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

- 3 mm hex key with angle
- Standard Olympus 3 mm hex key

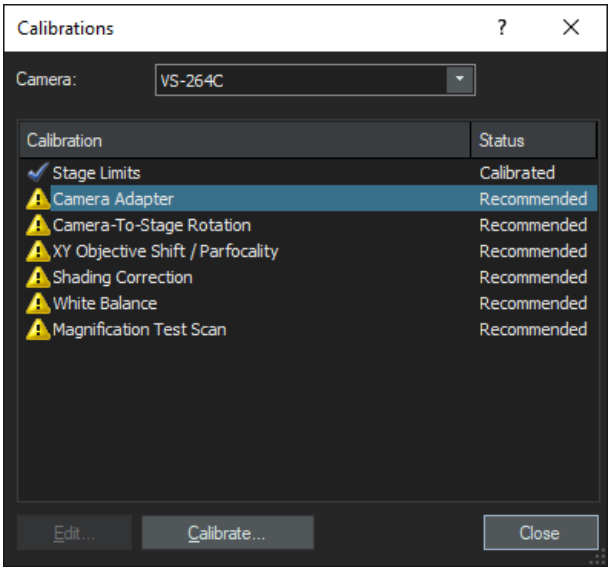
- The iDS color camera is mounted and calibrated ex-works.
  The parfocality might change due to temperature changes. Therefore do not check this calibration with a "cold" system (e.g. directly after assembling the system). Wait at least 30 min with the system switched on to heat it up.
- If you use objectives with a cover slip 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 standard for the focus distance is +/- 20µm. However the closer you get to zero the better.

Voraussetzungen

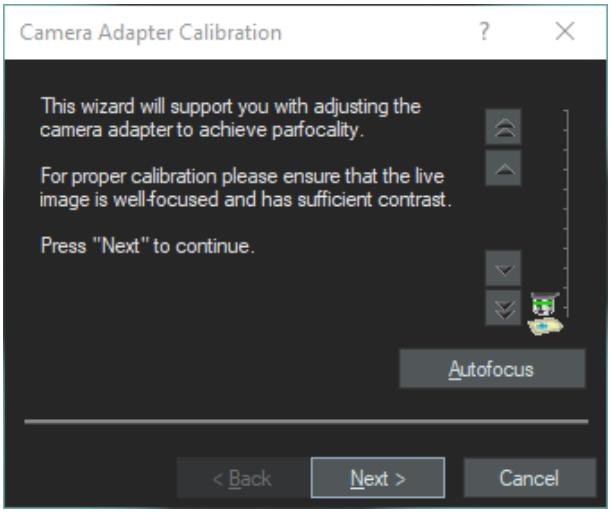
- ✓ In case your VS200 kit contains an objective with a cover slip correction collar ask the customer which coverslips they are using and note the thickness.
- ✓ Before you start with the camera adapter calibration make sure that the coverslip correction ring of the objective, for example 40x UPlanXApo, 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.

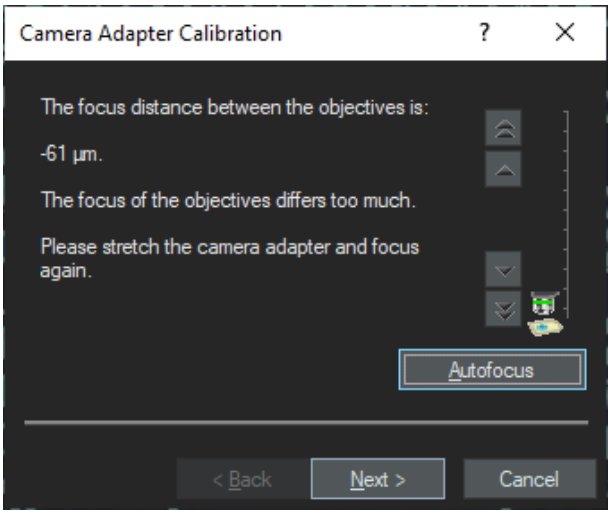


2. The system will use the objective with the highest magnification and switch to live mode. If the image is not in focus click the [Autofocus] button to perform an autofocus.

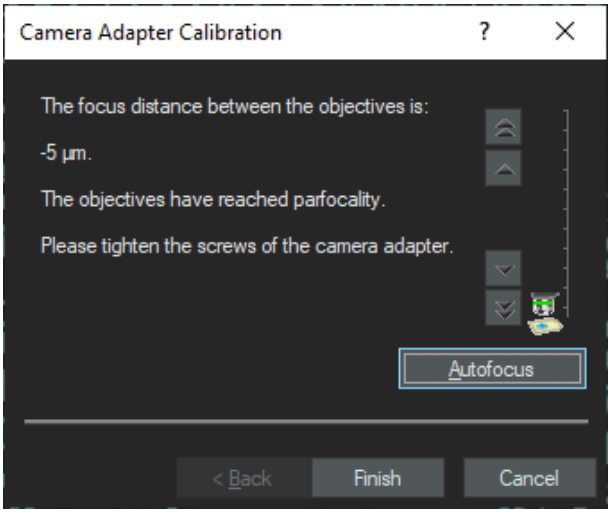


3. Select an area on the calibration slide were it is possible to focus with both the 40x and 2x objectives like in the screenshot below.

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 standard (+/- 20µm or better).

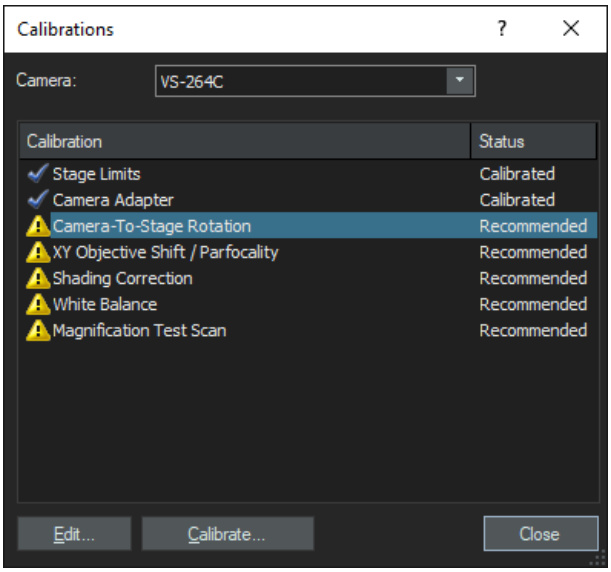


9. Click the [Finish] button to finalize the calibration process.

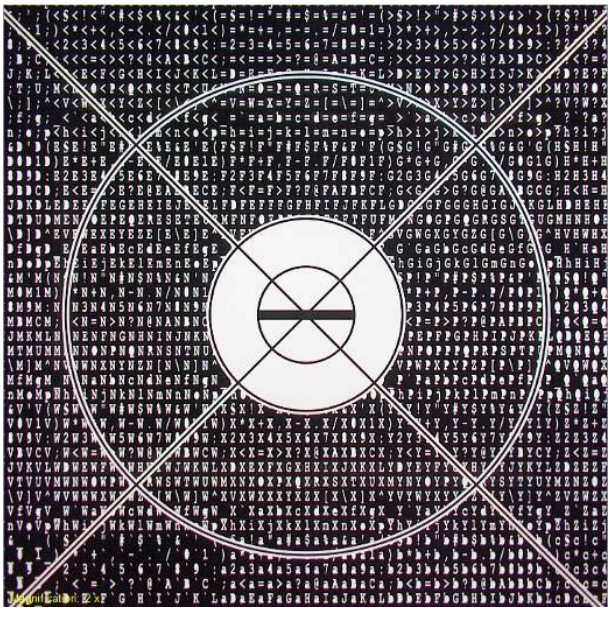
20.3 Camera-To-Stage Rotation

The standard for the camera-to-stage rotation is +/- 0.1°. However the closer you get to zero the better.

1. Select the [Camera-To-Stage Rotation] entry.

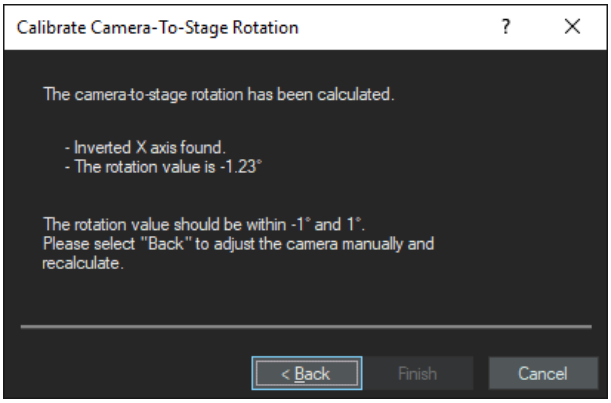


2. Click the [Calibrate] button to start the wizard.
3. Use the stage navigator to move to the center of the calibration slide as shown in the image below.



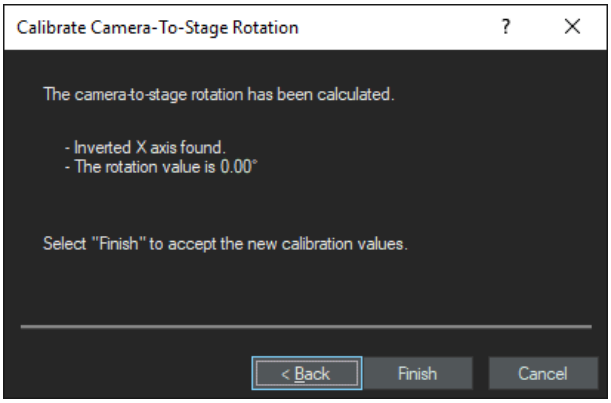
4. Select the 10x or 20x objective from the menu bar.

5. If the value is beyond the acceptable rotation angle, loosen the headless hex screw used to fix the TV 0.63 adapter at the flange of the beam splitter.



6. After a slight rotation, tighten the screw again.
   - » If the value is negative, tighten the screw counter clockwise.
   - » If the value is positive, tighten the screw clockwise.

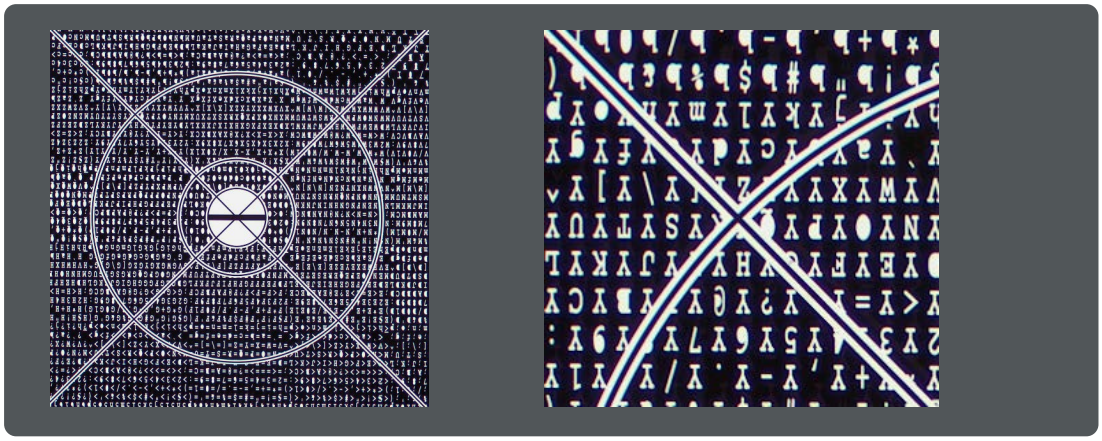
   Click the [Back] button in the window. Then click the [Autofocus] button in the next dialog box to perform the test again.
7. Adjust until the standard is met.



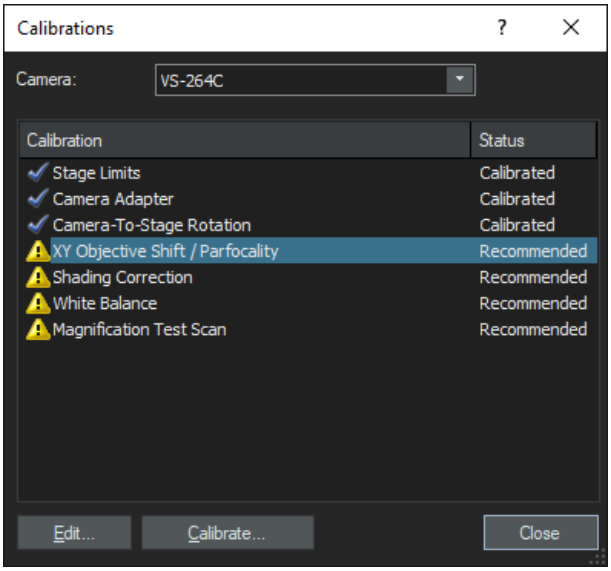
8. Finalize the process by clicking the [Finish] button.

20.4 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.

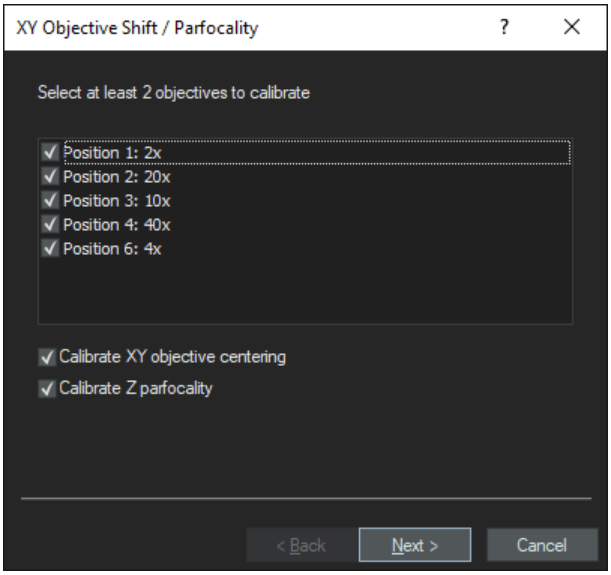


1. In the [Calibrations] dialog box select the [XY Objective Shift / Parfocality] entry.



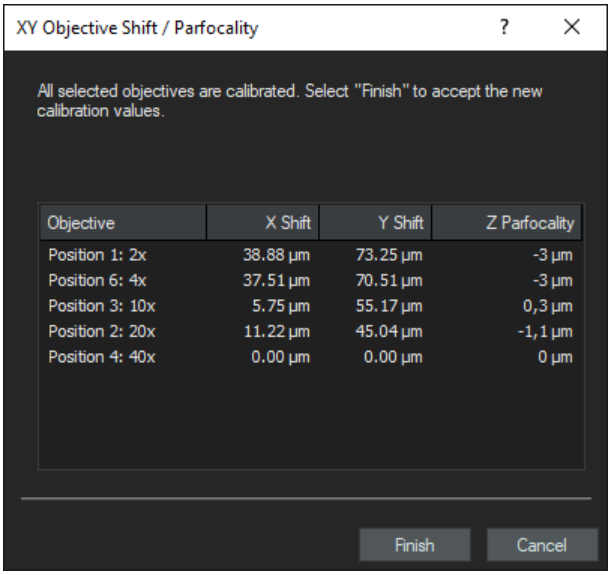
2. Click the [Calibrate] button to start the wizard.

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 objective should not exceed 20µm.

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

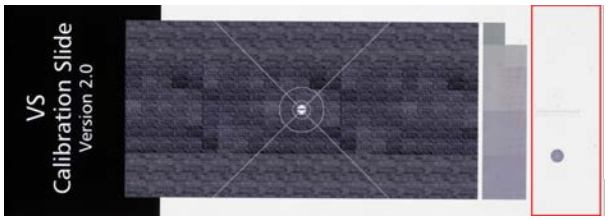
8. Finalize the calibration by clicking the [Finish] button.

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 Immersion Objectives auf Seite 38.

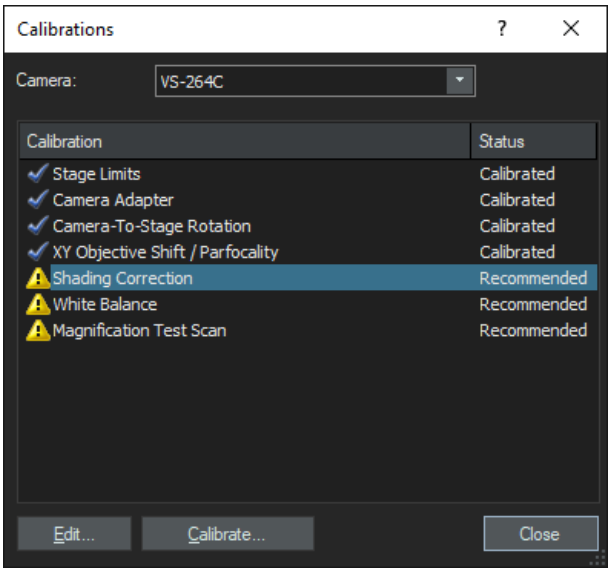
20.5 Shading Correction (Brightfield)

Use the stage navigator to move to the 'empty' area of the calibration slide.

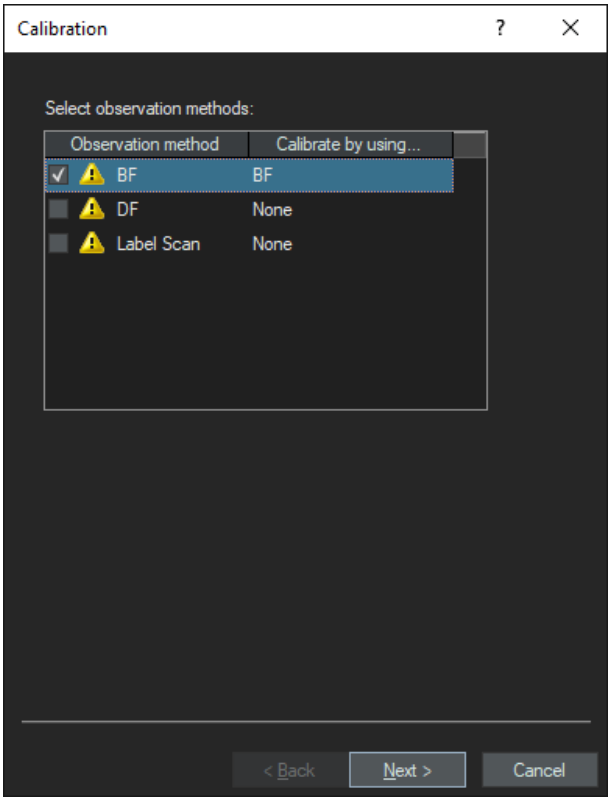


The slide must be very clean (free of dust particles which will disrupt the shading correction procedure).

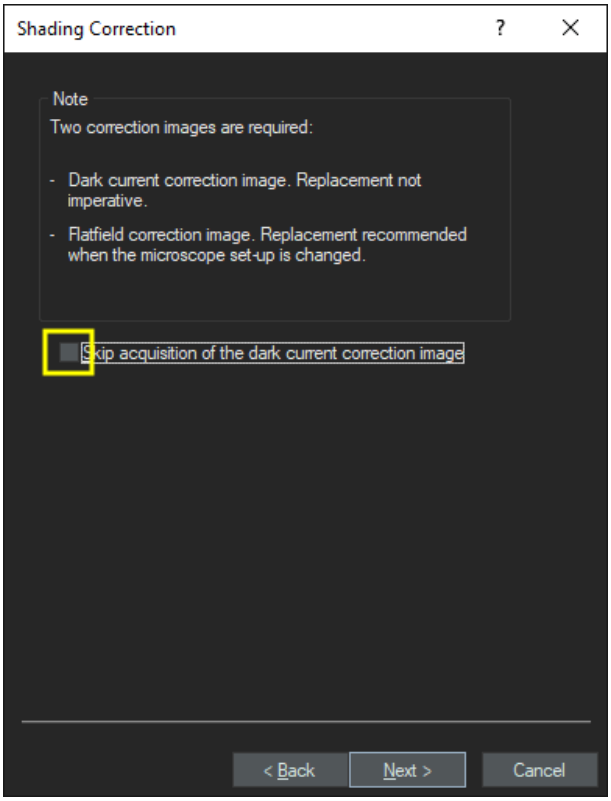
1. In the [Calibrations] dialog box select the [Shading Correction] entry and click the [Calibrate] button.



