ZAFH – PHOTON^{n}

PHOTONische Verfahren in neuen Dimensionen

Hochschule Reutlingen
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Reutlingen Research Institute

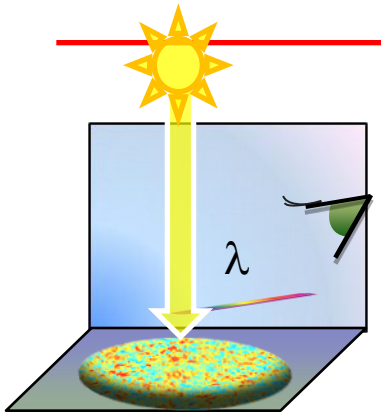
Multimodale Spektroskopie und Multivariate Datenanalyse

2. Aalener Photoniktag

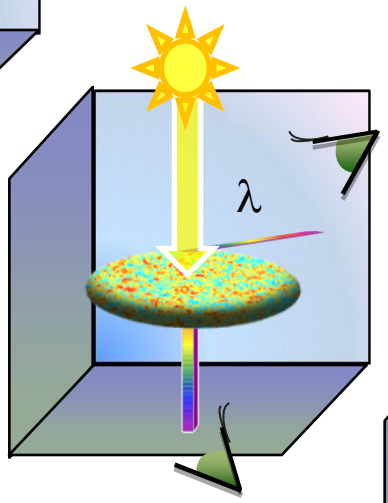
Agenda

- Toolbox Spektroskopie und Chemometrie
- Beispiel Farbstoffgemisch und Kristallisation
- Beispiele aus der Biotechnologie
- Beispiel spektrales Imaging
- Photonendiffusion und
  Multipoint Spektroskopie

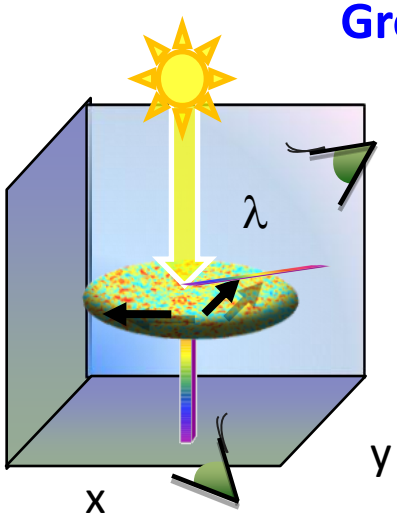
Toolbox: Optische Spektroskopie



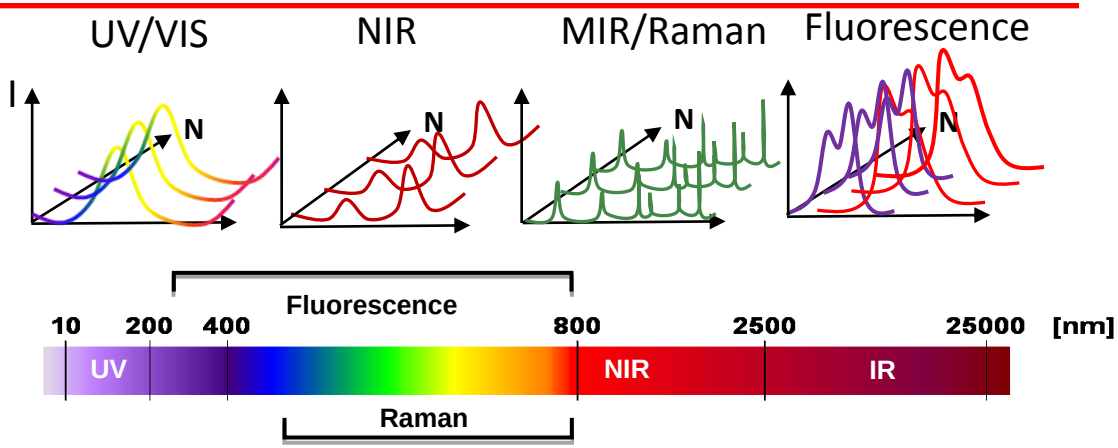
Reflexion



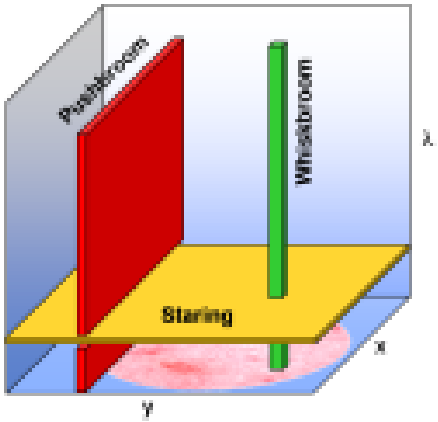
Transmission



Ortsaufgelöst (x,y)



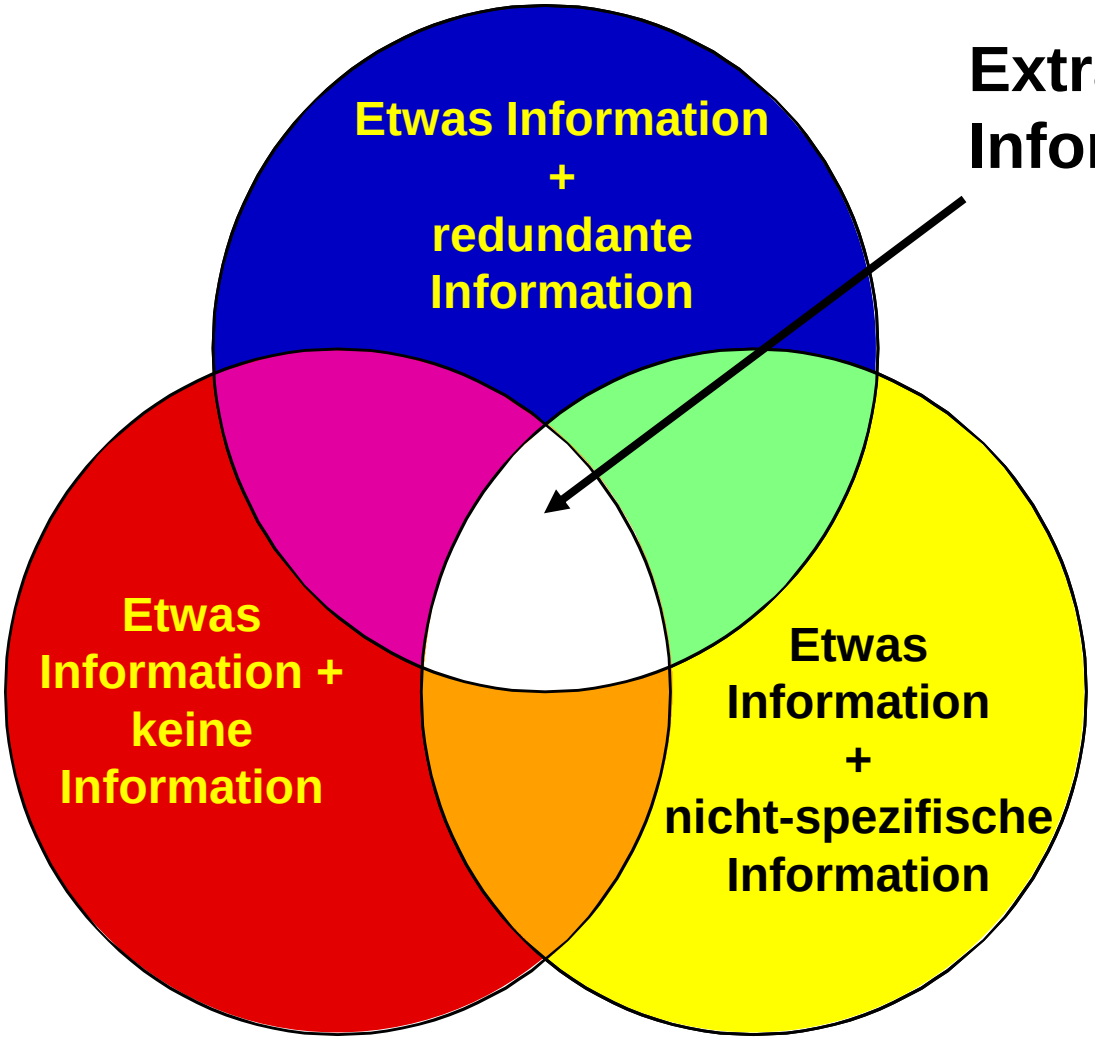
Großer Spektralbereich



Hyperspectral imaging

unterschiedliche Konfiguration

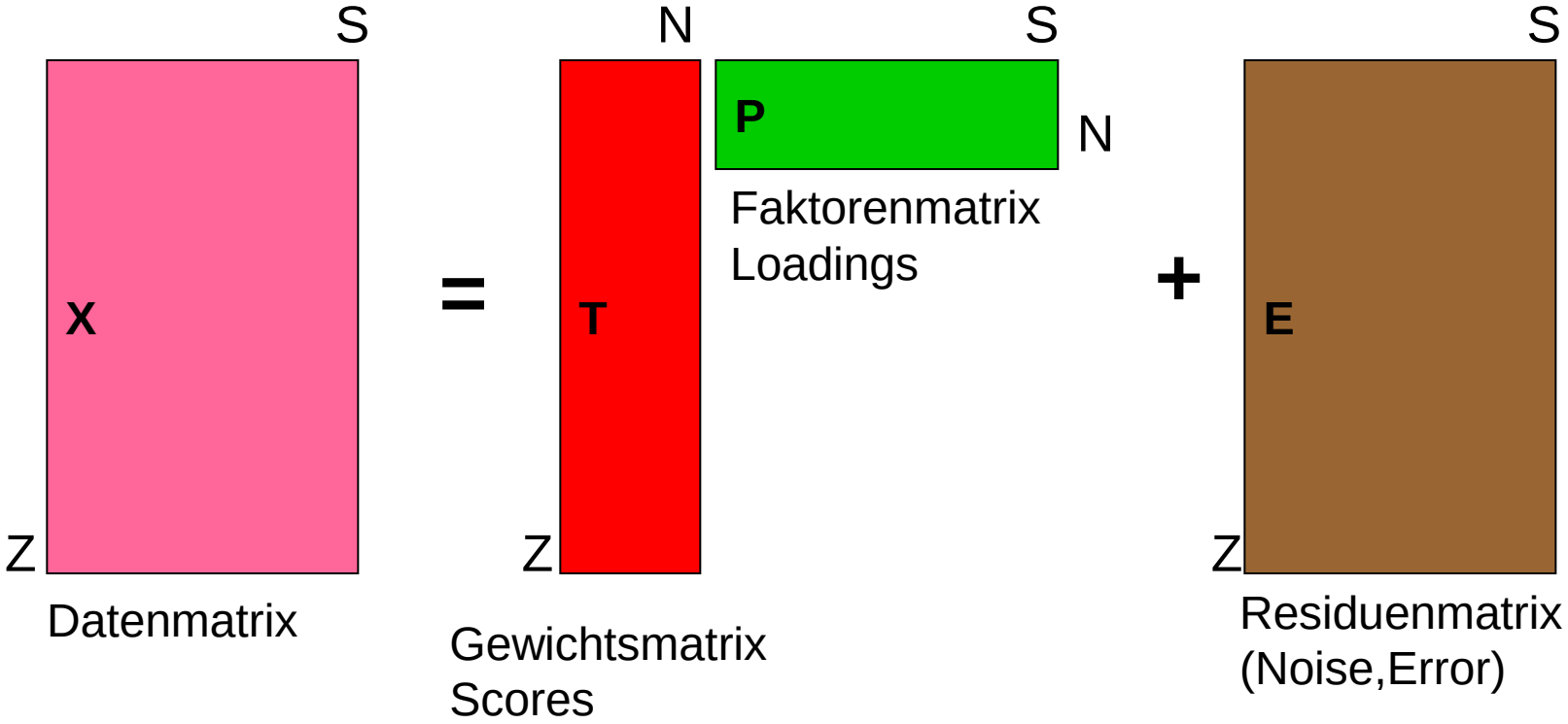
Multivariate Datenanalyse



Extrahiere die nützliche Information

- Univariate Daten Analyse
- Explorative multivariate Daten Analyse (PCA, etc.)
- Multivariate Regression und Klassifizierung (PLS, MCR, MIA, RBF, Kohonen……..)
- Optische Hauptkomponenten-analyse

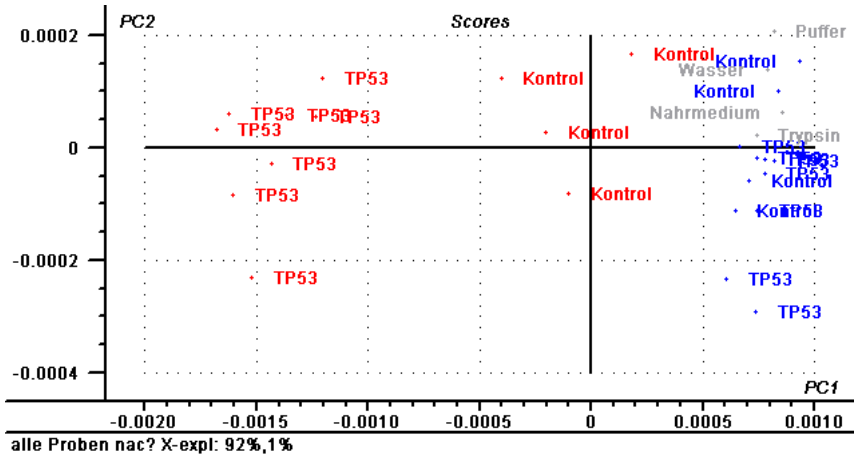
Prinzip der PCA



Besipiel: Gliobastomzellen (ATR-FTIR)

