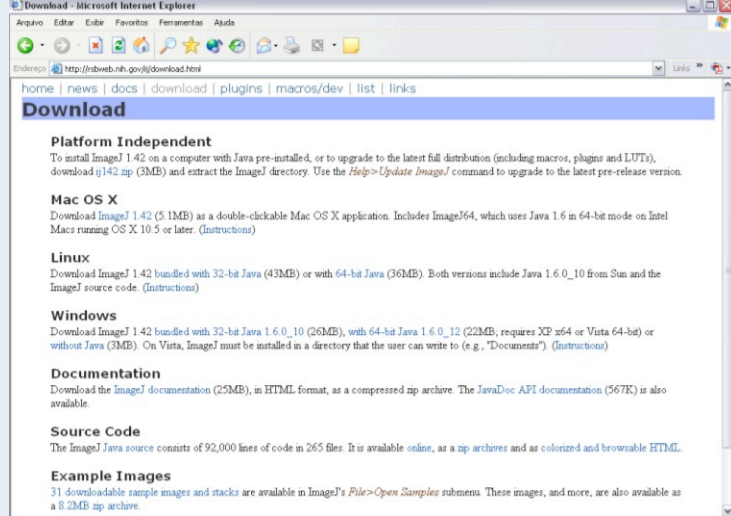


Levan Tutorial - Step by Step

Morphological criteria of the plugin

Arm Ratio	Levan et al. (1964)	Green & Sessions (1991)	Levan plugin
1.0	M (Metacentric)	m (Metacentric)	m (Metacentric)
$1.0 \leq x < 1.7$	m (Metacentric)		
$1.7 \leq x < 3.0$	sm (Submetacentric)	sm (Submetacentric)	sm (Submetacentric)
$3.0 \leq x < 7.0$	st (Subtelocentric)	st (Subtelocentric)	A (Acrocentric)
$7.0 \leq x < \infty$	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>

Platform Independent
To install ImageJ 1.42 on a computer with Java pre-installed, or to upgrade to the latest full distribution (including macros, plugins and LUTs), download the 1.42 zip (2MB) and extract the ImageJ directory. Use the [http://rsbweb.nih.gov/ij/download](#) to upgrade to the latest pre-release version.

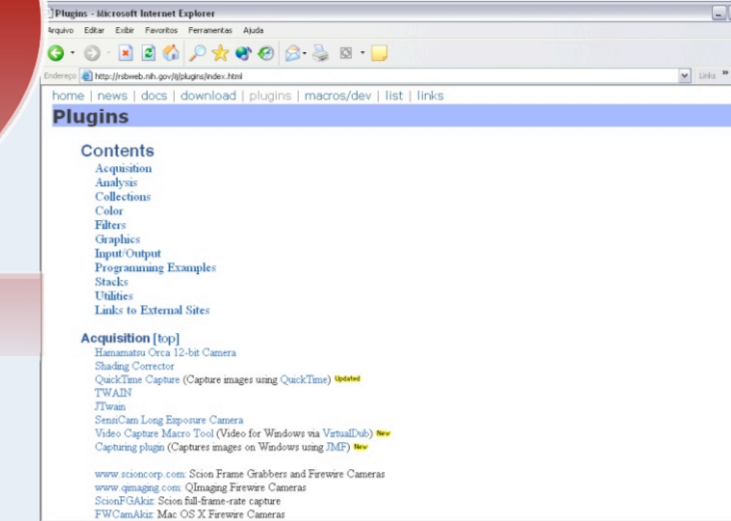
Mac OS X
Download ImageJ 1.42 (5.1MB) as a double-clickable Mac OS X application. Includes ImageJ64, which uses Java 1.6 in 64-bit mode on Intel Macs running OS X 10.5 or later. (Instructions)

Linux
Download ImageJ 1.42 bundled with 32-bit Java (43MB) or with 64-bit Java (93MB). Both versions include Java 1.6.0_30 from Sun and the ImageJ source code. (Instructions)

