

## EnMAP Field Guides Technical Report

### Measuring Leaf Chlorophyll Content with the Konica Minolta SPAD-502Plus

Andreas Süß, Martin Danner, Christina Obster,  
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Recommended citation:

Süß, A., Danner, M., Obster, C., Locherer, M., Hank, T., Richter, K. (2015):  
Measuring Leaf Chlorophyll Content with the Konica Minolta SPAD-502Plus –  
Theory, Measurement, Problems, Interpretation. *EnMAP Field Guides Technical  
Report, GFZ Data Services.*

DOI: <http://doi.org/10.2312/enmap.2015.010>

## **Imprint**

**EnMAP Consortium**

**GFZ Data Services**

Telegrafenberg  
D-14473 Potsdam

Published in Potsdam, Germany  
December 2015

<http://doi.org/10.2312/enmap.2015.010>



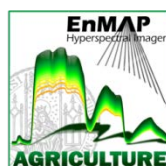
# EnMAP Field Guides

## Technical Report

### Measuring Leaf Chlorophyll Content with the Konica Minolta SPAD-502Plus

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Supported by:



Federal Ministry  
for Economic Affairs  
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# 1 Introduction

## 1.1 Definitions

Chlorophylls (Chl) are a dominant factor controlling leaf optical properties of healthy green vegetation and are thus an essential part of the photosynthetic process. They harness light energy from the sun to store it as chemical energy (Richardson et al., 2002). Within the cross-section of healthy green leaves, chlorophyll pigments are located in the chloroplasts of palisade parenchyma cells. In general, chloroplasts occur more frequently towards the upper side of palisade cells. For this reason the upper leaf surface appears darker compared to the bottom surface side (Jensen, 2007). The amount of chlorophyll in leaves is normally expressed in terms of either concentration ( $\mu\text{g Chl/g tissue}$ ) or content ( $\mu\text{g Chl/cm tissue}$ ) and can vary significantly in value among different plant kingdoms and growing stages (Taiz et al., 2007). If optical methods for measuring leaf chlorophyll content are applied, index values (e.g. SPAD-Value) are commonly used to specify the relative leaf chlorophyll content, but not absolute chlorophyll content or concentration (Richardson et al., 2002).

The most significant plant pigments for oxygenic conversion of light energy are *chlorophyll a* and *chlorophyll b* (e.g. Jensen, 2007; Taiz et al., 2007). Figure 1-1 presents the corresponding absorption spectra of chlorophyll a and b pigments. Maximum absorption of chlorophyll a occurs at 0.43 and 0.66  $\mu\text{m}$ , whereas the highest chlorophyll b absorption is located at 0.45 and 0.65  $\mu\text{m}$  Jensen (2007). In general, absorbance rates of chlorophyll a pigments are higher compared to chlorophyll b absorption. Besides, the relative lack of chlorophyll absorption in the regions of the red and blue light makes leaves appear green to our eyes (Taiz et al., 2007). Apart from chlorophyll, there are other pigments within the palisade mesophyll cells: in particular the carotenoid pigment class consisting of *xanthophylls* and *carotenes* (s. Figure 1-1). The abundance of chlorophyll a and b absorption obscures the effects of those pigments. When the tissue dies, chlorophyll rapidly disappears and the above mentioned pigments gain in relative importance (Matile et al., 1999).

