Detection of Seed Vigor Based on Optical Methods

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Optics is an ancient science, with the advancement of laser technology, optical engineering, and information technology, optical detection technology has gained a resurgence. The theoretical basis for the application of modern optical methods to the detection of seed vigor is that light irradiation on the surface of seeds will produce reflection, scattering, and transmission phenomena, accompanied by changes in parameters such as frequency, phase, and polarization state. In different vigor stages, the contents and content of nucleic acid, starch, enzyme, and protein inside the seed have a predictable change, and the reflected, scattered, and transmitted light information generated by the interaction with the light field can inform the internal composition and quality of the seed. Existing optical detection methods for seed vitality mainly include machine vision detection, near-infrared spectroscopy, hyperspectral detection, Raman spectroscopy, fluorescence spectroscopy, and seed exhalation gas spectroscopy.

Keywords: : seed vigor ; spectral detection technology ; image ; seed vigor ; optical

1. Machine Vision Method

Machine vision technology has advanced to integrate many research fields, such as image acquisition, image processing, and machine learning. During research on the detection of seed vigor, researchers have found that seed vigor is not only related to the genetic characteristics of the seeds but also closely related to the appearance and physical characteristics of the seeds, such as size, color, and texture. Machine vision technology working in the visible light band is essentially a detection method based on appearance features. It mainly simulates the human eye through image sensors such as charge-coupled imaging capture devices (CCDs), which collect images of seeds and then use computer technologies such as edge detection and feature extraction to simulate human vision. This provides for the extraction of physical information such as shape features, color features, and texture features of seeds, which can be combined with standard germination experiments of seed vigor. The combination of these techniques establishes the basis for a machine vision detection model of seed vigor to evaluate the status of seed vigor.

Since the 1990s, studies have launched in-depth research on the application of machine vision technology in agriculture. For example, McCormac et al. developed a lettuce seed vigor detection system based on machine vision technology [1]. The results of the study showed that the image analysis technique has the potential to determine the vigor of seeds. In China, scholars have successively developed similar methods for pepper seeds [2], which exploits a machine vision detection system for the vigor of perilla, wheat, and other seeds. The accuracy rate of the detection results is usually between 80 and 90%, and this system provides the basis for an online detection application for vegetable seed vigor [3].

Traditional machine vision technology mostly works in the visible light band. With the advancement of infrared devices, infrared thermal imaging technology has significantly advanced. As an extension of traditional machine vision technology, infrared methods can be applied to the field of non-destructive detection of seed vitality. Because of the influence of water absorption, respiration, and other metabolic activities of seeds during seed germination, the surface temperature will exhibit small changes which infrared thermal imaging technology can quantify. These temperature changes are closely related to seed vitality [4], thus distinguishing the seed differences in temporal vitality intervals [5].

Analyzing the existing research literature, the current problems in the application of machine vision technology in the field of seed vigor detection focus mainly on the fact that, when assessing seed vigor indicators, current machine vision methods extract vigor-related parameters from only the physical parameters of the seed surface features. However, the volume of indicative information is relatively small, and there is room for improvement. Generally speaking, with the advantage of inexpensive key equipment components and relatively mature algorithms, machine vision technology has significant promise for further development.

2. Infrared Spectroscopy Technology

With the help of the interaction between light and matter, spectroscopy can extract the internal vigor components and content information of seeds, so it has greater application potential in the detection of seed vigor. Among various spectroscopic techniques, near-infrared spectroscopy (NIRS) has been extensively studied. NIRS belongs to the class of imaging techniques known as molecular absorption spectroscopy. The principle is that specific functional groups in different molecules produce specific absorption indicators at specific wavelengths, which can be exhibited as absorption spectra in broadband light sources. As mentioned above, during the storage of seeds, the internal protein, starch, fat, and cellulose components will change, which will cause changes in seed vigor. The above substances are rich in hydrogen-containing groups such as C-H (aliphatic hydrocarbon), C-H (aromatic hydrocarbon), C=O (carboxyl group), OH (hydroxyl group), and N-H (amino group). Because of abundant absorption properties, one can identify obvious differences in absorption wavelength and intensity [6]. For example, Jin et al. extracted the difference in near-infrared spectra between viable rice seeds and inactive seeds [7], which more intuitively reflects the above characteristics; the results are shown in **Figure 1**.

