Zeiss ZEN – performing a maximum intensity projection of a Z-stack

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Zeiss Microscopy makes a free version of their ZEN software available for installation on Windows PCs. The software can be obtained from the UA Imaging Cores in Marley or LSN. Our instructions for installing the software can be found at: http://microscopy.arizona.edu/learn/printable-materials One of the oddities of the ZEN software is that there are two versions of the software that look very similar. ZEN Black was built for controlling laser-based instruments and ZEN Blue for controlling widefield microscopes. Their feature sets are not identical.

Maximum intensity projection

A maximum intensity projection[i] is a scientific visualization technique that takes 3D data (in our case a Z-stack of microscope images, either confocal or SIM) and turns it into a single 2D image. The projection takes the brightest pixel (voxel) in each layer and displays that pixel intensity value in the final 2D image. Commonly the projection is created so that it is being viewed "down" into the image stack along the Z axis. Alternatively, the projection can be created along any single axis or a combination of axes. For example, the rotation movies created by software are really a series of maximum intensity projections shown at slightly different angles, which (when played back) look like a 3D display of the object data rotating in space.

Maximum intensity projection images can be useful for displaying data that is sometimes difficult to see, or display, in a single plane image. Sometimes only a few adjacent image planes out of an entire image stack need to be added together to convey the information. There are a few caveats:

- Spatial resolution (XY, or orthogonal to the projection axis) will most likely be compromised. This may be an acceptable choice, but users need to be aware of this possibility.
- In complicated 3D structures, a maximum intensity projection may not improve clarity, but instead may increase confusion as objects are flattened out on top of each other. The more z planes used for the projection, the higher the likelihood of this being a problem. Objects in the 3D volume that did not "touch" each other may appear to do so in the final 2D image.
- From a publication ethics standpoint, this technique should be reported when using the projection image in a scientific article or grant application. It is helpful for the reader/editor to know how many images and/or the Z thickness that was used to create the projection, along with any special settings, and the software version used to create the image.
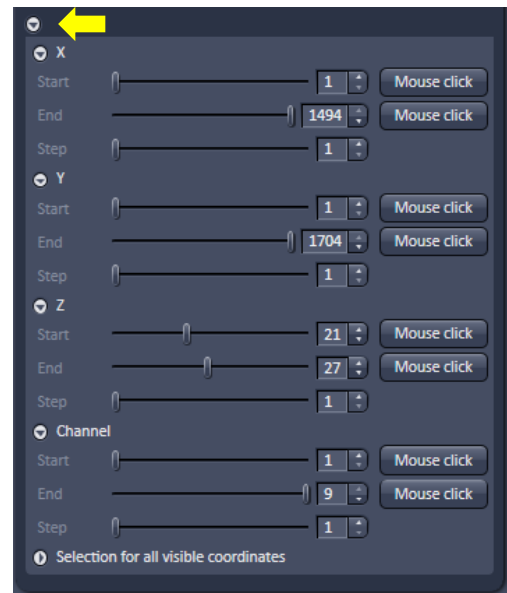
ZEN Black – maximum intensity projections (easy)

Open your Z-stack CZI image in ZEN Black. *This can be done on any of the core microscopes or data processing workstations in UA Imaging Cores Marley or LSN. Alternatively, if you have ZEN black installed on a lab computer you can do this on the lab PC.*

Rather than performing a projection of an entire image stack, perhaps only a few layers will best show the object(s) of interest.

