LongAxis Manual

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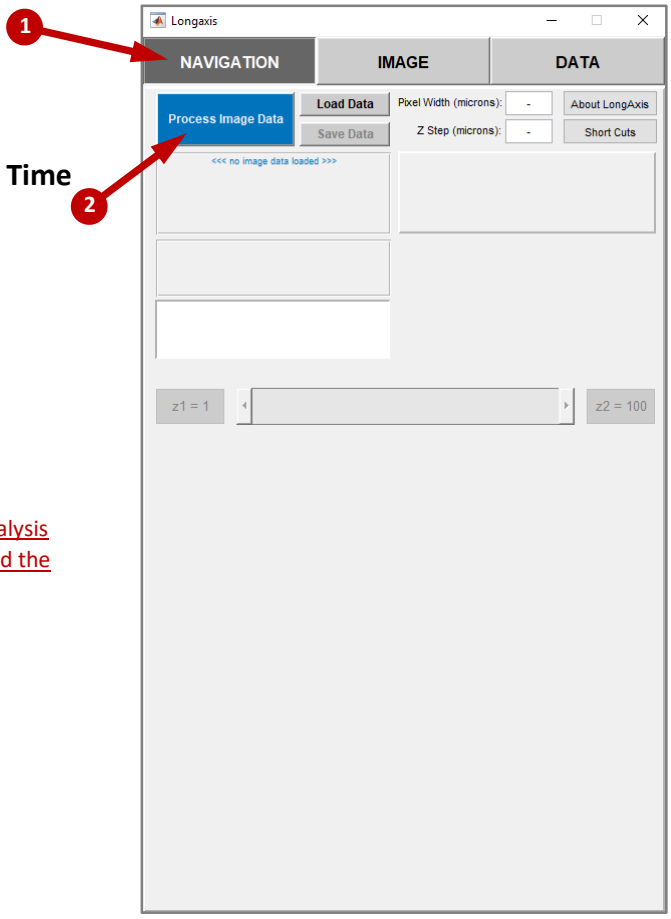
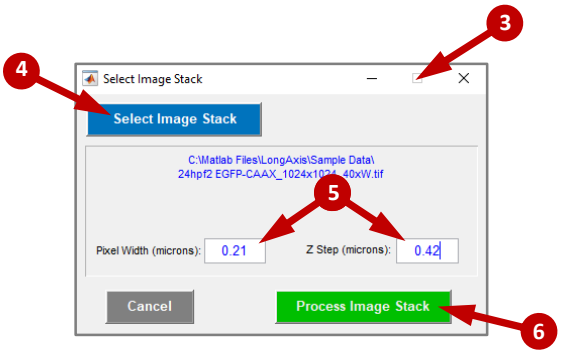
A. Starting LongAxis

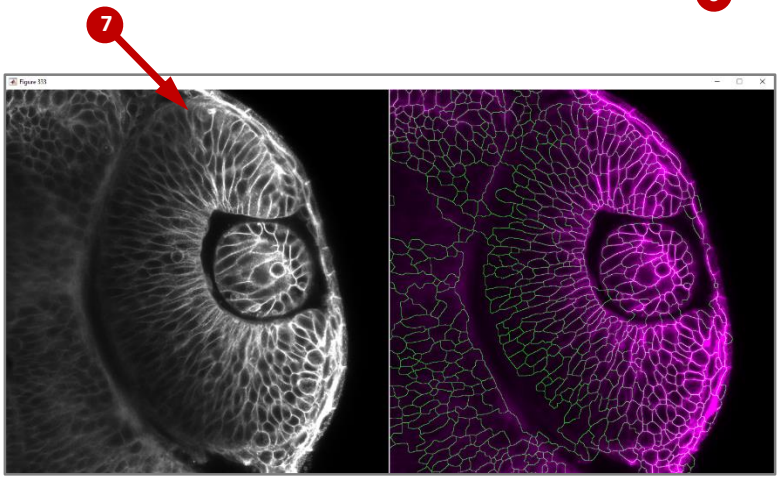
- Open MATLAB.
- Type "longaxis" at the prompt and LongAxis should open.

