Multidimensionale Mikroskopie

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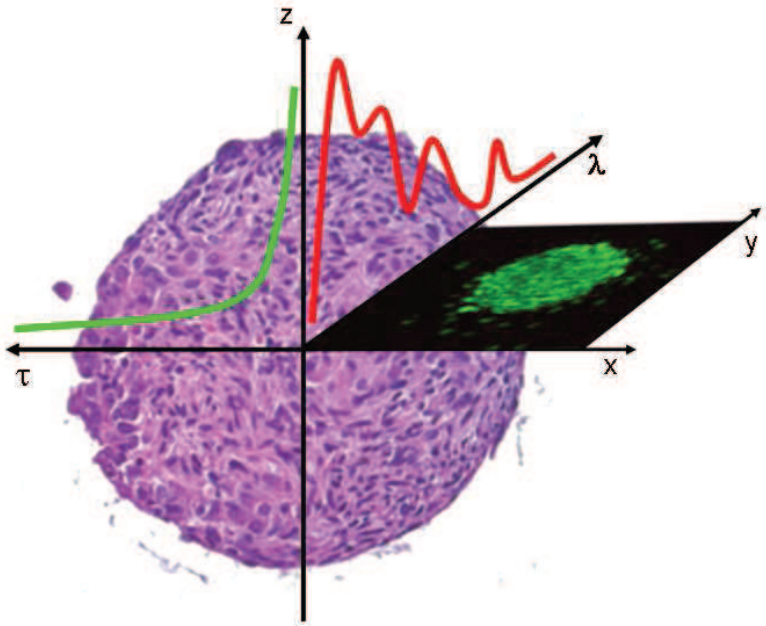
Multidimensionale Mikroskopie

- 3D Mikroskopie
- Spectral Imaging
- Fluorescence Lifetime Imaging
- (Polarization Imaging)



Anwendungen:

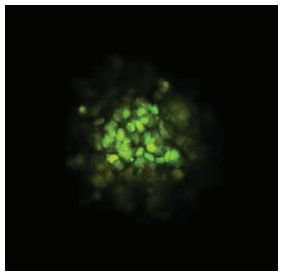
- Krebs
- Neurodegenerative Krankheiten

Tiefenauflösung in der Mikroskopie lebender Zellen

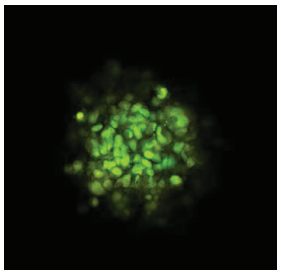
- Selektion von Fokusebenen -

Methoden:

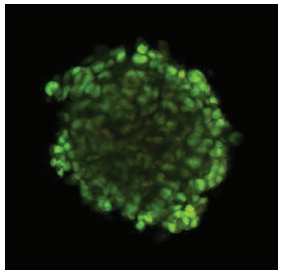
- Laser-Scanning-Mikroskopie: Ausblenden



z = 10 µm

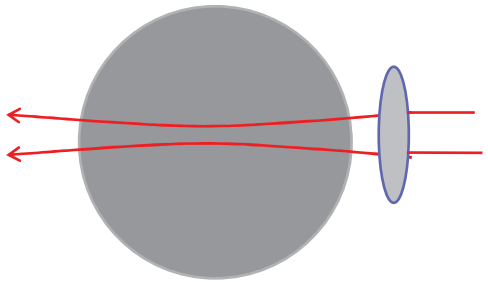


z = 15 µm



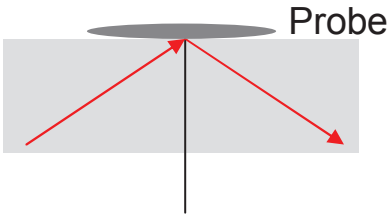
z = 60 µm

- Strukturierte Beleuchtung: Berechnung

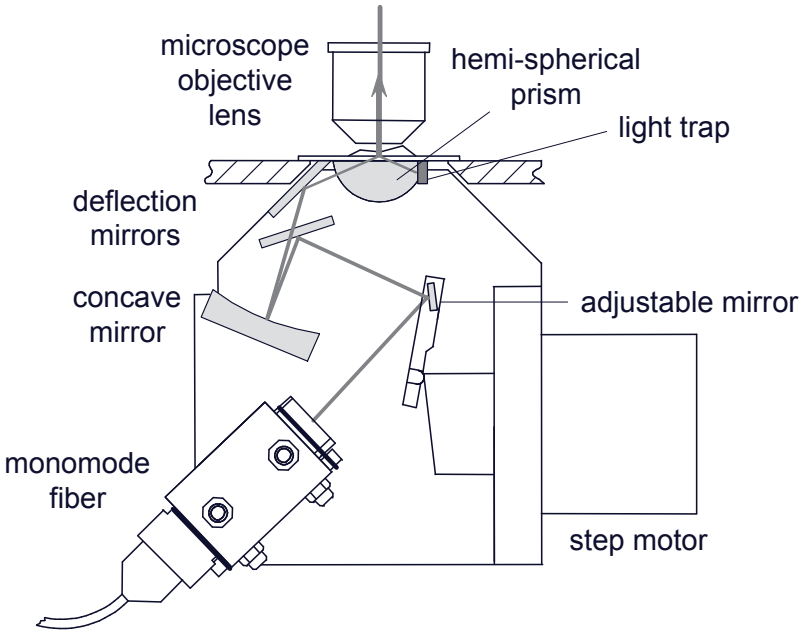


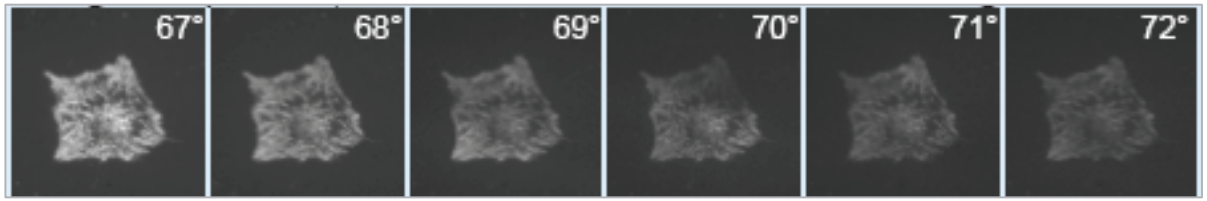
- Lichtscheibenmikroskopie: Selektive Belichtung

