Mitglied der Helmholtz-Gemeinschaft

Institut für Bio- und Geowissenschaften
IBG-2: Pflanzenwissenschaften

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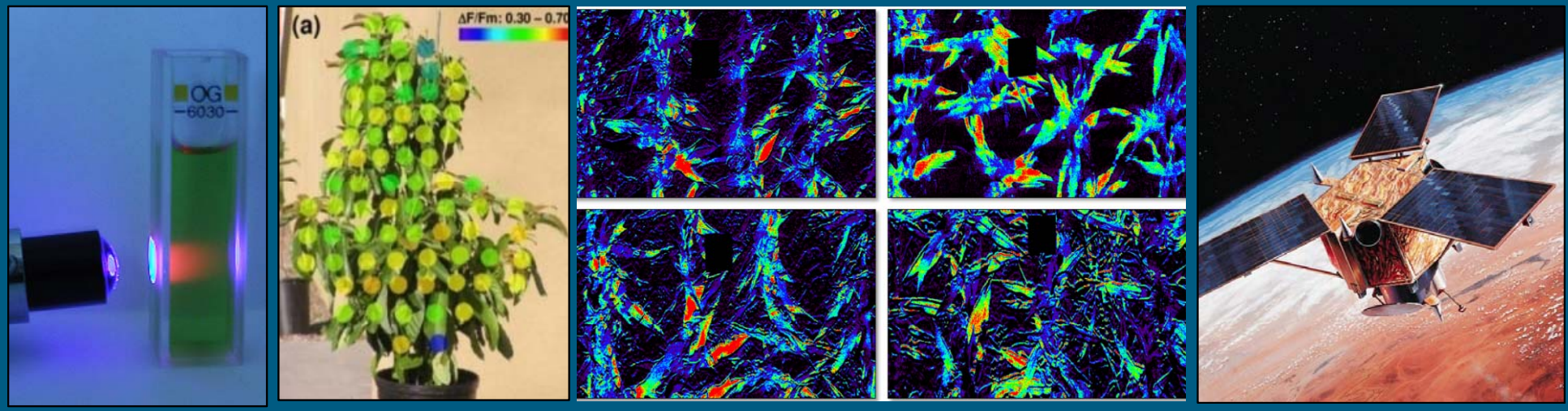
Hyperspectral imaging as a tool for plant phenotyping



Uwe Rascher • EPPN summer school

July 5th 2013

Forschungszentrum Jülich

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Budget: 380 Mio. €

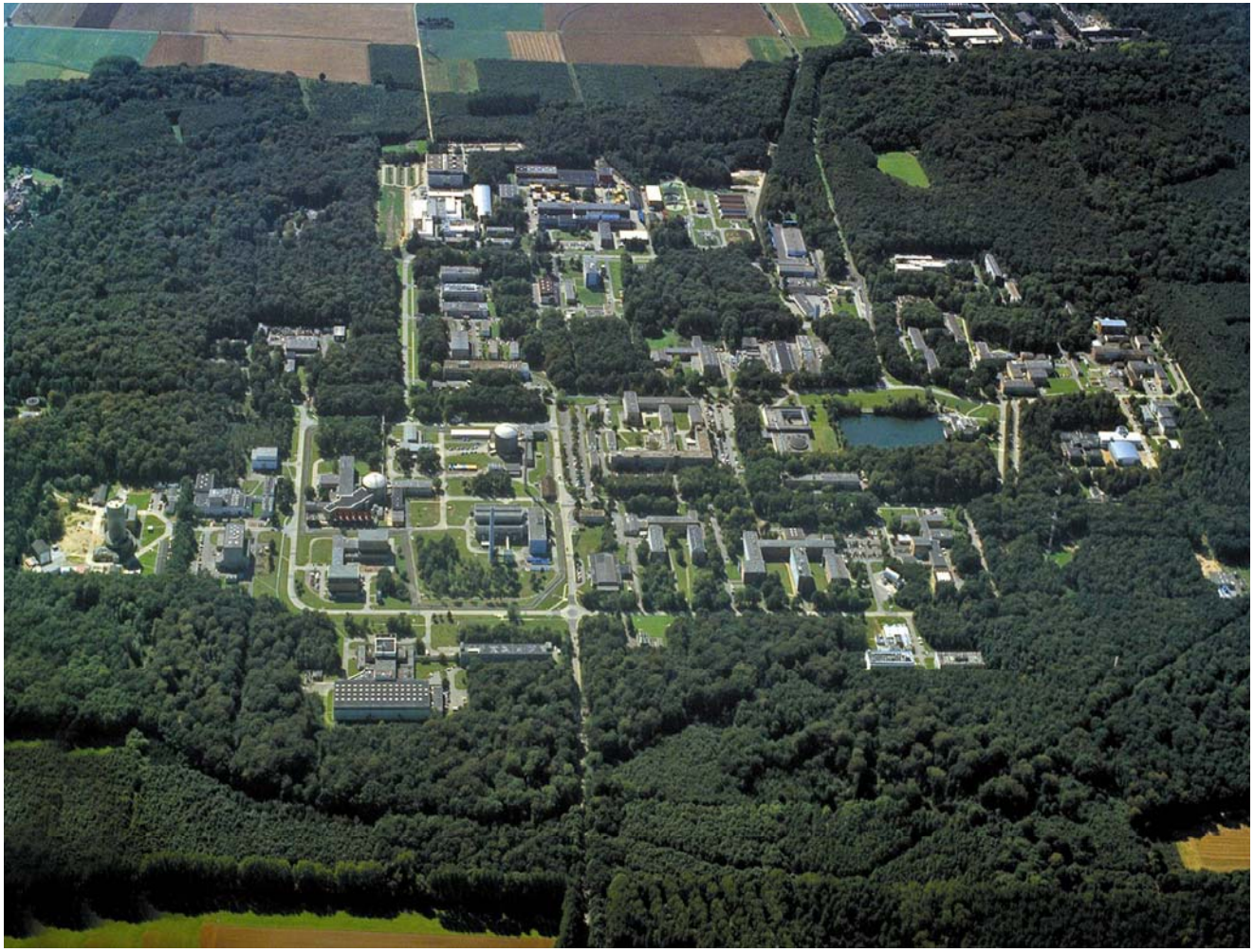
Third party funding: 95 Mio. € (16 Mio. Industry)

Employees: 4.300

Scientists: 1.500

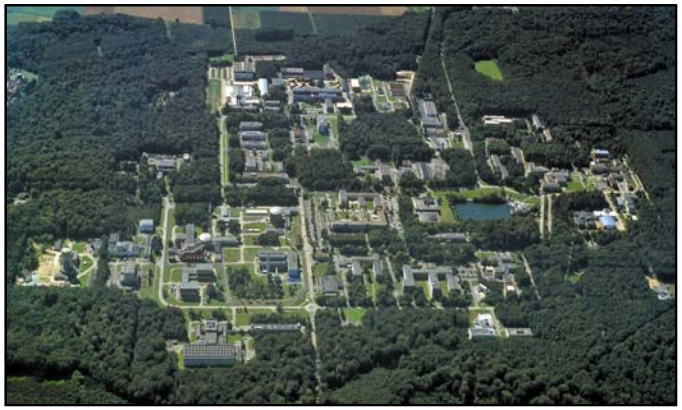
+ 900 guest scientists per year

8.500 patents



Forschungszentrum Jülich
IBG-2: Plant Sciences

www.fz-juelich.de/ibg/ibg-2



140 Employees ● 45 Scientists ● 25 PhD students

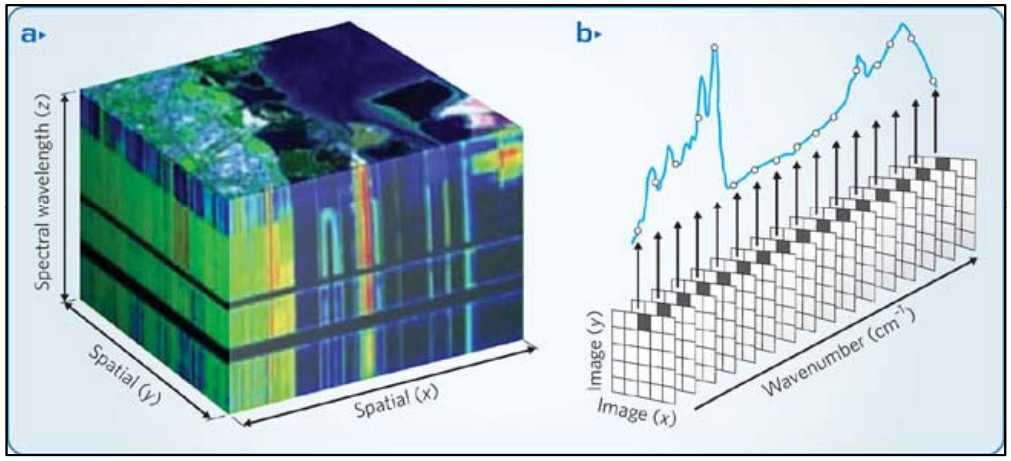
- Bioeconomy
- Plant Phenotyping
- Adaptation to Climate Change
- Sustainable Bioproduction
- From basic research to application

Outline: Hyperspectral Phenotyping

1) Some principles on light

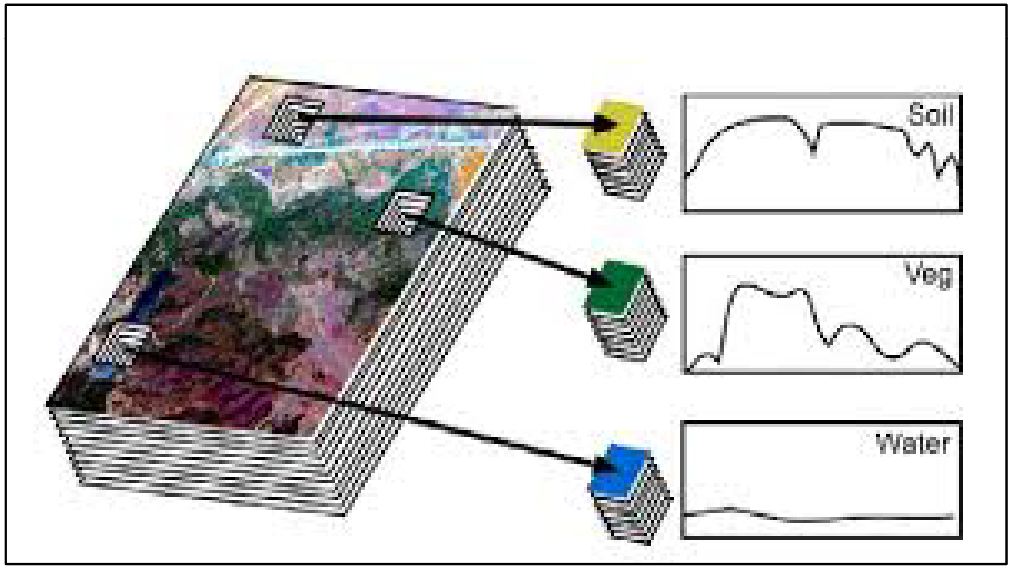
Outline: Hyperspectral Phenotyping

1) Some principles on light
2) ‘Hyperspectral imaging’ or ‘Imaging Spectroscopy’ of plants



Outline: Hyperspectral Phenotyping

1) Some principles on light
2) ‘Hyperspectral imaging’ or ‘Imaging Spectroscopy’ of plants
3) How to extract quantitative data – vegetation indices and advanced methods



Outline: Hyperspectral Phenotyping

1) Some principles on light
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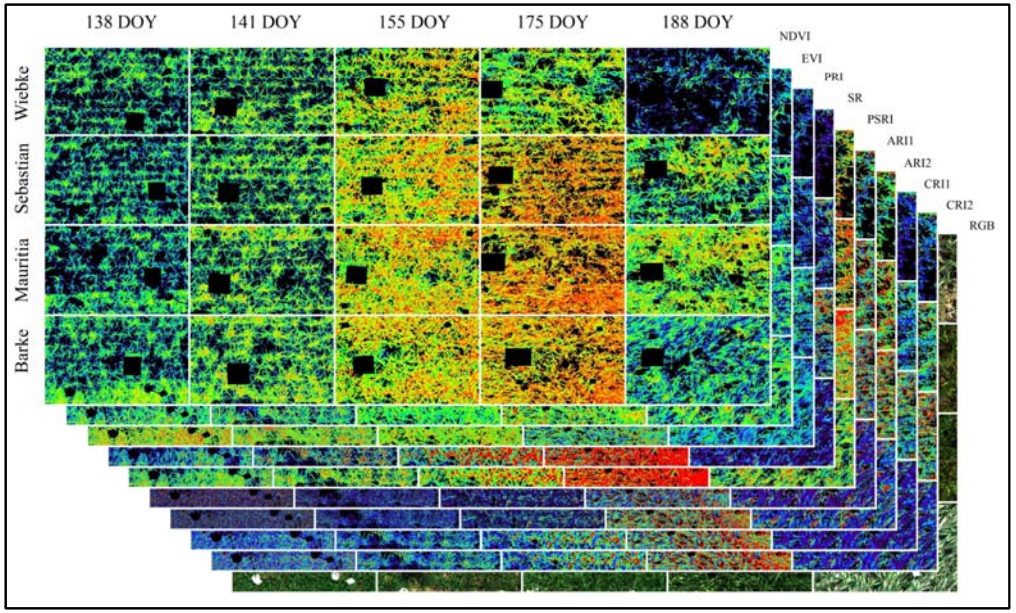
3) How to extract quantitative data – vegetation indices and advanced methods
4) Examples from the real world



Outline: Hyperspectral Phenotyping

1) Some principles on light
2) ‘Hyperspectral imaging’ or ‘Imaging Spectroscopy’ of plants
3) How to extract quantitative data – vegetation indices and advanced methods
4) Examples from the real world
5) Linking hyperspectral with fluorescence

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Some principles of light

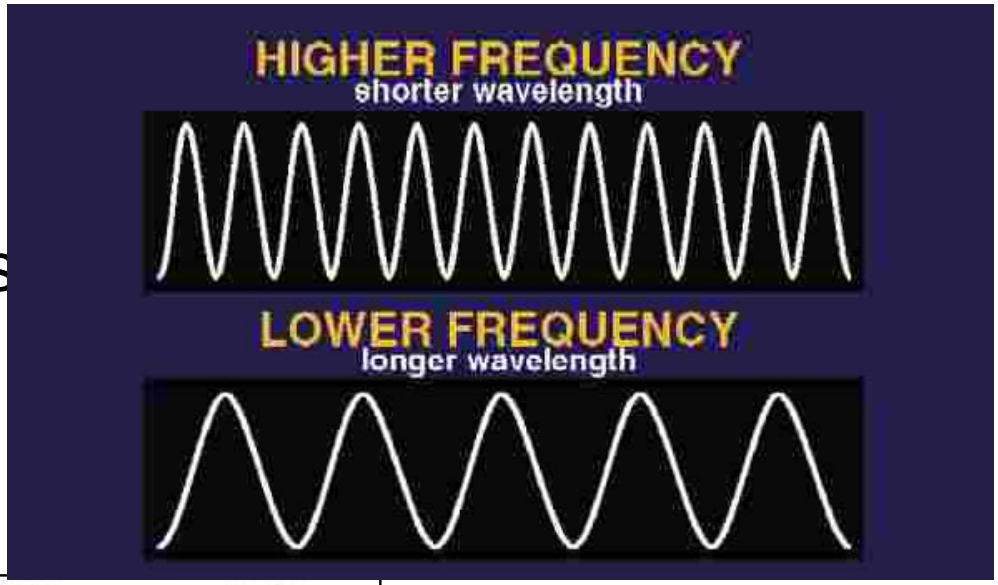
1. Wavelength vs. Frequency
2. Absorption by pigments

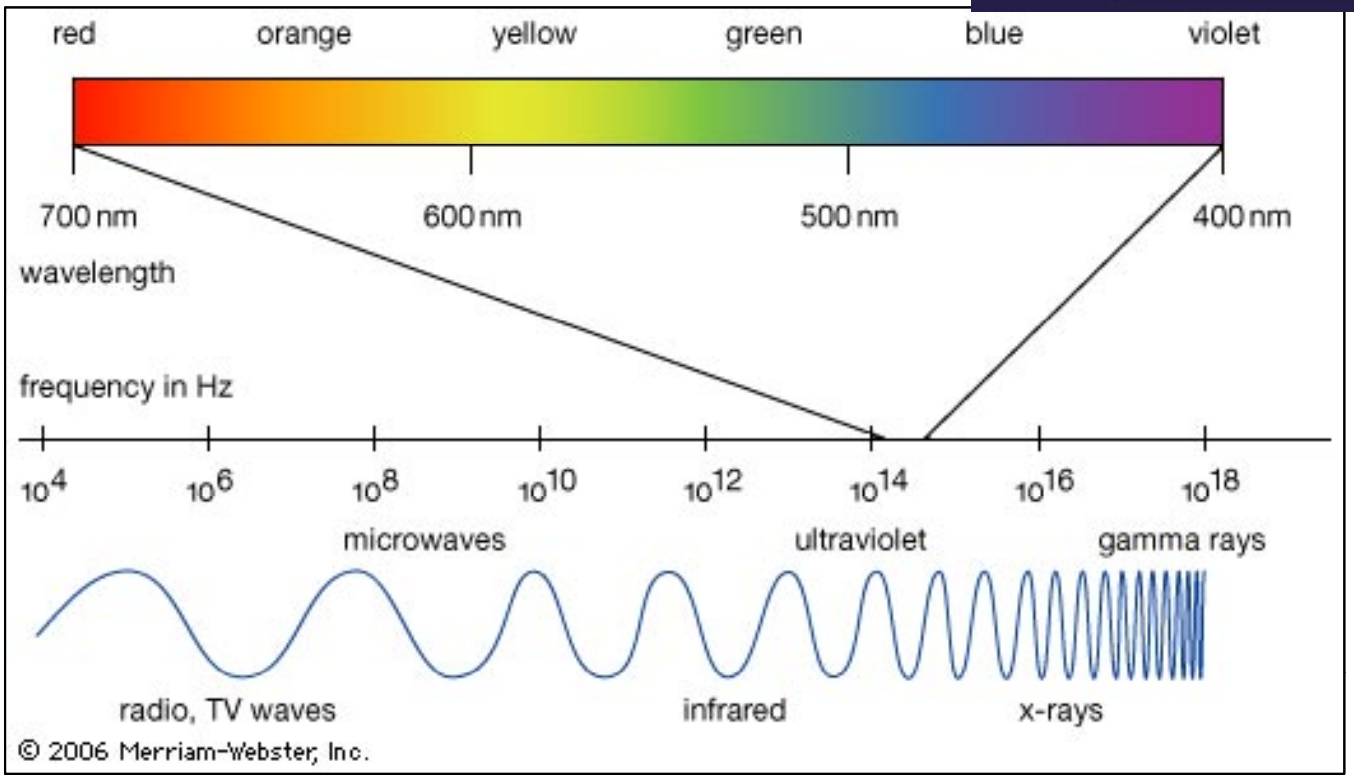
4. Juli 2013

Some principles of light

Light consists of photons, which properties are best described by wave functions

Light is an electromagnetical wave





$$c = \lambda \cdot f$$

c: light speed (300 000 km/s)

