

USER'S GUIDE



MULTIPLEX® 3

Hand-held multi-parameter optical sensor

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1. Description

Multiplex® 3 is a hand-held multi-parameter optical sensor based on noncontact leaf and fruit autofluorescence measurements. It is operating on a long-life rechargeable battery.

Based on 15 years of research on plant autofluorescence, the Multiplex® 3 sensor provides accurate and complete information on the physiological state of the crop.

Advantages of the technology are:

- simultaneous acquisition of several optical signatures;
- real-time non contact sensing;
- active sensing, measurements even possible at night;
- no preparation of the plant.

Multiplex® 3 provides optical signatures of:

- chlorophyll content;
- content of constitutive and induced polyphenolics;

Thanks to its proprietary acquisition system and software, the Multiplex® 3 sensor is completely autonomous for easy and rapid measurement in the fields. The graphic LCD with touch screen is a friendly user interface and a real-time data display.

The measurements and pre-processed data are directly stored in the instrument memory (SD card), and can be transferred to a computer for further statistical analysis and presentation.

The instrument allows the acquisition of a large number of data in a short time, thus opening new tracks for plant research.

An incorporated GPS module allows geo-referencing of the data and mapping.

2. Applications

Associated with other agronomic data, Multiplex® 3 is designed to provide input for decision support systems (DSS) for a range of crops, concerning:

- fertilisation needs (nutrients spraying);
- crop quality forecast.

As a stand alone sensor it is a valuable tool for ecophyiological research, for a rapide acquisition of information on sample chlorophyll and flavonoid contents. Associated to a computer with its kinetics software it is a high performance, large surface, multi-wavelength fluorometer (variable chlorophyll fluorescence, PSI/PSII emission ratio, blue-green fluorescence, chlorophyll fluorescence excitation ratios - FER).

3. Measurement type

Samples	Any plant material: leaves, coniferous needles, crops, turf, fruits, vegetables, grains, etc.
Measured parameters	 Epidermal UV absorbance by FER method: flavonol content Epidermal Visible absorbance by FER method: anthocyanin content Chlorophyll fluorescence emission ratio: chlorophyll content UV-excited Blue-Green fluorescence (BGF) Other fluorescence ratios
Measurement area	< 10 cm diameter
Measurement distance	10 cm
Light sources	Light Emitting Diodes (LED) ; pulsed operation 4 excitation channels: UV, Blue, Green and Red
Detectors	Silicon photodiodes 3 detection channels: Yellow (or Blue), Red and Far-Red

4. General Description

• Multiplex®

The Multiplex® 3 sensor is composed of two parts:

- the optical head which contains optical sources (UV, blue, green and red) and detectors (blue-green, red and far-red (NIR)) (Figure 1);
- the battery with its charger (Figure 2).





Figure 1: the Multiplex®

Figure 2: the battery and the charger

Accessories

The different available accessories with the Multiplex® 3 sensor are:

- the GPS antenna (Figure 3);
- the charger;
- the anodized aluminium holders (Figure 4);
- the anodized aluminium black mask (Figure 5).



Figure 3: the GPS antenna



Figure 4: Accessory for grape berries (50 cells and 100 cells)



Figure 5: Black mask for better target definition (for example grape clusters)

5. Getting started



Figure 6: Battery connector and power switch First, plug the battery in the optical head (right connector - Figure 6).

Check that the SD card (FAT formatted) is inserted on the left edge of the acquisition system (Figure 7).

Finally, press the power switch on the left of the optical head (Figure 6).



Figure 7: SD card and USB connector

The GPS antenna is connected to the sensor through a standard SMA connector (Figure 8).



Figure 8: GPS connector



Figure 9: Multiplex in use.



Figure 10: Multiplex in front use.

6. Using the Multiplex®

When the sensor starts, you can see the *main* screen (Figure 11). Measurements are performed by pressing the trigger button on the handle.

Please note that measurements can only be performed when you are in the *main* screen configuration.



Figure 11: main screen

When the measure has finished, four values are displayed by default on the screen (Figure 12):

- Flav (Flavonol content);
- Anth (Anthocyanin content);
- SFR_R (Simple Fluorescence ratio Chlorophyll dependent);
- NBI_G (Nitrogen Balance Index).

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Figure 12: first acquisition

You can access to all the data by pressing one of the four values on the touch screen (Figure 13). Please note that ALL the data are stored into an ASCII-file (spreadsheet-ready format) on the SD-card.

