Software Guide

**ZEN 2 (blue edition)**

The Shuttle & Find Module
1 Introduction

Illustration 1: SEM / LM system for correlative microscopy

The Shuttle & Find module in ZEN 2 is used for the relocation of sample positions in two different microscopes, e.g. a light microscope and a scanning electron microscope (SEM), and the correlation of two images to one merged image. This technique is called correlative microscopy or just “CorrMic”. It is used to combine the two worlds of scanning electron microscopy and light microscopy and brings it together in one image.

The samples can be mounted in special designed correlative holder systems (with three correlative calibration markers) from ZEISS. Also user-defined holder systems with three calibration markers can be used. Biological samples are mainly deposited on cover glasses or on TEM grids. In contrast to biological samples, the shape and size of material samples vary strongly. In respect to these requirements, the correlative holders were designed accordingly.

Illustration 2: Example of a correlative ZEISS sample holder
2 User Interface and Functions

2.1 Shuttle and Find tool

Here you choose and calibrate your sample holders.
### 2.1 Shuttle and Find tool

<table>
<thead>
<tr>
<th>Option</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enable Shuttle and Find</td>
<td><strong>Activated:</strong> The Shuttle and Find tool is active. You are able to start the correlative workflow by selecting and calibrating the sample holder. Deactivate the checkbox if you don’t want to use Shuttle and Find within your experiments.</td>
</tr>
<tr>
<td>Sample holder</td>
<td>Here you see the name and preview of the selected sample holder.</td>
</tr>
<tr>
<td>Select... button</td>
<td>Opens the <strong>Select Template</strong> dialog. There you select the preferred sample holder or define new holder templates, see Selecting the sample holder.</td>
</tr>
<tr>
<td>Calibrate... button</td>
<td>Opens the Sample Holder Calibration Wizard. There you can calibrate the selected sample holder.</td>
</tr>
<tr>
<td>Apply to Image button</td>
<td>Only visible if the <strong>Show All</strong> mode is activated. Use this button only, when you forgot to calibrate the holder before you acquire the image. Applies a calibration to an acquired image. Do not remove the sample out of the correlative holder between image acquisition and calibration.</td>
</tr>
</tbody>
</table>

**Shuttle and Find tool for SEM**

Only visible if you have started the ZEN 2 SEM software.

The tool window is adapted to the requirements of the correlative workflow on a SEM. Therefore three additional buttons are available.

Illustration 5: Shuttle and Find tool in ZEN SEM software
### 2.2 S&F view

Besides the Shuttle and Find tool in the Left Tool Area, the S&F (Shuttle & Find) view is visible in the Center Screen Area of the ZEN software. If the S&F view is selected, the S&F tab and S&F Correlation tab will appear as specific view options under the image area.

<table>
<thead>
<tr>
<th>Button</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scale bar</td>
<td>Adds a scale bar to the snapped (acquired) image.</td>
</tr>
<tr>
<td>Annotation bar</td>
<td>Adds an annotation bar to the snapped (acquired) image.</td>
</tr>
<tr>
<td>Select...</td>
<td>By clicking on this button a dialog opens to select parameters for the annotation bar. You can select max. 9 parameters for the annotation bar.</td>
</tr>
</tbody>
</table>

*Illustration 6: Shuttle and Find View*
2.2.1 S&F tab

Here you find helpful options and tools to draw in and relocate regions of interests (ROIs) or points of interest (POIs) within the sample image.

2.2.1.1 Options

To show the section in full, click on the arrow button.

Illustration 7: S&F tab - Options

<table>
<thead>
<tr>
<th>Options</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mirror Image</td>
<td>Here you can mirror the image display horizontally or vertically by using the two buttons. The orientation of the images depends on the microscope (upright/inverted) and orientation of the sample holder.</td>
</tr>
<tr>
<td>Keep tool</td>
<td><strong>Activated:</strong> Keeps the current tool active. That’s helpful if you want to draw in more than one ROI/POI.</td>
</tr>
<tr>
<td>Auto color</td>
<td><strong>Activated:</strong> Uses a new color for each new graphical element which is drawn in.</td>
</tr>
<tr>
<td>Snap to Pixel</td>
<td><strong>Activated:</strong> Draws in graphical elements using the pixel grid.</td>
</tr>
<tr>
<td>Use fine calibration value</td>
<td>Active only, if you have defined an offset, see Fine Calibration of the sample holder. <strong>Activated:</strong> Uses the measured fine calibration. The defined offset value is only valid for the loaded image. If another image is loaded or if you close the dialogue, the offset value will be deleted.</td>
</tr>
<tr>
<td>Double click in image to move stage</td>
<td><strong>Activated:</strong> Moves the stage to the position you have double-clicked on.</td>
</tr>
</tbody>
</table>
### 2.2 S&F view