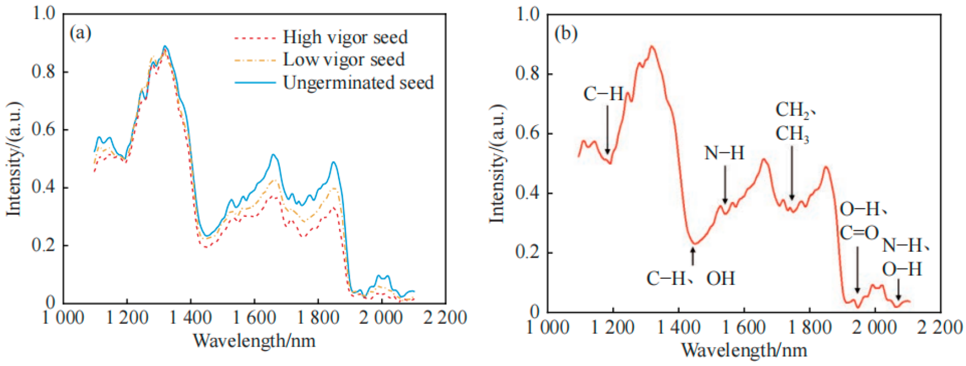

Figure 1. Near-infrared absorption spectrum of rice seeds; (**a**) The average curves of transmission spectra of rice seeds with different vigor characteristics; (**b**) Near-infrared absorption spectrum of high vigor seeds.

As shown in **Figure 1**, the spectra of viable and non-viable seeds have significant peak/valley differences in the bands of 1150–1220 nm, 1410–1450 nm, 1510–1540 nm, 1660–1800 nm, 1910–1950 nm, and 1980–2100 nm. The valleys represent groups such as amino N-H, hydroxyl O-H, and carbonyl C=O, respectively (**Figure 1**b). It can be seen that the changes in protein, starch, fat, and other components during the aging process of seeds can be characterized by this difference of infrared absorption spectrum, so as to extract identification characteristics of seed vitality.

Infrared spectroscopy, as a rapid non-destructive testing method, has previously attracted the attention of scholars earlier. For example, Tigabu et al. first applied near-infrared spectroscopy analysis technology to the detection of seed vigor. Their experiment obtained pine seeds with different vigor parameters that varied through accelerated aging. With the help of infrared spectroscopy technology, they successfully distinguished aged and unaged pine seeds. The accuracy rates of pine seed identification at the same level reached 80%, 90%, and 75%, respectively [8]. Al-Amery et al. used near-infrared spectroscopy to measure the standard germination rate and seed vigor of soybean seeds. By establishing a partial least squares prediction model, the seed vigor grades were classified, and two kinds of soybean seeds with low vigor and high vigor were identified. Note that the corrected rates were 80%~100% and 96.3%~96.6%, respectively. This is also the first non-destructive prediction model of near-infrared spectrum soybean seed vigor provided by the academic field [9]. NIRS technique has been reported for its application on seed non-destructive evaluation with pepper [10], watermelon [3], and tomato [4]. These studies helped to establish the viability of NIRS models of seed quality and have confirmed their application for seed quality evaluation.

Compared with the traditional infrared spectroscopy technology, the Fourier transform infrared spectroscopy technology that has recently emerged provides more powerful spectral analysis capabilities. For example, Larios et al. used Fourier transform infrared spectroscopy to study the vigor of soybean seeds and established a recognition model using modeling methods such as support vector machines. Their results showed that, under laboratory conditions, the correct rate of classification and identification of soybean seeds with different vigor could reach 100% [11].

In October 2006, the first near-infrared spectroscopy conference was held in China. This meeting became a milestone in the history of China's near-infrared technology development. Since then, a number of excellent research reports on the application of infrared spectroscopy to seed vigor have emerged. For example, Ling et al. used near-infrared supercontinuum laser spectroscopy to study three kinds of rice with different seed vigor, and the results showed that the accuracy of the prediction results reached more than 95% [12]. Fan et al. used near-infrared spectroscopy [13], studied the vigor of wheat seeds, and the results showed that the prediction accuracy of the model was not less than 84%, indicating that the near-infrared spectroscopy technology has the potential to predict the vigor of wheat seeds. Overall, near-infrared spectroscopy technology is widely used in wheat, rice, corn, soybean, and other crops, fir, Masson pine, Slash pine, Loblolly pine, and other forest seeds, as well as tomatoes, in the study of vigor detection of vegetable seeds [14][15].

Summarizing the existing problems in the application of infrared spectroscopy technology to the detection of seed vitality has identified the following aspects. On the one hand, infrared spectroscopy technology is susceptible to interference from working environment factors, and the extraction of spectral characteristics often needs to consider the noise caused by factors such as temperature and moisture interference. The correction of relevant environmental factors thus needs to be considered, which results in a relatively large modeling workload. On the other hand, the material components in the seeds are rich and complex, and interference factors such as the overlap and superposition of near-infrared spectral absorption peaks have always existed, which also affected the quantitative detection ability of infrared spectroscopy to a certain extent.

