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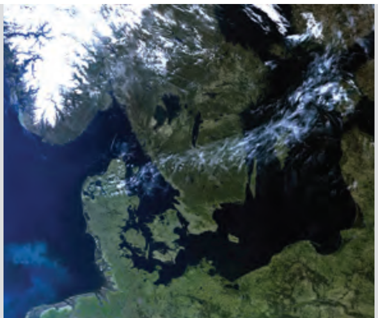

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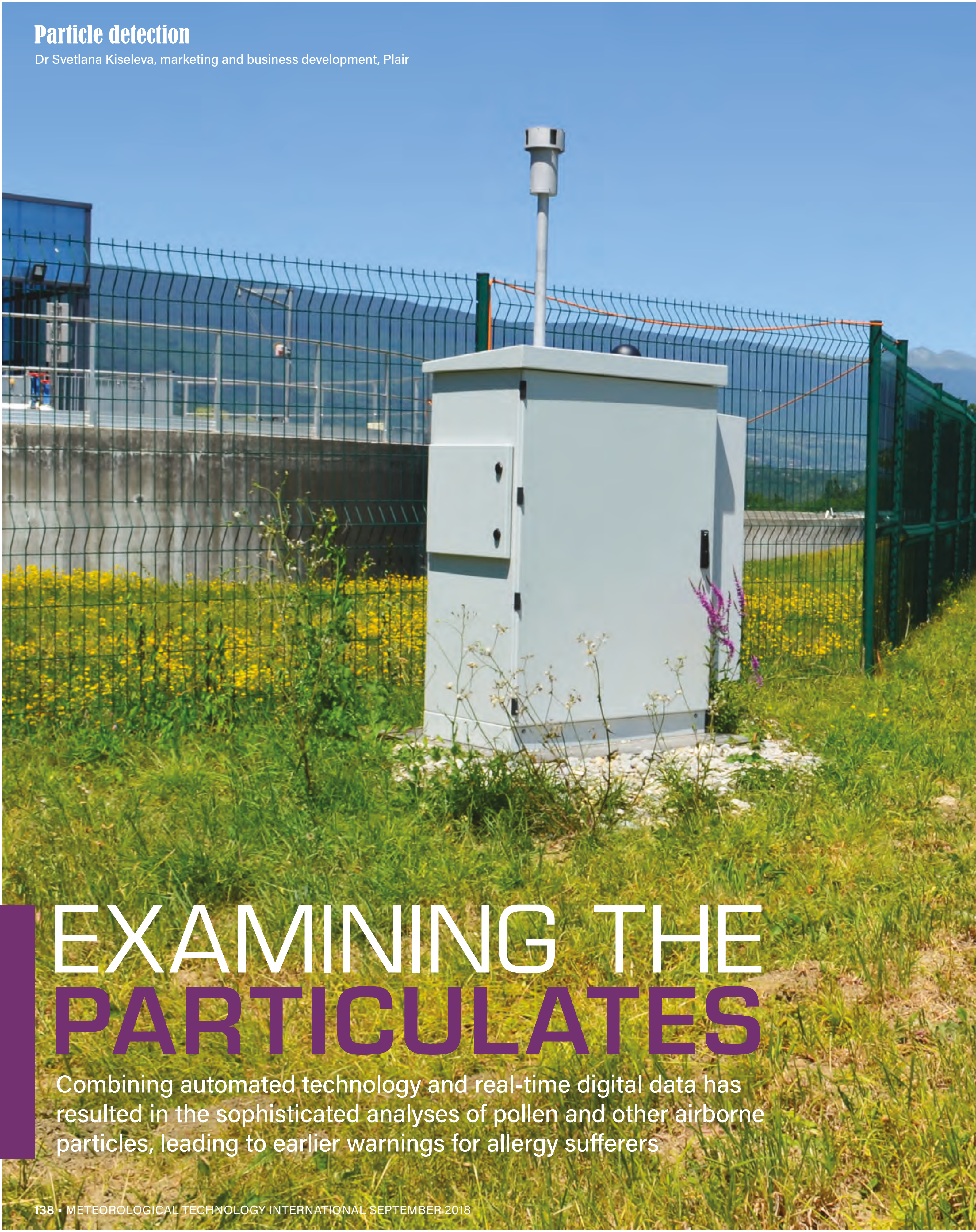
Dr Svetlana Kiseleva, marketing and business development, Plair



EXAMINING THE PARTICULATES

Combining automated technology and real-time digital data has resulted in the sophisticated analyses of pollen and other airborne particles, leading to earlier warnings for allergy sufferers

A network of Rapid-E instruments from Plair is currently performing successfully in Switzerland

Traditional methods of detecting aeroallergens are slow and labor-intensive, taking two to eight days to produce results. Automated methods, in contrast, can be sensitive, precise and quick. The increasing prevalence of allergies and asthma caused by pollen has led to a greater need for automated aeroallergen monitoring, to provide timely information and accurate predictions. Continuous monitoring and real-time reporting of pollen concentrations in the air can help susceptible individuals better prepare to avoid exposure.

