The purpose of this document is to introduce the concept of Scalings and guide the user through creating and using Scalings with AxioVision 4.X.X.  Any questions or concerns can be directed to the ZEISS Product and Application Support group at 1-800-509-3905 or support@zeiss.com

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## What are Scalings?

The term Scalings describes the information the software needs to determine the width and height of individual pixels ("picture elements") in a digital microscope image. In the case of Z-stack images, there is an additional scaling dimension: the "thickness" of an individual slice and the "voxels" ("volume elements") have a third dimension in addition to width and height, i.e. thickness.

## Why are Scalings needed?

Scaling information allows you to calculate the size of an image. For example the image width equals the number of pixels in x-direction [pixels] multiplied by the pixel size in x-direction [μm / pixel] and the image height equals the number of pixels in y-direction [pixels] multiplied by the pixel size in y-direction [μm / pixel].

In addition to calculating the overall size of an image, scaling information allows the user to measure structures in an image and to assign scale bars.

Without the pixel sizes, AxioVision is unable to acquire MosaiX images since the motorized scanning stage needs accurate information on the size of acquired images in order to scan and correctly align the images of a given area.

Finally, scaling information is required for deconvolution processing.

## What are the system components that determine Scalings?

The pixel size of a digital microscope image is determined by the following microscope components and camera parameters:

1) The magnification of the objective used.

2) The magnification factor of the optovar/tube lens or the optovar cube in the reflector turret.

3) The magnification factor of the camera adapter(s) used.

4) The pixel size of the digital camera's CCD sensor.

5) The binning mode used on the digital microscope camera.

6) And finally, in the case of a stereomicroscope, the zoom factor.

# How do I acquire Measured Scalings?

Using the AxioVision Scalings Wizard, you can acquire images of a stage micrometer and calculate the sizes of the pixels for each of the images, for all available objectives, optovar / tube lens or zoom settings, and camera modes. Each of these definitions can be saved as a Scalings file. Scalings generated this way are referred to as **Measured Scalings**.

Start by creating images of a stage micrometer using all of the different system component configurations for which you wish to create Scalings. You can save these images in a dedicated folder or leave them open in AxioVision.

