

WSC 7

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Russia

In-line Monitoring of Yeast Fermentation with 2D-Fluorescence Probe

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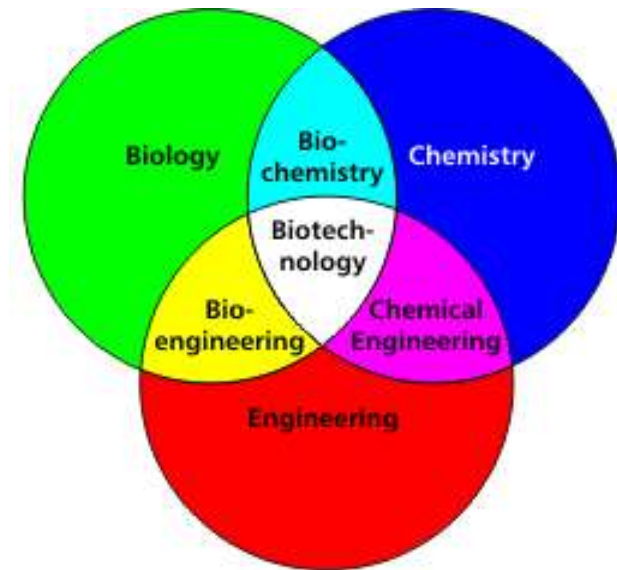
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- Process monitoring and control in biotechnology
 - current state
 - problems and trends
 - fluorescence and 2D-fluorescence applications
- Yeast fermentation process monitoring
 - new 2D-fluorescence probe
 - new approaches to 2D-fluorescence data analysis
- Conclusions

- Biotechnology is...
 - *“any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use”*
- A few examples
 - ancient biotechnologies
 - *beer, wine, lactic fermentation*
 - drug production (“red” biotechnology)
 - *antibiotics, insulin etc.*
 - industrial applications (“white”)
 - *wastes treatment, biofuel production*
 - many other
 - *agricultural (“green”), aquatic (“blue”)*
- The significance of biotechnologies in the modern world is very high and is constantly growing



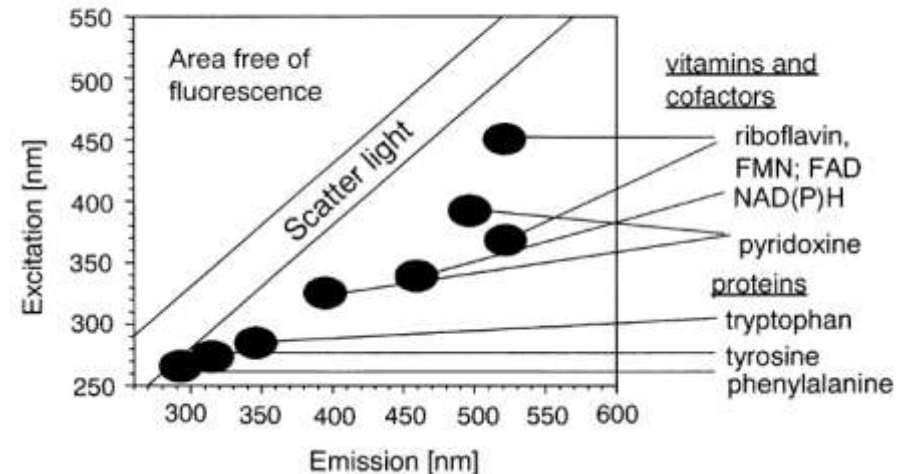
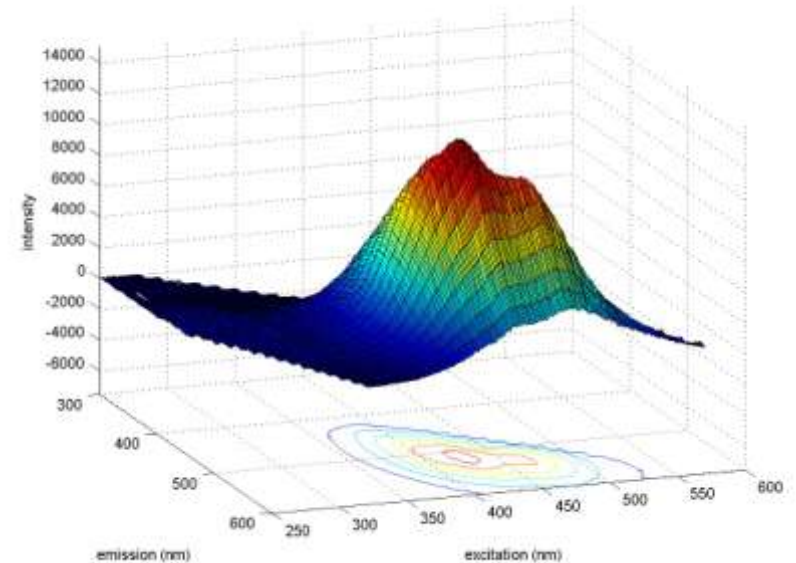
- Monitoring needs
 - medium composition
 - culture cell count and their viability/ metabolic state
 - process maturity (end point)
- Conventional methods
 - in-/ on-line sensors
 - t° , pH, $[O_2]$, $[CO_2]$, turbidity
 - off-line analysis
 - *product or substrate concentration, viscosity, cell count & viability, metabolic products*
 - => insufficient level of process control
- Up-to-date trends
 - development of in-line monitoring techniques
 - growing application optical (spectroscopic) sensors



- Fluorescence and 2D-fluorescence
 - very sensitive and selective
 - biogenic fluorophores ($10^{-5} - 10^{-6}$ M)
 - *proteins*
 - *vitamins*
 - **(!) intracellular (co-)enzymes**

