



CORRESCOPY™
CORRELATIVE IMAGING

USER'S MANUAL

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Please read these instructions carefully and keep them in a safe place for later reference.

1. Example of the Correscopy set

Correscopy sample holder
(e.g., CS1-F5)



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Microscope adapters
(e.g., LM_R160110,
SEM_P3, M50)

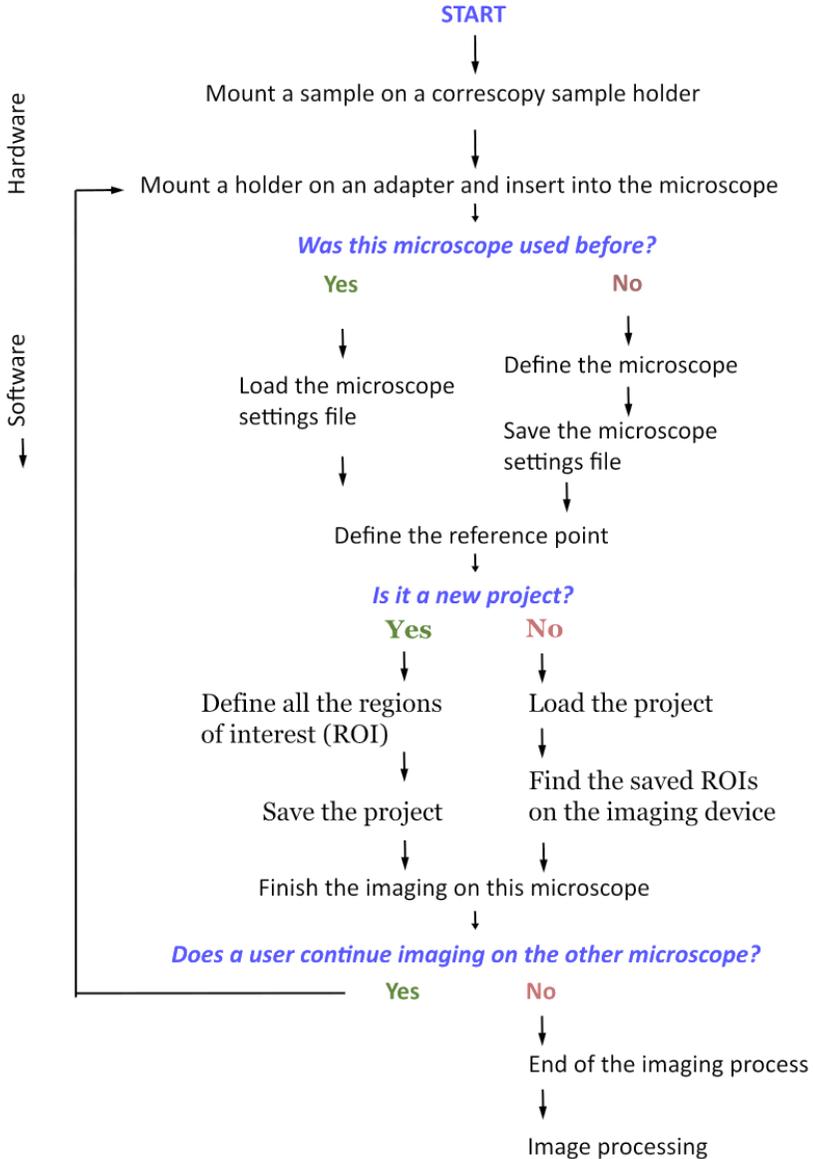


+

Software
with license on USB drive



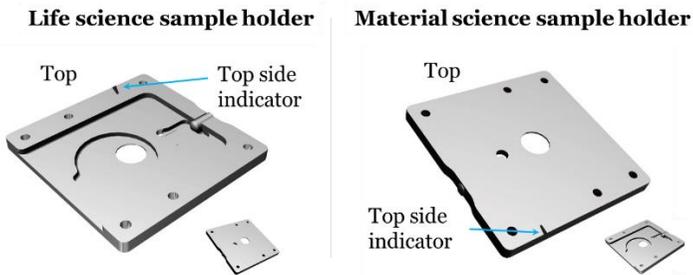
2. The imaging workflow



3. Correscopy sample holder and microscope adapters

3.1. Sample holder

Depending on the desired application there are 2 types of the sample holders, with focus of life science and material science research. The difference between them is on which side the reference crystal is installed and visible for surface (non-transmission) imaging methods (e.g. SEM). The type of the holder can be determined by looking at the location of the top side indicator which is present on the top surface of the sample holder (like presented on the figure below).



3.2. Mounting a sample on the correscopy sample holder

It is crucial that the sample remain fixed on the stage throughout the whole imaging process.

Multiple ways can be applied to immobilize the sample on the holder.

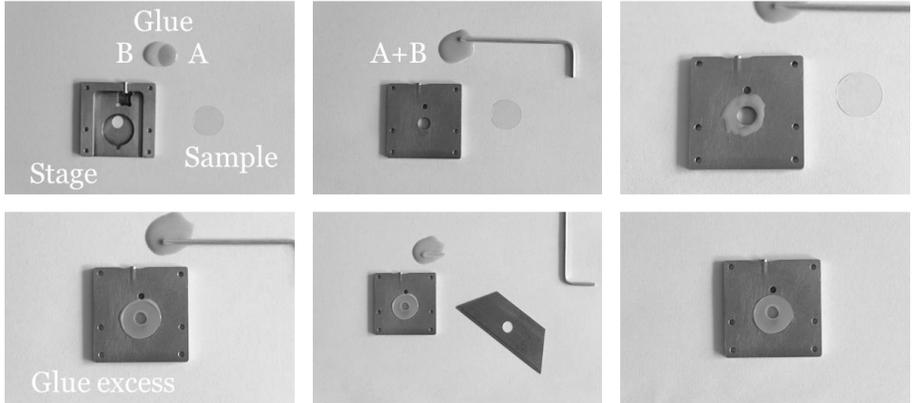
- Double sided tapes (e.g. carbon tape),
- Metal springs mounted on the middle holes (accessory ports) on the sample holder (springs are not provided),
- Variety of glues and pastes.

