Quantification of Microscopic Optical Scattering – A Potent Indicator of Environmental Stressor-Induced Tissue Structural Deformations in Biological Specimen

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Abstract

Light scattering serves as a non-invasive tool for monitoring structural deformities at tissue level by utilizing the change in refractive index (tissue optical property), without requiring dyes or labels. Using computerized analysis of polarized microscopy images, this study aimed at quantifying the scattering intensities in the plant specimen *Alternanthera philoxeroides* under normal, dehydrated, and rehydrated states to identify localized, real-time responses to hydration changes. The study showed that scattering intensity may serve as a potential non-destructive, real-time biomarker for plant tissue hydration and structural integrity under stress, using *Alternanthera philoxeroides* as a model.

Keywords: Image Analysis, Tissue Optical Scattering, Polarized Microscopy, Pixel Intensity Quantification, Water Stressor

Introduction

In biological microscopic analysis, light scattering serves as a powerful, non-invasive probe that reveals the hidden structural complexity of living systems without the need for chemical stains or labels [1]. Scattering occurs at the interfaces of cellular components like cell walls, nuclei, and mitochondria, where there is a mismatch in refractive indices [2]. Consequently, the intensity and pattern of scattered light act as a direct map of a tissue's physical organization and density. In plant and animal studies, this phenomenon allows researchers to monitor real-time physiological changes, such as turgor pressure fluctuations or the onset of disease, by observing how the optical properties shift [3]. By quantifying these scattering signals, microscopy transcends mere visualization, turning light into a mathematical tool for assessing tissue health and structural integrity at a subcellular level.

Water is a fundamental component of plant life, serving as a structural scaffold and a vital medium for metabolic processes. The maintenance of cellular hydration is critical for sustaining turgor pressure, which in turn preserves the anatomical integrity of tissues [4]. Environmental stressors, particularly desiccation, induce significant structural and physiological changes that can impair plant survival and productivity [5]. Understanding these dynamic responses to water stress and subsequent recovery is essential for advancing plant biophysical research.

Traditional methods for assessing plant water status often involve invasive or destructive techniques that lack real-time spatial resolution. However, recent advances in optical imaging have highlighted light scattering as a promising parameter for cellular organization. Since light scattering in biological tissues is driven by refractive index gradients at cellular and subcellular interfaces, any shift in water content within vacuoles and intercellular spaces inevitably alters the tissue's optical profile. This study explores the potential of optical scattering intensity, quantified through digital image processing, as a non-destructive biomarker for tissue structural status. By examining the scattering profiles of various tissue layers—specifically the epidermis, cortex, and cambium ring—under normal, dehydrated, and rehydrated conditions, the study aims to identify localized sensitivities to water stress. Through quantitative analysis of pixel intensity values and point maxima, the work provides a plausible approach for monitoring the physiological adaptability of plant tissues in response to fluctuating water availability.

