Microscopy Image Analysis Software for Medical Applications

MIASMA

Parasite Counting 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.

Parasite Counting Analysis

Image SXM contains routines that have been written to identify and count malaria parasites. In the following pages the process by which the parasites are identified is outlined and the effects of user input are 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 and allow the user to use reasonable judgement in selecting input options.

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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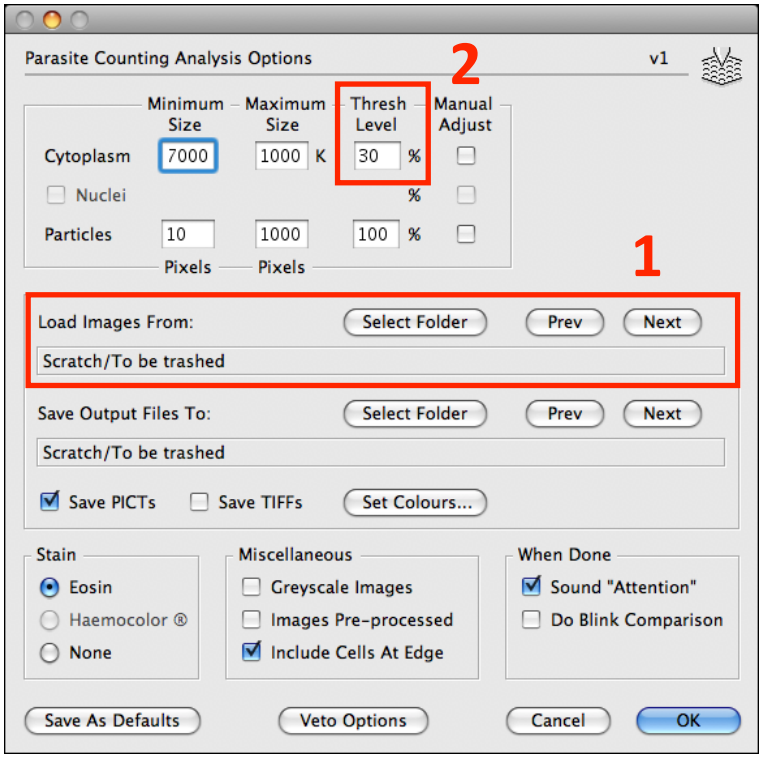
The Analysis Process

The raw (TIFF) images are loaded from a folder selected by the user [1], or a set of folders within the folder selected by the user. The images are processed folder by folder.

Firstly, images within the selected folder (the top folder) are processed. Secondly, images within any sub-folders are processed.

A maximum of 256 images in each of 32 folders can be processed at a time.



(Note that the PCA Options dialog box has a number of elements in common with that created for Particulate Matter Analysis (PMA). Some of these elements are not appropriate for PCA are so are dimmed or disabled. In future versions the dialog box may be tidied up a bit.)

For each image, the following generic processing is carried out...

- create an image that makes cytoplasm look dark (cf background)
- threshold at value specified in dialog box [2] — see p3
- identify the outline of the cells

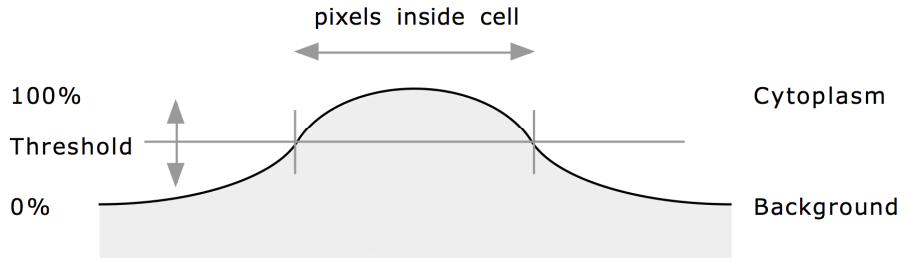
  [outlying cells is not necessary for the counting analysis, but helps when]

  [comparing the original image with the maps of identified parasites]
- create an image that makes parasites look dark (*cf* cytoplasm)
- threshold at fixed level (adjusted by user–specified value in dialog box)
- optionally allow user to adjust threshold manually
- invert the image and threshold again to find interior 'holes' in parasites

The algorithm that creates the images and generates the threshold levels that discriminate between parasites and cytoplasm is described in Appendix 1.

