LEAF-STATE-ANALYZER
LSA-2050

Manual

2.179/11.2023
1. Edition: February 7, 2024
LSA_2050_01.docx

Heinz Walz GmbH · Eichenring 6 · 91090 Effeltrich · Germany
✆ +49 9133 7765-0 · 🖷 +49 9133 5395
✉ info@walz.com · 🕸 www.walz.com

CONTENTS

1 Safety Instructions

1.1 General Safety Instructions

- Read and follow safety and operating instructions prior to operation of the device. Pay attention to all safety warnings.
- The LEAF-STATE-ANALYZER 2050 (LSA-2050) is designed for outdoor use. However, avoid exposing the LSA-2050 to dust, sand, and dirt as much as possible.
- Avoid taking measurements in precipitation. The device is not waterproof.
- Ensure that neither liquids nor foreign bodies get inside the LSA-2050.
- Do not put the LSA-2050 near sources of heat.
- The LSA-2050 must not be dropped. Open battery compartment only in dry and clean environment.
- Use only type AAA (Micro) batteries.
- Keep USB-C socket clean.
- Do not open the housing of the LSA-2050. There are no serviceable parts inside. The device may only be repaired by the manufacturer.

1.2 Special Safety Instructions

- The optical components of the upper and lower leaf clamps are covered with a fragile quartz disc. Avoid exerting any force on these quartz discs.
- Do not measure moist or wet samples. Wipe samples dry before measuring.
- When operating with the lower part removed, cover open electric contacts with the special electronics lid (see Fig. 4, page 8).
- The LSA-2050 is a highly sensitive measuring system which should be only used for research purposes, as specified in this manual. Follow the instructions of this manual to avoid potential harm to the user and damage to the instrument.
- The LSA-2050 can emit very strong light! To avoid harm to your eyes, never look directly at the LEDs of the sample clip.

2 Introduction

The LEAF-STATE-ANALYZER LSA-2050 is a handheld device for non-invasive leaf analysis. The measuring device employs three different approaches to probe the plant health status: (1) the extent of protection from ultraviolet and strong visible radiation, (2) the chlorophyll concentration, and (3) the maximum photochemical quantum yield of photosystem II, F_V/F_M. In summary, the LEAF-STATE-ANALYZER LSA-2050 provides a picture of stress effects and a plant's ability to cope with stress.

General Features

The LEAF-STATE-ANALYZER LSA-2050 measures radiation screening by the efficiency of fluorescence excitation. The four different excitation wavebands employed can be related to four pigment groups: UV-B and UV-A to hydroxycinnamic acids and flavonoids, respectively [1], blue to carotenoids [2], and green to anthocyanins [3]. Absorbance values indicating relative flavonoid and anthocyanin concentration are provided.

Chlorophyll concentration is measured by the Cerovic method [4]. The method excels by high response even at high chlorophyll concentrations.

Photosystem II is analyzed by the well-proven PAM fluorescence/saturation pulse method [5].

With each measurement, GPS data, leaf orientation, and the direction of sun radiation are logged.

[1] Bilger W, Veit M, Schreiber L, Schreiber U (1997) Measurement of leaf epidermal transmission of UV radiation by chlorophyll fluorescence. Physiol Plant 101: 754–763. https://doi.org/10.1111/j.1399-3054.1997.tb01060.x

[2] Nichelmann L, Schulze M, Herppich WB, Bilger W (2016) A simple indicator for non-destructive estimation of the violaxanthin cycle pigment content in leaves. Photosynth Res 128: 183-193. https://doi.org/10.1007/s11120-016-0218-1

[3] Cerovic ZG, Moise N, Agati G, Latouche G, Ben Ghozlen N, Meyer S (2008) New portable optical sensors for the assessment of winegrape phenolic maturity based on berry fluorescence. J Food Compos Anal 21: 650-654. https://doi.org/10.1016/j.jfca.2008.03.012

[4] Cerovic ZG, Masdoumier G, Ben Ghozlen N, Latouche G (2012) A new optical leaf-clip meter for simultaneous non-destructive assessment of leaf chlorophyll and epidermal flavonoids. Physiol Plant 146: 251–260. https://doi.org/10.1111%2Fj.1399-3054.2012.01639.x

[5] Schreiber U, Schliwa U, Bilger W (1986) Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. Photosynth Res 10: 51-62. https://doi.org/10.1007/bf00024185

3 LSA-2050: Extent of Delivery

Item	Order Number
LEAF-STATE-ANALYZER	LSA-2050
Four-position battery charger	000190101116
2 x 4 eneloop AAA rechargeable batteries	000160101989
Protection platelet (covers electrical contacts when operated without the lower clip jaw)	000247001714
Fluorescence standard	000240315214
Black standard for offset determination	000247001814
Allen wrench 2.5 mm	000160201201
USB C to USB A Cable	000130606258
Carrying Case	LSA-2050/T
Software LSA-2050 on USB flash drive	
LSA-2050 Manual	

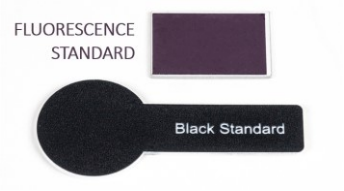


Fig. 1: Fluorescence and Offset Standards

3.1 LSA-2050 Description

The tip of the LSA-2050 LEAF-STATE-ANALYZER is formed by a bipartite measuring head (Fig. 2). The heads form a clip in which a sample or standard (Fig. 1) are placed. The clip can be opened by pressing the lever on the bottom side of the device (Fig. 4).

The upper “Emitter Detector Head” holds the photodiode detector, and 5 LEDs emitting in the UV or the visible range (Fig. 3). A far red and a near infrared LED is situated in the lower “FR, NIR Emitter”. The emission by these LEDs has to pass the sample to reach the photodiode. The sample’s chlorophyll concentration is derived from the beam attenuation by the sample.

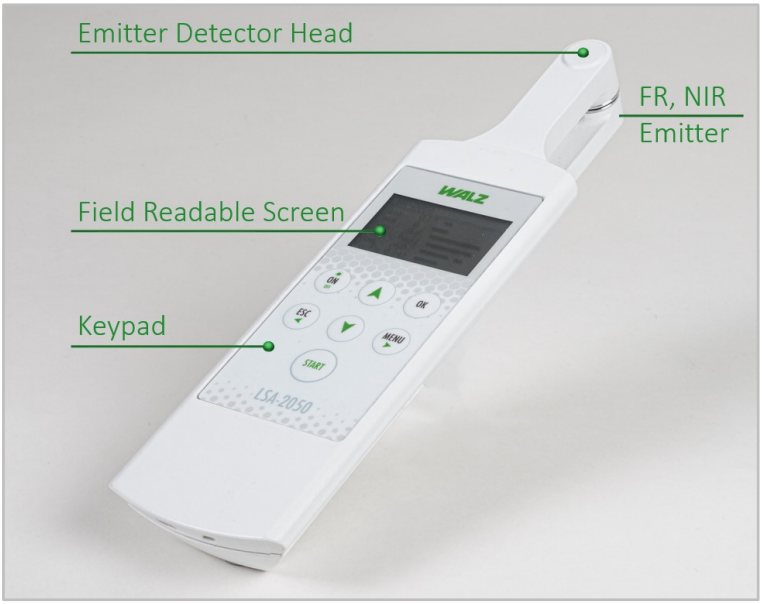


Fig. 2: LSA-2050 Overview

The emitter detector head excites and detects fluorescence from the same side. The sample properties investigated are the state of photosystem II and screening of ultraviolet and visible radiation.

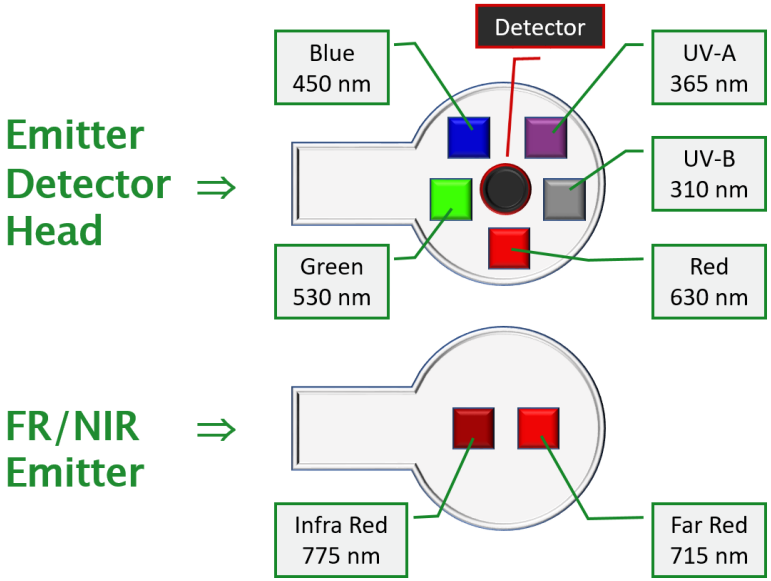


Fig. 3: LSA-2050 Measuring Head

To examine bulky samples (e.g. fruits), the FR, NIR Emitter can be removed (Fig. 4). It is obvious that chlorophyll concentrations cannot be measured with this configuration. However, it is possible to use the F_0 fluorescence as a measure for changes in the concentration of chlorophyll.

