SEQUIP

IN PROCESS PARTICLE-SIZE POPULATION

Co-Application Story

ORM Measurement

Sequip S + E GmbH

&

usePAT

accurate measuring solutions

SIEMENS

Ingenuity for life

Content

Introduction

The show case demonstrates the ability of the Sequip **2D ORM PSD** particle probe to analyze fermenting yeast cells. In the challenging test environment of a bioreactor with a heavily gassed fermentation, the ultrasound of the PAT add-on "sonic**catch** by usePAT" supports the probe in the measurement, as the disturbing influence of air bubbles and the conditionally optimal installation modality of the probe obscure the desired yeast cell signal.

The online process monitoring and control set up by SIMATIC PCS 7 and SIMATIC SIPAT offers the possibility to collect, store and model the improved measured values and to derive and implement direct measures for steering the process. This enables direct monitoring of the active fermentation culture and its control without the detour of measuring several critical process parameters in its environment. Direct measurement of product concentration enables more efficient overall process performance or new insights into the process.

Realization of the Requirements and the Sequip ORM Measurement-Method

The analysis system is based on an **optical back-reflection measurement** (ORM).

The **main features** of this ORM measurement principle are:

1. **Inline In-Situ** with a focus in front of the window in the process medium to be measured
2. **Real-time measurement** directly in the process– 24/7,
3. **Autofocus** for sensors,
4. **Sensors** with **various** focal depth,
5. a **highly stable** laser-based lightsource,
6. clear, high-resolution signal evaluation,
7. **Single-mode** optical fibers for transporting the light signals,
8. **Precise** detection and sizing of the **individual particles** passing the focus.

With the Sequip **ORM** technology, the **measurement results** are obtained exclusively from the data of the **individual** particles.

Each particle that passes the **focal zone** before the window in the original process medium is counted and assigned to the corresponding fraction width.

Due to this focal zone and the single-mode technique, the so-called "multiscattering" can be **eliminated** signal-wise.

Thus, a representation of the real distribution of the exactly focused particle sizes is obtained **without the aid** of a manipulable standard distribution.

The ORM software **documents** the obtained data. Thus, a **permanent** representation of the process course becomes possible. This acquired data can also be accessed again at a later time.

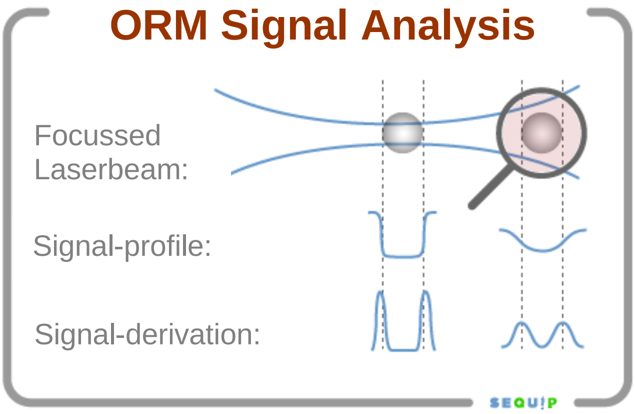

Figure 1: see magnifier (particles outside the focus zone)

The derived signal of these particles located outside the focal zone has a too soft and flat curve.

Particles coupled in at the edge of the focus are rejected by the evaluation electronics for being off-center signals due to their signal pattern.

With the **Sequip** **ORM** measurement method, all particle diameters can be measured that exhibit a difference in refractive index between the object to be measured and the carrier phase.

This applies to measurements in liquid, dry, gaseous media as well as in aerosol phases.

