# MATO (Measurement and Analysis tools)

# User Guide

## 1. Introduction

Plant morphological characteristics, which are the external expression of genes, are not only important for studying the evolution of plant species but also serve as a basis for human efforts to improve plants. Molecular phylogeny brings new vitality to classical taxonomies such as morphological anatomy and cytotaxonomy (Maliogka V.I. et al., 2018; Gokhman V.E., 2022; Faraut T., 2008). The superimposition of morphological and micromorphological information onto a phylogenetic framework is extremely useful for understanding the direction of evolutionary change and defining taxonomic hierarchy. Many phylogenetic studies rely on combining morphological characteristics with phylogenetic analysis to uncover the evolutionary patterns and processes of species evolution (Henderson A., 2006). In cytotaxonomy, the physical appearance of chromosomes is known as the "karyotype." It represents the structural and functional organization of the nuclear genome at the macroscopic level. The constancy of the karyotype ensures that the same genetic material is transmitted to the next generation, while variation in the karyotype allows for ecological differentiation and adaptation (Vimala, 2021; Gokhman, 2022).

MATO (Measurement and Analysis Tools) is an improved version of the popular KaryoType software, which is used for the measurement of macromorphology, micromorphology, and cytotaxonomy. Some of the main functions of MATO include:

- Karyotype analysis: measurement of chromosome traits (such as arm length or total chromosome length), automatic grouping of chromosomes, calculation of chromosome asymmetry coefficient, and drawing of ideograms.
- General morphometric analysis: Length, Size, Count, Angle, and Color are the five basic tools that can be used to measure traits.
- Image correction tools: tools for correcting horizontal, vertical, perspective, and white balance issues in images.

MATO supports various image formats, including JPG, JPEG, TIF, and PNG, and all measurement processes can be saved and repeated. In addition to being useful for systematic taxonomic research, it can also be applied in other fields such as ecology, pathology, and other disciplines that require morphological measurement.

## 2. Installing

**Download:** https://github.com/sculab/MATO

**Installation:**

MATO is provided as a zip file that can be easily unzipped and run on your computer. Simply unzip the package to a location of your choice and double-click on the folder to launch 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.

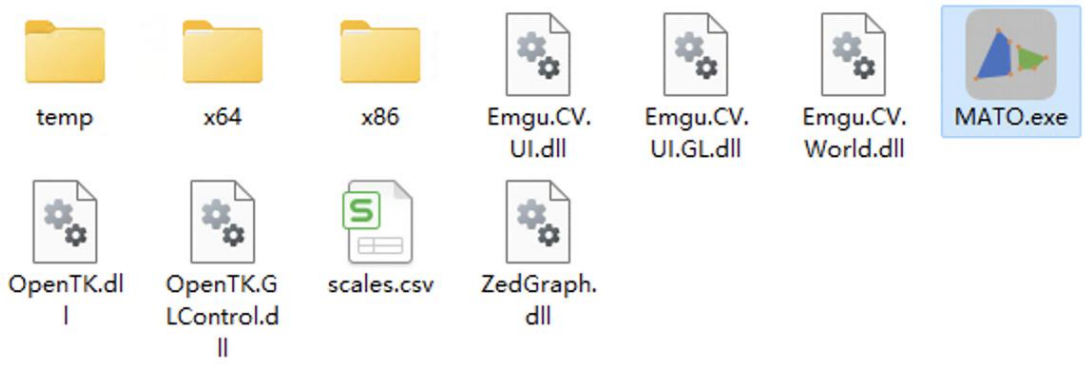
macOS: MATO is packaged with Wineskin to run on MacOS and supports both Intel CPU and M1. Please try to use the latest version of MacOS. Due to limited conditions, MATO has not been tested on all MacOS versions.

Linux: As there are many different distributions of Linux, a direct runtime package for MATO is not available. However, you can package MATO for Windows using Wine, or run it on a virtual machine.

**Note: It's important to note that in Windows, you should not run the program directly from the zip package, or remove the MATO.exe file from the folder and try to run it alone. Doing so may cause an error such as "file cannot be found." To avoid this issue, make sure to unzip the package and run the program from the unzipped folder.**

## 3. Main functions

### 3.1 Running MATO




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

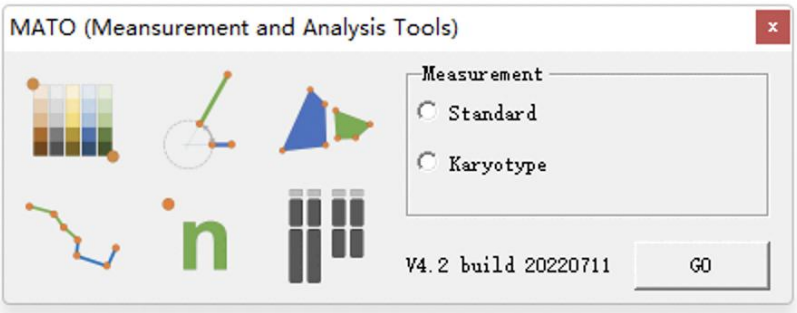
On macOS, unzip the downloaded package, place the MATO.app in the Application folder, and double-click MATO.app to run it.

**Note: If you encounter an error message stating that "MATO.app is damaged and can't be opened. You should eject the disk image," you may need to run following command at the first time run MATO:**

