MATO (Measurement and Analysis tools)

User Guide

1、Introduction

As the external expression of genes, plant morphological characteristics are not only the most important evidence to study the evolution of plant species, but also the foothold for human to reform plants. Molecular phylogeny endows classical taxonomy such as morphological anatomy, and cytotaxonomy with new vitality (Maliogka V.I. et al., 2018; Gokhman V.E., 2022; Faraut T., 2008). Superimposition of morphological/ micromorphological information onto a phylogenetic framework has immense utility in elucidating direction of evolutionary change and delineation of taxonomic hierarchy. Most phylogenetic studies need to combine morphological characteristics with phylogenetic analysis to deduce the evolutionary patterns and processes of species evolution (Endress P.K. et al., 2000; Buzgo M. et al., 2004; Henderson A., 2006). In cytotaxonomy, the phenotype of chromosome is called "karyotype" and represents the structural and functional organization of the nuclear genome at the macroscopic level. Its constancy ensures transfer of the same genetic material to the next generation, while variation enables ecological diferentiation and adaptation (Vimala, 2021; Gokhman, 2022).

MATO (Measurement and Analysis tools), an improved version of the widely used KaryoType, was developed for the measurement of macromorphology, micromorphology, and cytotaxonomy. The updated KaryoType 3.0 added a basic analysis module related to morphology, and the software interface was changed to "Karyotype" and "Standard", thus the name was changed to MATO. The main functions of MATO include:

- Karyotype analysis: measurement of chromosome traits (arm length or total chromosome length etc.), automatic grouping of chromosomes, calculation of chromosome asymmetry coefficient, drawing of ideogram.
- General morphometric analysis: Length, Size, Count, Angle and Color are the five basic tools that can be used to measure traits.
- Image correction tools: Horizontal correction, Vertical correction, Perspective correction, White balance correction.

MATO supports jpg, jpeg, tif, png and other image formats, and all measurement

processes can be saved and reproduced. It can not only be used for systematic taxonomic research, but also play an important role in ecology, pathology and other research disciplines requiring morphological measurement.

2、Installing

Download Link: http://mnh.scu.edu.cn/soft/blog/KaryoType/index.html

It is available for Windows and macOS.

Installation instructions:

MATO is distributed as a zip package. You just need to unzip the zip package to any location on your computer and double-click on the folder to run the program.

Windows: Please make sure Microsoft® .NET 3.5 Framework have be installed on your computer. The .NET framework is part of Windows, but versions may vary from system to system. The .NET framework 3.5 package is available for free on Microsoft's official website. Please install it before you use MATO to prevent it from not working.

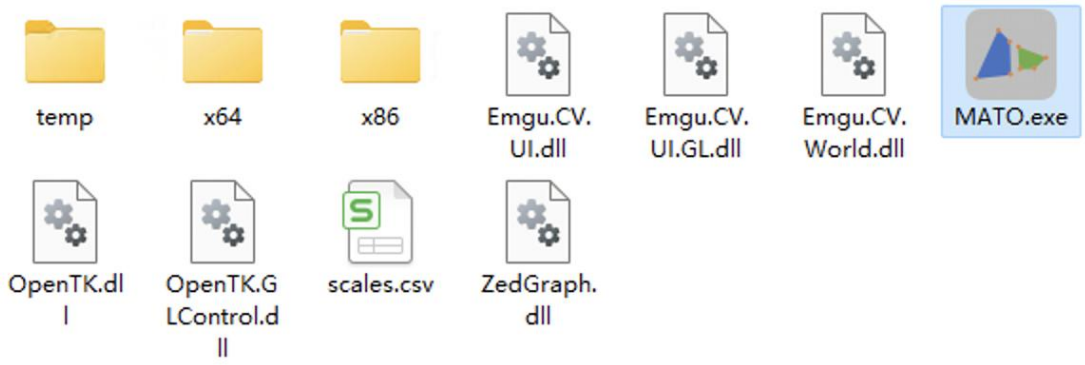
MacOS: MATO is packaged with Wineskin to run on macOS and supports both Intel CPU and M1. Please try to use the latest version of MacOS. Due to limited conditions, MATO has not been tested on all macOS versions.

Linux: Because there are so many distributions of Linux, MATO does not provide a direct runtime package. You can package MATO for Windows yourself using Wine, or run it on a virtual machine.

Note: In windows, do not run the program directly in the zip package, and do not remove the MATO.exe from the folder alone, the "file cannot be found" error will occur.

3、Software functions

3.1 executive program

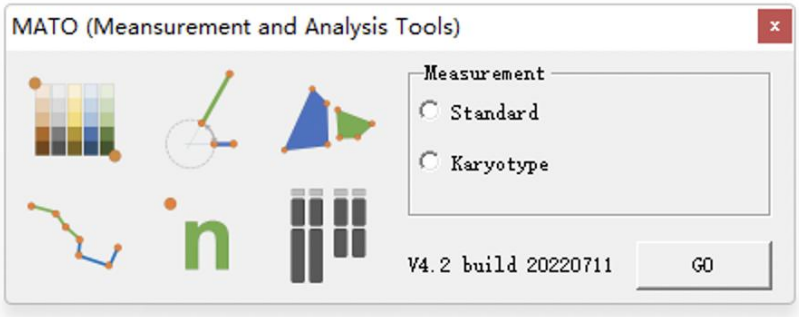


On the Windows operating system, double-click MATO.exe after decompressing the downloaded package.

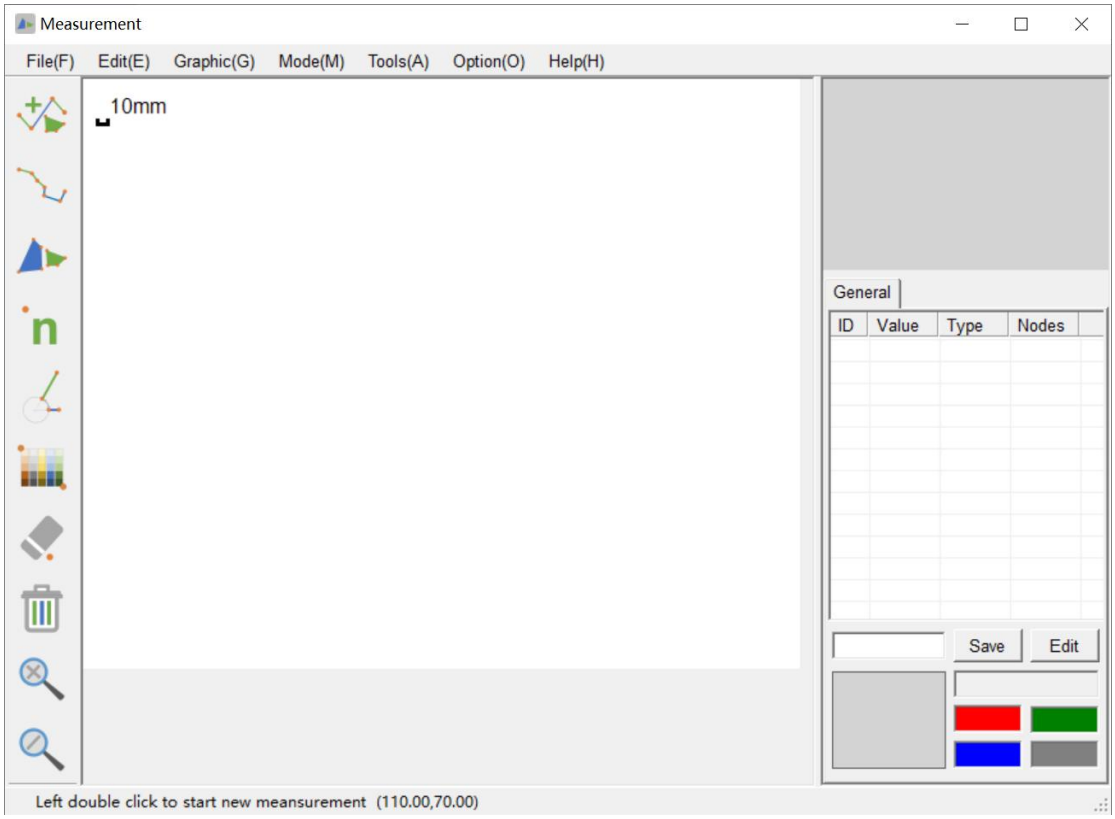
On macOS, unzip the downloaded package, place the MATO.app in the Application folder, and double-click MATO.app to run it.

