



The Impact of different Leaf Surface Tissues on active 3D Laser Triangulation Measurements

JAN DUPUIS, Bonn, STEFAN PAULUS, Aachen, ANNE-KATRIN MAHLEIN, HEINER KUHLMANN & THOMAS EICHERT, Bonn

Keywords: 3D laser scanning, laser intensity, tissue penetration, optical properties of leaf surfaces, plant disease detection

Summary: Laser scanning devices used for plant phenotyping have shown their ability to penetrate the plant surface. This results in an interaction with the leaf tissue and in absorption of the emitted laser. The use of the intensity of reflection, measured from the backscattered laser ray, enables a more profound analysis of the geometric accuracy as well as an inspection of the plant's physiological condition. We show the comparison of two triangulation-based 3D laser scanners with different wavelengths, 658 nm (red) and 405 nm (blue), providing the intensity as additional information. By analysing the interaction of both laser sensors with separated leaf tissue contents it is possible to locate the origin of the measured signal and to evaluate the geometric accuracy of the point cloud. Furthermore, differences in the physiology of the plant as well as surface altering plant diseases like powdery mildew can be identified using the intensity of reflection. The use of the combination of a blue and a red laser scanner for high precision 3D imaging of the plant surface is shown, as well as its applicability for the analysis of plant tissue composition, the stage of leaf senescence and for a detection of plant diseases is demonstrated. Finally, the intensity of the red laser showed a high interpretability regarding the tissue composition while the blue laser provided a high geometric accuracy.

Zusammenfassung: Einfluss von unterschiedlichen Blattschichten auf 3D Laser-Triangulation. Bei der Phänotypisierung von Pflanzen mittels Laserscannern hat sich gezeigt, dass der ausgesandte Laser in die Pflanzenstruktur eindringt und dort von den photoaktiven Bestandteilen teilweise absorbiert wird. Die Nutzung zusätzlicher Messinformationen wie der Intensität des Messsignals ermöglicht es, eine detailliertere Aussage über den Ursprung der Reflexion und somit auch über die geometrische Genauigkeit zu treffen. Zusätzlich können Aussagen über den physiologischen Zustand der Pflanzen abgeleitet werden. Die Studie nutzt zwei 2D-Lasertriangulationssensoren mit unterschiedlichen Wellenlängen, 658 nm (rot) und 405 nm (blau), mit deren Hilfe die Interaktion verschiedener Laserfarben mit separierten Blattbestandteilen untersucht wird. Durch Einbeziehen der Intensität ist es möglich, den Ursprung der Reflexion zu lokalisieren und systematische Abweichungen der resultierenden Punktwolke aufzudecken. Darüber hinaus unterstützt die Intensität die Analyse des physiologischen Zustands der Pflanze und ermöglicht die Detektion oberflächenverändernder Pflanzenkrankheiten wie dem Echten Mehltau. Letztlich kann gezeigt werden, dass die Kombination zweier Laserfarben sowohl die Interpretierbarkeit als auch die geometrische Genauigkeit der gemessenen Punktwolke verbessert.

1 Introduction

Highly accurate 3D plant measurements have become an important tool for plant phenotyping (OMASA et al. 2007). 3D measurements are performed at different scales from airplanes, with fixed-positioned terrestrial laser scan-

ners and with experimental close-up laser setups. The scales of the analysed objects comprise the canopy structure (NÆSSET & BJERKNES 2001), single trees (PFEIFER et al. 2004) and single plants on organ level (PAULUS et al. 2013, PAULUS et al. 2014b, WAHABZADA et al. 2015), respectively.

In particular, high throughput phenotyping focuses on the monitoring of plant and organ development (HONSDORF et al. 2014) as well as on the derivation of phenotypic parameters from processed point clouds (PAPROKI et al. 2012, BELLASIO et al. 2012). Industrial measuring systems using active laser triangulation were commonly applied for plant measurements, providing a spatial resolution and geometrical accuracy of sub-millimetres (PAULUS et al. 2014a, WAGNER et al. 2010). This is essential for the tracking of growth and smallest geometrical changes due to environmental conditions like nutrient availability or abiotic and biotic stress (PAULUS et al. 2014d).

Evaluating the accuracy of 3D plant measurements is extremely challenging, because no highly accurate reference systems are available. For industrial purposes high accurate reference artifacts can be provided that enable the analysis of the sensor quality (DUPUIS & KUHLMANN 2014). Furthermore, recent research has shown that the plant-sensor interaction is not negligible (PAULUS et al. 2014c). Sensor properties like laser wavelength and exposure time have a significant impact on the resulting 3D point cloud. The received signal, backscattered from different layers of the plant structure, is partially absorbed by chlorophyll and, in the worst case, it results in a sensor signal that is not evaluable.

The physical effect of the penetration of laser light into the surface structure of different materials as well as the depth of the penetration is well known in dermatology (BAROLET 2008) and photodynamic therapy (MUSTAFA & JAAFAR 2012). In this context, it is of high importance to treat the human skin in defined tissue depths. For wavelengths between 300 nm and 900 nm, experiments and simulations pointed out that longer wavelengths lead to larger physical penetration (BAROLET 2008). Furthermore, spectral analysis has shown that the absorption by plant tissue strongly depends on the used wavelength (FIORANI et al. 2012). As established commercial 3D measuring devices mainly use a red laser light that lies in the absorption maximum of chlorophyll, the use of a different wavelength should improve the scanning result and the interpretability (STROTHMANN et al. 2014). The work of WEI et al. (2012) showed the application of up to four

laser wavelengths in a multi-wavelength canopy LiDAR for the detection and classification of biochemical concentrations, e.g. withered vs. healthy leaves on canopy level.