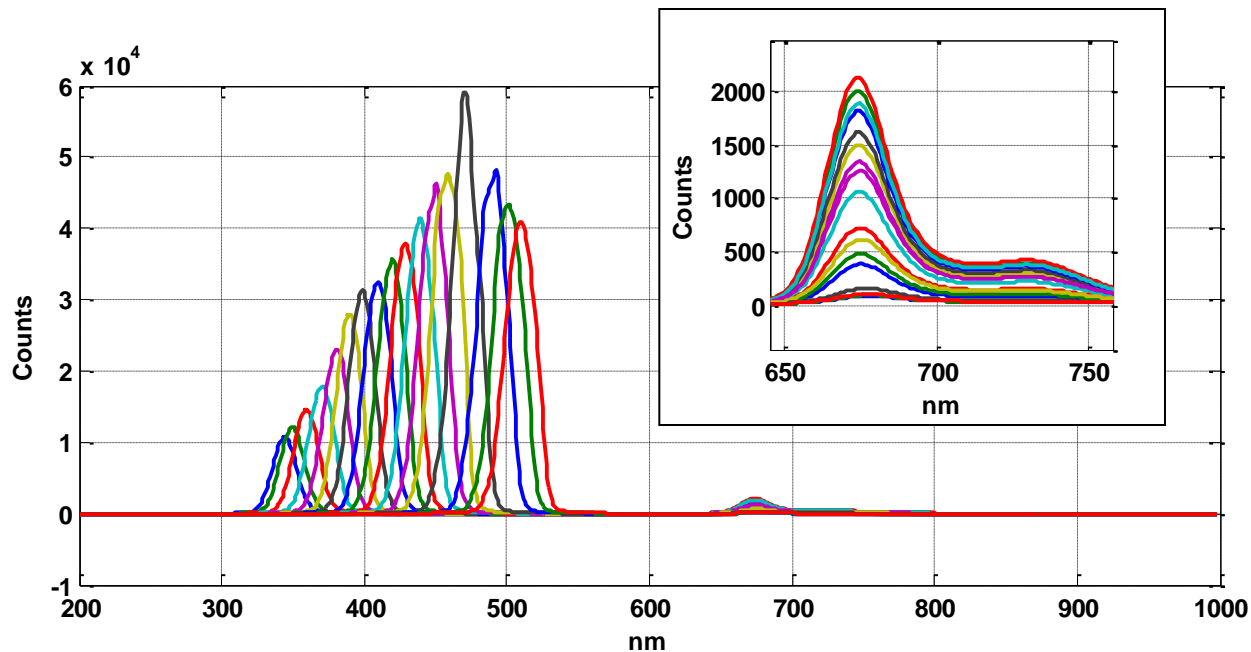
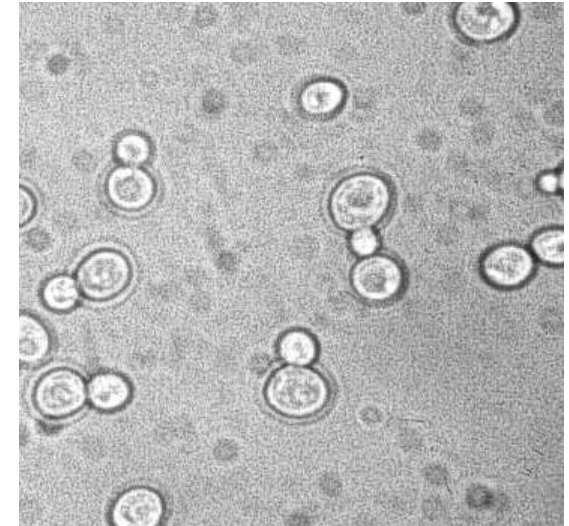
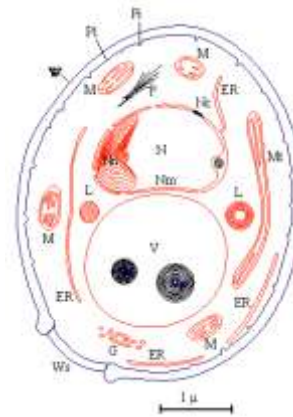
- Historical reference
 - first biological application in 1957 (Duysens)
 - splash of the research in 1980-90s
 - [still very few industrial implementations]
 - new wave of activity in last 5-10 years (chemometrics!)

- Problems and challenges
 - a lot of affecting (complicating) factors
 - *geometry, source instability, excitation and emitted light absorption and scattering, background fluorescence, cascade and filter effects, etc...*
 - complexity of metabolic processes
 - *difficult to interpret*
 - data analysis
 - *3-way methods*



cited from: Skibsted et al. *J. Biotech* **88** (2001) 47

- Research objectives
 - test monitoring of a well-known fermentation process (brewing)
 - *Saccharomyces cerevisiae*
 - with a new 2D-fluorescence probe
 - *Lighthouse Probe™* technology
 - try new approaches to 1D- and 2D-fluorescence data analysis



- Equipment

- 5L laboratory fermenter (Bioengineering)
- monochromator (J&M)/ diode laser 365nm
- CCD-detector (J&M)
- fluorescence probe (J&M)
- sensors

