2nd Call for Funding: John Ferguson – University of Nottingham.

October 8, 2020 (updated October 8, 2020)
Project Title *Leveraging optical topography for rapid reconstructions of 3D leaf surfaces to address key questions in plant biology*.

Total Fund Requested – £25,000.00

Project Summary

Introduction and Overview

The surfaces of leaves are critical for regulating plant-ecosystem interactions. Micro-morphological structures, such as ridges, veins, stomata, and trichomes define overall leaf surface texture. This texture and the constituent components contribute to the capacity for plants to tolerate abiotic and biotic stress. For example, variation in stomatal patterning (Dutton *et al*. 2019; Tateda *et al*., 2019) and overall leaf surface texture (Ritpitakphong *et al*., 2016; Sanchez-Lopez *et al*., 2018) has been linked to fungal and bacterial disease resistance. These traits are also key for defining responses to environmental stress. Cuticular wax, for example, is a major component of overall surface texture (Zeisler-Diehl *et al*. 2018) and facilitates resistance to high light intensities and high temperatures (Shepherd & Griffiths, 2006). Moreover, stomatal and trichome morphology are key determinants of plant-water-relations, drought tolerance, and thermoregulation (Leakey *et al*., 2019; Urban *et ai.*, 2017).

Presently there are no platforms publicly available or otherwise in the UK or globally for characterising the 3D surfaces of leaves (Haus *et al.*, 2015). Gold standard methodologies for quantifying individual components of the leaf surface are well defined and widely employed, however they are typically low-throughput and lack 3D resolution. This represents a fundamental phenotyping bottleneck to be addressed by the phenomics and phenotyping community. The development of a high-throughput platform for scanning and reconstructing 3D leaf surfaces would open up much-needed avenues for novel integrative plant biology research and crop improvement efforts. We envisage applications for 3D plant surface analysis to be in high demand and to be significantly varied in their scope, e.g. optimising gas exchange for photosynthesis, trichome properties, palaeobotany, microbiome analysis, wax deposition, and plant defence and protection.

With this project, we will develop the use of emerging and novel optical surface topographic measurement techniques for rapid reconstruction of 3D leaf surfaces. Various measurement techniques, such as focus variation, coherence scanning interferometry and confocal microscopy, will be explored to evaluate the most suitable methodologies, using surface feature analysis methods previously developed by the Manufacturing Metrology Team at the University of Nottingham, the leading UK group in this area, for novel manufacturing methods.

To this end, we will evaluate the use of specialist characterisation and feature-extraction software, such as DigitalSurf MountainsMap, for quantifying overall leaf surface texture as well as a suite of additional micro-morphological traits (Table 1; Figure 1). MountainsMap is the current leading software choice found in industrial surface characterisation and analysis. This project will generate outputs to validate our approaches and put in place a plan to establish the first world centre for 3D plant surface analysis.

Many 2D-resolution microscopy-based methodologies exist for the extraction of singular leaf surface features. These are widely employed in multiple branches of plant biology research; however, they are typically low throughput such as stomatal frequency and cell size. The technology we are proposing to employ to characterise 3D leaf textures will address this low throughput bottleneck, whilst additionally facilitating the simultaneous obtainment of novel 3D-resolution traits of significant interest to multiple areas of plant and crop research (Table 1). Consequently, this platform has the potential to provide a high-throughput and all-encompassing means of phenotyping leaf surfaces that will produce large amounts of data that are widely applicable. They are typically lab- based methods using desktop or free-standing instruments. Details of the *optical surface texture systems* instruments available on Jubilee campus are found here: https://www.nottingham.ac.uk/research/groups/advanced-manufacturing-technology-research-group/research/manufacturing-metrology-team/facilities.aspx.

