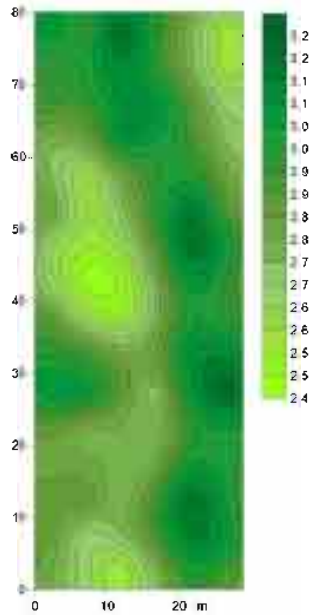
Optical sensors based on plant fluorescence

Zoran G. Cerovic

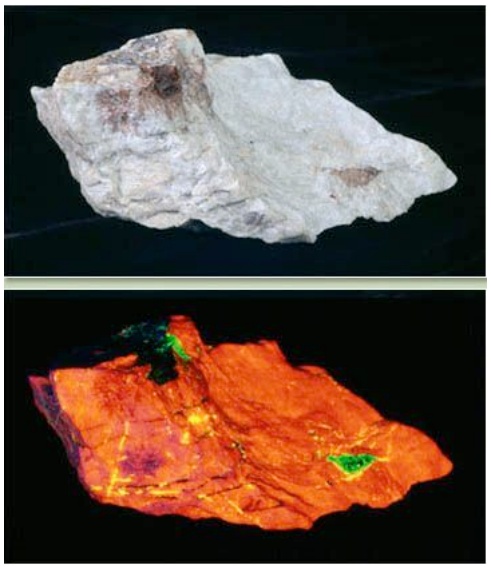
CNRS, Univ. Paris-Sud, Orsay, France

zoran.cerovic@u-psud.fr

"to see the invisible"

