`Biophysical and Engineering Contributions to Plant Research`

Artmann GM. , I. Digel, P. Linder, and Mrs. Aysegül Temiz Artmann

University of Applied Sciences Aachen, Institute for Bioengineering, Heinrich Mussmann Strasse 1, D-52428 Juelich, Tel.+49 241 6009 53866, E-mail: artmann@fh-aachen.de, Digel@fh-Aachen.de, Linder@fh-Aachen.de, and a.artmann@fh-aachen.de, Admin. Office: Kayser@fh-aachen.de.

Abstract: In the past decades, much scientific attention has been spent to the development of biomedical engineering devices applicable to humans in health and disease. At the same time, biology of eukaryotic cells including stem cells developed to an extend that nowadays we are able to think about curing diseases which we could not think of any time before. Many of us, scientists as well as engineers, are proud of these developments. Our Institute of Bioengineering at Juelich, a city with strong background in bioengineering research located close to the city of Aachen, Germany (www.biomedtech.de) contributed its share. We did it with dedication and profound background in engineering and natural sciences.

Introduction: Mankind needs food, drinking water, and healthy environments to live in. In the past few years our Institute for Bioengineering shed parts of it´s attention to technological solutions in crop sciences, plant growths, and plant disease identification and -fighting. We intend to apply our background to palm tree research, palm tree gender differentiation, (the RPW pest combating) and palm tree disease recognition. The topics of our talk will include 1) automated scanners and data achieved so far of date palm tree leaves. This will include judging the potential of such scanners to differentiate date palm tree gender and phenotype. Plants including date palm trees display a huge variety of geometries, boundary conditions, necessities. Any plant scanner, if portable or not, not only lives from its hardware design but very much and to a huge extend from the science and engineering one puts into the ´brain´ of such machines. Thus, automated scanner generations designed for certain applications as date palm tree research must be custom designed and can only be marketed as such. One of the key goals will be finding technological solutions in particular for palm tree research which are applicable in the field (plantations). Considering such goal, one of our plant scanners was developed as portable machine, capable of scanning leaves (date palm tree leaves) in any plantation and location. With a little wider focus in plant research we will talk on implications of automated C3/C4 plant differentiation (rice/corn), on water uptake of plants, time constants of water transport in leaves, plant leave volume and geometry determination for water uptake and precise biomass quantification as well as for plant stress research. Finally, we would like to emphasize that combining engineering, biophysics and plant research is a most valuable tool for a better living, a healthy environment and sufficient food for all people on our planet.

Automated Plant Leaf Scanners - Methods and Techniques

Any classification for gender or phenotype based on leaf analysis, respectively, requires the identification of parameters sensitive to differences between two different types of samples. Thus, experiments based on very different technology have to be carried out before designing a scanner to observe whether a particular parameter and method is of interest for such classification. Surface topology detection is a very first method which can be carried out. Principally it can be achieved by scanning a leave passing through a Laser line light path from above the leave and at the same time from below. Mounting both profiles together a cross-section of a strip of a leaf (70µm wide) can be detected. Using appropriate software a 3D Image of this leaf can be reproduced on the computer screen. Such image can be analyzed further for characteristic surface features potentially usable for plant differentiation purposes. Such analysis would imply the assumption that different internal structures are represented at the surface of a particular leaf (figure 1).