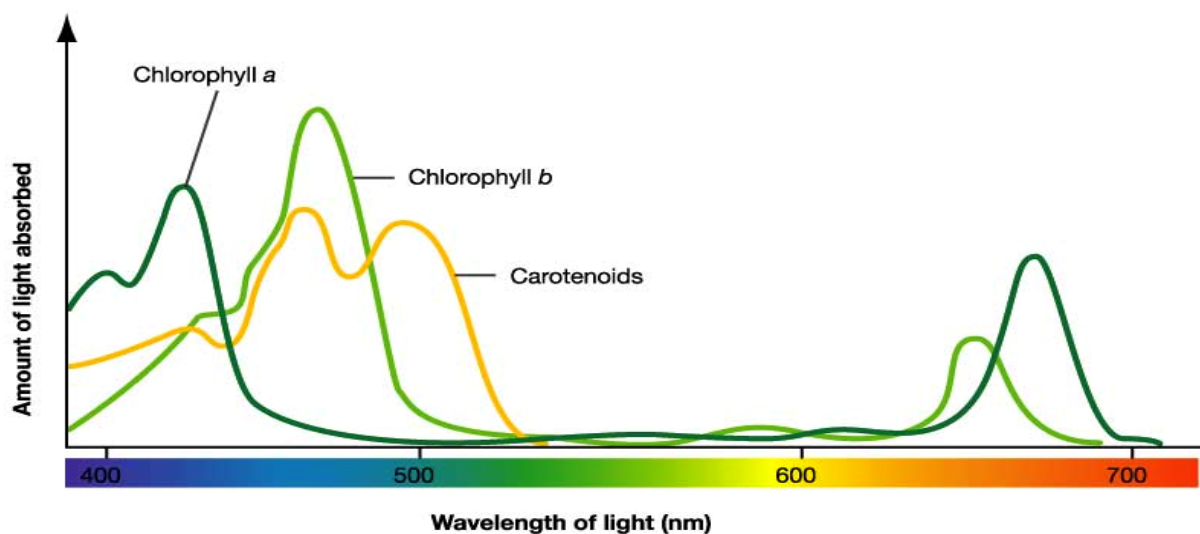


Figure 1-1: Absorption spectra of Chlorophyll a, b and carotenoids (UIC 2004).

## **1.2 Areas of Application**

Leaf chlorophyll content is directly associated with the efficiency and capacity of the photosynthetic apparatus and hence provides useful information about photosynthetic potential and primary production for land managers and eco-physiologists. A further area of application arises from the fact that plant senescence, several diseases and many long-term stress factors directly lead to changes in pigmentation caused by a decrease in chlorophyll content which is equal to an increase in relative carotenoid concentrations. Chlorophyll content was also found to be directly related to nitrogen status, considering that most of the leaf nitrogen is integrated in chlorophyll. In consequence, nitrogen status can be indirectly assessed by measuring leaf chlorophyll content so that useful information can be provided to adjust the rate of nitrogen fertilization (Richardson et al., 2002).

Generally, non-destructive techniques to estimate chlorophyll content of vegetation are of significant importance to agricultural management operations, particularly in the area of precision farming (Gitelson et al., 2003). The scientific interest is verified by Kaufman et al. (2010), showing that chlorophyll content is amongst the parameters with the highest frequency within investigations of agricultural hyperspectral studies. Those investigations are strongly dependent on quick non-destructive and accurate in situ reference measurements. Some eligible technologies and methods respectively are presented as followed.

## **1.3 Measurement and Devices**

Over the last decades, various optical techniques to assess leaf chlorophyll content quickly and non-destructively have been developed (e.g., Markwell et al., 1995, Richardson et al., 2002). Those methods, which are based on the absorbance or reflectance of certain wavelengths of light by intact leaves, more recently substituted the traditional wet chemical extraction methods. However, such extraction techniques are still required for obtaining benchmark values to evaluate the performance of optical methods. Some recommended techniques of Chlorophyll extraction are described in Hiscox and Israelstam (1979) or Barnes et al. (1992).

Optical methods generally output a chlorophyll index by measuring absorbance or reflectance of light in sample leaves. The following section describes currently applied measurement techniques and devices to measure chlorophyll content. A detailed evaluation of different methods to estimate foliar chlorophyll content is presented in Richardson et al. (2002).

