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# Field phenotyping infrastructure in a future world—Quantifying information on plant structure and function for precision agriculture and climate change.

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***Abstract.*** *Phenotyping in the field is an essential step in the phenotyping chain. Phenotyping begins in the well-defined, controlled conditions in laboratories and greenhouses and extends to heterogeneous, fluctuating environments in the field. Field measurements represent a significant reference point for the relevance of the laboratory and greenhouse approaches and an important source of information on potential mechanisms and constraints for plant performance tested at controlled conditions. In this paper, we present a range of methods deployed within the German Plant Phenotyping Network (DPPN, www.dppn.de), focusing on plant architecture, photosynthesis, and water relations. Specialized field platforms (a) test innovative phenotyping technologies; (b) provide access to semi-controlled field installations to support breeding approaches for future CO2–concentrations (breed-FACE); and (c) study the translation of phenotypic properties from controlled environments to stands in the field. In this paper, we report that stereo imaging allows the quantification of canopy structure; active thermography estimates leaf water content and provides information on transpiration conditions: sun induced (SIF) and light-induced fluorescence transient (LIFT) techniques allow us to estimate remotely photosynthesis at canopy and leaf-to-plant level, respectively. Regarding photosynthesis, because the Fluorescence Explorer was recently selected, SIF will be measured by the next European Space Agency satellite Earth Explorer mission. All methods will be tested further and incorporated into (semi-)automated systems of sensors positioned in the field, introducing a promising portfolio to measure plant traits in field phenotyping and to enhance our understanding of relevant traits under natural conditions now and in the future.*

***Keywords.*** *Field Phenotyping, Free Air CO2 enrichment (FACE), active thermography, Light Induced Fluorescence Transients, Stereo Imaging , Fluorescence Explorer*

## Introduction

In the past 10 years, plant phenotyping has become one of the major research areas in plant sciences. Nevertheless, the gap between genotype and phenotype remains particularly large (Deery et al. 2014; Furbank & Tester 2011). Breeders and farmers need accurate and reliable sensing technologies to support their strategic decisions with detailed spatial and temporal information on plant growth to predict future plant development and yield (Auernhammer 2001). These methods must be able to cover large field areas within short times, to deliver the necessary data to the breeder and farmer in almost real time. The problem under field conditions is that a large number of plant phenotypes result from almost endless permutations of environmental conditions. To counter this challenge, we require high-throughput phenotyping techniques that can screen thousands of plants in order to identify relevant breeding traits (Fiorani & Schurr 2013). The demand for enhanced technical and scientific capabilities has resulted in various activities worldwide, where, on the one hand, research institutions and universities develop phenotyping infrastructure, and, on the other hand, networks to enhance the concepts behind phenotyping (Pieruschka & Lawson 2015; Großkinsky et al. 2015).

In this presentation, we focus mainly on the concepts and technical developments for field phenotyping of plant shoot dynamics at Forschungszentrum Jülich, Germany, within the German Plant Phenotyping Network (DPPN). Plant shoot dynamics can be divided into functional and structural traits. Water content and photosynthetic activity are thought to be two important functional traits, for which we have selected and developed infrastructure not only to quantify these traits but also to manipulate field conditions to measure the changes in traits of different genotypes in the environment. This presentation is not a full review of the field phenotyping methods available. Instead, it selects a small set of sensors, in which the quantitative information on plant structure and function could be applied for precision agriculture in a continuously changing climate.

## Structural and Functional Plant Traits

### Canopy structure

Researchers have known for a long time that structure and function are linked in natural and agronomic systems.For example decreasing the height of the plant resulted in higher yield and at a smaller scale in corn the leaf angle distribution greatly influences light interception and yield in densely planted canopies (Ford et al. 2008). For use in the field, we selected the stereo imaging approach recently developed by Biskup et al. (2007) and Müller-Linow et al. (2015). The hardware requires two carefully placed cameras that can obtain images simultaneously. Processing these images results in a 3-dimensional depth point cloud that allows classification of different canopy elements. After this information is fitted into a plant model, we can calculate relevant structural parameters, such as canopy area, leaf angle distribution, the number of leaves, and the ratio between leaves and fruits (Rascher et al. 2010, Müller-Linow et al. 2015). The short time to obtain a picture (the shutter time of the camera) allows operation under field conditions, where plants are sessile but their leaves are almost always moving due to wind. Furthermore, it is a relatively cost-efficient approach that with adjusted algorithms for automatic trait detection is also a promising tool for precision agriculture.