As a proof-of-concept, we will employ this platform to quantify the 3D leaf surfaces of multiple varieties of wheat and relate variation in surface texture and stomatal patterning and morphology to variation in stomatal and boundary layer resistance to photosynthesis as assessed through infra-red gas exchange analysis (proof-of-concept I). Additionally, we will utilise links with long-standing collaborators in research areas outside of plant physiology, e.g. pathology, palaeobotany, and root biology, to explore further uses of these technologies for plant and crop research (proof-of-concept II). These proof-of-concept studies are detailed below.

This pilot project is an interdisciplinary collaboration between the School of Biosciences and the Faculty of Engineering (Department of Mechanical Materials and Manufacture Engineering). We have assembled a team with unique expertise needed to coordinate the interdisciplinary activities. PI Dr John Ferguson is an Early Career Researcher (3 years post-PhD) at the University of Nottingham with expertise in plant ecophysiology and quantitative genetics. He has significant expertise in developing high throughput phenotyping tools to support rapid phenotype-to-genotype studies. Recently, he coordinated a multi-institutional and multi-disciplinary collaborative project across the US to develop a vertically integrated phenotyping pipeline for collating traits associated with water use efficiency in economically important grass species. Erik Murchie is Professor in Applied Plant Physiology and will apply his expertise acquired in managing interdisciplinary projects in photosynthesis and canopy modelling. Richard Leach is Professor in Metrology and a world leader in surface topography measurements. Dr Adam Thompson and Dr Lewis Newton are Research Fellows within the Manufacturing Metrology team in the Faculty of Engineering.

To successfully complete this pilot project, we require funding for: minimal plant growth space, resources for physiological and microscopy work, and postdoctoral time (Fully Biosciences; Part- or Full-time). The role of the postdoctoral researcher to be hired is hugely critical to the successful completion of this pilot project. We will seek to hire someone with a biological sciences background and extensive experience in computer vision and image analysis. We have postdocs in place who have the relevant interdisciplinary expertise and may be available to take this up e.g. Dr Jonathan Gibbs. It is envisaged that they will be based at Biosciences but embedded within the Manufacturing Metrology Team in order to quickly learn and develop the surface analytical skills required for feature extraction in order to apply them to plant 3D surfaces efficiently. Without these funds, it will not be possible to perform this work.

