In situ Real-Time Monitoring of Bioprocesses using Process Analytical Technology

Bioprocessing & Process Development Seminar Series
BTEC, NC, October 2011

METTLER TOLEDO

Outline

- Brief overview of Mettler Toledo
- *In situ* probe technologies for real-time measurement
- Applications
  - Upstream - *In situ* mid-IR for real-time monitoring of culture medium
  - Downstream - *In situ* particle characterization for monitoring:
    - flocculation for clarification of cell culture harvests
    - protein crystallization
- Summary
- Acknowledgments

Mettler Toledo International

Laboratory

Indus

Weighing

Pipettes

AutoChem

etail

Liquid Analysis

Thermal Analysis

In situ probe technologies for real-time measurement

- Information in real-time for real-time process understanding and control
- No need for sampling and/or sample preparation for further analysis

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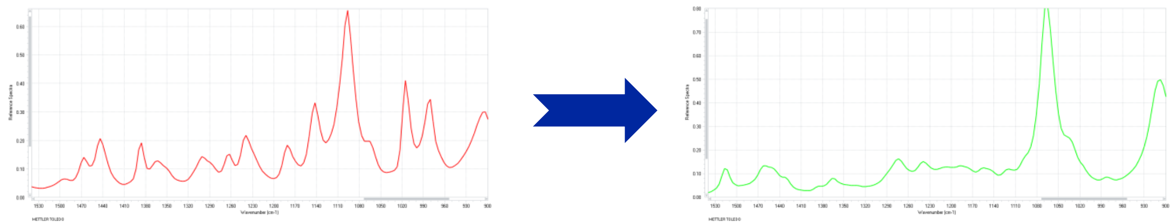
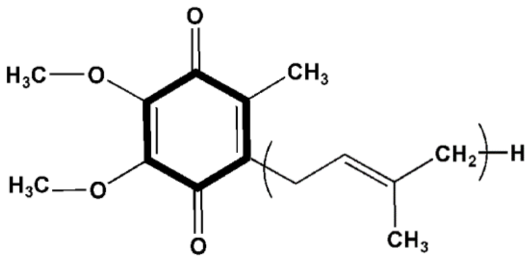
ReactIR™ - Reaction Analysis System

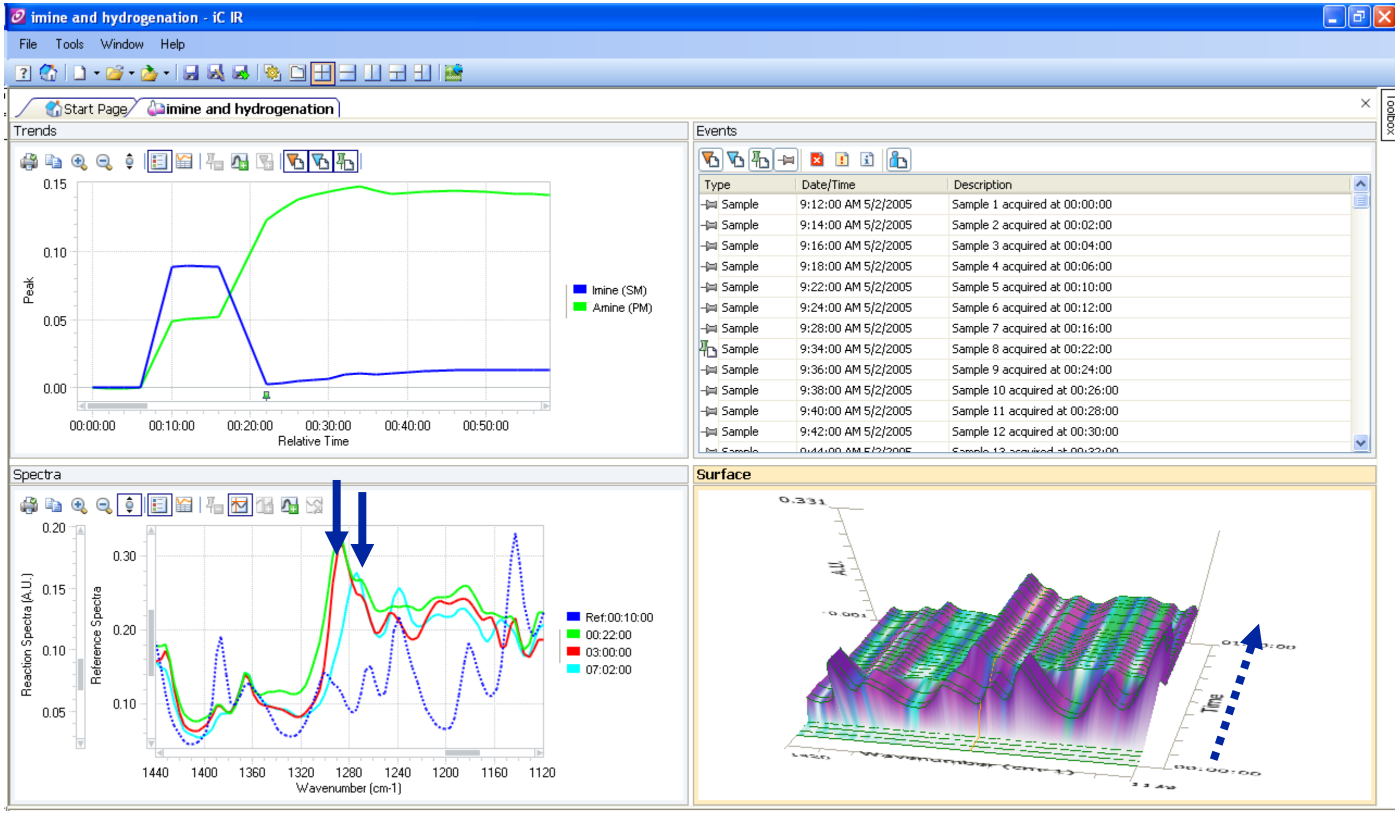




- ReactIR™ is a fully optimized and integrated mid-infrared, *in-situ* reaction analysis system.

- ReactIR™ records a “molecular video” of a chemical reaction. It shows, in real-time molecular bonds breaking and forming.

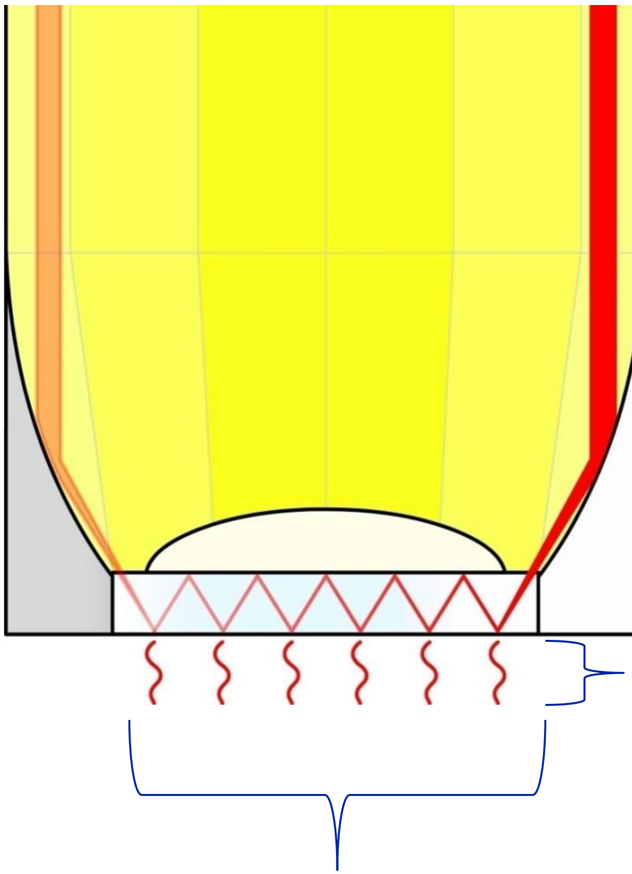
ReactIR™ - Follows Reaction Progression



- The mid-infrared spectrum contains specific information related to each component in the reaction.
- By monitoring these signals using iCIR™, the ReactIR™ gives real-time feedback on reaction progress without having to take samples for off-line analysis.

