AnimalCol user manual

v 2.1.0

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I. General

AnimalCol is a user-friendly program whose aim is to facilitate coloration-related image analysis. It has been designed by and for biologists to handle numerous pictures at the same time while ensuring robustness and repeatability in color analysis.

AnimalCol is a Python-based software, designed for the Windows operating system. The AnimalCol installer can be downloaded from: http://vchiara.eu/index.php/animalcol or https://github.com/VioletteChiara/AnimalCol/releases

II. Projects

In AnimalCol, you will always be working inside a project. An AnimalCol project can be seen as a collection of images, each associated with various information. A project is associated with a ".acl" file, which can be open by the program.

1. Create a new project

To create a new project, go to the main menu "Project > New" and then specify the location and name of the project. From this moment a project will be created, where you can work and add new images.

2. Managing the projects

To save an open project, go to the main menu "Project > Save". This will replace the last saved version of the project with the current one.

Tip: *The shortcut <Ctrl + S> will also save the current project.*

The option "Project > Save as" will allow you to save the project under a new name, while keeping the old version accessible and unmodified.
To open a saved project, select "Project > Open" option.

⚠ ***Warning:*** *The program does not allow to have two different projects open at the same time. Note that opening a new project will automatically close the current one.*

III. Images

1. Managing the images

Once a project is created, images can be added using the "Images > Add new images" option from the main menu. The program will then ask for the location of the images. It is always possible to add more images later, using the same option. Images can also be removed from the project using the "Images > Remove current image" and "Images > Remove images" options.

Images can be in various formats (jpeg, png, tif, etc.) with no requirements for resolution or quality. There is no limit to the number of images in a project, but large projects may increase processing time and slow down the computer.

2. Image display

The current image is displayed in the left panel of the program, while thumbnails of all images are available in the bottom right corner. To move from one image to another, the buttons "Previous image" and "Next image" can be used. Another option is to use the scrollbar on the right of the thumbnails to display different images. Clicking on one of the thumbnails makes it the current image.

3. Interacting with the current image

Once an image is displayed in the left panel, several interaction options are available.

a. Zoom

To have a better view of a particular area, you can Zoom-in by hold the <Control> key + click where you want to zoom. You can also hold the <Control> key and click and drag to do zoom in a rectangular area. If the rectangle display is red, it means that the selected area is not suitable for a zoom-in. This may be because the selected area is too small or includes outside the image. To zoom out, hold the <Control> key + right click.

b. Scale definition

Defining the scale of an image will allow you to obtain data in international system units instead of pixels. By defining the scale of the images, you can also obtain uniform results across images.

To define the scale, move the scale points in each image (top left corner of the image) towards a known distance. Then write in the top right corner the real-life distance between these two points (Figure 1). Note that AnimalCol will use the same units for later analysis. For example, if you indicate the real-life distance in mm, then results data will be expressed in mm or mm^2.

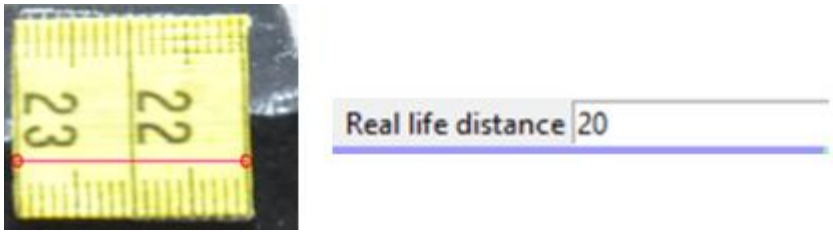

Figure 1. Illustration of how to place the scale points and corresponding real-life distance.

⚠ ***Warning:*** *The real-life distance used for the scale must be the same for all the images within the same project.*

c. Manual definition of targets

In AnimalCol, a target for analysis is defined as a Region of Interest (ROI) on the image. Most of the time, it is the element from which you want to extract information (see Figure 2 for illustration).

