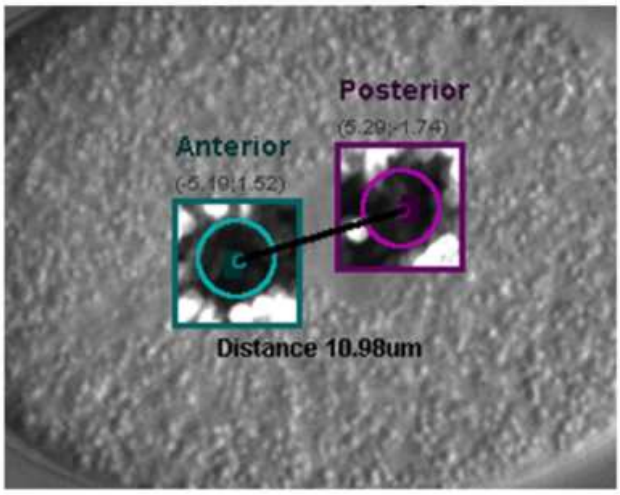
Automated Centrosome Tracking

Structure and Interactions of Molecular Biosystems (SIMBIO) Team

Plasticity and Evolution of the cell division Team



User Guide

Adresses/Contact

CLUET David, Research Ingeneer

Structures and Interactions of Molecular Biosystems: Computer simulations and experimental biology (SIMBIO).

david.cluet@ens-lyon.fr

SPICHTY Martin, Team Leader

Structures and Interactions of Molecular Biosystems: Computer simulations and experimental biology (SIMBIO).

martin.spichty@ens-lyon.fr

DELATTRE Marie, Team Leader

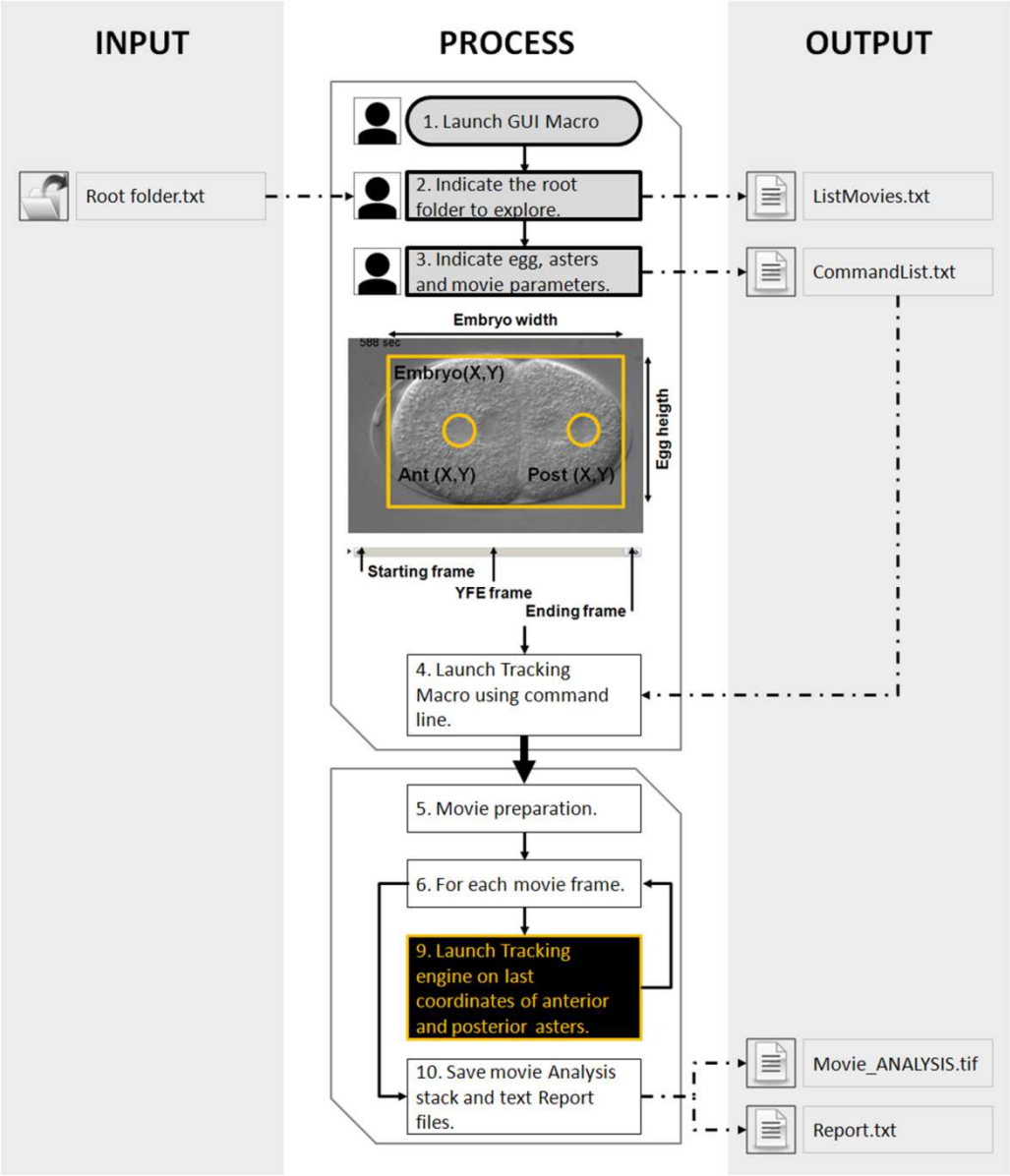
Plasticity and evolution of the cell division.

marie.delattre@ens-lyon.fr

Laboratory of Molecular and Cellular Biology (LBMC)
Ecole Normale Supérieure de Lyon
46, Allée d'Italie
69364 Lyon cedex 07
FRANCE

TABLE OF CONTENT

Architecture of the ACT macro



The Graphic User Interface (top panel) and the tracking program (bottom panel) are presented. The steps requiring user intervention are indicated with a specific icon and bold edge. The parameters that the user has to enter are indicated on the embryo image (3). The input and output flows are represented as dotted lines.

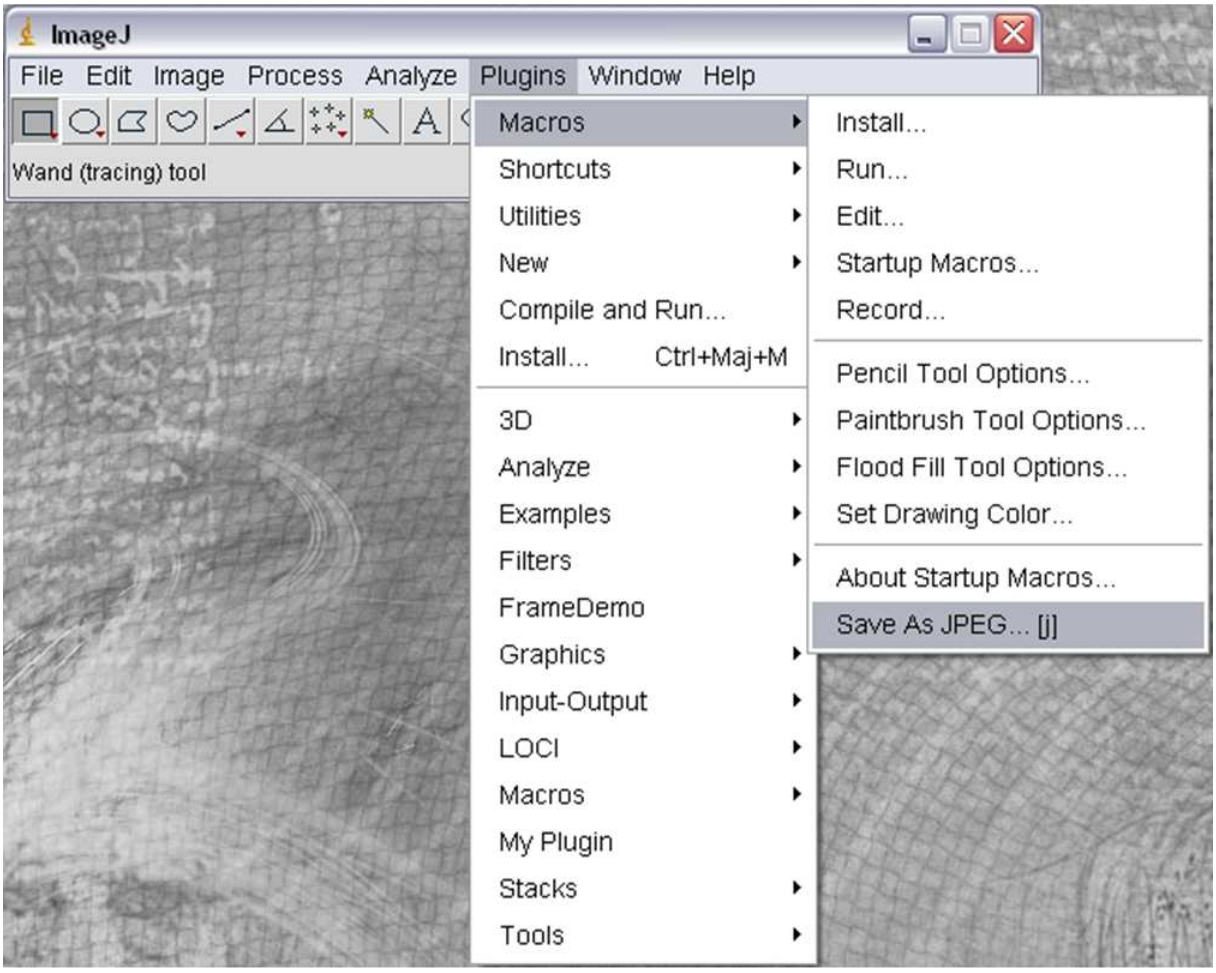
Procedure for Installation

Step 1 : Launch ImageJ software

First launch the **ImageJ program**. If you do not have it already installed on your computer, you can download it at this address: http://rsbweb.nih.gov/ij/download.html.

Note: *For the analysis of **movies** compressed as **.mov** you will require to install the **QuickTime Opener plugin** that you can find here:* http://rsbweb.nih.gov/ij/plugins/movie-opener.html.

Step 2 : Basic configuration of ImageJ

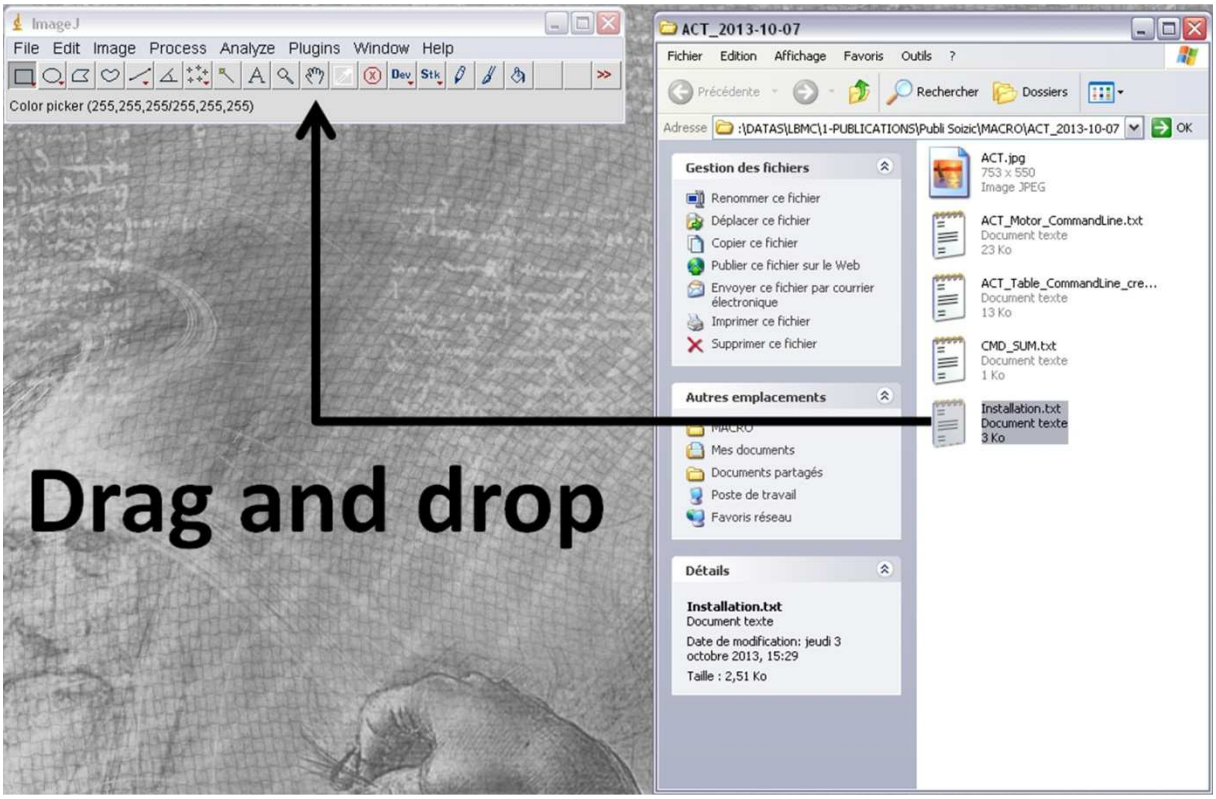


By default your ImageJ program has no shortcut to the ACT macro. The objective of this **installation procedure** is to transfer **automatically** the ACT macro and all its required files within ImageJ "macro" subfolder.

Finally, for convenient usage, **shortcuts** will be automatically generated within the **Plugins/Macros menu**.

If you have already a version of ACT, the **installation procedure** will overwrite it, making easy **updating** of your system

Step 3 : Initiate the installation macro

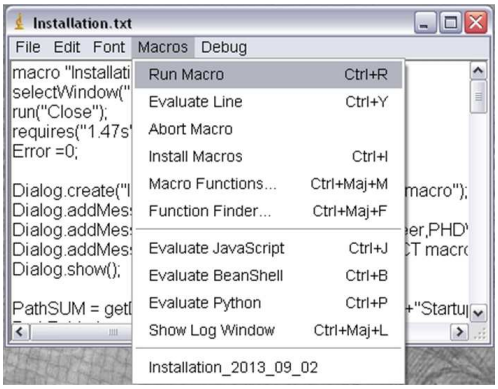


Open the folder you downloaded from our web site. It contains the current version of the ACT macro and the **Installation.txt** file.

Drag this file and **drop** it on **ImageJ command bar**.

