



## **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 non-contact 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 ecophysiological 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

<b>Samples</b>	Any plant material: leaves, coniferous needles, crops, turf, fruits, vegetables, grains, etc.
<b>Measured parameters</b>	<ul style="list-style-type: none"><li>• Epidermal UV absorbance by FER method: flavonol content</li><li>• Epidermal Visible absorbance by FER method: anthocyanin content</li><li>• Chlorophyll fluorescence emission ratio: chlorophyll content</li><li>• UV-excited Blue-Green fluorescence (BGF)</li><li>• Other fluorescence ratios</li></ul>
<b>Measurement area</b>	< 10 cm diameter
<b>Measurement distance</b>	10 cm
<b>Light sources</b>	Light Emitting Diodes (LED) ; pulsed operation 4 excitation channels: UV, Blue, Green and Red
<b>Detectors</b>	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

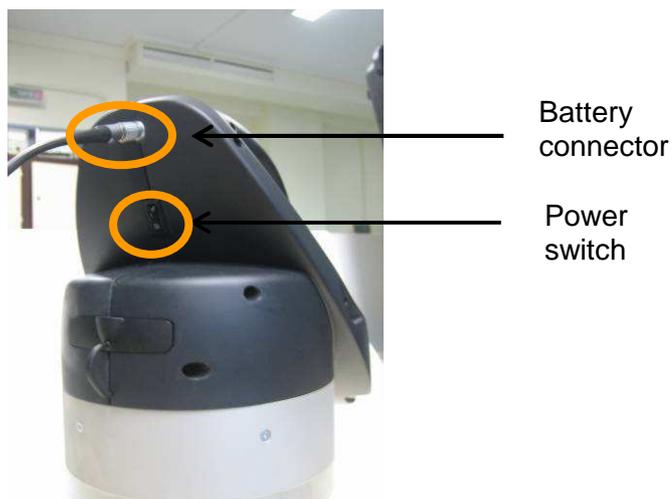


Figure 6: Battery connector and power switch

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.

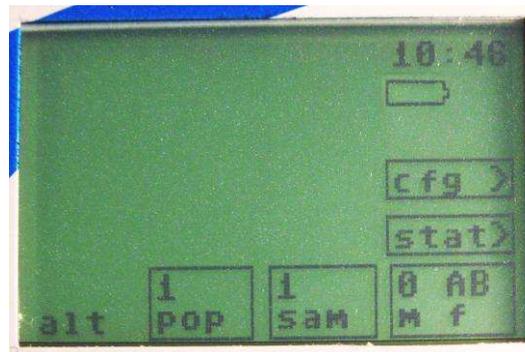


Figure 11: *main* screen

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).

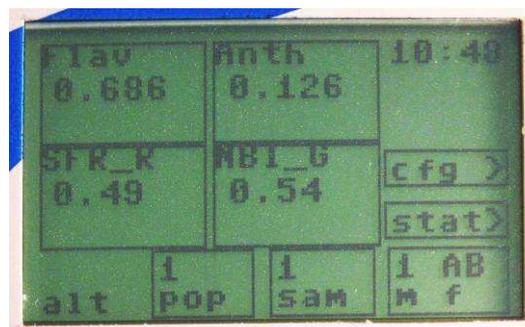


Figure 12: first acquisition

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.

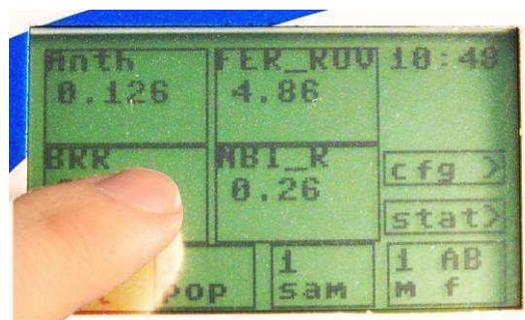


Figure 13: touch screen

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*.

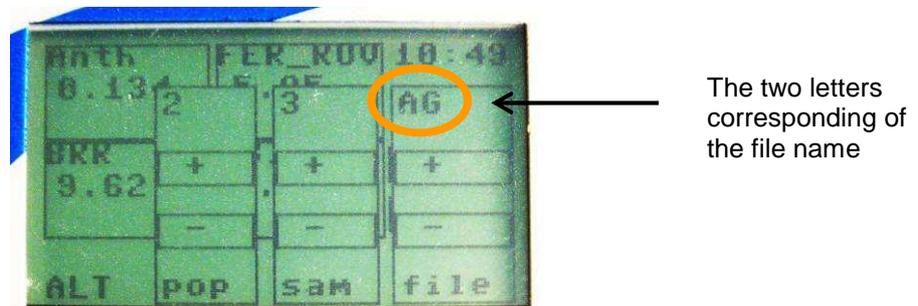
- *Organize your data during the experiment*

