ZAFH – PHOTON^{n}

PHOTONische Verfahren in neuen Dimensionen

Hochschule Reutlingen

Multidimensionale Mikroskopie

Multispektrales Imaging

1. Aalener Photoniktag

Projektleitung: Prof. Dr. R.W. Kessler
Kooperation mit Hochschulen AA und MA, (FU) sowie ILM

Agenda

- Konzept und Versuchsaufbau
- Chromosomenkaryotypisierung
- Modell
- Nahfeldspektroskopie

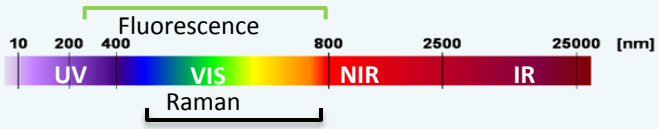
Agenda

- **Konzept und Versuchsaufbau**
- Chromosomenkaryotypisierung
- Modell
- Nahfeldspektroskopie

Multi – Modal – Spatially – Resolved - Spectroscopy

Multi-Information Spectroscopy

A ⇒ λ



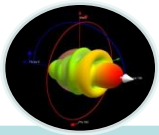
B ⇒ A + Setup geometry

C ⇒ A + B + 2D-scan

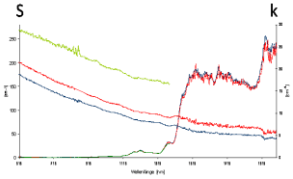
Reutlingen Research Institute (RRI) - Process Analysis Technolo
-

Direct linkage

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

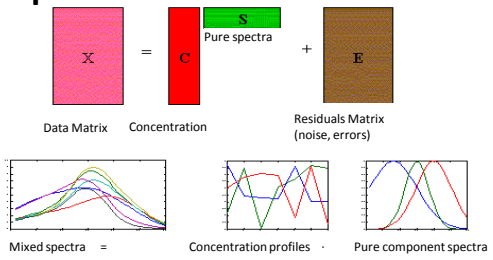


Science Based Analysis



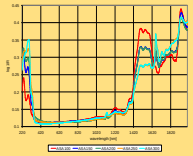
- Theory
- Models
- Approximations

Reduced Number of calibration Samples



Multivariate Curve Resolution (MCR)

Raw Spectra

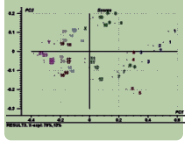


Data Pretreatment

- Derivatives
- Normalizations
- SNV, EMSC...

Data Analysis

PCA, PCR, PLS, SIMCA



Chemometrics / Multivariate Data Analysis

Pure Statistics?!

PHOTON^n

Chemical Imaging: Techniken

Flexibilität

Pushbroom Imaging

Simultan (x, λ)

Sequentiell (y)

Staring Imaging

Simultan (x, y)

Sequentiell (λ)

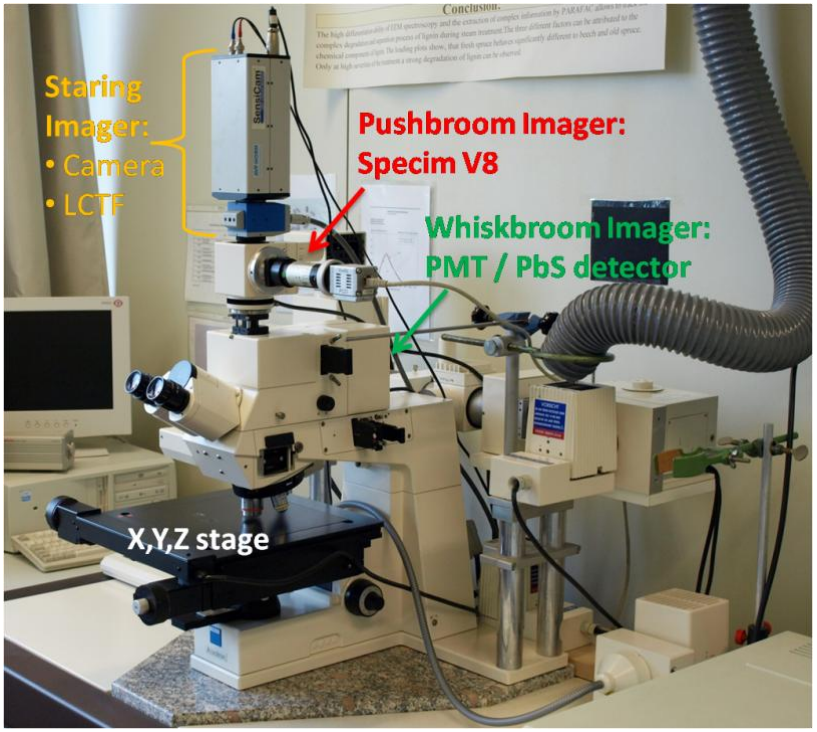
Whiskbroom Imaging

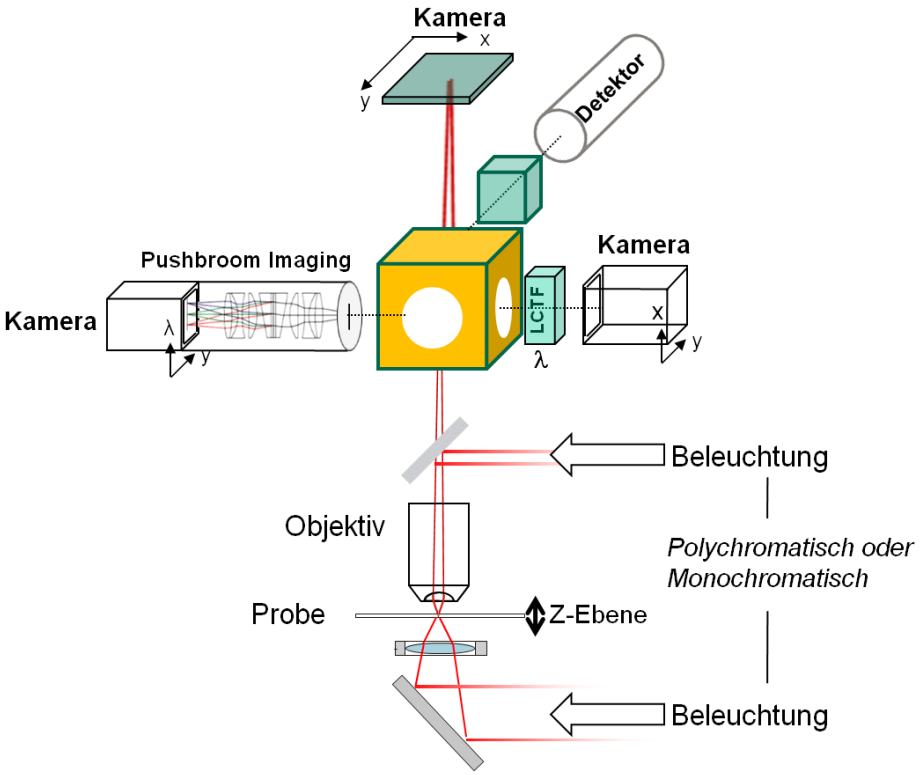
Sequentiell (x, y, λ)

Real Time

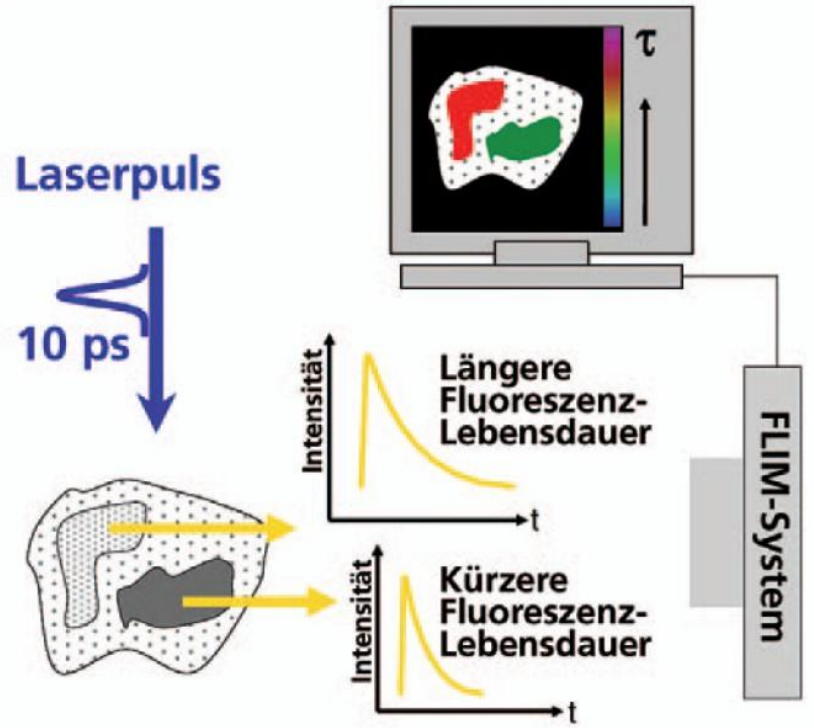
Micro Chemical Imaging von UV bis NIR, x, y, z Koordinate inklusive 2D-Fluoreszenz, Raman und FLIM,

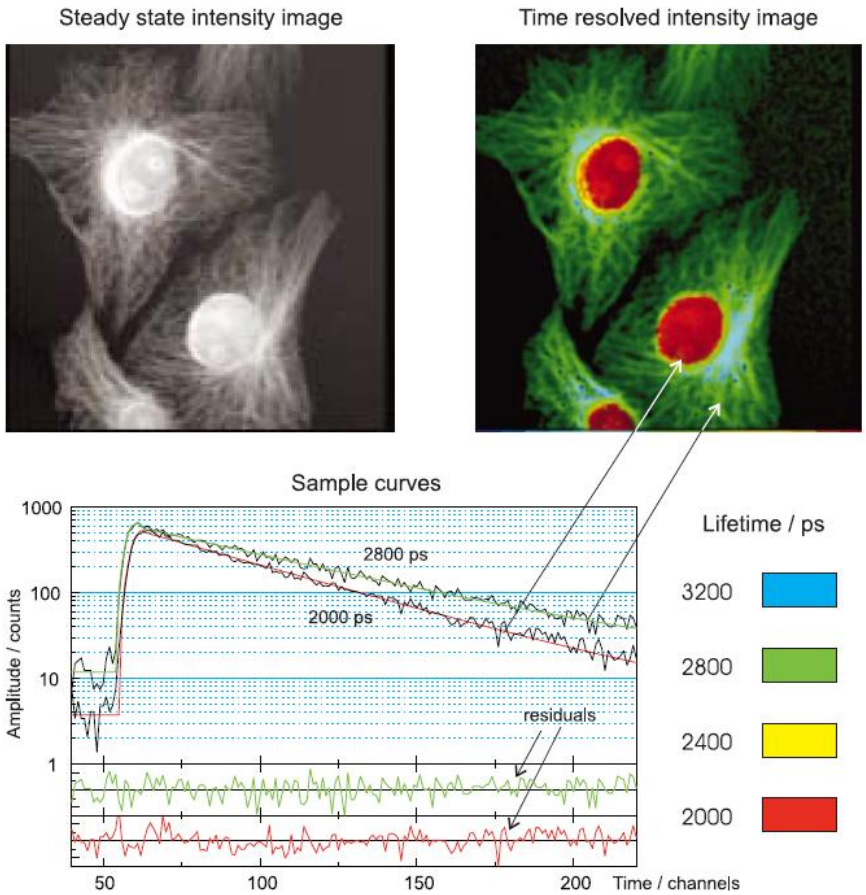
Whiskbroom-, Staring- und Pushbroom-Imaging vereint





