

## Graphing sneeze or contraction behaviour using Image J

The procedure in Image J involves:

- 1) **generating a macro** by a) opening an image, b) selecting Plugin>Macro>Record, which opens up a 'recorder' window;
  
- 2) **recording the macro** for image processing:
  - a) select the rectangular selection tool and draw a rectangle over the image to create a ROI,
  - b) crop the image to that region >Image>Crop,
  - c) threshold the image >Image>Adjust>Threshold>Autot>Set>OK,
  - d) measure the area fraction, >Analyze>Set Measurements>, check the Area Fraction box (no other measure is needed), >Analyze>Measure. This action sends the measurement to a results window. Save the results to a folder by choosing >File>Save as>[choose your folder and type the file name].
  - e) Stop the macro recording in the 'recorder' window by clicking >Create. A Macro.ijm window opens with the macro, click >File>Save as>[choose a folder and file name for the macro].
  
3. **Run the macro** on a set of images: a) in the main ImageJ toolbar choose >Process>Batch>Macro, to open the batch process window. Select the folder which contains the image set to be analysed, and the folder where the data and the processed images will be placed. Copy and paste the macro.ijm file from above into the window, and click >Process. The image set will be analysed and the area fraction for each image will be calculated and placed in a .csv file, which can be opened for graphing or further analysis in Excel or other programs.