V1.0

User's Manual

OpenCASA

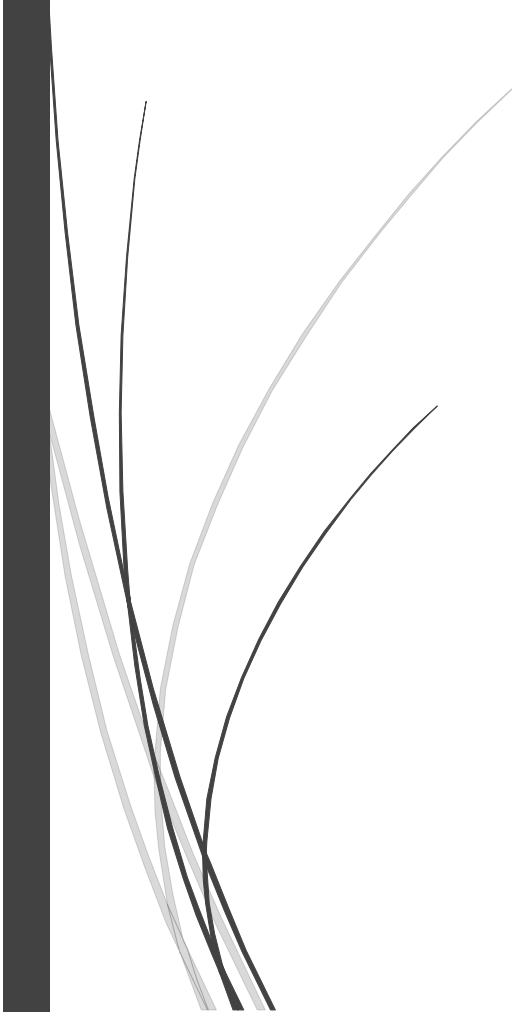


Table of Contents

Specifications

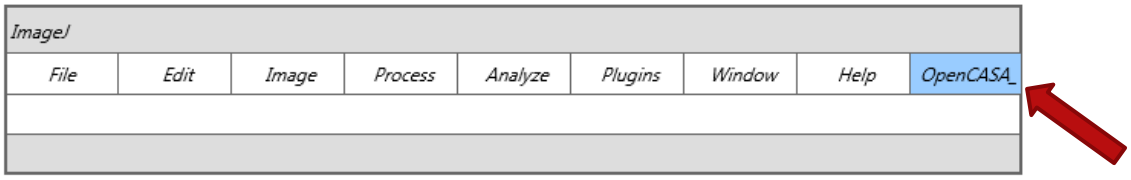
This program has been developed and tested on Windows 7 (64-bit) using Imagej v1.49q and Java 1.8.0_101 (64-bit). There are no specific requierements to use this plugin but a special attention of RAM memory is suggested when video analysis is carried on. At least 5GB of heap memory size is recommended, but it depends on the size of the files. One good estimation could be to use a heap memory size of 2.5 times the size of the heaviest file that is going to be analyzed. Information about how to increase memory on ImageJ can be found following this link: https://imagej.net/Troubleshooting#OutOfMemoryError. For all tests, only videos in AVI format and images in JPEG or PNG format were used. The plugin has not been tested on Linux or MAC platforms.

Installation

For users

First of all, it is necessary to have installed ImageJ. The latest version of the program can be downloaded from https://imagej.nih.gov/ij/download.html. We recommend to download the 64-bit bundle with the latest java version.

To install OpenCASA plugin on ImageJ, we recommend to follow these instructions: https://imagej.net/Installing_3rd_party_plugins: just drag and drop the .jar file into the ImageJ menu and select the destination folder. Once this is done, the OpenCASA_ option in the menu bar will be added.