### Plant water status

The effect of drought on plants is directly related to the plant water status. However, non-invasive quantification of plant water status in the field is not straightforward. In the lab and under controlled conditions, we have further developed the active thermography method, which applies a heat pulse to a leaf then monitors the change in leaf temperature with a thermal camera. The time constant derived from the transient heating and cooling of the leaf corresponds to the leaf heat capacity. Higher leaf water content correlates with higher leaf heat capacity. Albrecht et al. had found in measurements of several species a clear correlation between destructively measured leaf water content and the time constant of cooling (Albrecht et al. in prep.).

## Photosynthesis

Currently, the most widely used technique to quantify the efficiency of photosynthesis exploits the fluorescence signal of chlorophyll. Chlorophyll fluorescence is red (690 nm) to near infrared (740 nm) light re-emission following light absorption by photosynthetic pigments (chlorophylls and carotenoids) in plants. The principle underlying the use of chlorophyll fluorescence as an indicator of plant photosynthetic status is relatively straightforward. Absorbed light energy excites chlorophyll molecules, and de-excitation of this energy is mainly attained through three competing processes: photosynthesis, radiative loss of photons or chlorophyll fluorescence, and non-radiative thermal energy dissipation (non-photochemical quenching, NPQ). Because these three energy dissipation processes compete for excitation energy, changes in one process (e.g. photosynthesis) will affect the other two. Therefore, by measuring chlorophyll fluorescence, we can derive information on NPQ and photosynthesis (Porcar-Castell et al. 2014).