Fluorescence Lifetime Imaging (FLIM)



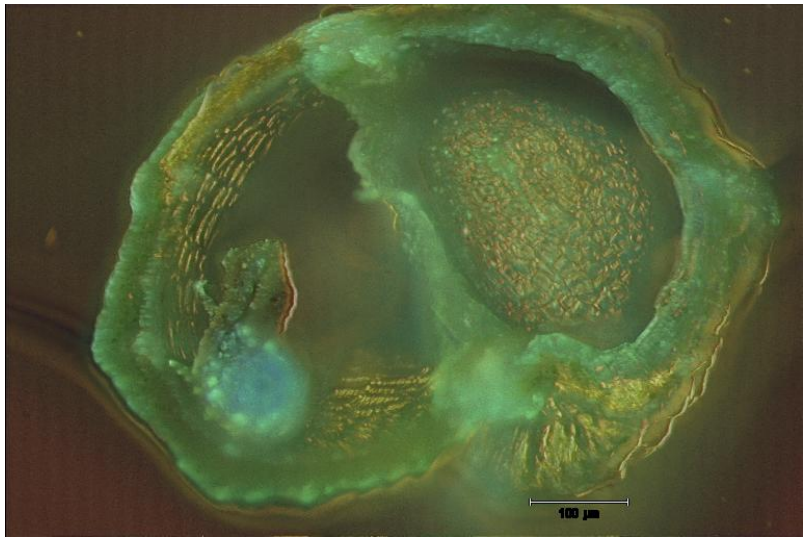


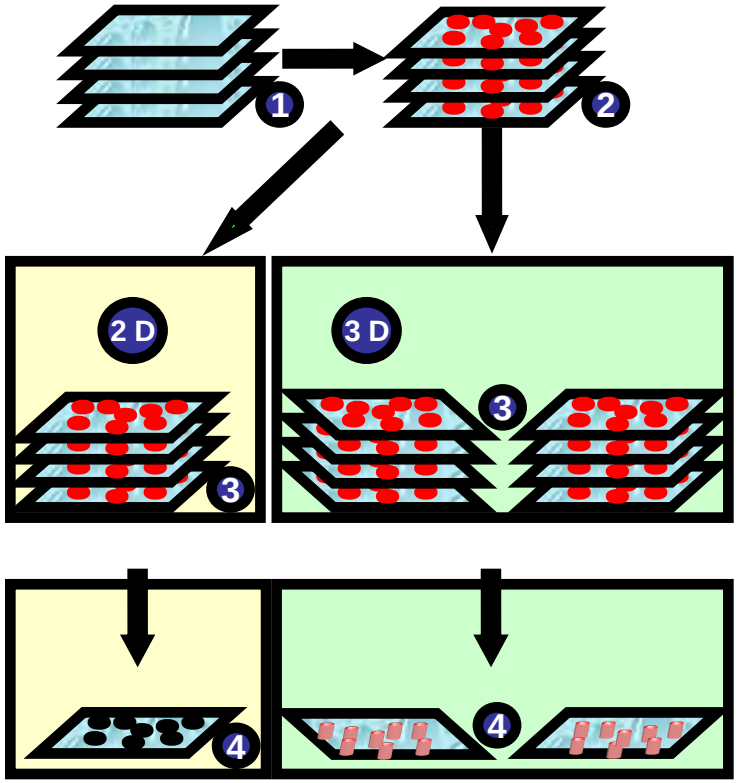
Kontrastgewinn über Fluoreszenz-Lebensdauer

Micro Chemical Imaging von UV bis NIR, Fluoreszenz in x-, y- und z-Ortskoordinate

Optische Tomographie

1. Aufnahme von Bildern als z-Stapel
2. Superposition & Interpolation
3. Allen Video Kontrast
4. 2D / 3D Darstellung

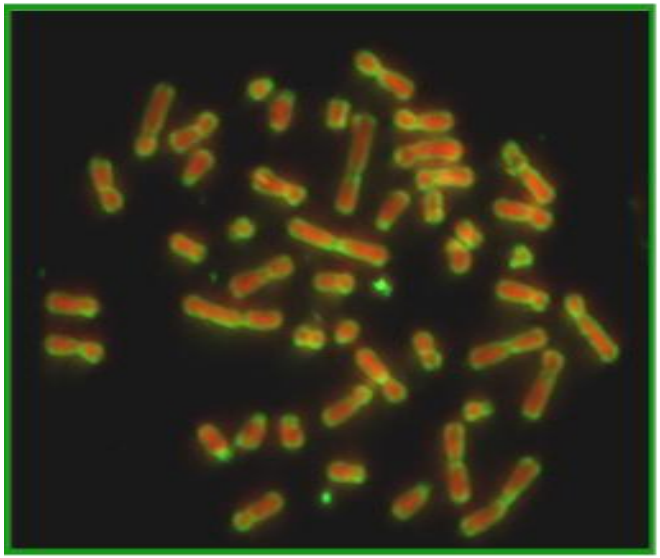




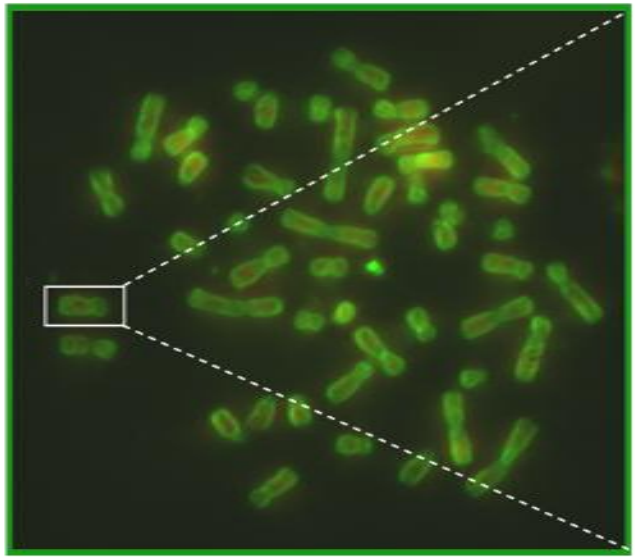
Agenda

- Konzept und Versuchsaufbau
- **Chromosomenkaryotypisierung**
- Modell
- Nahfeldspektroskopie