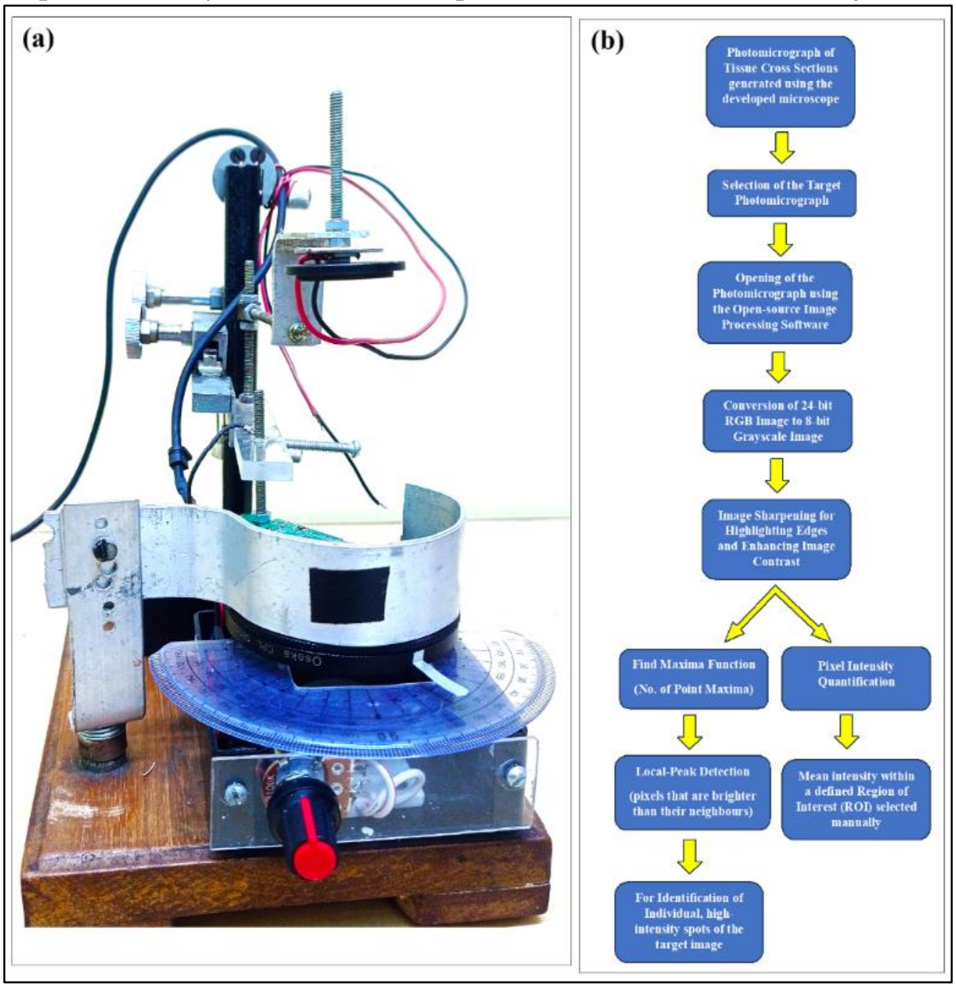
Methodology

Study Procedure

For the present study, the biological specimen was observed under a developed polarizing microscope (Fig. 1) and further subjected to image processing using the ImageJ open-source software [6]. The biological sample used for analysis was tissue cross sections of the plant *Alternanthera philoxeroides* (Alligator weed), (Mart). Griseb. The photomicrographs of the tissue cross sections were accessed from our data repository https://github.com/royshibsankar-biophysics/microscopic_scattering. The specimens were exposed to three experimental conditions – normal, dehydrated in air for 15 min and rehydrated for 15 min. For the study, a total of 12 photomicrographs were analysed.

Image Processing and Data Analysis

For performing image analysis, standard image processing steps were performed. The images were first converted to binary image (8-bit) followed by image sharpening. Sharpening was done to increase the contrast and accentuate details of the image. This was followed by computing point maxima using the find maxima function of the software. The 'find maxima' function determines the local maxima in an image and creates a binary (mask-like) image of the same size with the maxima, or one segmented (marked) particle per maximum [7]. After this, the analysis of the maximum pixel intensity with respect to its background is performed on the selected area of the image. Following this, the mean values of the computed point maxima and pixel intensity under the three experimental conditions were subjected to ANOVA



statistical analysis in order to find out the presence of any significant difference in the scattering intensity when observed under the developed microscope.

Fig. 1: (a) The developed polarizing microscope; (b) Flow chart representing the outline of the image processing steps

Results

In the study both, inter-nodal and nodal tissue regions of the plant specimen were subjected to image analysis. Microscopic observation and further image processing revealed when a tissue section was maintained under normal water medium there was no structural deformity of the tissue layers. Moreover, epidermis, cambium ring and the pericycle showed a prominent optical scattering under microscope. When the tissue section was dehydrated in air for 15 min (without any water medium) a marked deformity of the tissue structures was observed along with prominent reduction in scattering intensity of the tissue layers. However, when the tissue section was rehydrated the tissue layers regained normal structure and the scattering intensity was also similar to that of normal tissue section (even at times higher than that of normal condition). The scattering intensities of the tissue layers under the three conditions – normal, dehydrated and rehydrated was quantitatively studied in terms of pixel value (range of 0 to 255). The maximum pixel value was noted in rehydrated condition, followed by normal condition and dehydrated state.

