SIGNAL PROCESSING DEVELOPMENTS FOR

A MERCURY LAMP BASED FILTER FLUORESENSOR

Diploma paper

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ABSTRACT

A system for fluorescence signal processing and simultaneous optical inspection is described. The equipment is based on a high pressure mercury lamp and a rotating filter wheel. The primary objective of the instrument is early cancer detection using characteristic fluorescence from the tumour-seeking agent hematoporphyrin derivative. A dimensionless contrast function is monitored making the system insensitive to target distance and surface topography. The successful operation of the system is presented and discussed.

1 INTRODUCTION AND THEORY

This paper presents an instrument for fluorescence detection and characterization. Mainly the medical application of malignant tumour detection is viewed, but other applications should be possible.
Chapter 1.1 discusses the phenomenon of fluorescence. Chapter 1.2 deals with a tumour marker called HPD, and the following chapter explains how it is used in practice. It also presents a short description of some fluorescence diagnostic systems.

1.1 Fluorescence in molecules

1.1.1 Photoluminescence

Photoluminescence is the name of light emission caused by absorption of light. The notion includes phosphorescence and fluorescence. This work is based upon the phenomenon of fluorescence light.

1.1.2 Energy states in a molecule

An atom presents discrete energy states due to the situation of its electrons. The diagram of energy levels is characteristic for every atom.
A molecule presents vibrational and rotational movements giving additional energy. This allows the molecule to possess more available energy states than an atom.
The energy separation for different modes of rotation is about 10^{-5} eV, while it is about 10^{-3} eV for vibration. This should be compared with the separation of two electronic states, which is typically 1 eV.

1.1.3 Energy bands

For molecules in solution the rotational levels are broadened compared with free molecules. They merge together into one continuous band for each vibrational level. This occurs due to interactions with other molecules, as the distance between molecules is much shorter in solution than in a gas.
Vibrational states in complex organic molecules, like hematoporphyrin, also form energy bands rather than levels. This makes the fluorescence spectrum continuous instead of discrete.

1.1.4 Energy distribution in molecules

The equilibrium population in the energy levels are distributed according to the Boltzmann distribution:

$$N = N_0 \exp(-E/kT)$$

Here k is the Boltzmann constant, T the temperature, E the energy and N the number of particles. As the first electronically excited state is usually of the order of 1 eV above the ground state and kT at room temperature is about 1/40 eV the electronic absorption spectrum is normally due entirely to transitions originating from the ground electronic state.
With the exception of the free ions, molecules have an even number of electrons. This means the resultant spin quantum number must be zero in the ground state according to the Pauli exclusion principle. The state is called a singlet state if the spin is zero. If an electron is excited to an upper state the spin of the excited electron may be parallel or antiparallel to the electron remaining in the lower level. If they are parallel the resultant spin quantum number is 1/2 + 1/2 = 1. This is a triplet state.

When the molecule is situated in an external magnetic field the spin vector then may have the three values -1, 0 or 1. This gives rise to a small splitting of the energy states of the excited electrons.

1.1.5 Transitions

A photon is able to interact with an atomic or molecular system if there is an energy interval which corresponds to the energy of the photon. Transitions discussed below are shown in Fig. 1. Following electronic excitation to a higher state the molecule undergoes vibrational relaxation. By internal conversion and collisions the molecule goes from a low vibrational level to a high vibrational level in a lower electronic state and loses its vibrational energy quickly. Transitions consisting in internal conversions and collisions are radiationless. Fluorescence radiation also appears from the lowest vibrational level of the excited electronic state. The molecule can also undergo a photo-chemical reaction when raised to an excited state.
Radiative transitions between singlets and triplets are theoretically forbidden in the first order approximation, but the weak spin-orbit coupling gives a small transition probability. The triplet states can be populated by singlet-triplet transitions. When a triplet state decays radiatively to the ground state the light emission is called phosphorescence and is characterized by a life time about a hundred times longer than the fluorescence due to the small probability for a triplet to singlet transition. This can be studied in Fig. 2.

1.1.6 The Franck-Condon principle

The fluorescence light spectral distribution will depend on the vibrational level distribution. Regardless of how the excitation is performed the molecules are normally nonradiatively transferred

to the lowest vibrational state of the first excited singlet state from where fluorecence occurs. This explains why absorbed photons of different energy often give rise to similar fluorescence.
Transitions from excited electronic states do not necessarily terminate at the lowest vibrational level in the state below, according to the Frank-Condon principle:

> The interatomic distance does not change during a light emission or absorption.

The energy levels of a molecule change during the vibration. In Fig. 3 the energy is plotted as a function of the distance between the atoms in a diatomic molecule. If the molecule absorbs a photon when the atoms are at a distance r_1 the electron makes transition 1. After some collisions (transition 2) the electron will be transferred to the lowest level in the excited state. During these radiationless transitions the interatomic distance has changed to r_2 and the corresponding lower level energy has increased. The combined effects of the increased lower level energy and the radiationless transitions result in emission of photons (corresponding to transition 3) with less energy than those absorbed, if excited from the lowest level (Stokes' law). The number of emitted photons is proportional to the number of absorbed ones.

1.1.7 Fields of application

The phenomenon of fluorescence can be used in many analytical ways. Fluorescence is a useful tool in chemistry and combustion research to qualitatively and quantitatively determine the presence of various species in a sample as well as in biochemistry and biophysics to measure certain processes and process rates. General accounts of fluorescence spectroscopy and it's application to the life sciences are given in references 1-3.

Fluorescence diagnostics have a wide field of application in medicine. Laser excitation is mostly used. This is also called laser-induced fluorescence (LIF). In this work though, the excitation light is obtained from a mercury lamp.
Either the fluorescence emission from tissue itself or from an earlier injected substance can be utilized for diagnostic purposes. A common application is fluorescence microscopy. Another field is laser-induced autofluorescence from blood vessels, which discriminates artherosclerotic plaques from normal vessel wall[4,5]. Fluorescence diagnostics may also be used to distinguish healthy teeth from caries. However, most research on LIF in medicine has been focused in the application of malignant tumour diagnostics, using the tumour marker HPD.

1.2 The tumour marker hematoporphyrin derivative, HPD

1.2.1 Chemical structure

Porphyrins are naturally occurring dyes which consist of chained pyrol rings, as illustrated in Fig. 4 a. Porphyrins are able to bind small molecules like oxygen, nitrogen, carbon dioxide, nitrogen monoxide etc. They are important in biological systems included in enzymes, chlorophyll and hemoglobin for instance. Porphyrins are often photodynamically active *in vivo*.
Hematoporphyrin (HP) is a metabolic product from hemoglobin. Its empirical formula is $C_{34}H_{38}O_6N_4$ and its molecular weight is 598.7. The structure of HP is shown in Fig. 4 b.
By a special treatment of HP with acetic acid, according to Lipson[6,7], one obtains Hematoporphyrin derivative (HPD). This agent has proved to be much more photodynamically efficient than HP. In 1960 HPD was used for tumour localization for the first time by Lipson. The purified photodynamically active component of HPD has been found to be dihematoporphyrin ether (DHE), and this is the substance which is used at present. Often the denomination HPD is used also for the purified component DHE.

1.2.2 Alternative agents

Comparative studies between HP, HPD, DHE and some other similar substances (polyhematoporphyrin ester, PHE, and tetra-sulphonated phthalocyanine, TSPC for example) have been made, with respect to their potential for tumour demarcation, using LIF[4]. DHE exhibits the best properties in this respect. However, there are some drawbacks connected with DHE compared to HP. DHE does not exhibit best tumour discrimination until 2-3 days after injection, and due to the photosensitivity to sunlight the patient cannot expose himself to sunlight until 4-6 weeks after injection. HP, on the other hand gives rise to a demarcation of a malignant tumour only 2 hours after injection. It is not possible to use HP for photodynamic therapy, which means there are no problems of photosensitivity to sunlight associated with this agent. The contrast between a malignant tumour and normal tissue is not at all as distinct for HP as it is for DHE.
Research is continued to find an agent with optimum tumour localizing properties and with as few undesirable side-effects as possible. At present only DHE (and the former used impure HPD) is approved for clinical investigations.

1.2.3 The properties of HPD

HPD has three important properties. It

* accumulates in malignant tissue
* fluoresces characteristically when excited
* causes necrosis of the tissue when irradiated with light at a proper wavelength.

1.2.3.1 Accumulation in malignant tissue

HPD has a different uptake and excretion in malignant versus normal tissue after the HPD is injected intravenously. After 2-3 days the ratio between the HPD concentration in tumour versus in normal tissue is 5-10.

1.2.3.2 Fluorescence characterization of HPD

When HPD is irradiated with light of a proper wavelength it will fluoresce with a characteristic spectrum in the red region. Absorption and emission spectra are shown in Fig. 5 a and b. The excitation and emission spectra are affected by the solvent and pH, and shifts of tens of nanometers can occur.

1.2.3.3 Photodynamic therapy

The excited HPD molecules can, alternatively, transfer their acquired energy to oxygen molecules. This energy transfer to oxygen molecules takes place from the long-lived lowest triplet HPD state to which radiationless transitions can occur. Oxygen molecules are promoted from their ground $X\ ^3\Sigma_g$ to the $a\ ^1\Delta_g$ state as illustrated in Fig. 6.
Oxygen is an exception from the rule that substances normally are in a singlet state in their ground level. Non-excited oxygen molecules are in a triplet state.
Singlet oxygen is known to be a strong toxic agent, which violently oxidizes the surrounding (tumour) tissue. This laser-induced chemical process, which is referred to as HPD-PDT (hematoporphyrin derivative - photodynamic therapy) is normally performed with laser light at 630 nm, where the tissue has a much better light transmission than at shorter wavelengths and where the HPD molecules still absorb[8]. HPD has been used to evaluate the potential of photodynamic therapy of malignant tumours for about

ten years now, and a general permission for this modality of treating malignant tumours is expected to be granted soon.

1.2.4 Tumour demarcation using HPD

Fluorescence monitoring of tissue is a promising technique for non-intrusive real-time diagnostics. In Fig. 7 laser-induced fluorescence spectra from an induced cancer tumour and the surrounding muscle tissue are shown. The curves are corrected for system nonuniform spectral detection efficiency. A nitrogen laser (λ = 337 nm) was used to induce the fluorescence, which was analyzed with an optical multichannel analyzer system. 48 hours before the sacrifice of the test animal, DHE (the purified active component of HPD) solution had been administered intravenously at a concentration of 5 mg/kg body weight.

The characteristic dual-peaked red fluorescence due to the selectively retained HPD molecules is easily recognized in the tumour spectrum. The muscle spectrum has a weaker HPD signal, but a stronger blue autofluorescence.

Characteristic signal intensities *A*, *A'*, *B*, *C* and *D* are indicated. Since the red background light (*A' - A*) may be relatively intense, it is important to subtract the background. An easy way is to subtract the *D* signal which will be a good approximation.

To distinguish tumour from the surrounding muscle, a suitable dimensionless contrast function (*F*) is

$$F = (A' - D)/B.$$

B, *A'*, *A*, and *A/B* when scanning a human epidermal tumour are shown in Fig. 8. These data are obtained using the OMA system presented in chapter 1.3.2.2.

Monitoring a dimensionless quantity has important advantages, in particular in endoscopic (fibre optic) applications:

* Immunity to distance variations
* Immunity to surface topography
* Immunity to variations in excitation or detection efficiency
* Immunity to wavelength-independent attenuation.

The characteristic signals *A'*, *B*, and *D* give all information needed. The rest of the spectra mediates very little information of interest. This knowledge is the base of the instrument presented in this paper.

1.3 Fluorescence diagnostic systems

1.3.1 Short general description

Fluorescence diagnostic instruments may be classified as imaging or non-imaging ones[9]. In fluorescence imaging, a picture of the varying intensity (and perhaps colour) of the fluorescence is obtained. Non-imaging instruments give a signal which is related to the fluorescent light intensity from the area excited. They may detect one narrow band of wavelength, two or more bands or the entire emission spectrum. Instruments may also permit imaging by reflected light under white light illumination (or excitation source illumination), a necessity for guiding non-imaging fluorescence instruments in endoscopic examinations. White light background would normally destroy fluorescence contrast, which means one has to find a way of combining the guiding white light illumination and the observation of fluorescence.
It is very important that the excitation system does not

contribute to any background at the wavelengths detected. The fluorescence light is in the order of a thousand times weaker than the reflected excitation light. Mercury arc lamps with UV-transmitting filters, blocking the visible spectrum, can be used when observing the red fluorescence. Lasers give more power and better signal-to-background ratio. Even a laser may need a filter to further attenuate the light overlapping the detected fluorescence spectrum.
Multi mode fibre optic light guides are used in endoscopic applications, and even for convenience for surface excitation. Many light guides made of glass or plastic absorb violet light, and they also fluorescence themselves, so it is advisable to use fused silica or fused quartz as fibre material.

1.3.2 Examples of systems

At the Mayo clinic[10] a non-imaging system based on a mercury arc lamp and a rotating wheel to alternate between the fluorescence and reflected light has been developed. The violet 405 nm mercury line is used for excitation. The fluorescence signal controls an audio oscillator with pitch varying as the signal strength. There is no compensation for distance, hence care and skill are required to position the endoscope probe while scanning to locate a tumour. Autofluorescence is not subtracted. This is the forerunner to the instrument dealt with in this paper, but a number of improvements are made.
At the University of California in Santa Barbara, UCSB[11], both a non-imaging and an imaging system are developed. Both systems use a violet krypton ion laser (410 nm) for excitation. Red DHE fluorescence and autofluorescence is measured, and it is possible to subtract autofluorescence background. In the non-imaging system the ratio of red to green, which is independent of distance, is calculated and displayed on a digital meter and also indicated by a variable pitch oscillator. The endoscope is guided by switching rapidly to a reflected image under white light illumination. In

the imaging system the fluorescence is observed visually or with a video camera or photographic camera. It also allows rapid switching between fluorescence and reflected white light views.

At Tokyo Medical College[12] a non-imaging system also based on a violet krypton ion laser is used. The fluorescence spectrum is recorded with a spectrograph (polychromator and intensified photo diode array, optical multichannel analyzer). Guidance is accomplished by rapidly switching to the reflected light view under white light illumination.

A group in West Germany[13] uses two excitation wavelengths from a krypton ion laser, by switching mirrors, to handle the background subtraction. The violet light (405 nm) excites both DHE and autofluorescence, while the blue light (470 nm) excites about as much autofluorescence but very little DHE emission. The blue-excited image is subtracted from the violet-excited image, and the net image is mostly from DHE.

Here in Lund another non-imaging system apart from the one presented in this paper has been developed. The spectrograph with polychromator, intensified photo diode array and optical multichannel analyzer uses a nitrogen laser for excitation. A system like this is called an OMA system (Optical Multichannel Analyzer).

An imaging system also based on a nitrogen laser is under development. Computer processing gives information on the DHE fluorescence spectrum. The short gating of the microchannel plate image intensifier in combination with the pulsed laser makes it possible to operate under white light illumination. The pulsed laser is so intense that the fluorescence light during the short excitation pulse has a magnitude exceeding the white light background.

1.3.3 The present system

Two diploma papers have already been written in Lund about earlier versions of this system. All versions use a mercury lamp for excitation and a rotating wheel with filters to select desired wavelengths. The wheel also has windows to let all the white light from the mercury lamp through for visual inspection and guidance. The frequency of the rotation is chosen high enough not to cause visual flicker. An audio oscillator with varying pitch indicates the fluorescence signal strength.

The first version[14] used a simpler chopper wheel allowing only one excitation wavelength and two detection wavelengths. This means it was not possible to both subtract autofluorescence and calculate the ratio between red to green. The latter alternative was chosen to obtain distance independence. The signals were treated in an eight-bit EPROM one-chip computer equipped with an A/D-converter. This choice makes it difficult to try out the optimal performance, as the EPROM is not easily modified. This system gave unstable results and low reliability due to insufficient suppression of the white light in the chopper wheel. Also the stability of the amplifier was not completely satisfactory.

With the experience from the first work another chopper wheel was constructed for the second version[15]. This wheel makes it possible to excite at two wavelengths and to detect three fluorescence wavelengths, which means both the distance independence and the autofluorescence subtraction is obtained.

The filters of the wheel have basically the same optical efficiency. Otherwise it would have been necessary to introduce a coefficient, k, in front of the signal D in the contrast function. This coefficient is to be set by evaluating $A - kD = 0$ for the case of no free standing red peak.

A small mini computer, Sinclair Spectrum, was chosen for signal processing. The amplifier of this system was very unstable and it was not possible to get any reliable results.

The present equipment consist in the same chopper wheel as used for the second version, and a common personal computer for signal

processing. A new amplifier is constructed. This makes it possible to eventually get reliable results. The personal computer makes it possible to easily optimize the performance and allows more flexibility in signal processing.

Advantages for this system compared with other systems are:
The mercury lamp has

* plenty of light in the vicinity of the two wavelengths utilized (405 nm, 365 nm), due to strong lines in the Hg spectrum

* a quasi-continuous spectral distribution due to pressure broadening. This quasi continuum is used for white light guidance

* a long lifetime

* much lower price than a laser.

The whole system is

* easy to run and understand for a non-professional in physics, due to the simplicity in the way of presenting the results

* serviceable as inspection in white light is possible at the same time as processing

* handy as the photomultiplier tube is fully enclosed, which in combination with background subtraction means that it is possible to leave the room illumination on

* flexible as both differential excitation and detection can be used. The chosen wavelengths can easily be altered by exchanging the filters, which implies that the equipment also may be used in other application areas, for example in industrial applications.

Disadvantages for this system are:
The mercury lamp

* must operate in a lamp housing due to the explosion hazard

* produces, due to the radiation below 200 nm, a small quantity of ozone. This substance can irritate the mucous membranes

* is inefficient when coupling the light into an optical fibre. This is to be compared with the case for a laser where practically all light emitted is gathered into the fibre.

The whole system

* is in spite of its flexibility not as flexible as an OMA system

* makes some noise due to the rotating wheel.

2 DESCRIPTION OF THE EQUIPMENT IN DETAIL

A principal scheme of the fluorescence-monitoring system is given in Fig. 9. The excitation light is taken from the mercury lamp. It passes a Pyrex plate, which absorbs the infra-red light to protect the optics, further via the optical quartz fibre and a ten times microscope objective to the rotating wheel. Here the light passes an interference filter to select the excitation wavelength and continues via another ten times microscope objective and an optical fibre to the object to be examined.
The fluorescence light is collected in another optical fibre bringing it back to the wheel, where the interesting fluorescence wavelengths are selected with interference filters, and via a collecting lens to the photomultiplier tube.
The signal is fed to the amplifier and the gated integrator which are enclosed in a protecting box. The amplified and integrated signal is fed to the computer, which processes the signals and also controls the amplifier and gates the integrator.
The signal processing consist in evaluating the contrast function which is discussed in chapter 1.3.1. The calculated function value is then transformed into a sound from the loudspeaker in the computer, the larger magnitude of the function value the higher pitch. When pressing a button the program interrupts reading the signals. Then the 100 last calculated values is written to the screen and to a file.
Chapter 2.1 deals with the rotating wheel. Chapter 2.2 describes the electronic unit: the amplification of the trigger signal from the wheel, the fluorescence signal amplification and the integrator. Chapter 2.3 describes the communication between the wheel, the electronic unit and the computer in detail. In chapter 2.4 the software is explained, and in Appendix A the program is listed. In Appendix B data about the integrator card is given.

2.1 The rotating wheel

The chopper wheel, which has been designed by Stefan Andersson-Engels at the Department of Physics in Lund, is shown in Fig. 10. In reality there is an angle between the fluorescence and the excitation areas, but pictured in this way it is easy to see what happens simultaneously.

The outer circle is for illumination, either with white light (the two large sector holes), or with the excitation wavelengths 365 nm and 405 nm. The 365 nm light induces the autofluorescence but is not as good for DHE excitation, and vice versa for the 405 nm light. The optimum must be to combine the two.

In the inner circle the fluorescence detection filters are situated. As described in the theory section the wavelengths 470 nm, 600 nm and 630 nm are chosen to form the contrast function. First, for every filter, the background light is measured before the transmission of the excitation light. Then the signal is detected. When white light is transmitted through the outer circle no detection is made.

The chopper wheel is driven by a motor and rotates at 16 2/3 Hz, that is one turn takes 60 ms. Each background is read for 0.8 ms and each signal for 1.3 ms. The filters are easily replaceable in case the instrument is used for another application or in case they break.

To give the computer a trigger signal an optical trigger sensor called GP 411, consisting in a photo diode and a phototransistor, is used. It is situated in a specially designed hole in the wheelhouse, facing the rotating wheel. The edge of the wheel is black, except in connection to every fluorescence filter where there is a metallic spot. One of the spots is wider than the others to tell the filters apart. At the metallic spots the light from the diode is reflected onto the phototransistor which gives a signal. The phototransistor signal is fed to a small amplification card for this purpose, situated in the electronic unit. A fluorescence signal and the trigger signal from the wheel, displayed on an oscilloscope, is shown in Fig. 11.