- Totalreflexionsmikroskopie

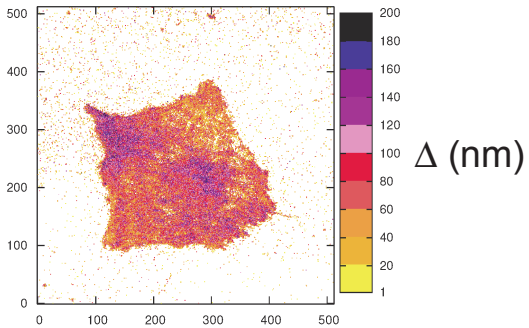


Tiefenauflösende Totalreflexionsmikroskopie (TIRFM)



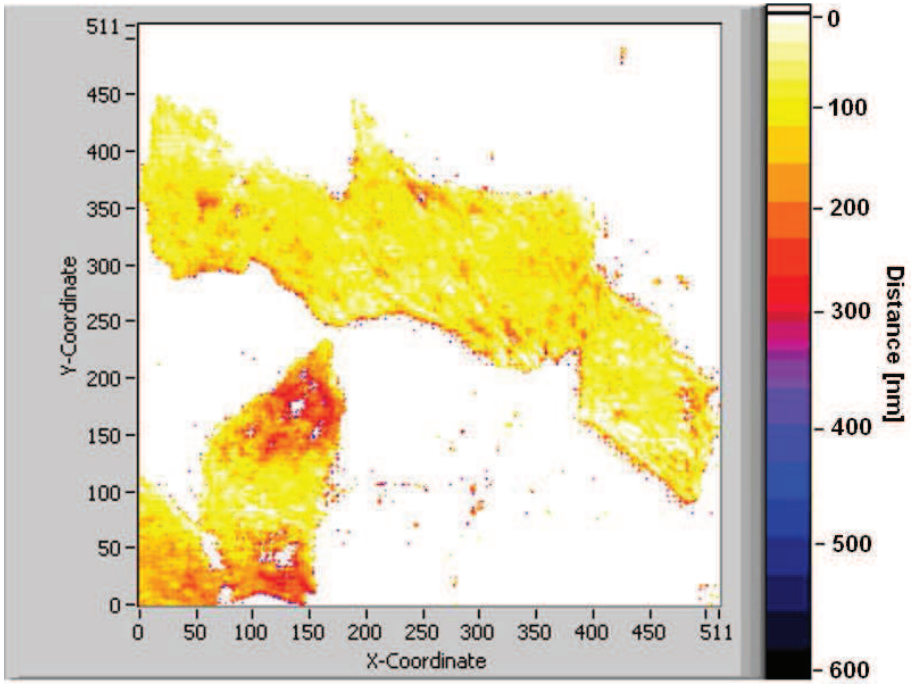


Zell-Substrat-Topologie:

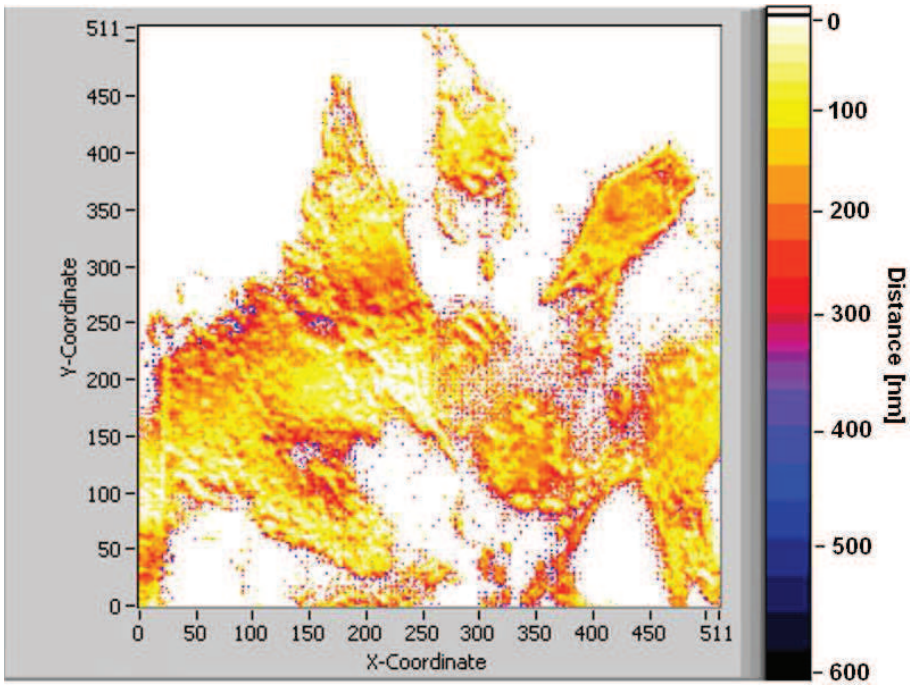


Zell – Substrat – Topologie (U251-MG Glioblastomzellen)

