

FOAMS

Version 1.0.4

User guide

by
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Funded by



a program from the



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FOAMS PROGRAM GUIDE

1. INTRODUCTION

FOAMS (Fast Object Acquisition and Measurement System) is a Matlab®-based program designed to help geologists wishing to initiate in vesicle texture studies. FOAMS is built around two main graphical user interfaces (GUI) that use simple push buttons to facilitate most operations. The user needs to be prepared with sets of images in either binary or grayscale format and understanding the program requires reading both this manual and the journal article it is based on (Shea et al., 2010). Because FOAMS is still in its early stages, it will certainly undergo substantial modifications throughout the next years; hence your input on program malfunction, errors or possible improvements is deeply appreciated.

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Thank you for your patience using our program,

The authors,

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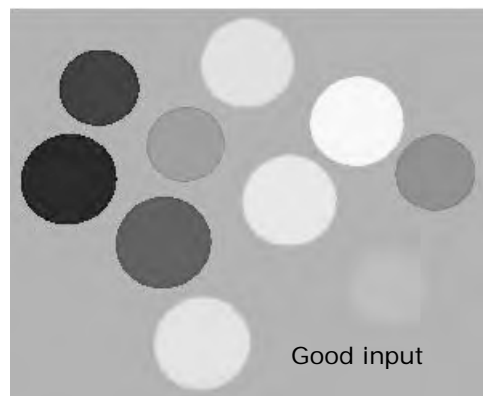
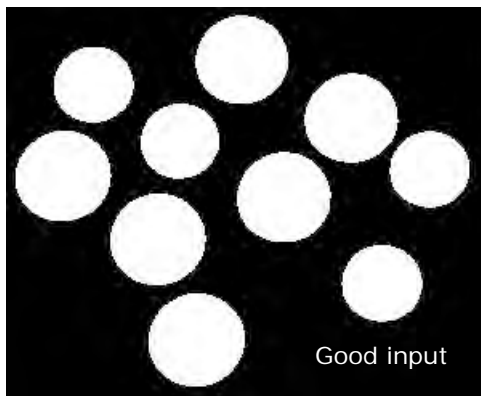
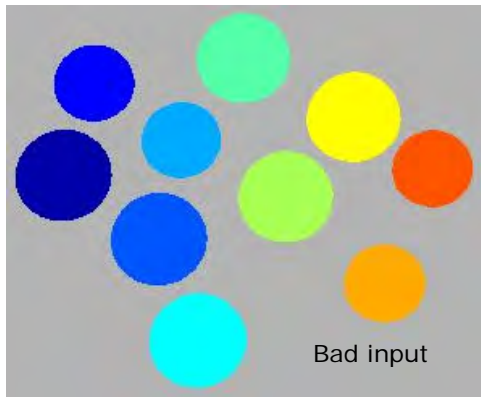
2. FOAMS INPUT

The data that FOAMS uses for analysis are grayscale images. The shades of gray are used for object recognition. Therefore, all black objects (bubbles here) will be treated as one group, and dark gray (crystal phase 1) or light gray (glass) will be treated as other. The program analyzes one object per run only; this means that only one shade of gray will be selected for size analyses.

Examples:

- Color image: three layers Red, Green and Blue, *erroneous input*.
- Binary images: bubbles are 100% black (pixel shade~digital number DN=0), glass is 100% white (DN=255): *good input*
- Grayscale image: bubbles are black (DN=0), glass is dark grey (DN=100), olivines are medium gray (DN=180) and oxides are white (DN=255): *good input*
- Grayscale image: bubbles are blackish ($0 < DN < 30$), glass is dark grayish ($15 < DN < 140$), etc...*erroneous input*.

Most importantly, the objects you wish to analyze are to be fed into the program as uniform gray shaded areas. Image treatment operations to obtain uniform objects are easily achieved using Adobe Photoshop (see Image processing guide included in the FOAMS package).



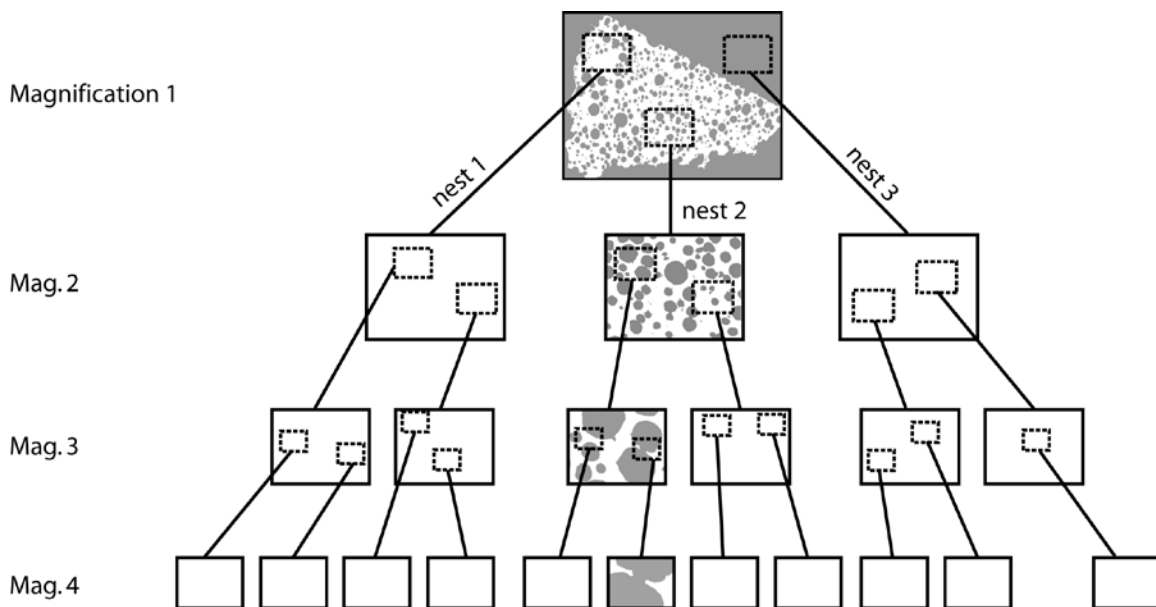
3. OPERATION MANUAL: INITIAL INTERFACE

a. Overview of image nest configuration

Typically, vesicle texture analysis is performed using images of varying magnifications to resolve all size ranges. FOAMS allows the user to input as many as 20 images with their corresponding magnifications.

Example:

- One large image of small magnification (5X)
- Three images at a larger magnification (25X)
- Six images at even larger magnification (100X)
- Eleven images at the highest magnification (500X)



b. Starting FOAMS

To **start** FOAMS' graphical user interface (GUI), you may either double click on 'init.fig' or open it from the Matlab program itself. In the latter case, go to New→GUI and click on the 'Open existing GUI' tab. Browse for the 'init.fig' within your matlab directory and open. If you loaded the program interface from Matlab, to actually start FOAMS you need to push on the green triangle button ("run").

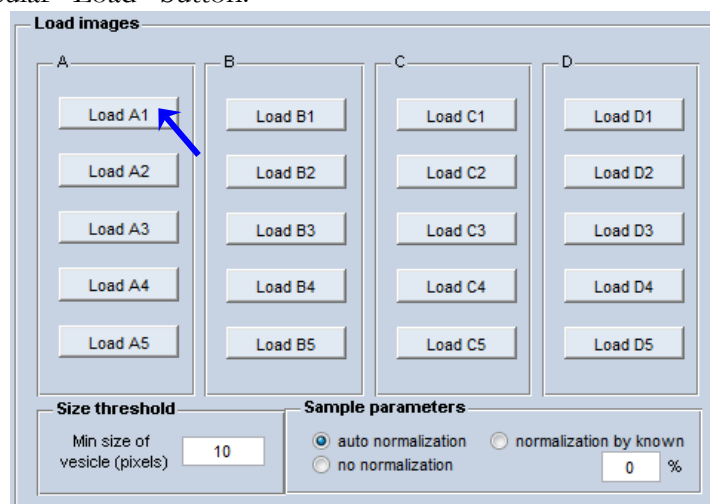
The dataset included with the program is within the "Examples" folder, and includes 13 grayscale images of Vesuvius pumice. The numbers at the end of each filename correspond to the scale factors for each image (i.e. 43, 208, 830, or 4150 pixels/mm which depend on the imaging instrument used). The set comprises:

- one scanned image (43 pixels/mm)
- two 25X images (208 pixels/mm)
- three 100X images (830 pixels/mm)
- seven 500X images (4150 pixels/mm)

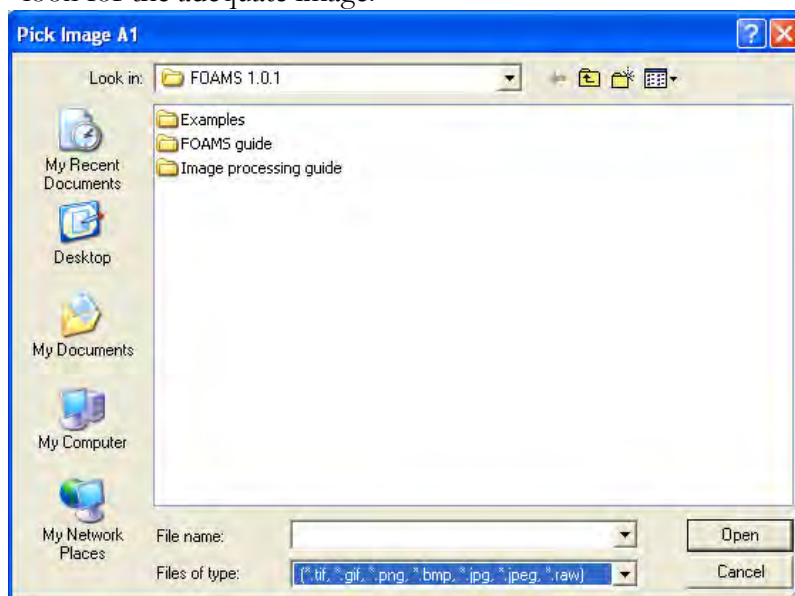
While not essential, it is recommended to place the images to analyze within this folder, since it makes them easier to access through the running program.

c. Loading your images

To load the images, press on the buttons on the upper right portion of the interface. The buttons are distributed as a 5-by-4 grid and correspond to the image grid displayed on the main interface. Hence, for each image there is one particular “Load” button:



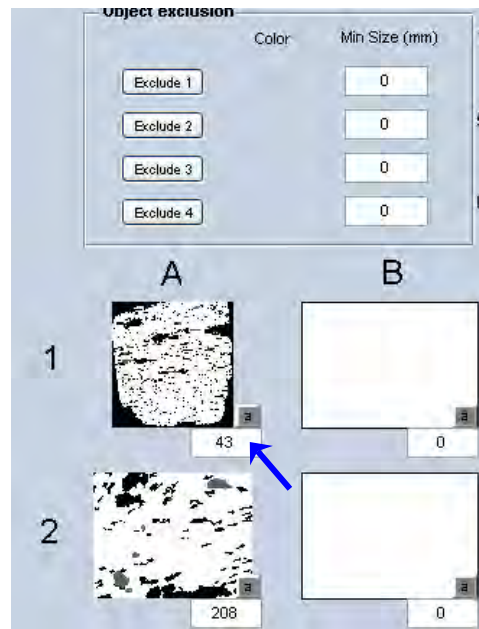
A click on one of the buttons generates a dialog box which invites the user to look for the adequate image:



Images may be loaded in any order with evidently a minimum of 1 image in order for the program to run.

d. Scale factors

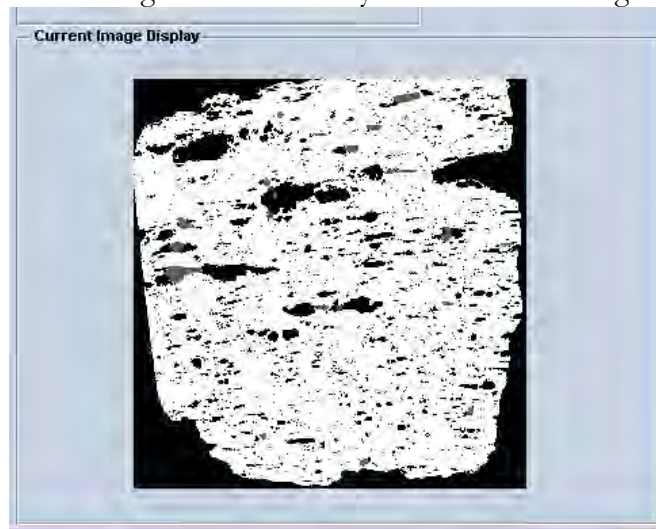
Either when each image is loaded or when all the images are loaded, the user must type in the different scale factors for the magnifications utilized (e.g. 4150 or 5020 pix/mm for 500X with our SEM) and leave 0 values in all those which are not used:



Note: The dimension scale used by default in FOAMS is the millimeter (mm). All outputs are therefore generated with values in mm.

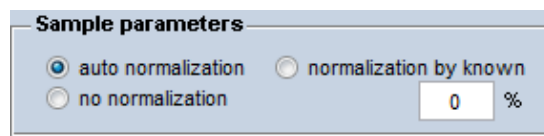
e. Active display window

At any time, the user may view any particular image on the larger “Current Image Display” box by clicking on the little gray “a” buttons at the bottom right corner of each loaded image. This effectively “activates” the image.

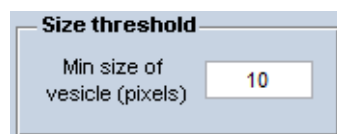


f. Vesicularity input and minimum diameter

There are three options allowing the user to normalize the vesicle volume distributions obtained through FOAMS. This normalization can be useful in cases where vesicle volumes corrected through stereology yield totals that are well over 100% (frequent in the most vesicular clasts). To display realistic VVDs, each bin will be normalized by either a known vesicularity (measured independently through density, “3D vesicularity”), or by the vesicularity FOAMS measures within the images (“2D vesicularity”). Within the init interface, these various possibilities are available as three ‘radio buttons’, namely **auto normalization**, **no normalization**, and **normalization by known**. If the first option is selected (auto normalization), the calculated vesicle volume distributions will be normalized by the value calculated from an integration of measured image vesicularities (i.e., “2D vesicularity”, method by Thomas Giachetti). The second leaves the VVDs unchanged from the volume values calculated after stereological conversion, and the last normalizes by the independently measured density-derived value (entered by the operator). For the Vesuvius pumice included as an example, the density-derived vesicularity is 77%.



In the “minimum size of object” box you should type an estimate of what is the smallest size diameter in pixels that you consider to represent and actual object in your highest images at each magnification (for the Vesuvius example, 5 pixels is a minimum).



To determine which minimum diameter to use, refer to the accompanying article (Shea et al., 2010).

g. Quick image processing

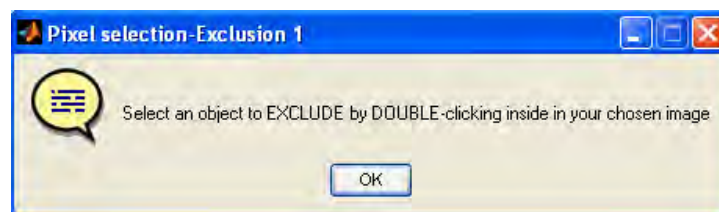
The Quick image process panel can be used to do quick operations of converting all your loaded images into binary or grayscale while playing with the threshold (0 to 1). If not satisfied by the grayscaling of binarization, press the ‘undo’ button; otherwise, push ‘confirm’ when satisfied. We however recommend using Photoshop for most image rectification as such simple functions do not allow much user control. In addition, all the objects in the images need to be perfectly homogeneous in the shade of gray in order for the results to be truthful.



