Multiparametric classification of particles as a pathway to oversize analysis in complex fluids via SPES technology

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INTRODUCTION

A quantitative multiparametric analysis of grains in watercolor pigments via Classizer™ ONE and SPES patented method is reported as a case study of a more general approach for multicomponent complex fluids.

Paints are mixture of microscopic and sub-microscopic grains, the pigment particles that provide the colour, suspended in a liquid that allows the paint to be applied and finally dries, leaving the paint on the support ideally unchanged even for centuries. The liquid itself is a mixture of components and additives having physical and chemical role in defining the paint before and after use. Shelf life, manufacturing costs, handling of paint, and final appearance are examples of issues traded off by optimizing the liquid. Grains are milled so that a given mass is divided into smaller and smaller particles, thus increasing the surface area. This increases the quality of the paint but also requires more liquid to wet the pigment. Depending on the material the pigments are made of, dispersants can be added to reduce clustering or clumping.

Pigments are usually manufactured to a well-defined particle size, or with a given particle size distribution, depending on the material. It affects the paint features like transparency, saturation, but also staining and even shelf life. Sizes are from smaller than 0.5 µm up to approximately 5 µm. Generally, white, and black pigments are among the smallest, although non-trivial clustering phenomena characterize the particle suspensions of these paints.

From a physical point of view, refractive index plays a major role in determining the optical properties of pigments. They change from 1.4 for phthalocyanines up to effective 2.5-2.8 of venetian red and titanium white.

Measuring size and refractive index of pigments is of utmost importance for characterizing them and for improving product formulation.

PARTICLE ANALYSIS METHOD

Among the several methods currently adopted for particle sizing, optical ones have unique advantages. Therefore, they 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 resulting data.

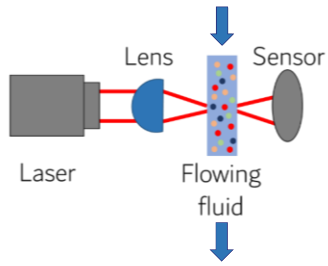
Figure 1 EOS Classizer™ ONE – front panel – inlet/outlet and 7" HMI

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

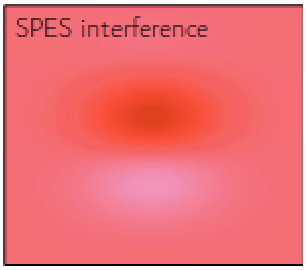
EOS Classizer™ ONE provides data well beyond the traditionally optical approaches. EOS Classizer™ ONE discriminates, counts, and analyses single particles through their optical properties. It provides the user with several pieces of information such as: particle size distribution of the single populations, absolute and relative numerical concentrations, particle stability, information about 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.

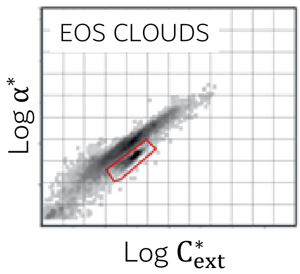


Particles are driven by a laminar fluid flow (liquid or gas depending on the application/CLASSIZER™ version) through the waist region of a tightly focused laser beam.



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 two features are directly related to the real $\Re e\, S(0)$ 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™ ONE 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 and sample, EOS Classizer™ ONE covers a dynamic range of 0.1 – 20 µm, concentration range of 1E5-1E7 ptc/mL @ 0.5-5ccm. 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 SW1.4.39 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 EXAMPLE

EOS Classizer™ ONE is exploited to characterize a suspension of Chinese white watercolour pigments suspended in pure water.

SPES data for the whole population are presented first. A PSD is recovered by best fitting refractive index to data and assuming particles as spheres, as commonly done in traditional instruments. A preliminary oversize analysis is obtained. Further, the main population is considered in more details, evidencing three subpopulations with different optical properties. By separating them, three PSDs are obtained. The corresponding oversize analysis shows remarkable differences with the former. Moreover, the subpopulations exhibit clear differences in terms of the refractive indexes. A careful analysis of the populations suggests the presence of clustering.

