



DSENS

Professur für
Diagnostische
Sensorik



Universität Augsburg
Fakultät für Angewandte
Informatik

07 Hyperspectral imaging and other spectroscopic techniques

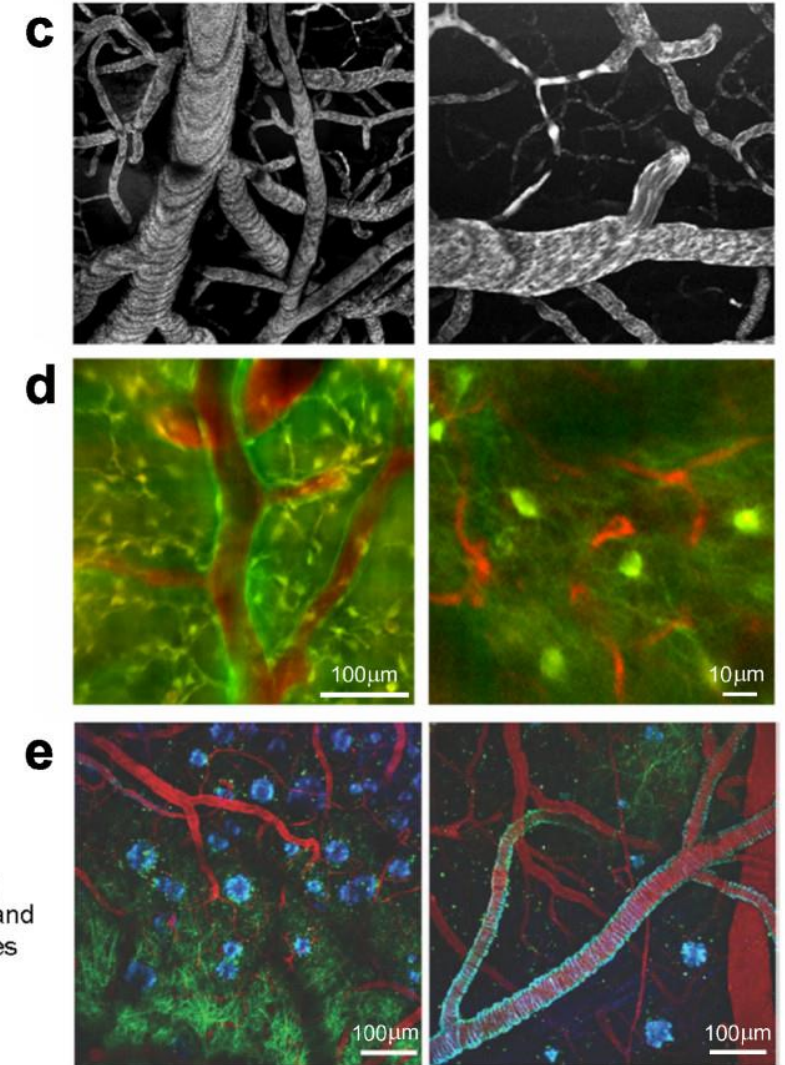
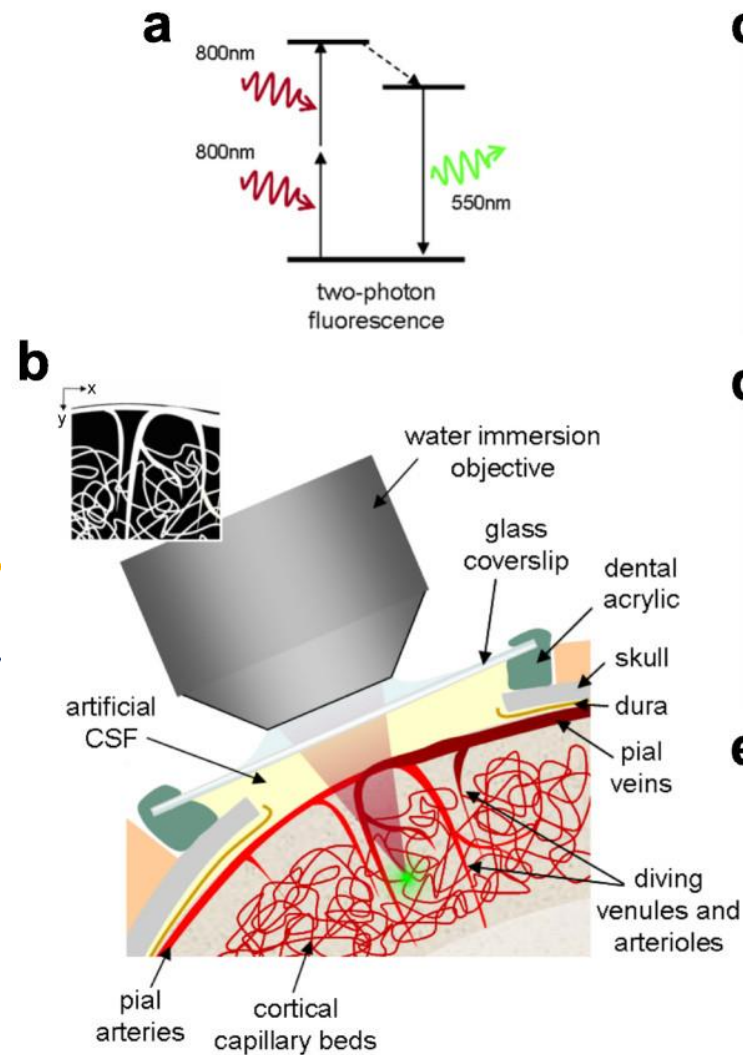
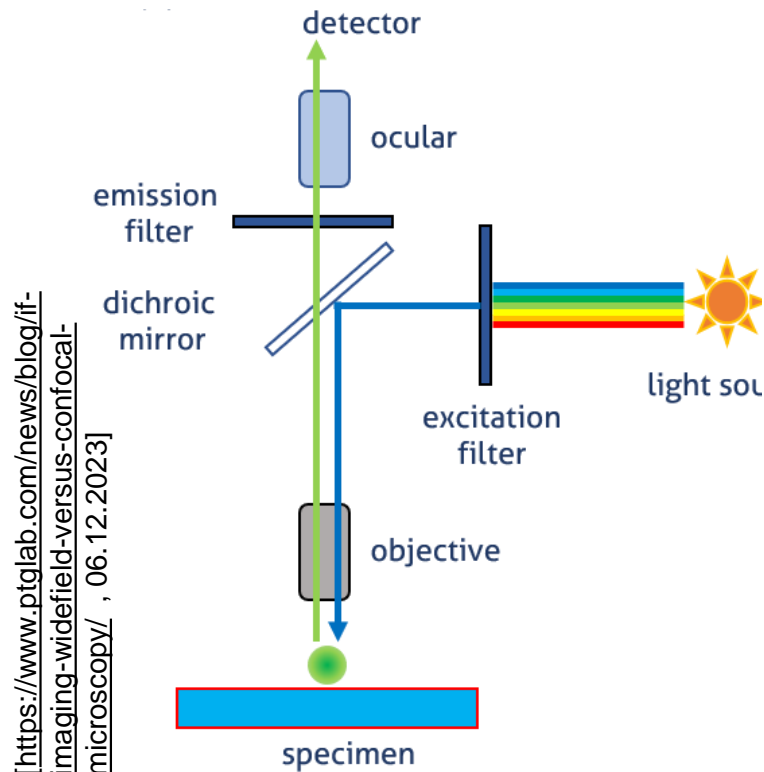
Professur für Diagnostische Sensorik