With the Correscopy set a two component removable glue is provided. “A” - blue and “B” – yellow components (as shown on the side) must be mixed, until it gets on a uniform color in 1:1 ratio. Ensure uniform glue distribution to avoid potential leakages in case of using wet sample. Press the sample uniformly to the stage in order to avoid an unnecessary



tilt of the sample. After the imaging the stage can be cleaned from glue and reused.

How to slick the sample using provided 2 component glue:



Note: The glue cures also in the wet environment within a few minutes. It is resistant to common solvents like ethanol or acetone.

Note: An amount of glue in full droppers (10 ml) is enough to fix more than 100 coverslips.

Caution: Do not glue the sample (substrate) near the edges of the stage, it may prevent correct sample holder mounting on some adapters.

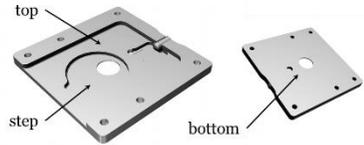
Caution: While using the high resolution and vibration sensitive methods (like AFM), the glue may cause vibration of the sample due to its elasticity. It is recommended to use different means to immobilize the sample.

Regardless of which type of the sample holder type is used, there are a few alternative ways of mounting a sample.

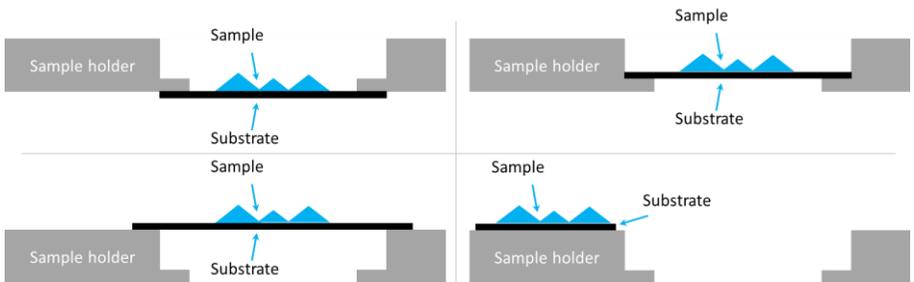
In order to do it correctly one must design an imaging process beforehand – which imaging devices and techniques are going to be used. The sample can be placed at any position of the sample holder facing any direction, all depends on the imaging process.

Sample can be mounted on:

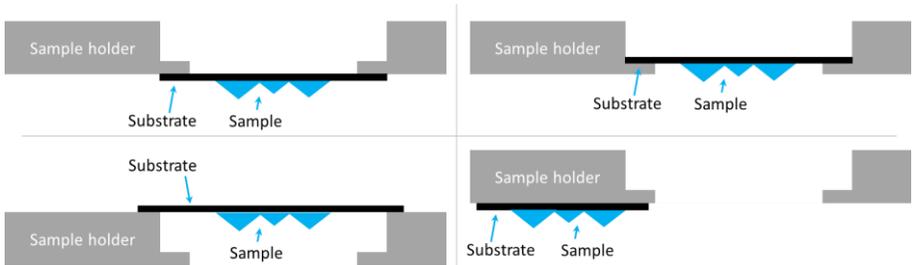
- a) top of the sample holder
- b) sample holder step
- c) bottom of the sample holder



Each of them has its own advantages and must be considered while mounting the sample. Regardless the type of the sample holder, the sample can be placed in 4 distinctive locations (partial cross-section of the sample holder):



Similarly, these locations can be used when sample is facing the other direction:



Note: *the solution does not require specific substrate for the sample. It can be for example a transparent coverslip – for most biological applications, or a metal plate – for material science oriented research, etc..*

When using samples, which must remain wet during the imaging, a small amount of liquid can be applied on the top of the sample. The small well for liquid can be created by mounting the sample on the holder in the area with the hole or by adding provided hydrophobic glue around the sample on the top creating the liquid drop. Moreover, in order to keep the liquid for extended time, an additional coverslip can be used to cover the sample, either on the step or on the top surface (it limits the liquid evaporation rate). The additional coverslip covering the liquid can also be glued to the surface creating closed volume which fully prevents the evaporation, however its removal without damaging the sample may be a difficult task.

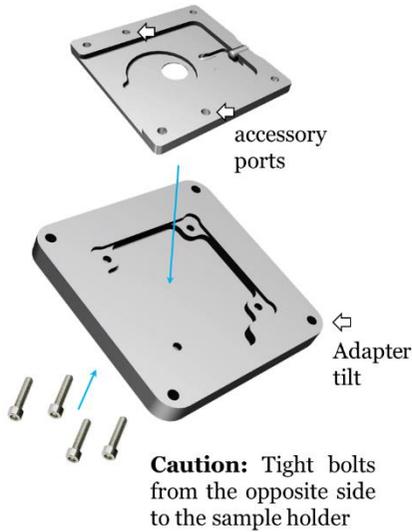
Caution: *Using the additional coverslip may cause damage or removal of weakly adherent samples (e.g. cells) to the substrate surface when removing it.*

The stage provides additional ports for the accessories, like a flow chamber (under development), which can be simply attached using 2 bolts. The most delicate and the main part of the stage is a reference crystal which is located on the CS stage. It is important to keep the crystal clean and free of cracks or edge chips.