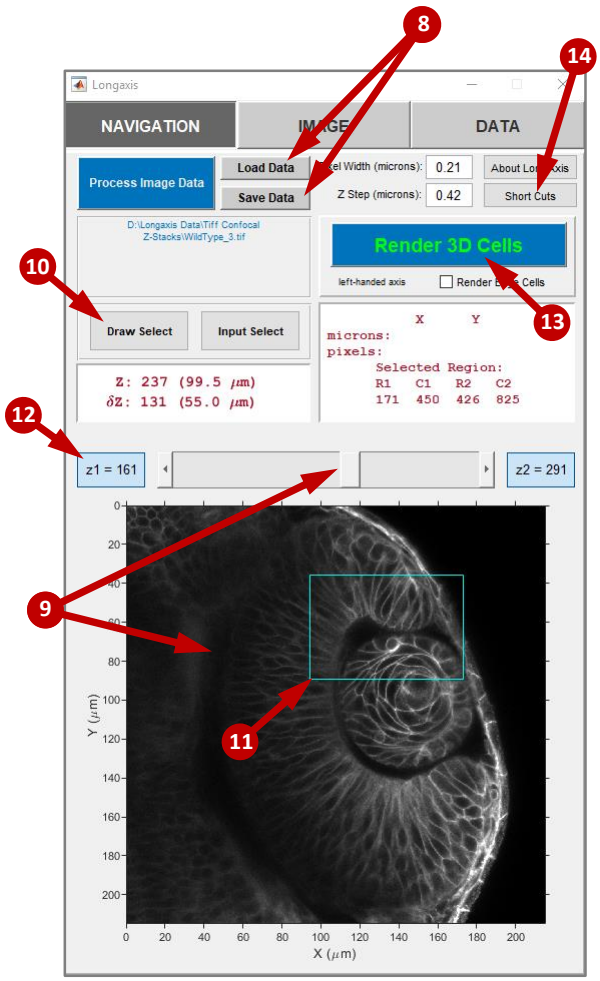
B. Loading and Processing Confocal Z-stack for the First Time

1. Click on **Navigation** tab.
2. Click on **Process Image Data** button,
3. and **Select Image Stack** window will open.
4. Select file location of 8bit grayscale z-stack.
5. Input pixel size and z-step size in microns and hit <enter>.
6. Click **Process Image Stack** button after it becomes enabled.
7. LongAxis will now start the 2D image processing. *This could take 5-30 minutes depending on data file size and your computer's processing speed*.
8. When 2D processing is done the **Save Data** button will be enabled. Click **Save Data,** and save processed image data. For subsequent analysis of the same data set, you can skip the 2D processing and quickly load the saved processed data set into LongAxis using **Load Data**.