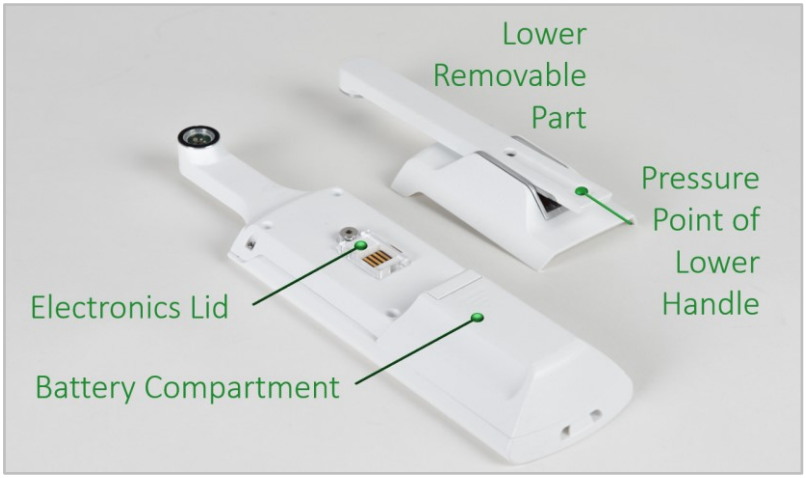


Fig. 4: LSA-2050 Modified for Bulky Samples

4 Accessory

4.1 Darkening Bags LSA-2050/DB

This accessory is designed for darkening of leaves in the field. Dark-acclimation is prerequisite to measure the maximum photosystem II quantum yield, F_V/F_M. The bags consist of light-tight material. Chlorophyll concentration can be determined through a central hole. The bags are available in three sizes. For details see Section 7.4.1, page 49.

A

B

Fig. 5: Darkening Bags LSA-2050/DB

A: Darkening bags are available in three sizes. The LSA-2050/DB represents a set of bags, and includes three bags of each size. **B**: Each bag has a central hole. For dark-acclimation, the hole is covered on both sides by flaps.

5 Operation

The LSA-2050 is controlled by 7 keys. The function of these keys is outlined in Fig. 6. The symbols on the keys and the instructions in the software make the operation of the unit self-explanatory.

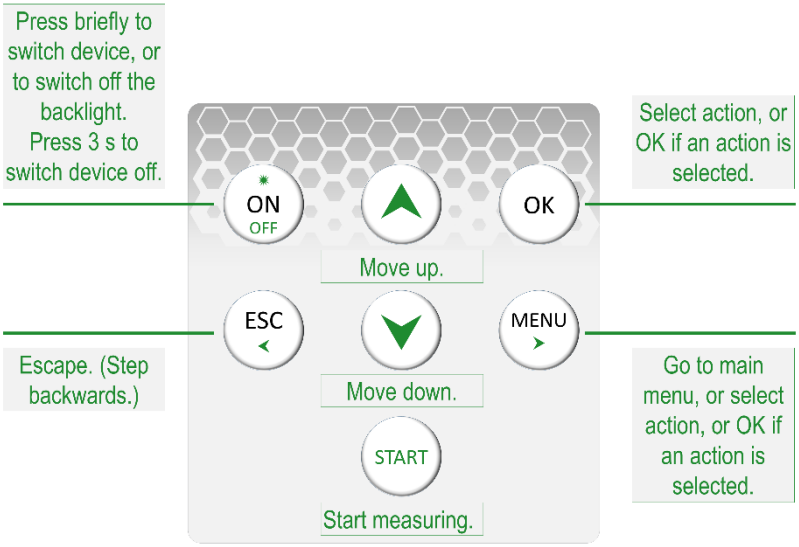


Fig. 6: Keys

The button START is only active when the window “LSA-2050” or “SAT Pulse Data” is visible.

5.1 Quick Start

- Switch ON.
- Check UTC Offset Time. (MENU→Settings→Device Settings (see Table 10, page 17).
- Click MENU, select “Calibration” and perform all calibration procedures.
- Click ESC twice and start measuring.

Note: Default settings apply unless changed by the user. Defaults settings are: (1) All tests active, (2) chlorophyll concentration is calculated with the calibration curve for C_3 leaves (Table 19, page 34) and it is given in nm/cm^2, and (3) the mesophyll reference is from upper leave sides of *Hylotelephium telephium* (Fig. 15, page 38).

5.2 General Instructions

5.2.1 Main Windows

After system start, LSA-2050 window appears (Table 1). The window displays the apparent transmittance at the four different wavelengths of excitation light, the chlorophyll concentration, and the maximum photochemical yield of photosystem II, F_V/F_M. See Section 5.3 (page 26) for comments on these data.

The device status is indicated by symbols in the top right corner of the LSA-2050 window. The same information is given in the other two main windows: “Sat Pulse Data” and “Geospatial Data” (Table 3 and Table 4, respectively). The meaning of the status symbols is explained in Table 2 (page13). Note that measurements can only be started from the three main windows.

Table 1: LSA-2050 Window		
LSA-2050 Window		
T_{310}	0.17	
T_{365}	0.58	
T_{450}	0.98	
T_{530}	0.96	
C_{CHL}	47.1	
F_V/F_M	0.762	

Table 2: Status Indicators	
	Battery charge status. From left to right, full to low charge. Flashing, change batteries.
	LED Status. Left, visible ON. Right, UV ON.
	GPS status. Left, ON. Right, location determined.

The F_V/F_M value is also shown in the Sat Pulse Data window together with F_0 and F_M fluorescence levels ($F_V=F_M-F_0$), and the fluorescence transient induced by the saturation pulse.

Table 3: Sat Pulse Data		
Sat Pulse Data		
F_V/F_M	0.762	
F_0	0.58	
F_M	0.98	
2023-09-18	17:41:43	

The window Geodata provides longitude and latitude (first line, number followed by W or E, and number followed by N or S, respectively). The position is calculated from signals from GPS satellites which are received by the on-board GPS receiver. The number of satellites that are picked up is indicated in line 2 (#Sat). The second line also displays the height of the current

position in m. From position and world time (UTC), the current sun azimuth (Sun Az.) and sun elevation (sun El.) is derived.

Leaf Azimut (Leaf Az.) and Leaf Slope (Leaf Sl.) are determined using gravity, magnetic field, acceleration and rotation data from the LSA-2050 instrument.

By combining the information on sun and leaf position, the angle of incidence is derived (A. o. I.). This number describes the angle at which sunlight strikes the leaf surface. The cosine of the angle of incidence is called surface incidence (Inc.). The surface incidence is of ecophylsiological relevance because the relative intensity of sun radiation at the leaf surface is porportional to this value.

Chapter 6.3 (page 41) summarizes all hardware involved in geodata acquisition. The same chapter explains the terminology used in geodata.

Table 4: Geo Data

Geo Data			
12.345678 E		12.345678 N	
Height	400	#Sat	15
Leaf Az.		Sun Az.	135
Leaf Sl.	45	Sun El.	60
A. o. I.	60	Inc.	50
2023-09-18		17:41:43	

5.2.2 Main Menu

The Main Menu (s) can be accessed from any of the three main windows by pressing the MENU button.

Table 5: Main Menu	
Main Menu	
Settings	→
Calibration	→
Create New File	→
Memory	→
Device Info	→

5.2.2.1 Settings

The first item of the main menu leads to the menu Settings (Table 6). In any of the submenus of Settings, choose item by up and down keys. Then make selection by the pressing the OK key.

Table 6: Settings	
Settings	
Active Tests	→
Chl. Settings	→
Mesophyll Type	→
Device Settings	→

In Settings, the item “Active Tests” permits deselecting test procedures (Table 7).

Notes to Geodata.

- To generally save energy, or when battery power is low, switch off geodata. Geodata sensors consume a lot of energy.
- At the time of printing this manual, export of geodata is not implemented in the LSA-2050 software.

Table 7: Active Tests	
Active Tests	
Chl. Concentration	on/off
F_V/F_M	on/off
Screening	on/off
Geo Data	on/off

The item Chl. Settings allows changing the unit of chlorophyll concentration, and the calibration curve used to calculate chlorophyll concentrations (see Section 6.1, page 31). The selection of the unit for chlorophyll concentration here does not affect the exported data: both nmol/cm^2 and µg/cm^2 are provided. Changing the calibration curve puts the next measurement in a new file.

Table 8: Chl. Settings	
Chl. Settings	
nmol/cm^2	X
µmol/cm^2	
C_3 Type	X
C_4 Type	

The mesophyll reference used for radiation screening evaluations can be selected in the Mesophyll Type item (see 6.2, page 35). As in the case of calibration curves, changing the calibration curve creates a new file.