λ: Wavelength (nm)

f: Frequency (1/s or Hz)

Some principles of light: light from a plant's perspective

98% of the electromagnetic radiation on the surface of our earth is between 300 und 3000 nm

Ultraviolet radiation (UV) [280 – 370 nm]

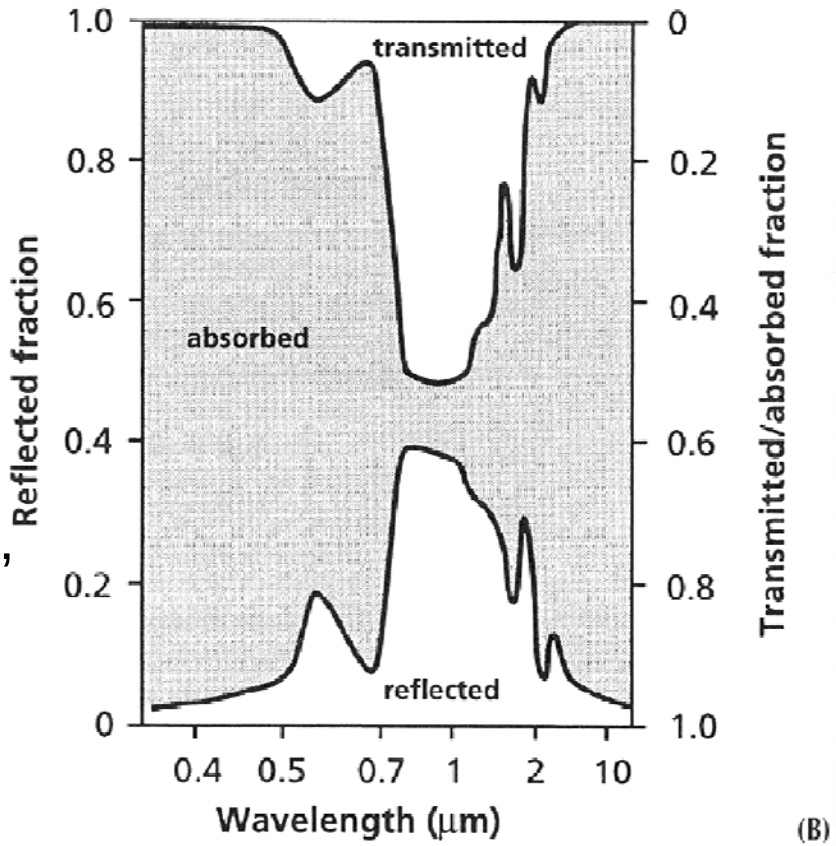
Photosynthetically active radiation [370 – 700 nm]

- PAR: "Photosynthetic active radiation"
- PFD: "photone flux density"
- PPFD: "photosynthetic photone flux density"

Infrared radiation

- Near Infrared (near-IR, 700 – 3000 nm)
- Long wavelength IR (> 3000 nm)

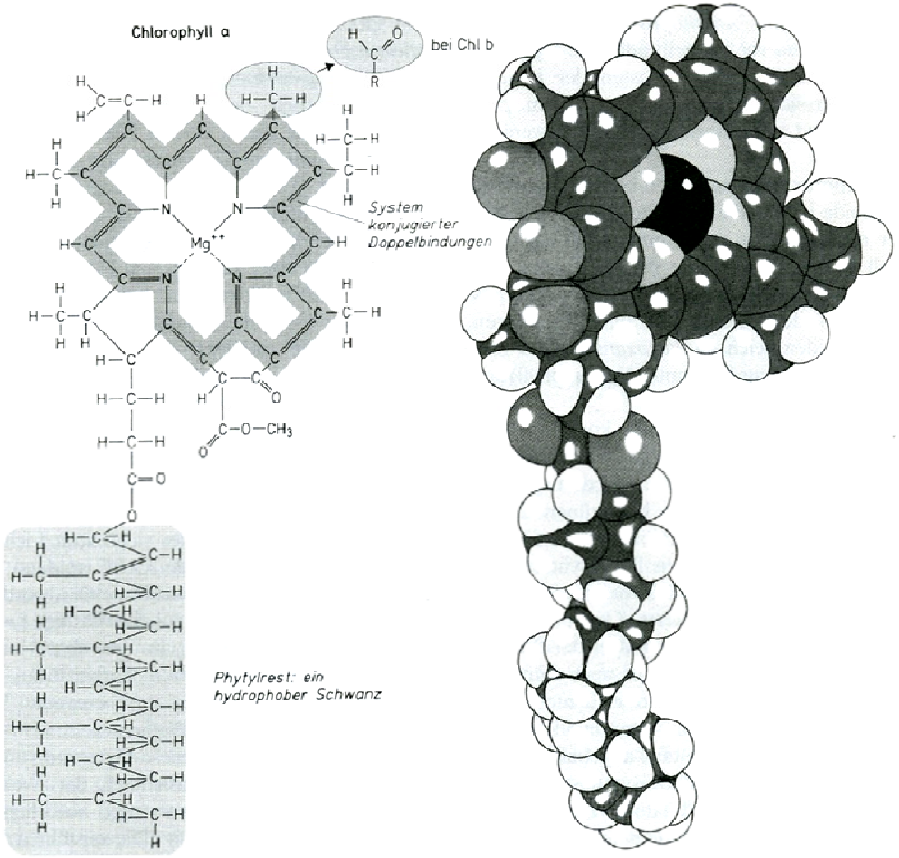
Thermal radiation [8000 – 16000 nm or 8 – 16 µm]

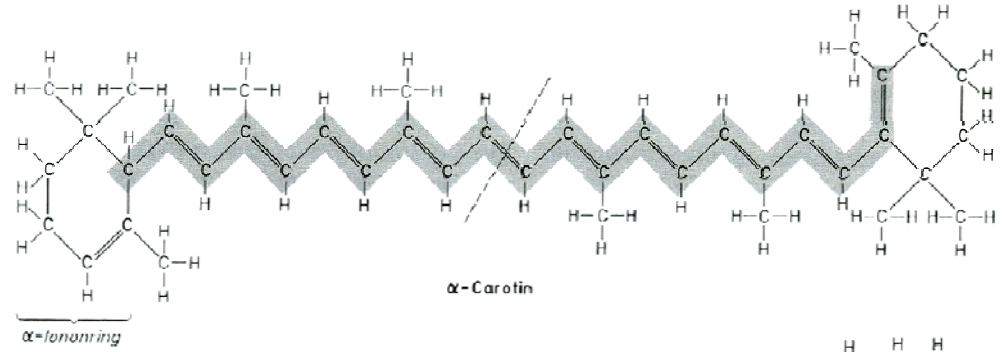


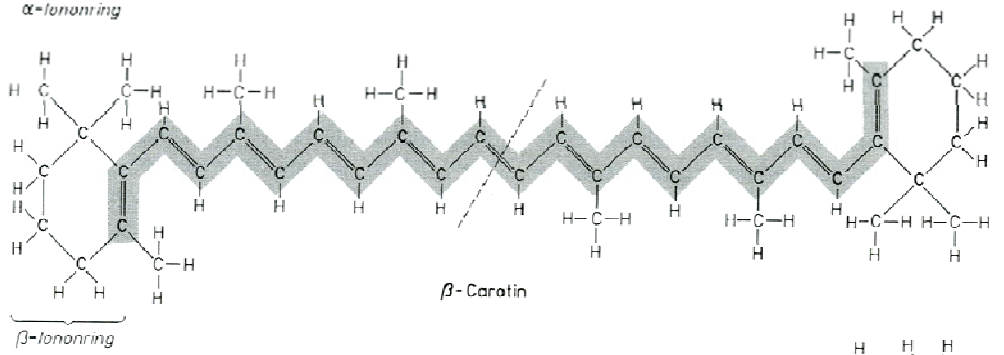
(B) Wavelength spectrum of absorbed, transmitted, and reflected irradiance (% of total) by a leaf (Gates 1965). Copyright Ecological Society of America.

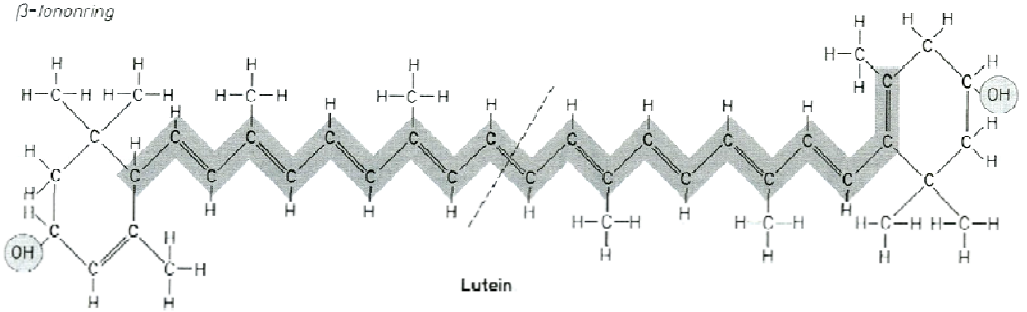
Some principles of light: Light absorption by pigments

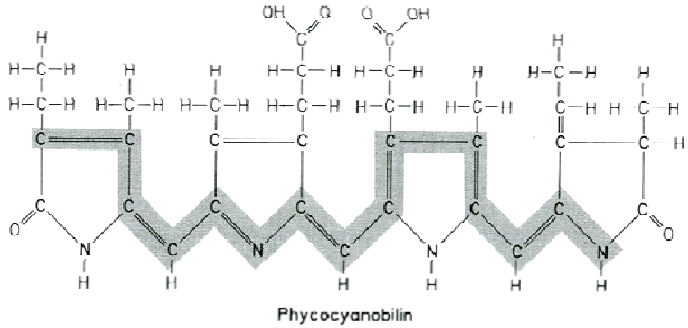
All pigment of the photo-synthetic apparatus have conjugated π-electron systems





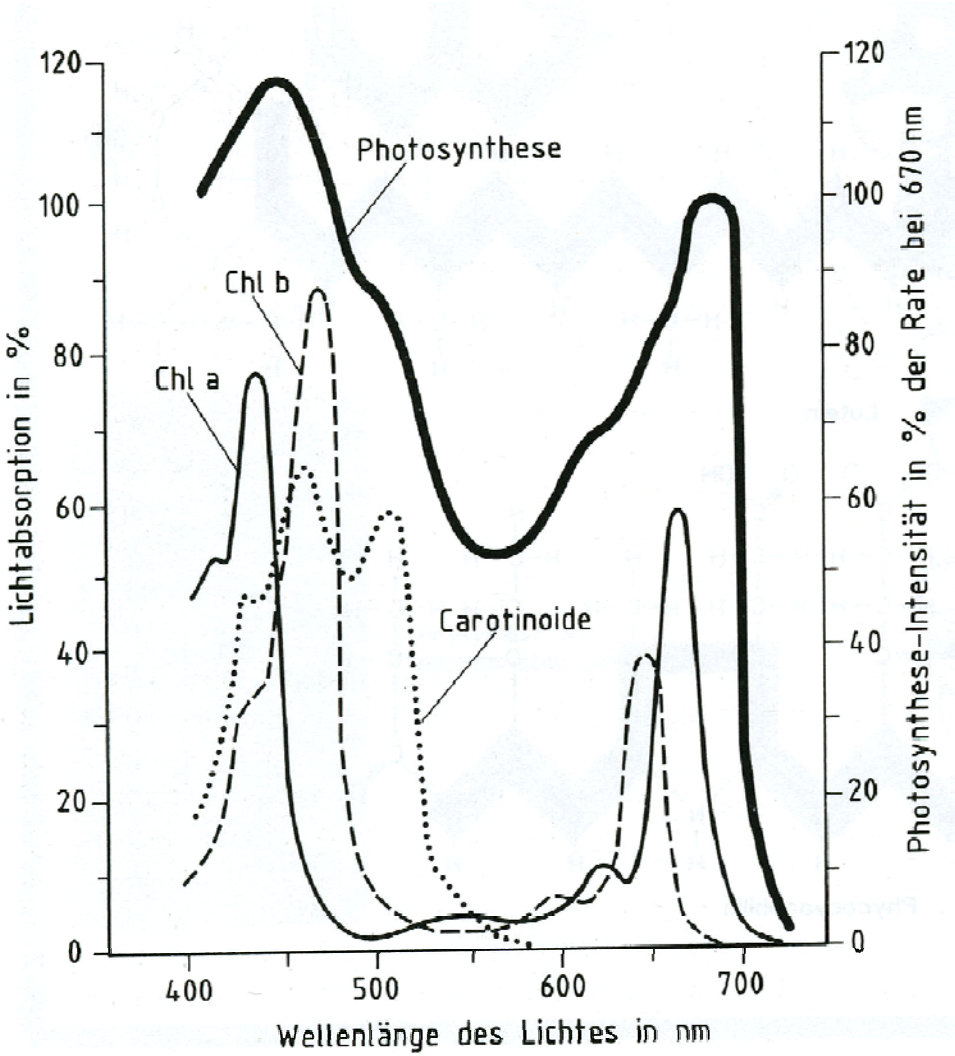






Some principles of light: Light absorption by pigments

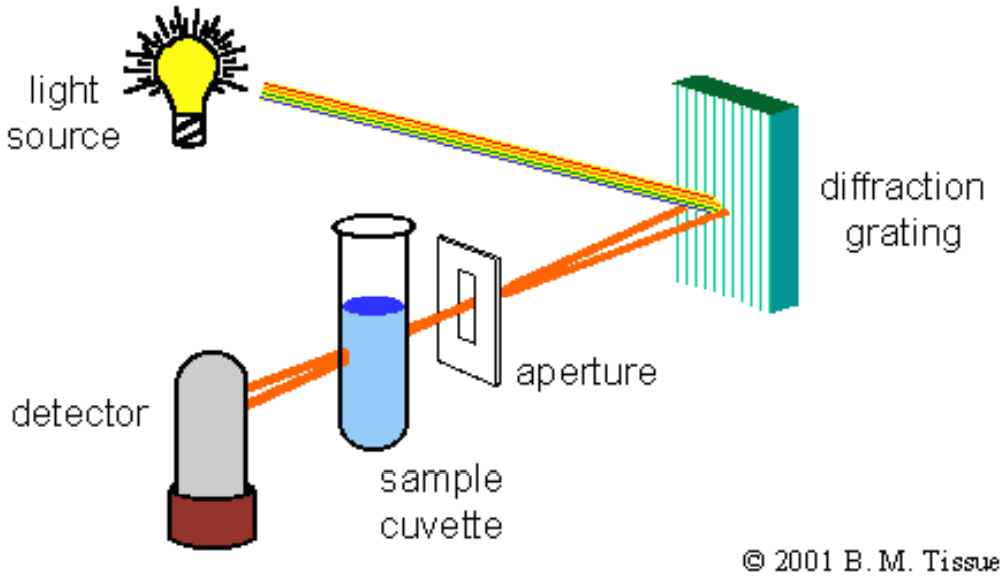
- Plant photosynthetic pigments absorb light between 380 – 700 nm
- Each pigment has it's characteristic spectral absorption features (spectral fingerprint)
- Absorption features are used in classical spectros-copy to quantify substances



•*Lüttge, Kluge, Bauer, Abb. 8-6*

Some principles of light: Classical spectroscopy

- Plant photosynthetic pigments absorb light between 380 – 700 nm
- Each pigment has it's characteristic spectral absorption features (spectra fingerprint)
- Absorption features are used in classical spectroscopy to quantify substances



Imaging Spectroscopy

1. Reflection, Transmission, Absorption
2. Imaging Spectroscopy of leaves, plants and canopies

4. Juli 2013

3. [synonym: Hyperspectral Imaging]

