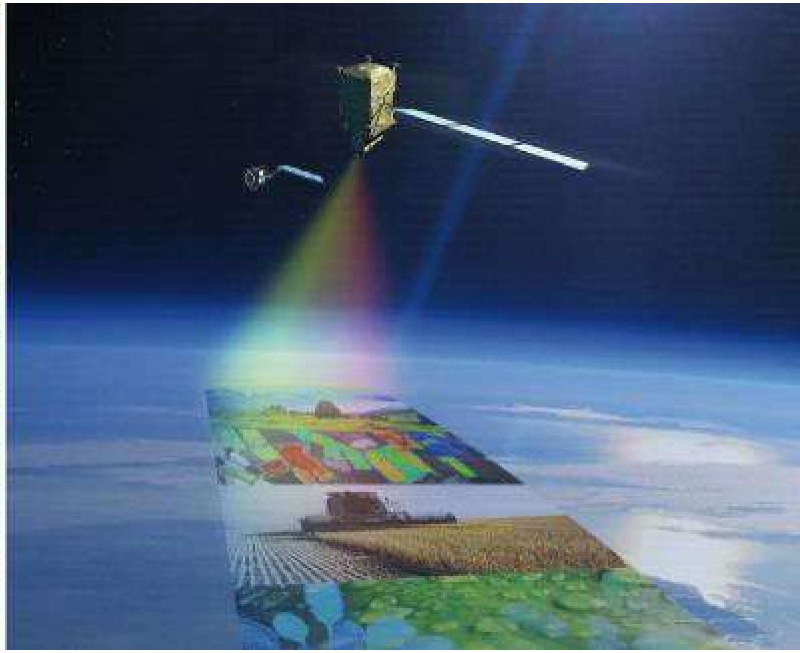




MEASURING, SCALING AND UNDERSTANDING SOLAR-INDUCED FLUORESCENCE FROM THE LEAF TO THE CANOPY AND FIELD SCALE - OVERVIEW ON OUR CURRENT KNOWLEDGE TO RELATE ACTUAL CANOPY PHOTOSYNTHESIS TO LEAF-LEVEL REGULATION AND STRESS RESPONSE

Uwe Rascher – Forschungszentrum Jülich, IBG-2: Plant Sciences

20. SEPT 2023

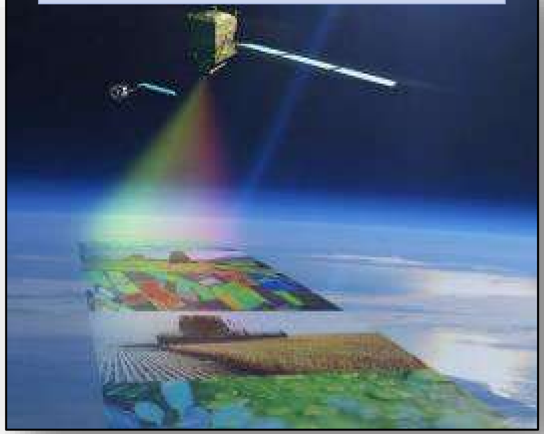
Juliane Bendig | Jim Buffat | Antony Castro | Ruonan Chen | Sofia Choza Farias | David Herrera | Ireneus Kleppert | Deepthi Konche | Caspar Kneer | Oliver Knopf | Julie Krämer | Vera Krieger | Onno Muller | Huaiyue Peng | Juan Quiros-Vargas | Saja Salattna | Theresa Sandmann | Bastian Siegmann

Outline

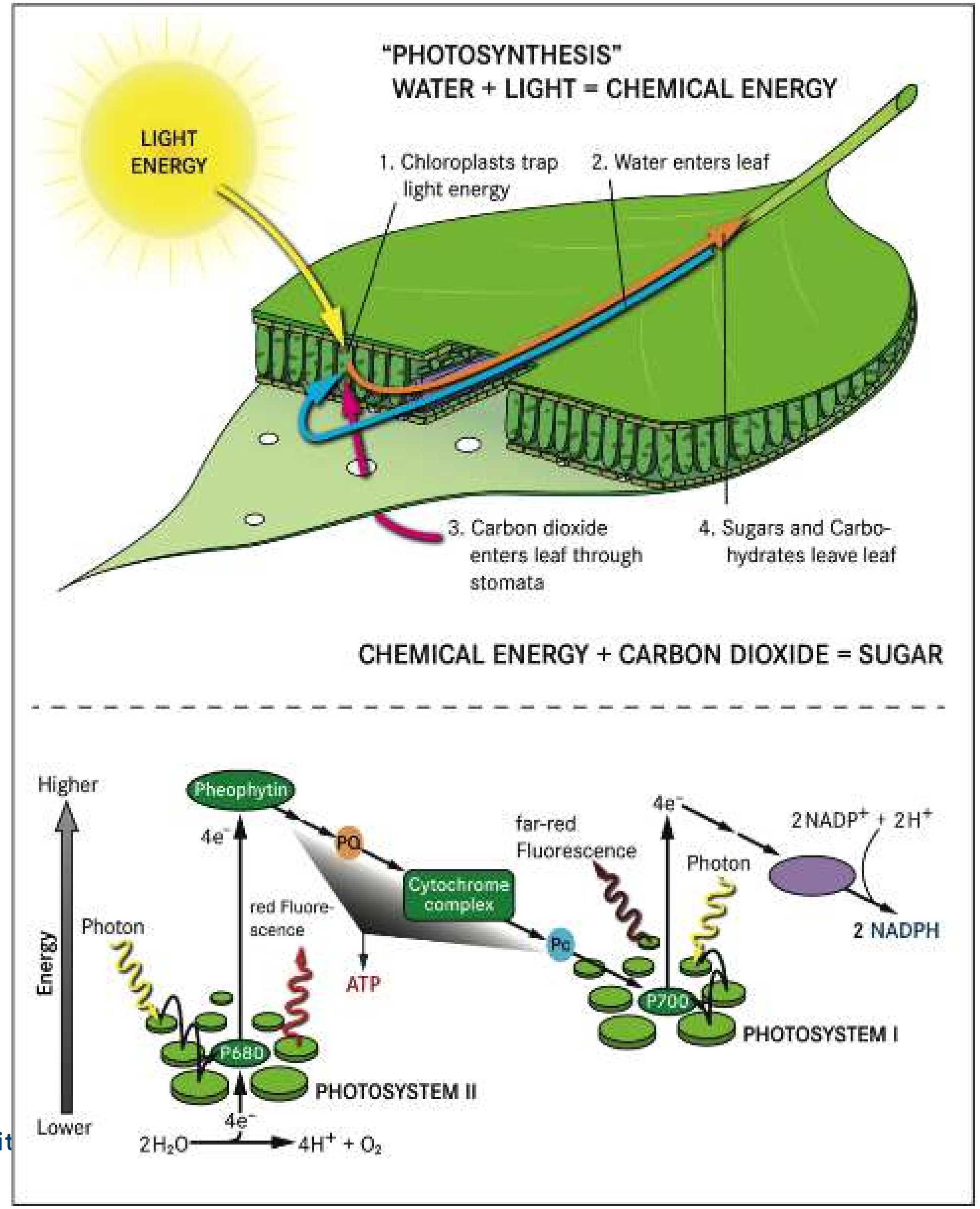
- ❑ Focus on the past 12 years of experimental data on solar-induced fluorescence (SIF)
- ❑ SIF to improve our understanding of the regulatory dynamics of photosynthesis – mechanistic knowledge is needed to related fluorescence to functioning of photosynthesis
- ❑ Evolution of instruments and retrievals to measure SIF (and other fluorescence parameters) from the leaf, top-of-canopy, UAV, and aircraft
- ❑ Testing & discussion of two main FLEX hypothesis
  - ❑ SIF is the most direct proxy for actual rates of photosynthesis and improves forward modelling of carbon uptake and evapotranspiration
  - ❑ SIF is able to early detect vegetation stress
- ❑ SIF beyond satellite observations – plant breeding & sensor guided agricultural management

Mechanistic understanding

FLEX products



Chlorophyll fluorescence – an indicator for photosynthetic efficiency



- Photosynthesis is a highly regulated process that involves a cascade of electron transfers (*Light reaction*) to fuel carbon fixation (*Calvin cycle*)
- Fluorescence is emitted from the cores of the photosynthetic machinery: Photosystems I and II

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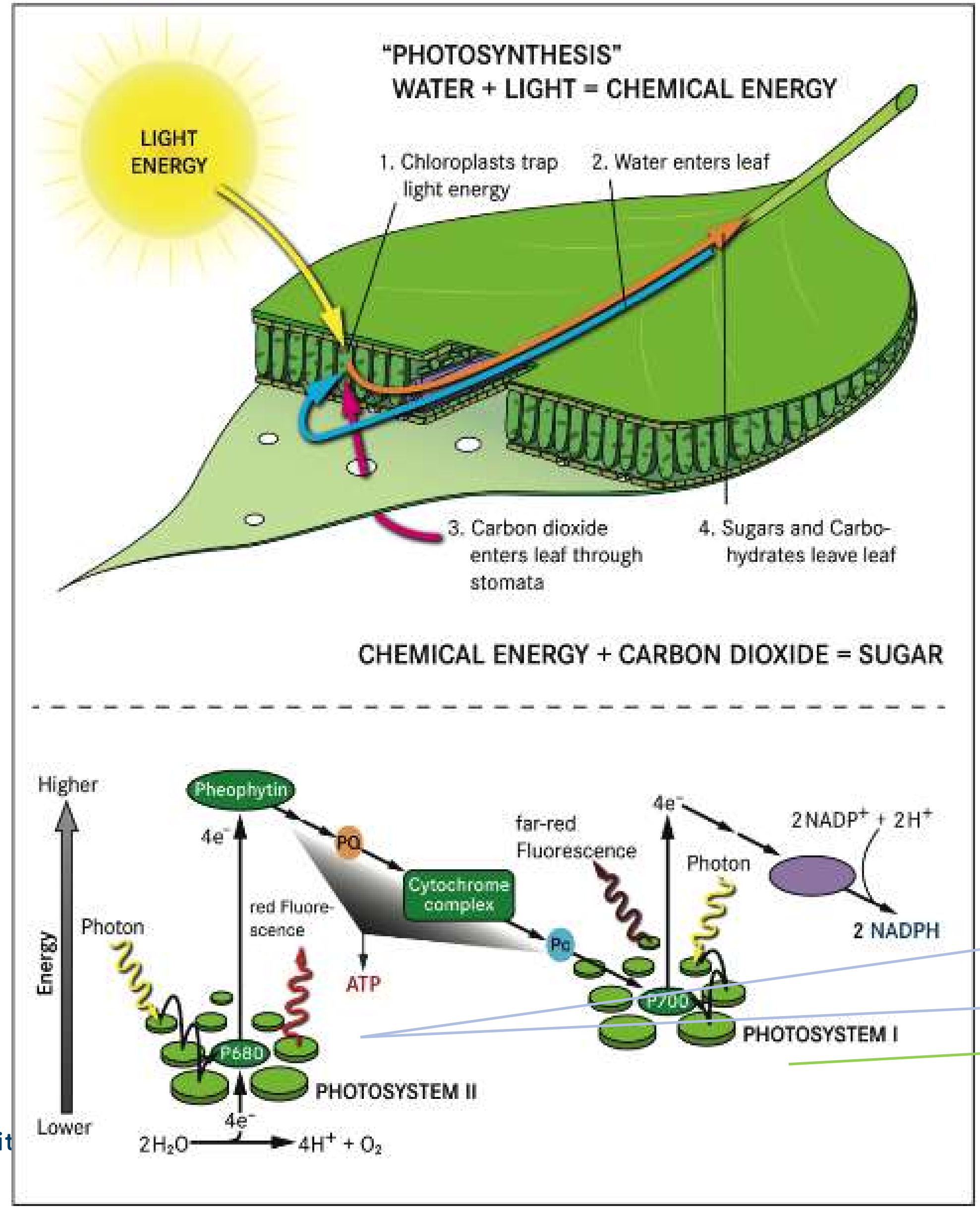
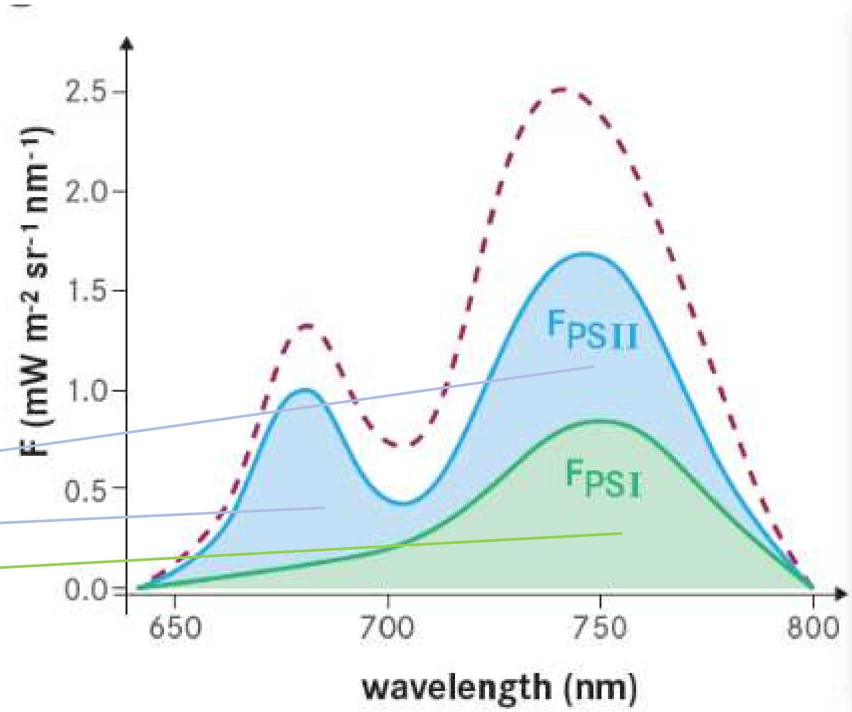
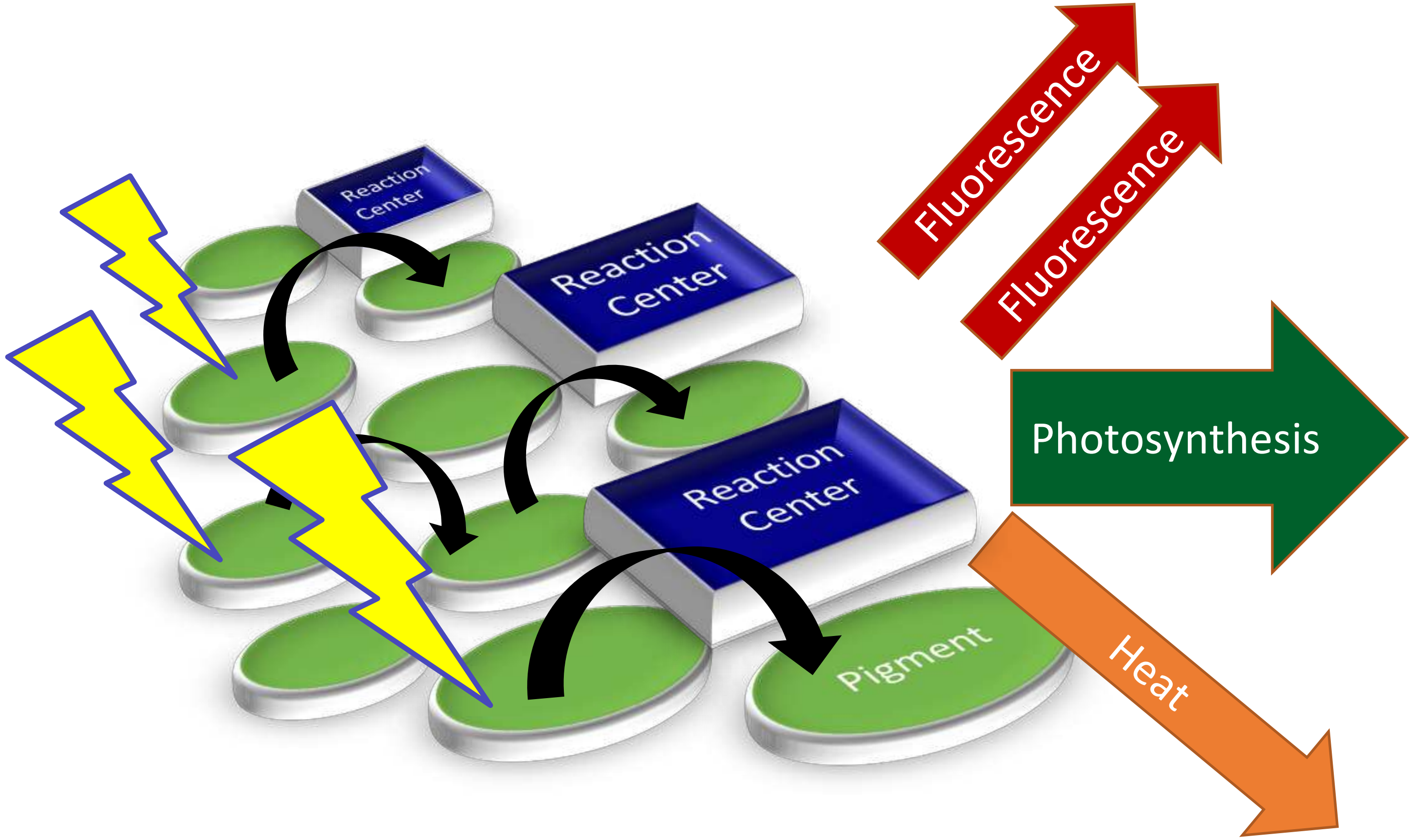
Chlorophyll fluorescence – an indicator for photosynthetic efficiency



- Photosynthesis is a highly regulated process that involves a cascade of electron transfers (*Light reaction*) to fuel carbon fixation (*Calvin cycle*)
- Fluorescence is emitted from the cores of the photosynthetic machinery: Photosystems I and II
- Two-peak feature of fluorescence



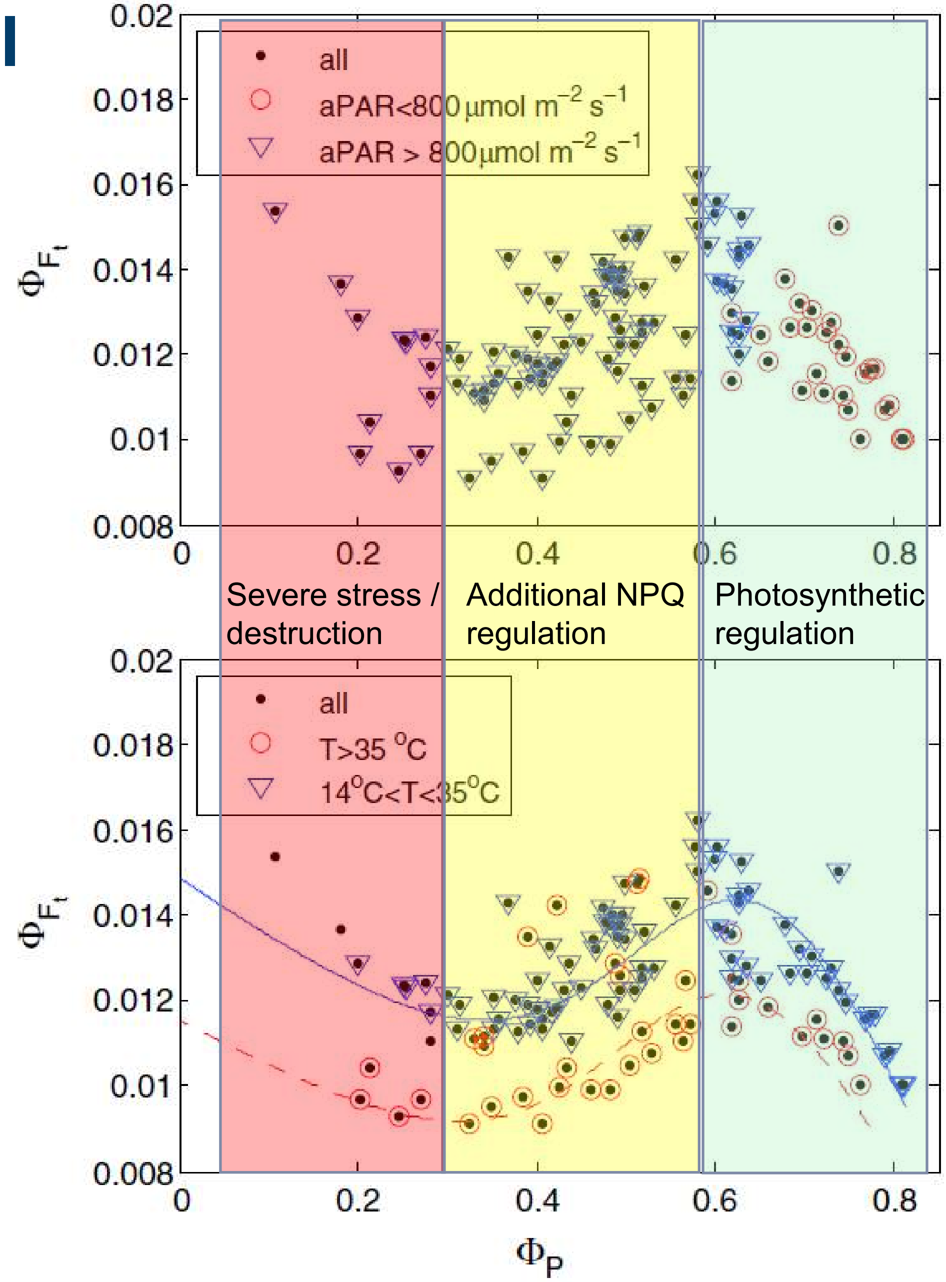
Chlorophyll fluorescence – an indicator for photosynthetic efficiency