Prof. Sebastian Zaunseder, sebastian.zaunseder@uni-a.de
16.12.24, Augsburg

Introduction

Motivation

- Background



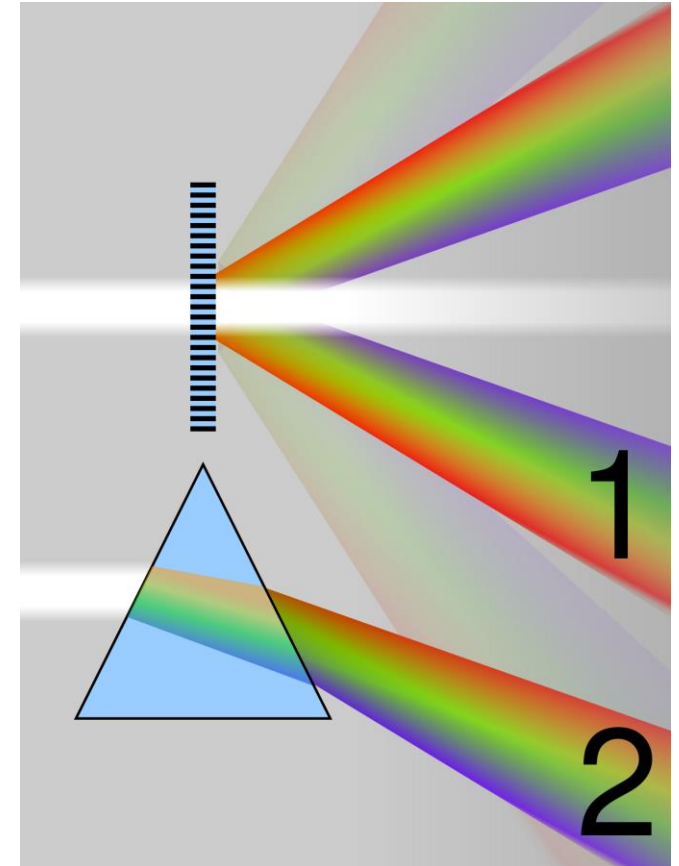
[<https://www.ptglab.com/news/blog/if-imaging-widefield-versus-confocal-microscopy/> , 06.12.2023]

[<https://bmcsystbiol.biomedcentral.com/articles/10.1186/1752-0509-2-74> , 06.12.2023]

Introduction

Motivation

- Definitions hyperspectral, imaging, spectroscopy
- Spectroscopy always refers to multiple wavelengths
 - (Conventional) Spectroscopy vs. spectroscopic imaging: single spot vs. spatial resolution
 - Difference multi- and hyperspectral
 - Multispectral: 4 to 10 spectral bands (no exact definition)
 - Hyperspectral: 10 up to 100 with specialty cases going into 1000
- In the end, light needs to be separated into wavelengths
- Separation approaches → required for all techniques
 - Dispersive elements (prism, diffraction grating)
 - Filters (Dichroic mirrors, tuneable filters / bandpass filters)
 - Tunable illumination



https://en.wikipedia.org/wiki/Diffraction_grating#/media/File:Comparison_refraction_diffraction_spectra.svg

Introduction

Motivation

[Karim2022]

Feature	Monochrome	RGB	Spectroscopy	Multispectral	Hyperspectral
Spatial information	Yes	Yes	No	Yes	Yes
Band Numbers	1	3	From several dozens to hundreds	3 to 10	From several dozens to hundreds
Spectral information	No	No	Yes	Limited	Yes
Multiconstituent information	No	Limited	Yes	Limited	Yes
Sensitivity to minor components	No	No	Yes	Limited	Yes

Introduction

Outline

I Introduction

1 Hyperspectral imaging basics

2 (Absorption) Hyperspectral imaging applications

3 Overview on related concepts

S Summary

01

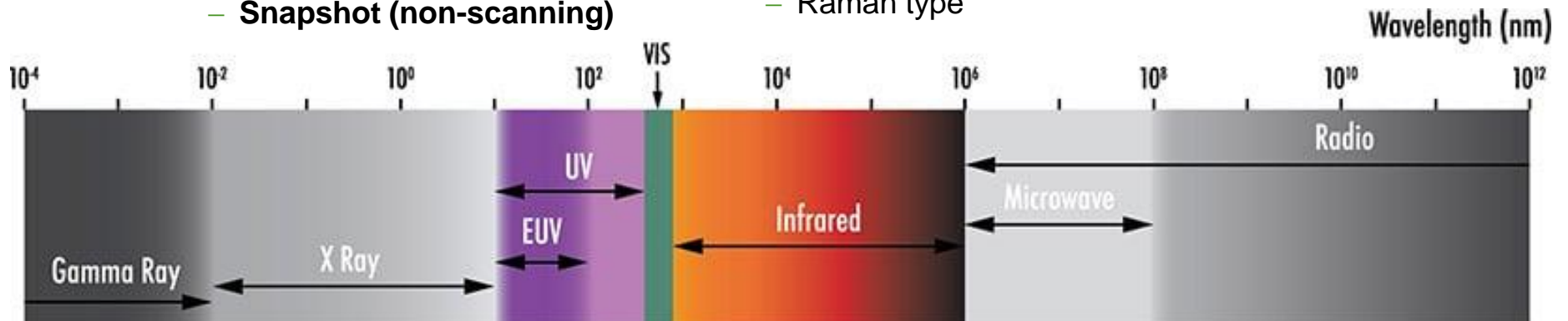
HYPERSENSPECTRAL IMAGING BASICS

Hyperspectral imaging basics

Basics - taxonomies

- General problem: a 2D sensor needs to capture a 3D data structure → any spectral discrimination has to take place before the light is captured by the sensor pixels (compare Bayer filter array)
- Possible taxonomies
 - According to wavelength range
 - Ultraviolet
 - Visible
 - Near-infrared
 - Midinfrared
 - **According to hyperspectral data collection**
 - Whiskbroom (scanning)
 - Pushbroom (scanning)
 - Spectral Scanning (scanning)
 - Snapshot (non-scanning)
 - According to measurement type
 - „Absorption type“ (absorption, reflection, scattering, transmission)
 - Fluorescence type
 - Raman type
 - According to spectral dispersion mode
 - Prisms/grating types
 - Tunable filter types
 - Single-lens imaging types

