tomviz: a basic user guide for 3D reconstruction.

User guide written by Barnaby D.A. Levin & Robert Hovden.
The tomviz project is a collaboration between many scientists and software developers: Yi Jiang, Shawn Waldon, Peter Ercius, Elliot Padgett, Corey Quammen, Chris Harris and Barnaby Levin. The team is led by Marcus D. Hanwell and Utkarsh Ayachit at Kitware, Inc., and David A. Muller and Robert Hovden at Cornell University under DOE Office of Science contract DE-SC0011385.

First, make sure you download *tomviz* from the website www.tomviz.org. The raw data used in this guide has been published, and is described in detail in *Nature Scientific Data*, **3**, 160041 (2016). This guide is compatible with tomviz version 0.9.1 and above, and also version 0.9.0-34-gef2fa18 (the nightly build available as of August 15th 2016).

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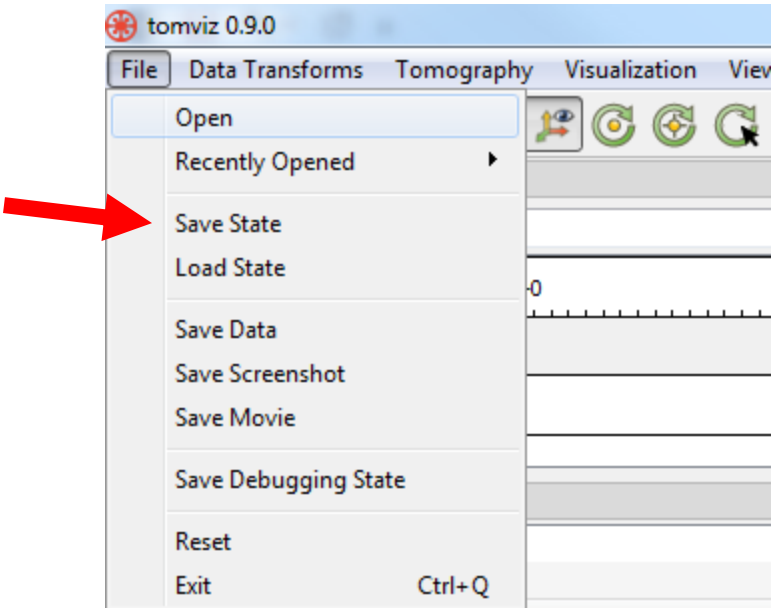
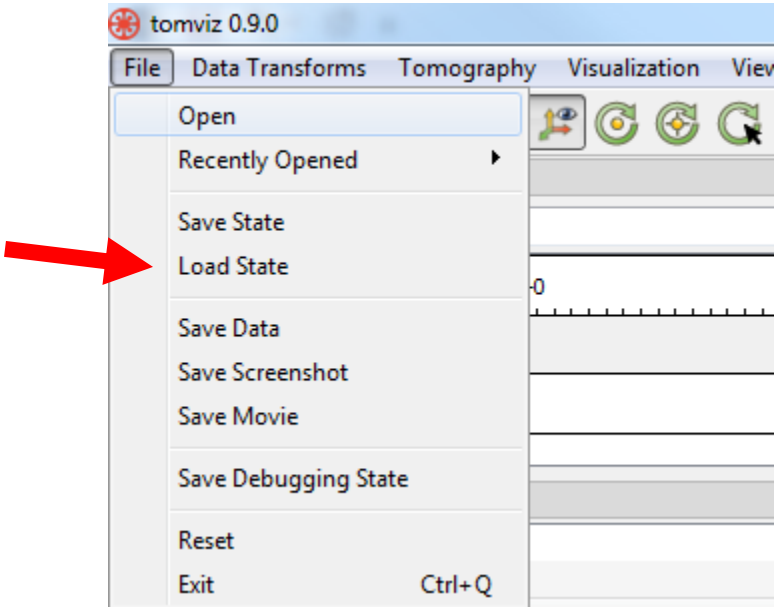
Before You Start – Saving Your Work.

Tomographic reconstruction and visualization can be a lengthy process. To avoid losing work, we recommend saving your state often by going to the File tab and clicking "Save State". This saves a .tvsm file, preserving all of your work.

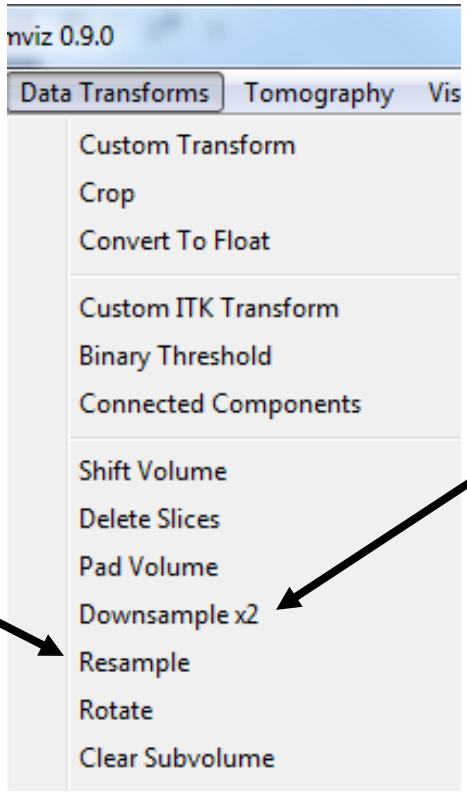


.tvsm files can be loaded into *tomviz* by going to File and clicking "Load State". Then you can continue from exactly where you left your work.



Before You Start – File Sizes.

Working with 3D datasets requires a lot of memory. For example, a 1024x1024x1024 32-bit dataset will occupy ~4 GB of memory. Transforming and visualizing such a large dataset will require even more memory! If you are using that a powerful computer with lots of RAM and multiple cores, this is fine, but if not, you will need to downsize! To downsize a *volume*, go to the Data Transforms tab, and select "Downsample x 2".
For volumes, Downsample x2 will scale the data along all three spatial dimensions, reducing the memory required to 1/8 of the original data.
To downsize a *tilt* series, use the function "Resample". This allows you to downsize the X and Y image dimensions, while leaving the tilt dimension alone. It is recommended to downsample a tilt series before running a reconstruction if the resulting volume will occupy too much RAM.

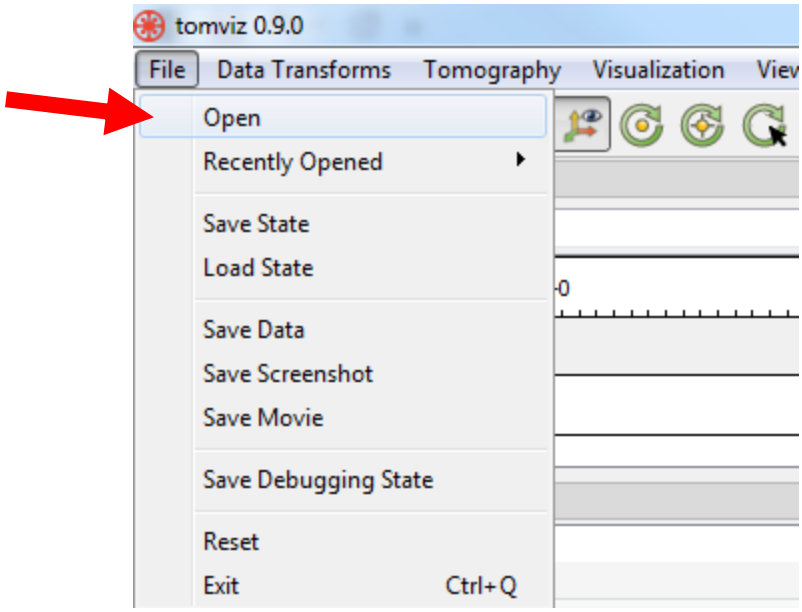


Use Resample to downsize *tilt series*. data.
(Or scale volumes by a factor of your choice).

Use Downsample x2 to downsize *volumes*, and save yourself computer memory, and computation time!

1.0 – Loading Datasets.

To load data into *tomviz*, simply go to the File tab, and select Open. Then navigate to the data that you would like to load into *tomviz*.