Chlorophyll molecules emit fluorescence. The intensity of the fluorescence signal is a function of light intensity, the concentration of chlorophyll and the efficiency of photosynthesis (functioning of photosynthesis)

Steady-state fluorescence and photosynthetic efficiency are non-linearly related – leaf level

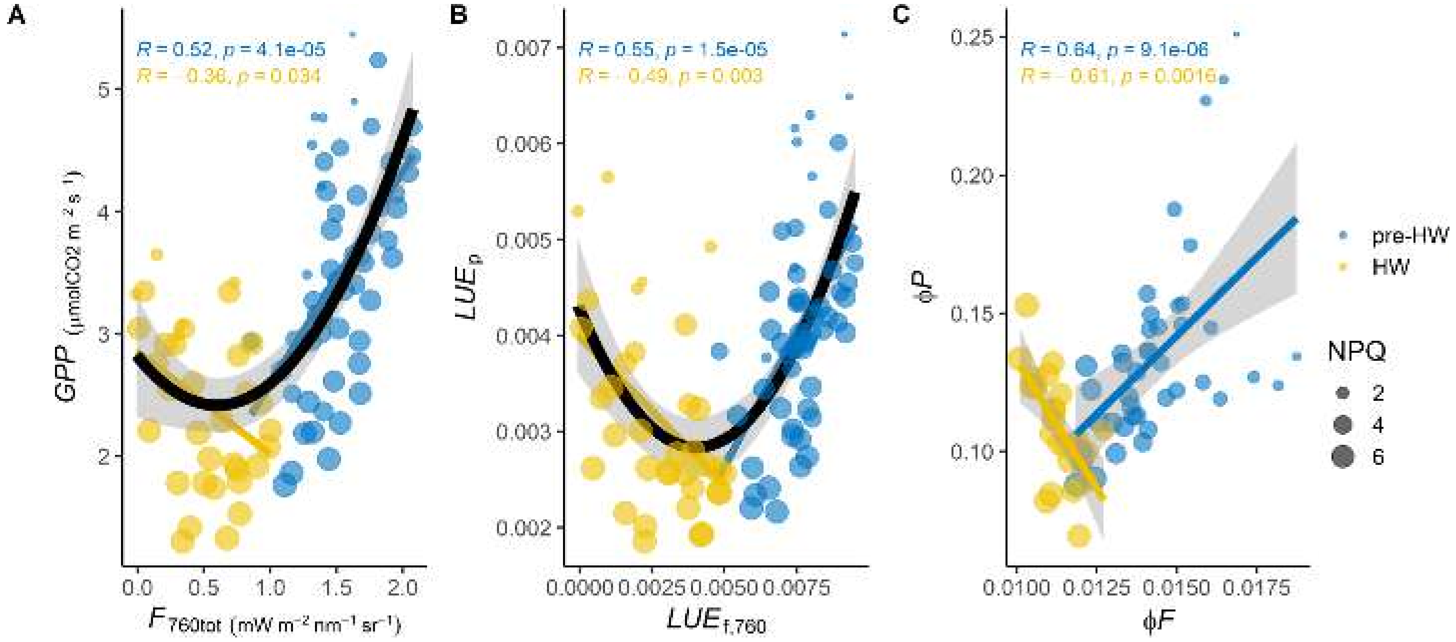
- The relationship between solar-induced fluorescence and efficiency of photosynthesis is not linear
- Light intensity and the degree of non-photochemical energy dissipation influence the relation between solar-induced fluorescence and photosynthesis



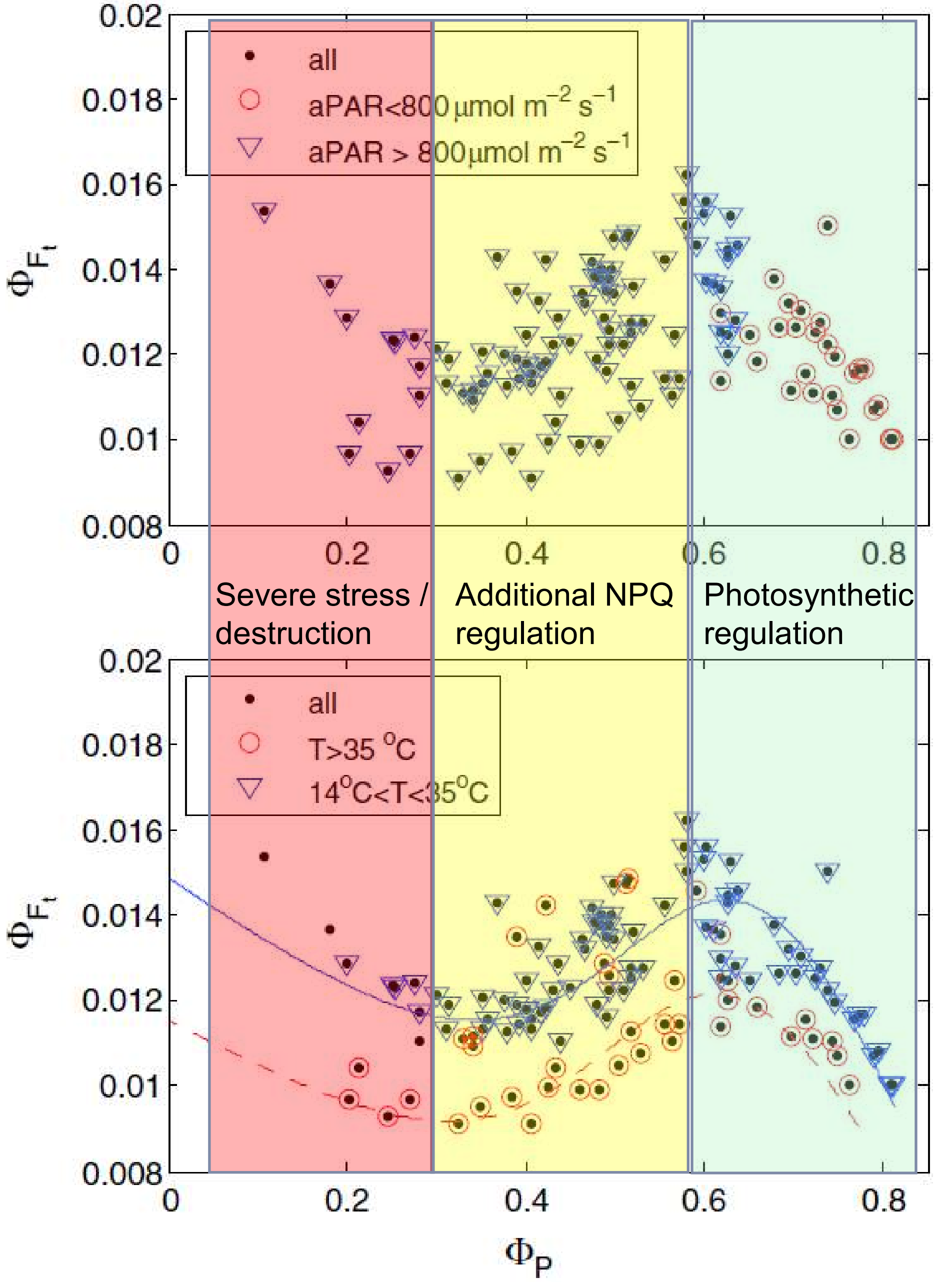
van der Tol C., Berry J.A., Campbell P.K.E. & Rascher U. (2014) Models of fluorescence and photosynthesis for interpreting measurements of solar-induced chlorophyll fluorescence. *Journal of Geophysical Research - Biogeosciences*, *119*, 2312-2327.

Steady-state fluorescence and photosynthetic efficiency are non-linearly related

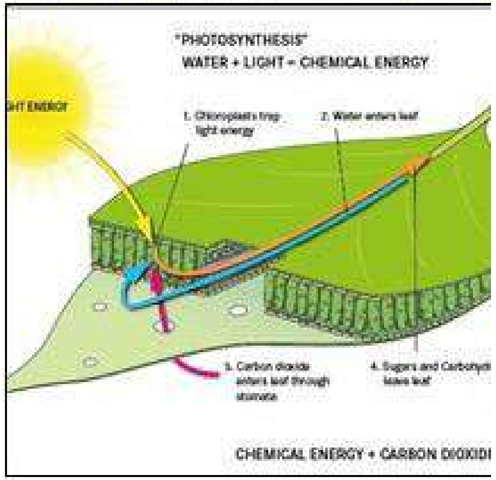
- Models predicted a three-phase relationship between SIF and photosynthetic efficiency
- Heat wave in Span (summer 2018) demonstrated the tipping point between dynamically regulated photo-synthesis and severe stress (canopy level)



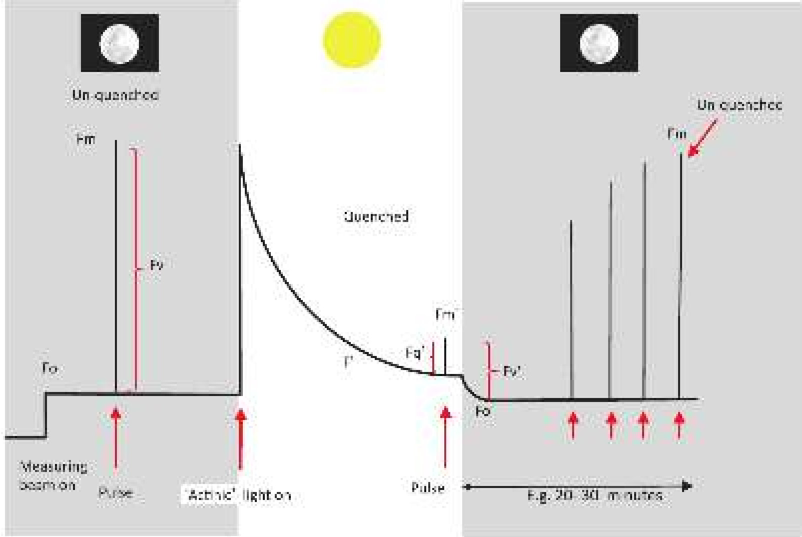
Martini et al. (2022) Heatwave breaks down the linearity between sun-induced fluorescence and gross primary production. *New Phytologist*, 233, 2415–2428



Fluorescence techniques are the most widely used approaches to investigate photosynthesis



- Leaf level measurements to understand the functional link between photosynthetic regulation and fluorescence emission
- Various instruments available and currently ~750 Papers published per year (ISI core collection).
- Most of these methods use active approaches, such as PAM, saturating light pulses or lasers induced fluorescence transients



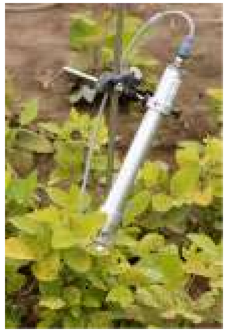
Murchie E. & Lawson T. (2013) Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. *J. Exp. Bot.*, 64, 3983–3998.

Murchie E. et al (2018) Measuring the dynamic photosynthome. Annals of Botany, 122, 207-220, doi: 10.1093/aob/mcy087.

Rascher U. et al. (2010) Sensing of photosynthetic activity of crops. In Precision Crop Protection - the Challenge and Use of Heterogeneity. Springer Science+Business Media B.V., doi 10.1007/978-90-481-9277-9_6.

Evolution of relevant instruments to quantify fluorescence and photosynthesis from the leaf to the field

Active



Passive (SIF)

FLUO-Wat

Air-FLOX

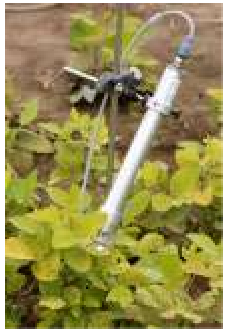
SIF-Cam

HyPlant

Leaf | top-of-canopy | UAVs | aircraft

Evolution of instruments: PAM and LIFT

Active



Passive (SIF)

FLUO-Wat

Air-FLOX

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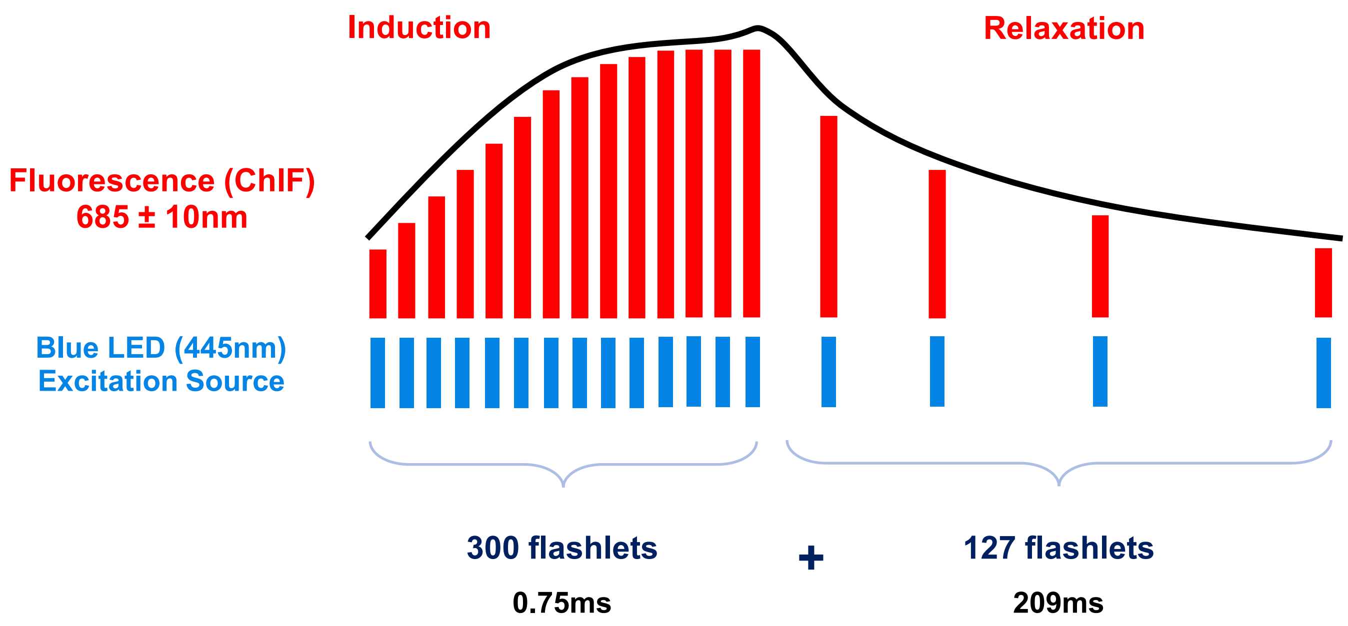
HyPlant

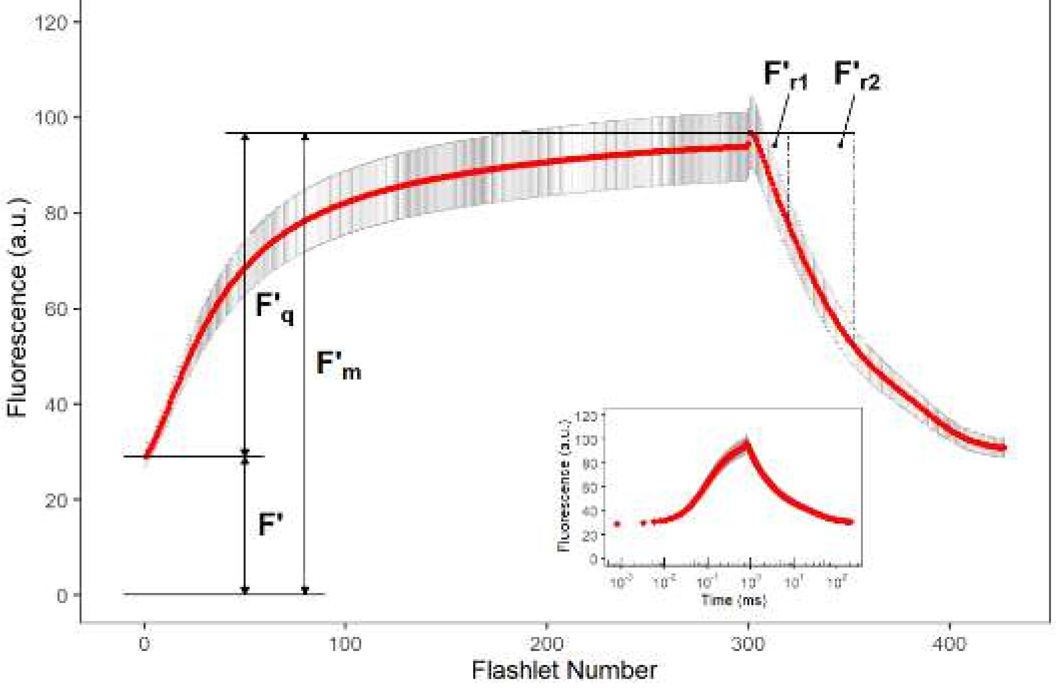
Leaf top-of-canopy UAVs aircraft

Evolution of instruments: PAM and LIFT

Q_A Flash approach: excitation protocol to reduce Q_A



Schematic Profile of a Light-Induced Fluorescence Transient Obtained with the Q_A Flash Protocol



Keller B. et al. (2019) Maximum fluorescence and electron transport kinetics determined by light induced fluorescence transients (LIFT) for photosynthesis phenotyping. *Photosynthesis Research, 140*, 221-233, doi: 0.1007/s11120-018-0594-9.

Keller B. et al. (2022) Toward predicting photosynthetic efficiency and biomass gain in crop genotypes over a field season. *Plant Physiology, 188*, 301-317; doi: 10.1093/plphys/kiab483.

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LIFT: Proximal sensing drought response in durum wheat

dos Santos N.Z. et al. (2021) High-throughput field phenotyping reveals genetic variation in photosynthetic traits in durum wheat under drought. *Plant, Cell & Environment, 44*, 2858-2878, doi: 10.1111/pce.14136.

