Sensing the Dynamic Response of Photosynthesis to Abiotic Stressors with Forced Oscillating Light

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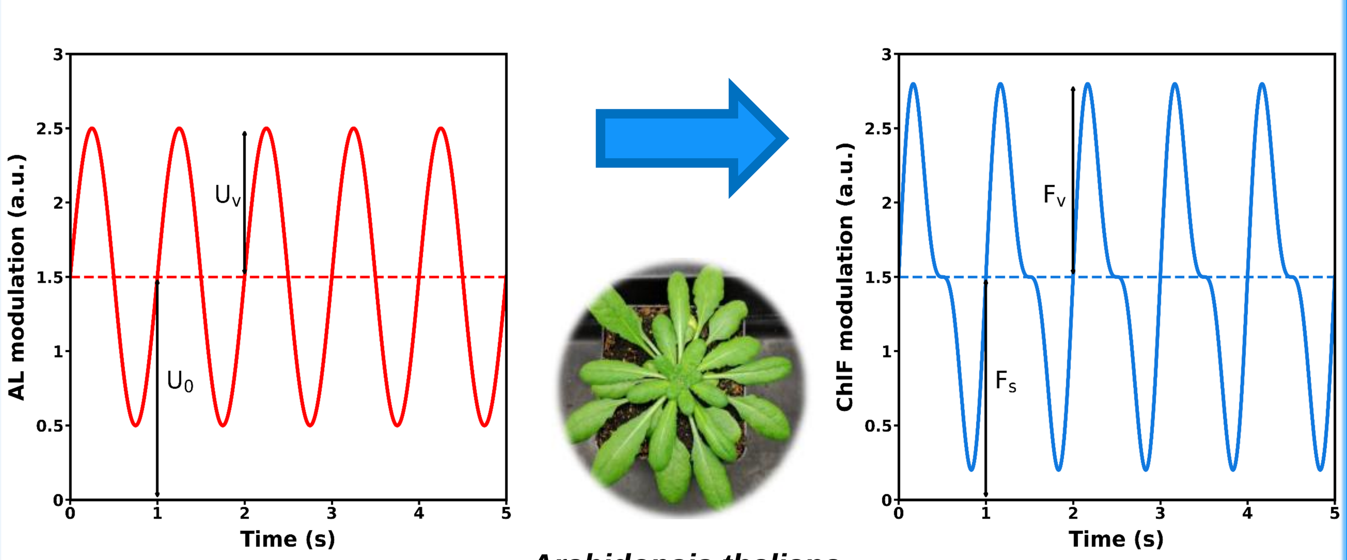
▶ Introduction and Rationale

- In nature, solar irradiance is characterized by **variable fluctuations** in time and intensity.
- Photosynthetic organisms evolved a range of **adaptive mechanisms** to cope with these **dynamic light environments**.
- The response of photosynthesis is typically studied in **steady-state** conditions, such as in **constant light** and with **dark - light** or **low light - high light** transitions.
- To systematically investigate photosynthesis in **non-steady-state** conditions, a frequency-domain chlorophyll fluorescence **(ChlF)**[1],[2] measurement protocol was established. Actinic light was modulated as a **harmonic function** at **selected frequencies**, generating **oscillating (sinusoidal) light**.
- This method was implemented to measure the photosynthesis response in plants exposed to **High Temperature (HT) or High Light (HL)**.
- **Advantage:** This method allows to detect fingerprints of dynamic acclimation in **light-adapted** plants **(no need for dark-adaptation)**.