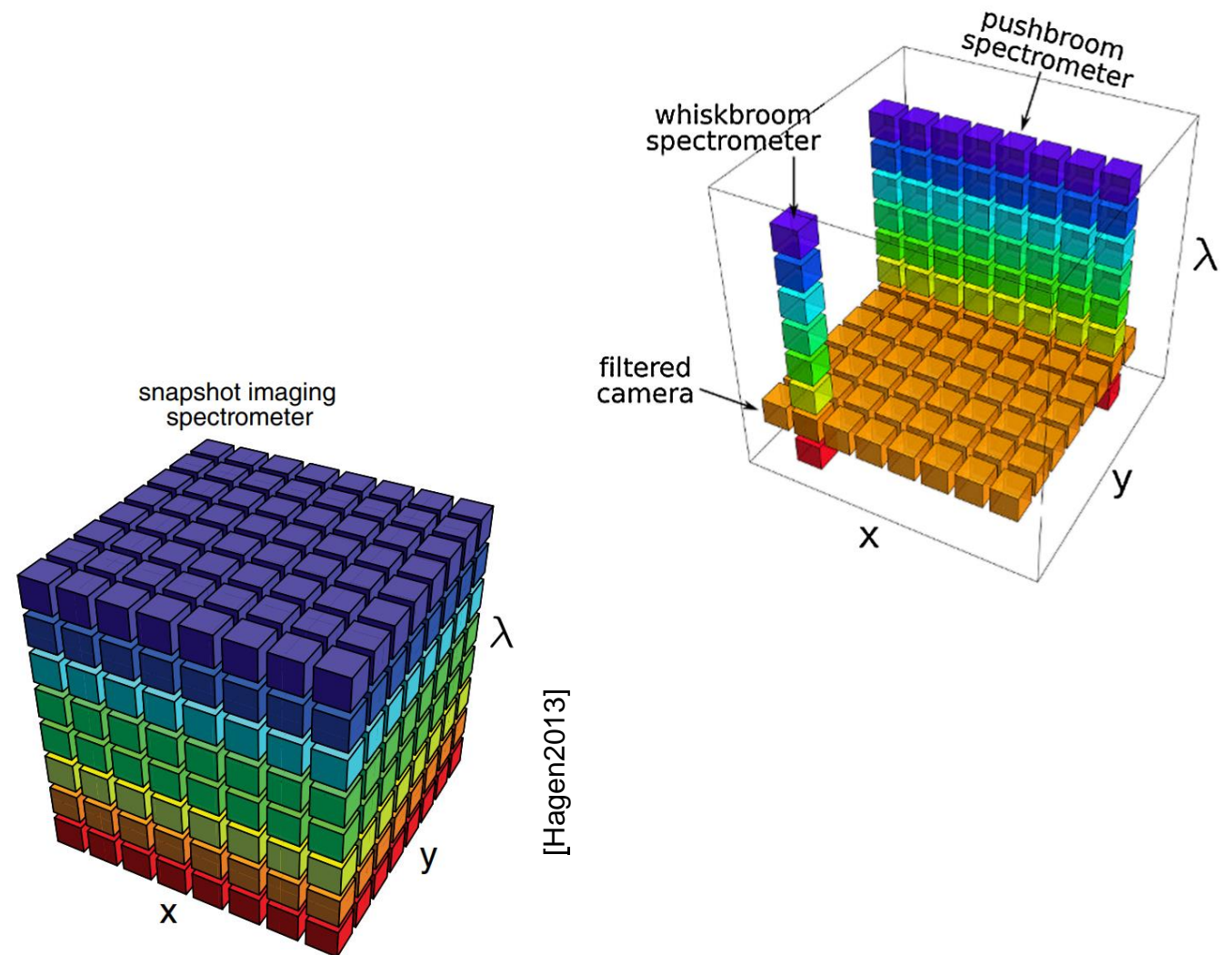
[<https://www.edmundoptics.com/knowledge-center/application-notes/imaging/hyperspectral-imaging-and-multispectral-imaging/>, 24.10.2023]



Hyperspectral imaging basics

Collection modes

- Whiskbroom (point scanning)
- Pushbroom (line scanning)
- Spectral Scanning (staring)
- Snapshot (full registration at once)
- Computational /indirect approaches

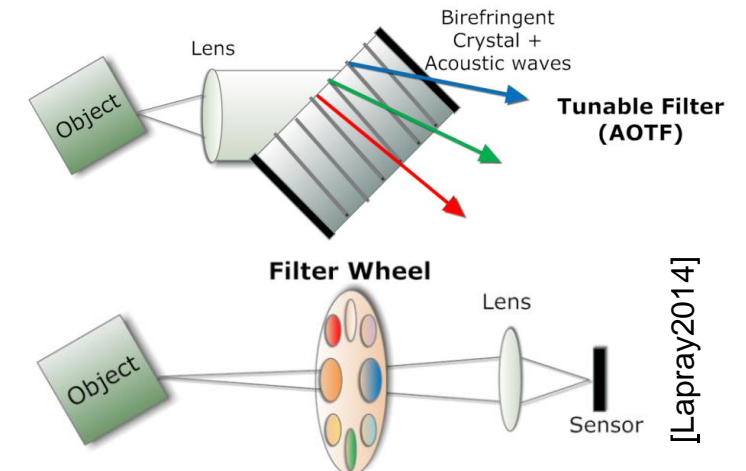
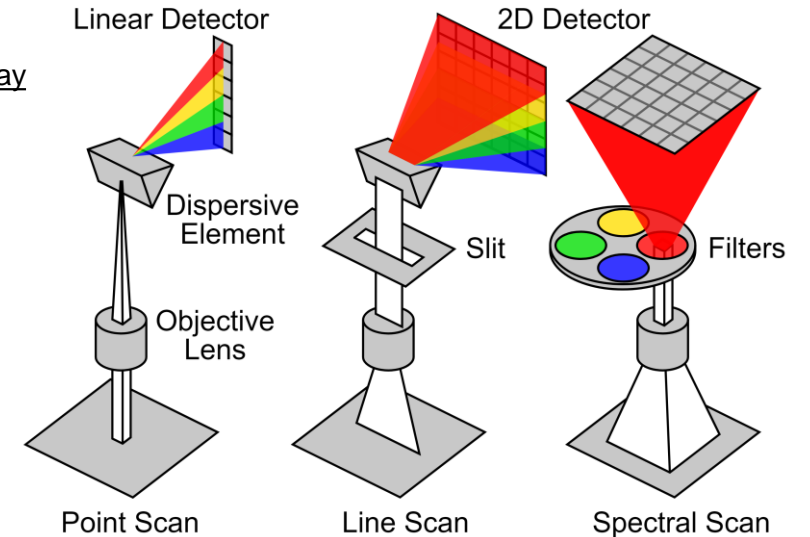


Hyperspectral imaging basics

Collection modes

- Whiskbroom (point scanning)
 - Spectrum at a single location
 - Either translational or rotational scanning
 - Light path does not change
 - Slow operation (→ motion artifacts) and precise positioning required
- Pushbroom (line scanning)
 - Scene along one spatial dimension is captured (by narrow slit aperture)
 - Perpendicular axis is used for spectral discrimination
 - Either translational or rotational scanning to capture second dimension (→ motion artifacts)
- Spectral Scanning (staring)
 - 2D grayscale image of one color band
 - Sequential color band acquisition (→ motion artifacts)
 - Simple optics and detectors, tuneable/exchangeable filters or light source

[<https://collab.dvb.bayern/display/TUMzfp/Hyper-and-multispectral-imaging>, 13.12.2023]