2.2 The electronic unit

2.2.1 The box design

Fig. 12 shows the front of the box. Beginning from the left there is:

* the power switch and a photo diode indicating on and off;

* a voltage supply, +15 V, 0 V and -15 V if needed for simulating a signal, for example;

* STOP, a button which is to be pressed when interrupting the program. It generates a logical signal to the computer called STOP;

* the PC cable which mediates all logical signals between box and computer;

* GAIN; the control signal from the computer to the fluorescence amplifier, with which the computer sets the gain;

* PMT which of course is the principal signal from the photo multiplier tube. The amplified and integrated signal is fed from OUT to the computer;

* TRIG PC which is a trigger signal set from the computer and functions as an external trigger to an oscilloscope if desired. It makes displaying of the signals from one chopper wheel revolution easy;

* TRIG CHOPPER; the trigger from the rotating wheel;

* GATE, with which the computer sets the integration. This goes via the PC cable, but it may be interesting to be able to present it on an oscilloscope. When exchanging the

personal computer, an adjustment of the software to the speed of the computer is needed, and then it is necessary to display the GATE signal on an oscilloscope;

* AMPL'D SIGNAL; the amplified, but not integrated, signal, which also could be interesting to complete the picture;

* The CHOPPER cable which goes to the chopper wheel and includes the voltage supply for the photomultiplier tube and the communication with the optical trigger sensor.

2.2.2 The amplification of the trigger signal from the wheel

The small amplification module for the trigger signal is shown in Fig. 13. It consists in two transistors of the type BC 547. The first one is a so called emitter follower. It is a current amplifier. The second one is a voltage amplifier. When a metallic spot passes the optical trigger sensor, the photo diode illuminates the phototransistor, that is it gets a base current. This means the phototransistor will be conductive, and the collector - the upper point of the transistor - will be low compared to V^+. Then the base of the first BC 547 transistor is low, and it will not conduct. This means the base of the second transistor is also low, and the TRIG CHOPPER will be high. The point in having two transistors instead of one is that it is possible to have a higher input resistance as viewed from the optical trigger sensor.
The amplified trigger signal is connected to the computer via a Zener diode, which cuts the signal to a nice square wave. The pulses are 5 volts high and about 1 ms long. The longer pulse has a duration of about 10 ms.

2.2.3 The fluorescence signal amplification

The amplifying module consist in three operational amplifiers called μA 741. The module is shown in Fig. 14.
The first stage from the left functions as a current - voltage converter. It inverts the signal and does not amplify the signal much.
The second operational amplifier from the left is also inverting. The amplification of this stage is controlled by the computer via a photo diode and a photoresistance cell. The voltage signal (GAIN), set by the computer, is fed to a BC 547 transistor, which gives a current to the photo diode. The diode illuminates the photoresistance cell and the resistance varies (Clas Ohlson AB, Insjön, article #: 22 - 1420). This is the resistor which, parallel-coupled with a common resistor of 1 MΩ, determines the gain of this amplifier.
The last amplifier is a stabilizer. It assures a low output resistance.

2.2.4 The integrator

The gated integrator module (Model 4130) is obtained from Evans Associates, Berkeley, California. The integrator card is controlled by the RESET and the GATE signal given from the computer. The reset signal short-circuits the integrating capacitor a very short period to empty it before the integration start. The GATE signal tells the integrator when to integrate the signal. Negative logic is used. The complete description is to be found in Appendix B.

2.3 The communication between wheel, electronic unit and computer

2.3.1 The PC cable

A survey over the PC cable is given below:

Signal	C-Pin	Colour	A-Pin	Logical	Register	Bit
-GATE	1	brown	1	-strobe	prctrl	0
-RESET	2	white	14	-autofeed	prctrl	1
+TRIG PC	3	yellow	16	+init printer	prctrl	2
+TRIG CHOP	4	grey	11	-busy	prstat	7
GROUND	5	green	20	----		
-STOP	6	orange	10	+acknowledge	prstat	6

* Signal: The sign in front of the signal name indicates negative or positive logic. For example, the integrator card uses negative logic for the GATE. This means the signal is normally high and goes low when gating;

* C-Pin is the pin number on the 9-pin box contact;

* Colour indicates the colour of the inner cable;

* A-Pin is the pin number on the 25-pin printer adapter, that is where the signals are connected to the computer;

* Logical is the usual name of the logical variable in the computer. The sign of - indicates that the logical variable is inverted in the computer;

* Register is the input/output port: prctrl - printer control port - address 03BE, or prstat - printer status port - address 03BD;

* Bit is the number of the bit where the signal is set.

2.3.2 The CHOPPER cable

A survey of the CHOPPER cable is given below:

Signal	C-Pin	C-Colour	W-Colour
OPTICAL TRIGGER SENSOR:			
photo diode	1	brown	brown
ground	4	white	white
photo transistor	2	grey	violet
PHOTO MULTIPLIER TUBE:			
+15 V	5	yellow	red
ground and control	3	green	white

* C-Pin is the pin number on the box contact;

* C-colour is the colour of the inner cable;

* W-colour is the corresponding colour from the wheel.

Control is a control signal to the photo multiplier tube which is set to ground.

2.4 The computer processing

2.4.1 The ADDA interface card

The PC ADDA-12 card (FPC-010) is an analogue-digital/digital-analogue high performance data conversion card operating at a twelve-bit resolution. It uses I/O port address $270 - $277 in the personal computer. I/O port address $2F0 -$2F7 is alternatively selectable depending on a jumper (JP1).
The card has one D/A channel for twelve-bit data transfer from digital to analogue representation in either unipolar or bipolar operation, depending on the jumper (JP2) setting. As in this work the D/A channel has been used not only for the positive control

signal to the computer (GAIN), but also for simulating the current from the photomultiplier (PMT), the jumper is set to bipolar operation. The current setting time is 500 ns. There is at most a non-linearity of 0.2 % in performance.
The card also includes sixteen unipolar A/D channels for twelve-bit analogue to digital data conversion. Here only one channel (CH1) is used. Six different signals and backgrounds are processed, but they are separated in time and they are not processed in separate channels.
The voltage range is adjustable by a potentiometer on the card (VR) from 0 V to 9 V, unipolar operation. Here the voltage range is adjusted just to manage maximum output from the integrator. As the card uses twelve-bit representation the maximum digital value is 4095. The integrator card reaches maximum approximately at 4035, which will be discussed later. A successive approximation method is used, and the conversion takes 60 μs.
The 25-pin female D-type connector includes following signals:

Pin	Signal	Pin	Signal
1	GND	14	GND
2	D/A OUT	15	0.5 V
3	5 V	16	GND
4	GND	17	A/D CH15
5	A/D CH0	18	A/D CH14
6	A/D CH1	19	A/D CH13
7	A/D CH2	20	A/D CH12
8	A/D CH3	21	A/D CH11
9	A/D CH4	22	A/D CH10
10	A/D CH5	23	A/D CH9
11	A/D CH6	24	A/D CH8
12	A/D CH7	25	0.12 V
13	12 V		

For this application only pins 2, 6, 14 and 16 are used.

2.4.2 Description of performance

The computer (JOINT Personal Computer, IBM compatible) gates the integrator, adjusts the gain, processes the amplified and integrated signals and presents the results.
Six filters passes every revolution. Each filter is read twice according to Fig. 10 - the background once and the actual fluorescence signal once.
The computer gets the trigger signal from the wheel just before every filter. First the program waits for the longer trigger signal which acts as a reference signal and initiates every revolution. Then the program counts down according to a loop with a length specially set to every filter - the trigger signal proved to be difficult to adjust perfectly the same for all filters.
The integration is performed, controlled from the computer. Then the background is read via the ADDA interface card. This card converts the analogue signal to a digital value, processable by the computer. After a small delay the signal is measured in the same way, but during a longer integration period due to the wheel geometry. The difference in integration time is compensated for and the background is subtracted.
All signals every revolution are compared with the largest former value and the largest one is stored. The frequency of the gain adjustment is set to be once for every evaluation of the contrast function average. This frequency is easily changed in the program.
The gain is set to adjust the fluorescence signal amplification to a satisfactory magnitude. As earlier discussed the integrator reaches its maximum first, before the ADDA card, at the digital value of approximately 4035. To have some margin the interval 2500 - 3800 is chosen as a satisfactory magnitude for the output signal from the integrator. If the stored largest signal (big) between two gain adjustment occasions does not fit into this interval the control signal (GAIN) is to be changed, and by that the current

gain factor (g0) is also changed. A fitting new gain (gnew) is calculated as:

$$gnew = (3000 * g0) / big$$

where 3000 is chosen as a target for the fluorescence signal value after the adjusted amplification. Then the control signal to the amplification module (called GAIN) is calculated according to a calibration curve showing the gain against the control signal. See further chapter 2.4.1.

Once every revolution the contrast function value $(A' - D)/B$ is calculated. These values are added together until it is time to evaluate the average. If the denominator is less than or equal to 0 and the numerator is greater than 100 the function value is supposed to be at maximum and the value of 20 is added to the sum. If the denominator is less than or equal to 0 and the numerator is less than or equal to 100 the fluorescence signal is supposed to be diminutive and unreliable and the value of zero is added to the sum.

At a frequency of every tenth revolution the average of the contrast function value is evaluated and stored. This frequency is easily changed in the program. A sound from the loudspeaker in the computer is also set, the larger magnitude of the ratio, the higher pitch . If the current largest signal between two gain adjustment occasions, big, is less than or equal to 2000, the fluorescence signals are supposed to be too weak to be reliable and the sound is interrupted and the "average value" of zero is stored. These limits and the limits set for the denominator and numerator could be subject to some discussion.

The program is interrupted by pressing the STOP button on the box. Then the last 100 average values of the contrast function are listed on the screen and into a file.

2.4.3 The software

The program is written in Turbo Pascal 4.0. A revolution takes 60 ms which means the program has to be fast to manage all calculations and conditional comparisons during the periods between filter reading. The program is written without procedures and functions due to the short available time.
Interrupts automatically set from the computer take time. These interrupts are turned off with a *INLINE* command before starting to read the filters. Also all compiling options available in Turbo Pascal, like automatic range checking, stack checking etc are turned off.
The complete program is listed in Appendix A. First there is an initiation section. Then the first three filters are read. Between the third and the fourth filter there is a longer time available due to the white light window on the wheel. Here the gain adjustment is placed.
The contrast function value is calculated and added together at the end of every revolution. The rest of the function processing is made between the third and the fourth filters the revolutions just after those where the gain adjustment is made. There is not time both to adjust the gain and to process the contrast function the same revolution. The filter reading loop in the program is interrupted by pressing the STOP button on the box. This is a logical signal which goes via a printer port to the computer according to chapter 2.3.1.
At the end of the program the program turns the automatic computer interrupts on again with an *INLINE* command. Then it lists the last 100 average values of the contrast function on the screen and into a file. If the file is to be saved it is necessary to change the name of the file in the program, otherwise it will be overwritten the next run.

3 RESULTS

3.1 The amplifier and integrator processing

The linearity of the fluorescence signal amplifier has been tested with splendid results, see Fig. 15 a and b. They show the output signal, OUT, from the integrator, against the input signal (PMT). The input signal is this time simulated by the computer via the D/A output, the x-axis showing the corresponding digital value. Fig. 15 a is plotted when a low constant gain is set, that is the higher control signal value of the two (according to chapter 2.2.3). Fig. 15 b corresponds to a higher constant gain. As earlier mentioned, the voltage range of the ADDA card in the computer is set just to manage the maximum value from the integrator card. Fig. 15 b shows that the integrator reaches maximum approximately at the digital value 4035. Fig. 16 a and b shows the output signal (OUT) against the control signal to the amplifier (GAIN), Fig. 16 a and b corresponding to a weak and a strong fluorescence signal respectively. This is not a linear relation, but as only the ratio between different fluorescence signals is studied and not the absolute values, this is not an important inconvenience. The main thing is that the output signal varies continuously with the control signal. As before, Fig. 16 b asserts the reached maximum value of the integrator.
The gain, that is the output signal divided with the input signal, compensated for the fact that the zero point is at the digital value of 2048 (see chapter 2.4.1), is plotted against the control signal in Fig. 17 a and b. Fig. 17 a and b correspond to a low and a high constant fluorescence signal, respectively. The interesting point is to compare these curves to confirm that, for every control signal, high signals encounter the same gain factor as low signals. The x-axis ranges for both diagrams differ as a consequence of the integrator reaching maximum. At the left edge of the curves the output signals have reached roughly 4035 and the curves flatten out. Fig. 17 c shows the approximate accordance between the two former curves at the interval where the x-axes

coincide. This agrees with the earlier proved linearity of the amplifier.
Fig. 17 a and b act as calibration curves for the gain setting. An extrapolation is made in Fig. 17 a for this purpose. According to the curves the control signal (called contr in the program) is set in the program as follows:

```
if gnew > 187 then contr = 2700
else if gnew > 170 then contr = 2725
else if gnew > 140 then contr = 2750
else if gnew > 110 then contr = 2775
else if gnew >  88 then contr = 2800
else if gnew >  55 then contr = 2850
else if gnew >  35 then contr = 2900
else if gnew >  24 then contr = 2950
else if gnew >  18 then contr = 3000
else if gnew >  14 then contr = 3050
else if gnew >  12 then contr = 3100
else if gnew >  10 then contr = 3150
else if gnew >   8 then contr = 3200
else if gnew >   7 then contr = 3250
else if gnew >   6 then contr = 3350
else if gnew >   5 then contr = 3450
else if gnew >   4 then contr = 3650
else contr = 3850
```

The range 2700 - 3850 for the control signal is chosen with consideration to these curves. The simulated signals are here chosen to be at the magnitude of the weakest and the strongest fluorescence signals in a test sample of a crude oil. The tumour fluorescence signals seems to be somewhat weaker which suggests an extension downwards for the control signal range and by that a possibility to set a higher gain factor.

3.2 Detection of malignant tumours

A study of a rat muscle with a cancer tumour, the tumour capsule and the surrounding muscle exposed by removing the hairy skin of the leg, has been done with very good results. To evaluate 100 contrast function average values, stored with a frequency of every tenth revolution, it takes 60 s. 60 seconds scans have been made over different parts of the tumour in Fig. 18 a and b. Discrete movements of the optical fibre tips have been performed with approximately 10 s delays in Fig 18 c. The contrast function illustrates the significant differences between tumour and muscle, and also the tumour edges show a difference in contrast function value compared to muscle and tumour.

3.3 Discussion

This third version of the non-imaging point-monitoring fluorescence system in Lund, using the experience from former systems, shows considerably enhanced performance. The dimensionless contrast function in combination with differential excitation and detection should make it possible to detect early tumours with a low dose of HPD injected.

To find the optimized contrast function, a menu-based extension of the computer program is planned, which will make it possible to choose between all different combinations of the six signals. At the moment the discussed contrast function value is evaluated simply by adding together the fluorescence signal contributions from the two different excitation wavelengths.

It is asserted that the mercury lamp gives enough light, and the choice of using a common personal computer for the processing proved to be successful. This facilitates a wide-spread use of this system as the prize should be reasonable.

A further improvement visualized is a presentation of the results as a curve plotted directly on the screen or into a file. This will simplify the understanding of the results.

Of course the equipment has to be tested further to determine if it performs satisfactory also on a tumour that is not as well prepared as the one tested upon. The possibility of using this equipment for other, for example industrial, applications, also has to be analyzed.

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REFERENCES

[1] J.R. Lakowicz, "Principles of Fluorescence Spectroscopy", Plenum Press, New York (1983).

[2] G.M. Barenboim, A.N. Domanskii, K.K. Turoverov, "Luminescence of Biopolymers and Cells", Plenum Press, New York (1969).

[3] S. Udenfriend, "Fluorescence Assay in Biology and Medicine", Vol. I and Vol. II, Academic Press, New York (1962 and 1969).

[4] S. Andersson-Engels, J. Ankerst, A. Brun, Å. Elner, A. Gustafson, J. Johansson, S.-E. Karlsson, D. Killander, E. Kjellén, E. Lindstedt, S. Montán, L.G. Salford, B. Simonsson, U. Stenram, L.-G. Strömblad, K. Svanberg, and S. Svanberg, "Tissue Diagnostics using Laser-Induced Fluorescence", Ber. Bunsenges. Phys. Chem. **93**, pp. 335-342 (1989).

[5] S. Andersson-Engels, A. Gustavson, J. Johansson, U. Stenram, K. Svanberg, S. Svanberg, "Laser-Induced Fluorescence used in Localizing Atherosclerotic Lesions" (1988). To appear in Lasers in Med. Sci.

[6] R.L. Lipson, "The photodynamic and fluorescent properties of a particular hematoporphyrin derivative and its use in tumor detection", Master's Thesis, University of Minnesota (1960).

[7] R.L. Lipson, E.J. Baldes, A.M. Olsen, "The use of a derivative of hematoporphyrin in tumor detection", J. Nat. Cancer Inst. **26** (1961).

[8] S. Andersson-Engels, J. Johansson, D. Killander, E. Kjellén, M. Olivo, L.O. Svaasand, K. Svanberg, S. Svanberg, "Photodynamic Therapy alone or in Conjunction with Near-Infrared Light-Induced Hyperthermia in Human Malignant Tumors. A Methodological Case Study." SPIE **908**, pp. 116-125 (1988).

[9] A.E. Profio, "Review of fluorescence diagnosis using porphyrins", SPIE **907**, pp. 150-156 (1988).

[10] J.H. Kinsey, D.A. Cortese, "Endoscopic system for simultaneous visual examination and electronic detection of fluorescence", Rev. Sci. Instrum. **51** (10), pp. 1403-1406 (1980).

[11] A.E. Profio, D.R. Doiron, J. Sarnaik, "Fluorometer for endoscopic diagnosis of tumors", Med. Phys. **11** (4), pp. 516-520 (1984).

[12] K. Aizawa, H. Kato, et al., "A new diagnostic system for malignant tumors using hematoporphyrin derivative, laser photoradiation and a spectroscope", Porphyrin Localization and Treatment of Tumors (D.R. Doiron and C.J. Gomer, Eds.), Alan R. Liss, New York, pp. 227-238 (1984).

[13] R. Baumgartner, H, Fisslinger, et al., "A fluorescence imaging device for encoscopic detection of early stage cancer - instrumental and experimental studies", Photochem. and Photobiol. **46** (5), pp. 759-764 (1987).

[14] P.S. Andersson, S. Montán, T. Persson, S. Svanberg, S. Tapper, "Fluorescence endoscopy instrumentation for improved tissue characterization", Med. Phys. **14** (4), pp. 633- 636 (1987).

[15] C. Arfwidsson, "A Computer-Controlled Fluorescence-Monitoring System", Diploma Paper, Lund Reports on Atomic Physics LRAP-79 (1987).

[16] P.S. Andersson, E. Kjellén, S. Montan, K. Svanberg, S. Svanberg, Lasers in Med. Sci. **2**, p.41 (1987).

[17] D.R. Doiron, E. Profido, R.G. Vincent, T.J. Dougherty "Fluorescence Bronchoscopy for Detection of Lung Cancer", Chest **76**, p. 27 (1979).

[18] J.H. Kinsey, D.A. Cortese, H.L. Moses, R.J. Ryan, E.L. Branum, "Photodynamic Effect of Hematoporphyrin Derivative as a Function of Optical Spectrum and Incident Energy Density", Cancer Res. **41**, p. 5020 (1981).

[19] S. Andersson-Engels, J. Johansson, D. Killander, E. Kjellén, M. Olivo, L.O. Svaasand, K. Svanberg, S. Svanberg, L.I.A. ICALEO **60**, p. 67 (1987).

FIGURE CAPTIONS

Fig. 1: Schematic diagram of energy transitions in large molecules[16].

Fig. 2: Phosphorescence light generation[15].

Fig. 3: Illustration of the Franck-Condon principle: The interatomic distance does not change during a light emission or absorption.

Fig. 4 a and b: Schematic structure of porphyrin and Hematoporphyrin, respectively.

Fig. 5 a: Absorption spectrum of HPD[17].

Fig. 5 b: Emission spectrum of HPD[18].

Fig. 6: The molecular basis for photodynamic therapy[4].

Fig. 7: Laser-induced fluorescence spectra from an induced cancer tumour and the surrounding muscle[14].

Fig. 8: Fluorescence data obtained for a human epidermal tumour using the OMA system developed in Lund[19].

Fig. 9: Principal scheme of the present fluorescence-monitoring system.

Fig. 10: Schematic of the chopper wheel.

Fig. 11: A fluorescence signal and the trigger signal from the wheel displayed on an oscilloscope.

Fig. 12: A display of the front of the box.

Fig. 13: Schematic of the small amplification module for the trigger signal from the wheel.

Fig. 14: Schematic of the fluorescence amplification module.