### **Hand-held Chlorophyll Absorbance Meters**

In general, hand-held chlorophyll meters measure absorbance by the leaf of two different wavelengths in the spectral domain of red and near-infrared. As an output, they calculate index-values (e.g. SPAD-value, CCI-value) that specify leaf chlorophyll content. In the domain of red light, strong chlorophyll absorption occurs and the near-infrared light measurement is made to record differences in between the leaf structure. It should be taken into account that the accuracy of leaf chlorophyll content estimation declines (s. Figure 1-2) with increasing chlorophyll content (Richardson et al., 2002). Some of the most well-known devices are the CCM-200 (Opti-Sciences, Tyngsboro, Massachusetts, USA) and the SPAD-502 (Minolta Camera Co., Osaka, Japan). The latter device is described further in the following chapters. A detailed description of the theoretical principles of hand-held chlorophyll absorbance meters is found in Markwell et al. (1995).

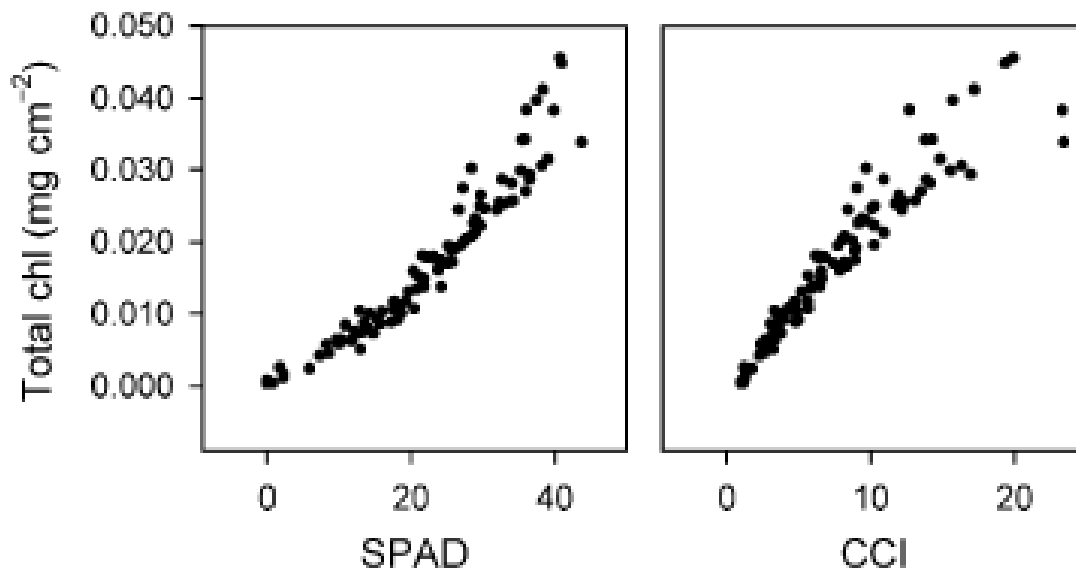


Figure 1-2: Scatter plots indicating the relationship between SPAD and Chlorophyll Content Index (CCI) calculated by the Minolta SPAD-502 and Opti-Sciences CCM-200 hand-held chlorophyll meters compared to total chlorophyll (Richardson et al., 2002).

