BIOLIGHT STUDIO IN ROUTINE UV/VIS SPECTROSCOPY

UV/Vis Spectroscopy is a technique that is widely used to characterize, identify and quantify chemical compounds in all fields of analytical chemistry. The main advantages of UV/Vis Spectroscopy are simple and easy to use instruments and the general applicability to many substances that absorb light in the wavelength range between 200nm and 1000nm. UV/Vis Spectroscopy plays an important role in the field of water- and environmental analysis, biotechnology, food analysis, clinical chemistry pharmaceutical analysis and many more applications. Almost in any analytical discipline you will find applications for UV Visible Spectroscopy - in many cases for quantification purposes.

UV Vis Spectroscopy plays an especially important role in routine analytics due to its simple applicability and manifold application fields. Routine analytics often means the quantification of a certain compound often with a certain sample pre-treatment before the actual analytical step. Routine analysis also means that there are many samples to be analyzed on a regular base using the same analytical methodology. This often required s calibration with a series of calibration standards and the establishment of a calibration curve.

In general these basic technologies are applied in UV Visible Spectroscopy, from which the analytical results can be determined:

Scan - Recording of absorbance or transmission data as a function of the wavelength. This technique mostly is used to characterize and quantify pure substances, often in the field of pharmaceutical and chemical analysis.

Quantitative Analysis - Determination of the concentration of a compound based on a previously recorded calibration curve. In this case the absorbance data are recorded as a function of concentration. This technique often employs single wavelength measurement and is frequently used for colorimetric determination of substances after a specific chemical reaction producing a colored compound. Quantitative analysis is frequently used in water analysis, clinical chemistry and all kinds of analytical chemistry and comprises the most important application of UV/Visible spectroscopy.

Kinetics - Recording of absorbance data at one (or several) fixed wavelength(s) over a certain time period as a function of time. This technique mostly is used to quantify enzyme activity or substrate concentration as a result of a (bio-)chemical reaction.

Result calculation often requires that certain calculations are applied to the raw absorbance (or transmittance) data. Typically many samples have to be measured, by lab technicians who are often not so experienced with the specific requirements and theoretical backgrounds of UV/Vis spectroscopy. Therefore it is crucial to use a flexible software package, which easy and safe to

use - despite its complexity. *BioLight Studio* is such a software package which is dedicated to calculate results from UV/Vis spectroscopy data and store them in a save data-base.

In this report it will be shown, how easy it is to obtain results from UV/Vis measurements, when using the powerful *BioLight Studio* software package - especially when it comes to routine applications. Therefore 3 typical UV/Vis measurement procedures are shown below - where absorbance is recorded as a function of wavelength (Scan), absorbance as a function of concentration (Quantitative analysis) and absorbance as a function of time (Enzyme Kinetics).

EASE-TO-USE SOFTWARE PACKAGE: POWERFUL AND DESIGNED FOR ROUTINE UV/VISIBLE SPECTROSCOPY

Software is the key: The widespread use of UV/Vis spectroscopy for different applications, at the same time applying different analytical methodology (techniques), requires a powerful and flexible, application oriented software package. The *BioLight Studio* is powerful, flexible and simple to use software package designed for routine UV/Vis spectroscopy. The *BioLight Studio* was designed for intuitive operation from the very start: At the most direct level, this offers the researcher an easy-to-use, walk up system. Open a method, click on 'Start', and you're up and running. Measure samples untill you are finished, then click on stop. All data from the current sample batch is saved automatically in a single multifile: No more searching the hard disk to find which data belongs to an experiment, because all the data from the current experiment is stored conveniently in one file.
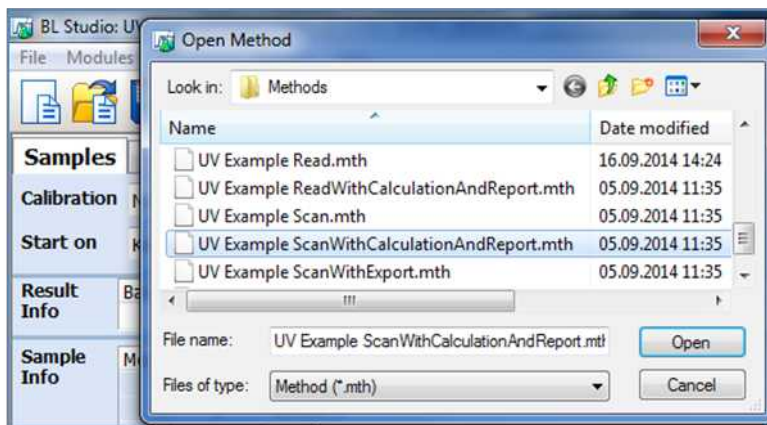
The typical UV/Vis analysis procedure using BL Studio is shown below. This demonstrates how simple and easy method setup and routine measurement can be, when using *BioLight Studio*. The examples below show typical situations using UV/Vis Scan, Concentration measurement and kinetic analysis. The examples shown below are rather basic and include quite simple calculations. The intention is just to give an understanding of the principal possibilities. Please keep in mind, that *BioLight Studio* is extremely powerful and basically allows any kind of result calculation used in UV/Visible Spectroscopy - certainly more complex as in the examples shown below.