1. In ZEN Black, in the PROCESSING tab (left side), within the METHOD window, choose COPY ▶ SUBSET.
2. Select your Z stack data using the select button
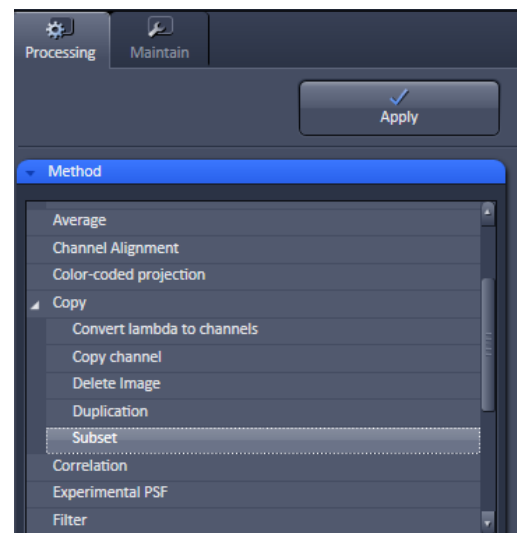
3. If you do not see the options shown in the image here, look for and click on the small options circled arrow (see yellow arrow in image).
4. Instead of the 53 slices in this example Z stack, only 6 were chosen using the Z option.
5. Click APPLY.
6. A new (smaller) data file is created. You may want to save this to the computer and name the subset.
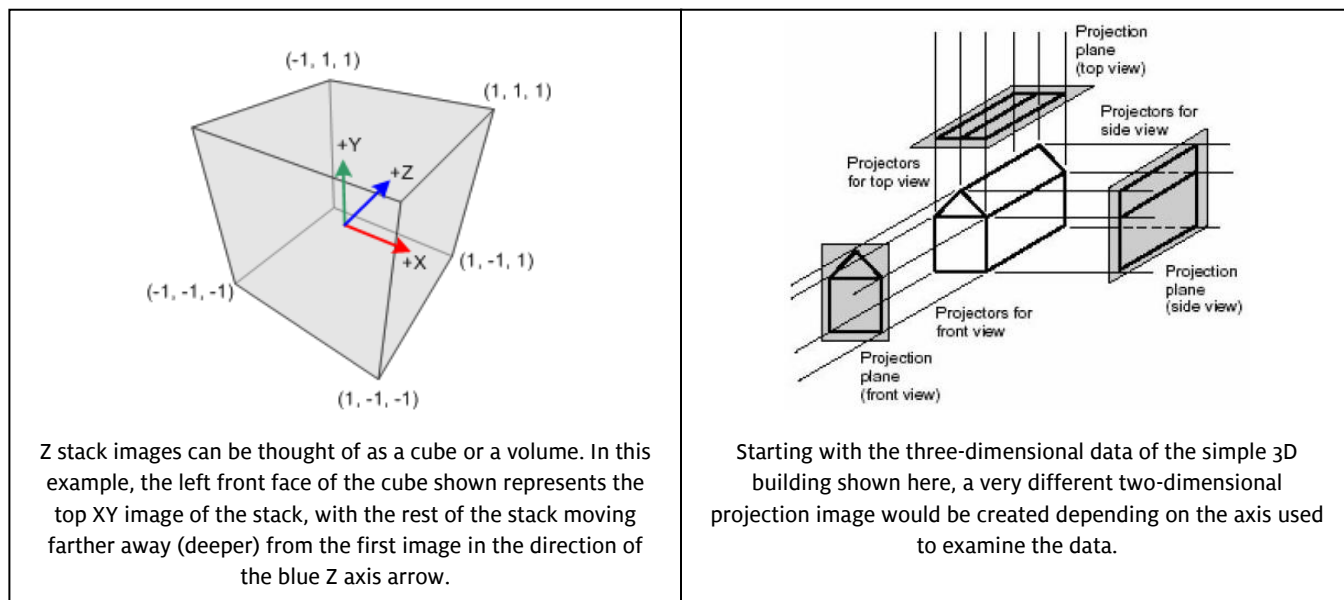
To create a maximum intensity projection of the subset you just created, see the following steps.

1. In the PROCESSING tab (left side), within the METHOD window, choose MAXIMUM INTENSITY PROJECTION.
2. This option in ZEN Black will only project along the Z axis.
3. Select your subset data.
4. Click APPLY.
5. Save the new projection as a separate file. If the software does not offer to name it as a maximum intensity projection, you should name it appropriately so as to not confuse this image with single plane image data at a later time.

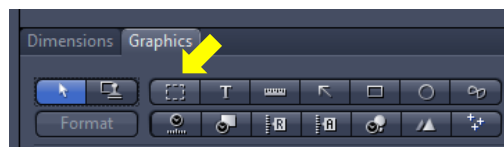## ZEN Blue - maximum intensity projection (more complicated, with more options)

It is helpful to think about three-dimensional data as a volume when using ZEN Blue. We can create very different 2D images, depending on which axis is used for creating the projection of the 3D data. (*sources for the images shown below* [ii])

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Z stack images can be thought of as a cube or a volume. In this example, the left front face of the cube shown represents the top XY image of the stack, with the rest of the stack moving farther away (deeper) from the first image in the direction of the blue Z axis arrow.	Starting with the three-dimensional data of the simple 3D building shown here, a very different two-dimensional projection image would be created depending on the axis used to examine the data.

Open your Z-stack CZI image in ZEN Blue.

Rather than performing a projection of an entire image stack, perhaps only a few layers will best show the object(s) of interest.

1. In ZEN Blue, in the PROCESSING tab (left side), within the METHOD window, choose UTILITIES ▶ CREATE IMAGE SUBSET.
2. In IMAGES & DOCUMENTS (far right side), make sure that you have selected (highlights the file) the correct data set.
3. You can crop a region of interest (REGION), to look at a smaller area (XY) within the Z-stack. You will need to use the selection tool (see yellow arrow) in the graphics tab in ZEN Blue. This tab can be found directly below the image on the left side.
4. You can subset the channels used in the projection, perhaps selecting just one or two colors to avoid complicating the final image. This is done in CHANNELS.
5. You can select a range of Z planes to subset, using the Z-POSITION option.
6. You can, if you are feeling ambitious, combine several of these options together when creating a subset. Perhaps a smaller XY region, of only two (out of four) channels, using just five Z-planes.
7. Once you are ready, click APPLY.
8. A new (smaller) data file is created. You may want to save this to the computer and name the subset.

To create a maximum intensity projection of the subset you just created, see the following steps.