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Combining LIFT and FLOX to understand the functional meaning of solar-induced fluorescence

Poster from Deepthi Konche & Sofia Choza-Farías

Poster from Oliver Knopf & Juan Romero

Evolution of instruments: Mobile FLOX

Active

Passive (SIF)

FLUO-Wat

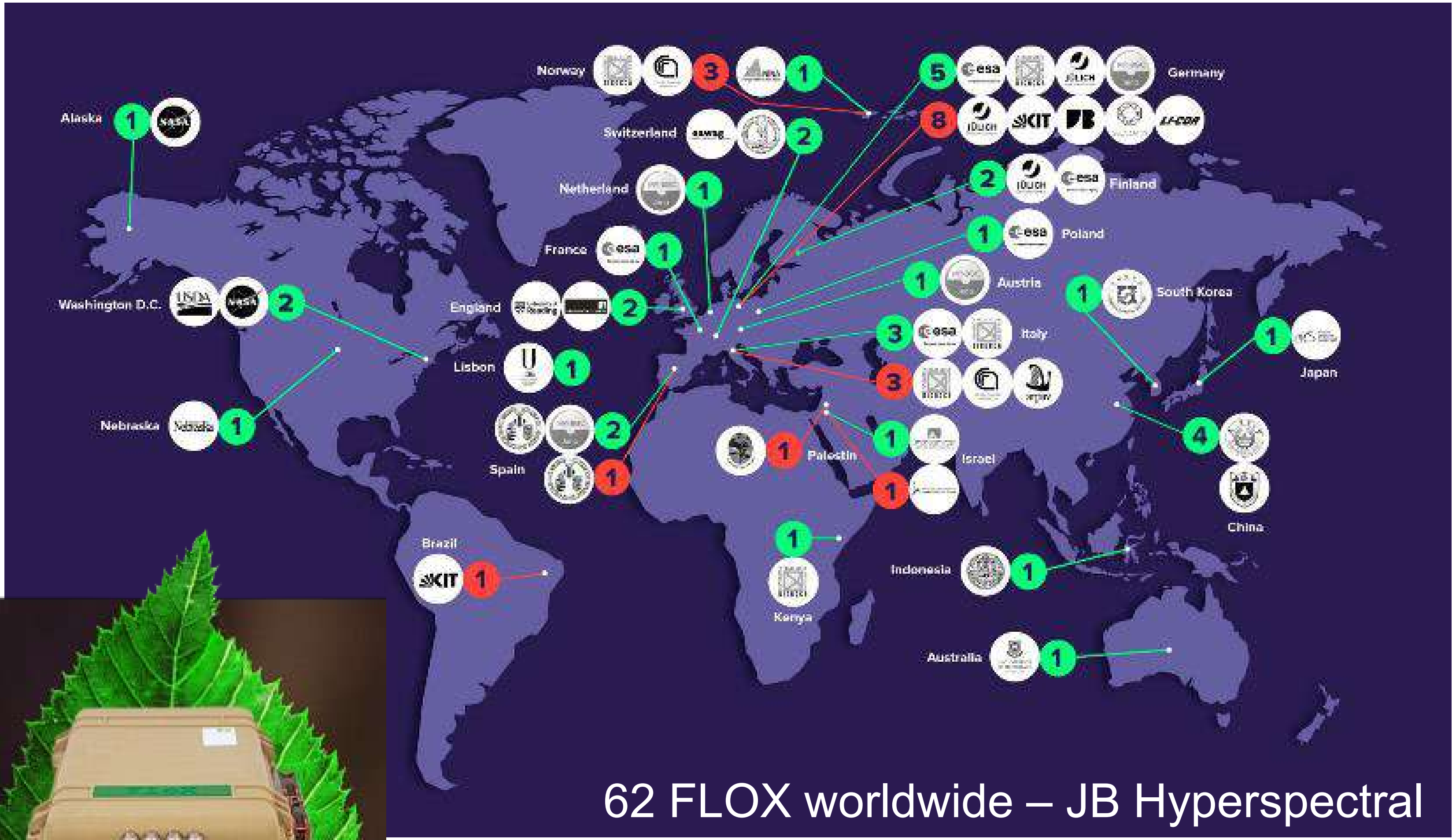
Air-FLOX

SIF-Cam

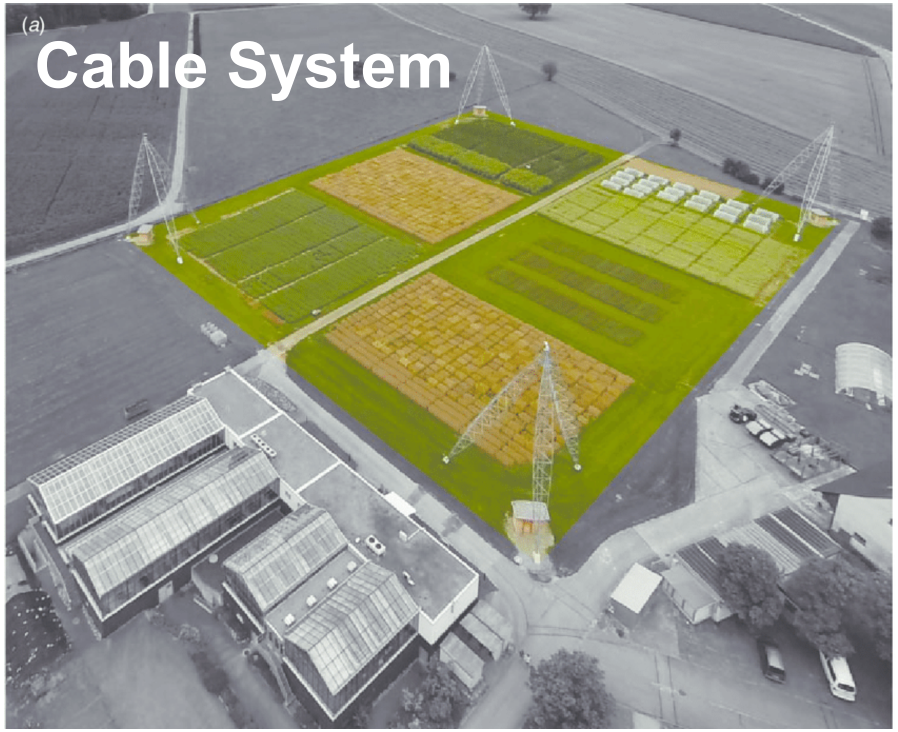
HyPlant

Leaf — top-of-canopy — UAVs — aircraft

Some new technical developments for the leaf-to-canopy scale: FLOX systems – stationary system and mobile instrument



FLOX systems – stationary system and mobile instrument – FLOX integrated and tested in sensor positioning systems



Used in various poster presentations

Evolution of instruments: HyScreen

Active

Passive (SIF)

FLUO-Wat

Air-FLOX

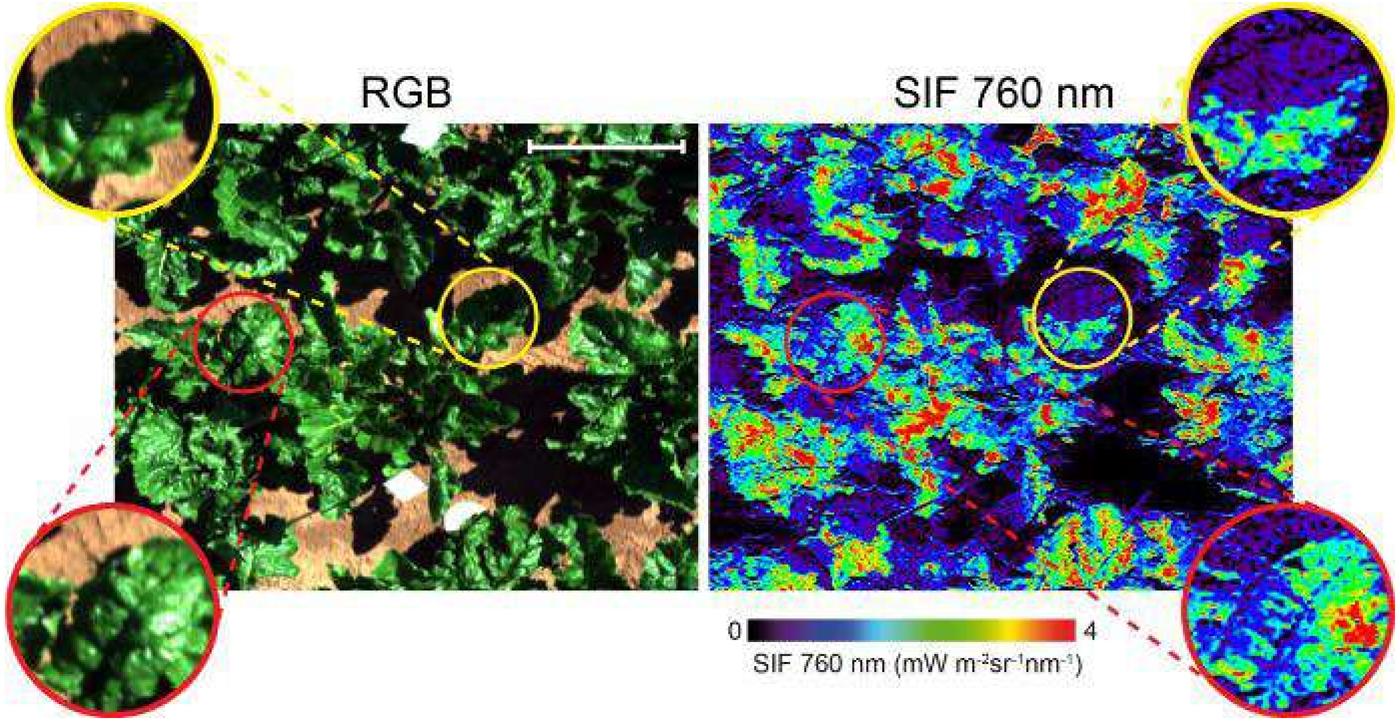
SIF-Cam

HyPlant

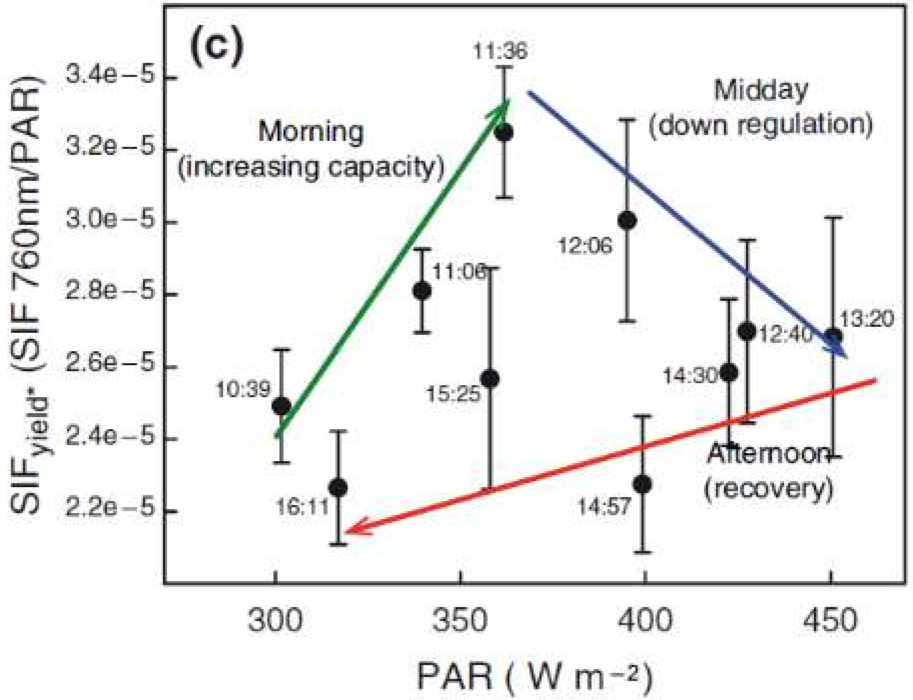
Leaf → top-of-canopy → UAVs → aircraft

Steady-state fluorescence and photosynthetic efficiency are non-linearly related – leaf to canopy level

- Mapping of sun-induced fluorescence on the ground to understand interplay of the variations of light intensity within natural canopies and the 3-dimensional leaf display



Diurnal course of sun-lit leaves of upper canopy



Pinto et al. (2016) *Plant, Cell and Environment, 39*, 1500–1512

Pinto et al. (2017) *Remote Sensing, 9, 415, doi: 10.3390/rs9050415*

Steady-state fluorescence and photosynthetic efficiency are non-linearly related – leaf to canopy level

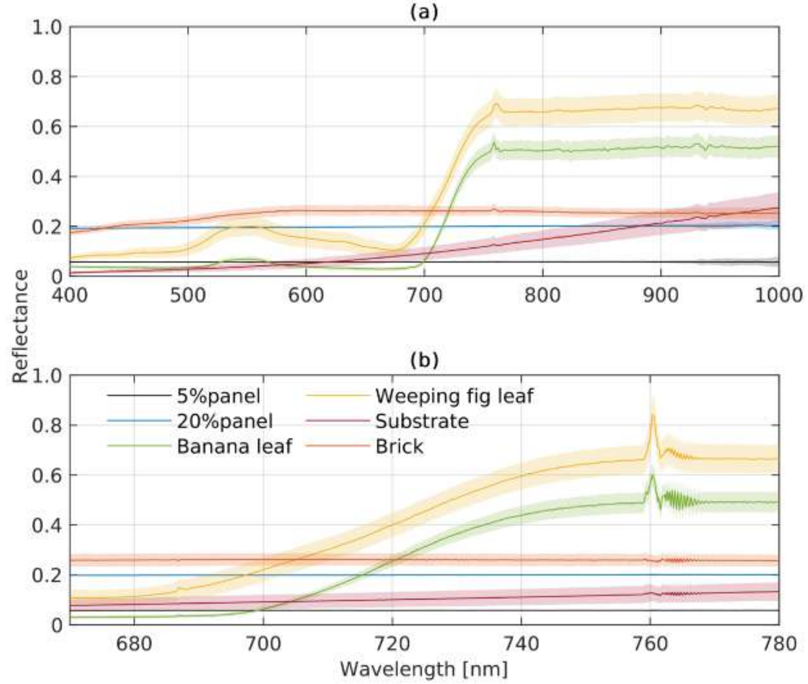
sensors

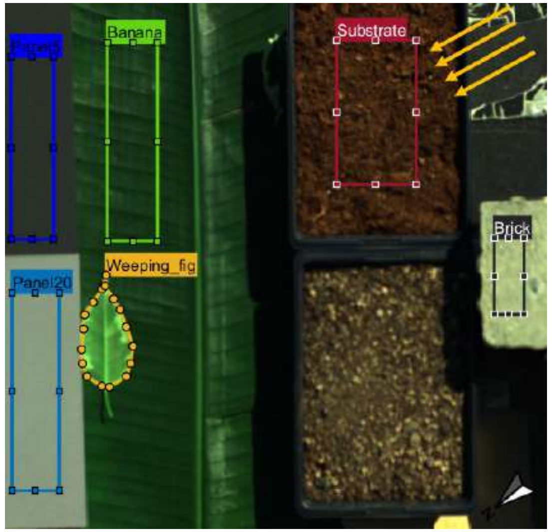
MDPI

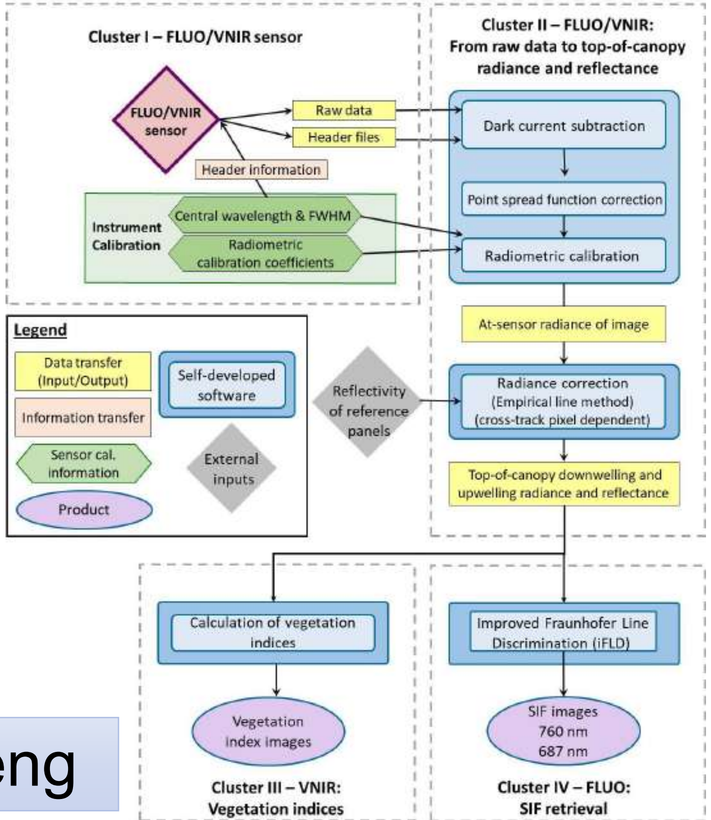
Article

HyScreen: A Ground-Based Imaging System for High-Resolution Red and Far-Red Solar-Induced Chlorophyll Fluorescence

Huaiyue Peng [1,*], Maria Pilar Cendrero-Mateo [2], Juliane Bendig [1], Bastian Siegmann [1], Kelvin Acebron [1], Caspar Kneer [1], Kari Kataja [3], Onno Muller [1] and Uwe Rascher [1]