1. Select **Measure** and **Scalings** from the menu bar, or select **Scalings** and **Advanced** from the Workarea.

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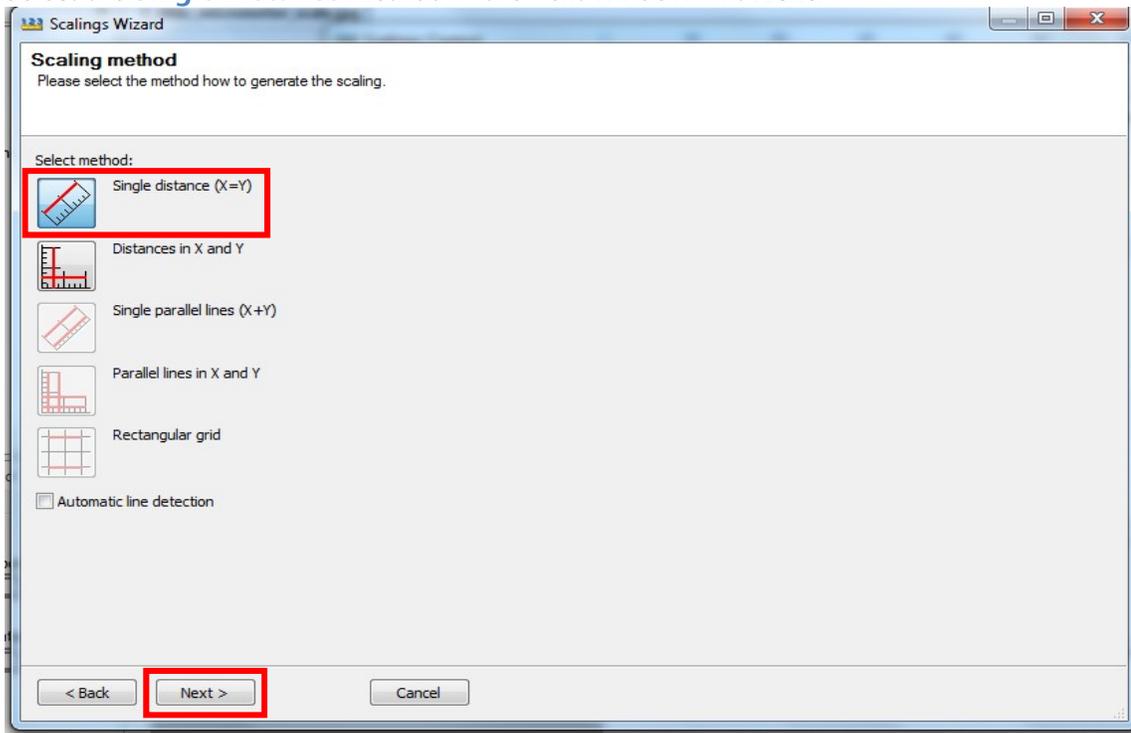Product and Application Support