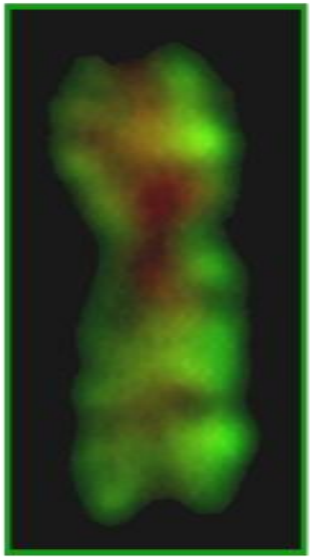
Chromosomen Dunkelfeldbeleuchtung 65R0



Ohne Trypsinierung



Mit Trypsinierung

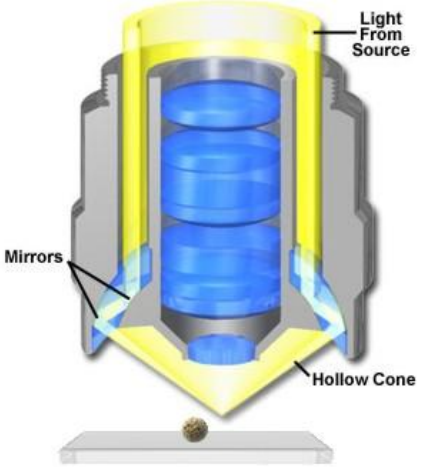


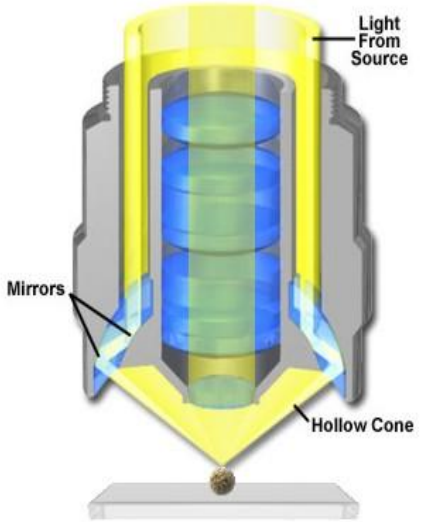
Chromosom 17

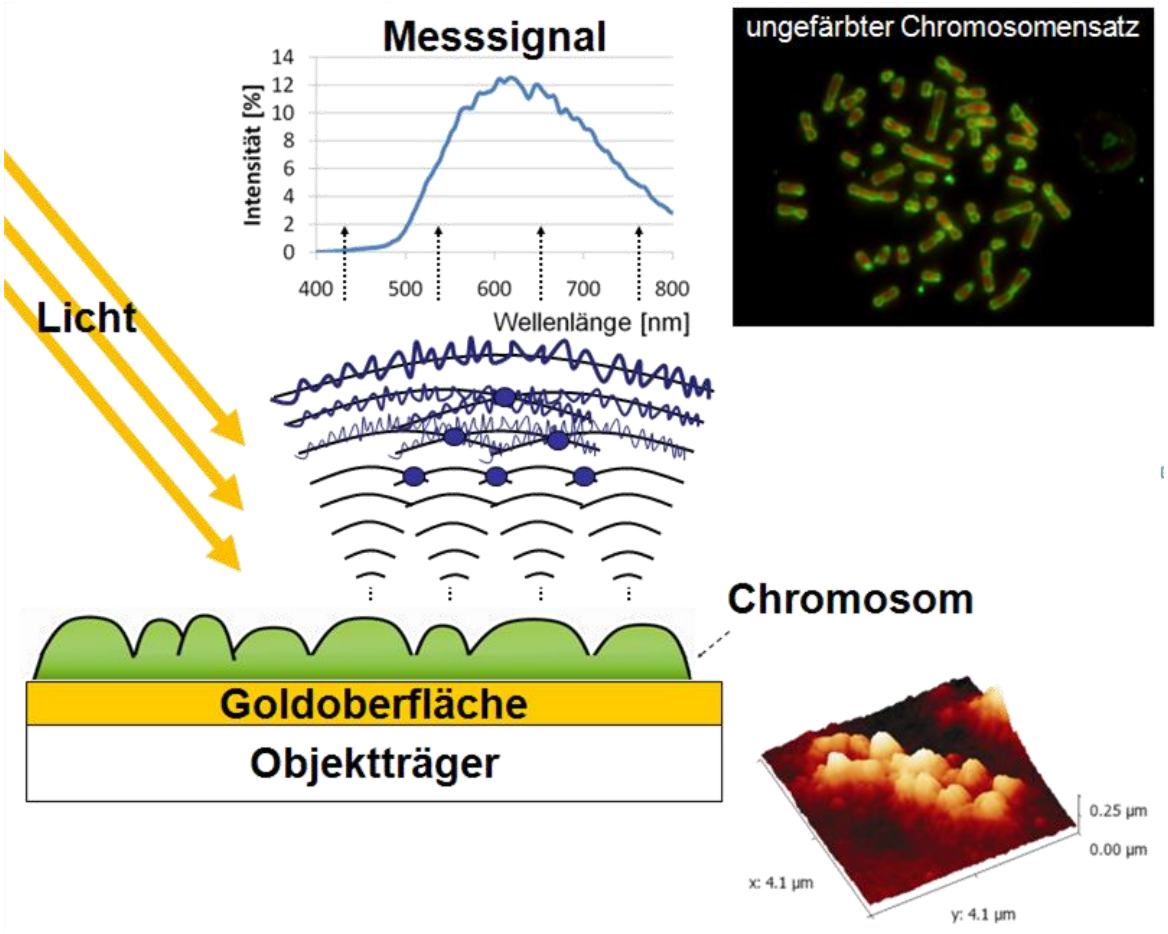
Verringerung der Schichtdicke

- Überlagerung von Interferenzeffekten
- Spezifische Bandenstruktur

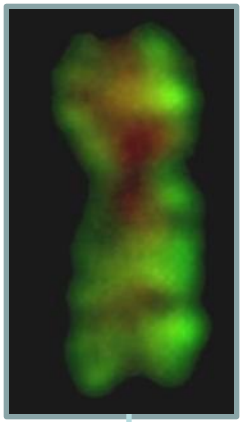
Dunkelfeldabbildung





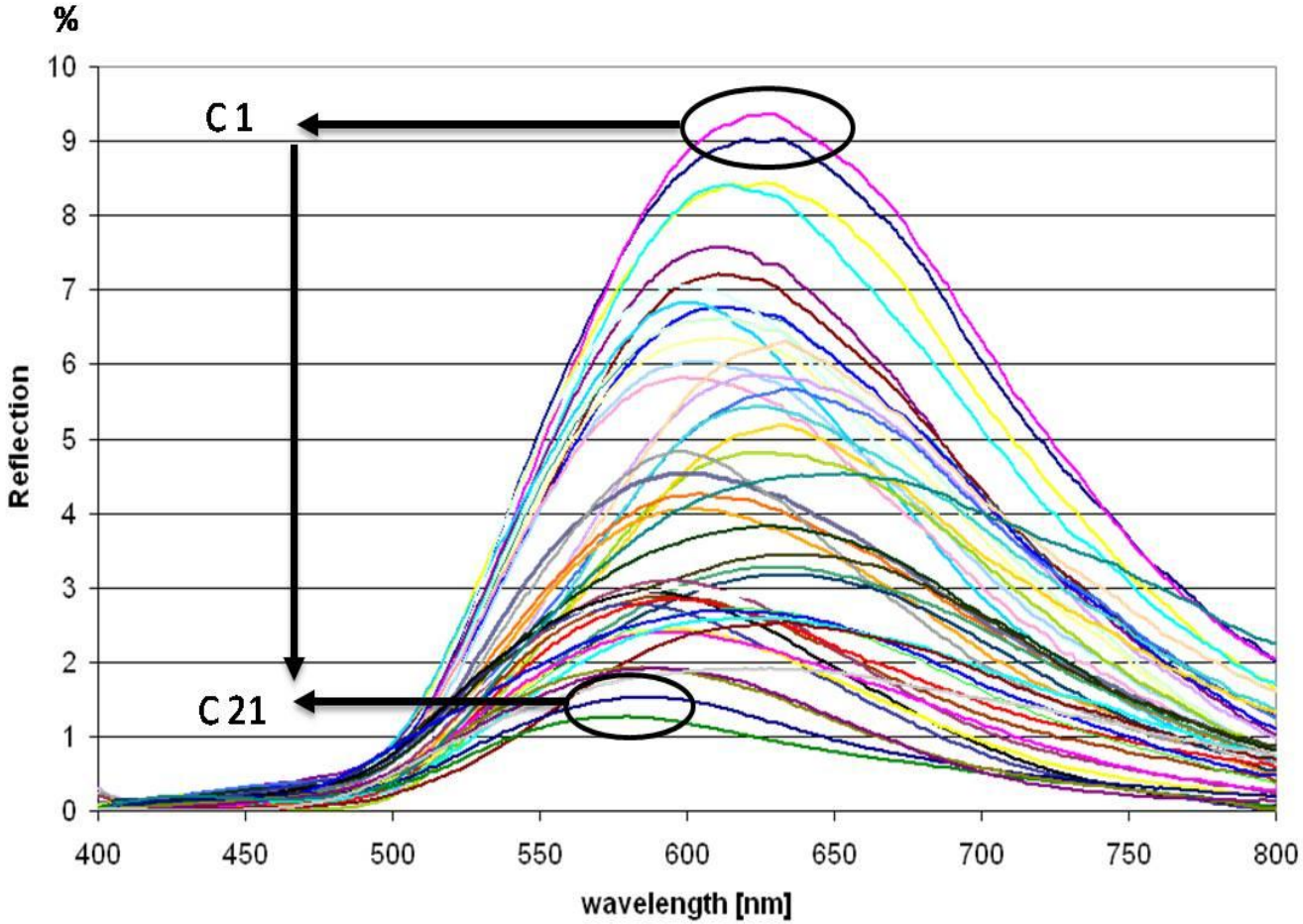


Dunkelfeld VIS Spektroskopie

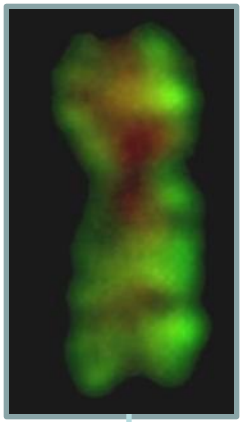


Messfeld

Einzelspektren der Chromosomen

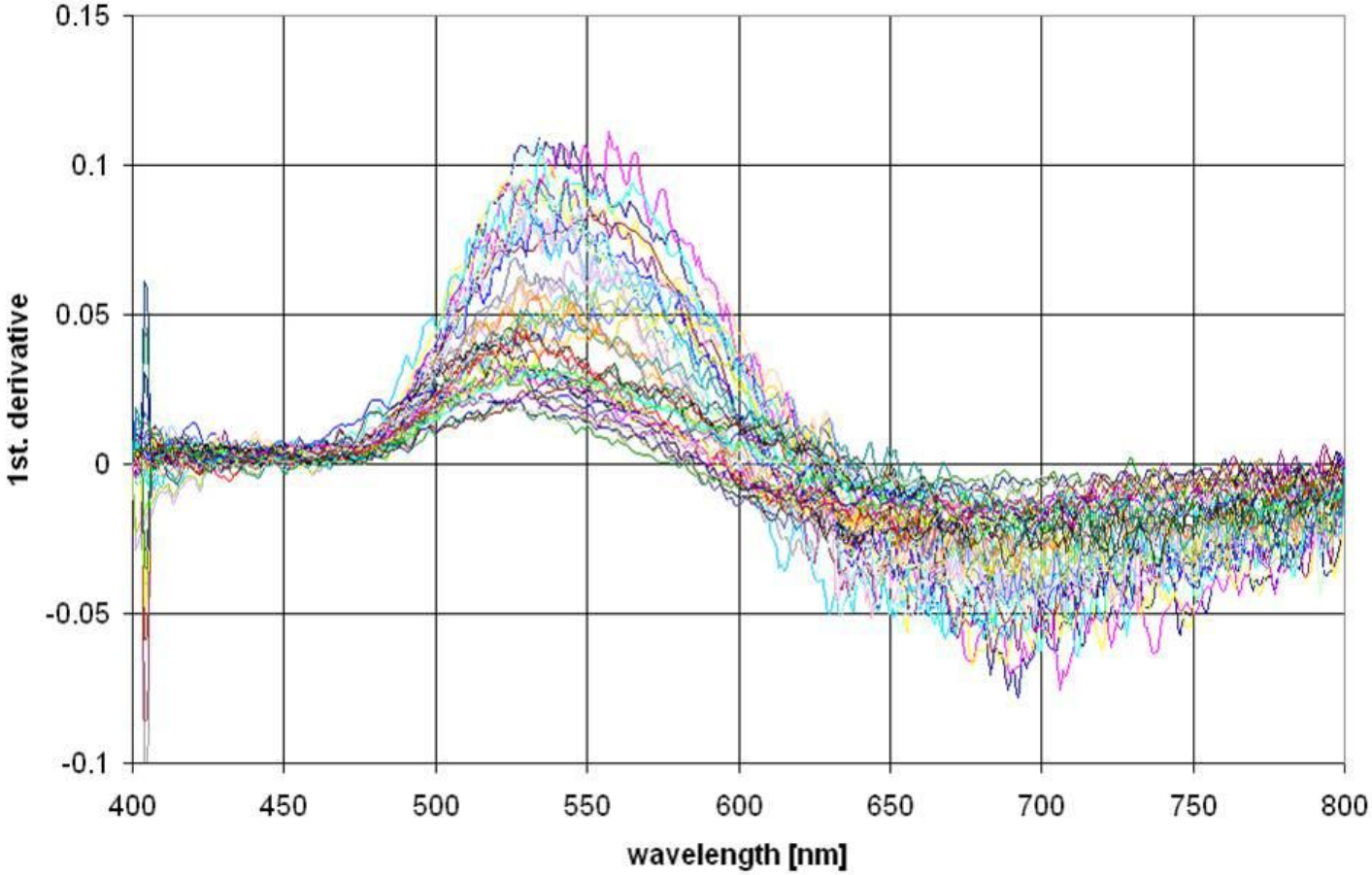


Dunkelfeld VIS Spektroskopie

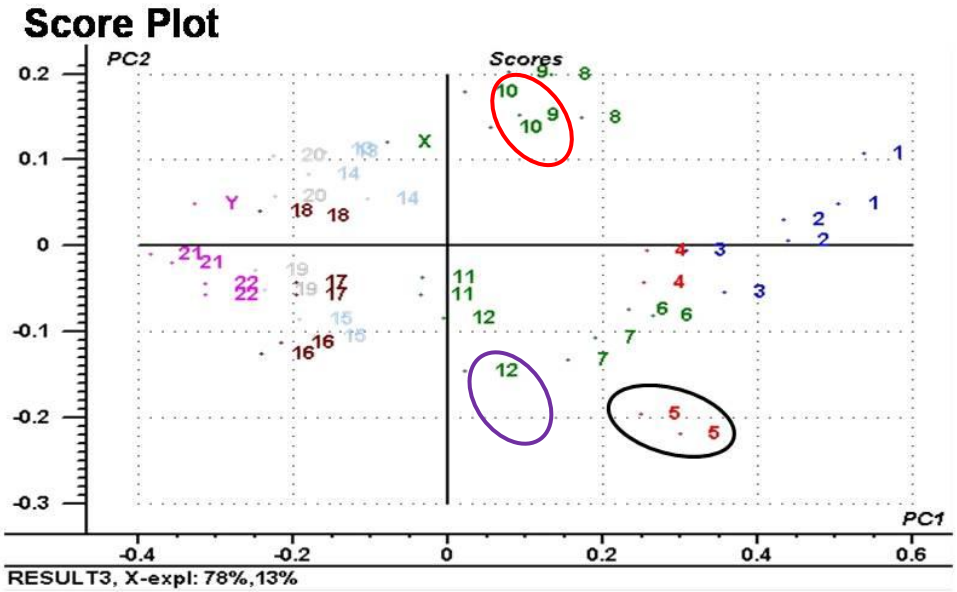


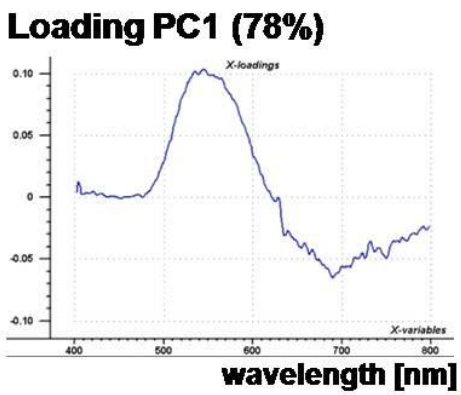
measuring field

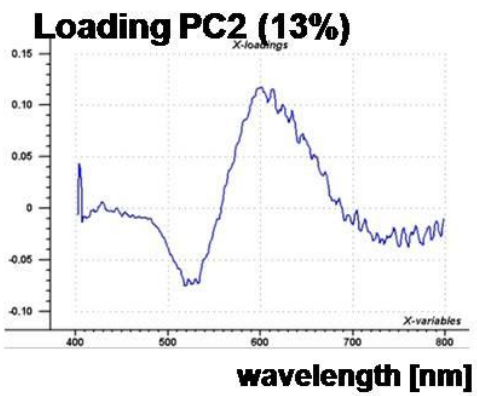
1. Ableitung der Spektren



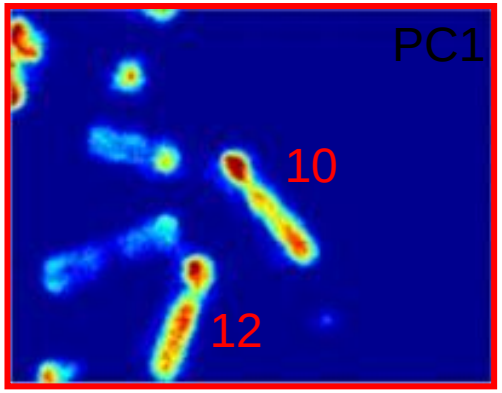
Hauptkomponentenanalyse (PCA)

