Continuous SPES Flow Analysis (CFA-SPES)

INTRODUCTION

Multiparametric Continuous Flow Analysis (CFA) of submicron and micrometric particles is an analytical approach aimed to the automatic time resolved and continuous measure of particles suspended in a flowing fluid. CFA becomes widely used in many research and quality control processes, eg. for the in-line/on-line monitoring of particles in pilot synthesis reactors or for the particle characterisation using synergic methods on the same flowing complex sample of complex industrial, biological, and environmental interest. This results in a high-resolution data set of particle qualitative and quantitative time resolved information.

State of the art CFA analysis methods in various application areas need the analysis of heterogeneous, unfiltered, and / or impurified samples regardless of their complexity and heterogeneity. Analyses retrieve to the user information on particles with a defined time resolution, eg. each second, and last from few minutes up to hours, or indefinitely until the monitored process ends. Complexities to be faced and hardly managed by traditional methods are due to the presence of particles with different optical properties, such as different refractive index, different internal structure (e.g., core-shell, mesoporous), different shapes (e.g., rods, plates), and, finally, the presence of impurities or synthesis residues. Same considerations must be adopted when the behaviour of a heterogeneous formulations has to be studied, and thus optimised while analysing the particles directly in target fluid where surrounding particles prevent an effective analysis via traditional particle analysis and sizing methods.

PARTICLE ANALYSIS METHOD

Among the several methods currently adopted, optical ones have unique advantages, and therefore, have brought light scattering into the forefront of analytical methods in many scientific and industrial applications. Unfortunately, the number of parameters typically affecting the scattering properties of a given particle is such that the basic measure of the scattering power (or even the power removal from a light beam -extinction- from one particle) is far from being enough to recover something more than a rough estimate of its size. Things change appreciably when considering a collection of many scatterers, with the immediate drawback of introducing the need for mathematical inversion and ill-posed problems to interpret experimental real data.

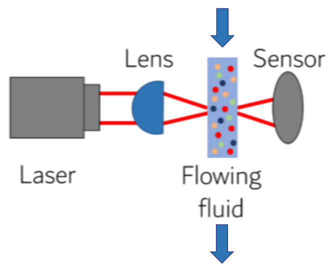
EOS Classizer™ ONE particle analyser is based on patented Single Particle Extinction and Scattering (SPES) method. It introduces a step forward in the way light scattering is exploited for single particle characterization.

Figure 1 EOS Classizer™ ONE

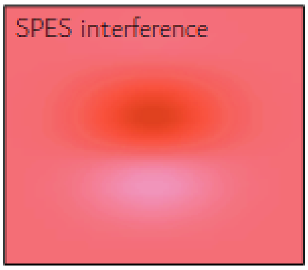
EOS Classizer™ ONE provides data that go beyond the traditionally optical approaches. EOS Classizer™ ONE discriminates, counts, and analyses single particles through their optical properties. It retrieves to the user several pieces of information such as: particle size distribution of the single observed populations, absolute and relative numerical concentrations, particle stability, information on optical particle structure and oversize. Classizer™ ONE works offline and online/real-time, enabling to verify consistency of intermediate and final formulations with target QbD, SbD, and Quality Control target expectations.

SPES TECHNOLOGY IN A NUTSHELL

The patented Single Particle Extinction and Scattering (SPES) method is based on a self-reference interferometric measurement of the scattered wavefront in the forward direction by a single illuminated particle.



Particles are driven by a laminar fluid flow (liquid or gas depending on the application/CLASSIZER™ version) through the waist region of a tightly focused laser beam.

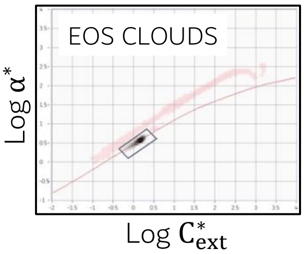


The intense transmitted beam interferes with the faint scattered wavefront in the far field, thus superimposing the two waves with the same curvature. This causes the interference pattern to exhibit intensity modulations on the spatial scale of the beam itself.