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**

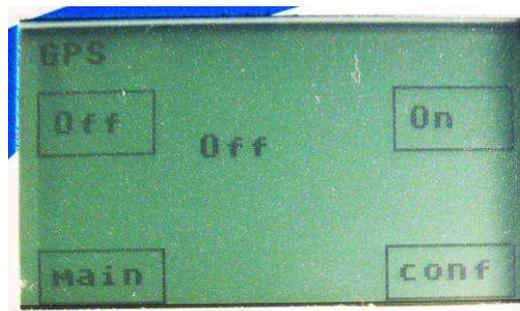
The name of the current file is: YYMMDDXX.TXT, defined as follows:

- 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*



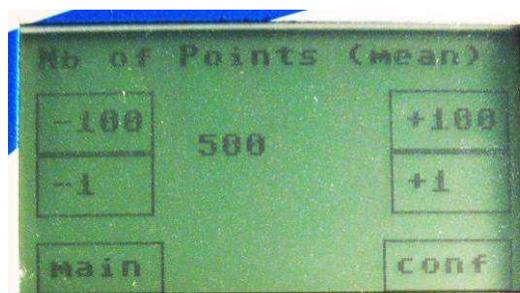
**Figure 15: activating the GPS module**

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.



**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*

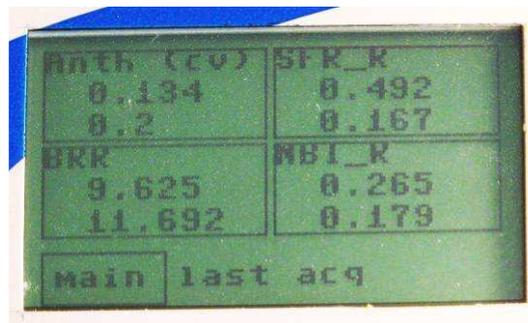


Figure 17: Statistics of the last acquisition

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*

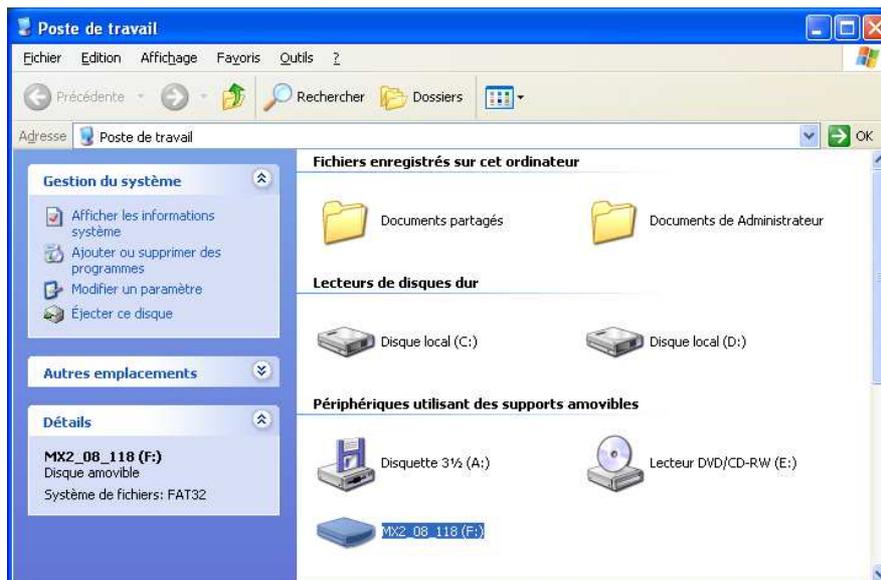


Figure 18: SD card into the workstation

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.

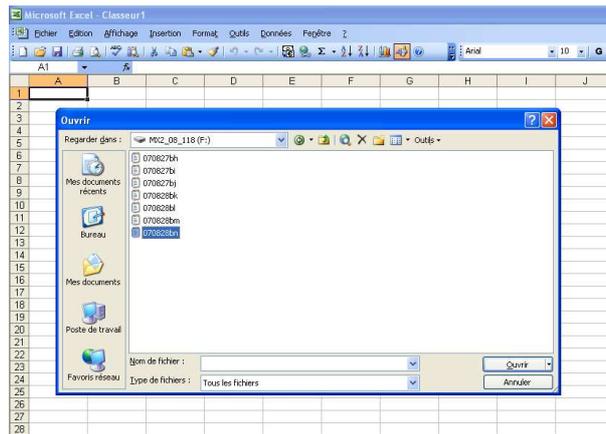


Figure 19: selecting the file through the spreadsheet

Use the two steps Importation Assistant:

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

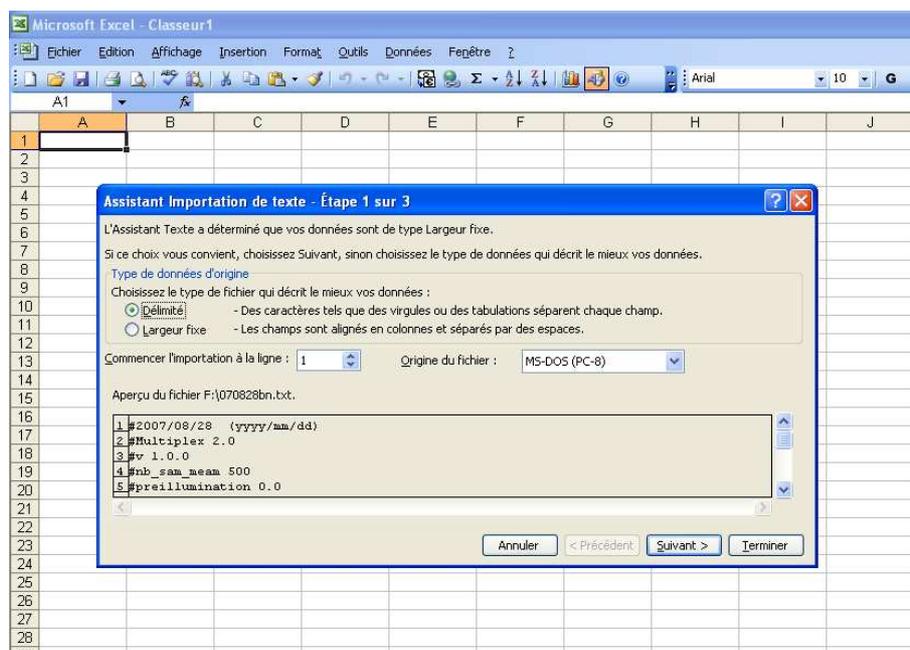


Figure 20: select 'delimitating'

- Use the 'space' as delimiter then click next (Figure 21) ;
- Click finish.

