Authors response to reviewer's comments.

First of all, we would like to thank the reviewers for their detailed comments to the paper. We used their comments and criticism to improve the manuscript. Below, we provide point-by-point response to the issues raised by the reviewers.

Reviewer 2

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.

We respectfully disagree. The studies were indeed following their own paths, primarily reflecting the differences in the three independent projects supporting the work of the teams. It is also important that there is no previous experience with Rapid-E and no "good practice" could therefore be followed – development of such practice is one of the purposes of this paper. The main added value was that the different approaches resulted in similar (but not completely identical) skills of the pollen recognition. The differences in the skills were, where possible, traced to the different approaches, ANN construction and data pre-processing. As a result, all groups participating in the paper were able to assess efficiency of their approaches and look into alternative ways of analysis. Now, we would like to share the findings with others, who are just considering to enter the field of online pollen monitoring. We hope that the revised paper is more clear in this regard.

2. The paper is quite lengthy and can be condensed considerably, improving the readability of the paper and preventing reader fatigue.

We have reviewed the paper structure and shortened it

3. How do the results compare to the Poleno method that integrates both image recognition and laser fluorescence?

There was no comparison with POLENO monitor, it was not the purpose of the paper to compare different devices. We were building the procedures for Rapid-E and analyse the "absolute" skills of recognition that can be obtained with the Rapid-E technology

4. The timing of the fluorescence 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 analysed 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.

The recall rate for fluorescence is indeed one of the significant concerns. For the confusion tables, however, the recall rate was not important: the initial number of pollen noticed by the scattering laser is not used in the analysis, i.e. the algorithms only dealt with fluorescent particles. The point is explicitly commented in the revised paper as a note to Table 1.

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?

This seems to be improbable, in fact, shaking pollens off the inflorescences and storing them in a package may rather increase the number of aggregates and small-size debris. This is why we put attention to microscopic analysis confirming that the fraction of these additions is very small.

6. What was the accuracy of the particle morphology step for preclassifying pollen vs non-pollen? Is there any selection bias just based on particle size and therefore pollen type?

In fact, the pollen-nonpollen pre-classification (so-called "pollen mode" of Rapid-E) is solely based on a requirement of the particle being larger than 5 um of optical diameter. Therefore, introduction of bias at this step is very improbable.

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.

As stated above, there is no guidance of optimal practices of ANN construction for Rapid-E. Therefore, all participating groups were building their own approaches and all have tried several options before arriving at the ones shown in the paper. Harmonization effort is certainly in need but was left for the future when the problem of compatibility of the devices themselves is solved. In the Siauliai case, various options have been tested for the neural network design. The three layers gave the best results. To avoid overtrainnig, we used regularization layers that are currently often combined: dropout and batch normalization. The 1D convolutional layers are suitable when the entire signal is homogenous. Pollen fluorescence peaks at different wavelengths are different, so it seemed expedient to use a fully-connected layer.

Introduction Generally, the introduction starts off quite clear, but at the end it needs more structure to clarify the goals of the paper.

The goals of the paper have been refined

8. line 52: "Hirst-type pollen traps" needs a bit more introduction on its methodology, before discussing its limitations.

An outline of the Hirst device is added

9. line 72: Is there any literature on the evaluation of the Poleno device, that needs referencing?

Not really. This is a very new device and no consistent evaluation has been published.

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?)?

Clarification is added, goals have been refined, as well as the roles of each centre. As stated above, the centres are operating within completely independent projects, which initiated certain diversity in the approaches.

11. line 78: what are “the Rapid-E data”?
Clarified

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.
Clarification is added, goals have been refined, as well as the roles of each centre. As stated above, the centres are operating within completely independent projects, which initiated certain diversity in the approaches.

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 definition of ‘the best classification’?
Clarification added

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?
The selection of the group was opportunistic: the Swiss team had the Rapid-E and Hirst devices working next to each other during full seasons in the previous years, so it was possible to put the time series next to each other. However, the paper does not have a goal of the Rapid-E – Hirst comparison. The provided time series were added to discuss the “false positive” identifications. We made it clearer in the revised version

15. line 96: which range do you refer to?
Clarified: changed to “this saturation level will not be reached in realistic ambient conditions”

16. line 103-104: Do I understand correctly, that this follow-up analysis is then comparable to the Hirst-type of analysis?
Well, to some extent. The geometry of the slide and flow is strongly different but the principle is the same: impaction on a sticky slide followed by the microscopic 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.
In this section, we did not derive conclusions, only described the device. The pollen mode as the default one is brought up later when we describe the setup of the experiment.

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.
The data analysis sections and the experiment description sections have been switched

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.
The experimental section has been rearranged to provide the overall picture first and then specificity of each site.

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)?
Indeed, the system does not come with any classifier to any of the machines, plus the machines proved to be substantially different.

21. line 117: "the pollen recognition algorithm" is not introduced yet. This is due to comment 14.
The sentence is reformulated

22. line 125: "repeated twice", so in total 3 times?
No, just twice. The word "repeated" is replaced with "performed"

23. line 144: What does "practically identical" mean? So, the same classifier was trained (again) and the experiment repeated twice?
No, just the practical side, i.e. the pollen exposure. Clarified.

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?
This is indeed just reflecting the pollen availability in Serbia.

25. line 158: "For practical reasons" is a bit vague.
The meaning clarified: "Low ambient concentration of coarse particles allowed a less laborious approach: pollen calibrations in Payerne …."

26. line 160: What were the threshold-based criteria? To what parameter was the threshold applied?
Clarified

27. line 234: Why was a threshold of 10um used instead of 5?
Partly, for the historical reasons, following the work of Crouzy et al. For the tested pollens it is the same: they all are much larger. But the tighter threshold was effective in eliminating the ambient particles, which, albeit in low numbers, were still reaching the device.

28. lines 258: “Results obtained in Siauliai”. Shouldn’t there then be a chapter on the results of the other two sites?
The results from all three sites are collected together

29. lines 259-265: These research question should be presented in the introduction, not in the results section.
Moved to introduction

30. line 270: Why have the spectra been normalized, isn’t the amplitude a characteristic in itself?
Each spectrum was normalised separately prior to any further analysis to eliminate the effect of imperfect hit of the pollen by the UV laser impulse. Normalization allows to reduce variation of fluorescence amplitude for one pollen taxon at fixed wavelength.

31. line 270-274: It is not clear what is tested statistically: difference between genera or within genera? The Student test is not appropriate for testing data that is highly inter-correlated, like a spectrum.
In the shortened paper, we removed the Student test remark as a weakly-connected part of the analysis.

32. line 272: “The uncertainties [. . .] were a fraction of a %..” This sentence is not clear to me: you mean less than 1%, but that is not true.
Corrected to “percentage”.
33. line 273: “statistically significant difference”, what was the differences and what was the p-value?
Updated to $p < 0.001$.

34. line 323: Which of the 8 test data tests have been used here, what is n? (idem for section 3.2.2.)
In Siauliai experiment with each genus consisted of up to 8 sample tests (approximately 5 mg of pollen/test). n of registered data by Rapid-E and analysed in research is indicated in Table 1 in column “Fluorescent particles”. They were all subjected to the recognition analysis, together.

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.
The sentence is changed to “showed similar recognition skills”

Technical Corrections:
36. line 372: 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.
Thanks you! All corrected

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.
Corrected

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
Thank you! All corrected

Reviewer 3

General comments

(1) adding a column indicating the percentage of pollen that were deemed above the fluorescence threshold would be of help in table 1. Was this consistent from sample to sample? Ie Was approximately the same fraction of each pollen type deem fluorescent in each calibration test?
The column has been added, together with the comment that the fractions were not exactly the same.

(2) how reflective of pollen in the atmosphere would the test pollen be? The collection method used in Payerne seems to significantly different than that seen in the other sites.
The basic idea was to use as fresh pollen as possible but after drying it somewhat. This is generally consistent with what is happening in the environment because pollen grains tend to loose water during the first minutes of the atmospheric transport providing that the weather is good and relative humidity is comparatively low – also the conditions facilitating the pollen release.

(3) Was the compressed air, zero grade air from a cylinder or from a compressor for the Siauliai tests?
Zero grade air from the cylinder.

(4) the instrument was run in pollen mode between 5-100 micron. Can this be changed? The majority of pollen is far larger than 5 micron. What is the expected lifetime of the instrument? Increasing the lower size threshold would likely extend it. Were the authors interested in fractionated pollen also?
The lower bound of 5 micrometres is the factory setting, hardcoded into hardware. The lifetime of the instrument is yet-unknown but estimates circle around 3-4 years depending on the level of pollution, amount of calibration exercises, etc. None of our instruments reached its end till today.

(5) L189: Do the authors have any idea how many pollen particles are not classified due to partly or fully missed the particle if the deep-UV laser fired at a wrong moment of time?
In the table 1, the "fluorescent" particles compare to "total particles" is the fraction of the sufficiently good hits.

(6) L196: Is saturation of the fluorescence spectra still a possibility? Were some pollen more likely to cause this than others?
The devices behaved differently: the saturation was more a problem in Siauliai than in other labs. But after exclusion of the first spectrum, the impact of this problem became small.

(7) why was fluorescence lifetime not used in the Siauliai data analysis
It has been noticed that the lifetime signal was often saturated in-between the rise and fall sections when the value remains constant over some time.
(8) how was the threshold of the particle fluorescence intensity level (> 1500 units) determined? This would be interesting for the reader and is generally discussed for other instruments? What was undertaken at the other sites?
This threshold is an empirical parameter and it had to vary between the devices. Somehow, the strength of the signal was substantially different between the labs. This was one of the reasons for normalization of the spectra. We added a clarifying sentence in the revised paper.

(9) for the Swiss data analysis, why was the optical size corresponding to 10 micrometers estimated? Was it simply due to the practicality of not having 10 micron PSLs?
Partly, for the historical reasons, following the work of Crouzy et al. For the tested pollens it is the same: they all are much larger. But the tighter threshold was effective in eliminating the ambient particles, which, despite in low numbers, were still reaching the device.

(10) The beginning of the results section has both general and site specific research questions (Lithuania). This seems out of place (consider moving to intro).
The results from all three sites are collected together

(11) Consider cutting section 3.2.1. Recognition using scattering images only to a sentence and removing table 2 or moving it to a supplemental section. It does not add to the results and is far and away the weakest procedure.
We have moved the details of the separate recognitions with scattering and fluorescence only into an Annex but still consider this analysis useful as it shows the relative importance of each information channel. Therefore, a few sentences comparing these channels are retained in the revised paper.

(12) Should there be results sections for the other two sites as well? Or is the results section an amalgam of the other sites also. Currently it reads like the results originate only from Siauliai
The results from all three sites are collected together

(13) A very interesting observation that Festuca pollen was seen with the signal amplitude growing during the first 500 ns (Figure 8) do the author have any suggestion as to why this is so?
Unfortunately, no, this is just the empirical fact.

(14) A line on chemical interactions and degradation should be added L 405
A sentence is added

(15) A brief mention of the work undertaken by the WIBS instrument should be discussed in 4.3. Comparison with other studies on pollen recognition
A short discussion is added. We added information to Introduction and 4.3 section.

(16) authors have suggested lifetime could be utilized to discriminate between pollen I feel this should be discussed, for example "O'Connor et al Using spectral analysis and fluorescence lifetimes to discriminate between grass and tree pollen for aerobiological applications Anal. Methods, 2014,6, 1633-1639"
Thank you for the reference! We have added the reference and corresponding discussion.

(17) What are the R2 values between the Rapid E and the Hirst? Is the Hirst a true reflection of what is in the atmosphere?
The R2 values are provided. Hirst is certainly not the true reflection but the best we have for last 60 years

(18) Could large fungal spores or clumps of fungal spores act as an interferant in the Rapid E for its current task?
No, they will not. During next steps of calibrations, not shown in this paper, we included spores and the first impression is that the difference is substantial, first of all, in fluorescence spectra, which do not depend on agglomeration.

(19) Did only the Swiss site compare the Rapid E to a Hirst type trap? If yes why? If not, why are the results talked not about? This would be a good way to evaluate the ANN at each sampling site.
Not only but the Payerne group has the longest experience with the Plair device (albeit with its previous version). Therefore, they faced the problem of the false-positives, which is discussed in this section.

(20) Does the Rapid E come with any classifier? Or is it incumbent on the purchaser to develop their own? If the creator has an algorithm 5-10% better than seen here why is this not part of the commercial instrument?
In theory, it can have it. However, none of our devices were equipped with it. In all cases, the algorithm is closed and requires calibration to adapt to the specific machine. All machines are provided as "experimental devices", with reduced warranty period and some features disabled. However, the conclusions of the paper imply that the details of such algorithm are not too relevant: pre-processing and pre-filtering the input datasets may have stronger influence on the recognition quality than setup of the ANN.

Specific comments
L19 specialized rather specialization
L65 “it has become a necessity to develop new methods enabling the information on airborne pollen to become available in real-time”
L66 were related
L87 experiments
L128 fitted rather than fit
L136 a new bottle
L137 “previously sampled” rather than “blown”
L142 clarify for the reader what you mean by busy slides
L212 which aimed
L312 a challenging task
Thank you! All corrected

On behalf of authors
Ingrida Šaulienė

Automatic pollen recognition with the Rapid-E particle counter: the first-level procedure, experience and next steps

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Abstract. Pollen-induced allergy is among the most-prevalent non-contagious diseases, with about a quarter of European population sensitive to various atmospheric bioaerosols. In most European countries, pollen information is based on a weekly-cycle Hirst-type pollen trap method. This method is labour-intensive, requires narrow ~~specialization~~ specialized abilities and substantial time, so that the pollen data are always delayed, subject to sampling- and counting-related uncertainties. Emerging new approaches to automatic pollen monitoring can, in principle, allow for real-time availability of the data with no human involvement.

The goal of the current paper is to evaluate the capabilities of the new Plair Rapid-E pollen monitor and to construct the first-level pollen recognition algorithm. The evaluation was performed for three devices located in Lithuania, Serbia and Switzerland, with independent calibration data and classification algorithms. The Rapid-E output data include multi-angle scattering images and the fluorescence spectra recorded at several times for each particle reaching the device. Both modalities of the Rapid-E output were treated with artificial neural networks (ANN) and the results were combined to obtain the pollen type. For the first classification experiment, the monitor was challenged with a large variety of pollen types and the quality of many-to-many classification was evaluated. It was shown that in this case, both scattering- and fluorescence- based recognition algorithms fall short of acceptable quality. The combinations of these algorithms performed better exceeding 80% accuracy for 5 out of 11 species. Fluorescence spectra showed similarities among different species ending up with three well-resolved groups: (*Alnus*, *Corylus*, *Betula* and *Quercus*), (*Salix* and *Populus*), and (*Festuca*, *Artemisia*, *Juniperus*). Within these groups, pollen is practically non-distinguishable for the first-level recognition procedure. Construction of multi-steps algorithms with sequential discrimination of pollen inside each group seems to be one of possible ways forwards. In order to connect the classification experiment to existing technology, a short comparison with the Hirst measurements is presented and an issue of the false-positive pollen detections by Rapid-E is discussed.

Key words: pollen observations, real-time monitoring, artificial neural networks (ANN), scattering, fluorescence

1 Introduction

Pollen of many wind-pollinated plants has specific proteins that cause human allergy (Valenta et al., 1992; Bousquet et al., 2006; Radauer and Breiteneder, 2006; Bousquet et al., 2015; Choual et al. 2018), particularly affecting children (Skoner 2001; Höst et al., 2003; Douladiris et al., 2018). Stress due to contact with the pollen-contained allergen can cause an allergic reaction or exacerbate some related diseases (Leynaert et al., 2000; Devillier et al., 2017; Poethko-Müller et al., 2018). Allergy impairs the quality of life of about 30% of the world population (Akdis et al., 2015). In most of European countries, national organizations of various kinds provide information about pollen concentration in the air, publish pollen prognosis and issue warnings. The bulk of such efforts are based on retrospective pollen observations and climatological pollen calendars. Most observers use Hirst-type volumetric pollen traps where airborne particles (>5 μm) are collected on a rotating drum covered by Melinex tape. ~~pollen traps and s~~Samples are identified by a microscopic analysis (Galán et al., 2014; Buters et al., 2018). This method is labour-intensive, tedious, requires narrow specialization abilities and incorporates significant uncertainties – e.g. (Oteros et al., 2017). In addition, due to the manual treatment of the collected samples and weekly cycle of the trap the data are always delayed from a few days up to a few weeks. However, timely data about pollen concentration in the air are also needed for improving the accuracy of tools for personalized medicine (for example, PASYFO app, http://www.pasyfo.lt, POLLEN app, http://www.polleninfo.org, NORKKO forecast and app http://www.norkko.fi, etc.) (Bousquet et al., 2017; Horgan and Pazzagli, 2017; Pereira et al., 2018, Tabatabaian and Casale 2018). It can be also used for informing people about current pollen concentration in the air. Finally, real-time data are needed for short-term pollen forecasts with statistical and atmospheric dispersion models (Sofiev et al, 2013, 2015, 2017, Prank et al., 2016; Ritenberga et al., 2016; Zink et al., 2017).