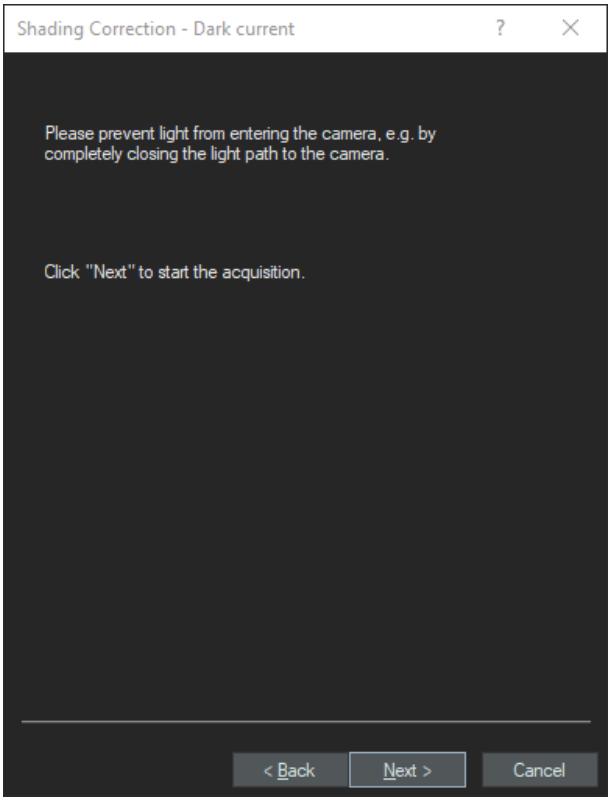
2. Select the [BF] entry as the observation method and proceed with [Next].



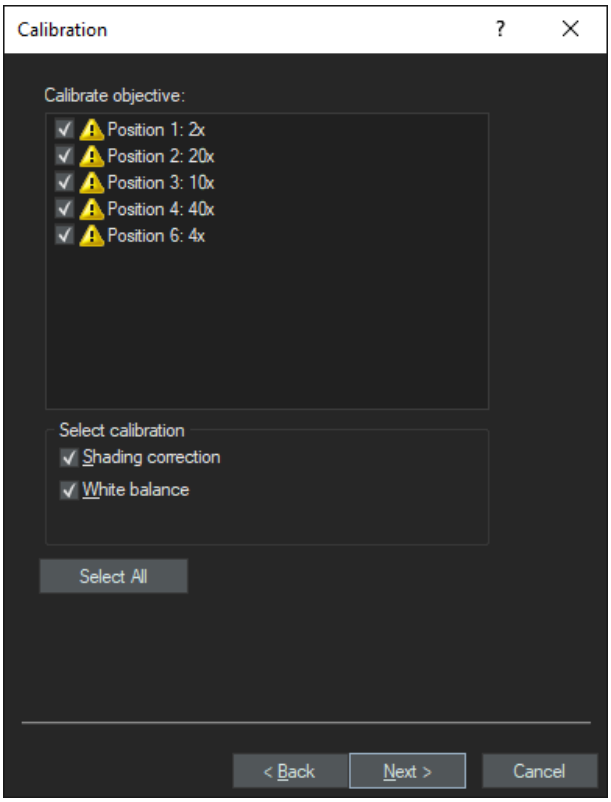
3. When you carry out the calibration for the first time do not skip the acquisition of the dark current correction image. For all future calibrations you can skip the acquisition of the dark current correction image.



4. Proceed with [Next].



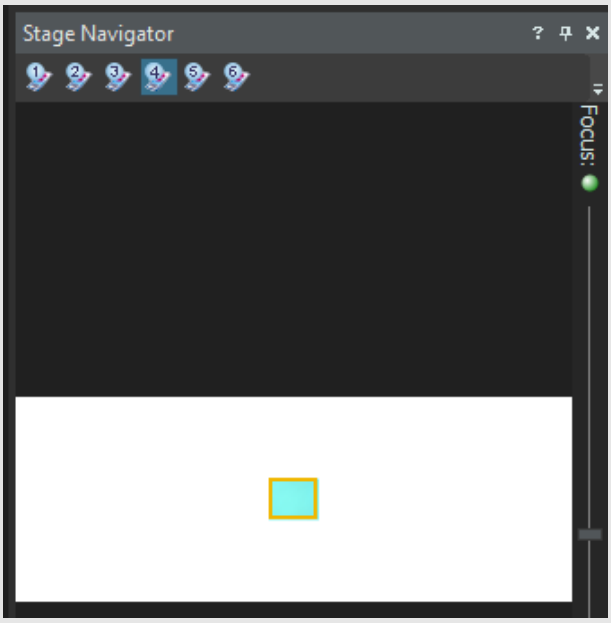
5. After the dark current correction image calibration, select all objectives. Immersion objectives are calibrated in the same wizard. However the calibration wizard will calibrate all dry objectives first and subsequently select the immersion medium objective(s).



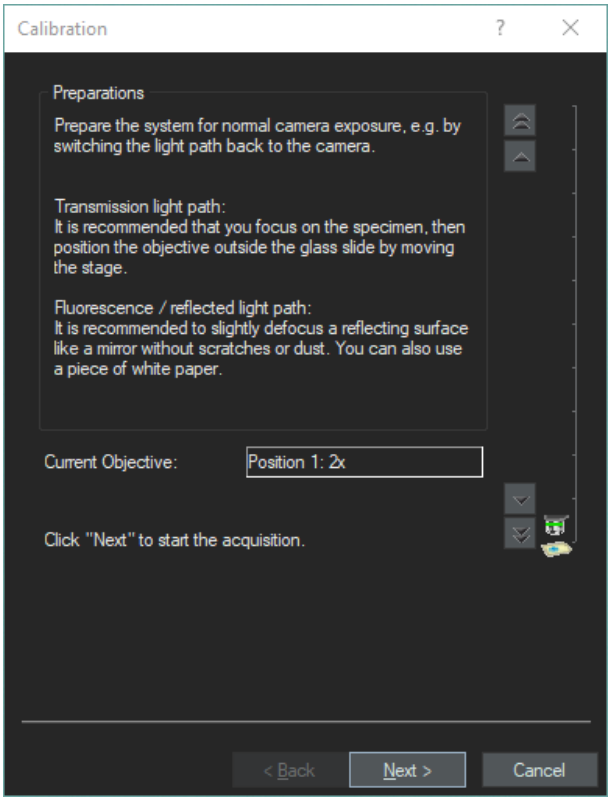
6. Make sure that the options for [Shading correction] and [White balance] are checked.

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.

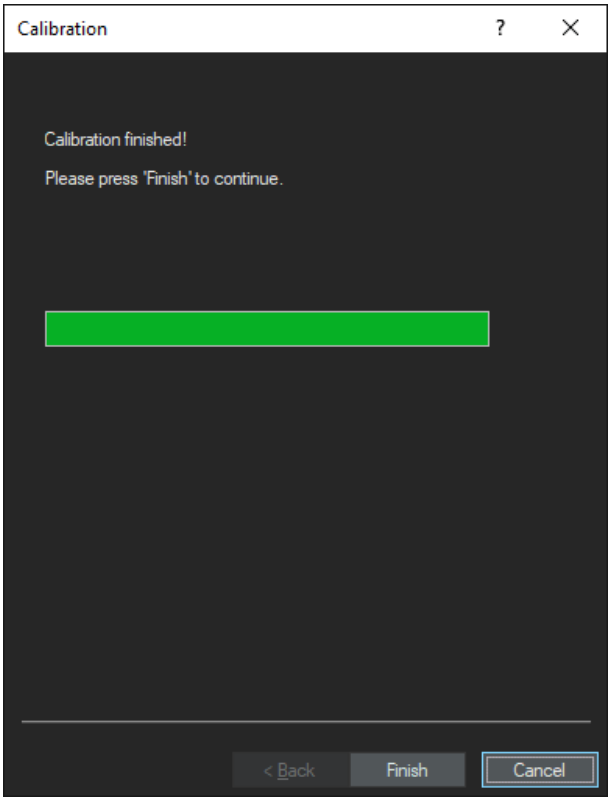


» After the acquisition is complete, the calibration process automatically moves to the next objective.

> The shading correction for objectives with a magnification equal to or higher than 10x should be done on a sample glass slide with cover slip. 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 Immersion Objectives auf Seite 38.

20.6 Shading correction of the label area

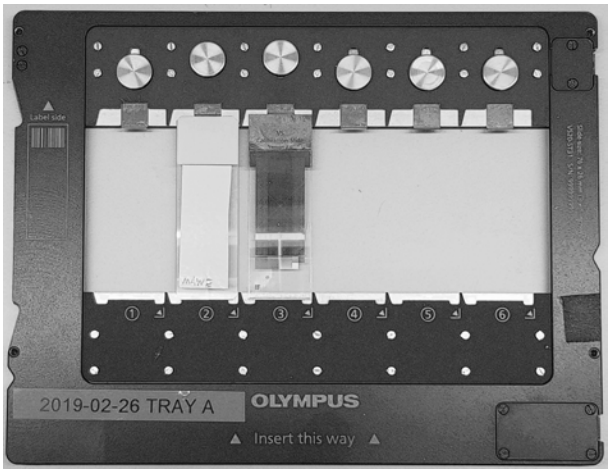
20.6.1 Preparation of calibration slide

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.

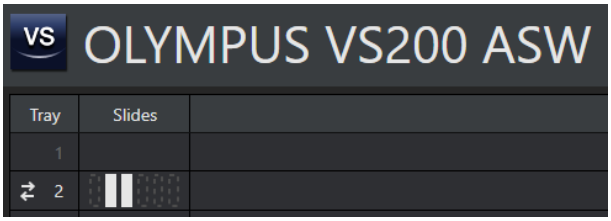
Example of a slide with an empty sticker

1. Insert the VS-calibration slide into position 3 of the slide tray and the prepared label-shading-correction slide in position 2.

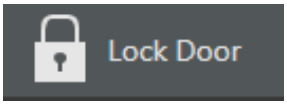


2. Insert the tray by clicking the [Exchange Trays] button on the start page of the VS200 ASW software.

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

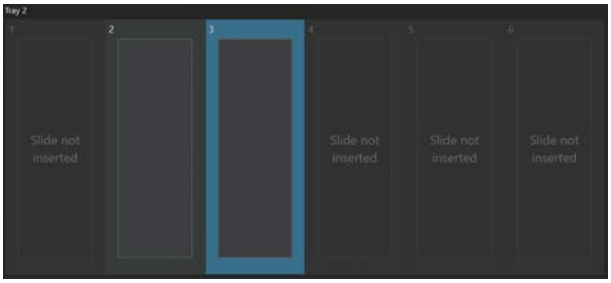


3. Click the [Lock Door] button in the VS200 ASW software to lock the door.

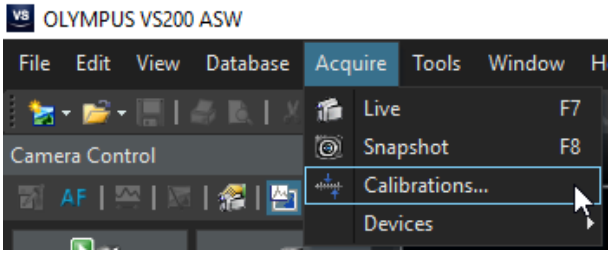


4. Click the [Select Slide for Calibration] button on the start page of the VS200 ASW software.

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



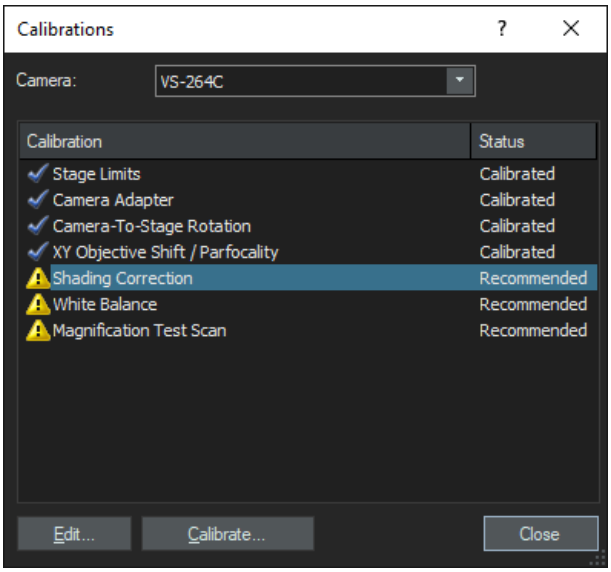
6. In the [View] layout select the [Acquire] > [Calibrations] command.



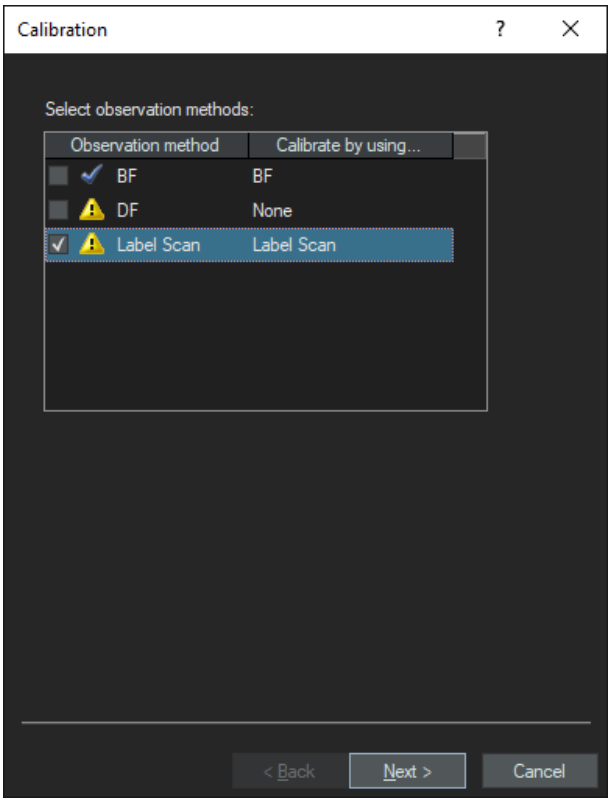
» The [Calibrations] dialog box opens.

Start the calibration

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

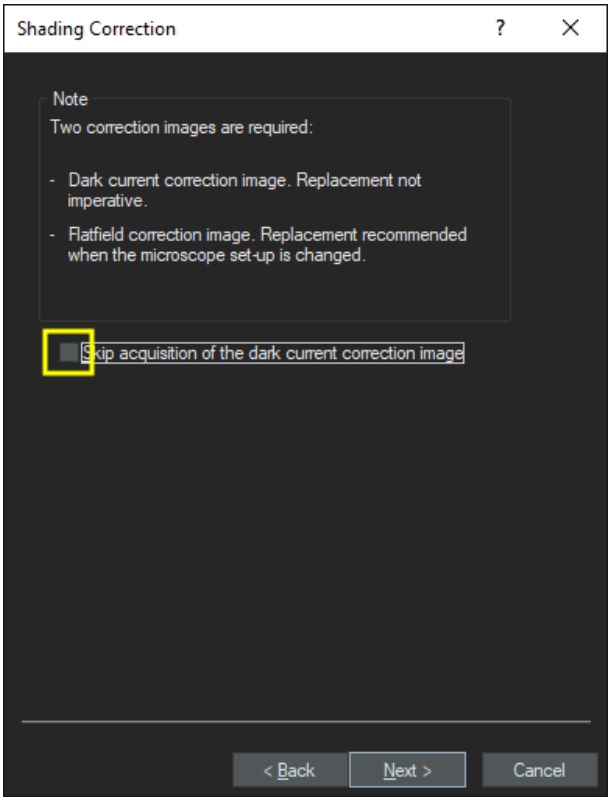


2. Select the [Label Scan] entry for the observation method and proceed with [Next].

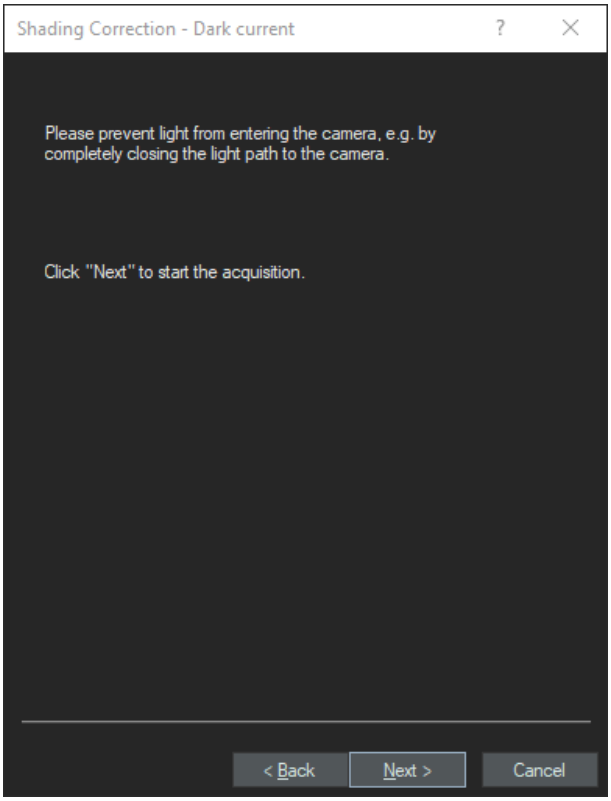


3. When you carry out the calibration for the first time do not skip the acquisition of the dark current correction image. For all future calibrations you can skip the acquisition of the dark current correction image.

   Proceed with [Next].

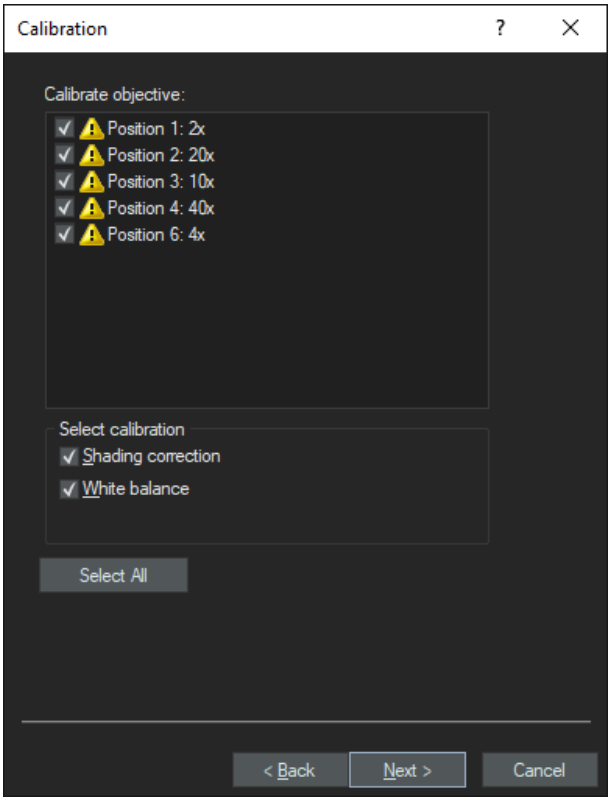


4. Proceed with [Next] to start the acquisition process



5. Proceed with [Next].

6. After the dark current image calibration select all non-immersion objectives. Immersion objectives shall be calibrated separately. Proceed with [Next].

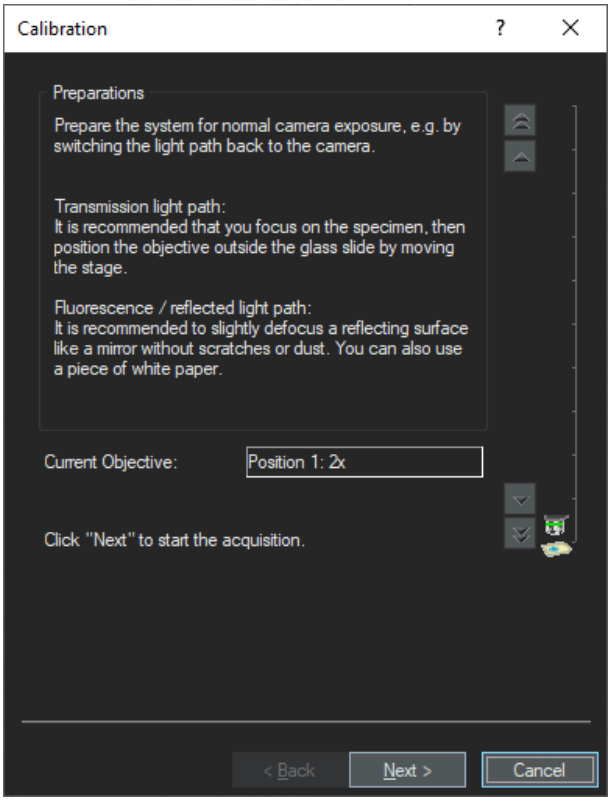


> IMPORTANT
>
> Actually, the label shading correction must only be performed for the 2x objective. However you should do it for all other objectives as well as otherwise there will always be an exclamation mark next to the entry.

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.