<table>
<thead>
<tr>
<th><strong>Options</strong></th>
<th><strong>Description</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Refocus after stage movement</td>
<td><strong>Activated:</strong> The software calculates the correct z-value for re-focusing out of the image and the marker calibration information.</td>
</tr>
<tr>
<td>Move the stage to load position before xy movement</td>
<td><strong>Activated:</strong> Moves the stage to the load position before the stage moves.</td>
</tr>
<tr>
<td>Show splitter view</td>
<td><strong>Activated:</strong> Activates <strong>Splitter Mode</strong> in the <strong>Center Screen Area</strong>.</td>
</tr>
</tbody>
</table>

#### 2.2.1.2 Regions, Find and Dimensions

**Illustration 8:** S&F tab - Regions, Find, Dimension

**Regions and Find tool bar**

<table>
<thead>
<tr>
<th><strong>Button</strong></th>
<th><strong>Description</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Select</td>
<td>By clicking on this button you activate the selection mode (button is marked blue) to select the ROIs or POIs in the image area (if drawn in). If you are in another mode, you can switch back to the selection mode using this button.</td>
</tr>
<tr>
<td>Draw Region of Interest</td>
<td>By clicking on this button you can draw in a rectangular ROI (<strong>Region of Interest</strong>) into the acquired image.</td>
</tr>
<tr>
<td>Draw Point of Interest</td>
<td>By clicking on this button you can draw in a POI (<strong>Point of Interest</strong>) / marker point into the acquired image.</td>
</tr>
</tbody>
</table>
2 User Interface and Functions | 2.2 S&F view

<table>
<thead>
<tr>
<th>Button</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Center</td>
<td>Moves the stage to the center of the loaded image. The center of the field of view corresponds to the center of the loaded image.</td>
</tr>
<tr>
<td>ROI / POI</td>
<td>Moves the stage to the selected ROI / POI. The center of the field of view corresponds to the center of the selected ROI / POI.</td>
</tr>
<tr>
<td>Show stage position</td>
<td>Shows the current stage position as a rectangle in the loaded image.</td>
</tr>
</tbody>
</table>

**Dimension section**

Here you see coordinates and dimensions of the selected graphical element in the list. If the *Scaled pixel* checkbox is activated, the unit is µm, otherwise Pixel.

- Parameter \(X\): Shows the horizontal position (x coordinate) of the center of the graphical element.
- Parameter \(Y\): Shows the vertical position (y coordinate) of the center of the graphical element.
- Parameter \(W\): Shows the width of the graphical element.
- Parameter \(H\): Shows the height of the graphical element.

**Graphical elements list**

Here you see the list of all ROI / POI which are drawn in. The following table describes the list columns and its functions:

<table>
<thead>
<tr>
<th>List columns</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eye symbol</td>
<td>Shows or hides the ROI / POI in the image.</td>
</tr>
<tr>
<td>Lock symbol</td>
<td>Locks a ROI / POI to prevent changes.</td>
</tr>
<tr>
<td>Type</td>
<td>Displays the icon for the tool type (ROI/POI). To format a graphic element, double-click on the icon. The <strong>Format Graphic Elements</strong> dialog opens.</td>
</tr>
<tr>
<td>ID</td>
<td>Only visible if the <strong>Show All</strong> mode is activated. Displays the ID for the graphic element. To do this, activate the checkbox at the corresponding list entry.</td>
</tr>
</tbody>
</table>
### S&F view

#### List columns

<table>
<thead>
<tr>
<th>List columns</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Only visible if the <strong>Show All</strong> mode is activated. Displays annotations for a graphic element (ROI). To do this, activate the checkbox at the corresponding list entry. If you double-click on a row, the <strong>Format Graphic Elements</strong> dialog opens. Within this dialog, for example, you can change colors of the graphical element or choose other annotations you want to have displayed within the graphical element (e.g., focus position, exposure time).</td>
</tr>
<tr>
<td>M</td>
<td>Only visible if the <strong>Show All</strong> mode is activated. Displays measurement data for a graphic element. To do this, activate the checkbox at the corresponding list entry.</td>
</tr>
<tr>
<td>Name</td>
<td>Displays the name of the graphic element. To change the name, double-click in the Name field. Then enter the text of your choice.</td>
</tr>
</tbody>
</table>

#### 2.2.2 S&F Correlation tab

Here you find all functions to overlay (correlate) two images.