Figure shows SPES data for the whole sample diluted in pure water. Data from thousands of grains are collected in a few minutes. Grey tones are proportional to relative numerical particle concentration. Number concentration is not of interest here, so that we mainly focus at studying the features of the SPES CLOUDS, the location in the 2D SPES CLOUDS being an optical fingerprint of the sample.

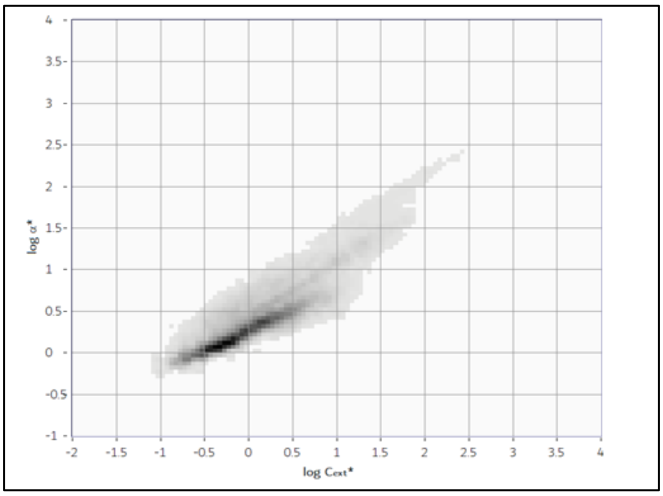


Figure 1 EOS CLOUDS histogram for the Chinese White watercolor. Grey tones are proportional to relative numerical concentration.

Experimental results are then automatically compared to conventional dielectric spheres (Mie theory) by fitting to data the best RI, here resulting n=2.00. Notice that we can safely assume the grains to be as purely dielectric (no absorption) for the white pigment analysed here. The effective, average RI is in accordance to the expected one. Each particle is sized from its S(0) value with the RI extracted from data. EOS Classizer™ ONE provides the user with the Numerical Particle Size Distribution and other statistical values as AVG, CV, and quantiles (see *Figure*). Oversizers obtained from this size distribution are reported in Table I: 2% of the sample is measured to be larger than 1.0 μm, 0% is larger than 2.0 μm.

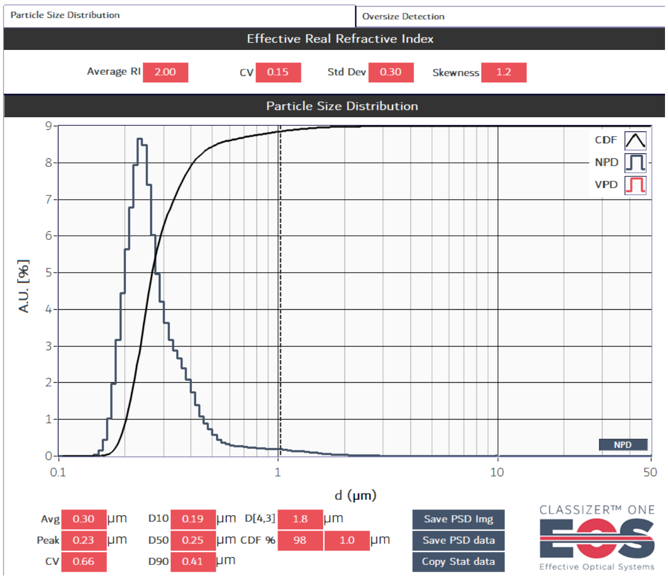


Figure 2 Numerical Particle Size Distribution obtained from data in Fig. 1. Average particle diameter retrieved by EOS Classizer™ is 0.30 μm @ measured n=2.00. Mie scattering model is considered for particle sizing.