As the approach to information and personal responsibility for health is changing, ~~it becomes necessary to develop new methods enabling the information on airborne pollen available in real time~~ it has become a necessity to develop new methods enabling the information on airborne pollen to become available in real-time. The first attempts to obtain automated information ~~are~~ were related to image recognition technologies (Bennett, 1990). Their development was accompanied by the formation of more potential possibilities (Ronneberger et al., 2002; Landsmeer, 2009). Currently, two types of technologies seem to be the most-suitable for taxon-level classification of pollen: based on image recognition and laser-fluorescence (or their combinations). Image-based technologies are used in detectors, such as BAA500 (Hund Wetzlar, https://www.hund.de); the laser fluorescence-based approach is implemented in WIBS device (http://www.dropletmeasurement.com), PA-300 and Rapid-E (Plair, http://www.Plair.ch), whereas the new Poleno device (Swi~~s~~ssens, https://swisens.ch/) aims at integration of both features. The Hund- and Plair- manufactured devices were used in limited-scale scientific studies: Oteros et al. (2015) for BAA500 and Crouzy et al. (2016) for PA-300 and showed promising results. However, the large-scale evaluation and calibration suitable for European-scale applications are yet to be concluded (Oteros et al., 2015, Crouzy et al., 2016).

The goal of the current paper is to evaluate the capabilities of the new Plair Rapid-E pollen monitor and to construct and evaluate the first-level pollen recognition algorithms using particle ~~the Rapid-E~~ scattering and fluorescent data from the Rapid-E. The ~~fluorescence-laser based technology was used~~ key questions to answer were~~the following questions~~:

- can we identify different pollen genera using the Rapid-E data?
- can we identify different species within the same pollen genus?
- what is the recognition accuracy for the most-common pollen types in ~~Lithuania~~Europe?

The experiment was performed in Siauliai (University of Siauliai and Finnish Meteorological Institute), Novi Sad (BioSense Institute of University of Novi Sad) and Payerne (Federal office of meteorology and climatology MeteoSwiss) with three newly acquired experimental Rapid-E devices. The devices were provided with local pollen samples and several ~~Below, we present in details the procedure followed in Siauliai and resulting extensive assessment of the device performance. Numerous of experiments~~ pollen recognition algorithms have been constructed independently ~~were carried out~~ in each ~~of the three~~ center.~~s~~ This organization of the study allowed accounting for ~~until an algorithm could identify the selected pollen.~~ variability of the actual ~~All devices are different and the same algorithm cannot be successfully used due to the~~ technical characteristics of the individual devices and an absence of "good practice" for such type of measurements. The best classification results ~~is then~~ compared across the centres formed the basis of the Result section of this paper~~with the best first-level classification of same or similar pollen morphotypes made independently in Novi Sad and Payerne~~. Finally, outputs of the MeteoSwiss classifier ~~in a monitoring setup~~ are ~~presented and~~ compared with airborne pollen data collected with ~~by using~~ the Hirst~~-~~type pollen trap ~~data~~. The provided time series were used ~~added~~ to discuss the "false positive" identifications important for the operational context.

2 Methods

2.1. Description of the measurement instrument

The new Rapid-E instrument designed and produced by Plair S.A is the successor of the first-generation particle analyser PA-300 used by Crouzy et al. (2016). It is a particle counter, i.e. it analyses all particles coming to its inlet one-by-one. Operation of the instrument is based on two physical principles: scattering of near-UV laser beam and deep-UV laser-induced fluorescence (Kiselev et al., 2011; 2013). Multi-angle scattering is used for determination of the particle's morphology, such as size and shape. The fluorescent light is analysed for its spectrum and lifetime. The instrument constantly takes in the ambient air through the air inlet on the top of its panel. Sample air flow is up to 2.8 litres per minute with the counting rate of up to 4500 particle detections per minute, i.e. the theoretical saturation level is 1.6 10^6 particles m^{-3}. Since according to the device provider the smallest observable particle is 0.5µm in diameter, this ~~range is sufficient for practically all~~ saturation level will not be reached in realistic ambient conditions.

The sampled air enters the nozzle, which creates a laminar flow in the measurement zone. Particles interact with 400 nm laser light source and the scattered light is captured by twenty-four time-resolving detectors distributed at different angles. The information on chemical properties of the particles is obtained by a powerful deep-UV laser (320 nm) source that induces fluorescence. Its spectrum (32 measuring channels within spectral range of 350-800 nm, 8 sequential acquisitions with 500 ns retention) and lifetime (4 particular bands) are recorded and used for the particle identification (Figure 1).

The threshold of the particle fluorescence intensity ~~level~~ (> 1500 units) was empirically determined as a cut-off level for sufficiently recorded pollen grains. T~~for this research and t~~he spectra were~~as~~ subsequently

normalized ~~due~~ to eliminate the difference in the signal strength ~~of the signal was substantially different~~ between the ~~measurement~~ instruments.

Rapid-E has an embedded mechanism for collecting the particles, which passed through the registration chamber, onto sticky slides for the follow-up microscopic analysis.

The device has several modes of operations. Since the deep-UV laser has a limited resource, the 400 nm scattering image is used for prior estimation of the particle morphology and deciding if it can be pollen. In the Pollen mode, the device ignites the deep-UV laser only for 5-100 micrometer particle size range (used in ~~for~~ this study~~research~~). ~~Solely based on a requirement of the particle being larger than 5 um of optical diameter the Pollen mode was used in pollen-nonpollen pre-classification.~~ Another mode allows detecting particles in the range of 0.5-100 micrometers for spores, particulate matter and bacteria identification. However, the expected lifetime of the deep-UV laser is much shorter in this mode, especially in polluted atmosphere.

2.2. Data processing and recognition ~~analysis~~ methods ~~in~~

2.2.1. Siauliai

Both modalities of the Rapid-E output (scattering image and the fluorescence spectra) were processed independently with artificial neural networks (ANN) and the scores were merged to obtain the final classification result.

Both ~~The~~ scattering and fluorescence signals (Figure 1) ~~image has a peculiarity requiring special treatment.~~ significantly ~~D~~depend~~ing~~ on the particle position with regard to the laser beam while passing through it. In particular, the~~, the~~ apparent particle size (~~scatering~~scattering) and the fluorescence intensity ~~of the particle deduced from the Rapid-E scattering image~~ varied between ~~for different~~ the recordings ~~(Figure 1)~~. Apart from that, 15-50% of particles are missed by the deep-UV laser. Therefore, pre-processing included: (i) identification ~~at the beginning,~~ of a characteristic template of 44x20 pixels from the scattering image ~~was identified. The template was used~~ to localize the features characteristic for each pollen type~~,~~; (ii) particles with insufficient fluorescence intensity are filtered out (Table 1); (iii) ~~most similar areas in the scattering images.~~fluorescence spectrum was normalised, (iv) ~~Because the Rapid-E device in Siauliai often gives saturated short-wavelength fluorescence spectrum~~ at the first time moment, only 16 of 32 ~~half of the possible~~ wavelengths were included in the feature vector to exclude the saturated short-wavelength fluorescence bands. ~~A convolutional neural network was trained for pollen recognition from such images.~~

Several ANNs were created. One of the best-performing networks included only scattering and fluorescence signals taking them separately and disregarding the noisy lifetime component.

ANN for scattering images consists of two convolutional blocks for the feature extraction and two fully connected layers for classification (Figure 2). Every convolutional block consists of the 2D convolutional layer, the batch normalization layer, the ReLU activation layer, and the maxpooling layer. One mask of the convolutional layer has size of 5x5. The convolutional layer of the first block has 16 filters, and the one of the second block has 32 filters. The maxpooling layer selects the maximal response from the area of 2x2. At the output of the second convolutional block, the size of the feature vector is 1760. The first

fully connected layer has 256 neurons. The second fully connected layer classifies these vectors to the number of pollen classes chosen for the calibration. The ANN was trained using the cross-entropy loss criterion.

~~The fluorescence analysis also starts from pre-processing. Similarly to the scattering image, particle position with regard to the laser beam influenced the features (first of all, intensity) of the fluorescence. Apart from that, the deep-UV laser is activated for a short period of time triggered by the 400 nm scattering image analysis, i.e. it can partly or fully miss the particle if fired at a wrong moment of time. As a result, only half of pollen grains recorded by the 400 nm laser produce reasonable intensity of the fluorescence (Table 1).~~

The fluorescence spectrum was processed by a multilayer perceptron ANN (Figure 2~~3~~) with~~.~~

~~Because the Rapid-E device in Siauliai often gives saturated short-wavelength fluorescence spectrum at the first time moment, only half of the possible wavelengths were included in the feature vector. Subsequent reduction of the light intensity allowed inclusion of all 32 values starting already from the second time moment. D~~dropout and batch normalization layers ~~are~~ used for ~~ANN~~ regularization. This ANN also was trained using the cross-entropy loss criterion.

Results of two ANN were fused by summing scores of every pollen type.

~~With both networks, c~~Tare ~~was taken to follow-up t~~he training process was monitored ~~and~~ to avoid over-fitting the networks~~. Despite the large volume of the samples (Table 1), certain over-fitting was possible after many training cycles~~ – see the Discussion section.

2.2.2~~3~~. ~~Data analysis methods in~~ Novi Sad

All Rapid-E signals (i.e. scattering, ~~spectrum~~ fluorescence and life time) were transformed into images and jointly processed by a single ANN (Figure 4~~3~~). Its architecture considers the same input dimensions of every image, and since the scatter signal could vary in the number of acquisitions, each image's width was equalised by finding its centre of mass and either cutting or zero-padding to fit to 24x70 pixels. The dynamic range of each image was reduced by replacing each pixel value with its logarithm, which resulted in enhancing of the low intensity pixels. Images from temporally resolved spectrum data and all bands of the life time data were used unprocessed.

Similarly to Šiauliai, particles with the ~~Only the data of sufficient strength were deemed suitable for the analysis. Experiments in Novi Sad indicated that the threshold of the particle~~ fluorescence intensity less than ~~level to be >~~ 1500 units at the Rapid-E scale ~~for~~ at all ~~least one emitted~~ wavelengths were filtered out. In addition, particles with calculated optical size out of the range 5-100 micrometers were filtered out using the manufacturers size approximation, depending on the sum of the scattering image. Size is 0.5 micrometers if the sum is less than 5500000. If the sum is between 5500000 and 500000000, the size is given by the 9.95e-01*np.log(3.81e-05*x)-4.84e+00. Finally, if the sum is greater than 500000000, the size is given by 0.0004*x**0.5 - 3.9.

Each~~Since there are three sources of information, the data fusion which aimed to create an architecture that would allow the gradient to flow through the whole network, so that the back-propagation can be done updating the weights for each distinct source. Each~~ input signal ~~image~~ is analysed ~~in the ANN goes through~~ with its own chain~~framework,~~ consisting of 2D convolutional layers, replication padding layers, ReLU activation functions, batch normalization layers, max pooling and the dropout layers, together

combination of which we will call forming the convolutional block (Figure 3). The ANN was trained using negative log-likelihood (NLL) loss and the Stochastic Gradient Descent with the learning rate of 0.001 and the momentum of 0.9.

For scattering images, we used two convolutional blocks. The first convolutional layer of the first scattering block had 10 filters with the kernel size of 5x5 while , and the second one had 20 filters with the kernel size of 3x3. For the spectral images, the convolutional layer of the first block had 50 filters with the kernel size of 5x5, and the one of the second block had 100 filters with the kernel size of 3x3. For the lifetime images, the first convolutional layer had 70 filters with kernel size of 7x1, the second one had 140 filters with kernel size of 5x1 and the one of the final block had 200 filters with the kernel size of 3x3. At the output of the final convolutional block, the sizes of the feature vectors for scattering image, fluorescence spectrum and lifetime are 1800, 1600 and 1400, respectively. Each feature vector is passed through one fully connected layer with 50 neurons. Those features were concatenated resulting in the feature vector of dimension of 150. The size of the second (lastand the final) fully connected layer was of the size of the number of classes, after which the samples were classified with the log-softmax activation function.

The ANN was trained using negative log-likelihood (NLL) loss and the Stochastic Gradient Descent with the learning rate of 0.001 and the momentum of 0.9.

2.2.3. Data analysis methods in Payerne: classifier and aspects related with operational use

At the pre-processing stage, all three signalsThe device outputs w were first normalized with their using the respective maxima of the signals: the maximal scattering intensity, the maxima of each of the four lifetime bands and the maximal fluorescence. For scattering, the image was in additionally centered and cut to a 24 x 100 shape. Extra filtering was imposed retaining only Only calibrations with optical size above 10 micrometers were retained, and a fluorescence signal in a range and spectrum compatible with single pollen grains was kept (see Crouzy et al., 2016, for examples of spectra). The optical size corresponding to 10 micrometers was estimated by comparing the integral of the scattering signal of 5 micrometer PSLs with the integral of the scattering signal for *Urtica* and *Parietaria* pollen grains.

For scattering ANN, 5x5 convolutions were applied with 32 filters, ReLU activation, and the pooling layers with operated on a 2x2 window. For lifetime, 1D convolution was applied with ReLU activation, with windows size 10x1 and with 10 filters. For the spectrometer, asymmetric 2x4 convolution was applied with 8 filters with ReLU activation. The ANN was trained using the Adam optimizer and categorical cross-entropy as loss function (Figure 54). In order to retain flexibility, additional features were inserted before the final fully-connected layers after: features computed from the raw signals indeed showed promising results in [30] Crouzy et al. (2016): . For the moment, only the maximum and the integral of the scattering together with the maxima of each of the four lifetime bands and the maxima of the first three spectrum acquisition were used.

Even if high expectations on the performance of the classifier are met, problems are bound to occur in the form of false-positive detections. Even a few percent of the error in discriminating between two pollen types can lead to problematic drifts. For example, Birch pollen concentrations regularly exceed 1000 particles per cubic meter in Switzerland in spring. If just 2% of these are mis-interpreted as, e.g., Ambrosia pollen, the false concentration of 20 grains per cubic meter would be already significant for allergy

~~analysis. In order to cope with this, we introduced two methods.~~ ~~Firstly, events with classifications below a certain threshold were disregarded, as was done in (Crouzy et al., 2016) where a reduction of sampling of 20% lead to an increase in precision of about 10%.~~

2.2.4. Additional filtering of false-positives in operational context

Even if high expectations on the performance of the classifier are met, problems are bound to occur in the operational applications due to false-positive detections. For example, Birch pollen concentrations regularly exceed 1000 pollen m^{-3} in Switzerland in spring. If just 2% of these are mis-interpreted as, e.g., Ambrosia pollen, the false concentration of 20 grains per cubic meter would be already significant for allergy analysis. In order to cope with this, extra steps were introduced in Payerne.

Additional filtering was applied disregarding the events with classifications quality below a certain threshold as was done in (Crouzy et al., 2016) where a reduction of sampling of 20% lead to an increase in precision of about 10%.

For the operational monitoring, at least a few events with extremely good classification score were required during the same or two preceding days to accept the middle-confidence recognition of the specific pollen type. This condition is applied uniformly over the pollen season to verify what pollen taxa are present in the air.

~~Secondly, we require in operational monitoring at least a few events with extremely good classification score to occur on the day considered or on the two preceding days, this condition is applied uniformly over the pollen season to check which pollen taxa are present in the air. We preferred this method over the expert supervision or calendar rules due to the fact that it bases only on measurement.~~

2.~~2~~ 3. The scheme of the experiment

In this chapter, we present in details how ~~study~~ the ~~baseline (groundwork)~~ calibration experiment ~~of how Rapid-E can identify different species of pollen genera or pollen within the genus~~ was implemented in Siauliai, followed ~~(Lithuania). The~~ by the description of specifics of the setups in ~~unique results of~~ Novi Sad ~~(Serbia)~~ and Payerne ~~(Switzerland) experiments were integrated into the accuracy evaluation in different geographic territories~~. ~~The~~ Most-importantly, ~~pollen we used in the study was~~ only ~~typical~~ pollen characteristic for each ~~particular~~ location was used. ~~For c~~Comparison of the results were based on ~~purposes, the selected~~ pollen types belonging to the same plant families ~~that are~~ found in all three locations.

2.3.1 Siauliai

The experiment in Siauliai was carried out with 14 pollen morphotypes, the tested amounts of which are given in Table 1. Three genera (*Salix*, *Acer*, *Pinus*) were represented by two plant types. All 14 plants are naturally widespread in Lithuania and their airborne pollen is abundantly recorded annually (Šaulienė et al., 2016). ~~These particles were provided to the device one set after another splitting the recordings to~~

~~training and test subsets. After that, the pollen recognition algorithm was calibrated using training subsets and challenged with pollen from the test subsets with no stratification or a-priori other information provided. Each-to-each confusion table was the main quality measure.~~

Pollen was taken from the plant inflorescences collected during the vegetation period in April-August of 2018 during the days with intense pollen release. The collected material was put in air-permeable paper bags and dried at a temperature of 40°C until the maximum release of pollen from the inflorescences. Vibratory Sieve Shaker ANALYSETTE 3 PRO was used for gentle shaking the pollen grains out of the inflorescences. The extracted pollen was stored in Petri dishes at +4°C.

Each experiment was ~~repeated~~ performed twice and consisted of up to ~~eight~~ 8 sample tests, ~~each~~ using approximately 5 mg of pollen per sample test. The number ~~n~~ of grains registered in the scattering signal ~~data by Rapid-E and analysed in research~~ is indicated in Table 1 as "Total particles", whereas ~~in~~ the column "Fluorescent particles" shows the number of grains with usable fluorescent and lifetime signals.

The experiments were carried out in laboratory conditions with a self-designed manual exposure method (Figure ~~2~~5). In order to isolate the environment of the experiment from the ambient particles, a plastic (PET) bottle was fitted~~fit~~ tightly to the Rapid-E inlet. One of the bottle walls was cut open and two holes of ~15 cm^2 were covered with a household air filter. The filter fabric was tested to hold ~99% of particles larger than 1 micrometre in diameter without any noticeable disturbance of the air inflow into the device. The pollen was injected into the upper part of the bottle by inserting the pipette tip with the pollen sample into the narrow cut in the bottle and then gently blowing the air through the pipette. With the sampling rate up to 2.8 litres per minute, Rapid-E was collecting the pollen grains from the bottle within a few tens of seconds. This simple scheme enabled reducing the environmental sample contamination by up to 5 times compared to the unfiltered air in the lab. Each new experiment used a new bottle and the nozzle of the instrument was cleaned, thus ensuring the removal of previously ~~blown~~ sampled pollen.