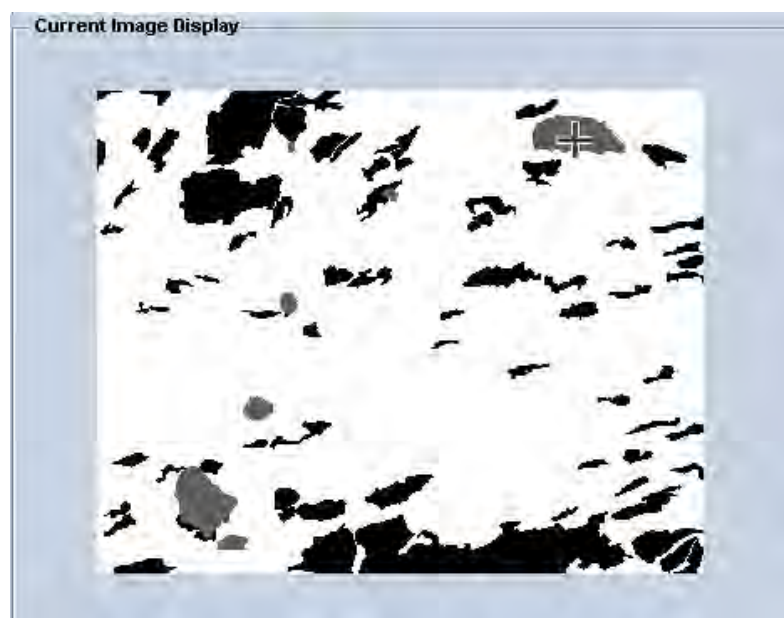
b. Object exclusion

In many cases, vesicle analysis involves discarding crystal phases which have grown prior to vesiculation to obtain number densities representing the initial density of nucleation. An object exclusion panel located on the upper left corner of the interface allows the user to exclude up to four object-types (i.e. up to four different shades of gray).

To perform this operation, the user makes one of the images containing the object to exclude "ACTIVE" by clicking on the corresponding "a" button in its bottom right corner. Then, the user clicks on the "Exclude 1" button, and a dialog box will appear:



On the active image panel window, the user now selects any of the objects with its characteristic color simply by double clicking (or clicking once and pressing enter on the keyboard).



If the right object has been clicked, it corresponding shade of gray should appear next to the Exclude 1 button:

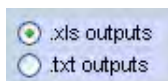


Note: Everytime you exclude an object or double-click in an active window, you must assume that the window then becomes 'inactive'. Hence, for each additional exclusion ensure that you 'activate' your selected image first by clicking in the lower right 'a' button.

As shown in the illustration above, it is also possible to enter a minimum size (in mm) for the excluded objects. For instance, if a mineral phase such as plagioclase grew in the storage region before vesiculation was initiated, but also grew during ascent, there will be a whole range of size populations which should not be included. Hence, the user may decide to select a threshold of 0.03 mm (limit between microlites and microphenocrysts) to ensure that only the larger ones are discarded.

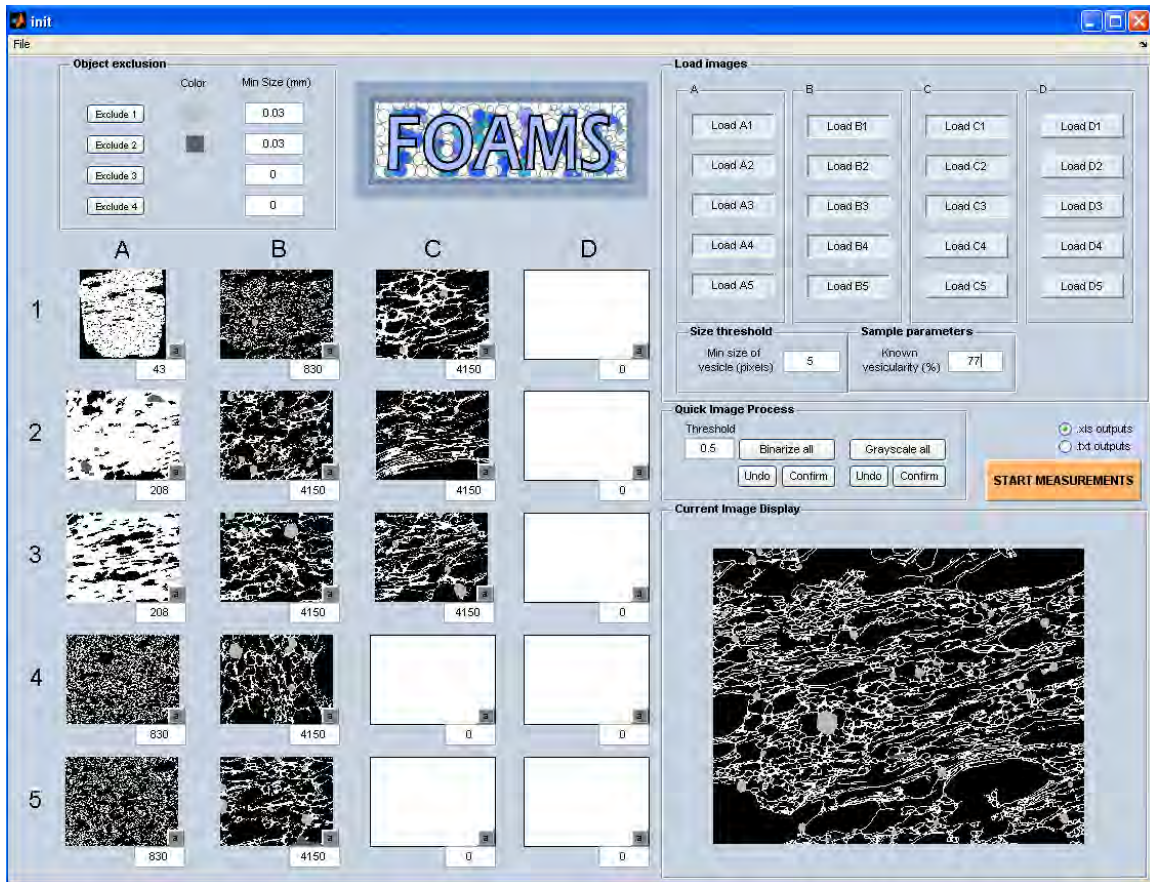
i. Choice of Output file type

In terms of outputs, two format choices are offered: either measurements and calculations can be saved as tab delimited text files, or as Excel® spreadsheets. The latter format increases the computational time compared to .txt formats.



j. Launching vesicle analysis

The next step involves selecting the objects to actually analyze (vesicles) in the same manner as for objects which were being discarded. IMPORTANTLY, before doing anything else the user should activate the image he wants to use to select vesicle objects. Like before, a click on the “a” button on any image makes it active in the main image window. If the user loaded the images given as an example, his interface window should look like this:



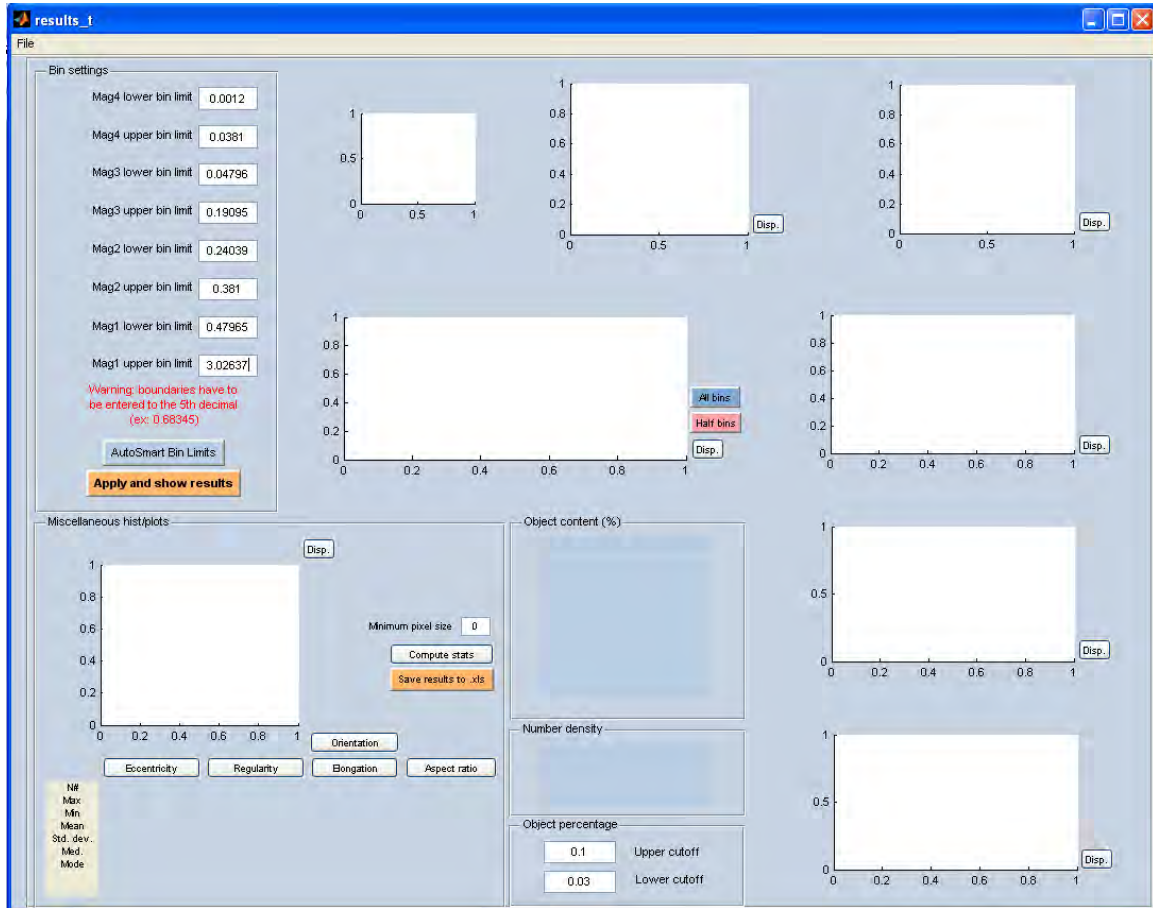
When all the inputs have been verified by the user, the “Start measurements” button is pushed. Like before, a dialog box should appear asking to double click on an object pixel in the zoom panel. In the case displayed above, the user will double click ANYWHERE on the image represented by a black pixel.