- Fluoreszenzmarker Laurdan -



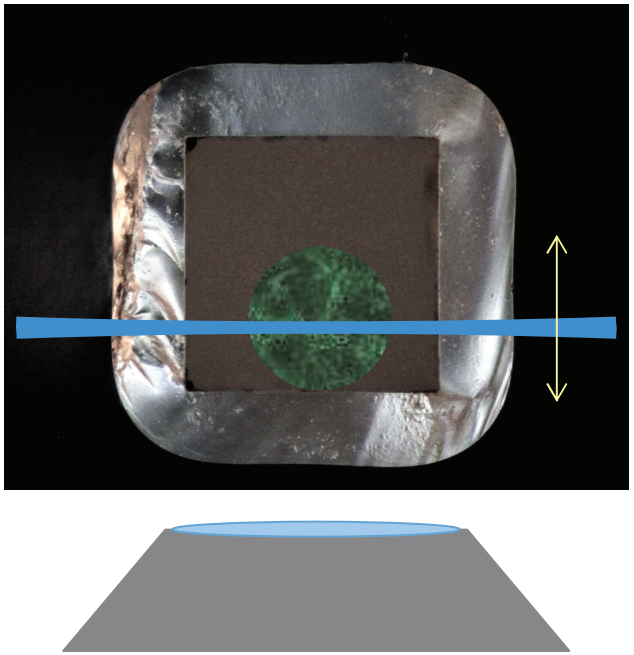
Tumorzellen

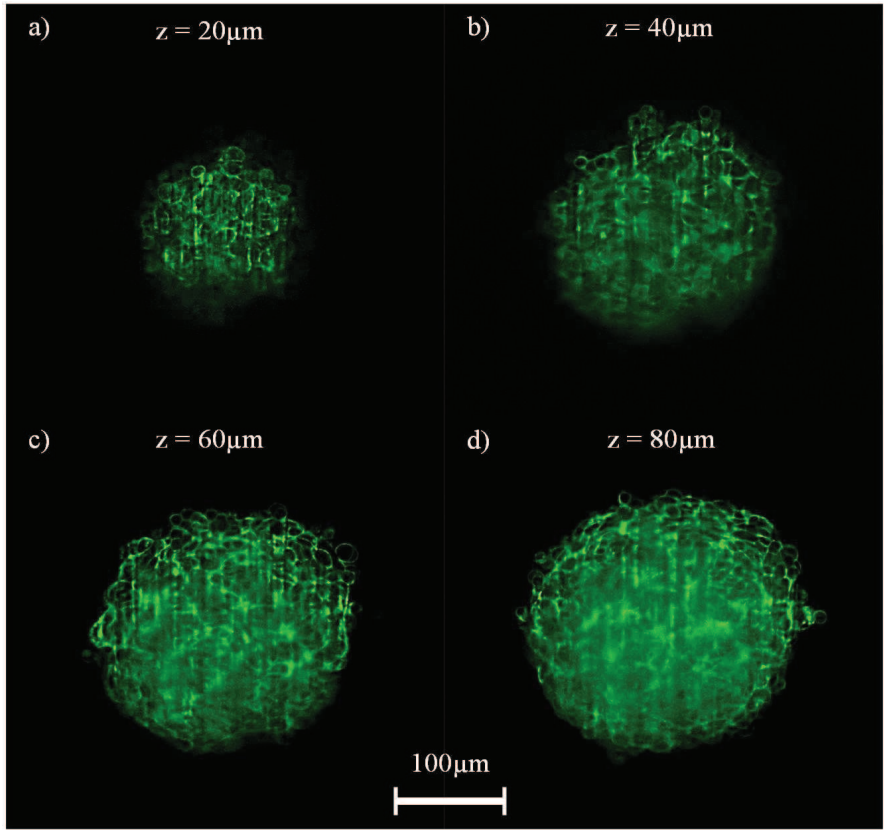


Zellen mit aktiviertem Supressorgen TP53

M. Wagner et al., J. Vis. Exp. 2012, 68, pii: 4133. doi: 10.3791/4133.

Lichtscheiben-Mikroskopie (SPIM)