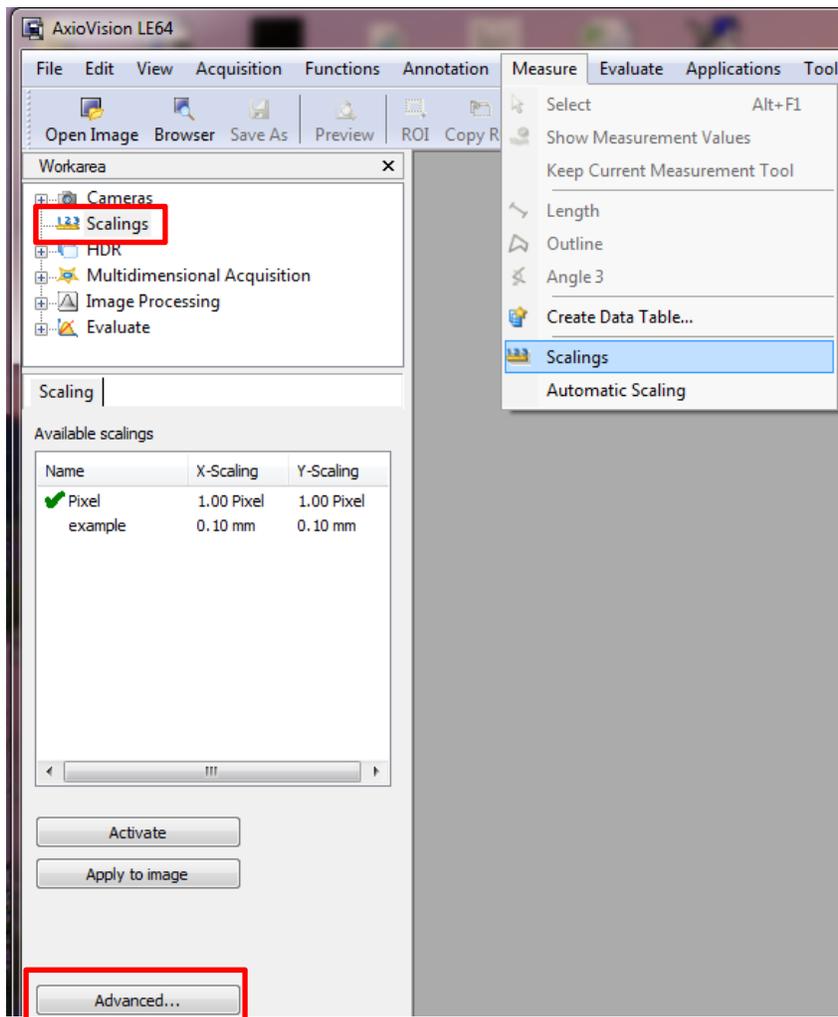
2. The **Scalings Control** window displays all the available Scalings for your user account. Select **New** to start the Scalings Wizard.
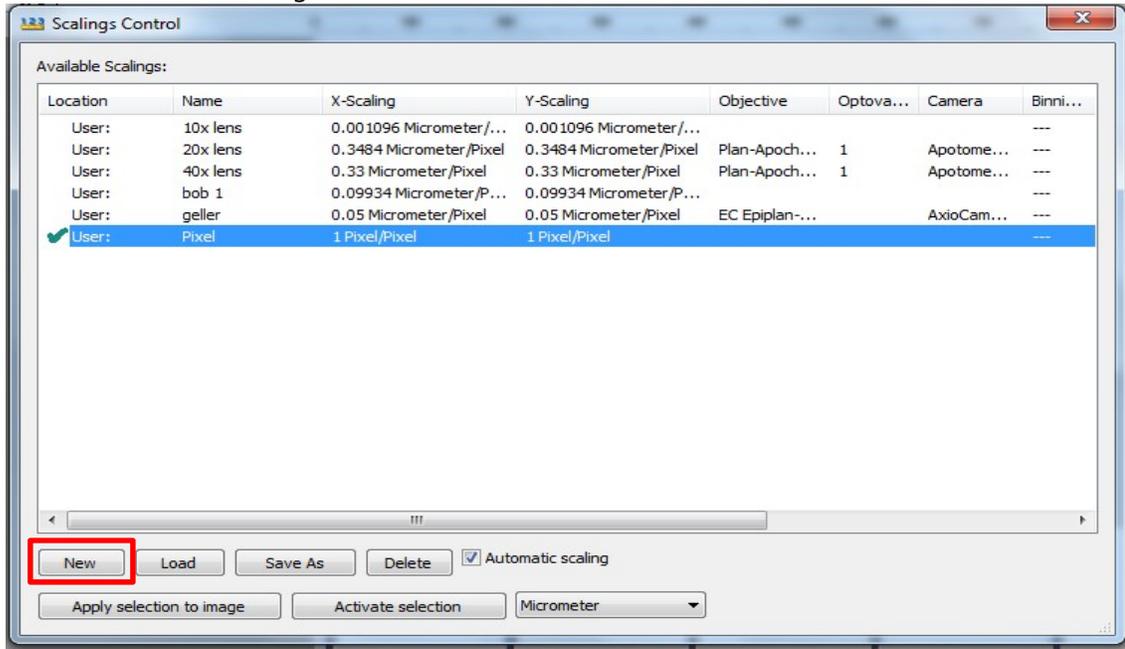