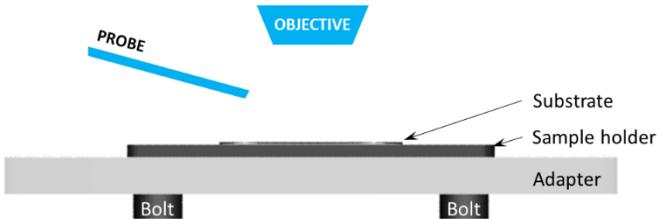
Note: *For scanning electron microscopy users, the reference crystal on the stage is made out of transparent electrical conducting material, hence it is not required to coat a metal layer on it.*

3.3. Mounting sample holder on the adapters

The sample holder is provided with a number of adapters which allow to mount the sample on various imaging devices (the choice depends on a user and an application). The sample holder is mounted using 4 bolts on the adapters.

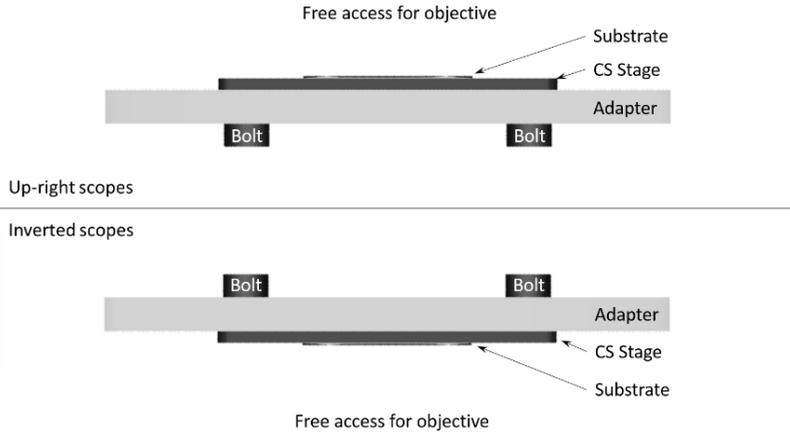


This solution provides a firm connection between all the elements and also a flat surface without any protruding elements towards the objective/detector/probe ensuring safe usage of the imaging device.



allowing easy access of e.g. objectives to a sample (as shown in the next figure).

Some of the adapters (like adapters for light microscopy) allow to mount the sample holder on two sides. It can be utilized depending on the imaging needs. For example correlation of the up-right and inverted optical microscope

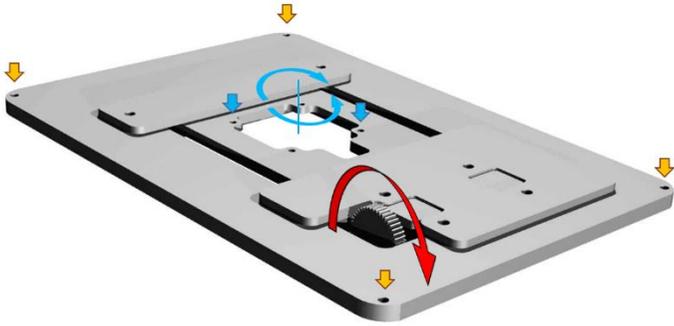


The sample holder can be mounted on the adapter in 4 different orientations (varied by 90 deg) depending on the needs.

Note: *The total number of different ways of mounting the sample holder on the adapter is 8 (facing 2 different directions (up or down) in 4 different orientations).*

Some of the adapters (e.g. LM_# – for light microscopy) provide a rotation function which is used for the calibration of the sample holder with microscope stage. The rotation of the stage is provided by a scroll as shown below. Moreover, if the stage or sample has a tilt, it can be levelled by use of levelling bolts on the sides of some adapters.

In the cases where the rotation is not provided on the microscope adapter the rotation can be done using the rotation of the microscope stage.



4. The Correscopy software

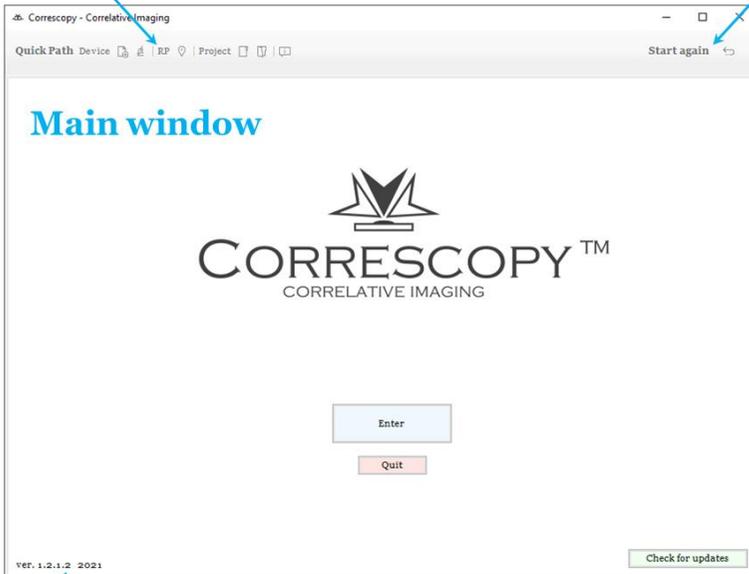
4.1. The Main window

The application consists of two parts, the main window and the quick path menu. Quick path is a tool bar at the top of the main window area. In the quick path menu is dedicated for experienced users who can quickly navigate through the software without going through all the steps included in standard protocol.

All the steps are displayed in the main window area, where all the user instructions are user's input fields are shown.