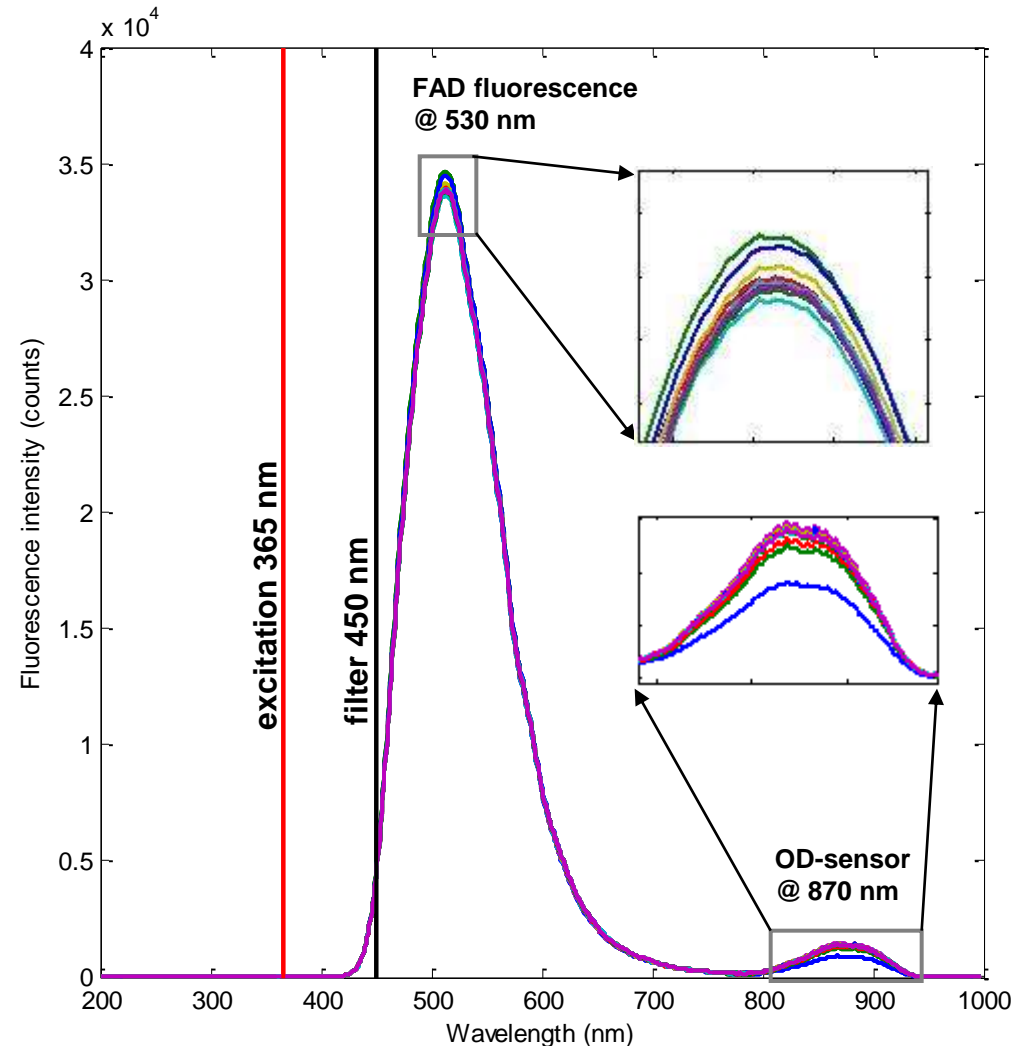


- Monitored parameters

- temperature (in-line)
- pH (in-line)
- pO2 (in-line)
- turbidity (in-line)
- Cell count (off-line)
- Glucose (off-line)

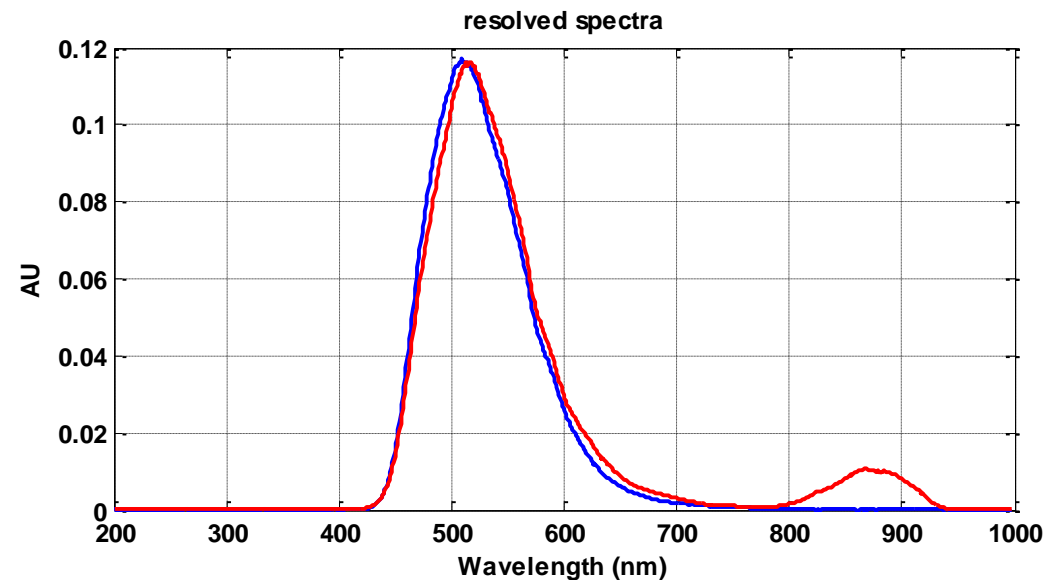
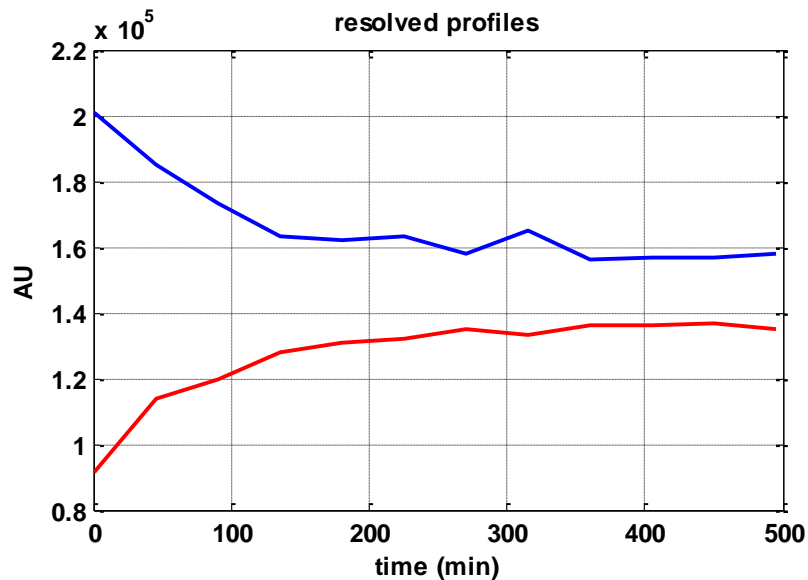
Use of additional data (1)