Table 9: Mesophyll Type	
Mesophyll Type	
Sedum upper side	X
Sedum lower side	
Tulipa upper side	
Kalanchoe upper side	

Device Settings configures the idle time after which the device is automatically switched off. You can also turn off the beep that accompanies keystrokes.

The UTC offset is the difference (hours:minutes) between Co-ordinated Universal Time (UTC) and your local time. A list of UTC offset values is provided by Wikipedia: https://en.wikipedia.org/wiki/List_of_UTC_offsets. Enter the correct UTC offset to set the LSA-2050 to your local time.

Table 10: Device Settings	
Device Settings	
Auto Off	15:00
Beeper	on/off
UTC Offset	on/off
Load Defaults	→

The default settings, which are activated by the command „Load Default" are outline in Section 5.1 (page 12).

5.2.2.2 Calibration

Prior to measurements, three calibration procedures must be performed: Offset Values, Absorbance, and Fluorescence (Table 11). The fourth calibration (Orient. Sensor) is not needed for leaf analysis but for information on sun exposure of the leaf.

Offset Values determine the background signal of the device, Absorbance calibration measures the intensity of the FR and NIR LEDs without sample. Fluorescence calibration determines the signal with the Walz fluorescence standard (see Section 6.2, page 35). All calibration values are documented in the exported data (Section 5.2.3, page 20).

Table 11: Calibration

Calibration	
Offset Values	→
Absorbance	→
Fluorescence	→
Orient. Sensor	

Sensors involved in determining the leaf position are calibrated in the window “Orient Sens. Calibration” (Table 12). Three orientation sensors are calibrated. The sensors and the meaning of the displayed numbers (Heading, Pitch, Role) are introduced in Section 6.3 (page 41).

Table 12: Orient. Sens. Calibration

Orient. Sens. Calibration		
Gyro	■■■■■	Heading
Accel	■	Pitch
Mag	■	Role
	Instruction text.	

To calibrate orientation sensors, follow the instructions displayed in the lower part of the window. Calibration of the gyroscope occurs first. Horizontal bars indicate the progress of calibration. Calibration switches automatically from Gyroscope to accelerometer. In most cases, accelerometer calibrates the

magnetometer simultaneously. When asked to describe the shape of the number 8, do so with the device tilted.

5.2.2.3 Create New File

The command Create New File will be executed with the next measurement.

5.2.2.4 Memory

In the Memory window, you can scroll through stored measurements by the up and down key. Within a measurement, the fluorescence transients can be accessed by the MENU key. To leave the Memory window, press ESC.

5.2.2.5 Device Info

The window LSA-2050 Info reports the charge status of the battery. When connected to a computer, the charge status indicates 75%. The device and software specific information is needed when reporting errors to the Walz staff.

Table 13:	**LSA-2050 Info**
LSA-2050 Info	
Battery: 80% S/N: LSAN0101 Firmware: 22/2271 Built 24-01-25 23:21:12 Prof: 50-10-20 05:00:21	

5.2.3 Downloading Data

Install the LSA software (e.g, LSA-2050-v1.0-Installer.exe) located on the Walz USB flash by drive double clicking on the file. You can also copy the LSA installer to your computer and install it from there.

The software installation window opens (Fig. 7). Read the instructions carefully and select the appropriate option for your computer configuration. Click Install. A link to the LSA software is added to the program list in the Windows Start menu. Depending on choice, an LSA desktop icon was generated.

Check the Walz website regularly for software updates!

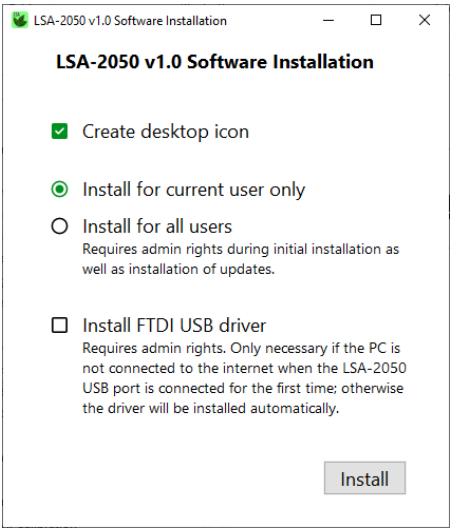


Fig. 7: Installation Window

To download data, remove cover of battery compartment (see Fig. 8). Connect LSA-2050 and computer using the USB-C to USB-A cable. Execute the LSA software from the LSA desktop icon (double click) or from the program list in the Start menu (single click). The download window that appears will list all the files that are stored on the LSA-2050 (Fig. 9, page 22).

Fig. 8: Access to USB-C Port

In the download window, left-click once on the file to be downloaded. This action open a file dialogue box. Choose file name and directory, and click Save. A green checkmark indicates that the file has been successfully downloaded, that is, an Excel file has been created. A red cross indicates a download error.

The Excel file contains two sheets called “Measure” (Fig. 10, page 23) and “SAT Chart” (Fig. 11, Page 24). The initial view of the sheet “Measure” displays the sample properties. Table 14 (page 25) explains the abbreviations used for the sample properties. The SAT Chart sheet contains the fluorescence transients from which the F_0 and F_M levels are derived (Fig. 11, page 24). Instructions on how to graph this data are given in the figure legend.

The sheet “Measure” also contains the data used for the calculations of the sample properties. The legend of Fig. 10 elucidates how these data can be displayed. The data on which the calculations are based comprise the original signal levels, the offset values and various calibration data (see Table 15, page 25; Table 16, page 26).

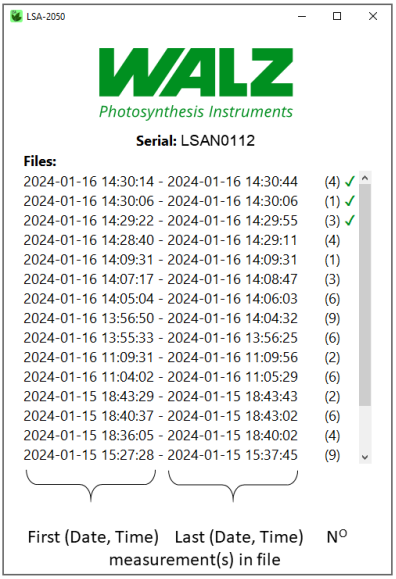


Fig. 9: LSA Download Window

	A	B	C	R	S	T	U	V	W	X	Y	AD	AE	AF
1	**Date**	**Time**	**Type**	**F_V/F_M**	**MesRef**	**Q_{310}**	**Q_{365}**	**Q_{450}**	**Q_{530}**	**A_{FLAV}**	**A_{ANTH}**	**Model**	**nmol/cm²**	**µg/cm²**
2			Offset									C3 Coeff Q	328	294
3			Reference		Sedum telephium	0.530	1.053	1.015	0.818			C3 Coeff L	195	175
4	24-01-16	13:30:31	Sample	0.648		0.177	0.602	0.974	0.990	0.220	0.004		230.61	206.40
5	24-01-16	13:30:38	Sample	0.583		0.159	0.576	0.923	0.998	0.240	0.001		219.62	196.56
6	24-01-16	13:30:44	Sample	0.560		0.156	0.573	0.916	0.999	0.242	0.001		219.74	196.67
7	24-01-17	09:30:46	Sample	0.715		0.181	0.585	1.017	0.988	0.233	0.005		220.19	197.07
8	24-01-17	09:31:13	Sample	0.646		0.161	0.574	0.991	0.979	0.241	0.009		212.48	190.17
9	24-01-17	09:31:22	Sample	0.598		0.148	0.560	0.954	0.973	0.252	0.012		205.04	183.51
10	24-01-17	09:31:29	Sample	0.584		0.151	0.556	0.947	0.977	0.255	0.010		204.50	183.02
11	24-01-17	09:31:40	Sample	0.606		0.150	0.552	0.962	0.968	0.258	0.014		193.13	172.85
12	24-01-17	09:31:48	Sample	0.586		0.149	0.551	0.955	0.963	0.259	0.016		192.06	171.90

Fig. 10: Measure Chart

“ Measure Chart “ created by the LSA software. Green-bordered buttons give access to original data. Click on the [+] above column R to display the data used for calculation of screening and F_V/F_M. Click on the [+] above column AD to see the data used for calculation of chlorophyll concentration. Click [2] in the top left corner to see all date. Click [1] in the top left corner to return to the original view. For details see Table 14, Table 15, and Table 16.