BIOLIGHT STUDIO FOR SCANNING UV VISIBLE SPECTRA - SETUP A SCAN METHOD FOR SPECTRAL PURITY MEASUREMENT

UV/Vis spectroscopy is a frequently used technique to determine the purity of a pharmaceutical or biological sample. A typical example is the purity determination of a DNA preparation. This is a frequently used technique during the process DNA purification, to find out whether there is a protein contamination in the sample. For this a UV-spectrum is recorded and the absorbance redings at 260nm and 280nm are determined. The absorbance ratio A260nm/A280nm is used as a qualitative measure of DNA purity. When the ratio lies between 1.7 and 2.0, the DNA preparation is pure. Lower ratio indicates protein impurity.

This is the procedure, using the "Development" module of *BioLight Studio*: First open an pre-set method. In this case the best is the method "UV Example ScanWithCalculationAndReport.mth"

Figure 1: Selection of pre-set methods. For the example given here, the method "UV Example Scan with Calculations and Report" is chosen.

This pre-set method already has the basic structure, needed for the purity determination. It records a spectrum (Scan), allows to perform calculations on the spectrum and will give a report, containing all the relevant information.

The method setup page will show 5 different tabs:

1. Sample - this allows to define the sample information and enter sample ID and sample specific information

2. Acquisition - this allows to define the instrument parameters for acquiring the UV/Vis spectrum

3. Calculation - this allows to define the calculations to be applied to the spectrum recorded

4. Presentation - this allows to configure the application specific report

5. Manual Control - this allows to perform manual, live measurements to optimize instrument parameters

Figures 2-5 shown below, are typical screenshots illustrating the how straight-forward it is to enter specific parameters and calculations for the given example-application DNA-purity measurement.
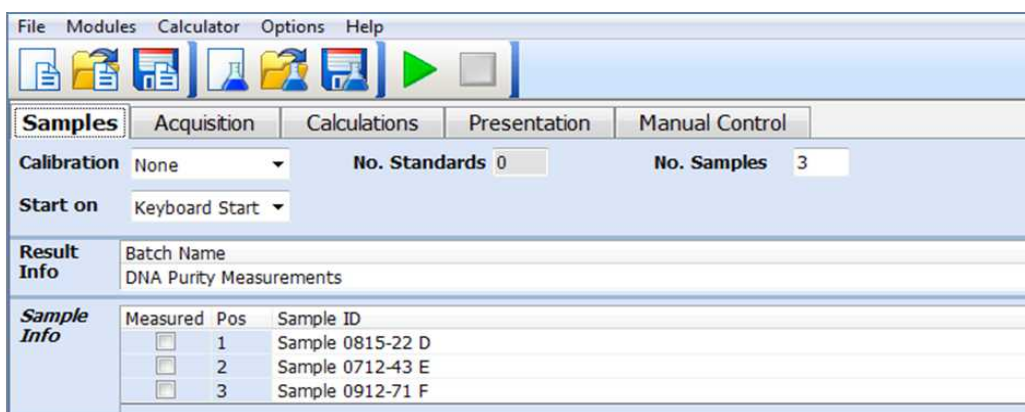
Figure 2: The "Sample" Tab allows to define the number of samples to be measured, including sample information and sample ID together with additional documentation of sample and batch.
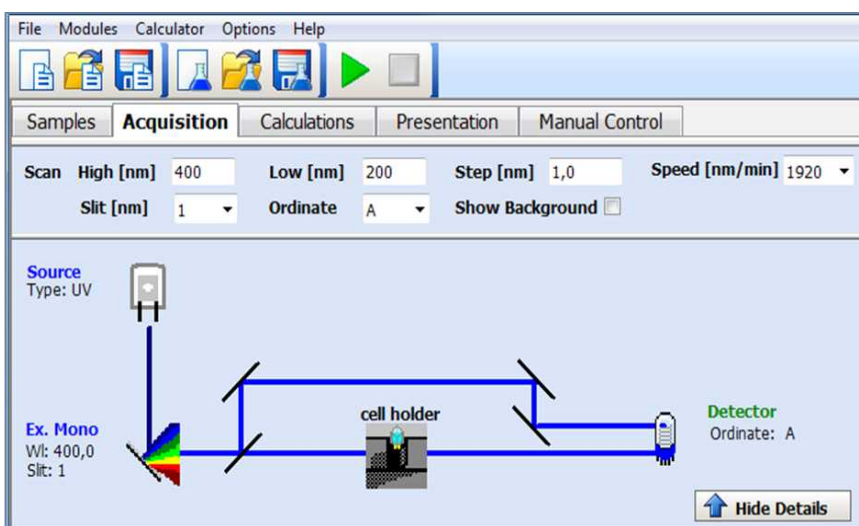