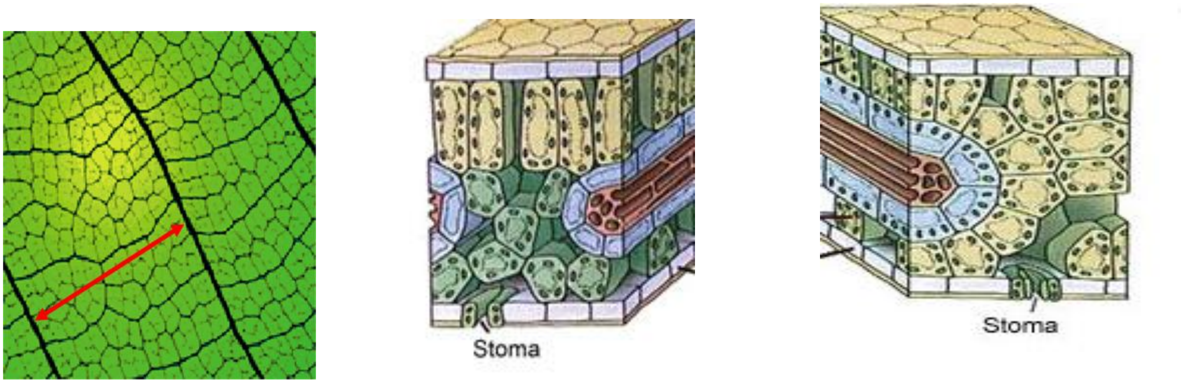

Figure 1: Left) Scheme of a plant leaf. The red arrow indicates the vascular bundle distance. Typical pattern (black) as well as chlorophyll distributions (green) appear between bundles. After pattern analysis based on image analysis and neural network applications characteristics of such pattern may lead to a differentiation between two types of plants the leaves were harvested from. Middle) Scheme of the internal structure of a C3 plant leave (rice) as compared to a C4 plant leave (right). Differences between the internal structures may appear as typical pattern on surfaces of plant leaves enabling to differentiate plant leaves using scanning procedures.

Regular bright field microscopy may be chosen as well for plant differentiation (figure 2, above left). This requires a leaf to be permeable for regular light which is not always the case. However, if applicable, such technique is very useful to identify internal leaf structures. However, not only methods determine the complexity of a scanner software. A leaf may be incorrectly introduced into the scanner. A handy software needs to be able to recognize and correct such matter in order to calculate correct distances of for example vascular bundle distance (figure 2,3).

Methods like auto fluorescence microscopy may be also helpful (figure 3). Chlorophyll exhibits a wonderful auto-fluorescence. Such images would enable detecting pattern of chlorophyll distribution. Overlaying such images with topographic images in the computer hows for example chlorophyll distributions relative to vascular bundles. Additionally, very new technology may be applied as Optical Coherence Tomography. Such imaging shows cross sections through a leaf at a resolution of 10 µm (figure 3, right).

Thus, the set of methods implemented into the scanner defines its purpose and applicability. There is no scanner for everything at once. Very detailed pre-investigations will have to be performed in order to reach two goals 1) to detect what you want to detect, and 2) to use the most appropriate method in order to make the scanner as inexpensive as possible.

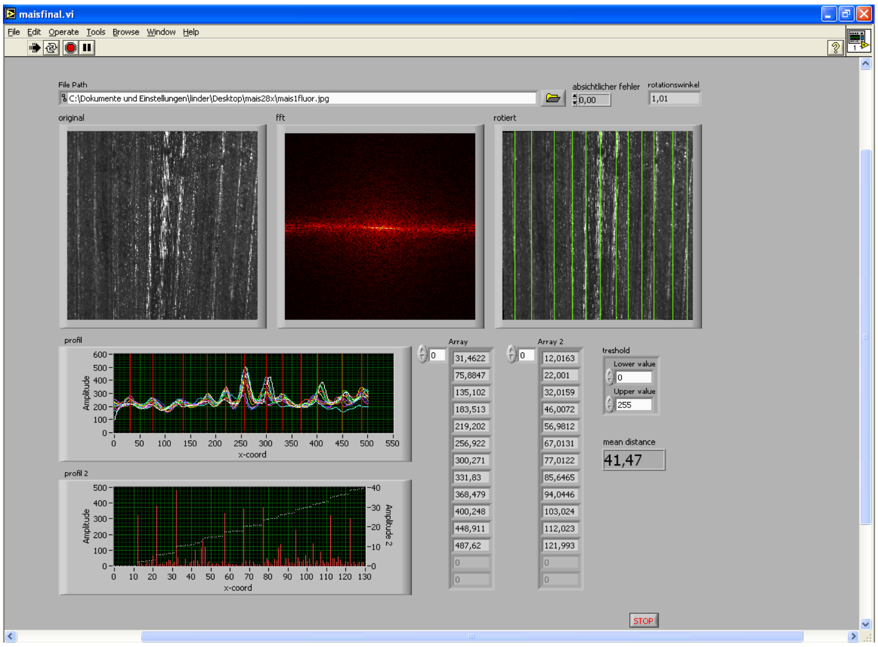

Figure 2: Screenshot of a plant scanner for the detection of distances between vascular bundles. Images 1-5 from top, left to right, and to below: 1) Transmitted light image through a leaf (white lines indicate a vascular bundle, 2) 2D Fourier Transformed Image (angle to the horizontal line of the image indicates the inclination angle of the leaf with respect to the Laser line which should be oriented perpendicular to the leaf. 3) Image corrected for an inclination angle of 90° relative to the Laser line and vascular bundles (green lines), 4) maximum detection for vascular bundles, and 5) location of the bundles. Below, right, the average vascular bundle distance, 41.47 µm, is shown.