Apart from these physical effects, based on a metrological viewpoint, there is the question how the surface penetration and the spectral properties of plants affect the results of commercial laser scanning devices. For materials such as marble, it has been shown that the distance measurement is affected systematically, which results in a reduced measurement accuracy (DUPUIS & KUHLMANN 2014).

Therefore, we show a combined 3D laser scanning approach using two commercial triangulation-based laser scanners with different wavelengths (405 nm and 658 nm), providing the intensity of the backscattered laser ray as additional information. In contrast to PAULUS et al. (2014c), where the analysis was based on the point density using several scans with different sensor settings, the intensity enables the direct and repeatable analysis of the reflectance depth of the signal and a better understanding of the interaction of the laser with the leaf surface structures. Based on the intensity values we are able to locate the origin of the received signal and to analyse the accuracy of the 3D point cloud. Furthermore, we show that an intensity based analysis provides more profound insights into the stage of leaf senescence of the plants combined with the geometrical surface information in 3D.

2 Measuring Setup

Laser scanning experiments were performed using a measuring system comprising a 2D laser triangulation sensor (LTS), working according to the light-section method (DONGES & NOLL 1993), and a moving table. A linear guidance system, combined with a stepper motor (isel Germany AG, Eichenzell, Germany) that actuates the moving table, supplements the 2D laser sensor with the third dimension (Fig. 1A, B), i.e. the scanner is mounted in a fixed position above the moving table and the measured object is moved through its vertical laser plane. The table was moved stepwise, i.e. the measurement was performed when the object under test was static. According to investigations

using special reference artifacts, the precision of the measuring system is $\sigma = 20 \mu\text{m}$ (standard deviation) (DUPUIS & KUHLMANN 2014).

The measuring system was originally equipped with a commercial laser sensor (scanCONTROL 2700-100, MICRO-EPSILON MESSTECHNIK GmbH & Co. KG, Ortenburg, Germany) providing a wavelength of 658 nm (red). Latest developments in sensor technology allow the use of an alternative wavelength of 405 nm (blue) in a commercial high-precision laser sensor. Thus, we decided to extend the measuring system with an extra laser line scanner (gapCONTROL 2911-100 BL). To overcome interferences between the two sensors the measuring system was only equipped with one sensor during the measurement and the sensors were exchanged afterwards.

The two-dimensional field of view of both sensors is limited to a window of 100 mm \times 100 mm at a spatial resolution of 5 μm in distance and $\sim 150 \mu\text{m}$ in the lateral position. The theoretical accuracy for the distance measurement provided by the manufacturer is 15 μm for the red and 12 μm for the blue laser sensor.

In addition to the access to Euclidean coordinates and the geometrical shape of plants' surface, the data analysis is based on the intensity that is provided by both laser scanners. This additional information is determined as the maximum amount of reflected laser light collected in each column of the CCD-array. The intensity is given as a relative value in %, which describes the amount of photons in one pixel element compared to the maximum possible amount of photons. Thus, using a fixed exposure time, these intensity values can be interpreted as reflectance values of the measured surface for every 3D point.

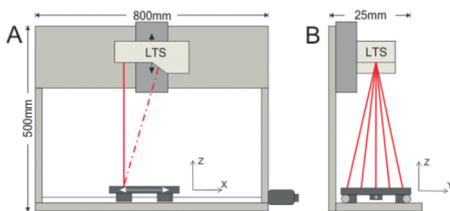


Fig. 1: The technical setup of the measuring system in front (A) and side view (B), LTS = laser triangulation sensor (DUPUIS & KUHLMANN 2014).

3 Impact of different Leaf Tissues

The main objective of this study is to present the impact of the characteristics of leaf or plant surfaces on measurements performed by an active 3D laser triangulation sensor. The experiments focus on the interaction of the laser sensor with the leaf surface and on the way in which this will affect the measurement accuracy. They were inspired by a previous publication (PAULUS et al. 2014c) where the data interpretation was based on the point density measured at different exposure times ranging from the minimum to the maximum limit of the laser sensor. In the present study, an intensity-based data analysis was utilized, thus it was possible to analyse single measurements using one fixed exposure time. However, measurements were performed up to 10 times to prove their repeatability. As all repetitions follow the same behaviour, representative results for one measurement were shown. The exposure time was manually set to a level with a minimum amount of over- and underexposure of the CCD-array to achieve highest geometric measurement accuracy and to minimize systematic uncertainties (DUPUIS & KUHLMANN 2014). However, as we used natural objects with various reflective properties, over- or underexposure cannot be excluded entirely.

3.1 Measurability of Epidermis

In theory, the epidermis, a single layer of cells that forms the boundary between the plant and the environment, is lacking chlorophyll and, thus, is almost transparent compared to the mesophyll cells. To visualize the effect of the physical penetration depth on the measured point cloud, different numbers of epidermal stripes extracted from leek leaves (*Allium porrum* L.) were scanned with both lasers. The physical penetration of the laser beam into the surface structure causes a systematic deviation of the distance measurement and, therefore, the measured surface does not represent the real surface. This deviation, afterwards called measured penetration depth, may differ from the physical penetration depth and is described for different types of laser sensors and

different surfaces, e.g. terrestrial laser scanners (GORDON 2008) and laser triangulation sensors (DUPUIS & KUHLMANN 2014).

