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Development and assessment of a multi-sensor platform for precision phenotyping of small grain cereals under field conditions

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Contents

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Abbreviations

AC	Alternating current
AGM	Absorbant glass mat
BBCH	Biologische **B**undesanstalt, **B**undessortenamt und **CH**emische Industrie
BLUEs	Best linear unbiased estimates
BM	Biomass
cM	Centimorgan
CMOS	Complementary metal oxide semiconductor
CPU	Central processing unit
DC	Direct current
DGPS	Differential global positioning system
DH	Doubled haploid
ES	Estimation set
FBPP	Field based precision phenotyping
GPS	Global positioning system
HSI	Hyperspectral imaging
LAI	Leaf area index
LC	Light curtain
LD	Linkage disequilibrium
LDS	Laser distance sensor
MC-QTL	Multiple-line cross quantitative trait loci
MV	Multivariate analysis
N	Nitrogen
NIR	Near infrared
NTP	Network time protocol
PC	Personal computer
PCA	Principal component analysis
QTL	Quantitative trait loci
REML	Restricted maximum likelihood
RTK	Real time kinematic
Tof	Time-of-flight
TS	Test set

1. General Introduction

The human population is expected to exceed nine billion by 2050 which corresponds to an increase of about 40% as compared to 2012 (Bruinsma, 2009). Due to this increase in world population in combination with changing food habits, i.e., higher meat consumption in newly industrialized countries, the agricultural production needs to be increased by 70% by 2050 to achieve food security for the future (Bruinsma, 2009). Notably, this scenario does not yet take into account the increasing demand for agricultural products for bioenergy production. Consequently, the constantly increasing human population, changes in eating habits and the predicted climate change represent major challenges for the global agriculture that must be faced in the coming decades (Bruinsma, 2009; Fischer, 2009).

Ninety percent of this required increase in crop production has to be achieved by higher crop yields on areas already in use, the remainder coming from the exploitation of additional arable land (Bruinsma, 2009). To achieve higher yields, new high-yielding crop genotypes must be established by plant breeding (Bruinsma, 2009; Fischer, 2009; Furbank and Tester, 2011). The impact of the expected climate change on global rainfall and temperatures will lead to an increase in abiotic stress in the future (Fischer, 2009). Consequently, the development of high-yielding crop cultivars adapted to the changing environmental conditions represents a major component to achieve food and energy security for future generations and presents a tremendous challenge for plant breeding (Takeda and Matsuoka, 2008).

1.1 The phenotyping bottleneck

Whereas recently developed genomic approaches promise to substantially increase progress in plant breeding (Edwards et al., 2013), the ability to assess the phenome of plants in the field has changed little in the last decades. To obtain robust phenotypic data for traits of agricultural importance, like for example abiotic stress tolerance or grain yield, field trials across multiple environments and seasons are required (Furbank and Tester, 2011). Currently available phenotyping methods are, however, labor and time consuming, not totally objective and often destructive (Montes et al., 2007). Consequently, phenotypic data collection is restricted with regard to the number of plots that can be assessed, the traits that can be evaluated and, due to the destructiveness, the ability to measure the phenotypic changes during the life cycle of the plants. The latter is of particular severity because many traits of biological and agricultural importance are controlled by complex dynamic regulatory mechanisms (Wu and Lin, 2006). For example, biomass accumulation is affected by plant development over time but traditional methods to assess this trait were based on measurements of single time points disregarding the developmental dynamics of trait formation. Thus, plant phenotyping is widely recognized as one of the main bottlenecks in the development of improved crop varieties (Xu and Crouch, 2008; Furbank, 2009; Myles et al., 2009; Araus et al., 2012). Consequently, there is an urgent need for the development of non-destructive phenotyping methods to measure diverse traits under field conditions with a higher quality and quantity compared to state-of-the-art techniques and to enable phenotyping of traits not amenable to traditional approaches (Montes et al., 2007; White et al., 2012; Cobb et al., 2013).

1.2 State-of-the-art phenotyping techniques in plant breeding

The phenotyping methods and procedures currently applied in plant breeding in Germany are specified in the "Richtlinie für die Durchführung von landwirtschaftlichen Wertprüfungen und Sortenversuchen" of the Federal Plant Variety Office (http://www.bundessortenamt.de). Beside information about sowing, fertilization and tending strategies, the guideline provides detailed instructions to record growth parameters, pest infestation and finally yield parameters at harvest. Most of the parameters have to be collected manually by experts, e.g., plant height is determined by using graduated measuring rods, tiller density by counting the spikes within a defined area, and biomass yield is assessed destructively by using a field chopper, precluding the monitoring of biomass accumulation during the life cycle of the plants (for further information refer to the guideline of the Plant Variety Office). Taken together, state-of-the-art phenotyping methods have severe limitations, thus hampering selection gain in plant breeding.

1.3 Remote sensing methods in agriculture

The global agricultural sector, faced with the challenges ahead, will more and more become a high-technology sector using various electronic and information technologies to increase the agricultural production and to reduce the negative impacts on the environment. There are various reports in the literature about remote sensing technologies to measure different plant parameters in agriculture. Most of these reports are focused on the development of methods to support decision making in precision agriculture and only a few assess the ability of remote sensing technologies for precision phenotyping in plant breeding. The main difference between these two fields of application is the required spatial resolution and accuracy of trait determination. Precision agriculture aims at inter- and intra-field variations to optimize fertilizer and herbicide application rates. Spatial resolutions of a few square meters and measuring accuracies enabling parameter classifications into a few classes are common practice and sufficient to support decision making. By contrast, precision phenotyping aims to reveal even little phenotypic variations between different genotypes grown in plots of 5-

10 m^2 or even of single plants to identify the most promising genotypes. Consequently, precision plant phenotyping has higher requirements regarding the spatial resolution and the measuring accuracies as compared to remote sensing methods in precision agriculture.
A brief overview of methods applied in agriculture with their corresponding potentials and constraints for precision phenotyping and selection in plant breeding is provided in the following section.

1.3.1 Remote sensing methods with morphological selectivity

Sensors that assess morphological parameters of plants like laser rangefinders, 3D Time-of-Flight cameras, stereo imaging systems and light-curtains were applied to measure traits like for example plant height or tiller density.