Two scattering features are sampled to follow the evolution of the intensity modulations during the passage of each single particle through the beam: i) the global attenuation given by the particle which removes a small fraction of the incoming power; ii) the fringes given by the partial constructive and destructive interference, proportional to the amplitude of the complex forward adimensional scattered field S(0). These features are directly related to the real $\Re\, S(0)$ and the imaginary $\Im m\, S(0)$ components of S(0), as stems from the Optical Theorem [H. C. van de Hulst, Light Scattering by Small Particles, 1981].

The Extinction Cross Section $\Re e\, S(0) = C^*_{ext} = \frac{k^2}{4\pi} C_{ext}$ **and the Polarizability** $\Im m\, S(0) = \alpha^* = k^3\, \alpha$, where $k = 2\pi n/\lambda$ is the wave number in the medium n at wavelength λ, **are thus retrieved for each single detected, validated, and counted particle** thanks to a robust Pulse Shape Analysis scheme and proprietary algorithms, without adopting ill-posed problems, like the inversion or deconvolution (other optical parameters could be alternatively retrieved, eg. particle optical thickness ϱ).

In a few minutes SPES/ CLASSIZER™ creates the unique **EOS CLOUDS**: a 2D histogram which is the optical fingerprint of the sample.



Heterogeneous samples produce simultaneously different clouds for each particle population, which can be individually selected, analyzed, and compared. Particle size distribution, numerical concentration, oversize, and other insights are retrieved accordingly to the selection, to the whole sample, or for each time frame acquired in CFA mode. Statistical approaches as PCA are furthermore viable to extract unique information typically inaccessible nowadays.

Added-value information is provided thanks to **SPES** and **EOS Classizer™ ONE** unique data and analysis libraries:

- **Optical Classification,** Absolute **Particle Size Distribution, Numerical Concentration** of each single population irrespectively of polydispersity/composition.

- Quality Control of particle **porosity, wetting**, **aspect ratio**, **payload**, **impurities**, **scraps**, and **shelf-life without intermediate steps** (purification/filtration).

- Measurement of **particle behavior and formulation stability** directly in real **heterogeneous non-filtered target biological, industrial, or environmental fluids**.

- Hi-Resolution **Continuous Flow Analysis**, also coupling SPES information with other analytical devices as cFFF separators, small chemical reactors, and pilot line.

- Statistical approaches as **Oversize Measure** and **PCA** for Hi-Quality Batch-2-Batch analysis and out-of-specifics identifications in product formulation and production.

Depending on the system configuration and sample, EOS Classizer™ ONE standard analysis covers a dynamic range of 0.1 – 20 µm, concentration range of 1E5-1E7 ptc/mL @ 0.5-5ccm. EOS Classizer™ ONE in CFA mode measures particles in 0.1-2 µm or 1-20 µm range depending on the settings. A SPES CFA measurement can last from a few seconds to an arbitrary time defined by the user. In case of ergodic samples or processes, two or more runs can be merged in a single acquisition. External sample manager and autosampler are available.

This document presents representative examples of applications of EOS Classizer™ ONE and does not cover all the cases where the patented SPES method solves the particle identification, classification, and characterisation of challenges in heterogeneous samples and complex liquids. EOS software release SW1.4.42 is used for the data analysis and generation of the figures.

For a general introduction to SPES data with standard samples, as polystyrene spheres, please refer to the Application Note AN001/2021, available for free online at EOS website: www.eosinstruments.com/publications/

APPLICATION EXAMPLES

CFA capabilities of EOS Classizer™ ONE and SPES method are exploited and presented for the inline / online characterisation of water-based particle suspensions of general interest in flowing fluids

Three case studies are considered in this application note:

1) relative time-varying concentrations of a mix of particles having the same size but different optical properties.

2) time resolved characterisation of a secondary population of particles injected in a constant stream of filtered solvent.

3) merging of two subsequent CFA SPES data of an ergodic sample acquired using the two available size ranges.