Anth	FE	R_RUV	10:49
8.125	4	. 86	
BKR	AB	1_k	cfg)
	0	26	stat)
20	P	1 sam	i AB M f

Figure 13: touch screen

In case of weak ambient-light condition, you can switch on the backlight of the screen by pressing the time button on the top-right edge of the screen. Backlight uses much power, so limit its use only to set up the instrument. Also *do not use backlight during measurement*.

• Organize your data during the experiment

Data can be organised by using group specification as file, population and sample (Figure 14).

Each time you start Multiplex®, a new file is systematically created.

The first measurement (noted as measure 1) will belong to the population 1 and the sample 1. These numbers are available on the *main* screen.

If you want to change of file, population or sample, you can touch their button respectively, by pressing the touch screen (file, sample or population button).



Figure 14: organisation of the data

The name of the current file is: YYMMDDXX.TXT, defined as follows:

- YY the year;
- MM the month;
- DD the day;
- XX the two letters which correspond to the file name. These letters are displayed on bottom-right edge of the *main* screen (Figure 13).

You can use the ALT key to decrease and increase sample (*sam*), population (*pop*) and file. Press '-' or '+' to change value and then ALT key again to come back on *main* screen (Figure 14).

The measure number is always incremented and can not be changed by the user. It is set to zero when you change sample, population or file.

• Configuration Menu



Figure 15: activating the GPS module

To set time and date, you have to use the internal GPS module (Figure 15). First, plug the GPS-antenna and place it outdoor (GPS does not work indoors). Switch then to the configuration section by pressing *conf* on the right of the screen. Scroll configuration sub-menus by pressing *conf*, start the GPS in the GPS sub-menu. This may take more than one minute in case of 'cold-start' of the GPS-module.

Time and date are automatically set right for local area if enough satellites and signals are detected. Return to the *main* screen by pressing *main*. Time should be correctly displayed in the top right-hand corner (cf.Figure 11).

If you don't need geo-referenced data, you can now stop the GPS-module because it slows the measurement.

Please note that time and date are saved until you start again the GPS-module and the GPS-module is switch-off by default.

Nb of Points (mean)
-100 580	+100
-1	+1
	Conf

Figure 16: configuration of the number of excitation flashes to obtain one measurement

In the *conf* menu, you can set up the measurement parameters. First, the 'Nb of Points' is the number of light excitation flashes averaged to obtain one measurement. The measurement is the mean of 500 excitations by default.

However, the number of excitation flashes can be decreased to increase the rate of acquisition, e.g. in the continuous mode of operation. This is useful for measurement in movement.

The number of flashes can also be increased for more stringent measurements. For most samples 500 is well adapted (0.5 s for a measure).

In case of a great number of measures, you can switch off the 'full display' to speed up measurements. In this case, only one value will be displayed on the *main* screen, still all data are stored on the SD card.

Please note that all the configuration parameters are set back to default at each reset.

• Statistics Menu

Anth (co) 0,134 0,2	SFR_R 0.492 0.167
9.625 11.692	NBI_R 0.265 0.179
main last	acq

Figure 17: Statistics of the last acquisition

The statistics menu (Figure 17) allows you to have a quick and simple view of the variability of the measurements during the experiment.

The Multiplex® gives you the standard deviation for each signal of the last acquisition.

7. Use of Multiplex® with a Computer

• Data export



Figure 18: SD card into the workstation

At the end of the measurements, you can download your data to your favourite spreadsheet software. Data are stored in ASCII format, columns separated by spaces.

Note that there is no need to use data format converters.

The SD card is automatically detected when it is insert into a generic memory card reader (Figure 18).

Open then the Multiplex® 3 data file in your favourite spreadsheet as is shown in Figure 19.