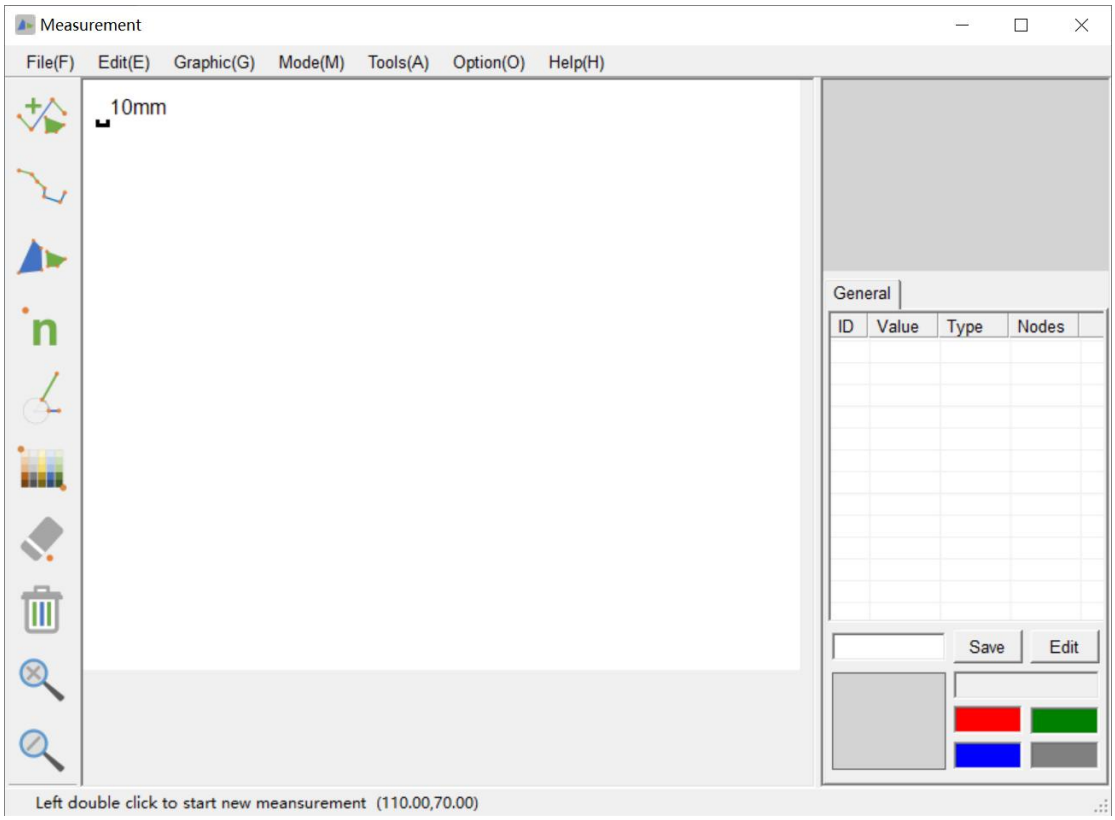
xattr -cr /Applications/MATO.app

### 3.2 Software Interface

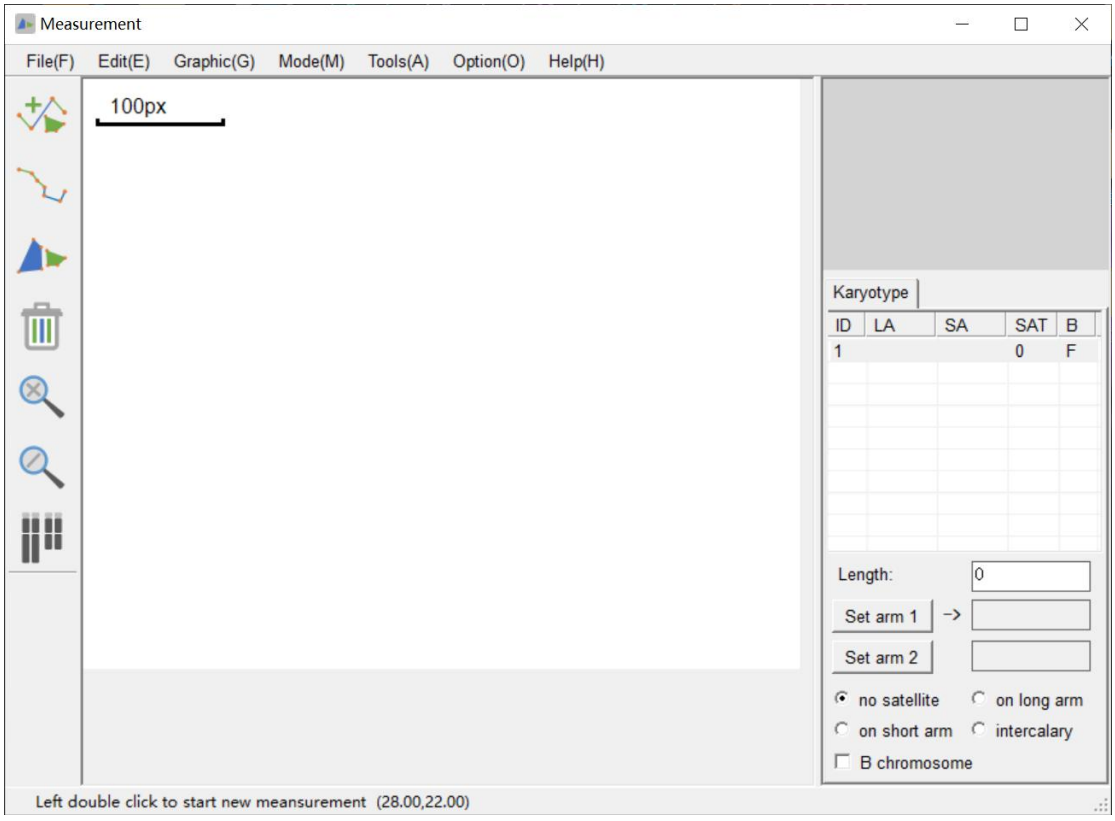
After startup, the software interface is as follows:




After selecting Standard, the following interface will be displayed:

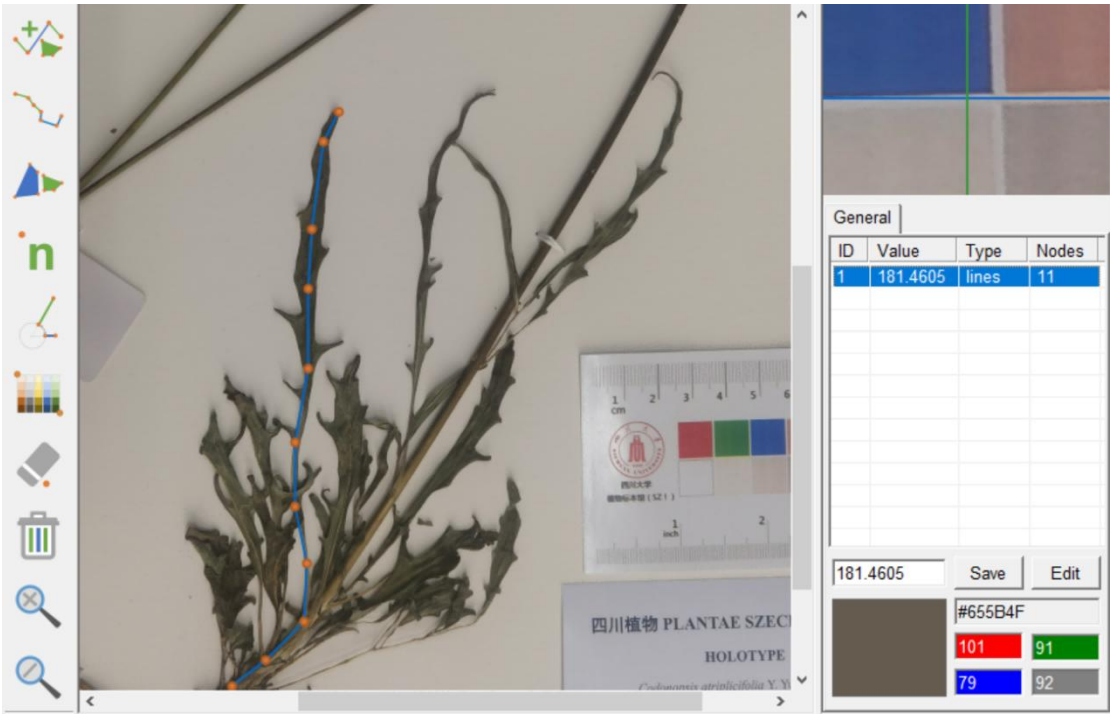



When selecting Karyotype:




Main menu:


Software operation area:




### 3.3 Main function module

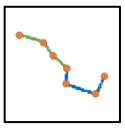
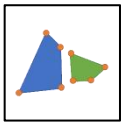
#### 3.3.1 Menu Bar

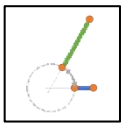

- **File:** The following options are available in the File menu: Load, Save, and New. Use Load to import an image or previously saved measurement data, use Save to save the current measurement data (in csv or txt format) or image (in jpg, png, or bmp format), and use New to clear the current operation and return to a blank state.
- **Edit:** The Edit tool is used to modify your measurement data, with the options to insert a new item, delete a selected item, or clear a selected item..
- **Graphic:** The Graphic menu is used to process imported images. The Rotate function can flip the image (90 ° , center symmetry, and axis symmetry), the Gray Scale function can convert the image to grayscale, and the Crop Image

function can cut out a portion of the image. The remaining four Correction functions (Vertical Correction, Horizontal Correction, Perspective Correction, White Balance Correction) can be used to correct issues that affect measurements, such as uneven or skewed images, perspective errors, and white balance errors (see 4.3 for more information on these correction functions)..

- **Mode:** There are two modes available for selection: Standard and Karyotype. The Standard modes is used for basic measurements, while the Karyotype modes is used for karyotype measurements..
- **Tools:** The Scales option can be used to set the ruler. The other two features, Group and Combine Results, are only available in Karyotype mode. The Group function can be used to automatically group chromosomes, and the Combine Results function can be used to merge the results of multiple measurements.
- **Option:** The display, size, and color of points and lines can be customized in this area. You can also choose to disable double-clicking and left-dragging.
- **Help:** The author's contact information and software references are displayed in this area.

### 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.

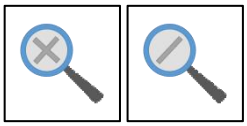
❖  (**Color**): Measure the color and display it in the color value box on the lower right.