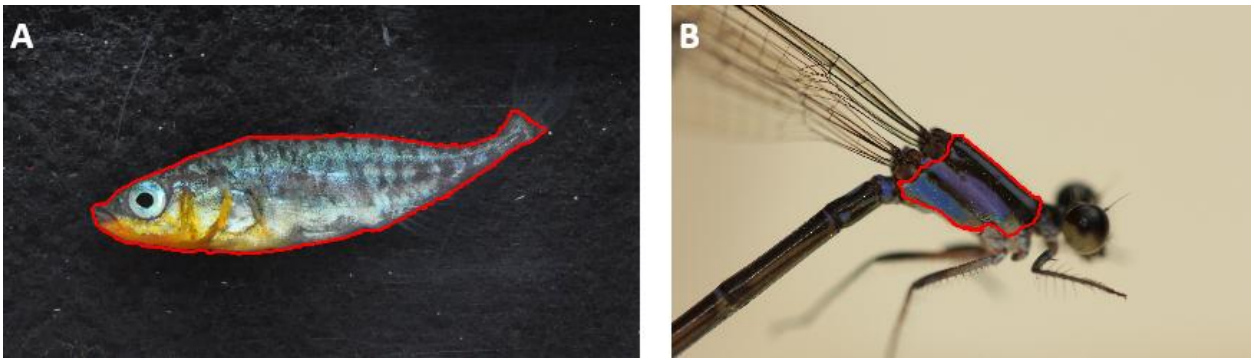

Figure 2. Illustration of how the ROI should be drawn according to the aim of the user. A) If one wants to know what proportion of the fish body surface is red, the Target is the fish itself. B) if one wants to know the average hue value of the damselfly thorax, then the Target is the thorax only.

> ***Tip:*** *It is possible to have more than one target per image. In that case, AnimalCol will analyze them separately.*

AnimalCol interface allows to directly draw the Targets on the current image. For this, two tools are available: the Pencil and the Polygon. To select one or the other, use the user panel on the right of the interface (see Figure 3).

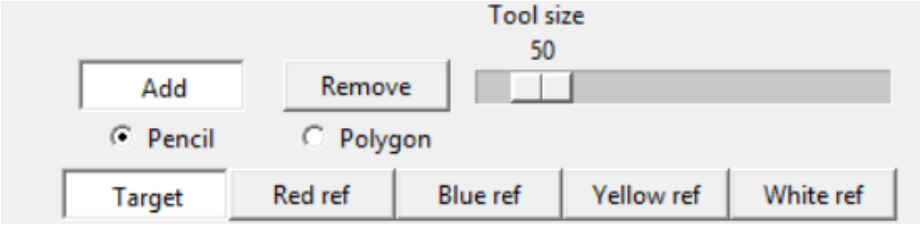

Figure 3. Tool options for drawing of the targets and color references.

The Pencil tool draws a continuous line around the mouse cursor while holding the left click, and the width of the line can be adjusted using the "Tool size" slider. If you use the right click instead of the left, the Pencil will work as an eraser and remove drawn areas. If the option "Remove" is selected instead of "Add", the opposite will happen (The left click will erase, while the right click will draw).

The Polygon tool allows you to select the target by clicking repetitively on the image to place points along the outline of the target, and then clicking on the starting point to finish the

selection. Like for the Pencil tool, using the “Remove” option instead of the “Add” one will erase from the selected area inside the Polygon. Figure 4 illustrates the difference between the two methods.

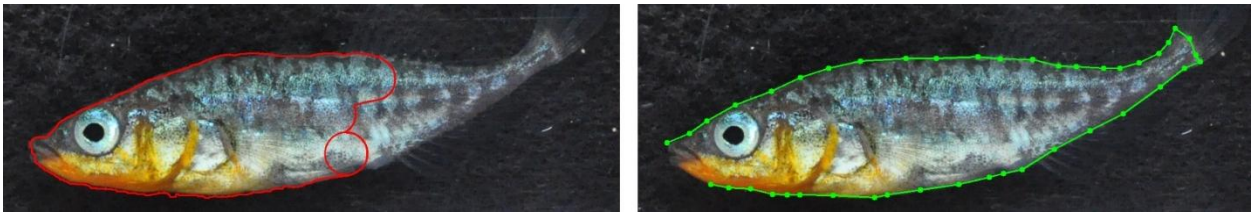

Figure 4. Illustration of the two target selection methods, on the left the Pencil is being used, while on the right the Polygon is being used.

