ZAFH – PHOTON^{n}

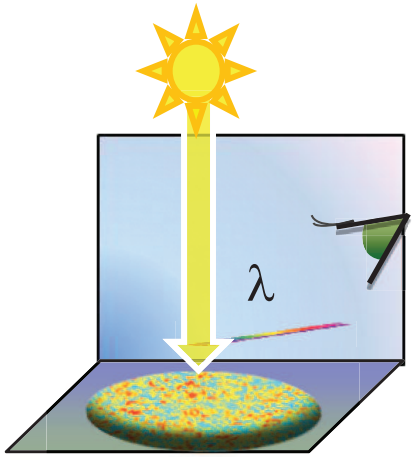
PHOTONische Verfahren in neuen Dimensionen

Hochschule Reutlingen
Prof. Dr. Rudolf Kessler

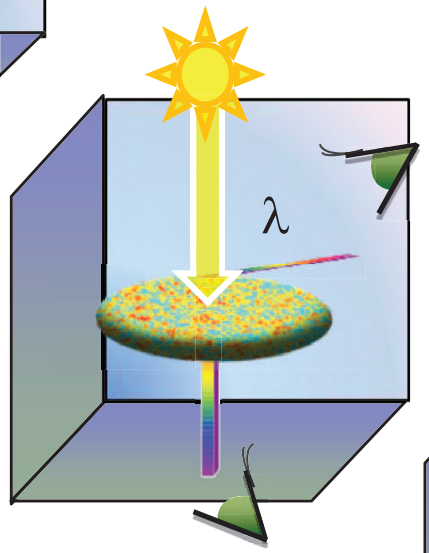
Multimodale Spektroskopie zur *markierungsfreien* Charakterisierung mikro- und nanoskaliger Strukturen: *Beispiel Chromosomen*

4. Aalener Photoniktag

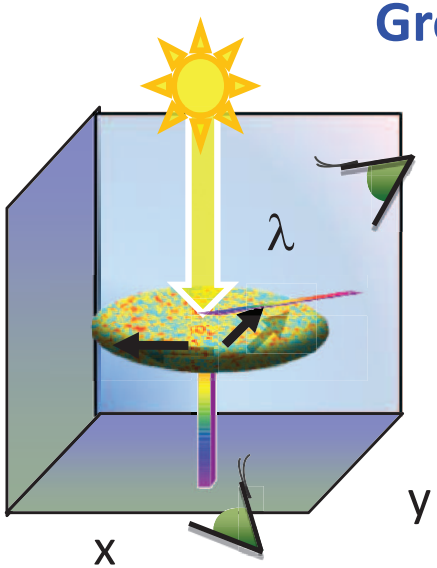
Toolbox: Multimodale Optische Spektroskopie



Reflexion

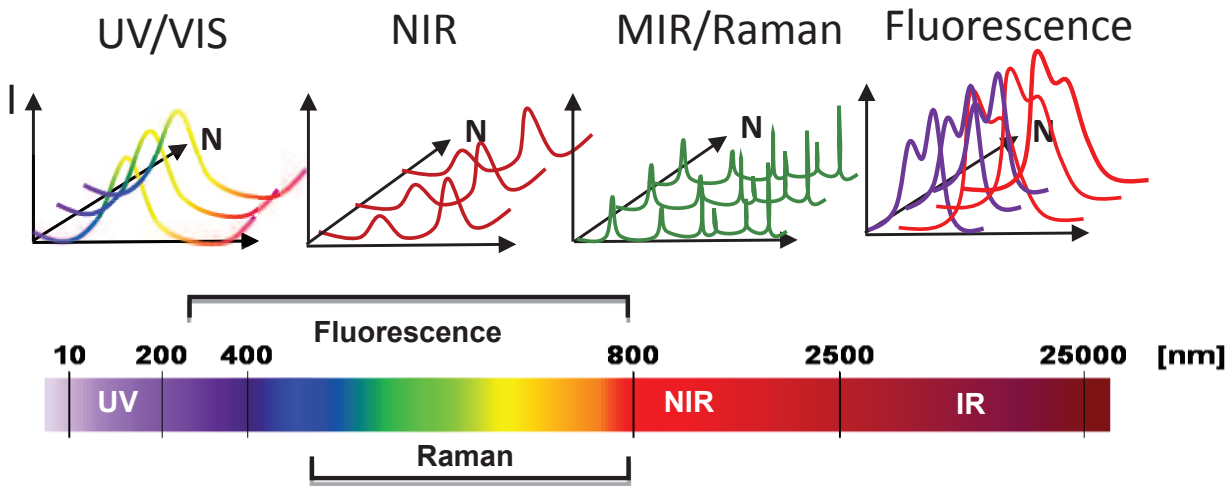


Transmission

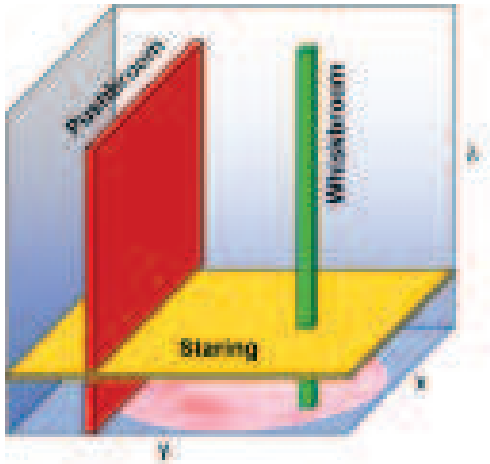


Ortsaufgelöst (x,y)

unterschiedliche Konfiguration

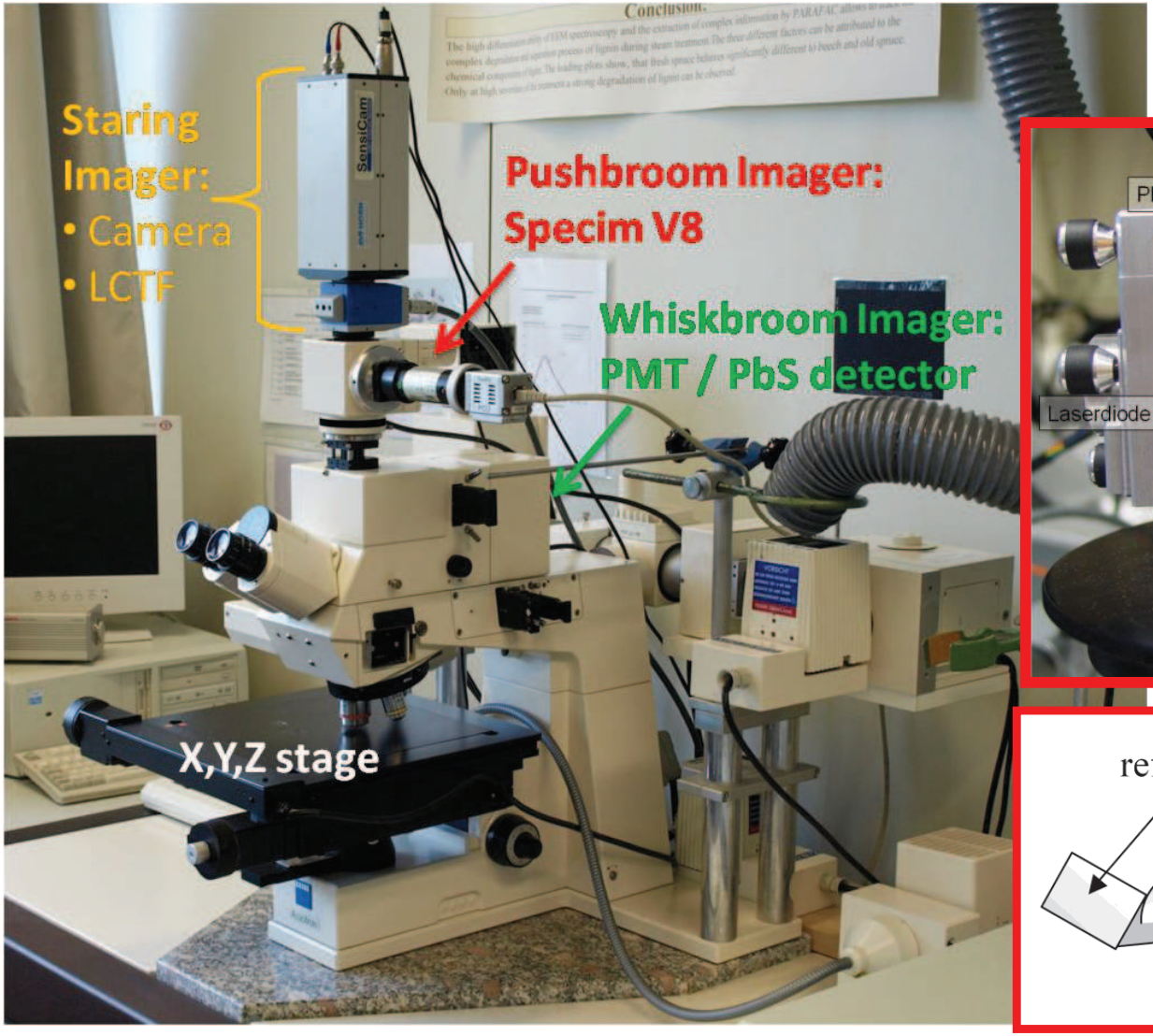


Großer Spektralbereich

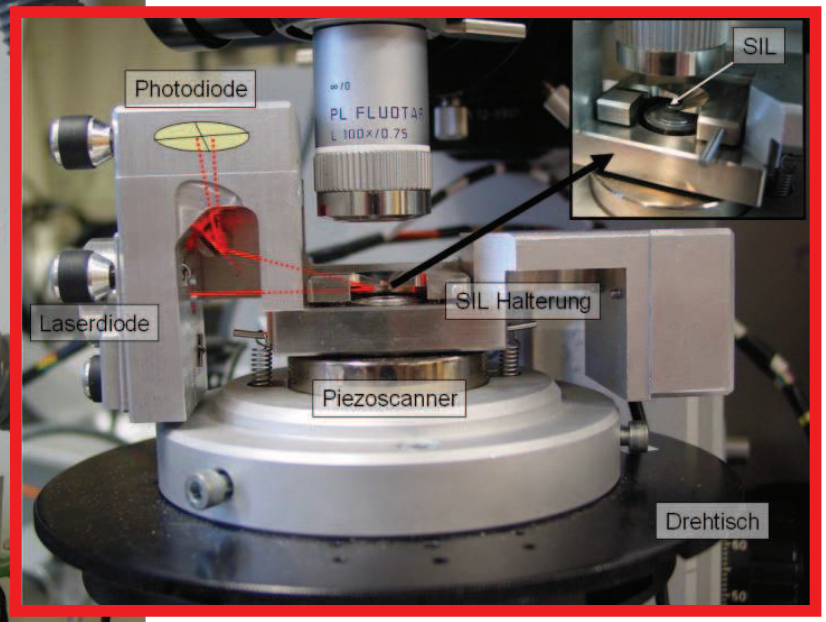


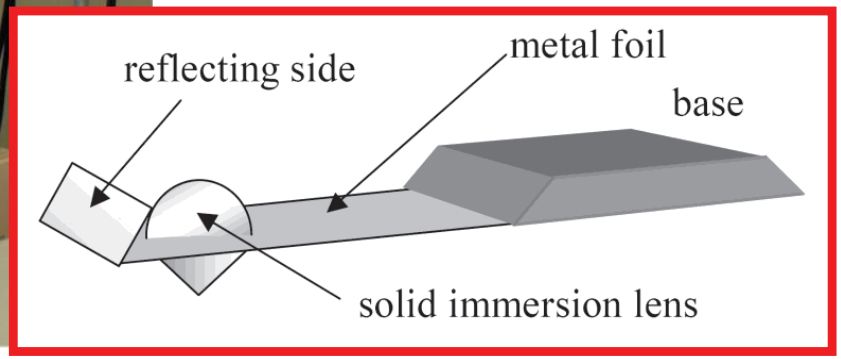
Hyperspectral imaging

Multimodal Chemical Imaging System UMSP und MPM: UV-, Vis-Spektroskopie, 2D-Fluoreszenz