Fig. 15 a, b: Diagrams showing the linearity of the fluorescence signal amplifier. The A/D converted output signal from the integrator (OUT) is plotted against the simulated PMT signal. Fig. a and b correspond to a low and a high constant gain, respectively.

Fig. 16 a, b: Diagrams of the output signal (OUT) against the control signal (GAIN) to the amplifier, Fig. a and b corresponding to a weak and a strong fluorescence signal, respectively.

Fig. 17 a, b: Diagrams of the gain (the output signal divided with the input signal, compensated for the fact that the zero point is at the digital value of 2048) against the control signal (GAIN). Fig. a and b correspond to a weak and a strong fluorescence signal, respectively.

Fig. 17 c: A comparison of Fig. 18 a and b.

Fig. 18 a, b, c: The successful contrast function data obtained from a rat tumour using the present system.

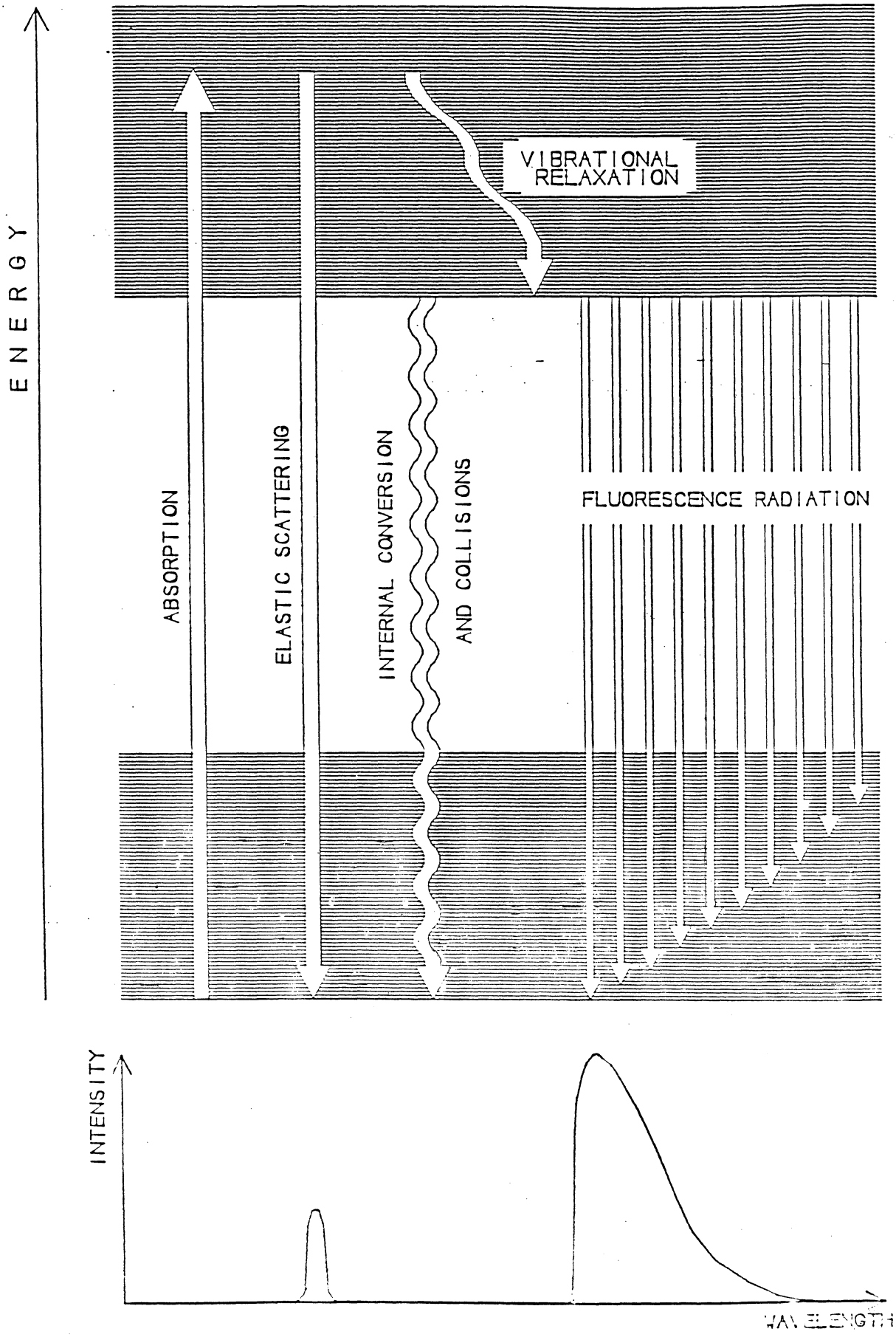

ENERGY
VIBRATIONAL RELAXATION
ABSORPTION
ELASTIC SCATTERING
INTERNAL CONVERSION
AND COLLISIONS
FLUORESCENCE RADIATION
INTENSITY
WAVELENGTH

Fig. 1

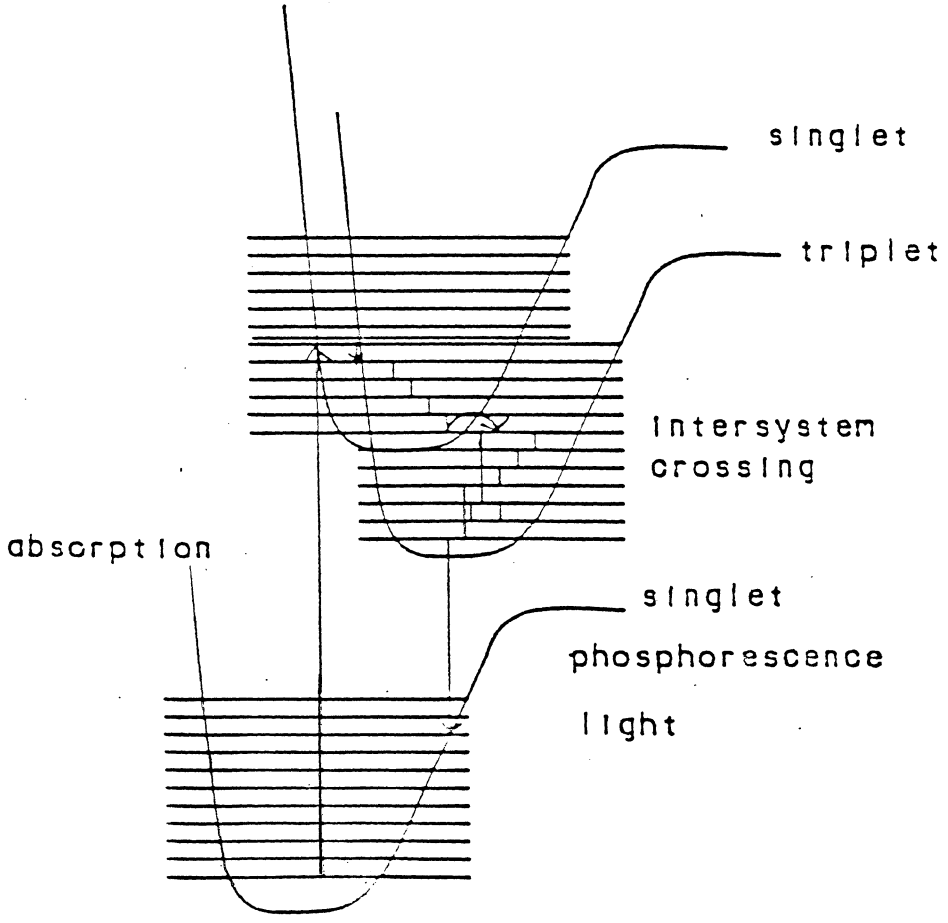

singlet
triplet
intersystem
crossing
absorption
singlet
phosphorescence
light

Fig. 2

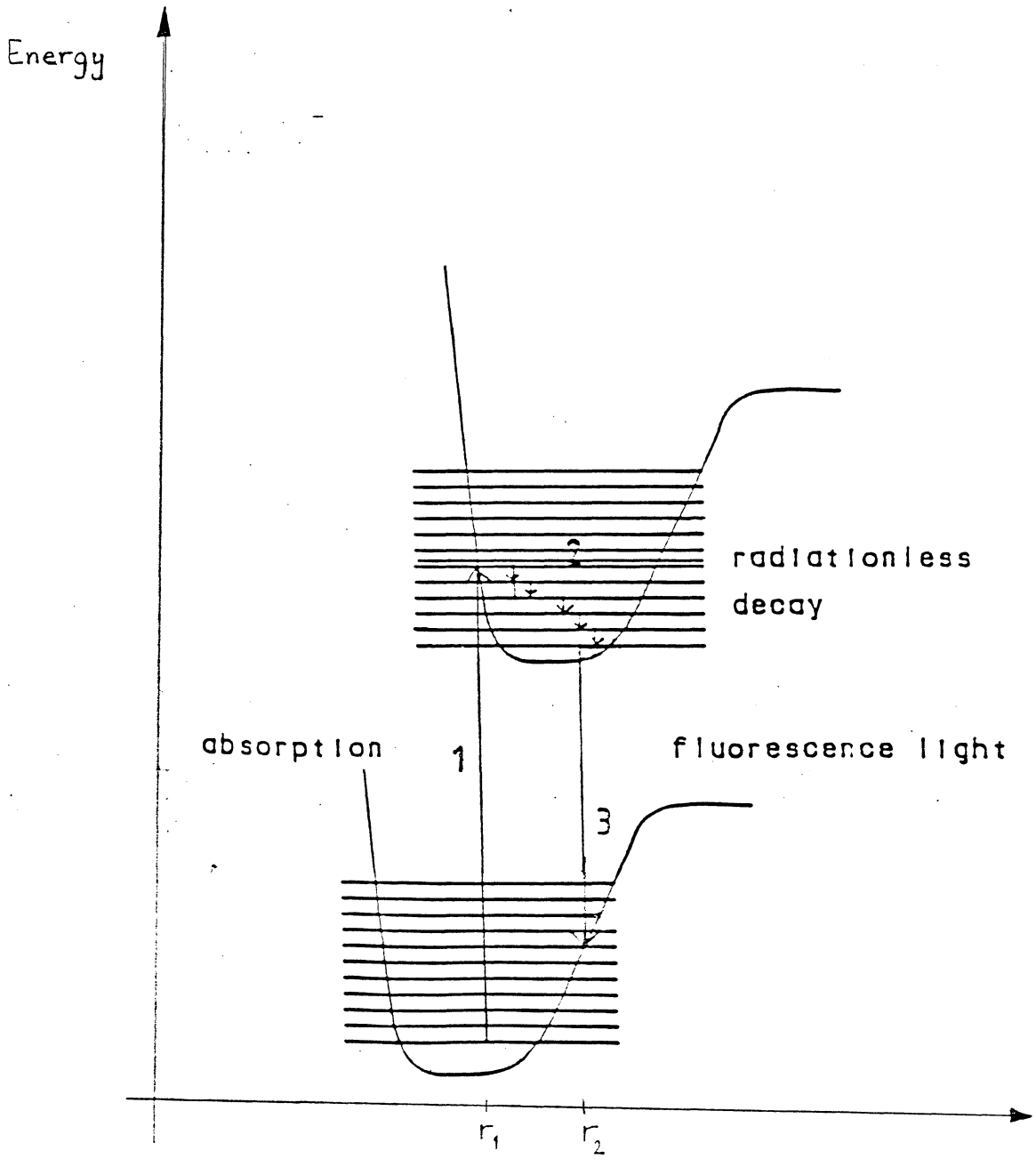

Energy
radiationless
decay
absorption
1
2
3
fluorescence light
r_1
r_2

Fig. 3

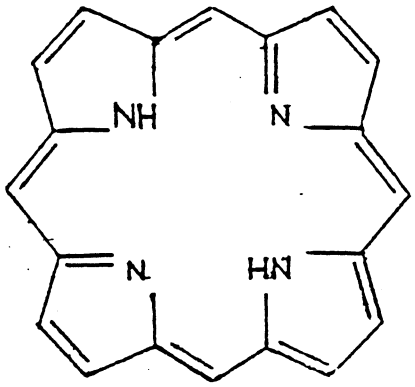

NH
N
N
HN

Fig. 4 a

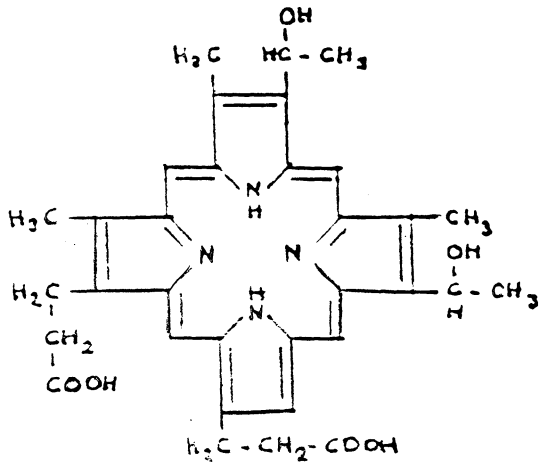

OH
H2C
HC - CH3
N
H
H3C
CH3
N
N
OH
H2C
C - CH3
H
H
N
CH2
COOH
H2C - CH2 - COOH

Fig. 4 b

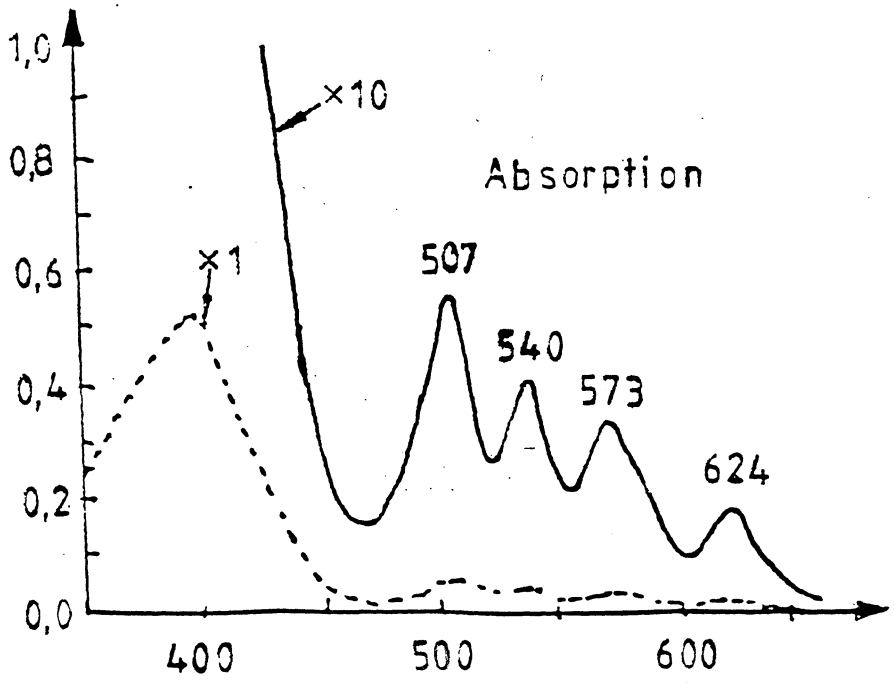

1,0
0,8
0,6
0,4
0,2
0,0
×10
×1
Absorption
507
540
573
624
400
500
600

Fig. 5 a

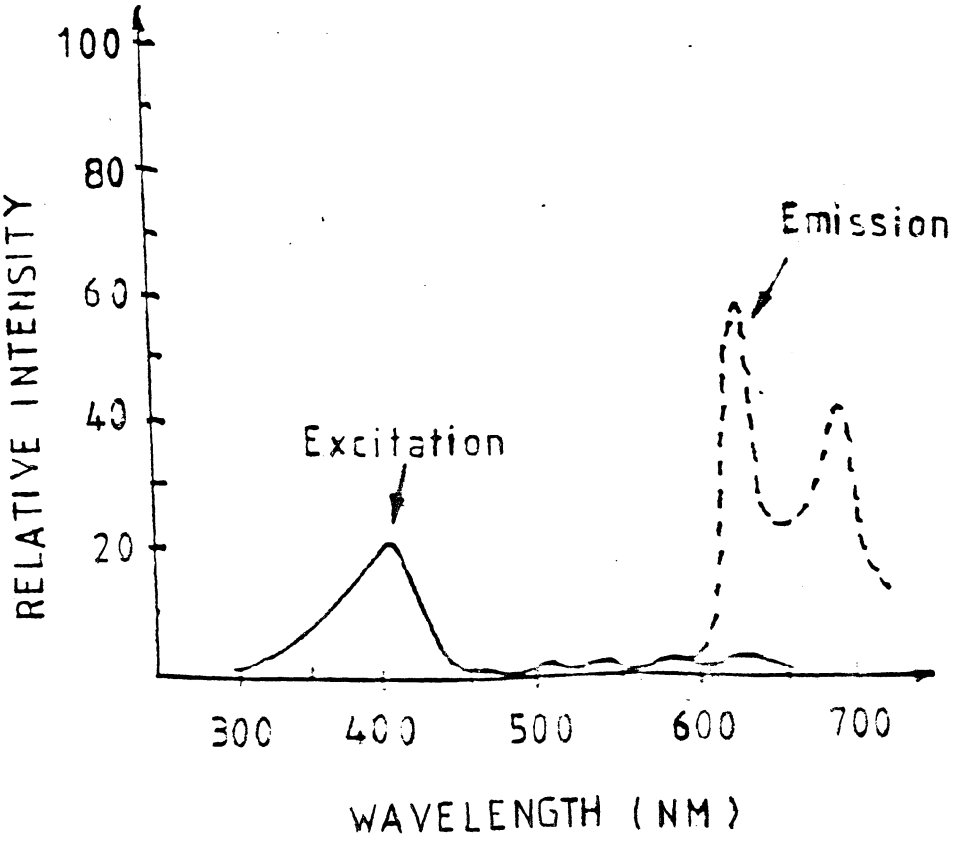

100
80
60
40
20
RELATIVE INTENSITY
Emission
Excitation
300
400
500
600
700
WAVELENGTH (NM)

Fig. 5 b

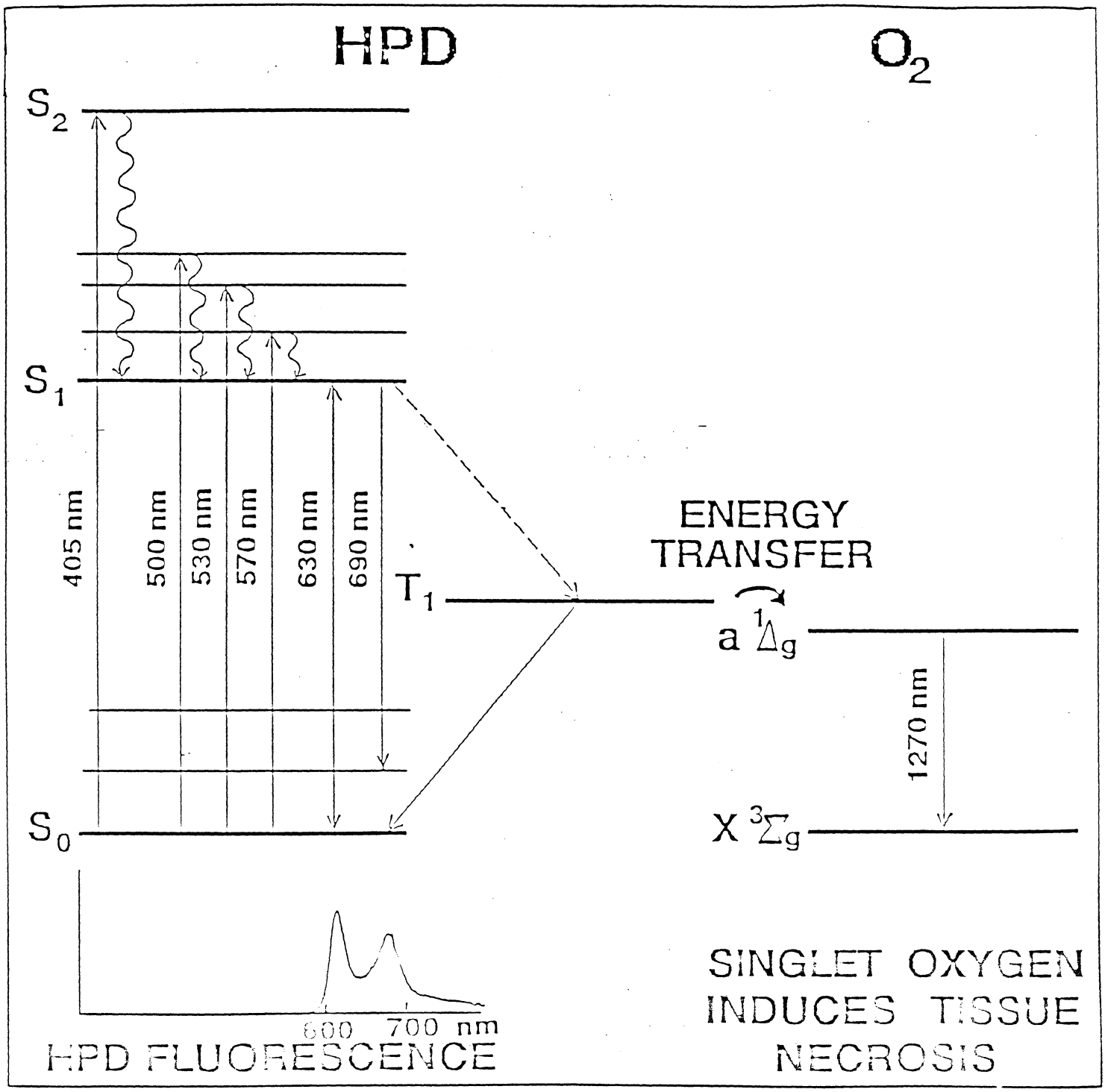

HPD
O_2
S_2
S_1
S_0
405 nm
500 nm
530 nm
570 nm
630 nm
690 nm
T_1
ENERGY TRANSFER
$a\ ^1\Delta_g$
1270 nm
$X\ ^3\Sigma_g$
600
700 nm
HPD FLUORESCENCE
SINGLET OXYGEN INDUCES TISSUE NECROSIS

