

Taking the pulse of a plant: dynamic laser speckle analysis of plants

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Abstract: Ideally, to achieve optimal production in agriculture, crop stress needs to be measured in real-time, and plant inputs managed in response. However, many important physiological responses like photosynthesis are difficult to measure, and current trade-offs between cost, robustness, and spatial measurement capacity of available plant sensors may prevent practical in-field application of most current sensing techniques. This paper investigates a novel application of laser speckle imaging of a plant leaf as a sensor with an aim, ultimately, to detect indicators of crop stress: changes to the dynamic properties of leaf topography on the scale of the wavelength of laser light. In our previous published work, an initial prototype of the laser speckle acquisition system specific for plant status measurements together with data processing algorithms were developed. In this paper, we report a new area based statistical method that improves robustness of the data processing against disturbances from various sources. Water and light responses of the laser speckle measurements from cabbage leaves taken by the developed apparatus are exhibited via growth chamber experiments. Experimental evidence indicates that the properties of the laser speckle patterns from a leaf are closely related to the physiological status of the leaf. This technology has the potential to be robust, cost effective, and relatively inexpensive to scale.

Key words: dynamic laser speckle analysis; local normal vector; leaf activity detection;
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0 Introduction

The idea of monitoring the dynamic properties of plant leaves via dynamic laser speckle analysis was initially proposed and investigated in our previous work^[1], inspired by earlier reports^[2-9] that the activity of a leaf surface depends on its physiological condition. The anatomical structure of a leaf is usually studied with a microscope, requiring destruction of the leaf or at least its separation from the plant. The laser speckle based "leaf sensor" permits statistical "observation" of micro-structural dynamics, (surface activities) on a living leaf non-destructively. This is not, as far as we are aware, achievable by any other existing sensor.

The distribution of laser speckle intensity is extremely sensitive to micro-motion of the scattering centres in a sample. Activity levels of the sample can be statistically quantified via dynamic analysis of the intensity fluctuation over a sequence of speckle images. This statistical method, known as dynamic laser speckle analysis, has applications in the measurement of sample activity of the viable materials^[10] such as plant seed activity analysis^[11], blood flow detection^[12-15], visualisation of tissue perfusion^[16], burn scar perfusion^[17] and works of art^[18]. Although dynamic laser speckle analysis has been widely investigated to measure sample activity, the use of this technique for plant sensing remains challenging. Specifically, influences of the laser speckle equipment on the physiological conditions of the leaf itself need to be minimised. This requires delicate design of the speckle acquisition apparatus in addition to the use of a specially developed data analysis algorithm. A prototype apparatus, the design and implementation of which were described in^[19], ensures minimal influences to leaf functions.

Current dynamic laser speckle analysis methods are limited in effectiveness for the analysis of leaf laser speckle for various reasons:

(1) The essential impossibility of control of the natural environment in doing field experiments. As a result data will inevitably contain artefacts resulting from changes to the environmental conditions.

(2) The low reflectivity of plant leaf surfaces forces a longer exposure time, resulting in more ambient light being collected along with the relatively low signal-to-noise ratio (SNR) laser speckle.

(3) Leaf activity appears to be significantly less than other samples considered in the literature, such as wet paint and seeds. Signal-to-ambient light ratios are lower in this context than in the other cases mentioned, and this may have more of an effect on the other methods than on ours.

All existing techniques for dynamic laser speckle analysis in the literature are based on statistics of speckle intensity^[10]. The larger the fluctuation of speckle intensity, the higher the dynamics of speckle intensity and the greater the causative micro-geometric variations of the samples. In general, to produce interference-free measurements using intensity based statistical methods requires uniform laser illumination and uniform reflectivity of the sample. In practice, a wide laser beam with a uniform magnitude may only be approximately obtained through a delicate optical setup (for example, a beam expander). For biological samples, in particular, surface reflectivity is non-uniform and can vary over time. In addition, in a field environment the intensity of laser speckle images may contain laser-illumination unrelated changes caused by the variability of solar radiation, even when an interference filter is used. As a result, the activity distribution of the sample calculated using intensity based approaches can present significant difficulties. It is crucial, then, to develop novel statistical methods that provide consistent measures of activity under complex and varying reflectivity and lighting conditions. In the literature^[20-21], a wavelet based entropy method WvEn for dynamic speckle analysis is proposed and learning from data to extract the most significant wavelets that correlate with the process

under detection is discussed. This method is able to emphasize the response of speckle dynamics to sample activity, however, it is difficult to apply it to our plant sensing application. As mentioned before, the low signal-to-ambient light ratio makes it difficult to choose a wavelet component that can guarantee retention of the information from speckle dynamics and consistently remove the influence of ambient light.

