Pulse selection system with electro-optic modulators applied to mode-locked cw lasers and time-resolved single photon counting

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An electro-optical pulse selection system for mode-locked cw lasers is described. The repetition rate of the exciting pulses can be chosen by electronically dividing the mode locker drive frequency. The shortest repetition time for pulse picking is 3 μ s. The optics, electronics, alignment procedure, and adaptation to the detection system are outlined. We use this equipment for measuring the decay of fluorescence and of emission anisotropy of biological molecules.

PACS numbers: 84.30.Wp, 42.80.Ks, 42.60.Kg

INTRODUCTION

When a laser is (actively) mode locked, the repetition rate of the pulses is determined by the cavity length of the resonator and the velocity of light in that cavity. With our argon ion laser, a CR 18 UV, mode-locking is achieved by driving an acousto-optic prism (Harris Corp.) with a 38.080-MHz signal. With proper settings this will yield a train of optical pulses with a repetition rate of 76.160 MHz and a full width at half maximum (FWHM) of the pulses of about 100 ps.

The frequency of 76.160 MHz is too high when the laser is used as a source of excitation for the analysis of fluorescent samples characterized with relatively long lifetimes (>3 ns). In those cases the fluorescence created by a particular laser pulse has not disappeared before the next pulse arrives. Reduction of the pulse rate can also be required to ensure complete relaxation of excited state population (triplet saturation) before the arrival of the following light pulse.

We use a nonlinear least square iterative convolution method in the time domain for data analysis¹ and one pulse out of the train is isolated at a particular time in order to ensure complete relaxation of the fluorescence. Depending on the requirements¹ the method of U. P. Wild et al.² can be used. In that case pulse picking is only necessary at much longer lifetimes.

The optical arrangement and properties of the modulator as a function of bias voltage and temperature are described. The drive electronics as well as the requirements for incorporation in the detection system are discussed. Also an application is shown.

I. OPTICS

The argon ion laser and the inherent optics are installed on a home-built 7.20 m long optical table consisting of two marble plates, each 3.60 m long, 60 cm wide, and 10 cm thick. These plates are carried by a steel construction, mounted on balloon-like rubber buffers from Firestone. The mechanical resonance

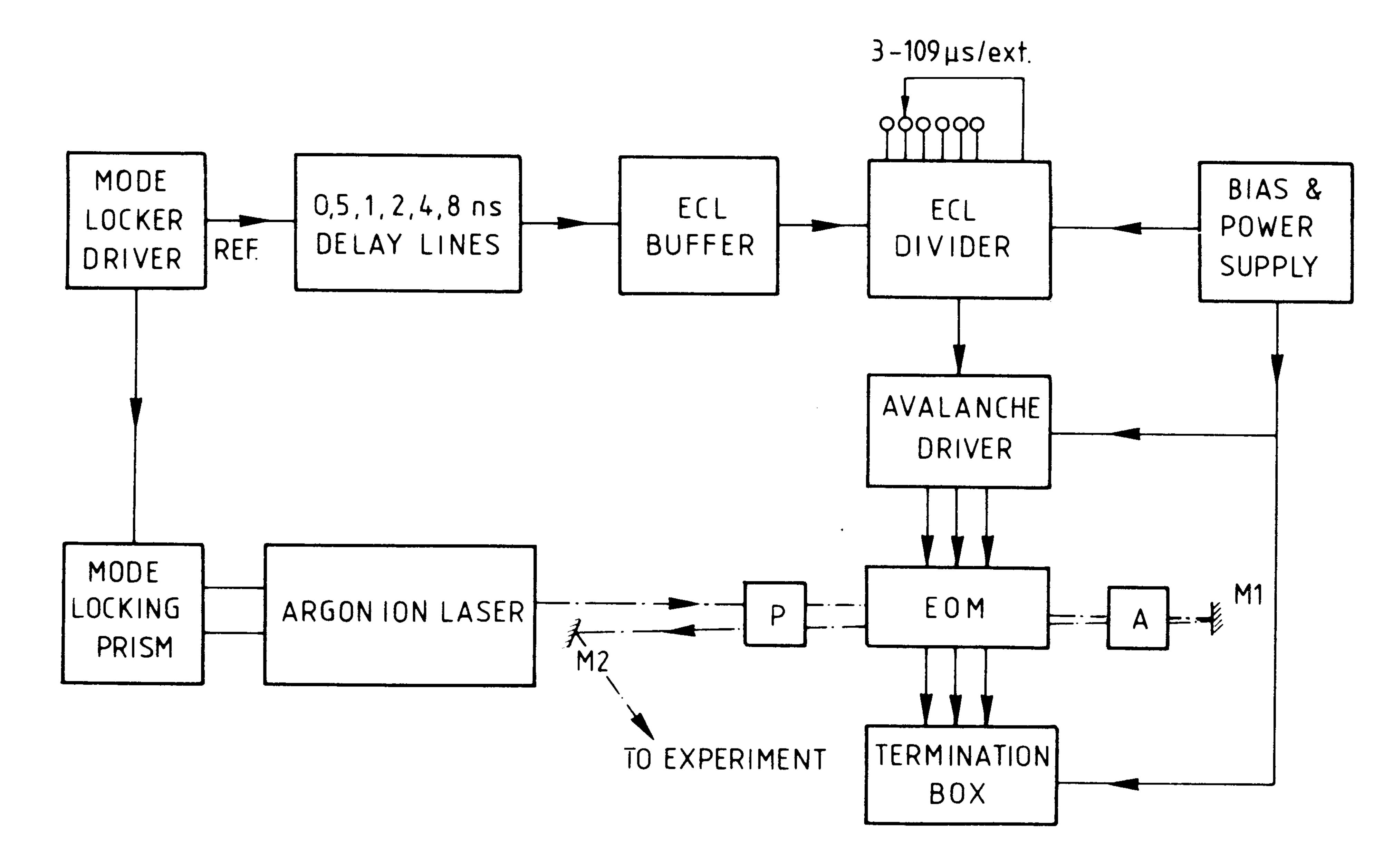
frequency of the table is about 1.5 Hz in the vertical sense. This is sufficiently low to reject the floor vibrations, occurring at 20-40 Hz.

Holes were drilled into the marble and into the holes brass female screw threads were glued. Triangular optical rails were fixed using these screw threads. The optics, sample housing, monochromator, detector housing, etc. are mounted on these rails. The laser beams are about 70 cm above floor level in order to prevent eye hazard.

For modulating the mode-locked pulse train we used low voltage electro-optic modulators (Coherent Associates, Inc.). Special types of models 22 and 28, respectively, are used for the visible and ultraviolet part of the spectrum. These transverse field modulators are rather sensitive to temperature changes. They are used in a room, thermostated to within 1°C. In order to suppress short term temperature variations of the crystals, especially during the alignment procedure, we have isolated the modulators with layers of foam and aluminum foil.

In operation the modulator is mounted in two Oriel model 1276 adjustable diameter optic holders, one at each end of the modulator. When the upper of the three lock screws of both holders are somewhat loosened, the modulator can be rotated without much displacement. The optic holders are mounted on two Ealing model 22-4899 transverse and vertical slide assemblies. By tuning the controls of these assemblies, the modulator can be moved with sufficient precision into the laser beam.

In order to improve the degree of polarization of the laser beam, and for analyzing the output beam of the electro-optic modulator (Fig. 1), two uncoated Glan—Taylor laser polarizers, from Halbo Optics³ are used: (model P10 with two exit windows for the rejected beams). The polarizers are mounted on constructions consisting of the Ealing model 22-8163 tilt and rotation platform for positioning, and the Ealing model 35-1742 360° rotation base for rotation of the polarizers. We have measured an extinction ratio of the polarizers up to 10^{-7} at 457.9 nm wavelength.



Block diagram of the experimental setup. P = optical polarizer, A = optical analyzer, M1 and M2 = mirrors, ECL = emittor coupled logic, and EOM = electro-optic modulator.

II. BIAS VOLTAGE

The transmittance of laser light at a particular wavelength through an aligned electro-optic modulator and analyzer combination in single pass gives rise to a sinusoidal curve as a function of the bias voltage across the crystals [Fig. 2(a)]. The difference in voltage between one point of this curve with maximum transmission and the next point with minimum transmission is defined as the half wave voltage $(V\lambda/2)$. This half wave voltage is linearly dependent on the wavelength of the laser light. The half wave voltages for model 22 are from 80 to 120 V for wavelengths from 450 to 650 nm. For the model 28 the half wave voltages range between 110 and 125 V for the UV lines of the argon ion laser. Figure 1 presents the schematics of a dual-pass configuration. The curve of transmittance vs bias voltage is a quadratic

representation, to be distinguished from that obtained with the single pass arrangement [Fig. 2(b)].

The bias voltages at which the modulator has minimum transmission depend on the optical alignment and on the temperature. If the temperature changes, the sinusoidal curve shifts along the voltage axis. A change in optical power or the presence of electronic modulation pulses in the modulator also gives rise to a shift. In pulse picking operations, the bias voltage is set at a point of minimum transmission. During the pass of the picked laser light pulse through the modulator, a voltage pulse of about one half wave voltage is generated with an electronic circuit, the triple avalanche driver.

The avalanche driver is directly coupled to the electrooptic modulator. The outputs of the power supplies for the driver are floating and their system ground is connected to the bias supply (Fig. 3). All voltages mentioned

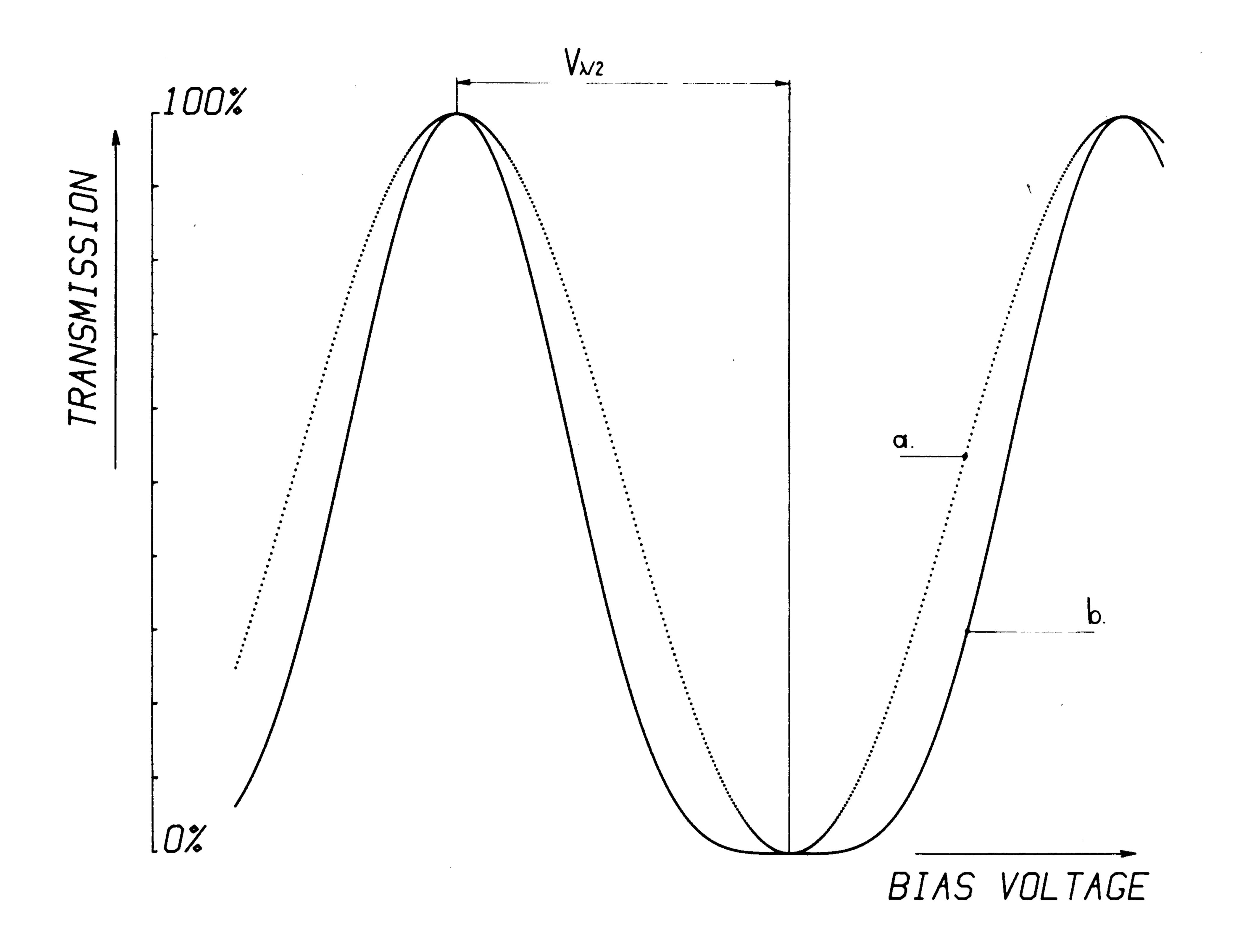


Fig. 2. Calculated curves of the transmission of an electro-optic modulator in (a) single pass, and (b) dual pass configuration as a function of the bias voltage across the crystals. The maximum transmissions are normalized to 100% and the point of zero bias voltage is arbitrary (see text).

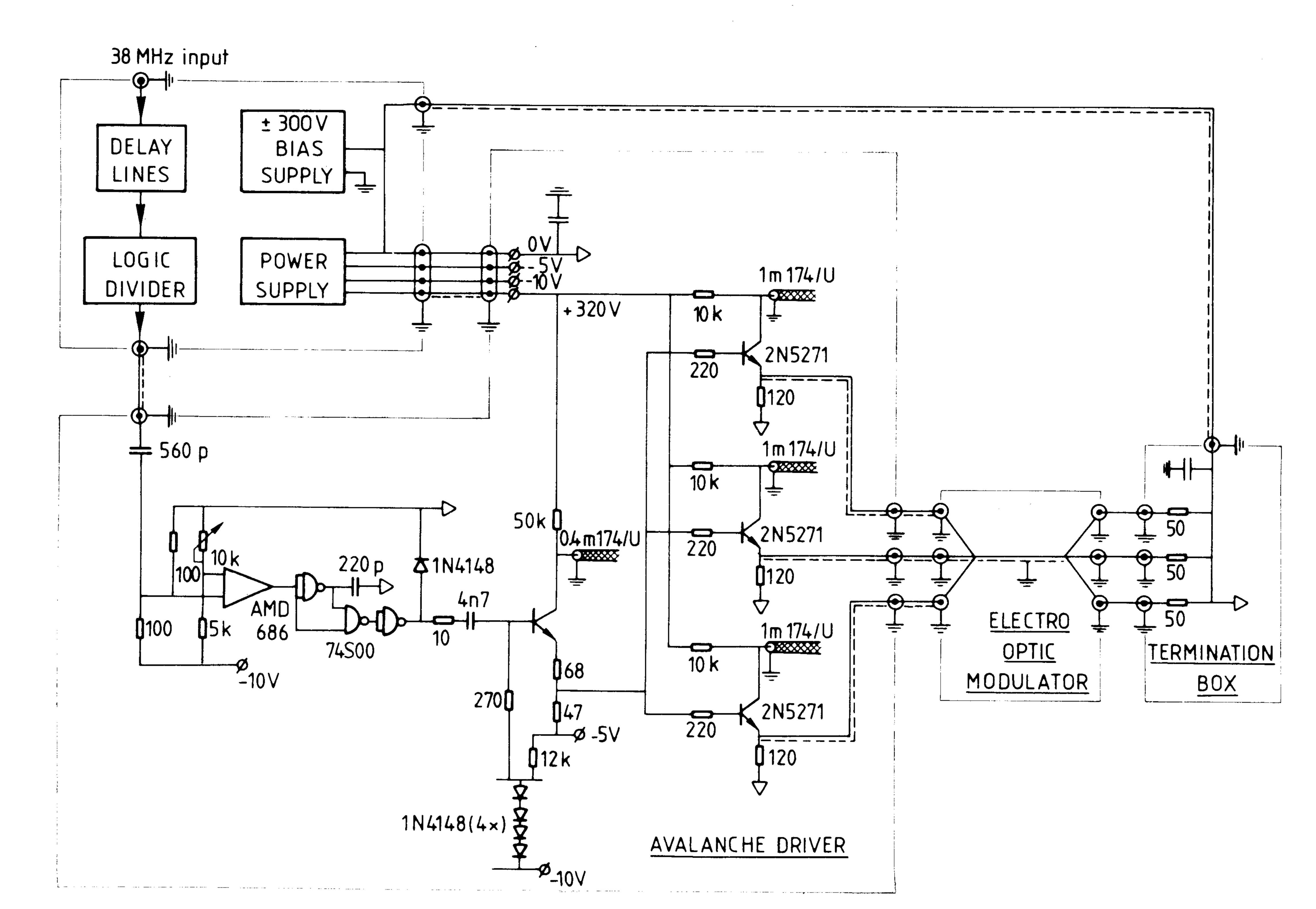


FIG. 3. Electrical diagram of the modulator driver. Resistors in ohms, capacitors in farad.

in Fig. 3 are relative to this bias voltage. Components nonessential for quick understanding are deleted from Fig. 3. The capacitors for decoupling the system ground (bias voltage) to the safety ground are composite capacitances to obtain a good decoupling over the full frequency range of the circuitry. The bias supply consists of a ten-turn potentiometer (300 k Ω) connected to a 300-V, 5 mA zener stabilized power supply. The bias output is connected to the wiper and to one side of this potentiometer. A double pole switch is used for polarity changing.

III. ELECTRONICS

The main part of the modulator drive electronics is the triple avalanche driver. The driver consists of three avalanche transistors, Motorola 2N5271, each driving a 50- Ω load with 10 ns pulses, via interconnections in the electro-optic modulator (Fig. 3). There are three input and three output female BNC-connectors on the electro-optic modulator. The inner conductors of the connectors are connected to one side of a pair of electro-optic crystals, the outer conductors are connected to the other side. Because of the low impedance (50 Ω /3) a sufficiently low RC time is obtained for nanosecond switching.

The repetition rate of the pulses driving the modulator is determined by the dividing of the mode-locker drive frequency (38.080 MHz). An emitter coupled logic (ECL) divider is used because it has less time jitter than other logic. The range of the dividends varies from 128 to 4096 which selects repetition intervals from 3.36 to 109 μ s, respectively. An external trigger signal also can be used for non-mode-locked experiments.

In both modes of operation the avalanche driver is

triggered with an ECL square wave signal via capacitive coupling. The signal is differentiated and then discriminated by a comparator type 686 from Advanced Micro Devices, followed by a pulse shaping circuit with a Schottky TTL device 74S00. The output of this circuit is a 10-ns logic pulse, which triggers the transistor 2N2222A, set up in avalanche mode. The output pulse of this avalance stage triggers the triple avalanche driver with the transistors type 2N5271.

The generated output pulse is a positive 120-V, 10 ns FWHM pulse with about 2 ns rise time when the modulator is connected. The maximum repetition rate of the pulses is limited by the charging time of the capacitance at the collectors of the avalanche transistors. We use delay lines as capacitors to get a semirectangular pulse shape. The capacitance of the RC combination is then determined by the required length of the delay line, in this case about 1 m for 10 ns pulses. The minimum charging resistance is limited by the properties of the avalanche transistors.

If the collector current of the avalanche transistors in the nontriggered mode is increased, both the power dissipation of the transistor and the probability of self-triggering are increased. The latter effect can be reduced by connecting the base of the avalanche transistor to a negative voltage (-5 V) with a low resistance. In addition the collector to emitter avalanche voltage rises and once triggered, a higher output pulse voltage is the result.

For rapid charging, a small resistance between the +320 V power supply and the collector and delay line is necessary. With a given maximum collector current in the nontriggered situation, this yields a power supply voltage as close as possible to the collector to emitter avalanche voltage. This is limited by the variation in avalanche voltages of the transistors.

Because of lower output power requirements for the avalanche stage with the 2N2222A, and shorter delay line, the collector resistor has a higher value. Then the base of the transistor can be kept at a smaller negative voltage as in the case of the triple output stage. Thus the 2N2222A can be triggered with the output of a logic integrated circuit.

Because of the lower half wave voltage for most wavelengths the output pulse of 120 V can be attenuated with three 50 Ω attenuator circuits. The minimum duration of the output pulses of the triple avalanche drivers is determined by the transit time of the electrical pulse through the modulator (about 5 ns in the case of the model 22).

We have carried out experiments with other transistors in avalanche mode in the triple driver stage, but the 2N5271 showed the best performance, especially with respect to time jitter of the output pulse relative to the trigger pulse. It gave a reliable performance when driving the base of the transistor in the way described and with effective cooling of the components. Because the collector of the 2N5271 is connected to its case and parasitic capacitance on the collector has to be avoided, forced air cooling is chosen.

IV. ALIGNMENT PROCEDURE

In the single pass arrangement of the electro-optic modulator, the ratio between minimum and maximum transmission upon changing the bias voltage varies from $1 \div 300$ to $1 \div 1000$, depending on the wavelength. This extinction ratio is measured using a system consisting of a beam splitter, neutral density filters, diaphragms, a bandpass filter, a standard side window photomultiplier tube, and an electrometer.

Because we want to use a dynamic range as large as possible for fluorescence measurements, we use an experimental setup with the modulator in the dualpass arrangement. Then extinction ratios as high as $1 \div 200,000$ can be measured. In Fig. 1 a block diagram of the setup is shown. The laser beam successively passes the polarizer, the modulator, and the analyzer (single pass). Then the laser beam is reflected by a mirror (M1) that is placed close to the analyzer, passing again through the analyzer, modulator, and polarizer (dual pass). Finally, the beam is deflected to the experiments by another mirror, M2.

If scattered laser light, or reflections from optical surfaces, are reflected back towards the laser cavity, mode-lock action can be disturbed. Thus when positioning the modulator setup, it is important that no external reflections enter the cavity. When modulating a mode-locked laser beam, the beam is minimized with the aid of the bias voltage of the modulator, and only one in a hundred or more mode-locked pulses is transmitted. There was no detectable distortion of the mode-locked pulse when the setup was properly aligned.

The displacements of the paths of the beam and the reflected beam in the modulator has to be very small.

When this is not the case two higher minima (instead of one) appear in the curve of transmittance vs bias voltage. After proper alignment one minimum will appear when the angle between the two beams is sufficiently small (about 1.5 mrad). In our setup the modulator is placed at a distance of about 3 m from the laser output mirror. The distances within the polarizer—modulator—analyzer combination are as short as possible. The mirror that deflects the modulated beam towards the experiments (M2) is positioned close to the laser, and the distance between modulated and unmodulated beams near this mirror M2 is kept as small as possible, about 5 mm.

Before the alignment procedure is started, the laser has to be adjusted and be in a thermal stationary state. Next, the polarizer and analyzer are placed in such a way, that the modulator (on mounts) can later be placed in between. For simplicity the single-pass arrangement is built up first. In principle the analyzer can be aligned crossed or parallel with respect to the polarizer, but we use both polarizer and analyzer parallel to the laser polarization direction.

A diaphragm is then placed in front of the laser. The beams reflected from the optical surfaces are projected on this diaphragm by adjusting the controls of the mounts. The reflections of the (uncoated) polarizer and analyzer are directed to a point just at one side of the center of the diaphragm, opposite to the beam used for the experiment. The polarization direction relative to that of the laser is not critical. The polarization direction of polarizer and analyzer are matched by monitoring the spot on the ceiling, minimizing the intensity of the rejected beam from the analyzer. When polarizer and analyzer are adjusted in this way, their mounts are kept in this position during the rest of the procedure.

The modulator must be placed between polarizer and analyzer with the direction of the electric fields across the crystals rotated 45° with respect to the polarization direction of the incoming light. To determine the electric field direction the end caps of the modulator have to be removed and when mounting these caps again, the alignment with respect to the crystals should be checked by looking through the modulator. Whilst the end caps are removed, possible fluid leakage of the cell can also be detected.

The angle of 45° between the direction of polarization of the incoming laser beam and the modulating electric field gives two equal resolved components of the light, polarized parallel and perpendicular to the direction of the electric field. In this situation the modulating electric field will influence only one resolved component. So a maximum difference in effect is obtained and a maximum extinction can be measured when the electric field is varied one half wave voltage, yielding a 90° rotation of the polarization of the light leaving the modulator.

When the modulator is placed in this way, only minor adjustments are necessary for proper alignment. Now the alignment is continued by maximizing the transmission by adjusting the bias voltage, and by positioning

and rotating the cell. Because the settings will influence each other, this sequence of actions has to be repeated several times, and this also applies to the rest of the procedure. When the maximum transmission is reached, this should be noted, since this value has to be close to the value after final alignment. During this alignment there is no output from the avalanche driver, only the bias voltage is tuned.

After the cell is tuned to maximum transmission, the bias voltage is varied to find a minimum in the curve of transmission vs bias voltage. This minimum is first optimized by a small rotation of the modulator, and then by adjustment of the bias voltage, the point of minimum transmission is checked again. Then the other position controls are tuned. Upon tuning one of the controls a clear minimum must be found in the characteristic of transmission vs a control setting. Otherwise, there is too much offset, and maximum transmission has to be checked again. Finally, finding an absolute minimum, maximum transmission and also the extinction ratio can be checked.

When measuring high extinction ratios it is important to reject background light by placing diaphragms in the light path and positioning the center of the beam in the center of the diaphragm. The latter operation is important when measuring the extinction ratio of the polarizer and analyzer: Possibly the beam is somewhat refracted by the analyzer. It is also illustrative to see the projection of a beam, modulated by slowly changing the bias voltage of the modulator close to the point of minimum transmission.

When, after repeating this procedure several times, an optimum extinction ratio is found, the bias voltage is set for minimum transmission. Then the triple avalanche driver can be triggered with the output of

the ECL divider. In principle the procedure as described can be done with the laser either in mode-locked or in cw operation. With the laser mode locked, the avalanche pulses in the modulator have to be adjusted, so that they are coincident with the mode-locked laser pulses. This can simply be done by choosing a delay line (Figs. 1 and 3) for maximum light throughput. After the triple avalanche stage is switched on, the bias voltage must be repeatedly adjusted over a period of 15 minutes because of temperature drift. During this adjustment the driver is switched off, for as short a time as possible.

V. ADAPTATION TO DETECTION SYSTEM

For fast data collection it is advantageous to have a frequency of excitation pulses as high as possible. The first limitation to this frequency is the minimum time necessary for total relaxation of the sample itself. When the fluorescence is detected with time-resolved photon counting, there exists a maximum frequency of the detected photon counts relative to the frequency of the excitation pulses, caused by the statistical effect of pulse pileup (we use a 5% rule, see also Refs. 4 and 7). For attenuating the laser beam to the required power, we use neutral density filters.

When measuring nanosecond and subnanosecond fluorescence lifetimes, the maximum data collection frequency is also limited by the single photon counting detection electronics. When the time to amplitude converter (TAC) is started with the repetition rate of the excitation pulses and stopped with fluorescence photon pulses [Fig. 4(a)], two different limiting effects have to be taken into account.

Firstly, there is the reset time of the TAC occurring after every start pulse. Because of the 5% rule, 95% of

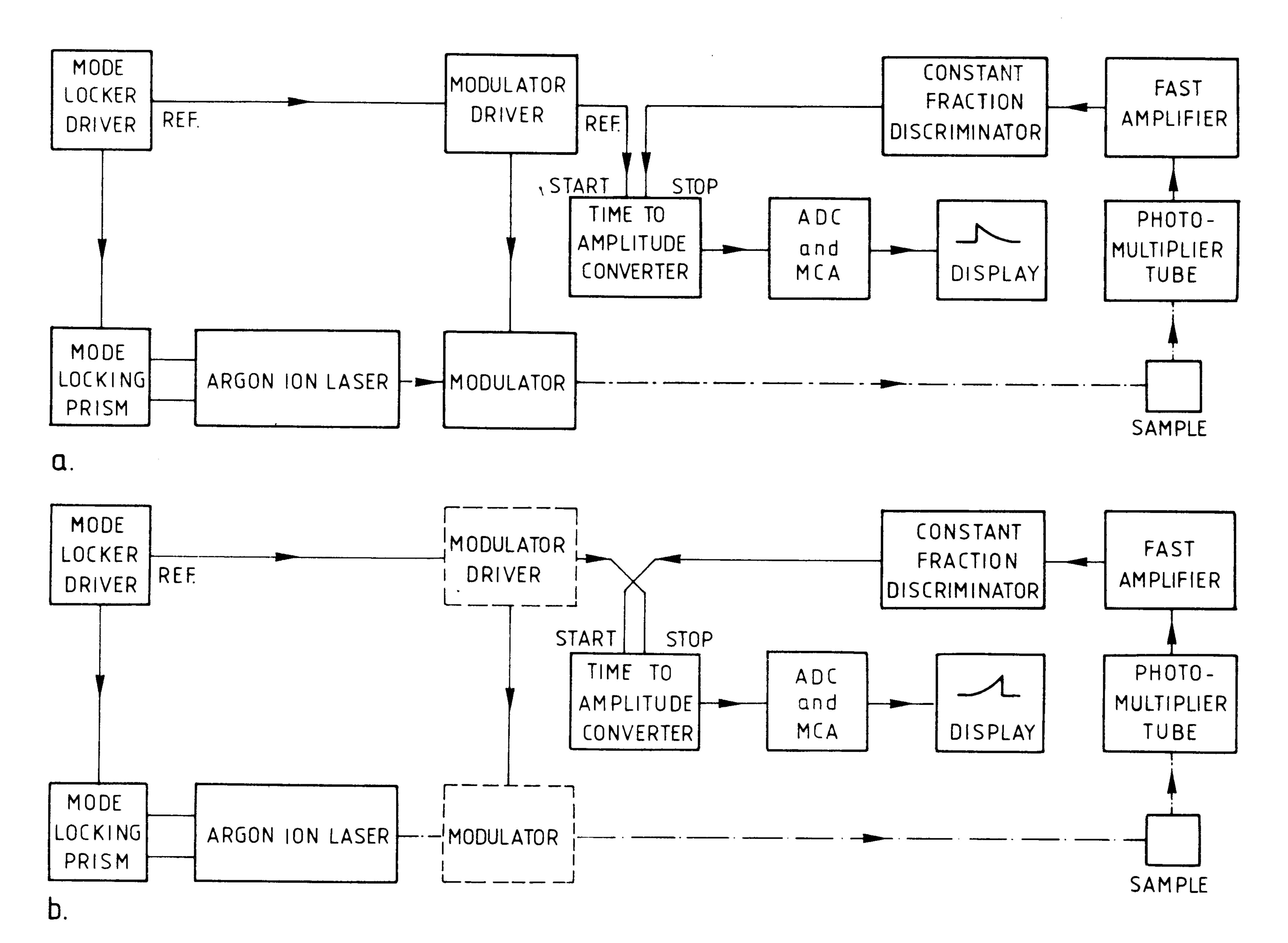


FIG. 4. Block diagrams for fluorescence decay measurements with (a) normal, and (b) reversed start—stop sequence.

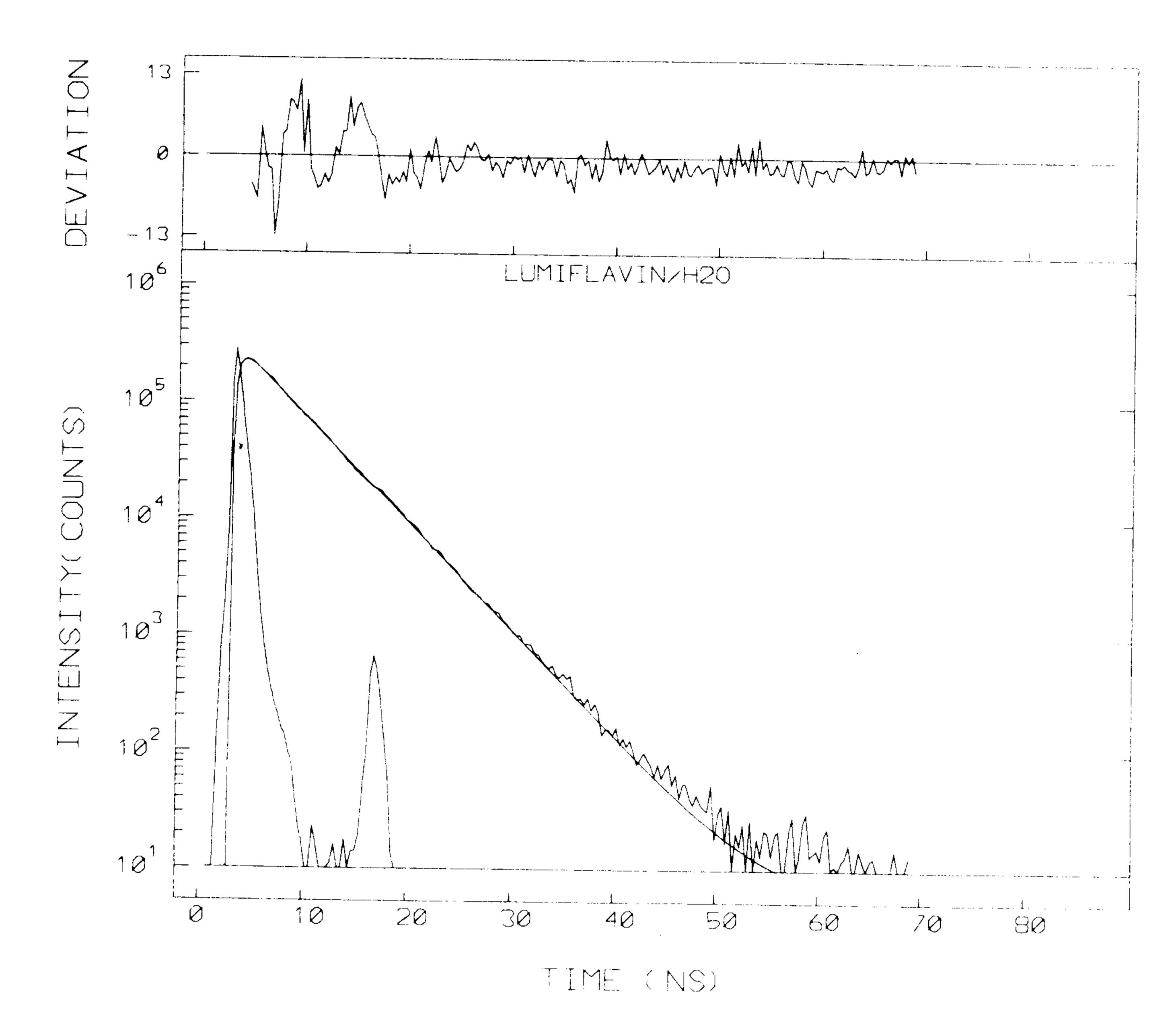


Fig. 5. Example of fluorescence decay measurement. 5.0 μM 3-methyllumiflavin in water; $\lambda_{\rm exc} = 457.9$ nm, $\lambda_{\rm em} = 531$ nm.

the sequences are nonstopped. In fast TAC's this reset time is 1 μ s, in the slower types 5 μ s. This results in a maximum start rate (= excitation rate) of about 1 MHz or 200 kHz, respectively. Using the 5% rule these start rates correspond with maximum stop rates of 50 or 10 kHz, respectively. (The time window of the TAC is taken to be relatively small.)

Secondly, when a data count is generated after a sequence stopped within the TAC time window, the conversion time for TAC, analog to digital converter (ADC), and multichannel analyzer (MCA), is at least 10 μ s. The signal transit time in the photomultiplier tube, fast amplifier, and constant fraction discriminator is in the range of tens of nanoseconds and is also negligible in this case.

There is a probability for the occurrence of two or more photon counts during the 10 μ s conversion time of the stopped sequence. On this time scale a Poisson distribution can be expected for the fluorescence photon counts as a function of time. With the above mentioned maximum stop rates of 50 and 10 kHz, the probability for more than one photon count within the 10 μs time interval is 10% and 0.5%, respectively. This loss of data will hardly distort the decay profile. Only the occurrence of two or more photon counts within the TAC time window (ns range) gives rise to pulse pileup.

Suppose now that the TAC is started with fluorescence photon pulses and stopped with the train of the excitation pulses [Fig. 4(b); start and stop reversed]. The data collection frequency (= start frequency in this case) is, in addition to the maximum permitted excitation rate for the given fluorescence lifetime and the 5% rule, only limited by the maximum percentage of data loss. So now the limitation to the maximum excitation rate is no longer the reset time of the TAC, but the total conversion time of the chain: photomultiplier tube, fast amplifier, constant fraction discriminator, TAC, ADC, and MCA.

The main part of the above mentioned 10 μ s is the conversion time of ADC and MCA, and so in the Fig. 3(b) setup, it is important to have fast types of these electronic devices. This setup is also very useful when a "slow" TAC is used (as in our case, an Ortec model 457 with a reset time of 4.5 μ s), and of course when high excitation rates can be reached, as with mode-locked cw lasers. Possible disadvantages of the method are the reversal of the time scale on the display and the fact that a certain experience in insertion of delay lines is required.

When using electro-optic modulators for pulse picking, the choice of the frequency of the excitation pulses is limited. Next to the fundamental frequency of 76.160 MHz, the repetition frequency of 297 kHz (i.e., 38.08/128 MHz) can be employed. In both cases we use the setup of Fig. 4(b). Only when decay times become longer than a hundred nanoseconds do we use the setup of Fig. 4(a).

VI. APPLICATION

We have reported on the use of the mode-locked laser as excitation source, without the use of the pulse selection system, to investigate biological fluorophores characterized by short lifetimes in the range of 0.1-3.0ns.^{5,6} In this contribution we want to present the results of similar experiments obtained with the pulse selection system. As test sample 5 μ M 3-methyllumiflavin dissolved in water is chosen. In Fig. 5, the data are presented on a semilogarithmic scale to facilitate quick comparison and to determine the contrast ratio. In the figures are shown the laser pulse measured by the detection system at 457.9 nm, the fluorescence response (531 nm) of the sample, and the computed convolution product of laser pulse with a single exponential function fitted with $\tau = 4.6$ ns. On top of the graphs the relative difference between computed and experimental fluorescence decay is shown (see Ref. 5 for more details of fitting and data presentation).

If attention is focussed on the laser pulse itself, it can' be noted that an afterpulse is present. The afterpulse is due to processes inside the photomultiplier tube (Philips XP 20208) and coincides in our case with the time of arrival of the next pulse of the train. After time intervals of multiples of 13.1 ns, however, it can easily be seen that all subsequent pulses are suppressed. Disregarding the afterpulse the lower limit of the observed extinction ratio can be set at least at 25,000, since the number of counts does not exceed 10 at times >20 ns after the first pulse. The graphical representation of the pulse clearly illustrates the performance of the system. The laser pulse is measured within a time frame of 87 ns (256 channels, 0.339 ns/channel). The fluorescence response in this example is a straight monoexponential decay with no or hardly any distortions induced by the laser pulse. Analysis is started at the peak of the fluorescence response and extended over 200 channels. Practically no deconvolution is required in this example.

VII. DISCUSSION

The pulse selection system has the objective of selecting one of a train of mode-locked laser pulses without affecting the original. The first alignment of the modulator is a lengthy procedure, but once installed, the system is relatively easy to use. The maximum repetition rate of the pulse picker matches the maximum frequency of excitation pulses for optimum data collection efficiency.

The ultimate goal of the system is to apply this technique to a variety of measurements: analysis of decay of fluorescence and of emission anisotropy, time-resolved luminescence spectroscopy, and fluorescence rejection in Raman spectroscopy.

ACKNOWLEDGMENT

A similar system has been previously built at the laboratory for Physical Chemistry of the University of Amsterdam by Dick Bebelaar. We want to thank him for his kind cooperation during the implementation of our system and for critically reading the manuscript. The

authors are indebted to Simon Maasland who assembled the optical mounts, to R. Baldew for typing, to C. Rijpma for drawing the figures, and to Dr. G. F. W. Searle for his interest and correction of the English text. Drs. T. J. Schaafsma and C. Veeger are acknowledged for their constructive interest. Part of this work has been carried out under the auspices of the Netherlands Foundation for Chemical Research (S.O.N.) with financial aid from the Netherlands Organisation for the Advancement of Pure Research (Z.W.O.).

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