

Effortless Counting of Sorted *Culicoides* from Light-Traps

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Counting *Culicoides* from sorted light traps may be tedious when numerous samples are being studied. Despite advancements in artificial intelligence (AI) and the availability of AI-based applications for object counting, the technique remains costly or difficult to implement in a straightforward manner. We propose a simple, cost-free, and easy-to-implement method for counting *Culicoides* which relies on the utilization of the freeware **IMAGEJ (FIJI)**.

The method can be used on a computer with **IMAGEJ** installed or directly on a **cell phone** using the **IMAGEJ.JS** program.

Culicoides specimens are placed in a small amount of alcohol, on a white dish.



How does it work ?



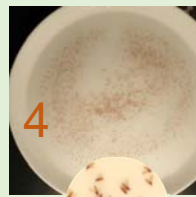
Take your photo with a cell-phone and import it in ImageJ or ImageJ .JS



In ImageJ, use the command Filter GAUSSIAN BLURR



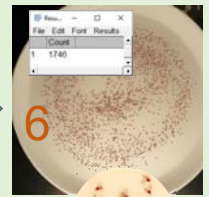
Apply the command Filter MEDIAN



Apply the command SUBTRACT-BACKGROUND



Delineate the counting area with the FREEHAND TOOL



Apply the command FIND MAXIMA to count the insects

We have developed a small MACRO that includes all the steps with their corresponding parameters to facilitate image processing.

The MACRO is particularly useful with IMAGE.JS on a phone, where screen manipulation with the menu bar is difficult.

Example result

- Manual counting with stereomicroscope: 1750
- Automatic counting with ImageJ : 1746

Agreement of ImageJ counting with exact manual counting using a stereomicroscope was evaluated using **Bland-Altman analysis** (Fig. 1) and **Passing-Bablok regression** (Fig. 2) using 91 pairs of counts from 10 to 2200.

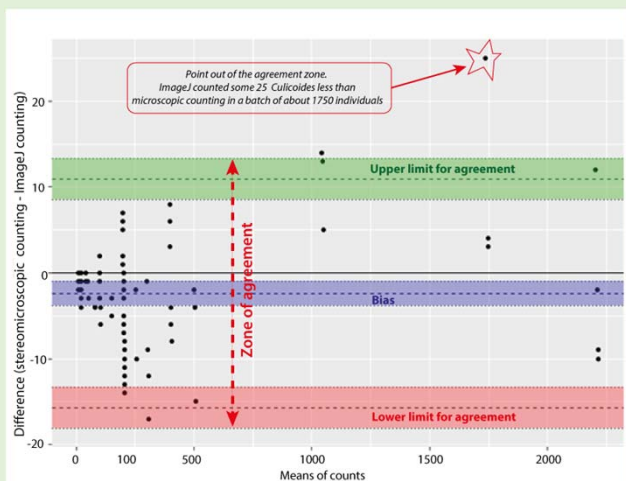


Fig. 1. Bland-Altman plot for comparison of stereomicroscopic and ImageJ countings

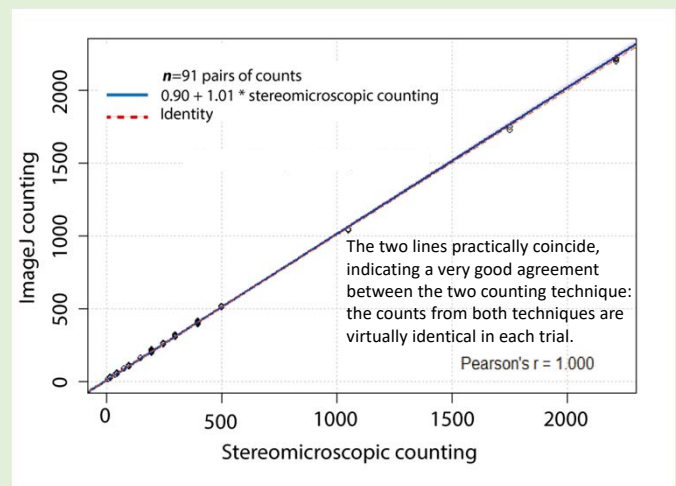


Fig. 2. Passin-Bablok Regression Fit

Conclusion

With a clean sample where individuals are not overcrowded, the ImageJ method is as precise as standard manual counting and can thus be used in routine analysis.

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For details on parameters for ImageJ commands, and optimal conditions of use of our method, please refer to this document.