3.2 Software Interface

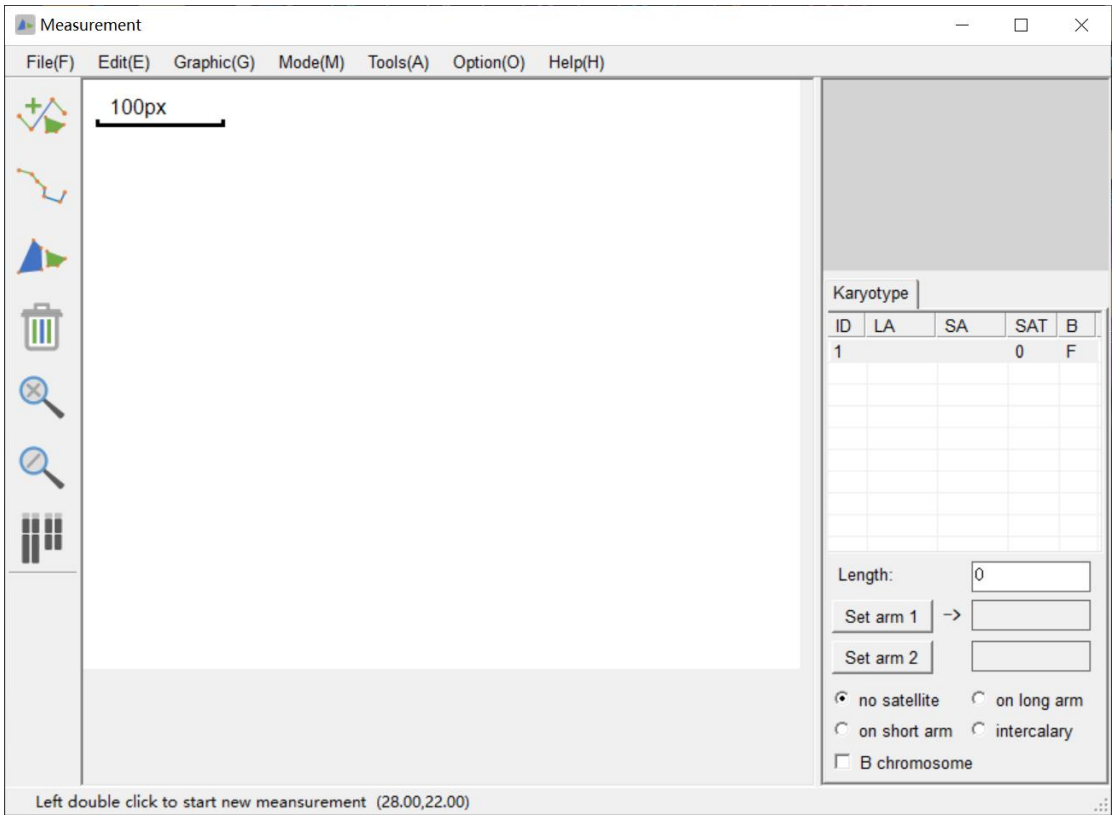
After startup, the software interface is as follows:



After selecting Standard, the following interface is displayed:

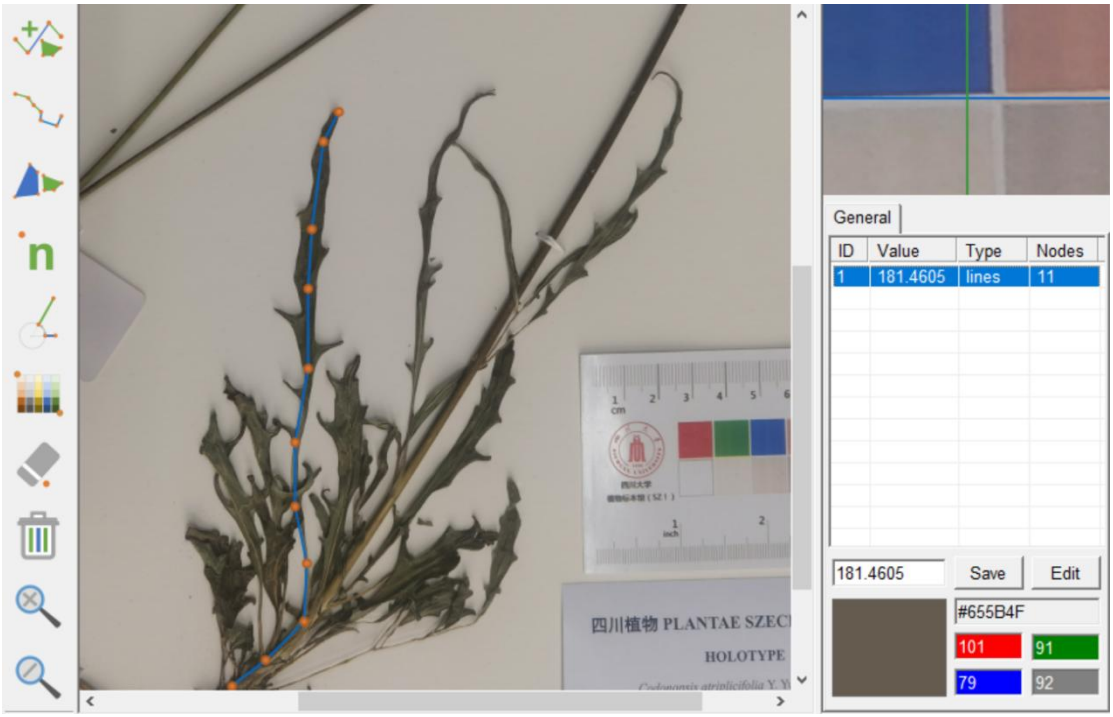


When selecting Karyotype:



Software function area:

Software operation area:



3.3 Main function module

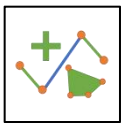
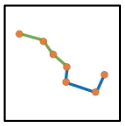
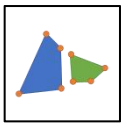
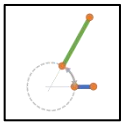
3.3.1 Menu Bar

- **File**: The following contents include Load, Save and New. **Load** is used to import the image or previously saved measurement data, **Save** is used to save the current measurement data (csv, txt format) or image (jpg, png, and bmp format), and **New** is used to clear the current operation and return to the blank state.
- **Edit**： Used to edit your measurement data, with **Insert New Item**, **Delete Selected Item**, **Clear Selected Item** three functions.
- **Graphic**： Used to process imported images. **Rotate** can flip the Image (90°, center symmetry and axis symmetry), **Gray Scale** can turn the image into gray and white color, **Crop Image** can cut out the image. The remaining four Correction functions (**Vertical Correction**, **Horizontal Correction**, **Perspective**

Correction, **White Balance Correction**) is used to correct problems that affect measurements such as uneven or skewed images, perspective, white balance errors, etc. (see 4.3 for details).

- **Mode**: Selecting modules, there are two modules can be selected, **Standard** and **Karyotype**, which are used for base and karyotype measurements respectively.
- **Tools**: The **Scales** below can be used to set the ruler. The other two features are only available in the Karyotype mode, where **Group** can be used to group chromosomes automatically and **Combine Results** can be used to combine the results of multiple measurements.
- **Option**: You can set the display, size and color of point and line here; Whether to disable double click and left drag.
- **Help**: The author's contact information and software references are displayed here.

3.3.2 Function of operation area

-  (**New**): Create a data ID. After the ID is created, the length is the default value.
-  (**Length**): Measure length.
-  (**Size**): Measure area.
-  (**Count**): Take count of something.
-  (**Angle**): Measure angle.

