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Automatic Image Processing

AIM

Measuring Collembolan body size using stereo microscope is a complicated and time-consuming process. In case of alive animals the measurable length may vary as they move, and as a consequence it is necessary to repeat both taking pictures and measuring individuals.

Our aim was to develop a device which can be used for automatic body size measurements of living microarthropods in an easy and less time-consuming way, both in the laboratory and on the field.

CollScope DEVICE

The device consist of a

- **camera:** 3.3 MP color CCD camera(TCC-3.3ICE-N, ICX412AQ Sony CCD),
- **lens :** a Tamron Mega-pixel Machine Vision CCTV (23FM25SP Focal length 25mm, Aperture 1.4)
- **sample holder:** PMMA with a view field of 48mm 637mm, and uniform white backlight illumination.

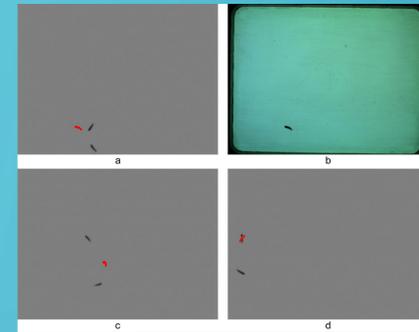
Resolution: one pixel : 24.6 micrometers * 24.6 micrometers

Housing: anodized aluminum to avoid the influence of external light

PHOTOGRAPHING SOFTWARE

For this photographing process we developed a Delphi software, which simplified the image capturing and file storing processes.

The software automatically captures ten consecutive images of each collembolan individual over a 30 second period in 3 second intervals and stores the images in JPEG format.



Secondary difference images produced by the CollScope image analysis software application. In the resulting pictures individuals appear in three representations: red patches correspond to the animal's spot involved in further calculations, while dark grey patches show its previous and next positions (a). Original photo (b), Collembolan bending in a crescent on the image or split during the image processing (c and d).

CollScope Image Analysis Software Application

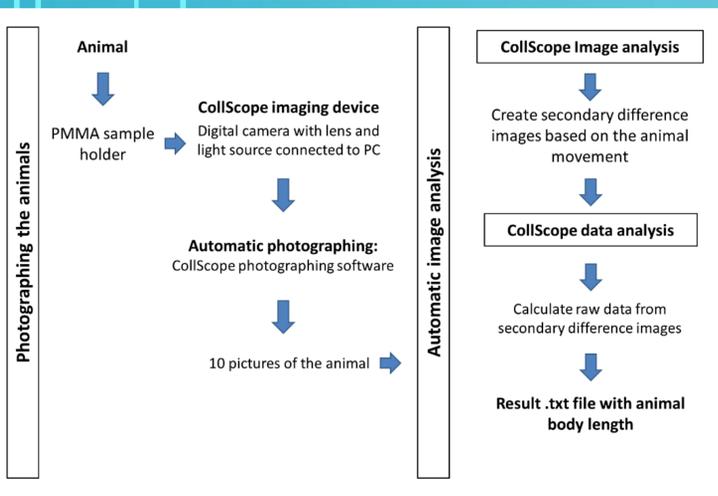
Alive individuals are identified based on their movements, everything else treated as background or noise. Pixel extraction method was used, resulting in patches corresponding to the moving animal.

Image J plug-in

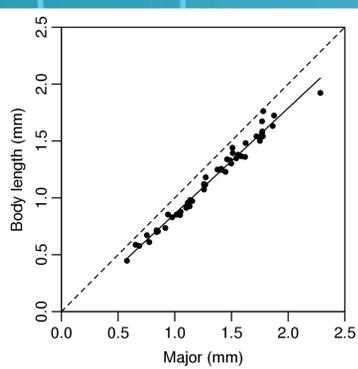
We wrote a plug-in for the open source image processing package ImageJ, which operates with differences of images.

Only particles within the 0.01 mm²–10 mm² range are included in the image analysis.

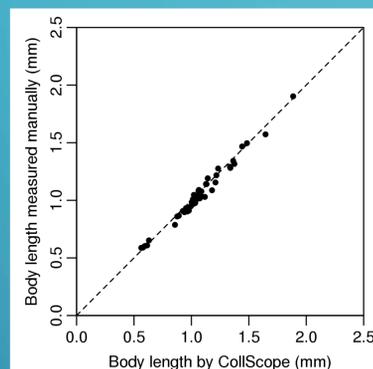
The CollScope image analysis macro can be freely downloaded from Supplement S2 in Bánszegi, O., Kosztolányi, A., Bakonyi, G., Szabó, B., & Dombos, M. (2014). New Method for Automatic Body Length Measurement of the Collembolan, *Folsomia candida* Willem 1902 (Insecta: Collembola). *PLoS one*, 9(6), e98230.



