

EXAMINATION OF ACCURACY AND RESOLUTION OF A HAND-HELD SPECTROMETER

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ABSTRACT:

Several applications for the examination of plants by use of hand-held spectrometers exist in the field of environmental monitoring and precision agriculture. Portable instruments render possible the measurement of the intensity of radiation in the range of approximately 350 nm to 950 nm with a spectral resolution of about 3 nm. Thus, they can be used for the spectral analysis of chemical elements in a laboratory, e.g. for chlorophyll analysis, but also for the mass screening of spectral reflectance of plants or parts of them in field work. Although field measurements cannot compete with a laboratory analysis concerning precision and reliability of the results, their application in a study is justified at least for coarse measurements but especially under the aspect of cost reduction. The reliable application of a hand-held spectrometer requires the examination of the spectral resolution and the accuracy of the intensity measurements. This paper describes a test procedure for a spectrometer based on calibrated cuvettes which are used for testing the state and the change of the instrument. The spectrometer is then applied in a study which aims at the detection of chemical elements, e.g. nutrients or heavy metal, in plants.

1. INTRODUCTION

The pressure on the environment caused by the emission of polluting substances has increased drastically since the Industrial Revolution. In areas with high industrial density the strain has already reached vast dimensions. Especially the pollution of soil in industrial waste land or in areas close to industrial plants can be extremely high so that a decontamination has to be carried out for any type of usage of the areas.

Here, two different groups of contaminants have to be distinguished:

- organic contaminants (oil, fat, military waste) and
- metal, especially heavy metal like cadmium or lead.

While for organic contaminants several well approved methods of decontamination are readily available, the extraction of metal from contaminated soil can only be accomplished by expensive procedures like extraction with acids, Fiedler 2001, or Phytoremediation.

1.1 The Phytoremediation project

Phytoremediation, i.e. the extraction of pollutants from soil by using plants, is an interesting alternative because the method offers several positive features:

- In-situ application,
- suitability for less or medium contaminated areas,
- low costs and
- maintenance of soil functionality,
- but as a major disadvantage
- decontamination of soil takes several years up to decades.

During such long periods of time the areas have to be monitored with suitable methods. Here, mobile hyper-spectral scanners can be applied for documenting the progress of Phytoremediation under qualitative and quantitative aspects.

The application of spectrometers in the research and industrial environment is increasing since several years. Due to expanding possibilities and falling prices a number of new applications came up:

- Online process control,
- Quality control,
- Color management,
- Qualitative and quantitative analysis in chemistry, pharmacy and biochemistry,
- Precision farming,
- Ground truth in Remote Sensing and
- Medicine.

The Institute for Applied Photogrammetry and Geoinformatics is currently participating in a research and development project which examines the performance of Phytoremediation measures. There, hyper-spectral sensors are applied for the detection of the effects and influence of specific chemical elements on the color of plants.

1.2 The sensor

Due to latest developments in the consumer sector several low-cost sensors with prices less than 3000 € are readily available. These hyper-spectral sensors in the VIS- to NIR-range are able to capture data in the range from up to 300 nm to 1000 nm with a resolution of some 3 to 5 nm with an accuracy of 5 to 10 %. This information is given by manufacturers and must be

examined and tested for application and compared with information of other spectrometers and photometers. By this, a combination and connection of different spectral data is achievable.

For monitoring purposes in phyto-remediation applications especially the following questions came up:

- With which test setup and by what means can the sensors be examined ?
- Which standard must be reached in order to compare different sensors ?
- Is the spectral sensitivity and the data of the sensor stable over a longer range of time ?

Currently, in the monitoring process a sensor of the manufacturer TriOS used having the following specifications.

Spectral range: 350 nm – 950 nm
 Detector type: Channel silicon photodiode array
 Spectral sampling: 3,3 nm / pixel
 Usable channels: 190
 Accuracy : Better than 6 – 10 %

This VIS-Scanner is an independent and compact hyper-spectral sensor in the range of visible light. It has been designed for outdoor use under unfavorable conditions. A ZEISS spectrometer has been integrated as OEM-component in the system. Grid and sensor are rigidly connected while the standard SMA-connector of the light-wave conductor is separated from the system in order to avoid damage of grid and sensor due to external influences on the connector. Data is transferred using an external interface which also accomplishes the electrical connection with a voltage from 12 V DC to 240 V AC. When connecting the sensor to a computer with wire lengths of up to 50 m the connection either to a serial port or by using a serial to USB converter the connection to USB 1.1 or USB 2.0 standard is easily possible.



