

## **Produkční biologie Bi8030 podzimní semestr 2014**

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Optical properties of leaves and its implication in understanding plants functional properties and primary production

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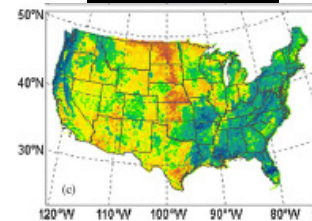
Email: [mishra.k@czechglobe.cz](mailto:mishra.k@czechglobe.cz)

# Spatial Scales of Inquiry:

Span 13-14 orders of Magnitude



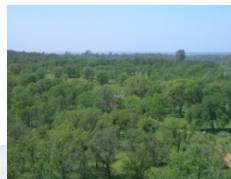
**Globe: 10,000 km ( $10^7$  m)**



**Continent: 1000 km ( $10^6$  m)**



**Landscape: 1-100 km**



**Canopy: 100-1000 m**



**Plant: 1-10 m**



**Leaf: 0.01-0.1 m**

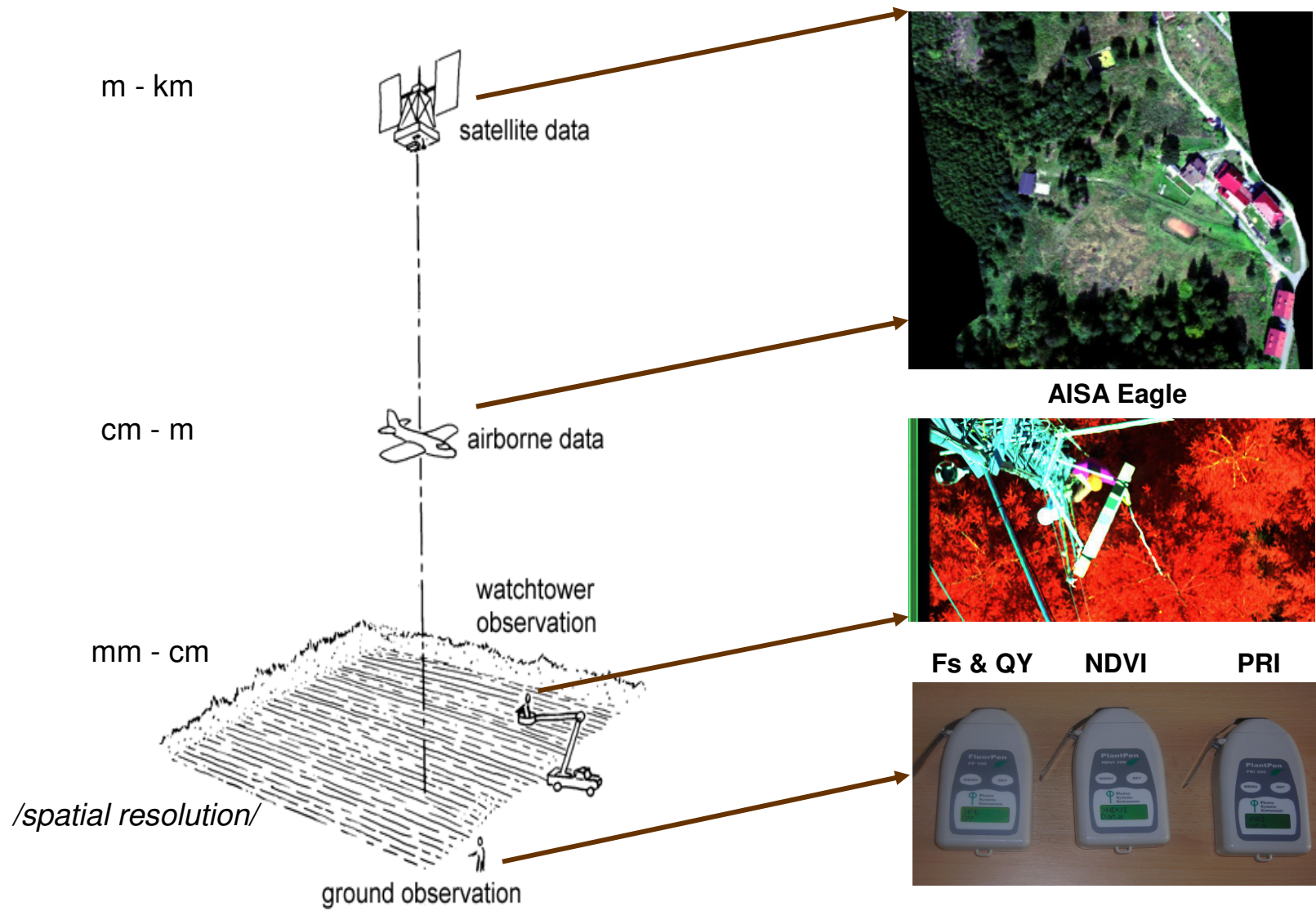


**Stomata:  $10^{-5}$  m**

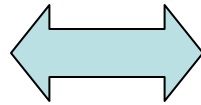


**Bacteria/Chloroplast:  $10^{-6}$  m**

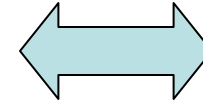
# Multi-scale monitoring



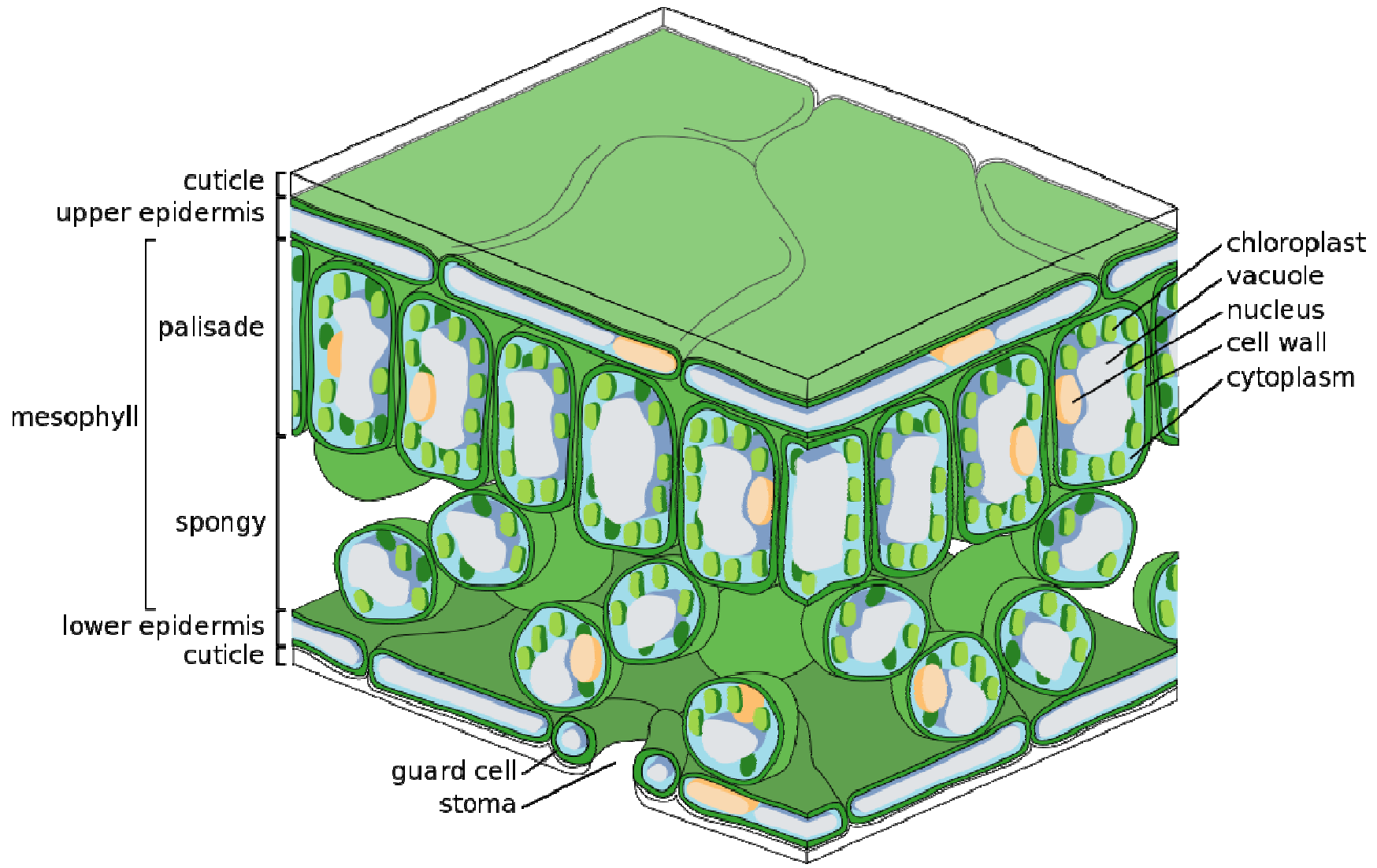
# Plant leaves and its function



- Producing food and oxygen through photosynthesis
- Balancing water loss
- Regulating gas exchange
- Transporting products of photosynthesis



# Diagram of the internal structure of a leaf



# Cross section of leaves

## Cuticle:

## Upper epidermis:

Preventing water loss, providing an extra layer between the outside and inside of the leaf.