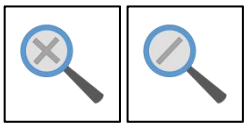
❖  (**Color**)：Measure the color and display it in the color value box on the lower right.

❖  (**Remove node**)：Undo the previous point.

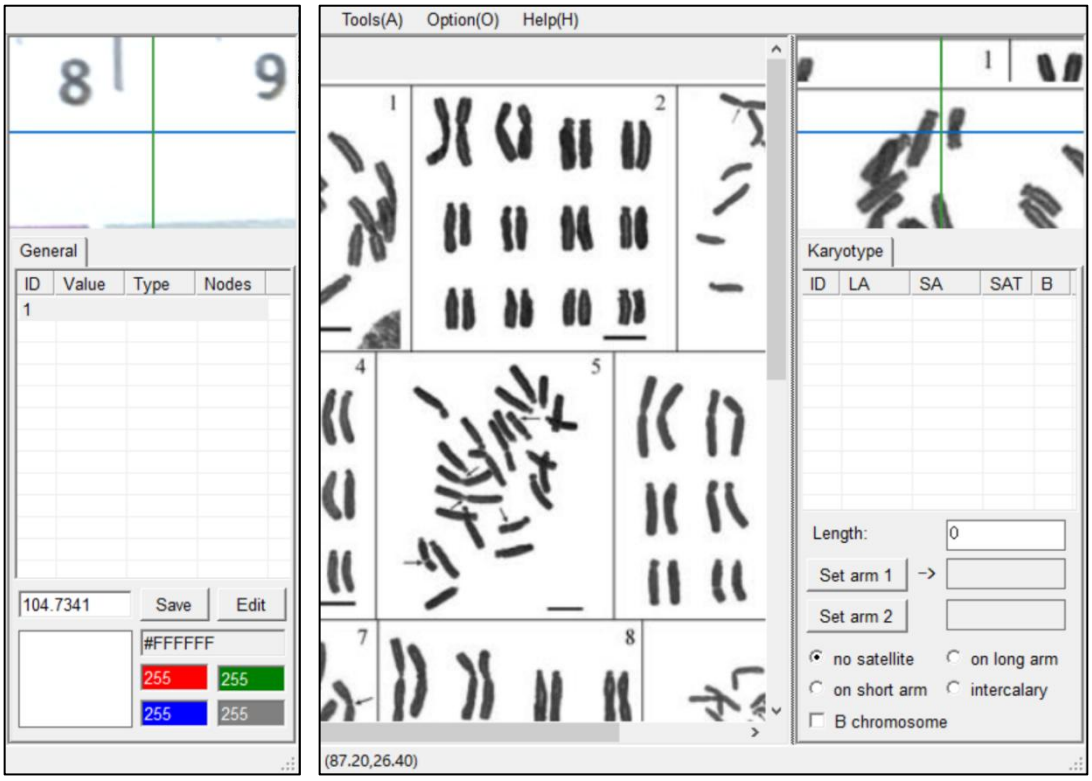
❖  (**Delete**)：Delete the last row of data.

Note: The Delete here are from the bottom up. If you want to delete the previous data, use Edit-Delete selected item in the menu bar.

❖  (**Group**)：Draw a ideogram.

❖  (**Zoom In/Out**)：Zoom the picture in or out.

3.3.3 Data area function



Standard mode (see above left):

- **ID**: Displays the ID number of the measurement step you are currently operating.
- **Value**： Displays the values that you measured in this step. The real-time measured Value is displayed in the lower data box. Click "Save" and it will be displayed in Value, indicating that the value has been saved as the record of this ID.
- **Type**： Displays the type of measurement you are currently measuring, lines as a unit of length, size as a unit of area, count as a count, angle as an Angle, and color as a color.
- **Nodes**： Displays the total number of clicks for your current measurement step.
- Others： The **Save** and **Edit** functions manipulate the data being measured; The color value box below shows the color of the point you are measuring.

Karyotype mode (see above right)：

- **ID**: Displays the ID number of the measurement step you are currently operating.

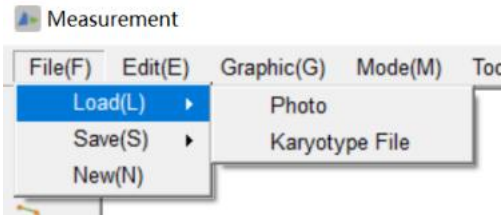
- **LA**：The abbreviation of long arm, save the value of the long arm.
- **SA**：The abbreviation of short arm, save the value of the short arm.
- **SAT**：The abbreviation of satellite chromosome. Satellite chromosomes or SAT chromosomes is an abbreviated form of 'Sine- Acido- Thymonucleico' meaning without thymonucleic acid as it do not get stain with Feulgen reaction. Satellites are present as secondary constrictions at the tip of chromosomes that carries NOR (nucleolus organising region) i.e. site for nucleolus formation. They represent a heterochromatin region containing less DNA than RNA. When you select no long arm after measurement, the value will automatically change to 1. If no short arm is selected, the value automatically changes to 2. When intercalary is selected, the value automatically changes to 3.
- **B**：The abbreviation of B chromosome (also known as accessory, supernumerary, or extra chromosome). F indicated that there was no B chromosome, and T indicated that there was B chromosome. When the "B chromosome" column was checked, this value was automatically changed to T.
- **Others**：Length- Shows the value you are currently measuring (note: The value units measured before calibration are not ruler units.) ; Set arm 1/2- Sets the Length of the arm, which is the value displayed by the current length. No satellite- Select this item if the measured chromosome has no satellite. This option is selected by default. No long arm- Target chromosome to be measured Select this item if no long arm is available. No short arm- Target chromosome for measurement Select this item if no short arm is available. Intercalary- Select this item if the measured target chromosome has intermediate deletion; B chromosome- Select this parameter if the measured chromosome was B chromosome.

4、Analyze examples

4.1 Examples of karyotype measurement and analysis

4.1.1 Open an image

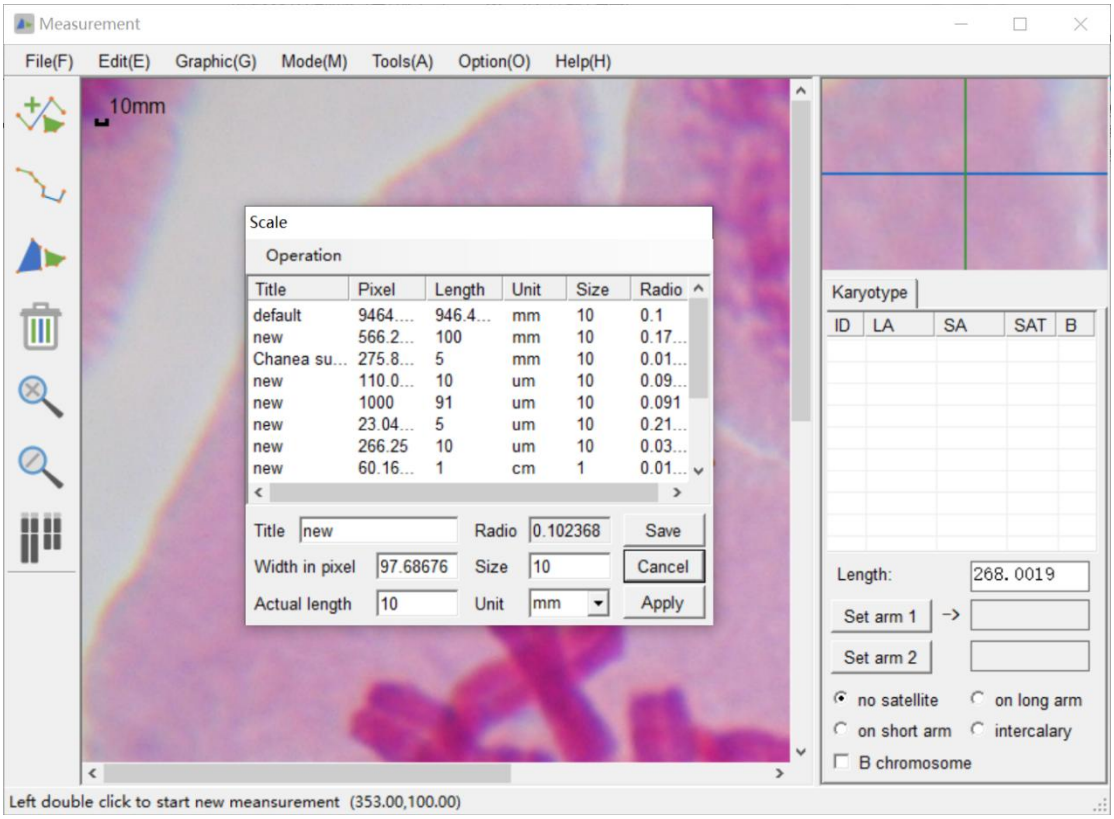
Open MATO, select the mode of Karyotype, click File-Load-Photo, find the target image in the folder, and select Open.



