

This manuscript “Classification accuracy and compatibility across devices of a new Rapid-E+ flow cytometer” describes the evaluation of a new instrument, the Rapid-E+, upgraded from a previous model made by Plair SA, and its ability to monitor pollen compared alongside a manual Hirst-type sampler. The necessary training of a classification algorithm to distinguish pollen types is detailed and lab evaluation is followed up by field evaluation, and cross-comparison with instruments at other sites to assess method generalisability. The study is thorough and comprehensive, looking into the detail of the different modalities of data obtained for different pollen types across different instruments.

The manuscript is of rigorous scientific quality and reports findings that are useful in this field to further the advancement of automated pollen monitoring. It is written and presented concisely and generally clearly, with ample supporting information in the Appendices. There are only some minor technical points that I would address before continuing to publication.

Please see below for specific comments by line.

Abstract

Line 22: I would use the term ‘instrument’ instead of ‘monitor’.

Introduction

Line 29: “Buters et al. 2022”

Line 30: “monitoring instruments”

Materials and Methods

Line 49-50: Not sure in this sentence exactly how the Rapid-E+ compares to the Rapid-E. Perhaps alter to “In particular the Rapid-E+ samples at a faster flow rate of 5 l min⁻¹ (compared to 2.8 l min⁻¹ for the Rapid-E), and records all particles passing through a 447 nm scattering laser into 4 size bins (>0.3 μm, >0.5 μm, >1 μm, >5 μm) unlike the Rapid-E which...?” (does the Rapid-E not have different size bins?)

Line 55-56: “also allows for adjusting the gain of the fluorescence spectrum and lifetime detectors”

Line 72: “Three Rapid-E+ air flow cytometers were involved in this study.”

Line 72: “...in Novi Sad, Serbia, ...”

Line 73: “the Novi Sad laboratory” is very nondescript. Details about the organisation that runs the Novi Sad laboratory may be helpful, and the environment?

Line 78: “The test period allowed for the exploration of measurement performance of the automatic bioaerosol monitoring instrument in a variety of conditions characteristic of the Pannonian Plain in [where?]. This region contains a large diversity of pollen and fungal spores...” This sentence was quite long so I suggest splitting it into two, e.g. where I have done so.

Line 82: “the period of seasonal allergies” – perhaps a little more description specifically as to what these seasonal allergies are in this place?

Line 83: “when large quantities of ragweed pollen are recorded in the air”

Line 85: “the main features of diurnal variations”

Line 89: “Reference pollen for training was collected locally.”

Line 98: “to ensure identity” - could you explain this better?

Line 102: “exposed to pollen using the Swisens Atomizer”

Line 103: “expose pollen to the Novi Sad and Osijek devices.

Line 106: “validating”

Line 109: Could say “colocated” instead of side-by-side.

Results and discussion

Line 201: Are these precision, recall and F1 scores averaged across scores for each pollen classification? If so, just mention they are averaged to avoid confusion, if not, I am unsure how the score differs from the discrimination of pollen from “other”.

Line 207: By ‘the classification algorithm with high accuracy’ do you mean the one that achieved F1 score of 0.86 as opposed to 0.84? Or simply that the algorithm managed to distinguish these pollen types with high accuracy, regardless as to which? Perhaps it may be better to write something like one of the following, depending on which you meant to avoid confusion...

“It is interesting to note that the latter classification algorithm (with merged classes) distinguished *Urtica* and *Parietaria* from *Brousonetia* despite these pollen grains being morphologically similar.”

Or

“It is interesting to note that the classification algorithm distinguished *Urtica* and *Parietaria* from *Brousonetia* with high accuracy, despite these pollen grains being morphologically similar.”

Fig. 2: The numbers and names are a bit small and blurry, would be good to make the characters a little larger if possible.

Line 226: what are the exact dates referred to here?

Line 235: Best to define PSLs in brackets for good measure as it is mentioned for the first time in this manuscript.

Line 241: At a glance, this sentence was a little confusing, I would correct it to something like: “Automatic detections of total pollen, as well as *Juglans*, *Morus* and *Ambrosia*, have a statistically significant positive correlation with...”

Line 243: “for most pollen classes” or “for most of the pollen classes”

Line 245: Perhaps rephrase as, for example, “Pollen grains that occur simultaneously in the air had a clear tendency to be confused amongst each other, which was expected...”

Line 261: “As demonstrated for the Rapid-E, this problem also exists for the Rapid-E+.”

Line 278: I would probably start a new sentence and replace the second i.e. before ‘different timing...’ with something else. This sentence is a bit confusing and long. Is it saying that since some pollen classes were comparable across devices, the differences observed across others shouldn’t be due to doing lab work at different times and different methods of pollen exposure to the instrument? Or are you saying each lab followed the same procedures so it shouldn’t be an issue?

Fig. 5 writing font too small and am unsure what I am looking at in 5D, can labels be added to the x, y and colour axes?

Fig. 6 again writing font too small.