Quick Path menu

Restart button



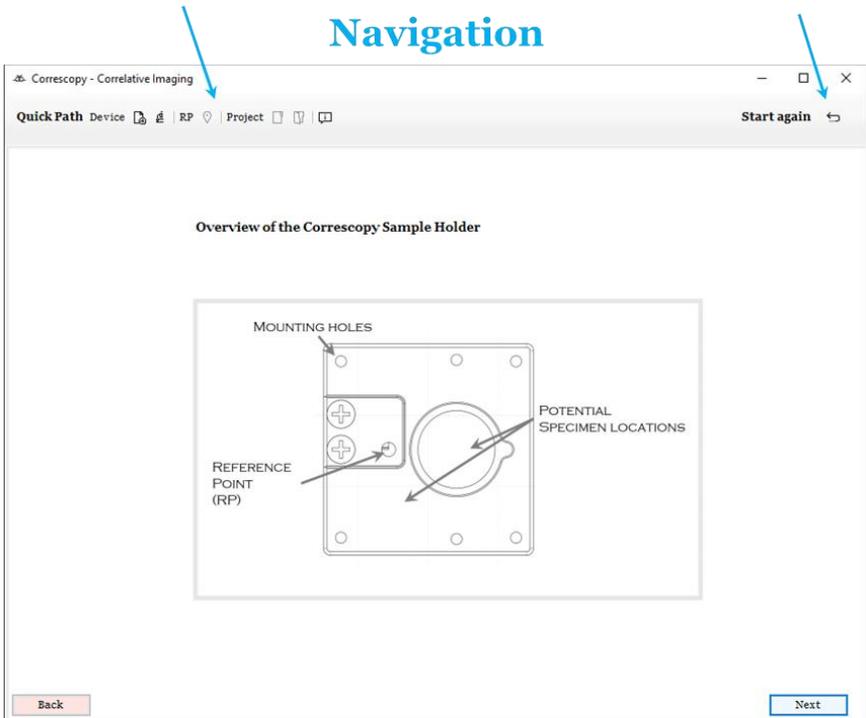
Software version

4.2. Software navigation

Depending on the path a user follows (standard or quick) the navigation buttons are displayed in different areas – a user can switch paths during the process. The quick path becomes available only after accepting terms and conditions of use. To move forward or backward use the navigation buttons on the bottom of the main window (back / next button). The restart of the whole process can be done by pressing the “restart button” causing the reset of all application parameters and collected user data.

Quick Path buttons

Restart button

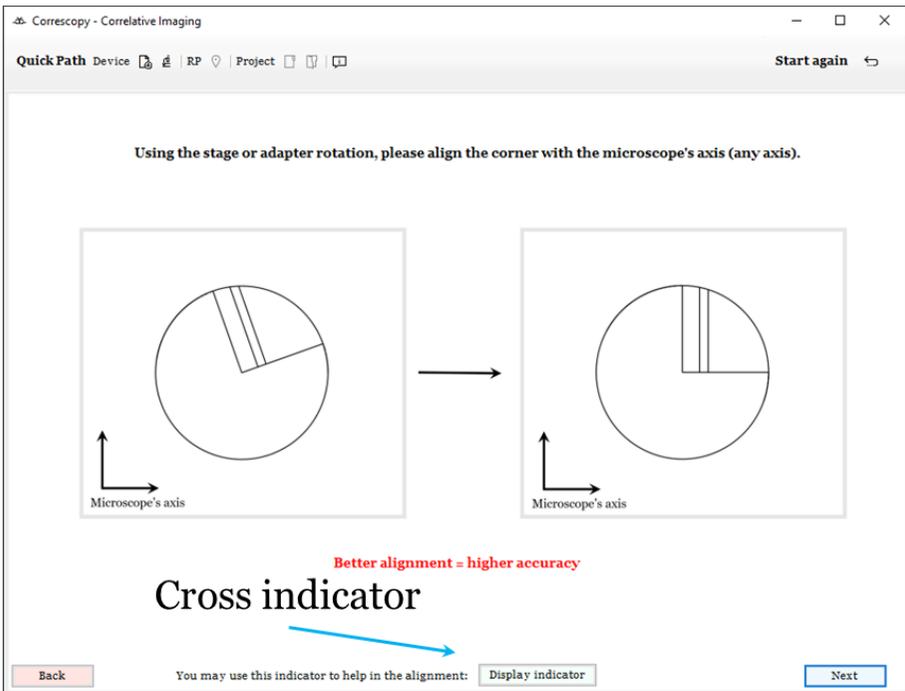


Back / Next step buttons

Caution: Before you move further please reset the digital image rotation to avoid confusion.

4.3. Correscopy sample holder calibration with a microscope

The first and the most important part which directly affects the accuracy of the localization of the region of interest is the calibration of the Correscopy sample holder with the microscope. To do it correctly, the user must align the edges of the reference crystal corner parallel to the microscope's stage X and Y axis (as shown below). To change the orientation of the sample holder against the microscope axis the microscope's stage rotation or microscope's adapter rotation (if provided) can be used. Once the alignment is done, to verify it, please move the stage along one of the axis and observe the distance from the crystal's edge. If the distance is not changing, the alignment is done correctly.