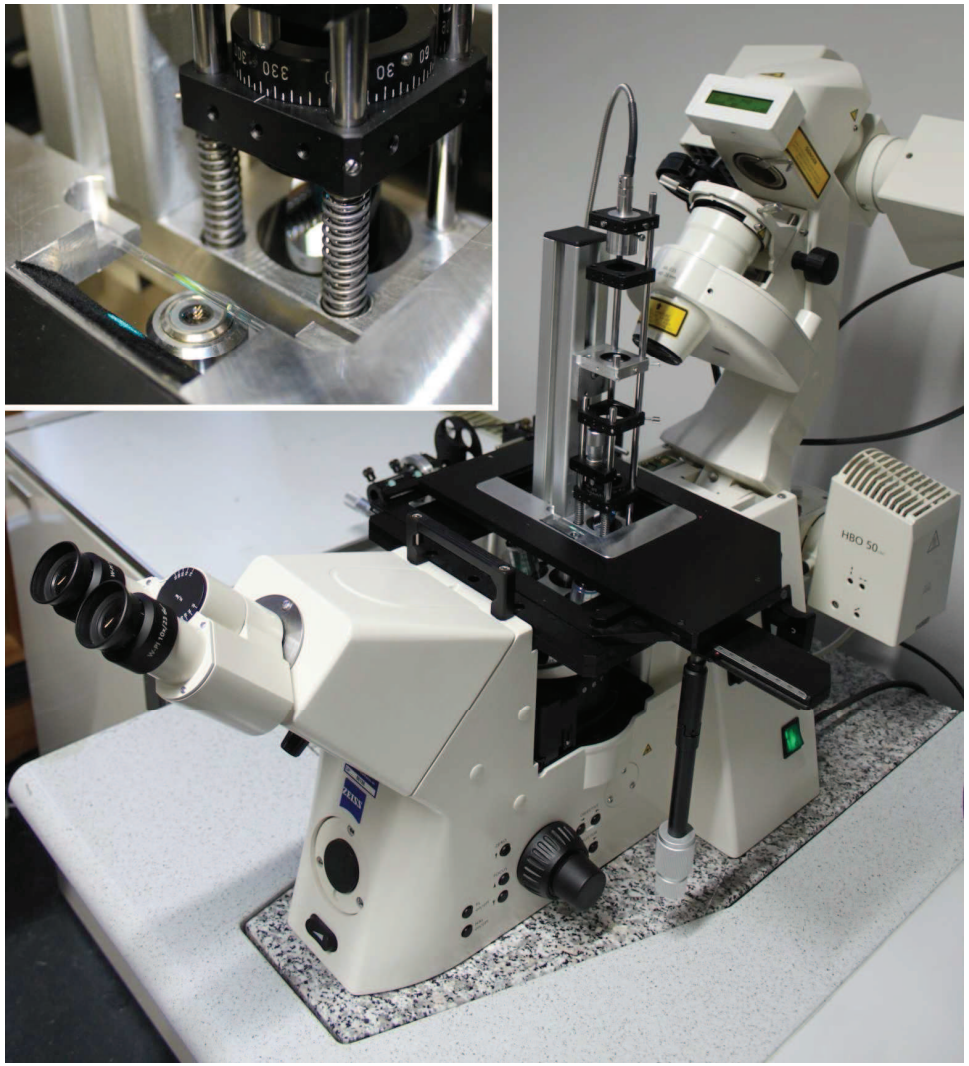




Tiefenauflösung: $\Delta z \approx 10$ µm

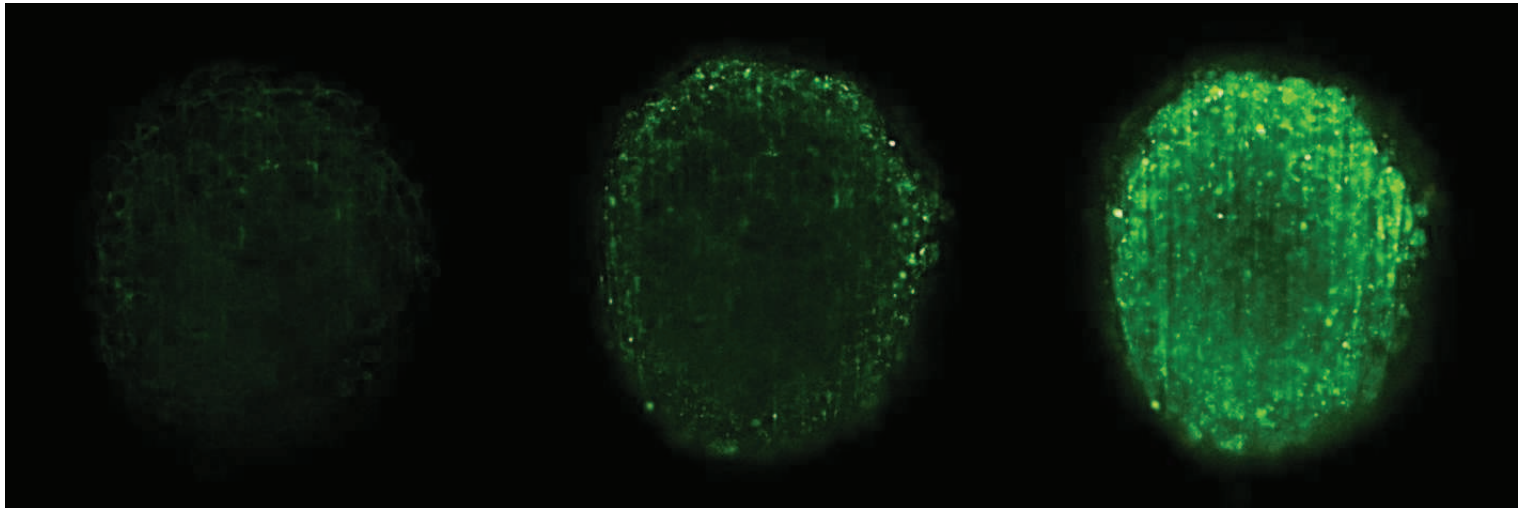
Vorteile:
- Geringe Lichtdosis: nur untersuchte Probenbereiche werden beleuchtet
- Kombination mit konventioneller Mikroskopie
- Kombination mit Mikrofluidik → Wirkstoffforschung