**Options**

To show the section in full, click on the **arrow** button.

![Illustration 9: S&F Correlation tab](image)
### User Interface and Functions

#### 2.2 S&F view

<table>
<thead>
<tr>
<th>Option</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Transform</strong></td>
<td>Here you select which image will be transformed. During transformation a pixel in the overlay image is calculated by using pixels of the two original images that shall be overlaid / merged.</td>
</tr>
<tr>
<td><strong>Interpolation</strong></td>
<td>Here you can select one of the following interpolation methods:</td>
</tr>
<tr>
<td><strong>Nearest Neighbor</strong></td>
<td>The gray value of the resulting pixel in the overlay image is made of a pixel which is located next. This interpolation method is very fast.</td>
</tr>
<tr>
<td><strong>Linear</strong></td>
<td>The resulting or calculated pixel in the overlay image is assigned to a gray value, which is the result of a linear combination of gray values derive from pixels located nearby (in the original image).</td>
</tr>
<tr>
<td><strong>Cubic</strong></td>
<td>The calculated pixel in the overlay image is assigned to a gray value, which is calculated by means of a polynomial function using gray values of pixels in the original images; these pixels are located nearby the calculated pixel.</td>
</tr>
<tr>
<td><strong>Mirror image</strong></td>
<td>Here you can mirror the image horizontally or vertically. Therefore simply click on the corresponding button. Mirroring an image is necessary, when the loaded image shows a different orientation than the live image.</td>
</tr>
<tr>
<td><strong>Show Correlation</strong></td>
<td><strong>Activated</strong>: Opens the correlated image in a new image document / new container.</td>
</tr>
<tr>
<td><strong>Set correlation points</strong></td>
<td>Enables you to set 6 points (3 points in each image) as correlation markers in a row, see Correlating two loaded images [31].</td>
</tr>
<tr>
<td><strong>Reset</strong></td>
<td>Deletes all correlation points in the images.</td>
</tr>
<tr>
<td><strong>Create Correlation</strong></td>
<td>Active only, if all correlation points are set in both images. Creates a correlative overlay image. A third image container with the correlated image will be opened in the Center Screen Area and the Show Correlation checkbox will be activated automatically.</td>
</tr>
</tbody>
</table>
2.3 Sample Holder Calibration Wizard

With the Sample Holder Calibration Wizard you calibrate your selected correlative sample holder. The wizard is opened via the Shuttle and Find tool. Make sure that you have activated the Shuttle and Find tool and selected a sample holder, see Selecting the sample holder [p. 21].
### 2.3.1 General Options

![Sample Holder Calibration Wizard Options](image)

**Illustration 10: Sample Holder Calibration Wizard Options**

<table>
<thead>
<tr>
<th>Option</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Save marker images</td>
<td><strong>Activated:</strong> the marker images are saved during the calibration. The images can be used to check the calibration afterwards. Click on the Select Folder (...) button to select a storage folder.</td>
</tr>
</tbody>
</table>
| Move the stage to load position before x/y movement | **Activated:** the stage will move to load position before moving to the next correlative calibration marker.  
In case of using an AxioObserver, the objective revolver moves to load position. |
| Automatic movement to next marker            | **Activated:** By clicking on the Next button within the wizard the stage moves automatically to the next calibration marker. |
| Use Autofocus at each marker position       | This option is active only if the Automatic movement to next marker position checkbox is activated.  
**Activated:** the focus is adjusted automatically after moving to the next marker position. |
### 2.3 Sample Holder Calibration Wizard

**Option** | **Description**  
---|---  
Use automatic marker detection | **Activated:** The software will try to detect the small calibration marker automatically.  
Use settings for marker detection | This option is active only if the **Use automatic marker detection** checkbox is activated.  
 | **Activated:** shows settings for marker detection (see description below). Here you select the properties of the calibration markers.  

#### Settings for marker detection

Only visible if the **Use settings for marker detection** checkbox is activated.