### Reflectance spectroscopy

Portable full range spectrometers like the UniSpec Spectral Analysis System (PP Systems, Haverhill, Massachusetts, USA) or the ASD FieldSpec 3 field spectrometer (ASD inc., Boulder, Colorado, USA) detect reflection for many contiguous bands of the ultraviolet, visible, and near-infrared wavelengths (Richardson et al., 2002). By measuring leaf reflectance, different narrow band indices (e.g. hNDVI (Oppelt and Mauser, 2004) or PRI3 (Gamon et al., 1992)) or a hyperspectral analysis of certain areas of absorption (e.g., chlorophyll absorption integral (CAI) (Oppelt, 2002)) can be applied that have been proven to be well correlated with leaf chlorophyll content. Compared to hand-held chlorophyll absorbance meters, reflectance spectroscopy detects much more spectral data generating a great number of spectral information for interpretation. However, a proper selection out of these abundant information sometimes poses a key problem for researchers. Nevertheless, investigations from Richardson et al. (2002) show that using reflectance indices to estimate chlorophyll content at the leaf level can be more accurate in certain cases than measurements with hand-held chlorophyll absorbance meters. Yet, it should be considered that reflection measurements require comprehensive calibration and is thus more complex, prone to errors and time consuming. In consequence, comparable simple hand-held chlorophyll absorbance meters are more preferred for in situ measurements within the scope of remote sensing campaigns (Richardson et al., 2002)



## 2 Data Collection

### 2.1 Theory: Measurement Principle

The SPAD-502 Plus (Konica Minolta, Inc.) determines the chlorophyll concentration by measuring the leaf absorbance in red and near-infrared regions. The light is emitted by two LEDs with peak wavelengths at 650 nm and 940 nm. These LEDs emit light in sequence from the emitting window to a photodiode detector when the measuring head is closed. When the light passes through the sample leaf in the measuring head, a certain amount transmits through the leaf and the transmitted light strikes the receptor and is converted into electrical signals. With these absorbance values the SPAD-502 Plus calculates a company defined SPAD (Soil Plant Analysis Development) value by division of light transmission intensities at 650 nm by 942 nm. This numerical SPAD value specifies the relative content of chlorophyll within the sample leaf (Konica Minolta Optics, 2012).

### 2.2 Technical Accomplishment

#### 2.2.1 Preparation (Handling the Device)

The SPAD-502 Plus is a hand-held device that is used for accurate, quick and non-destructive in situ measurements of chlorophyll concentrations for numerous plant species (Richardson et al. 2002). The claimed accuracy of the SPAD-502 is  $\pm 1.0$  SPAD units. The device weighs 200g and has a compact form for high portability. The measuring area is 2mm x 3mm. It can be used during rainfall due to its water-repellent construction. It is, however, not completely waterproof. Power consumption is low and a set of two AA-batteries provides enough energy for approximately 20 000 measurements.

The memory of the device is sufficient for 30 measurements and the data in memory can be recalled or deleted at a later time. Average values of all memory data can be automatically calculated. When the memory is full, the oldest data in memory is deleted with the remaining getting shifted to the next place. The memory gets cleared at latest when the device is switched off. There are different keys on the SPAD to perform certain operations on data in memory. Overall, the following functions exist:

|                       |   |
|-----------------------|---|
| <b>AVERAGE</b>        | Calculates the average value of all data in memory.                                 |
| <b>ALL DATA CLEAR</b> | For deleting all data in memory, the key must be pressed twice.                     |
| <b>DATA RECALL</b>    | Recalls the data stored for the previous data number onto the display for checking. |
| <b>1 DATA DELETE</b>  | Deletes only displayed data; helpful to delete incorrect data.                      |

The SPAD-502plus also includes a reading checker. It can be used to check that the device is operating normally. It should be applied periodically for best accuracy. The *depth stop* (s. Figure 2-1) can be used to set the maximum depth to which samples can be inserted into the sample slot to keep the measuring point constant. This is especially useful for measuring small leaves. To set the position of the depth stop, squeeze both of its sides against the edge of the measuring head and slide it to the desired position. The depth stop can be moved within the range of 0 to 6mm from the center line. When not in use, remove the depth stop, turn it around and replace it on the measuring head. Figure 2-1 shows all parts and functions of the device, Figure 2-2 illustrates its display.

## Names of parts



Figure 2-1: Survey of SPAD-502plus (Konica Minolta Optics, 2012).