▶ Materials and Methods

Harmonic modulation

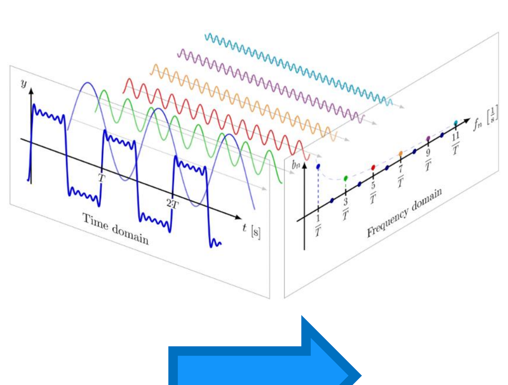


$U_t = U_0 + U_v \sin(2\pi ft)$

Arabidopsis thaliana **(Columbia-0)**

$F_t = F_s + F_v(f) \sin[(ft +/- \Phi(f)]$

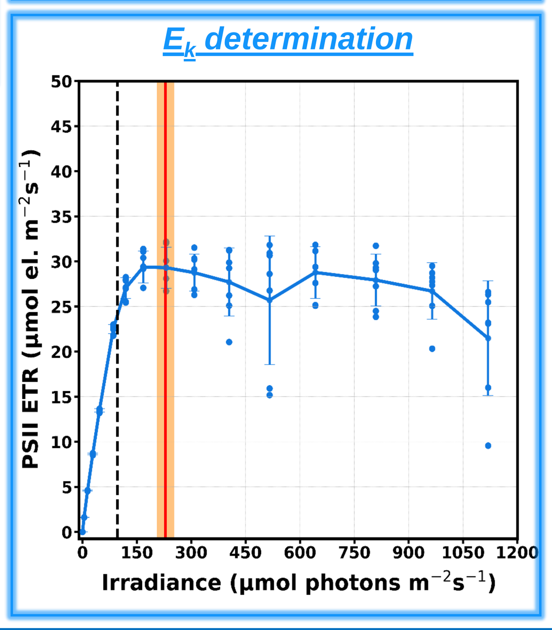
Fast Fourier Transform (FFT)



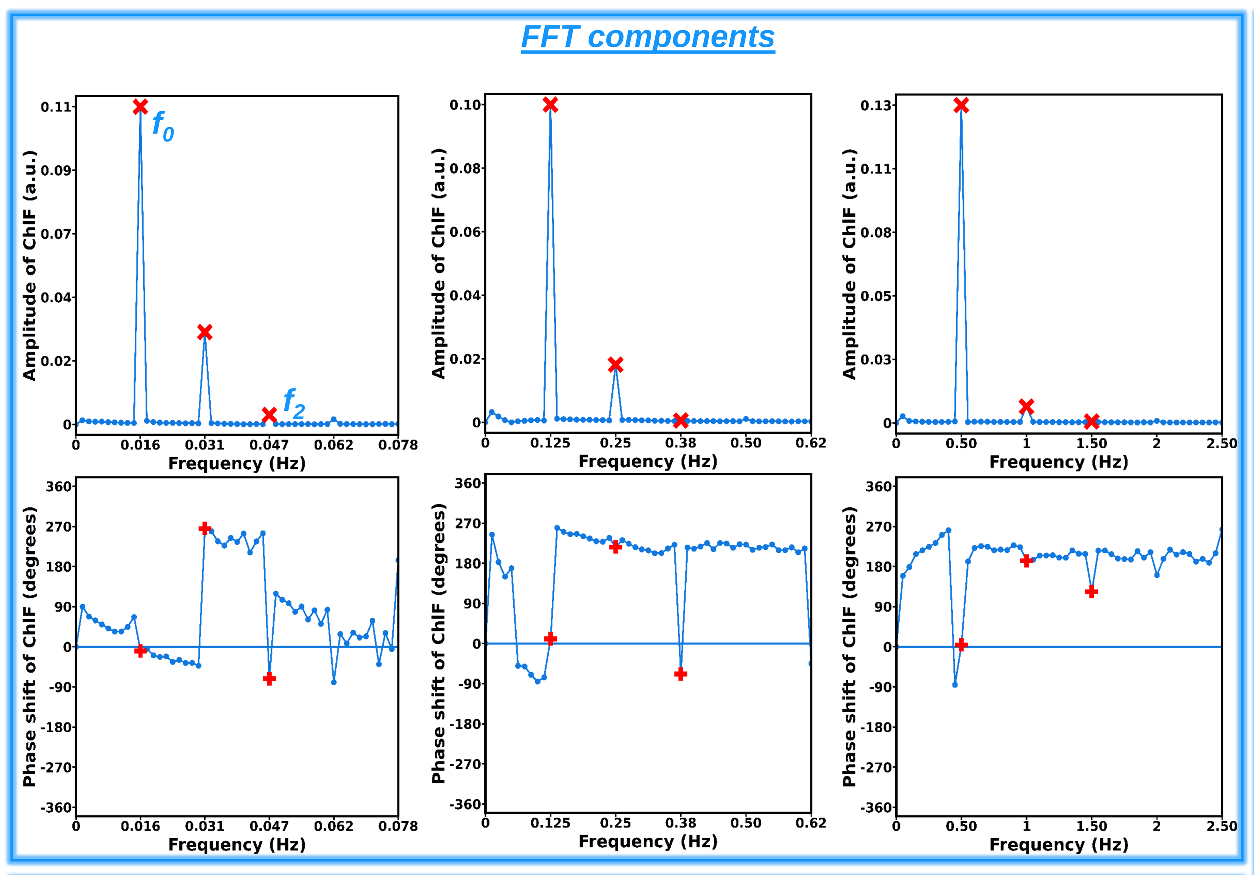
$$ReX[k] = \sum_{i=0}^{N-1} x[i]\cos(2\pi k i/N)$$