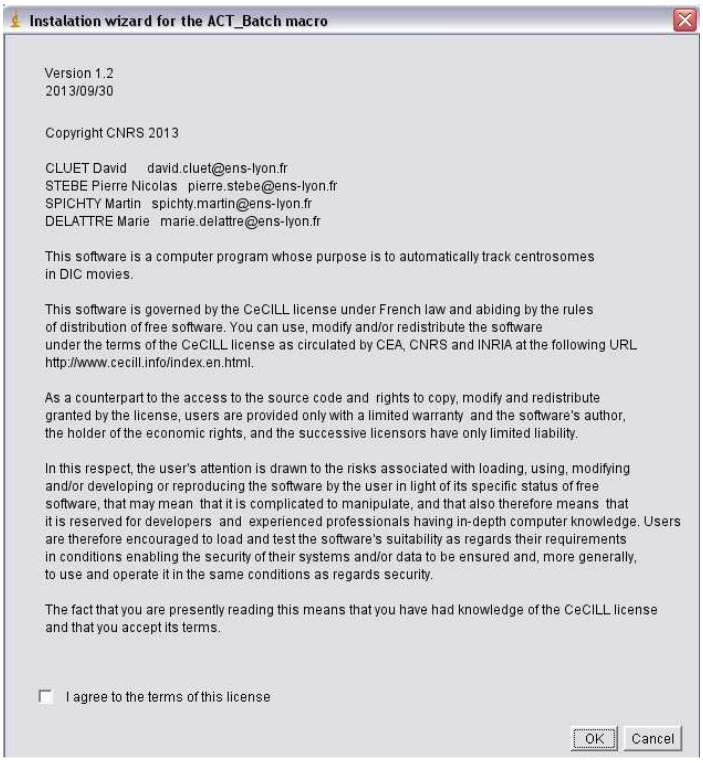
A new window will automatically pop up.

Step 4: Run the installation macro



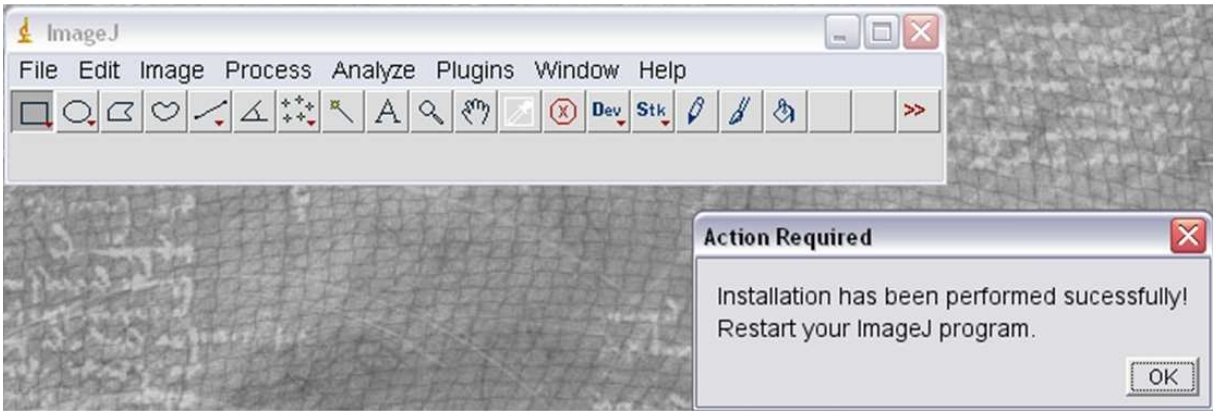
Use in the menu bar the **Macros/Run Macro command**.

Step 5 : Validation by the user of the installation procedure



The previous window disappears and **License greement window** of the installation program is displayed
Check the license agreement box.
Click on **OK** to **proceed** with the installation.
Click on **Cancel** to **abort** the process.

Step 6 : Installation termination



Once you have clicked on OK, the **installation** is performed in **few seconds**.

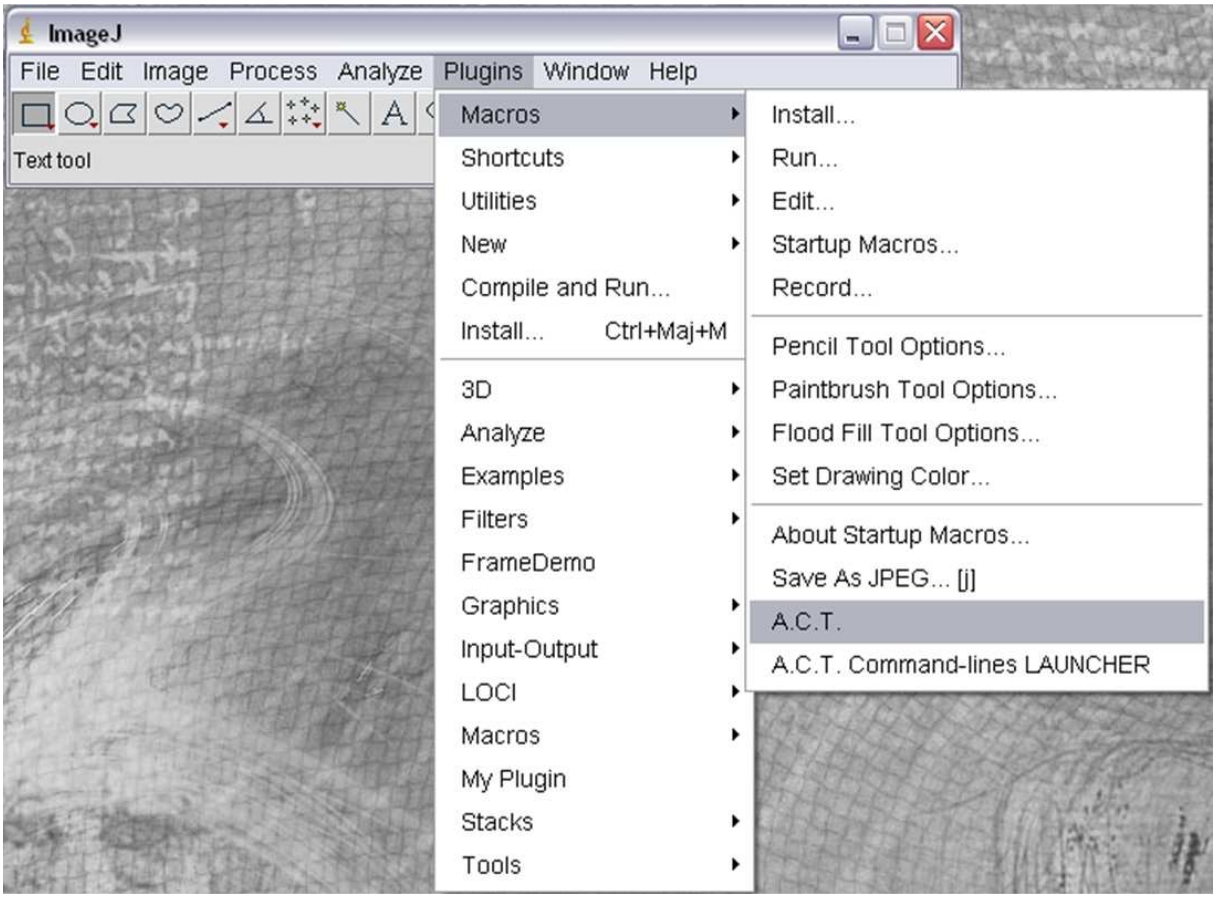
At the end of the process the program inform you of the **success** of the installation.

Quit ImageJ to **update** the "startup macro file" and see the **newly created shortcuts**.

If you perform an **updating** of **ACT**, you can **already use** it without restarting ImageJ.

Note: *If **files** were **missing** in your original folder the program will **inform you**. In such case **verify** that all files displayed in step 2 are present. Else **redownload** the installation folder from web page.*

Step 7 : Enhanced configuration of ImageJ



Now the ACT macro and its components are correctly installed.

Once **ImageJ** is **restarted** you can see **two** new **shortcuts** in the Plugin/Macros menu:

1. **A.C.T.**
2. **A.C.T. Command-Lines LAUNCHER**

The **first one** will allow you to launch the **graphic user interface** of ACT, **pre-process** movies and **analyze** them **batch wise**.

The **second one** will permit **direct submission** of **particular command lines** (for example to perform automated tracking on only certain movies).

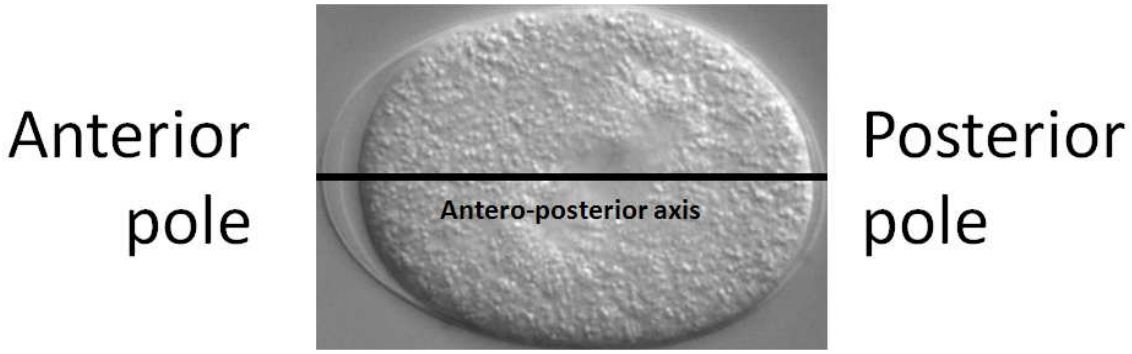
Pre-treatment of a movie and command line creation

In this section we will detail the **guided pre-processing** of the movie allowing the **downstream automated tracking** of the centrosomes.

For this tutorial the **procedure** is **detailed** for only **one movie**.

In the case of **several movies** the procedure is a **loop** between **steps 7 and 15**. The **batch wise pre-processing** can be performed for a specific root folder **at several times**. The ACT macro **keeps record** of all the pre-process movies and their generated **command lines** when you exit. You will **restart** next time with the **next movie**.

Movie preparation



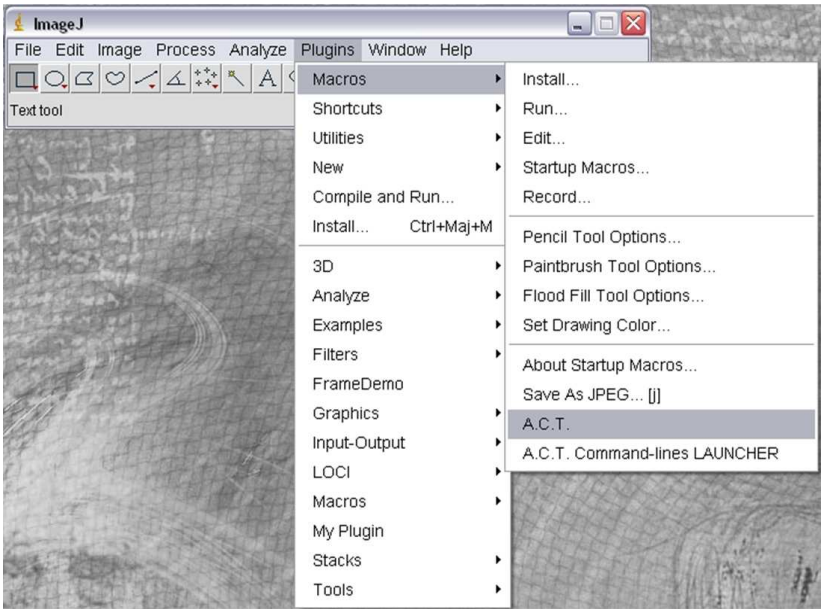
In order to **facilitate** the batch-wise **pre-treatment** of your movies, we recommend you to **orientate** your **embryo**'s images **as** presented **above**.

A **standardized** orientation of the embryos will allow you to **use** the **same parameters** (boundary box type in particular) for all your movies and then skip the advance settings step (see Step 15).

If the **posterior** centrosome of your movie is on the **left** side, use the "**Retrograde**" boundary box for this one and not for the anterior centrosome, **contrary** to what is described in the **example below**.

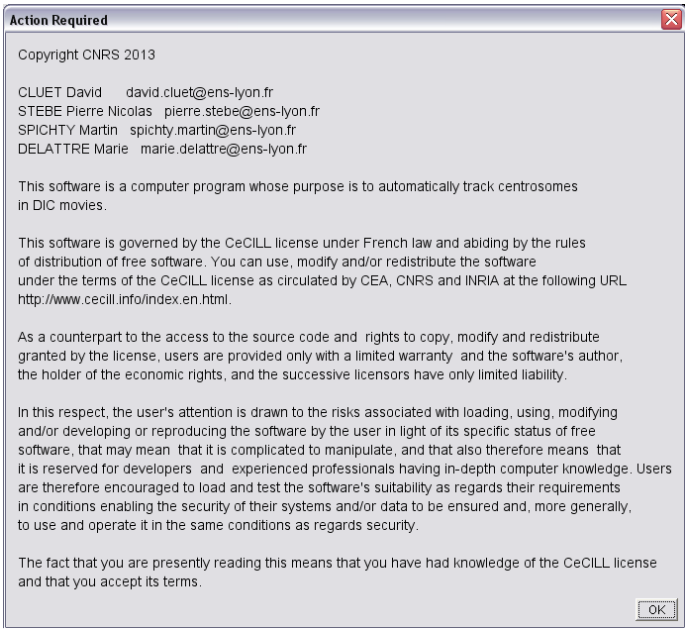
For **optimal** antero-posterior **positioning** of the **centrosomes** make sure that this axis is **parallel** to the **top** and **bottom edges** of the movie.

Step 1: Initiate ACT macro



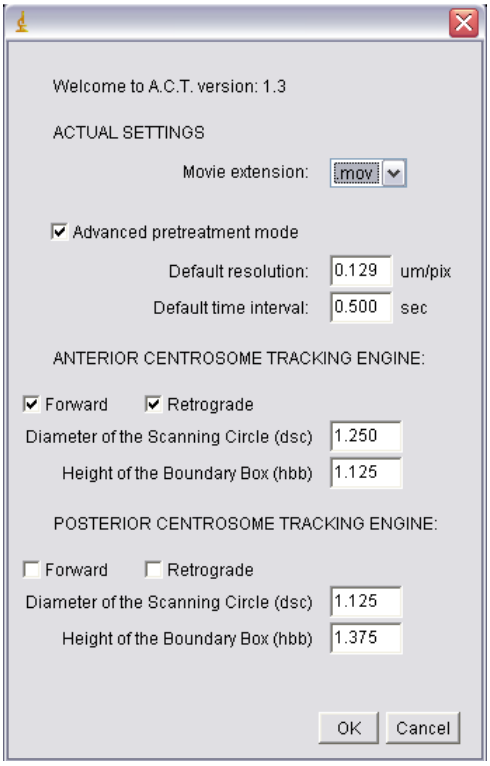
Initiate the **ACT** macro using the **shortcut** present in the Plugins/Macros menu.