Figure 1: Mobile hyper-spectral sensor TriOS

1.3 Spectral data and calibration configuration

If directional interdependencies can be neglected the result of any measurement with a spectrometer is intensity in a defined band of wavelengths within a period of time. As long as the measurement device does not acquire or consider the incident light on a surface from which reflected or transmitted light is to be measured, the data is not comparable to other measurements which are taken with different devices or under different conditions. This is due to the fact that reflection or transmission

of light depends on the incident radiation, cf. Weisensee 1992. Thus, spectral measurements must be standardized, e.g. according to DIN 5036. For the standardization of transmittance measurements either the spectral transmittance factor or the spectral optical density can be applied.

$$\tau(\lambda) = \frac{\phi_{e\lambda\rho}}{\phi_{e\lambda}} \quad (1)$$

τ spectral transmittance
 $\phi_{e\lambda\rho}$ transmitted spectral radiant power
 $\phi_{e\lambda}$ incident spectral radiant power

$$D(\lambda) = \lg \frac{1}{\rho(\lambda)} \quad (2)$$

D spectral optical Density

The above formulae show that the value that is independent of incident radiation is the logarithm of the ratio of incident and transmitted radiation. Thus, for every field measurement of an object surface also a measurement of the incident light is required. In laboratory work a calibrated light source is sufficient for a larger measurement campaign if it can be assumed that besides the sensor also the light source remains constant.

In order to avoid environmental influences on the sensor a closed system consisting of the following parts has been build up:

- electric and thermal stabilized tungsten-halogen light source,
- shielded cuvette container with SMA-adapter,
- sensor and
- fiber optic cable.



Figure 2: Concept of intensity measurement in a closed system.

Four different cuvettes with defined properties are currently in use for testing and calibration purposes of the system, cf. ISO 9000-9004. One filter (in the following referred to as F1, see table 1) consists of holmium oxide glass (Ho₂O₃) which has several narrow absorption peaks in the ultra-violet, visible and near infra-red band. Furthermore, three neutral glass filters (F2 – F4, cf. table 2) which are characterized by high evenness and stability and a constant transmittance in the visible range are used.

1	2	3	4	5
279,30	360,90	453,60	536,40	638,00

Table 1: Calibrated wavelengths (Peaks) of the holmium oxide cuvette F1.

No.	440nm	465nm	546nm	590nm	635nm
F2	0,268	0,237	0,238	0,255	0,255
F3	0,499	0,457	0,469	0,500	0,486
F4	0,964	0,899	0,926	0,960	0,915

Table 2: Spectral optical density of the calibrated cuvettes (F2 – F4).

2. MEASUREMENTS

A spectral measurement always follows the same scheme:

1. Measurement of light source
2. Measurement of cuvette
3. Measurement of light source

This sequence guarantees for the stability of the light source and the sensor. Measurements with a duration of several hours have shown a constant behavior of the system.

2.1 Results of the reference measurement

The first measurement of the holmium oxide cuvette showed the following results: (The figure of the spectral optical measurement is shown in Appendix A)

Theoretical peaks of holmium-oxide	Measured peaks	Differences
279 nm	Not measurable	Not measurable
361 nm	362 nm	- 1 nm
453 nm	450 nm	+ 3 nm
536 nm	537 nm	- 1 nm
638 nm	639 nm	- 1 nm

Table 3: Absorption peaks of the holmium oxide cuvette

The comparison between a priori known values and measured absorption peaks shows maximum deviations of approximately 3 nm. This corresponds with the manufacturers information of the spectral resolution of the sensor so that the calibration which transforms sensor position into wavelengths can be accepted.

Measured intensities have then been tested by using the neutral glass filters, tab. 4 and Appendix B.

Measurements

Wavelength	n(F2)	n(F3)	n(F4)
440	0,282	0,514	0,987
465	0,255	0,474	0,923
546	0,254	0,480	0,937
590	0,268	0,506	0,972
635	0,268	0,492	0,929

Differences

Wavelength	n(F2)	n(F3)	n(F4)
440	-0,014	-0,015	-0,023
465	-0,018	-0,017	-0,024
546	-0,016	-0,011	-0,011
590	-0,013	-0,006	-0,012
635	-0,013	-0,006	-0,014

Table 4: Results of the neutral glass filters

The results are convincing in the whole visible spectral range and they are in accordance with the manufacturers information

but a systematic offset of $-0,014$ is statistically significant. Nevertheless, considering this offset does not improve the derived spectral optical density data to a remarkable amount.