> ***Tips:*** *If Targets are on a homogeneous background, they can also be found automatically using the automatic target detection option to speed up the target detection process (see IV. Automatic target detection).*

d. Manual definition of color references

AnimalCol allows you to also select color references. This step is not mandatory but may improve the control of between pictures homogeneity. The process of color reference selection is similar to the one for target selection, except that the corresponding color tool must be selected (see Figure 2, bottom line of buttons).

IV. Automatic target detection

When working with numerous images, manual selection of the targets may be time consuming. Thus, AnimalCol implements an automatic target detector. This detection method is based on background segmentation and works only with homogenous background that differs in color from the target.

To use this option, select in the main menu “Detection > Automatic target detection”.
A new panel will open, displaying the currently selected image.
Each time you click on the background around your target the color chart in the right part of the panel (called “Background color”) will add the new color selection. All the pixels with color characteristics falling inside this color chart will be considered as part of the background and excluded from a potential target selection. If the program finds a suitable target, it will be highlighted in pink (see Figure 5). Pixels that may belong to the target are highlighted in transparent red. If those are part of the background, clicking on them will add their color to the background color chart and exclude them from the potential target. Use the “Reset” button to reset this color chart to 0.

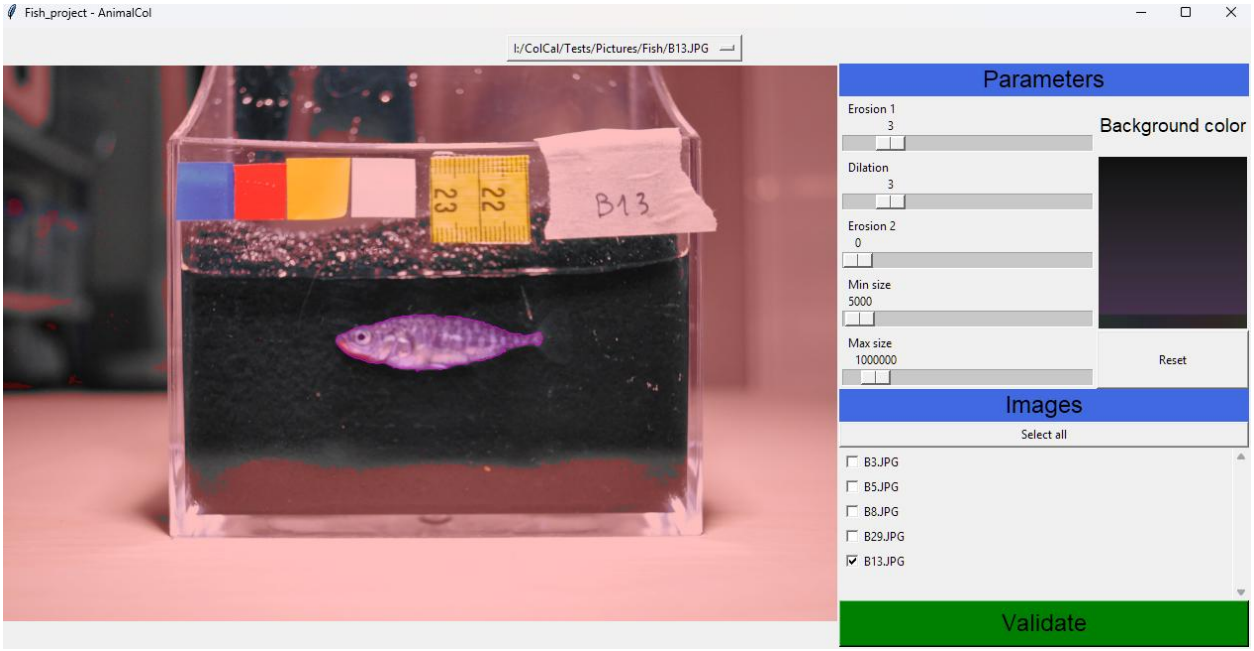

Figure 5. Panel of the automatic target detector.