Particle Size Distribution | Oversize Detection

Estimate of Oversize Measured/Scaled

Dimensional Class	Meas Num Conc [ptc/mL]	Scaled Num Conc [ptc/mL]	Relative Num Conc [%]	Expert Advise on Statistics
overall	8.54E+6	8.54E+6	100%	-
> 0.5 μm	5.18E+5	5.18E+5	6%	-
> 0.7 μm	2.89E+5	2.89E+5	3%	-
> 1.0 μm	1.46E+5	1.46E+5	2%	-
> 2.0 μm	1.73E+4	1.73E+4	0%	-
> 5.0 μm	5.22E+1	5.22E+1	0%	-
> 10.0 μm	0.00E+0	0.00E+0	0%	-

Table *1* Oversizers obtained from data reported in Fig.1.

More insight can actually be obtained by looking into data through the EOS Classizer™ ONE software application, by taking advantage from the unique single particle and multiparametric approach of EOS Classizer™ ONE. As it is suggested by Figure 1, the EOS CLOUS are neither narrowly nor uniformly distributed. By selecting specific regions one can evidence details and analyse subsets of experimental data with remarkable insight and comprehension of the system under study. For example, in *Figure* two EOS CLOUDS are analysed separately.

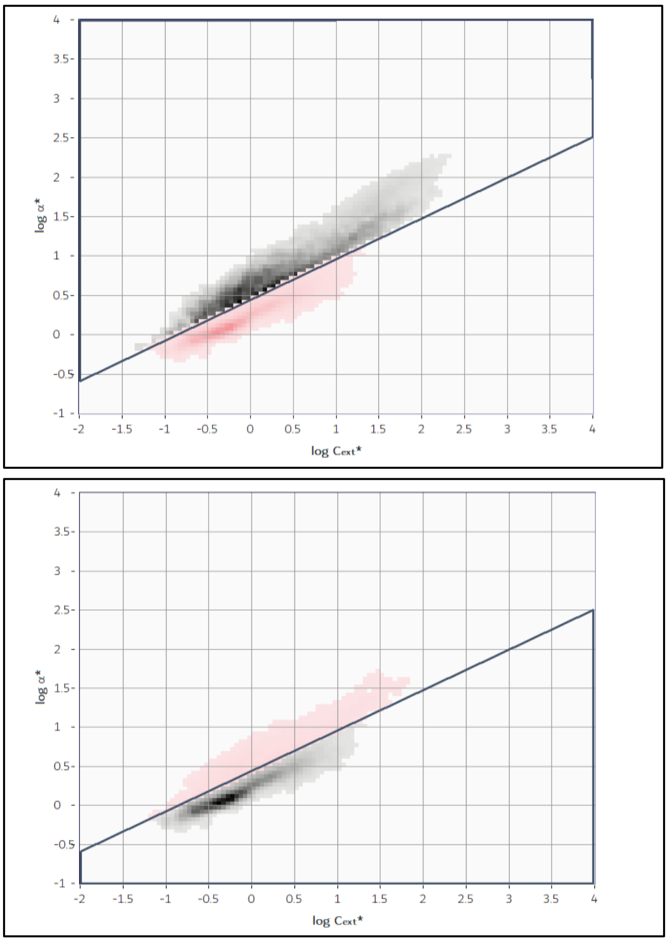


Figure 3 Two complementary EOS CLOUDS extracted from data reported above. Two principal and separated populations are detected by imposing a sharp border separating the upper population (top panel), and the lower (bottom panel). Now the best fitted refractive index increases to n=2.10, as evidenced by the red line (adapted from Mie theory).