Technological progress in hardware and software over the last 20 years has made pollen counting and classification more efficient. Despite global trends in automation, however, some industries are still reluctant to move to alternative methods. In fact, users often consider these methods as black boxes because of their complex technology. Companies developing solutions based on automated pollen monitoring need to adapt to these barriers while delivering reliable high-resolution data.

There are two common methods of automatic pollen monitoring: one based on automated imaging and the other based on flow cytometry using fluorescence. While other existing and emerging technologies for rapid identification, such as polymerase chain reaction or DNA analysis, provide accurate results, they cannot produce uninterrupted measurements and so are unsuitable. Moreover, they require a person to operate them and to replace saturated chips. Until now, image recognition methods have only been successful for detecting pollen, while flow cytometry can be applied to analyze numerous types of particles simultaneously. For example, it can measure fine and coarse particles together with airborne pollen. In addition, methods based on fluorescence flow cytometry can measure fungal spores and bacteria.

Rapid-E, or Real-Time Airborne Particle Identifier, which corresponds to an advanced fluorescence-based flow cytometer (Figure 1), is produced by biotechnology company Plair. The instrument's patented, proprietary technology relies on advanced laser analysis through scattering and fluorescence, and its machine learning algorithms classify pollen species automatically. The system has been tested on more than 15 species of tree pollen, including birch, alder, hazel, pine, cypress, oak, ash, plantain, plane, hornbeam and elm, and numerous weeds and grasses. New pollen types can be added through the calibration process.

Aside from producing real-time measurements, Rapid-E also collects samples which can be verified manually. The process is similar to how traditional microscopy is undertaken, using manual identification of samples under a microscope.

QUALITY CONTROL

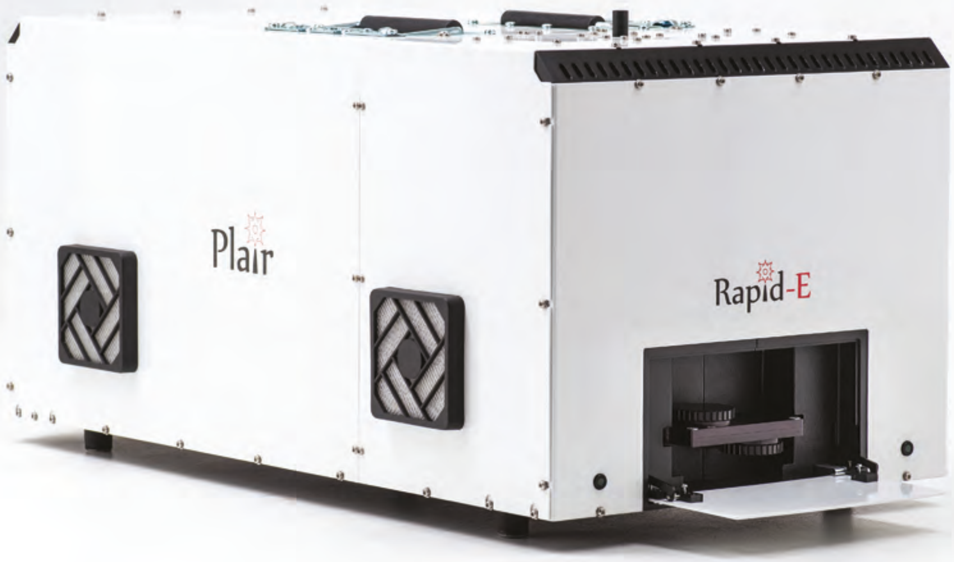
Ever since the idea of using fluorescence-based flow cytometry to monitor pollen was first introduced, some users have raised a weakness – that these instruments were unable to provide backup images for subsequent quality control or analysis. To address this need, Plair has introduced a mechanism to perform on-demand sampling following online measurements of scattering and fluorescence.

Rapid-E can collect multiple samples of airborne particles intended for lab-based analysis at a later stage. Users can install up to 10 independent filters in Rapid-E's internal airflow, and collect microparticles during user-defined time spans. The filters can easily be used for further in vitro analysis and replaced afterwards. Glass slides used for traditional microscopy serve as impaction media in the sampling mechanism (Figure 2).

Examples of airborne particles captured by Rapid-E's sampling mechanism and analyzed under a microscope are shown in Figure 3. They reveal that Rapid-E can efficiently sample biological particles, from small fungal spores to large pollen grains. Large air pollutants of more than 5µm in diameter can also be observed.

