INTRODUCTION

Submicron and micrometric polymeric particle dispersions are common in a multitude of applications and products such as pharmaceutical, personal care, food, ceramics, pigments, inks, and cements. A proper dispersion of the particles is necessary to avoid sedimentation, instability, or product failure due to aggregation, oversize, and aging.

Bottom-up Quality-by-Design formulation, top-down Safe-by-Design approaches and product manufacturing require a reliable method to analyse the different particulate populations in all the intermediate formulation steps and in the final product. This operation must be achieved regardless of the complexity and heterogeneity of the sample. These complexities 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 shape (e.g., rods, plates), and, finally, the presence of impurities or synthesis residues. The same considerations must be adopted when the formulation's behaviour is studied, and thus optimised while analysing the particles directly in target fluids. In this case, the presence of other particles typically prevents a reliable and repeatable analysis via traditional approaches.

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.

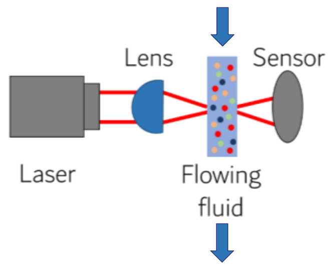
EOS Classizer™ ONE particle analyser is based on patented Single Particle Extinction and Scattering (SPES) method. It introduces a step forward in the way light scattering is exploited for single particle characterization.

Figure 1 EOS Classizer™ ONE – front panel – inlet/outlet and 7” HMI

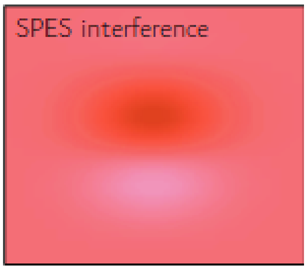
EOS Classizer™ ONE provides data that go beyond the traditionally optical approaches. EOS Classizer™ ONE discriminates, counts, and analyses single particles through their optical properties. It retrieves to the user several pieces of information such as: particle size distribution of the single observed 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.

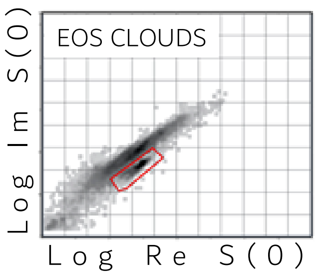


Particles are driven by a laminar fluid flow (liquid or gas depending on the application/CLASSIZER™ version) through the waist region of a tightly focused laser beam.



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 interference fringes during the passage of a particle through the beam: i) a global beam attenuation given by the extinction cross section of the particle, removing a small fraction of the incoming power; ii) the fringes given by the partial constructive and destructive interference, proportional to the amplitude of the forward a-dimensional scattered field S(0). These features are directly related to the real *Re* S(0) and the imaginary *Im* S(0) components of complex field S(0), as stems from the Optical Theorem [H. C. van de Hulst, Light Scattering by Small Particles, 1981].



Im S(0) and *Re* S(0) are retrieved for each single detected and counted particle thanks to a robust Pulse Shape validation scheme, without adopting ill-posed problems like the inversion or deconvolution (note: other optical parameters could be alternatively retrieved instead of S(0) components, as particle extinction cross section C_{ext}, particle polarizability α, or particle optical thickness ϱ).

In a few minutes SPES 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. Statistical approaches as PCA are furthermore possible for extracting valuable 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 CF3 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.

EOS Classizer™ ONE, based on patented SPES method, is the ideal solution for improving colloids formulations and for verifying product consistency with the target Quality-by-Design final expectations.

Depending on the system configuration, EOS Classizer™ ONE covers a dynamic range of 0.2 – 20 µm, concentration range of 1E5-1E7 ptc/mL @ 0.5-5ccm. External auto-dilution sampler and autosampler 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 colloids formulation and the manufacturing challenges.

APPLICATION EXAMPLES

The first example of application is the analysis of polystyrene (PS) spheres typical employed as standards in particle analysis and instrument calibration and validation.