Laser rangefinder: There are various experiments using laser rangefinders for *in situ* measurements of different plant characteristics. Preckwickel et al. (2004) used a laser distance sensor based on the triangulation principle to measure in vertical direction from top view into the plants to determine the height of wheat under field conditions. Ehlert et al. (2008) used the same approach to determine crop biomass under field conditions. In another publication Ehlert et al. (2010) investigated the suitability of a multilayer, multi echo laser scanner to characterize winter wheat under field conditions. It was shown that crop stand parameters like plant height and biomass can be calculated based on the reflection height of the laser beam. Saeys et al. (2009) investigated the potential of two different laser scanners (a high- and a low-frequency sensor) fixed in front of a combine harvester and different data analysis methods to determine crop stand density and crop volume for automatic feedrate control. Gebbers et al. (2011) evaluated a new approach for leaf area index mapping (LAI) of winter wheat, winter rye and oilseed rape based on laser rangefinders mounted on a vehicle.
These studies revealed that laser rangefinders are well suited to measure simple traits like plant height and to collect information associated with crop and dry biomass density of cereals under field conditions. However, the accuracies of the developed methods for crop and dry biomass density determination were not high enough to enable selection in plant breeding.

Depth cameras: There are also a few reports about depth cameras to collect morphological information of plants. Klose et al. (2009) confirmed the suitability of 3D Time-of-Flight cameras (3D Tof) for the characterization of plants under field conditions. In another study, Klose et al. (2012) evaluated the use of 3D Tof cameras to measure plant height and to count the leaves of single maize plants in the field. Möller et al. (2011) reported the application of 3D Tof cameras to measure the height of small grain cereals under field conditions. Jeon et al. (2009) used a stereo vision system to capture three-dimensional images of plants in the field for plant-weed detection. Chéné et al. (2012) reported the suitability of a low cost depth camera from the entertainment industry (Microsoft® Kinect®) for indoor leaf curvature measurements.

These studies have shown that depth cameras are suitable for field-based plant phenotyping. Image and non-image based data analysis methods can be applied to the datasets, e.g. to count the leaves or to measure the leaf curvature, to measure the height or to determine a projected volume of plants in the field. Though the data of depth cameras contains detailed morphological information about the habitus of the plants, extensive studies about the suitability of depth cameras for yield estimation are missing in the literature.

Light curtain: Another technology that enables the collection of morphological information of plants originates from safety applications in the industry, the so-called light curtain imaging technique. Light curtain imaging is a robust technology to capture binary images of plants under field conditions. Although these images contain various information about plant and stand parameters, for example plant height, tiller density, lodging or information about the size and orientation of cereal spikes, the only reports employing light curtains are on the determination of plant height.

Dzinaj et al. (1998) used, among other sensors, a light curtain imaging system to determine the height of plants for crop-weed detection under field conditions. Fender and Hanneken (2005) reported the operation mode of light curtains and possible applications of the technique. Stewart et al. (1999) investigated the suitability of light curtains to measure the height of cotton plants under field conditions for site-specific crop management. Busemeyer et al. (2010) used light curtain imaging to determine the height of small grain cereals and Montes et al. (2011) to measure the height of maize under field conditions.

1.3.2 Remote sensing methods with spectral selectivity

Beside these morphological selective systems, there are various experiments investigating the applicability of sensors with spectral selectivity like simple spectrometers, multi- and hyperspectral imaging systems, infrared thermography cameras or even simple color cameras to obtain phenotypic information of crop plants under field conditions.

Digital color cameras: Gang et al. (2007) used a digital camera measuring from top view onto the canopy to estimate the vegetation cover of wheat in the field. The study revealed that automatic crop cover estimation is possible on the basis of digital camera data. The crop cover was highly correlated with the leaf area index but showed only moderate results as predictor for crop yield. Li et al. (2010b) used a digital camera measuring from top view onto the canopy to calculate the canopy cover of wheat which

represents the proportion of detected green pixels in an image. In the calculation a pixel was counted as green when the soil adjusted vegetation index (SAVIgreen; for more information see Huete, 1988) was bigger than zero. The study revealed that canopy cover has a good correlation to nitrogen content and leaf area index but, nevertheless, only moderate correlation to above-ground biomass.
In conclusion digital cameras appear promising as a standalone system to measure for example the leaf area index or to give an approximation of nitrogen content but their potential to measure traits which, from a technical point of view are more complex, like for example dry biomass yield under field conditions, appears limited.

Thermal infrared imaging: Another technology based on spectral reflectance measurements with a high potential for crop improvement, especially in the field of drought stress adaption, is thermal infrared imaging. Differences in canopy temperature contain information about the stomatal conductance of plants and can thus be used to obtain information about the water stress tolerance of different genotypes. However, most of the currently available studies have investigated the suitability of thermal infrared imaging to detect drought stress for irrigation scheduling (Grant et al., 2007; Zia et al., 2009). Even though Jones et al. (2009) suggested thermal imaging as a promising tool for the selection of drought-adapted genotypes in plant breeding, there are only a few reports about this promising field of application (Romano et al., 2011; Zia et al., 2013).

Spectrometer and spectral imaging systems: There are various reports about spectrometer and spectral imaging systems for non-destructive determination of different crop parameters under field conditions. These systems were applied to measure the nitrogen status (Hansen and Schjoerring, 2003; Li et al., 2010a; Mistele and Schmidhalter, 2010; Erdle et al., 2011), the crop cover nutrient accumulation (Zhao et al., 2010), disease infections (Moshou et al., 2005; Bauriegel et al., 2011; Moshou et al., 2011), crop yield (Aparicio et al., 2000; Ferrio et al., 2005; Gutierrez et al., 2010b; Erdle et al., 2011; Perbandt et al., 2011; Erdle et al., 2013) and the crop water status (Thiel et al., 2010; Gutierrez et al., 2010a) under field conditions.
These studies revealed that spectrometers have a great potential to measure crop water status, the nitrogen status and crop cover nutrient accumulation. The reports about yield estimation using this approach illustrated that there are correlations between several spectral indices and yield parameters but also that the prediction accuracies are insufficient to select high-yielding genotypes based on this information.