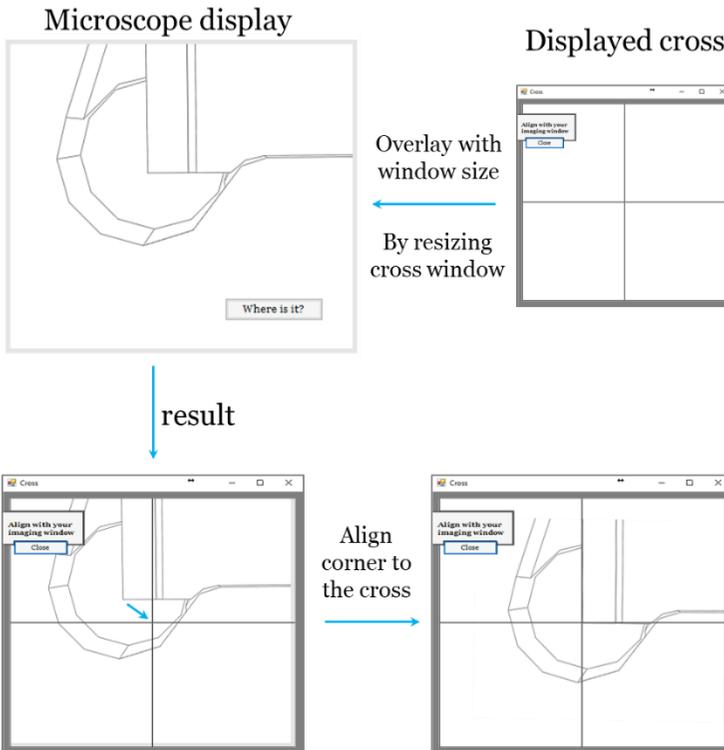
Caution:

- Do not use software image rotation for the calibration!
- If the alignment mark is not visible but the surface of the crystal is in focus, it means that the microscope's focal plane is on the other surface of the crystal. Only one side of the crystal has the alignment mark on it.

- *Please put attention as sometimes the detector/camera of the microscope is rotated. In that case focus on the distance between the edge of the crystal and the fixed point (like mouse cursor), if it is constant while moving along the axis, the edge and axis are parallel.*

Advise:

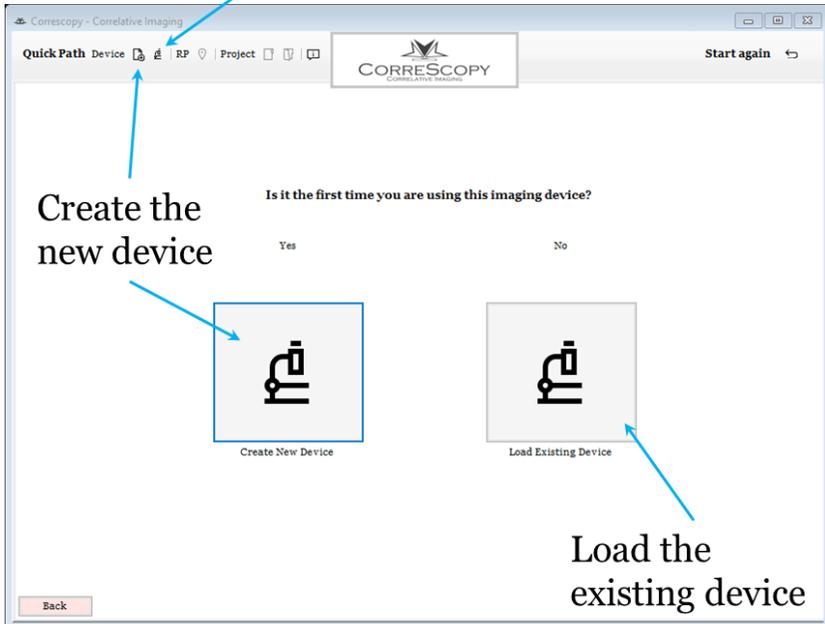
To make it easier, you can use the center cross lines displayed on the microscopes screen or use the provided cross indicator (if you run the Correscopy software on the microscopes PC), as displayed below. To align the displayed cross from the provided software, overlay the “cross” window evenly with the field of view as shown below. After the overlaying, a user must align the reference point (corner) with the cross indicator.



4.4. The Correscopy software calibration with the microscope

The next step is to calibrate the microscope with the Correscopy software. To do it, a user may define the imaging device or load the device file which was already created before. Thanks to this calibration the Correscopy software will learn what microscope the user is using for the imaging session.

Load the existing device



Create the new device

Load the existing device

4.4.1. Create a new device file

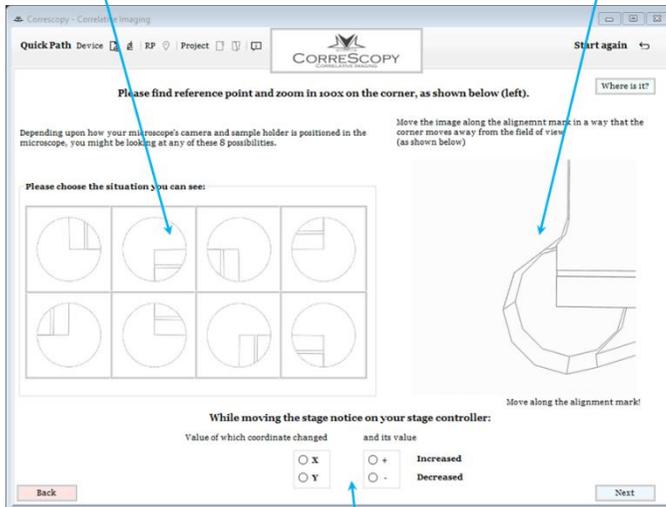
Imaging devices have various sample stages which work in various coordinate systems. In this step a user defines how a coordinate system is set on the particular imaging device.

Determining coordinates of the device

In the first step, the user needs to define the orientation of the reference crystal which he can observe on the screen (left).

The coordinate system must be defined for both X and Y axis including their directions (if coordinates values increase or decrease). To do so, bring the reference point (crystal's corner) into the field of view. Then move the stage as presented in the animation (right), while moving the stage a user must note on the imaging device which coordinate and how coordinates values changed. The change must be defined in the lower part of the window.