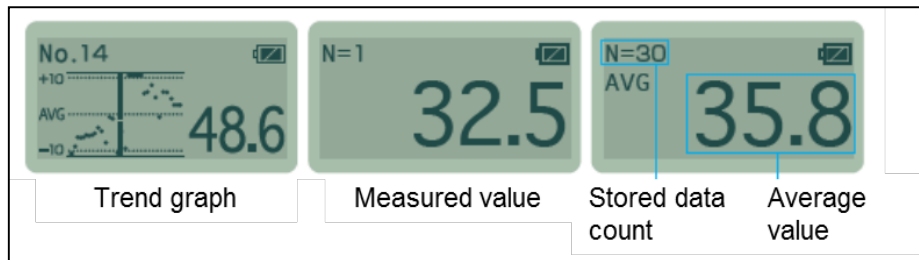


Figure 2-2: Display of SPAD-502plus (Konica Minolta Optics, 2012).

For the SPAD Chlorophyll meter there is no special preparation apart from the calibration. Calibration is necessary whenever the meter is switched on. During calibration the two LEDs emit light sequentially without any sample leave in the measuring head. The received light is converted into electrical signals and the ratio of their intensities is calculated.

### 2.2.2 Measurement

#### Measurement procedure

- (1) At first turn power switch ON.
- (2) Without a sample in the sample slot, press on the finger to close the measuring head. Hold it close until a beep sounds and the display shows that calibration is complete. If a series of beep sounds and “ERROR” appears on the display, calibration has not been completed properly. The most probable reasons are that the measuring head had been opened too early or had not been closed entirely. If the display shows “ERROR” and “E-U”, the windows of the measuring head may be dirty.
- (3) Insert the sample of plant leaf you want to measure into the receptor window (s. also Figure 2-3). The sample has to cover the window completely. Do not attempt to measure extremely thick parts, such as the veins of a leaf.
- (4) Close the measuring head with your fingers. Hold it closed until a beep sounds and the measured value appears on the display. The measurement will automatically be stored in the memory.
- (5) Execute several measurements and press “Average”. If you want to remove a certain measurement from the memory before averaging, use the “DATA RECALL” button or the “1 DATA DELETE” button if you want to delete just the last measurement



Figure 2-3: Example for measuring with SPAD-502plus.

Further descriptions about the SPAD-502plus can be found in the product manual (Spectrum Technologies, 2011).

### 2.3 Sampling Strategy

First of all, measurements with the SPAD-502plus chlorophyll meter require no special environmental conditions and can be taken at any time, in any weather and at any developmental stage of plants. The overall objective of the sampling strategy is to represent the heterogeneity adequately within the Elementary Sampling Unit (ESU) (Martinez et al., 2009). The area of the ESU corresponds to the spatial

resolution of the remote sensing sensor and must already be clearly defined in the process of preparation of each field campaign. Since distinct heterogeneities often exist within a single plant, several measurements are indispensable to obtain representative values of chlorophyll content. The sampling strategy applied with the SPAD-502plus chlorophyll meter at the Department of Geography at the Ludwig-Maximilian-University Munich is the followed:

| Sampling strategy  |  |
|--|--|
| <ul style="list-style-type: none"> <li>Overall, measurements on five to ten leaves per plant are realized.</li> </ul>  | <ul style="list-style-type: none"> <li>When selecting sample leaves, do pay attention that leaves be fully intact, clean, dry, green and free of signs of disease or damage.</li> </ul>                            |
| <ul style="list-style-type: none"> <li>The selection of leaves shall be representative with respect to the heterogeneities within each sample point. Leaves at the top of the canopy usually contain more chlorophyll than those near the ground.</li> </ul> | <ul style="list-style-type: none"> <li>Special parts of the leaf, like the midrib, major veins or particularly thick parts, should not be placed in the measuring chamber (Spectrum Technologies, 2011)</li> </ul> |
| <ul style="list-style-type: none"> <li>The number of individual measurements to be averaged per leaf depends on its size and uniformity. A minimum of five measurements is recommended.</li> </ul>   |  |

The average of all leaf measurements at one plant represents one sampling point. To ensure the right sampling position within the ESU is kept, the position is located in the field using a handheld GPS receiver.