For developers

To develop with ImageJ in Eclipse, it is recommended to follow the instructions specified in: https://imagej.net/Developing_ImageJ_in_Eclipse. Briefly, to set up and run an existing ImageJ project in eclipse, it is necessary to follow these four steps:

1. Install the Java Development Kit
2. Install and configure Eclipse
3. Clone the source code
4. Import the source code

Note: the source code is included in the .jar file. It is possible to extract it using a common software like 7zip or WinRAR. Once the source code has been extracted, for small changes, the plugin can be modified and compiled using the embedded java compiler in ImageJ located on the *Plugins* menu (in order to be sure that all changes have been updated, it is recommended to remove, previously the compilation, all .class files included in both folder and subfolders of OpenCASA plugin).

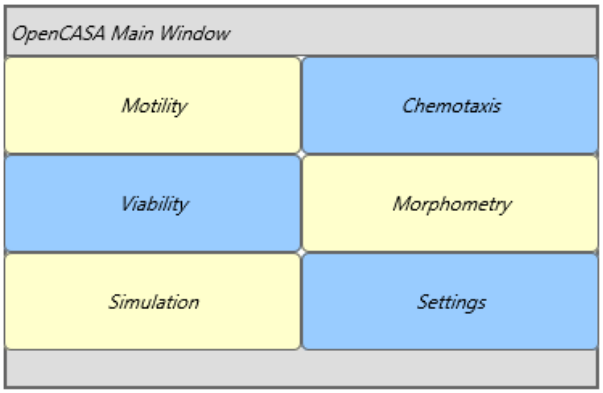
Tip: In order to increase the heap memory in eclipse, after the project has been set up, in the menu bar go to Run->Run Configuration, find the name of the class you have been running, select it, click the Arguments tab and then add:

-Xms5120M –Xmx5120M

where 5120 is the 5 gigabytes of memory that you want to assign (in megabytes). Remember that a heap memory size of 2.5 times the size of the heaviest file that is going to be analyzed is recommended.

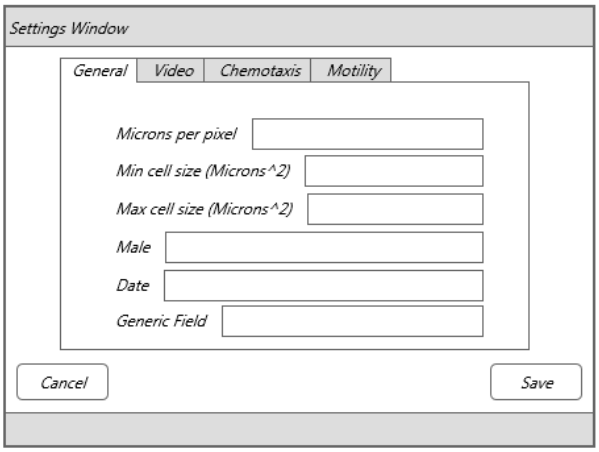
Main Window

The OpenCASA main window consists in a set of buttons corresponding each one with one functionality or module. In this version, five modules were implemented (Motility, Chemotaxis, Viability, Morphometry and Simulation), and a Settings menu was added in order to configure the parameters for each analysis.



Settings

In this window, the user can modify the value of a few parameters related with the input data and the analysis. The window is divided in four tabs. In the General tab, the user can set a few parameters related with the scale (it depends on the objective used to capture images or videos), the size of the cells or other fields like male identifier or the date of the analysis. In the Video tab, the user can set a few parameters related with the video analysis. In the Chemotaxis tab, the user can modify a few parameters involved in the chemotaxis analysis, such as the gradient direction or the number of bootstrapping resamples used for bootstrapping analysis. Finally,



in the Motility tab, the user can set the parameters related to cells in movement, like the vcl filter used to classify the cells in motile/non motile, or the value that determines when a cell has progressive motility or not.

The parameters that the user can configure for each category are specified in the following tables:

General

Microns per pixel	This is the ratio of microns per pixels.
Minimum cell size	Minimum cell size to be detected (in microns).
Maximum cell size	Maximum cell size to be detected (in microns).
Male	(optional) Identifier of the male that is analyzed.
Date	(optional) Date of the analysis.
Generic Field	(optional) This generic field can be used to add extra info to the final report.