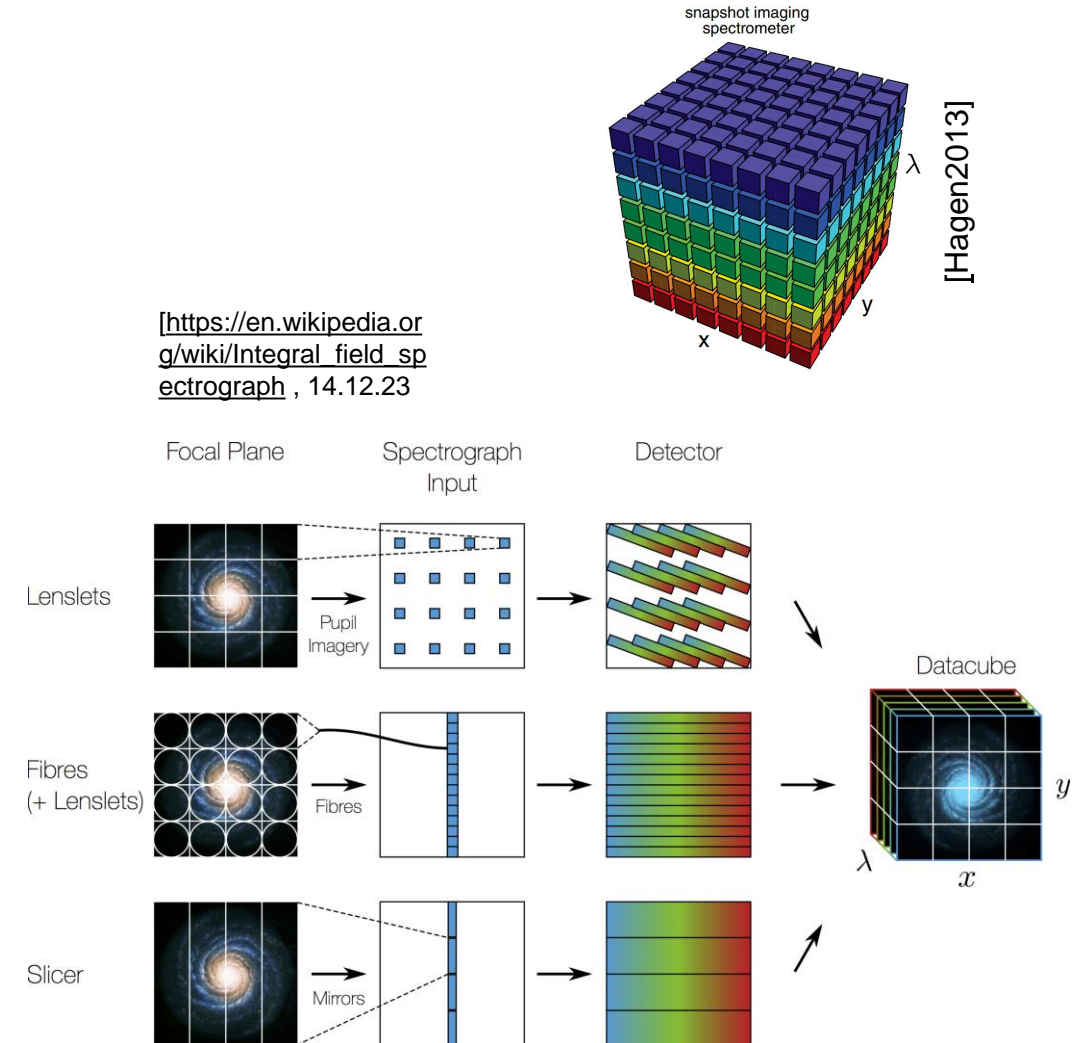


[Lapray2014]

Hyperspectral imaging basics

Collection modes

- Snapshot
 - Combination of image-division and dispersion (wavelength-discriminating) optical elements in front of the sensor and data processing.
 - Techniques
 - Integral Field Spectrometry (IFS; split the image into multiple parts, either by a slicer mirror assembly, a fiber bundle or an lenslet array, to be rearranged, dispersed and projected on the sensor)
 - Multispectral beamsplitting (MSBS; filters and microlenses)
 - Spectrally resolving detector arrays (SRDA; also called mosaic filter-on-chip cameras; place an array of color filters directly on the sensor pixels (similar to Beyer filters)
 - ...
- Software / indirect approaches
 - Multi-camera imaging (using RGB)
 - Sparse sampling (spatial or spectral reconstruction from few support points) and other algorithmic reconstruction approaches



02

(ABSORPTION)
HYPERSENSPECTRAL
IMAGING
APPLICATIONS

(Absorption) Hyperspectral imaging applications

Overview

- Technique in early stage → no/rare routine use in clinics but
 - Wide possibilities
 - Many current research works
- Available information
 - Tissue / blood composition assessment (including oxygenation, hemoglobin concentration, tissue classification, ...)
 - Perfusion assessment
- Medical applications
 - Assessment of skin disease and wounds
 - Peripheral arterial disease
 - Tumor detection/segmentation
 - Hyperspectral microscopy
 - ...

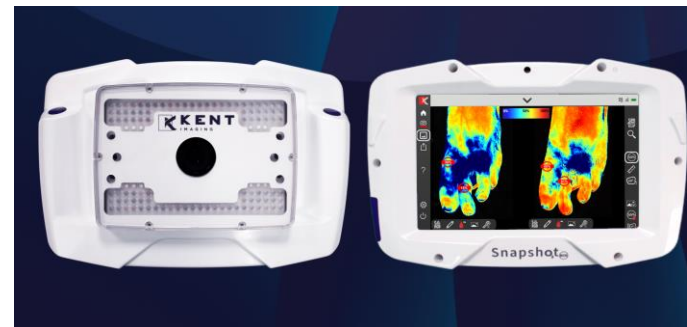
[<https://www.kentimaging.com/news-articles/kent-imaging-announces-the-launch-of-snapshotnir-40> , 13.12.2023

Clinical HSI system Tivita (pushbroom system by Diaspective Vision)



[<https://diaspective-vision.com/produkt/tivita-2-0/> , 13.12.2023]

Kent SNAPSHOTNIR 4.0*



(Absorption) Hyperspectral imaging applications

Overview – Analytic procedure (opposed to purely data driven)

- Background (absorption by media; for scattering similar relations hold)

$$\ln\left(\frac{I_0}{I(d)}\right) := A(\lambda) = \mu_a \cdot d = \sum_{n=1}^N \epsilon_n(\lambda) \cdot c_n \cdot d$$

- Common handling in HSI → scattering assumed a wavelength independent factor (e.g. [Miclos2015])

$$A(x, y, \lambda) := -\log_{10}\left(\frac{I_t(x, y, \lambda) - B(x, y, \lambda)}{I_c(x, y, \lambda) - B(x, y, \lambda)}\right) = G(x, y) + \sum_{n=1}^N \epsilon_n(x, y, \lambda) \cdot c_n(x, y, \lambda)$$

$I_c(x, y, \lambda)$... *white reference*, $G(x, y)$... *Scattering factor*, $B(x, y, \lambda)$... *black reference*

- Example from „Wavelength and model selection for hyperspectral imaging of tissue oxygen saturation” using HSI [Chen2015]

$$\begin{aligned} A(x, y, \lambda) &= \epsilon_{HbO_2}(\lambda) \cdot c_{HbO_2}(x, y) + \epsilon_{Hb}(\lambda) \cdot c_{Hb}(x, y) \\ A(x, y, \lambda) &= \epsilon_{HbO_2}(\lambda) \cdot c_{HbO_2}(x, y) + \epsilon_{Hb}(\lambda) \cdot c_{Hb}(x, y) + \epsilon_{melanin}(\lambda) \cdot c_{melanin}(x, y) \\ A(x, y, \lambda) &= \epsilon_{HbO_2}(\lambda) \cdot c_{HbO_2}(x, y) + \epsilon_{Hb}(\lambda) \cdot c_{Hb}(x, y) + G(x, y) \\ A(x, y, \lambda) &= \epsilon_{HbO_2}(\lambda) \cdot c_{HbO_2}(x, y) + \epsilon_{Hb}(\lambda) \cdot c_{Hb}(x, y) + \epsilon_{melanin}(\lambda) \cdot c_{melanin}(x, y) + G(x, y) \end{aligned}$$

→ MBL3C model is the most suitable for the assessment of StO₂ (best wavelengths [516–580] nm)

