NANOCOLOR® PC Software

for

Spectrophotometers

Version 4.0

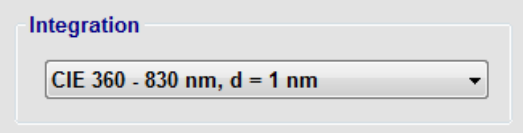
Rev. 13 (October, 2010)

Instructions for Colour Measurement

Software Manual Addendum I

Important Notice

All colour scales have been meticulously calibrated by MACHEREY-NAGEL. The calibrations were performed strictly following the recommendations of the International Commission on Illumination CIE (*CIE 15:2004 3rd Ed.*). A **wavelength range from 360 to 830 nm in 1 nm intervals** was used for the calculation (see figure).



The use of different scan ranges and intervals can affect the determination of colour values and lead to false results.

All calibrations were performed against distilled water as ZERO standard.

CAUTION! Please cover the cuvette slot to perform colour measurements!

All *NANOCOLOR*® photometers were developed for the analysis of rapid tests in cuvettes and do not need slot cover. Every possible influence due to external light is compensated so that an error-free measurement can be performed.

In colour measurements, however, the normally insignificant residual errors add up 4500 times per each colour measurement!
This can lead to a total error of up to 5%, especially when working with fluorescent light!

For that reason, please cover the cuvette slot for each colour measurement!

Error Considerations

The ***NANOCOLOR***® PC-Software for Spectrophotometer calculates the standard deviation for all colour parameters, based on Gauss' error propagation law. This standard deviation is shown in parentheses behind the measured value, like 1,52(0,04). The software automatically **rounds off** all measured values to the number of significant decimals.

The maximum deviation of 0,005 E for a measured value of 1,000 E, which is indicated in the photometer specifications, is used for the calculation of the standard deviation. Based on this maximum deviation, a standard deviation of about 0,003 E can be estimated.

Consequently, the following mean deviations must be expected:

L* : (±0,05)	a*, b* : (±0,3)	X,Y,Z : (±0,04)	x,y,z : (±0,0007)
u*, v* : (±1)	ICUMSA : (±1)	EBC : (±0,08)	ASBC : (±0,03)
Hess-Ives : (±0,9)	Yellowness Index : (±0,4)		Klett : (±5)

1 Introduction

The ***NANOCOLOR*® PC software for Spectrophotometers** version 4.0 from MACHEREY-NAGEL offers the possibility to conduct colour measurements. When performing a colour measurement, the specific colour of a solution is determined with the aid of a spectrophotometer. Measuring the colour may be a simple task for the user, but determining the value or indicator number of a colour is an extensive and complicated task. To correctly use all the functions of the software, specific knowledge in colour measurement is necessary. Chapter 1 of this manual explains the fundamentals of colour measurement. Users who are familiar with colour measurement and just want to learn the software functions can read from chapter 2, page 20.

1.1 Colour Measurement

There are two main reasons why the colour is measured in production processes. The first case is when the colour of a product is considered a quality criterion. The product must have a specific colour within a very narrow range of variation (e.g. beer). In the second case, the product is supposed to be colourless but shows a colouration indicating some quality deficit (contamination, oxidation or yellowing) which can only be tolerated between defined limits (for example in sugar, fuel, clarified wastewater). In this case, the colour measurement is carried out with the aim of describing the colouration of the product and quantifying it with one or more numbers. In practice, there are different ways to achieve this: a) with colour scales b) named colours and c) colour spaces.

1.1.1 Colour Scales

The oldest and simplest method to determine the colour of a sample is using colour scales. Here, the sample is compared with a series of coloured standard solutions or coloured glass filters. The result corresponds to the standard solution or filter with the most similar colour, or can be expressed as "a colour between standards 3 and 4". These kinds of colour scales are large in number and include the USP and Ph.Eur. scales, the Iodine scale, the Hazen/PtCo scale, the Gardner scale, among others.

Colour scales have crucial disadvantages. Even if you use a photometer to eliminate every subjective interpretation of the result, colour scales remain a "visual comparison" method. Photometers do not calculate the colour of the sample in the colour scale used, they just compare measured values with a calibration table and indicate which is the "closest" value in the scale (see Chapter 2.4, page 36). Strictly speaking, the colouration of the sample must match exactly the colouration of the calibration solution. According to the DIN standard for the iodine colour evaluation, "if the colouration of the sample does not match the iodine colouration, the iodine colour scale may not be used."

Other colour scales, such as the EBC for beer or the ICUMSA for sugar, are based on a measurement of the absorbance at one or a few wavelengths. The disadvantage of these methods is that colour deviations at wavelengths other than the test wavelength are not noticed.

Colour scales are therefore unsuitable to make an absolute determination of the colour of a sample.

1.1.2 Named Colours

Different colour classification systems have been developed. The most known are the Munsell, NCS®, Pantone®, RAL®, and HKS® colours. There are colour books or cards available in every system. The determination of the colour is done by visual comparison. The advantage of colour systems is that every colour can be named (e.g. ***7.5 R 6/16*** in the Munsell system). Many of these systems were developed by paint manufacturers. This makes it possible, for example, to buy HKS® colours in cans. As you can recognise by the registered trade mark symbol "®", most of these systems were created for commercial purpose. The manufacturers normally offer small reflection photometers which measure the colour on solid samples and indicate the closest colour in their systems.

Only the American Munsell colour system has been designated by the standardisation body ASTM as the standard for colour comparison. Since the calibration data of the near 1900 Munsell colours have been published, the ***NANOCOLOR*® PC software for Spectrophotometers** is able to indicate the closest Munsell colour in each colour measurement. With respect to the other systems, calibration data do not exist or cannot be used due to copyright reasons. What all systems have in common is the very limited number of approx. 2000 colours. For this reason, they are not appropriate for colour measurement. Indicating the "closest" colour of a colour system may be useful, if the measured colour is going to be printed by a printing press.

1.1.3 Device-dependent Colour Spaces

Normally, colour spaces are three dimensional spaces with a colour component on every axis. The colour of an object is defined by combining these three elements. Every computer user has heard about the RGB colour space. Every colour that can be displayed on the monitor is obtained by mixing red, green and blue lights. For example, a RGB colour like {150, 220, 60} is composed of 150 parts of red, 220 parts of green and 60 parts of blue. The colour is generated by mixing these three primary lights (additive colour model = the colour is generated by adding light). Unfortunately, it is not possible to print with lights. Inkjet printers use pigment-based or dye-based inks which absorb light. When mixing printing inks, the colour is obtained as a result of light being "absorbed" (subtractive colour model). The most known colour space may be the CMYK space. By mixing the primary colours cyan, magenta and yellow

(in offset printing, black is also used as a 4th colour), every colour can be represented.

The number of colours that can be represented by mixing lights is not the same as the number of colours that can be represented by mixing printing inks: the RGB and the CMYK colour spaces present big differences.

Why device-dependent? Because nobody has defined a standard for what is red, green and blue, or cyan, magenta and yellow. This is the reason why a colour like {150, 220, 60} will be displayed differently on monitors of different manufacturers. The ***NANOCOLOR*® PC software for Spectrophotometers** alone has 15 different RGB colour spaces (and there are considerably more).

So, an objective determination of the colour of a sample is only possible using an absolute colour space.

1.1.4 Absolute Colour Spaces

An absolute colour space is a three-dimensional co-ordinate system containing all colours that the human eye can perceive. Every colour clearly corresponds to a point in the co-ordinate system. The position of a colour in the colour space only depends on the spectral composition of the light used and the absorption characteristics of the sample. All absolute colour spaces are based on the XYZ colour space created by the International Commission on Illumination (CIE).

Figure 1 shows the CIE XYZ colour space as a Yxy transformation (see Chapter 1.3, page 13).

The values X,Y and Z are called standard colour values or tristimulus values.

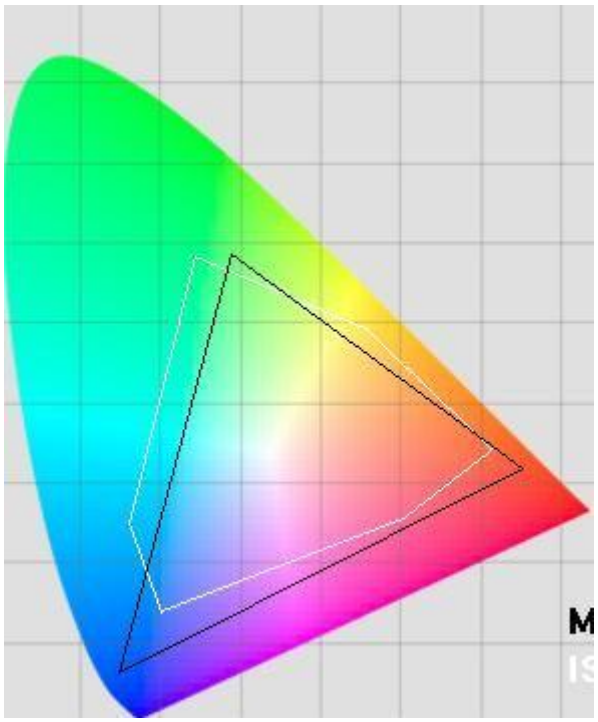


Fig. 1: Yxy projection of the XYZ colour space

The x co-ordinate represents the red component of the colour and the y co-ordinate represents the green component. The z co-ordinate is perpendicular to the xy plane and represents the lightness (brightness). This chromaticity diagram contains (in schematic form) all visible colours. The pure spectral colours (e.g. the colour at 560 nm) are on the outer curved boundary of the diagram. The black triangle represents schematically the colour gamut of a device using the RGB colour space. As it can be observed, a monitor can display considerably fewer colours than the human eye can see. The white polygon represents the colour gamut of a CMYK device.

Important: this diagram is very often misunderstood! It should be always remembered that this is a very schematic representation. Of course, you can also see the colours located outside both gamuts on a monitor or a printout. The reason is that it is impossible to create digital pictures containing the true XYZ colours. All digital pictures are GIF or JPG files that contain the RGB colour space. If it were possible to pack the XYZ colours in a JPG file, all colours outside of the black triangle would appear black on the monitor! (If this page were printed, then everything outside the white polygon would be black too!) The diagram just illustrates the nature of XYZ colours that can be seen in the real world, but cannot be displayed by a monitor.

1.2 Colour Measurement in an Absolute Colour Space

When measuring a colour in the XYZ colour space, the visually perceived colour of a solid is determined with reflected light or, as is usually the case in photometry, with transmitted light. The measurement is carried out with the aim of quantifying or describing with numbers the "visually perceived colour", making it clearly reproducible.

Many decisive factors are involved in the way we perceive colours. Figure 2 shows a simple test arrangement.

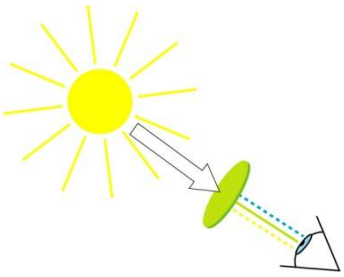


Fig. 2: Measurement with transmitted light

The figure above shows three of the factors that are involved in colour perception.

1.2.1 The Light Source

As you can see in the sequence shown in Fig. 2, the first factor that influences colour perception is the light source. The spectral energy distribution of light affects the way the eye perceives a colour. A pure white object would seem white by daylight, slightly yellow with a bulb light and slightly red by sunset. Thus, the spectral energy of the light source used for the illumination (or transillumination) of the sample plays a decisive role. The International Commission on Illumination CIE has defined two standard light sources: *Standard Illuminant A* and *Standard Illuminant D65*. *Standard Illuminant A* represents the light of a 100 W bulb with tungsten filament (colour temperature approx. 2856 K). *Standard Illuminant D65* represents the daylight (D = Daylight) with a colour temperature of approx. 6500 K. There are also other **(non CIE)** standard daylight types like D50, D55 and D75, as well as *Illuminant C* (average daylight with a colour temperature of 6800 K) and *Illuminant E* (equal energy spectrum).

