Microscopy Image Analysis Software for Medical Applications

MIASMA

Microcirculation Analysis

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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.

Microcirculation Analysis

Image SXM contains routines that have been written to process and analyse video images of blood flow in capillaries. In the following pages the process by which the video images are stabilised and the blood flow speeds calculated is outlined. These notes are not intended to be comprehensive documentation, but should be enough to give the user an idea of how the processing and analysis 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

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Overview of Microcirculation Analysis

- The microcirculation videos to be analysed comprise ~500 frames (20 s @ 25 frames/s).
- In each frame blood cells may appear as isolated objects or as unresolved collections of blood cells. Motion of the cells defines the blood vessels, which are not themselves imaged. Vessels in which there are no moving cells are 'invisible'.
- To determine the speed of blood flow in a vessel a cell (or group of cells) must be followed from frame to frame. Knowing the spatial scale of the image and the time interval between frames, the movement of a cell in pixels per frame can be converted to a speed in μm/s.
- If the video is unstable then it is nearly impossible to be sure that a cell is identified correctly from one frame to the next. A 'triage pass' is made of the full video to assess which parts can used for the microcirculation analysis. If the field of view changes significantly, or the image goes out of focus, or artefacts appear in the image (such as air bubbles) then the unaffected part of the video is extracted for analysis. This 'good' segment needs to be at least 4 seconds long and reasonably stable.
- The video is then stabilised. Each frame is translated, magnified or demagnified, rotated and corrected for skew distortion so that the capillary network appears to be relatively static over the duration of the video, even though the cells are moving.
- With a stable video, the quantification of the blood flow can proceed. Following the motion of all cells over all frames allows the vessel network to be mapped out. This network is broken into separate segments (as the speed of blood flow cannot be defined at a junction between vessels). Each segment has a diameter which is noted so that vessel densities and blood flow speeds can be categorised for capillaries of $d < 20\,\mu m$ or $d < 10\,\mu m$.
- Each vessel segment, which is probably not particularly straight, is straightened to make subsequent flow speed analysis easier. Vessel segments must be at least 50 μm long for analysis to give meaningful results.
- For each vessel in turn, the cells visible in each frame are displayed in a 'space–time' plot. If the cells are imaged clearly, this plot exhibits lines or streaks whose orientation is related to flow speed. If the cells are static then the streaks are aligned with the time axis of the diagram. Cells with high speeds produce streaks at larger angles with respect to the time axis. The speed is calculated every second and if the vessel is longer than ~ 100 μm then speeds can also be calculated at different positions along the vessel length.

Results

The flow speed data for each vessel is reduced to mean values and 'scores' that can be compared with those produced by manual analysis:

SVD Small Vessel Density = $\frac{\text{number of times a vessel crosses a set of grid lines}}{\text{total length of grid lines in mm}}$

PPV Percentage of Perfused Vessels

A vessel is defined as perfused if P > 1 where

P = 0	no flow at any time
P = 1	flow for < 50% of the time
P = 2	flow for > 50% of the time
P = 3	flow all the time

PVD Perfused Vessel Density = SVD * PPV

MFI Microvascular Flow Index (aka Microcirculatory Flow Index)

The MFI score for a vessel is defined as

MFI = 0	flow absent
MFI = 1	flow intermittent
MFI = 2	flow speed sluggish
MFI = 3	flow speed OK

Menu Location

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

Analyze > Specialist Analysis > MIASMA > Microcirculation 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 MCA extensively will probably prefer to have it available directly from the menu bar. If you press the option and control keys and select the MCA 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.

Video stabilisation

In the triage pass the video is checked every 25 frames to follow the progress of drift. The video segment is considered 'good' if the drift remains less than 32 pixels in any direction.

After triage, the good segment the video is inspected every 5 frames. Four 128 x 128 pixel regions of interest (ROIs) are correlated to equivalent ROIs in a reference frame (the middle frame of the video). The cross–correlation images give the relative movement of each quadrant of the frame. These are then used to correct every frame of the video for the distortion of the vessel network which is a mixture of:

- Translation – all quadrants drift by the same amount
- Magnification – all quadrants move outward or inward with respect to the frame centre
- Rotation – all quadrants move perpendicularly to a line drawn to the frame centre
- Skew distortion – each quadrant moves independently of the others

Stabilising a video takes ~1 second per video frame.

Vessel straightening

To facilitate the extraction of the pixel intensity data from the curvilinear vessels, each vessel is first straightened using an algorithm developed originally for images of chromosomes.

See http://www.liv.ac.uk/~sdb/Papers/2003-CR-11-83.pdf for the method used.

Flow speed calculations

- The space–time plots are scaled such that 1 second = 64 pixels along the time axis
- An unsharp mask filter is applied to enhance the contrast of streaks
- The orientation of the streaks is determined using 64 x 64 pixel autocorrelation images
- The co-tangent of the orientation angle gives the flow speed

Vessel processing and flow speed calculations take ~3 seconds per vessel.

Definitions

In calculations of PPV and MFI scores:

No flow	⇒	speed <	25 µm/s
Sluggish	⇒	speed <	100 µm/s
Maximum valid speed		=	1500 µm/s

These thresholds are arbitrary and were set after inspection of a few videos of control subjects.

Execution time

Analysis of a 'typical' video comprising ~200 useful frames and ~50 vessels takes ~5 minutes. Analysis of 100 'typical' videos takes ~10 hours.

Appendix 2

History of changes to Microcirculation Analysis routines in *Image SXM*

v1	First public release of MCA code (Image SXM v189)	23 Aug 2009
	Public release of *Image SXM* v190	14 Apr 2010
v2	Added colour flow map	25 Apr 2010
	Public release of *Image SXM* v191	23 Dec 2010
v3	Minor modification to display of video filename	1 Jan 2011
	Public release of *Image SXM* v192	18 Apr 2011
v4	Redfined MFI scores of 0 (= absent) and 1 (= intermittent)	
	MFI = 0 : Number of measurements of non-zero flow ≤ one–quarter of total	
	MFI = 1 : Number of measurements of non-zero flow ≤ two–thirds of total	
	AVI opened as 788 x 576 pixels	7 May 2011
v5	Use arithmetic and geometric means for flow speeds	15 May 2011
	Public release of *Image SXM* v193	28 Apr 2012

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v5 May 2012