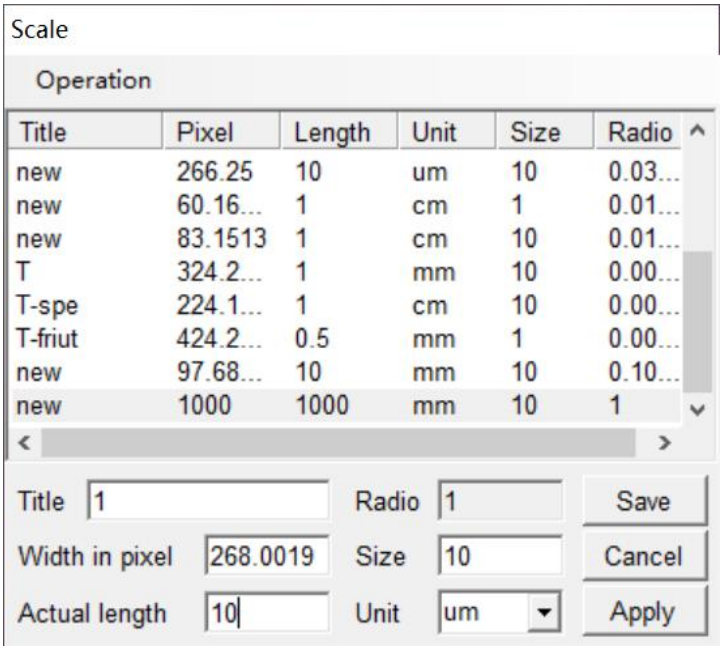
4.1.2 Create and set a ruler

After importing the image, use "Length" in the upper left corner to measure the pixel value of the ruler in the image, as shown below 268.0019.

Then copy the measured values and click Tools-Scales in the menu bar.



Then click Operation-new Scale in the upper left corner to create a new ruler and name it in the "Title" column. We then paste the measurement "268.0019" into the "Width in pixel" column and modify the remaining parameters. In this example, the Actual length of the ruler is 10 microns, so we set the "actual length" to 10 and change the "Unit" to um. After setting, click Save to save and Apply to correct the ruler.



After setting the ruler, we can see that the correct ruler appears in the upper left corner. If you do not want to display it, click Option-Show-Scale Axis in the upper

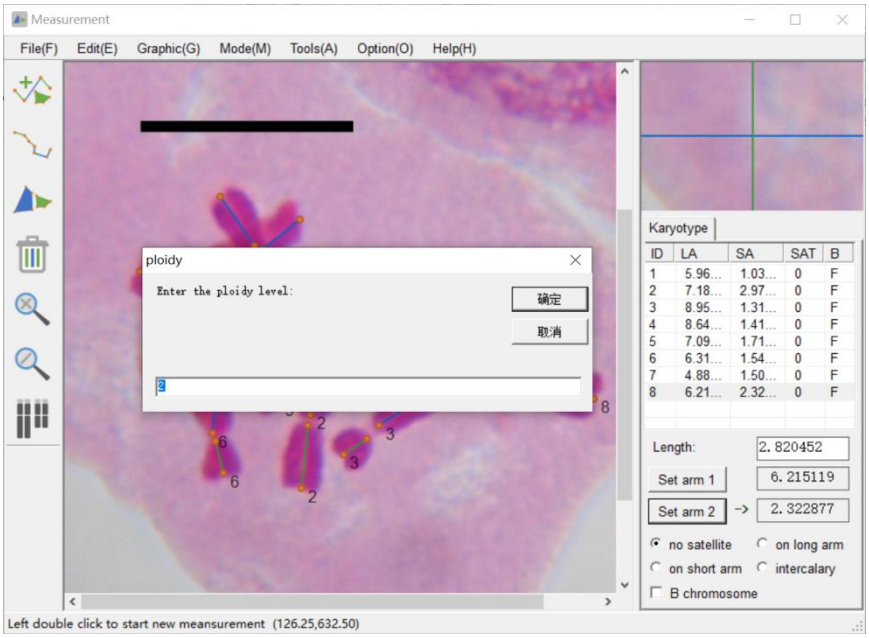
menu bar to close it. We can verify the correction result of the ruler by using "Length" again to measure the length of the ruler in the figure. See the following figure, the measurement result is "9.999999", and the ruler is successfully set.

4.1.3 Chromosome measurement

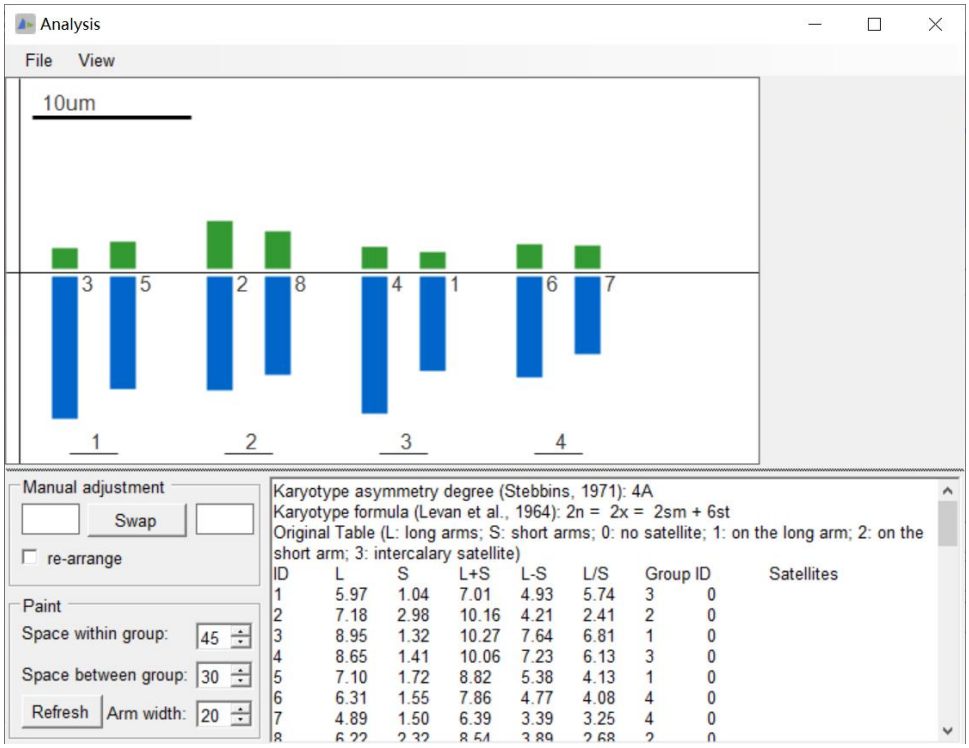
Click "New" in the upper left corner to create a measurement ID, and then click "Length" for measurement. Click the left mouse button to add a measurement point, and click the right button to stop the measurement line segment. If the measured part is longer, click "Set arm 1" to save it in LA; if it is shorter, click "Set arm 1" to save it in SA. See the instructions in 3.3.3 for the special options below.