$$ImX[k] = -\sum_{i=0}^{N-1} x[i]\sin(2\pi k i/N)$$

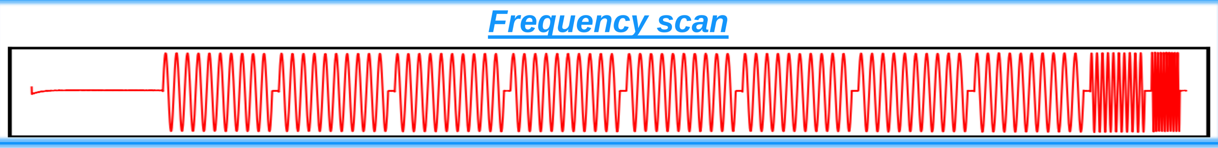
E_k determination



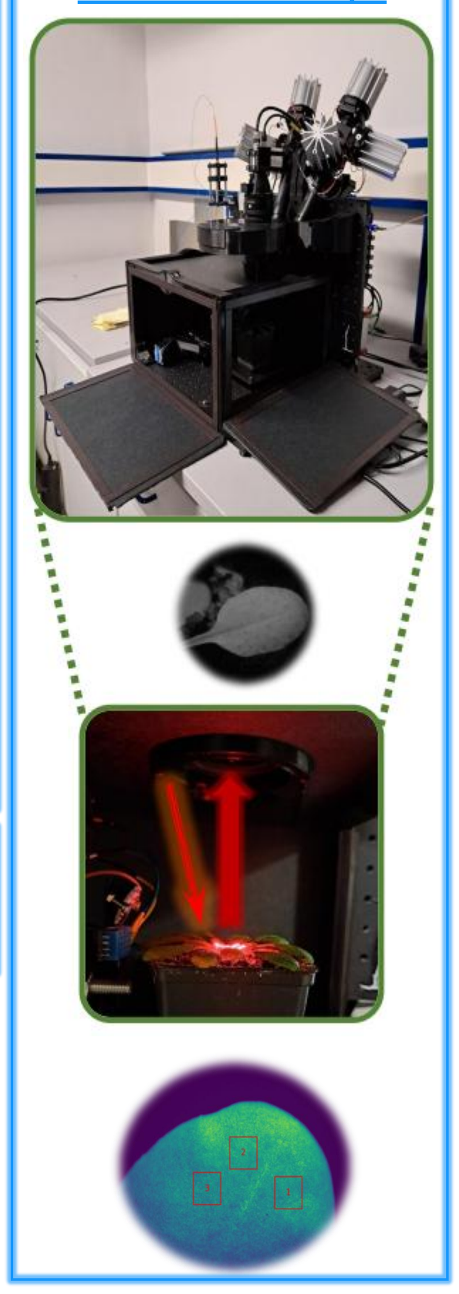
FFT components



Frequency scan



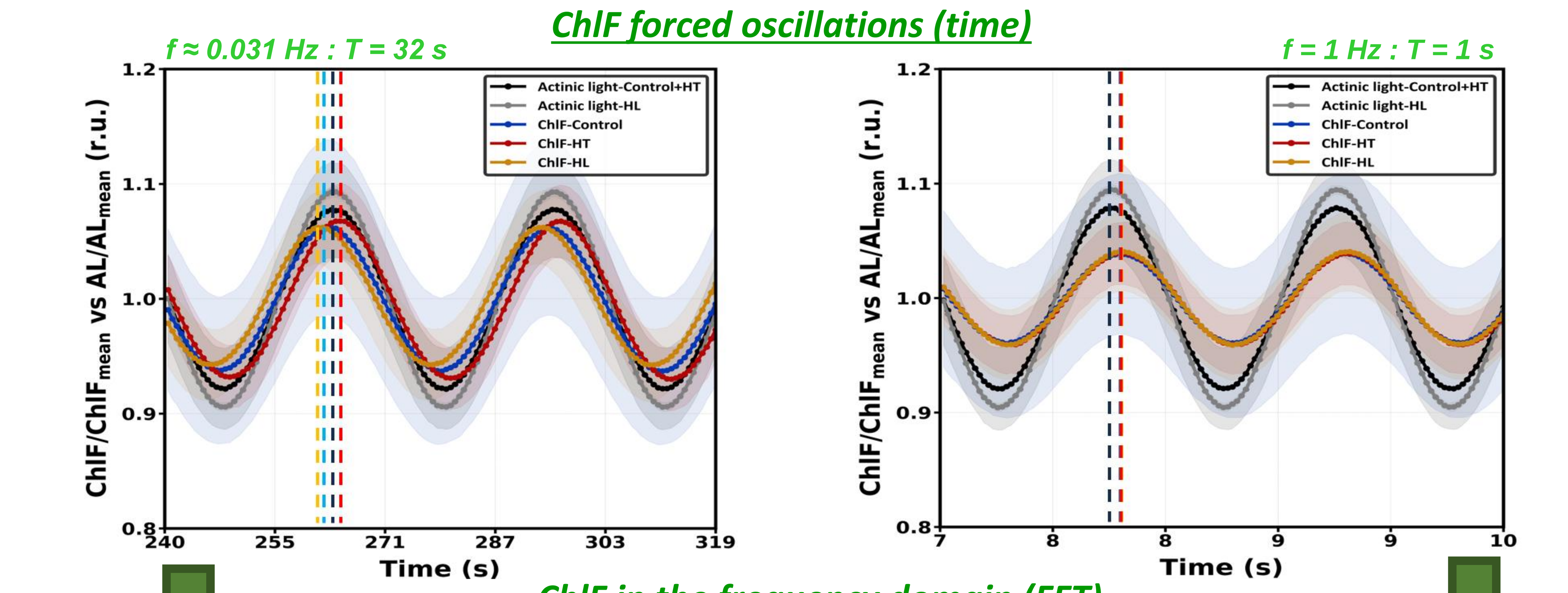
DREAM Macroscope



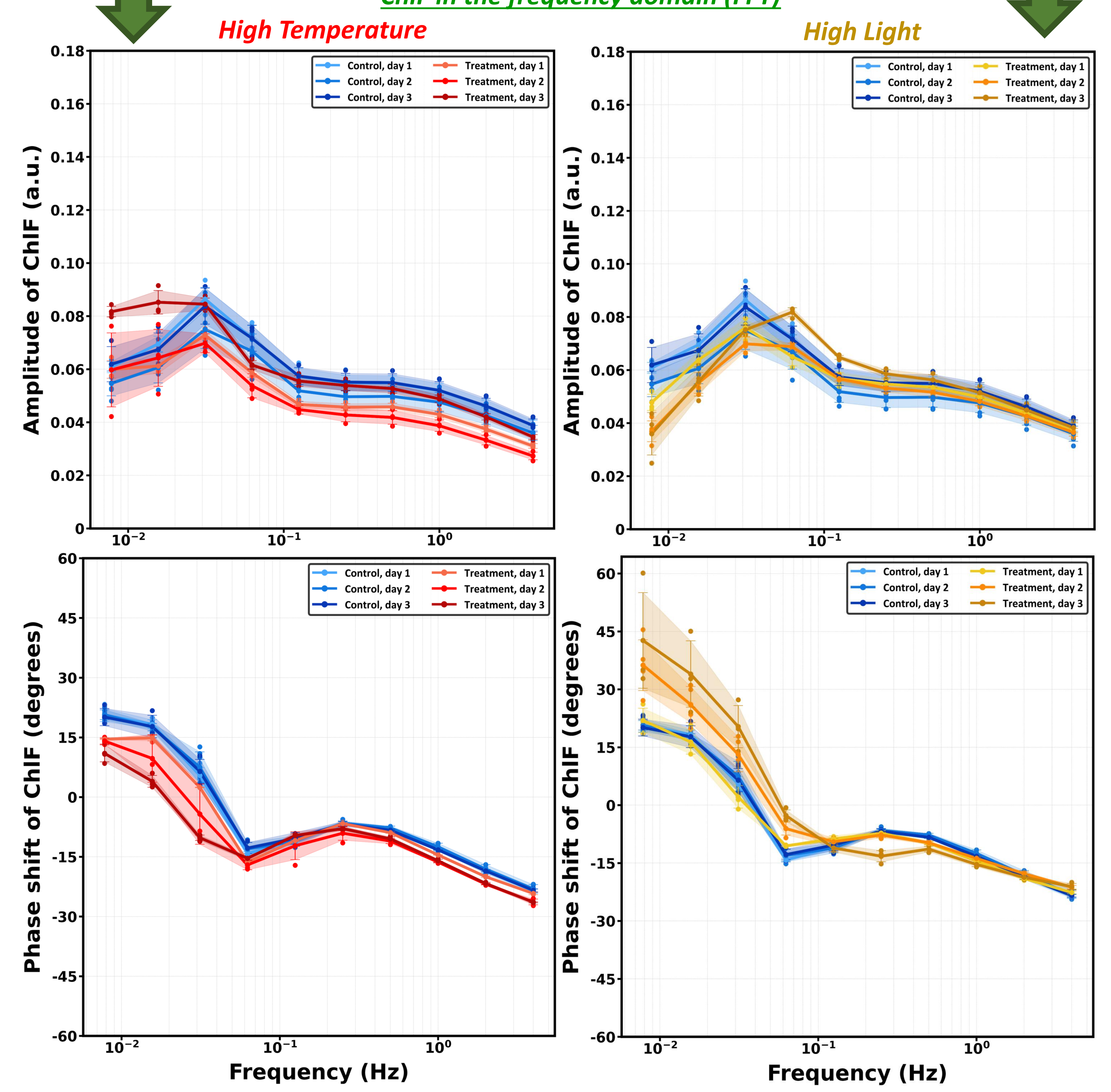
- **Control:** irradiance ≈ **80-90 µE m^{-2} s^{-1}**; air temperature ≈ **22-24°C** (light) and ≈ **16-17°C** (dark); leaf temperature ≈ **22-24°C** during measurements.