The average of all sampling points within the ESU represents the average SPAD-Value within the corresponding sampling unit. A general pattern for the positioning of sample points is represented in Figure 2-4. The number of measurements in each field should be regarded as a compromise between all requirements of representativeness and available time and staff resources.

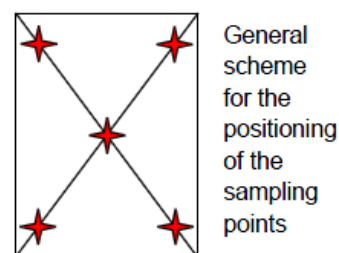


Figure 2-4: GPS positioning of sampling points (left) and general pattern for the positioning of sampling points within a test field (right).

It should be remembered that the memory can only store up to 30 measurements and they are lost when the instrument is switched off; so it is inevitable to record the measuring results on field sheets.

## 2.4 Sources of Errors and Uncertainties

It is important to check regularly whether the measuring chamber is clean and the rubber seal surrounding the chamber is intact and clean. Otherwise light may leak into the chamber causing incorrect readings.

As described in Chapter 2.3, the choice of suitable leaves is important to obtain reliable measurements. Environmental factors have no impact on measuring quality; only the sample leaves have to be dry. To avoid sunlight affecting the measurement, do not expose the instrument to direct sunlight during measurement.

In the following several errors, their possible reasons and solutions are described:

|                 |  |
|-----------------|--|
| <b>Problem</b>  | <b>Device is ON, but display is blank</b>  |
| <b>Reason</b>   | Batteries are dead or incorrectly installed  |
| <b>Solution</b> | <ul style="list-style-type: none"> <li>▪ Insert (new) batteries correctly</li> </ul> |

|                 |   |
|-----------------|---|
| <b>Problem</b>  | <b>Measurements cannot be taken even if measuring head is closed</b>    |
| <b>Reason</b>   | Calibration process has been skipped                                    |
| <b>Solution</b> | <ul style="list-style-type: none"> <li>▪ Perform calibration</li> </ul> |

|                 |  |
|-----------------|--|
| <b>Problem</b>  | <b>Measured values disappear from memory</b>   |
| <b>Reason</b>   | Memory is full or device had been switched OFF   |
| <b>Solution</b> | <ul style="list-style-type: none"> <li>▪ Only 30 measurements can be stored at a time. Memory is erased when the power switch is turned to OFF.</li> </ul> |

|                 |   |
|-----------------|---|
| <b>Problem</b>  | <b>The measured value varies even though the same area of the leaf is measured</b>  |
| <b>Reason</b>   | Measuring window is dirty or wet  |
| <b>Solution</b> | <ul style="list-style-type: none"> <li>▪ Clean measuring window and repeat calibration</li> </ul>   |
| <b>Reason</b>   | Sample leaf is not in correct position  |
| <b>Solution</b> | <ul style="list-style-type: none"> <li>▪ Position of the sample has to be under the center line and completely covering the receiving window</li> </ul>         |
| <b>Reason</b>   | Sample leaf has many veins  |
| <b>Solution</b> | <ul style="list-style-type: none"> <li>▪ Measure several areas of the leaf and use the average value</li> </ul>   |
| <b>Reason</b>   | Measuring head has not been closed correctly or opened too early  |
| <b>Solution</b> | <ul style="list-style-type: none"> <li>▪ Close measuring head correctly and hold it completely closed until measurement is complete (one beep sound)</li> </ul> |
| <b>Reason</b>   | Measuring under direct sunlight   |
| <b>Solution</b> | <ul style="list-style-type: none"> <li>▪ Shade the SPAD with your body</li> </ul>   |

## 3 Data Elaboration

### 3.1 Required Software

FieldScout software is used to communicate with the data logging SPAD meter. The software allows the user to download the meter as well as to select GPS options for data collection. The software requires a serial port to communicate with the meter. Newest additional equipment is a built-in data logger. This equipment allows compiling readings for statistical analysis and the communication with a PC or portable GPS receiver. So you can geo-reference the measurements and download the data into mapping software.