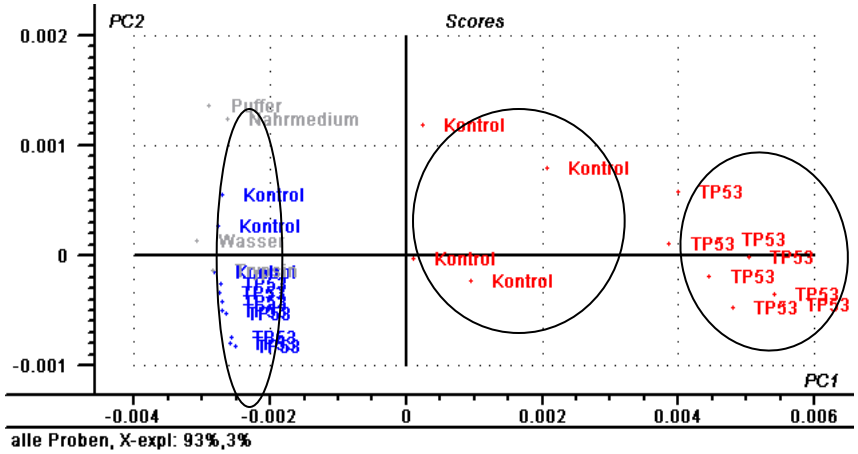
3000 - 2800 cm^{-1}

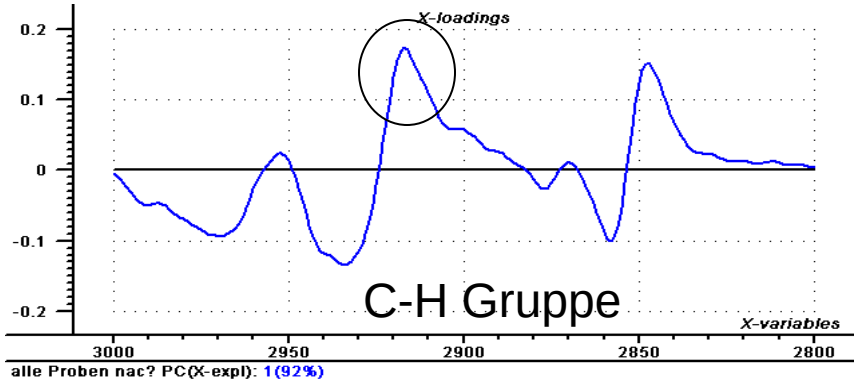
1. Ableitung mit 5 Glättungspunkten

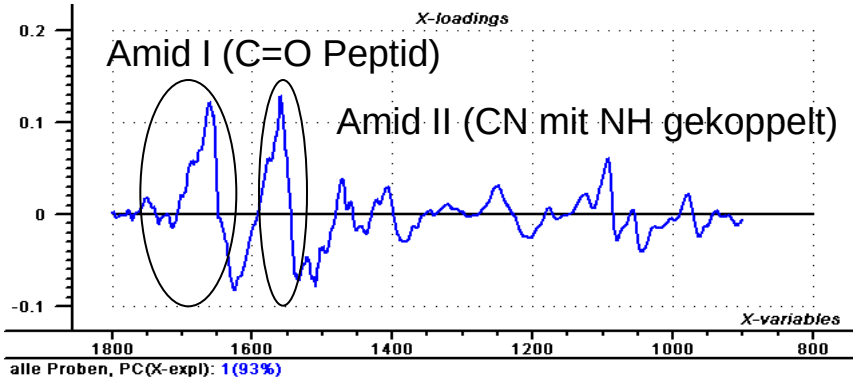


1800 - 900 cm^{-1}

1. Ableitung mit 5 Glättungspunkten







Multivariate Curve Resolution: MCR: Integration von Wissen

<u>Ziel</u>: Herausrechnen der Reinkomponenten

Im Prinzip möglich über PCA oder PLS
aber:
Reiner mathematischer Ansatz
Referenzwerte und Kalibrierung nötig

Kalibrierungsfreie Lösung:

Multivariate Curve Resolution (MCR) - ALS
Mathematischer Ansatz + physikalische und chemische Nebenbedingungen

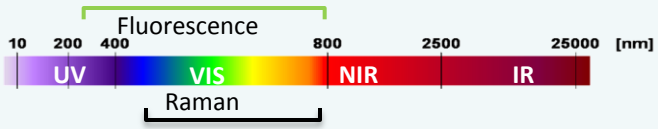
"Natürliche" Nebenbedingungen für die MCR

- **Nichtnegativität**
  - Spektren und/oder Konzentrationsprofile müssen positiv sein
- **Unimodalität**
  - einige Konzentrationsprofile oder Spektren haben nur ein Maximum
- **Selektivität**
  - es ist bekannt, welche der Komponenten zu einem bestimmten Zeitpunkt vorhanden ist. (Reaktant, Produkt)
- **Geschlossenheit (Massenkonstanz)**
  - die Summe der Konzentrationen aller beteiligten Komponenten ist eine bekannte Konstante (z.B. die Massenbilanzgleichung bei bekannten Reaktionen)

Multi – Modal – Spatially – Resolved - Spectroscopy

Multi-Information Spectroscopy

A ⇒ λ



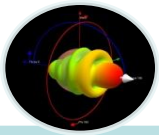
B ⇒ A + Setup geometry

C ⇒ A + B + 2D-scan

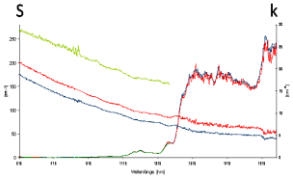
Reutlingen Research Institute (RRI) - Process Analysis Technolo
-

Direct linkage

- Kubelka-Munk K + S
- Radiative Transfer μ_a + μ_s + g
- (Maxwell Equations)

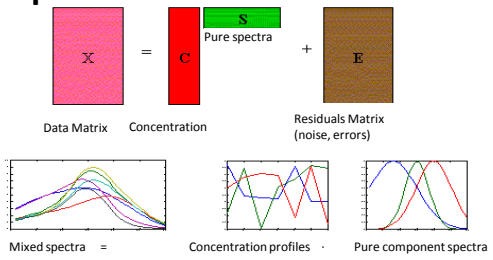


Science Based Analysis



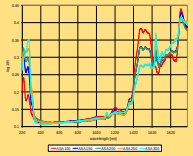
- Theory
- Models
- Approximations

Reduced Number of calibration Samples



Multivariate Curve Resolution (MCR)

Raw Spectra

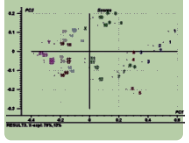


Data Pretreatment

- Derivatives
- Normalizations
- SNV, EMSC...

Data Analysis

PCA, PCR, PLS, SIMCA



Chemometrics / Multivariate Data Analysis

Pure Statistics?!

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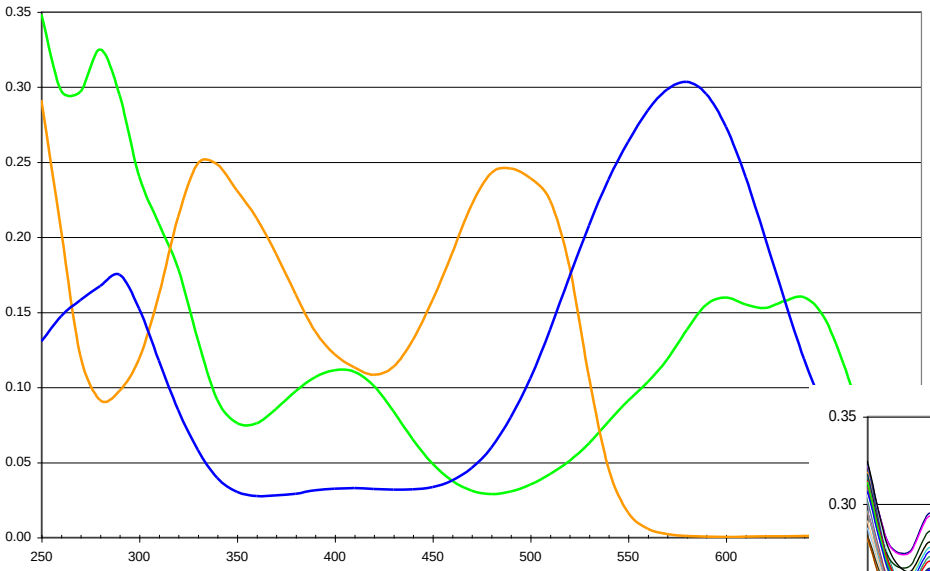
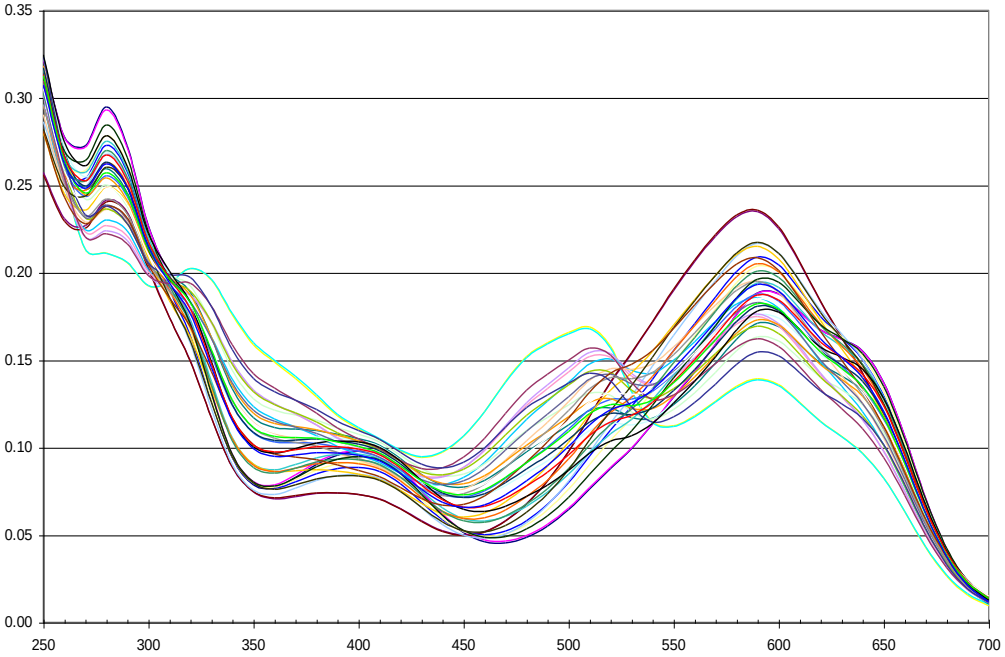
Beispiel: Mischung aus 3 Einzelfarben

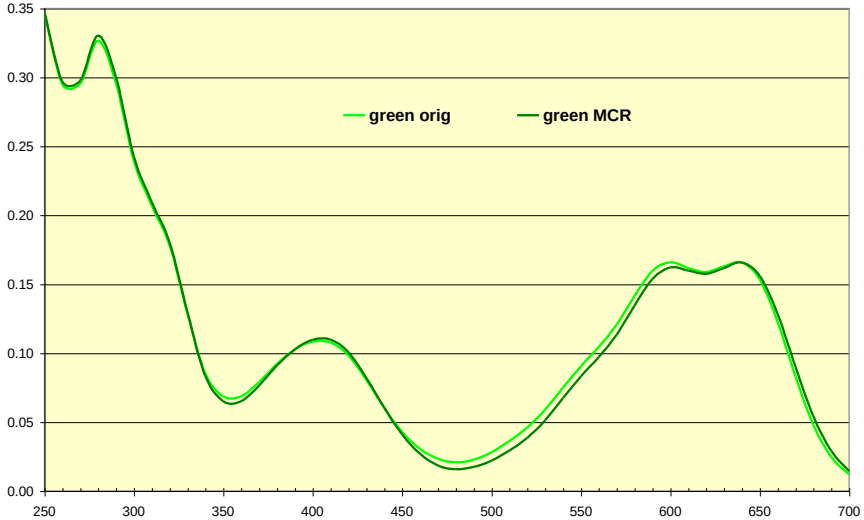


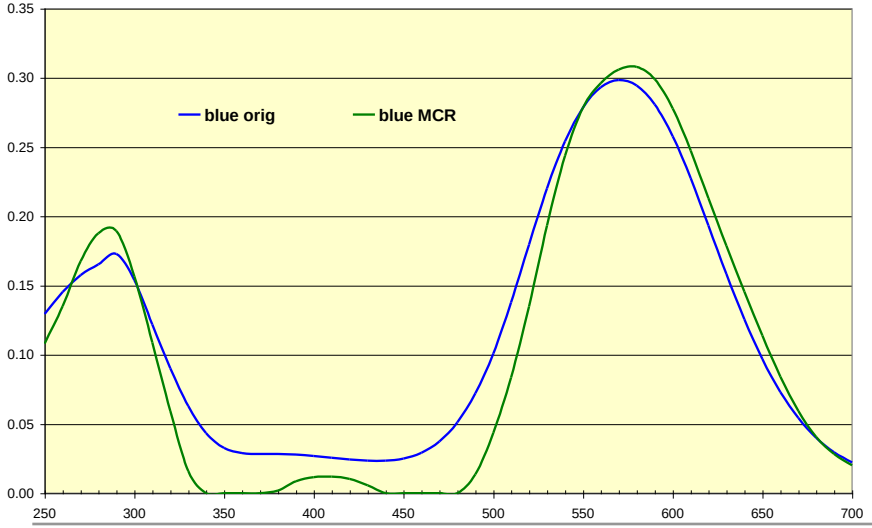
UVVIS-Spektren von 3 Farbstoffen (grün, orange, blau)