1) relative time-varying concentrations of a mix of particles having the same size but different optical properties

In this case study a system of two pumps is used to create a constant flow with a time-varying mix of particles. As described in Figure 2, an aqueous suspension of 600nm PS particles @ 3.5E6 ptc/mL is loaded in syringe A, an aqueous suspension of 600nm PMMA particles @ 3.5E6 ptc/mL is loaded in syringe B. CFA SPES data is acquired and retrieved with 1 second time resolution.

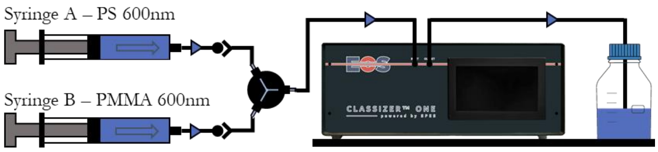


Figure 2 Simplified layout of the scheme used for this case study. Two syringes loaded with two different species of polymeric particles are pushed with an inverse time-varying velocity so the sum of the two flows is 2 mL/min. The flows are mixed, and the sample is measured using an EOS Classizer™ ONE system. In this case the sample go to drain after the analysis, but it can be recovered for further tests if needed.

The test starts with the one Syringe A flowing the liquid at a rate of 2ccm. This flow is then reduced step by step every few minutes, simultaneously increasing the flow of the syringe B so that the total flow is maintained constant at 2 ccm. In Figure 3 we show data of the whole acquisition: the EOS CLOUDS, the particle size distributions, and the numerical particle concentration refer to the integral of the data acquired during the whole run. Two clouds are detected corresponding to two different particle populations with different optical properties.

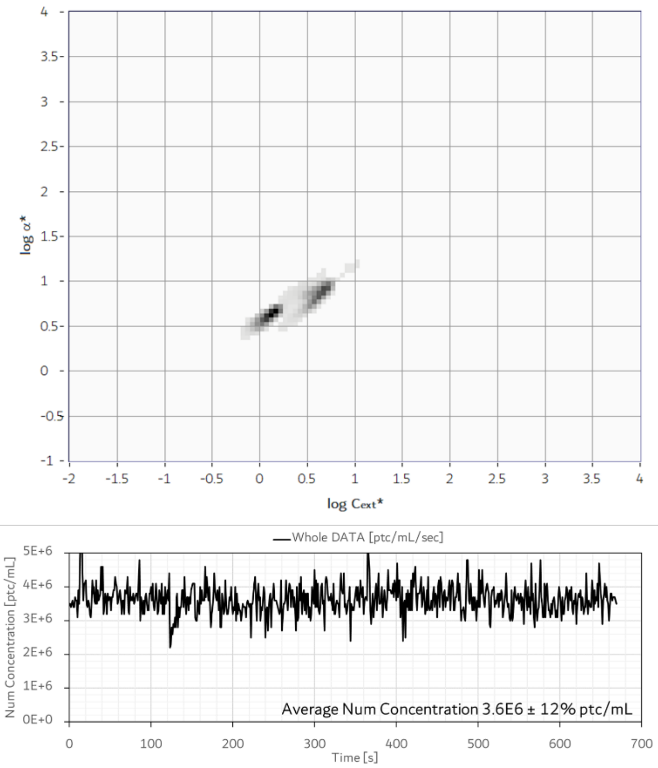


Figure 3 (top) EOS CLOUDS histogram corresponding to the whole CFA run. Two clouds are detected with different optical properties. (bottom) time evolution of the numerical particle concentration, 1 sec time resolution. The numerical concentration is stable and agrees within experimental error with expected values.

In Figure 3, bottom panel, the time evolution of the numerical particle concentration is reported with 1 second time resolution. The average value is 3.6E6±12% ptc/mL.

EOS Classizer™ ONE and SPES method allow a high value and unique analysis of each single particle population detected, classifying, and extracting temporal resolved data in function of the optical properties. In Figure 4 the results of the classification based on optical properties of the particle populations are presented. The time evolution of numerical particle concentration and other statistical information from the selections performed in the two-dimensional histograms are retrieved from the data set.