Repeat this process with different images for which you want to apply this target detection until you are satisfied with the result (use the drop-down menu on the top of the panel to move from one image to another).

You can use Erosion and Dilation tools to smooth the target contour or remove small anomalies. Size filter can be used to restrict element size to be considered as targets (i.e. the target surface must be larger than the min size and smaller than the maximum size).

Finally, in the bottom right section "Images" of the panel, select the photographs for which you want to apply the automatic target detection, then click the "Validate" button.

> ***Tip:*** *If the contours of the target are not well defined, they can be adjusted manually (see III.3.c. Manual selection of the targets).*

V. Particles

It is often interesting to identify areas within an image that share similar color characteristics. In AnimalCol, these areas are called particles and are regions within the target. Specifically, a particle is any area inside the target whose color properties—hue, saturation, and value—fall within the ranges defined by the user (See Figure 6 for illustration).

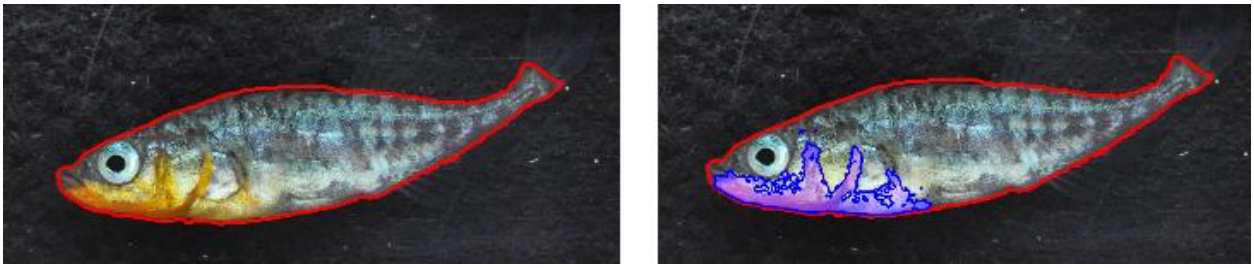

Figure 6. Illustration of the functioning of the particles. The left image is the target before particle selection. In the right we can see, highlighted in blue the particles found by AnimalCol that matches with the user color range requirements.

1. Defining color range

Defining the particle color range can be done by three different methods.

a. Selection from the color chart

In the "Color selection" panel (Figure 7A): click and drag in the color wheel to select the Hue range of interest, idem for Saturation and Value.