1.3.3 Sensor and data fusion for improved plant phenotyping

Most available remote sensing methods are based on the determination of plant parameters using only one type of sensor and one single parameter derived from sensor raw data. These concepts show promising results for the determination of simple traits, like e.g. plant height, but show clear constraints in prediction accuracy of (from a technical point of view) more complex traits, like e.g. dry biomass. To overcome this limitation, Fender and Hanneken (2005) suggested the concept of sensor and data fusion (Mitchell, 2007) to enhance the accuracy of phenotypic measurements of complex traits. Nevertheless, reports about sensor and data fusion for improved plant phenotyping of complex traits under field conditions are scarce.

Ruckelshausen et al. (1999) reported the combined use of light curtains, CMOS-cameras, triangulation and ultrasonic as well as pressure sensors for crop-weed detection. Montes et al. (2011) evaluated the potential of light-curtains and spectral reflection sensors to measure the biomass of maize at an early stage of development. However, multi-sensor platforms to measure multiple traits and the application of multi-sensor fusion concepts to determine complex traits under field conditions for cereal crops are lacking.

1.4 Objectives of this study

The aim of this study was the development and assessment of a multi-sensor platform for precision phenotyping of small grain cereals under field conditions. In particular the objectives were to

(i) develop the phenotyping platform, including the mechanical construction, the hard- and software design for multi-sensor data collection, the implementation of a phenotyping procedure for data collection and analysis and the assessment of data collection robustness of the phenotyping procedure during field operation,

(ii) develop and evaluate data reduction models for the different types of sensors to achieve parameters with selectivity to plant height as a simple trait and dry biomass yield as example for a complex trait,

(iii) develop and calibrate sensor fusion models for dry biomass yield determination and to evaluate the benefits of sensor fusion compared to single sensor approaches, and

(iv) to assess the suitability of the developed precision phenotyping platform in applied plant breeding and its performance in combination with genomics.

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2. Publication 1:

BreedVision — A Multi-Sensor Platform for Non-Destructive Field-Based Phenotyping in Plant Breeding

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Abstract

To achieve the food and energy security of an increasing World population likely to exceed nine billion by 2050 represents a major challenge for plant breeding. Our ability to measure traits under field conditions has improved little over the last decades and currently constitutes a major bottleneck in crop improvement. This work describes the development of a tractor-pulled multi-sensor phenotyping platform for small grain cereals with a focus on the technological development of the system. Various optical sensors like light curtain imaging, 3D Time-of-Flight cameras, laser distance sensors, hyperspectral imaging as well as color imaging are integrated into the system to collect spectral and morphological information of the plants. The study specifies: the mechanical design, the system architecture for data collection and data processing, the phenotyping procedure of the integrated system, results from field trials for data quality evaluation, as well as calibration results for plant height determination as a quantified example for a platform application. Repeated measurements were taken at three developmental stages of the plants in the years 2011 and 2012 employing triticale (× Triticosecale Wittmack L.) as a model species. The technical repeatability of measurement results was high for nearly all different types of sensors which confirmed the high suitability of the platform under field conditions. The developed platform constitutes a robust basis for the development and calibration of further sensor and multi-sensor fusion models to measure various agronomic traits like plant moisture content, lodging, tiller density or biomass yield, and thus, represents a major step towards widening the bottleneck of non-destructive phenotyping for crop improvement and plant genetic studies.

3. Publication 2:

Precision phenotyping of biomass accumulation in triticale reveals temporal genetic patterns of regulation

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Abstract

To extend agricultural productivity by knowledge-based breeding and tailor varieties adapted to specific environmental conditions, it is imperative to improve our ability to assess the dynamic changes of the phenome of crops under field conditions. To this end, we have developed a precision phenotyping platform that combines various sensors for a non-invasive, high-throughput and high-dimensional phenotyping of small grain cereals. This platform yielded high prediction accuracies and heritabilities for biomass of triticale. Genetic variation for biomass accumulation was dissected with 647 doubled haploid lines derived from four families. Employing a genome-wide association mapping approach, two major QTL for biomass were identified and the genetic architecture of biomass accumulation was found to be characterized by dynamic temporal patterns. Our findings highlight the potential of precision phenotyping to assess the dynamic genetics of complex traits, especially those not amenable to traditional phenotyping.

4. Publication 3:

Multiple-line cross QTL mapping for biomass yield and plant height in triticale (x *Triticosecale* Wittmack)

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Abstract

Triticale shows a broad genetic variation for biomass yield which is of concern for a range of purposes, including bioenergy. Plant height is a major contributor to biomass yield and in this investigation, we studied the genetic architecture underlying biomass yield and plant height by multiple-line cross QTL mapping. We used 647 doubled haploid lines from four mapping populations that have been analyzedin four environments and genotyped with 1710 DArT markers. Twelve QTL were detected for plant height and nine for biomass yield which cross-validated explained 59.6% and 38.2% of the genotypic variance, respectively. A major QTL for both traits was found on chromosome 5R which likely corresponds to the dominant dwarfing gene *Ddw1*. Moreover, we identified epistatic QTL for plant height and biomass yield which, however, contributed only little to the genetic architecture of the traits. In conclusion, our findings illustrate the potential of genomic approaches for a knowledge-based improvement of biomass yield in triticale.

5. General Discussion

Precision phenotyping platforms are of paramount importance to assess the phenome of crops under field conditions (Furbank and Tester, 2011, p. 635). Especially the collection of data on the plants' responses to environmental cues as well as information on traits not amenable to classical phenotyping, promise to revolutionize crop phenotyping. Consequently, precision phenotyping platforms could assist plant breeders to develop new, high yielding and environmentally adapted cultivars. However, operational field-based precision phenotyping (FBPP) platforms that enable the characterization of plants with sufficient spatial resolution for breeding purposes are still lacking. While vast amounts of information can nowadays be gathered with new genomic approaches, this so-called phenotyping bottleneck has in recent years more and more moved into the focus of the plant scientific community. Several recent publications point to the need of FBPP platforms for plant genetic studies and review currently available concepts and methods (Montes et al., 2007; Takeda and Matsuoka, 2008; Xu and Crouch, 2008; Houle et al., 2010; Furbank and Tester, 2011; White et al., 2012). White et al. (2012, p. 103) emphasized that the core challenge in the development of FBPP platforms is the adaption of available remote sensing technologies to an appropriate spatial resolution for the characterization of diverse crops and traits in plant breeding. In addition, the systems need to be flexible and reliable and should permit the evaluation of hundreds to thousands of plots within a few hours. To meet these requirements it was suggested that FBPP platforms comprise the following six components:

1. Remote sensor systems for raw data generation
2. A carrier vehicle for instrument positioning in the field
3. A physical system for hardware integration
4. Analytical methods for reference value generation
5. Methods for data management and analysis
6. Integrated management protocols to increase data reliability

In the following chapters relevant aspects of the different components of the FBPP platform developed in this study are discussed as well as the suitability of the platform for application in plant breeding and genomic studies.