Fig. 6

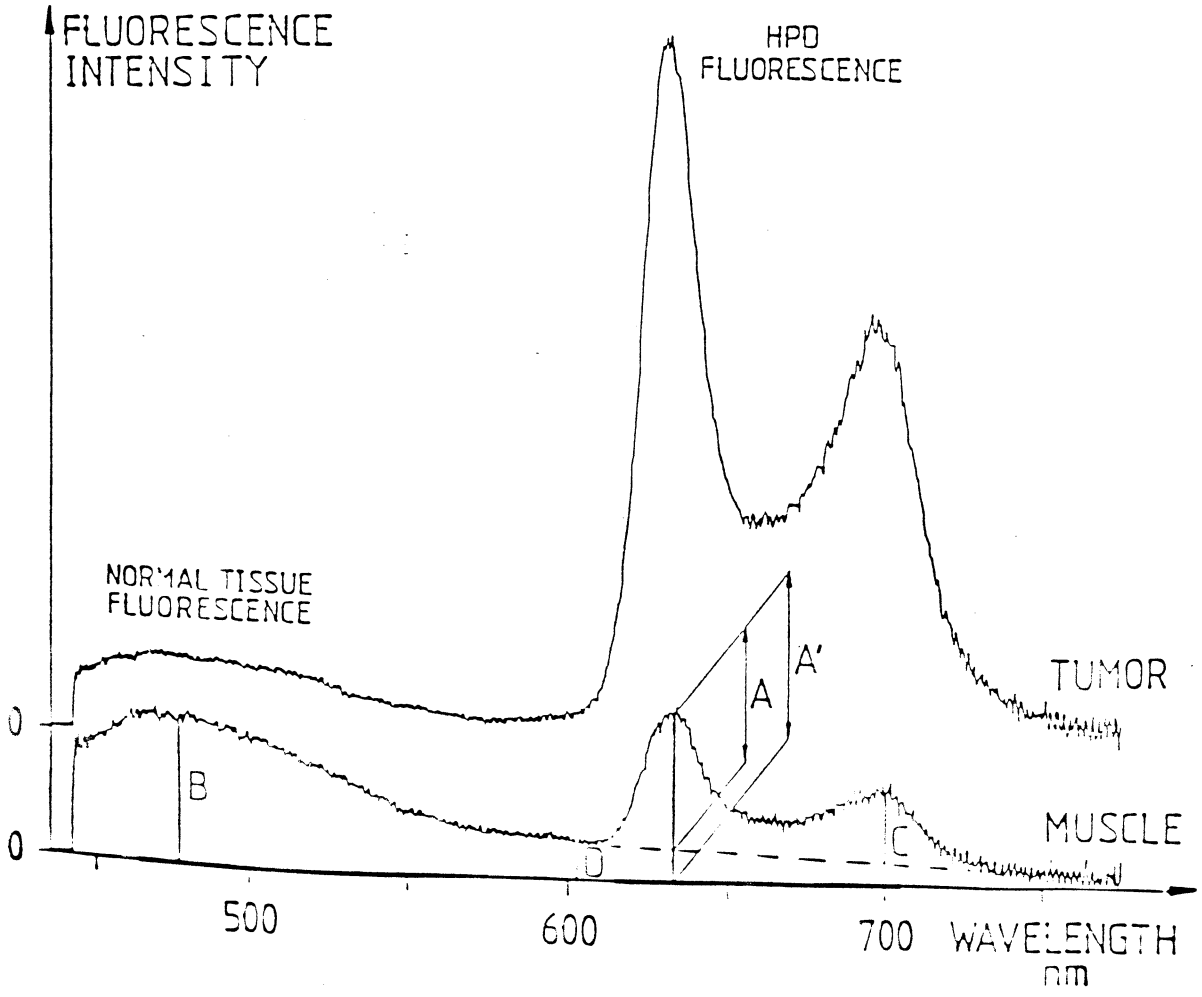

FLUORESCENCE
INTENSITY
HPD
FLUORESCENCE
NORMAL TISSUE
FLUORESCENCE
A'
A
TUMOR
B
MUSCLE
C
0
0
500
600
700
WAVELENGTH
nm

Fig. 7

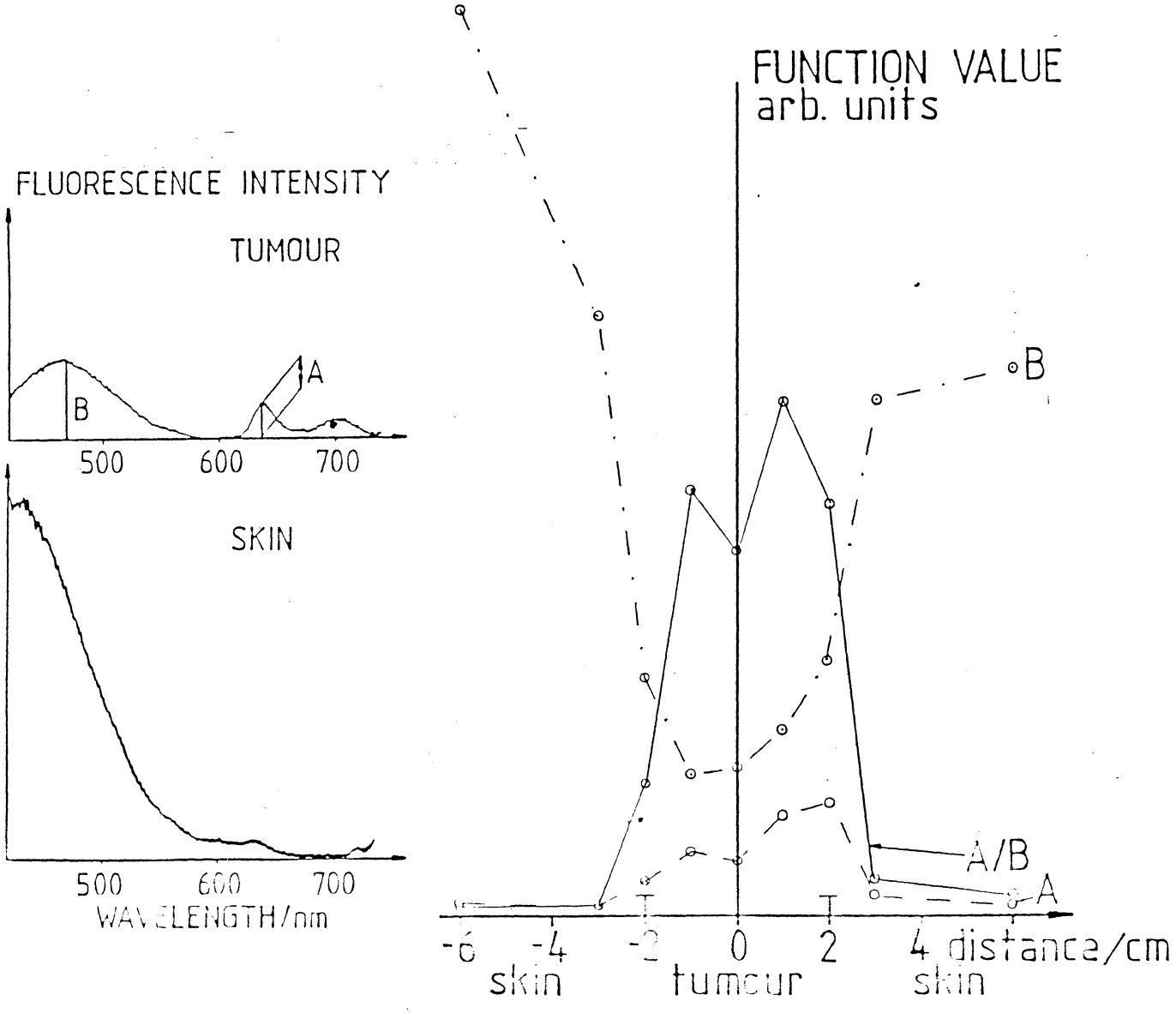

FLUORESCENCE INTENSITY
TUMOUR
A
B
500
600
700
SKIN
500
600
700
WAVELENGTH/nm
FUNCTION VALUE
arb. units
B
A/B
A
-6
-4
-2
0
2
4 distance/cm
skin
tumour
skin

Fig. 8

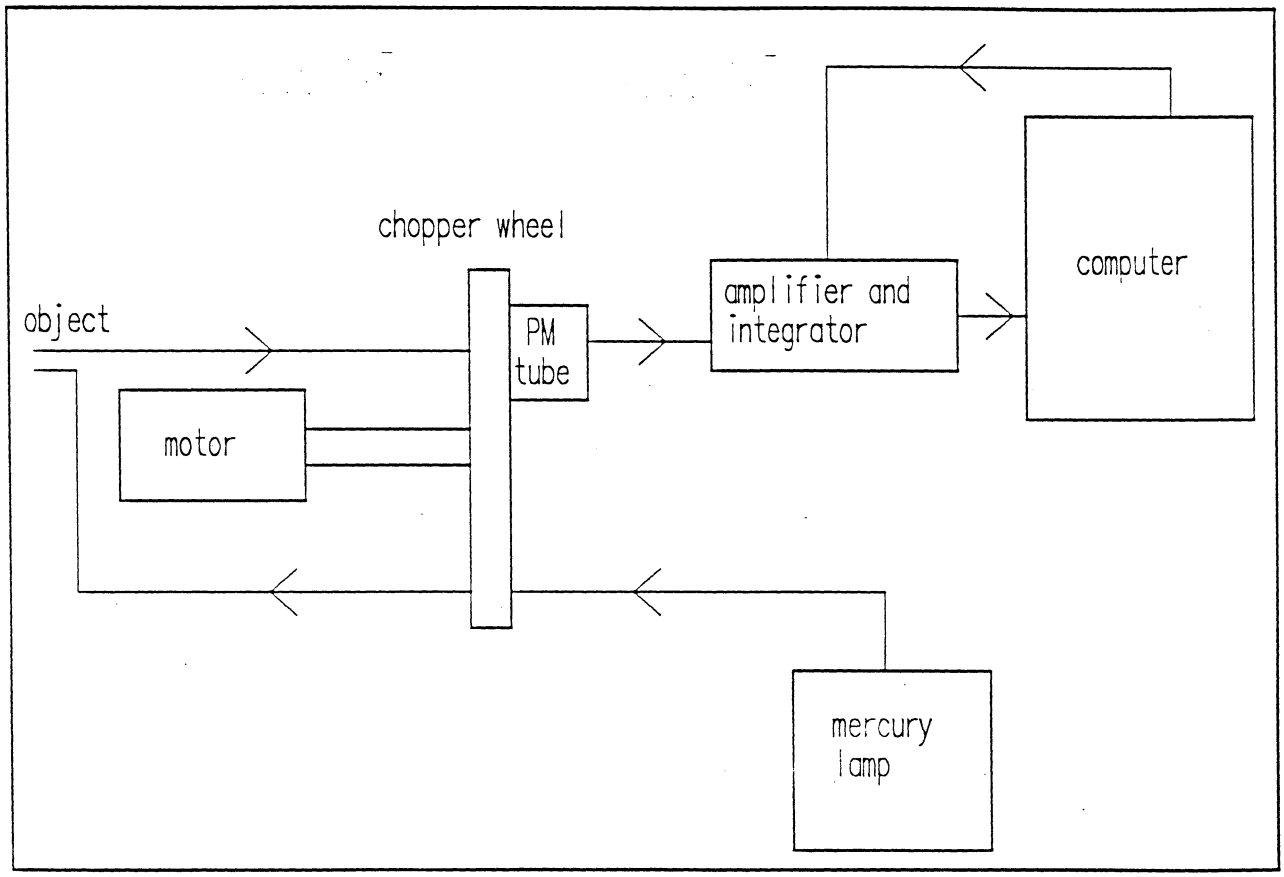

chopper wheel
object
PM
tube
amplifier and
integrator
computer
motor
mercury
lamp

Fig. 9

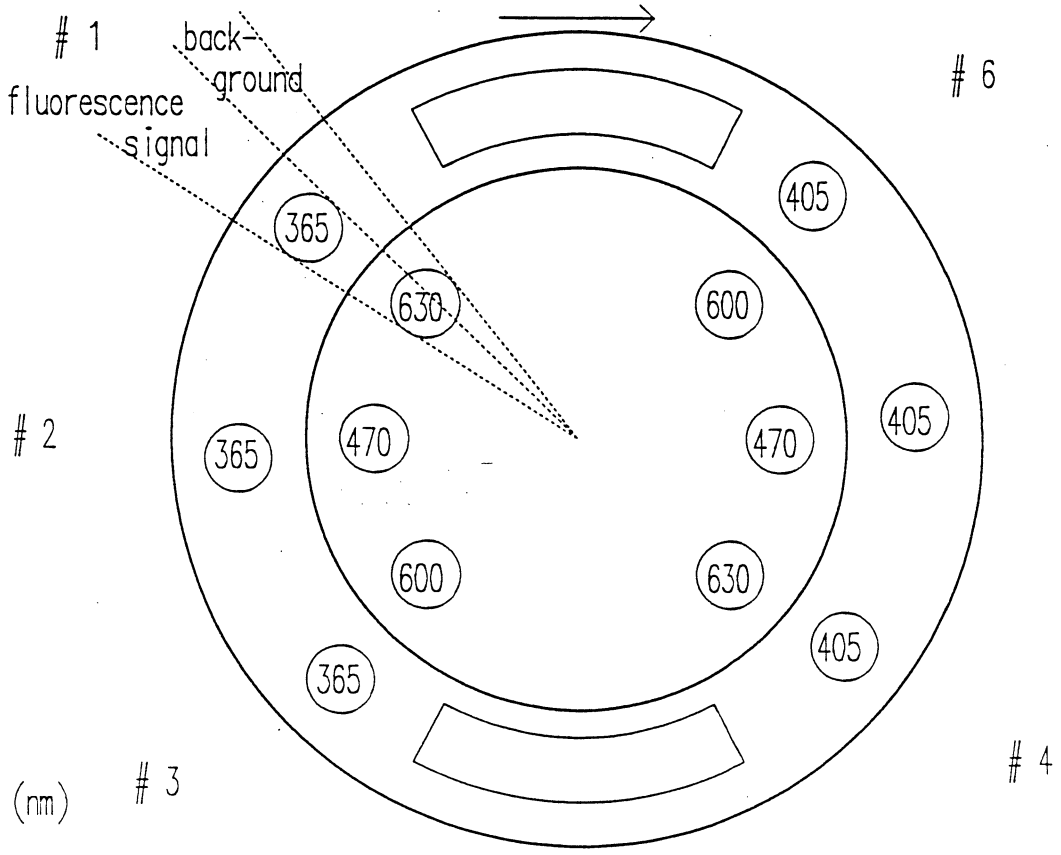

1
back-
ground
fluorescence
signal
6
405
365
630
600
2
365
470
470
405
5
600
630
405
365
(nm)
3
4

Fig. 10

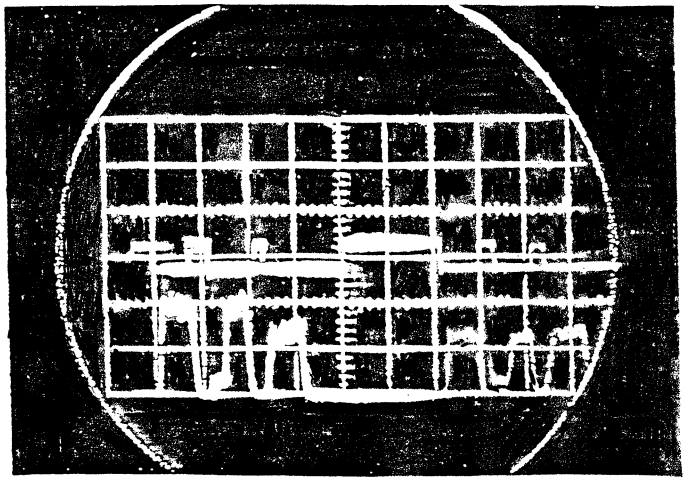


Fig. 11

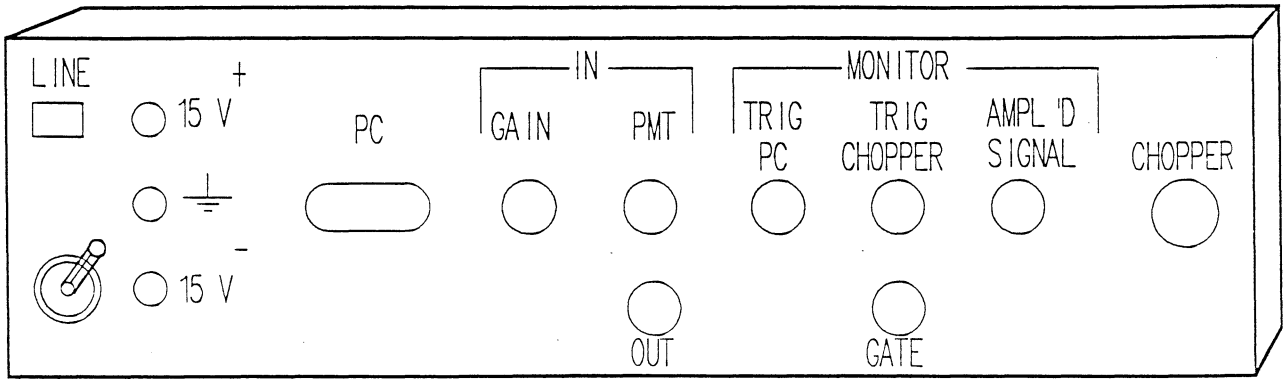

LINE
+
15 V
PC
IN
GAIN
PMT
MONITOR
TRIG
PC
TRIG
CHOPPER
AMPL 'D
SIGNAL
CHOPPER
15 V
-
OUT
GATE

Fig. 12

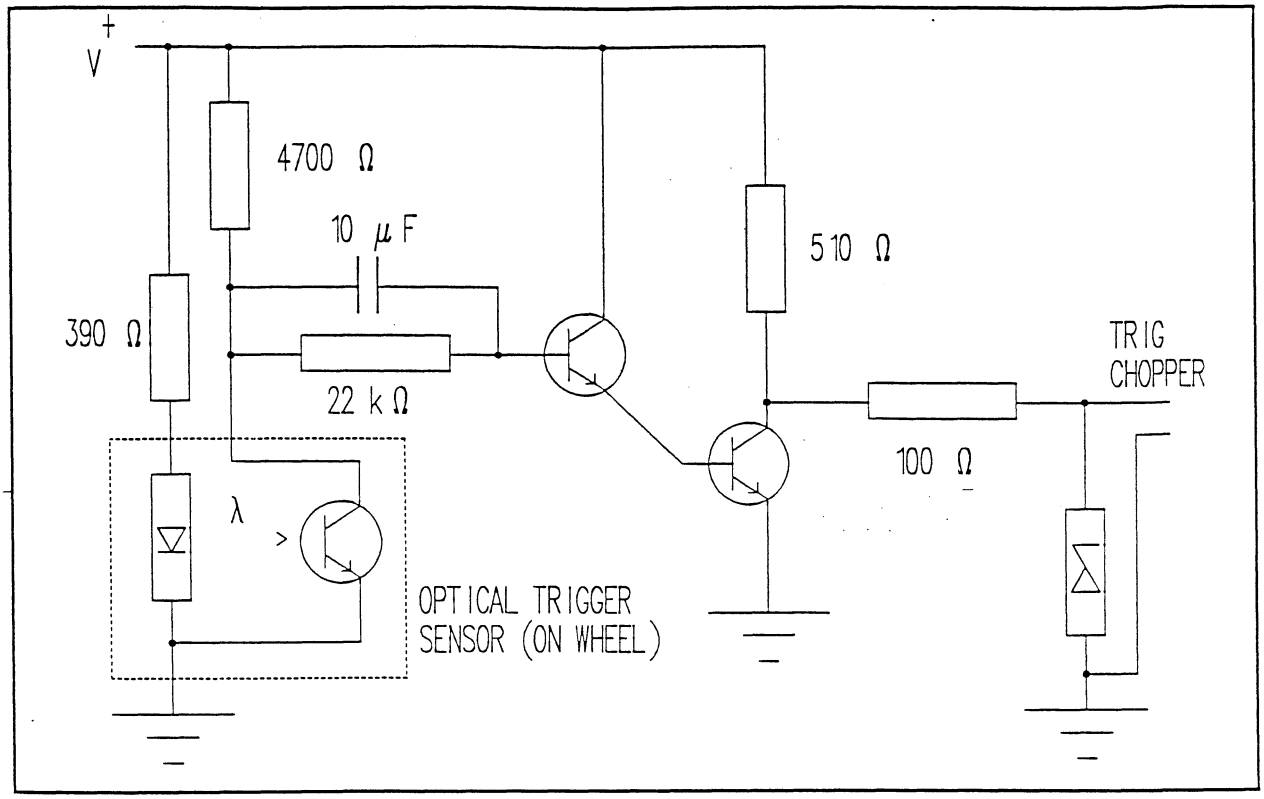

+
V
4700 Ω
10 μF
390 Ω
22 k Ω
510 Ω
100 Ω
TRIG
CHOPPER
λ
OPTICAL TRIGGER
SENSOR (ON WHEEL)

