MULTIPARAMETRIC DETERMINATION OF YEAST CELL VIABILITY VIA SPES TECHNOLOGY

INTRODUCTION

The cell viability is defined as a percentage of live cells in a whole population. The determination of yeast cell viability is commonly used to assessing the impact of various types of stressors in toxicity research and in industrial microbiology studies. The yeast *Saccharomyces cerevisiae* is a very useful model organism used for studies of cyto- or genotoxicity under different kinds of chemical, physical, or environmental factors. The analysis of the cell viability is also very important for industrial processes where microorganisms are used, as food, beverage, and biofuel.

The EOS Classizer™ ONE provides an accurate estimation of the cell viability regardless the presence of secondary populations and impurities. In this app notes, the *Saccharomyces cerevisiae* yeast is suspended in ethanol and hydrogen peroxide solutions as examples of stress factors. Monitoring of cell viability over time is performed via CFA-SPES tool. Multiparametric Principal Component Analysis SPES-PCA provides Batch-to-Batch QC analysis.

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.

EOS Classizer™ ONE particle analyzer equipped with EOS standard liquid sample manager LMS01™ - front view

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.

For a general introduction to SPES data please refer to the Application Note AN001/2021, available online along with other application notes and example of applications at EOS website: www.eosinstruments.com/publications/

APPLICATION EXAMPLES

The topics covered in this document are:

1. Optical classification of a yeast aqueous suspension.
2. Viability time monitoring of yeast in ethanol solutions.
3. Batch-to-Batch Principal Component Analysis for a reliable and unique determination of the yeast viability.

Optical classification of an aqueous suspension of yeast – EOS CLOUDS of live and dead cells

From the optical point of view, yeast cells are sightly elliptic particles with a narrow size distribution peaked at around 4µm. They could be considered a quite monodisperse sample, thus the corresponding EOS CLOUD would be also narrow in the C_{ext}-α plane. Alive cells and dead cells present a small but measurable difference both in size and in effective refractive index.

A yeast cell can have an uncontrolled accidental death or a regulated one (apoptosis). In both cases the cell shrinks itself by about 25-28% of his volume [Kato S. et al. "Single-Cell Time-Lapse Observation Reveals Cell Shrinkage upon Cell Death in Batch Culture of Saccharomyces cerevisiae", mBio. 2021; 12(6): e03094-21]. Due to the loss of liquid, the overall refractive index of the particle increases.

This effect can be clearly observed and studied with SPES technique. In comparison with current fluorescence kit available on the market, SPES has mayor advantages:

- User does not count single particles via a microscope. Automatically a reliable measure of ten thousand counts is performed.

- It is not time consuming: each single measure of this application note lasts less than few minutes.

- It does not have any other cost of expensive consumables.

- It does not need any type of sample treatment, except for a simple and automatable pre-dilution with e.g. water.

-It is quite simple to have an inline/online monitoring in an industrial process.

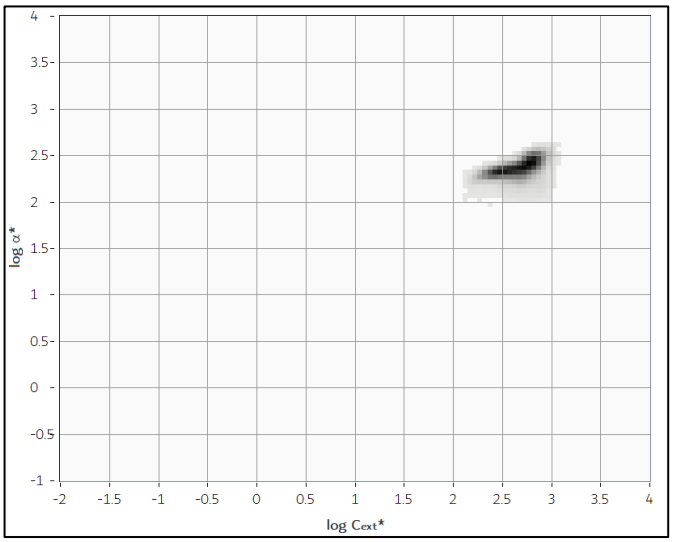

Figure 1 EOS CLOUDS of alive yeast cells in aqueous suspension.