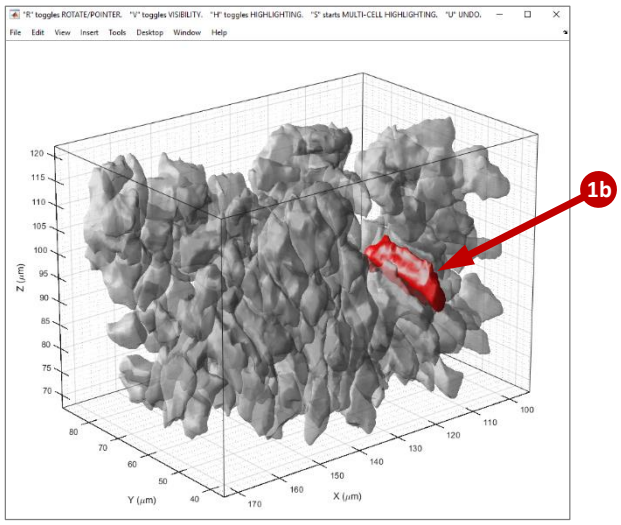


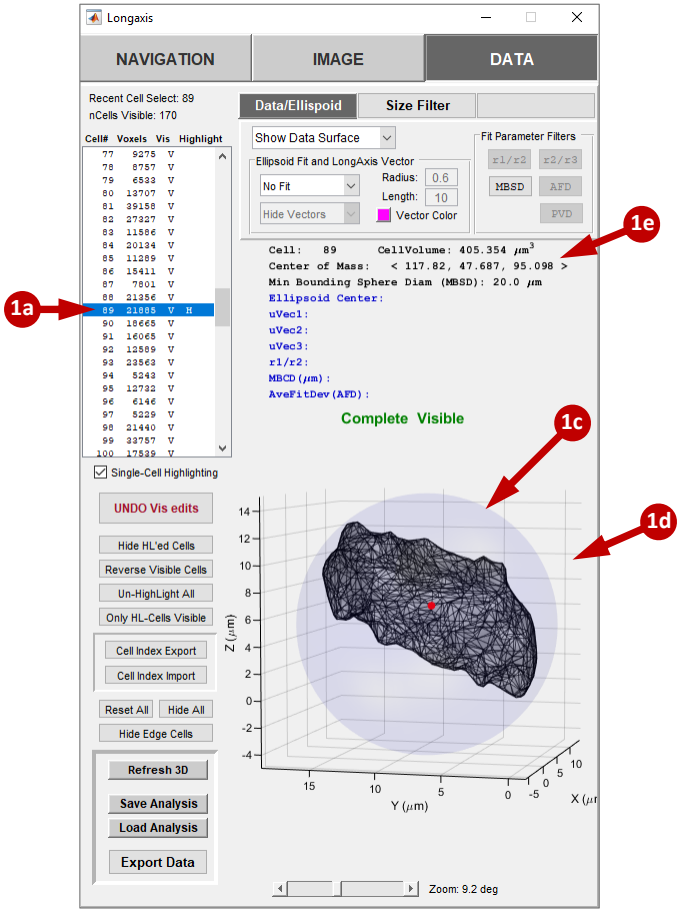
C. Select 3D Rendering Volume

9. Move **Z-slider** until a representative sample of the image is displayed.
10. Click on the **Draw Select** button.
11. Move cursor to data-viewing window and define the XY region of interest by drawing a rectangle (left-mouse click when done).
12. Move Z-slider up and down and set upper and lower z boundaries by pressing **z1** and **z2** buttons (press again to reset).
13. After XY and Z boundaries are set, press the **Render 3D Cells** button.
14. After rendering is completed, click on **Short Cuts** button for a list of key-presses that will make interacting with the rendering faster and easier.

D. Cell Selection

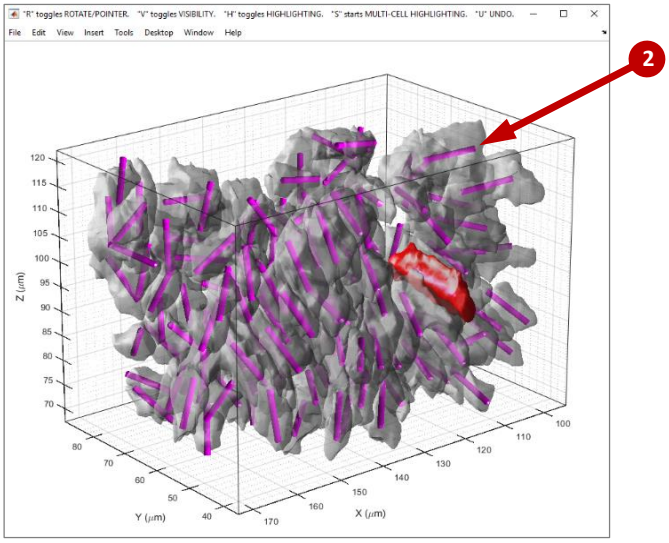
1. Clicking on a cell in the rendered object list **(1a)**, or a cell in the rendering figue **(1b)**, will highlight the cell in both locations as well as display it in the cell-display-figure **(1d)**. The blue tinted sphere around the rendered cell represents the Minimum Bounding Sphere Diameter (MBSD) **(1c)**. Pressing <V> on the keyboard will control the visibility of the cell, and <H> controls highlighting. Visibility defines which cells are included in data exports, histograms, and convergence point calculations. Currently selected cell states are also displayed **(1e)**.

