LEAF-STATE-ANALYZER
LSA-2050

Manual

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Contents

1 SAFETY INSTRUCTIONS 3

1.1 General Safety Instructions 3
1.2 Special Safety Instructions 4

2 INTRODUCTION 5

3 LSA-2050: EXTENT OF DELIVERY 7

3.1 LSA-2050 Description 10

4 ACCESSORIES 13

4.1 Darkening Bags LSA-2050/DB 13
4.2 Darkening Clips LSA-2050/DLC 14

5 OPERATION 15

5.1 Quick Start 16
5.2 Detailed Instructions 16
5.2.1 Main Windows 16
5.2.2 Main Menu 19
5.2.2.1 Settings 20
5.2.2.2 Calibration 22
5.2.2.3 Create New File 24
5.2.2.4 Memory 24
5.2.2.5 Marker 26
5.2.2.6 Device Info 26
5.3 LSA-2050 Software 26
5.3.1 Installing Software 26
5.3.2 Connecting the LSA-2050 to Computer 28
5.3.3 Adjusting Time 28
5.3.4 Downloading Data 30
5.3.5 Excel File 30

6 COMMENTS ON RESULTS 37

6.1 F_V/F_M 37
6.2 Screening 38
6.3 Chlorophyll Concentration 39
6.4 Nitrogen Balance Index (NBI), ... 40

7 DOCUMENTATION 43

7.1 *Darkening Clip LSA-2050/DLC* 43
7.2 *Chlorophyll Calibration* 45
7.2.1 *Plant Species* 45
7.2.2 *Growth Conditions* 45
7.2.3 *Chl. Measurements* 46
7.2.4 *Correlations* 47
7.3 *Chlorophyll and Small Leaves* 50
7.4 *Screening* 51
7.4.1 *Mesophyll Reference Factor RF(λ)* 53
7.4.2 *Calibration and Measurement* 56
7.5 *Geodata* 57

8 SPECIFICATIONS 63

8.1 *LEAF-STATE-ANALYZER LSA-2050* 63
8.1.1 *General Design* 63
8.1.2 *Measuring Modules* 64
8.1.3 *Geospatial Data* 64
8.1.4 *Walz Calibration Standards LSA-2050/STD* 65
8.1.5 *Viewing area reduction kit* 65
8.2 *Carrying Case LSA-2050/T* 66
8.3 *Battery Charger* 66
8.4 *Accessories* 66
8.4.1 *Darkening Bags LSA-2050/DB* 66
8.4.2 *Darkening Clips LSA-2050/DLC* 67

9 GUARANTEE 69

9.1 *Manufacturer's Guarantee* 69
9.2 *Conditions* 69
9.3 *Instructions* 70
9.4 *Applicable law* 71

10 INDEX 73

11 LIST OF FIGURES 75

12 LIST OF TABLES 77

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 the 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. 5, page 12).
- 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 four 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, and (4) the state of nitrogen supply. 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 evaluates radiation screening by measuring the intensity of chlorophyll fluorescence excited by constant radiation of specific wavelengths. The excitation intensity used is low. This is to avoid variations in the fluorescence quantum yield caused by energy quenching processes.

The four different excitation wavebands employed can be related to radiation absorption of 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.

The nitrogen balance index (NBI) is calculated according to another paper by Cerovic and colleagues [5] as the ratio of chlorophyll divided by epidermal flavonoids.

Photosystem II is analyzed by the well-proven PAM fluorescence/saturation pulse method [6].

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] Cerovic ZG, Ben Ghozlen N, Milhade C, Obert M, Debuisson S, Le Moigne M (2015) Nondestructive Diagnostic test for nitrogen nutrition of grapevine (Vitis vinifera L.) based on Dualex leaf-clip measurements in the field. J Agric Food Chem 63 (14): 3669–3680

[6] 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 plate (covers electrical contacts when operated without the lower clip jaw)	000247001714
Walz LSA-2050 set of standards	LSA-2050/STD
Viewing area reduction kit Adhesive rings to reduce the diameter of the optical window to 6 mm. Centering tool for the adhesive ring to reduce the diameter of the optical window. Pres-on tool for the adhesive ring to reduce the diameter of the optical window.	 000247003214 000247003014 000247003114
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	

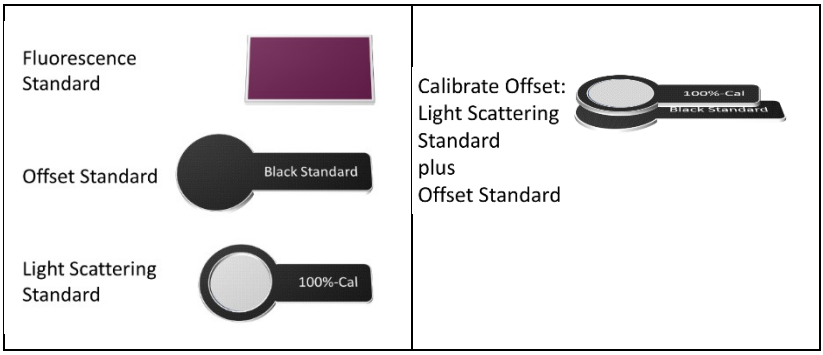

Fig. 1: Fluorescence and Offset Standards

Calibration with the fluorescence standard is required to measure radiation screening (see Section 7.4, page 51). The signals in the presence of a sandwich of the light scattering standard (top) and the black standard (bottom) are considered as instrument offset which are subtracted from the corresponding sample signals. The light scattering standard is used to calibrate the 100% state (absence of chlorophyll) under light scattering conditions similar to a leaf.

To correctly measure chlorophyll contents, the entire optical window of the FR, NIR Emitter must be covered by the sample. In case of small samples, the optical window can be narrowed by a reduction ring which reduces the optical window to a spot of 6 mm diameter.

Fig. 2 describes how the ring is mounted using a special centering device. For mounting, the lower part of the LSA-2050 must be disconnected (compare Fig. 5).

The reduction ring fits exactly in the center of the centering device, which itself fits on the measuring window of the lower part of the LSA-2050.

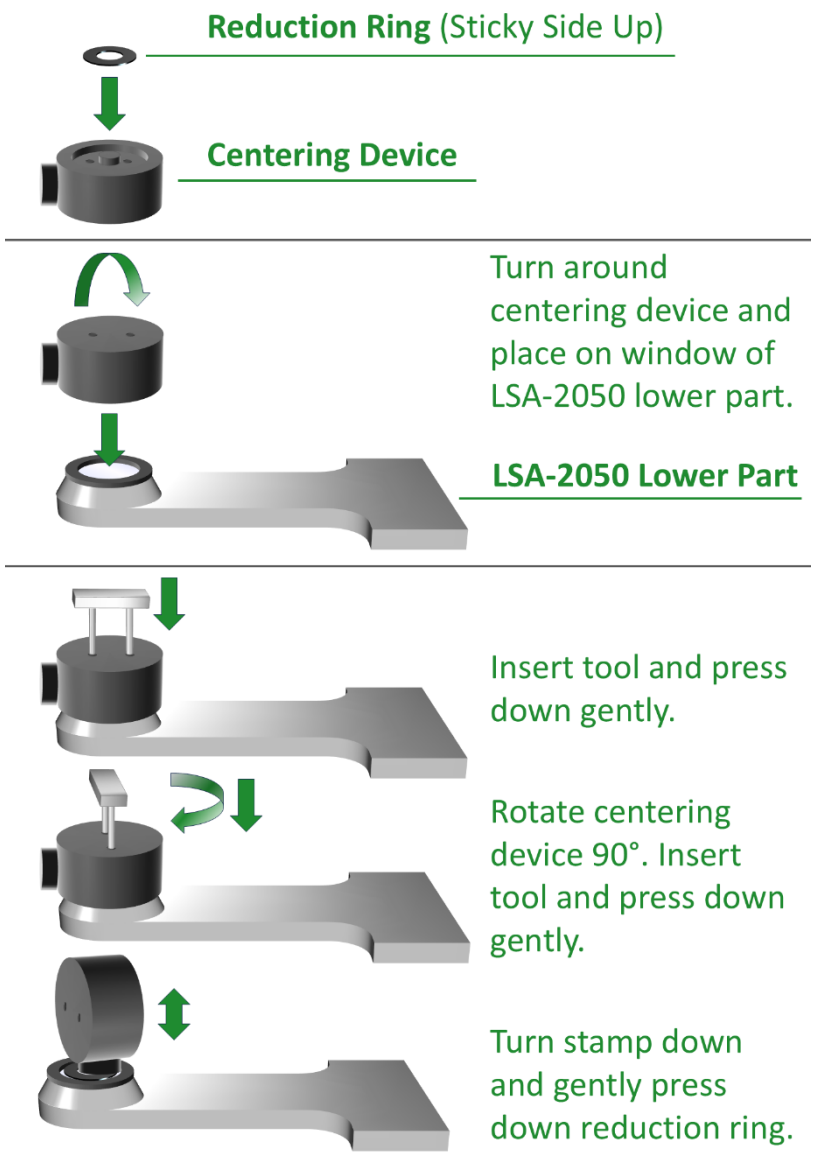

Fig. 2: Reduction Ring Mounting

The ring reduces the diameter of the measuring window to 6 mm. The ring must be placed on the optical window of the lower LSA-2050 part (FR, NIR emitter, see Fig. 3 for a device description, see also Fig. 19, page 51).

3.1 LSA-2050 Description

The tip of the LSA-2050 LEAF-STATE-ANALYZER is formed by a bipartite measuring head (Fig. 3). The heads form a clip in which a sample or standard (Fig. 1) is placed. The clip can be opened by pressing the lever on the bottom side of the device (Fig. 5).

The upper “Emitter Detector Head” holds the photodiode detector, and 5 LEDs emitting in the UV or the visible range (Fig. 4). A far red and a near infrared LED is situated in the lower “FR, NIR Emitter Head”. The emission by these LEDs has to pass through the sample to reach the photodiode. The sample’s chlorophyll concentration is derived from the beam attenuation by the sample.

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.