2.2 Results of the repetition of the measurements

During field work the instrument is subject to environmental strain of climate like temperature and humidity, vibration and others. So, after four months of use in laboratory and field work a review of the calibration of the sensor has been undertaken. In order to minimize error sources in the repetition the same fiber optics have been used in the same sequence as in the first measurement.

Theoretical peaks of holmium-oxide	Measured peaks	Differences
279 nm	Not measurable	Not measurable
361 nm	359 nm	+ 2 nm
453 nm	448 nm	+ 5 nm
536 nm	537 nm	- 1 nm
638 nm	639 nm	- 1 nm

Table 5: Results of the holmium oxide cuvette after 4 months

The results did not show significant differences to the previous measurements. The narrow banded absorption peaks of holmium oxide have been found again with differences in the order of magnitude of the spectral resolution of the sensor. Also, the systematic offset of the ordinate has been repeatedly determined with $-0,016$.

Measurements

Wavelength	n(F2)	n(F3)	n(F4)
440	0,288	0,516	0,987
465	0,260	0,476	0,928
546	0,256	0,482	0,942
590	0,269	0,508	0,977
635	0,269	0,495	0,934

Differences

Wavelength	n(F2)	n(F3)	n(F4)
440	-0,020	-0,017	-0,023
465	-0,023	-0,000	-0,029
546	-0,018	-0,013	-0,016
590	-0,014	-0,008	-0,017
635	-0,014	-0,009	-0,019

Table 6: Results of the neutral glass filters after 4 months

2.3 Measurement of chlorophyll

The reason for considering the concentration of chlorophyll in plants in a Phytoremediation project results from the influence of pollutants, e.g. heavy metals like lead or cadmium, on the production of specific kinds of chlorophyll in plants. Thus, chlorophyll can serve as an indicator for the occurrence of pollutants in soil and is expected to allow for the estimation of

quantities of such material that has been extracted from soil by plants.

Chlorophyll is a colorant which is produced by plants for the use of light energy. It consists of two parts: a non-polar part with which chlorophyll is fixed to the membrane of chloroplasts and a complex polar part from which the color results, (<http://www.net-lexikon.de/Chlorophyll.html>).

There are different types of chlorophyll with different absorption spectra and occurrence:

- Chl a: blue-green, chlorophyta (green alga, land plants)
- Chl b: green, chlorophyta (green alga, land plants)
- Chl c: yellow-green, phaeophyta (brown alga)
- Bacteriochlorophyll b: cyano bacteria

In photosynthesis the energy of light is transferred to an electron in the active center between two coupled chlorophyll a-molecules, while chlorophyll b is mainly responsible for collecting and transferring light to the active center.

In land plants only chlorophyll a and b is produced. So suitable techniques have to be applied for the extraction of the molecules out of the leaves. Firstly, the leaves are cut up small and under addition of calcium carbonate (CaCO_3) and acetone triturated in a cooled mortar. After the first destruction of the leaf structure the breakup is continued by adding further acetone. The material is finally centrifuged for ten minutes. The clear, deep-green supernatant which contains all pigments is decanted to a cuvette for the transmission measurement.

The chlorophyll concentration in $\text{mg}\cdot\text{ml}^{-1}$ results from the measured transmission of the probe at specific wavelengths from (Arnon 1949 and Porra 2002):

$$\begin{aligned} CHL(a) &= (11,75 * E_{662}) - (2,35 * E_{645}) \\ CHL(b) &= (18,61 * E_{645}) - (3,96 * E_{662}) \end{aligned} \quad (3)$$

For control purposes the probes have been measured by the TriOS sensor and a calibrated laboratory photometer (Perkin Elmer UV/Vis Spectrometer Lambda 20 with Software UVWinlab). With the sensor system and the formulae described above the results shown in Appendix C have been achieved.

The results comply with the expected accuracy of less than 10 % of the chlorophyll concentration. This proves that the sensor system and the measurement setup used is suitable for this task. Furthermore, it can be observed that considering the offset of -0,014 in the transmission values does not improve the results significantly.

