Zen Scalings
Product and Application Support
April 2014

The purpose of this document is to introduce the concept of Scalings in ZEN 2012 and guide the user through creating and using Scalings with ZEN 2012. Any questions or concerns can be directed to the ZEISS Product and Application Support group at 1-800-509-3905 or support@zeiss.com

What are Scalings?
The term Scalings refers to 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?
The scaling information allows you to calculate the size of the 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 also allows the user to measure structures in an image and to assign scale bars.

Without the pixel sizes ZEN is unable to acquire Tile 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 ZEN 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.

> 1. In the status bar (the bottom of the ZEN window), under Scaling, deselect the Automatic checkbox. Then, click the gear icon next to it and select *Create New Scaling.*
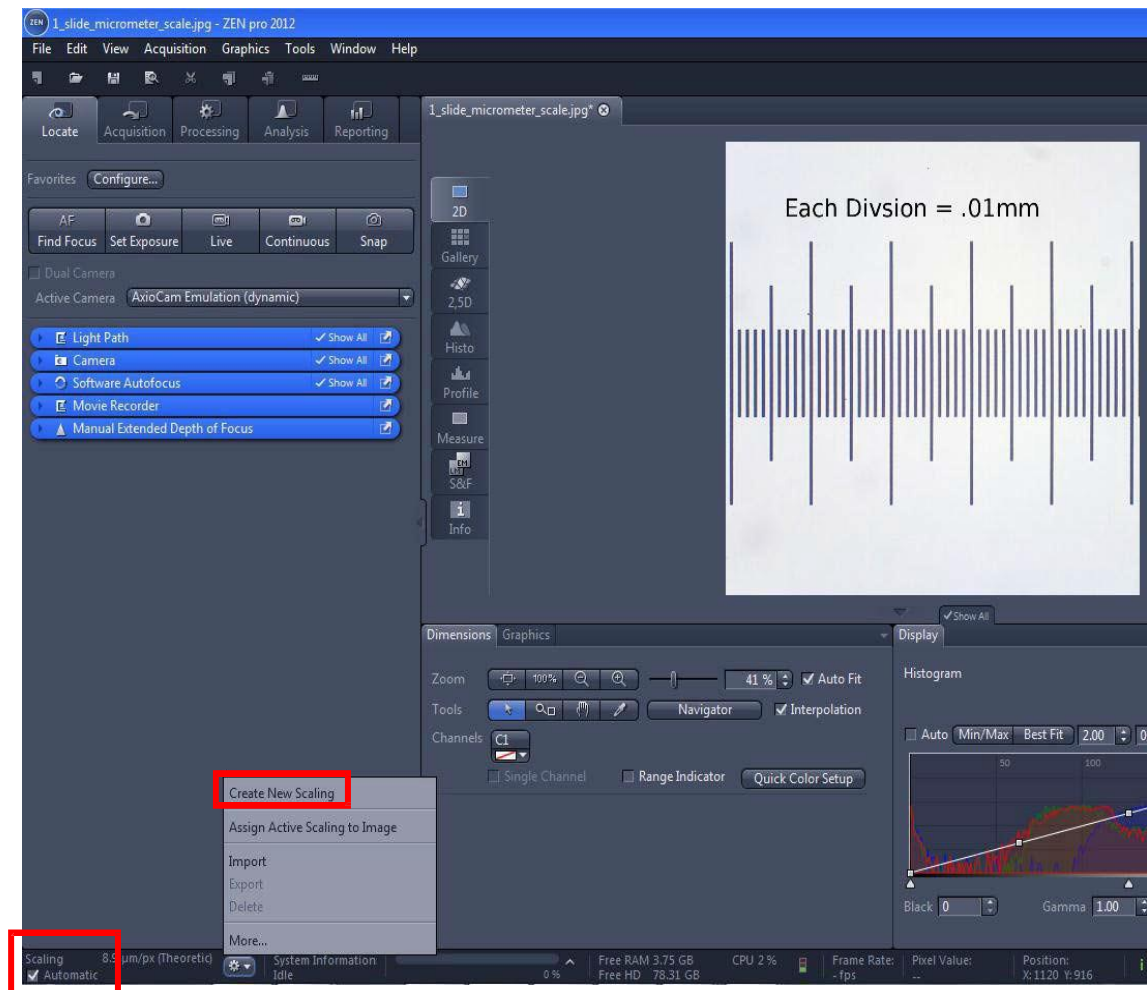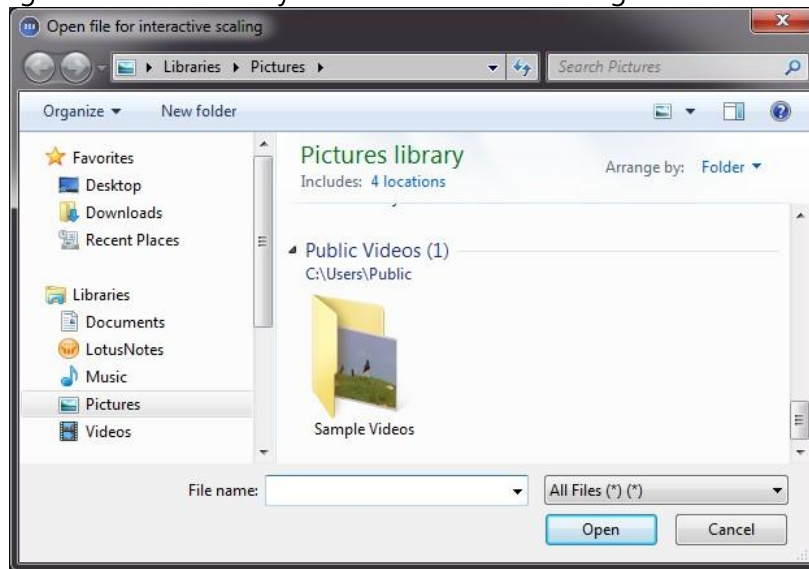
2. At this point ZEN will prompt you to load the saved micrometer image taken using the component configuration for which you wish to create Scalings.

