Biochrom Anthos 2010

Microplate Reader

User's Manual

biochrom

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Introduction and Intended Use

The Biochrom Anthos 2010 Microplate Reader is for microplate-based applications requiring endpoint or kinetic absorbance measurements from 400-750 nm in optically clear flat-bottomed 96-well plates.

The Biochrom Anthos 2010 Microplate Reader is intended for general laboratory and research use only.

1 Declaration of conformity

Product Name:	Absorbance Microplate Reader Biochrom Anthos 2010
Manufacturer:	Biochrom Ltd Cambridge.
Address:	22 Cambridge Science Park
	Cambridge, CB4 0FJ, England

The above mentioned product complies with the regulations defined in the following guidelines:

Number:	73/23/EWG and its modifications
Title:	Low Voltage Directive

Compliance of above mentioned product with the regulations of the guideline No. 73/23/EWG is proven by adherence to the following harmonized European standards:

Reference number:	**EN 61010-1/1993 +A2/1995**
	EN 1658/1996

The above mentioned product complies with the regulations defined in the following guidelines:

Number:	**89/336/EWG and its modifications**
Title:	**Electromagnetic Compatibility**

Compliance of above mentioned product with the regulations of the guideline No: 89/336/EWG is proven by adherence to the following harmonized European standards:

Reference number:	**EN 50 081-1 1992**
	EN 50 082-1 1997

This declaration certifies the compliance with above mentioned guidelines; however, does not include assurance for properties of the product.

Safe operation requires use of the instrument by an operator informed of the safety instructions detailed with this manual as well as familiarity of good laboratory practices.

2 Safety Information

All warnings and cautions in this document include an exclamation point, a lightning bolt, or a light burst symbol framed within a triangle. Please pay special attention to the specific safety information associated with these symbols.

A WARNING calls attention to a condition or situation that may result in injury to the user.

A CAUTION calls attention to a condition or situation that may damage or destroy the product or the user's work.

Warning and Caution Symbols

The exclamation point symbol is an international symbol, which serves as a warning to read all safety instructions before attempting to install, use, maintain, or service the instrument. When this symbol is displayed in this manual, pay special attention to the specific safety information associated with the symbol.

Electrical Safety

To prevent electrically related injuries and property damage, properly inspect all electrical equipment prior to use and immediately report any electrical deficiencies. Contact a Biochrom service representative for any servicing of equipment requiring the removal of covers or panels.

This symbol indicates the potential of electrical shock existing from a high voltage source. Prevention of harm from electrical shock requires the reading and understanding of all safety instructions prior to installation, maintenance, and service.

2.1.1 Please note these particular electrical safety concerns:

- ✓ Voltages dangerous to human life are present in this device. Before removing any covers disconnect the device from the power source.
- ✓ Ensure that the power cord supplied with the unit is used.
- ✓ The power cord may only be inserted in a socket outlet provided with a protective ground (earth) contact. Extensions cords should not be used with the instruments.

- ✓ Do not replace fuses without disconnecting the instrument from first the main power cord. Ensure that only fuses with the required rated current and of the specified type are used for replacement. The use of makeshift fuses and the short-circuiting of fuse holders is prohibited.

- ✓ When the instrument is connected to the main power source, do not open the cover of the instrument or otherwise tamper with the parts of the instrument. The device shall be disconnected from all voltage sources before it is opened for adjustment or repair

- ✓ Only a qualified service engineer should adjust or repair the instrument.

- ✓ Use the equipment only in the intended manner otherwise the protection provided by the equipment may be impaired.

2.1.2 Chemical and Biological Safety

Normal operation of the Biochrom Anthos 2010 Microplate Reader may involve the use of materials that are toxic, inflammable, infectious or otherwise biologically harmful. When using such materials, observe the following precautions:

- ✓ Handle infectious samples or dangerous materials in accordance with good laboratory practices.

- ✓ Wear protective clothing when using the instrument including but not limited to laboratory gloves, laboratory coat and safety glasses

- ✓ Observe all cautionary information printed on the original solutions containers prior to their use.

- ✓ Dispose of all waste solutions safely and in accordance with the policies and practices of your facility.

- ✓ Operate the Biochrom Anthos Microplate Reader in accordance with the instructions outlined in this manual.

- ✓ Use appropriate precautions when using pathological, toxic, or radioactive materials. Consult the MSDS.

- ✓ Use an appropriately contained environment when using hazardous materials.