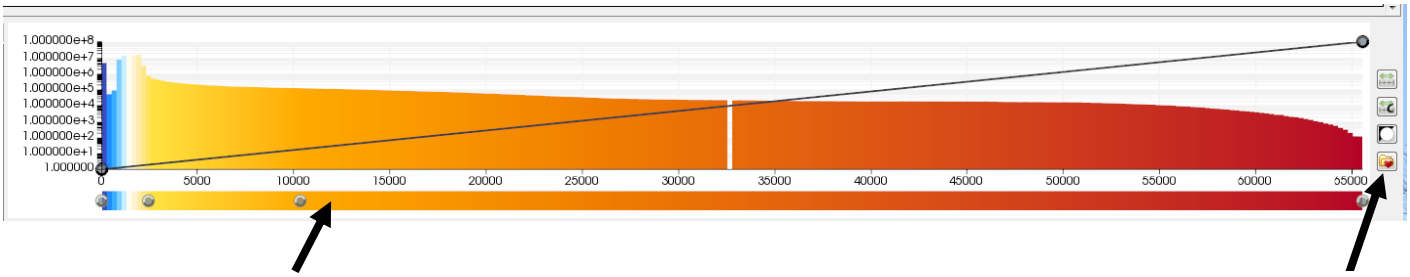


In this guide, we're going to start by loading a tilt series dataset called "tiltser_Co2P_endmisaligned.tif". This file contains a tomographic tilt series of 76 images of a Co_2P nanoparticle acquired at 2^o tilt increments.

1.1 Changing Colormap and Background.

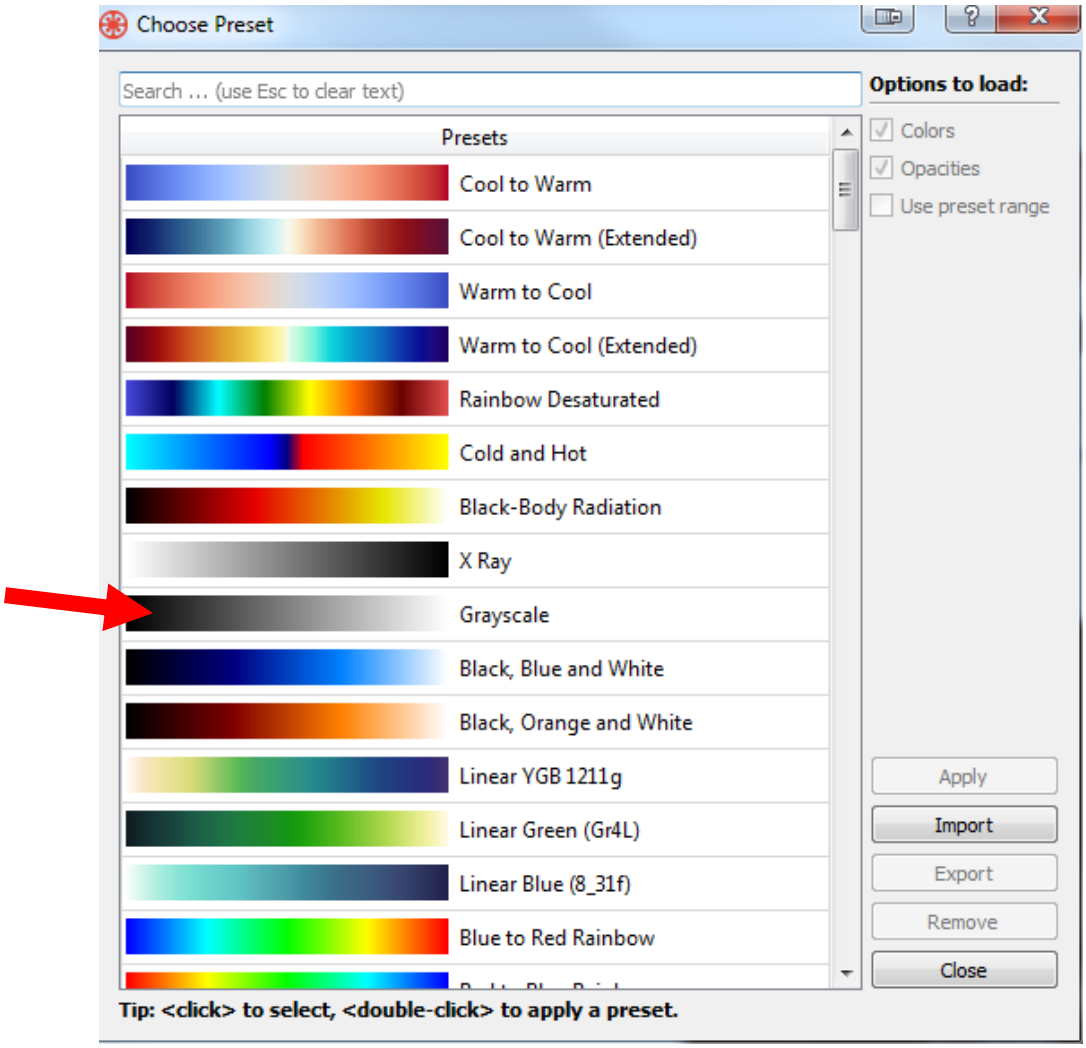
Once you have your file loaded up, you may choose to change the display colors. You can change the background by right-clicking on the background surrounding the data, and choosing "Set Background Color".

When data is loaded, you will see a histogram of the colors used to plot the values of intensity in the image display (a 'color map'). You can change the color map used to display the data either by choosing a preset map from the menu to the right, or interactively by manually selecting colors at points of reference on the bar below the color map. New points of reference can be added by clicking on the color bar, and can be moved by clicking and dragging on them. Points of reference can be removed by selecting them, and then hitting the "Delete" key. In this guide, we use the preset "Grayscale" color map to display tilt series data, as this is the format used during acquisition in the electron microscope.



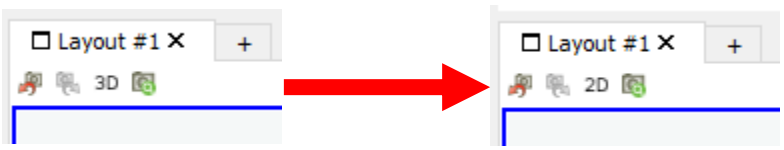
Manually adjust color map.

Choose preset color map.

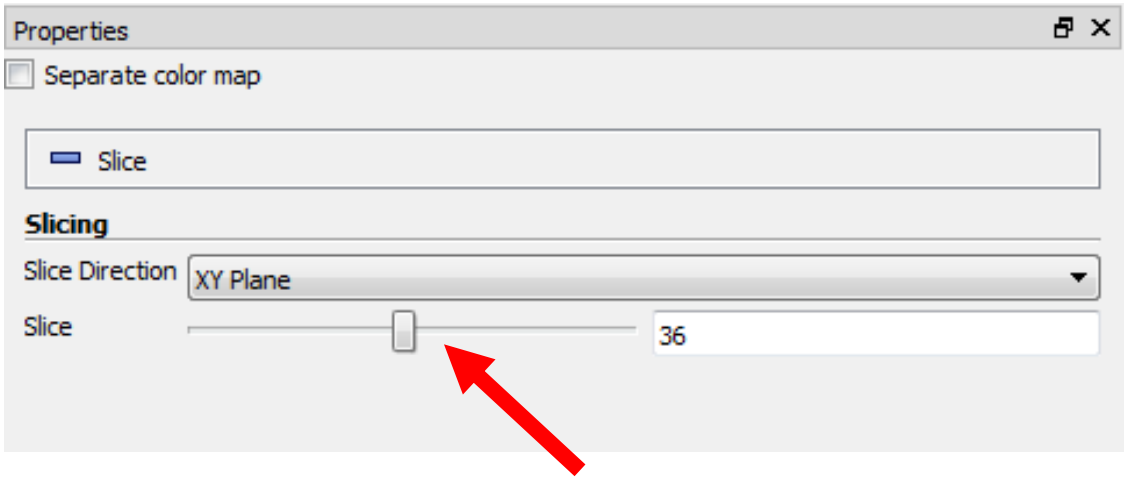


2.0 – Aligning a Tilt Series.

When data is loaded into *tomviz*, it is automatically displayed by the orthogonal slice method. This is the most convenient way to view tilt series data, as each image in the tilt series is displayed as a separate slice. To remove the 3D visual effects, and make it easier to see how well the tilt series is aligned, click the "3D" button under Layout, to change to 2D viewing mode.



To scroll through the tilt series images, use the slider in the "Properties" box in the lower left of the display, or manually enter the number of the image in the stack that you want to view.



On scrolling through the images in tiltser_Co2P_nonalignedend.tif, it is apparent that first 59 images are aligned to a common tilt axis, but the final 17 images are not.