In order to address these issues, a normal vector based dynamic laser speckle analysis technique was proposed in Reference [22]. It differs from intensity-based dynamic speckle analysis techniques in that (1) the variation of local normal vectors (rather than intensities) of speckle images forms the basis for quantification of the activity of a sample; (2) statistics in the normal vector space of speckle images enables both temporal variation and local topography of speckle intensity surfaces to be included in the dynamic speckle statistics. In the proposed method, the temporal maps of the directions of normalised normal vectors across a sequence of laser speckle images are considered as the activity statistics of a sample. Computational procedures for obtaining the temporal maps from a sequence of speckle images are presented. The effectiveness and robustness of the proposed method is demonstrated using a paint drying experiment and an example of activity detection on a plant leaf. Simulation examples presented in this section show that the proposed method can work robustly relative to a wide range of experimental condition changes while the intensity based methods cannot.

The combination of the prototype apparatus and normal vector based processing methods forms a prototype laser speckle based plant sensor, which is used in the proof-of-concept experiments presented in this paper. The principle of operation and the design and implementation of the new laser speckle based plant sensor are described in Section 2. A new area vector based speckle analysis method is proposed in Section 3. In order to validate the effectiveness of the

sensing method, the physiological responses of leaves to changes in leaf water status and light are measured at the leaf level in Sections 4. Finally, conclusions are made in Section 5.

1 Method

Leaf water status is an important factor that affects many of the physiological responses of a leaf. Stomatal aperture, which controls CO_2 entering a leaf, depends on the water status of the leaf [3,23]. Stomatal closure caused by water stress limits the source of CO_2 for photosynthesis and constrains photosynthetic rate. Water is also one of the inputs to the light reactions in photosynthesis. Light also significant factors in photosynthesis and strongly affect stomatal conductance. Photosynthesis rate and stomatal conductance positively depend on photosynthetic active radiation (PAR). Stomatal changes to the anatomical structure^[3,6-8,24] of a plant leaf arise when a plant deals with variations of water and light conditions. The laser speckle analysis technique has the potential to detect these responses and, in turn, has the potential to lead to a novel laser speckle based sensing technique for measuring plant physiological responses.

1.1 Principle of operation

Plant leaves have evolved a range of strategies to deal with abiotic factors. They can respond to changes in such factors to avoid death or optimise growth to some extent. The changes that may have an effect on laser speckle patterns from the leaf include:

(1) Stomata aperture

Stomata open in the daytime in C_3 plants. The functional role of stomata is as an opening in the leaf surface to permit atmospheric CO_2 to diffuse into the leaf. During the process of photosynthesis, the CO_2 gas is fixed into carbohydrates (sugars)^[25]. This is a fundamental process in plants that allows them to grow and store energy. Alongside the intake of CO_2 , water vapour from within the plant can escape into the atmosphere^[26]. To reduce the effects of this dilemma, known as the transpirational compromise^[27], the plant

can minimise the loss of water to the atmosphere by continuously adjusting its stomata opening [23]. Quantification of the opening and closing of stomata is obtainable through measurement of the stomata aperture.

(2) Turgidity of epidermal and mesophyll cells

Leaf water potential is a measure of the "available water" within a plant. As leaf water availability reduces, the leaf water potential becomes more negative [23], resulting leaf stress. Reductions in leaf water potential result in reduced leaf cell turgidity [5], and changes in turgidity of mesophyll and epidermal cells can also directly affect guard cells and therefore stomatal conductance. Experimental evidence shows that leaf water potential is, in addition, dependent on light and temperature [23]. Reductions to leaf cell turgidity can cause cell division and cell expansion to diminish. This in turn can negatively impact on plant growth.

(3) Leaf temperature (transpiration cooling)

As a consequence of reduced stomatal aperture, the process of increasing or limiting transpiration cooling can impact leaf temperature. Leaf water status and light also affect leaf temperature, and changes to leaf temperature influence mesophyll shape.

(4) Water transportation

Plant water transportation occurs in xylem vessels, a significant composite of veins. Water deficiency reduces the intensity of water transportation, and thereby reduces the micro-motion of veins.

Changes in the anatomical structure of plant leaves result in changes to their activity. In principle, then, measurement of changes to leaf activity can provide indicators of the plant physiological status. This underpins the principle of using the dynamic laser speckle analysis technique as a plant sensor.

1.2 Design and implementation of a prototype apparatus

To validate the concept, a laser speckle imaging system, built for laser speckle experiments on plant

leaves, was developed (see Figure 1). A laser diode, operating at 650 nm, was placed in front of the abaxial leaf surface to illuminate it, resulting in the production of laser speckle patterns, registered by a CCD digital camera (CCE-B013-U) with a 12 mm-focal-length lens (M1214-MP). A control module was built to synchronise the flash of the laser diode with the strobe signal generated by the camera shutter. This design eliminated unnecessary illumination and hence reduced the influence of laser radiation on the status of leaf samples. A computer linked to the control module managed the entire speckle measurement process. Consecutive speckle images were collected by the computer and processed using the dynamic speckle analysis algorithms newly developed.