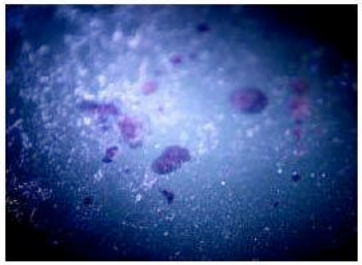


Fluorescence under UV light



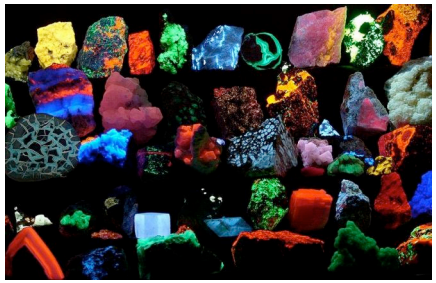
VIS

UV

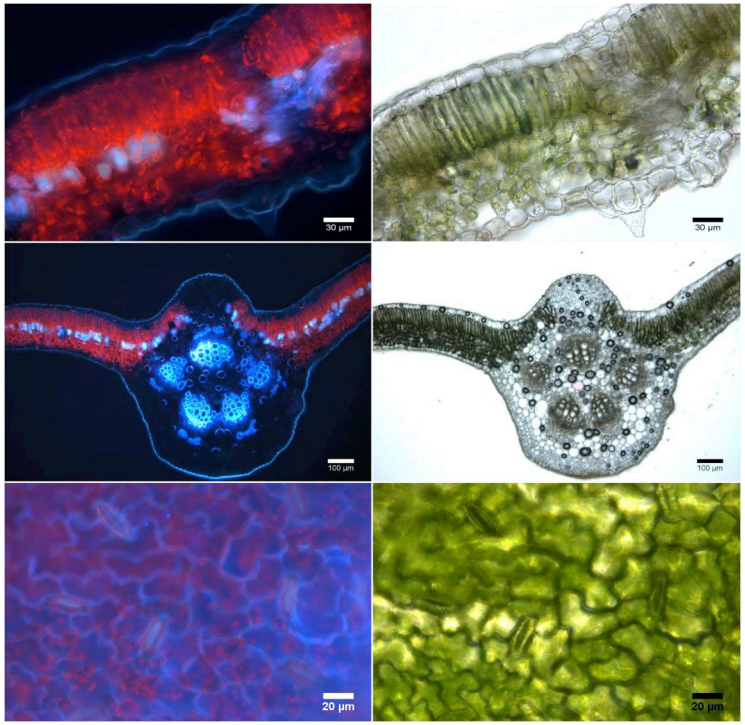


Mineralogy

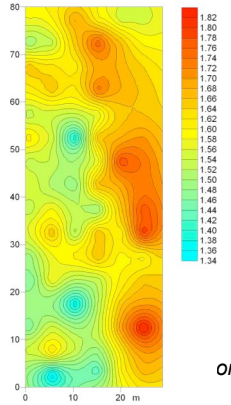
Forensics



UV VIS

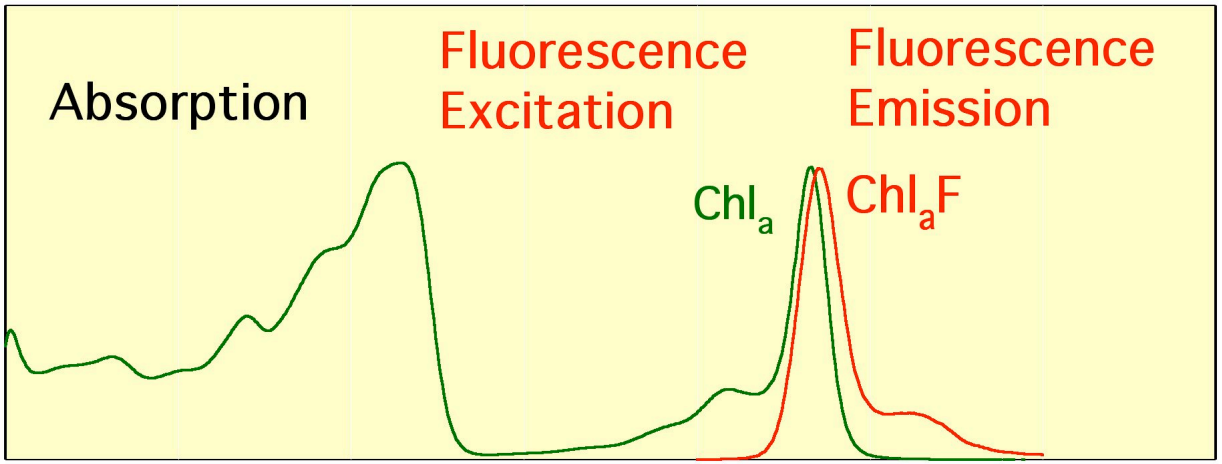


Plants & Agriculture



Chlorophyll fluorescence

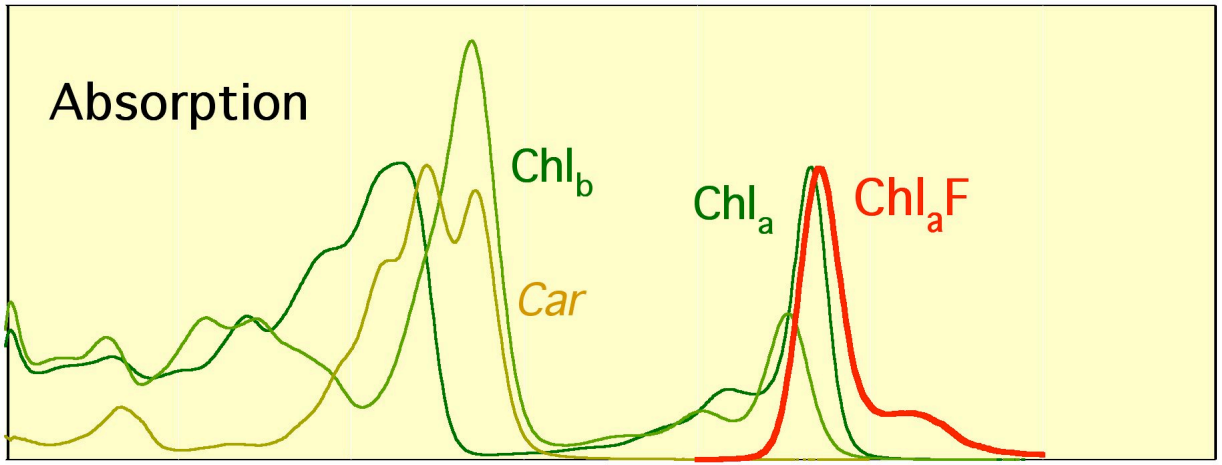
Chlorophyll *a* in solution



Cerovic et al. (1999) Agronomie, ***19:*** *543*

Chlorophyll fluorescence

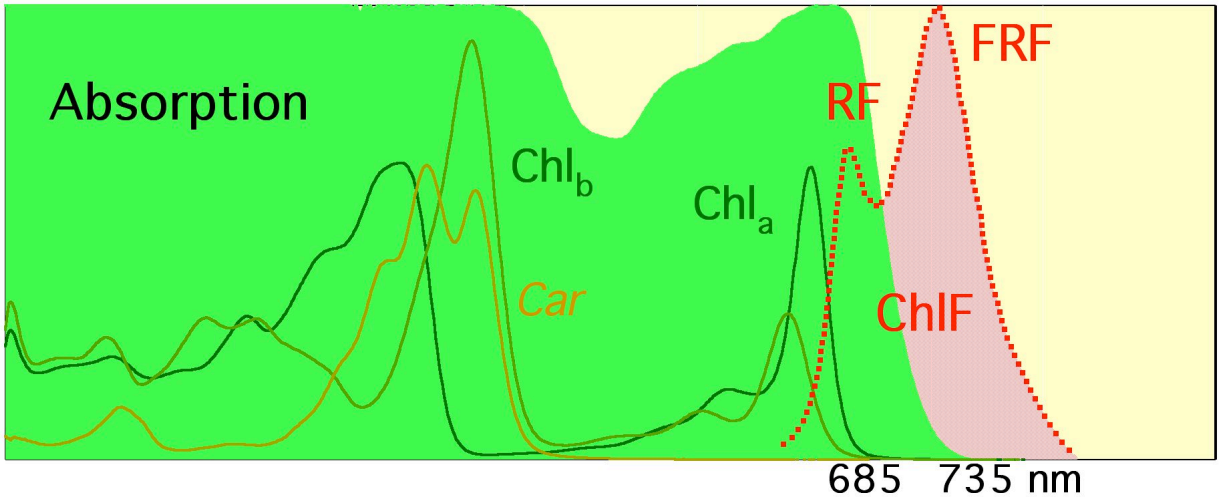
Chlorophyll *a* in solution



Cerovic et al. (1999) Agronomie, ***19:*** *543*

Chlorophyll fluorescence

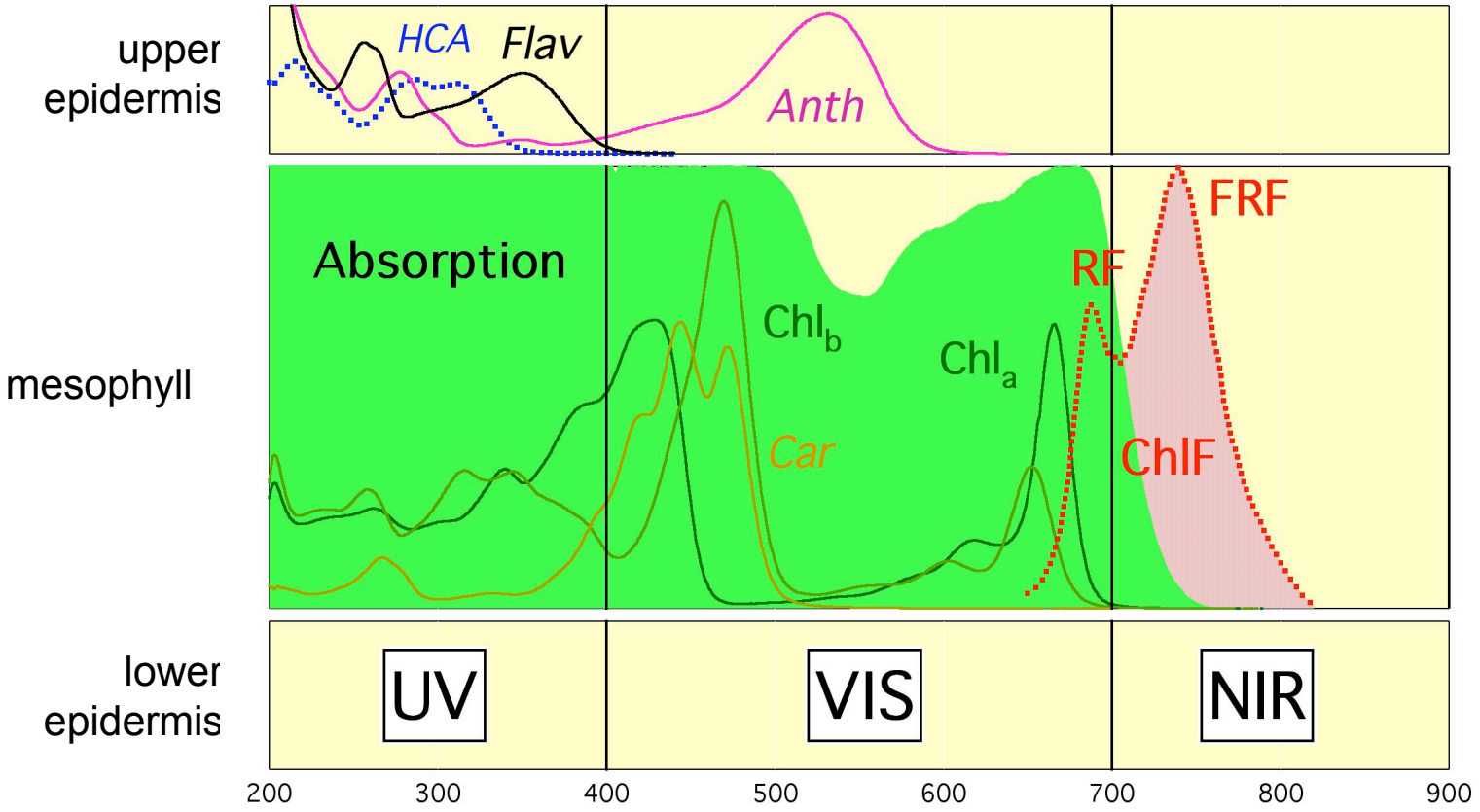
Chlorophyll *a* in solution



UV Chlorophyll in vivo NIR

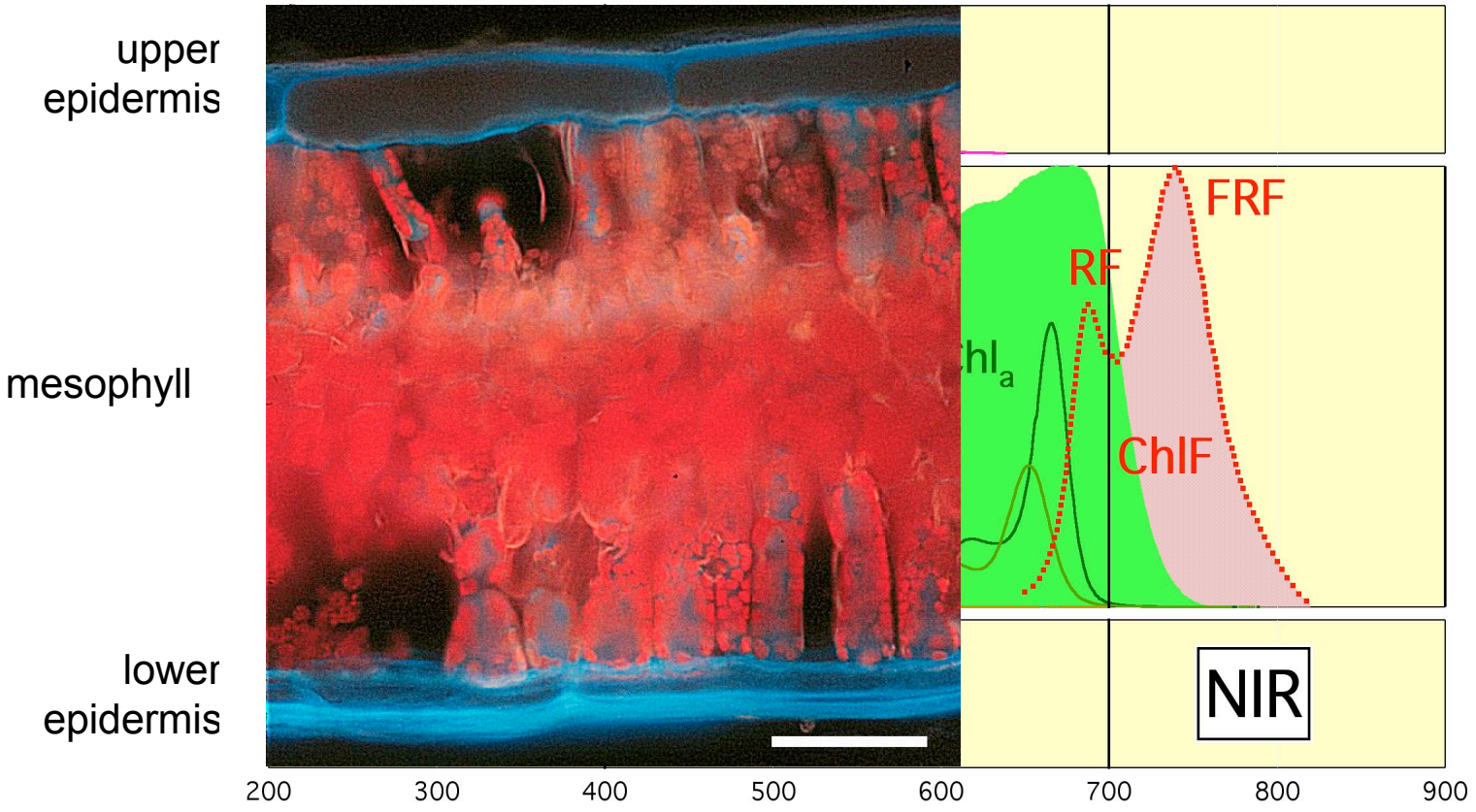
Cerovic et al. (1999) Agronomie, ***19:*** *543*

Chlorophyll fluorescence



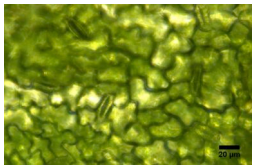
*Cerovic et al. (1999) Agronomie, **19:** 543*

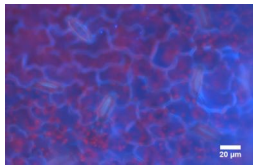
Chlorophyll fluorescence



Cerovic et al. (1999) Agronomie, ***19:*** *543*

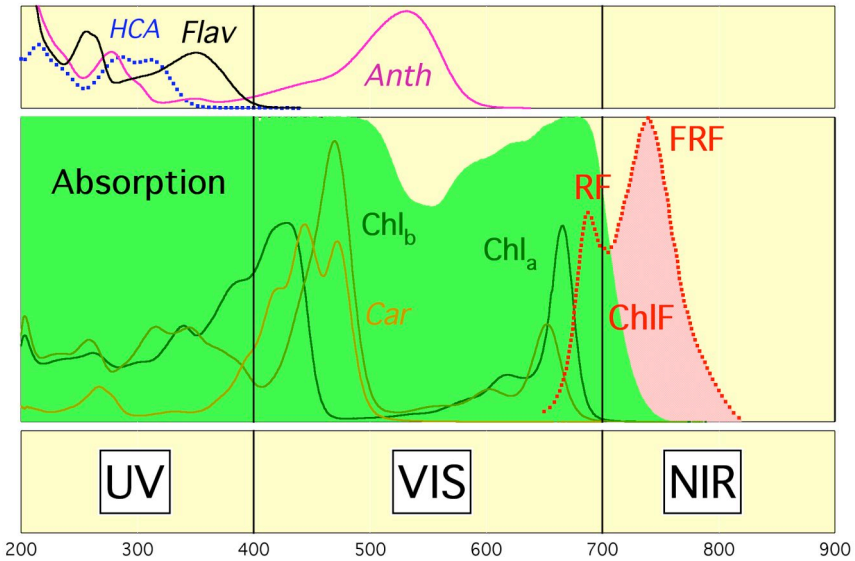
Nomenclature and type of plant fluorescence

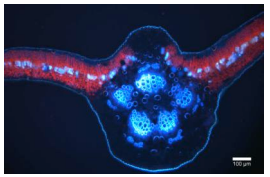




Chlorophyll Fluorescence = ChlF
Red Fluorescence = FRF (685)
Far-Red Fluorescence = FRF (735)

FRF_G
emission_excitation





Blue-Green Fluorescence = BGF

ChlF_VIS | ChlF_UV | BGF_UV

FRF_VIS

"variable" fluorescence

Stress

PAM sensors
Imaging

FRF/RF

emission ratio

Chl

MiniVeg
Laser-N-Detector
Multiplex

FRF_R/FRF_UV

excitation ratio

Flav

UV-A PAM
Dualex
Multiplex

BF_UV/FRF_UV

"blue" fluorescence

HCA

Multiplex
Imaging

Fungi

Proximal sensing of vegetation

ACTIVE

artificial light source

Reflectance or **Fluorescence**

Type of functioning

PASSIVE

the Sun as light source

Reflectance or Fluorescence

Working distance

contact — small — medium — large

leaf clips — Multiplex — LIDAR — aircraft CASI — MERIS satellite

(Fibre optics)

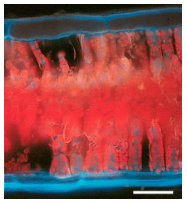
Spectral Domain

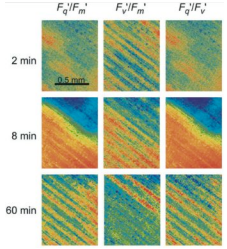
UV — visible — NIR — SWIR — thermal — radio

Phen Car Anth Chl LMA Prot H_2O

Imaging

Parallel imaging,
CDD cameras,
microscopy, macroscopy
(only as illustration)

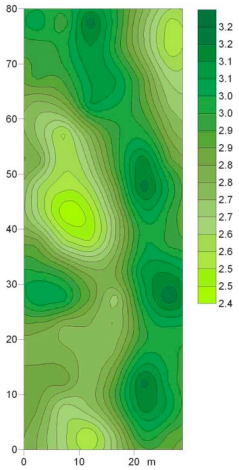




Not adapted
for fluorescence
from a distance

Push-broom imaging (scanning),
DAD sensors,
spectro-microscopy, remote sensing

Raster imaging (scanning),
Single point measurement,
microscopy, macroscopy
Proximal sensing



"Variable" ChlF

Stress

excitation	emission
Single source	Single detector
Visible (G, O, R) laser, LED	RF FRF ChlF

Variation of ***fluorescence yield***
- with time or light intensity
- Photosynthesis related

Limitation:
need for pre-darkening

STRESS
photoinhibition
freezing
chilling
heat
nutrition
salinity
drought

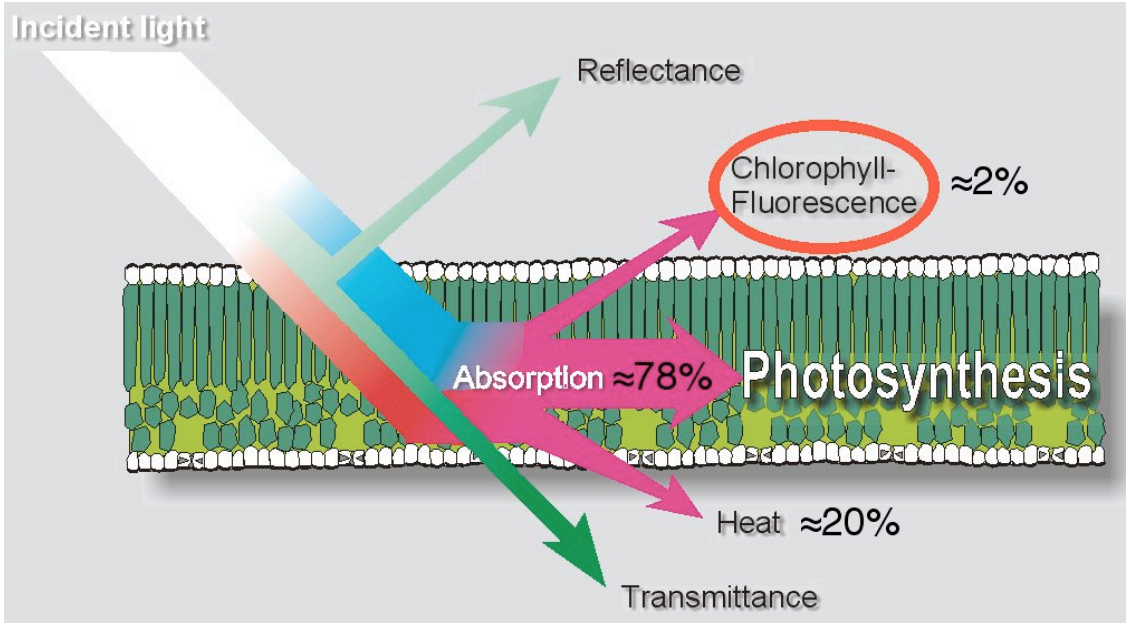
Chlorophyll a Fluorescence. A Signature of Photosynthesis. (2004)
Papageorgiou G.C. & Govindjee (Eds.) Springer, Dordrecht

