# Chitin + Lutidine Bijels

Apply the following modules to each channel (Channel 1: chitin, channel 2: lutidine):

* Deconvolution
  + Input: raw image
  + Iterations: 10
  + Method: blind
  + Mode: confocal
* Median Filter
  + Input: deconvolution output
  + Interpretation: 3d
  + Iterations: 5
* Normalize Grayscale
  + Input: median filter output
  + Interpretation: xy planes
  + Range mode: percentile
* Interactive Thresholding
  + Input: normalize grayscale output
* Remove Small Spots
  + Input: thresholding output
  + Interpretation: xy plane
  + Size: 100
* Remove Small Holes
  + Input: remove small spots output
  + Interpretation: xy plane
  + Size: 50
* Opening
  + Input: remove small holes output
  + Type: ball
  + Size: 5
* Resample
  + Input: opening output
  + Average: 3,3,1
* Generate Surface
  + Input: Resampled output
  + Settings: uncheck all
* Surface View
  + Input: generated surface
  + Draw Style: transparent
  + Colors: constant
  + Colormap: Chitin – yellow, lutidine – red
  + Base Trans: 0.5
* Bounding Box
  + Input: a generated surface
  + Line Width: 5
  + Colour: black
* Scalebars
  + Input: a generated surface
  + Axis Options: only frame – x-axis
  + Unit: Microns
  + Color: black
  + Line Width: 3
  + Font Color: black
  + Custom Size: on
* Auto Skeleton
  + Input: resampled output
* Area
  + Input: resampled output
  + Interpretation: 3d
* Average Object Thickness
  + Input: resampled output
  + Model: cylinder rod model
* Volume fraction
  + Input: resampled output
  + Interpretation: 3d
* Isosurface
  + Input: opening output
  + Down Sample: on
  + Average: 6,6,6
* Extract Surface
  + Input: isosurface
* Remesh Surface
  + Input: extracted surface
  + Desired Size: %100
* Curvature (x2)
  + Input: remeshed surface
  + Method: on vertices
  + Output: (1) mean curvature (2) Gaussian curvature
* Surface View (x2)
  + Input: curvature outputs
  + Colour Map: physics (update range)
* Normals (x2)
  + Input: surface views
  + Options: check arrows
* AND Image
  + Input: resampled images
* Volume fraction
  + Input: And image output
* Bounding Box Volume
  + Input: a resampled image

For 3d samples:

1. Set up modules as above.
2. Export ‘auto skeleton’ data into excel files.
3. Copy data from bounding box volume, area, average object thickness and volume fractions into excel file. Calculate characteristic domain and chitin&lutidine volume fraction/chitin or lutidine volume fraction.
4. To get connectivity data for each channel:
   1. Open excel file with auto skeleton data.
   2. Node count is the total number of nodes (all coordination numbers).
   3. Average coordination number is the mean of the coordination number greater than 1.
   4. Tails not on bounding box are the number of nodes with coordination number 1 whose x,y,z coordinates are not on the bounding box.
   5. Tail percentage is the tails not on bounding box/node count.

For 4d samples:

1. Set up modules as above.
2. Update the text file ‘TimeStepScript’ for desired time steps, sample name, threshold value and pathways for saving data.
3. Copy and paste (or use tcl module) to the tcl console in Avizo.
4. Repeat steps 2-3 for the set thresholded value +/- 10.
5. Open ‘Analyze\_Chitin\_Bijel.m’ and ‘analyze\_chitin\_bijel\_script.m’ in matlab.
6. In ‘analyze\_chitin\_bijel\_script.m’, update lines 15-21 and file pathway. Run the program. Formatted results will be in the specified output file.

Note: ‘analyze\_chitin\_bijel\_script.m’ can be modified for 3d images by setting ‘start\_tstep’ and ‘end\_tstep’ to 1, and ‘tstep\_length’ to 0, as well as saving data in the same structure as the time series samples.

As of now, the ‘TimeStepScript’ does not generate the isosurface and curvatures.

Data gathered for each channel and threshold:

* Time step and time (4d)
* Area
* Average object thickness
* Volume fractions
* Chitin&&lutidine / chitin || lutidine volume fraction ratios
* Characteristic domain
* Average coordination number
* Tails not on bounding box
* Node count
* Tail percentage
* Snapshots of overlaid surfaces