Lichtscheibenmikroskopie: Laboraufbau



Lichtscheibenmikroskopie: Aufnahme eines Fluoreszenzmarkers

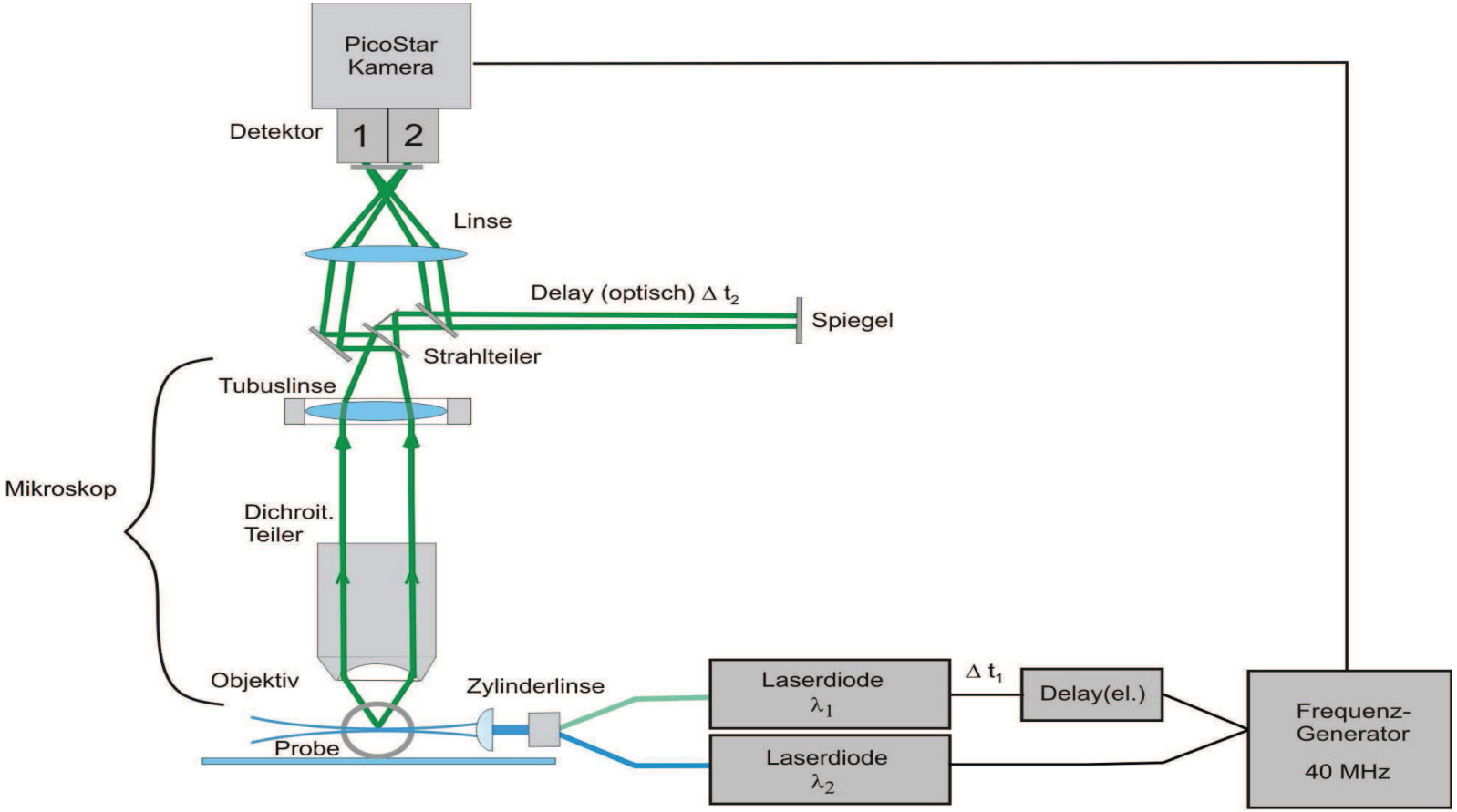
t = 0 min t = 5 min. t = 30 min..



Acridin Orange (5 µM in Zellkulturmedium)