5.1 Selection of sensor systems

As described in the introduction, there are various remote sensing systems available to collect morphological and spectral information of plants in the field. Nevertheless, most of the available studies report the application of single systems for decision making in precision agriculture (Hansen and Schjoerring, 2003; Preckwinkel, 2004; Bredemeier and Schmidhalter, 2005; Ehlert et al., 2008; Ehlert et al., 2009; Saeys et al., 2009; Ehlert et al., 2010; Li et al., 2010; Mistele and Schmidhalter, 2010; Thoele and Ehlert, 2010; Bauriegel et al., 2011; Gebbers et al., 2011; Moshou et al., 2011; Perbandt et al., 2011; Cao et al., 2012; Mahlein et al., 2012) and only a few were tested for their applicability in plant breeding (Ferrio et al., 2005; Gutierrez et al., 2010; Montes et al., 2011; Romano et al., 2011; Comar et al., 2012).

The aim of this study was the development of a flexible FBPP platform that enables the non-invasive phenotyping of small grain cereals. Dry biomass yield was chosen as a complex trait to evaluate the performance of the FBPP platform. This requires sensors with morphological selectivity for crop volume estimation and sensors with selectivity to plant density. According to these requirements the sensor systems specified in Table 3-1, suitable for measurements under field conditions, were selected and integrated into the platform.

Table 5-1 Sensor systems integrated into the FBPP platform with corresponding selectivity and amenable traits

Typ of Sensor	Selectivity	Amenable traits/parameters
Laser distance sensors	Morphological	Plant height, tiller density, biomass yield
3D Time-of-Fligh cameras	Morphological	Plant height, projected volume, biomass yield
Light curtain imaging	Morphological	Plant height, coverage density, biomass yield
Hyperspectral imaging	Spectral	Moisture content

5.2 Carrier vehicle and Sensor module

The physical system of FBPP platforms can be divided into a carrier vehicle and a mechanical construction for sensor and hardware integration. Both components are important for the quality of the whole phenotyping procedure since they are affecting phenotypic data at the very beginning of the process. Thus particular attention has to be paid to the physical design of the FBPP platform.

According to White et al. (2012) possible types of carrier vehicles for field-based phenotyping, each with its specific pros and cons, are high clearance tractors (Schleicher et al., 2003; Ehlert et al., 2008; Mistele and Schmidhalter, 2010; Montes et al., 2011; Andrade-Sanchez et al., 2012), crane and linear-moving systems (Colaizzi et al., 2002; Kostrzewski et al., 2003; Haberland et al., 2010), cable robots (White and Bostelman, 2011), manned (French et al., 2007; Lamb et al., 2011) and unmanned aircrafts (Hunt, JR. et al., 2005; Córcoles et al., 2013) and tethered aerostats (Jensen et al., 2007; Ritchie et al., 2008). For the FBPP platform established here, a high clearance tractor with a chassis clearance of 80 cm pulling a trailer with a chassis clearance of 120 cm was chosen for sensor transportation and positioning in the field. This carrier can carry a high payload facilitating the combination of many sensor systems.

The field trials conducted in this study demonstrated the ability of the developed mechanical concept to deliver high quality sensor raw data, quantified by the high technical repeatability of the system during repeated measurements. Nevertheless, extensive tests also revealed a few constraints of the platform. Plants with a height above 80 cm were pushed down by the tractor and plants higher than 120 cm were also pushed down by the carrier trailer. Although the plants were not damaged during the measurements in most of the cases, the contact of the plants with the platform led to movements of the canopy affecting sensor raw data quality. In addition, uneven field conditions caused galloping movements of the trailer, affecting the positions of the sensor systems to the canopy and the total length of the tractor plus trailer resulted in a rather large turn radius compared to a tractor based system without a trailer. The current system might thus be improved by a direct adaption of the sensor module in the middle of a tractor with a higher ground clearance.

The physical system for hardware integration in this study, hereinafter referred to as sensor module, is a separate mechanical construction shaded with a black canvas which was adapted to the trailer of the carrier vehicle. The sensor module provides flexible mounting options for the different types of sensors, thus enabling sensor integration with defined offsets which is the basis for sensor fusion and multiple trait determination. The module protects the sensors from direct solar irradiation and adverse weather conditions which is essential to generate high quality data even under changing weather conditions. In addition, all electronic components for data collection are integrated into the module making it adaptable to different carrier vehicles and usable as a standalone system for example for test measurements in the laboratory.

The excellent calibration results for trait determination and the high technical repeatability of the system corroborate the robustness and suitability of the developed sensor module for FBPP, even under changing field and weather conditions. Nevertheless, the extensive field trials revealed the potential to optimize the clamping systems of the light curtains. The construction, consisting of a separate clamping system for the transmitter and receiver each with a width of 80 mm, has a fixed distance between the transmitter and receiver of 340 mm penetrating the canopy during measurements. The width of the clamping systems require a customized plot design and the construction penetrating the canopy, which is currently realized without crop lifters, induced lodging in some cases, especially in developmental stages of the plants after grain filling. Consequently, the redesign of the light curtain clamping system with a reduced width, an adjustable distance between transmitter and receiver and integrated crop lifters may further improve the phenotyping quality and would enable the use of the platform without the need for a specific plot design.

5.3 Data management and analysis

The output of FBPP platforms is strongly dependent on the structure and quality of the data management and analysis methods of the system (Bhave et al., 2007; Cobb et al., 2013). Novel and innovative sensor technologies for FBPP, as applied in our platform, generate massive datasets with varying data formats and frame rates for every different type of sensor. It is important to have the capability to record and manage these vast amounts of data in a way which offers the opportunity for sensor and data fusion and finally empowers value extraction. Furthermore, value extraction itself requires customized sensor models and algorithms. Consequently, the quality of the phenotyping output can only be as valuable as the analysis methods applied on sensor raw data.