Figure reports the results of separate sizing obtained from Figure the two EOS CLOUDS. Data show appreciable differences among the size distributions of the two populations composing the watercolour suspension. The bottom population can now be safely analysed in terms of spherical, or at least isometrical, grains. Quantitatively speaking, the effective refractive index fitted to data now increases to n=2.20, indicating the presence a grain suspension compatible with the specifications of the white watercolour. The corresponding size distribution shows an average size of 0.24 µm, with a D90 of 0.32 µm, still in agreement with the expectations. On top of that, an additional population exists with smaller RI (n=1.50), larger sizes (Avg=0.71 µm, D90=1.2 µm). Notice that the results of the corresponding oversize analyses reported in Table II and Table III are completely different and remarkably changed with respect to the results obtained with the former analysis. While the lower population is compressed to small sizes, 2% only over 0.5 µm, the upper one shows 18% larger than 1.0 µm. Normalizing to the number concentration of the whole population, the latter amounts to 3.5% of the total. The difference essentially comes from the RI values that are now correctly estimated from experimental data.

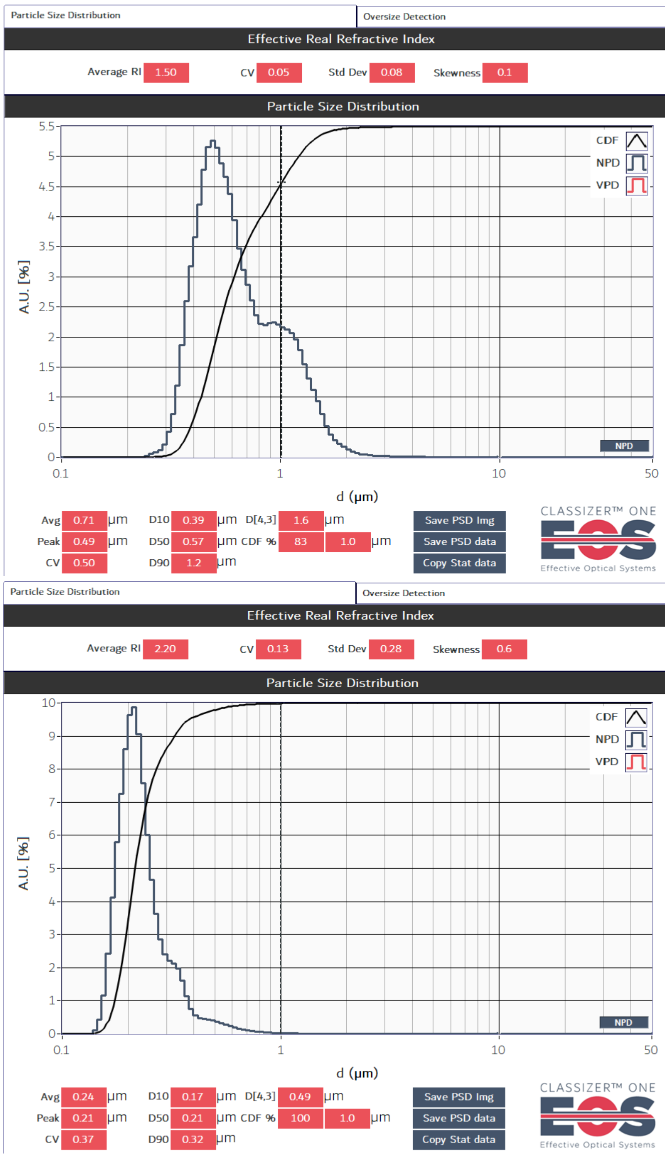


Figure 4 EOS CLOUDS extracted from data reported in Fig.1 show evidence of a heterogeneous suspension composed by compact, high RI grains (bottom) mixed with a suspension of smaller refractive index particles (top) with appreciably larger sizes.

Estimate of Oversize Measured/Scaled				
Dimensional Class	Meas Num Conc [ptc/mL]	Scaled Num Conc [ptc/mL]	Relative Num Conc [%]	Expert Advise on Statistics
overall	1.65E+6	1.65E+6	100%	-
> 0.5 µm	1.10E+6	1.10E+6	67%	-
> 0.7 µm	5.97E+5	5.97E+5	36%	-
> 1.0 µm	2.97E+5	2.97E+5	18%	-
> 2.0 µm	1.04E+4	1.04E+4	1%	-
> 5.0 µm	2.04E+2	2.04E+2	0%	-
> 10.0 µm	0.00E+0	0.00E+0	0%	-

Table II Oversize analysis results for the population within the upper EOS CLOUDS.