Figure 2 shows SPES data for a sample of 0.5µm PS spheres dispersed in filtered water at a nominal numerical concentration of 1E6 ptc/mL. About 4 mL of sample have been analysed at 5ccm using a lab syringe pump. About 8000 validated particles populate the SPES CLOUDS histogram and are employed for the quantitative analysis. The grey tones of the cloud are proportional to relative numerical particle concentration. Location of data in the 2D SPES CLOUDS is an optical fingerprint of the sample.

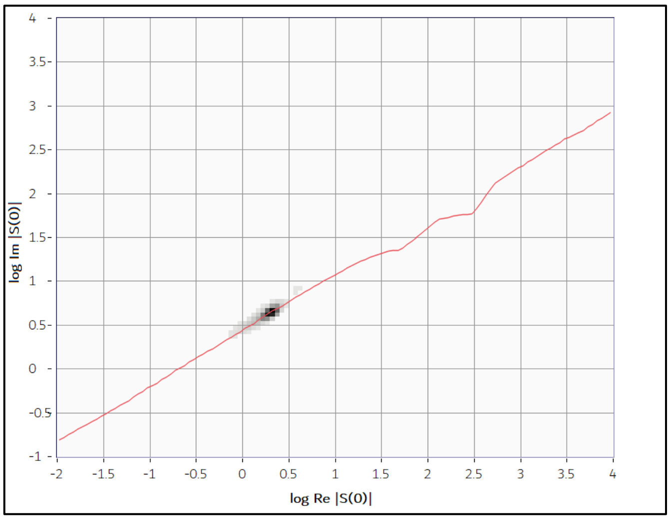


Figure 2 EOS CLOUDS histogram for a sample of PS 0.5µm spheres dispersed in milliQ-grade water. Position of experimental data (grey tones proportional to relative numerical concentration). Red line represents expected SPES position for PS spheres with different sizes.

Experimental data are compared to theoretical expected positions in the histogram for dielectric spheres of different sizes and refractive indexes. Different approaches can be tempted, as tailored Mie or DDA. The most compatible effective refractive index is thus automatically determined by EOS Classizer™ ONE, in this case as n=1.60, in agreement within experimental error with theoretical value at λ=640nm. Once retrieved the effective refractive index, particles are individually sized comparing their S(0) values with expected ones for spheres of different diameters. EOS Classizer™ ONE provides to user the Numerical Particle Size Distribution and other statistical values as AVG, CV, and quantiles (see *Figure 3*).

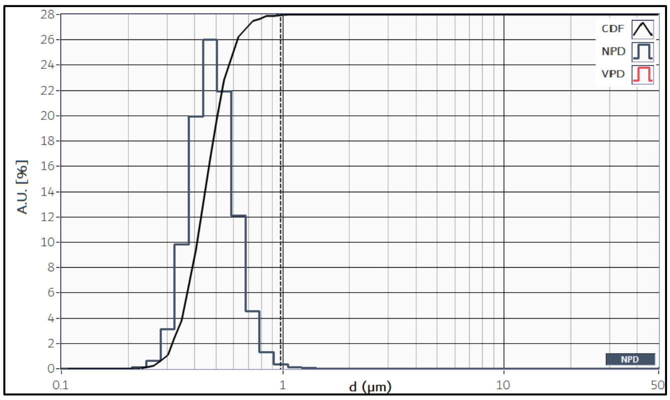


Figure 3 Experimental Numerical Particle Size Distribution of calibrated PS 0.5µm spheres dispersed in milliQ-grade water. Average particle diameter retrieved by EOS Classizer™ is 0.49 µm @ measured n=1.60.

Sizing accuracy of EOS Classizer™ ONE can be verified by analysing monodisperse PS sample standards of different nominal sizes. *Figure 4* shows Numerical Particle Size Distributions PSD and a Cumulant Distributions of polystyrene PS samples of different diameters. For the samples presented, the effective refractive index n is automatically defined by EOS Classizer™ as 1.58-1.62, in agreement within experimental error with the theoretical expected value at λ=640nm. For only PS 2.1µm *n* is manually set to 1.60 due to Mie oscillations [H.C. van de Hulst, Light Scattering by Small Particles, 1981].