- Measurement
 - excitation with a diode laser 365 nm
 - filter at 450 nm (80%)
- Process
 - duration 500 min (aeration 300 min)
- Monitoring
 - in-line
 - *pH, O₂, turbidity (OD@870)*
 - off-line
 - *CC, glucose*
- Complications
 - strong background fluorescence
 - *yeast extract is present*
 - variable (unknown) excitation signal
 - variable turbidity
 - filter spectrum



- ALS MCR on full spectra
 - (does not work for the fluorescence spectra alone!)
 - non-negativity constraints
 - 2 components

- Interpretation of results
 - red: fluorescence that depends on the cell count
 - => *intra-cellular*
 - blue: does not depend on the biomass
 - => *extra-cellular biofluorophores, excitation, medium*
- **(!) Additional NIR-signal may help resolving subtle effects**



- Process profile correlation table
 - 3 profiles
 - *cell count (CC)*
 - *optical density (OD) sensor*
 - *MCR-resolved profile*
 - OD and MCR are well-correlated
 - => **MCR-profile has better correlation with CC, than OD (standard technique)**

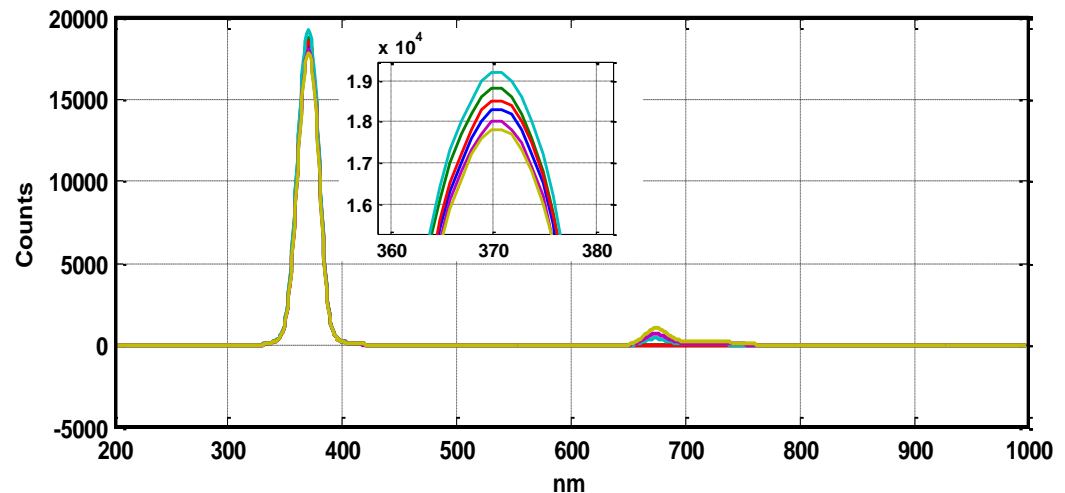
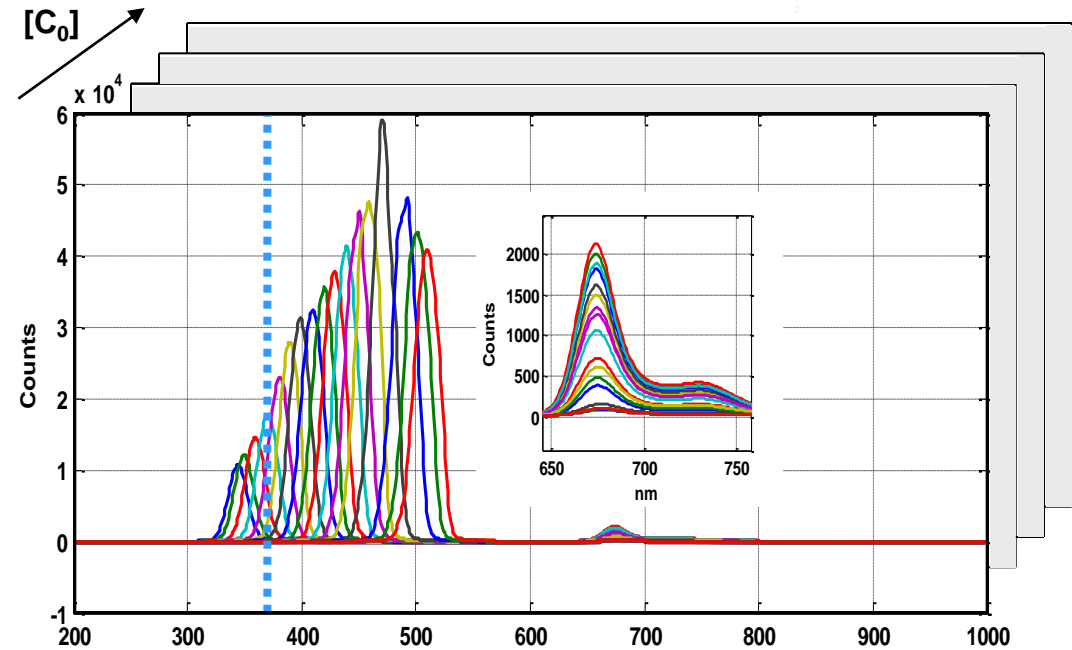
[r]	CC	OD	MCR
CC	1		
OD	0.880	1	
MCR	0.894	0.984	1