## Mesophyll.

### -palisade layer

Chloroplast; **photosynthesis, food & O<sub>2</sub>**

### -spongy layer

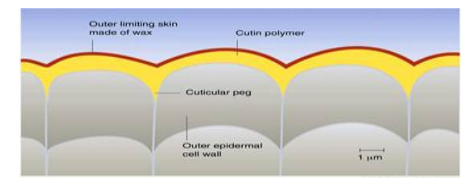
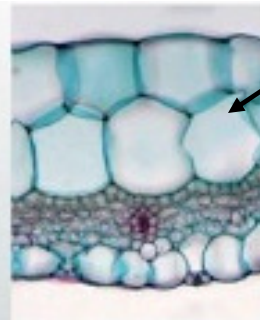
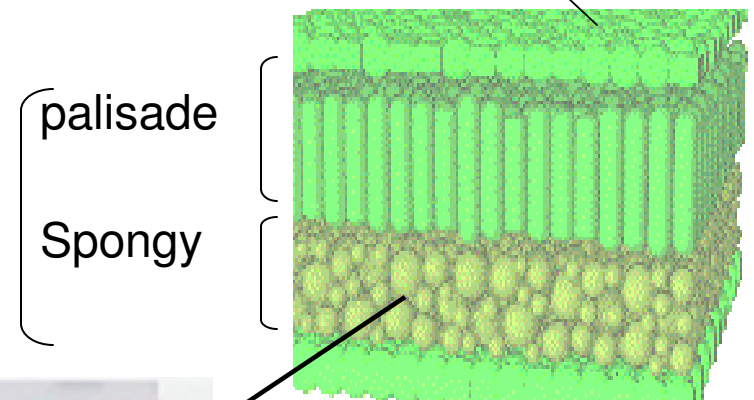
vascular bundles: xylem and phloem

## Lower Epidermis

Stomata



Upper cuticle



# Leaves: Cuticle & Epidermis

## Waxy layer: (hydrophobic)

- prevent water loss,
- first defense against pests and pathogen
- reflect UV light
- self cleaning (Lotus)-

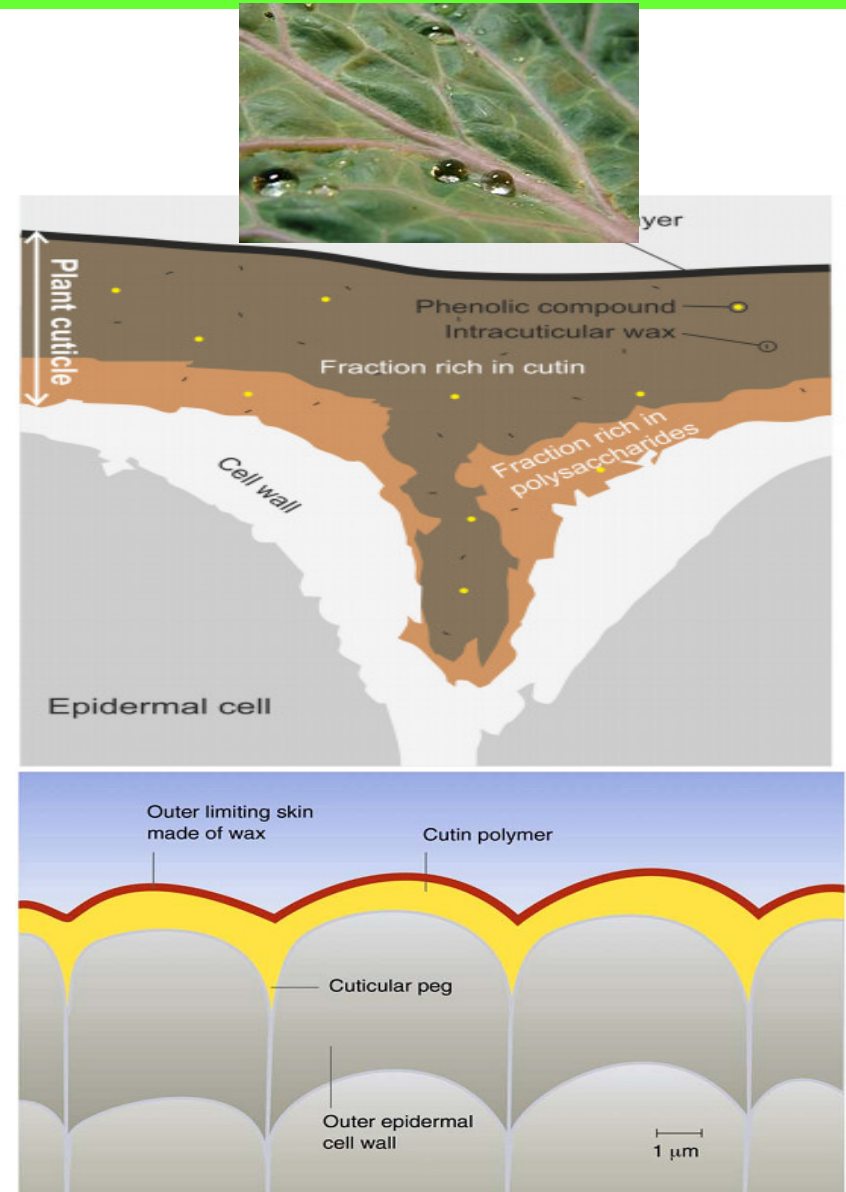
Deposition of pathogens

Sunlight blocking particles

## Phenolics:

- UV screening,
- Antioxidant,
- changes with environment, stresses

Heredia-Guerrero et al.(2014)





## Localization of different subgroups of phenolics at tissues and cellular level

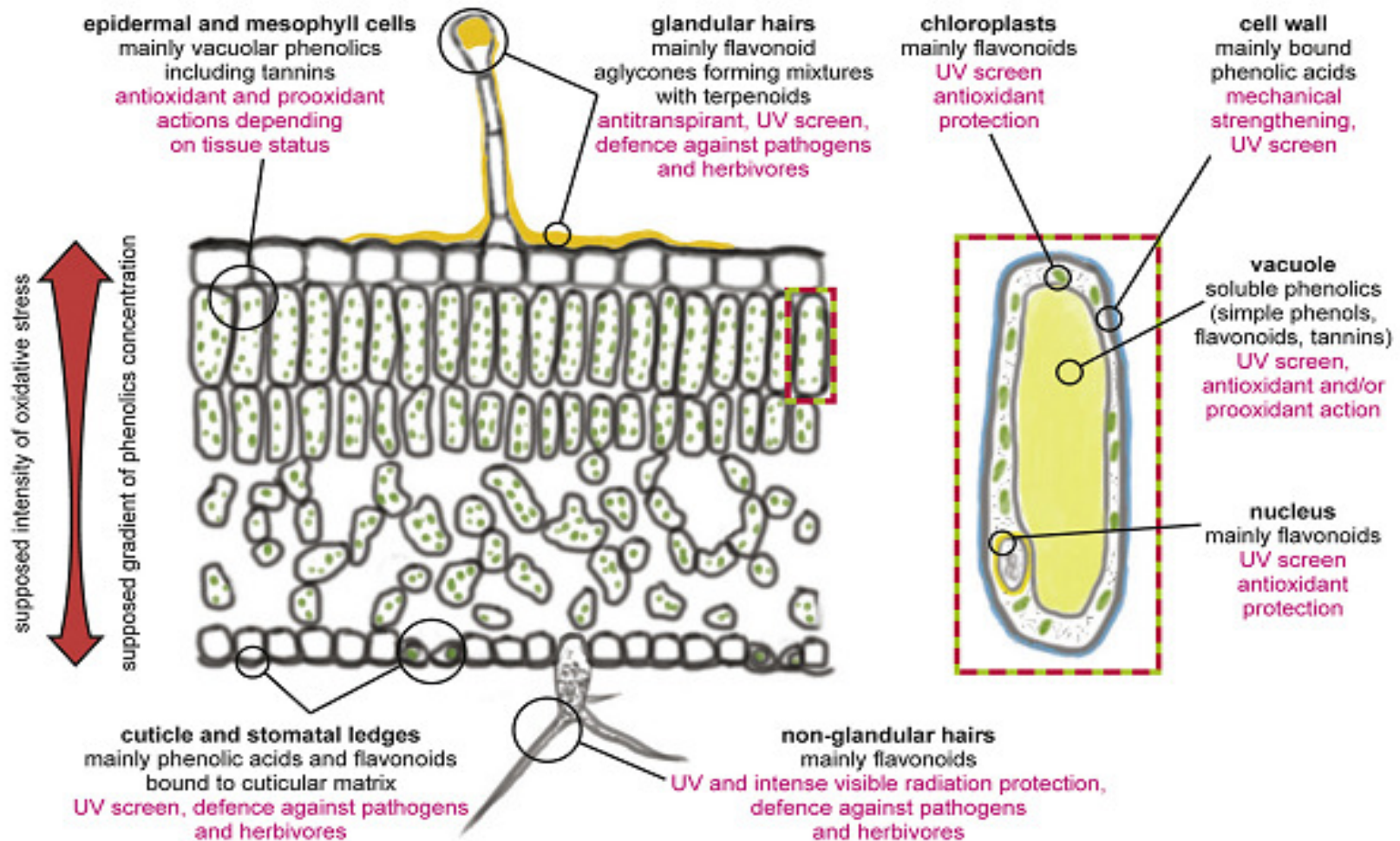


Fig. 2. Schematic representation of typical anatomical features, often occurring in hypostomatic leaves, showing the localization of the different subgroups of phenolics at tissue and cellular level. The main roles of phenolics of each feature are shown in magenta. Two-headed arrow shows the hypothetical gradient of oxidative stress and hypothetical concentration of phenolic compounds across mesophyll tissues. For details, see text.



# Spectral regions of absorption maxima of compounds in leaves epidermis

**Table 1.** Spectral regions of absorption maxima of major classes of phenolics (aglycones in methanol). Compiled from Jurd (1957), Harborne (1989), Mabry, Markham & Thomas (1970) and from our own measurements. In most cases, glycosylation of these compounds shifts the Band I maximum towards shorter wavelength

UV-absorbing compound	Absorption maxima (nm)									
Simple phenols	266–295									
Phenolic acids	235–305									
Hydroxycinnamic acids <sup>a</sup>	227–245								310–332	
Chlorogenic acid	244								329 <sup>b</sup>	
Caffeic acid	244								324 <sup>b</sup>	
Ferulic acid	234								320 <sup>b</sup>	
Sinapic acid	235								319	
Stilbenes			300–310						320–330	
Spectral bands <sup>d</sup>	240	260	280	300	320	340	360	380	Vis <sup>a</sup>	

<sup>a</sup>The spectral data for hydroxycinnamic acids are given for the E- (*trans*-) isoform of the acids, which is preponderant *in vivo*.

<sup>b</sup>Chlorogenic, caffeic and ferulic acid have a pronounced shoulder (inflection) at 299 nm.

<sup>c</sup>For isoflavones and flavanones band I is a minor band; it is of a much smaller amplitude than band II, or the band I of flavones or flavonols.

<sup>d</sup>The UV region is divided into 20-nm-wide spectral bands with the central wavelength indicated (Vis, visible).

## Spectral regions of absorption maxima of compounds in leaves epidermis

Flavonoids	Band II (benzoyl)			Band I (hydroxycinnamoyl)					
Isoflavones	255–265			(310–330) <sup>c</sup>					
Flavanones	275–290			(310–330) <sup>c</sup>					
Flavones <sup>3</sup>	250–270			30–350					
Apigenin	267			336					
Luteolin	267			349					
Flavonols	250–270			350–390					
Kaempferol	266			367					
Quercetin	255			370					
Chalcones	240–260			365–390					
Aurones <sup>3</sup>	240–270			390–430					
Anthocyanins	267–275			475–545					
Spectral bands <sup>d</sup>	240	260	280	300	320	340	360	380	Vis <sup>a</sup>

<sup>a</sup>The spectral data for hydroxycinnamic acids are given for the E- (*trans*-) isoform of the acids, which is preponderant *in vivo*.