Mischungen unterschiedlicher Konzentrationen

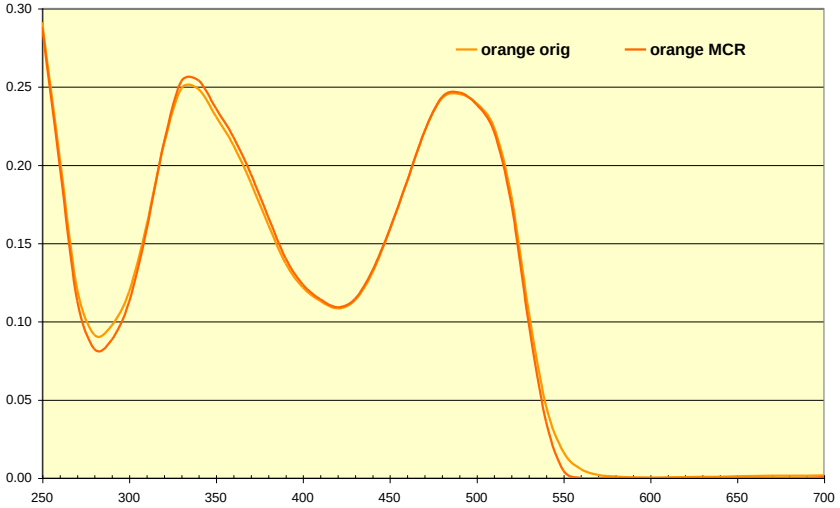
Extraktion der Reinspektren mit MCR





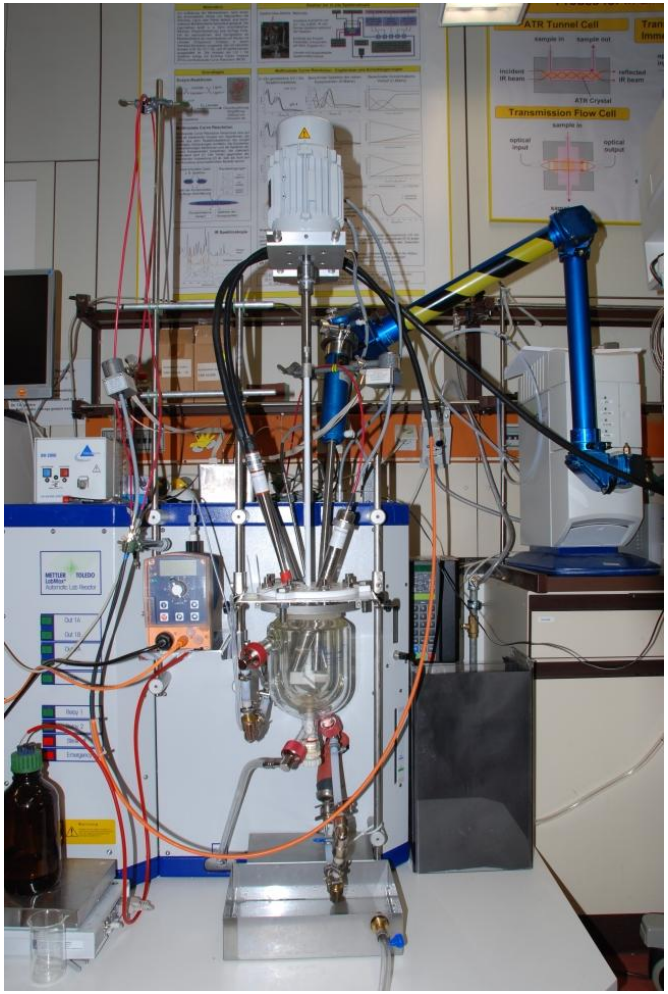
Eingabe: Mischungsspektren

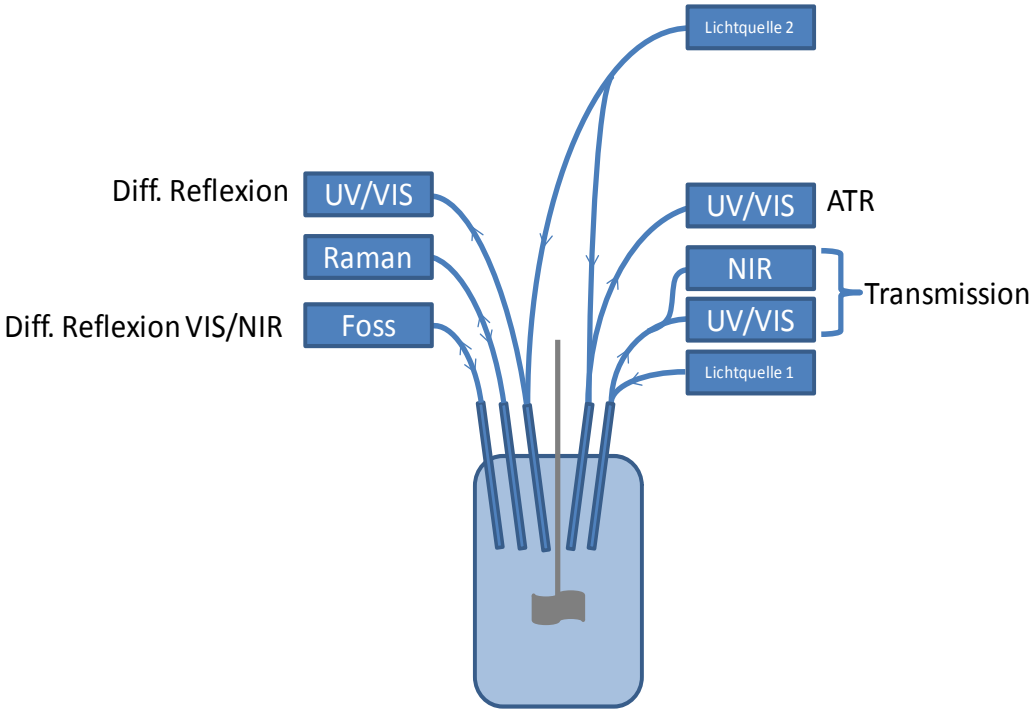
Nebenbedingung: Nichtnegativität



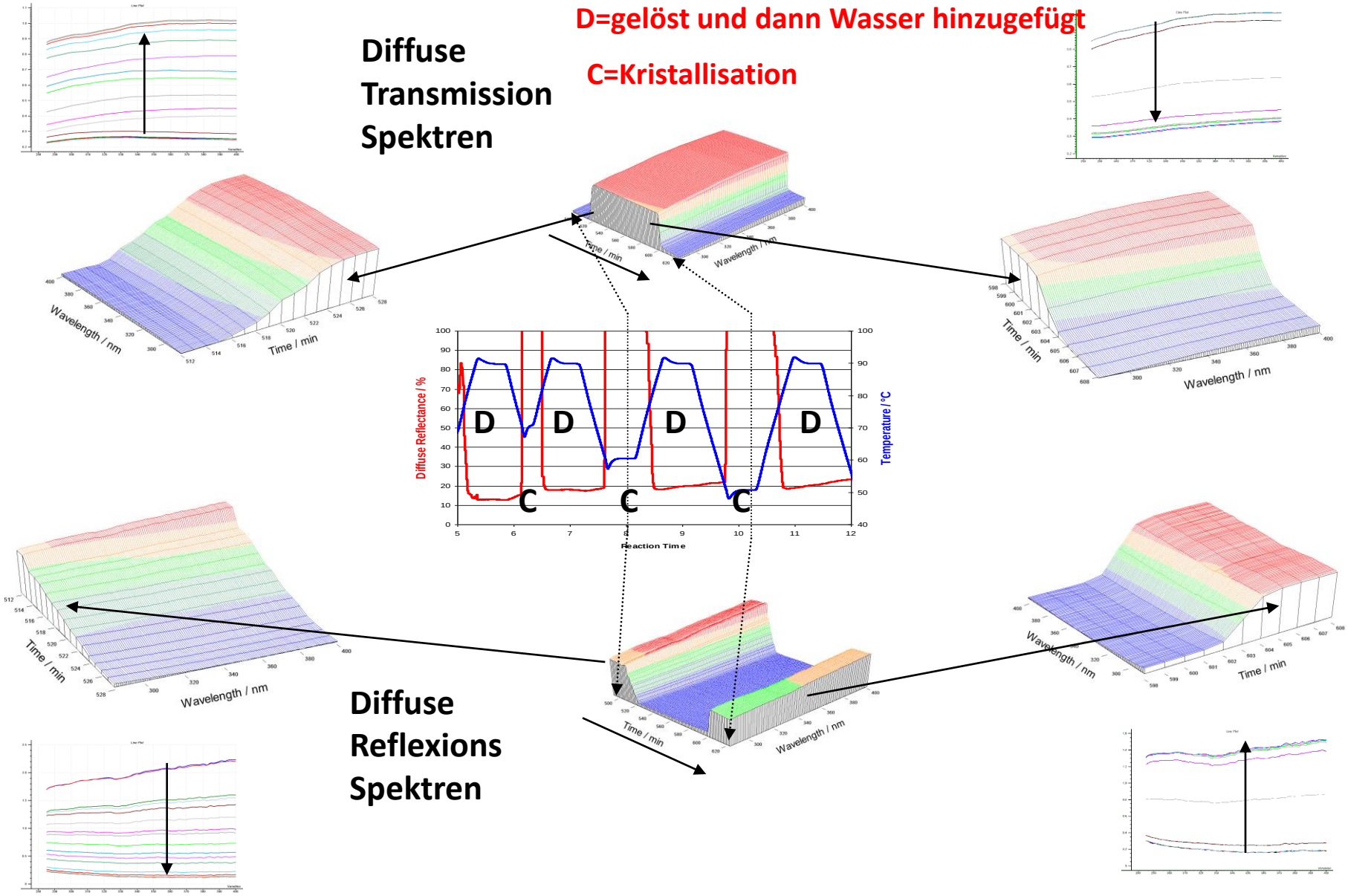
Ergebnis:
Spektren der Reinkomponenten
und zugehörige Konzentrationen

Beispiel Kristallisation: Messaufbau

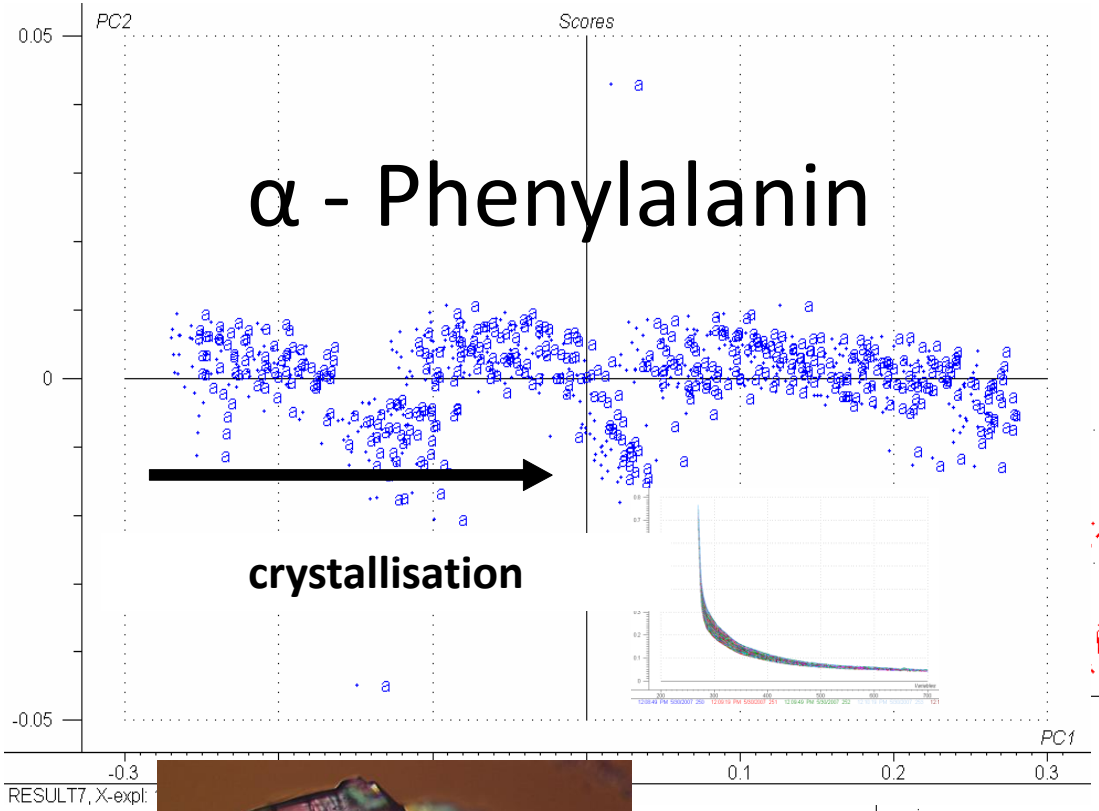


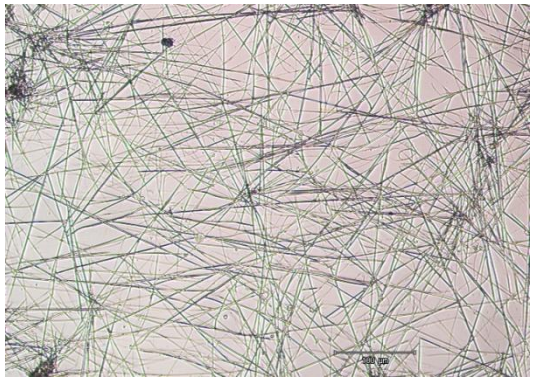


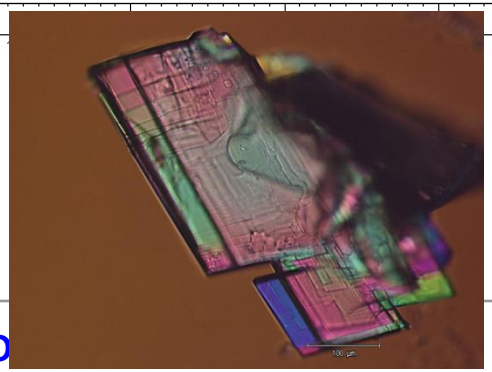
Bestimmung der metastabilen Phase: alpha-Phenylalanne



