

Verification of the Eppendorf BioSpectrometer® with Hellma® Filters F2, F3, F4 Using a Control Chart

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Introduction

It is advisable to calibrate photometers regularly using certified and traceable reference materials [1]. For this purpose, Eppendorf offers own Reference Filter Sets which are traceable to NIST®. Companies that are obliged to use reference materials which have been certified by an accredited body can test their instruments using the DAkkS certified filters by Hellma Analytics®.

This Short Protocol describes the measuring procedure with the reference filters F2, F3, F4 from Hellma Analytics which are used to determine the photometric accuracy. The analysis is done by a control chart [2] bases on an Excel® file and which allows the easy evaluation of photometric calibration data.

Material and Methods

Materials

- > Eppendorf BioSpectrometer (basic, kinetic, fluorescence)
- > Hellma Analytics F0 Filter (empty filter mount, order no. 666-F0) and F2, F3, F4 Filters (Neutral density glass filters, order nos. 666-F2, 666-F3, 666-F4) or glass filter sets (order no. 666-S000 or 666-S002) including DAkkS certificate
- > Control chart F2 F3 F4 (www.eppendorf.com)
- > Option for data transfer from the Eppendorf BioSpectrometer to the computer (USB stick, USB cable, or via ethernet)

Method

Data generation with the Eppendorf BioSpectrometer:

1. Measurement:
Choose method "Multi λ" (Main Groups → Absorbance / Sub Groups → Multi λ) in the Eppendorf BioSpectrometer (Figure 1) and set the wavelengths in the "Check parameters" section to 440 nm, 465 nm, 546* nm, 590 nm, 635 nm (Figure 2). Insert the Filter 0 into the cuvette shaft and press the "Blank" key before performing the measurement with the Filter F2, F3, F4 filter by pressing the "Sample" key.
2. Data export:
For convenient "copy-paste" of data, export the results to a computer as an Excel file (Format XLS) (Figure 3). Export is possible by using either a USB stick, a USB cable, or via Ethernet cable (prerequisites of Eppendorf BioSpectrometer: software version 4.3.0 or higher and ethernet port).

* Filter specifications cover a measurement at 546.1 nm which originates from the emission spectrum of a mercury-vapor lamp. Eppendorf BioSpectrometers show a bandwidth of ≤ 4nm and are therefore not designed to be checked at this wavelength. The measurement is done at 546 nm instead.