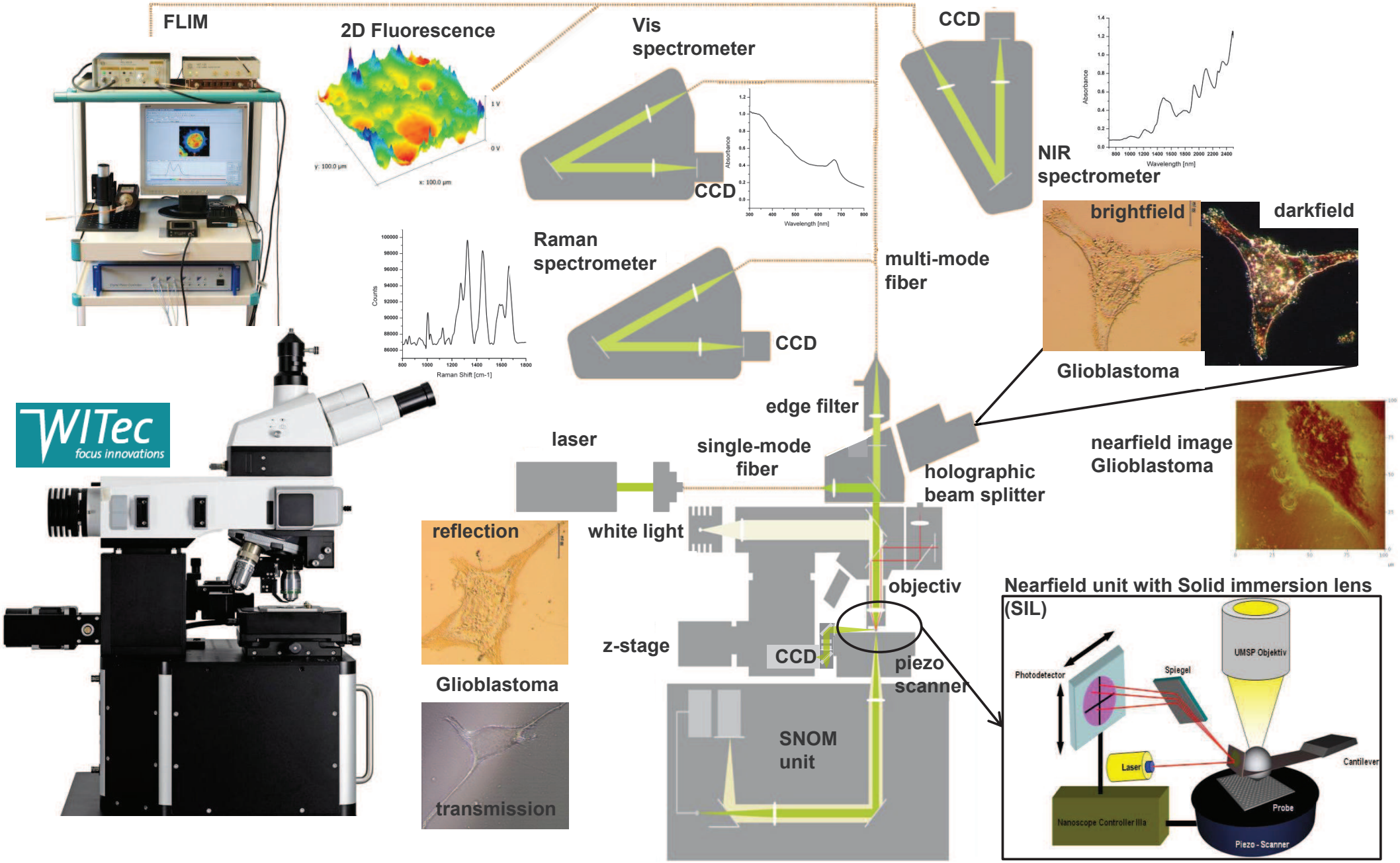


Nahfeld Einheit





Multimodal Spatially Resolved (Near Field) Spectroscopy



Wichtig:

Spektroskopie erfasst

ALLE chemischen Informationen über die Messung der Absorption von Licht

ALLE morphologischen Informationen (Textur der Partikel, Kolloide, etc.) über die Messung der Streuung von Licht

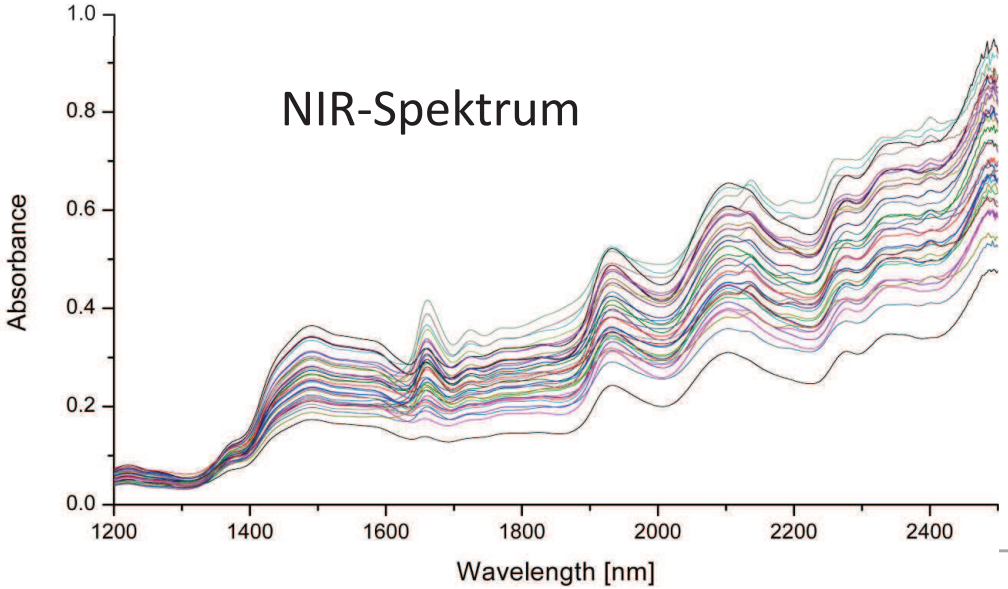
PROBLEM: Überlagernde, vielfältige Informationen

→ **Information (mathematisch) verdichten und extrahieren**

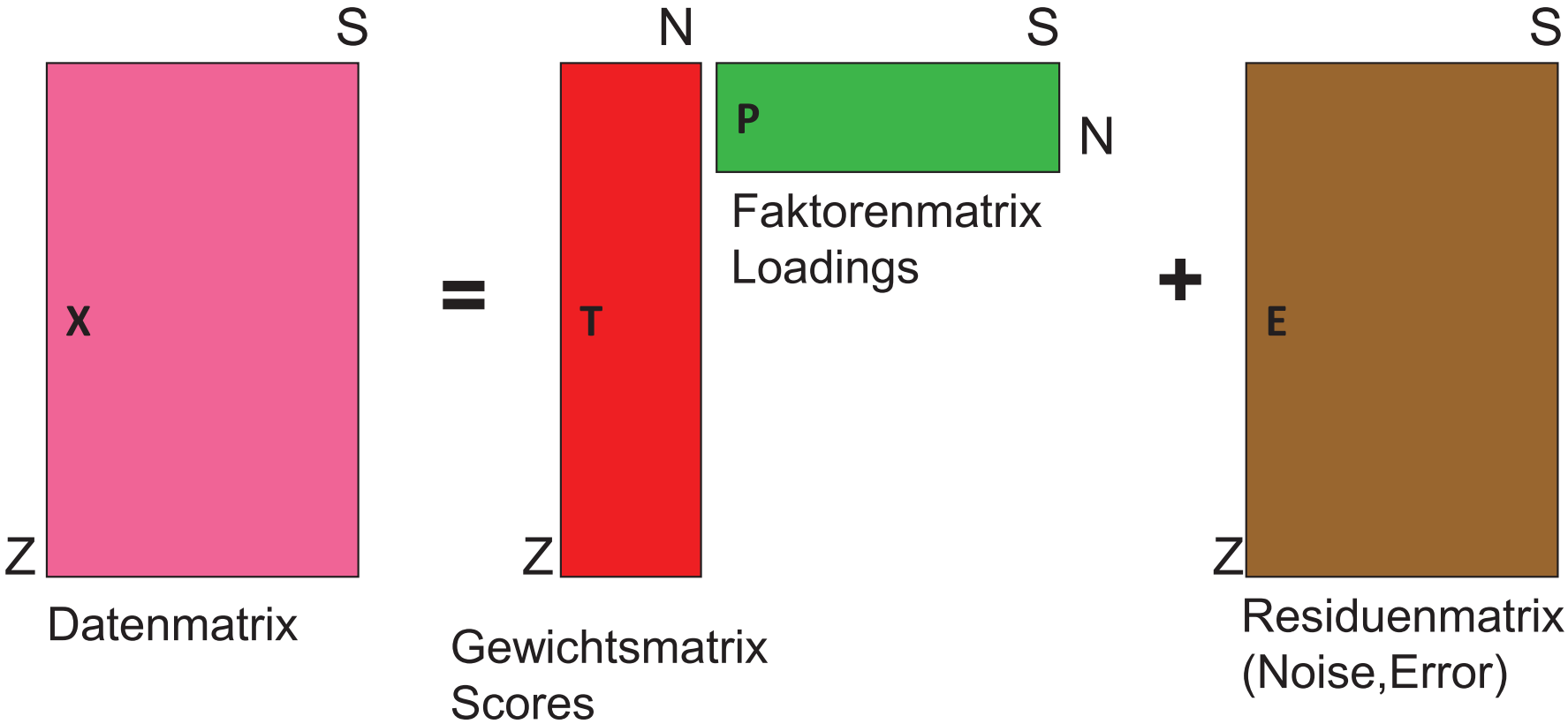
Toolbox Chemometrie = Mustererkennung

ERKENNEN (berechnen) der maximalen Varianz!!!!!!!!!!!
= Principal Component Analyse (PCA)

Hauptkomponente 1:
Ein Kopf mit Augen und Mund
Hauptkomponente 2: Unterschiede in der Mundform
Hauptkomponente 3:
Unterschiede in der Augenform
Hauptkomponente 4:
Unerklärter Anteil (Ausreißer??)



Prinzip der PCA



Multivariate Curve Resolution (MCR): Integration von Wissen und Nebenbedingungen

Agenda

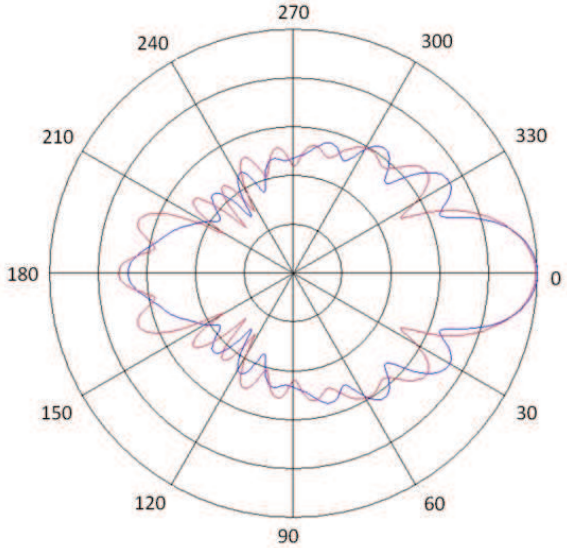
- Streulichtspektroskopie
- Mie Streuspektren von Modell-Partikeln, Partikel-Aggregaten und von Chromosomen
- Vielfachstreuung von Partikeln
- Vielfachstreuung von Chromosomen
- Neue Konzepte

Model System Particles: Straylight Spectroscopy:

single particle

- size, Δn, etc.

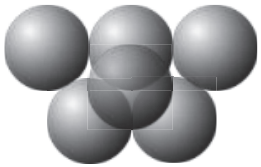
e.g. Mie-Scattering



+
complexity

particle

assemblies

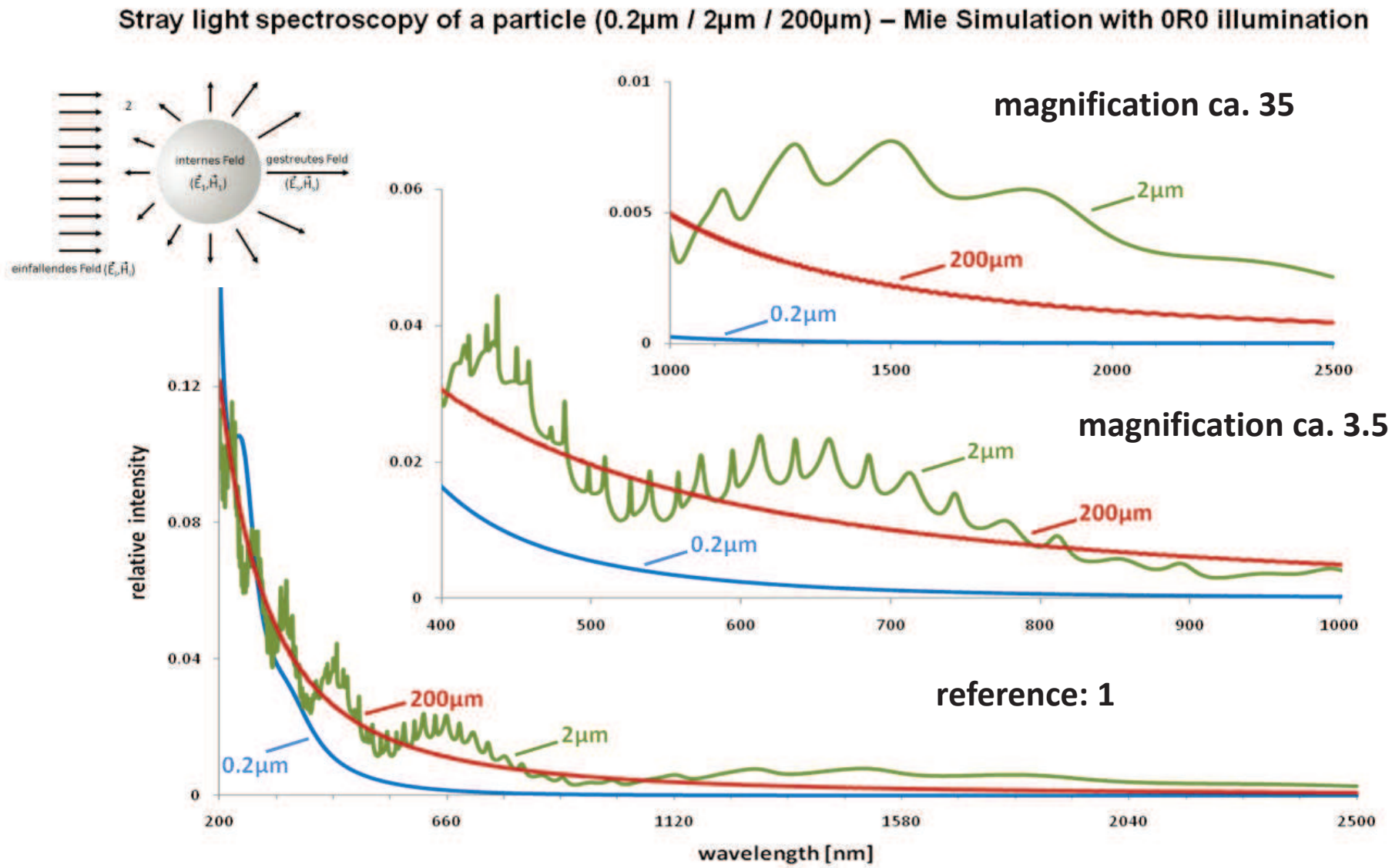


- size, Δn, etc.
- number, geometry
- topology

?