Imaging Spectroscopy of plants and vegetation

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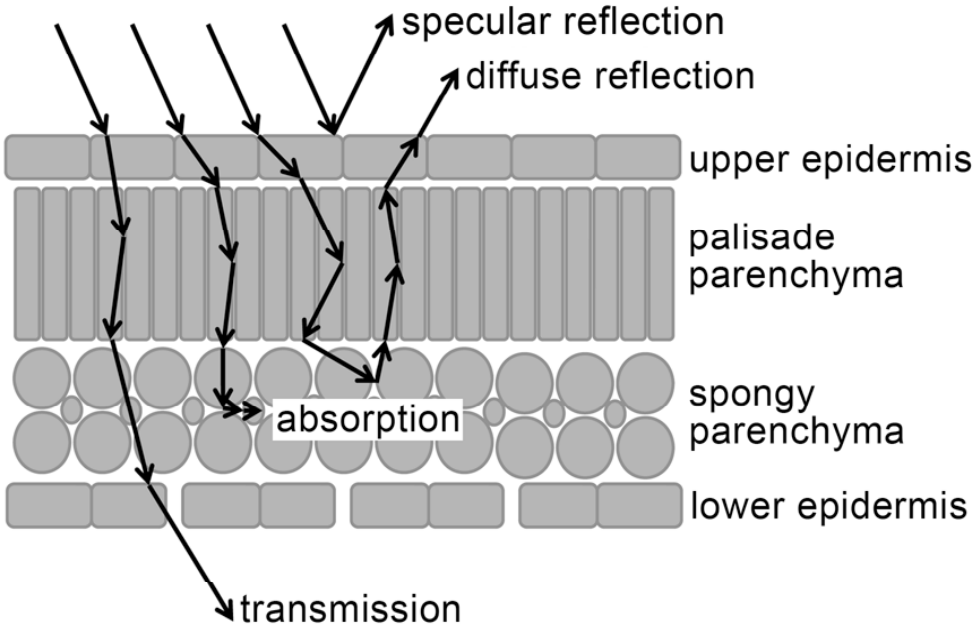
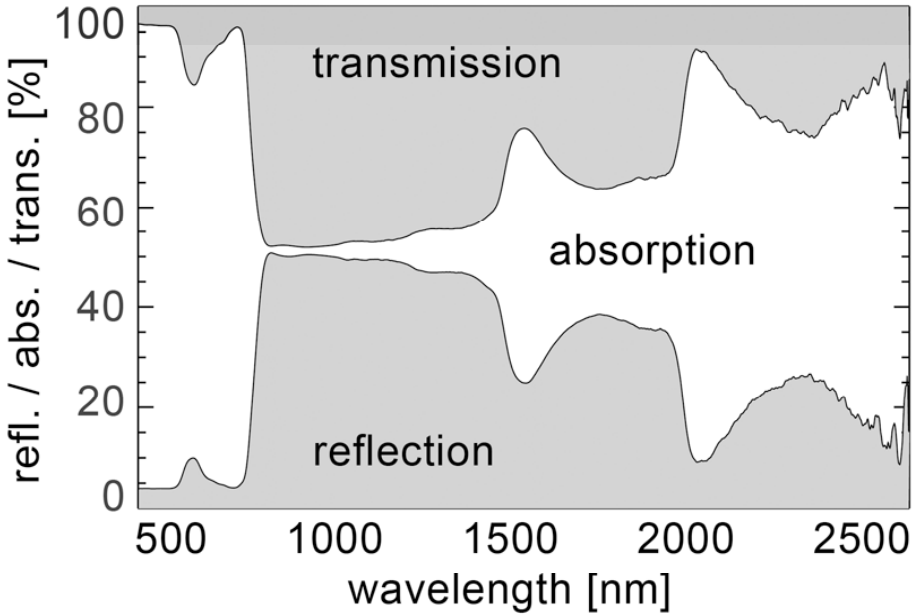
Absorption, transmission and reflection of photons is primarily determined by plant pigments and constituents





Imaging Spectroscopy of plants and vegetation

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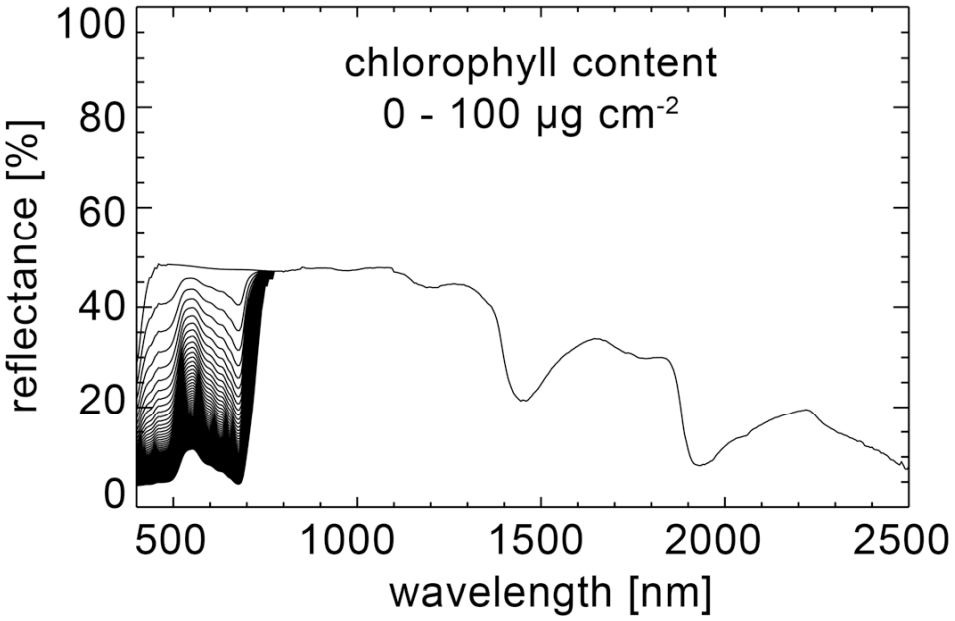
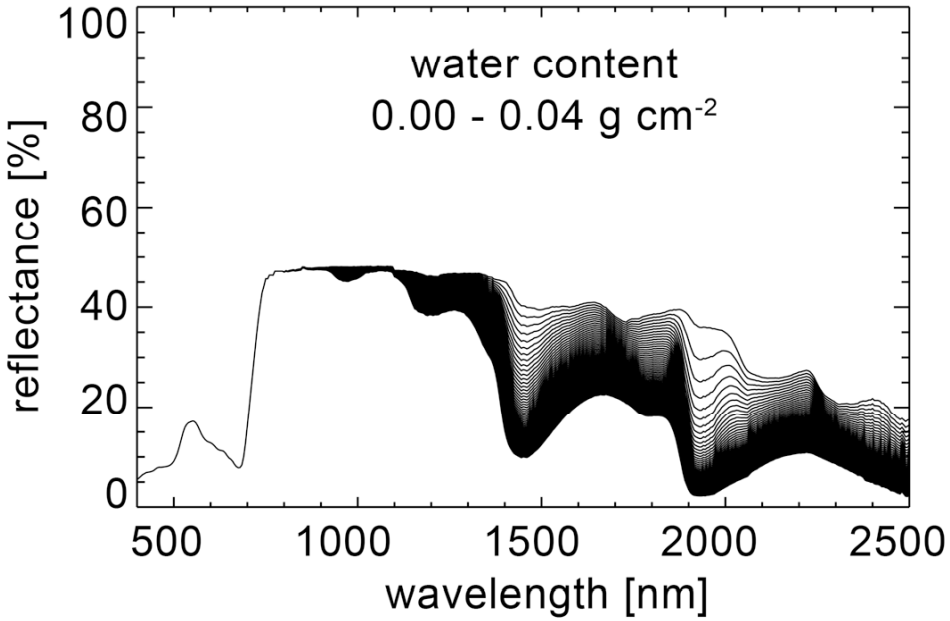
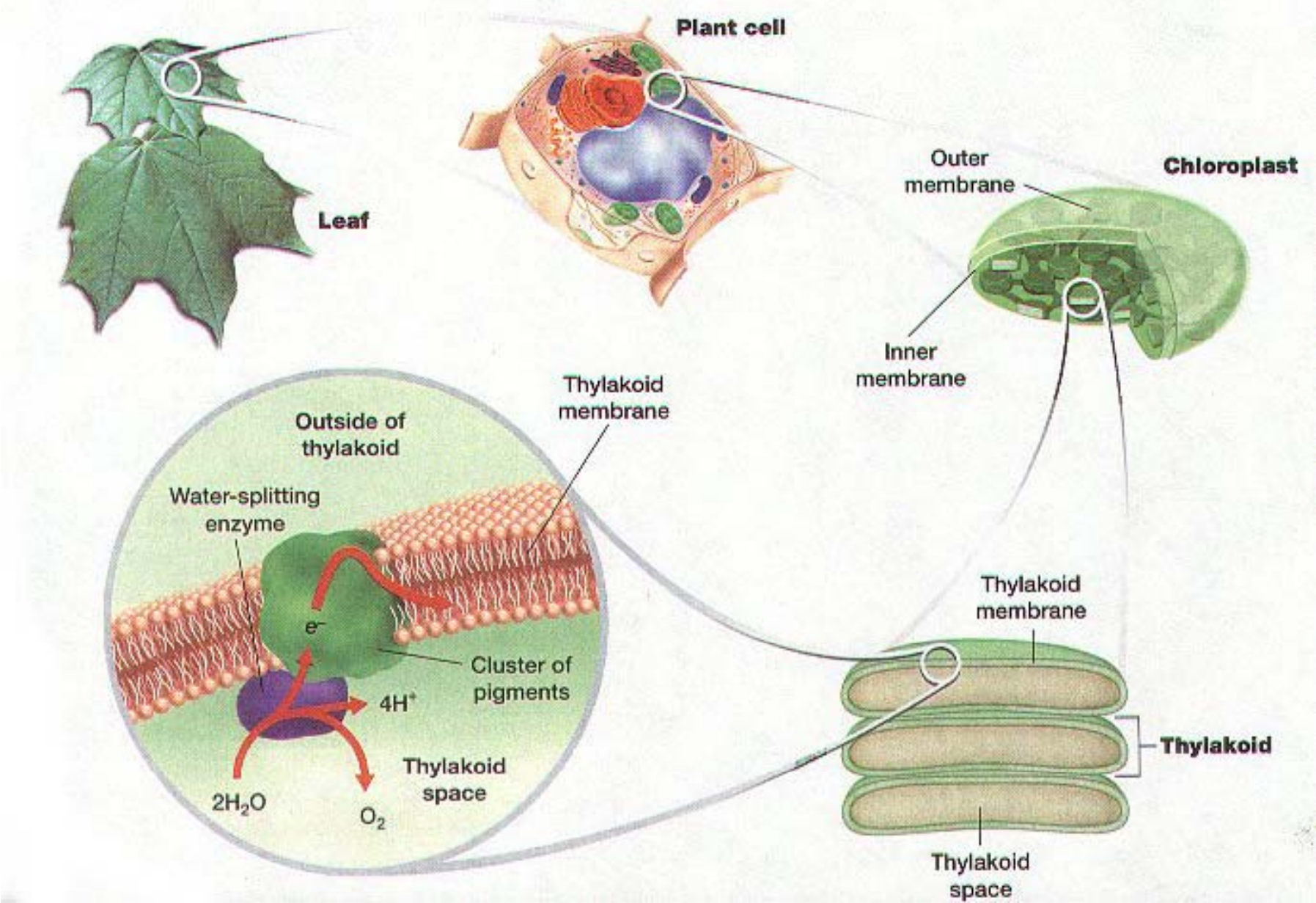
Chlorophyll, carotenoid, water and cellulose are the main parameters that determine reflectance of plant surfaces



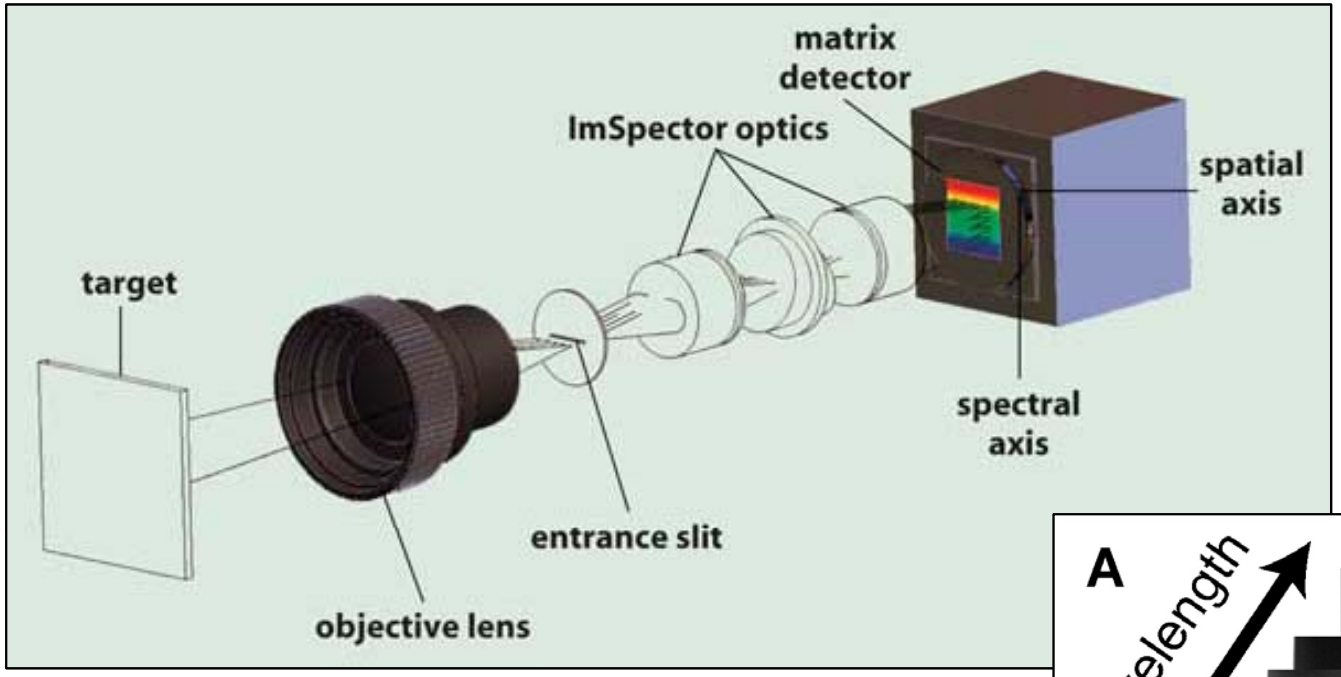


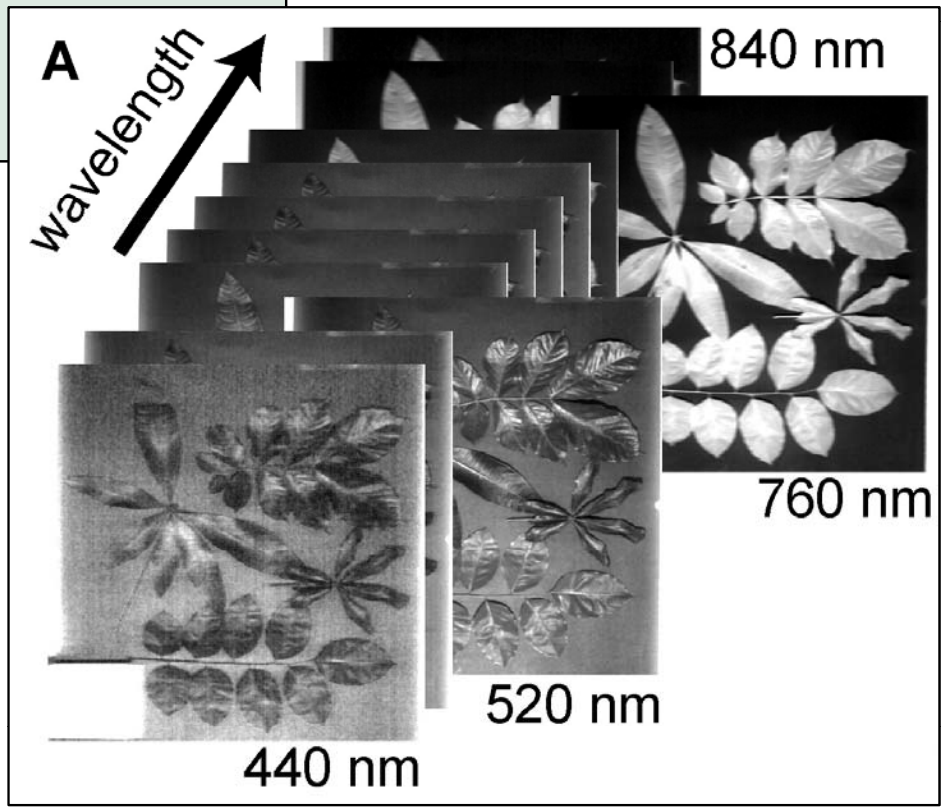
Rascher et al. (2010) *Precision Crop Protection, Springer, ISBN: 978-90-481-9276-2*, pp 87-100