- ✓ Wash your hands thoroughly after handling test fluids. If equipment has been in contact with hazardous substances, it must be disinfected prior to shipment in accordance with the effective provisions.

2.1.3 Moving Parts

- ✓ Do not touch the plate during movement of the plate transport (risk of injury to the user or damage to the instrument).
- ✓ Keep the area around the microplate reader clear of clutter.

3 Installation and Use

Unpacking the Instrument and System Setup

The original packing has been especially designed to protect the instrument during transportation. It is therefore recommended to keep the original carton with its foam parts and the accessories box for re-use in case of future shipments. Warranty claims are void if improper packing causes transport damages!

Unpacking the Instrument

Check the box for any visible damage during transportation. In case of damage inform your supplier immediately and keep the damaged packing

Place the device on a suitable working surface

Remove the transportation lock (foam part) from the plate holder

Connect the serial cable to PC

Connect power cable to standard mains plug

Powering on the Instrument

Switch on main switch (rear left side).

The power indicator is located on the front left side of the unit. There are three different instrument states indicated by the power indicator:

Green light: Instrument is ready for operation.

Green flashing: A problem during initialization was encountered. The instrument is not ready for use.

Not illuminated: Instrument is not ready for operation.

Software Installation and Quick Start Guide

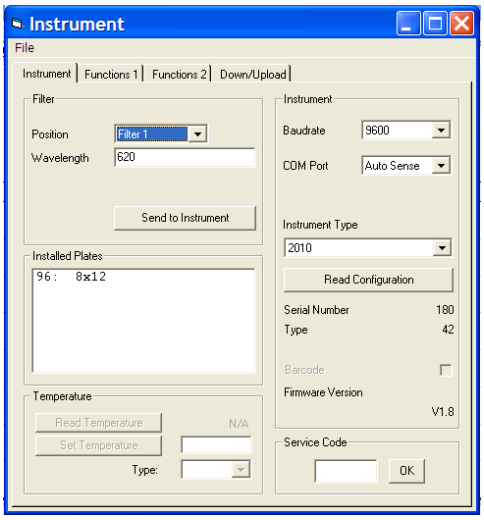
ADAP 2.0 Basic is supplied with all Anthos 2010 Microplate Instruments. The software is used for reader control for all endpoint and kinetic assays. Resulting data can be easily copied and pasted as a matrix into your software program of choice.

1. **To turn on the instrument**: Connect instrument to a power source using the appropriate power cord. Turn on the instrument using the switch at the back of the instrument.

2. **To connect the instrument to a PC**: Connect to the PC via serial port to serial port or a serial to USB port adaptor. Determine the communication port (com) used by the instrument. In the Start menu of the PC, go to Control Panel\System\Hardware\Device Manager\Ports.

3. **To connect instrument to ADAP software:** Insert the CD supplied with the instrument into PC, install ADAP. Open ADAP. ADAP will prompt for a user ID and password. Login using the pre-set ID and password: sadmin/sadmin. Once logged as sadmin, specific user IDs, passwords and administrative rights may be set.

4. Select Setup/Instrument in the task bar. A dialogue box will open:



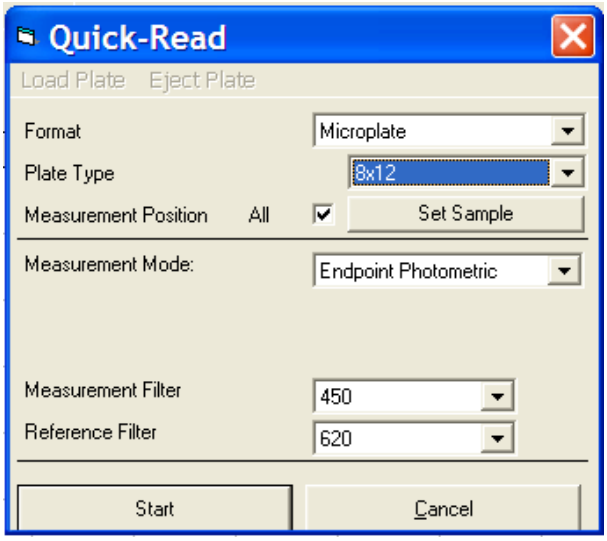
Under the **Instrument** tab, select:

Baudrate: 9600

COM Port: select port or Auto Sense

Instrument Type: 2010

5. To confirm that the instrument is connected with the computer, select the **Read Configuration** button. The serial number of the instrument should now appear in the **Setup/Instrument** dialogue box along with compatible plate types. Select **File\Save** to save settings.



