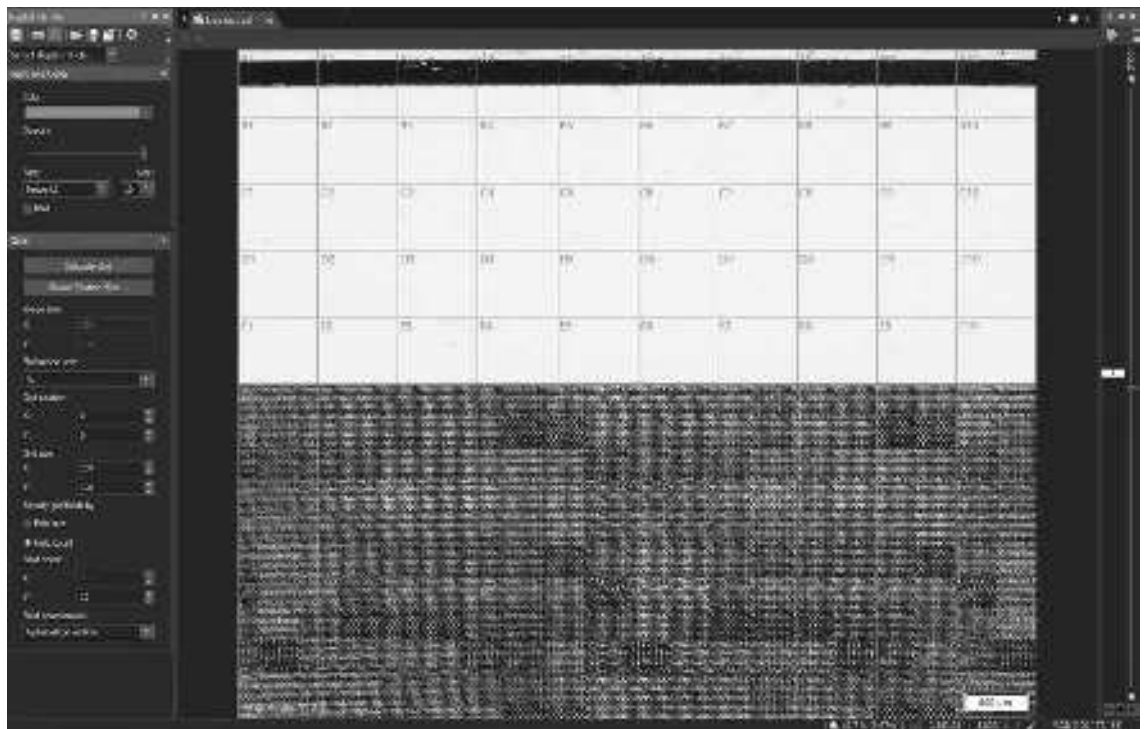
7. The system allows a deviation of 0,05° which will be calculated and corrected during the [Magnification Test Scan] calibration. If you notice at this step a large angle deviation, refer to the troubleshoot chapter to learn how to adjust the color camera rotation. See Color camera rotation adjustment on page 267.

23.6   XY Objective Shift / Parfocality

To carry out a proper XY shift/parfocality calibration use the position of the calibration slide shown in the screenshot below.
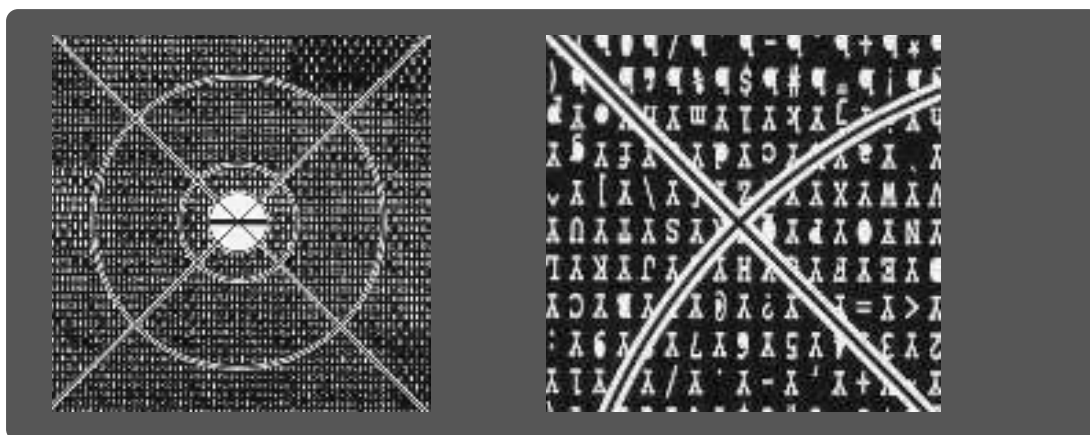
1. In the [Calibrations] dialog box select the [XY Objective Shift / Parfocality] entry.
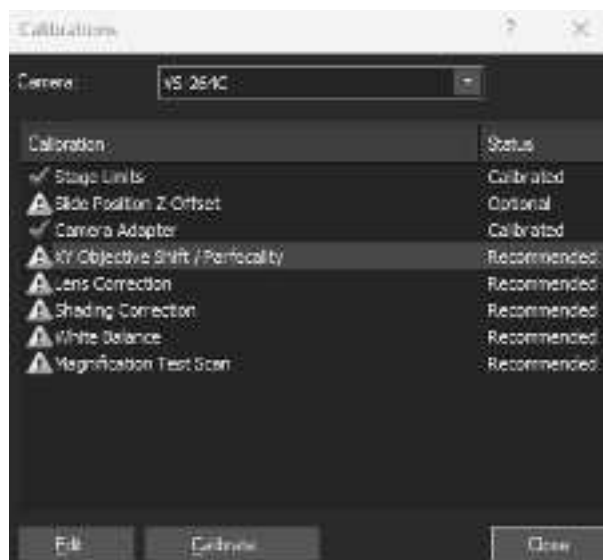
2. Click the [Calibrate] button to start the wizard.

3. Select all objectives. Immersion objectives are calibrated in the same wizard. However the calibration wizard will calibrate all dry objectives first and subsequently select the immersion medium objective(s).
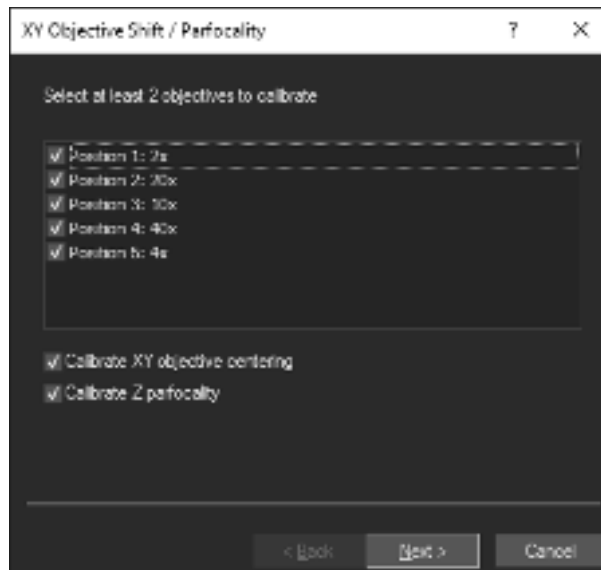
4. Make sure that the functions [Calibrate XY objective centering] as well as [Calibrate Z parfocality] is selected.

5. Proceed with [Next].

6. If the image seems not to be in focus perform an autofocus.

7. The wizard will automatically select all of the objectives that are present and calculate the correction factors.
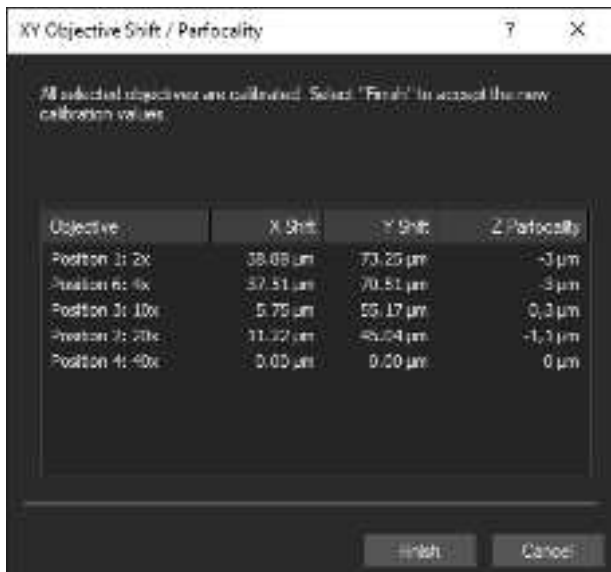
💡 If the Z parfocality for the 40x, 20x and 10x objectives is below 4µm everything is fine. The Z parfocality distance for the 2x and 4x objective should not exceed 20µm.

If the values do not match the standard please check whether all objectives are screwed in completely and whether the calibration slide is clean. Repeat the calibration.

8. Finalize the calibration by clicking the [Finish] button.

! **ATTENTION**
In case you have calibrated an immersion medium objective as well clean the objective and remove immersion medium residues from the calibration slide. See Cleaning the immersion objective on page 226.

23.7 Lens Correction (Brightfield)

On the calibration slide opposite the label area you find an area with check-erboard grids in different sizes. Next to each grid there is a number (see the circle in the example image) for the objective magnification from 4x to 60x/100x which has to be used for the calibration.

1. In the [Calibrations] dialog box select the [Lens Correction] entry and click the [Calibrate] button.

2. Select all available objectives. Immersion objectives are calibrated in the same wizard. However the calibration wizard will calibrate all dry objectives first and subsequently select the immersion medium objective(s).
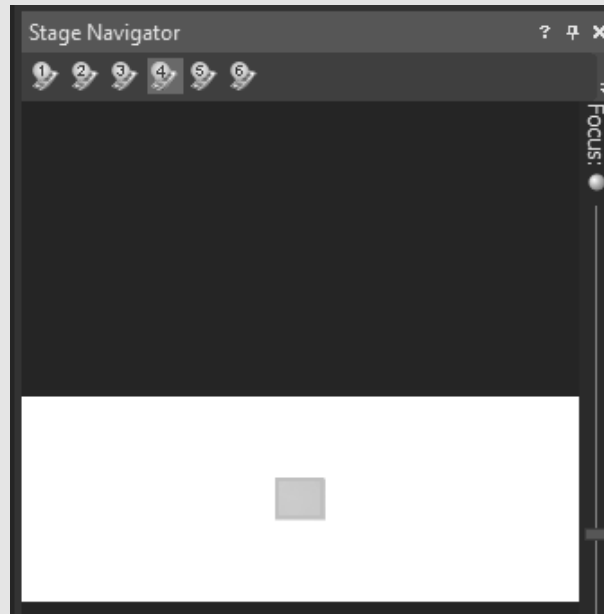
3. Use the stage navigator or drag the VS calibration slide to the required checkerboard area while holding down the [Ctrl] key.