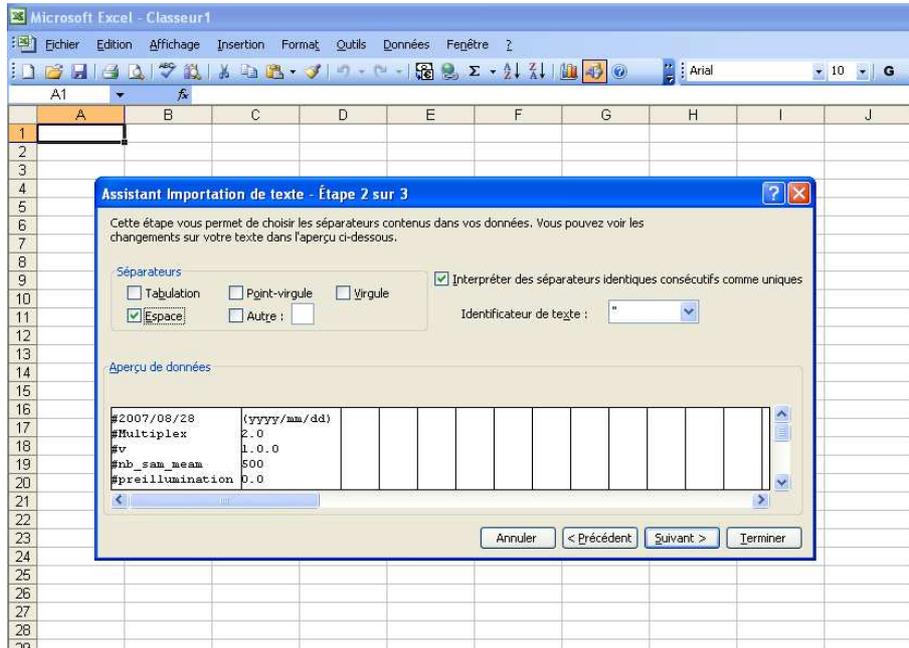


Figure 21: select 'space' as separator

- *Data organisation*

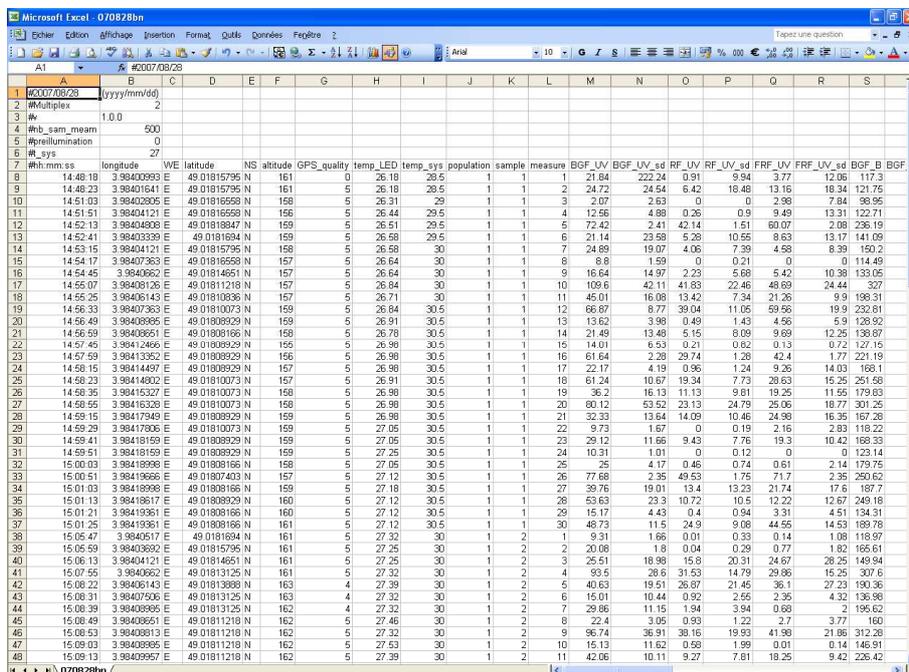


Figure 22: data in the spread sheet

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.

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

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

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

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

<b>RATIO</b>	<b>Description</b>	<b>Formula</b>
<b>SFR_G</b>	Simple Fluorescence Ratio (Green Exc.)	$FRF_G / RF_G$
<b>SFR_R</b>	Simple Fluorescence Ratio (Red Exc.)	$FRF_R / RF_R$
<b>BRR_FRF</b>	Blue-to-Red Fluorescence Ratio (UV Exc.)	$BGF_{UV} / FRF_{UV}$
<b>FER_RUV</b>	Fluorescence Excitation Ratio (Red & UV Exc.)	$FRF_R / FRF_{UV}$
<b>FLAV</b>	Flavonols	$\log(FER_{RUV})$
<b>FER_RG</b>	Fluorescence Excitation Ratio (Red & Green Exc.)	$FRF_R / FRF_G$
<b>ANTH</b>	Anthocyanins	$\log(FER_{RG})$
<b>NBI_G</b>	Nitrogen Balance Index (SFR_G / FER_RUV)	$FRF_{UV} / RF_G$
<b>NBI_R</b>	Nitrogen Balance Index (SFR_R / FER_RUV)	$FRF_{UV} / RF_R$

**Table 2: Multiplex® 3 ratios**

## 8. Battery Charge

The Multiplex® 3 instrument is operated using one standard rechargeable Li-ion 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

<b>Samples</b>	Any plant material: leaves, coniferous needles, crops, turf, fruits, vegetables, grains, etc. <ul style="list-style-type: none"> <li>• Epidermal UV absorbance by FER method: flavonol content</li> </ul>
<b>Measured parameters</b>	<ul style="list-style-type: none"> <li>• Epidermal Visible absorbance by FER method: anthocyanins content</li> <li>• Chlorophyll fluorescence emission ratio: chlorophyll content</li> <li>• UV-excited Blue-Green fluorescence (BGF)</li> <li>• Several other fluorescence ratios</li> </ul>
<b>Measurement area</b>	< 10 cm diameter
<b>Measurement distance</b>	10 cm
<b>Acquisition</b>	9000 acquisitions per second
<b>Acquisition modes</b>	One shot mode Continuous mode
<b>Light sources</b>	Light Emitting Diodes (LED); pulsed operation 4 excitation channels: UV, Blue, Green and Red
<b>Detectors</b>	Silicon photodiodes 3 detection channels: Yellow (or Blue), Red and Far-Red
<b>Storage capacity</b>	128 Mo (1 Go optional) = 1 million multi-parametric data (12 spectroscopic data, GPS location, time, temperature)
<b>User Interfaces</b>	3.2" (160 x 80) graphic LCD panel with touchscreen Sound warning
<b>Data Interfaces</b>	USB port for data transfer Serial port for external GPS link Data transfer compatible with Excel sheets
<b>Temperature range</b>	5 – 35°C (operation)
<b>Power source</b>	External Li-ion rechargeable battery
<b>Power autonomy</b>	10 hours
<b>Charging time</b>	2 hours
<b>Total weight</b>	2.5 kg (without battery)
<b>Optical-head size</b>	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**

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91893 Orsay Cedex

France

Tel : +33 1 69 35 87 47

Fax : +33 1 69 35 88 97

e-mail : [support@force-a.fr](mailto:support@force-a.fr)

## **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.