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

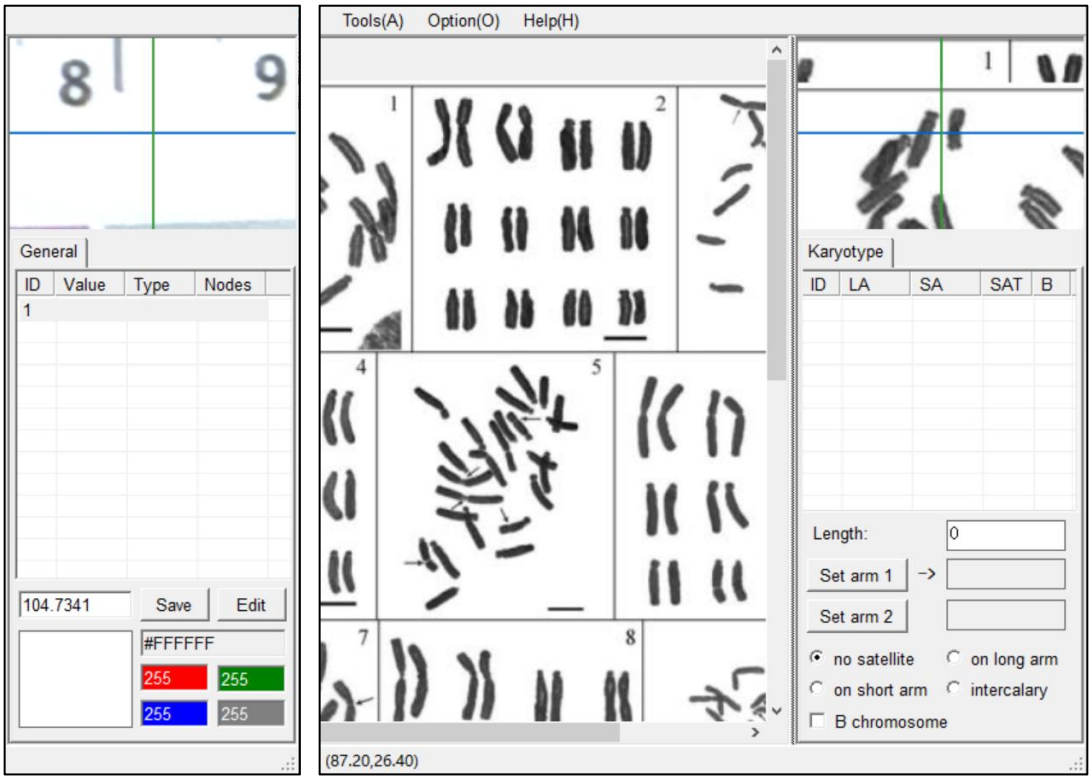
❖  (**Delete**): Delete the last row of data.

**Note: The Delete function removes items from the bottom up. To delete 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**: This area displays the ID number of the measurement step you are currently working on.
- **Value:** This area displays the values that you have measured in this step. The real-time measured value is displayed in the lower data box. When you click "Save," the value will be displayed in the Value column, indicating that it has been saved as a record for this ID.
- **Type:** This area displays the type of measurement you are currently performing: 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:** This area displays the total number of clicks for your current measurement step.
- **Others:** The **Save** and **Edit** functions allow you to manipulate the data being measured, while the color value box below shows the color of the point you are measuring.

Karyotype mode (see above right):

- **ID:** This area displays the ID number of the measurement step you are currently working on. Right-click to adjust the sequence.

  **Note: If you need to measure multiple cells of the same species and finally combine them, it is recommended to arrange all the measurement results from the largest to the smallest or from the smallest to the largest after the single cell measurement is completed, otherwise the final combination result will be wrong. Right-click to select automatic sorting.**
- **LA:** The abbreviation "LA" stands for "long arm" and is used to save the value of the long arm.
- **SA:** The abbreviation "SA" stands for "short arm" and is used to save the value of the short arm.
- **SAT:** The abbreviation "SAT" stands for "satellite chromosome." Satellite chromosomes are a type of chromosome that are distinguished by a secondary constriction at the tip and are characterized by the presence of nucleolus organizing regions (NORs). They contain less DNA than RNA and are typically found in heterochromatin regions. When you select "no long arm" after measurement, the value will automatically change to 1. If you select "no short arm," the value will automatically change to 2. When "intercalary" is selected, the value will automatically change to 3.
- **B:** The abbreviation "B" stands for "B chromosome" (also known as an accessory, supernumerary, or extra chromosome). "F" (False) indicates that there is no B chromosome, while "T" (True) indicates that there is a B chromosome. When the "B chromosome" column is checked, the value is automatically changed to "T".
- **Others:** The "Length" field shows the value you are currently measuring (note: the value units measured before calibration are not ruler units). The "Set arm 1/2" option allows you to set the length of the arm, which is the value displayed by the current length. "On satellite" should be selected if the measured chromosome has no satellite (this option is selected by default). "On long arm" should be selected

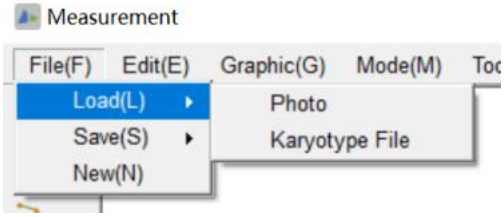
if the target chromosome being measured has no long arm. "On short arm" should be selected if the target chromosome being measured has no short arm. "Intercalary" should be selected if the target chromosome being measured has an intermediate deletion. "B chromosome" should be selected if the chromosome being measured is a B chromosome.

## 4. Examples

### 4.1 Examples of karyotype measurement and analysis

#### 4.1.1 Open an image

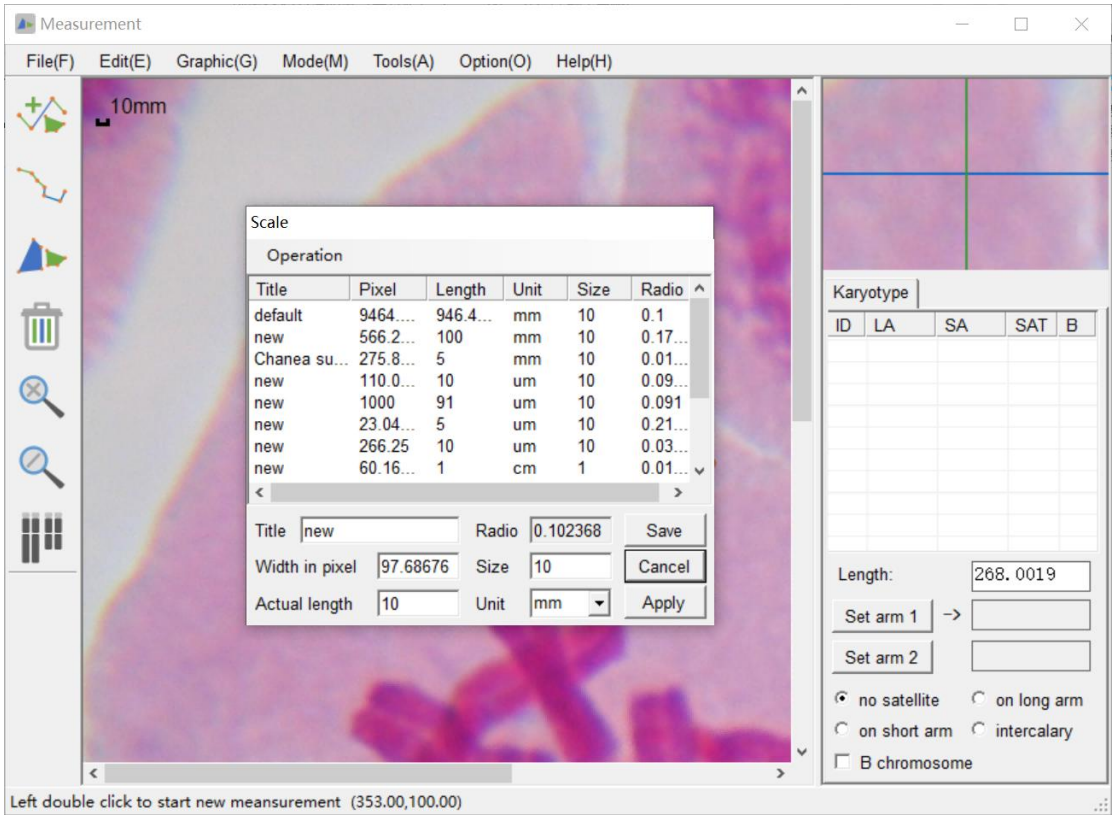
To open MATO, select the Karyotype mode, click "File->Load->Photo," navigate to the target image in the folder, and select "Open."