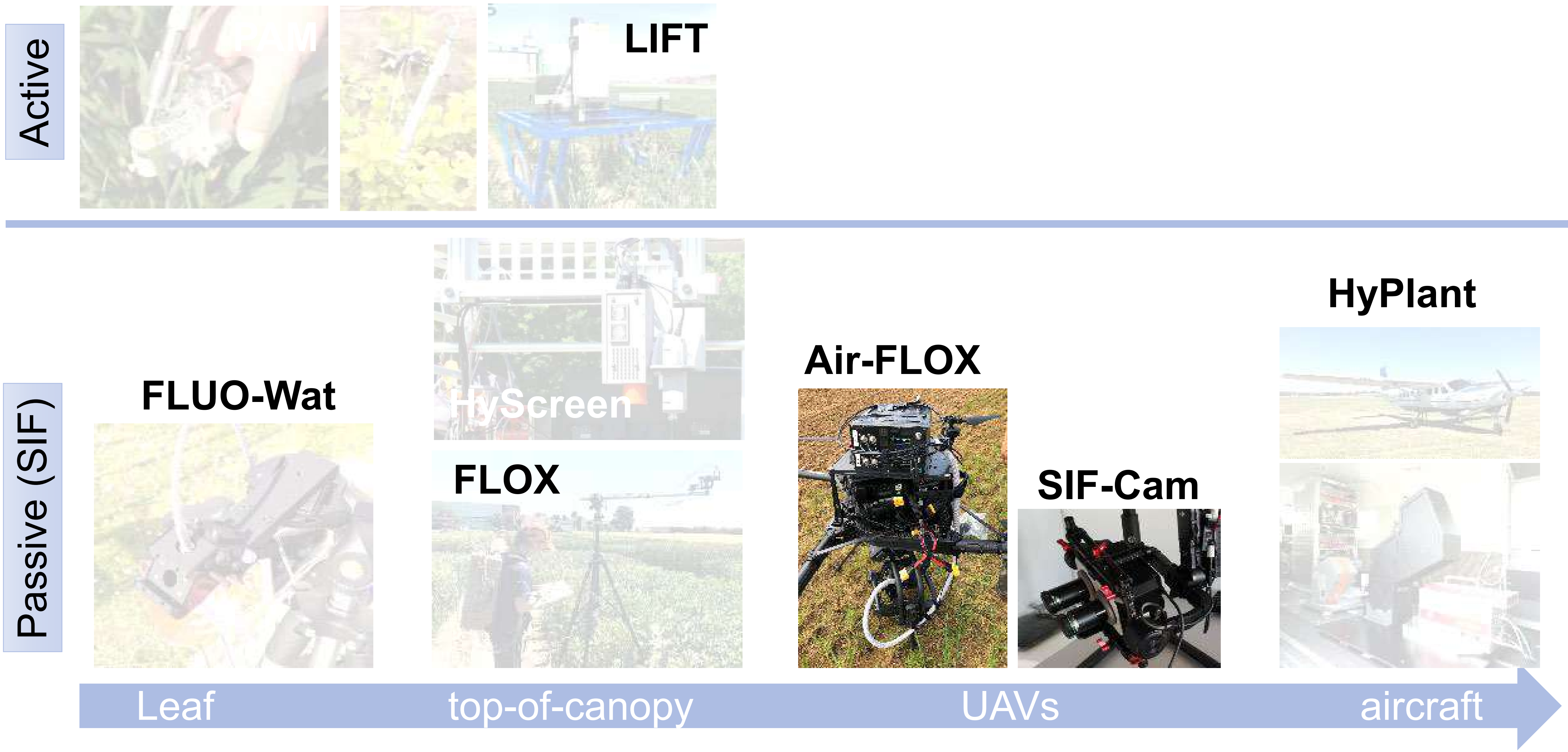




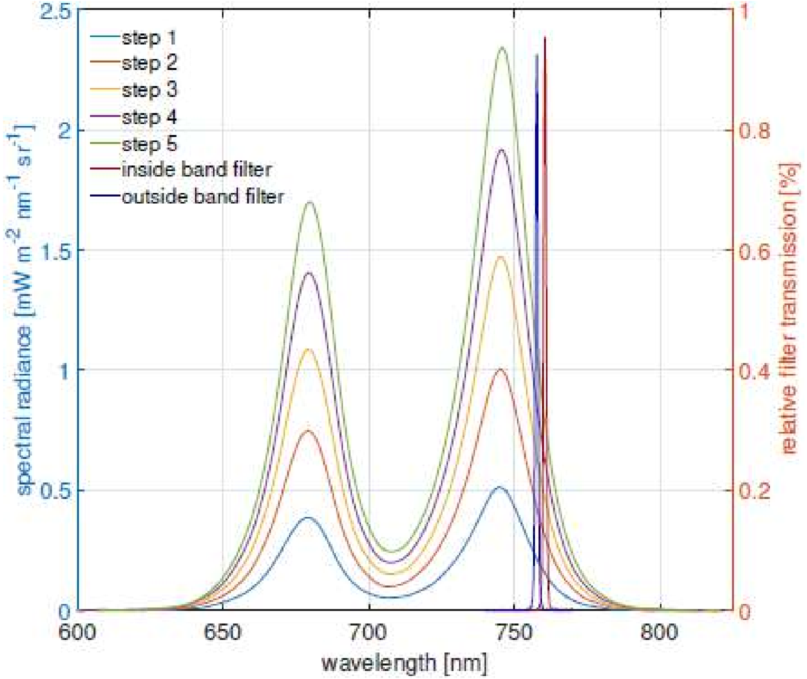


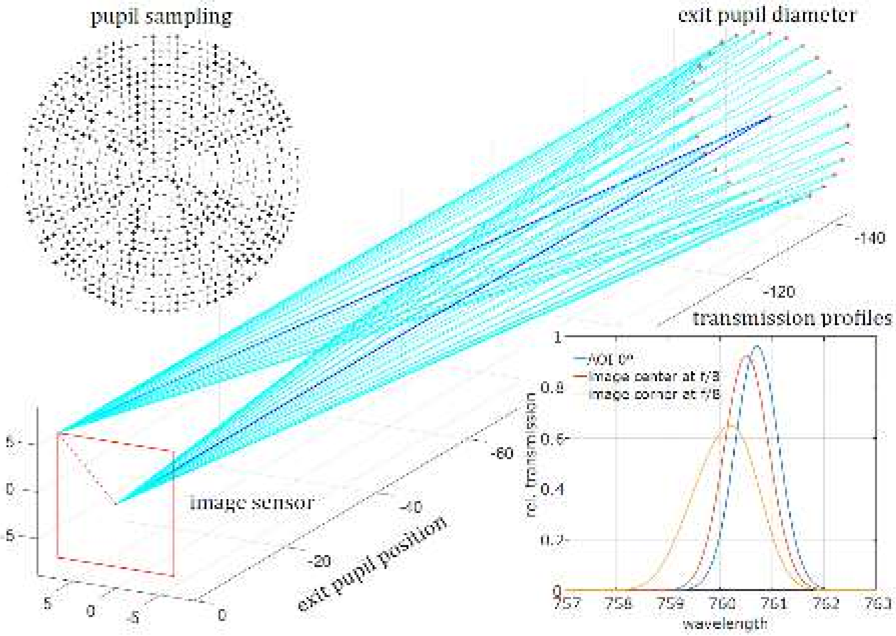
Poster from Huaiyue Peng

Evolution of instruments: AirFLOX & SIF-Cam



Enabling UAVs to measure SIF quantitatively – SIF camera





Cameras	Resolution (full)	2048 x 2048
	Sensor size [mm]	13.3 x 13.3 / 18.8 (diag.)
	Dynamic range [db]	87
	Binning	2x2, 4x4
System	FOV (full) [deg]	29.8 (hor. & ver.) / 41.2 (diag.)
	Storage [GB]	SSD: 500 / microSD: 128
	Power consumption [W]	36 max.
	Weight [kg]	1.89

- Two ultra-narrowband interference filters
  - Central wavelengths (CW) at 757.9 and 760.7 nm (out- and inside band filter)
  - FWHM < 1.2 nm & well positioned in optical path
  - Peak transmission of > 90 %

Kneer et al. (2023) A snapshot imaging system for the measurement of solar-induced chlorophyll fluorescence – addressing the challenges of high-performance spectral imaging for mapping SIF. *MDPI – Sensors,*

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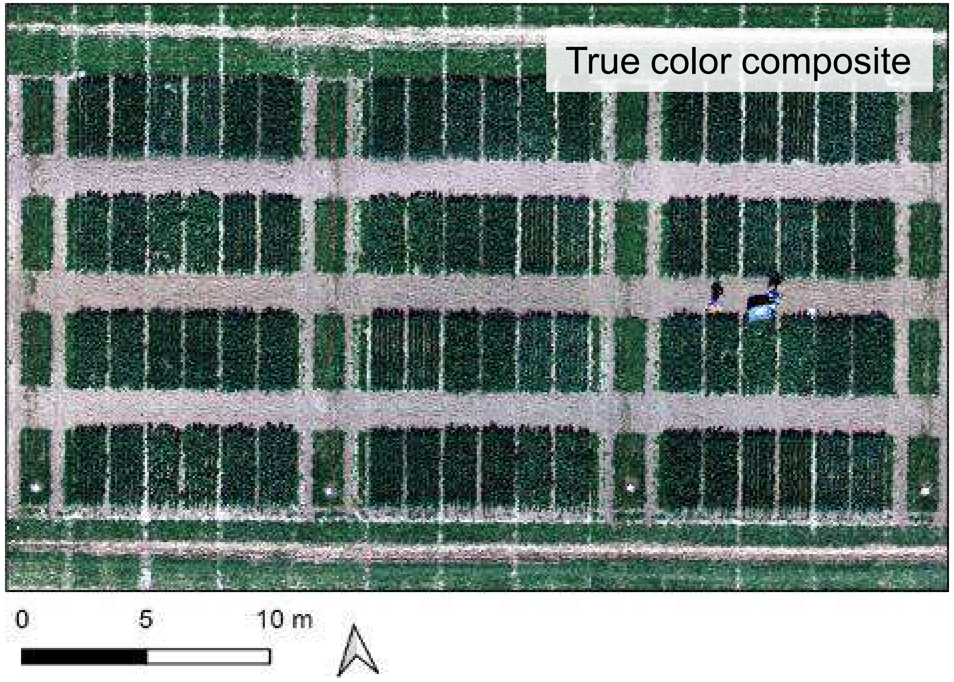
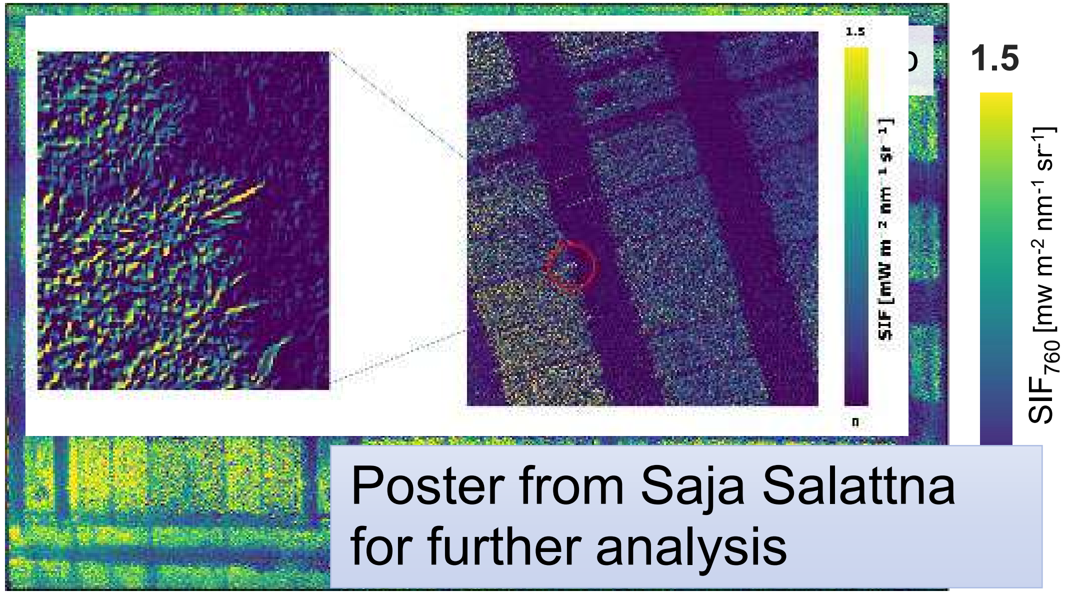
Enabling UAVs to measure SIF quantitatively – SIF camera

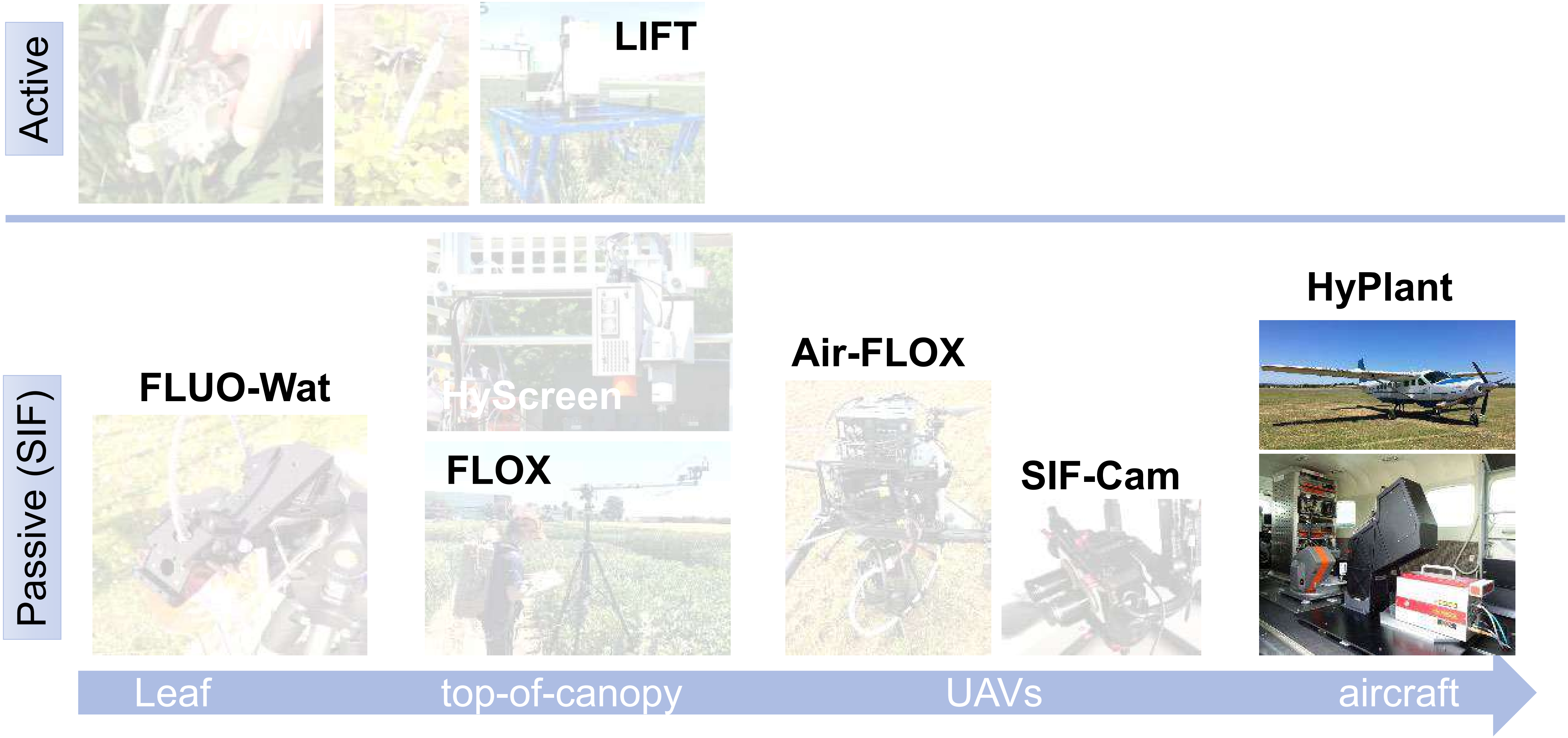




Poster from Saja Salattna for further analysis

Integrated system of SIFcam & AirFLOX was operational end of Aug 2023

Evolution of instruments: HyPlant



Airborne sensor for FLEX satellite mission: *HyPlant* (in combination with TASI and LiDAR)

HyPlant 3

- DUAL module (380 – 2500 nm)
  VIS/NIR: 3-4 nm FWHM, 1.7 nm SSI, SNR >510
  SWIR: 13 nm FWHM, 5.5 nm SSI, SNR >1100
- FLUO module (670 – 780 nm)
  0.25 nm FWHM, 0.11 nm SSI, SNR >210

TASI-600

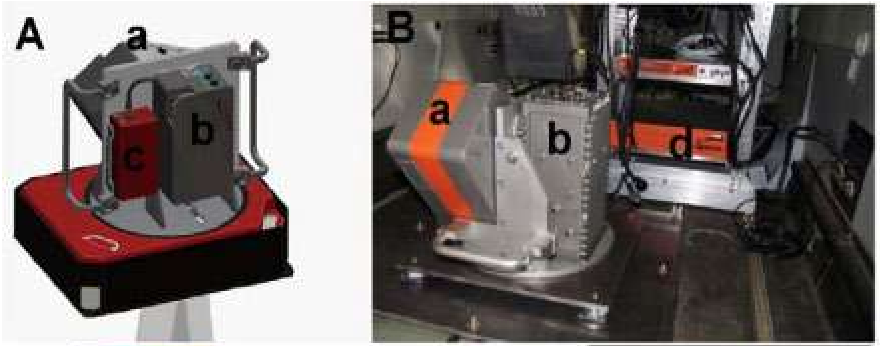
- Hyperspectral thermal sensor (8 – 11.5 µm)

Riegl LMS-Q780

- full-waveform airborne LiDAR

Airborne sensor for FLEX satellite mission: *HyPlant* (in combination with TASI and LiDAR)

- *HyPlant* sensor is operational since 2012 and was fundamentally upgraded in 2018 ⇨ *HyPlant_3*
- *HyPlant_3* is consolidated and used in campaigns since 2016 having improved radiometric performance and geometric accuracy (Siegmann et al. 2019)



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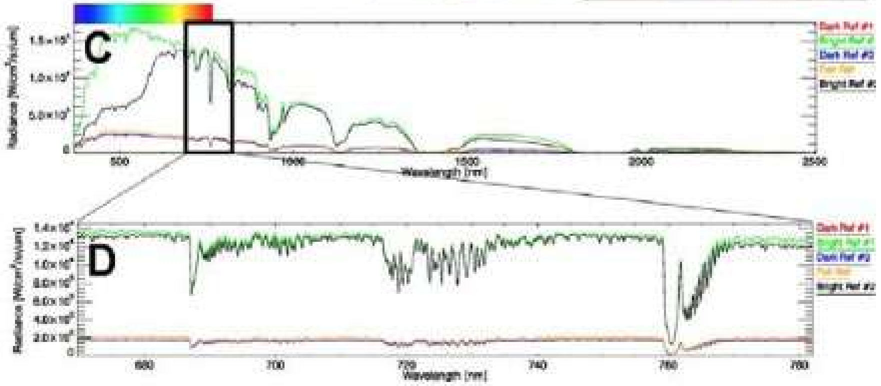
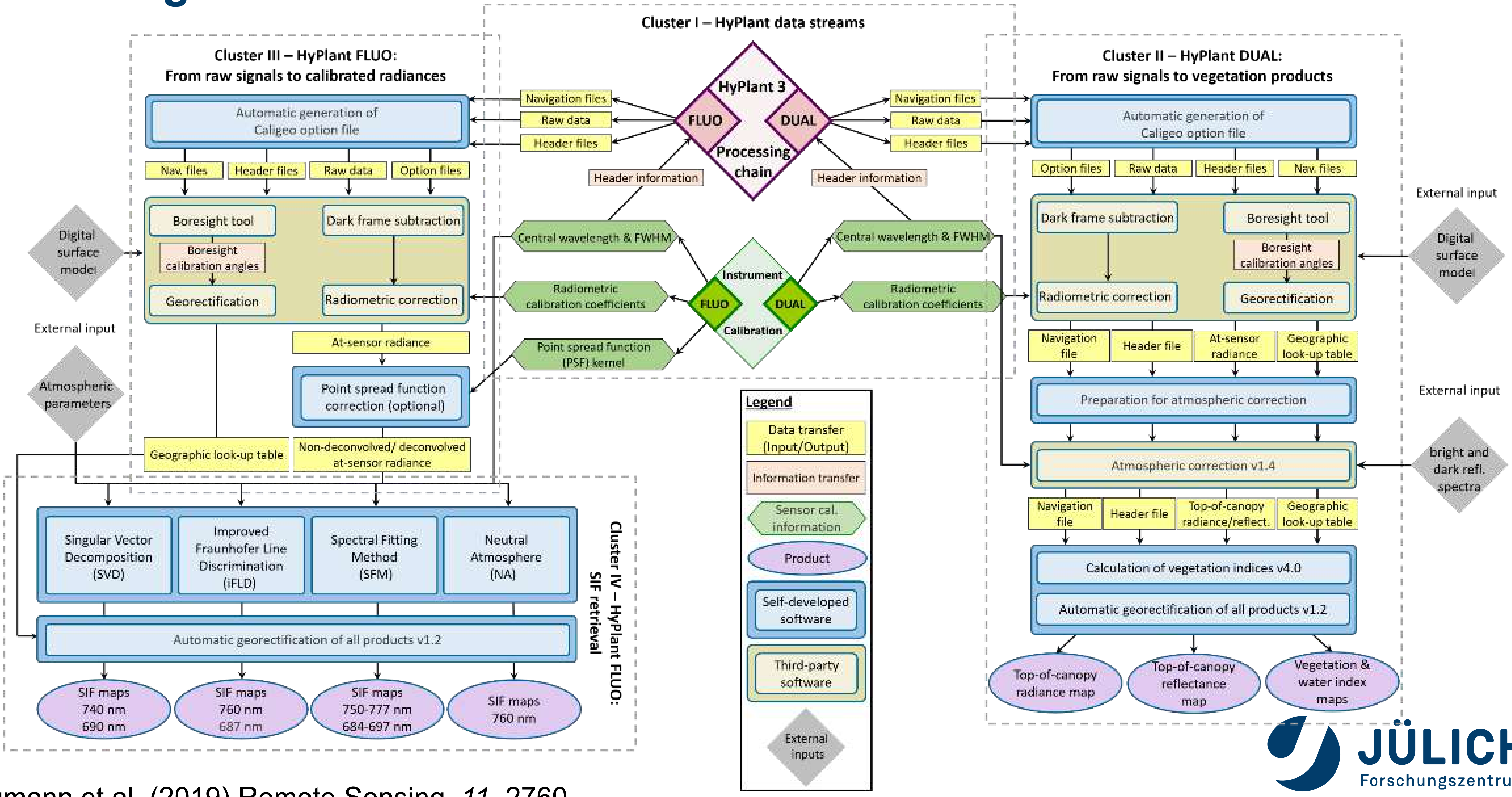


Rascher et al. (2015) *Global Change Biology, 21,* 4673–4684; doi: 10.1111/gcb.13017

Siegmann et al. (2019) The high-performance airborne imaging spectrometer *HyPlant* – From raw images to top-of-canopy reflectance and fluorescence products: Introduction of an automatized processing chain. *Remote Sensing, 11*, article no. 2760, doi: 10.3390/rs11232760.

HyPlant processing scheme is operational and delivers SIF products according to three retrieval schemes & surface reflectance



Siegmann et al. (2019) Remote Sensing, *11*, 2760.

