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Computer screen as a programmable light source for visible absorption characterization of (bio)chemical assays

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Received (in Cambridge, UK) 31st October 2002, Accepted 27th November 2002
First published as an Advance Article on the web 11th December 2002

Visible absorption features suitable for color recognition and micro-plate reading of a standard bioassay are performed by the combination of a computer screen used as a programmable light source and a web camera as detector. The method provides in this way a highly available platform for 'home tests' or 'self-tests', where the requirement is to monitor well defined assays and the use of economical instrumentation is advantageous.

Numerous chemical or biochemical assays make use of visible absorption measurements to read the information they codify.[1–4] Typically bioassays are arrays of samples with different transmittance spectra which suffer changes upon target reactions, which are evaluated with dedicated instruments (microplate readers for instance) which minimally comprise a two dimensional position controlled light source of tuneable wavelength (embedded monochromator or discrete filters) and modulated intensity (depending on the arrangement or detectors).

The assays are not universal, but optimised to identify well defined target processes instead, in which case the evaluation of visible absorption 'features' (named this way instead of spectra since this work deals with pattern recognition, as in electronic noses[5,6] where a collection of response details, so called 'features', qualify a sample) is mainly reduced to discriminate between a small set of substances (*e.g.* by their colours) and then to determine their corresponding relative concentration.

In this work we especially consider emerging applications as 'home tests' or 'self-tests'[7] where the existing instrumentation is too expensive or complex, making necessary a more versatile and simpler approach. In consonance with this goal we present a method to measure visible absorption features using a programmable large area light source provided by broadly available computer screens, working in the simplest case with a web camera as detector. The ability for colour recognition is demonstrated with common colour dyes. A biochemical example of a cell viability test using a micro-plate format assay is also provided.

Visible absorption features of common dyes (blue aniline, orange acridine and phenolphthalein), contained in a plastic cuvette (light shielded to prevent ambient illumination), were obtained placing the samples between the computer screen and a light detector. In this case a metal-oxide-semiconductor (MOS) capacitor, chosen as an example that demands light intensity modulation to operate, but any other light detector like a web camera (measuring total light intensity) would conform with the experimental arrangement (Fig. 1).

The light source is supplied by a video file played (with any media player) on a region of the computer screen (a Nokia 447 Xi cathode-ray-tube (CRT) monitor operating at a refresh frequency of 85 Hz, with a resolution of 1024×768 pixels), which sequentially displays 256 different colours (colour indexes 0 to 255) sorted according to the perception of the visible spectrum.

The transmittance of the sample is calculated as the ratio of the intensity with the sample (I) and with the solvent alone (I_0) and such value (or the corresponding absorbance, $-\log(I/I_0)$) represented as a function of the color index leads to a distinctive feature which is correlated with the standard visible absorption spectra (Shimadzu UV-1601PC, operating at a resolution of 2 nm per step) as depicted in Fig. 1.

Accordingly, it is possible to distinguish coloured substances within a constrained set of alternatives, as commonly occurs in microtitrerplate work.

Intensity modulation, is intrinsically provided by the CRT screen instead of the typical chopping wheels. In these screens a sweeping electron beam excites a matrix of phosphorous dots coating the screen on its inner side. These phosphorous dots are arranged in sets of red, green and blue emitting blobs which comprise the individual pixels, which once excited by the electron beam, decay in intensity until the next excitation cycle (given by the screen refresh frequency), inducing in this way a