	A	B	C	D	E	F	G	H	I	J	K
1	**Date**	**Time**		0 ms	50 ms	100 ms	150 ms	200 ms	250 ms	300 ms	350 ms
2											
3											
4	24-01-16	13:30:31		471	471	471	471	932	1300	1374	1398
5	24-01-16	13:30:38		571	571	571	571	1229	1422	1423	1427
6	24-01-16	13:30:44		599	599	599	599	1230	1405	1402	1408
7	24-01-17	09:30:46		425	425	425	425	879	1493	1556	1572
8	24-01-17	09:31:13		550	550	550	550	1365	1596	1600	1607
9	24-01-17	09:31:22		633	633	633	633	1399	1606	1611	1614
10	24-01-17	09:31:29		655	655	655	655	1397	1605	1614	1616
11	24-01-17	09:31:40		618	618	618	618	1382	1596	1601	1609
12	24-01-17	09:31:48		649	649	649	649	1383	1595	1598	1608

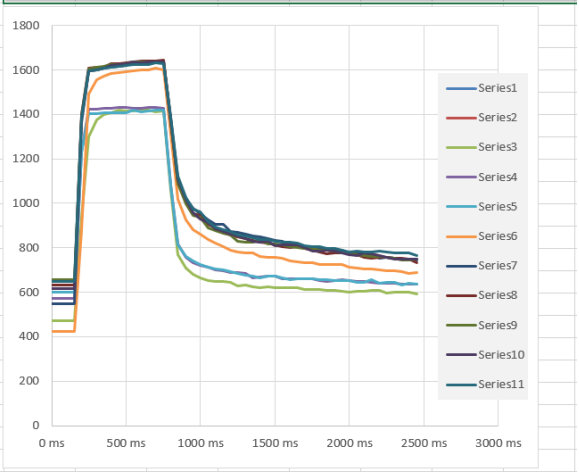


Fig. 11: SAT Chart

The “SAT Chart” contains the data of fluorescence transients of F_V/F_M determinations. To graph the data, select time (row 1) and fluorescence data (row 4 to row n) from column D to column BA. The entire time row is conveniently selected by a left-click in the first time cell (D1), followed by first simultaneously pressing Shift + Ctrl and then the right arrow key. Fluorescence data can be successively added by pressing the Shift key and then the down arrow key. All data can be selected by left click in cell D1, holding down the Shift + Ctrl, followed by pressing the right arrow key and the down arrow key. After data selection, go to window “Insert”, and pick “insert scatter (x, y) graph” in the section “Charts”..

Table 14: Results		
Column	**Title**	**Comment**
R	**F_V/F_M**	Maximum photochemical quantum yield of photosystem II.
T U V W	**Q_{310}** **Q_{365}** **Q_{450}** **Q_{530}**	Apparent UV-B, UV-A, blue light, and green light screening, respectively. (Fluorescence excited at 310 nm, 365 nm, 450 nm, and 530 nm, respectively, relative to fluorescence excited at 630 nm.)
X	**A_{FLAV}**	The A_{FLAV} (absorbance by flavonoids) is derived from Q_{365} and can be considered as proportional to the concentration of flavonoids involved in UV-A screening.
Y	**A_{ANTH}**	The A_{ANTH} (absorbance by anthocyanins) is derived from Q_{530} and can be considered as proportional to the concentration of anthocyanins involved in green light screening.
AE	**nmol/cm²**	Concentration of Chl *a* + Chl *b*
AF	**µg/cm²**	Concentration of Chl *a* + Chl *b*

Table 15: Calibration Data			
Row	**Title**		**Comment**
2	**I_{310}** **I_{365}** **I_{450}** **I_{530}** **I_{615}**	**I_{Fo}** **I_{Fm}** **I_{700}** **I_{770}**	Background signal (Offset) for all LEDs and methods. System property determined by calibration.
3	**I_{310}** **I_{365}** **I_{450}** **I_{530}** **I_{615}**	**F_{310}** **F_{365}** **F_{450}** **F_{530}** **F_{615}**	I_λ: Signal at wavelength λ measured with the fluorescence standard. F_λ: I_λ corrected by the background signal.
3	**Q_{310}** **Q_{365}** **Q_{450}** **Q_{530}**		Mesophyll reference factors (see 6.2.1, page 38).

Table 15:		Calibration Data
2 and 3	**nmol/cm²** **µg/cm²**	Coefficients for calculating the molar and weight concentrations of Chl *a* + Chl *b* (see Section 6.1.4, page 34). In column AD, C3 and C4 indicate which calibration curve was used to calculate concentrations; Coeff Q is the coefficient of the quadratic term, and Coeff L is the coefficient of the linear term of the calibration curve (See Section 6.1, page 31).

Table 16: Source Data Data from row 4 onwards	
Title	**Comment**
I_{Fo} F_o I_{Fm} F_m	I_{Fo}, raw fluorescence signal of the dark leaf. I_{Fm}, raw fluorescence signal of the leaf exposed to a strong (saturating) light pulse. F_o and F_m are the corresponding signals after offset correction. F_V/F_M in Table 14 (page 25) equals 1- F_o/F_m.
I_{310} F_{310} I_{365} F_{365} I_{450} F_{450} I_{530} F_{530} I_{615} F_{615}	I_λ: Signal at wavelength λ measured with the leaf. F_λ: I_λ corrected by the background signal.
I_{700} I_{770}	Signal induced by radiation transmitted by the leaf at 700 nm and 770 nm, respectively.
Transmittance	I_{700}/I_{770} of the sample normalized to I_{700}/I_{770} of calibration. All data were offset-corrected prior to calculation.
Absorbance	$-\log_{10}$ Transmittance.

5.3 Comments on Results

This section shortly treats meaning and interpretation of the parameters obtained with the LSA-2050 LEAF-STATE-ANALYZER. Cited literature is listed right after the text.

5.3.1 F_V/F_M

Using low temperature (77 K) fluorescence technique, Kitajima and Butler (1975) have introduced the fluorescence ratio of F_V/F_M as a measure of the maximum yield for primary photochemistry of photosystem II. With the same technique, Björkman and Demmig (1987) have determined this value to be around 0.83 in many species with C_3 photochemistry.

This finding has been confirmed by PAM fluorescence, and it has additionally been shown that the F_V/F_M of plants having C_4 NADP-ME photochemistry (e.g. maize) can be as low as 0.76 (Pfündel 1998, Pfündel et al. 2013).

When F_V/F_M values are clearly smaller than the maximum F_V/F_M, photoinhibition of photosystem II is likely (Maxwell and Johnson 2000). This means that a part of the photosystem II in the sample has damaged reaction centers.

For correct F_V/F_M measurements, it is important that leaves are fully dark-acclimated. Depending on species and growth conditions, dark times can range between 10 minutes and many hours. The dark time for F_V/F_M measurements must be determined experimentally.

Björkman O, Demmig B (1987) Photon yield of O2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. Planta 170, 489–504 (1987). https://doi.org/10.1007/BF00402983

Kitajima M, Butler WL (1975) Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone. Biochim Biophys Acta – Bioenergetics 376: 105-115. https://doi.org/10.1016/0005-2728(75)90209-1

Maxwell K, Johnson GN (2000) Chlorophyll fluorescence—a practical guide, *Journal of Experimental Botany* 51: 659–668. https://doi.org/10.1093/jexbot/51.345.659

Pfündel E (1998) Estimating the contribution of Photosystem I to total leaf chlorophyll fluorescence. Photosynthesis Research 56, 185–195. https://doi.org/10.1023/A:1006032804606

Pfündel EE, Klughammer C, Meister A, Cerovic ZG (2013) Deriving fluorometer-specific values of relative PSI fluorescence intensity from quenching of F_0 fluorescence in leaves of *Arabidopsis thaliana* and *Zea mays*. Photosynth Res 114, 189–206. https://doi.org/10.1007/s11120-012-9788-8

5.3.2 Screening

The fundamental articles of radiation-screening substances in the leaf are listed in Section 6.2, page 35. As has been introduced by Bilger et al. (1997), the LSA-2050 outputs fluorescence quotients (Q_λ) as a measure for the extent of screening. The quotient ranges from 0 to 1, where 1 signifies full transparence.

As a rule of thumb, leaves are sufficiently protected against UV-B radiation when the Q_{310} is 0.1 or smaller. Similar efficient screening has been reported for green light screening (Q_{530}) by anthocyanins (Nichelmann and Bilger 2017).

The fluorescence quotients (Q_λ) can be viewed as transmittance values. Transmittance can be converted to absorbance, which ideally is proportional to the concentration of screening compounds (Goulas et al. 2004).

The LSA-2050 converts the quotients Q_{365} and Q_{530} into absorbance data which are supposed to be proportional to the concentration of flavonoids and anthocyanins, respectively. These absorbance values reflect the total concentration in the leaf only if the screening pigments are almost exclusively located in the epidermis which is probed. This requirement seems to be met, e.g., in grapevine but not in barley (Kolb and Pfündel 2005).