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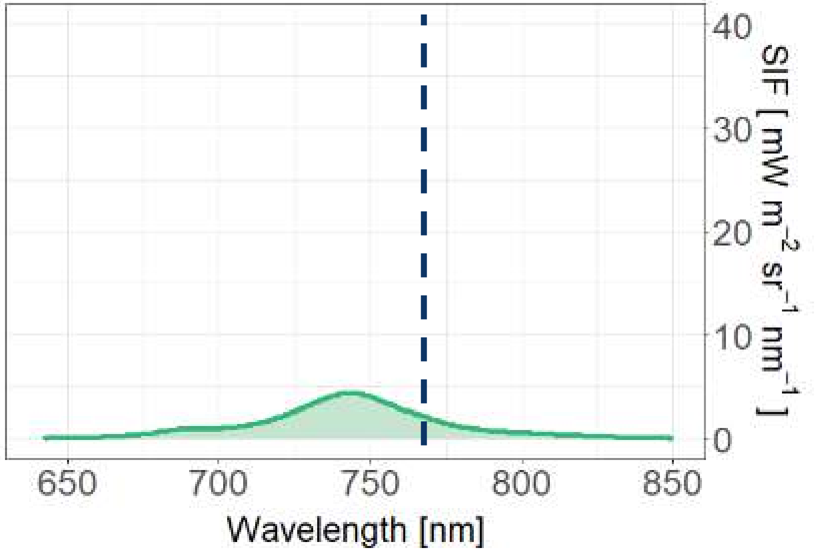
Translating top-of-canopy SIF to the leaf-level by normalizing for canopy structure

SIF at canopy-level (directional)

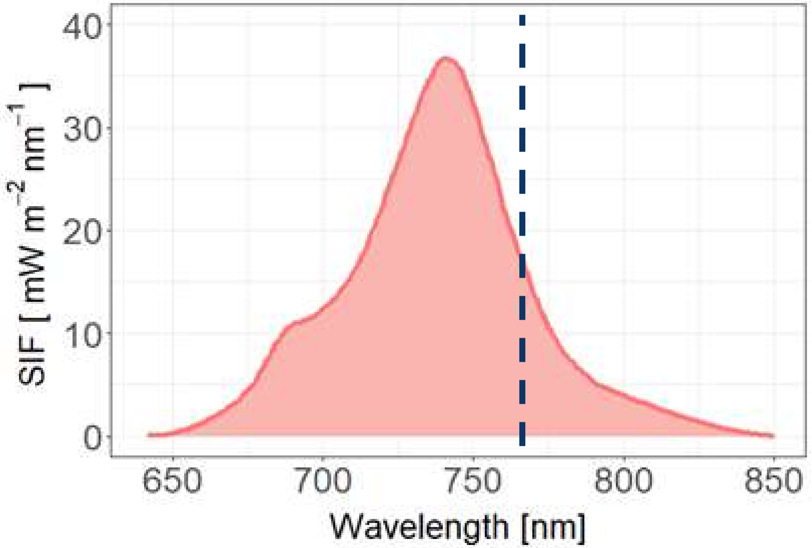
down-scaling

SIF escape fraction (f_{esc})

SIF at leaf-level (hemispherical)



$$f_{esc} \approx \frac{\pi \, SIF_{canopy}}{SIF_{leaf}}$$

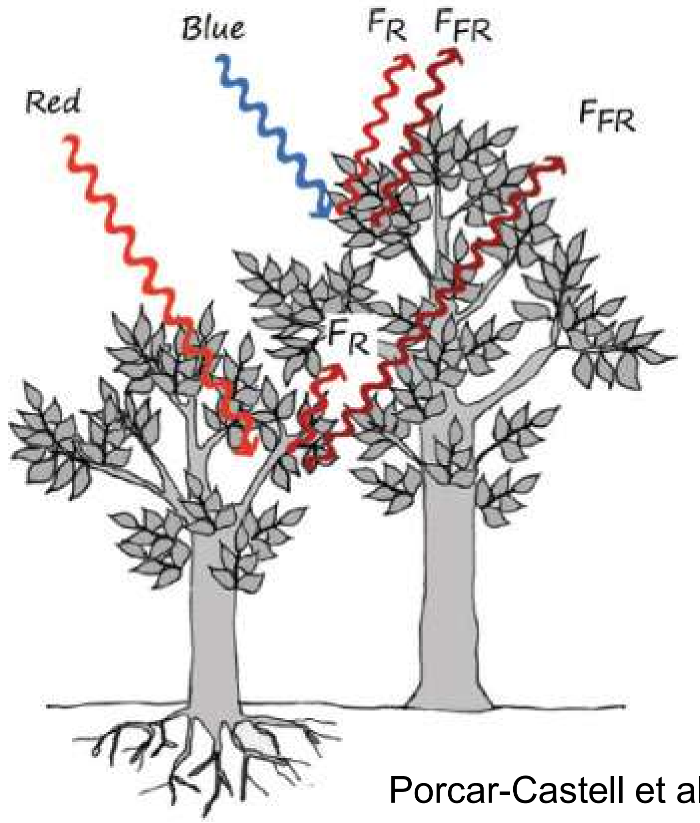


Poster from Antony Castro
Poster from Bastian Siegmann

Downscaling of SIF from TOC to leaf level – normalizing for canopy structure

- Fluorescence correction vegetation index (FCVI) (Yang et al. 2020)
- Near-infrared reflectance of vegetation (NIRv) index (Badgley et al., 2017)
- NIRvH1 and NIRvH2 (Zeng et al. 2021)

$$f_{esc} \approx \frac{\text{index}}{\text{fAPAR}}$$



Porcar-Castell et al. (2014)

f_{esc} = SIF escape fraction
index = FCVI, NIRv, NIRvH1 or NIRvH2
fAPAR = fraction of photosynthetically active radiation

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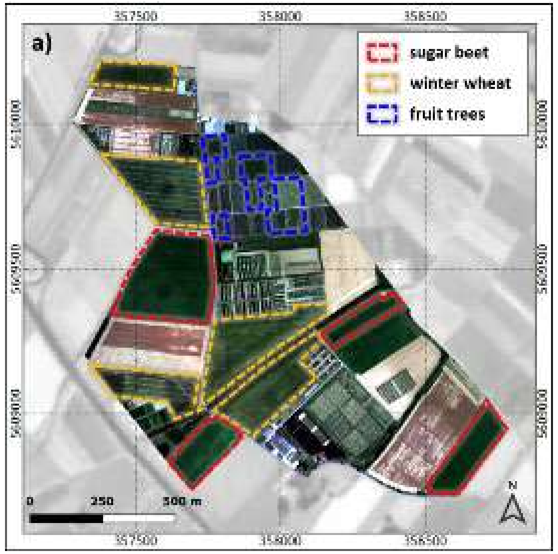
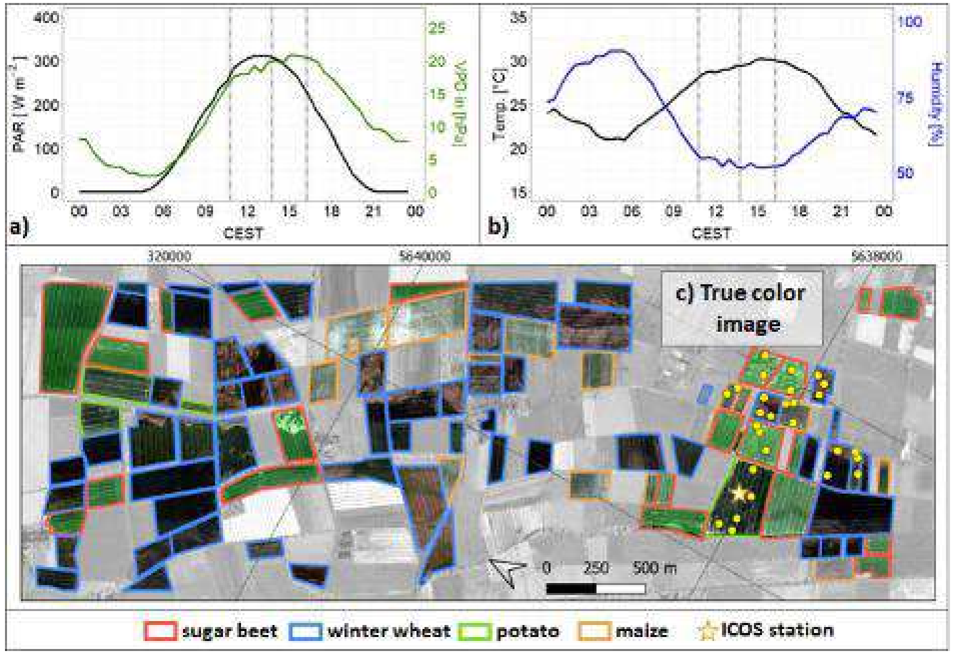
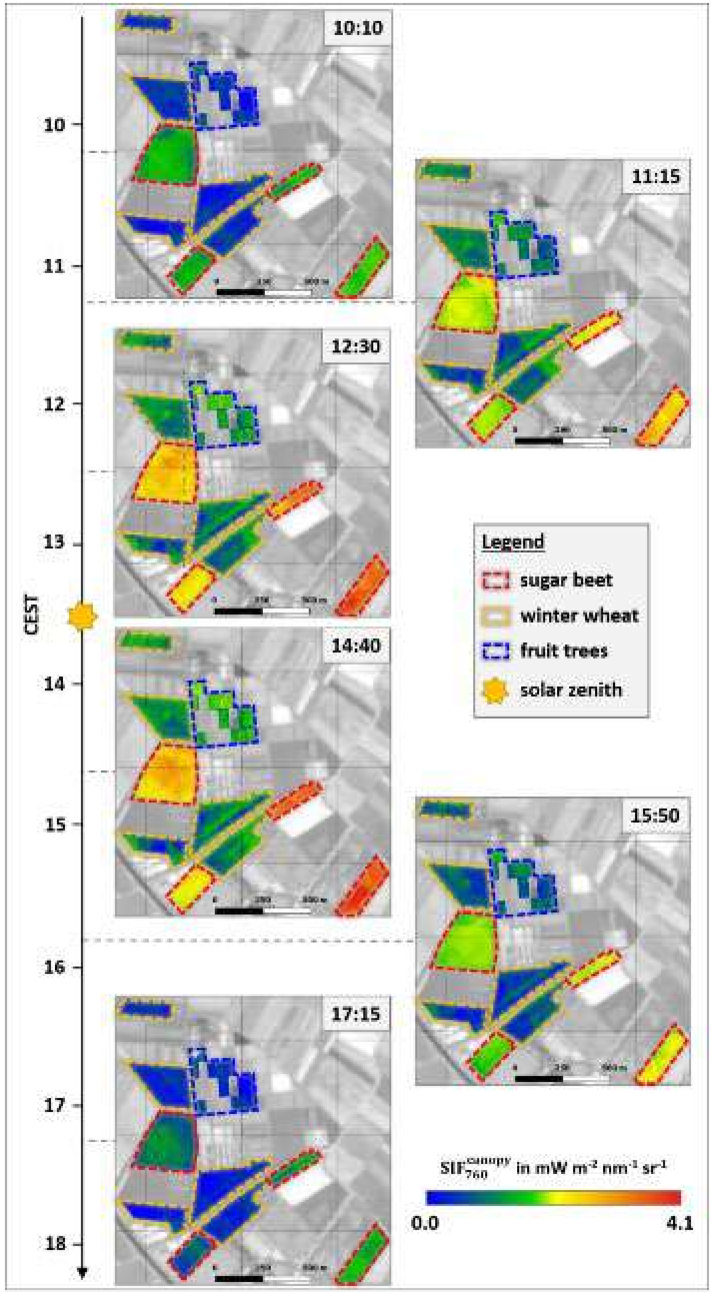
Downscaling of SIF from TOC to leaf level – normalizing for canopy structure

- Diurnal course over heterogeneous fields of experimental research campus (Campus Klein-Altendorf, Uni Bonn)
- Normalization with escape fraction or FCVI reveals leaf-level processes and the dynamic regulation of photosynthesis
- Separating canopy structural from leaf functional processes







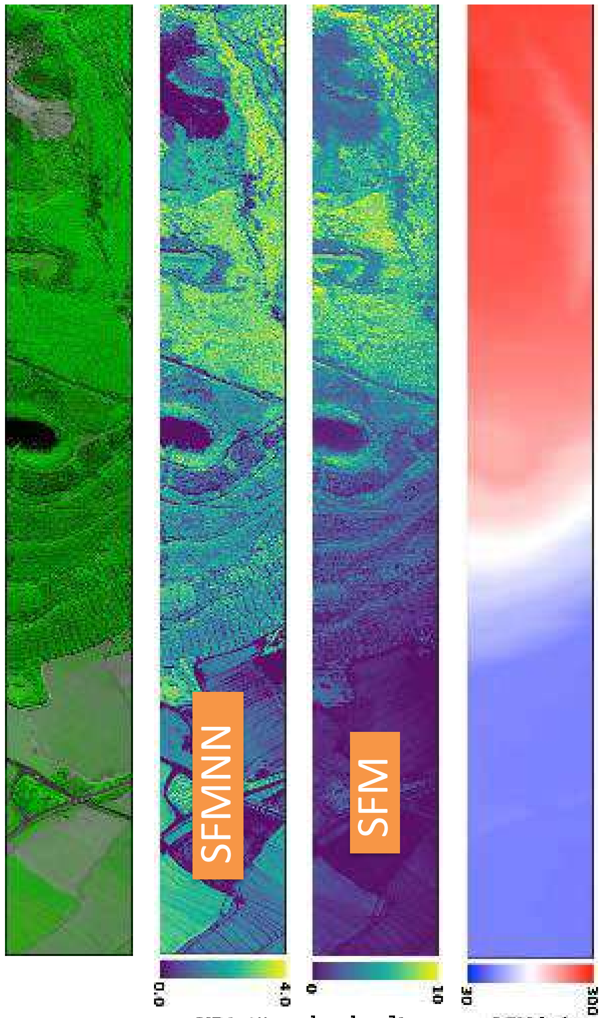
Poster from Bastian Siegmann

Time series over several years (2016-2023) from HyPlant available

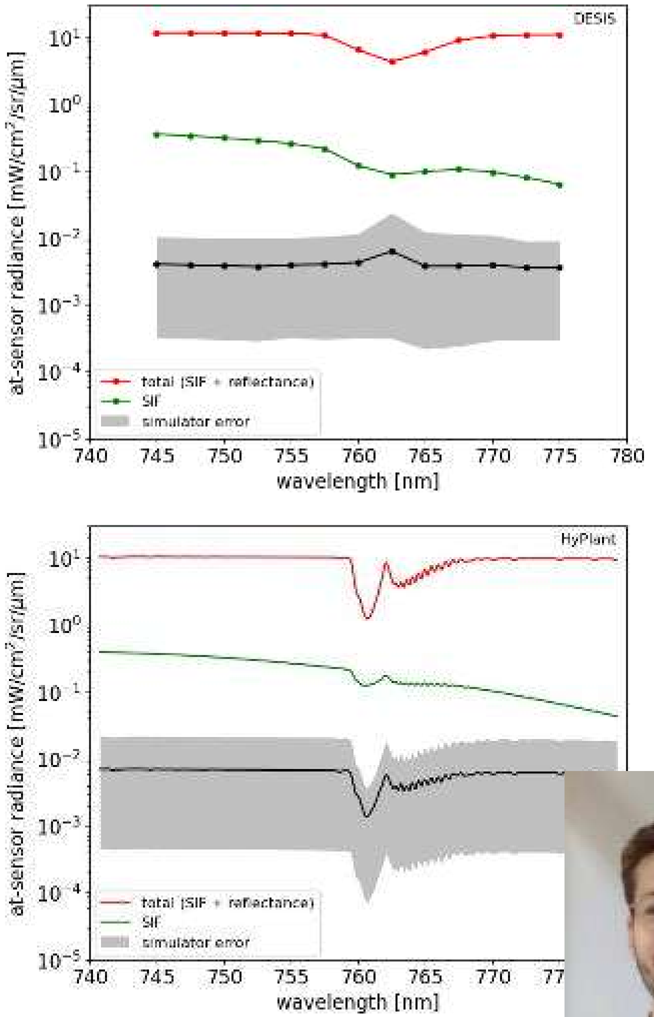
Siegmann et al. (2021) Downscaling of far-red solar-induced chlorophyll fluorescence of different crops from canopy to leaf level using a diurnal data set acquired by the airborne imaging spectrometer HyPlant. *Rem. Sens. Environ.*, 264, article no. 112609

Complementing the physically-based retrievals with machine learning

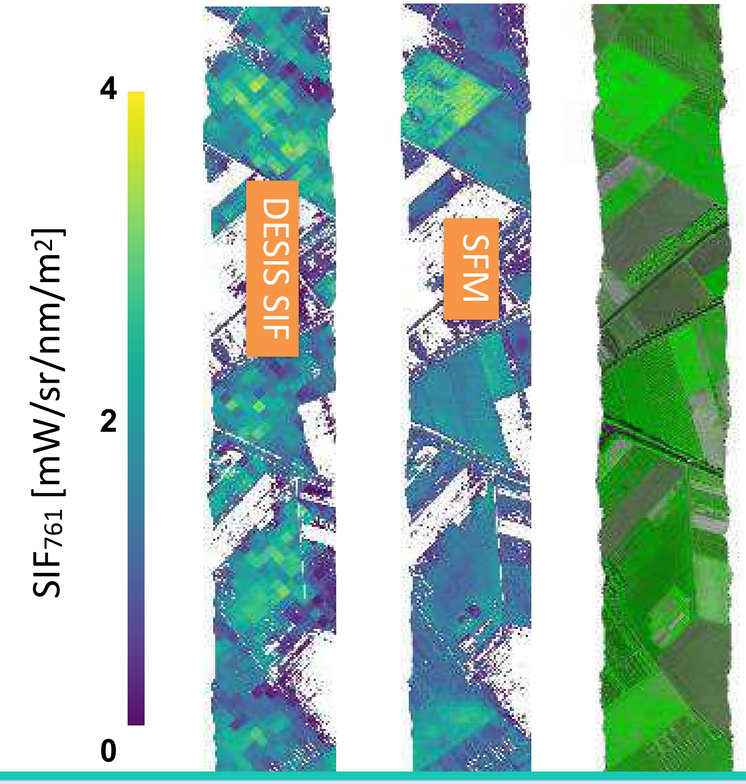
Self-Supervised Spectral Fitting



Simulation & Emulation



SIF prediction for DESIS

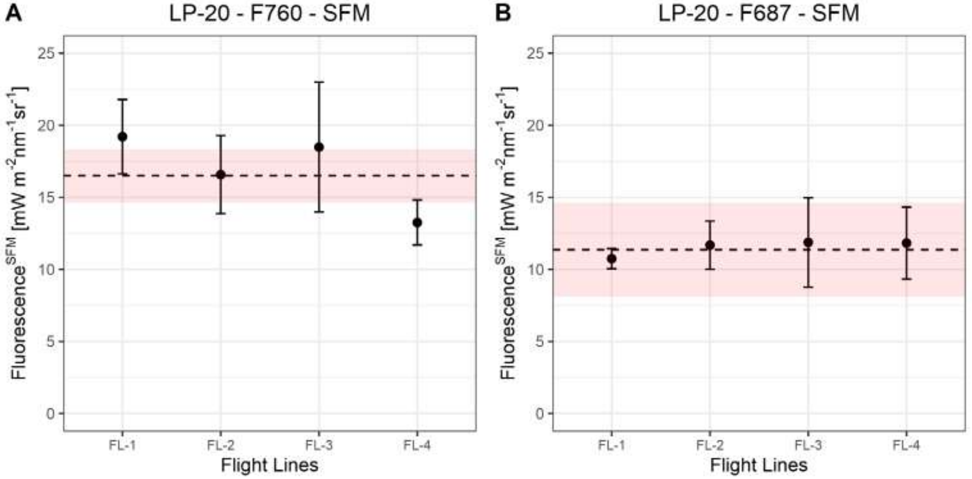


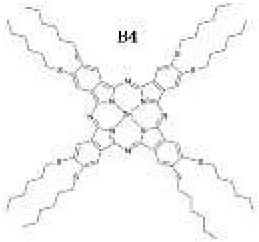
Poster from Jim Buffat & talk Stefan Meier

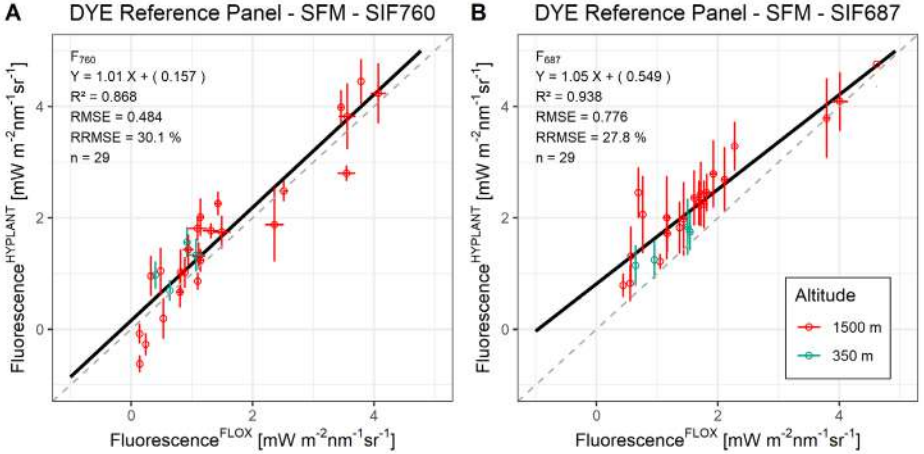
Deep learning-based SIF retrieval for HyPlant & DESIS. Integrating physical and data-driven approach