All light sources mentioned above can be used with the ***NANOCOLOR*® PC software for Spectrophotometers V 4.0,** as well as user-defined light sources (see Chapter 3.5, page 48).

How is it possible to use different light types, if the photometer has only one halogen lamp?

Photometers measure absorbance or transmission giving only a relative measured value (i.e. how many fractions of energy are absorbed by the sample at abc nm). They do not tell you how much energy falls on the sample at abc nm. The measuring principle they use, i.e. to subtract the zero value from the measured value (in two-beam devices, the reference beam from the measuring beam), eliminates all information about the spectral energy distribution of the light source. If photometric data is going to be used for the colour measurement, the information about the spectral energy distribution of the light source must be recalculated from data contained in tables. The data tables of the different light sources are stored in the software.

Fig. 3 shows the spectral energy distribution **S(λ)** of Illuminants A, D65 and C. The relative energy values are standardised, so that 560 nm correspond to a value of 100.

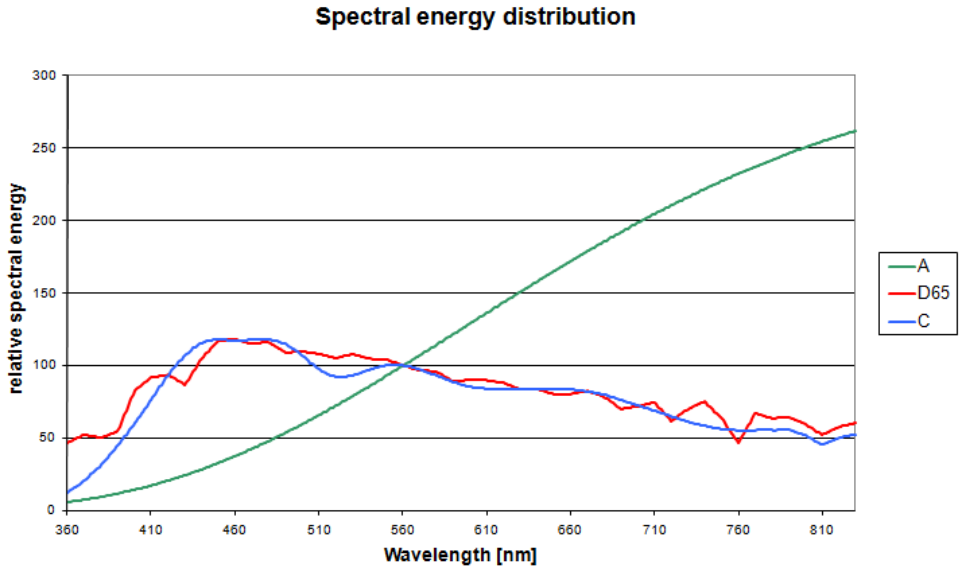


Fig. 3: Spectral energy distribution of Illuminants A, D65 and C

The spectral energy distribution functions **S(λ)** for all light sources are saved in a table in 1 nm intervals, which is included in the ***NANOCOLOR*® PC software for Spectrophotometers V 4.0**.

1.2.2 The Sample

Back to figure 2, the second and most important factor that influences colour perception is the sample itself. The sample absorbs more or less energy at different wavelengths depending on its chemical nature. If the absorption ranges are situated on the visible spectrum, the sample will appear coloured. The wavelength-dependent absorption function **T(λ)** is measured with the ***NANOCOLOUR***® Spectrophotometer in transmission (I / I_0) from 360 to 830 nm.

1.2.3 The Eye

The third factor that influences colour perception is the eye. In the retina of the eye, you can find photoreceptor cells: rods and cones. Rods are light-sensitive and therefore responsible for day/night vision. They have no role in colour vision. Unlike rods, cone cells allow the perception of colour. According to their sensitivities, they can be roughly classified in red, green and blue cones. The wavelength-dependent sensitivity curves of these three cell types have been measured and are well known. The data tables are also included in the software. In specialised literature, the sensitivity functions (colour matching functions) of the red, green and blue cones are called **x(λ)**, **y(λ)** and **z(λ)**, respectively. Figure 4 shows the relative sensitivity of these three cone types.

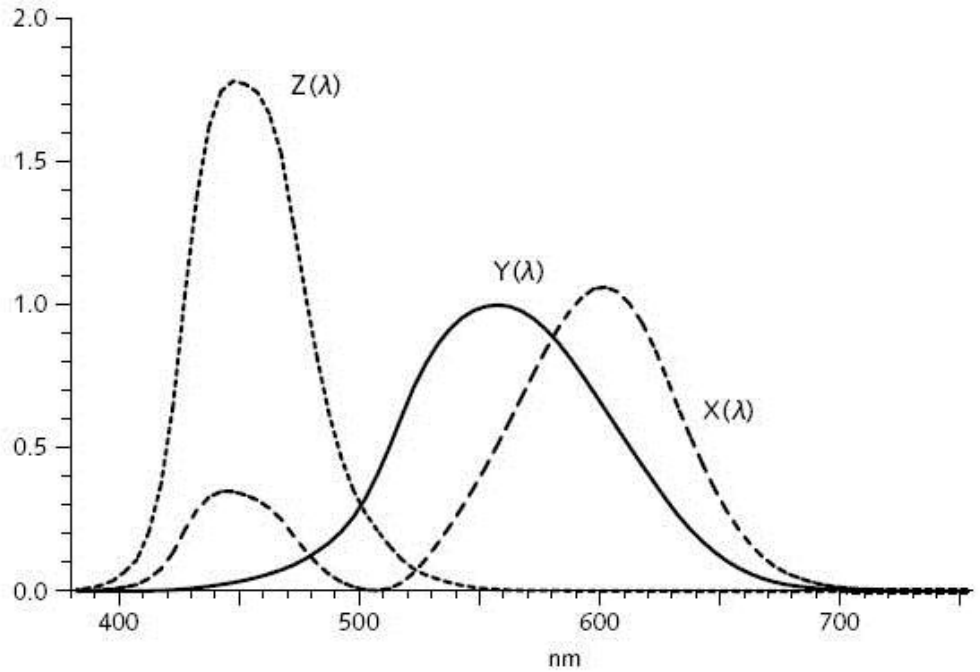


Fig. 4: Relative sensitivity of the red, green and blue cone pigments

The "influence of the eye" does not mean "personal sensation or interpretation of colour"! It refers only to the – physically measurable – reaction of the photopigments in the retina.

1.2.4 The Observer

The fourth factor which influences colour perception is the observer or, better said, what the observer sees. Study results demonstrated that colour perception is influenced by the size of the observed object. If you put two objects of the same colour side by side, the first with an area of 1 cm^2 and the second with an area of 10 cm^2, and look at them from the same distance, you will perceive them as differently coloured. This is due to the non-uniform distribution of the cones in the retina: in the centre of the retina they are closely spaced and become sparser towards the periphery. For this reason, the International Commission on Illumination has defined two standard observers: the *CIE 1931 2°* Standard Observer and the *CIE 1964 10°* Standard Observer. 2° and 10° refer to the viewing angle at which the coloured sample is observed. 2° stands for the 1 cm^2 area and 10° for the 10 cm^2 area. This means in practice that there are different sensitivities for the cone cells: **x(λ)**, **y(λ)**, **z(λ)** and $\mathbf{x(\lambda)_{10}}$, $\mathbf{y(\lambda)_{10}}$ $\mathbf{z(\lambda)_{10}}$. The International Commission on Illumination recommends using the subscript 10 in the functions when working with the *CIE 1964 10°* Standard Observer.

Both options can be adjusted in the ***NANOCOLOR*® PC software for Spectrophotometers V 4.0**.

1.3 Calculating the Standard Colour Values XYZ

The first step in a colour measurement is the determination of the standard colour values. A wavelength scan from 360 nm to 830 nm is performed (see *CIE 15:2004 3rd Ed., 6.1*) and the transmission is measured in 1 nm intervals. The result is shown directly as the function **T(λ)**. The function **S(λ)** - as said before - depends on the type of light source and the required data is stored in the software. Depending on the observer that has been set, the software will use the functions **x(λ)**, **y(λ)** and **z(λ)** or $\mathbf{x(\lambda)_{10}}$, $\mathbf{y(\lambda)_{10}}$ and $\mathbf{z(\lambda)_{10}}$. Hence, the standard colour values are calculated as follows (the example shows formulas 1 to 3 for a 2° observer):

$$X = K * \int_{360}^{830} S(\lambda) * T(\lambda) * x(\lambda)\ d\lambda$$

$$Y = K * \int_{360}^{830} S(\lambda) * T(\lambda) * y(\lambda)\ d\lambda$$

$$Z = K * \int_{360}^{830} S(\lambda) * T(\lambda) * z(\lambda)\ d\lambda$$

Formulas 1-3: Calculation of standard values

The integrals are standardised so that the resulting value for Y without any absorption is 100% (**T(λ)** = 1). Hence, the factor K is calculated as follows (see formula 4):

$$K = 100 \; / \int_{360}^{830} S(\lambda) * y(\lambda) \; d\lambda$$

Formula 4: Calculation of the standardisation factor

The use of **chromaticity co-ordinates** instead of standard colour values (take note of the **small letters** in the formulas) is very frequent. The calculation is made with the following formulas 5 to 7:

$$x = \frac{X}{X + Y + Z}$$

$$y = \frac{Y}{X + Y + Z}$$

$$z = \frac{Z}{X + Y + Z}$$

Formulas 5-7: Calculation of chromaticity co-ordinates

Since by definition **x + y + z = 1**, the value of z is often not indicated in the literature.

The values of **X**, **Y** and **Z** or **x**, **y**, and **z** obtained with this kind of colour measurement describe mathematically, in an exact way, the visual sensation of an average human eye. For an exact description or definition of a colour, these values are more than enough.

They have only **one crucial disadvantage**: Nobody can imagine a colour with the expressions 34.12, 12.45 or 45.64. To circumvent this disadvantage, the values measured in the standard colour space are transformed to another colour space that corresponds to the colour theory, of which the co-ordinates can be represented. Several colour spaces are common and differ basically in their mathematical transformation.

1.3.1 The CIE L*a*b* Colour Space

The most used colour space is the CIE L*a*b* colour space, also called CIELAB, or simply, Lab. In this three-dimensional space, "L" represents the colour-independent lightness (brightness). "a" denotes the red-green axis where positive values indicate a shift towards red and negative values a shift toward green. Along the "b" axis are placed the complementary colours yellow (positive values) and blue (negative values).

To calculate the L*a*b* values, you need the standard colour values of the light source used. They are marked with a subscript (e.g. $\mathbf{X_n}$). That means, the colour values of the light source correspond to the colour of the ZERO standard. The standard colour values of the different light sources are saved in the PC software in tabular form. They can be calculated with the formulas 1 to 3 or 4 to 7. The formulas 8 to 12 are applicable to the parameters L*, a* and b*. The condition for ($\mathbf{X/X_n}$) is also valid for ($\mathbf{Y/Y_n}$) and ($\mathbf{Z/Z_n}$). Since it is not possible to write asterisks with the function generator, the degree symbol (°) has been used in the formulas (L° corresponds to L*).

$$L^\circ = 116 * f(Y/Y_n) - 16$$

$$a^\circ = 500 * [f(X/X_n) - f(Y/Y_n)]$$

$$b^\circ = 200 * [f(Y/Y_n) - f(Z/Z_n)]$$

$$f(X/X_n) = (X/X_n)^{(1/3)} \quad wenn \quad (X/X_n) > (24/116)^3$$

$$f(X/X_n) = (841/108) * X/X_n + 16/116 \quad wenn \quad (X/X_n) \leq (24/116)^3$$

*Formulas 8-12: CIE L*a*b* system*

$f(Y/Y_n)$ and $f(Z/Z_n)$ are calculated analogously to $f(X/X_n)$.