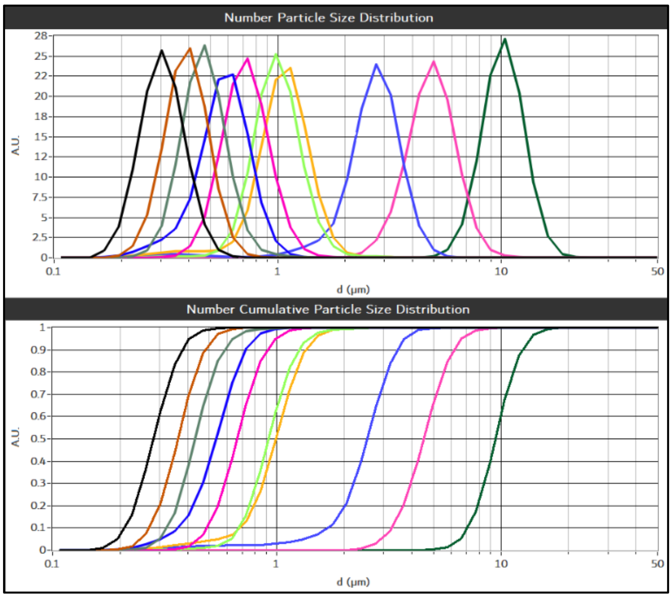


Figure 4 Numerical Particle Size Distributions and Cumulant Distributions of PS samples of 0.3µm, 0.46µm, 0.5µm, 0.6µm, 0.7µm, 1µm, 1.1µm, 2.1µm, 5µm and 10µm diameters dispersed in milliQ water.

Figure 5 shows the PSD and the Cumulant Distributions of PMMA samples of different sizes. The effective *n* is automatically defined by EOS Classizer™ as 1.49-1.52 for all the three samples, in agreement within experimental error with the theoretical expected value at λ=640nm.

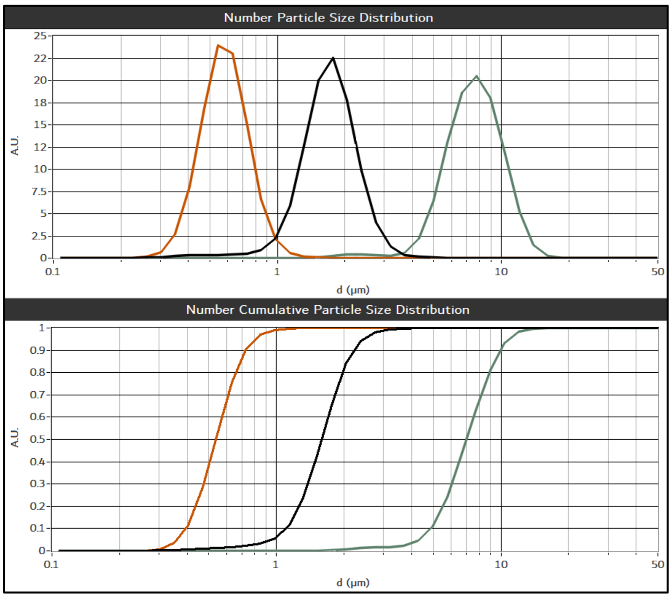


Figure 5 Numerical Particle Size Distributions and Cumulant Distributions of PMMA samples of 0.6µm, 1.8µm and 8µm diameters dispersed in filtered water. Average refractive index *n* is automatically calculated by EOS Classizer™ software as 1.49 @ *λ=640nm*.

Reproducibility is another aspect of capital importance for analytical methods, especially for batch-2-batch QC applications. SPES measurements of analytical replicates of submicron polystyrene particles are useful for the quantification and the validation of the reproducibility within the sample preparation error as shown in Figure *6*.