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<sup>d</sup>The UV region is divided into 20-nm-wide spectral bands with the central wavelength indicated (Vis, visible).

## How to measure compounds in the Leaf epidermis?

-Extraction of leaf cuticle and measurements of absorbance and transmittance

Krauss et al. (1997) Plant, Cell, and Environment 1997

### **Problems in measuring epidermis contents by leaf extraction:**

leaf structures on UV absorption, and the local distribution of phenylpropanoids in leaves are lost by the extraction procedures.

**ChlF excitation ratio (FER) = ChlF yields [UV / blue-green excitations]**

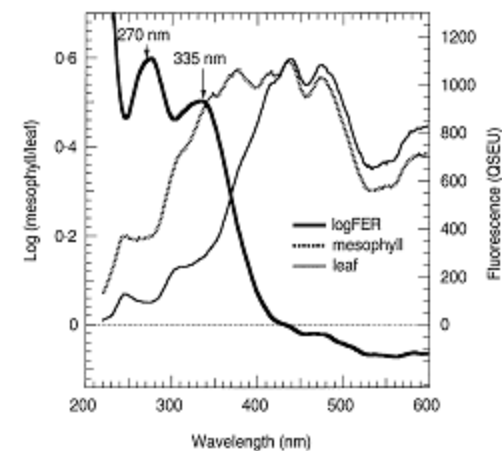
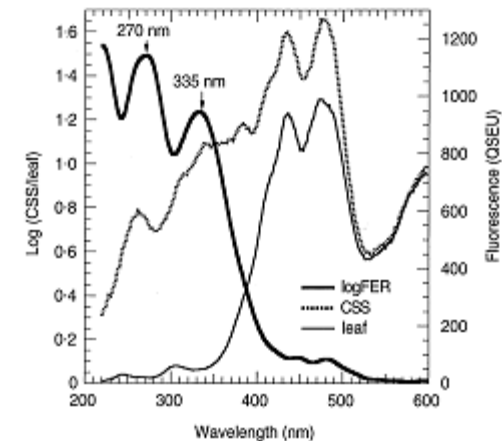
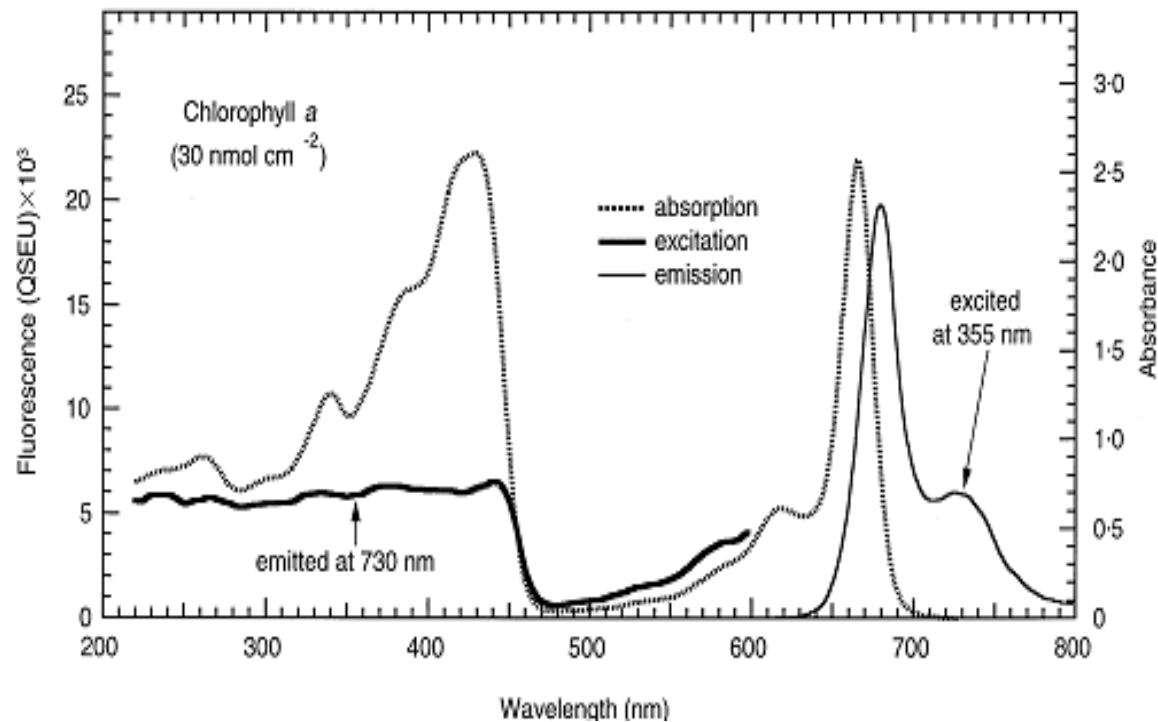
**Log(FER)= absorbance of leaf epidermal**

Cerovic et al.(2002) Plant Cell and Environment, 25: 1663-1676

## How to measure compounds in the cuticle?

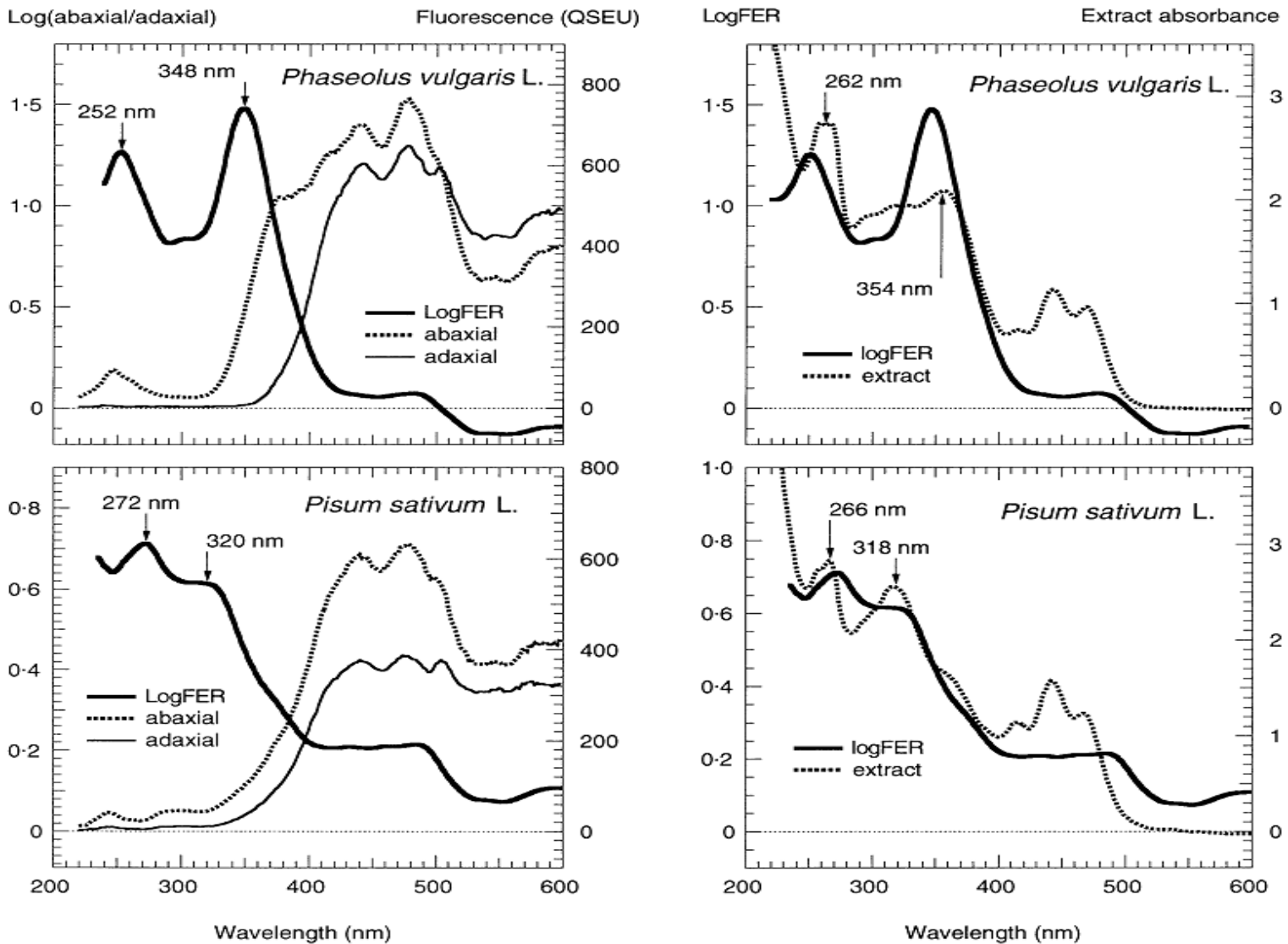
**ChIF excitation ratio (FER) = ChIF yields [UV / blue-green excitations]**

**Log(FER) = absorbance of leaf epidermal**



Cerovic et al.(2002) Plant Cell and Environment, 25: 1663-1676

# FER can measure absorbance of leaf epidermis



Cerovic et al.(2002) Plant Cell and Environment, 25: 1663-1676



# Leaves: Mesophyll & Lower Epidermis

## palisade layer

- cells are tall,
- closely packed to absorb maximum light.
- many chloroplasts.
- most photosynthesis takes place in the palisade cells.

