FINAL SCRIPT: APPROVED FOR FILMING

Submission ID #: 69530
Scriptwriter Name: Poornima G
Project Page Link: https://review.jove.com/account/file-uploader?src=21206698

Title: High-Throughput, In-Field Screening of Photosynthetic Efficiency in Crop Plants Using an Autonomous Robot

Authors and Affiliations:
Nicolin Caflisch[1], Andreas Hund[1], Onno Muller[2], Achim Walter[1], Beat Keller [1]

[1]Crop Science, Institute of Agricultural Sciences, Swiss Federal Institute of Technology Zurich
[2]Plant Sciences, Institute of Bio-and Geosciences, Forschungszentrum Jülich

Corresponding Authors:
Beat Keller beat.keller@usys.ethz.ch

Email Addresses for All Authors:
Nicolin Caflisch nicolin.caflisch@usys.ethz.ch
Andreas Hund andreas.hund@usys.ethz.ch
Onno Muller o.muller@fz-juelich.de
Achim Walter achim.walter@usys.ethz.ch
Beat Keller beat.keller@usys.ethz.ch

Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **NO**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes**

3. **Filming location:** Will the filming need to take place in multiple locations? **NO**

4. **Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. NO

Current Protocol Length
Number of Steps: 18
Number of Shots: 30 (17 SC)

Introduction

INTRODUCTION:

1.1. **Beat Keller:** We investigate photosynthesis to identify highly efficient and resilient varieties, and the underlying beneficial alleles, under fluctuating field conditions.
   1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

1.2. **Beat Keller**: Photosynthesis is mostly assessed through time-consuming manual PAM or gas-exchange measurements, which limits the detection of seasonal dynamics and the screening of high genetic diversity.
   1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

1.3. **Nicolin Caflisch**: Automated LIFT measurements throughout the season reveal physiological heterogeneity within plot canopies and, importantly, distinct photosynthetic efficiencies between genotypes.
   1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

1.4. **Nicolin Caflisch**: Our approach captures real-time photosynthesis measurements non-invasively and autonomously within milliseconds, enabling rapid compa rison of photosynthetic responses across full seasons and diverse genotypes.
   1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

1.5. **Beat Keller**: Future work will integrate LIFT with genomic, thermal, and 3D canopy data to more precisely predict photosynthesis, stress resilience, and canopy productivity.
   1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Protocol

2. Setting Up the Robot and Conducting LIFT Measurements

Demonstrators: Nicolin Caflisch, Beat Keller

2.1. To begin, install the camera tripod next to the experimental field **[1]**. Place the PPFR sensor on top of the tripod **[2-TXT]**, and set the data logger and power bank on the ground below the tripod **[3]**.

2.1.1. WIDE: Talent setting up a camera tripod at the edge of the experimental field.

2.1.2. Talent mounting the PPFR sensor securely on top of the tripod. **TXT: PPFR: Photosynthetic Photon Flux Rate**

2.1.3. Talent placing or pointing to the data logger and power bank side by side on the ground below the tripod.

2.2. Now, mount the LIFT *(lift)* sensor assembly on the front of the robot, positioning it at a height of approximately 60 centimeters above the crop canopy **[1]**.

2.2.1. Talent aligning and securing the LIFT sensor assembly onto the front panel of the robot at the specified height.

2.3. Place the laptop computer, car battery, and power inverter on top of the robot **[1]**. Connect the power inverter to the car battery **[2]**, then connect the inverter to the LIFT sensor assembly **[3]**.

2.3.1. Talent pointing to the laptop, car battery, and inverter securely on the robot's top platform.

2.3.2. Talent connecting the power inverter input cables to the car battery terminals.

2.3.3. Talent plugging the LIFT sensor assembly cable into the output port of the power inverter.

2.4. Next, position the GNSS antenna kit on top of the robot **[1]**, connect the antenna kit to the laptop computer **[2]**, and start the GNSS logger desktop client and adjust the desired settings **[3-TXT]**.

2.4.1. Talent placing the GNSS antenna kit flat on top of the robot.

2.4.2. Talent plugging in the antenna cable into the laptop.

2.4.3. SCREEN: Show the GNSS logger desktop client interface being opened and

activated on the laptop. 2025-12-08 09-37-35_step_2-4-3_high_resolution_NC.mp4 00:02-00:19. **TXT: Do not look directly into the excitation beam**

2.5. The quantity directly measured by the LIFT sensor is the chlorophyll fluorescence yield, which is the increase in total chlorophyll fluorescence due to the excitation beam for each flashlet **[1]**. To calculate the quantum efficiency of the photosystem II *(2)*, use the given formula **[2]**. The total duration of one chlorophyll fluorescence measurement, including the relaxation phase, is around 21 milliseconds, with the 300 flashlet excitation phase lasting only 750 microseconds **[3]**.