Figure 19: selecting the file through the spreadsheet

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• Choose the datatype as 'delimited' then click next (Figure 20);

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Figure 20: select 'delimitating'

- Use the 'space' as delimiter then click next (Figure 21) ;
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Figure 21: select 'space' as separator

• Data organisation

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14	14:53:15	3.98404121 E	49.01815795	N 15	8 5	26.58	30	1	1	7	24.89	19.07	4.06	7.39	4.58	8.39	150.2	2
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27	14:00:01	2 00410100 E	49.01000929	N 15	0 5	27.20	20.5		1	24	10.31	4.17	0.46	0.12	0.61	2.14	170.74	, c
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38	15:05:47	3 9840517 E	49 0181694	N 16	1 6	27.32	30	1	2	1	9.31	1.66	0.01	0.33	0.14	1.08	118 90	7
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41	15:07:55	3 9840662 E	49.01813125	N 16	1 5	27.32	30	1	2	4	93.5	28.6	31.53	14.79	29.86	15.25	307.6	à
42	15:08:22	3 98406143 E	49.01813888	N 16	3 4	27.39	30	1	2	5	40.63	19.51	26.87	21.45	36.1	27.23	190.36	1
43	15:08:31	3.98407506 F	49.01813125	N 1P	3 4	27.32	30	1	2	6	15.01	10.44	0.92	2.55	2.35	4.32	136.96	3
44	15:08:39	3.98408985 F	49.01813125	N 1P	2 4	27.32	30	1	2	7	29.86	11.15	1.94	3.94	0.68	2	195.6	ź
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47	15:09:03	3.98408985 E	49.01811218	N 16	2 5	27.53	30	1	2	10	15.13	11.62	0.58	1.99	0.01	0.14	146.9	(–)
48	15:09:13	3.98409957 E	49.01811218	N 16	2 5	27.39	30	1	2	11	42.06	10.11	9.27	7.81	18.25	9.42	226.42	2
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Figure 22: data in the spread sheet

Each file begins with a header of five comment lines that specify the type of measurement (Figure 20). Each column corresponds to one signal and each line corresponds to one measurement (Figure 20).

The first data column is the time, then the x/y position of the measurement in degrees, the altitude and the GPS-quality if GPS-module is switch on. They are followed by the LED and system temperature.

Finally, the data are displayed: signal value and standard deviation for each signal and ratio (Table 1 and table 2). For example: FRF_UV for 'Far-Red fluorescence excited by UV and FRF_UV_sd for the standard deviation of the measurement obtain from n excitation flashes (n = 500 by default).

Only for the Flav and Anth signals, you will find coefficient of variation (Flav_cv) rather than standard deviation.

SIGNAL	Emission	Excitation
BGF_UV	Blue-Green Fluorescence	UV
RF_UV	Red Fluorescence	UV
FRF_UV	Far-Red Fluorescence	UV
BGF_B	not in use	not used
RF_B	not in use	not used
FRF_B	not in use	not used
BGF_G	Reflected Blue-Green light	Green
RF_G	Red Fluorescence	Green
FRF_G	Far-Red Fluorescence	Green
BGF_R	not in use	Red
RF_R	Red Fluorescence	Red
FRF_R	Far-Red Fluorescence	Red

Table 1A: Multiplex® 3 signals with blue filter configuration

SIGNAL	Emission	Excitation
BGF_UV	Yellow Fluorescence	UV
RF_UV	Red Fluorescence	UV
FRF_UV	Far-Red Fluorescence	UV
BGF_B	Yellow fluorescence	Blue
RF_B	Red Fluorescence	Blue
FRF_B	Far-Red Fluorescence	Blue
BGF_G	Reflected Yellow-Green light	Green
RF_G	Red Fluorescence	Green
FRF_G	Far-Red Fluorescence	Green
BGF_R	Reflected Yellow-Red light	Red
RF_R	Red Fluorescence	Red
FRF_R	Far-Red Fluorescence	Red

Table 1B: Multiplex® 3 signals with yellow filter configuration

RATIO	Description	Formula
SED C	Simple Fluorescence Ratio	EDE C/DE C
3F K_ G	(Green Exc.)	FKF_07 KF_0
SED D	Simple Fluorescence Ratio	EDE D/DE D
5F K_K	(Red Exc.)	TKI'_K / KI'_K
DDD FDF	Blue-to-Red Fluorescence Ratio	RCE IIV / EDE IIV
DKK_FKF	(UV Exc.)	
FFD DIW	Fluorescence Excitation Ratio	EDE D/EDE IN/
FER_RUV	(Red & UV Exc.)	
FLAV	Flavonols	log(FER_RUV)
FFD DC	Fluorescence Excitation Ratio	EDE D/EDE C
FER_KG	(Red & Green Exc.)	FRF_K7 FRF_O
ANTH	Anthocyanins	log(FER_RG)
NDI C	Nitrogen Balance Index	EDE IIV/DE C
NDI_G	(SFR_G / FER_RUV)	FKF_UV/KF_U
NDI D	Nitrogen Balance Index	EDE IN//DE D
INDI_K	(SFR_R / FER_RUV)	rkr_uv / kr_k

Table 2: Multiplex® 3 ratios

8. Battery Charge

The Multiplex® 3 instrument is operated using one standard rechargeable Liion battery. A level indicator on the LCD screen is used to monitor the battery charge level (Figure 11). When the low battery message is displayed on the LCD screen, recharge the battery by connecting it to the external power supply.