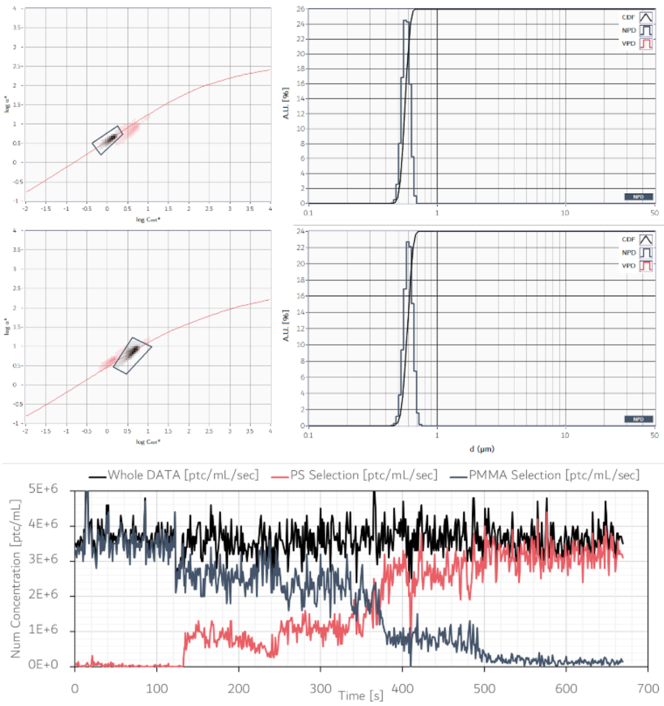


Figure *4* (top left) selection of EOS CLOUDS corresponding to the first particle population detected; (top right) particle size distribution from the corresponding selection. Size and RI values are in good agreement with values expected for 600nm PMMA particles; (middle left) selection of EOS CLOUDS corresponding to the second particle population detected; (middle right) particle size distribution from the corresponding selection. Size and RI values are in good agreement with expected ones (600nm PS particles); (bottom) time evolution of the numerical particle concentrations for the whole sample (black), for the particle population in the top selection (PMMA, red) and middle selection (PS, gray blue).

The relative time changes of the numerical concentration of the two populations agree with the expected values imposed by the pumping system.

2) Time resolved characterisation of a secondary population of particles injected in a constant stream of filtered solvent

In this case study a single pump pulls a pure filtered solvent @ 2 ccm flow rate. Aliquots of samples are injected in the line thought a valve, as represented in Figure 5.

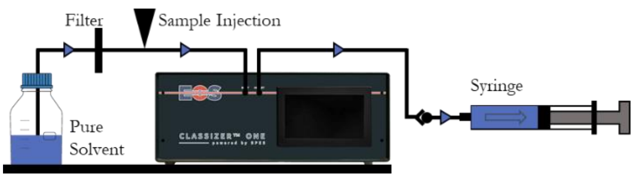


Figure 5 Simplified layout of the scheme used for this case study. A single syringe pump pulls a pure filtered solvent @ 2ccm flow. Small aliquots of samples are injected in the line thought a valve.

This scheme is representative of analytical cases as, e.g., on-line / in-line monitoring of particle contamination during continuous flow processes and for measuring samples injected using an external autosampler (e.g., for analytical LC, HPLC, or FPLC). Note. The system can run in "slave mode" to start/stop acquisitions via an external 5V trigger. Feel free to contact info@eosinstruments.com for further information and evaluate customizations of EOS Classizer™ ONE external communication protocols.

For the present example, two samples are injected at two different time points with two time intervals. The first sample injected is a suspension of ceria CeO2, the second a mix of CeO2 and silica SiO2. In Figure 6 the time evolution of the numerical particle concentration is shown. Two concentration peaks corresponding to two sample injection are detected.