1.3.2 The CIE L*u*v* Colour Space

The formulas 13 to 17 can be applied to the parameters L*, u* and v*.

$$u' = \frac{4X}{X + 15Y + 3Z}$$

$$v' = \frac{9Y}{X + 15Y + 3Z}$$

$$L^\circ = 116 * f(Y/Y_n) - 16$$

$$u^\circ = 13 * L\ (u' - u'_n)$$

$$v^\circ = 13 * L\ (v' - v'_n)$$

*Formulas 13-17: CIE L*u*v* system*

$f(Y/Y_n)$ is calculated as indicated in 4.7.1.

1.3.3 The Hunter Lab Colour Space

The parameters L, a, and b can be calculated with the formulas 18 to 20. In this space also, the L axis represents the lightness, along the a-axis are the red-green colours and along the b-axis the yellow–blue colours.

$$L = 100 * \sqrt{Y/Y_n}$$

$$a = K_a * \frac{X/X_n - Y/Y_n}{\sqrt{Y/Y_n}}$$

$$b = K_b * \frac{Y/Y_n - Z/Z_n}{\sqrt{Y/Y_n}}$$

Formulas 18-20: Hunter Lab system

The factors K_a and K_b depend on the light type. They are shown in table 2.

Light source	Ka	Kb
A	185,20	38,40
C	175,00	70,00
D65	172,30	67,20
D50	173,51	58,48
D55	172,47	64,72
D75	172,22	71,30
E	176,68	64,96

Table 2: Factors K_a and K_b

1.3.4 Interpretation of the CIE L*a*b* Colour Space

Since all mentioned colour spaces are similar and the most widely used is the CIE L*a*b* colour space, it will be explained in this section. In this space, the three standard colour values XYZ are translated to the L*, a* and b* co-ordinates. Hereby, L* is normalised to the values 0 to 100, corresponding to a percentage scale (%). The L* co-ordinate describes only the lightness and does not contain any colour-related information. L*=100% means 100% light and L*=0 means *no light* (black).

The a* and b* coordinates are not normalised, so that theoretically they can go from +infinite to –infinite. In practice, i.e. with real colours, you find values between -120 and +120. These co-ordinates contain the colour information. If a* = 0 and b* = 0, there is no colour. This means, the sample is light or dark grey, depending on its L* value (L* 100 = white, L* 0 = black).

On the a* axis are located the complementary colours red and green: positive a* values represent reddish colours and negative values indicate greenish ones. A more positive a* provides a more reddish tone, and a more negative a* provides a greener tone.

The same applies to the b* axis with the colours yellow and blue. Consequently, we can affirm that a* and b* describe the colour tone (the spectral energy distribution) and L* indicates the luminance (the intensity of light reflected or transmitted by an object).

Figure 5 shows the CIE L*a*b* colour space.

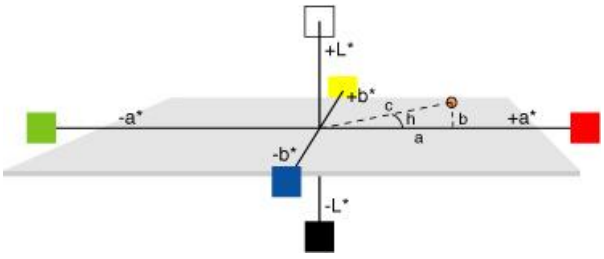


*Fig. 5: The CIE L*a*b* colour space*

Very frequently, the Lab system is translated to polar co-ordinates, namely the L*C*h system. Here, L* defines lightness, C* specifies chromaticity and h denotes the colour angle or hue (see Fig. 5).

1.3.5 Colour Difference

In quality control applications, absolute colours are not measured. More interesting is how much the colour of the product differs from a reference colour. This can be determined by measuring "how far away" one colour from another is. Unlike the colour description, which is always expressed with three numbers, the colour difference can be indicated only with one value called ΔE.

Several methods have been defined for the calculation of the ΔE value. The most used is the formula defined by the CIE in 1976 and shown in the example below. The PC software also works with other ΔE values such as CIE 1994, CIE 2000, CMC and DIN 99 (see Chapter 2.2, page 20).

Since colour spaces are three-dimensional co-ordinates systems, the colour difference can be defined as "the distance vector between two colour points in space". The definition of the CIE 1976 ΔE value is shown in the formulas 21 to 24.

$$\Delta E = \sqrt{\Delta L^{\circ\,2} + \Delta a^{\circ\,2} + \Delta b^{\circ\,2}}$$

$$\Delta L^{\circ} = L^{\circ}_{Probe} - L^{\circ}_{Ref}$$

$$\Delta a^{\circ} = a^{\circ}_{Probe} - a^{\circ}_{Ref}$$

$$\Delta b^{\circ} = b^{\circ}_{Probe} - b^{\circ}_{Ref}$$

Formulas 21-24: Colour difference ΔE

Figure 6 illustrates the relation mentioned above. On the right side of the figure, you can see two colour points: • and •, with ΔE drawn as a vector between them (green line). In practice, usually the distance and not the orientation of the vector is specified.

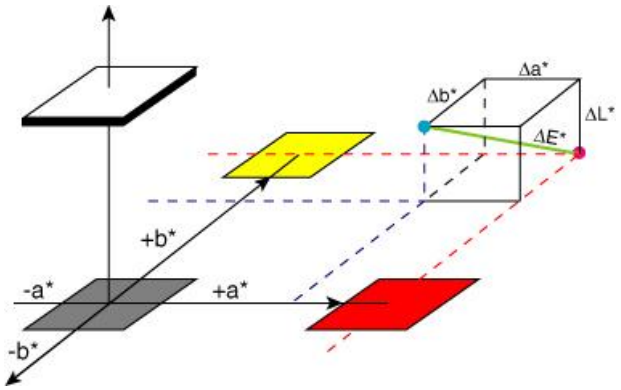


Fig. 6: Colour difference ΔE

The formulas 21 to 24 allow you to calculate the colour difference ΔE in the CIE L*a*b* colour space according to CIE 1976. It is also possible to determine ΔE in the CIE L*u*v* and Hunter Lab colour spaces with the software, this is done by selecting the respective colour space.

2 Performing Colour Measurements

Chapter 2 of this supplement explains how to measure the colour of translucent liquid samples with the ***NANOCOLOR*® PC software for Spectrophotometers V 4.0**. To perform a colour measurement, no preparation of the sample or of the reagents is required. The only requisite is that the sample must be absolutely clear. This means that all samples which are going to be measured must be filtered with a 0.45 µm membrane filter (e.g. ***CHROMAFIL*®**). This has to be done because turbid particles can influence, to some extent, the visually perceived colour.

2.1 Feasible Measurements

The version 4.0 of the ***NANOCOLOR*® PC software for Spectrophotometers** makes possible the determination of the following parameters:

Colour spaces: standard colour values XYZ, chromaticity co-ordinates xyz, CIE L*a*b*, CIE L*Ch, CIE L*u*v*, Hunter Lab, RGB, CMYK, HSB, HSL, YUV

Colour systems: Munsell

Colour differences: ΔE CIE 1976, ΔE CIE 1994, ΔE CIE 2000, ΔE CMC(1:1), ΔE CMC(2:1), ΔE DIN99, measurement with as many standards as desired

Colour scales: Hazen/APHA/PtCo, Iodine, ICUMSA, EBC, ASBC, Yellowness Index, Hess-Ives, European Pharmacopoeia, Gardner, ADMI, ASTM, Saybolt, Klett

Important parameters that can be used for colour measurement:

CIE observers: CIE 1931 2°, CIE 1964 10°

Light sources: A, C, D65, D50, D55, D75, E, user-defined (see Chapter 3.5)

2.2 Basic Settings

Before you perform a colour measurement, you have to configure some basic settings. Some of the parameters used for the measurement cannot be changed once the *Colour Measurement* window appears. When the colour window is closed, the settings can be changed via the menu command *System/Colour management*. This command opens the window for the basic settings (see Figure 7).

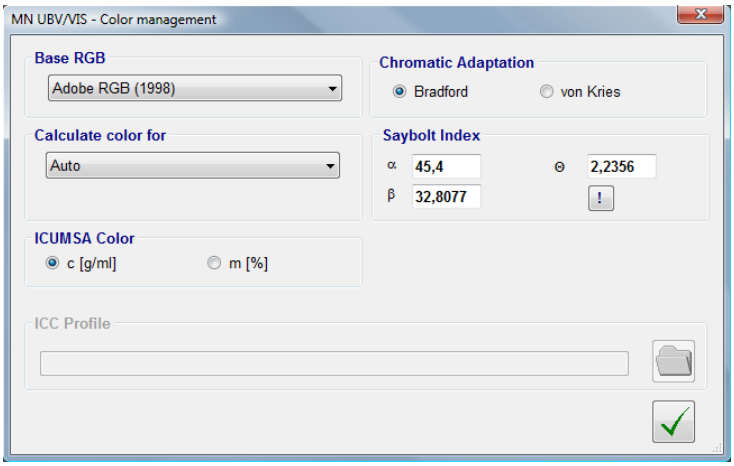


Fig. 7: Basic settings window

In the list box of the *Base RGB* window area, you can choose which RGB system is going to be used. In practice, there are many different RGB spaces, but the most popular is the Adobe RGB (1998), which has almost become a standard. **This setting affects the conversion of CIE XYZ values into RGB and CMYK colour values**. All RGB spaces differ in the definition of the primary colours and the reference light. The Adobe RGB (1998) uses the D65 light.

In the *Chromatic Adaptation* window area, it is possible to set the calculation method used for the conversion of measured colour values when the light source changes. In praxis, two models are widely used: the *von Kries* and the *Bradford* method. The Bradford method is more accurate and is the standard method.

In the third window area, *Calculate colour for*, you can set the path length of the cuvette with the solution to be measured. The cuvette slot of the ***NANOCOLOR*®** $^{UV}/_{VIS}$ spectrophotometer accommodates 10 mm, 14 mm, 20 mm, 40 mm and 50 mm cuvettes. This function also allows the measurement of colours with an optical path length of 60 to 150 mm. If this parameter is set to *Auto* (default setting), the calculation is made with the path length of the inserted cuvette. **This function only affects the colour measurement, if the *RGB* check box in the *Colour Measurement* window is selected** (see Chapter 2.3, page 22).

With the text boxes in the *Saybold Index* window area, you can adapt the calculation of values in the Saybolt colour scale if necessary. To set the default values again, click on the small button with the exclamation mark.

In the lowest window area, *ICUMSA Colour*, you can define whether you are going to work with the concentration of the sugar solution (option *c [g/ml]*) or with the solid content (option *m [%]*) in ICUMSA colour measurements.

2.3 The Measurement

In the main window of the ***NANOCOLOR*® PC software for Spectrophotometers**, click on the menu command *Measure/Colour Measurement* (see Figure 8).

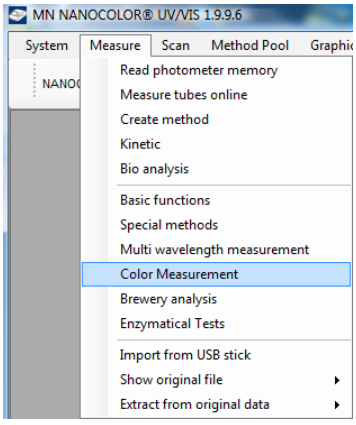


Fig. 8: Menu command Colour Measurement

The software opens the *Colour Measurement* window (see Figure 9). On the right section of the window, you can see the text box for the measurement protocol. On the left, you can find 5 areas where you can make settings. Below these areas, there are buttons to execute different commands.