Quality and level of contamination of the samples was manually controlled by using the sticky slides. The presence of non-pollen particles (debris from the remnants of inflorescences ~~etc~~etc.) was verified to be substantially less than 1% by the visual inspection of a subset of the calibration events. Abundance of pollen aggregates (several pollens stuck together) was also low but their reliable identification by microscopic analysis was more difficult because of ~~busy~~ thick layer of pollen on slides. The calibration was performed in the Pollen mode, which excluded particles smaller than 5 μm of optical size.

2.3.2 Novi Sad

The scheme of the pollen exposure experiment was ~~practically identical~~ similar to that in Siauliai ~~and Novi Sad~~. The ~~pollen~~ exposure was conducted on the roof by fitting the PET bottle to the sampling pipe after removing the Sigma-2 inlet. Manual microscopic analysis of sticky slides was used to confirm the quality of samples and absence of non-pollen debris and pollen agglomerates. The device was also in Pollen mode, i.e. it filtered out particles smaller than 5 μm of optical size.

Classification was tested for ~~same or similar~~ an adjusted set of pollen morphotypes accounting for the availability of the fresh material during the study season. In particular, *Juniperus* was replaced by *Taxus* and *Festuca* was replaced by *Cynodon* and *Dactyl~~i~~is* aiming to assess the degree of discrimination between different grass genera. Similarly, *Picea* and *Cedrus* pollen were used for assessing differences between the same pollen morphotype. Only *Acer negundo* was analysed as it is the only *Acer* pollen that

is recorded regularly in Serbia. *Fraxinus* (including both *F. excelsior* and *F. ornus*) was added to the test as it is commonly recorded throughout spring season.

2.3.3 Payerne

~~For practical reasons, pollen calibrations in~~ Low ambient concentration of coarse particles allowed a less laborious approach: pollen calibrations in Payerne were performed by directly blowing the material into the Sigma 2 inlet, without protection from contamination. The details of the procedure are described in (Crouzy et al., 2016). ~~Thresholds based criteria were~~ pollen size and this was ~~used to discriminate single pollen grains from debris, dust or agglomerates.~~ In order to obtain a reasonable panel of the relevant pollen types, 60 calibrations were performed for 21 different taxa. Focus was set on repeating calibrations, if possible under varying conditions. Only fresh pollen was used and time between collection and calibration was reduced to a minimum (range: 15-120 minutes). The presence of agglomerates and debris was investigated by collecting histograms of the optical size and of the fluorescence intensity of the recorded events. Cut-offs were introduced accordingly, in order to retain only single pollen grains. The device was also in Pollen mode, i.e. filtered out particles smaller than 5 µm of optical size.

~~2.3 Data analysis methods in Siauliai~~

~~Both modalities of the Rapid-E output (scattering image and the fluorescence spectra) were processed independently with artificial neural networks (ANN) and the scores were merged to obtain the final classification result.~~

~~The scattering image has a peculiarity requiring special treatment. Depending on the particle position with regard to the laser beam while passing through it, the apparent size of the particle deduced from the Rapid-E scattering image varied for different recordings (Figure 1). Therefore, at the beginning, a characteristic template of 44x20 pixels was identified. The template was used to localize the most similar areas in the scattering images. A convolutional neural network was trained for pollen recognition from such images. ANN for scattering images consists of two convolutional blocks for the feature extraction and two fully connected layers for classification (Figure 3). Every convolutional block consists of the 2D convolutional layer, the batch normalization layer, the ReLU activation layer, and the maxpooling layer. One mask of the convolutional layer has size of 5x5. The convolutional layer of the first block has 16 filters, and the one of the second block has 32 filters. The maxpooling layer selects the maximal response from the area of 2x2. At the output of the second convolutional block, the size of the feature vector is 1760. The first fully connected layer has 256 neurons. The second fully connected layer classifies these vectors to the number of pollen classes chosen for the calibration. The ANN was trained using the cross-entropy loss.~~

~~The fluorescence analysis also starts from pre-processing. Similarly to the scattering image, particle position with regard to the laser beam influenced the features (first of all, intensity) of the fluorescence. Apart from that, the deep-UV laser is activated for a short period of time triggered by the 400 nm scattering image analysis, i.e. it can partly or fully miss the particle if fired at a wrong moment of time. As a result, only half of pollen grains recorded by the 400 nm laser produce reasonable intensity of the fluorescence (Table 1).~~

~~The fluorescence spectrum was processed by a multilayer perceptron ANN (Figure 4).~~

Because the Rapid-E device in Siauliai often gives saturated short-wavelength fluorescence spectrum at the first time moment, only half of the possible wavelengths were included in the feature vector. Subsequent reduction of the light intensity allowed inclusion of all 32 values starting already from the second time moment. Dropout and batch normalization layers are used for ANN regularization. This ANN also was trained using the cross-entropy loss. Results of two ANN were fused by summing scores of every pollen type.

With both networks, care was taken to follow up the training process and to avoid over fitting the networks. Despite the large volume of the samples (Table 1), certain over-fitting was possible after many training cycles – see the Discussion section.

2.4. Data analysis methods in Novi Sad

All Rapid-e signals (i.e. scatter, spectrum and life time) were transformed into images and jointly processed by a single ANN (Figure 5). Its architecture considers the same input dimensions of every image, and since the scatter signal could vary in the number of acquisitions, each image's width was equalised by founding its centre of mass and either cutting or zero-padding to fit to 24x70 pixels. The dynamic range of each image was reduced by replacing each pixel value with its logarithm, which resulted in enhancing of the low intensity pixels. Images from temporally resolved spectrum data and all bands of the life time data were used unprocessed.

Only the data of sufficient strength were deemed suitable for the analysis. Experiments in Novi Sad indicated that the threshold of the particle fluorescence intensity level to be > 1500 units at the Rapid-E scale for at least one emitted wavelength.

Since there are three sources of information, the data fusion was needed aiming to create an architecture that would allow the gradient to flow through the whole network, so that the back-propagation can be done updating the weights for each distinct source. Each input image in the ANN goes through its own framework, consisting of 2D convolutional layers, replication padding layers, ReLU activation functions, batch normalization layers, max pooling and the dropout layers, combination of which we will call the convolutional block. The ANN was trained using NLL loss and the Stochastic Gradient Descent with the learning rate of 0.001 and the momentum of 0.9.

For scattering images, we used two convolutional blocks. The first convolutional layer had 10 filters with the kernel size of 5x5, and the second one had 20 filters with the kernel size of 3x3. For the spectral images, the convolutional layer of the first block had 50 filters with the kernel size of 5x5, and the one of the second block had 100 filters with the kernel size of 3x3. For the lifetime images, the first convolutional layer had 70 filters with kernel size of 7x1, the second one had 140 filters with kernel size of 5x1 and the one of the final block had 200 filters with the kernel size of 3x3. At the output of the final convolutional block, the sizes of the feature vectors for scattering image, fluorescence spectrum and lifetime are 1800, 1600 and 1400, respectively. Each feature vector is passed through one fully connected layer with 50 neurons. Those features were concatenated resulting in the feature vector of dimension of 150. The second and the final fully connected layer was of the size of the number of classes, after which the samples were classified with the log-softmax activation function.

~~2.5 Data analysis methods in Payerne: classifier and aspects related with operational use~~

~~The device outputs were first normalized using the respective maxima of the signals: the maximal scattering intensity, the maxima of each of the four lifetime bands and the maximal fluorescence. For scattering, the image was in addition centered and cut to a 24 x 100 shape. Only calibrations with optical size above 10 micrometers were retained, and a fluorescence signal in a range and spectrum compatible with single pollen grains was kept (see (Crouzy et al., 2016) for examples of spectra). The optical size corresponding to 10 micrometers was estimated by comparing the integral of the scattering signal of 5 micrometer PSLs with the integral of the scattering signal for *Urtica* and *Parietaria* pollen grains. For scattering, 5x5 convolutions were applied with 32 filters, ReLU activation, and the pooling layers operated on a 2x2 window. For lifetime, 1D convolution was applied with ReLU activation, with windows size 10x1 and with 10 filters. For the spectrometer, asymmetric 2x4 convolution was applied with 8 filters with ReLU activation. The ANN was trained using the Adam optimizer and categorical cross-entropy as loss function (Figure 6). In order to retain flexibility, additional features were inserted before the final fully-connected layers: features computed from the raw signals indeed showed promising results in [30]. For the moment, only the maximum and the integral of the scattering together with the maxima of each of the four lifetime bands and the maxima of the first three spectrum acquisition were used.~~

~~Even if high expectations on the performance of the classifier are met, problems are bound to occur in the form of false-positive detections. Even a few percent of the error in discriminating between two pollen types can lead to problematic drifts. For example, Birch pollen concentrations regularly exceed 1000 particles per cubic meter in Switzerland in spring. If just 2% of these are mis-interpreted as, e.g., Ambrosia pollen, the false concentration of 20 grains per cubic meter would be already significant for allergy analysis. In order to cope with this, we introduced two methods. Firstly, events with classifications below a certain threshold were disregarded, as was done in (Crouzy et al., 2016) where a reduction of sampling of 20% lead to an increase in precision of about 10%. Secondly, we require in operational monitoring at least a few events with extremely good classification score to occur on the day considered or on the two preceding days, this condition is applied uniformly over the pollen season to check which pollen taxa are present in the air. We preferred this method over the expert supervision or calendar rules due to the fact that it bases only on measurement.~~

3. Results obtained in Siauliai

~~The above technology was used to answer the following questions:~~

- ~~can we identify different pollen genera using the Rapid-E data?~~
- ~~can we identify different species within the same pollen genus?~~
- ~~what is the recognition accuracy for the most-common pollen types in Lithuania?~~

The analysis was started from a semi-qualitative consideration of the fluorescence spectra, primarily aiming at demonstration of the capabilities and the limitations of the approach and preliminarily assessing the principal possibility to construct a reliable particle recognition algorithm.

3.1. Qualitative comparison of the fluorescence spectra of different pollen species

3.1.1. Comparison of fluorescence spectra of different species of the same genus

The experiment included three genera, for which we collected pollen from different species (Table 1): *Salix*, *Pinus*, and *Acer*. Their fluorescence spectra are shown in Figure 7, where the solid lines represent the normalized mean spectrum and shadows show the standard deviation range. ~~We also computed the Standard Error of the Mean value and performed Student tests to evaluate the significance of the difference between the mean spectra within genera.~~ The uncertainties of the mean spectra were a fraction of a ~~%~~ percentage leading to the statistically significant difference ($p<0.001$) at all wavelengths for both *Pinus* and *Acer* mean spectra and even for some wavelengths of the *Salix* spectra.

Despite statistically significant differences between the mean spectra, the sample standard deviation (shadowed ranges in Figure ~~7~~6) was quite large. Therefore, it was not possible to distinguish between *Salix alba* and *Salix fragilis*. The normalised spectra *of Pinus sylvestris* and *Pinus mugo* coincided at the maximum value of the amplitudes at the wavelength of 460 nm but the mean amplitude of the *Pinus sylvestris* spectrum was higher in short-wave range (< 450 nm). At the longer wavelenghts (480-550 nm) the amplitude was higher for the *Pinus mugo* pollen. However, these differences were well inside the sample standard deviation. The difference between the species of the *Acer* genus was the most-pronounced and, even taking the sample variability into account, these were the ones that could be distinguished. The *Acer pseudoplatanus* spectrum showed higher amplitude than *Acer negundo* in the short-wave range and lower in the central part of the spectrum (400-520 nm).

Therefore, two out of three tested genera allowed, in principle, an inter-genus species classification using the pollen fluorescence spectrum. However, the differences between them were evidently too small for the multi-species algorithm considered in the current paper. Practical work was therefore left for the follow-up studies.

3.1.2 Comparison of fluorescence spectra of species of different genera

The study included 11 different pollen genera (Table 1), whose spectra are shown in Figure 7~~8~~ for recordings at every 500 ns starting from the first pulse reaching the detector. For all species, the most intense fluorescence was observed for the wavelengths from 390 to 570 nm, with different locations of the maximum and with different amplitude. For example, the highest mean intensity of fluorescence was recorded for the *Artemisia* pollen: it exceeded >7000. Meanwhile, the amplitudes of *Betula* and *Quercus* reached more than 4000. In all cases, the first pulse had a wider wavelength range than the subsequent ones. The amplitudes of already the second recording (500 ns from the first pulse) was close to zero for wavelengths longer than 600 nm.

In addition, Figure ~~8~~ 7 shows that not only the intensity of the first signal between separate genera differs, but the shape of the second recording is also specific, which is significant for the identification of the pollen morphotype. For example, the difference in fluorescence intensity of *Salix* pollen between the first and second signals was larger than for other tested taxa. Tests with *Festuca* pollen actually showed that, unlike all other species, the signal amplitude grows during the first 500 ns resulting in the absolute maximum intensity of the spectrum registered at the second recording, 500 ns after the fluorescence is induced.

The qualitative analysis of the data was continued by grouping the data according to similarity of the fluorescence spectrum of the first recording (Figure ~~9~~8).

Alnus, *Corylus* and *Betula* plants are in one taxonomic family, and our results indicate that their pollen has a similar fluorescence spectrum. Interestingly, according to the similarities of the fluorescence spectra, *Quercus* pollen appeared in the same group with *Betulaceae*, although the maximum value of its mean of the normalised spectrum was the lowest in the group.

Another group in which the pollen fluorescence curves have similar shapes also consists of pollen of woody plants: *Populus* and *Salix*. They also bloom at a similar time; therefore their precise identification is an important but, as seen from Figure ~~9~~8, a challenging task. The tested grass pollens form a separate group, which however also included pollen of the woody plant *Juniperus*. This group is characterised by the high mean amplitude in short (< 400 nm) wavelength range.

3.2 Recognition skills

The key practical question for the Rapid-E application in the daily pollen monitoring is the accuracy of the pollen type classification~~, which we~~ presented below via the confusion matrices. In these matrices, rows represent the actual type of pollen and columns are the assigned type. All values are in %, the sum of values over each row is 100%: every pollen has to be assigned to some type.

~~Rapid-E provided the scattering and fluorescence data arrays. The recognition procedure in Siauliai was built independently for each of them with subsequent fusion of the results (Tables 2-4).~~

~~3.2.1. Recognition using scattering images only~~

~~In the confusion Table 2, the classification is based on the scattering images. The overall recognition accuracy was at an unimpressive level of 44%.~~

~~Only very large and specific *Pinus* pollen was recognised correctly in 76% cases. In 13% of cases it was mis-classified as *Festuca*. The accuracy similar to the second best species *Artemisia* was obtained for recognising the *Festuca* morphotype. *Corylus*, *Alnus* and *Betula* pollen were frequently confused inside the *Betualceae* family. *Salix* pollen was confused with the *Populus* pollen, which identification accuracy is the lowest in this test. Other pollen morphotypes tested in the experiment are identified correctly in less than 50% of cases.~~

~~3.2.2. Recognition using fluorescence spectra~~

~~The results of pollen identification using the fluorescence spectrum are better than those of the scattering-based recognition. The total accuracy reaches 67% and, as seen from Table 3, pollen of *Pinus*, *Artemisia*, *Acer*, *Festuca*, *Juniperus* and *Salix* are well distinguished.~~

~~Grouping the genera, one can notice that the highest percentage of confusion was again within the *Betulaceae* family. *Betula* was confused with *Alnus* in 29% of cases. Due to confusion with *Alnus* and *Corylus*, about a fifth of the *Quercus* pollen was not recognized correctly. *Populus* could also be attributed to a multitude of pollen types. In particular, in 17% cases it was attributed to *Salix* and in 9% – to *Artemisia*.~~

~~The individual species of *Betulaceae* family were the worst identifiable from their fluorescence but compared to recognition from the scattering image (Table 2) the results are better. An analogous situation was in the case of *Salix*: identification from the fluorescence spectrum was more accurate than that based on the scattering images. The method also significantly improved identification of *Juniperus* and *Acer* pollen in comparison with the scattering images: 36% and 84%, 29% and 84% respectively.~~

3.2.~~3~~1. ~~Combined identification~~ Comparison of the confusion tables obtained in Novi Sad, Payerne and Siauliai

~~Rapid-E provided the scattering and fluorescence data arrays. The recognition procedure in Siauliai was built independently for each of them with subsequent fusion of the results (Tables 2-4).~~

~~Table 4 2 presents the outcome of the combined identification using both the scattering image and the fluorescence spectra. With the exception of *Alnus*, the combination of the identification methods showed better recognition skills than each of the methods separately.~~ ~~Overall, the improvement over individual methods was ~23% compared to scattering images and ~7% compared to fluorescence.~~

~~Overall, 6 out of 11 tested pollen genera were identified with the accuracy better than 75%. The best results (> 91% of correct classification) were achieved for *Pinus* pollen. The pollen of the plants of *Betulaceae* genus was also less confused than with the separate methods, but the recognition of the individual species of this family was still poor. The fusion of scattering image and fluorescence algorithms significantly reduced confusion of *Festuca* and *Pinus* pollens. A particular improvement was obtained for *Acer* and *Juniperus* (> 50%) comparing to the scattering-based classification. Gain over the fluorescence-only method was the largest for *Quercus*, which recognition improved by 14%. The identification of other pollen types improved by 2-10%.~~

The recognition procedure in Siauliai was built independently for scattering and fluorescence signals with subsequent fusion of the results. The tables for the individual components are presented in Annex 1. Table 2 presents the outcome of the combined identification using both the scattering image and the fluorescence spectra. With the exception of *Alnus*, the combination of the identification methods showed better recognition skills than each of the methods separately. Overall, the improvement over individual methods was ~23% compared to scattering images and ~7% compared to fluorescence.