3. If you have a saved micrometer image taken using a system component configuration for which you wish to create Scalings, click **File** to select the image. If the image is open in AxioVision, click **Gallery**. Once the appropriate image is loaded, hit **Next.**



Each division = .01mm
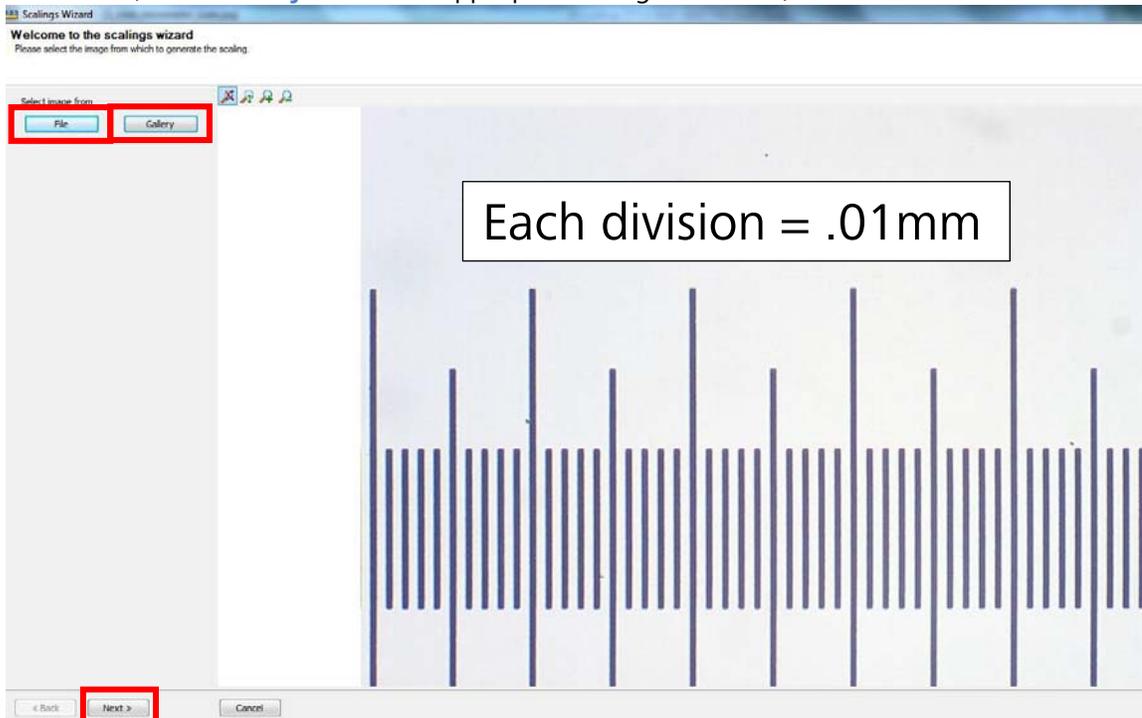
4. Select the **Single Distance** method in the next window. Hit **Next**.

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5. Draw a line between two points on the image and enter that known distance in **Assign range: X-Direction**. AxioVision will calculate the pixel length using that information.