ReactIR™ Method of Measurement

Attenuated Total Reflectance (ATR) sensor in the probe tip



Why ATR for *in situ* monitoring applications?

- Pathlength = (depth of penetration) x number of reflections = 12 μm
- No interference from bubbles, solid, mixing, etc.
- Selective information on the **liquid phase**
- Beer's Law: A=abc
- a & b are constants so A is proportional to concentration!

a – **absorptivity**, extinction coefficient
b – optical **path length** into the sample (cm)
c – solute molar **concentration** (mol/L)

In situ monitoring of culture medium

Online monitoring of nine different batch cultures of *E. coli* by mid-infrared spectroscopy, using a single spectra library for calibration

Jonas Schenk, Carla Viscasillas, Ian W. Marison, Urs von Stockar

Journal of Biotechnology 134 (2008) 93–102

- Objectives
  - Investigate the possibility of using a single spectra library to predict the concentration of principal metabolites for cultures on different media.
  - Test the robustness of the library with respect to chemically undefined complex media, including additives such as peptone and yeast extract.

Online Monitoring of Nine Different Batch cultures of *E. coli* by mid-infrared spectroscopy, using a single spectra library for calibration

Jonas Schenk[a], Carla Viscasillas[a], Ian W. Marison[b], Urs von Stockar[a]
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b. School of Biotechnology, Dublin City University, Glasnevin, Dublin 9, Ireland

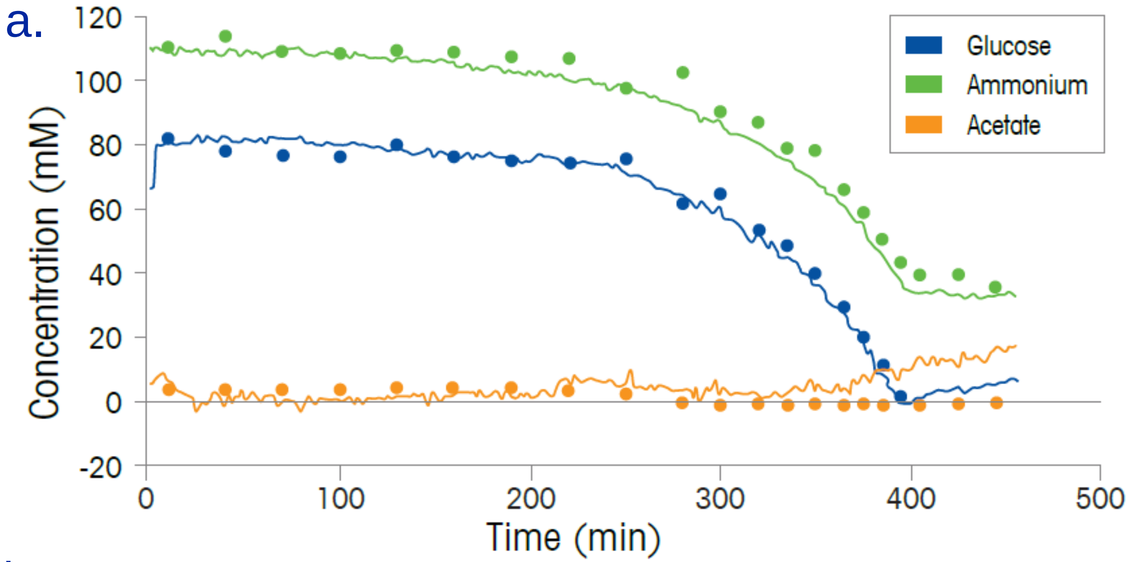
Journal of Biotechnology 134 (2008) 93–102

In situ monitoring of culture medium

Experimental

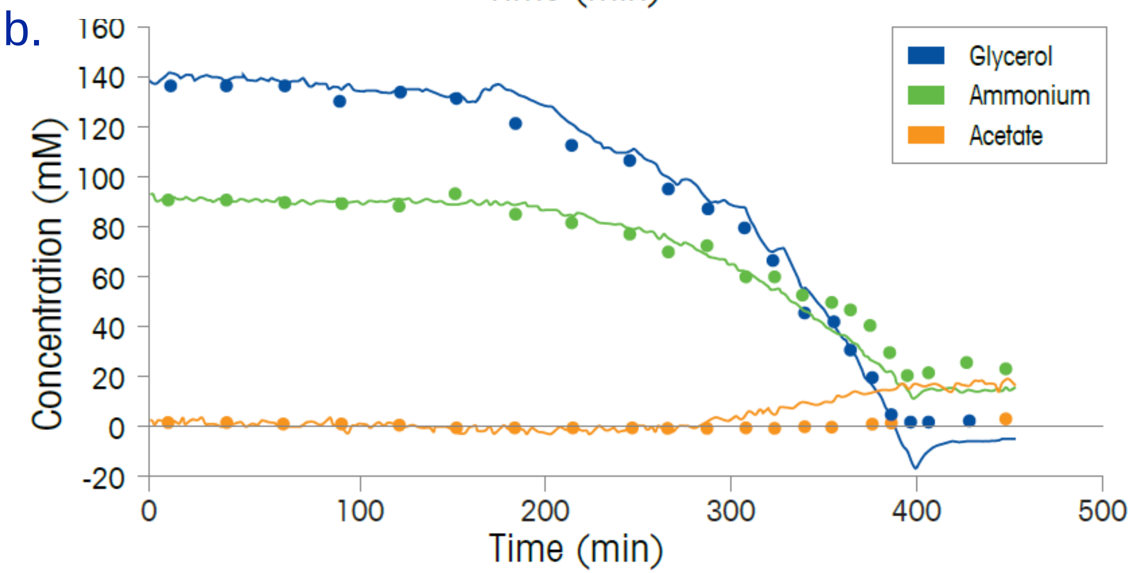
- Spectral library components were:
  - glucose, glycerol, ammonium and acetate.
  - two "drift spectra", to eliminate signal intensity drifts
- *E. coli* was cultured on:
    - glucose
    - glycerol
    - glucose + glycerol
- Concentration of the analytes of interest, glucose, glycerol ammonium ion and acetic acid, were obtained by enzymatic analysis kits (R-Biopharm, Germany).
- All mid-IR spectra were obtained with a ReactIR™ spectrometer, with DiComp ATR sensor.
- Concentrations were calculated by applying traditional least squares regression.

In situ monitoring of culture medium



a. Glucose, ammonium and acetate concentration profiles measured offline (circles) and online by ReactIR™ (corresponding lines), during batch growth on glucose of the *E. coli* culture.

b. Glycerol, ammonium and acetate concentration profiles measured offline (circles) and online by ReactIR™ (corresponding lines), during batch growth on glucose of the *E. coli* culture.