T. Bruns et al., 2012, J. Biomed. Opt. 17(10), 101518.

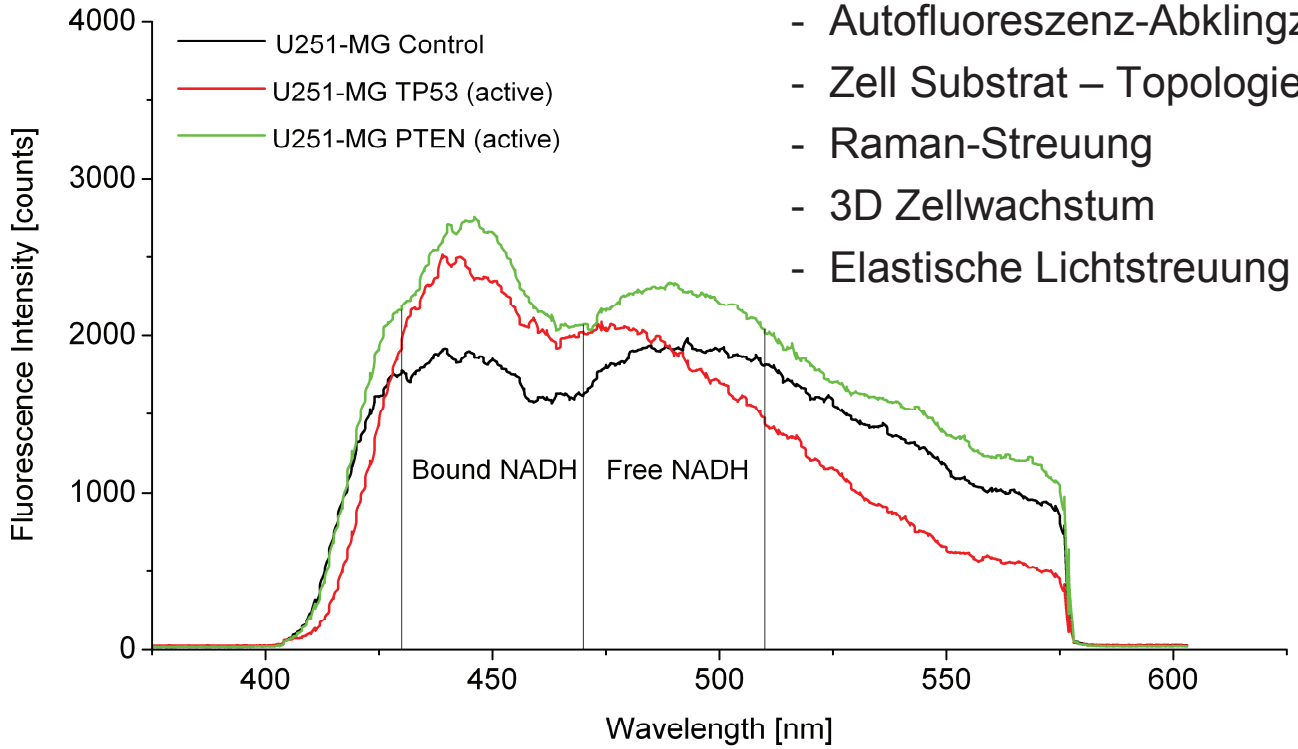
Lichtscheibenmikroskopie: Ratio Imaging



Land Baden-Württemberg: Innovatives Projekt

Markerfreie Methoden zur Erkennung von Krebszellen

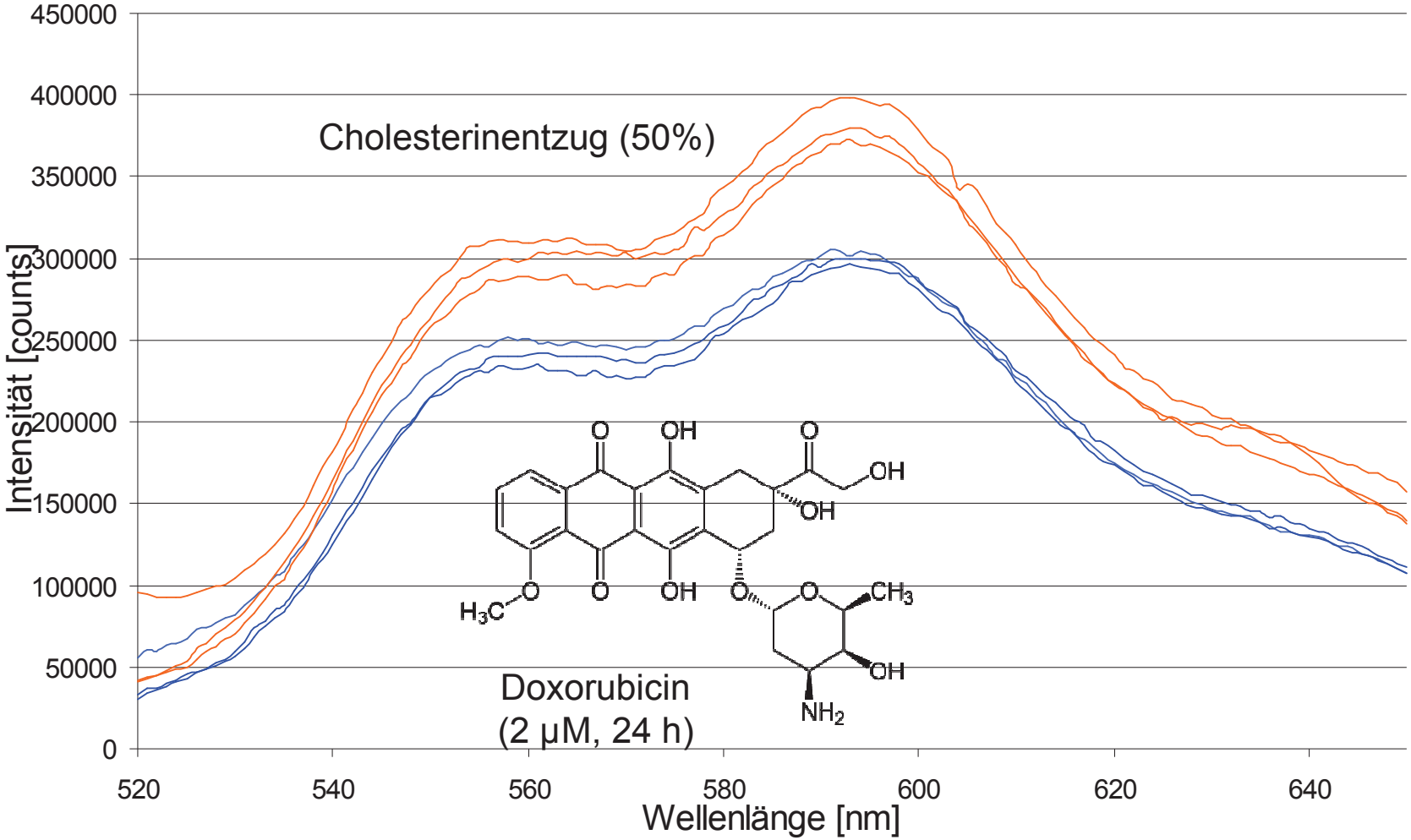
- Autofluoreszenzspektren
- Autofluoreszenz-Abklingzeiten
- Zell Substrat – Topologie
- Raman-Streuung
- 3D Zellwachstum
- Elastische Lichtstreuung (?)