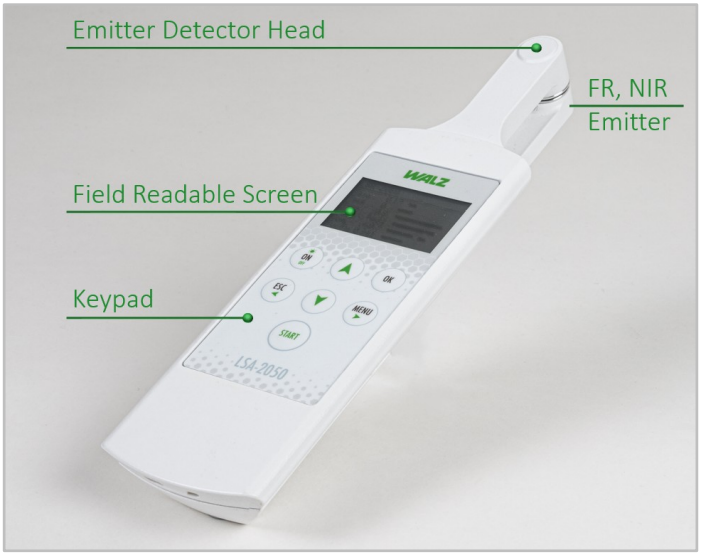

Fig. 3: LSA-2050 Overview

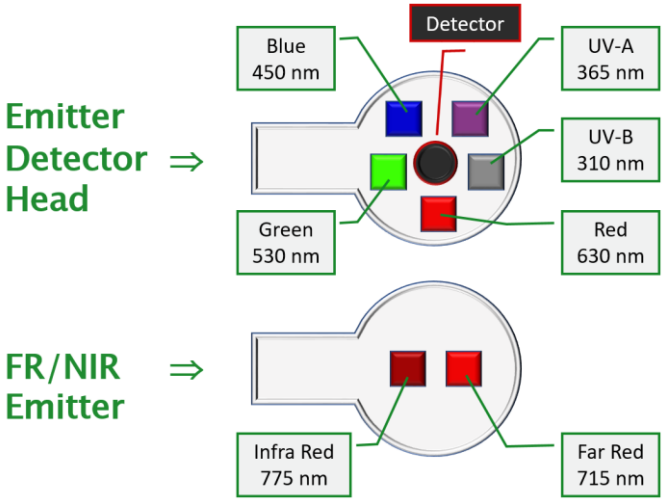

Fig. 4: LSA-2050 Measuring Head

To examine bulky samples (e.g. fruits), the FR, NIR Emitter can be removed (Fig. 5). 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.

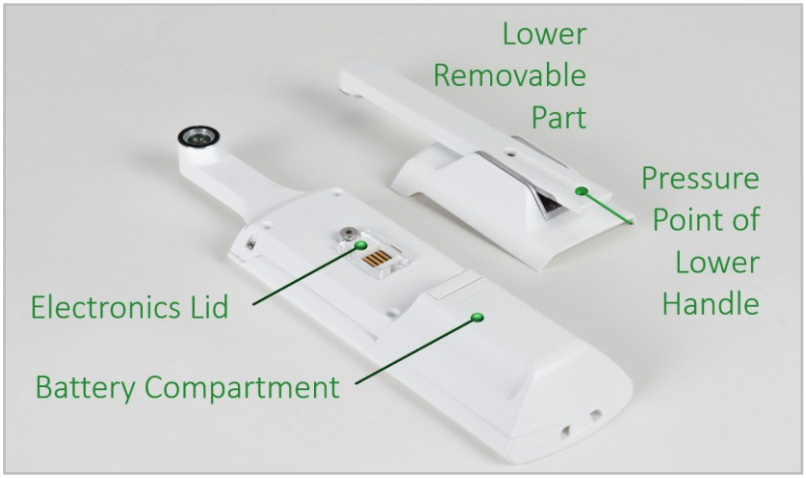

Fig. 5: LSA-2050 Modified for Bulky Samples

4 Accessories

4.1 Darkening Bags LSA-2050/DB

This accessory is designed for darkening 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 8.4.1, page 66.

A

B

Fig. 6: 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.

4.2 Darkening Clips LSA-2050/DLC

Alternative to the darkening bags, darkening clips can be used for dark acclimation of a sample area prior to the measurements.

Both jaws of the clip possess a hole so that the measuring light for chlorophyll determination can pass through the sample. For dark acclimation, these holes are closed by the shutters.

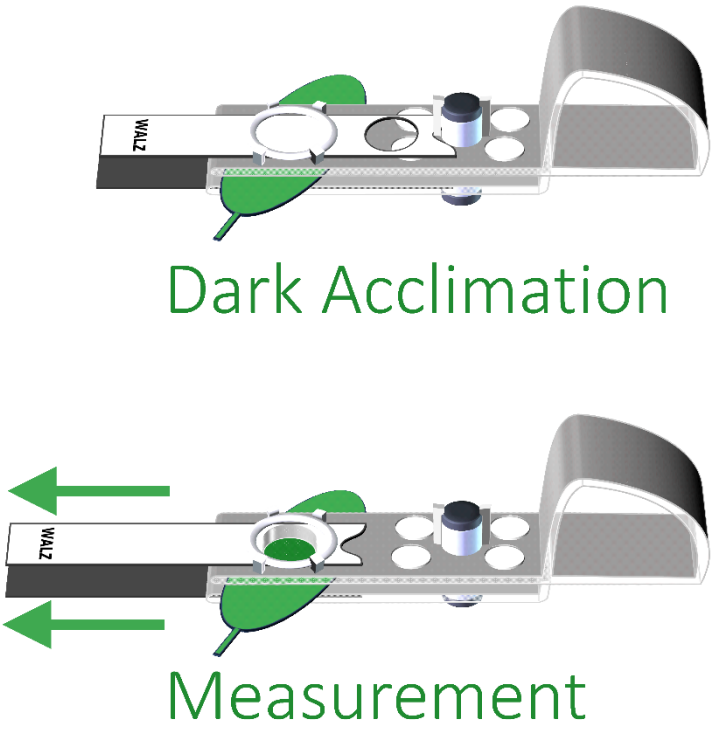

Fig. 7: Darkening Clip LSA-2050/DLC

Upper drawing: Shutters slid in for dark acclimation. **Lower drawing:** Shutters drawn out for measurement.

5 Operation

The LSA-2050 is controlled by 7 keys. The function of these keys is outlined in Fig. 8. The symbols on the keys and the instructions in the software make the operation of the unit self-explanatory.

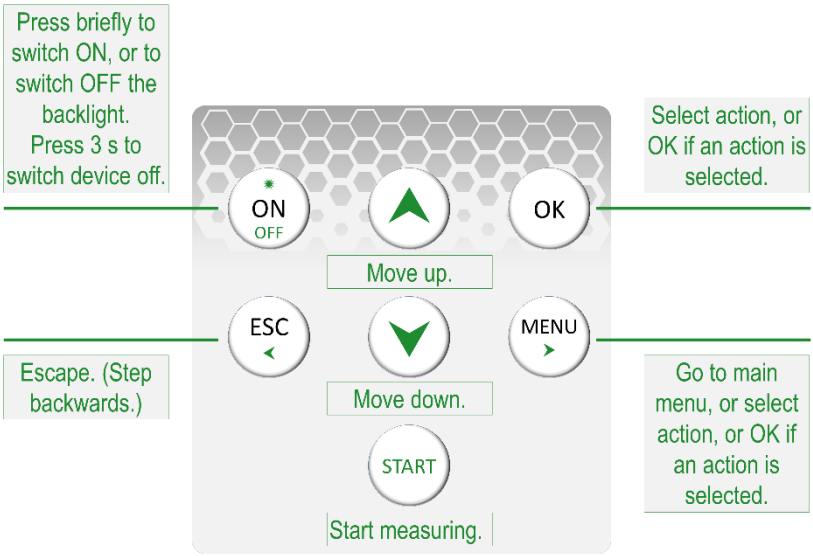

Fig. 8: Keys

The START button is only active when one of the four main windows is visible (see Section 5.2.1, page 16).

5.1 Quick Start

- Connect LSA-2050 to computer. Install and launch LSA-2050 software. Set time zone (see 5.3.3, page 28).
- On the LSA-2050, click the MENU button, select “Calibration”, and perform all four calibration procedures. Follow exactly the instructions on the screen.
- Start measuring.

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 28, page 49) and it is given in nmol/cm^2, and (3) the mesophyll reference is from lower leave sides of *Valerianella locusta* with modified 530 nm mesophyll reference (“Valerianella mod. Lower side”, Table 10, page 21).

5.2 Detailed Instructions

5.2.1 Main Windows

After system start, the LSA-2050 window appears (Table 1).

Table 1: LSA-2050 Window

LSA-2500		1 A
Q_{310}	0.13	
Q_{365}	0.11	
Q_{450}	0.81	
Q_{530}	1.00	
C_{CHL}	86.6	
F_V/F_M	0.689	

The LSA-2050 window displays the apparent transmittance at the wavelengths 310, 365, 450 and 530 nm (Q_{310}, Q_{365}, Q_{450}, and Q_{530}, respectively). The window further shows the chlorophyll concentration in the unit selected in the menu “Chl. Settings” (Table 9, page 21), and the maximum photochemical yield of photosystem II, F_V/F_M. See Section 6 (page 37) for comments on this data.

The top line indicates the sample identifiers, and the device status (see Table 2, page 17). The same information is given in the other three main windows: “Sat Pulse Data”, “N Status”, and “Geospatial Data” (Table 3 to Table 5, respectively). The sample identifiers are also displayed in the memory windows (5.2.2.4, page 24).

Measurements can only be started when one of the four main windows is selected.

Table 2: Main Windows Top Line	
Numbers 1 - 100	Running numbering for measurements within a file, starting with 1.
Capital letters A to Z	Sample identifier within a file. Select letter in Main Menu (Table 6, page 19).
	Battery charge status. From left to right, full to low charge. Flashing, change batteries.
	LED Status. Left, visible ON. Right, UV ON. No Status LED, OFF.
	GPS status. Left, ON. Right, location determined.

In the N Status Window, the Q_{365} and Q_{530} values are converted into absorbance data (A_{FLAV} and A_{ANTH}, respectively). The NBI (nitrogen balance index) corresponds to the ratio of chlorophyll content to A_{FLAV} (see Section 6.4, page 40).

Table 3: N Status Window

N Status		1 A
NBI	61.2	
A_{FLAV}	0.82	
A_{ANTH}	0.07	
2024-03-20	15:28:41	

The F_V/F_M value of the window “LSA-2050” 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 a saturation pulse.