- ReactIR™ profiles of the carbon source, ammonium and acetate matched well with the offline concentration.
- Monitoring to low concentrations
  - 30mM glucose = 5.4g/L

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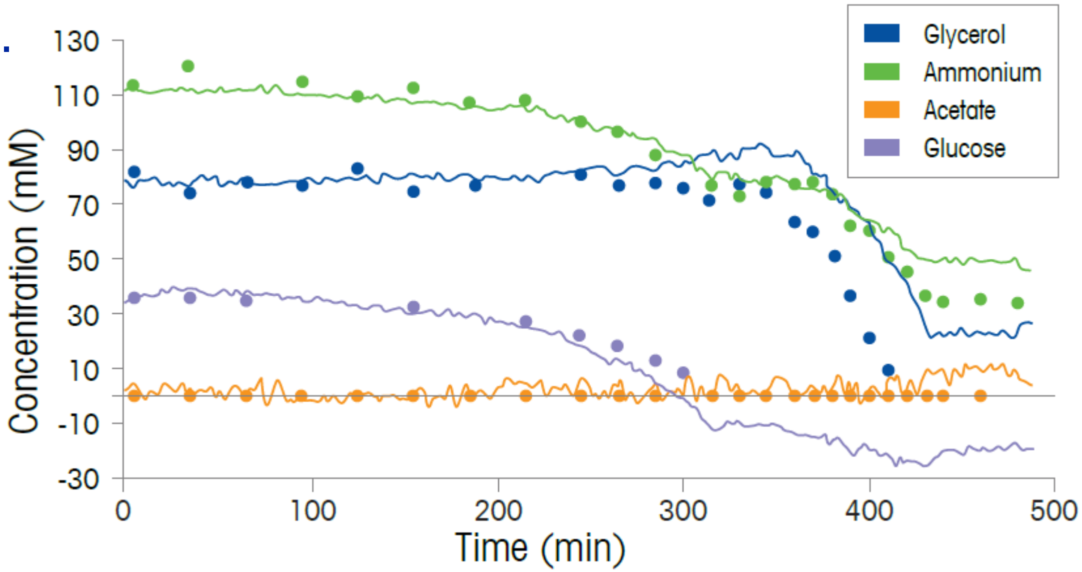
In situ monitoring of culture medium

c.



c. Glucose, glycerol, ammonium and acetate concentration profiles measured offline (circles) and online by ReactIR™ (corresponding lines), during batch growth on glucose and glycerol of the *E. coli* culture.

- For the batch with the medium containing both glucose and glycerol, ReactIR™ profiles were in agreement with the actual concentrations during the glucose phase, but then diverged significantly in the later stage.
- Divergence explained to be due to the two carbon sources being similar in structure and functional groups.
- Library-based modeling is accurate for single carbon source systems, it is only qualitatively accurate for media where two or more substrates have similar functional groups.

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In situ monitoring of culture medium

- Robustness of the spectral library was challenged by culturing *E. coli* in "undefined" media.
  - Concentrations obtained with ReactIR^{TM} were in excellent agreement with offline data (data not shown). It was concluded that the spectral library approach is robust enough to tolerate the addition of unknown compounds.

In situ monitoring of culture medium

Conclusions

- Eliminates the need for offline sampling and sample preparation.
- Carbon source depletion in batch cultures
- Applied to controlling addition of carbon source in fed-batch and continuous cultures
- Traditional least squares regression modeling, using a single library of six spectra as a calibration set, was as accurate and predictive as the conventional chemometrics approaches, for online *E. coli* batch culture.
  - This is significant in reducing calibration time.
- This library approach does not apply PCR, making it amenable to those not experts in process modeling.

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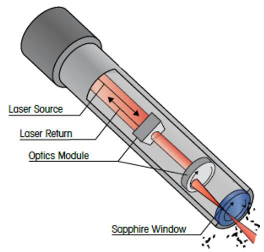
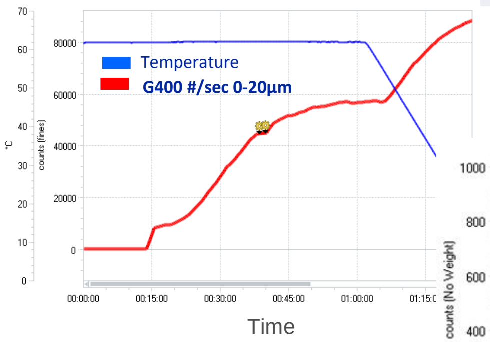
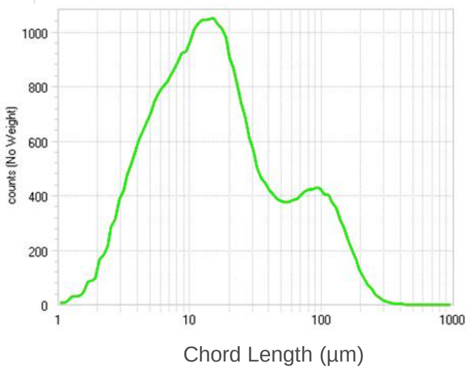
In-Situ Particle Characterization Tools