(Absorption) Hyperspectral imaging applications

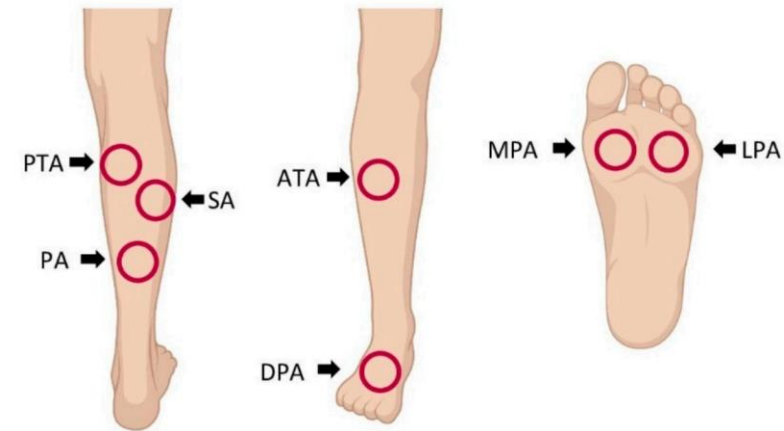
Peripheral arterial disease (PAD)

■ Peripheral arterial disease (PAD)

- Vascular disorder with abnormal narrowing of arteries
- Symptoms: pain, wounds
- Risk factors: smoking, high blood pressure, high sugar,

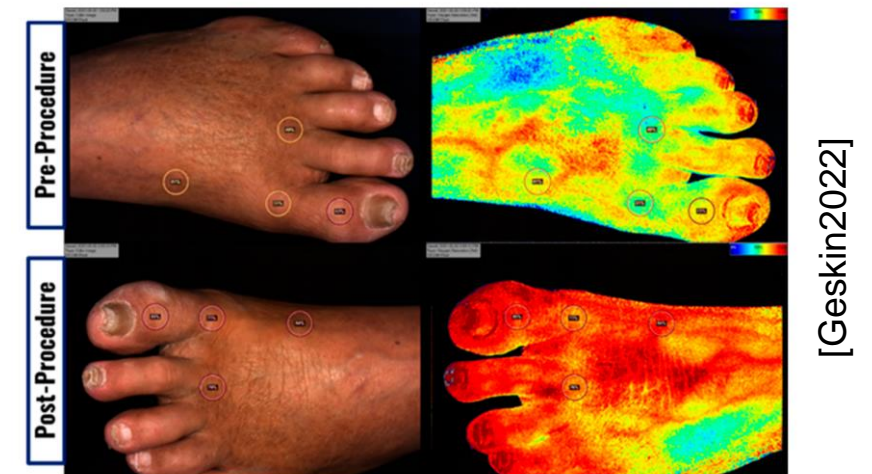
■ Assessment by HIS

- [Grambow2022]
 - Main idea: assessment of angiosomes after surgery (angiosome is a three-dimensional unit of skin and underlying tissues vascularized by a source artery)
 - Finding (e.g.): increase in tissue oxygenation StO₂ and NIR perfusion index
- [Geskin2022]
 - Main idea: Assessment of foot microcirculation as well as macrovascular and microvascular correlation
 - Increases in tissue oxygenation StO₂ and Oxyhemoglobin HbO₂ ; no correlation between used parameters to assess macro- and micro



PTA Posterior tibial artery, SA sural artery, PA peroneal artery, ATA anterior tibial artery, DPA dorsal pedal artery, MPS medial plantar artery, LPA lateral plantar artery

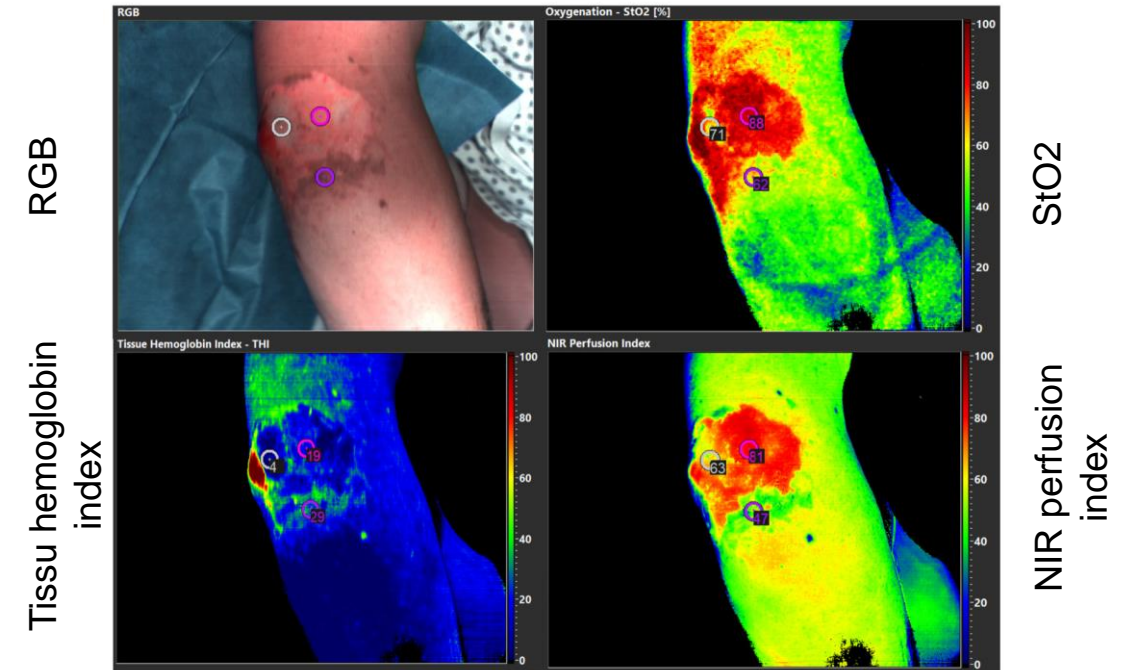
[Grambow2022]



(Absorption) Hyperspectral imaging applications

Assessment of skin disease and wounds

- Wounds show variable compositions and perfusion pattern
- HSI is very well suited as spatial assessment is fundamental
- Multiple HSI based parameter relevant (tissue oxygenation, total oxyhaemoglobin, total deoxyhaemoglobin, total haemoglobin,...)
- Findings
 - [Promny2022] Significant differences between healthy skin and wound areas (of variable strength)
 - E.g. Burn depth assessment possible



[Promny2022]