Table 4: Sat Pulse Data

Sat Pulse		1 A
F_V/F_M	0.689	
F_0	382	
F_M	1230	
2024-03-20	15:28:41	

The window Geodata provides longitude and latitude (first line to the left, number followed by W or E; first line to the right, number followed by N or S, respectively), and height above sea level in meter. These position data is calculated from signals from GPS satellites which are picked up by the on-board GPS receiver. The number of available satellites is indicated in line 2 (#Sat). From position and world time (Coordinated Universal 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 relevance because the effective intensity of sun radiation at the leaf surface is porportional to this value.

Chapter 7.5 (page 57) summarizes all hardware involved in geodata acquisition. The same chapter explains the geodata terminology.

Table 5: Geodata

Geodata		1 A	
12.345678 E		12.345678 N	
Height	400	#Sat	15
Leaf Az.	76	Sun Az.	135
Leaf Sl.	45	Sun El.	60
A. o. I.	60	Inc.	50
2024-03-20		17:41:43	

5.2.2 Main Menu

The Main Menu can be accessed from any of the main windows by pressing the MENU button on the instrument.

Table 6: Main Menu

Main Menu	
Settings	→
Calibration	→
Create New File	→
Memory	→
Marker	**A**
Device Info	→

5.2.2.1 Settings

The first item of the main menu leads to the menu “Settings” (Table 7). Choose a submenu by up and down keys. Select by pressing OK.

Table 7: Settings

Settings	
Active Tests	→
Chl. Settings	→
Mesophyll Type	→
Device Settings	→

The item "Active Tests" option allows to switch tests on and off (Table 8). To save energy, or when battery power is low, switch off geodata. Geodata sensors consume a lot of energy.

Table 8: Active Tests

Active Tests	
Chl. Concentration	on/off
F_V/F_M	on/off
Screening	on/off
Geodata	on/off

The item “Chl. Settings” permits changing the unit of chlorophyll concentration, and the calibration curve used to calculate chlorophyll concentrations (see Section 7.1, page 43). The selection of the unit for chlorophyll concentration does not affect the exported data: both nmol/cm^2 and µg/cm^2 are exported. Changing the calibration curve creates a new file with the next measurement.

Table 9: Chl. Settings	
Chl. Settings	
nmol/cm^2	X
µmol/cm^2	
C_3 Type	X
C_4 Type	

The item “Mesophyll Type” opens a list of the available mesophyll references. The selected mesophyll reference (up/down keys followed by OK) is used to evaluate radiation screening (see 7.4, page 51). Changing the mesophyll reference creates a new file.

Default mesophyll reference is “Valerianella mod.”, which consists of UV and blue mesophyll references (310, 365, and 410 nm) of epidermis-free lower leaf sides of *Valerianella locusta*. The green (530 nm) reference value was obtained from upper sides of intact control leaves lacking anthocyanins (Table 30, page 55).

Table 10: Mesophyll Reference		
Mesophyll Reference		
Hylotelephium	Upper side	
Hylotelephium	Lower side	
Kalanchoe	Upper side	
Spinacia	Lower side	
Tulipa	Upper side	
Valerianella	Lower side	
Valerian. mod.	Lower side	X

The first item of the menu “Device Settings” allows adjusting the idle time after which the device is automatically switched off. The second item turns the beeper of the instrument on or off. Note that both the beginning and end of a measurement are signaled by a beep.

„Load Default" activates default settings which are outlined in Section 5.1 (page 16).

"Clear Memory" command erases all data.

Table 11:	Device Settings
Device Settings	
Auto Off	15:00
Beeper	on/off
Load Defaults	→
Clear Memory	→

5.2.2.2 Calibration

Prior to measurements, three calibration procedures must be performed: Offset Values, Absorbance, and Fluorescence (Table 12). The fourth calibration (Orient. Sensor) is not needed for leaf analysis but for information on sun exposure of the leaf.

Table 12:	Calibration
Calibration	
Offset Values	→
Absorbance	→
Fluorescence	→
Orient. Sensor	→

"Offset Values" determine the background signal of the device, with the sandwich light diffusing/black standard in place (see Fig. 9, page 23). "Absorbance" calibration measures the intensity of the FR and NIR LED in the presence of the Walz light diffusing standard. Fluorescence calibration determines the signal with the Walz fluorescence standard. All calibration values are documented in the exported data (Section 5.3.1, page 26).

Note that measurements with the LSA-2050/DLC Clip require that calibration is performed with this clip (Fig. 10).

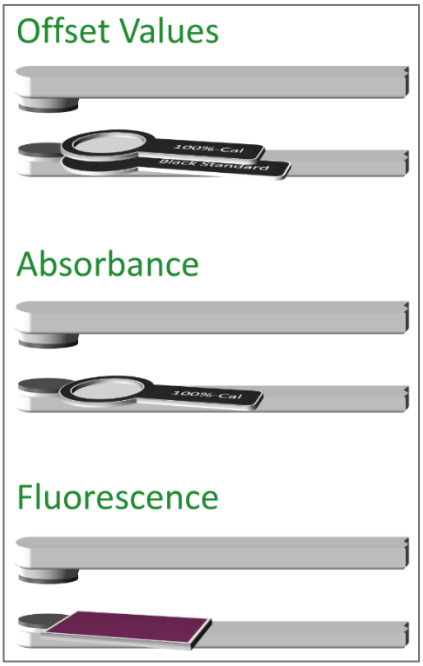

Fig. 9: Calibration without LSA-2050/DLC Clip

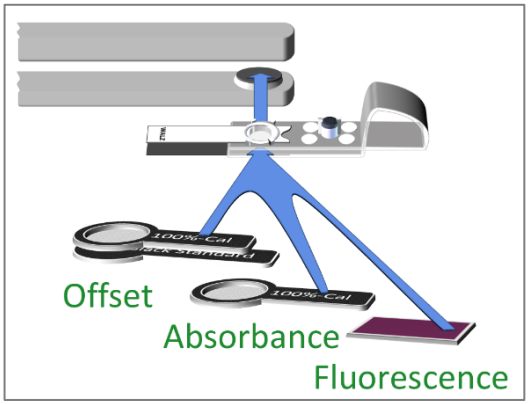

Fig. 10: Calibration with LSA-2050/DLC Clip

The geodata sensors are calibrated in the window “Orient Sens. Calibration” (Table 13). Three orientation sensors are calibrated. The sensors and the meaning of the displayed numbers (Heading, Pitch, Role) are introduced in Section 7.5 (page 57).

Table 13: Orient. Sens. Calibration

Orient. Sens. Calibration		
Gyro	[redacted]	Heading
Accel	[redacted]	Pitch
Mag	[redacted]	Role
Various instruction texts.		

To calibrate the 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, calibration of accelerometer and magnetometer occur simultaneously. When separate magnetometer calibration is required, you will be 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. Consecutive numbering of measurements starts newly.

5.2.2.4 Memory

The Memory item permits scrolling through stored measurements using the up and down keys. For each data set, the initial view is the LSA-2500 window. Pressing the Menu (➤) key sequentially shows the Windows “N Status”, “Sat Pulse”, and

"Geo-data". ESC (◄) returns to the previous view (Table 14 to Table 17).

Table 14: Memory Window LSA-2050

Memory	1 A	
Q_{310}	0.13	
Q_{365}	0.11	
Q_{450}	0.81	
Q_{530}	1.00	
C_{CHL}	86.6	
F_V/F_M	0.689	

Table 15: Memory Window N Status

Memory	1 A	
NBI	61.2	
A_{FLAV}	0.82	
A_{ANTH}	0.07	
2025-10-17	11:29:49	

Table 16: Memory Window Sat Pulse Data

Memory	1 A	
F_V/F_M	0.689	
F_0	382	
F_M	1230	
2025-10-17	11:29:49	

Table 17: Memory Window Geodata

Memory	1 A		
12.345678 E		12.345678 N	
Height	400	#Sat	15
Leaf Az.	76	Sun Az.	135
Leaf Sl.	45	Sun El.	60
A. o. I.	60	Inc.	50
2025-10-17	11:29:49		

5.2.2.5 Marker

The marker identifies different measurements within a file. To change marker, go to line “Marker”, select Marker with right arrow, select character with up/down keys.

Table 18:	Select Marker
Main Menu	
Settings	→
Calibration	→
Create New File	→
Memory	→
Marker	A
Device Info	→

5.2.2.6 Device Info

The window “LSA-2050 Info” reports the charge status of the batteries. When connected to a computer, with batteries installed, the charge status indicates 100%. The device and software specific information is needed when reporting errors to the Walz staff.

Table 19:	LSA-2050 Info
LSA-2050 Info	
Battery: 80%	
S/N: LSAN0101	
Firmware: 22/2311	
Build:	24-05-07 15:16:59
Prog:	24-05-17 08:32:16

5.3 LSA-2050 Software

5.3.1 Installing Software

Always check if you use the latest software that is available here: https://www.walz.com/downloads/?filter=lsa-2050.

Execute the LSA software installer (e.g., LSA-2050-v1.0x-Installer.exe) located on the Walz USB flash drive by 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. 11). Read the instructions carefully and select the appropriate option for your computer configuration. The FTDI USB driver is needed for communication with the LSA-2050 device. 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 is generated.

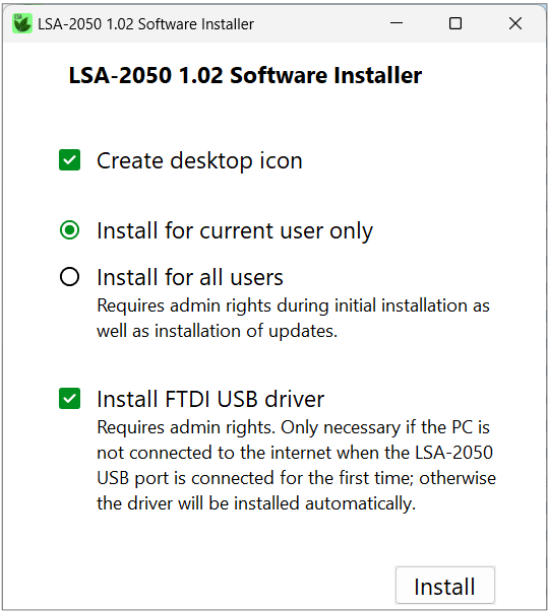

Fig. 11: Installation Window

5.3.2 Connecting the LSA-2050 to Computer

Remove cover of the battery compartment to gain access to the USB-C port of the LSA-2050 (Fig. 12).