## -photosynthesis,

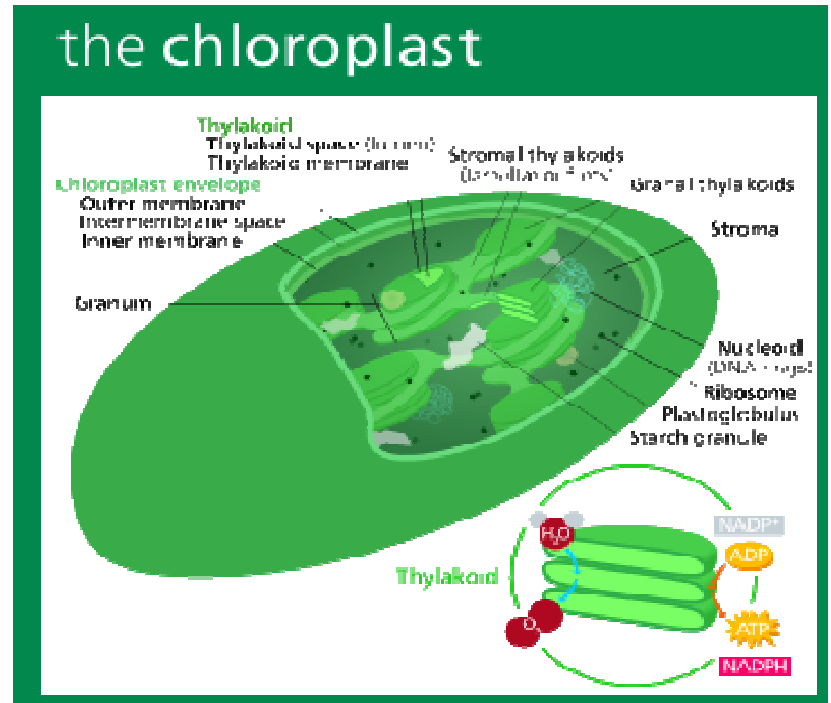
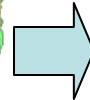
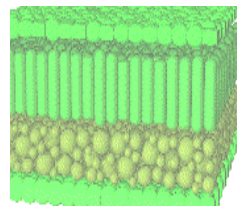
- food

-O<sub>2</sub>

## spongy layer

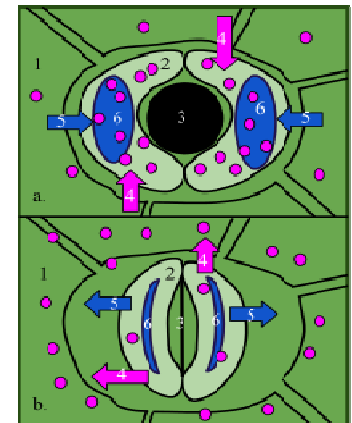
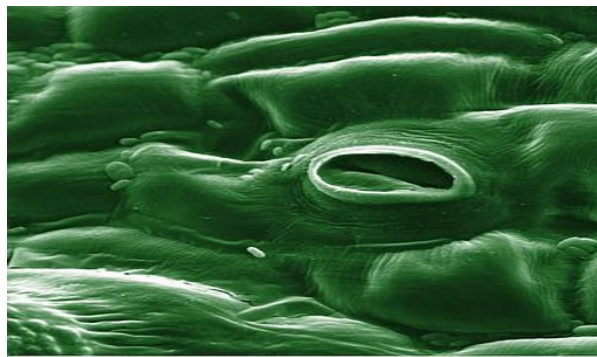
vascular bundles:

- xylem & phloem



## Lower Epidermis

Stomata



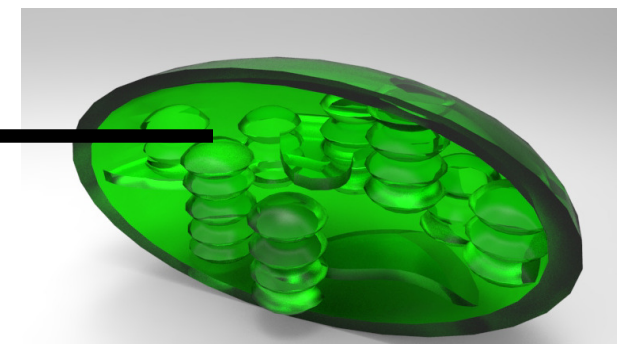
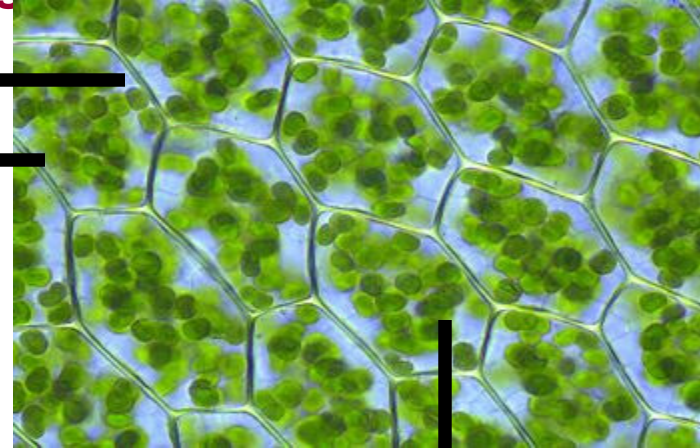
# Biochemical leaf composition

## A typical cell of green fresh leaf contains:

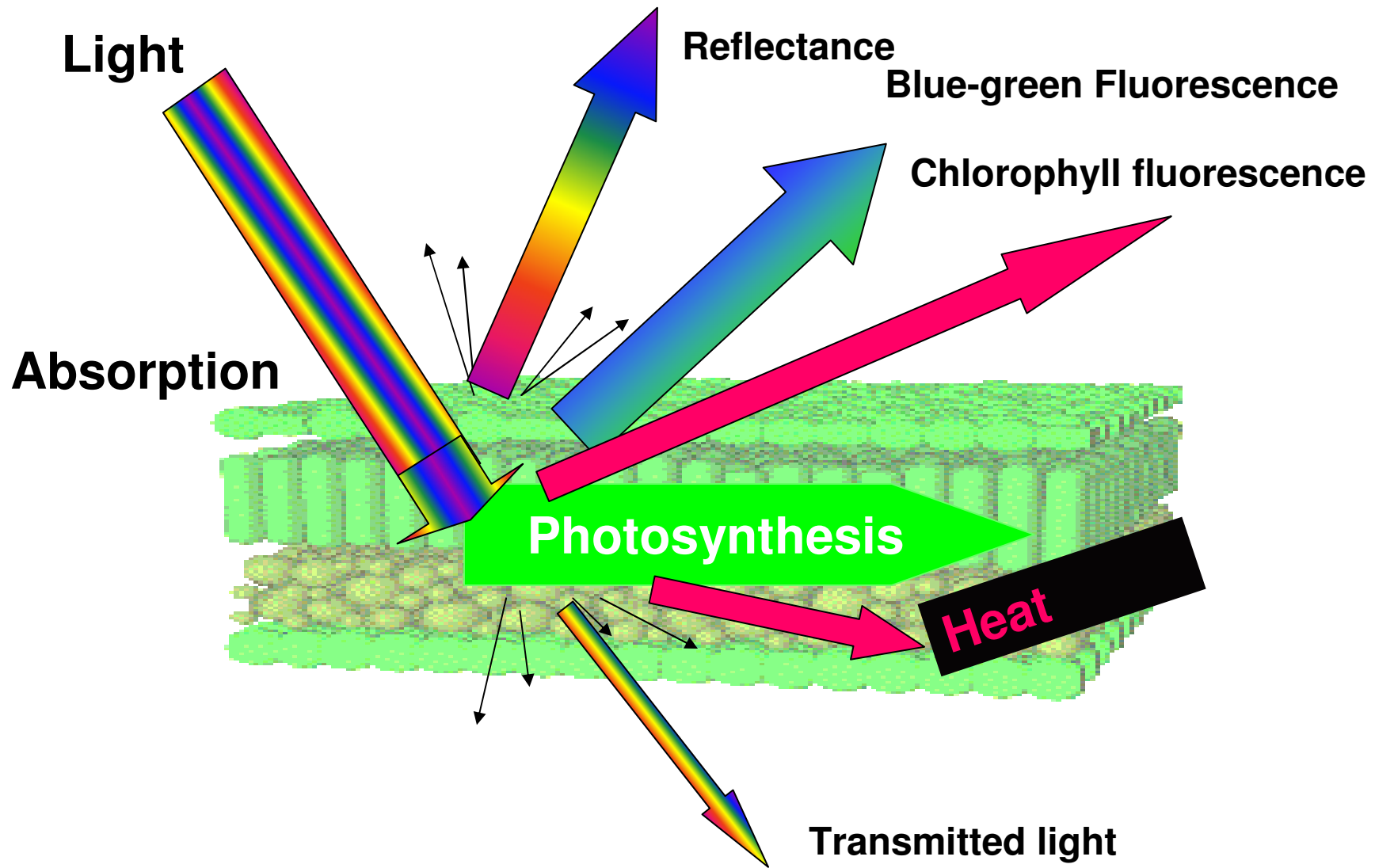
-Water (vacuole) 90-95%

-dry matter : 5-10 %

- Cellulose 15-10 %
- Hemicellulose 10-30%
- Proteins 10-20%]
- Lignin 5-15%
- Starch 0.2-2.7%
- Sugar
- Chloroplasts (Chlorophyll *a*, *b*)
- Other pigments (Carotenoids, anthocynin, brown pigments, Flavons, etc.)

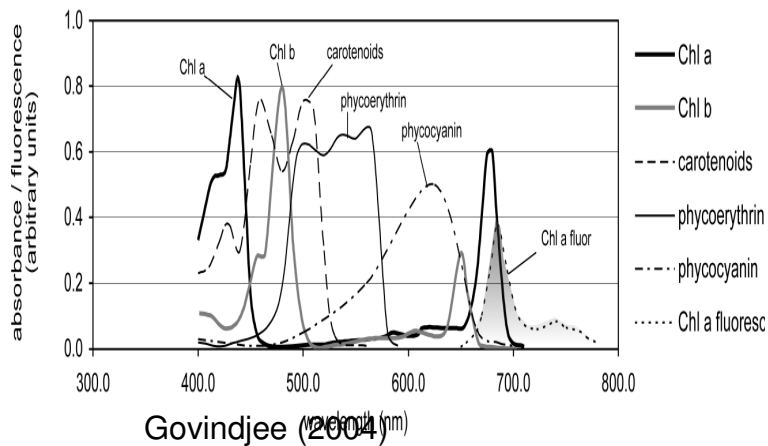
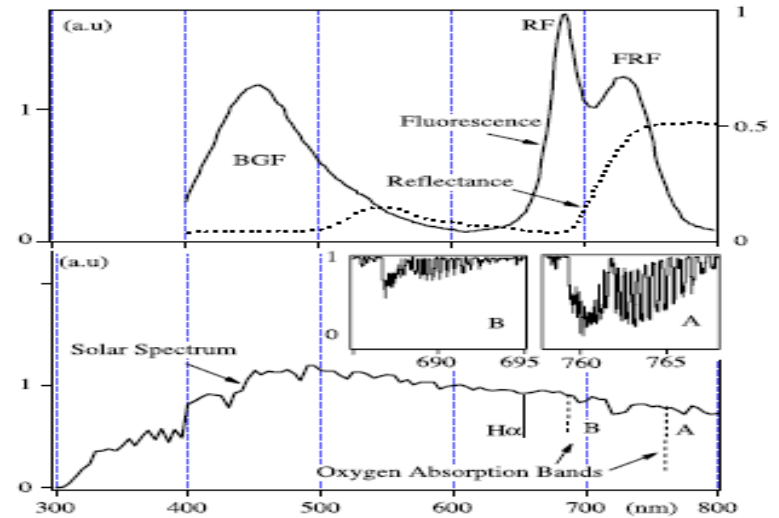
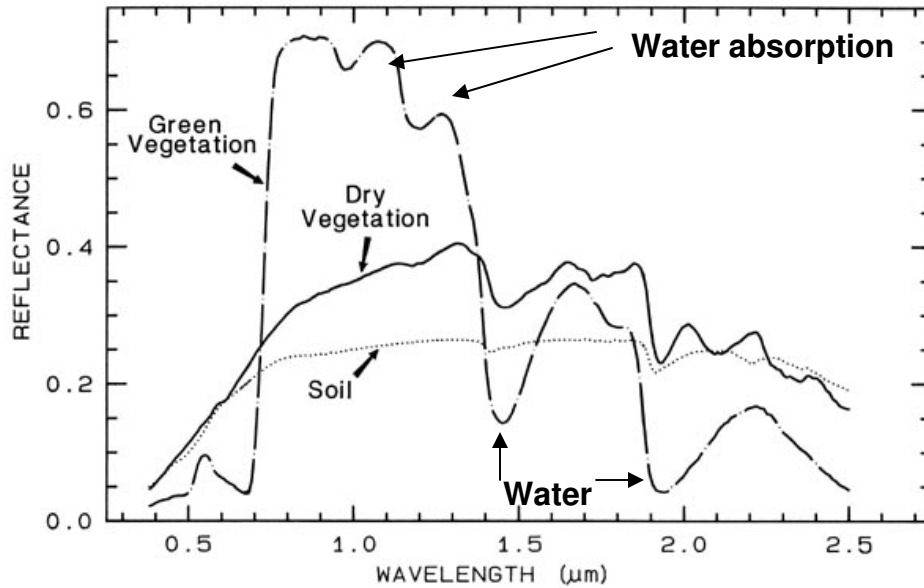