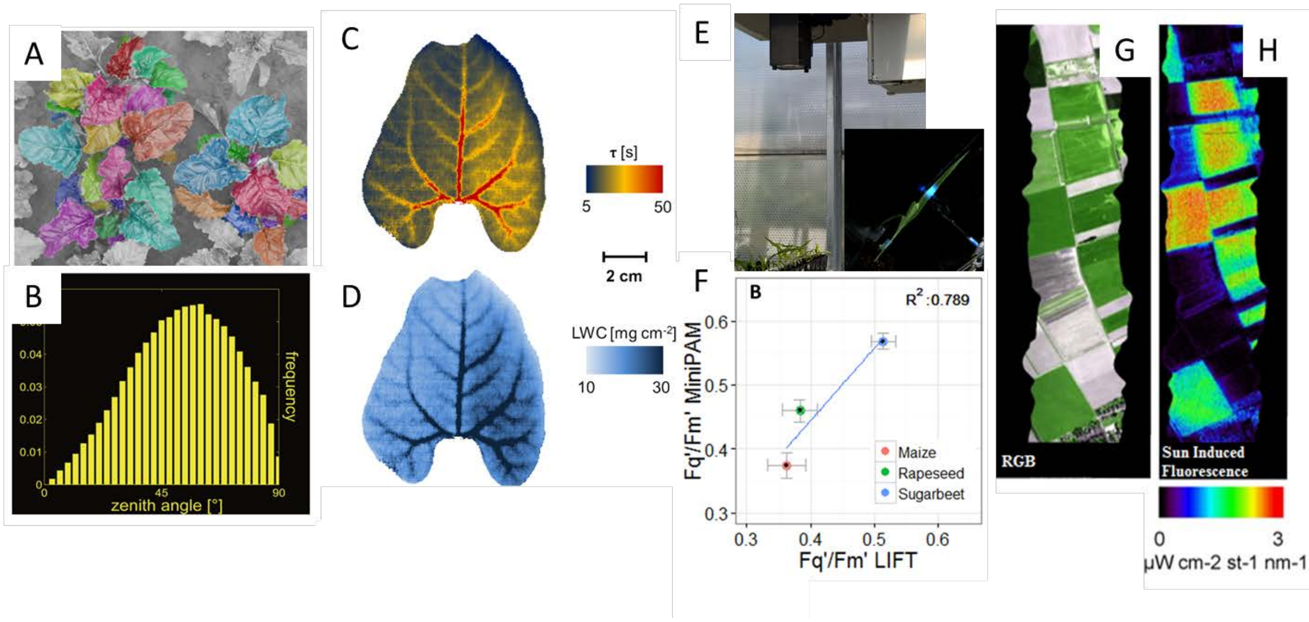


**Fig 1. Examples of different plant trait quantification methods. (A) Estimation of the leaf angle distribution from a 3d-reconstruction of a sugar beet canopy by a stereo camera set-up with single leaf segmentation, which is used for further, individual leaf surface modeling. (B) The leaf angle distribution of the zenith angle of the previous reconstruction; the zenith angle ranges from 0° (flat leaf surface) to 90° (erected surface) and can derived locally or for the complete leaf (modified after Muller-Linow et al. 2015). (C) Spatial map of τ and derived LWC of a young primary bean leaf. τ is given by color scale with blue being low τ values (5 s) and red being τ-values up to 50s. (D) LWC is indicated by blue-intensity scale, where light blue is LWC of 10 mg cm$^{-2}$ and dark-blue 30 mg cm$^{-2}$. (E) Picture of Light Induced Fluorescence Transient (LIFT) method in the upper left corner measuring corn, and in the inset, the blue light (445 nm) pulse on the leaf in the dark. (F) LIFT measurements Fv'/Fm' compared to MiniPam for maize, rapeseed and sugar beet Error bars represent standard error (n=24, 2 varieties per species with 2 replicates). (G) Airborne maps of different vegetation and sun-induced fluorescence (SIF) from an agricultural area in western Germany. The flight line was recorded on 23 August 2012 from 600 m height at 13:50 local time (UTC +2 hours), which was approximately 1 hour after solar noon. (A) For the pseudo-RGB image reflectance bands at 696, 708, and 677 nm are used for the red, green, and blue channel of the image. (H) Sun-induced fluorescence (F760) was calculated using the 3FLD method with an empirical correction of effective transmittance in the relevant wavebands**

Over the last 30 years, methods actively measuring light saturation have been prominent in detecting fluorescence; the most used method is pulse amplitude modulation (PAM) (Schreiber 2004). However, the application of a saturating pulse in close vicinity of the leaf limits its application in the field at high throughput. In the last 10 years, the light-induced fluorescence transient (LIFT) method (Kolber et al. 2005; Pieruschka 2010) was developed, allowing measurements of fluorescence parameters from a distance of several meters. This "pump-and-probe" technique has been developed and further improved for applications in the field (Kolber et al. 2005; Rascher & Pieruschka 2008; Pieruschka et al. 2014; Raesch et al. 2014). LIFT is now delivering the first fluorescence signals from a distance that allows us to determine the efficiency of photosynthesis as well as electron transport kinetics parameters (Keller et al. in prep).

For large-scale studies, passive techniques retrieve chlorophyll fluorescence emission from solar irradiance and vegetation-emitted radiance by using the absorption bands in surface solar irradiance (termed sun-induced fluorescence). The most important atmospheric absorption bands in the fluorescence emission region are two oxygen absorption bands: O2-A at 761nm and O2-B at 687 nm. The Fraunhofer Line Discrimination (FLD) principle makes it possible for us to retrieve chlorophyll fluorescence emission in these absorption lines (see Meroni et al. 2009 for an in depth review). Using this technique, we can estimate absolute variations in the sun-induced fluorescence intensity from leaf up to regional scales. At canopy scale, high-resolution point spectrometers can retrieve sun-induced fluorescence emission in the oxygen absorption lines (Rossini et al. 2010; Burkart et al. 2015). For larger scales, a recently released airborne sensor allows the exact quantification of sun-induced fluorescence from a research aircraft (Rossini et al. 2015; Rascher et al. 2015). This sensor, called HyPlant, delivered novel information in large field trials. An example of a HyPlant flight line covering a large agricultural area close to the Forschungszentrum Jülich is shown in figure 1H (adapted from Rascher et al. 2015). On a global scale, the European Space Agency (ESA) recently selected the FLuorescence EXplorer (FLEX) mission to measure sun-induced fluorescence in their new Earth Explorer 8. We expect great progress in this method in the coming years and that it will make it possible to map vegetation health and stress globally.