In Figure *1* it is represented the EOS CLOUD of a suspension of alive yeast cells in filtered tap water. The cloud is narrow and monodisperse accordingly to expected values. The EOS Classizer™ software automatically retrieves an effective RI of 1.41, which is compatible with values of cells. The average size is 3.9µm, D50 is 3.7µm, and D[4,3] is 4.4µm (Figure *2*).

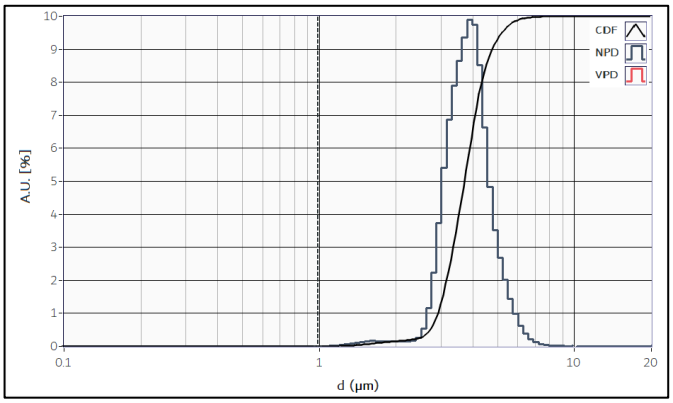

Figure 2 Typical PSD of alive yeast cells in aqueous suspension

To accelerate the natural death rate of yeast cell, a 20% percentage in volume of pure ethanol 96° is added to the aqueous suspension of yeast cells (Figure 3).

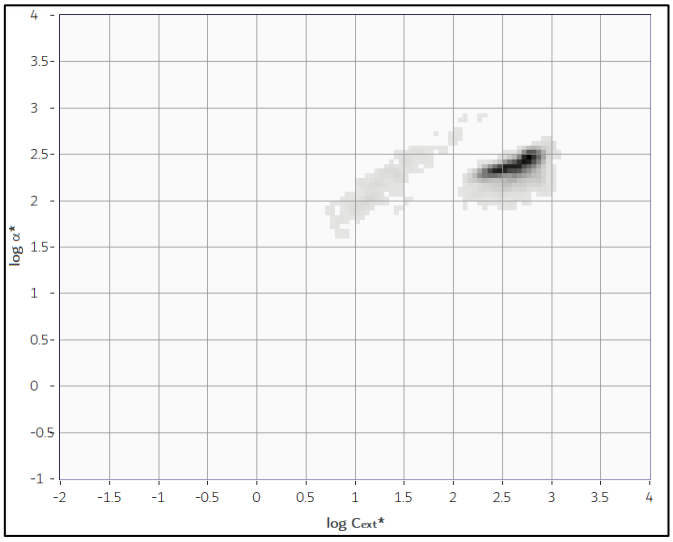

Figure 3 EOS CLOUDS of alive yeast cells in 20% ethanol solution.

The presence of impurities in ethanol creates a mild cloud of data which do not interfere with the classification, counting, and analysis of the main yeast population. The optical properties of the yeast remain unchanged at the start. The initial concentration is 1.7×10^5 ptcs/mL.

The yeast suspension is monitored in time measuring every five minutes. After 30 minutes the yeast cells have dead and their optical properties shifted to a position in the EOS CLOUD compatible with smaller particles with slightly higher effective refractive index (Figure 4).

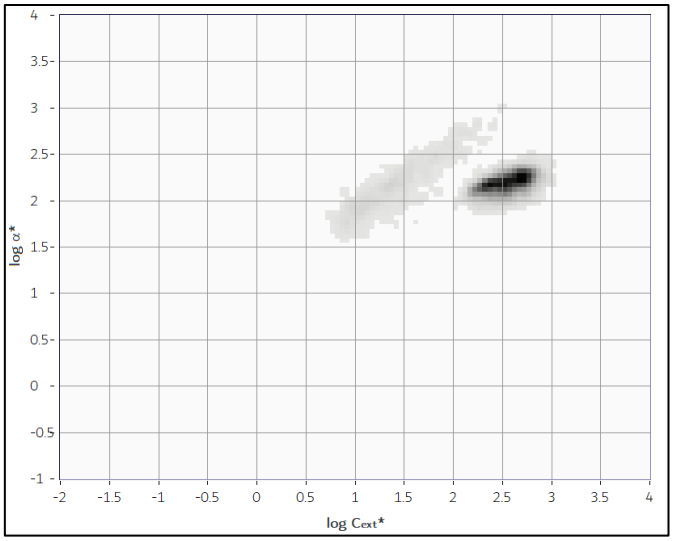

Figure 4 EOS CLOUDS of dead yeast cells in 20% ethanol solution after 25 minutes.