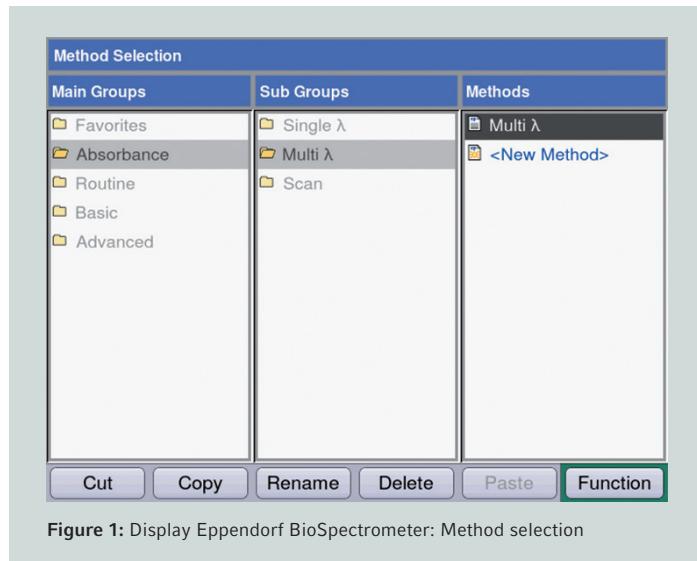


Figure 1: Display Eppendorf BioSpectrometer: Method selection

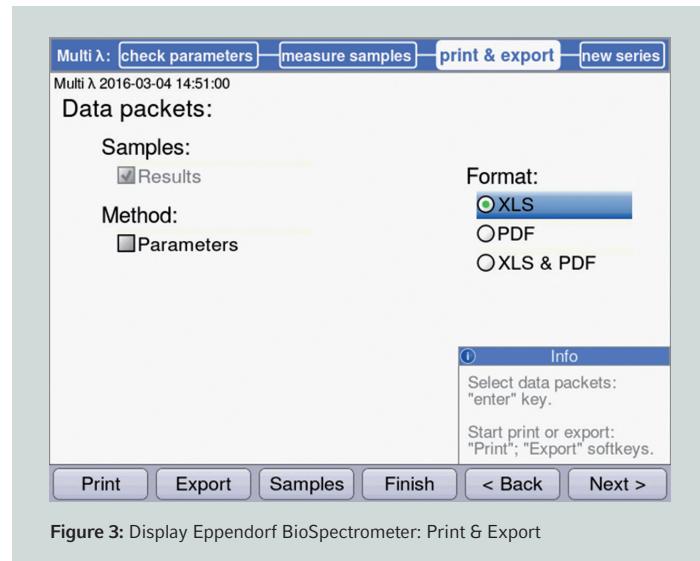


Figure 3: Display Eppendorf BioSpectrometer: Print & Export

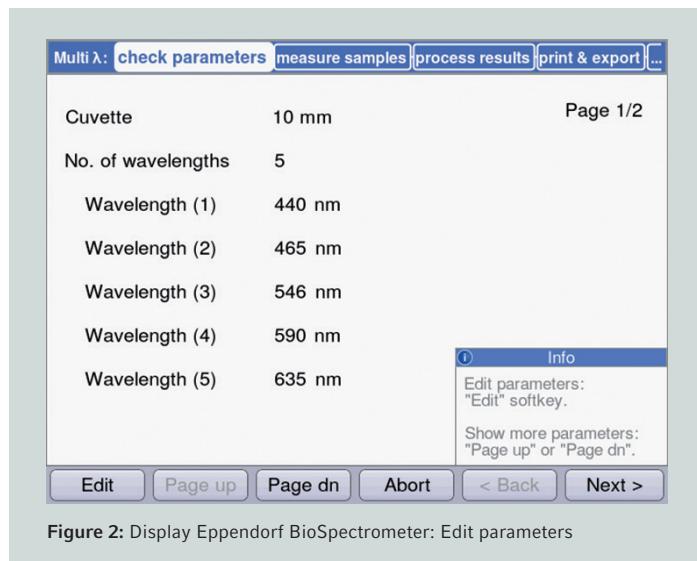


Figure 2: Display Eppendorf BioSpectrometer: Edit parameters

Data evaluation with the control chart:

The control chart Excel file consists of the following tabs (Figure 4):

> Explanation: Instruction how to fill the chart "F2, F3, F4 numbers"

> F2, F3, F4 numbers: Control charts for filters F2, F3, F4 for data analysis

> F2, F3, F4 charts: Graphic display of results (over time)



Figure 4: Control chart F2_F3_F4 Excel file - Tabs

1. Completing the control chart:

- The following data have to be entered into the tabs “F2, F3, F4 numbers” which represent the actual control chart.
- A short instruction is found in the tab “explanation”.
- Serial number of the F2, F3, F4 filters
 - Absorbance target values and measurement uncertainty (stated on the DAkkS certificate)
 - Optional into F2 numbers only: Information on laboratory, device type and serial number
 - Values for the filters F2, F3, F4 which have been measured by the Eppendorf BioSpectrometer (Figure 5)

2. Evaluation:

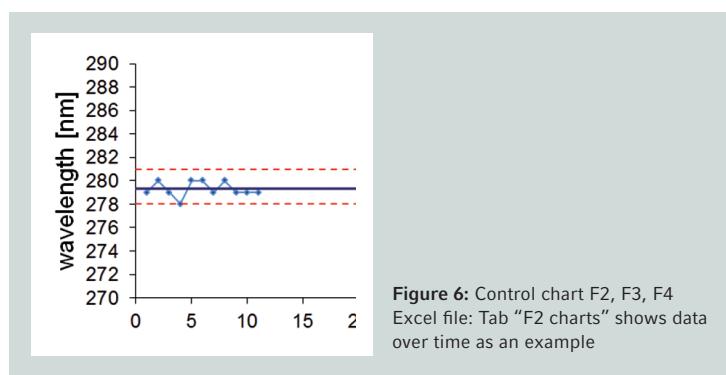
- F2 (F3, F4) numbers: Values which are inside the limits stay black whereas values outside the upper/lower limit will be marked red in this tab. The limits are calculated from measurement uncertainty and device specification. In case of red numbers, please contact your local Eppendorf service organization regarding a check of the device.
- F2 (F3, F4) charts: This tab offers a graphic overview about the measured values as well as the limits and can be used to monitor calibration values over time (Figure 6).

The figure shows three screenshots illustrating the transfer of peak absorbance values. The top-left screenshot shows the Eppendorf BioSpectrometer software interface with measured values for wavelengths λ1 to λ5. The top-right screenshot shows an Excel table titled 'Measurement of neutral density glass filter F3 (0.5A) with Eppendorf BioSpectrometer (basic, fluorescence, kinetic)'. The bottom screenshot shows an Excel spreadsheet with the transferred data.

wavelength (certificate)	440,00	465,00	546,10	590,00	635,00	nm
target value (certificate)	0,493	0,462	0,479	0,535	0,537	A
measurement uncertainty (certificate)	0,0028	0,0028	0,0028	0,0028	0,0028	A
upper limit	0,5259	0,4947	0,5121	0,5681	0,5697	A
lower limit	0,4603	0,4291	0,4465	0,5025	0,5041	A

laboratory	0					
device	BioSpectrometer basic					
serial number	0					
date	440 nm	465 nm	546,1 nm	590 nm	635 nm	comment
02.03.2016	0,493	0,462	0,495	0,516	0,524	

Figure 5: Transfer of peak absorbance values manually from Eppendorf BioSpectrometer or alternatively via “copy-paste” from exported Excel file to control chart Excel file tab “F3 numbers”



Literature

- [1] Weiss N. Better safe than sorry: Verification of spectrophotometers for accurate and reliable measuring results.
Eppendorf White Paper No. 33. www.eppendorf.com
- [2] Control chart F2, F3, F4: www.eppendorf.com/biospectrometer-control-charts

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