Fig. 12: Access to USB-C Port

Connect to the computer using the Walz USB-C to USB-A cable (included in delivery) or a commercially available cable. Execute LSA-2050 software. Outdated firmware on the LSA-2050 device will automatically be updated. Then, the download window appears.

5.3.3 Adjusting Time

Click on the gear icon on the download window, to set your local time zone. Instructions are given in the legend of Fig. 13.

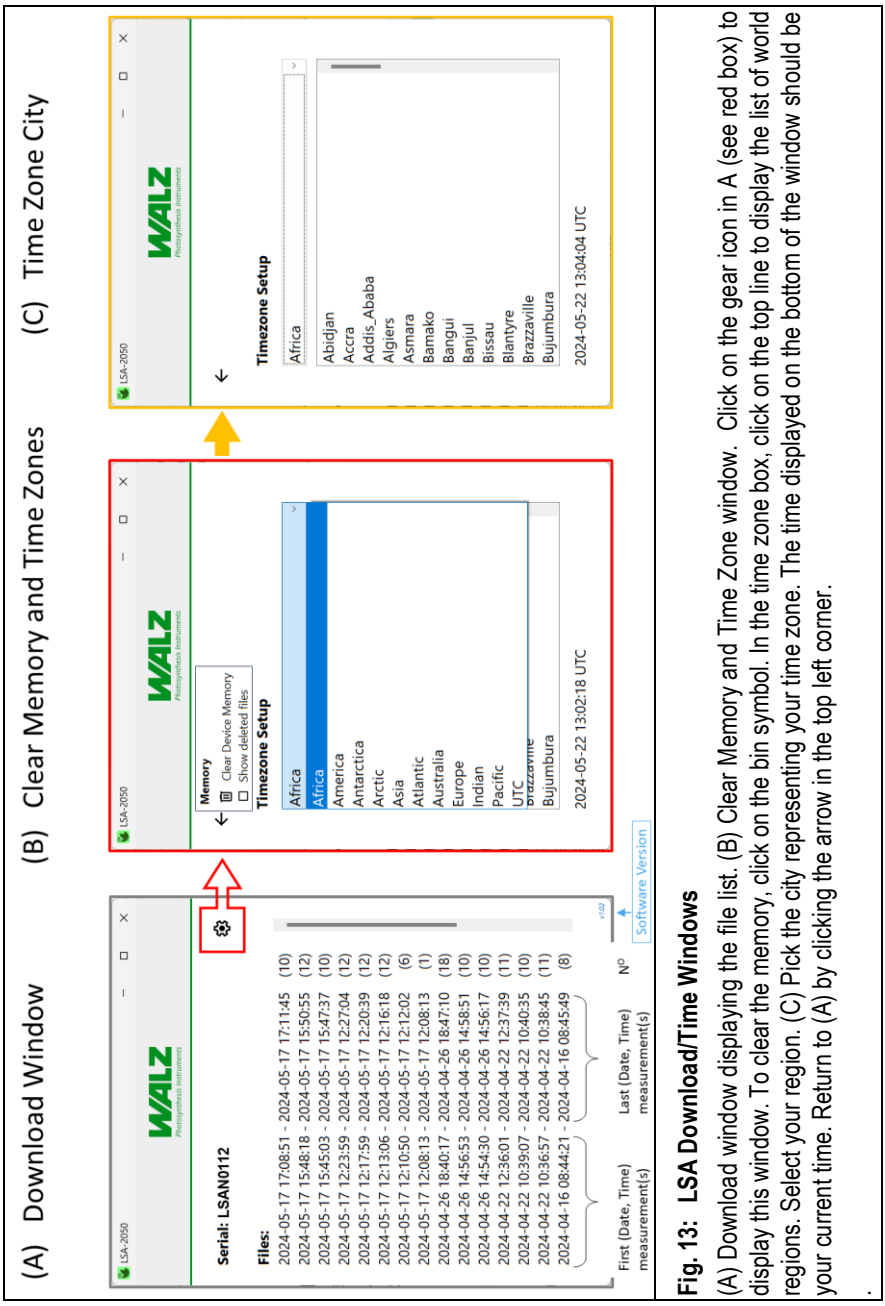

Fig. 13: LSA Download/Time Windows

(A) Download window displaying the file list. (B) Clear Memory and Time Zone window. Click on the gear icon in A (see red box) to display this window. To clear the memory, click on the bin symbol. In the time zone box, click on the top line to display the list of world regions. Select your region. (C) Pick the city representing your time zone. The time displayed on the bottom of the window should be your current time. Return to (A) by clicking the arrow in the top left corner.

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5.3.4 Downloading Data

The download window lists all the files that are stored on the LSA-2050 (Fig. 13, page 29). Left click once on the file to be downloaded. This action opens 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.

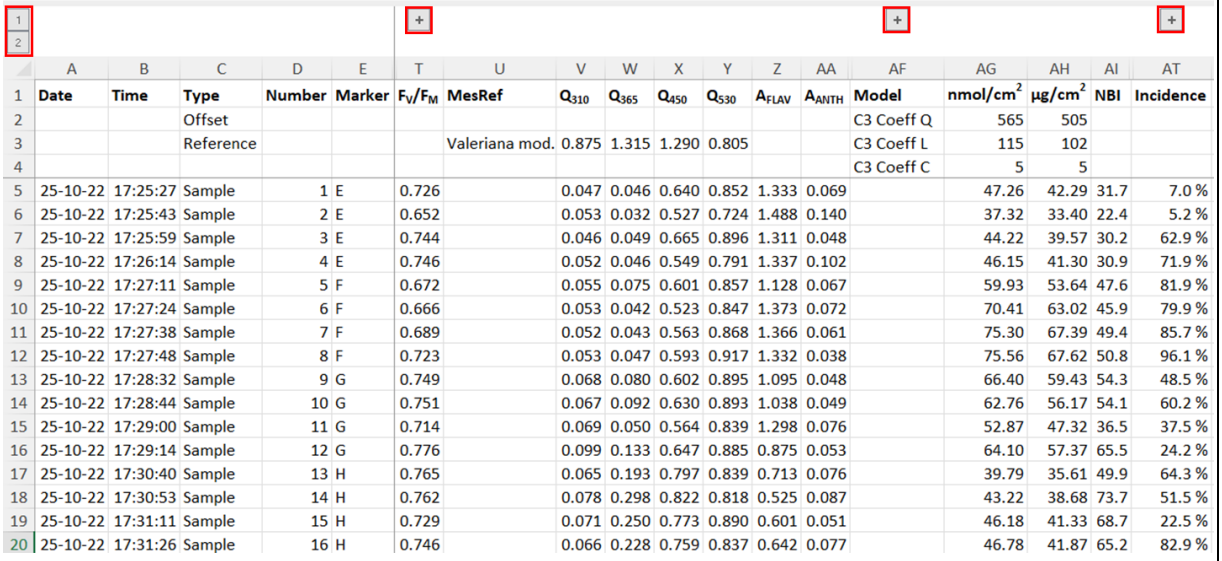
5.3.5 Excel File

The Excel file contains two sheets called “Measure” (Fig. 14, page 31) and “SAT Chart” (Fig. 15, Page 32). The initial view of the sheet “Measure” displays the basic sample properties and the incidence value (see Fig. 26, page 61). Table 20 (page 33) explains the abbreviations used.

The SAT Chart sheet contains the fluorescence transients from which the F_0 and F_M levels are derived (Fig. 15, page 32). Instructions on how to graph this data are given in the figure legend.

The sheet “Measure” contains all data used for the calculations of the sample properties, and of the incidence value. The buttons to display all data are highlighted by a green frame in Fig. 14.

The [2] button on the top right corner fully unfolds the Excel spreadsheet, the [+] above column R reveals all fluorescence data (Table 21, page 34 and Table 22, page 35), the [+] above column AD reveals all absorption data of chlorophyll determination, and the [+] above column AR reveals all geodata. All data are explained in Table 21, page 34 to Table 23, page 35.



	A	B	C	D	E	T	U	V	W	X	Y	Z	AA	AF	AG	AH	AI	AT
1	**Date**	**Time**	**Type**	**Number**	**Marker**	**F_V/F_M**	**MesRef**	**Q_{310}**	**Q_{365}**	**Q_{450}**	**Q_{530}**	**A_{FLAV}**	**A_{ANTH}**	**Model**	**nmol/cm²**	**µg/cm²**	**NBI**	**Incidence**
2			Offset											C3 Coeff Q	565	505		
3			Reference				Valeriana mod.	0.875	1.315	1.290	0.805			C3 Coeff L	115	102		
4														C3 Coeff C	5	5		
5	25-10-22	17:25:27	Sample	1	E	0.726		0.047	0.046	0.640	0.852	1.333	0.069		47.26	42.29	31.7	7.0 %
6	25-10-22	17:25:43	Sample	2	E	0.652		0.053	0.032	0.527	0.724	1.488	0.140		37.32	33.40	22.4	5.2 %
7	25-10-22	17:25:59	Sample	3	E	0.744		0.046	0.049	0.665	0.896	1.311	0.048		44.22	39.57	30.2	62.9 %
8	25-10-22	17:26:14	Sample	4	E	0.746		0.052	0.046	0.549	0.791	1.337	0.102		46.15	41.30	30.9	71.9 %
9	25-10-22	17:27:11	Sample	5	F	0.672		0.055	0.075	0.601	0.857	1.128	0.067		59.93	53.64	47.6	81.9 %
10	25-10-22	17:27:24	Sample	6	F	0.666		0.053	0.042	0.523	0.847	1.373	0.072		70.41	63.02	45.9	79.9 %
11	25-10-22	17:27:38	Sample	7	F	0.689		0.052	0.043	0.563	0.868	1.366	0.061		75.30	67.39	49.4	85.7 %
12	25-10-22	17:27:48	Sample	8	F	0.723		0.053	0.047	0.593	0.917	1.332	0.038		75.56	67.62	50.8	96.1 %
13	25-10-22	17:28:32	Sample	9	G	0.749		0.068	0.080	0.602	0.895	1.095	0.048		66.40	59.43	54.3	48.5 %
14	25-10-22	17:28:44	Sample	10	G	0.751		0.067	0.092	0.630	0.893	1.038	0.049		62.76	56.17	54.1	60.2 %
15	25-10-22	17:29:00	Sample	11	G	0.714		0.069	0.050	0.564	0.839	1.298	0.076		52.87	47.32	36.5	37.5 %
16	25-10-22	17:29:14	Sample	12	G	0.776		0.099	0.133	0.647	0.885	0.875	0.053		64.10	57.37	65.5	24.2 %
17	25-10-22	17:30:40	Sample	13	H	0.765		0.065	0.193	0.797	0.839	0.713	0.076		39.79	35.61	49.9	64.3 %
18	25-10-22	17:30:53	Sample	14	H	0.762		0.078	0.298	0.822	0.818	0.525	0.087		43.22	38.68	73.7	51.5 %
19	25-10-22	17:31:11	Sample	15	H	0.729		0.071	0.250	0.773	0.890	0.601	0.051		46.18	41.33	68.7	22.5 %
20	25-10-22	17:31:26	Sample	16	H	0.746		0.066	0.228	0.759	0.837	0.642	0.077		46.78	41.87	65.2	82.9 %

Fig. 14: Measure Chart

Original Excel spreadsheet created by the LSA software. Click on the red bordered buttons for more data. The + button above column T displays the data used for calculation of screening and F_V/F_M. Click on the + above column AF to see the data used for calculation of chlorophyll concentration. All geodata become visible by clicking on the + above column AF. The 2 in the top left corner to makes all data visible. Click 1 in the top left corner to return to the original view. For details see Table 20 to Table 23.