Figure 3: The "Acquisition" Tab allows to define the instrument parameters for acquiring the UV/Vis spectrum. In the specific case an absorbance scan range from 400 to 200 nm is selected which covers basically the UV range of the spectrophotometer used.
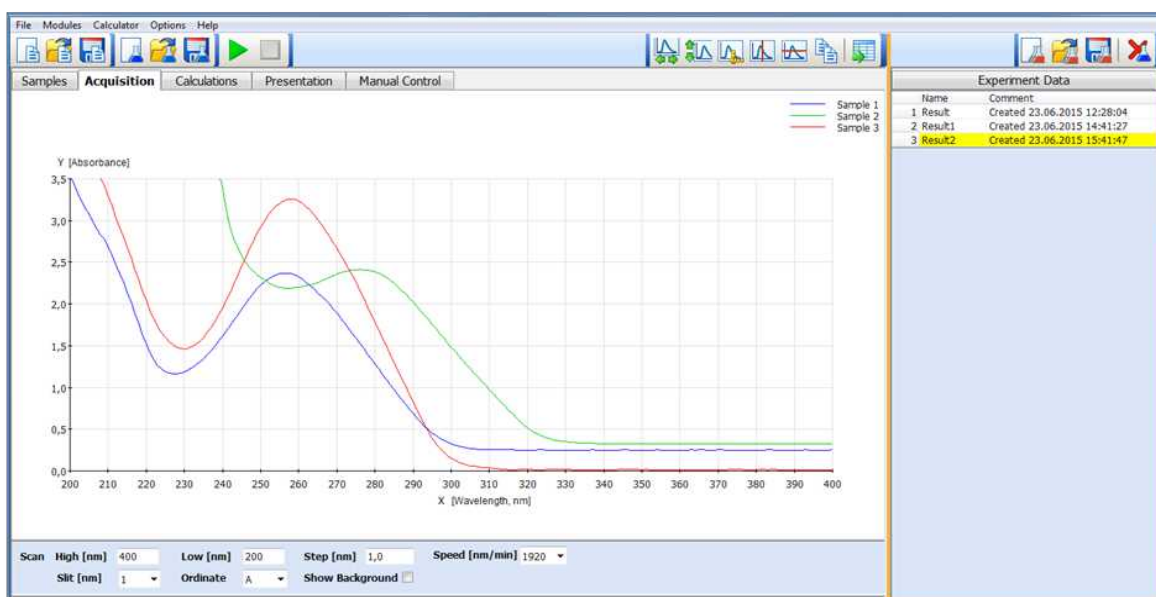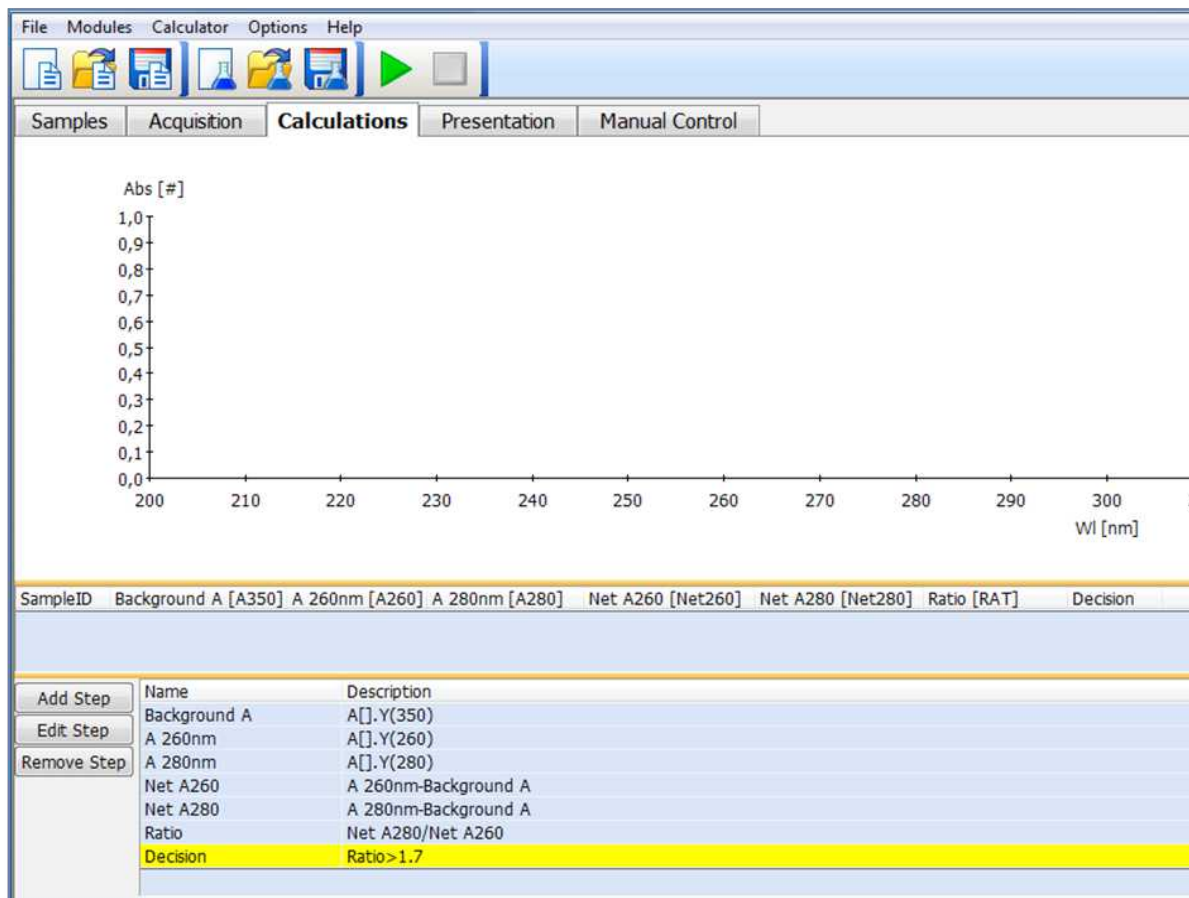
Figure 5: The "Calculations" Tab allows to define the calculations to be applied to the spectrum recorded. The calculations include all kind of arithmetic operations to be applied to the spectra, including filters or derivative spectroscopy. In addition it allows to extract data from the spectra, like peaks (minimum/maximum), slope calculation, area calculation or display of single data points, for example absorbance values at specified wavelengths.