To charge the battery: connect the battery charger to the 1200 mA - 29.4 V DC input of the battery (Figure 2).

During the battery charge process the LED indicator of the charger is red. The LED light turns green at the end of the charging process. The maximum charge time is approximately **three hours**.

Note: The Multiplex® 3 shouldn't be used during the battery charge process.

9. Troubleshooting

Please note that the Multiplex® program won't start if the SD card is absent.

10. Specifications

Samples	Any plant material: leaves, coniferous needles,
	 Epidermal UV absorbance by FER method: flavonol content
Measured	 Epidermal Visible absorbance by FER method: anthocyanins content
parameters	 Chlorophyll fluorescence emission ratio: chlorophyll content
	 UV-excited Blue-Green fluorescence (BGF)
	Several other fluorescence ratios
Measurement area	< 10 cm diameter
Measurement distance	10 cm
Acquisition	9000 acquisitions per second
Acquisition modes	One shot mode Continuous mode
Light sources	Light Emitting Diodes (LED); pulsed operation 4 excitation channels: UV, Blue, Green and Red
Detectors	Silicon photodiodes 3 detection channels: Yellow (or Blue), Red and Far-Red
Storage capacity	128 Mo (1 Go optional) = 1 million multi-parametric data (12 spectroscopic data, GPS location, time, temperature)
User Interfaces	3.2" (160 x 80) graphic LCD panel with touchscreen Sound warning
Data Interfaces	USB port for data transfer Serial port for external GPS link Data transfer compatible with Excel sheets
Temperature range	5 – 35 [°] C (operation)
Power source	External Li-ion rechargeable battery
Power autonomy	10 hours
Charging time	2 hours
Total weight	2.5 kg (without battery)
Optical-head size	16 cm Diameter x 15 cm Height

11. Maintenance

• Batteries

To ensure a long lifetime of the batteries please refer to the instructions described in the chapter's *battery charge*.

• Environmental Conditions

Multiplex® 3 was designed to be used directly in the field under different environmental conditions. The absorbance measurements are not sensitive to the ambient light. The instrument is resistant to the natural humidity of the plant material.

• Mechanical Vibrations and Shocks

Avoid mechanical shocks when you use the Multiplex® 3. The sensor contains several optical components that are sensitive to shocks.

• Storage Conditions

Always turn off the power switch to off when you have finished the measurements. Charge the batteries before a long period of storage of the Multiplex® 3.

Cleaning Operation

Clean the Multiplex® 3 instrument each time you return in the laboratory from the field, using a slightly wet dust-cloth with a water and alcohol mixture. Use a compressed air bomb to remove the dust from the optical parts. Apply gently the air pressure.

12. Warranty

The Multiplex® 3 carries a one-year warranty (from date of shipment) against material and/or workmanship defects, when used under normal operating conditions.

Any attempt by an unauthorized person to alter or repair the product voids the warranty.

Multiplex® 3 is a device for data acquisition. FORCE-A is not responsible for the interpretation of the data resulting from measurements.

In case of malfunction, contact FORCE-A office to obtain a return authorization number. The material should be returned to:

FORCE-A Centre Universitaire Paris-Sud ORSAY Bât. 503 91893 Orsay Cedex France

Tel : +33 1 69 35 87 47 Fax : +33 1 69 35 88 97 e-mail : <u>support@force-a.fr</u>

13. Claims

To obtain warranty service, contact FORCE-A office, with a description of the problem, transportation and insurance prepaid to FORCE-A. FORCE-A will, at its option, repair or replace the defective product free of charge. However, if FORCE-A determines that the failure is caused by misuse, alterations, accident or abnormal condition of operation or handling, you will be billed for the repair and the repaired product will be returned to you, transportation prepaid.