Kolb CA, Pfündel EE (2005) Origins of non-linear and dissimilar relationships between epidermal UV absorbance and UV absorbance of extracted phenolics in leaves of grapevine and barley. Plant, Cell & Environment 28: 580-590. https://doi.org/10.1111/j.1365-3040.2005.01302.x

Nichelmann L, Bilger W (2017) Quantification of light screening by anthocyanins in leaves of *Berberis thunbergii*. Planta 246: 1069–1082 (2017). https://doi.org/10.1007

5.3.3 Chlorophyll Concentration

Leaf chlorophyll concentrations respond to nutrient availability and various stress factors including pollution or herbivory (Agathokleous et al. 2020). Relationships between leaf chlorophyll and the leaf nitrogen status have been shown (Evans 1989, Lu et al. 2020, Xiong et al. 2015). That nitrogen fertilization can elevate leaf contents of chlorophyll and nitrogen has been demonstrated (Prsa et al. 2007, Muhammad et al. 2022).

In summary, the leaf chlorophyll concentration can detect stress effects and insufficient nitrogen supply. Because the leaf chlorophyll concentration varies between species, and the relationships between chlorophyll and nitrogen are variable, universal chlorophyll values for healthy plant cannot be given. However, by analyzing comparable plants after different treatments, the measurement of chlorophyll concentration becomes a highly meaningful stress detector.

Agathokleous E, Feng ZZ, Peñuelas J (2020) Chlorophyll hormesis: Are chlorophylls major components of stress biology in higher plants? Sci Total Environ 726, 138637. https://doi.org/10.1016/j.scitotenv.2020.138637

Evans JR (1989) Photosynthesis and nitrogen relationships in leaves of C3 plants. Oecologia 78: 9-19. https://doi.org/10.1007/bf00377192

Lu X, Ju W, Li J, Croft H, Chen JM, Luo Y, Yu H, Hu H (2020). Maximum carboxylation rate estimation with chlorophyll content as a proxy of Rubisco content. J Geophys Res Biogeosci 125: e2020JG005748. https://doi.org/10.1029/2020JG005748

Muhammad I, Yang L, Ahmad S; Farooq S, Al-Ghamdi AA, Khan A, Zeeshan M, Elshikh MS, Abbasi AM, Zhou X-B (2022) Nitrogen fertilizer modulates plant growth, chlorophyll pigments and enzymatic activities under different irrigation regimes. Agronomy 12: 845. https://doi.org/10.3390/agronomy12040845

Prsa I, Stampar F, Vodnik D, Veberic R (2007) Influence of nitrogen on leaf chlorophyll content and photosynthesis of 'Golden Delicious' apple. Acta Agric Scand B Soil Plant Sci 57: 283-289. https://doi.org/10.1080/09064710600982878

Xiong D, Chen J, Yu T, Gao W, Ling X, Li Y, Peng S, Huang J (2015). SPAD-based leaf nitrogen estimation is impacted by environmental factors and crop leaf characteristics. Sci Rep 5: 13389. https://doi.org/10.1038/srep13389

6 Documentation

6.1 Chlorophyll Calibration

6.1.1 Plant Species

Three plant species were investigated (Table 17). The species were chosen to represent monocot (*T. aestivum*, *Z. mays*) and dicot leaf anatomy (*H. anuus*). The species also represent different types of photosynthesis with different Chl *a*/Chl *b* concentration ratios (Table 17).

Table 17: Plant Species used for Chl Calibration

Species	Group	Type of Photosynthesis	Chl *a*/Chl *b*
Common sunflower (*Helianthus annuus*) Asteraceae	Dicot	C_3	Normal
Common wheat (*Triticum aestivum*) Poaceae	Monocot	C_3	Normal
Corn (*Zea mays*) Poaceae	Monocot	C_4 NADP-ME	Elevated

6.1.2 Growth Conditions

Five different growth conditions were used to achieve different chlorophyll contents in leaves (Table 18).

Table 18: Growth Conditions

Treatment	Fertilization*	Additional Lighting**	Location
1	None	Yes	Greenhouse
2	1 g / pot	Yes	Greenhouse
3	3 g / pot	Yes	Greenhouse
4	None	No	Greenhouse
5	3 g / pot	No	Greenhouse→Field***

*40 days after sowing (June 13, 2022): Compound fertilizer ("Blaukorn") added, consisting of 12% N, 8% P_2O_5, 16% K_2O, 3% MgO, 23% SO_3, 0.02% B, 0.06% Fe, 0.01% Zn. Pots: 17.5 cm x 17.5 cm x 17 cm containing 4.5 liter soil (compost : sand : clay = 2:1:1.

**400 W high-pressure sodium vapor lamps mounted 100 cm above the planting table, providing about 250 µmol m^{-2} s^{-1} from 06:00 until 18:00, additionally to natural PPFD.

***42 days after sowing (June 15, 2022): Moving to the field.

6.1.3 Chl measurements

Measurements took place 55 days after sowing (June 28, 2022) in dim light. First, the spots to be measured were marked. Then, attached leaves were sequentially probed with three prototypes of the LSA-2050 using the wavebands introduced by Cerovic et al.:

Cerovic ZG, Masdoumier G, Ben Ghozlen N, Latouche G (2012) A new optical leaf-clip meter for simultaneous non-destructive assessment of leaf chlorophyll and epidermal flavonoids. Physiol Plant 146: 251–260. https://doi.org/10.1111%2Fj.1399-3054.2012.01639.x)

The marked spots were then punched out with a 1 cm diameter cork borer and the leaf disks were frozen in liquid nitrogen. The frozen disks were shipped on dry ice to the laboratory where they were stored at -80°C until pigment analysis.

Photosynthetic pigments were extracted with 100% acetone as described in Bethmann et al. 2019):

Bethmann S, Melzer M, Schwarz N, Jahns P (2019) The zeaxanthin epoxidase is degraded along with the D1 protein during photoinhibition of photosystem II. Plant Direct 3: 1–13. https://doi.org/10.1002%2Fpld3.185

Individual pigments were separated and quantified by high-performance liquid chromatography (HPLC, Fig. 12) according to Färber et al. 1997.

Färber A, Young AJ, Ruban AV, Horton P, Jahns P (1997) Dynamics of xanthophyll-cycle activity in different antenna subcomplexes in the photosynthetic membranes of higher plants (The relationship between zeaxanthin conversion and nonphotochemical fluorescence quenching). Plant Physiol 115: 1609-1618. https://doi.org/10.1104/pp.115.4.1609

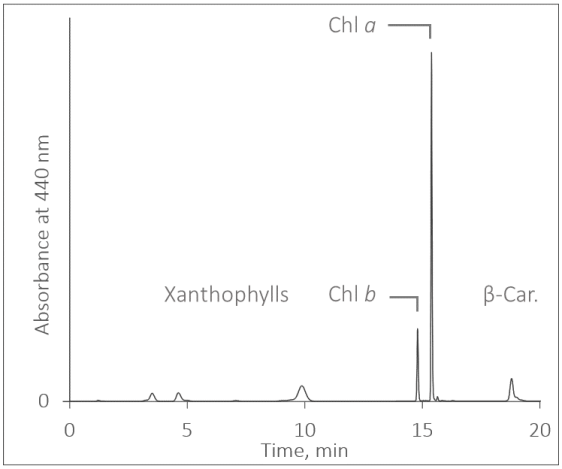
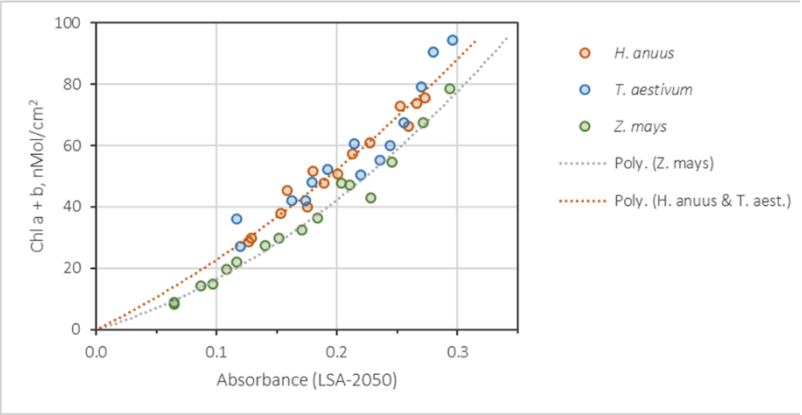


Fig. 12: Chromatogram for Pigment Determination

Table 19: Chl Correlations

Species	Chl *a* + *b*, nMol/cm²	Chl *a* + *b*, µg/cm²
H. anuus & *T. aestivum*	y = 328 x² + 195 x R² = 0.923	y = 294 x² + 175 x
Z. mays	y = 472 x² + 117 x R² = 0.981	y = 422.37x² + 104.98x

x=absorbance as measured by a LSA-2050.
Regressions were forced to extrapolate to zero.



6.1.4 Correlations

Curvilinear functions with high coefficients of determination were observed for the relationships between total chlorophyll content (Chl *a* + Chl *b*) and absorbance measured by the LSA-2050 device (Table 19). The data of the two species with C_3 photosynthesis were similar, suggesting that chlorophyll determination is not affected by the different anatomy of monocot and dicot leaves.