The average polarizability α* of the dead yeast cells went from 2.35 to 2.19. For the cells in Figure 4 the EOS Classizer™ software retrieves an effective refractive index of 1.44 and an average particle size of 3.3µm, a D50 of 3.1µm and a D[4,3] of 3.9µm (Figure *5*), corresponding to a cell volume reduction of 30%.

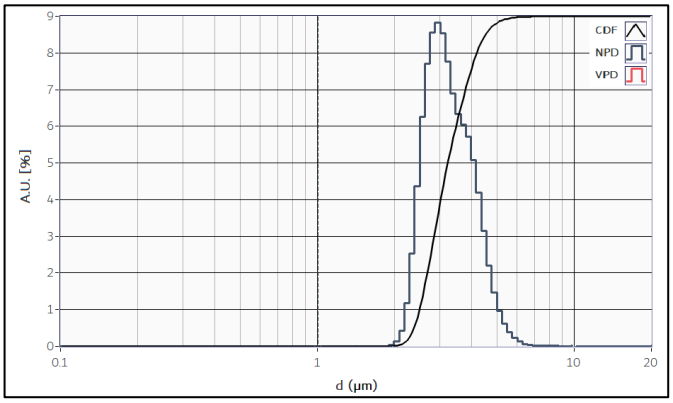

Figure 5 PSD of dead yeast cells in 20% ethanol solution after 25 minutes.

As a reference of the capability of Classizer ONE of classifying the two different types of cells (alive/dead), in Figure *6* it is represented the EOS CLOUDS of an aqueous mix of alive and dead cells. As it clearly visible, the system classifies and quantify two separated clouds corresponding to the two population of cells.

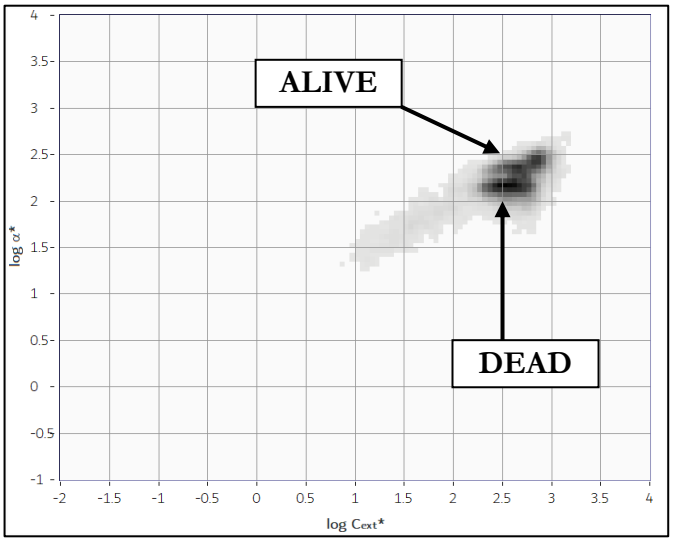

Figure 6 EOS CLOUDS of a mix of a sample with alive yeast cells and dead yeast cells.

By selecting the lower population (as presented in Figure *7*), the software retrieves a refractive index of 1.45, an average dimension of 3.4µm, a D50 of 3.2µm and a D[4.3] of 4.1µm. The percentage of particles selected is the 45% of the whole sample. The selection or alive cell is the 35% of the whole sample. The other contributions to the EOS CLOUDS histograms are related to the impurities detected and counted in suspension.

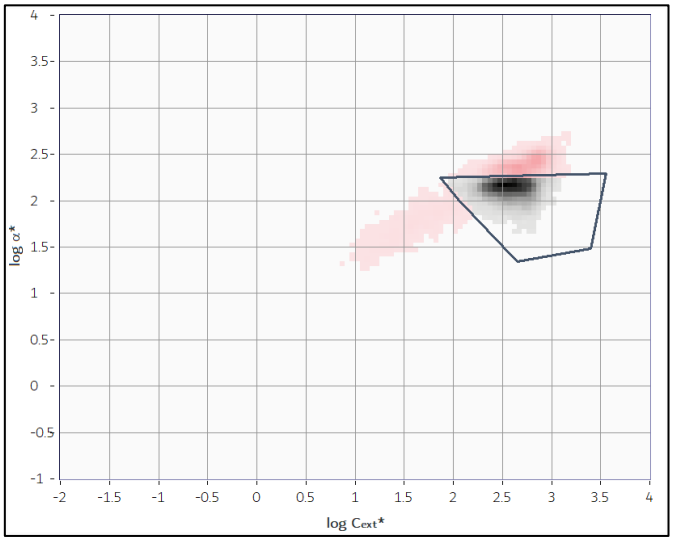

Figure 7 Example of a selection of the dead yeast cells in the suspension.