Received: 27 April 2020 | Revised: 7 July 2020 | Accepted: 15 July 2020

DOI: 10.1111/iwj.13474

ORIGINAL ARTICLE

IWJ WILEY

Hyperspectral imaging in wound care: A systematic review

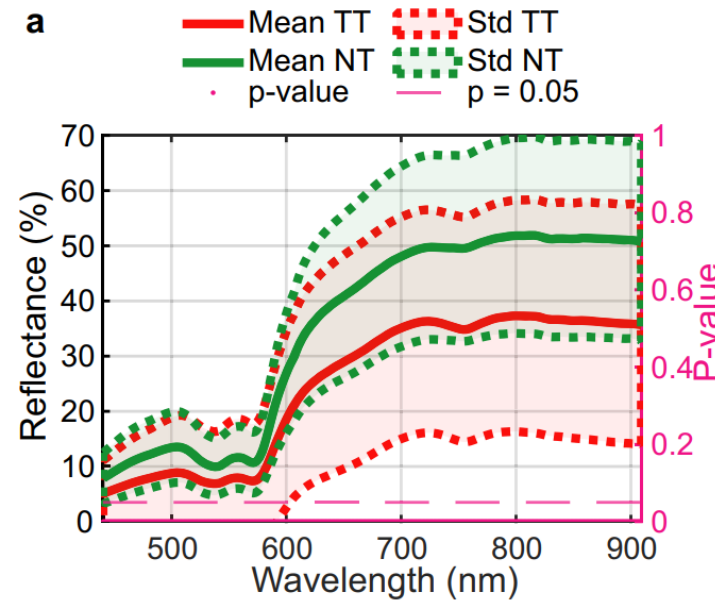
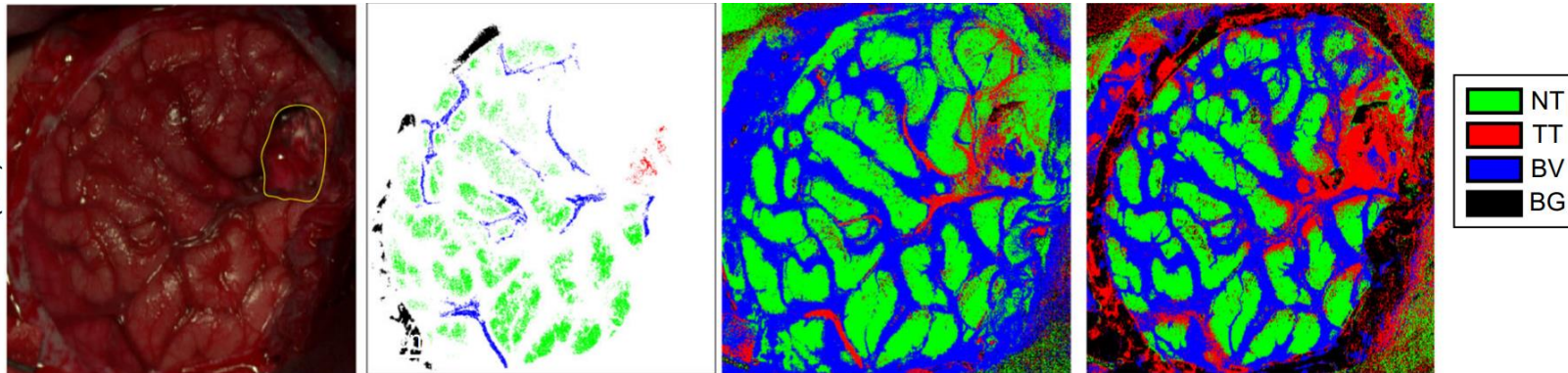
(Absorption) Hyperspectral imaging applications

Tumor detection/segmentation

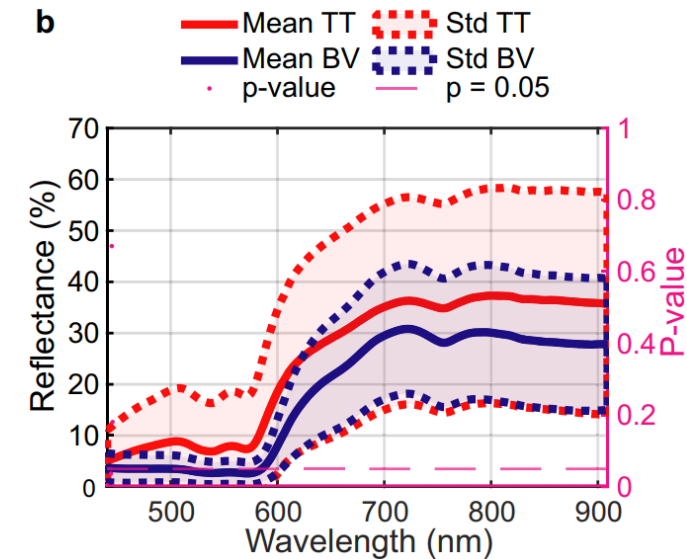
- Tumor section is a (often) life saving intervention
- In brain tumors, segmentation is highly critical (opposing demands)
- Typically, machine learning approaches to distinguish tissue
- Findings

[Leon2023]

Op42C2
Astrocytoma
(G2)



Reflection characteristics
from tumor tissue (TT)
and normal tissue (NT)



Reflection characteristics
from tumor tissue (TT)
and blood vessel (BV)

03

OTHER SPECTROSCOPIC TECHNIQUES

Other spectroscopic techniques

Overview

- Multiple spectroscopic techniques available
- In principle, all techniques can be applied as spot (spectroscopy) or areal (hyperspectral imaging) measurements
- „Type“ is fundamental difference
 - „Absorption type“ (absorption, reflection, scattering, transmission)
 - Fluorescence type
 - Raman type

[Keiser2022, page 295]

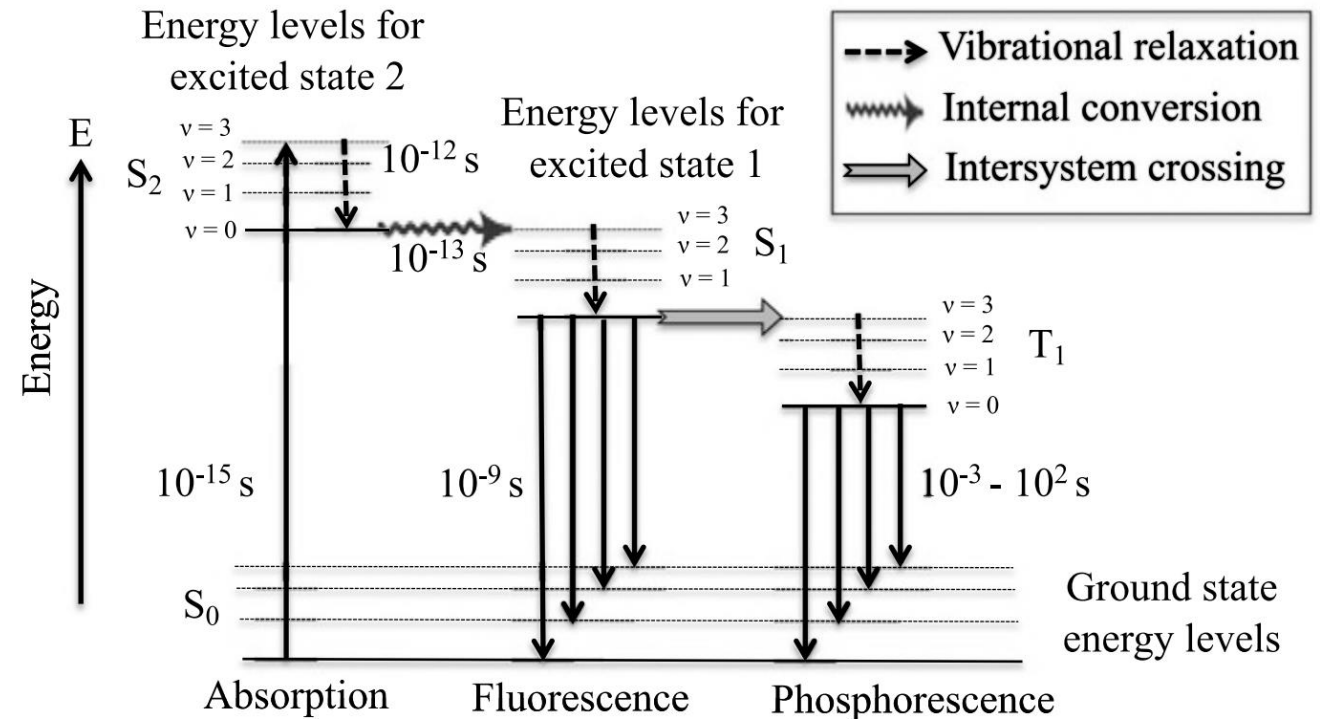
Spectroscopic technique	Description and function
Fluorescence spectroscopy	Based on examining the fluorescence spectra of molecules to determine their basic molecular behavior characteristics, to identify infectious diseases, and to perform noninvasive biopsies
Fluorescent correlation spectroscopy (FCS)	Examines spontaneous fluorescent intensity fluctuations to determine concentrations and diffusion coefficients of molecules and large molecular complexes
Elastic scattering spectroscopy (ESS)	Also called <i>diffuse reflectance spectroscopy</i> and <i>light scattering spectroscopy</i> ; based on analyzing the relative intensity of elastic backscattered light to distinguish diseased from healthy tissue
Diffuse correlation spectroscopy (DCS)	A noninvasive technique that probes deep into tissue to measure blood flow by using the time-averaged intensity autocorrelation function of the fluctuating diffuse reflectance signal
Raman spectroscopy	A non-invasive, label-free biomedical optics tool for evaluating the chemical composition of biological tissue samples (variations: CARS; time-resolved; wavelength-modulated)
Surface-enhanced Raman scattering (SERS) spectroscopy	Combines Raman scattering effects with surface plasmon resonance to identify a molecular species and to quantify different targets in a mixture of different types of molecules
Coherent anti-Stokes Raman scattering (CARS) spectroscopy	A nonlinear optical four-wave-mixing process for label-free imaging of a wide range of molecular assemblies based on the resonant vibrational spectra of their constituents
Stimulated Raman scattering (SRS) spectroscopy	Uses two laser beams to coherently excite a sample for straightforward chemical analyses
Photon correlation spectroscopy (PCS)	Uses dynamic light scattering to measure density or concentration fluctuations of small particles in a highly diluted suspending fluid to examine sizes and movements of scattering particles
Fourier transform infrared (FTIR) spectroscopy	Precisely measures light absorption per wavelength over a broad spectral range to identify materials, determine their constituent elements, and check their quality
Brillouin scattering spectroscopy	Optical technique for noninvasively determining the elastic moduli or stiffness of materials