Leaf-light interactions

Stress

Reflected & back-diffused
(Reflectance = Ir/Io)

visible (PAR = 48 % of Solar irradiance)



biomass = 3 %

Heat = 97 %

Transmitted & forward diffused
(Transmittance = It/Io)

Absorbed
(absorptance = 1 - R - T)
(absorbance = - log T)

Portable field fluorometers

"Variable" ChlF

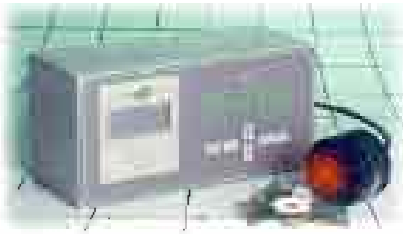
Stress

PAM-2000
Portable Chlorophyll
Fluorometer

for investigating
photosynthesis in the field,
greenhouse and laboratory

MINI-PAM
Photosynthesis Yield Analyzer

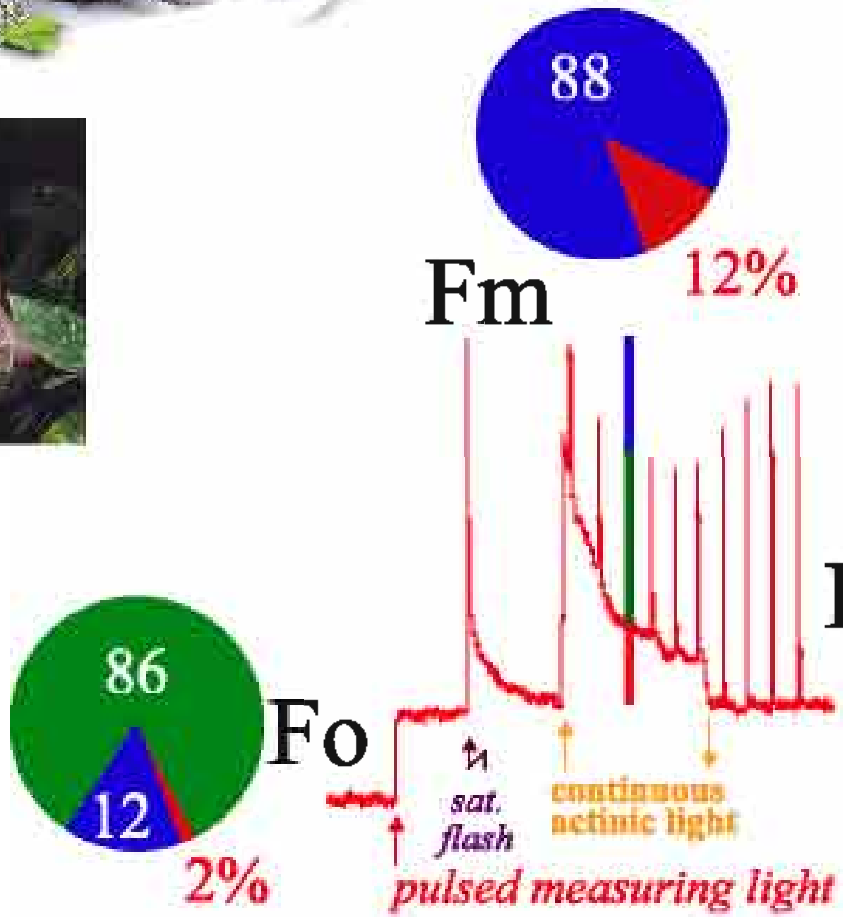
the ultimate answer to portability
and ease of operation



PEA, Hansatech, UK

EARS-PPM,
Netherlands

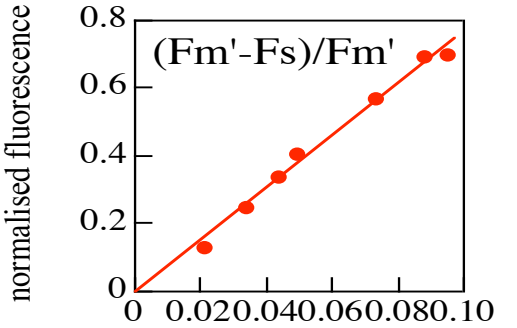
FluorPen FP 100, PSI



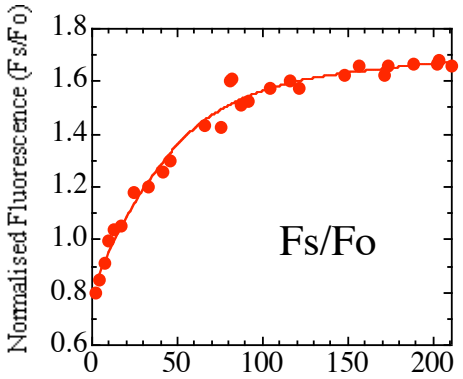
LIDARs & micro-LIDARs

Stress

Laser-PAM LURE

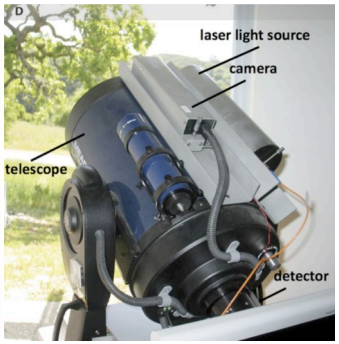


*Genty et al. (1989) BBA, **990:** 87*

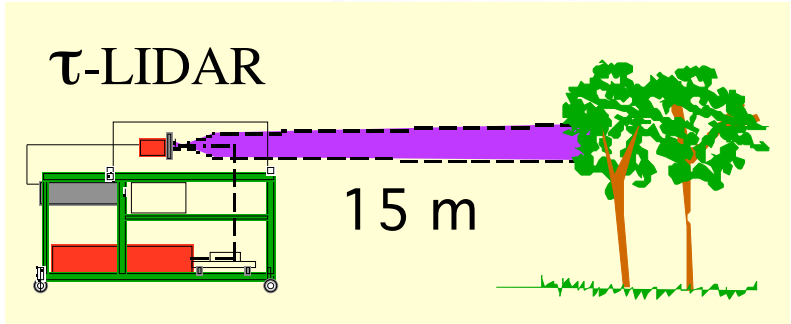


*Flexas et al. (2000) RSE, **73:** 283*

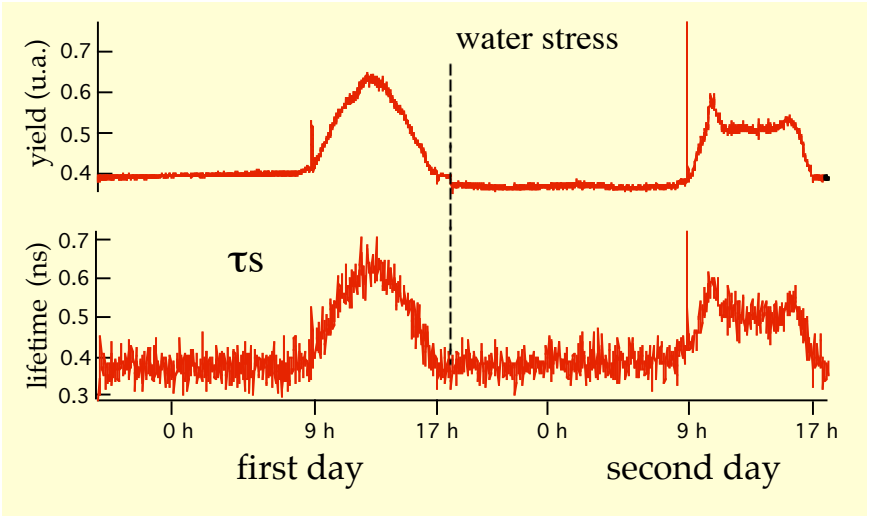
LEAF



LIFT



Lifetime LIDAR LURE



*Cerovic et al. (1996) RSE, **58:** 311*

ChlF emission ratio

Chl

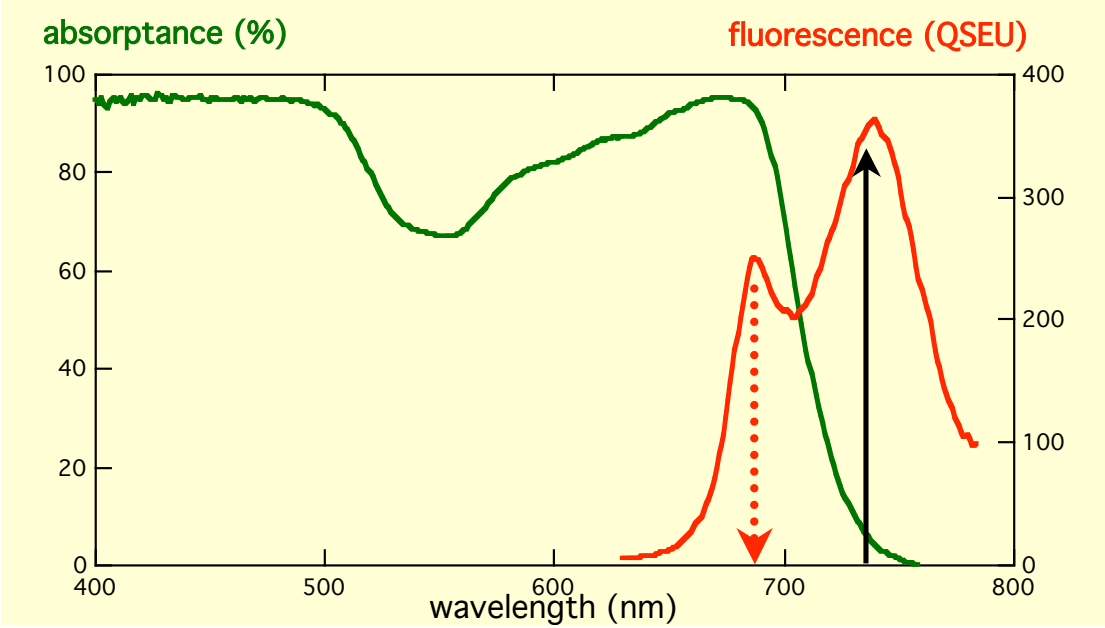
excitation	emission
Single source	**Dual** detector
Visible (G, O, R) laser, LED	RF/FRF

Chlorophyll quantification
• nitrogen nutrition related
• (cover fraction)

Limitation:
influence of "variable" ChlF

Chlorophyll estimation from fluorescence reabsorption

Chl



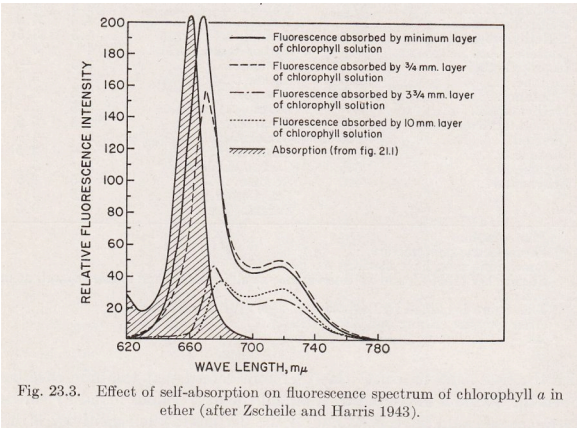


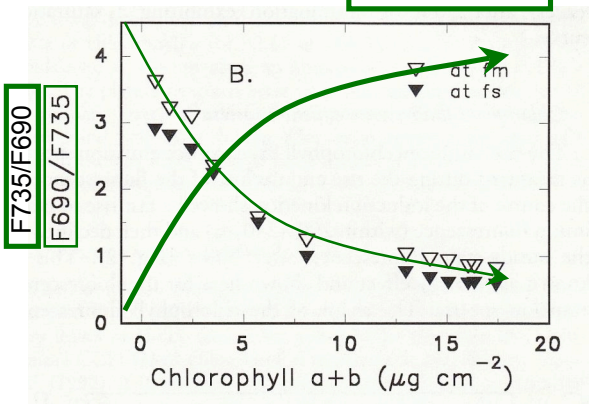
Fig. 23.3. Effect of self-absorption on fluorescence spectrum of chlorophyll *a* in ether (after Zscheile and Harris 1943).