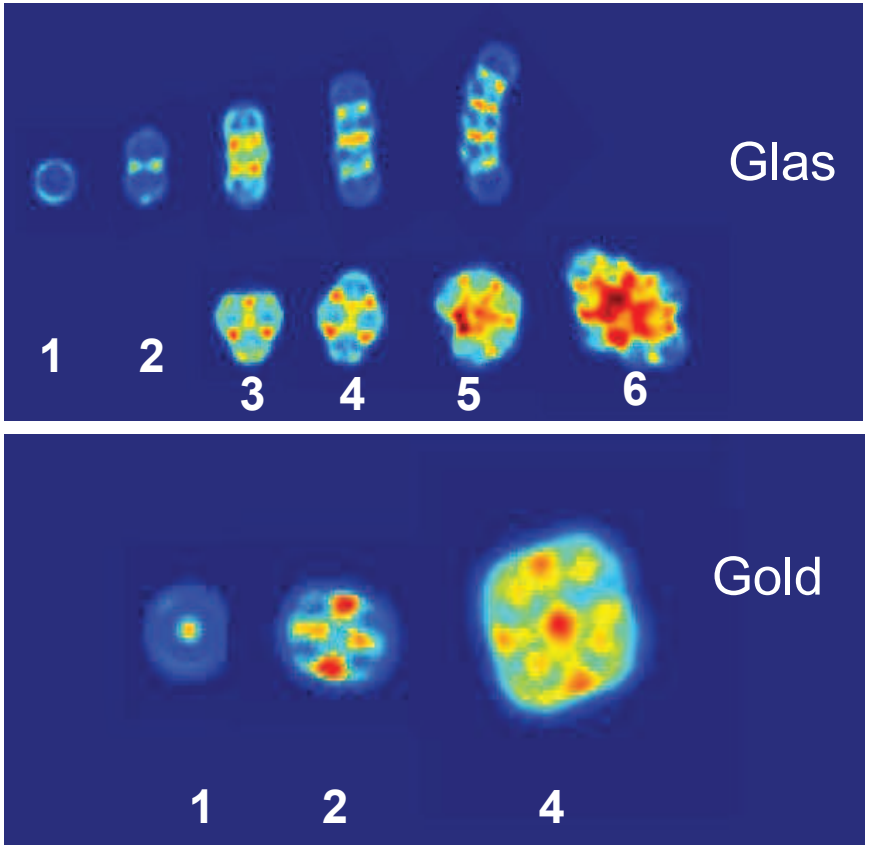
e.g. FDTE simulations???

Mie Calculations of Stray Light Spectra of a Single Particle

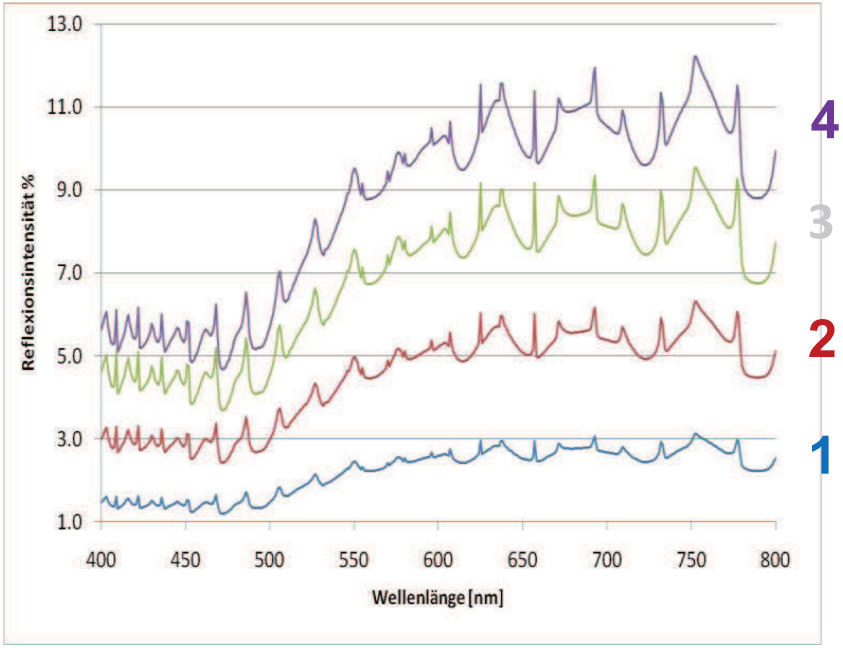


Mie Scattering of Particle Assemblies: Center Point Measurements

890 nm PS Particles on Glas und Gold



RGB pictures in dark field arrangement



PC1: 55% sample preparation

PC2: 31% number of particles

PC3: 9% angular or linear assembly

PC4: 4% contact area

e.g. Chromosomes = Nanostructured „Particle“ Assembly

metaphase-chromosomes

or **cancer cells**

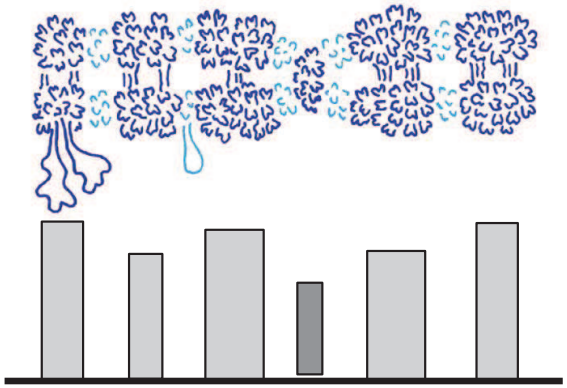
= complex systems

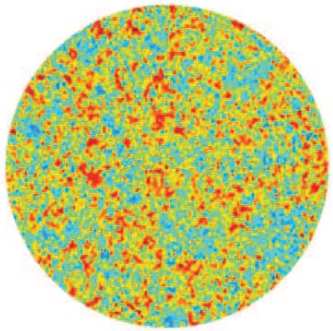
complexity
increases

e.g. tablets, powders

multiple scattering systems

„Bar-Code System“ Chromosome





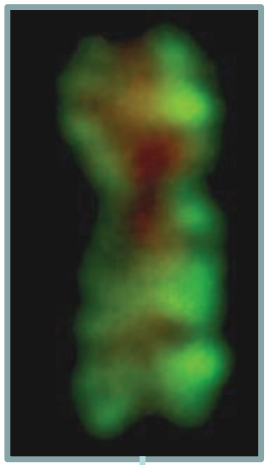
- size, centromer position
- morphology, texture
- banding structure,
- chemical composition

Theory??????????

scatter vs absorption

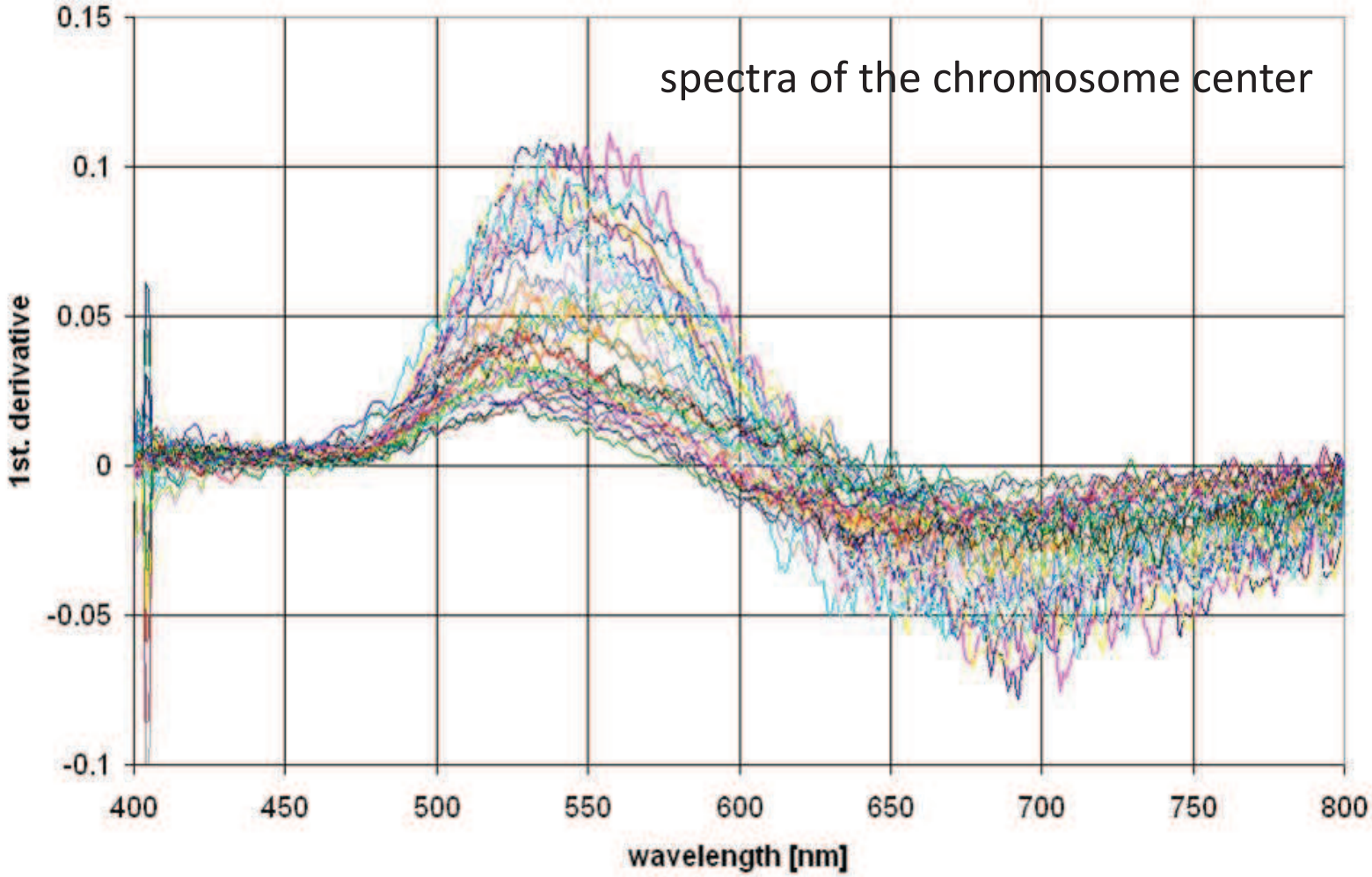
(KM, RTE, Maxwell etc.)