Other spectroscopic techniques

Fluorescence spectroscopy

- Fluorescence
 - Three stage process
 - Fluorophores are molecules that allow fluorescence
 - Jablonski diagram visualizes processes
- In fluorescence, emission may generally relax to a variety of vibrational levels ($v=n$) of the ground state (S_0) \rightarrow large bandwidth (i.e. range of possible wavelengths) of emitted photons
- Stokes shift: wavelength difference between excitation and emission (emission wavelength is smaller)

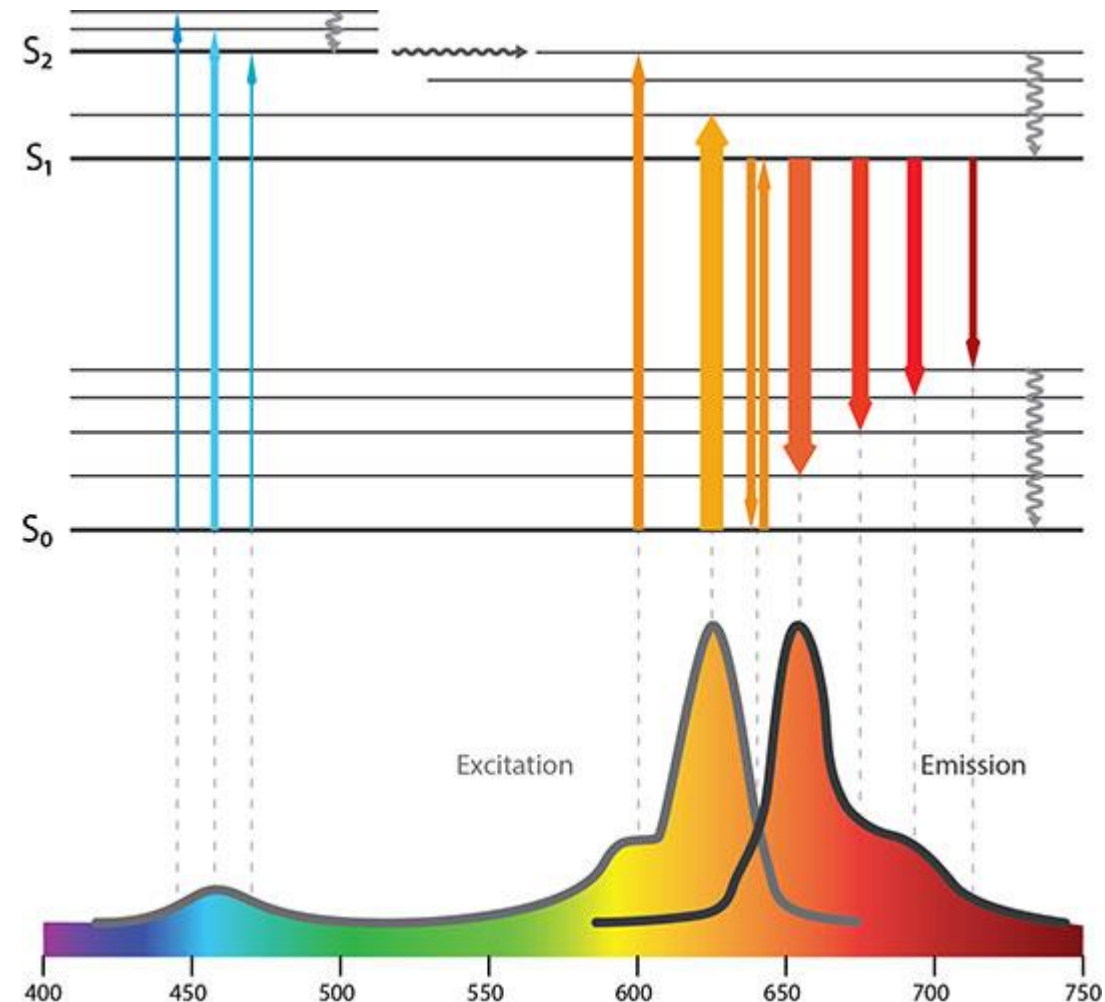
Jablonski diagram for fluorescence and phosphorescence



Other spectroscopic techniques

Fluorescence spectroscopy

- Example of a excitation/emission spectrum
 - Depends on observed atoms/molecules
 - Emission can be broad
- Fluorescence correlation spectroscopy
 - Used to observe particle movements
 - Variation of fluorescence over time is observed (slow processes)



