ARBOR TOOLS MANUAL (V1.06)

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Overview

The arbor tools program is designed to assist in segmenting and analyzing arbors that are contained in greyscale TIF stacks. This program uses the Treestoolbox for part of its tree analysis (Copyright (C) 2009 - 2018 Hermann Cuntz).

Setup and installation

Standalone program

If you have an old version of the software installed, be sure to uninstall this first before proceeding. Run the provided installer executable; the program will walk you through installation. As part of this installation a Matlab runtime is required. The installer will automatically download/install the runtime or prompt you to manually install it.

Directly from app

You can also run directly from the app itself in Matlab (.mlapp file). It requires the Treestoolbox, so make sure that folder is in the current folder you are running from. I also recommend installing the latest version of Matlab, as there may be newer functions included in the code that will not work with older versions of Matlab. This program was developed/tested using Matlab R2021a.

Computer recommendations

Recommended specifications for a computer running this program (processing can be quite slow otherwise):

- Minimum 8GB RAM (16 recommended).
- Dedicated graphics card (helps in handling the volume visualization).
- A CPU with 14nm or smaller fabrication (many CPUs made after 2014) designed for desktop use.

Extraction mode

Workflow for extraction mode:

1. Put all the TIF stacks you want to process into a single folder with no other files present.
2. Be sure these TIF stacks are named so that the timepoint follows the first lowercase t. For example:
    a. 233 t0 TA001.tif: The program searches for the first lower case t, then reads up to six digits until it hits an empty space. This is used to order the files by timepoint for both extraction and analysis mode, so be sure they are named like this.
3. Open the program and set the XY size microns field. This is the X and Y size of the stack in microns. Scaling to account for Z differences is done later in batch analysis mode.
4. Press the “Select folder” button and when prompted select the folder you setup in steps one and two.
5. The program will automatically load the first image, but if desired change the “Select file” dropdown to extract from another file. The program will load the image once the file is selected.

6. Press the “Crop stack” button. A loading bar will pop up as it generates fibermetric filter enhanced projections of the stack. This can take up to a minute depending on the computer being used. There are two cropping modes:
    a. Polygonal cropping = You draw a polygon point by point on three 2D projections to create a 3D mask. Everything outside that 3D mask is set to zero. The bounding box of the convex hull of this 3D mask is used to crop the original stack. Crops all Z levels like simple crop does. This method is more time consuming, but produces better results compared to simple crop.
        i. When drawing, a single left click places a point.
        ii. Backspace deletes the most recently placed point.
        iii. Double left click to connect where your mouse is to the first point and complete the polygon. Alternatively, click the first point you placed (cursor will change to a circle as you hover over the first point).
        iv. Holding shift makes the line snap in 15-degree increments as you move your mouse.
        v. You can left click outside the bounds of the image to set a vertex at the closest border of the image.
    b. Simple cropping = You draw and resize a single rectangle on the top down 2D projection.
        i. Left click and hold + drag to create a rough box around the region to crop. You can resize the box using the controls on the box face centers + corners. Once you are happy with the cropping box, double left-click inside the box to set that

crop window. The program crops out everything outside the box and grabs all the Z levels within it.

7. The crop window should close, and the Z projection should appear on the main program. Additionally, the controls for segmentation should appear.
8. Segmentation settings are as follows:
    a. a, b, and c: The size of a window used in filtering. A good starting point is abc = 5x5x3. Smaller windows pick up more detail but also can grab erroneous noise.
    b. Delta, epsilon, and gamma: Used as part of the P filter (Lang et al 2011) to alter segmentation strictness. Higher values will increase the strictness of segmentation and reject more voxels (arbor vs not arbor).
        i. Negative values can be used to try and fix gaps in the arbor, especially with prescale zero. In this case, try using Delta/Epsilon = 8 and gamma = -0.005.
        ii. Positive values are often used in combination with a prescale > 0. When using a prescale of two for example, start with delta/epsilon = 10 and gamma = 0.25.
    c. Prescale: A scaling (multiple of 2) that can be used to improve the segmentation and fix discontinuities. Using this slows the processing down dramatically, especially at a prescale of four or six. Try using zero initially. Increasing the prescale also removes some fine detail from the extracted arbor.
    d. Closing element size: The size of the box window used to close small gaps. If you notice that the arbor you have is nearly accurate but has small gaps this can be used to fix it. This can also connect noise to the main body of the arbor, so be careful about larger values. For example, if a noise volume is within five voxels of the arbor and you pick a closing element size of five or greater, that noise will be connected to the arbor erroneously. **If you need to use this use the smallest possible value to preserve as**