	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

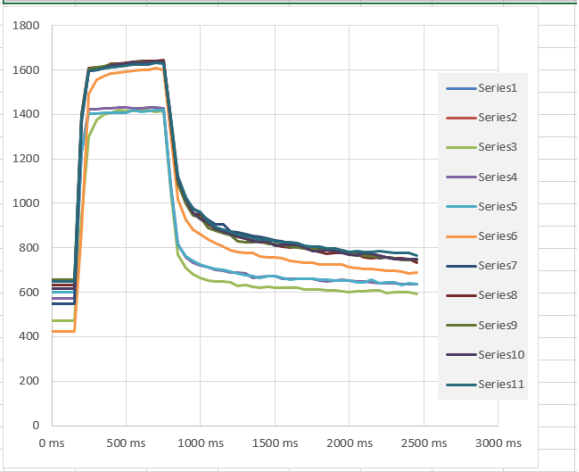


Fig. 15: SAT Chart

The “SAT Chart” contains the data of fluorescence transients used for 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 20: Results		
Column titles of the initial view of the Excel spreadsheet created by the LSA-2050 software (see Fig. 14, page 31)		
Column	**Title**	**Comment**
A	**Date**	Date in the format YY-MM-DD
B	**Time**	Time in the format hh:mm:ss
C	**Type**	Indicator of data source.
D	**Number**	Consecutive numbering of measurements.
E	**Marker**	Sample identifier.
T	**F_V/F_M**	Maximum photochemical quantum yield of photosystem II.
V W X Y	**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.)
Z	**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.
AA	**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.
AG	**nmol/cm²**	Concentration of Chl *a* + Chl *b*
AH	**µg/cm²**	Concentration of Chl *a* + Chl *b*
AI	**NBI**	Nitrogen balance index.
AT	**Incidence**	Cosine of the angle at which solar radiation hits the leaf.

Table 21: Calibration Data

Click [+] or [2] to see all data (compare Table 20, page 33).

Row	Title	Comment
2 Offset	I_{310} I_{Fo} I_{365} I_{Fm} I_{450} I_{530} I_{700} I_{615} I_{770}	Background signal (Offset) for all LEDs and methods (system property determined by calibration).
3 Reference	I_{310} F_{310} I_{365} F_{365} I_{450} F_{450} I_{530} F_{530} I_{615} F_{615}	I_λ: Original signal at wavelength λ measured with the fluorescence standard. F_λ: I_λ corrected by the background signal.
3 Reference	Q_{310} Q_{365} Q_{450} Q_{530}	Mesophyll reference factors (see 7.4.1, page 53).
2, 3, and 4	**nmol/cm²** **µg/cm²**	Coefficients for calculating the molar and weight concentrations of Chl *a* + Chl *b* (see Section 7.2.4, page 47). In column AF, “C3” and “C4” indicate which calibration curve was used to calculate concentrations; “Coeff Q” is the coefficient of the quadratic term, “Coeff L” is the coefficient of the linear term of the calibration curve, and “Coeff C” is a constant (See Section 7.1, page 43).

Table 22: Source Data 01

Data from row 4 onwards. Click + or 2 to see all data (compare Table 20, page 33).

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 20 (page 33) 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 was offset-corrected prior to calculation.
Absorbance	$-\log_{10}$ Transmittance.

Table 23: Source Data 02

Data from row 4 onwards. Click + or 2 to see all data (compare Table 20, page 33).

Title	Comment
Satellite #	Number of GPS satellites.
DoP	Dilution of precision. The number indicates the precision with which the GPS determines a position (<1, high precision. >20, very poor confidence).
Latitude **Longitude**	Coordinate parameters for a point on the Earth surface given in decimal degrees.
Height	Height above sea level in meters.
Leaf Az.	Leaf azimuth. See Fig. 25, page 60.
Leaf Sl.	Leaf slope. See Fig. 24, page 60.
Sun Az.	Sun azimuth. See Fig. 23, page 59.
Sun Elev.	Sun elevation. See Fig. 23, page 59.
AoI	Angle of incidence. See Fig. 26, page 61.
Incidence	Cosine of angle of incidence. See Fig. 26, page 61.

6 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.

6.1 F_V/F_M

Kitajima and Butler (1975) have measured at low temperature (77 K), the effect of a fluorescence-quenching chemical on the fluorescence ratio of F_V/F_M. They have mathematically derived that the F_V/F_M is a measure for 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 photochemistry can be as low as 0.76 (Pfündel 1998, Pfündel et al. 2013). Guidi et al. (2019) have provided a compilation of F_V/F_M values in various species.

When F_V/F_M values are significantly lower 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

Guidi L, Lo Piccolo E, Landi M (2019) Chlorophyll fluorescence, photoinhibition and abiotic stress: does it make any difference the fact to be a C3 or C4 Species? Front Plant Sci 10:174. https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2019.00174/full

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

6.2 Screening

The fundamental articles of radiation-screening substances in the leaf are listed in Section 7.4, page 51. 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 (no screening).

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

6.3 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 reflect 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

6.4 Nitrogen Balance Index (NBI),

The NBI has been defined as the concentration ratio of chlorophyll to UV-absorbing phenolics, mainly flavonoids (see papers below). The NBI was formulated on the basis that the chlorophyll content increased with nitrogen supply and the phenolic content tended to decrease in parallel. Because of these opposing relationships, the NBI potentially responds more sensitive and robust to the plant nitrogen status than the individual concentrations of chlorophyll and phenolics.

The NBI is expected to increase with nitrogen supply. The relationship between NBI and nitrogen status, however, may vary between species and even cultivars of the same species. Therefore, universal NBI values indicating optimum nitrogen supply are not available but must be experimentally established. However, once the NBI for optimal nitrogen supply has been established for a plant culture, the NBI can be a valuable tool for matching fertilization to crop requirements.

Cartelat A, Cerovic ZG, Goulas Y, Meyer S, Lelarge C, Prioul J-L, Barbottin A, Jeuffroy M-H, Gate P, Agati G, Moya I (2005) Optically assessed contents of leaf polyphenolics and chlorophyll as indicators of nitrogen deficiency in wheat (Triticum aestivum L.). Field Crops Res 91: 35–49. https://doi.org/10.1016/j.fcr.2004.05.002

Cerovic ZG, Ben Ghozlen N, Milhade C, Obert M, Debuisson S, Le Moigne M (2015) Nondestructive Diagnostic test for nitrogen nutrition of grapevine (Vitis vinifera L.) based on Dualex leaf-clip measurements in the field. J Agric Food Chem 63 (14): 3669–3680. https://doi.org/10.1021/acs.jafc.5b00304

7 Documentation

7.1 Darkening Clip LSA-2050/DLC

When using the Darkening Clip LSA-2050/DLC for measurements, instrument calibration must be performed with this clip. Relative to calibration without the clip, the clip reduces offset values between 33 and 25% except the offsets at 700 and 770 nm which changed little (Table 24). The clip did not affect the fluorescence signals but reduced the signal of both chlorophyll measuring lights by about 70%.

When properly calibrated, the data obtained with clip agreed reasonably well with data obtained without clip. Data measured with the clip were maximally about 10% higher than that measured without clip (**Table 25**). When data obtained with clip are plotted against data without clip, linear relationships were observed with high coefficient of determination (R^2, Fig. 16).

Table 24: LSA-2050/DLC Effect on Offset and Calibration

	Darkening Clip		I_{Fo}	I_{Fm}	I_{310}	I_{365}	I_{450}	I_{530}	I_{615}	I_{700}	I_{770}
Offset	Without	Mean (n=8)	47.5	47.5	74.5	46.1	21.6	81.3	82.4	1.8	1.4
		95% CI	2.8	2.8	2.6	3.0	1.1	5.6	7.1	0.4	0.4
	With	Mean (n=11)	32.0	32.0	62.6	33.0	15.2	54.5	70.0	1.8	1.3
		95% CI	2.1	2.1	1.4	1.4	1.1	4.2	4.5	0.3	0.3
Fluorescence and chlorophyll	Without	Mean (n=8)			1268	1288	1262	1254	1227	792	899
		95% CI			7	9	3	3	8	29	27
	With	Mean (n=11)			1252	1270	1254	1229	1215	244	288
		95% CI			7	8	3	1	5	4	8

Table 25: With Clip/Without Clip Data Ratios							
With Clip/Without Clip	**F_V/F_M**	**Q_{310}**	**Q_{365}**	**Q_{450}**	**Q_{530}**	**nmol/cm²**	**NBI**
Mean (n=126)	1.02	1.07	1.05	1.03	1.01	1.09	1.11
95% CI	0.01	0.02	0.02	0.01	0.01	0.03	0.04

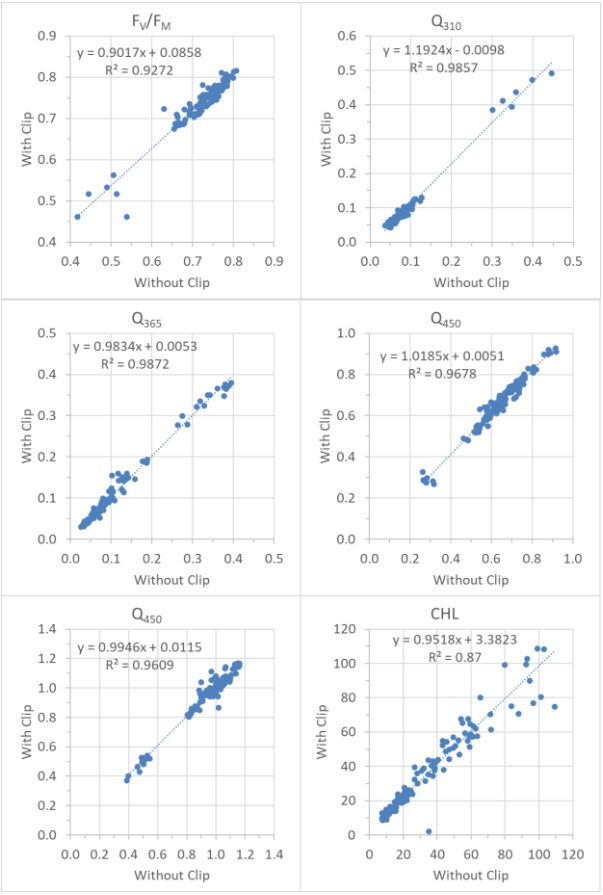


Fig. 16: Data with Clip Plotted against Data Without Clip

The results of linear regression analyses are shown.