Each division = .01mm



Properly drawn line

Improperly drawn line
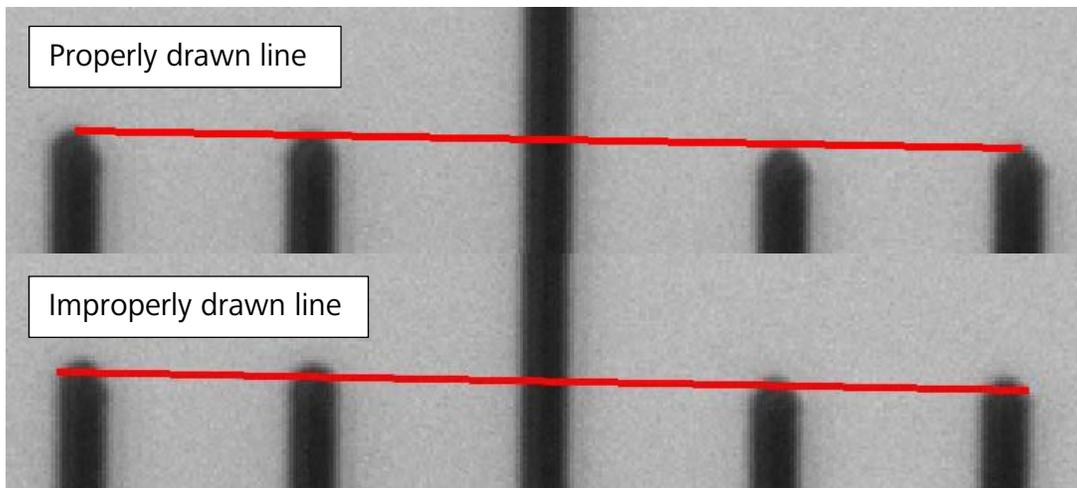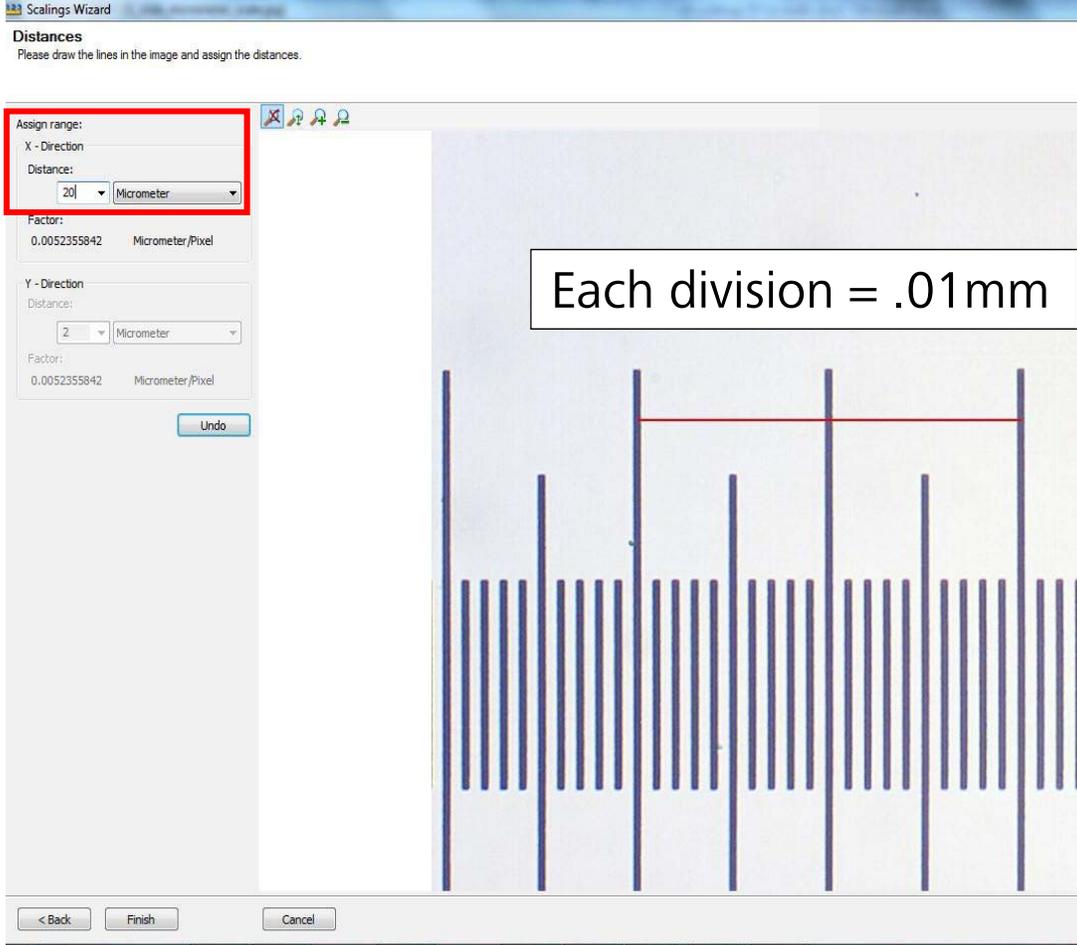
6. Click **Finish**. Enter a name for the Scaling in the window that pops up and click **OK.**
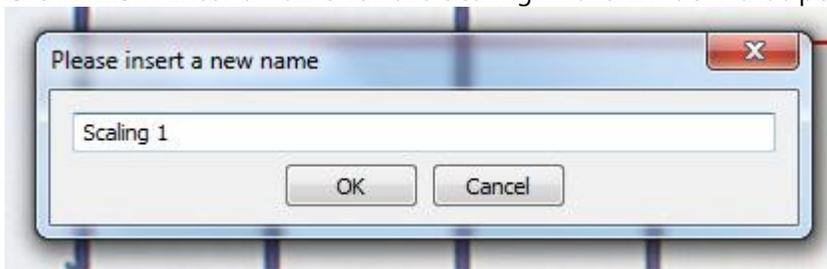