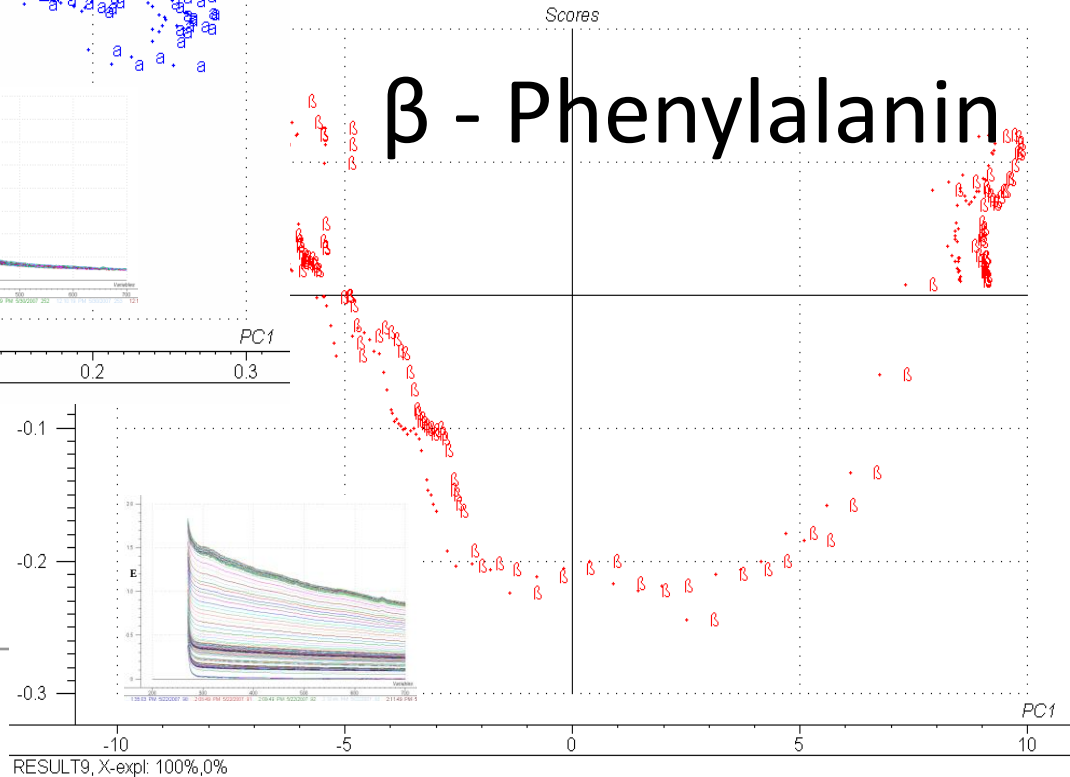
Kristallisation: Multivariate Charakterisierung



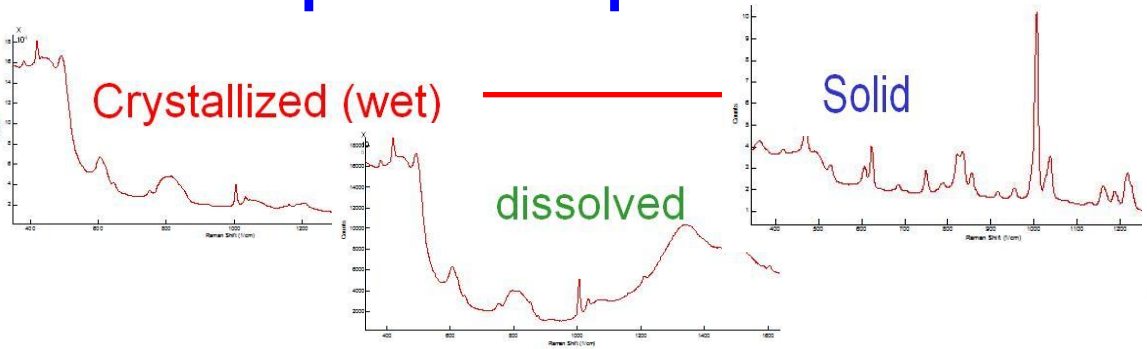


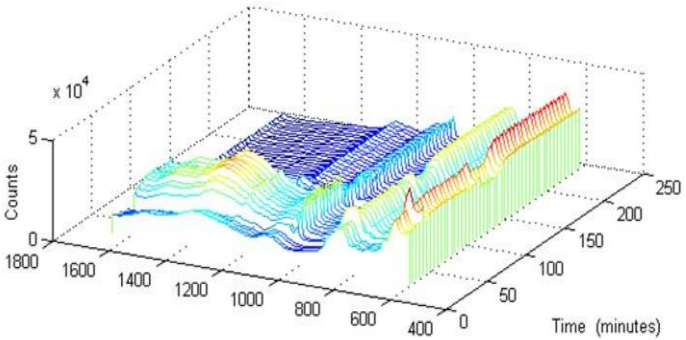


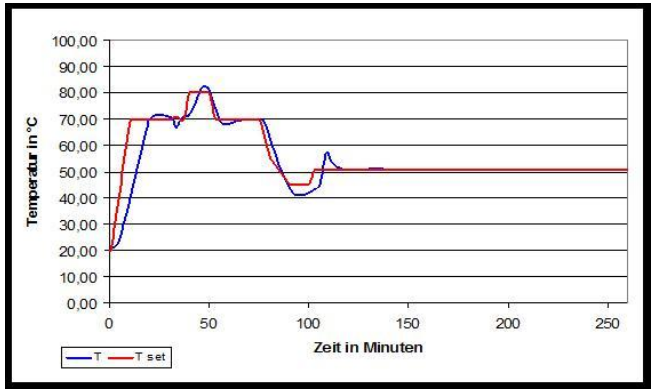
PHOTO

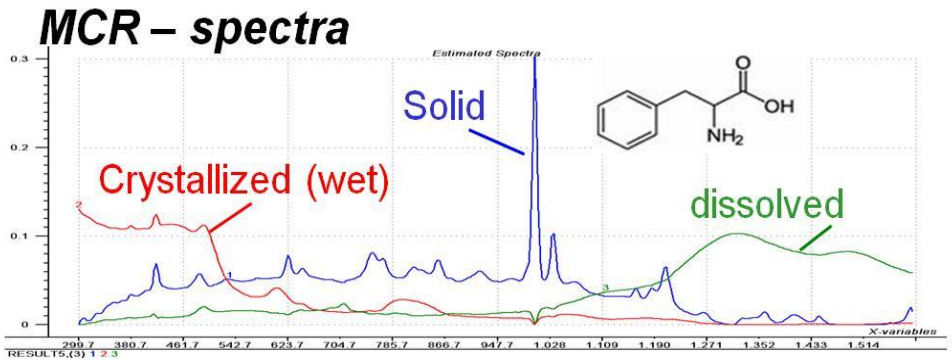


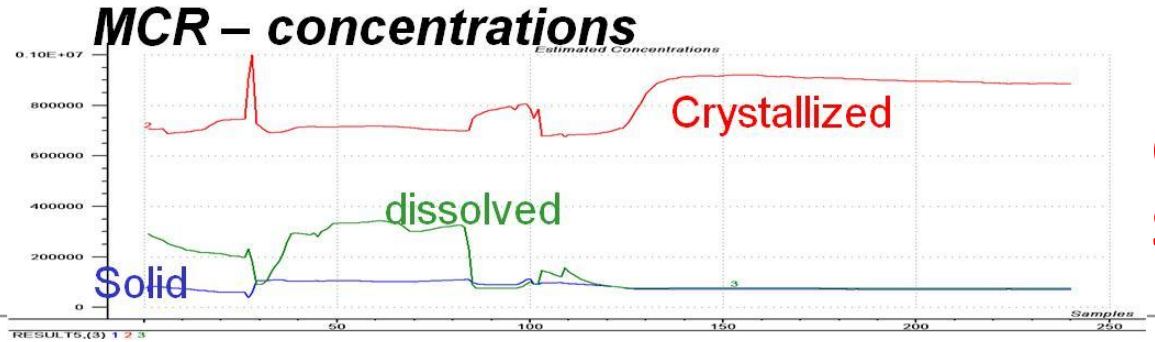
Raman Spektroskopie MCR: Kristallisation von Phenylalanin











Kaiser Optical System

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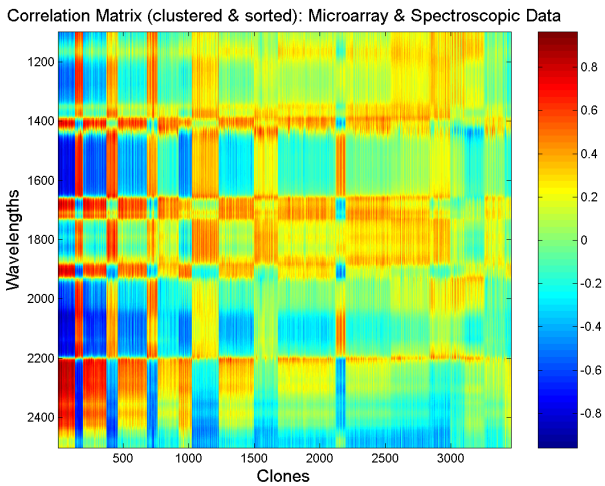
Microarray und Spektroscopische Daten

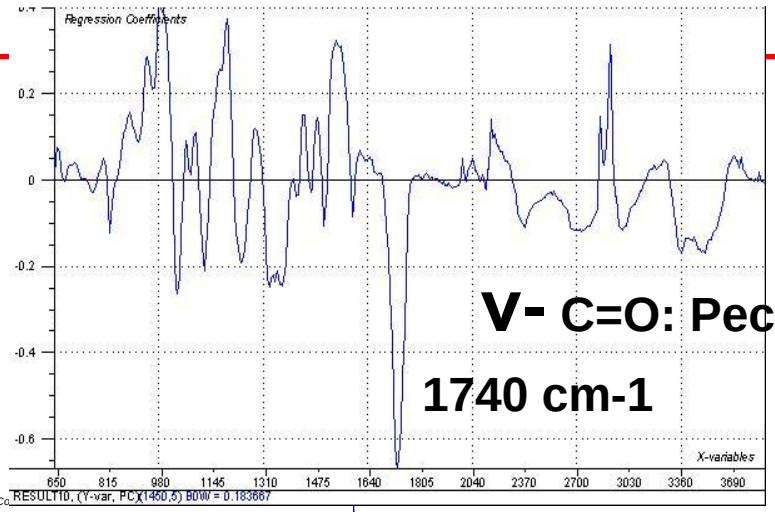
PLANT RESEARCH INTERNATIONAL

PRI (Reijmers, Maliepard et al.) IAF (Kessler and Kessler)

Clone 27A3: 1450 nm (Water??)