4. If you are calibrating the 2x objective, use the checkerboard pattern for the 4x magnification and position it in the center of the field of view.

5. A warning will appear notifying the user that the pattern has to be located in the center of the image. Click [OK] and continue with the calibration.

6. Perform an autofocus and proceed with [Next].

» The VS200 system shows the progress during the acquisition. This calibration may take a few minutes.
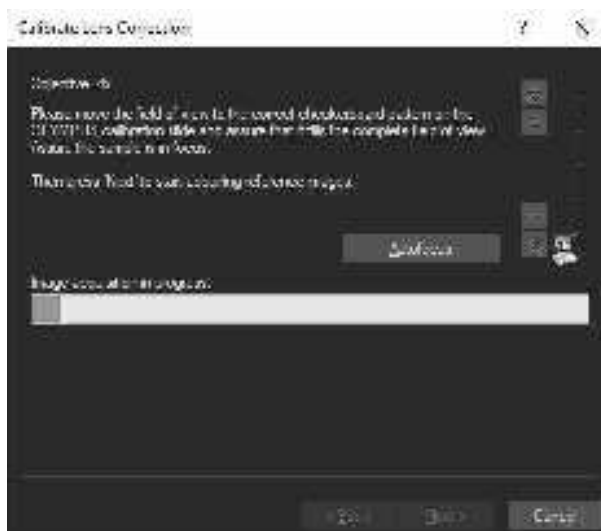
7. Do the same for all other objectives.
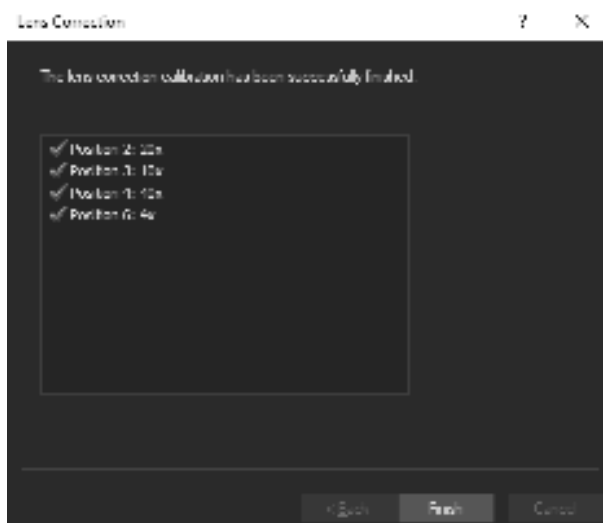
8. Finalize the process by clicking the [Finish] button.

💡 If you see the message below please check if you are doing the calibration on the correct checkerboard area.
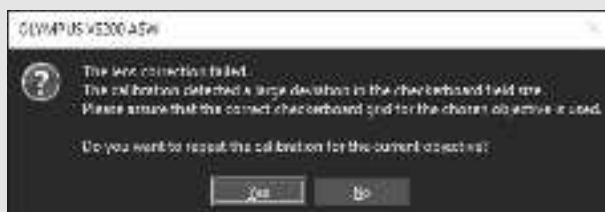
⚠ **ATTENTION**
In case you have calibrated an immersion medium objective as well clean the objective and remove immersion medium residues from the calibration slide. See Cleaning the immersion objective on page 226.

23.8   Shading Correction (Brightfield)

> 💡 The Koehler position of the system is calibrated ex works. However, it might be necessary to at least check the calibration position. To do so, please refer to chapter .

Use the stage navigator or drag the 'empty' area of the calibration slide while holding down the [Ctrl] key.

> 💡 The slide must be very clean (free of dust particles which will disrupt the shading correction procedure).

1. In the [Calibrations] dialog box select the [Shading Correction] entry and click the [Calibrate] button.
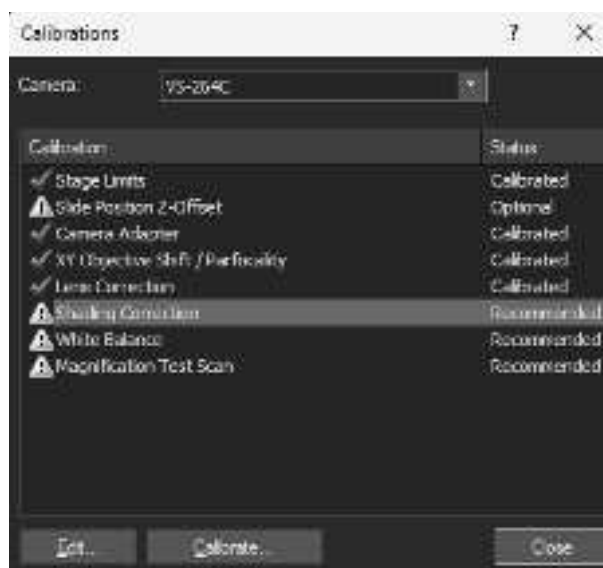
2. Select the [BF] entry as the observation method and proceed with [Next].
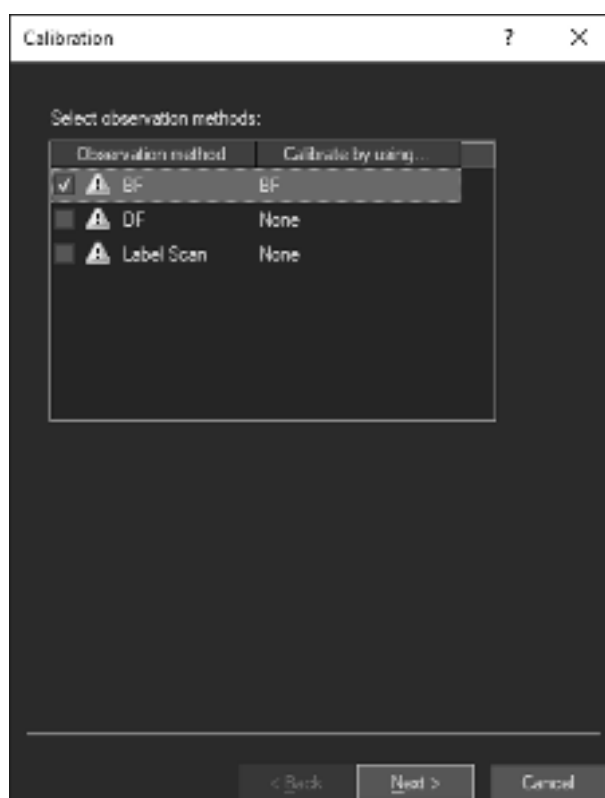
3. When you carry out the calibration for the first time **do not** skip the acquisition of the dark current correction image. For all future calibrations you can skip the acquisition of the dark current correction image.

4. Proceed with [Next].

5. After the dark current correction image calibration, select all objectives. Immersion objectives are calibrated in the same wizard. However the calibration wizard will calibrate all dry objectives first and subsequently select the immersion medium objective(s).
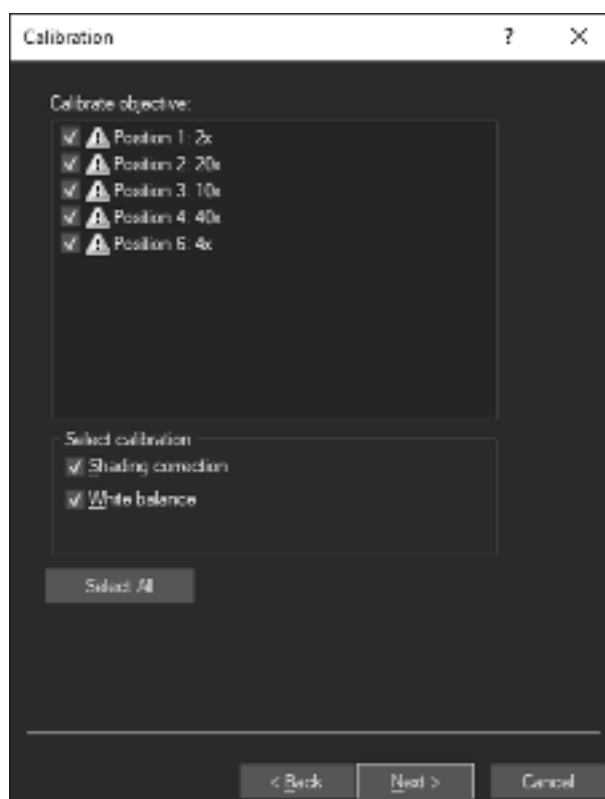
6. Make sure that the options for [Shading correction] and [White balance] are checked.

7. Proceed with [Next].

> 💡 The shading correction for the 2x and 4x objectives has to be done on an empty tray position. Use the stage navigator to move e.g. to position 4. Subsequently go back to the position where the calibration slide is inserted.

8. Click the [Next] button to start the image acquisition process for the 2x objective.
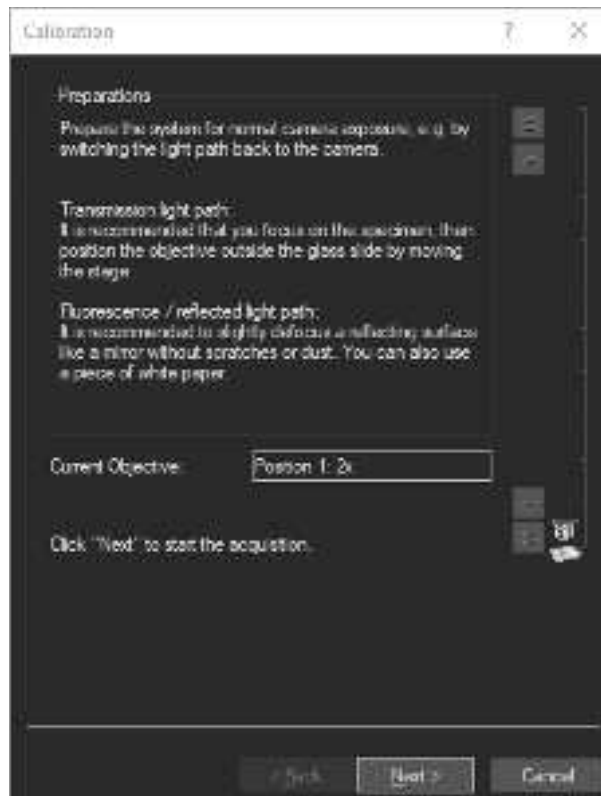
» After the acquisition is complete, the calibration process automatically moves to the next objective.

> 💡 The shading correction for objectives with a magnification equal to or higher than 10x should be done on a sample glass slide with coverslip. You will receive good results if you use the VS-calibration slide.