3. Hyperspectral Imaging Technology

Machine vision technology and spectroscopy technology have their own outstanding advantages. How to realize the combination of the advantages of the two has naturally become the goal pursued by the developers of optical analysis instruments. Thanks to the successful development of high-performance imaging spectrometers, hyperspectral imaging (HSI) has become a reality. Each pixel of HSI can obtain a piece of multi-spectral data, which can reflect the material composition information at a certain position on the target surface; by combining a large number of pixels, seed image information can be reconstructed to achieve the effect of map integration. HSI technology can effectively integrate image technology and spectral technology, increase the dimension of information detection, and thus can analyze seed sample information more comprehensively [16].

Hyperspectral imaging technology was first applied in the field of geographic information remote sensing and gradually applied to the field of seed vigor detection in the past ten years. For example, Baek et al. used hyperspectral imaging technology to quickly and non-destructively distinguish the viability of soybean seeds, and the test accuracy was close to 100% [17]. Ambrose et al. used hyperspectral imaging technology in the 400–2500 nm band to identify two corn seeds with different vigor levels, and the recognition accuracy of the established models reached 97.6% and 95.6%, respectively [18]. Perez et al. used hyperspectral imaging technology to study the seed vigor of the Japanese juniper, and the results showed that hyperspectral imaging technology could be used to effectively predict the vigor of the Japanese juniper [19].

In China, relevant scholars have also carried out a lot of research on the application of hyperspectral imaging technology to the detection of seed vitality. The research targets include crops such as rice, wheat, soybean, and corn. Some progress has also been made in the construction of discriminant models. Pang et al. used hyperspectral imaging technology to study four kinds of corn seeds with different vigor and constructed a seed vigor identification model by using various modeling methods such as a support vector machine and extreme learning machine. The results showed that the identification of different vigor seeds was accurate. The rate reached over 90% [20]. Zhang et al. used hyperspectral imaging technology to detect wheat seed vigor and established a seed vigor detection model using spectral data sets at different positions on the seed surface. The results showed that the established vigor detection model had high accuracy [21]. Li et al. detected the vigor of rice seeds based on hyperspectral imaging technology, and the results showed that the accuracy of classification and identification of seeds with different vigor could reach 94.44% [22].

As analyzed above, compared with machine vision technology and near-infrared spectroscopy technology, hyperspectral imaging technology has the ability to extract the characteristics and internal components of samples at the same time. Therefore, hyperspectral imaging technology has gradually become an active direction for seed vigor detection. At the same time, the analysis of the existing research also found that hyperspectral imaging research generally has a series of problems, such as high spectral data dimensionality, data redundancy and interference information, and complex construction of seed vitality mapping models, which still need to be improved by domestic and foreign scholars.

4. Tunable Diode Laser Absorption Spectroscopy

Spectral technology can not only directly detect seed components but also approach the challenge of seed vitality detection by detecting gas components produced during seed metabolism. Respiration is a comprehensive indicator of plant seed metabolism. Most seeds need to complete life activities through respiration, such as cell division and differentiation during early seed germination. Scholars have carried out a lot of research on the correlation between seed respiration and seed vigor, and Woodstock et al. discovered there is a significant positive correlation between seed respiration rate and germination rate [23]. Kalpana et al. [24] found the respiration of seeds showed a positive correlation between strength and vigor, and the above research conclusions become the theoretical basis for judging seed vigor by using the components of seed respiration.

In the presence of oxygen, seed respiration manifests as aerobic respiration, which consumes O_2 and releases CO_2. Under anaerobic conditions, seeds decompose by storing glucose themselves to produce ethanol and CO_2. Therefore, by detecting the change in CO_2 gas concentration produced by seed respiration, the seed vitality can be judged through the information on seed respiration intensity. Scholars at home and abroad have tried to use different gas sensors to detect the CO_2 produced in the process of seed respiration and judge the vigor of seeds. Among various CO_2 sensors, Tunable Diode Laser Absorption Spectroscopy (TDLAS) has better detection accuracy and lower limit than electrochemical or semiconductor sensors and can accurately detect the CO_2 concentration produced by seed respiration, and thus has been favored by researchers. The principle of TDLAS technology exploits a distributed feedback laser (Distributed Feedback Laser, DFB) by adjusting the DFB operating temperature and the size of the driving current and adjusting the wavelength of the laser output laser so that the laser is “selectively” absorbed by the gas to be measured. Macroscopically, the laser intensity is weakened, and the concentration of the gas to be measured can be reversed by using the change of the laser intensity. TDLAS technology has excellent single gas molecule selectivity and can achieve online response; combined with optical absorption cell technology and wavelength modulation technology, its detection limit can reach hundreds of ppt levels.