Once the right pixel color has been selected a wait bar appears while the measurements are being performed. On any current PC or Mac, this process may take anywhere from 30-120 seconds depending on the number of images, number of vesicles, and on which of the two output modes is chosen (Excel spreadsheets or tab delimited text files).

4. OPERATION MANUAL: RESULTS INTERFACE

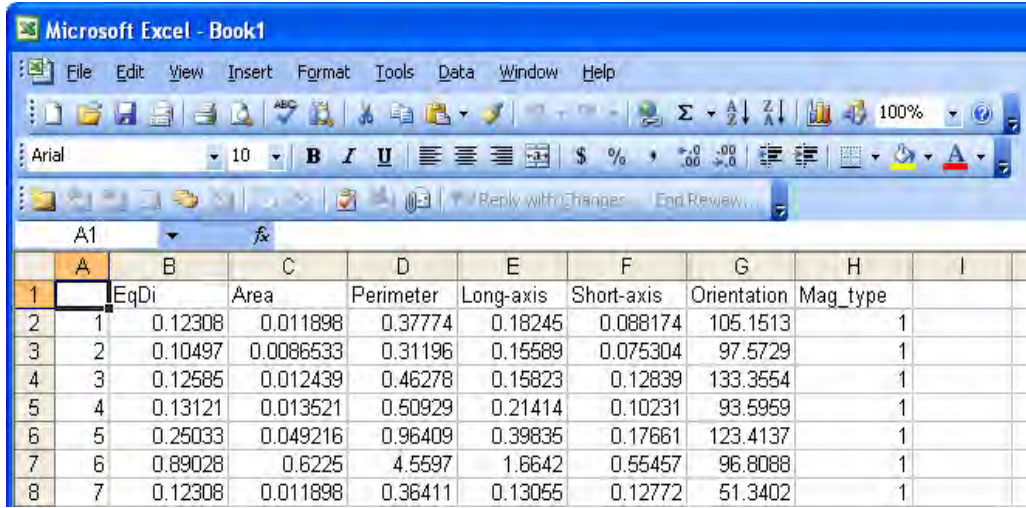
a. Results GUI window

Once the wait bar disappears, a new GUI called 'results' is displayed:



By now two outputs will have been created in the FOAMS directory named “Raw_data” and “NA_mag”, which contain the individual equivalent diameters and the number of vesicles per area for each size range. The minimum bin size (or minimum diameter) is defined by the ratio of the minimum number of pixels representing a vesicle (e.g. 5 pixels in this example) to the scale factor (e.g. 4150 pix/mm here). Each subsequent bin is larger by a factor of $10^{0.1}$ (i.e. geometric binning).

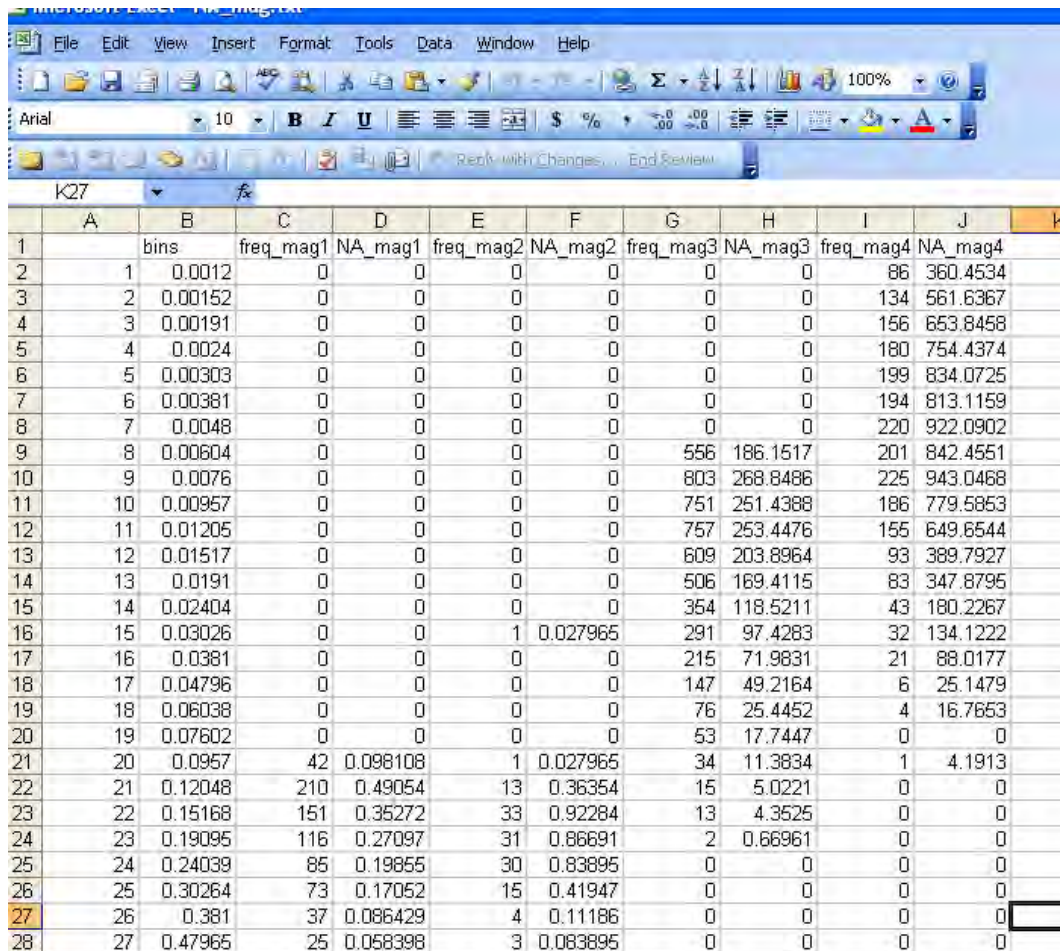
b. Verifying the measurements and selecting cutoffs



	A	B	C	D	E	F	G	H	I
1		EqDi	Area	Perimeter	Long-axis	Short-axis	Orientation	Mag_type	
2	1	0.12308	0.011898	0.37774	0.18245	0.088174	105.1513	1	
3	2	0.10497	0.0086533	0.31196	0.15589	0.075304	97.5729	1	
4	3	0.12585	0.012439	0.46278	0.15823	0.12839	133.3554	1	
5	4	0.13121	0.013521	0.50929	0.21414	0.10231	93.5959	1	
6	5	0.25033	0.049216	0.96409	0.39835	0.17661	123.4137	1	
7	6	0.89028	0.6225	4.5597	1.6642	0.55457	96.8088	1	
8	7	0.12308	0.011898	0.36411	0.13055	0.12772	51.3402	1	

The Raw_data file contains not only the measured equivalent diameter of each vesicle but also the area, its perimeter, and the long-axis, short-axis and orientation of best-fit ellipses to each individual. The last column displays in which magnification the object was measured.