The relationship of the C_4 plant differed from that of the two C_3 plants: the same absorbance value yielded a lower Chl *a* + Chl *b* concentration compared to the relationship for C_3 plants. This originates in relatively low abundance of Chl *b* in the C_4 plant in

combination with the fact that the LSA-2050 measures mainly Chl *a* light absorption (compare Fig. 13).

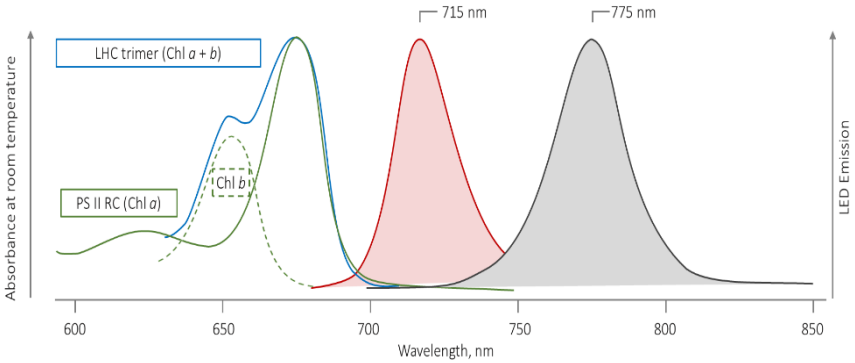


Fig. 13: Chlorophyll determination wavelengths

The LSA-2050 mainly measured Chl *a* light absorption because the Chl *b* absorbance spectrum does not overlap meaningfully with the emission spectrum of the sample LED. Red line and pink-filled: emission spectrum of the sample LED. Dark-grey line and grey-filled: emission spectrum of the reference LED. Green line: absorbance spectrum of the photosystem II reaction center containing only Chl *a*. Blue line: absorbance spectrum of the trimer of the light harvesting complex of photosystem II containing Chl *a* and Chl *b*. Green dashed line: approximate absorption spectrum of Chl *b*. Absorbance spectra of photosynthetic complexes redrawn after Mendes-Pinto MM, Galzerano D, Telfer A, Pascal AA, Robert B, Ilioaia C (2013) Mechanisms underlying carotenoid absorption in oxygenic photosynthetic proteins. Biol Chem 288: 18758 –18765. https://doi.org/10.1074%2Fjbc.M112.423681

6.2 Screening

Screening of photosynthetic pigment-protein complexes against UV-B and UV-A radiation by phenolics was non-invasively measured according to:

Bilger W, Veit M, Schreiber L, Schreiber U (1997) Measurement of leaf epidermal transmission of UV radiation by chlorophyll fluorescence. Physiol Plant 101: 754–763. https://doi.org/10.1111/j.1399-3054.1997.tb01060.x

Cerovic and coworkers have extended the Bilger method into the visible range by using green light to assess light screening by anthocyanins.

Cerovic ZG, Moise N, Agati G, Latouche G, Ben Ghozlen N, Meyer S (2008) New portable optical sensors for the assessment of winegrape phenolic maturity based on berry fluorescence. Journal of Food Composition and Analysis 21: 650-654. https://doi.org/10.1016/j.jfca.2008.03.012

Nichelmann and coworkers have made a further step by introducing blue light to probe screening by carotenoids that are not or only partially active in photosynthetic light harvesting.

Nichelmann L, Schulze M, Herppich WB, Bilger W (2016) A simple indicator for non-destructive estimation of the violaxanthin cycle pigment content in leaves. Photosynthesis Research 128: 183-193. https://doi.org/10.1007/s11120-016-0218-1

The LSA-2050 integrates these three approaches by measuring screening as apparent transmittance at UV-B, UV-A, blue, and green wavelengths with red as reference beam. For UV-A and green radiation, transmittance is converted into absorbance, to provide a relative number for the concentration of flavonoids and anthocyanins, respectively, which are active in light screening. Converting transmittance into absorbance for obtain a proxy for phenolics concentration has been introduced by Goulas et al. (2004):

Goulas Y, Cerovic ZG, Cartelat A, Moya I (2004) Dualex: a new instrument for field measurements of epidermal ultraviolet absorbance by chlorophyll fluorescence. Appl Opt 43, 4488-4496. https://doi.org/10.1364/ao.43.004488

The LED combinations used together with the main classes of pigments absorbing at probing wavelengths are compiled in Table 20. The LED emission spectra a shown in Fig. 14.

Table 20: LED Combinations

LED Pair	Main class of screening pigments
310 nm vs. 630 nm	Hydroxycinnamic acids
365 nm vs. 630 nm	Flavonoids
450 nm vs. 630 nm	Carotenoids
530 nm vs. 630 nm	Anthocyanins

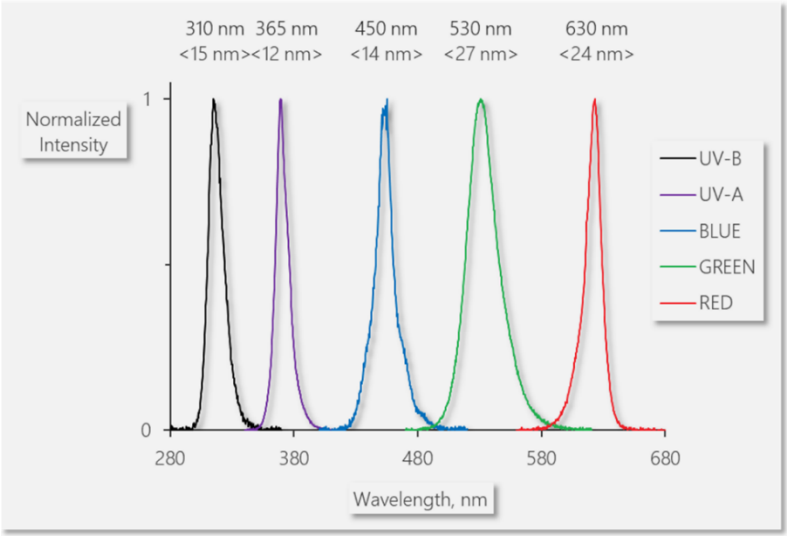


Fig. 14: Emission spectra of LEDs involved in screening measurements

Emission spectra normalized to the same maximum are shown. For each spectrum, peak wavelength, and full width at half maximum (<FWHM>) is indicated.

6.2.1 Mesophyll Reference Factor *MF*

To estimate the radiation screening of the leaf mesophyll, measurements of intact leaves must be related to measurements of unscreened mesophyll tissue. For this, three plant species with easily removable epidermis were selected (Fig. 15, Table 21). For two of the species, only the upper leaf side was examined, but for *H. telephium*, both the upper and the lower leaf sides were probed.

Table 21: Plant Species used as Mesophyll Reference
The epidermis of all leaves was removed by hand, the exposed mesophyll was rinsed with tap water and carefully dabbed dry.

Species, family	Leaf side
Hylotelephium telephium, Crassulaceae	Upper
Hylotelephium telephium, Crassulaceae	Lower
Tulipa spec., Liliaceae	Upper
Kalanchoe daigremontiana, Crassulaceae	Upper

Fig. 15: Leaf of *H. telephium* with Epidermis Partially Removed

MES, free mesophyll. EP, epidermis.

For all cases, mesophyll ratios, $MR(\lambda)_{MES}$, were established as defined by Eq. 1.

$$MR(\lambda)_{MES} = (I(\lambda)/I(630))_{MES} \quad \text{Eq. 1}$$

where $I(\lambda)$ is the fluorescence intensity, excited at wavelength λ or at 630 nm. The λ represents one of the four wavebands: UV-B, UV-A, blue, or green, and 630 is the peak wavelength of the red LED (Fig. 14, page 37).

The same ratios were also established for the fluorescence standard of the LSA-2050. Dividing the mesophyll ratio by the corresponding standard ratio yields the mesophyll reference factor, $RF(\lambda)$:

$$RF(\lambda) = \frac{(I(\lambda)/I(630))_{MES}}{(I(\lambda)/I(630))_{STD}} \quad \text{Eq. 2}$$

Table 22: Mesophyll Reference Factors

Mean values and 95% confidence intervals (95% CI) are given. *RF*(λ) is defined by Eq. 2.

Species	Leaf side	n		*RF*(310)	*RF*(365)	*RF*(450)	*RF*(530)
H. telephium	Upper	51	Mean 95% CI	0.573 0.026	1.105 0.024	1.055 0.015	0.809 0.006
H. telephium	Lower	30	Mean 95% CI	0.863 0.048	1.285 0.040	1.160 0.017	0.807 0.008
T. spec.	Upper	40	Mean 95% CI	0.690 0.039	0.852 0.049	1.023 0.012	0.807 0.007
K. daigremontiana	Upper	26	Mean 95% CI	0.689 0.061	1.208 0.041	0.937 0.030	0.844 0.047

The Reference Factor *RF*(λ) is device-independent. This is because different intensities of the LSA-2050 LEDs affect the intensity ratios measured with mesophyll and with the standard in the same way.