The basic settings for the colour measurement are displayed at the lower right edge of this window, for example *Adobe RGB (1998), dE: CIE-76, Auto*, as shown in Figure 9 (see Chapter 2.2, page 20).

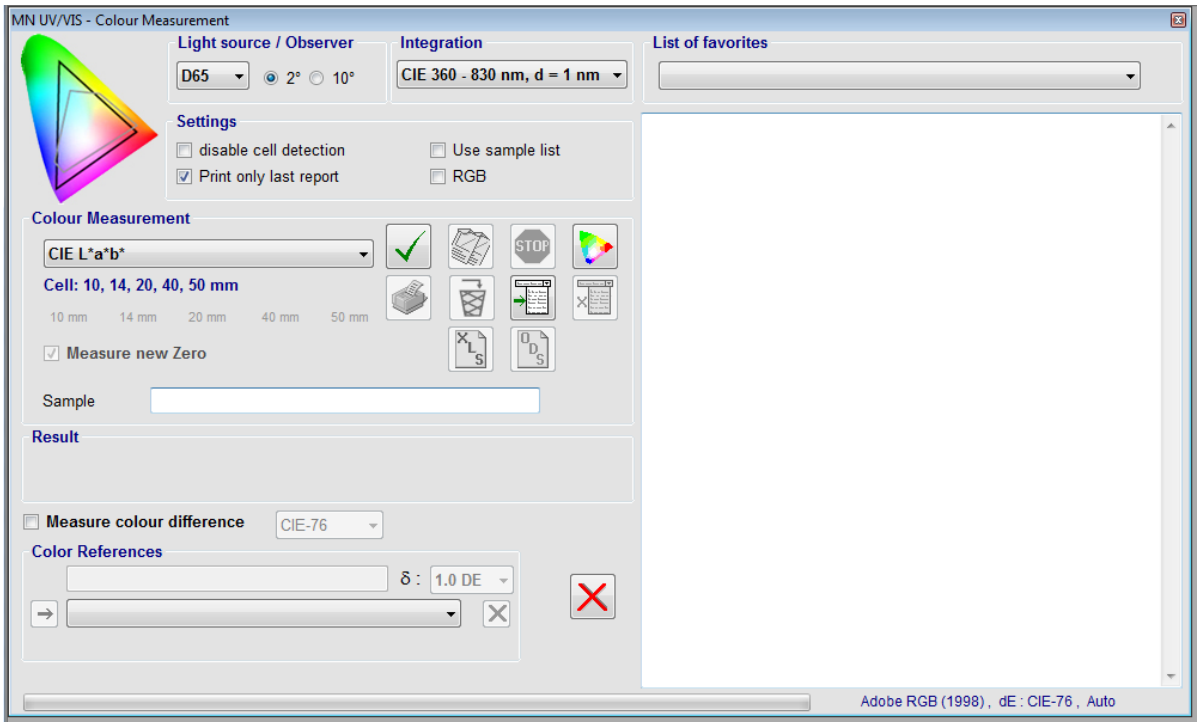


Fig. 9: Colour Measurement window

In the *Integration* area, on top of the window, you can define the integration range for the colour measurement. Following the CIE specifications, the default setting is 360-830 nm. This is the recommended setting and therefore highlighted bold. Alternatively, you can also choose a range of 360-780 nm, however, the time saving is insignificant. To make it possible to directly compare with other colour measurement devices, other settings can be made. However, these settings **do not conform** to the CIE.

In the *Light source / Observer* area there are the options *2°* und *10°* as well as the drop-down list for the light type. Select the CIE standard observer and the required light source (see Chapter 1.2.1, page 10). The most important light sources, i.e. the CIE Standard Illuminants, are **highlighted bold**. Select the option *Custom* if you want to use your own light type (see Chapter 3.5, page 48).

Just below is the *Settings* window area with the four option checkboxes *Disable cell detection* , *Use sample list*, *Print only last report* and *RGB*. If you are working with disposable semi-micro or standard cuvettes recessed on both sides, choose the option *Disable cell detection* **since these cuvettes do not activate the photometer's contact buttons for the cuvette recognition**. If a sample list has been created (see software manual, Chapter 8.10), activate the *Use sample list* checkbox.

Every time you perform a measurement, an individual measurement protocol is created. If several measurements are carried out consecutively, the respective protocols are adjoined and shown in the text field on the right hand side of the window. A click on the "print" or "copy to clipboard" buttons will print or copy all protocols. If you choose the option *Print only last report*, only the protocol of the last colour measurement will be printed or copied.

At the right of this window area is the *RGB* option button. If this option is activated, the XYZ colour values are translated to the RGB, HSB, HSL, YUV and CMYK colour spaces and, where possible, the closest Munsell colour is determined. The measurement can be carried out with standard cuvettes and with path lengths greater than 50 mm (see Chapter 2.2 page 20). If this function has been activated, each time a measurement is performed a new button showing the measured colour appears in the *Colour Measurement* window area.

In the *Colour Measurement* area, you can specify which colour scales/spaces are to be determined (see Fig. 10).

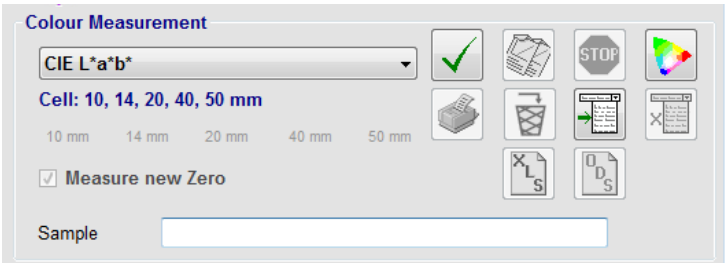


Fig. 10: Selecting the colour scale

If some list entries are not enabled, this is because the colour values are being prepared for future software versions. Regardless of the selected setting, XYZ, xyz and L*a*b* are always calculated.

The selection of a colour space or scale can affect the settings of the *Light source / Observer* window area

Below the drop-down list for the colour space, the allowed cuvette sizes for the selected option are shown in blue letters. Below this, there is a line with several cuvette sizes showing those in green where a zero calibration has already been performed. As you can see in Fig. 11, a zero measurement was conducted with the 14 mm ***NANOCOLOR*®** test tube

Once you have selected a colour space, enter a name, number or description for your sample in the *Sample* text box. The *Sample* text box is a compulsory field: when the software security is set to high, the colour measurement will not be carried

out if no sample designation is entered. If you are working with a sample list, the software automatically chooses a value for this box.

The option *Measure new Zero* will be enabled only after the first measurement. As long as the cuvette type is not changed or no new zero reference for the next measurement is needed, you can accomplish a measurement series with just one zero calibration. If a new zero calibration is needed, select the option *Measure new Zero*.

You will also find a series of buttons in the *Colour Measurement* window area (see Fig. 11).

Fig. 11: Buttons

If you want to delete the protocol, click the  button. The storage as original file will not be affected by this action.

To print the protocol click the  button. This button will be active only if a measurement has been performed.

The second button  copies the protocol to the clipboard. This button will be active only if a measurement has been performed.

The  button starts the colour measurement. In the first measurement, you will be requested to insert a blank cuvette in the photometer. After the zero value has been measured, insert the first sample cuvette in the photometer. As soon as the result is shown, you can measure further samples. Alternatively, you can press the **[ENTER]** key on your PC keyboard when the cursor is in the *Sample* text box.

The  and  buttons are disabled until a first measurement has been performed. With these buttons you can easily copy your colour measurement results to EXCEL or OPENOFFICE.

The  button is displayed in this window area only if the *RGB* option checkbox has been activated before carrying out the measurement. The colour of the button depends on the colour of the sample. When the button is clicked, a measurement protocol with the RGB, HSB, HSL, YUV and CMYK values is created and printed.

This protocol includes a coloured field. A printout on a colour printer allows a direct visual comparison of the measured colour. With this function you can create colour charts very easily.

The printed colour can differ from the real colour. Exact results can only be obtained with specially calibrated printers and monitors!
The printed colour depends on the RGB colour space settings.

The  button shows the CIE chromaticity diagram (see Figure 12). This diagram can vary depending on the light source and observer set. The RGB space used for the measurement is drawn in the diagram as a black triangle with indication of its respective white reference point. If a colour measurement has already been performed, the last measured value is also shown as a vector starting from the white point. When the mouse is moved over the chromaticity diagram, the colour co-ordinates and the RGB values of the current mouse position are shown in the title line of the window.

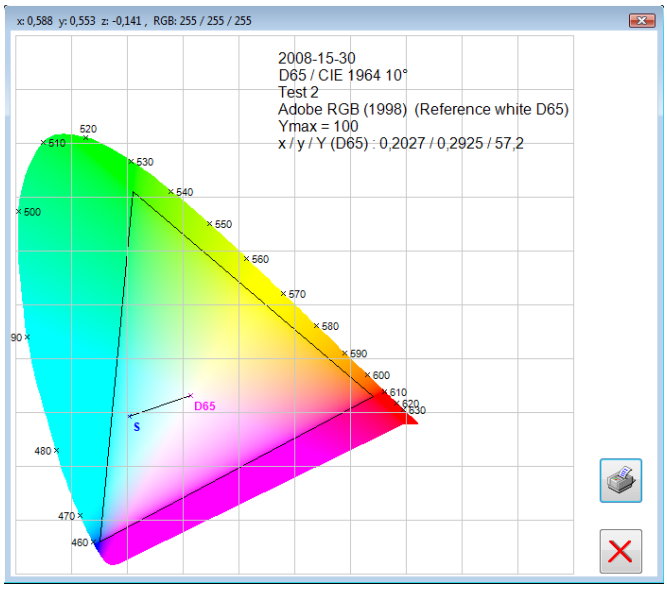


Fig. 12: CIE chromaticity diagram

The chromaticity diagram can either be printed or copied to the clipboard via the menu command *Graphic*, or saved as a file. If you want to see what happens with your measured point in case of other L*a*b* or Yxy values, enter different values in the respective lists situated at the right hand side section of the window. **Changes made in this window do not affect the measured values!**

The button  stores the selected setiings and colour scale as favourite in your list of favorites. Using favourites simplifies regular measurements. You find the list of favourites in the frame *List of favourites* in the upper right edge of the colour measurement window. The button  deletes the selected entry from the list of favourites. Therefor you need administrators rights.

At the lower right hand edge of the window, you can find the functions *Measure colour difference* and *Colour References*. These functions allow you to determine the distance between the measured colour and a reference colour, and are explained in Chapter 2.3.2, page 25.

The button with the red **X** closes the *Colour Measurement* window. When this window is closed, all measurement protocols are saved as original files (see software manual, Chapter 3.13, page 47).

The result of a colour measurement is displayed as shown in Figure 13. Figure 14 shows the protocol of another measurement.