#### 4.1.2 Create and set a ruler

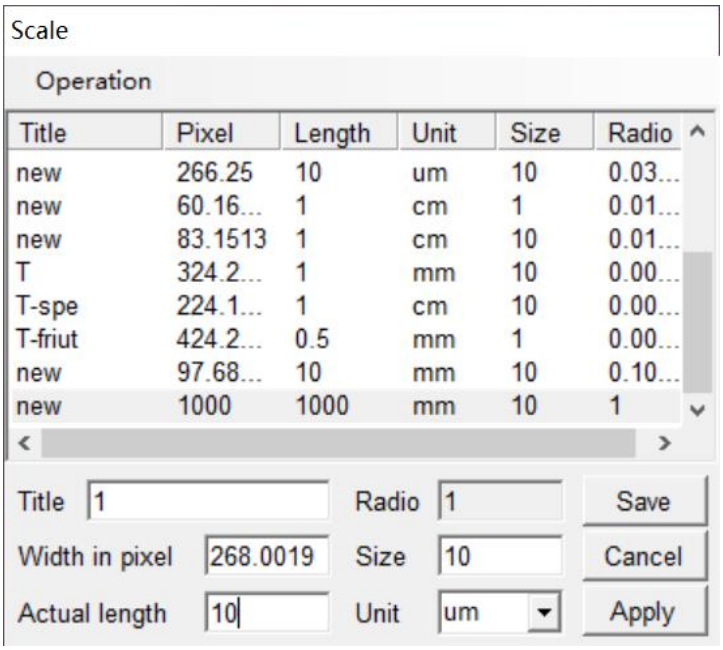
After importing the image, use the "Length" tool in the upper left corner to measure the pixel value of the ruler in the image, as shown below (268.0019).


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, you 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. You can verify the correction result of the ruler by using "Length" again to measure the length of the ruler in the image. In the following figure, the measurement result is "9.999999", indicating that the ruler has been successfully set.

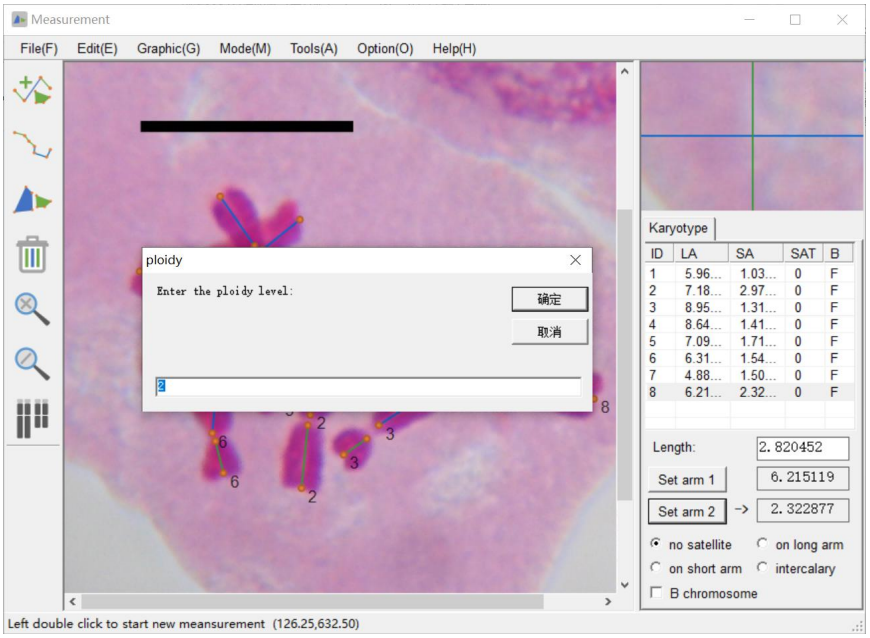

### 4.1.3 Chromosome measurement

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 mouse 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 2" to save it in SA. Refer to the instructions in 3.3.3 for the special options below.

**Note: If you need to measure multiple cells of the same species and finally combine them, it is recommended to arrange all the measurement results from the largest to the smallest or from the smallest to the largest after the single cell measurement is completed, otherwise the final combination result will be wrong. See 4.1.6 for detailed steps.**

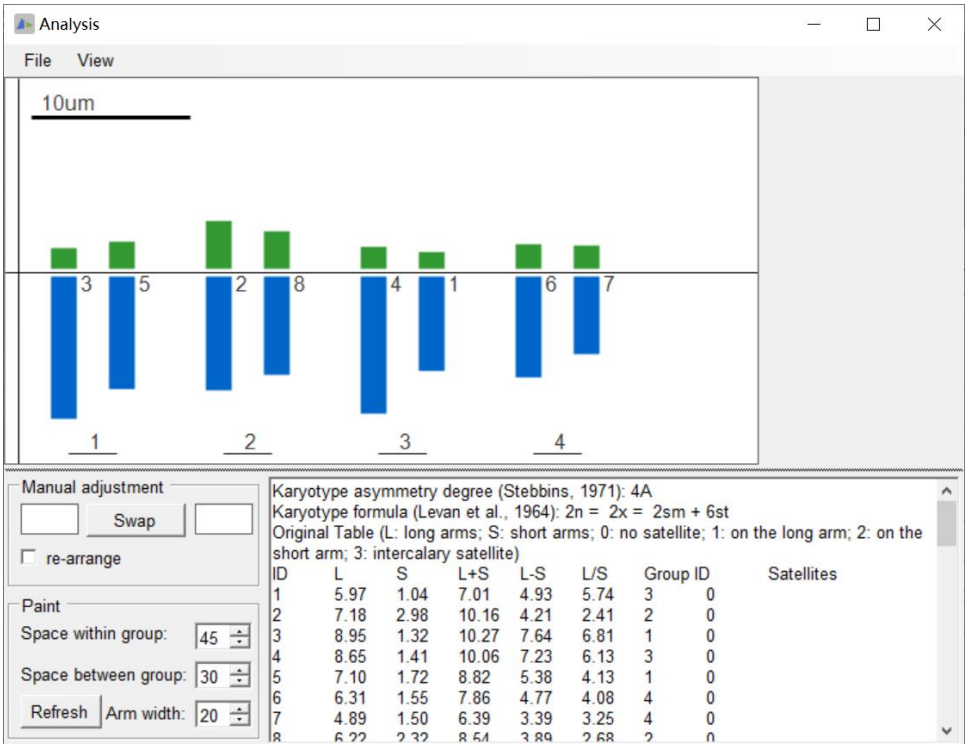

### 4.1.4 Chromosome grouping and Ideogram drawing

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

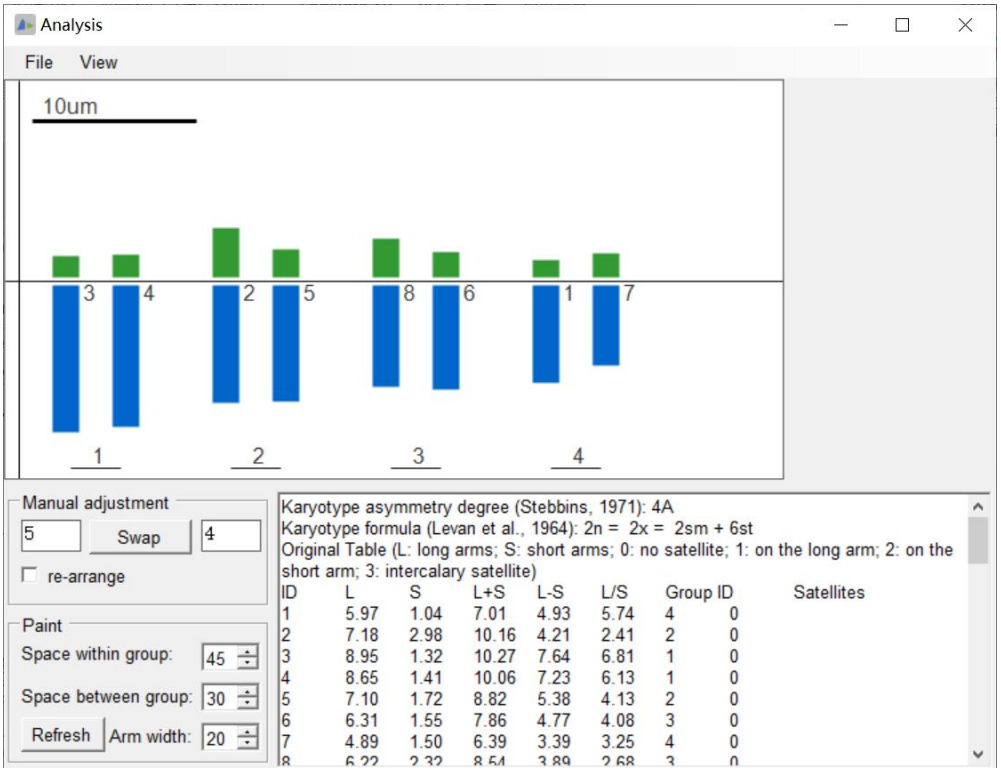



Then the ideogram and analysis results will automatically pop up. If you think there is an error in the grouping result, you can manually adjust it through "Manual

adjustment". For example, "4" and "5" can be swapped by filling in the blank spaces with 4 and 5 respectively, clicking "Swap," and they will be swapped.