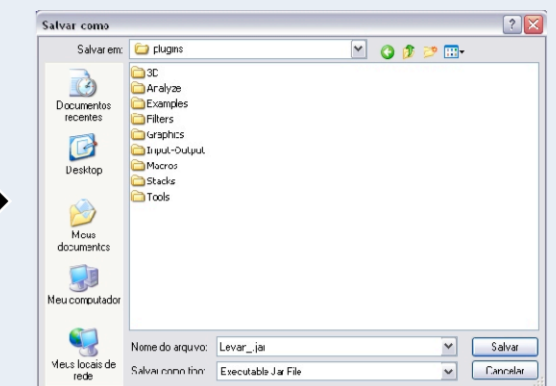
Windows
Download ImageJ 1.42 bundled with 32-bit Java 1.6.0_30 (20MB), with 64-bit Java 1.6.0_30 (22MB), requires XP SP3 or Vista SP1 or Windows 7 (32-bit or 64-bit). On Vista, ImageJ must be installed in a directory that the user can write to (e.g., "Documents"). (Instructions)

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

2 Download and install the Levan plugin:

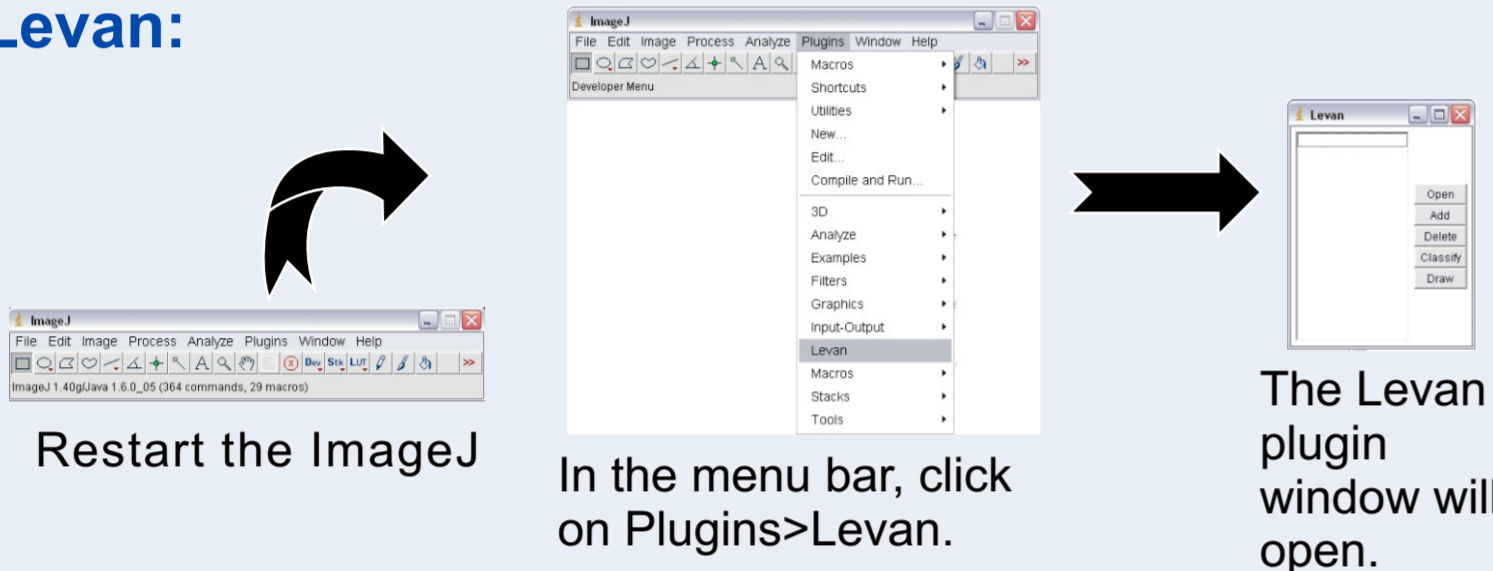


Download the Levan_.jar file in <http://rsbweb.nih.gov/ij/plugins/index.html>



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

3 Open Levan:

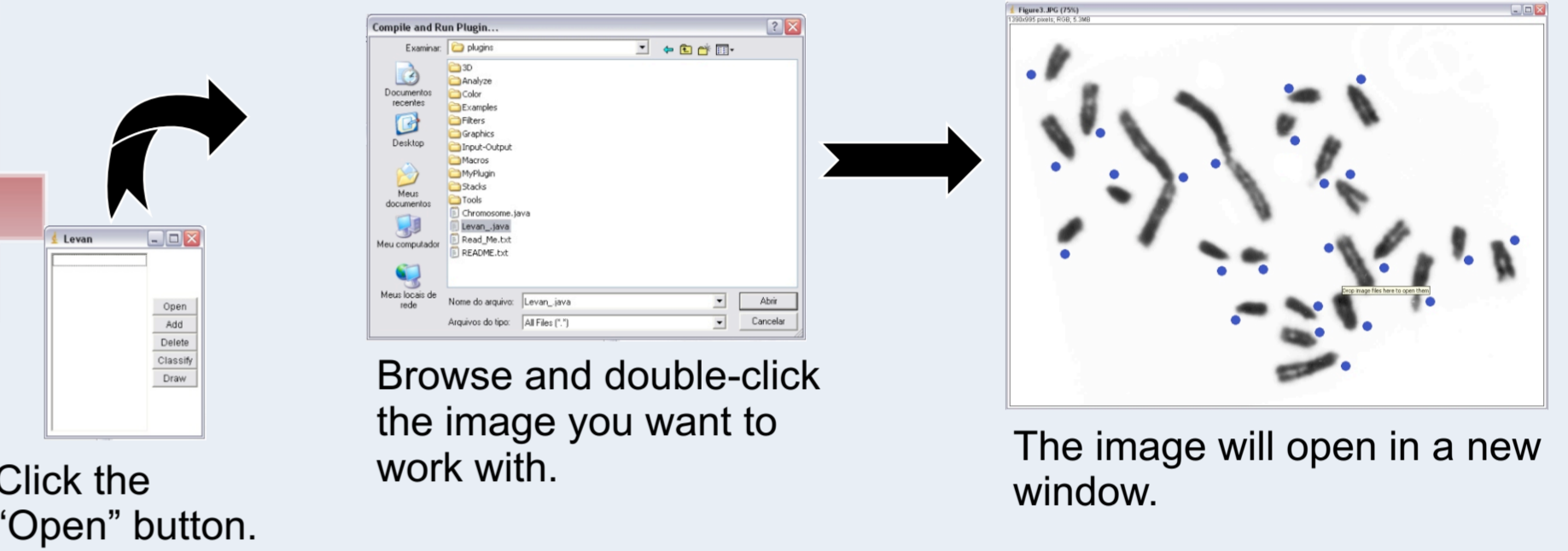


Restart the ImageJ

In the menu bar, click on Plugins>Levan.

The Levan plugin window will open.