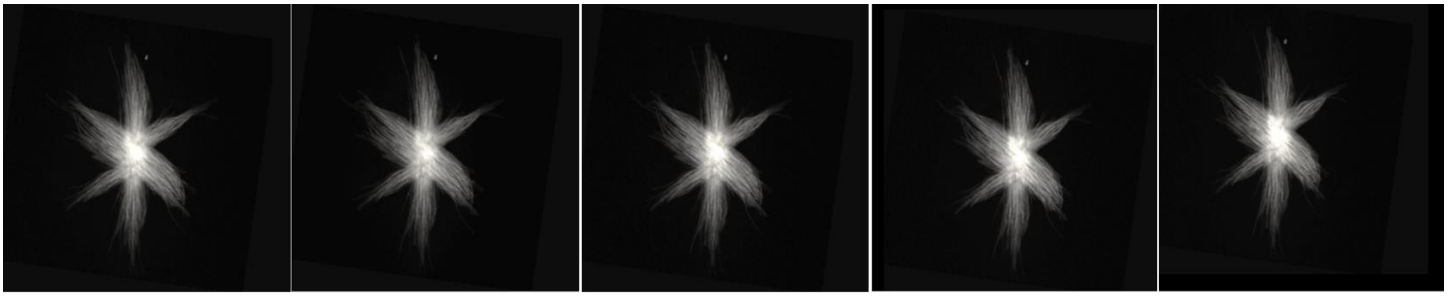
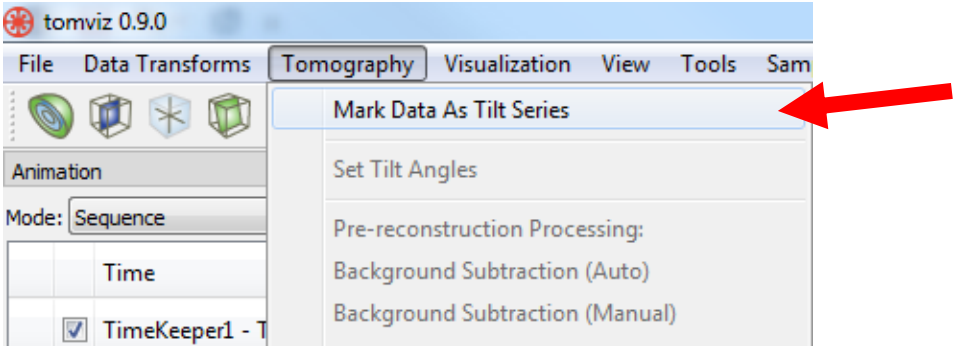


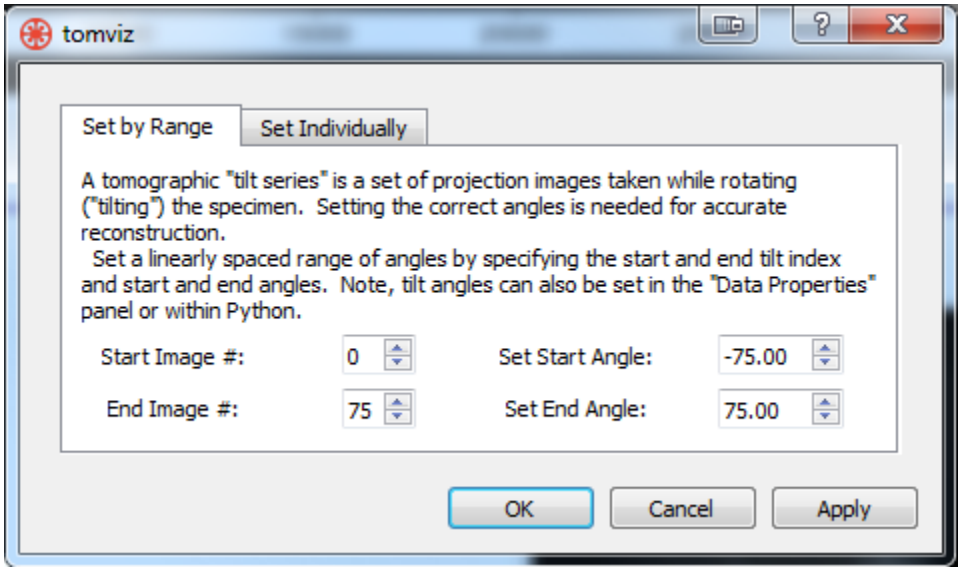
Image 57 Image 58 Image 59 Image 60 Image 61

These images must all be aligned before the tilt series can be reconstructed accurately to show the 3D structure of the particle in the images.

In order to align tilt series images in *tomviz, we must first explicitly tell the software to mark the data as a tilt series*. You can do this by going to the Tomography tab, and selecting "Mark Data as Tilt Series".



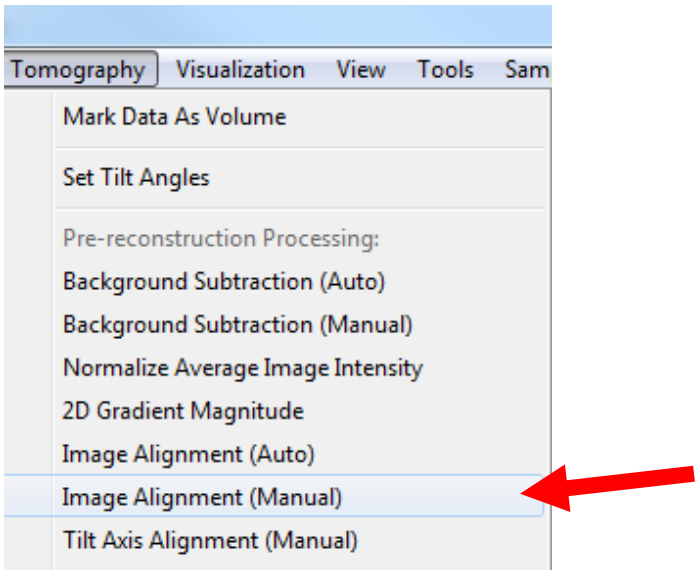
A dialog box will appear asking for some properties of the tilt series. Start Image # and End Image # let you choose the range of images in the data to use to form the tilt series. To use all of the images in the data, simply leave these at the default settings. Set Start Angle and Set End Angle require you to input the starting and ending angles of the microscope goniometer for the tilt series. By default, the software assumes equal tilt increments of 2^{o}, which is correct for the data used in this example. For tilt series acquired at non-equal tilt increments (for example by equal slope tomography), the tilt corresponding to each image can be entered manually.

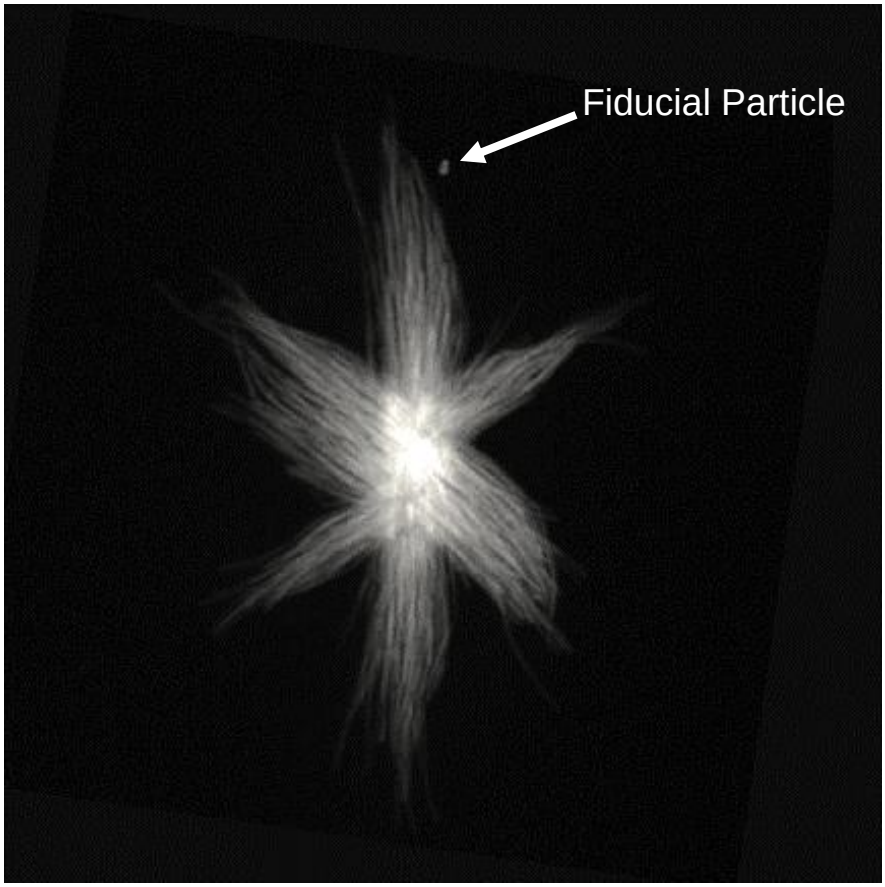


Once the properties of the tilt series are assigned, the software gives us access to tools for aligning the data, and generating 3D reconstructions. Go to the Tomography tab again. You will see that there are two main options for aligning the images in the tilt series, “Image Alignment (Auto)”, and “Image Alignment (Manual)”.