6. **To measure a plate:** Go to **Reading/Quick** or the **R** button in the menu bar.

In the **Quick-Read** dialogue box: Confirm that the correct format and plate type are selected.

Select **All** in **Measurement Position** to measure the entire plate.

Select **Endpoint Photometric** for basic measurements using a measurement and reference filter.

Note: It is important to use a reference filter to account for optical inference from the plate.

7. Place plate in the plate transporter. Select **Start**. Absorbance measurements will appear in the open matrix in ADAP. When prompted, enter a plate ID. Data can be exported to data analysis software using the **Copy** icon. Data will paste as a matrix with filter wavelength, with time and date.

Consult the ADAP user's manual in for additional information.

TECHNICAL DETAILS	
Photometric method	Transmission photometer
Light Source	Tungsten halogen lamp
Photodetector	Silicon photodiode
Wavelength range	400 - 750 nm
Resolution	0.001 OD

4 Technical Information

Table 1

Measurement range	0.000 – 2.500 OD
Accuracy	2% at 1.000 OD
Linearity	<±0.75% and ±0.005 OD from 0.100 OD to 2.500 OD
Reproducibility	<±0.3% at 1.000 OD
Reading speed	45 seconds single wavelength endpoint measurement
Standard filters	405, 450, 492, 620 nm
Additional filters	Optional. Filter wheel holds up to 8 filters
Power requirement	90 -130V,180-250VAC (autosensing) 47-63Hz
Serial interface	9 pin (RS232)
Quality control	Automatic calibration, autolamp adjustment, status report
Dimensions (wxhxd)	32.6 x 17.3 x 43.5 cm (12.8 x 6.8 x 17.1 inches)
Weight	6.6 kg (14.6 lb)

Technical Specification

Table 2 Rated Operating Conditions

RATED OPERATING CONDITIONS	
Warming-up time	None required
Operating voltage	90V - 130V, 180V – 250V auto-sensing
Fuses (user exchangeable):	2 parts. 1A TH250VAC, slow blow
Built in Fuse (on power supply):	1 parts 2A TH250VAC, fast blow
Ambient temperature	15°C - +40°C (operation); -25°C - +50°C (storage)
Relative humidity	15 - 85% non-condensing (operation) < 95% non-condensing (storage)
Air pressure tolerance	54.000 - 106.000 Pascal
Height over sea level (operation):	up to 2000 m
Installation Category	II
Pollution Degree	2
Wavelength accuracy	2 nm
Spectral width at half maximum intensity	8 nm
Outer lighting influences	Precaution, avoid direct sunlight
Consumption	2010: 50VA, fuse 1A T
Operating voltage	90-130V, 180-250V
Frequency range	47 - 63 Hz (auto-sensing)

5 General features

Measurement Mode:

Single, dual and multi-wavelength and kinetics measurements with absorbance data output in OD on display.

Integrated quality control

- ✓ Post-sample filtering to eliminate ambient light.
- ✓ Automatic calibration prior to each measurement.
- ✓ Plate centering system positions all flat-bottomed 96-well microplate conforming to ANSI SBS 1_2004 accurately under the optical path.

Scope of supply

- ✓ Instrument
- ✓ Serial Cable
- ✓ 4 filters preinstalled in the filter wheel 405, 450, 492, 620 nm
- ✓ Dust-cover
- ✓ CD containing user's manual, ADAP Basic Software and ADAP operating manual.
- ✓ Spare fuses
- ✓ Power cord

Please Note Only use the power cable supplied with the instrument or a power cable with protective earth connection carrying the CE-mark.

6 Warranty Terms and Conditions

This warranty refers to the obligations of Biochrom and can only be amended upon the written consent of Biochrom Ltd.

A warranty period of 12 months shall be granted to the original buyer of the Biochrom Anthos 2010 Microplate Reader.