Accuracy and precision



Relationship between the M values obtained from the image analysis and body length measured manually. The brokenline corresponds to $y = x$. The continuous line shows the prediction used in the calculations ($n = 50$ individuals). "Major" (Mhis parameter corresponds to the major axis of the ellipse fitted to each patch of the picture. The matching between the classic manual measurement and our newly developed method was significantly correlated (Pearson correlation: $r = 0.99$; $n = 50$; $p < 0.001$)



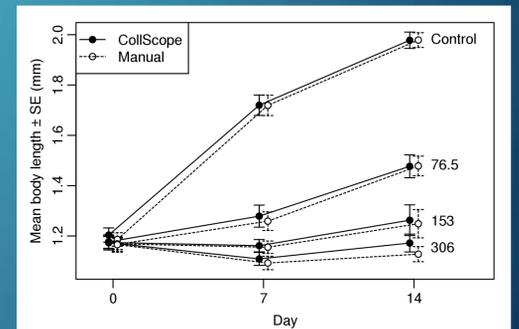
Relationship between the body length estimated by CollScope and measured manually ($n = 50$ individuals). The broken line corresponds to $y = x$. Body length measurements did not differ between the two methods over the experiment (LRT, $x^2 = 1.36$, $df = 1$, $p = 0.244$).

Conclusion:

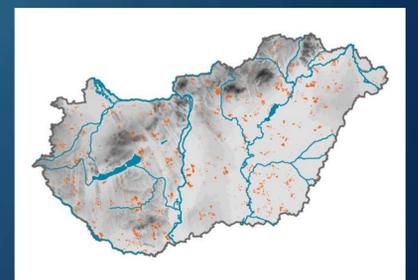
The automated measurement of collembolan body sizes is highly correlated with the traditional manual measurements (estimated measuring accuracy 0.05 mm). Size data measured by CollScope or manually did not differ significantly. Furthermore the new software package decreased time consumption of the measurements to 42% when tested on 35 animals.

Application of the method in a standard ecotoxicology test

A standard growth test on *F. candida* was conducted to investigate the accuracy of the new method. Control, and three nominal cadmium concentrations were used in the standard ecotoxicological test. Body length measurement has been performed on the same individual with both methods and the results showed no significant differences between the methods. This new method can be used for constant monitoring of Collembolan growth throughout the exposure period in ecotoxicological experiments. there was no difference between treatment groups at the beginning of the experiment (day 0: ANOVA, $F_{3,56} = 0.29$, $p = 0.833$), whereas body lengths were significantly different on days 7 and 14 (ANOVAs, day 7: $F_{3,47} = 58.27$, $p < 0.001$; day 14: ANOVA, $F_{3,44} = 59.95$, $p < 0.001$)



Application in Country Scale Survey in Hungary



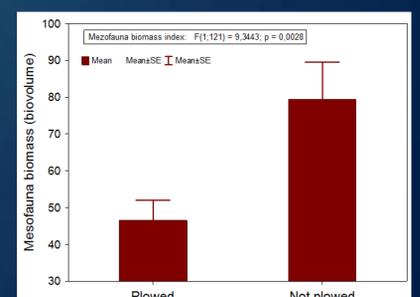
We used the device in a country-scale survey in Hungary.

Summary of the soil survey:

- ≈ 300 farms, on 250 000 ha
- Soil mesofauna was sampled in 100 farms and 200 parcels.
- Only on loamy soils
- Cropping system – only winter cereals

Soil microarthropods were extracted alive by Berlese funnels and then the abundance and body sizes were estimated by using CollScope.

As an example of the results we showed that the mesofaunal biomass estimate measured in CollScope was significantly lower on ploughed sites compared to not ploughed ones. Physical disturbance of the soil had a negative effect on mesofaunal biomass



For more see:

Bánszegi, O., Kosztolányi, A., Bakonyi, G., Szabó, B., & Dombos, M. (2014). New Method for Automatic Body Length Measurement of the Collembolan, *Folsomia candida* Willem 1902 (Insecta: Collembola). *PLoS one*, 9(6), e98230.

Acknowledgement:

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