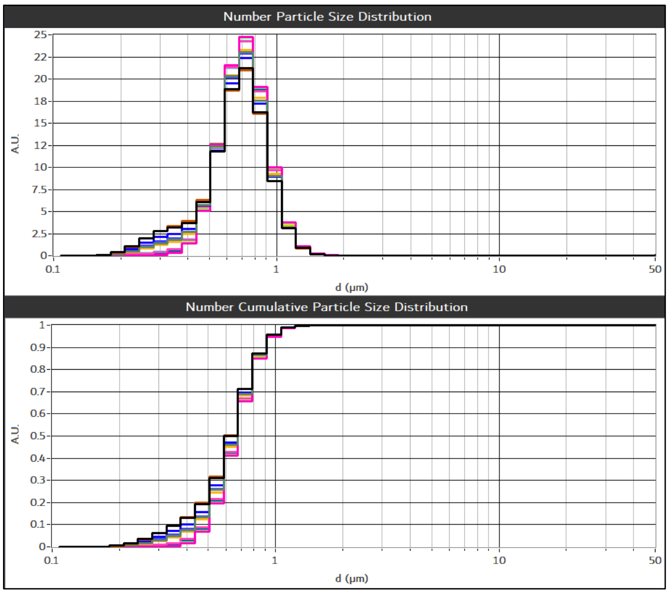


Figure 6 SPES reproducibility test. Sixteen analytical replicates of PS calibrate spheres of 0.7µm, diluted 20000 times in milliQ-grade water at 5E6 ptc/mL from an initial bulk concentration of 1E11 ptc/mL. (top) Particle Size Distribution, (down) Cumulant Particle Size Distributions.

As first and main value proposition, EOS Classizer™ ONE is a particle analyser capable of discriminating and analysing separately populations in optically heterogeneous sample. Notwithstanding, thanks to its single particle approach, an estimate of the numerical particle concentration is provided for each particle population detected, improving the range of possibilities of added value information retrieved by patented SPES method.

Figure 7 shows the reproducibility of the estimate of numerical concentration via SPES method. Results for 16 analytical replicates of 0.7µm PS samples diluted 20000 times from an 1E11 ptc/mL bulk sample are presented.

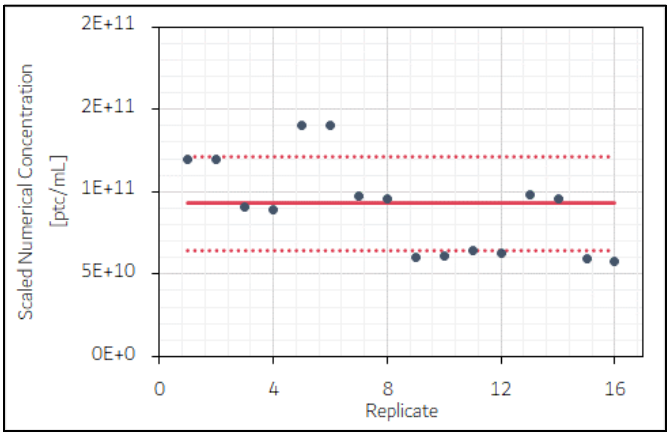


Figure 7 Reproducibility of the concentration estimations of the 16 analytical replicates of 0.7µm PS samples presented in Figure 6. Note that the concentration values are multiplied by the 20000 times dilution factor to retrieve the bulk value of the numerical particle concentration.

An estimate of the accuracy can be performed also by comparing the expected numerical concentration, based on the nominal concentration of the sample bulk and dilution done of samples of different diameters and the relative experimental measured values, as reported in *Figure 8*.

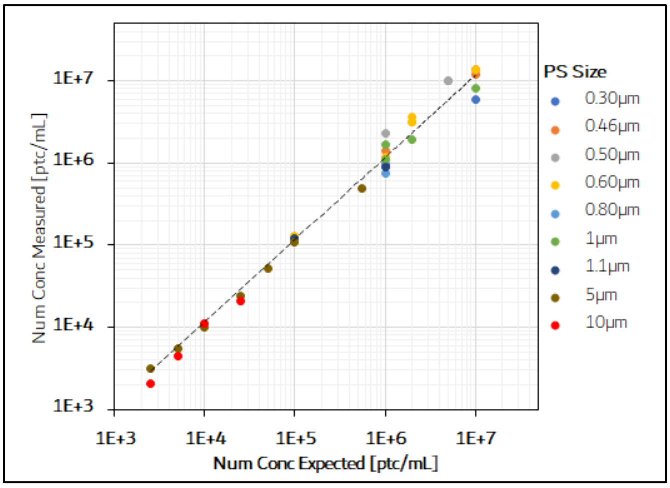


Figure 8 Estimate of the accuracy of the numerical concentration analysis of the EOS Classizer™ ONE. Polystyrene samples of different size and concentration are considered.