The adjusted results are shown below.




**Note: Many of the karyotype asymmetry parameters in the result file are out of date. We have retained them but do not recommend their use. See the details in**

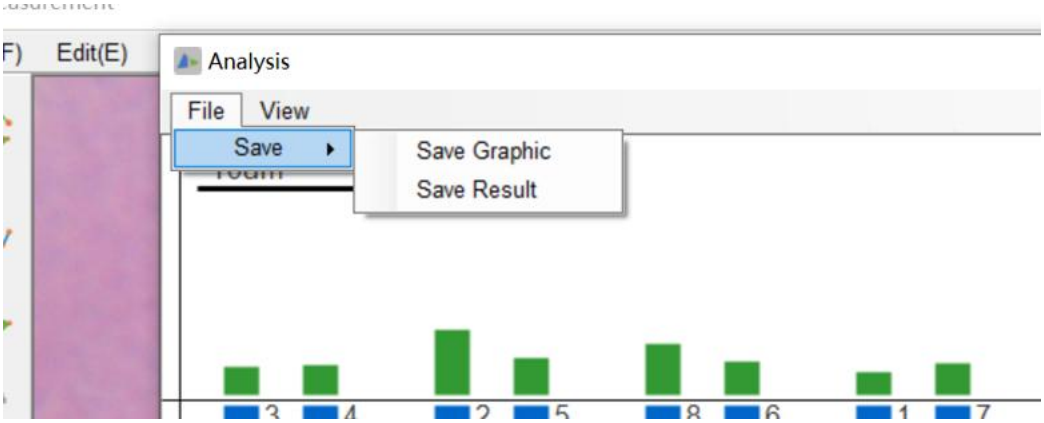
**the result file. The references are as follows:**

Astuti G., Roma-Marzio F., Peruzzi L., 2017. Traditional cytotaxonomic studies: can they still provide a solid basis in plant systematics? Flora Medit. 27: 91–98.

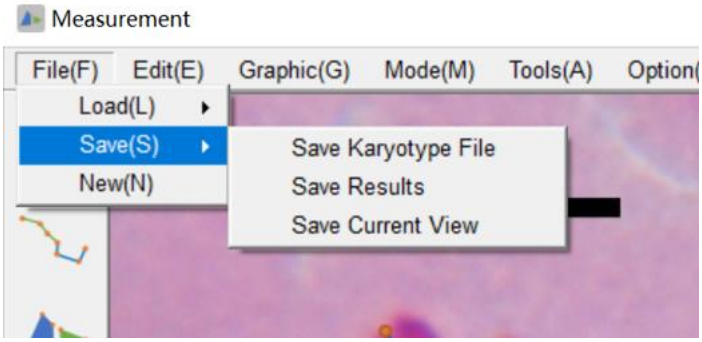
Peruzzi L., Eroğlu H.E., 2013. Karyotype asymmetry: again, how to measure and what to measure? Comp. Cytogen. 7(1): 1–9.

### 4.1.5 Data saving and loading

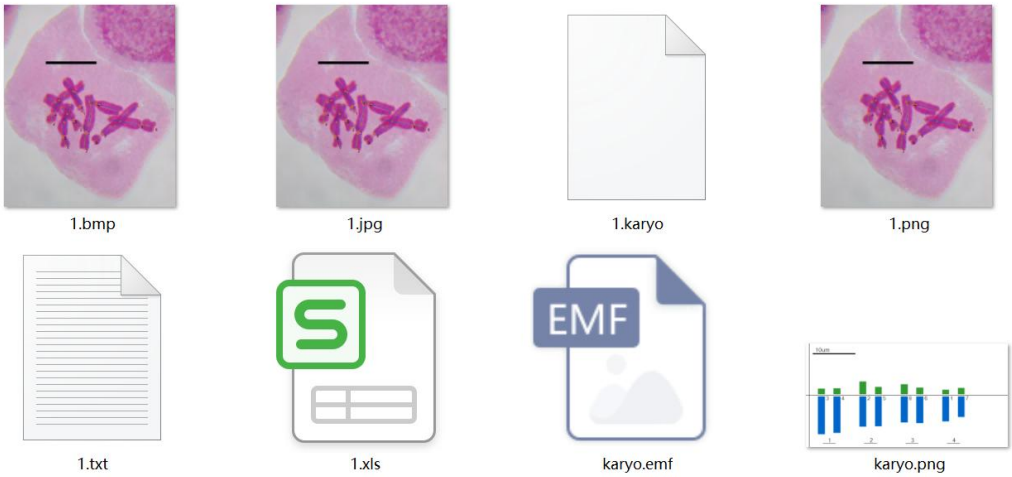
Click "File->Save" at the top left of the analysis results page, and two options will appear. "Save Graphic" is the ideogram that saves the upper-left corner; "Save Result" saves the analysis result in the lower right corner. The ideogram can be saved in png or emf format; the analysis results can be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



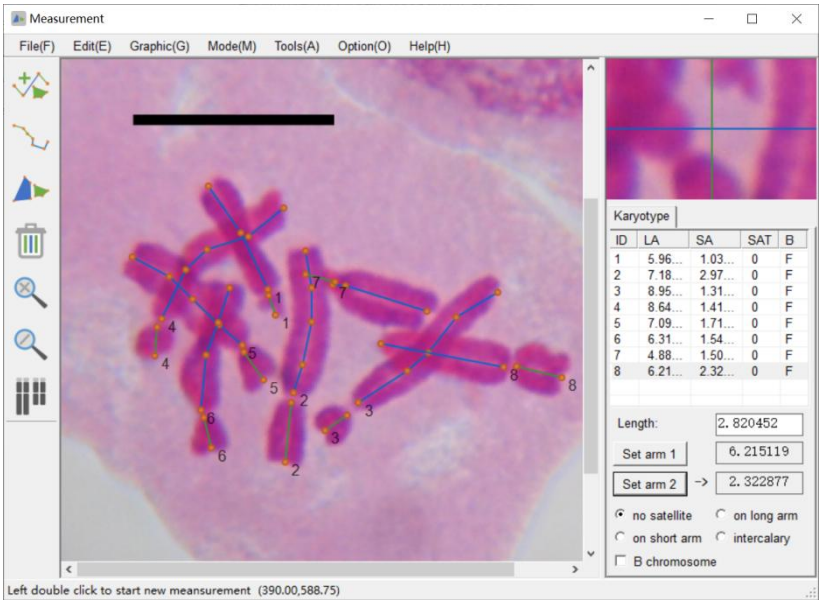

Close the analysis results page, click "File->Save" in the menu bar, and you will see three options. "Save Results" is also an analysis result file in xls or txt format, which is the same as the above "Save Result" option. "Save Current View" is used to save the current operation image in jpg, png, or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




Overall, the karyo file is the most important, and other results can be exported from it. The saved result file looks like this.