Rabinowitch (1951) Book

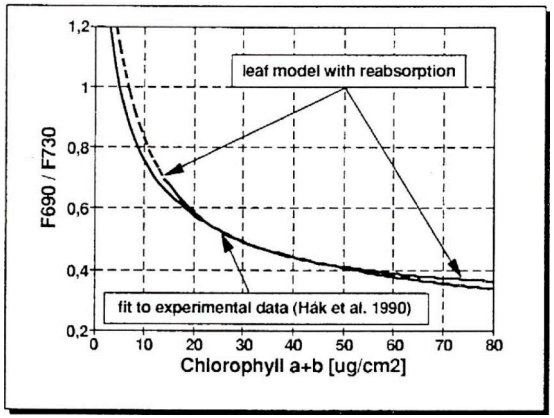
685 735

RF FRF

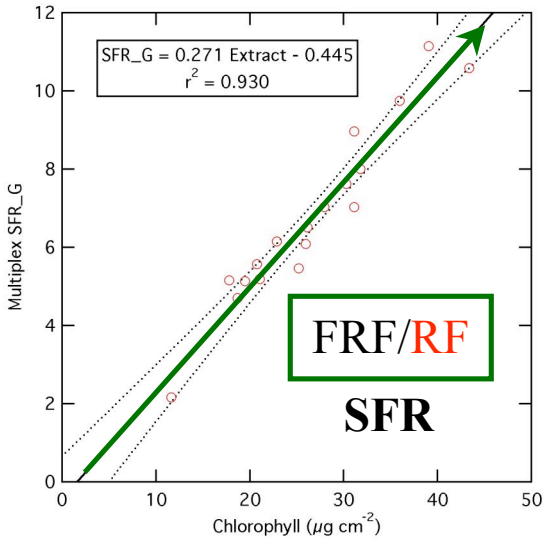
FRF/RF



*Babani et al. (1996) JPP, **148:** 471*



*Dahn et al. (1992) EARSeL Adv. Remote Sens., **1:** 12*



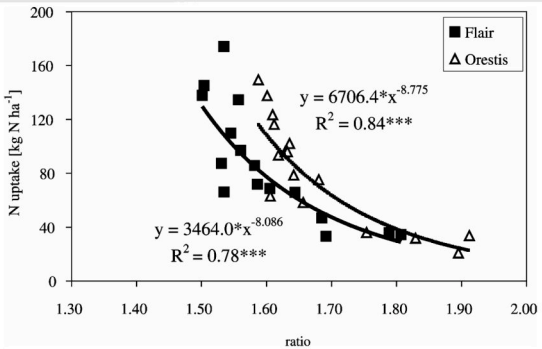
Cerovic et al. (2008) unpublished

Tractor mounted sensors

Chl

Laser-N-Detector (Planto)

maps ?



Schächtl et al. (2005) Prec. Ag. 6: 143

MiniVeg (Fritzmeier)

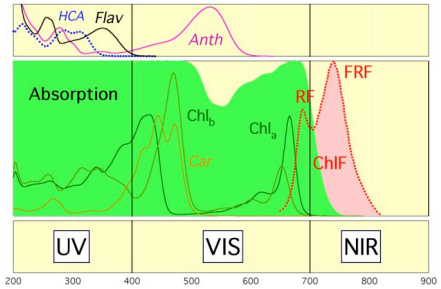
ChlF excitation ratio

Flav

excitation

Dual source

UV & Visible
laser, LED



emission

Single detector

FRF
ChlF

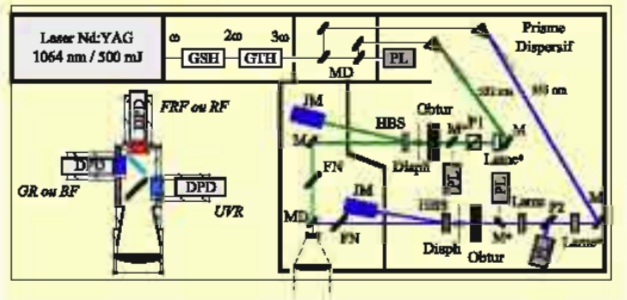
UV-absorption by the screening effect
• Phenolic compounds quantification
• (Green absorption)

Limitation:
UV sources

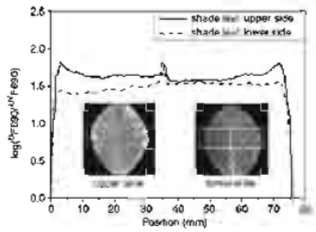
ChlF screening method

Flav

Instruments

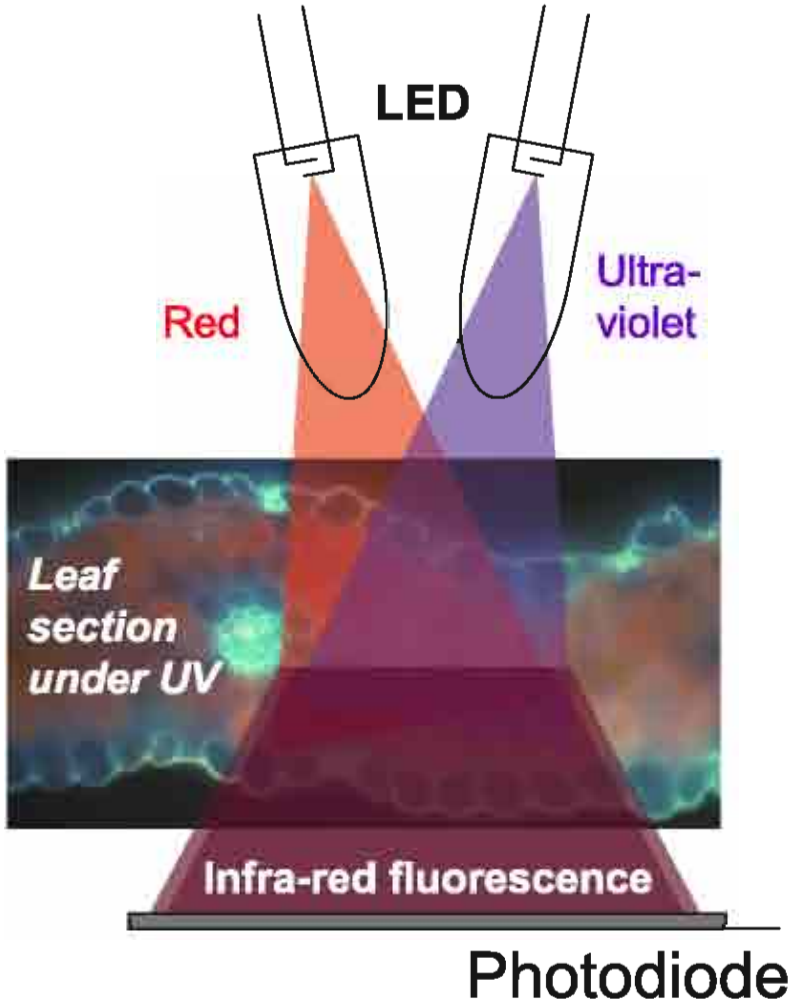


Xe-PAM Bilger et al. (1997), Cerovic et al. (1993)
UV-A-PAM Bilger et al. (2001), Kolb et al. (2005)
DE-FLIDAR Ounis et al. (2001)
Super-ACO Cerovic et al. (2002)
Dualex Goulas et al. (2004)
CCD Camera Lenk & Buschmann (2006)
Multiplex Cerovic et al. (2007, 2009)

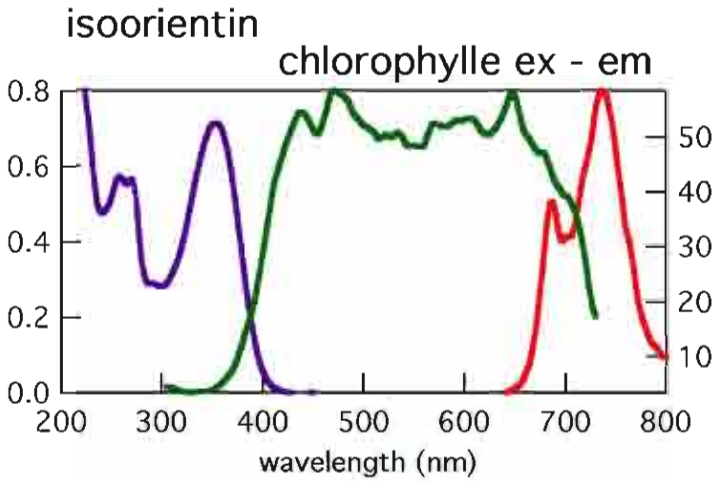


Dualex FLAV - based on ChlF screening method

Flav



*Goulas et al. (2004) Applied Optics **43**, 4488-4496*

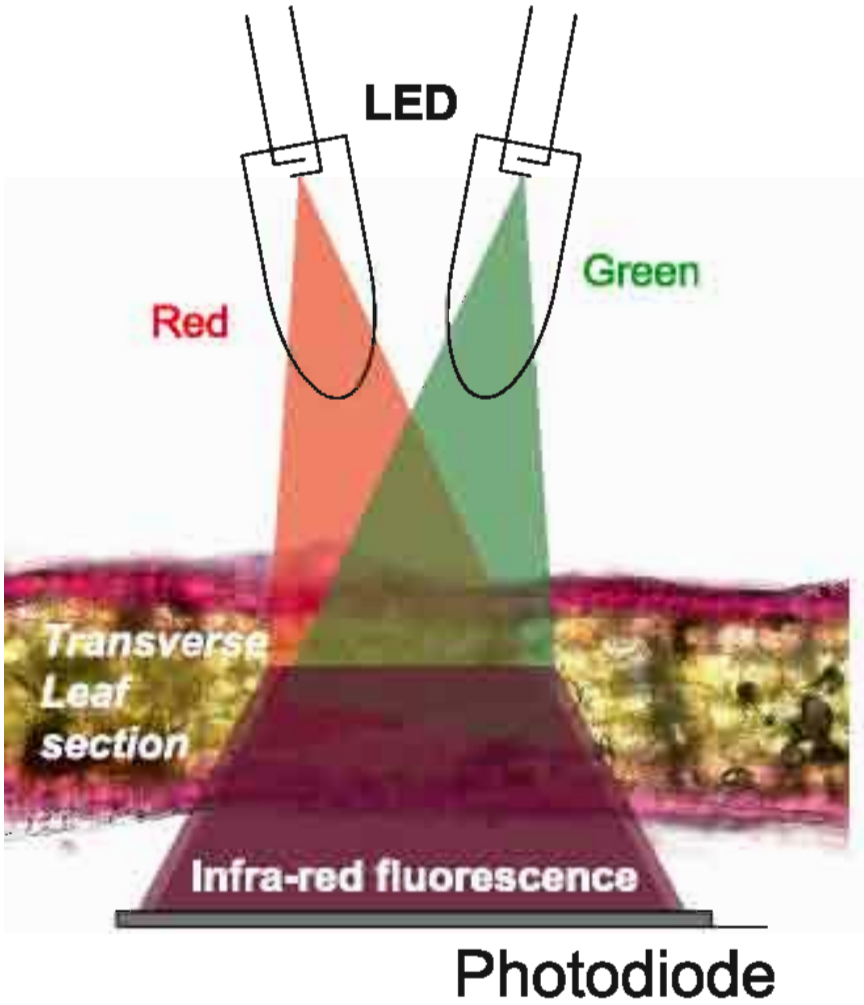


Flavonoids are present in the epidermis. They absorb UV radiation and screen the mesophyll.