Estimate of Oversize Measured/Scaled				
Dimensional Class	Meas Num Conc [ptc/mL]	Scaled Num Conc [ptc/mL]	Relative Num Conc [%]	Expert Advise on Statistics
overall	6.27E+6	6.27E+6	100%	-
> 0.5 µm	1.43E+5	1.43E+5	2%	-
> 0.7 µm	3.41E+4	3.41E+4	1%	-
> 1.0 µm	7.66E+3	7.66E+3	0%	-
> 2.0 µm	0.00E+0	0.00E+0	0%	-
> 5.0 µm	0.00E+0	0.00E+0	0%	-
> 10.0 µm	0.00E+0	0.00E+0	0%	-

Table III Oversize analysis results for the population within the lower EOS CLOUDS.

Focusing on data within the upper EOS CLOUDS, see Figure 2, additional segregation can be argued. We then consider the data subset represented in the upper part of *Figure* and repeat the approach adopted so far. Results are reported in *Figure* , *Figure* , and Table IV and V with the same criteria adopted above.

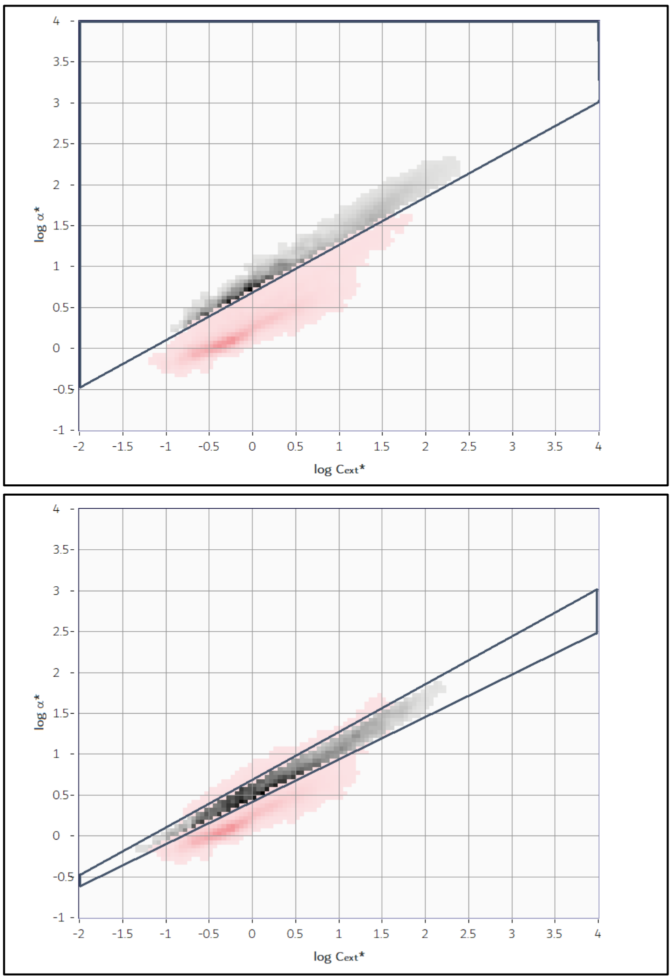


Figure 5 EOS CLOUDS extracted from data reported in the upper panel of Fig.3. A narrow population is still evident with n=1.61 (bottom).

Results show a remarkable segregation of data even in this subset of the EOS CLOUDS. This indicates the presence of two populations with refractive index n=1.57 (lower) and n=1.40 (upper). Again, these values influence the PSDs and the oversize analysis, as reported in Tables IV and V. Notice that in this case the average sizes change into 0.59 µm and 1.2 µm respectively, the D90 values into 0.94 µm and 2.0 µm. Differences in the oversizers are really exaggerated now, with 9% and 45% over 1 µm. Normalizing to the total population again, this fraction corresponds to about 2% of the total, meaning that oversizers are distributed in both these populations.