TDLAS technology has been mainly used in industrial field detection, atmospheric greenhouse gas monitoring, and other fields, while there are relatively few research reports on seed breath detection in the agricultural field. However, in recent years, scholars at home and abroad have strengthened research work in related fields. In China, Jia and others used TDLAS technology [25], and the CO_2 released during the respiration process of corn seeds was detected; combined with the results of the standard seed vitality germination experiment, the results showed that the average value of the correlation coefficient between the CO_2 concentration produced by respiration and the vitality index was 0.975, and the respiration intensity strongly correlated with the vitality index. These experimental results also show that, while limited by the existing device level of TDLAS technology, it is currently difficult to accurately calculate the respiration intensity value of seeds under high dynamic resolution. This affects research progress of the detection of seed respiration intensity, which indicates the need for future research.

Note that current research on seed respiration and seed vigor is mainly qualitative, and a large number of seed respiration detection and seed germination experiments still need to be carried out to quantitatively study the correlation between seed respiration intensity and seed vigor. On the other hand, although existing studies have shown that the respiration intensity of seeds such as corn, rice, and Chinese fir has a certain positive correlation with their germination rates, there are still some scientific issues in the process that have not been clearly understood, such as in the same genetic strain. Specific issues, such as the quantitative relationship between seed respiration intensity and seed vigor and the quantitative relationship between seed vigor and respiration intensity among different genetic lines, still need to be continuously studied.

5. Other Optical Detection Technologies

Fluorescence detection technology, Raman detection technology, photoacoustic spectroscopy detection technology, and other advanced optical detection methods have also been investigated for the development of non-destructive detection of seed vitality.

The application of chlorophyll fluorescence detection technology in the field of seed viability detection has also received attention in recent years. Anhui Institute of Optics and Fine Mechanics, CAS, has studied fluorescence detection techniques for years and developed a system of material composition three-dimensional platform. It has been used in digital agriculture research (**Figure 2**).

Figure 2. Material composition three-dimensional platform.

When the visible or near-infrared wavelength beam irradiates the seed epidermis, the chlorophyll on the seed coat will release energy in the form of fluorescence. By measuring the fluorescence spectrum released by the chlorophyll on the seed coat, relevant information on the seed vitality level can be obtained. It is confirmed that the smaller the peak signal of the fluorescence spectrum of the seed epidermis is, the lower the chlorophyll content is, and the higher the vigor of the seed is. Because this method is only specific to chlorophyll, it can reduce the influence of other optical radiation noise on the signal, so this method has certain technical advantages. Jalink and others first adopted fluorescence detection technology [26], studied the vigor of cabbage (*Brassica oleracea* L.) seeds, and confirmed that the chlorophyll fluorescence signal was negatively correlated with seed vigor. The analysis of existing research reports also found that the chlorophyll fluorescence detection method still needs to solve the technical difficulties, such as poor seed vigor grading effect due to the influence of chlorophyll content differences in seed samples.

Raman spectroscopy is a scattering spectrum technique that can provide detailed information about molecular vibrations. Since Raman peaks are usually clear and emerge in a relatively narrow band (only a few wavenumbers), it is useful in the feature identification and analysis of organic macromolecules. Raman methods have unique technical advantages and have formed the basis for a standard Raman analysis test instrument. Anhui Institute of Optics and Fine Mechanics, CAS, has carried out research on Raman Spectroscopy detection techniques. A handhold Raman Spectroscopy detection device which can be used for seed Raman spectroscopy detection is shown in **Figure 3**.