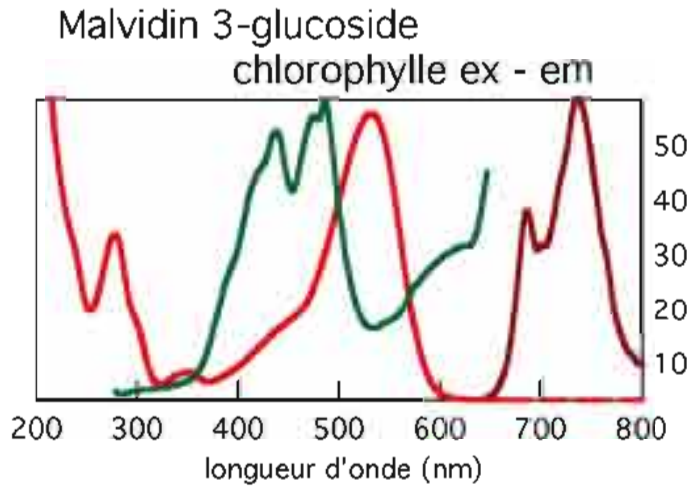
Chlorophyll from the mesophyll emits near-IR fluorescence measurable on both sides of the leaf.

The Dualex ANTH

Flav



acc. Goulas et al. (2003) Patent WO03029791



When anthocyanins are present in the epidermis they absorb green light and screen the mesophyll.

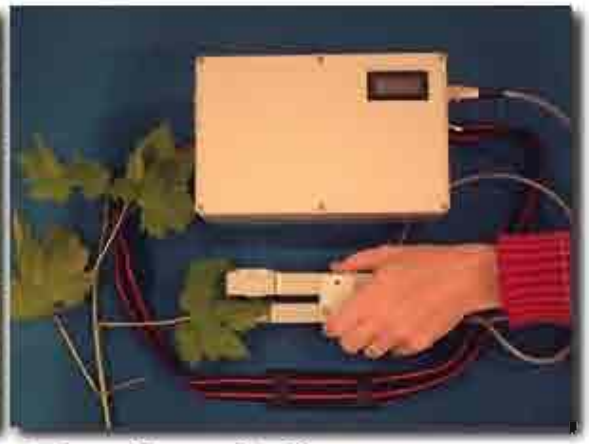
Chlorophyll from the mesophyll emits near-IR fluorescence measurable on both sides of the leaf.

Dualex evolution

Flav

Dualex 1 - Field (2002)

Dualex 2 (2003)



Dualex 3.2 (2004)

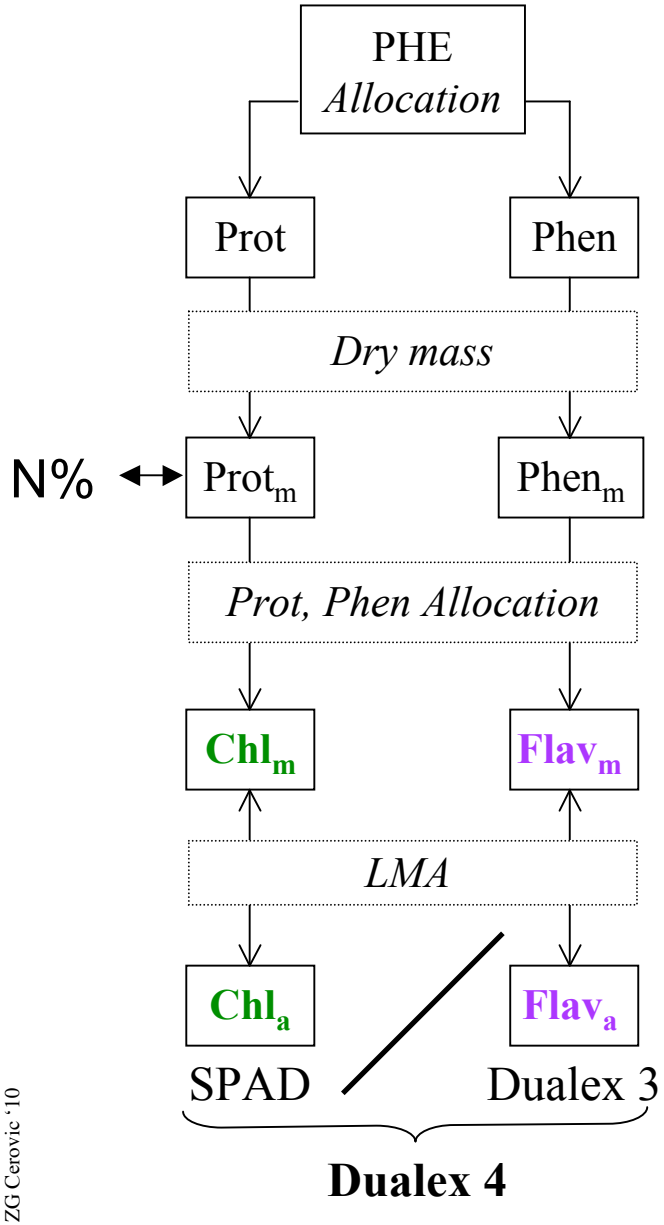
Dualex 1 - Lab (2001)

Dualex 4 (2009)

Dualex 3.3 (2005)

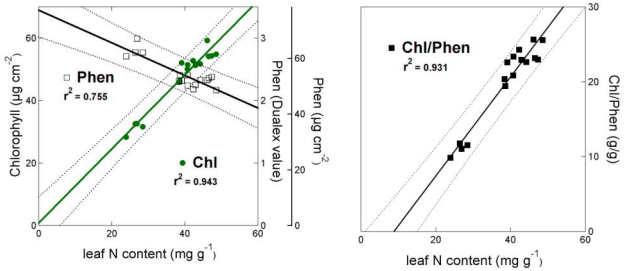
The Chl/Phen ratio: Nitrogen Balance Index (NBI)

Flav

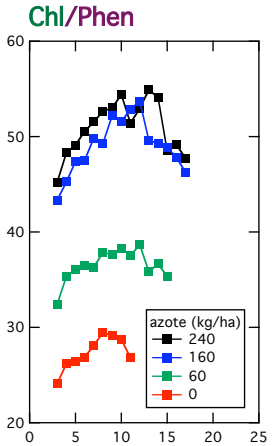


Three Beneficial effects

1. The **opposite dependence** on nitrogen increases the dynamic range



2. The **parallel dependence** on leaf age decreases leaf position influence



3. The ratio of **two surface-based measurements** avoids the influence of LMA

Blue-green fluorescence

HCA

excitation	emission
Single source	**Dual** detector
UV laser, LED	B/G B/FRF

Alternative source of information
- Leaf phenology
- Presence of pathogen

Limitation:
multiple potential fluorophores
temperature variation

Autofluorescence of wheat leaves under UV-excitation

HCA

Variable ChlF
Photosynthesis

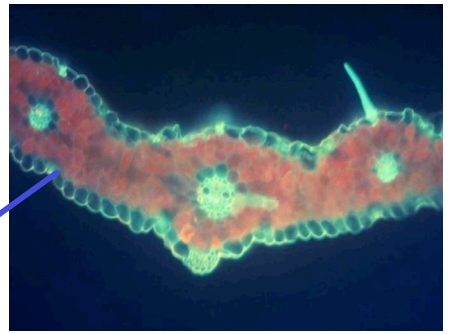
Stress

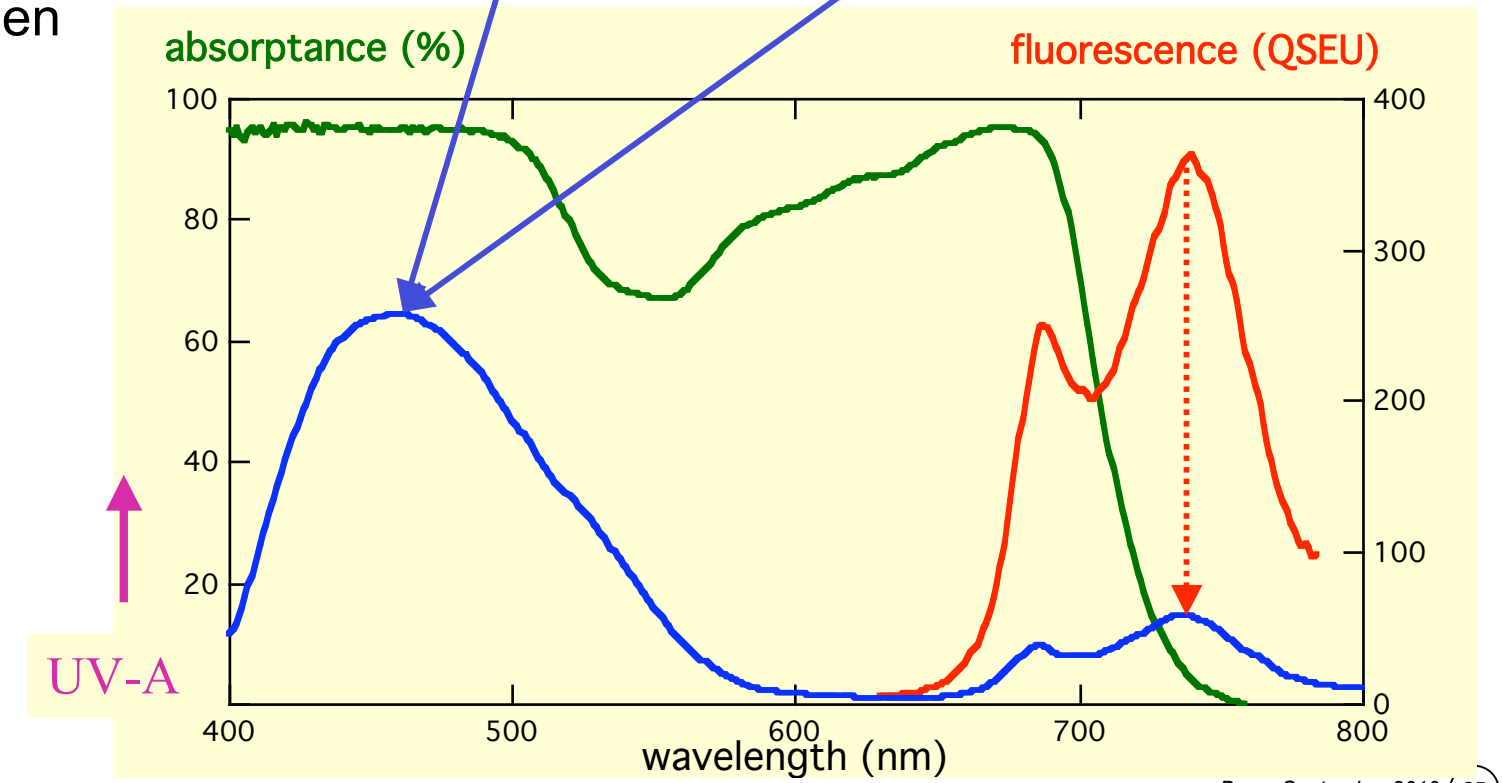
RF/FRF ratio
Chl content

Chl

ChlF UV-screen
Phenolics

BGF
Structure



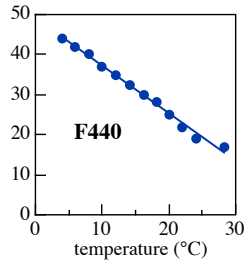


Plant and pathogen fluorescence

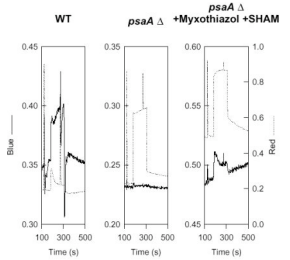
HCA Fungi

- Temperature
- Redox state [NAD(P)H]
- Water stress
- Leaf development
- Nutrition