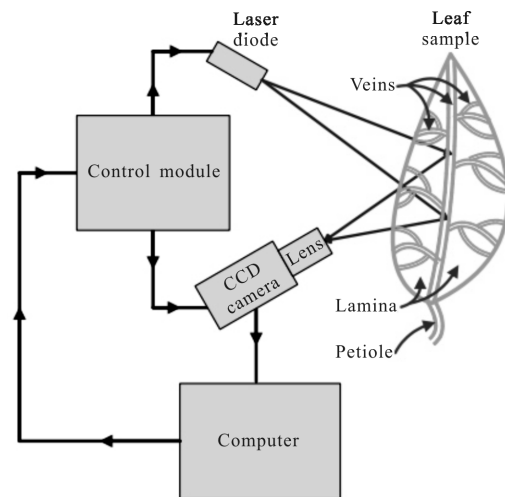


Fig.1 A schematic of the laser speckle imaging system consisting of a 650 nm laser diode, a CCD camera and a control module, illustrating the region of laser illumination and camera imaging on a leaf

In the current prototype, samples need to be held firmly because of the responsiveness of laser speckle to optical geometry, though not firmly enough to affect the physiological response of the leaf, especially when observations are over a long period.

Figure 2 illustrates the implementation of the prototype sensor and how the leaf sample was clamped on the sensor. The leaf clamp was built using the gasket seals on the gas chamber of the

LiCor-6400. The seals have been proven to be able to maintain the health of the clamped leaf. The seals were clamped by two magnets which provided enough force to hold the leaf firmly without damaging it. The magnet clamp made the installation of the leaf sample extremely quick and easy, thus it reduced the possibility of damaging the leaf during installation. This leaf clamp unit was tested for a week, where the clamped leaf was as healthy as a free leaf, and no physical damage was observed visually. The leaf clamp was held about 1 cm above the case to make the boundary layer condition of a clamped leaf close to that of other free leaves on the plant.



Fig.2 A view of the experimental plant and the leaf clamping system on the laser speckle imaging apparatus placed inside the chamber

2 Data processing enhancement

As discussed previously, existing dynamic laser speckle analysis methods cannot be adopted to measure activity of plant leaves. In our previous work^[22], a normal vector based statistical method for dynamic speckle analysis is proposed. A fundamental difference between current approaches and the normal vector based approach for dynamic speckle analysis is that the former are performed in the image intensity space whereas the latter is in the normal vector space of laser speckle images.

The underlying idea is the following:

(1) A measurement sequence for a fixed image point should reflect the relative intensities between the adjacent sample images and between that point and its

neighbouring points for every images.

(2) A time sequence of the local normal vectors of a fixed image point satisfies the above measurement requirement. In particular, the sequence of normal vector directions are quantities that depend on the corresponding relative intensities between adjacent images and also carry the local topographic information about the image point in the speckle surface.

(3) Similar statistical techniques to those used for the intensity based methods may be used for the normal vector based dynamic laser speckle analysis. We expect to get more insights into the properties of a time-varying sample via the proposed statistical methods than via the intensity based methods.

The normal vector based dynamic laser speckle analysis methods can address the statistics bias issue caused by non-uniform reflectivity, non-uniform illumination, and uncertain ambient light. However, the processing result is subject to the accuracy of local normals estimated from discrete speckle surfaces. The normal vector voting method can accurately estimate the local normals of a smooth discrete surface, but the error increases as the coarseness of the discrete surface increases. Thus the speckle images are smoothed prior to estimating the normal vectors, which extends processing time and consequently impairs real-time performance.

In order to guarantee the smoothness of a speckle surface, the aperture of the imaging system needs to be adjusted according to the distance from the camera to the sample and to the resolution of the camera. This can result in a small aperture when a laser speckle collection device with an ordinary optical system (An ordinary optical system refers to an optical set-up of a portable dimension and a camera of ordinary resolution) is used, which reduces the spatial resolution of activity measurement. In addition, when the aperture is small, long exposure time and high imaging sensor sensitivity are usually needed in order to detect enough scattered laser light energy. Long exposure time limits the application of the device for

measuring very active samples, and high imaging sensor sensitivity reduces the signal-to-noise ratio.

The normal vector based dynamic laser speckle analysis methods are robust under complex lighting conditions. This is because activity is quantified by calculating the dynamics of the local topography (Represented by local normals) of speckle images. In this section, a new representation of the local topography is proposed to address the coarseness issue discussed previously. The new method is compared with the normal vector based methods via processing dynamic speckle images of small speckle size to demonstrate the improvement, especially in processing coarse speckle images.

2.1 Algorithm

The principle of the new representation method is demonstrated in Figure 3. Figure 3 (a) displays the neighbourhood system of the vertex v_i (the central black round dot). The black grid denotes the pixel grid of the image at the level of the intensity of the pixel v_i , where the closest eight neighbouring pixels $\{v_j, j=1,2, \dots, 8\}$ of v_i are represented by the empty round dots. This grid is called the imaging grid at v_i in the rest of this section. The grey filled round dots $\{v_{j,int}, j=1,2, \dots, 8\}$ denote the intensity of the neighbouring pixels which along with v_i form a triangulated surface illustrated by the more densely dashed lines.