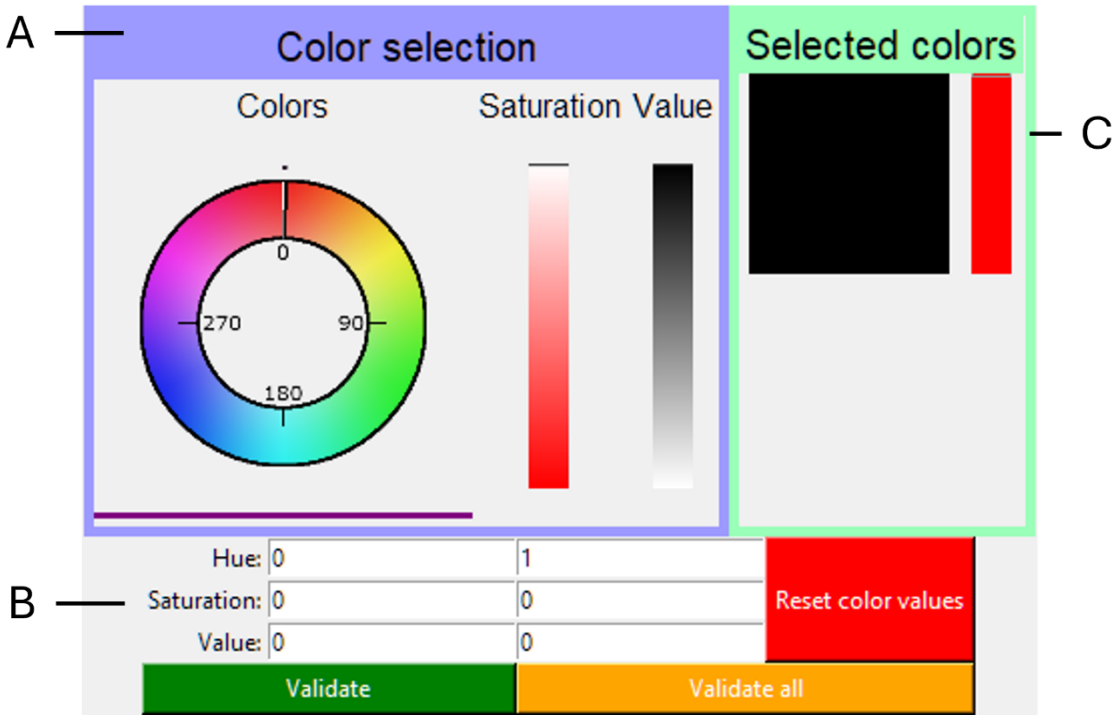

Figure 7. Options used to define the range of Hue, Saturation and Value for the particles. A) Selection based on color chart, B) Entries for value manual input, C) All colors falling within the selected range.

b. Manual entry of the HSV values

Specify the minimum and maximum Hue, Saturation, and Value in the Entries bellow the "Color Selection" panel (see Figure 7B). Only particles within these ranges will be selected.

c. Using the color picker tool

To facilitate color selection, the final option allows you to directly specify on the current image which colors of pixel should be included in the particles. To use the color picker, hold the <Shift> key and left click on a pixel in the image that you want to include the particle range. The range will then expand to include that pixel. Repeat this process as many times as necessary until the color range includes all the colors of interest. Conversely, if you want to exclude pixels from the selection, hold the <Shift> key and right click on the image.

2. Validate and Export Particles

The "Validate" button bellow the "Color selection" panel calculates and displays the selected particles for the current image, while "Validate All" performs the calculation for all images in the project (see Figure 7). Particles will be displayed in blue on the image (Figure 6). Once the calculation is complete, you can export the results by selecting from the main menu "Detection > Export Particles", then choosing the location where the result file will be saved.

⚠ ***Warning:*** *If the computer is too slow when defining targets or particles, disable the Auto-update of particles option in the "Options" menu to improve performance.*

VI. Results data file

The exported **.csv file** provides a summary of the collected data. It can be opened with standard spreadsheet applications such as **Microsoft Excel** or **LibreOffice Calc**. The file uses "." as decimal delimiter and ";" as column separator.

The table contains the following columns:

File:
The name of the image file.

Type:
This indicates the source of the data in the corresponding row. Possible values are:

- **Target**: All pixels inside the target region (ROI).
- **All_particles**: All pixels belonging to particles contained within the target.
- **Particle**: Pixels belonging to a single particle.
- **Reference color**: Pixels belonging to a reference color ROI.

Target_ID:
The ID of the target associated with the data.

- If the image contains a single target, this value is always 0.
- Reference color entries have no Target ID, since they are independent of targets.

ID:
A unique identifier for individual **Particles** and **Reference colors**. This column is empty for other types.

Area:
The surface area of the pixel group, expressed in the squared units defined by the user.

Mean_Hue:
The average hue of the pixel group. Since hue is circular (0° = 360°), this value is computed using circular statistics (*circmean* from *scipy.stats*). For more information: https://docs.scipy.org/doc/scipy/reference/generated/scipy.stats.circmean.html).

Mean_Saturation/Value:
The average saturation or value of the pixel group.

For more information about the interpretation of these color characteristics:
https://www.lifewire.com/what-is-hsv-in-design-1078068