Looking into the detailed structure of the photosynthetic machinerie



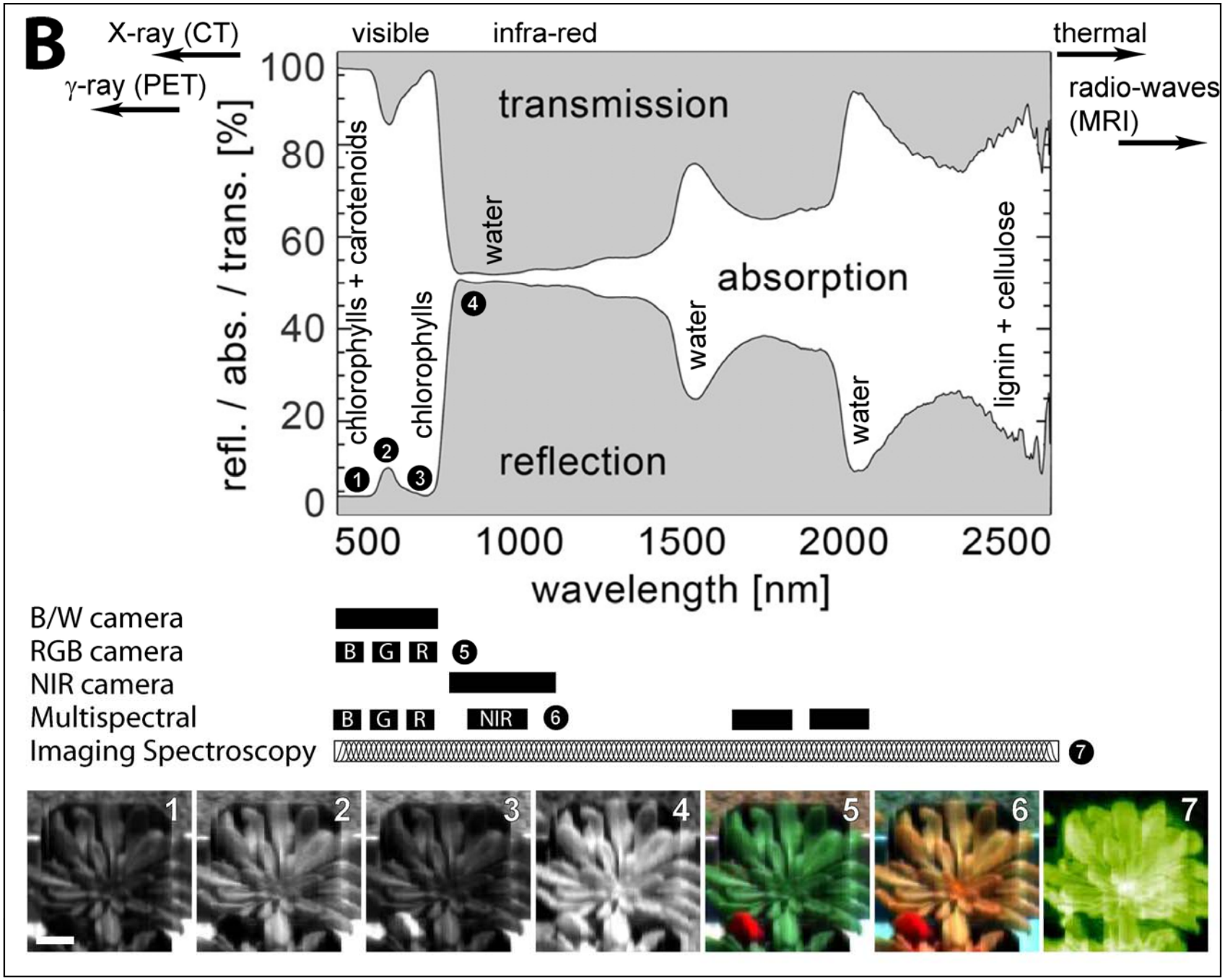
Imaging Spectroscopy of plants and vegetation





Rascher et al. (2007) *Photogrammetric Engineering and Remote Sensing*, *73*, 45-56

Imaging Spectroscopy of plants and vegetation

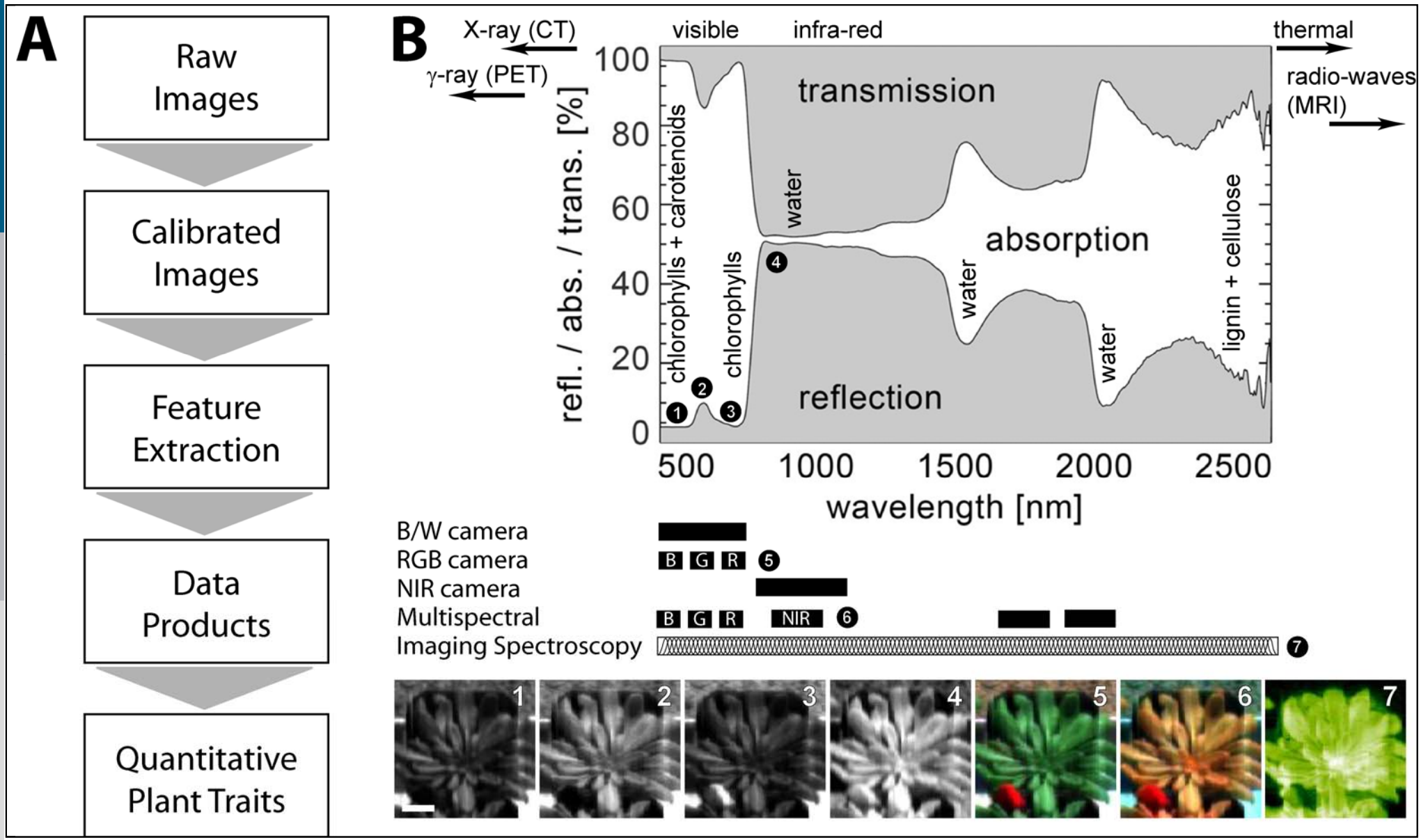


Fiorani et al. (2012) *Current Opinion in Biotechnology, 23*, 227-235.

How to extract quantitative data

1. vegetation indices
2. advanced methods (support vector machines and spectral unmixing)

Imaging Spectroscopy of plants and vegetation



Fiorani et al. (2012) *Current Opinion in Biotechnology, 23*, 227-235.

Vegetation indices: a simple way to measure plant ecosystems

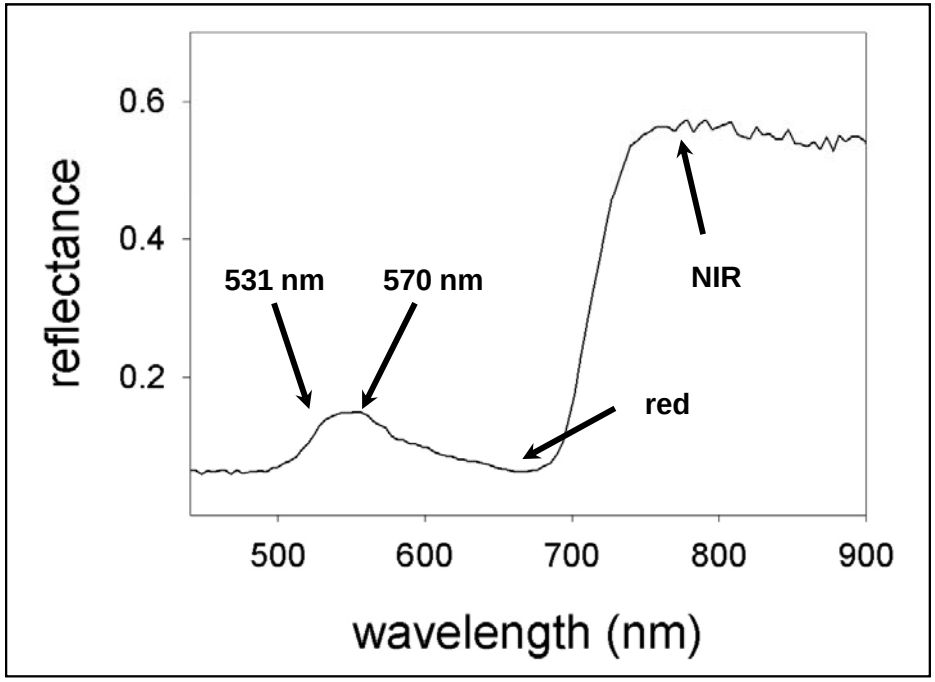
- SR (Simple ratio): correlation with chlorophyll content or leaf area index
- NDVI: correlation with chlorophyll content or leaf area index
- PRI (Photochemical Reflectance Index): correlation with photosynthetic efficiency

$$SR = \frac{R_{NIR}}{R_{red}}$$

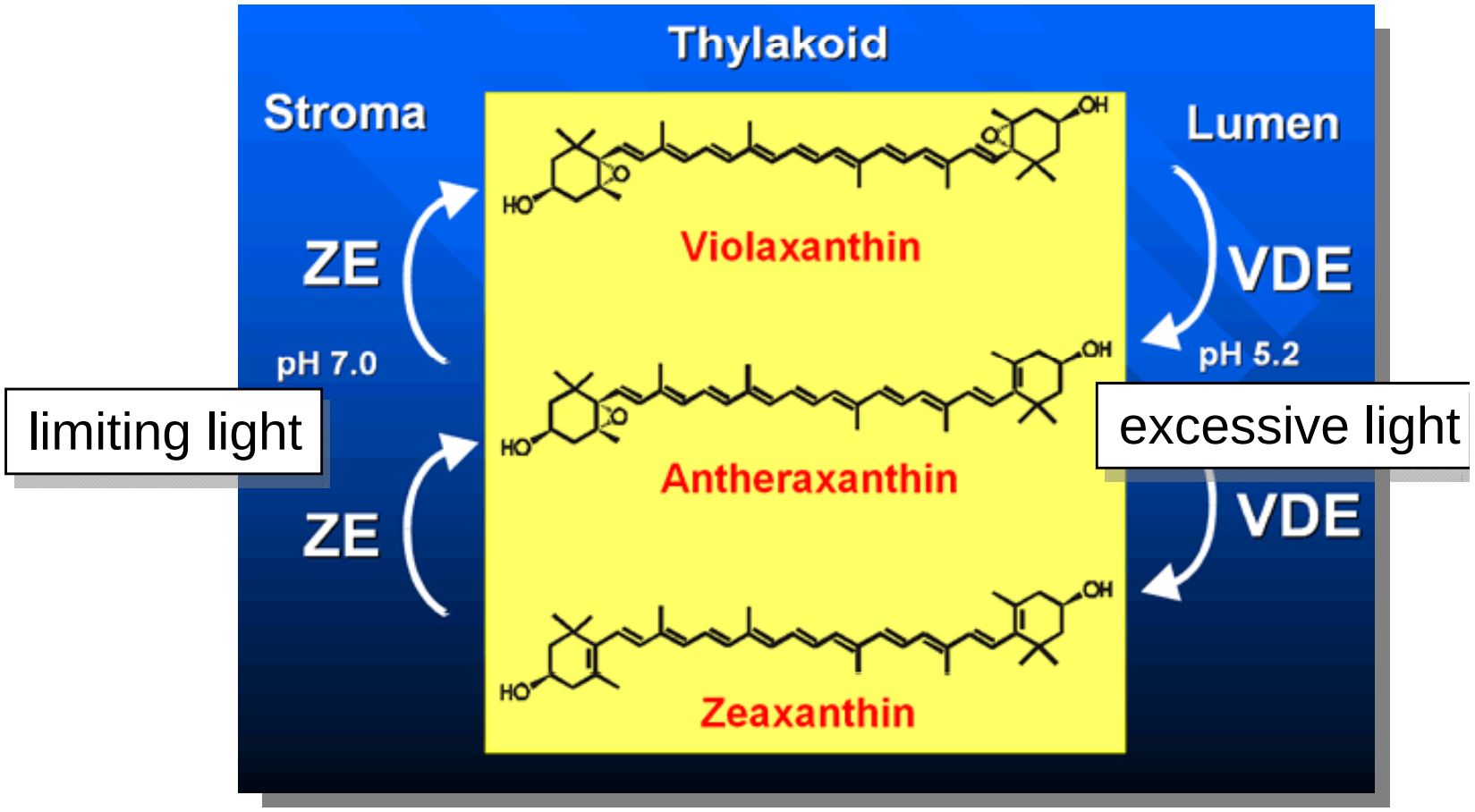
$$NDVI = \frac{R_{NIR} - R_{red}}{R_{NIR} + R_{red}}$$

$$NDVI = \frac{R_{780} - R_{670}}{R_{780} + R_{670}}$$

$$PRI = \frac{R_{570} - R_{531}}{R_{570} + R_{531}}$$

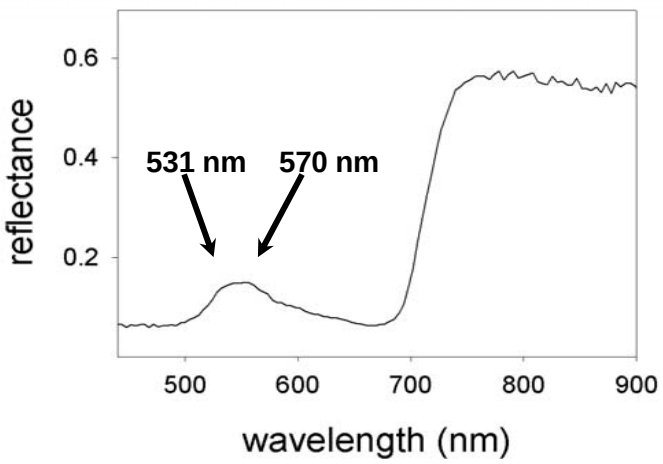


The Xanthophyll Zyklus a dynamic mechanism to protect from excess light

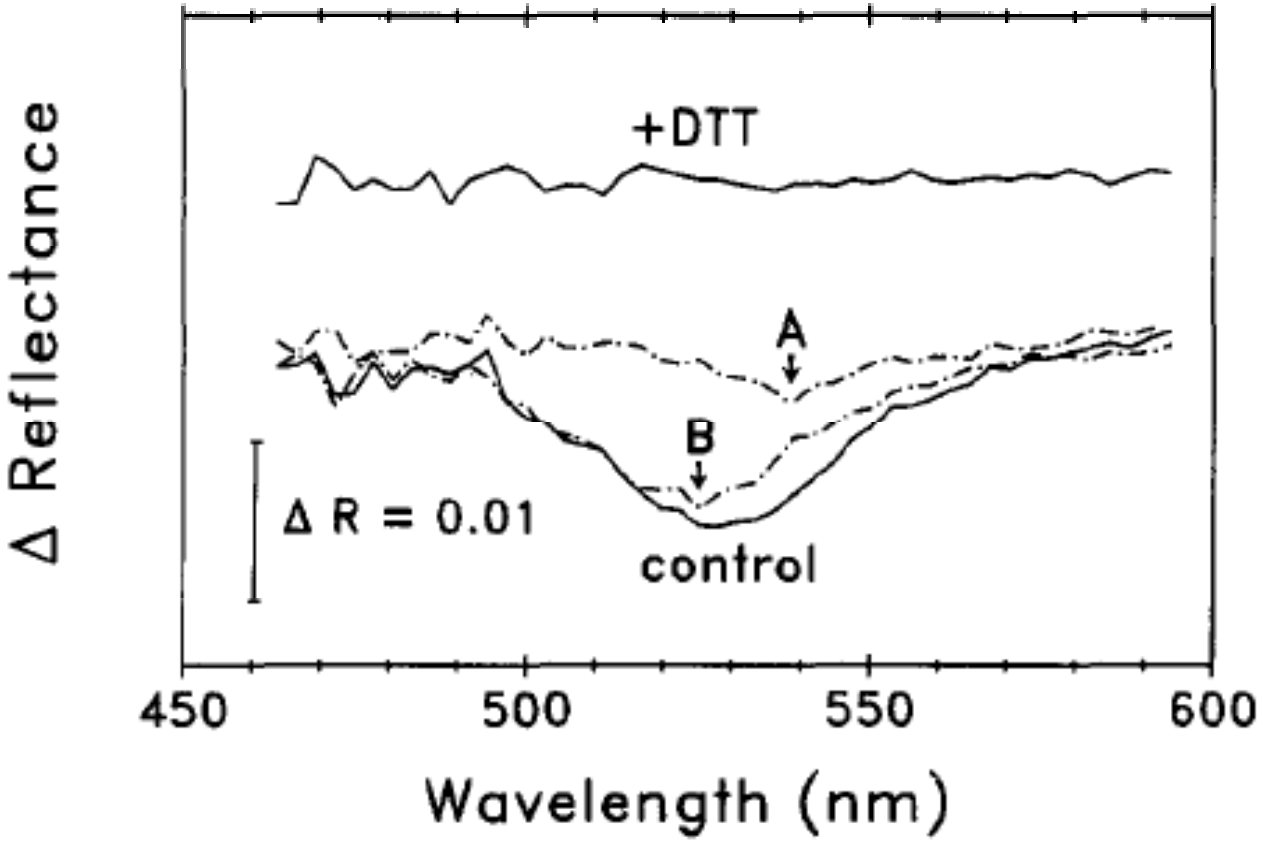


Dynamic changes in pigment composition that can be measured in the green spectral region