- PLS regression
 - X-matrix
 - *Fluorescence region (Fluor)*
 - *NIR-sensor signal region (NIR)*
 - *full spectrum (Full)*
 - y-vector
 - *cell count*
 - => **full spectrum produces the best prediction**

	Fluor	NIR	Full
RMSE/10 ⁷	1.25	1.34	1.03
R-square	0.906	0.891	0.936
LV	4	3	3

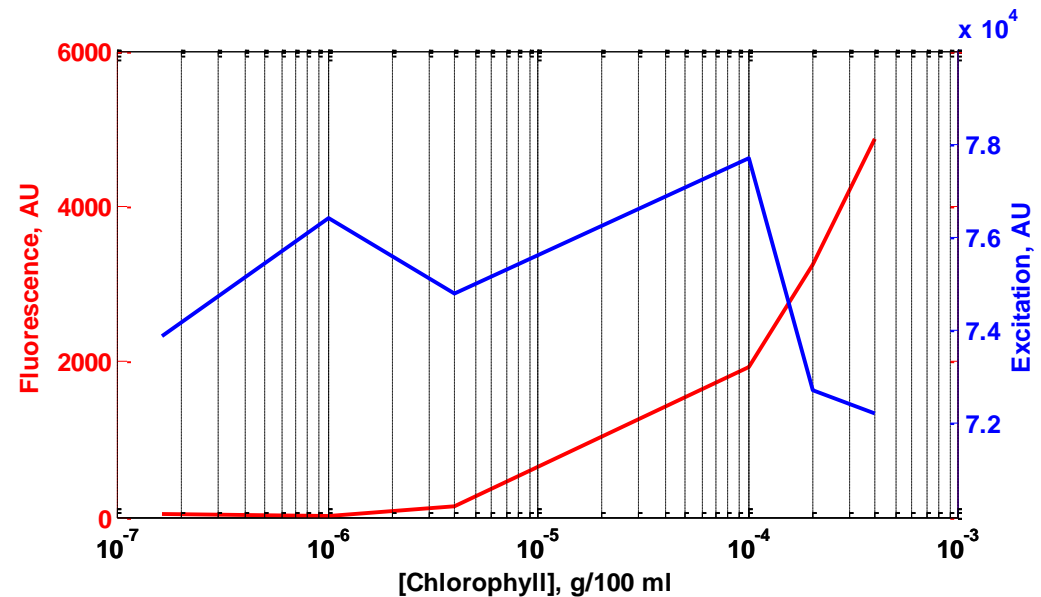
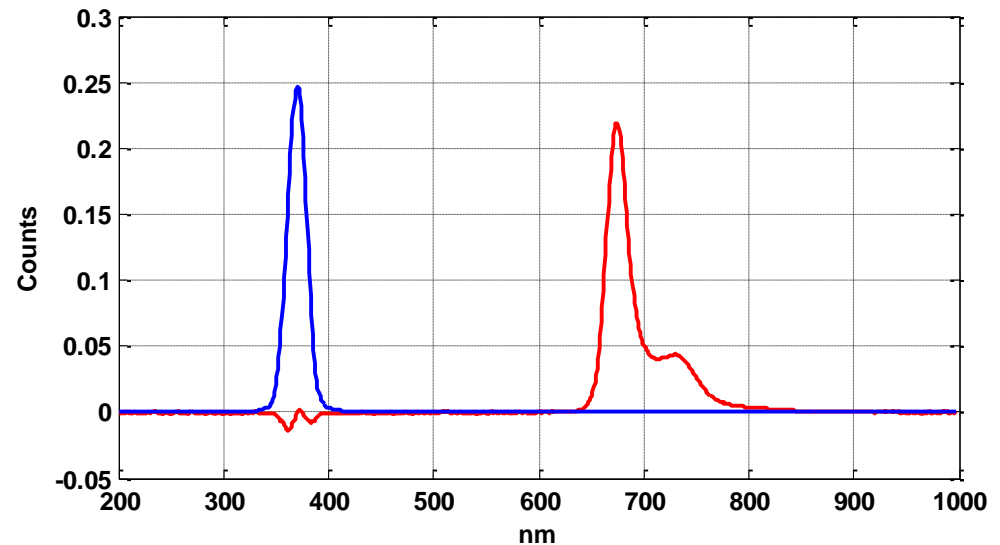
Use of excitation light (1)

- *Chlorophyll a* concentration series
 - 3-way process data emulation
- Excitation light
 - very intensive
 - is typically avoided
 - *filtered out during the measurement*
 - *cut off before the analysis*
- Can it be useful for the data analysis?
 - compensate for variations
 - *excitation light intensity*
 - *optical properties of the medium*
- Simplified data ----->
 - only excitation at 370 nm used
 - excitation peak is variable
 - *5-10% amplitude*