much accurate data as possible (if closing element size of five works, try four to see if that also works).

9. Click the “Apply Segmentation” button once you have picked your settings. Once processing is finished a new window should open showing the labeled volume.
10. Pan around in this new window using the mouse wheel for zoom and left click + drag to rotate the volume.
11. Repeat steps ten through twelve as needed to get the volume to a state where each arbor is one label (color) with as few gaps or erroneous projections as possible. Gaps are ok but should be avoided.
12. Once you have confirmed the volume, use the volume removal panel to remove any small or erroneous volumes. Checking the “Keep?” box for a given volume will leave it when you press the “Remove all but selected volume(s)”.
13. Pressing the “Remove all but selected volume(s)” button will re-open the volume with only what you chose to keep.
14. Now switch to the ‘Skeletonization’ tab and press the ‘Skeletonize’ button a minimum branch length of 0 and smoothing size of 3 (defaults). A new window will open showing this new skeleton overlaid on a transparent arbor. Alter the settings as needed until you are happy with the skeletonization.
    a. The ‘erode volume’ should be left on unless it causes problems. This erodes the entire binary array until just before the number of volumes changes in order to fix skeleton loops that don’t exist. It also improves endpoint detection.
15. Once a skeleton has been generated, the “Write data file” will appear. Press this button once you are finished creating the volume/skeletonizing to create the .mat file.
    a. The mat file will be put into the same folder as the TIF image stacks you picked.