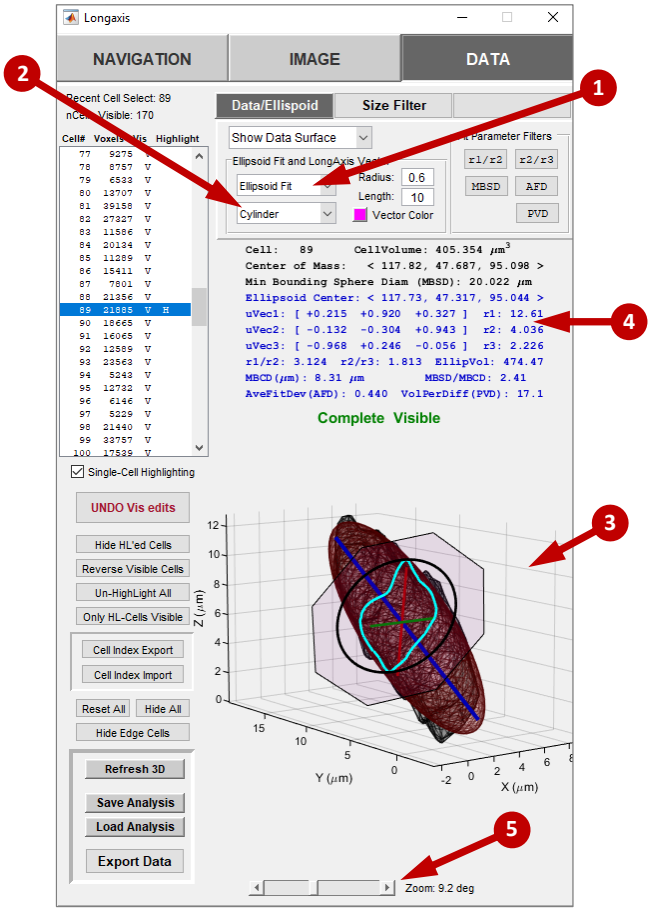




E. Adding Ellipsoid Fit and Displaying Longaxis Vectors

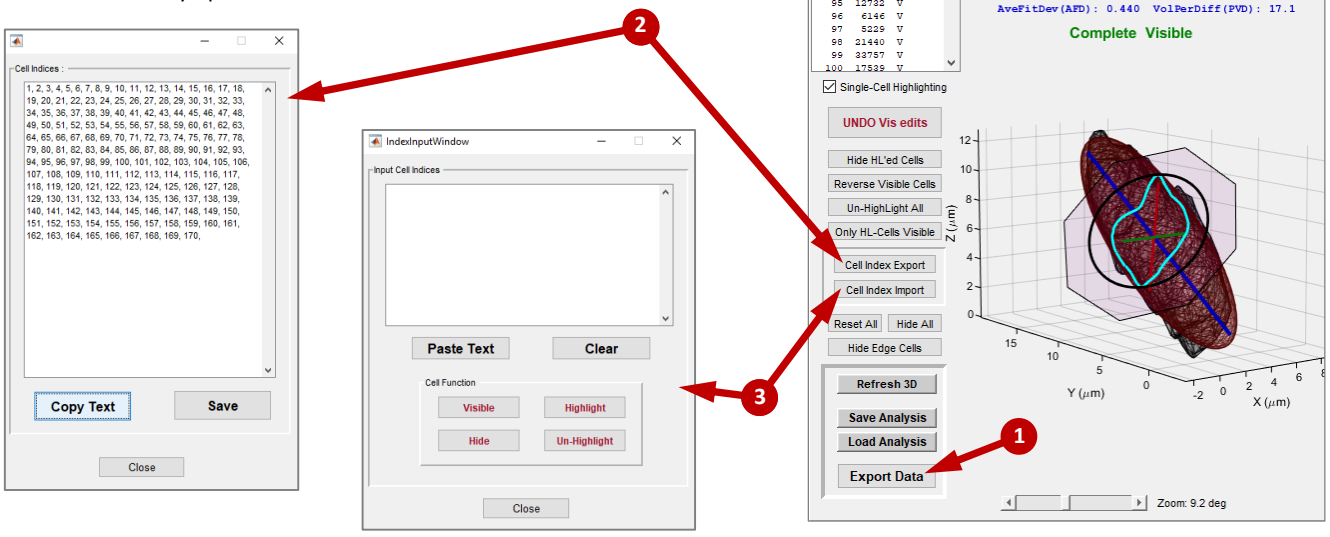
1. Turn on **ellipsoid fit** by selecting "Ellipsoid Fit" under fit selection menu.
2. Display ellipsoid **long-axis vectors** by selecting "cylinder".
3. When "Ellipsoid Fit" is turned on the cell-display-figure shows: Ellipsoid overlay **(red)**, ellipsoid longaxis **(blue line)**, and Minimum Bounding Circle Diameter (MBCD) **(black)**. The MBCD is calculated in the plane **(pink octagon)** that runs through the cell's center of mass and is perpendicular to the ellipsoid-fit's long axis.
4. Ellipsoid-fit stats are shown in blue.
5. The zoom control for the cell-display-figure is expressed in terms of the view angle (degrees). *Small angles are far, and large angles are close*.