9. Proceed with all other objectives in the same way.

10. Click the [Finish] button to finalize the calibration process.
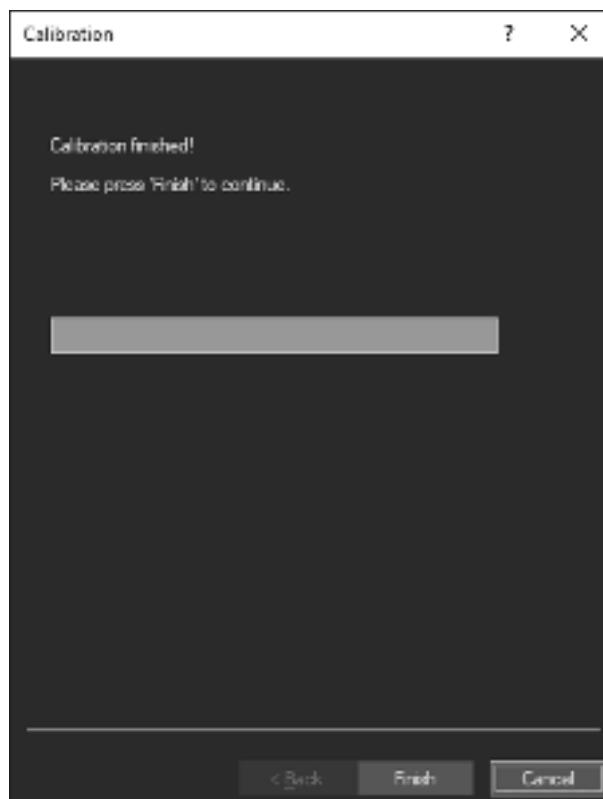
> **ATTENTION**
> In case you have calibrated an immersion medium objective as well clean the objective and remove immersion medium residues from the calibration slide. See Cleaning the immersion objective on page 226.

23.9 Shading correction of the label area

23.9.1 Preparation of calibration slide

For the shading correction calibration of the label scan you need to prepare a proper slide.

If a label printer is used, take an empty sticker and stick it on a normal glass slide as shown in the image below. If the customer does not use a label printer take a piece of white copy paper and glue it onto the slide.

Example of a slide with an empty sticker

1. Insert the VS-calibration slide into position 3 of the slide tray and the prepared label-shading-correction slide in position 2.
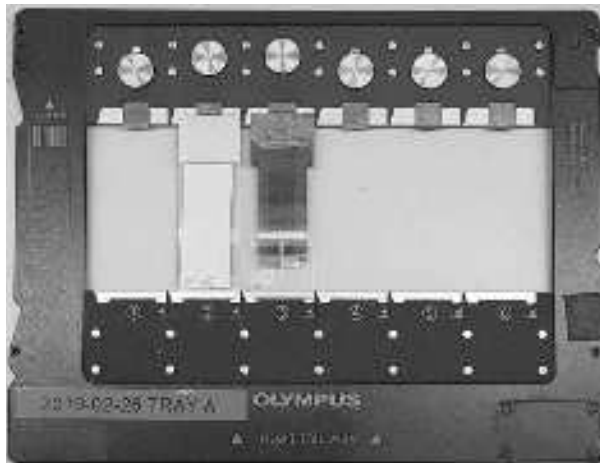
2. Insert the tray by clicking the [Exchange Trays] button on the start page of the VS200 ASW software.

If you use the VS200 loader put the tray in e.g. position 2.

3. Click the [Lock Door] button in the VS200 ASW software to lock the door.

4. Click the [Select Slide for Calibration] button on the start page of the VS200 ASW software.

5. Load the tray (either manually or using the VS200 loader) by clicking the [Load Slide and Calibrate] button.

6. In the [Manual Control] layout select the [Acquire] > [Calibrations] command.

» The [Calibrations] dialog box opens.

Start the calibration

1. In the [Calibrations] dialog box select the [Shading Correction] entry and click the [Calibrate] button.
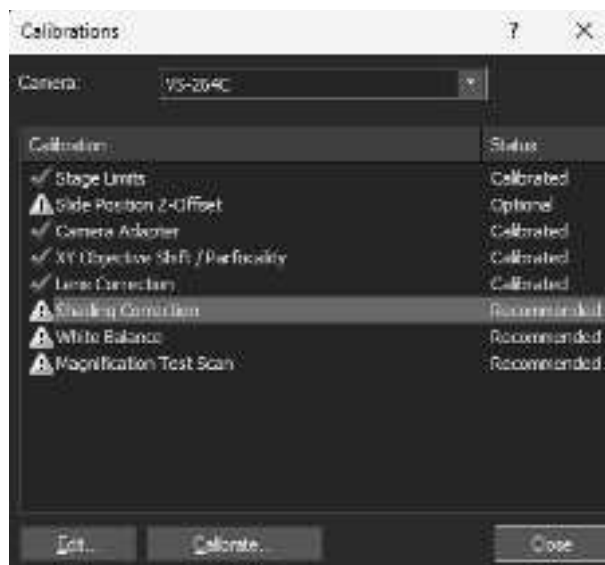
2. Select the [Label Scan] entry for the observation method and proceed with [Next].
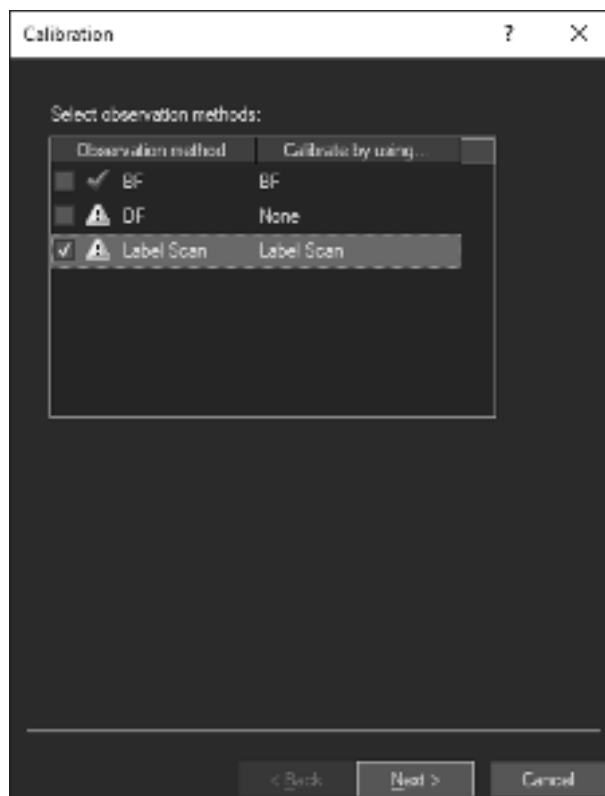
3. When you carry out the calibration for the first time **do not** skip the acquisition of the dark current correction image. For all future calibrations you can skip the acquisition of the dark current correction image.

Proceed with [Next].
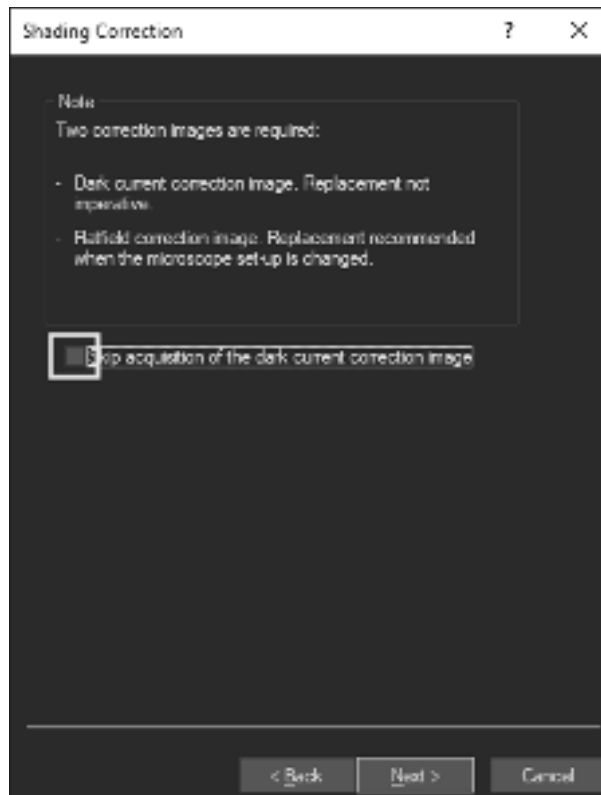
4. Proceed with [Next] to start the acquisition process

5. Proceed with [Next].

6. After the dark current image calibration select all non-immersion object-ives. Immersion objectives shall be calibrated separately. Proceed with [Next].
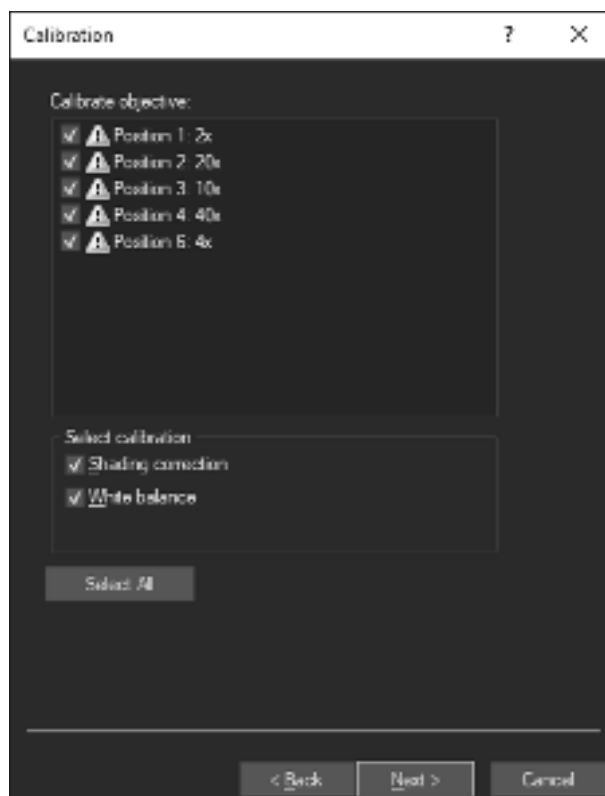
> ○ **IMPORTANT**
>
> Actually, the label shading correction must only be performed for the 2x objective. However you should do it for all other objectives as well as otherwise there will always be an exclamation mark next to the entry.

7. Use the stage navigator to move e.g. to position 2 (where you placed the calibration slide for the label). Make sure you are in the center of the label sticker.

8. Click the [Next] button to start the image acquisition for the 2x objective.

9. Focus on the empty label sticker and click the [Next] button to proceed. Use the focus up and down buttons like shown in the image below.