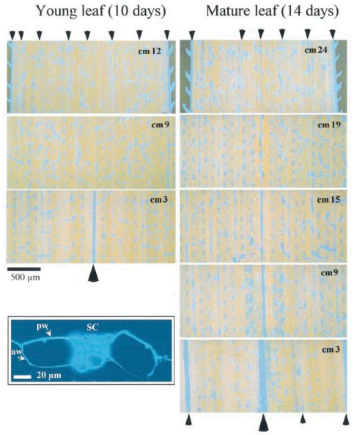
- Pathogen on leaf
- Leaf response
- Field infection



I. Goulas (1992) thesis

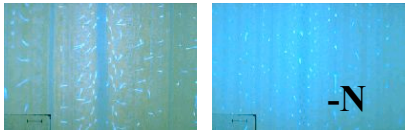


Cournac et al. (2002)
Plant Physiol. ***129****, 1921*

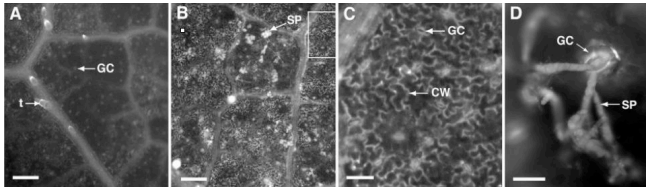


Meyer et al. (2003)
J. Exp Bot ***54****, 757*

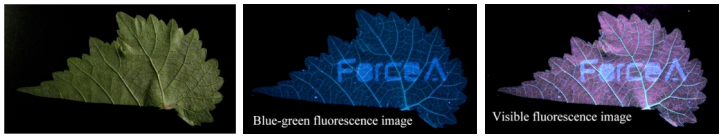
cf. Chappelle et al. (1984)
Appl. Optics ***23****, 134*



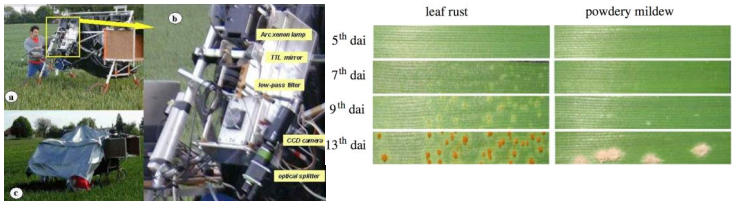
Z.G. Cerovic (2005)
unpublished



Poutaraud et al. (2007)
J. Agric. Food Chem. ***55****, 4917*



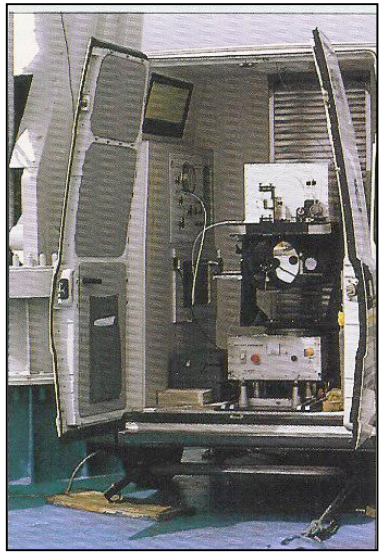
G. Latouche (2010)
unpublished



Kuckenberg et al. (2009)
Prec. Ag. ***10****, 34*

Bravo et al. (2004)
Ag. Eng. Intl. FP 04008

UV-LIDARs

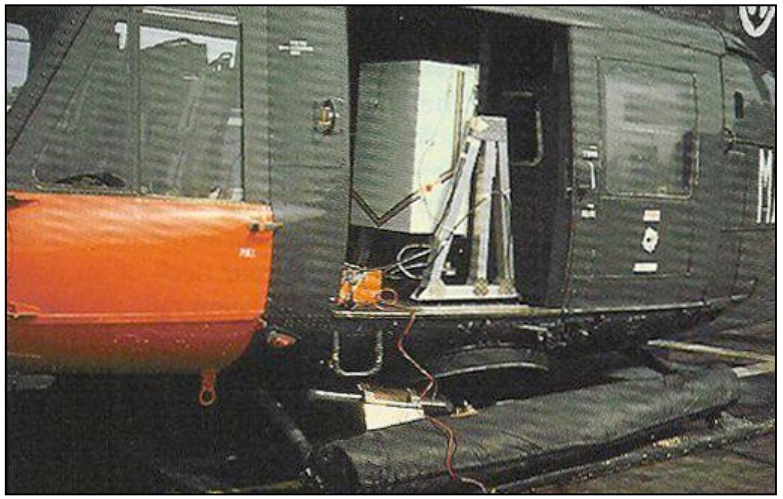


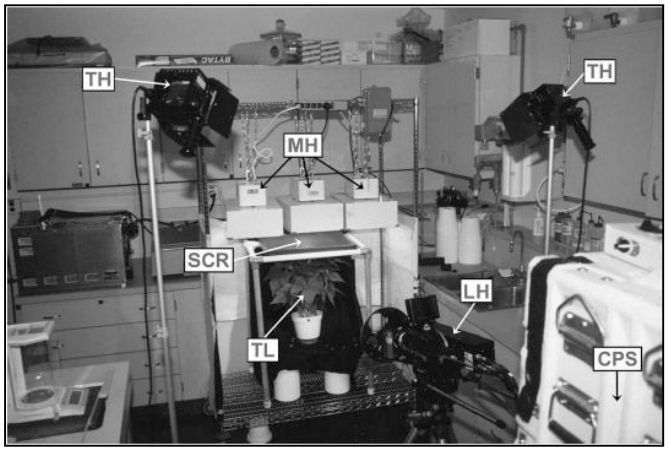
XeCl Excimer lasers
308 nm

FLIDAR-3
IROE-CNR
Firenze

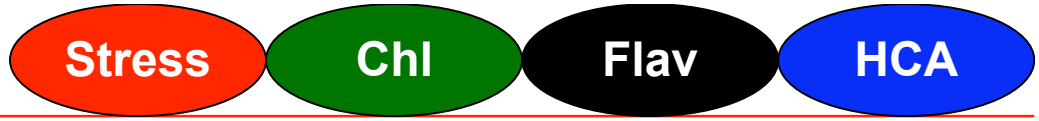
Optimare + Oldenburg University

Frequency tripled YAG lasers 355 nm





Excitation Emission Matrix



excitation

Multiple source

Lamp +
Monochromator
LED

emission

Multiple detection

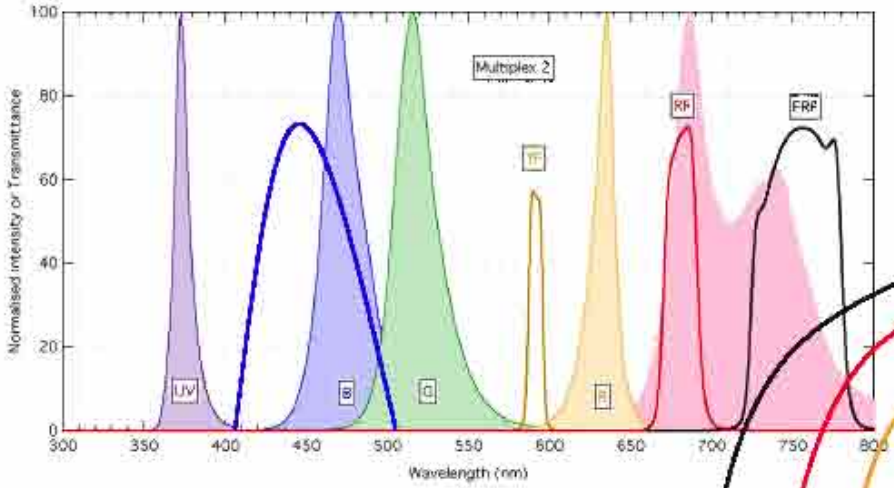
Monochromator
+ *detector* *(PM, PD)*
(Spectrograph)
Multi-detectors

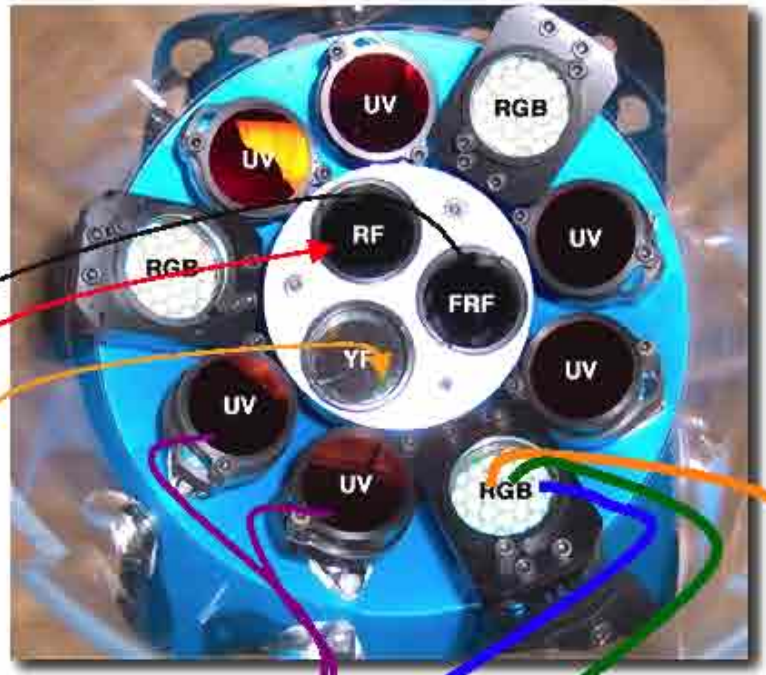
Complete information
• Quantification
• Statistics

Limitation:
often laboratory set-ups
slit entrance

The Multiplex sensor

Stress Chl Flav HCA

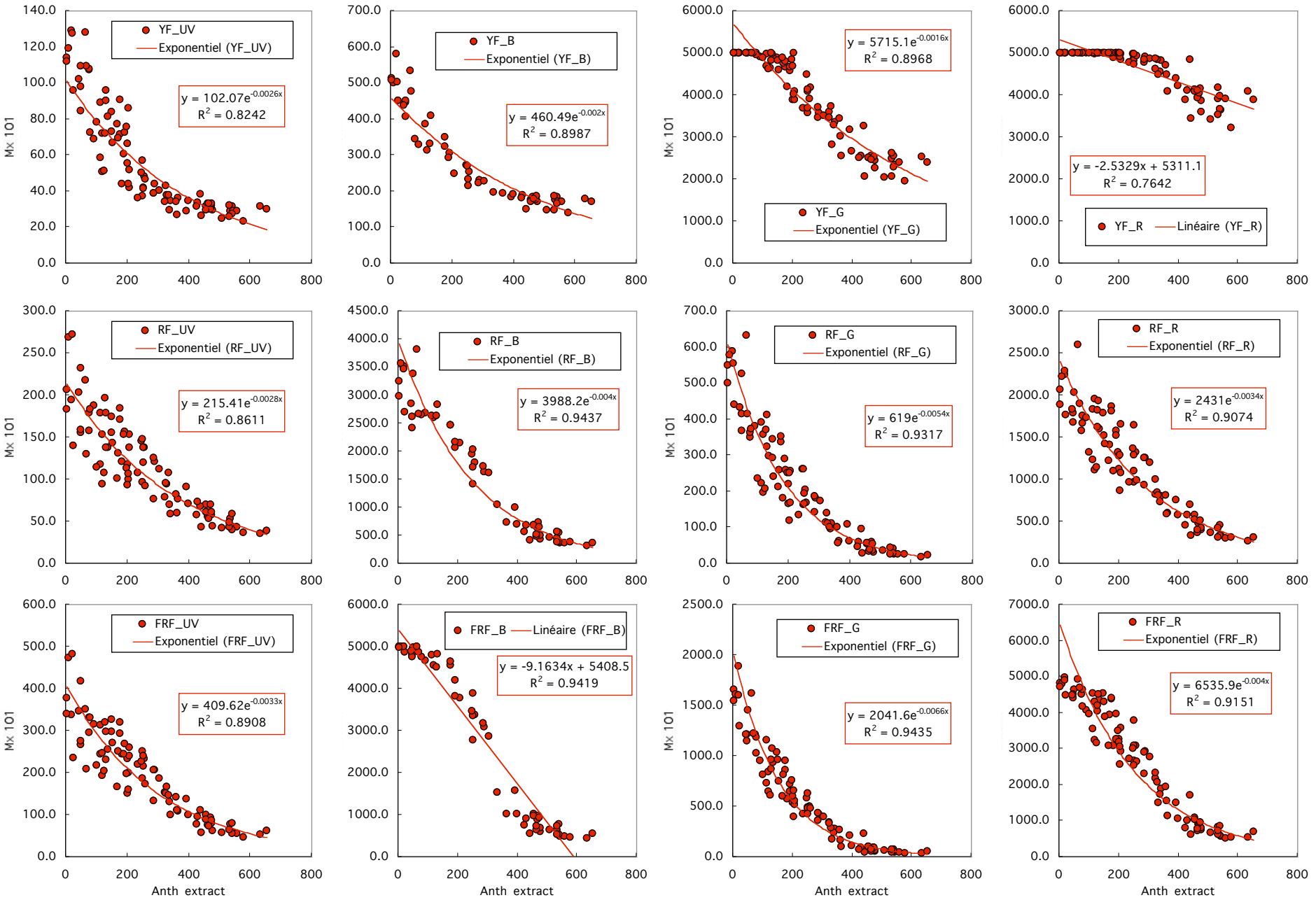




Emission (nm)	Excitation			
	UV	Blue (B)	Green (G)	Red-Orange (R)
YF (590)	YF_UV	YF_B = *R*	YF_G = *R*	YF_R = *R*
RF (685)	RF_UV	RF_B	RF_G	RF_R
FRF (735)	FRF_UV	FRF_B	FRF_G	FRF_R