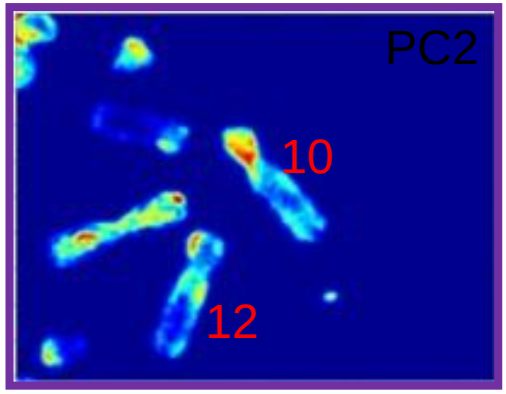




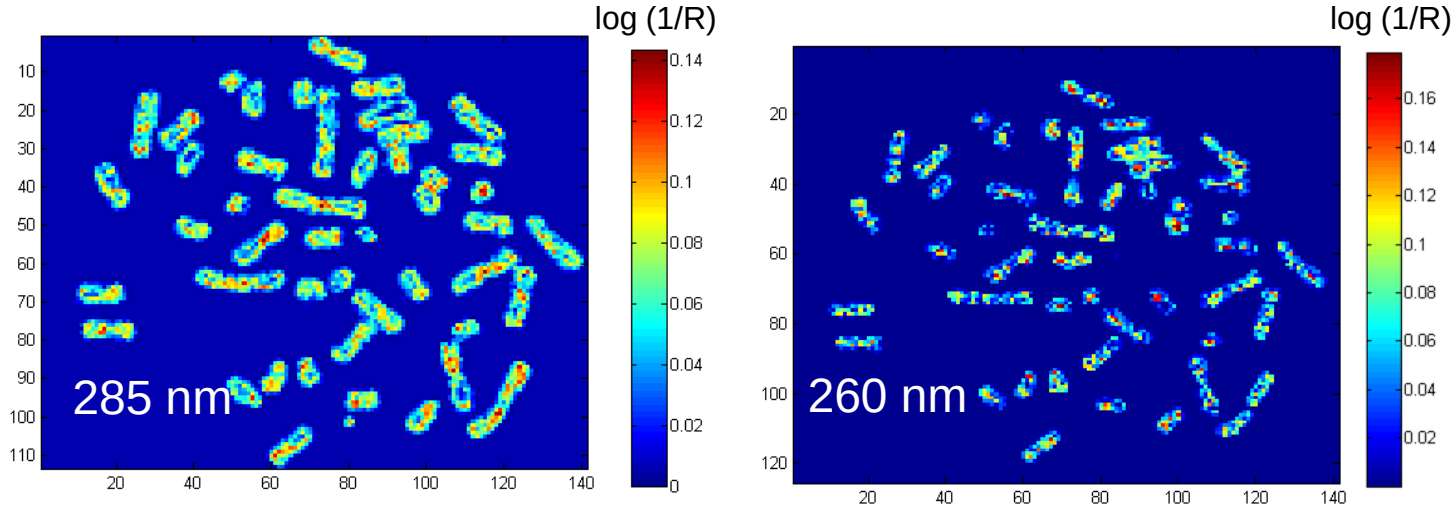


Staring Imaging

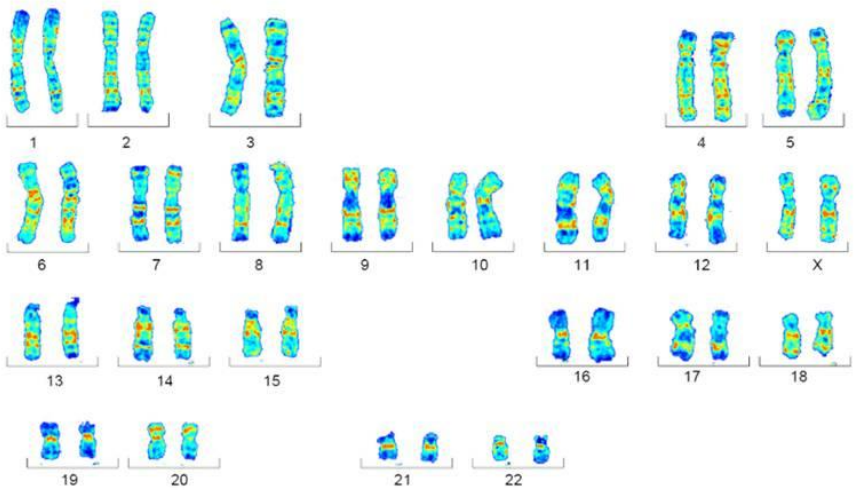




UV Imaging

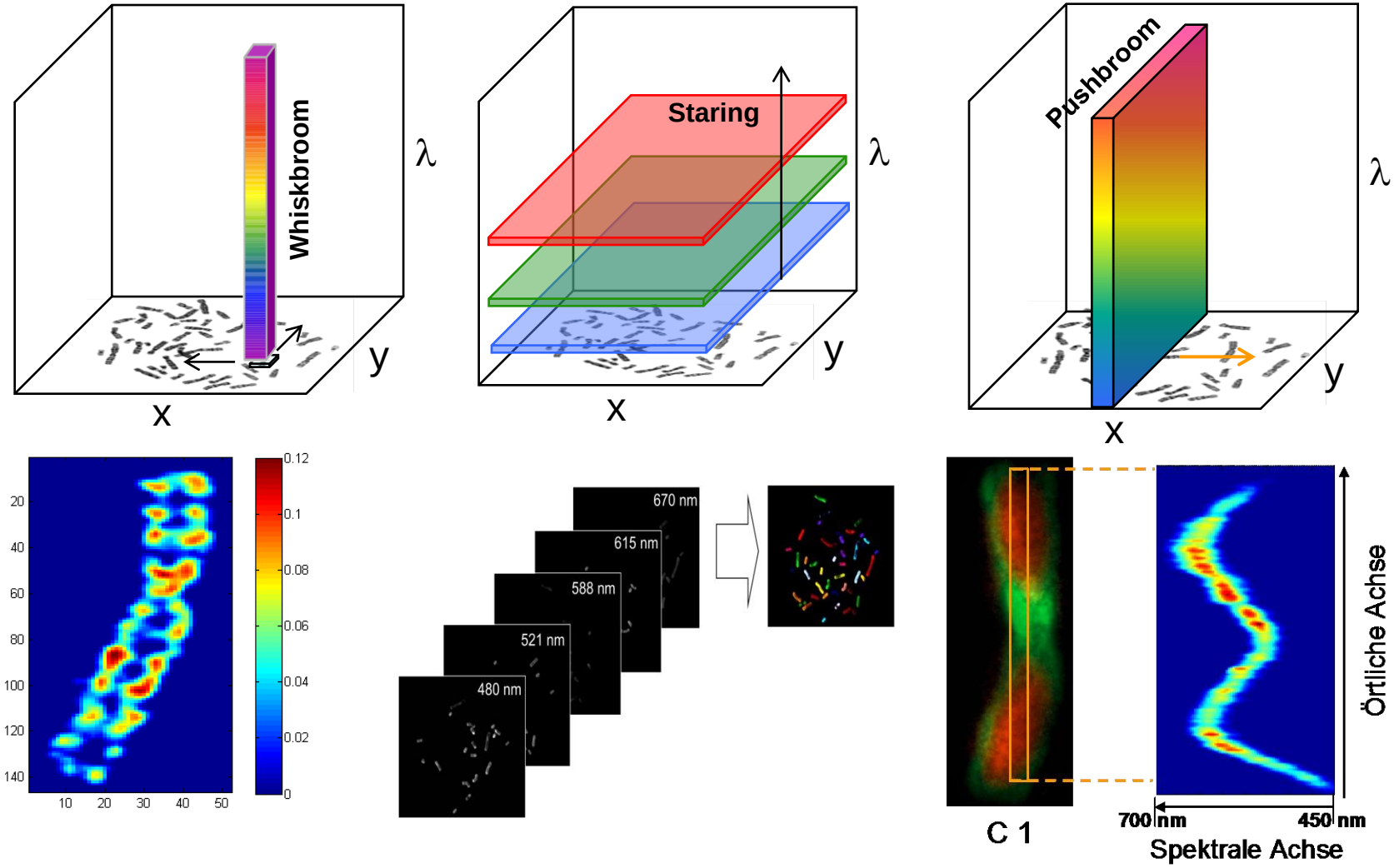


Protein



DNA

Chemical Imaging an Chromosomen



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

Agenda

- Konzept und Versuchsaufbau
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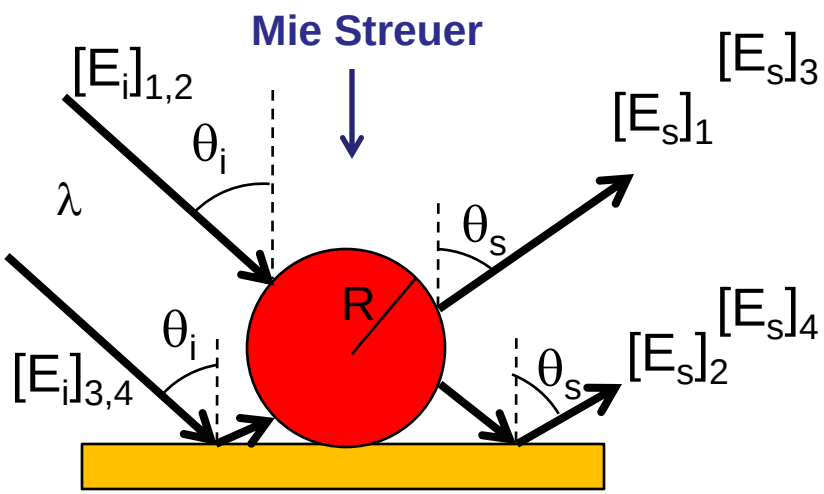
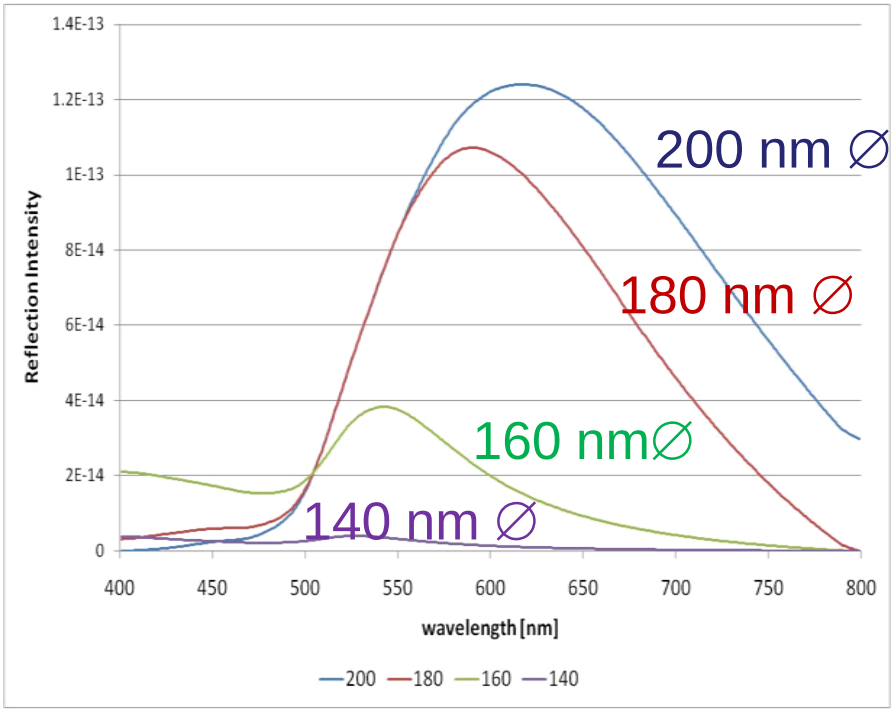
- **Modell**
- Nahfeldspektroskopie