Viability time monitoring of yeast dispersed in ethanol solutions

The Classizer can perform a real-time monitoring of an inline production process to determine sample differences for an indefinite time with the CFA (Continuous Flow Analysis) addon. In this example, from an initial sample of living yeast cells in Milliq-grade water, different percentages of ethanol are added and then continuously measured for 20 minutes. An area was then selected on the two-dimensional histogram compatible with the position of the living cells, to be able to calculate the percentage of measured particles that fall inside and inside this area. The Figure *8* shows the results.

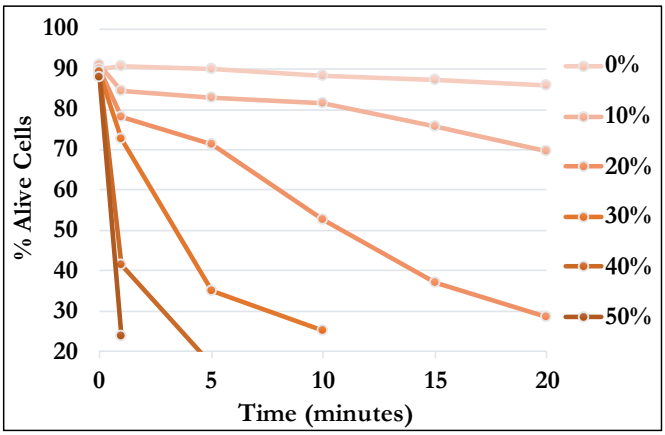

Figure 8 Evolution of living cells in a mix of water / ethanol in increasing percentages of the latter. The Y-axis represents the percentage of particles considered as living cells. A small percentage of the counts are given by impurities, which therefore fall outside the selected area, even in the case of a sample without ethanol.

Form the results it is possible to evince that the higher the percentage of ethanol, the faster the cells die, as expected. In the same way the Classizer can monitor the viability of a production process without unreliable and time-consuming traditional techniques.

Batch-to-Batch Principal Component Analysis for a reliable and unique multiparametric determination of the yeast cell viability

Modern industry in the food market relies on quality control to guarantee the robustness of their productive process. EOS software provides a useful add-on to automate the analysis of a big ensemble of data. Each measure is a batch control which is confronted with a starting dataset. Combining PCA (Principal Component Analysis) for data reduction with a supervised-learning algorithm it is possible to make a very fast batch to batch control. For a complete introduction to PCA analysis with SPES data please refer to the Application Note AN005/2022.

The starting dataset is made by sample which the composition of dead and alive cells in known. The EOS algorithm defines two different areas for alive and dead sample. This is called the training set (see Figure 9).

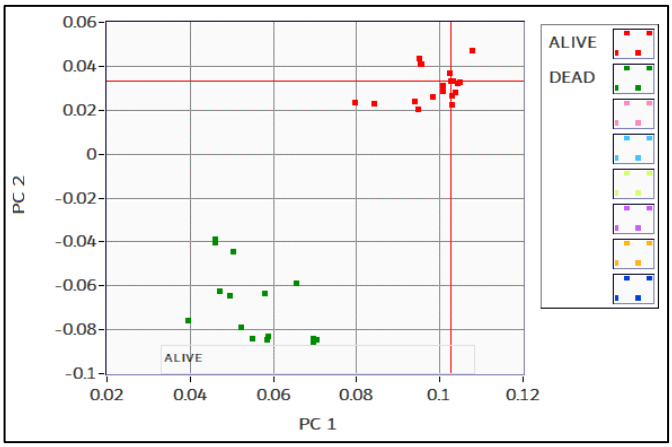

Figure 9 Example of PCA-SPES of batches of alive and dead yeast cells. Two clear separated clouds of points are represented meaning that the method is able to discriminate the two cases of batches.

Thus, the user may test the SPES-PCA capability of classification by running the algorithm adding a new dataset of 21 unknown samples, labelled as "UNKN", and made by various percentages of both dead and alive yeast cells (see Figure 10).