Step 2 : Welcome window



The **welcome window** is displayed and the program prompts you to **accept** the **License Agreement**.

Step 3 : Main menu and parameters configuration



You access then to the **main menu** in which you can choose the **global parameters** for the **current analysis process**.

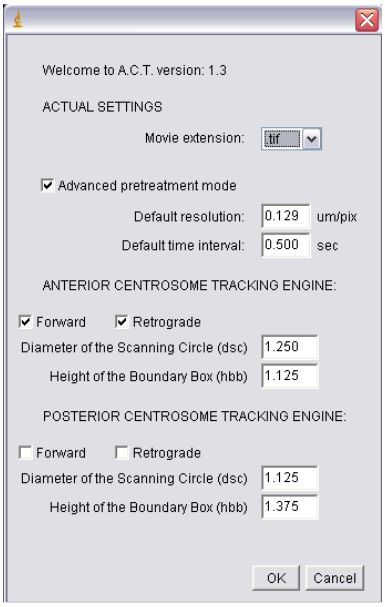
1. **Type of movie** (.mov, .tif, or .stk).
2. If the **advance settings** will be **proposed** for **each movie**.
3. The **pixel** and **time resolution** of the movies.
4. The **tracking parameters** for the **anterior centrosome**.
5. The **tracking parameters** for the **posterior centrosome**.

Validate Forward *to perform* ***tracking*** *from the* ***beginning*** *of the movies,* ***else*** *it will be performed from the* ***end****.*

Validate Retrograde *to use the* ***anterior*** *centrosome* ***optimized boundary box*** *(trajectrory toward the left side of the movie), else the program will use the posterior centrosome optimized one (trajectrory toward the right side of the movie).*

The ***size*** *of the* ***scanning circle*** *and the* ***boundary box*** *is* ***independent*** *for both centrosomes. The current values correspond to the* ***optimized*** *ones for* ***our input movies.***

Step 4 : Choice of the extension of the movies to analyze

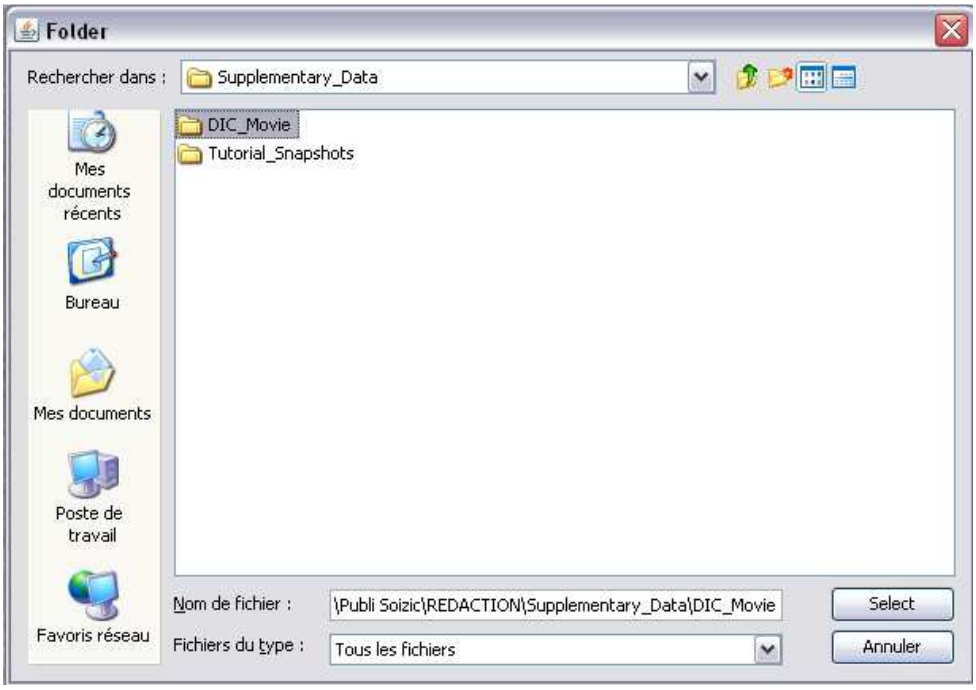


Once you have entered **your parameters** click on **OK**.

If you want to **abort** click on **Cancel**.

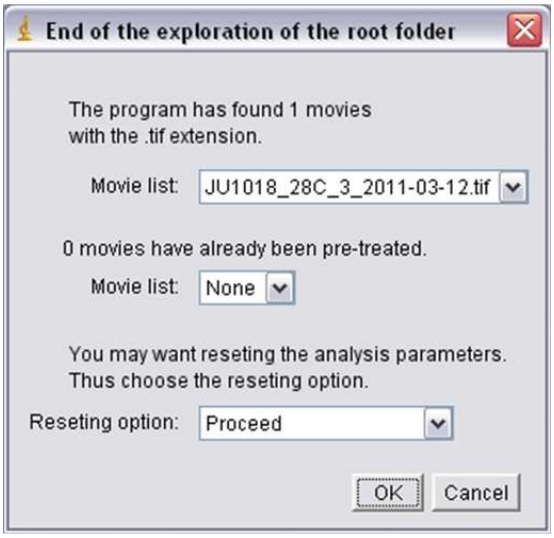
Here we present you the **settings** to analyze **a tif movie** with our **classical parameters**.

Step 5 : Choice of the root folder containing the movies



The program then **prompts** you to **indicate the root folder** containing your movies. **All movies** with the correct **extension** will be **found**, **whatever** the **arborescence** of your folder is.

Step 6 : List of the found movies

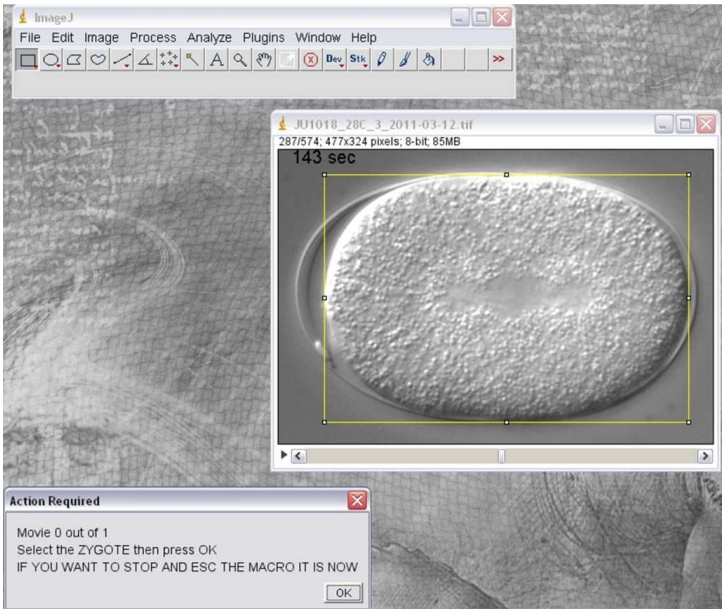


The **program** then **indicates** you how many **movies** it **identified** with the extension you specified in step 4. You have access to all the **movie names** by clicking on the first list form.

The program also indicates you **how many** have already **been pre-processed** (here none) and allow you to access to their names. This will allow you to **monitor your progression** in a consequent batch pre-processing performed during **several sessions**.

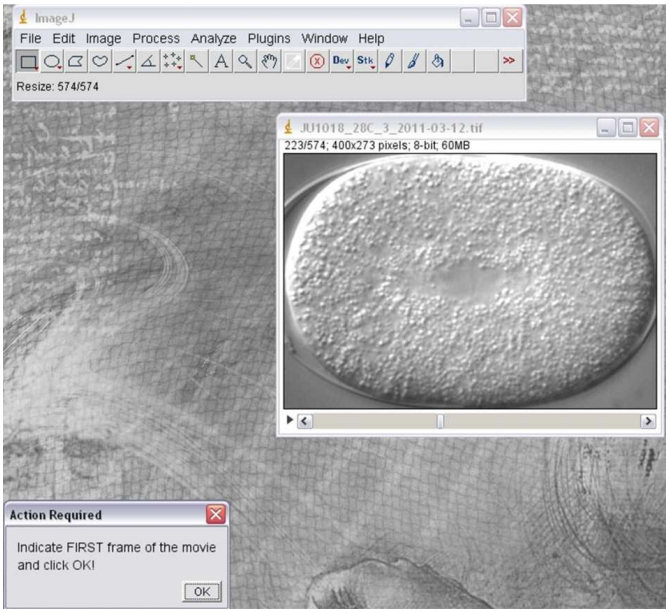
The last form allows you to choose between **proceeding** to the next step or **reset all command lines** (you will then restart the pre-processing with the first movie).

Step 7 : Drawing of the embryo's fitting rectangle



The **first movie** is then **automatically displayed** and the macro prompts the user to position a **rectangle fitting** the shape of the embryo.

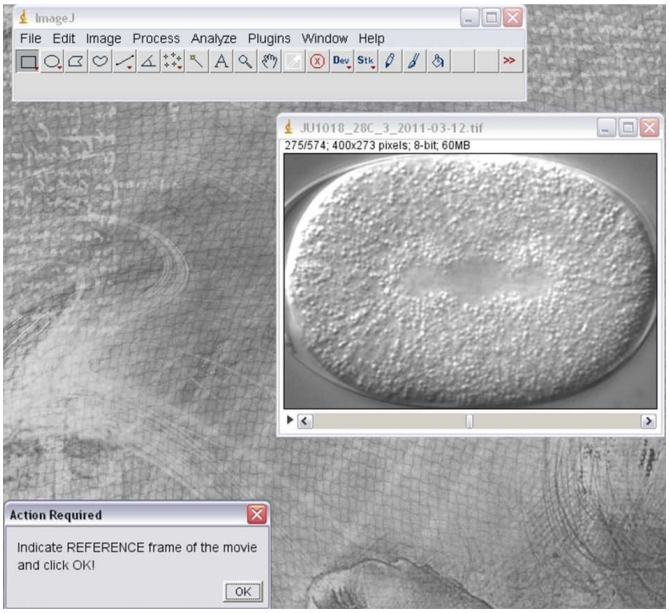
Step 8 : Identification of the first frame to be analyze



Using the **movie slider bar**, display the frame which will become the **starting point** of the analysis and click on **Ok**.

Note: To perform an **optimal tracking** in forward direction the **centrosomes** have to be **clearly expanded**.

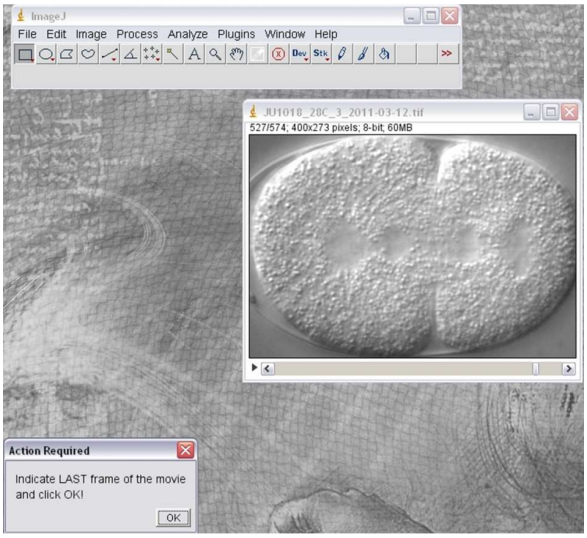
Step 9 : Identification of the frame corresponding to your favorite event



Position then the movie on the **frame** corresponding to the **beginning** (t0) of your **favorite event** (YFE, for example chromosome segregation, first occilation…) and click on **Ok**.

Note: *Depending on the **quality** of the **movie** you might have an **earlier t0** than the **starting frame**. The program can handle it as it generates **absolute time references** to your **t0**.*

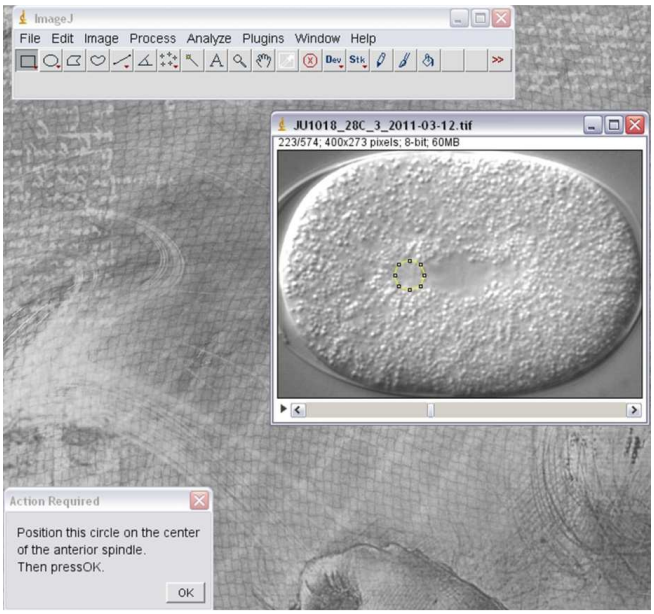
Step 10 : Identification of the last frame to be analyzed



Finally, indicate the **last frame** to be analyzed and click on **Ok**.

Note: *For an* ***optimal tracking*** *in* ***reverse*** *direction, try to* ***avoid*** *excessive* ***centrosome flattening****.*

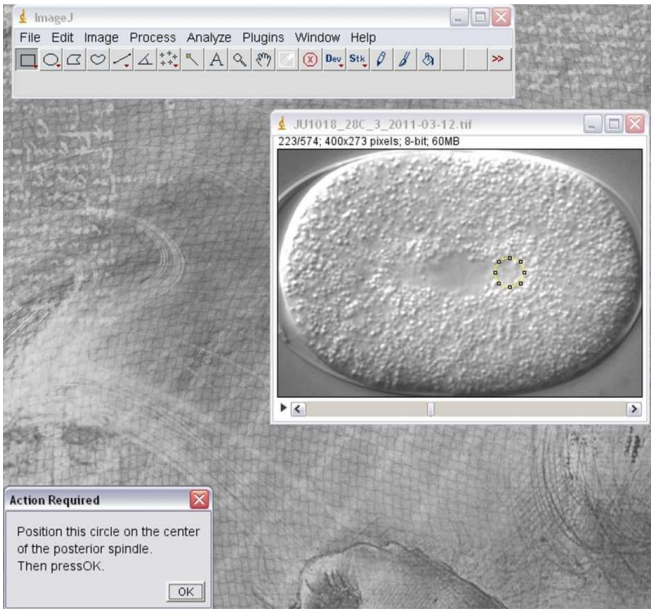
Step 11 : Positioning of the anterior centrosome on the first frame



The program the displays a **circle** on the **first frame** of the movie. **Place** it on the **anterior centrosome** and click on **Ok**.

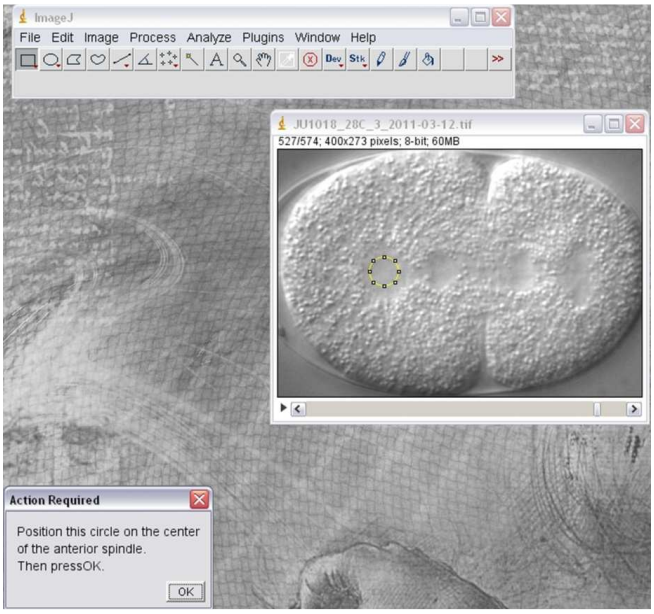
Note: If during the manipulation you **deform** and/or **delete** it, you can **redraw** one circle using the **draw ellipse tool** of image. Only the **center** of the **circle** if important!

Step 12 : Positioning of the posterior centrosome on the first frame



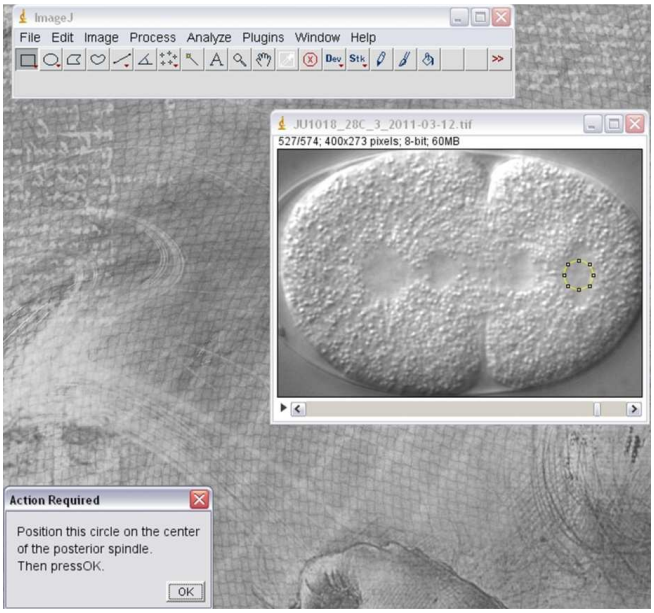
Reproduce this operation for the **posterior centrosome**

Step 13 : Positioning of the anterior centrosome on the last frame



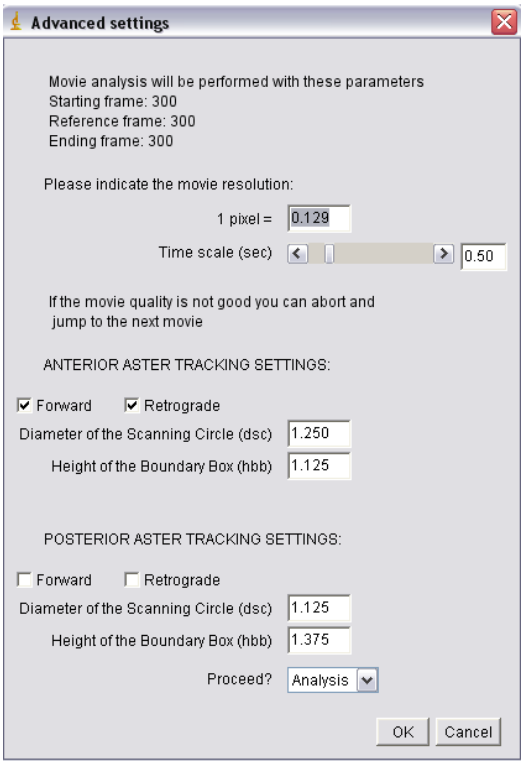
The program then **displays** the **last frame** to be analyzed. Place the **circle** on the **anterior aster** and click on Ok.

Step 14 : Positioning of the posterior centrosome on the last frame



Repeat this procedure for **posterior centrosome**.

Step 15 : Advance settings



If at **step 4** you have **checked** the "**advance settings**", box this final **window** is **displayed**.

It allows you to use **different parameters** for each **movie** (resolution, time scale…).

The **last field** allows you to **proceed** through **analysis** or **jump** to **next movie** without creating a command line. This could be helpful to **avoid analysis** on a movie with a bad quality.

Structure of the command line file

Command line file

The **file** containing the **command lines** is a simple **text file** (.txt). Each **line** is **separated** from the next one using the **"\n"** character.

By **default** the **first** line is **empty** and will not be read by the ACT command line launcher, allowing the advanced user to **add valuable comments** within the file without affecting the automated tracking process.

Arguments of a command line

The generated **command lines** are composed of **27 arguments** separated with the character **"\t".**

Argument	*Description*	*Type*
1	Absolute path of the movie file	String
2	Reference X position of the zygote	Integer
3	Reference Y position of the zygote	Integer
4	Width of the zygote	Integer
5	Heigth of the zygote	Integer
6	Movie resolution	Float
7	Starting frame of the movie	Integer
8	Reference frame of the event	Integer
9	Ending frame of the movie	Integer
10	Timegap	Float
11	X coordinate of anterior aster in 1st frame	Float
12	Y coordinate of anterior aster in 1st frame	Float
13	X coordinate of anterior aster in last frame	Float
14	Y coordinate of anterior aster in last frame	Float
15	Sens of analysis for anterior aster	Boolean
16	Box type for anterior aster	Boolean
17	Circle size for anterior aster	Float
18	Profile size for anterior aster	Float
19	X coordinate of posterior aster in 1st frame	Float
20	Y coordinate of posterior aster in 1st frame	Float
21	X coordinate of posterior aster in last frame	Float
22	Y coordinate of posterior aster in last frame	Float
23	Sens of analysis for posterior aster	Boolean
24	Box type for posterior aster	Boolean
25	Circle size for posterior aster	Float
26	Profile size for posterior aster	Float
27	Movie extension	String

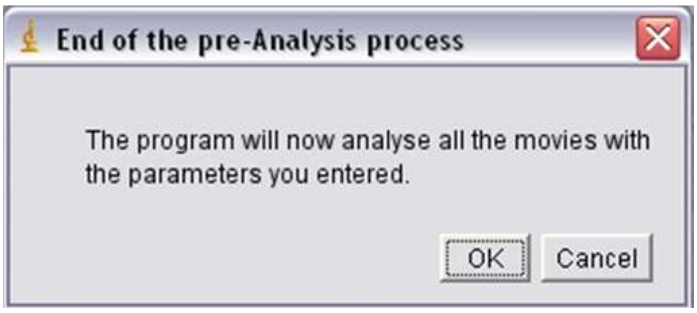
Job submission

The **movie files**, for which the ACT program was designed for can be heavy. **Batch-wise analysis** of such files can require an **extensive amount** of **memory** and **CPU load**.

The ACT program was designed to allow the scientist to **run analyses** at his **discretion** when it is more convenient for him (overnight, during the week-end,…). Thus the user has several options to launch the automated tracking of the centrosomes n his movies.

We will describe here the **different procedures** available.

Immediate submission



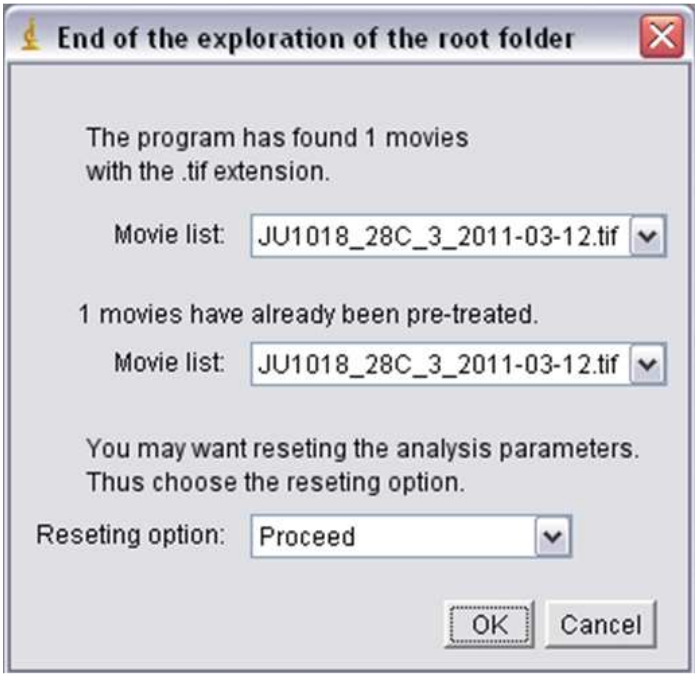
Once all movies present in your root folder have **pre-treated** the program informs you that the batch-wise **analysis** can be **immediately** performed.

Click on **Ok** to **start** the automated tracking.

Click on **Cancel** to **exit** the program to perform it later.

Delayed submission

Step 1 : Initiate ACT macro



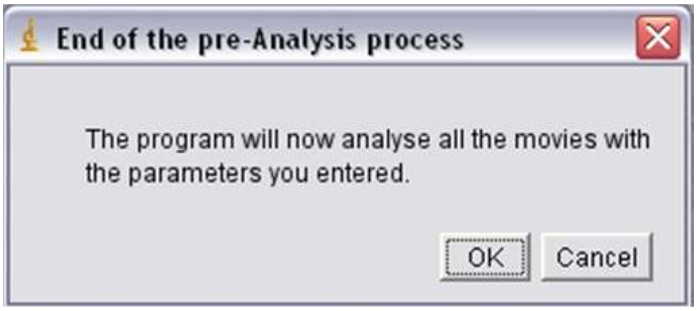
If you have **quit** the program at the **end of the pre-treatment process**, launch the **ACT macro** (as described in "*Pre-treatment of a movie and command line creation/Step 1*"), and re-specify the **path** of the root **folder**.

The program will verify that **all** your **movies** have been correctly **pretreated** (the list in the two forms are the same).

Click on **Ok**.

Note: *The **parameters** displayed on the **main menu** will have **no impact** on your command lines as they have been already saved with their specific settings.*

Step 2 : Activate the automated command line launcher



As all your movies have been pre-treated you **access directly** to **job submission** window.

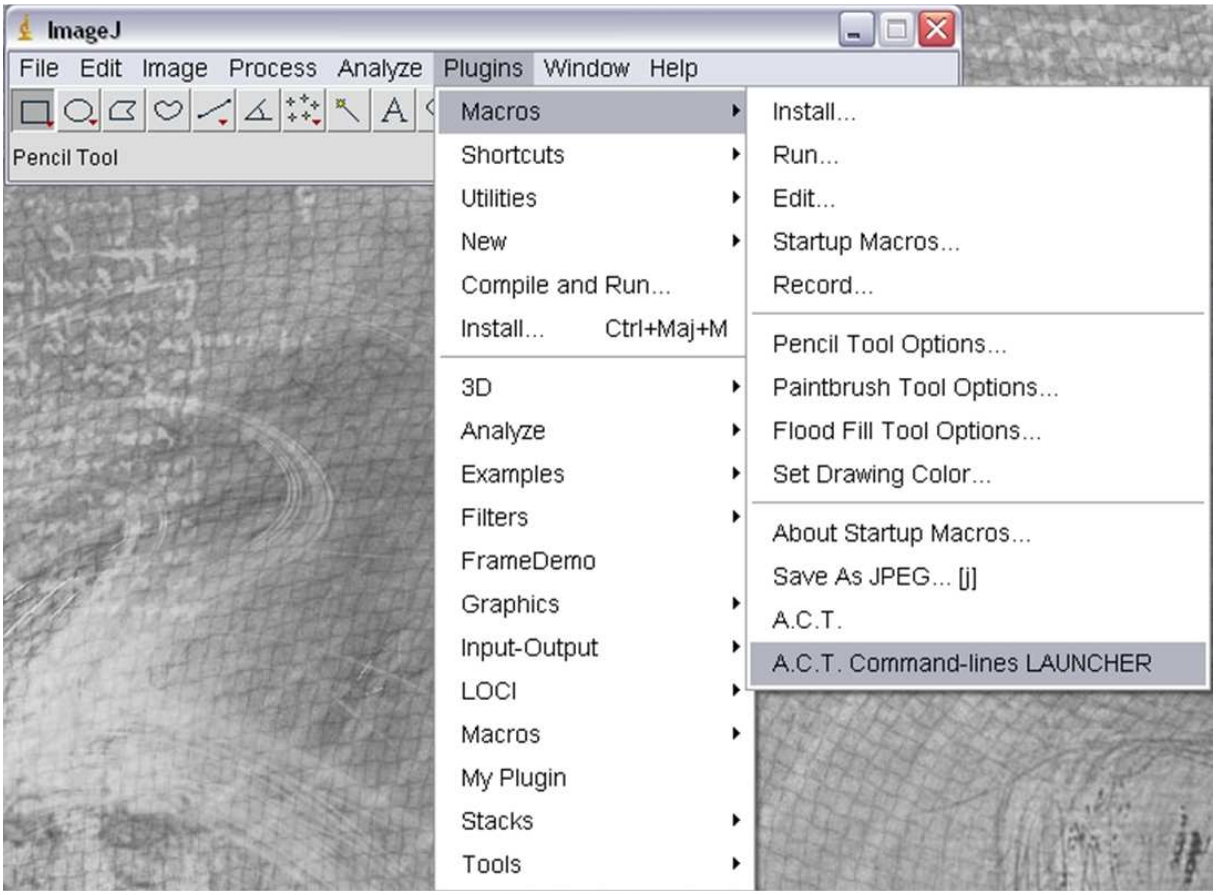
Click on **Ok** to start the **automated analysis**.

Direct submission of a command line file

For **advanced users**, it could be **convenient** to use a manually **created/modified** command-line **file** (for example to analyze only certain movies within a root folder, or after having changed certain parameters within the command line file).

For these purpose you have **direct access** to the command lien **launcher** of ACT.

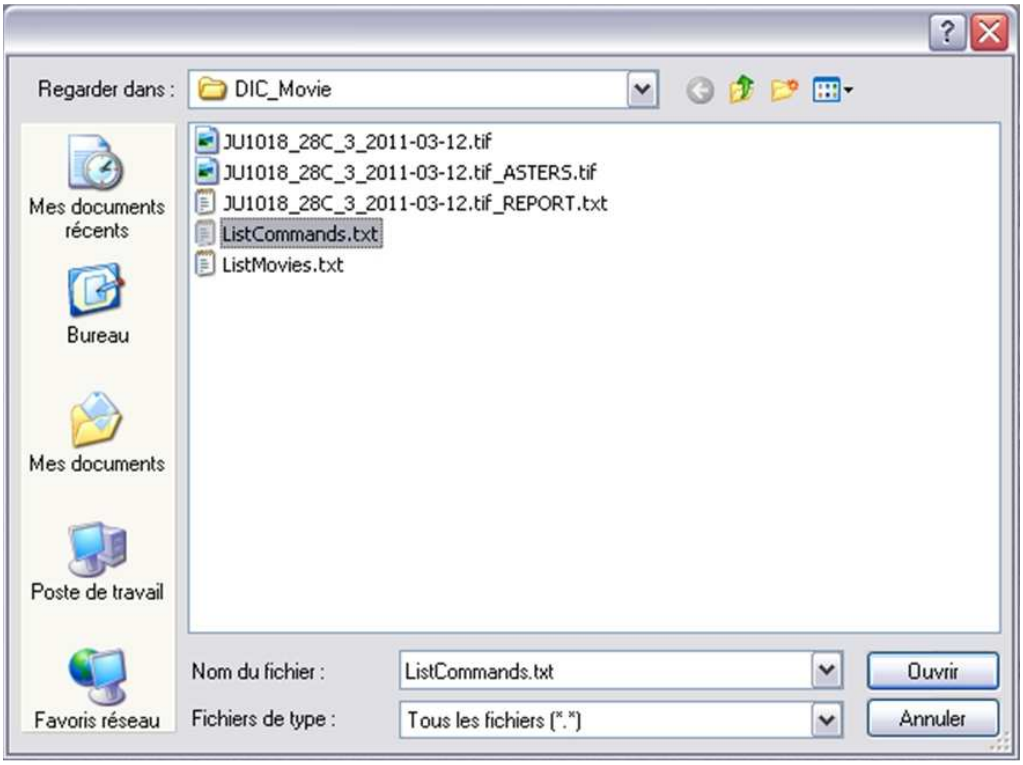
Step 1: Initiate the command line launcher



First **initiate** the **ACT command-lines launcher** from the **Plugins/Macros** menu.

Note: *In this submission mode the **program** will **not check** that all the movies present in a root folder have a **corresponding command line**.*

Step 2: Choice of the command line file

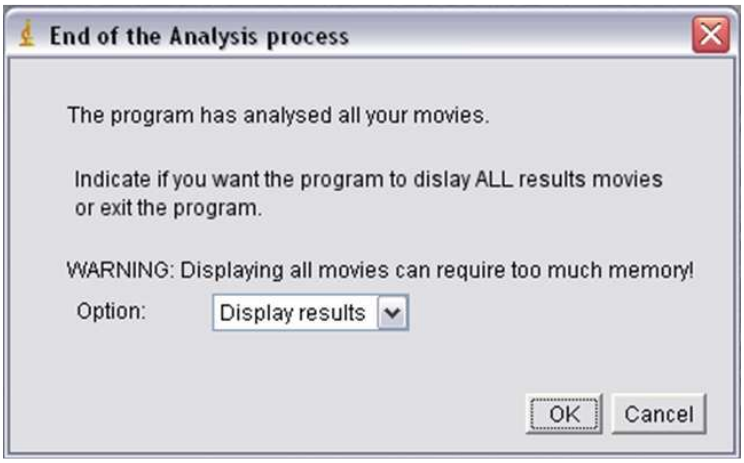


The program then **prompts** you to **specify** which command line **file to use.**

Once the file is **selected**, the automated **tracking immediately starts**.

Note: ***While*** *the command line file is a* ***.txt file*** *and respects the* ***structure*** *previously described, the* ***name*** *and/or* ***relative path*** *to the movies location has* ***no impact****. Indeed, the absolute path of each movie is specified in its command line.*

End of a job



Once all the t**rackings** have been **performed**, the program (if you use ACT and not ACT command line launcher) propose you to **display all generated** result **movies**.

Be **aware** that displaying these movies requires **tremendous usage** of your **computer memory**. Use it only for small batch (<10 movies).

In the list form, if you choose "**exit the macro**" and click on **Ok**, you will **quit the program**.

You can **access manually** to the **output files** (movie and report) generated. They are **saved** in the same **subfolde**r that the **original movie**.

Outputs

Files generated

For each **root folder** analyzed**, two files** are generated during the pre-treatment process:

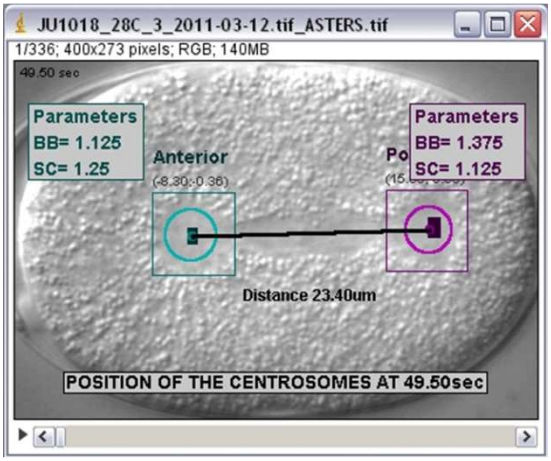
- "**ListMovies.txt**", which contains the path of all movies detected within the root folder with the correct extension.
- "**ListCommands.txt**", that is the command line file generated.

For each analyzed movies (here an example with only one movie, "JU1018_28C_2011-03-12.tif"), **two output files** are created with the **movie name as suffix**:

- A "**_ASTERS.tif**" **movie** which displays graphs of the trajectory of the centrosomes and presents frame by frame the tracking tools (scanning circle, boundary box and focus square) as the immediate detected position of the centrosomes.
- A "**_REPORT.txt**" **table** containing all the parameters of the tracking and the resulting coordinates of the centrosomes.

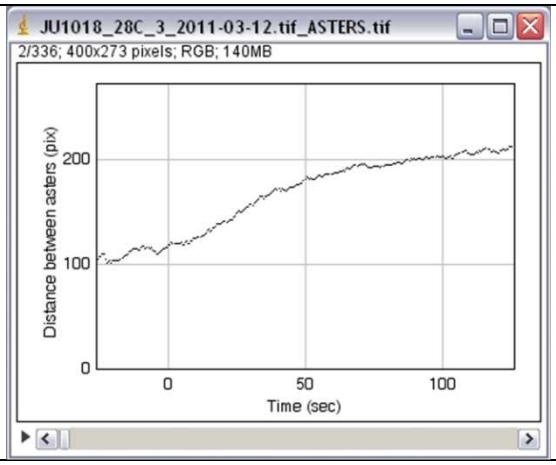
Overview of the output movie

Overview frame



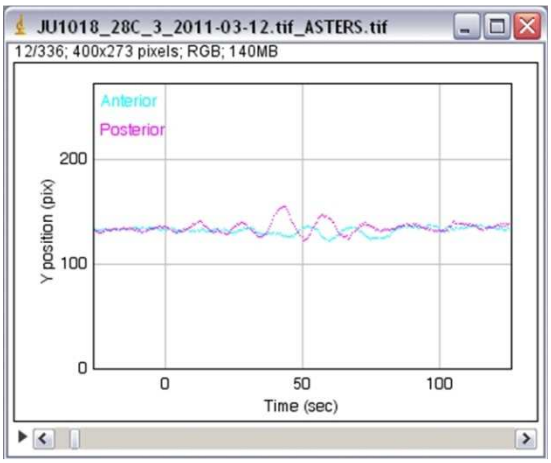
The **first frame** of the report movie **resumes** the **parameters** for both centrosomes tracking. Their **detected position** is presented for the **median frame** of the analyzed movie

Graph presenting the elongation of the spindle during the tracking



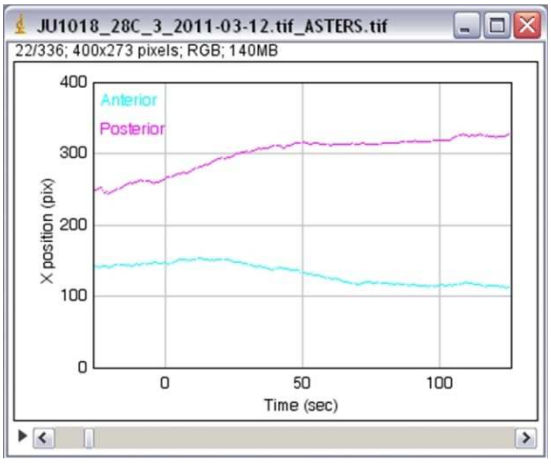
Next a **graph** presenting the **distance** (expressed in pixels) between the **centrosomes** as a **function** of **time** (t=0 being your reference frame) is displayed.

Graph presenting the evolution of the Y coordinate of the centrosome during the tracking



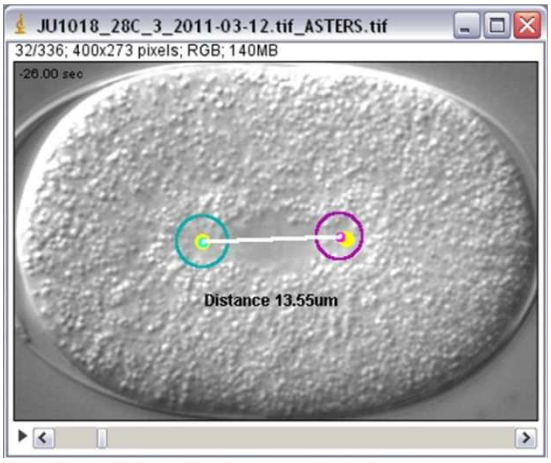
The next graph to be displayed presents you the **position** (in pixels) of the **centrosomes** on the **transversal axis** of the embryo.

Graph presenting the evolution of the X coordinate of the centrosome during the tracking



A similar graph is then displayed but presenting you the **position** (in pixels) of the **centrosomes** on the **antero-posterior axis** of the embryo.

First analyzed frame of the movie



The movie will the **display** all the **analyzed frames**.

The coordinates of the **detected centrosomes** are represented as **plain dark cyan** or **magenta circles** for the anterior and posterior centrosome respectively. The size of their respective **scanning circle** is represented with **empty circles** with the same color code

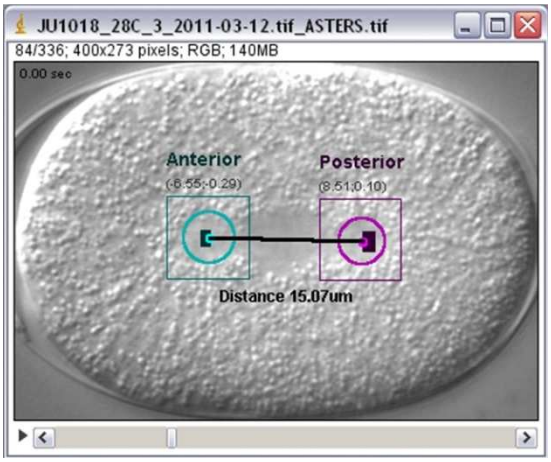
On the first one the **manually indicated position** of the centrosomes is represented as **yellow circles**.

For the **anterior** centrosome this position corresponds to its **initial tracking position**.

For the **posterior** centrosome, as the analysis has been performed in the backward position, the center of this yellow circle corresponds to the "**optimal**" position it **should have reached**.

Note that the **spindle elongation** is represented as a **line between** the two tracked positions of the **centrosomes**, with the length (in micrometer) displayed below. As the displayed frame is **before** the **reference** (t=0) one, this line is **white**. Moreover the **time** corresponding to the frame relatively to the reference one is presented in **seconds** on the **top left corne**r of the movie

Reference frame of the movie (t=0)

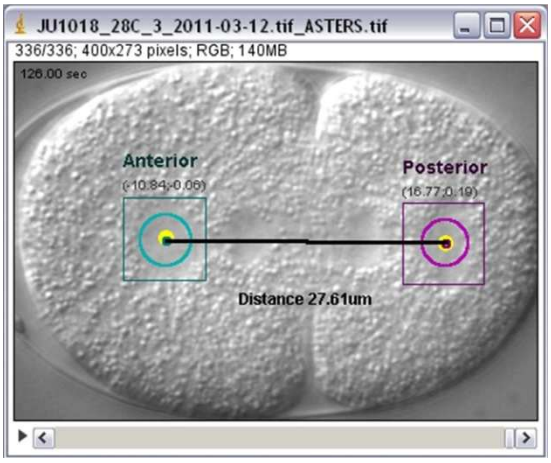


On the **subsequent frames**, the **focus square** (empty square), the **scanning circle** (empty circle), **boundary box** (filled rectangle), and **detected position** (filled circle) are represented with the same color code (cyan for the anterior and magenta for the anterior).

Moreover their **positions** (in micron) compared to the **center** of the **embryo** are **displayed** above the focus square.

For the **presented frame** we reached the **reference one**. Thus the line representing the splindle elongation is turning from **white to black**. So you easily know if you are before or after your t0 frame by looking to the color of the “spindle elongation line”.

Last frame of the analysis movie



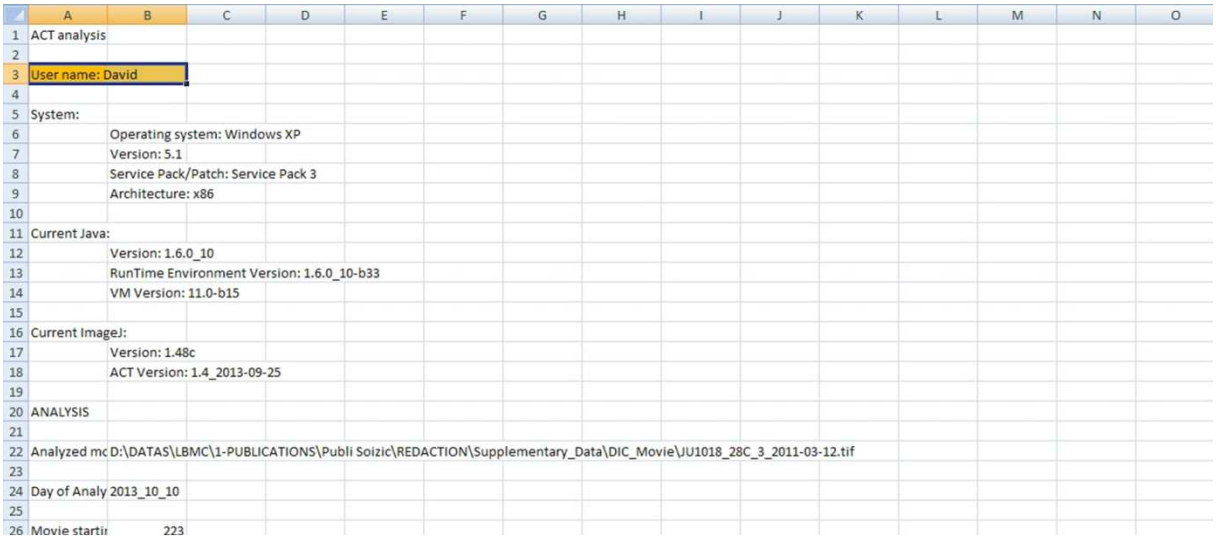
The **last frame** of the report movie presents the last **analyzed** frame. As for the first analyzed frame, the **manually** indicated **positions** of the centrosomes are represented in **yellow**.

For the **posterior** centrosome it is **initial** position for the tracking. For the **anterior** it represents the position it **should reach**.

Overview of the output txt table file.

The **output table file** has been developed to allow a **maximal** recording of the **process parameters** but permit to have the **position** of the **centrosomes formatted** for **direct use**.

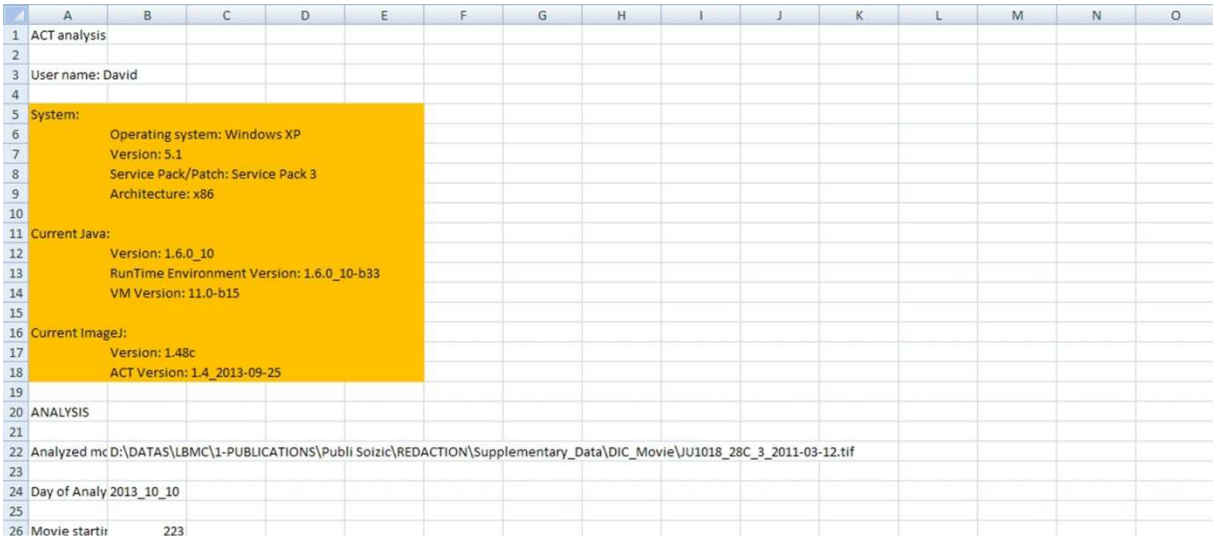
User name



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	ACT analysis														
2															
3	User name: David														
4															
5	System:														
6		Operating system: Windows XP													
7		Version: 5.1													
8		Service Pack/Patch: Service Pack 3													
9		Architecture: x86													
10															
11	Current Java:														
12		Version: 1.6.0_10													
13		RunTime Environment Version: 1.6.0_10-b33													
14		VM Version: 11.0-b15													
15															
16	Current ImageJ:														
17		Version: 1.48c													
18		ACT Version: 1.4_2013-09-25													
19															
20	ANALYSIS														
21															
22	Analyzed mc	D:\DATAS\LBMC\1-PUBLICATIONS\Publi Soizic\REDACTION\Supplementary_Data\DIC_Movie\JU1018_28C_3_2011-03-12.tif													
23															
24	Day of Analy	2013_10_10													
25															
26	Movie startir	223													

The first displayed information is the **user name** (i.e. name of the session used to perform the analysis).

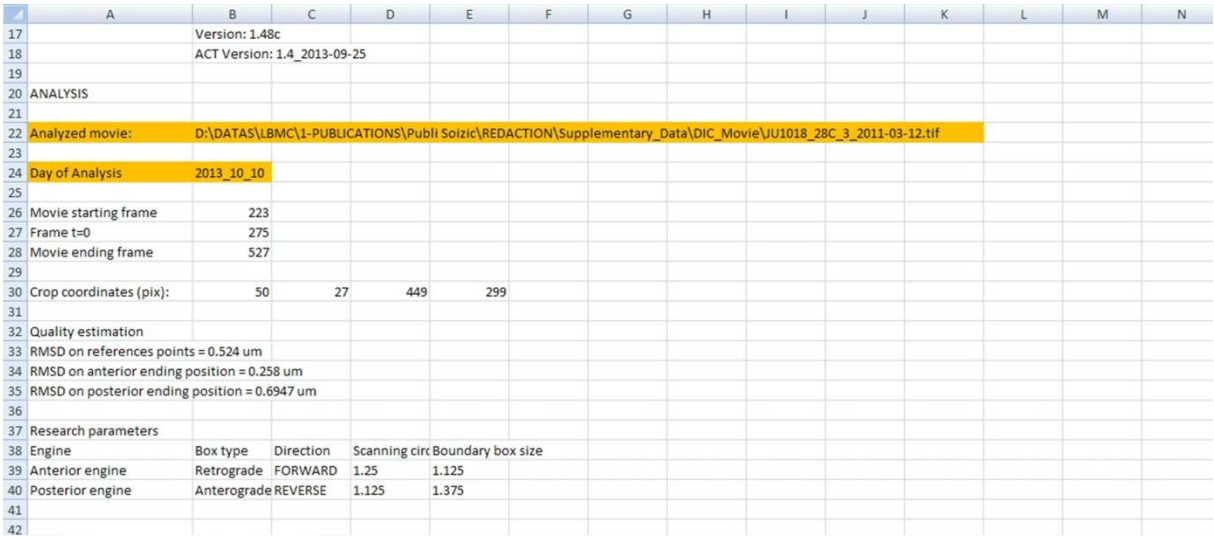
System configuration



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	ACT analysis														
2															
3	User name: David														
4															
5	System:														
6		Operating system: Windows XP													
7		Version: 5.1													
8		Service Pack/Patch: Service Pack 3													
9		Architecture: x86													
10															
11	Current Java:														
12		Version: 1.6.0_10													
13		RunTime Environment Version: 1.6.0_10-b33													
14		VM Version: 11.0-b15													
15															
16	Current ImageJ:														
17		Version: 1.48c													
18		ACT Version: 1.4_2013-09-25													
19															
20	ANALYSIS														
21															
22	Analyzed mc	D:\DATAS\LBMC\1-PUBLICATIONS\Publi Soizic\REDACTION\Supplementary_Data\DIC_Movie\JU1018_28C_3_2011-03-12.tif													
23															
24	Day of Analy	2013_10_10													
25															
26	Movie startir	223													

The **current configuration** (OS, Java, ImageJ and ACT version) of the computer used is then presented. This information can be used in case of abherent results to **determine** potential **divergence** between OS/Java/ImageJ **updates**.

Movie identification and date of automated tracking



	A	B	C	D	E	F	G	H	I	J	K	L	M	N
17		Version: 1.48c												
18		ACT Version: 1.4_2013-09-25												
19														
20	ANALYSIS													
21														
22	Analyzed movie:	D:\DATAS\LBMC\1-PUBLICATIONS\Publi Soizic\REDACTION\Supplementary_Data\DIC_Movie\JU1018_28C_3_2011-03-12.tif												
23														
24	Day of Analysis	2013_10_10												
25														
26	Movie starting frame	223												
27	Frame t=0	275												
28	Movie ending frame	527												
29														
30	Crop coordinates (pix):	50	27	449	299									
31														
32	Quality estimation													
33	RMSD on references points = 0.524 um													
34	RMSD on anterior ending position = 0.258 um													
35	RMSD on posterior ending position = 0.6947 um													
36														
37	Research parameters													
38	Engine	Box type	Direction	Scanning circ	Boundary box size									
39	Anterior engine	Retrograde	FORWARD	1.25	1.125									
40	Posterior engine	Anterograde	REVERSE	1.125	1.375									
41														
42														

The next fields are the **absolute path** of the analyzed movie and the **date** of the analysis.

Movie cutting datas

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
17		Version: 1.48c												
18		ACT Version: 1.4_2013-09-25												
19														
20	ANALYSIS													
21														
22	Analyzed movie:	D:\DATAS\LBMC\1-PUBLICATIONS\Publi Soizic\REDACTION\Supplementary_Data\DIC_Movie\JU1018_28C_3_2011-03-12.tif												
23														
24	Day of Analysis	2013_10_10												
25														
26	Movie starting frame	223												
27	Frame t=0	275												
28	Movie ending frame	527												
29														
30	Crop coordinates (pix):	50	27	449	299									
31														
32	Quality estimation													
33	RMSD on references points = 0.524 um													
34	RMSD on anterior ending position = 0.258 um													
35	RMSD on posterior ending position = 0.6947 um													
36														
37	Research parameters													
38	Engine	Box type	Direction	Scanning circ	Boundary box size									
39	Anterior engine	Retrograde	FORWARD	1.25	1.125									
40	Posterior engine	Anterograde	REVERSE	1.125	1.375									
41														
42														

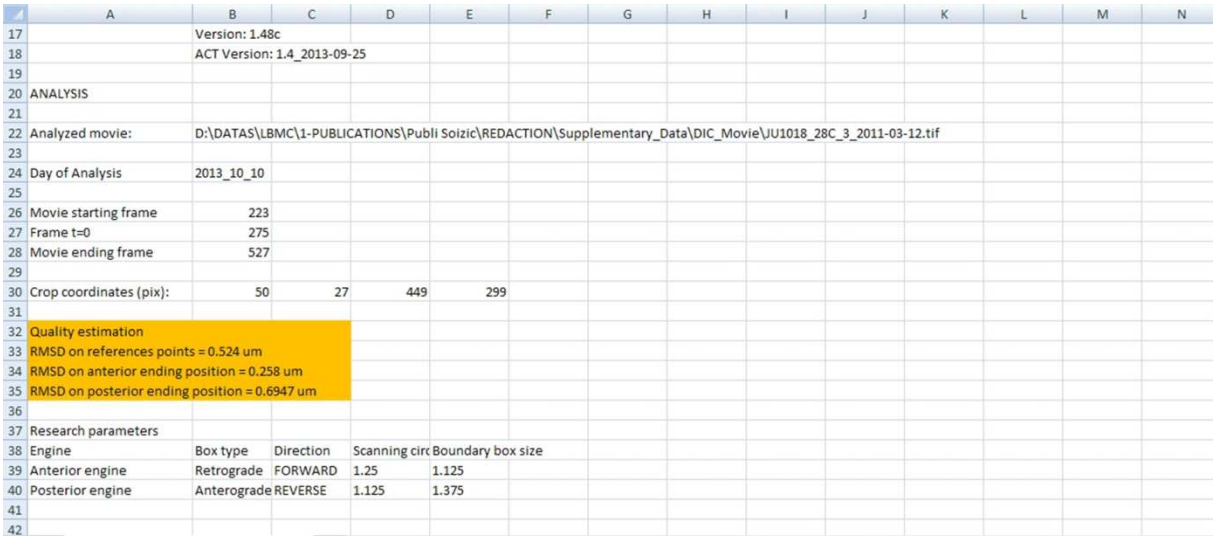
As a **reminder** the **starting**, **reference** and **ending** frame numbers of the analysis within the **original movie** are presented.

Coordinates of the rectangle fitting to the embryo

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
17		Version: 1.48c												
18		ACT Version: 1.4_2013-09-25												
19														
20	ANALYSIS													
21														
22	Analyzed movie:	D:\DATAS\LBMC\1-PUBLICATIONS\Publi Soizic\REDACTION\Supplementary_Data\DIC_Movie\JU1018_28C_3_2011-03-12.tif												
23														
24	Day of Analysis	2013_10_10												
25														
26	Movie starting frame	223												
27	Frame t=0	275												
28	Movie ending frame	527												
29														
30	Crop coordinates (pix):	50	27	449	299									
31														
32	Quality estimation													
33	RMSD on references points = 0.524 um													
34	RMSD on anterior ending position = 0.258 um													
35	RMSD on posterior ending position = 0.6947 um													
36														
37	Research parameters													
38	Engine	Box type	Direction	Scanning circ	Boundary box size									
39	Anterior engine	Retrograde	FORWARD	1.25	1.125									
40	Posterior engine	Anterograde	REVERSE	1.125	1.375									
41														
42														

The **upper left** (ul) and **lower right** (lr) corners of the **embryo**'s fitting **rectangle** are indicated as **absolute pixel coordinates** on the **original movie** (Xul, Yul, Xlr, Ylr).

Basic estimation of the quality of the automated tracking



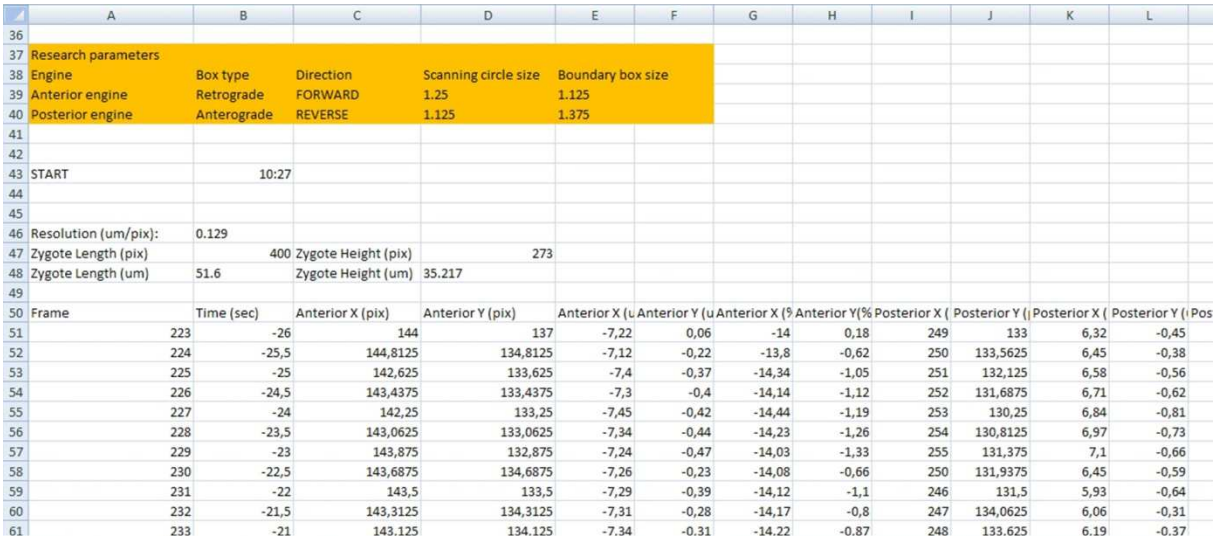
	A	B	C	D	E	F	G	H	I	J	K	L	M	N
17		Version: 1.48c												
18		ACT Version: 1.4_2013-09-25												
19														
20	ANALYSIS													
21														
22	Analyzed movie:	D:\DATAS\LBMC\1-PUBLICATIONS\Publi Soizic\REDACTION\Supplementary_Data\DIC_Movie\JU1018_28C_3_2011-03-12.tif												
23														
24	Day of Analysis	2013_10_10												
25														
26	Movie starting frame	223												
27	Frame t=0	275												
28	Movie ending frame	527												
29														
30	Crop coordinates (pix):	50	27	449	299									
31														
32	Quality estimation													
33	RMSD on references points = 0.524 um													
34	RMSD on anterior ending position = 0.258 um													
35	RMSD on posterior ending position = 0.6947 um													
36														
37	Research parameters													
38	Engine	Box type	Direction	Scanning circ	Boundary box size									
39	Anterior engine	Retrograde	FORWARD	1.25	1.125									
40	Posterior engine	Anterograde	REVERSE	1.125	1.375									
41														
42														

In order to help the user to determine the **quality** of the **analysis** this report indicate the **RMSD** (Root Mean Square Deviation) for **both** and **separated anterior** and **posterior** centrosomes at their **final tracked position** compared to the **manual** indicated ones.

The **lower** these values are, the **better** the final position is.

Note: *This quality evaluation has to be **associated** with a **visual inspection** of the report movie, as we observed in rare **loss and recatching** of one centrosome during the tracking (especially when the **focus is unstable**). Moreover if the **starting frame** is **too early**, the **posterior** centrosome can be too **deviated** in the **last couple of frames**, when the **majority** of the **tracking** process give **correct** positions.*

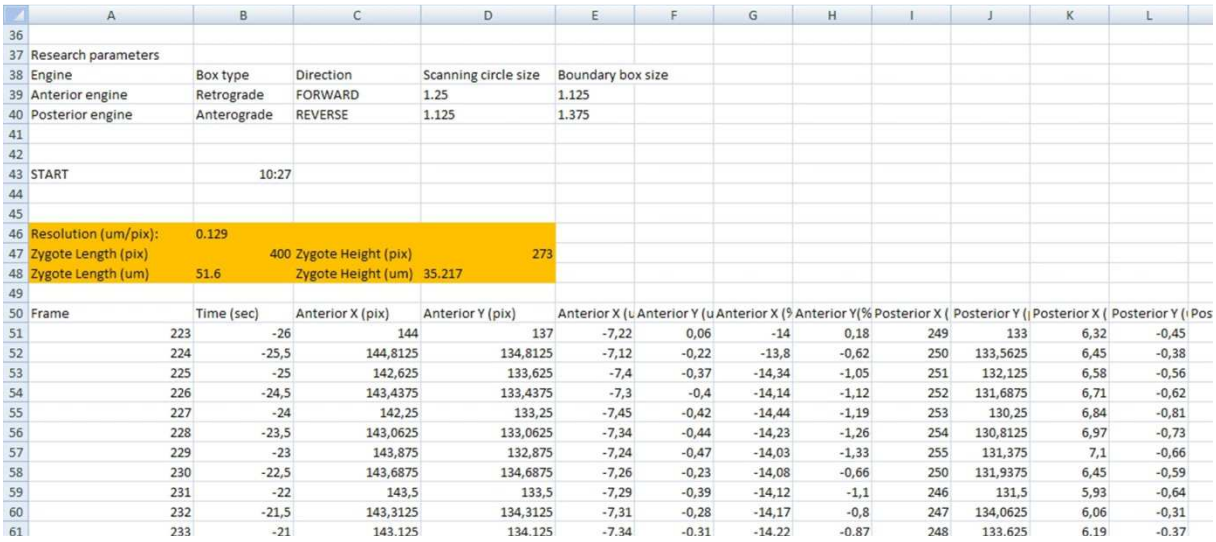
Tracking parameters



	A	B	C	D	E	F	G	H	I	J	K	L
36												
37	Research parameters											
38	Engine	Box type	Direction	Scanning circle size	Boundary box size							
39	Anterior engine	Retrograde	FORWARD	1.25	1.125							
40	Posterior engine	Anterograde	REVERSE	1.125	1.375							
41												
42												
43	START	10:27										
44												
45												
46	Resolution (um/pix):	0.129										
47	Zygote Length (pix)	400	Zygote Height (pix)	273								
48	Zygote Length (um)	51.6	Zygote Height (um)	35.217								
49												
50	Frame	Time (sec)	Anterior X (pix)	Anterior Y (pix)	Anterior X (u	Anterior Y (u	Anterior X (%	Anterior Y(%	Posterior X (	Posterior Y ((	Posterior X (	Posterior Y ((Pos
51	223	-26	144	137	-7,22	0,06	-14	0,18	249	133	6,32	-0,45
52	224	-25,5	144,8125	134,8125	-7,12	-0,22	-13,8	-0,62	250	133,5625	6,45	-0,38
53	225	-25	142,625	133,625	-7,4	-0,37	-14,34	-1,05	251	132,125	6,58	-0,56
54	226	-24,5	143,4375	133,4375	-7,3	-0,4	-14,14	-1,12	252	131,6875	6,71	-0,62
55	227	-24	142,25	133,25	-7,45	-0,42	-14,44	-1,19	253	130,25	6,84	-0,81
56	228	-23,5	143,0625	133,0625	-7,34	-0,44	-14,23	-1,26	254	130,8125	6,97	-0,73
57	229	-23	143,875	132,875	-7,24	-0,47	-14,03	-1,33	255	131,375	7,1	-0,66
58	230	-22,5	143,6875	134,6875	-7,26	-0,23	-14,08	-0,66	250	131,9375	6,45	-0,59
59	231	-22	143,5	133,5	-7,29	-0,39	-14,12	-1,1	246	131,5	5,93	-0,64
60	232	-21,5	143,3125	134,3125	-7,31	-0,28	-14,17	-0,8	247	134,0625	6,06	-0,31
61	233	-21	143.125	134.125	-7.34	-0.31	-14.22	-0.87	248	133.625	6.19	-0.37

The **parameters** used to **track** the two centrosomes are then indicated

Resolution of the movie and dimensions of the embryo



	A	B	C	D	E	F	G	H	I	J	K	L
36												
37	Research parameters											
38	Engine	Box type	Direction	Scanning circle size	Boundary box size							
39	Anterior engine	Retrograde	FORWARD	1.25	1.125							
40	Posterior engine	Anterograde	REVERSE	1.125	1.375							
41												
42												
43	START	10:27										
44												
45												
46	Resolution (um/pix):	0.129										
47	Zygote Length (pix)	400	Zygote Height (pix)	273								
48	Zygote Length (um)	51.6	Zygote Height (um)	35.217								
49												
50	Frame	Time (sec)	Anterior X (pix)	Anterior Y (pix)	Anterior X (u	Anterior Y (u	Anterior X (%	Anterior Y(%	Posterior X (	Posterior Y ((	Posterior X (	Posterior Y ((Pos
51	223	-26	144	137	-7,22	0,06	-14	0,18	249	133	6,32	-0,45
52	224	-25,5	144,8125	134,8125	-7,12	-0,22	-13,8	-0,62	250	133,5625	6,45	-0,38
53	225	-25	142,625	133,625	-7,4	-0,37	-14,34	-1,05	251	132,125	6,58	-0,56
54	226	-24,5	143,4375	133,4375	-7,3	-0,4	-14,14	-1,12	252	131,6875	6,71	-0,62
55	227	-24	142,25	133,25	-7,45	-0,42	-14,44	-1,19	253	130,25	6,84	-0,81
56	228	-23,5	143,0625	133,0625	-7,34	-0,44	-14,23	-1,26	254	130,8125	6,97	-0,73
57	229	-23	143,875	132,875	-7,24	-0,47	-14,03	-1,33	255	131,375	7,1	-0,66
58	230	-22,5	143,6875	134,6875	-7,26	-0,23	-14,08	-0,66	250	131,9375	6,45	-0,59
59	231	-22	143,5	133,5	-7,29	-0,39	-14,12	-1,1	246	131,5	5,93	-0,64
60	232	-21,5	143,3125	134,3125	-7,31	-0,28	-14,17	-0,8	247	134,0625	6,06	-0,31
61	233	-21	143.125	134.125	-7.34	-0.31	-14.22	-0.87	248	133.625	6.19	-0.37

The **pixel resolution** in **µm** and the **embryo's dimensions** are presented as the subsequent **coordinates** of the **centrosomes** are for some formats directly **calculated** from **these values**

Frame number and time value

	A	B	C	D	E	F	G	H	I	J	K	
50	Frame	Time (sec)	Anterior X (pix)	Anterior Y (pix)	Anterior X (um)	Anterior Y (um)	Anterior X (%)	Anterior Y(%)	Posterior X (pix)	Posterior Y (pix)	Posterior X (um)	Posterio
51	223	-26	144	137	-7,22	0,06	-14	0,18	249	133	6,32	
52	224	-25,5	144,8125	134,8125	-7,12	-0,22	-13,8	-0,62	250	133,5625	6,45	
53	225	-25	142,625	133,625	-7,4	-0,37	-14,34	-1,05	251	132,125	6,58	
54	226	-24,5	143,4375	133,4375	-7,3	-0,4	-14,14	-1,12	252	131,6875	6,71	
55	227	-24	142,25	133,25	-7,45	-0,42	-14,44	-1,19	253	130,25	6,84	
56	228	-23,5	143,0625	133,0625	-7,34	-0,44	-14,23	-1,26	254	130,8125	6,97	
57	229	-23	143,875	132,875	-7,24	-0,47	-14,03	-1,33	255	131,375	7,1	
58	230	-22,5	143,6875	134,6875	-7,26	-0,23	-14,08	-0,66	250	131,9375	6,45	
59	231	-22	143,5	133,5	-7,29	-0,39	-14,12	-1,1	246	131,5	5,93	
60	232	-21,5	143,3125	134,3125	-7,31	-0,28	-14,17	-0,8	247	134,0625	6,06	
61	233	-21	143,125	134,125	-7,34	-0,31	-14,22	-0,87	248	133,625	6,19	
62	234	-20,5	142,9375	133,9375	-7,36	-0,33	-14,27	-0,94	245	133,1875	5,8	
63	235	-20	141,75	134,75	-7,51	-0,23	-14,56	-0,64	246	132,75	5,93	
64	236	-19,5	142,5625	132,5625	-7,41	-0,51	-14,36	-1,44	247	133,3125	6,06	
65	237	-19	143,375	134,375	-7,3	-0,27	-14,16	-0,78	248	132,875	6,19	
66	238	-18,5	144,1875	135,1875	-7,2	-0,17	-13,95	-0,48	249	134,4375	6,32	
67	239	-18	145	135	-7,1	-0,19	-13,75	-0,55	250	134	6,45	
68	240	-17,5	145,8125	133,8125	-6,99	-0,35	-13,55	-0,98	251	134,5625	6,58	
69	241	-17	145,625	133,625	-7,01	-0,37	-13,59	-1,05	252	134,125	6,71	
70	242	-16,5	145,4375	134,4375	-7,04	-0,27	-13,64	-0,76	253	133,6875	6,84	
71	243	-16	145,25	134,25	-7,06	-0,29	-13,69	-0,82	254	134,25	6,97	
72	244	-15,5	145,0625	135,0625	-7,09	-0,19	-13,73	-0,53	255	134,8125	7,1	
73	245	-15	145,875	134,875	-6,98	-0,21	-13,53	-0,6	256	135,375	7,22	
74	246	-14,5	146,6875	134,6875	-6,88	-0,23	-13,33	-0,66	257	134,9375	7,35	
75	247	-14	144,5	133,5	-7,16	-0,39	-13,88	-1,1	258	134,5	7,48	

The **coordinates** of the **centrosomes** are presented as **function** of two parameters. The first one is the **absolute frame number** in the **original movie** (column A). The second is the **time** in seconds (column B) as calculated using the reference frame number as t=0.

Absolute position of the anterior centrosome

	A	B	C	D	E	F	G	H	I	J	K	
50	Frame	Time (sec)	Anterior X (pix)	Anterior Y (pix)	Anterior X (um)	Anterior Y (um)	Anterior X (%)	Anterior Y(%)	Posterior X (pix)	Posterior Y (pix)	Posterior X (um)	Posterio
51	223	-26	144	137	-7,22	0,06	-14	0,18	249	133	6,32	
52	224	-25,5	144,8125	134,8125	-7,12	-0,22	-13,8	-0,62	250	133,5625	6,45	
53	225	-25	142,625	133,625	-7,4	-0,37	-14,34	-1,05	251	132,125	6,58	
54	226	-24,5	143,4375	133,4375	-7,3	-0,4	-14,14	-1,12	252	131,6875	6,71	
55	227	-24	142,25	133,25	-7,45	-0,42	-14,44	-1,19	253	130,25	6,84	
56	228	-23,5	143,0625	133,0625	-7,34	-0,44	-14,23	-1,26	254	130,8125	6,97	
57	229	-23	143,875	132,875	-7,24	-0,47	-14,03	-1,33	255	131,375	7,1	
58	230	-22,5	143,6875	134,6875	-7,26	-0,23	-14,08	-0,66	250	131,9375	6,45	
59	231	-22	143,5	133,5	-7,29	-0,39	-14,12	-1,1	246	131,5	5,93	
60	232	-21,5	143,3125	134,3125	-7,31	-0,28	-14,17	-0,8	247	134,0625	6,06	
61	233	-21	143,125	134,125	-7,34	-0,31	-14,22	-0,87	248	133,625	6,19	
62	234	-20,5	142,9375	133,9375	-7,36	-0,33	-14,27	-0,94	245	133,1875	5,8	
63	235	-20	141,75	134,75	-7,51	-0,23	-14,56	-0,64	246	132,75	5,93	
64	236	-19,5	142,5625	132,5625	-7,41	-0,51	-14,36	-1,44	247	133,3125	6,06	
65	237	-19	143,375	134,375	-7,3	-0,27	-14,16	-0,78	248	132,875	6,19	
66	238	-18,5	144,1875	135,1875	-7,2	-0,17	-13,95	-0,48	249	134,4375	6,32	
67	239	-18	145	135	-7,1	-0,19	-13,75	-0,55	250	134	6,45	
68	240	-17,5	145,8125	133,8125	-6,99	-0,35	-13,55	-0,98	251	134,5625	6,58	
69	241	-17	145,625	133,625	-7,01	-0,37	-13,59	-1,05	252	134,125	6,71	
70	242	-16,5	145,4375	134,4375	-7,04	-0,27	-13,64	-0,76	253	133,6875	6,84	
71	243	-16	145,25	134,25	-7,06	-0,29	-13,69	-0,82	254	134,25	6,97	
72	244	-15,5	145,0625	135,0625	-7,09	-0,19	-13,73	-0,53	255	134,8125	7,1	
73	245	-15	145,875	134,875	-6,98	-0,21	-13,53	-0,6	256	135,375	7,22	
74	246	-14,5	146,6875	134,6875	-6,88	-0,23	-13,33	-0,66	257	134,9375	7,35	
75	247	-14	144,5	133,5	-7,16	-0,39	-13,88	-1,1	258	134,5	7,48	

The position of the **anterior centrosome** expressed as **absolute pixel** coordinates **within** the embryo's **fitting rectangle** are presented column **C** (X) and **D** (Y). The same values for the posterior can be found in columns I and J.

Position of the anterior centrosome in µm

	A	B	C	D	E	F	G	H	I	J	K	
50	Frame	Time (sec)	Anterior X (pix)	Anterior Y (pix)	Anterior X (um)	Anterior Y (um)	Anterior X (%)	Anterior Y(%)	Posterior X (pix)	Posterior Y (pix)	Posterior X (um)	Posterio
51	223	-26	144	137	-7,22	0,06	-14	0,18	249	133	6,32	
52	224	-25,5	144,8125	134,8125	-7,12	-0,22	-13,8	-0,62	250	133,5625	6,45	
53	225	-25	142,625	133,625	-7,4	-0,37	-14,34	-1,05	251	132,125	6,58	
54	226	-24,5	143,4375	133,4375	-7,3	-0,4	-14,14	-1,12	252	131,6875	6,71	
55	227	-24	142,25	133,25	-7,45	-0,42	-14,44	-1,19	253	130,25	6,84	
56	228	-23,5	143,0625	133,0625	-7,34	-0,44	-14,23	-1,26	254	130,8125	6,97	
57	229	-23	143,875	132,875	-7,24	-0,47	-14,03	-1,33	255	131,375	7,1	
58	230	-22,5	143,6875	134,6875	-7,26	-0,23	-14,08	-0,66	250	131,9375	6,45	
59	231	-22	143,5	133,5	-7,29	-0,39	-14,12	-1,1	246	131,5	5,93	
60	232	-21,5	143,3125	134,3125	-7,31	-0,28	-14,17	-0,8	247	134,0625	6,06	
61	233	-21	143,125	134,125	-7,34	-0,31	-14,22	-0,87	248	133,625	6,19	
62	234	-20,5	142,9375	133,9375	-7,36	-0,33	-14,27	-0,94	245	133,1875	5,8	
63	235	-20	141,75	134,75	-7,51	-0,23	-14,56	-0,64	246	132,75	5,93	
64	236	-19,5	142,5625	132,5625	-7,41	-0,51	-14,36	-1,44	247	133,3125	6,06	
65	237	-19	143,375	134,375	-7,3	-0,27	-14,16	-0,78	248	132,875	6,19	
66	238	-18,5	144,1875	135,1875	-7,2	-0,17	-13,95	-0,48	249	134,4375	6,32	
67	239	-18	145	135	-7,1	-0,19	-13,75	-0,55	250	134	6,45	
68	240	-17,5	145,8125	133,8125	-6,99	-0,35	-13,55	-0,98	251	134,5625	6,58	
69	241	-17	145,625	133,625	-7,01	-0,37	-13,59	-1,05	252	134,125	6,71	
70	242	-16,5	145,4375	134,4375	-7,04	-0,27	-13,64	-0,76	253	133,6875	6,84	
71	243	-16	145,25	134,25	-7,06	-0,29	-13,69	-0,82	254	134,25	6,97	
72	244	-15,5	145,0625	135,0625	-7,09	-0,19	-13,73	-0,53	255	134,8125	7,1	
73	245	-15	145,875	134,875	-6,98	-0,21	-13,53	-0,6	256	135,375	7,22	
74	246	-14,5	146,6875	134,6875	-6,88	-0,23	-13,33	-0,66	257	134,9375	7,35	
75	247	-14	144.5	133.5	-7.16	-0.39	-13.88	-1.1	258	134.5	7.48	

The position of the **anterior centrosome** expressed as **distance** (in **µm**) from the **center** of the **embryo** are presented column **E** (X) and **F** (Y). The same values for the posterior can be found in columns K and L.

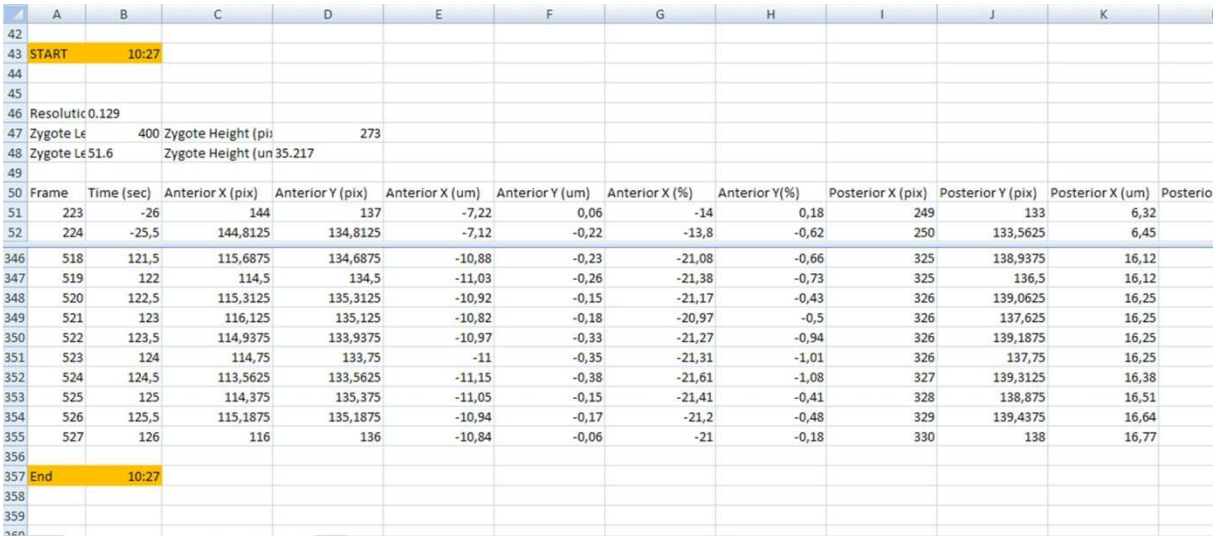
Position of the anterior centrosome expressed as %

	A	B	C	D	E	F	G	H	I	J	K	
50	Frame	Time (sec)	Anterior X (pix)	Anterior Y (pix)	Anterior X (um)	Anterior Y (um)	Anterior X (%)	Anterior Y(%)	Posterior X (pix)	Posterior Y (pix)	Posterior X (um)	Posterio
51	223	-26	144	137	-7,22	0,06	-14	0,18	249	133	6,32	
52	224	-25,5	144,8125	134,8125	-7,12	-0,22	-13,8	-0,62	250	133,5625	6,45	
53	225	-25	142,625	133,625	-7,4	-0,37	-14,34	-1,05	251	132,125	6,58	
54	226	-24,5	143,4375	133,4375	-7,3	-0,4	-14,14	-1,12	252	131,6875	6,71	
55	227	-24	142,25	133,25	-7,45	-0,42	-14,44	-1,19	253	130,25	6,84	
56	228	-23,5	143,0625	133,0625	-7,34	-0,44	-14,23	-1,26	254	130,8125	6,97	
57	229	-23	143,875	132,875	-7,24	-0,47	-14,03	-1,33	255	131,375	7,1	
58	230	-22,5	143,6875	134,6875	-7,26	-0,23	-14,08	-0,66	250	131,9375	6,45	
59	231	-22	143,5	133,5	-7,29	-0,39	-14,12	-1,1	246	131,5	5,93	
60	232	-21,5	143,3125	134,3125	-7,31	-0,28	-14,17	-0,8	247	134,0625	6,06	
61	233	-21	143,125	134,125	-7,34	-0,31	-14,22	-0,87	248	133,625	6,19	
62	234	-20,5	142,9375	133,9375	-7,36	-0,33	-14,27	-0,94	245	133,1875	5,8	
63	235	-20	141,75	134,75	-7,51	-0,23	-14,56	-0,64	246	132,75	5,93	
64	236	-19,5	142,5625	132,5625	-7,41	-0,51	-14,36	-1,44	247	133,3125	6,06	
65	237	-19	143,375	134,375	-7,3	-0,27	-14,16	-0,78	248	132,875	6,19	
66	238	-18,5	144,1875	135,1875	-7,2	-0,17	-13,95	-0,48	249	134,4375	6,32	
67	239	-18	145	135	-7,1	-0,19	-13,75	-0,55	250	134	6,45	
68	240	-17,5	145,8125	133,8125	-6,99	-0,35	-13,55	-0,98	251	134,5625	6,58	
69	241	-17	145,625	133,625	-7,01	-0,37	-13,59	-1,05	252	134,125	6,71	
70	242	-16,5	145,4375	134,4375	-7,04	-0,27	-13,64	-0,76	253	133,6875	6,84	
71	243	-16	145,25	134,25	-7,06	-0,29	-13,69	-0,82	254	134,25	6,97	
72	244	-15,5	145,0625	135,0625	-7,09	-0,19	-13,73	-0,53	255	134,8125	7,1	
73	245	-15	145,875	134,875	-6,98	-0,21	-13,53	-0,6	256	135,375	7,22	
74	246	-14,5	146,6875	134,6875	-6,88	-0,23	-13,33	-0,66	257	134,9375	7,35	
75	247	-14	144.5	133.5	-7.16	-0.39	-13.88	-1.1	258	134.5	7.48	

The position of the **anterior centrosome** expressed as **distance** (in **%** of **width** or **height**) from the **center** of the **embryo** are presented column **G** (X) and **H** (Y). The same values for the posterior can be found in columns M and N.

Note: *The **spindle elongation** expressed in **pixels**, **µm** or **%** of the width of the embryo can be found in the columns **O**, **P** and **Q** respectively.*

Starting and ending time of the entire tracking process



	A	B	C	D	E	F	G	H	I	J	K
42											
43	START	10:27									
44											
45											
46	Resolutic	0.129									
47	Zygote Le	400	Zygote Height (pi	273							
48	Zygote Le	51.6	Zygote Height (un	35.217							
49											
50	Frame	Time (sec)	Anterior X (pix)	Anterior Y (pix)	Anterior X (um)	Anterior Y (um)	Anterior X (%)	Anterior Y(%)	Posterior X (pix)	Posterior Y (pix)	Posterior X (um)
51	223	-26	144	137	-7,22	0,06	-14	0,18	249	133	6,32
52	224	-25,5	144,8125	134,8125	-7,12	-0,22	-13,8	-0,62	250	133,5625	6,45
346	518	121,5	115,6875	134,6875	-10,88	-0,23	-21,08	-0,66	325	138,9375	16,12
347	519	122	114,5	134,5	-11,03	-0,26	-21,38	-0,73	325	136,5	16,12
348	520	122,5	115,3125	135,3125	-10,92	-0,15	-21,17	-0,43	326	139,0625	16,25
349	521	123	116,125	135,125	-10,82	-0,18	-20,97	-0,5	326	137,625	16,25
350	522	123,5	114,9375	133,9375	-10,97	-0,33	-21,27	-0,94	326	139,1875	16,25
351	523	124	114,75	133,75	-11	-0,35	-21,31	-1,01	326	137,75	16,25
352	524	124,5	113,5625	133,5625	-11,15	-0,38	-21,61	-1,08	327	139,3125	16,38
353	525	125	114,375	135,375	-11,05	-0,15	-21,41	-0,41	328	138,875	16,51
354	526	125,5	115,1875	135,1875	-10,94	-0,17	-21,2	-0,48	329	139,4375	16,64
355	527	126	116	136	-10,84	-0,06	-21	-0,18	330	138	16,77
356											
357	End	10:27									
358											
359											

Finally, the **times** in hour and minute when the **analysis started** and **finished** are indicated before and after the table of coordinates (lines 43 and here 357 respectively)