» After the acquisition is done the calibration process automatically moves to the next objective.

10. Proceed with all other objectives in the same way.

11. Click the [Finish] button to finalize the calibration process.
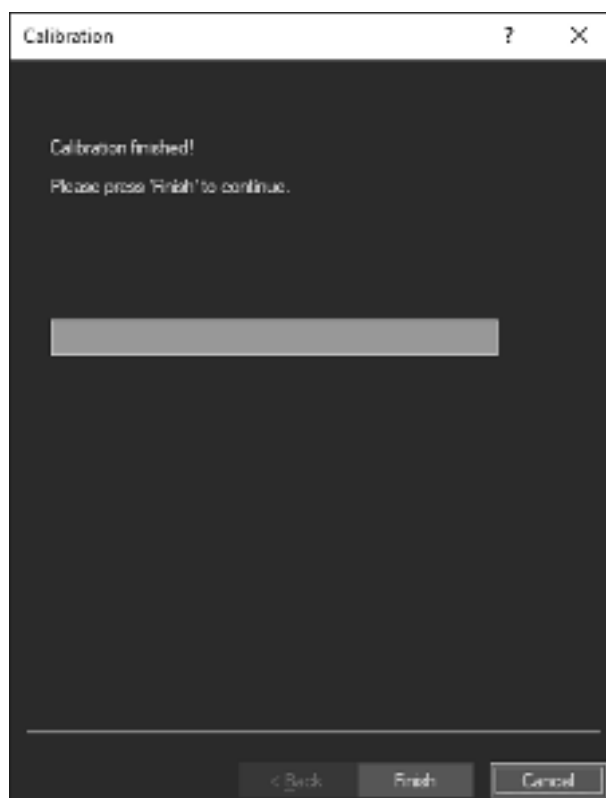
23.9.2   Shading correction for polarization (Pol)

> 💡 The result of the shading correction for polarization is dependent on the value for the [Polarization Angle Changer] which is set in the Polarization observation method. See Setup polarization (Pol) observation method on page 125. If you change the angle you subsequently have to redo the shading correction.

1. In the [Calibrations] dialog box select the [Shading Correction] entry and click the [Calibrate] button.
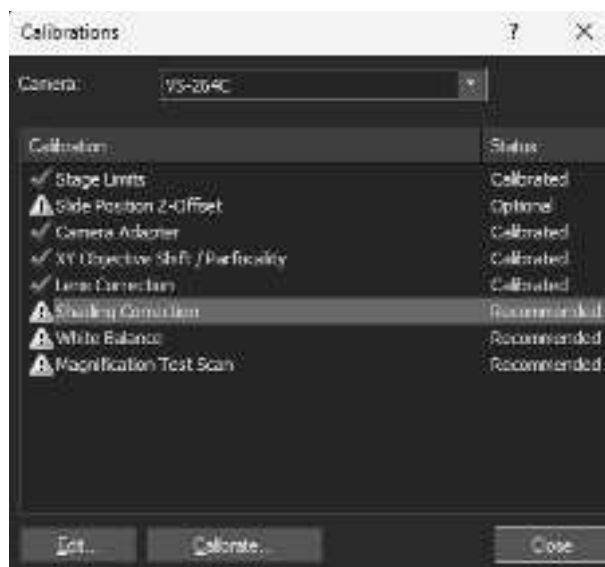
2. Select the [Polarization] observation method.
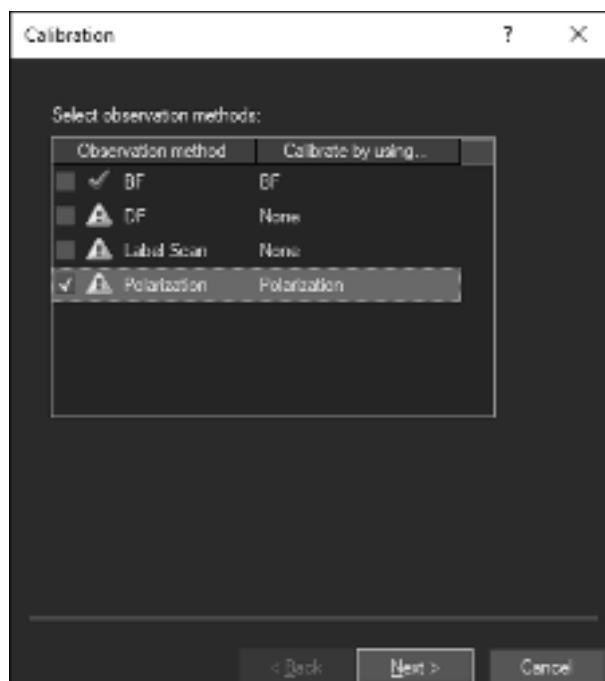
3. Skip the acquisition of the dark current correction image.
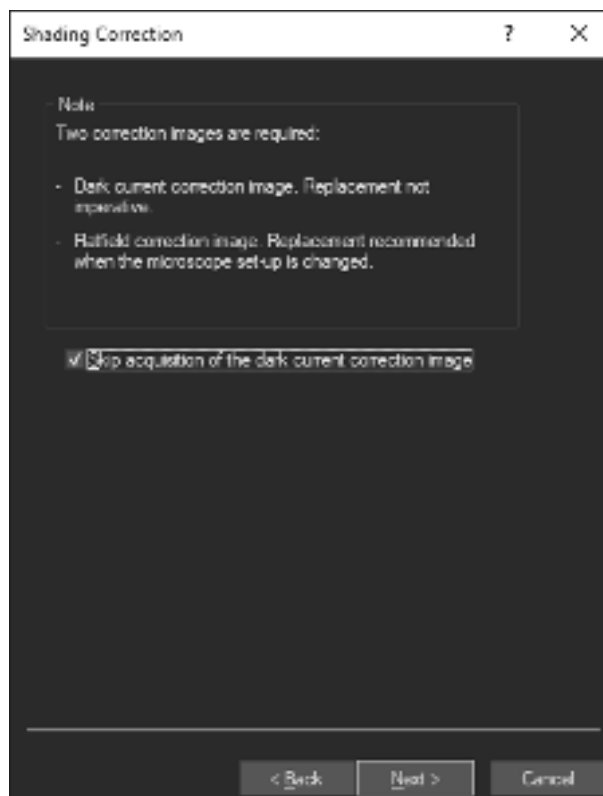
4. Select all objectives.
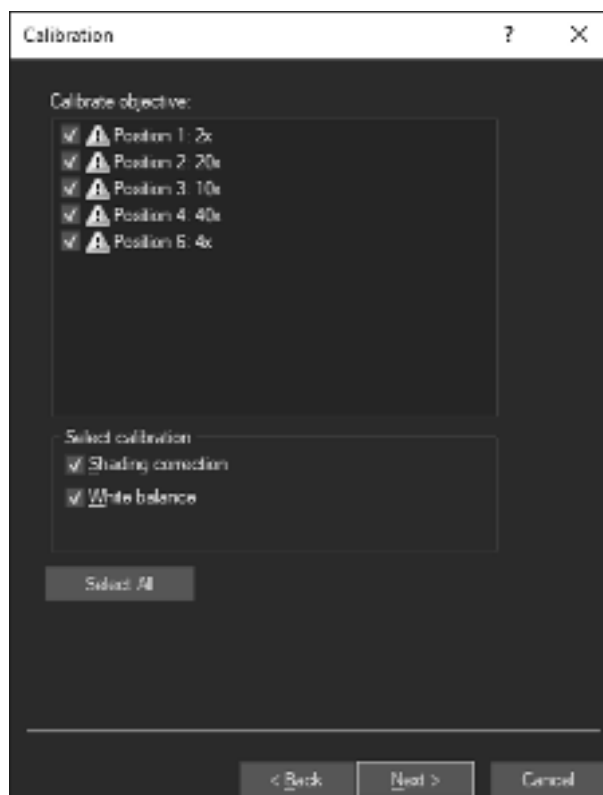
5. Make sure that the options for [Shading correction] and [White balance] are checked.

> The shading correction for the 2x and 4x objectives has to be done on an empty tray position. Use the stage navigator to move e.g. to position 4. Subsequently go back to the position where the calibration slide is inserted.
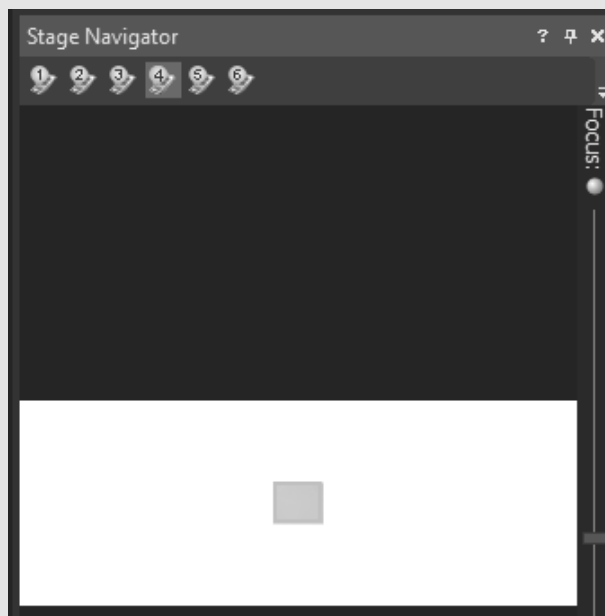
6. Click the [Next] button to start the image acquisition process for the 2x objective.
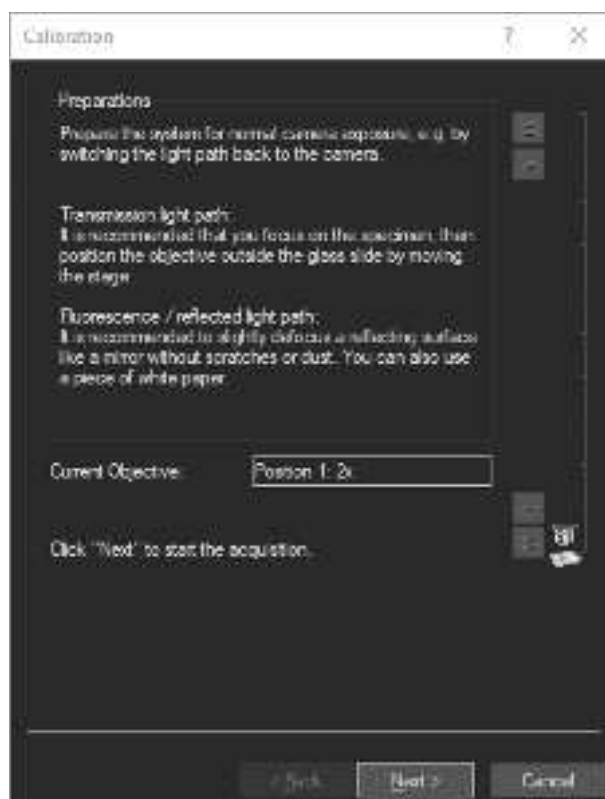
» After the acquisition is complete, the calibration process automatically moves to the next objective.