4 Open an image using Levan:

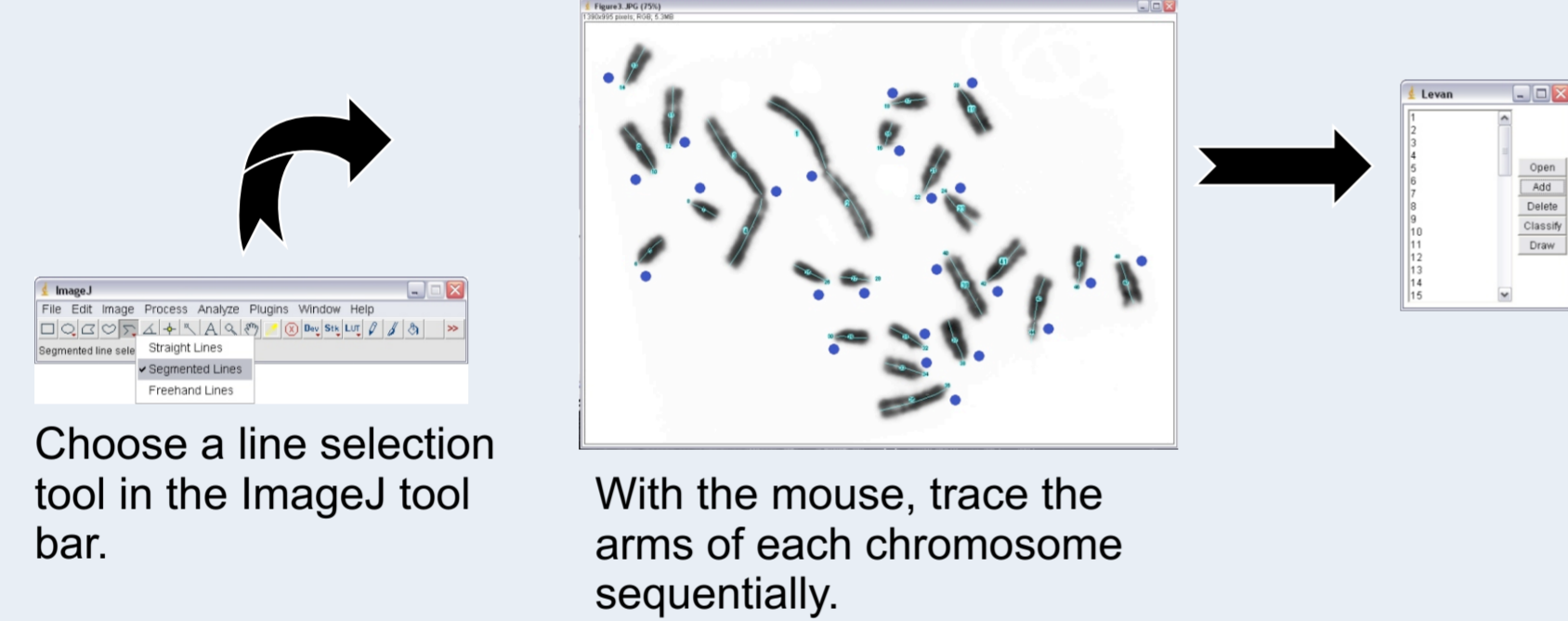


Click the "Open" button.

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

The image will open in a new window.

5 Trace the chromosome arms:

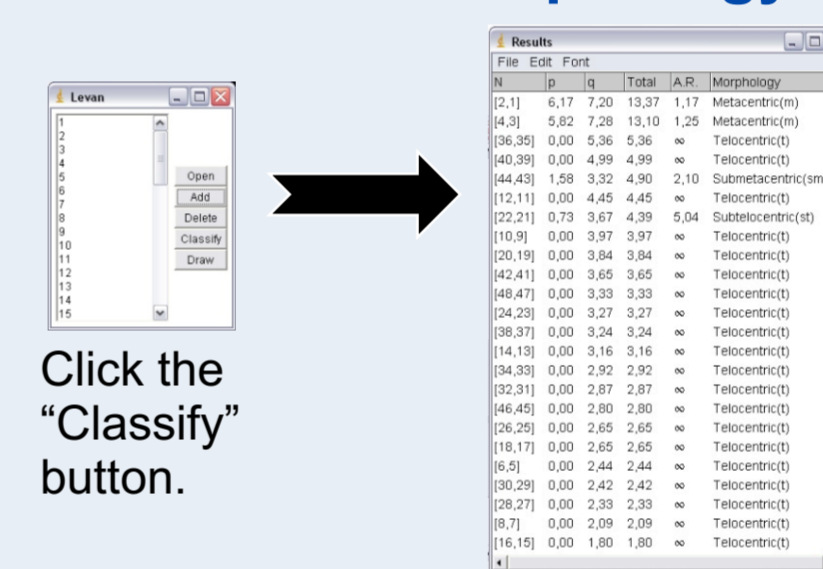


Choose a line selection tool in the ImageJ tool bar.