FBRM® Technology

Focused Beam Reflectance Measurement

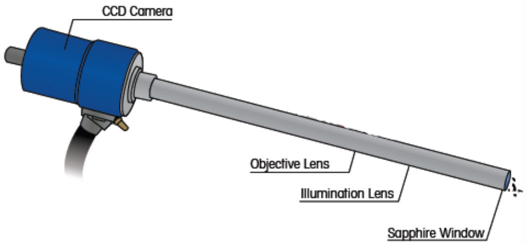


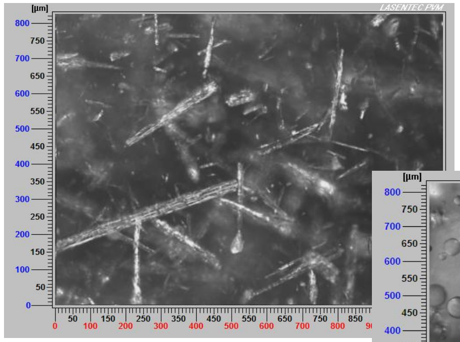


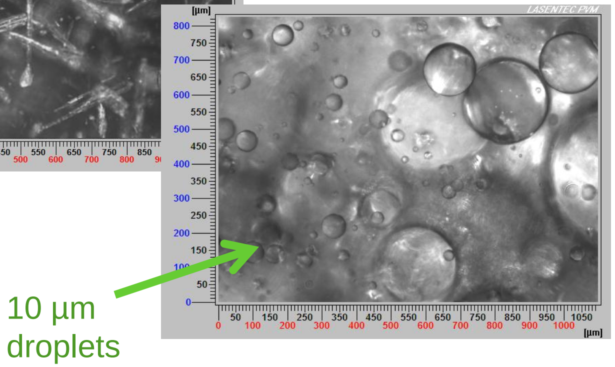


PVM® Technology

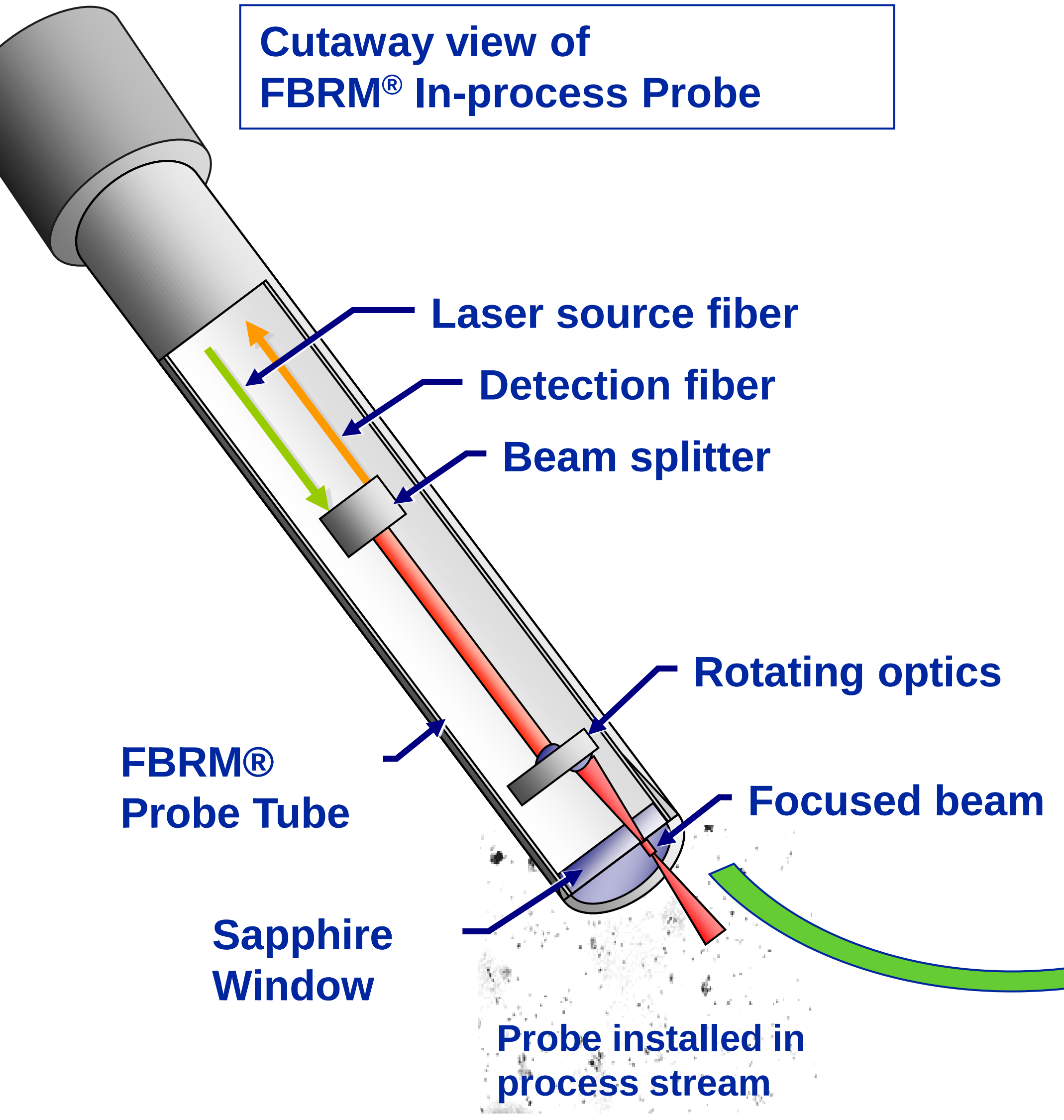
Particle Vision and Measurement



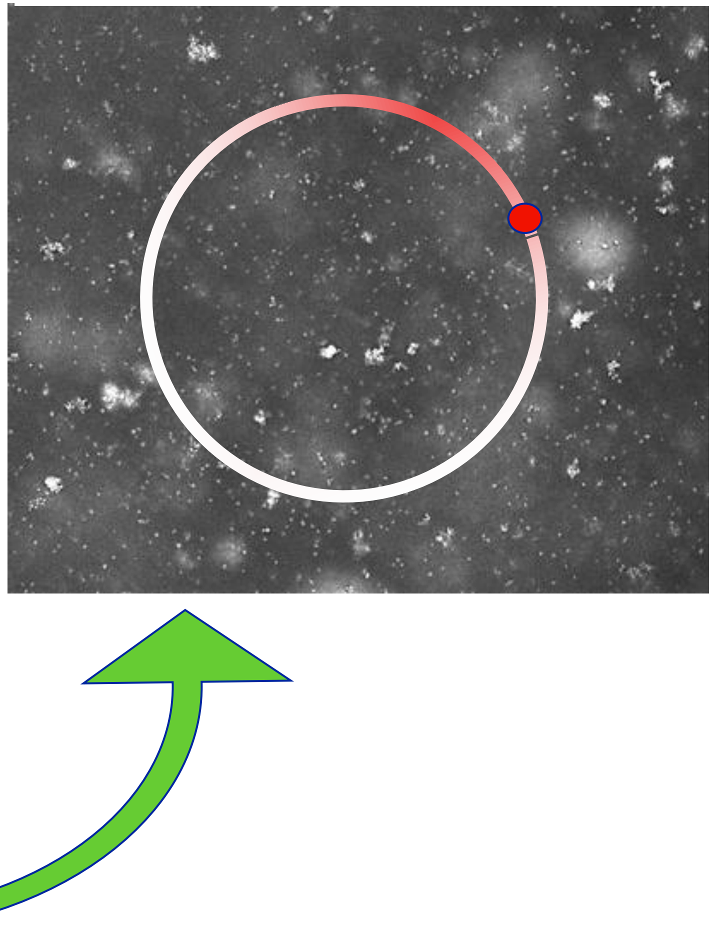




The FBRM® Method of Measurement

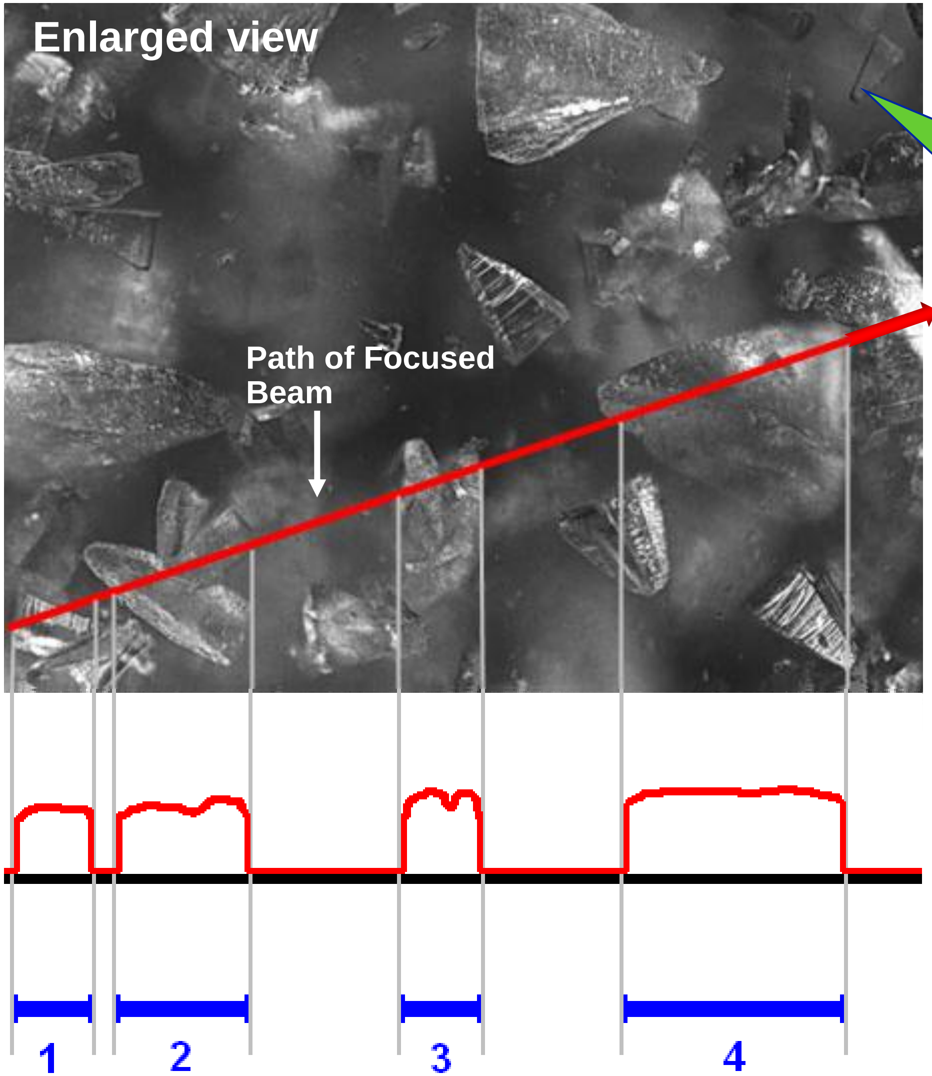


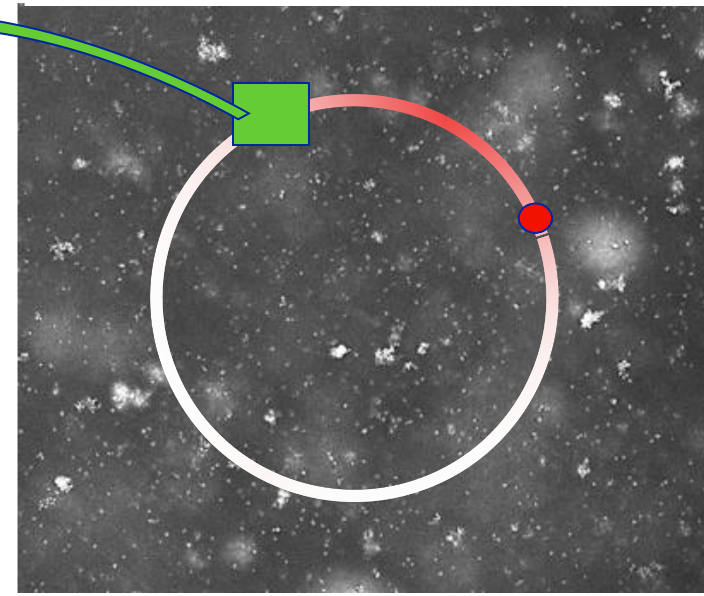
PVM® image illustrating the view from the FBRM® Probe Window