Image Alignment (Auto) applies an algorithm which cross-correlates the features of each image in the stack, shifting them to maximize the overlap of these features.

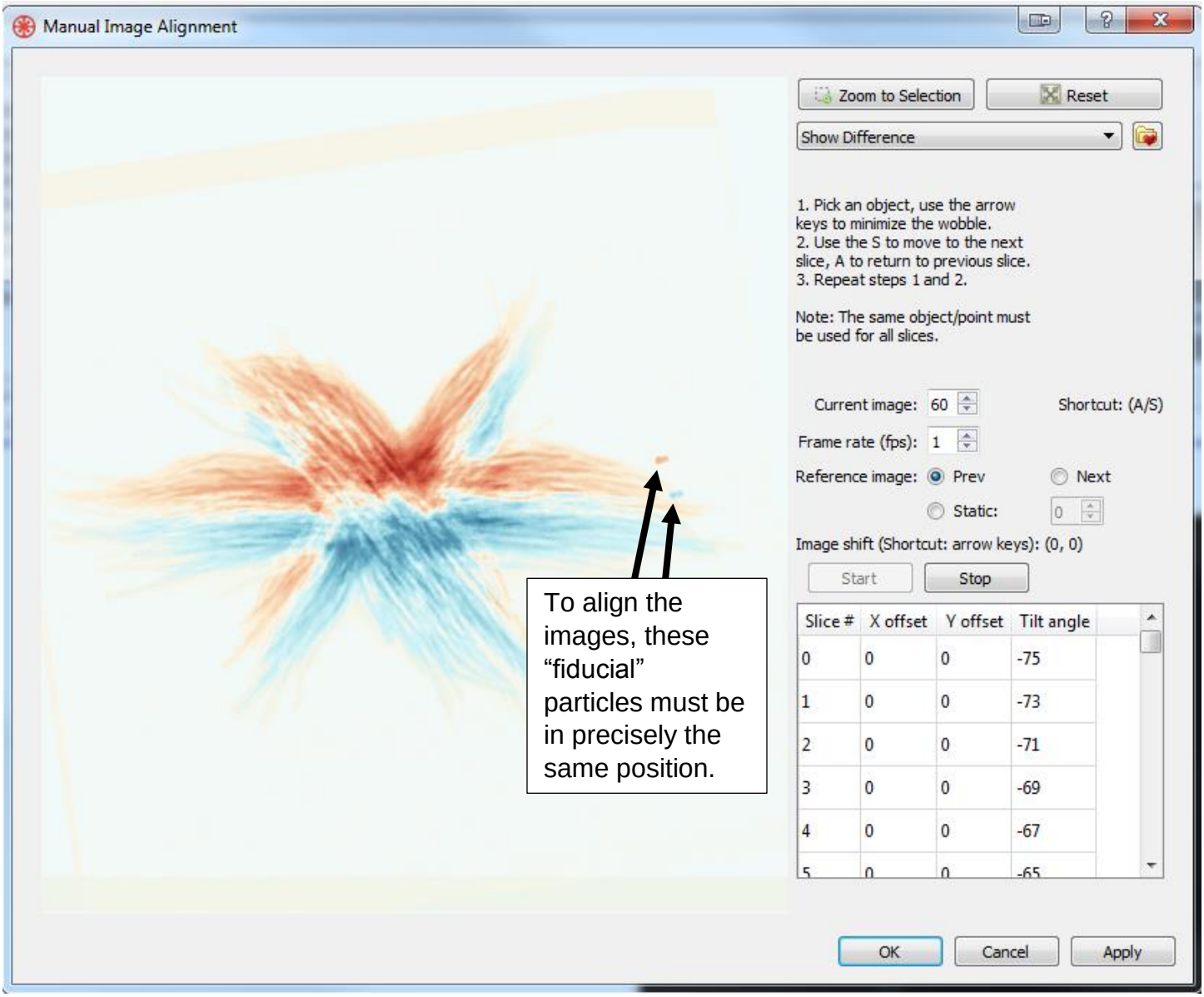
Image Alignment (Manual) allows the user to align the data by manually shifting the images so that the position of a small “fiducial” feature is the same in every image. In our data, two small gold nanoparticles above and to the right of the Co_2P nanoparticle can be used as fiducials. Manual alignment to very small fiducial particles is a very accurate, and commonly used alignment method, and so this is the method we shall proceed with.


Tomography
Visualization
View
Tools
Sam
Mark Data As Volume
Set Tilt Angles
Pre-reconstruction Processing:
Background Subtraction (Auto)
Background Subtraction (Manual)
Normalize Average Image Intensity
2D Gradient Magnitude
Image Alignment (Auto)
Image Alignment (Manual)
Tilt Axis Alignment (Manual)


Fiducial Particle

2.1 Manual Alignment.

On selecting Image Alignment (Manual) from the Tomography tab, a dialog box pops up with a range of tools to help you align the data. There are two display modes. "Toggle Images" flashes between two images that are being aligned at a frame rate specified by the user. "Show Difference" maps the difference in value between two images being aligned, plotted on a color scale specified by the user (Blue to Red shown below),
Images can be aligned to the previous image in the tilt series, the next image in the series, or to a fixed reference image in the series. You can use your keyboard's arrow keys to shift the images until the fiducial particles line up, or you can enter shifts manually into the shift array in the lower right of the dialog box. You can zoom in to the area containing the fiducial particles in order to align them more accurately.



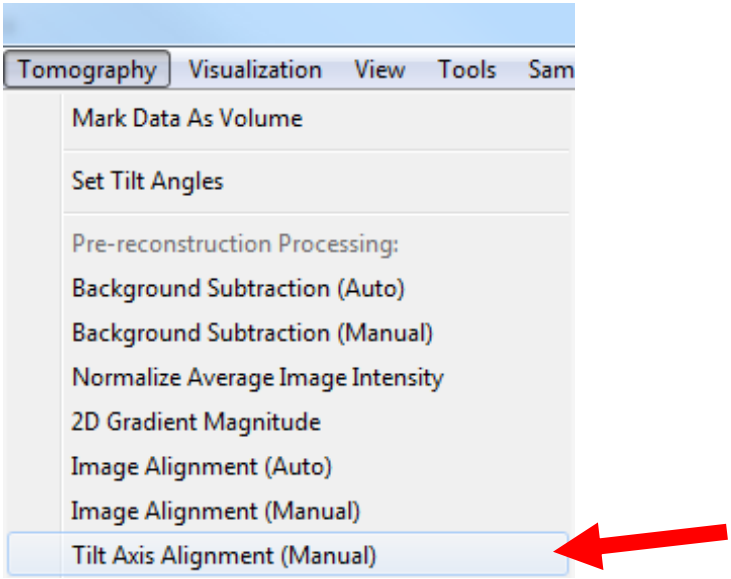
Once the fiducial particles are aligned to the same position in each image, the precise tilt axis must be found.

SAVING YOUR WORK ALERT!
Aligning data can take a long time. Make sure you save your work after you do an alignment.

Your first pass at alignments won't always get it perfectly right. Repeat at least one or two more times to refine your alignment before heading on to the next step.