Dunkelfeld Simulationen

Simulation von PS-Partikel mit modifizierter Mie-Theorie





$$[E_s]_1 = A_0 A(\pi-\theta_i-\theta_s)$$

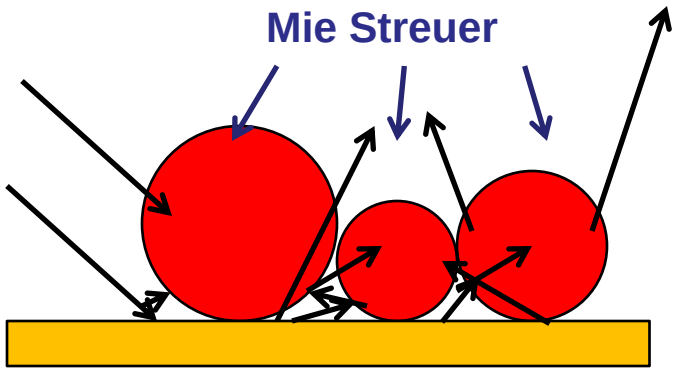
$$[E_s]_2 = A_0 A(\theta_i-\theta_s)\, A_0\, r^*(\theta_s) \exp[i\varphi(\theta_s]$$

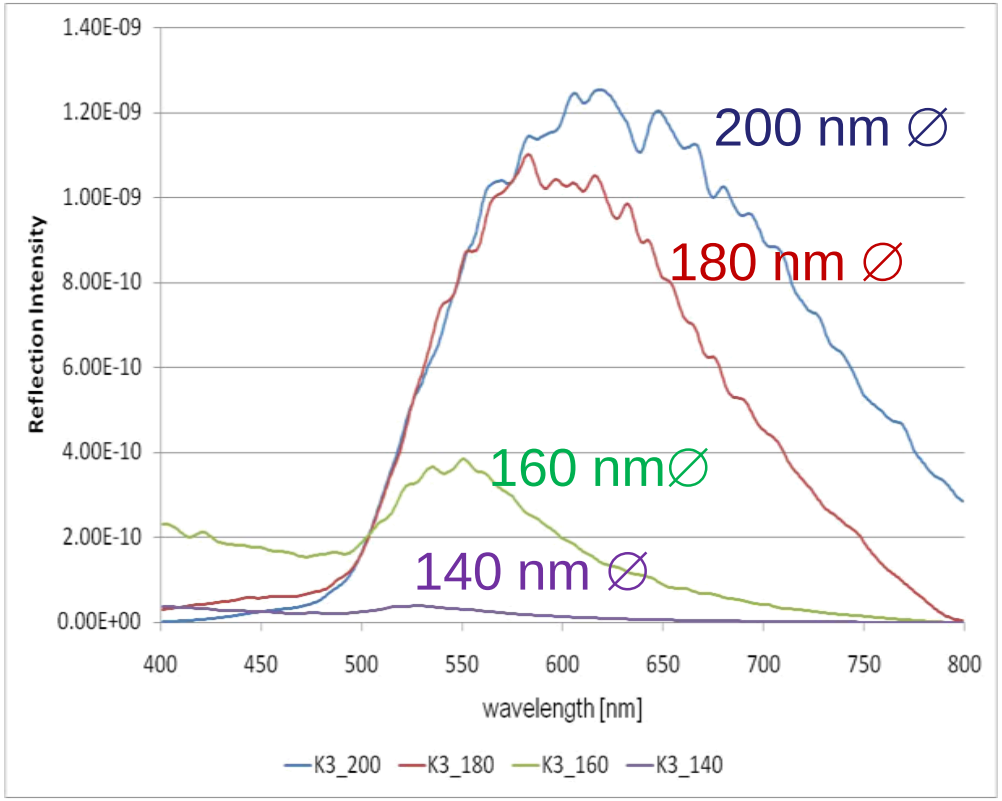
$$[E_s]_3 = A_0 A(\theta_i-\theta_s)\, A_0\, r^*(\theta_i) \exp[i\varphi(\theta_i]$$

$$[E_s]_4 = A_0 A(\pi-\theta_i-\theta_s)\, A_0\, r^*(\theta_i)\, A_0\, r^*(\theta_s) \exp[i\varphi(\theta_i) + i\varphi(\theta_s)]$$

Dunkelfeld Simulationen

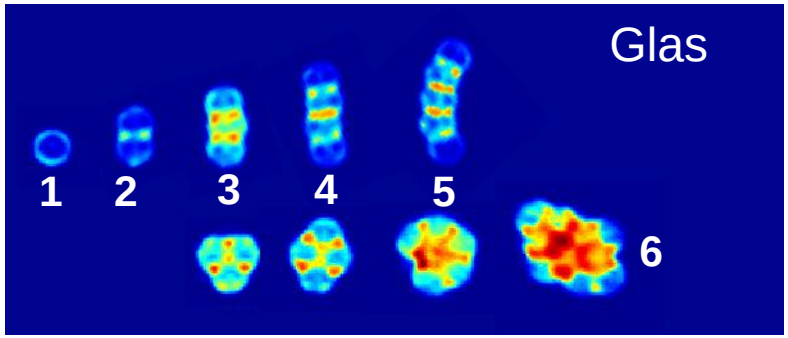
Simulation von PS-Partikel mit modifizierter Mie-Theorie

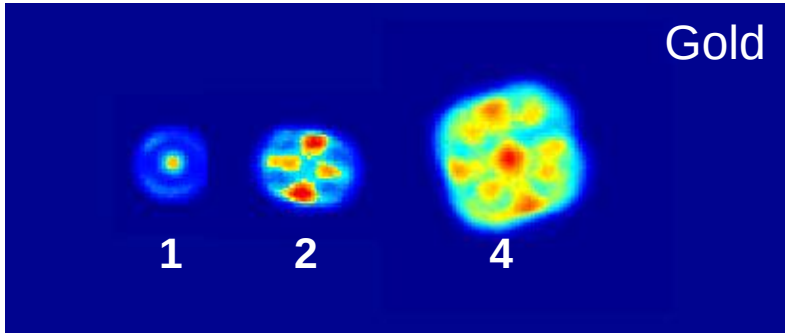




Dunkelfeld Messungen

890 nm PS Partikel auf Glas und Gold





Spectra of 2.9 µm particles

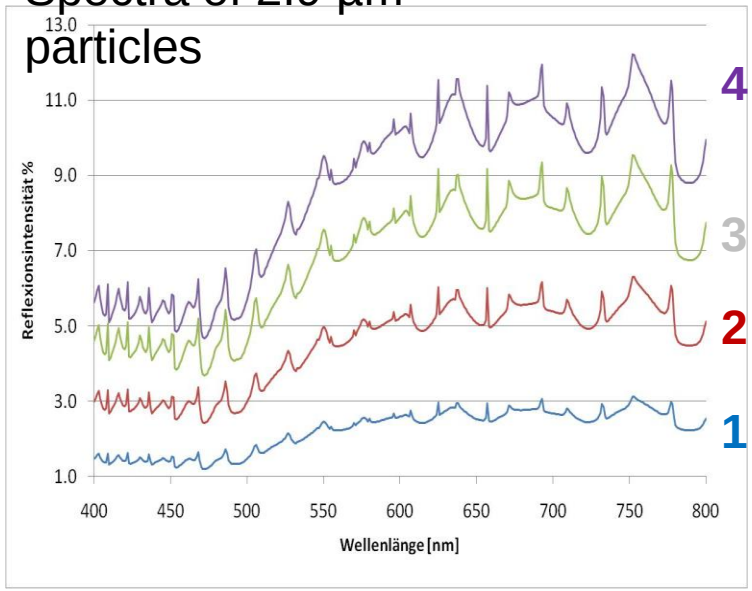
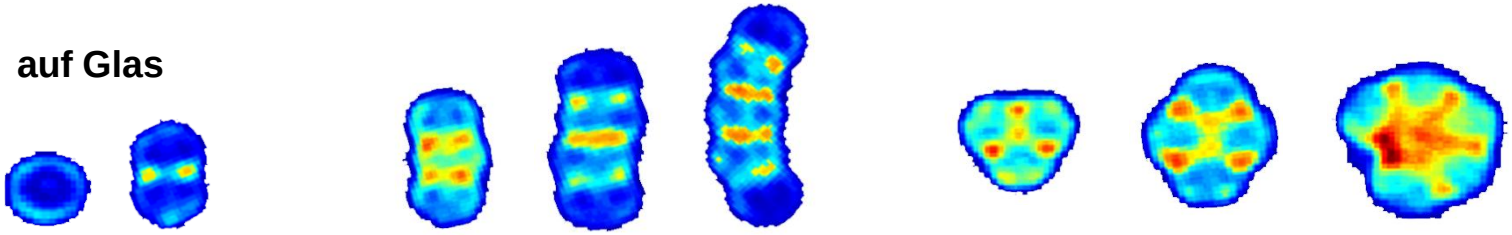


Image von strukturierten Partikeln

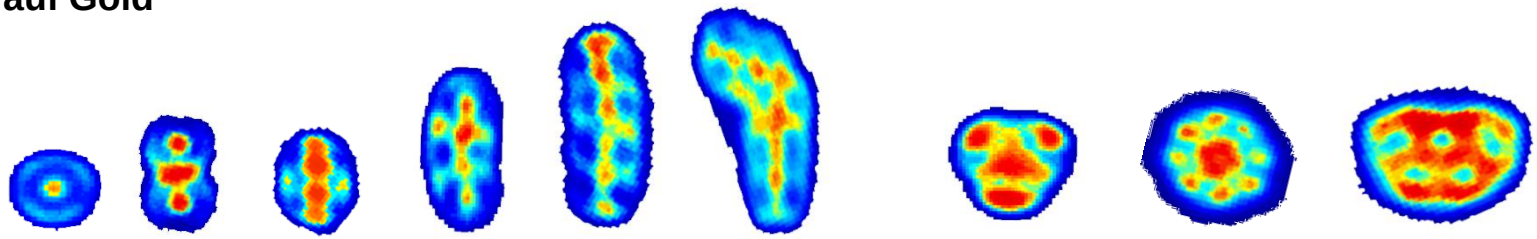
0.8 µm Partikel

auf Glas



G1 G2 G3L G4L G5L G3NL G4NL G5NL

auf Gold



Au1 Au2 Au2a Au3L Au4L Au5L Au3NL Au4NL Au5NL

→ **Reflektion auf Gold ist ca. 7 mal höher als wie auf Glas**

→ **Der Einfluss der Goldoberfläche auf die Spektren nimmt zu , wenn**
a) die Komplexität (Phasengrenze) der Morphologie zunimmt
b) die Kugelgröße kleiner wird