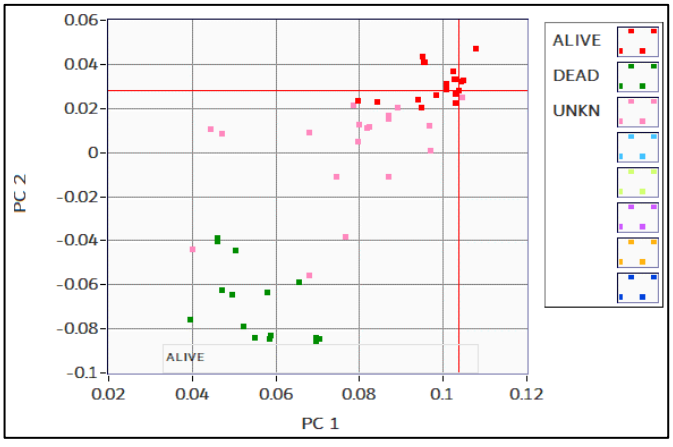

Figure 10 Example of SPES-PCA of batches of alive, dead, and alive+dead yeast cells. The algorithm classifies the novel UNKN samples comparing the data to the trained set represented in Figure 9.

The system is able to estimate if a novel unknown sample is more similar to the population of alive cells or to the populations of dead cells.

The next step is to use machine learning to automatically detect which lot has more or less alive cells through the K-nearest neighbour method. The software suggests using a k-value of five as presented in Figure 11.

LOT001.spes.2Dhist.txt	DEAD
LOT002.spes.2Dhist.txt	ALIVE
LOT003.spes.2Dhist.txt	ALIVE
LOT004.spes.2Dhist.txt	ALIVE
LOT005.spes.2Dhist.txt	ALIVE
LOT006.spes.2Dhist.txt	ALIVE
LOT007.spes.2Dhist.txt	ALIVE
LOT008.spes.2Dhist.txt	ALIVE
LOT009.spes.2Dhist.txt	ALIVE
LOT010.spes.2Dhist.txt	ALIVE
LOT011.spes.2Dhist.txt	ALIVE (far
LOT012.spes.2Dhist.txt	ALIVE
LOT013.spes.2Dhist.txt	DEAD
LOT014.spes.2Dhist.txt	ALIVE

Figure 11 Results of the K-nearest neighbor method on the UNKN samples represented in Figure 10

Five out of twenty-one samples were labelled and classified as batches compatible to dead cells set.

CONCLUSIONS

The EOS Classizer ™ ONE and SPES patented method provide a novel unique solution for the determination of the cell viability of yeast solutions regardless the presence of impurities and secondary populations. SPES data provide physical and statistical information, as particle size distribution, effective refractive index, an estimate on the behavior and stability. Monitoring over time of the cell viability is performed via Continuous Flow Analysis CFA-SPES tool. A multiparametric Batch-to-Batch analysis is done via Principal Component Analysis SPES-PCA tool.

RELEVANT PUBLICATIONS AND REFERENCES

Presentation of Single Particle Extinction and Scattering (SPES) method for particle analysis
AN001-2021 Analysis of Polymeric Particle Mixes via SPES Technology – an 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 Batch-To-Batch Consistency Via Multiparametric SPES Principal Component Analysis PCA

Classizer™ ONE + Sample Managers & Autosampler
AN008-2022 Automatic Liquid Sample Management and System Cleaning with EOS LMS01™ and LMA01™

AN009-2022 Standardize SPES Operative Procedure and improve throughput of Liquid Samples via EOS LAS01™

Example of SPES application to aggregates
AN003-2021 Addressing the Issue of 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 Particles by Mean of the 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

AN015-2022 Classification of Oil and Oil Mixes Emulsions via SPES Technology

Examples of SPES application to particle analysis and behavior characterization in biotech applications
AN011-2021 Quantitative Classification of Particles in Biological Liquids via SPES Technology

AN016-2021 Multiparametric Determination of Yeast Cell Viability via SPES Technology

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

Examples of SPES application to inks and pigments
AN018-2022 Classification of Inks and Pigments via SPES Technology

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)

AN013-2022 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
Cremonesi L *et al.*, «Multiparametric optical characterization of airborne dust …. », Env Int 123 (2019)

AN010-2023 Multiparametric Optical Characterization of Airborne Particles via Patented SPES/SPES² Technologies

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