INSTRUMENTATION SCIENCE & TECHNOLOGY, 28(3), 189–204 (2000)

TWO-DIMENSIONAL CHROMATOGRAPHIC ARRAY DETECTOR USING LASER INDUCED FLUORESCENCE WITH AUTORANGING

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ABSTRACT

A two-dimensional chromatographic detector, featuring rapidly acquired spectra and employing laser induced fluorescence with a CCD camera, is described. The detector response is linear over four orders of magnitude and features a detection limit of less than 10^{-9} M for anthracene. The normal wavelength range recorded is 250 nm, extending from about 335 to 585 nm using the 325 nm line of a He-Cd CW laser. Spectral acquisition times range from 1 to 10 seconds. An autoranging feature extends the linear dynamic range of the detector to over 5 million. The detector is most useful for the analysis of naturally fluorescing materials in complex mixtures.

INTRODUCTION

Two-dimensional detectors, i.e., those that take data related to concentration, such as absorbance, as a function of both time and another variable, such as wavelength, are becoming increasingly popular for the following reasons: these (1) provide qualitative information which helps in peak identification, (2) allow the easy selection of the variable, *e.g.* wavelength, of maximum sensitivity, (3) allow increased selectivity, and (4) provide the means to test peak purity. On the negative side, two-dimensional detectors: (1) acquire large amounts of data, much of which is often of little use, (2) are often noisier and less sensitive than their one-dimensional counterparts, and (3) in some cases, may not be able to adequately follow narrow chromatographic peaks. Mass spectrometers and photodiode array detectors operating in the absorbance mode are two familiar two-dimensional detectors. Fetzer and Biggs have reviewed full-spectrum chromatographic detectors.[1]

Detectors based on the measurement of fluorescence are some of the most sensitive and specific available to chromatographers. In addition, fluorescence detection is non-destructive. Thus, it may seem surprising that, until very recently, there were no true two-dimensional fluorescence detectors available commercially. With the excep-

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tion of the new Hewlett Packard (Palo Alto, CA USA) Model 1100 detector, all commercial fluorescence detectors operated either at single excitation and emission wavelengths or could scan only in the stopped-flow mode. The more sophisticated detectors did allow changes in both the excitation and emission wavelengths during an experiment to allow maximization of sensitivity. Several reasons for the lack of such detectors may be postulated: (1) Most compounds are non-fluorescent and, thus, must be derivatized to be observed. Since the differences in spectral properties of labelled compounds are small or non-existent, a two-dimensional detector offers no advantage. (2) Samples that are fairly simple, containing only a few fluorescent components, can be easily analyzed by the much simpler detectors that operate in the stopped flow mode. (3) Samples that are well characterized and whose composition is relatively constant can be determined at high sensitivity and selectivity by detectors with programmable wavelength capability. (4) The low light levels involved with fluorescence require very sensitive detection systems which can be expensive.

Two-dimensional fluorescence detectors are most useful for samples that contain many naturally fluorescing compounds where stopped flow scanning is impractical and where qualitative information is available and useful. Good examples would be environmental or petroleum samples that contain polycyclic aromatic hydrocarbons (PAHs) which are naturally fluorescent. Mass spectrometry is often used as a detector for such samples, but it cannot distinguish structural isomers, either those with different positional substitution or different ring structures. Fluorescence easily distinguishes PAH isomers with different ring structures, *e.g.* anthracene and phenanthrene, and can sometimes distinguish isomers with different substitution patterns, e.g,. 1- and 2-methylnaphthalene, by small shifts in peak wavelengths.

Several approaches can be applied to the rapid, continuous acquisition of full spectra for two-dimensional detection. These fall into two main categories – (1) the use of a single detector element such as a photomultiplier tube (PMT) in conjunction with a rapid wavelength scanning device and (2) the use of an array of detectors which continuously monitors some portion of the emission spectrum and can be read-out at selected intervals. Smalley and McGown have reviewed the types and uses of fast scanning fluorescence detectors.[2]

Several groups have used rapid scanning monochromators with PMT detectors to implement two-dimensional detection.[3-7] These systems employ commercial spectro-fluorometric instruments, sometimes modified, to obtain emission spectra. These suffer from the disadvantages of mechanical scanning – relatively slow data acquisition, gear backlash, component wear, etc. In addition, the acquired spectra show time distortion since the sample is changing while the wavelength is being scanned. The slower the scan rate and the sharper the peak, the greater the distortion. Guiteras' group has used special software to correct for this,[6,7] but it is not clear whether this correction would be successful when used on a complex sample with many overlapping peaks. One advantage of this approach is that these instruments use a polychromatic light source with an excitation monochromator and thus can acquire either excitation or emission spectra or can change excitation wavelength during an experiment to maximize sensitivity. In the stopped-flow mode, these can acquire an excitation-emission matrix and provide three dimensional detection. The detector used by Ferrer *et al*. for the analysis of the EPA PAH priority pollutants could scan 100 nm in 2.7 seconds, used four different excitation wavelengths at various times during the chromato-gram, and provided a limit of detection for anthracene of 1 pg injected.[6]

The only commercial rapid scanning detector available, the Hewlett Packard 1100 Series detector, also operates on the above principles. It has fixed 20 nm excitation and emission bandwidths and scans at a rate of 28 ms per step with steps variable from 1 to 20 nm. Thus, to scan 250 nm with a 5 nm step, requires about 2 s. The wide bandwidth will cause loss of detail in spectra with sharp peaks (see below).

Acousto-optic tunable filters have been shown to provide rapid scanning capability,[8-10] but, to date, have not been used in rapid scanning fluorescence detectors to our knowledge.

Array detectors have several advantages: (1) they provide spectra that are not time-skewed (although they are time-averaged), (2) they are integrating in nature in that they observe all wavelengths continuously during acquisition, and (3) being solid state, they can acquire data very rapidly, although data read-out can take a significant amount of time. The silicon-intensified-target vidicon was the first array detector to be applied to fluorescence detection in chromatography.[11] These acquire spectra quite rapidly (*e.g.* 33 ms) but require signal averaging to increase dynamic range and improve detection limits. Vo-Dinh *et al*. report a detection limit for anthracene of about 5×10^{-10} M for a spectrum covering 62.5 nm, but this required an acquisition time of 66 s.[12]

Photodiode arrays, which are universally used in full-spectrum absorbance detectors, have also been applied to fluorescence detection.[13,14] The low sensitivity and poor dynamic range of these devices, which is not as much of a problem for an absorbance detector, limits their use in fluorescence detection. Cecil and Rutan used an intensified photodiode array to improve sensitivity.[15] They obtained a detection limit of about 1×10^{-7} M for anthracene in the flow cell for an acquisition time of 0.1 s and a wavelength range of 200 nm.

Charge transfer devices, which may be either charge coupled devices (CCDs) or charge injection devices (CIDs), have been widely applied in analytical instrumentation due to their low noise, high sensitivity, and large linear range. The characteristics and applications of these devices have recently been reviewed.[16] CCDs and CIDs have been used, for example, to obtain spectral data from chemiluminescent systems,[17] in laser-Raman instruments,[18] for materials condensed in water droplets,[19] and of compounds at the water-air interface.[20] They have also been employed to obtain the fluorescence lifetime parameters of crude oils.[21] The largest number of applications would seem to be as detectors for capillary electrophoresis (CE), an application very similar to chromatographic detection. However, reports of CCDs used in chromatographic detectors are surprisingly rare.

Rapid scanning, full-spectrum, fluorescence detectors normally use either high power xenon arc lamps or lasers as the excitation source. The xenon lamps require an excitation monochromator, but provide the ability to change the excitation wavelength easily. Lasers provide monochromatic, coherent radiation and require less complicated optics but offer only fixed wavelength excitation. Multi-line lasers, *e.g.* argon-ion lasers, are available, but changing the wavelength of such lasers is not usually quickly accomplished. Tunable lasers, titanium sapphire[22] and dye lasers,[23] have been used in applications with CCD arrays, but these are pulsed lasers. Continuous-wave (CW) lasers are preferred as excitation sources since there is less danger of saturation and non-linear response.[24] For this reason, argon-ion and helium-cadmium lasers are the most popular excitation sources in laser induced fluorescence (LIF).

We describe, below, a simple but sensitive chromatographic detector designed for use in detecting PAHs in complex petroleum and environmental samples. The detector uses a laser for excitation, a small polychromator for dispersion of the emission spectrum, and a CCD array for detection. In addition, the detector monitors the laser output to correct for any temporal drift. One problem often encountered with complex samples from various sources is the inability to properly set the system gain prior to the experiment to maintain high sensitivity yet avoid detector saturation and loss of data. The detector described has autoranging circuitry which almost completely alleviates this difficulty.

EXPERIMENTAL

Instrument Arrangement

The block diagram of the detector is shown in Figure 1. The detector, in its original form, did not include the sapphire window or shutter in the laser beam or their associated interface and computer expansion card. Excitation radiation is provided by a dual wavelength He-Cd laser, Model IK5451R-E (Kimmon Electric Co., Englewood CO, USA). This laser provides a 1 mm diameter beam of CW radiation with 10 mW of power at 325 nm and 50 mW at 442 nm. Both wavelengths are available simultaneously, but, normally, filters are employed to select a single value. The 325 nm wavelength is, by far, the most useful for the analysis of PAHs.

The flow cell is simply a 12 mm section of square Supracil silica tubing, type WQS-101 (Wilmad Glass Co., Buena NJ, USA), with internal dimensions of 1 mm per side. Thus, it has a volume of 12 mL. It is mounted in a brass chamber with HPLC fittings at the inlet and outlet and sealed with small Teflon gaskets. One millimeter holes are drilled in the chamber to allow access to the laser beam. Fluorescence emission is

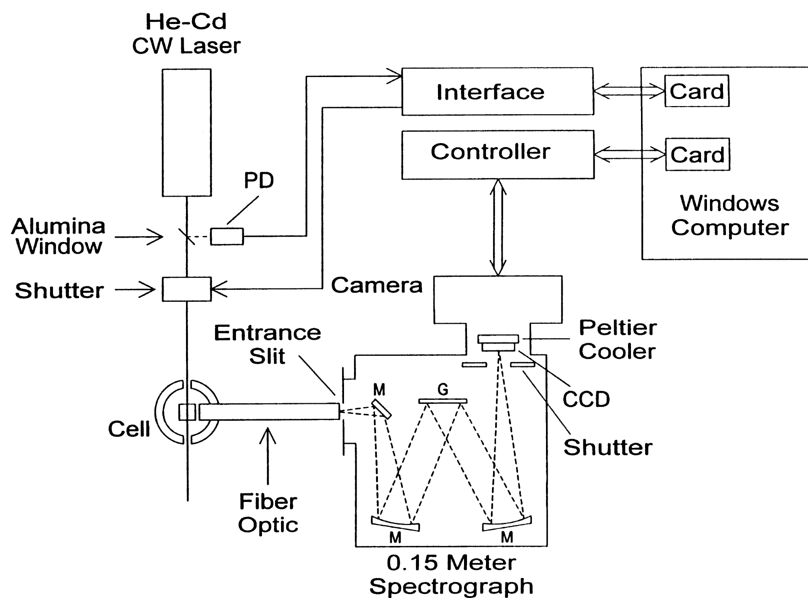

Figure 1. LIF detector block diagram.

observed at right angles to the laser beam. The emitted light is transferred from the cell to the polychromator via a high grade fused silica fiber optic bundle, #77403 (Oriel Corp., Stratford CT, USA). This bundle has a 0.66 mm diameter circular aperture at the cell and a 2.5 mm by 0.135 mm rectangular aperture at the polychromator entrance slit. This arrangement avoids the use of lenses or mirrors in either the excitation or emission beam.

The polychromator is a 0.15 m spectrograph, Spectra Pro Model 150 (Acton Research Corp., Acton MA, USA) with focal plane dimensions of 10 mm high by 25 mm wide. This polychromator uses a variable entrance slit and two interchangeable gratings. Our instrument contains a 600 g/mm grating which provides a linear dispersion of 10 nm/mm (250 nm wavelength range at the focal plane) and a 300 g/mm grating for 20 nm/mm (500 nm wavelength range). The 600 g/mm grating is the one normally selected.

The CCD camera and its controller and computer interface card are all supplied by Roper Scientific Inc., Trenton NJ, USA. The camera, Model TE/CCD-1024-EM/1, contains an EEV 256 by 1024 pixel CCD (approximately 6 mm by 25 mm active area) which is specially coated for ultraviolet response and can be cooled to about -50ºC by a Peltier cooler. The controller, Model ST-130S, employs a 16-bit, 100 kHz analog-to-digital converter (ADC) for data acquisition. With over 1000 pixels in the wavelength dimension, it might be expected that the resolution would be as high as 0.25 nm with the 600 g/mm grating. In fact, the best resolution obtainable is about 0.8 nm and this decreases as the entrance slit is opened. In order to obtain maximum sensitivity, the entrance slit is opened until just before spectral distortion is observed for PAHs such as anthracene and pyrene, which have fine structure in their fluorescence spectra.

The computer is a Model PC756-A9 (Digital Equipment Corp., Maynard MA, USA). This uses a 486 processor running at 66 MHz and contains 16 MB of memory. The operating system used is Windows 3.11.

This arrangement is similar to one used by Goodpaster and McGuffin[25] to study fluorescence quenching in PAHs. The major difference is that we have built autoranging into our detector.

Autoranging

Two drawbacks to the original arrangement, as described above, became apparent soon after it was put into use. The first problem was drift in laser intensity, which could be as much as 20% per hour. This drift did not interfere with qualitative work, but was unacceptable for quantitative analysis. The problem was easily solved by monitoring the laser intensity and correcting for drift in software. A 1 mm thick by 10 mm diameter anti-reflection coated alumina window, #02WSA004 (Melles Griot, Napean ON, Canada), was placed at 45º in the laser beam (Figure 1) and reflected about 1% of the laser light onto a UV sensitive photodiode (PD), similar to #UV-005E (UDT Sensors Inc., Hawthorne CA, USA). The photodiode signal was integrated, converted with a 12-bit ADC, and input to the computer.

The second drawback was the inability to estimate, prior to an experiment, the correct detector gain (exposure time) to use for the various sample types which we exam-

ine. An entire 60 minute chromatogram could be wasted by an incorrect gain setting. Thus, it was decided to devise an autoranging system to avoid this problem. Several approaches might be possible in designing such a system. First, the excitation intensity might be varied in inverse proportion to the emission light intensity available. This is not easily achieved when using a laser as excitation source. Second, the gain of the amplifier used to read out the pixels might be adjusted as the signal varied. There is no access to this amplifier in the controller, so this avenue of attack was not available. Besides, changing amplifier gain will not avoid pixel saturation at high light levels, which is a major problem with array detectors. Third, the acquisition time could be varied, either by varying the total acquisition time or by holding the acquisition time constant and varying the shutter open time, adjusting the exposure for the emission light intensity available.

The shutter supplied with the camera has an aperture as large as the CCD array, since it controls the light directly falling on the array. Since it is so large, it operates relatively slowly (about 30 ms) and, furthermore, due to its method of opening and shutting, it exposes different areas of the CCD array to various amounts of light. This distorts the spectra obtained at short exposure times. In addition, the control software provided by the manufacturer does not allow the shutter time to be varied during an experiment.

Thus, it was decided to employ a second shutter to control the excitation laser beam (Figure 1) while leaving the camera shutter open at all times. Since the laser beam is only 1 mm in diameter, the shutter used to control it can be very small and fast. All areas of the CCD array are exposed equally and no spectral distortion is introduced. A Model 846 HP bistable electronic shutter (Newport Instruments Canada, Mississauga, ON) was selected. This has an aperture of 5 mm and a response time of 3 ms when operated with pulses of 30V. This device has the advantage that it does not require coil current to hold it open, but it does require pulses of opposite direction to open and close. Thus, its opening response time is the same as its closing response time. Since the shutter completely opens and closes a 5 mm aperture in 3 ms, it will shutter the 1 mm laser beam in a time equal to or less than 0.6 ms. Experiments performed down to a shutter time of 4 ms indicate that the shutter response is linear.

A simplified diagram of the autoranging electronics is shown in Figure 2. All digital logic used in the detector, with the exception of the 8253 programmable timer chip, is LS TTL logic. The CCD controller is run in the external trigger mode, so that one signal from the Experiment Clock controls all experiment timing. The Experiment Clock is driven by a 1.000 MHz crystal oscillator and consists of six decade counters in series followed by a binary and a decade counter in parallel. Certain of the outputs of these latter counters are manually selected and control the rate at which spectra are acquired (from one per second to one every 10 seconds). This portion of the circuit was designed and built prior to implementing autoranging and would be done differently if done again, using a second 8253 programmable timer chip with computer control of the master timing signal.

A pulse from the Experiment Clock signals the CCD controller to start spectral acquisition and sets the Start/Stop RS Flip-Flop (FF), enabling Timer 0 and Timer 1 of the 8253 timer chip. Timer 0, operated in Mode 3 (square wave generator), is a prescaler that divides the 1.000 MHz input signal to a more usable value. This timer is usually set to output a 5.000 kHz signal. Timer 1, operated in Mode 5 (hardware triggered

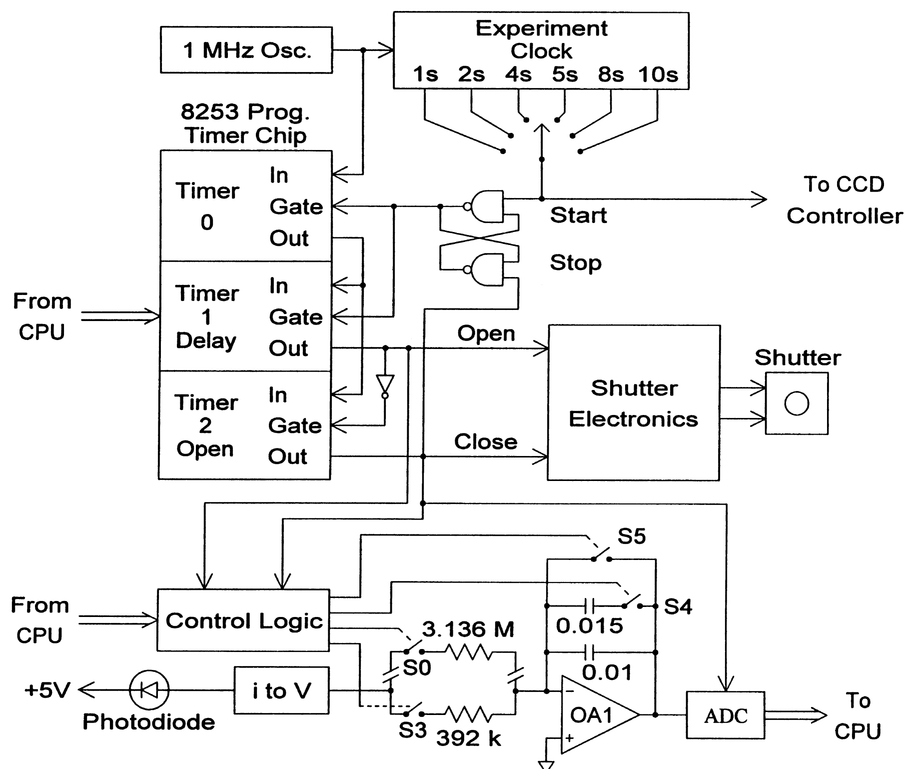

Figure 2. Detector interface block diagram.

strobe), provides a delay between the beginning of spectral acquisition and the opening of the external shutter. When Timer 1 counts down, its output signal opens the shutter and enables Timer 2. Timer 2, also operated in Mode 5, controls the shutter open time. When it counts down, its output signal closes the shutter and resets the Start/Stop FF. This cycle repeats with the next Experiment Clock pulse. Autoranging is accomplished by changing the shutter open time through Timer 2.

Figure 3 shows the shutter control electronics in some detail. Q1 and Q2 are 2N5845 transistors, Q3 to Q6 are TIP111 transistors, and D1 to D4 are 1N4002 diodes. An output signal from Timer 1 causes monostable multivibrator 1 (M1) to produce a 3 ms pulse, turning on Q3 and Q6 and causing the shutter to open. An output signal from Timer 2 causes M2 to produce a 3 ms pulse, turning on Q5 and Q4 and causing the shutter to close, since current flows through the shutter in the opposite direction to that for a Timer 1 signal. The integrated photodiode signal, which tracks the laser intensity, must also be considered in the autoranging scheme. Since the spectral acquisition rate (as set by the Experiment Clock) is constant, the photodiode signal could be integrated over this time with no changes made for autoranging. A better arrangement would be to integrate this signal only during the time the shutter is open, thus tracking the laser intensity only when it is being used to excite the chromatographic effluent. This method is employed in our system. However, the integrated signal must be adjusted

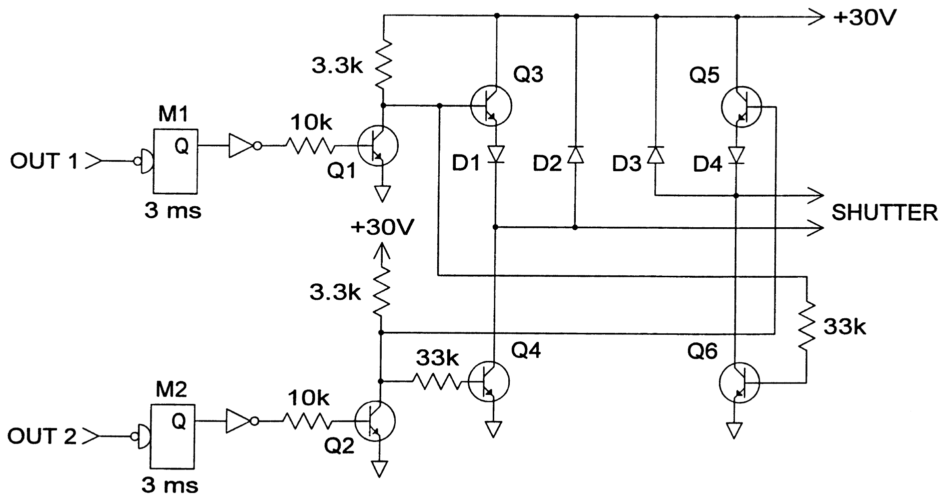

Figure 3. Detailed circuit for the shutter drive electronics.

for changes in shutter time. This is accomplished by using a variable gain gated integrator. It was decided that autoranging would be done by changing shutter times by a factor of two in each of eight steps. Thus, the gated integrator must also change gain by a factor of two in eight steps.

The gated integrator is shown at the bottom of Figure 2. The current from the photodiode is converted to a voltage through a conventional operational amplifier current-to-voltage converter (i to V) with manually selected ranges of 10, 20, 40, 80, 100, 200, 400, and 800 V/mA (not shown in Figure 2). This signal is integrated by OA1 (Burr-Brown #BB 3523, Tuscon, AZ USA). The integration time constant is controlled by selecting various values of input resistor and feedback capacitor through high speed electronic analog switches (S0 to S4, Analog Devices #ADG201HS, Norwood, MA USA). The four resistor values (only two shown in Figure 2) increase in steps of 2.000, while the capacitor value changes by a factor of 16.00. The highest gain value is achieved with S3 closed and S4 open, the lowest with both S0 and S4 closed.

The capacitance and resistance values of the gated integrator are specially matched. The gated integrator gain is increased as the shutter open time is decreased, providing no change in photodiode signal unless the laser intensity varies. A 12-bit ADC (Analog Devices #AD574A) converts the output of the integrator and inputs this value to the computer.

The control logic for the gated integrator is shown in more detail in Figure 4. A three bit gain code is output by the computer and stored in the 74LS175 4-bit D Latch. The two least significant bits of this code are decoded by the four three-input NAND gates and select the integrating resistance. The most significant bit selects the integrating capacitance. In the reset state, switch S5 is closed and switches S0 to S3 are open (S4 may be open or closed, depending on the gain code). When Timer 1 opens the shutter, it also sets both FF1 and FF2, closing one of S0 to S3 (depending on the gain code)

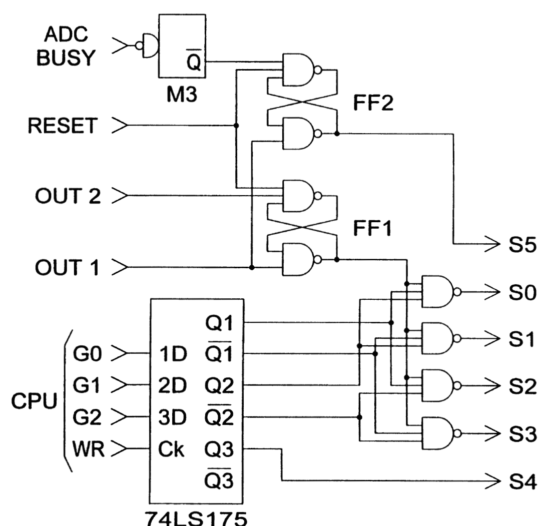

Figure 4. Detailed circuit for the laser intensity monitoring control logic.

and opening S5, starting integration. When Timer 2 closes the shutter, it also resets FF1 opening S0 to S3 and halting integration but not resetting the integrator. At this time, the integrator acts as a track and hold amplifier, maintaining a steady signal during conversion. When conversion is complete, the ADC busy signal triggers M3, resets FF2 and closes S5, resetting the integrator.

All digital signals between the interface and the computer are transferred through a 48-bit digital input/output card (Model CIO-DIO48, Techmatron Instruments, Laval PQ, Canada).

Software

The software provided by the camera manufacturer, which operates in the Windows environment, was written for spectroscopy, not chromatography. It is capable of acquiring a set of spectra in time and displaying them as acquired. However, it cannot display the chromatogram as it is acquired nor can it display single wavelength chromatograms (the equivalent of mass chromatograms with a mass spectrometric detector). It is totally incapable of providing control for any other equipment, such as the autoranger. The manufacturer does provide (at additional cost) a set of dynamic link libraries (DLLs) that are guaranteed to link properly only to programs written in Microsoft Visual C++. Thus, the data acquisition and autoranger control program was written in Microsoft Visual C++, V1.52 for Windows V3.11.

The time between spectra is set manually using the Experiment Clock (see above), all other timing functions, including autoranging, are under software control. The longest shutter open time is set to be about 0.5 s less than the time between spectra and the camera acquisition time is set to a value slightly greater than the sum of the shortest delay time (which is always 10 ms) and the longest shutter open time. This guaran-

tees that the camera starts acquiring before the shutter opens and continues to acquire for a short time after the shutter closes.

In order to keep the shutter time always centered at the same location during the period of the Experiment Clock, the delay time must be lengthened as the shutter time is decreased. If this is not done, the time between data points changes with autoranging. These ideas are illustrated in the timing diagrams shown in Figure 5. Since both delay time and shutter time are under computer control, this is easily accomplished in software.

Autoranging, which is really nothing more than predicting the future from a knowledge of the present and past, is more complex with two-dimensional data, since the decision to change gain must be based on an estimation of how all points in a spectrum, not just the most intense point, may change in the future. It may be that the intensity at the wavelength of maximum intensity is decreasing while the intensity at another wavelength is increasing dramatically. This could occur on the tailing edge of one chromatographic peak when a second, overlapping peak just begins to emerge.

Our method of autoranging assumes a linear growth model for chromatographic peaks. Let "NS" represent the most recently acquired spectrum, "PS" the spectrum acquired just before NS, and "FS" the estimate of the future spectrum to be used for autoranging. Then

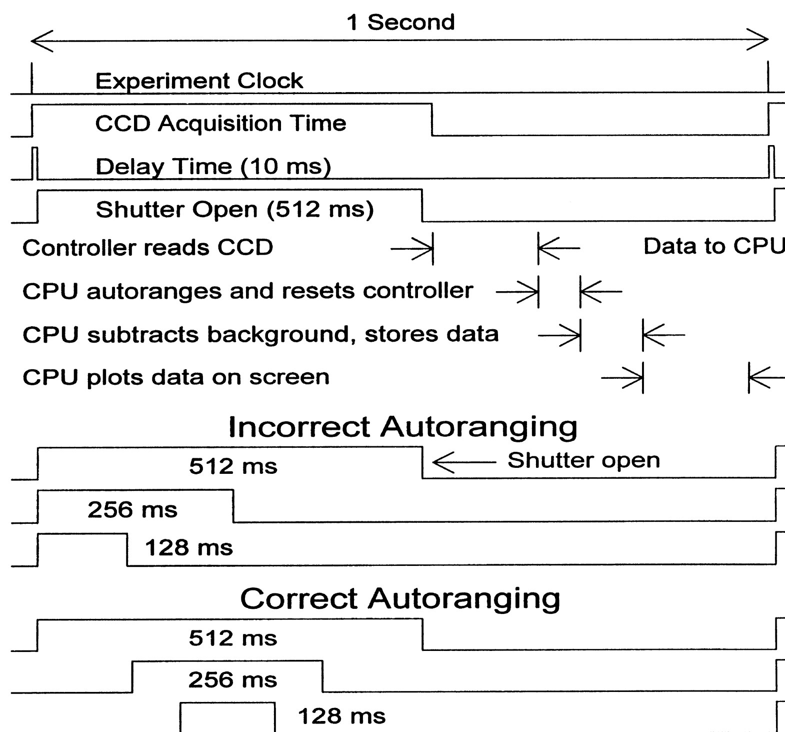

Figure 5. Data acquisition timing diagram.

$$FS = NS + NS(NS - PS)/PS = NS^2/PS.$$

Once FS has been calculated, the highest intensity in this spectrum is located. This value, FS_{MAX}, is then divided by 48000 to give a multiplication factor, MF,

$$MF = FS_{MAX}/48000$$

The autoranger gain code (AGC) used by the interface is the number of right shifts (integer divisions by 2) required to reduce MF to zero. Software never allows AGC to be less than zero or greater than seven. Since the full scale value for our ADC is 65535, the use of 48000 in the above equation causes autoranging to occur when the signal reaches approximately 75% of the full scale value. It should be noted that all spectra are stored in long integer (32-bit) format and that the converted values are shifted left by the AGC value in effect when the spectrum was acquired. Autoranging is done on non-background-subtracted data, requiring that PS must be temporarily stored in a non-background-subtracted form. This method has the advantage of being able to change the gain code by more than a single value at any autoranging step. Background subtraction is also complicated by an autoranging scheme that uses variations in the exposure time. There are two types of background, dark background and laser background. The dark background consists of pixel read noise and charge that is built up in the total absence of light (similar to the dark current in a PMT). The former is independent of either exposure time or acquisition time. The latter, in our system, depends on acquisition time, not exposure time. Thus, in a given chromatographic experiment, the dark background is constant. Laser background consists of the Rayleigh (elastic) and Raman (inelastic) scattering from the solvent and background fluorescence from impurities in the cell walls and in the solvent. Laser background varies linearly with exposure time. In an experiment where background is to be subtracted, the dark and laser backgrounds at the longest exposure time are determined prior to the experiment. During the experiment, but after the data have been left-shifted by the value of AGC, the dark background is also left-shifted by the value of AGC and is then subtracted from the data. Finally, laser background is subtracted from the data without alteration. This procedure corrects for the change in laser background during autoranging.

This works well for isocratic chromatography. In solvent-programmed experiments the scattering from the solvent and the fluorescence from solvent impurities will change with time. This requires that a series of laser background spectra be acquired separately at different solvent compositions and used for subtraction. We have not implemented this type of background subtraction yet.

RESULTS AND DISCUSSION

The quality of the fluorescence spectra taken by our LIF detector is demonstrated for anthracene in Figure 6. Also included for comparison is a spectrum of anthracene taken at the same concentration and over the same wavelength range by the new Hewlett Packard Model 1100 fluorescence detector. Fluorescence intensity is listed in "ADC counts," the value taken directly from the ADC and properly adjusted by the AGC. The intensities for the Model 1100 have been normalized for convenience of display. The conditions for spectral acquisition with the two instruments were maintained as similar as possible; however, the excitation wavelength was 325 nm for the LIF detector and 250 nm for the commercial detector (recommended by the manufacturer). The commercial

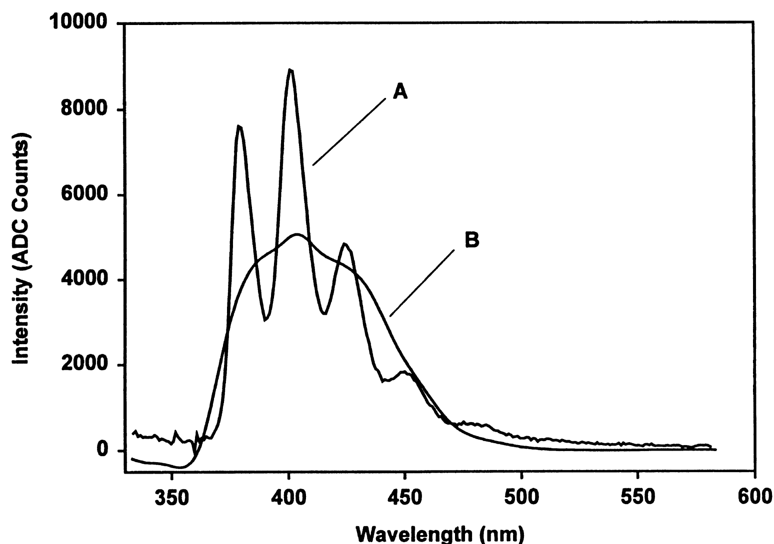

Figure 6. Fluorescence spectrum of anthracene: 5.0×10^{-8} M in acetonitrile, 3.5 s exposure time, data taken every 2 nm. Curve A: LIF detector, excitation wavelength 325 nm; Curve B: Hewlett Packard Model 1100 detector, excitation wavelength 250 nm.

detector was not able to produce a satisfactory spectrum at an excitation wavelength of 325 nm.

Sensitivity, spectral resolution, and chromatographic resolution are interconnected parameters. Increasing the slit setting (the spectral bandwidth) increases sensitivity but decreases spectral resolution. The Model 1100 detector uses constant excitation and emission bandwidths of 20 nm, achieving high sensitivity at the expense of spectral resolution. The slit on the LIF detector was set to 0.50 mm, producing a spectral bandwidth of 5.5 nm, which is sufficient to give good sensitivity yet maintain satisfactory spectral resolution. Increasing the acquisition time also increases the sensitivity but decreases the chromatographic resolution. Our system takes spectra from periods of one to ten seconds. At the highest data rate, which is sufficient for almost all liquid chromatographic purposes, the exposure time varies from 512 ms to 4 ms with autoranging. The shutter operating time will not allow faster data rates with eight-step autoranging.

Figure 7 is an example of spectra taken near the detection limit, again comparing the LIF detector with the Model 1100 detector. It should be noted that the LIF spectrum is not smoothed, while Model 1100 spectra are always smoothed. All spectra in both Figures 6 and 7 are background subtracted. This is done by the control software for the LIF detector as the spectra are acquired. This option is not available with the Model 1100 detector. Thus, background spectra were collected for the Model 1100 and background subtraction was performed off-line. This did not produce entirely satisfactory results, as indicated by the negative values observed for the Model 1100. It is not clear what caused this behavior.

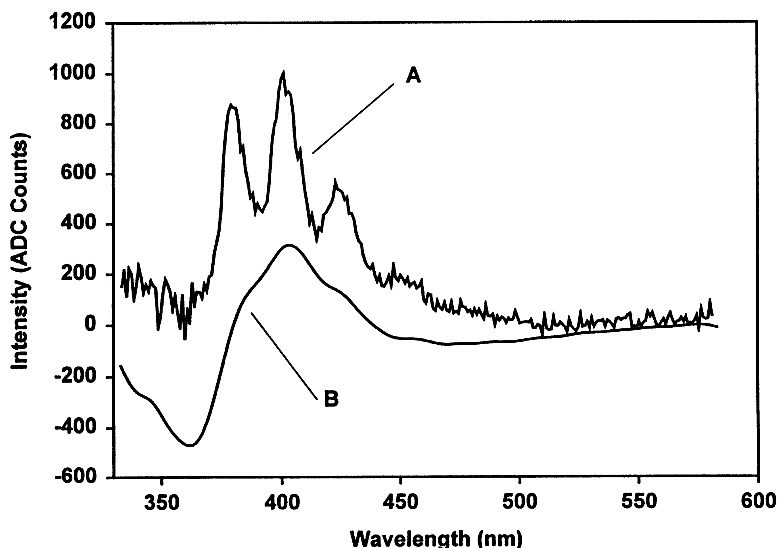

Figure 7. Fluorescence spectrum of 5.0×10^{-9} M anthracene; other conditions the same as Figure 6.

The detection limit for the conditions used in Figure 7, exposure time of 3.5 sec and slit width of 0.50 mm (equivalent to a bandwidth of 5.5 nm) is below 1×10^{-9} M for anthracene in acetonitrile for the LIF detector. The detector response in linear from the detection limit to above 1×10^{-5} M anthracene.

To reduce file size (by a factor of four) four columns of pixels are usually binned (combined) along the wavelength axis. This does not appreciably affect spectral resolution, since there are still 256 data points to cover a wavelength range of 250 nm.

Figures 8 and 9 demonstrate the autoranging abilities of our system. Figure 8 is the full chromatogram of a heavy gas oil supplied by Syncrude Canada Ltd. and run in the reversed phase mode on a Model 1100 Hewlett Packard liquid chromatograph with a quaternary pump. The solvent program started with 50:50 (v/v) water:acetonitrile (AN) and ended with 100 % dichloromethane (DCM). The maximum intensity measurable in this experiment was about 3.36×10^{7} counts (65535x128x4 – the value of 65535 is the maximum full scale count of the ADC, the value of 128 arises from the 8-step autoranging, and the value of 4 from the binning of four columns of pixels). Thus, the autoranger will have used both its highest and lowest gain (longest and shortest exposure time) during this chromatogram. Figure 9 shows the first 1000 seconds of the same chromatogram at a much higher magnification where the gain would have been the highest. The peak that goes off scale at 500 sec (the first peak to do so) in Figure 9 is the same as the very first peak to be visible in Figure 8. Several single spectrum noise events, possibly cosmic rays, which were obviously not spectral data, have been removed from the data of Figure 9. There are, obviously, peaks observed in Figure 9

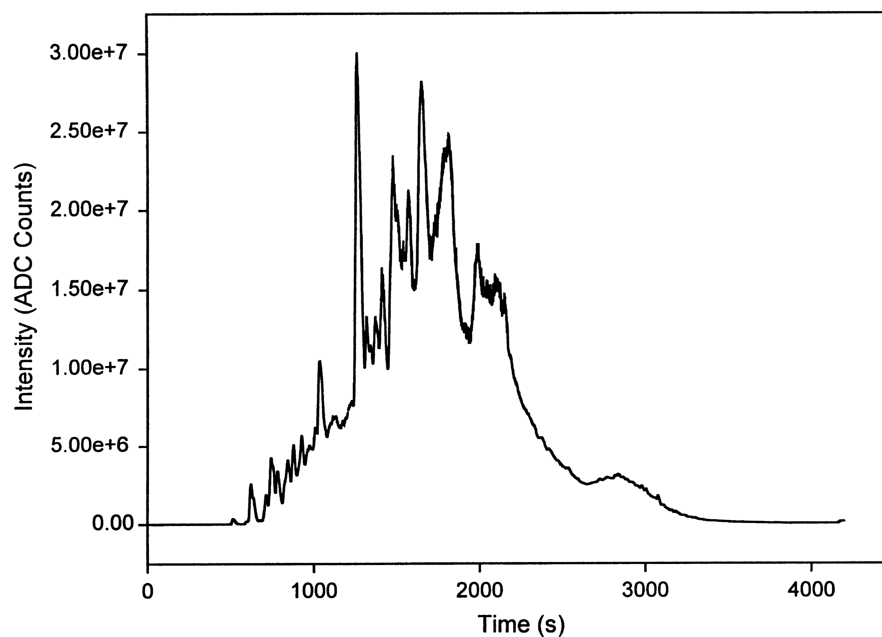

Figure 8. HPLC chromatogram of a Syncrude heavy gas oil sample (343 - 542ºC): Vydac C_{18} reversed phase column, 4.1 mm by 25 cm; 1.0 mL/min flow rate; 20ºC; 50:50 (v/v) water:AN to 100% AN at 30 min, 100% AN to 100% DCM from 40 to 60 min.

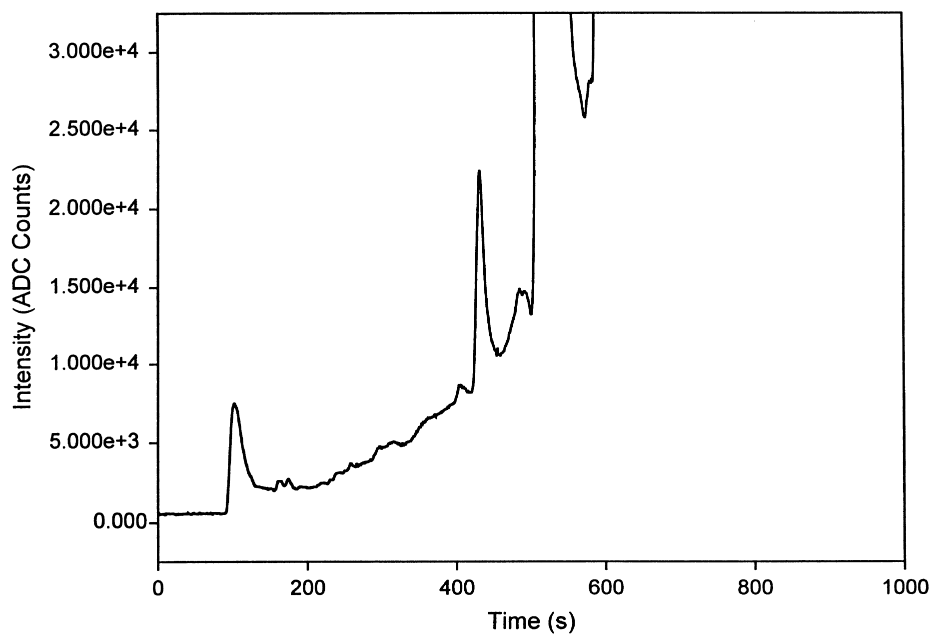

Figure 9. First 1000 s of the chromatogram shown in Figure 8.

that would not be observed without autoranging, yet the data from the intense peaks were not lost either.

ACKNOWLEDGMENTS

The authors wish to acknowledge Robert D. Guy and Michael Potvin for providing the chromatographic data and the data for the Model 1100 detector and Dalhousie University and the Natural Sciences and Engineering Research Council of Canada for financial support.

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Received June 29, 1999
Accepted July 20, 1999
Author's Revisions December 12, 1999
Manuscript 1218