Label Free Straylight Spectra of Chromosomes

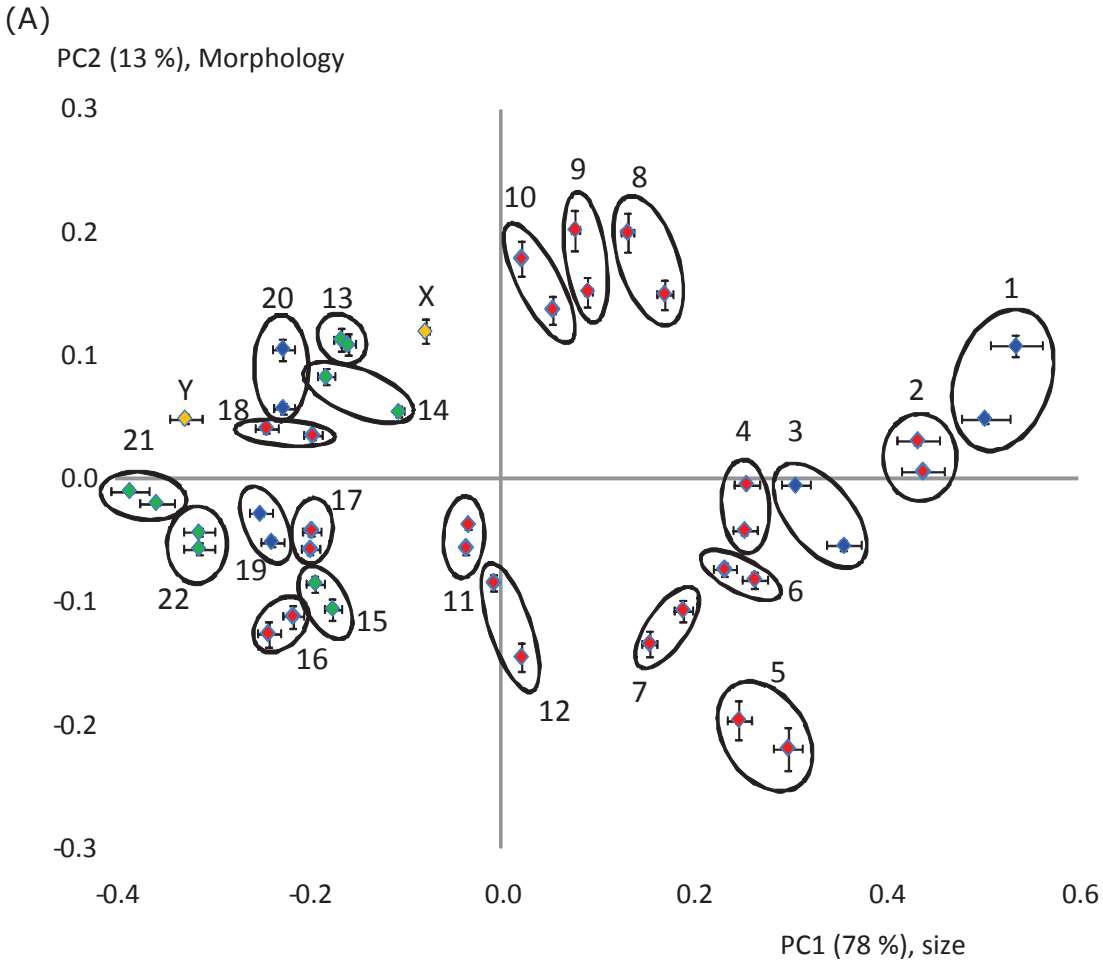


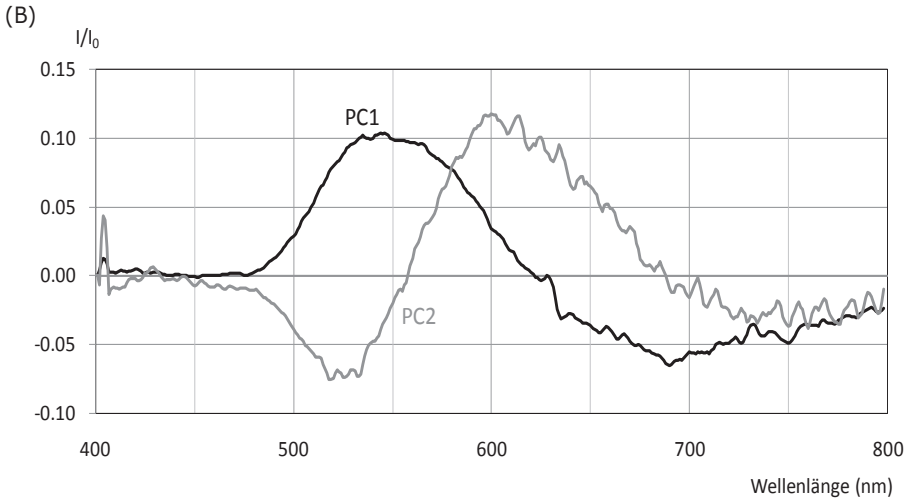
measuring field

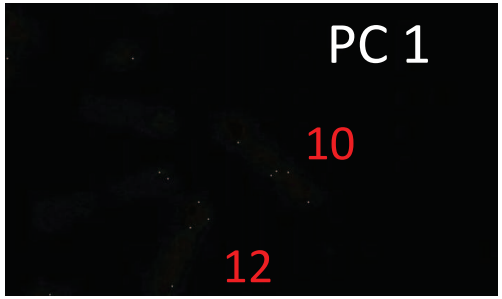
1st derivative of each chromsome spectra