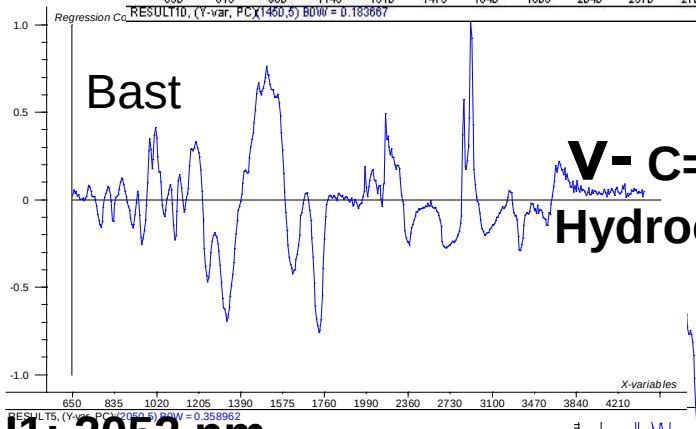
(Variety contrast: hypothetical protein)



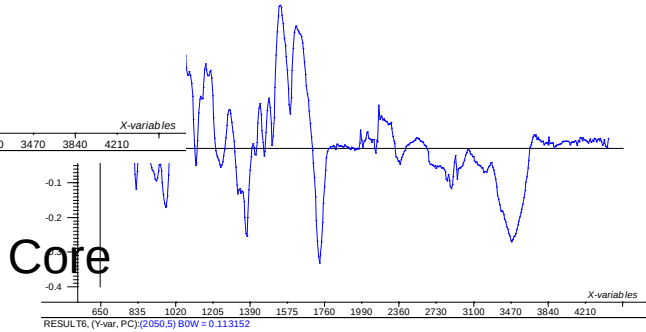


V- C=O: Pectine

1740 cm-1



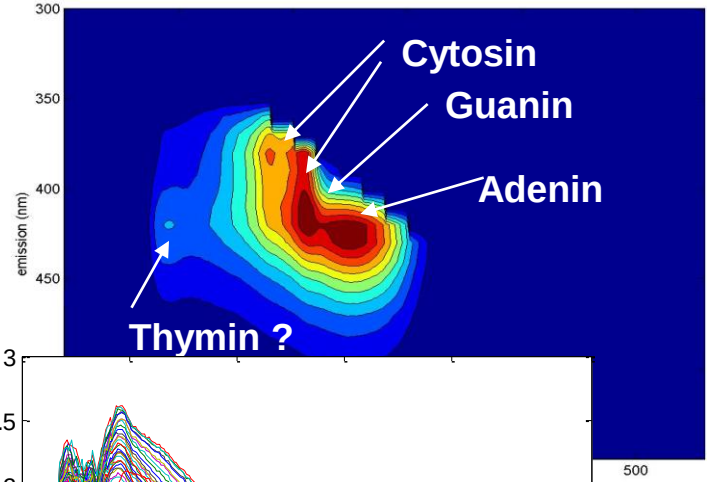
V- C=C/ v- C-H/: : Lignin, Hydrocarbons, Fats

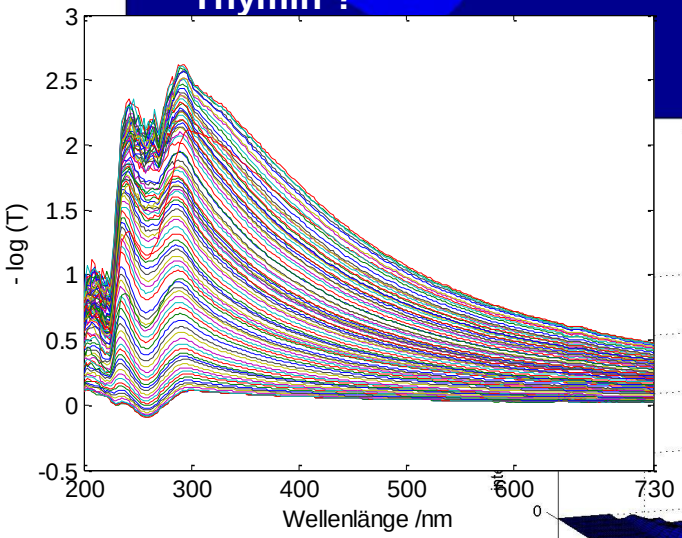


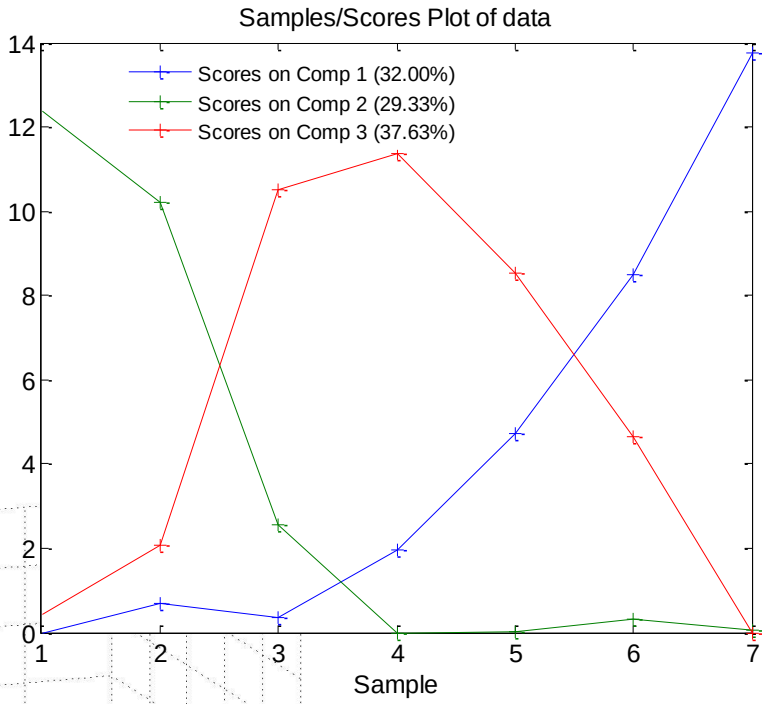
Clone 11C11 and inversely 4H1: 2052 nm

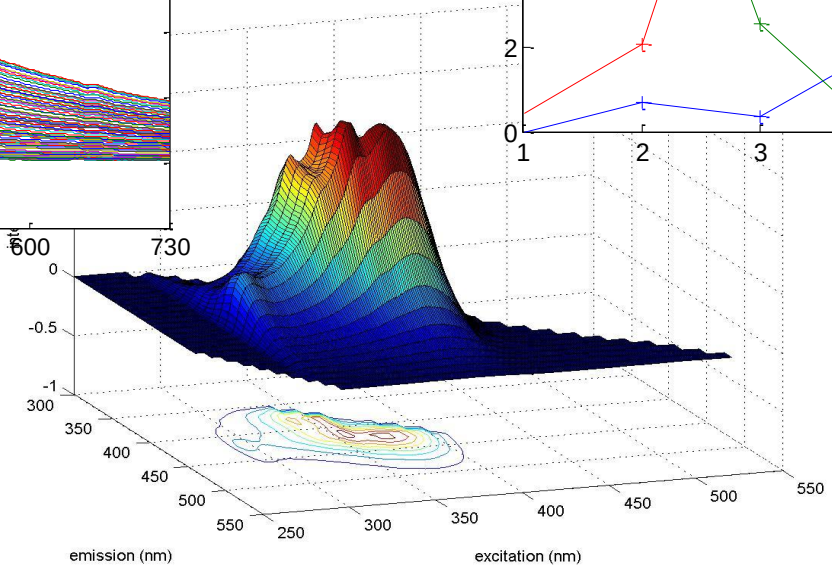
Core-Bast Contrast: S-adenosyl-methionine synthetase 2 / bacterial-induced peroxidase precursor.

E. Coli Fermentation









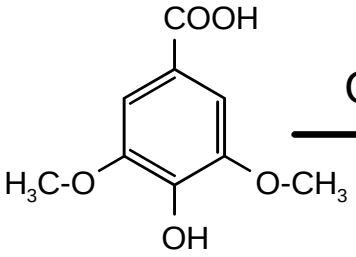
Enzymatische Reaktion mit Laccasen

O_2 Laccase Lignin

H_2O Laccase ^{ox} Lignin ^{ox}

O_2 Laccase Mediator^{ox} Lignin

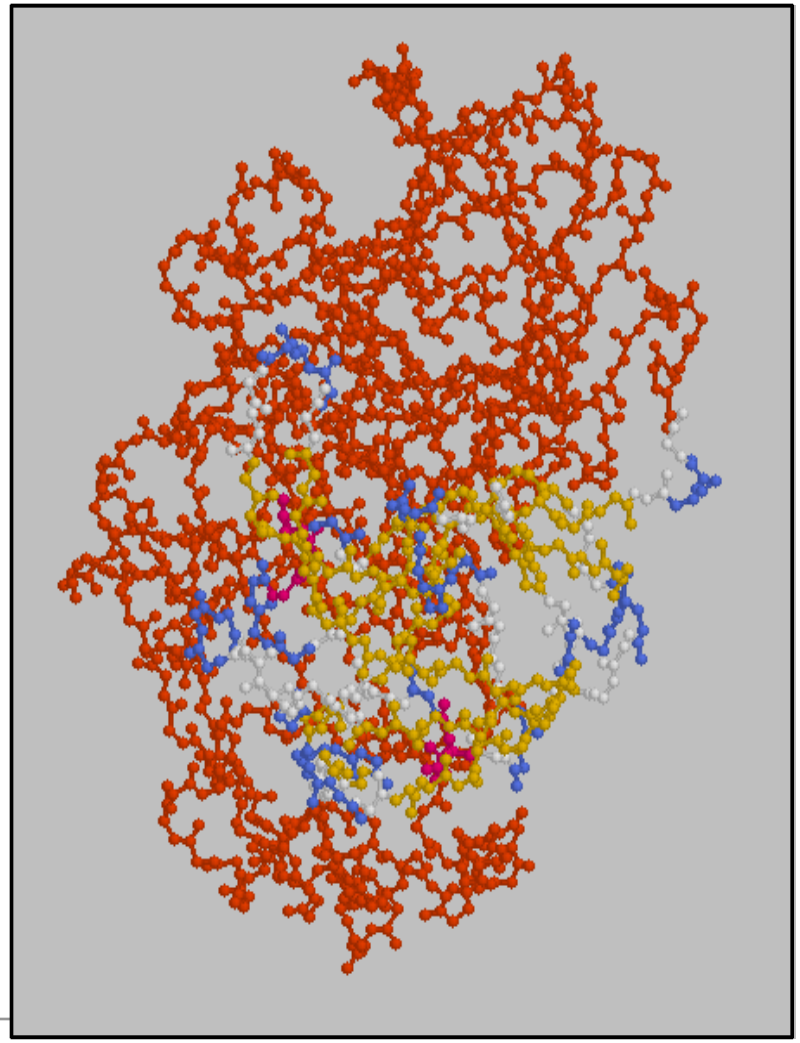
H_2O Laccase ^{ox} Mediator Lignin ^{ox}



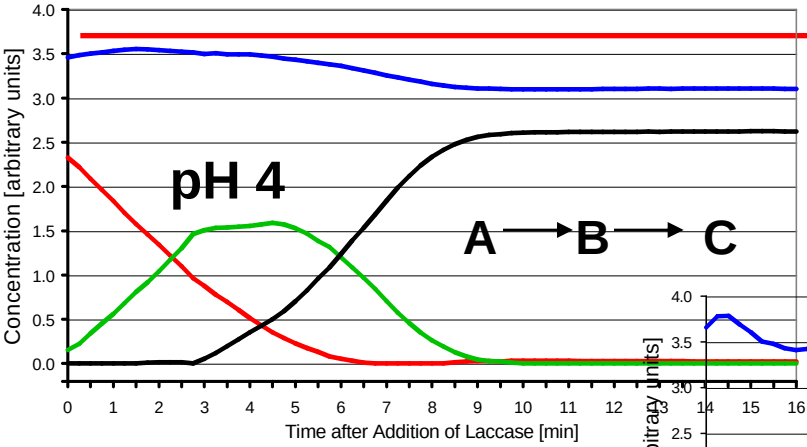
Syringic Acid

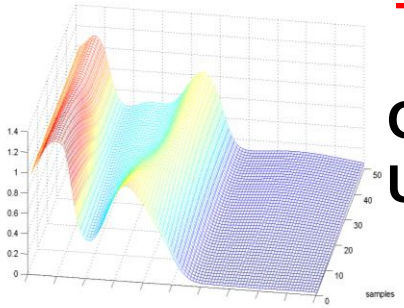
O_2, Laccase →

Decarboxylation, Ring Opening, Formation of Quinons etc.

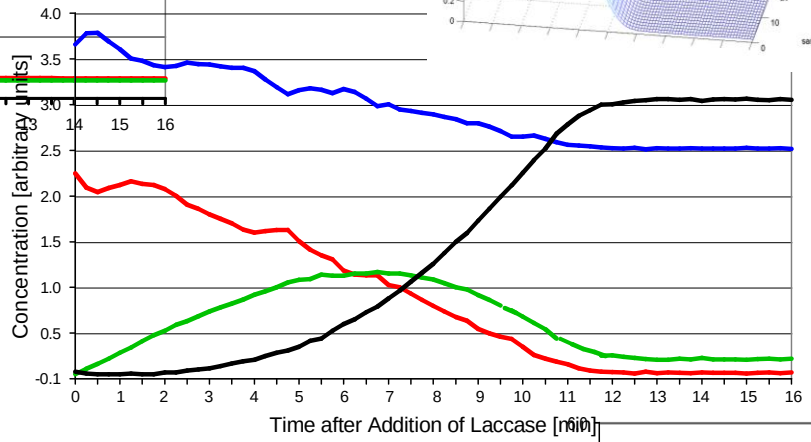


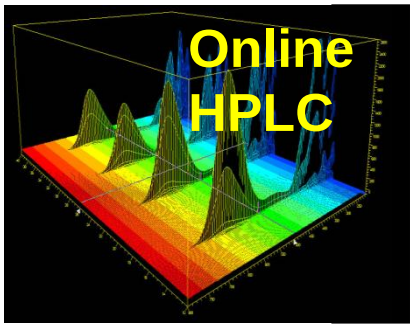
Kinetik und Intermediate von Laccase Abbauprodukten