Video

Frame rate	Frame rate of the video (frames / second).
Minimum track length	Trajectories with less length will not be considered (microns).
Maximum displacement between frames	Maximum displacement that a cell could carry on between two following frames (microns).
Window size	Length of the rectangle window used in the moving average method to calculate the average path (frames).
Print XY Coordinates	This option allows the user to save the x-y coordinates of each trajectory. Only available for file analysis (not directories) in both chemotaxis and motility analysis.

Chemotaxis

Chemotaxis gradient direction	Gradient direction (degrees).
Chemotaxis cone's amplitude	Gradient amplitude (degrees).
Number of bootstrapping resamples	Used to calculate the OR threshold in bootstrapping analysis.
Angle delta	Used when the frame rate of the recordings is too fast. For high frame rates, cells don't move enough between two following frames, so this parameter is used to calculate the angle between frame t and frame (t + angle delta). This parameter is not a strict decimator factor, in the context of signal processing.
Compare opposite directions	If this parameter is set to true, the analysis only takes into account angles in both the gradient and opposite directions as positive and negative displacements respectively, and ignore the rest. Otherwise, the module takes into account all angles in all directions.

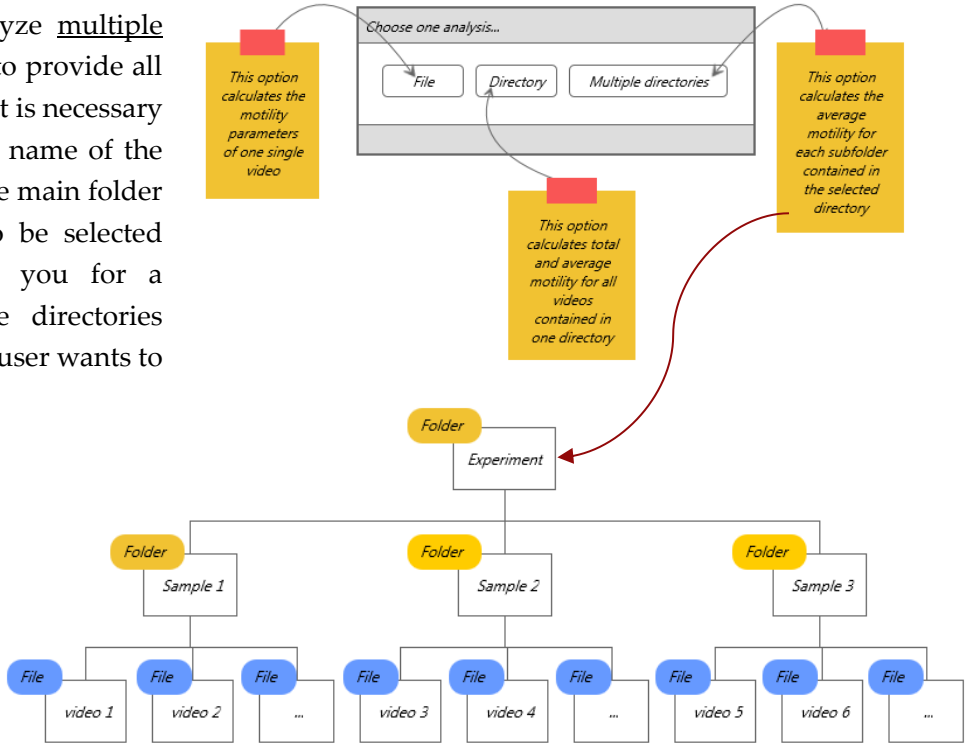
Motility

Progressive motility	Threshold used to determine when a cell has progressive motility or not. The value means % of STR motility parameter.
Minimum VCL	Minimum curvilinear velocity to consider a trajectory as motile.
VCL lower threshold *VCL upper threshold*	Only for file analysis. These parameters are used together to classify trajectories in three categories depending on their vcl value: Slow – Medium – Fast. Tracks below lower threshold are tagged as SLOW. Between lower threshold and upper threshold are tagged MEDIUM, and trajectories above upper threshold are tagged as FAST.