# Schematic diagram representing interaction of plant's leaf with light



Energy conservation:  $R(I) + T(I) + A(I) = 1$

# Reflectance and fluorescence signals

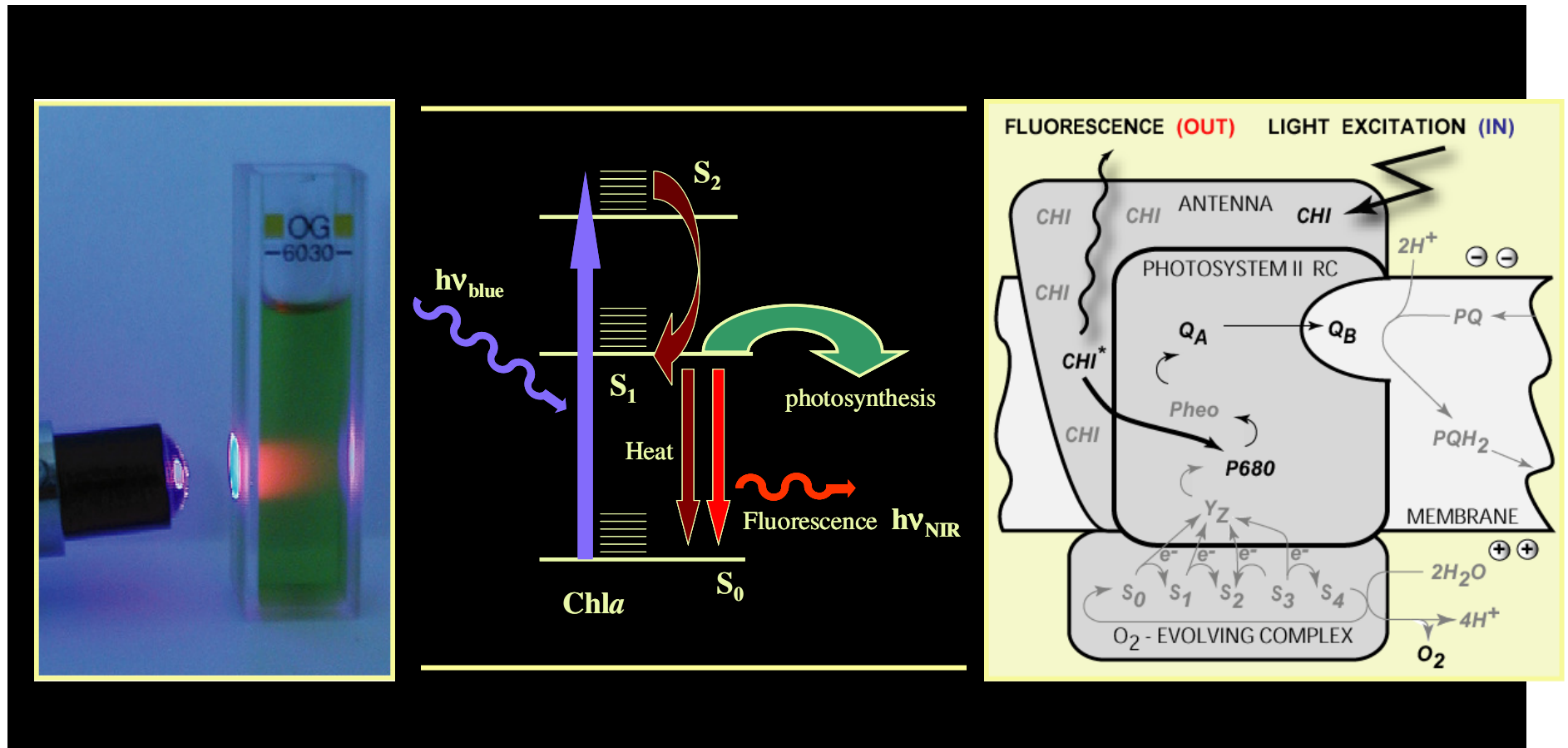


Govindjee (2004)

Reflectance is being measured at various level and many vegetation indexes has been derived.

Steady state Fluorescence at Fraunhofer lines will be measured in proposed FLEX mission

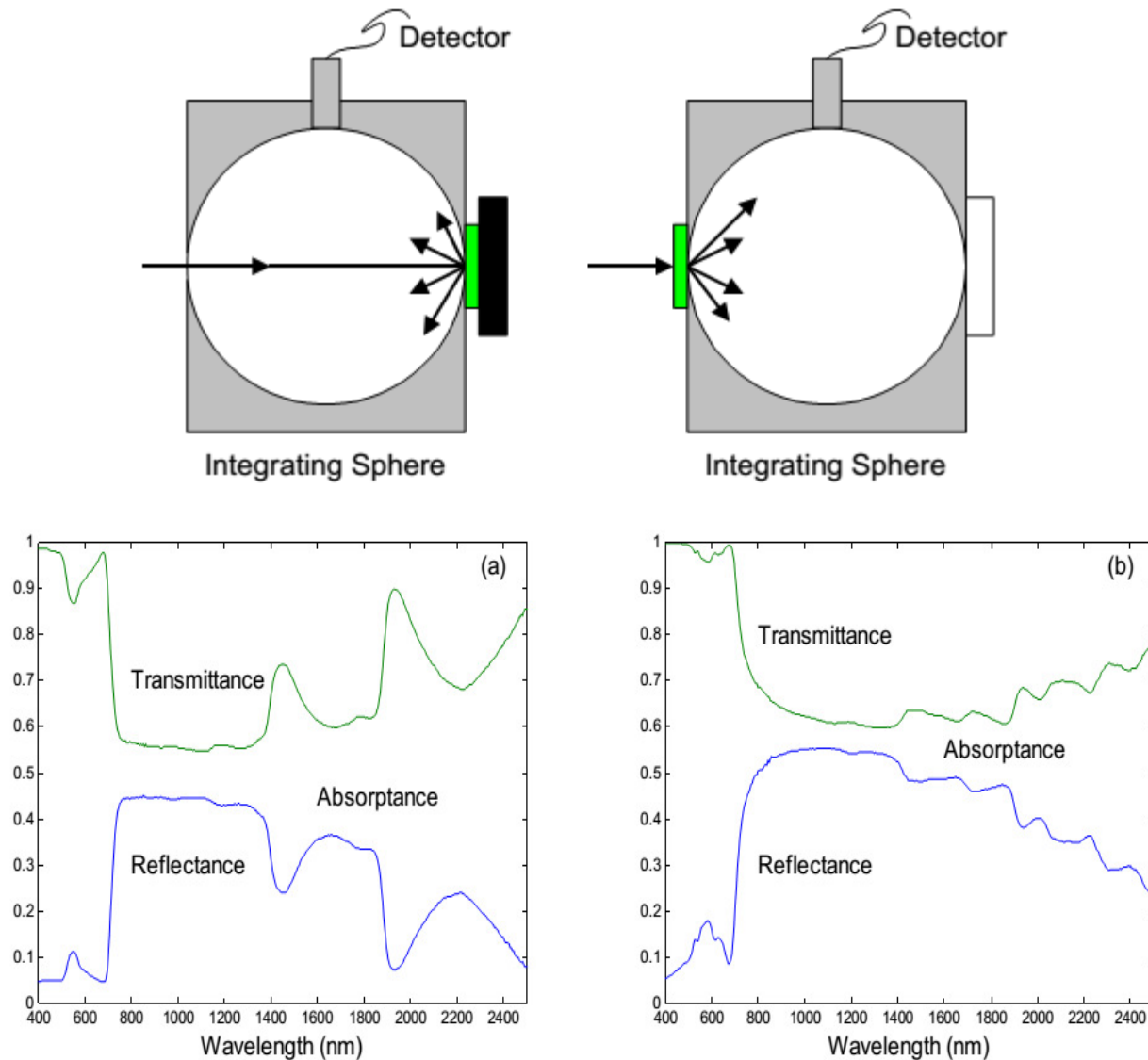
# Chlorophyll fluorescence competes with photosynthesis for excitation energy