In case of a polydisperse sample in size, the cloud of data in EOS CLOUDS elongates along the diagonal of the histogram as presented in *Figure 9*. Red line represents the expected theoretical trend n=1.40. Thickness of the cloud in the other direction represent the homogeneity of particle structure. The experimental elongated and narrow cloud presented in *Figure 9* is typically observed with emulsions.

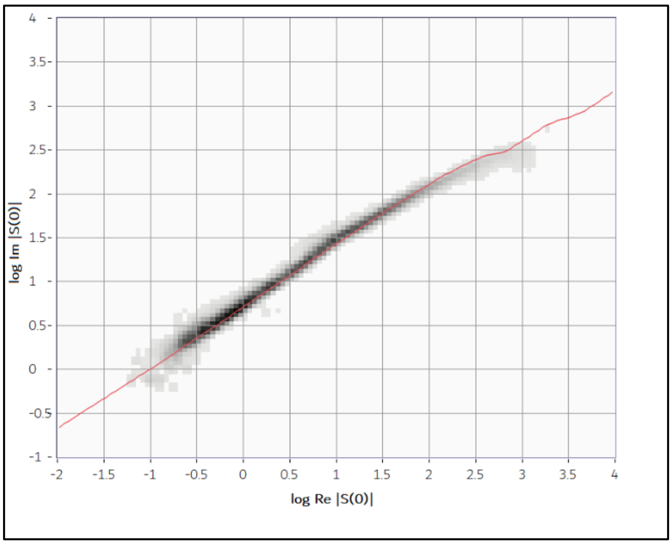


Figure 9 EOS CLOUDS of a polydisperse emulsion. Theoretical refractive index is n=1.40, measured refractive index is n=1.39.

In case of heterogeneous samples in terms of the sizes and/or of the refractive indexes, secondary populations could limit, or even preclude any reliable approaches with traditional analytical methods. Thanks to the SPES patented multiparametric approach, EOS Classizer™ ONE discriminates particles basing on optical properties. Heterogeneous samples produce simultaneously more clouds for each particle population which can be easily individually selected, analysed, and compared.

Figure 10 and *Figure 11* present two examples of heterogeneous samples with more than one component. Two clouds corresponding to optical different particles are detected and represented on the EOS CLOUDS in both the cases. In the first one, SPES experimental data of a mix of PS and PMMA particles with same size are presented. In the second one, the detection of PS submicron particles mixed with an emulsion of castor oil is has been evaluated.

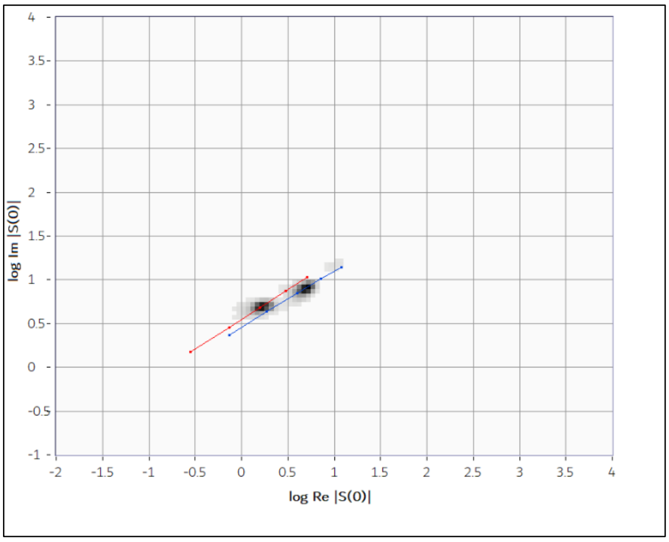


Figure 10 Example of EOS CLOUDS for a heterogeneous sample of PMMA 600nm and PS 600nm submicron particles. Two separate clouds are detected and can be selected and analyzed separately, as well as for the absolute and relative concentration of each particle population. Red line and blue line are expected trends for PMMA and PS, respectively.