The data collection and management system developed in this study proved to be a robust and flexible system, well suited to satisfy the demands for precision phenotyping applications. Data is stored in a separate table for every type of sensor in a MySQL database. Each incoming single dataset of all different types of sensors has its own time- and position stamp, offering the opportunity for sensor and data fusion in an offline plot-based data analysis procedure to determine complex traits, like for example dry biomass yield.

An automated data analysis software tool, including sensor and trait specific algorithms for value extraction, calibration and trait determination, was developed with the software package MATLAB (The Mathworks, Natick, USA). The developed tool was successfully used to analyze data of about 15,000 plots to determine plant height, dry matter content and dry biomass yield.

Plant height, for example, can be regarded as simple trait that can be determined with information gathered from a single sensor. Thus, plant height can be determined using data of the 3D Tof camera measuring from top view (Möller et al., 2011), the laser distance sensor measuring from top view (Busemeyer et al., 2010) or the light curtains penetrating the canopy, respectively. The excellent calibration result of height determination based on light curtain data achieved in this study, reaching a very high accuracy (R^2=0.97) and technical repeatability (R^2=0.99), is an example of measuring simple traits by using a single type of sensor for plant characterization. In addition, the result corroborates the high suitability and robustness of light curtain imaging for FBPP. Another trait amenable with a single type of sensor in this study was the determination of dry matter content which is physically based on moisture determination by NIR hyperspectral imaging. Although the trait was calculated based on data from a single type of sensor, it is from a technical point of view a complex trait to be measured under field conditions. State-of-the-art remote sensing methods for moisture determination are based on NIR spectrometers which spatially integrate over a few square centimeters, thus including reflectance information of plants and soil. Due to the noise induced by light reflected from the soil, measurement accuracies for *in vivo* plant characterizations of these state-of-the-art remote sensing methods are limited. Hyperspectral imaging is an innovative technology offering the ability to separate plant from soil which helps to avoid the influence of light reflected by the soil. The excellent calibration results for dry matter content achieved in this study, reaching a very high accuracy (R^2=0.97) and technical repeatability (R^2=0.98), demonstrate the suitability and robustness of hyperspectral imaging for FBPP.

Non-destructive determination of dry biomass yield is, from a technical point of view, more complex to measure because it is affected by several components. Dry biomass yield determination requires information about the volume and the average density of the plants. Consequently, a robust and precise calculation requires fusion of different but complementary types of sensors, collecting information about morphological and spectral properties of the canopy. The calibration results obtained in this study increased significantly by combining different types of sensors which confirmed the advantages of sensor and data fusion to measure complex traits. In the developed model, data of the light-curtains, laser distance sensors and 3D Tof cameras were used to approximate the volume of the plants and dry matter content extracted from hyperspectral imaging data was used for plant density approximation. The excellent calibration results of dry biomass yield achieved in this study, reaching a very high accuracy (R^2=0.92) and technical repeatability (R^2=0.99), demonstrate the suitability and robustness of the platform and sensor fusion methods to measure dry biomass yield under field conditions. Nevertheless, the developed models have limitations because the underlying algorithms are based on a simplified model, assuming the plants to be cylindrical bodies including the volume of all different components of the plants. However, the habitus of the plants, which is composed of the stems, the leaves and the spikes, is much more complex than described in the model. The contribution of the different components to dry biomass yield is different and varies over time which is also not considered in the model. For example, the surface of the leaves is bigger than the surface of the stems but the latter possess a higher volume. Consequently, the effect of the leaves on the average penetration depth of the different types of sensors is bigger than the effect of the stems on this parameter. Thus, variations in the ratio between stem and leaf area may lead to noise during volume estimation between different genotypes. In addition, changes in leaf morphology induced by biotic or abiotic stress may also lead to noise during volume estimation, even between plants with identical genotype. Consequently, a differentiation between the leaves, stems and spikes might be a promising optimization for plant volume estimation, thus improving the accuracy of biomass determination. Light curtain data or hyperspectral imaging data may be used to count and to determine the size of the ears. Different light source intensities of the light curtains may be used to differentiate between leaves and stems of the plants, assuming the ability of the light curtains to penetrate the leaves with high light intensities. In addition, new sensor technologies like ultrasonic rangefinders or high resolution color images might help to enhance biomass determination accuracy. In conclusion, the developed multi-sensor fusion model for *in situ* dry biomass determination is well suited for FBPP but, nevertheless, there is the potential to increase measurement robustness and accuracy by integrating further data extraction algorithms and new sensor technologies.

5.4 Data reliability

FBPP platforms have to deal with many different disturbances which can significantly influence the phenotyping output of the platform. These disturbances can be divided into three different categories:

1. Factors which have a direct influence on the sensor raw data generation
2. Factors which temporally change the condition of the plants
3. Effects of the environment to the habitus of the plants

Disturbances of the first category, for example dust, dirt or direct solar irradiation, can cause abnormal sensor raw data values, thus irreversibly affecting data quality at the beginning of the phenotyping procedure. Potential adverse effects of this type of disturbance were considered during the mechanical construction of the phenotyping platform and by a manual control of correct sensor function during measurements. To avoid the influence of direct solar irradiation, the sensors' field of view was shaded with a canvas. Sensor raw data quality can be assessed online with the graphical user interface of the platform. The system permanently analyses the incoming sensor raw data values concerning plausibility and the status of every sensor is visualized and reported as non-functioning (red color), problematic (yellow) and functional (green). This visualization of the sensor status is an essential tool to increase data quality and reliability and was successfully used in the developed platform as confirmed by the high technical repeatability of trait determination.

The second category contains factors which temporally change the condition of the plants, for example strong wind or heavy rain as factors which may induce lodging. In addition, a mechanical influence of the phenotyping platform which may also lead to movements of the plants in the sensors' field of view or lodging. Environmental disturbances causing lodging of the plants cannot be controlled by the FBPP platform and lead to a decrease of measurement accuracy. Prior to the third harvest (BM3) of the calibration experiment in 2011, heavy rain and strong wind damaged a few plots of the experiment which affected the measurement accuracy and technical repeatability as compared to the calibration results of the two previous measurement time points (BM1 and BM2). A promising optimization would be the development of methods to detect damaged plots and to quantify the degree of lodging. This information could be used to select and exclude irregular plots from further analysis or to generate separate calibrations. Disturbances induced by the mechanical construction of the FBPP platform should be minimized by continuous optimization of the platform. An increase of ground clearance and the integration of crop lifters to the clamping system of the light curtains would be promising optimizations to further increase data reliability of the platform.

