Hyperspectral reflectance system for plant diagnostics

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Abstract. The article presents a hyperspectral reflectance system. The spectral range of the system varies from 480 to 900 nm with a spectral resolution of approximately 5 nm and a spatial resolution of at least 0.1 mm. The developed system is able to measure the reflectivity of objects, resulting in a two-dimensional image with full spectral information in each pixel (“hypercube”). The article describes the procedure for image calibration and correction with respect to system artifacts caused by optics and lighting conditions across the entire spectrum. The operation of the hyperspectral imaging system was demonstrated on the samples of the Siberian 12 wheat leaves, healthy and affected by powdery mildew (Blumeria graminis (DC) Speer). The proposed use of the hyperspectral reflectance system is to conduct research in early detection and localization of agricultural plants lesions.

1. Introduction
Fast non-invasive methods of detection and identification of diseases and other stressful conditions of plants are an urgent problem in agriculture [1, 2]. The detection of diseases or stress by clearly visible symptoms, which are often manifested at the middle and late stages of the pathological process development and based on visual detection, have disadvantages compared to the spectrometric approach [3]. Methods of spectral visualization of the plant leaves reflectivity represent a significant potential in the environmental monitoring of crops and assessment of the physiological state of plants at the early development stages of the pathological process [4]. In particular, spectral visualization methods are better suited for detecting local effects in a sample. Thus, Lu and Chen [5] showed that the use of imaging methods may require only a few basic spectral ranges (multispectral) to detect diseases. However, the optimal spectral ranges depend not only on the object of study, but also on a number of other factors, such as the type of disease and condition of the object of study, the stage of the lesion, and so on. A variety of factors indicates to the need to create a detection system that can both visualize and detect areas of damage from hyperspectral images.

Hyperspectral imaging methods have already been adapted in many scientific disciplines, ranging from microscopic studies to the use of aerial remote sensing [6, 7, 8]. In general, these systems measure reflectivity in the spectral range, varying from the visible (VIS) to the short-wave infrared (SWIR) range.
of the solar spectrum. The volume of hyperspectral images consists of three-dimensional data that
contains spatial information with a high spectral resolution (10 nm) of the spectrum at each pixel point.

There are two approaches to obtaining the cube of spatial and spectral hyperspectral data. The first
approach is to capture a complete spatial picture in each spectral range to form a three-dimensional
image cube. An example of this approach is the use of multiple bandpass filters, a liquid crystal tunable
filter, or an acousto-optical tunable filter [9, 10].

The second approach is the slotted scanning method, in which a line of spatial information with a
full spectral range per one spatial pixel is captured sequentially to compose spatial-spectral data [7].

In accordance with these approaches, we have developed a hyperspectral reflectance system capable
to perform reflectometric measurements of plant leaves. The purpose of this article is a detailed
description of the hyperspectral imaging system, including its calibration and image correction.

2. Hyperspectral reflectance system

2.1. System design

The hyperspectral reflectance system uses linear scanning techniques that take into account different
sample sizes and CMV2K LS150+ VIS-NIR hyperspectral camera by Photonfocus. A special feature of
the camera is an interference filter consisting of a set of bands of different spectral transmittance ($\lambda_1, \lambda_2, \ldots \lambda_N$), and a scanning mode of operation.

The modular design of the unit combines the main components together, providing easy access to
replace/update parts, including the camera, optics and lighting peripherals. The frame of the installation
was constructed from an anodized aluminum profile, which ensures the rigidity of the platform.

2.2. System construction

Figure 1 shows the general installation diagram. The installation includes a hyperspectral camera
Photonfocus MV1-D2048x1088-HS05-96-G2-10, fixed at a height corresponding to the size of the
frame 20x10 cm; a coordinate table on which the object of study is placed; 2 searchlights with halogen
lamps installed on the sides.

The spectral bandwidth of the camera is divided into 149 channels, in the range from 475 to 900 nm,
the spatial resolution of the camera is 2048x1088 pixels, the viewing angle is 14°x7°. The camera is
used with a pair of wide-band filters that are necessary to cut off the side interference peaks leading to
interference in the operating range: the shortwave (Shortpass) one cuts the spectrum after 900 nm and the long-wave (Longpass) one cuts the spectrum to 475 nm.

The camera has 192 channels, including 64 channels in the visible VIS (470-600 nm) and 128 channels in the near-infrared NIR (600-900 nm) bands. Within each area, the channels are organized in a wedge-shaped pattern, in which each band spans 5 rows of pixels. The areas are separated from each other by an empty interface zone of 120 lines. After processing, there are 149 independent channels, and the rest partially or completely duplicate the information received from them.