The Photochemical Reflectance Index (PRI) correlate with photosynthetic efficiency (on the leaf level)

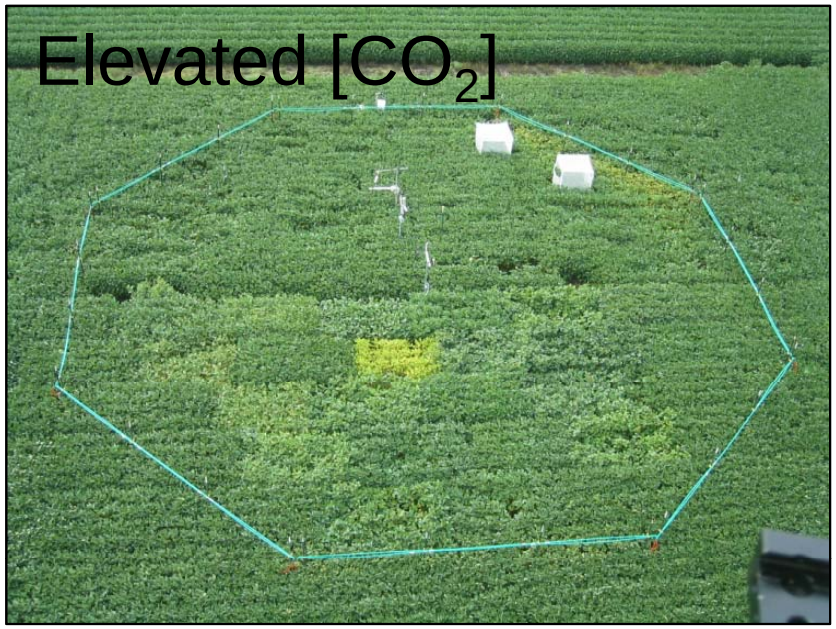


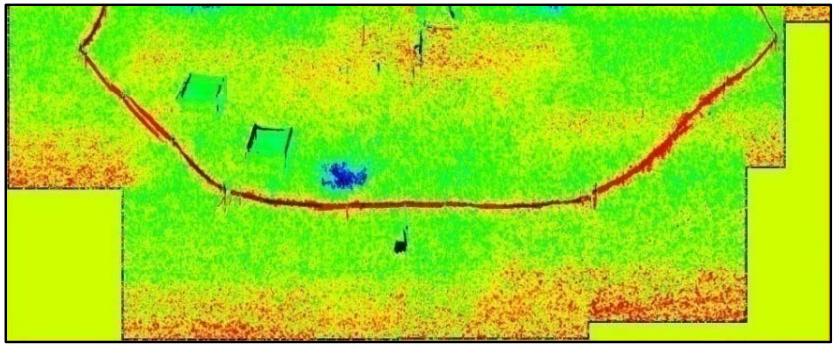
$$PRI = \frac{R_{570} - R_{531}}{R_{570} + R_{531}}$$



Gamon et al. (1990) *Oecologia*, 85, 1-7

….but not necessarily on the canopy / field level

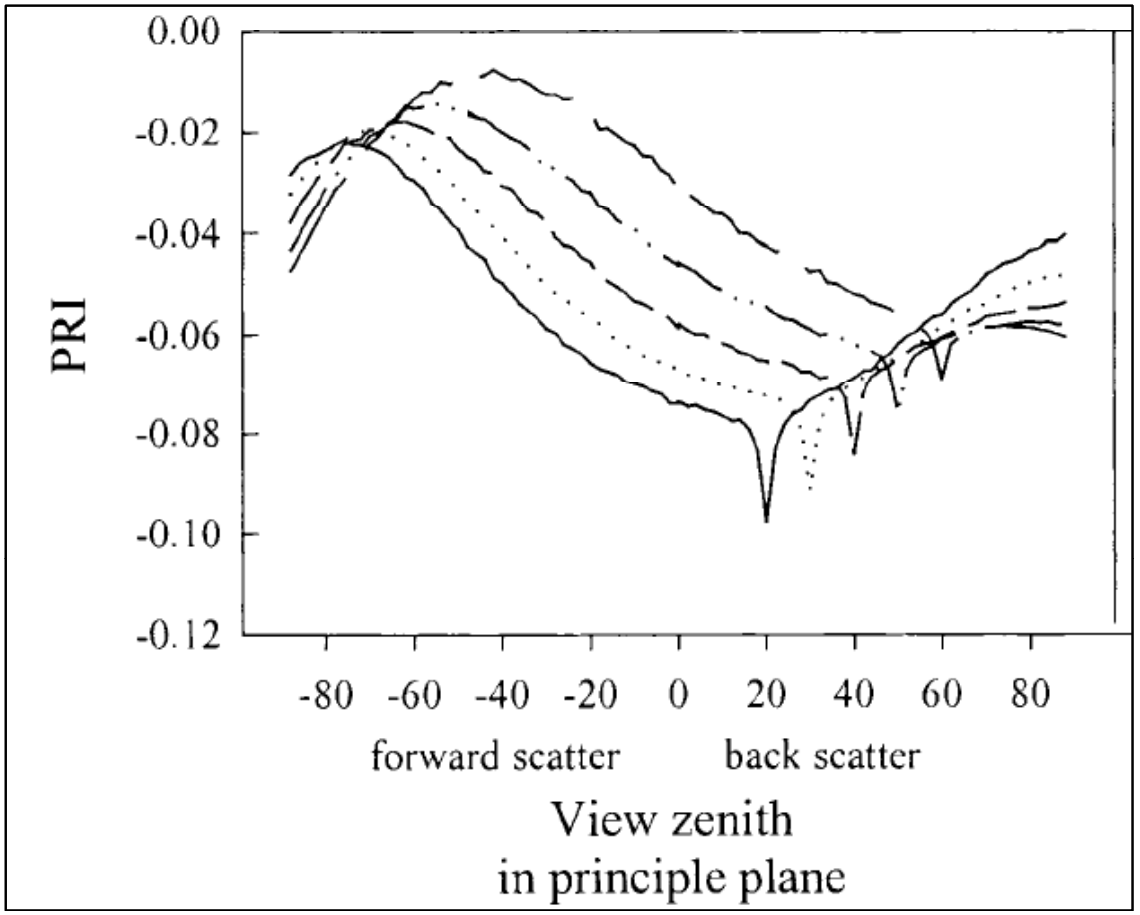




PRI

-0.026 0.082

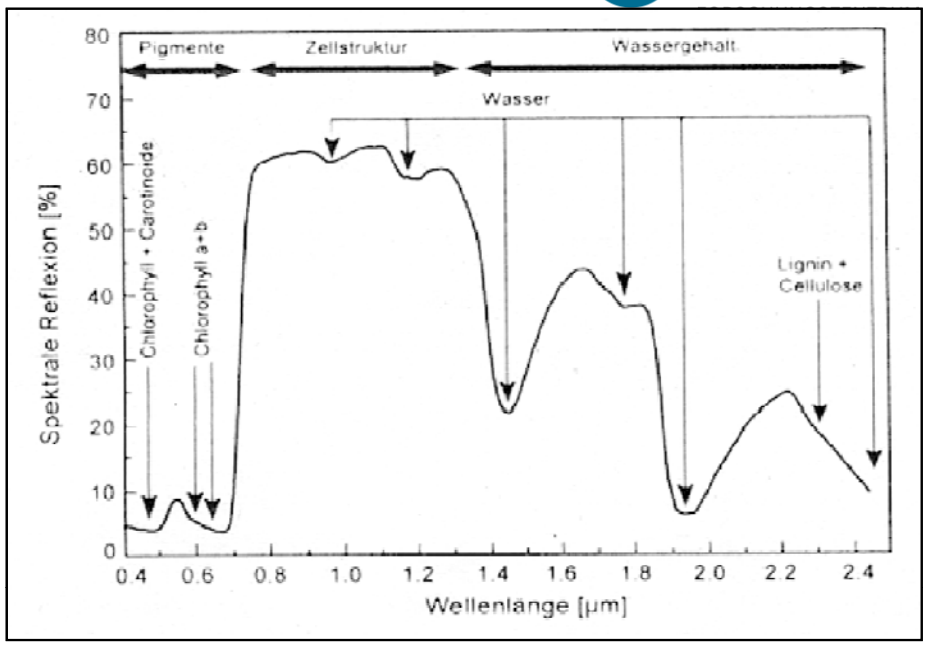
Rascher and Pieruschka (2008) *Precision Agriculture, 9, 355-366*



Barton & North (2001) *Remote Sensing of Environment* 78, 264-273

Please think and don't use vegetation indices naively

There is a large number of vegetation indiced



EVI: Enhanced Vegetation Index (similar to NDVI including blue reflectance)

Red Edge Indices (quantify the slope of red absorption)

NDNI: Normalized Difference Nitrogen Index (Nitrogen absorption at 1510 nm)

NDLI: Normalized Difference Lignin Index (Lignin absorption at 1754 nm)

CAI: Cellulose Absorption Index (cellulose absorption at 2000 – 2200 nm)

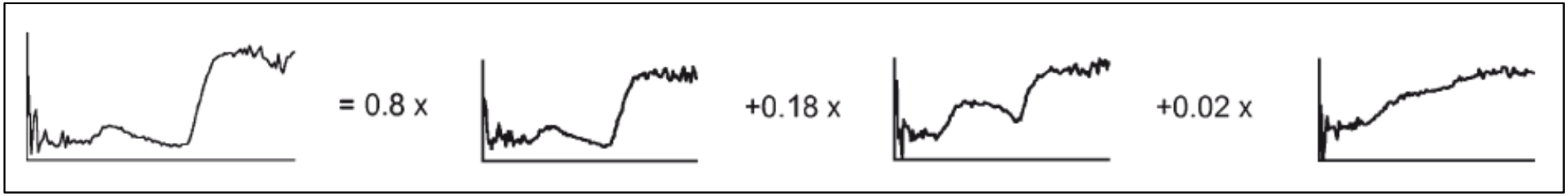
PSRI: Plant Senscence Index (correlates with seneszens and fruit ripening)

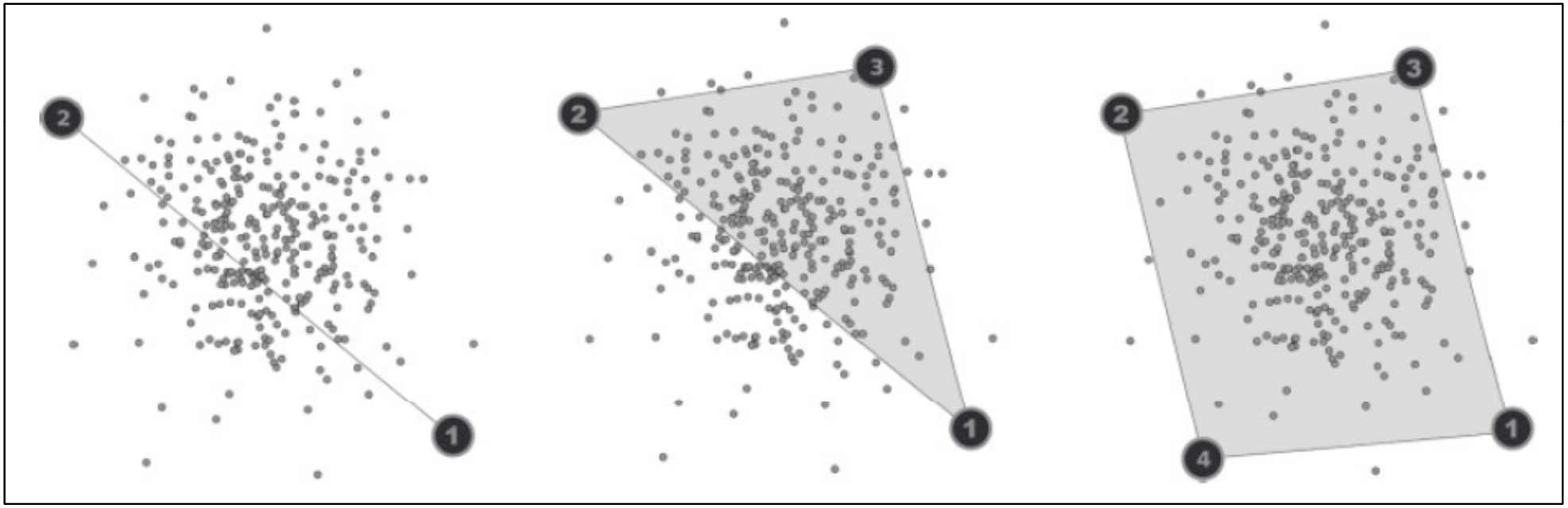
CRI1 / CRI2: Carotenoid Reflectance Index (yellow spectral region, correlates with carotinoid / chlorophyll ratio)

ARI1 / ARI2: Anthocyanin Reflectance Index (yellow and red spectral region, correlates with anthocyanin / chlorophyll ratio)

NDWI: Normalized Difference Water Index (canopy water content)

Beyond Indices: Taking the full spectrum into account

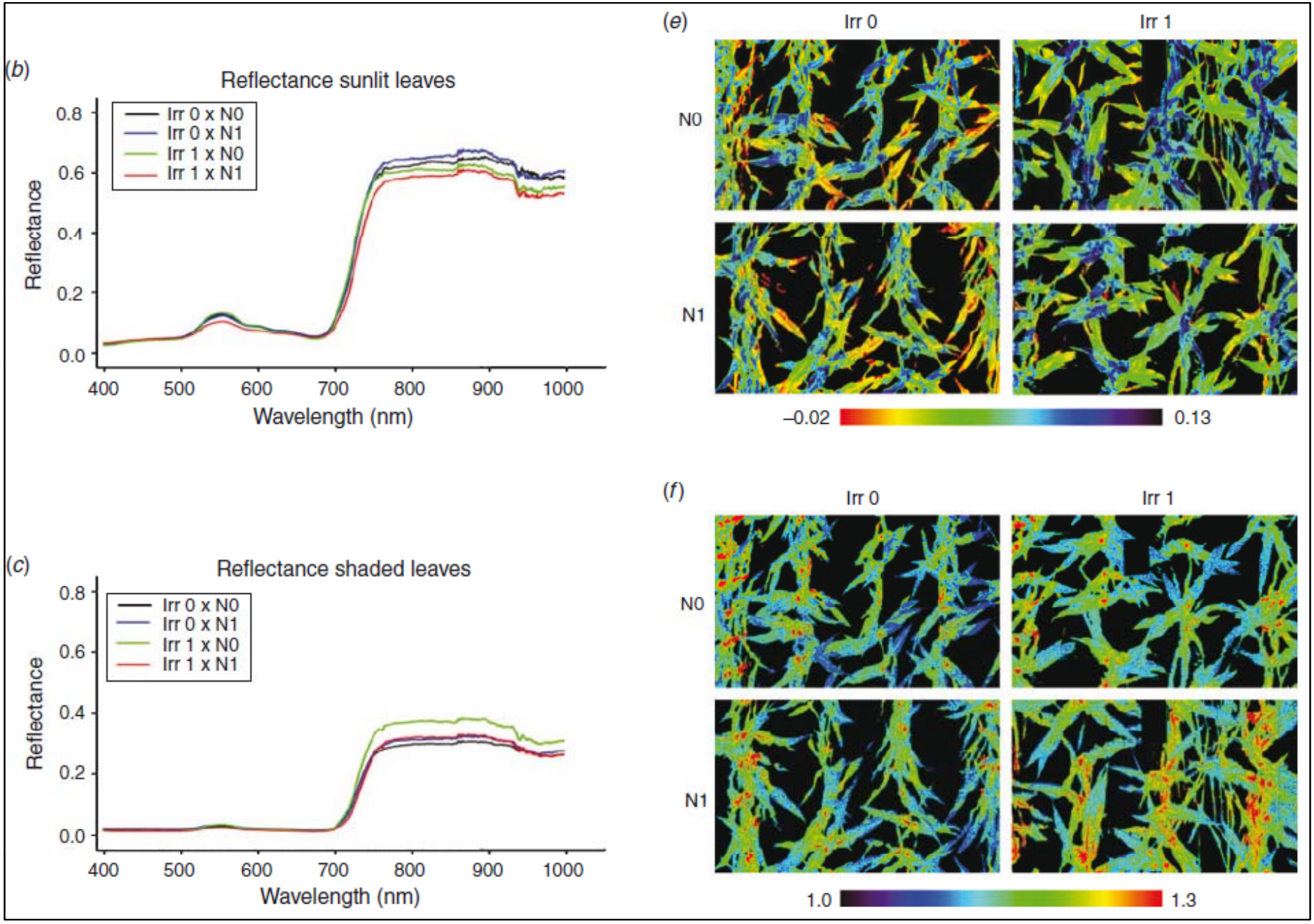




‘Endmember unmixing’ with the help of ‘support vector machines’ allows to quantify each spectrum in respect to predefined cases.