The epidermal strips were separated carefully using a razor blade and were attached subsequently in a flat way on a microscope slide made of glass (Fig. 2A–C). As we were interested in the measured depth of penetration, three different test objects were created having an increasing number of epidermal strips of one to three layers.

From the measured point clouds, regions representing the epidermal layer were selected manually and a best-fit plane was estimated using a least-square approach (MIKHAIL & ACKERMANN 1976). The underlying microscope slides were prepared with black stripes that represent an impenetrable pattern on the glass surface. Based on this setup, measurements without the epidermal stripes (Fig. 2D) would result in a point distribution as illustrated in Fig. 2E. In this case, the estimated plane would lie approximately in the middle of the microscope slide and the residuals would represent the pattern of the black stripes.

Scanning the epidermal stripes attached on top of the slides (Fig. 2F) could theoretically create two different results. The first one, shown in Fig. 2G, will be achieved, if the epidermis is impenetrable. In this case the point cloud represents the real geometric surface, as it is expected for blue laser sensor. The second possible outcome, shown in Fig. 2H, will be similar to the point cloud in Fig. 2E, but attenuated, and will be achieved if the epidermis is transparent or semi-transparent. Based

on these considerations one can assume that if the pattern of the black stripes is visible in the residuals of the best-fit plane, the laser scanner will be able to penetrate the epidermis and to measure the underlying surface tissues.

Fig. 3 shows the point clouds of both laser colours for the different numbers of epidermal layers. The colour information represents the residuals of the best-fit plane. In Fig. 3A, the stripe pattern is clearly visible – comparable to the theoretical pattern in Fig. 2E – with a maximum positive deviation from the estimated plane of ~ 0.5 mm (blue stripes). The regions with red colour constitute points with a maximum negative deviation from the plane, i.e. the distance measured by the sensor is larger, which in turn is attributable to a physical penetration of the laser light through the epidermis and the microscope slide. Fig. 3A illustrates the measurement of one epidermal layer with the red laser sensor. Accordingly, one epidermal layer measured with the red laser scanner leads to a transmission of the laser light. A similar but weaker effect is visible in case of two or three epidermal layers (Fig. 3B, C).

The measurements performed with the blue laser sensor show a different behaviour (Fig. 3D–F). The black strips are only visible in case of one epidermal layer with a deviation of about 0.1 mm. Even parts of the regions between these strips provide a very small deviation value. These smaller deviations can be related to a much smaller physical and measured penetration depth. Furthermore, the results for two and three epidermal layers do not show

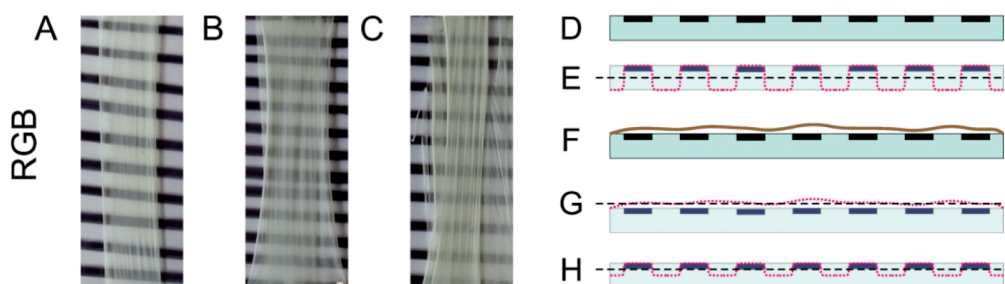


Fig. 2: Experimental setup for the evaluation of the penetration depth. A to C show an RGB image of the different numbers of epidermal layers (one to three). D and E represent the profile of the microscope slide with the stripe pattern and the theoretic scanning result without an epidermal layer, respectively. F to H show the case with one epidermal layer and two possible scanning results. The dashed line represents the best-fit plane.

the control pattern anymore, suggesting that the received signal is backscattered on top of or inside the second epidermal layer.

To compare the results to the finding of PAULUS et al. (2014c), where the exposure time had

a significant impact on the measured penetration depth, the epidermal stripes were scanned using different exposure times varying from 0.35 ms to 0.8 ms. As illustrated in Fig. 4A, B, for the red laser sensor, the measured pen-

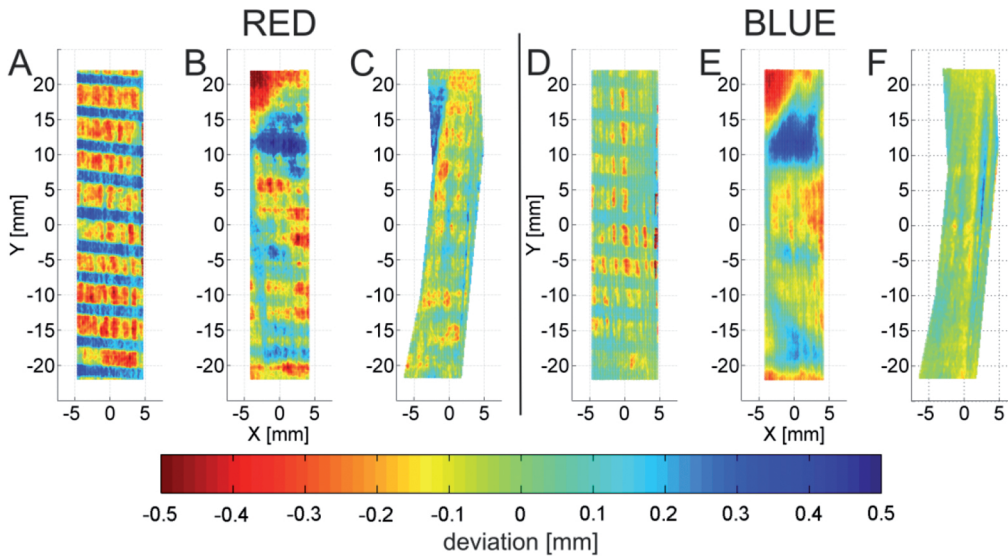


Fig. 3: Evaluation of the penetration of the red and the blue laser scanner at a constant exposure time. All point clouds were rotated to the x-y-plane and coloured using the deviation from the best-fit plane. A to C illustrate the coloured point cloud for one, two and three epidermal layers measured with the red and D to F measured with the blue scanner, respectively.