This warranty shall lose effect if:

- ✓ Biochrom Anthos 2010 Microplate Reader is not used in the defined scope of application.
- ✓ Biochrom Anthos 2010 Microplate Reader has obviously been damaged by external influences which are not in accordance with the provisions for the nominal range of use.
- ✓ Biochrom Anthos 2010 Microplate Reader has been modified or parts exchanged by a person other than Biochrom personnel or an authorized servicing agent.
- ✓ The warranty seals on the housing of the instrument are broken.
- ✓ The parts and subassemblies are implemented, which are not original from Anthos.
- ✓ Biochrom Anthos 2010 Microplate Reader serial number is no longer legible, has been removed or altered.
- ✓ Biochrom Anthos 2010 Microplate Reader has not been installed in accordance with the instructions supplied.
- ✓ Biochrom Anthos 2010 Microplate Reader has been damaged during return transport due to wrong packing (e.g. not in original packing material).
- ✓ Biochrom Anthos 2010 Microplate Reader was damaged due to improper operation and outside of the use described in this manual.

If a warranty is brought into operation, Biochrom will repair or replace any defects, which have resulted from faulty material or during production as it sees fit. No costs shall arise for the client (except the cost of shipping the instrument.

All components found in the original equipment, or an adequate and full compatible alternative shall be available for a period of 5 years after manufacture.

7 Liability

In its original condition the instrument meets all safety regulations for a risk-less operation.

Anthos cannot be liable for damages or any resulting costs caused by unauthorized alterations, repairs or modifications of the equipment.

8 Maintenance

Approved parts

Except for the parts shown in the following list, only parts supplied by Anthos or an authorized Biochrom Distributor may be installed in or used with the Anthos 2010:

Fuses: as specified in Table 2 **Rated operating conditions**

Cleaning and Disinfection

All parts of the reader that come into contact with potentially infectious material must be treated as potentially infectious areas. Authorized trained personnel in a well-ventilated room while wearing disposable gloves, protective glasses and clothing should perform the cleaning and disinfection procedures

<u>The following materials are recommended for cleaning and disinfection of the reader:</u>

- ✓ Non-lint tissues.
- ✓ Protein degrading mild detergent (e.g. Mucasol™ / Rogypon™) or saline solution (0.9%NaCl).
- ✓ Ethanol (70%) or chlorine solution (1500 ppm).

Note: Never spray directly into the interior of the reader.

8.1.1 Clean the reader regularly and immediately after spillage. This has to be done with due care and attention. Always observe laboratory safety rules and regulations. Do not use force when cleaning the reader.

Wipe off spills immediately with soft tissue.

Avoid build up of dust on the instrument and wipe off visible dust.

Cover the instrument with the dust cover if not in use.

The following cleaning procedure should be performed:

1. Switch off the reader.
2. Carefully wipe off the entire reader with non-lint tissues that have been moistened in a protein degrading mild detergent or a saline solution.
3. Carefully wipe off the entire reader with non-lint tissues that have been moistened in ethanol or a chloric solution.
4. Put non-lint tissues that have been moistened in ethanol or a chlorine solution onto the plate transport mechanism and let it soak for ± 30 minutes.
5. When a chlorine solution has been used, carefully wipe off the entire reader with non-lint tissues that have been moistened in water.
6. Dry the reader by wiping it off with non-lint tissues.

8.1.2 Disinfection

Before the reader is returned to the distributor it must be disinfected and a disinfection certificate must be completed.

The following procedure must be used for disinfecting the reader:

1. Switch off the reader and disconnect it from the mains power supply.
2. When used, disconnect the reader from any accessories (printer and PC).
3. Carefully wipe off the entire reader with lint-free tissues that have been moistened in a protein degrading mild detergent or a saline solution.
4. Carefully wipe off the entire reader with non-lint tissues that have been moistened in ethanol or a chlorine solution.
5. Put non-lint tissues that have been moistened in ethanol or a chlorine solution onto the plate transport mechanism and let it soak for ± 30 minutes.
6. When a chlorine solution has been used, carefully wipe off the entire reader with non-lint tissues that have been moistened in water.
7. Dry the reader by wiping it off with non-lint tissues.
8. Pack the reader in its original packaging.
9. Complete a disinfection certificate and make a copy of the certificate.

- Enclose the disinfection certificate in the reader package and attach the copy to the outside of the package so that it is clearly visible.

Changing a Fuse

In case of malfunction, the fuses (in the mains filter next to the mains socket on the rear of the device) can be checked and replaced.

Disconnect the instrument from mains by unplugging the power cable.

Open fuse-carrier next to the mains socket with a screwdriver

Remove fuse

Insert spare fuse included in supply and specified earlier in this manual

Close fuse-carrier.

Turn device on and check functioning. In case of malfunction, call a service technician.