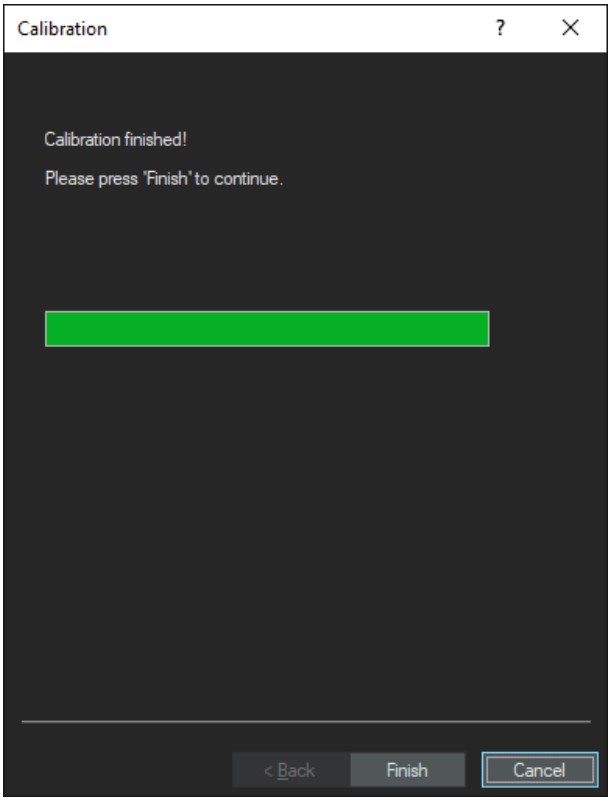
9. Focus on the empty label sticker and click the [Next] button to proceed. Use the focus up and down buttons like shown in the image below.



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

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

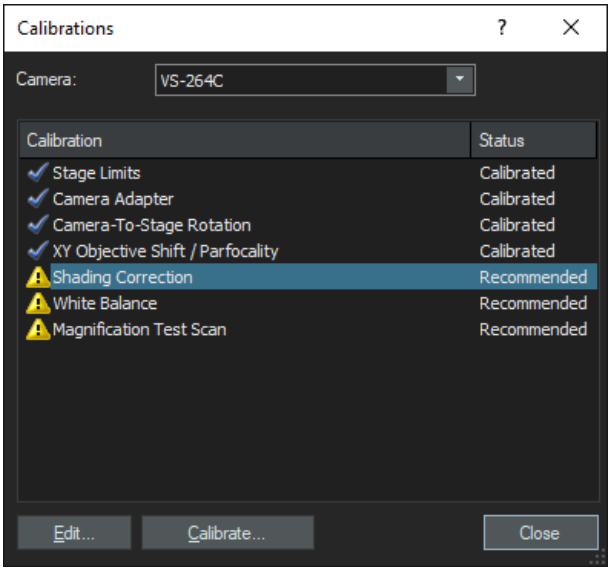
11. Click the [Finish] button to finalize the calibration process.



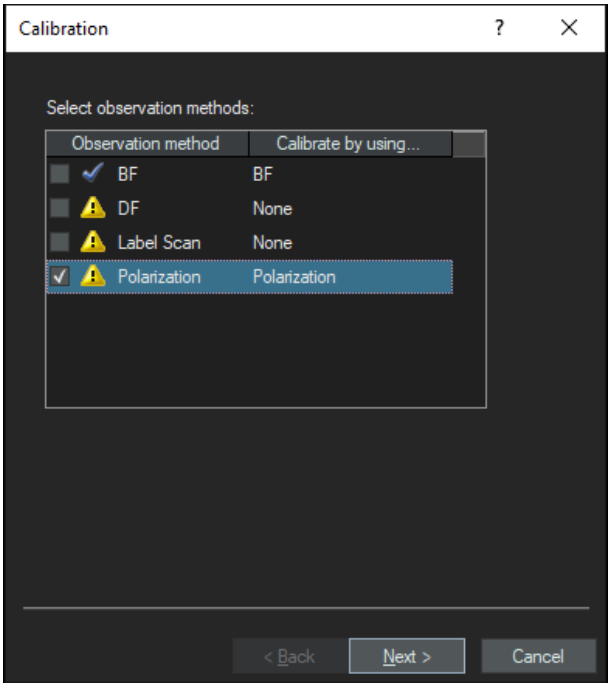
20.6.2 Shading correction for polarization (Pol)

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 auf Seite 94. If you change the angle you subsequently have to redo the shading correction.

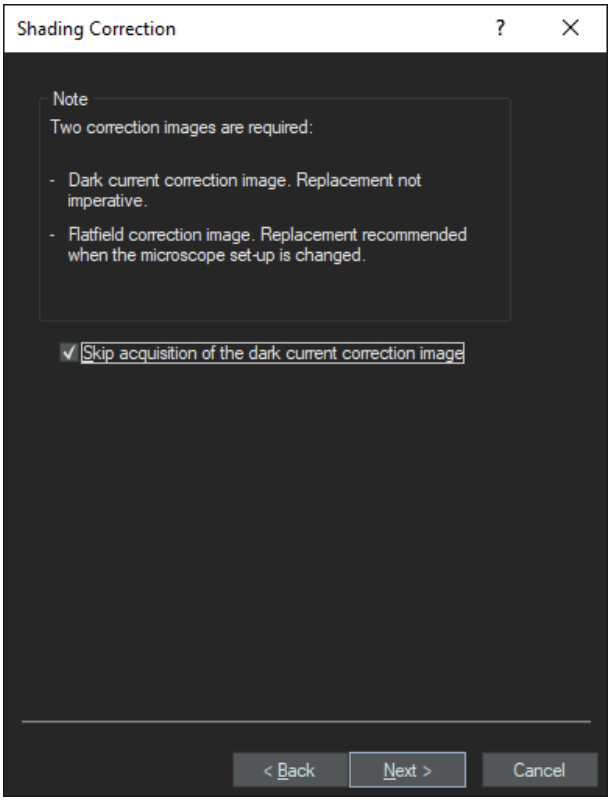
1. In the [Calibrations] dialog box select the [Shading Correction] entry and click the [Calibrate] button.



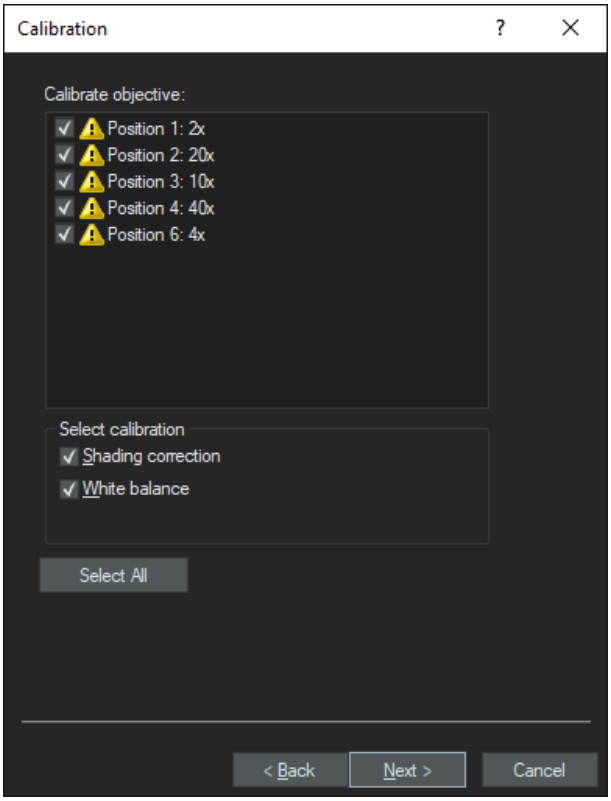
2. Select the [Polarization] observation method.



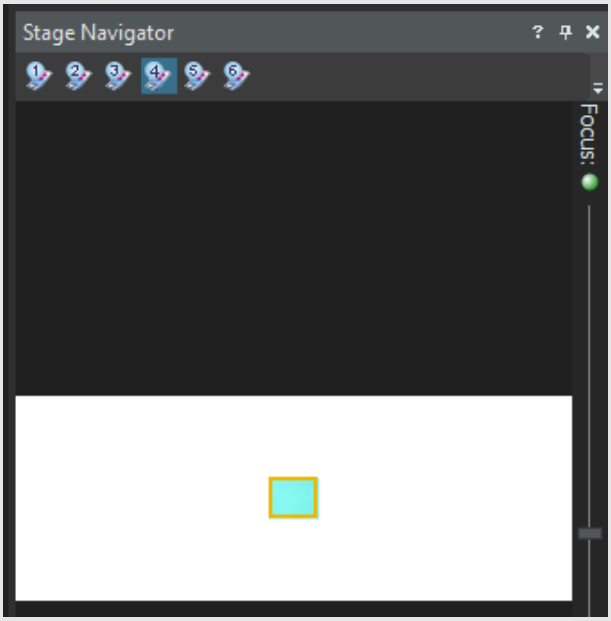
3. Skip the acquisition of the dark current correction image.



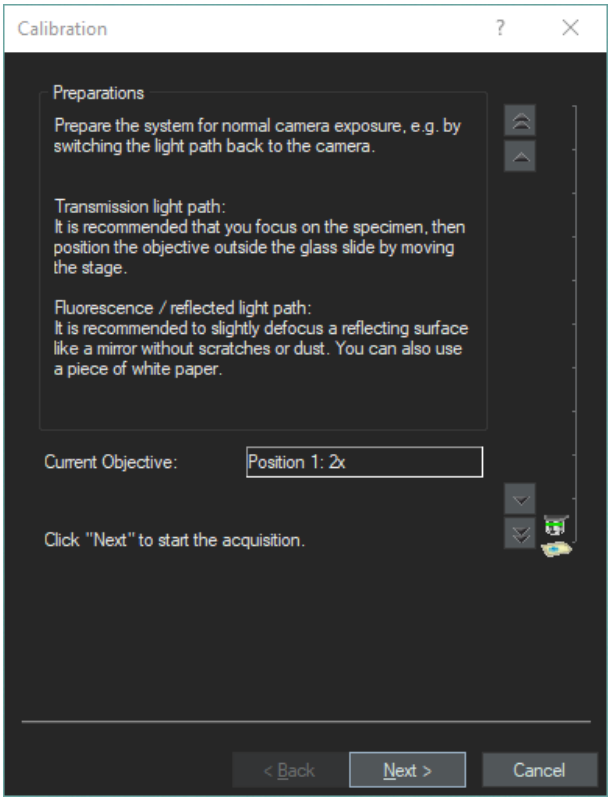
4. Select all objectives.



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.
>
> 
>

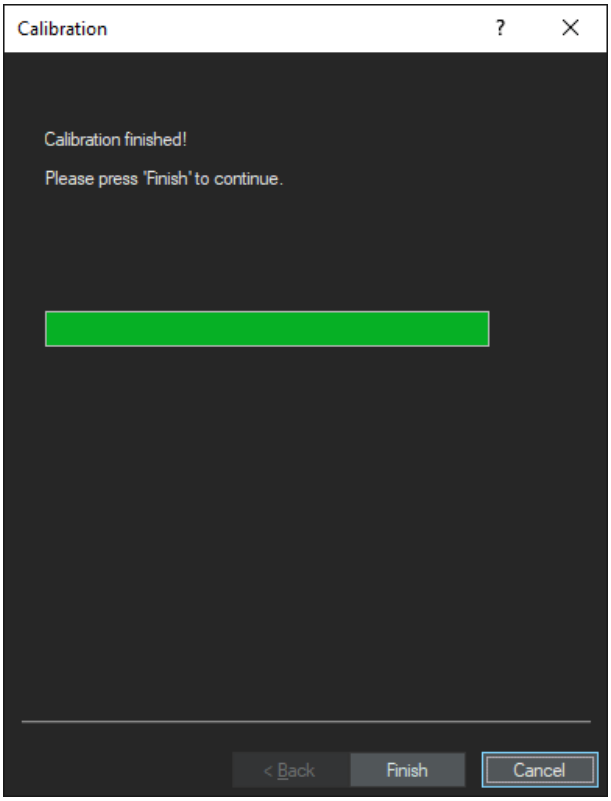
6. Click the [Next] button to start the image acquisition process for the 2x objective.



» 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 cover slip. 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.

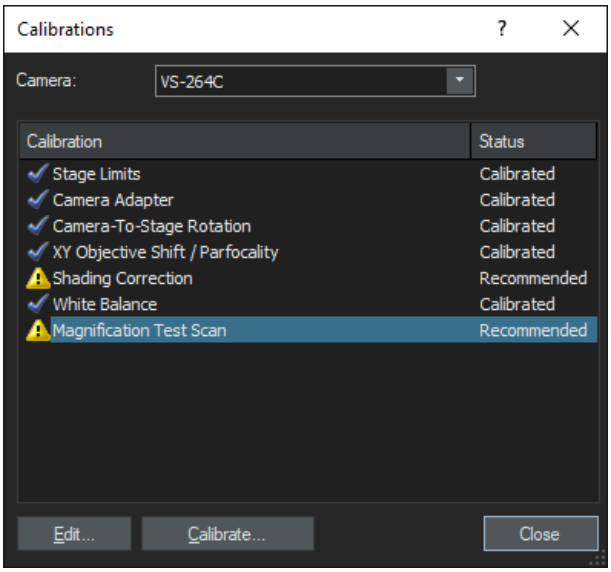


ATTENTION

In case you have calibrated an immersion medium objective as well clean the objective and remove immersion medium residues from the calibration slide.
See Immersion Objectives auf Seite 38.

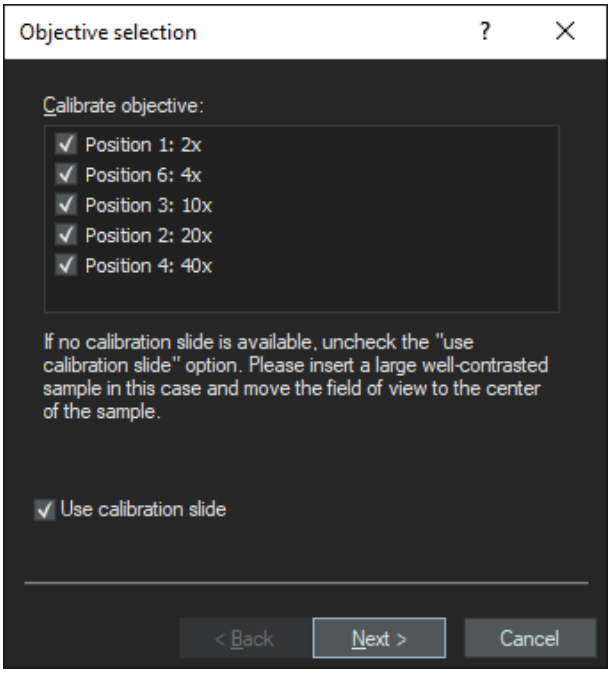
20.7 Magnification Test Scan

1. In the [Calibrations] dialog box select the [Magnification Test Scan] entry and click the [Calibrate] button.

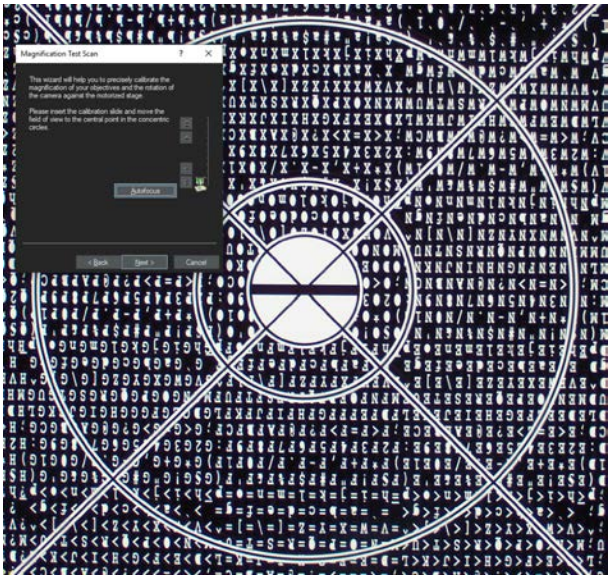


2. Now you can select whether you want to perform the magnification test scan for all objectives or only for certain ones. Immersion medium objectives are calibrated in the same wizard. However the calibration wizard will calibrate all dry objectives first and subsequently select the immersion medium objective(s).

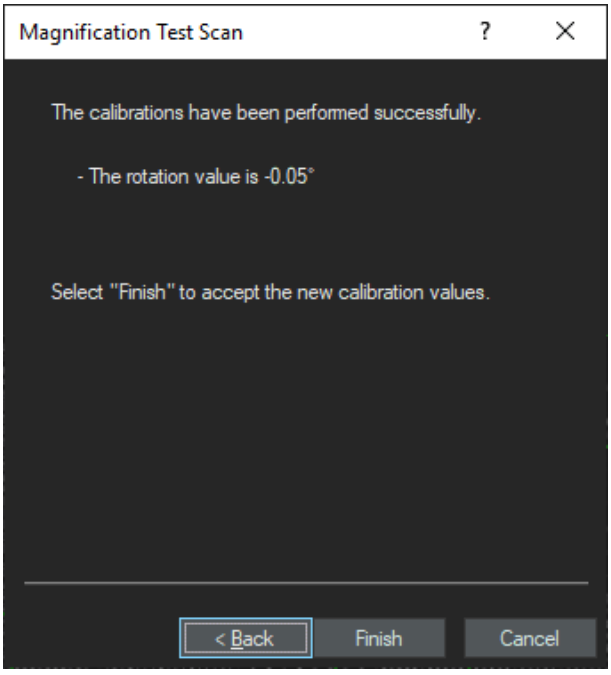
   Select the [Use calibration slide] check box.



3. Use the stage navigator to move to center (cross hair) of slide and auto-focus.



4. Proceed with [Next].
5. Click the [Finish] button to finalize the calibration process.

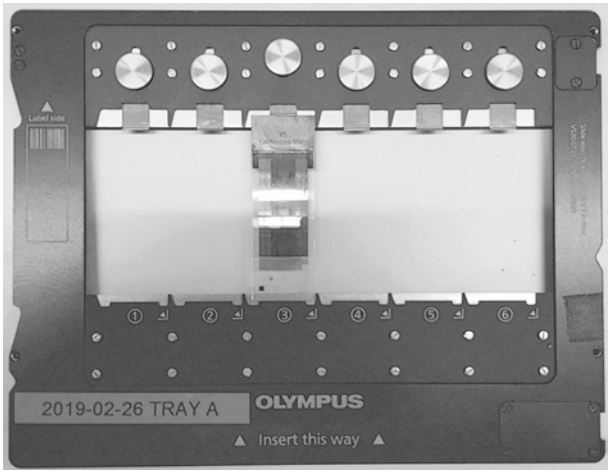


In case the calibration value is not within the standard go back to the [Camera-To-Stage Rotation] calibration and redo all calibrations.

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 Immersion Objectives auf Seite 38.

21 Additional calibrations for a fluorescence system

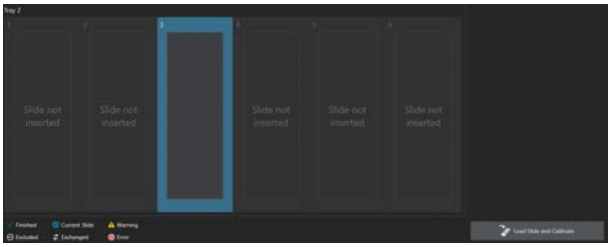
1. Insert the VS-calibration slide into position 3 of the slide tray.



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.

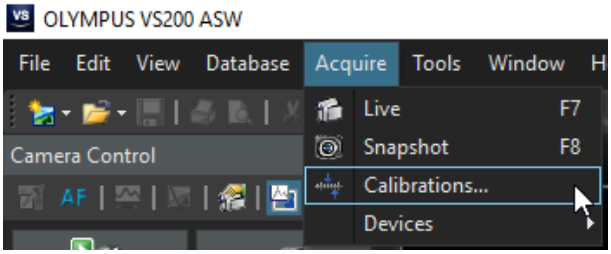
4. Load the tray (either manually or using the loader) by clicking the [Load Slide and Calibrate] button.



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

5. In the [Manual control] layout select the [Acquire] > [Calibrations] com-

mand.



21.1 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 min with the system switched on to heat it up.
- If you are using objectives with a cover slip correction collar, it is recommended to adjust it prior to all subsequent calibrations.
- The limit for the focus distance is +/- 20µm. However the closer you get to zero the better.

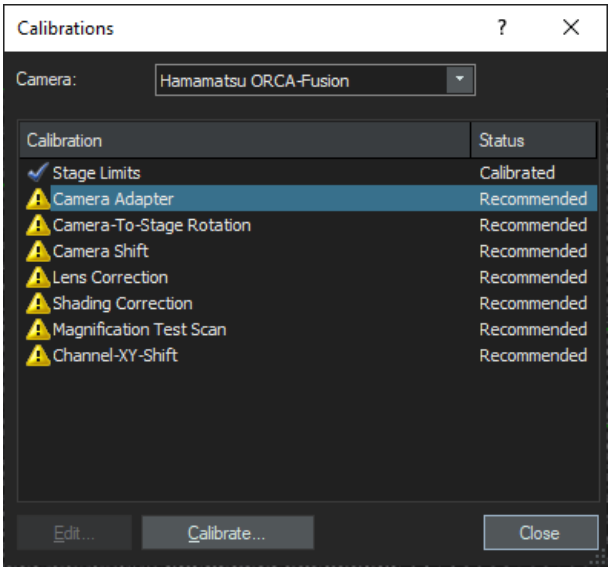
21.1.1 Camera adapter U-FFWO T3

This example describes the calibration for an ORCA Flash 4.0. The calibration for the VS-304M as well as the ORCA Fusion VS200 can be done in the same way.