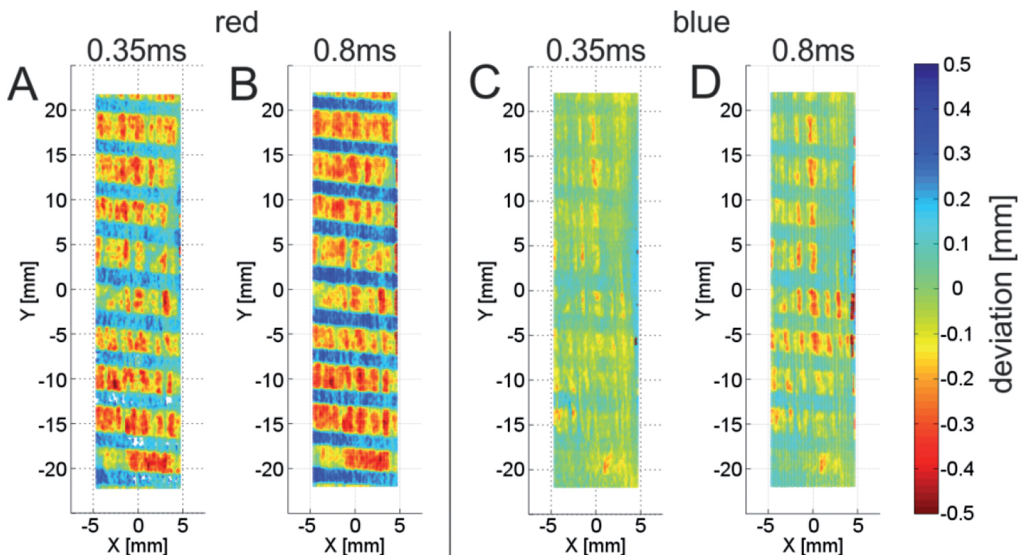


Fig. 4: Deviations from the best-fit plane for one epidermal stripe using the red and the blue laser sensors at different exposure times.

etration depth was slightly increased when scanning with longer exposure times, which is apparent from the larger deviations from the best-fit plane. A similar but slightly weaker effect was achieved for the blue laser sensor (Fig. 4C, D).

To explain this effect the physical shape of the backscattered laser line has to be taken into account. Due to a constant luminous power of the emitted laser, the physical penetration depth should be constant depending on the wavelength (BAROLET 2008). However, in case of semi-transparent materials, like the epidermis, the signal originating inside the surface structure is weakened. The exposure time controls the amount of backscattered laser light that is collected by the CCD-array elements. In other words, the longer the exposure time, the more light is collected. As a consequence, when longer exposure times are used, the weakened signal originating from inside the surface structure is additionally collected by the CCD-array and the centre of gravity of the received laser line is displaced. This displacement results in a longer distance measurement (DUPUIS & KUHLMANN 2014) and, therefore, in a larger measured penetration depth.

3.2 Measuring Photoactive Plant Tissues

In a second experiment the impact of the chlorophyll located in the mesophyll layer on the laser measurements was investigated. To simulate measurements of the mesophyll layer without any impact of cuticle and epidermis, pieces of filter paper were soaked with different concentrations of leaf pigments extracted from sword fern leaves (*Nephrolepis exaltata* (L.) Schott). The pieces were arranged in order visually by colour, measured with both laser sensors and the actual chlorophyll (type a and b) as well as carotenoid concentrations were determined after extraction with 10 ml of 80% acetone as described in PORRA et al. (1989).

The impact of the leaf pigments on the received signal was determined by analysing the intensity values obtained for each 3D point. Therefore, the regions of the paper pieces were extracted manually from the point cloud

using MATLAB® 2009b (The MathWorks Inc., Natick, MA, USA). To overcome possible variations of the chlorophyll distribution inside the filter paper, one intensity value for each region representing the averaged intensity for all points inside the region was determined for data analysis.

Fig. 5 shows an RGB image and the results of the experiment. To compensate the different physical power of both lasers (8 mW for the blue laser vs. 10 mW for the red), we chose different exposure times (0.75 ms for the red and 1.25 ms for the blue laser) to obtain nearly the same averaged intensity at the lowest chlorophyll concentration.

Regarding the colour coded point clouds in Fig. 5, as expected, both sensors interacted with chlorophyll. With an increasing chlorophyll content from 5.8 up to 24.7 $\mu\text{g}\cdot\text{cm}^{-2}$ (first ten stripes: A-1 to A-7 and B-1 to B-4) both laser colours provided a linear decreasing trend ($R^2 > 0.95$) with nearly the same slope of $-2.8\%\cdot\text{cm}^2\cdot\mu\text{g}^{-1}$ for the blue laser and of $-2.6\%\cdot\text{cm}^2\cdot\mu\text{g}^{-1}$ for the red one (Fig. 6).