Fig. 13

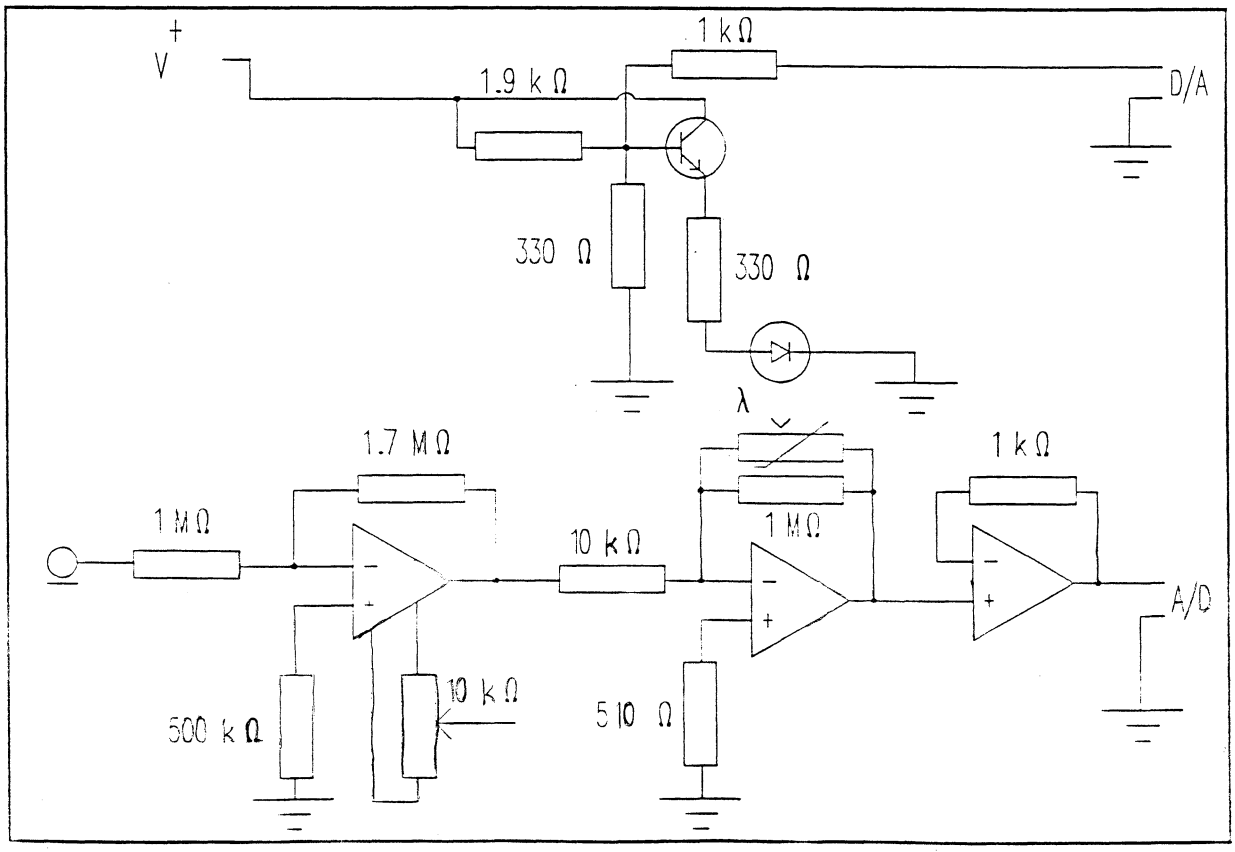

+
V
1 k Ω
1.9 k Ω
D/A
330 Ω
330 Ω
λ
1.7 M Ω
1 k Ω
1 M Ω
10 k Ω
1 M Ω
A/D
10 k Ω
500 k Ω
510 Ω

Fig. 14

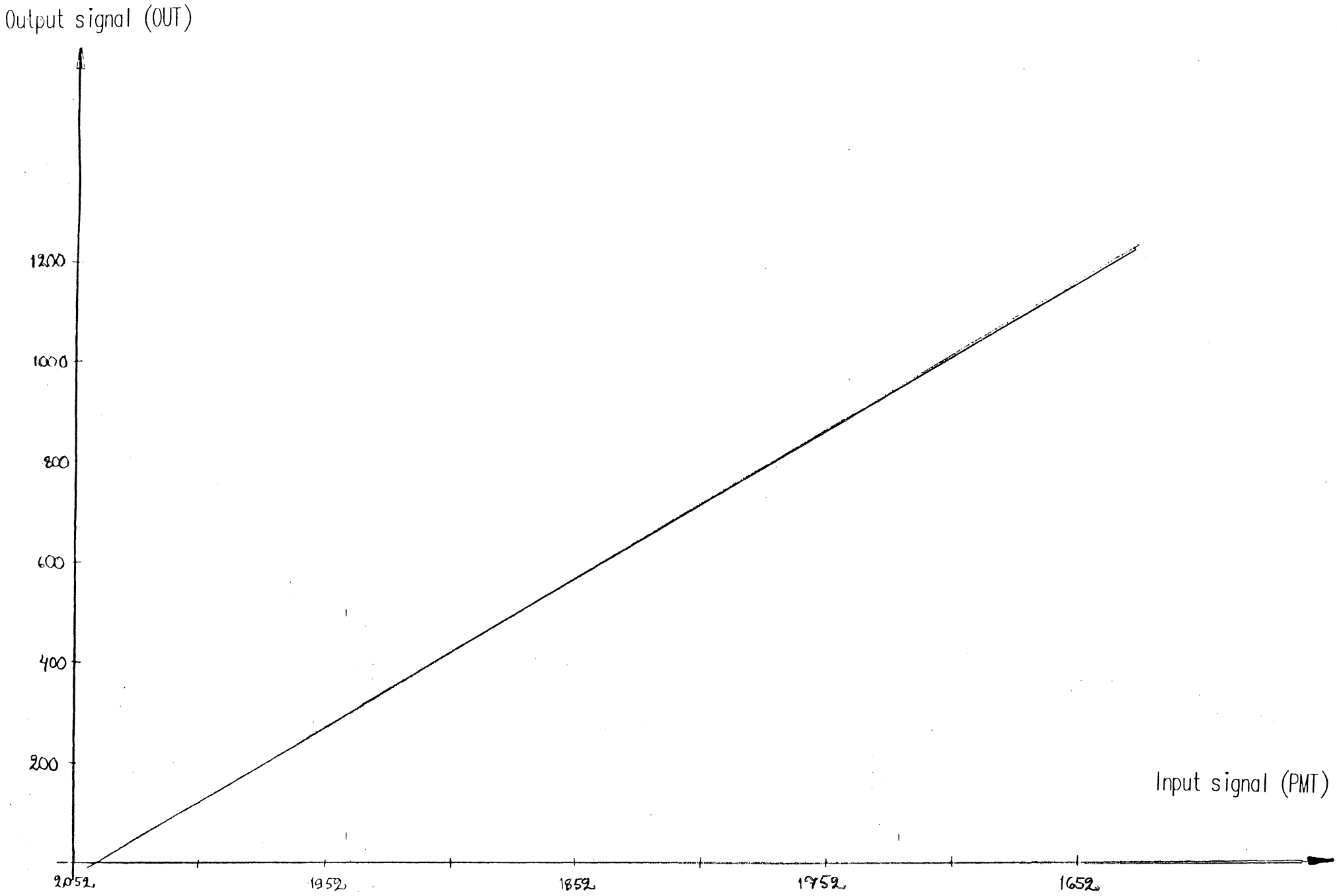

Output signal (OUT)
1200
1000
800
600
400
200
2052
1952
1852
1752
1652
Input signal (PMT)

Fig. 15 a

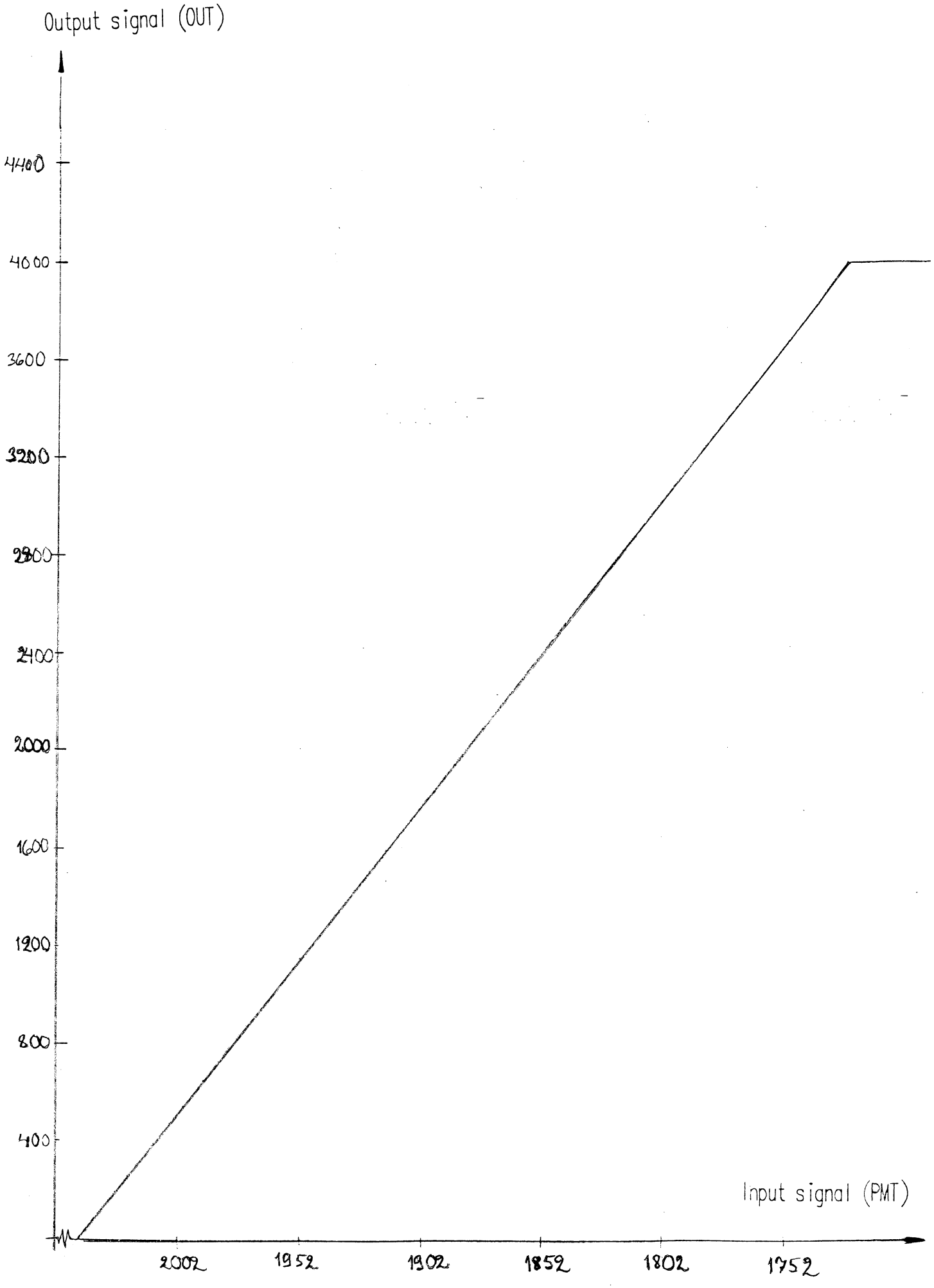

Output signal (OUT)
4400
4000
3600
3200
2800
2400
2000
1600
1200
800
400
Input signal (PMT)
2002
1952
1902
1852
1802
1752

Fig. 15 b

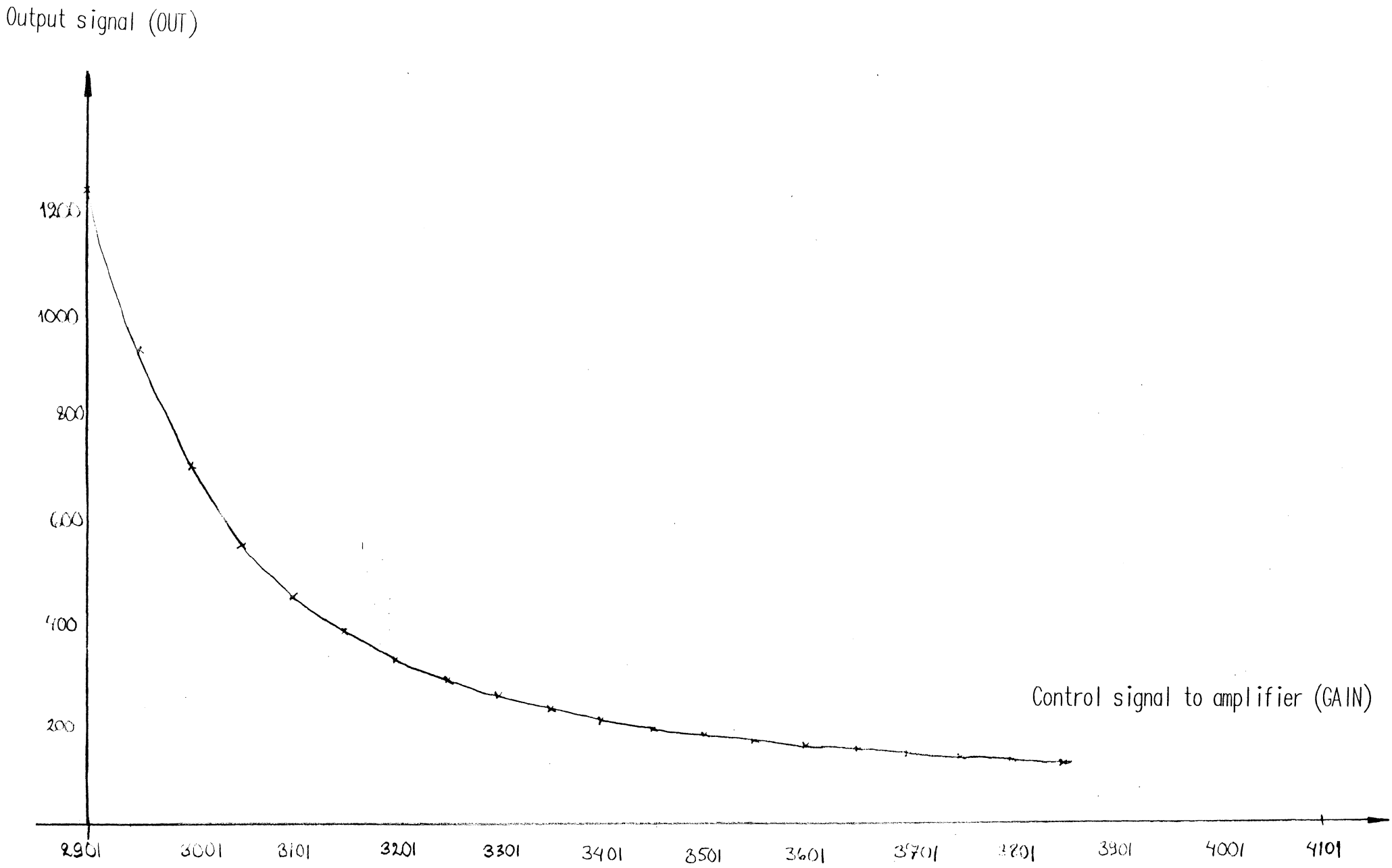

Output signal (OUT)
1200
1000
800
600
400
200
2901
3001
3101
3201
3301
3401
3501
3601
3701
3801
3901
4001
4101
Control signal to amplifier (GAIN)

Fig. 16 a

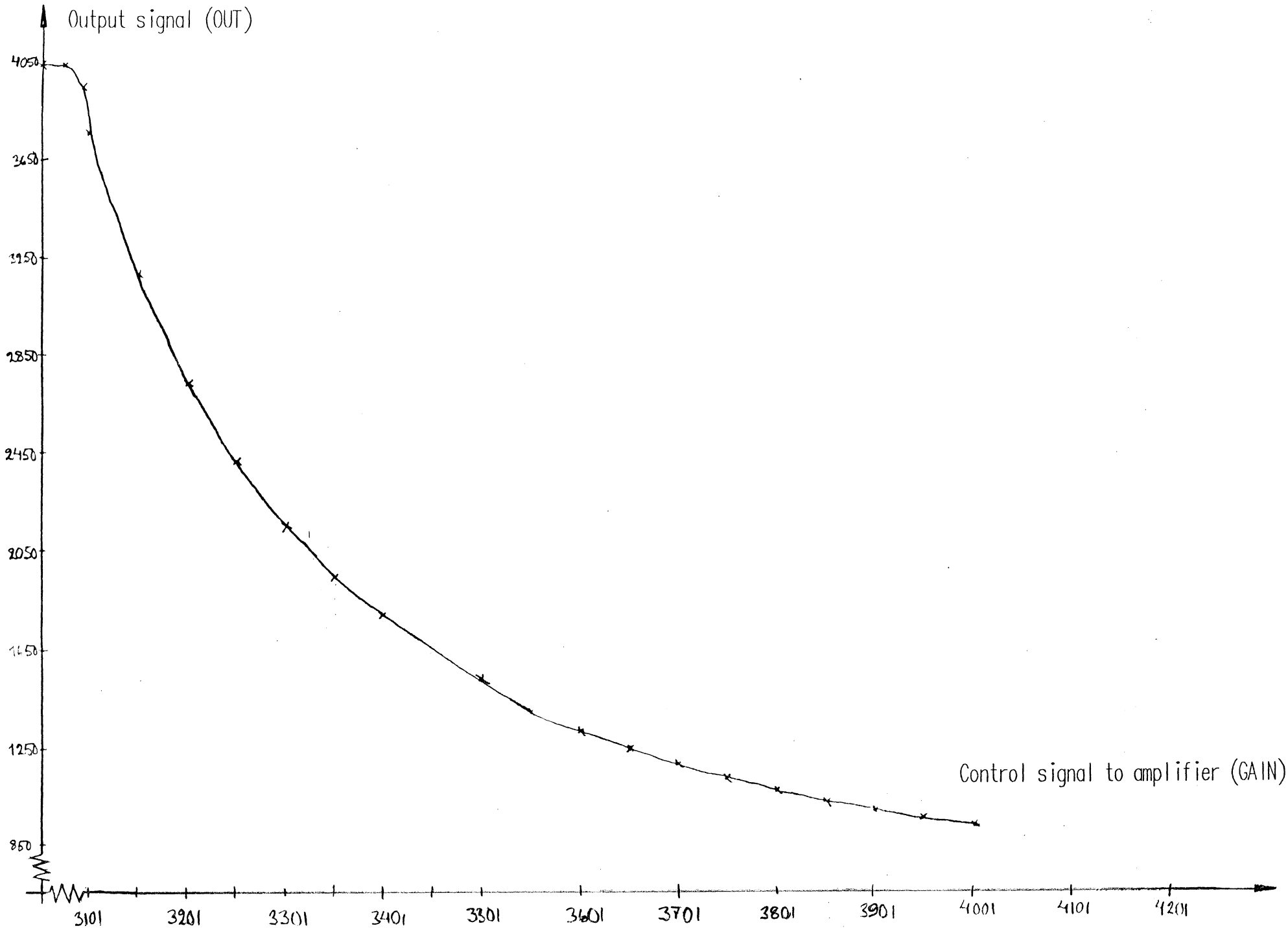

Output signal (OUT)
4050
3650
3250
2850
2450
2050
1650
1250
850
3101
3201
3301
3401
3501
3601
3701
3801
3901
4001
4101
4201
Control signal to amplifier (GAIN)