3. In the window that opens, use the crosshairs to draw a reference line between two points on the image. You can activate Automatic Line Detection to calculate with sub-pixel accuracy the distance between the corresponding rulings on the stage micrometer, regardless of how accurately you've drawn the reference line.
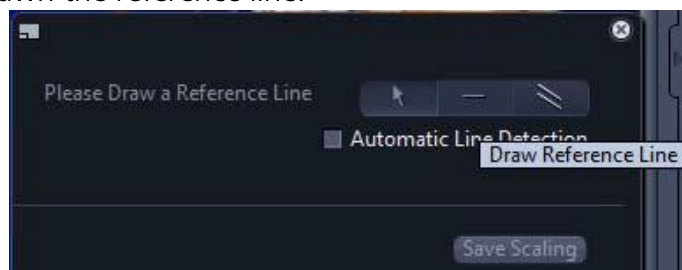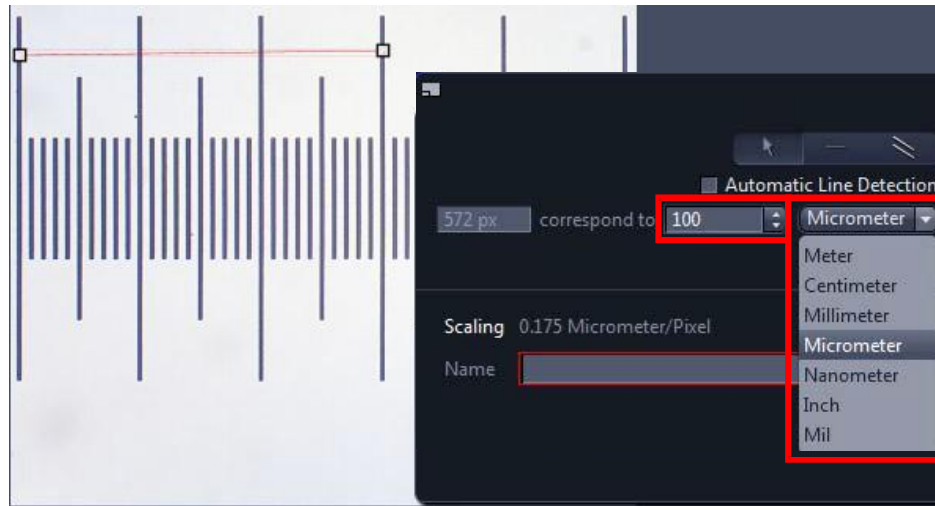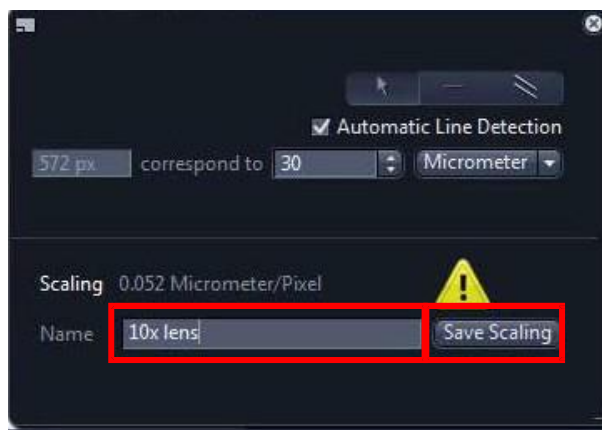
4. After you've drawn the reference line, enter the known distance of the line in the appropriate fields.