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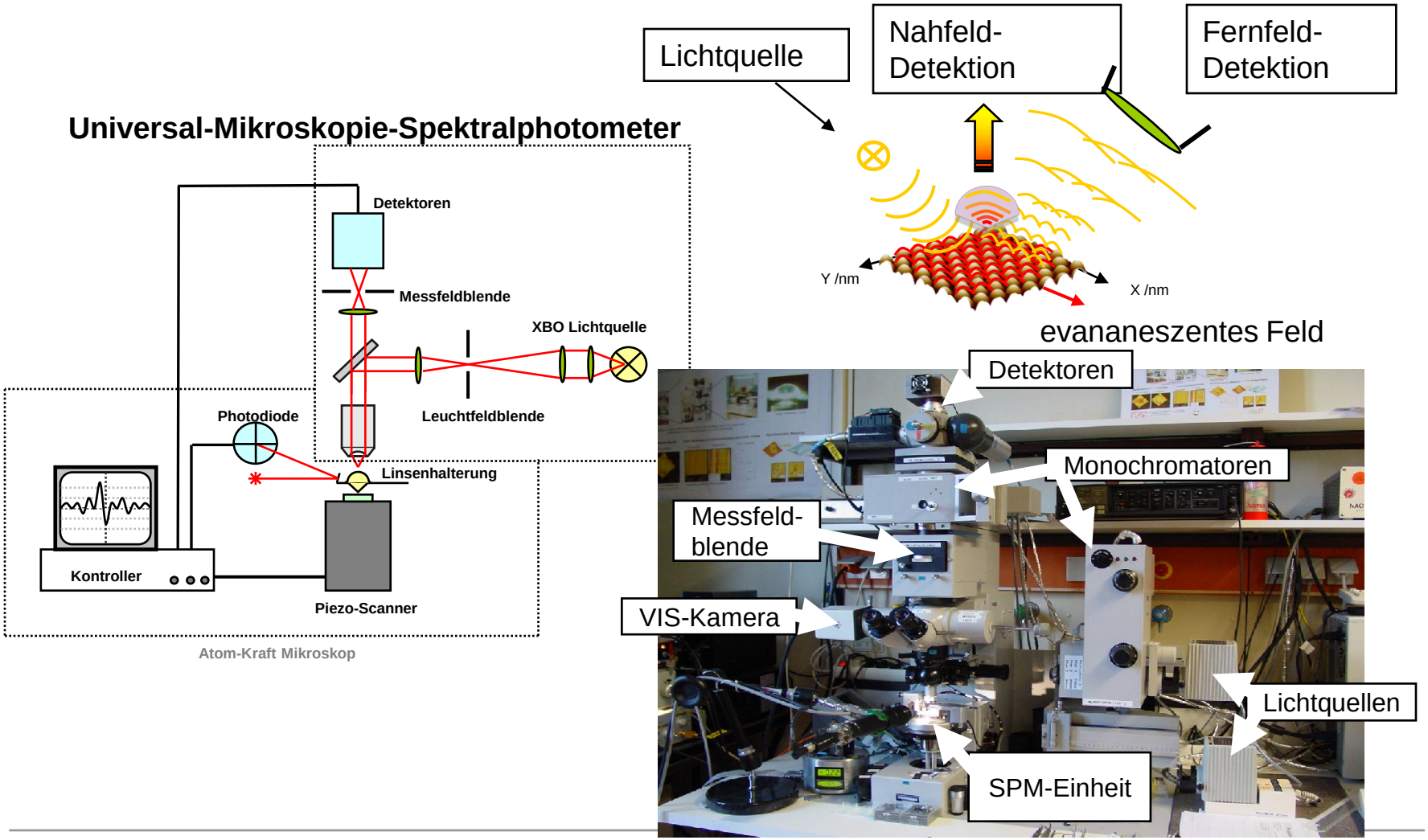
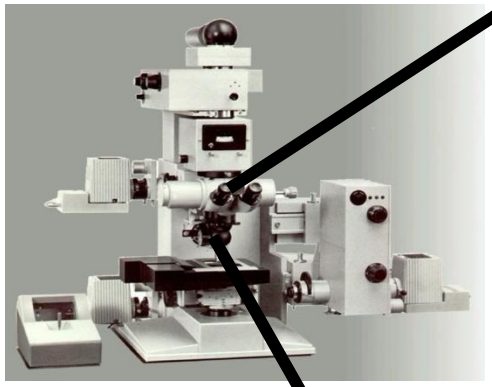
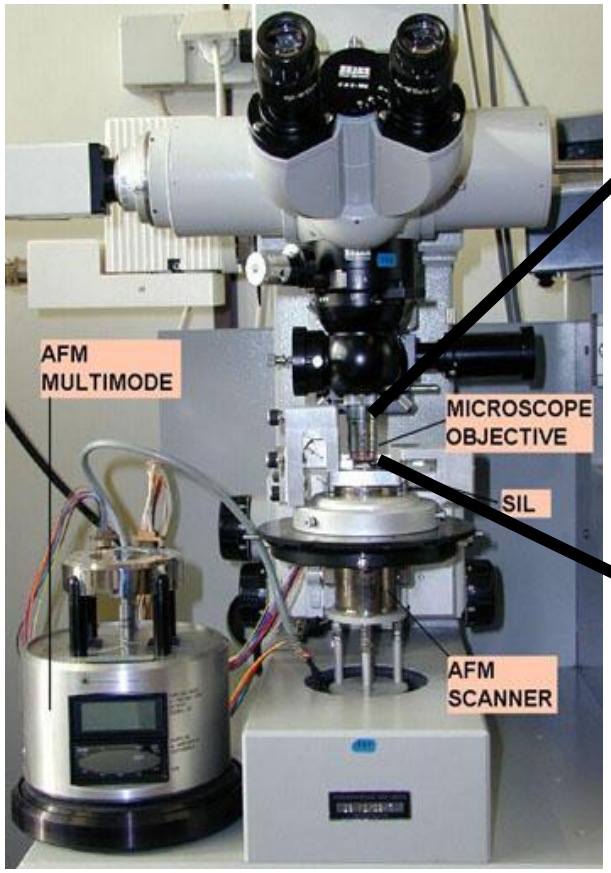
- Konzept und Versuchsaufbau
- Chromosomenkaryotypisierung
- Modell
- **Nahfeldspektroskopie**

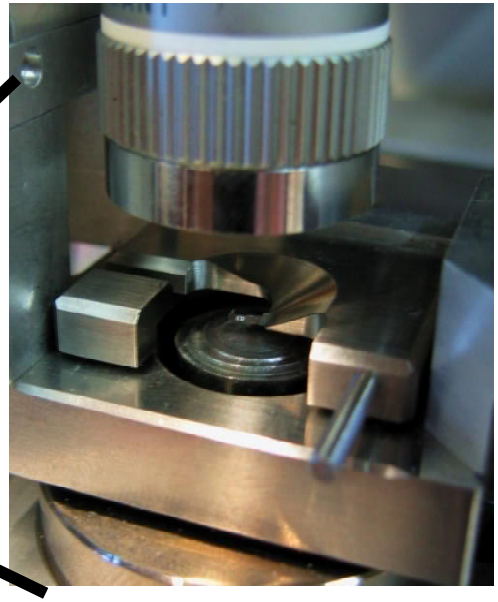
Nahfeld-Imaging

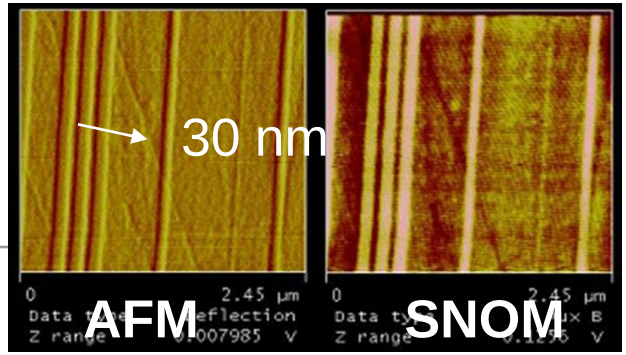


Nahfeld UV/VIS, Fluoreszenz und Raman mit Festkörperimmersionslinse und Mikro-Spektralphotometry