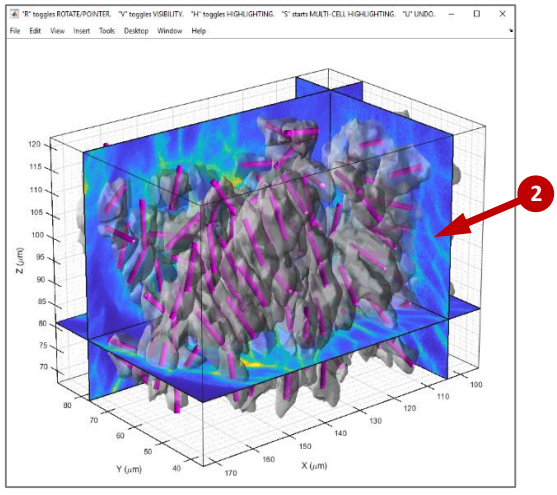
F. Exporting Data and Cell Index Import/Export

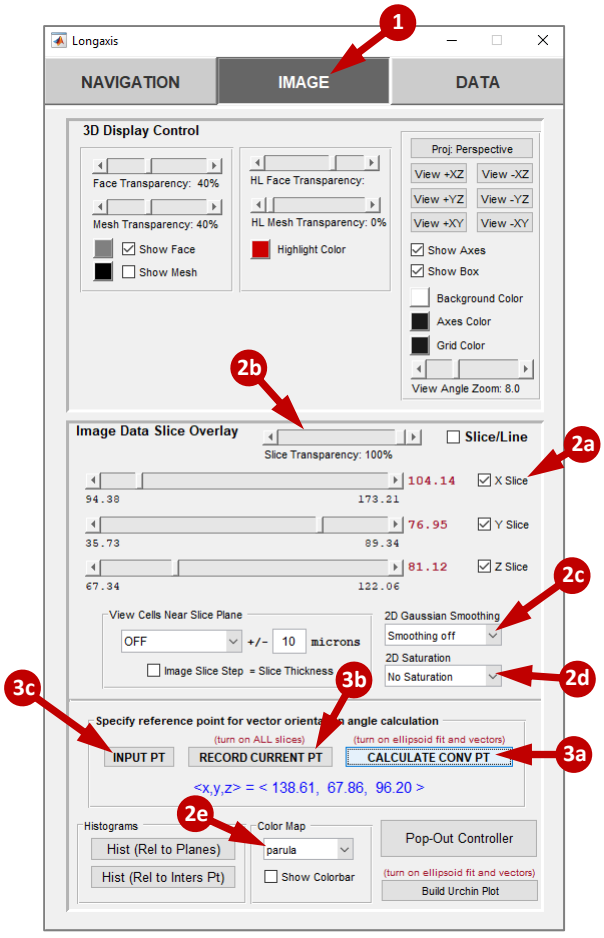
1. Pressing **Export Data** will create a text file (easily imported into MS Excel) that contains the rendered cell measurements **(D1e)**. If **Ellipsoid Fit (E1)** and **Cylinder (E2)** are turned on, the ellipsoid meaurements are included in the exported data file **(E4)**. *(note: orientation angles will only be calculated if there is a reference point specified [see G3])*
2. The **Cell Index Export** button will provide a list of all the current visible/selected cells. *It is recommended copying these index numbers into a MS Word document and labeling them according to their region (example: lens, retina,...).*
3. The **Cell Index Import** button allows you to input cell index numbers and control visibility options for them.