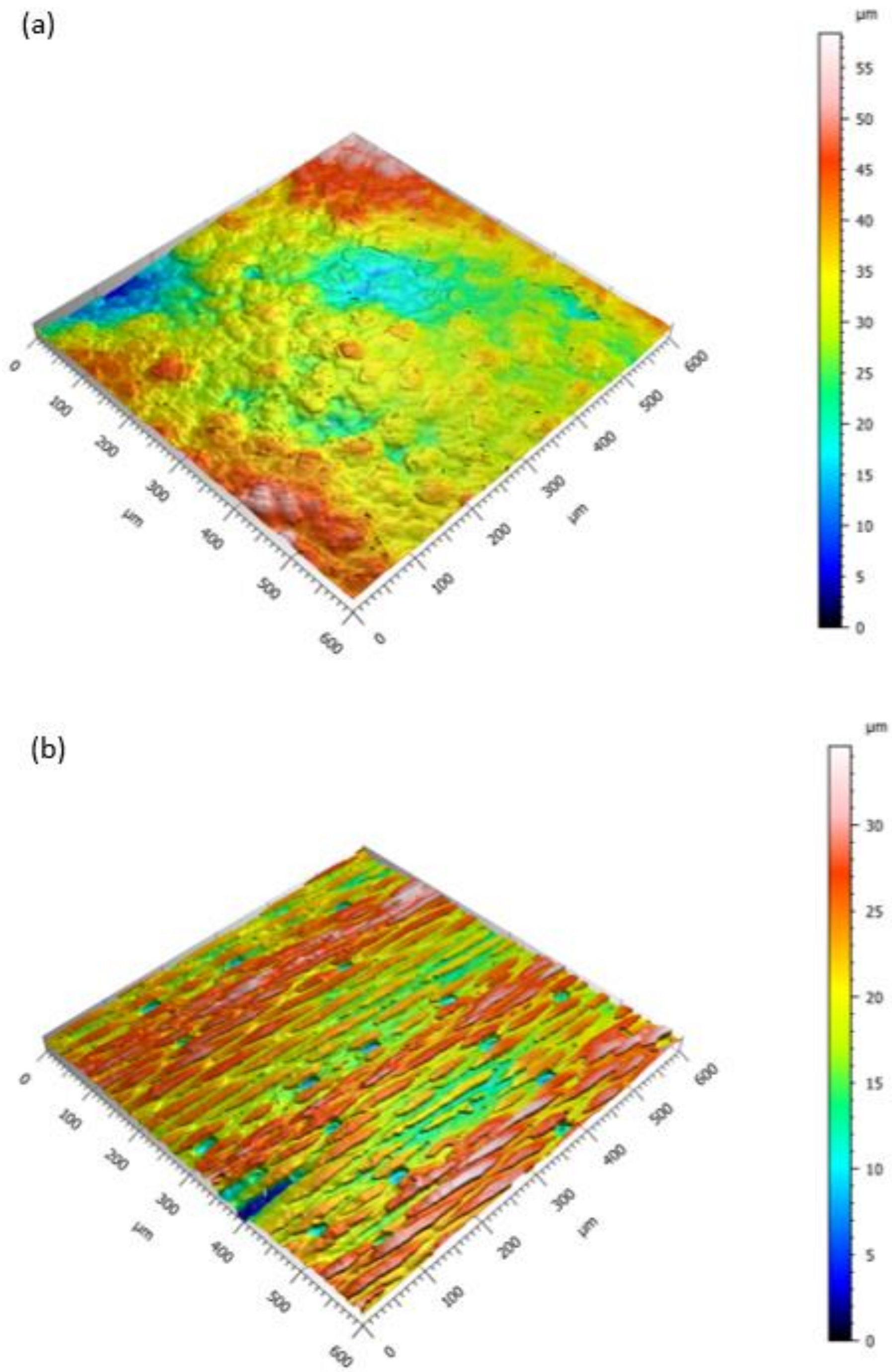

Figure 1. Testing of optical topographic techniques for reconstructing 3D surfaces of (a) dicotyledonous and (b) monocotyledonous leaf surfaces. Reconstructions were made at the University of Nottingham via scanning of fresh leaf material. The Depth map is shown for each image and stomatal pores and their altered depth with respect to the leaf surface are clearly visible.

Proof-of-concept I: Linking leaf surface physiology to stomatal and boundary layer limitations to photosynthesis

To demonstrate the capacity of the proposed platform for expediting integrative plant biological research through high-throughput phenotyping, we have designed an experimental application of the platform that will facilitate an improved understanding of major leaf morphological constraints to photosynthetic assimilation. Fixing atmospheric CO_2 via photosynthesis is the fundamental basis of crop growth and yield. In most plant species, CO_2 is fixed within leaf mesophyll cells. The pathway of CO_2 to mesophyll tissue within leaves is facilitated by stomata. Consequently, stomatal characteristics play a pivotal role in defining the rate at which CO_2 can transverse this pathway (Bertolino *et al* 2019; Figure 2b). Additionally, the texture of the leaf surface affects this rate due its impact on the windspeed of the air over the leaf surface (known as the boundary layer), which in turn impacts the conductance of CO_2 from the atmosphere to the leaf (Schreuder *et al* 2001; Figure 2a). The boundary layer is also important in the regulation and exchange of carbon uptake for water loss, i.e. intrinsic leaf water use efficiency, a key trait that influences crop productivity.