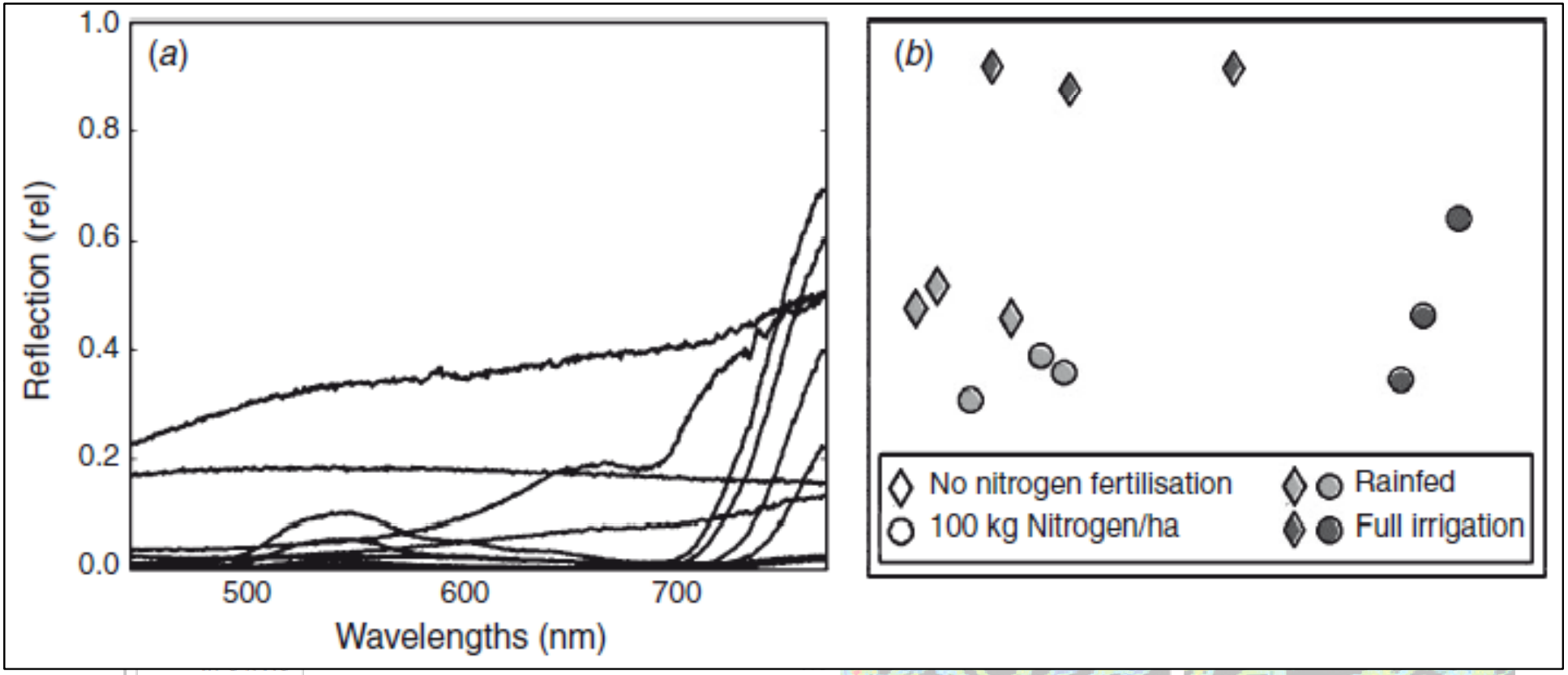
Römer et al. (2012) *Functional Plant Biology, 39*, 878–890

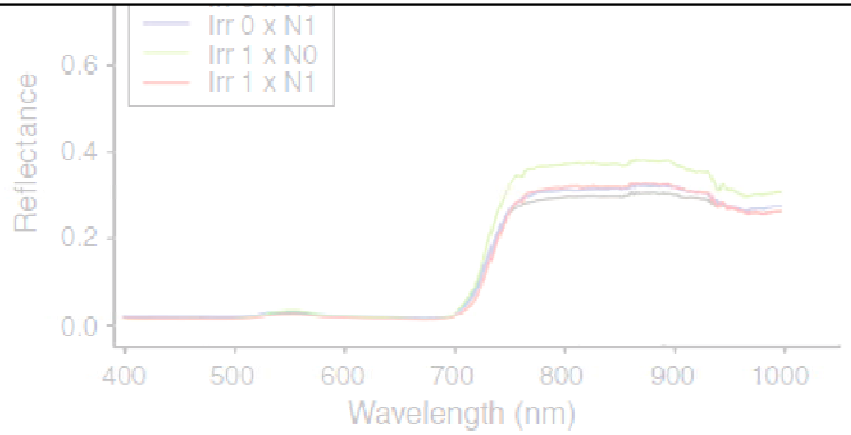
Beyond Indices: Taking the full spectrum into account

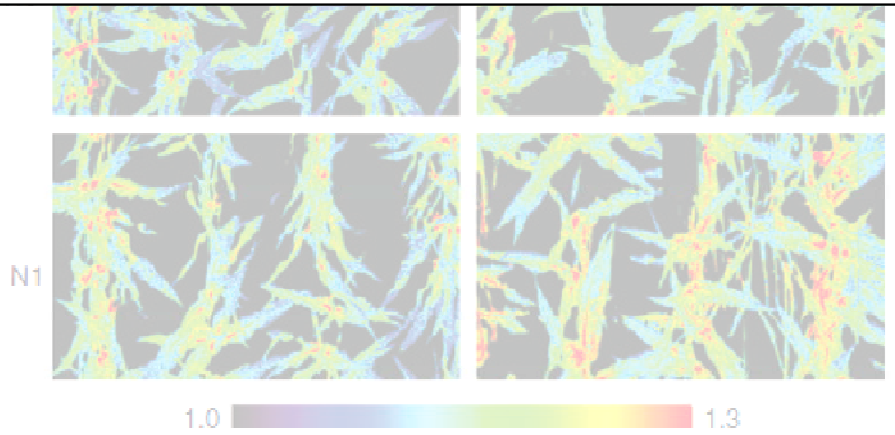


Römer et al. (2012) *Functional Plant Biology, 39*, 878–890

Beyond Indices: Taking the full spectrum into account





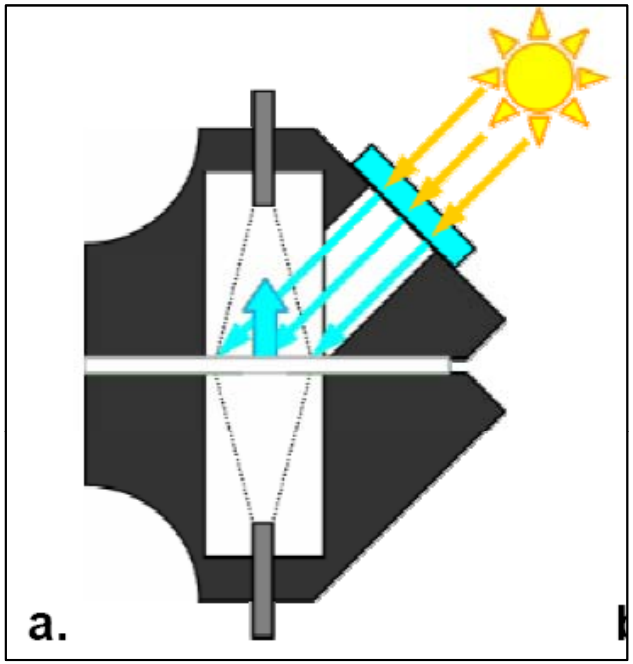


Römer et al. (2012) *Functional Plant Biology, 39*, 878–890

Some examples from the real life

1. Leaf level – clip on
2. Leaf level: high presicion measurements
3. Canopy level

Leaf clip-on: simultaneous recording of reflectance, transmittance and absorbance



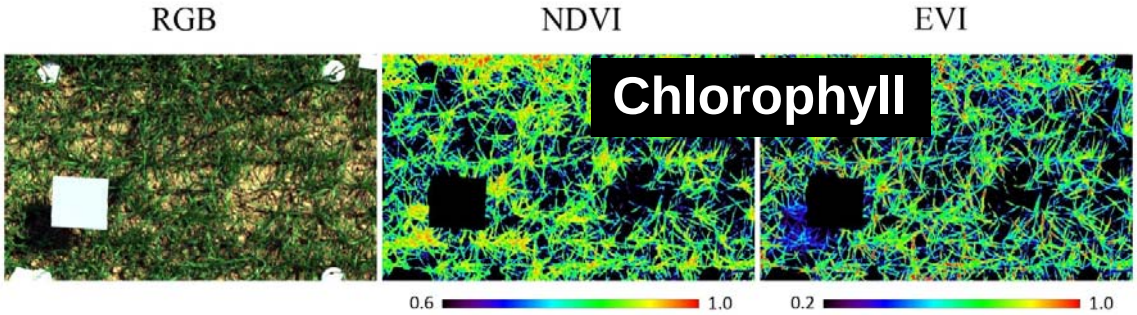
Imaging Spectroscopy on the canopy level

Corn, wheat, barley and sugar beet

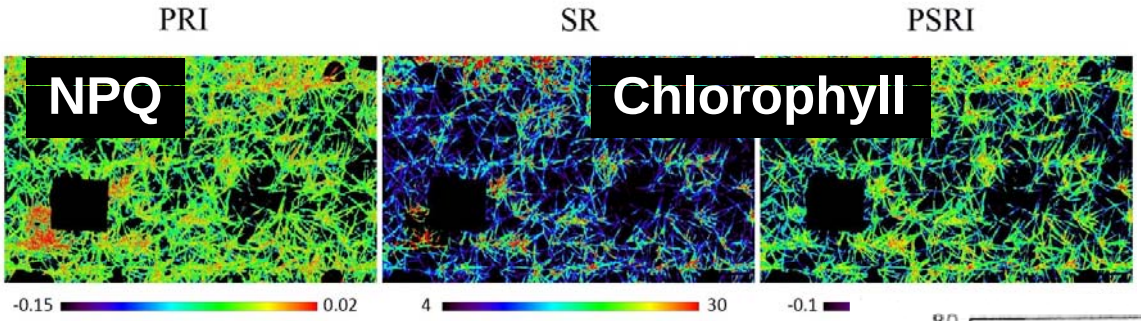
4-7 meters distance

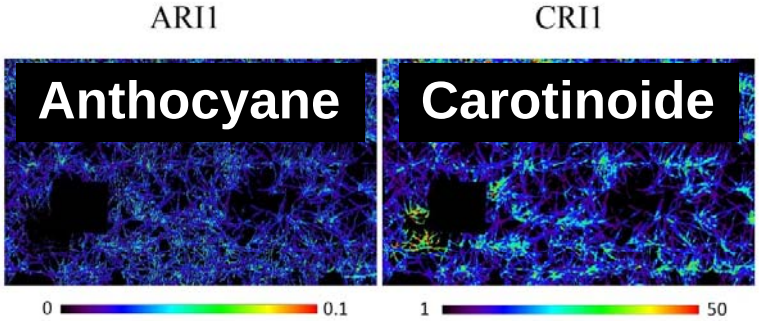
1.5 x 2.7 meter plots

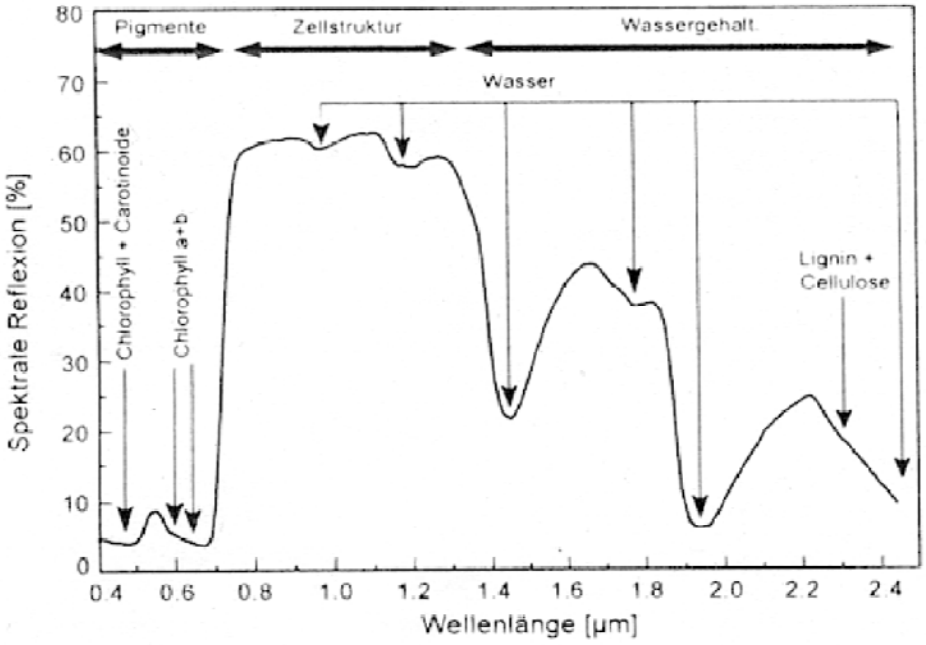
Imaging Spectroscopy on the canopy level



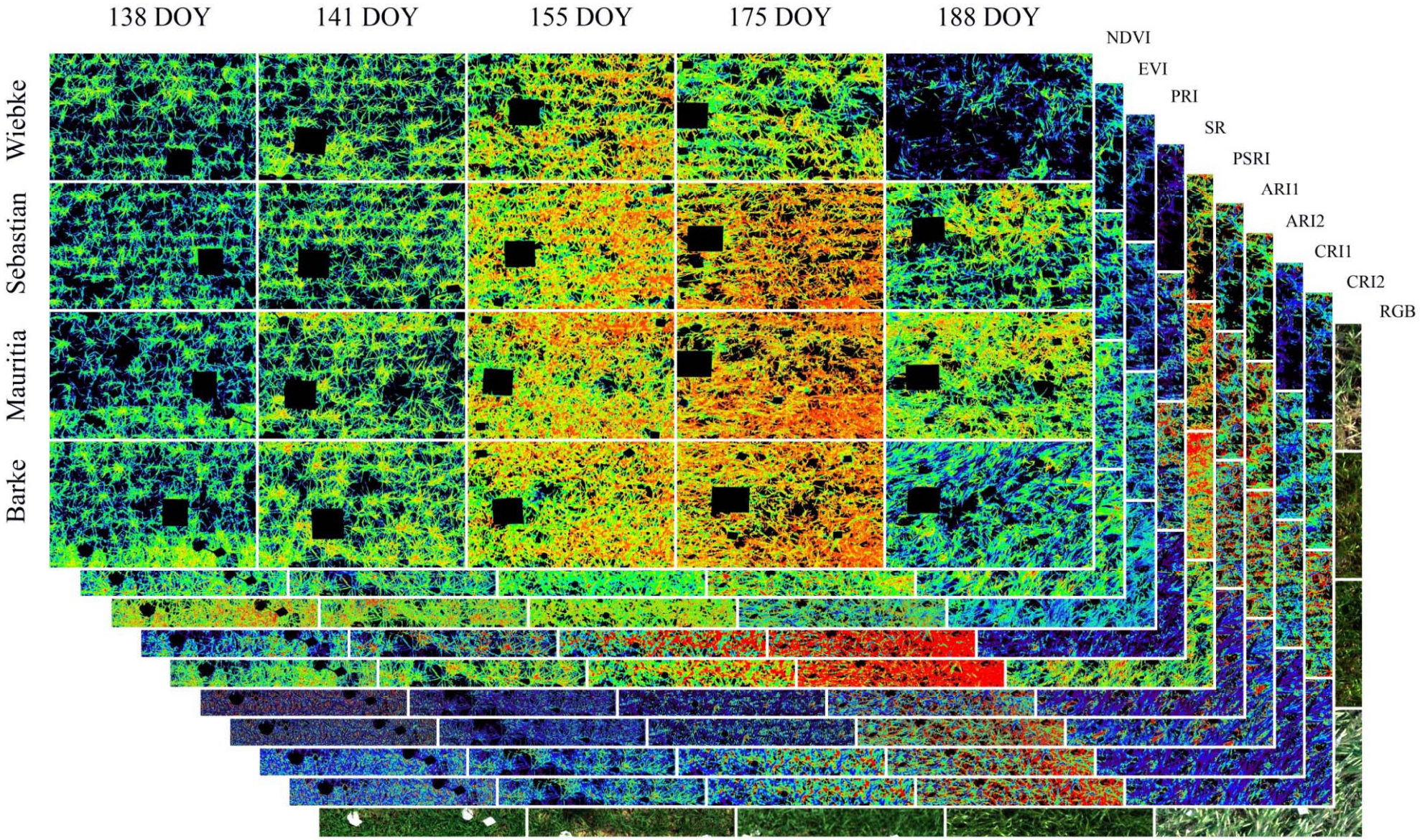
Wellenlängen-spezifische Trennung von Struktur- und Funktionsparametern