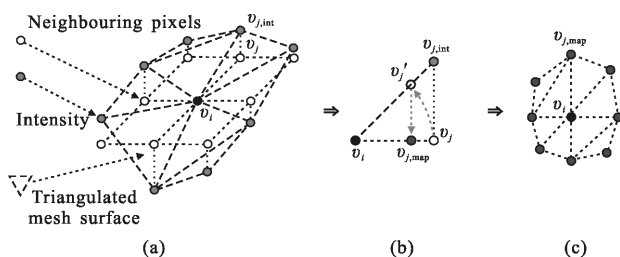


Fig.3 Schematic of the construction of an area vector. (a) the neighbouring system of v_i and the triangulated mesh surface formed by the intensity of v_i and its neighbours. (b) the procedure of mapping $v_{j,int}$ to $v_{j,map}$ in the imaging grid at v_i . (c) the triangulated mesh surface constructed by v_i and the mappings of the intensity of its neighbours. The areas of the triangles form a vector called the area vector

The intensities of the neighbouring vertices of v_i are mapped to the imaging grid at v_i as shown in Figure 3 (b). A point v_j' is found in the segment between v_i and $v_{j,int}$ such that $|v_i v_j'| = |v_i v_j'|$. Then the mapping of $v_{j,int}$ to the imaging grid at v_i is defined by the projection $(v_{j,map})$ of v_j' on $v_i v_j$. The position of $v_{j,map}$ is determined by,

$$|v_i v_{j,map}| = \frac{|v_i v_j'|^2}{\sqrt{|v_i v_{j,int}}|^2 + |v_i v_j|^2}} \quad (1)$$

v_i and $\{v_{j,map}, j=1,2,\dots,8\}$ form a new triangulated surface shown in Figure 3 (c). The areas of the eight triangles produce an 8-dimensional feature vector of the local topography at v_i . This feature vector is called the area vector. The degree of fluctuation of the area vector is quantified as the activity at the point in a sample that corresponds to v_i . The point-wise activity distribution of a sample can be obtained by applying this process to every pixel in a sequence of dynamic speckle images from the sample. This method is named as the area vector based dynamic speckle analysis method.

Similar with Reference [22], the weighted generalised difference (WGD), approximated entropy (ApEn), and sample entropy (SaEn) can be modified to quantify the fluctuation of the area vector. ApEn, and SaEn can be calculated given a distance function, such as the absolute intensity difference in the intensity based methods and the angle between two normal vectors in the normal vector based methods. In the area vector based methods, the distance function is defined by,

$$D(A_k, A_l) = \frac{\|A_k - A_l\|_2}{(\|A_k\|_2^2 + \|A_l\|_2^2)^{\frac{1}{d}}} \quad (2)$$

where A_k is the area vector at frame k ; $\|\cdot\|_2$ stands for the 2-norm, d is a scale and speckle-size dependent parameter. The WGD, ApEn, and SaEn of a sequence of area vectors calculated using this distance function are named as area vector based WGD (AVBGD), area vector based ApEn (AVBApEn), and area vector based SaEn (AVBSaEn), respectively.

2.2 Effect of speckle size on speckle statistics

The small-speckle-size dynamic speckle images

taken from plant leaves are processed using both normal vector based sample entropy (NVBSaEn)^[22] and AVBSaEn methods. The analysis results are compared to demonstrate the improvements of the area vector based methods in processing coarse speckle images. Figure 4 shows one of the small-speckle-size speckle images taken from a leaf. The activity distributions of the leaf calculated using the normal vector and area vector based algorithms are compared in Figure 5. It is difficult to distinguish the veins from the lamina in the distributions (Figure 5(a)) computed using the normal vector based methods. The statistics of the normal vectors is severely disturbed by the errors in the normal vectors estimated from coarse speckle surfaces. Compared with the normal vector based methods, the area vector based methods provide more physiologically reasonable activity distributions of the leaf. The main vein is the most active part of the leaf area within the region of interest, and the activity distributes nearly uniformly across it. The sub veins are also distinguishable from the lamina. In addition, the activity of the veins illustrates a positive correlation with the thickness of the veins.