In case of higher concentrations, a break can be found in the linear function and both sensors exposed a slightly different behaviour.

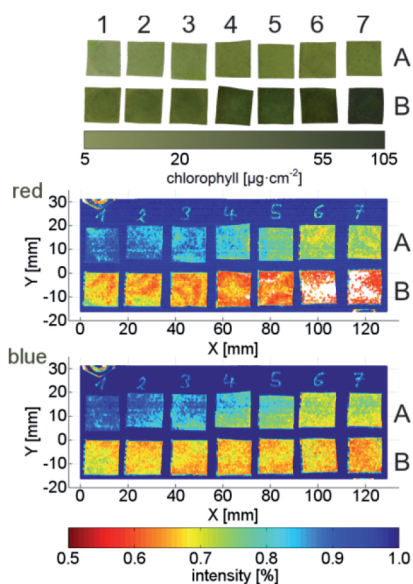


Fig. 5: Investigation of the interaction of extracted chlorophyll ($\text{chl}_A + \text{chl}_B$) with the laser of both laser scanners. The chlorophyll content ranged from 5.8 $\mu\text{g}\cdot\text{cm}^{-2}$ up to 102 $\mu\text{g}\cdot\text{cm}^{-2}$.

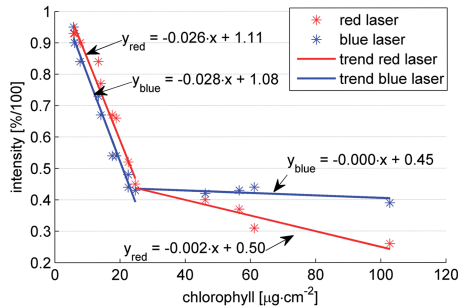


Fig. 6: Decrease of the intensity with an increasing chlorophyll content.

While the intensity of the red laser was still decreasing with a much smaller slope, the intensity of the blue sensor remained approximately constant (B-5 to B-7). This difference could probably be linked to the different physical penetration depths of the two lasers. As already shown in the previous experiment, the physical penetration depth of the blue laser is smaller compared to the red one. This leads to the assumption that the blue laser interacted with chlorophyll in the upper layers of the filter paper, while the red laser penetrated deeper into the filter paper. This resulted in an interaction with more chlorophyll and therefore to a stronger relative absorption.

4 Discussion

Transferring the results of sections 3.1 and 3.2 to a real plant surface leads to the following assumptions:

Scanning with the red laser wavelength (658 nm) enables the sensor to penetrate the leaf surface and to interact with deeper plant tissues. This interaction leads to the partial absorption of the emitted laser ray by the chlorophyll located in the mesophyll layer and therefore to lower intensity values. Thus, the derived point cloud should provide a higher measurement noise, caused by the low intensity of the signal, and should be systematically displaced, because the signal is backscattered in the mesophyll layer.

Using the blue laser wavelength (405 nm) should lead to different result. Due to the smaller physical penetration depth the laser should at best not be able to interact with the

chlorophyll, which in turn results in a chlorophyll-independent intensity. This should result in a lower measurement noise without a systematic displacement, i.e. the geometric accuracy is higher compared to the red one.

Nevertheless, analysing the intensity of the red laser sensor should increase the interpretability of the point cloud because variances in the chlorophyll content should alter the measured intensity values.

5 Possible Applications

In this section the results obtained in section 3 are transferred to real plants and possible applications are presented, where the intensity of the received signal can be used to characterize the physiology of the plant.

5.1 Stages of Leaf Senescence

In the first application we present how the intensity of the received signal is affected by the different stages of leaf senescence. Therefore, leaves of bottlebrush buckeye (*Aesculus parviflora* Walter) containing areas with different stages of leaf senescence ranging from healthy (green) to chlorotic (yellow) and necrotic (brown) tissues (Fig. 7A) were scanned. We expected the different stages to result in different intensity values in case of a penetration of the laser line into the mesophyll layer.

Using the red laser sensor, chlorotic and necrotic areas of the bottlebrush buckeye leave caused different intensity values, due to changes in chlorophyll concentration. Healthy areas with higher chlorophyll content resulted in lower intensity values, while chlorotic and necrotic regions provided higher intensity values (Fig. 7B).

A different result was achieved when scanning with the blue laser. Changes in chlorophyll content were not visible in the intensity distribution, what implies that the received signal originated on top of the leaf surface and the measured penetration depth was minimized. This in turn leads to a higher geometric accuracy.

Compared to classical photogrammetric approaches where the stages of leaf senes-

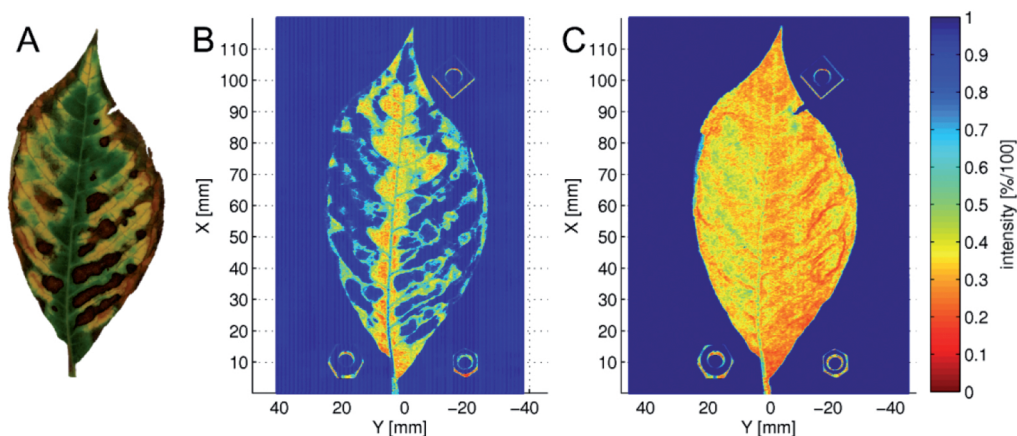


Fig. 7: The leaf of a bottlebrush buckeye (A) was scanned with both laser scanners. The resulting point clouds were rotated to the x-y-plane and coloured according to the intensity values (red laser: B, blue laser: C).