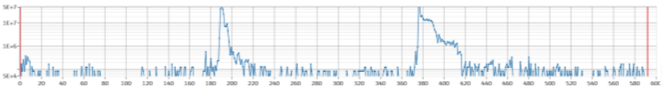


Figure 6 Time evolution of the numerical particle concentration, 1 second time resolution. Two peaks corresponding to two sample injection are detected.

Temporal selections can be performed: in Figure 7 and in Figure 8 we report the results for the first peak and second peak shown in Figure 6.

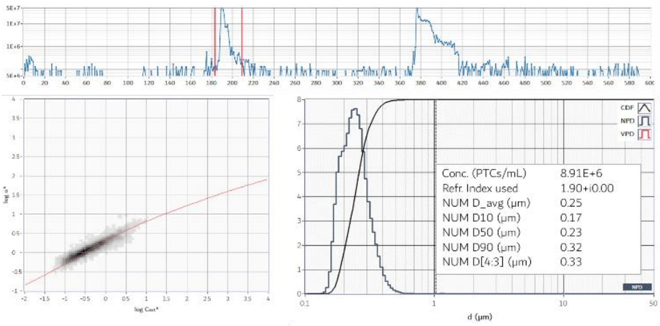


Figure 7 (top) Temporal selections of particles detected during the first peak, as represented by the two vertical red lines; (bottom left) EOS CLOUDS corresponding to the first peak. Optical properties are compatible with a RI of 1.9, in agreement with expected value for CeO2 @ λ640nm within experimental error (red line, tailored Mie theory for spherical particles with n = 1.90 @ λ640nm); (bottom right) particle size distribution and statistical information of particles represented in the left and corresponding to the first peak. Size and RI agree with expected values.

A similar analysis for the time selection of the second peak presents two well separated clouds, corresponding to two different particle populations. These could be further classified and analyzed separately by making a further selection in the EOS CLOUDS, as presented in Figure 8.

Note. Traditional methods fail in discriminating two or more particle populations in a peak, resulting in an analytical limitation even after a separation, e.g., via Flow Field Factionary FFF. The unique added value of EOS Classizer™ ONE surpasses this traditional issue thanks to its SPES multiparametric approach.

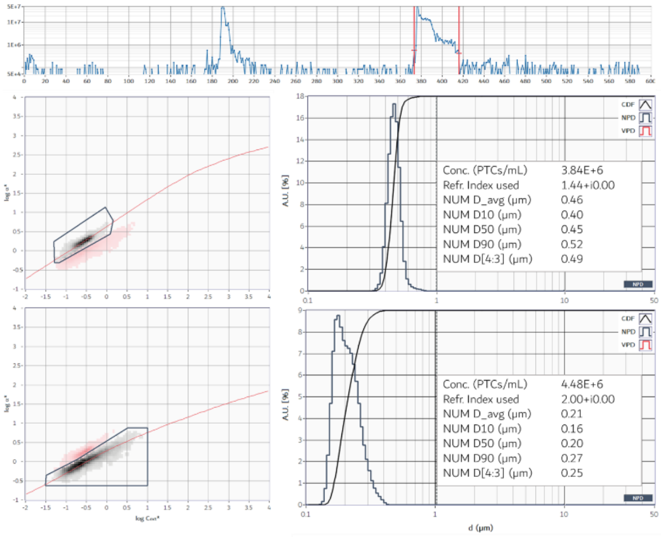


Figure 8 (top) Temporal selections of particles detected during the second peak, as represented by the two vertical red lines. Two particle populations are detected and thus analyzed separately by making a further selection in the EOS CLOUDS 2D histogram; (middle left) EOS CLOUDS corresponding to the selection of the first particle population detected in the second peak. Optical properties are compatible with a RI of 1.44, in agreement with expected value for SiO2 within experimental error (red line, tailored Mie theory for spherical SiO2 @ λ640nm); (middle right) particle size distribution and statistical information of particle represented in the left and corresponding to the first peak. Size and RI agree with expected values; (bottom left) EOS CLOUDS corresponding to the selection of the second particle population detected in the second peak. Optical properties are compatible with a RI of 2.00, in agreement with expected value for CeO2 within experimental error (red line, tailored Mie theory for spherical CeO2 @ λ640nm); (bottom right) particle size distribution and statistical information of particle represented on the left and corresponding to the first peak. Size and RI agree with expected values.