As shown in the figure above, '1.karyo' can be re-imported by going to “File->Load->Karyotype” File in the menu bar. The page that opens in Karyotype mode contains all the previous operations, which can be reviewed and continued. (Note: At this time, the Scale is still the ruler used in the last measurement. Remember to select the correct ruler set before continuing the measurement)



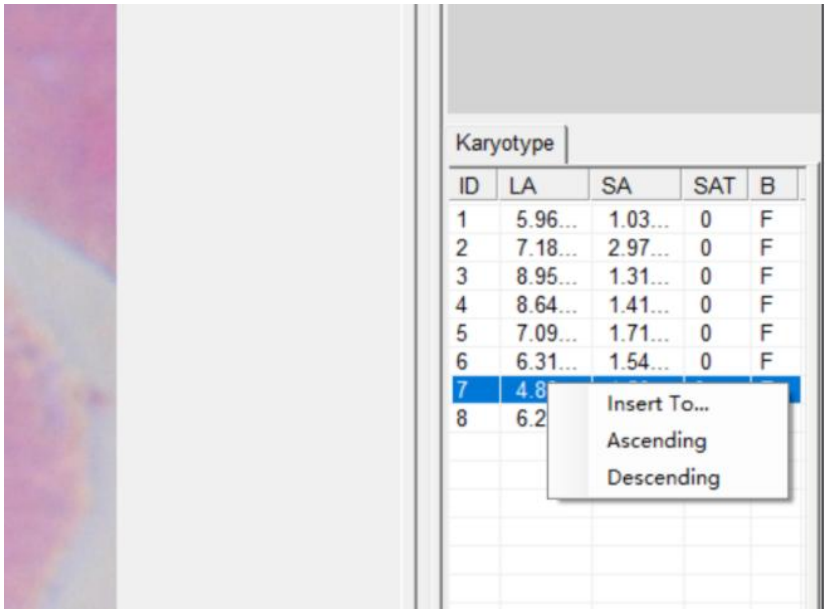

### 4.1.6 Combine multiple measurements

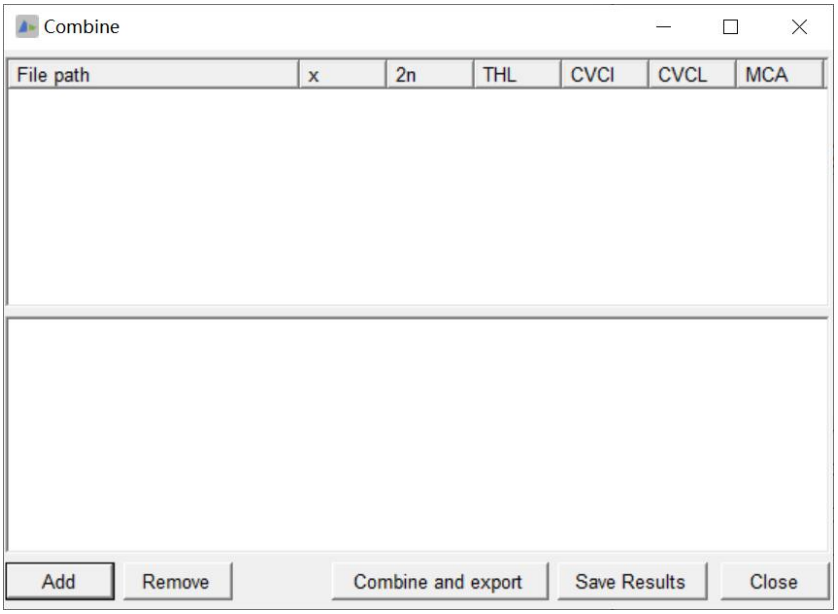
When measuring chromosomal karyotypes, we often measure multiple different cells. MATO can combine all of the results with just one click and summarize them into a single result.

**Notes: If you need to measure multiple cells of the same species and finally combine them, it is recommended to arrange all the measurement results from**

**the largest to the smallest or from the smallest to the largest after the single cell measurement is completed, otherwise the final combination result will be wrong.**

Click the right mouse button on the right functional area, and you can choose to sort all the measured data with one key. The size order of the data that needs to be merged must be uniform (both Ascending or Descending), otherwise, it will lead to serious error.




To combine the results of multiple measurements, follow these steps:

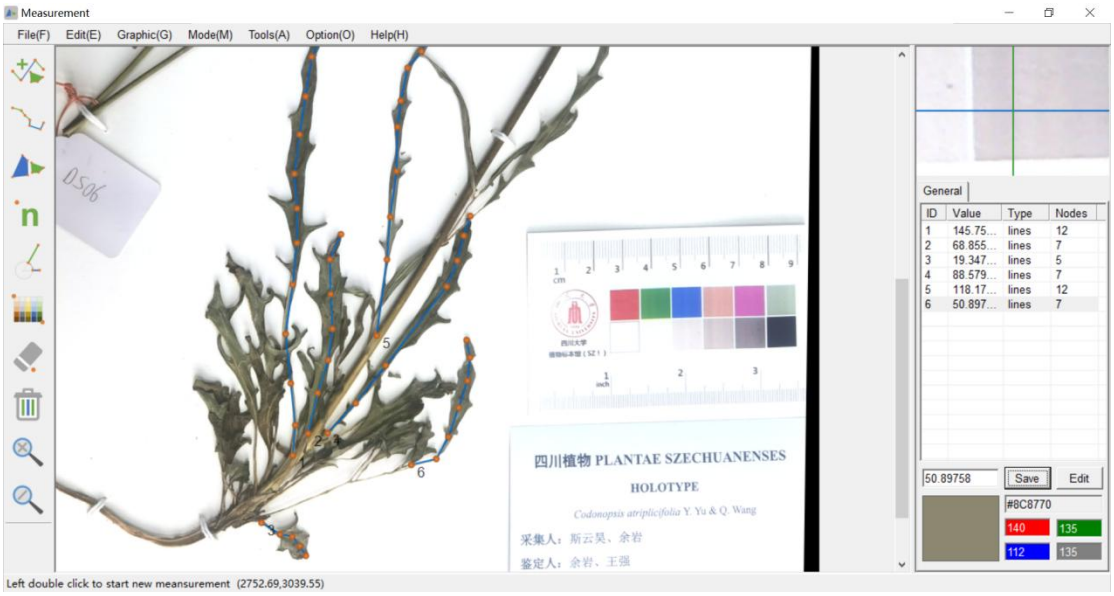
- From the menu bar, click Tools->Combine Results to bring up the action box.
- Click the Add button in the lower left corner to add the measurements (xls or txt files) in sequence.

- Click Combine and Export to merge the results and save them as a karyotype file.
- Open the karyotype file through MATO to export the karyotype diagram and other required data.
- Click Save Results to save the merged results directly as an xls or txt text file.

### 4.2 Examples of morphological Measurement

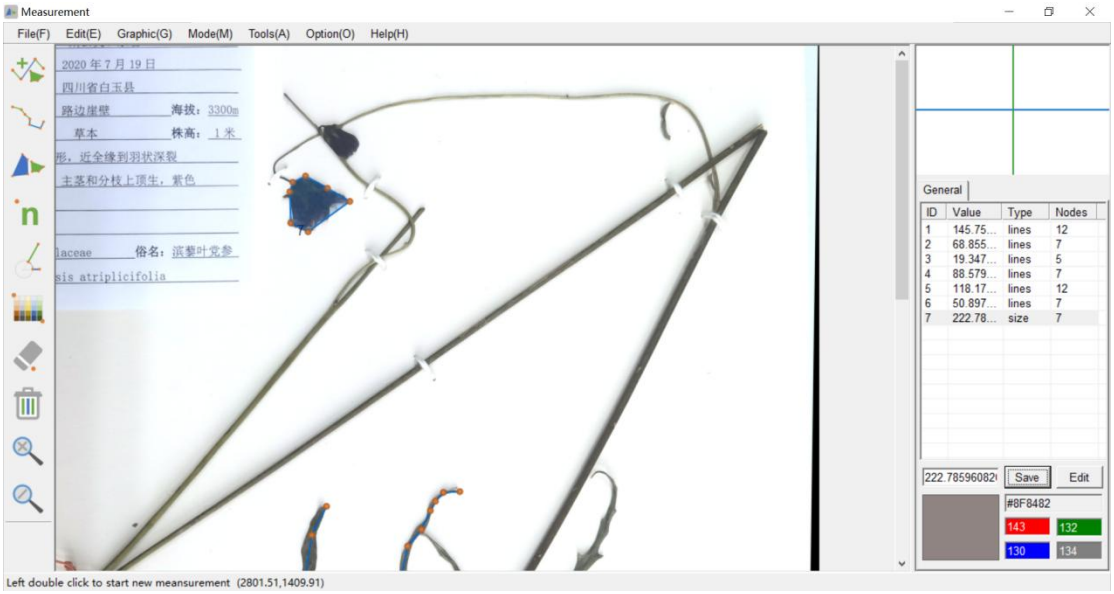
#### 4.2.1 Length measurement

To measure the length of an object in an image, select the second "Length" tool on the left. To begin measuring, click the left mouse button to select a point and the right mouse button to end the measurement. (Note: It is important to calibrate the scale before performing any measurements. See 4.1.2 for more information on scale calibration.)