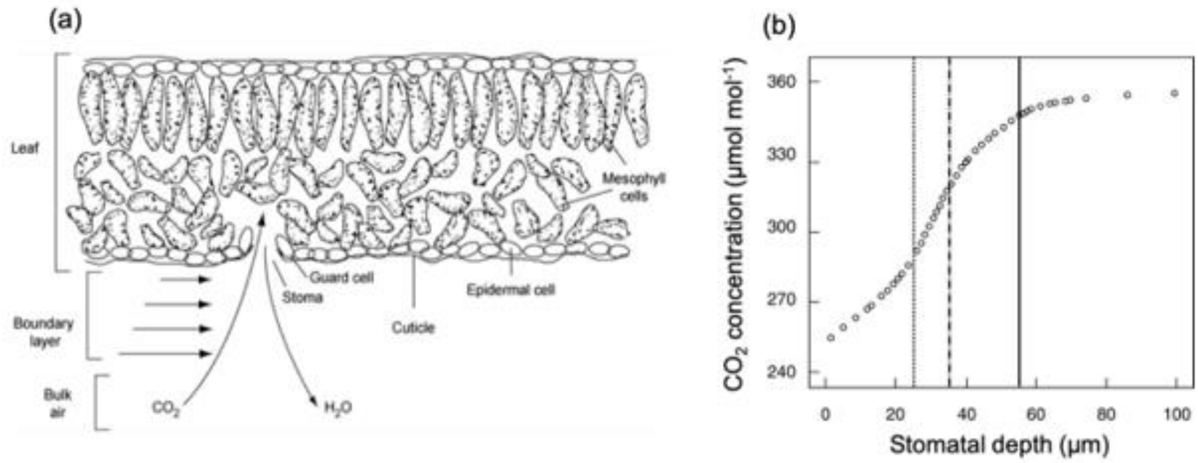

Figure 2. The impact of leaf surface physiology on photosynthesis. (a) Schematic demonstrating how the diffusion of atmospheric CO_2 into the leaf is impacted by boundary air flow (Figure from: Chapin III *et al*. (2011). As leaf surface texture becomes rougher, the boundary layer becomes more turbulent, reducing CO_2 diffusion (Schreuder *et al*. 2000). (b) The increase in the CO_2 diffusion gradient with increasing stomatal depth (Figure from: Roth-Nebelsick 2007). Stomata exist in depressions within the epidermis, known as crypts. As crypts become more depressed, the diffusion gradient of CO_2 increases, thereby limiting photosynthesis.

The photosynthetic limitations imposed by the combined effect of stomatal and boundary layer resistance (*l*) can be graphically modelled, such that the percentage reduction in photosynthesis attributed to *l* can be calculated (Figure 3; Bernachi & Long 2003). Despite the feasibility of determining *l* through gas exchange methods, there have been very few published studies that attempt to quantify how stomatal or leaf surface texture characteristics impact *l*. This is important because underexplored 3D features of leaf surfaces, e.g. trichomes, stomatal pitting, and surface texture, are hypothesised to influence gas exchange. Developing an understanding of the stomatal and leaf surface features that constrain the rate of flow of CO_2 to the mesophyll will allow for future crop improvement efforts that directly target these features to maximise photosynthesis and therefore growth and yield.