> 💡 The shading correction for objectives with a magnification equal to or higher than 10x should be done on a sample glass slide with coverslip. You will receive good results if you use the VS-calibration slide.

7. Focus on the part of the slide that contains the sample and subsequently move to a very clean area to acquire the shading image.

8. Proceed with all other objectives in the same way.

9. Click the [Finish] button to finalize the calibration process.

> ⚠ **ATTENTION**
> In case you have calibrated an immersion medium objective as well clean the objective and remove immersion medium residues from the calibration slide. See Cleaning the immersion objective on page 226.

23.10   Magnification Test Scan

1. In the [Calibrations] dialog box select the [Magnification Test Scan] entry and click the [Calibrate] button.
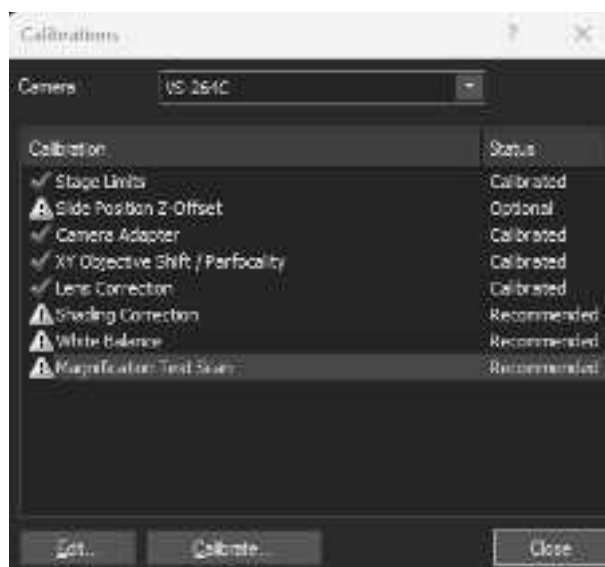
2. Now you can select whether you want to perform the magnification test scan for all objectives or only for certain ones. Immersion medium objectives are calibrated in the same wizard. However the calibration wizard will calibrate all dry objectives first and subsequently select the immersion medium objective(s).

   Select the [Use calibration slide] check box.
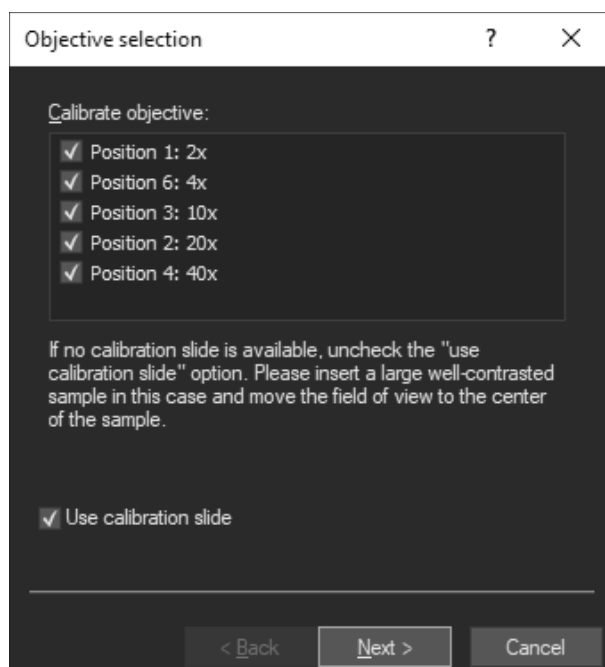
3. Use the stage navigator to move to the center (crosshair) of the slide and then autofocus.

4. Proceed with [Next].

5. Click the [Finish] button to finalize the calibration process.
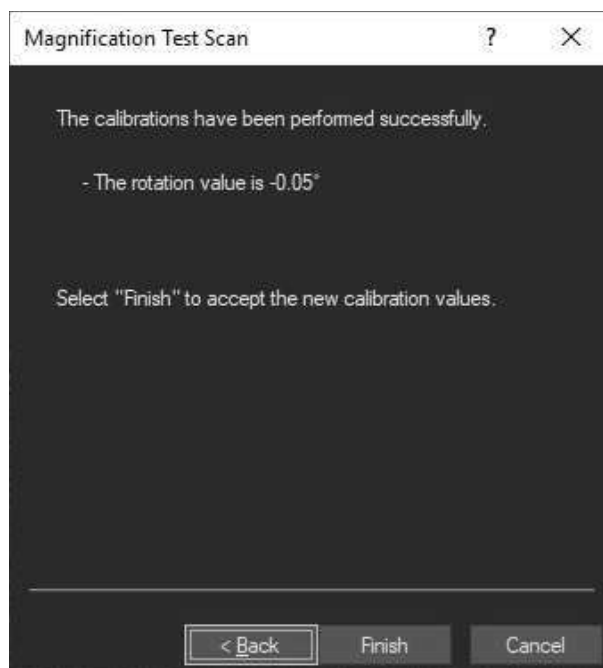
> 💡 If the calibration value is not within the standard refer to the troubleshooting chapter. See Color camera rotation adjustment on page 267.

> **!** **ATTENTION**
> In case you have calibrated an immersion medium objective as well clean the
> objective and remove immersion medium residues from the calibration slide.
> See Cleaning the immersion objective on page 226.

24 Additional calibrations for a fluorescence system

1. Insert the VS-calibration slide into position 3 of the slide tray.

2. Click the [Exchange Trays] button on the start page of the VS200 ASW software to insert the tray.

3. Click the [Select slide for calibration] button.

4. Load the tray (either manually or using the VS200 loader) by clicking the [Load Slide and Calibrate] button.

   » The software switches to the [Manual Control] layout.

5. In the [Manual Control] layout select the [Acquire] > [Calibrations] command.

24.1   Camera Adapter

> ☿ » The parfocality might change due to temperature changes. Therefore do not perform this calibration with a "cold" system (e.g. directly after assembling the system). Wait at least 30 minutes with the system switched on to heat it up.
>
> » If you are using objectives with a coverslip correction collar, it is recommended to adjust it prior to all subsequent calibrations.
>
> » The limit for the focus distance is +/- 20µm.

24.1.1   Camera adapter U-FFWO T3

> ☿ This example describes the calibration for an ORCA-Flash 4.0. The calibration for the VS-304M as well as the ORCA-Fusion and ORCA-Fusion BT can be done in the same way.

> 🔧 » Small hex key to lock the tubus
> » Hex key to open the filter wheel
> » Small spanner to adjust the height of the tubus
> » Spanner to fix the tubus

> ☿ The acceptable tolerance for the focus distance is +/- 20 µm.

1. In the [Calibrations] dialog box select the [Camera Adapter] entry and click the [Calibrate] button.

2. Use [Ctrl] + mouse wheel to fine focus.
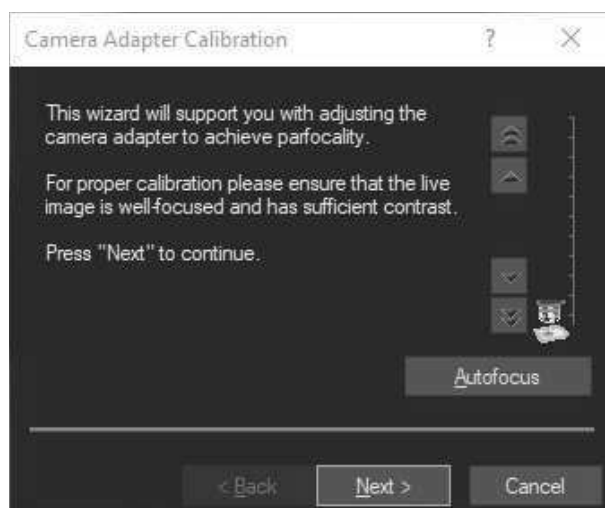
3. Use the stage navigator or the [Ctrl] + left click to move to a similar position on the calibration slide like shown in the picture below.

4. Perform an autofocus.

5. If the focus differs too much you need to manually adjust the U-FFWO T3 adapter.
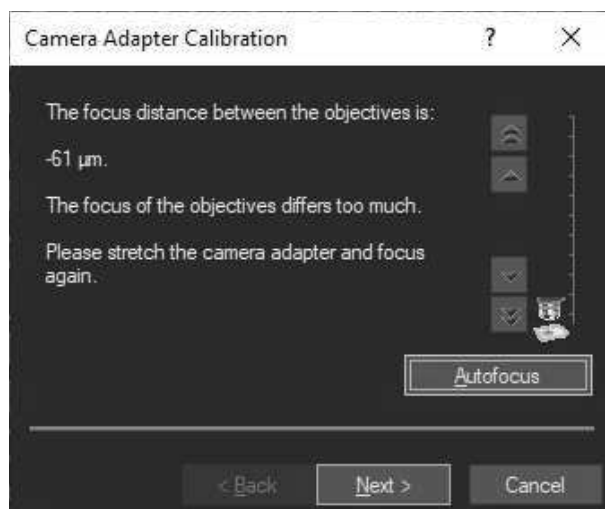
Adjust the camera adapter

1. Release the camera rotation locking screw. Hold the camera with one hand to prevent camera rotation. Use the small wrench to adjust parfocality. Turning the small hex screw (**3**) will move the camera up or down. Turn clockwise if the calibration wizard shows a positive distance (shorten the camera adapter), turn counter-clockwise if the calibration wizard shows a negative distance (stretch the camera adapter).
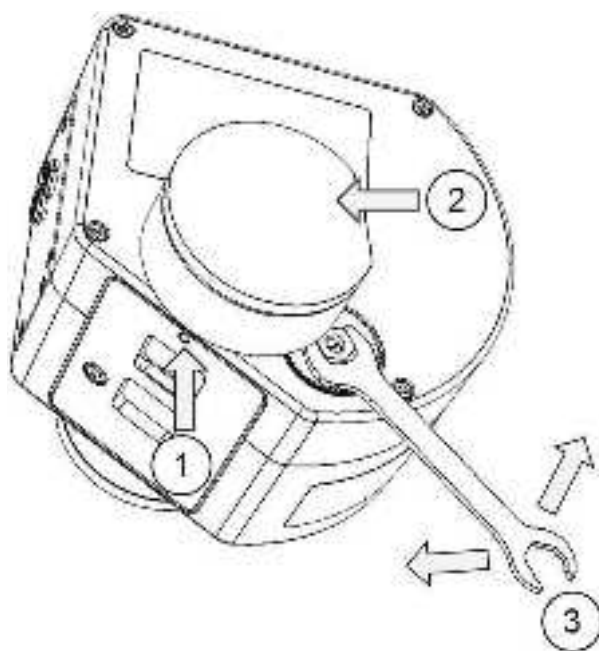
(1)	Release locking screw.
(2)	Keep hold of camera to prevent rotation.
(3)	Adjust parfocality.