In the specific case of DNA purity measurement 6 subsequent calculations are defined. First the background absorbance at 350nm is extracted from the spectrum, then the absorbance values at 260nm and 280nm. In the following steps the net absorbance values are calculated: NetA260=A260-A350 and NetA280=A280-A350. Then the ratio of the net absorbance values is determined: (Net A260)/(NetA280). This ratio is a measure of the DNA purity. The final step of the calculations is a decision. This gives the final answer: When the ration is bigger than 1.7, the Sample is pure DNA. If the ratio is lower, the DNA is contaminated.

BioLight Studio includes a very powerful Report generator. It just works like editing a MS Word document - and therefore it is very easy to create an application specific report template - containing all relevant information and even customer specific bitmaps - like the company logo. As a result you will have a customized, application specific report, representing all the data you want to show. The report template is created under the "Presentation" tab.
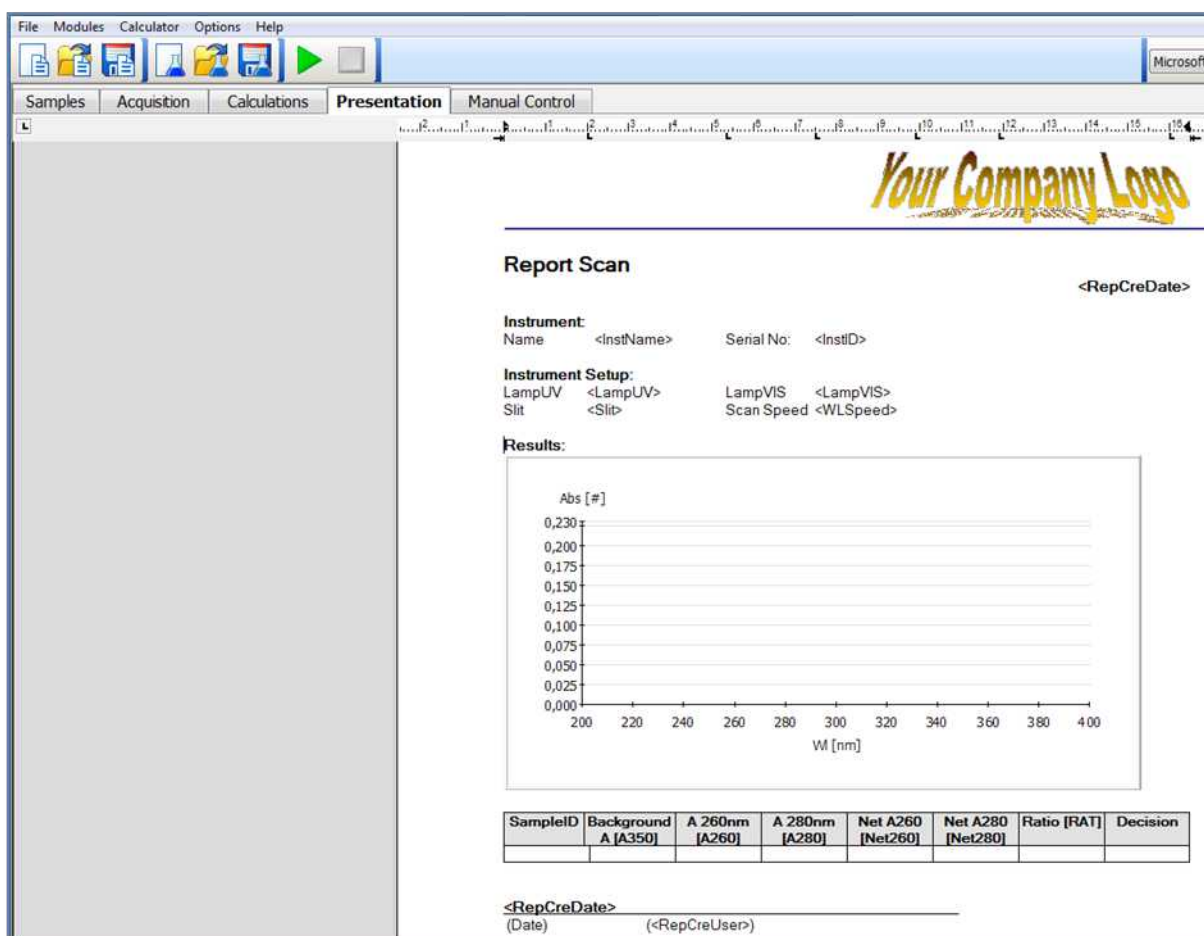
Figure 6: "Presentation" tab - this allows to configure a template for the application specific report. It is very simple to set-up. Just like in Microsoft Word you can create a template where you define which kind of data and results should be included in the report. It also allows to include bitmaps and graphics - like your company's logo.