Overall, 6 out of 11 tested pollen genera were identified with the accuracy better than 75%. The best results (> 91% of correct classification) were achieved for *Pinus* pollen. The pollen of the plants of *Betulaceae* genus was identified comparatively well but the recognition of the individual species of this family was poor.

The overall accuracy was very similar for Siauliai and Novi Sad and somewhat better for Payerne, partially owing to the stricter filtering of the raw data. Although it is difficult to make exact comparison of the confusion tables between the studies, it still sheds some light on the overall performance and also

highlights the similarities and differences between the regions. Comparing the Tables 24 and 35, one can see that the difference in the recognition quality is about 10% for most of species, being practically identical for *Betula* (~50% in both studies) and *Quercus* (~60%). Somewhat higher skills in Novi Sad were obtained for *Corylus*, *Alnus* and *Populus* while in Siauliai higher skills were reached for *Acer* and *Artemisia*. It is interesting to note that the confusion between the two chosen grass pollen morphotypes in Novi Sad was not notable and for these genera the Rapid-E data have certain discrimination potential. One can therefore conclude that the multi-species discrimination algorithms applied in these studies showed similar recognition skills. It should be stressed however that the training of the ANNs were completely independent and used the local pollen grains. Therefore, the similar recognition quality does not imply similar pollen in these regions.

As mentioned earlier (Section 2.2.3), the calibration procedure used at MeteoSwiss was slightly different than in Novi Sad and in Siauliai. In addition, the focus at MeteoSwiss was more towards operational applications. As a consequence, only a subset of the 60 calibrations was used to train the classifier. Only taxa with high relevance for monitoring or for which very good calibrations were available were selected. It was noticed that increasing the number of taxa could worsen the problem of false positive detections (see below). An optimum for monitoring purposes was found when using 10 taxa. The performance of the corresponding classifier is shown in Table 4. It is interesting to note that, as expected, most errors occur within the *Betulacae* family, with an extremely low recall for *Alnus*. It was hypothesised that, although calibrations were repeated, the classifier may to some extend recognize the conditions under which the calibration was performed and quality of the sample. Obtaining a classifier working only on the generic features of the taxa is a very difficult task. A holistic validation procedure, going from the analysis of device raw outputs (Section 3.1) analysis to the comparison with reference measurements (Section 4.5), is therefore essential.~~Overall, the improvement over individual methods was ~23% compared to scattering images and ~7% compared to fluorescence.~~

4 Discussion

~~**4.1. Comparison of the confusion tables obtained in Novi Sad, MeteoSwiss (Payerne) and Siauliai.**~~

~~The study in Novi Sad was performed for the largely similar set of species using practically identical experimental part but independent analytical procedures and different pollen material for calibration. Although it is difficult to make exact comparison the confusion tables between the studies, it still sheds some light on the overall performance and also clearly highlights the similarities and differences between the regions. Comparing the Tables 4 and 5, one can see that the difference in the recognition quality is about 10% for most of species, being practically identical for *Betula* (~50% in both studies) and *Quercus* (~60%). Somewhat higher skills in Novi Sad were obtained for *Corylus*, *Alnus* and *Populus* while in Siauliai higher skills were reached for *Acer* and *Artemisia*. It is interesting to note that the confusion between the two chosen grass pollen morphotypes in Novi Sad was not notable and for these genera the Rapid-E data have certain discrimination potential. One can therefore conclude that the multi-species discrimination algorithms applied in these studies performed in a showed similar recognition skillsvery similar way. It should be stressed however that the training of the ANNs were completely independent~~

~~and used the local pollen grains. Therefore, the similar recognition quality does not imply similar pollen in these regions.~~

~~As mentioned earlier (Section 2.2.3), the calibration procedure used at MeteoSwiss was slightly different than in Novi Sad and in Siauliai. In addition, the focus was set at MeteoSwiss towards testing the ability to monitor relevant taxa. As a consequence, only a subset of the 60 calibrations performed was used to train the classifier. Taxa with a high relevance for monitoring or for which very good calibrations were available were selected. It was indeed observed that increasing the number of taxa could worsen the problem of false positive detections (see below). An optimum for monitoring purposes was found when using 10 taxa. The performance of the corresponding classifier is shown in Table 64. It is interesting to note that, as expected, most errors occur within the *Betulacae* family, with an extremely low recall for *Alnus*. Note that this confusion table should be understood as a measure of the ability of the classifier to distinguish between calibrations. As such, although calibrations were repeated, the classifier may to some extend recognize the conditions under which the calibration was performed and the quality of the sample. Obtaining a classifier working only on the generic features of the taxa is a very difficult task. A holistic validation procedure, going from the analysis of device raw outputs (Section 3.1) analysis to the comparison with reference measurements (Section 4.5), is therefore essential.~~

4.12. Over-training – a problem?

The problem of potential over-training was addressed from two directions: via the standard training ~~–~~vs ~~–~~test datasets evaluation, and via an explicit verification of homogeneity of the datasets.

4.12.1. Performance in the training and test datasets

Prior to starting the ANN training, all datasets were split to the training and test subsets. The test subset in Siauliai consisted of 1000 particles picked at the end of every calibration event while all other particles were used for training. The Siauliai ANN training continued until saturation of the recognition quality for the training dataset (see example in Figure 9~~10~~), thus including the overfitting range. The maximum performance of the fluorescence-based recognition was obtained at the epoch of ~900, after which the over-fitting gradually picked up. Therefore, the ANN parameters after this epoch were taken as the study outcome. For the scattering-image-based training, a similar consideration suggested the epoch 3500 as the optimum.

For Novi Sad (Figure 10~~1~~), the training was stopped before the overfitting picked up and thus the parameters of the last trained epoch 3000 were used.

4.12.2. Test of homogeneity of the calibration datasets

One of the concerns regarding the fluorescence-based technology is the stability of the spectra for different conditions of pollen grains, which are affected by ambient humidity, temperature, time they spent in the air, chemical interaction and degradation, etc. Full-scale evaluation of this problem lies beyond the scope of this paper. Here, we only present a brief check demonstrating that ~~this~~ it was~~is~~ not the major issue.

As stated in the methodological section, the calibration set for each pollen type in Siauliai consisted of up to 8 independent calibration sessions. For four species, these sessions ~~sometimes~~ were performed in different days and thus with pollen of different age. A simple check of homogeneity of the fluorescence spectra is then to use the data of one of these days as the training set and those from another day as the test subset. Substantial difference in the recognition quality would point at the inhomogeneous data.

~~This experiment was performed for only 4 species, which had such multi-days calibration sets. Therefore, the problem was significantly simpler: to d~~Distinguishing ~~only between~~ these 4 species is simpler than ~~instead of~~ 11 but ~~in the above Siauliai tables 2-4. However, the~~ important ipart was the difference between the training and test recognition quality.

Comparing the upper and lower rows of Figure 1~~2~~1, one can see that for the above epochs (3500 for scattering- and 900 for fluorescence-based ANNs), the quality of recognition for the training subset (one day) and test subset (another day) differ by <5% for all 4 species. Therefore, we conclude that the conditions during the different days of calibration did not affect the homogeneity of the dataset.

4.2~~3~~. Comparison with other studies on pollen recognition

During recent years, a number of attempts to obtain information about pollen concentration in the air in real time have been undertaken. However, even the most-successful tests carried out with WIBS-4 (O'Connor et al., 2014), Hund BAA500 (Oteros et a., 2015), Yamatronics KH-3000-01 (Kawashima et al., 2017), and Plair PA-300 (Crouzy et al., 2016) devices, strongly advancing the pollen monitoring field, left open the questions of scalability and replicability of the results. They also did not touch the topics related to application of the tested systems in the operational context. Application of yet-another new device – Plair Rapid-E – in our study was pursuing, apart from the scientific objectives, the operational implementation as a mid- to long-term goal. However, having tested 14 different pollen morphotypes, we found that significant work is still needed.

One of the challenges to the automatic monitors is the rich mixture of pollen types in Europe that all pose significant allergenic threat. This makes it particularly difficult for the monitors to satisfy the needs of allergic people and allergologists – unlike in many other regions. For instance~~Thus~~, *Cryptomeria japonica* ~~is the species that has been~~ identified ~~in the automated pollen identification system~~ more than 10 years ago by Kawashima et al. (2007) ~~and~~ is still the main pollen type recognised by that system (Kawashima et al., 2007; Wang et al., 2013; Wang et al., 2014; Takahashi et al., 2018). However, it seems to be more or less sufficient for that region.

Varying level of allergenicity of species within a single genus or a family raises the question if the intra-genus classification is possible. Hirst-based manual techniques do not allow it: pollen grains are too similar in the microscopic analysis. Our results show that such level of identification is not immediately possible using Rapid-E information either~~ – at least the multi-species discrimination algorithm is not sufficiently sensitive~~. In particular, our data demonstrated that the fluorescence spectra of the *Salix alba* and *Salix fragilis* species were ~~all but~~almost identical. More promising were the experiments with *Pinus* and *Acer* (Figure ~~4~~6) and some grasses (Table 3~~5~~) where the work should be continued with different identification algorithms built for these very species after their separation from other pollen types. Other genera should also be tested.

~~We also found out that the f~~Fluorescence spectra can be similar not only between species of a particular genus but also between different families. Several ~~We found several~~ groups of otherwise unconnected species manifested ~~, whose~~ very similar spectra, ~~are similar~~ to a degree that di~~does~~ not allow their reliable differentiation ~~with the first-level multi-species algorithm~~ (Figure ~~9~~8). Similar results were obtained in the studies conducted by D. J. O'Connor with co-authors (2011). They assessed the fluorescence spectrum of pollen of *Betulaceae* family and stated that "birch and alder spectra closely resemble each other although there is a possibility that the birch pollen is less fluorescent than alder". Our results show that in the case of *Alnus*, the fluorescence amplitudes are higher than of *Betula*~~,~~ but the spectra are indeed similar. Similar spectra of *Salix* and *Populus* pollen (Figure 7) also resulted in poor differentiation between them. At the same time, the degree of confusion was higher for *Populus* than for *Salix* ~~, which was recognized much better than *Populus*~~ (Tables 2 – 4). This is in agreement with the results obtained with Hund BAA500 by Oteros et al. (2015), who identified *Salix* pollen as the worst of ~~all~~ pollen types analysed ~~in their work~~ (Oteros et al., 2015). The BAA500 algorithm is based on recognition of the particle shape, which can be weakly related to the scattering images in our study – the very part that showed substantial confusion of almost all studied pollens with *Salix* and *Populus*.

Crouzy et al. suggested that a non-zero fluorescence amplitude around 600 nm wavelength is incompatible with pollen from the *Betulaceae* family (*Alnus*, *Carpinus*, *Corylus* and *Betula*) but could possibly be observed for grass pollen (*Dactylis* and *Phleum*) (Crouzy et al., 2016). Our results support this suggestion and in addition the test in Novi Sad shows that ANN could show some discriminatory power between *Dactylis* and *Cynodon*. Noteworthy, recognition of the herbaceous plants (*Festuca*, *Artemisia*) was considerably better than that of pollen of *Betulaceae* family also in Siauliai (Table ~~4~~2).

~~In general, our results strongly suggest that combination of recognitions based on scattering images and fluorescence spectra have the highest potential as they exploit very different features of the pollen grains and can serve as complementary methodologies. This approach showed the highest overall recognition accuracy exceeding 70%. The lifetime of the fluorescence was explicitly included as a separate set of variables in Novi Sad and Payerne and implicitly used in Siauliai via incorporation of the spectra taken in different moments.~~

One can note that the ~~above~~ recognition accuracy of this study (just above 70%) is in an apparent contradiction with the ~~published~~ results of ~~(~~Crouzy et al.~~,~~ (2016), where the skills were significantly higher: 91% was obtained with PA-300. However, there are several important differences between the approaches. Firstly, the pre-filtering of the particles is substantially stricter and ~~in the procedure of Crouzy et al. (2016) than in Siauliai and Novi Sad and~~ about 20% of classifications ~~outputs~~ were filtered out as uncertain (failed the threshold of the classification quality). Secondly, the accuracy of the recognition depends significantly on the number of pollen morphotypes used for the test (8 by Crouzy et al). In an extreme case, automated discrimination of just one species (*Cryptomeria japonica*) from non-pollen particles using KH-3000 was high already 10 year ago (Kawashima et al., 2007; Kawashima et al., 2017). Similarly, the high fraction of BAA500 true positive counts (93,3%) against manual analysis of individual species by Oteros et al. (2015) went down to 65% ~~as in our study~~ when the recognition of 13 pollen morphotypes was requested. It took an additional training of the algorithm to raise it up to the same 72% as in our study. Finally, it should also be noted that PA-300 delivers fewer parameters than Rapid-E, possibly making it difficult to identify the important combinations in the raw signal in a single-level

many-to-many identification task. Application of additional levels of the discrimination filters can ~~substantially~~ improve the results.

4.~~3~~4. Possible ways to improve the recognition skills

The dependence of the recognition quality on the number of categories is one of the directions of the future research. It may be possible to consider independent groups of pollens that never (or very rarely) appear in the air at the same time – but it can make the algorithm place-specific. It is vital however to obtain improvement of the algorithm for reliable separation of pollens that can be in the air together (e.g., *Betulaceae*, *Quercus*, and the like).

Considering improvements of the recognition algorithms, Matsuda and Kawashima (2018) suggested the "extract window" method of analysis of the scattering images, which enabled to distinguish unique ranges of light scattering intensities for each taxon ~~of pollen~~. However ~~of that study but~~, the reliability of the algorithm is known only for 5 pollen morphotypes. Development of this and similar approaches for the Rapid-E scattering images may eventually improve this line of analysis and, subsequently, push up the overall scores.

Since the output of the ANN can be transformed to give a vector of probabilities, where each element *i* of the vector represents the probability that the sample belongs to class c_i, we expect improvement of the classification accuracy if we demand that the classification occurs only if the highest probability in that vector is greater than some probability threshold, but with the price of discarding the samples below the defined threshold. This direction was initially explored by Crouzy et al (2016) and showed high potential: discarding 20% of samples led to an increase of precision of about 10% (see also Section 2.2.4). ~~The future studies will encompass this challenge of losing samples while introducing probability threshold.~~

As a more radical approach, one can challenge the ~~solo~~ usage of ANNs without a-priori relations derived from physical or chemical features of each pollen type. Even generic considerations of scattering and fluorescence theories might hint on quantities, which show enhanced contrast in comparison with the raw data. The idea was tried in the Payerne algorithm and showed its potential.

4.~~5~~5 Lessons from the comparison of the Hirst and Rapid-E measurements

~~Even if high expectations on the performance of the classifier are met, problems are bound to occur in the form of false-positive detections. Even a few percent of the error in discriminating between two pollen types can lead to problematic drifts. For example, Birch pollen concentrations regularly exceed 1000 particles per cubic meter in Switzerland in spring. If just 2% of these are mis-interpreted as, e.g., Ambrosia pollen, the false concentration of 20 grains per cubic meter would be already significant for allergy analysis. In order to cope with this, we introduced two methods. Secondly, we require in operational monitoring at least a few events with extremely good classification score to occur on the day considered or on the two preceding days, this condition is applied uniformly over the pollen season to check which pollen taxa are present in the air. We preferred this method over the expert supervision or calendar rules due to the fact that it bases only on measurement.~~

Comparison of the Rapid-E of MeteoSwiss with the operational Hirst measurements in Payerne from February to June 2018 extended the results of Crouzy et al. (2016) to more important taxa (Figure ~~13~~12),

but also showed that robust determination of the sampling still needs to be achieved. In order to obtain pollen concentrations, large particles presenting bimodal fluorescence spectra with position and intensity of maxima compatible with the observations made from calibrations (see Section 3.1) were first selected. Then, the classifier presented in Section 2.~~5~~ 2.3 was applied. The effective sampling of Rapid-E is the result of a series of physical and algorithmic processes: the sampling efficiency of the Sigma-2 head, the imperfect targeting by lasers and the drop-offs due to the below-~~the~~ threshold ~~signal during the~~ classification ~~step~~. In Figure ~~13~~12, the Rapid-E data are scaled with species-dependent factors (constant over the season) bringing the seasonal mean to that of the Hirst time series. The issue deserves attention since, as shown by the Novi Sad results, tightening the thresholds improves the recognition skills but increases the drop-offs at the recognition stage. Sampling with *Poaceae* is the highest, *Pinaceae* present a 2% decrease of sampling and *Betula* presents a 33% decrease in sampling with respect to *Poaceae*. False positive are a significant issue with *Fraxinus*: ~~due to~~ the ~~necessary activation thresholds~~ sampling is dramatically reduced (75%) for higher fluorescence thresholds. As a consequence of those limitations, the results presented here should not be taken as a complete demonstration of operational capabilities.

The suppression of the false-positive detections as described in Section 2.~~5~~ 2.4 worked quite efficiently but still an evident false-positive event resulting from the *Betula* misinterpretation as *Poaceae* is visible in the beginning of April. Further work is required to completely remove such events, and, as a last resort, expert supervision could be used in an operational setup.

4.~~5~~6. Opinion of the Rapid-E producer

During the work, we have been in periodic contact with the Plair company regarding features and issues of the Rapid-E devices used by our groups. Having the paper finalised, we asked their feedback.

D.Kiselev, Plair: “Our impression concerning the presented material is mixed. While I see some positive and encouraging results, my main critics would be addressed to your calibration sets, which cleaning and filtering falls short of the actual needs. Our results are 5-10 % better without overfitting the data or other special processing. Time series Plair gets for "problematic pollens" like Betula, Corylus and Alnus are actually very good. Our goal is to obtain high quality time series calculated in real-time by the instruments and the good calibration is essential for that.”