ChlF can be used as a non-invasive reporter to study photosynthetic yield

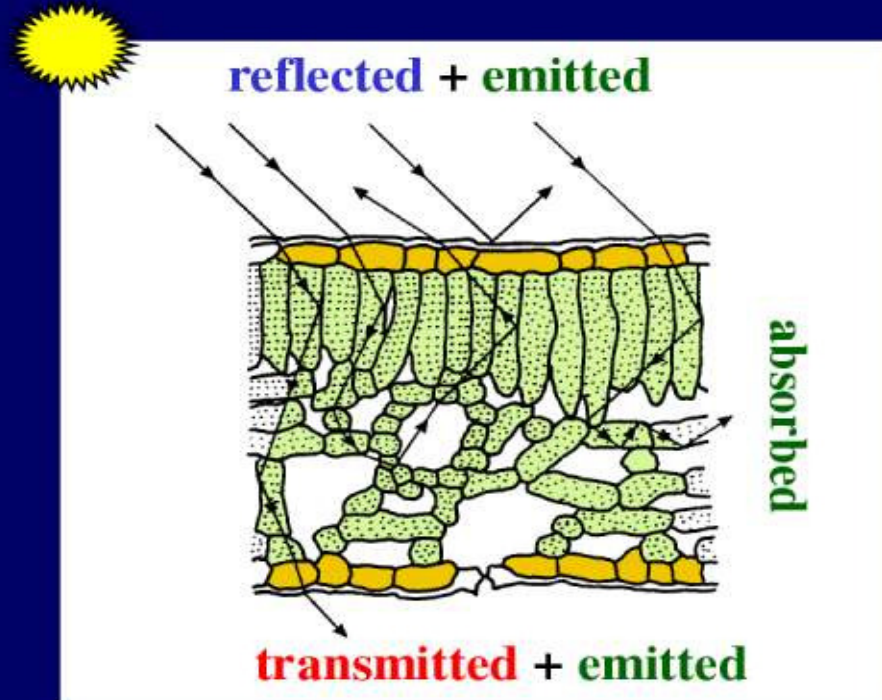


# Integrating sphere for accurately measurements of reflectance



**Fig. 2:** Reflectance and transmittance spectra of (a) fresh and (b) dry poplar leaves

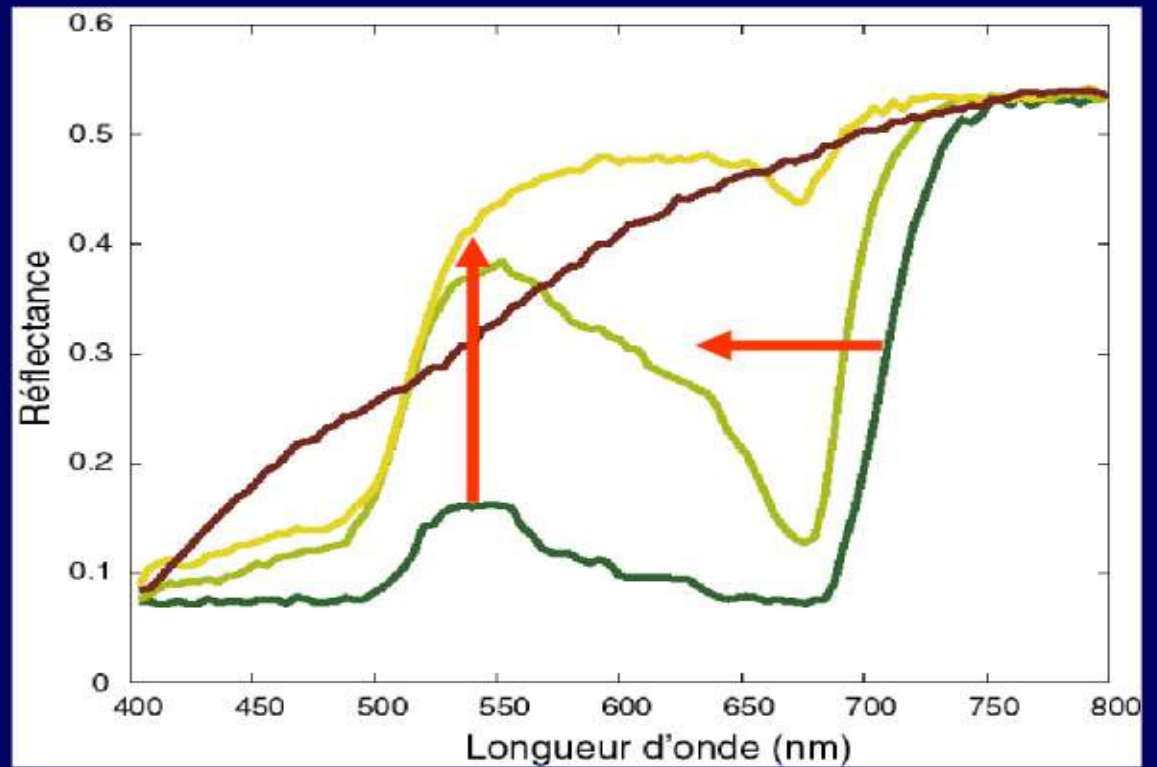
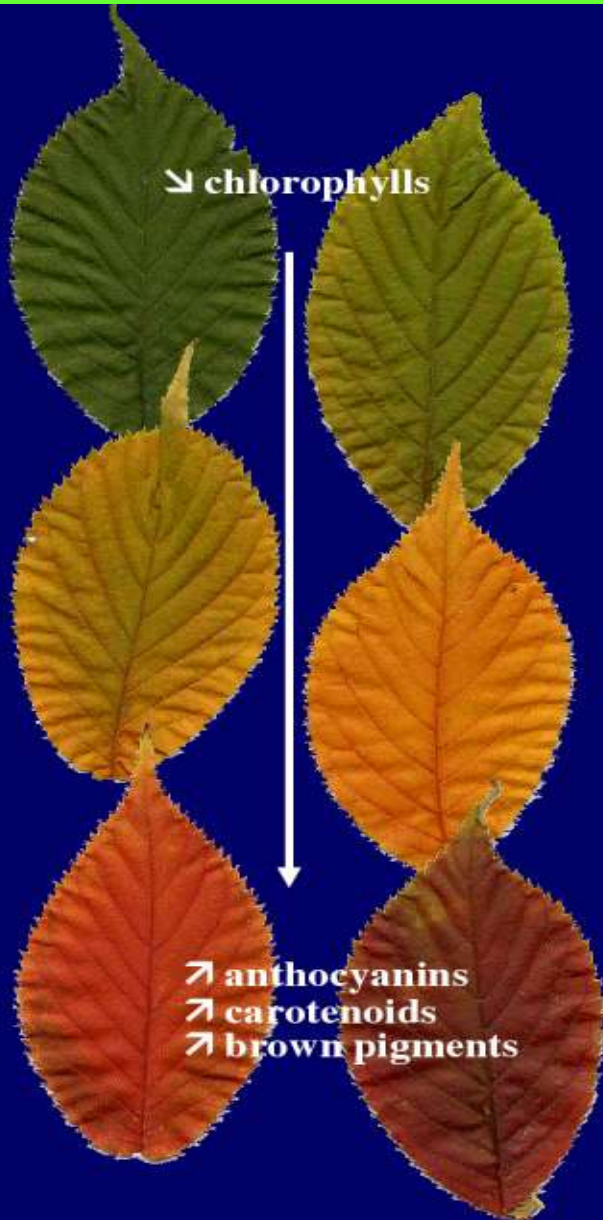
# Propagation of photons in leaf



T.R. Sinclair, M.M. Schreiber & R.M. Hoffer, 1973, Diffuse reflectance hypothesis for the pathway of Solar radiation through leaves, *Agronomy Journal*, 65:276-283

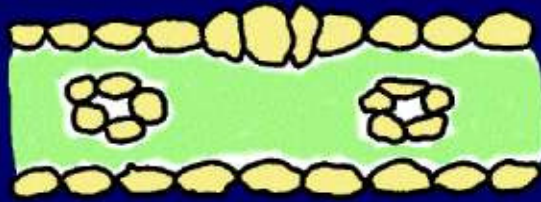
## Spectral properties: effect of leaf pigments

### Spectral properties: effect of leaf pigments

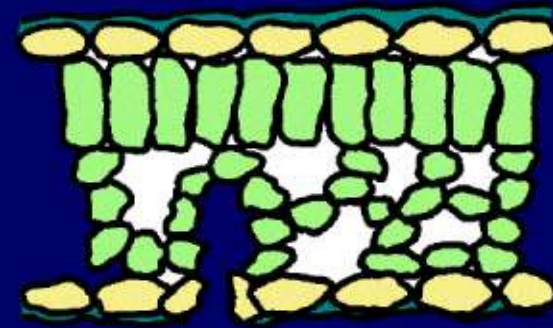
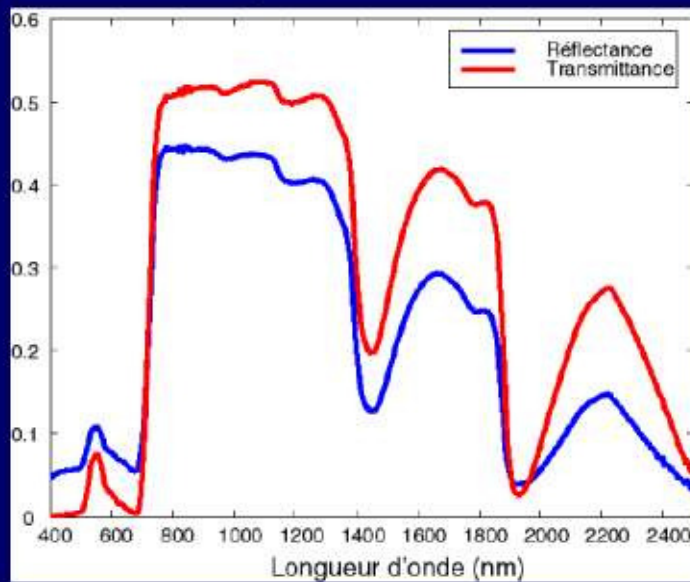




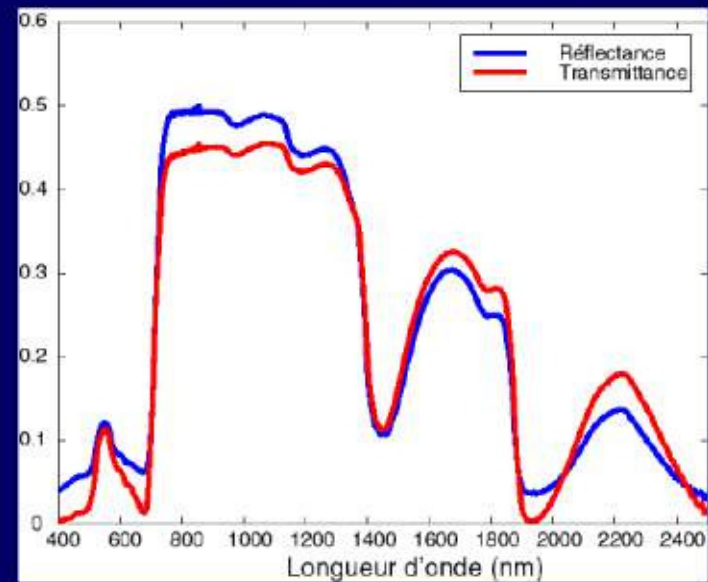
## Spectral properties: effect of leaf internal structure



corn (*Zea mays*)



sunflower (*Helianthus annuus*)