1. In the PROCESSING tab (left side), within the METHOD window, choose GEOMETRIC ▶ ORTHOGONAL PROJECTION.
2. In IMAGES & DOCUMENTS (far right side), make sure that you have selected (highlights the file) the correct data set.
3. The options for the software tool are explained in greater detail on the next page.
4. To accomplish a similar projection to what was done in the example above using ZEN Black, choose PROJECTION PLANE | FRONTAL (XY).
5. Leave the METHOD here at MAXIMUM.
6. Since you have already created a subset of your Z stack, you do not need to change the settings for START POSITION or THICKNESS.
7. Click APPLY.
8. Save the new projection as a separate file. If the software does not offer to name it as a maximum intensity projection, you should name it appropriately so as to not confuse this image with single plane image data at a later time.
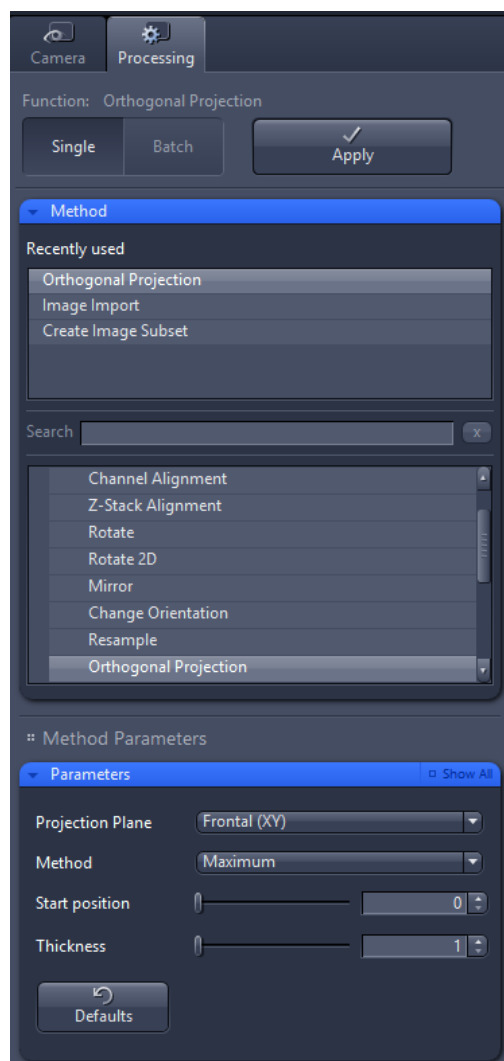
REFERENCE: *Orthogonal Projection* [iii]

With this method you can extract specific parts of the image of three-dimensional images. This is accomplished with three alternative projection planes, frontal in the XY direction, sagittal in YZ direction or transverse in XZ direction as seen from the observer of the image. You can choose between different projection methods, all have in common is that the pixels are analyzed by the observer along an imaginary projection beam. You can also determine the thickness of the projection planes, and thus the projection depth.

Parameter	Description
Projection Plane	Here you choose the type of the projection plane (Frontal X/Y, Transverse (X/Z), Sagittal (Y/Z).
Method	
- Maximum	Uses the brightest pixel along the projection beam.
- Minimum	Uses the darkest pixel along the projection beam.
- Average	Calculates the average of all pixel along the projection beam.
- Weighted average	This method is related to the calculation of the extended depth of focus. It prefers structures with more lateral sharpness along the projection beam. The output image contains more significant details.
- Standard deviation	Calculates the standard deviation of pixel grey values along the projection beam.
Start position	Here you adjust the starting position of the project plane (in pixel units or z-stack positions depending on the chosen projection plane). The maximum range results automatically of the size of the input image.
Thickness	Here you adjust the thickness of the cutting plane (in pixel or z-stacks depending on the chosen projection plane). The maximum range results automatically of the size of the input image.

Other options for 3D data

Both ZEN Black and ZEN Blue are capable of creating 3D volume renderings that can be outputted to movies. These software tools are very computationally intensive (even for the powerful computers we have in the cores), and they require a bit of practice before a user can create satisfactory results. If you are interested, the cores have Zeiss' software manuals available for both versions of ZEN and the core managers can also see about requesting a Zeiss technical specialist to come show us how to use this software.

The UITS Research Computing group has licensed copies of the powerful 3D and 4D analysis and visualization software packages IMARIS and AMIRA. The software is available to campus researchers on a workstation computer located in the computer center. Contact the IT Visualization Consulting group to learn more. For more information about these software packages, see: https://it.arizona.edu/documentation/visualization-software

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[i]   https://en.wikipedia.org/wiki/Maximum_intensity_projection
[ii] Left image: https://www.davrous.com/2013/06/13/tutorial-series-learning-how-to-write-a-3d-soft-engine-from-scratch-in-c-typescript-or-javascript/ Right image: http://cs.brown.edu/stc/summer/viewing_history/viewing_history_18.html
[iii] Italicized text is quoted from the Zeiss ZEN Blue 2.5 software help file, see also the user guide, section 5.10.4.10.