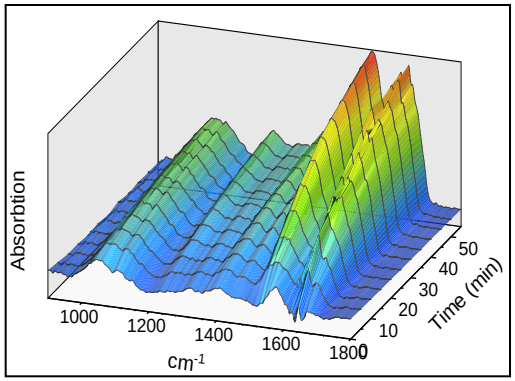




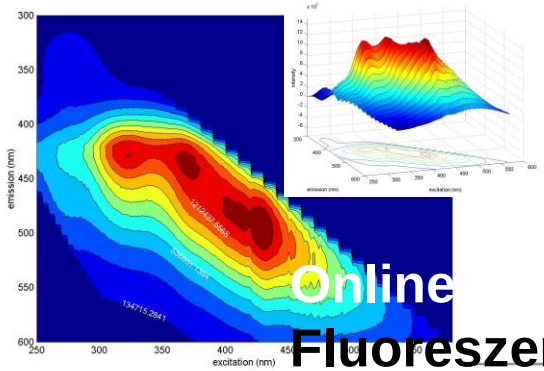
Online
UV/VIS/NIR



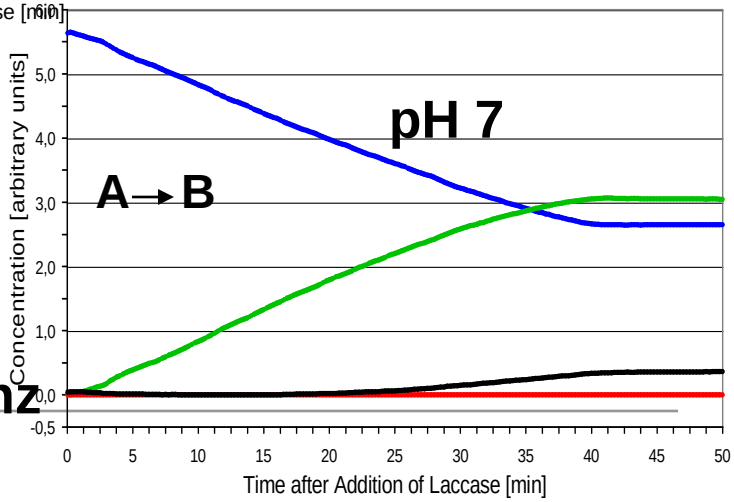




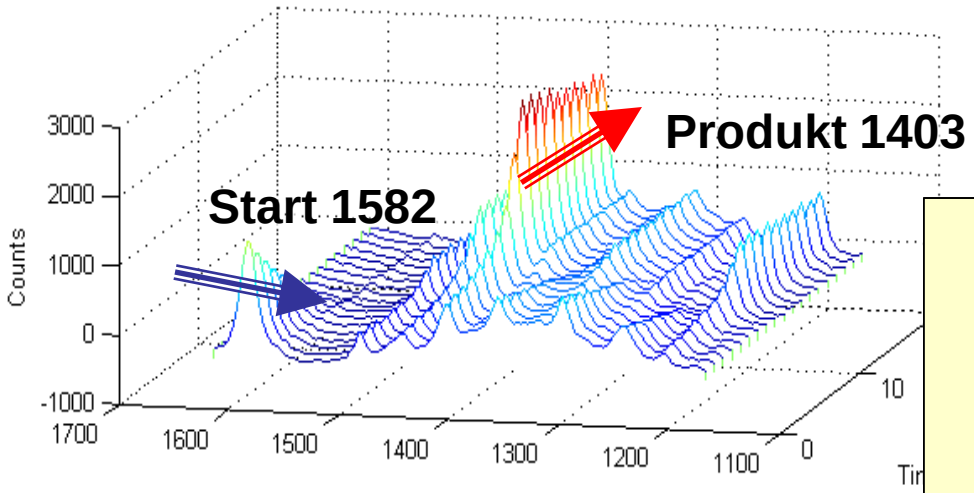
Online
FTIR/Raman

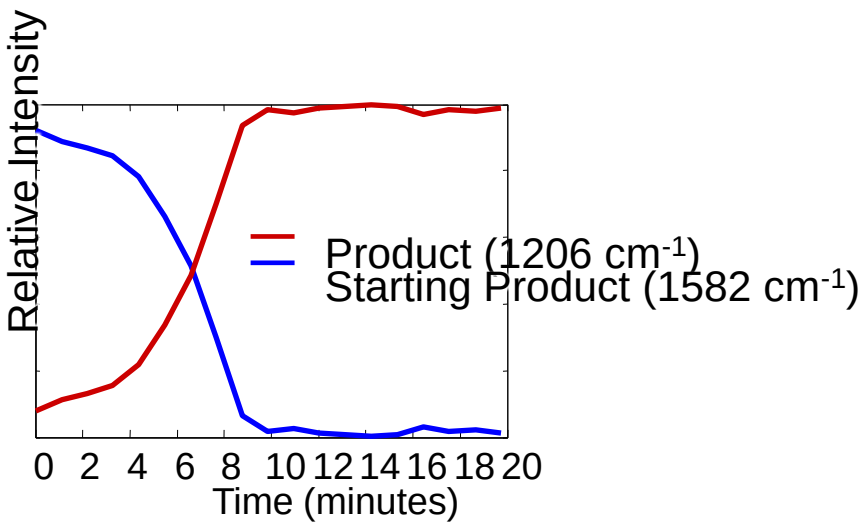


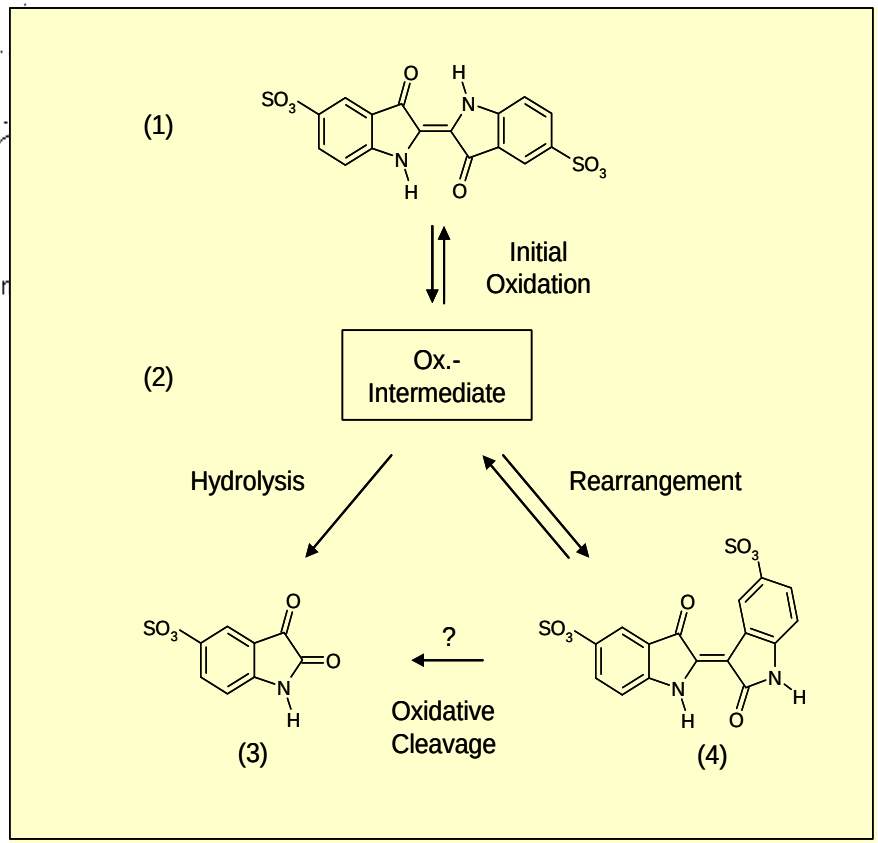
Online
Fluoreszenz



Insitu Ramanspektroskopie



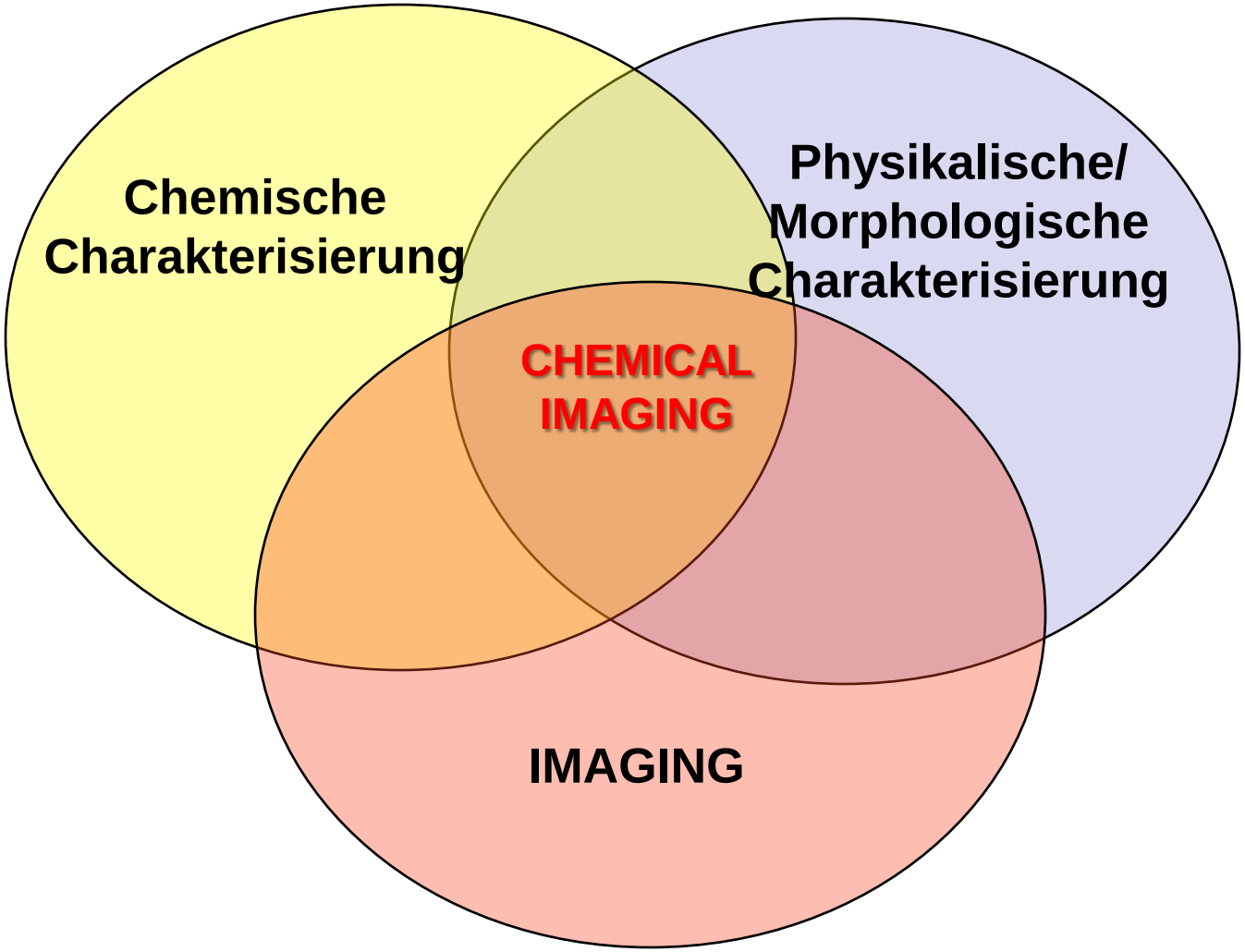




Agenda

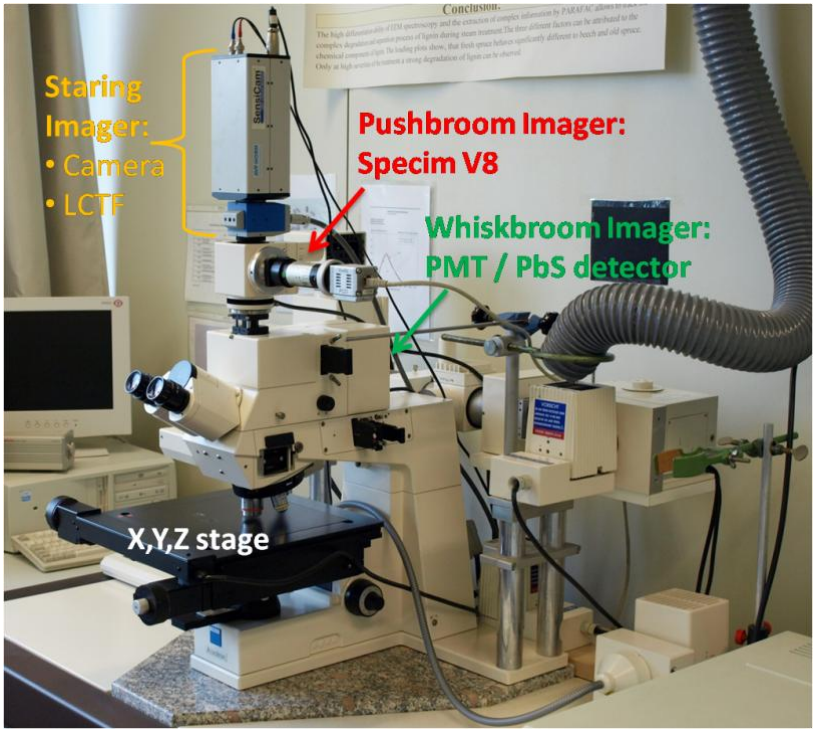
- Toolbox Spektroskopie und Chemometrie
- Beispiel Farbstoffgemisch und Kristallisation
- Beispiele aus der Biotechnologie
- Beispiel spektrales Imaging
- Photonendiffusion und Multipoint Spektroskopie