The FBRM® Method of Measurement

PVM® image illustrating the view from the FBRM® Probe Window

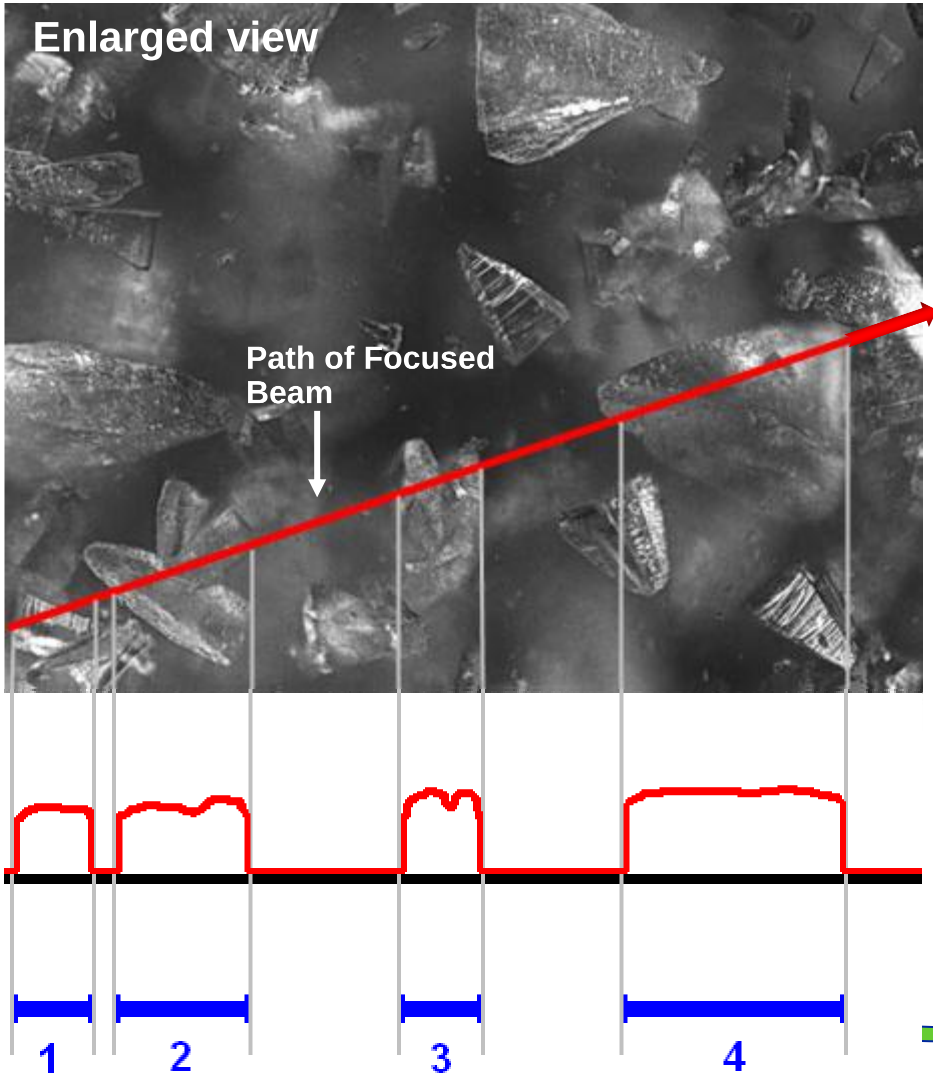


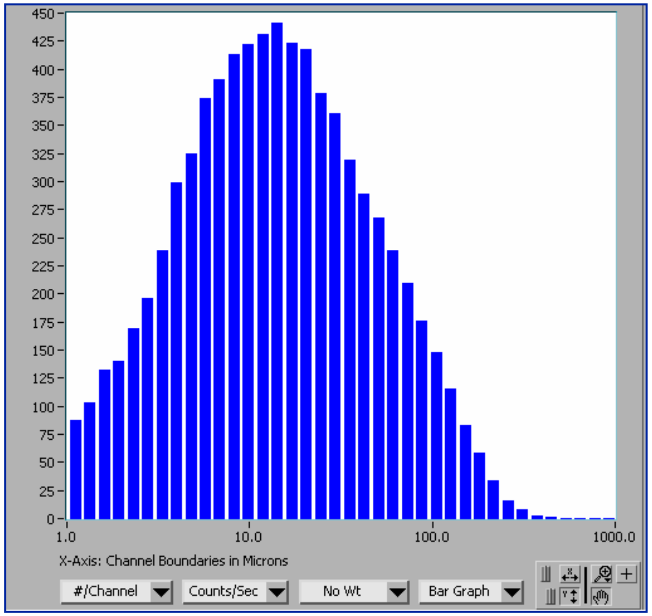


Probe detects pulses of Backscattered light

And records measured Chord Lengths

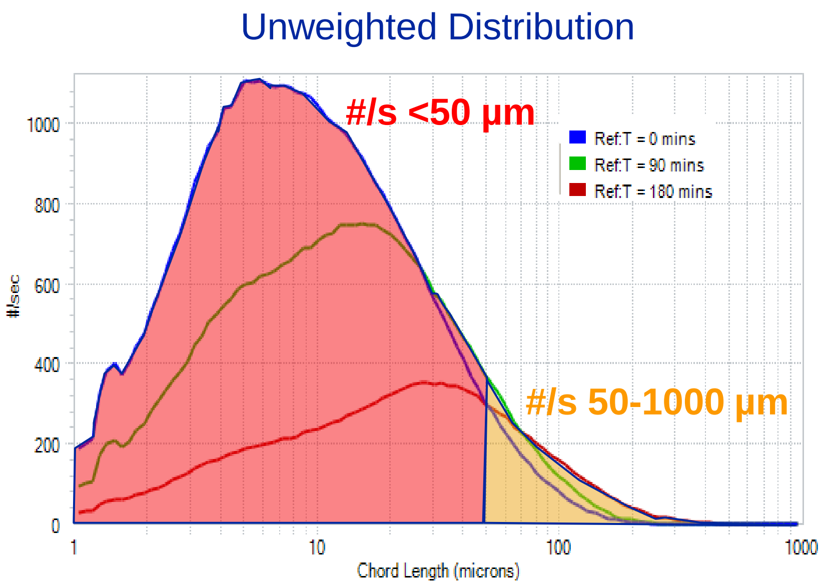
The FBRM® Method of Measurement

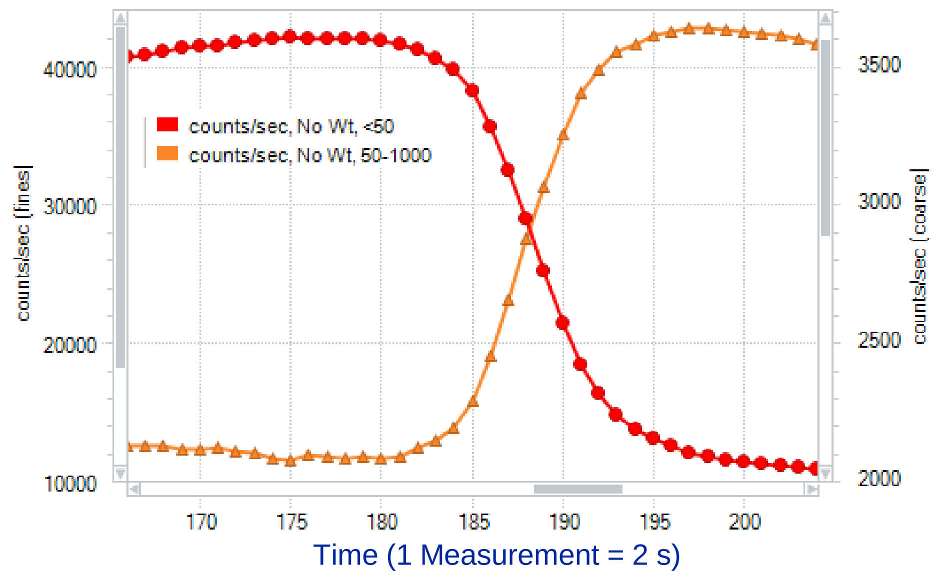


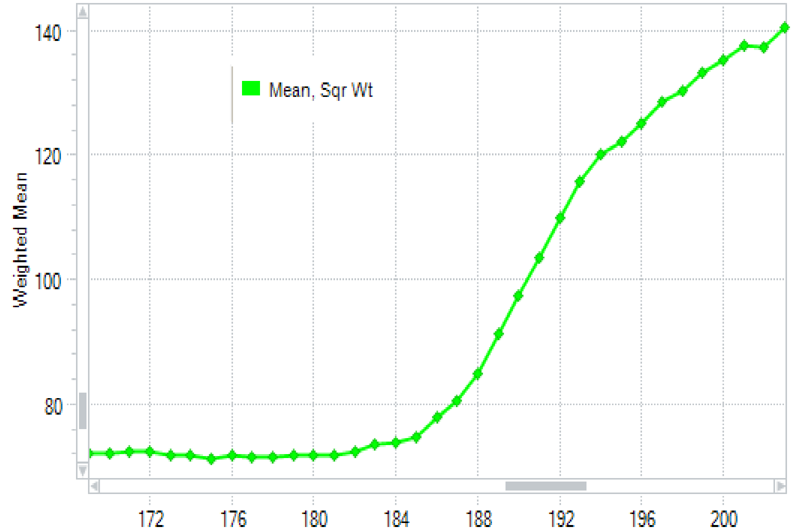


Thousands of Chord Lengths are measured each second to produce the FBRM® Chord Length Distribution :

Tracking real-time changes with precision







- 0.5µm to 3000µm in a single unit without altering the system
- Trended Statistics over time for specific user defined size ranges
- No calibration necessary to measure fine and coarse particles

Flocculation