We agree with importance of the calibration datasets; the procedures ensuring their quality are described in the Methodology section and further explained in the discussion above. Noteworthy, our groups were working largely independently using local pollen and original methods of the data collection and processing. Therefore, the similarities in the observed features provide additional support for our conclusions. ~~Unfortunately, the Plair company declined to reveal any details of procedures and datasets substantiating their message.~~ Unfortunately, details of the Plair analysis were not available when the paper was prepared. Therefore, independent evaluation of that algorithm against the common criteria described in this paper was not possible.

5 Conclusions

We conducted the first analysis of the pollen monitoring capabilities of the new automatic pollen detector Plair Rapid-E. Using the very limited data pre-processing and basic ANN classification it was shown that,

if comparatively large number of pollen types is considered, stand-alone scattering- and fluorescence-based recognition algorithms fail to produce reliable results for majority of species. The combination of these algorithms performed better exceeding 80% accuracy for 5 out of 11 species. Therefore, this combination can be considered as the first-stage classification of pollen types. It should be followed by more in-depth discrimination efforts, including also life time of florescence into the classification model, etc.

The fluorescence spectra showed similarities among several tested species ending up with three groups: (*Alnus*, *Corylus*, *Betula* and *Quercus*), (*Salix* and *Populus*), and (*Festuca*, *Artemisia*, *Juniperus*) – as identified from the Siauliai data. The classification between the groups was comparatively easy ~~and reliable but distinguishing pollens inside the groups turned out more problematic~~.

Attempts to distinguish between the species of the same genus showed certain potential for some genera but more work is needed.

The results obtained in Siauliai and Novi Sad with very similar experimental setup but independent analysis, showed comparable results confirming the overall conclusions. They also pointed out at certain limitations of replicability of the raw data features between the devices, which will require an additional conversion step to make them compatible. In this line, the comparison performed at MeteoSwiss shows a reasonable potential for automatic monitoring of important taxa, however it is not clear to which extent algorithms can be transposed from one device to another.

The in-depth discussion and improvement of the methodology and the extension to more taxa goes beyond the scope of this paper. We decided to communicate early the current results, as well as the methods developed independently by the three teams currently working with the Rapid-E counters, in order to stimulate parallel developments by the user community of the Rapid-E devices. The emergence of such community is a good opportunity to address generalization and replicability of the device-specific results. We also believe that moving from expert supervision or calendar methods to the approach presented here and bas~~ed~~~~ing~~ only on device outputs for, e.g., elimination of false-positive detections could be of help for other automatic monitoring systems.

Among the main challenges to be resolved in the future work, the most important ones are:

- to obtain reliable recognition skills at least for the pollen types that can be in the air at same time
- to reach full replicability of the algorithms and results across the different copies of the same monitors (we are thankful to the Plair team for suggesting the scripts addressing this problem, which are now under evaluation)
- to resolve specific questions related to the algorithm construction and training – including the minimal sample volume, problems of over- and under-fitting, preprocessing and pre-filtering of the data, false-positive identifications, etc.

Successful resolution of these questions will open the way for wide applications of the automatic particle counters for pollen observations.

Code and data availability

All data and algorithms presented in the paper are experimental and subject to further development. They are available for research purposes on-request basis from the authors of the manuscript. Work is in progress to harmonise the algorithms and make them public together with the data via open software and data repositories. Possibility of GPL-type license is being evaluated.

Author Contributions:

All the authors made significant contributions to this study. Conceptualization M.S.; Writing – Original Draft Preparation, Review & Editing I.Š., M.S., L.Š, B.S., B.C., B. Cr.; Methodology G.V., I.Š, L.Š; Data analysis and visualization G.D; L.V; M.S; Experiment in Novi Sad P.M., S.B., M.P., B.S; Experiment in Payerne B.C., B. Cr.

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References

Akdis, C. A., Hellings, P. W., and Agache, I. (Eds.): European Academy of Allergy and Clinical Immunology. Global atlas of allergic rhinitis and chronic rhinosinusitis, EAACI, Zürich, 2015.

Bennett, K. D.: Pollen counting on a pocket computer, New Phytol., 114, 275–280, https://doi.org/10.1111/j.1469-8137.1990.tb00399.x, 1990.

Bousquet, J., Caimmi, D. P., Bedbrook, A., Bewick, M., Hellings, P. W., Devillier, P., Arnavielhe, S., Bachert, C., Bergmann, K. C., Canonica, G. W., Chavannes, N. H, Cruz, A. A., Dahl, R., Demoly, P., De Vries, G., Mathieu-Dupas, E., Finkwagner, A., Fonseca, J., Guldemond, N., Haahtela, T., Hellqvist-Dahl, B., Just, J., Keil, T., Klimek, L., Kowalski, M. L., Kuitunen, M., Kuna, P., Kvedariene, V., Laune, D., Pereira, A. M., Carreiro-Martins, P., Melén, E., Morais-Almeida, M., Mullol, J., Muraro, A., Murray, R., Nogueira-Silva, L., Papadopoulos, N. G., Passalacqua, G., Portejoie, F., Price, D., Ryan, D., Samolinski, B., Sheikh, A., Siroux, V., Spranger, O., Todo Bom, A., Tomazic, P. V., Valero, A., Valovirta, E., Valiulis, A., VandenPlas, O., van der Meulen, S., van Eerd, M., Wickman, M., and Zuberbier, T.: Pilot study of mobile phone technology in allergic rhinitis in European countries: the MASK-rhinitis study, Allergy, 72, 857–865, https://doi.org/10.1111/all.13125, 2017.

Bousquet, J., Schunemann, H.J., Fonseca, J., Samolinski, B., Bachert, C., Canonica, G. W., Casale, T., Cruz, A. A., Demoly, P., Hellings, P., Valiulis, A., Wickman, M., Zuberbier, T., Bosnic-Anticevitch, S., Bedbrook, A., Bergmann, K. C., Caimmi, D., Dahl, R., Fokkens, W. J., Grisle, I., Lodrup Carlsen, K., Mullol, J., Muraro, A., Palkonen, S., Papadopoulos, N., Passalacqua, G., Ryan, D., Valovirta, E., Yorgancioglu, A., Aberer, W., Agache, I., Adachi, M., Akdis, C. A., Akdis, M., Annesi-Maesano, I., Ansotegui, I. J., Anto, J. M., Arnavielhe, S., Arshad, H., Baiardini, I., Baigenzhin, A. K., Barbara, C.,

Bateman, E.D., Beghé, B., Bel, E. H., Ben Kheder, A., Bennoor, K. S., Benson, M., Bewick, M., Bieber, T., Bindslev-Jensen, C., Bjermer, L., Blain, H., Boner, A. L., Boulet, L. P., Bonini, M., Bonini, S., Bosse, I., Bourret, R., Bousquet, P. J., Braido, F., Briggs, A. H., Brightling, C. E., Brozek, J., Buhl, R., Burney, P. G., Bush, A., Caballero-Fonseca, F., Calderon, M. A., Camargos, P. A. M., Camuzat, T., Carlsen, K. H., Carr, W., Cepeda Sarabia, A. M., Chavannes, N. H., Chatzi, L., Chen, Y. Z., Chiron, R., Chkhartishvili, E., Chuchalin, A. G., Ciprandi, G., Cirule, I., Correia De Sousa, J., Cox, L., Crooks, G., Costa, D. J., Custovic, A., Dahlen, S. E., Darsow, U., De Carlo, G., De Blay, F., Dedeu, T., Deleanu, D., Denburg, J. A., Devillier, P., Didier, A., Dinh-Xuan, A. T., Dokic, D., Douagui, H., Dray, G., Dubakiene, R., Durham, S. R., Dykewicz, M. S., El-Gamal, Y., Emuzyte, R., Fink Wagner, A., Fletcher, M., Fiocchi, A., Forastiere, F., Gamkrelidze, A., Gemicioğlu, B., Gereda, J. E., González Diaz, S., Gotua, M., Grouse, L., Guzmán, M. A., Haahtela, T., Hellquist-Dahl, B., Heinrich, J., Horak, F., Hourihane, J. O. B., Howarth, P., Humbert, M., Hyland, M. E., Ivancevich, J. C., Jares, E. J., Johnston, S. L., Joos, G., Jonquet, O., Jung, K. S., Just, J., Kaidashev, I. P., Kalayci, O., Kalyoncu, A. F., Keil, T., Keith, P. K., Khaltaev, N., Klimek, L., Koffi N'Goran, B., Kolek, V., Koppelman, G. H., Kowalski, M. L., Kull, I., Kuna, P., Kvedariene, V., Lambrecht, B., Lau, S., Larenas-Linnemann, D., Laune, D., Le, L. T. T., Lieberman, P., Lipworth, B., Li, J., Louis, R., Magard, Y., Magnan, A., Mahboub, B., Majer, I., Makela, M. J., Manning, P., De Manuel Keenoy, E., Marshall, G. D., Masjedi, M. R., Maurer, M., Mavale-Manuel, S., Melén, E., Melo-Gomes, E., Meltzer, E. O., Merk, H., Miculinic, N., Mihaltan, F., Milenkovic, B., Mohammad, Y., Molimard, M., Momas, I., Montilla-Santana, A., Morais-Almeida, M., Mösges, R., Namazova-Baranova, L., Naclerio, R., Neou, A., Neffen, H., Nekam, K., Niggemann, B., Nyembue, T. D., O'Hehir, R. E., Ohta, K., Okamoto, Y., Okubo, K., Ouedraogo, S., Paggiaro, P., Pali-Schöll, I., Palmer, S., Panzner, P., Papi, A., Park, H. S., Pavord, I., Pawankar, R., Pfaar, O., Picard, R., Pigearias, B., Pin, I., Plavec, D., Pohl, W., Popov, T. A., Portejoie, F., Postma, D., Potter, P., Price, D., Rabe, K. F., Raciborski, F., Radier Pontal, F., Repka-Ramirez, S., Robalo-Cordeiro, C., Rolland, C., Rosado-Pinto, J., Reitamo, S., Rodenas, F., Roman Rodriguez, M., Romano, A., Rosario, N., Rosenwasser, L., Rottem, M., Sanchez-Borges, M., Scadding, G. K., Serrano, E., Schmid-Grendelmeier, P., Sheikh, A., Simons, F. E. R., Sisul, J. C., Skrindo, I., Smit, H. A., Solé, D., Sooronbaev, T., Spranger, O., Stelmach, R., Strandberg, T., Sunyer, J., Thijs, C., Todo-Bom, A., Triggiani, M., Valenta, R., Valero, A. L., Van Hage, M., Vandenplas, O., Vezzani, G., Vichyanond, P., Viegi, G., Wagenmann, M., Walker, S., Wang, D. Y., Wahn, U., Williams, D. M., Wright, J., Yawn, B. P., Yiallouros, P. K., Yusuf, O. M., Zar, H. J., Zernotti, M. E., Zhang, L., Zhong, N., Zidarn, M., and Mercier, J.: MACVIA-ARIA Sentinel NetworK for allergic rhinitis (MASK-rhinitis): the new generation guideline implementation, Allergy, 70, 1372–1392, https://doi.org/10.1111/all.12686, 2015.

Bousquet, J., Van Cauwenberge, P., Ait Khaled, N., Bachert, C., Baena-Cagnani, C. E., Bouchard, J., Bunnag, C., Canonica, G. W., Carlsen, K. H., Chen, Y. Z., Cruz, -A. A., Custovic, -A., Demoly, -P., Dubakiene, -R., Durham, -S., Fokkens, -W., Howarth, -P., Kemp, -J., Kowalski, -M. L., Kvedariene, V., Lipworth,- B., Lockey,- R., Lund, -V., Mavale-Manuel,- S., Meltzer,- E. O., Mullol, J., Naclerio, R., Nekam, -K., Ohta, -K., Papadopoulos, -N., Passalacqua, -G., Pawankar, -R., Popov, -T., Potter -P., Price, -D., Scadding, -G., Simons, -F. E. R., Spicak, -V., Valovirta, -E., Wang, -D.-Y., Yawn, B., and Yusufand, O.: Pharmacologic and anti-IgE treatment of allergic rhinitis ARIA update (in collaboration with GA2LEN), Allergy, 61, 1086–96, https://doi.org/10.1111/j.1398-9995.2006.01144.x, 2006.

Buters, J. T. M., Antunes, C., Galveias, A., Bergmann, K. C., Thibaudon, M., Galán, C., Schmidt-Weber, C., and Oteros, J.: Pollen and spore monitoring in the world, Clin Transl Allergy, 8, 9, https://doi.org/10.1186/s13601-018-0197-8, 2018.

Choual, I., Radu, C., Khayath, N., Beck, N., Schoettel, F., Jacob, A., Domis, N., de Blay, F. J., and MD: Comparison of symptoms during a conjunctival provocation test (CPT) and a controlled exposure to birch pollen in the Strasbourg Environmental Exposure Chamber (EEC) (ALYATEC), J. Allergy Clin. Immunol., 141, AB236, https://doi.org/10.1016/j.jaci.2017.12.748, 2018.

Crouzy, B., Stella, M., Konzelmann, T., Calpini, B., and Clot, B.: All-optical automatic pollen identification: towards an operational system, Atmos. Environ., 140, 202–212, https://doi.org/10.1016/j.atmosenv.2016.05.062, 2016.

Devillier, P., Wahn, U., Zielen, S., and Heinrich, J.: Grass pollen sublingual immunotherapy tablets provide long-term relief of grass pollen-associated allergic rhinitis and reduce the risk of asthma: findings from a retrospective, real-world database subanalysis, Expert Rev Clin Immunol, 13, 1199–1206, https://doi.org/10.1080/1744666X.2017.1398082, 2017.

Douladiris, N., Garib, V., Tejkl, M. F., Valenta, R., Papadopoulos, N. G., and Linhart, B.: Detection of genuine grass pollen sensitization in children by skin testing with a recombinant grass pollen hybrid, Pediatr Allergy Immunol, https://doi.org/10.1111/pai.12991, 2018.

Galán, C., Smith, M., Thibaudon, M., Frenguelli, G., Oteros, J., Gehrig, R., Berger U., Clot, B., Brandao R., and EAS QC working group: Pollen monitoring: minimum requirements and reproducibility of analysis, Aerobiologia, 30, 385–395, https://doi.org/10.1007/s10453-014-9335-5, 2014.

Horgan, D., and Pazzagli, M.: Prevention, Early Dialogue and Education in the Personalised Healthcare Era, Biomed Hub., 2, 7–7, https://doi.org/10.1159/000479492, 2017.

Høst, A., Andrae, S., Charkin, S., Diaz-Vázquez, C., Dreborg, S., Eigenmann, P. A., Friedrichs, F., Grinsted, P., Lack, G., Meylan, G., Miglioranzi, P., Muraro, A., Nieto, A., Niggemann, B., Pascual, C., Pouech, M. G., Rancé, F., Rietschel, E., and Wickman, M.: Allergy testing in children: why, who, when and how?, Allergy, 58, 559–569, 2003.

Kawashima, S., Clot, B., Fujita, T., Takahashi, Y., and Nakamura, K.: An algorithm and a device for counting airborne pollen automatically using laser optics, Atmos. Environ., 41, 7987–7993, https://doi.org/10.1016/j.atmosenv.2007.09.019, 2007.

Kawashima, S., Thibaudon, M., Matsuda, S., Fujita, T., Lemonis, N., Clot, B., and Oliver, G.: Automated pollen monitoring system using laser optics for observing seasonal changes in the concentration of total airborne pollen, Aerobiologia, 33, 351–362, https://doi.org/10.1007/s10453-017-9474-6, 2017.

Kiselev, D., Bonacina, L., and Wolf, J. P.: A flash-lamp based device for fluorescence detection and identification of individual pollen grains, Rev Sci Instrum, 84, 033302, https://doi.org/10.1063/1.4793792, 2013.

Kiselev, D., Bonacina, L., and Wolf, J. P.: Individual bioaerosol particle discrimination by multi-photon excited fluorescence, Opt Express, 19, 24516–24521, https://doi.org/10.1364/OE.19.024516, 2011.

Landsmeer, S. H., Hendriks, E. A., De Weger, L. A., Reiber, J. H., and Stoel, B. C.: Detection of pollen grains in multifocal optical microscopy images of air samples, Microsc. Res. Tech., 72, 424–430, https://doi.org/10.1002/jemt.20688, 2009.

Leynaert, B., Neukirch, C., Liard, R., Bousquet, J., and Neukirch, F.: Quality of life in allergic rhinitis and asthma: a population-based study of young adults, Am. J. Respir. Crit. Care Med., 162, 1391–1396, https://doi.org/10.1164/ajrccm.162.4.9912033, 2000.

Matsuda, S., and Kawashima, S.: Relationship between laser light scattering and physical properties of airborne pollen, J Aerosol Sci, 124, 122–132, https://doi.org/10.1016/j.jaerosci.2018.07.009, 2018.

O'Connor, D. J., Iacopino, D., Healy, D. A., O'Sullivan, D., and Sodeau, J. R.: The intrinsic fluorescence spectra of selected pollen and fungal spores, Atmos. Environ., 45, 6451–6458, https://doi.org/10.1016/j.atmosenv.2011.07.044, 2011.

O'Connor, D. J., Healy, D. A., Hellebust, S., Buters, J. T., and Sodeau, J. R.: Using the WIBS-4 (Waveband Integrated Bioaerosol Sensor) technique for the on-line detection of pollen grains, *Aerosol Sci. Technol.*, *48*, 341-349, https://doi.org/10.1080/02786826.2013.872768, 2014.

Oteros, J. Buters, J., Laven, G., Roseler, S., Wachter, R., Schmidt-Weber, C., and Hofman, F.: Errors in determining the flow rate of Hirst-type pollen traps, Aerobiologia, 33, 201–210, https://doi.org/10.1007/s10453-016-9467-x, 2017.

