

Verification of the Eppendorf BioSpectrometer[®] with Hellma[®] Filter F1 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 filter F1 from Hellma Analytics which is used to determine the wavelength accuracy of peaks. The analysis is done by a control chart [2] based 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 F1 Filter (Holmium oxide glass filter, order no. 666-F1) or glass filter set (order no. 666-S000) including DAkkS certificate
- > Control chart F1 (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 "Scan" (Main Groups → Absorbance / Sub Groups → Scan) in the Eppendorf BioSpectrometer (Figure 1) and set the range of the scan in the parameter section to 250 - 650 nm (Figure 2) in the "Check parameters" section. Insert the Filter 0 into the cuvette shaft and press the "Blank" key before performing the measurement with the Filter F1 by pressing the "Sample" key.

2. Process results:

After the measurement, the absorbance spectrum of the F1 filter is shown which includes various peaks (Figure 3). Since the peak detection of the Eppendorf BioSpectrometer (via soft key "Peaks") uses an automated algorithm, it may be difficult to identify the peak maxima of the smaller peaks directly in the device. Therefore we recommend to use the control chart which offers a tool for evaluation. The export function can be used to transfer the data.

3. Data export:

For convenient peak identification export the results to a computer as an Excel file (Format XLS) and choose under "Samples" the option "Graph data" (Figure 4). 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).