4.1.4 Chromosome grouping and Ideogram drawing

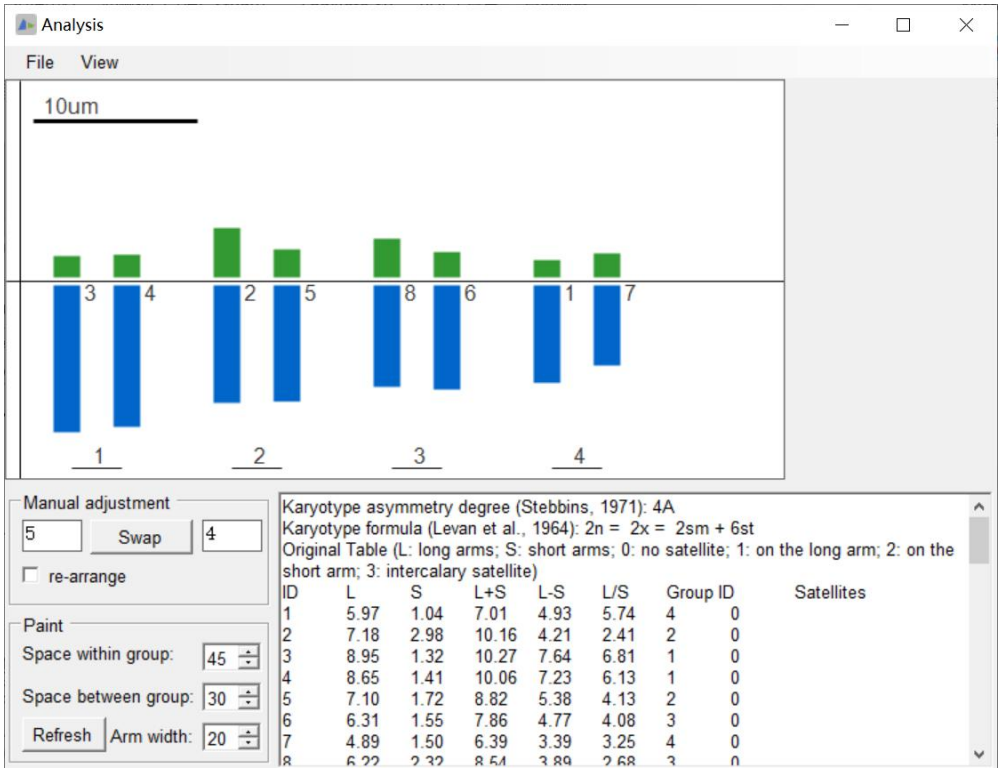
After all chromosomes are measured, the Group function can be used to automatically group the measured data and draw an ideogram directly, and conduct karyotype analysis. Click on "Group" in the bottom left corner (Tools-Group in the menu bar can also be used), set the species ploidy in the prompt box that pops up, and click OK.



Then the ideogram and analysis results automatically pop up. If you think there is an error in the grouping result, you can manually adjust it through "Manual adjustment", for example, "4" and "5" can be swapped, fill in the blank space with 4 and 5 respectively, click "Swap" and they are swapped.

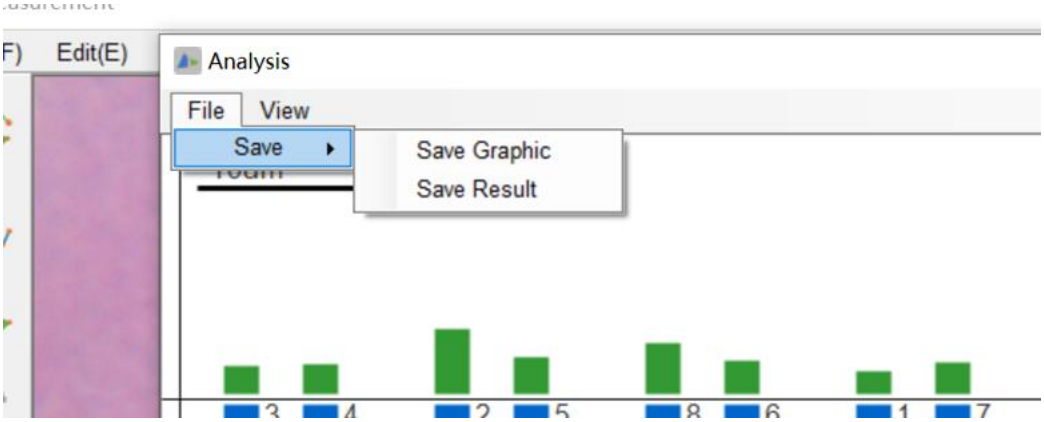


The adjusted results are as follows.

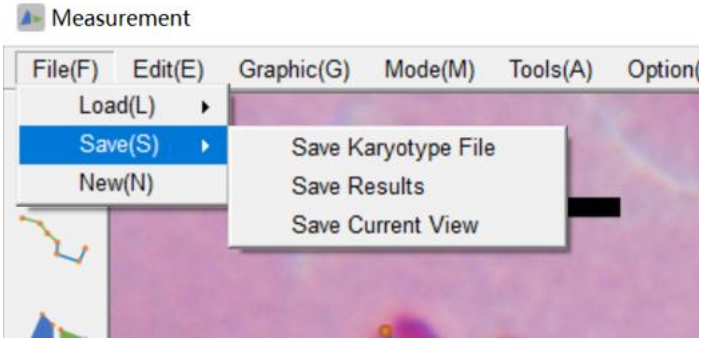


4.1.4 Data saving and loading

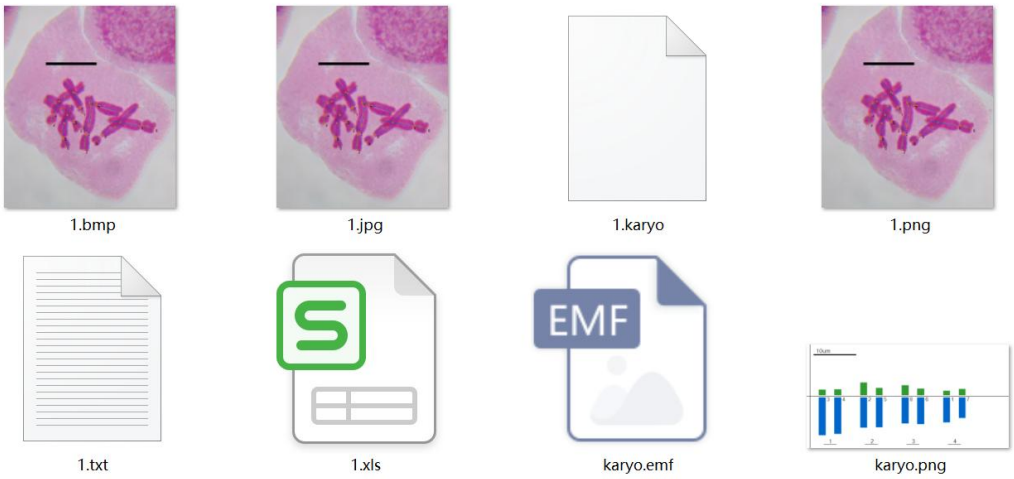
Click File-Save at the top left of the analysis results page, and two options appear. The "Save Graphic" is the kernel graph that saves the upper-left corner; Save Result saves the analysis result in the lower right corner. The core diagram can be saved in png or emf format; The analysis results can be saved in xls or txt format.