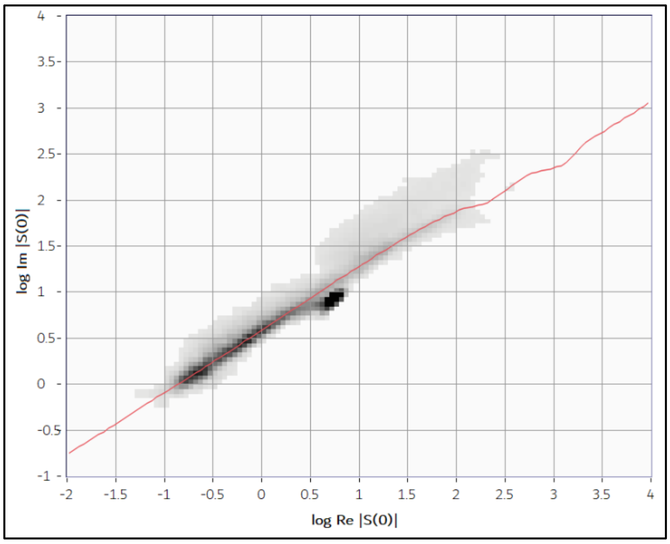


Figure 11 Example of EOS CLOUDS for a heterogeneous sample of castor oil emulsion with polystyrene 0.6µm spheres as traceable particles. Two principal and separated populations are detected. Red line represents expected size trend for droplets of castor oil refractive index.

User can select and/or crop the data in EOS CLOUD, easily drawing a blue polygon as presented in *Figure 12*.

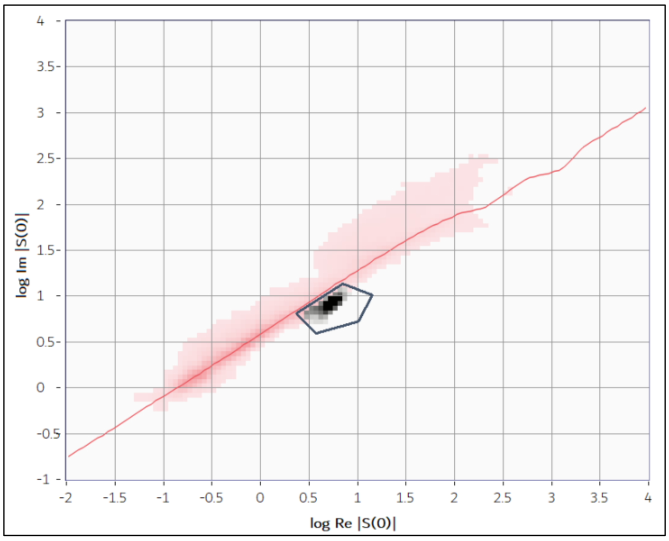


Figure 12 Example of a selection of an area on the EOS CLOUDS to focus and refine particle analysis on a fraction of the whole particulate system detected (blue closed line). This capability can be used to analyze single components, tails in polydisperse distributions, and impurities.

As the area is defined, EOS Classizer™ software focuses the analysis considering just the particles enclosed in the selection. Firstly, it redefines the most adequate optical properties of the particles. Thus, parameters as the particle size distribution and the numerical concentration, are retrieved to the user as presented in *Figure 13*.