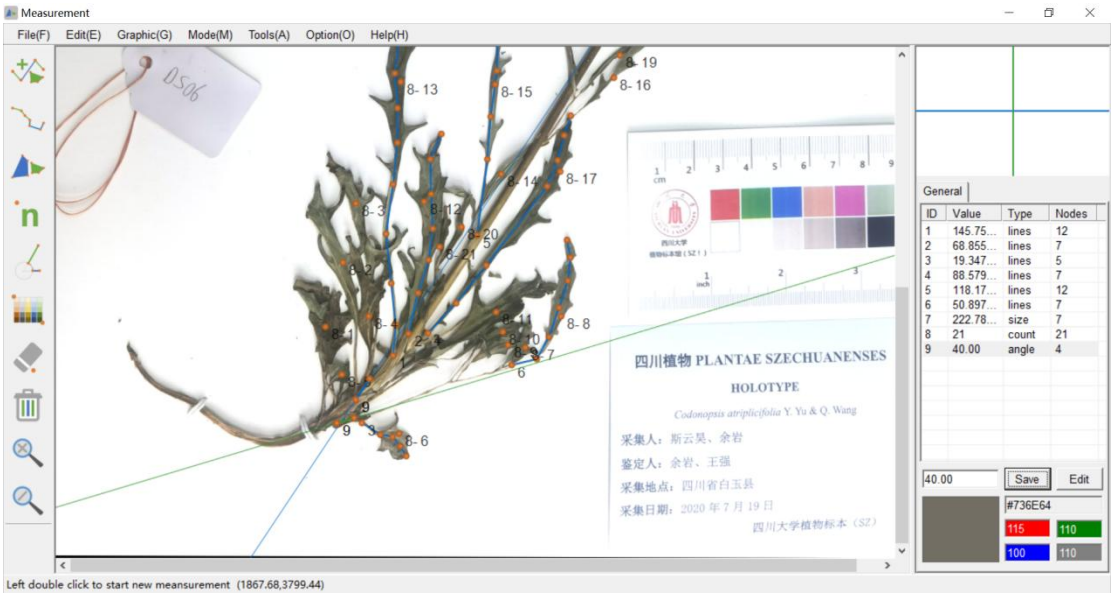

#### 4.2.2 Size measurement

To measure the size of an object in an image, click the third "Size" tool on the left. To begin measuring, click the left mouse button to select a point and the right mouse button to end the measurement. (Note: It is important to calibrate the scale before performing any measurements. See 4.1.2 for more information on scale calibration)




### 4.2.3 Angle measurement

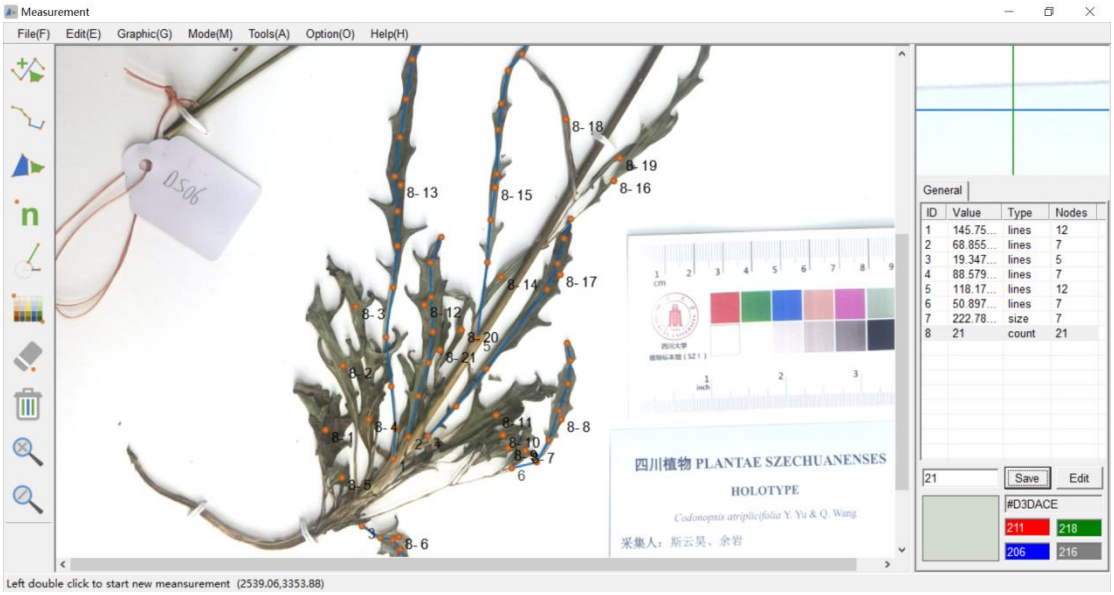
To measure the angle between two intersecting lines in an image, click the fifth "Angle" tool on the left. To measure the angle, click four points with the left mouse button to draw two intersecting lines. For example, if you wanted to measure the angle between the trunk and a small branch, you could roughly estimate that the angle is about 40 ° .




### 4.2.4 Count

To count objects in an image, click the fourth "Count" tool on the left. To begin counting, click the left mouse button, then click the left mouse button to select each point and the right mouse button to finish. The counting process is displayed as an ID

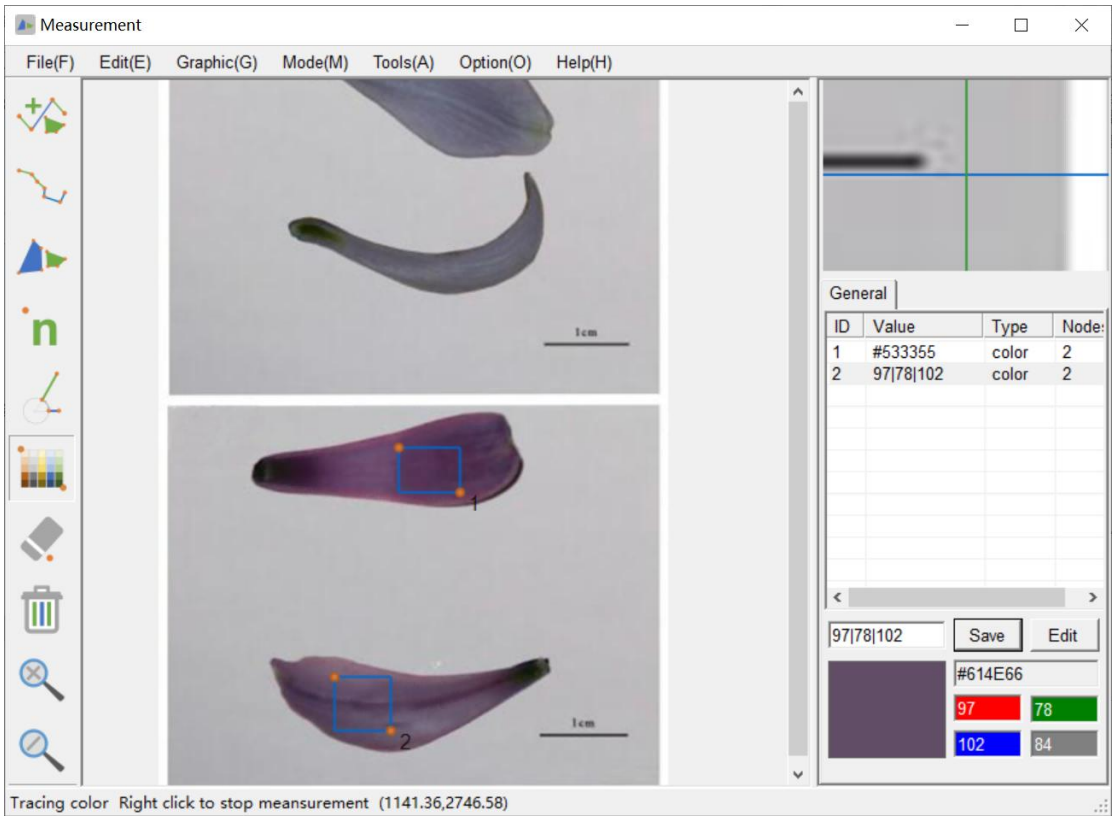
number and dot sequence number in the diagram. For example, if the specimen in this case has 21 leaves, the counting process would show 21 dots.