2. Lock the parfocal setting by tightening the counternut. To do so, pull the large wrench clockwise to fasten the counternut while holding the small wrench steady.
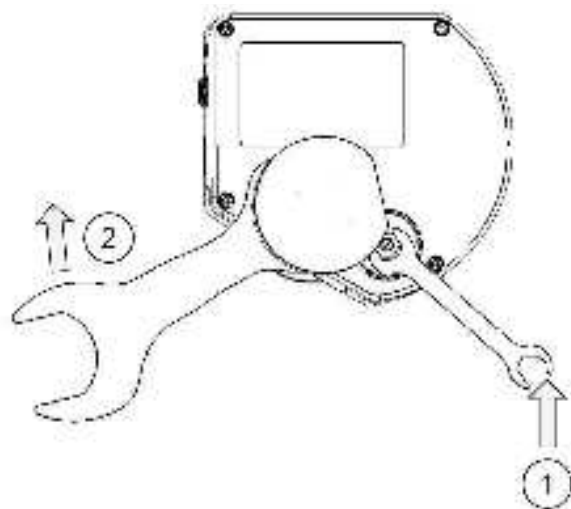
(1)	Hold steady.
(2)	Fasten clockwise.

3. Execute an autofocus again and check the final result.
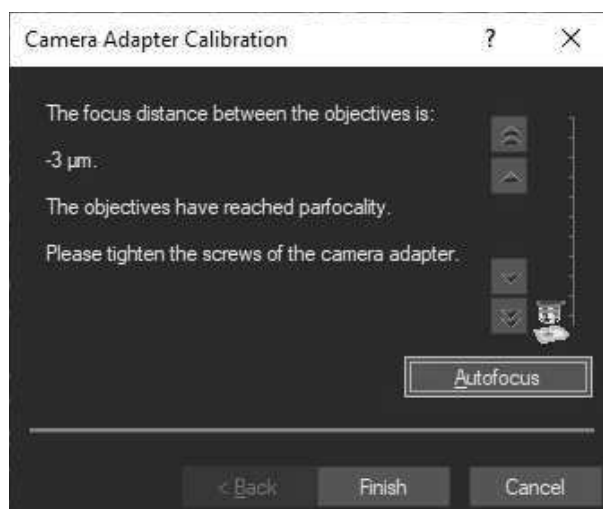
4. Repeat until the camera adapter parfocality meets the acceptable tolerance.

5. Finalize the process by clicking the [Finish] button.

Make sure the camera is roughly at the correct angle at the end of the calibration, with the letter matrix up-side-down and roughly parallel to the horizontal axis. Fine angle adjustment will be done within the calibration [Camera alignment].
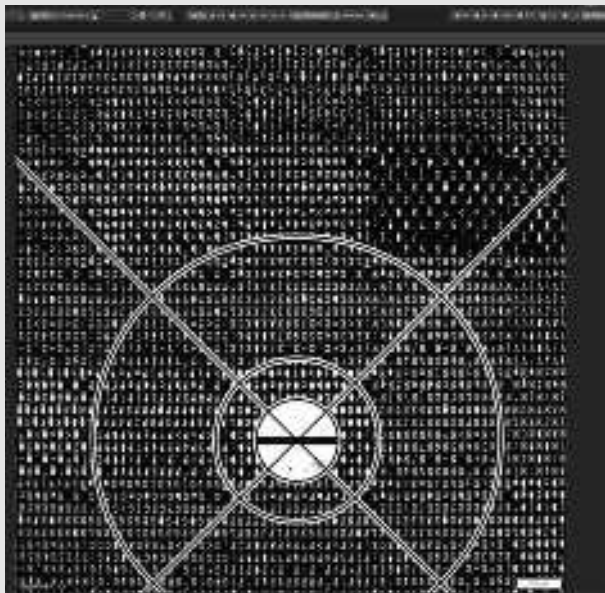
24.2 Camera Alignment

This calibration is essential to precisely adjust the camera rotation of the monochrome camera. In this process the 20x UPlanXApo objective is automatically selected. The standard for the camera alignment rotation is +/- 0.01°.

1. In the [Calibrations] dialog box select the [Camera Alignment] entry.
2. Click the [Calibrate] button.
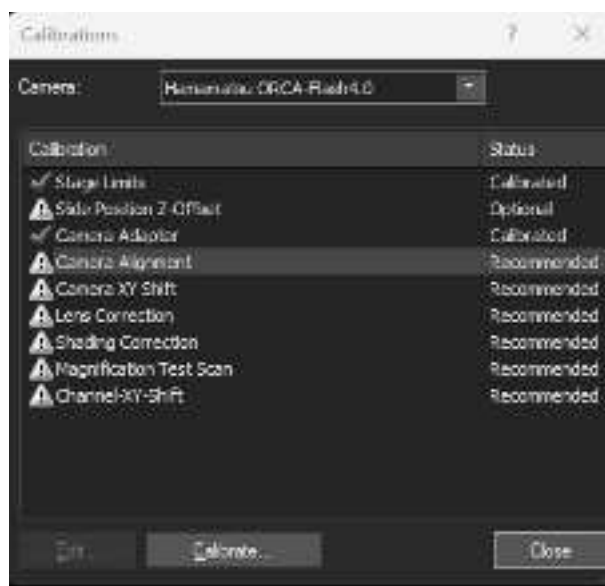
» The system will automatically move to the center of the calibration slide and display the center position.
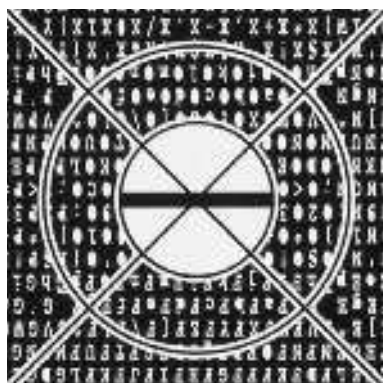
3. Recheck the focus before you proceed with [Next].
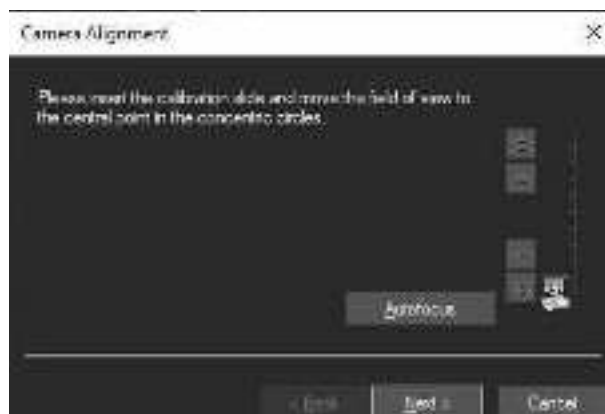
» The system automatically moves to the 20x checkerboard position on the calibration slide.
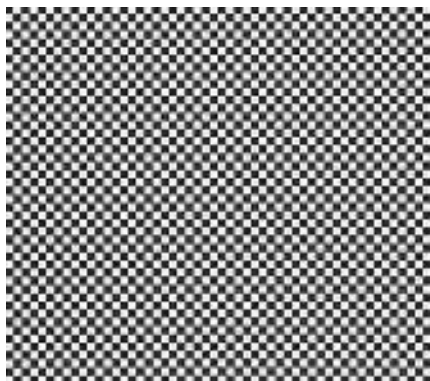
4. Check the focus and proceed with [Next].
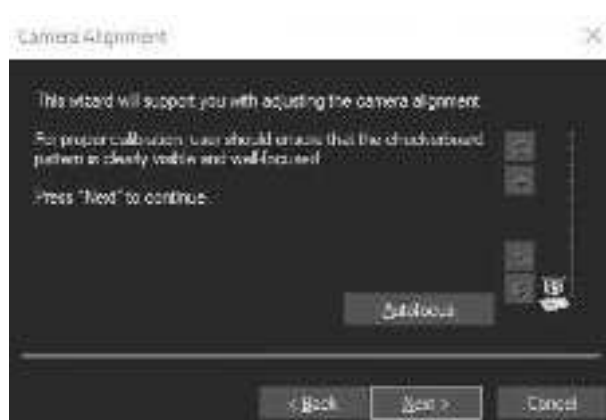
» The VS200 system switches into live mode and displays a window showing the current rotation angle (the angle value might fluctuate a little bit).
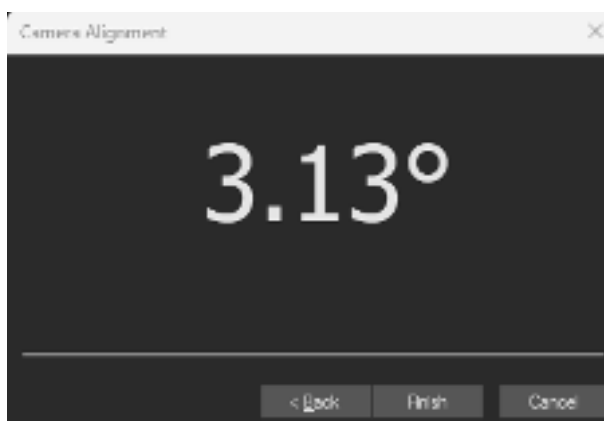
If the rotation is out of standard:

1. Release the locking screw **(1)**.
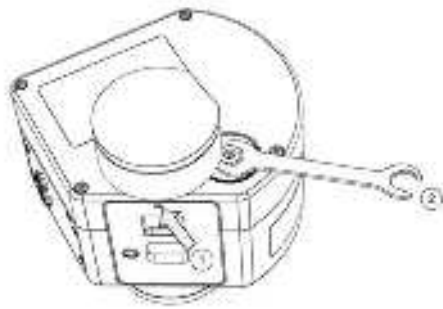
2. Adjust the rotation by turning the brass hex nut using the small spanner **(2)**.

   » The camera should be able to rotate freely.

   » Turn counter-clockwise if the rotation angle is negative and clockwise if the rotation angle is positive.

3. Rotate and check the Live-Rotation-Angle until the standard (+/- 0.01°) is achieved.

> 💡 If you are only using a TV 1.0x adapter, turn the camera clockwise if the value is negative and counter-clockwise if the value is positive.

4. Tighten the locking screw **(1)**.
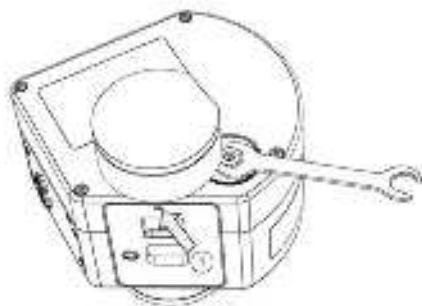
5. Press [Finish] to accept the calibration.