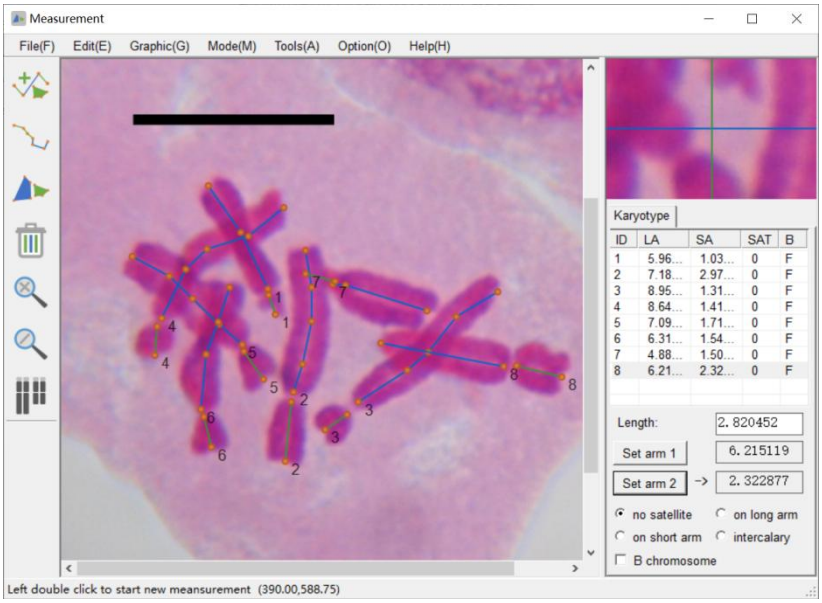
Close the analysis results page, click File-Save in the menu bar, and you see three options. "Save Results" is also an analysis Result file in xls or txt format, which is the same as the above "Save Result" result. "Save Current View" is to save the current operation image. The formats can be jpg, png or bmp. The "Save Karyotype File" is a file that saves all current operation information in karyo format.



In general, the karyo file is the most important, and other results can be exported by it. The saved result file is as follows.



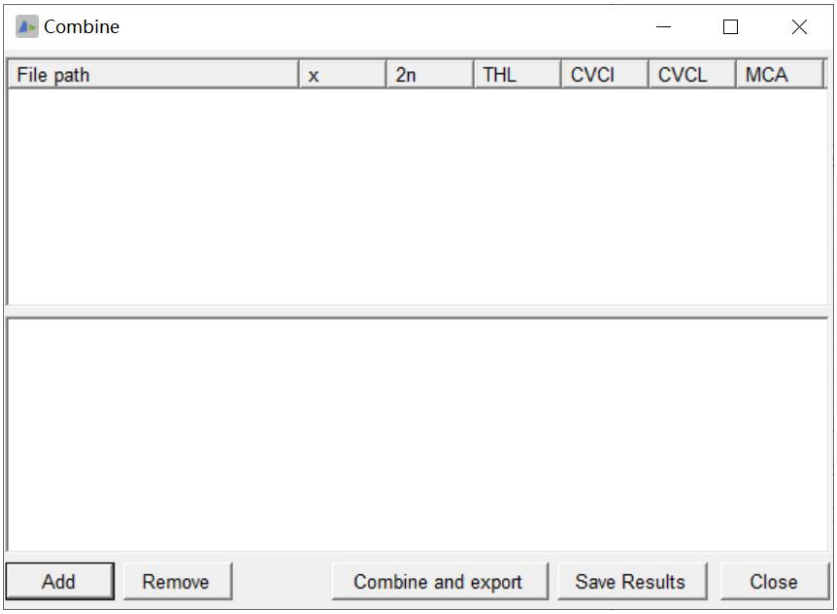
In the figure above, 1.karyo can be re-imported by the File-Lord-Karyotype File in the menu bar. The page that comes to Karyotype contains all the operations before exiting, which can be checked and continued to be measured. (Note: At this time, Scale is still the ruler used in the last measurement. Remember to select the ruler set during the measurement after import, and then continue the measurement.)



4.1.5 Combined multiple measurements

When we measure chromosomal karyotypes, we tend to measure many different cells. MATO can combine all the results in one click and summarize them into one result.

Click Tools-Combine Results from the menu bar to bring up the action box.

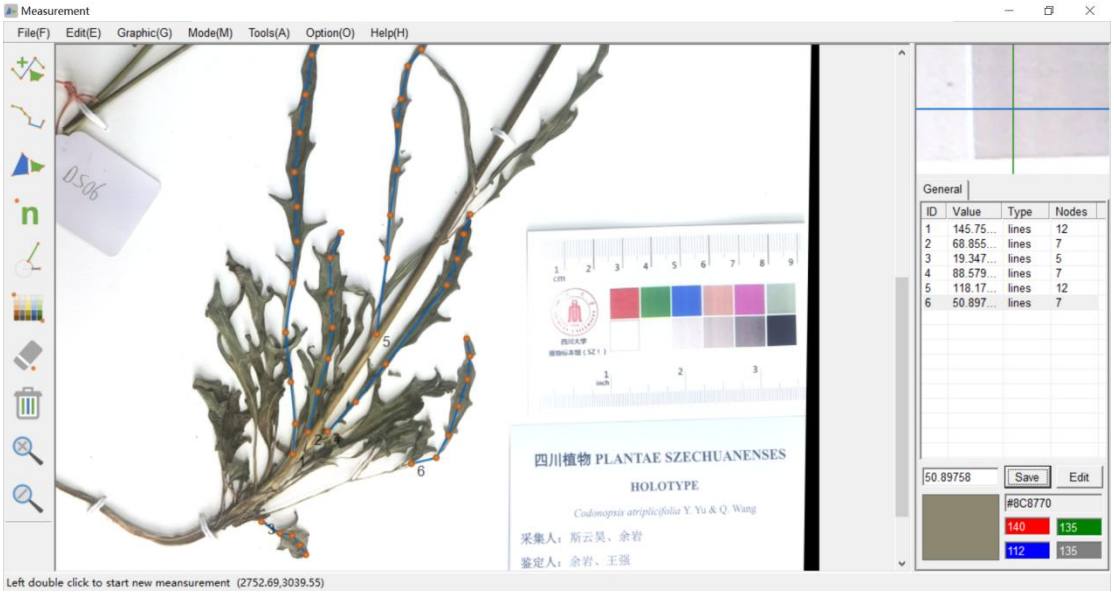


You can click Add in the lower left corner and add the measurements (xls or txt file) in sequence, then Combine and export to get the merge result, which can be saved as an karyotype file and opened through MATO to export the karyotype diagram and the rest of the required data. Click Save Results to save the merged results directly as an xls or txt text file.

4.2 Examples of morphological Measurement

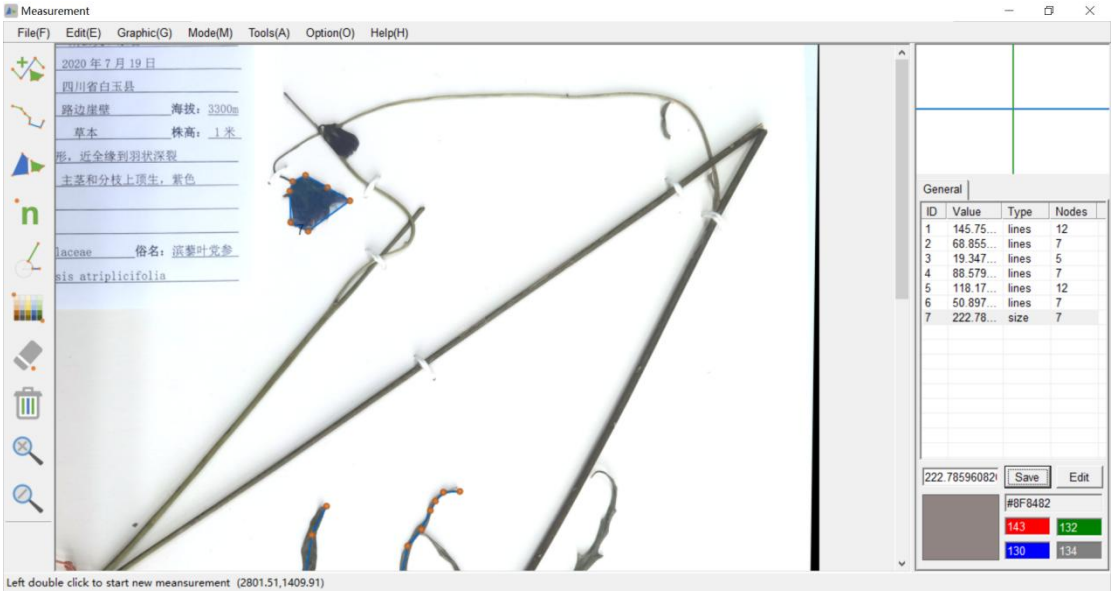
4.2.1 Length measurement

Select the second "Length" tool on the left and measure the length data. Left - click to select a point, right - click to end. (Note: Scale should be carried out before measurement, see 4.1.2)