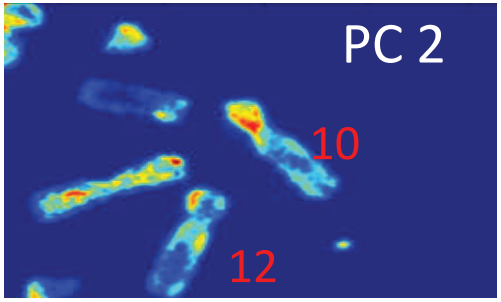


Markerfree Karyotyping: Morphology

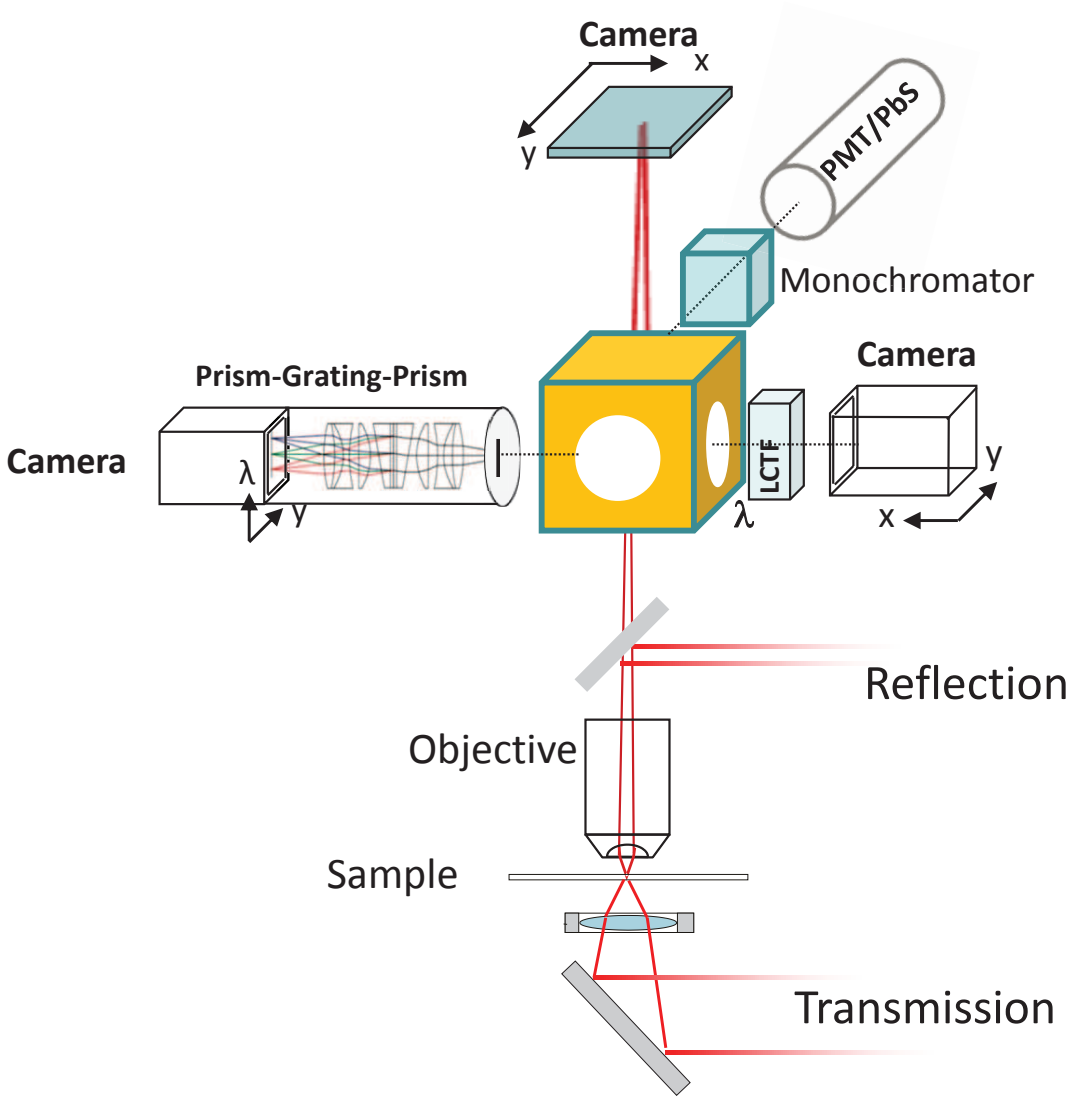


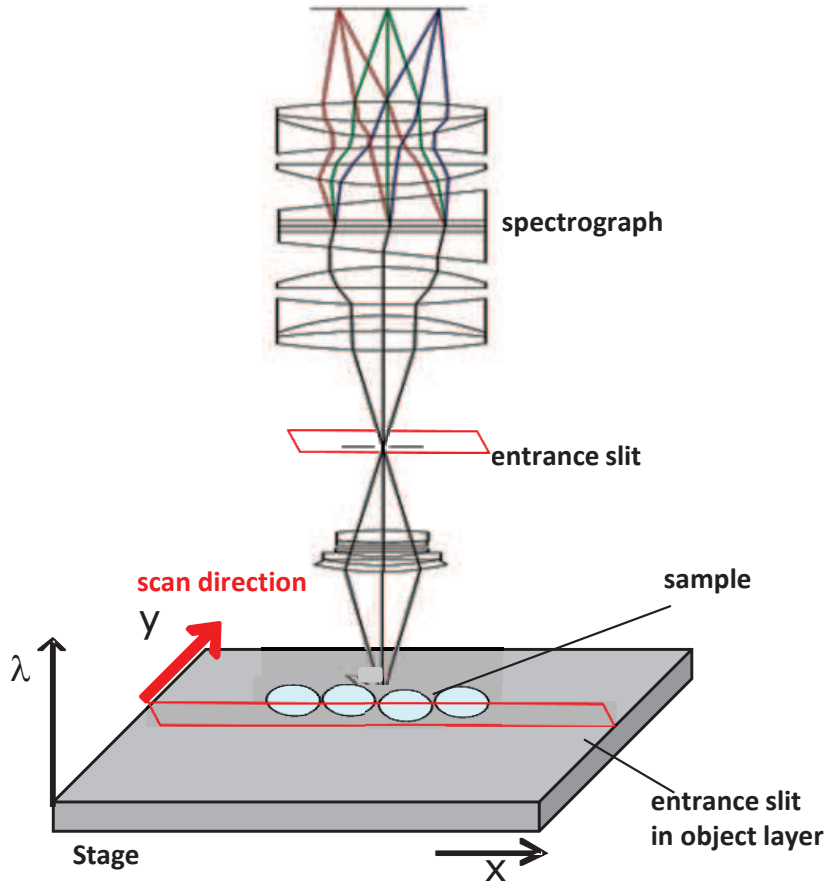




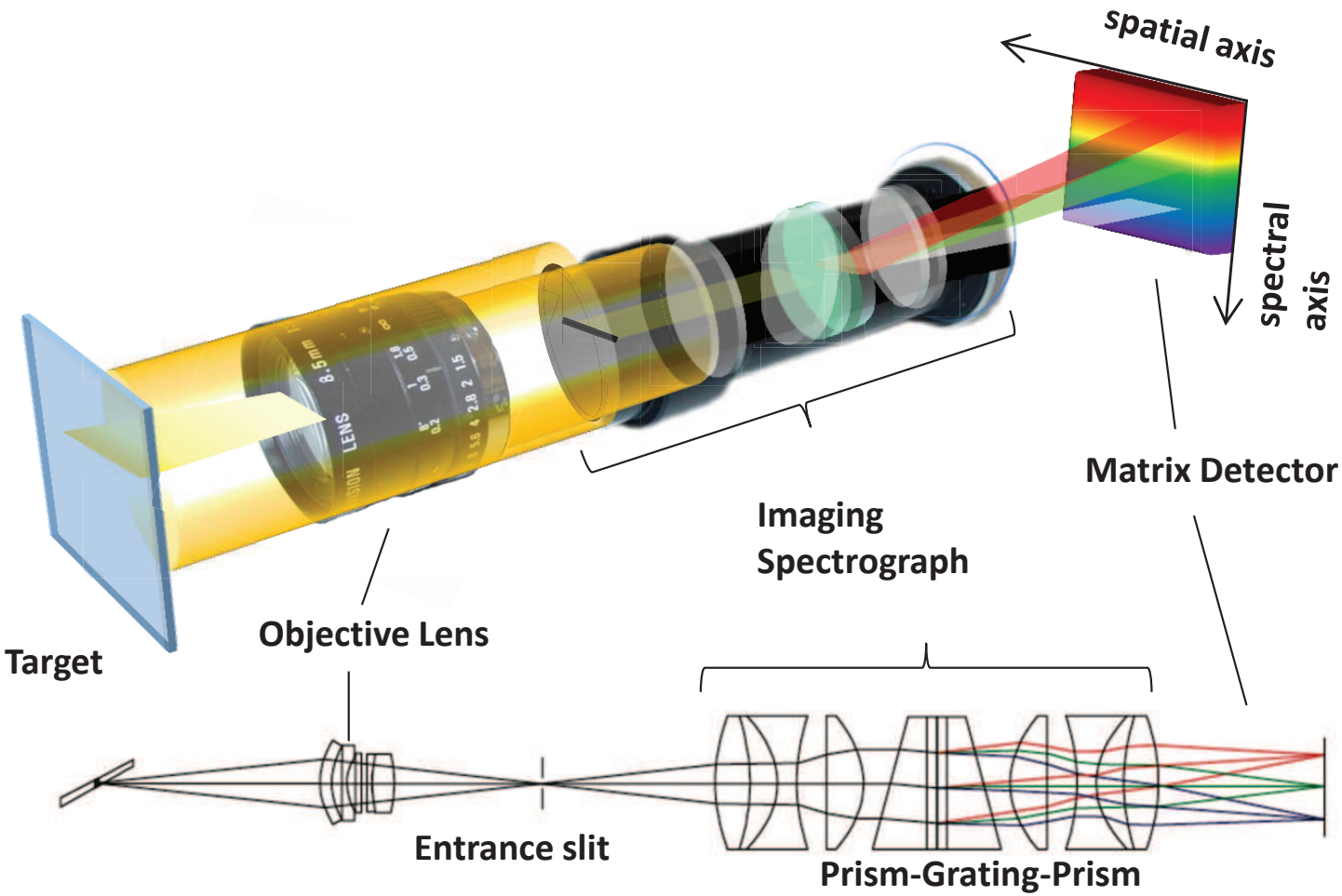


Real Life Measurements of Chromosomes by Pushbroom Imaging

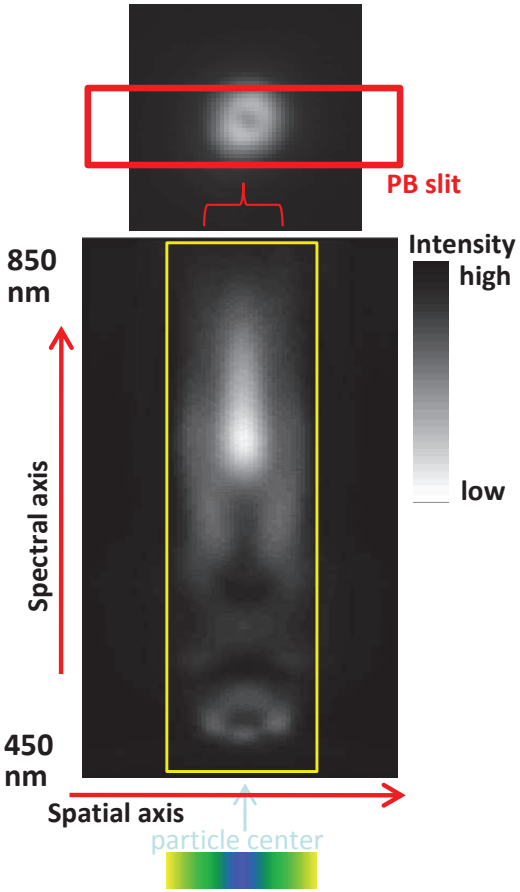


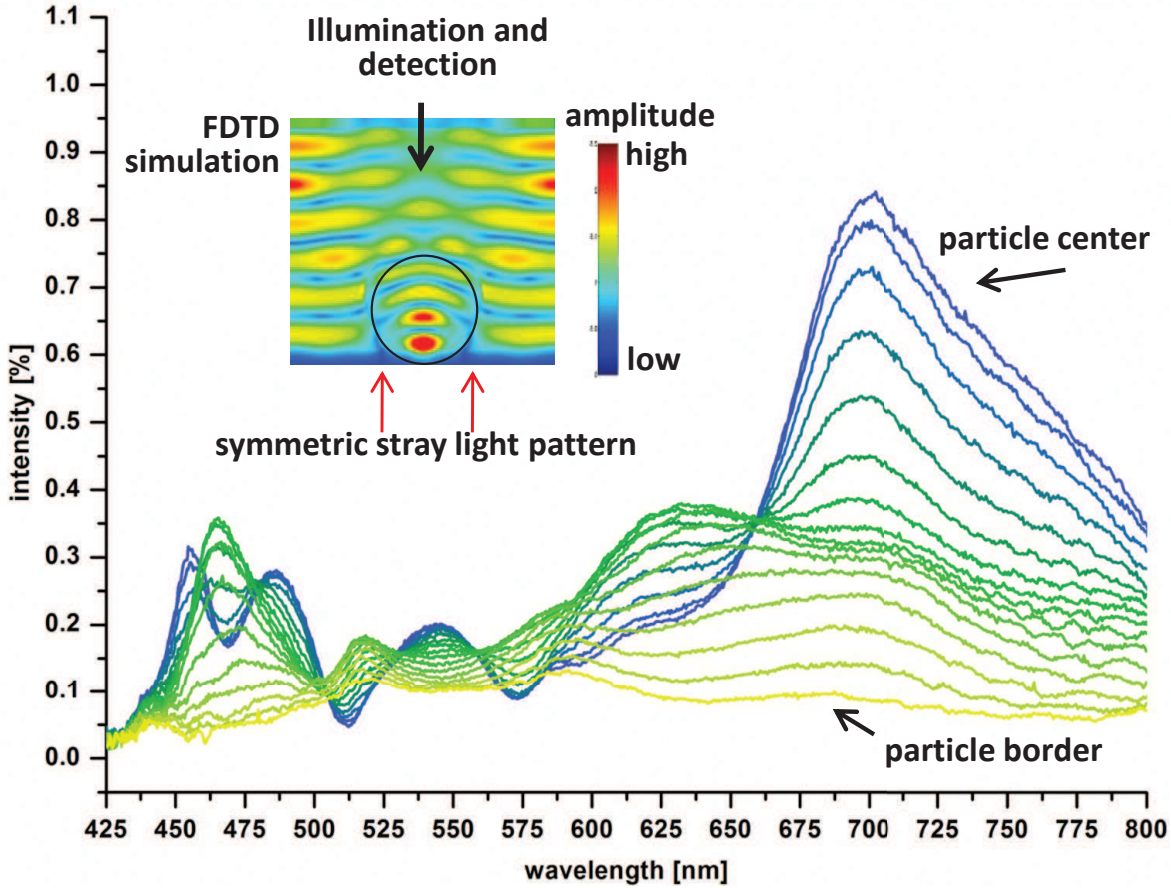


Pushbroom Imager



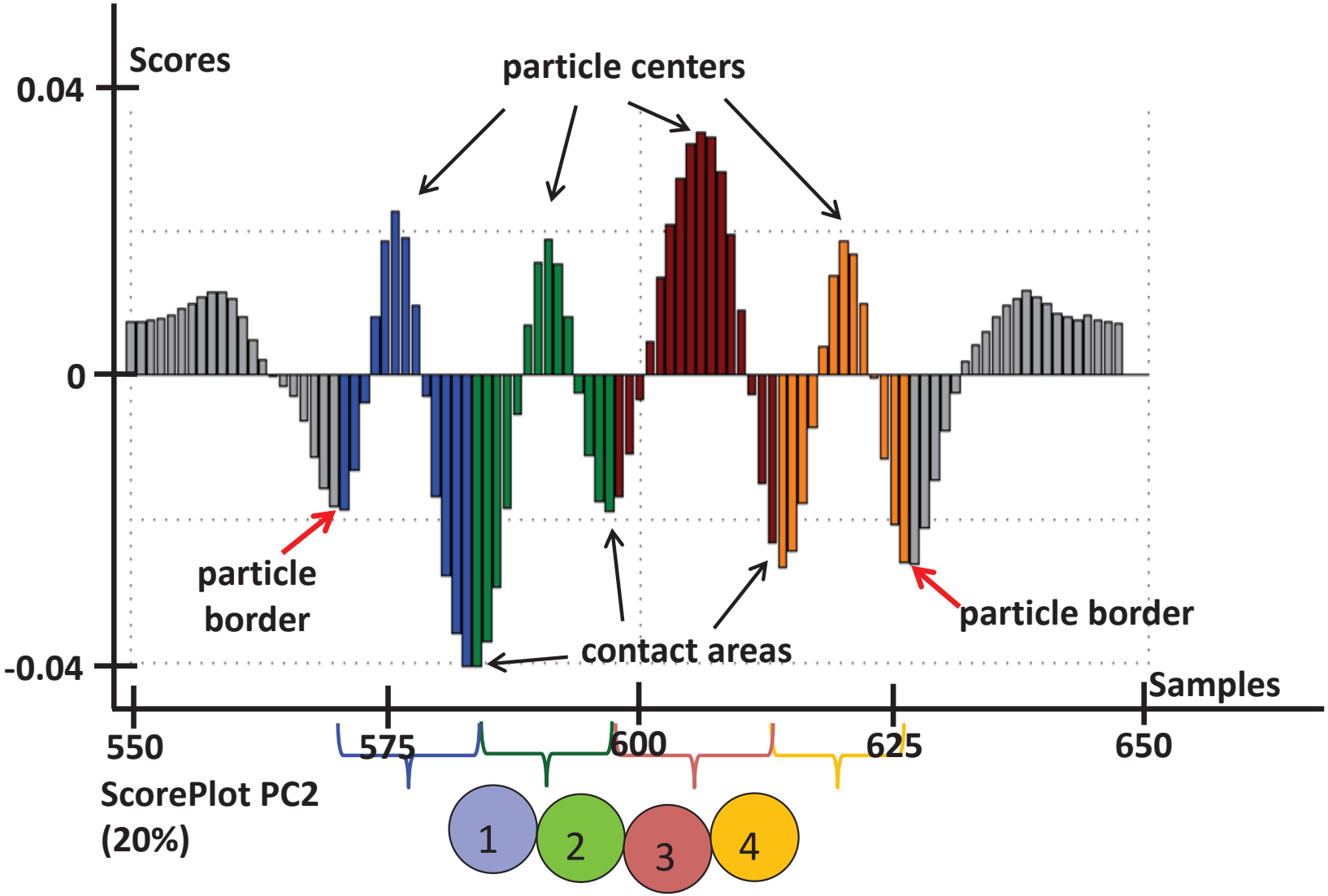
Pushbroom Imaging of a single particle



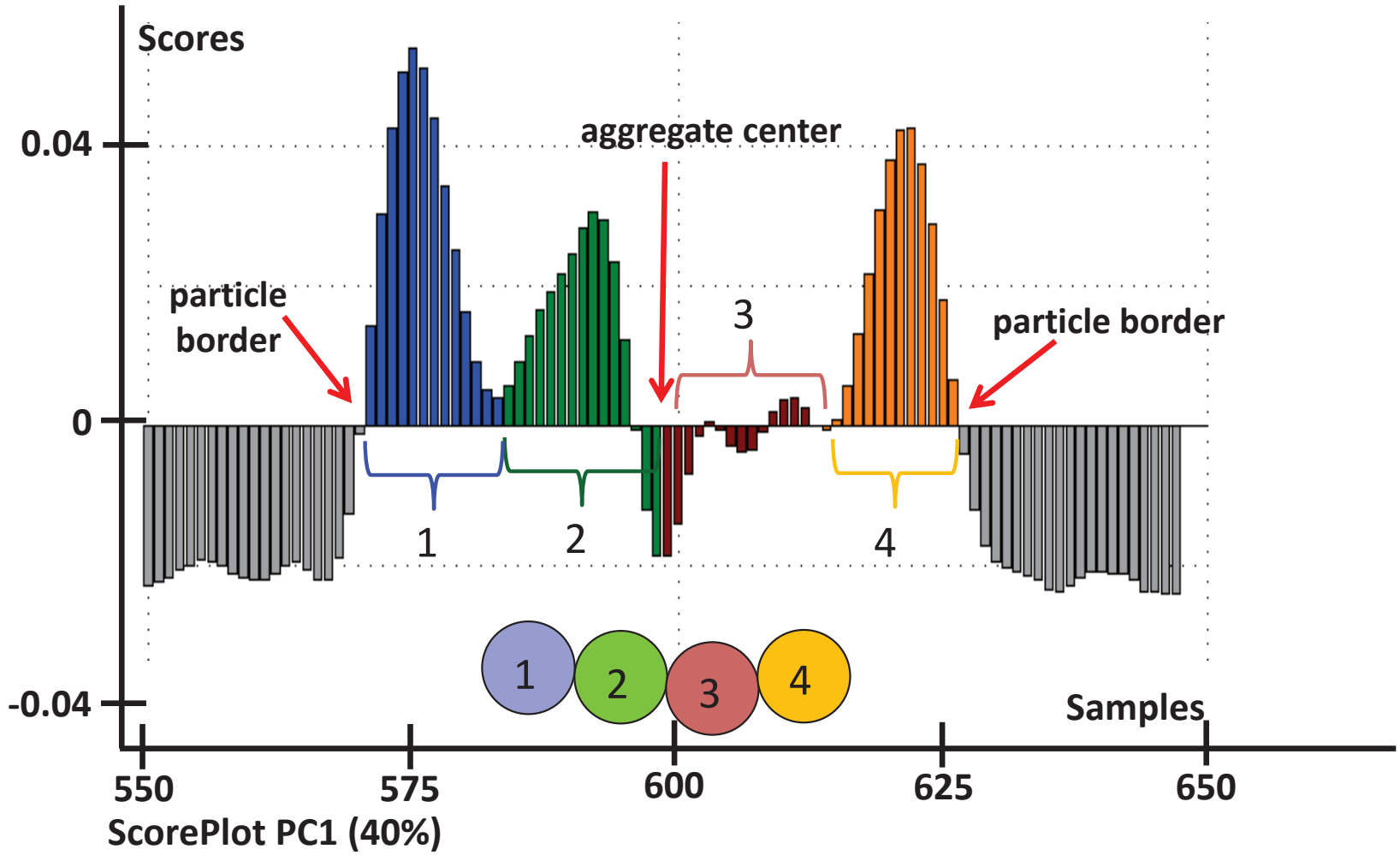


FDTD simulation parameters: λ=600nm; n=1.6; k=0

Pushbroom Imaging of a four particle array - PC 2 (20%)