#### Downloading data to PC with FieldScout

- (1) Connect the cable to the computer and into the meter.
- (2) Turn the meter on. For 10 seconds, the meter will be in *COMMUNICATION MODE* and the green LED flashes. Press the *DOWNLOAD* button. If the meter receives data while in *COMMUNICATION MODE*, it will remain in that mode until the meter is turned off. If the meter is not contacted within 10 seconds, it will proceed to *MEASUREMENT MODE* (indicated by a steadily glowing LED). Turn the meter on and off to return to *COMMUNICATION MODE*.
- (3) After the data has been downloaded, the software will prompt you to save the file. Name the file and select the location where you want to save it (the default location is the FieldScout directory). The software will then give you the option of clearing the internal data logger. Select the appropriate response. When the download is complete, the meter can be disconnected from the cable.

### 3.2 Correction Methods

The SPAD-502 output represents leaf chlorophyll content, expressed in arbitrary units. They should resemble absolute concentrations of Chl in  $\mu\text{g}/\text{cm}^2$ , but may not easily be treated as such. Every SPAD instrument is equipped with an individual calibration filter so that two devices might return different values when sampling the same leaf (Markwell et al., 1995). This even applies to working with two devices of the same model type. For that reason, a calibration is recommended in order to calculate Chl contents from intern SPAD values prior to any interpretation. Many authors have approached this problem by setting up regression curves of different mathematical correlations. A selection of these is shown in Table 3-1.



**Table 3-1: Different calculation methods for the leaf chlorophyll content as a function of intern SPAD values.**

| Author                 | Type   | Crop  | R <sup>2</sup> | Unit                   | Regression formula (Chl = ...)             |
|------------------------|--------|-------|----------------|------------------------|--|
| Monje & Bugbee (1992)  | Polyn. | All   | 0.97           | [µg/cm <sup>2</sup> ]  | $1.034 + 0.308 \cdot SV + 0.11 \cdot SV^2$ |
| Markwell et al. (1995) | Polyn. | All   | 0.96           | [µmol/m <sup>2</sup> ] | $10.6 + 7.39 \cdot SV + 0.114 \cdot SV^2$  |
|                        | Expon. | All   | 0.94           | [µmol/m <sup>2</sup> ] | $10^{SV^{0.265}}$                          |
| Uddling et al. (2005)  | Expon. | All   | 0.84           | [µg/cm <sup>2</sup> ]  | $6.91 \cdot e^{0.0459 \cdot SV}$           |
|                        | Expon. | Wheat | 0.89           | [µg/cm <sup>2</sup> ]  | $5.99 \cdot e^{0.0493 \cdot SV}$           |

Although these statistical correlations appear to be quite high in every case, they do lead to an estimation of *different* Chl contents for the *same* SPAD values (as shown in Figure 3-1). The only plausible reason for that is an application restriction of the literature empirical formulas to the sample they were derived from.