3. CONCLUSIONS

When acquiring spectral information from plants the relevant parameter is the ratio of incident and reflected or transmitted illumination. For field work the problem of simultaneous measurement of both intensities has to be solved or a closed sensor system has to be used.

By using calibrated cuvettes and fiber optic cables spectral resolution and intensity accuracy of a sensor system can be easily verified in the visible range by a laboratory test. Fiber optic cables must be used in a constant order and direction to avoid any effects or irregularities in the optical pathway.

The use of low-cost spectrometers normally accomplishes the required accuracy and reliability for applications with plants like precision agriculture or phyto-remediation. The quality of the instrumentation allows for a long term use in field-work. There, a rapid test with defined material with a known spectral behavior (spectral standard) can be carried through.

Further research work has to be done to analyze some effects concerning the spectral behavior of the light source. Within the limits of accuracy of a highly precise laboratory photometer the light source remained constant for a longer observation epoch. Nevertheless, differences in the intensities were noticeable between different epochs.

4. LITERATURE

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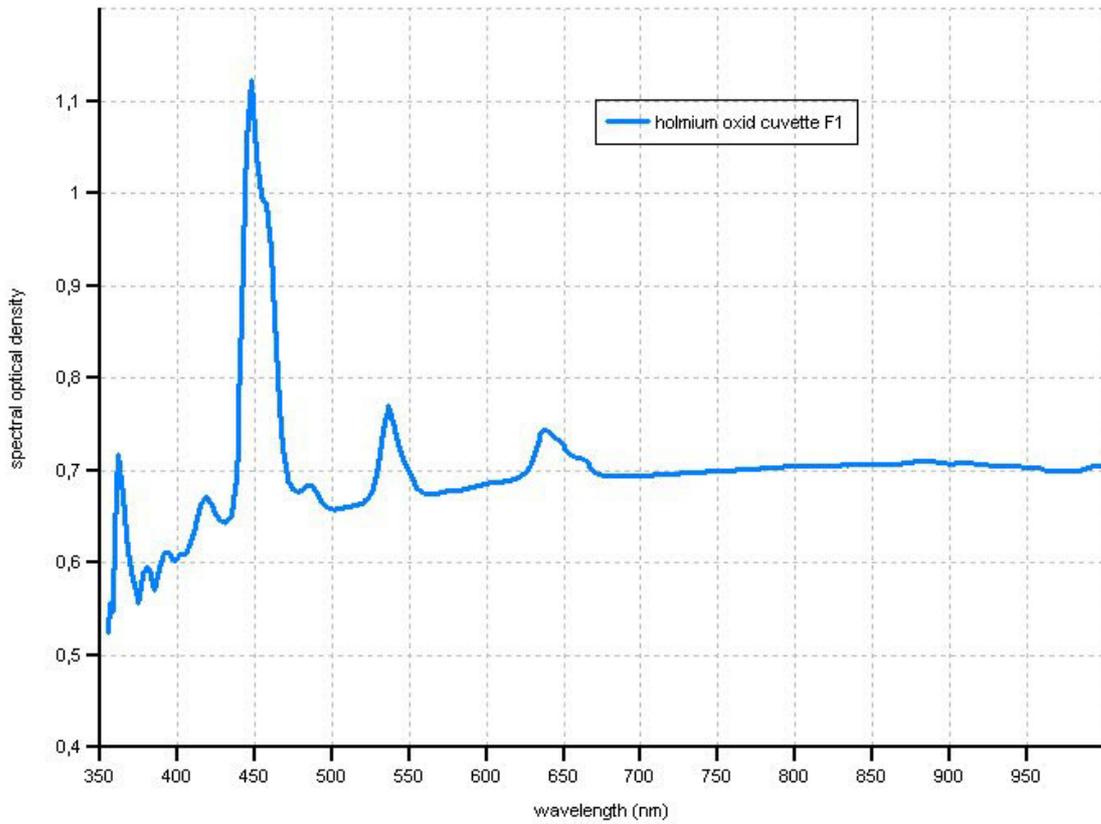
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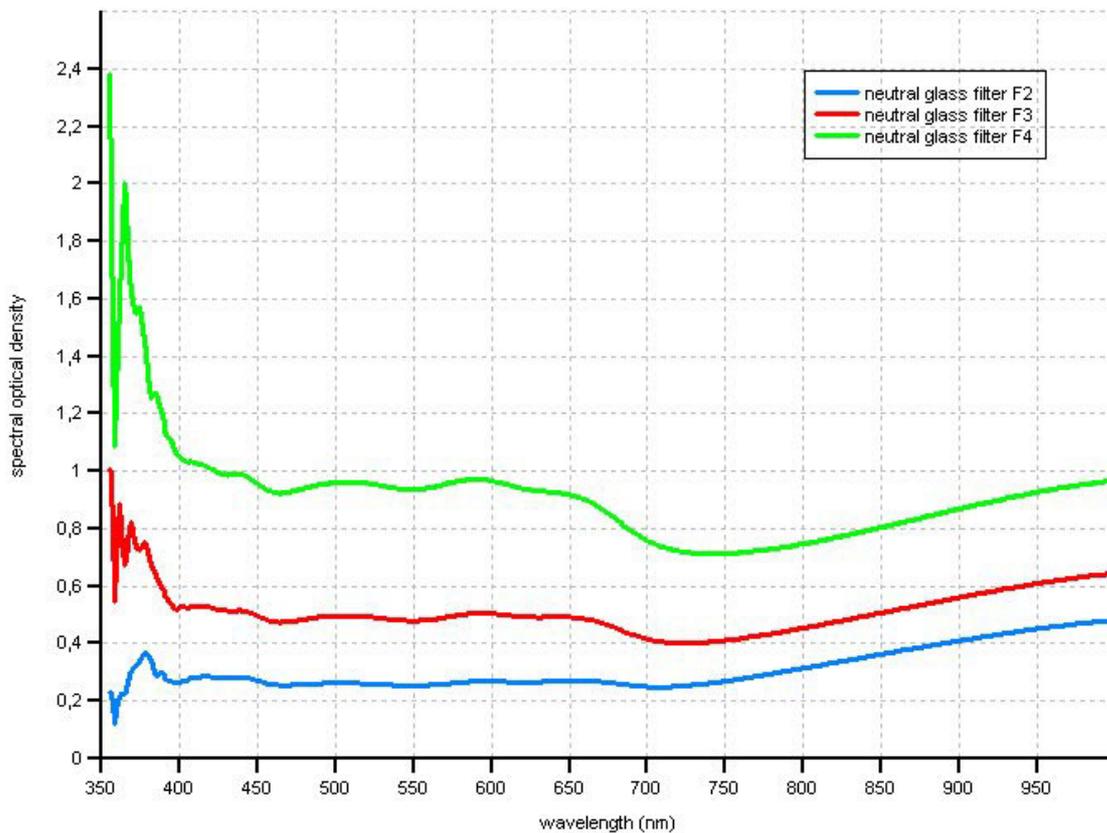
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APPENDIX A: SPECTRAL OPTICAL DENSITY MEASUREMENT OF THE HOLMIUM OXIDE CUVETTE