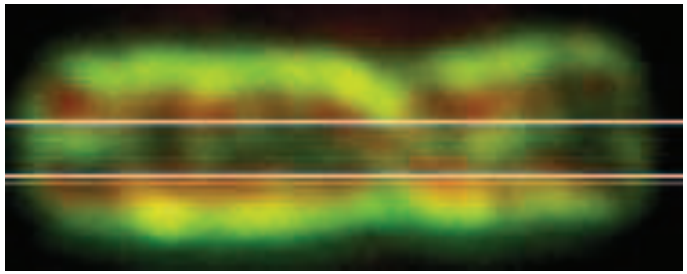


Pushbroom Imaging of a four particle array - PC 1 (40%)



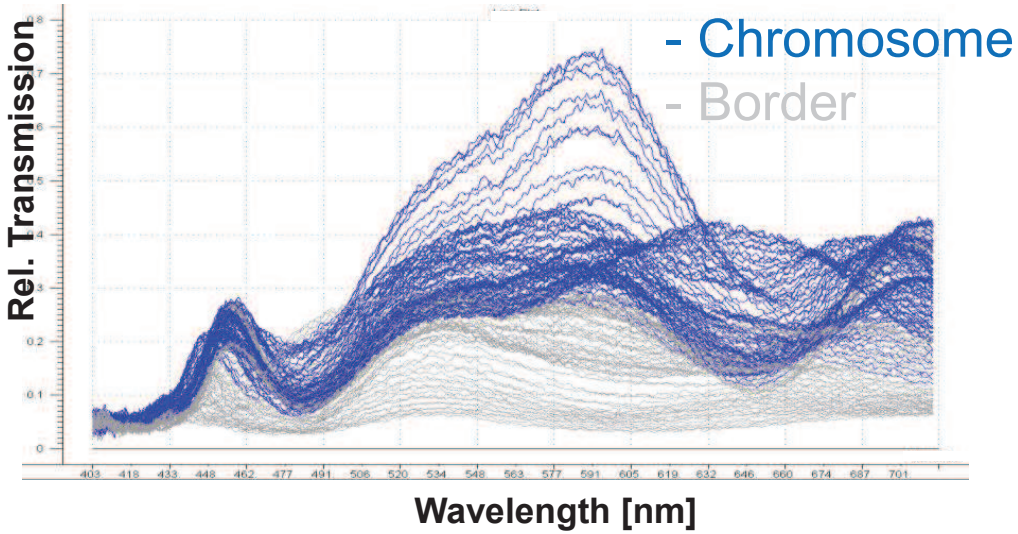
New MCR Karyotyping of a Chromosome Pushbroom Imaging (focus on center area)