Animation with the Definition of the reference crystal orientation required movement of the crystal



Definition of the microscope stage coordinates

Caution: Make sure, the stage movement is exactly the same as on the animation!

Advice:

You can note down the values of the coordinates before and after each movement. From the noted coordinates you can see which coordinated changed (X or Y, the order does not matter) and if its value increased or decreased.

Example:

Before the movement

X = 1234

Y = 5678

After the movement

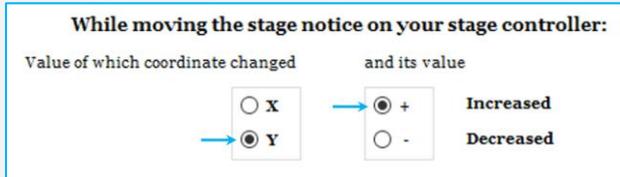
X = 1234

Y = 6123

From the table we can see that X did not change its value, while Y did.

Y coordinate changed from 5678 to 6123, we can see that its value increased.

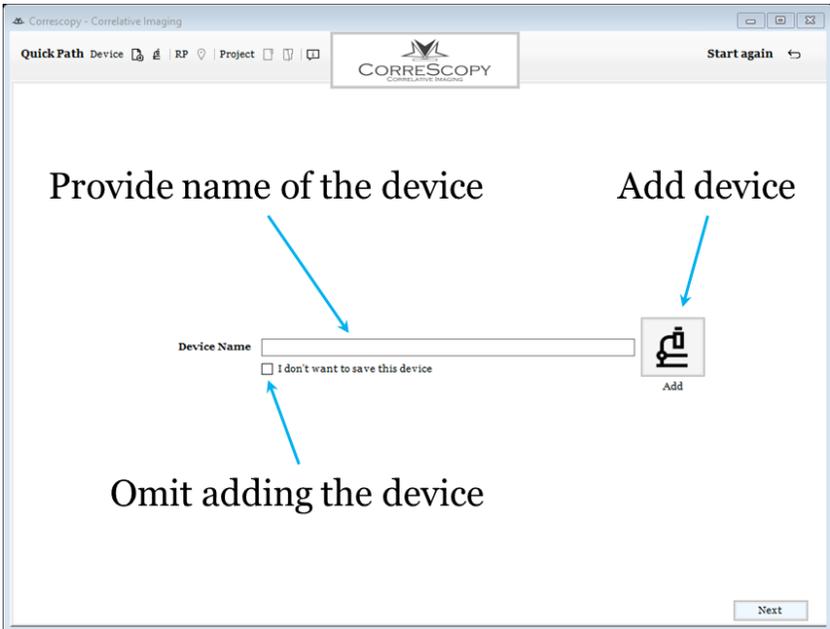
In the coordinates definition on the bottom of the window we indicate: Y and increased.



After the first coordinate is set, the other one must be set in the similar process in the next step, but for the second axis.

Save the device properties into the device library

After the Software and imaging device calibration is done, it can be saved and reused during next imaging sessions on this device. However, a user can also omit this step by checking the checkbox below the device name.



4.4.2. Load the existing device data

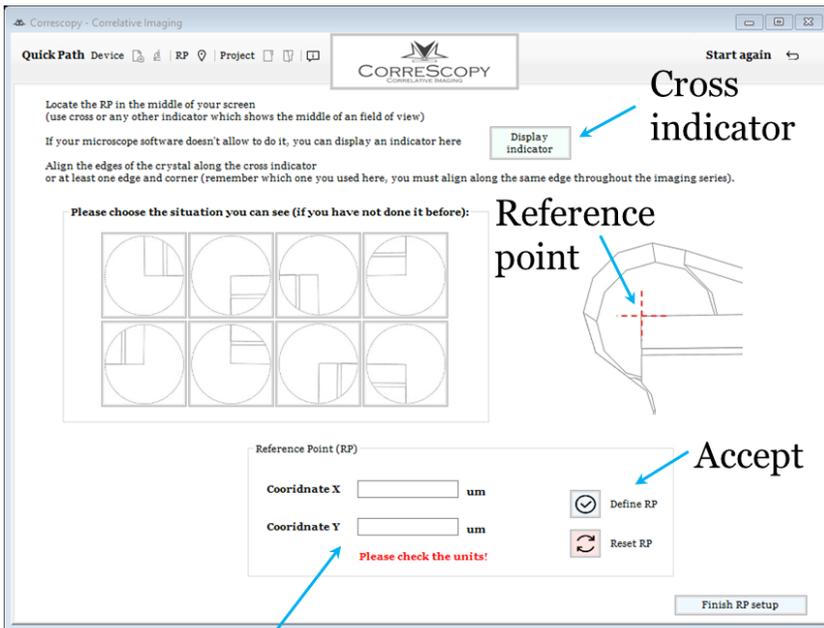
When a user decides to load the existing device calibration settings, the open dialog window will pop up asking to locate the file containing device parameters. The device parameter files have *.csd file extension.

Caution: *The sample holder must be installed on the imaging device in the exactly same way as it was when saving the calibration data file.*

4.5. Define the reference point

Once all the calibration steps are done, the user needs to define the coordinates of the reference point. The reference point (crystal’s corner) must be located again and ideally located in the center of the image.

The cross indicator (showing the very center of the field of view) can be displayed from the device manufacturer software or from the provided software. However, to use provided cross indicator, the software must be installed on the device’s computer (where the image is displayed).



Reference point coordinates
(as displayed on the microscope)

After the reference point is aligned, the coordinates (X and Y) must be saved into the software and accepted by the “define RP” button.