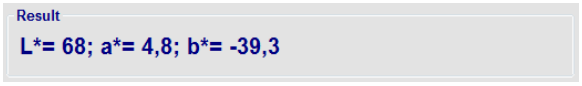


Fig. 13: Result of the colour measurement

Color Measurement

Geometry : 0/180, Method : integral (CIE), 360 - 830 nm, d = 1 nm
Light source : D65 , Observer : CIE 1931 standard colorimetric observer (2°)

Operator : WieczorrekC
Computername : NB-VISTA
Photometer : NUV0027
Date : 07.25.2008, Time : 09:51:11
Cuvette : 14 mm, Mesurement No. : 2
Sample : Sample blue 2

CIE 1976 (L*a*b*) Color Space according to DIN 5033, CIE S 002, CIE Publication 15:2004
Standard color values X: 37,5 (0,2) ; Y: 38 (0,2) ; Z: 84,9 (0,2)
Standard color fractions x: 0,234 (0,0009) ; y: 0,2366 (0,001) ; z: 0,529 (0,002)
L* : 68 (0,2) ; a* : 4,8 (0,8) ; b* : -39,3 (0,3)
Cab* : 39,6 (0,4) ; hab : 277° (2) °

Fig. 14: Protocol of a single measurement

The result can be converted to another colour scale without conducting a new measurement (if physically possible/reasonable) just by choosing the required colour scale in the *Colour Measurement* window area (see Figure 15).

*Fig. 15: Conversion from L*a*b* values to the Hess-Ives scale*

In the same way, L*a*b* colour values can be converted to different light sources just by selecting the required light source in the drop-down list.

2.3.1 Measuring the Colour Difference

To calculate the colour difference between two colours, proceed as follows:

Make the required settings in the *Integration* and *Light source / Observer* window areas. Select in the drop-down list below *Colour Measurement* the options CIE-L*a*b*, CIE L*u*v*, Hunter-Lab or DIN99. Enter a designation for your sample in the *Sample* text box and click the  button or press the **[ENTER]** key on your PC keyboard. The software starts the measurement and requests the blank cuvette be inserted in the photometer. After measuring the blank-value, the software requests that the blank cuvette be removed and the sample cuvette be inserted. If you want to measure the colour difference with respect to a second sample, choose the required ΔE type (the example shows CIE-76) and activate the *Measure colour difference* option button above the *Colour References* window area (see Figure 16).

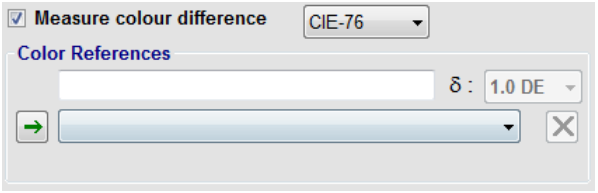


Fig. 16: Measurement of the colour difference

Now, enter a designation for the second sample and click the  button again. The second measurement is performed and the result is calculated as shown in Figure 17.

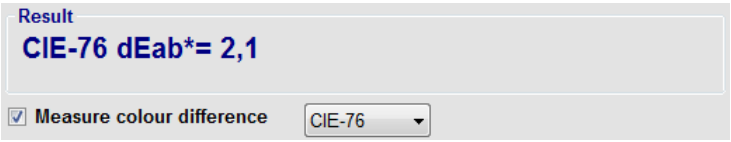


Fig. 17: Result of the colour difference measurement

To avoid confusion, the method used to determine the ΔE value is always displayed. In our case, ΔE was calculated according to CIE-76. If you want to see other ΔE values, you do not need to perform another measurement, just select the required method in the list (see Figure 18).

Fig. 18: Displaying another ΔE value

From now on, every time a new sample is measured, the software will determine the colour difference with respect to the first sample, i.e. the first sample is always the reference sample.

2.3.2 User-defined Colour References

If you regularly had to measure the colour difference of a series of samples with regard to a reference, for example compare the colour of a charge with the colour of a product specification, the procedure described in Chapter 2.3.1 would be very tedious, as for every sample series you would have to measure the reference. The *Colour References* function was conceived for these kinds of applications. Measure your reference sample and save the colour values. The next time you want to make a measurement with these values, you do not need to measure a reference again but just enter the name of the saved reference and conduct only the measurement of the samples.

2.3.2.1 Defining a Colour Reference

If your colour standard is defined by a reference substance and the statistical colour distribution of your samples is well known, you can create a colour reference from a single measurement.

Perform a normal colour measurement of your reference sample. As soon as the result is displayed (see Figure 12), enter an unambiguous name for your reference in the text box below *Colour References*, as shown in Figure 19.

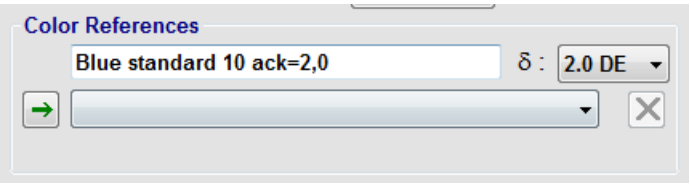


Fig. 19: Entering the name of a colour reference

After entering the name, select a DE value from the list δ . Now, the software will insert the text "ack = 2.0". This function allows you to define a tolerance value. If the measured ΔE value is less than the defined "ack" value, the result is shown in **green**, otherwise in **red letters**. The default value "2.0" can be modified as required. Then, click the button with the green arrow below the text box (see Figure 19). This will save the colour values of your reference in the references database and make it appear in the selection list (see Figure 20). The L*a*b* values of the selected reference are displayed.

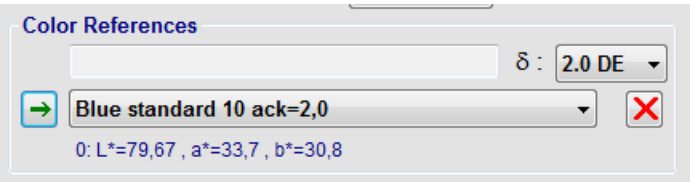


Fig. 20: Reference saved

In this way, you can define as many colour references as you want. Once a new reference has been saved, the button with the green arrow is automatically disabled. It will be activated again, if a further measurement is carried out. The button with the red X is now enabled. This button allows you to delete the actual reference from the database. **This function can only be executed, if the user has administrator access rights!**

2.3.2.2 Create a colour reference from a sample pool

If your samples show a distribution of colour values, e. g. lemonades made of natural fruit juice with seasonal differences in colour quality or different origin, you have to determine your colour reference from the samples colour distribution.

Measure about 10 representative samples from different LOTs. After the last measurement, press the [CTRL] key on your computer keyboard and click simultaneously on the  button. The DE analysis windows opens, see figure 21:

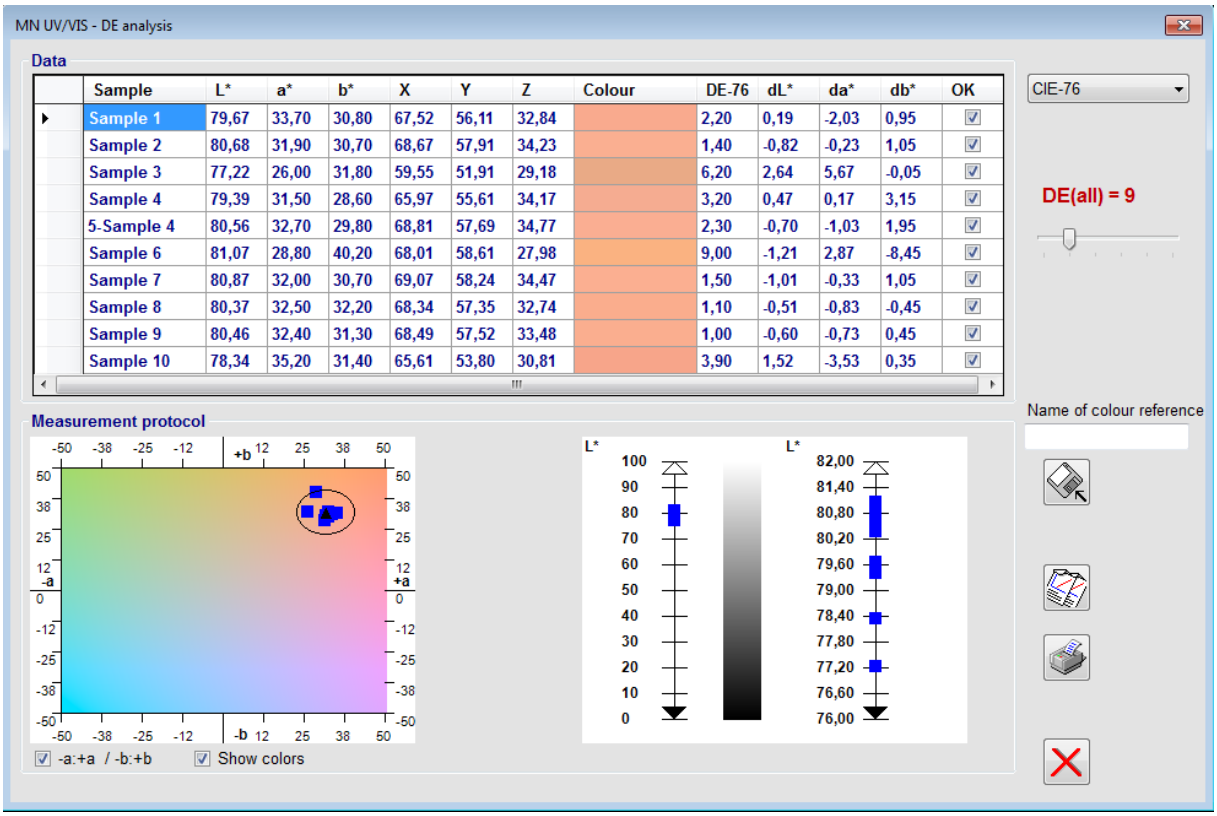


Fig. 21 : DE analysis window

At the top of the window you find the data table of your measurement. The 8th column displays the measured color (the colour impression depends on the quality of your scree/display). In the upper right edge of the window you can select the colour difference formula to be used. Below you see the maximum DE value of your samples to the median colour.

The frame *Measurement protocol* displays the measured colours in the CIE a,b plane as blue squares. The median colour is displayed as black triangle. The black circle represents the maximum colour difference to the median colour.

The samples values are very narrow distributed, to get a clear euation of your samples it is necessary to spread the scales of the a,b plane. Switch of the button *-a:+a / -b:+b*. The software displays now only the required part of the a,b-plane, see figure 22:

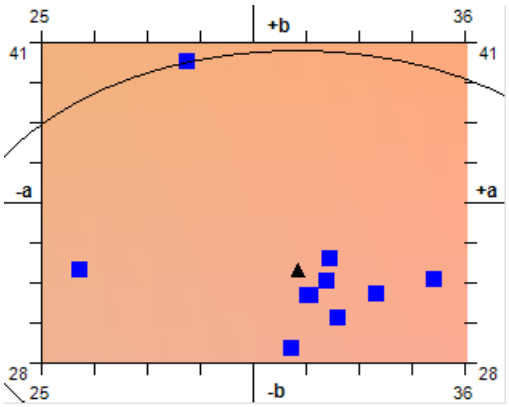


Fig. 22 : spreaded a,b-plane

In this scale the samples pool seems to contain two outliers. Remove the outliers by disable then OK button in the data table, see figure 23. To find the correct sample, just click on the row header (the field with the little black triangle): The corresponding value is marked red in the charts.