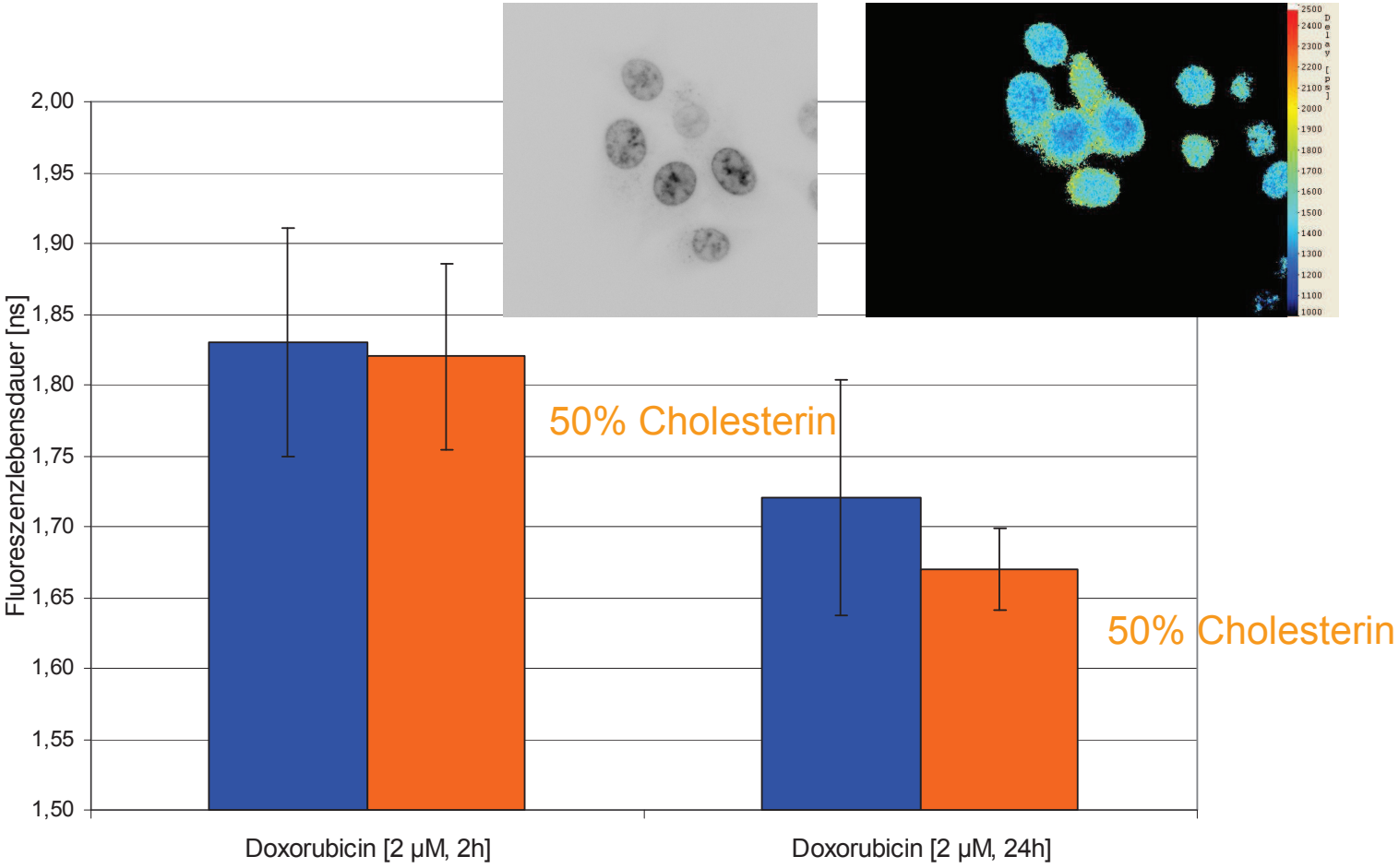
BMBF und BW – Stiftung gGmbH

P. Weber et al., 2012, J. Biomed. Opt. 17(10), 101508.

MCF-7 Brustkrebszellen: Aufnahme eines Zytostatikums

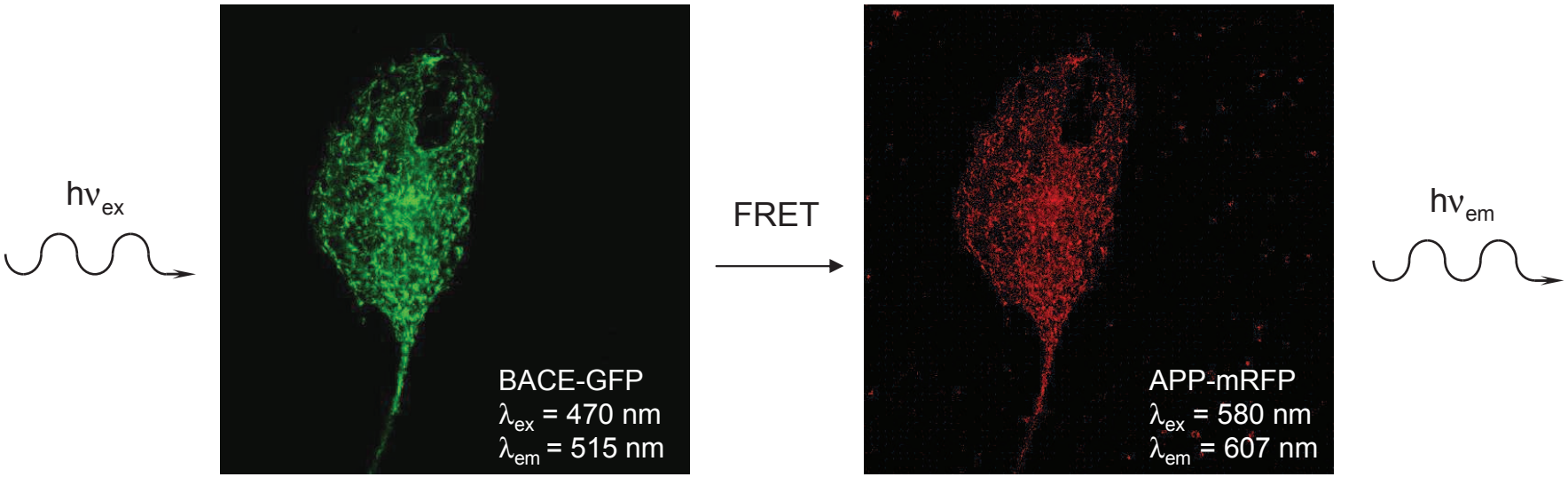


Doxorubicin: Fluoreszenzlebensdauer



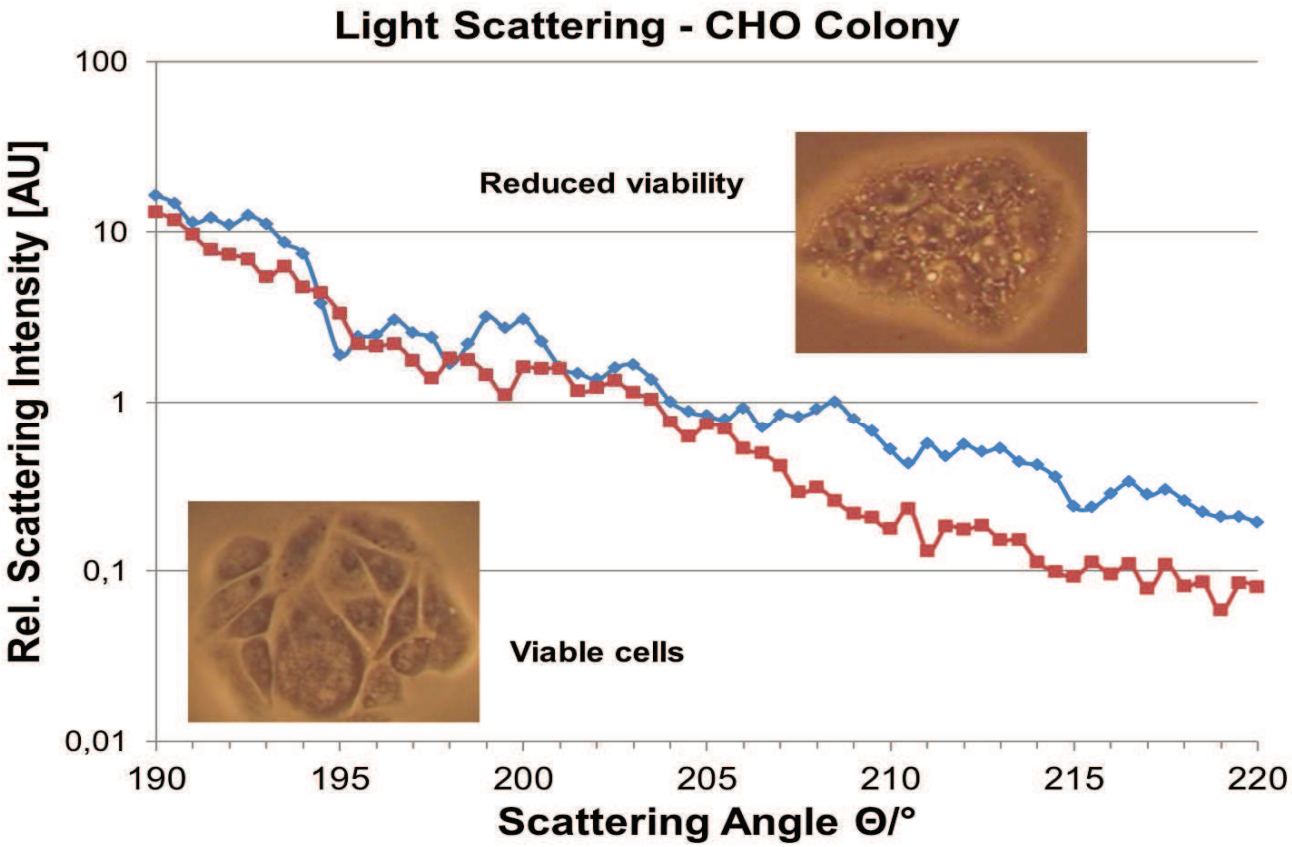
Perspektive: Spektrales Imaging

- Mikroskopie mit spektraler Signatur
- Biomedizinische Materialien
- 3 D Zellkulturen
- u. a. neurodegenerative Erkrankungen