Setting Thresholds

For cytoplasm the threshold level specified in the dialog box relates to the range between the background intensity and the mean cytoplasm intensity.



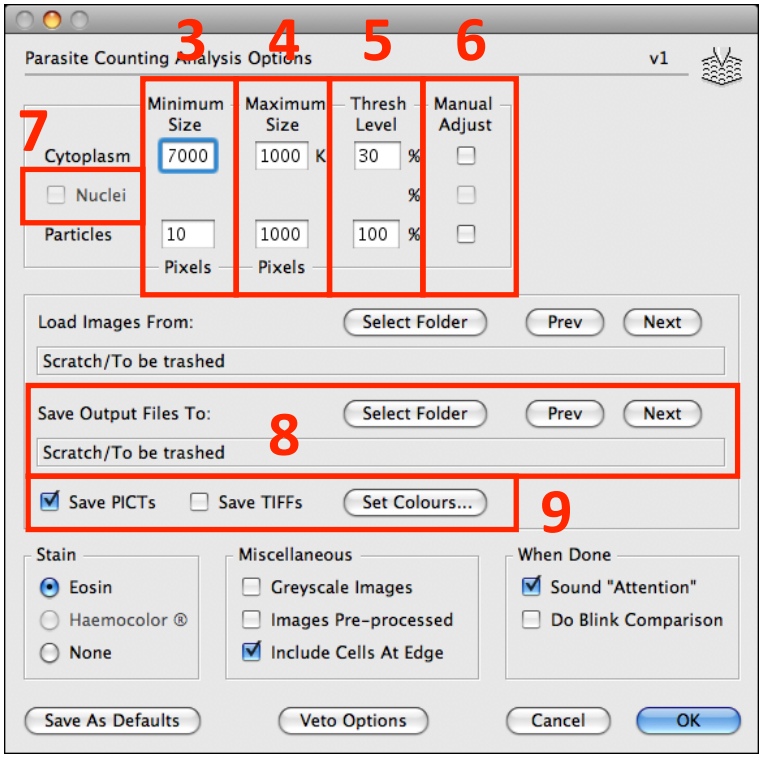
The default value of 50% should be appropriate for many analyses. Set the threshold to a lower value (~ 25%) to include more of the cytoplasm edges or to a higher value (~ 75%) to include only the denser regions. These settings do NOT affect the parasite count.

For parasites the threshold level specified in the dialog box is expressed as a percentage of the corresponding threshold levels determined by the analysis algorithms. These should be changed from the default values of 100% only if there is a consistent under/overestimate of the parasite count (as determined by, for instance, the Blink Comparator).

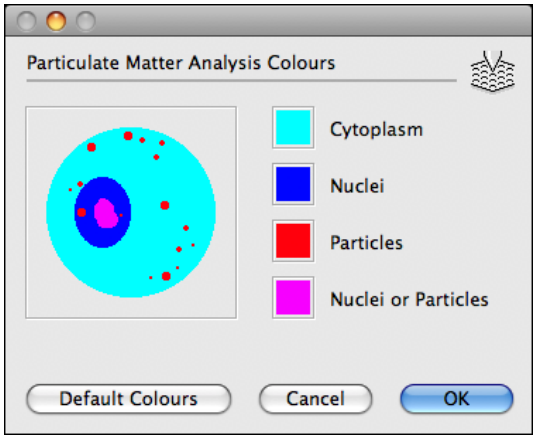
Selecting Options

Lower [3] and upper [4] limits to the sizes of cells and parasites that are detected can be set. The sizes are specified in pixels for the minimum size and either pixels or thousands of pixels for the maximum size.

Thresholds for cytoplasm and parasites are set here [5]. The value for cytoplasm affects the measured areas of cells that have spread out (see p2). The value for parasites is a percentage of the value calculated by the program, and should only be changed from 100% if the values calculated by the program are consistently wrong.