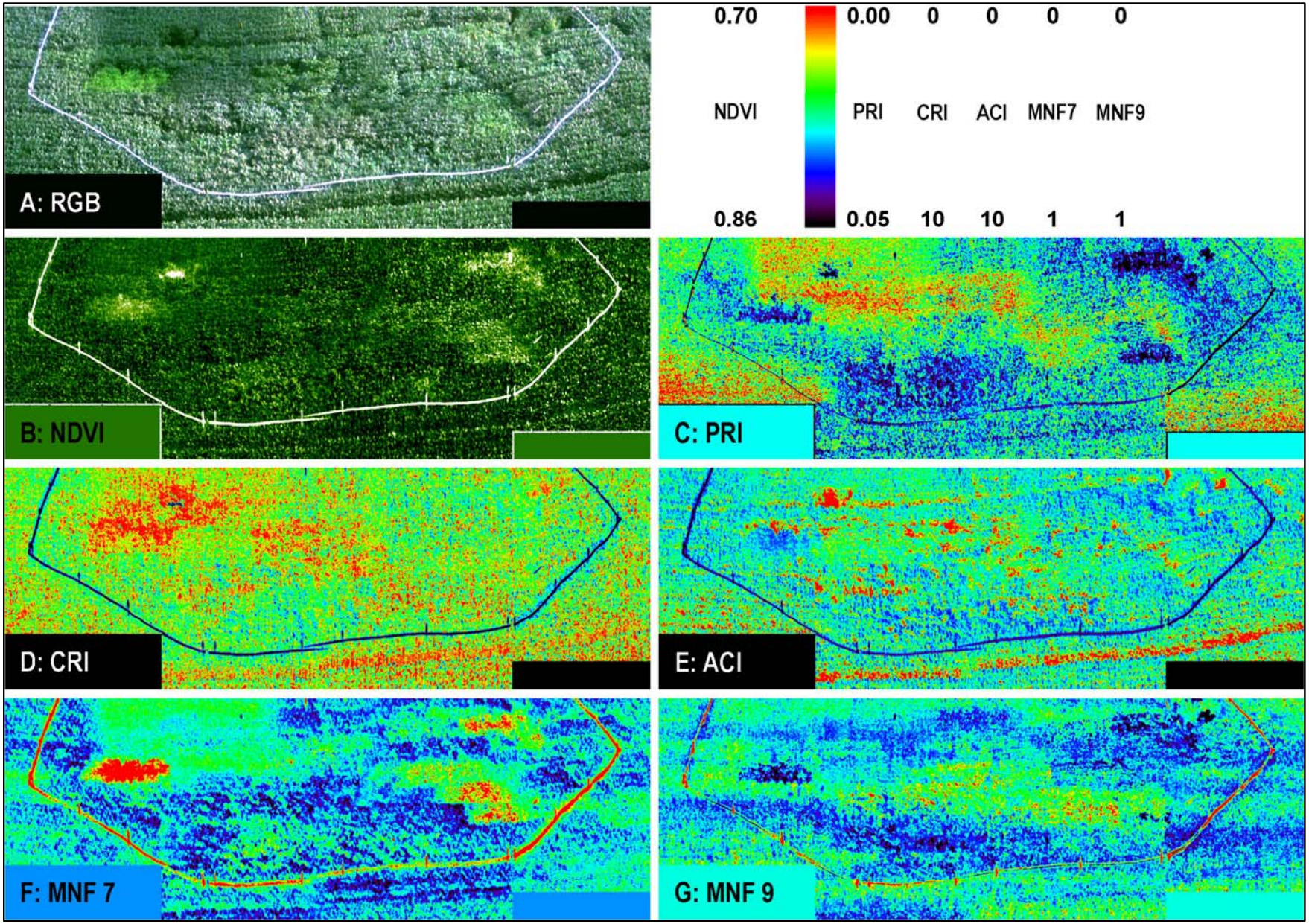


Imaging Spectroscopy on the canopy level: seasonal and cultivar differences



Jansen et al (in press) *Advances in Genomics of Plant Genetic Resources*, Springer

Imaging Spectroscopy on the canopy level: Influence of cultivars and elevated CO_2

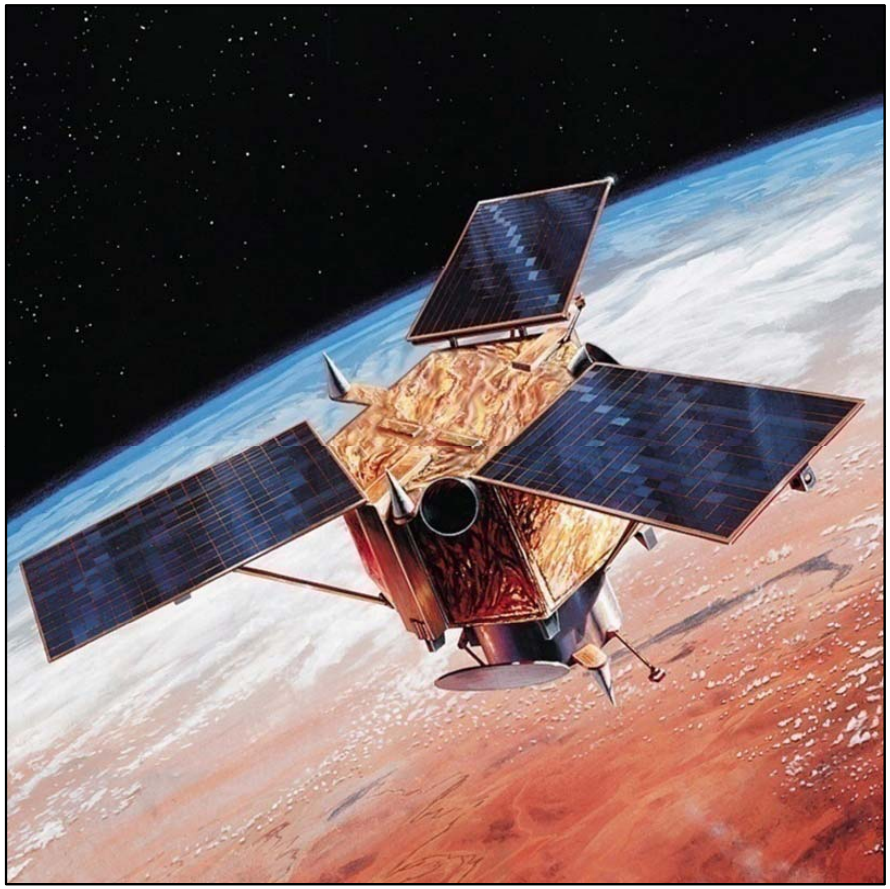


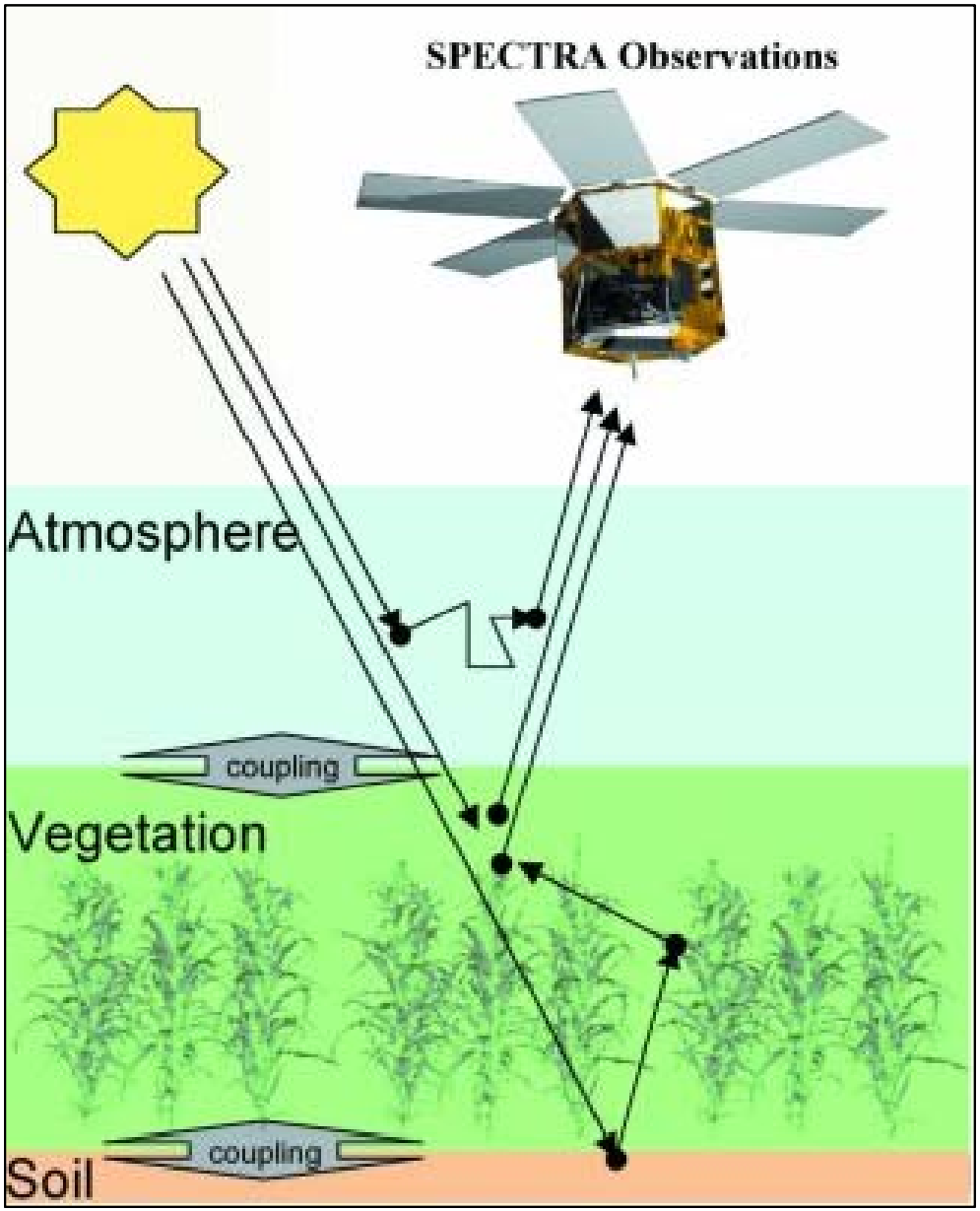
Rascher et al. (2011) *Functional Plant Biology, 38,* 968-983

Linking spectroscopy with fluorescence

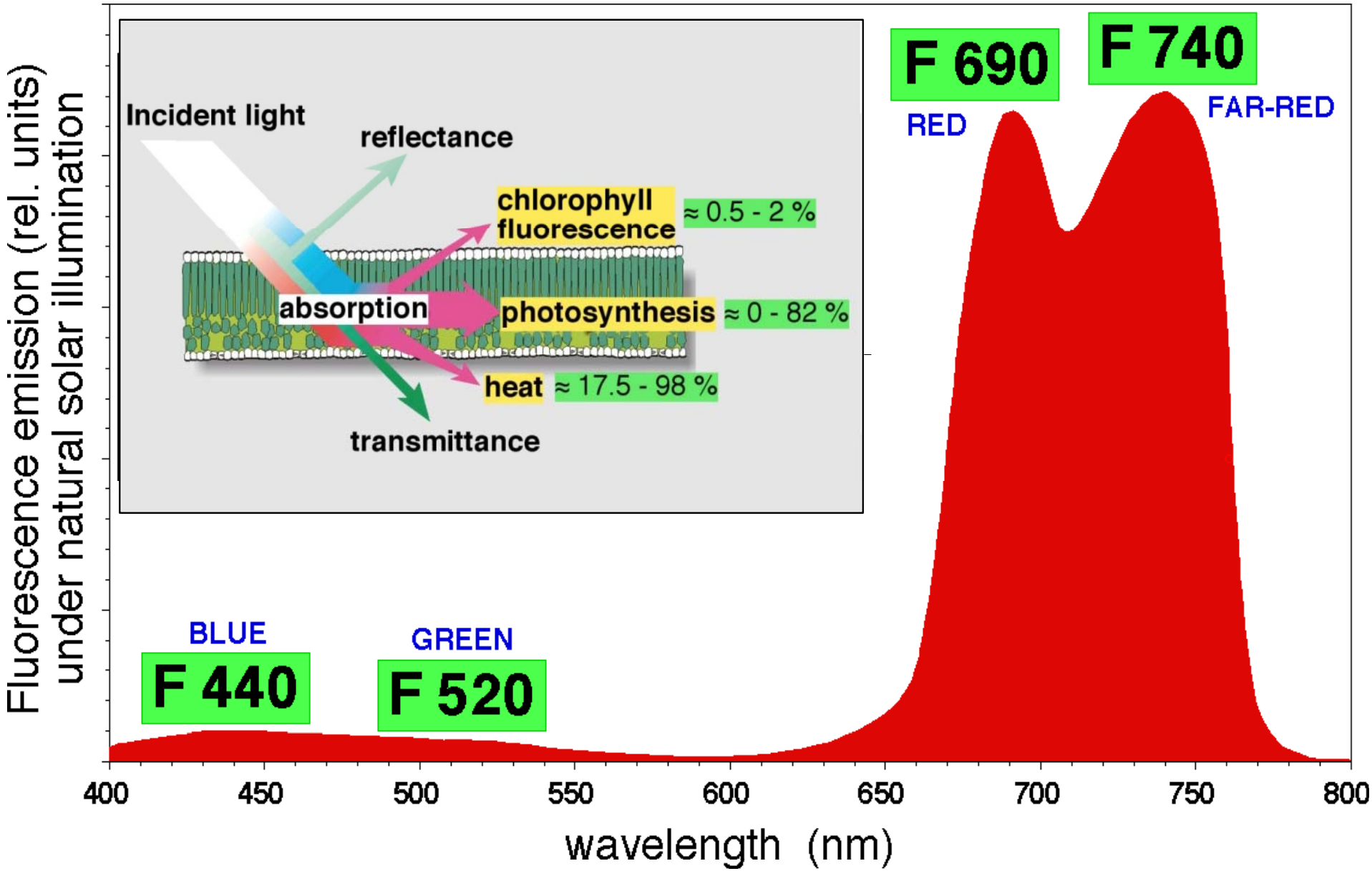
1. Concept of sun-induced fluorescence

Mapping photosynthesis from space: hyperspectral reflectance - fluorescence





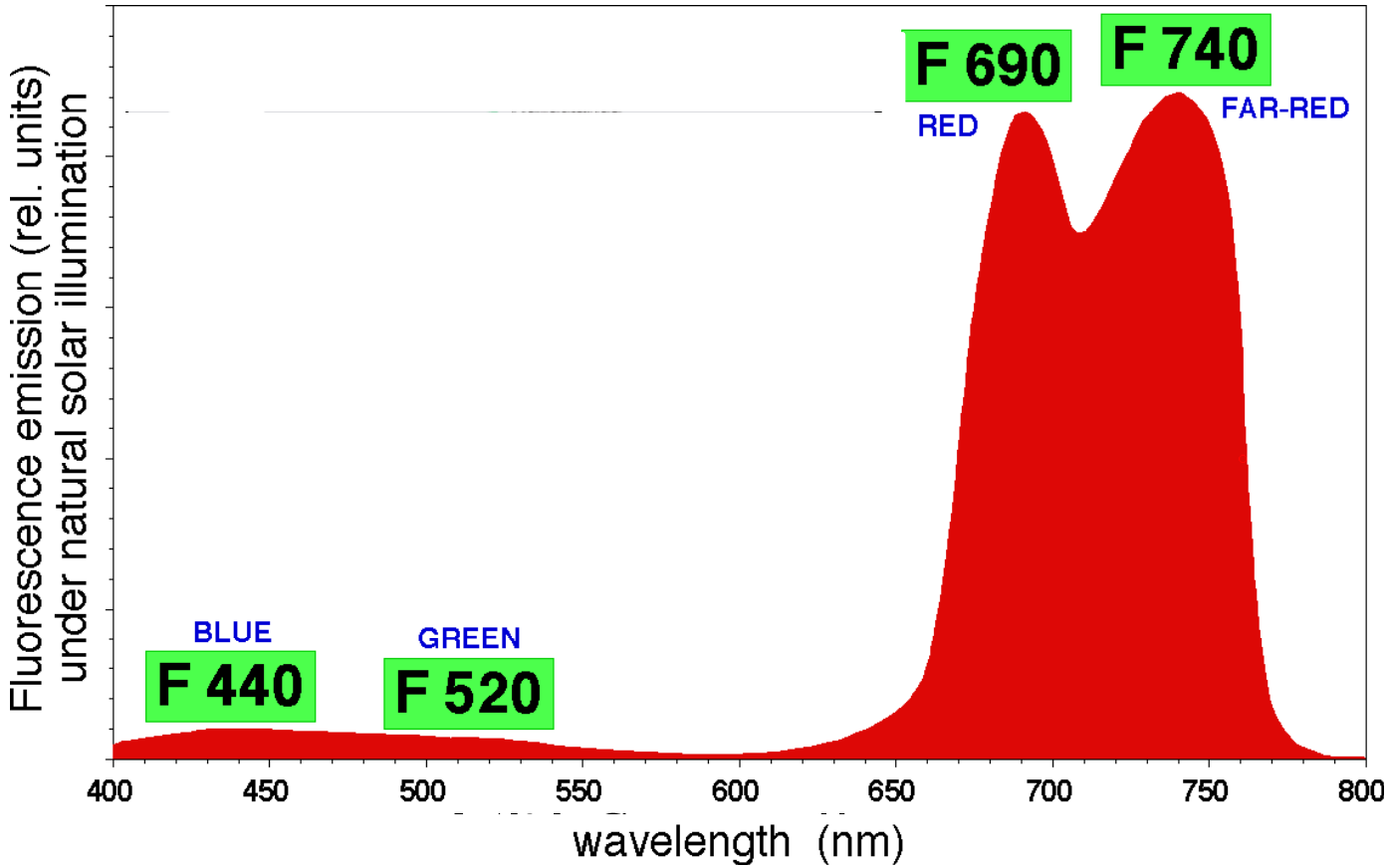
Chlorophyll Fluorescence (1)



courtesy of C. Buschmann

Chlorophyll Fluorescence (2)