20 µs flashes
4 excitations
3 emission channels
Repeated 500 times
6000 measurements
0.5 s per sample
In situ
Under daylight

Multiplex signals (grapes maturation)

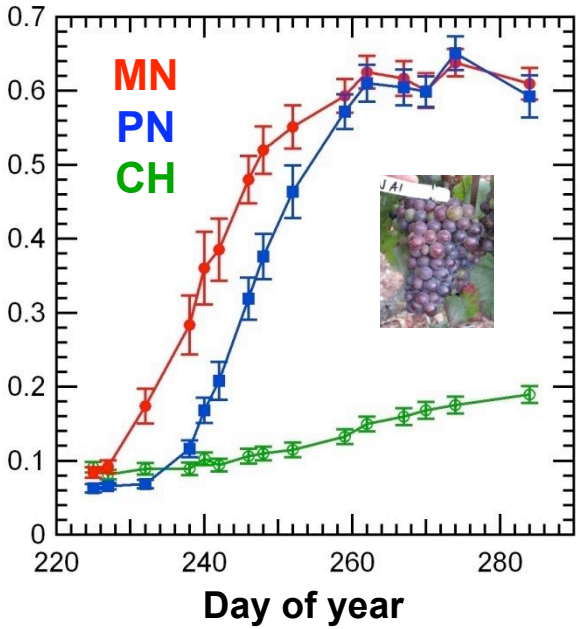


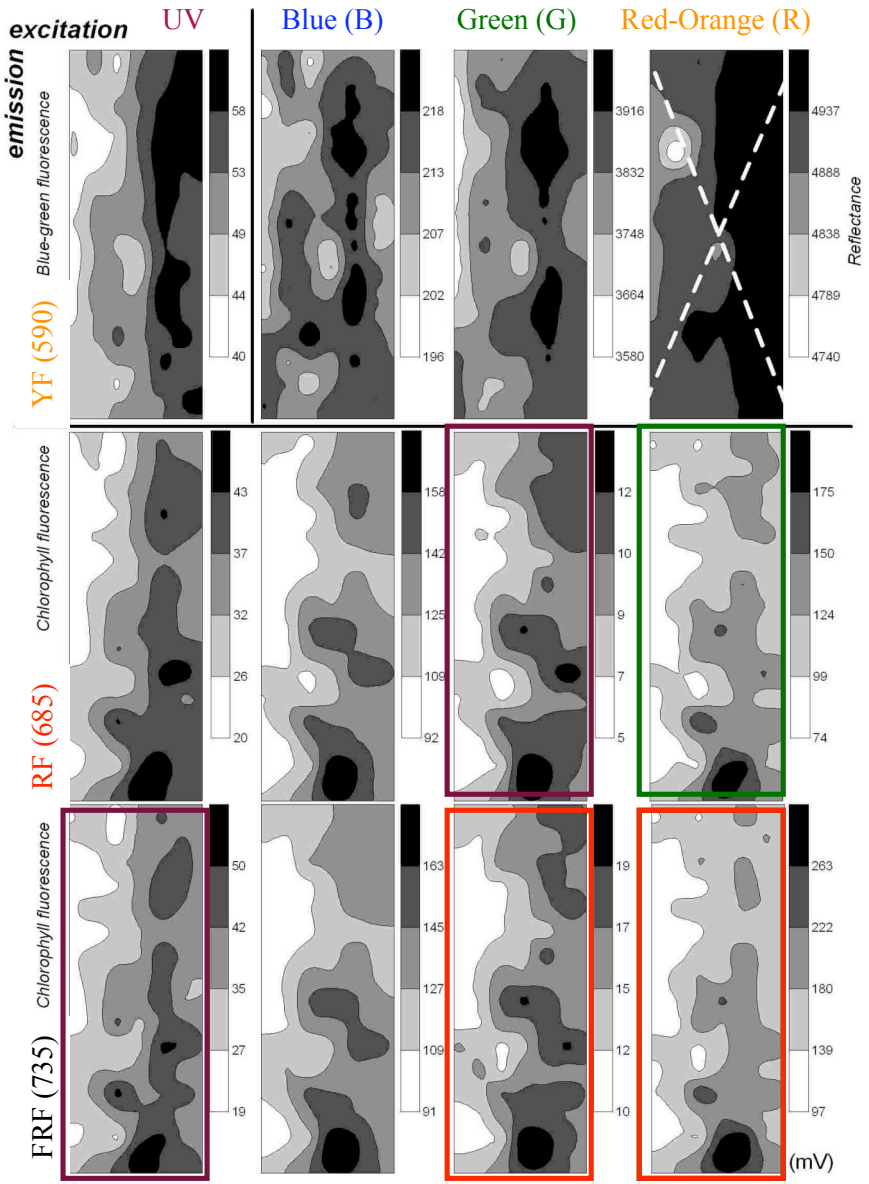
The Multiplex signal matrix: time & space

Grape maturation kinetics

40 marked clusters

ANTH_RG (Mx units)





Grape at harvest

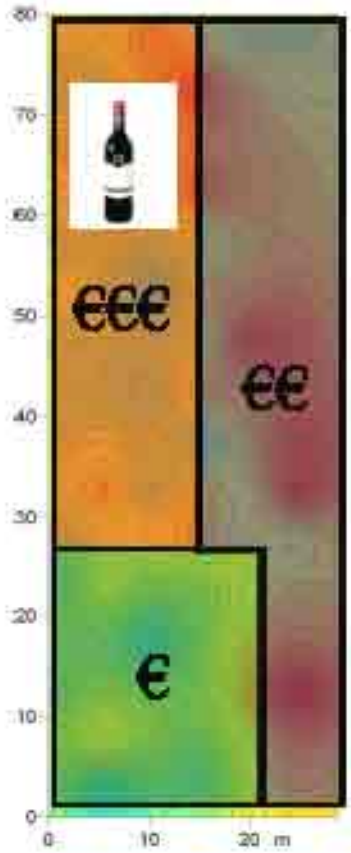
Mapping / Zoning / Block selection

Anth Chl

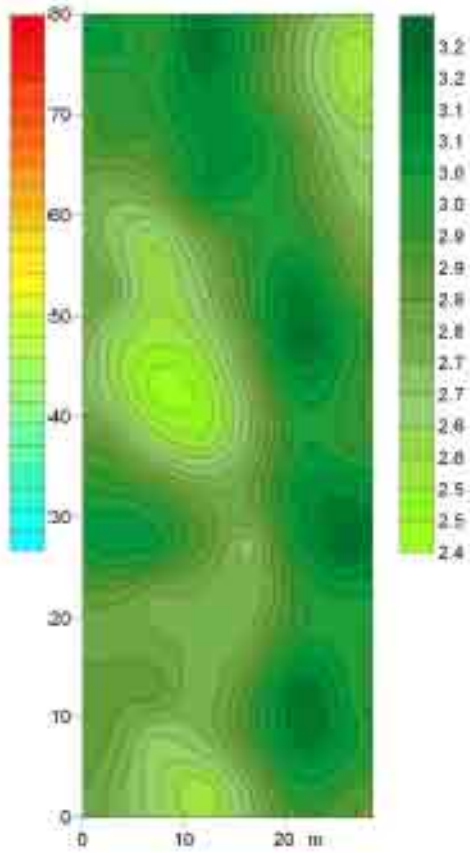
Mounted Multiplex

Vine mapping
(N status, Chl)
Grape quality zoning

ANTH Map
Couhins 2007



CHL Map
Couhins 2008



Precision agriculture: Multiplex NBI mapping

wheat

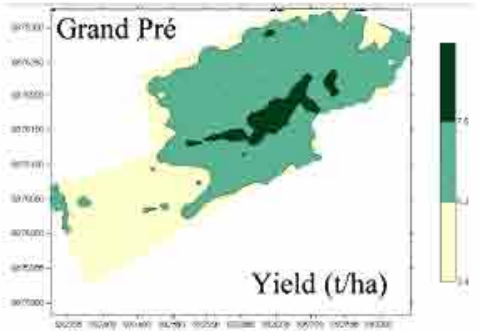
Martinon et al. (2010)
10th ICPA, Denver