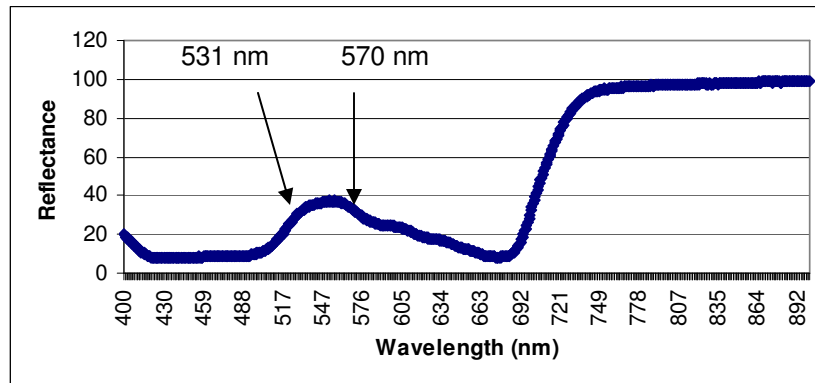


## VEGETATION INDEX: Two wavelength dependent Indexes

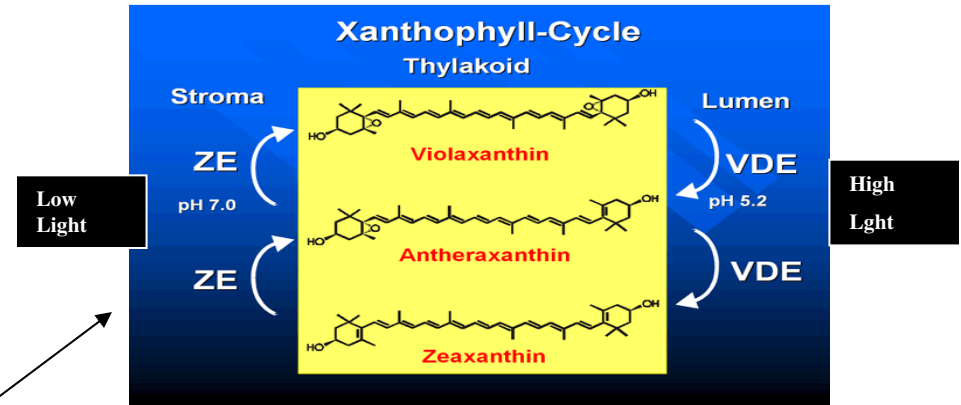
Normalized Difference Vegetation Index (NDVI)	$NDVI = (R_{NIR} - R_{red}) / (R_{NIR} + R_{red})$	Rouse <i>et al.</i> (1974)
Renormalized Difference Vegetation Index (RDVI)	$RDVI = (R_{800} - R_{670}) / \sqrt{(R_{800} + R_{670})}$	Rougean and Breon, (1995)
Simple Ratio Index (SR)	$SR = RNIR/R_{red}$	Jordan (1969); Rouse <i>et al.</i> (1974)
Modified Chlorophyll Absorption in Reflectance Index (MCARI1)	$MCARI1 = 1.2 * [2.5 * (R_{800} - R_{670}) - 1.3 * (R_{800} - R_{550})]$	Haboudane <i>et al.</i> (2004)
Modified Chlorophyll Absorption in Reflectance Index (MCARI2)	$MCARI2 = \frac{1.5 * [2.5 * (R_{800} - R_{670}) - 1.3 * (R_{800} - R_{550})]}{\sqrt{(2 * R_{800} + 1)^2 - (6 * R_{800} - 5 * \sqrt{R_{670}}) - 0.5}}$	Haboudane <i>et al.</i> (2004)
Improved SAVI with Self-Adjustment Factor L (MSAVI)	$MSAVI = \frac{1}{2} [2 * R_{800} + 1 - \sqrt{(2 * R_{800} + 1)^2 - 8 * (R_{800} - R_{670})}]$	Qi <i>et al.</i> (1994)
Optimized Soil-Adjusted Vegetation Index (OSAVI)	$OSAVI = (1 + 0.16) * (R_{800} - R_{670}) / (R_{800} + R_{670} + 0.16)$	Rondeaux <i>et al.</i> (1996)
Greenness Index (G)	$G = (R_{554}) / (R_{677})$	-
Modified Chlorophyll Absorption in Reflectance Index (MCARI)	$MCARI = [(R_{700} - R_{670}) - 0.2 * (R_{700} - R_{550})] * (R_{700} / R_{670})$	Daughtry <i>et al.</i> (2000)
Transformed CARI (TCARI)	$TCARI = 3 * [(R_{700} - R_{670}) - 0.2 * (R_{700} - R_{550})] * (R_{700} / R_{670})$	Haboudane <i>et al.</i> (2002)
Triangular Vegetation Index (TVI)	$TVI = 0.5 * [120 * (R_{750} - R_{550}) - 200 * (R_{670} - R_{550})]$	Broge and Leblanc (2000)
Zarco-Tejada & Miller	$ZM = (R_{750}) / (R_{710})$	Zarco-Tejada <i>et al.</i> (2001)
Simple R. Pigment Ind. (SRPI)	$SRPI = (R_{430}) / (R_{680})$	Peñuelas <i>et al.</i> (1995)
Normalized Phaeophytinization Index (NPQI)	$NPQI = (R_{415} - R_{435}) / (R_{415} + R_{435})$	Barnes <i>et al.</i> (1992)
Photochemical Reflectance Index (PRI)	$PRI = (R_{531} - R_{570}) / (R_{531} + R_{570})$	Gamon <i>et al.</i> (1992)
Normalized Pigment Chlorophyll Index (NPCI)	$NPCI = (R_{680} - R_{430}) / (R_{680} + R_{430})$	Peñuelas <i>et al.</i> (1994)
Carter Indices	$Ctr1 = (R_{695}) / (R_{420})$ $Ctr2 = (R_{695}) / (R_{760})$	Carter (1994) Carter <i>et al.</i> (1996)
Lichtenthaler Indices	$Lic1 = (R_{800} - R_{680}) / (R_{800} + R_{680})$ $Lic2 = (R_{440}) / (R_{690})$	Lichtenthaler <i>et al.</i> (1996)
Structure Intensive Pigment Index (SIPI)	$SIPI = (R_{800} - R_{450}) / (R_{800} + R_{650})$	Peñuelas <i>et al.</i> (1995)
Gitelson and Merzlyak	$GM1 = R_{750} / R_{550}$ $GM2 = R_{750} / R_{700}$	Gitelson & Merzlyak (1997)



# Photochemical reflectance index (PRI) Normalized Differential Vegetation Index (NDVI)



Change in De-epoxidation state of Xanthophylls cycle could be detected by measuring PRI



$$PRI = (R_{531} - R_{570}) / (R_{531} + R_{570})$$

$R_{531}$  is sensitive to change in Xanth's Cycle Epoxidation state  
 $R_{570}$  is a reference, unaffected by Xanth's Cycle Epoxidation state.

Quite high variability in day, hours, minutes and even second.

Seasonal variability is also seen

$$NDVI = (R_{\text{far-red}} - R_{\text{red}}) / (R_{\text{far-red}} + R_{\text{red}})$$

- PRI - a way of dissipating excess light to protect the photosynthetic apparatus (Gamon et al. 1990).
- conversion of violaxanthin to zeaxanthin through xanthophyll cycle.

## VEGETATION INDEX: Three wavelength dependent Indexes

$$R^{-1} \propto R_{\infty} = a / b_b$$

Gitelson et al (2006)

$a$  = Sum of the Absorption coefficients for the pigments of interest  $a_p$  and other pigments  $a_0$

$b_b$  = Backscattering coefficient

$$[R(\lambda_1)^{-1} - R(\lambda_2)^{-1}] \times R(\lambda_3) \propto a_p$$

$R(\lambda)_1$  = Reflectance maximally sensitive to the absorption of particular pigments  $a_p$

$R(\lambda)_2$  = Reflectance where the absorption is much lower than  $R(\lambda)_1$

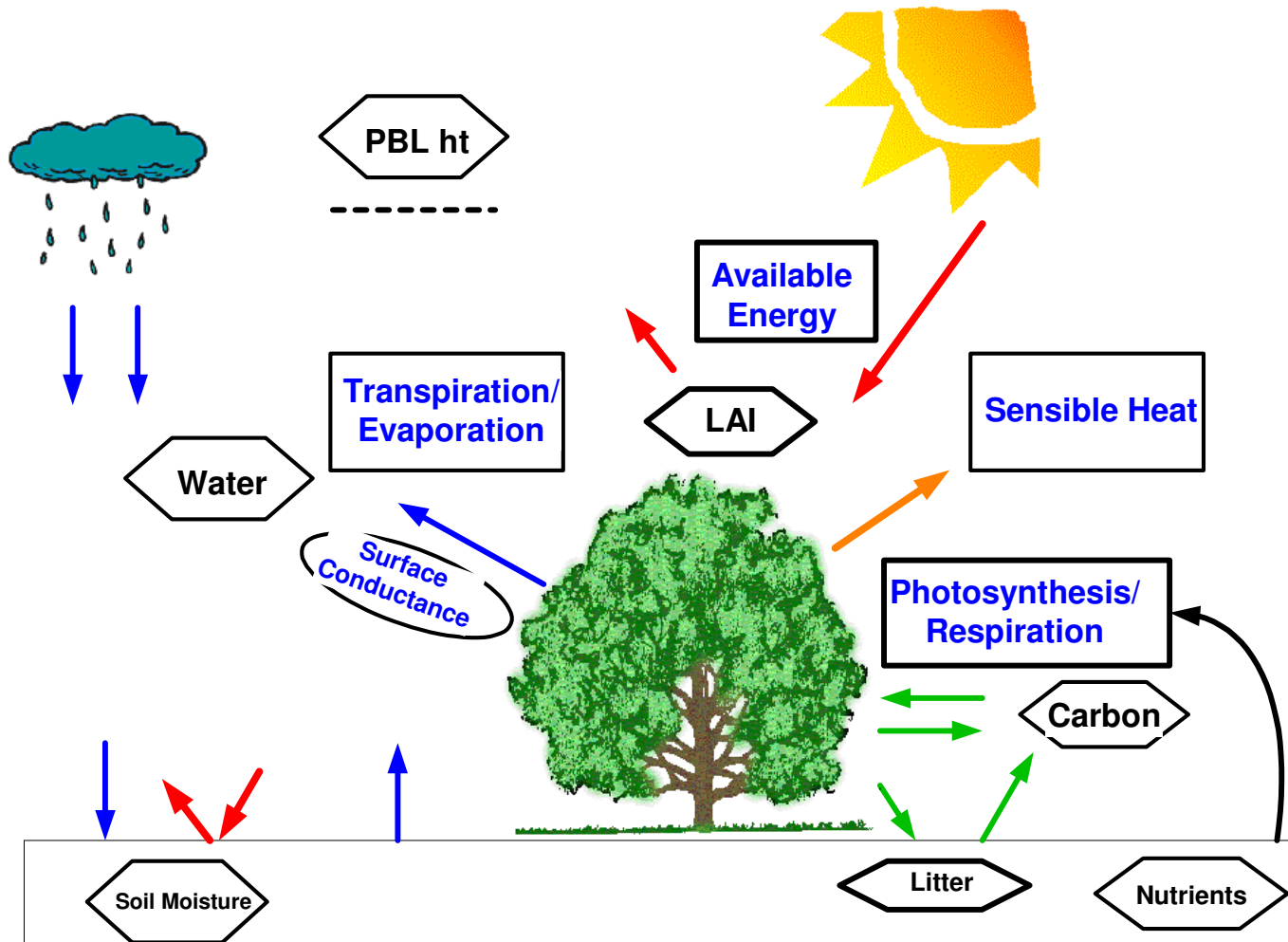
$R(\lambda)_3$  = Reflectance where backscattering controls the reflectance