7.2 Chlorophyll Calibration

7.2.1 Plant Species

Four plant species were investigated (Table 26). The species were chosen to represent monocot (*T. aestivum*, *Z. mays*) and dicot leaf anatomy (*H. annuus, N. tabacum*). The species also represent different types of photosynthesis with different Chl *a*/Chl *b* concentration ratios (Table 26).

Table 26: Plant Species used for Chl Calibration

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

7.2.2 Growth Conditions

In case of the species *H. annuus*, *T. aestivum*, and *Z. mays,* (A, B, and D, in Table 26) varying chlorophyll contents were achieved by five different growth conditions (Table 27). In another experiment, leaves of *N. tabacum* cv. Samsun NN were probed. These plants had senescing leaves with very low chlorophyll contents.

Table 27: Growth Conditions

H. annuus, *T. aestivum*, and *Z. mays*			
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.

7.2.3 Chl. Measurements

In the first experiment (species A, B, and D, in Table 26), 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. (2012).

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):

Individual pigments were separated and quantified by high-performance liquid chromatography (HPLC, Fig. 17) according to Färber et al. (1997).

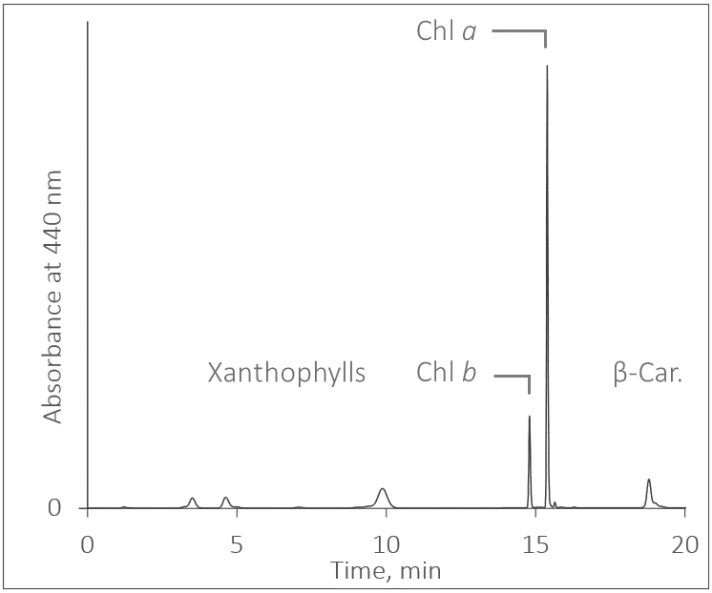


Fig. 17: Chromatogram for Pigment Determination

In *N. tabacum*, pigment extraction was as the forgoing experiment, but chlorophyll quantification was spectrometrically according to Lichtenthaler and Buschmann (2001).

7.2.4 Correlations

The data of the three species with C_3 photosynthesis could be described by a single function. Curvilinear relationships 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 28).

The relationship of the C_4 plant differed from that of the C_3 species: the same absorbance value yielded a lower Chl *a* + Chl *b* concentration compared to the relationship for C_3 plants. This may originate in relatively low abundance of Chl *b* in the C_4 plant and the fact that the LSA-2050 measures mainly Chl *a* light absorption (compare Fig. 18). Also, the optical properties of the C_4 Kranz anatomy could have affected the correlation for *Z. mays*.

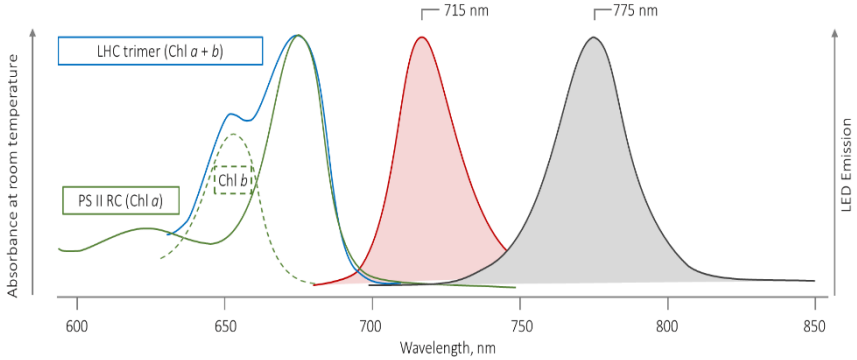
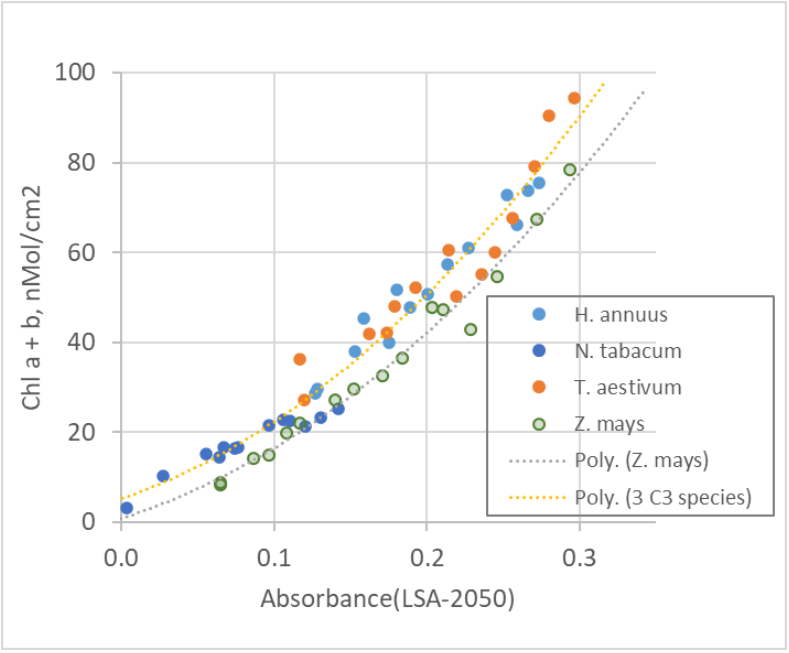


Fig. 18: Chlorophyll determination wavelengths

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 et. al (2013)

Table 28:	**Chl. Correlations**	
Species	Chl *a* + *b*, nMol/cm^2	Chl *a* + *b*, µg/cm^2
H. annuus, *N. tabacum*, *T. aestivum*	$y = 565x^2 + 115x + 5$ $R^2 = 0{,}964$	$y = 505x^2 + 102x + 5$
Z. mays	$y = 501x^2 + 106x + 1$ $R^2 = 0.981$	$y = 449x^2 + 95x + 1$

x=absorbance as measured by an LSA-2050.



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

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)

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

Lichtenthaler HK, Buschmann C (2001) Chlorophylls and carotenoids - Measurement and characterisation by UV-VIS. Current Protocols in Food Analytical Chemistry (CPFA), John Wiley, New York, 2001, Supplement 1 pp. F4.3.1eF4.3.8.
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

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

7.3 Chlorophyll and Small Leaves

To measure chlorophyll concentration on small leaves, the emitter aperture can be reduced to a spot of 6 mm diameter without affecting the chlorophyll measurements.

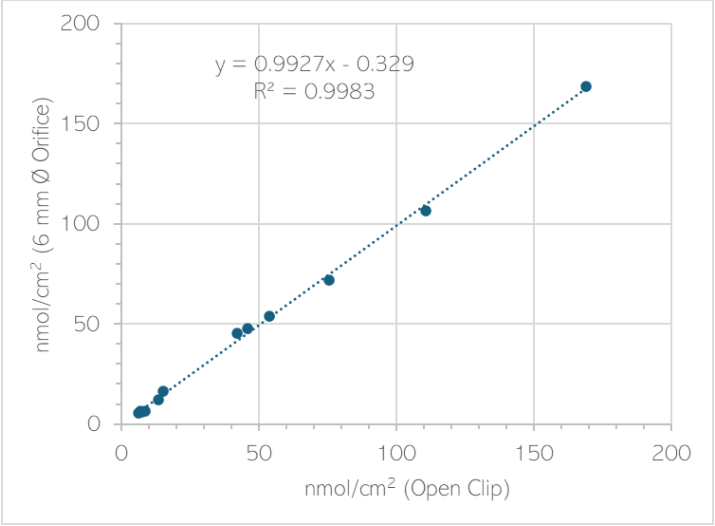


Fig. 19: Chlorophyll Determination with Small Leaves

Various samples were probed with the default configuration of the LSA-2050, and then again with the emitter opening reduced to a spot of 6 mm diameter. The graph shows the latter data plotted against the corresponding former data. The straight line, its equation and the R^2 result from linear regression.

7.4 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 taken 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 29. The LED emission spectra a shown in Fig. 20.