- **HT:** irradiance ≈ **100-110 µE m^{-2} s^{-1}**; air temperature ≈ **41-42°C** (light) and ≈ **25-26°C** (dark), with intermediate steps at ≈ **36-38°C**; leaf temperature ≈ **22-24°C** during measurements.
- **HL:** irradiance at ≈ **500-550 µE m^{-2} s^{-1}**, with intermediate steps at ≈ **250-260 µE m^{-2} s^{-1}**; air temperature ≈ **22-24°C** (light) and ≈ **16-17°C** (dark); leaf temperature ≈ **22-24°C** during measurements.
- Photoperiod was **12h/12h light/dark**.
- **Mature plants** were exposed to **HT** or **HL** for **three consecutive days** and measured every day at **+4 hours** in the light period.
- A novel **macroscopic fluorescence imaging system** was implemented **(Dr. Ian Coghill, CNRS, Paris, France, DREAM)**.
- **$\lambda_{excitation}$ = 645 nm; $\lambda_{detection}$ = 690 nm (50 nm bandwidth).**
- A specific illumination protocol was applied, based on **the light saturation coefficient E_k[3]**. $U_0 \approx 2.5\ E_k$ and $U_v \approx 0.25\ E_k$.
- **Frequency-scan** from ≈ 0.008 Hz (T = 128 s) to **4 Hz** (T = 0.25 s).