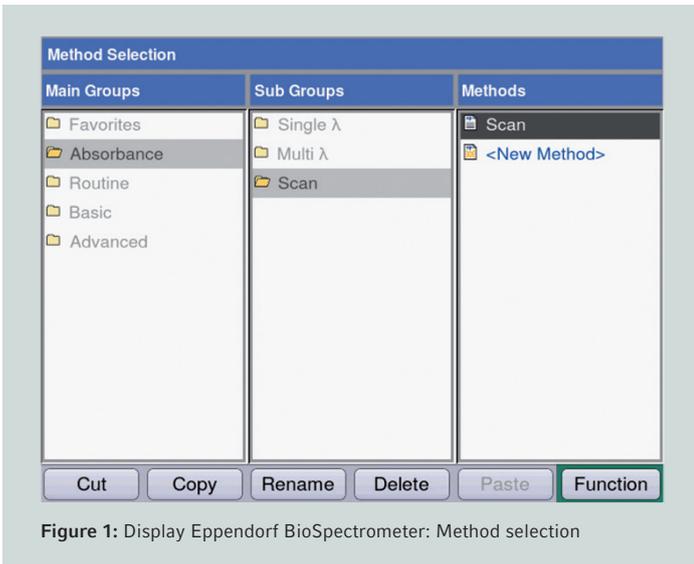


Figure 1: Display Eppendorf BioSpectrometer: Method selection

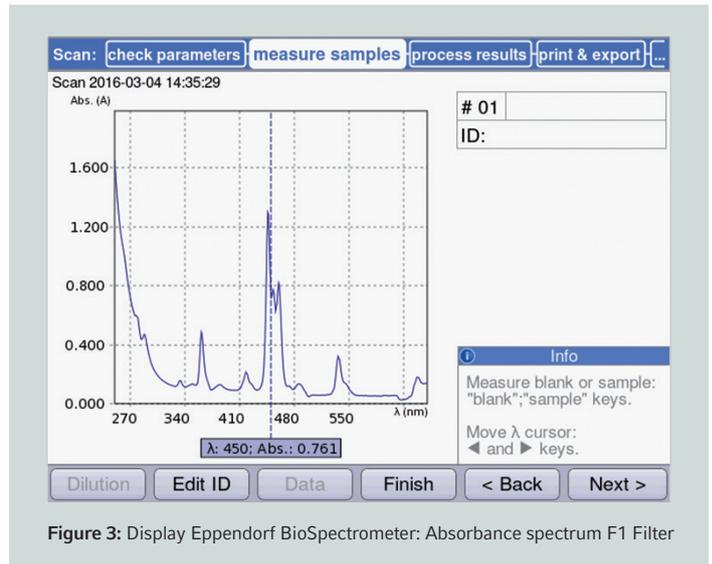


Figure 3: Display Eppendorf BioSpectrometer: Absorbance spectrum F1 Filter

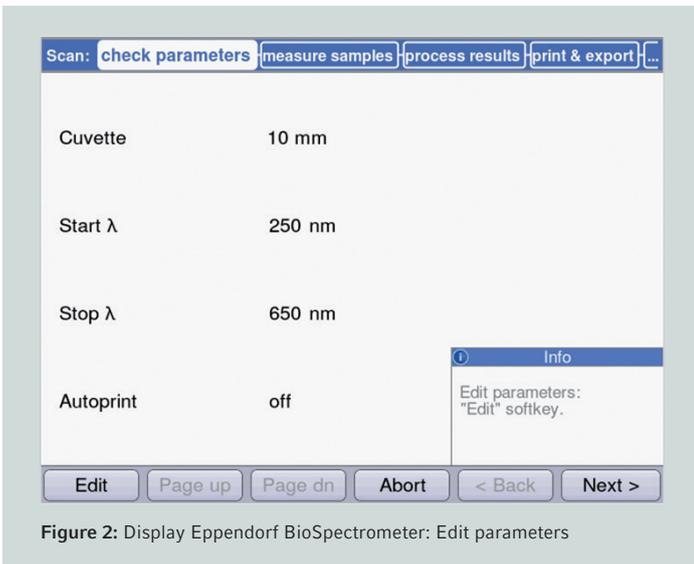


Figure 2: Display Eppendorf BioSpectrometer: Edit parameters

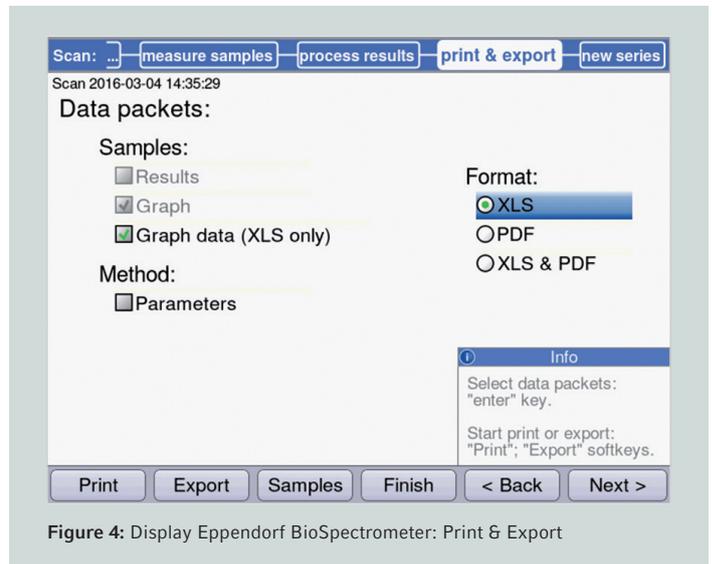


Figure 4: Display Eppendorf BioSpectrometer: Print & Export

Data evaluation with the control chart

The control chart Excel file consists of the following four tabs (Figure 5):

> Explanation: Instruction how to fill the chart "F1 numbers"

- > F1 numbers: Control chart for filter F1 for data analysis
- > F1 charts: Graphic display of results (over time)
- > Evaluate Excel files: Tool for the identification of peaks of the measured data



Figure 5: Control chart F1 Excel file: Tabs

1. Determination of peak maxima:

Open the Excel file from the Eppendorf BioSpectrometer and the Control Chart. Copy the measured absorbance values 250 – 650 nm (graph data) from the Eppendorf BioSpectrometer file into the column of the tab “evaluate Excel file”. For each of 5 data groups a peak will be indicated automatically (Figure 6).

2. Completing the control chart:

The following data have to be entered into the tab “F1 numbers” which represents the actual control chart. A short instruction is found in the tab “explanation”.

- a) Serial number of the F1 filter
- b) Wavelength target values and measurement uncertainty (stated on the DAkkS certificate)
- c) Optional: information on laboratory, device type and serial number
- d) Wavelengths of the peak maxima (from tab “evaluate Excel file”, Figure 6)

3. Evaluation:

- a) F1 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.
- b) F1 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 7).

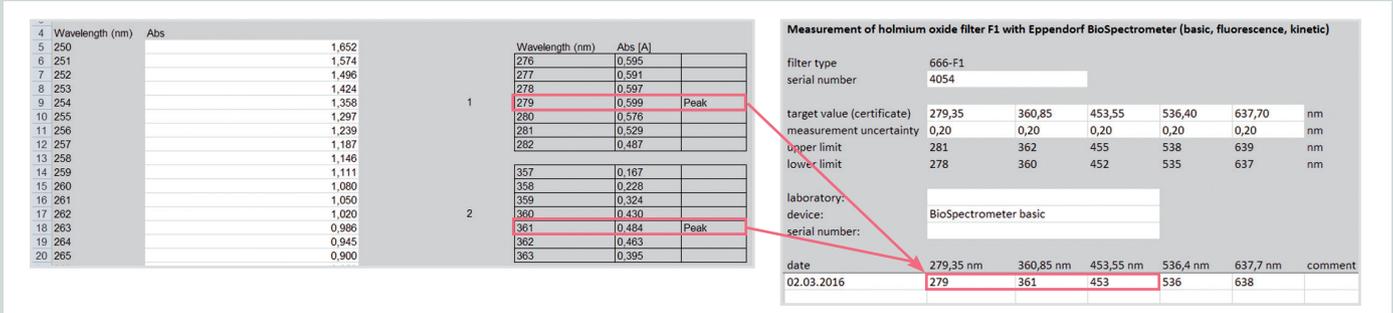


Figure 6: Control chart F1 Excel file: Transfer of peak absorbance values from tab “Evaluate Excel file” to tab “F1 numbers”

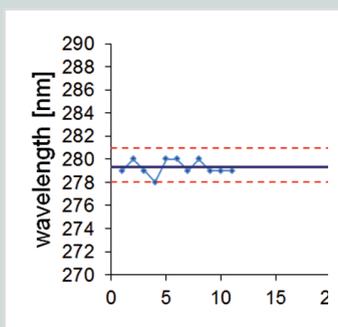


Figure 7: Control chart F1 Excel file: Tab “F1 charts” shows data over time as an example

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 F1: www.eppendorf.com/biospectrometer-control-charts

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