Disconnect the instrument from the power source. Move the instrument to the edge of a table so that it stands on three feet.

With a socket wrench unscrew the four hexagonal bolts on the bottom of the instrument holding the upper housing. Do not turn the instrument upside-down because the transport system could become misaligned.

8.1.3 Mathematical Error Messages

- **A**

  Addition: is displayed if the argument left or right of the + is missing.

  Argument not in domain of function (Undefined result): is displayed when an Argument of a formula is out of the defined range of this operation (e.g. Log (-1)).

- **C**

  Calculation of well XNN: (state window) - is displayed if the calculation of the transformation is erroneous.

  Correlation too low: (state window) - is displayed if the quantitative evaluation is erroneous.

  Cut-Off Values: (state window) - is displayed if the qualitative evaluation is erroneous (e.g.: calculation of the cut-off values; cut-off values are not increasing).

- **D**

  Division: is displayed if the argument left or right of the / is missing.

  Division by zero: is displayed when a mathematical operation results in a division by zero.

- **F**

  Formula Error: (state window) - is displayed if the evaluation formula is erroneous (e.g. replicate elimination; calculation of the test validation).

  Function: e: is displayed if the argument of the Exp function is missing.

  Function: ln: is displayed if the argument of the Ln function is missing.

  Function: log (base 10): is displayed if the argument of the Log function is missing.

  Function: power: is displayed if one or both arguments of the Pow function are missing.

- **I**

  Incorrect number-format: is displayed when a number with more than one comma is entered (e.g. 12.34.5).

  Inversion: is displayed when the argument after NOT is missing (e.g. NOT()).

- **L**

  Left parenthesis expected: is displayed when the left parenthesis of a formula is missing.

- **M**

  Mathematical error: (state window) - is displayed if the quantitative evaluation is erroneous (e.g. linear regression; 4-paramter-fit).

  Multiplication: is displayed if the argument left or right of the * is missing.

- **N**

  Number length exceeded: is displayed when number is longer the 14 characters..

- **O**

  Overflow: is displayed when the result of a mathematical operation exceeds the maximum of the positive range (e.g. Exp (1000)).

- **P**

  Parameter expected: XXX: is displayed if the argument left or right of an operator is missing (e.g.: 1>).

  Precondition: is displayed when the result of a mathematical operation is out of the specified range (e.g. the response value for a 4-parameter-fit is less or equal the lower asymptote).

- **R**

  Result: is displayed if the entered formula gives more or less the one result.

Result is a singularity: is displayed when the result of a mathematical operation would be a singularity (e.g. Pow (0, -2)).

Right parenthesis expected: is displayed when the right parenthesis of a formula is missing.

- **S**

  Spline: (state window) - is displayed if the quantitative evaluation is erroneous (e.g. curve not definite).

  Standard-Point Calculation: (state window) - is displayed if the quantitative evaluation is erroneous (e.g.: calculation; monotony).

  Subtraction: is displayed if the argument left or right of the - is missing.

- **T**

  Total loss of significance: is displayed when the digits behind the comma are not correct.

  Type mismatch: is displayed when the entered formula is not correct (e.g.: (X>0.5)AND(1))

- **U**

  Underflow: is displayed when the result of a mathematical operation exceeds the maximum of the negative range (e.g. Log (-1)).

  Unknown character: X: is displayed when the formula contains an unknown character (e.g.: X*~); this is only possible, when the instrument is controlled with an external PC.

- **V**

  Variable not found: is displayed when formula contains a variable, which is not defined.

8.1.4 Device and Communication Error messages

- **sb1.1 FILTER ERROR**

  If the fw value in NORMAL MEASUREMENT command (reefer chapter 10.7) does not match the fw from the anthos 2010/2020 lists, the FILTER ERROR flag and ALL ERROR flag are set. This is the information that the requested filter is not installed in the anthos 2010/2020. Also a movement of the filter wheel during a NORMAL MEASUREMENT command or an error in the SET FILTER command can cause this error.

- **sb1.2 TRANSPORT INIT ERROR**

  The error may occur during the transport initialization and is reported through this status flag. It indicates that the transport system is not in the load position. A defective motor, transport mechanism or sensor could be possible reasons for this error.

- **sb1.3 PRINTER BUSY ERROR**

  Before a byte is transmitted to the printer the BUSY line is checked. A 0.5 seconds timeout is set for waiting for the printer. Possible reasons: printer is OFF-LINE, no paper.