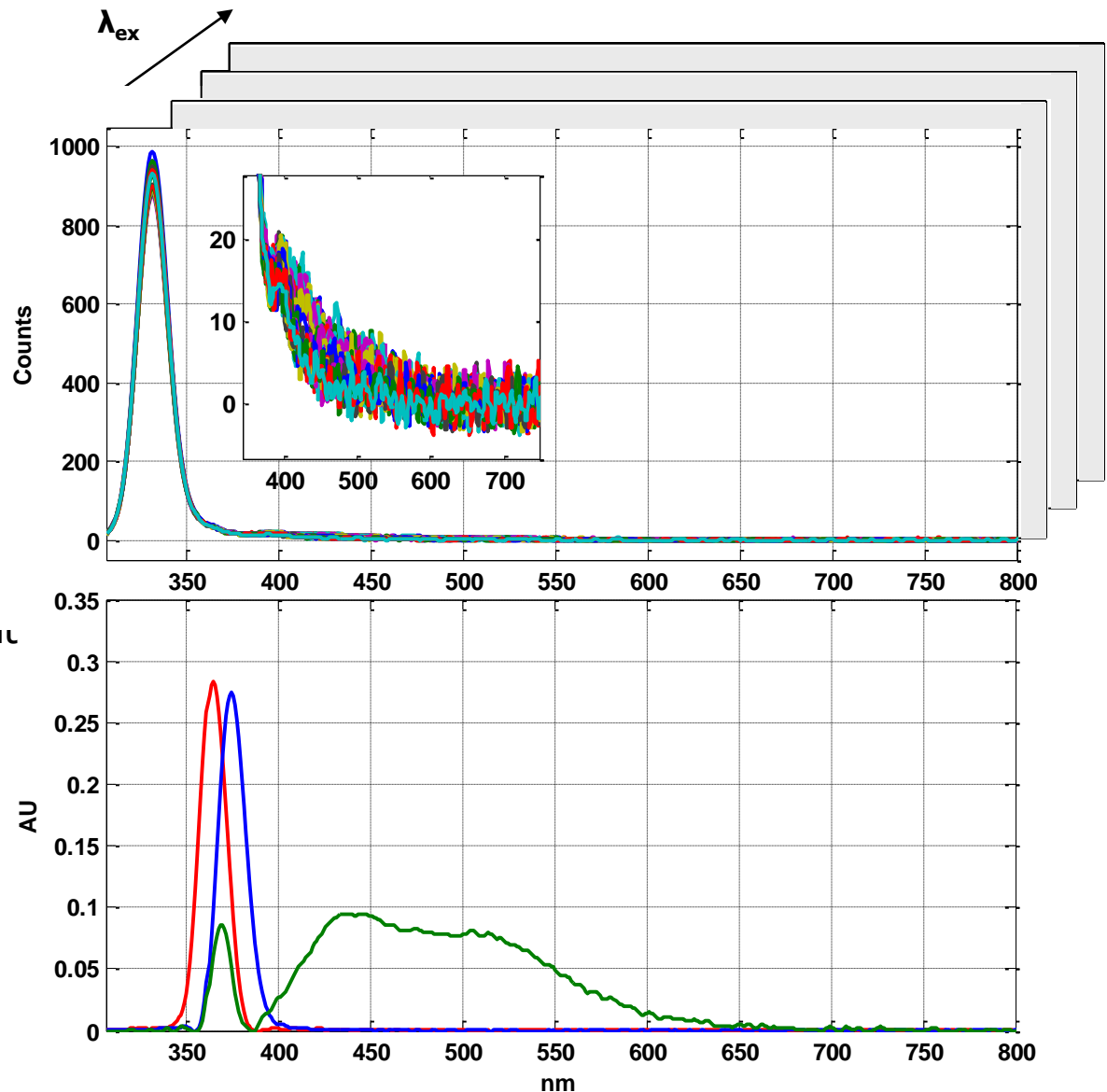
Use of excitation light (2)

- ALS MCR
 - on spectra excited @370 nm
 - without non-negativity constraints
 - 2 components
- Result
 - excitation and fluorescence signals are perfectly resolved
 - => **excitation light (and possibly medium) variability is separated**
- Correlation coefficients
 - $[C_0]$ with $I_{fl}(674nm)$
 - $r=0.972$
 - $[C_0]$ with $[C_{resolved}]$
 - $r=0.982$

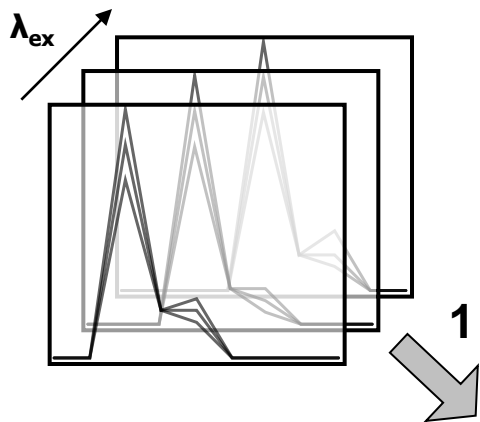


Use of data augmentation (1)

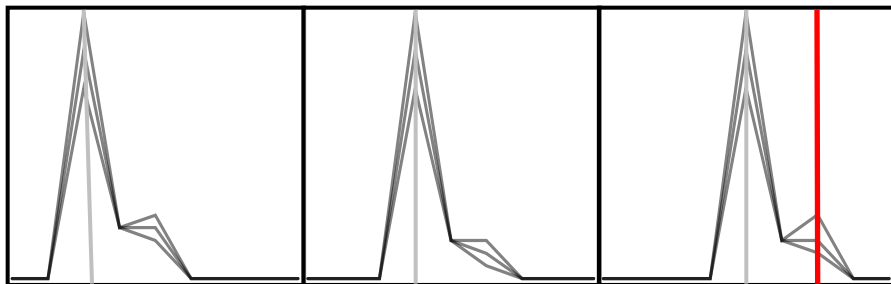
- Fermentation process
 - *S. cerevisiae*
 - 360 min
- 2D-fluorescence spectra
 - 832 x 45 x 39
 - excitation light is present
 - very weak fluorescence
 - overlap
- Data analysis
 - PARAFAC (!?)
 - neither resolution of data at a single excitation
 - correlation with the excitation signal
 - two unresolved peaks
 - How to get the process profile(s) and spectra of fluorophores?