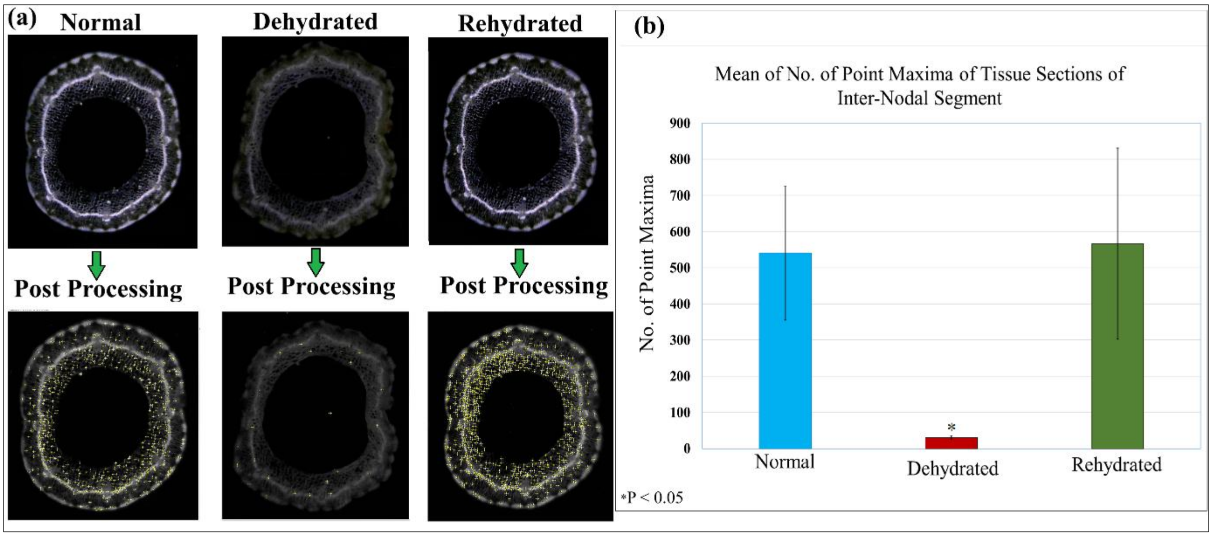


Fig. 2: (a) Images of the photomicrographs of the Inter-nodal sections before and after image processing highlighting the no. of point maxima under the three experimental conditions; (b) Graphical representation of the mean values of the no. of point maxima computed for each of the experimental conditions