cence can be easily identified using the RGB values, the laser measurements provide a direct and highly accurate access to the 3D geometry of the plant or a leaf. Photogrammetric approaches using Structure from Motion (SfM) and Multi View Stereo (MVS) also enable the reconstruction of coloured 3D point cloud from digital images (PAPROKI et al. 2012, ROSE et al. 2015). However, these approaches provide lower point densities and also a lower geometric accuracy.

Using laser scanning and the intensity of reflection enables an easy and accurate way to get access to the 3D structure and the stage of leaf senescence of single plant organs.

5.2 Detection of Plant Diseases

The detection of plant diseases is of high relevance in plant research. The integration of the detection by non-invasive sensors in an automated canopy measurement process or in a high-throughput phenotyping approach would support agricultural praxis and breeding processes (MAHLEIN et al. 2012a).

In this experiment, we illustrate how the intensity values can support a detection of diseased leaf areas. Therefore, norway maple leaves (*Acer plantanoides* L.) diseased with powdery mildew (*Sawadarea tulasnei* (Fuckel) Homma 1937) were scanned at a fixed expo-

sure time and analysed based on the resulting intensity values. Because powdery mildew modifies the leaf surface in terms of colour and structure, it should thus change the reflective properties. Fig. 8 shows the pattern of damage caused by powdery mildew in a microscope RGB image and semi-thin sections of the leaf surface, prepared and observed

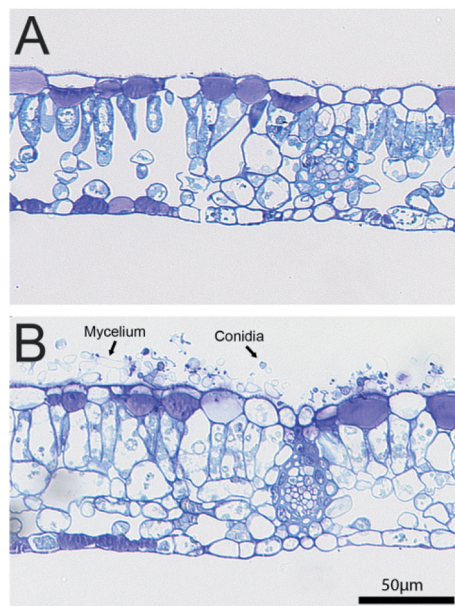


Fig. 8: Semi-thin sections of healthy (A) and diseased (B) plant tissues of a maple leaf.

with a photomicroscope according to MAHLEIN et al. (2012b).

It can be observed from Fig. 8B that the mycelium and the conidia of the fungus alter the rather smooth surface structure in an irregular way. Thus, the emitted laser is backscattered at the rough surface in several directions which is similar to a diffuse reflection, e.g. provided by commercial coating spray. For this reason, we expected higher intensity values at the diseased regions and a lower intensity at the healthy regions.

For the quantification of the disease severity the ratio of diseased and healthy leaf area was calculated by a basic threshold. The point cloud was projected into the x-y-plane and every point was assigned to one pixel in order to generate a 2D greyscale image. Due to the flat attachment of the leaf to the x-y-plane and the vertical alignment of the laser plane, the uncertainties caused by the projection are quite small. In the greyscale image the leaf was manually extracted from the background and the infected pixels were estimated using a threshold value. The disease severity in % was calculated from the estimated amount of diseased pixels in relation to the total amount of leaf pixels.

The results of the threshold approach were compared to manually labelled data of RGB images using the free image processing software GIMP (GIMP 2.8.6, www.gimp.org).

The coloured point clouds illustrated in Fig. 9B, C confirm the theoretical assumption. Regions diseased by powdery mildew pro-

vide much higher intensity values compared to healthy regions. The quantification of the relative disease severity using an empirically chosen threshold value of $x = 200$ which corresponds to an intensity value of $\sim 79\%$, resulted in a relative disease severity of 38% , compared to 40% manually labelled by an expert. The difference could be addressed to uncertainties in the manual labelling process.

Due to the higher intensity values, diseased regions provide a lower local measurement noise. By combining these spatial features together with the intensity (as a spectral feature) and by using a more complex machine learning approach such as support vector machines, an automated and more precise classification should be possible.

In contrast to the results presented in section 5.1, both laser colours showed nearly the same intensity distribution and the powdery mildew diseased leaf areas were visible for both scanners (Fig. 9B, C). This behaviour can be addressed to the fact that powdery mildew changes the structure of the leaf surface as shown in Fig. 8B.