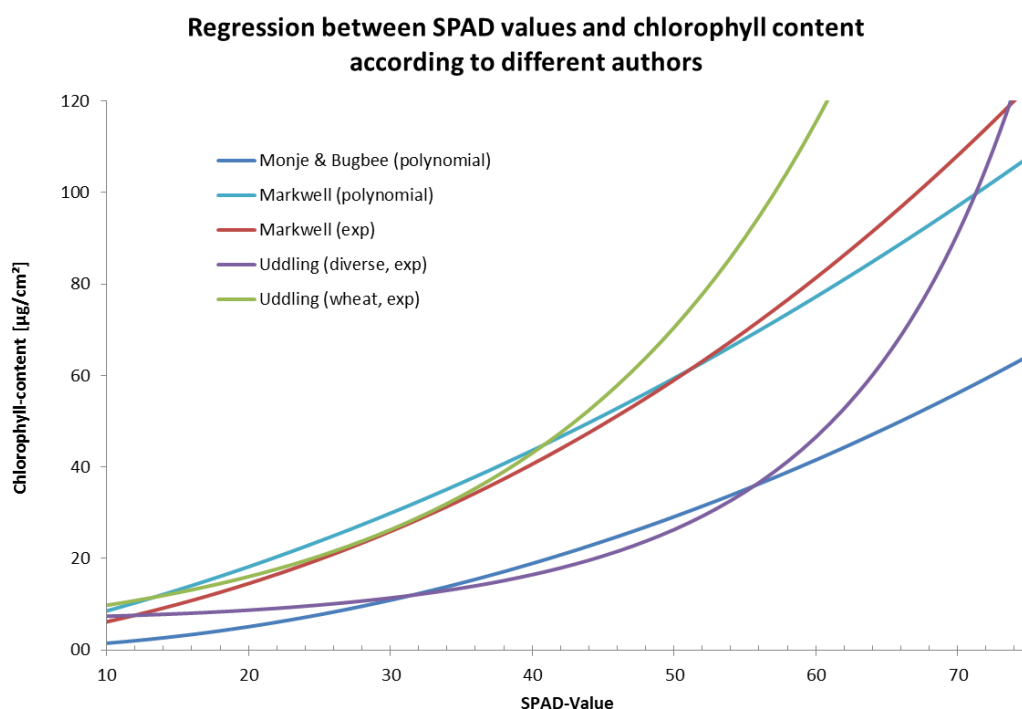


Figure 3-1: Illustration of the correlation between measured SPAD values and the ones resulting from different calculation methods as examples.

Consequently, in order to obtain realistic data from your SPAD-502, it may be a good choice to calibrate it by in vitro determination of chlorophyll content. Laboratory data will reveal actual concentrations of chlorophyll which then need to be referred to corresponding SPAD values. The more pairs of values established over a wide range of vital and senescent leaves, the more accurate the resulting regression function will be. It is recommended to find such relations for each plant species individually. It is of course possible to perform a complete field campaign with one SPAD instrument first and process these data further at a later date, when the function is set and validated.

Such a calibration campaign was conducted in 2014 at the LMU München as part of the preparation for an exhaustive field campaign in the EnMAP context (Gessner, 2014). 25 leaves of winter wheat plants were sampled, covering a range between SPAD = 18 and 52. The testing of different types of mathematical relationships between in vivo and in vitro measurements delivered the results of Table 3-2.

**Table 3-2: Overview of the results for the SPAD calibration campaign for winter wheat.**

| <b>Function type</b>         | <b>Regression formula</b>    | <b>R<sup>2</sup></b> |
|------------------------------|------------------------------|----------------------|
| <i>Linear with intercept</i> | $0.82 \cdot SV + 6.87$       | <i>0.54</i>          |
| <i>Linear through origin</i> | $0.98 \cdot SV$              | <i>0.52</i>          |
| <i>Logarithmic</i>           | $27.66 \cdot \ln SV - 60.76$ | <i>0.57</i>          |
| <i>Exponential</i>           | $12.23 \cdot e^{0.0279 SV}$  | <i>0.61</i>          |

Although correlations were lower in comparison to the studies listed in Table 3-1, they fitted better for this experimental data set. The statistical results could be further improved up to  $R^2 = 0.84$  by eliminating three outliers. After all, the linear model with intercept = 0.0 can be approximated by a simple identity function. This means that in this case SPAD values could immediately be interpreted as chlorophyll concentrations in  $\mu\text{g}/\text{cm}^2$ .

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