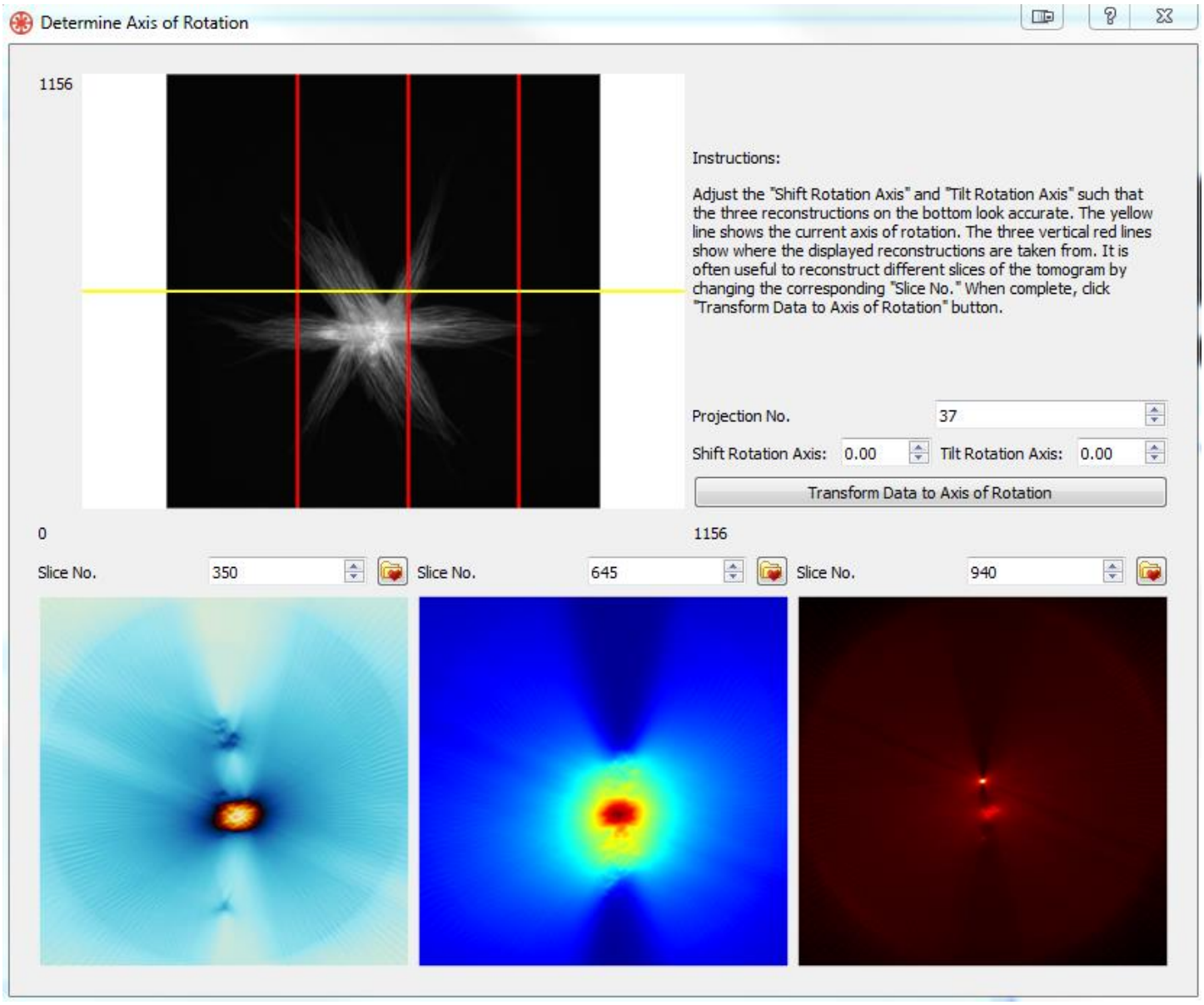
2.2 Determining Precise Tilt Axis.

In addition to aligning all of the images, the software needs to know the position and angle of the tilt axis in order to reconstruct the data. To find the tilt axis, go to the Tomography tab again and select "Tilt Axis Alignment (Manual)".



This brings up another dialog box. Here, you choose 3 slices (shown by the red lines), and the software computes a quick reconstruction showing you what each of those slices through the 3D volume will look like with your current tilt axis alignment. You should put one of your slices on a fiducial particle, and place the other two on interesting features of the sample if possible.
Adjust the angle and position of the tilt axis (yellow line) to minimize artifacts. Eg. The fiducial nanoparticles should be roughly spherical, but will appear crescent shaped if the tilt axis is in the wrong position.
The color map for each reconstructed slice can be changed separately to aid users in searching for artifacts.

Once satisfied that the tilt axis is correct, click "Transform Data to Axis of Rotation". Wait for the software to transform the data, and then proceed to reconstruction.


Determine Axis of Rotation
1156
Instructions:
Adjust the "Shift Rotation Axis" and "Tilt Rotation Axis" such that the three reconstructions on the bottom look accurate. The yellow line shows the current axis of rotation. The three vertical red lines show where the displayed reconstructions are taken from. It is often useful to reconstruct different slices of the tomogram by changing the corresponding "Slice No." When complete, click "Transform Data to Axis of Rotation" button.
Projection No.
37
Shift Rotation Axis:
0.00
Tilt Rotation Axis:
0.00
Transform Data to Axis of Rotation
0
1156
Slice No.
350
Slice No.
645
Slice No.
940

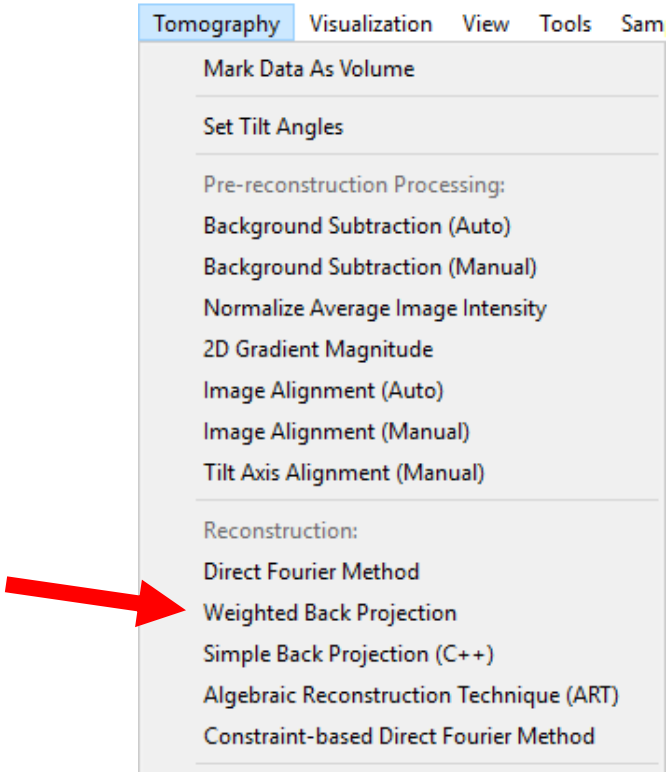
3.0 – Basic 3D Reconstruction.

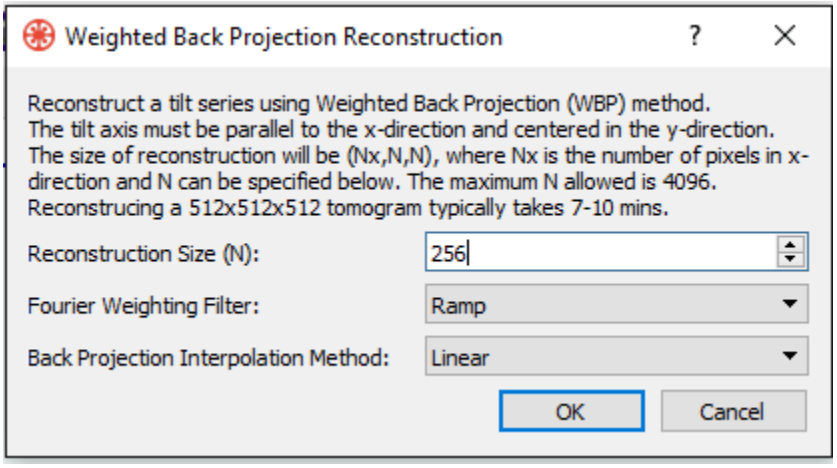
Now you're onto the most computationally intensive part of the process, so downsize your data if you don't have a powerful processor and a ton of RAM (see page 3).

SAVING YOUR WORK ALERT!
Some of the more complex reconstruction algorithms can take a long time to run, so make sure you've saved your data before you attempt a reconstruction.

There are many different algorithms for reconstructing a 3D object from a tilt series. One of the more basic methods is Simple Back Projection, which essentially projects 2D images back into three dimensions, and compares the projections acquired at different angles to try to recover the structure of the 3D object.
Another basic algorithm is the Direct Fourier method. This algorithm takes advantage of the Central Slice Theorem, that each 2D projection image of a 3D object, acquired at each different tilt has a corresponding plane in the 3D Fourier transform of the 3D object. Collecting enough 2D images of the object at enough tilt angles allows you to reconstruct the object's 3D Fourier transform. Applying an inverse 3D Fourier transform recovers the 3D structure of the object.
Other algorithms, such as Weighted Back Projection build on these more basic algorithms to try to produce high quality reconstructions.