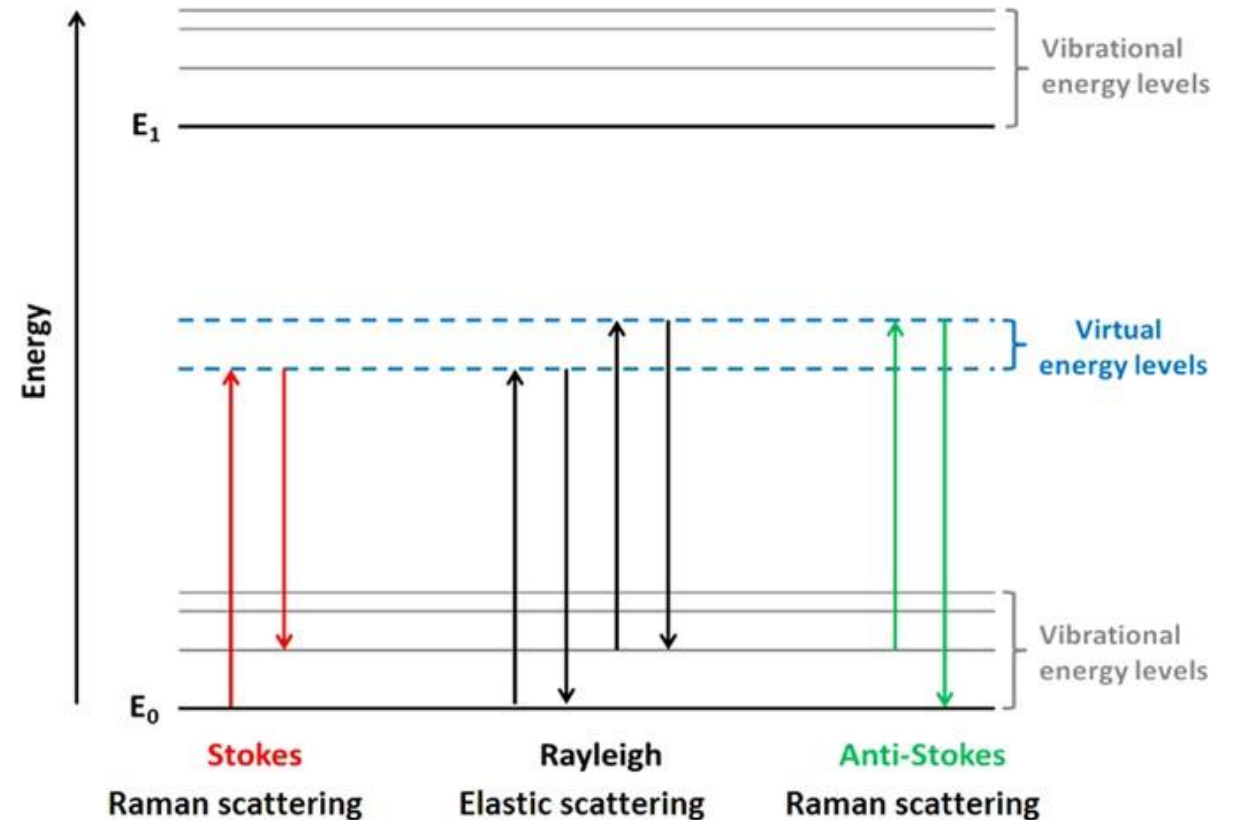
[<https://www.enzolifesciences.com/science-center/techniques/2019/december/what-is-the-difference-between-fluorescence-phosphorescence-and-luminescence?/> , 14.12.23]

Other spectroscopic techniques

Raman spectroscopy

- Raman scattering: inelastic scattering
- Raman-Effect vs. Rayleigh scattering: part of the scattered photons will undergo a shift in frequency
- General difference in Raman scattering: Stokes & Anti-Stokes scattering

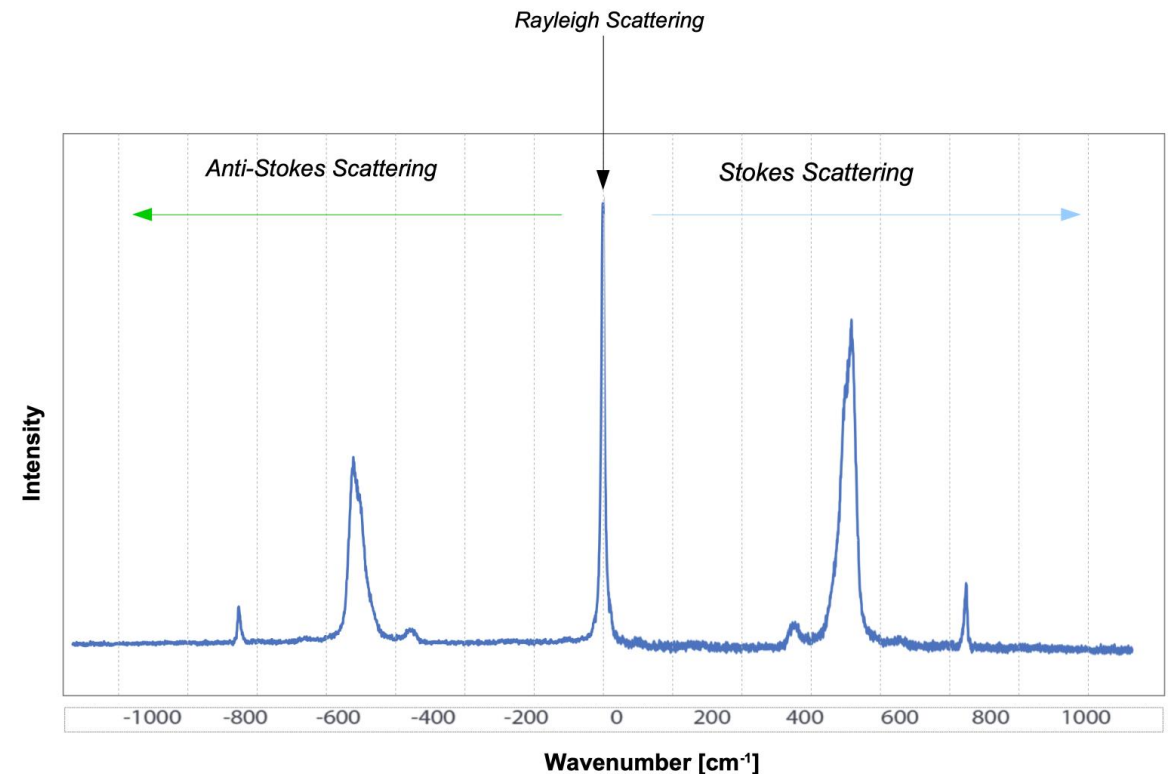
[<https://www.nature.com/articles/s41536-017-0014-3/figures/1> 08.11.2023]



Other spectroscopic techniques

Raman spectroscopy

- Results „similar“ to fluorescence: novel (broadband) spectral components + exciting wavelength
- Application condition differ as scattering is required



[<https://collab.dvb.bayern/pages/viewpage.action?pageId=70096837> , 13.12.2023]

S

SUMMARY

Summary

Key messages

- Spectroscopy refers to the acquisition of multiple wavelengths
- Multiple spectroscopic techniques available; generally, spectroscopic measurements can be done as a spot measurement and spatially resolved
- Multi- and hyperspectral imaging combine ideas from imaging (2d recordings) and spectroscopy (multiple wavelengths)
- Fluorescence spectroscopy exploits the property of certain materials to emit light at certain wavelengths upon absorption of light at another wavelength
- Raman spectroscopy exploits the property of certain materials to vary the wavelength upon scattering (inelastic scattering)

Summary

Literature

[Geskin2022] G. Geskin, M. D. Mulock, N. L. Tomko, A. D’asta, and S. Gopalakrishnan, “Effects of Lower Limb Revascularization on the Microcirculation of the Foot: A Retrospective Cohort Study,” *Diagnostics*, vol. 12, no. 6, pp. 1–13, 2022, doi: 10.3390/diagnostics12061320.

[Grambow2022] E. Grambow *et al.*, “Evaluation of Hyperspectral Imaging for Follow-Up Assessment after Revascularization in Peripheral Artery Disease,” *J. Clin. Med.*, vol. 11, no. 3, 2022, doi: 10.3390/jcm11030758.

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[Saiko2020] G. Saiko, P. Lombardi, Y. Au, D. Queen, D. Armstrong, and K. Harding, “Hyperspectral imaging in wound care: A systematic review,” *Int. Wound J.*, vol. 17, no. 6, pp. 1840–1856, 2020, doi: 10.1111/iwj.13474.

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