BMBF-Antrag „Multimodales spektrales Imaging“ (R. Kessler, H. Schneckenburger + Partner)

Perspektive: Mikrostrukturanalyse



BW – Stiftung gGmbH: Forschungsprojekt Mistral (ILM + HS Aalen)

Mitarbeiter und Partner

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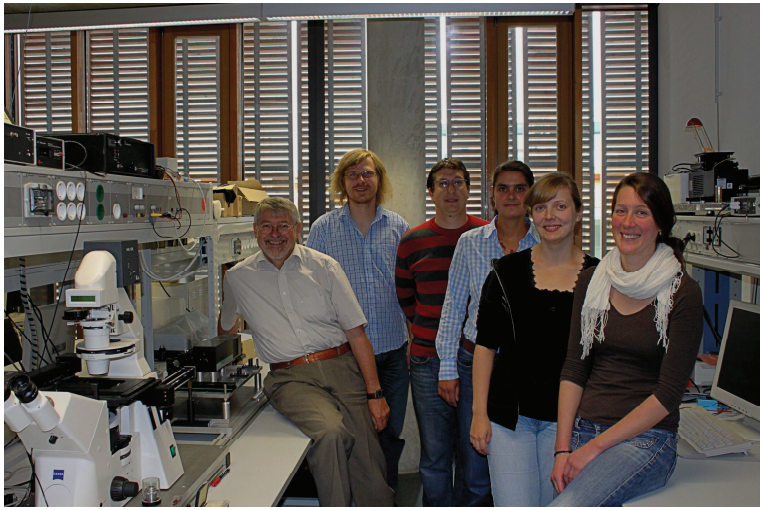
H. Schneckenburger

ILM:
R. Wittig, W.S.L. Strauß, A. Kienle,
T. Rothe, R. Hibst



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ZAFH-Photon^{n}

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