**Table: Spectral bands for retrieving pigment content from leaf reflectance**

Pigment	$\lambda_1$	$\lambda_2$	$\lambda_3$
Chlorophylls, Anth-free	540 – 560	760 – 800	760 – 800
Chlorophylls, Anth-free	690 – 720	760 – 800	760 – 800
Chlorophylls, Anth-cont	690 – 720		760 – 800
Carotenoids	510 – 520	540 – 560	760 – 800
Carotenoids	510 – 520	690 – 710	760 – 800
Anthocyanins	540 – 560	690 – 710	760 – 800

Three wavelength dependent indices proved to be very useful for tracking of particular pigments.

# System Complexity: Interconnection of Key Ecosystem Processes



## Remote sensing of vegetation products scaling of biophysical processes : A challenge

---

**Leaf area index (LAI)** = Leaf area per unit ground surface area  
= *leaf area / ground area, m<sup>2</sup> / m<sup>2</sup>*  
~ projected leaf area m<sup>2</sup>

**Fraction of absorbed photosynthetic active radiation (fPAR)**  
= *radiation absorbed by vegetation for photosynthesis*  
*/ total incoming PAR between 400–720 nm*

- Primary productivity of photosynthesis
- evapotranspiration
- reference tool for [crop](#) growth

# How to measure LAI?

## Direct Methods

- Area harvest (grasslands, agriculture)
- application of allometric equations to stand diameter data,
- leaf litterfall

*collect leaves during **leaf fall in traps** and compare to area measurements. Leaf area is measured using a scanner and image processing software collect leaves from the canopy and conduct measurements. Is **destructive**, especially with evergreens due to the difficulties and destructiveness of direct methods for determining LAI, they are mostly used as a reference for indirect methods that are easier and faster to apply. disadvantages include:*

***Destructive; time consuming; expensive, especially if the study area is large***

## Indirect Methods

*indirect contact LAI measurements* (very subjective and labor intensive)

***plumb lines inclined point quadrates***

*indirect non-contact measurements* (typically preferred)

***hemispherical photography: estimate LAI from analyzing upward looking fisheye photographs taken beneath the plant canopy***





## How to measure LAI?

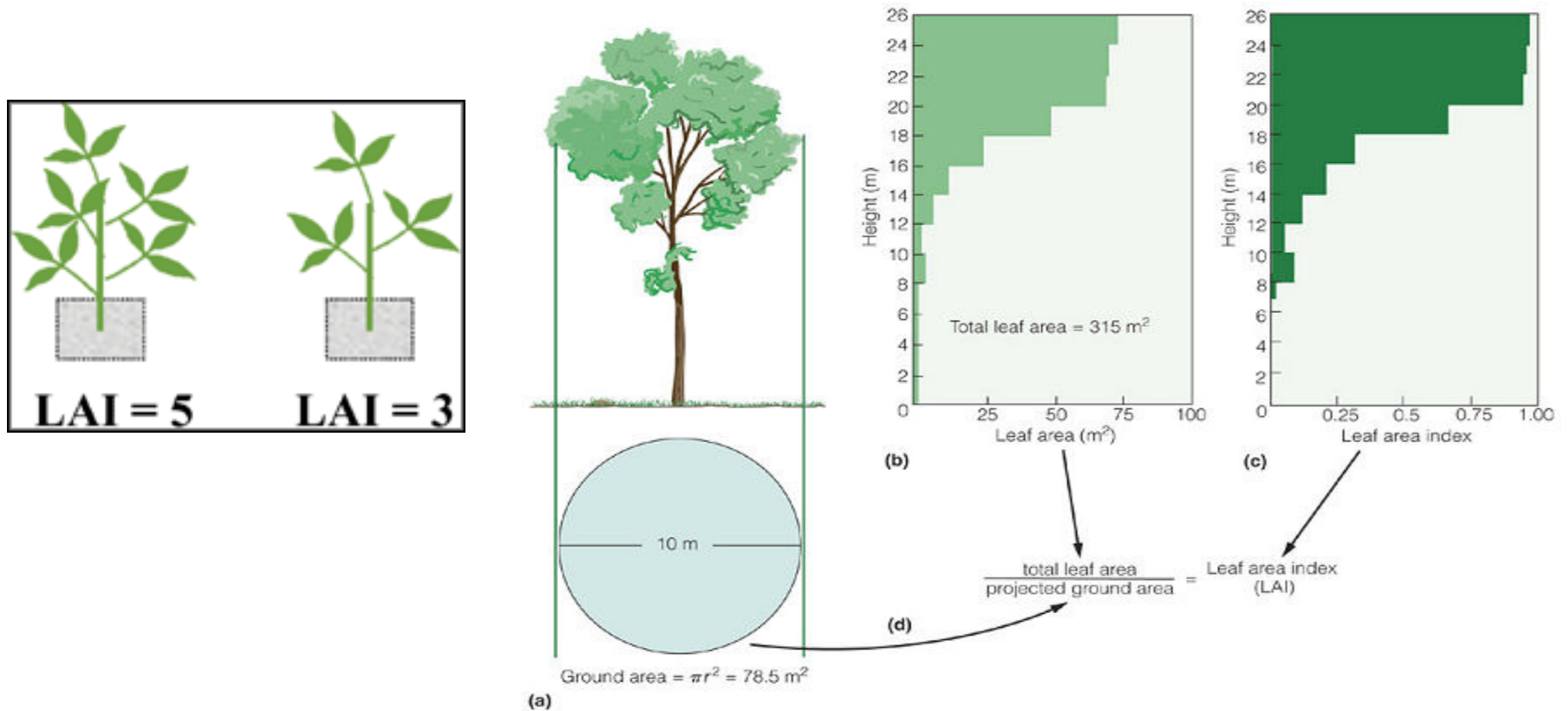
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Beer's Law predicts light transmission through a turbid medium, in terms of the relative light transmission ( $I/I_0$ ), as an exponential function of leaf area index (L) and a proportionality constant (k); k reflects the geometric influence associated with the angle between leaves and the sun:

$$I/I_0 = \exp(-kL)$$

# Remote sensing of vegetation products scaling of biophysical processes : A challenge

**Leaf area index (LAI) = Leaf area per unit ground surface area**  
*= leaf area / ground area, m<sup>2</sup> / m<sup>2</sup>)*

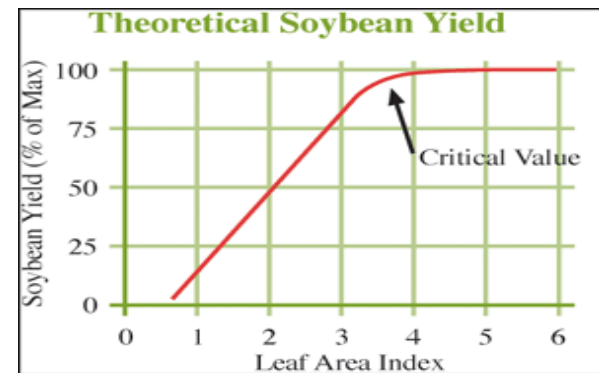
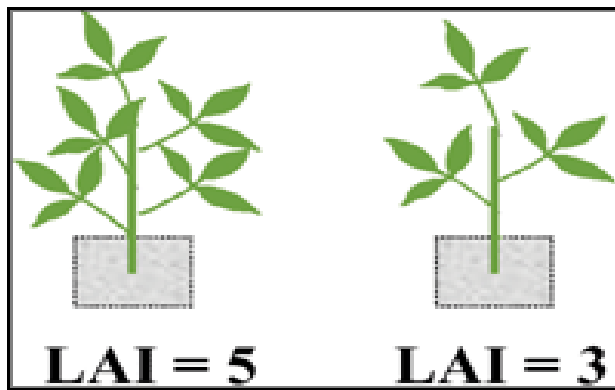


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LAI ranges from zero (bare ground) to over 10 (dense conifer forests)

# Remote sensing of vegetation products scaling of biophysical processes : A challenge

**Leaf area index (LAI) = Leaf area per unit ground surface area**  
*= leaf area / ground area, m<sup>2</sup> / m<sup>2</sup>)*



**LAI**

## How to measure LAI?

---



**pinus trees**  
LAI = 2 - 4)



**temperate deciduous forest**  
LAI = 3 - 5



**tropical rain forest**  
LAI= 6-10





## Productivity Estimation

- NDVI has been applied in innumerable studies for estimation of vegetation biomass, greenness, primary production, dominant species, leaf area index (LAI), fraction of absorbed photosynthetically active radiation (fAPAR)

$$fAPAR = a * NDVI_{toc} + b$$

$$APAR = fAPAR * PAR$$

**GPP can be calculated from remotely sensed NDVI**

$$GPP = \epsilon \sum n(a * NDVI + b) PAR$$

$$NPP = GPP - R_{(autotrophic)}$$

Net ecosystem exchange of CO<sub>2</sub> (g C m<sup>-2</sup> time<sup>-1</sup>) **NEE = GPP - R<sub>total</sub>**

R<sub>total</sub> is the sum of all autotrophic and heterotrophic respiration over some time period (Hunt et al. 2002)

Direct and indirect methods for estimation of LAI, fAPAR, and modeling NPP of different types of terrestrial ecosystem  
Gower et al. (1999)

The TERRA, EOS-AM platform was launched on December 19, 1999 and started to provide global primary production (namely, MOD17) on the 8-day interval with nominal 1-km resolution beginning on Feb 24, 2000 (Zhao et al. 2005).

Turner et al. (2006) tested the performance of NPP and GPP on nine sites varying widely in biome types and land use, and concluded an over estimation at low productivity sites and underestimation at higher productivity site of these parameters while comparing satellite and eddy co-variance technique.