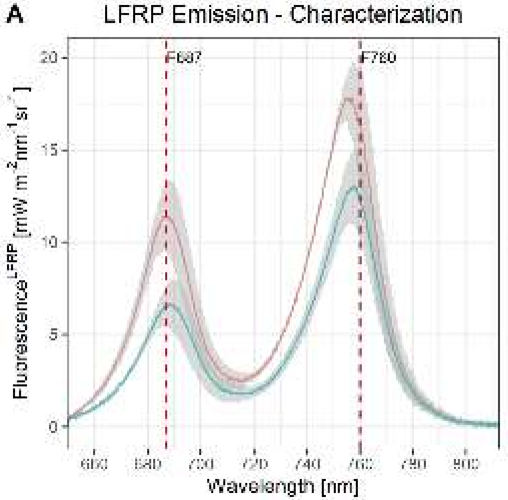
Active and passive reference targets to validate the SIF signal and to facilitate comparability across instruments, sites and time

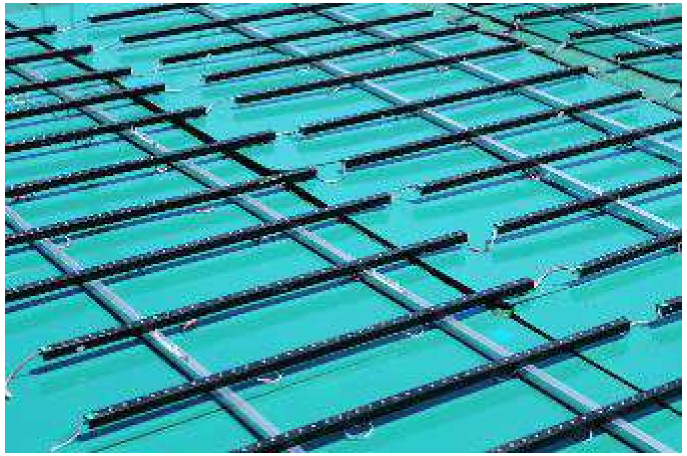
- *Active* reference panels add a known SIF-like signal ⇨ relevant for determining absolute error
- *Passive* reference panels (using fluorescing dye) can be scaled to large areas ⇨ relevant for uncertainty of retrieval







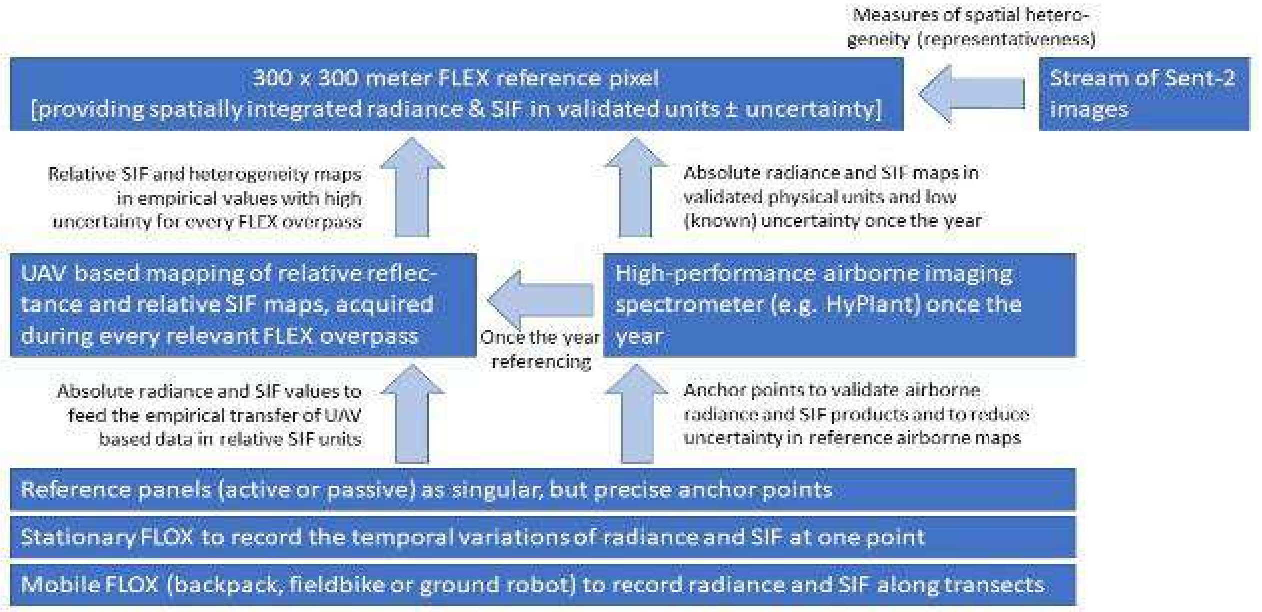




Poster from David Herrera and talk from Bastian Siegmann

Active and passive reference targets to validate the SIF signal and to facilitate comparability across instruments, sites and time

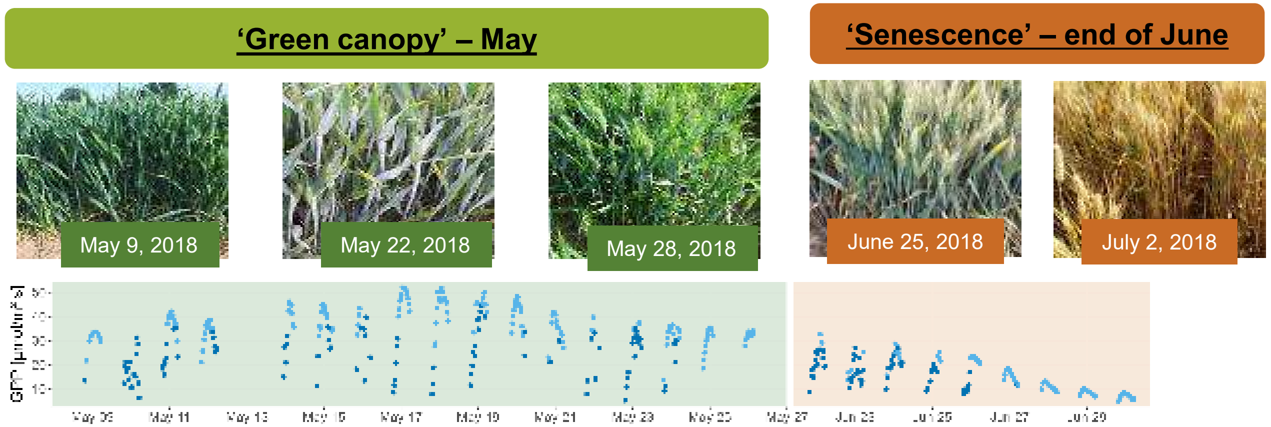
- *Active* reference panels add a known SIF-like signal ⇨ relevant for determining absolute error
- *Passive* reference panels (using fluorescing dye) can be scaled to large areas ⇨ relevant for uncertainty of retrieval
- Comparability across sensors, sites and different measurements along the seasonal cycle

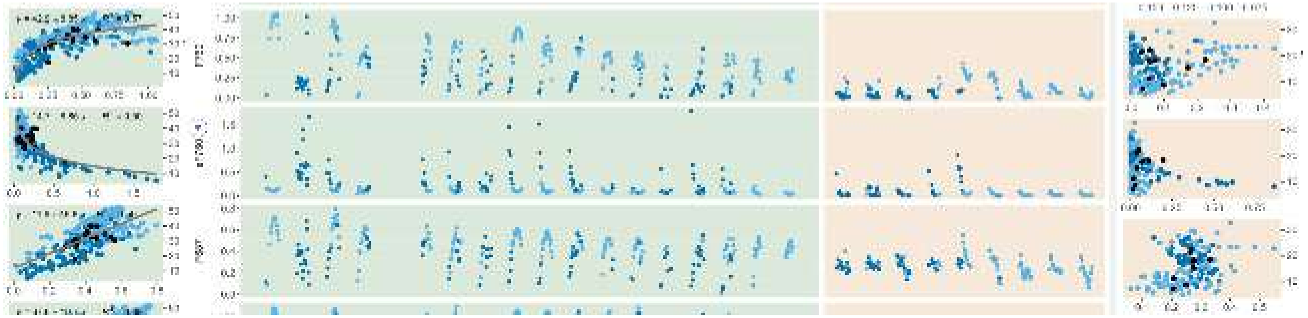


Poster from David Herrera and talk from Bastian Siegmann

Hypothesis 1: SIF is related to GPP and improves forward modelling

- Yes, SIF helps to improve forward modelling of GPP (shown in various studies), but
- there are time windows (in the seasonal cycle), where structural changes are the first order effect (e.g. during early canopy growth and senescence)
- SIF shows greatest improvements in dense canopies and when photosynthesis operates below its maximum capacity (times of stress)

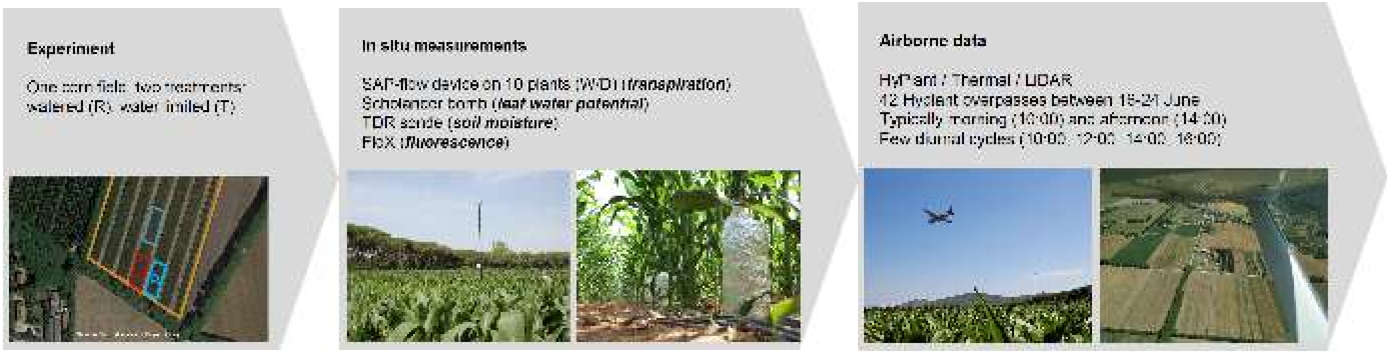


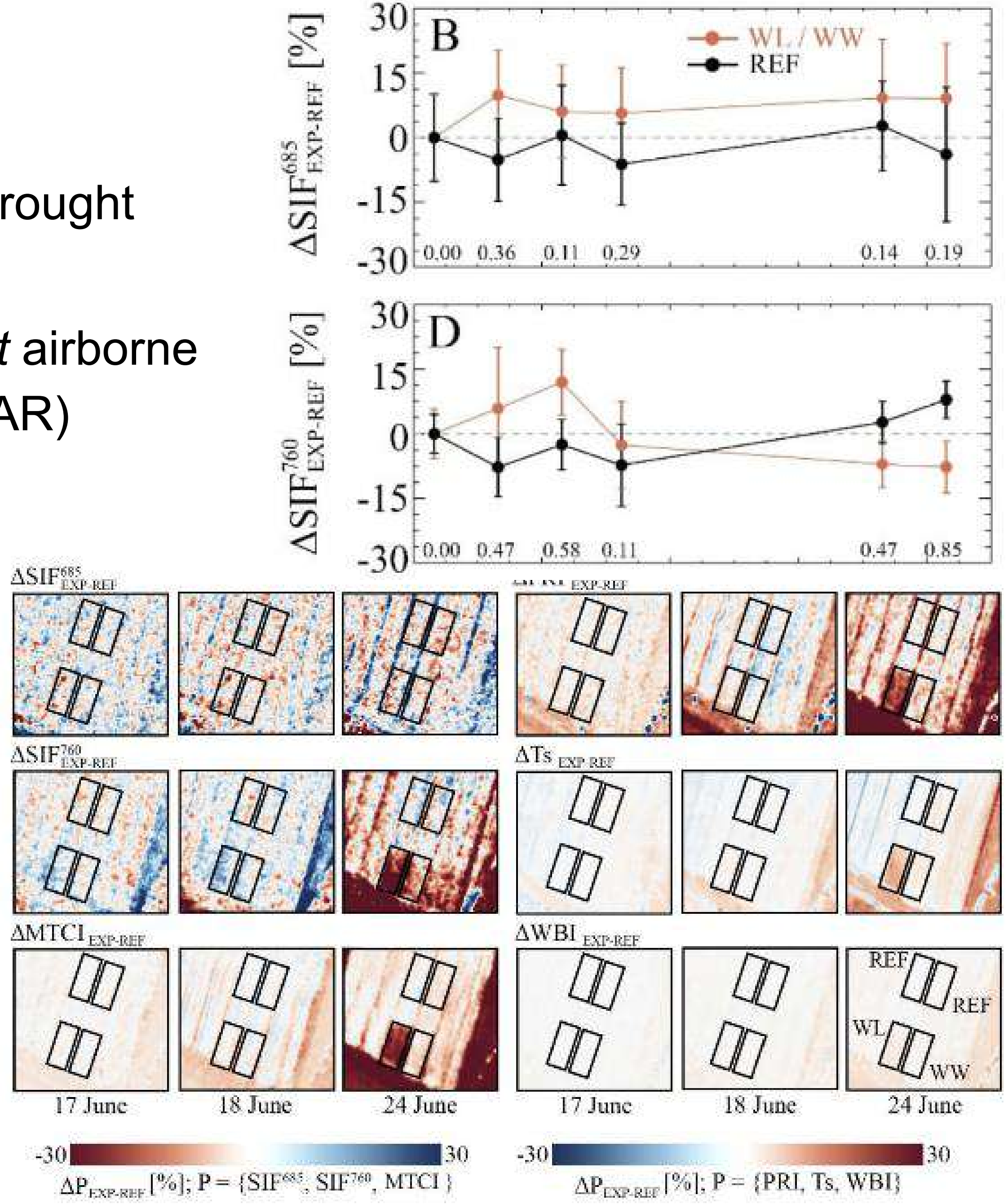


Poster from Vera Krieger
Presentation of Ruonan Chen

SIF to early detect vegetation drought

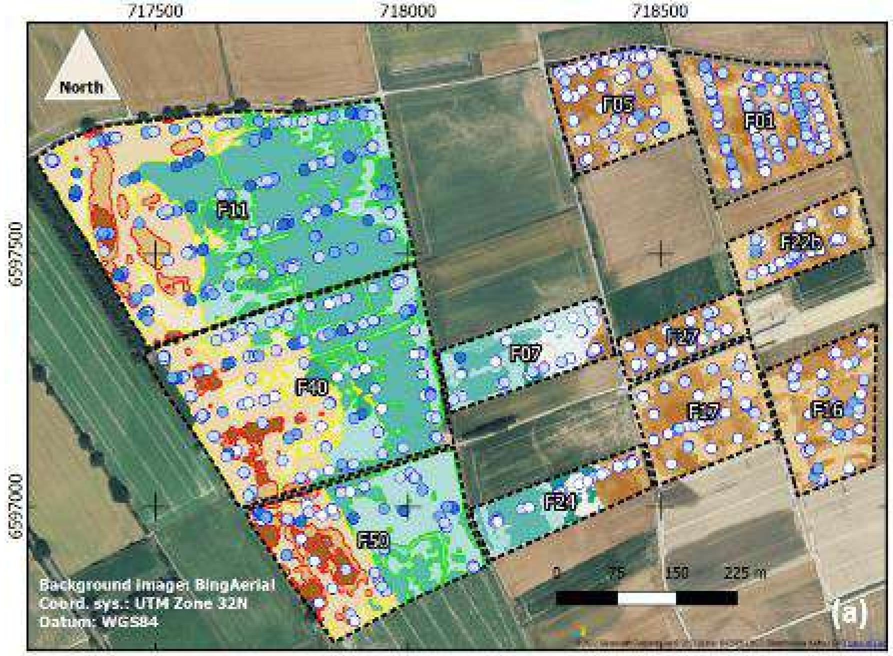
- Controlled drought experiment in Italy with quantitative measurements during the continuous development of drought response
- Complete data set using the full potential of the *HyPlant* airborne package (SIF, hyperspectral reflectance, thermal & LiDAR)
- SIF has the potential to detect early, 'pre-visual' signs of drought stress (complementing thermal and hyper-spectral data)



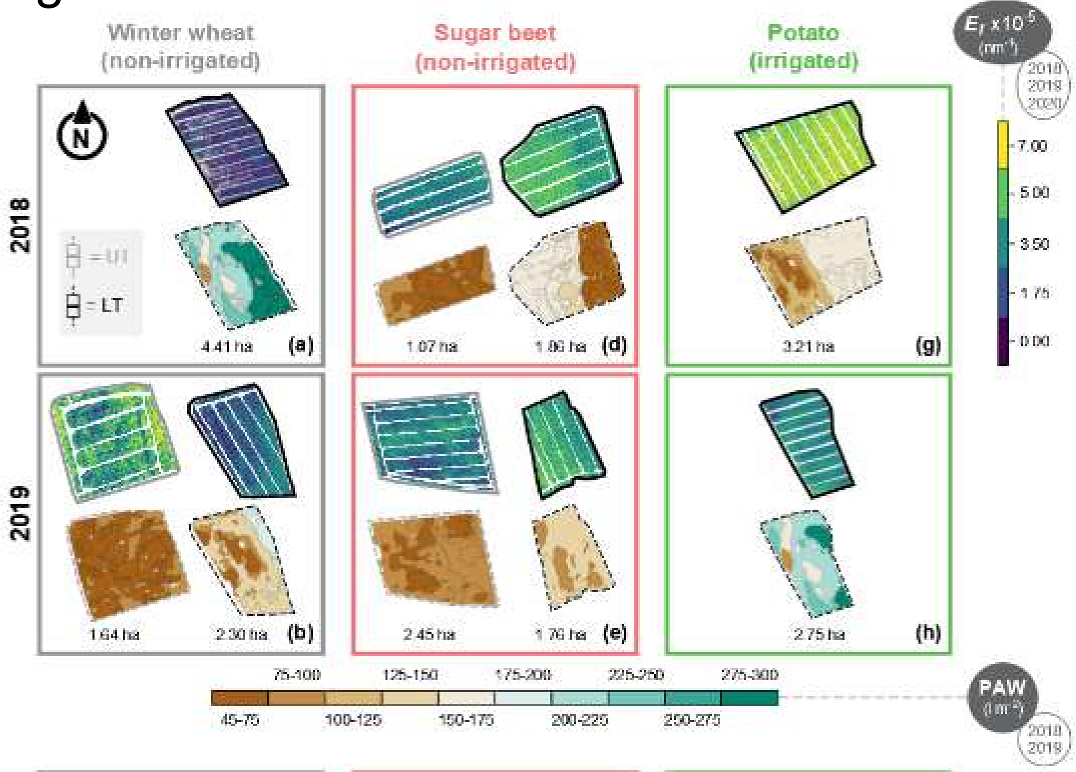


Damm A., et al. (2022) Response times of remote sensing measured sun-induced chlorophyll fluorescence, surface temperature and vegetation indices to evolving soil water limitation in a crop canopy. *Rem. Sens. Environ.*, 273, article no. 112957

SIF to detect vegetation drought (limitation of soil plant available water)



- ➢ Controlled drought experiment in Italy with quantitative and continuous ground and HyPlant measurements showed the potential to detect early, 'pre-visual' signs of drought stress (complementing thermal and hyper-spectral data)
- ➢ New extended study: SIF emission efficiency (ε_{SIF}) is related to plant available water (PAW) in the root zone, demonstrating the functional mechanism of early drought sensitivity



von Hebel C et al. (2018) Understanding soil and plant interaction by combining ground-based quantitative electromagnetic induction and airborne hyperspectral data. *Geophysical Research Letters, 45*, 7571-7579, doi: 10.1029/2018GL078658.