<table>
<thead>
<tr>
<th>Option</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threshold marker detection: high – low</td>
<td>A low threshold for marker detection is used when the dimensions of the correlative L markers cannot be recognized precisely, e.g. when the sample holder is slightly filthy.</td>
</tr>
<tr>
<td>Marker color</td>
<td>Here you select the color of the markers displayed in the live image.</td>
</tr>
<tr>
<td></td>
<td><strong>White:</strong> the marker is displayed white on a dark background.</td>
</tr>
<tr>
<td></td>
<td><strong>Black:</strong> the marker is displayed dark on light background.</td>
</tr>
<tr>
<td></td>
<td><strong>Auto:</strong> the marker color is set automatically.</td>
</tr>
<tr>
<td>Marker orientation</td>
<td>Here you need to set the orientation of the L-markers on your sample holder. Click on the corresponding button to select the orientation of the calibration marker which you can see in the live image</td>
</tr>
</tbody>
</table>

If you click on the **Next** button you will move to the next step of the wizard.
2.3.2 Calibration functions

In steps 2-4 of the wizard you will be guided through the calibration procedure.

Illustration 11: Sample Holder Calibration Wizard

<table>
<thead>
<tr>
<th>Option</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Holder position</td>
<td>Move to Position 1 button</td>
</tr>
<tr>
<td></td>
<td>Moves the stage to marker position 1. This is possible only if the first position was set before and x/y coordinates are given.</td>
</tr>
<tr>
<td></td>
<td>Current button</td>
</tr>
<tr>
<td></td>
<td>Only visible for marker position 2 and 3.</td>
</tr>
<tr>
<td></td>
<td>Moves the stage to the current marker position. This is possible only if the current position was set before and x/y coordinates are given.</td>
</tr>
<tr>
<td>Stage movement to the next marker</td>
<td>Here you can change the movement of the stage in x or y direction. This is necessary if during calibration the stage moves in the wrong direction.</td>
</tr>
<tr>
<td>Marker position</td>
<td>By clicking on the Set button, the actual marker position will be confirmed.</td>
</tr>
</tbody>
</table>
3 The Shuttle & Find Workflow

3.1 Settings and image acquisition with the light microscope

Before acquiring an image with the light microscope and using it for correlative microscopy, it is necessary to make general settings e.g. stage calibration, camera orientation, calibrating objectives and setting the correct scaling. Please notice that we do not describe all these topics within this guide as we focus on the Shuttle and Find workflow only.

Furthermore we will not describe basic functionality of ZEN 2 software in this guide, like program layout or general image acquisition topics. If you want to read more about these topics, please read the online help of the software.

3.1.1 Mounting the sample holder to the LM

Illustration 12: Mounting of cover glasses or TEM grids

1. Place the cover glass (2) in the suitable sample holder and fix it.

In case of using the holder Life Science Cover Glass 22x22:
- Remove the clamping frame (1) using tweezers.
- Insert the cover glass (2) in the sample holder (3).
- Slide in the clamping frame into the sample holder until the clamps are clicking into place (4).

In case of using the holder **Life Science for TEM grids**:
- Lift the spring of the appropriate position and turn it sidewards (5).
- Insert the TEM grid (6) into the provided holding spot of the holder and fix it with the spring (7).

*Illustration 13: Inserting a sample holder*

2 Insert the sample holder (1) into the mounting frame of the microscope stage in the following way:
- For inverted stands, see (3).
- For upright stands, see (2).
3.1.2 Starting the LM software

For correlative microscopy with light microscopes ZEN 2 software has to be installed. In addition you need to licence the Shuttle and Find module.

**Procedure**

1. Start ZEN 2 by clicking on the corresponding program icon on your desktop. Following window will appear.

2. Click on the ZEN pro or ZEN system button to start ZEN 2.

The software will start now. Make sure that you have activated the Shuttle and Find module in the menu **Tools | Modules Manager**...
In the **Left Tool Area** click on the *Acquisition tab* and open the **Shuttle and find** tool.

You have successfully started the software. Now you can start working with the **Shuttle and Find** module.
3.1.3 Selecting the sample holder

**Prerequisites**
- You are in the Shuttle and Find tool.

**Procedure**

1. Within the Shuttle and Find tool activate the checkbox.

2. Click on the Select… button to open the Select Template dialog and to choose the correlative holder you want to use. Different types of correlative holders are available, see Appendix Correlative Sample Holders [34].