Additional information can be obtained from the time evolution of the numerical particle concentration, that is the duration of the sample injection. The first peak lasts about 15 seconds, the second 45 seconds, in agreement with the two different injection times imposed during the experiment. Note: EOS Classizer™ ONE retrieves the particles detected with a linear time response that is faster that the reported time resolution (1 second). This allows to detect and monitor fast transients, as well as the transit of an air bubble lasting less than one second.

3) Merging of two subsequent CFA SPES data of an ergodic sample acquired using the two available size ranges

In CFA mode, EOS Classizer™ ONE measures particles either in the size range 0.1-2µm or 1-20µm, depending on the acquisition settings. In case of ergodic samples or processes, two runs (or more) can be performed, and the results merged and analysed as a single acquisition, as described in Figure 9.

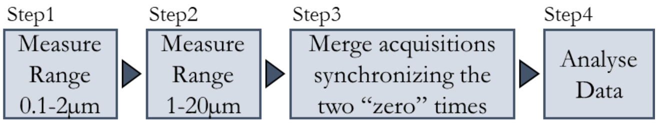


Figure 9 Example of CFA data merging. Data acquired with the same sample and acquired by repeating the analysis in the two available CFA particle size ranges are merged. The new data set can be analyzed as a single run to monitor and compare particles in the extended size range. Merging data can also be performed with subsequent runs on of the same samples in the same size range and other conditions / combinations.

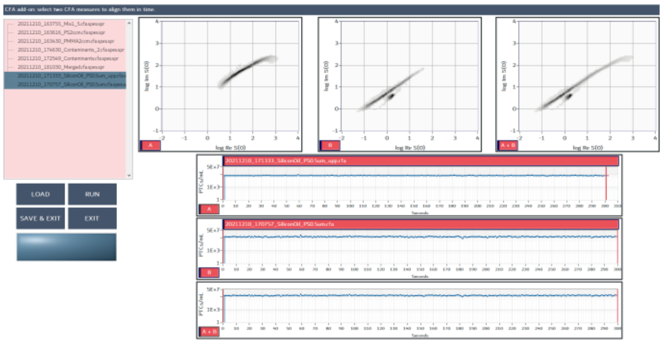


Figure 9 A screenshot of the EOS Classizer software. Two data sets are selected. The system allows to impose two zero/start points and two end points for the two acquisitions. Data of the two acquisitions are merged resulting in a third file with the merged acquisition. In this example a mix of polydisperse silicon droplets and 500nm PS spherical particles are measured in the lower and in the upper size range, then merged in one whole acquisition.

Resulting data from merging can be analyzed and exported using all the tools available for the standard CFA acquisitions, thus taking advantage from the added value of the Classizer™ ONE over its entire available particle size range. In Figure 10 an example of the merged data is shown.

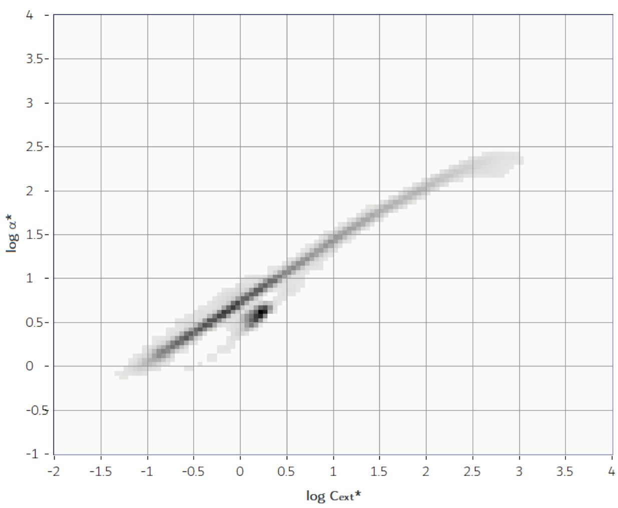


Figure 10 Merged data of two acquisitions a mix of polydisperse silicon droplets and 500nm PS spherical particles presented in Figure 9.