The Measurement Principle

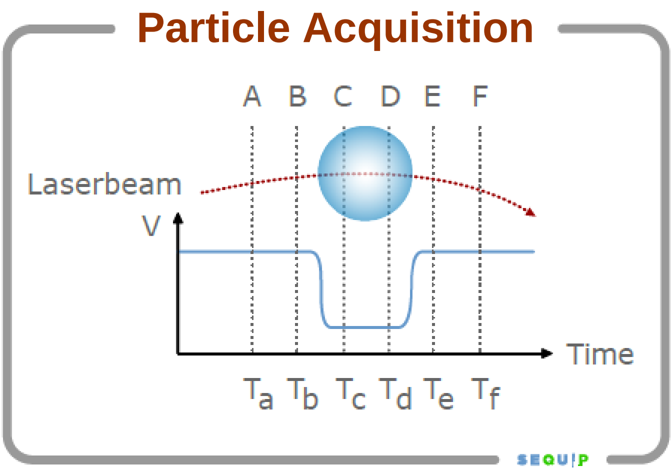

Figure 2: The in-situ **Sequip** **ORM** systems work on the basis of the patented technology with the **ToF** (Time of Fly) method, among others.

The measurement software determines the size of the **focused** particles from the time required to sweep them.

The sum of the measurement signals provides a statistically **robust** and real representation of the size distribution.

The shape of the selective focal space in the **undiluted** medium **directly** in the process with the aid of the back reflection signal represents the special characteristic of the **Sequip** **ORM** technology.

Inline Monitoring of Fermentation Processes

The use-case together with usePAT and Siemens aimed at generating unique and qualified data from the process, providing selective information about the medium, the microorganisms and their products.

Ultrasonic fields are used to catch cells directly in the focus of a PAT probe (sonic**catch**), resulting in in-situ sample preparation and enabling sensitive measurement of microorganisms without damaging them. usePAT uses its ultrasonic technology in the "sonic**catch**" and "sonic**wipe**" products to provide accurate measurements in industrial liquids by improving signal intensity on the one hand, and to keep them continuously clean on the other.

The Siemens LivingLab provides the necessary actual implementation of a biotechnological process for this project. There, the fermentation process is monitored and controlled online and in real time using a set of sensors and analyzers directly connected to a control system (SIMATIC PCS 7) and a Process Analytical Technology (PAT) tool (SIMATIC SIPAT). All data, univariate data and multivariate spectroscopic data are collected, contextualized and form the basis to perform Advanced Process Contol with an embedded control system, such as the SIMATIC PCS 7 used in the LivingLab.

Specifically, a fermentation with yeast S-33 was set up aerobically in the reactor under 20-fold aeration. Integrated in the ultrasonic trap sonic**catch** by usePAT for "sorting" the particulate fractions, a **Sequip 2D ORM PSD** measurement was introduced into the process through the reactor lid.