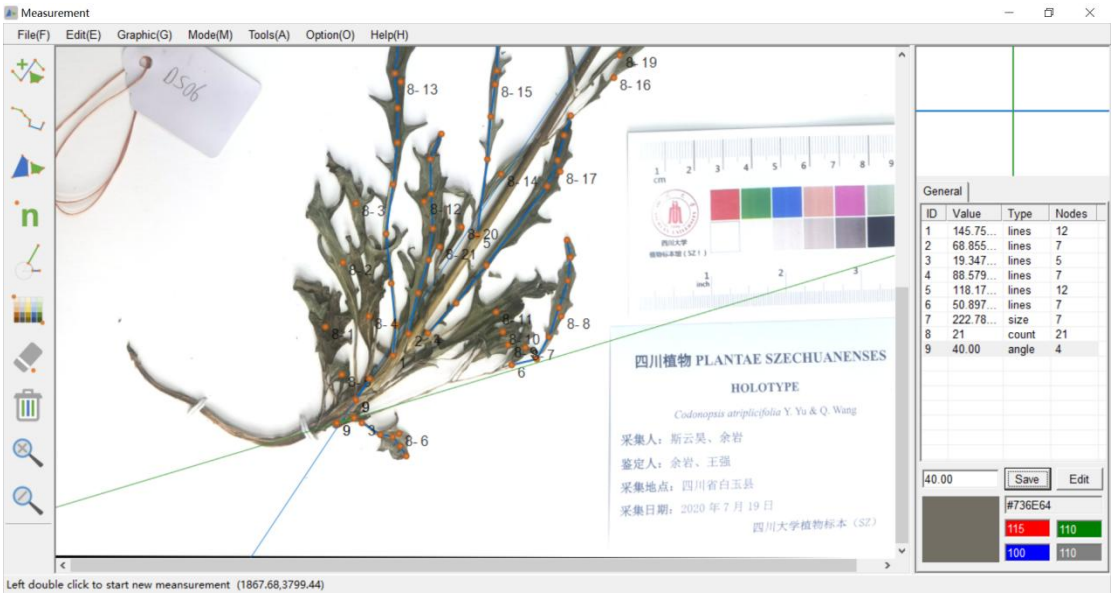
4.2.2 Size measurement

Click the third left "Size" tool to measure the area, left click to select the point, right click to end. (Note: Scale should be carried out before measurement, see 4.1.2)



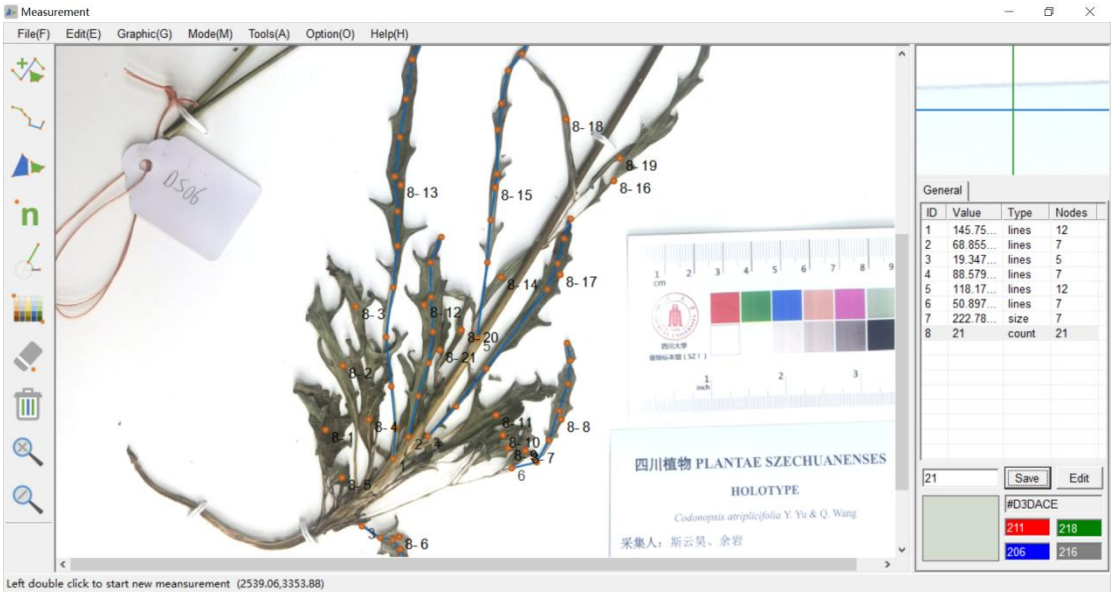
4.2.3 Angle measurement

Click the fifth left "Angle" tool to measure the area. The tool measures angles by clicking four points with the left mouse button to make two intersecting lines. The Angle between the trunk and a small branch is roughly measured at 40°.



4.2.4 Count

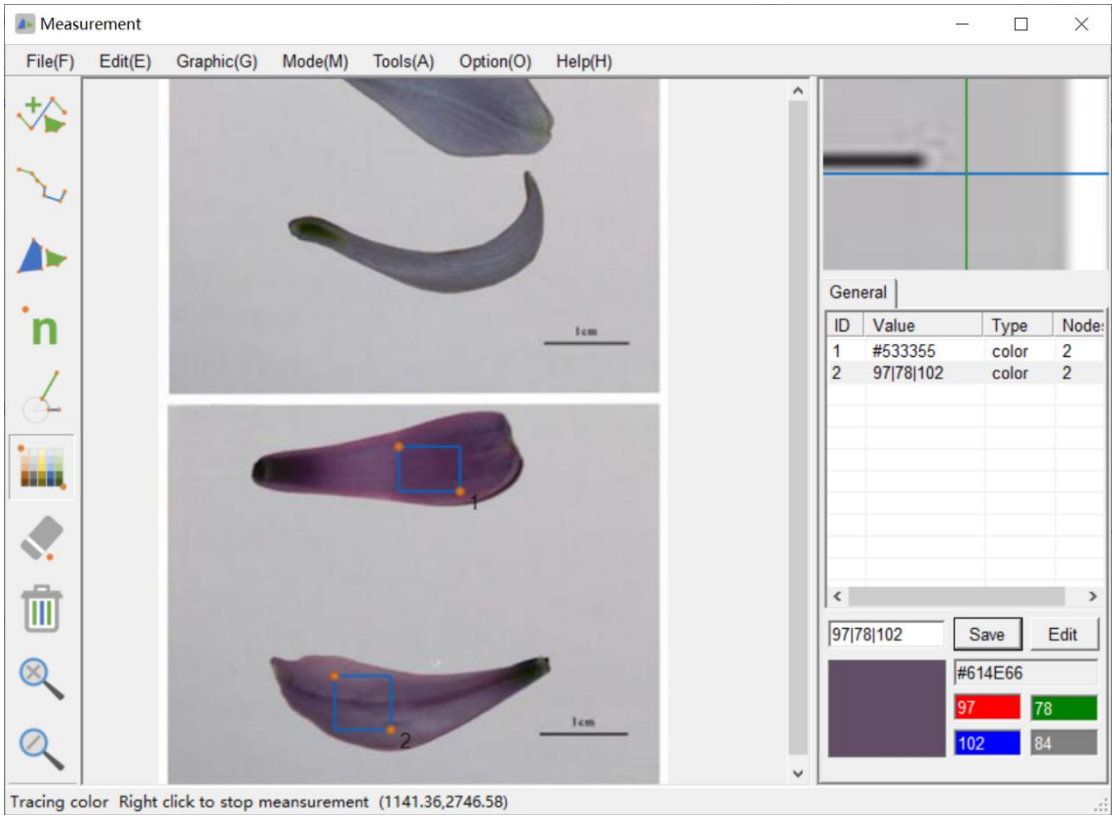
Click the fourth "Count" tool on the left to count. The left mouse button is clicked to count, the left mouse button is clicked to select the point, and the right click is finished. The counting process is shown in the diagram as an ID number - dot sequence number. This specimen, for example, has 21 leaves.