7. Repeat Steps 1-6 for all other system component configurations for which you want Scalings.

Scalings are saved in the Scaling folder (C:\Users\*Username*\Documents\Carl Zeiss\Data\Scaling) as files with file extension **.zvsc**. You can make a Scaling available for all users by moving the file to the C:\Users\**All Users**\Documents\Carl Zeiss\Data\Scaling folder. Each user will then be able to see a list of these Scalings in his or her Scalings Control window.

# How do I acquire Theoretical Scalings?

AxioVision always checks whether a valid Measured Scaling file is available and, if so, uses this file. If you have no Measured Scalings, for certain configurations or no Measured Scalings altogether, you can still apply Scalings to your images. AxioVision can extrapolate Scalings from existing Measured Scalings and/or calculate them using theoretical data.

1. If your system components are motorized and/or encoded, AxioVision can automatically calculate Theoretical Scalings, provided all the system components that determine Scalings (page 2) are configured in **MTB20XX Configuration X.X.X.X** (MTB).
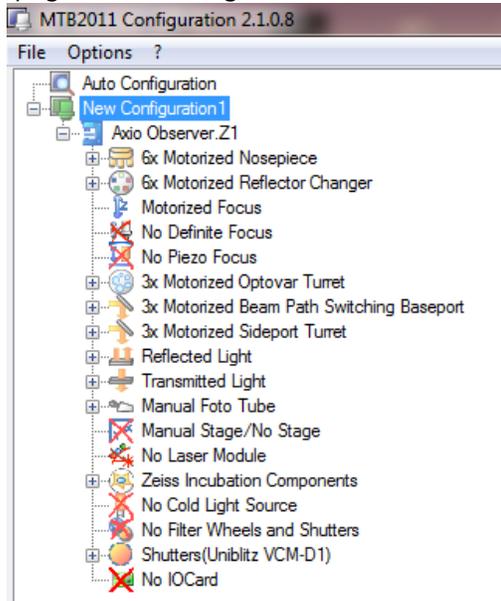


2. If some/all the system components that determine Scalings (page 2) are manual, configure MTB with an **Other Stand** and all the relevant system components.

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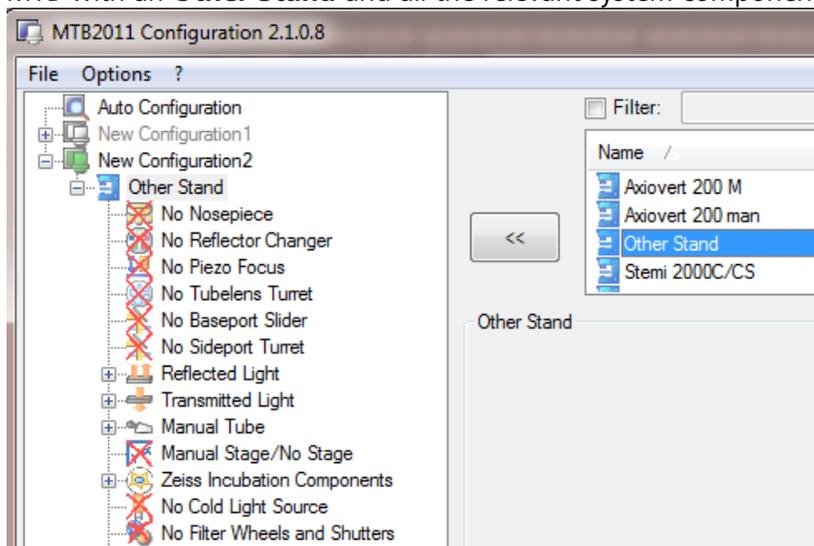
3. In the case that you have created a Measured Scaling for a certain system configuration but want to use a somewhat different configuration without creating a new Measured Scaling (e.g. adding camera binning or changing an objective to a system configuration that is otherwise identical), AxioVision can extrapolate a Theoretical Scaling (e.g. for that objective magnification or the generated binned image). The Measured Scaling off which an extrapolated Theoretical Scaling is created is marked with a green dot in the **Scalings Control** window to indicate that it is a "parent" of a dependent Scaling.