The third category contains the effects of the environment to the habitus of the plants. Cohort validations conducted in this study, using data of year 2011 for calibration and data of year 2012 for validation and *vice versa*, revealed that the prediction of dry biomass yield across locations is feasible. Nevertheless, the bias observed in the cohort validations illustrates the influence of the environment to the habitus of the plants. A calibration using data of multiple locations and years will increase absolute cross environmental measurement accuracy and is a promising optimization to decrease the effect of this third category of disturbances.

In conclusion, FBPP platforms have to deal with many different disturbances which require adapted mechanical constructions, online tools for data quality analysis during field operation and data analysis methods to assess and to increase the reliability of the phenotyping output.

5.5 Suitability of the platform for plant breeding and genomics

In comparison to traditional phenotyping approaches, FBPP platforms have to enable the screening of a higher number of plants per time unit with less labor capacities, provide a higher accuracy of phenotypic data measurements, or have to enable the determination of traits that so far have been impossible to measure with routine phenotyping methods in plant breeding.

Employing traditional phenotyping methods every trait of interest has to be determined separately which is both labor and time intensive. The determination of dry biomass yield is, for example, a destructive procedure and requires two technicians, a field chopper for harvest and a drying oven to measure the moisture content of the plants. The throughput per day is restricted to about 500 plots with an area of five square meters. The determination of plant height also requires two persons and the throughput per day is restricted to about 1,500 plots. In contrast to this very labor and time consuming traditional methods, the developed FBPP platform enables the screening of more than 2,000 plots per day, allows for multiple traits being determined simultaneously and, due to its non-destructiveness, enables repeated measurements of the same plots even for traits which by traditional approaches can only be assessed destructively. The latter is of great interest to assess growth dynamics, a trait currently not amenable with state-of-the-art phenotyping techniques. The already high throughput of the platform is based on a measurement velocity of about 0.5 ms^{-1} and a field design with 25 plots per row. Consequently, a further increase of the platform throughput can be achieved by an increase of the number of plots per row which results in a decrease of the time required to change between rows and by an increase in measurement velocity. Whereas the former has no effect on data quality, the latter is affecting the resolution of the light curtain, laser distance sensor and spectral imaging data and the quality of the 3D Tof camera data. Since the data analysis methods for plant height, dry matter content and dry biomass yield determination are based on statistical methods, estimating average values for each plot, a further increase of measurement velocity might be possible without a significant decrease in measurement accuracy. Nevertheless, an increase of measurement velocity without an appropriate adaptation of measurement frequency of the different types of sensors will decrease the applicability of image analysis methods to determine for example tiller density or to collect more detailed information about the habitus of the plants.

According to Cobb et al. (Cobb et al., 2013) modular designs of FBPP platforms are essential to increase flexibility of phenotyping in the future and to make platforms applicable for plant breeding. In this context, important aspects considered in the development of the platform in this study are the ability to integrate new sensor technologies without the need to change the entire data collection system, the possibility to integrate new algorithms in the data analysis pipeline for value extraction and the potential to generate new calibrations, even by non-expert users. The latter is of particular interest because it ensures a sustainable application of the platform in plant breeding, offering the possibility to extend available calibrations with data of additional locations and years and to generate new calibrations for other crop species and traits.

To assess the suitability of the platform for plant breeding and for genomic approaches, two experiments were performed: (1) a calibration experiment to assess the accuracy of the FBPP, i.e., the agreement between estimated and observed phenotypic values and (2) an experiment aimed at evaluating the heritability of traits assessed with the FBPP, i.e., the repeatability of measurements of the same genotype across replications, locations and years. For a successful application in plant breeding the FBPP must combine a high accuracy and a high heritability of the measurements. As described above, the accuracies obtained in Experiment 1 were very high. In Experiment 2 a large population consisting of 647 doubled haploid lines derived from four families was grown in field trials and phenotyped with the platform at three developmental stages at two different locations in two consecutive years. The platform yielded high heritabilities for dry biomass for all three developmental stages (BM1=0.78; BM2=0.84; BM3= 0.79) which illustrates its immense potential for application in applied plant breeding. Experiment 2 was also used to evaluate the potential of the platform for genomic approaches and to dissect the genetic architecture of biomass accumulation. To this end, the plants were genotyped with molecular markers. These genotypic data were analyzed together with the phenotypic data to detect quantitatiev trait loci (QTL), i.e., chromosomal regions affecting the trait. Two and nine QTL were detected for biomass yield applying two different QTL mapping approaches. In addition, this study revealed that the genetic architecture of biomass accumulation is controlled by dynamic temporal patterns, a novel finding that depended on the repeated measurements as facilitated by the FBPP. Taken together, the results from the two experiments illustrate the high suitability of the platform for plant breeding as well as in combination with genomic approaches.

5.6 Concluding remarks and outlook

The developed tractor pulled FBPP platform, comprised of a carrier trailer for system positioning on the field and a sensor module for sensor integration, turned out to be a very robust, flexible and reliable system for field-based applications. The data collection and analysis software of the platform permits the collection of sensor raw data with a throughput of more than 2,000 plots per day and an automated trait determination even by non-expert users. Extensive calibration studies revealed the benefit of sensor and data fusion to determine complex traits, like for example dry biomass yield. The excellent calibration results of plant height and biomass yield, the ability to measure multiple traits simultaneously, the non-destructiveness of the procedures, the flexibility of the platform, very high heritabilities of predicted biomass yield, as well as promising results in combination with genomic approaches, corroborate the high potential of the platform to revolutionize plant phenotyping under field conditions.

Nevertheless the study also revealed the potential for optimization and extensions which should be considered in further developments of the platform. Promising aspects are

- the optimization of the light curtain clamping system,
- the integration of the sensor module in the middle of a high clearance tractor,
- the increase of sensor measurement frequencies to achieve a higher throughput with equivalent data quality,
- the optimized use of already available and the integration of additional innovative sensor technologies to enhance volume estimation in the available biomass prediction model and to extend the measurements towards novel traits and
- the development of new algorithms and calibrations for novel traits and crop species.