Fig. 16 b

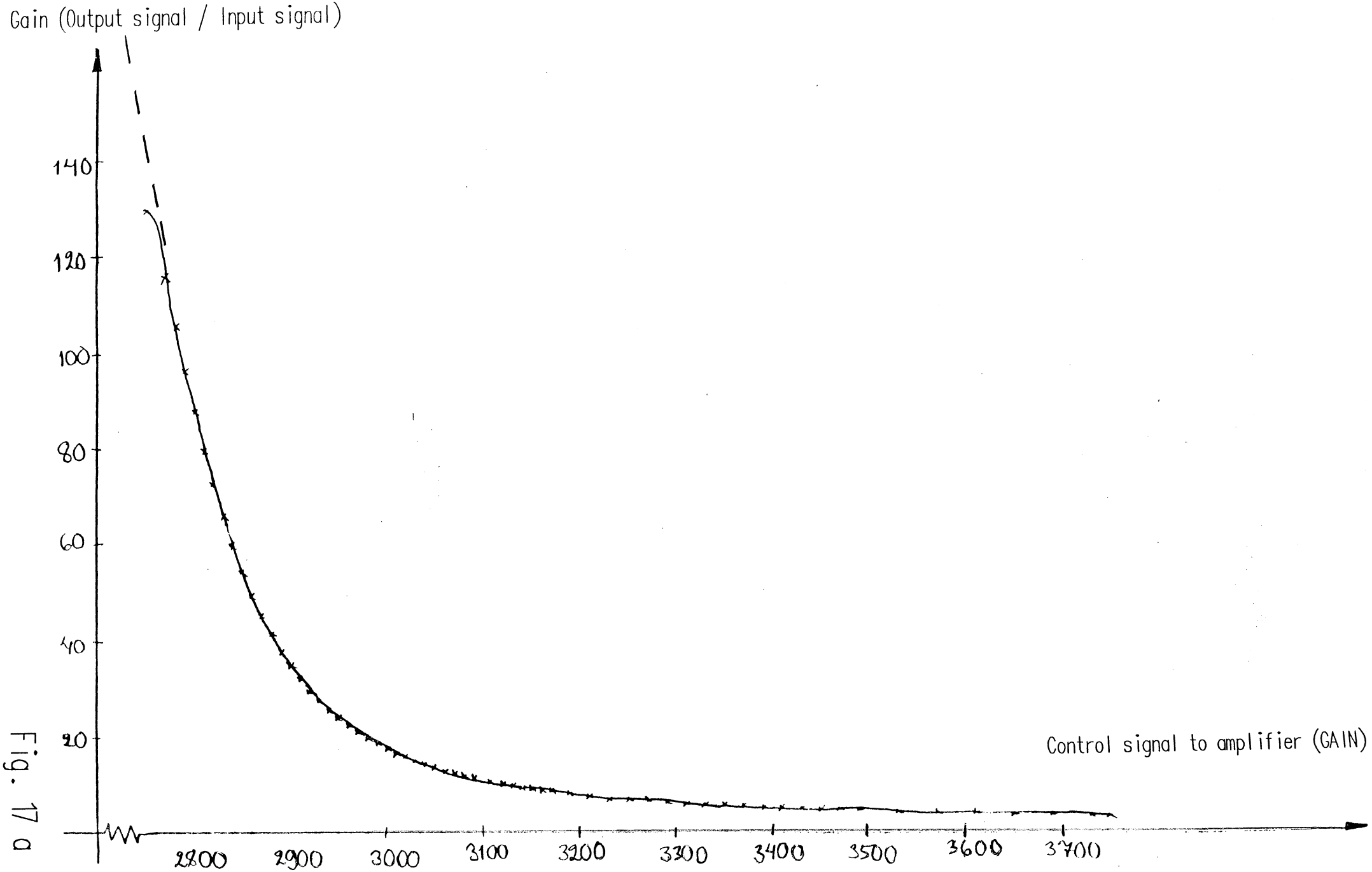

Gain (Output signal / Input signal)
140
120
100
80
60
40
20
2800
2900
3000
3100
3200
3300
3400
3500
3600
3700
Control signal to amplifier (GAIN)

Fig. 17 a

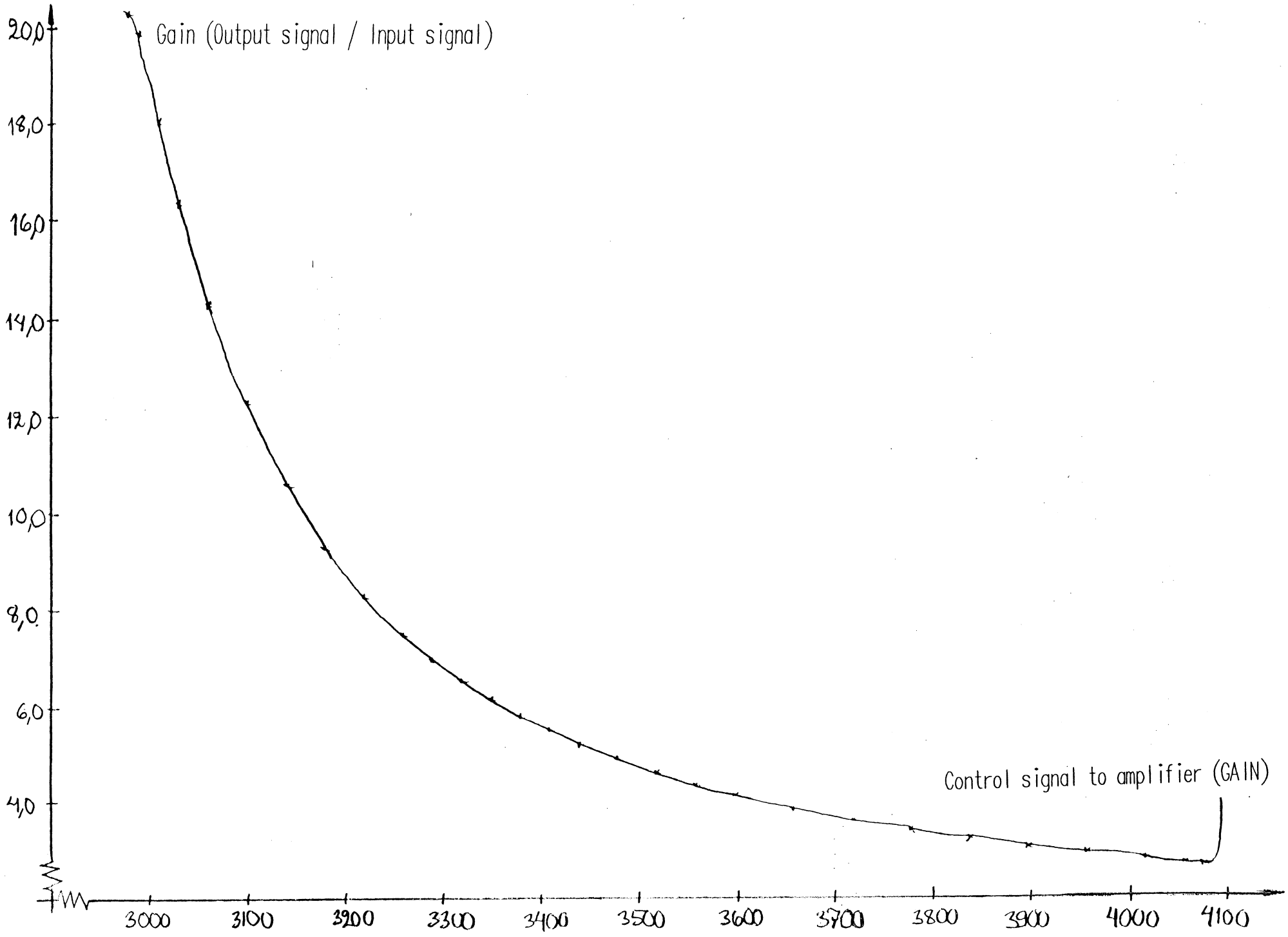

Gain (Output signal / Input signal)
20,0
18,0
16,0
14,0
12,0
10,0
8,0
6,0
4,0
3000
3100
3200
3300
3400
3500
3600
3700
3800
3900
4000
4100
Control signal to amplifier (GAIN)

Fig. 17 b

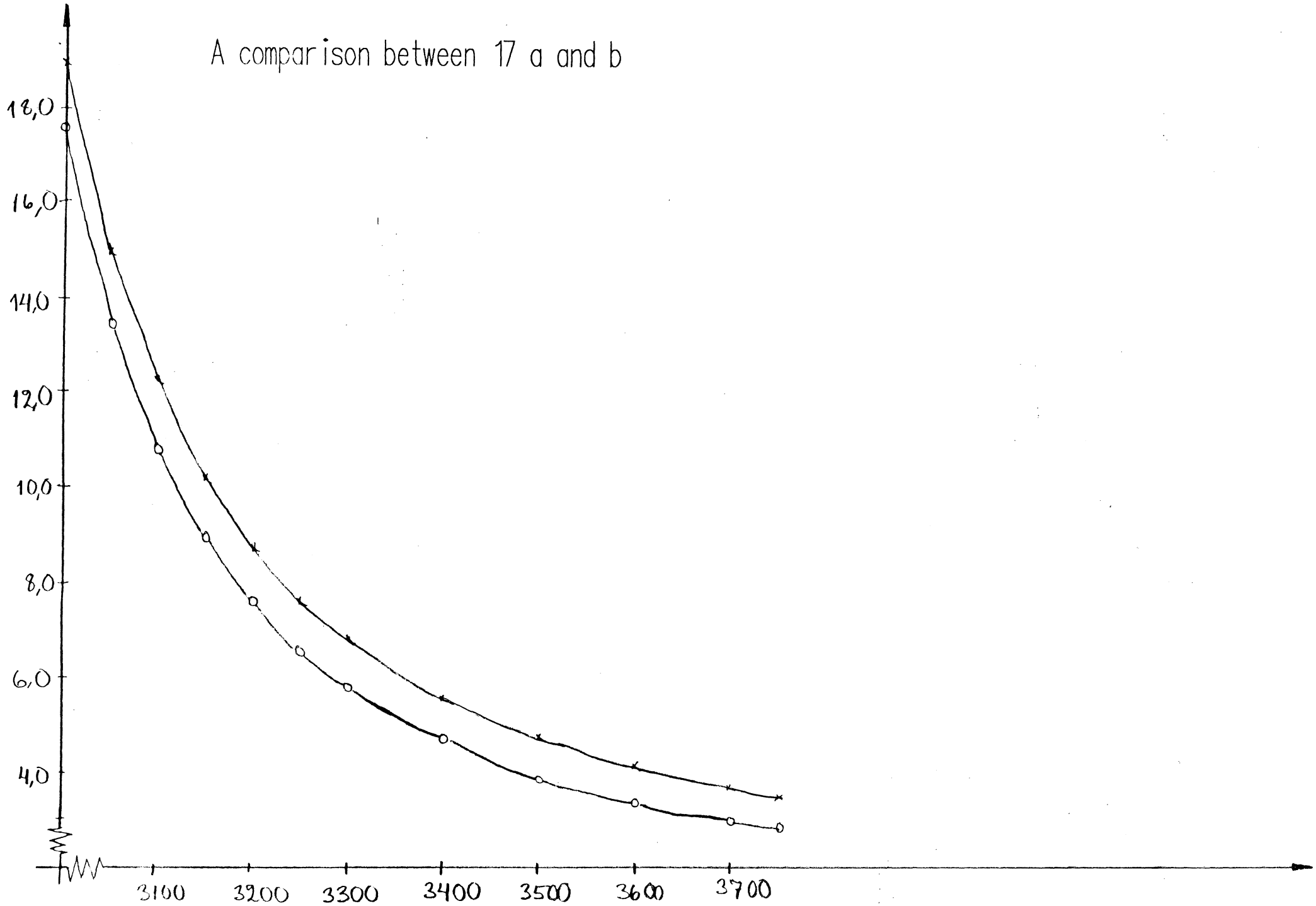

A comparison between 17 a and b
18,0
16,0
14,0
12,0
10,0
8,0
6,0
4,0
3100
3200
3300
3400
3500
3600
3700

Fig. 17 c

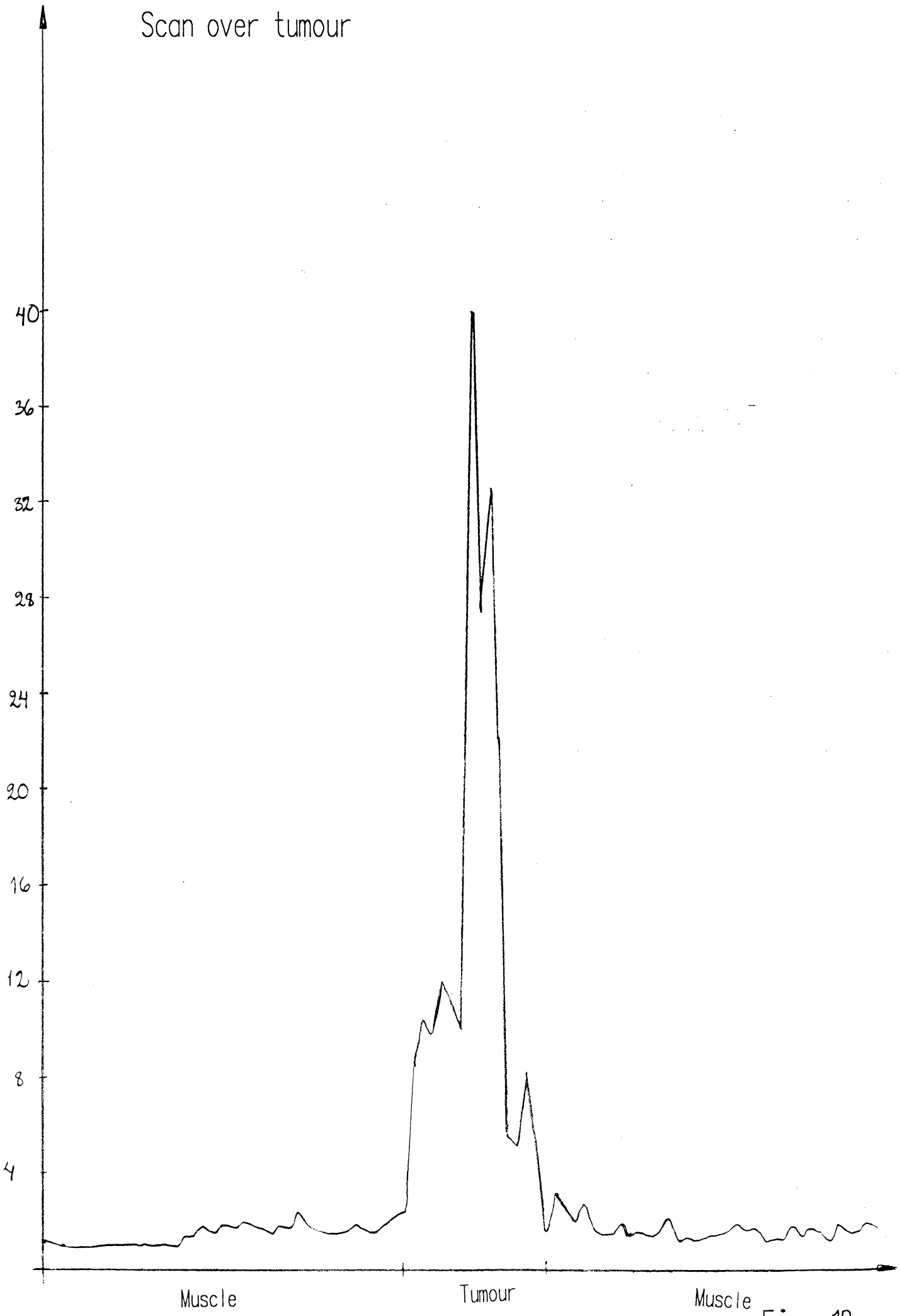

Scan over tumour
40
36
32
28
24
20
16
12
8
4
Muscle
Tumour
Muscle

Fig. 18 a

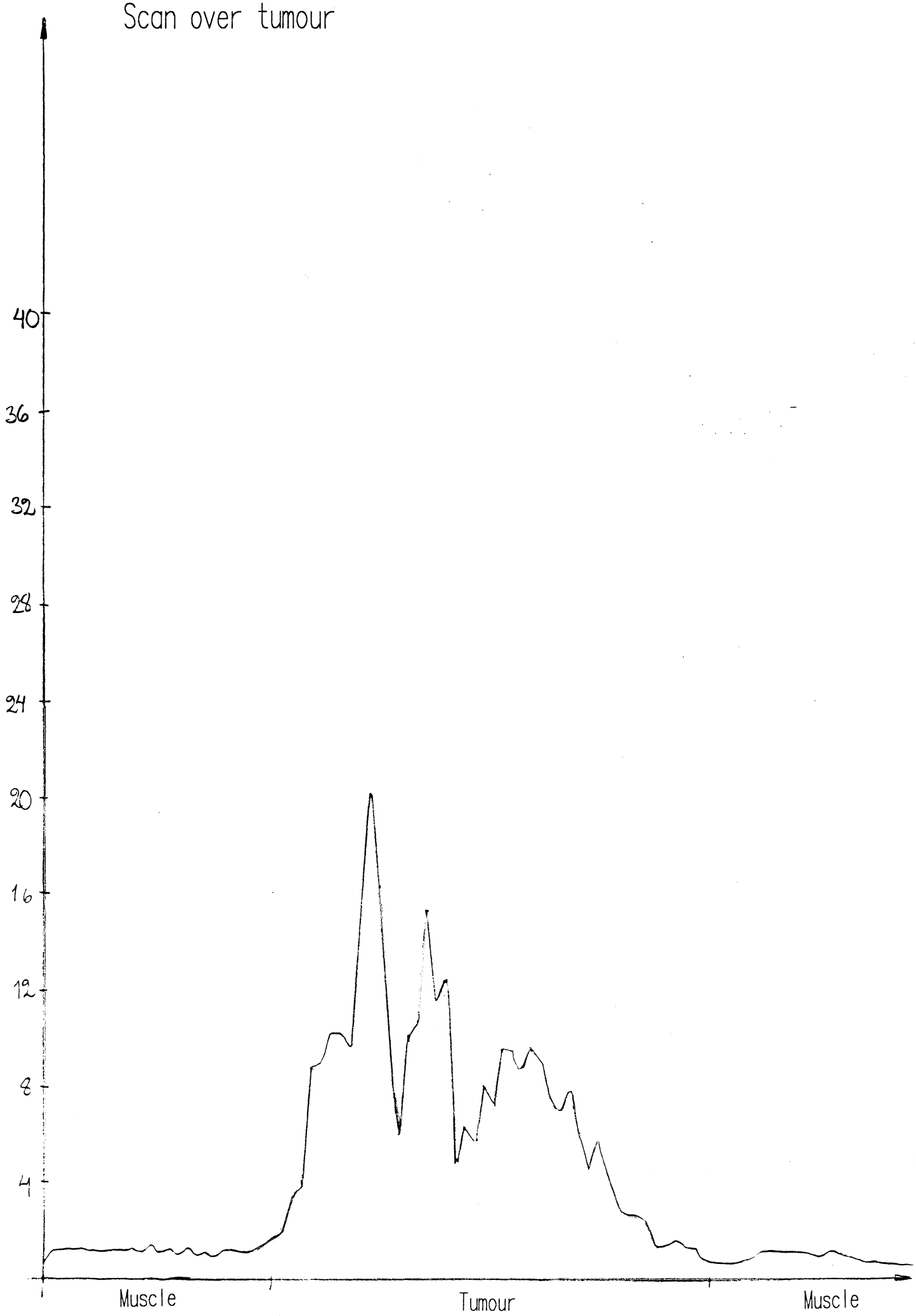

Scan over tumour
40
36
32
28
24
20
16
12
8
4
Muscle
Tumour
Muscle

Fig. 18 b

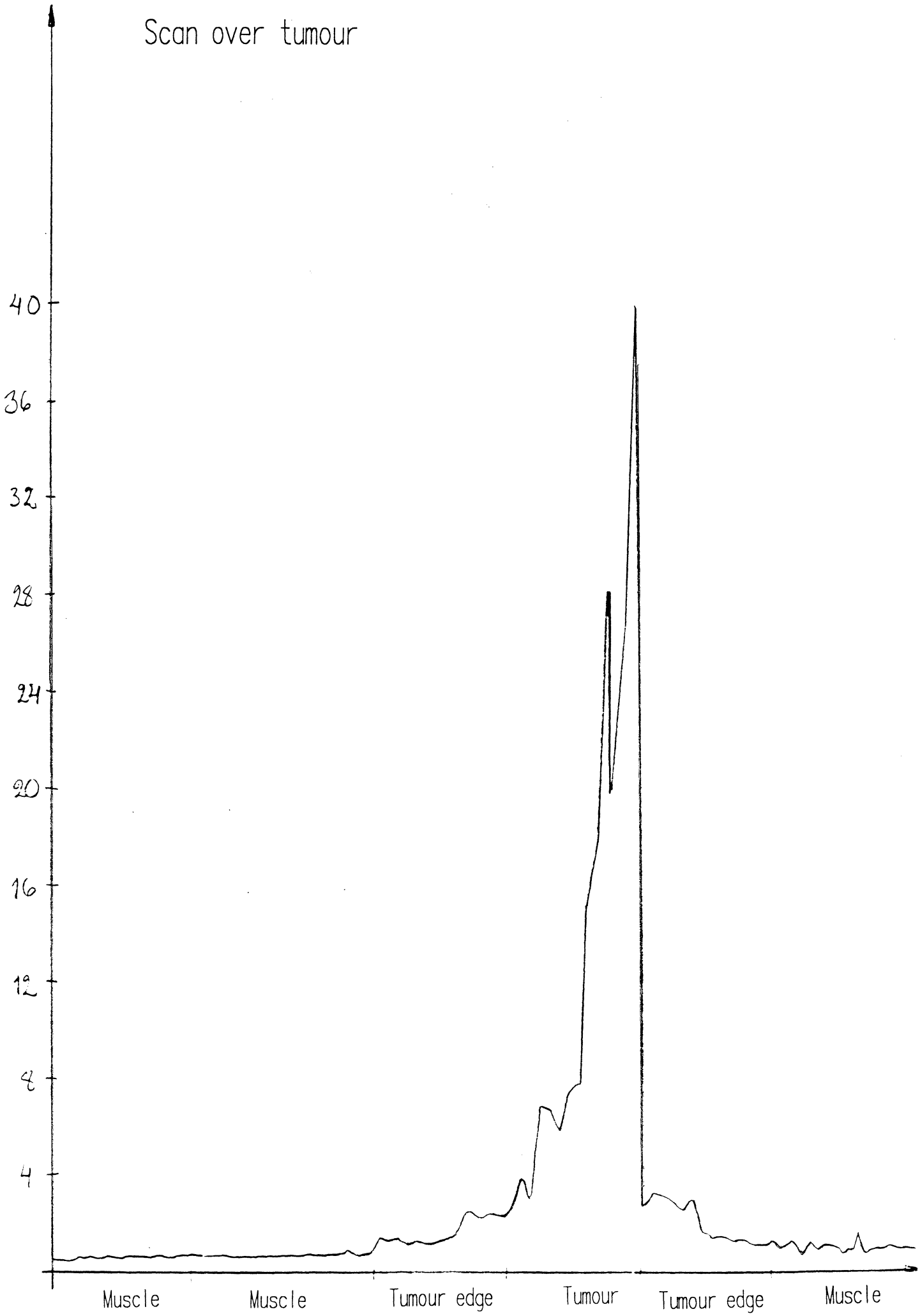

Scan over tumour
40
36
32
28
24
20
16
12
8
4
Muscle
Muscle
Tumour edge
Tumour
Tumour edge
Muscle