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.

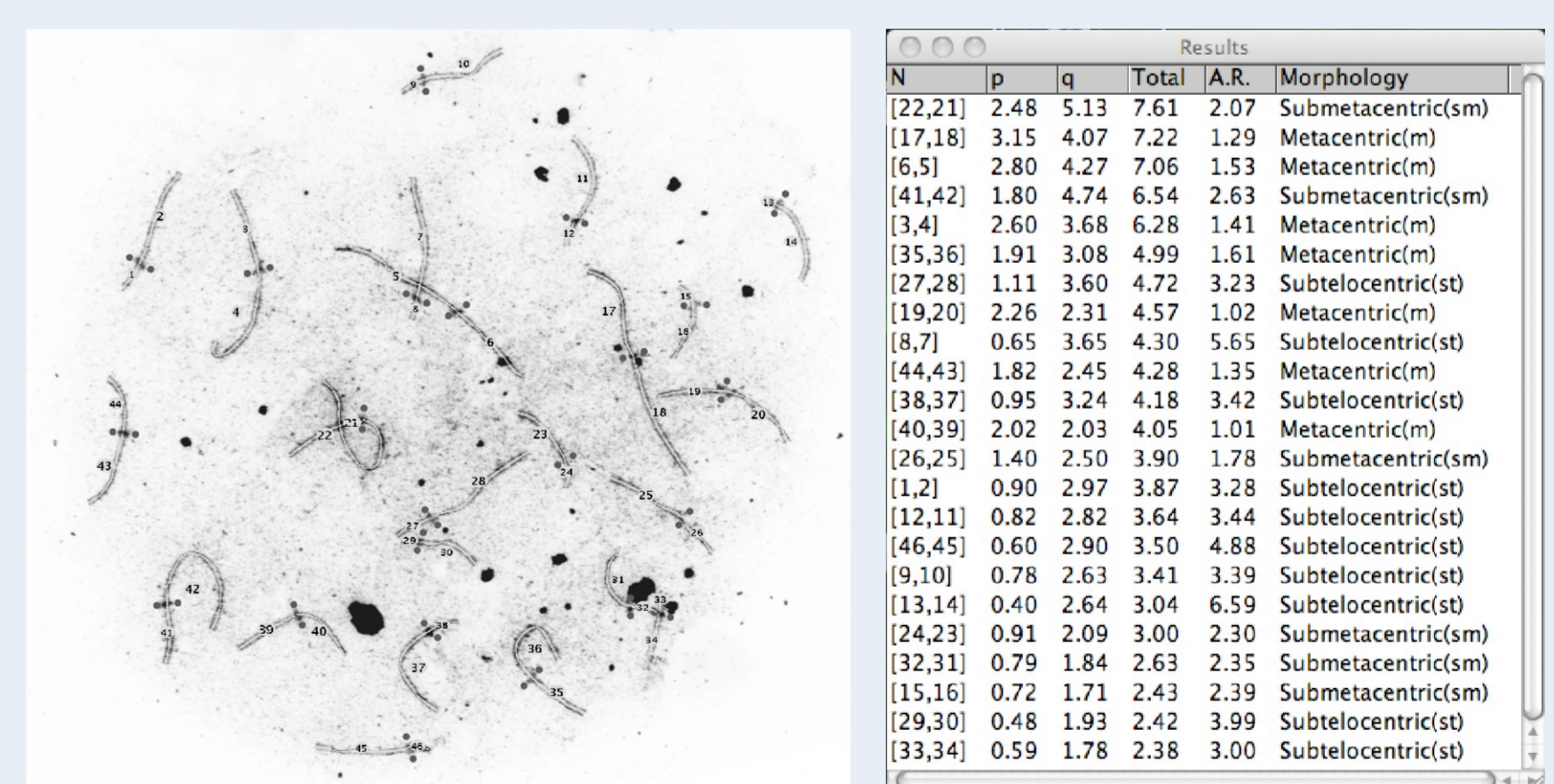
6 View the chromosome morphology:



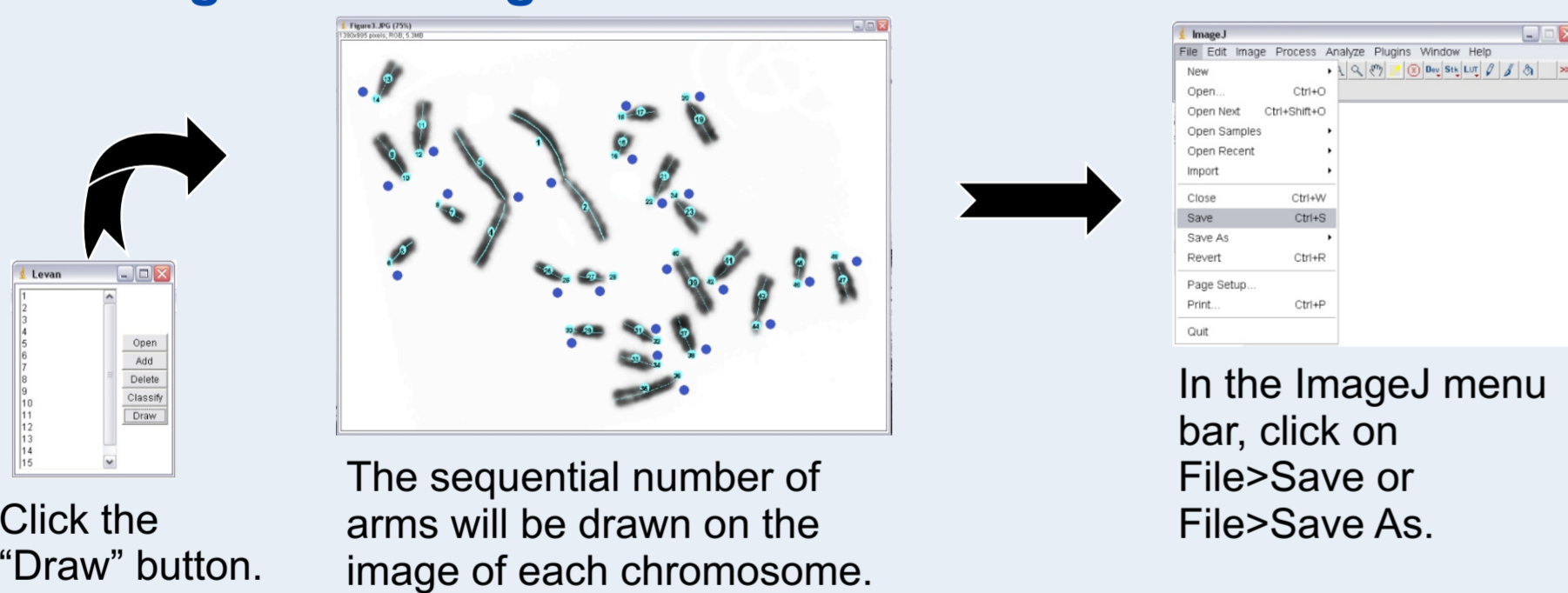
Click the "Classify" button.

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

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



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.

Tips to use the Levan plugin:

- remember that Levan uses the ImageJ original tools ("Selection Lines"); they have specific instructions, which can be viewed in the ImageJ manual;
- for complex curved or bend lines, such as observed in a synaptonemal complex structure or axial elements of pachytene nuclei, we recommend the use of the "Segmented Line" tool; this tool allows the user to easily redefine traced lines;
- 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.
- 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.
- 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);
- 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⁾, Y. & Zacaro, A. A. (2009)

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