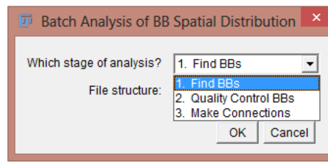
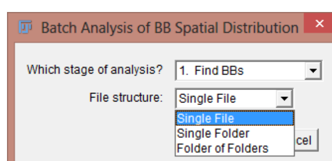
Tetrahymena Analysis Routine Instructions (Galati et. al., Biology Open, 2015)

Below are instructions for using the ImageJ/FIJI analysis routine that automatically measures the organization of Tetrahymena basal bodies.

1. Generate single channel or multi-channel TIFF stacks of Tetrahymena where at least one channel is represents a homogeneously bright (i.e., "robust") basal body marker, such as centrin. To get calibrated spacing information regarding basal body organization, the images should be captured using a camera with calibrated pixel sizes.
2. Download the ImageJ macro file . Also make sure that your version of ImageJ contains the XXX plugins, which are required by the analysis routine.
3. Open the macro file in ImageJ or FIJI's script editor.
4. Run the macro, which operates in Batch mode so images will be hidden while the analysis routine is running.
5. The analysis routine is divided into three stages which need to be run sequentially to generate a basal body organization analysis. So the first prompt asks the user to choose which stage of the analysis they would like to run.



   a. 1. Find BBs – Crops and orients the cell and finds its cortical basal bodies. The pixel coordinates for these features are stored in a .txt file that is automatically generated in the same folder where the image is located.
   b. 2. Quality control BBs – Displays a composite of the raw basal body image (red) and the centroids of the found basal bodies (green) and generates an interactive cursor for editing basal bodies. First, the user scrolls through the stack and left-clicks near each extraneous basal body to remove it. Next, the user left clicks near each missed basal body to add it. The revised pixel coordinates are updated in the .txt file.
   c. 3. Make connections – Uses an iterative process to find the anterior partner of each basal body in the .txt file and identifies the location of each basal body relative to cellular polarity cues. The final data is saved as .xls file in the same folder with the image and the .txt folder. This data can be sorted or analyzed using your favorite method (Excel, MatLab, etc.).
6. The analysis routine works on single images, folders of images or folders of folders of images. So the second prompt asks the user to choose the file structure for the analysis.



a. If the user chooses a folder or a folder of folders, the routine assumes that the folder only contains the relevant image files.

2. If the user has chosen to “1. Find BBs”, the routine will then ask for the user to input basic image information. This information includes the channel number that contains the homogenous, or “robust”, basal body marker, the total number of channels in the image and the name of each channel (if there are less than 4 channels, leave the unused channels blank).

Batch Analysis of BB Spatial Distribution
How many channels are your images? 2.0
Which channel is the robust BB marker? 1.0
Channel 1 Centrin
Channel 2 Poc1
Channel 3
Channel 4
OK Cancel

3. If the user has chosen “2. Quality control BBs” or “3. Make connections”, the user does not need to re-enter the image information since it is automatically stored within the .txt file that was generated by “1. Find BBs”.

Recommended workflow:

1. Capture a few sample images that represent the typical quality of your images using a confocal microscope.
2. Run “1. Find BBs” and “2. Quality control BBs” on these images to see if the routine is doing a reasonable job of finding basal bodies.
3. If the routine is working well, capture a full data set and run “1. Find BBs” on the entire data set using either “Single Folder” or “Folder of Folders”. This DOES NOT require the user to be present, so the routine will march through each file automatically.
4. After “1. Find BBs” has completed for the entire data set, run “2. Quality control BBs” one file at a time since this DOES require the user to be present.
5. After each file in the data set has been manually corrected and confirmed, run “3. Make connections” on the entire data set using either “Single Folder” or “Folder of Folders” since this DOES NOT require the user to be present, so the routine will march through each file automatically.

Basal body organization output file:

1. *Volume (pix)*- The volume of the 3D convex hull that includes each basal body in the cell.
2. *Volume (unit)*- The calibrated volume of the 3D convex hull (μm^3).
3. *Surface (pix)*- The surface area of the 3D convex hull that includes each basal body in the cell.
4. *Surface (unit)*- The calibrated surface area of the 3D convex hull (μm^2).
5. *X,Y,Z*-The X,Y and Z voxel coordinates that define the peak pixel intensity of each basal body.
6. *Ant_X,Ant_Y, Ant_Z*-The X,Y and Z voxel coordinates that define the peak pixel intensity for each basal body’s anterior partner.

1. *BB_Distance*- The distance between the basal body and it's anterior basal body partner.
2. *Normalized_Pole_Distance*- The distance between the basal body and the anterior pole of the cell. The distance is normalized such that the anterior pole itself is 0 and the posterior pole itself is 1.
3. *BB_Angle_Deviation*- The 3 angle between the anterior pole, the basal body and the basal body's anterior partner. The basal body is the vertex.
4. *Angular_Displacement*- The angle between the oral apparatus, the basal body, and the point on anterior-posterior axis closest to the basal body. The point along the anterior posterior axis is the vertex.
5. *_Int*- The background subtracted intensity of the basal body.
6. *_Partner_Int*- The background subtracted intensity of the basal body's anterior partner.
7. *_Partner_Int_Ratio*- The log2 transformed ratio of _Int:_Partner_Int.