Motility module

This module allows the user to analyze a single or multiple recordings of cells in movement, and extract a set of motility parameters for each sample. The program accepts videos in AVI format. There are three options: analyze a file, a directory or multiple directories.

If the user wants to analyze multiple directories, it is necessary to provide all the data in a specific way. It is necessary to create a folder with the name of the experiment. This will be the main folder and the one that have to be selected when the program asks you for a directory in the multiple directories option. All folders that the user wants to analyze have to be added as a subfolders of the experiment directory, as it is represented in the figure on the right. Each subfolder has to contain all AVI videos that will be analyzed.



Results

Depending on the selected analysis, this module shows different results, summarized in the following table:

File	This analysis returns two reports: one with all motility parameters for each tracked cell, and an average report with the mean values of each parameter.
Directory	This analysis returns two reports: one with all motility parameters for each tracked cell, and an average report with the mean values of each parameter, one row for each video contained in the directory.
Multiple directories	This analysis return one report: for each subfolder, the average motility parameters is calculated. The repost contains one row for each subfolder.

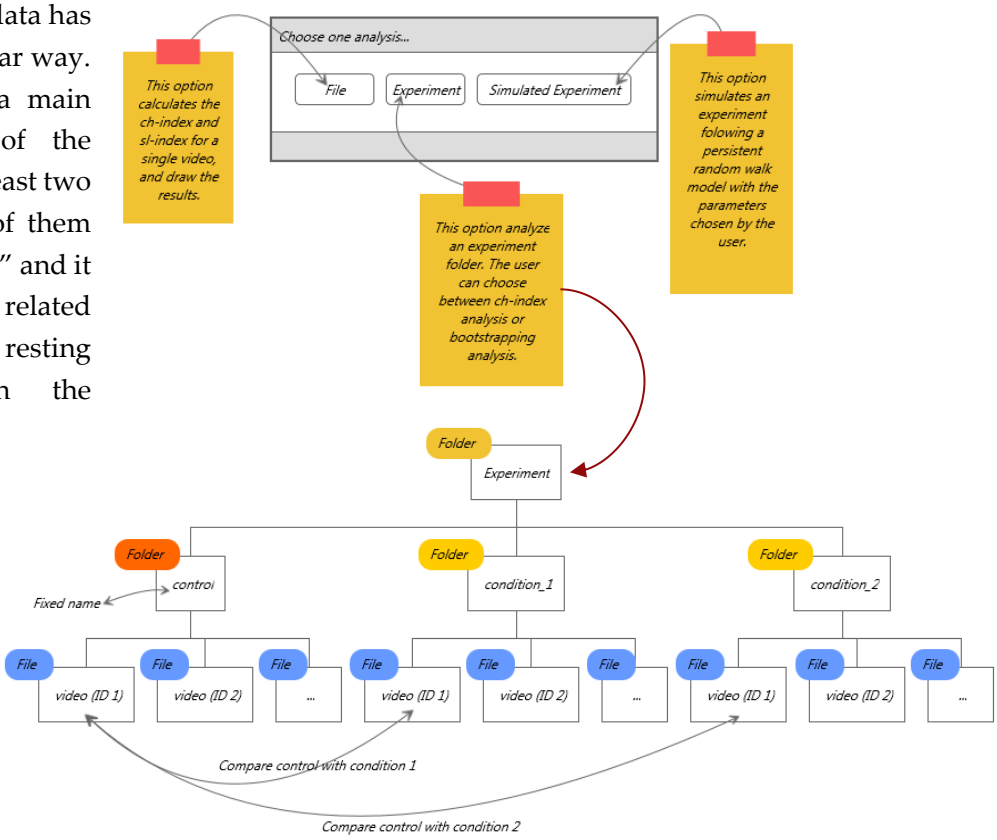
Parameters involved

General	Video	Chemotaxis	Motility
All	All	-	All

Chemotaxis module

This module allows the user to carry on a chemotaxis analysis in a single or multiple recordings. The program accepts files in AVI format. There are three options: analyze a file, a directory or simulate multiple samples. In both directory and multiple simulations, the user can choose between two different analyses: ch-index analysis and bootstrapping analysis.