Fig. 18 c

APPENDIX A - The computer program

```
program lumisond;
uses crt;

var
   backgr : real;
   s1,s2,s3,s4,s5,s6 : real;
   busy,n,l,ltimer,a,antyary,i,intloopb,intloops,dummy : integer;
   t,timer1,timer2,timer3,timer4,timer5,timer6,loopmlnbs : integer;
   adhigh,adlow,dahigh,dalow,fors:integer;
   fil : text;
   contr : integer;
   constgain : boolean;
   ffreq,setgain,sg,sg_1,ffreq_1: integer;
   sign : array[1..6] of real;
   v,b,m : integer;
   big,g0,gnew : real;
   numer,denom : real;
   func : real;
   fvek : array[0..99] of real;
   hz : word;
   ch : char;

const

   adport     = $270;
   prctrlport = $3be;
   prstatport = $3bd;

{************************************************************************}
{                    INITIATION                                          }
{************************************************************************}


begin
  ltimer := 65;                          {delay loop index to find the}
                                         {long trig signal}

  port[adport] := 1;                     {A/D Channel 1 is chosen}

  writeln('Press any button to start');
  writeln;


  constgain := false;                  {Default is that the gain is to }
                                       {be set from the computer}

  contr := 3200;                       {Initiation of control signal}
  g0 := 8;                             {Initiation of gain}
  big := 0;

  writeln('If the gain is changed the new control signal will be');
  writeln('listed below (default control signal = 3200, the lower');
  writeln('control signal the larger gain and vice versa):');
  writeln;

{================ GAIN setting to amplication module ==================}

  dalow := contr and $FF;
  dahigh := (contr shr 8) and $F;
  port[adport + 7] := dahigh;
  port[adport + 6] := dalow;

{======================================================================}


{  writeln('Hur lnge vnta p behandlat filter?');
  readln(timer1);
  writeln('Hur lnge integrera?');
  readln(intloops);}


  timer1 := 40;                          {Delay loop indexes for the delay}
  timer2 := 38;                          {between the chopper trig signals}
  timer3 := 72;                          {and the filters}
  timer4 := 42;
  timer5 := 31;
  timer6 := 40;

  intloopb := 35;                    {Loop index for background integration}
  loopmlnbs := 15;                   {Delay between background and signal}
  intloops := 60;                    {Loop index for signal integration}


  func := 0;                             {The contrast function}

  ffreq := 10;                           {The default frequency for }
                                         {the contrast function evaluation}
                                         {Cannot be evaluated more often}
                                         {than every second revolution if}
                                         {the gain is not constant, because}
                                         {there is not time both to evaluate}
                                         {func and to set the gain during}
                                         {the same revolution}

  setgain := 1;                          {The default frequency for }
                                         {the gain adjustment in relation}
                                         {to the ffreq, that is default: }
                                         {the gain is set once (1) for}
                                         {every time func is evaluated}

  sg := (ffreq * setgain);             {The frequency in number of turns}
                                       {for the gain adjustment}

  ffreq_1 := ffreq - 1;

  sg_1 := sg - 1;

  a := -1;              {Loop index for the gain adjustment. Set to -1}
                        {just to make sure the gain is not adjusted }
                        {the first revolution}

  v := -1;              {Loop index for the sound setting}

  m := -1;              {Loop index for the func storage}

{======================================================================}
{==Turning off automatic interrupts in computer in order to speed up====}

  inline($FA);
```

```
{=========================================================================}

{*************************************************************************}
{*************************************************************************}
{                        START OF INFINITE LOOP                           }
{*************************************************************************}
{*************************************************************************}

    ch := readkey;

    while (port[prstatport] and $40) <> 0 do begin

{====================Looking for long trig============================}

     repeat
        l := ltimer;
        while ((port[prstatport] and $80) = 128) do;
        while ((port[prstatport] and $80) = 0) and (l <> 0) do
        l := pred(l);
     until (l = 0);

{=========================================================================}

{*************************************************************************}
{                          FIRST FILTER                                   }
{*************************************************************************}

     while ((port[prstatport]and $80) = 0) do;

     t := timer1;                                {wait for first background}
     repeat
        t := pred(t);
     until t = 0;

{=======================Background number 1===========================}

     port[prctrlport] := 6;                          {reset the integrator}
     port[prctrlport] := 6;                          {and give a trig signal}
     port[prctrlport] := 0;                          {to the oscillator}

     port[prctrlport] := 1;                          {integrate background 1}
     i := intloopb;
     repeat
        i := pred(i);
     until i = 0;
     port[prctrlport] := 0;

     dummy := port[adport + 3];                      {clear A/D register}

{---------------------A/D conversion of background 1------------------}

     n := 7;
     repeat
        dummy := port[adport + 4];
        n := pred(n);
             until n = 0;
     n := 7;
     repeat
        dummy := port[adport + 5];
        n := pred(n);
     until n = 0;

     adhigh := port[adport + 2];
     adhigh := adhigh and $F;
     adhigh := adhigh shl 8;

     adlow := port[adport + 1];

{-------------------------------------------------------------------------}
     backgr := (adhigh or adlow)*60/35;

{=========================================================================}

     t := loopmlnbs;                    {wait between background and signal}
     repeat
        t := pred(t);
     until t = 0;

{=======================Signal number 1===============================}

     port[prctrlport] := 2;                          {reset the integrator}
     port[prctrlport] := 2;
     port[prctrlport] := 0;


     port[prctrlport] := 1;                             {integrate signal 1}
     i := intloops;
     repeat
        i := pred(i);
     until i = 0;
     port[prctrlport] := 0;

     dummy := port[adport + 3];                         {Clear A/D register}


{---------------A/D conversion of signal 1----------------------------}

     n := 7;
     repeat
        dummy := port[adport + 4];
        n := pred(n);
     until n = 0;
     n := 7;
     repeat
        dummy := port[adport + 5];
        n := pred(n);
     until n = 0;

     adhigh := port[adport + 2];
     adhigh := adhigh and $F;
     adhigh := adhigh shl 8;
     adlow := port[adport + 1];

{-------------------------------------------------------------------------}
     sign[1] := adhigh or adlow;

     s1 := sign[1] - backgr;

{*************************************************************************}
{                        SECOND FILTER                                    }
```

```
{*************************************************************************}
     while ((port[prstatport]and $80) = 128) do;
     while ((port[prstatport]and $80) = 0) do;

     t := timer2;                                {wait for second background}
     repeat
        t := pred(t);
     until t = 0;

{=========================Background number 2============================}

     port[prctrlport] := 2;                       {reset the integrator}
     port[prctrlport] := 2;
     port[prctrlport] := 0;

     port[prctrlport] := 1;                       {integrate background 2}
     i := intloopb;
     repeat
        i := pred(i);
     until i = 0;
     port[prctrlport] := 0;

     dummy := port[adport + 3];                   {clear A/D register}

{-----------------A/D conversion of background 2-----------------------}
     n := 7;
     repeat
        dummy := port[adport + 4];
        n := pred(n);
     until n = 0;
     n := 7;
     repeat
        dummy := port[adport + 5];
        n := pred(n);
     until n = 0;

     adhigh := port[adport + 2];
     adhigh := adhigh and $F;
     adhigh := adhigh shl 8;
     adlow := port[adport + 1];

{-------------------------------------------------------------------------}
     backgr := (adhigh or adlow)*60/35;

{=========================================================================}
     t := loopmlnbs;                    {wait between background and signal}
     repeat
        t := pred(t);
     until t = 0;

{=========================Signal number 2================================}

     port[prctrlport] := 2;                       {reset the integrator}
     port[prctrlport] := 2;
     port[prctrlport] := 0;

     port[prctrlport] := 1;                        {integrate signal 2}
     i := intloops;
     repeat
        i := pred(i);
     until i = 0;
     port[prctrlport] := 0;

     dummy := port[adport + 3];                    {Clear A/D register}


{----------------A/D conversion of signal 3----------------------------}

     n := 7;
     repeat
        dummy := port[adport + 4];
        n := pred(n);
     until n = 0;
     n := 7;
     repeat
        dummy := port[adport + 5];
        n := pred(n);
     until n = 0;

     adhigh := port[adport + 2];
     adhigh := adhigh and $F;
     adhigh := adhigh shl 8;
     adlow := port[adport + 1];

{-------------------------------------------------------------------------}
     sign[2] := (adhigh or adlow);

     s2 := sign[2] - backgr;

{*************************************************************************}
{                        THIRD FILTER                                     }
{*************************************************************************}

     while ((port[prstatport]and $80) = 128) do;
     while ((port[prstatport]and $80) = 0) do;

     t := timer3;                                {wait for third background}
     repeat
        t := pred(t);
     until t = 0;

{=========================Background number 3============================}

     port[prctrlport] := 2;                       {reset the integrator}
     port[prctrlport] := 2;
     port[prctrlport] := 0;
```

```
     port[prctrlport] := 1;                          {integrate background 3}
     i := intloopb;
     repeat
        i := pred(i);
     until i = 0;
     port[prctrlport] := 0;

     dummy := port[adport + 3];                       {clear A/D register}

{---------------A/D conversion of background 3-------------------------}

     n := 7;
     repeat
        dummy := port[adport + 4];
        n := pred(n);
     until n = 0;
     n := 7;
     repeat
        dummy := port[adport + 5];
        n := pred(n);
     until n = 0;

     adhigh := port[adport + 2];
     adhigh := adhigh and $F;
     adhigh := adhigh shl 8;
     adlow := port[adport + 1];

{---------------------------------------------------------------------}
     backgr := (adhigh or adlow)*60/35;

{=====================================================================}

     t := loopmlnbs;                  {wait between background and signal}
     repeat
        t := pred(t);
     until t = 0;

{=======================Signal number 3================================}

     port[prctrlport] := 2;                           {reset the integrator}
     port[prctrlport] := 2;
     port[prctrlport] := 0;


     port[prctrlport] := 1;                            {integrate signal 3}
     i := intloops;
     repeat
        i := pred(i);
     until i = 0;
     port[prctrlport] := 0;

     dummy := port[adport + 3];                      {Clear A/D register}


{---------------A/D conversion of signal 3-----------------------------}

     n := 7;
     repeat
        dummy := port[adport + 4];
        n := pred(n);
     until n = 0;
     n := 7;
     repeat
        dummy := port[adport + 5];
        n := pred(n);
     until n = 0;

     adhigh := port[adport + 2];
     adhigh := adhigh and $F;
     adhigh := adhigh shl 8;
     adlow := port[adport + 1];

{---------------------------------------------------------------------}

     sign[3] := adhigh or adlow;

     s3 := sign[3] - backgr;

{*********************************************************************}
{                        GAIN ADJUSTMENT                              }
{*********************************************************************}

     for b := 1 to 6 do
        if sign[b] > big then big := sign[b];

     a := (a + 1)  mod sg;

     if (not constgain) and (a = sg-1) then begin
        if ((big < 2500) and (big > 10)) or (big > 3800) then begin
           gnew := (3000*g0)/big;
           if       (gnew > 187) then begin
              contr := 2700;
              gnew := 200;
           end
           else if (gnew > 170) then contr := 2725
           else if (gnew > 140) then contr := 2750
           else if (gnew > 110) then contr := 2775
           else if (gnew >  88) then contr := 2800
           else if (gnew >  55) then contr := 2850
           else if (gnew >  35) then contr := 2900
           else if (gnew >  24) then contr := 2950
           else if (gnew >  18) then contr := 3000
           else if (gnew >  14) then contr := 3050
           else if (gnew >  12) then contr := 3100
           else if (gnew >  10) then contr := 3150
           else if (gnew >   8) then contr := 3200
           else if (gnew  >  7) then contr := 3250
           else if (gnew  >  6) then contr := 3350
           else if (gnew  >  5) then contr := 3450
           else if (gnew  >  4) then contr := 3650
           else                      contr := 3850;
           write(contr,'  ');
           dalow := contr and $FF;
           dahigh := (contr shr 8) and $F;
           port[adport + 7] := dahigh;
           port[adport + 6] := dalow;
           g0 := gnew;
        end;
        big := 0;
     end

{*********************************************************************}
```

```
{        If not GAIN adjustment there is time to set the sound        }
{ The contrast function (func) is evaluated at the end of the revolution}
{*****************************************************************}

     else if (v = ffreq-1)  then begin
        m := (m + 1) mod 100;
        if big > 2000 then begin
           fvek[m] := func/ffreq;
           hz := round(fvek[m] * 100) + 300;
           sound(hz)
        end
        else  begin                              {If big <= 2000 there}
           fvek[m] := 0;                         {is no reliable signal}
           nosound;
        end;
        func := 0;
     end;

{=================================================================}

     v := (v + 1)  mod ffreq;

{*****************************************************************}
{                        FOURTH FILTER                            }
{*****************************************************************}

     while ((port[prstatport]and $80) = 128) do;
     while ((port[prstatport]and $80) = 0) do;

     port[prctrlport] := 2;                          {reset the integrator}
     port[prctrlport] := 2;
     port[prctrlport] := 0;

     t := timer4;                               {wait for fourth background}
     repeat
        t := pred(t);
     until t = 0;

{=======================Background number 4=======================}

     port[prctrlport] := 1;                        {integrate background 4}
     i := intloopb;
     repeat
        i := pred(i);
     until i = 0;
     port[prctrlport] := 0;

     dummy := port[adport + 3];                    {Clear A/D register}

{---------------A/D conversion of background 4-------------------}

     n := 7;
     repeat
        dummy := port[adport + 4];
        n := pred(n);
     until n = 0;
     n := 7;
     repeat
        dummy := port[adport + 5];
        n := pred(n);
     until n = 0;

     adhigh := port[adport + 2];
     adhigh := adhigh and $F;
     adhigh := adhigh shl 8;
     adlow := port[adport + 1];

{-----------------------------------------------------------------}
     backgr := (adhigh or adlow)*60/35;

{=================================================================}

     t := loopmlnbs;                    {wait between background and signal}
     repeat
        t := pred(t);
     until t = 0;

{=======================Signal number 4===========================}

     port[prctrlport] := 2;                          {reset the integrator}
     port[prctrlport] := 2;
     port[prctrlport] := 0;


     port[prctrlport] := 1;                          {integrate signal 4}
     i := intloops;
     repeat
        i := pred(i);
     until i = 0;
     port[prctrlport] := 0;

     dummy := port[adport + 3];                      {Clear A/D register}

{---------------A/D conversion of signal 4----------------------}

     n := 7;
     repeat
        dummy := port[adport + 4];
        n := pred(n);
     until n = 0;
     n := 7;
     repeat
        dummy := port[adport + 5];
        n := pred(n);
     until n = 0;

     adhigh := port[adport + 2];
     adhigh := adhigh and $F;
     adhigh := adhigh shl 8;
     adlow := port[adport + 1];

{-----------------------------------------------------------------}

     sign[4] := adhigh or adlow;

     s4 := sign[4] - backgr;

{*****************************************************************}
{                        FIFTH FILTER                             }
{*****************************************************************}
```

```
     while ((port[prstatport]and $80) = 128) do;
     while ((port[prstatport]and $80) = 0) do;

     t := timer5;                                  {wait for fifth background}
     repeat
        t := pred(t);
     until t = 0;

{=======================Background number 5============================}

     port[prctrlport] := 2;                          {reset the integrator}
     port[prctrlport] := 2;
     port[prctrlport] := 0;

     port[prctrlport] := 1;                        {integrate background 5}
     i := intloopb;
     repeat
        i := pred(i);
     until i = 0;
     port[prctrlport] := 0;

     dummy := port[adport + 3];                     {Clear A/D register}


{---------------A/D conversion of background 5-------------------------}

     n := 7;
     repeat
        dummy := port[adport + 4];
        n := pred(n);
     until n = 0;
     n := 7;
     repeat
        dummy := port[adport + 5];
        n := pred(n);
     until n = 0;

     adhigh := port[adport + 2];
     adhigh := adhigh and $F;
     adhigh := adhigh shl 8;
     adlow := port[adport + 1];

{-----------------------------------------------------------------------}
     backgr := (adhigh or adlow)*60/35;

{=======================================================================}

     t := loopmlnbs;                {wait between background and signal}
     repeat
        t := pred(t);
     until t = 0;

{=======================Signal number 5================================}

     port[prctrlport] := 2;                          {reset the integrator}
     port[prctrlport] := 2;
     port[prctrlport] := 0;

     port[prctrlport] := 1;                          {integrate signal 5}
     i := intloops;
     repeat
        i := pred(i);
     until i = 0;
     port[prctrlport] := 0;

     dummy := port[adport + 3];                      {Clear A/D register}


{---------------A/D conversion of signal 5-----------------------------}

     n := 7;
     repeat
        dummy := port[adport + 4];
        n := pred(n);
     until n = 0;
     n := 7;
     repeat
        dummy := port[adport + 5];
        n := pred(n);
     until n = 0;

     adhigh := port[adport + 2];
     adhigh := adhigh and $F;
     adhigh := adhigh shl 8;
     adlow := port[adport + 1];

{-----------------------------------------------------------------------}

     sign[5] := adhigh or adlow;

     s5 := sign[5] - backgr;

{***********************************************************************}
{                       SIXTH FILTER                                    }
{***********************************************************************}

     while ((port[prstatport]and $80) = 128) do;
     while ((port[prstatport]and $80) = 0) do;

     t := timer6;                                  {wait for sixth background}
     repeat
        t := pred(t);
     until t = 0;

{=======================Background number 6============================}

     port[prctrlport] := 2;                          {reset the integrator}
     port[prctrlport] := 2;
     port[prctrlport] := 0;

     port[prctrlport] := 1;                        {integrate background 6}
     i := intloopb;
     repeat
        i := pred(i);
     until i = 0;
     port[prctrlport] := 0;
```

