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Master End Project – Raman scattering of fermentation particles

In this project you will be able to gain experience with state-of-the-art Raman spectroscopy and automatic bioreactor monitoring, which is highly relevant for bioprocess engineering and bioprocess development research in industry and academia. You will design and perform an experimental study to determine the influence of bubble and cell density on Raman spectroscopy signals to ensure robust model development for real-time monitoring applications.

Main project objectives:

- Experimentally characterize the Raman signal attenuation by bubbles and cells
- Investigate spectra pre-processing methods to correct signal attenuation by broth particles

Project background:

Within bioprocessing, the determination of cell density and nutrient concentrations is mostly based on manual sampling and offline analysis. This provides a delayed and partial view of the process, and the bioprocessing industry is searching for process analytical technologies that provide real-time in-situ information and improve process characterization. Optical monitoring techniques such as Raman spectroscopy allow non-invasive and non-destructive process monitoring and can be employed to observe essential process parameters.

Raman spectroscopy is a vibrational spectroscopy technique with a high molecular specificity that has seen a large increase in applications over the last decade [1]. Spectra can be acquired continuously during fermentation, and each spectrum comprehends the full chemical composition of the culture broth. To extract useful information from the spectral data, elaborate pre-processing methods and multivariate analytical strategies are employed. Data pre-processing is an essential part of Raman spectroscopy, and aims to remove undesired signal variation while focusing on spectra variation linked to the parameters of interest [2].

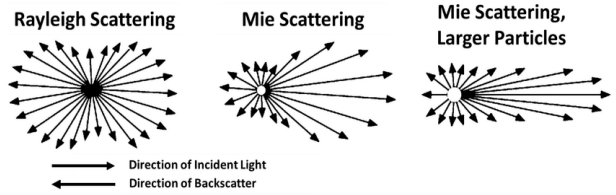

Figure 1: *Types of light scattering based on particle size [3]. Molecules and nanoparticles scatter light according to the Rayleigh theory, larger particles show Mie and optical scattering.*

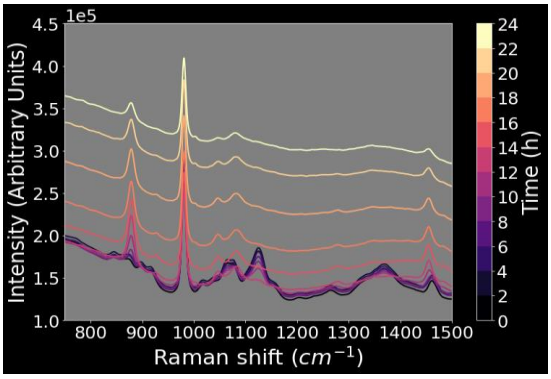

Figure 2: *Unprocessed Raman spectra acquired during 24 hours of S. cerevisiae fermentation. The background signal intensifies together with the increase of biomass.*

Whereas the intensity of Raman scattering is proportional to the molecule concentration, non-linear disturbances can alter the spectral data and complicate data pre-processing and subsequent analysis [4]. Within the bioreactor, air bubbles and cells influence light scattering which attenuates the Raman signal (**Figure 1**). The increase in cell density often results in an increasing background signal (**Figure 2**), and the effect is poorly characterized in literature. A deeper understanding of this light attenuation will help to develop more suitable data pre-processing methods and can lead to new modeling approaches for quantifying cell density with Raman.

References:

1. Esmonde-White, K.A., et al., *Raman spectroscopy as a process analytical technology for pharmaceutical manufacturing and bioprocessing.* Analytical and bioanalytical chemistry, 2017. **409**(3): p. 637-649.
2. Bocklitz, T., et al., *How to pre-process Raman spectra for reliable and stable models?* Analytica chimica acta, 2011. **704**(1-2): p. 47-56.
3. Nave, C.R. *Blue Sky*. 2012; Available from: http://hyperphysics.phy-astr.gsu.edu/hbase/atmos/blusky.html#c4.
4. Pelletier, M.J., *Quantitative analysis using Raman spectrometry.* Applied spectroscopy, 2003. **57**(1): p. 20A-42A.