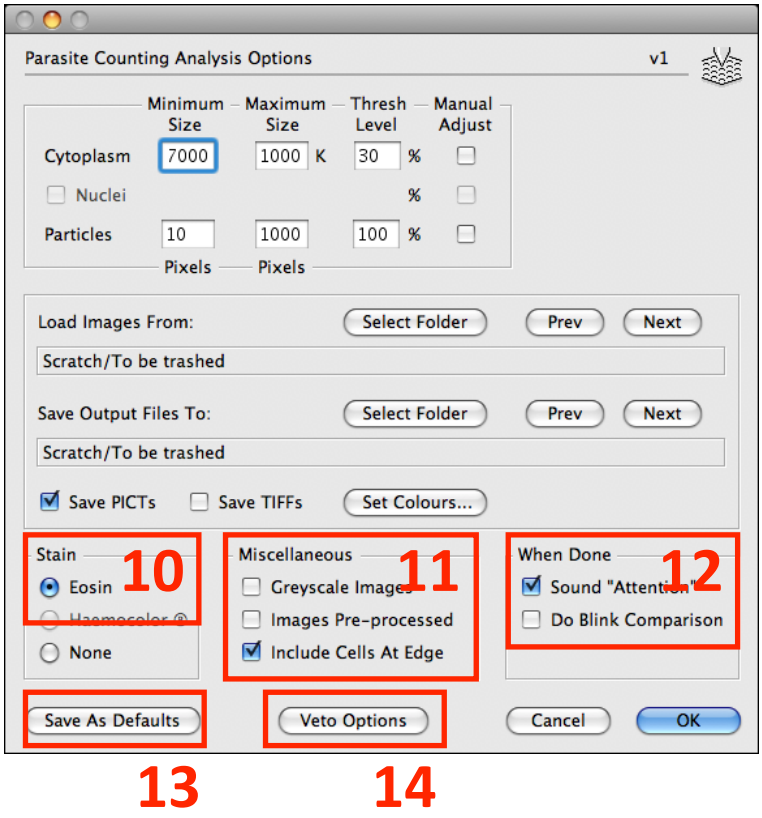
Thresholds calculated by the program can be manually adjusted by the user on an image-by-image basis [6]. The threshold level is displayed in the Info window and the values before and after user adjustment are recorded in the log file. If you are unhappy with an image even after making these manual adjustments, press the 'V' key to veto that image from the calculation of the mean value of the parasite count. Cell nuclei are ignored completely in the analysis and so item [7] is dimmed.



Output files (see p6) are saved to a folder selected by the user [8]. Optionally, colour 'maps' showing which pixels were identified as cytoplasm, nuclei or particles (parasites) can be saved as PICT or TIFF images [9]. The colours used can be specified in a separate dialog box [9] (left).

If the cells have been stained using eosin then select this item [10]. These analysis routines may not work with any other stain unless the code is modified — email Steve Barrett <S.D.Barrett @ liv.ac.uk> if you use Haemocolor ® or a different stain.

Select Greyscale Images [11] if the images are 8-bit greyscale (rather than 24-bit RGB colour) and contain parasites but no cells.



The Blink Comparator [12] can be selected to launch when the analysis is complete so that you can carry out a visual check of the results.

The Save As Defaults button [13] saves the Preferences file with the current settings of the dialog box so that these will be the default values next time Image SXM is launched.

Some images may be vetoed from the calculation of the average parasite count [14]. See p6 for details.

Output Files

Three text files are generated for each analysis run

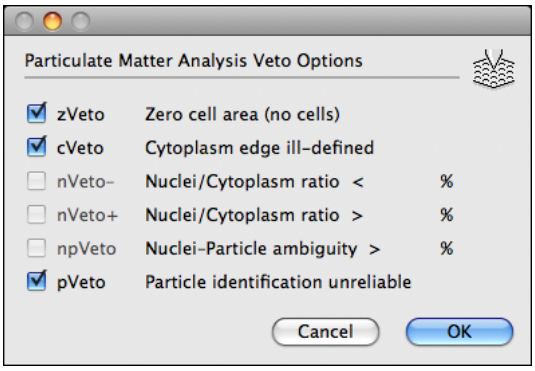
Log-yymmddhhmm.txt
PM-A-yymmddhhmm.txt
PM-G-yymmddhhmm.txt

where yymmdd are the date and hhmm the time at which the analysis ended. The log file contains full information of the analysis parameters and the parasite count in each image. The PM-A and PM-G files contains just the (arithmetic and geometric) mean parasite counts and their standard deviations for each folder of images analysed. If the analysis fails to complete due to an error, the file 'Log-Crash.txt' records the results for all folders analysed up to the time at which the error occurred.