Caution: *In case of the damaged crystal corner:*

- *Damaged edge, the alignment can be done with one edge of the crystal (in this case a user must remember which edge was used for alignment, hence the same edge must be used on the other imaging device).*
- *Damaged corner, the reference point can be set at other location like the corner between the edge and alignment mark*

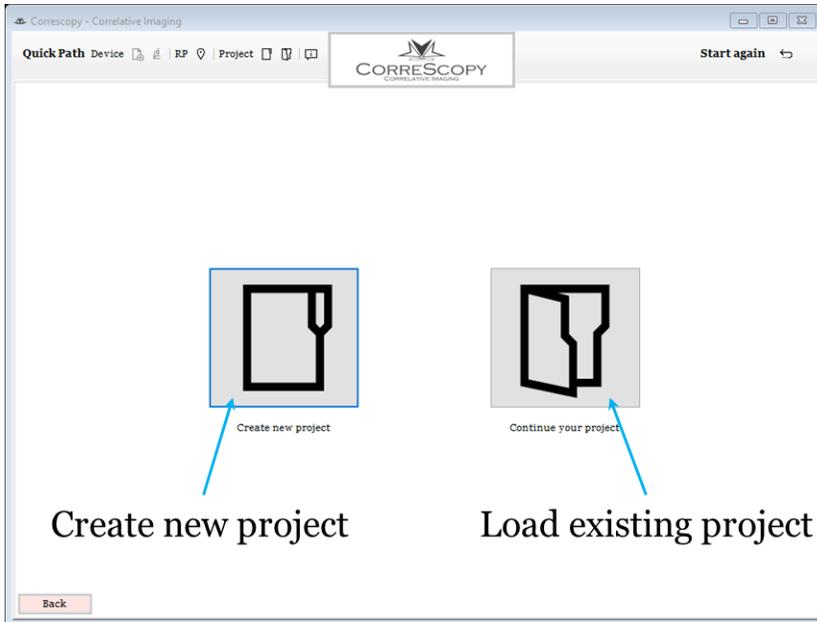
Note: *It is recommended to capture the image of the reference point for the later imaging session.*

4.6. Project definition

After successful setting of the device and the reference point, either a new project can be created or the existing project can be continued.

New project – define the regions of interest on the first microscope

Continue project – saved regions of interest will be located on next microscopes

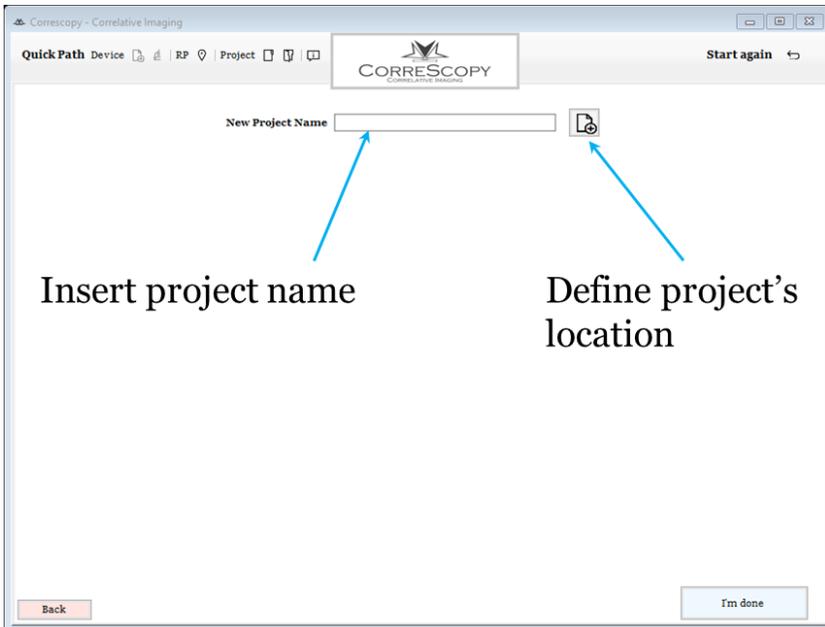


4.6.1. Create a new project

AT THIS POINT START IMAGING THE SAMPLE AND SEARCH FOR INTERESTING REGIONS

When creating a new project, the project name and the file location must be defined first. After setting them, the definition of user's ROIs becomes visible.

Note: the hole in the sample holder for transmitted signals is located along the alignment mark of the reference crystal.



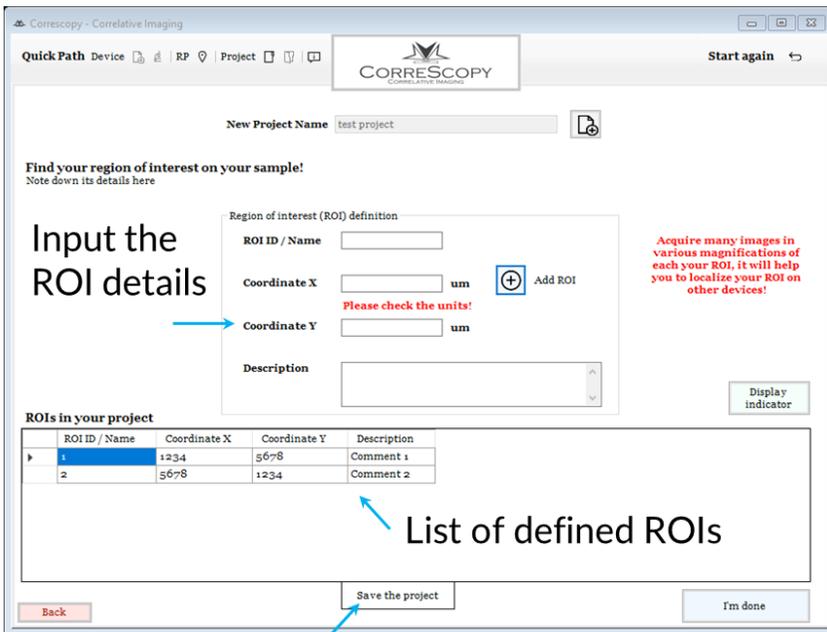
After locating the ROI, a user must provide the software with all the required details, such as the name, the ROI coordinates (read from a device) and the comments, then press 'add the ROI' button. The added ROI is saved into the table below which stores all the ROIs a user is interested in withing the project.