- **sb1.4 PRINTER ACKNOWLEDGE ERROR**

  Indicates no ACK response from printer for transmitted characters. Only the GET PRINTER STATUS command checks the ACK signal. A switched-off printer can be the reason for this error!

- **sb1.5 AD TIMEOUT ERROR**

  Means that there is no signal from ADC Chip. This indicates a hardware error.

- **sb1.6 LAMP ERROR**

  The MAX. measurement is done with diode values between 60 000 to 65 000. If it is not possible to reach this lamp energy level the LAMP ERROR is reported. Possible reasons:

  1.) The lamp, lamp connections and/or the lamp regulation are defected.

  2.) The transport system is not in the load position.

  3.) The filter wheel is not in the correct position.

  4.) The lamp energy value is not correct (an error, which may have been coming up during the SET FILTER command, or an EEPROM error).

- **sb1.7 Y SENSOR INTERRUPT ERROR**

  The movement of the transport system is not correct (i.e. the location of the wells is not correctly in the optical path).

- **sb2.0 MEASUREMENT TIMEOUT ERROR**

  The motor of the transport system does not move or a transport sensor is defected.

- **sb2.1 Watch-Dog ERROR**

  Set by the Watchdog reset routine. Indicates an unknown, usually undetermined system error.

- **sb2.2 TO LOW MOTOR SPEED ERROR**

  The transport speed is too slow.

- **sb2.3 DELTA MAX TO HIGH**

  The lamp energy is not stable.

- **sb2.4 START POSITION ERROR**

  The transport system is not in the load position. The transport INITIALIZE command should be repeated!

- **sb2.7 ALL ERROR**

  This is a common error status bit which is logical OR of all the individual error status flags. This is a read only status bit useful for a quick check if any error status flag is set. The GET STATUS command clears this and other error status bits.

1. Appendix:

Helpful Hints for Successful Microplate Assays

- ✓ Always insert the plate with well A1 on the upper left position.
- ✓ Keep plates clean: measurements in microplates are made through the surface of the plate and thus the surface should be as free as possible of debris, scratches and smudges. Wipe the plate clean with a lint-free tissue if necessary.
- ✓ Measurements may be affected by uneven or heterogeneous well contents. Visually inspect the plate for foaming, uneven meniscuses, bubbles or particles in the well, all of which can interfere with absorption. Centrifuge the plate if necessary to obtain a homogeneous solution in the wells.
- ✓ Use a reference measurement for reducing measurement error caused by interference from the plate material or contamination. (Use a reference wavelength at which your molecule of interest does not absorb. Many assays recommend a reference measurement at 620 nm.)

- ✓ When using plate frames for column/row strips or single wells (breakable strips) check that all strips and wells are pushed down completely and are level with the frame.

- ✓ Keep the bottom of the dry. If liquid has contaminated the well bottoms or if condensation is present, dry the surface with a lint-free tissue.

Use optically clear microplates with flat wells for the best results. Other microplates can be used as well: consult the Biochrom website for a list of microplates that are approved for use with the Anthos 2010.

Principles of Photometry Measurement

Light is electromagnetic wave radiation. Rays from 100nm to 400nm are defined as the ultraviolet spectrum of light. Only rays in the range from 400nm to 780nm are visible for the human's eye, rays with longer wavelengths are called infrared. Color impressions are caused by reflection of electromagnetic waves striking the surface of material substances. Substances absorb the complementary spectrum of their visual perceptible color. Hence green plants look green since they absorb red light (light of a wavelength perceived as red, e.g.: round about 750nm). A photometer is an optoelectronic measuring device to determine the amount of light absorbed at a specific wavelength. Therefore a photometer can be used to determine the concentration of a certain light-absorbing substance in a colored solution.

8.1.5 Absorbance Measurements

Experimental measurements return the value of transmission (**T**):

Transmission is defined as the percentage ratio between the total available light energy at the detector (measured through air) and the residual luminous energy after sample transmission at a specific wavelength.

$$\mathbf{T = I/I_0}$$ (I.....Light intensity after passing through the sample

I_0.... Initial light intensity)

Transmission has no linear relation to concentration; therefore **A**bsorbance (also called **O**ptical **D**ensity) is calculated from Transmission by the formula:

$$\mathbf{A = OD = -\log T}, \text{ or } \mathbf{A = OD = \log (1/T)}$$

Practically, the logarithmic relationship between transmission and absorbance means that if a solution has 10% transmission then doubling the concentration of the absorbing material using an identical path length results in 1% transmission. If the solution became 2 fold more concentrated the transmission would be 0.1 %. Where as the absorbance reading for the 1x solution would be 1 OD and the 2x solution would be 2 OD.