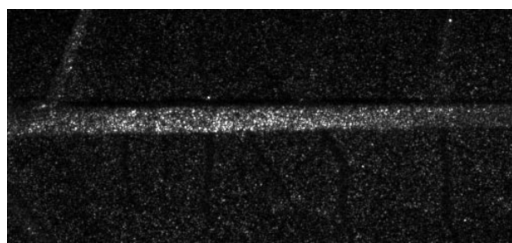


Fig.4 One of the small-speckle-size speckle images taken from a leaf

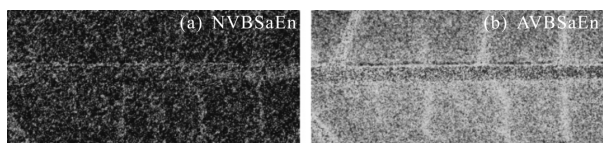


Fig.5 Comparison of normal vector and area based methods when processing small-speckle-size speckle images. (a) the processing results using the normal vector based methods. (b) the processing results using the area vector based methods. The normal vector based methods fail to detect the veins because of the coarse discrete speckle surface. In contrast, the area vector based methods deliver physiologically reasonable distributions of the activity of the leaf

The distance function (Equation 2) defined for the area vector based methods contains a scale and speckle-size dependent parameter d . To date d needs to be altered manually to obtain robust and consistent results for different speckle sizes and intensity scales. Further consideration and investigations are needed to decide whether there is a universal method for calculation of the dynamics of the area vectors, or if there is a method to determine the optimal d according to the size and scale of the speckle. In addition, the speckle size of the experimental data in the next section is large enough for normal vector based methods. Hence at this stage, the experimental data are still processed using the more universal normal vector based methods.

3 Result

In this section the new plant sensing method based on dynamic laser speckle analysis is validated experimentally. In order to study one factor at a time and isolate the impact of water deficiency on leaf activity, it is necessary to minimise as many of these effects as possible. The experiments were performed in Conviron Controlled Environmental Cabinets, CMP5000, BioLab, Industrial Technologies, Australia located at Dookie College, University of Melbourne. The first experiment measures the extreme response of leaf activity to leaf water content. The extreme light response is investigated in the second experiment.

3.1 Measuring water response

The measurement of leaf water response using dynamic laser speckle analysis has been reported in our previous paper^[19]. Thus the experiment scenario and results are briefly described. Readers can refer to Reference [19] for more details.

3.1.1 Experimental scenario

Two healthy leaves were selected from two brassica oleracea (cabbage) plants two months post germination at the phenological stage of 8–10 fully expanded leaves were employed during this experiment. The photosynthetically active radiation

(PAR) was adjusted to $800 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. The temperature in the chamber was controlled to be $28 \pm 0.5 \text{ }^\circ\text{C}$. The relative humidity in the cabinet was controlled to be $40\% \pm 2\%$.

The two plants were deprived of water until leaf wilt was observed. Then 100 speckle pattern frames of 450×200 pixels each were recorded every 5 minutes from two sample leaves (1 per plant) at 30 fps for 12 h. At 12:10 pm, 3 h after the commencement of measurements, the plant was watered sufficiently for it to recover from water stress. This scenario can represent and emphasize the impacts of water status of a leaf on the activity of the leaf better. Comparing with controlling a plant under development of water deficit, irrigating a water stressed plant is a more sudden stimulation to the water status of the leaves. This can result in a significant response of leaf activity in a shorter time. Although monitoring the activity of a leaf during the development of water deficit is closer to the future application of the sensor, it takes much longer to observe the response of leaf activity. It is very difficult to ensure that the leaf does not respond to other impacts during the experiment even if in a controlled growth cabinet. This can make the experimental results difficult to be interpreted. Inducing a leaf response in a short time reduces the possibility of the leaf status being affected by other factors. In the proof of concept perspective, monitoring the leaf from wilted status to full hydration is more appropriate.

3.1.2 Experimental results

Figure 6 shows the time evolution of average vein activity of the first and the second leaf samples. The average vein activity was summarised from the results calculated using NVBSaEn over a vein area which was determined from the optical image of the leaf via the Hessian based tubular object extraction algorithm^[29]. The horizontal and vertical axes represent the absolute time and degree of speckle dynamics produced using different algorithms. The night (including simulated sunrise and sunset) is indicated

by grey background. The watering time point is marked using a vertical dashed line (magenta). In the two replicates (leaves 1 and 2), the vein activity increases gradually with the rise of leaf water content in the first 5 h after being watered and reaches a stable level as the leaf becoming water hydrated. Such an observation agrees with the underpinning physiological principle of a leaf.

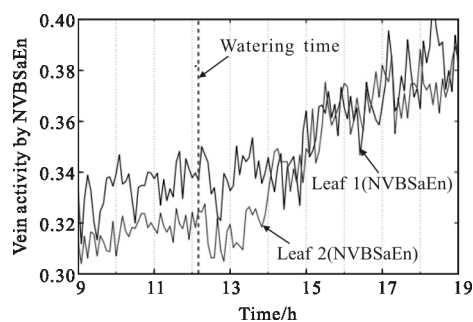


Fig.6 Time evolution of the mean vein activity of the first leaf (black line) and second leaf (gray line) evaluated by NVBSaEn

3.2 Measuring light response

The physiological status of a leaf responds not only to the water status of the leaf, but also to weather conditions such as temperature, humidity, wind speed, light, etc^[3,25,30-32]. It is reasonable to hypothesise that activity of a leaf may also respond to stimulations like light and temperature. However, it is inappropriate to investigate these responses with conventional intensity based dynamic laser speckle analysis algorithms because they provide inconsistent results under changing lighting conditions^[22]. Thanks to the development of the normal vector based methods and when experimentation is conducted under controlled environmental conditions in a growth chamber, it is possible to study light and temperature responses to leaf activity.