2.5.1. SCREEN: Visualize the excitation pattern and increase in chlorophyll fluorescence signal per flashlet in the LIFT sensor software. 2025-12-08 10-03-55_step_2-5-1_high_resolution_NC_short.mp4 00:02-00:09

2.5.2. TEXT ON PLAIN BACKGROUND:

Fq'/Fm' = (Fm' - F') / Fm'

Where, F': The ChlF yield of the 1st flashlet

Fm': The average of the ChlF yields of the 301st and the 302nd flashlets

2.5.3. SCREEN: Highlight the data showing the 750 microsecond excitation phase and the complete 21 millisecond measurement cycle. 2025-12-08 10-22-20_step_2-5-3_high_resolution_NC.mp4, 00:02-00:23. NOTE from the videographer: The 750 microsecond excitation phase is shown with the mouse from 00:09 to 00:12. The complete 21 millisecond measurement cycle is shown with the mouse from 00:14 to 00:22. To show the 21 millisecond measurement cycle, the mouse is deliberately moved beyond the right margin of the plot because the x axis of the plot only goes until 7.5 milliseconds

2.6. Next, manually drive the robot to the beginning of the first row of plots in the experimental field using the remote controller **[1]**.

2.6.1. Talent guiding the robot forward with the remote controller toward the first plot row.

2.7. Activate the measuring script for both the LIFT sensor and the spectrometer in continuous mode **[1]**.

2.7.1. SCREEN: Show the desktop interface where the measuring script is launched, and continuous mode is selected for both devices. 2025-12-08 09-52-29_step_2-7-1_high_resolution_NC.mp4, 00:02-00:24. NOTE from the videographer: Please do not cut before 00:24 so that those watching the video see that the excitation pattern in the bottom-left pane appears only after START was pressed.

2.8. Start the autonomous robot navigation at a velocity of 0.5 meters per second using the robot control website **[1]**.

2.8.1. SCREEN: Show the robot control website interface, with the speed set to 0.5 meters per second and the start command executed. NOTE: No screen video, step was filmed off the smartphone display by the videographer using his camera.

2.9. During measurement, periodically check the plot representing the chlorophyll fluorescence yield over time in the LIFT sensor desktop client to confirm the expected shape **[1]**. If signals appear too weak, adjust the sensor gain accordingly **[2]**.

2.9.1. SCREEN: Display the real-time chlorophyll fluorescence yield plot within the LIFT sensor software interface. 2025-12-08 10-41-06_step_2-9-1_2-9-2_high_resolution_NC_ChlF_vs_flashlet_number_best.mp4, 00:03-00:03.

2.9.2. SCREEN: Show the gain adjustment panel in the interface, with talent increasing the gain slider. 2025-12-08 10-41-06_step_2-9-1_2-9-2_high_resolution_NC_ChlF_vs_flashlet_number_best.mp4, 00:04-00:24. NOTE from the videographer: At the beginning, the gain is too low (1), and the plot appears not very smooth. Then the gain is adjusted to an adequate value (100), resulting in a smooth plot with the correct shape. After 00:14, the gain is set to a too high value (500), resulting in a plot with a poor shape. In the end, the gain is again set to an apropriate value (100), resulting in a smooth plot with the correct shape. Showing what happens if the gain is set too high was not requested in the script, but it would be good to do so. If you do not want to show what happens if the gain is set too high (since it was not requested in the script), cut at 00:14.

2.10. Periodically hold the white reference panel under the excitation beam at the height of the crop canopy **[1]** while the robot is turning at the field border **[2]**.

2.10.1. Talent placing the white reference panel directly under the excitation beam while standing beside the turning robot.

2.10.2. Show the robot driving through the rows and turning at the end of the row.

3. Data Integration and Preprocessing

Demonstrator: Beat Keller

3.1. Use the data.table *(data table)* package in R to read in the LIFT transient and spectral data

from all files ending in _data.csv *(data-C-S-V)* and _spectral.csv *(spectral-C-S-V)* **[1]**.

3.1.1. SCREEN: Display the R console with the script reading multiple *_data.csv and *_spectral.csv files using the data.table package. R_start.mp4, 00:06-00:21 and R_end.mp4, 00:20-00:24.

3.2. Read in the GNSS data, weather data, the .geojson *(geo-J-son)* plot maps, and the experimental design file **[1]**.