Oteros, J., Pusch, G., Weichenmeier, I., Heimann, U., Möller, R., Röseler, S., Traidl-Hoffmann, C., Schmidt-Weber, C., and Buters, J. T.: Automatic and online pollen monitoring, Int. Arch. Allergy Immunol., 167, 158–166, https://doi.org/10.1159/000436968, 2015.

Pereira, A. M., Jácome, C., Almeida, R., and Fonseca, J. A.: How the Smartphone Is Changing Allergy Diagnostics, Curr Allergy Asthma Rep, 18, 69, https://doi.org/10.1007/s11882-018-0824-4, 2018.

Poethko-Müller, C., Thamm, M., and Thamm, R.: Allergic rhinitis and asthma among children and adolescents in Germany. Results of the cross-sectional KiGGS Wave 2 study and trends, Journal of Health Monitoring, 52–56, http://doi.org/10.25646/5557, 2018.

Prank, M., Sofiev, M., Siljamo, P., and Kauhaniemi, M.: Increasing the Number of Allergenic Pollen Species in SILAM Forecasts, in: Air Pollution Modeling and its Application XXIV, edited by: Steyn, G. D., Chaumerliac, N., Springer International Publishing, Cham, Switzerland, 313–317, https://doi.org/10.1007/978-3-319-24478-5, 2016.

Radauer, C., and Breiteneder, H.: Pollen allergens are restricted to few protein families and show distinct patterns of species distribution, J. Allergy Clin. Immunol., 117, 141–147, https://doi.org/10.1016/j.jaci.2005.09.010, 2006.

Ritenberga, O., Sofiev, M., Kirillova, V., Kalnina, L., and Genikhovich, E.: Statistical modelling of non-stationary processes of atmospheric pollution from natural sources: example of birch pollen, Agric For Meteorol, 226, 96–107, https://doi.org/10.1016/j.agrformet.2016.05.016, 2016.

Ronneberger, O., Schultz, E., and Burkhardt, H.: Automated pollen recognition using 3D volume images from fluorescence microscopy, Aerobiologia, 18, 107–115, https://doi.org/10.1023/A:1020623724584, 2002.

Šaulienė, I., Šukienė, L., Kainov, D., and Greičiuvienė, J.: The impact of pollen load on quality of life: a questionnaire-based study in Lithuania, Aerobiologia, 32, 157–170, https://doi.org/10.1007/s10453-015-9387-1, 2016.

Skoner, D. P.: Allergic rhinitis: definition, epidemiology, pathophysiology, detection, and diagnosis, J. Allergy Clin. Immunol., 108, S2–S8, https://doi.org/10.1067/mai.2001.115569, 2001.

Sofiev, M., Berger, U., Prank, M., Vira, J., Arteta, J., Belmonte, J., Bergmann, K. C., Chéroux, F., Elbern, H., Friese, E., Galan, C., Gehrig, R., Khvorostyanov, D., Kranenburg, R., Kumar, U., Marécal, V.,

Meleux, F., Menut, L., Pessi, A. M., Robertson, L., Ritenberga, O., Rodinkova, V., Saarto, A., Segers, A., Severova, E., Sauliene, I., Siljamo, P., Steensen, B. M., Teinemaa, E., Thibaudon, M., and Peuch V. H.: MACC regional multi-model ensemble simulations of birch pollen dispersion in Europe, Atmos Chem Phys, 15, 8115–8130, https://doi.org/10.5194/acp-15-8115-2015, 2015.

Sofiev, M., Ritenberga, O., Albertini, R., Arteta, J., Belmonte, J., Bonini, M., Celenk, S., Damialis, A., Douros, J., Elbern, H., Friese, E., Galan, C., Oliver, G., Hrga, I., Kouznetsov, R., Krajsek, K., Magyar, D., Parmentier, J., Plu, M., Prank, M., Robertson, L., Steensen, B. M., Thibaudon, M., Segers, A., Stepanovich, B., Valdebenito, A. M., Vira, J., and D. Vokou: Multi-model ensemble simulations of olive pollen distribution in Europe in 2014, Atmos Chem Phys, 17, 12341–12360, https://doi.org/10.5194/acp-2016-1189, 2017.

Sofiev, M., Siljamo, P., Ranta, H., Linkosalo, T., Jaeger, S., Rasmussen, A., Rantio-Lehtimaki, A., Severova, E., and Kukkonen, J.: A numerical model of birch pollen emission and dispersion in the atmosphere. Description of the emission module, Int J Biometeorol, 57, 45–58, https://doi.org/10.1007/s00484-012-0532-z, 2013.

Tabatabaian, F., and Casale, T. B.: Allergic rhinitis management: what's next?, Expert Rev Clin Immunol., 14, 191–196, https://doi.org/10.1080/1744666X.2018.1438270, 2018.

Takahashi, Y., Kawashima, S., Suzuki, Y., Ohta, N., and Kakehata, S.: Enrichment of airborne Japanese cedar (Cryptomeria japonica) pollen in mountain ranges when passing through a front accompanying temperate low pressure, Aerobiologia, 34, 105–110, https://doi.org/10.1007/s10453-017-9494-2, 2018.

Valenta, R., Vrtala, S., Ebner, C., Kraft, D., and Scheiner, O.: Diagnosis of grass pollen allergy with recombinant timothy grass (Phleum pratense) pollen allergens, Int. Arch. Allergy Immunol., 97, 287–294, https://doi.org/10.1159/000236135, 1992.

Wang, Q., Gong, X., Suzuki, M., Lu, S., Sekiguchi, K., Nakajima, D., and Miwa, M.: Size-segregated Allergenic Particles Released from Airborne Cryptomeria japonica Pollen Grains during the Yellow Sand Events within the Pollen Scattering Seasons, Asian J. Atmos. Environ., 7, 191–198, https://doi.org/10.5572/ajae.2013.7.4.191, 2013.

Wang, Q., Nakamura, S., Gong, S., Suzuki, M., Nakajima, D., Takai, Y., Lu, S., Sekiguchi, K., and Miwa, M.: Release behaviour of Cryptomeria japonica pollen allergenic cry j 1 and cry j 2 in rainwater containing air pollutants, Int J Dev Sustain, 9, 42–53, https://doi.org/10.2495/SDP-V9-N1-42-53, 2014.

Zink, K., Kaufmann, P., Petitpierre, B., Broennimann, O., Guisan, A., Gentilini, E., and Rotach, M. W.: Numerical ragweed pollen forecasts using different source maps: a comparison for France, Int J Biometeorol, 61, 23–33, https://doi.org/10.1007/s00484-016-1188-x, 2017.

Table 1. Pollen used for testing the identification capabilities of the instrument in Siauliai

Plant group	Total particles counted by 400 nm laser	Fluorescent particles *	
		Number	Percentage of particles with sufficient fluorescence level
Festuca	21808	12205	56
Artemisia	15521	13370	86
Corylus	14858	10865	73
Alnus	13692	10486	77
Betula	20676	12089	58
Salix alba	15383	13431	87
Salix fragilis	12942	10401	80
Populus	15340	10963	71
Acer negundo	11832	8647	73
Acer pseudoplatanus	11030	7372	67
Juniperus	17926	10404	58
Quercus	17677	8934	51
Pinus sylvestris	14224	8537	60
Pinus mugo	13399	8287	62

* the particle fluorescence intensity level> 1500 at the Rapid-E scale for at least one emitted wavelength. The initial number of pollen noticed by the scattering laser is not used in the analysis. The algorithms were based on data of fluorescent particles. Calibration datasets were normalised.

~~**Table 2.** Confusion table for pollen taxa identification by using ANN based on scattering image. Multiclass accuracy 44 %~~

~~Plant genus~~	~~*Festuca*~~	~~*Artemisia*~~	~~*Corylus*~~	~~*Alnus*~~	~~*Betula*~~	~~*Salix*~~	~~*Populus*~~	~~*Acer*~~	~~*Juniperus*~~	~~*Quercus*~~	~~*Pinus*~~
~~*Festuca*~~	~~52~~	~~1~~	~~1~~	~~2~~	~~1~~	~~3~~	~~3~~	~~5~~	~~5~~	~~9~~	~~19~~
~~*Artemisia*~~	~~2~~	~~58~~	~~1~~	~~1~~	~~4~~	~~13~~	~~3~~	~~4~~	~~9~~	~~3~~	~~2~~
~~*Corylus*~~	~~3~~	~~1~~	~~38~~	~~20~~	~~20~~	~~1~~	~~1~~	~~5~~	~~1~~	~~9~~	~~1~~
~~*Alnus*~~	~~4~~	~~3~~	~~21~~	~~29~~	~~19~~	~~3~~	~~1~~	~~6~~	~~1~~	~~11~~	~~2~~
~~*Betula*~~	~~4~~	~~4~~	~~19~~	~~15~~	~~37~~	~~3~~	~~1~~	~~5~~	~~1~~	~~9~~	~~2~~
~~*Salix*~~	~~5~~	~~8~~	~~1~~	~~0~~	~~5~~	~~51~~	~~13~~	~~3~~	~~6~~	~~7~~	~~1~~
~~*Populus*~~	~~16~~	~~4~~	~~0~~	~~1~~	~~1~~	~~16~~	~~28~~	~~4~~	~~16~~	~~8~~	~~6~~
~~*Acer*~~	~~8~~	~~5~~	~~6~~	~~7~~	~~4~~	~~4~~	~~5~~	~~29~~	~~4~~	~~23~~	~~5~~
~~*Juniperus*~~	~~16~~	~~19~~	~~0~~	~~0~~	~~0~~	~~4~~	~~10~~	~~2~~	~~36~~	~~3~~	~~10~~
~~*Quercus*~~	~~7~~	~~2~~	~~10~~	~~6~~	~~3~~	~~3~~	~~3~~	~~14~~	~~2~~	~~48~~	~~2~~
~~*Pinus*~~	~~13~~	~~2~~	~~0~~	~~1~~	~~1~~	~~1~~	~~1~~	~~1~~	~~3~~	~~1~~	~~76~~

~~Predicted label~~

~~True label~~

Table 3. Confusion table for pollen taxa identification using fluorescence spectrum. Multiclass accuracy 67 %

Plant genus	*Festuca*	*Artemisia*	*Corylus*	*Alnus*	*Betula*	*Salix*	*Populus*	*Acer*	*Juniperus*	*Quercus*	*Pinus*
Festuca	**84**	2	0	0	0	0	1	5	3	0	5
Artemisia	2	**80**	0	0	0	1	3	6	8	0	0
Corylus	2	0	**53**	15	7	6	1	0	1	14	1
Alnus	1	0	14	**45**	19	3	4	1	1	10	2
Betula	3	1	7	29	**38**	3	4	0	1	12	2
Salix	0	2	7	2	3	**72**	9	0	1	4	0
Populus	2	9	3	2	2	17	**53**	4	4	3	1
Acer	6	3	1	0	1	0	2	**84**	2	1	0
Juniperus	6	5	0	0	1	0	3	0	**84**	0	1
Quercus	1	0	12	13	9	6	2	0	1	**55**	1
Pinus	11	0	0	0	1	0	0	0	1	1	**86**

Predicted label

True label

Table 42. Confusion table obtained in Siauliai. based on two ANNs fused by summing scores of every pollen type

Accuracy: 73 %

Plant genus	*Festuca*	*Artemisia*	*Corylus*	*Alnus*	*Betula*	*Salix*	*Populus*	*Acer*	*Juniperus*	*Quercus*	*Pinus*
Festuca	**88**	1	0	0	0	0	2	5	2	0	2
Artemisia	2	**86**	0	0	0	2	1	4	5	0	0
Corylus	2	0	**63**	17	8	1	0	0	0	9	0
Alnus	1	0	15	**53**	18	2	1	0	0	9	1
Betula	3	1	9	30	**47**	1	1	0	1	6	1
Salix	1	1	2	1	2	**78**	10	0	1	4	0
Populus	3	6	1	1	1	18	**58**	3	3	5	1
Acer	5	2	1	1	1	0	2	**86**	1	1	0
Juniperus	4	4	0	0	0	0	3	1	**87**	0	1
Quercus	2	0	9	10	5	4	1	0	0	**69**	0
Pinus	7	0	0	0	0	0	0	0	1	0	**91**

Predicted label

True label

Table ~~.~~ ~~53~~. Confusion table obtained in Novi Sad. Accuracy 74% (obs different number of species)

Plant genus	*Dactilis*	*Cynodon*	*Corylus*	*Alnus*	*Betula*	*Salix*	*Fraxinus*	*Populus*	*Acer*	*Artemisia*	*Taxus*	*Quercus*	*Picea*	*Cedrus*
Dactilis	**78**	3	0	3	0	2	0	4	5	0	1	0	0	0
Cynodon	4	**70**	0	0	0	6	1	0	0	12	5	0	2	0
Corylus	0	0	**64**	6	12	1	10	0	0	0	4	2	0	0
Alnus	1	2	6	**72**	3	2	3	2	1	2	6	3	0	0
Betula	1	0	25	5	**51**	3	3	0	0	1	1	10	1	0
Salix	3	1	0	2	1	**80**	3	2	2	2	1	3	1	0
Fraxinus	0	0	7	1	3	2	**79**	1	0	0	4	3	0	0
Populus	5	3	1	4	0	4	3	**71**	1	1	3	4	1	0
Acer	8	1	0	2	1	4	0	0	**73**	0	1	9	1	0
Artemisia	1	5	0	2	1	4	0	1	0	**84**	0	1	1	0
Taxus	0	3	0	2	0	0	0	0	0	0	**93**	1	1	0
Quercus	1	0	4	5	8	9	2	1	1	1	1	**63**	4	0
Picea	3	3	0	2	4	3	0	1	1	7	1	13	**61**	0
Cedrus	0	0	0	0	0	1	0	1	0	1	0	0	1	**95**

True label

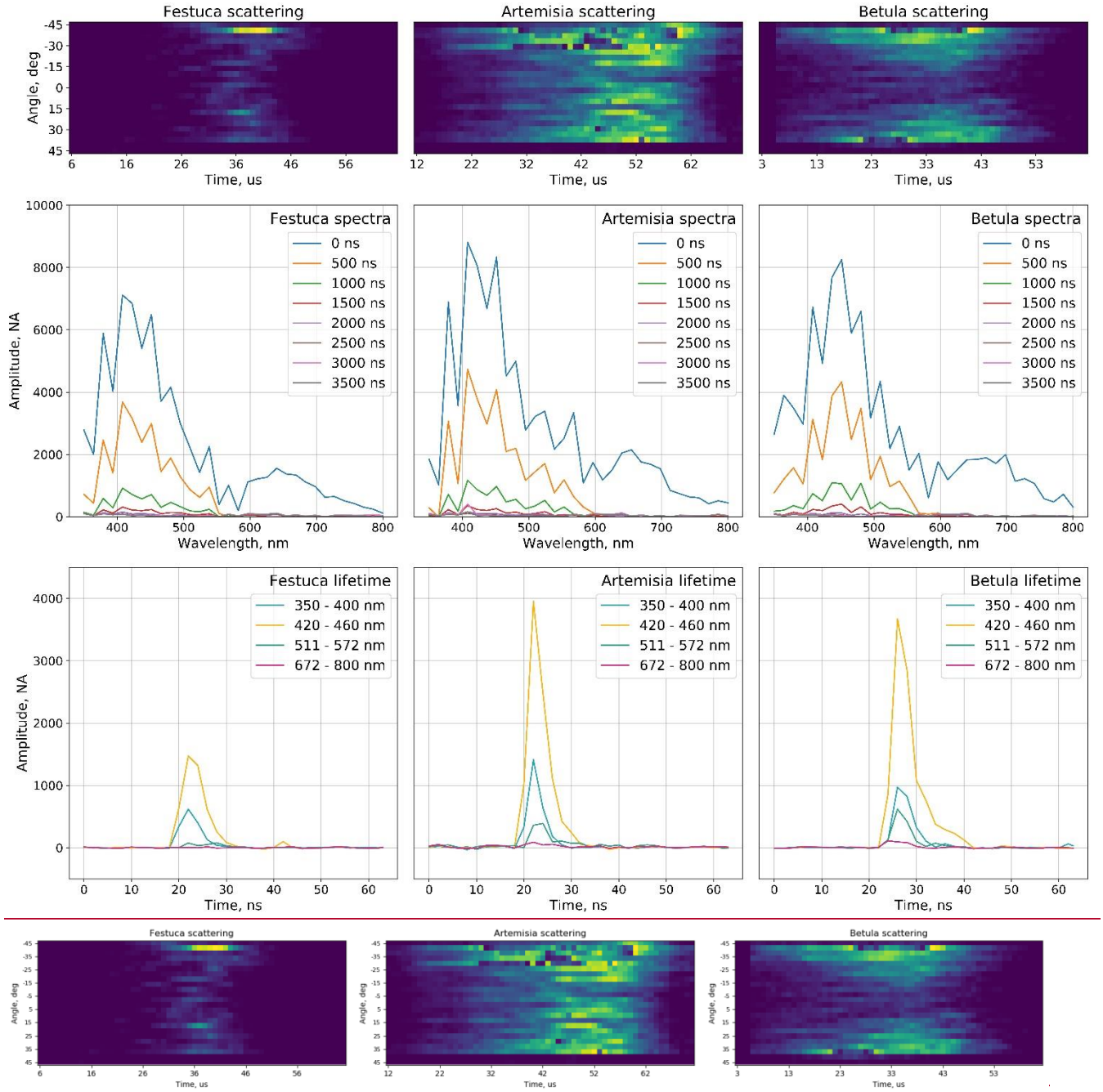
Predicted label

Table ~~6~~ ~~4~~ Confusion table obtained at MeteoSwiss, Payerne. Accuracy 80% ~~(obs different number of species)~~