PLAIRGRID DASHBOARD

Rapid-E's PlairGrid online dashboard enables users to activate and run the sampling module remotely. They can define the date and timespan to perform sampling for each



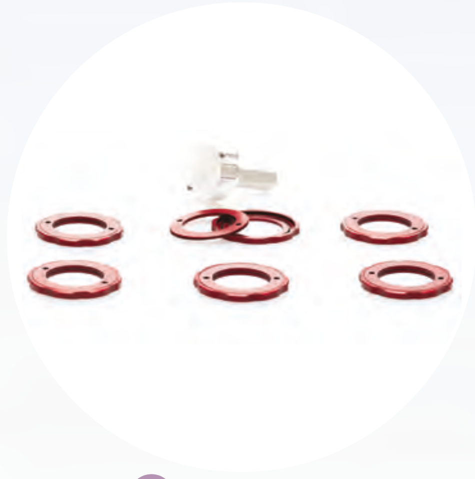


Figure 1: Rapid-E detects numerous airborne particles, including pollen, fungal spores and air pollution

Figure 2: Rapid-E's filter sampling mechanism uses glass slides to collect and sample airborne particles

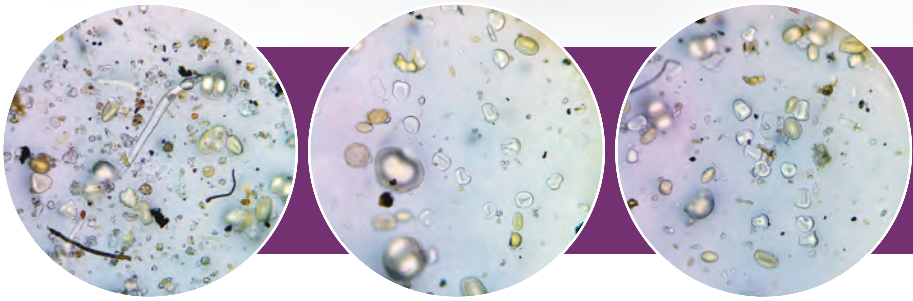


Figure 3: Typical images, obtained with Rapid-E's sampling mechanism, of tree pollen species, pollutants, and fungal spores on filters

filter. Up to 10 independent samplings can be launched automatically from a distance without intervention on the instrument.

Applications of on-demand sampling include quality control of calibrations on various pollen species, a posteriori analysis of pollutants with chemical methods, and identification of fungal spores using microscopy or molecular tools, among other research applications.

SIMULTANEOUS MEASUREMENT

Besides its use for sophisticated pollen analysis, fluorescence-based flow cytometry can also measure air pollutants, such as fine and coarse particles. Timely measurements of all airborne particles provide a better picture of atmospheric aerosol concentrations. Indeed, such measurements are in high demand for healthcare-related research, as the combination of pollen with other particulate matter (PM) can lead to more severe allergic reactions.

To answer this need, Plair recently implemented the functionality of PM measurements in Rapid-E, which runs in parallel with its sophisticated scattering and spectroscopic analysis. Four standard parameters are now reported to the instrument's dashboard and the online PlairGrid dashboard: PM1, PM2.5, PM10 and total particulate matter. Introducing this feature was a natural step, since Rapid-E can detect airborne particles down to 0.5µm. Performed independently, PM measurements consider all particles passing though the instrument whether or not fluorescence measurement is performed.

For online measurements, Rapid-E can be adjusted to execute full particle analysis only on a selected particle size, ranging from 0.5µm to 100µm. A good example is configuring the instrument to count pollen grains (full analysis of all particles larger than 5µm) as well as to measure PM. In this case, the volume of data and usage of sensitive ultraviolet components are optimized, allowing for long-period and consistent monitoring with Rapid-E.

OPPORTUNITIES FROM AUTOMATION

Automated technology leads to efficient and speedy airborne allergen analysis and enables the production of continuous and timely data. Rapid-E can count pollen in real time, and identify and classify multiple species while measuring other particulates. It delivers uniform digital data that can be stored, shared and disseminated further among users and the susceptible population. Such data helps to develop a common pollen database and global forecasting system. The data's real-time availability permits the identification of trends in pollen emission, which is still not possible through traditional sampling methods.

What's more, in addition to real-time observations, Rapid-E preserves the advantages of traditional sampling. The instrument can collect a wide range of airborne particles on filters, including pollen, spores and particulates. The filters can then be analyzed under a microscope and stored for later analysis. Such samples can be used for quality control to verify real-time analysis or to check calibrations.

The current and potential benefits of advanced pollen monitoring are wide-ranging and significant. Timely information assists allergy experts and patients in determining exposure levels, and helps pharmaceutical companies to optimize stock and advertisements, and even to develop targeted medication. Analysis over the long term could contribute to research on climate change's influence on flowering stages and pollen allergenicity. ∎