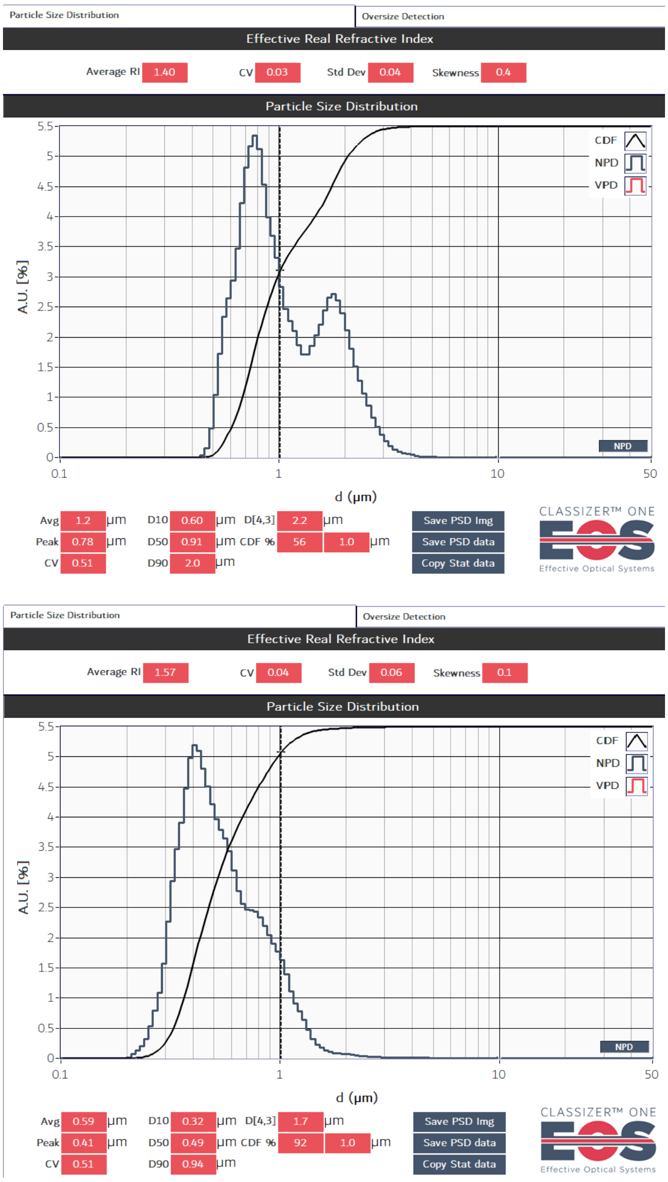


Figure 6 PSD obtained by fitting to data in Figure 5 the S(0) values obtained for dielectric spheres for different RI (Mie theory). Notice that the upper population requires a RI as small as n=1.40, the lower n=1.57.

Estimate of Oversize Measured/Scaled

Dimensional Class	Meas Num Conc [ptc/mL]	Scaled Num Conc [ptc/mL]	Relative Num Conc [%]	Expert Advise on Statistics
overall	3.71E+5	3.71E+5	100%	-
> 0.5 µm	3.69E+5	3.69E+5	99%	-
> 0.7 µm	2.97E+5	2.97E+5	80%	-
> 1.0 µm	1.68E+5	1.68E+5	45%	-
> 2.0 µm	4.13E+4	4.13E+4	11%	-
> 5.0 µm	7.25E+1	7.25E+1	0%	-
> 10.0 µm	0.00E+0	0.00E+0	0%	-

Table IV Oversize analysis for the upper part of the EOS CLOUDS.