Once you've suitably downsized your data, try running the Weighted Back Projection algorithm for yourself to get a 3D reconstruction of the Co_2P particle! Just go to Tomography, and click "Weighted Back Projection", and then set parameters in the dialog box to run the algorithm.

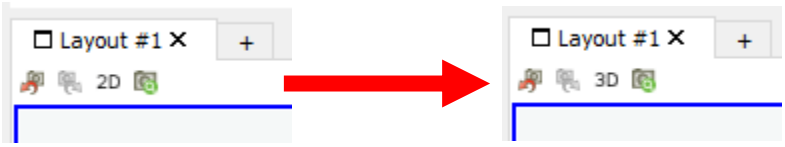




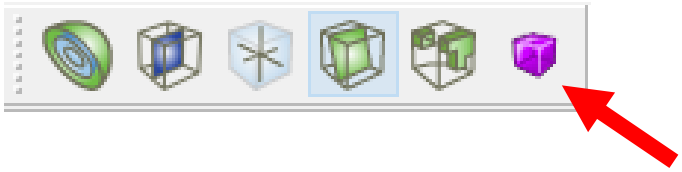
Depending on how powerful your computer is, this could take a while, so make sure you have something else to do while you wait!

4.0 Visualizing 3D Volume Data

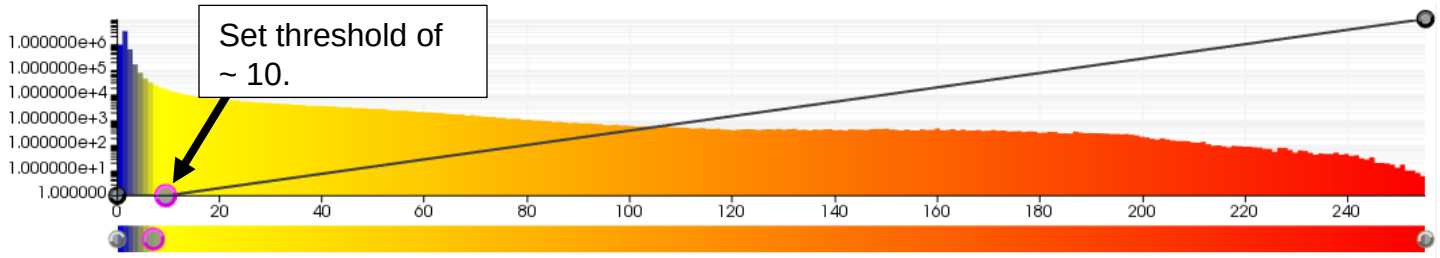
Once you have a 3D reconstruction, you can visualize the 3D structure of the particle. If the viewing mode under Layout is still in 2D, set it back to 3D by clicking on "2D".



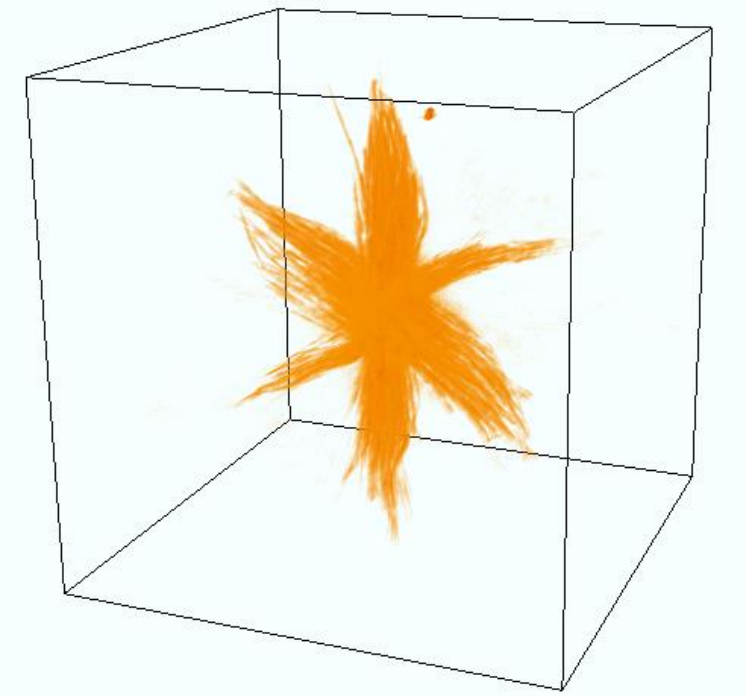
Volume rendering is a simple and popular method of displaying 3D objects. To get a volume render of your data in *tomviz*, click the purple cube on the main toolbar.



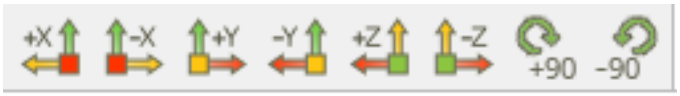
Initially, all intensities in the volume will be displayed on a linear scale, but you can set a threshold so that the 3D object is more visible by clicking on and dragging part of the dark line on the color map.



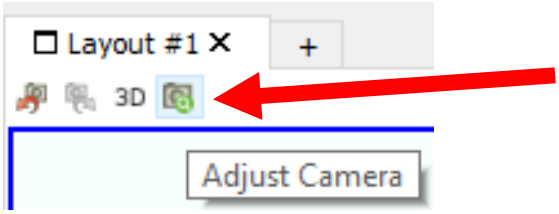
The 3D image of the particle will be visible in the Layout section of the screen.



You can use the mouse to alter the angle of view of the particle and zoom in and out. You can go to specific on-axis viewing angle, or rotate in 90° increments by clicking the axis buttons on the man toolbar.



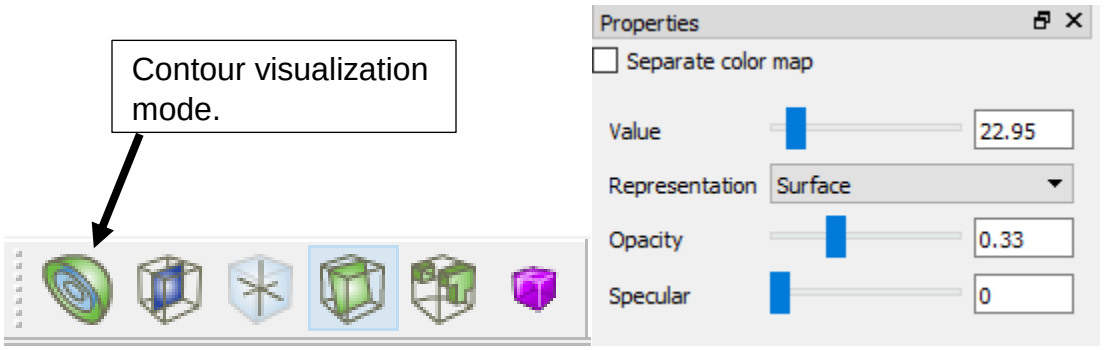
For precise viewing angles and magnifications, you can use the "Adjust Camera" dialog to enter values.

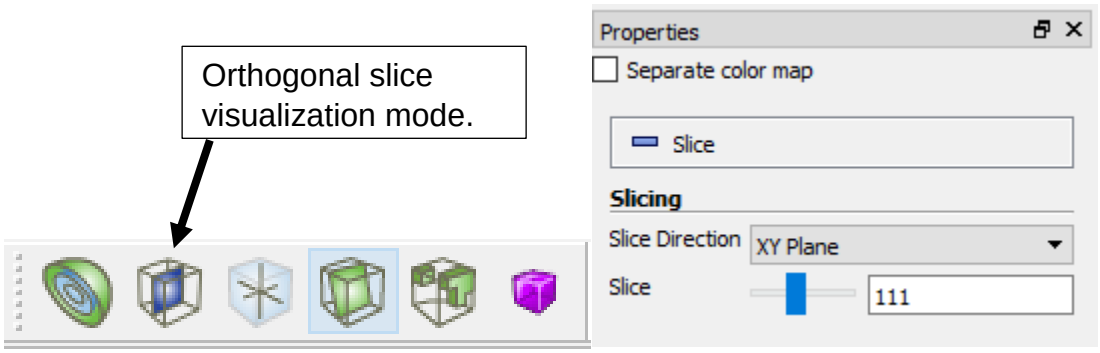


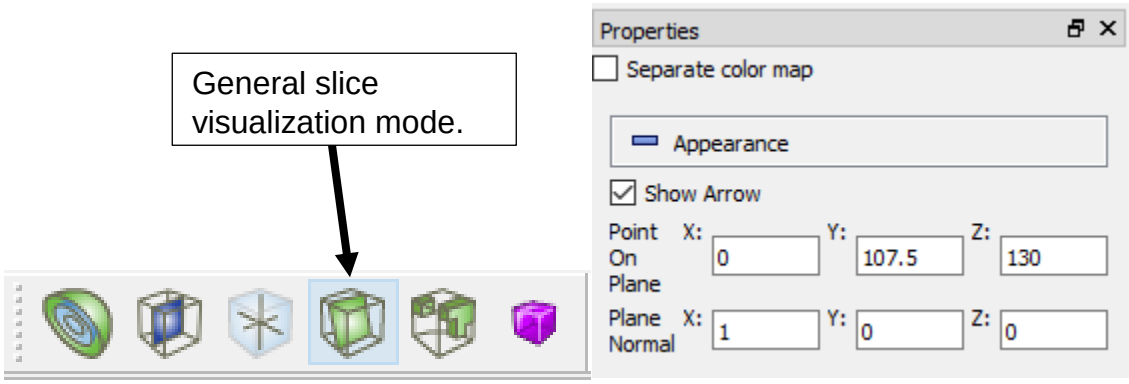
Interacting with the data in 3D should give you a full sense of the 3D structure of the particle.