4.2.4 Get the color

Click the sixth "Color" tool on the left to get the color. There are six options for measuring content. We can measure grayscale value, RGB value, separately measure R/G/B or measure hexadecimal color code. These can be selected from the menu bar under Option-Color Channel.

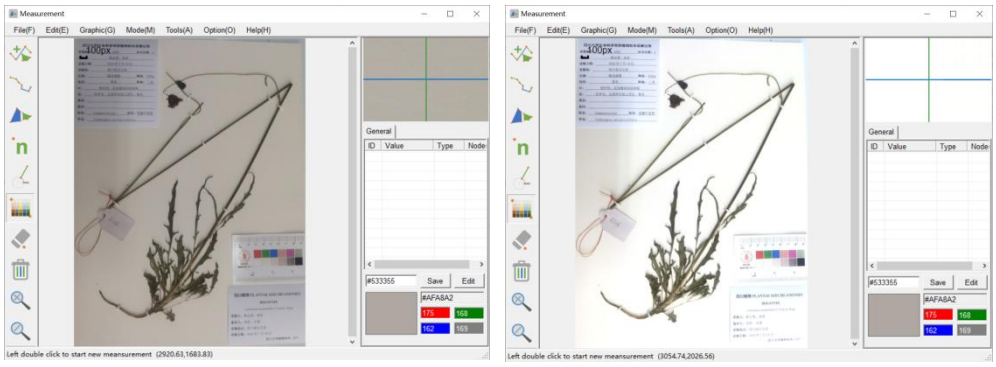
Click the box twice with the left mouse button to select a rectangle. The color value displayed is the mean value within the range.



4.3 Image correction example

4.3.1 White Balance Correction

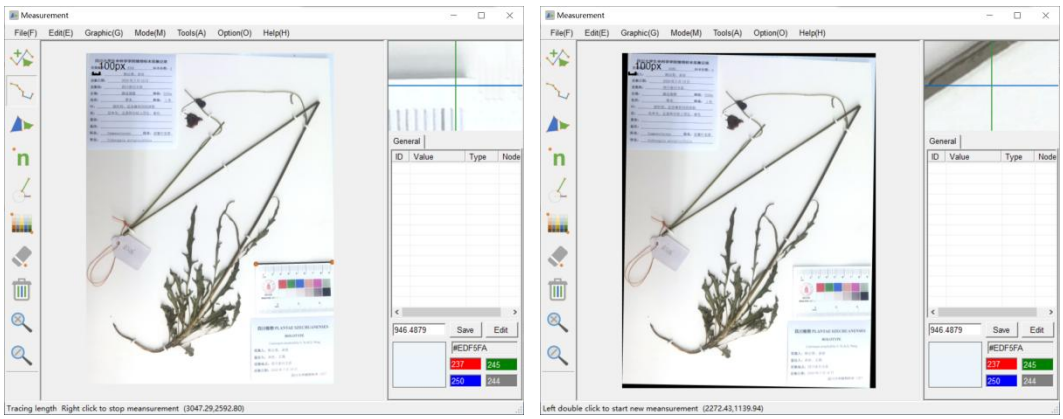
After importing the image, click the part of the image that should be white. (You can see the color in the lower right corner.) Then click Graphic-White Balance Correction to correct the white balance.



before and after

4.3.2 Horizontal Correction

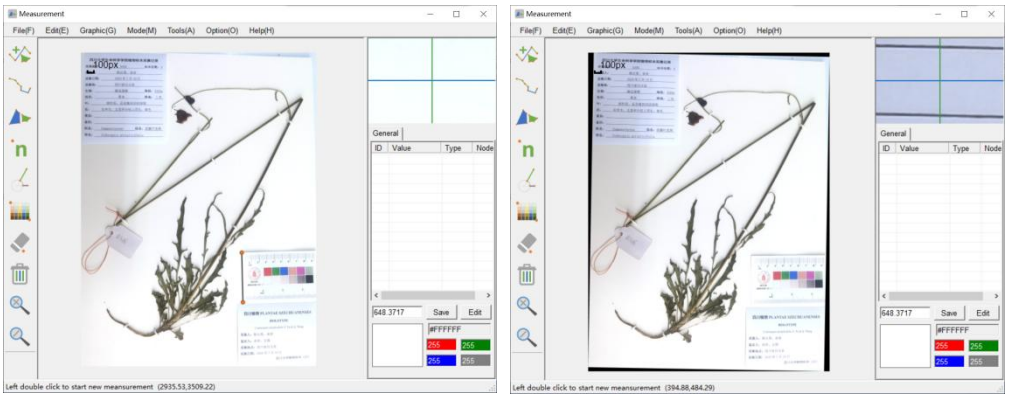
After importing the image, use "Length" to pull a line segment with the level you have confirmed. Then click Graphic-Horizontal Correction to correct the Horizontal Correction. In the example we assume that the colorimetric calipers are horizontal.



before and after

4.3.3 Vertical Correction

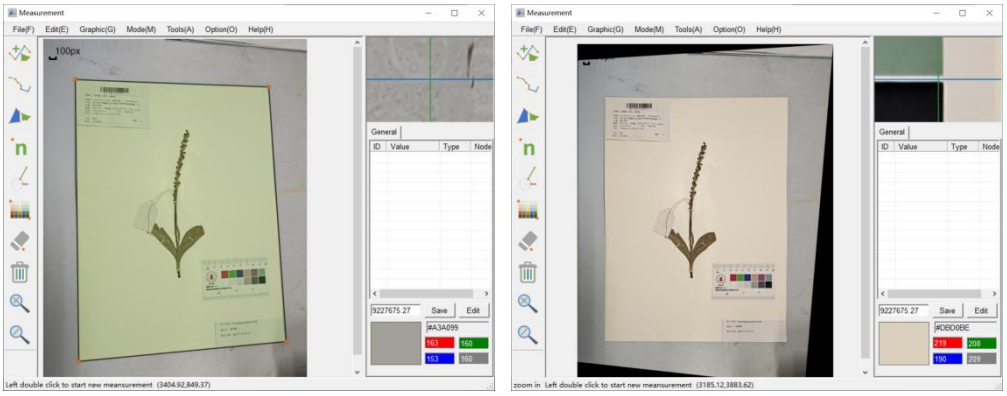
After importing the image, use "Length" to pull a line segment that you confirm is vertical, then click Graphic-Vertical Correction to correct the vertical offset. We also assume that the edges of the color card are vertical.



before and after

4.3.4 Perspective Correction

After importing the picture, use the "Size" tool to find the original four points of the specimen to form a plane, and then use the Graphic-Perspective Correction for geometric correction. In the dialog box that is displayed, enter a similar and correct aspect ratio. For example, the herbarium sheet in this example is 30cm*40cm, so the width of 2533 is changed to 3000, and the length of 3330 is changed to 4000.



before and after

5、Q&A

Q1: What are the shooting requirements for the images to be determined?

A1: The image should be clear, unobstructed and as complete as possible, preferably at the same Angle. In addition, the image should come with a ruler or reference for image preprocessing, for example, a properly placed specimen color card.

Q2: When do you need to correct an image?

A2: When the image to be measured has obvious too dark light, color offset, skew, not level, you can correct the image to reduce the error.

Q3: When do you need to adjust automatic grouping?

A3: When you feel that there is something obviously wrong with the automatic grouping of the karyograph, you can manually adjust the results to make them more reasonable. It is also worth noting that it is best to measure chromosomes from small to large or from large to small in order to combine subsequent results.