Plant genus	*Alnus*	*Betula*	*Carpinus*	*Cupressus*	*Fagus*	*Fraxinus*	*Corylus*	*Pinus*	*Poaceae*	*Taxus*
Alnus	**27**	27	1	0	0	1	43	0	0	1
Betula	1	**83**	2	0	0	4	7	0	0	1
Carpinus	0	13	**74**	0	0	2	3	0	6	1
Cupressus	0	3	1	**84**	0	0	1	2	1	8
Fagus	0	2	3	1	**88**	0	1	1	2	3
Fraxinus	0	12	2	0	0	**78**	2	1	2	3
Corylus	4	8	0	0	0	0	**87**	0	0	0
Pinus	0	0	0	0	0	0	0	**98**	0	2
Poaceae	0	3	8	1	0	1	0	1	**82**	4
Taxus	0	0	0	1	0	0	0	1	0	**97**

True label

Predicted label


Festuca scattering
Artemisia scattering
Betula scattering
Angle, deg
Time, us
Festuca spectra
Artemisia spectra
Betula spectra
0 ns
500 ns
1000 ns
1500 ns
2000 ns
2500 ns
3000 ns
3500 ns
Amplitude, NA
Wavelength, nm
Festuca lifetime
Artemisia lifetime
Betula lifetime
350 - 400 nm
420 - 460 nm
511 - 572 nm
672 - 800 nm
Time, ns

Festuca scattering
Artemisia scattering
Betula scattering
Angle, deg
Time, us

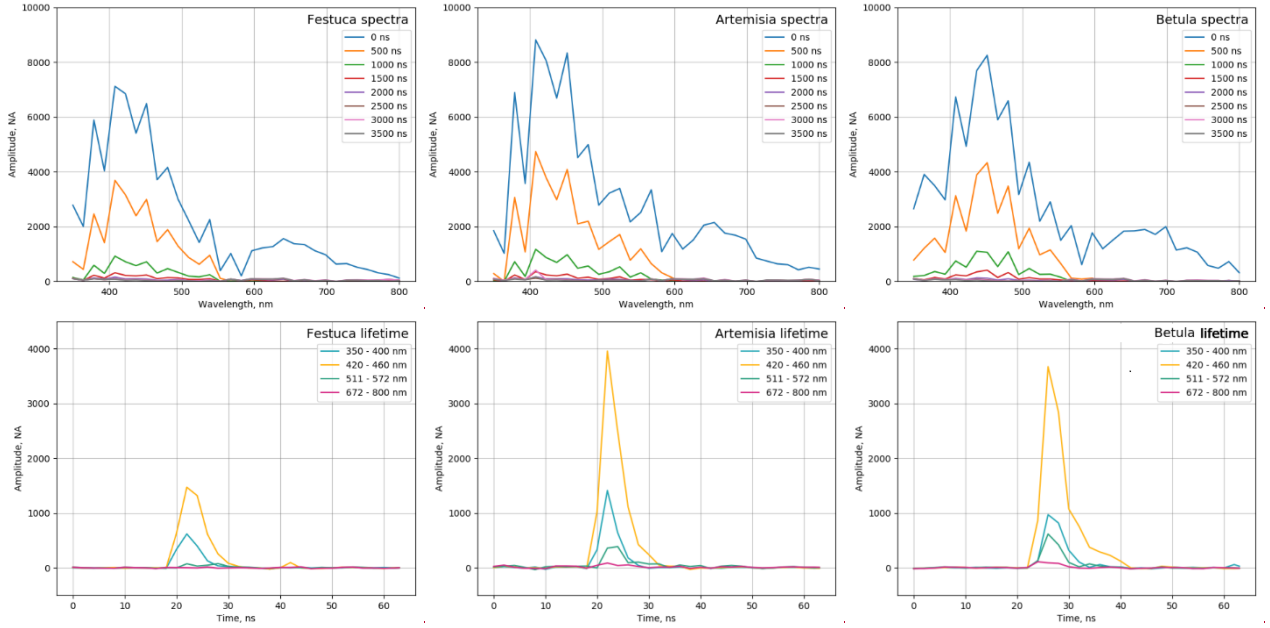
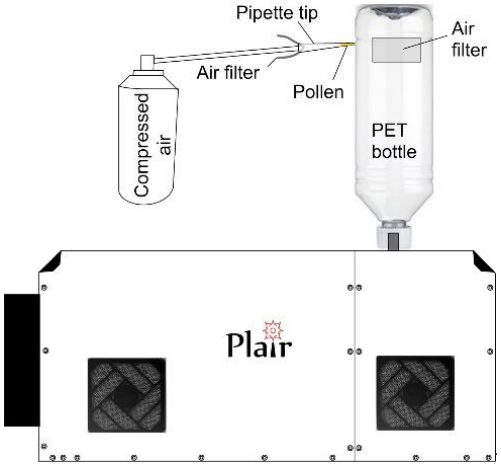


Figure 1. Examples of scattering images, fluorescence spectra and lifetimes of selected pollen types



~~**Figure 2.** The scheme of the experiment for identification of pollen~~

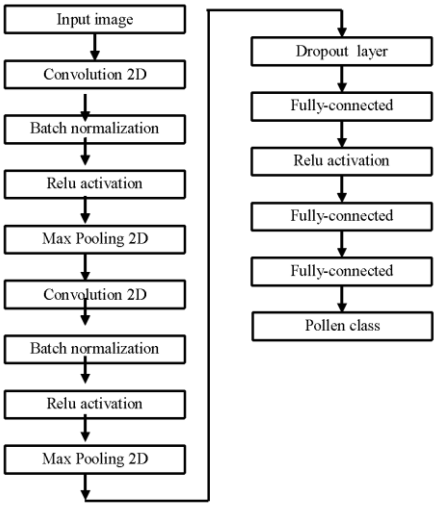


Figure 3. Neural network for pollen classification from the scattering image

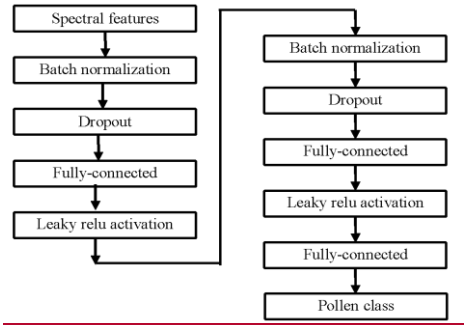


Figure 4. Neural network for pollen classification by fluorescence spectrum

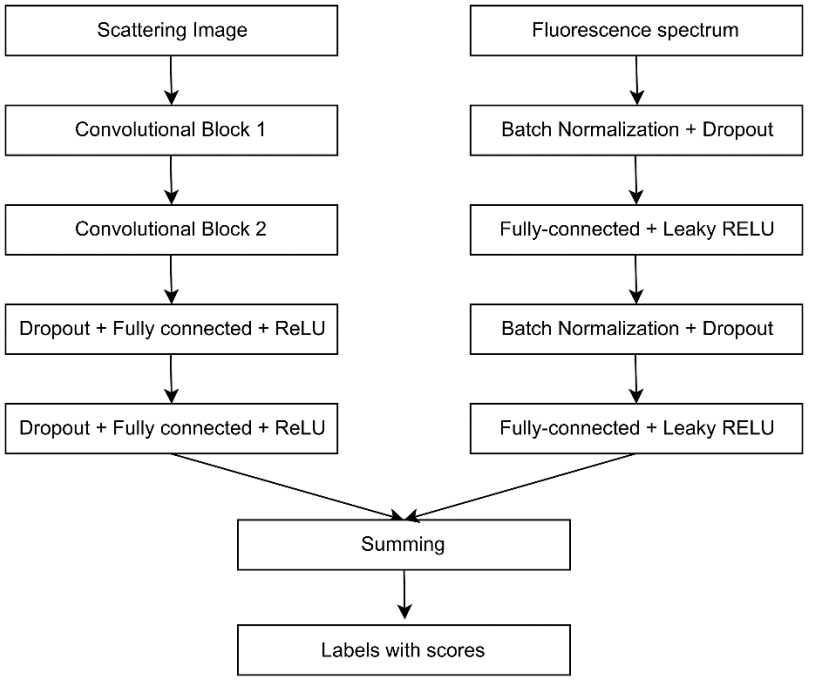


Figure 32. Neural network for pollen classification in Šiauliai based on separately treated scattering and fluerescence signals

1400

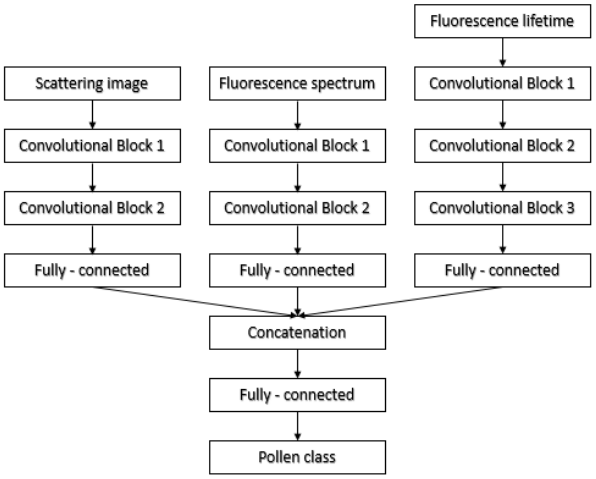
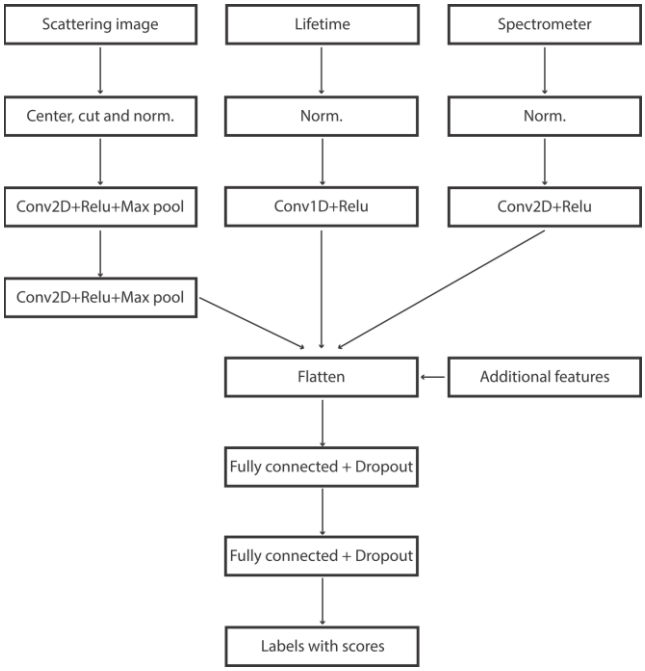


Figure ~~5~~3. Neural network for pollen classification in Novi Sad using ~~by~~ all three signals



1405

Figure ~~4~~6. Neural network used for classification at Payerne~~MeteoSwiss~~

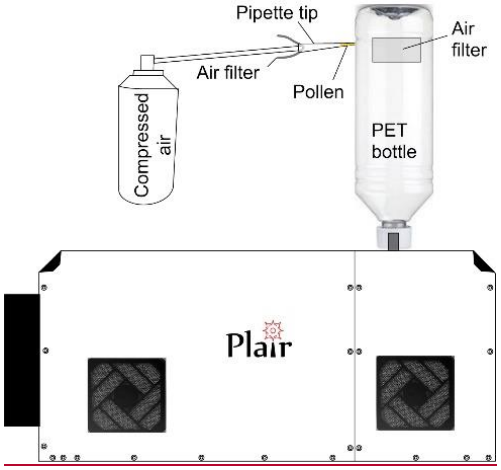


Figure 5. The scheme of the experiment for identification of pollen

1410

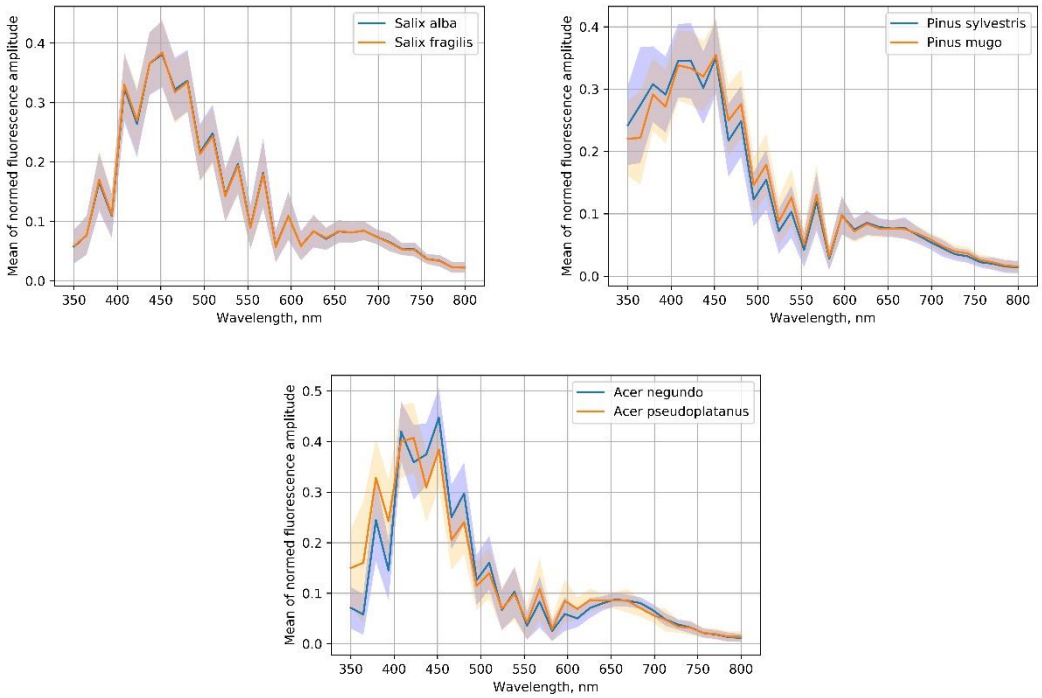
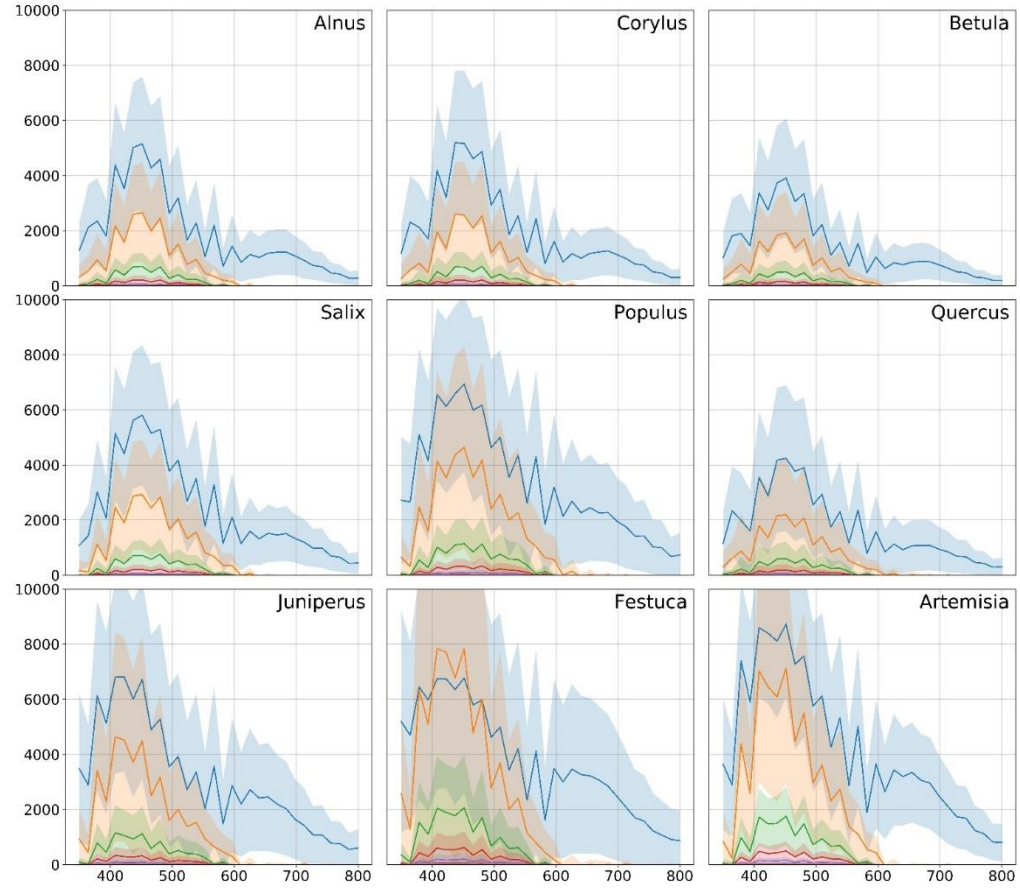
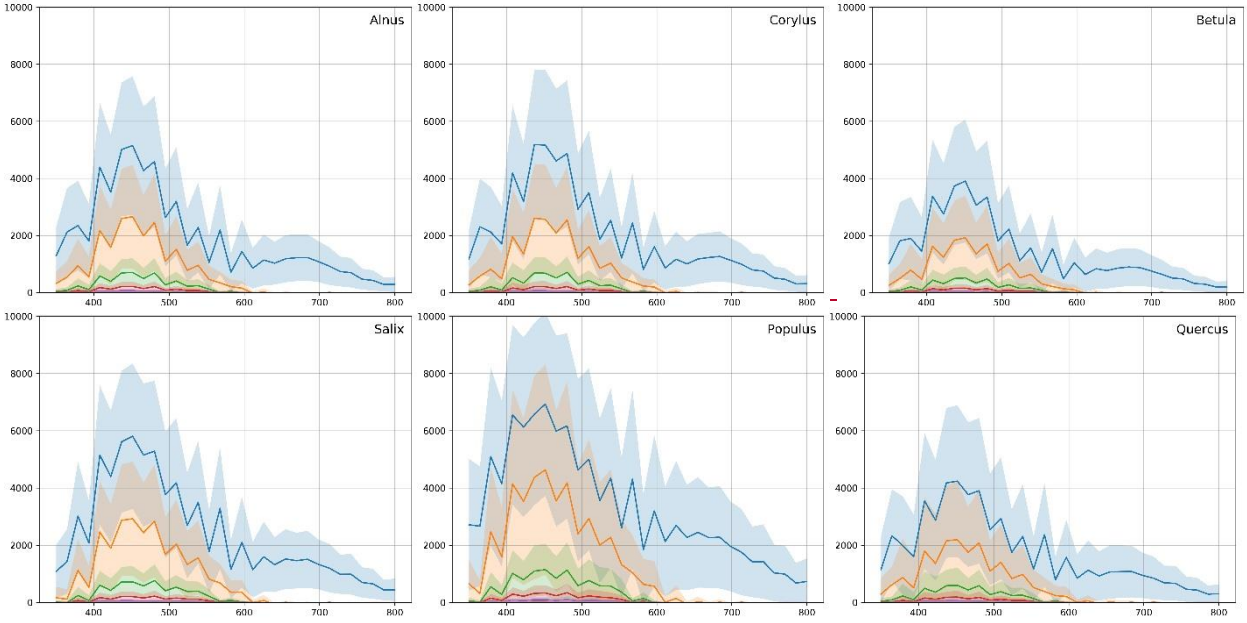