In the specific case the report template created for the DNA-purity measurement includes a header with your company logo, information about the instrument and measurement conditions. It includes a place-holder for the graphics (Spectra). Then it includes the table with all the numerical results and finally date and time, including operator's name and a place for the signature after printout.

This report template is stored with the method and will be populated with the actual measurement data after the samples analysis.

In routine applications the lab technician will call the BioLight Studio "Routine" module and start the respective Scan method. This method is protected and the parameters cannot be changed by a normal operator. Usually the operator will enter certain sample information and run the method. Typically the spectra will be displayed live and the measurement data will be stored in a data-base - in just one simple file at a defined location. A report will be created based on the template which had been created in the "Development" module. In the DNA purity determination method shown above the report will look as shown in figure 7 below.
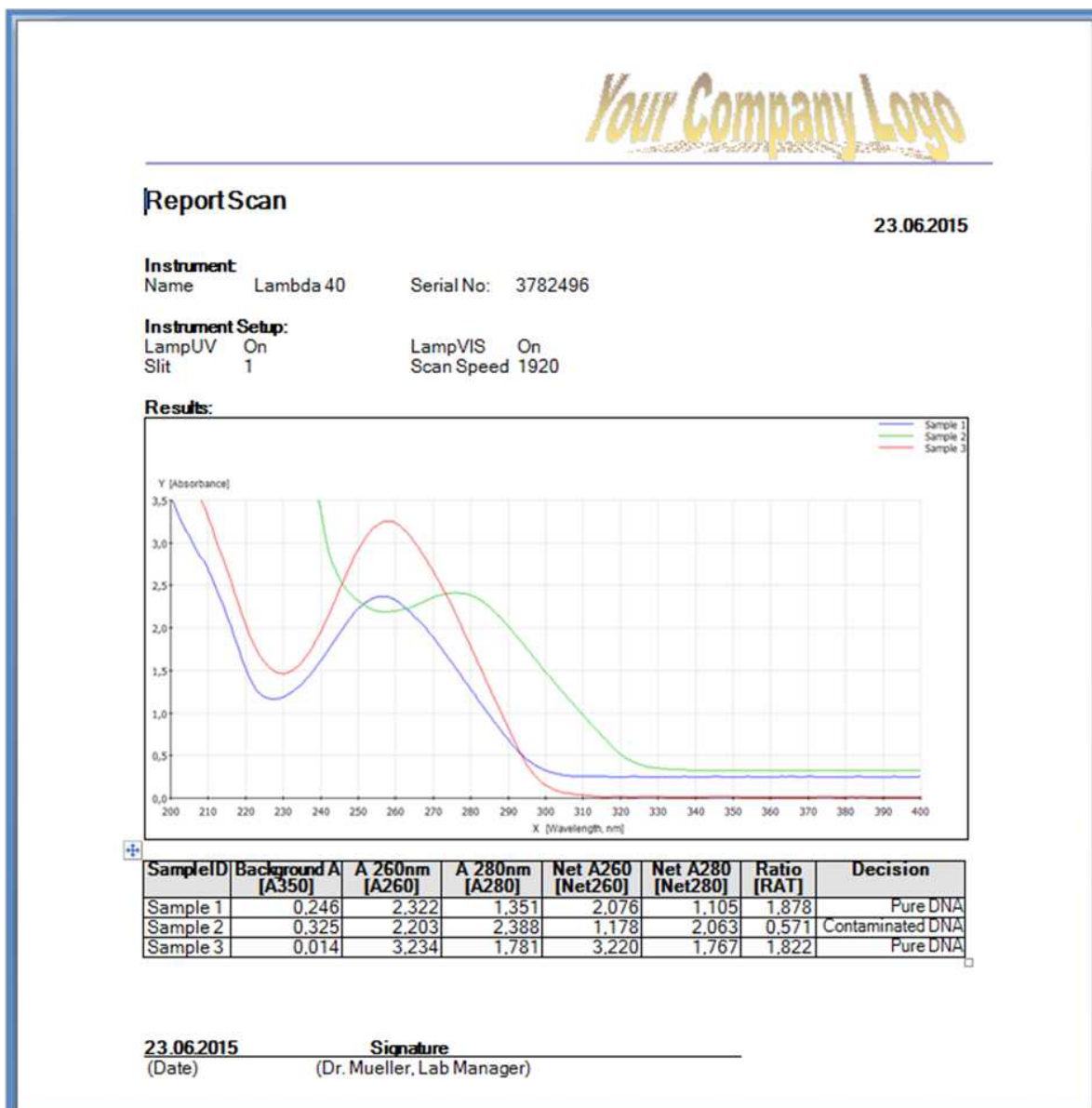
Figure 7: Report for DNA purity measurement, created from the method shown above using the template as shown in figure 6. The report contains the spectra and the results from the calculations based on the spectra. It also includes the final answer: When the Ratio is below a certain limit the output says "contaminated DNA" when it is above the limit, the answer is "Pure DNA".