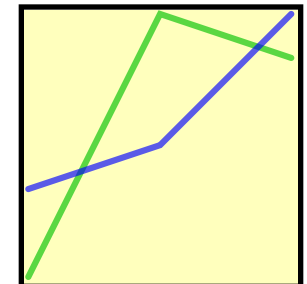
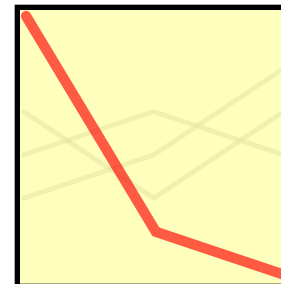
Use of data augmentation (2)



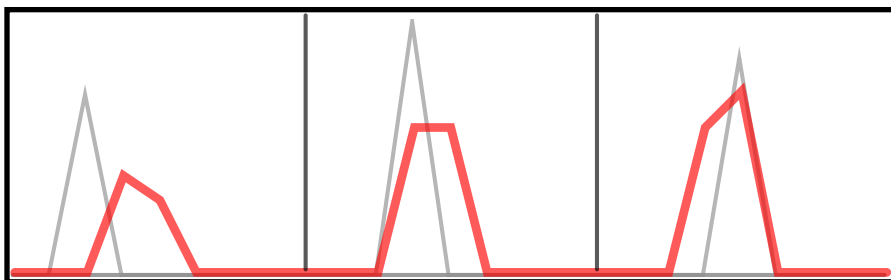
1. Augmentation with common time (selected ex. wavelengths)
2. Curve resolution (SIMPLISMA) on augmented data
 - => common **process profile(s)**
3. de-augmentation of resolved fluorescence spectra
4. MCR on de-augmented fluorescence profiles
 - => individual fluorophore **excitation and emission spectra**