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Quirós-Vargas et al. Spatial relation between solar-induced chlorophyll fluorescence and plant available water in the root zone. *Rem. Sens. Environ.*, to be submitted

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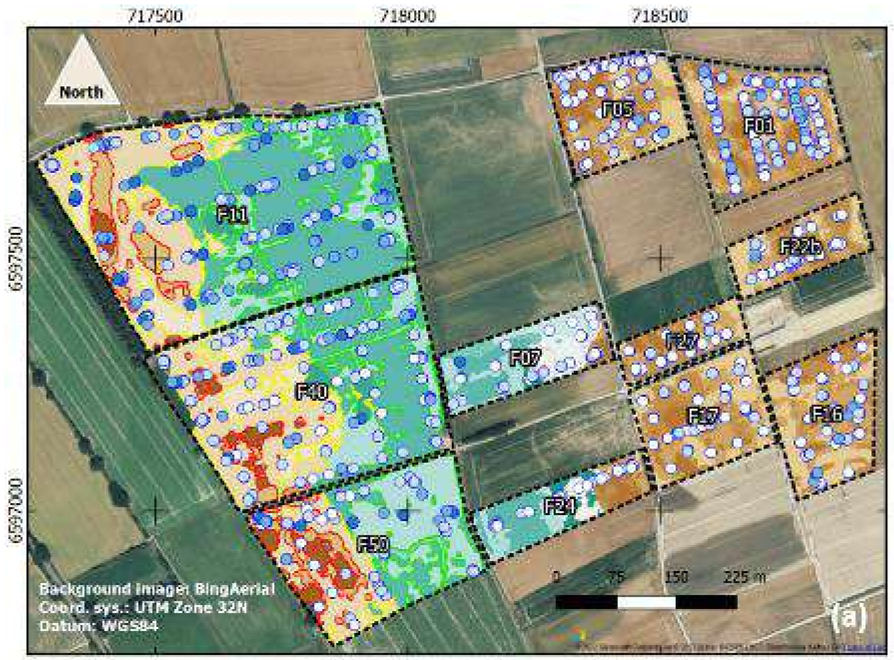

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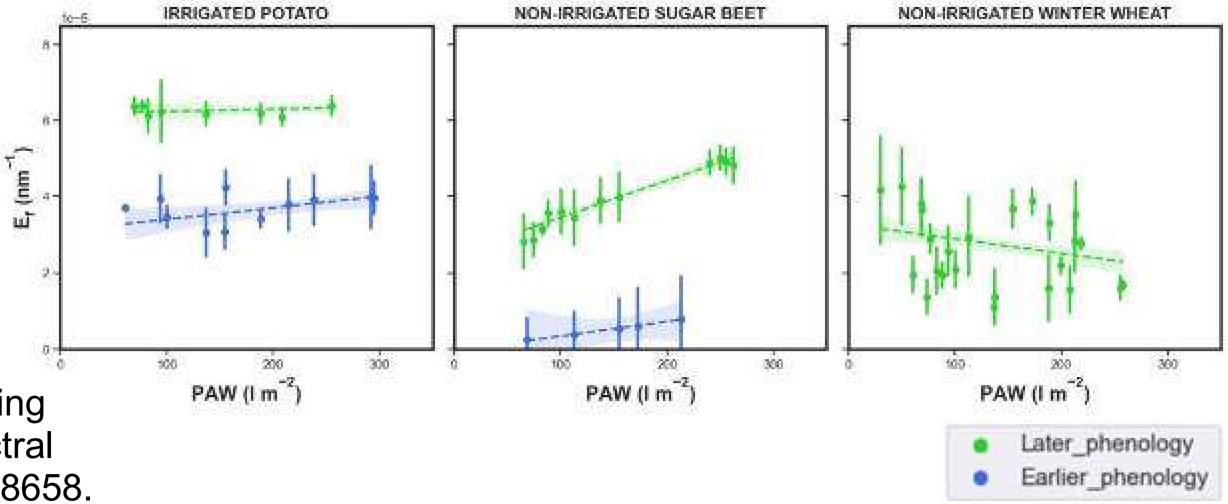

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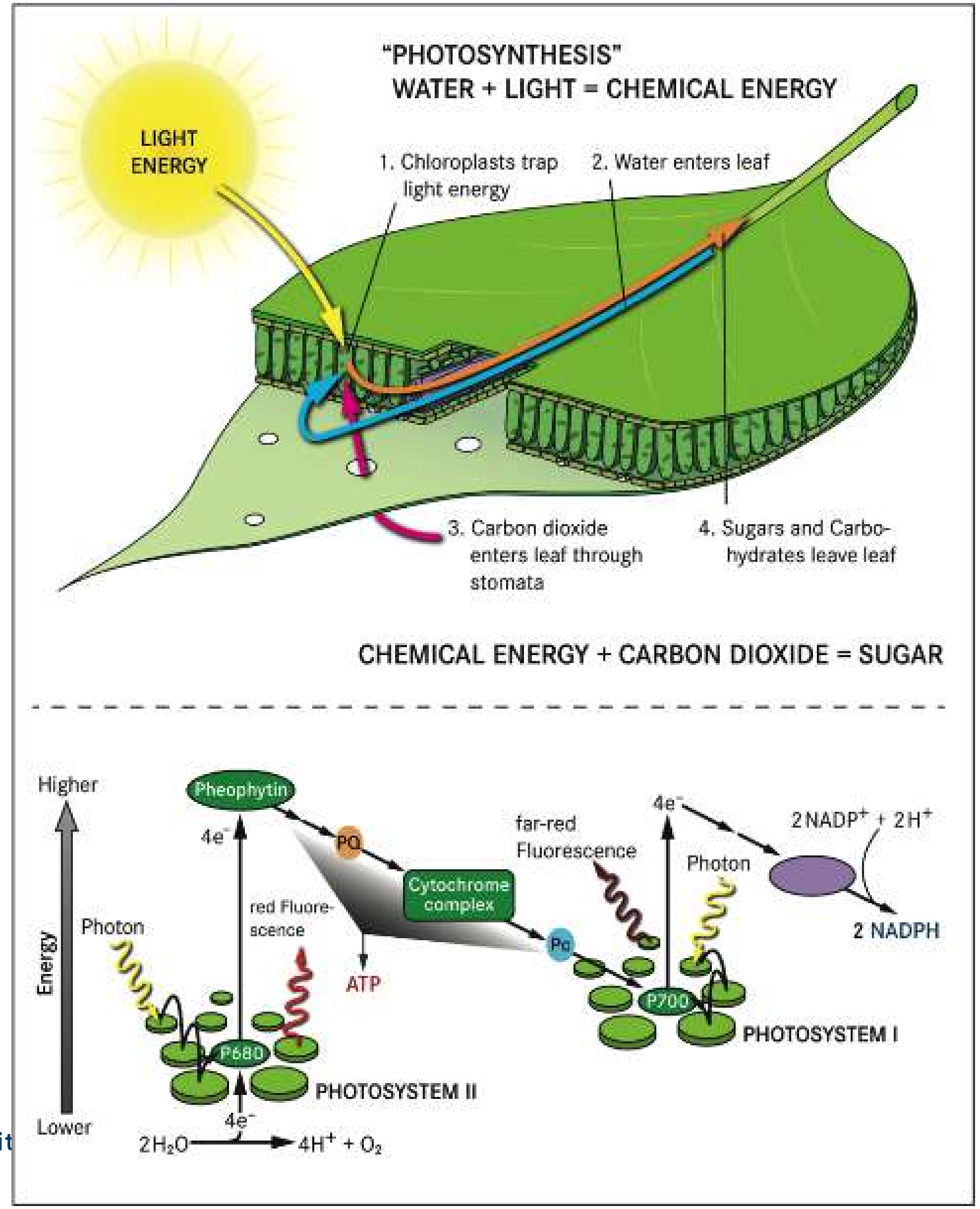
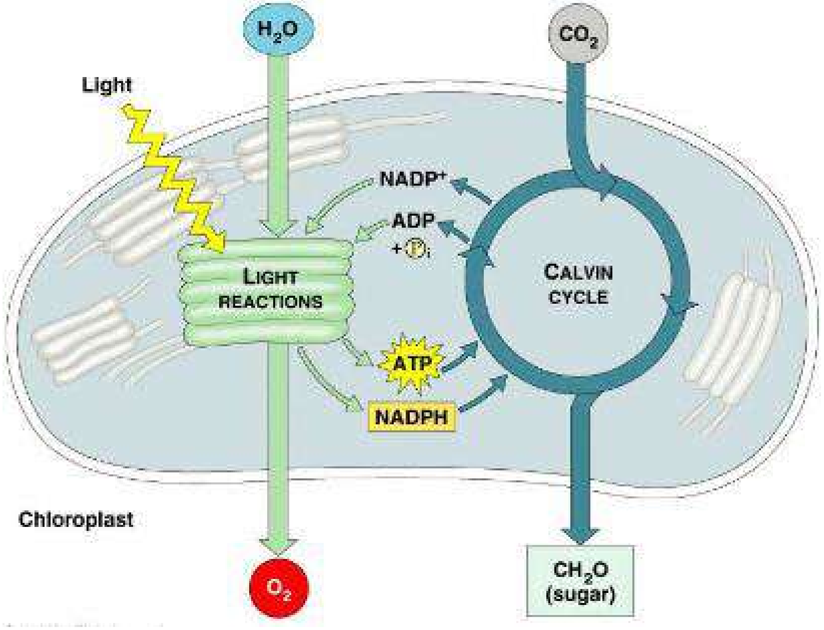
JÜLICH
Forschungszentrum

The origin of fluorescence – an indicator for photosynthetic efficiency



- Photosynthesis is a highly regulated process that involves a cascade of electron transfers (*Light reaction*) to fuel carbon fixation (*Calvin cycle*)
- Fluorescence is emitted from the cores of the photosynthetic machinery: Photosystems I and II
- Energy of ligh-reactions is used to biochemically fix CO_2 from the atmosphere



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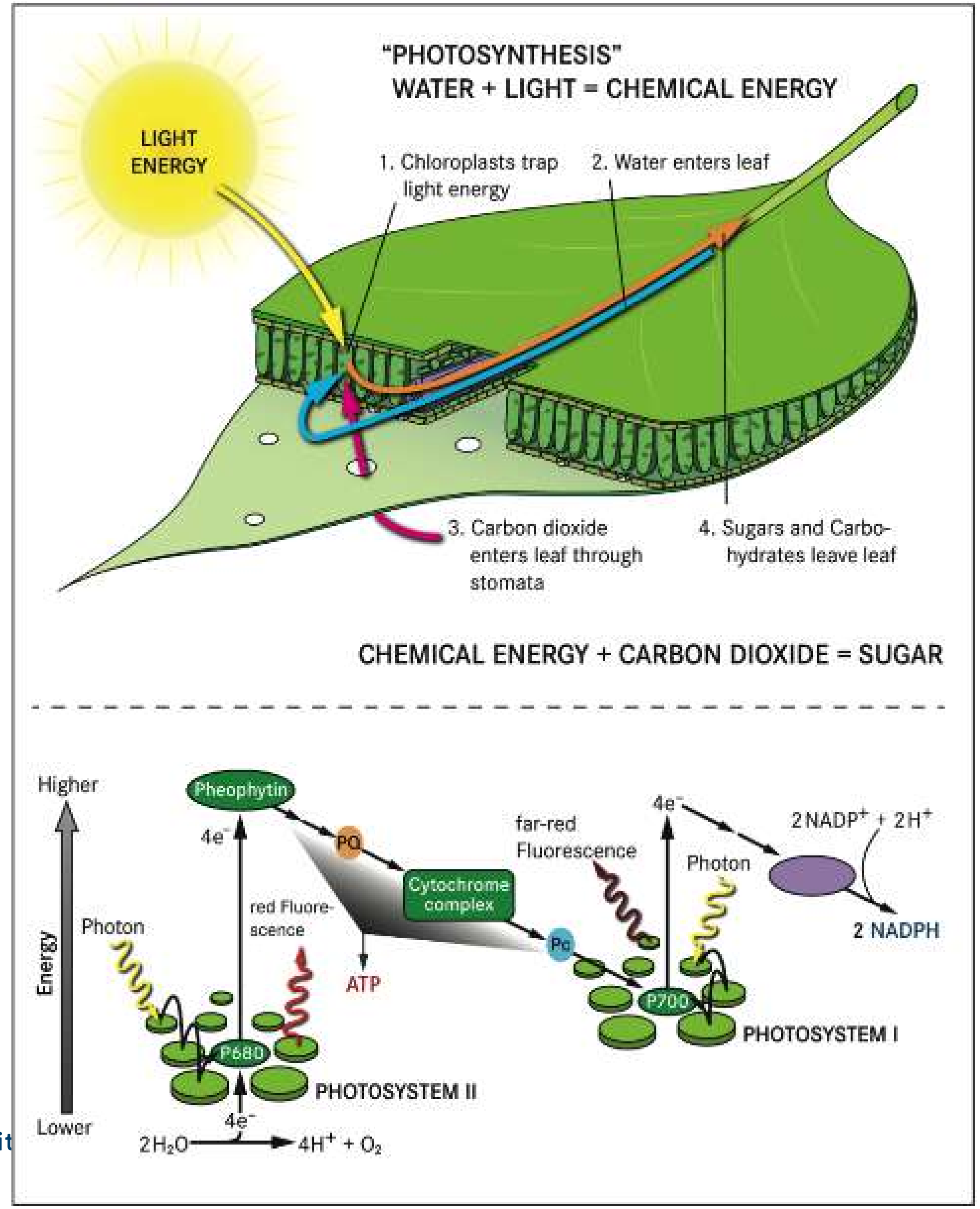

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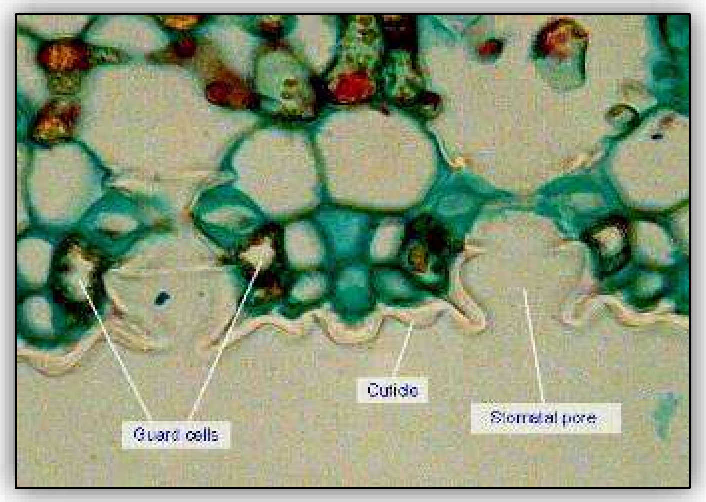
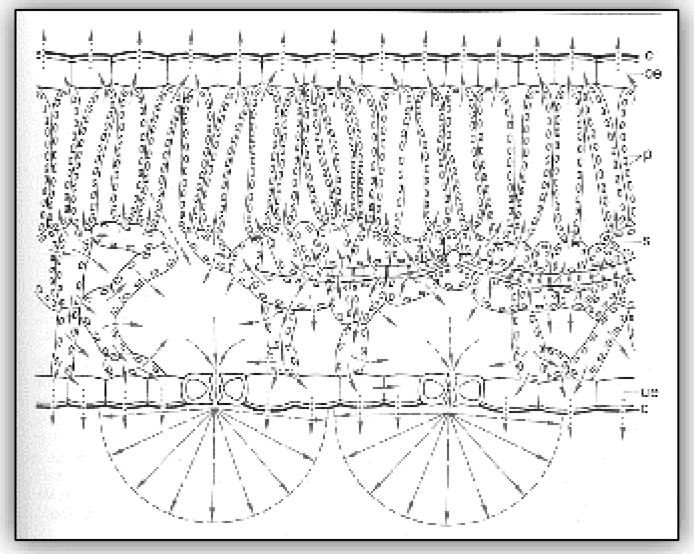
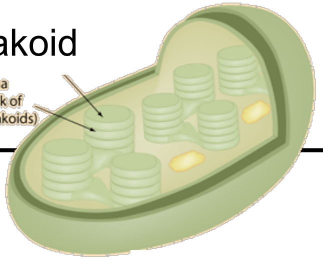
- Fluorescence is emitted from the cores of the photosynthetic machinery: Photosystems I and II
- Energy of ligh-reactions is used to biochemically fix CO_2 from the atmosphere.
- And there are stomata… the maybe ‚most important cells on earth‘ [Joe Berery]





Quantifying the relation between (i) photochemical energy transport, (ii) non-photochemical energy dissipation and (iii) fluorescence

Thylakoid



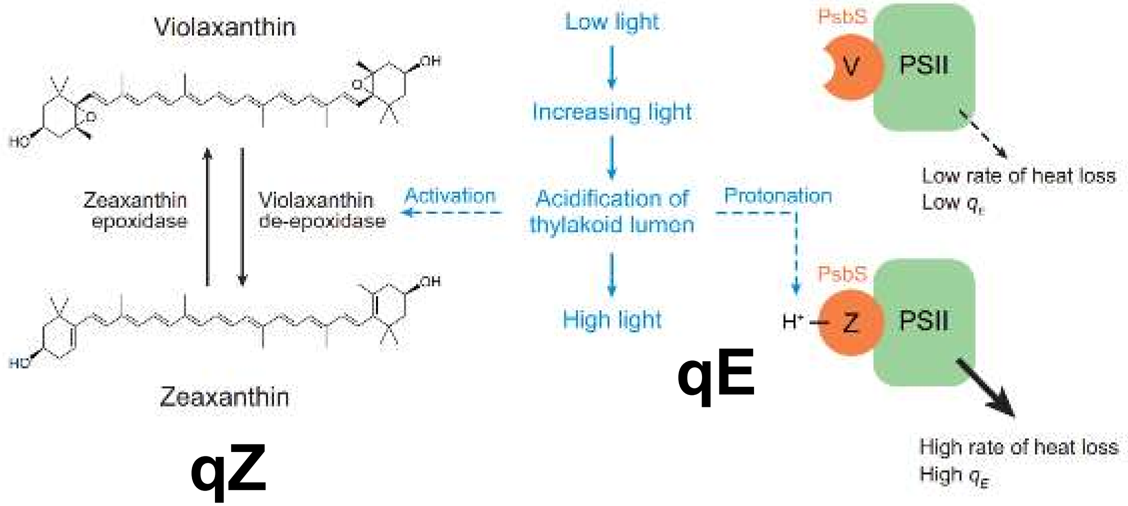
Chloroplast

Photoprotective

vs

Photodamage

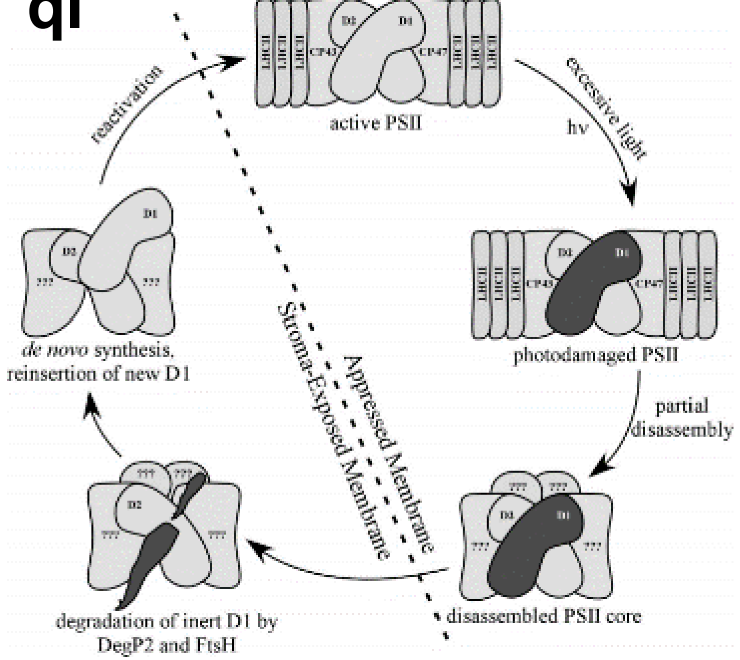
+OEC?



qZ

qE

qI



Baker, 2008. Annu. Rev. Plant Biol.