The “NA_mag” file shows calculated bins as rows and two columns for each magnification: the number of objects measured for each bin size and the resulting number density per image area (NA_mag):



	A	B	C	D	E	F	G	H	I	J	K
1		bins	freq_mag1	NA_mag1	freq_mag2	NA_mag2	freq_mag3	NA_mag3	freq_mag4	NA_mag4	
2	1	0.0012	0	0	0	0	0	0	86	360.4534	
3	2	0.00152	0	0	0	0	0	0	134	561.6367	
4	3	0.00191	0	0	0	0	0	0	156	653.8458	
5	4	0.0024	0	0	0	0	0	0	180	754.4374	
6	5	0.00303	0	0	0	0	0	0	199	834.0725	
7	6	0.00381	0	0	0	0	0	0	194	813.1159	
8	7	0.0048	0	0	0	0	0	0	220	922.0902	
9	8	0.00604	0	0	0	0	556	186.1517	201	842.4551	
10	9	0.0076	0	0	0	0	803	268.8486	225	943.0468	
11	10	0.00957	0	0	0	0	751	251.4388	186	779.5853	
12	11	0.01205	0	0	0	0	757	253.4476	155	649.6544	
13	12	0.01517	0	0	0	0	609	203.8964	93	389.7927	
14	13	0.0191	0	0	0	0	506	169.4115	83	347.8795	
15	14	0.02404	0	0	0	0	354	118.5211	43	180.2267	
16	15	0.03026	0	0	1	0.027965	291	97.4283	32	134.1222	
17	16	0.0381	0	0	0	0	215	71.9831	21	88.0177	
18	17	0.04796	0	0	0	0	147	49.2164	6	25.1479	
19	18	0.06038	0	0	0	0	76	25.4452	4	16.7653	
20	19	0.07602	0	0	0	0	53	17.7447	0	0	
21	20	0.0957	42	0.098108	1	0.027965	34	11.3834	1	4.1913	
22	21	0.12048	210	0.49054	13	0.36354	15	5.0221	0	0	
23	22	0.15168	151	0.35272	33	0.92284	13	4.3525	0	0	
24	23	0.19095	116	0.27097	31	0.86691	2	0.66961	0	0	
25	24	0.24039	85	0.19855	30	0.83895	0	0	0	0	
26	25	0.30264	73	0.17052	15	0.41947	0	0	0	0	
27	26	0.381	37	0.086429	4	0.11186	0	0	0	0	
28	27	0.47965	25	0.058398	3	0.083895	0	0	0	0	

As mentioned in the manuscript (Shea et al., 2009), prior to proceeding with stereological conversion and final size distribution computations, it is necessary to define the different size boundaries which will be covered by each magnification (not applicable if only one magnification is used). This is done in FOAMS either by simply pushing on the “AutoSmart Bin Limits” button (which calculates the transitions by minimizing the difference in number density between two successive NA vs. L curves), or by entering them individually in the upper left panel of the new results interface. For our example, this is what FOAMS calculates in terms of bin boundaries →

Bin settings

Mag4 lower bin limit 0.0012

Mag4 upper bin limit 0.0381

Mag3 lower bin limit 0.04796

Mag3 upper bin limit 0.19095

Mag2 lower bin limit 0.24039

Mag2 upper bin limit 0.381

Mag1 lower bin limit 0.47965

Mag1 upper bin limit 3.02637

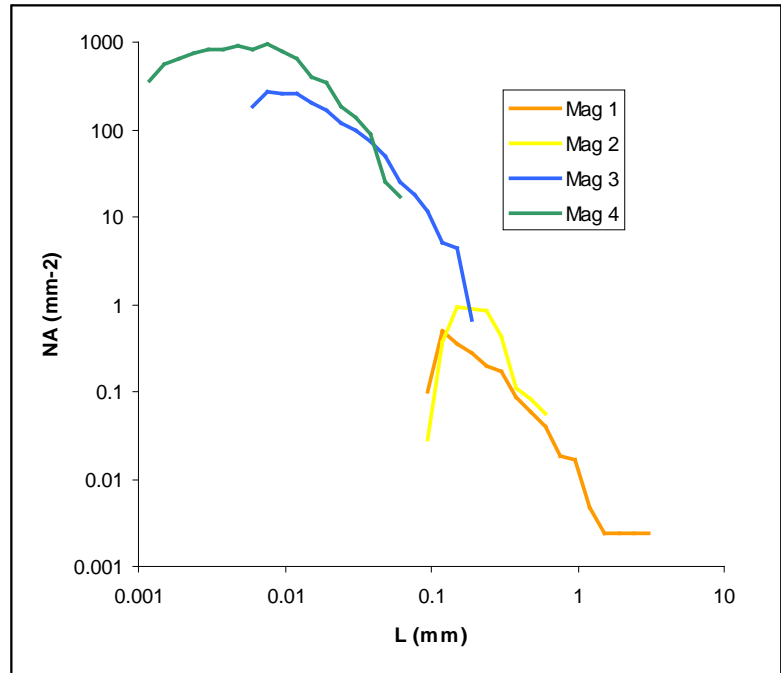
Warning: boundaries have to be entered to the 5th decimal (ex: 0.68345)

In the spreadsheet, these limits (unit is mm) correspond to:

	A	B	C	D	E	F	G	H	I	J	K
1		bins	freq_mag1	NA_mag1	freq_mag2	NA_mag2	freq_mag3	NA_mag3	freq_mag4	NA_mag4	
2	1	0.0012	0	0	0	0	0	0	86	360.4534	
3	2	0.00152	0	0	0	0	0	0	134	561.6367	
4	3	0.00191	0	0	0	0	0	0	156	653.8458	
5	4	0.0024	0	0	0	0	0	0	180	754.4374	
6	5	0.00303	0	0	0	0	0	0	199	834.0725	
7	6	0.00381	0	0	0	0	0	0	194	813.1159	
8	7	0.0048	0	0	0	0	0	0	220	922.0902	
9	8	0.00604	0	0	0	0	556	186.1517	201	842.4551	
10	9	0.0076	0	0	0	0	803	268.8486	225	943.0468	
11	10	0.00957	0	0	0	0	751	251.4388	186	779.5853	
12	11	0.01205	0	0	0	0	757	253.4476	155	649.6544	
13	12	0.01517	0	0	0	0	609	203.8964	93	389.7927	
14	13	0.0191	0	0	0	0	506	169.4115	83	347.8795	
15	14	0.02404	0	0	0	0	354	118.5211	43	180.2267	
16	15	0.03026	0	0	1	0.027965	291	97.4283	32	134.1222	
17	16	0.0381	0	0	0	0	215	71.9831	21	88.0177	
18	17	0.04796	0	0	0	0	147	49.2164	6	25.1479	
19	18	0.06038	0	0	0	0	76	25.4452	4	16.7653	
20	19	0.07602	0	0	0	0	53	17.7447	0	0	
21	20	0.0957	42	0.098108	1	0.027965	34	11.3834	1	4.1913	
22	21	0.12048	210	0.49054	13	0.36354	15	5.0221	0	0	
23	22	0.15168	151	0.35272	33	0.92284	13	4.3525	0	0	
24	23	0.19095	116	0.27097	31	0.86691	2	0.66961	0	0	
25	24	0.24039	85	0.19855	30	0.83895	0	0	0	0	
26	25	0.30264	73	0.17052	15	0.41947	0	0	0	0	
27	26	0.381	37	0.086429	4	0.11186	0	0	0	0	
28	27	0.47965	25	0.058398	3	0.083895	0	0	0	0	
29	28	0.60384	17	0.039711	2	0.05593	0	0	0	0	
30	29	0.76019	8	0.018687	0	0	0	0	0	0	
31	30	0.95702	7	0.016351	2	0.05593	0	0	0	0	
32	31	1.2048	2	0.004672	0	0	0	0	0	0	
33	32	1.5168	1	0.002336	0	0	0	0	0	0	
34	33	1.9095	1	0.002336	0	0	0	0	0	0	
35	34	2.4039	1	0.002336	0	0	0	0	0	0	
36	35	3.0264	1	0.002336	0	0	0	0	0	0	