Estimate of Oversize Measured/Scaled

Dimensional Class	Meas Num Conc [ptc/mL]	Scaled Num Conc [ptc/mL]	Relative Num Conc [%]	Expert Advise on Statistics
overall	1.17E+6	1.17E+6	100%	-
> 0.5 µm	5.85E+5	5.85E+5	50%	-
> 0.7 µm	3.01E+5	3.01E+5	26%	-
> 1.0 µm	1.01E+5	1.01E+5	9%	-
> 2.0 µm	5.16E+3	5.16E+3	0%	-
> 5.0 µm	1.42E+2	1.42E+2	0%	-
> 10.0 µm	0.00E+0	0.00E+0	0%	-

Table V Oversize analysis for the lower subpopulation.

The presence of such large oversizers can be questioned and better interpreted stemming from the same data set. Still, the unique capabilities of the EOS Classizer™ ONE and the analysis tool makes the difference. First, notice that the large size comes from the low RI value. Such RI is better compatible with non-compact objects rather than homogeneous grains, the average polarizability being affected by the void space within the volume occupied by the analysed particle. By approaching the analysis with the Mean Field Approximation (Chylek et al. 1988; Bohren and Huffman 2008) and the Lorentz-Mie method, further information can be retrieved about the Average Filling Factor of the particles. The EOS software tool provides this information as an estimate of the solid volume contained within non-compact particles, as aggregates, agglomerates, or even mesoporous particles. For the particles under study, with an effective RI n=1.40, we can impose for the bulk material n=2.10 (for solvent, water, n=1.33). The MFA computation tool retrieves a filling factor of approximately 10%, meaning that, on average, 90% of the composition of each particle is water. Otherwise, if a lower refractive index is adopted, so that the aggregates are assumed to be fully composed by the lightest particles (bottom panel in Figure 5), the filling factor increases to 25%, with 75% of the volume filled by water. Likely, the actual case could be in the middle, with aggregates composed by a mixture of grains of the two species and water. In any case one can conclude that the largest particles, measured within the population corresponding to the EOS CLOUD with the lowest RI (upper panel Figure 5), are actually aggregates or clusters composed by smaller grains. Therefore, oversizers analysis can be better interpreted by distinguishing the largest grains composed by the pigment, from the largest objects with intermediate RI, 2% larger than 1 μm, and fluffy clusters. Notice that, very likely, the latter objects are responsible for the apparent oversizers encountered with the first, rough analysis, bringing to 3% of the objects larger than 1 μm. The analysis performed so far clearly shows that the oversize analysis can be heavily affected by remarkable errors if no information is available that allow to interpret data in the correct way.

Experimental results clearly show that EOS Classizer™ ONE and SPES technology can provide unique insights on pigment characterization, well beyond particle sizing. This information are the key aspects for the evaluation of the physical properties of pigment grains (and their behavior in the paint, in this specific case).

The independent measurement of different EOS CLOUDS for the component of a formulation or a complex mix of particles can be of utmost importance and utility to identify the optical properties of the single population and inferring their nature, as it occurs here for aggregates. The results of this analysis can effectively prove changes in particle behavior stability, and properties due to the surrounding media.

CONCLUSIONS

The capability of EOS Classizer™ ONE and SPES patented method in discriminating single particle basing on their optical properties is of capital importance with heterogeneous systems, as pigments ultimately are. In particular, the advantage is rampant and unique when particles have to be analysed to tailor the effectiveness of the product formulation or to study features like stability, the behavior of a single component, etc.

SPES data provide physical and statistical information, as particle size distribution, effective refractive index and numerical concentration, as well as insight into the particle internal structure. Applications range from the study of the behaviour of particles in target heterogeneous media to tailor the formulation, to the improvement of wetting properties of the grains depending on the surfactant, that impacts on both the shelf life and the final appearance once painted. Oversize analysis can be performed accurately separating different populations, also in presence of impurities. Scraps and out-of-spec can be monitored in intermediate 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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