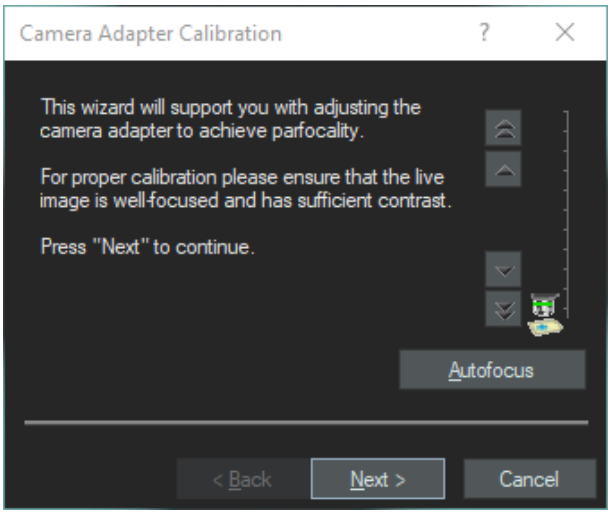
- Small hex key to lock the tubus
- Hex key to open the filter wheel
- Small spanner to adjust the height of the tubus
- Spanner to fix the tubus

The standard for the focus distance is +/- 20 µm. However the closer you get to zero the better.

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

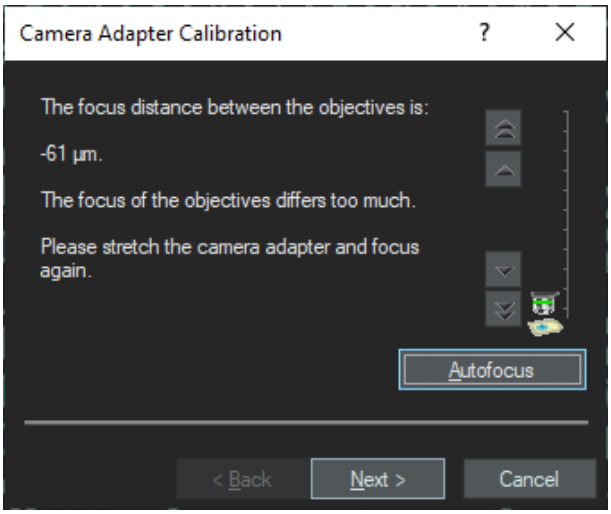


2. Use [Ctrl] + mouse wheel to fine focus.



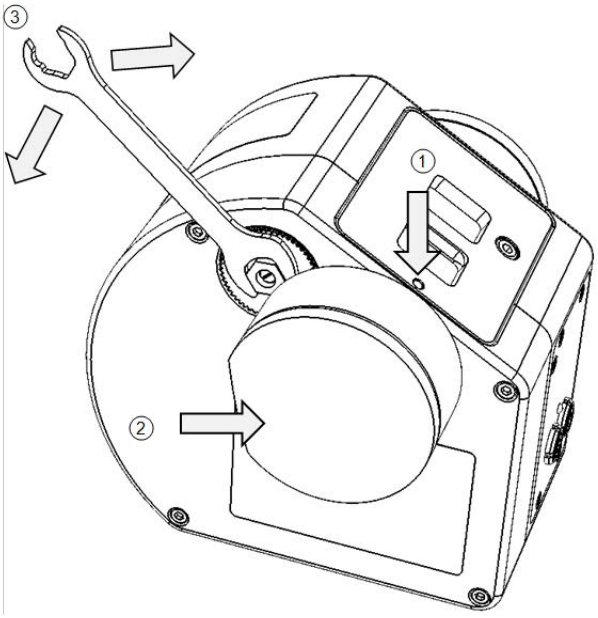
3. Use the stage navigator to move to a similar position on the calibration slides to that shown in the image below.

4. Perform an autofocus.
5. If the focus differs too much you need to manually adjust the U-FFWO T3 adapter.



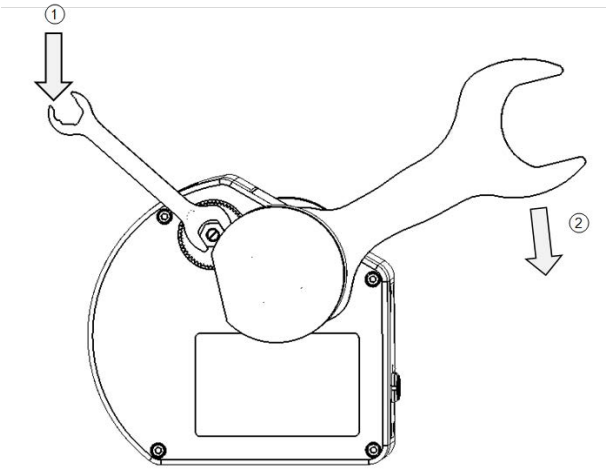
Adjust the camera adapter

1. Release the camera rotation locking screw. Hold the camera with one hand to prevent camera rotation. Use the small wrench to adjust parfocality of the camera until the image on the screen is in focus.



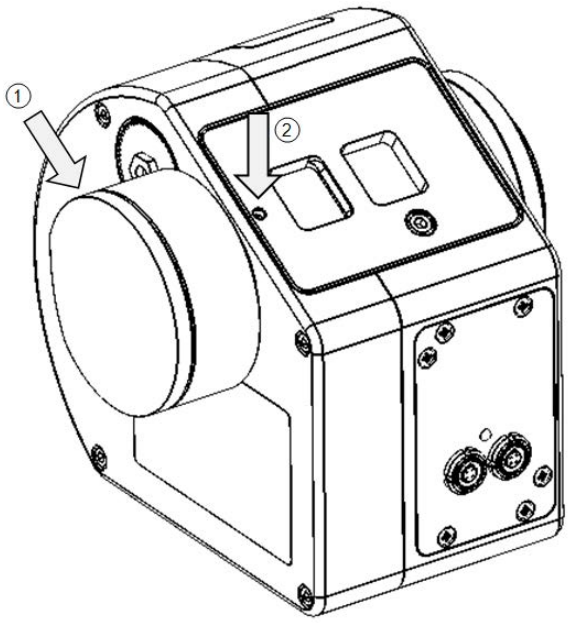
(1)	Release locking screw.
(2)	Keep hold of camera to prevent rotation.
(3)	Adjust parfocality.

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.



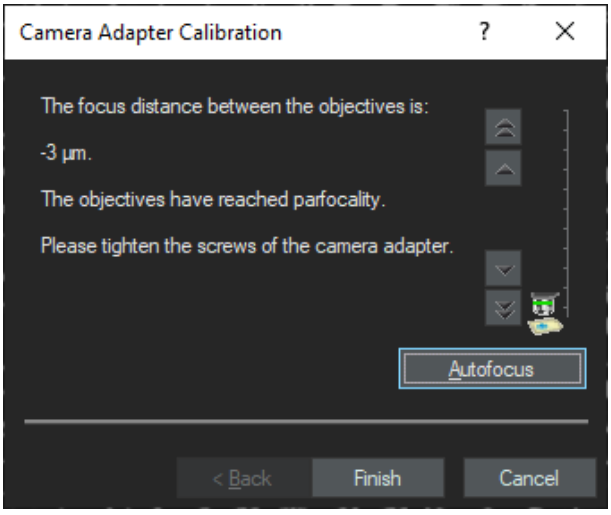
(1)	Hold steady.
(2)	Fasten clockwise.

3. Adjust the camera rotation by hand and tighten the camera rotation locking screw.



(1)	Rotate camera.
(2)	Tighten locking screw.

4. Execute an autofocus again and check the final result.

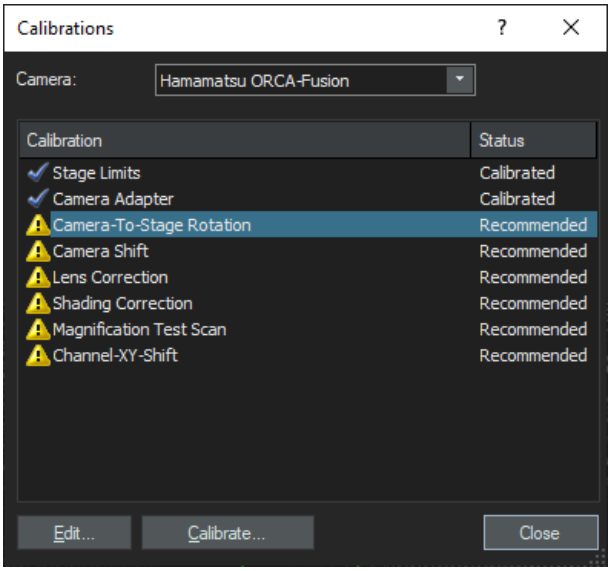


5. Repeat until the rotation value meets the standard.
6. Finalize the process by clicking the [Finish] button.

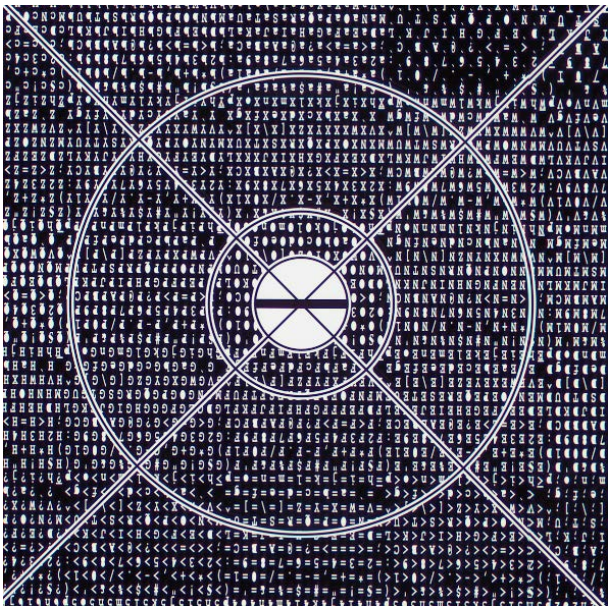
21.2 Camera-To-Stage Rotation

The standard for the camera-to-stage rotation is +/- 0.1°. However the closer you get to zero the better.

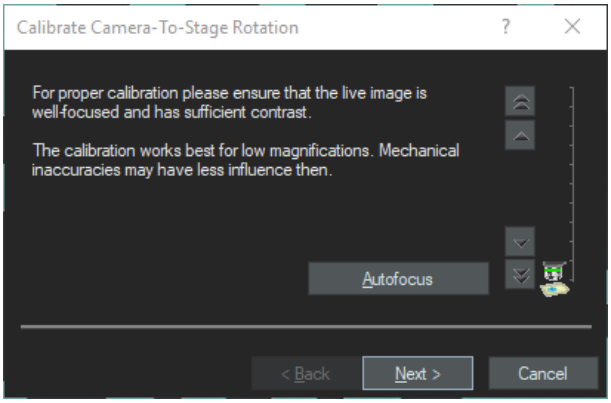
1. In the [Calibrations] dialog box select the [Camera-To-Stage Rotation] entry and click the [Calibrate] button.



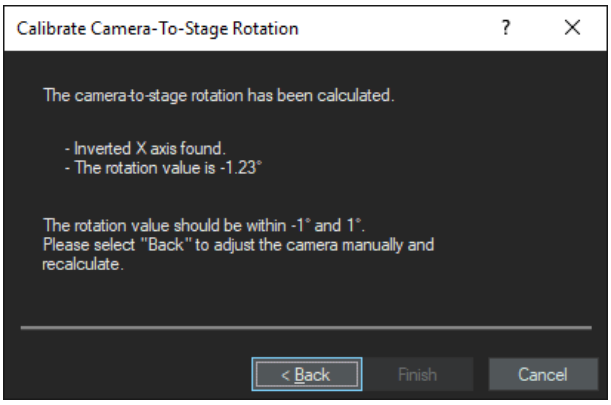
2. Use the stage navigator to move to the center of the calibration slide.



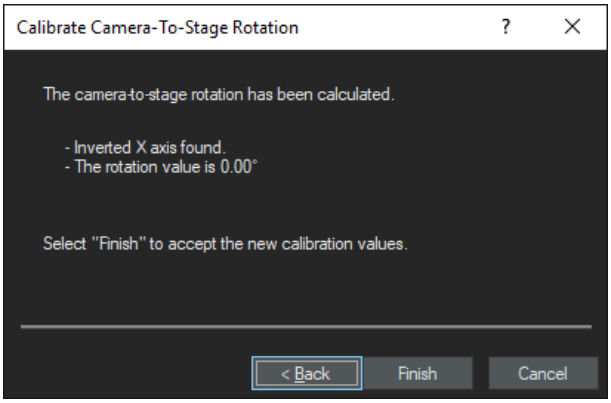
3. Select the 10x objective from the menu bar and perform an autofocus.



4. If the value is beyond the acceptable limits, loosen the screws in the beam splitter flange to rotate the U-FFWO together with the camera.



5. After a slight rotation, tighten the screws again and click the [Back] button in the dialog box.
6. Then click the [Autofocus] button in the next dialog box to perform the test again.
7. Repeat until the value meets the standard.



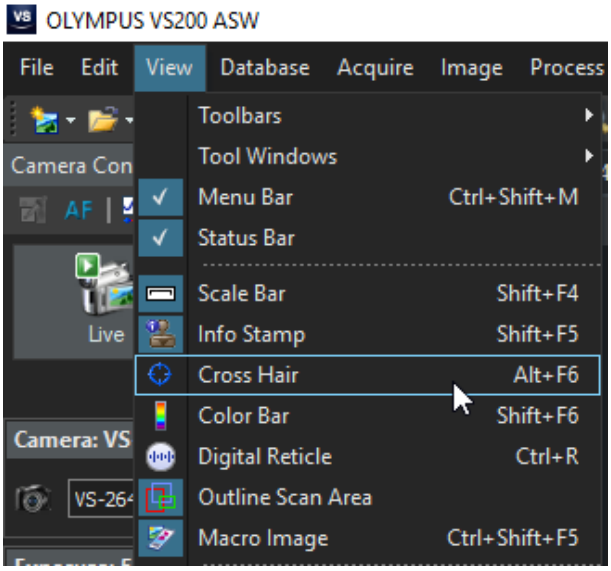
8. Finalize the process by clicking the [Finish] button.

21.3 Camera shift

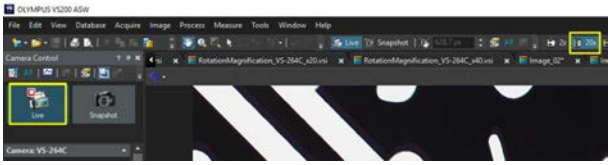
The camera shift calibration corrects the shift between the brightfield and monochrome cameras. It is a software-based correction.

Measure the camera shift between the two cameras

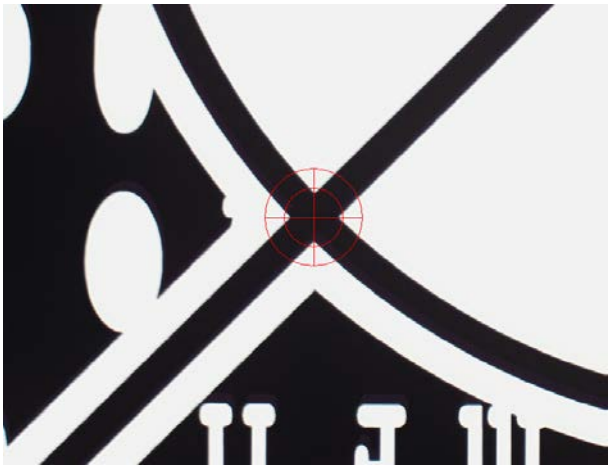
1. Open the [Manual control] layout.
2. Display the cross hair by selecting the [Cross Hair] entry in the [View] menu.



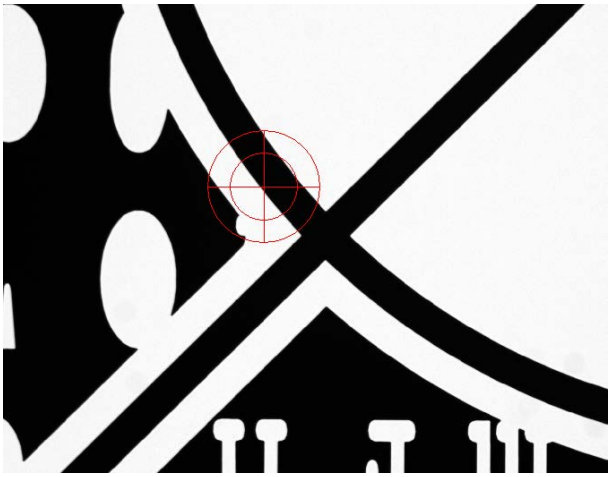
3. Select the 20x objective.
4. Switch to live mode with the BF observation method selected.



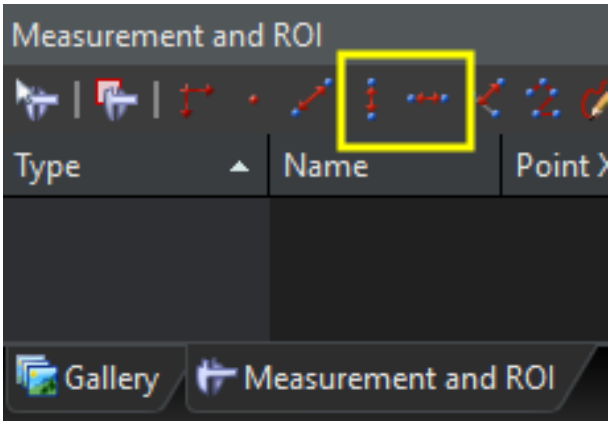
5. Use the stage navigator to move the stage so that the cross of the calibration slide is in the middle of the cross hair.



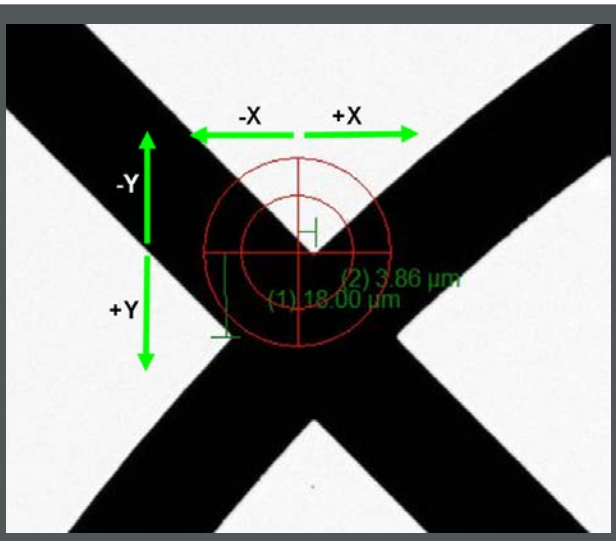
6. Fine focus with [Ctrl] + mouse wheel.
7. Switch off live mode and change to the BFMono observation method.
8. Switch on live mode again.
   » You might recognize a shift.



9. Switch to the [View] layout, go to the [Measurement and ROI] tab and select either the [Vertical Line] button or the [Horizontal Line] button.



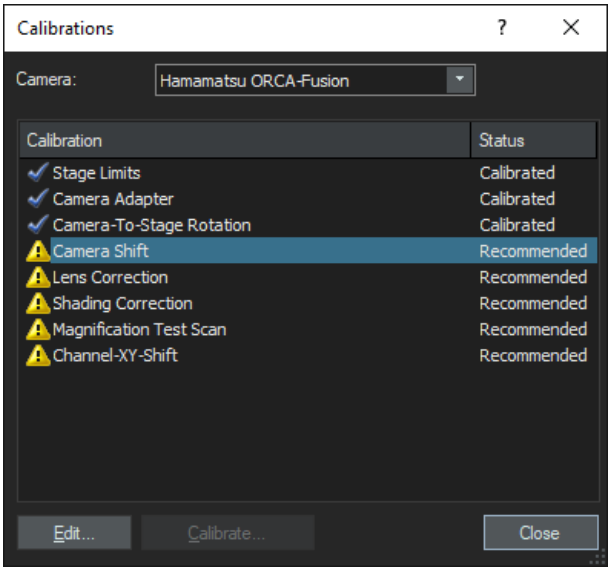
10. Measure the shift between the cross hair and the center structure and note the X and Y values.



In this case the monochrome camera is shifted up an to the left. To adjust the shift the cross hair needs to be 'shifted' to the right (+X) and down (+Y).

11. Remember these values (+3.86µm in X and +18.00µm in Y) to enter them in the [Camera Shift] dialog box in the following steps.

