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*Technical communication*

# LED array spectrophotometer for measurement of time resolved difference spectra in the 530–600 nm wavelength region

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**Abstract**

A new type of computer controlled spectrophotometer is described which is based on an array of independent, monochromatic pulsed light sources consisting of light emitting diodes (LED) equipped with narrow band interference filters. The LEDs are sequentially pulsed at a high repetition rate. The absorbance information at specific wavelengths is sampled in the $\mu$s-time range, using a computer-controlled, highly selective technique of synchronous amplification. A first prototype of this LED Array Spectrophotometer allows simultaneous recording of kinetic changes at 16 different wavelengths in the range from 530 to 600 nm, with a time resolution of 1 ms/point. Special features of the new type of spectrophotometer are: Weak integrated measuring light intensity, high signal/noise ratio even with scattering samples like intact leaves, active baseline adjustment by LED current regulation, computer control of system operation and data analysis. To deconvolute the complex absorbance changes in the cytochrome $\alpha$-band region, 'standard spectra' of the major components are stored in computer memory and used for curve fitting of difference spectra and kinetic changes. As an example of application, the light-induced absorbance changes in a heat-pretreated spinach leaf are analysed. The system effectively separates specific absorbance changes of C550, cyt *f*, cyt $b_{559}$ and cyt $b_{563}$ from a large background of non-specific changes.

*Abbreviations:* DCMU – 3-(3, 4-dichlorophenyl-)1, 1-dimethylurea; DAD – diaminodurol; DNP-INT – 2, 4-dinitrophenylether of 2-iodo-4-nitrothymol; ANT-2p – 2-(3-chloro-4-trifluoromethyl)-anilino-3, 5-dinitrothiophene; RAM – random access memory; LED – light emitting diode; HBW – half bandwidth

## Introduction

Absorbance spectroscopy has been contributing significantly to the elucidation of photosynthetic mechanisms, in particular with respect to light-induced redox changes of thylakoid membrane associated electron carriers, as e.g., cytochromes, plastoquinone, flavoproteins and P700. Most information so far has been obtained by dual-wavelength spectroscopy, as introduced by Chance (reviews by Chance 1972, Böhme and Böger 1978) or by repetitive flash spectroscopy (often single-beam operation), as introduced by Witt and co-workers (reviews by Witt et al. 1965, Witt 1971). With both of these 'conventional methods', at a given time only one particular measuring wavelength (with or without a reference wavelength) passes through the sample. Measuring wavelength in general is changed by mechanical scanning of a monochromator, with the time for one scan normally being too long for kinetic resolution of the process under investiga-

tion. In order to obtain combined spectral and kinetic information, any change of interest has to be repeated at least as many times as wavelength points are desired. Such approach becomes problematic when the observed absorbance changes are complex, so that for proper analysis a large number of wavelength points are required, and when the photosynthesizing samples undergo slow physiological changes during the period required for accumulation of a sufficient number of wavelength responses. This aspect is particularly troublesome in experiments with intact leaves or intact chloroplasts, and when the highly complex absorbance changes in the green wavelength range are studied.

In principle, modern solid-state detector technology has provided a means for synchronous detection of absorbance changes at a large number of wavelengths: Recently so-called photodiode-array detectors have become commercially available. In spectrophotometers equipped with diode-array detectors, polychromatic light passes through the sample and the transmitted light first is dispersed into its monochromatic components before these can be received by the corresponding, individual photodiode detectors positioned in a linear array at the polychromator exit. This measuring principle is well suited for non- or weakly-scattering samples which are not particularly light-sensitive. With leaves and intact chloroplasts, however, which are highly scattering and for which the integrated measuring light intensity has to be low, kinetic spectroscopy with diode array detectors appears problematic.

Probably the most sophisticated spectrophotometer based on the diode array principle was developed by Uhl et al. (1984), using 70 large area photodiodes in a purpose-optimized grating spectrograph. Whereas this system was reported to display outstanding properties with weakly scattering samples of bacteriorhodopsin, its performance with highly scattering leaves necessarily will be less favorable. Also, with a spectral resolution of 10 nm, it would not qualify for separation of narrow band cytochrome absorbance changes.

We have developed a new type of computer-controlled spectrophotometer based on an array of independent, monochromatic light sources, which allows the almost simultaneous recording of rapid absorbance changes at 16 different wavelengths. In this communication we will describe the measuring principle and basic components of the new type of spectrophotometer. The various steps involved in computer-aided data analysis will be outlined using an example of light-induced absorbance changes in the green spectral region ($\alpha$-band of cytochrome absorption) of a heat-pretreated leaf, which displays a large background of non-specific, apparent absorbance changes.

## The measuring principle

It was the aim of the present project to develop a measuring system combining the following properties:

a) Low integrated measuring light intensity.
b) Simultaneous recording of absorbance changes at 16 different wavelengths.
c) Time resolution of 1 ms.
d) Computer-controlled system operation and data analysis.
e) High signal/noise ratio even with highly scattering samples.
f) Capacity of resolving absorbance changes in the order of $10^{-4}$ units.
g) Spectral resolution of 2–5 nm half band width (HBW).
h) Relative insensitivity against non-specific background changes.

We have reached this aim on the basis of a new measuring principle, which may be outlined as follows:

The sample is irradiated with modulated measuring light originating from 16 **independent monochromatic pulse light sources** which are triggered in cyclic sequence at a high repetition rate. LED-pulses of 6 $\mu$s length are applied every 12.5 $\mu$s, such that a full cycle of 16 wavelengths takes 200 $\mu$s. In this way, at any given moment the incident measuring light is monochromatic and, hence, weak in intensity, and still there is (when evaluated with a time resolution of 1 ms) practically simultaneous information on the changes at all 16 wavelengths. The signals corresponding to the different wavelengths are separated by computer-controlled sample & hold and multichannel technology. With this ap-

proach, a single, large area photodetector can be mounted directly behind the sample, where it collects with a large acceptance angle most of the transmitted and scattered measuring light. As a result, a high signal/noise ratio is achieved even with highly scattering samples. Non-modulated background signals, e.g., by ambient daylight or actinic light leaking through the filter protecting the photo-detector, can be eliminated by selective pulse amplification. For the given purpose, light-emitting-diodes (LED) are particularly suited as pulsed measuring light sources, because of the following properties:

- Steep on/off switching characteristics
- High stability and durability
- Low power consumption
- High luminous density
- Low heat dissipation
- Easily regulated light output.

Narrow emission-bands (2–5 nm HBW) can be selected from the overall LED-emission (about 25 nm HBW) by interference filters. With the help of a computer, each individual LED can be current regulated to provide a corresponding transmittance signal which matches a programmed, standard value, such that a flat baseline is automatically obtained. This is important for high digital resolution of small absorbance changes, which then can be further analysed by the computer.

On the basis of this measuring principle, we have developed a LED Array Spectrophotometer for the wavelength region between 530 and 600 nm, which was optimized for analysis of cytochrome absorbance changes in the $\alpha$-band region and for simultaneous measurements of chlorophyll fluorescence and P700 absorbance changes. The design of this measuring system is outlined in the following section.

## Design of the LED Array Spectrophotometer

Figure 1 shows a block-diagram, depicting the basic functional components of the new LED Array Spectrophotometer. The light from 16 individual LEDs is filtered through 16 different narrow-band interference filters and focussed on 16 separate fiberarms via fiber cones. The joint fiberarms are statistically mixed. In addition, in order to optimize randomization of the 16 measuring light wavelengths, a mixing quartz rod is placed between the joint end of the fiberoptics and the sample which is contained in a standard $1 \times 1$ cm cuvette. Mixing rods also link the sample with the detector unit and with the fiberoptics connecting to the actinic light sources and the emitter-detector units for measurements of modulated chlorophyll fluorescence (ED 101, Walz) (Schreiber 1986, Schreiber et al. 1986) and P700 (ED 800 T, Walz) (Schreiber et al. 1988). The fiberoptics were custom made by Schölly (Fiberoptic GmbH, Denzlingen, FRG). The LEDs, with peak emissions between 550 and 600 nm, were selected from the following types of Stanley Hi-superbright LEDs: HBF 5566 X (pure green), HPG 5566 X (green), HPY 5566 X (yellow, 570) HAY 5566 X (yellow, 580) and HAA 5566 X (orange). Interference filters with 1.2 nm HBW were obtained from Omega Optical Inc. (Brattleboro, Vermont, USA); they were used for all except the shortest three wavelengths (535–545 nm, i.e., out of range of the narrow-band cytochrome changes). For the latter wavelengths, where LED emission is relatively weak, interference filters with 6 nm HBW (Type A7-0.5 Schott, Mainz, FRG) were used. The actual spectral resolution is determined by the combination of filter HBW, LED angular radiation pattern and fiberoptics acceptance angle. It was measured with the help of a spectrofluorimeter (SPF-500, Aminco) and amounted to mean values of 2.5 nm for the Omega Optical-filters and 6.5 nm for the Schott-filters. The fibercones (type 038, Schott, Wiesbaden, FRG) accept monochromatic light from the interference filters through an active cross section of 4.5 mm and focus this on 2.4 mm diameter individual fiberarms, resulting in an effective increase of measuring light intensity by a factor of 2.5. Integrated measuring light intensity at the sample amounts to about 5 mW $m^{-2}$. For continuous actinic illumination a laboratory-built halogen lamp (Type Xenophot XLX 64634 Osram) or fiber-illuminators (Type KS 1500, Schott or FL 103, Walz) is used. Single-turn over flashes can be obtained from a discharge flash lamp (XST 103, Walz) or from a tunable laser (Type FL 200, Lambda Physik) pumped by an eximer laser (Type EMG 101 MSC, Lambda Physik). To

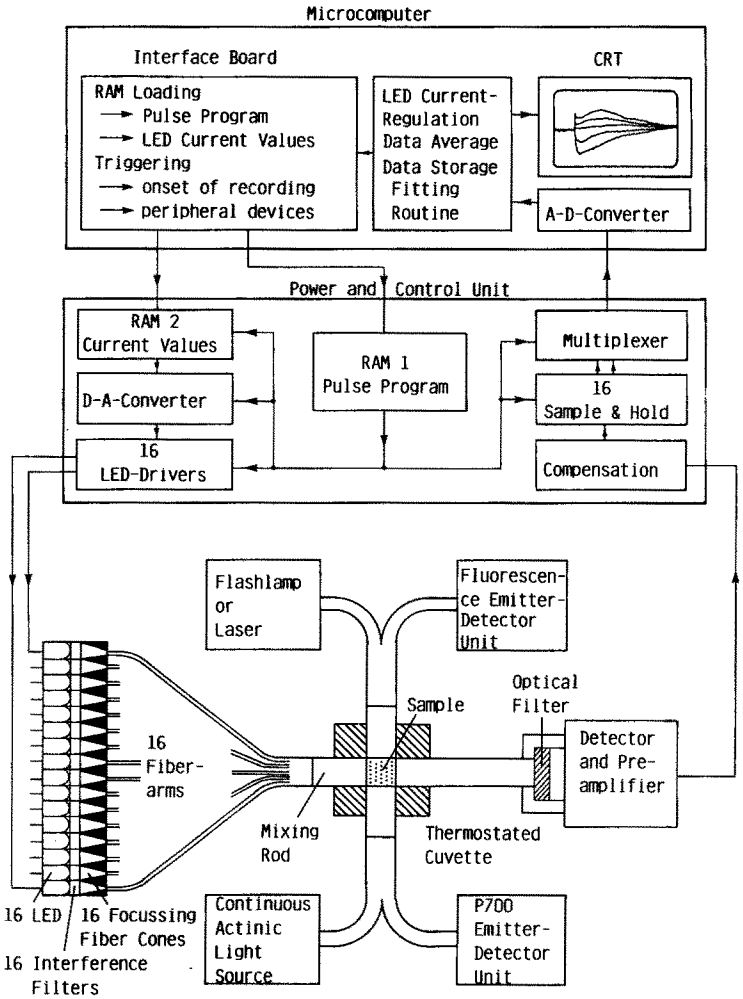


*Fig. 1.* Block-diagram of newly developed LED Array Spectrophotometer integrated with the aid of branched fiberoptics into a multipurpose measuring system including units for monitoring modulated fluorescence and P700-absorbance changes. For details see text.

remove most of the actinic light the detector is protected by an optical filter (e.g., Schott BG 39, when Schott RG 630 is used for actinic illumination). A silicon PIN-photodiode (type S 1723, Hamamatsu) is used as photodetector. The pulsed measuring light response is pre-amplified with a laboratory-built AC-coupled pulse-amplifier. Up to a ratio of 1:1000 between integrated measuring light and background light intensities, there is no disturbance by non-modulated background light, e.g., stray actinic light or ambient day light.

Synchronous generation of LED measuring pulses and signal processing is controlled by a Power and Control Unit (laboratory-built) and a Microcomputer (Commodore PC 40, with numerical co-processor, and laboratory-built interface board). Within the Power and Control Unit central functions are carried out by two read & write memories (RAM 1 and RAM 2), which are programmed via the computer. With RAM 1 pulse frequency and width is determined, controlling 16 individual LED-drivers and synchronously operating the corresponding 16 individual Sample & Hold units and the Multiplexer, from which a multiplexed analog signal is transfered to the computer via an A-D Converter (AM 6112, Advanced Microdevices). Before the train of pre-amplified pulse signals is processed by the RAM 1-controlled Sample & Hold units, the

AC-signals are rectified synchronously with the pulse program and compensated by a constant DC-compensation signal, under the control of the computer. Within RAM 2, the appropriate current values are stored which were calculated by the computer, to give a flat baseline. Synchronously with the cyclic addressing of individual LED-Drivers by RAM 1, the corresponding addresses in RAM 2 and of the D-A Converter are enabled.

The Microcomputer, containing a laboratory-built, purpose-optimized Interface Board, fulfills the central functions of coordinated control of pulse illumination and signal processing: Loading of RAM 1 with the pulse program and of RAM 2 with the LED current values. Also various trigger lines, e.g., for onset of recordings and triggering of peripheral devices, are under computer-control. By LED current regulation a flat baseline is obtained before onset of a recording: The computer regulates the rectified and compensated individual pulse signals at 16 different wavelengths to zero by appropriate adjustment of the current values loaded into RAM 2. Computer software was developed for data averaging, such that the signal/noise ratio can be increased. The data are stored on hard disc. Software for a fitting routine was developed to deconvolute measured spectra into the contributions of several specific components. 'Standard spectra' of these specific components were determined under favorable conditions and are stored in hard disc memory (see following section). Hard copies of the data displayed on the CRT-monitor screen are obtained with a printer (NEC Pinwriter P7 plus).

**Standard spectra and curve fitting routine**

In the wavelength range between 530 and 600 nm, for which the first prototype of a LED Array Spectrophotometer was designed, a considerable number of thylakoid components show absorbance changes. These include P515, C550, cyt *f* (i.e., cyt $c_{554}$), cyt $b_{559}$, cyt $b_{563}$, plastocyanin and P700. In addition, broad band apparent absorbance changes caused by light scattering (Latimer and Rabinowitch 1959, Heber 1969), the sieve- or flattening-effect (Duysens 1956) and sample dilution (upon addition of chemicals in solution) are taking place. In intact chloroplasts and leaves under close to in vivo conditions where most of these changes may overlap, an unambiguous identification of specific absorbance changes is problematic. However, special conditions can be found which allow almost selective measurement of some specific absorbance changes, such that a collection of 'standard spectra' may be obtained, which can be stored in computer memory and used for fitting analysis. We have measured such 'standard spectra' with the new LED Array Spectrophotometer for P515, C550, cyt *f*, cyt $b_{559}$ and cyt $b_{563}$. In Fig. 2 these 'standard spectra' (except that for P515) are depicted. In order to obtain a satisfactory signal/noise ratio an appropriate number of spectra were averaged. The data points are connected by lines obtained by 'spline-interpolation', fitting curve segments to polynomial functions of third degree. The specific conditions under which the depicted 'standard spectra' were recorded, were as follows:

– C550. Flash-induced absorbance difference spectrum of PS II particles, isolated from spinach chloroplasts according to Berthold et al. (1981), in presence of ferricyanide (2 mM) and DCMU (10 $\mu$M). Average of 80 curves, obtained with 4 different samples. Single turnover saturating flashes were applied with dark-intervals of 60 s.

– cyt *f*. Far-red induced absorbance difference spectrum of osmotically shocked spinach chloroplasts in presence of valinomycin (0.4 $\mu$M), nigericin (0.4 $\mu$M), DAD (4 $\mu$M), Na-ascorbate (60 $\mu$M), DNP–INT (10 $\mu$M) and methylviologen (0.2 mM). Average of 25 curves, obtained with one sample. 12 s dark-time was given between 5 s far-red illumination (2 W $m^{-2}$; RG 715, Schott).

– cyt $b_{559}$. Flash-induced difference spectrum of osmotically shocked chloroplasts in presence of valinomycin (0.4 $\mu$M), nigericin (0.4 $\mu$M), DAD (0.1 mM), Na-ascorbate (1.5 mM) and ANT-2p (1 $\mu$M). Average of 10 curves, obtained with one sample and illumination with single turnover saturating flashes at 3 min intervals.

– cyt $b_{563}$. Far-red induced difference spectrum

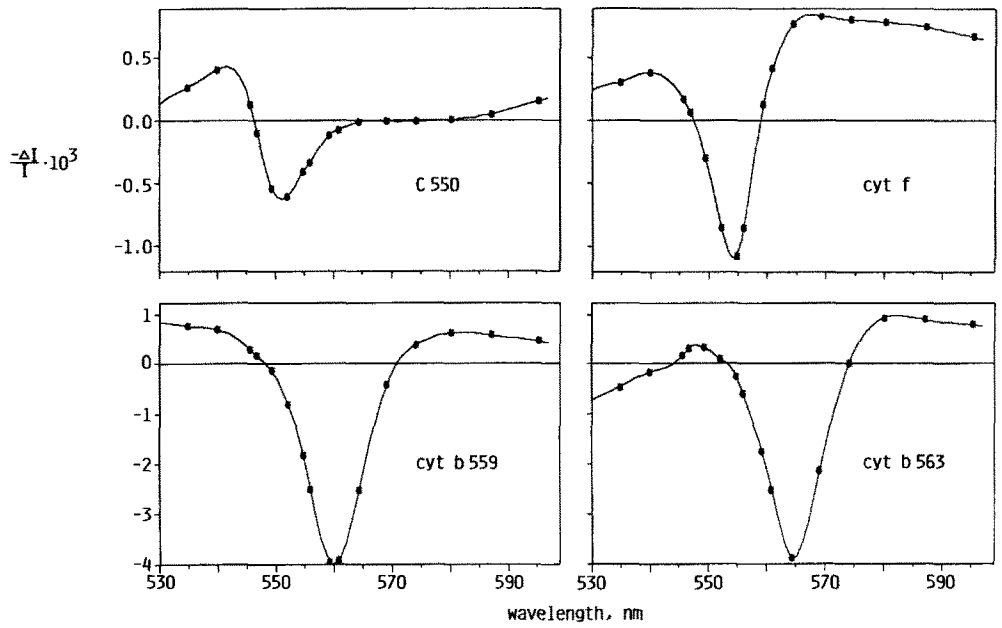


*Fig. 2.* Standard spectra of specific absorbance changes measured under favorable conditions and used for the curve fitting routine. See text for conditions.

of osmotically shocked chloroplasts in presence of valinomycin (0.4 $\mu$M), nigericin (0.4 $\mu$M), DAD (0.2 mM), Na-ascorbate (3 mM), Na-dithionite (5 mM) and DCMU (10 $\mu$M). Average of 10 curves obtained with one sample. 4 min dark-time between 15 s far-red illumination (2 W $m^{-2}$).

It may be expected that these 'standard spectra' still show small deviations from the ideal specific absorbance changes attributable to the various components. If such information arises from future work, this can be readily accounted for by correction of the 'standard spectra'. As compared to the four specific absorbance changes considered above, the other possible changes are displaying relatively broad difference spectra, which in the given wavelength region sum up to a rather flat, curved line. This so-called 'background signal' can be described in good approximation by polynomial functions of zero to second degree. For fitting of the absorbance changes obtained with a particular experiment, any combination of the stored standard spectra as well as the degree of the polynomial function can be chosen.

The curve fitting routine developed for the LED Array Spectrophotometer allows the fitting of difference spectra as well as of the kinetic changes of individual components. A full recording consists of 16 times 544 data points. Hence, in principle, the information for 544 difference spectra is stored. However, in practice this number is reduced by the choice of appropriate intervals, for which the data points are averaged, so that the signal/noise ratio is improved. Each resulting data point of the fitted kinetics of one particular component is corresponding to the amplitude of this component in a fitted difference spectrum at the given time (or within the given interval). Due to this approach, the obtained kinetic information may be considered unique, because it is derived not only from the changes at the peak and a reference wavelength (as in dual wavelength spectroscopy) but from the amplitude changes of the complete difference spectra. In principle, there is also additional kinetic information on three types of background signals, as the three parameters determining the polynomial functions are calculated for every time interval. However, in most applications this information is too unspecific to be of analytical value.

## Example of application

The following example of application may illustrate some of the main properties of the new LED Array Spectrophotometer. It shall be demonstrated that this system is capable of discriminating small specific absorbance changes against a large background of non-specific changes. For this purpose, the measurement of light-induced absorbance changes in a heat-pretreated, intact spinach leaf was chosen as an example.

Figure 3 shows the original kinetic traces recorded at 16 different wavelengths with a time resolution of 20 ms/point. Each trace represents the average of 9 recordings, for each of which a new leaf sample was used. The individual traces are positioned with respect to the wavelength axis at somewhat variable intervals, as determined by the availability of appropriate interference filters. At time zero, continuous actinic light is switched on. It is apparent that light-induced changes at all 16 wavelengths are rather similar, i.e., they are dominated by large absorbance changes with a flat difference spectrum in the given wavelength region. However, already in this three-dimensional presentation of the kinetics, at the end of the traces the difference spectrum of the more specific absorbance

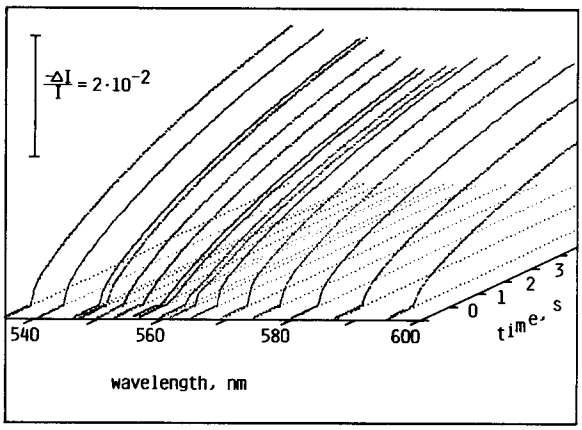


*Fig. 3.* Original kinetic traces of light-induced absorbance changes in a heat-pretreated spinach leaf. Actinic light: 30 W $m^{-2}$, RG 645 Schott. Heat-pretreatment: 5 min at 48°C. Average of 9 recordings, for each of which a new leaf sample was used. Leaf segments were placed at a 45° angle in the cuvette to allow actinic illumination.

changes can be noticed (minimum around 555 nm). This is more clearly shown in the two-dimensional presentation of the difference spectra (Fig. 4). The symbols represent data points and the lines show the computer-fitted spectra derived from 'standard spectra'-components (see section on **'Standard spectra and curve fitting'** and further description of analysis below). The success of the deconvolution procedure can be

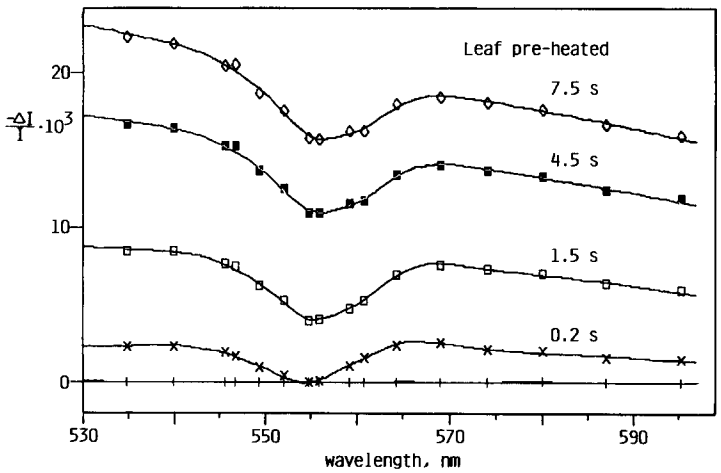


*Fig. 4.* Time-resolved absorbance difference spectra upon a dark-light transition of a heat-pretreated spinach leaf. Two-dimensional presentation of data in Fig. 3 for four different times after onset of illumination (as indicated). Symbols represent measured data points and lines show calculated, computer-fitted spectra. The baseline at the 0-level (cross symbols) was obtained by averaging the first 50 data points before onset of actinic illumination.

judged from the deviation of the calculated lines from the measured data points. The cross-symbols at the 0-line represent the baseline, obtained by averaging of the first 50 data points before onset of actinic illumination. The time values marking the four depicted difference spectra corresponds to medium values of 20 points (with 0.2 s) and 50 points (with 1.5, 4.5 and 7.5 s).

A reference wavelength can be chosen and the absorbance change occurring at this wavelength is then subtracted from the changes at all other wavelengths. This leads to normalization of the time-resolved difference spectra at the reference wavelength and to the elimination of wavelength-independent, apparent absorbance changes. In this way, an artificial isosbestic point is created at the chosen reference wavelength. As shown in Fig. 5, after choosing a reference wavelength, the absorbance changes can be presented on a more expanded scale and are now more easily compared. The difference spectra suggest that cyt *f* becomes rapidly oxidized, as indicated by bleaching around 554 nm in the 0.2 s spectrum, and that there is a slower oxidation of cyt $b_{559}$ (bleaching around 559 nm).

To obtain quantitative information on the contribution of cyt *f* and cyt $b_{559}$ oxidation to the overall difference spectra, further analysis by the curve fitting routine is required. As outlined above (see section on **Standard spectra and curve fitting routine**) a number of standard spectra are stored in computer memory and software is available for fitting the measured spectra with the sum of these standard spectra and of a polynomial function of zero to second degree, describing broad band absorbance changes occurring in the given wavelength region ('background signal'). Figure 6 shows the outcome of curve fitting analysis for the 4.5 s difference spectrum, which is indicated by the data points (solid squares). For best fitting, the computer calculated a straight line rising towards lower wavelengths as representing the 'background signal'. In the given example, C550 did not show any appreciable absorbance change 4.5 s after onset of illumination and there was no P515-change apparent. The membrane potential is known to be particularly sensitive towards heat-stress (Bilger 1987, Bilger and Schreiber 1990, this issue). Besides oxidation of cyt *f* and cyt $b_{559}$, which already was apparent from the overall difference spectra (see Fig. 5), curve fitting reveals also some cyt $b_{563}$ reduction.

Eventually, in Fig. 7, the complete deconvoluted kinetics of C550, cyt *f*, cyt $b_{559}$ and cyt $b_{563}$ changes are shown. For every point of these kinetics the contribution of the four standard spectral component was determined by the curve fitting routine. The resulting kinetic traces are

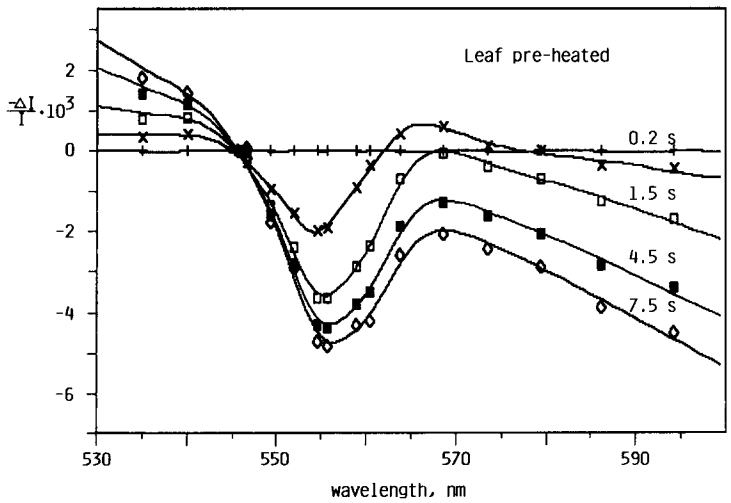


*Fig. 5.* Time-resolved absorbance difference spectra normalized at a reference wavelength to eliminate non-specific changes. Data of Figs. 3 and 4 are presented at an expanded ordinate scale. Reference wavelength: 545.5 nm

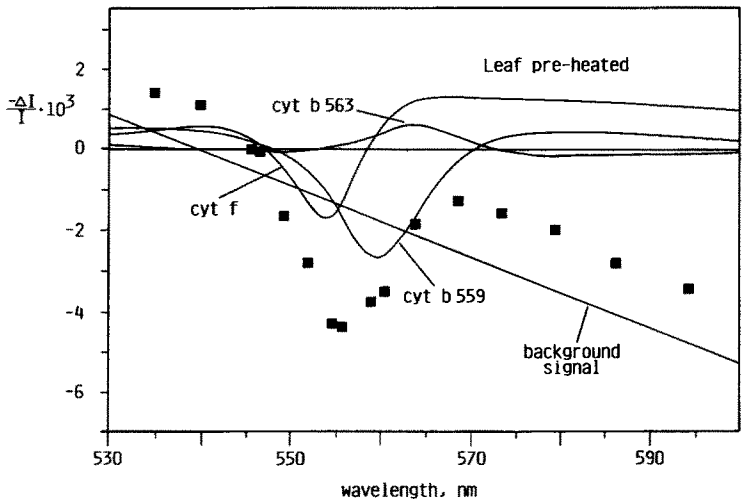


*Fig. 6.* Deconvoluted spectral contributions (lines) to overall measured absorbance difference spectrum (square points) at 4.5 s following onset of actinic illumination. Deconvolution was achieved by curve fitting on the basis of the 4 'standard spectra' shown in Fig. 2 and of a first degree polynomial function for the background signal. No contribution of a C550 change was apparent under the given conditions in the chosen time interval and the heat-treated leaf did not show a P515 change

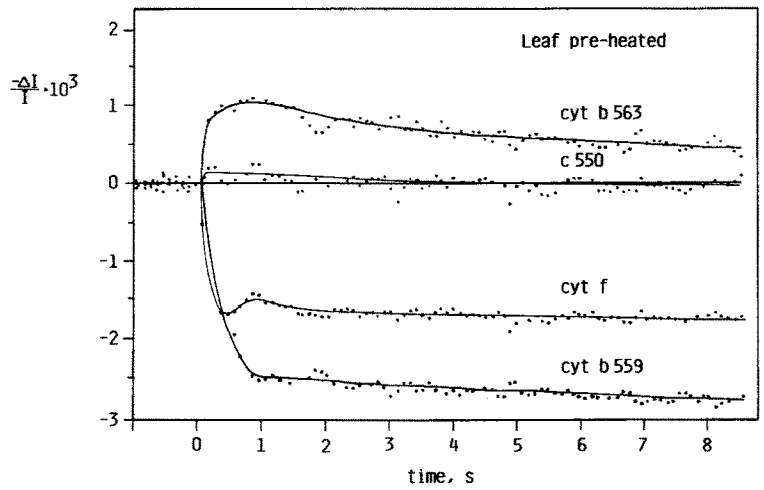


*Fig. 7.* Deconvoluted kinetics of light-induced absorbance changes in a heat-treated leaf attributable to specific components. The points represent calculated values, the lines are hand-fitted. For every time value, the points correspond to the deconvoluted specific contribution at the respective peak wavelength.

unique in that they represent changes in concentration of the various components, as resulting from the calculated changes in absorbance at the respective peak wavelengths. Positive signals reflect reduction, negative signals oxidation. For this reason, the amplitudes of the various traces are not directly comparable. To evaluate the information on the induction kinetics of the pre-heated leaf, presented in Fig. 7, comparison with the corresponding kinetics of a control leaf is helpful (see Fig. 8). In the control, substantial P515 changes occur, and therefore curve fitting was carried out taking also a standard P515 difference spectrum into consideration (not shown). Significant differences are apparent between induction kinetics of control and pre-heated leaf: A negligibly small cyt $b_{559}$ reduction in the control is contrasted by a large cyt $b_{559}$ oxidation in the pre-heated leaf. In the control, cyt *f* shows rapid, transient oxidation and re-

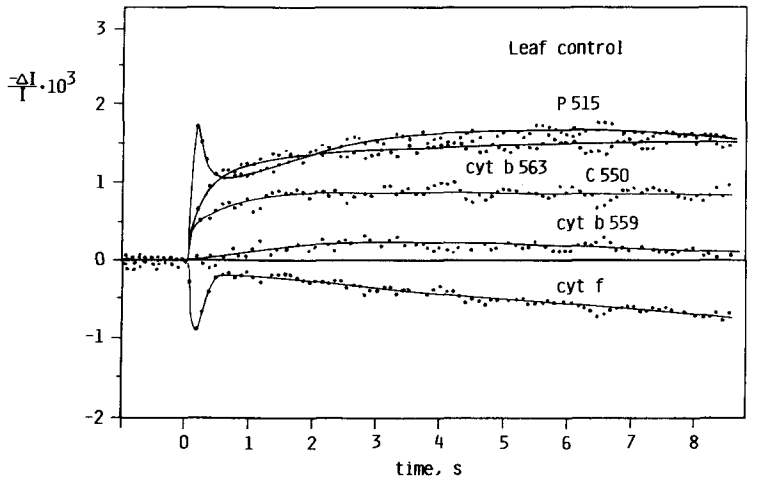


*Fig. 8.* Deconvoluted kinetics of light-induced absorbance changes in a control leaf. Compare with corresponding changes in Fig. 7 for a heat-treated leaf. Note: Contrary to the heat-stressed leaf, in the control leaf a substantial P515 change was apparent. The 'standard spectrum' used for deconvolution of the P515-change was obtained from single turnover flash responses in presence and absence of $10^{-6}$ M valinomycin (not shown).

reduction before it becomes slowly partially oxidized again. After heat pretreatment, cyt *f* becomes more extensively oxidized, with only minor transient re-reduction. While a pronounced, biphasic change in C550 is seen in the control leaf, there is only a very small C550 change in the stressed leaf. There is cyt $b_{563}$ reduction in both types of leaf, but the extent of reduction is larger in the control. A more detailed discussion of the data is out of scope of this presentation.

## Conclusions

With the analysis of light-induced absorbance changes in a heat-pretreated leaf, an example of application was chosen which puts exceptionally high demands on the sensitivity and selectivity of the measuring system. First, the transmittance through a leaf is rather low (about 0.01 in the green region) and strongly scattered. Second, in a heat-pretreated leaf the light-induced scattering increase is strongly enhanced (Weis 1981, Bilger 1987). Still, even in this application the kinetic recordings obtained with the new LED Array Spectrophotometer are quite satisfactory, allowing detailed insights into the relative changes of C550, cyt *f*, cyt $b_{559}$ and cyt $b_{563}$. With isolated chloroplasts, the signal/noise ratio obtained with the new system is considerably higher than with leaf samples (Klughammer and Schreiber, in preparation).

Main aspects for the exceptional performance of the new system are the measuring principle and the intensive data analysis. By measuring almost simultaneously at 16 different wavelengths, in a single run a very large amount of spectral and kinetic information is obtained which then can be submitted to detailed computer analysis. With this procedure, it is unavoidable that the signal/noise ratio at a particular wavelength is lower than with a single- or dual-beam measurement. However, this can be improved by data averaging, if necessary. At present, the signal/noise ratio is mainly limited by the relatively low light output of LEDs in the green spectral region. When compared to conventional dual-wavelength spectroscopy, a major advantage of the new measuring principle is that the information on the various spectral components is practically simultaneous, and, hence, time dependent changes of the sample under investigation do not prevent the reliable measurement of difference spectra. In principle, with any transient change obtained by dual-wavelength measurement, this can only be ascribed to a particular component (e.g., cyt $b_{559}$) if the corresponding difference spectrum is derived, a

requirement which is time-consuming and not always fulfilled in practice. With the new measuring system, this problem has been satisfactorily solved. Therefore, it may be expected that this system will lead to new insights into the mechanisms of photosynthetic electron transport not only using intact leaves, but intact chloroplasts and thylakoids as well. In particular, the complex and still enigmatic reactions at the cyt $b/f$ complex and of cyt $b_{559}$ offer an interesting field of application for the LED Array Spectrophotometer. These reactions participate in important regulatory mechanisms in vivo, which have to be studied under conditions where numerous background signals may overlap the specific changes of known components. Whilst the matrix computer analysis of Rich et al. (1987) is suitable for studies with isolated complexes of known composition, this method becomes problematic for in vivo studies when unknown background signals cannot be avoided.

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