Some images may be vetoed from the calculation of the average parasite count. These are indicated in the log file with the following flags:

Flag	Reason for veto	
zVeto	zero cytoplasm area (no cells)	
cVeto	cytoplasm edge ill–defined	
nVeto–	nuclei too small as fraction of cytoplasm	[n/a for PCA]
nVeto+	nuclei too large as fraction of cytoplasm	[n/a for PCA]
npVeto	discrimination (nuclei /parasites) ambiguous	[n/a for PCA]
pVeto	identification of parasites ambiguous	
uVeto	veto by user when manually inspecting images	

Each veto can be enabled/disabled individually and the criteria fine–tuned in the Veto Options dialog box.



If the pVeto is disabled but the analysis determines that an image is underexposed (too many dark pixels) or overexposed (too many bright pixels) then this will be indicated in the log as *UnEx* or *OvEx*. The image will be included in the calculation of the average even though the parasite count may be unreliable.

Using the Analysis Routines

I would recommend that you start by analysing just a few typical images to establish whether or not the default settings are appropriate. Either copy a few images into a test folder, or analyse one image at a time by pressing the shift key before selecting the Parasite Counting Analysis item from the Cells menu (the menu item changes to 'PC Analysis for Single Image'). You can then analyse the rest of your images in one of two ways:

i) Run the analysis on one or more folders containing your images with all the settings at the default values. On automatic, the analysis will take a few seconds per image. The time remaining to complete the analysis is indicated in the Info window in the bottom left corner of the screen. Go and have a cup of tea.

   When the analysis is complete, use the Blink Comparator to check each image with the output 'map' showing where pixels were identified as cytoplasm and parasites. The Blink Comparator toggles between the original image and its corresponding map so that you can judge whether or not the identification was satisfactory.

   Note whether or not there is any consistent under/overestimate of the parasite count. If there is, adjust the parasite threshold level (see p4) and run again. Lowering (raising) a threshold level will count more (less) pixels and hence increase (decrease) the measured area of an object.

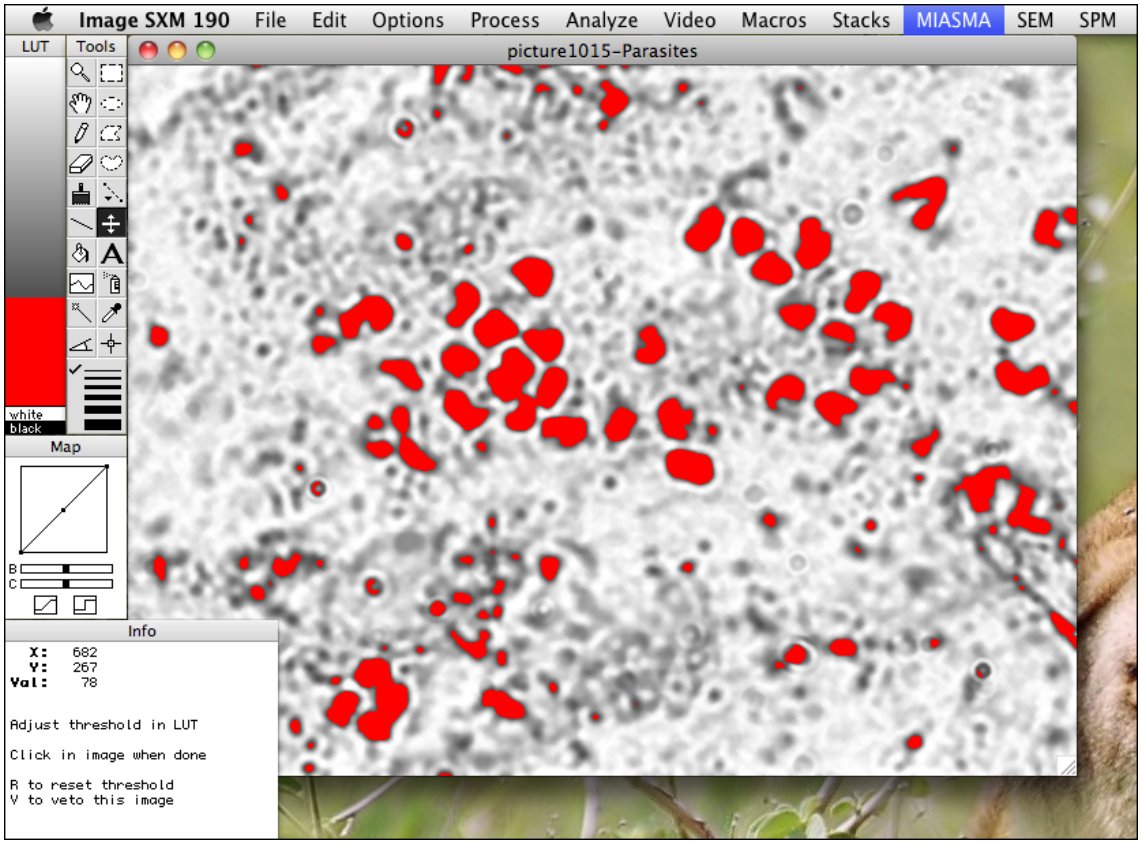
   If there is no consistent under/overestimate of the parasite count but some objects in some images have been identified incorrectly, either:

   (a) remove the files from the folder and re-run the analysis, or

   (b) re-run the analysis with the Manual Adjust option selected and either adjust the threshold until you are happy with the parasite count or veto the image.

ii) Run the analysis with the Manual Adjust options selected. For each image, the analysis will pause and allow you to adjust the thresholds that determine the areas of cytoplasm and parasite count. Adjust the threshold until you are happy with the parasite count or veto the image. Both the automatic and the manually adjusted threshold values are recorded in the log file.

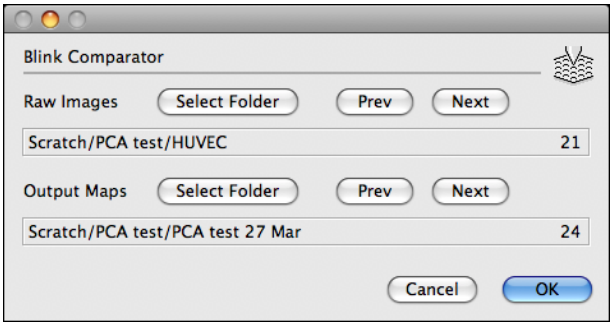
Manual adjustment of a threshold is carried out by dragging the red/grey boundary line the the look-up table (LUT) window whilst looking at the effect this has on the pixels in the image.



When you are happy with the threshold, click in the image. An image can be vetoed by pressing 'V' at any time during manual threshold adjustment.

Blink Comparator

For a quick way to check whether or not the PCA routines have done their job correctly use the Blink Comparator to jump back and forth (blink) between the original images and the corresponding colour maps of cytoplasm and parasites.



Select the folders containing the original images and the output maps using the 'Select Folder' buttons, or the Prev/Next (or, with the option key, Up/Down) buttons to jump quickly between folders. The number at the end of the folder path name shows how many images are in the folder — a quick check that you have selected the correct folder(s). When blinking, use the Tab or Space keys to blink between images, or the Caps Lock key to blink continuously every half second. Use the arrow keys to move to the next image in a folder. Instructions are summarised in the Info window.

Menu Location

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

Analyze > Specialist Analysis > MIASMA > Parasite Counting 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 PCA extensively will probably prefer to have it available directly from the menu bar. If you press the option and control keys and select the PCA 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 2

History of changes to Parasite Counting Analysis routines in *Image SXM*

v1	First public release of PCA code (*Image SXM* v186)	20 Apr 2008
v2	Added routine to handle (greyscale) images of parasites with no cells Improved algorithm for touching parasite multiplicity	11 May 2008
v3	Improved handling of structured background in images of parasites with no cells Minor changes to user interface: 'Particles' → 'Parasites' in dialog box Max parasite size → 99999 pixels and item box enlarged accordingly Enabled single–image analysis even if load folder not specified Deleted histogram of results from log file	2 Jun 2008
	Public release of *Image SXM* v187	4 Oct 2008
	Public release of *Image SXM* v188	14 Feb 2009
	Public release of *Image SXM* v189	23 Aug 2009
v4	Changes to recognition algorithm to reduce false negatives and false positives Public release of Image SXM v190	14 Apr 2010
v5	Change to optimum threshold for greyscale images 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

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