24.3   Camera XY Shift

> 💡 The camera shift calibration corrects the shift between the brightfield and monochrome cameras. It is a software-based correction.

Measure the camera shift between the two cameras

1. In the [Calibrations] dialog box, select the [Camera XY Shift] entry and click the [Calibrate] button.
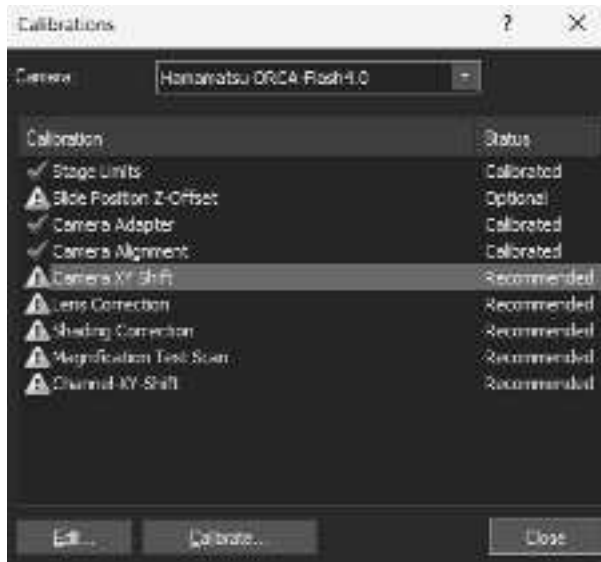
2. Use the stage navigator or left click on the center of the concentric circles while holding down the [Ctrl] key to move to the center of the slide.
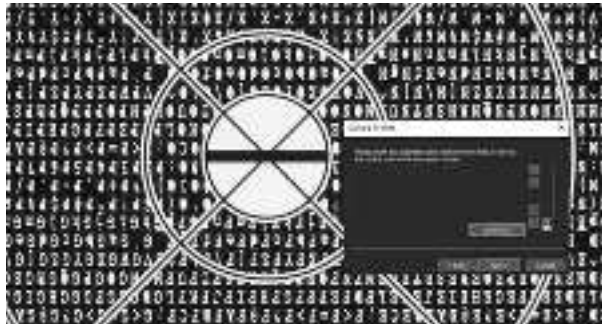
3. Proceed with [Next].

4. Perform an autofocus and proceed with [Next]. This will be done once on BF and once on BFMono.

5. Click [Finish] to confirm the results of the calibration.
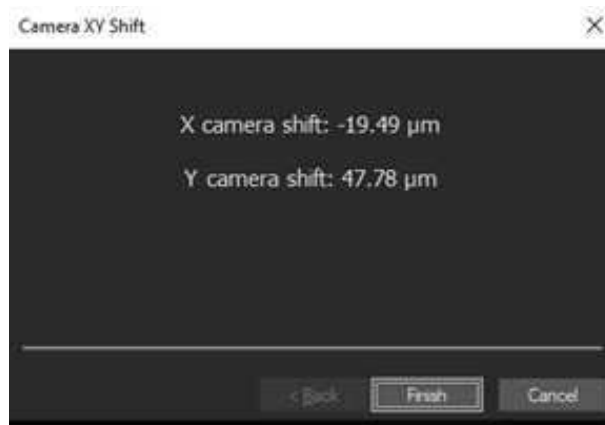
24.4   Lens Correction (Fluorescence)

> 💡 Do not perform lens correction when SILA is installed.
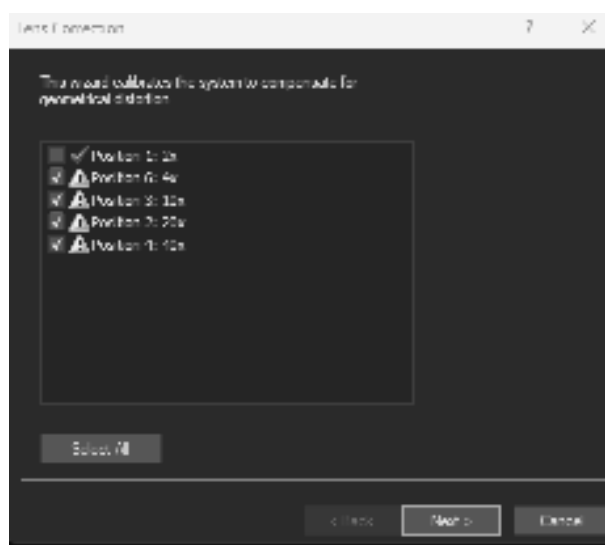

On the calibration slide opposite the label area you find an area with checkerboard grids in different sizes. Next to each grid there is a number (see the circle in the example image) for the objective magnification from 4x to 60x/100x which has to be used for the calibration.
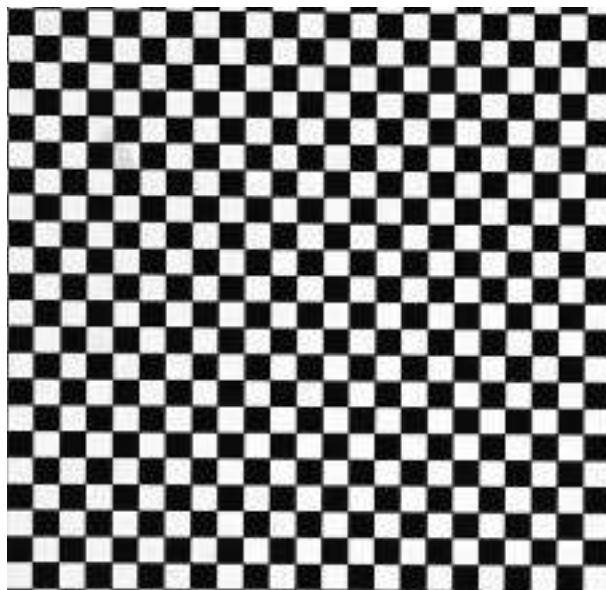
1. In the [Calibrations] dialog box select the [Lens Correction] entry and click the [Calibrate] button.

2. Select all available objectives. Immersion objectives are calibrated in the same wizard. However the calibration wizard will calibrate all dry object-ives first and subsequently select the immersion medium objective(s).

3. Use the stage navigator or drag the VS calibration slide to the required checkerboard area while holding down the [Ctrl] key.
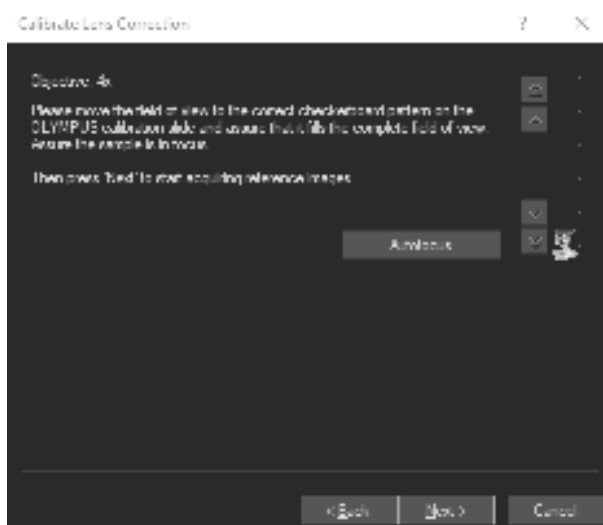

4. If you are calibrating the 2x objective, use the checkerboard pattern for the 4x magnification and position it in the center of the field of view.
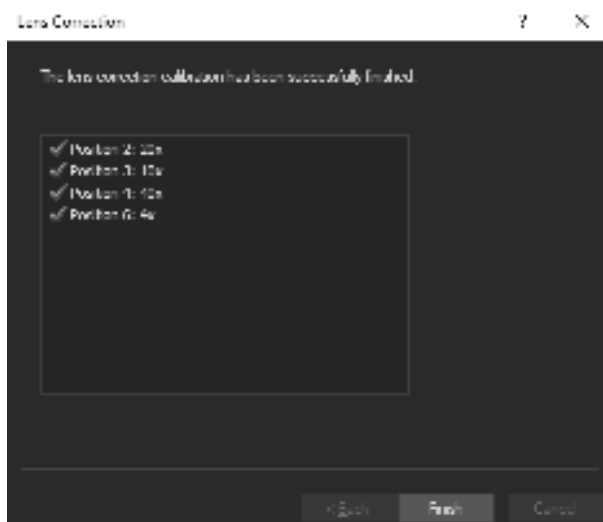

5. A warning will appear notifying the user that the pattern has to be located in the center of the image. Click [OK] and continue with the calibration.

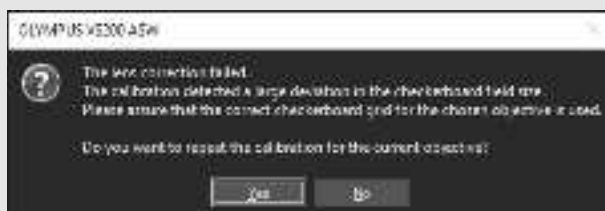6. Perform an autofocus and proceed with [Next].