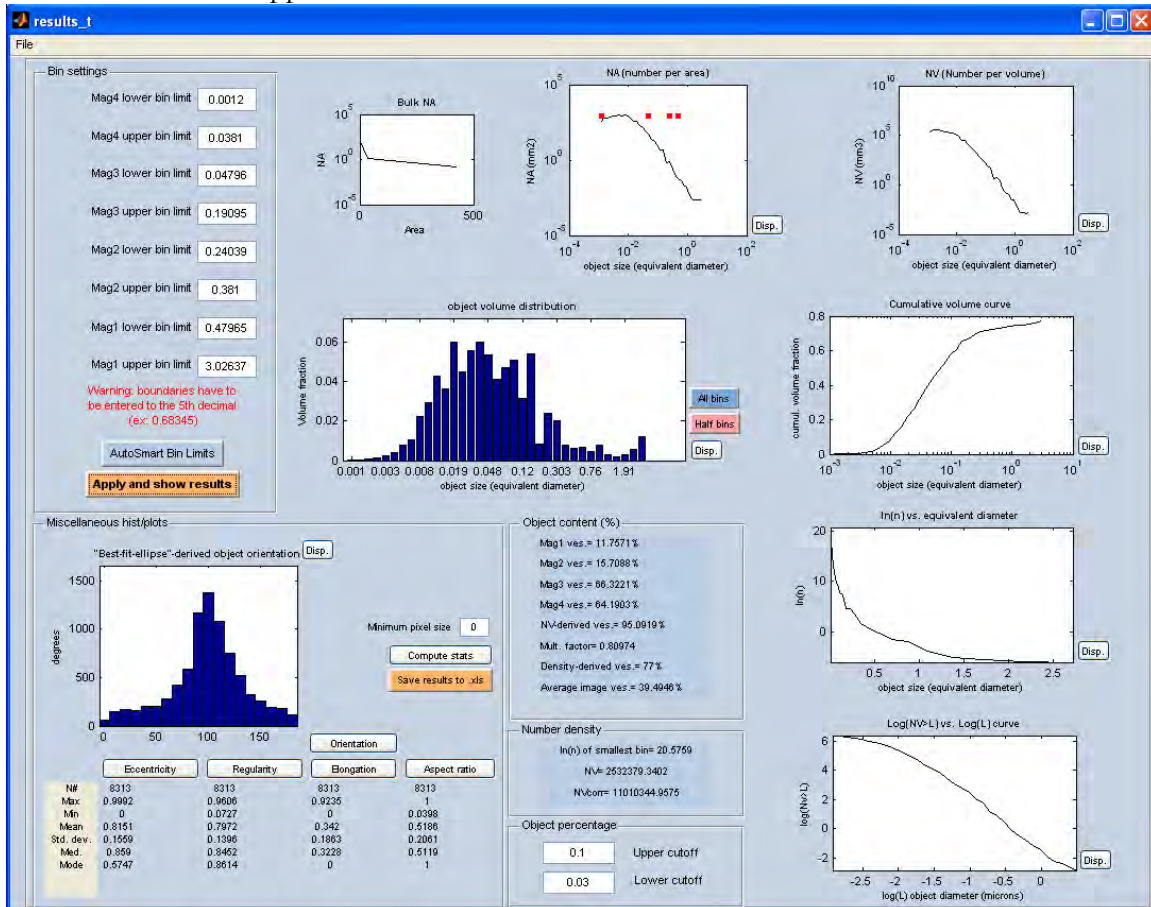
We always recommend making an actual NA vs. L plot at this point to ensure that the boundaries chosen are truly the best possible:

When better bin cutoffs between the curves are discovered, they should be directly entered in the appropriate cases in the interface with a 5 decimal precision (same as in the output data files).



c. Result plots

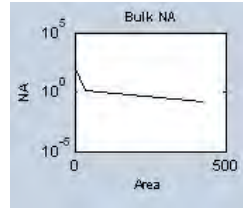
After the cutoffs are entered, the user simply presses 'Apply and show results' and a new wait bar should appear, lasting on average 15-40 seconds. All resulting plots should then appear in the interface:



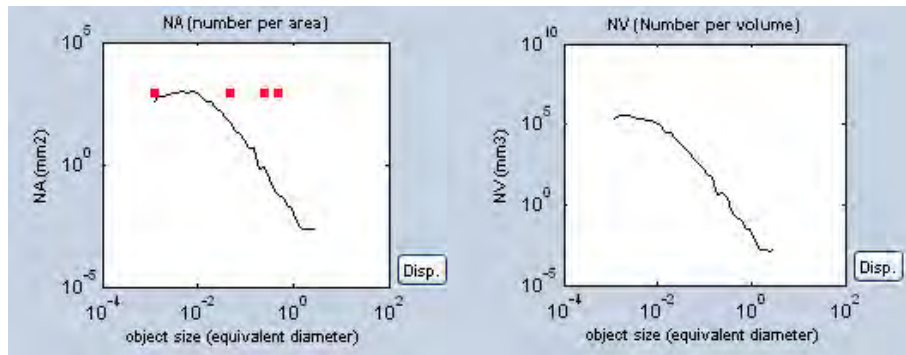
None of the plot windows should be blank. Each plot can be visualized in a separate window by pushing on “disp” buttons on the lower right corner. In this way, all plot properties can be modified, and graphs can be saved in any desired format.

In detail, the plots represent:

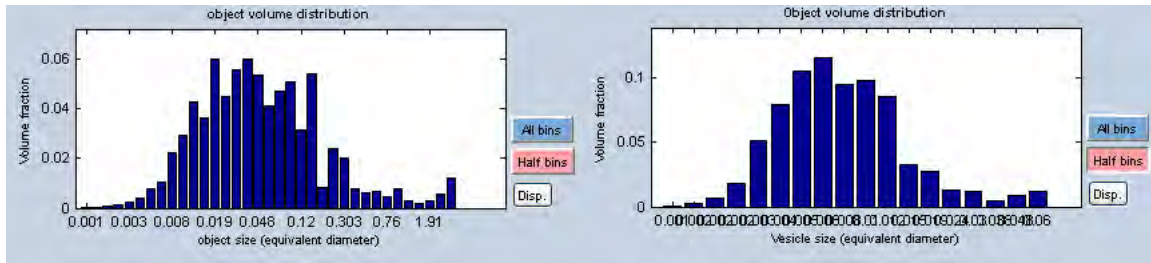
- **Bulk NA vs. Area plot:** This small graph allow to visualize and ensure than the number density of objects decreases with increasing image magnification (or area covered by the magnification).



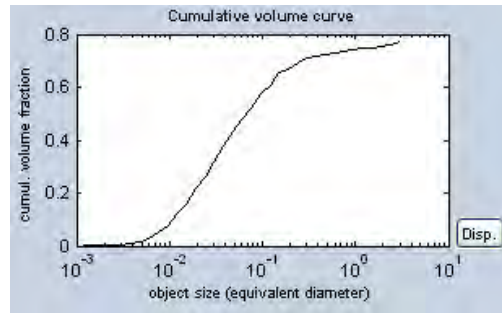
- **N_A and N_V vs. L plots:** These allows to visualize not only cutoffs (red squares) as well as the curve resulting from the choice of the different cutoffs (it should ideally be as smooth as possible, and shaped like an upside down hook) but also the curve resulting from stereological conversion of N_A to N_V (they should not look very different).



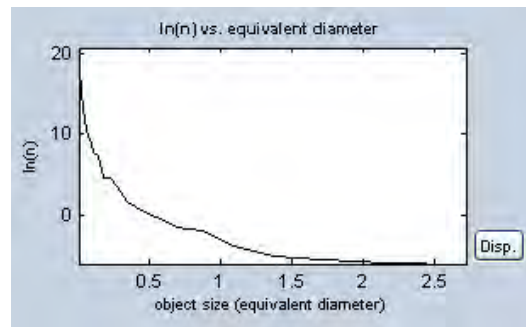
- **Volume fraction vs. L (VVD) plots:** These are particularly useful to observe mode variations within size distributions. This plot, like the others, can be visualized separately using “disp”, but also has the option to either display the full range of bins, or to divide them in two (all bins vs. half bins). To switch between the two views, only one button is pressed (and stays pressed) and the other one must stay “unpressed”.