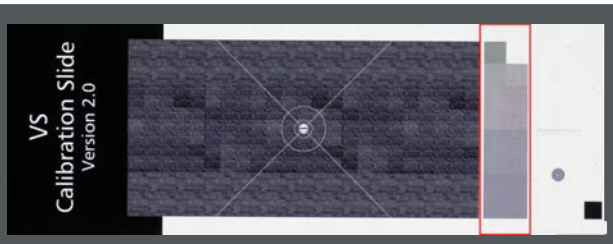
12. In the [Calibrations] dialog box select the [Camera Shift] entry and click the [Edit] button.



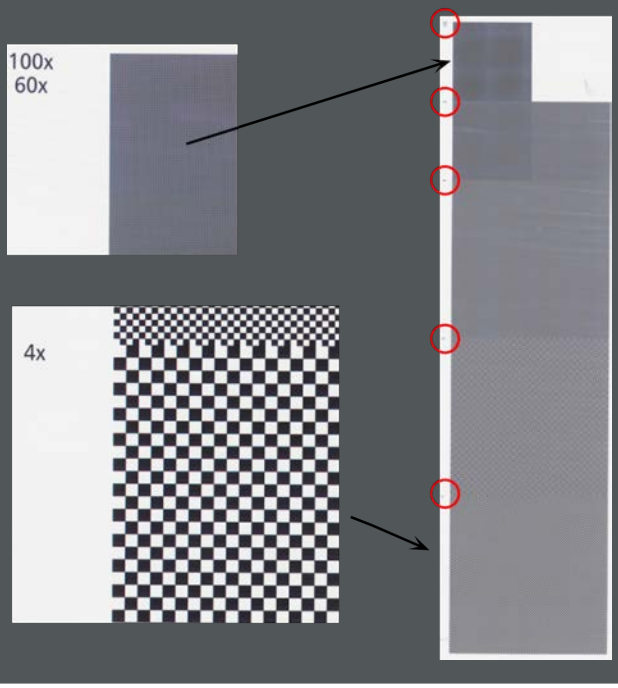
13. In the [Camera Shift] dialog box be sure to select the correct objective (20x) and enter the values you measured manually to align the two cameras being used.

14. Click the [OK] button to finish the calibration process.

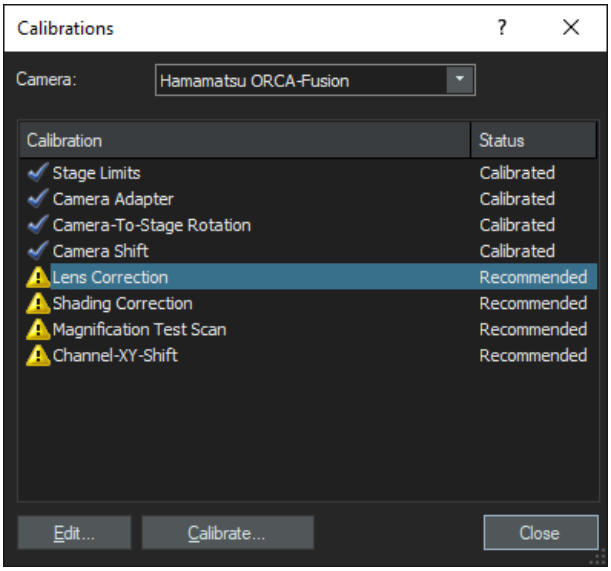
21.4 Lens Correction



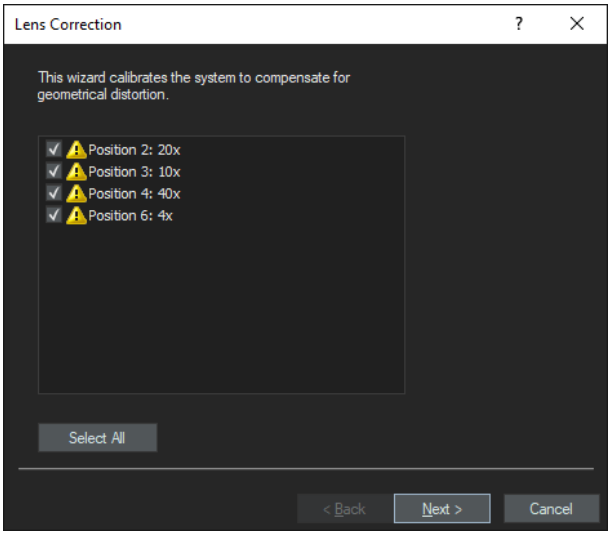
On the calibration slide opposite the label area you find an area with checkerboard grids in different sizes. Next to each grid there is a number (red circle in the example image) for the objective magnification from 4x to 60x/100x which has to be used for the calibration.



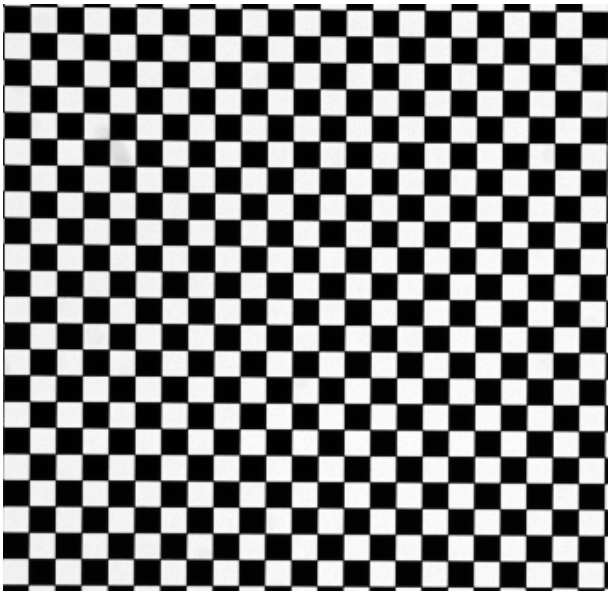
1. In the [Calibrations] dialog box select the [Lens Correction] entry and click the [Calibrate] button.



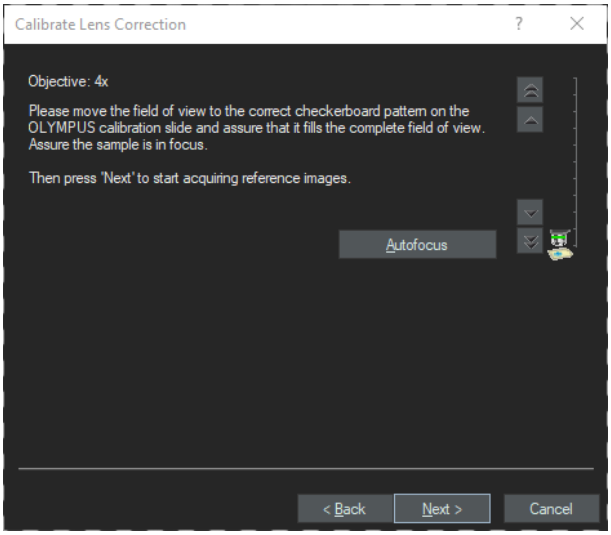
2. Select all available objectives. Immersion are calibrated in the same wizard. However the calibration wizard will calibrate all dry objectives first and subsequently select the immersion medium objective(s).



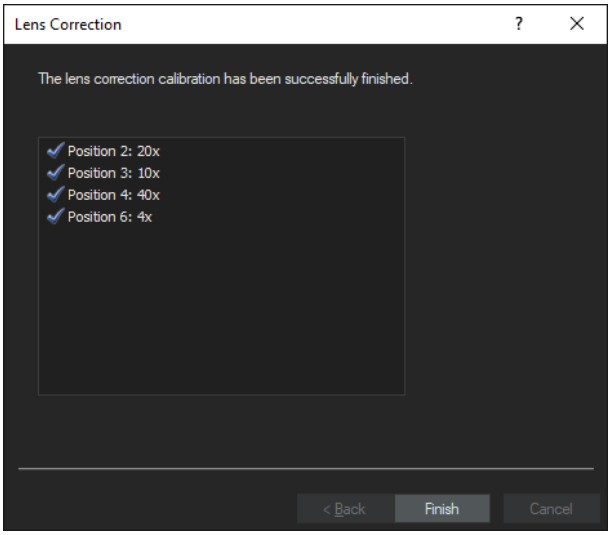
3. Use the stage navigator to move the VS calibration slide to the correct checkerboard area.



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

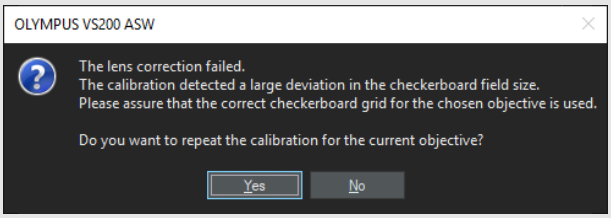


5. Do the same for all other objectives.



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

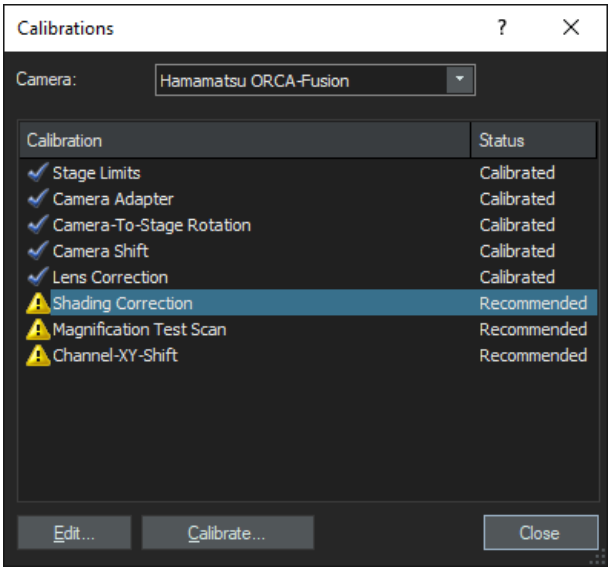
If you see the message below please check if you are doing the calibration on the correct checkerboard area.



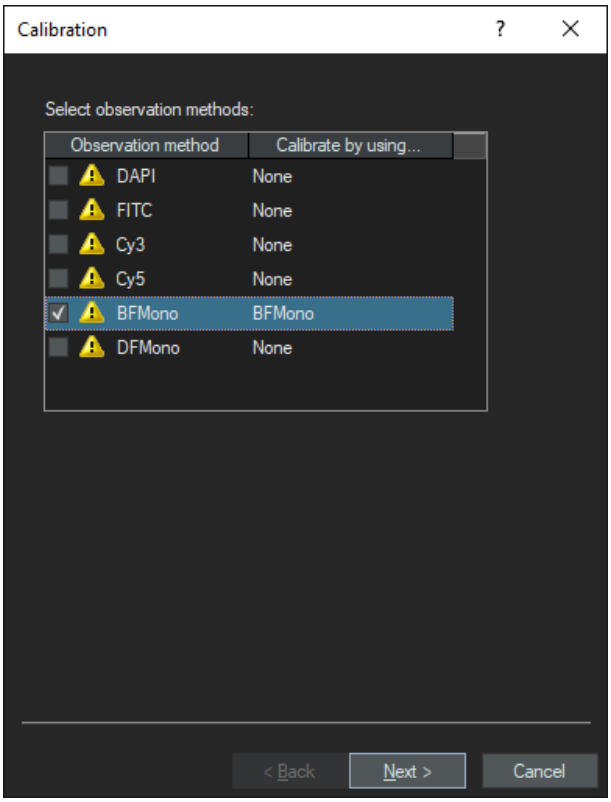
ATTENTION
In case you have calibrated an immersion medium objective as well clean the objective and remove immersion medium residues from the calibration slide.
See Immersion Objectives auf Seite 38.

21.5 Shading correction BFMono

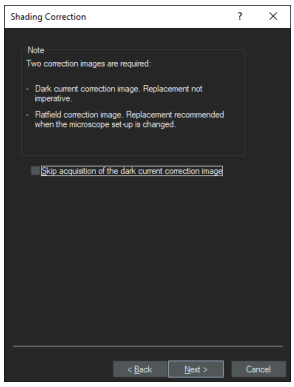
1. In the [Calibrations] dialog box select the [Shading Correction] entry and click the [Calibrate] button.



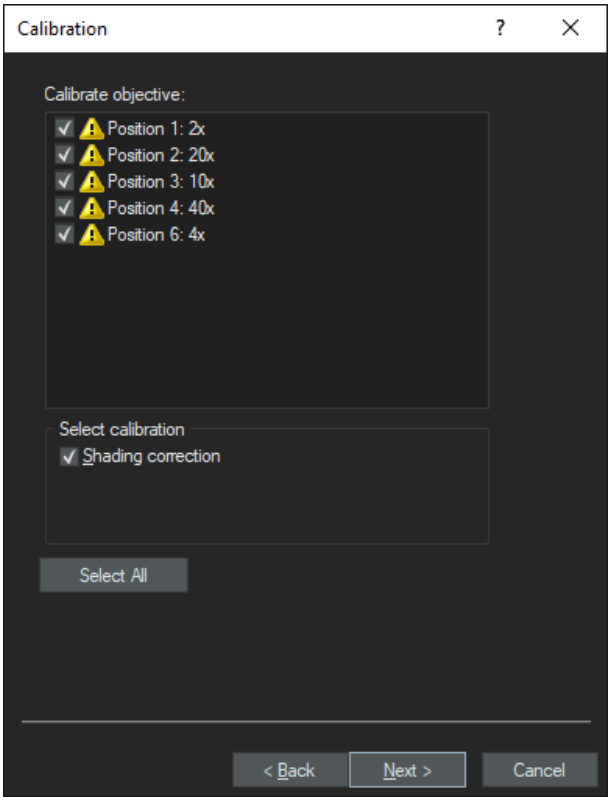
2. Select the [BF Mono] observation method and proceed with [Next].



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.



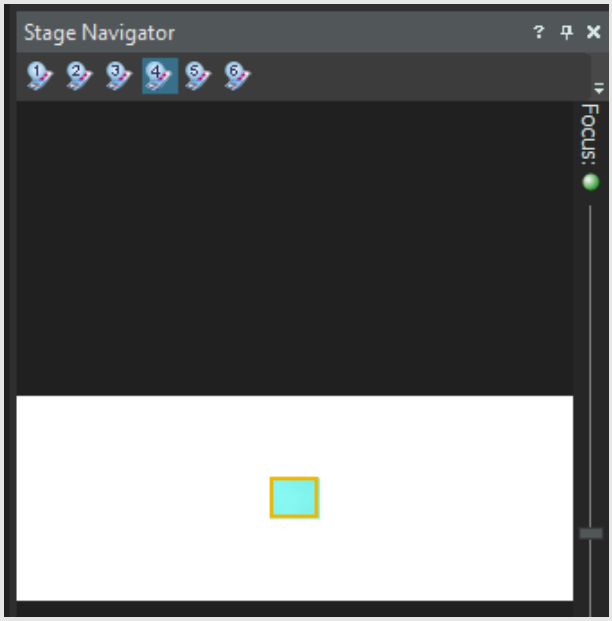
4. After the dark current image calibration select all objectives. Immersion objectives are calibrated in the same wizard. However the calibration wizard will calibrate all dry objectives first and subsequently select the immersion medium objective(s).



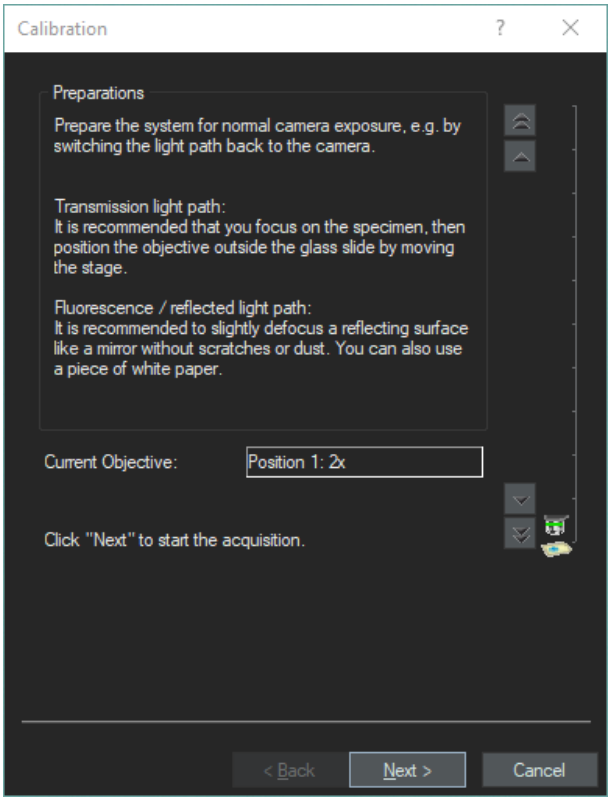
5. Make sure the [Shading correction] check box is selected
6. Proceed with [Next].

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.



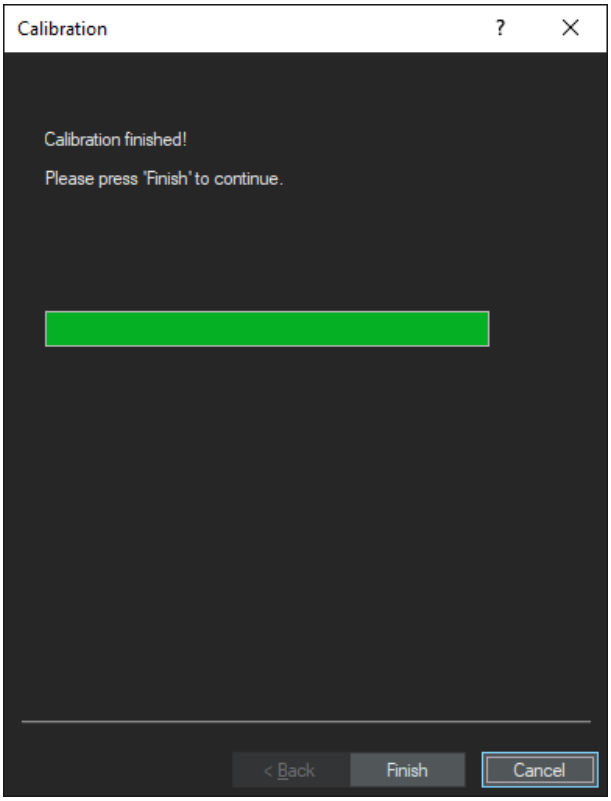
» After the acquisition process is finished, the calibration process automatically moves to the next objective.

IMPORTANT

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

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

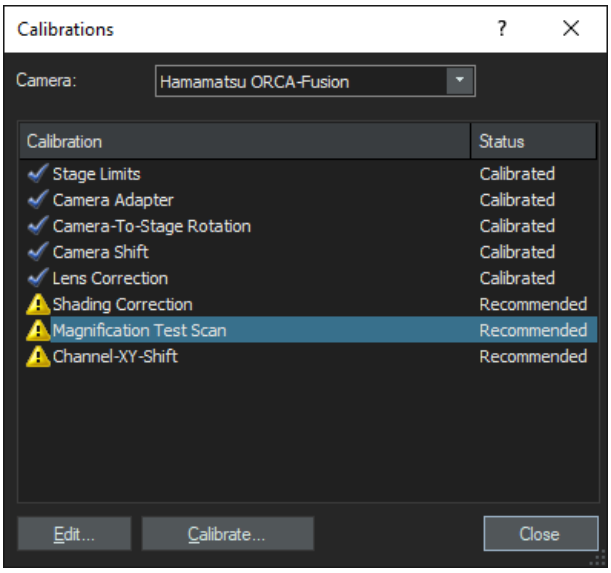
9. Finalize the process by clicking the [Finish] button.



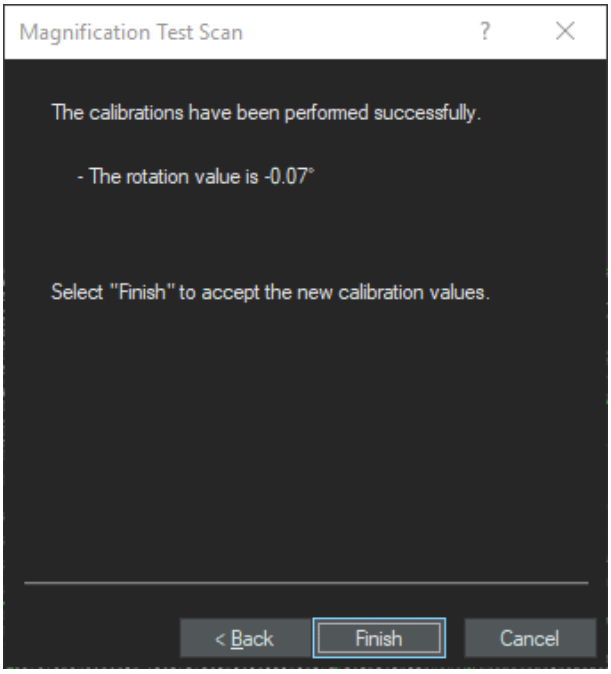
ATTENTION
In case you have calibrated an immersion medium objective as well clean the objective and remove immersion medium residues from the calibration slide.
See Immersion Objectives auf Seite 38.