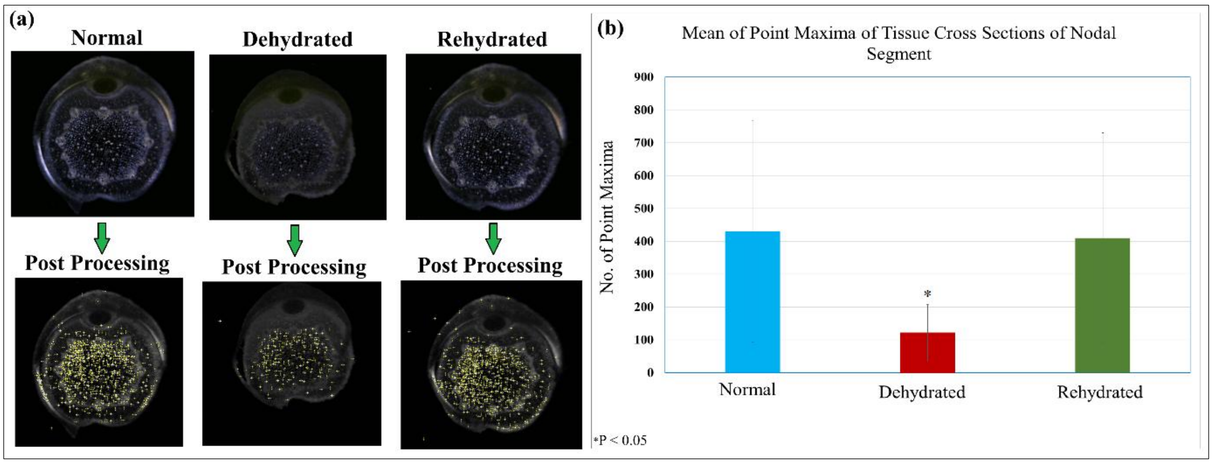
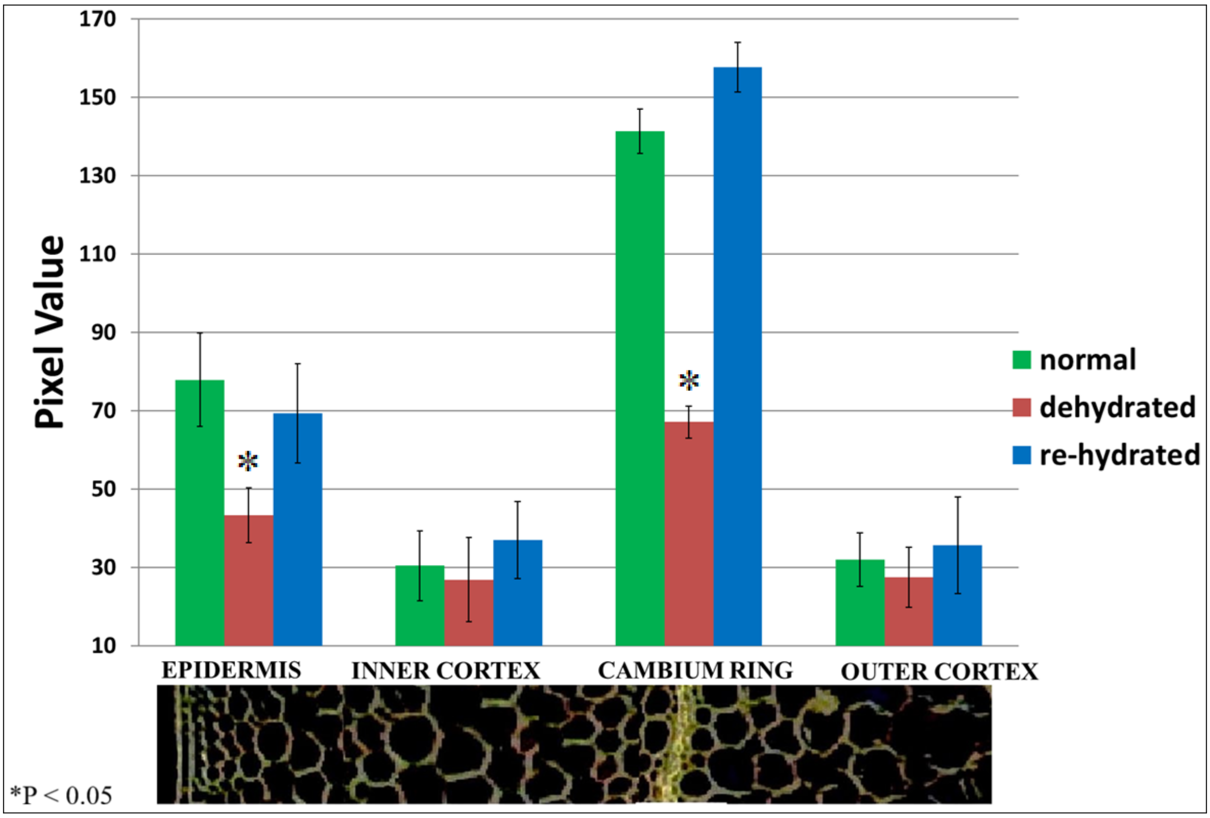


Fig. 3: (a) Images of the photomicrographs of the Nodal sections before and after image processing highlighting the no. of point maxima under the three experimental conditions; (b) Graphical representation of the mean values of the no. of point maxima computed for each of the experimental conditions

Moreover, among the four tissue layers studied – epidermis, outer cortex, cambium ring and inner cortex, the cambium ring showed the highest pixel value. After statistical analysis it was found that in both nodal and inter-nodal region, under the dehydrated condition the mean value of no. of point maxima was significantly lower ($P<0.05$) compared to the normal and rehydrated condition (Fig. 2 and Fig. 3). It was also found that there existed a significant difference in the mean value of pixel intensity among the three experimental conditions only



in the epidermis and cambium ring region ($P<0.05$) (Fig. 4). However, those of outer and inner cortex did not show any significant difference.