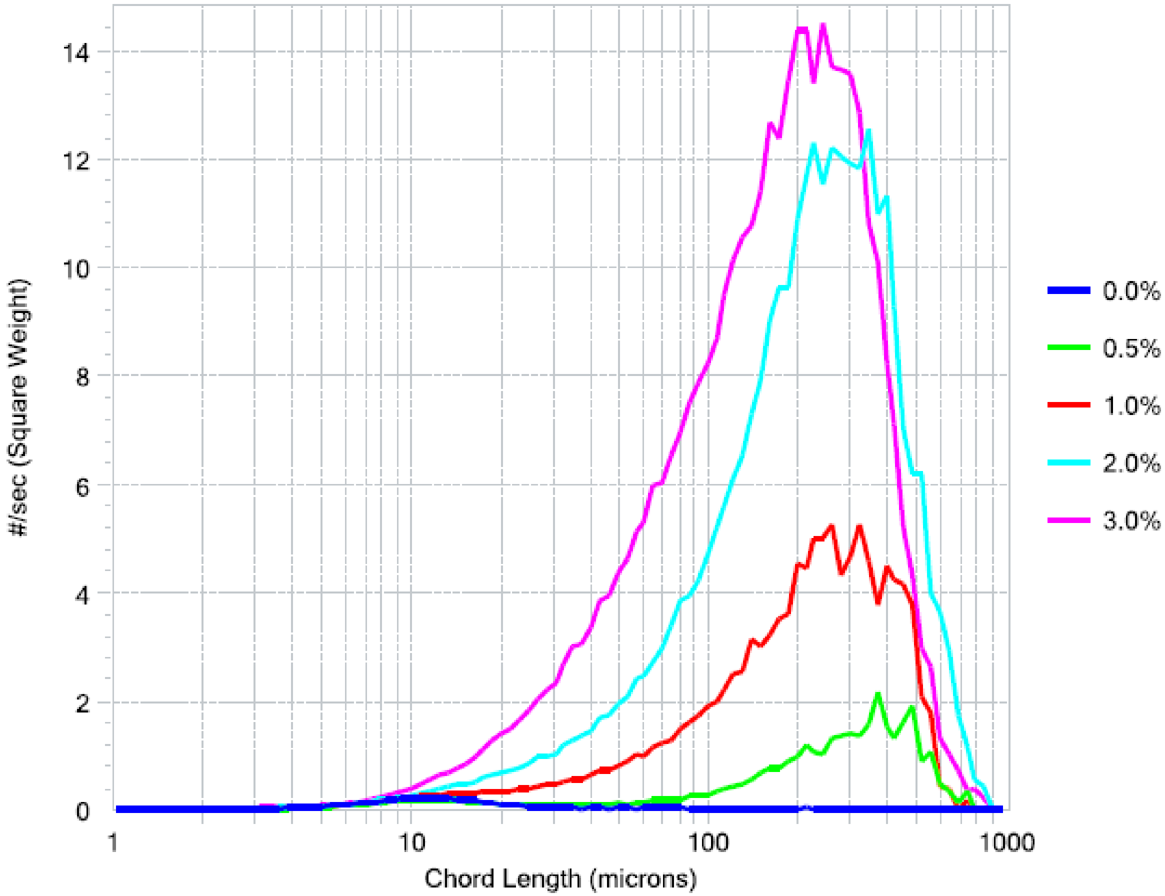
- Flocculation has been extensively used for the clarification of wastewater, chemicals and food
- Flocculants aggregate fine particles, settling them from the liquid phase and reducing the turbidity of the solution
- Flocculation is becoming more widely used for downstream processing of bioprocesses
  - Reduce filtration amounts
  - followed by centrifugation, depth filtration and absolute filtration
- Examples of flocculating agents reported

chitosan	diallyl dimethyl ammonium chloride (DADMAC)
acrylamide acrylate copolymers	quaternized polyamines

- For bioprocesses the flocculating agent should be:
  - non-mammalian (reduce the risk of prion contamination in therapeutic applications)
  - non-toxic and low in heavy metals
  - inexpensive