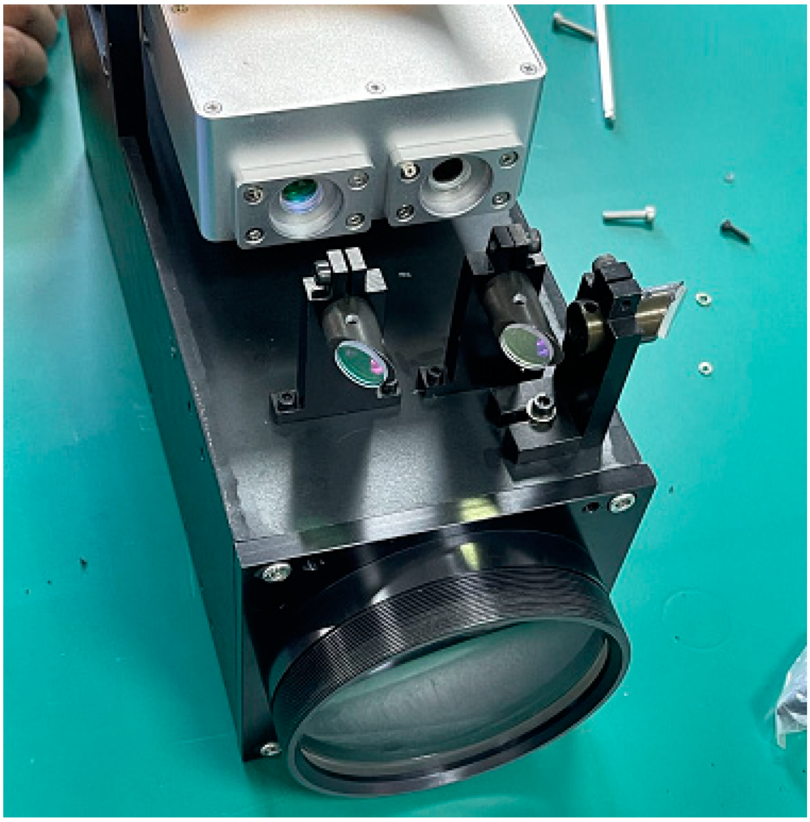

Figure 3. A handhold Raman Spectroscopy detection device.

As Raman spectroscopy shows great potential for rapid analysis, its related work in the field of general agricultural detection is also developing rapidly. More typically, Ambrose et al. used a Raman spectrometer (1700–3200 cm^{-1}) to identify viable and inactive corn seeds [27], with an accuracy rate above 93%. The analysis of existing research reports also found that the Raman scattering signal is weak, which plagues its application in the field of seed viability detection, and related problems still need breakthroughs in key devices.

Photoacoustic spectroscopy technology (Photoacoustic spectrometry technology, or PAS) is a detection technology based on a photoacoustic effect. Its detection principle is that the light source irradiates and heats the target. The ambient air is induced to vibrate weakly and generate sound waves, and the photoacoustic spectrum of the target can be obtained by detecting the weak sound wave signals. Different from traditional absorption spectroscopy, PAS does not directly detect the spectrum based on photon characteristics but detects the periodic heat flow in the non-radiative excitation phenomenon after the sample is illuminated, which changes the laser modulation frequency to adjust the light transmission. This measurement reveals the depth of the seed so that it can detect the material composition of the sample at different depths. Pardo et al. used photoacoustic spectroscopy technology [28] and carried out vegetable seed vitality detection research; their test results show that the light absorption of unaged seeds is higher, and the results show that the scheme of predicting seed vigor by photoacoustic spectroscopy is feasible. In general, photoacoustic spectroscopy is still a relatively new cutting-edge technology, and it is worth investing more resources in research. The current research developments of seed vigor detection procedures based on modern optical methods are assembled in **Table 1**.

Table 1. Current research developments of seed vigor detection procedures using modern optical methods.

Detection Method	Main Principle	Working Light Band	Core Device	Typical Application
Machine Vision Technology	**Optical Imaging of Seeds**	**Visible light/near-infrared**	**CCD Imaging device**	**Perilla, Wheat, etc.** [1][2][3]
Infrared Spectroscopy	**Infrared Absorption Spectrum of Organic Matter**	**Near-infrared**	**Infrared spectrometer**	**Pine seeds, soybeans, wheat, rice, corn, etc.** [8][15][29][30][31]
Hyperspectral Imaging Technology	**Reflection/Absorption Spectrum of Seed Surface**	**UV to NIR**	**Broadband light source + imaging spectrometer**	**Pepper, Rice, Avocado etc.** [7][22][32][33]

Detection Method	Main Principle	Working Light Band	Core Device	Typical Application
Turnble Laser Absorption Spectroscopy	**Infrared Absorption Spectrum of Organic Matter**	**Near-infrared to mid-infrared**	**Tunable laser + lock-in amplifier Tunable laser + lock-in amplifier**	**Maize [23], pigeonpea [24]**
Raman spectrometer	**Raman Frequency Shift Spectroscopy of Organic Compounds**	**UV to Visible Light**	**Pulse laser + Raman spectrometer**	**Maize [34]**
Photoacoustic spectroscopy	**Photoacoustic Effect Excited by Matter Without Radiation**	**UV/Visible**	**Laser + weak acoustic wave detector**	**Lettuce, leaf beets, etc. [27]**
Fluorescence spectroscopy	**Fluorescent Effect of Organic Matter**	**UV to visible Light**	**Fluorescence Spectrometer**	**Cabbage and other vegetable seeds [10]**

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