▶ Results and Conclusion

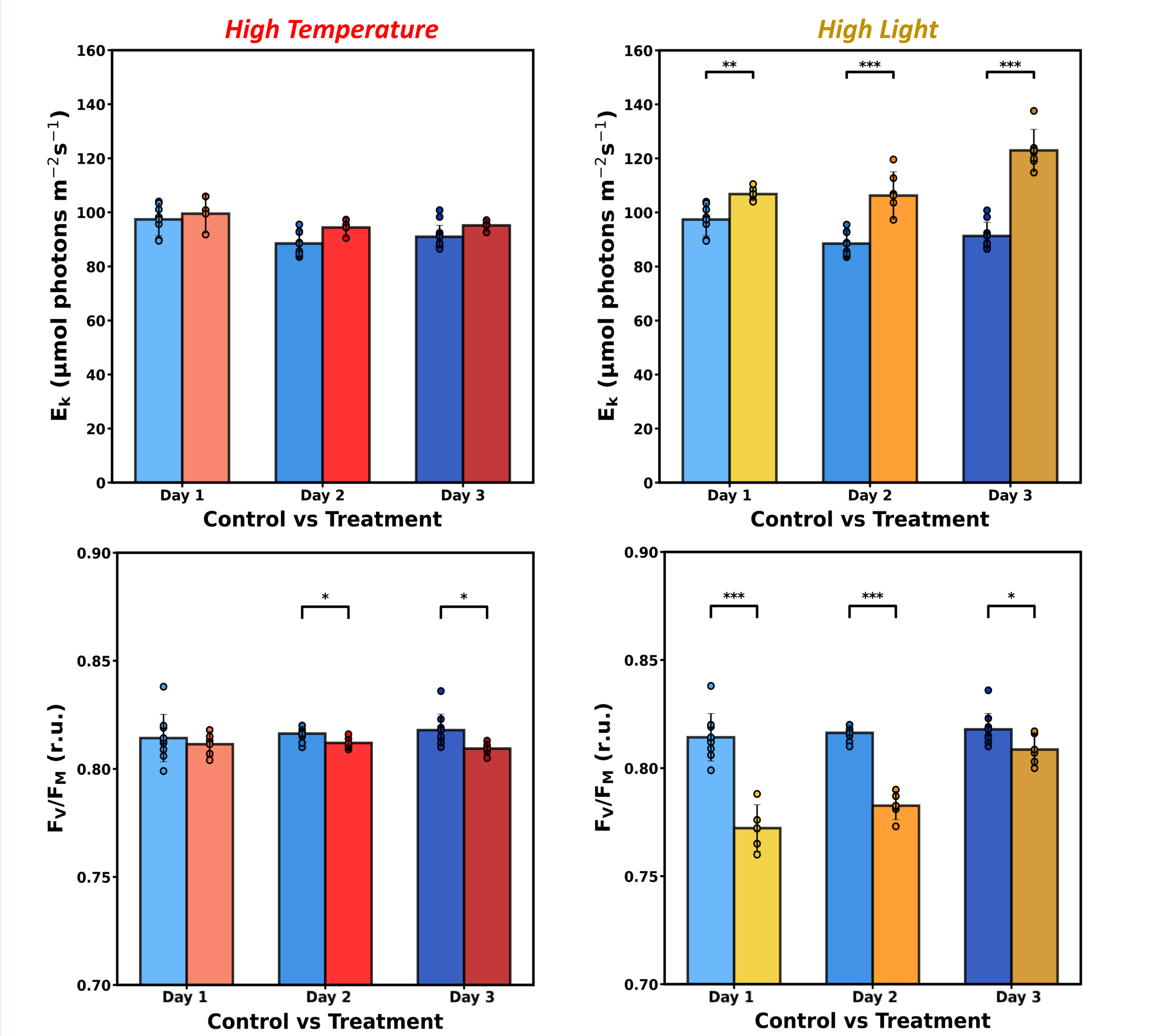
ChlF forced oscillations (time)



ChlF in the frequency domain (FFT)



ChlF in the time domain



Conclusion: The frequency-domain ChlF analysis can detect **frequency-specific fingerprints of stress response** in **light-adapted** plants without **dark adaptation**.

▶ Outlook

- **Machine learning** algorithms to categorize stress responses in a **fast and reliable** manner (collaboration with **SONY CSL, DREAM**).
- Application of the method to **crop plants under relevant growth environments**.
- Exploration of **various stress scenarios**, such as nutrient deficiency, drought, biotic stress, and combination of multiple stress factors.

[1]: Nedbal, L., & Lazar, D., (2021). Photosynthesis dynamics and regulation sensed in the frequency domain. *Plant Physiology*, 187: 646–661. doi:10.1093/plphys/kiab317.
[2]: Niu, Y. et al. (2023). Plants cope with fluctuating light by frequency-dependent nonphotochemical quenching and cyclic electron transport. *New Phytologist*, 239: 1869–1886. doi: 10.1111/nph.19083.
[3]: Consalvey, M. et al. (2005). PAM fluorescence: a beginners guide for benthic diatomists. *Diatom Research*, 20(1):1-22. https://doi.org/10.1080/0269249X.2005.9705619.