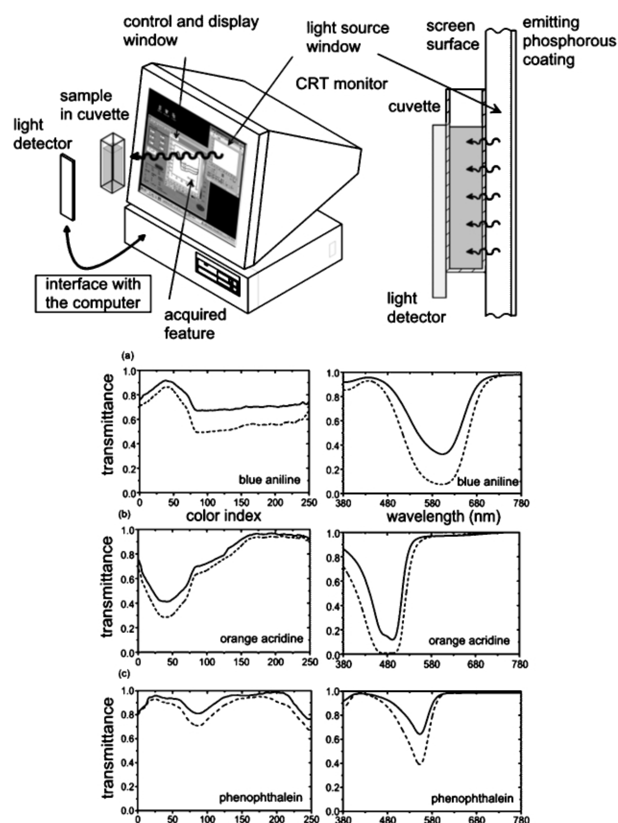

Fig. 1 Diagram of the experimental arrangement, showing the sample in a cuvette, placed in front of a region of the computer screen used as a light source displaying a sequence of colours, which is captured by the light detector upon absorption through the sample. The lateral sides of the cuvette are shielded with aluminium foil to prevent background illumination. a) Absorption feature of blue aniline obtained by computer screen illumination (left) and standard visible absorption spectra of the same substance (right), b) orange acridine and c) phenolphthalein. In a) and b), the solid lines correspond to concentrations of 50 μM and dashed lines to 100 μM, meanwhile in c) they correspond to phenolphthalein tracing the pH of 10 mM and 100 mM of ammonia in water respectively.

DOI: 10.1039/b210677a

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modulation of the emitted intensity able to generate the required transient photocurrent in MOS detectors.

The colour of the emitted light from each pixel finally depends on the contribution of each primary according to:[8]

$$C(\lambda) = A_R R(\lambda) + A_G G(\lambda) + A_B B(\lambda) \qquad (1)$$

where $C(\lambda)$ represent the spectral distribution of any colour and $R(\lambda)$, $G(\lambda)$ and $B(\lambda)$ the primary colour spectral distributions, and A_R, A_G A_B are the relative amounts of each primary required to match the perception of a given colour. For each colour index there is a triplex (A_R A_G A_B), and the whole sequence of 255 triplexes mimic the perception of the visible spectrum.

Since each primary colour is polychromatic, colours composed according to eqn. (1) are also polychromatic. So, the displayed sequence provides illumination under 256 different light sources (say particular spectral distributions) which, however suffice to generate distinctive low resolution transmission (absorption) features.

Although the present source is far from monochromatic, it is possible estimate the resolving power ($RP = \lambda / \Delta\lambda$), by assuming the full intensity of the primary green emission as representative of the source, as if it were the output of a wide slit monochromator. In this case $RP \sim 6.3$ (corresponding to a FWHM = 84.9 nm for a wavelength 533.9 nm), meanwhile a standard spectrophotometer allows bandwidths of 2 nm. Considering that the sequence of 256 colours used in these experiments is composed of only three primaries it might be possible that some of the colours are redundant, which would imply a further simplification in the procedure.

In the second example, an alternative approach to the two-dimensional scanning used in conventional micro-plate readers is provided by exploiting the intrinsic large area light source of the screen, which enables single shot recording of the whole array that makes the measurement time independent of the number of wells. Two-dimensional micro-positioning, within a resolution of ~ 200 μm, is also possible by displacing the source on the screen.[9]

In the presence of functional mitochondria, living cells have the ability to metabolise the yellow coloured salt MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) into a blue coloured product. MOLT-4 cells cultured in RPMI 1640, 10% FCS, 100 U ml^{-1} penicillin, 0.1 mg ml^{-1} streptomycin and 2 mM L-glutamine were exposed to increasing concentrations of doxorubicin or daunorubicin. After an incubation of 72 h, MTT at a concentration of 5 mg ml^{-1} was added. The cells were incubated for an additional 4 h and the MTT was dissolved in 5% SDS.[10]

The columns of the array correspond to different concentrations of the tested drugs, control refers to the cells but without drugs, and blank is without cells. In the rows are repeated experiments; duplicate samples for danorubicin and triplicates for doxorubicin.

The whole micro-plate (shielded to prevent ambient illumination) is simultaneously illuminated with the screen facing the samples and the resulting image is recorded with a web camera (Logitech QuickCam Pro3000) operating at a resolution of 320 × 240 pixels per frame.

The MTT assay was evaluated with a micro-plate spectrophotometer (Spectra Max 340, Molecular Devices) operating at a wavelength of 650 nm, which in the case of the computer screen is mimicked using a pure red screen (rgb = 255 000 000), and the web camera acquires one frame per second (30 frames in this case to average intensities) in synchronism with the illumination (directly controlled by the software provided by the manufacturer, QuickCam version 6). This allows the a standard video file (AVI format) to be composed as a compact output from the measurement which is analysed off line with a specially developed software that extracts the intensity of each well and calculates the corresponding transmittance.

The detection limit in the example in Fig. 1 was ~ 10 μM, mainly due to the poor quality of the MOS detector (a simple device in house) but just using the web camera instead this limit easily reaches nM concentrations which are suitable for practical assay evaluation (Fig. 2).

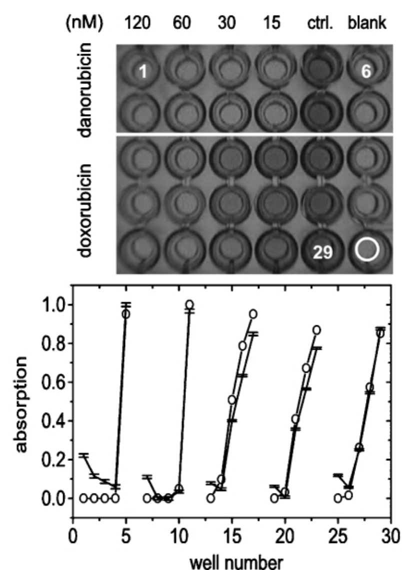

Fig. 2 Region of 30 wells used for evaluation. The picture is one single frame (320 × 240 pixels) of the video file recorded with a web camera and displayed in grey tones codifying transmitted intensity. The graphic displays the normalised absorbance of these 30 wells, where open dots correspond to the commercial micro-plate spectrophotometer and error bars to the computer screen measurement. The intensity of each well is computed as the average over 254 pixels composing its image (inside an area defined by a white circle as indicated in well 30) over the number of frames in the video.

As in the case of electronic noses,[5,6] where a composition of unspecific signal features allows distinctive chemical recognition, the absolute value of the absorbance is not the most important factor, but rather the consistent conformation of relative absorption pattern instead. Accordingly, the results are represented as the normalised absorption (Fig. 2), which show good agreement with the standard measurement.

The errors displayed in Fig. 2 correspond to differences in absorbance, mainly due to local variations of cell concentration within each well. Repeatable results must be expected from other screens following the same procedure, since the measured values are a ratio between intensities which make the result more robust to the peculiarities of different screens.

The choice of a computer screen as a light source is a trade off between its versatility or availability and its physical characteristics. However even at the present stage of development it suffices for numerous important uses as home testing or self-testing where regular monochromators, intensity modulation elements or two-dimensional micro positioning would make the system too complex or unaffordable.

Finally since not only CRT screens, but also LCD-TFT (liquid crystal displays), TV monitors or any active display such as in last generation palm tops or mobile telephones are suitable for these measurements, the same approach can be implemented as a highly mobile platform.

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