Flocculation

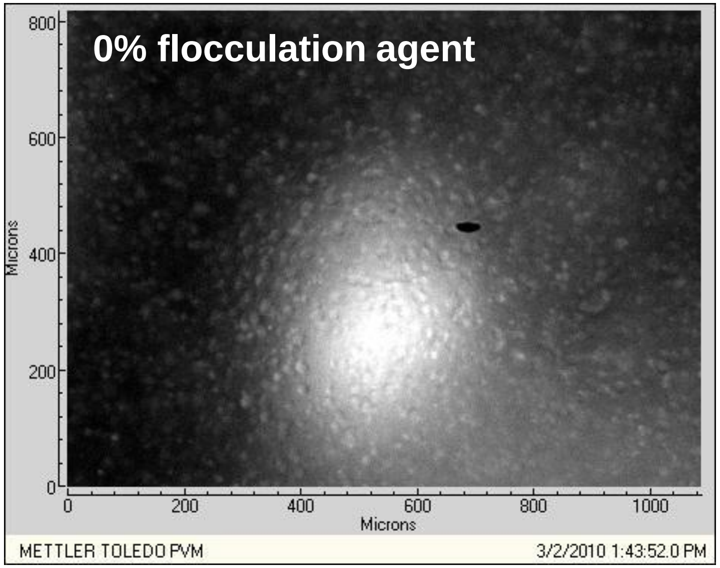
- Objective
  - Understand the effect of flocculation agent concentration

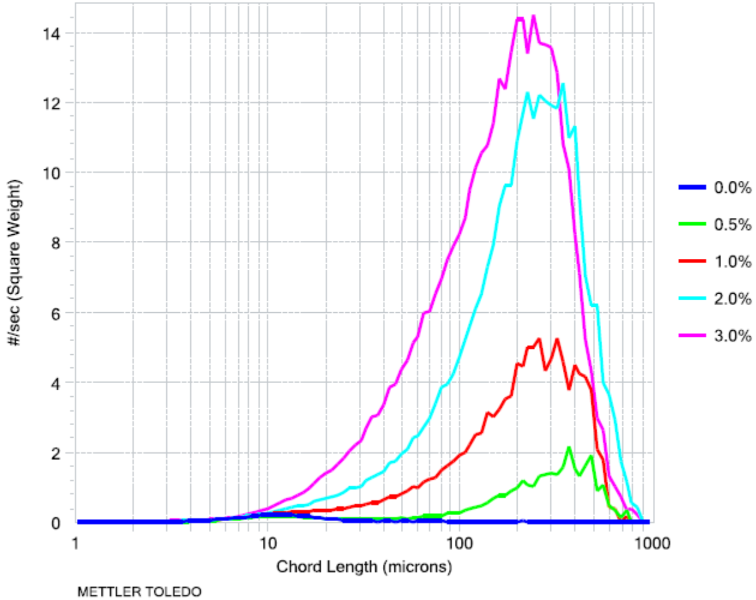


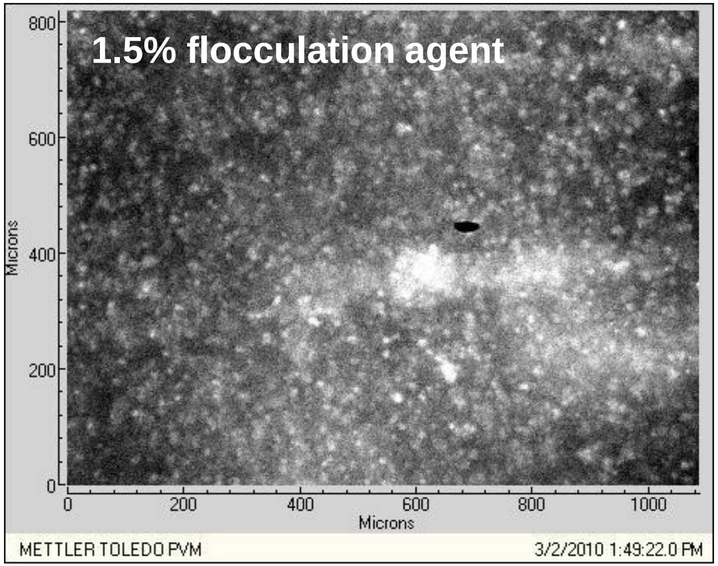
- Effect of concentration of flocculation agent – FBRM data
  - At 3%, the distribution starts to shift to the left due to settling, or maybe chord splitting.
  - FBRM tracks the degree of change at each concentration.