The coordinate table of the system consists of guides with linear bearings along which the platform moves and a stepper motor with a ball-screw transmission that allows positioning the platform with the object of study with an accuracy of ±5 microns.

The movement of the coordinate table and the supply of external pulses to the camera is controlled by a microcontroller (figure 3). When the control signal is received on the stepper motor driver, it is offset by a specified angle, this is performed by micro-displacement of the coordinate table by a step relative to the original position. At the same time, a control signal is sent to the pulse generator board, from which a 1.5-microsecond pulse is transmitted to the camera and starts shooting the image frame. This is repeated until the subject is fully scanned. Power is provided from three voltage sources of 12V and 9V. The 12V voltage sources provide power to the camera and stepper motor, and the 9V voltage source provides power to the scanning system's microcontroller control.

The installation appearance is shown in figure 2.

2.3. System operation
An imaging system is a laboratory system designed for indoor use. The study samples are placed on a white coordinate table. To get a data set, the following steps are required: 1) to calibrate the camera to a white background, 2) to calibrate the camera to a black background, 3) to get a hyperspectral image.

In order for each point of the research object to pass through all 192 channels, it is necessary to shoot in scanning mode. The microcontroller moves the coordinate table to a specified distance at a specified speed (the speed was adjusted in advance based on the specified exposure time of the CCD camera's matrix and the data transfer rate), while an external pulse of 1.5 microseconds is transmitted to the camera via a special CameraLink interface to start shooting the image frame. This process lasts until the entire object of research passes through all 192 channels. The resulting set of images is recorded in a separate folder on the computer, and then collected using special software into a hypercube (a three-dimensional array of registered values in x, y, and λ coordinates) in the standard ENVI HDR format.

2.4. Software
The camera is controlled using software based on MS Windows - PF_GEVPlayer, which is supplied with the camera. We have developed the specialized software and algorithmic tools that allow to automate the configuration of the scanning system, which significantly reduces the time for adjusting its parameters, process the obtained hyperspectral data with the formation of a hypercube, perform its visualization and interactive analysis.

3. System of calibration and image correction
Calibration and correction of data for a hyperspectral imaging system is difficult due to the imperfect spectral response of individual filters, as well as the need to organize a scanning mode of shooting. Instrument artifacts, such as non-uniform CCD responses, illumination, and optics, degrade data performance and quality. The calibration and adjustment procedure makes it possible to significantly compensate for a number of such errors.

One of the calibration tasks was to develop a method for fine-tuning the scanning system, which consists of the following procedures:

- determining the scan step;
- determining the width of the linear section of the characteristic curve of the CCD matrix;
• determining the shooting parameters (shutter speed, aperture);
• registering three calibration images.

Due to the design features of the hyperspectral (HS) camera, the survey objects must be photographed with a shift between neighboring images of 5 pixels corresponding to the same wavelength $\lambda_i$. To determine the scanning step with the required accuracy, a black-and-white template has been developed, which is a set of strips oriented perpendicular to the scanning direction. The template images were registered in different positions of the scanner and the offset between them in pixels was determined using the correlation method. This made it possible to automate the detection of the scan step. At each iteration, the scanning step was doubled, the template image was registered, and the image shift relative to the previous one was determined using the correlation method. This allowed to calculate the scanning step corresponding to 5 pixels of linear offset, with an accuracy of 1/200 pixels.

Getting the correct hyperspectral values requires that the pixels related to the object of study remain in the linear region of the characteristic curve of the CCD matrix. To determine this range, it is proposed to shoot a white sheet of paper and dark images in pairs with a closed lens with a uniformly increasing shutter speed. Then subtract the dark values from the obtained white sheet brightness values, and approximate the result using the least squares method. It was found that the linear section starts near zero and is stored with acceptable accuracy to a value of 900 units out of 1024 obtained by a 10-bit ADC.

When shooting, it is necessary that the values of the recorded images do not exceed the linear range, including in the simplest version of two-frame calibration (white sheet and dark image). However, for objects with a brightness significantly lower than that of the white sheet, this requirement significantly reduces the part of the dynamic range that falls on the object of study. In this case, it is recommended to calibrate using three frames: take the white sheet (and the corresponding dark frame) at a shutter speed less than that used when shooting the object of study, and when calibrating, substitute the value of the white sheet with the corresponding coefficient equal to the ratio of exposures. Thus, the calibrated radiation intensity of the object is equal to

$$\nu = \frac{(\rho - \rho_0,\tau)}{(\rho_{\text{ref}},\tau_\text{ref} - \rho_0,\tau_\text{ref})}\tau$$

where $\rho$ is the recorded radiation intensity of an object captured with an exposure $\tau$, $\rho_0,\tau$ is the dark frame, taken with an exposure $\tau$, $\rho_{\text{ref}}$ is the exposure rate of a white sheet with exposure $\tau_{\text{ref}}$, $\rho_0,\tau_{\text{ref}}$ is a dark frame with an exposure $\tau_{\text{ref}}$.