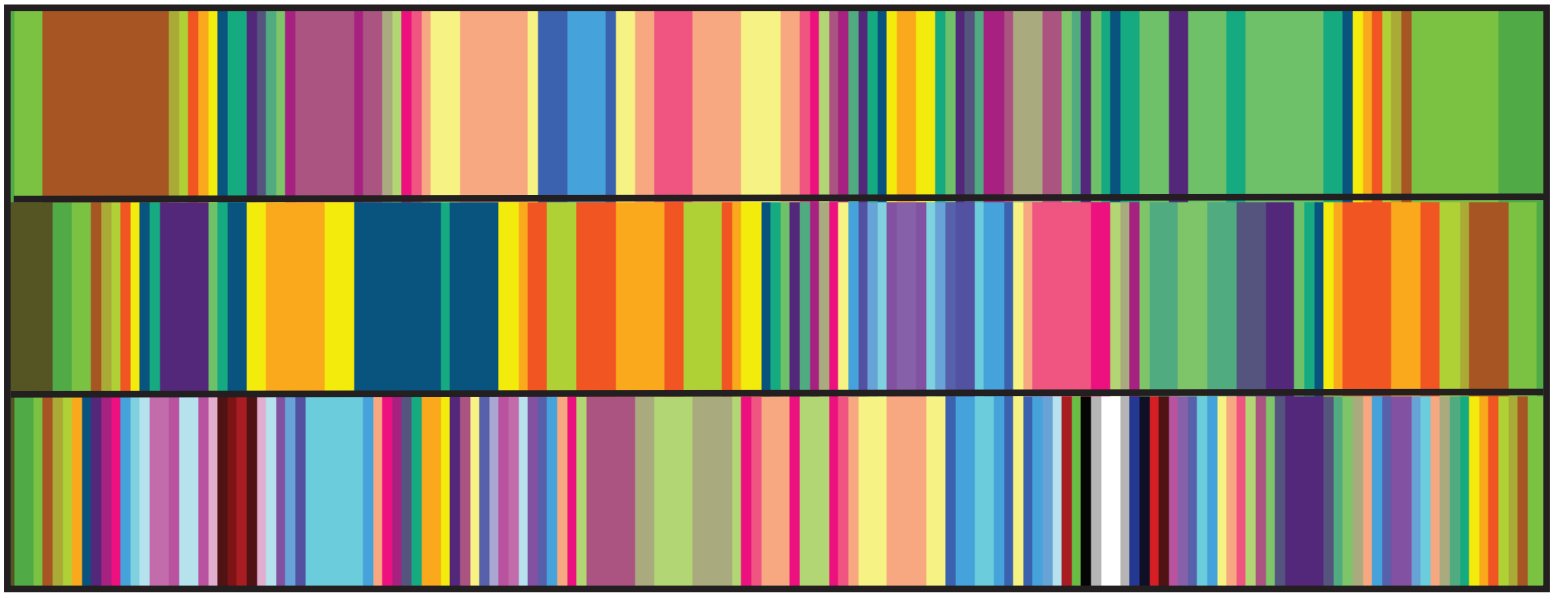
RGB-Image of Chromosome



Pushbroom Slit

resolution in x-axis: 64.5nm per pixel





MCR Component 1

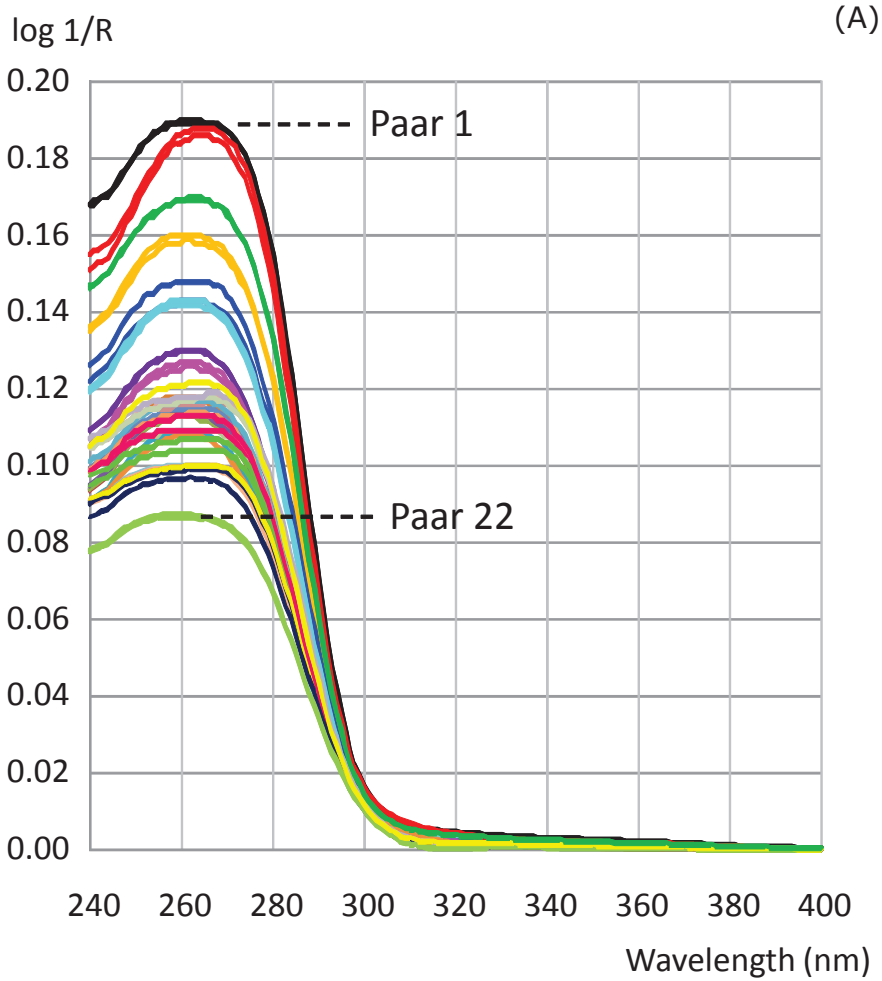
MCR Component 2

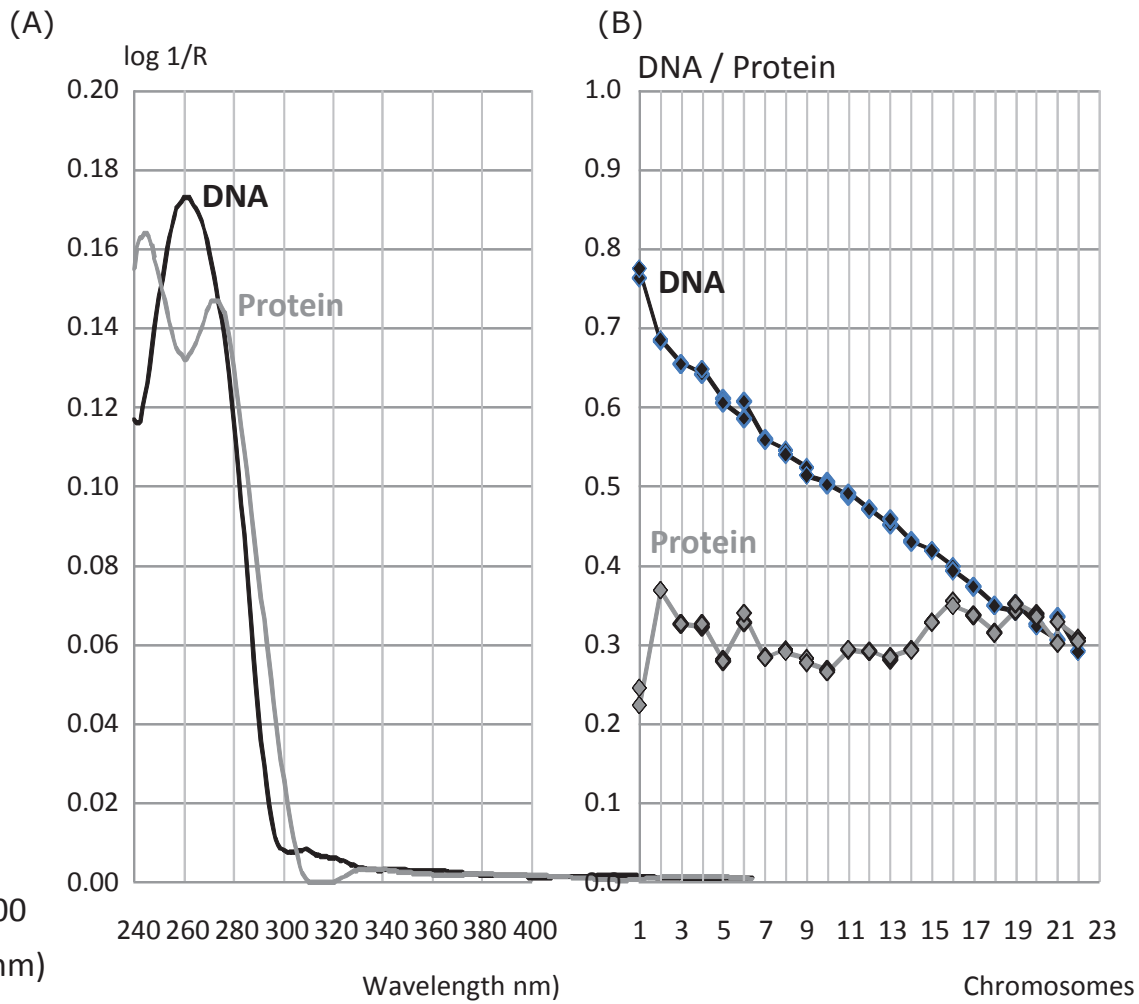
MCR Component 3

false colour karyotype

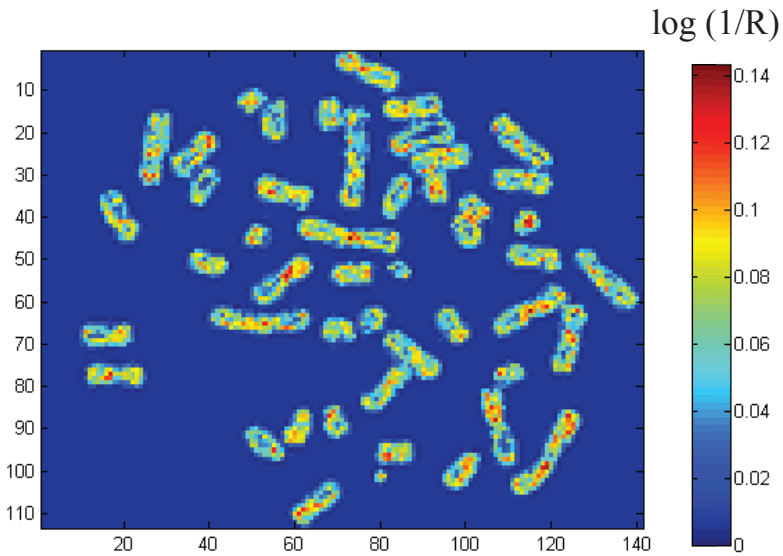
Markerfree Karyotyping: Chemistry

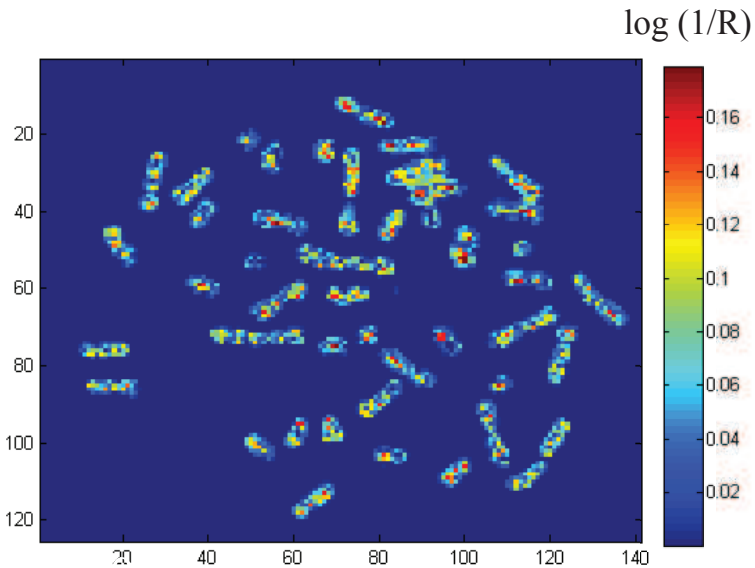
UV-Absorption Spectra (Bright Field Transmission) and MCR





UV Imaging


log (1/R)
10
20
30
40
50
60
70
80
90
100
110
20
40
60
80
100
120
140
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0


log (1/R)
20
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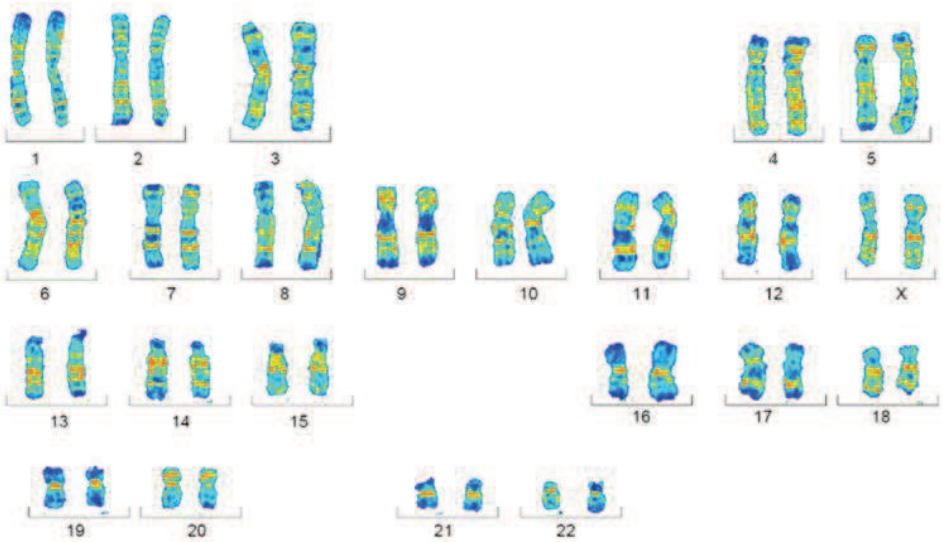
0.04
0.02


1
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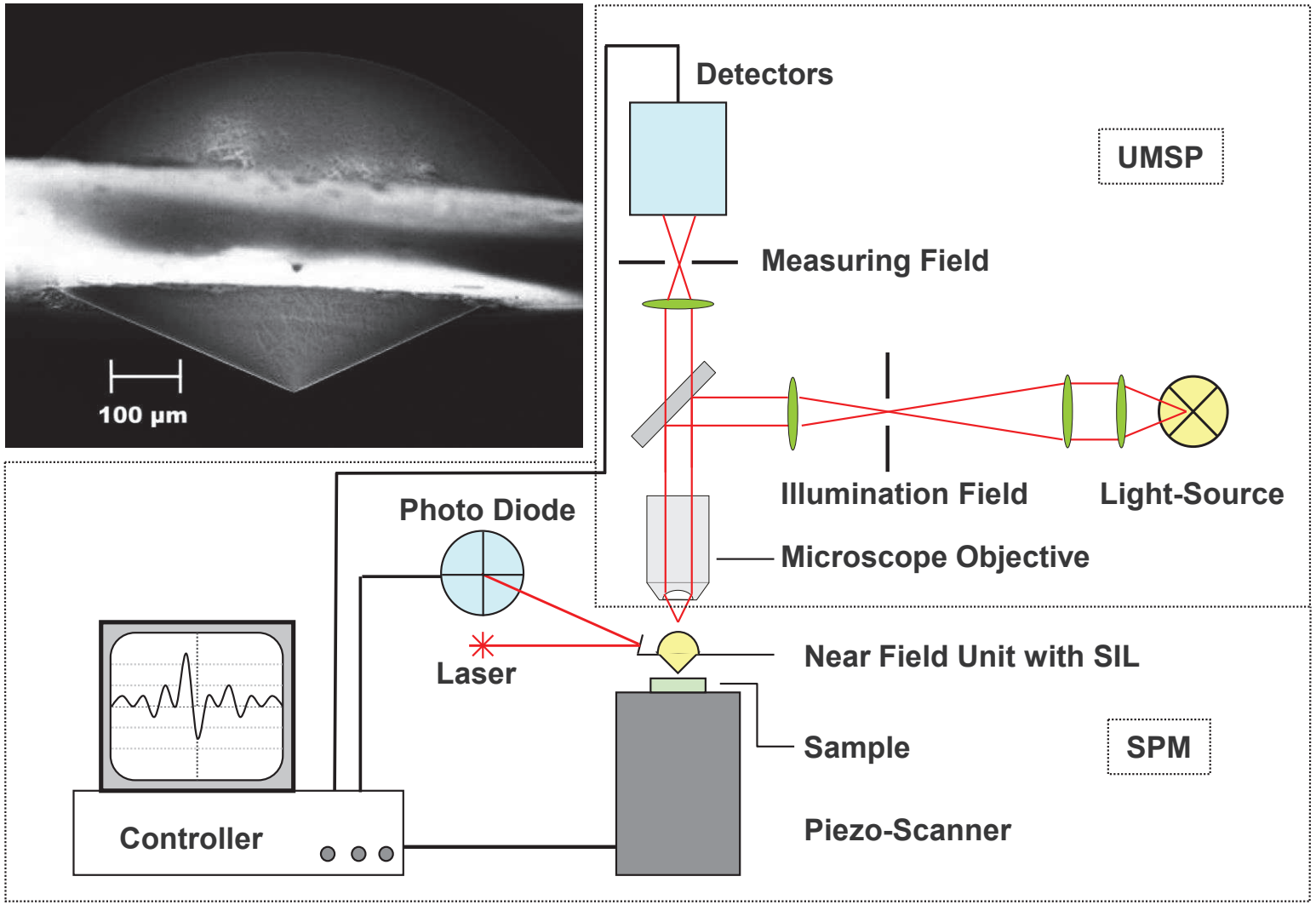
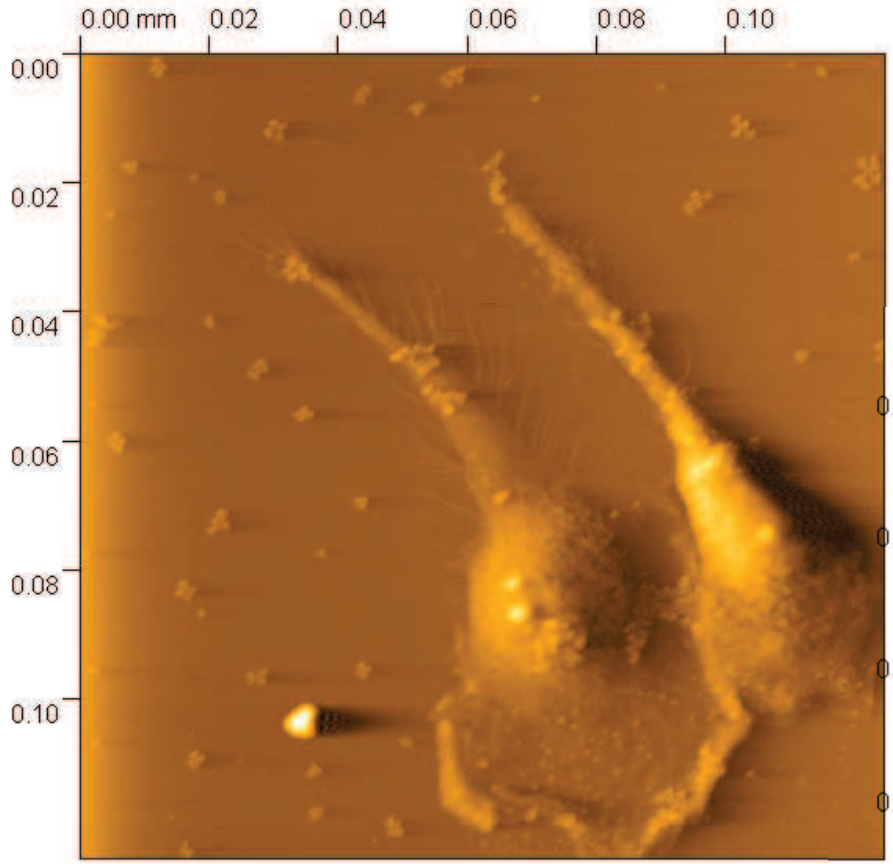
22