7. Do the same for all other objectives.

8. Finalize the process by clicking the [Finish] button.

> 💡 If you see the message below please check if you are doing the calibration on the correct checkerboard area.
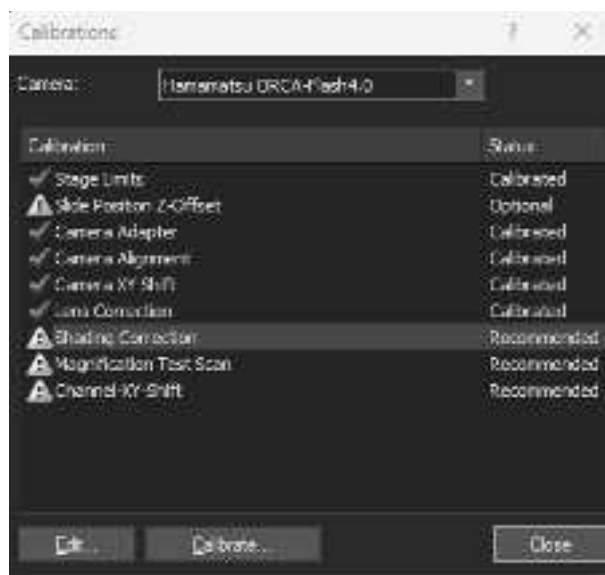>
>

> **ATTENTION**
> In case you have calibrated an immersion medium objective as well clean the objective and remove immersion medium residues from the calibration slide. See Cleaning the immersion objective on page 226.
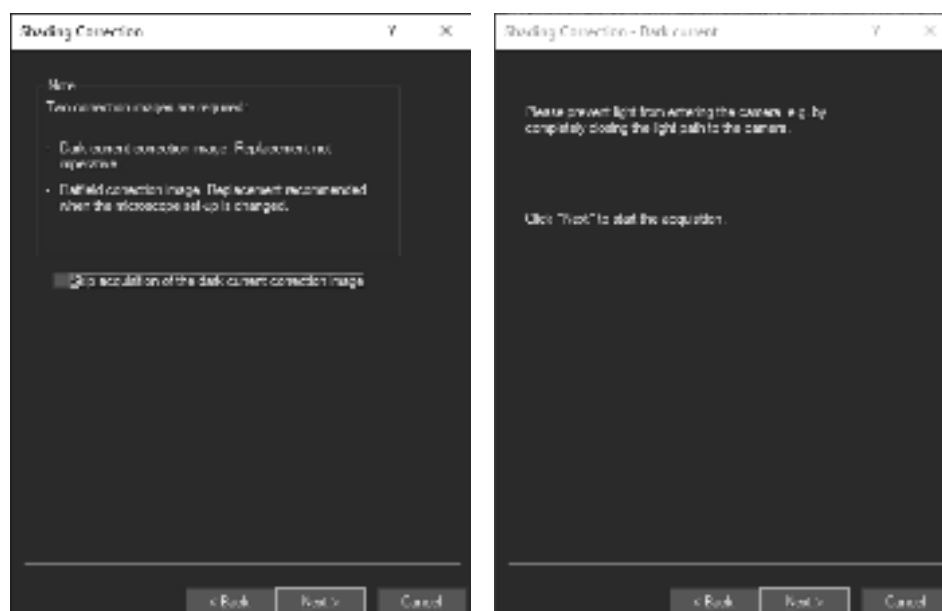
24.5 Shading Correction BFMono

1. In the [Calibrations] dialog box, select the [Shading Correction] entry and click the [Calibrate] button.

2. Select the [BFMono] observation method and proceed with [Next].
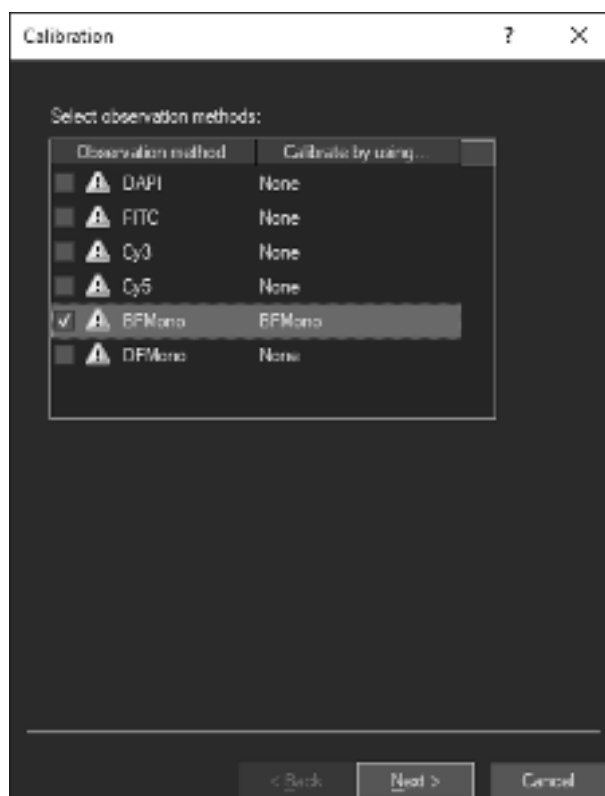
3. If you are calibrating an out of the box system (no shading correction was done before) start with the acquisition of the dark current correction image.

4. After the dark current image calibration select all objectives. Immersion objectives are calibrated in the same wizard. However the calibration wizard will calibrate all dry objectives first and subsequently select the immersion medium objective(s).
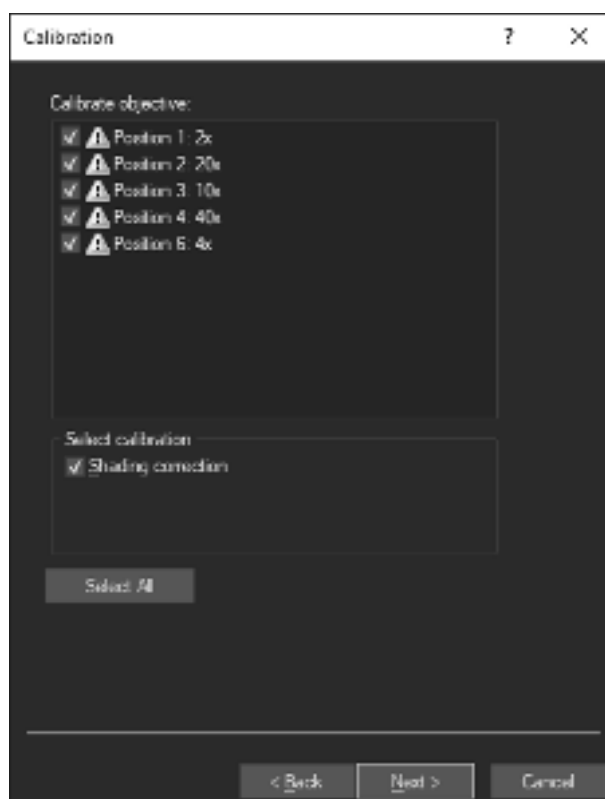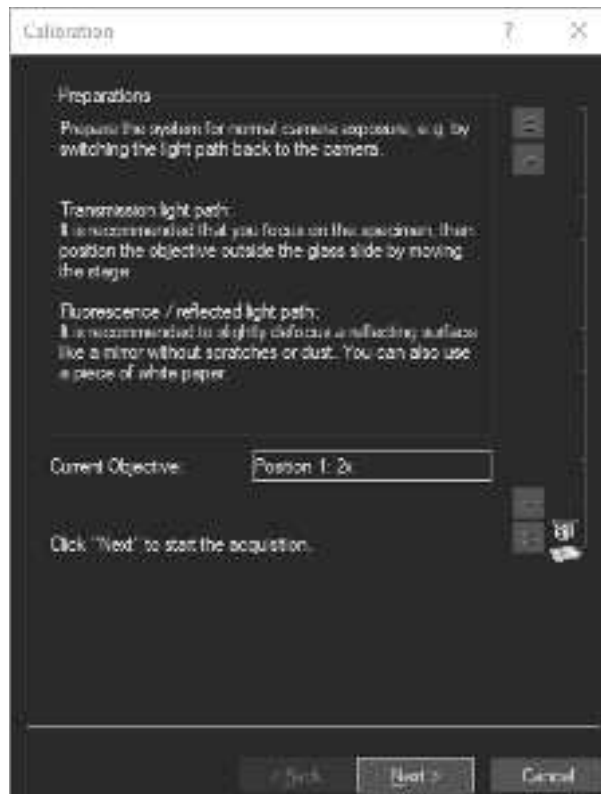

5. Make sure the [Shading correction] check box is selected
6. Proceed with [Next].

> **IMPORTANT**
>
> The shading correction for the 2x and 4x objectives has to be done on an empty tray position. Use the stage navigator to move e.g. to position 4.
>
>
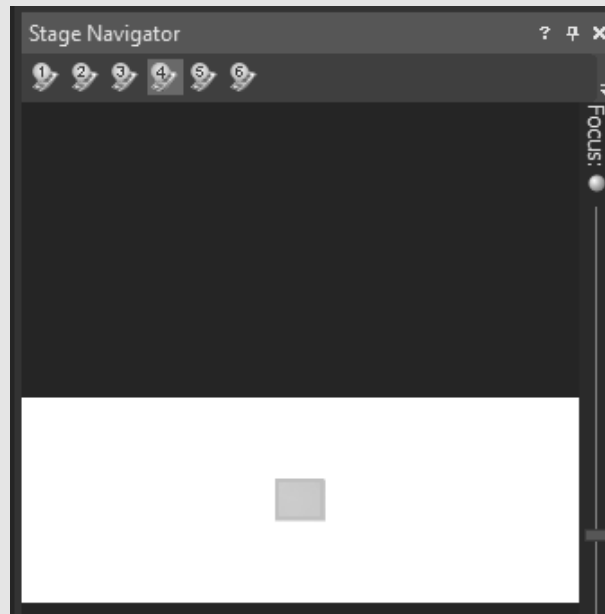
7. Click the [Next] button to start the image acquisition process for the 2x objective.

» After the acquisition process is finished, the calibration process auto-matically moves to the next objective.

> 💡 IMPORTANT
>
> The shading correction for objectives with a magnification equal to or higher than 10x should be done on a sample glass slide with coverslip. You will receive good results if you use the VS-calibration slide.

8. Proceed with all other objectives in the same way.

9. Finalize the process by clicking the [Finish] button.
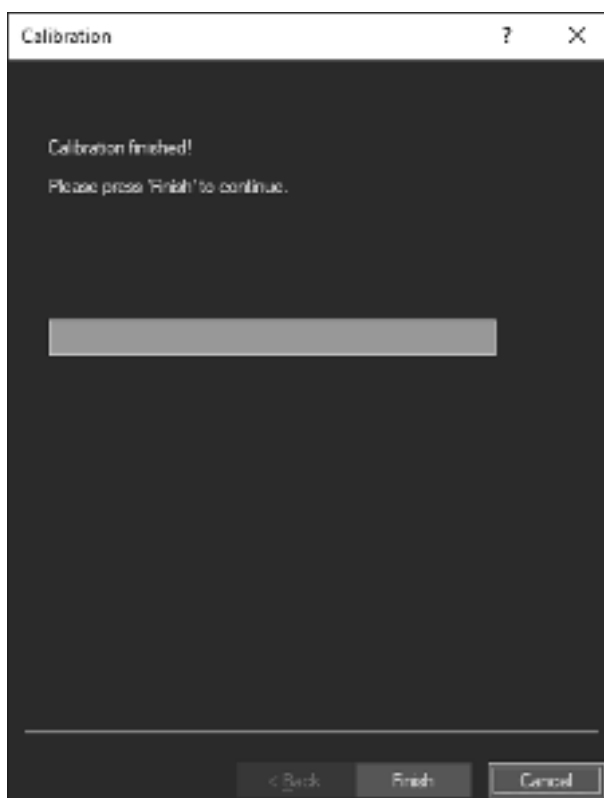
> **ATTENTION**
> In case you have calibrated an immersion medium objective as well clean the objective and remove immersion medium residues from the calibration slide. See Cleaning the immersion objective on page 226.

24.6 Magnification Test Scan

1. In the [Calibrations] dialog box select the [Magnification Test Scan] entry.