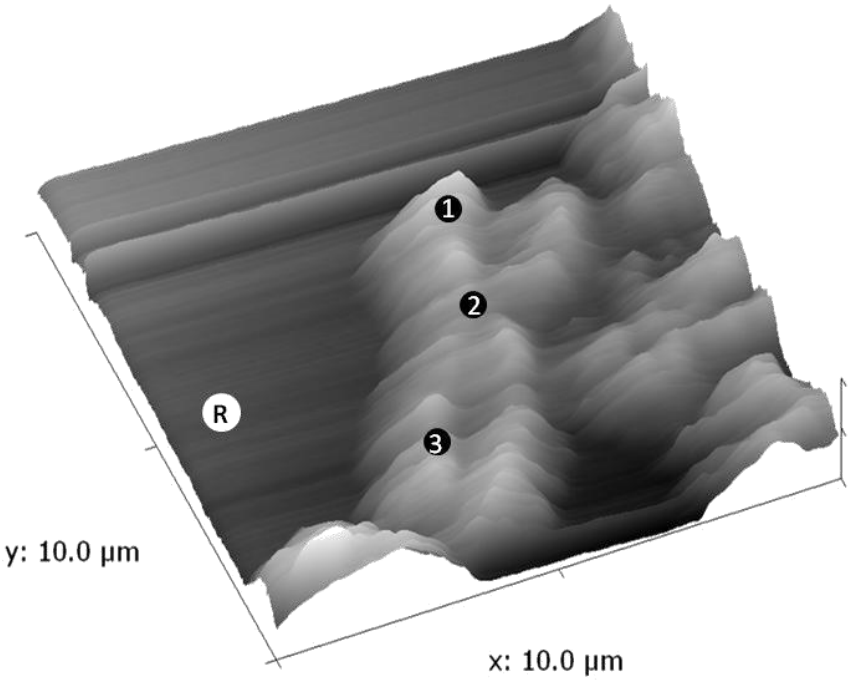


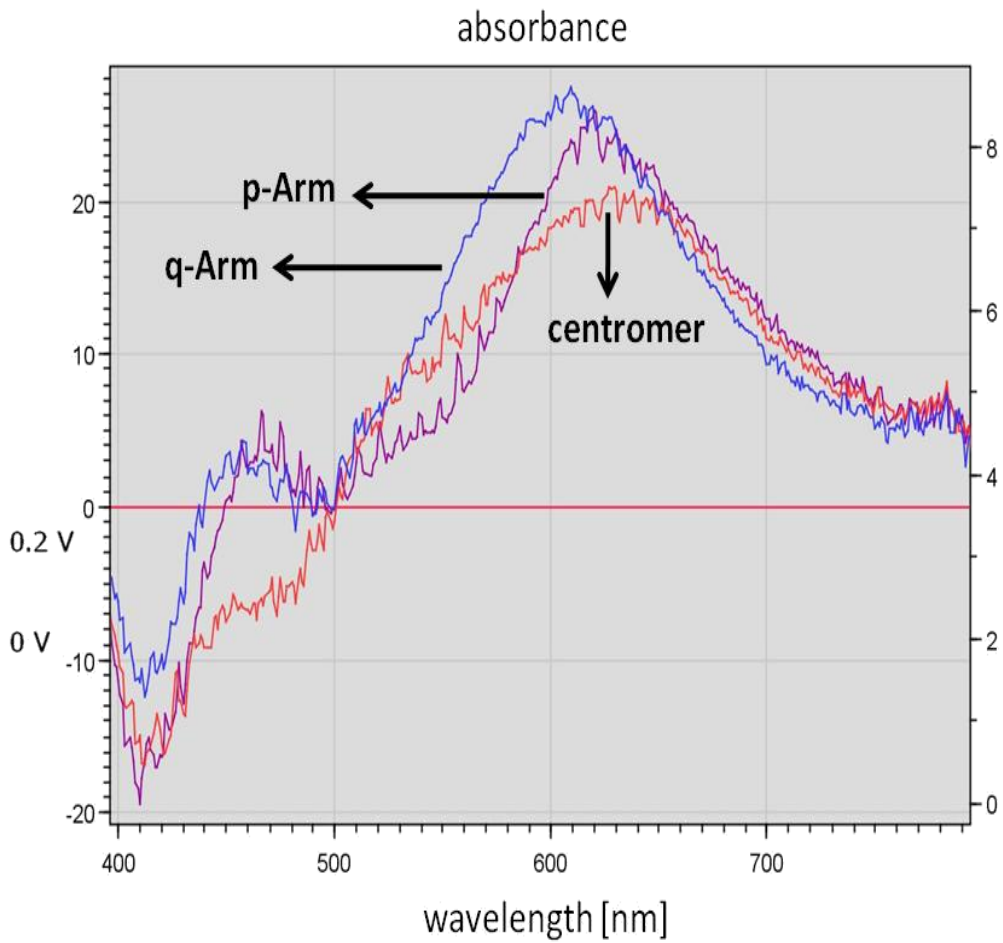






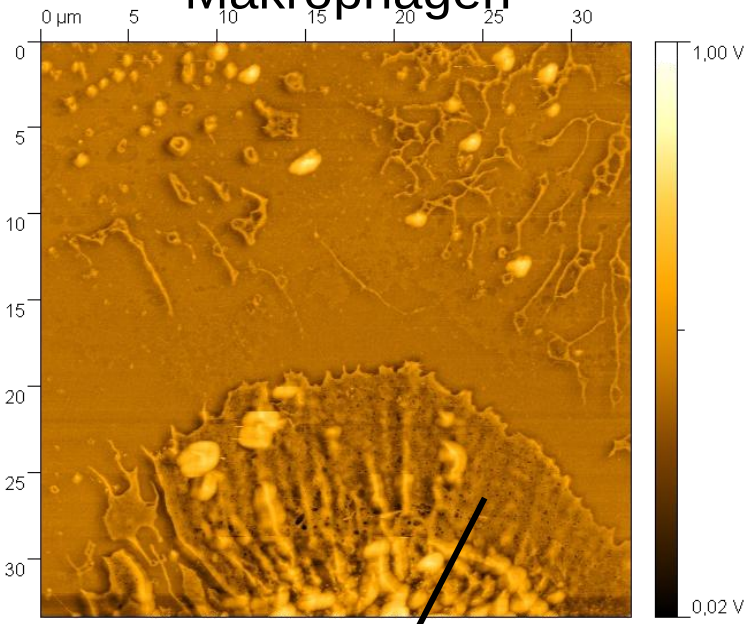
Nahfeld Spektroskopie (SIL-SNOM)

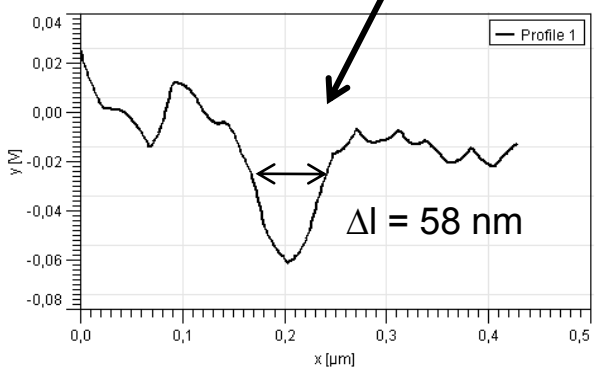




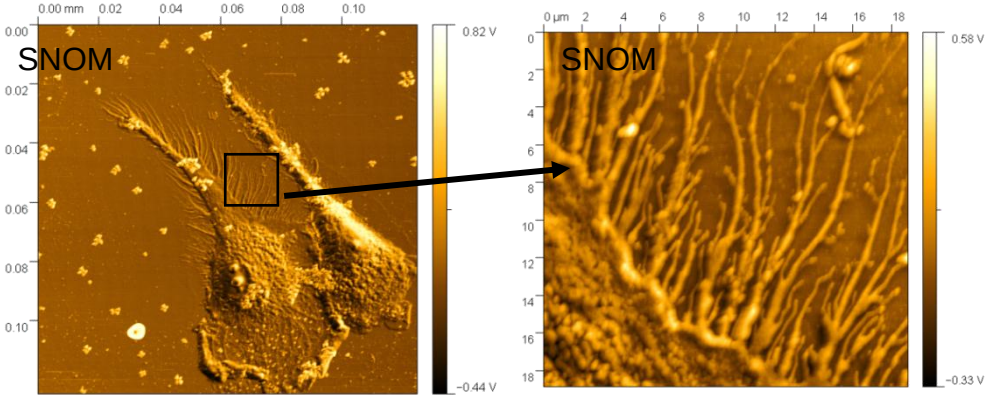
SNOM - Beispiele

Makrophagen

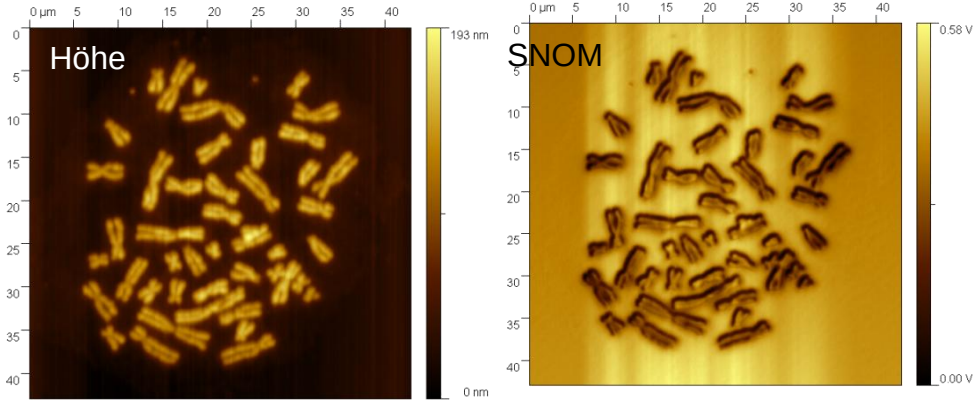




Dendritische Zellen



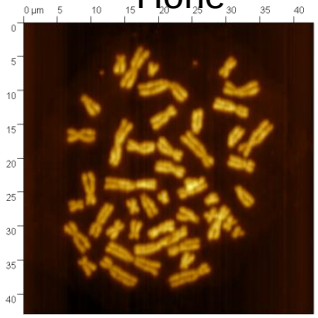
Chromosome



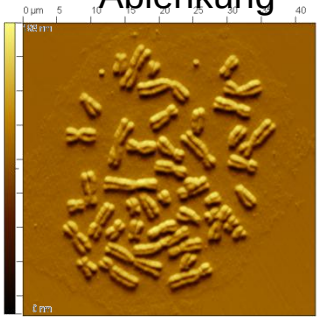
SIL-SNOM Beispiele

Chemical Imaging an Chromosomen

Höhe



Ablenkung



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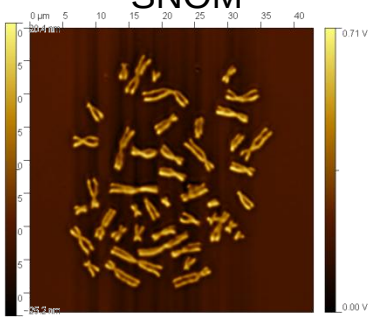
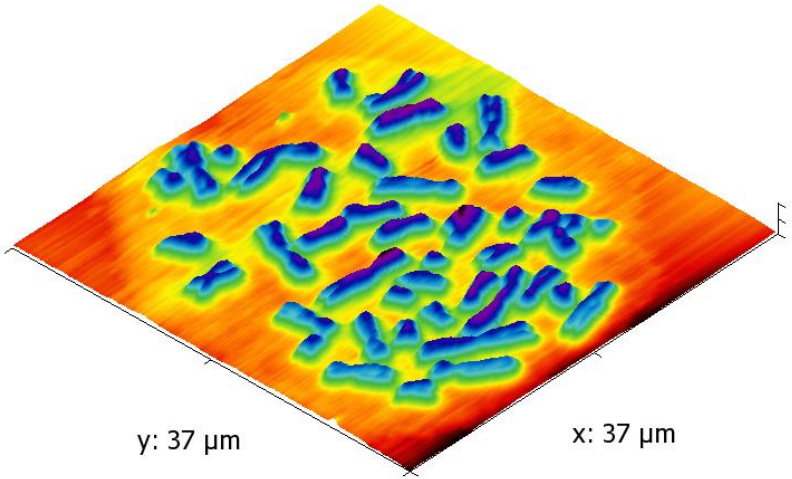
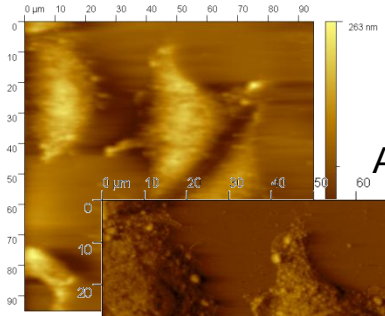