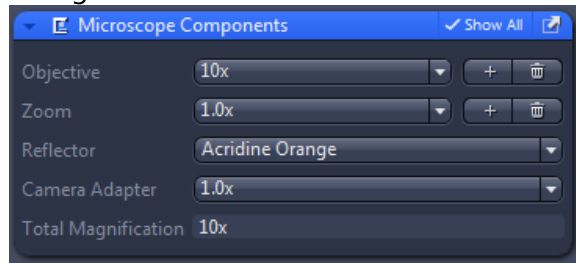5. Name the Scaling and hit *Save Scaling*.

6. Repeat Steps 1-5 for all other system component configurations for which you want Measured Scalings. Make sure to match your Measured Scaling files with your **Microscope Components** configuration in the left toolbar.


In the status bar, you can activate Automatic Scaling to have ZEN apply the relevant Measured Scaling automatically for a given system configuration and use it for subsequent image acquisitions.

If you create Scalings while logged in as an Administrator, they are saved in the Scalings folder (C:\ProgramData\CarlZeiss\ZEN\Users\admin\Documents\Scalings) as files with file extension .czsc. Available Scalings are listed in the dropdown menu in the status bar.
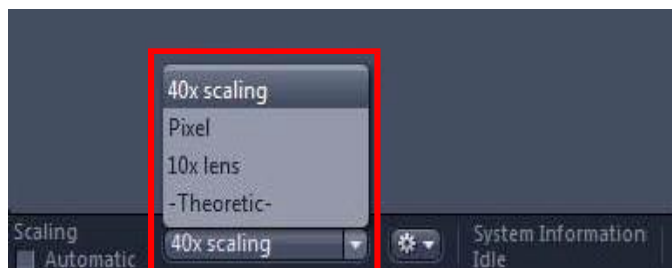
The pixel size will read "Measured" in parentheses for Measured Scalings and "Theoretic" for Theoretical Scalings.
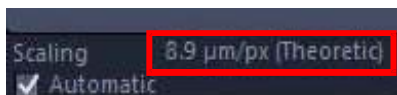
How do I acquire Theoretical Scalings?

For newly acquired images ZEN determines the scaling from the configuration set for the microscope and camera. Data acquired in another session or imported from another system has associated metadata that contains information about the scaling per pixel.