21.6 Magnification Test Scan

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



2. Click the [Calibrate] button.
   » The magnification test scan for the monochrome camera is identical with the magnification test scan for the color camera. See Magnification Test Scan auf Seite 136.
3. The resulting value should be within a deviation of +/- 0.1 degree.



In case the calibration value is not within the standard go back to the [Camera-To-Stage Rotation] calibration and readjust the rotation. This will also effect the shading correction, however. Refer to chapter Shading Correction (Brightfield) auf Seite 115 to redo the shading correction.

21.7 Shading correction for fluorescence observation methods

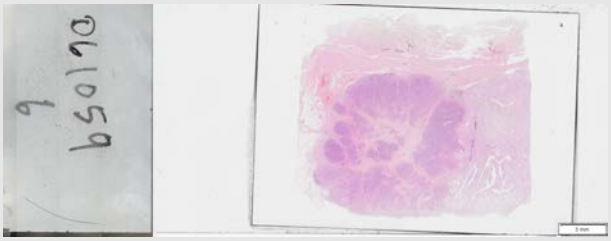
The shading correction for DAPI, FITC, CY3 and Cy5 can be done on a 'normal' thin, homogenous and large H&E sample using the autofluorescence like in the example below.

However also fluorescence samples provided by the customer can be used for shading correction.

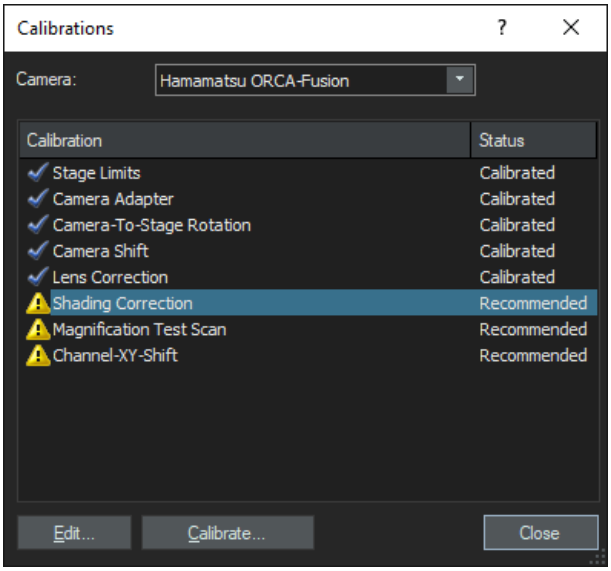
To perform the shading correction for CY7 a real CY7 stained sample is necessary as CY7 does not show any autofluorescence.

However shading correction is also sample dependent. Especially the thickness of the sample might have an influence on the correction.

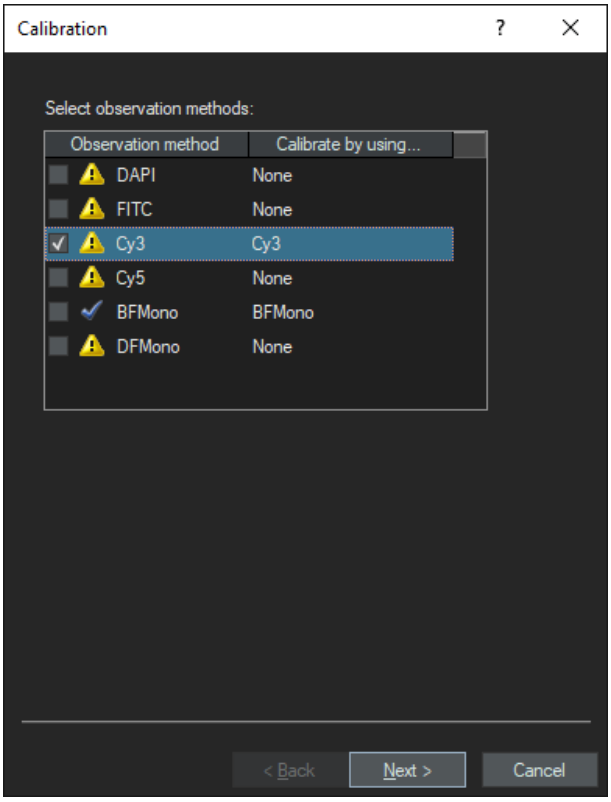
It could be necessary to repeat the shading correction if different samples are used.



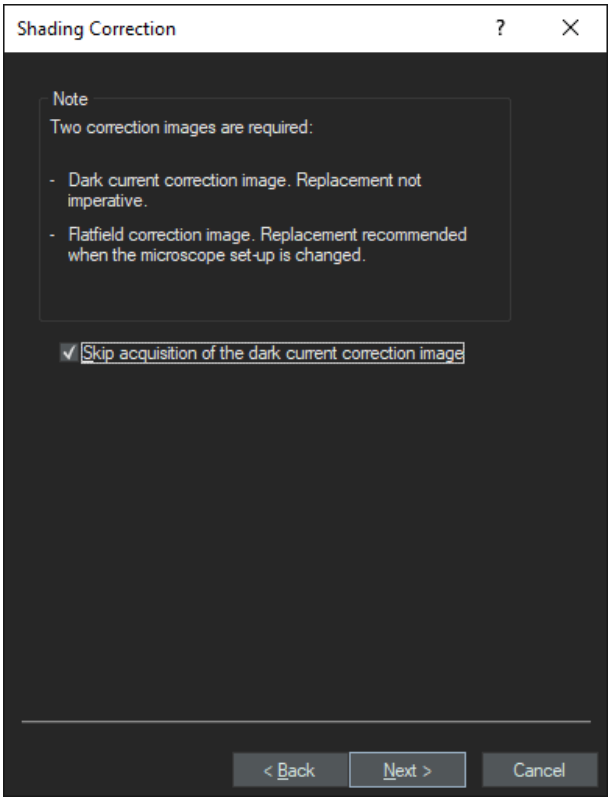
1. Put a similar slide into a tray and load the tray.
2. In the [Calibrations] dialog box select [Shading Correction] entry.



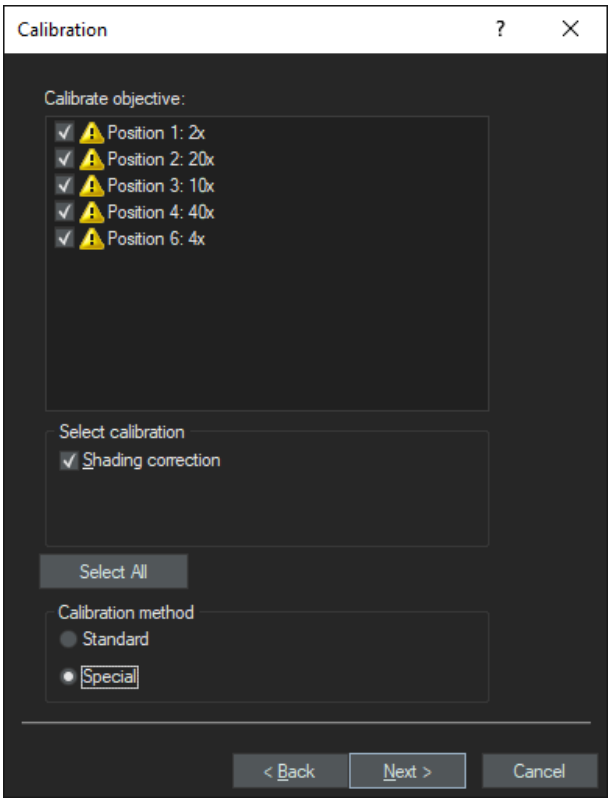
3. Select e.g. the [Cy3] observation method and click [Next].



4. Always skip the acquisition of the dark current correction image.

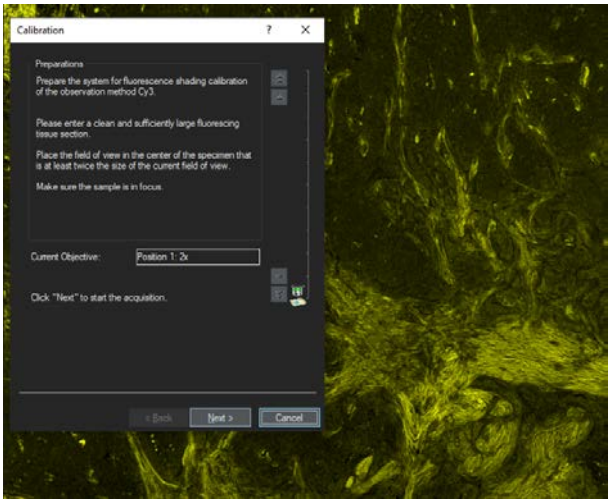


5. Select all objectives. Immersion objectives are calibrated in the same wizard. However the calibration wizard will calibrate all dry objectives first and subsequently select the immersion medium objective(s).
6. It is very important to select in the [Calibration method] group the calibration method [Special]. Only the calibration method [Special] will work on a real sample. The calibration method [Standard] requires different samples and is less effective. Also make sure that the checkbox [Shading correction] is checked.

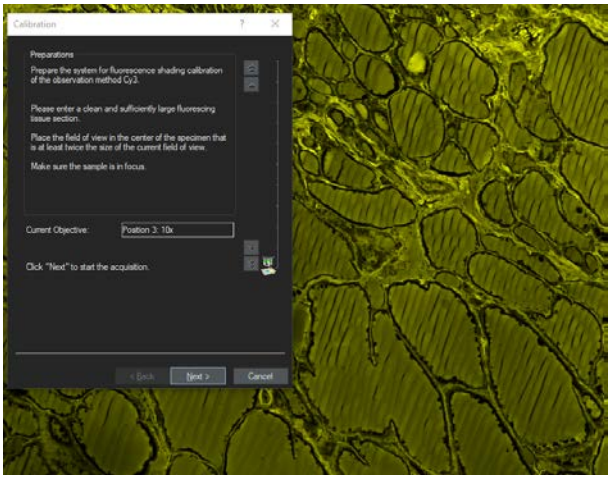


7. Proceed with [Next].
   » The system switches into live mode.

8. Select an area on the slide large enough for a 2x field of view and focus the sample via 'Ctrl' + mouse wheel and proceed with [Next].



For objectives equal or higher than 10x try to find a sample area which is quite homogenous.



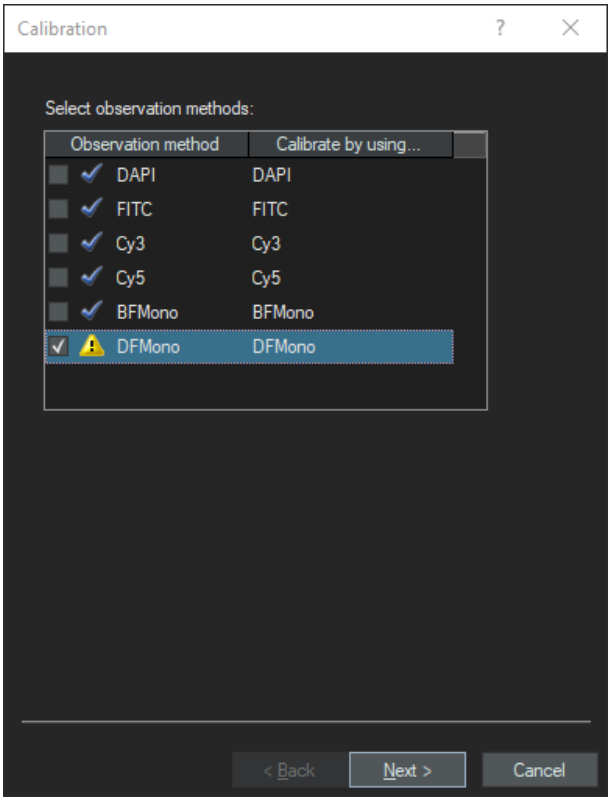
9. Repeat these steps for all other selected objectives.
10. Continue the shading correction for all other fluorescence observation methods.

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 Immersion Objectives auf Seite 38.

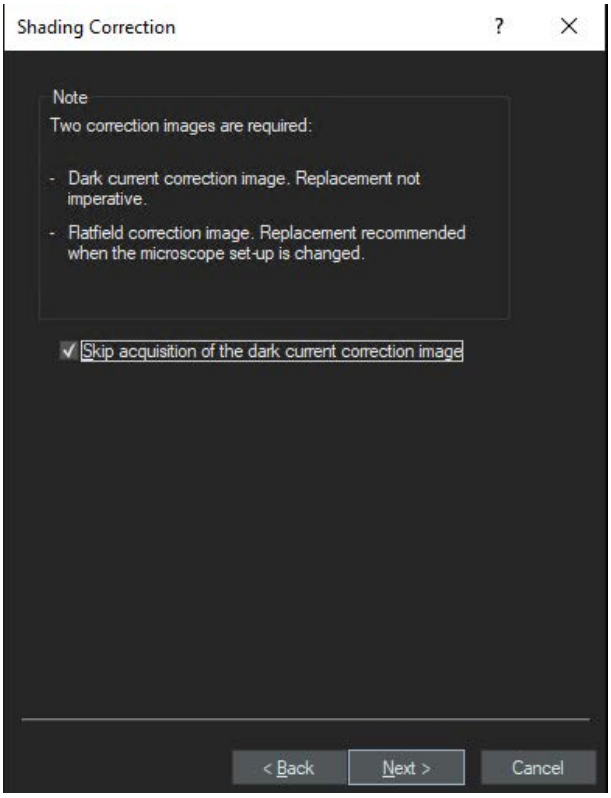
21.8 Shading correction for darkfield (DFMono)

- The darkfield observation method can only be used with 4x and 10x objectives.
- To perform shading correction for DFMono use the checkerboard sections on the VS-calibration slide.

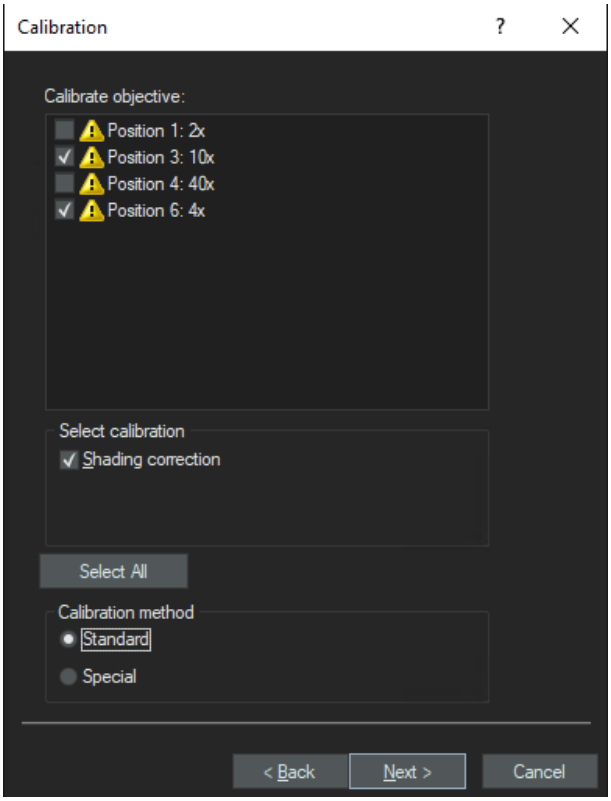
1. In the [Calibrations] dialog box, select the [Shading Correction] entry and click the [Calibrate] button.
2. Select the [DFMono] observation method and proceed with [Next].



3. Skip the acquisition of the dark current correction image. Proceed with [Next].



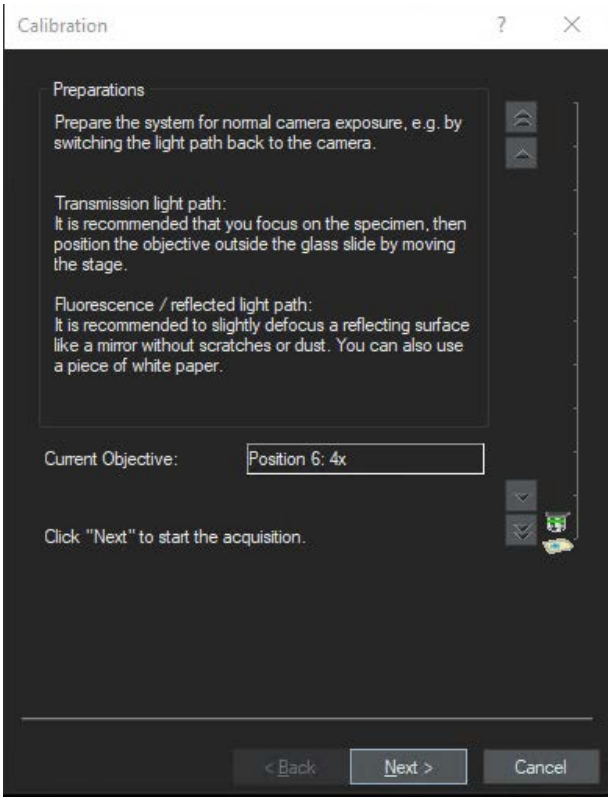
4. Select only the check boxes for the 4x and 10x objectives.
   Make sure that the [Shading correction] check box is selected.
   If necessary, select the [Calibration method] > [Standard] option.
   Proceed with [Next].



» The system will activate the live mode with the 4x objective used.

5. Use the [Stage Navigator] tool window to navigate to the 20x checkerboard and focus the sample.
6. Defocus by going down (-100 to 200µm in Z) until you see a homogenous image (no checkerboards) anymore.

7. Click the [Next] button to start the shading correction.



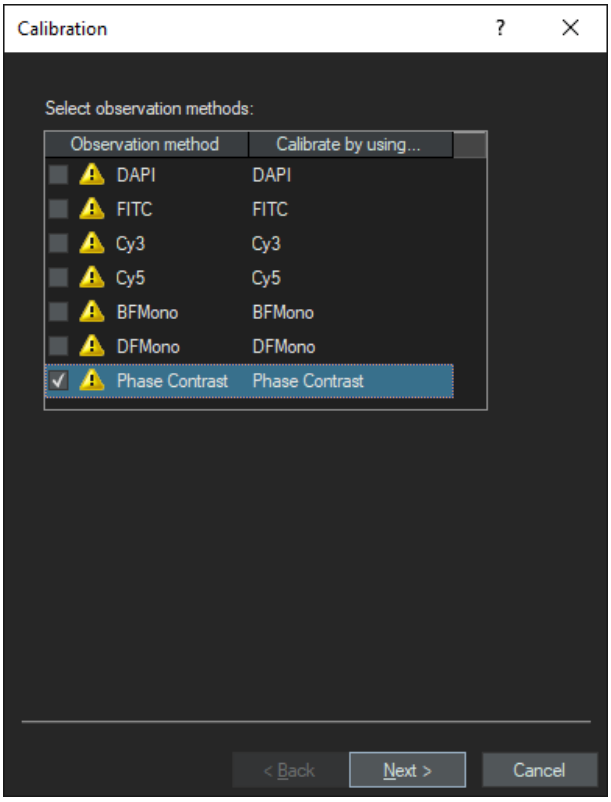
8. Continue in a similar way with the 10x objective.
9. For the 10x objective, go to the 100x/60x checkerboard area and defocus by going down 40µm.
10. Click the [Finish] button to finalize the shading correction.

21.9 Shading correction for PhaseContrast (PH)

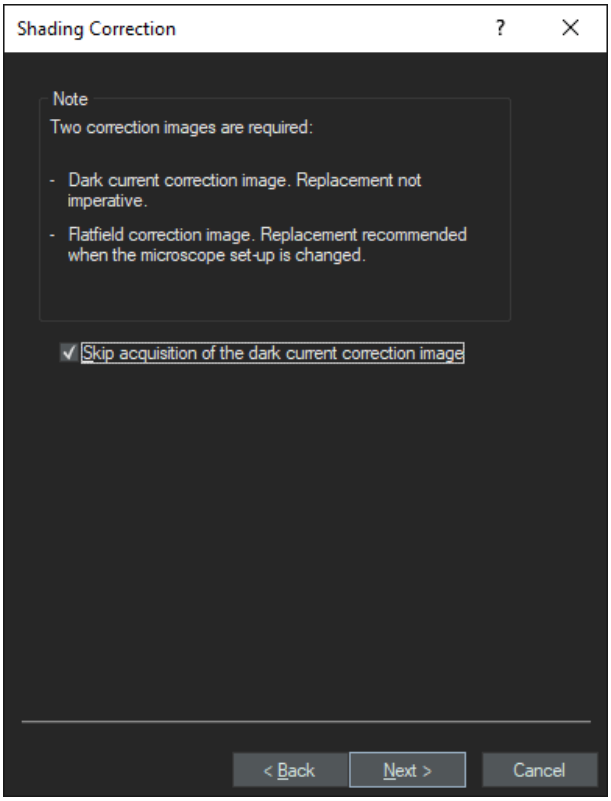
Prepare a slide with some sample on it for focusing. The rest of the slide should be empty and very clean.