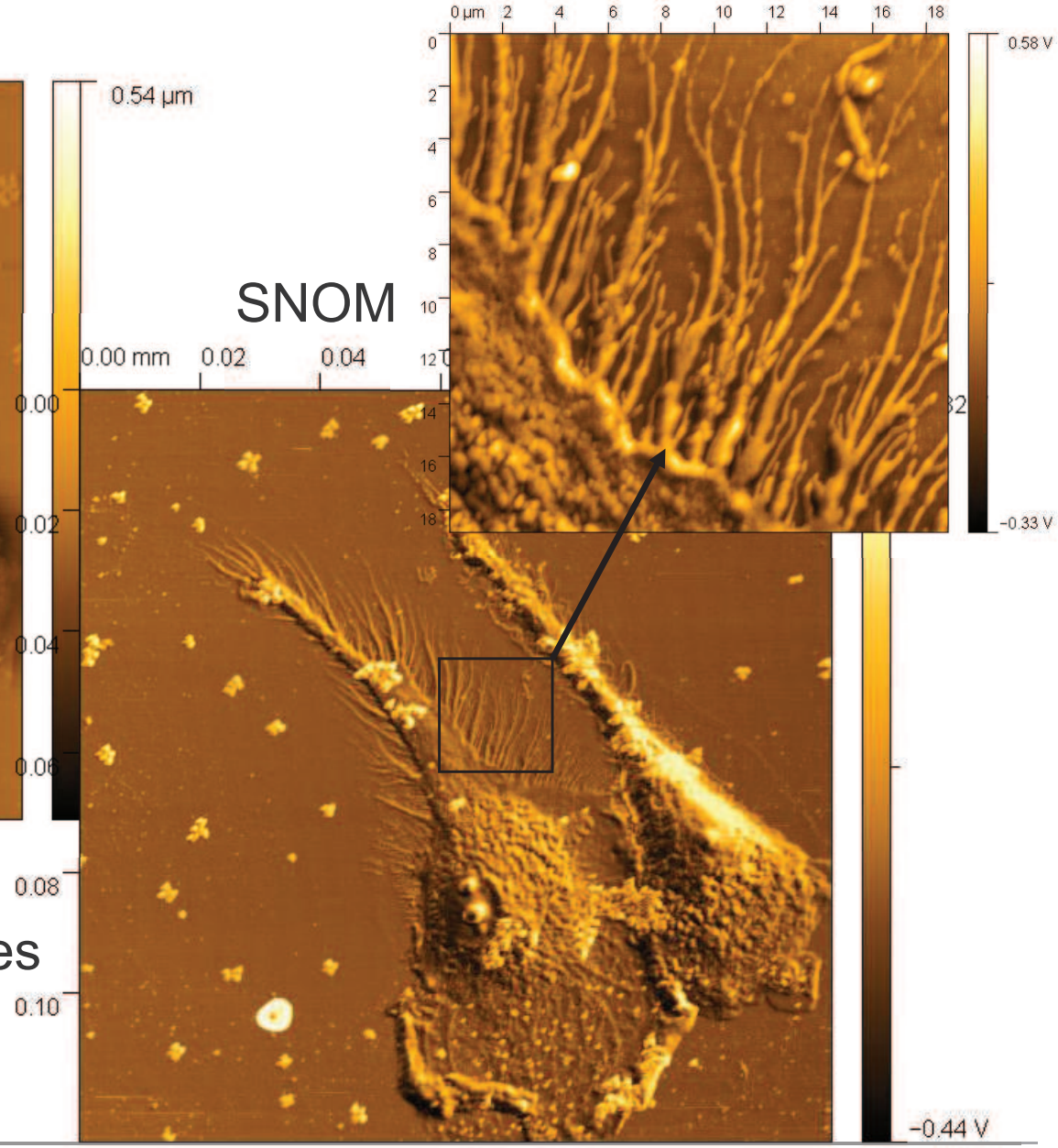
Near Field: System Integration: „Standard“ Microscope: Solid Immersion Lens



Polychromatic VIS-Imaging

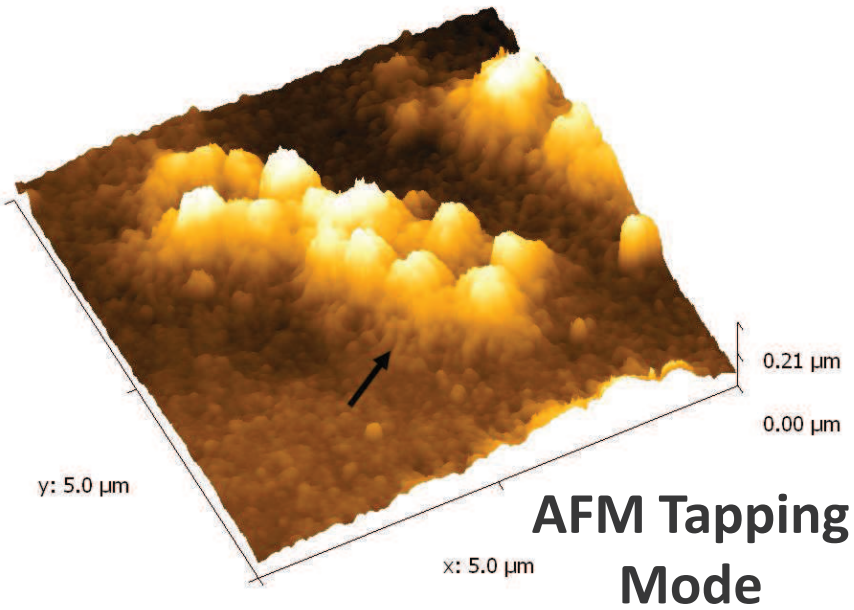


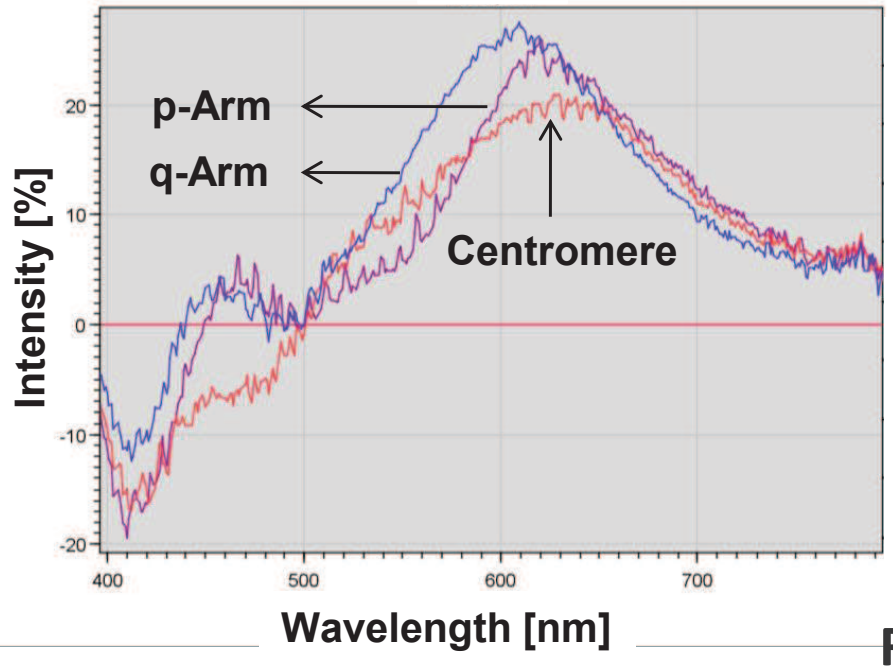
Deflection



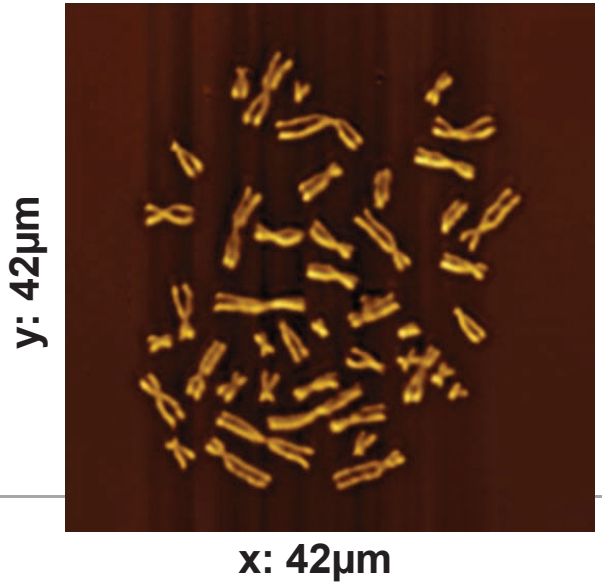
Monocytes with dendritic branches

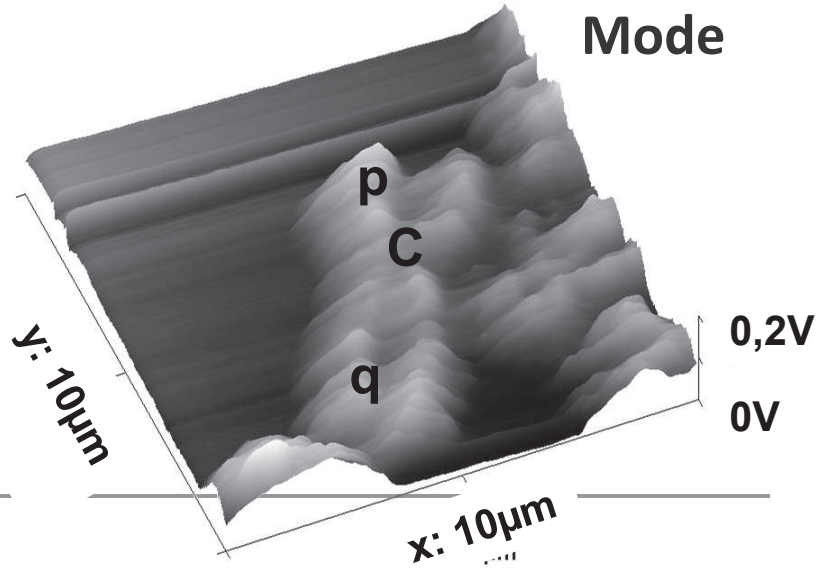
Near-field Spectra of Chromosome Sub-Structures





SNOM-Image of chromosome set. Nanoscale spectra are acquired along one chromosome for characterisation of small sub-structures.





Conclusion: Simultaneous Information with a single Shot!

Measured Data	Levels of Information
+ Intensity distribution over wavelength	⇒ size
+ Global interference pattern	⇒ morphology
+ Local interference pattern	⇒ topology
+ Characteristic absorption & scattering	⇒ DNA and protein ratio

→ simple single spectrum with a diode array spectrometer or pushbroom imaging system

→ Integrated into a standard lab microscope

Methodenorientiertes Lehr- und Forschungszentrum

Hochschule Reutlingen
Reutlingen University

Process Analysis and Technology: PA&T

(ca. 5 – 7 Professoren, interdisziplinär)

Intern. Master of Science in Englisch

Projektorientiertes Lernen

Operational Excellence

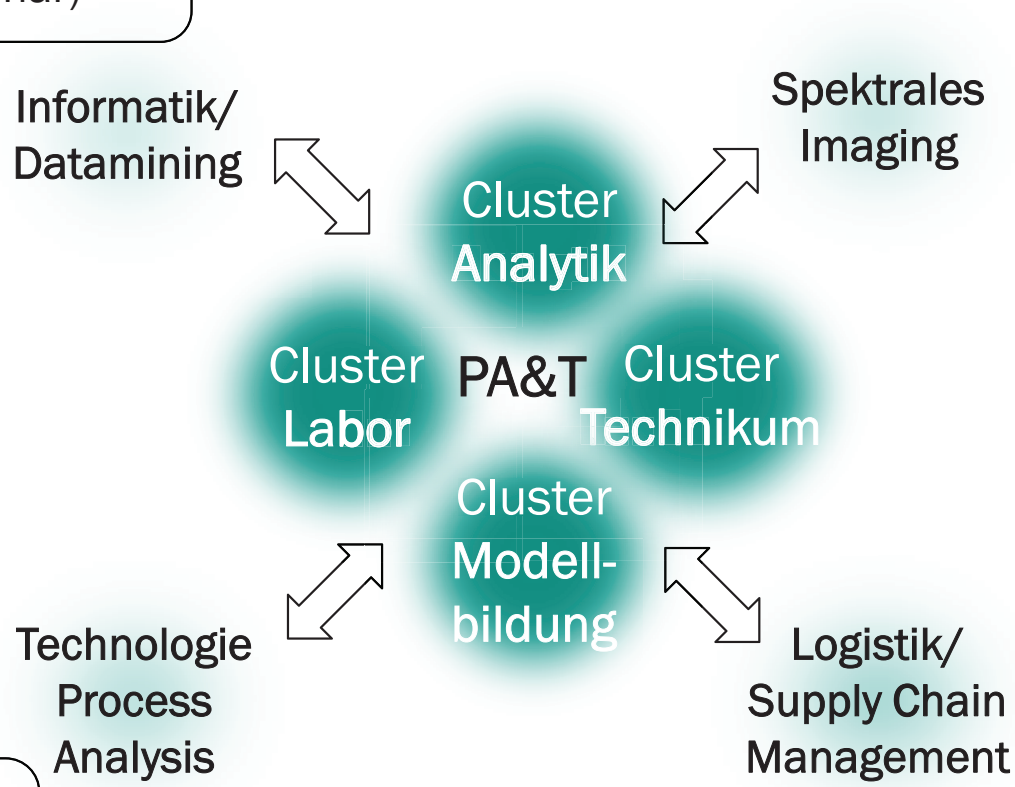
fakultätsübergreifend

z.B.: Complexity Management, Innovation Management, Supply Chain Management, Process Analysis, Process Technology

Forschungs - /Promotionskolleg

Biophotonics and Spectral Imaging

zusammen mit Universitäten, auch International



ZAFH-Photon^{n}

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Europäischer Fonds für regionale Entwicklung
und das Land Baden-Württemberg