BIOLIGHT STUDIO FOR QUANTITATIVE ANALYSIS

Most common application of UV/Vis Spectroscopy is the quantitative measurement at a single wavelength. This allows to determine the concentration of an analyte. According to Beer-Lambert's law, the concentration is a linear function of the absorbance measured at a certain wavelength. Often the compound to be analyzed is chemically derivatized, resulting in a colored compound which can be measured using Visible Spectroscopy. Sometimes the concentration is calculated from the absorbance reading by just multiplying the readout with a simple factor. In

BioLight Studio this can be very easily done with a method, similar to the DNA purity measurement, shown above.

More frequently a calibration curve is established using reference solutions with known analyte concentrations. The respective calibration curve can be established with *BioLight Studio* and it will automatically be used for measurement of unknown samples.

Figure 8: Calibration curve, established from the absorbance readings of 4 reference solutions("standards") with known concentrations. In this example a quadratic curve fit was selected, not forced to go through 0. BioLight Studio calculates the quadratic curve function and - in this example - obtains a perfect fit with a correlation coefficient of 1.0. The values of the references are displayed with a green cross, the six unknown samples measured are displayed with a blue cross in the graph.

In routine applications the lab technician will call the *BioLight Studio* "Routine" module and start the respective Quantitation method. This method is protected and the parameters cannot be changed by a normal operator. Usually the operator will enter certain sample information and run the method. Typically the Calibration curve will be displayed live on the screen together with the measurement data. These will automatically be stored in a single file in d protected data-base. A report will be created. In the example quantitation method shown above, the report might look as shown in the figure 9 below.
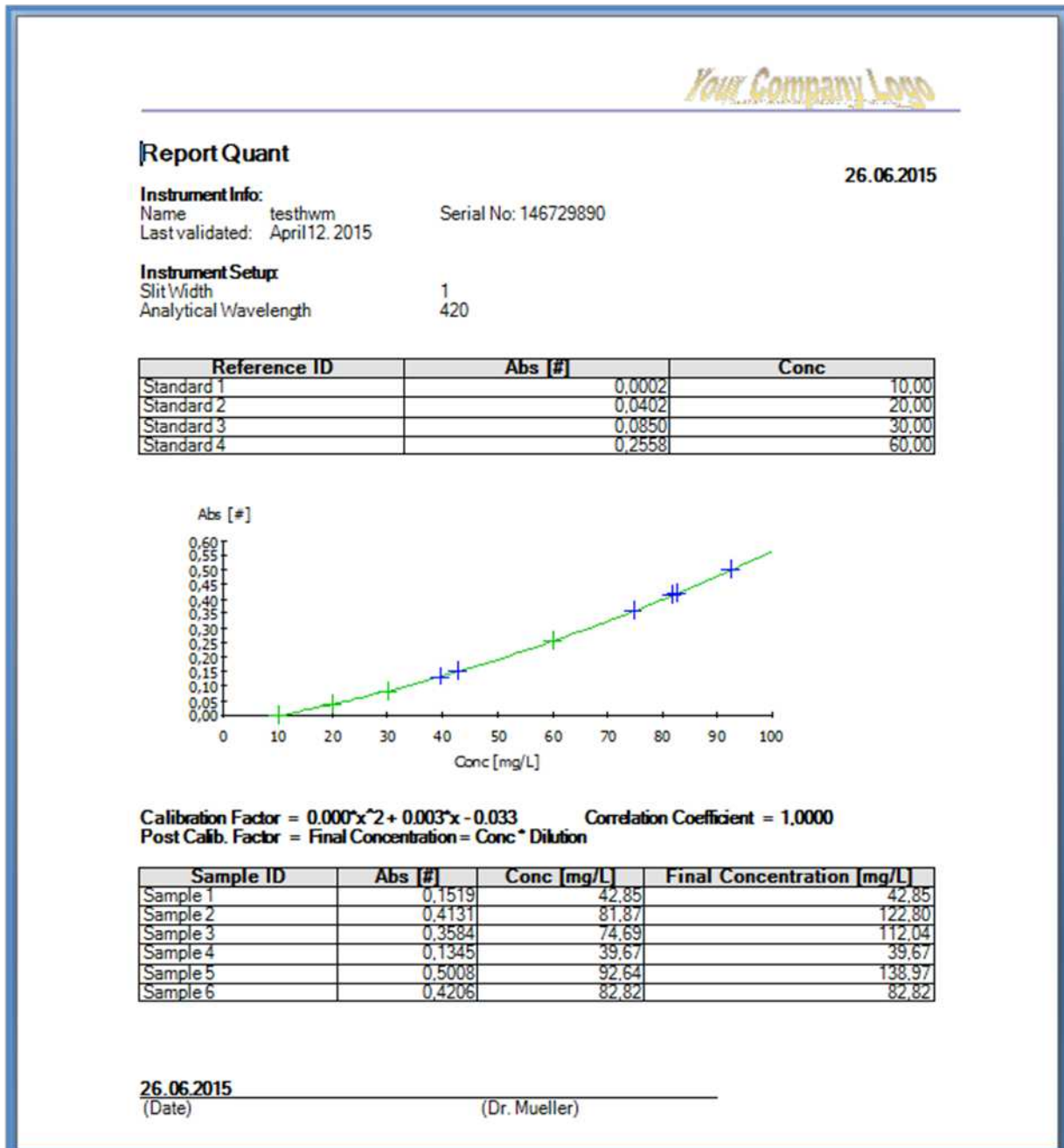
Report Quant