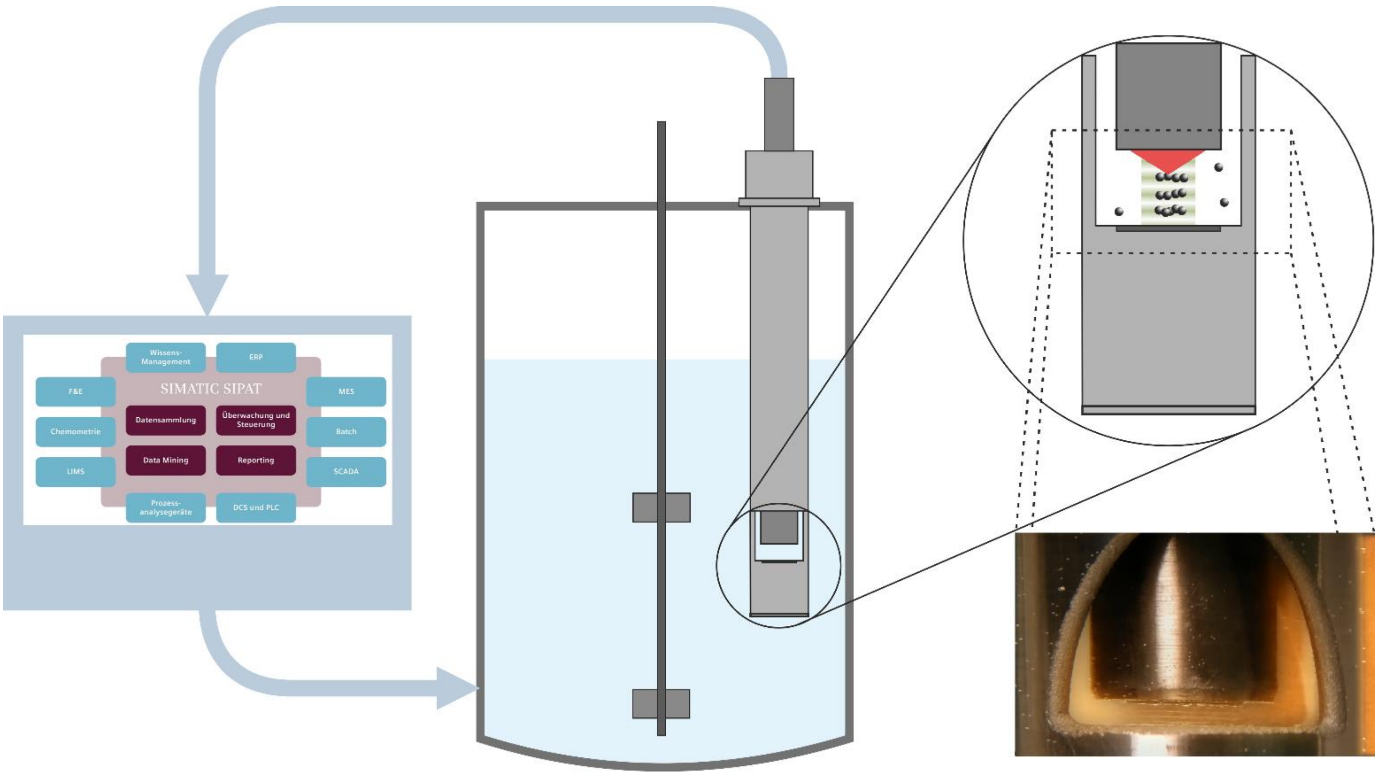

Figure 3: Set-up of the use-case in the LivingLab. The in-situ **Sequip ORM** particle probe was installed in the reactor together with the ultrasonic trap sonic**catch** by usePAT. SIPAT manages the process data from the SIMATIC PCS7 process control system.

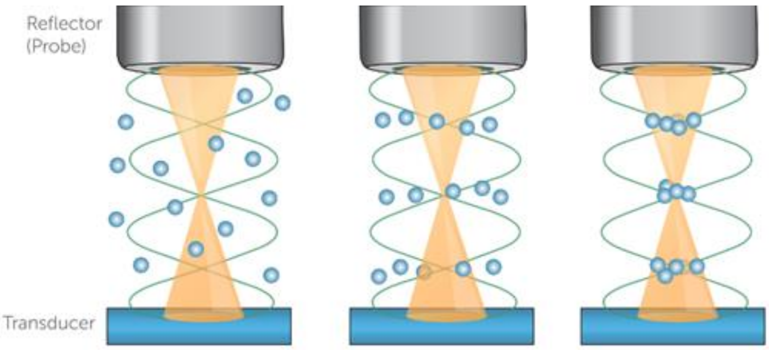

Figure 4: Schematic principle of a standing ultrasonic wave between piezo ultrasonic transducer of sonic**catch** and **Sequip ORM** particle probe as reflector for trapping the particles to make the measurement more sensitive.

Focusing on Cells

Due to the strong aeration in the reactor of the LivingLab Vienna and the conditionally optimal vertical installation of the probe, the yeast cell signal was temporarily "burdened by noise". Activating the ultrasound amplifies the detected spectrum of the yeast cells so that they become more measurable. At the same time, the signal level is reduced by the air bubbles and outgassing present in many applications. These are now more than clearly structured by the ultrasound and kept away from the measuring range of the probe. This improves the noise behavior in the Sequip application and even increases it further with good reproducibility.