However, taking a closer look at the intensity distributions of both scanners, some differences can be detected regarding the main and smaller leaf veins. In the intensity image of the red laser sensor (Fig. 9B) the main leaf vein and some smaller veins were imaged with high intensity values ($\sim 90\%$) due to lower chlorophyll content. A different result was obtained from the blue scanner. Small leaf veins were not visible in the intensity image

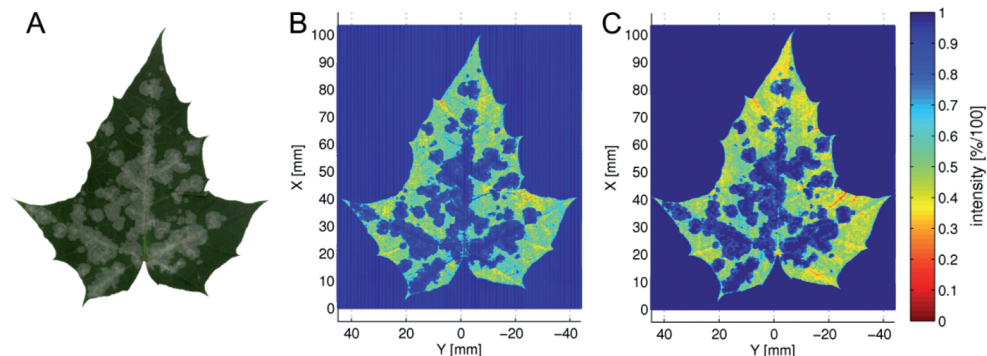


Fig. 9: Detection of the mildew infection of a maple leaf (RGB image: A). The point clouds of the red (B) and the blue (C) laser scanner were rotated to x-y-plane and coloured using the intensity values.

and the main leaf vein was imaged with a low intensity (~50%). This leads to the assumption that the principle behaviour is the same as obtained in the previous experiments. However, powdery mildew changes the reflective properties of the leaf surface in a way that it can be identified with both laser colours.

Summing up, this experiment pointed out that the parameter intensity of reflection supports the detection of plant diseases like powdery mildew without using additional sensors like RGB- or hyperspectral cameras. The simple approach using a threshold to identify infected regions illustrated the significance of the intensity values for plant analysis.

6 Conclusion

Using two commercial state-of-the-art 3D laser triangulation sensors with different wavelengths (658 nm and 405 nm) and providing the intensity of the received signal as additional measuring information gives a great advantage for the metrological and functional analysis of plant measurements. Systematic deviations caused by penetration of the laser into the leaf's surface were found for the red laser sensor, while the blue laser sensor was able to measure the real surface of the plant. Thus, from a metrological viewpoint the blue laser sensor provides higher geometric accuracy. However, despite the lower geometric accuracy, using the intensity values of the red laser sensor measured for every 3D point uncovers detailed and highly resolved information about changes of the stage of leaf senescence, tissue content or plant diseases. Thus, a combination of these laser wavelengths enables a precise imaging of the 3D surface structure of plants together with an analysis of the underlying surface tissues. As we used commercial 3D scanning systems, our experimental results can directly be transferred to existing phenotyping platforms using similar laser scanning systems.

Acknowledgements

The authors also want to express their gratitude to BIRGIT KRETSCHMANN for proofreading

the manuscript. This work could be carried out due to the financial support of the German Federal Ministry of Education and Research (BMBF) within the scope of the competitive grants program "Networks of excellence in agricultural and nutrition research – CROP.SENSE.net" (Funding code: 0315529).

JD, SP, TE, AKM and HK designed the study. JD, SP and TE interpreted the measured data and drafted the manuscript. Measuring and programming was carried out by JD and SP. Extracting the chlorophyll and analysing its content in the filter paper was performed by TE. The microscope images and the profiles of the leaf surfaces were prepared and analysed by AKM. HK directed the research and gave initial input. All authors read and approved the final manuscript.

References

- BAROLET, D., 2008: Light-Emitting Diodes (LEDs) in Dermatology. – *Seminars in Cutaneous Medicine and Surgery* **27** (4): 227–238.
- BELLASIO, C., OLEJNIČKOVÁ, J., TESAR, R., ŠEBELA, D. & NEDBAL, L., 2012: Computer reconstruction of plant growth and chlorophyll fluorescence emission in three spatial dimensions. – *Sensors* **12** (1): 1052–1071.
- DONGES, A. & NOLL, R., 1993: *Lasermesstechnik. Grundlagen und Anwendungen*. – Hüthig Verlag, Heidelberg.
- DUPUIS, J. & KUHLMANN, H., 2014: High-Precision Surface Inspection: Uncertainty Evaluation within an Accuracy Range of 15µm with Triangulation-based Laser Line Scanners. – *Journal of Applied Geodesy* **8** (2): 109–118.
- FIORANI, F., RASCHER, U., JAHNKE, S. & SCHURR, U., 2012: Imaging plants dynamics in heterogenic environments. – *Current Opinion in Biotechnology* **23** (2): 227–235.
- GORDON, B., 2008: *Zur Bestimmung von Messunsicherheiten terrestrischer Laserscanner* (Doctoral Dissertation). – Technische Universität Darmstadt.
- HONSDORF, N., MARCH, T.J., BERGER, B., TESTER, M. & PILLEN, K., 2014: High-throughput phenotyping to detect drought tolerance QTL in wild barley introgression lines. – *PloS One* **9** (5): e97047, doi: 10.1371/journal.pone.0097047.
- MAHLEIN, A.-K., OERKE, E.-C., STEINER, U. & DEHNE, H.-W., 2012a: Recent advances in sens-