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6. Summary

The growing world population, changing food habits especially to increased meat consumption in newly industrialized countries, the growing demand for energy and the climate change pose major challenges for tomorrow's agriculture. The agricultural output has to be increased by 70% by 2050 to achieve food and energy security for the future and 90% of this increase must be achieved by increasing yields on existing agricultural land. Achieving this increase in yield is one of the biggest challenges for the global agriculture and requires, among other things, an efficient breeding of new, higher-yielding varieties adapted to the predicted climate change. To achieve this goal, new methods need to be established in plant breeding which include efficient genotyping and phenotyping approaches of crops. Enormous progress has been achieved in the field of genotyping which enables to gain a better understanding of the molecular basis of complex traits. However, phenotyping must be considered as equally important as genomic approaches rely on high quality phenotypic data and as efficient phenotyping enables the identification of superior lines in breeding programs. In contrast to the rapid development of genotyping approaches, phenotyping methods in plant breeding have changed only little in recent decades which is also referred to as phenotyping bottleneck. Due to this discrepancy between available phenotypic and genotypic information a significant potential for crop improvement remains unexploited. The aim of this work was the development and evaluation of a precision phenotyping platform for the non-invasive measurement of crops under field conditions. The developed platform is assembled of a tractor with 80 cm ground clearance, a carrier trailer and a sensor module attached to the carrier trailer. The innovative sensors for plant phenotyping, consisting of several 3D Time-of-Flight cameras, laser distance sensors, light curtains and a spectral imaging camera in the near infrared reflectance (NIR) range, and the entire system technology for data acquisition were fully integrated into the sensor module. To operate the system, software with a graphical user interface has been developed that enables recording of sensor raw data with time- and location information which is the basis of a subsequent sensor and data fusion for trait determination. Data analysis software with a graphical user interface was developed under Matlab. This software applies all created sensor models and algorithms on sensor raw data for parameter extraction, enables the flexible integration of new algorithms into the data analysis pipeline, offers the opportunity to generate and calibrate new sensor fusion models and allows for trait determination. The developed platform facilitates the simultaneous measurement of several plant parameters with a throughput of over 2,000 plots per day.

Based on data of the years 2011 and 2012, extensive calibrations were developed for the traits plant height, dry matter content and biomass yield employing triticale as a model species. For this purpose, 600 plots were grown each year and recorded twice with the platform followed by subsequent phenotyping with state-of-the-art methods for reference value generation. The experiments of each year were subdivided into three measurements at different time points to incorporate information of three different developmental stages of the plants into the calibrations. To validate the raw data quality and robustness of the data collection and reduction process, the technical repeatability for all developed data analysis algorithms was determined. In addition to these analyses, the accuracy of the generated calibrations was assessed as the correlations between determined and observed phenotypic values. The calibration of plant height based on light curtain data achieved a technical repeatability R_w^2 of 0.99 and a correlation coefficient R_C^2 of 0.97, the calibration of dry matter content based on spectral imaging data a R_w^2 of 0.98 and a R_C^2 of 0.97. The generation and analysis of dry biomass calibrations revealed that a significant improvement of measurement accuracy can be achieved by a fusion of different sensors and data evaluations. The calibration of dry biomass based on data of the light curtains, laser distance sensors, 3D Time-of-Flight cameras and spectral imaging achieved a R_w^2 of 0.99 and a R_C^2 of 0.92. The achieved excellent results illustrate the suitability of the developed platform, the integrated sensors and the data analysis software to non-invasively measure small grain cereals under field conditions.

The high utility of the platform for plant breeding as well as for genomic studies was illustrated by the measurement of a large population with a total of 647 doubled haploid triticale lines derived from four families that were grown in four environments. The phenotypic data was determined based on platform measurements and showed a very high heritability for dry biomass yield. The combination of these phenotypic data with a genomic approach enabled the identification of quantitative trait loci (QTL), i.e., chromosomal regions affecting this trait. Furthermore, the repeated measurements revealed that the accumulation of biomass is controlled by temporal genetic regulation.

Taken together, the very high robustness of the system, the excellent calibration results and the high heritability of the phenotypic data determined based on platform measurements demonstrate the utility of the precision phenotyping platform for plant breeding and its enormous potential to widen the phenotyping bottleneck.

7. Zusammenfassung

Die stetig wachsende Weltbevölkerung, sich ändernde Ernährungsgewohnheiten hin zu vermehrtem Fleischkonsum in Schwellenländern, der stetig wachsende Energiebedarf sowie der Klimawandel stellen große Herausforderungen an die Landwirtschaft von morgen. Um eine gesicherte Lebensmittel- und Energieversorgung zu gewährleisten muss die landwirtschaftliche Produktion bis 2050 um 70% gesteigert werden, wobei 90% dieser Steigerung durch eine Erhöhung der Erträge auf bereits bestehenden landwirtschaftlichen Flächen erzielt werden muss. Diese erforderliche Ertragssteigerung ist eine der größten Herausforderungen für die weltweite Landwirtschaft und bedarf unter anderem einer effizienten Züchtung neuer, an den Klimawandel angepasster, ertragsreicherer Sorten. Um eine ausreichende Steigerung der Erträge sicherstellen zu können müssen neue Methoden in der Pflanzenzucht etabliert werden, welche auf einer effizienten Geno- sowie Phänotypisierung der Pflanzen basieren. Im Bereich der Genotypisierung gab es in den letzten Jahrzehnten große Fortschritte, wodurch ein enormer Wissenszuwachs über die molekulare Basis komplexer Merkmale erzielt werden konnte. Trotzdem ist der Bereich der Phänotypisierung als ebenso wichtig anzusehen, da genetische Untersuchungen unter anderem von der Qualität phänotypischer Daten abhängen und qualitativ hochwertige phänotypische Daten die Selektion überlegener Linien in der Pflanzenzucht verbessern können. Im Vergleich zur Genotypisierung gab es jedoch im Bereich der Phänotypisierung in den letzten Jahrzehnten nur wenig wissenschaftlichen Fortschritt. Durch dieses Missverhältnis zwischen der Qualität phänotypischer und genotypischer Informationen bleibt somit ein erhebliches Potential an neuen Erkenntnissen unentdeckt. Das Ziel dieser Arbeit war die Entwicklung und Bewertung einer Präzisionsphänotypisierungsplattform zur zerstörungsfreien Charakterisierung von Energiegetreide in der Pflanzenzucht, um den aktuell bestehenden Flaschenhals bei der Umsetzung neuer Zuchtmethoden zu weiten.