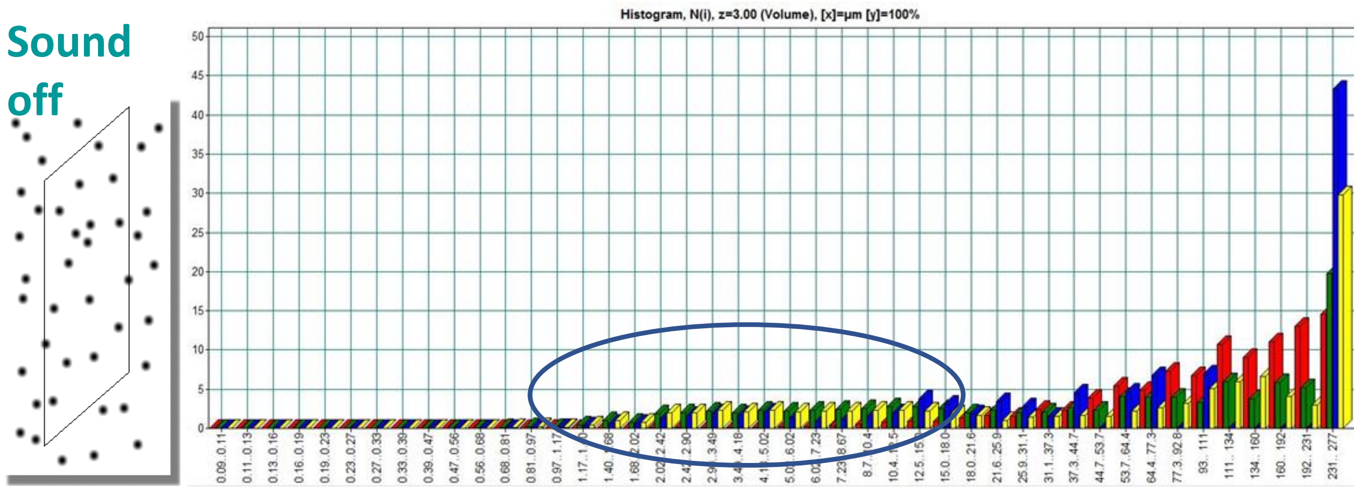

Figure 5: Histogram volume distribution of particle sizes - without ultrasound, the yeast fraction (marked in blue) disappears almost completely behind the bubble carpet.

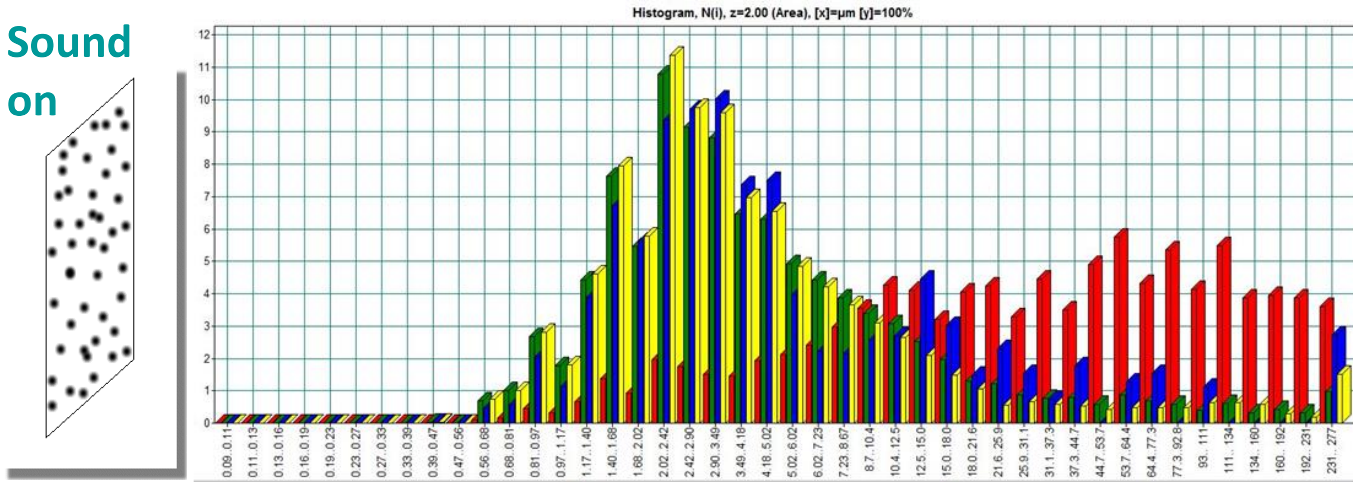

Figure 6: The histogram in length distribution shows again how the yeast cells become measurable over the bubble carpet through excitation by ultrasound.