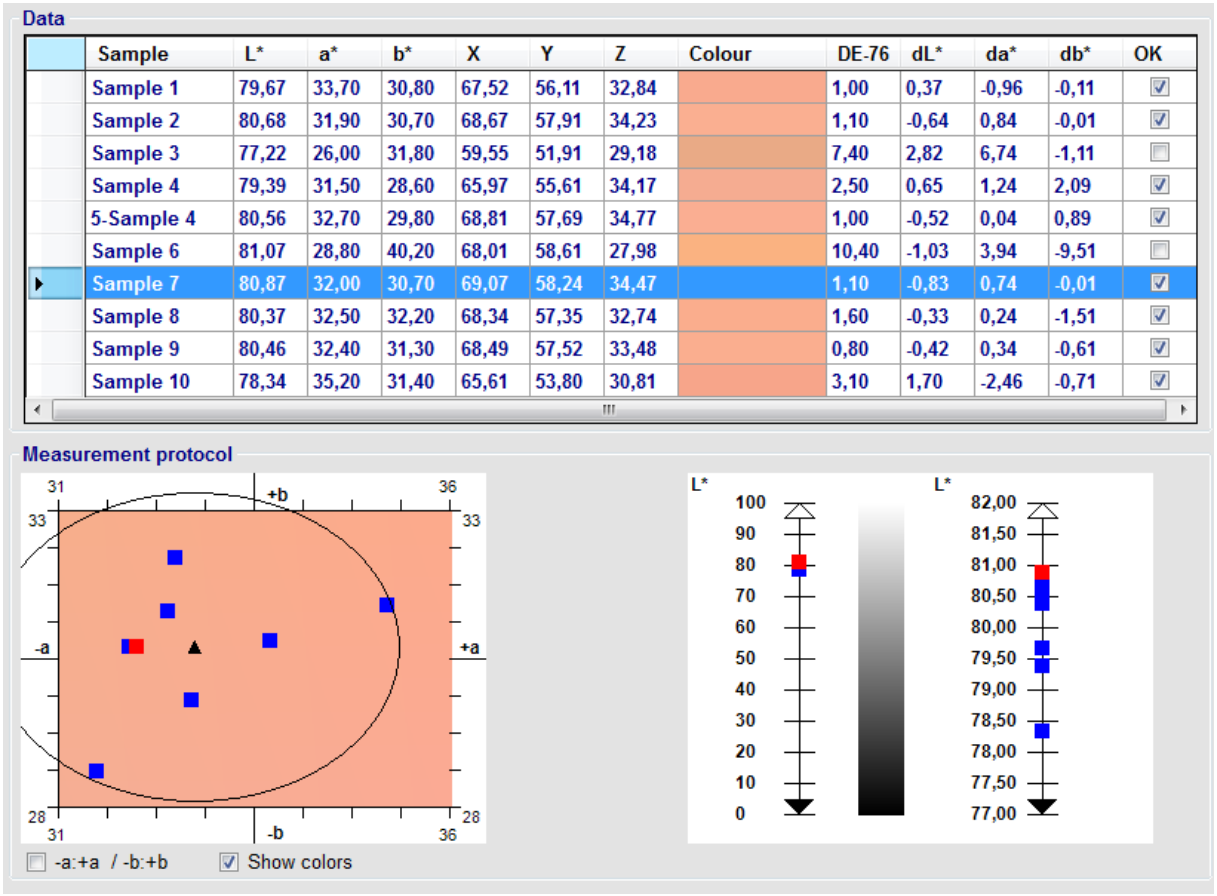


Fig. 23 : Remove outliers and find corresponding samples

The median colour is calculated automatically and displayed in the chart in the lower left edge of the window. In this example the DE values decreases from 9 to 3.1. It can be manually adjusted by using the DE slider in the upper right edge of the window, see figure 24:

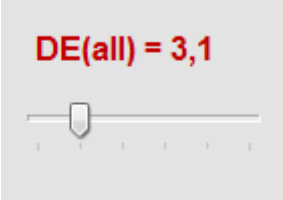


Fig. 24 : Manual correction of DE

Now enter an unambiguous name for your reference into the text box *Name of colour reference* and click on the button  .Your reference is saved into the colour reference list of the colour measurement window.

The button  copies the measurement protocol into the Windows clipboard and the button  prints the protocol.

2.3.3 Measurement against a User-defined Colour References

Start the PC software and open the Colour Measurement window via the menu command *Measure/Colour Measurement*.
Activate the *Measure colour difference* button, as shown in Figure 27. Select a reference in the list of the *Colour References* window area (see Figures 25 and 26).

Fig. 25: List of saved references

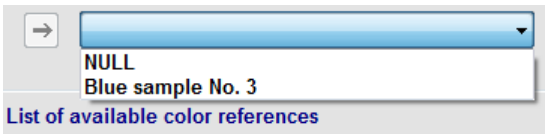


Fig. 26: Available colour references

By default, a reference with the name NULL (Zero) is already defined in the software. The values of this reference correspond to the colour of distilled water with Standard Illuminant C, Standard Observer 2°, integration from 360 to 830 nm and L*a*b* colour space. The colour values of the selected reference are displayed at the lower edge of this window area.

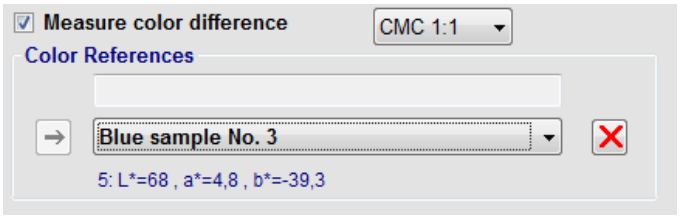


Fig. 27: Measurement against a reference

Now, enter the sample designation in the *Sample* text box and click the button. Then, insert the blank cuvette in the photometer. After the blank measurement has been completed, insert the sample cuvette. The result is displayed as shown in Figure 28.

Color Measurement

Geometry : 0/180, Method : integral (CIE), 360 - 830 nm, d = 1 nm
Light source : D65 , Observer : CIE 1964 standard colorimetric observer (10°)

Operator : WieczorrekC
Computername : NB-VISTA
Photometer : NUV0027
Date : 07.25.2008, Time : 10:01:56
Cuvette : 14 mm, Mesurement No. : 4
Sample 0: Sample blue 2
Sample 1: Sample blue 4

CIE 1976 (L*a*b*) Color Space according to DIN 5033, CIE S 002, CIE Publication 15:2004
Standard color values X10: 42,7 (0,2) ; Y10: 46,5 (0,2) ; Z10: 88,5 (0,2)
Standard color fractions x10: 0,2403 (0,0009) ; y10: 0,2618 (0,0009) ; z10: 0,498 (0,002)
L*10 : 73,9 (0,2) ; a*10 : -4,1 (0,7) ; b*10 : -32,6 (0,3)
Cab*10 : 32,9 (0,4) ; hab10 : 263° (2) °

REF : Blue sample No. 3
dL* : 5,9 ; da* : -8,9 ; db* : 6,7
dCab* : -6,7; dhab : -14,2° ; dHab* : -4,5
CMC 1:1 dEab* : 8,65

Fig. 28: Measurement result

The name of the used reference is also indicated in the measurement protocol.

2.4 Calculating Values in other Colour Scales

The values of the colour scales used in this PC software are calculated in different ways. Some colour scales are based on a measurement of the absorbance at one or a few wavelengths. Other scales are calculated from CIE L*a*b* or XYZ values. Furthermore, there are scales for which no mathematical definition is given, as they are only based on visual comparison.

2.4.1 Colour Scales based on the Absorbance

The EBC (**E**uropean **B**rewery **C**onvention) colour scale, the ASBC (**A**merican **S**ociety of **B**rewing **C**hemists) colour scale, the Hess-Ives colour scale, the ICUMSA (**I**nternational **C**ommission for **U**niform **M**ethods of **S**ugar **A**nalysis) colour scale and the Klett colour scale **(see also Chapter 2.7, page 42)** are based on a measurement of the sample absorbance.

2.4.1.1 EBC and ASBC Colour Scales

The colour of beer is calculated from the measured absorbance at 430 nm in 10 mm cuvettes (according to EBC MEBAK 2.13.2) as follows:

$$EBC = E430 * 25 * F$$

Formula 25

Here, F is the dilution factor of the beer sample. The ASBC colour can be derived directly from the EBC colour:

$$ASBC = EBC * 0{,}375 + 0{,}46$$

Formula 26

Distilled water is used as ZERO reference.

2.4.1.2 Hess-Ives Colour Scale

The Hess-Ives colour is calculated from four absorbances at 640, 560, 470 and 460 nm as follows:

$$H-I = \frac{(R + G + B) * 6}{d}$$

$$R = 43{,}45 * E_{640}$$

$$G = 162{,}38 * E_{560}$$

$$B = 22{,}89 * \frac{E_{460} + E_{470}}{2}$$

Formulas 27 - 30

Here, d is the optical path length in mm.

2.4.1.3 ICUMSA Sugar Score

The colour of sugar solutions is calculated from the absorbance at 420 nm (according to ICUMSA GS1/3-7) as follows:

$$ICUMSA\,420 = 1000 * \frac{E_{420,\ 50\,Brix}}{c * b}$$

Formula 31

Here, c is the concentration of the sugar solution in g/ml and b is the optical path length in cm. **Distilled water is used as ZERO reference.**

2.4.1.4 Klett Colour Scale

The Klett colour is calculated from the absorbance of the sample at 417 nm and with an optical path length of 50 mm, as follows (**see also Chapter 2.7, page 42**):

$$Klett = 484{,}23 * E_{417}$$

Formula 32

Distilled water is used as ZERO reference.

2.4.2 Colour Scales based on Lab or XYZ Values

For the calculation of the Gardner colour index, there is a conversion method from CIE L*a*b* values according to DIN EN ISO. **This is the only colour scale with a calculation method defined by a standard!** Other scales also based on L*a*b* values are the ASTM and the Saybolt colour scales. The ADMI colour values are determined from the ΔE values between a platinum-cobalt solution and water.

2.4.2.1 Gardner Index

The Gardner scale can be used for all types of colourations. It goes from 1 (brightest value) to 18 (darkest value) and is measured in 10 mm cuvettes. The Gardner index is defined by the CIE chromaticity co-ordinates x and y. The integer values are defined by a calibration and the decimals by a complicated calculation with the x and y values of two subsequent measured points. For more information consult EN ISO 4630-2.

Distilled water is used as ZERO reference.

2.4.2.2 ASTM Colour Scale

The calculation of the ASTM colour is based on the CIE XYZ standard colour values, this scale being thus appropriate to measure all types of colourations. This scale ranges from 0.5 (brightest value) to 8 (darkest value) in 0.5 intervals. If the measured value lies between two values on the scale, the result will be the darkest value preceded by an "L" (lower). The pre-set calibration is valid for 32.5 mm cuvettes. If 20 mm or 50 mm cuvettes are used, the software automatically converts the measured values to a 32.5 mm path length.

$$ASTM = 0{,}25 + 0{,}8695 * (\Delta X + \Delta Y + \Delta Z)$$

$$\Delta X = -\log\left(\frac{X}{98{,}074}\right)$$

$$\Delta Y = -\log\left(\frac{Y}{100}\right)$$

$$\Delta Z = -\log\left(\frac{Z}{118{,}232}\right)$$

Formulas 33-36

Distilled water is used as ZERO reference.

2.4.2.3 Saybolt Colour Scale

The Saybolt colour scale can be used for all types of colourations. It ranges from -16 (brightest value) to +30 (darkest value) and is measured in 50 mm cuvettes. The Saybolt colour value is calculated from CIE L*a*b* values as follows:

$$Saybolt = \alpha + \left(\frac{\beta}{\log_{10}\Delta E - \Theta}\right)$$

$$\alpha = 51{,}1 \quad \beta = 44{,}5 \quad \Theta = 2{,}55$$

$$\Delta E = \sqrt{(100 - L^{\circ})^2 + a^{\circ 2} + b^{\circ 2}}$$

Formulas 37-40

Here, the parameters α, β, Θ can be replaced by user-defined calibrations (see Chapter 2.2, page 20).

Distilled water is used as ZERO reference.