3.2.1. SCREEN: Show all the corresponding data files being imported into R, including visual confirmation of .geojson maps and experimental layout. R_end.mp4, 00:27-00:48.

3.3. Extract the quantum efficiency of the photosystem II *(2)* ratio from each recorded LIFT transient **[1]**.

3.3.1. SCREEN: Show the R script parsing LIFT transient data and extracting Fq'/Fm' from each record. R_end.mp4, 00:50-01:20.

3.4. Then, determine the robot's heading based on consecutive GNSS positions **[1]**. Merge the GNSS data with the experimental design using the assigned plot identifiers to associate spatial locations with treatment and replication information **[2]**.

3.4.1. SCREEN: Display a GNSS dataset with sequential positions used to calculate robot heading. R_end.mp4, 00:50-01:20.

3.4.2. SCREEN: Show a merged dataset linking GNSS coordinates to plot identifiers, treatment types, and block designations. R_end.mp4, 01:35-01:47.

3.5. Filter the white reference measurements from the spectral reflectance dataset **[1]** and merge these measurements with the PPFR data using timestamps to create a lookup table that links spectral reflectance to different incident light intensities **[2]**.

3.5.1. SCREEN: Display filtered rows from the spectral dataset showing only white reference entries. R_end.mp4, 01:48-01:52.

3.5.2. SCREEN: Show the creation of a timestamp-aligned lookup table combining spectral reflectance and PPFR data. R_end.mp4, 01:54-01:59.

3.6. Now, apply corrections to the raw spectral reflectance data using the generated lookup table **[1]**.

3.6.1. SCREEN: Display the spectral dataset before and after correction based on the

lookup values. R_end.mp4, 02:02-02:05.

3.7. Extract genotype-specific photosynthetic response trends from the outlier-filtered chlorophyll fluorescence dataset with the model **[1]**.

3.7.1. TEXT ON PLAIN BACKGROUND:

$Fq'/Fm' = \beta_0 + \beta_1 \text{ Date} + \beta_2 (\text{Heading} \times \text{Hour}) + \beta_3 (\text{Genotype} \times \text{PPFR}) + \beta_4 \text{ MTCI} + \beta_5 \text{ PPFR} + \varepsilon$

Where, β_3 can be the slope or 'response of Fq'/Fm' to increasing incident PPFR

3.8. Estimate the slope of the genotype-by-PPFR interaction term using the emtrends *(E-M-trends)* function in R to quantify genotype-specific irradiance response **[1]**. The model includes MTCI to account for variations in chlorophyll content and canopy structure **[2]**.

3.8.1. SCREEN: Show the R script running emtrends with a focus on the β_3 slope term for Genotype × PPFR. R_end.mp4, 02:05-02:11.

3.8.2. SCREEN: Highlight the summary output of the model, with slope estimates linked to each genotype. R_end.mp4, 02:14-02:25.

Videographer's NOTE: Additional soybean sequences during summer (the common time for executing this protocol)

PXL_20250627_093607575.TS_screen.mp4

PXL_20250723_142759363.TS.mp4

PXL_20250723_095824228.TS.mp4

PXL_20250723_072241842.TS

PXL_20250723_112844824.TS

Videographer's NOTE: Additional soybean sequences during summer: Dr. Andreas Hund (Co-Author) must be credited in the video when using these sequences. https://polybox.ethz.ch/index.php/s/7sRgAPEAMtMtoHa
Parts of this material is openly available under: https://oc-aem-dist-downloads.ethz.ch/mh_default_org/oaipmh-cq5/d15c9377-ca72-480d-91fe-18b6f3949ceb/104cee7b-ee0b-44db-9320-9c294e975df4/LIFTRoverBeat_V05_FIN.mp4

Results

4. Results

4.1. The georeferenced dataset revealed strong spatial patterns in both quantum efficiency of photosystem II *(2)* **[1]** and normalized difference vegetation index across the experimental fields due to different genotypes grown in the plots and likely additional soil and field heterogeneity **[2]**.

4.1.1. LAB MEDIA: Figure 2. *Video editor: Zoom in on the top panel showing Fq'/Fm' values*

4.1.2. LAB MEDIA: Figure 2. *Video editor: Zoom in on the bottom panel showing NDVI*

4.2. Row-wise spatial patterns were partially explained by the heading direction of the field robot causing shading of the target leaves at a specific time of the day **[1]**.