ZEN always checks whether a valid Scalings 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 use apply Scalings to your images. ZEN can extrapolate Scalings from existing Measured Scalings and/or calculate them using theoretical data. Scalings generated this way are referred to as **Theoretical Scalings.**

1. If your system components are motorized and/or encoded, ZEN 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).
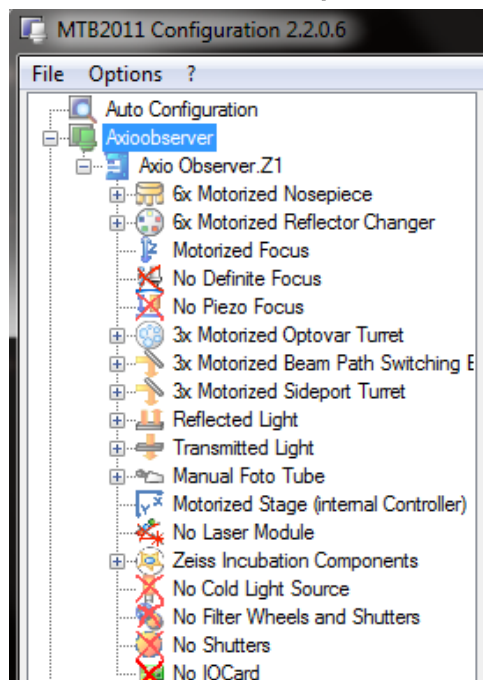
2. If some/all the system components that determine Scalings (page 2) are manual, configure MTB with an Other Stand and all the relevant system components.

3. In the case that you have created a Measured Scaling for a certain system configuration but want to use a slightly different configuration without creating a new Measured Scaling

(i.e. adding camera binning), ZEN can extrapolate a Theoretical Scaling for the generated binned image.

In the status bar, you can activate Automatic Scaling to have ZEN apply the relevant Theoretical Scaling automatically for a given system configuration and use it for subsequent image acquisitions.

How can I change the unit of measurement?

Click the gear icon in the status bar and select More. Select the desired unit of measure from the Units dropdown menu for the Scaling you wish to apply to the image, click the gear icon under Available Scaling, and select Assign Scaling to Image. ZEN will apply the change to subsequent image acquisitions.
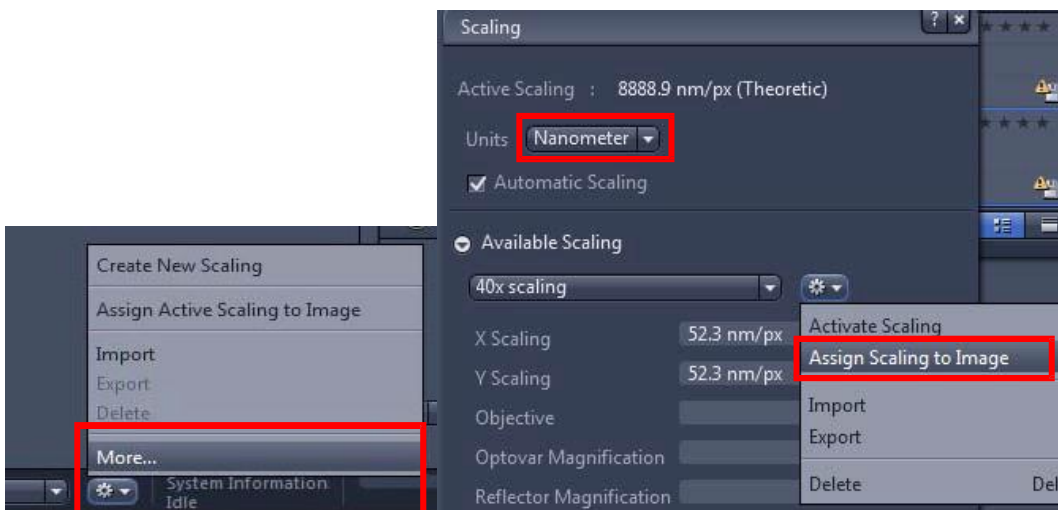
To change the unit of measure in Scalings already applied to an image, navigate to the Info tab of the image and click the Edit button next to the Scaling information under Image Dimensions. You can then change the units in the Scale Unit dropdown menus in the Edit Scaling window.