## Specialized field platforms

The aforementioned sensors are tested in selected agricultural field trials. The first stage of trials entails preparing a reference collection of major crops species, with clearly different genotypes at sufficient replication. The high concentration of field phenotyping sensors being tested in one field additionally gives the possibility to compare sensors. Moreover, a full set of environmental sensors is placed in each sub-field, which is essential for field phenotyping. The other field platform that is being prepared, and where the aforementioned sensors are also used, focuses on the need to breed under future CO2 concentrations. With increasing CO2 in the atmosphere and the direct link through photosynthesis to plant performance, it is pivotal to determine whether or not there is genetic variation in crop species' yield and seed quality in response to elevated CO2. Therefore we established a breed-Free Air CO2 Enrichment (FACE) system in the agricultural field with the unique combination of multiple, non-invasive field phenotyping sensors.

## Positioning Systems

Whereas it is most important that field phenotyping methods are able to measure relevant plant traits under various environmental conditions, throughput is also dependent on the positioning of the sensors in the field. The positioning systems are adjusted to the developmental stage of the sensors and use for future applications. For example, we use a cherry picker to position sensors above the canopy at various heights. The field4cycle (Fig. 2B) lets us position sensors above the canopy and drive through plots at relatively low cost and easy operation by multiple users. A fully automated FieldCOP (Fig. 2C) that can drive through the field using GPS and steering itself autonomously with a universal mounting platform for various sensors above the canopy. These different positioning systems provide the necessary information prior before we position systems in precision agriculture, such as the tractor-based sensors and UAVs, which we have also included in our positioning system portfolio.

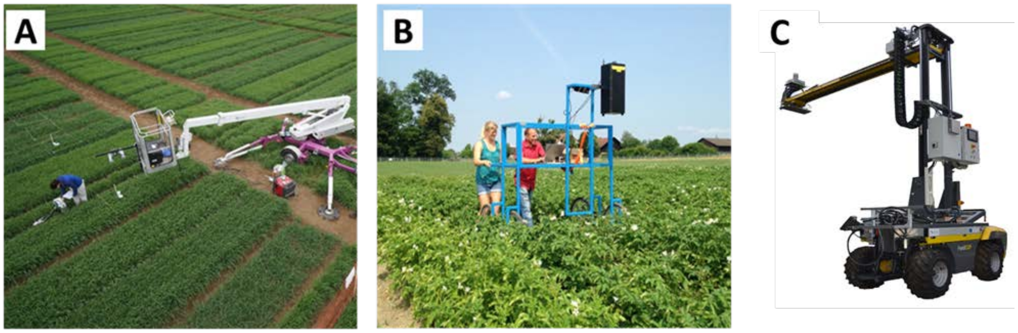


**Fig 2. Examples of positioning systems. A) FieldLIFT is capable of performing measurement from 1 to 10 meters above the ground, has a payload of 200 kg and an articulated arm that provides great flexibility to cover different positions above the canopy. (B) Field4Cycle is a welded steel structure with four bicycle wheels using the width of existing tracks alongside plots, in the current versions 1.5 or 3 m in width. It can drive over vegetation of up to 1.5 m high and can position sensors at adjustable height up to 1.5 above the top of the vegetation. (C) FieldCOP can reach 4m above the top of the plants and can penetrate 3 m inside the canopy. Additionally, it has a payload of 12 kg and can work autonomously in the field.**

## Conclusions

The methods, field platforms, and positioning-systems described in this paper are examples of the field phenotyping infrastructure at Forschungszentrum Jülich. Combined together, they are a promising portfolio for field phenotyping to measure plant traits and to enhance our understanding of relevant traits under natural conditions now and in the future. There are many field phenotyping examples worldwide that exist or are currently being developed. They may differ in their methods but all successful systems for field phenotyping will be equally important for precision agriculture in order to increase the yield and seed quality in the current and future climate.

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