2.4.2.4 ADMI Colour Scale

The ADMI colour scale was developed, like the PtCo colour scale, for the measurement of hexachloroplatinate solutions. In this scale, the measurement is performed in 50 mm cuvettes. To offer the possibility of measuring solutions with a colouration other than one ranging from yellow to orange, the calibration is not based on the CIE L*a*b* values but on the ΔE values of a PtCo solution against water. Thus, a solution with an ADMI value of 50 has the same ΔE value as a Hazen/APHA/PtCo standard solution of 50 mg/l Pt, but can be red, green or blue coloured. The ADMI values are not calculated by comparison with the PtCo calibration, but as polynomial up to the 4th degree.
Distilled water is used as ZERO reference.

2.4.2.5 Yellowness Index

The Yellowness Index is calculated from the CIE XYZ values. Although suitable for all types of colourations, it was defined only for yellow samples. The Yellowness Index is calculated as follows:

$$Y-I = \frac{100 * (C_X * X - C_Z * Z)}{Y}$$

Formula 41

White samples have a YI = 0. Yellow-red samples have positive YI values. Here, the parameters C_X and C_Z depend on the selected light source and the observer.

Distilled water is used as ZERO reference.

2.4.2.6 Hazen/APHA/PtCo Colour Scale

The Hazen/APHA/PtCo colour scale ranges from colourless (< 1) to light yellow-orange (500). The Hazen colour value is defined as the concentration of hexachloroplatinate in a hydrochloric acid/water solution and is expressed in mg/l of Pt. The measurement is carried out in 50 mm cuvettes. The Hazen/APHA/PtCo colour scale is calculated from the Yellowness Index according to ASTM D 5386-05.
Distilled water is used as ZERO reference.

2.4.3 Visual Colour Scales

In the Iodine and the European Pharmacopoeia colour scales, the colour is determined only by visual comparison. The software makes an approximation of the measured CIE L*a*b* values with a calibration table (estimation).

2.4.3.1 Iodine Colour Scale

The Iodine colour scale ranges from colourless through yellow to dark brown and 10 mm cuvettes are used for the measurement. The colour values are indicated according to DIN 6162 and range from 10 (light yellow) to 100 (brown). This standard prescribes the use of potassium iodide solutions with an iodine concentration of 10 mg, 20 mg, 30 mg, 40 mg, etc. per 100 ml for the visual comparison with the sample. The requisite for using the Iodine colour scale is that the sample must have a colouration "similar" to the iodine colour. The Iodine colour value is expressed as mg of iodine per 100 ml solution.
Distilled water is used as ZERO reference.

2.4.3.2 European Pharmacopoeia Colour Scale

The European Pharmacopoeia has defined three colour standards for red (cobalt chloride/HCl), blue (copper chloride/HCl) and yellow (iron(III)chloride/HCl) from which five colour series for the colours red, brown, brown-yellow, yellow and yellow-green are mixed. These are further diluted. Usually, lighter colours are measured. The colour value is expressed, for example, as R3 or GY5, where the letters indicate the colour series (R = red, B = brown, BY = brown-yellow, Y = yellow, GY = green-yellow) and the number the solution according to Ph. Eur. 2.02.02. Number 1 is always used for the solution with the strongest colour. The "B" series ranges from B1 to B9, all the others range only to 7. It is very difficult to establish a difference between the light coloured solutions B9, GY7 and Y7, even using a photometer. The European Pharmacopoeia colour value is measured in 50 mm cuvettes.
Distilled water is used as ZERO reference.

2.5 Colour Measurement with Visual Scales

The following chapter illustrates how the ***NANOCOLOR*® PC software for Spectrophotometers** compares the colour of a sample with a visual colour scale. The Iodine Colour Scale (ICS) serves as an example. The same procedure applies for the iodine colour scale and the Ph. Eur. colour scales (see Chapter 2.4.3).

Figure 29 shows the calibration function of the iodine colour scale based on CIE L*a*b* values. The blue points represent the potassium iodide solutions according to DIN, of which the iodine concentration in mg per 100 ml solution is indicated next to the points. The diagram is a projection on the a*b* plane. The calibration function is a helix in 3D-space. When the ICS = 100, L* has a value around 50, increasing to approx. 100 when ICS = 1.

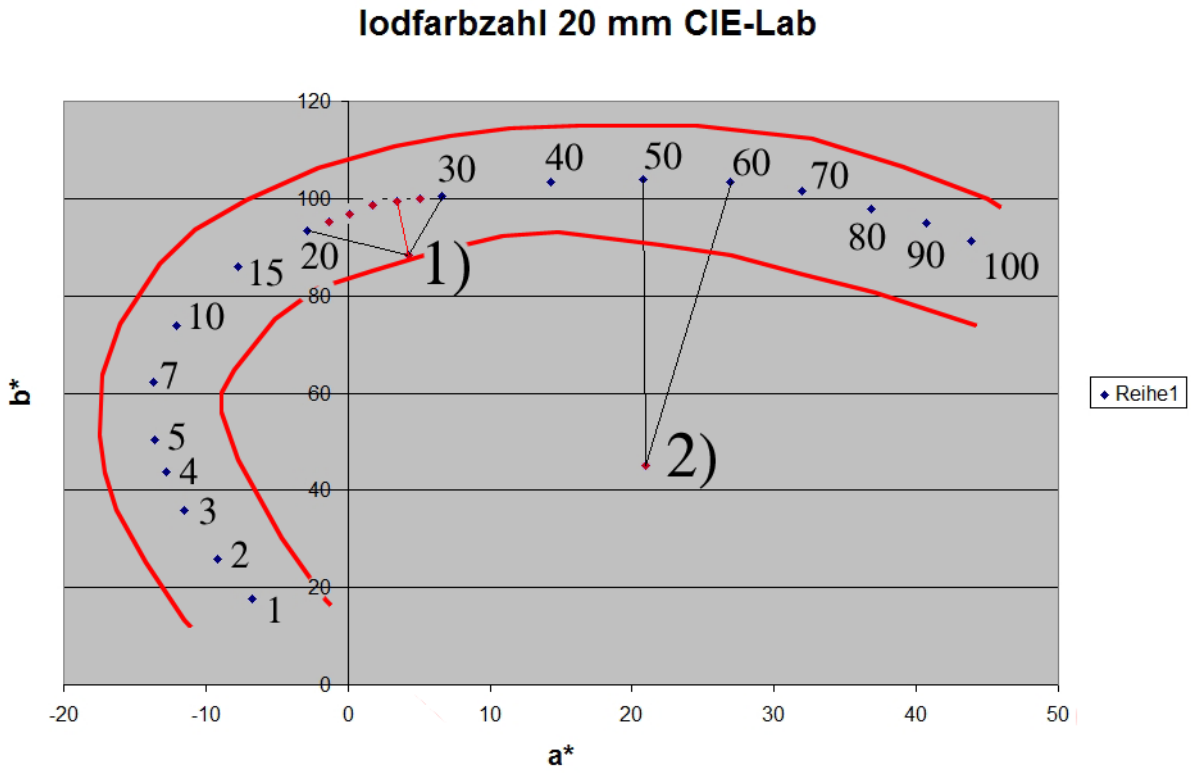


*Fig. 29: Calibration of the iodine colour scale with CIE L*a*b* values*

The ***NANOCOLOR*® PC software for Spectrophotometers** calculates a 20% tolerance range, that is represented by red lines in the chart. When the measured value for the sample is within this range, the software determines the two nearest values in the calibration function. In the chart, these are the two points connected with point 1) by the black lines. Thus, the colour value of the sample lies between ICS 20 and ICS 30. The software then divides the distance between ICS 20 and ICS 30 into 10 equal segments (marked by the red points) and looks for the nearest point to the measured value (red line). This point is given as the result and expressed, for example, as "**ICS = 26**".

When the measured value for the sample is outside the 20% tolerance range, as is the case with point 2), a message like **"Colouration does not correspond to the iodine colour, closest value is ICS = 50"** will appear. The user must then decide, if it makes sense to perform a measurement using the Iodine Colour Scale when the sample has a colouration such as in point 2). According to the DIN EN standards for the iodine and Hazen colour evaluations, if the colouration of the sample does not match the series colouration, the scale may not be used.

Each time a colour value of b* < 0 and/or a* < -25 is measured, the message **"Out of range"** will appear.

2.6 Particularities of the European Pharmacopoeia Colour Scale

If the Ph. Eur. colour scale is selected, after clicking the  button the software opens a windows where you can choose the colour series to be used for the measurement (see Figure 30).

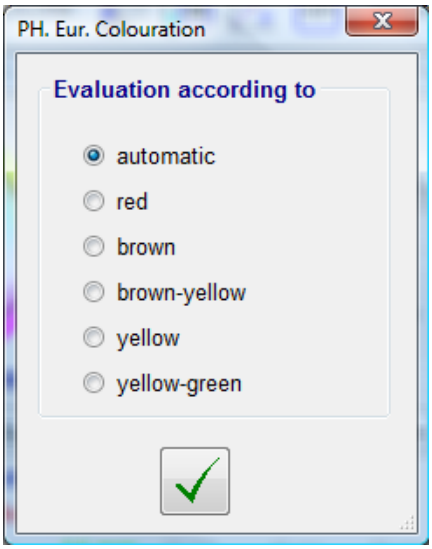


Fig. 30: Choosing the Ph. Eur. colour series

To make a comparison of your sample with all colour series, choose the option *automatic*. Otherwise you can select one of the options *red*, *brown*, *brown-yellow*, *yellow* or *yellow-green*. The result is displayed as shown in Figure 31.

Fig. 31: Ph. Eur. colour measurement result

In the example above, the sample has a Ph. Eur. colouration between the yellow 6 and the brown-yellow 6 standard colour solutions, being the lowest ΔE value between both 1,0 ΔE units. If the sample solution had a colouration closer to the BY6 standard solution, the result would be e.g. **Ph. Eur. = BY-6, delta = 1,25**.

2.7 Particularities of the Klett Colour Scale

The Klett Colour Scale is one of the oldest photometric colour scales. It is measured with an American Klett-Summerson photometer. These photometers have been manufactured since 1930 nearly unmodified and can still be bought today as a new device. They work with simple glass filters. In contrast to the earlier European photometers, the measurement result is not expressed in transmission or absorbance units but in an **arbitrary logarithmic scale** from 1 to 1000, namely the Klett scale. This scale is based on the Beer-Lambert's law, thus being proportional to the concentration. The glass filters of the Klett-Summerson photometers are sorted according to the wavelength of highest transmission and have designations like "KS-42". If the Klett Colour Scale is selected, a new list with the available filters appears below the colour scale selection list (see Figure 32).

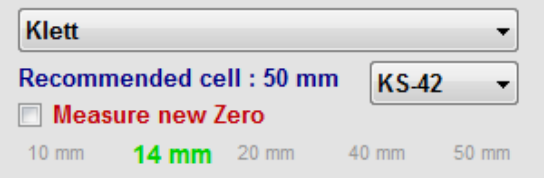


Fig. 32: Setting the Klett-Summerson filter

The KS-42 filter shows maximum transmission values at about 420 nm and is therefore used as the standard Klett filter.

Due to their properties, coloured glass filters have a relatively wide transmission range. The KS-42 filter, for example, allows light to pass through from 400 to 450 nm. **Hence, the original Klett value is determined with an integral measurement over a range of about 50 nm.**
The ***NANOCOLOR*® PC software for Spectrophotometers** attempts to meet this filter property by calculating an approximation with asymmetric Gauss curves.

Common colour measurement devices, on the contrary, determine the Klett colour value from the absorbance measured at the wavelength with the highest transmission of the respective filter used (417 nm for the KS-42 filter).

The software displays the Klett colour value according to both methods (see Figure 33).

Fig. 33: Klett colour values

The first value shown in Figure 29, *Klett 417 nm = 357*, is based on the absorbance measurement and the second value, *KS-42 = 361*, is based on the integral measurement method. In the example above, both values are extremely close. Here, a Pt-Co solution was measured for the calibration of both methods. If the sample has a colouration that deviates from the Pt-Co colouration, the values obtained can be clearly different, as shown in Figure 34 for a chromate solution.

Fig. 34: Absorbance and integral measurement methods

Till today, Klett photometers are used in bioanalytics for cell growth monitoring. In these kinds of applications, <u>not</u> an absorbance measurement, but a stray light measurement is performed. Since the stray light measurement depends on the geometry of the used device, the Klett values determined by the *NANOCOLOR*® PC software for Spectrophotometers <u>should not</u> be used for cell growth monitoring!

3 Appendix

3.1 Measurement Methods and References

In every colour measurement, the standard colour values and the chromaticity co-ordinates are calculated as defined by the International Commission on Illumination in its *CIE Publication 15:2004, 3rd Edition*.

The following values are directly calculated from the X, Y, Z or the x, y, z data:

XYZ, xyz, L*a*b* and L*u*v* according to *CIE 15:2004, 3rd Edition*

Hunter Lab according to *HunterLab Application Note „Hunter Lab Colour Scale", August-1-15, 1996 Vol. 8, No. 9*

Gardner colour according to *DIN EN ISO 4630-2*

Yellowness index according to *ASTM E313* and *ASTM D1925* (out of date)

Hazen/APHA/Pt-Co colour according *ASTM D 5386-05, ASTM E 313-05*

RGB, CMYK, YUV, HSB, HSL according to *www.brucelindbloom.com*

ASTM colour according to *ISO 2049*

Saybolt colour according to *ASTM D156, D6045*
ADMI according *APHA / AWWA / WEF Method 2120 F (2001)*

The following values are calculated from the absorbance at specific wavelengths:

ICUMSA sugar score according to the *ICUMSA Methods GS1/3-7*, *GS2/3-9* and *GS2/3-10*

EBC beer colour according to *Brautechnische Analysenmethoden (Methods of Analysis used in Brewery) Bd. 2*, EBC 9.4, MEBAK 2.16.2

ASBC beer colour

Hess-Ives colour according to DGK F 050.2

The following values are calibrated against CIE L*a*b* values:

Iodine colour according to *DIN 6162*

Ph. Eur. colouration according to *Ph. Eur. 2.2.2*

3.2 Differences with other Colour Measurement Devices

Most of the commercial devices perform colour measurements only within a wavelength range from 360 to 780 nm, some of them only to 720 nm, in 10 nm intervals.

***NANOCOLOR*®** Spectrophotometer strictly adheres to the recommendations of the CIE, i.e. 360-830 nm, in 1 nm intervals. For this reason, the measured L*a*b* values can **slightly** differ from those obtained with other commercial devices. These values are not wrong, they are more precise. On average, the following differences could be observed:

L* below 1%
a* up to 2% in red solutions, otherwise below 1%
b* up to 5% in green solutions, otherwise approx. 1%

3.3 Measuring Speed

The ***NANOCOLOR*®** Spectrophotometers are not a colour measurement device but a full-fledged UV/VIS resp. VIS spectrophotometer, colourimeter and nephelometer. These devices were not developed for fast, but for precise and versatile work. Since the ***NANOCOLOR*®** Spectrophotometer follows the CIE specifications exactly, the measurement takes more time than with other colour measurement devices. One colour measurement (of a blank or a sample) takes with an integration from 360-830 nm about 30 seconds.

3.4 Different Combinations of Light Source/Observer/Cuvette Size

For an absolute colour measurement, the use of all cuvettes sizes, light sources and observers is allowed, as long as the set parameters are shown in the protocol (see CIE publication).

It stands to reason that different combinations of light sources, observers and cuvette sizes will lead to different measurement results!

Table 1 shows the permissible values for the light source, the observer and the cuvette size in each colour scale/space. The cuvette size indicates the internal diameter i.e. the optical path length. The 14 mm cuvette is the ***NANOCOLOR*®** test tube, whereas all others are rectangular cuvettes. The last column of the table, "Specified ID/mm", contains the path length prescribed by the respective standard (see Chapter 3.1). Some colour values can be measured with all cuvette sizes, even if the standard recommends a special size. A dash (-) in the Specified ID column indicates either that there is no size recommended by the standard or that the calculation is made by dividing the result by the path length. A dash (-) in the columns *Observer* or *Light Source* means that the colour value is calculated from the absorbance and not from colour standard values. In this case, this setting is irrelevant.

For an exact colour measurement, the 14 mm test tube is not recommended.

Type of Measurement	Observer	Illuminant	Cuvette ID/mm	Specified ID/mm
Standard colour values	2°, 10°	A, C, D65, D50, D55, D75	10, 14, 20, 50	-
Chromaticity co-ordinates	2°, 10°	A, C, D65, D50, D55, D75	10, 14, 20, 50	-
CIE L*a*b*	2°, 10°	A, C, D65, D50, D55, D75	10, 14, 20, 50	-
CIE L*u*v*	2°, 10°	A, C, D65, D50, D55, D75	10, 14, 20, 50	-
Hunter Lab	2°, 10°	A, C, D65, D50, D55, D75	10, 14, 20, 50	-
PtCo/Hazen/APHA	2°	C	10, 14, 20, 50	50
Iodine	2°	C	10, 14, 20, 50	10
Gardner	2°	C	10	10
Hess-Ives	-	-	10, 14, 20, 50	-
Yellowness Index	2°	C	10, 14, 20, 50	-
Yellowness Index E313	2°, 10°	D65, C	10, 14, 20, 50	-
EBC beer colour	2°	C	10	10
ASBC beer colour	2°	C	10	10
ICUMSA sugar score	-	-	10, 14, 20, 50	10, 20, 50
ADMI	2°	D65	10, 14, 20, 50	10, 50
ASTM	2°	C	10, 14, 20, 50	32,5
Saybolt	2°	C	10, 14, 20, 50	50
Ph. Eur.	2°	C	50	12, 16
Klett	2°	C	10, 50	-

Table 1: Permissible combinations of light source, observer and cuvette size

3.5 User-defined Light Sources

The ***NANOCOLOR*®** PC Software for Spectrophotometers V 4.0 offers the possibility to perform measurements with user-defined light sources. To perform a measurement with a user-defined illuminant, the spectral data of this illuminant must be available in 1 nm intervals. In the *cielab* sub-directory of the software, you will find the file *custom_radiation.txt*. Open this file with a text editor program. The first lines of the file are shown as follows:

```
ILLUMINANT_INFORMATION_FILE
ILLUMINANT_NAME: D65
CIE_1931_OBSERVER: 95.04;100;108.88
CIE_1964_OBSERVER: 94.81;100;107.32
360; 46.6383
361; 47.1834
362; 47.7285
363; 48.2735
364; 48.8186
365; 49.3637
```

This file contains the data of Illuminant D65 as an example. Replace the D65 by the name of your choice. In the third line, the numbers 95.04; 100; 108.88 must be replaced by the standard colour values for the 2° observer. Enter in the fourth line the standard colour values for the 10° observer.

In the following 470 lines, enter the values for the relative spectral energy distribution of the new light source.

To use the newly-defined light source for a colour measurement, activate the *Custom* option in the *Light source / Observer* window area.

If the software finds the *custom_radiation.txt* file in the *cielab* sub-directory, the data will be then used for the colour measurement.

3.6 Demonstration of CIE colour curves

To give an better understanding of the CIE colour measurement you can display the colour curves together with the measured transmission curve. Click on the button  . The software opens the colour spectrum window, see figure 35:

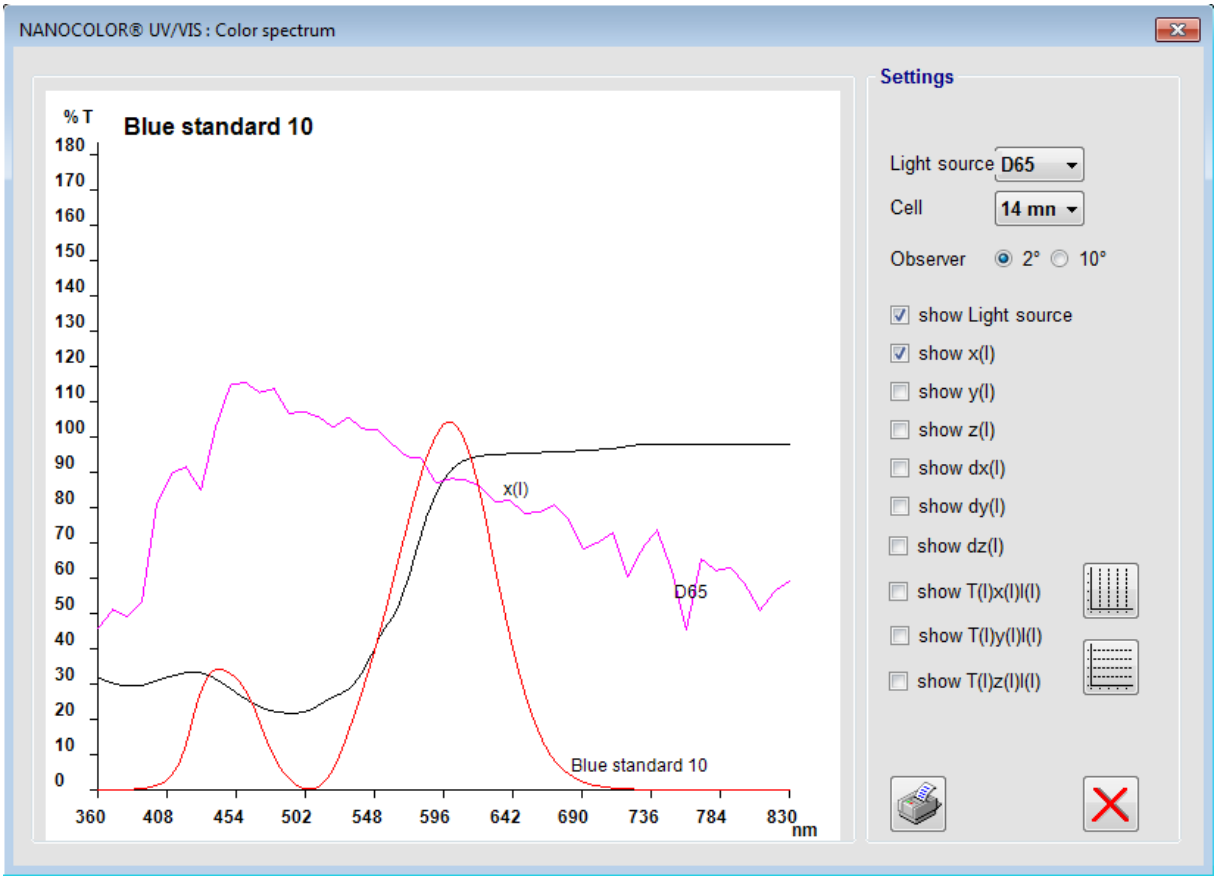


Fig. 35 : Colour spectrum window

The frame *Settings* allow you to select the light source to be displayed and the current CIE observer. The list *Cell* enable you to select the cell width used to

calculate the transmission. The switch Show Light source displays the curve of the selected light source. In the example it is D65 represented by the pink zigzag curve.

The switches *show x(l)* , *show y(l)* and *show z(l)* display the three CIE standard observer stimuli curves.

The switches *show dx(l)* , *show dy(l)* and *show dz(l)* display the three CIE deviation observer stimuli curves, used to calculate metameric indices.

The last three switches display the multiplied functions

F(l) = light source x transmission x selected observer stimuli

Used for the CIE X, Y, Z value calucation.