G. Specifying Reference Point and Overlay Data Slices

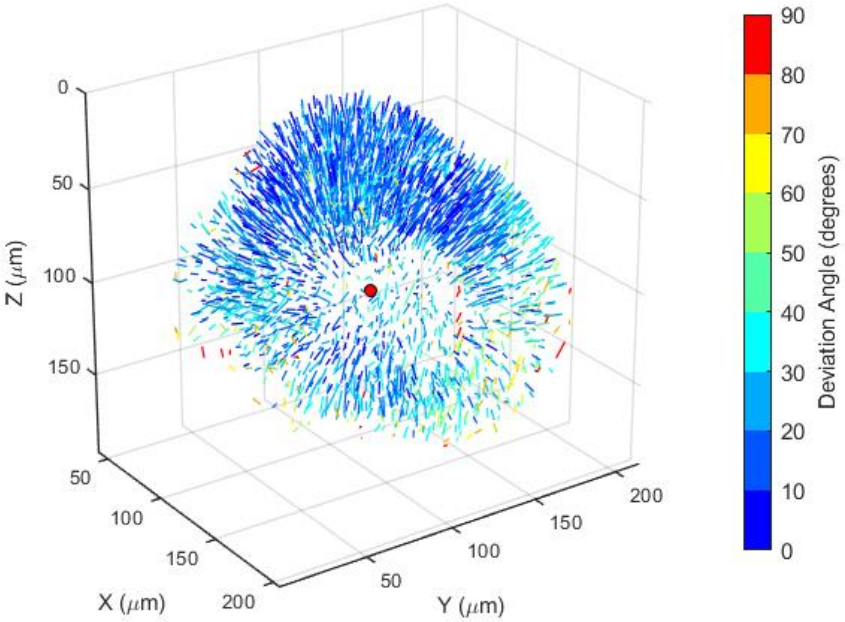
1. Select **IMAGE** panel.
2. Turning on X,Y,and Z slices **(2a)** will create image-data cross-sections overlaid with the rendered cells. Cross-sections also have transparency **(2b)**, smoothing **(2c)**, saturation **(2d)**, and colormap **(2e)** controls.
3. A reference point for orientation angle calculation can be calculated in three different ways:
   a. If **Ellipsoid Fit (E1)** and **Cylinder (E2)** are turned on, the **Calculate Convergence Point (3a)** button is enabled. The calculation uses all of the current visible cells.
   b. Pressing **Record Current Point (3b)** will record the point of intersection of all three image-data slices.
   c. Lastly, the reference point can also be input manually **(3c)**.

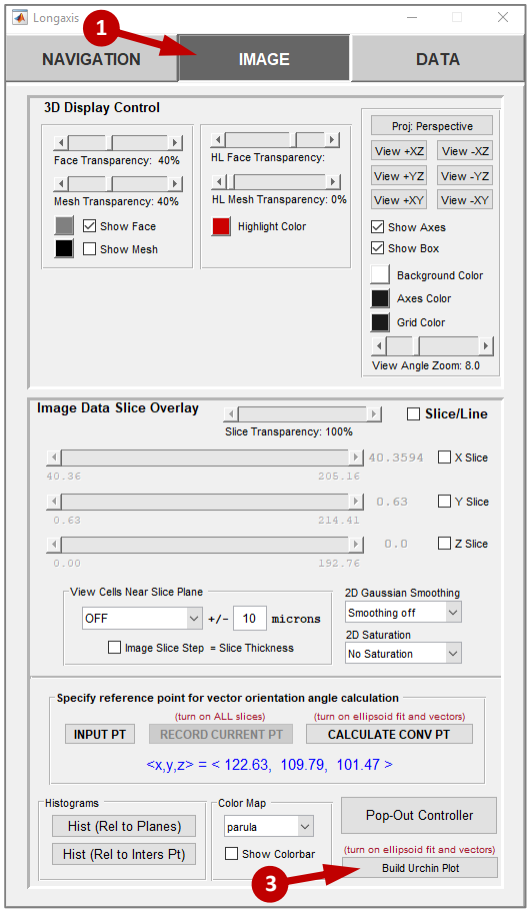




H. Creating Urchin Plots

1. Select **IMAGE** panel.
2. Turn on Ellipsoid Fit **(E1)** and Vectors **(E2)** and specify a reference point **(3a, 3b, or 3c)**.
3. Press **Build Urchin Plot** button.





I. Filtering

1. The visibility of cells can be filtered based on several parameters. *note: all filter functions (except MBSD) require the Ellipsoid Fit (E1) and longaxis vectors (E2) to be turned on.*
   a. **MBSD**: Filter cells based on their Minimum Bouding Sphere Diameter.
   b. **r1/r2** ratio:, cells can be filtered by the ratio of the fitted ellipsoid's r1 and r2 axis length.
   c. **r2/r3** ratio: (*Same as r1/r2 except the other axes combination*)
   d. **PVD**: Filter cells based on the percent volume difference of the cell and fitted ellipsoid.
   e. **MBSD/MBCD** ratio: Filter cells by the ratio of their Minimum Bounding Sphere Diameter and Minimum Bounding Circle Diameter.