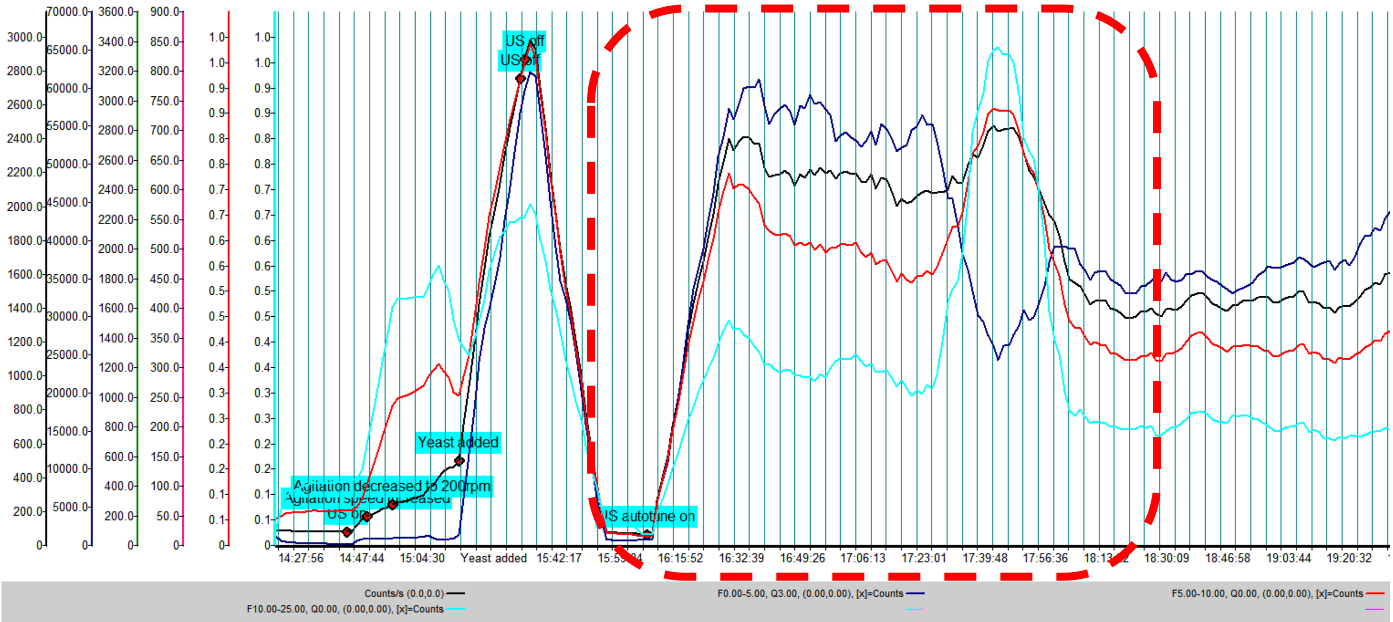

Figure 7: Initial parameters such as stirring speed and addition of yeast cells are evident in the signal (turquoise). The ultrasound is switched on at about 14:40, which starts the signal increase. Switching off the ultrasound ("UL off") at about 15:30 results in the following signal drop. Turning on ultrasound ("UL autotune on") causes the signal level to rise again. The glucose phase is shown in the section of the trend between 16:00 - 18:00 (highlighted in red).