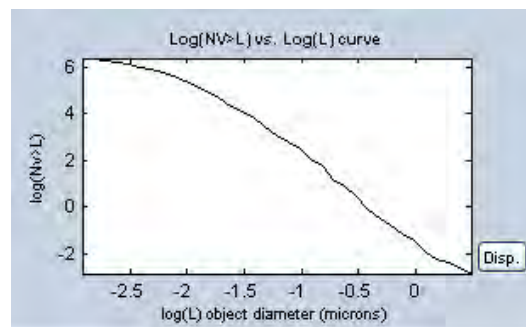
- **Cumulative Volume fraction (CVVD) plot:** Such plots are used in combination with VVDs to determine the participation of each size range in the total vesicularity of the sample.



- **$\ln(n)$ vs. L plots:** Used often in the literature to gather information on how number densities are distributed along the size interval.

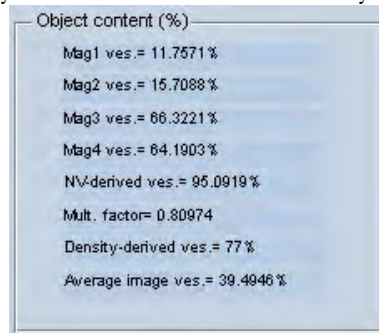


- **$\log(NV > L)$ vs. L plots:** Utilized to determine if number densities follow an exponential or power law distribution.

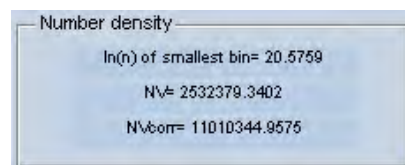


d. *Other useful information/plots*

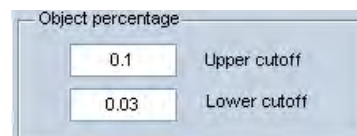
- **Object percentage information:** This data (also in the output files) comprises vesicularity measured for each magnification as well as the vesicularity derived after stereological conversion with the multiplication factor necessary to reach the true vesicularity.



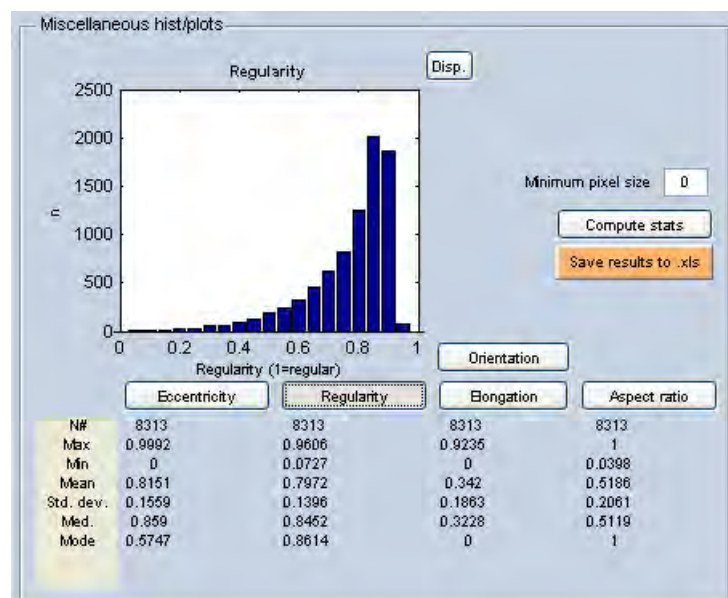
- **Number density information:** Provides $\ln(n)$ of the smallest bin measured in the $\ln(n)$ vs. L plots, as well as N_V and N_{Vcorr} (corrected for vesicularity).



- **Object percentage:** On the results interface, the two boxes “upper and lower cutoffs” are used to enter size intervals for which the user may want some 2D area fraction information (i.e. how many vesicles are larger than...).



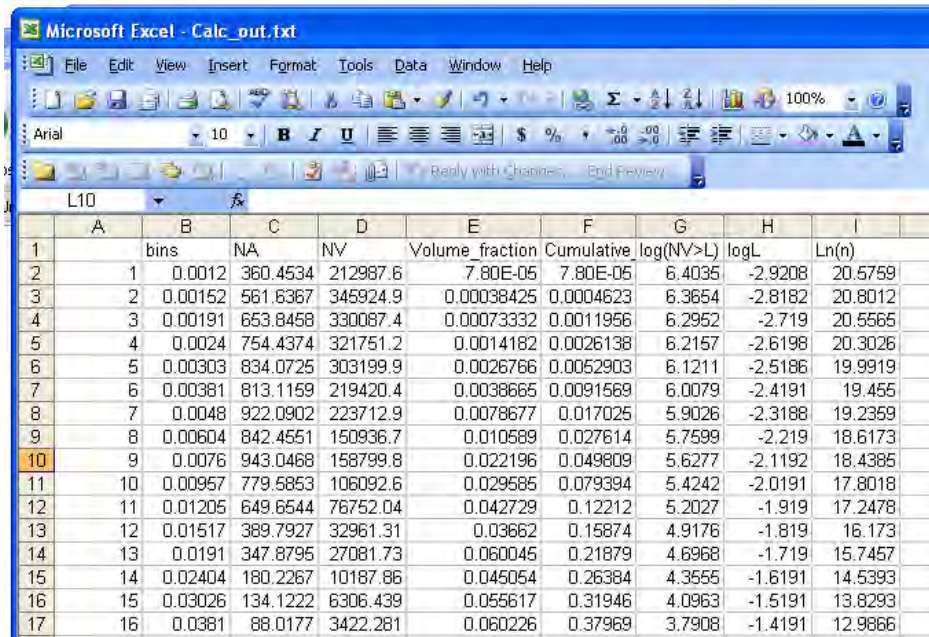
- **Miscellaneous plots/histograms** (aspect ratio, elongation, regularity...) and associated basic statistics may be useful to evaluate 2D shape factors and directionality in vesicle textures. The same configuration of pressed/unpressed buttons is used here, where a click on one leaves it in “pressed mode” and another click in “unpressed mode”.



The small box “Minimum pixel size” allows determining the influence of discarding smaller vesicles on the shape and orientation measurements; in other words, if minimum pixel is increased from 5 pixels to 20 pixels, smallest bubbles that are more prone to be poorly represented by a pixel compound are excluded. Hence the results should be statistically more robust. The results are displayed when the “Compute stats” button is pressed, and if the user wishes to save the data, a click on the “Save results to xls” button generates a spreadsheet in the main FOAMS directory.

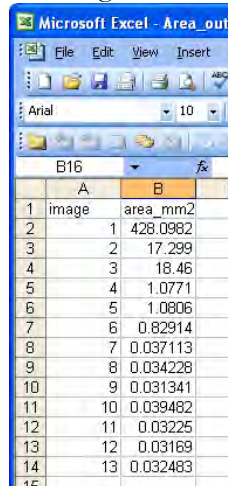
e. Output files

- **Calc_out:** Contains all the plot data (bins and corresponding N_A , N_V , Volume fraction, Cumulative volume fraction, $\log(NV>L)$ values, $\log(L)$ values and $\ln(n)$ values).