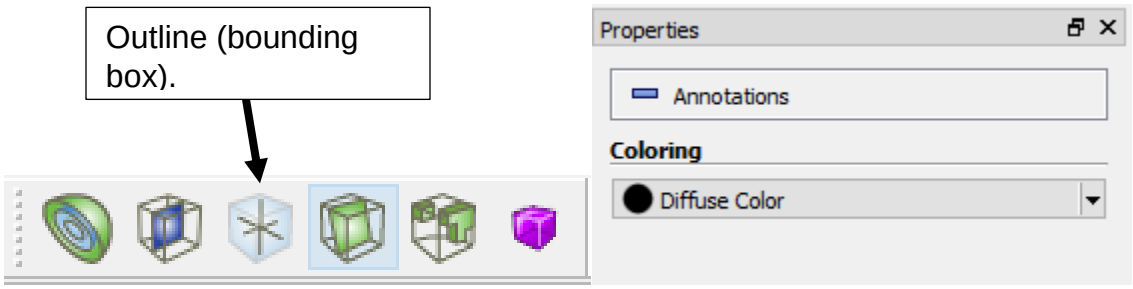
4.1 Different Visualization Modes

In addition to volume visualization, other popular modes for viewing the data include surface contours (which can give a glossier appearance than volumes), and 2D slices of the data, which can be helpful for showing specific features in the interior of the structure.
The parameters for each visualization mode can be set in the Properties box in the lower left of the display. You can also set the color of the outline (bounding box).

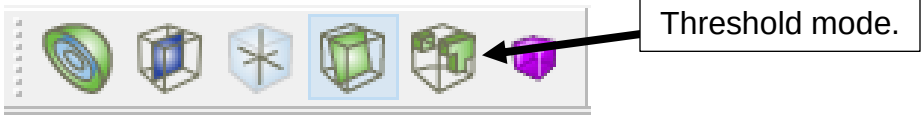






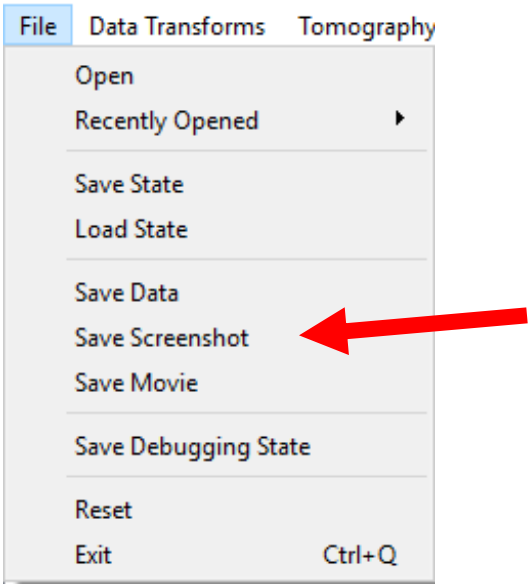


The Threshold visualization mode allows minimum and maximum values for contours to be set. This is currently very computationally intensive, and only recommended for high-end machines.

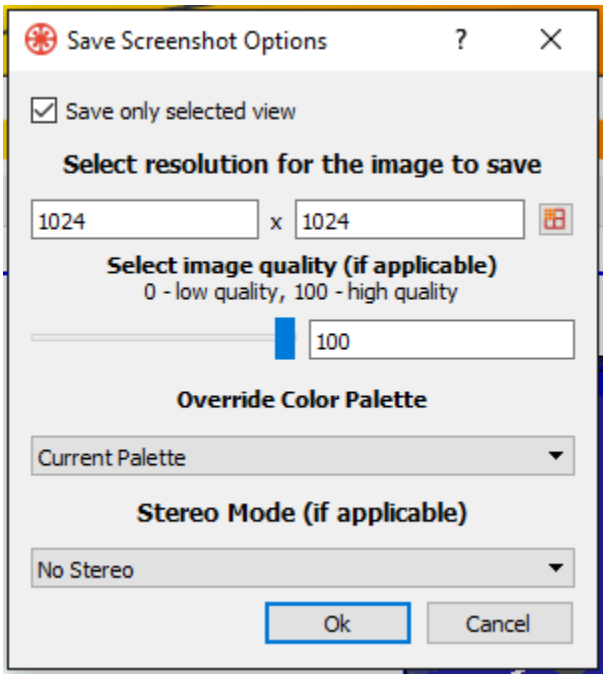


4.2 Saving Screenshots

To save a screenshot of a particular view, either for publication, or to share with colleagues, go to File and click "Save Screenshot".



In the dialog box, you can set the image dimensions, resolution, and other parameters.

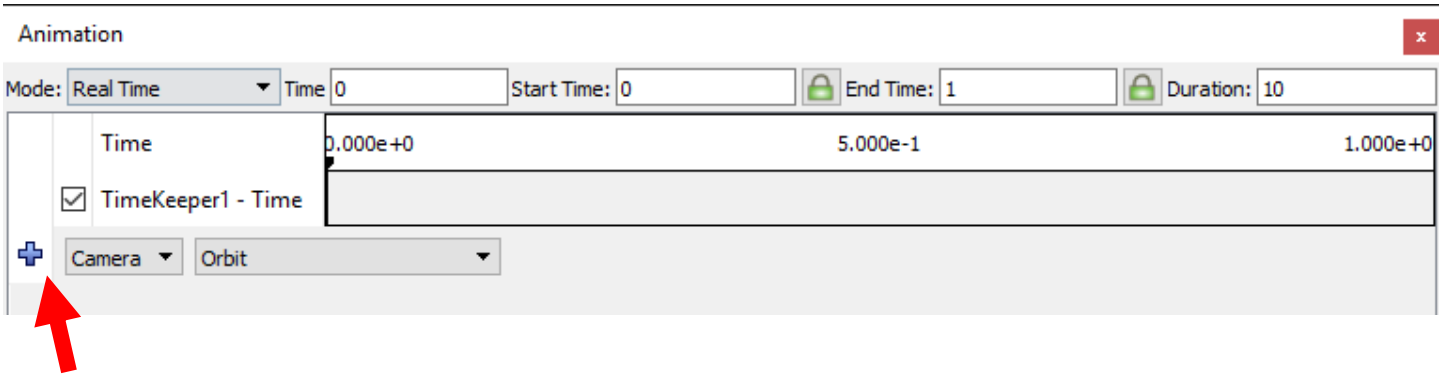


PNG, TIFF, JPEG, PPM and BMP file types are available for saved screenshots.