Figure 6~~7~~. Fluorescence spectra (first acquisition) of *Salix*, *Pinus* and *Acer* species

1415


Alnus
Corylus
Betula
Salix
Populus
Quercus
Juniperus
Festuca
Artemisia
0
2000
4000
6000
8000
10000
400
500
600
700
800


Alnus
Corylus
Betula
Salix
Populus
Quercus
0
2000
4000
6000
8000
10000
400
500
600
700
800

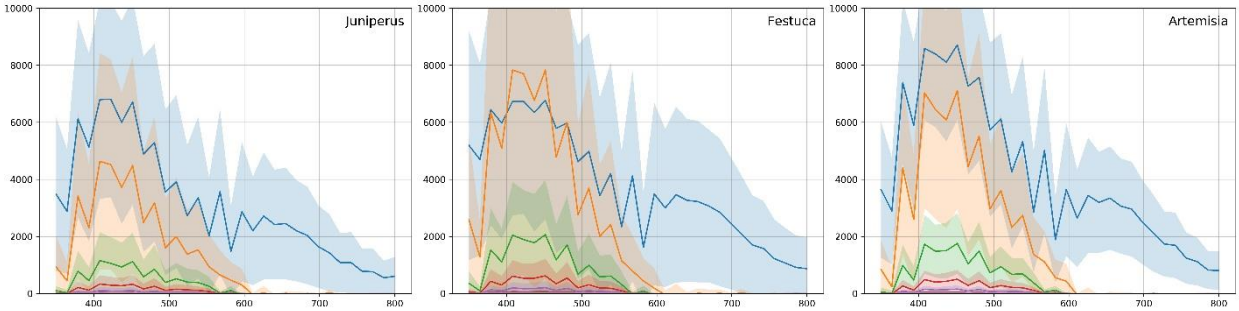


Figure ~~8~~7. Comparison of fluorescence indicators of the tested pollen. The blue line represents the first acquisition. All other lines are delayed acquisition by step of 500 ns from the last. Shadows show the standard deviation ranges for each acquisition. In the figures, the x-axis represents the wavelength, nm; the y axis shows the amplitude, NA

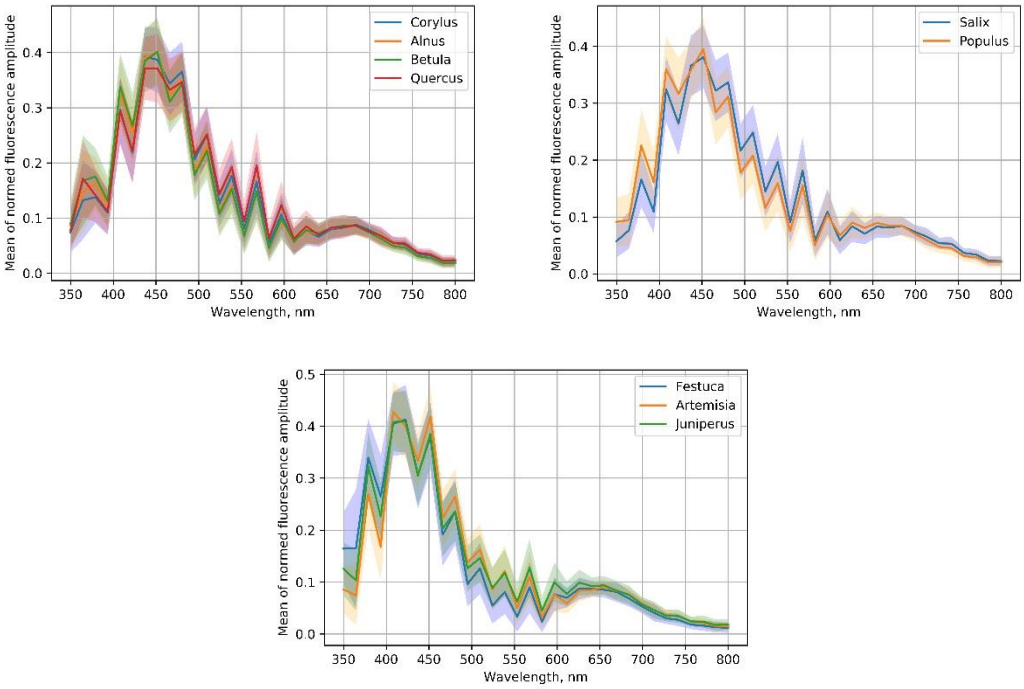


Figure ~~98~~. Groups with similar fluorescence spectra

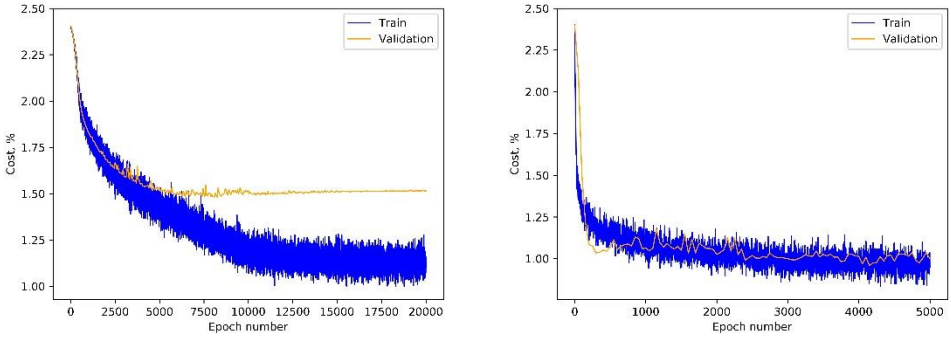
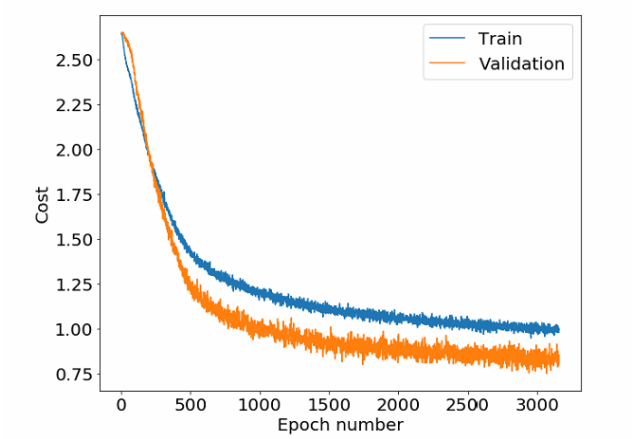
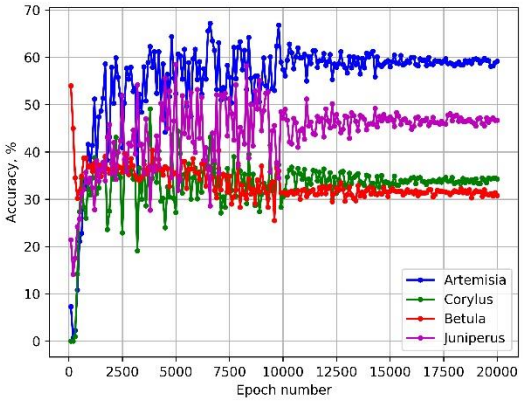


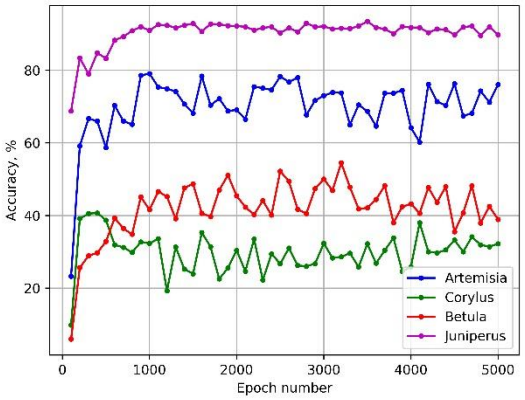
Figure ~~109~~. Siauliai ANN multi-species cost function for scatter (left) and fluorescence-(right) based recognition as a function of the training epoch.



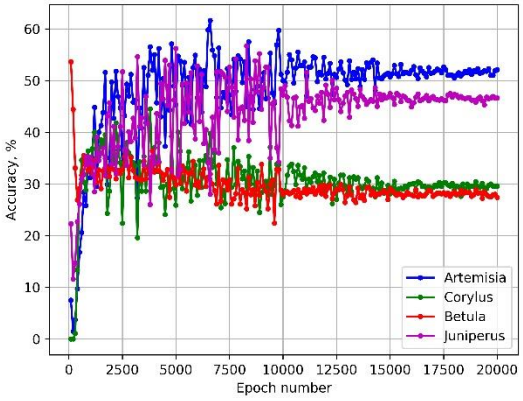
1435 **Figure ~~11~~10.** Novi Sad ANN overall cost as a function of the training epoch. The evaluation error is lower than the training error due to dropout (0.5) in each convolutional and fully connected layer, not used in the validation round.



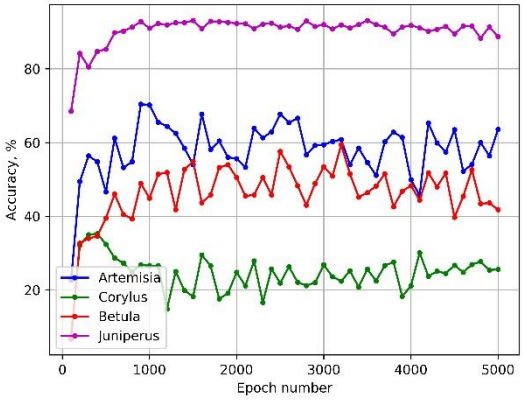
Scattering training accuracy



Fluorescense training accuracy



Scattering test accuracy



Fluorescence test accuracy

1440 **Figure ~~12~~11.** Performance of the Siauliai ANN for the test subset taken from different days than the calibration subset. Unit: %.

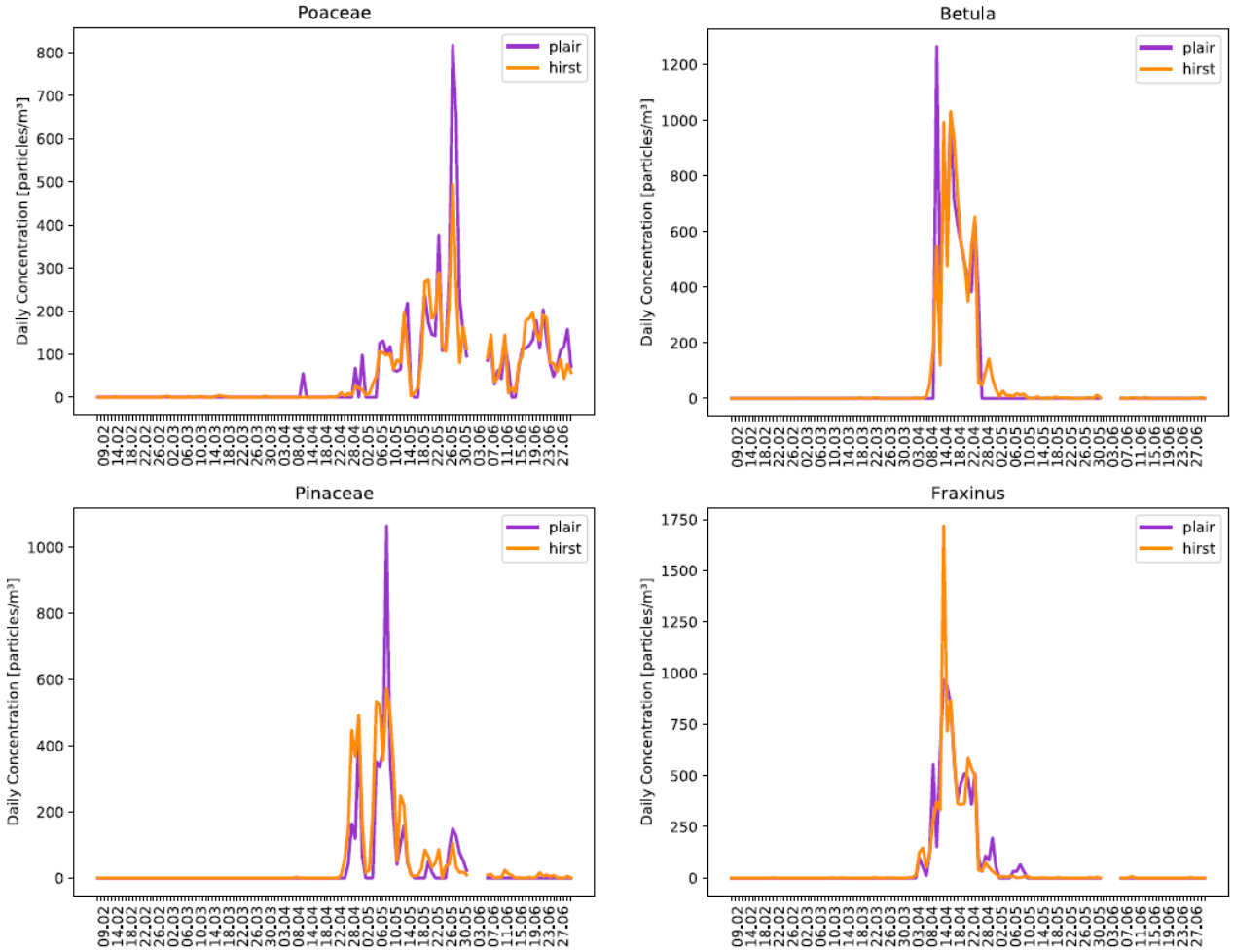


Figure ~~13~~12. Comparison between automatic (Plair Rapid-E) and manual (Hirst-type) pollen counts for *Betula*, *Fraxinus*, *Pinaceae*, *Poaceae*.

Annex 1

Table 2A1. Confusion table for pollen taxa identification by using ANN based on scattering image. Multiclass accuracy 44 %

Plant genus	*Festuca*	*Artemisia*	*Corylus*	*Alnus*	*Betula*	*Salix*	*Populus*	*Acer*	*Juniperus*	*Quercus*	*Pinus*
Festuca	**52**	1	1	2	1	3	3	5	5	9	19
Artemisia	2	**58**	1	1	4	13	3	4	9	3	2
Corylus	3	1	**38**	20	20	1	1	5	1	9	1
Alnus	4	3	21	**29**	19	3	1	6	1	11	2
Betula	4	4	19	15	**37**	3	1	5	1	9	2
Salix	5	8	1	0	5	**51**	13	3	6	7	1
Populus	16	4	0	1	1	16	**28**	4	16	8	6
Acer	8	5	6	7	4	4	5	**29**	4	23	5
Juniperus	16	19	0	0	0	4	10	2	**36**	3	10
Quercus	7	2	10	6	3	3	3	14	2	**48**	2
Pinus	13	2	0	1	1	1	1	1	3	1	**76**

Predicted label

True label

Table ~~3~~A2. Confusion table for pollen taxa identification using fluorescence spectrum. Multiclass accuracy 67 %

Plant genus	*Festuca*	*Artemisia*	*Corylus*	*Alnus*	*Betula*	*Salix*	*Populus*	*Acer*	*Juniperus*	*Quercus*	*Pinus*
Festuca	**84**	2	0	0	0	0	1	5	3	0	5
Artemisia	2	**80**	0	0	0	1	3	6	8	0	0
Corylus	2	0	**53**	15	7	6	1	0	1	14	1
Alnus	1	0	14	**45**	19	3	4	1	1	10	2
Betula	3	1	7	29	**38**	3	4	0	1	12	2
Salix	0	2	7	2	3	**72**	9	0	1	4	0
Populus	2	9	3	2	2	17	**53**	4	4	3	1
Acer	6	3	1	0	1	0	2	**84**	2	1	0
Juniperus	6	5	0	0	1	0	3	0	**84**	0	1
Quercus	1	0	12	13	9	6	2	0	1	**55**	1
Pinus	11	0	0	0	1	0	0	0	1	1	**86**

Predicted label

True label

1455