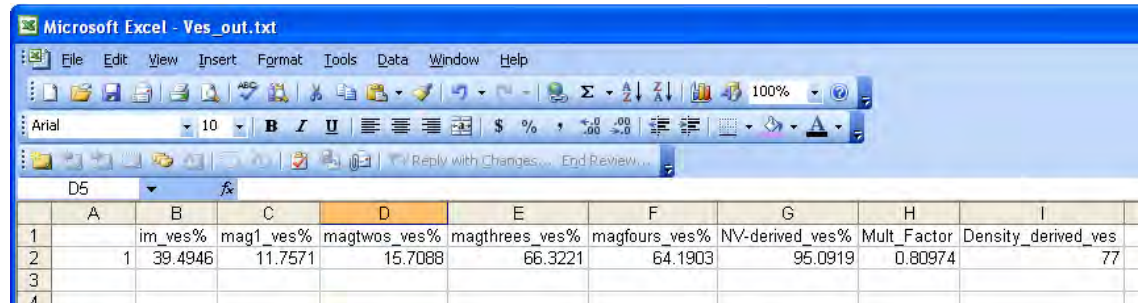
	A	B	C	D	E	F	G	H	I
1		bins	NA	NV	Volume fraction	Cumulative	$\log(NV>L)$	$\log L$	$\ln(n)$
2	1	0.0012	360.4534	212987.6	7.80E-05	7.80E-05	6.4035	-2.9208	20.5759
3	2	0.00152	561.6367	345924.9	0.00038425	0.0004623	6.3654	-2.8182	20.8012
4	3	0.00191	653.8458	330087.4	0.00073332	0.0011956	6.2952	-2.719	20.5565
5	4	0.0024	754.4374	321751.2	0.0014182	0.0026138	6.2157	-2.6198	20.3026
6	5	0.00303	834.0725	303199.9	0.0026766	0.0052903	6.1211	-2.5186	19.9919
7	6	0.00381	813.1159	219420.4	0.0038665	0.0091569	6.0079	-2.4191	19.455
8	7	0.0048	922.0902	223712.9	0.0078677	0.017025	5.9026	-2.3188	19.2359
9	8	0.00604	842.4551	150936.7	0.010589	0.027614	5.7599	-2.219	18.6173
10	9	0.0076	943.0468	158799.8	0.022196	0.049809	5.6277	-2.1192	18.4385
11	10	0.00957	779.5853	106092.6	0.029585	0.079394	5.4242	-2.0191	17.8018
12	11	0.01205	649.6544	76752.04	0.042729	0.12212	5.2027	-1.919	17.2478
13	12	0.01517	389.7927	32961.31	0.03662	0.15874	4.9176	-1.819	16.173
14	13	0.0191	347.8795	27081.73	0.060045	0.21879	4.6968	-1.719	15.7457
15	14	0.02404	180.2267	10187.86	0.045054	0.26384	4.3555	-1.6191	14.5393
16	15	0.03026	134.1222	6306.439	0.055617	0.31946	4.0963	-1.5191	13.8293
17	16	0.0381	88.0177	3422.281	0.060226	0.37969	3.7908	-1.4191	12.9866

- **Area_out:** Contains individual image areas



	A	B
1	image	area_mm2
2	1	428.0982
3	2	17.299
4	3	18.46
5	4	1.0771
6	5	1.0806
7	6	0.82914
8	7	0.037113
9	8	0.034228
10	9	0.031341
11	10	0.039482
12	11	0.03225
13	12	0.03169
14	13	0.032483
15		

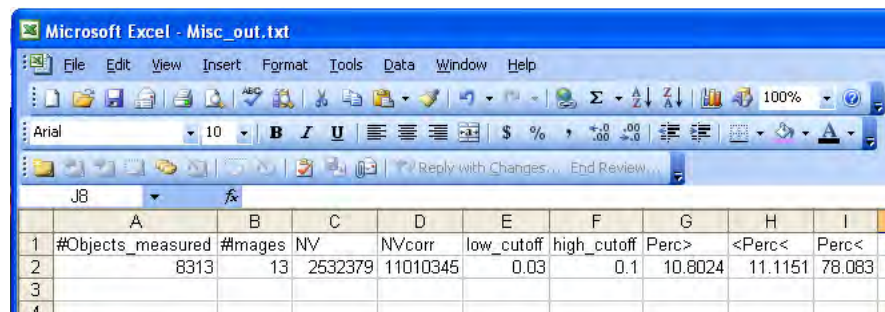
- **Ves_out**: Contains vesicularity information (2D image vesicularity, 2D individual magnification vesicularity, NV-derived vesicularity, multiplication factor, density derived vesicularity).



Microsoft Excel - Ves_out.txt

	A	B	C	D	E	F	G	H	I
1		im_ves%	mag1_ves%	magtwos_ves%	magthrees_ves%	magfours_ves%	NV-derived_ves%	Mult_Factor	Density_derived_ves
2	1	39.4946	11.7571	15.7088	66.3221	64.1903	95.0919	0.80974	77
3									
4									

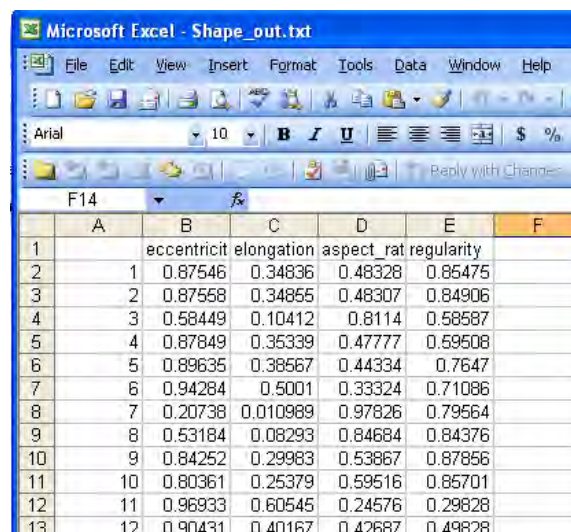
- **Misc_out**: Contains various information (number of objects measured, number of images, low and high cutoffs selected) and measurements (N_v , N_{vcorr} , and percentage of objects included within the cutoff intervals)



Microsoft Excel - Misc_out.txt

	A	B	C	D	E	F	G	H	I
1	#Objects_measured	#images	NV	NVcorr	low_cutoff	high_cutoff	Perc>	<Perc<	Perc<
2	8313	13	2532379	11010345	0.03	0.1	10.8024	11.1151	78.083
3									
4									

- **Shape_out**: Contains shape measurements (eccentricity calculated from best-fit ellipses, elongation, aspect ratio, and regularity, see Appendix in Shea et al., 2009).



Microsoft Excel - Shape_out.txt

	A	B	C	D	E	F
1		eccentricit	elongation	aspect_rat	regularity	
2	1	0.87546	0.34836	0.48328	0.85475	
3	2	0.87558	0.34855	0.48307	0.84906	
4	3	0.58449	0.10412	0.8114	0.58587	
5	4	0.87849	0.35339	0.47777	0.59508	
6	5	0.89635	0.38567	0.44334	0.7647	
7	6	0.94284	0.5001	0.33324	0.71086	
8	7	0.20738	0.010989	0.97826	0.79564	
9	8	0.53184	0.08293	0.84684	0.84376	
10	9	0.84252	0.29983	0.53867	0.87856	
11	10	0.80361	0.25379	0.59516	0.85701	
12	11	0.96933	0.60545	0.24576	0.29828	
13	12	0.90431	0.40167	0.42687	0.49828	

5. Known problems, issues with FOAMS

Because FOAMS has not been programmed by professionals (in case you hadn't noticed!!), there are numerous issues that derive from the difficulty of error-proofing associated with simple Matlab programs. As mentioned in the introduction, we welcome ALL feedback that may help to improve FOAMS.

Most errors during FOAMS runs will arise due to mistakes made by the user in terms of not following the code's expected "run-schedule". To ensure an error-free usage, be sure to follow step-by-step the instructions given above.

FATAL ERRORS (I.E YOU NEED TO RESTART THE PROGRAM ALTOGETHER) WILL MATERIALIZE WHEN:

- The images are not in the right format: these are 9 out of 10 times the answer to emails I get concerning FOAMS problems. It is crucial that the images are in the right format (**grayscale** and **not RGB**), and that each object is homogeneous in its grayscale level.
- The user chooses the wrong shade of gray either for selection of bubbles or selection of object to exclude. There is no way currently to go back once this choice is made and if you make that mistake unfortunately the program needs to be reset.
- Selecting pixel color for either vesicles or objects to exclude, no image was active in the "Current Image window" (you forgot to click on the small "a" button on one of the images). The cursor will appear but the selection will be blank and the program will eventually crash.
- The cutoffs boundaries are not successive; that is, if an entire bin separates two magnifications when entering them in the "results" interface instead of them immediately following each other, the program crashes.
- Sometimes the program may crash if between two runs the interfaces are not closed.