In these experiments, irrigation was frequent, and water was available from soil to the plants throughout the experimental period. Soil measurements were collected periodically to confirm this observation. It was assumed that plant water status was hydrated and

the plant was not water stressed. No visual signs of water stress were observed throughout the experiment. Temperature and light were changed independently to investigate how the activity of the leaves responded to changes to temperature and light conditions. Temperature was set via the temperature control function of the growth chamber. Light was controlled by toggling LED lamps on and off using a timer switch circuit. The laser speckle images from the leaves were recorded during fluctuations of temperature and light, and analyzed using the normal vector based dynamic laser speckle analysis methods. The dependence of the activity of the leaf samples on temperature and light conditions is illustrated and discussed in the rest of this chapter.

Light is a significant input signal that affects leaf physiological status^[33]. The photons that reach the chloroplast of plant leaves are a significant energy source for photosynthesis and for opening stomata^[31]. Generally speaking, the photosynthetic rate responds to light fluctuations faster than the stomatal conductance does^[34-37]. It is hypothesised that variation of the photosynthetic rate and the stomatal conductance of a leaf under a fluctuating lighting condition may result in changes to the activity of the leaf. In order to verify this hypothesis, a light response experiment was implemented in the growth chamber. The temperature in the chamber was maintained constantly. The LED light sources were turned on and off at specified time to create a fluctuating lighting condition.

In order to investigate the light-only impacts on activity of a leaf, the temperature in the growth chamber needs to be maintained during the experiment. Although the growth chamber has a temperature control function, ordinary light sources emit both visible and infrared radiation and consequently strongly affect temperature. In this experiment, LED lamps with cold operating temperature were used as in the experiment conducted by Chabrand et al^[37], to minimise the influence of the light sources on temperature.

3.2.1 Experimental scenario

The temperature inside the chamber was maintained at $23\pm 0.5\text{ }^{\circ}\text{C}$ during the experiment. Before the experiment and in the first thirty minutes of the experiment the LED lamps were off, and the plants were in darkness, which is considered a severe light stress situation for a C_3 plant. Then the LED lamps were toggled on and off every hour on three occasions. The time evolution of the photosynthetic active radiation (PAR) measured using SQ-110 is shown by the dashed line in Figure 7. Three replicates (three leaves from three individual brassica oleracea (cabbage) plants) were measured every three minutes under this treatment.

In this experiment the lighting condition was manipulated in an extreme way. This allows the observation of the most dramatic responses of leaf activity to changes in light under the limited number of LED lamps. As a proof of concept experiment this scenario achieves the maximal signal (response to changing light) to noise (response to unanticipated factors) ratio.

3.2.2 Experimental results

The influence of changing lighting conditions on vein activity is illustrated in Figure 7, where the average activity of the veins responds strongly to PAR. The sizes of the veins in the three replicates are different (3 mm wide in leaf 1 and 3, and 1.8 mm wide in leaf 2). The thicker vein shows a higher activity. Thus the activity levels of the three replicates are different while the trends are consistent.

We should mention that the light response of vein activity is not an artefact caused by light from the lamps. Conventional intensity based methods are responsive to lighting conditions, which causes biased and inconsistent results in a fluctuating lighting situation. On the other hand, the normal vector based dynamic speckle analysis methods ensure that the measurements taken under different lighting conditions are unbiased. In Figure 7, the activity increases or decreases dramatically at the second measurement after

the toggles of the lamps (indicated by the black dots) but not at the first measurement right after the toggles (indicated by the triangle dots). The activity levels measured immediately after the toggles are at the same level with those measured right before the toggles (indicated by the black dots). The instant changes of light did not affect the leaf activity immediately. Hence the results represent the physiological response of the activity of the leaf samples to light but not artefacts caused by the light from the lamps.