For directory analysis, the data has to be provided in a particular way. It is necessary to create a main folder with the name of the experiment, and to add at least two subfolders inside it. One of them has to be named as "control" and it will contain all recordings related to control condition. The resting subfolders will contain the corresponding files for a particular experimental condition.



When the user selects an experiment folder to be analyzed, the program compares automatically the control files with each condition separately. For this purpose, each control file has to share the same ID with its corresponding condition file. For example, if there is a control file names as "sample1.avi", there has to be one file with the same name for each condition folder, in order to allow the program to match and compare them.

Results

Depending on the selected analysis, this module shows different results, summarized in the following table:

Ch-index	For a single file analysis, the program returns two diagrams as a report, one corresponding for the straight line analysis, and other a rose diagram showing the circular histogram of the instantaneous angles between the cell displacement and the gradient direction. For experiments, the program returns a report summarizing all ch-index and sl-index calculated for each sample.

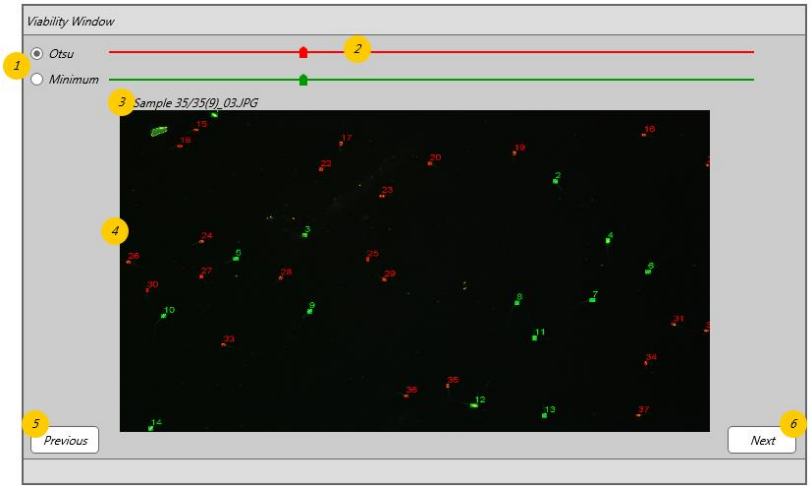
Bootstrapping	This analysis is only available for experiments. The program returns, for each experimental condition, the list of all analyzed samples, indicating which ones give positive and which ones not on chemotaxis, with 5%-confidence level. The threshold is calculated using all control samples included in the subfolder "control" and the number of bootstrapping resamples to calculate it can be configured in the settings menu.

Parameters involved

General	Video	Chemotaxis	Motility
All	All	All	Minimum VCL

Viability module

This module allows the user to count cells in two colors: red and green. There are two options: analyze one single image or multiple images, but both work in the same way. Once the program has load the first image, a window similar to this one is shown. This window has three different functional areas distributed in 6 components. The functionalities are explained in the following table:



Area	Component	Function
Intensity-based thresholding	1	Automatic threshold. Two possible methods to apply: Otsu and Minimum.
	2	Manual threshold. For each color, red and green, the user can select a different color intensity-based threshold.
Workspace	3	The current image name.
	4	The current image. The detected cells are shown with a unique numerical identifier for the current threshold. If the user clicks on this area, the raw unlabeled image is shown. This is useful to be sure that all cells are well classified.
Navigation	5	When a directory of images is analyzed, this button allows the user to navigate throw previous images.
	6	When a directory of images is analyzed, this button allows the user to navigate throw next images. Also, clicking on it, the results of the analysis are added to the report. This click is necessary even in a single file analysis or the last image of a directory.