Table 29: 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

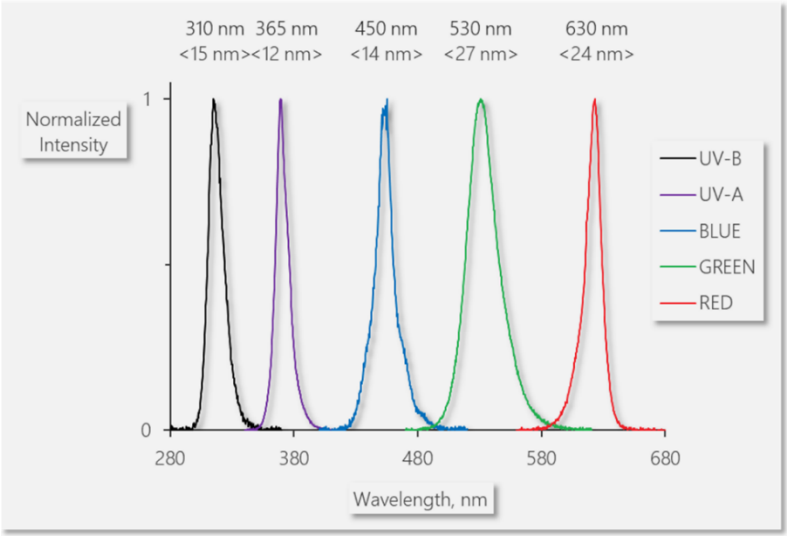


Fig. 20: Emission spectra of LEDs involved in screening measurements

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

7.4.1 Mesophyll Reference Factor *RF*(λ)

To estimate the radiation screening of the leaf mesophyll, measurements of intact leaves must be related to measurements of unscreened mesophyll tissue. For this, five plant species with removable epidermis were selected (Table 30, page 55). For *H.*

telephium, both the upper and the lower leaf sides were probed. For the other species, either the upper or the lower leaf side was probed.

Fig. 21: Leaf of *H. telephium* with Epidermis Partially Removed

MES, free mesophyll. EP, stripped 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, and $I(630)$ is the fluorescence intensity excited at 630 nm. The λ represents one of the four wavebands: UV-B, UV-A, blue, or green (Fig. 20, page 53).

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 30: Mesophyll Reference Factors

Mean values and 95% confidence intervals (95% CI) are given. *RF*(λ) is defined by Eq. 2. In the LSA-2050, the *RF*(λ) is denoted as "Mesophyll Reference". The *RF*(λ) can be selected via Main Menu→Settings→Mesophyll Type. In the Excel export file, the *RF*(λ) values are written in cells T3 to W3.

Species Family	Leaf side	n		*RF*(310)	*RF*(365)	*RF*(450)	*RF*(530)
Hylotelephium telephium *Crassulaceae*	Upper	51	Mean	0.573	1.105	1.055	0.809
			95% CI	0.026	0.024	0.015	0.006
Hylotelephium telephium Crassulaceae	Lower	30	Mean	0.863	1.285	1.160	0.807
			95% CI	0.048	0.040	0.017	0.008
Kalanchoe daigremontiana Crassulaceae	Upper	10	Mean	0.689	1.208	0.937	0.844
			95% CI	0.061	0.041	0.030	0.047
Spinacia oleracea Amaranthaceae	Lower	10	Mean	0.738	1.146	1.146	0.734
			95% CI	0.072	0.040	0.038	0.045
Tulipa spec. Liliaceae	Upper	40	Mean	0.690	0.852	1.023	0.807
			95% CI	0.039	0.049	0.012	0.007
Valerianella locusta Caprifoliaceae	Lower	16	Mean	0.875	1.315	1.290	0.688
			95% CI	0.055	0.053	0.064	0.017
Valerianella locusta mod Caprifoliaceae	Lower	16	Mean	0.875	1.315	1.290	0.805
			95% CI	0.055	0.053	0.064	0.025

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.

Among the reference factors measured, *Valerianella locusta*, lower leaf side, exhibited the highest *RF(310)*, *RF(365),* and *RF(450)* values (Table 30). These values agreed reasonably well with that obtained for *Hylotelephium telephium*, lower leaf side. The corresponding lower *RF*(λ) from the other samples might be caused by residual radiation screening after the epidermis was removed.

However, the *RF(530)* of *Valerianella locusta* was noticeably lower than that from the other samples resulting in Q_{530} values > 1 in control leaves without anthocyanins. Therefore, this *RF(530)* was replaced by a *RF(530)* established with intact control leaves of more than 10 species.

"*Valerianella locusta* mod" denotes the series of *RF*(λ) values of *Valerianella locusta* with *RF(530)* replaced by measurements with intact leaves (Table 30, lowest line). "*Valerianella locusta* mod" is the recommended default setting.

7.4.2 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 LED intensities of the LSA-2050 instrument used.

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

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

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

7.5 Geodata

Fig. 22 gives an overview of 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. 23, 59).

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. 24 (page 60) and Fig. 25 (page 60), respectively.

From the positions of sun and leaf, the angle at which sun radiation hits the leaf is derived (angle of incidence, Fig. 26, page 61). 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.

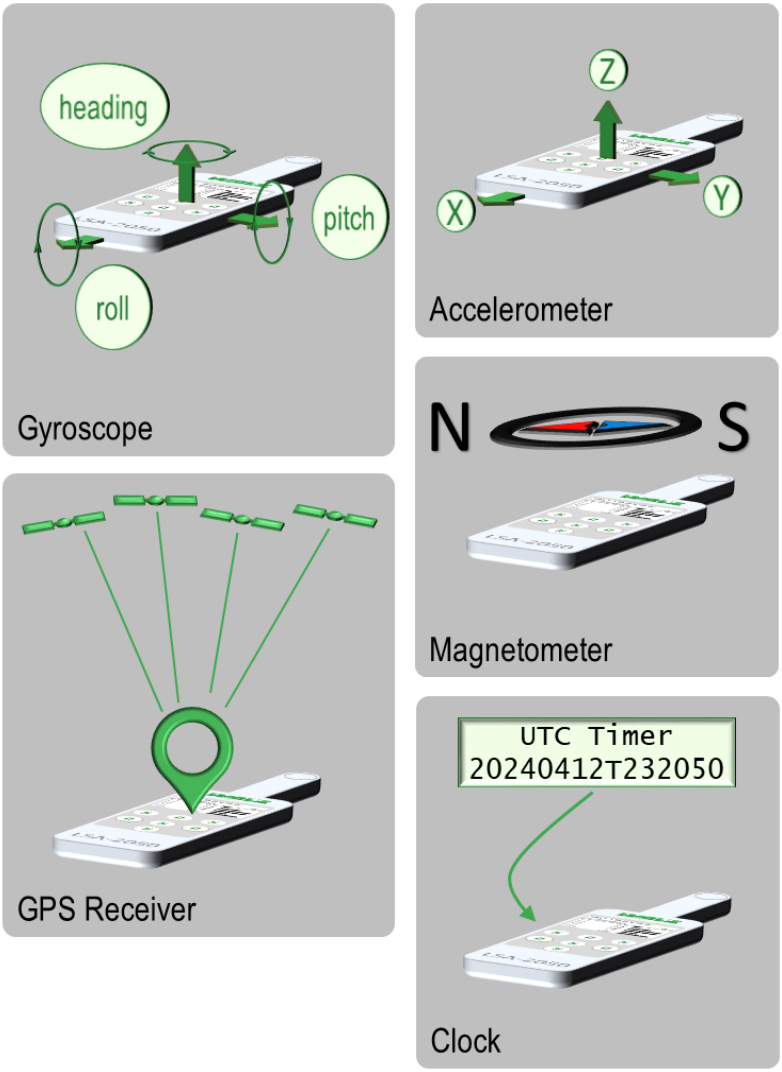


Fig. 22: Geodata Hardware

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.

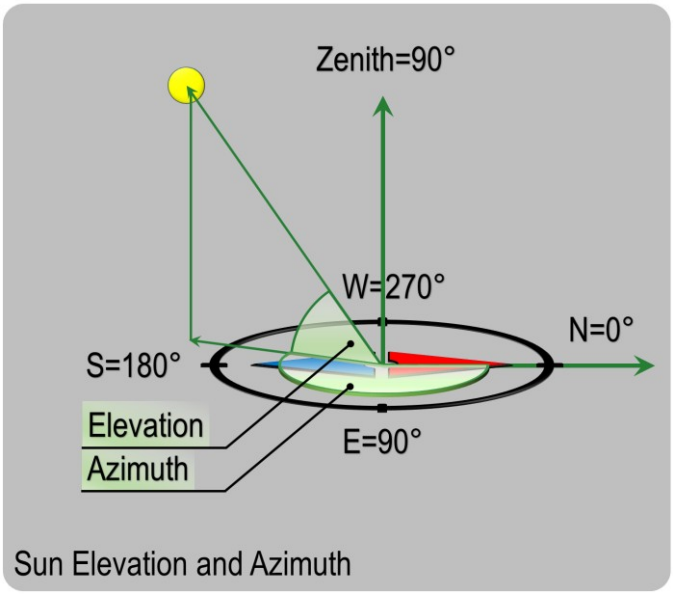


Fig. 23: Sun Position

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).

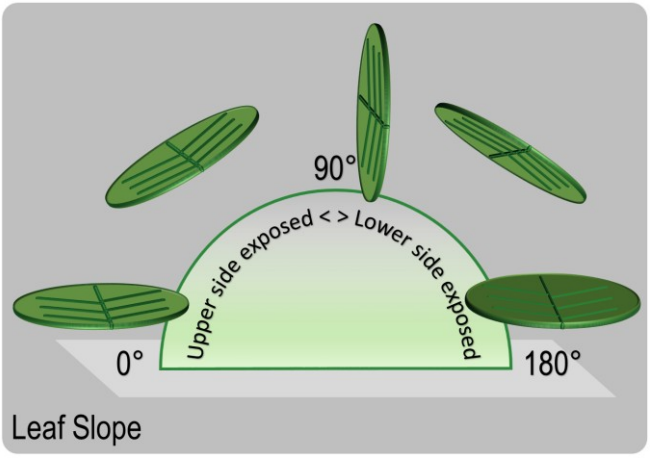


Fig. 24: Leaf Slope

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.