APPENDIX B: SPECTRAL OPTICAL DENSITY MEASUREMENTS OF THE NEUTRAL GLASS FILTERS (F2 - F4)



APPENDIX C: RESULTS OF CHLOROPHYLL MEASUREMENT

Photometer: Perkin Elmer UV/Vis Spectrometer Lambda 20 with Software UVWinlab

ID.	645nm	662nm	CHL a	CHL b	CHL (Perkin)
Licolly1A	0,4135	1,1073	12,04	3,31	15,35
Licolly1B	0,1990	0,5133	5,56	1,67	7,23
Licolly1C	0,0949	0,2656	2,90	0,71	3,61

Trios Sensor

Without offset

ID.	645nm	662nm	CHL a	CHL b	CHL (TriOS)	v (absolut)	v (%)
LIC 1A	0,4234	0,9916	10,66	3,95	14,61	0,74	4,82
LIC 1B	0,2137	0,4770	5,10	2,09	7,19	0,04	0,59
LIC 1C	0,0991	0,2423	2,61	0,88	3,50	0,11	3,16

Trios Sensor

With offset

ID.	645nm	662nm	CHL a	CHL b	CHL (TriOS)	v (absolut)	v (%)
		-0,014					
LIC 1A	0,4378	1,0060	10,79	4,16	14,96	-0,39	-2,57
LIC 1B	0,2281	0,4914	5,24	2,30	7,54	0,30	4,20
LIC 1C	0,1135	0,2567	2,75	1,10	3,84	0,23	6,43