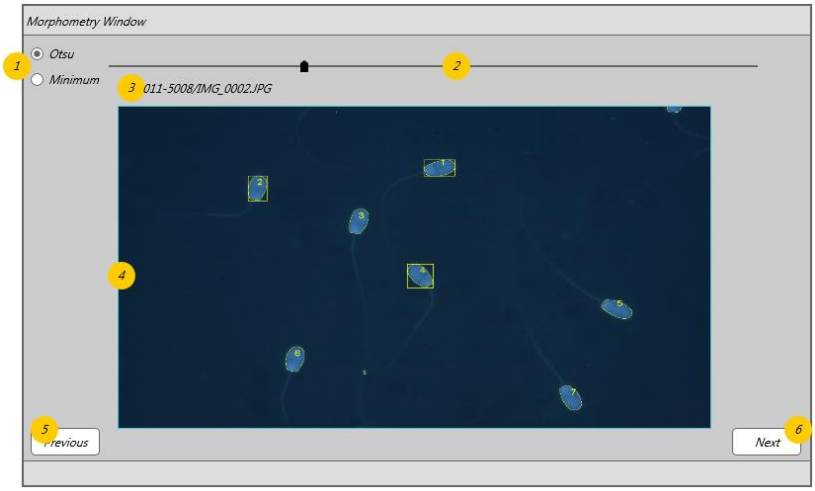
Results

This program returns a report with the number of cells of each type that have been counted. This report includes one row for each image.

Parameters involved

General	Video	Chemotaxis	Motility
All	-	-	-

Morphometry module



This module allows the user to calculate some morphometric parameters for a given cells. There are two options for the user but they work in the same way: analyze one single image or multiple images. Once the program has load the first image, a window similar to this one is shown. This window has three different functional areas distributed in six components. The functionalities are explained in the following table. When the user considers that one or multiple cells are well outlined, it is only necessary to click over them in order to add the corresponding morphometric parameters of these cells to the report.

Area	Component	Function
Intensity-based thresholding	1	Automatic threshold. Two possible methods to apply: Otsu and Minimum.
	2	Manual threshold. The user can select a different color intensity-based threshold.
Workspace	3	The current image name.
	4	The current image. The detected cells are shown with a unique numerical identifier for the current threshold. If the user clicks on this area, the raw unlabeled image is shown. This is useful to be sure that all cells are well outlined.
Navigation	5	When a directory of images is analyzed, this button allows the user to navigate throw previous images.
	6	When a directory of images is analyzed, this button allows the user to navigate throw next images.

Results

The program will show a report with various morphometric parameters from the selected cells (one row for each cell). The morphometric parameters calculated by the program are:

Parameter	Definition
Mean gray value	Average gray value of all pixels contained in the cell area (value between 0 and 255).
Area	Area of the cell (μm²).
Perimeter	Perimeter of the cell (μm).
Length	Length of the cell following the principal axis. Equivalent to Feret value (μm).
Width	Width of the cell following the secondary axis. Equivalent to Min_Feret (μm).
Ellipticity	$\frac{\text{Length}}{\text{Width}}$
Roughness	$\frac{4\ x\ \pi\ x\ Area}{Perimeter^2}$
Elongation	$\frac{Length - Width}{Length + Width}$
Regularity	$\frac{Length\ x\ Width\ x\ \pi}{4\ x\ Area}$

Parameters involved

General	Video	Chemotaxis	Motility
All	-	-	-

Simulation module

This module allows the user to create a video simulation of 100 cells following a persistent random walk model being attracted to the x-axis direction. Before the creation of a simulation, a dialog is shown to configure three parameters:

Parameter	Definition
Beta	This parameter is equivalent to the level of attraction. Bigger the parameter, stronger the attraction. The positive or negative sign of the parameter will determine the right/left direction of the cells, and a value of 0 means no attraction. A recommended range of this parameter is between [-5,5].
Responsiveness	This parameter indicates the percentage of the cells that are attracted. The rest of the cells follow a classical persistent random walk with no particular bias in their movement.
Length of the simulation	This parameter indicates the length (in frames) of the simulation.

Results

After setting these parameters, a video simulation is shown. The user can save the video as usual in ImageJ going to File menu -> Save As -> AVI…

Parameters involved

General	Video	Chemotaxis	Motility
-	-	-	-