# Segmentation of *Cloeon dipterum* gills

*Carlos Antonio Martín Blanco*

*31 de enero de 2019*

**AUTHORS: This pipeline was designed by Antonio Ruiz Sobrino. The macros and how to use them were made by Carlos Antonio Martín Blanco.**

**NOTE: This macros are made to be used in Linux, in order to use them in Windows they have to be slightly adapted.**

FIJI ImageJ 2.0.0-rc-61/1.51n; Java 1.8.0_66[64]bit Linux UBUNTU Version: 16.04.4 LTS (Xenial Xerus)

**DESCRIPTION:**

This pipeline consists of 5 different ImageJ macros that have to be called in order, and at least once to perform the segmentation. It needs a mother directory (for example called "TEST"), and a subdirectory called "selected" with the images to segment. It also needs a previously trained classifier model for the trainable weka segmentation, which is provided, and it is called "V5.model".

Macros names, and order to use:

- 1st - orient_crop.ijm
- 2nd - weka.ijm
- 3rd - correct_skeletonize.ijm // correct_skeletonize_2.ijm (optional)
- 4th - get_area.ijm
- 5th - Skeleton_Area.ijm

---

**MACROS explained:**

**orient_crop.ijm**

**Function:**

This macros sets the right scale, size, and orientation of the image for future analysis. It also creates the output directories for all the steps in the segmentation. It consist on two steps: the first is to orient the image, and the second one is to crop the image. The oriented image is saved in .tiff format in "steps/Oriented/" subfolder. The cropped image is saved in .tiff format in "steps/cropped" subfolder.

**Usage:**

When runned, it first asks to select the mother directory. Then it asks the user to orient the images or to skip this (skip only if the step has been already performed). If the user clicks ok, it calls the rotation of the image in order to orient the gill with the base of the tracheal tree at the bottom of the image. (NOTE: it is recommended to select preview) Then it asks the user to crop the image by clicking ok, or to skip this step for each image by clicking shift + ok in the dialog box, if the user cliks ok, it asks the user to select a point in the image for cropping, this point has to be the upper left corner for the desired cropped image. (NOTE: sometimes the user can fail at this step. Therefore the dialog box asking to skip the previous step or each image).

---

**weka.ijm**

**Function:**

This macros calls trainable Weka Segmentation using a previous trained classifier model to perform an automatic segmentation of the trachea of the gills. The segmented image is saved in .tiff format in "steps/classified" subfolder. (NOTE: this step never produces a perfect segmented image).

**Usage:**

It asks the user to select the mother directory, and then it asks the user to choose a model.

---

**correct_skeletonize.ijm**

**Function:**

This macros allows the user to correct the weka classified image, saving the corrected image in a subfolder in case it has to be revised again. Then it performs the skeletonization of the classified image. The corrected image is saved as .tiff in "steps/BnW/" and "steps/BnW_corrected/" subfolders. The skeletonized image is saved as .pgm in "Skeleton/" subfolder.

**Usage:**

First, it asks the user to select the mother directory. Then it opens the classified image from the previous step along with the cropped image from the first step ( This allows the user to see how well the classified image is), and it asks the user to correct the classified image if wanted or to skip this pressing shift and clicking ok on the dialog box. If the user clicks ok to correct the image, it adds the classified image to the cropped image with certain opacity (60%, this can be changed in the macros), then it selects the pencil tool and a new dialog box appears asking the user to connect the tracheal branches that are disconnected, and to click ok when finished. After this it asks to remove the false connected trachea or any other artifact in the image that is not a trachea and to click ok to finish. It does this with all the images and then it automatically performs the skeletonization.

**NOTE1**: for large artifacts the user can change the tool, for instance the brush tool. **NOTE2**: any pixel that contact by a corner is connected. **NOTE3**: in case that the user cannot continue the correction at the time, the user can just click ok without finishing and then use the optional macros to continue. This is correct_skeletonize_2.ijm

---

**correct_skeletonize_2.ijm**

**Function:**

This macros is an optional one. It can only be used if first correct_skeletonize.ijm has been used. It opens the previously corrected image along with the cropped image for revision, (or just in case that the correction was not fully performed). Then it performs the skeletonization on the new corrected image. The corrected image is saved as .tiff in "steps/BnW/" and "steps/BnW_corrected/" subfolders. The skeletonized image is saved as .pgm in "Skeleton/" subfolder.

**Usage:**

Same as the previous one.

---

**get_area.ijm**

**Function:**

This macros asks the user to get the area of the gills from the images in the cropped folder. It saves the area as .roi in "steps/Area/" subfolder.

**Usage: First it asks the user to select the mother directory.**

Then it asks to get the area for each image in the folder by clicking ok, or to skip the image clicking shift + ok. If the user clicks ok, it selects the polygon tool and asks the user to pinpoint the area of the gill, and once finished to click ok.

---

**Skeleton_Area.ijm**

**Function:**

This function combines the .roi file to the skeleton.pgm file to have a final file for analysis with Antonio's program. It saves the resulting image as .pgm in "Skeleton_Area/" subfolder.

**Usage:**

It just asks the user to select the mother directory.

.