Yokthongwattana and Melis, 2008. In Govindjee (Eds)

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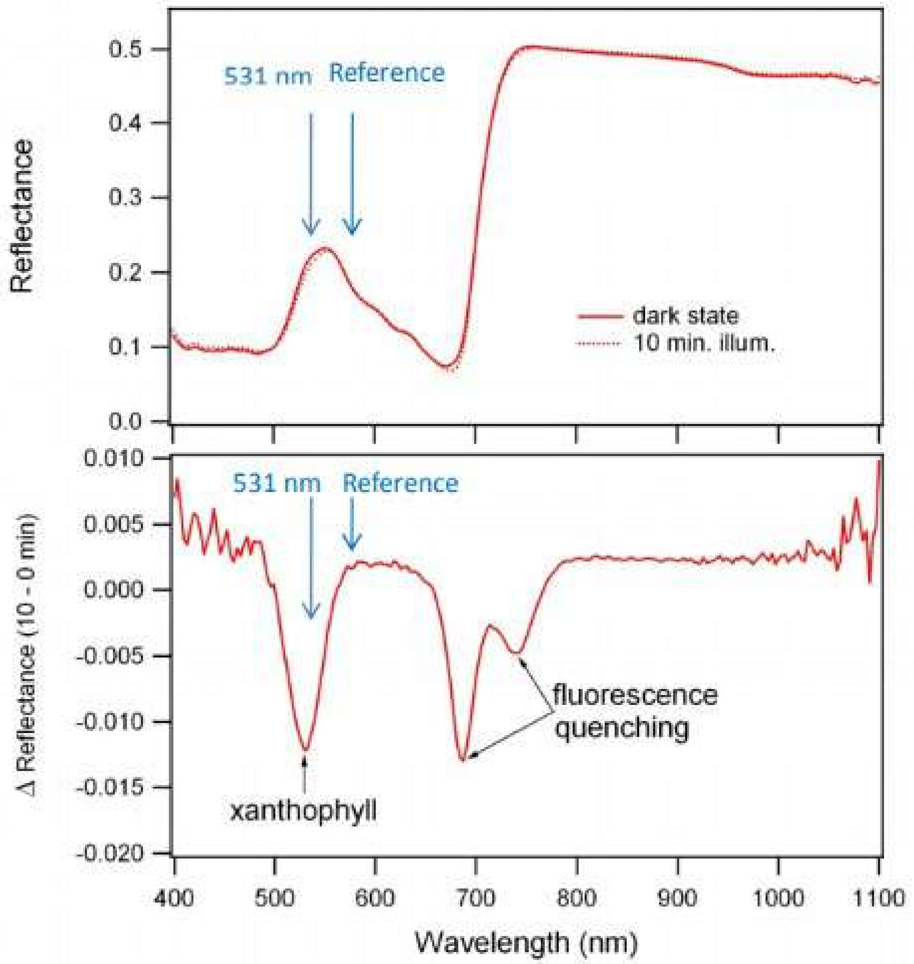
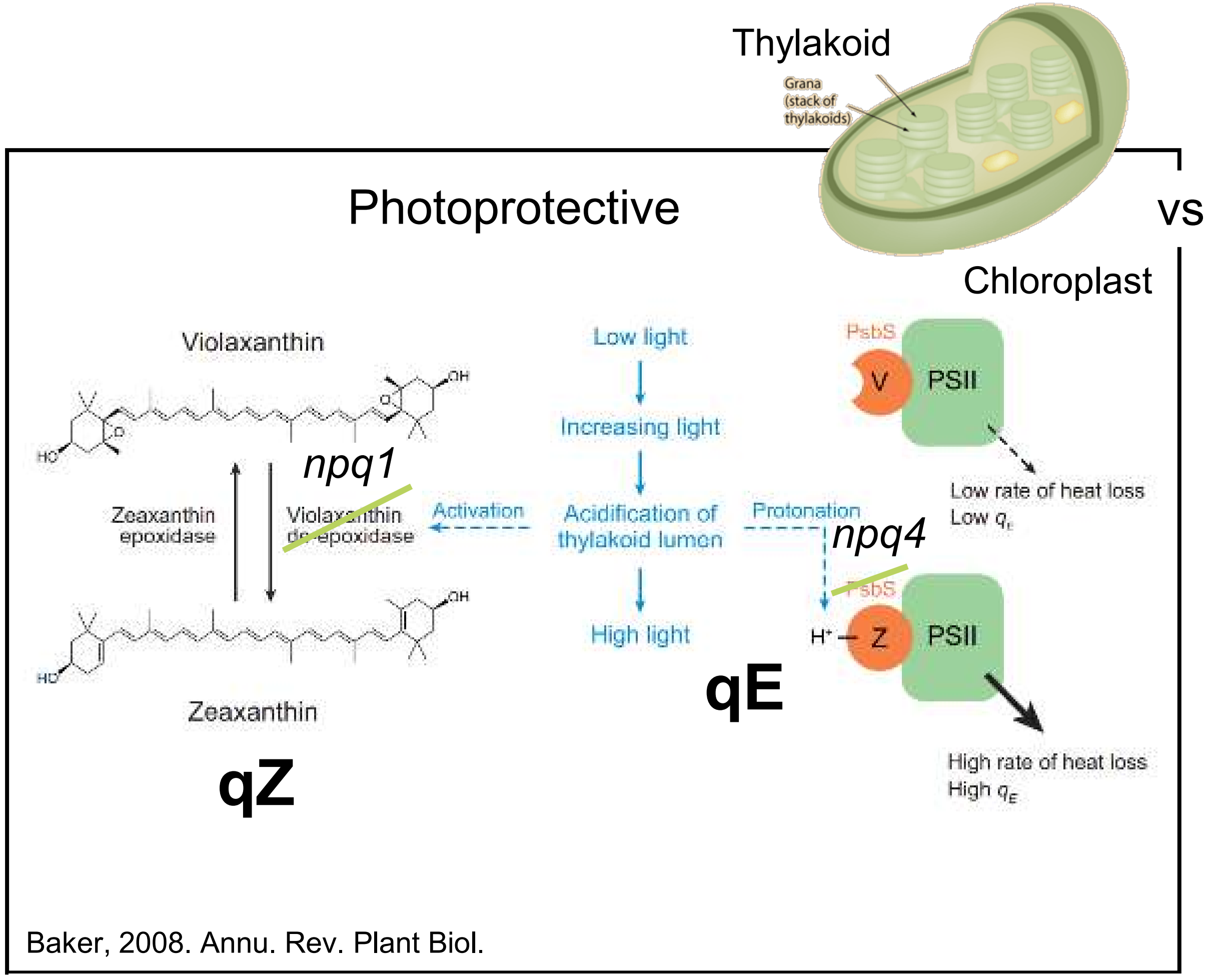


Acebron et al. (2021) Diurnal dynamics of non-photochemical quenching in Arabidopsis npq mutants assessed by solar-induced fluorescence and reflectance measurements in the field. *New Phytologist*, 229, 2104-2119, doi: 10.1111/nph.16984

$$NPQ = \frac{Fm - Fm'}{Fm'} \qquad PRI = \frac{(R_{531} - R_{570})}{R_{531} + R_{570}}$$

- NPQ can be measured by active fluorescence
- In remote sensing, PRI is widely used to exploits reflectance changes at 531 nm, to measure NPQ related to xanthophyll conversion
- However different mechanisms of NPQ, such as sustained photoprotection during overwintering and dynamic (diurnal) acclimation to high light

Quantifying the relation between (i) photochemical energy transport, (ii) non-photochemical energy dissipation and (iii) fluorescence



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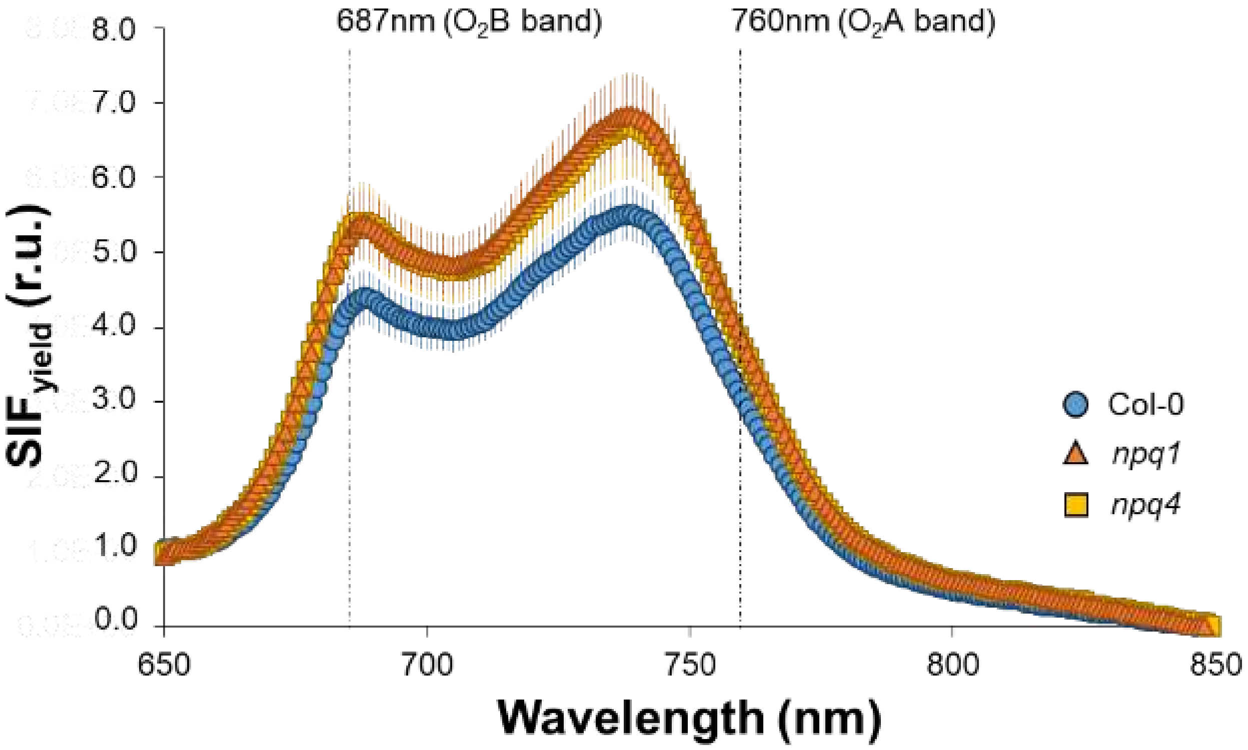
- NPQ mutants provide a very controlled test case to quantitatively understand the relation between specific non-photochemcial energy pathways and steady state fluorescence
- npq1: no conversion of violxanthin to zeaxanthin
- npq4: no binding of zeaxanthin to LHC of photosystem II
- In both mutants greatly reduced NPQ capacity

Quantifying the relation between (i) photochemical energy transport, (ii) non-photochemical energy dissipation and (iii) fluorescence

Passive method confirms increase in SIF emission if NPQ is reduced



- Similar increase in SIF_{yield} despite differences in function of NPQ mutation

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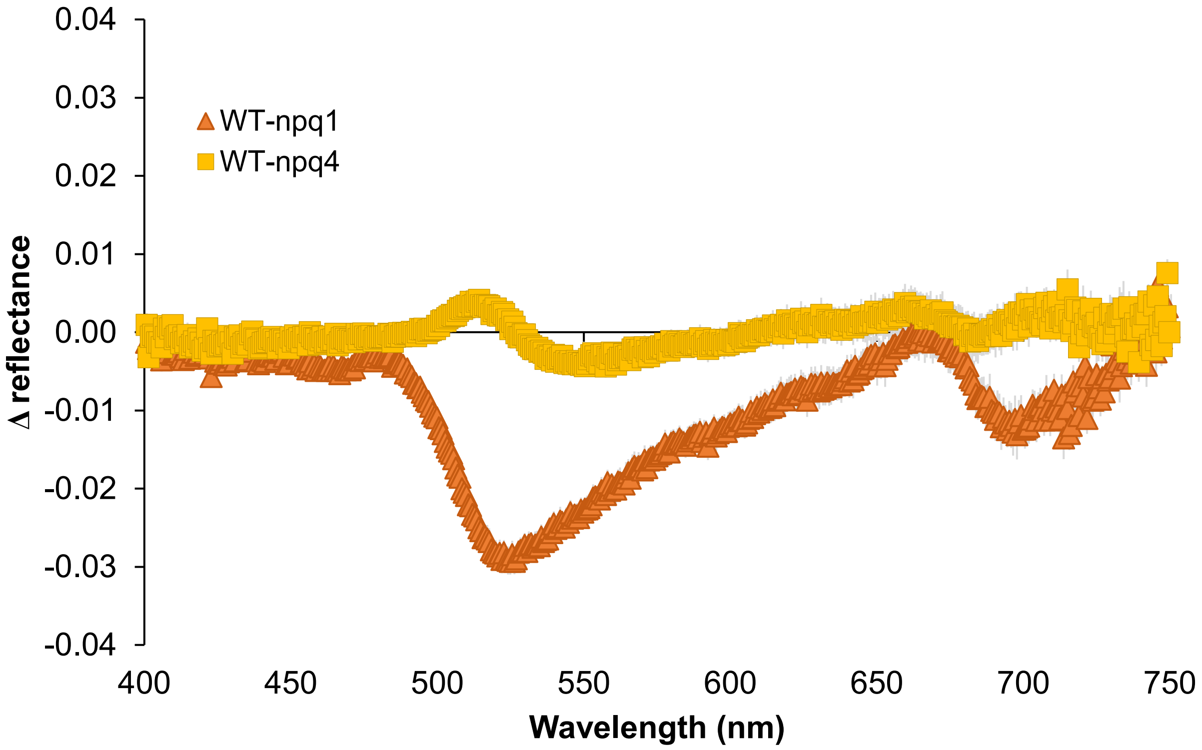

- Similar increase in SIF_{yield} despite differences in function of NPQ mutation
- SIF reflects NPQ deactivation in both mutants
- PRI only reflects zeaxanthin-related NPQ but not PsbS-dependent qE

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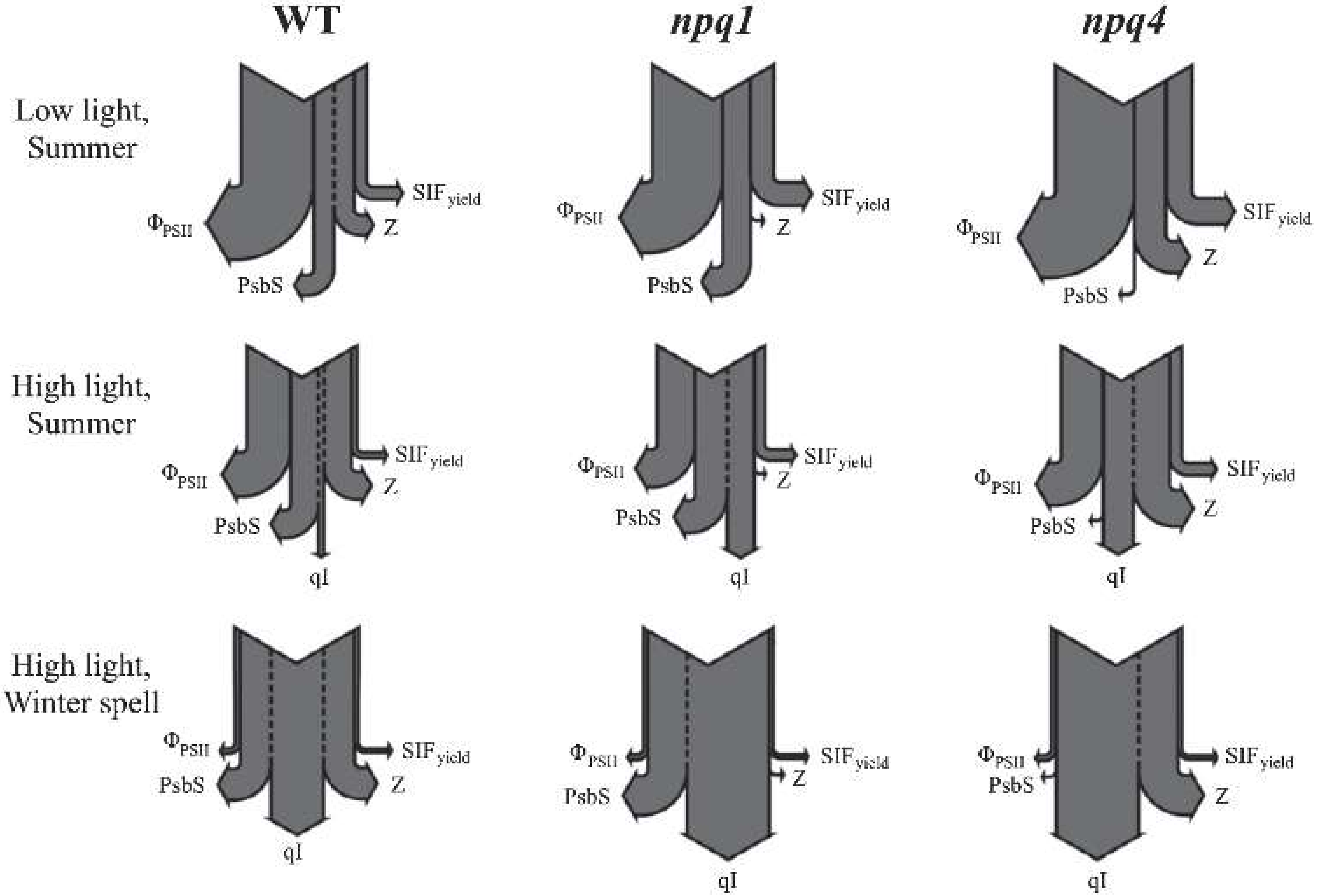

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- *npq1 and npq4* result in a similar increase in SIF_{yield} (PsbS and Zeaxanthin have the same quenching potential for SIF_{yield})
- Sustained photoinhibition may greatly quench SIF_{yield} in the winter. Important consideration in modelling photosynthesis in contrasting seasons and species (e.g. evergreens vs annuals).
- Dynamic (diurnal) changes in NPQ can only partly be tracked with PRI (only sensitive to one quenching pathway), NPQ mechanism affect the relationship between SIF_{yield} and photochemical yield

Conclusions and outlook

- Set of instruments (active and passive fluorescence) are in place and ready to be used to provide the mechanistic knowledge that is needed to related fluorescence to functioning of photosynthesis - Evolution of instruments and retrievals to measure SIF from the leaf, top-of-canopy, UAV, and aircraft
- Testing & discussion of two main FLEX hypothesis
  - SIF is the most direct proxy for actual rates of photosynthesis and improves forward modelling of carbon uptake and evapotranspiration
    Yes, but let's go beyond the fishing for general and linear correlations
  - SIF is able to early detect vegetation stress
    Yes, SIF will help to look into the mechanisms of stress response (may enable early detection)
- SIF beyond satellite observations – plant breeding & sensor guided agricultural management

Many thanks to the numerous partners

University of Zurich UZH
Centre de Recherche Public Gabriel Lippmann
University of Nebraska Lincoln
Specim Spectral Imaging
Carnegie Institution Department of Global Ecology
Extending the Frontiers of Science
Finnish Meteorological Institute
Institut Pierre Simon Laplace
Università degli Studi di Milano Bicocca
ITC University of Twente.
NASA
Goddard Space Flight Center
GFZ Helmholtz Centre Potsdam
metair
meteorological airborne observations measurements and expertise
Consiglio Nazionale delle Ricerche
Jülich Forschungszentrum
CzechGlobe
Helmholtz Centre for Environmental Research – UFZ
Max-Planck-Institut für Biogeochemie
Wageningen University & Research
Universität Bonn
PhenoRob
University of Tasmania
cnes
Hyperspectral Devices
University of Wollongong
European Commission
Joint Research Centre
Fondazione Clima e Sostenibilità
Vniversitat de València
cnrs
University of Cologne
Deutsche Forschungsgemeinschaft DFG
esa
Helmholtz Gemeinschaft
Bundesministerium für Bildung und Forschung
Bundesministerium für Ernährung und Landwirtschaft
cost European Cooperation in Science and Technology
Jülich Forschungszentrum

Two relevant announcements

Hyperspectral imaging of chlorophyll fluorescence across scales PART I: retrieval and modeling trends | PART II: sampling strategies and interpretation

Uwe Rascher, Shari Van Wittenberghe, Bastian Siegmann, Juliane Bendig, MªPilar Cendrero-Mateo

Two relevant announcements: *www.flex-mission.eu*

Collection of publications

Seminars & YouTube channel

User seminars on data products

Calls & Funding assistance

Background infor-mation & Newletter

Uwe Rascher

Juliane Bendig

Ireneusz Kleppert

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