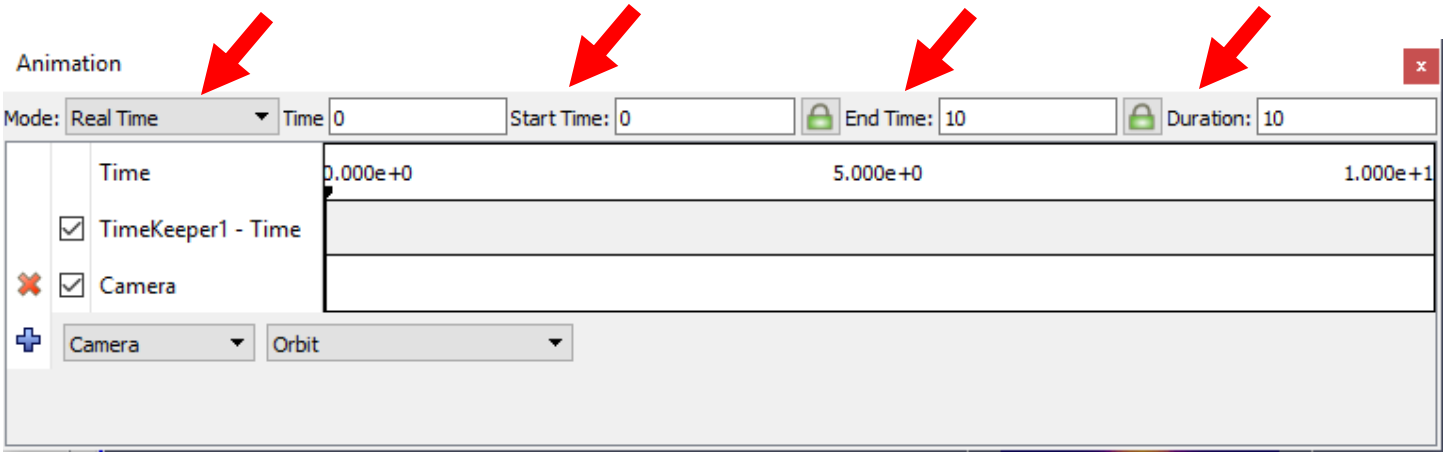
4.3 Creating and Saving Animations

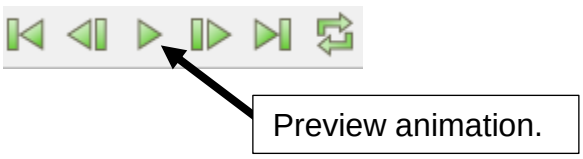
Animations can convey more of the 3D structure of an object than screenshots. *Tomviz* has an animation tool that allows users to create either simple, or intricate animations of their 3D structures, either to share with colleagues, or publish online.

The simplest animation is a 360° orbit about the object. To create an orbit, go to the animator tool, and choose "Camera" and then "Orbit" from the drop down menus. Then, click the blue "+" button to add this animation.

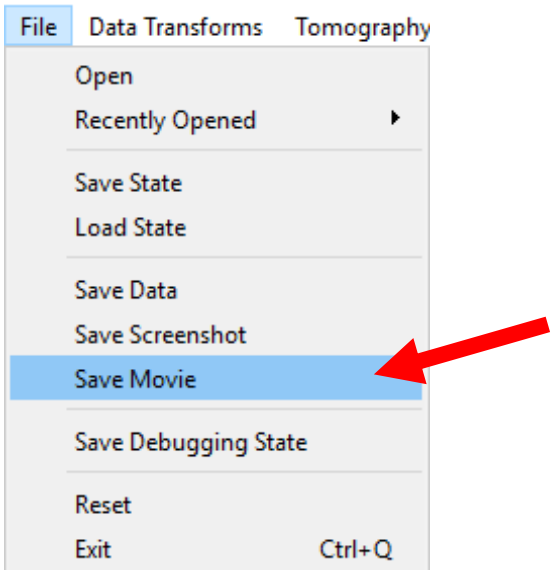


Once you've chosen animation type, you can set desired animation duration (in seconds) on the animation toolbar. You can preview animations using the animation buttons on the main *tomviz* toolbar. Setting animation mode to "Real Time" is helpful for previews of short animations.

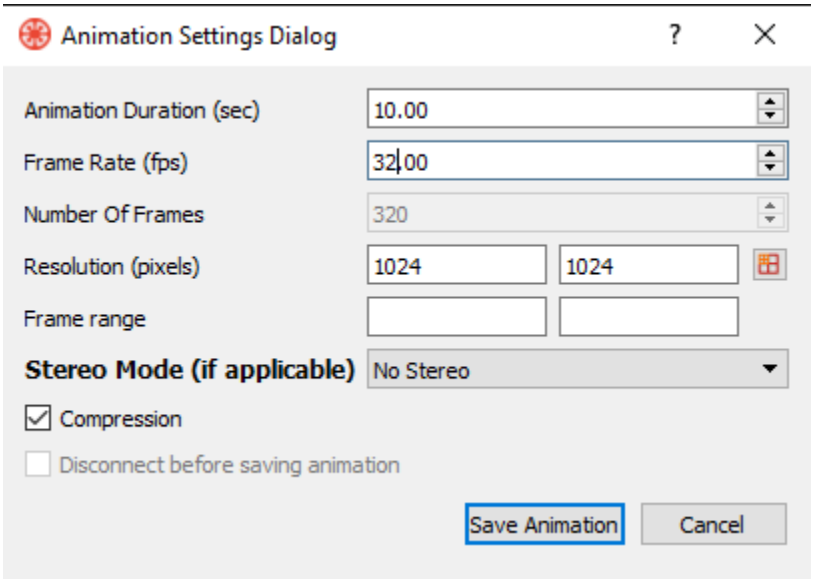




Once satisfied with your animation, you can save it by going to the File tab and clicking "Save Movie".



This brings up a dialog box which allows you to change the duration, and set the frame rate and resolution.

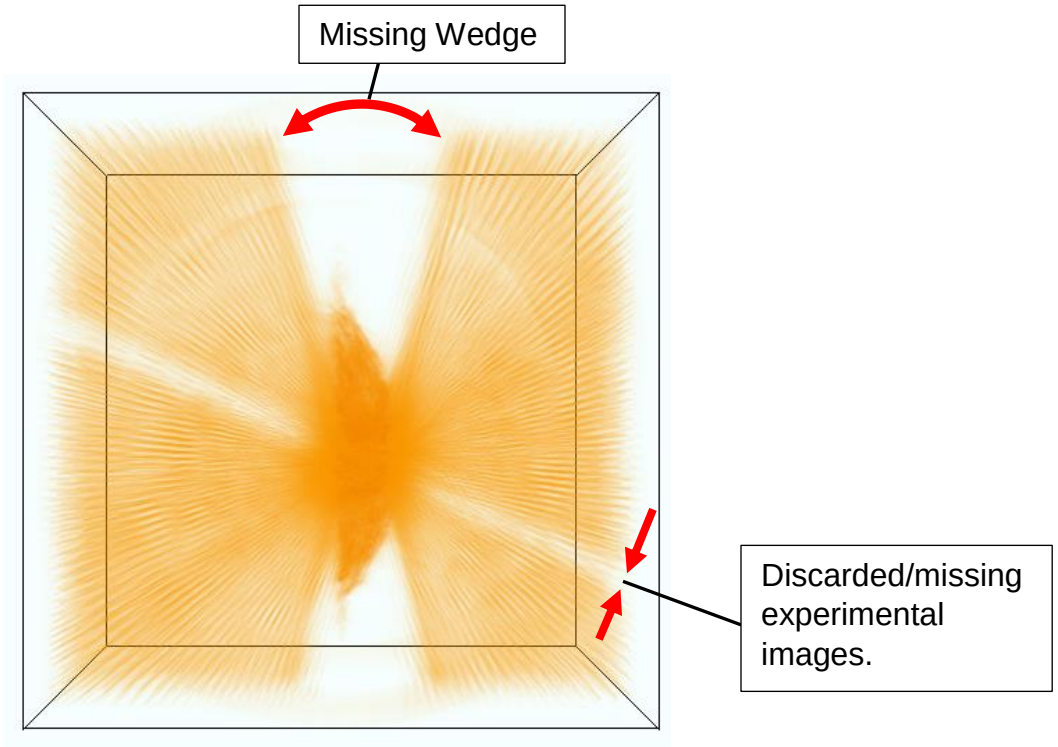


Animations can be saved in OGG and AVI format.

Advanced users can create more intricate animations using the "Interpolate Camera Positions" function. This allows you to create a custom path for the camera, passing through multiple user specified positions, with a user specified interval between them.

5.0 Advanced Reconstruction

In electron tomography, the geometry of most microscopes and specimen holders precludes a full 180 degree tilt range. Tilt ranges are typically limited to ~ 150 degrees. This results in a "missing wedge" of information, which manifests as artifacts in a 3D reconstruction.
The missing wedge in a weighted back projection reconstruction can be directly visualized by reducing the threshold intensity value displayed in the color map, and then viewing the object down the experimental tilt axis.



Various reconstruction algorithms have been devised to try to minimize missing wedge artifacts in electron tomography. In *tomviz*, the "Algebraic Reconstruction Technique" (ART), and the "Constraint Based Direct Fourier Method" are examples of such algorithms. These algorithms require high RAM and processing power to run quickly.

Advanced users can code their own algorithms into *tomviz* via Python. We are also aiming to have more reconstruction algorithms available a standard in *tomviz* in the near future, including the popular SIRT algorithm, and newer optimization based algorithms.