6.2.2 Calibration and Measurement

The calibration with the Walz fluorescence standard determines all four fluorescence ratios $(I(\lambda)/I(630))_{STD\_DEVICE}$. These ratios are device-dependent, because they are affected by the emission intensities of the LEDs.

Multiplying a ratio $(I(\lambda)/I(630))_{STD\_DEVICE}$ with the corresponding *RF*(λ) results in the "mesophyll reference" value $MR(\lambda)_{DEVICE}$. The $MR(\lambda)_{DEVICE}$ corresponds to the fluorescence ratio of the naked mesophyll adapted to the current LSA-2050 instrument.

$$MR(\lambda)_{DEVICE} = \left(\frac{I(\lambda)}{I(630)}\right)_{STD\_DEVICE} \cdot RF(\lambda) \qquad \text{Eq. 3}$$

The apparent screening is then the fluorescence ratio obtained with the sample, $(I(\lambda)/I(630))_{SAMPLE}$ divided by the corresponding mesophyll reference value:

$$Q(\lambda)_{SAMPLE} = \frac{(I(\lambda)/I(630))_{SAMPLE}}{MR(\lambda)_{DEVICE}} \qquad \text{Eq. 4}$$

Calculations of *Q*(λ) uses the *RF*(λ) selected (compare Table 22). The *RF*(λ) of *Hylotelephium* telephium works for most leaves, where upper leaf sides should use the *RF*(λ) from the upper side, and lower sides the *RF*(λ) from the lower side.

6.3 Geodata

Fig. 16 gives an overview on the components involved in providing geospatial information.

The GPS receiver obtains signals from satellites of the Global Positioning System. From satellite positions, latitude and longitude of the current position on Earth is calculated. The internal clock is set to UTC (Coordinated Universal Time) by an external timer like the Windows operating system. From the current position and UTC, the sun’s position relative to ground is determined (compare Fig. 17, 43).

The magnetometer determines north based on the earth's magnetic field. The accelerometer detects gravity and changes in velocity in XYZ directions, and the gyroscope measured rotations in these three directions. By integrating the data of these three sensors, the leaf position (slope and azimuth) is calculated. Leaf slope and azimuth are illustrated in Fig. 18 (page 44) and Fig. 19 (page 44), respectively.

From the positions of sun and leaf, the angle at which sun radiation hits the leaf is derived (angle of incidence, Fig. 20, page 45). The physiologically relevant number is the cosine of the angle of incidence, because it indicates the relative effective intensity of sun radiation at the leaf surface.

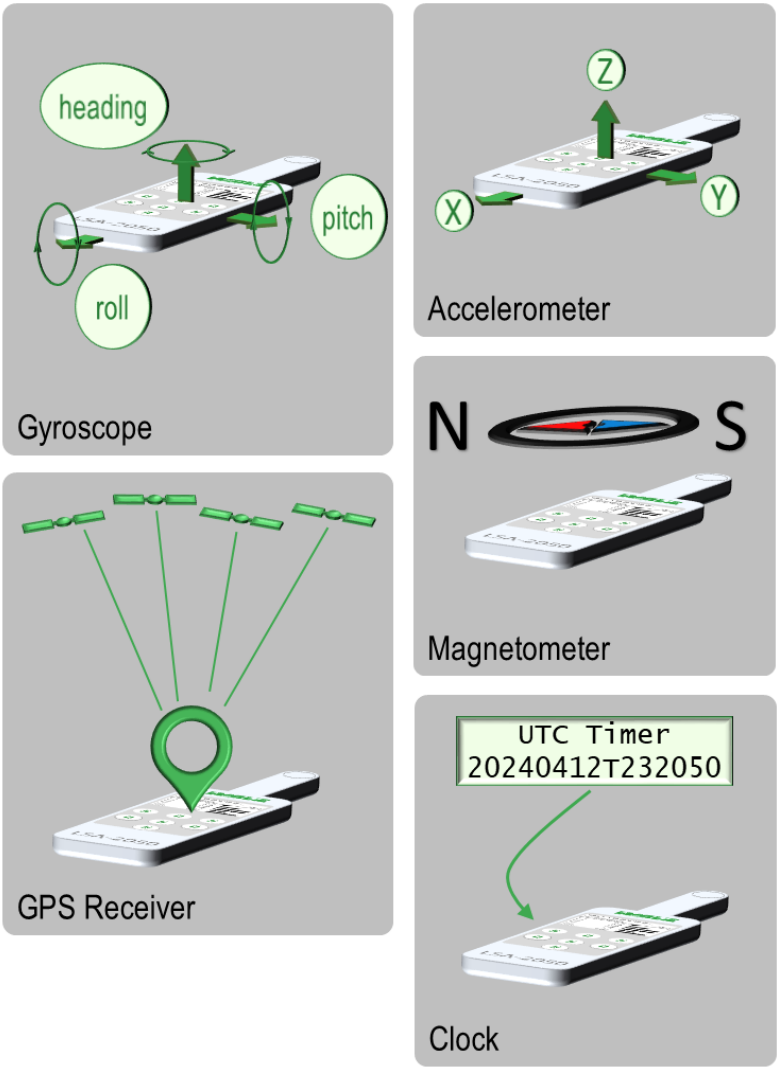


Fig. 16: Geodata Hardware

Devices and ways of geodata input. The gyroscope and the accelerometer measure rotation and velocity changes, respectively, in X, Y, and Z direction. The magnetometer records the earth magnetic field. The GPS receiver determines latitude and longitude of the current position. The UTC timer provides the world standard time.

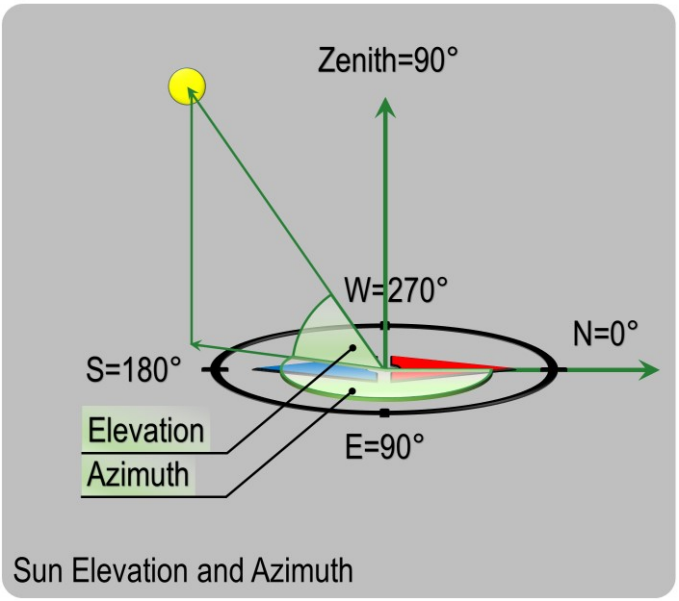


Fig. 17: Sun Position

The position of the sun relative to the Earth surface can be described by azimuth and elevation. Azimuth is the compass direction of the sun relative to the northern direction. With north being the zero point, the azimuth rises from 0° to 360° clockwise. Elevation is the sun's position above the horizon ranging from 0° (horizon) to 90° (zenith).

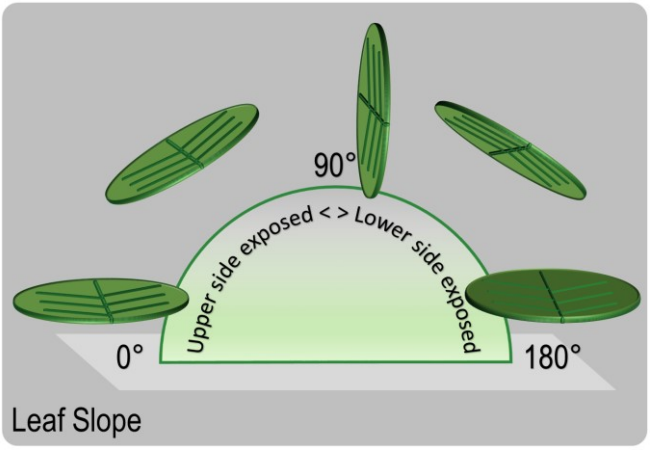


Fig. 18: Leaf Slope

The leaf slope is the leaf angle relative to the ground. 0° corresponds to the horizontal position of the leaf with top side up. 90° describes the vertical position and 180° is the horizontal position with the lower side up.