1. In the [Calibrations] dialog box select the [Shading correction] entry.
2. Click the [Calibrate] button.

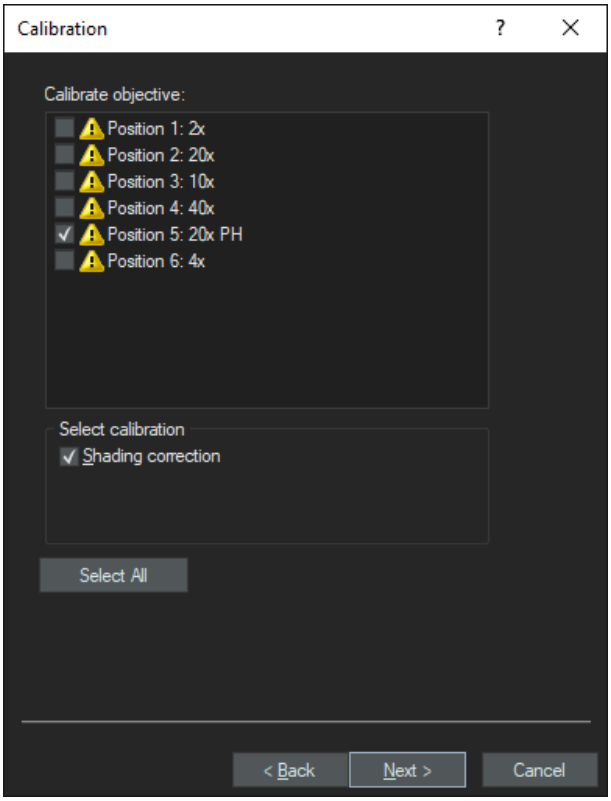
3. Select the [Phase Contrast] observation method.



4. Skip the acquisition of the dark current correction image.

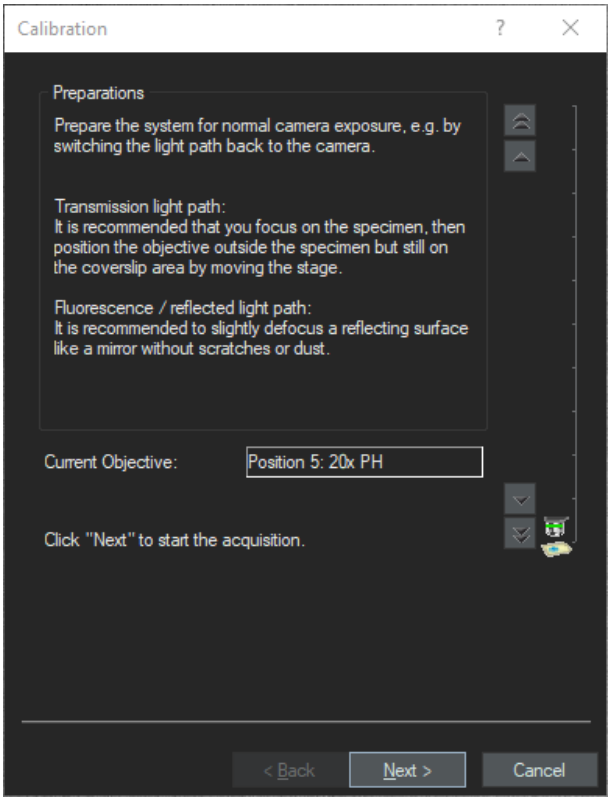


5. Only select the phase contrast objective(s). Make sure that the [Shading correction] check box is selected.

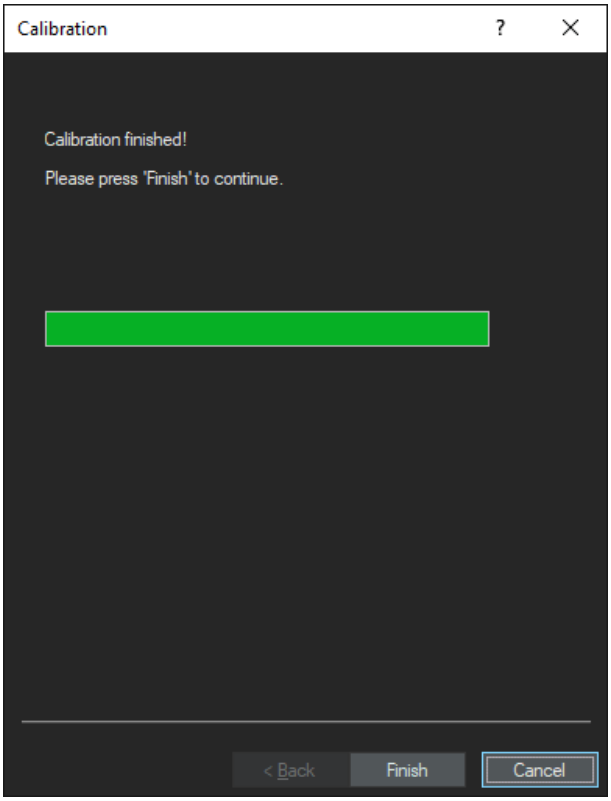


6. Focus on the part of the slide that contains the sample and subsequently search for a very clean area to acquire the shading image.

7. Proceed with [Next].



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



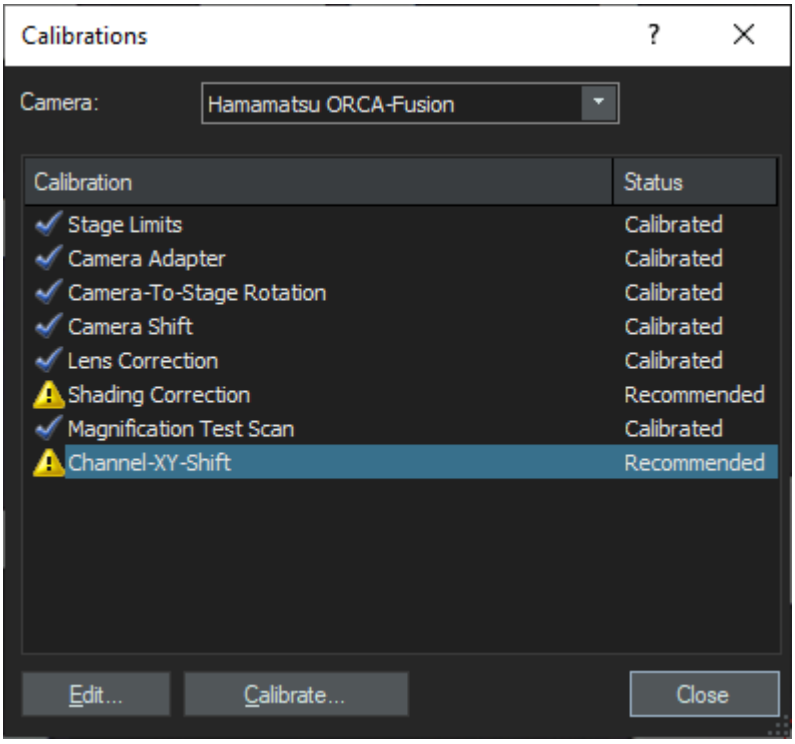
21.10 Channel-XY-Shift

The channel-XY-shift calibration is only needed if multiple fluorescence filters or dichroic mirrors in the IX3-RFACA are used.

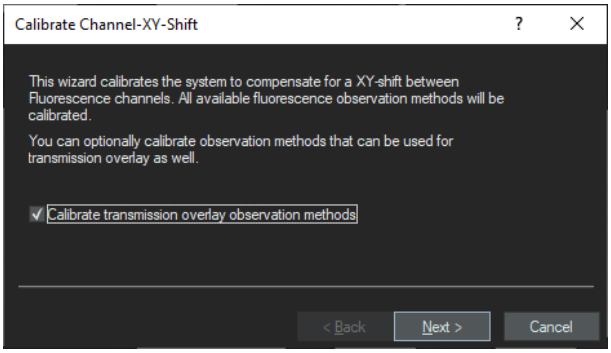
As this calibration depends on the customer's specific system configuration and customer's samples, the Olympus employee installing the system should ask for a dedicated sample slide. If such a sample slide is not available, the customer itself can do the calibration at any time.

It is important to correct a XY pixel shift induced by the different filter cubes.

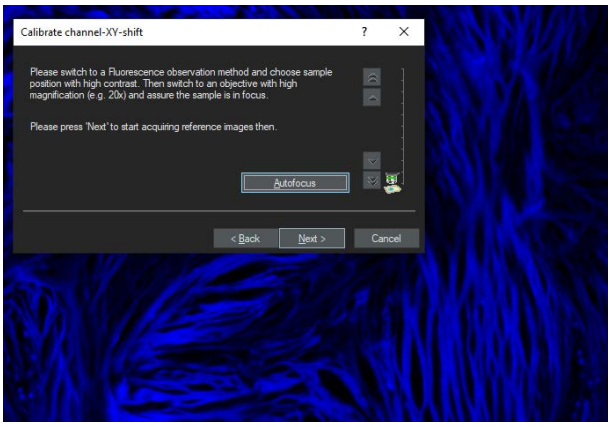
1. In the [Calibrations] dialog box select the [Channel-XY-Shift] entry.
2. Click the [Calibrate] button.



3. Select the [Calibrate transmission overlay observation methods] (bright field) check box.

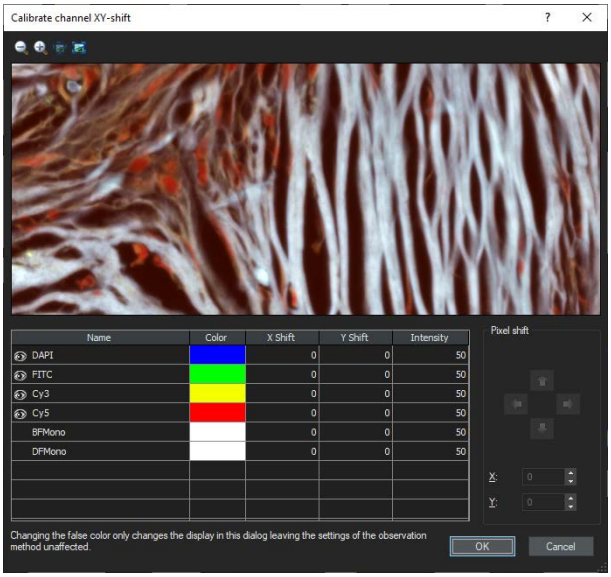


4. Put in a customer fluorescent sample which contains stainings for all fluorescent filters that should be calibrated. Use at least the 20x objective, but preferably the 40x objective.
5. Navigate to an area with high contrast and focus. Then click the [Next] button to start the acquisition process.

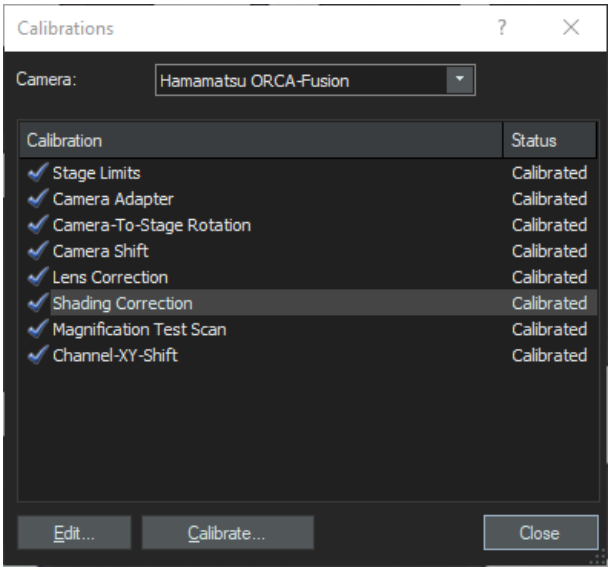


6. Once the image has been acquired, the [Calibrate channel XY-shift] tool window opens. The shift between the individual channels is corrected manually here.
   » The visibility of the channels can be changed by activating/deactivating the 'eye' icon next to the channel name.
7. Click on a channel e.g. FITC to activate it and then use the cursor key to move the FITC image around pixel by pixel to match the structure of the underlying DAPI image.
8. Proceed with the other channels the same way.

9. When you have finished, click the [OK] button to save the changes.



» All calibrations should have a blue tick now.



22 Cleaning the system

22.1 Cleaning the scanner

We recommend cleaning the scanner as required when you can see that it is dirty.

ATTENTION
Ingress of fluid can damage the devices.
An ingress of fluid can cause a short circuit or damage the scanner, individual components of the scanner, the loader, and connected devices.

- Before cleaning the VS200 system, disconnect it from the power supply.
- Protect the scanner, the loader, and individual components from dirt, water and ingress of fluids.
- Clean only the surface of the scanner, the loader and the individual components.

1. Disconnect the scanner from the power supply.
2. Clean the following components with a lint free cleaning cloth.
   - » The surface of the scanning stage
   - » Liquid dispenser head
3. For more pronounced marks, you can lightly wet the cleaning cloth with water or a mild solvent-free disinfectant or cleaning agent.
4. Clean the clamping mechanism only with a lint free cleaning cloth.
5. If the scanner or individual components were cleaned with a damp cloth, allow them to dry.

22.2 Cleaning the loader

ATTENTION
Ingress of fluid can damage the devices.
An ingress of fluid can cause a short circuit or damage the scanner, individual components of the scanner, the loader, and connected devices.

- Before cleaning the VS200 system, disconnect it from the power supply.
- Protect the scanner, the loader, and individual components from dirt, water and ingress of fluids.
- Clean only the surface of the scanner, the loader and the individual components.

1. Take all of the trays out of the loader.
2. Disconnect the VS200 system from the power supply.
3. Open the door of the loader.
4. Manually move the SCARA robot arm to the right.
5. Move the tray hotel to the loading position all the way at the front.

6. Clean the following components with a lint free cleaning cloth.
   - » Left side of the tray hotel
   - » Right side of the tray hotel
   - » The bottom panel of the tray hotel
7. For more pronounced marks, you can lightly wet the cleaning cloth with water or a mild solvent-free disinfectant or cleaning agent.
8. Push the tray hotel as far back as it will go to the back panel.
9. Manually move the SCARA robot arm to the left.
10. Clean the gripper only with a lint free cleaning cloth.
11. If the loader or individual components were cleaned with a damp cloth, allow them to dry.

22.3 Cleaning the trays

1. Clean the trays with a lint free cleaning cloth. For more pronounced marks, you can lightly wet the cleaning cloth with water or a mild solvent-free disinfectant or cleaning agent.

22.4 Cleaning the X-Cite Turbo

✓ The X-Cite Turbo light source is not available in all countries.

Cleaning of optics should only be attempted by qualified personnel using appropriate fluids and lens paper.

1. To clean the exterior of the unit, use a slightly dampened cloth and a simple water/ detergent solution only.

22.5 Cleaning the immersion objective

ATTENTION
Damage to the objectives and hardware
Objectives and hardware can get sticky after an immersion medium has been used. This can damage them.

- ▸ Clean the immersion objective after each use.

CAUTION
Quetschgefahr durch bewegte Komponenten im Inneren des VS200 Systems
Im Inneren des VS200 Systems bewegen sich mechanische Komponenten. Wenn Sie bei eingeschaltetem System im Inneren des Systems hantieren, können Hände und Finger gequetscht und Haare und Kleidung eingezogen werden.

- Achten sie darauf, dass Sie während der Reinigung der Immersionsobjektive keine Softwarefunktionen ausführen.

1. To clean lenses, remove dust by blowing them with a commercially available blower and wipe them lightly with cleaning paper (or a piece of repeatedly washed gauze). Only if they are stained by fingerprints or oils should you wipe them using cleaning paper slightly moistened with dehydrated alcohol sold at store

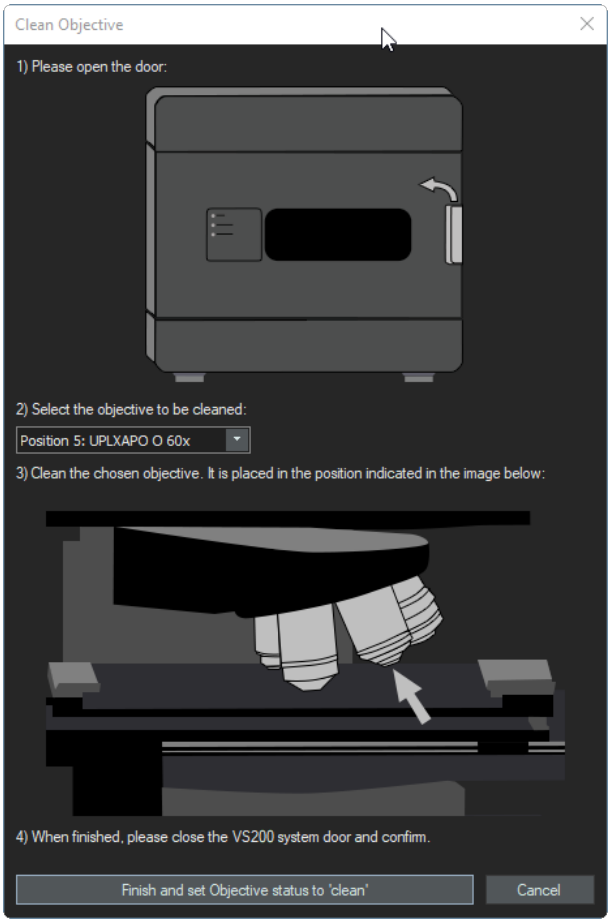
   CAUTION
   Dehydrated alcohol is highly flammable. Do not expose it to heat or flame, and do not turn off or on the power switch of various electrical apparatuses since ignition can be induced by just switching switches on and off. In addition, make sure that the room is well ventilated.

2. Do not use an organic solution to clean parts other than lenses. If a part is heavily stained, wipe it with a soft cloth slightly moistened with a diluted neutral detergent.

» Avoid cleaning lenses with the camera or the lenses removed. Doing so will cause the system to be uncalibrated. All calibrations need to be redone.

3. Click the [Clean Objective] button on the start page of the VS200 ASW software and follow the instructions in the dialog box.
   » The objective revolver will move up to grant more space.
   » It is not necessary to unmount an objective to clean it.

4. In the [Select the objective to be cleaned] list select the objective to be cleaned. If you have more than one immersion objective, also select the other objectives in the list.



3. To clean the objective use lens cleaning paper.
4. After cleaning the objective click on the [Finish and set Objective status to'clean'] button.

23 Additional software installation

23.1 OlyVIA

To install OlyVIA go to D:\OLYMPUS_SERVICE_ONLY_DO_NOT_DELETE\SetupOlyvia and execute the [setup.exe].

23.2 VS200 ASW Desktop

VS200 ASW and VS200 ASW DT cannot be installed on the same customer PC.

The installation requires a VS200 ASW DT licence key during the installation.

To install VS200 ASW DT on a different PC copy the setup files to the desired PC and execute the setup.exe. The setup files can be found on the VS200 ASW system PC under the follwing link: D:\OLYMPUS_SERVICE_ONLY_DO_NOT_DELETE\SetupMain

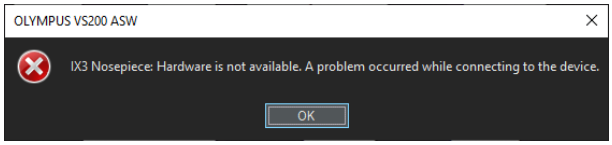
23.3 NetImage Server SQL (NIS-SQL) and Webinterface

The NetImageServer SQL is installed by Olympus Technical service only.

Also the Webinterface to utilize OlyVIAweb (HTML5-based webviewer) is installed and configured by Olympus Technical Service only.

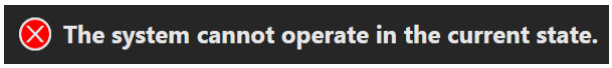
24 Troubleshooting

24.1 Hardware not available



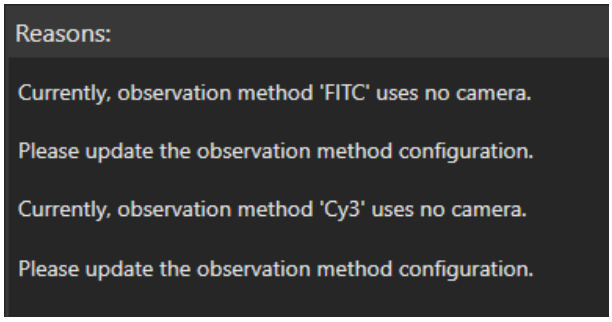
If the [IX3 Nosepiece: Hardware is not available] error message appears, do the following:

1. Close the VS200 ASW software.
2. Switch off the VS200 system!
3. Check cabling of IX3-RFACA.



24.2 "No camera" error

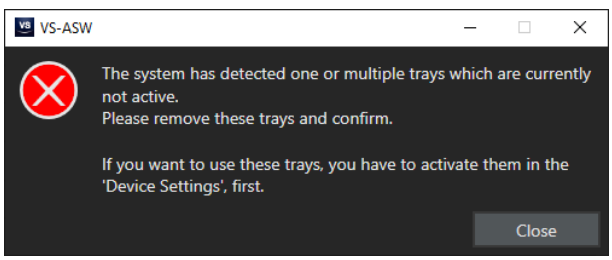
If you see the following error messages after switching to the monochrome camera you need to check whether the correct camera is assigned in the observation methods which use a monochrome camera. See Manual device configuration auf Seite 89.