If one layer of a certain material allows 10% transmission, doubling either path-length or concentration would bring down the transmission to 1% and doubling both would bring it down to only 0.01%

Absorbency is related in a linear way to layer thickness and sample concentration according to the "Bouger-Lambert-Beer" law:

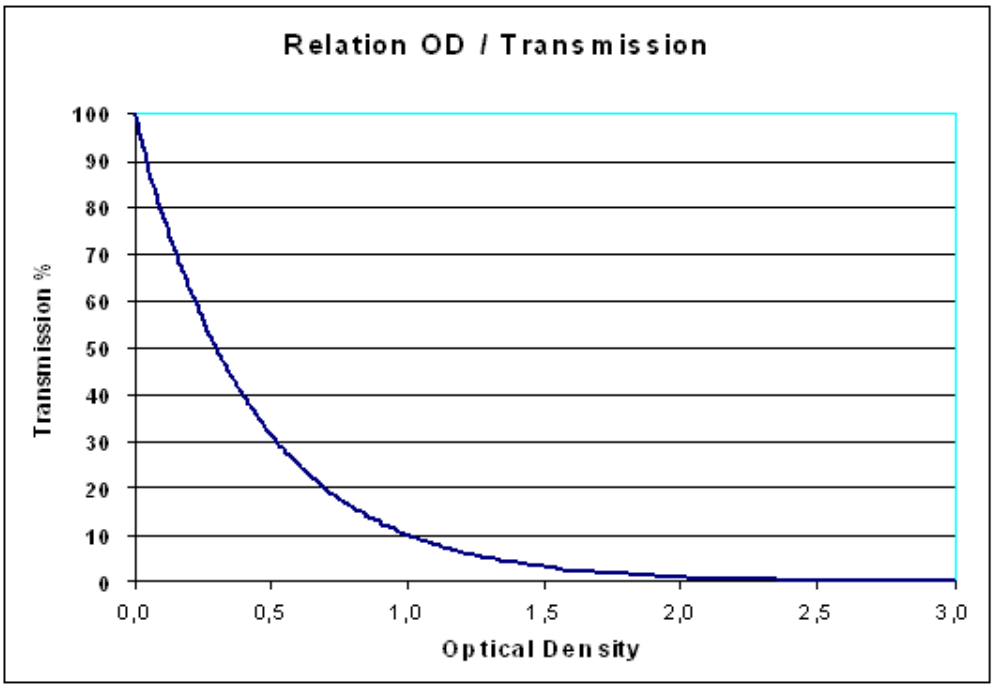
E = OD = c * d * e

(c = the concentration of the sample to be measured

d = the layer thickness of the sample to be measured

e = the molar extinction coefficient)

The following graph illustrates the relation between Absorbance and the light transmitted and measured at the detector:



It is important to keep in mind that the best measurement range of Absorbency is from 0.1 to 1 OD or 90% to 10% of transmission. Measurements above 2 OD deal with less than 1% of the original light and will therefore have lower resolution and accuracy.

8.1.6 Measurement at Specific Wavelengths

Each substance to be measured has a specific transmission profile that indicates its concentration and composition. A measurement with white light renders different sample concentrations in a certain spectral range only to a small amount and accordingly inaccurate. A higher significance can be obtained by using only that part of the light spectrum, which is relevant to prove the respective wavelength of the substance.

Due to this fact interference filters of a narrow banded wavelength spectrum are employed in this instrument. Each substance has a certain absorption spectrum. A measurement should be performed by selecting the correct filter for the maximum absorption of the sample, because in this way the best differentiability of various sample concentrations can be reached. We can also state that the differing absorption amplitude of two uniformly composed samples is a measure for the concentration. For this reason measurements in the flank area of the spectrum are not very accurate and therefore to be avoided. In this connection, it has to be taken into consideration that - when using interference filters – wavelength tolerances of +/- 2 nm from the nominal wavelength value may also result in inaccuracies. Each sample has an absorption minimum not specific to the measurement value, which can be deducted from the measurement value automatically by choosing a suitable reference wavelength. This kind of measurement is referred to as bichromatic.