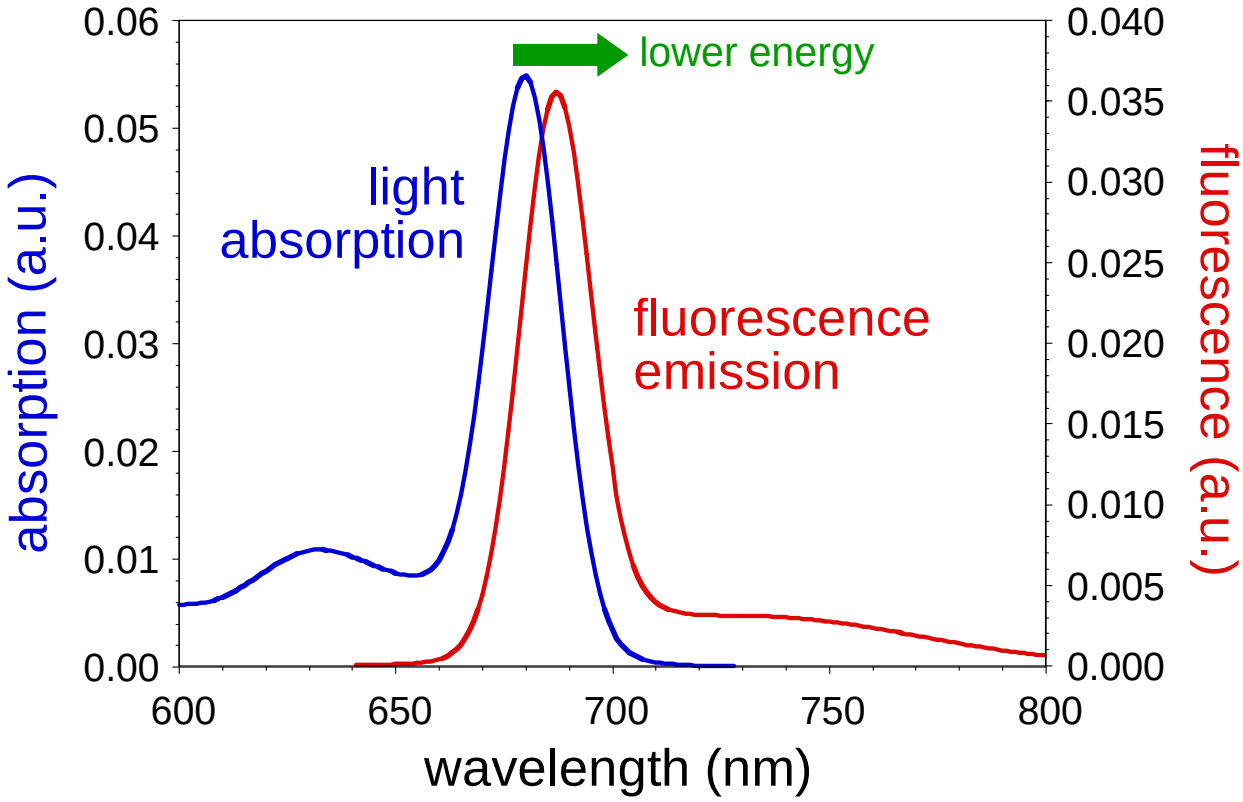
- Chlorophyll fluorescence is emitted from the core of the photosynthetic machinery and is directly correlated to efficiency of photosynthesis



courtesy of L. Nedbal

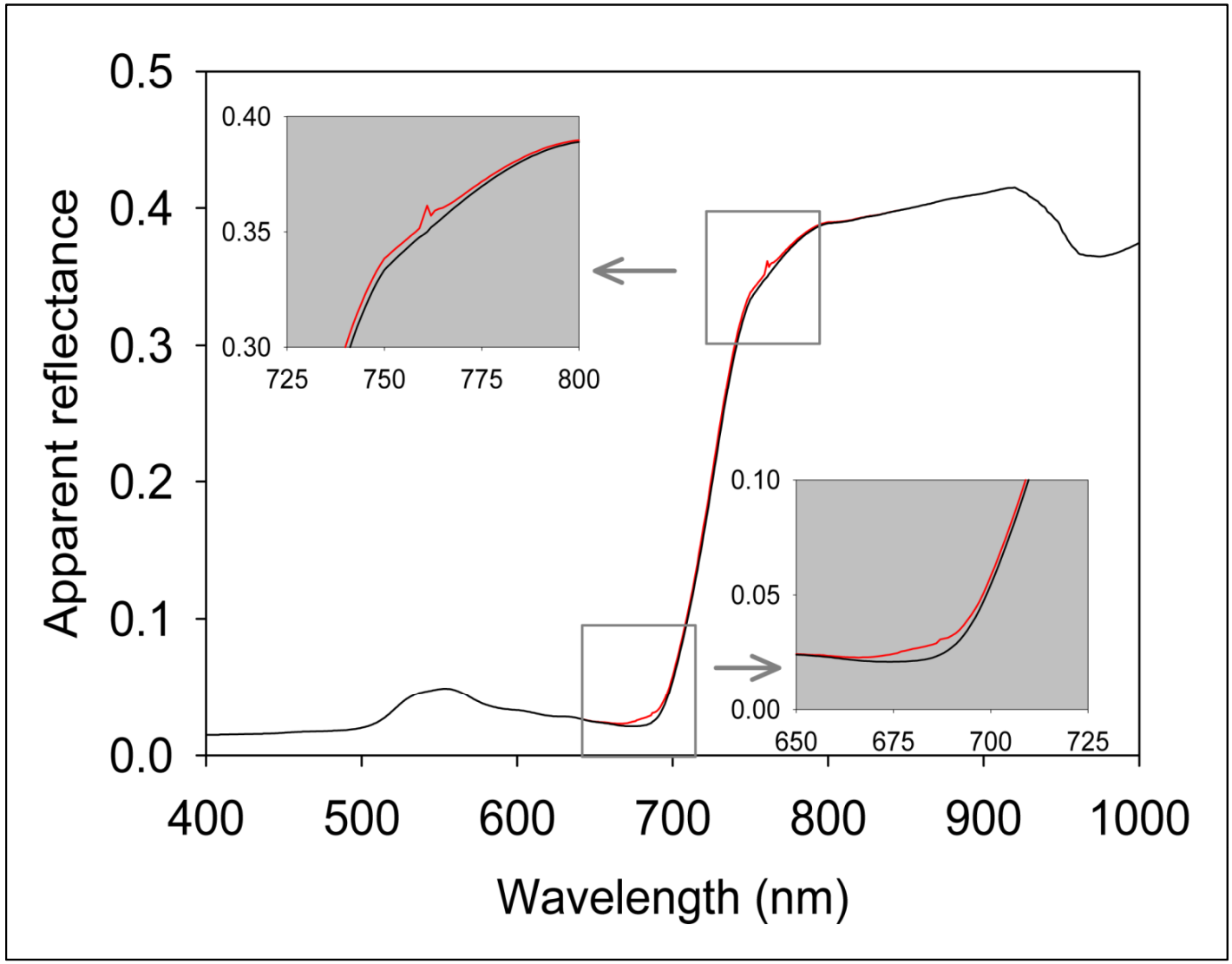
Chlorophyll Fluorescence (3)

- Chlorophyll fluorescence is emitted from the core of the photosynthetic machinery and is directly correlated to efficiency of photosynthesis
- The fluorescence signal is shifted to longer wavelengths



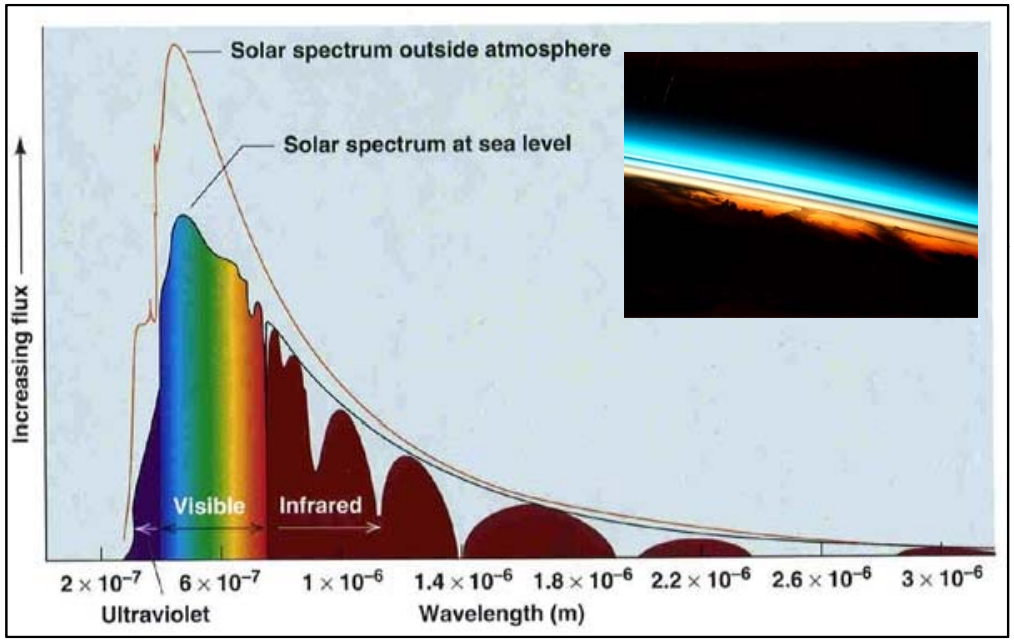
Chlorophyll Fluorescence (4)

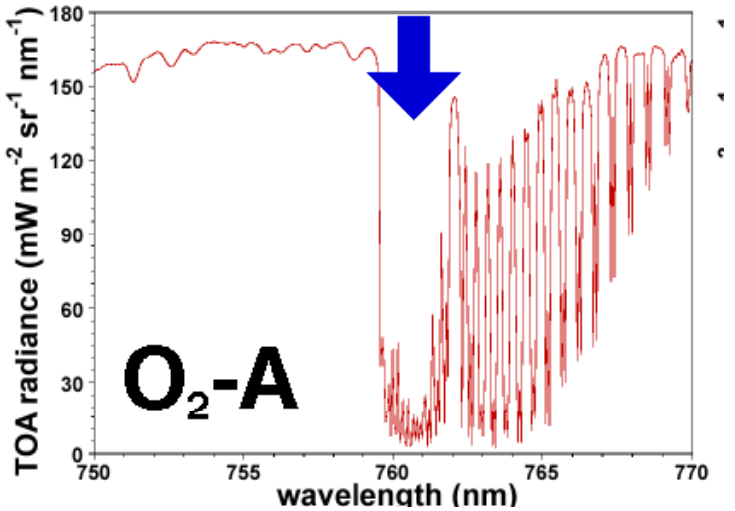
- However, the fluorescence signal adds only a small contribution to the reflected light and thus cannot not be measured with classic spectroscopy

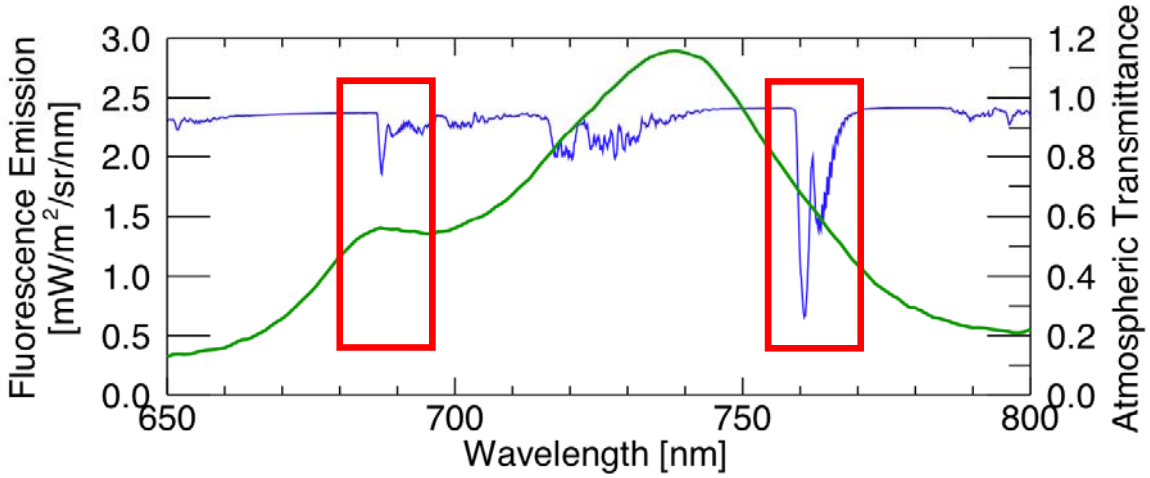


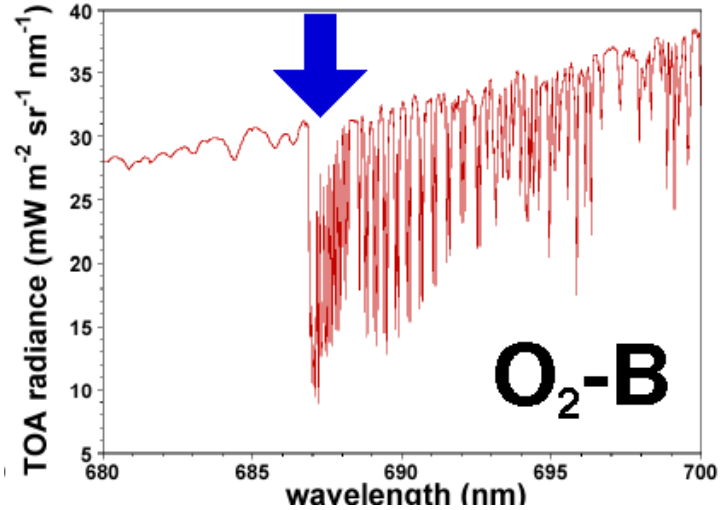
Fluorescence retrieval concept (1)

- Solar and earth atmosphere is a spectrally selective filter
- Two oxygen absorption bands (O_2-A and O_2-B) are at the right spectral region for fluorescence retrieval



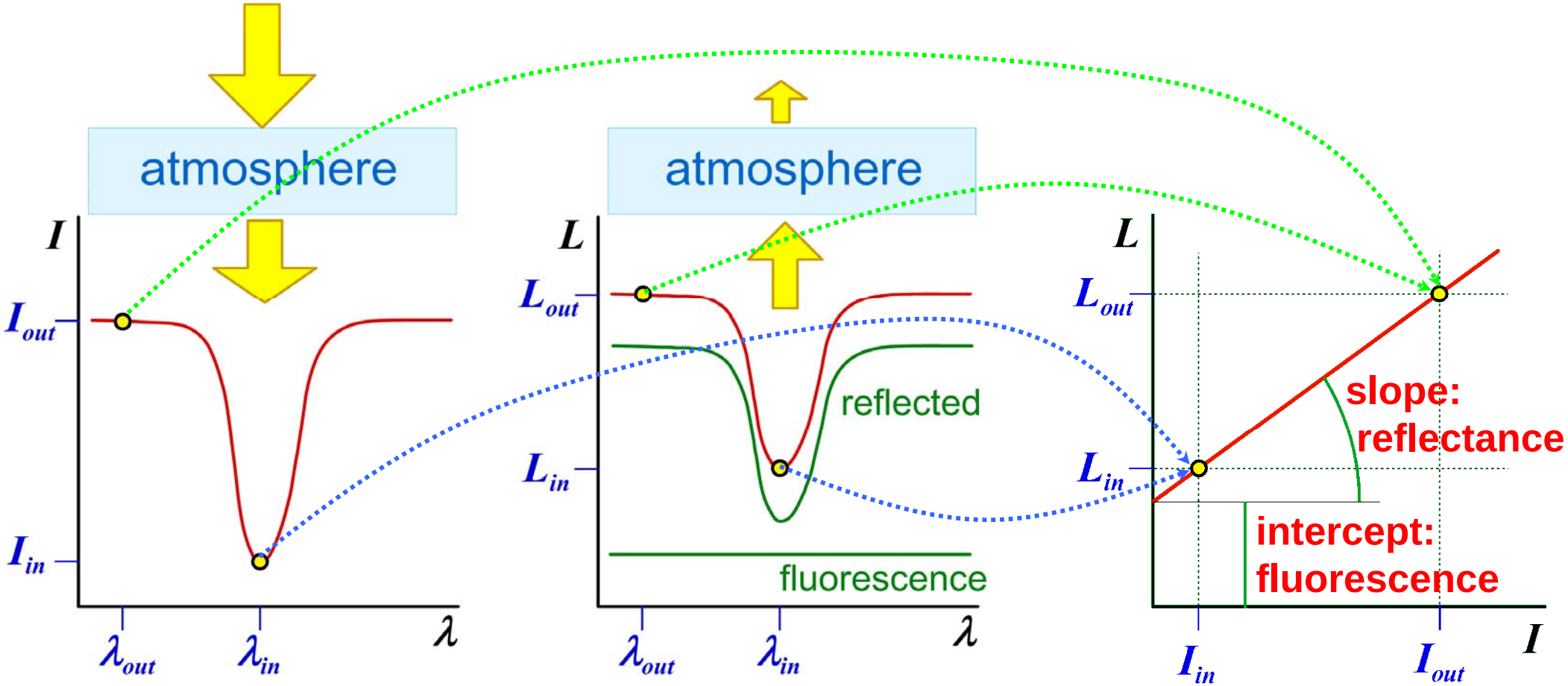






Fluorescence retrieval concept (2)

- Fluorescence can be retrieved in the relative dark atmospheric absorption bands according to the Fraunhofer Line Depth (FLD) method.



Meroni et al. (2009) *Remote Sensing of Environment, 113*, 2037-2051

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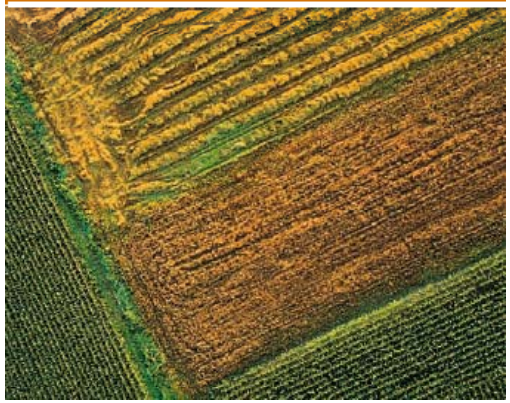

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Vielen Dank an:

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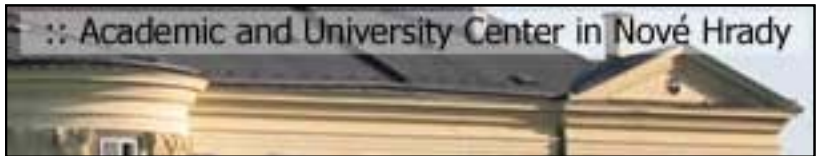

Lada Nedbal

Deutsche Forschungsgemeinschaft DFG

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