4.2.1. LAB MEDIA: Figure 3.

4.3. Linear mixed-effects modeling of the season-long responses of quantum efficiency of photosystem II to increasing incident photosynthetic photon flux rate or PPFR revealed pronounced field-level heterogeneity, explaining a substantial portion of the variance in the dataset **[1-TXT]**.

4.3.1. LAB MEDIA: Figure 4A. **TXT: During the summer, 36 soybean breeding lines were measured**

4.4. The extracted genotype-specific slopes showed clear differences among breeding lines **[1]**, with several lines exhibiting steeper or flatter response curves of quantum efficiency of photosystem II to increasing light intensities compared to the panel average **[2]**.

4.4.1. LAB MEDIA: Figure 4B. *Video editor: Highlight the colored dots in both top and bottom panels*

4.4.2. LAB MEDIA: Figure 4C. *Video editor: Show the colored lines in the middle (the lines indicate the slope)*

4.5. Three-dimensional canopy reconstructions using the MASt3R *(mas-T-3-R)* algorithm showed the potential of integrating physiological and structural phenotyping **[1]**.

4.5.1. LAB MEDIA: Figure 5. *Video editor: Highlight B and C*

1. Photosynthetic
   Pronunciation link: https://www.merriam-webster.com/dictionary/photosynthetic
   IPA: /ˌfoʊ.doʊ.sɪnˈθɛtɪk/
   Phonetic Spelling: foh·doh·sin·thet·ik
2. Chlorophyll
   Pronunciation link: https://www.merriam-webster.com/dictionary/chlorophyll
   IPA: /ˈklɔːr.ə.fɪl/
   Phonetic Spelling: klor·uh·fil
3. Fluorescence
   Pronunciation link: https://www.merriam-webster.com/dictionary/fluorescence
   IPA: /ˌflʊrˈɛs.ənts/
   Phonetic Spelling: floor·ess·uhns
4. Quantum
   Pronunciation link: https://www.merriam-webster.com/dictionary/quantum
   IPA: /ˈkwɑːn.təm/
   Phonetic Spelling: kwahn·tuhm
5. Photosystem
   Pronunciation link: https://www.merriam-webster.com/dictionary/photosystem
   IPA: /ˈfoʊ.toʊˌsɪs.təm/
   Phonetic Spelling: foh·toh·sis·tuhm
6. Spectrometer
   Pronunciation link: https://www.merriam-webster.com/dictionary/spectrometer
   IPA: /spɛkˈtrɑː.mə.tɚ/
   Phonetic Spelling: spek·trah·muh·ter
7. Autonomous
   Pronunciation link: https://www.merriam-webster.com/dictionary/autonomous
   IPA: /ɔːˈtɑː.nə.məs/
   Phonetic Spelling: aw·tah·nuh·muhs
8. Excitation
   Pronunciation link: https://www.merriam-webster.com/dictionary/excitation
   IPA: /ˌɛk.saɪˈteɪ.ʃən/
   Phonetic Spelling: ek·sai·tay·shuhn
9. Microseconds
   Pronunciation link: https://www.merriam-webster.com/dictionary/microsecond
   IPA: /ˈmaɪ.kroʊˌsɛk.ənd/
   Phonetic Spelling: my·kroh·sek·uhnd
10. GeoJSON
    Pronunciation link: No confirmed link found
    IPA: /ˈdʒiː.oʊˌdʒeɪ.sɑːn/
    Phonetic Spelling: jee·oh·jay·sahn
11. Genotype
    Pronunciation link: https://www.merriam-webster.com/dictionary/genotype
    IPA: /ˈdʒiː.nəˌtaɪp/
    Phonetic Spelling: jee·nuh·type

12. Irradiance
    Pronunciation link: https://www.merriam-webster.com/dictionary/irradiance
    IPA: /ɪˈreɪ.di.ənsˈ/
    Phonetic Spelling: ih·ray·dee·uhns
13. Heterogeneity
    Pronunciation link: https://www.merriam-webster.com/dictionary/heterogeneity
    IPA: /ˌhɛt.ə.rə.dʒəˈniː.ə.ti/
    Phonetic Spelling: het·uh·ruh·jee·nee·uh·tee
14. Phenotyping
    Pronunciation link: https://www.merriam-webster.com/dictionary/phenotyping
    IPA: /ˈfiː.noʊˌtaɪ.pɪŋ/
    Phonetic Spelling: fee·noh·type·ing
15. Algorithm
    Pronunciation link: https://www.merriam-webster.com/dictionary/algorithm
    IPA: /ˈæl.gəˌrɪð.əm/
    Phonetic Spelling: al·guh·rih·thuhm