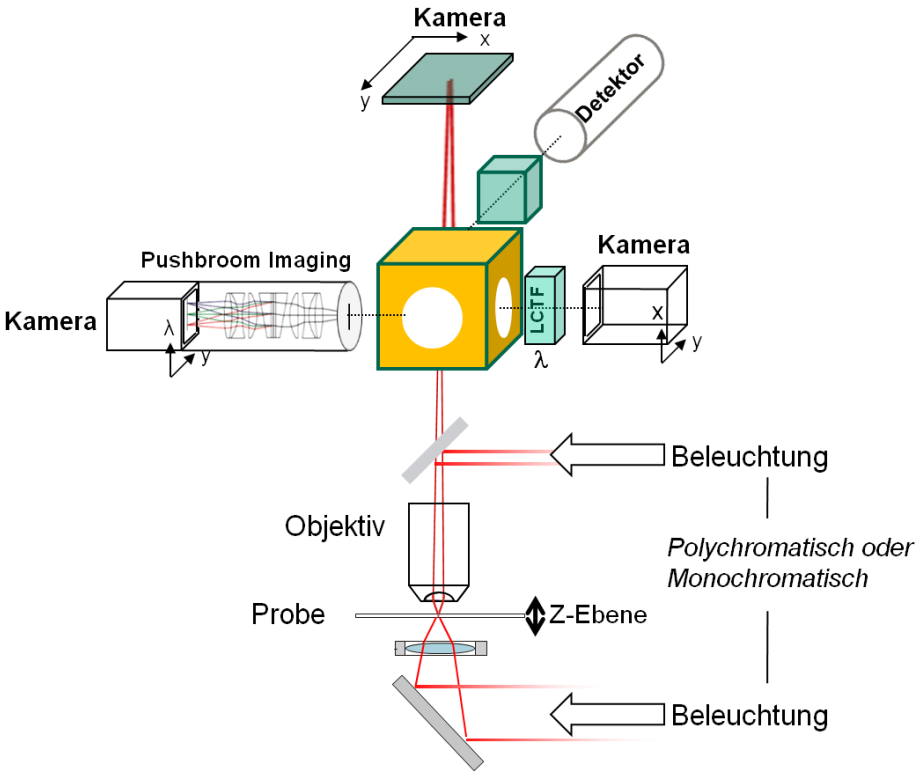
Chemical/ Spectral Imaging



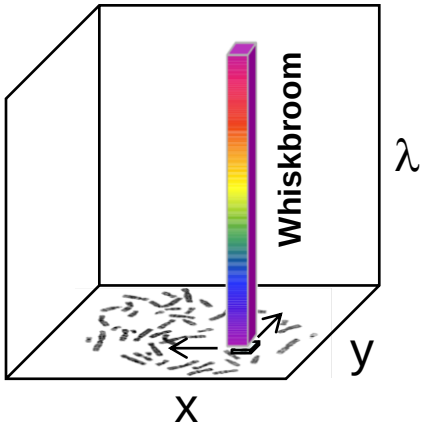
Micro Chemical Imaging von UV bis NIR, x, y, z Koordinate inklusive 2D-Fluoreszenz, Raman und FLIM,

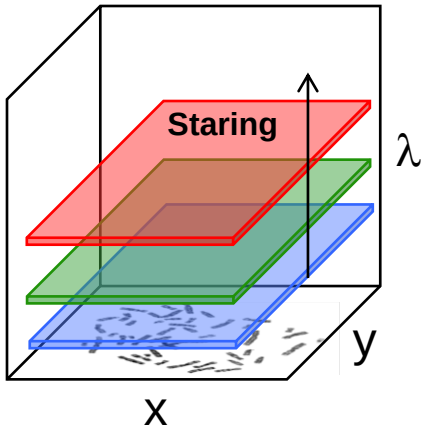
Whiskbroom-, Staring- und Pushbroom-Imaging vereint

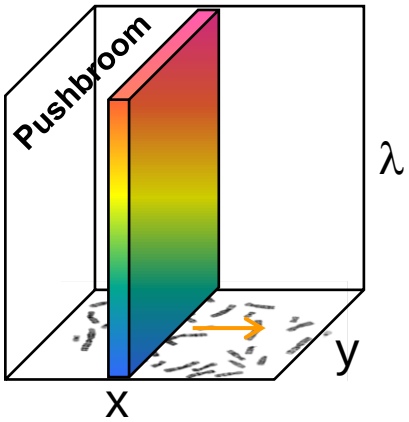




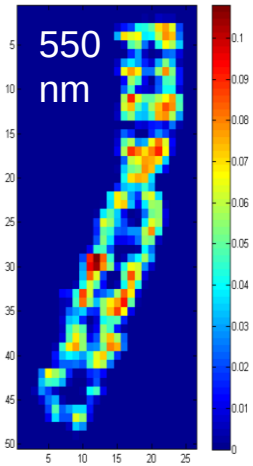
Chemical Imaging an Chromosomen

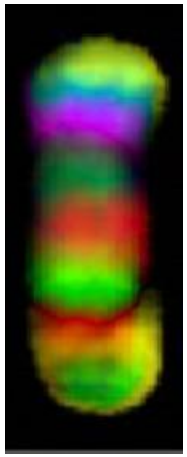


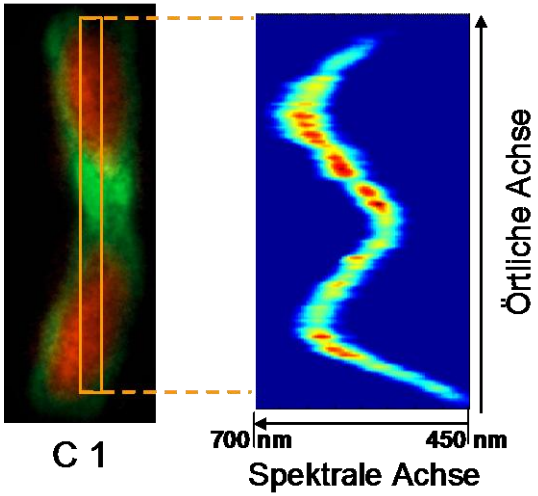




Beispiele







Giemsa gefärbt
Messung in Absorption

FISH gefärbt
Messung in Fluoreszenz

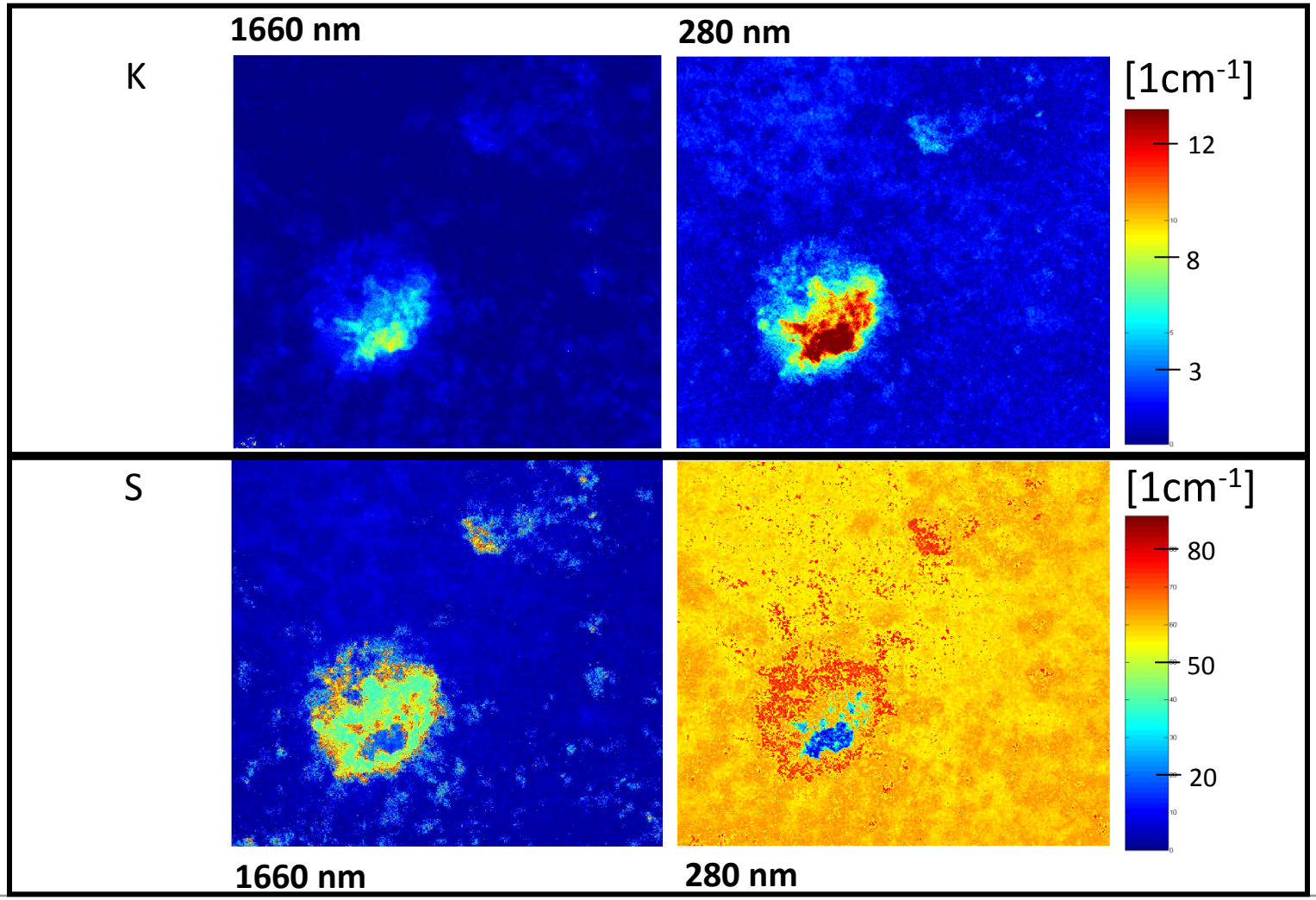
Ohne Markierung
Messung in Reflexion

Informationsebenen

Messungen

+ Intensität vs. Wellenlänge	$\Rightarrow$ Größe
+ Interferenzmuster (global)	$\Rightarrow$ Schichtdicke
+ Interferenzmuster (Verschiebung)	$\Rightarrow$ Morphologie
+ Interferenzmuster (lokal)	$\Rightarrow$ Topologie (Bänderung)
+ Absorption im UV Bereich	$\Rightarrow$ DNA & Protein Anteil