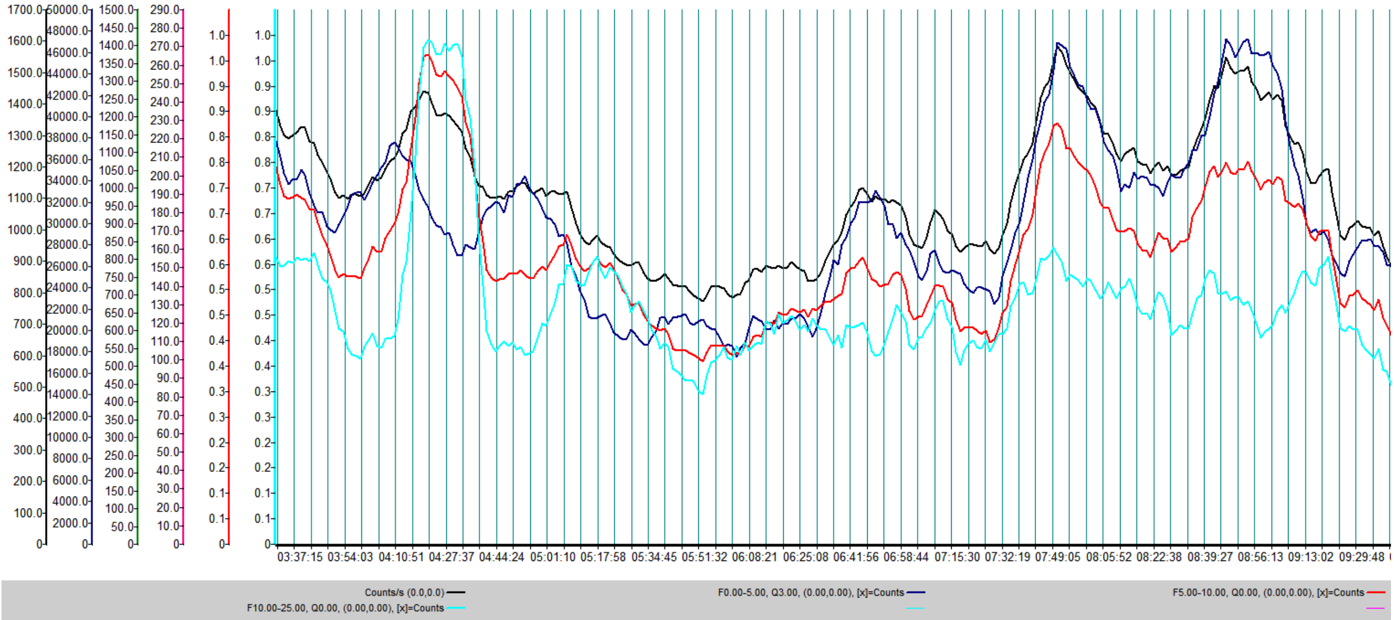

Figure 8: Since the primary C-source required for metabolism is no longer available, the cells trend of ethanol consumption begins in the "respiration phase", starting around 07:00 am on the following day. Also, the fine particle rate remained constant throughout the fermentation period, indicating non-destructive use of ultrasound in combination with live cells.

Preview

The application of particle probes in fermentation processes offers an alternative to spectroscopic probes. Monitoring the "viability" of the cells by means of particle size distribution analysis allows targeted statements to be made about the process status. The signal enhancement of the **Sequip** particle probe by the precise ultrasonic field of the sonic**catch** PAT-process fitting improves the process control of an active fermentation. Thanks to Siemens' integration of both PAT technologies into the superior SIMATIC SIPAT platform, this represents an innovative solution for biotech applications.

Appendix

Sequip Founder Partners and their technical Expertise

Sequip S + E GmbH was founded in 1993 with the aim of continuous inline measurement of particulate systems.

Sequip, was supported among others by the launching customers Bayer, BDF Beiersdorf, UNI Regensburg, Merck Darmstadt and Henkel.

When **Sequip** was founded, the main market requirement was to develop a system for measuring particle size distribution **based** on a **true** count rate of particles passing through the focus and **without** a standard distribution that could be mathematically manipulated.

The development ultimately led to the **Sequip** **ORM** measurement method.

Thus, the **Sequip** sensors should meet the following conditions:

1. The system should provide a robust statistical distribution by counting the individual particles directly and evaluating their size individually.

   The market requirement then, as now, was **accurate** determination of particle number and size distribution, including **simultaneous** display of fine and coarse particles, even with **changing** particle size systems,
2. **accurate** and **robust** evaluation with changing distributions of particle size systems from beaker to production vessel,
3. Acquisition of **agglomerates**,
4. **inline** suitable with **direct** installation in reactors and pipelines,
5. **directly** in the process and **originally concentrated** medium,
6. **without** preparative sample processing,
7. **autoclavable**,
8. **suitable** for hazardous conditions – workers safety

Starting in 2000, the implementation of these requirements led to the market launch of a groundbreaking technology that is supported by **numerous** patents.