Die entwickelte Plattform ist ein Gespann bestehend aus einem Hochradschlepper mit 80 cm Bodenfreiheit, einem eigens entwickelten Trägeranhänger und einem am Trägeranhänger befestigten Sensormodul. Die innovative Sensorik zur Pflanzenvermessung, bestehend aus mehreren 3D Time-of-Flight Kameras, Laserabstandssensoren, Lichtgittern und einem bildgebenden Spektralmessgerät im nahen infrarot (NIR) Bereich, sowie die gesamte Systemtechnik zur Datenaufnahme wurden vollständig im Sensormodul integriert. Zur Bedienung des Systems wurde eine Software mit graphischer Benutzeroberfläche entwickelt, die eine zeit- und ortsbezogene Aufnahme der Sensorrohdaten ermöglicht, was die Grundlage einer anschließenden Sensor- und Datenfusion zur Merkmalsbestimmung darstellt. Zur Datenauswertung wurde eine Software mit graphischer Benutzeroberfläche unter

Matlab entwickelt. Durch diese Software werden alle erstellten Sensormodelle und Algorithmen zur Datenauswertung auf die Rohdaten angewendet, wobei neue Algorithmen flexibel in das System eingebunden, Sensorfusionsmodelle erzeugt und kalibriert und Pflanzenparameter bestimmt werden können. Die entwickelte Plattform ermöglicht die simultane Vermessung mehrerer Pflanzenparameter bei einem Durchsatz von über 2000 Parzellen pro Tag.

Basierend auf Daten aus den Jahren 2011 und 2012 wurden umfangreiche Kalibrierungen für die Parameter Pflanzenhöhe, Trockensubstanzgehalt und Trockenmasse für Triticale erstellt. Zu diesem Zweck wurden in beiden Jahren Feldversuche mit jeweils 600 Parzellen angelegt, doppelt mit der Plattform vermessen und zur Referenzwertgenerierung im Anschluss konventionell phänotypisiert. In beiden Jahren wurden drei Messungen von jeweils 200 Parzellen zu drei verschiedenen Zeitpunkten durchgeführt, um Daten unterschiedlicher Entwicklungsstadien der Pflanzen für die Erstellung der Kalibrierungen zur Verfügung zu haben. Zur Validierung der Rohdatenqualität sowie der Robustheit der Datenreduktionsverfahren wurden zunächst für alle entwickelten Auswertungsalgorithmen basierend auf den Wiederholungsmessungen die technischen Wiederholbarkeiten bestimmt. Neben der Validierung der Rohdatenqualität wurden die Genauigkeiten der erstellten Kalibrierungen als Korrelation zwischen den Referenzwerten und den mit der Sensorplattform gemessenen Werten ermittelt. Die Kalibrierung der Pflanzenhöhe basierend auf Lichtgitterdaten erreicht eine technische Wiederholbarkeit R_w^2 von 0.99 und einen Korrelationskoeffizienten R_c^2 von 0.97, die Kalibrierung des Trockensubstanzgehalts basierend auf Spectral-Imaging Daten ein R_w^2 von 0.98 und ein R_c^2 von 0.97. Bei der Erstellung der Trockenmasse Kalibrierung konnte gezeigt werden, dass durch eine Fusion verschiedener Sensoren und Datenauswertungen eine signifikante Verbesserung der Messgenauigkeit erreicht werden kann. Die Kalibrierung der Trockenmasse basierend auf Daten der Lichtgitter, Laserabstandssensoren, 3D Time-of-Flight Kameras und des Spectral-Imaging erreicht ein R_w^2 von 0.99 und ein R_c^2 von 0.92. Die hervorragenden technischen Wiederholbarkeiten, sowie die exzellenten Genauigkeiten der entwickelten Kalibrierungen verdeutlichen die herausragende Eignung der entwickelten Plattform, der integrierten Sensoren und der entwickelten Datenaufnahme- sowie Datenauswertesoftware zur zerstörungsfreien Phänotypisierung von Getreide unter Feldbedingungen.

Der hohe praktische Nutzen der Plattform für die Pflanzenzucht sowie für genetische Studien konnte durch die wiederholte Phänotypisierung einer DH Population mit 647 doppelhaploiden Triticale Linien in vier Umwelten aufgezeigt werden. Die Pflanzen wurden mit der Plattform an drei verschiedenen Zeitpunkten phänotypisiert und die erzeugten Daten zeigten eine sehr hohe Heritabilität für Biomasse. Die Kombination dieser phänotypischen mit genotypischen Informationen in einer

Assoziationskartierungsstudie ermöglichte die Identifizierung von Regionen im Genom welche für quantitative Merkmale (QTL) kodieren. So konnten z.B. Regionen auf mehreren Chromosomen identifiziert werden, welche die Biomasse beeinflussen. Des Weiteren konnte durch Auswertung der wiederholten Messungen der Nachweis erbracht werden, dass die Biomasseentwicklung durch sich zeitlich ändernde genetische Mechanismen beeinflusst wird.

Die erreichte sehr hohe Robustheit des Systems, die exzellenten Kalibrierungsergebnisse und die hohen Heritabilitäten der mit der Plattform bestimmten phänotypischen Daten verdeutlichen die hervorragende Eignung des Systems zur Anwendung in der Pflanzenzucht und das enorme Potential der entwickelten Technologie zur Weitung des aktuell bestehenden Phänotypisierungs-Flaschenhalses.

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9. Appendix

Curriculum vitae

Name	Lucas Busemeyer
Date and Place of Birth	October 23, 1981 in Trier
School Education	1988-1992, elementary school (Grundschule Ruwer) 1992-2001, high school (Hindenburg Gymnasium, Trier) Abitur June 2001
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Professional Experience	04/06 – 08/06, Internship at the Fuel Cell Centre Rhineland-Palatinate 09/06 – 06/08, Student research assistant at University of Applied Sciences Trier in the interdisciplinary research project "proTRon"; Field of activity: Fuel cell development for a light weight vehicle 02/09 – 09/13, Scientific assistant at University of Applied Sciences Osnabrueck in the interdisciplinary research project "BreedVision"; Field of activity: Research in the field of plant phenotyping Since 10/13, Scientific assistant at the German Aerospace Center in Hamburg; Field of activity: Fuel cells for aviation

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Hamburg, im September 2014

Lucas Busemeyer