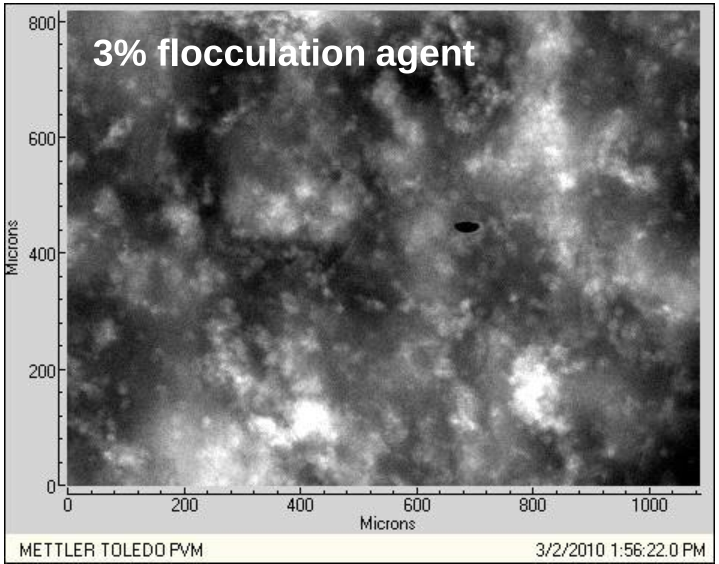
Flocculation

- Effect of concentration of flocculation agent – PVM images



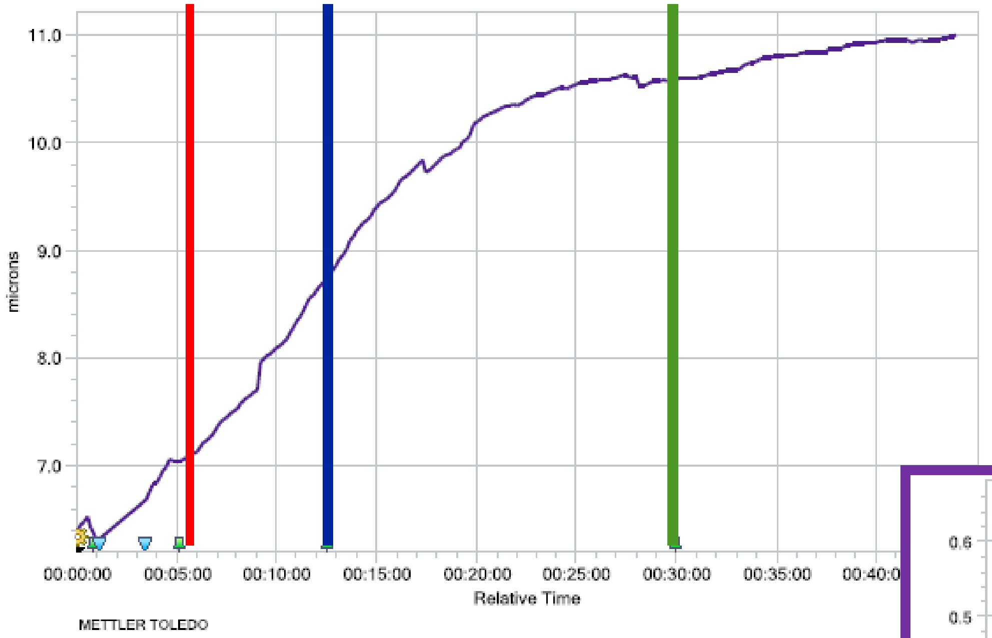




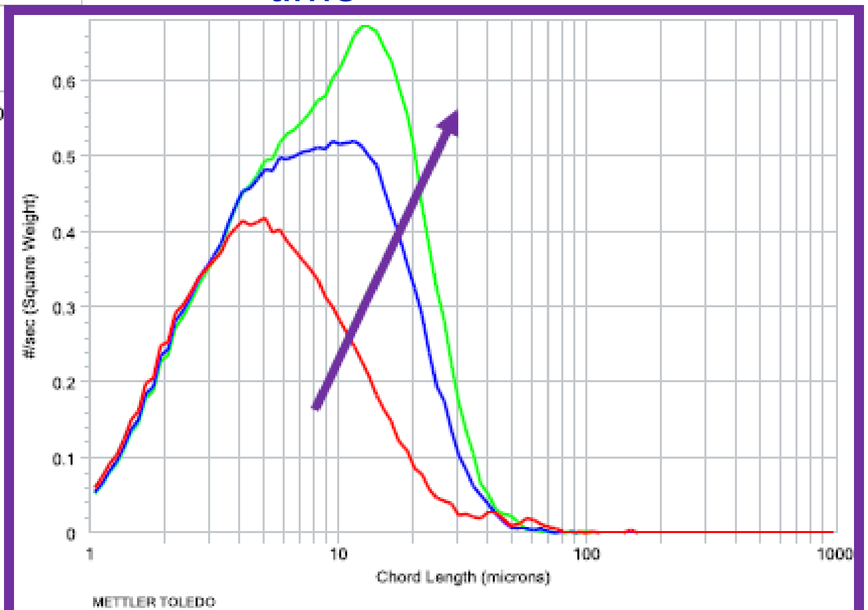


Flocculation

- Objective - tracking changes in solids concentration over time



- Centrate nucleation data
  - [Left] Solids increase significantly in size in the first 30 minutes.
  - [Below] Cord length distribution shifts to right over time



- FBRM can be used to optimize aging time to increase process efficiency

Flocculation

Conclusions

- Optimization of flocculant dosage can be achieved by using FBRM® *in situ* to track the difference in end-point, and hence ensure proper particle distribution.
- FBRM tracks the rate of flocculation to optimize aging time to ensure process efficiency
- Process development studies to determine the optimal flocculating agent and concentration
- Improve product recovery and reduce post-fermentation steps
- *In situ* monitoring allows for process control

Protein Crystallization

Protein crystals for the delivery of biopharmaceuticals

Basu, S. K. Govardhan, C. P. Jung, C. W.; Margolin, A. L.

Expert Opinion on Biological Therapy 2004, 4, 301-17.

- Advantages of crystalline proteins
  - higher bioavailability
  - increased ease of handling
  - improved stability
  - reduced physical and chemical degradation, and hence maintaining the protein's biological integrity during processing and storing
  - increased protection against proteolytic enzymes
  - may allow sustained release of the therapeutic agent, reducing the frequency of doses

Protein Crystallization

- Tracking protein crystallization process *in situ* and in real-time

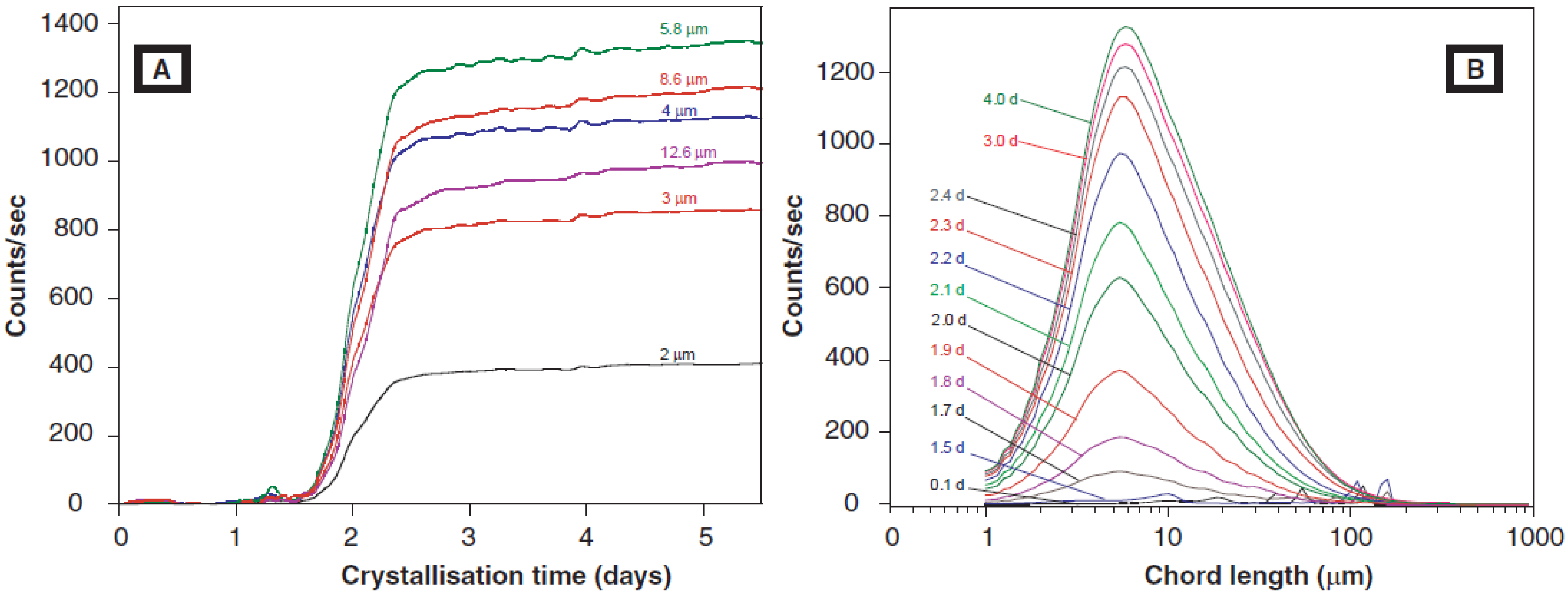


Figure 2. Close monitoring of protein crystallisation process *in situ*. A. Production crystallisation run of subtilisin Carlsberg (9 kg from 900 litre solution) followed by Lasentec FBRM® instrument. **B.** Crystal size distribution plots of the same manufacturing run in **A.** Increase in crystal concentration was readily apparent after ~ 0.5 days after tank equilibration. The final crystal size distribution (5.8 µm average chord length, lognormal distribution) is achieved in ~ 1 day after start of the process.

FBRM: Focused beam reflectance measurement.

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Protein Crystallization

Conclusions

- *In situ* crystallization process monitoring increased the efficiency of the process.
- Continued process monitoring provided insight into crystal breakage due to excessive agitation (data not presented).
- Improved batch-to-batch consistency in the manufacture of crystallized proteins.

Summary

- PAT tools are available for bioprocess monitoring
  - real-time monitoring and information to make decisions
  - improve batch-to-batch reproducibility, and increase product recovery

- Eliminates the need for sampling and sample preparation for offline analyses, which can be time consuming.

Other interesting published applications

- *In situ* real-time biomass monitoring
  - Monitor changes in biomass
  - Detect onset of apoptosis
- *In situ* real-time monitoring of product of bioprocess
- Drug therapy delivery systems
  - monitor breakdown of nanoparticle polymer and subsequent release of drug protein
- Biocalorimetry
  - Heat of fermentation as an indicator of biomass
- Formulations

Acknowledgements

- Biomanufacturing Training & Education Center
  - Bill Welsh

- Mettler Toledo AutoChem
  - Dave Johnson
  - Brian Wittkamp
  - Terry Redman
  - Benjamin Smith

Thank you for your attention.

Questions and Answers

- For further information on products and applications:
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