In addition to taking into account calibration frames, when forming a hypercube of hyperspectral data, it is necessary to recalculate the channel numbers into wavelengths by multiplying the measured spectrum by a pre-calculated calibration matrix. Each interference filter corresponds to one or (in some cases) several spectral transmission lines in the sensitivity region of the photodetector. The matrix of the corresponding transformation is provided by the manufacturer, however, the need to Refine it has been experimentally established. As an example, figure 3 shows the initial (figure 3 (a)) and corrected (figure 3 (b)) filter spectra for a wavelength of 518 nm using this matrix. Of course, the spectrum corrected using the manufacturer's matrix is better than the original one, but significantly inferior in quality, calculated using a more precisely selected combination of channels (figure 3(c)). In this case, 9 channels are used for the calculation, and the optimal weights for them are determined by the method of least squares.
Figure 3. Generation of correct data; (a) initial spectrum for wavelength 518 nm, (b) corrected using the matrix offered by the manufacturer, (c) spectrum calculated with more accurately selected combination of channels.

4. The use of the installation for analysis of wheat plants lesions

The developed scanning system and software-algorithmic tools for processing hyperspectral data allowed to obtain experimentally the spectral characteristics of wheat leaves of healthy and root-rot-affected cereals (pathogen *Bipolaris sorokiniana* Shoem.) and powdery mildew (pathogen *Blumeria graminis* (DC) Speer).

The research was carried out on wheat plants of the Siberian 12 variety that grew on a natural infectious background in the field (test ground of Siberian Federal Research Center of Agricultural Biotechnology of the RAS (SFRCAB of the RAS). Samples of wheat plants with different degrees of disease damage were selected in the tillering phase (for analysis of root rot spectra) and in the tube-
earing phase (for analysis of powdery mildew spectra). A representative sample of 40-45 plants in each variant of the experiment.

Figure 4 (a) shows the obtained spectral curves of the Siberian 12 wheat leaves, healthy and affected by powdery mildew (*Blumeria graminis*).

Analysis of reflective characteristics in certain areas of the spectrum allowed to establish the most informative PMI vegetation index for detecting powdery mildew on wheat plants (figure 4(b)).

![Figure 4](image)

**Figure 4.** (a) Spectral curves of wheat plants of varieties Siberian 12, healthy (green curve) and affected by powdery mildew (*Blumeria graminis*) (red curve); (b) Histograms of PMI values for healthy (green) and affected by powdery mildew (*Blumeria graminis*) (red color) samples of wheat of varieties Siberian 12.

5. Conclusion

We have developed and created a laboratory hyperspectral reflectance system based on the Photonfocus CMV2K LS150+ VIS-NIR hyperspectral camera with unique characteristics. The system is capable of receiving images in the area from 400 to 900 nm with a spatial resolution of 0.1 mm. The developed technique for fine-tuning of the scanning system consists of the following procedures: determining the scanning step; determining the width of the linear section of the characteristic curve of the CCD matrix; determining the shooting parameters (shutter speed, aperture); registering three calibration images. The scan step corresponding to 5 pixels of linear offset was determined with an accuracy of 1/200 pixels. The developed software and algorithmic tools allowed to automate the configuration of the scanning system, which significantly reduced the time for adjusting its parameters, processing the obtained hyperspectral data with the formation of a hypercube, and allowed us to perform data visualization and interactive analysis.

When testing the system, hyperspectral images of wheat leaves of the Siberian 12 variety, healthy and affected by root rot of cereals (the pathogen *Bipolaris sorokiniana* Shoem.) and powdery mildew (the pathogen *Blumeria graminis* (DC) Speer) were obtained. Based on image analysis, the PMI vegetation index was established to identify powdery mildew on wheat plants.

We believe that in the near future multispectral imaging will become an integral part of agricultural production for early detection and identification of crop lesions due to the speed of data collection and processing in real time. Hyperspectral reflectance systems are necessary for determining optimal spectral ranges and allow the development and application of algorithms for the detection and localization of crop lesions. We are convinced that our hyperspectral reflectance system is a universal platform capable of evaluating the reflectivity of objects with high spatial (at least 0.1 mm) and spectral (up to 5 nm) resolution.

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