Bestimmung von S & K in Chemical Imaging



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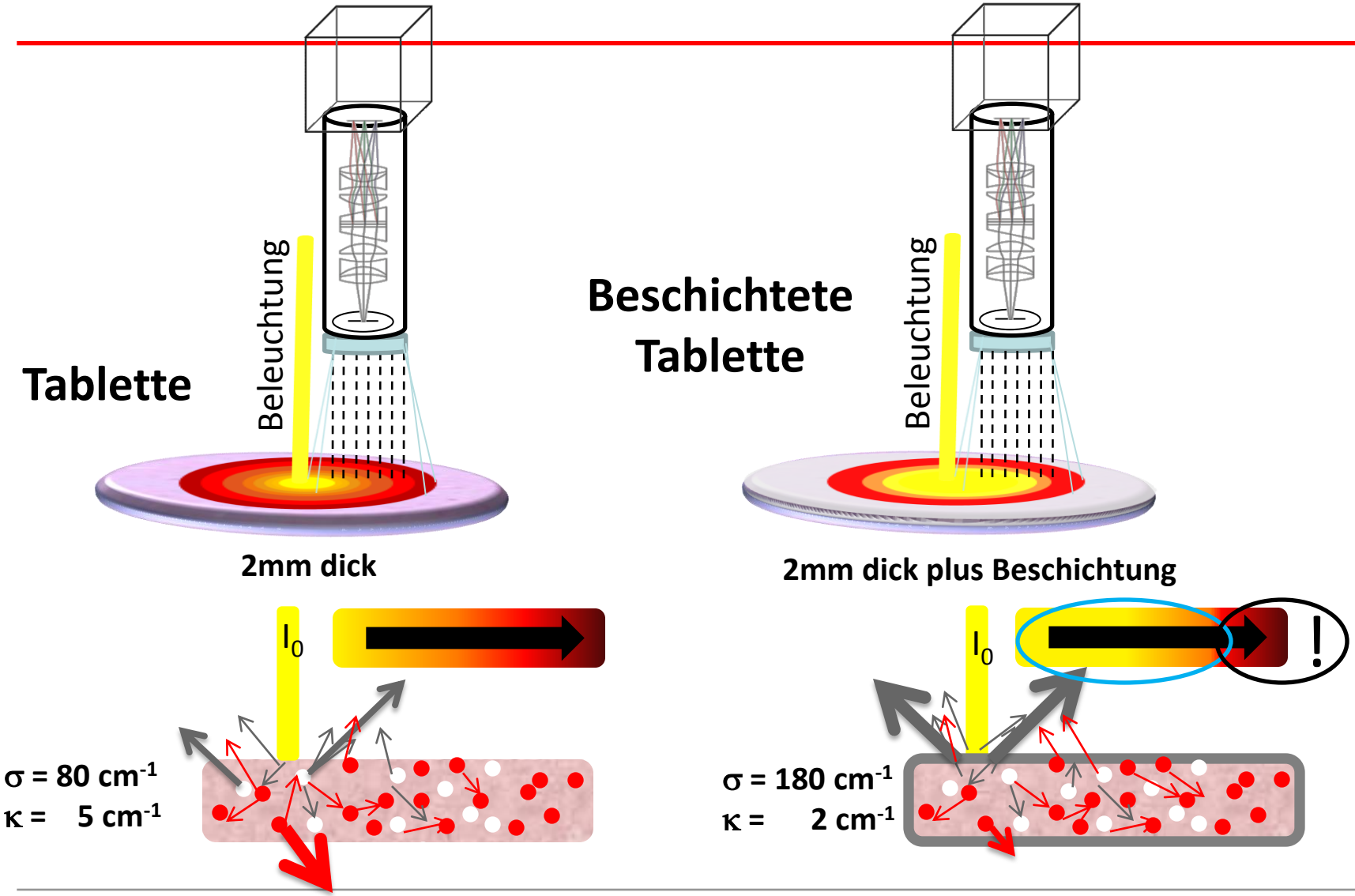
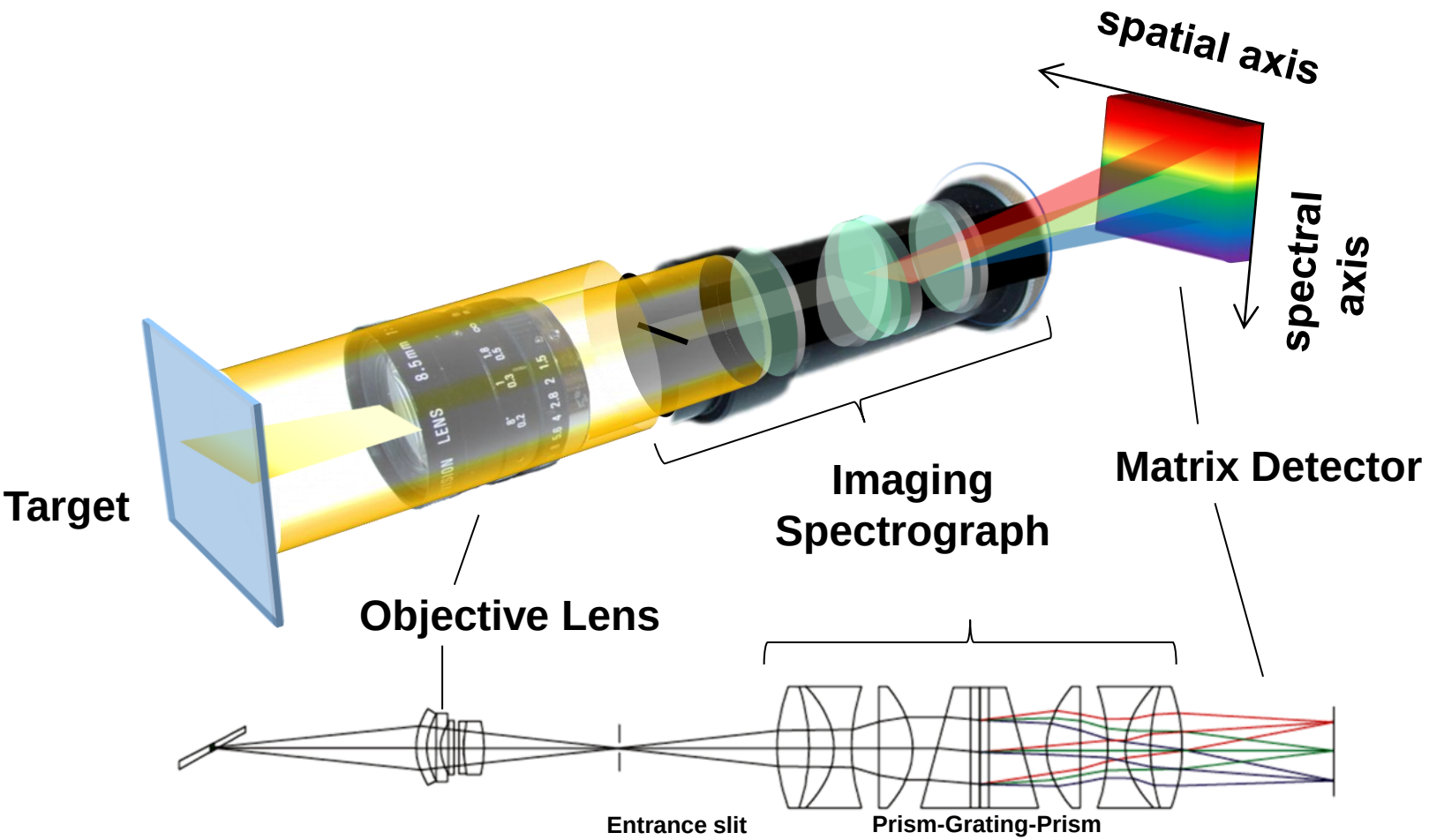
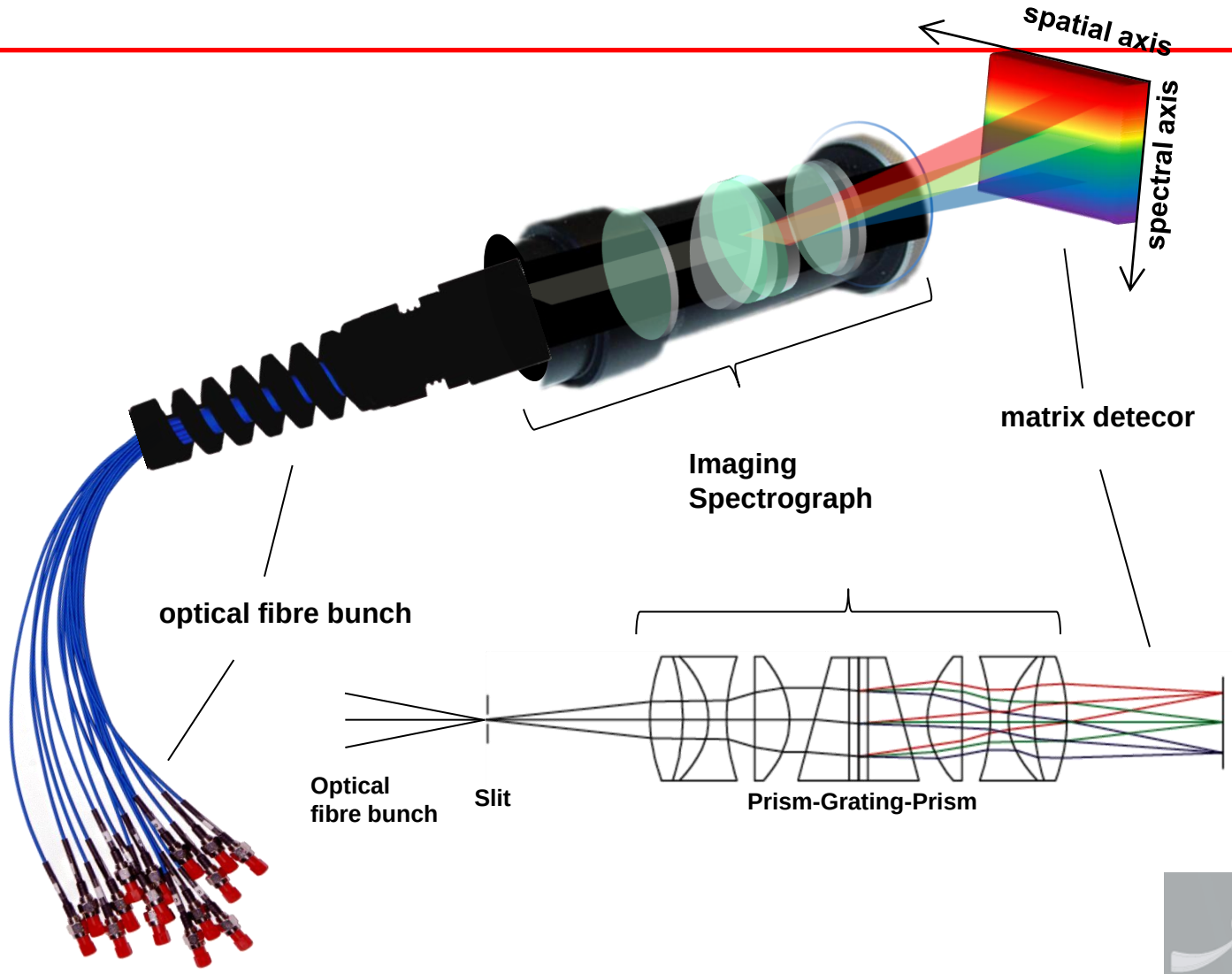
Photonendiffusion



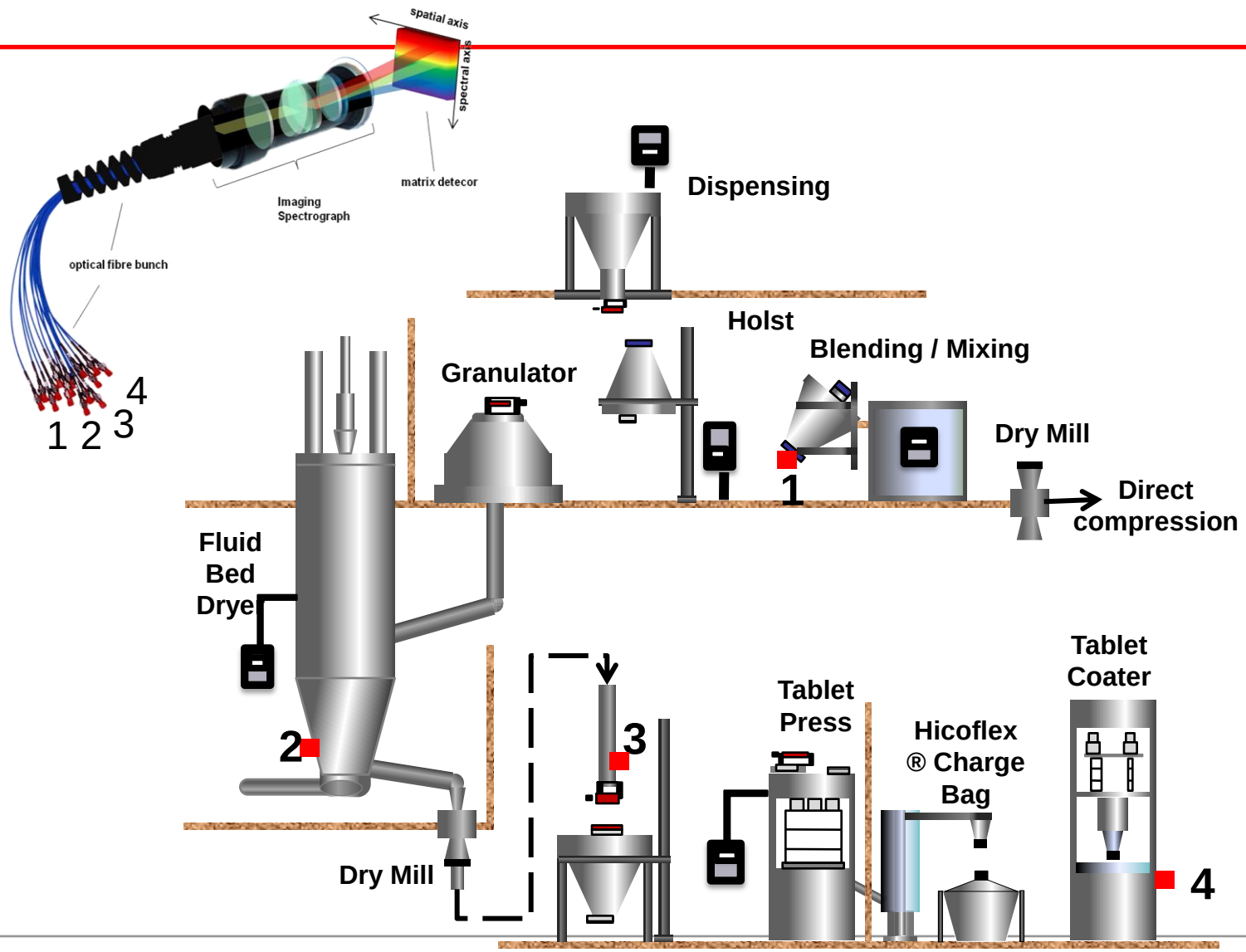
Pushbroom Imaging Technology (e.g. Specim)



Pushbroom Imager as a Multiplexing System



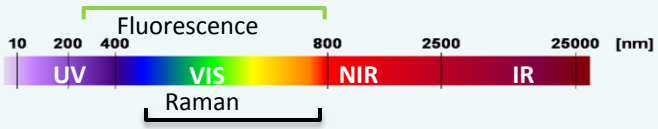
Multipoint Spektroskoie in Pharma Prozessen



Zusammenfassung

Multi-Information Spectroscopy

A ⇒ λ



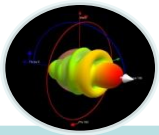
B ⇒ A + Setup geometry

C ⇒ A + B + 2D-scan

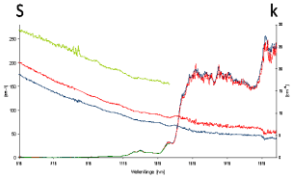
Reutlingen Research Institute (RRI) - Process Analysis Technolo
-

Direct linkage

- Kubelka-Munk K + S
- Radiative Transfer μ_a + μ_s + g
- (Maxwell Equations)

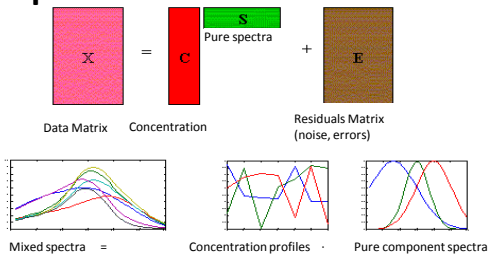


Science Based Analysis



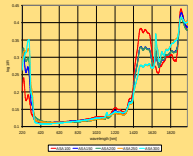
- Theory
- Models
- Approximations

Reduced Number of calibration Samples



Multivariate Curve Resolution (MCR)

Raw Spectra

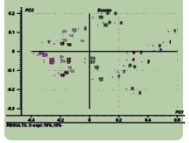


Data Pretreatment

- Derivatives
- Normalizations
- SNV, EMSC...

Data Analysis

PCA, PCR, PLS, SIMCA



Chemometrics / Multivariate Data Analysis

Pure Statistics?!