- ing plant diseases for precision crop protection. – *European Journal of Plant Pathology* **133** (1): 197–209.
- MAHLEIN, A.-K., STEINER, U., HILLNHÜTTER, C., DEHNE, H.-W. & OERKE, E.-C., 2012b: Hyperspectral imaging for small-scale analysis of symptoms caused by different sugar beet diseases. – *Plant Methods* **8** (1): 3, doi: 10.1186/1746-4811-8-3.
- MIKHAIL, E.M. & ACKERMANN, F.E., 1976: Observations and least squares. – IEP-A Dun-Donnelley Publisher, New York, NY, USA.
- MUSTAFA, F.H. & JAAFAR, M.S., 2012: Comparison of wavelength-dependent penetration depths of lasers in different types of skin in photodynamic therapy. – *Indian Journal of Physics* **87** (3): 203–209.
- NÆSSET, E. & BIERKNES, K.-O., 2001: Estimating tree heights and number of stems in young forest stands using airborne laser scanner data. – *Remote Sensing of Environment* **78** (3): 328–340.
- OMASA, K., HOSOI, F. & KONISHI, A., 2007: 3D lidar imaging for detecting and understanding plant responses and canopy structure. – *Journal of Experimental Botany* **58** (4): 881–898.
- PAPROKI, A., SIRAUTL, X., BERRY, S., FURBANK, R. & FRIPP, J., 2012: A novel mesh processing based technique for 3D plant analysis. – *BMC Plant Biology* **12**: 63.
- PAULUS, S., DUPUIS, J., MAHLEIN, A.-K. & KUHLMANN, H., 2013: Surface feature based classification of plant organs from 3D laserscanned point clouds for plant phenotyping. – *BMC Bioinformatics* **14**: 238.
- PAULUS, S., BEHMANN, J., MAHLEIN, A.-K., PLÜMER, L. & KUHLMANN, H., 2014a: Low-Cost 3D Systems: Suitable Tools for Plant Phenotyping. – *Sensors* **14** (2): 3001–3018.
- PAULUS, S., DUPUIS, J., RIEDEL, S. & KUHLMANN, H., 2014b: Automated Analysis of Barley Organs Using 3D Laser Scanning: An Approach for High Throughput Phenotyping. – *Sensors* **14** (7): 12670–12686.
- PAULUS, S., EICHERT, T., GOLDBACH, H.E. & KUHLMANN, H., 2014c: Limits of Active Laser Triangulation as an Instrument for High Precision Plant Imaging. – *Sensors* **14** (2): 2489–2509.
- PAULUS, S., SCHUMANN, H., KUHLMANN, H. & LÉON, J., 2014d: High-precision laser scanning system for capturing 3D plant architecture and analysing growth of cereal plants. – *Biosystems Engineering* **121**: 1–11.
- PFEIFER, N., GORTE, B. & WINTERHALDER, D., 2004: Automatic reconstruction of single trees from terrestrial laser scanner data. – 20th ISPRS Congress: 114–119, Istanbul, Turkey.
- PORRA, R.J., THOMPSON, W.A. & KRIEDEMANN, P.E., 1989: Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls and extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. – *Biochimica et Biophysica Acta (BBA)-Bioenergetics* **975** (3): 384–394.
- ROSE, J., PAULUS, S. & KUHLMANN, H., 2015: Accuracy Analysis of a Multi-View Stereo Approach for Phenotyping of Tomato Plants at the Organ Level. – *Sensors* **15** (5): 9651–9665.
- STROTHMANN, W., RUCKELSHAUSEN, A. & HERTZBERG, J., 2014: Multiwavelength laser line profile sensing for agricultural crop characterization. – *SPIE Photonics Europe*: 91411K–91411K.
- WAGNER, B., GÄRTNER, H., INGENSAND, H. & SANTINI, S., 2010: Incorporating 2D tree-ring data in 3D laser scans of coarse-root systems. – *Plant and Soil* **334** (1–2): 175–187.
- WAHABZADA, M., PAULUS, S., KERSTING, K. & MAHLEIN, A.-K., 2015: Automated interpretation of 3D laserscanned point clouds for plant organ segmentation. – *BMC Bioinformatics* **16**: 248.
- WEI, G., SHALEI, S., BO, Z., SHUO, S., FAQUAN, L. & XUEWU, C., 2012: Multi-wavelength canopy LiDAR for remote sensing of vegetation: Design and system performance. – *ISPRS Journal of Photogrammetry and Remote Sensing* **69**: 1–9.

Addresses of the Authors

M.Sc. JAN DUPUIS & Prof. Dr.-Ing. HEINER KUHLMANN, Universität Bonn, Institut für Geodäsie und Geoinformation, Nussallee 17, D-53115 Bonn, Tel.: +49-228-733571, Fax: +49-228-732988, e-mail: {dupuis}{kuhlmann}@igg.uni-bonn.de

Dipl.-Inform. STEFAN PAULUS, LemnaTec GmbH, Pascalstr. 59, D-52076 Aachen, e-mail: stefan.paulus@lemnatec.de

Dr. ANNE-KATRIN MAHLEIN, Universität Bonn, Institut für Nutzpflanzenwissenschaften und Ressourcenschutz, Abteilung Phytomedizin, Meckenheimer Allee 166a, D-53115 Bonn, e-mail: amahlein@uni-bonn.de

PD. Dr. THOMAS EICHERT, Universität Bonn, Institut für Nutzpflanzenwissenschaften und Ressourcenschutz, Abteilung Pflanzenernährung, Karlrobert-Kreiten-Str. 13, D-53115 Bonn, e-mail: t.eichert@uni-bonn.de

Manuskript eingereicht: September 2015

Angenommen: Oktober 2015