CONCLUSIONS

CFA capabilities of Classizer™ ONE and SPES patented method provide unique added value for time-resolved classification of particles suspended in flowing fluids.

This results in a high-resolution multiparametric analysis basing on single particle optical properties. This feature is of capital importance with heterogeneous fluids and systems of industrial, biological, and environmental interest where surrounding particles prevent an effective analysis via traditional methods EOS Classizer™ ONE and SPES patented method application ranges from the study of the behavior of particles, to the identification of secondary populations in flowing liquids.

RELEVANT PUBLICATIONS AND REFERENCES

Presentation of Single Particle Extinction and Scattering (SPES) method for particle analysis
AN001-2021 Analysis of Polymeric Particles via SPES Technology – a general introduction to SPES method

AN006-2021 Multiparametric Classification of Particles as a Pathway to Oversize Analysis in Complex Fluids via SPES Technology

Potenza MAC *et al.*, «Measuring the complex field scattered by single submicron particles », AIP Advances 5 (2015)

Example of CFA application of SPES technology
AN002-2021 Continuous SPES Flow Analysis CFA-SPES

Example of PCA application of SPES technology
AN005-2022 Multiparametric Principal Component Analysis of Heterogeneous Samples via SPES Technology

Classizer™ ONE with Sample Manager Autosampler
AN008-2022 Automatic Liquid Sample Management, Dilution, and System Cleaning with EOS Sample Manager

AN009-2022 Standardize SPES Operative Procedure of Liquid Samples Analysis via EOS Autosampler

Example of SPES application to aggregates
AN003-2021 Addressing the Issue of Particle Wetting and Clustering by means of SPES Technology

Potenza MAC *et al.*, «Single-Particle Extinction and Scattering Method …», ACS Earth Space Chem 15 (2017)

SPES application to non-spherical particles
AN004-2021 Addressing the Classification of Non-Spherical Particle by means of SPES Technology

Simonsen MF *et al.*, «Particle shape accounts for instrumental discrepancy in ice …», Clim. Past 14 (2018)

Example of SPES application to emulsions w/o payload in environmental waters
AN012-2021 Monitoring the Fate of a Lipid/ZnO Emulsion in Environmental Waters

Examples of SPES application to particle analysis and behavior characterization in biotech applications
AN011-2022 Quantitative Classification of Particles in Biological Liquids via SPES Technology

Sanvito T *et al.*, «Single particle extinction and scattering optical method unveils in real…", Nanomedicine 13 (2017)

Potenza MAC *et al.*, «Single particle optical extinction and scattering allows real time quantitative…», Sci Rep (2015)

Example of SPES application to oxide particles, abrasives, and industrial slurries w/o impurities
Potenza MAC *et al.*, «Optical characterization of particles for industries», KONA Powder and Particle 33 (2016)

AN002-2021 Analysis of Abrasives via SPES Technology

Example of SPES application to ecotoxicity analysis
Maiorana S *et al.*, «Phytotoxicity of wear debris from traditional and innovative brake pads», Env Int., 123 (2019)

Example of SPES application to aerosol analysis
Mariani F *et al.*, «Single Particle Extinction and Scattering allows novel optical …», J Nanopart Res 19 (2017)

Cremonesi L *et al.*, «Multiparametric optical characterization of airborne dust …. », Env Int 123 (2019)

Visit EOS website for further applications
www.eosinstruments.com

EOS S.r.l. – viale Ortles 22/4, 20139 Milano (Mi) – Italy
email: info@eosinstruments.com   Phone: +39 02 56660179

Distributed by