

# ***Interactive comment on “Automatic pollen recognition with the Rapid-E particle counter: the first-level procedure, experience and next steps” by Ingrida Šaulienė et al.***

**Anonymous Referee #2**

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The paper described an elaborate analysis of three different neural networks to classify pollen from scattering and fluorescence (and life time) imaging from three different centers in Europe, as a feasibility study. General Comments: 1. With three different research centers, three different training/test dataset, three different ANNs and three different experimental set-ups, it becomes unrealistic to make any meaningful comparisons. As a result, I'm afraid that the paper reads as if it were three separate studies intertwined, without any benefit of joining them into one. 2. The paper is quite lengthy and can be condensed considerably, improving the readability of the paper and preventing reader fatigue. 3. How do the results compare to the Poleno method that integrates both image recognition and laser fluorescence? 4. The timing of the flu-

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orescence imaging is apparently such that it captures only a very limited amount of particles detected by the 400nm laser. As presented in Table 1, the recall rate is not only low in general; the percentage of analyzed particles was varying considerably among plant groups, ranging from 51% (for *Quercus*) to 87% (for *Salix alba*). Therefore, this raises the concern whether the first step already introduces a bias towards certain pollen types. 5. Experiments were carried out with fresh samples of collected pollen grains of separate plants and dried. Could this have skewed the results, as this procedure may decrease the amount of damaged pollen and aggregates as compared to daily practice? 6. What was the accuracy of the particle morphology step for pre-classifying pollen vs non-pollen? Is there any selection bias just based on particle size and therefore pollen type? 7. The reason for choosing three different designs of neural networks is not clear to me. Especially the choices in designing the first network from Siauliai seem to be a bit 'ad hoc'. In the network for analyzing the scattering images, it is surprising that three fully-connected layers were needed and that batch normalization was combined with dropout. In the network for fluorescence, it is not clear why no 1D convolution layers were used. Specific Comments: Introduction Generally, the introduction starts off quite clear, but at the end it needs more structure to clarify the goals of the paper. 8. line 52: "Hirst-type pollen traps" needs a bit more introduction on its methodology, before discussing its limitations. 9. line 72: Is there any literature on the evaluation of the Poleno device, that needs referencing? 10. line 77: the goal to evaluate the Rapid-E is described in too general terms. What aspect will be evaluated: sensitivity & specificity (and compared to which gold standard?), reproducibility of the system, processing speed or general applicability (Is this why three different centers participated?)? 11. line 78: what are "the Rapid-E data"? 12. line 81-85: Apparently, the different centers had different tasks in this project, but it is not clear which. As I understand it from the text, the system was assessed only in Siauliai, but then it is not clear how Novi Sad and Payerne determined their best classification. 13. line 82: Why did you compare the best classifications from the three centers, because they have different training or different procedures or pollen population? And what is the defini-

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tion of 'the best classification'? 14. line 84: What was the hypothesis that led you to compare the Swiss classifier results to the Hirst data? And what is 'the Hirst' data? 15. line 96: which range do you refer to? 16. line 103-104: Do I understand correctly, that this follow-up analysis is then comparable to the Hirst-type of analysis? 17. line 109-110: What is the conclusion of this sentence? Is the 0.5-100um range not being used because of hardware life expectancy, or just because it does not apply to pollen? In the latter case, it is only logical to use the Pollen-mode. 18. line 111: Before entering the experiments section I would first expect a section on the ANN methods that do the classification, because these are evaluated in the experiments. 19. line 111: Chapter 2.2. The scheme of the experiment. Here I would expect first an overview of the different experiments that have been conducted, and especially the rationale behind them. As it is now, we dive into the details of the different experiments performed at the three sites, without a clue of the bigger picture. 20. line 113: What was the goal of this experiment (To test the accuracy) and what was the gold standard (separate purified samples of a particular plants)? Why was training needed in the first place (apparently the system does not come with a trained classifier for a specific area)? 21. line 117: "the pollen recognition algorithm" is not introduced yet. This is due to comment 14. 22. line 125: "repeated twice", so in total 3 times? 23. line 144: What does "practically identical" mean? So, the same classifier was trained (again) and the experiment repeated twice? 24. lines 150-156: It is not clear what the rationale is behind adjusting the test (and training?) set; is it to adapt to the local pollen population in Serbia or was it to test other hypotheses? 25. line 158: "For practical reasons" is a bit vague. 26. line 160: What were the threshold-based criteria? To what parameter was the threshold applied? 27. line 234: Why was a threshold of 10um used instead of 5? 28. lines 258: "Results obtained in Siauliai". Shouldn't there then be a chapter on the results of the other two sites? 29. lines 259-265: These research question should be presented in the introduction, not in the results section. 30. line 270: Why have the spectra been normalized, isn't the amplitude a characteristic in itself? 31. line 270-274: It is not clear what is tested statistically: difference between genera or within genera? The Student

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test is not appropriate for testing data that is highly inter-correlated, like a spectrum. 32. line 72: "The uncertainties [...] were a fraction of a %.." This sentence is not clear to me: you mean less than 1%, but that is not true. 33. line 273: "statistically significant difference", what was the differences and what was the p-value? 34. line 323: Which of the 8 test data tests have been used here, what is n? (idem for section 3.2.2.) 35. line 372: "very similar way". I don't think you can say that since both the training and test data were different, and the more classes are included the more difficult the classification by an ANN will be. Technical Corrections: 36. line 72: typo, Swissens -> Swisens 37. line 89: typo, "analyses" -> "it analyses" 38. line 126: typo, "with" ->"with a" 39. line 128: typo, "fit" -> "fitted" 40. line 136: typo's, "compare" -> "compared" and "used new"-> "used a new" 41. line 202: typo: Rapid-e ->Rapid-E 42. line 205: typo, "founding" -> "finding" 43. line 150: style, "same or similar pollen morphotypes" -> "an adjusted set of pollen morphotypes" 44. line 217: NLL (negative log-likelihood) in full 45. line 810: Figure 1, the font size in this figure are quite small and difficult to read in a printed version. 46. line 820: The results of the two ANNs in Figure 3 and 4 were simply summed, whereas the other centers used concatenation. Then it is more consistent to show Figures 3 and 4 in one figure, and connect the two networks with a summation component. Please use the same conventions as in figures 5 and 6; the network in Figure 3 can be shortened by using Convolution Blocks, like in Figure 5. 47. line 825: Figure 6, for consistency with Figure 5, the lifetime and spectrometer sub-network need to be swapped. 48. line 835: Figure 8, font size on the x- and y-axis is too small to read in printed version. 49. line 830: y-axis in Figure 7, normed ->normalized 50. line 845: y-axis in Figure 9, normed ->normalized

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