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AnimalCol: A tool for quantitative color analysis of digital images

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Software

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Summary

`AnimalCol` is a tool for analyzing animal coloration using standard digital photographs. It is a standalone Python program developed for the Windows operating system and comes with a straightforward installer. `AnimalCol` relies on several packages: OpenCV for image management and analysis, tkinter for the Graphical User Interface (GUI), and scipy for basic descriptive statistics of image characteristics. The `AnimalCol` code is open source and available at a GitHub repository (https://github.com/VioletteChiara/AnimalCol) where detailed guidelines are provided. A dedicated webpage is also available at http://vchiara.eu/index.php/animalcol, where the installer, guidelines and general information are provided.

Statement of need

Animal coloration has been extensively studied by biologists in both laboratory and field settings. For instance, skin, fur, feather, and scale colors play key roles in sexual signaling, establishing social status, protection from ultraviolet radiation, predator and parasite avoidance, or thermoregulation (Cuthill et al., 2017). Unlike microorganisms and plants, obtaining biological samples from animals often raises methodological and ethical issues. Therefore, photographic images have become increasingly useful resources for analyzing variation in animal coloration (Potash et al., 2020). To ensure accurate and consistent measurement of animal coloration, researchers typically standardize camera position and light conditions, and photograph specimens alongside color and scale references.

Some commercial programs (e.g., Las X and X-Rite Suite) and open-source programs (e.g., Image J and CellProfiler) also analyze color in digital images. However, many of these programs are designed for sophisticated analysis of high-resolution microscopic images or require additional plugins and customized settings. Thus, `AnimalCol` was developed to provide biologists with an open-source, stand-alone, and user-friendly tool for efficiently analyzing animal coloration from standard digital photographs.

Features

Features `AnimalCol` features a complete and user-friendly GUI (Figure 1) in which color hue, saturation (intensity) and value (brightness) charts are presented to help understand the technical characteristics of the loaded images. Users can define Regions of Interest (ROIs, Figure 1C) and set the scale directly on their images (Figure 1A). `AnimalCol` proposes a project-like organization, which allows users to load and analyze a number of images simultaneously within a single project.

`AnimalCol` also features a semi-automatic ROI detection based on background segmentation

to rapidly distinguish the target animal from the background. Users can also select color characteristics to find all matching regions on the image as particles (Figure 1D).

The program exports extracted data as a .csv file, which summarize: i) the ROI's area and mean color characteristics (Hue, Saturation, Value), ii) a summary of the same characteristic for all the particles, iii) a summary of the same characteristics for each individual particle.

Examples: Studying the sexual coloration in a fish and an insect

To illustrate the workflow of `AnimalCol`, we show the example of the analysis of red sexual coloration in male three-spined sticklebacks' cheek. The images used and presented in Figure 1 are from (Chiara et al., 2022) in which a preliminary version of `AnimalCol` was used. Here, we used `AnimalCol` to calculate for each male the area and ratio of red colored surface over the whole-body area.

Step 1. Project preparation

- Launch `AnimalCol` and create a new project (Project > New)
- Add the images to be used (Images > Add new images)

Step 2. Definition of the ROIs (Figure 1C)

- Click the Detection > Automatic target detection menu.
- Click on the background several times until the fish area is highlighted in pink. Tools of erosion, dilation and
- surface filters can also be used to improve results.
- Click the "Select all" button in the "Videos" section and validate.
- If necessary, manual correction of the ROIs can be done by clicking on the images.

Step 3. Definition of the Scale (Figure 1A)

- Move the two points on the top left corner of the images to set a known distance, for example using a reference scale on the image.
- Fill in the "Real life distance" entry on the top right corner of the program with the known distance between the two points.

Step 4. Particles detection and extraction

- Use the color chart to select the color of interest (in the example, hue 340–60, saturation 100–255, and value 25–255, Figure 1E).
- Click the "Validate all" button.
- In the main menu, select Detection>Export particles and select a destination location. The results will be saved as .csv file.

The ranking of the fish according to the red ratio can be seen on Figure 1.

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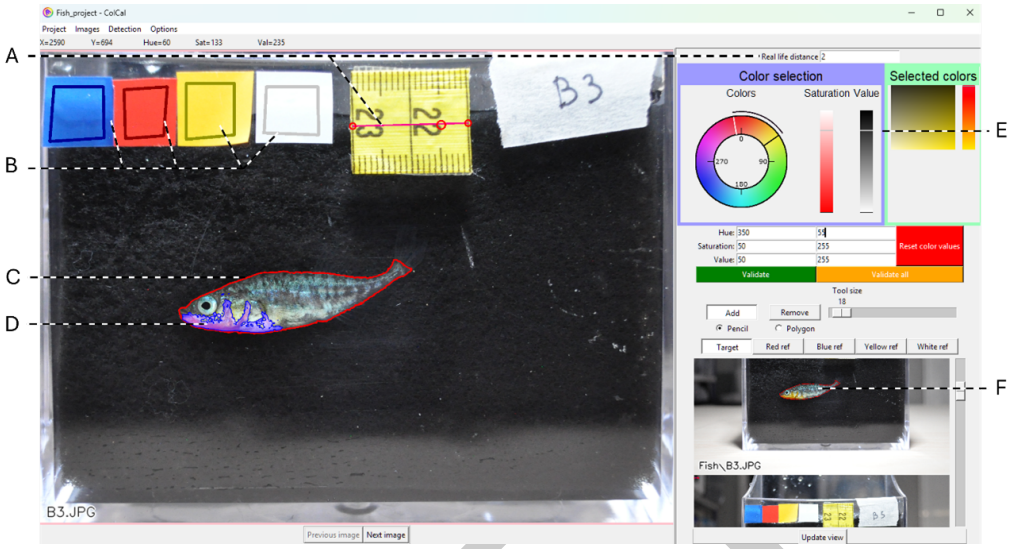

Figure 1: Illustration of AnimalCol interface. A) Scale information defined by user. B) Color references used to ensure that all pictures were taken in similar context. C) ROI delimitation. D) Particles found by the program that match the color settings. E) Color settings defined by the user. F) Image displayed in the miniature showing the picture before applying AnimalCol color selection.

`AnimalCol` can also be used for numerous purposes such as counting spots, calculating the mean hue value of ROIs, the intensity of specific areas, etc. In Figure 2, we illustrate how damselflies are ranked according to their thorax mean hue analyzed by `AnimalCol` (images from Sanmartín-Villar et al. (2017)).

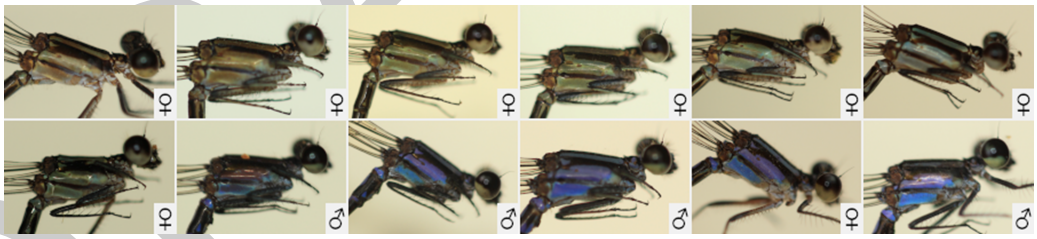

Figure 2: Damselflies ranked according to the AnimalCol results of the average hue value on their thorax (from left to right, and top to bottom). In this species, males have only a blue phenotype, while females have green or blue phenotype.

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