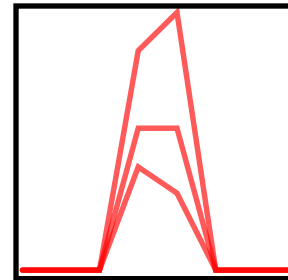
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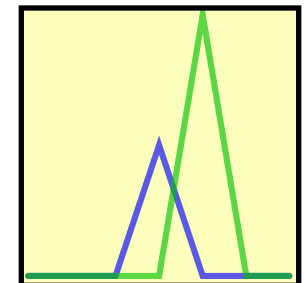
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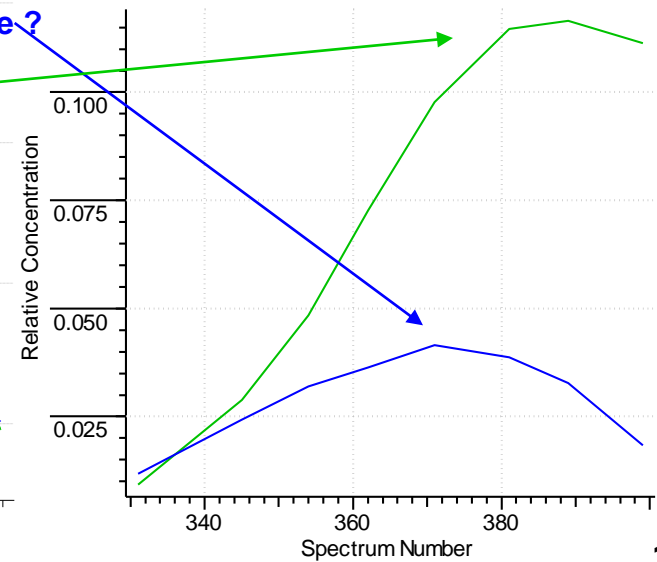
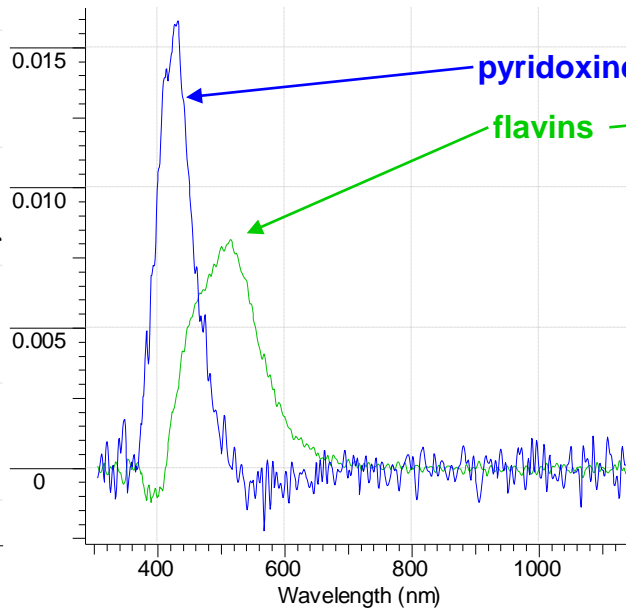
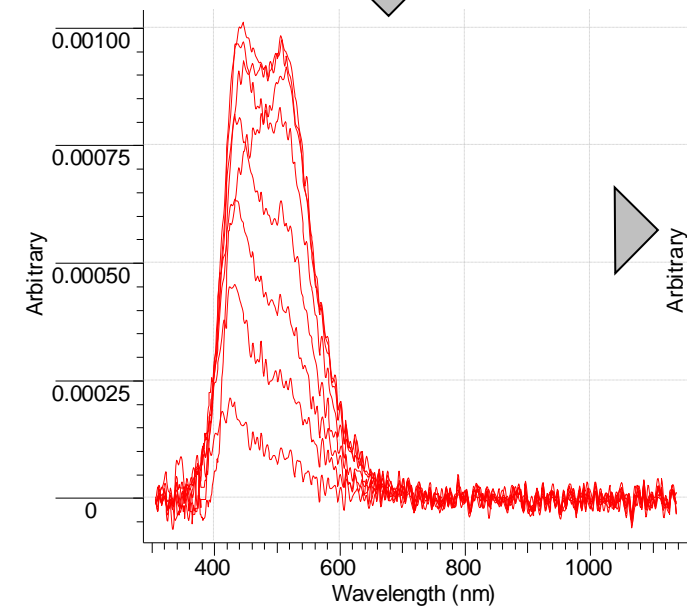
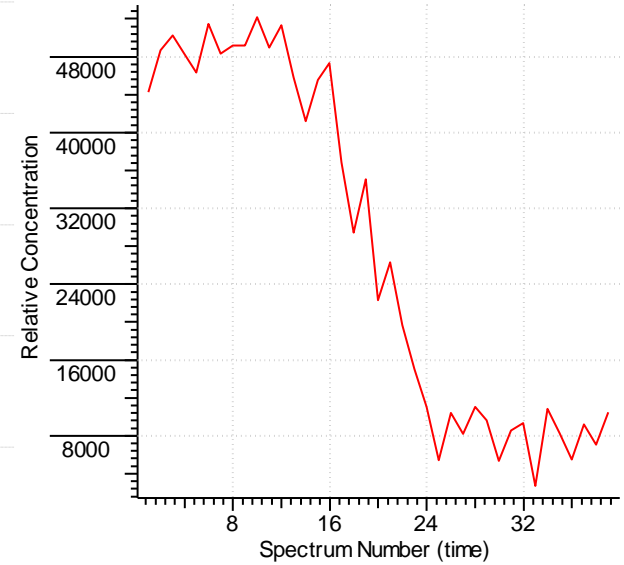
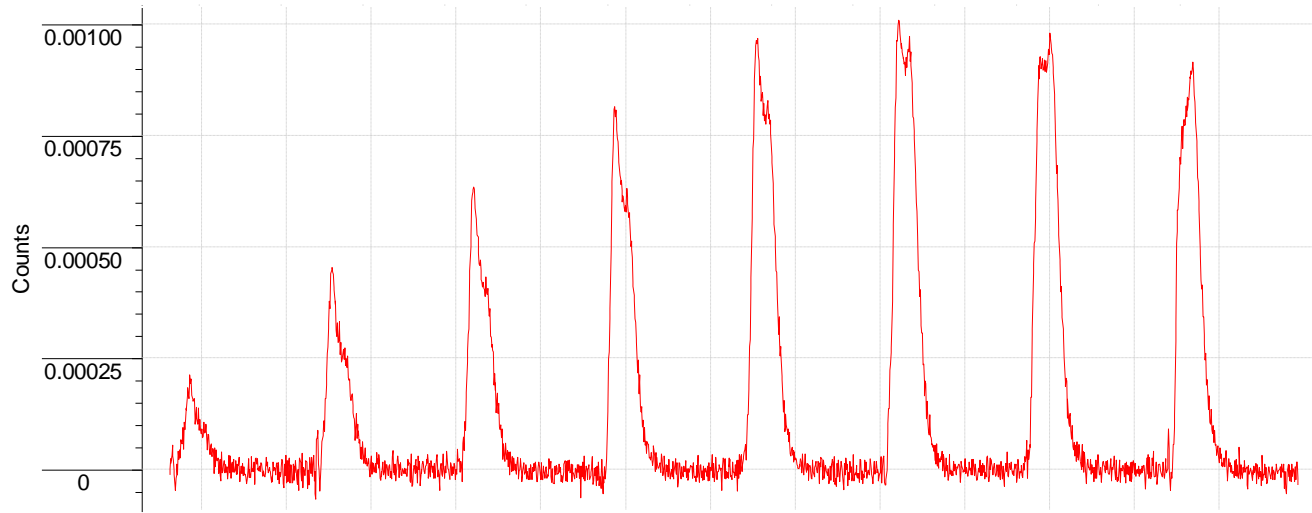


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Use of data augmentation (3)



- Fluorescence spectroscopy is an informative technique for the monitoring of biotechnological processes
 - high sensitivity and selectivity
 - intracellular biofluorophores (flavins, NADH, pyridoxin)
 - biomass growth, metabolic activity
- Lighthouse Probe
 - suitable for the acquisition of fluorescence spectra in the reflectance mode
 - further optimization is necessary to enhance the signal intensity in 2D-spectra
- New approaches to the acquisition and analysis of fluorescence data are suggested
 - simultaneous presence of NIR lighting (at ~ 800 nm) assists in data analysis
 - excitation light signal may be useful for the analysis
 - original algorithm for the resolution of process profile(s) as well as excitation-emission spectra of individual fluorophores has been suggested
 - resolved EE-spectra enabled the component identification

Acknowledgements



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