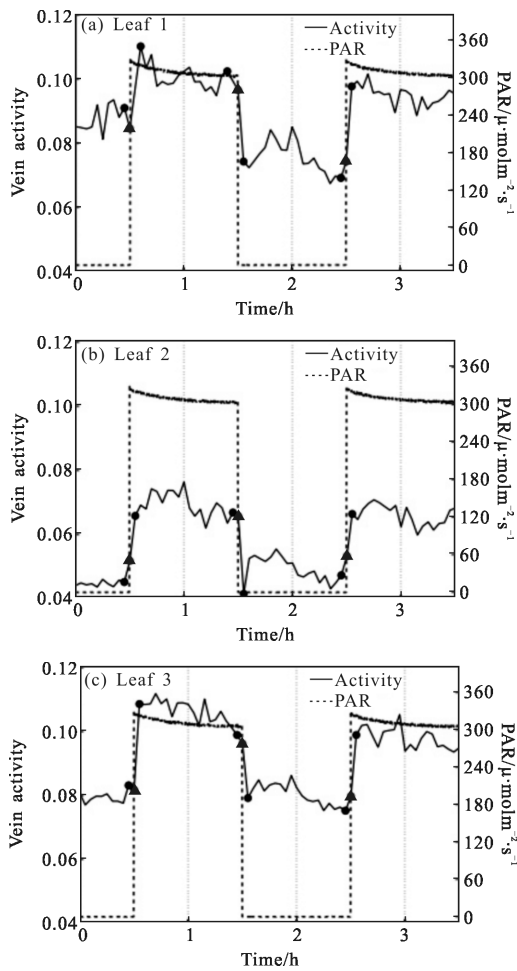


Fig.7 Time evolution of the mean vein activity of three living leaves during instant increases and decreases of PAR calculated using NVBSaEn. The horizontal axis represents time since the commencement of the experiment; the left vertical axis indicates vein activity assessed using different algorithms; and the right vertical axis represents PAR measured during the experiment. The full line is mean vein activity, and dotted line is PAR

4 Conclusion

In this paper the effectiveness of a new laser speckle based plant sensor was demonstrated. The responses of activity of functioning leaves to changes to water and light conditions were investigated by growth chamber experiments. Experimental results elucidated the potential to develop a new plant sensor based on laser speckle analysis on plant leaves. Several future works are needed to implement this technology as a commercial low-cost plant sensor. The sensing method needs to be tested on more impact factors of plant physiological status, such as temperature, CO₂ concentration, relative humidity, etc. The experiments in this paper tested the extreme responses of plant leaves.

The sensing method needs to be further validated under fine-grained changes in the impact factors of plant physiological status. Further, the design and implementation of the structure of the sensor need to be improved for field deployment.

References:

- [1] Zhong X, Wang X, Farrell P, et al. Modeling and classifying surface roughness via laser speckle statistics[C]//Proceedings of the 2011 International Conference on Signal and Information Processing, Shanghai China, 2011.
- [2] Jones Hamlyn G, Vaughan Robin A. Remote Sensing of Vegetation: Principles, Techniques, and Applications [M]. Oxford: Oxford University Press, 2010.
- [3] Buckley T N, Mott K A, Farquhar G D. A hydromechanical and biochemical model of stomatal conductance [J]. *Plant, Cell & Environment*, 2003, 26(10): 1767–1785.
- [4] Bowman William D. The relationship between leaf water status, gas exchange, and spectral reflectance in cotton leaves [J]. *Remote Sensing of Environment*, 1989, 30(3): 249–255.
- [5] Saliendra Nicanor Z, Sperry John S, Comstock Jonathan P. Influence of leaf water status on stomatal response to humidity, hydraulic conductance, and soil drought in betula occidentalis[J]. *Planta*, 1995, 196(2): 357–366.
- [6] Mohammad R Riahi, Hamid Latifi, Mohsen Sajjadi. Speckle correlation photography for the study of water content and