3. In the Select Template dialog select the correlative holder you want to work with. If you want use your own sample holders, click on the + (Add) button below the list and follow the instructions in the chapter Defining new sample holder templates [22].

4. Click on the Ok button to close the dialog.
You can now continue with the calibration of the sample holder, like it is shown in the chapter Calibrating the sample holder [p. 23]. The calibration of the sample holder is mandatory to acquire images.

### 3.1.4 Defining new sample holder templates

With this dialog you can define new correlative holders in addition to the existing holder templates. It is not mandatory to use correlative holders from ZEISS. User-defined correlative holders with 3 fiducial markers can be used as well.

**Procedure**

1. To open the dialog click on the **Add (+)** button in the **Select Template** dialog. This dialog can be opened via the Shuttle and Find tool.

   ![New Template dialog](image)

   The **New Template** dialog opens.

2. Type in a name for the new holder / sample carrier. An image of the new holder can be loaded as well.

3. Insert the distances (in millimeters) between the first and the second marker and between the second and third marker.

   The distances can be determined using the **Stage Control** dialog accessible via the **Light Path** tool on **Locate** tab. We recommend to do this before you start the New template dialog. Write down the distances to be prepared to enter them within the New Template dialog.

   1. Activate the live view in the Center Screen Area by clicking on the **Live** button in the Locate tab.

   2. Navigate the stage manually to the calibration marker on the sample holder by means of the joystick and note the x/y-coordinates of the marker.

   3. Repeat this procedure for all three markers and calculate the distances between marker 1 and marker 2 and between marker 2 and marker 3, respectively.
3 The Shuttle & Find Workflow | 3.1 Settings and image acquisition with the light microscope

Illustration 14: Stage Control dialog

3.1.5 Calibrating the sample holder

The correlative sample holders have three fiducial markers enabling a three point calibration (signed with the numbers 1-2-3). The calibration markers consist of one small (length 50 µm) and a large L-shape marker (length 1 mm). The bigger marker is used for coarse orientation, whereas the smaller marker is used for the calibration.

3.1.5.1 Preparing calibration

Procedure 1 Activate the live view in the Center Screen Area by clicking on the Live button in the Acquisition tab.

2 Navigate the stage manually to the first calibration marker on the sample holder (marked with No. 1) by means of the joystick. It is enough if you move the stage to the larger L-shaped calibration marker. The smaller marker will be detected automatically within the Sample Holder Calibration Wizard. To locate the marker positions we recommend to use a dry objective with low magnification (5x – 20x).
Click on the **Calibrate...** button, to open the **Sample Holder Calibration Wizard**.

![Sample Holder Calibration Wizard](image)

### 3.1.5.2 Setting the options

In step 1 of the wizard, the following options should be activated to follow our recommended workflow:

**Procedure 1** Check if the **Automatic movement to next marker** checkbox is activated.

This will automatically move the stage to the next marker position after you have set a marker position.

**Procedure 2** Check if the **Use automatic marker detection** checkbox is activated.

The software will try to find the correct positions of each marker automatically.
If you need to change the marker color, or check if the marker orientation is set correctly, activate the **Use settings for marker detection** checkbox to access these functions.

4. Click on **Next** to move to the next wizard step.

### 3.1.5.3 Calibrating the sample holder

**Procedure**

1. Click on the **Set** button to detect the first marker position.

   An automatic stage calibration will be performed. After the stage calibration, the system will try to detect the marker position of the small marker automatically.

   A message appears which asks if the marker was detected correctly.

2. Click on **Yes** to confirm the message.

   **Info**

   If the marker was not detected correctly, you have the possibility to set the marker position manually. Move the stage in that way, that the crosshair is located directly on the L marker and click on “Set”.

3. Click on **Next** to move to the next step of the wizard.

   The stage will automatically move to the next (coarse) marker position. If the stage moves into the wrong direction you can use the **invert X** / **invert Y** buttons to correct the movement direction.

4. Repeat the previous steps and set marker position 2 and 3 accordingly.

   After setting marker position 3 you will find a green check mark icon which shows you that the calibration was successful.

5. Click on the **Finish** button to save calibration and close the wizard.
3.1.6 Acquiring the LM image

Image acquisition can be done as you are used to do it within ZEN software. The file format for Shuttle and Find data is the common *.czi file format. Saved images can be loaded in ZEN via the menu File | Open.