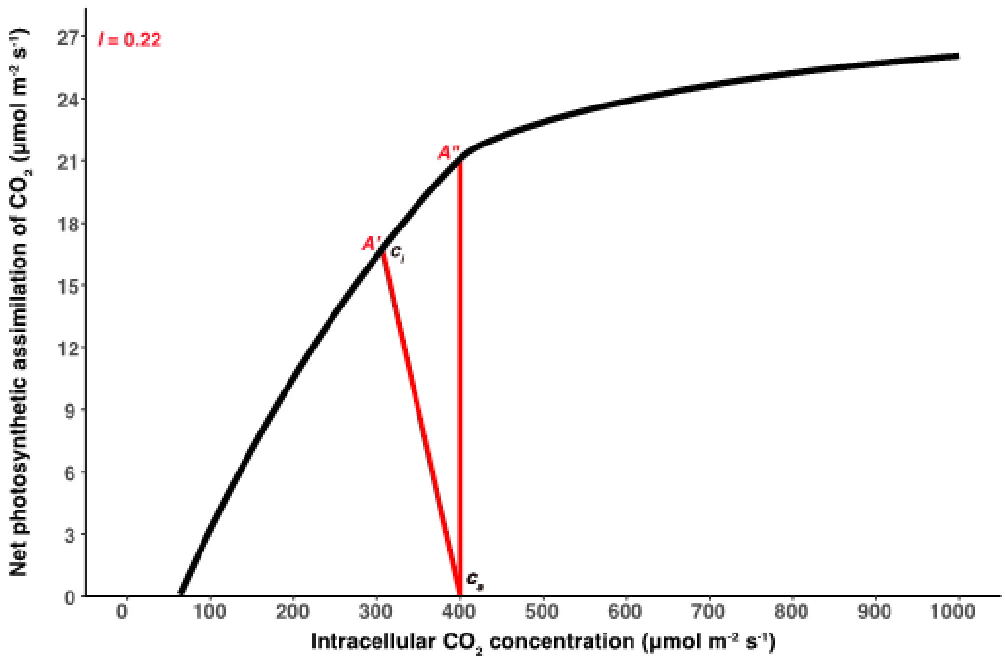

Figure 3. The response of net photosynthetic assimilation of CO_2 (*A*) to incrementally increasing intracellular CO_2 concentration (c_i) in wheat. The red supply functions denote the graphical method of estimating the limitation (*l*) imposed on A by stomata and the leaf boundary layer. Assuming lack of *l*, the supply function from atmospheric CO2 (~400 ppm) would intercept the A–c_i curve at A". However, *l* restricts CO_2 diffusion such that the supply function intercepts the A–c_i curve at A', which represents the operating rate of c_i. *l* can then be calculated as (A''-A')/A".

With this proof of concept study, we will demonstrate that we can (a) extract expected and novel features (Table 1) and (b) determine genetic variation for these features in wheat. To achieve this, we will employ above-described novel optical topographic techniques to scan and re-create the leaf surface tissue (Figure 1) from which *l* will be determined. Using specialised software, we will then characterise numerous traits pertaining to 2D and 3D stomatal characteristics and leaf surface texture from these reconstructions (Table 1). These will then be related back to *l* via standard regression and multivariate analyses to ascertain the extent to which singular features and combinations of features limit photosynthesis in wheat. We will use a set of ten varieties from the A.E. Watkins Collection that best represent the total genetic variation from this key germplasm resource. The Watkins Collection represents a highly suitable set of varieties for this project, since they harbour substantial novel genetic variation not present in elite breeding varieties.

Component of *l*	**Trait**	**3D / 2D**
Stomatal	Stomatal index	2D
Stomatal	Guard cell height, width length, density	2D
Stomatal	Stomatal density	2D
Stomatal	Pore depth	3D

Stomatal	Pore volume	3D
Stomatal	Maximum stomatal conductance value (Gs_{max})	2D
Boundary	Overall surface texture value	3D
Boundary	Vein width	2D
Boundary	Vein density	2D
Boundary	Vein height	3D
Boundary	Trichome height	3D
Boundary	Trichome width	3D
Boundary	Trichome density	3D

Table 1. List of proposed traits to be extracted from the 3D reconstruction of leaf surfaces. We will extract traditional 2D traits already obtainable from traditional 2D resolution methodologies at a much more rapid rate. Additionally, we will also obtain totally novel 3D parameters (highlighted in red) from the topographical reconstruction of leaf surfaces.

Proof-of-concept II: Exploration of the diverse applications of plant surface optical topography

We envisage applications for 3D plant surface analysis to be in high demand and

to be significantly varied in their scope, e.g. optimising gas exchange for photosynthesis, trichome properties, palaeobotany, microbiome analysis, wax deposition, plant defence and protection. To this end, we have established links and will continue to seek links with academics in these areas to carry out studies on material they provide, and which are relevant to genuine questions around leaf surface biology. These include the imaging of pathogens that utilise stomatal pores for entry (Rumiana Ray, Nottingham), the study of fossil and herbarium specimens (Barry Lomax and Susie Lydon, Nottingham), and the study of the root microbiome.