26.06.2015

Instrument Info:
Name   testhwm   Serial No: 146729890
Last validated: April 12. 2015

Instrument Setup:
Slit Width   1
Analytical Wavelength   420

Reference ID	Abs [#]	Conc
Standard 1	0,0002	10,00
Standard 2	0,0402	20,00
Standard 3	0,0850	30,00
Standard 4	0,2558	60,00

Calibration Factor = $0.000 \cdot x^2 + 0.003 \cdot x - 0.033$ Correlation Coefficient = 1,0000
Post Calib. Factor = Final Concentration = Conc * Dilution

Sample ID	Abs [#]	Conc [mg/L]	Final Concentration [mg/L]
Sample 1	0,1519	42,85	42,85
Sample 2	0,4131	81,87	122,80
Sample 3	0,3584	74,69	112,04
Sample 4	0,1345	39,67	39,67
Sample 5	0,5008	92,64	138,97
Sample 6	0,4206	82,82	82,82

26.06.2015
(Date)   (Dr. Mueller)

Figure 9: Report for a quantitative analysis using a calibration curve, created from the method specific template, containing company logo, instrument settings, Concentrations and Absorbance readings of the reference solutions, calibration curve with all respective information and a table with the concentration results of the unknown samples.

BIOLIGHT STUDIO FOR TIME-DEPENDENT MEASUREMENTS (KINETICS)

To determine the enzyme activity, an enzymatic reaction is followed over a certain time period, using an UV/Vis spectrometer at a defined wavelength and a defined temperature. The bigger the change of absorbance over time (dA/dt) is, the higher is the enzyme activity. In a routine lab therefore the slope (rate) of the resulting absorbance curve is calculated and then it is typically multiplied with a specific factor to obtain enzyme activity.This type of measurement can be easily performed when using *BioLight Studio* software package.

The procedure of method setup is analogue to the procedure shown before with the Scan- and Quantitation methods: As a base for a Kinetics experiment a "Timedrive" template is selected. Then the sample information is entered in the "Samples" tab. Next the acquisition parameters - like measurement wavelength and duration of the kinetic run - are defined under "Acquisition". On the "Calculations" Tab the respective calculations are defined. Finally a report template it set up under "Presentation". The methods then is saved and can be retrieved for the routine analysis - even by less educated operators.

Figure 10: Typical Enzyme kinetics graph, showing the increase of absorbance over time. This graph is shown in the "Caluclations" tab of the Timedrive method for enzyme activity determination. The experiment was carried out over 5 minutes (300 seconds) and the absorbance increased from 0.0A to approximately 0,7A. To obtain the desired result, two simple calculations are applied: In the first step the slope of the curve is determined. As the curve has some non-linear portions, the slope is only calculated in the range between 60 and 200 seconds. In the second step, the slope (rate) value is multiplied with an enzyme-specific factor (in this example it is 968,4) to give the result (=Enzyme Activity) of the singel sample measured in this example.