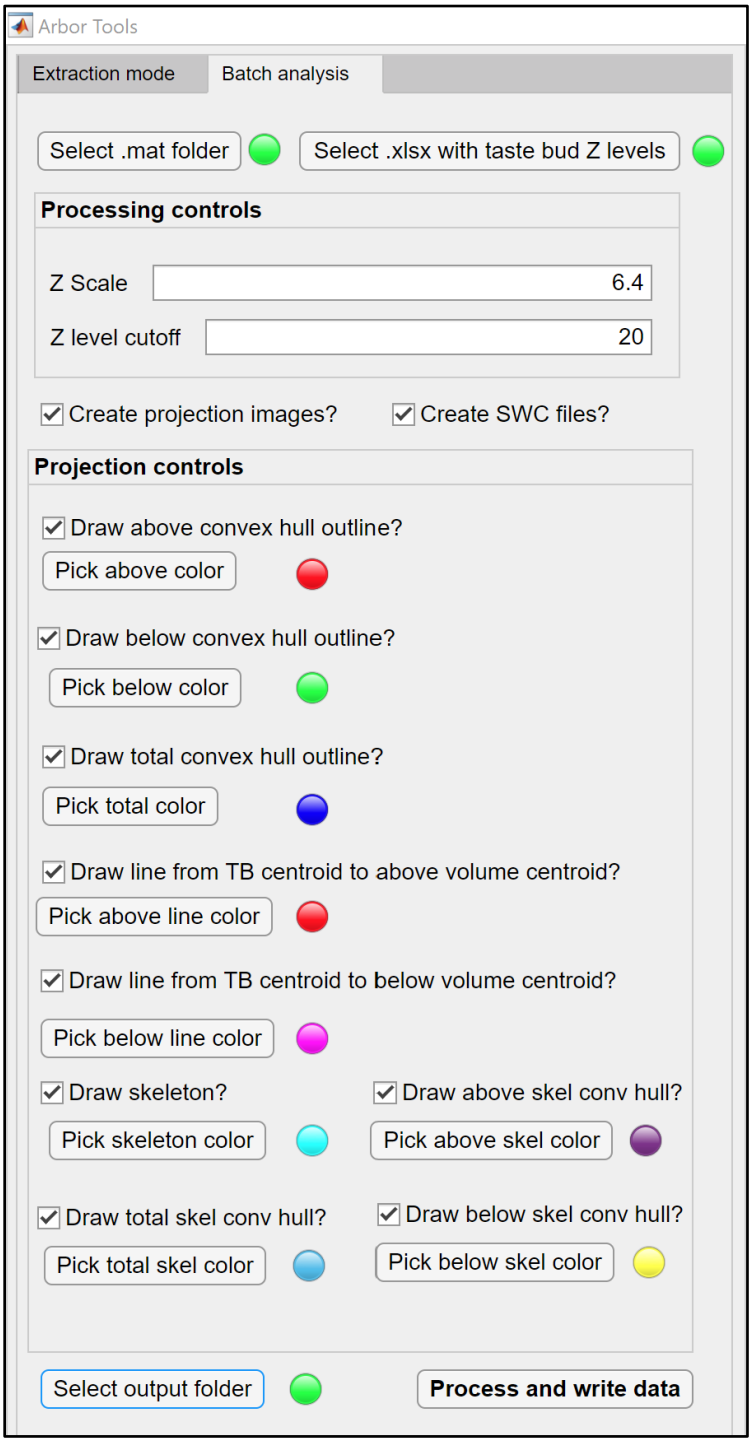
Batch analysis mode

Workflow for batch analysis mode:

1. Place all the .mat files you want to analyze into a single folder (the .mat files created by extraction mode).
    a. The program assumes all the mats present use the same XYZ scale. It also requires the naming that extraction mode does (see step 2a in extraction mode).
2. Create an excel file that contains the taste bud entrance Z levels for each file in the first row and no other data or text. Order them by timepoint (t0 is first entry, t12 second, etc.).
3. Create an empty folder for the output data.
4. Open the program and switch to Batch analysis mode, then click the “Select .mat folder” button and pick the folder you setup in step one.
5. Press the “Select .xlsx ...” button and select the excel file you created in step two.
6. Set the settings as desired, then press the “Select output folder” button to select the folder from step three. Once ready, click the “Process and write data” button to begin processing. This will take some time but can be canceled mid processing using the cancel button on the loading bar. If cancelled, a partial excel file will be generated.
    a. ZScale – Set this so that the final data/images have an XYZ scale of 1-1-1.
        i. If for example a stack had microns per pixel XYZ = [0.15625 0.15625 1], you would set your ZScale to 6.4 (1/0.16525). This scales the volume so that the 0.15625 microns per pixel value is valid in every dimension, not just XY.
    b. Z level cutoff – Everything beyond this many Z levels below the taste bud entrance (as set by what you picked in the excel file from step two) will be ignored (cleared of data when used in processing). This is applied before scaling (Z Scale).

c. The two options for drawing centroid lines draw a line from the taste bud entrance centroid to the above or below portion of the arbor volume (not the convex hulls).

d. Create SWC – Write a SWC file for the total, above, and below skeletons. These can be loaded by SNT in ImageJ/FIJI plus several other programs that deal with arbor skeletons.



Batch analysis error flags

These error flags will be in the excel data if something goes wrong (errFlag).