# How can I enable Automatic Scalings?

AxioVision can automatically apply the appropriate Measured Scaling or Theoretical Scaling to a given system configuration. This is referred to as **Automatic Scaling**.

1. If your system components are motorized and/or encoded and configured correctly in MTB, AxioVision can automatically apply the appropriate Scaling to a given configuration. Enable the **Automatic Scaling** option through **Measure** from the menu bar or through the **Automatic scaling** checkbox in the Scalings Control window.

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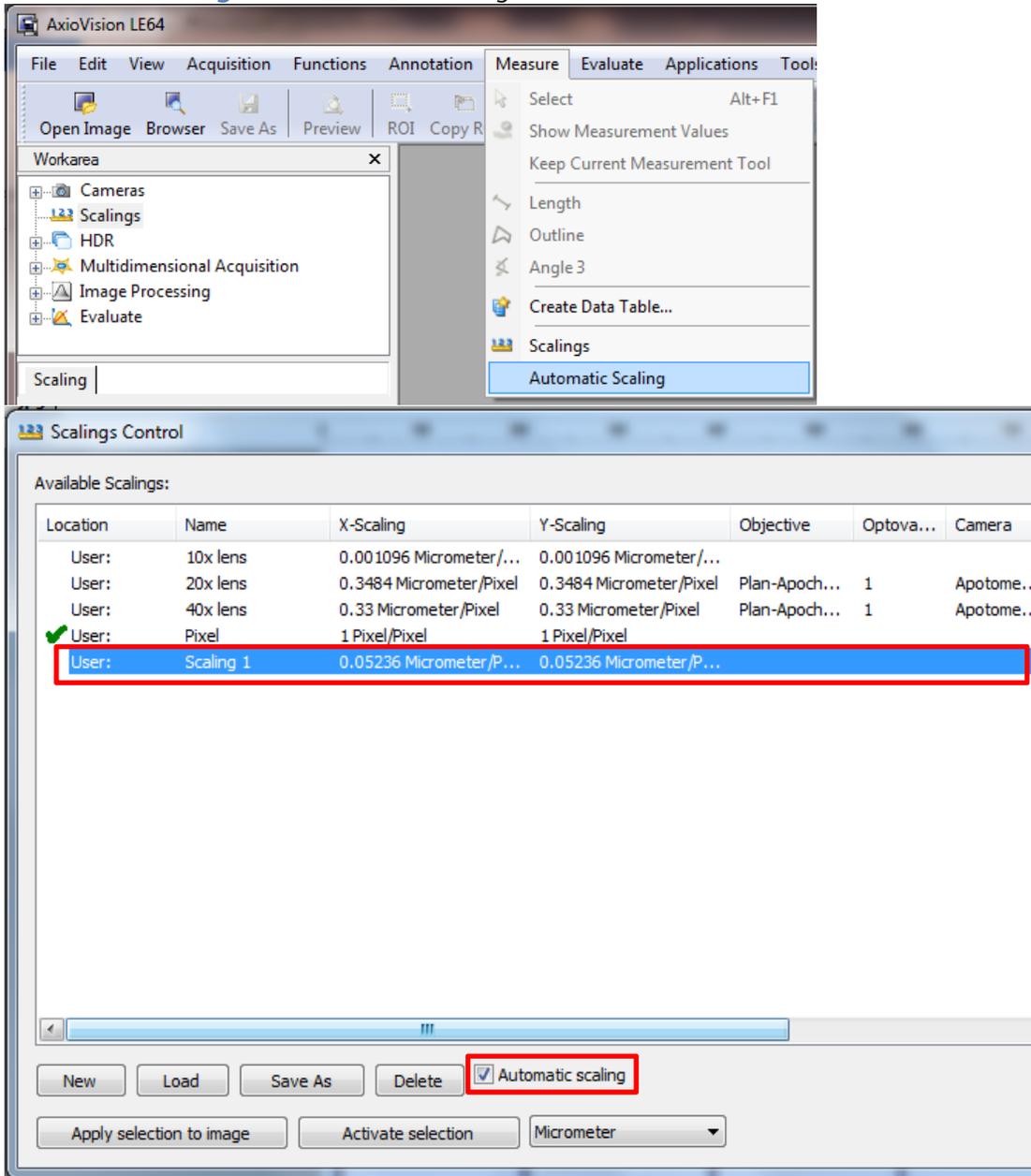
2. If AxioVision does not automatically recognize all system parameters and/or if some/all the system components that determine Scalings (page 2) are manual, you can manually apply a Scaling to a given configuration. Click on the appropriate Scaling and hit **Activate selection** in the **Scalings Control** window. The green checkmark move to the selected Scaling to indicate that this Scaling is the active Scaling that will be applied to all subsequent image acquisitions.
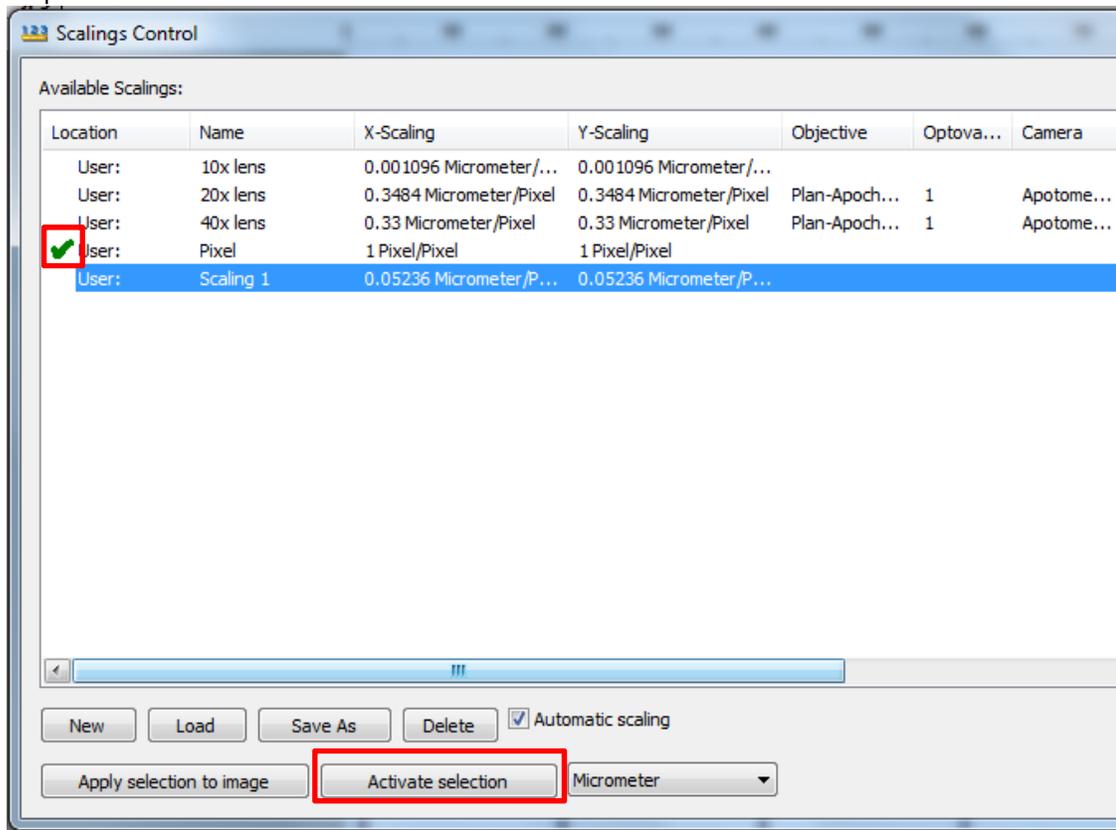