Fig. 4: Graphical representation of the mean values of the pixel intensity of the different tissue regions of the inter-nodal plant segment under the three experimental conditions

Discussion

The detection of local maxima through image processing techniques is considered an important strategy in histopathological studies for quantification of the phenotypic information of the tissue sections [8]. Maximum Finder plugin can help identify pixels that are significantly different from their neighbours [9]. In the study, the Maximum Finder plugin of the open-source software was used to identify the number of pixels significantly different from their neighbours. From the study, it was found that the same parameter may prove to be beneficial in understanding the hydration status of plants. It was also found that the scattering intensity (measured via pixel values) may serve as a biomarker for identifying plant tissue structural status. Due to the presence of heterogeneity and dynamicity of biological tissues, scattering of light is considered to be a crucial parameter. Scattering of light can be described as the process by which light rays are deflected in random directions when they encounter particles or irregularities in a medium [10]. This phenomenon occurs when light interacts with small particles, causing it to change direction. The amount and direction of scattering depend on the size of the particles relative to the wavelength of the light. The prominent reduction in scattering during dehydration likely resulted from the loss of water at the cellular interfaces [11]. When water leaves the vacuoles and intercellular spaces, there occurs a change in the refractive index, leading to an alteration in the optical pathways that normally scatter light [11]. Interestingly, the maximum pixel value was recorded in the rehydrated state, even surpassing the normal condition. This could suggest that rehydration leads to a temporary state of enhanced turgidity or a slight alteration in the cellular refractive index that enhances light scattering beyond its baseline level. The significant drop ($P<0.05$) in the mean value of point maxima during dehydration provides another interesting clue to the visual observations. The point maxima likely represent specific intracellular structures or interfaces that reflect light most strongly [12]. The fact that this decrease was significant in both nodal and inter-nodal regions confirms that the dehydration effect is a systemic response of the plant's vascular and protective tissues, rather than a localized anomaly.

Increased scattering often results in a higher intensity of backscattered light, leading to brighter pixel intensities [13]. From the study it was found that the mean values of the pixel intensity were maximum in case of normal and rehydrated condition and minimum in case of dehydrated condition. Interestingly, the data also revealed that not all tissue layers respond equally to water stress. It was found that the cambium ring (vascular bundle) emerged as the region to show the highest optical scattering with significantly ($P<0.05$) highest pixel intensity. As a region of active cell division and high metabolic density, the cambium likely has a different organelle composition or cell wall density, making it a primary optical marker for hydration levels [14]. Studies have also reported that the cambium ring (vascular bundle region) was most responsive to electrical stimulation as well [15]. Another study reported that in case of the plant *A. philoxeroides* the graviceptive starch sheath layer was found to be localized around the cambium ring region [16]. The statistically significant ($P<0.05$) difference in the epidermis and cambium, but not in the outer and inner cortex suggests that the epidermis and vascular regions are the first to react to environmental moisture changes. The findings coincide with earlier studies which reported that under minimum contrast mode of polarizing microscope, the epidermis layer and the vascular bundle region were the first to become visible, indicating maximum scattering tendency of these tissue layers of the plant stem [6].

Conclusion

In summary, the study confirms that optical scattering is a highly sensitive, non-destructive method for monitoring the dynamic hydration status of plant tissues. From the study it was found that there was a marked reduction in the mean point maxima value under dehydrated condition in comparison to that of normal and rehydrated states. The study also indicated a characteristic pattern in terms of the mean values of the pixel intensity across the different tissue layers of the inter-nodal stem regions. The mean pixel intensity values were significantly different ($P<0.05$) only with respect to the epidermis layer and the cambium ring (vascular bundle region) among the normal, dehydrated and rehydrated states. However, no such characteristic difference existed in the outer and inner cortical layers among the three experimental conditions. Therefore, from the study it may be stated that tissue optical scattering may act as an effective indicator of environmental stressor-induced structural deformations in plant specimens as well.

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Conflicts of Interest

The authors declare no conflicts of interest.

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