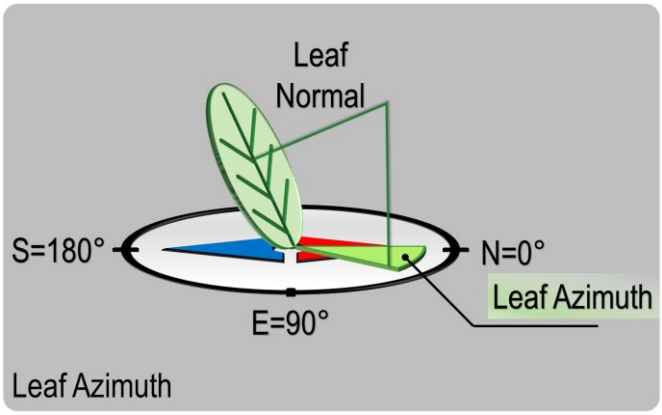


Fig. 19: Leaf Azimuth

The leaf azimuth is the compass direction of leaf normal relative to the northern direction. The range of the leaf azimuth is identical to that of the sun azimuth.

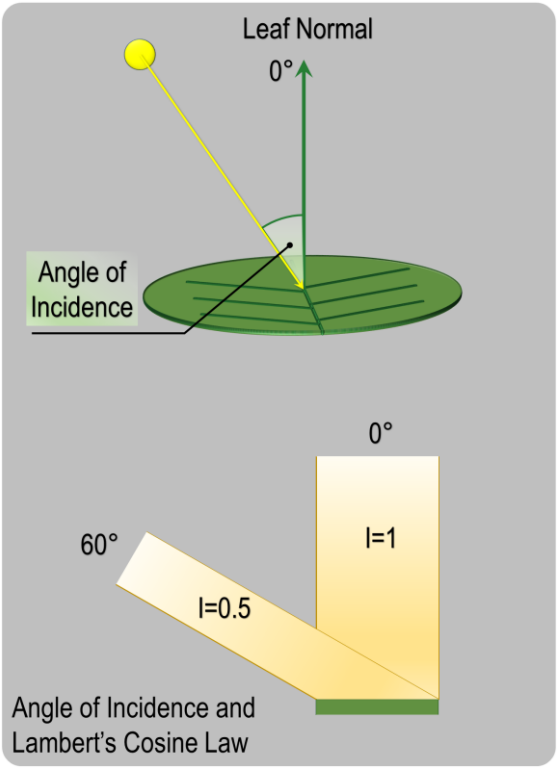


Fig. 20: Angle of Incidence

The angle at which the solar impinges on the leaf (angle of incidence) determines the relative effective radiation intensity on the leaf surface. This angle is defined as the deviation from the leaf normal, where perpendicularly and horizontally impinging radiation has angles of 0° and 90°, respectively. The effective intensity at leaf surface equals the cosine of the angle of incidence (Lambert's Cosine Law).

7 Specifications

Specifications are subject to change without notice.

7.1 LEAF-STATE-ANALYZER LSA-2050

a. General Design

Housing: Battery-powered handheld device consisting of a control unit and a sample clip, both made of painted polyamide 12 (PA 12). The control unit is equipped with a holder for four AAA-type batteries and a USB-C connector. Two metal flat springs press the clip jaws together. The lower clip jaw is removable.

Display: Backlit transflective B/W LCD display, 48 x 27 mm, 128 x 64 pixel

Control: Six control keys plus a separate START key to initiate a measurement

Data memory: Flash memory, 8 MB, providing memory for more than 30,000 data sets

Data transfer: USB-C port

Power supply: 4 AAA (Micro) rechargeable batteries (eneloop 1.2 V/2 Ah), 4 spare batteries, automatic power/off, battery charger (100 to 240 V AC, 50-60 Hz) for 4 batteries

Operating temperature: -5 to +45 °C, non-condensing

Dimensions: maximum 26.5 cm x 7.0 cm x 3.5 cm (L x W x H)

Weight: 240 g (without batteries)

b. Measuring Modules

Viewing area: Disk with 10 mm diameter

Upper clip jaw: Five LEDs are circularly arranged around a PIN photodiode, which is shielded from LED emission by a long-pass filter. A quartz glass disk closes the LED/photodiode compartment. Measuring light consists of 10 µs pulses given at 15 Hz except for F_M determinations (100 Hz). Typical maximum emission wavelength, full width at half maximum (FWHM), and integrated intensity at 15 Hz are: UV-B, 310 nm, 15 nm, 0.1 µmol m^{-2} s^{-1} (0.05 W m^{-2}). UV-A, 365 nm, 12 nm, 0.3 µmol m^{-2} s^{-1} (0.1 W m^{-2}). Blue, 450 nm, 14 nm, 0.1 µmol m^{-2} s^{-1}. Green, 530 nm, 27 nm, 0.1 µmol m^{-2} s^{-1}. Red, 630 nm, 24 nm, 0.1 µmol m^{-2} s^{-1}. The UV LEDs are only activated in the presence of a fluorescing sample.

Lower clip jaw: A far red LED (peak wavelength 715 nm, FWHM 25 nm) and a near infrared LED (peak wavelength 770 nm, FWHM 30 nm) are positioned in the center of the viewing are. The LEDs are covered by a light-diffusing disk and a quartz disk.

c. Geospatial Data

Devices are outlined in Fig. 16, page 42.

7.2 Carrying Case LSA-2050/T

Design: Padded plastic case with handle

Dimensions: 36.0 cm x 30.5 cm x 8.0 cm (L x W x H)

Weight: 920 g

7.3 Battery Charger

Design: Four position intelligent charger for AA or AAA Nickel Metal Hydride (NiMH) or Nickel Cadmium (NiCd) batteries

7.4 Accessory

7.4.1 Darkening Bags LSA-2050/DB

General Design: Set of three small, three medium and three large light-tight bags for dark acclimation of leaves of different sizes made of aluminum foil, colored on the outside. Each bag has a 2 cm diameter central hole for non-invasive determination of chlorophyll concentration. During dark acclimation, both sides of the hole are covered by metallized PET plastic flaps. The flaps magnetically attract each other which ensures that both lie tightly on the surface. The flaps are flexibly attached to the darkening bag at one end. The other, loose end is folded slightly outwards so that the sample clip of the LSA-2050 can be guided to the hole by folding open the flaps.

Dimensions: 100 mm x 70 mm, 150 mm x 100 mm, 180 mm x 120 mm (small, medium, and large size, respectively).

Weight: 3 g, 4 g, 5 g (small, medium, and large size, respectively)

8 Guarantee

All products supplied by the Heinz Walz GmbH, Germany, are warranted by Heinz Walz GmbH, Germany to be free from defects in material and workmanship for two (2) years from the shipping date (date on invoice).

8.1 Manufacturer's Guarantee

Under this Manufacturer's Guarantee ("Guarantee"), subject to the Conditions and Instructions below, Heinz Walz GmbH, Germany ("Manufacturer"), guarantees (§443 BGB) to the end customer and user ("Customer") that all products supplied by it shall substantially conform in material respects to the Specifications for 24 months from the delivery date (date on invoice). In this Guarantee, "Specifications" means the product's features (as may be amended by Manufacturer from time to time), which are set out under the headings "specifications" and/or "technical specifications" within the product's respective brochure, data sheet, or respective tab on the Manufacturer's website for such product, and which may be included with the documents for the product when delivered. In case of an eligible guarantee claim, this Guarantee entitles the Customer to repair or replacement, at the Manufacturer's option, and this Guarantee does not include any other rights or remedies.

8.2 Conditions

This Guarantee shall not apply to:

- Any defects or damage directly or indirectly caused by or resulting from the use of unauthorized replacement parts and/or service performed by unauthorized personnel.

- Any product supplied by the Heinz Walz GmbH, Germany which has been subjected to misuse, abuse, abnormal use, negligence, alteration or accident.

- Damage caused from improper packaging during shipment or any acts of God.

- Batteries, cables, calibrations, fiberoptics, fuses, gas filters, lamps (halogen, LED), thermocouples, and underwater cables.

- Defects that could reasonably have been detected upon inspection of the product when received by the Customer and not promptly noticed within ten (10) days to Heinz Walz GmbH.

- Submersible parts of the DIVING-PAM or the underwater version of the MONITORING-PAM have been tested to be watertight down to the maximum operating depth indicated in the respective manual. Guarantee shall not apply for diving depths exceeding the maximum operating depth. Further, guarantee shall not apply for damage resulting from improper operation of devices, in particular, the failure to properly seal ports or sockets.

8.3 Instructions

- To obtain guarantee service, please follow the instructions below:

- The Walz Service Information Form available at http://www.walz.com/support/repair_service.html must be completed and returned to Heinz Walz GmbH, Germany.

- The product must be returned to Heinz Walz GmbH, Germany, within 30 days after Heinz Walz GmbH, Germany has received written notice of the defect. Postage, insurance, and/or shipping costs incurred in returning equipment for guarantee service are at customer expense. Duty and taxes are covered by Walz.

- All products being returned for guarantee service must be carefully packed and sent freight prepaid.

- Heinz Walz GmbH, Germany is not responsible or liable for missing components or damage to the unit caused by handling during shipping. All claims or damage should be directed to the shipping carrier.

8.4 Applicable law

- This Guarantee is governed by German law. Place of jurisdiction is Bamberg, Germany.

9 Index

10 List of Figures

11 List of Tables