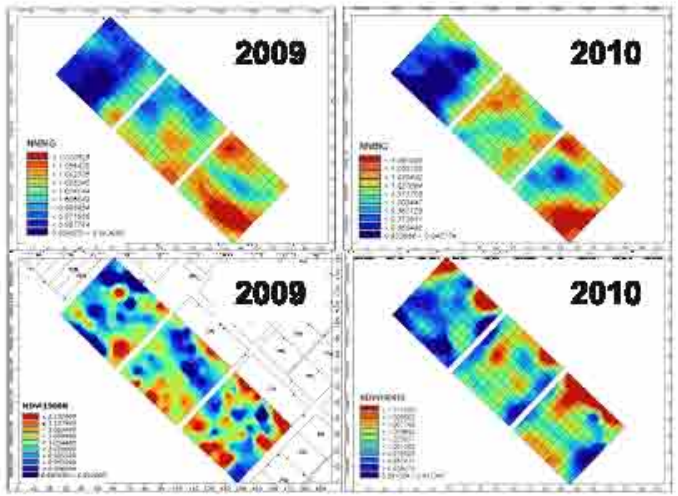
grapevine

Debuisson et al. (2010)
10th ICPA, Denver

Lejeune et al. (2010)
10th ICPA, Denver

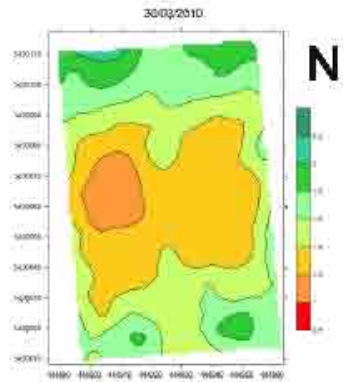
turf

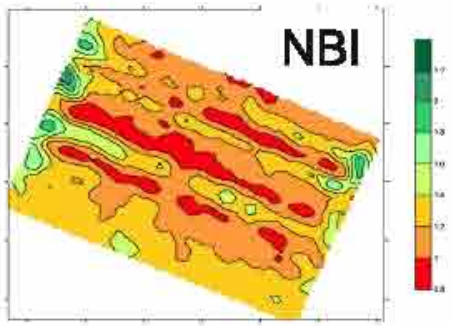




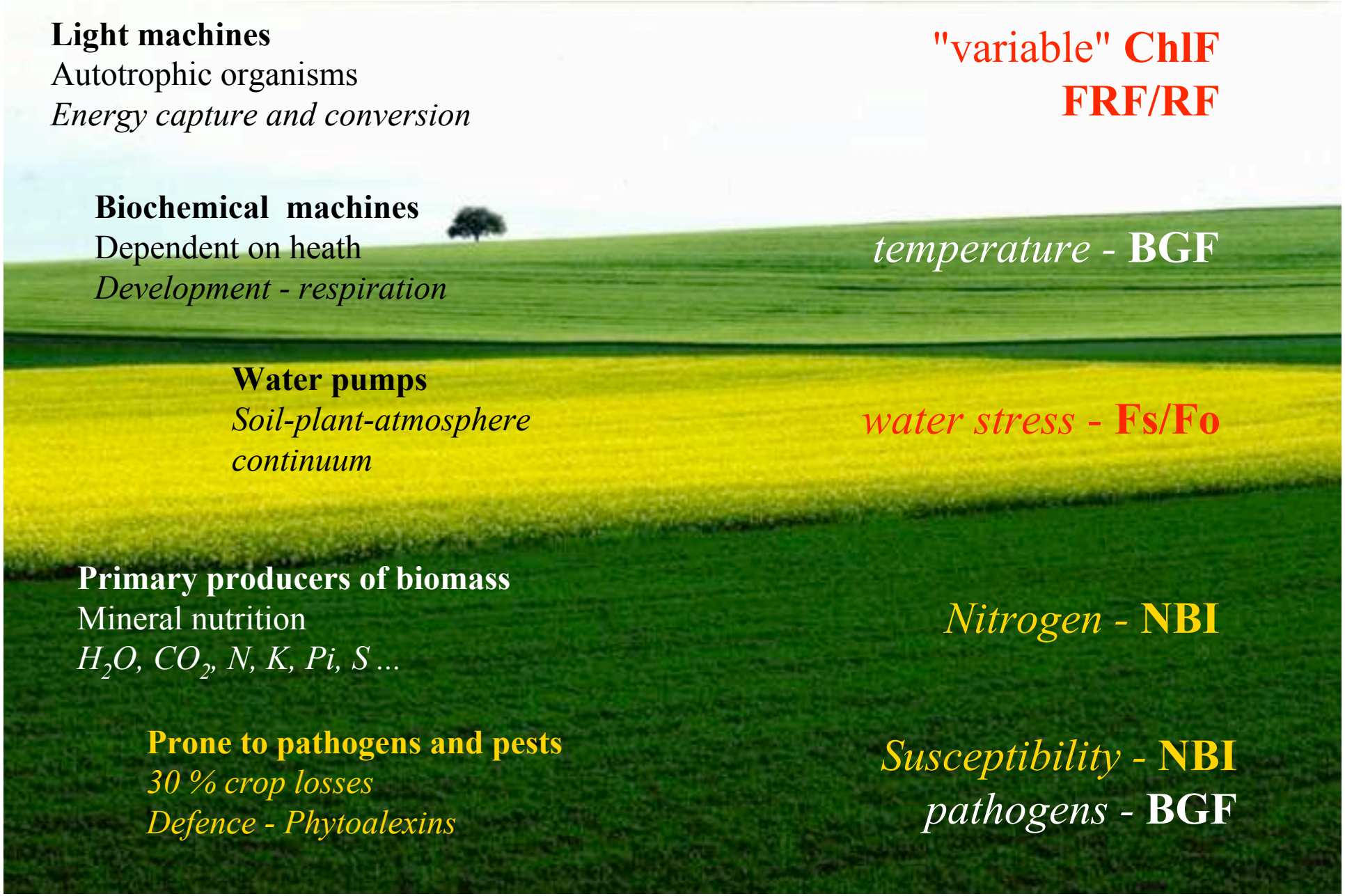
NBI $r^2 = 0.75$

NDVI $r^2 = 0.11$





Plant descriptors and fluorescence indices for agronomy



Plant Biospectroscopy team

Plant Ecophysiology Department
Ecology, Systematics and Evolution Laboratory
University Paris-Sud XI - CNRS UMR 8079

Zoran Cerovic

Sylvie Meyer

Gwendal Latouche

Sebastien Bellow

Naïma Ben Ghozlen

Jean-Marc Ducruet

Peter Streb

Touhami Rzigui

Constance Laureau

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Giovanni Agati (Firenze)
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Guy Samson (Trois-Rivières)
Nicolas Tremblay (Montreal)
Kathrin Bürling (Bonn)

International

Aurélie Cartelat (Paris)
Juliette Louis (Paris)
Erwin Dreyer (INRA-Nancy)
Philippe Gate (Arvalis-La Minière)
Yves Goulas (Palaiseau)
Ismaël Moya (Palaiseau)
Abderrahmane Ounis (Palaiseau)
Sébastien Debuisson (CIVIC-Epernay)

France

Jean-Luc Ayral
Nicolae Moise
Vincent Martinon
Marine Le Moigne
Sophie Lejealle
Guillaume Masdoumier

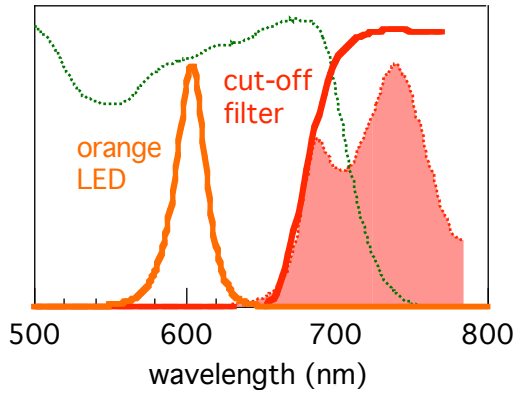
FORCE-A

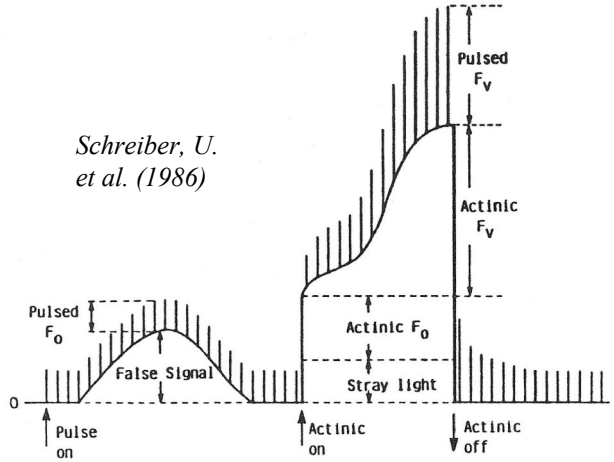
Passive vs. active fluorescence sensing

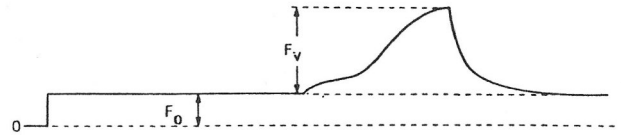
Stress

Active sensing

Short distance







Spectral and time selection

Low yield < 1%

Need to enrich the fluorescence signal

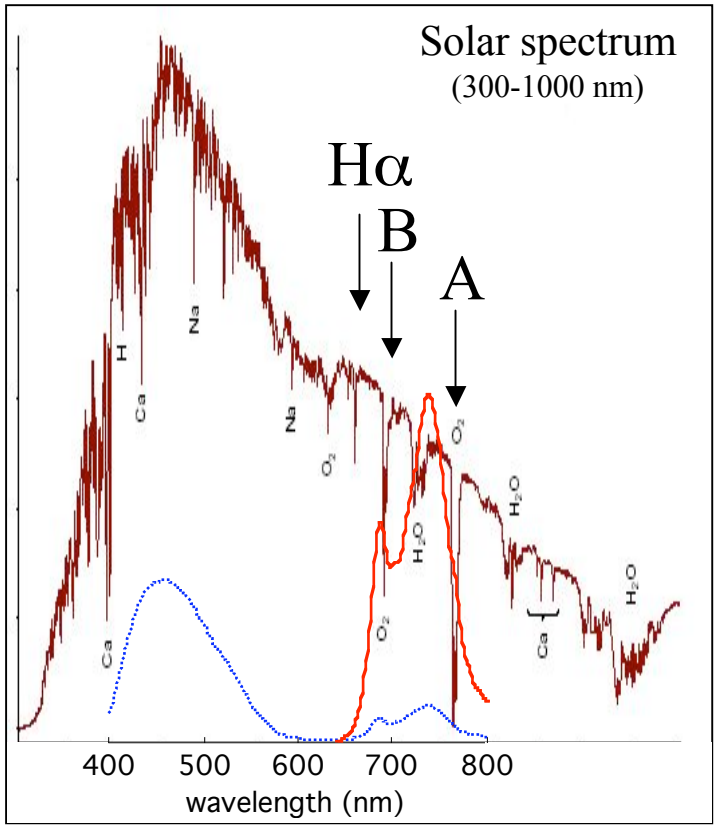
microsecond flashes

synchronised detection

Variations in fluorescence yield

Passive sensing

Long distance

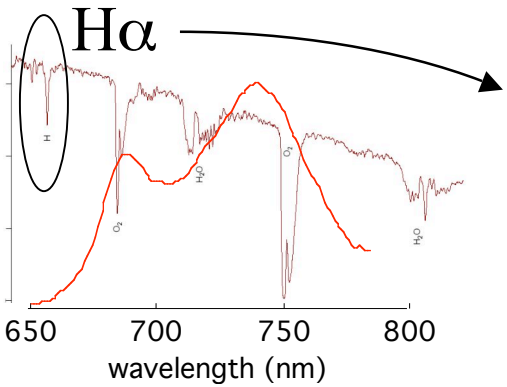


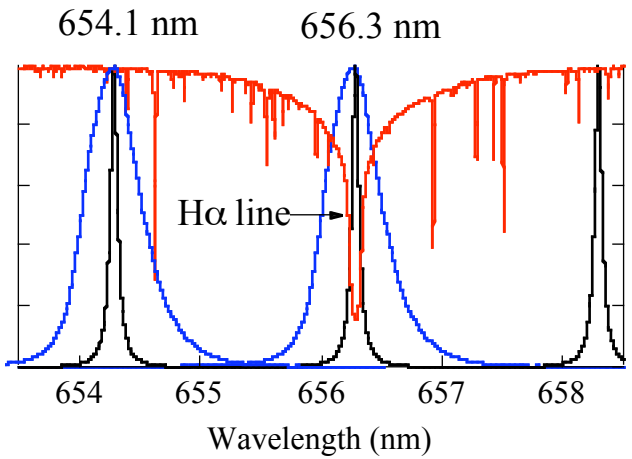
Fluorescence = **25** & **2.5** % of reflected light

Hyper-spectrale selection

FLD (Fraunhofer Line Discriminator) passive sensing

red fluorescence





projects

FLEX (ESA)

FLEXSAT (NASA)

Hα LURE-CNRS Orsay

FKD MKII

Plascyk J.A. (1975)
Opt. Eng. 14, 339-346

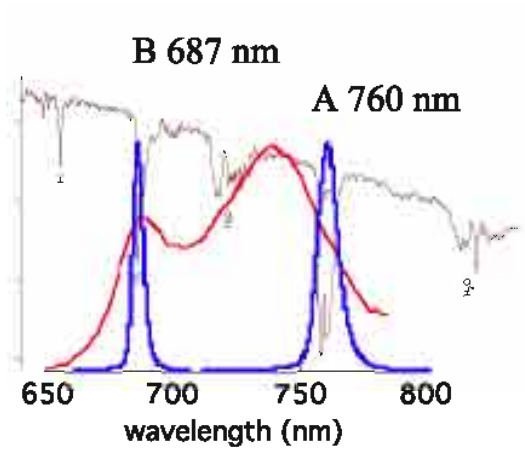
Moya I. et al. (2002)
CNES

FLD in the oxygen bands

Chl

red fluorescence

Ratio NIR/Red



Aerodyne Research USA

Kebabian P.L. et al. (1999)
Rev. Sci. Instrum. 70, 4386-4393.

PMFD LURE-CNRS Orsay

Moya I. et al. (1998) in Photosynthesis: Mechanisms and Effects (Garab, G., ed.), pp. 4265-4270, Kluwer Acad. Pub., Dordrecht.

AIRFLEX - LMD - CNRS

Moya I. et al. (2008)
unpublished