3. You can apply a Measured Scaling to an image that has already been acquired. This overwrites the image's previous Scaling but does not affect the **Automatic scaling** or **Activate selection** settings. This is not advisable if you are not certain about the configuration used to acquire the image and that the Scaling applied accurately reflects this configuration.
Open the image and navigate back to the Scalings Control window. Hit **Apply selection to image.**

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## How can I change the unit of measurement?

Select **Measure** and **Scalings** from the menu bar. Select the appropriate unit of measurement from the drop down menu at the bottom of the Scalings Control window. Click **Apply selection to image** to apply the change to the active image; click **Active selection** to apply the change to subsequent image acquisitions.
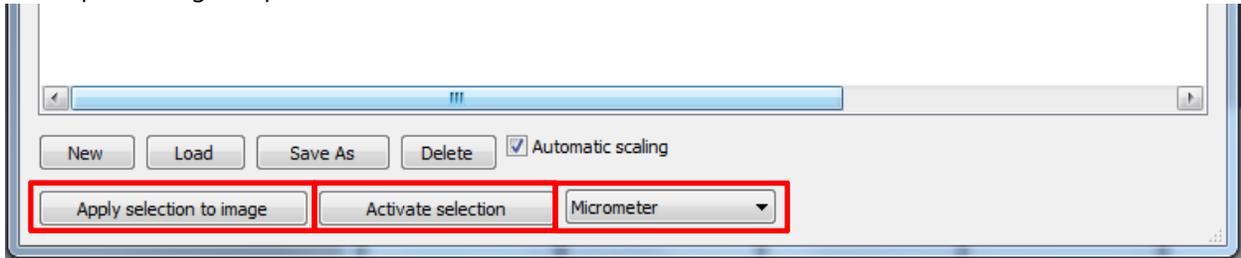


Note: You can only change the unit of measurement of an image with this method when using the same system computer used to acquire the image. If an image is opened in AxioVision on a different system computer or on an offline workstation, you must use Offline Scalings to change the unit of measurement.

## How can I generate Offline Scalings?

If you acquire an image without Scalings, you can assign Scalings afterwards provided you know all the system components that determine Scalings (page 2) that were used during acquisition. **Please note that this method is not recommended, especially for systems with third party components.**

1. Download:
   ftp://support.zeiss.com/PUBLIC/Application_Support/Offline_Scaling_Calculator.xlsx
   The username and password should be in the email in which this document was sent.
2. Follow the steps for acquiring Measured Scalings (pages 3-7), but load the image for which you wish to create offline scalings (step 3), draw a line of arbitrary length in the image (step 5), and enter "1" in the **Distance** field.
3. Use the Excel spreadsheet to determine the pixel size for the image by selecting the appropriate camera, camera adapter, objective lens, Optovar, and camera binning. Divide the number calculated from the spreadsheet (µm/pixel) by the **Factor** (Micrometer/Pixel) displayed in the Scalings Wizard (step 5). Type the result in the **Distance** field and hit **Enter**. The **Factor** now displayed in the scaling wizard should equal the one calculated using the spreadsheet. Finish step 6.
4. The created scaling will now appear on the Scalings Control window and can be applied to the image by selecting the Scaling and clicking **Apply selection to image**.

## Why does AxioVision throw a scaling-related error upon startup?

If the **Automatic Scaling** function is enabled, AxioVision monitors the system settings and displays this message if a valid Scaling is missing. This occurs when Scalings components are missing from or improperly configured in MTB; when AxioVision is started before the microscope is powered on; when the microscope does not communicate with MTB and, therefore, with the software. Check that the microscope is powered on, and that it is communicating with the software by selecting the microscope stand in MTB and clicking **Check Connection**. Restart AxioVision.
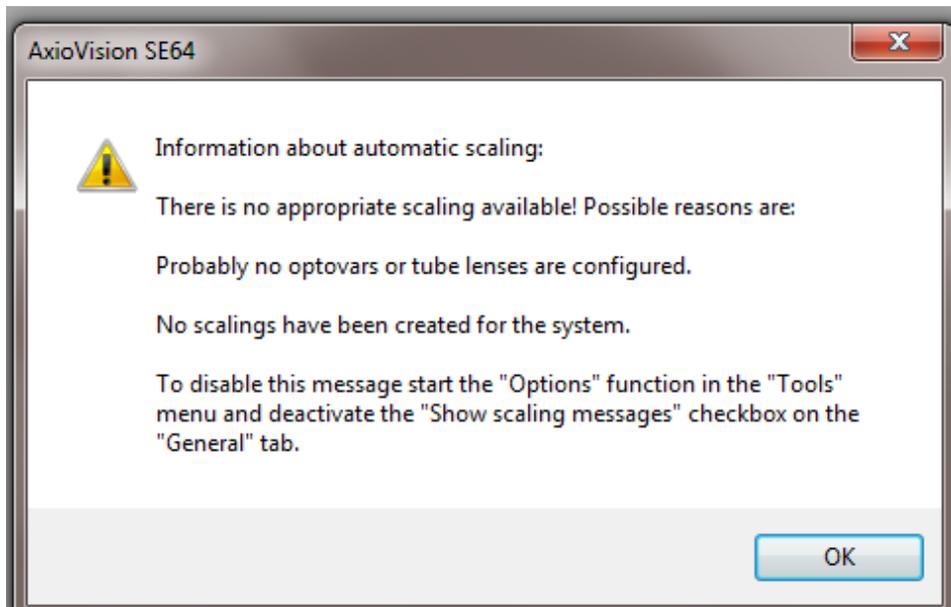


When this happens, a Scaling file with the name "Pixel" becomes active in the **Scalings Control** window.