In Routine Applications the lab technician will call the *BioLight Studio* "Routine" module and start the respective Timedrive method. This method is protected and the parameters cannot be changed by a normal operator. Typically the operator will enter certain sample information and run the method. The measurement data will be

stored in a single file in a protected data base and a report will be created. In the enzyme activity determination method shown above the report might look as shown in the figure below.
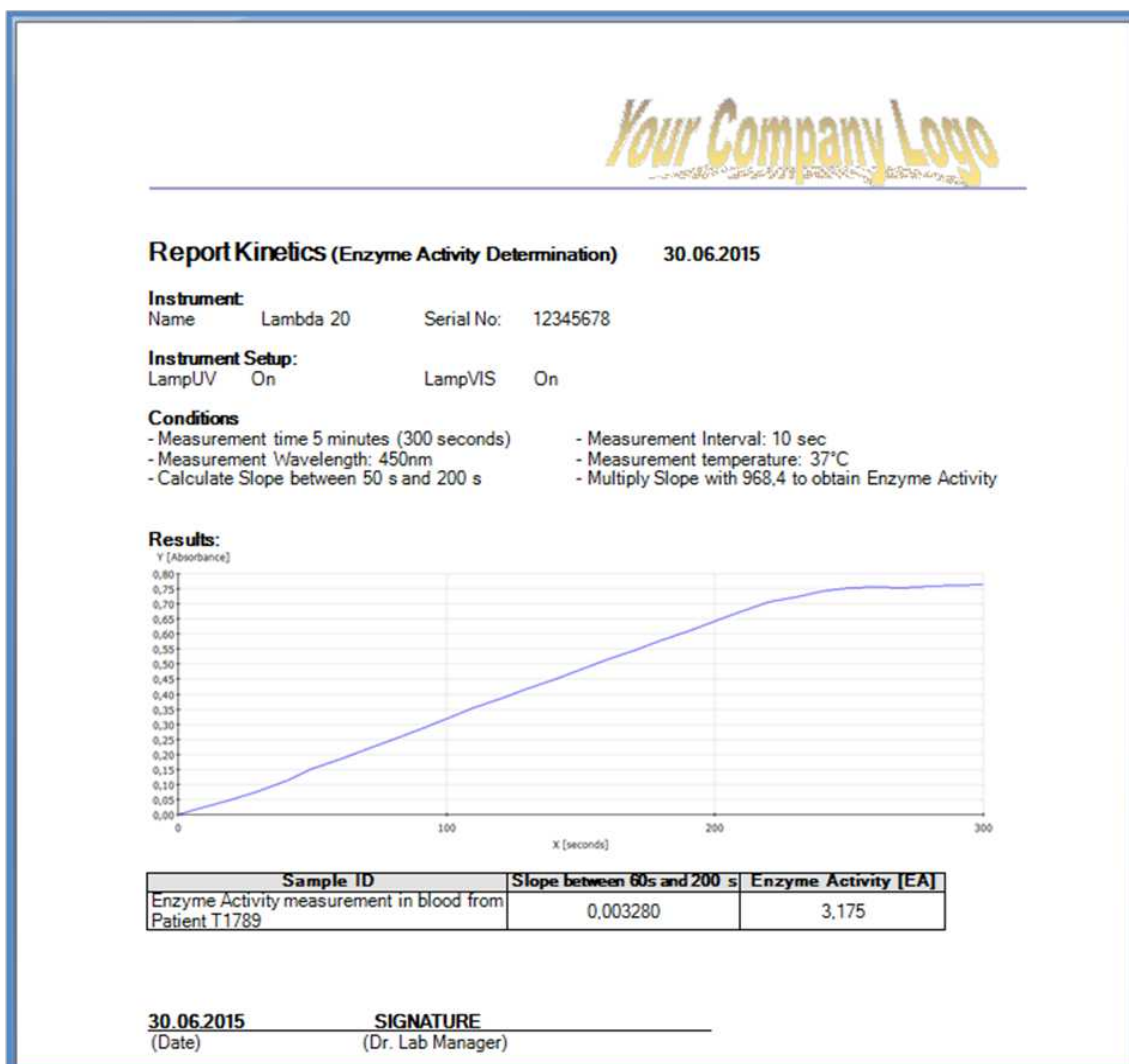

Figure 11: Report from a quantitative analysis using a calibration curve, created from the method specific template, containing company logo, instrument settings, Concentrations and Absorbance readings of the reference solutions, calibration curve with all respective information and a table with the concentration results of the unknown samples.

CONCLUSION

This report shows 3 typical routine applications as they are used in UV/Vis Spectroscopy applying 3 different analytical techniques: Wavelength Scan, Quantitation and Kinetics. All these techniques require relevant calculations to produce the final answer. The data shown here had been produced with the *BioLight Studio* software package, designed for routine analytics using UV/Vis spectroscopy. The software is very easy to use - despite its extreme flexibility and its potential to include complex calculations on the raw data and curves. *BioLight Studio* includes a flexible report generator which allows to generate an application- and customer specific report of the measurement data and all the results. *BioLight Studio* allows to set-up specific user profiles with different user rights. This is the basis for 21-CFR Part 11 compliance. Raw data, spectra and results are stored in a single file providing traceability and a comprehensive audit trail. The audit trail is organized as 'one record per day', thus allowing easy archiving of data. Altogether a dedicated spectroscopy software package - ideal for all kinds of routine applications of UV/Visible spectroscopy