- sap flow in plant leaves [J]. *Applied Optics*, 2006, 45(29): 7674–7678.
- [7] Tsukasa Matsuo, Hisashi Hirabayashi, Hiroaki Ishizawa, et al. Application of laser speckle method to water flow measurement in plant body [C]//Proceedings of the 2006 International Joint Conference on SICE–ICASE, 2006: 3563–3566.
- [8] Kawamura M, Ishizawa H, Horiguchi T, et al. Laser speckle pattern measurement for plant state monitoring [C]//Proceedings of the 2010 SICE Annual Conference, 2010: 2928–2932.
- [9] Wang Xuezi, Yang Weiping, Ashley Wheaton, et al. Automated canopy temperature estimation via infrared thermography: a first step towards automated plant water stress monitoring [J]. *Computers and Electronics in Agriculture*, 2010, 73(1): 74–83.
- [10] Rabal H J. Dynamic Laser Speckle and Applications [M]. New York: CRC Press, 2008.
- [11] Ricardo Arizaga, Nelly Luci, Marcelo Trivi, et al. Display of local activity using dynamical speckle patterns[J]. *Optical Engineering*, 2002, 41(2): 287–294.
- [12] Briers J David, Webster Sian. Laser speckle contrast analysis (lasca): a non-scanning, full-field technique for monitoring capillary blood flow[J]. *Journal of Biomedical Optics*, 1996, 1(2): 174–179.
- [13] Briers J David. Laser doppler, speckle and related techniques for blood perfusion mapping and imaging [J]. *Physiological Measurement*, 2001, 22(4): R35.
- [14] Miao Peng, Li Minheng, Fontenelle Hugues, et al. Imaging the cerebral blood flow with enhanced laser speckle contrast analysis (elasca) by monotonic point transformation [J]. *Biomedical Engineering, IEEE Transactions on*, 2009, 56(4): 1127–1133.
- [15] Miao P, Rege A, Li N, et al. High resolution cerebral blood flow imaging by registered laser speckle contrast analysis [J]. *IEEE Transactions on Biomedical Engineering*, 2010, 57(5): 1152–1157.
- [16] Forrester K R, Tulip J, Leonard C, et al. A laser speckle imaging technique for measuring tissue perfusion [J]. *IEEE Transactions on Biomedical Engineering*, 2004, 51(11): 2074–2084.
- [17] Stewart J B. Modelling surface conductance of pine forest[J]. *Agricultural and Forest Meteorology*, 1988, 43(1): 19–35.
- [18] Elaine Miles, Ann Roberts. Non-destructive speckle imaging of subsurface detail in paper-based cultural materials [J]. *Optics Express*, 2009, 17(15): 12309–12314.
- [19] Zhong Xu, Wang Xuezi, Nicola Cooley, et al. Normal vector based dynamic laser speckle analysis for plant water status monitoring [J]. *Optics Communications*, 2014, 313: 256–262.
- [20] Braga Jr R A, Horgan G W, Enes A M, et al. Biological feature isolation by wavelets in biospeckle laser images [J]. *Computers and Electronics in Agriculture*, 2007, 58(2): 123–132.
- [21] Nobre C M B, Braga Jr R A, Costa A G, et al. Biospeckle laser spectral analysis under inertia moment, entropy and cross-spectrum methods [J]. *Optics Communications*, 2009, 282(11): 2236–2242.
- [22] Zhong Xu, Wang Xuezi, Nicola Cooley, et al. Dynamic laser speckle analysis via normal vector space statistics [J]. *Optics Communications*, 2013, 305(313): 27–35.
- [23] Tuzet A, Perrier A, Leuning R. A coupled model of stomatal conductance, photosynthesis and transpiration [J]. *Plant, Cell & Environment*, 2003, 26(7): 1097–1116.
- [24] Gaëlle Damour, Thierry Simonneau, Hervé Cochard, et al. An overview of models of stomatal conductance at the leaf level [J]. *Plant, Cell & Environment*, 2010, 33(9): 1419–1438.
- [25] Susanna Von Caemmerer. Biochemical Models of Leaf Photosynthesis[M]. Australia: Csiro Publishing, 2000.
- [26] Driscoll S P, Prins A, Olmos Enrique, et al. Specification of adaxial and abaxial stomata, epidermal structure and photosynthesis to CO₂ enrichment in maize leaves [J]. *Journal of Experimental Botany*, 2006, 57(2): 381–390.
- [27] Belinda E Medlyn, Remko A Duursma, Derek Eamus, et al. Reconciling the optimal and empirical approaches to modelling stomatal conductance [J]. *Global Change Biology*, 2011, 17(6): 2134–2144.
- [28] Xavier Chone, Cornelis Van Leeuwen, Denis Dubourdieu. Stem water potential is a sensitive indicator of grapevine water status[J]. *Annals of Botany*, 2001, 87(4): 477–483.
- [29] Frangi A, Niessen W, Vincken K, et al. Multiscale vessel enhancement filtering [J]. *Medical Image Computing and Computer-Assisted Intervention –MICCAI'98*, 1998: 130–137.
- [30] James Collatz G, Timothy Ball J, Cyril Grivet, et al. Physiological and environmental regulation of stomatal conductance, photosynthesis and transpiration: a model that includes a laminar boundary layer [J]. *Agricultural and*

- Forest Meteorology*, 1991, 54(2): 107–136.
- [31] Leuning R. A critical appraisal of a combined stomatal-photosynthesis model for C_3 plants [J]. *Plant, Cell & Environment*, 1995, 18(4): 339–355.
- [32] Gabriel Katul, Stefano Manzoni, Sari Palmroth, et al. A stomatal optimization theory to describe the effects of atmospheric CO_2 on leaf photosynthesis and transpiration [J]. *Annals of Botany*, 2010, 105(3): 431–442.
- [33] Farquhar G D, von Caemmerer S, Berry J A. A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species [J]. *Planta*, 1980, 149(1): 78–90.
- [34] Kirschbaum MUF, Küppers M, Schneider H. Modelling photosynthesis in fluctuating light with inclusion of stomatal conductance, biochemical activation and pools of key photosynthetic intermediates [J]. *Planta*, 1997, 204(1): 16–26.
- [35] Uwe Rascher, Ladislav Nedbal. Dynamics of photosynthesis in fluctuating light [J]. *Current Opinion in Plant Biology*, 2006, 9(6): 671–678.
- [36] Kirschbaum MUF, Gross L J, Percy R W. Observed and modelled stomatal responses to dynamic light environments in the shade plant *alocasia macrorrhiza* [J]. *Plant, Cell & Environment*, 1988, 11(2): 111–121.
- [37] Silvère Vialet-Chabrand, Erwin Dreyer, Oliver Brendel. Performance of a new dynamic model for predicting diurnal time courses of stomatal conductance at the leaf level [J]. *Plant, Cell & Environment*, 2013, 8: 1529–1546.