Multispectral Imaging

From astronomy to microscopy

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# Photonics

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7.5 ECTS
Multispectral imaging

Lectures:
• Fundamentals of Spectroscopy
• Instrumentations
• Molecular Spectroscopy
• Image analysis
• Multivariate analysis
• Astronomical imaging
• Satellite imaging
• Atmosphere remote sensing/imaging
• Combustion Imaging
• Medical Imaging
• Visual science
• Magnetic resonance imaging
• Microscopy
• Nuclear imaging
Multispectral = many spectral bands

"Each pixel contains a spectrum"
Multispectral = many spectral bands

Optical microscopy

X-ray imaging
Spectral bands from the electromagnetic spectrum
Interactions between electromagnetic radiation and sample

- Sample is composed of atoms, molecules
- Electromagnetic radiation transfers energy
- By examining the resulting electromagnetic radiation after it has interacted with the sample - conclusions can be drawn about the object under study
Interactions between energy levels

Absorption

\[ E_1 \rightarrow E_2 \]

Emission

\[ E_1 \rightarrow E_2 \]
Basic arrangement for Multispectral imaging

Source
- Sun
- Lamps
- Lasers
- LEDs
- Synchrotron
- X-ray tube

Sample
- Plants
- Forests
- Tissue
- Cells
- Flames
- Chemical compounds
  etc.
  etc.

Analyzer
- Filters
- Spectrometers
- Monochromators

Detector
- Photodiodes
- Photomultiplier tubes
- CCD cameras
- CMOS cameras
- Image intensifiers
Astronomy
Supernova 1604

Multispectral (X-ray, optical and infrared) representation of the last supervova and the Milky Way in 1604
Coronal temperature diagnostics derived from multilayer observations with the multi-spectral solar telescope array

Image from the 211 Å Ritchey-Chrétien telescope

Image from the 1216 Å Ritchey-Chrétien telescope

Paul Boerner Dissertation thesis Stanford University July 2004
Remote sensing: Satellite imaging
Google Earth
Active or passive satellite imaging

Sensor system:
- Optical (passive)
- SAR (active)

Wavelength:
- 400–3000 nm
- 1–100 cm

Scattering medium:
- Leaf surface / interior
- Branches, trunks, ground

Absorbing medium:
- Chloroplasts, cell walls and cell water
- Negligible absorption
Push-broom imaging spectrometer
Reflectance from fresh or dry oak leaf

Reflectance spectra of leaves will be different dependent on its state
Satellite imaging of forests

Photosynthetic activity

Average rainfall

University of Arizona Terrestrial Biophysics and Remote Sensing Lab
Combustion diagnostics
Combustion diagnostics: UV-VIS-IR
Combustion diagnostics: Non-contact temperature measurements

Planck radiation
The spectral shape of the emission is temperature dependent

Planck's law
\[ I(\lambda) = \frac{2 \pi h c^2}{\lambda^5} \frac{1}{e^{\frac{hc}{\lambda k T}} - 1} \]

Wien's displacement law
\[ \lambda_{\text{max}} T = 2.898 \cdot 10^{-3} \text{K} \cdot \text{m} \]

Stefan-Boltzmann's law:
\[ I = \sigma T^4 \]
Combustion diagnostics: Non-contact temperature measurements

Temperature imaging using 2-D pyrometry

The ratio between the emission signals at two wavelengths is temperature dependent.

Still there is a line-of-sight limitation!
Combustion diagnostics: Laser-induced fluorescence

- Absorption
- Fluorescence
- Vibrational relaxation
Combustion diagnostics: Laser-induced fluorescence

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<th>CH</th>
<th>OH</th>
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<tr>
<td>Excitation (nm)</td>
<td>~387</td>
<td>~283</td>
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<tr>
<td>Detection (nm)</td>
<td>~430</td>
<td>~310</td>
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Medical Imaging
Medical imaging: X-ray
X-ray production
X-ray spectrum

Characteristic Radiation Spikes (Tungsten)
Contrast in X-ray images
X-ray computed tomography
Multispectral X-ray

The graph illustrates the variation of the mass attenuation coefficient ($\mu/\rho$) with energy (keV) for different materials. The materials include:

- **L-edge(s), Lead**
- **K-edge**
- **Iodine**
- **Bone**
- **Z = 53** (Iodine)
- **Lead**
- **Z = 82**
- **Soft tissue**
- **Fat**

The image on the right shows a multispectral X-ray scan of a human figure, highlighting different body parts in various colors.
Medical imaging: Optical imaging

• Why?
  • Non-ionizing
  • Energy carried by the visible/NIR radiation matches transitions in the outer electron shells in many molecular compounds > multiple sources of contrast
  • Characterize the tissue composition based on reflectance, transmission, fluorescence and scattering properties of the object under study
Medical optical imaging: UV-VIS-IR
Medical imaging: Optical imaging – absorption of light
Optical tomography of tissue chromophores
Optical tomography of tissue chromophores
Medical imaging: Contrast based on fluorescence
1. Administration of ALA or its esters
2. Build-up of PpIX
3. Diagnostics
4. Treatment

ALA-induced Protoporphyrin IX (PpIX) production

Haem

Protoporphyrin IX

Tumour

Blue laser

635 nm

Red laser
Fluorescence guided brain tumor resection

Brain tumor

normal tissue

infiltration zone

solid tumor

Fluorescence Intensity [a.u.]

0 1 2 3 4 5 6

Wavelength [nm]

500 550 600 650 700

Tumor border
Infiltration zone
Normal close
Normal far
Fit
Fluorescence guided bladder cancer delineation

Fluorescence diagnostics

Blue Light + Fluorescent tumour marker

Normal white light examination

Blue light examination
Medical imaging: Pre-clinical imaging

• Imaging of biological events within an experimental animal model (i.e. mice or rats)
• Relied heavily upon when investigating e.g.
  • Tumor growth
  • Pharmaceutical uptake
  • Pharmaceutical effect on tissue
  • ...

Fluorescence imaging instruments

PerkinElmer IVIS Spectrum
Fluorescence imaging: Targeting fluorophores

Targets a certain antigen on/in the cell

Over time the concentration will be higher in tissues where the antigen is over-expressed
Fluorescence imaging: Targeted fluorophores
Fluorescence imaging in 3D
Fluorescence imaging: Activatable fluorophores
Fluorescence imaging: Activatable fluorophores

Imaging of MMP-activation after induction of cerebral ischemia

MMP: Matrix metalloproteinases

A family of proteases that are involved in breakdown of extracellular matrix. E.g., wound healing, cell migration, angiogenesis, bone development as well as arthritis, intracerebral hemorrhage and metastasis.
Bioluminescence imaging

The firefly gene that encodes the *Luciferase* enzyme is infected into mice genome

*Luciferase* is an enzyme catalysing a reaction between *D-luciferin*, oxygen and ATP that yields light.

The expression of *Luciferase* can be tracked by imaging the green luminescence after administration of *D-luciferin*

\[
D\text{-}\text{luciferin} + \text{ATP} + \text{O}_2 \rightarrow \text{photon} \\
\text{Luciferase}
\]

The oxidation of *D-luciferin* produces light at 500-560 nm.
Microscopic imaging
Microscopic imaging

Angular Deflection Detection Regions

Sample

All incoming photons assumed at 0°

Camera

Beam Splitter

Reflective Objective (RO)

Fiber Ring Light (FRL)

R - Reflection
S - Scattering
T - Transmission

Camera

Beam Splitter

Reflective Objective (RO)

Fiber Ring Light (FRL)

R - Reflection
S - Scattering
T - Transmission
Microscopic imaging

From a multispectral image

We get a spectrum in each pixel

\[ x \]

\[ y \]

\[ UV \]

\[ \lambda \]

\[ IR \]

\[ \lambda \]

\[ \lambda \]
Microscopic imaging

- Microscopy of red blood cells
- Find which red blood cells that are infected with the malaria parasite
Microscopic imaging
Microscopic imaging
Microscopic imaging

Reflectance

Scattering

Transmittance
Multispectral imaging – from radiowaves to gammarays
Multispectral imaging – from astronomy to microscopy