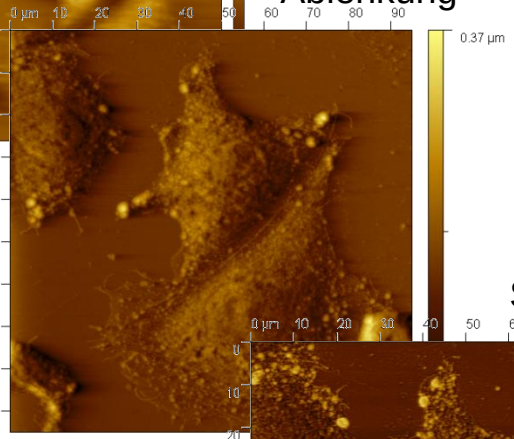


Chemical Imaging an CHO Zellen

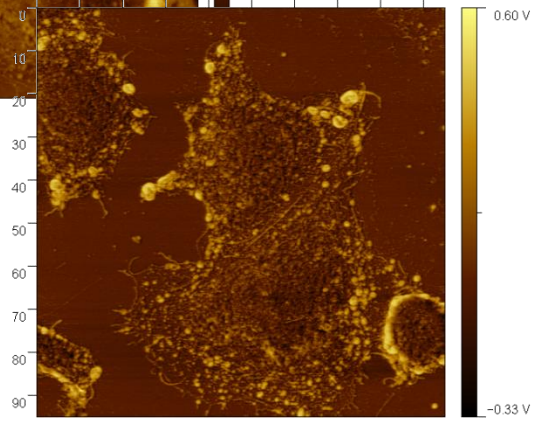
Höhe



Ablenkung



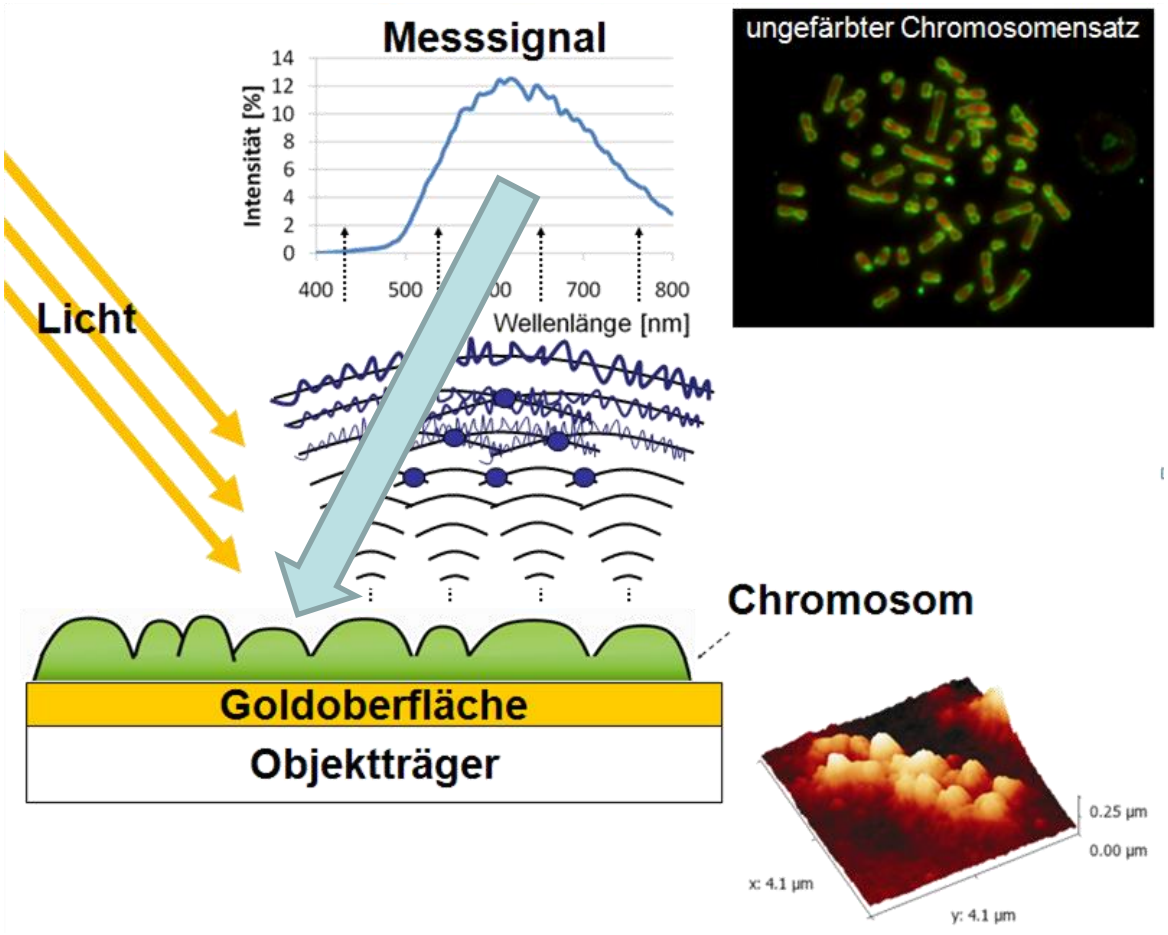
SNOM



Arbeits-, Zeit- und Kooperationsplan

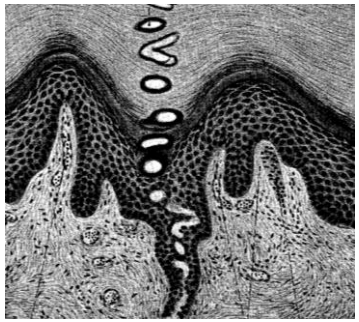
Monate	Arbeitspakete	PM	HS	Meilensteine	(Monat)
01-12	Etablierung von Anregungs- / Emissions-spektroskopie, Fluorescence Lifetime Imaging und Tiefenauflösung	12	RT	Funktionsfähige 6D-Mikroskopie	(12)
06-18	„Diffuse optical imaging“ an Modellsystem (Chromosomen)	06	RT (ILM)	Charakterisierung der Chromosomen ist mit diffus-optischer Abbildung möglich	(18)
19-27	Nutzung diffus-optischer Methoden und Anpas-sung der Auswerteverfahren (Zellkulturmodell)	09 06	RT MA	Validierung am biologischen Modell	(27)
25-36	Bioimaging am Alzheimer-Modellsystem	09 06	RT MA	Erfassung krankheitsspezischer Veränderungen	(36)

Diffuse Optical Imaging



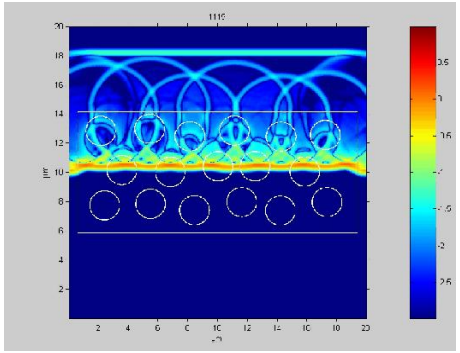
Lichtausbreitung in streuenden Medien - Theorie

Maxwelltheorie

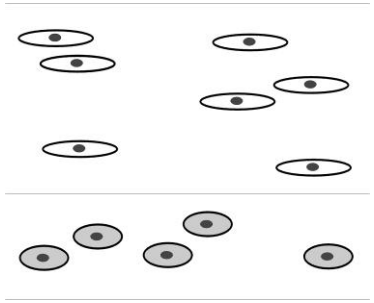


Mikroskopische Skala

Lösung:
FDTD, DDA, analytisch

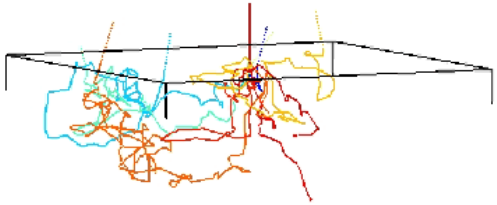


Transporttheorie

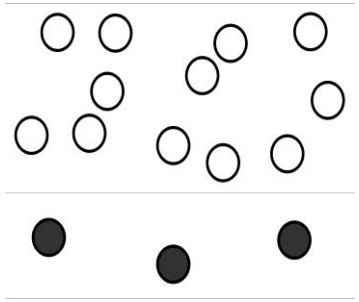


Makroskop. Skala

Lösung:
Monte Carlo Methode



Diffusionstheorie



Makroskopische Skala

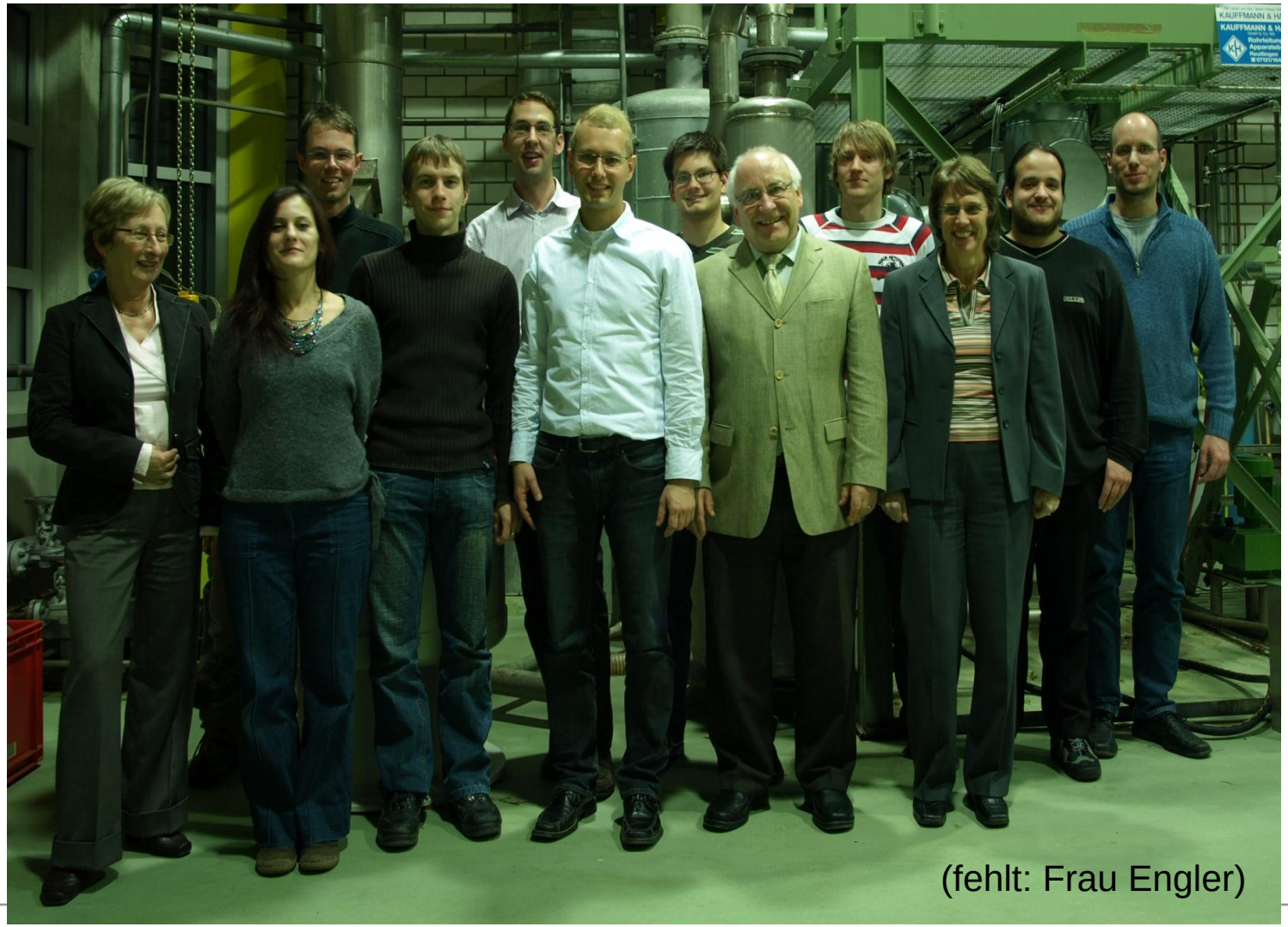
Analytische Lösungen

$$\phi_1(z,s) = \frac{\sinh[\alpha_1(z_b+z_0)]}{D_1\alpha_1} \times \frac{D_1\alpha_1\cosh[\alpha_1(l-z)] + D_2\alpha_2\sinh[\alpha_1(l-z)]}{D_1\alpha_1\cosh[\alpha_1(l+z_b)] + D_2\alpha_2\sinh[\alpha_1(l+z_b)]} - \frac{\sinh[\alpha_1(z_0-z)]}{D_1\alpha_1}, \quad 0 \le z < z_0, \tag{11}$$

$$\phi_1(z,s) = \frac{\sinh[\alpha_1(z_b+z_0)]}{D_1\alpha_1} \times \frac{D_1\alpha_1\cosh[\alpha_1(l-z)] + D_2\alpha_2\sinh[\alpha_1(l-z)]}{D_1\alpha_1\cosh[\alpha_1(l+z_b)] + D_2\alpha_2\sinh[\alpha_1(l+z_b)]}, \quad z_0 < z < l, \tag{12}$$

$$\Phi_i(\rho,z) = \frac{1}{(2\pi)^2}\int_{-\infty}^{\infty}\int_{-\infty}^{\infty}\phi_i(z,s)\exp[-i(s_1x+s_2y)]\mathrm{d}s_1\mathrm{d}s_2 = \frac{1}{2\pi}\int_0^{\infty}\phi_i(z,s)sJ_0(s\rho)\mathrm{d}s, \tag{14}$$

Mein Team



(fehlt: Frau Engler)