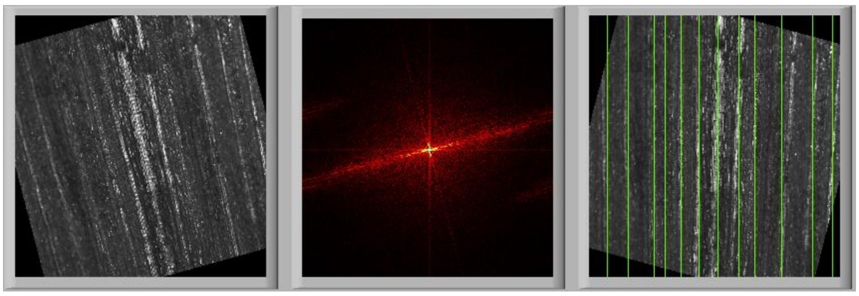

Figure 3: Same leaf as above inclined to the Laser line by 15°. Left: inclined original. Middle: 2D Fourier transformation, right: for orientation corrected image of the leaf with bundles (green).

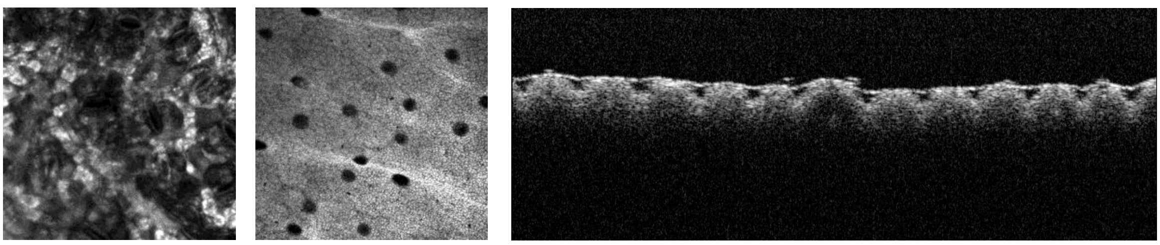

Figure 3: Left Image: Auto-fluorescence image (top view) of a fig tree leave. White: High chlorophyll content, Black: Vacuoles - no chlorophyll. Middle image: Birch tree leaf (view from below) showing stomata openings. Right image: Cross sectional image obtained with Optical Coherence Tomography (OCT). OCT is a non-invasive optical detection method resulting in cross sectional images of the leaf at

a resolution of ~ 10 µm. The photograph shows a cross section of a rice leaf. Black dot-areas above indicate the size and opening characteristics of stomata. The optical penetration depth depends on optical and leaf parameters and can reach up to 1 mm.

The convenience of handling a scanner also depends on the use of bar-code readers for plant identification (figure 4, middle), touch screen (figure 4, above), battery driven scanners, internet and USB connection for data storage and remote data analysis which theoretically could be done anywhere in the world. A scanner for greenhouse usage (figure 4, left) maybe larger but must be waterproof and withstand warm and humid environments. It should be easily transportable even on an uneven ground. Portable scanners for field studies must be robust, battery driven, easy to handle and easy to store and transport (figure 4, right).

Figure 4: Prototypes of plant scanners. Right: Phytoscan alpha 70, for green house studies (battery, internet, USB), Left: Mobile scanner prototype for field studies portable in a suitcase.

Conclusions: Tests with palm tree leaves have just started yet and scan data are in the process to be analyzed. The final goal of future project for palm tree gender and species recognition will be to develop optical scanning technology to be applied to date palm tree leaves for in–situ screening purposes. Depending on the software used and the particular requirements of the users the technology potentially shall be able to identify palm tree diseases, palm tree gender, and species of young date palm trees by scanning leaves. Being aware of the complexity of the subject we suggest a limit for a successful detection of ninety percent, thus, in nineteen percent of all detections the technology should allow a correct classification. This is an ambitious goal which will be approached by methods of contemporary high tech optical technology implanted in modern scanner machines as indicated above. Semi-automated scanners should identify micro pattern characteristics by extracting (neural network applications) characteristics of Date Palm Tree Leaves (Phoenix dactylifera L.) and shall be finally designed for field screenings. In parallel, molecular biological and genetic data should be extracted and analyzed. The final technology should be fast and applicable in-situ. There is still a road to go to reach such ambitious goals. However, if these inventions were successful then they could provide a significant progress in date palm tree culturing and date production. We are ready to take the challenge.