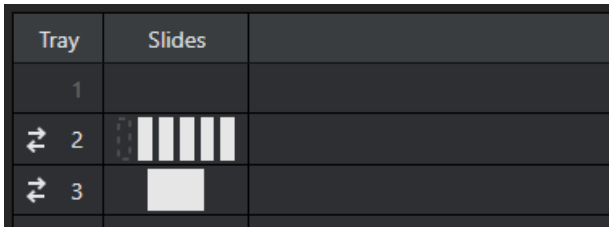


» In case you use an ORCA camera make sure that it is switched on.

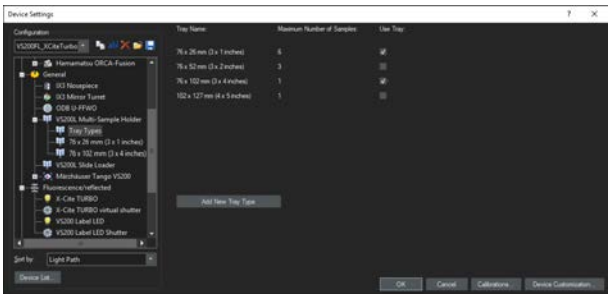
24.3 Tray not active

If you see the error message below you possibly inserted a new tray type without having it set up correctly in the VS200 ASW software first.



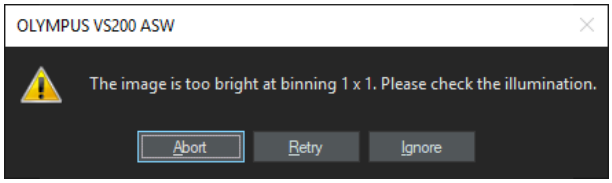


1. Remove the tray and switch to the [Manual control] layout.
2. Use the [Acquire] > [Devices] > [Device Settings] command to open the [Device Settings] dialog box.
3. Select the [VS200L Multi-Sample Holder] > [Tray Types] entry in the tree view.
4. Select the check box [Use Tray] next to the tray type you want to use.



24.4 Image too bright

If you see the following error message please adjust VS200 LED lamp voltages. See ORCA camera adjustments auf Seite 91.



24.5 Setting the Koehler illumination

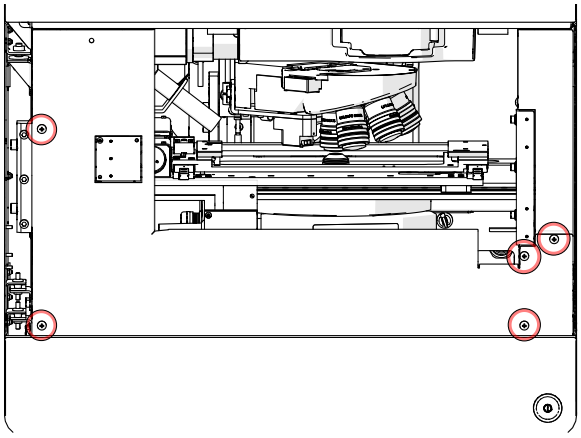
The units described below must be assembled and adjusted by Olympus. If these units are assembled or adjusted by the customer, the operations are not ensured.

This chapter describes how to set the Koehler illumination.

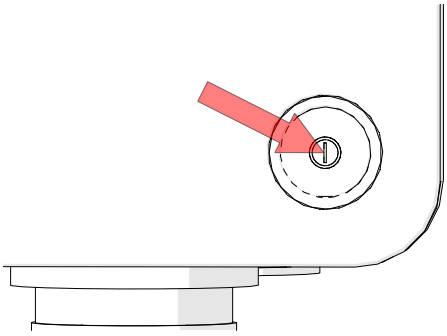
» Inbusschlüssel (Größe 2,5 mm und 3 mm mit Kugelkopf)

1. Open the door of the VS200 scanner.
2. Remove the lower tamper protection plate. To do so, loosen the 5 hex

screws (size 2.5 mm) indicated in the figure.



3. Use the main power switch to switch on the VS200 system.



CAUTION

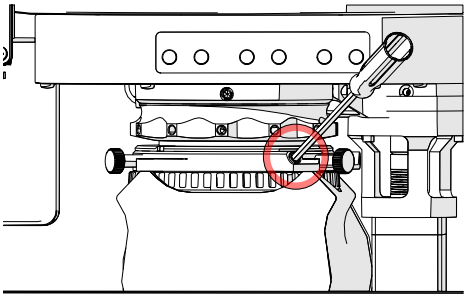
Crush hazard when initializing the VS200 system

Switching on the VS200 scanner and starting the VS200 ASW software will initialize all components. As a result, various components begin to move.

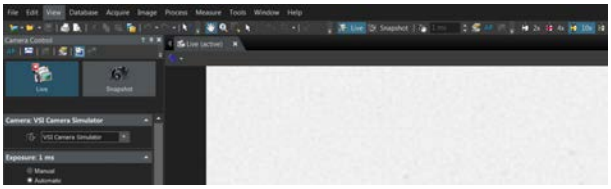
Gaps open and close when the stage moves. Hands and fingers can get crushed.

- Make sure that you are not within the stage's range of movement when it is moving.
- Try never to put your hands or fingers into any gaps.

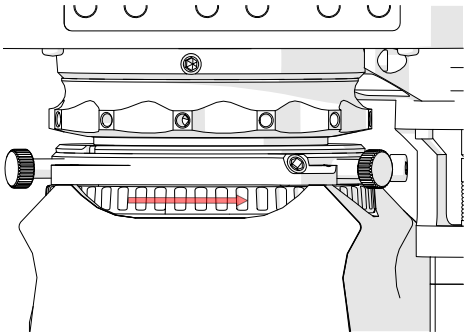
4. Release the fixation in the field stop. Lösen Sie dazu die in der Abbildung markierten INBUS-Schrauben (Größe 3 mm mit Kugelkopf).



5. Start the VS200 ASW software.
6. Take a tray with a slide in position 3.
7. On your software's start page, click the [Exchange Trays] button to load the tray.
8. Use the [Load Slide for Calibration] function to load the slide.
9. Make sure that the 2x objective is selected.
10. Starten Sie das Live-Bild in der VS200 ASWSoftware. To do so, go to the [Manual Control] layout.
11. In the [Camera Control] tool window, click the [Live] button to start the live image. Alternatively, you can also use the [F7] key.

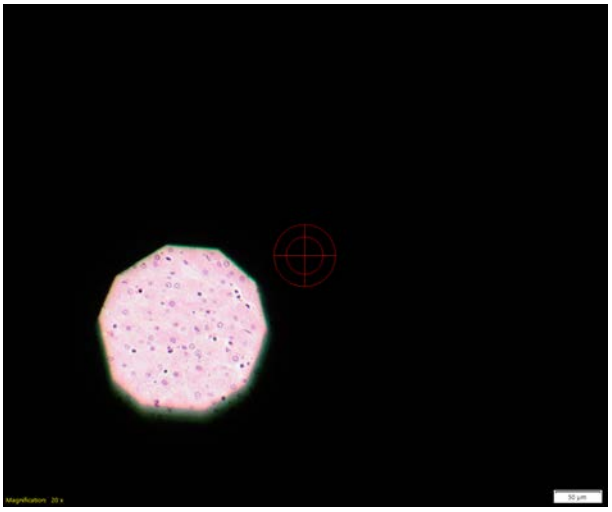


12. Carry out an autofocus.
13. Change to the objective with the magnification 10x or 20x.
14. Carry out an autofocus again.
15. Turn the adjustment ring indicated in the figure to close the field stop as far as possible.

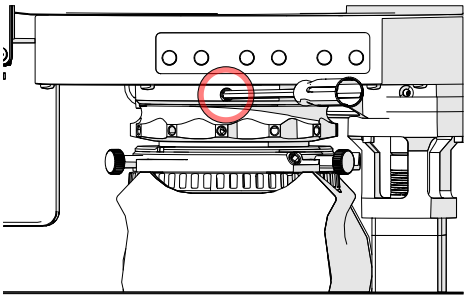


» You should now see a bright spot somewhere in the live image. The spot may appear polygonal (10 edges).
Display a cross hair in the live image to help with centering. To do so, use the [View] > [Cross Hair] command in the VS200 ASW software.

Alternatively, you can also use the [Alt + F6] keystroke.

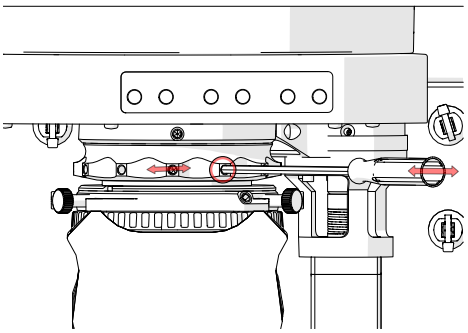


16. Release the field stop's focus setting. To do so, loosen the hex screw (size 3 mm) indicated in the figure.

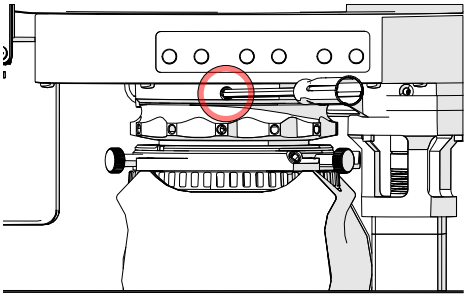


17. Focus the field stop. To do so, insert the hex key into one of the holes indicated in the figure. Move the hex key to the right and to the left to adjust the focus ring.

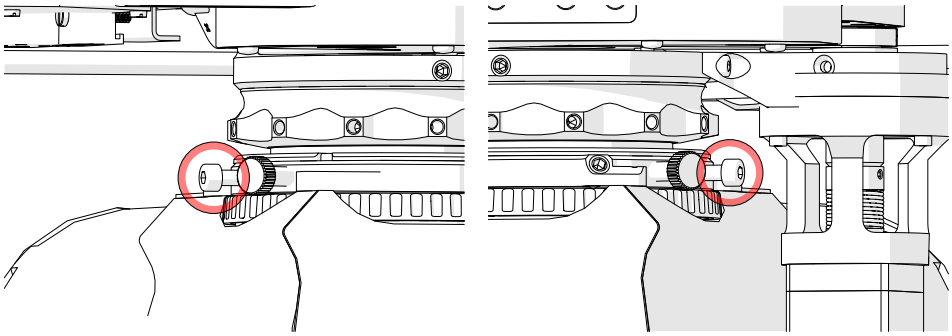
    Set the focus so that the edges of the closed field stop are sharp.



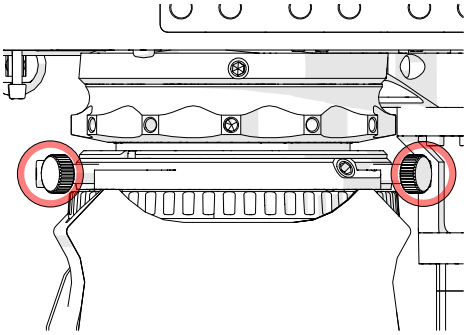
18. Tighten the field stop's focus setting. To do so, use the hex screw (size 3 mm) indicated in the figure.



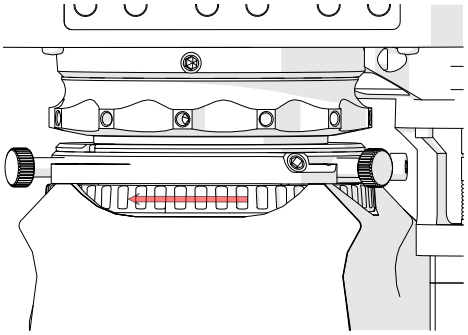
19. Loosen the 2 hex screws (size 3 mm) indicated in the figure. The screws should only have been screwed hand tight.
20. Use the two adjustment screws indicated in the figure to align the bright spot in the center of the cross hair.



21. Lock the field stop's focus setting. To do so, tighten the hex screw (size 3 mm) loosened before.
22. Open the field stop completely. To do so, turn the adjustment ring shown in the figure to the left as far as possible.



23. Lock the field stop. To do so, tighten the hex screw (size 3 mm) indicated in the figure.



24. Close the VS200 ASW software and wait until the VS200 scanner has reached its end positions.
25. Use the main power switch to switch off the VS200 system.
26. Montieren Sie den schwarzen Eingreifschutz mittels der sechs Schrauben wieder.
27. Close the door of the VS200 scanner.

25 Proper selection of the power supply cord

If no suitable power supply cord has been provided, please select an appropriate power supply cord with a certification mark by referring to the specifications and the table below.

Olympus is not responsible for damage caused by the use of uncertified power cords with Olympus devices.

Specifications

Voltage Rating	125 V Wechselstrom (für Gebiete mit 100-120 V) oder 250 V Wechselstrom (für Gebiete mit 220-240 V)
Rated current	min. 9,5 A
Temperature Rating	min. 60 °C
Length	max. 3,05 m
Fittings configuration	Kabel mit geerdetem Stecker. Gegenstück aufgeschweißte Kupplung gemäß IEC-Konfiguration.

Table 1 - Certification marks for power cords

The power cord has to have a certification mark from one of the bodies listed in table 1 or it must use a cable that has been tested by a body that is listed in table 1 or table 2. The plug has to have at least one certification mark shown in table 1. If you are unable to acquire a cord tested by one of the bodies listed in table 1 in your country, please use a cord that has been tested by a comparable body in your country.

Country	Agency	Certification Mark	Country	Agency	Certification Mark
Argentina	IRAM		Japan	JET, JQA	
Australia	SAA		Canada	CSA	
Belgium	CEBEC		Netherlands	KEMA	
Denmark	DEMKO		Norway	NEMKO	
Germany	VDE		Austria	ÖVE	
Finland	FEI		Sweden	SEMKO	
France	UTE		Switzerland	SEV	
United King-dom	ASTA BSI		Spain	AEE	
Ireland	NSAI		U.S.A.	UL	
Italy	IMQ				

Table 2 - HAR Flexible cables

Certification body	Printed or embossed harmonization mark (on the plug or cable insulation)	
Comité Électrotechnique Belge (CEBEC)	CEBEC	<HAR>
VDE Verband der Elektrotechnik Elektronik Informationstechnik e.V.	<VDE>	<HAR>
Union Technique de l'Électricité (UTE)	USE	<HAR>
Istituto Italiano del Marchio di Qualità (IMQ)	IEMMEQU	<HAR>
British Approvals Service for Cables (BASEC)	BASEC	<HAR>
N.V. KEMA	KEMA-KEUR	<HAR>
SEMKO AB Svenska Elektriska Materielkontroll-anstalten	SEMKO	<HAR>
Österreichischer Verband für Elektrotechnik (ÖVE)	<ÖVE>	<HAR>
Danmarks Elektriske Materielkontrol (DEMKO)	<DEMKO>	<HAR>
National Standards Authority of Ireland (NSAI)	<NSAI>	<HAR>
Norges Elektriske Materiellkontroll (NEMKO)	NEMKO	<HAR>
Asociación Electrotécnica Española (AEE)	<UNED>	<HAR>
Hellenic Organization for Standardization (ELOT)	ELOT	<HAR>
Instituto Português da Qualidade (IPQ)	np	<HAR>
Schweizerischer Elektrotechnischer Verein (SEV)	SEV	<HAR>
Elektriska Inspektoratet	SETI	<HAR>

Underwriters Laboratories Inc. (UL)	SV, SVT, SJ oder SJT, 3 X 18AWG
Canadian Standards Association (CSA)	SV, SVT, SJ oder SJT, 3 X 18AWG

26 Declarations of conformity and disposal

26.1 CE Conformity (Europe)

This system complies to the requirements of the following European directives:

- Low Voltage Directive 2014/35/EU
- EMC Directive 2014/30/EU
- Machinery Directive 2006/42/EC
- Radio Equipment Directive (RED) 2014/53/EU

This system complies with the requirements of Directive 2014/30/EU concerning electromagnetic compatibility according to Standard IEC/EN61326-1.

- Emission: Class A
- Immunity: Applied to industrial environment requirements. Operation of this equipment in a residential area may cause interference.

26.2 WEEE declaration (Europe)

In accordance with the European directive on Waste of Electrical and Electronic Equipment, this symbol indicates that the product must not be disposed of as unsorted municipal waste but should be collected separately. Refer to your local authority in the EU for return and/or collection systems available in your country.

26.3 RoHS Conformity (Europe)

This product conforms with the European Union directive on the restriction of the use of certain hazardous substances in electrical and electronic equipment 2011/65/EU.

26.4 FCC conformity (USA)

This device complies with Part 15 of the FCC regulations. Operation is subject to the following two conditions: (1) this device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

This device has been tested and found to comply with the limits for a Class B digital device, pursuant to Part 15 of the FCC Rules.

These limits are designed to provide reasonable protection against harmful interference in a residential installation. This device generates, uses, and can radiate radio frequency energy. If not installed and used in accordance with the instructions, it may cause harmful interference to radio communications.

However, there is no guarantee that interference will not occur in a particular installation.

If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures:

1. Reorient or relocate the receiving antenna
2. Increase the separation between the equipment and receiver
3. Connect the equipment into an outlet on a circuit different from that to which the receiver is connected
4. Consult the dealer or an experienced radio/TV technician for help

FCC warning	Changes or modifications not expressly approved by the party responsible for compliance could void user's authority to operate the equipment.

26.5 For Korea only

For Korea only

이 기기는 업무용 환경에서 사용할 목적으로 적합성평가를 받은 기기로서 가정용 환경에서 사용하는 경우 전파간섭의 우려가 있습니다.

26.6 China RoHS conformity (China)

Delete this text and replace it with your own content.

26.7 RFID

26.7.1 RFID (Korea)

본 기기는 통상 이용 상태의 경우 인체(머리, 몸통)와 20cm 초과하는 거리에서 사용되어야 합니다

26.7.2 RFID (USA)

FCC Supplier's Declaration of Conformity
Hereby declares that the product
Product name: Optical Microscope
Model Number: VS200
Confirms to the following specifications:
FCC part 15, Subpart B, Section 15.107 and Section 15.109
Supplementary Information:
This device complies with part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) This device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.
Responsible Party Name: Olympus Scientific Solutions Americas Corp.
Address: 48Woerd Ave Waltham, MA 02453, U.S.A.
Phone Number: 781-419-3900

Changes or modifications not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment".

FCC Part 15.19(a) [interference compliance statement], unless the following statement is already provided on the device label:

This device complies with part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) This device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

26.7.3 RFID (Canada)

ISED notice:

This device complies with Industry Canada's licence-exempt RSSs. Operation is subject to the following two conditions:

(1) This device may not cause interference; and

(2) This device must accept any interference, including interference that may cause undesired operation of the device.

Le présent appareil est conforme aux CNR d'Industrie Canada applicables aux appareils radio exempts de licence. L'exploitation est autorisée aux deux conditions suivantes :

1) l'appareil ne doit pas produire de brouillage;

2) l'appareil doit accepter tout brouillage radioélectrique subi, même si le brouillage est susceptible d'en compromettre le fonctionnement."

26.7.4 RFID (Taiwan)

第十二條 經型式認證合格之低功率射頻電機，非經許可，公司、商號或使用者均不得擅自變更頻率、加大功率或變更原設計之特性及功能。
第十四條 低功率射頻電機之使用不得影響飛航安全及干擾合法通信；經發現有干擾現象時，應立即停用，並改善至無干擾時方得繼續使用。
前項合法通信，指依電信法規定作業之無線電通信。低功率射頻電機須忍受合法通信或工業、科學及醫療用電波輻射性電機設備之干擾。

27 Support

Delete this text and replace it with your own content.

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OLYMPUS SOFT IMAGING SOLUTIONS GmbH

Johann-Krane-Weg 39, 48149 Münster, Germany

Distributed by

OLYMPUS CORPORATION

Shinjuku Monolith, 2-3-1, Nishi-Shinjuku, Shinjuku-ku, Tokyo 163-0914, Japan

OLYMPUS EUROPA SE & CO. KG

Wendenstrasse 14-18, 20097 Hamburg, Germany

OLYMPUS CORPORATION OF THE AMERICAS

3500 Corporate Parkway, P.O. Box 610, Center Valley, PA 18034-0610, U.S.A.

OLYMPUS CORPORATION OF ASIA PACIFIC LIMITED

L43, Office Tower, Langham Place, 8 Argyle Street, Mongkok, Kowloon, Hong Kong

OLYMPUS AUSTRALIA PTY LTD

3 Acacia Place, Notting Hill, 3168, Australia

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