After image acquisition the next step in the correlative workflow is to define / draw in ROIs / POIs in your image. Therefore you can use the Region tools on the S&F tab, see Regions, Find and Dimensions [9].

Illustration 16: LM image

3.2 Shuttle and Find sample positions at the electron microscope

Now you can transfer (Shuttle) the sample and the LM (Light Microscope) image file (.czi) to the SEM (Scanning Electron Microscope). There you can easily relocate (Find) the same sample positions and acquire a corresponding image within the ZEN 2 SEM software. Therefore exactly the same steps have to be done as for the light microscope.

3.2.1 Mounting the sample holder to the SEM

For imaging your sample in the SEM, insert the sample holder (2) in the special SEM adapter (1) and mount it to the SEM.
3 The Shuttle & Find Workflow | 3.2 Shuttle and Find sample positions at the electron microscope

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**Info**

The arrow of the sample holder has to face the arrow of the SEM adapter.

*Illustration 17: Sample holder mounted in SEM adapter*

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### 3.2.2 Starting the ZEN SEM software

For correlative microscopy with scanning electron microscopes **SmartSEM** and ZEN 2 SEM have to be installed. SmartSEM is still the control software of the scanning electron microscope. ZEN 2 SEM comes as an add-on for SmartSEM to perform correlative microscopy and using Shuttle&Find on a SEM.

**Prerequisites**

- You have started SmartSEM.

**Procedure**

1. Start ZEN 2 software by clicking on the corresponding program icon on your desktop.

   Following window will appear.

   ![ZEN 2 software window](image)

2. Click on the **SEM** button to start.
The Shuttle & Find Workflow

3.2 Shuttle and Find sample positions at the electron microscope

You will see the program interface with a reduced user interface comparing to ZEN 2. In the Left Tool Area the **SEM Acquisition** tab and the **Processing** tab are available only. On the SEM Acquisition tab you will find the **Shuttle and Find** tool which has 3 additional buttons at the lower part of the tool.

3.2.3 Selecting the sample holder

This step is exactly the same step like for the light microscope, so please read the chapter Selecting the sample holder [21] if you want to know the exact steps which you have to perform.

3.2.4 Calibrating the sample holder

Like the step before this step is exactly the same like for the light microscopy, so please refer to the chapter Calibrating the sample holder [23] for details.

**Info**

The calibration of the sample holder has to be done on both systems the LM and the SEM. Otherwise the relocation of your sample positions or ROIs / POIs stored in the image won’t be successful.
3.2 Shuttle and Find sample positions at the electron microscope

3.2.5 Acquiring the EM image

Procedure

1. Load your LM image in ZEN 2 SEM (.czi).

   The image will be displayed in the center screen area.

2. Activate the Live mode.

   You will see the Live image from the SEM. Notice that all settings for the SEM image have to be done within the SmartSEM software.

3. Activate the S&F View in Center Screen Area.

4. Go to the S&F tab.

Info

Note that for Shuttle & Find the beam shift must be switched off. The beam shift is deactivated in SmartSEM as follows:

- Call up the shortcut menu Center Point / Feature by right-clicking on the Stage property page.
- Select Center Point / Feature and select Stage only.
Check if the **Double click in image to move stage** and **Show splitter view** checkboxes are activated (default setting).

In the left image container you see the live image from the SEM. The right image container is empty.

Drag the loaded LM image from the **Images and Documents** gallery into the empty image container.

Now you can easily relocate sample positions by double clicking within the image or on the ROI/POI button (if ROI / POI are drawn in and selected) on S&F tab.

For image acquisition you have to use the **Snap** button within ZEN 2 SEM. Notice that we will not describe setup and image acquisition with the SEM. Please read the online help or user guide for the SEM software.

*Illustration 18: SEM and LM image*
3.2.6 Fine Calibration of the sample holder

The precision of relocation can be improved by determination of an offset value. This value describes the position offset between the loaded image and the live image. The defined offset value is only valid for the loaded image. If another image is loaded or if you close the dialogue, the offset value will be deleted.

Prerequisites
- An offset is visible when you try to relocate marker positions on the live image comparing to the LM image.

Procedure
1. Click on the Set Offset button.
   The stage moves to the selected marker position. Then a message appears which asks you to move the stage to the correct position.
2. Move the stage manually to the correct position by using the joystick.
3. Confirm the message by clicking on the OK button.

