Microscopy Image Analysis Software for Medical Applications

Lymphocyte Flow Analysis

Dr Steve Barrett, Department of Physics, University of Liverpool, UK

Overview of *MIASMA*

MIASMA is Microscopy Image Analysis Software for Medical Applications, the collective name for a number of projects involving image analysis in which I am collaborating with medics. I am the author of software for image analysis of scanning microscopy images, principally for applications in nanoscience and related disciplines. The software that I have written, and continue to develop and expand, is *Image SXM*. Although written for scanning microscopy applications, I have found that *Image SXM* is an excellent platform on which to develop specialist image analysis solutions for the specific needs of users, including those who obtain images from light microscopes. *MIASMA* is the result of a number of these specialist applications having some common ground and so benefiting from being considered as part of a larger, overarching project.

Lymphocyte Flow Analysis

Image SXM contains routines that have been written to identify and track lymphocyte cells in videos of flow experiments. In the following pages the process by which the cells are identified is outlined and the format of the output results is explained. These notes are not intended to be comprehensive documentation, but should be enough to give the user an idea of how the processing is carried out.

For more information on MIASMA see the web page http://www.liv.ac.uk/~sdb/MIASMA
For help using the other functions of *Image SXM* see http://www.ImageSXM.org.uk
If you have any problems using *Image SXM*, email me S.D.Barrett@liv.ac.uk

Overview of Lymphocyte Flow Analysis

- The video to be analysed can be loaded as a QuickTime movie, an AVI file or a TIFF stack.
- Selecting MIASMA > Lymphocyte Flow Analysis starts the analysis process.
- For each frame of the video the locations of static, or nearly static, cells are determined using an algorithm based on a Hough Transform.
- The speed of movement of each cell is calculated from one frame to the next (see Appendix 1 for details).
- After each frame has been analysed to determine the cell locations and speeds, all of the data are analysed to determine the arrest times for each cell. A cell is considered to be arrested if its speed is below an Arrest Rolling Time Cutoff (ARTC) value. Results are calculated for all ARTC values up to 2 seconds in increments of 1/25 second.
- The results are printed to a text output file 'LFA-yymmdd-hhmm.txt' where yymmdd is the date and hhmm the time at which the analysis ended.

Menu Location

By default the 'Lymphocyte Flow Analysis' menu item appears in the menu structure of *Image SXM* in a series of sub-menus:

Analyze > Specialist Analysis > MIASMA > Lymphocyte Flow Analysis

Most users of *Image SXM* will not use this menu item and so it is tucked away where it will not get in anybody's way. Those of you who intend to use LFA extensively will probably prefer to have it available directly from the menu bar. If you press the option and control keys and select the LFA sub-menu you will find an extra item 'Move This Menu To Menu Bar'. This creates a new 'MIASMA' menu in the menu bar, which will appear every time you run *Image SXM* (on that Mac). If you want to move it back, repeat the process.

Appendix 1

It is not necessary to read this appendix to use the analysis routines in *Image SXM*. The algorithms are described here only for background information, and are not in the public domain.

The Cell Detection Algorithm

The sequence of image processing operations applied to each frame of the video is:

- Background subtraction using a 2D rolling ball of radius 7 pixels
- Hough transform to pick out circles with a radius of 8 pixels
- Median filter to reduce the noise
- Unsharp mask filter
- Median filter to reduce the noise
- Set threshold = bkgd + 72 where
  bkgd = mean intensity of background
- Dilate black and white image to ensure all cells are counted
- Analyse particles (cells) to determine their coordinates
- For each cell found:
  - check if cell has been assigned a number in a previous frame
  - if this is a 'new' cell, assign it a number
  - compare cell coordinates with those in previous 3 frames
  - if necessary, fill in the gaps if a cell temporarily disappears
  - calculate the speed of cell movement with respect to previous frame

When each frame has been analysed, the arrest times are calculated.

Appendix 2

History of changes to Lymphocyte Flow Analysis routines in *Image SXM*

v1	First public release of LFA code (*Image SXM* v189)	23 Aug 2009
v2	New algorithm based on Hough transform to improve cell tracking Public release of *Image SXM* v190	14 Apr 2010
v3	Maximum number of cells increased from 256 to 9999 Data arrays changed from static to dynamic to allow for above Beta release of *Image SXM* v191-b β	1 Jun 2010
v4	Accounted for bi-directional flow Changed optimum threshold for USM of Hough transform Beta release of *Image SXM* v191-e β	19 Jul 2010
	Public release of *Image SXM* v191	23 Dec 2010
	Public release of *Image SXM* v192	18 Apr 2011
	Public release of *Image SXM* v193	28 Apr 2012

Dr Steve Barrett
S.D.Barrett @ liv.ac.uk

v4 May 2012