Caution: Use the same magnification for setting ROI location as it was used for setting reference point coordinates.

NOTE: It is highly recommended to acquire many images of each ROI in various magnifications. It will help significantly during next imaging sessions on different devices. As mentioned above the accuracy strongly depends on the user and the devices used, hence the ROI might be located slightly off the saved location. Moreover, a user is not able to memorize all the ROIs to make sure all found locations are exactly the same. The comparison between images acquired by different devices help in the final identification of the ROI.

The created table must be saved then by pressing the button ‘Save the project’ below. The table can be edited at any point of time, however, it should be done with extra care.

The cross indicator can also be displayed from this window to get the exact coordinates.

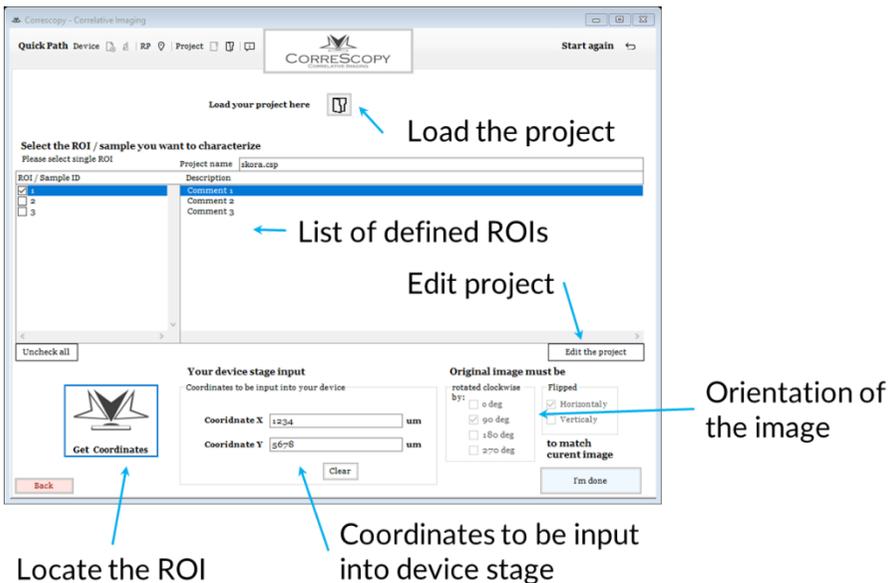


4.6.2. Load the existing project

In case of imaging the same sample on the next device, a user must load the project into the software. The project files with user ROIs have *.csp file extension. After loading the project, all user ROIs are displayed in the table (as shown below). By checking a single ROI row and pressing ‘Get coordinates’ button a user can get the X and Y coordinates which should be used to navigate the stage to locate saved ROI.

In case of any additional ROIs which were discovered on other device, or just in case of any mistakes, a user is able to edit the project (add/modify ROIs) at this stage by pressing “Edit project” button on the right side.

Note: the previously captured images of each ROI are crucial at this stage, they assure a user that imaging is done on exactly the same ROI.



The software also provides the information about the orientation between the originally saved images and currently viewed. To correctly orient the original image please first rotate it and then flip as indicated (strictly in this order).

5. Troubleshooting

Reference crystal edges have a lot of defects	Crystal edges could be damaged due to the improper handling of the sample holder. The device can be used correctly as long as the alignment can be done. It is recommended to replace the reference crystal or sample holder.
The corner of the reference crystal is damaged	On a very rare occasion when the corner's angle of the crystal is not 90° , the alignment can be done with a single edge of the crystal which is perfectly sufficient (in this case a user must remember which edge was used for alignment, hence the same edge is used on the other imaging device).
The alignment mark is missing at the surface of the crystal	The alignment mark is made on one side of the crystal (top). The device may be imaging the other surface (bottom). Change the focal plane to verify it.
Crystal's surface is charging heavily during SEM imaging	All crystal conduct small electrical currents while leaving the manufacturing site. However, the crystal may stop conducting due to the surface damage or contamination. To overcome it, a coating of the thin conductive layer can be applied on the crystal.
Sample holder does not fit into the adapter	The adapters' slot for CS stage is shaped so the stage fits in one setting. The slot has the additional space on one side.
The glue obstructs the objective	The glue should be applied only between the stage and the substrate. If the glue is outside the substrate's

rim on the stage, it can interfere with the microscope. Take a sharp blade and remove (cut) the excess of the glue.

A user cannot focus on the reference crystal surface

Make sure the used objectives have focal distance greater than the distance of the reference crystal surface from the top/bottom surface of the sample holder. It is recommended to use high focal distance lenses for the alignment/calibration purposes.

X and Y movement of the device stage is not sufficient to reach a predefined ROI

The maximum movement of the device stage should be determined before the imaging starts. A user should avoid the locations on the sample which cannot be reached by some of the devices included in the experiment.

The software crashes

Please report any problems with the software to Correscopy, bug fixes will be frequently released.

The device or the project file cannot be loaded as file is corrupted

If the data is important please send the file to Correscopy and we will try to help out.

A user cannot find the solution in this manual

Contact Correscopy for the solutions or for a softcopy of the manual's current version.



www.correscopy.com

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