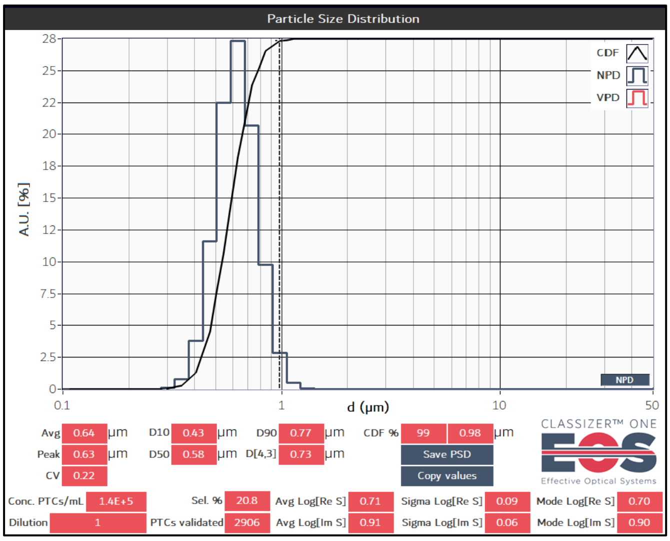


Figure 13 Numerical particle size distribution, cumulant size distribution and numerical concentration of the particles selected in Figure 12.

Note. EOS Classizer™ software compares automatically the SPES data with expected theoretical values and models for dielectric spheres to define the best effective refractive index of the system. If preferred or in special cases as e.g., absorptive particles, the user can always enter manually the values of the real and imaginary components of the refractive index to analyse and size the particles.

CONCLUSION

This capability of EOS Classizer™ ONE and SPES patented method of discriminating single particle basing on their optical properties is of capital importance with heterogeneous systems and when particle behaviour must be investigated in complex-but-real target media to tailor the product formulation and improve its effectiveness.

SPES data provides physical and statistical information, as particle size distribution and numerical concentration, as well as insight on the particle structure and stability. Applications ranges from the estimation of the number of aggregates per mL respect to the choice of the surfactant, e.g. for the improvement of the wetting of a powder or of the shelf life of a product, to the study of the behaviour of particles in target heterogeneous media to tailor the their formulation. Oversize analysis can be performed also in presence of impurities. Scraps and out-of-specifics can be monitored in intermediates and final formulation.

RELEVANT SPES PUBLICATIONS AND REFERENCES

Presentation of Single Particle Extinction and Scattering (SPES) method for particle analysis
Potenza MAC *et al.*, «Measuring the complex field scattered by single submicron particles », AIP Advances 5, 117222 (2015)

Example of SPES application to aggregates
Potenza MAC Holland *et al.*, «Single-Particle Extinction and Scattering Method Allows for Detection and Characterization of Aggregates of Aeolian Dust Grains in Ice Cores», ACS Earth Space Chem. 15 261-269 (2017)

Cremonesi L *et al.*, « Light extinction and scattering from aggregates composed of submicron particles», J Nanopart Res 22:344 (2020)

Examples of SPES application to non-spherical particles
Villa S *et al.*, «Measuring shape and size of micrometric particles from the analysis of the forward scattered field», Journal of Applied Physics 119, 224901 (2016)

Simonsen MF *et al.*, «Particle shape accounts for instrumental discrepancy in ice core dust size distributions», Clim. Past, 14, 601–608 (2018)

Example of SPES application to emulsion and polymeric particle analysis w/o payload
Potenza MAC *et al.*, «Single particle optical extinction and scattering allows real time quantitative characterization of drug payload and degradation of polymeric nanoparticles», Scientific Reports volume 5, 18228 (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 review 33 (2016)

Example of SPES application to particle analysis and behavior characterization in complex media
Sanvito T *et al.*, «Single particle extinction and scattering optical method unveils in real time the influence of the blood components on polymeric nanoparticles", Volume 13, Issue 8, Pages 2597-2603 (2017)

Example of SPES application to ecotoxicity analysis
Maiorana S *et al.*, «Phytotoxicity of wear debris from traditional and innovative brake pads», Environment Int., 123, 156-163 (2019)

Example of SPES application to aerosol analysis
Mariani F *et al.*, «Single Particle Extinction and Scattering allows novel optical characterization of aerosols», J Nanopart Res 19: 291 (2017)

Cremonesi L *et al.*, «Multiparametric optical characterization of airborne dust with Single Particle Extinction and Scattering», Environment International, 123, 156-163 (2019)

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