### 4.2.5 Get the color

To measure the color of an image, click the sixth "Color" tool on the left. There are six options for measuring the color content: grayscale value, RGB value, separate measurement of R/G/B, or measurement of hexadecimal color code. These options can be selected from the menu bar under "Option->Color Channel".

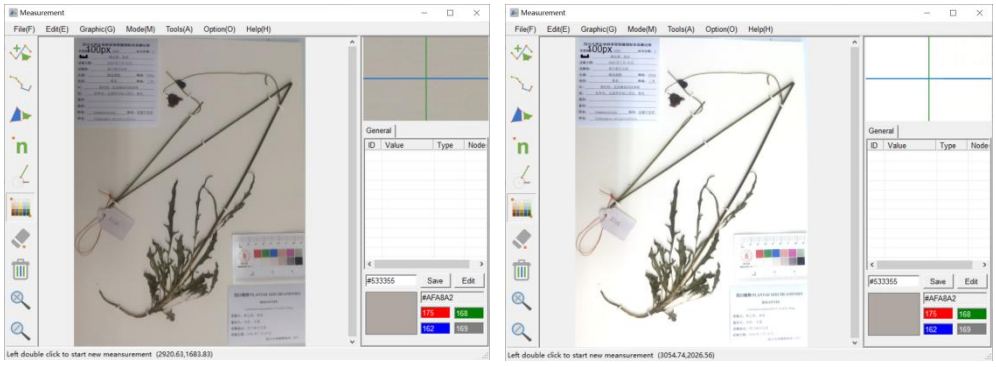
To select a rectangle, click the box twice with the left mouse button. The color value displayed is the mean value within the selected range.




### 4.3 Image correction example

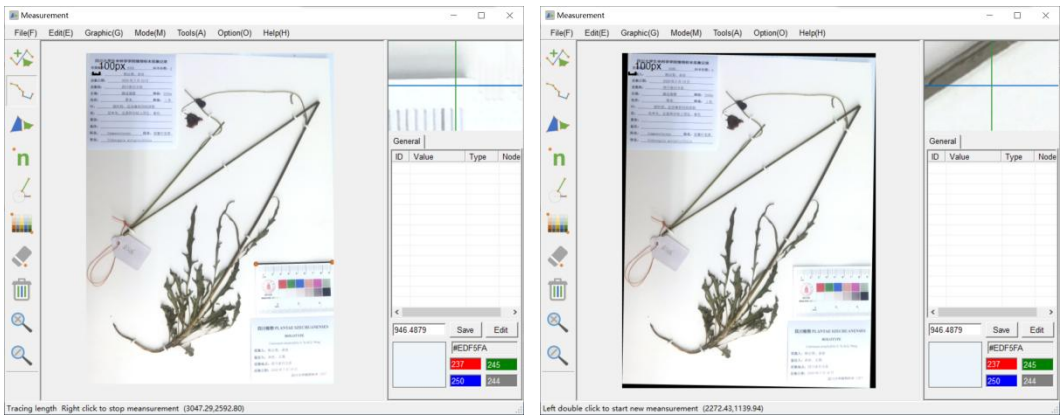
#### 4.3.1 White Balance Correction

To correct the white balance of the image, first import the image. Then, click on a part of the image that should be white (you can see the color in the lower right corner). Finally, click "Graphic->White Balance Correction" to apply the correction.



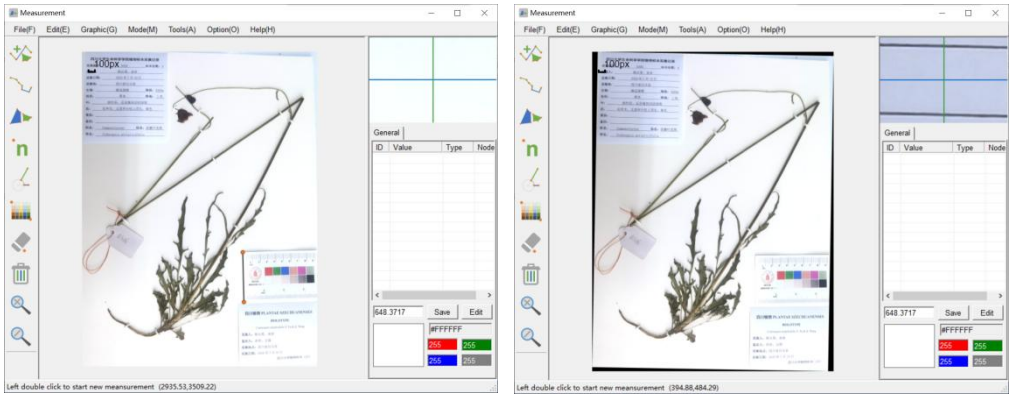
#### 4.3.2 Horizontal Correction

After importing the image, use the "Length" tool to draw a line segment that you confirm is vertical. Then, click "Graphic->Vertical Correction" to correct any vertical offset. It is assumed that the edges of the color card are vertical.



### 4.3.3 Vertical Correction

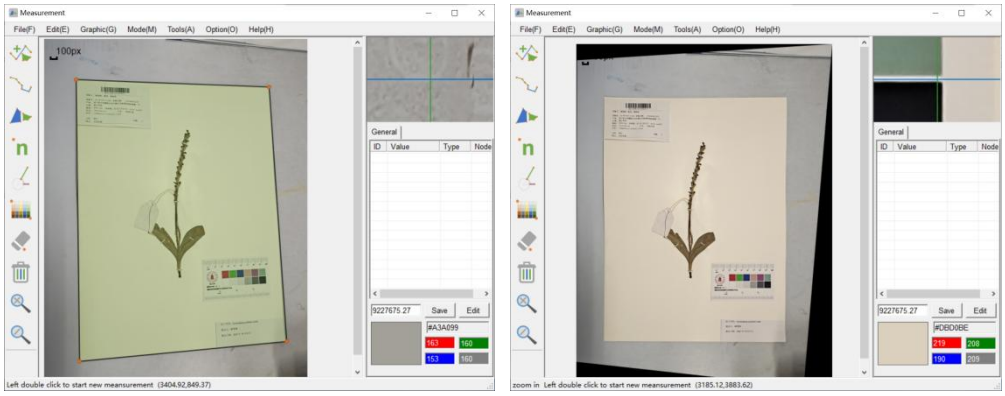
After importing the image, use "Length" to pull a line segment that you confirm is vertical, then click Graphic-Vertical Correction to correct the vertical offset. We also assume that the edges of the color card are vertical.



before and after

### 4.3.4 Perspective Correction

After importing the image, use the "Size" tool to locate the original four points of the specimen in order to create a plane. Then, use the "Graphic-Perspective Correction" tool to perform geometric correction. In the dialog box that appears, enter the correct aspect ratio. For example, if the herbarium sheet in this example is 30cm by 40cm, you can change the width of 2533 to 3000 and the length of 3330 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, ideally captured at the same angle. Additionally, it is helpful to include a reference object, such as a ruler or properly placed specimen color card, in the image to aid in image preprocessing. This can help ensure that the results of the analysis are accurate and reliable.

Q2: When do you need to correct an image?

A2: If the image to be measured appears too dark or has color offset, skew, or is not level, you can correct the image to reduce measurement error.

Q3: When do you need to adjust automatic grouping?

A3: If you notice that the automatic grouping of the karyograph is not accurate, you can manually adjust the results to improve their accuracy. It is generally best to measure chromosomes from small to large or from large to small to ensure that subsequent results can be combined effectively.