- 0 = No errors.
- 1 = Convex hull error for the entire segmentation volume (fewer than four points in entire segmentation volume, all the voxels are coplanar, etc.). This is usually due to either your taste bud Z level or Z level cutoff resulting in an analysis region well outside the arbor.
- 2 = As error code 1, but for the above portion of the segmentation volume.
- 3 = As error code 1, but for the below portion of the segmentation volume.
- 4 = As error code 1, but for the entire skeleton volume
- 5 = As error code 1, but for the above portion of the skeleton volume.
- 6 = As error code 1, but for the below portion of the skeleton volume.
- 7 = The mat data from extraction mode has a bug where the various stacks (original stack, segmented stack, etc.) do not all have the same number of Z levels. If this occurs, message David with the file and the extraction mode settings you used.

- As of version 1.06 the excel file will now contain partial data if the errFlag is 2, 3, 5, or 6. Flags 1, 4, or 7 result in no data present (all 0 in that row).
    - Note that this will result in invalid data for the affected region. If error flag 3 occurs for example, only the B- data is valid. The A- data (and by extension the T- data) for this time point will be arbitrary.
- If the selected tastebud Z level is empty, the program will automatically search for the nearest Z level to the requested Z level that has at least one pixel set true. This is done to allow tastebud centroid calculations even if error flags 2, 3, 5 or 6 occur.

Excel data overview

All measurements are in terms of micrometers (volumes and distances). T = Total, A = Above, B = Below. These numbers are based on the scaled volume (ZScale in processing controls). The arbor volume is scaled up using cubic interpolation, the skeleton is scaled using nearest neighbor interpolation. Be sure to note if the errFlag is nonzero (see section earlier in this document). Each column is as follows:

Time point – The time point for the current file (read from the filename).

Micron/Pix – The conversion factor used to scale from pixel distances and coordinates into microns. This number cubed is used as a conversion factor from number of voxels to cubic microns.

errFlag – A positive number indicates some error has occurred. See "Batch analysis error flags" section for more information.

Z level alteration – If this is set equal to 1, that means the chosen Z level (as read from the excel file) results in less than three layers above or below it (three is the minimum required). If less than three layers would be used, the program sets the Z level such that exactly three layers will be included.

T, A, and B - convH vol – The volume in cubic microns for the convex hull of the entire arbor, the convex hull above the taste bud entrance, and the convex hull below the taste bud entrance. The outline of these hulls can be drawn on the projection images.

T, A, and B - skel convH vol – Volume in cubic microns for the convex hull of just the skeleton. Can be drawn on projection images.

T, A, and B - XYZ widths – The extent in each dimension for the component listed in microns. This is calculated from the volume but should be identical to the extents of the convex hulls by the definition of a convex hull.

T, A, and B - centroid XYZ – The XYZ centroid in microns for each portion calculated from the arbors themselves (not the convex hulls).

T, A, and B - Volume – The volume in cubic microns of the arbor contained in the given portion (not the convex hull).

T, A, and B - Arbor length – Calculated using the Treestoolbox. Note that A+B is not always equal to T due to the way arbors are generated (T, A, and B are generated separately).

Above vs TB centroid distance – The distance in microns between the taste bud entrance centroid and the centroid of the above portion of the volume (not the convex hull). This can be drawn on the projection images.

Below vs TB centroid distance – As above, but from taste bud entrance to the below portion.

Generating TIF stacks of data and using ImageJ/FIJI to analyze them

Converting mat data to TIF stacks

Use the provided Matlab script 'batchMatToTifStacks.m' to convert data inside .mat files created by extraction mode into TIF stacks. The only toolbox required should be the Image Processing Toolbox. Make sure getFiles_F.m is also present in the same folder as this script. Simply enter your ZScale and ZCutoff as shown and run the script. It will prompt you for a folder containing the .mat data you want to convert, an excel sheet with taste bud Z levels (same as batch processing mode), and an output folder to place the image stacks in.

Using FIJI/ImageJ with these stacks

Open ImageJ and go to File -> Import -> Bio-Formats and select the TIF you want to load. On the next page, use default settings when prompted. This should load the TIF as a series of slices you can view.