Now you can repeat the relocation. The positions should be identical now.

3.3 Image Correlation

3.3.1 Correlating two loaded images

Prerequisites
- You have acquired and loaded two images containing S&F calibration data (e.g. LM / SEM) to be correlated. If the images are not oriented identically you can use the Mirror Image buttons under Options on the S&F Correlation tab.
- You see the two images next to each other (splitter view) in the center screen area. If not, drag your images from the Images and Documents gallery into the center screen area.

Procedure
1. Click on the Set correlation points button in the S&F Correlation tab.
   The curser will change to a pipette symbol.
2. Click in the left image to set a correlation point. Set all 3 marker points in the left image first, before you set the corresponding 3 markers in the right image. If a correlation point is set, a check mark icon will appear in front of the corresponding point.
   Make sure that the positions in both images are identical. After you have set all 6 points the cursor will be changed backwards from the pipette to the arrow.
3. Click on the Create Correlation button.
   The correlated image will be generated and opened in a new image container.
3.3 Image Correlation

Illustration 19: Correlated image

Tips & Tricks

- It is also possible to set each correlation point individually. Therefore under Left Image / Right Image click on the Arrow button behind a point (e.g. Point 1). Then click on the desired position within the image.

- To improve the accuracy of the identification you can zoom into the images by using the mouse wheel.

- To edit/move a point, click on the point you would like to move. When the point is marked with a dashed rectangle you are able to move the point by holding the left mouse button. Alternatively, below Left Image / Right Image click on the points Arrow button you want to move and click on a new position within the image.

3.3.2 Correlating a live image and loaded image

Prerequisites

- You have activated the Live mode.

Procedure

1. Select the S&F view in the Center Screen Area and click on the S&F Correlation tab.

   The splitter view will become visible in the Center Screen Area. In the left image container you see the live image.

2. Drag the corresponding LM image from the Documents and Images Gallery into the Center Screen Area.
3 Click on Set correlation points button to set the correlation points. Always start with setting 3 points in the left (live) image, then continue with setting the identical points (in the same order) in the loaded image.

4 After setting all 6 correlation points the image correlation will be performed automatically.

The correlated image will be visible in a third image container below the live image and the loaded image.
## Appendix

### 4.1 Correlative Sample Holders

<table>
<thead>
<tr>
<th>Name</th>
<th>Image</th>
</tr>
</thead>
<tbody>
<tr>
<td>Life Science cover glass 22x22</td>
<td><img src="image1" alt="Image" /></td>
</tr>
<tr>
<td>Life Science for TEM Grids</td>
<td><img src="image2" alt="Image" /></td>
</tr>
<tr>
<td>EVO - Life Science cover glass 22x22</td>
<td><img src="image3" alt="Image" /></td>
</tr>
<tr>
<td>MAT Flat Stubs a</td>
<td><img src="image4" alt="Image" /></td>
</tr>
</tbody>
</table>
### 4.1 Correlative Sample Holders

<table>
<thead>
<tr>
<th>Name</th>
<th>Image</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAT Flat Stubs</td>
<td><img src="image" alt="MAT Flat Stubs Image" /></td>
</tr>
<tr>
<td>MAT Universal A</td>
<td><img src="image" alt="MAT Universal A Image" /></td>
</tr>
</tbody>
</table>
### 4.1 Correlative Sample Holders

<table>
<thead>
<tr>
<th>Name</th>
<th>Image</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAT Universal B_A</td>
<td><img src="image" alt="Image of MAT Universal B_A" /></td>
</tr>
<tr>
<td>MAT Universal B_B</td>
<td><img src="image" alt="Image of MAT Universal B_B" /></td>
</tr>
</tbody>
</table>
4.2 Shuttle and Find with an EVO 10

To use Shuttle and Find (SW and correlative holders) with an EVO 10 make sure that the stage limits (for x, y and z) are set as follows:

![Stage Limits Table]

**NOTICE**

If you set a wrong orientation the stage cannot be moved to all correlative markers because of the stage limits for the EVO 10.

- The holder has to be mounted into the EVO in that the way that the correlative markers (1) and (2) have to be near the chamber door whereas marker (3) is located furthest from the chamber door (see **Mounting A/B**).
- If necessary, the SEM image can be rotated according to the LM image using the option **Scan Rotate** in SmartSEM.
4.2 Shuttle and Find with an EVO 10

Mounting A:

Mounting B: