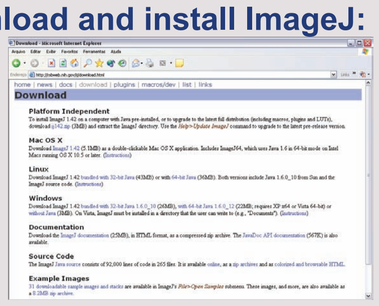
LEVAN Tutorial - Step by Step

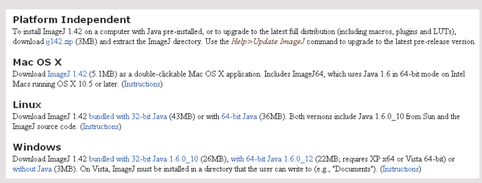
Morphological criteria of the plugin

Arm Ratio	Levan et al. (1964)	Green & Sessions (1991)	Levan plugin
1.0	M (Metacentric)	m (Metacentric)	m (Metacentric)
1.0 ≤ x < 1.7	m (Metacentric)		
1.7 ≤ x < 3.0	sm (Submetacentric)	sm (Submetacentric)	sm (Submetacentric)
3.0 ≤ x < 7.0	st (Subtelocentric)	st (Subtelocentric)	A (Acrocentric)
7.0 ≤ x < ∞	t (Acrocentric)	t (Telocentric)	t (Telocentric)
∞	T (Telocentric)		

1 Download and install ImageJ:

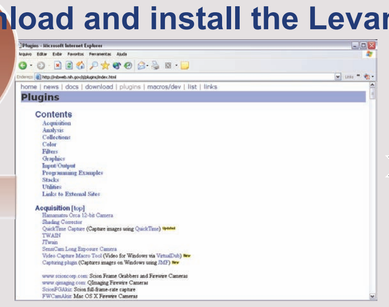


Download the ImageJ setup to your platform in the http://rsbweb.nih.gov/ij/download

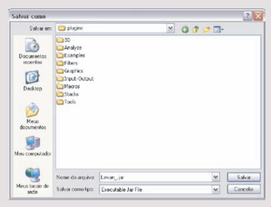


Each platform have a link for instructions to install the ImageJ.

2 Download and install the Levan plugin:

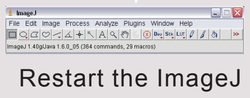


Download the Levan_.jar file in http://rsbweb.nih.gov/ij/plugins/index.html

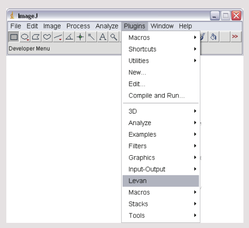


Save the Levan_.jar file in the ImageJ/Plugins folder.

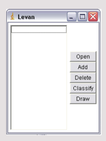
3 Open Levan:



Restart the ImageJ

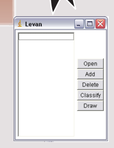


In the menu bar, click on Plugins>Levan.

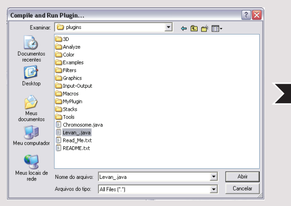


The Levan plugin window will open.

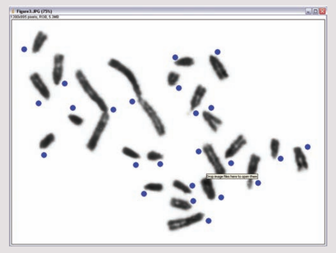
4 Open an image using Levan:



Click the "Open" button.

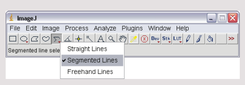


Browse and double-click the image you want to work with.

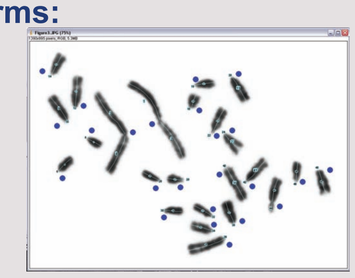


The image will open in a new window.

5 Trace the chromosome arms:



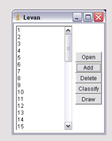
Choose a line selection tool in the ImageJ tool bar.



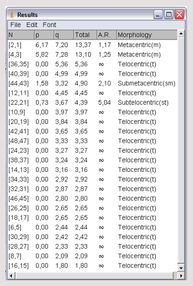
With the mouse, trace the arms of each chromosome sequentially.

If you used the freehand or the straight line selection tool, the arms will be automatically added to the Levan list of chromosome arms. Otherwise, to add chromosome arms using the segmented line selection tool, just use the "Add" button or the "U" key.

6 View the chromosome morphology:

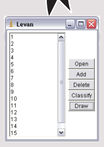


Click the "Classify" button.

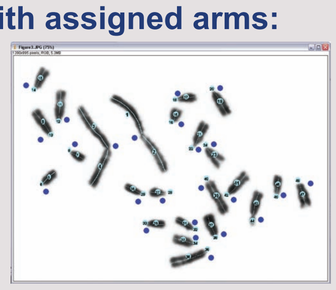


A "Results" window will open with the morphology information of each chromosome.

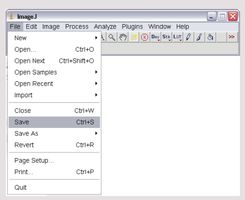
7 Save the image with assigned arms:



Click the "Draw" button.

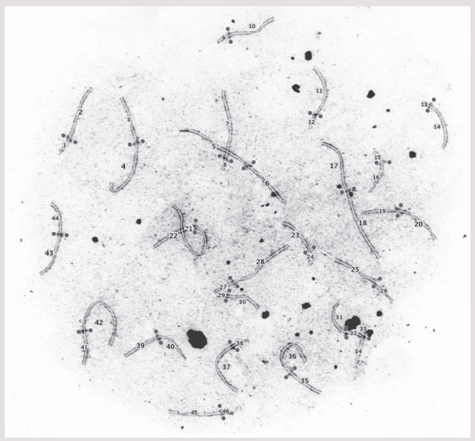


The sequential number of arms will be drawn on the image of each chromosome.



In the ImageJ menu bar, click on File>Save or File>Save As.

An example of a phachytene nuclei with 23 bivalents and the table "Results" showing the morphology of the chromosomes of each bivalent.



Results

N	p	q	Total	A.R.	Morphology
[22,21]	2.48	5.13	7.61	2.07	Submetacentric(sm)
[17,18]	3.15	4.07	7.22	1.29	Metacentric(m)
[6,5]	2.80	4.27	7.06	1.53	Metacentric(m)
[41,42]	1.80	4.74	6.54	2.63	Submetacentric(sm)
[3,4]	2.60	3.68	6.28	1.41	Metacentric(m)
[35,36]	1.91	3.08	4.99	1.61	Metacentric(m)
[27,28]	1.11	3.60	4.72	3.23	Subtelocentric(st)
[19,20]	2.26	2.31	4.57	1.02	Metacentric(m)
[8,7]	0.65	3.65	4.30	5.65	Subtelocentric(st)
[44,43]	1.82	2.45	4.28	1.35	Metacentric(m)
[38,37]	0.95	3.24	4.18	3.42	Subtelocentric(st)
[40,39]	2.02	2.03	4.05	1.01	Metacentric(m)
[26,25]	1.40	2.50	3.90	1.78	Submetacentric(sm)
[1,2]	0.90	2.97	3.87	3.28	Subtelocentric(st)
[12,11]	0.82	2.82	3.64	3.44	Subtelocentric(st)
[46,45]	0.60	2.90	3.50	4.88	Subtelocentric(st)
[9,10]	0.78	2.63	3.41	3.39	Subtelocentric(st)
[13,14]	0.40	2.64	3.04	6.59	Subtelocentric(st)
[24,23]	0.91	2.09	3.00	2.30	Submetacentric(sm)
[32,31]	0.79	1.84	2.63	2.35	Submetacentric(sm)
[15,16]	0.72	1.71	2.43	2.39	Submetacentric(sm)
[29,30]	0.48	1.93	2.42	3.99	Subtelocentric(st)
[33,34]	0.59	1.78	2.38	3.00	Subtelocentric(st)

Tips to use the Levan plugin:

a) remember that Levan uses the ImageJ original tools ("Selection Lines"); they have specific instructions, which can be viewed in the ImageJ manual;
b) for complex curved or bend lines, such as observed in a synaptonemal complex structure or axial elements of pachytene nuclei, we recomend the use of the "Segmented Line" tool; this tool allows the user to easily redefine traced lines;
c) for straight lines, such as observed in mitotic metaphase chromosomes, use the "Straight Line" tool; in this case, the lines are automatically added without the use of the "Add" button.
d) depending on the user skill to work with the mouse, use the "Freehand" tool; in this case, the lines are automatically added without the use of the "Add" button.
e) always remember that each chromosome should have two inputs in the plugin; in the case of very small-sized arms, always consider it with one click ("Freehand" or "Straight Line" tool) or one click followed with an "Add" ("Segmented Line" tool);
f) the plugin can be used to rank the total relative length of the morphological units of monocentric chromosomes without visible centromeres or holocentric chromosomes; in both cases, the user should desconsider the presented morphology classification.

Sakamoto[1, 2], Y. & Zacaro[2], A. A. (2009)

[2]Universidade Federal de Viçosa
Departamento de Biologia Geral - CCBS
Viçosa - MG - Brasil

[1]PIBIC/CNPQ Fellowship

For contact, please e-mail: ***levanplugin@ufv.br***

Citation

If you have decided to consider the use of this plugin in your work, please, do not forget to cite both ImageJ and the plugin LEVAN.
You may follow these citation examples for the LEVAN plugin:

a) text citation: Sakamoto & Zacaro (2009) or (Sakamoto & Zacaro, 2009);

b) reference citation: Sakamoto, Y. and Zacaro, A. A. 2009. LEVAN, an ImajeJ plugin for morphological cytogenetic analysis of mitotic and meiotic chromosomes. Initial version. An open source Java plugin distributed over the Internet from http://rsbweb.nih.gov/ij/

Examples for ImageJ citation can be found in http://rsbweb.nih.gov/ij/docs/faqs.html