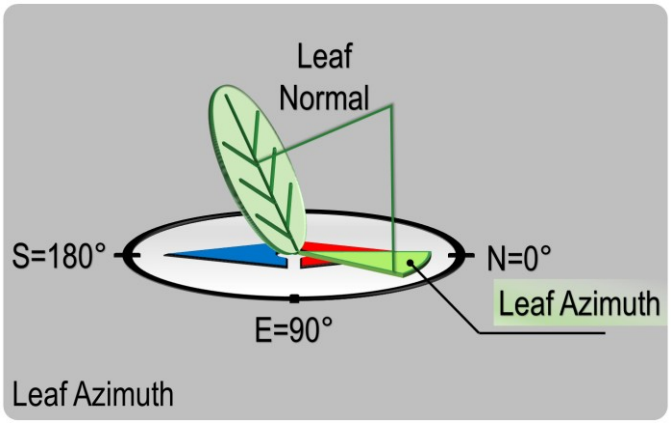


Fig. 25: Leaf Azimuth

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.

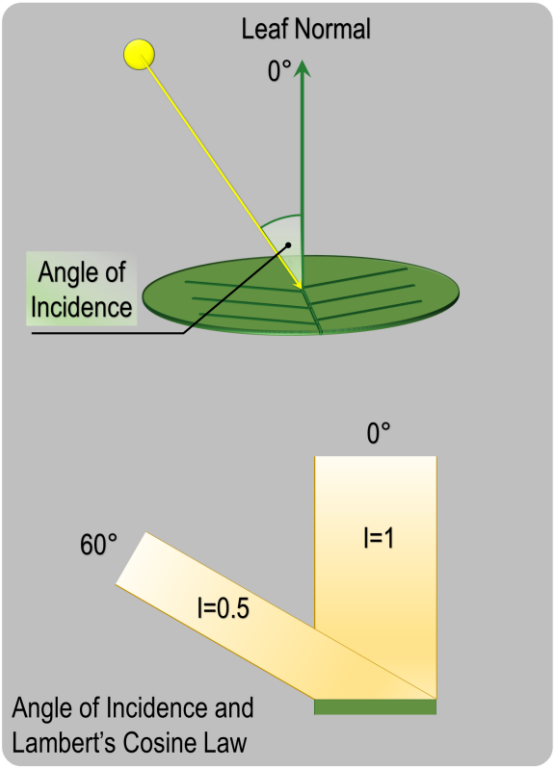


Fig. 26: Angle of Incidence

The angle at which solar radiation hits the leaf (angle of incidence) determines the relative effective radiation intensity on the leaf surface. This angle is defined as the deviation from the sheet normal, where perpendicular and horizontal incident radiation have angles of 0° and 90° respectively. The effective intensity at leaf surface equals the cosine of the angle of incidence (Lambert's Cosine Law).

8 Specifications

Specifications are subject to change without notice.

8.1 LEAF-STATE-ANALYZER LSA-2050

8.1.1 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)

8.1.2 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 area. The LEDs are covered by a light-diffusing disk and a quartz disk

8.1.3 Geospatial Data

A GPS receiver, plus accelerometer, gyro- and magneto-scope sensors add geospatial information to each measurement, including the angle at which sunlight impinges on the leaf. Devices are outlined in Fig. 22, page 58.

8.1.4 Walz Calibration Standards LSA-2050/STD

Fluorescence: Special fluorescence layer mounted on aluminum carrier

Offset: Non-fluorescent and untransparent black plastic

Scattering: Special plastic film for scattering of radiation

8.1.5 Viewing area reduction kit

Measuring area reduction ring 000247003214

Material: Polyvinyl chloride adhesive film

Dimensions: Outer diameter, 13 mm. Hole diameter, 6 mm

Weight: < 1 g

Centering tool 000247003014

Design: Cylinder made of polylactic acid, with recess for a measuring area reduction ring at one base, a small stamp on the side, and two openings for the plastic pegs of the pres-on tool.

Dimensions: Diameter, 23 mm. Height 15 mm.

Weight: < 1 g

Pres-on tool 000247003114

Design: Bar with two plastic pegs made of polylactic acid

Dimensions: 18 mm x 5 mm x 18 mm (L x W x H)

Weight: < 1 g

8.2 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

8.3 Battery Charger

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

8.4 Accessories

8.4.1 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)

8.4.2 Darkening Clips LSA-2050/DLC

General Design: One-piece construction made of polyamide (PA12), the two jaws of the clip are pressed together by the elasticity of the material and by two neodymium magnets, each jaw has a sliding shutter for pre-darkening and an 11 mm diameter opening for chlorophyll determination

Dimensions: 86 mm x 25 mm x 35 mm (L x W x H)

Weight: 10 g

9 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).

9.1 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.

9.2 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.

9.3 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.

9.4 Applicable law

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

10 Index

Accessories 66
Active Tests 20
Adjusting Time 28
Angle of Incidence 61
Anthocyanins 51
Battery 8, 63
Battery Charger 66
Bilger 6
C4 plant 48
Calibration 22
Calibration and Measurement 56
Calibration Data 34
Calibration Standards 65
Calibration with LSA-2050/DLC Clip 23
Calibration without LSA-2050/DLC Clip 23
Carotenoids 52
Carrying Case 66
Cerovic 6
Chl. Correlations 49
Chl. Measurements 46
Chl. Settings 21
Chlorophyll and Small Leaves 50
Chlorophyll Calibration 45
Chlorophyll Determination with Small Leaves 51
Chromatogram for Pigment Determination 47
Connecting the LSA-2050 to Computer 28
Contents 1
Create New File 24
Darkening Bags LSA-2050/DB 13, 66
Darkening Clip LSA-2050/DLC 14, 43
Darkening Clips LSA-2050/DLC 67
Data with Clip Plotted against Data Without Clip 44
Detailed Instructions 16
Device Info 26
Device Settings 22
Documentation 43
Downloading Data 30
Epidermis 54
Excel File 30, 31
Extent of Delivery 7
Flavonoids 53
Fluorescence and Offset Standards 8
FTDI USB Driver 27
Geodata 19, 57
Geodata Hardware 58
Geospatial Data 64
Growth Conditions 45
Guarantee 68
Hydroxycinnamic acids 53
Installing Software 26
Introduction 5
Keys 15
Lambert's Cosine Law 61
Leaf Slope 60
LED Combinations 53
Local Time 29
LSA 2050 Specifications 63
LSA Download/Time Windows 29
LSA-2050 Description 10
LSA-2050 Info 26
LSA-2050 Measuring Head 11

LSA-2050 Modified for Bulky Samples 12
LSA-2050 Overview ... 11
LSA-2050 Software ... 26
LSA-2050 Window ... 16, 25
LSA-2050/DLC Effect on Offset and Calibration ... 43
Main Menu ... 19
Main Windows ... 16
Main Windows Top Line ... 17
Marker ... 26
Measure Chart ... 31
Memory ... 24, 63
Memory Window Geodata ... 25
Memory Window LSA-2050 ... 25
Memory Window Sat Pulse Data ... 25
Mesophyll Reference ... 21
Mesophyll Reference Factor ... 53
Mesophyll Reference Factors RF ... 55
N Status Window ... 18
Operating Temperature ... 63
Operation ... 15
Orient. Sens. Calibration ... 24
Plant Species ... 45
Plant Species used for Chl Calibration 45
Quick Start ... 16
Reduction Ring Mounting ... 9
References ... 6
Results ... 33
Safety Instructions ... 3
SAT Chart ... 32
Sat Pulse Data ... 18
Schreiber ... 6
Screening ... 51
Select Marker ... 26
Settings ... 20
Sheet Measure ... 30
Source Data 01 ... 35
Source Data 02 ... 35
Specifications ... 63
Standards ... 8
Sun Position ... 59
Time Setting ... 29
USB Cable ... 8
UTC ... 29
UV-A ... 51
UV-B ... 51
Viewing Area ... 64
Viewing area reduction kit ... 65
Wavelengths Chl Determination ... 48
With Clip/Without Clip Data Ratios ... 44

11 List of Figures

Fig. 1:	Fluorescence and Offset Standards	8
Fig. 2:	Reduction Ring Mounting	9
Fig. 3:	LSA-2050 Overview	11
Fig. 4:	LSA-2050 Measuring Head	11
Fig. 5:	LSA-2050 Modified for Bulky Samples	12
Fig. 6:	Darkening Bags LSA-2050/DB	13
Fig. 7:	Darkening Clip LSA-2050/DLC	14
Fig. 8:	Keys	15
Fig. 9:	Calibration without LSA-2050/DLC Clip	23
Fig. 10:	Calibration with LSA-2050/DLC Clip	23
Fig. 11:	Installation Window	27
Fig. 12:	Access to USB-C Port	28
Fig. 13:	LSA Download/Time Windows	29
Fig. 14:	Measure Chart	31
Fig. 15:	SAT Chart	32
Fig. 16:	Data with Clip Plotted against Data Without Clip	44
Fig. 17:	Chromatogram for Pigment Determination	47
Fig. 18:	Chlorophyll determination wavelengths	48
Fig. 19:	Chlorophyll Determination with Small Leaves	51
Fig. 20:	Emission spectra of LEDs involved in screening measurements	53
Fig. 21:	Leaf of H. telephium with Epidermis Partially Removed	54
Fig. 22:	Geodata Hardware	58
Fig. 23:	Sun Position	59
Fig. 24:	Leaf Slope	60
Fig. 25:	Leaf Azimuth	60
Fig. 26:	Angle of Incidence	61

12 List of Tables

Table 1:	LSA-2050 Window	16
Table 2:	Main Windows Top Line	17
Table 3:	N Status Window	18
Table 4:	Sat Pulse Data	18
Table 5:	Geodata	19
Table 6:	Main Menu	19
Table 7:	Settings	20
Table 8:	Active Tests	20
Table 9:	Chl. Settings	21
Table 10:	Mesophyll Reference	21
Table 11:	Device Settings	22
Table 12:	Calibration	22
Table 13:	Orient. Sens. Calibration	24
Table 14:	Memory Window LSA-2050	25
Table 15:	Memory Window N Status	25
Table 16:	Memory Window Sat Pulse Data	25
Table 17:	Memory Window Geodata	25
Table 18:	Select Marker	26
Table 19:	LSA-2050 Info	26
Table 20:	Results	33
Table 21:	Calibration Data	34
Table 22:	Source Data 01	35
Table 23:	Source Data 02	35
Table 24:	LSA-2050/DLC Effect on Offset and Calibration	43
Table 25:	With Clip/Without Clip Data Ratios	44
Table 26:	Plant Species used for Chl Calibration	45
Table 27:	Growth Conditions	46
Table 28:	Chl. Correlations	49
Table 29:	LED Combinations	53
Table 30:	Mesophyll Reference Factors	55