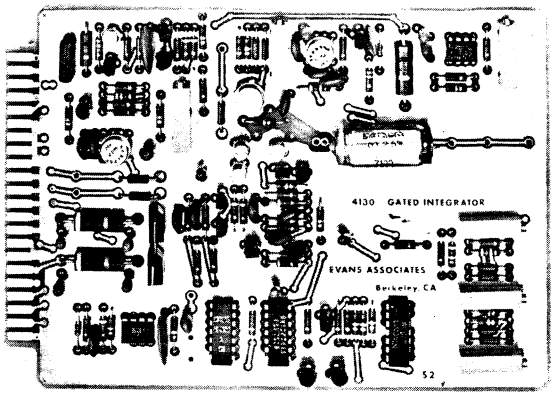
```
     dummy := port[adport + 3];                    {Clear A/D register}

{---------------A/D conversion of background 6-------------------------}

     n := 7;
     repeat
        dummy := port[adport + 4];
        n := pred(n);
     until n = 0;
     n := 7;
     repeat
        dummy := port[adport + 5];
        n := pred(n);
     until n = 0;

     adhigh := port[adport + 2];
     adhigh := adhigh and $F;
     adhigh := adhigh shl 8;

     adlow := port[adport + 1];

{---------------------------------------------------------------------}
     backgr := (adhigh or adlow)*60/35;

{=====================================================================}

     t := loopmlnbs;              {wait between background and signal}
     repeat
        t := pred(t);
     until t = 0;


{=======================Signal number 6================================}

     port[prctrl[port] := 2;                       {reset the integrator}
     port[prctrl[port] := 2;
     port[prctrl[port] := 0;

     port[prctrl[port] := 1;                       {integrate signal 6}
     i := intloops;
     repeat
        i := pred(i);
     until i = 0;
     port[prctrl[port] := 0;

     dummy := port[adport + 3];                    {Clear A/D register}

{---------------A/D conversion of signal 6-----------------------------}

     n := 7;
     repeat
        dummy := port[adport + 4];
        n := pred(n);
     until n = 0;
     n := 7;
     repeat
        dummy := port[adport + 5];
        n := pred(n);
     until n = 0;

     adhigh := port[adport + 2];
     adhigh := adhigh and $F;
     adhigh := adhigh shl 8;
     adlow := port[adport + 1];

{---------------------------------------------------------------------}

     sign[6] := adhigh or adlow;

     s6 := sign[6] - backgr;

{*********************************************************************}
{        EVALUATION OF FUNC (THE CONTRAST FUNCTION)                   }
{*********************************************************************}


     numer := (s1 + s4) - (s3 + s6);
     denom := (s2 + s5);
     if (denom > 0) then
        func := func + numer/denom       {If denom <= 0 and numer > 100}
     else if (numer > 100) then          {20 is added to func, chosen as }
        func := func + 20                {some sort of maximum value.}
     else                                {If denom <= 0 and numer <= 100}
        func := func + 0;                {0 is added to func as this means}
                                         {there is no signal}

{****************** END OF INFINITE LOOP *****************************}
   end;

{==============Turning on the interrupts again=========================}

   inline($FB);

{=====================================================================}

   nosound;
   if big > 4030 then writeln('overload');
   writeln;


{*********************************************************************}
{     PRINTING RESULTS ON SCREEN                                      }
{*********************************************************************}

   writeln('The contrast function is listed below:');
   for a := (m + 1) to 99 do begin
        write(fvek[a]:5:3,'  ');
   end;
   for a := 0 to m do begin
        write(fvek[a]:5:3,'  ');
   end;
   writeln;
   writeln;
   assign(fil,'c:\tp\petra\scan.dat');
   rewrite(fil);
   writeln(fil,m);
   for a := (m + 1) to 99 do begin
      b := a mod 8;
      write(fil,fvek[a]:8:3);
      if b = 0 then writeln(fil);
   end;
   for a := 0 to m do begin
      b := a mod 8;
```

```
      write(fil,fvek[a]:8:3);
      if b = 0 then writeln(fil);
   end;
   close(fil);
end.
```

APPENDIX B - The complete description of the integrator

GATED INTEGRATOR MODULE

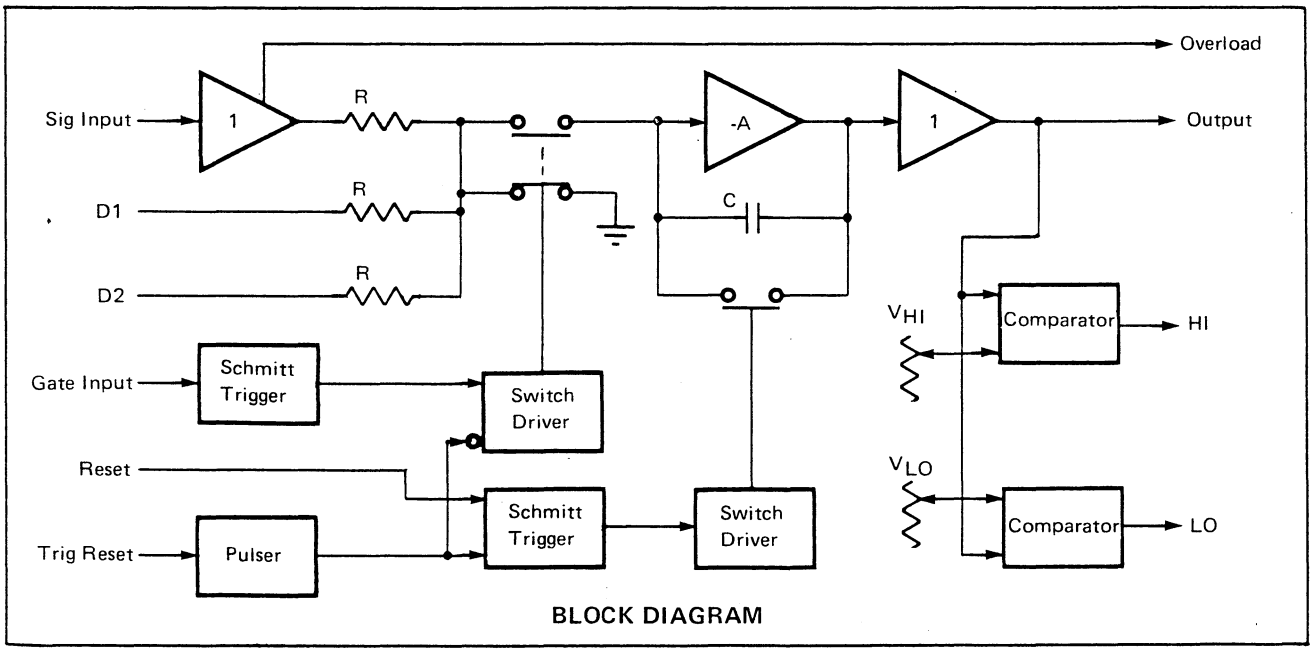
- 30 ns. Minimum Gating Time
- 1 pa. Maximum Leakage Current
- Fast Input Voltage Follower
- Adaptable to Boxcar Integration
- Automatic Baseline Correction (2 Modules)
- Automatic Limit Reset
- On-Board IC Voltage Regulators



The Model 4130 is a fast, low-leakage integrator having an input isolating gate which can be opened, under external control, for periods ranging from dc down to 30 ns. The output voltage closely approximates 1/RC x the time integral of the input voltage, with the integration proceeding only during open-gate intervals; the integral is held constant while the gate is closed. Two logic outputs indicate continuous comparisons (greater than or less than) of the integral with 2 voltage levels preset in with on-board potentiometers. A reset, returning the output voltage to zero, can be actuated with either a direct logic input, or a trigger applied to an internal pulser.

Signal input to the 4130 is accessible through a fast unity-gain buffer amplifier, and also through 2 direct connections. Each input is essentially independent of the others, and may be used to provide a separate input for zero offset, baseline correction, or feedback.

In addition to totalizing applications, the Model 4130 is especially well suited to boxcar integration in which sequential, or otherwise selected, segments of a repetitive signal are to be averaged over many cycles. Programmable time delays for boxcar applications are available in the EA Models 4141, 4145 modules.



BLOCK DIAGRAM

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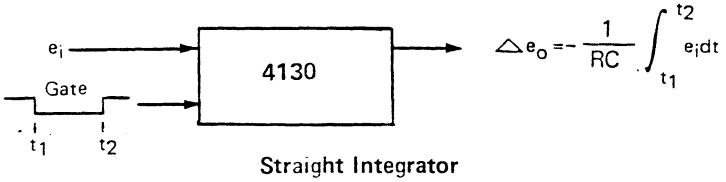
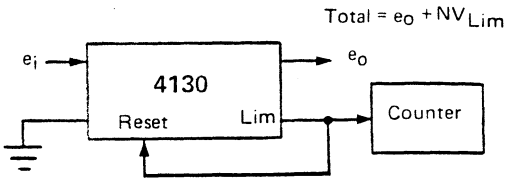
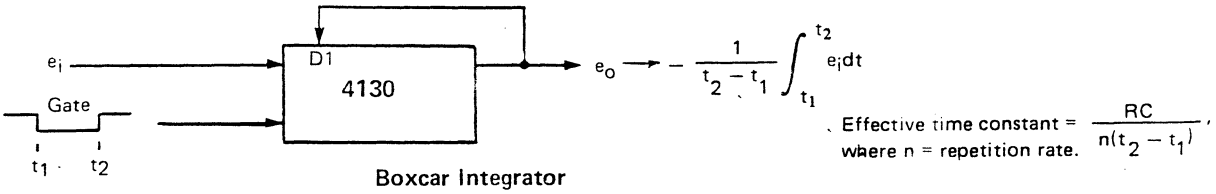
APPLICATIONS:



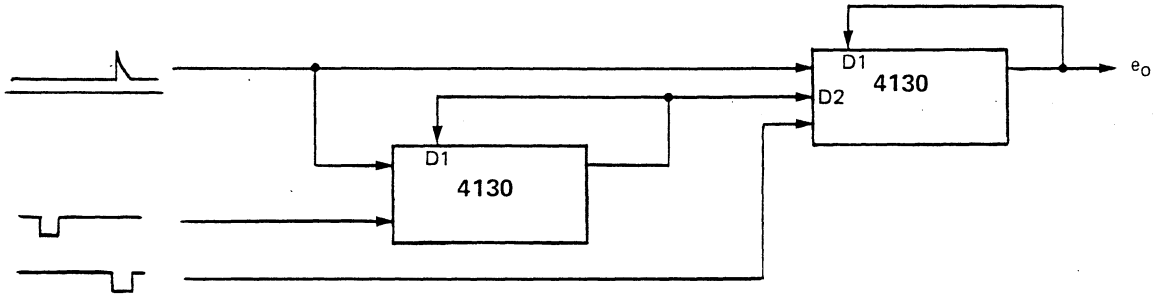
Straight Integrator



Long Term Integrator



Boxcar Integrator



Boxcar with Automatic Baseline Correction

SPECIFICATIONS @25°C:

INTEGRATING TIME CONSTANT

10^{-4} sec. ±10%. R=10K, C=10,000 pF. Board space allows user substitution of RC values covering 10^{-6} to 10^{-3} sec. Special order RC's available.

ANALOG OUTPUT

Max. swing, ±5v @10ma.

LEAKAGE

1 pa. max. with gate closed.

LINEARITY DEPARTURE

±.1% of full scale max.

DRIFT

70 μv/°C max, referred to output.

VOLTAGE FOLLOWER INPUT

Input impedance 10 megohms in parallel with <20 pF. AC or DC. 0.1 μF series capacitor on AC input. Rise time <4 ns. Temp. coeff. typically <10 μv/°C. Adjustment pot. to zero offset.

DIRECT INPUTS

Two inputs directly coupled to integrator junction through 10K resistors (other values can be substituted).

LOGIC INPUTS

Compatible with TTL, DTL, CMOS, and contact closure. Negative logic. Min. off voltage +2v (or open circuit). Min. on sink current, 5 ma. (or short circuit to ground).

1. Gate. Min. open time, 30 ns.
2. Reset. Places <100 ohms across integrating capacitor. Delay time to turnoff, <100ns.
3. Pulsed Reset. Negative input transition triggers 12 μs. resetting pulse. Other RC values can be substituted for longer pulses as desired.

LOGIC OUTPUTS

Negative logic. Open-collector transistor. Min. sink current typically >16 ma.

1. Overload. Indicates when input voltage follower swing exceeds ±3v
2. Hi, Lo. Indicates when integral output voltage exceeds preset values. Both Hi and Lo reference voltages adjustable over ±5v.

POWER REQUIREMENTS

+15v ± 1v, <150 ma. -15v ± 1v, <75 ma.

DIMENSIONS

.062" pc board. 4½"W x 6½"L. 7/8" clearance required on component side. Board edge mates to standard 2 x 22 pin connector on .156" centers (e.g., Cinch 50-44A-30).

OPERATING TEMP. RANGE

10° C.–40° C.

PRICE

$165 FOB Berkeley, CA.

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OPERATING NOTES

1. When used in the boxcar configuration as shown on the 4130 data sheet, the open-gate gain is determined by the ratio of 2 of the resistors R16, R17, R18 utilized as the feedback and input resistors respectively. With the 10k resistors supplied, the gain is approx. -1. Other values can be substituted; the integrating time constant is the product of C and the feedback R.

2. In the automatic baseline correction configuration, the gain of the 2nd compensating module should be equal to the ratio of the net forward gains referred to the 2 inputs of the 1st module. A potentiometer in the external feedback path of the 2nd module is recommended for exact nulling.

3. There is a small charge transferred with each gate transition. As the gate duration is decreased (below approx. 10 μs) this effect begins to contribute a measurable equivalent input error current which is constant for a given RC selection and gate width. This current can be effectively balanced out, if desired, by connecting V_X (pin 8) to any of the unused dc inputs, and adjusting R72 for zero OUTPUT (pin 10) with zero input. An external series resistor may be added for finer adj.

4. R15, R42, and R51 are local zeroing pots., and should require only minor and infrequent adjustment. The following procedure is recommended:
   a. Connect the OUTPUT (pin 10) directly to the DC SIG INPUT (pin 20).
   b. Ground the RESET (pin D).
   c. Locate R47 (1K, 1W), and attach a dc voltmeter (or scope) having a resolution of at least 0. 1mv. to the end of R47 nearest the center of the card.
   d. Adjust R42 for 0v.
   e. Remove voltmeter and attach it to OUTPUT (pin 10).
   f. Adjust R51 for 0v.
   g. Remove the ground at RESET (pin D).
   h. Ground the GATE (pin C).
   i. Adjust R15 for 0v. at the OUTPUT (pin 10).

5. R60 and R66 are the HI and LO limit adjustments respectively. A convenient arrangement for establishing the limit points is to ground the GATE (pin C), connect the OUTPUT (pin 10) directly to one of the dc inputs (D1, D2, or DC SIG INPUT), and connect V_X to another of the dc inputs. R72 can then be varied while monitoring the OUTPUT, and the desired limit established. Subsequently, R60, or R66, can be varied while HI (pin 2) and LO (pin B) logic status is observed. Note

OPERATING NOTES (Cont'd)

that HI and LO are open-collector outputs, and require resistor returns to a positive voltage in order to determine status.

6. The HI (pin 2) or LO (pin B) can be used for automatic reset by connecting either output directly to both RESET (pin D) and TRIG RESET (pin 4). No external output pull-up resistors are required in this arrangement. For complete resetting action C18 should be approx. .01C9. This configuration is useful for long term integration, and for generation of ramps and time delays.

7. When integrating from a very high impedance, or constant current, source, e.g., a collector electrode in vacuum, it is preferable to use the D1 (pin 12) or D2 (pin13) input. If, in addition, the voltage follower is not being used, it will be important to remove R18, since any path to ground during open-gate intervals will result in error current generated by input offset (note that even a 10 μv. offset with R18=10K produces 1 na.).

8. The integrating capacitor should be a film type, preferably polystyrene, teflon, or polycarbonate. Other film types, such as polyester, or mylar may be quite satisfactory where leakage resistance, temperature coefficient, or dielectric absorption are not critical. When a new capacitor is substituted, the board area inside the guard ring enclosing one capacitor terminal should be cleaned on both sides of the board with freon, or other flux remover. Caution: polystyrene capacitors can be irreversibly damaged upon contact with many solvents, including freon.

9. If the AC or DC SIG INPUT is not used, the DC SIG INPUT (pin 20) should be grounded to prevent pickup.

10. The module is sensitive to light in the guard ring area, and may exhibit leakage rates >1pa. under normal ambient light. It may be necessary to shade the board if minimum leakage is required.

11. CAUTION: Do not use a soldering gun when substituting components. AC line voltages can destroy the semiconductor devices. Use only soldering irons which are grounded through a 3-wire plug.

PIN CONNECTIONS

Bottom View of Connector

Solder side ←				Component side →
0 v	22	Z	0 v	
AC SIG INPUT	21	Y	0 v	
DC SIG INPUT	20	X	0 v	
0 v	19	W	0 v	
SPARE (Guarded)	18	V	0 v	
0 v	17	U	0 v	
SPARE	16	T	0 v	
SPARE	15	S	0 v	
NC	14	R	0 v	
D2 INPUT	13	P	0 v	
D1 INPUT	12	N	0 v	
NC	11	M	0 v	
OUTPUT	10	L	0 v	
NC	9	K	0 v	
V_x	8	J	0 v	
NC	7	H	0 v	
-15 v	6	F	-15 v	
+15 v	5	E	+15 v	
TRIG RESET	4	D	RESET	
OVERLOAD	3	C	GATE INPUT	
HI	2	B	LO	
0 v	1	A	0 v	

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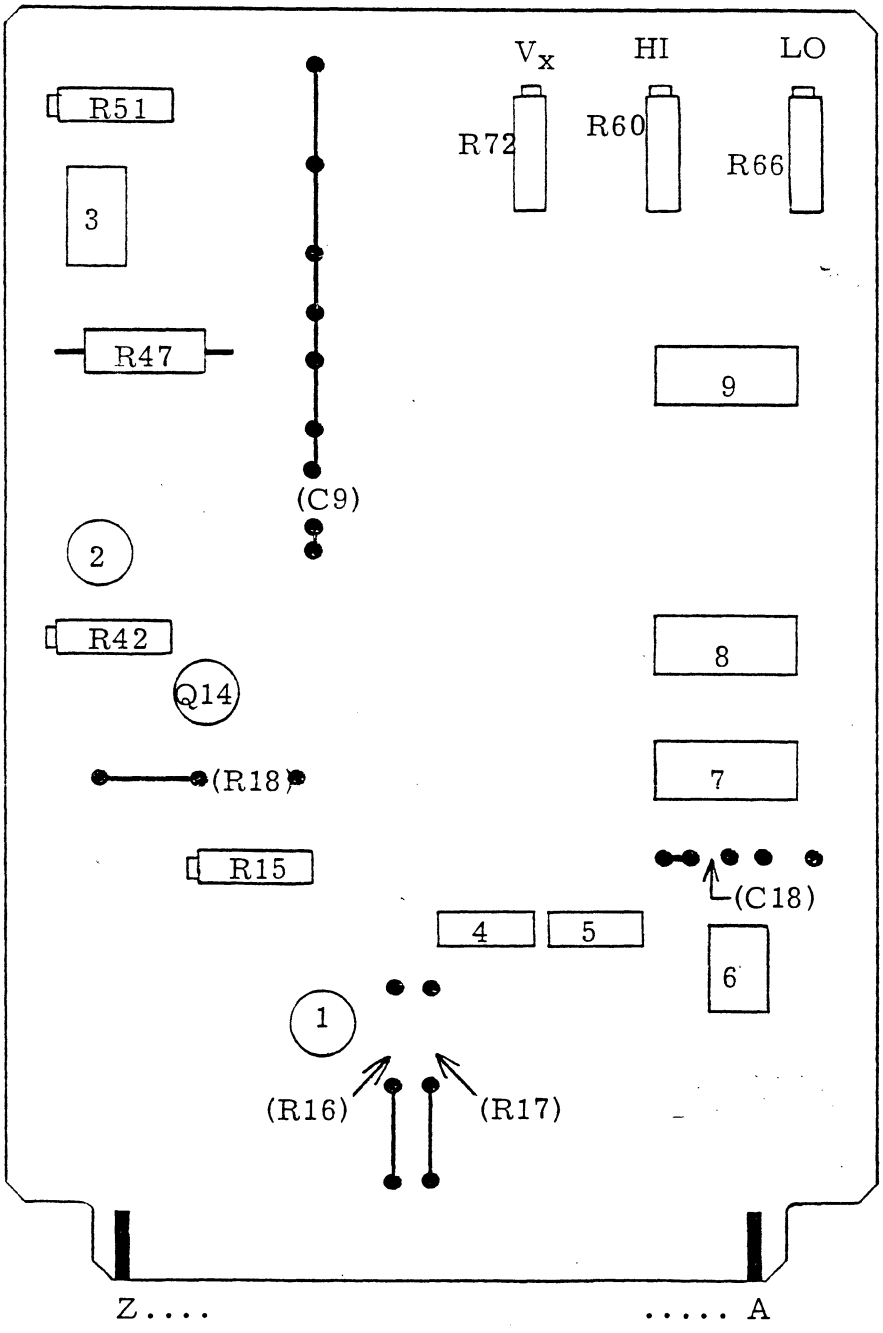
LOCATOR



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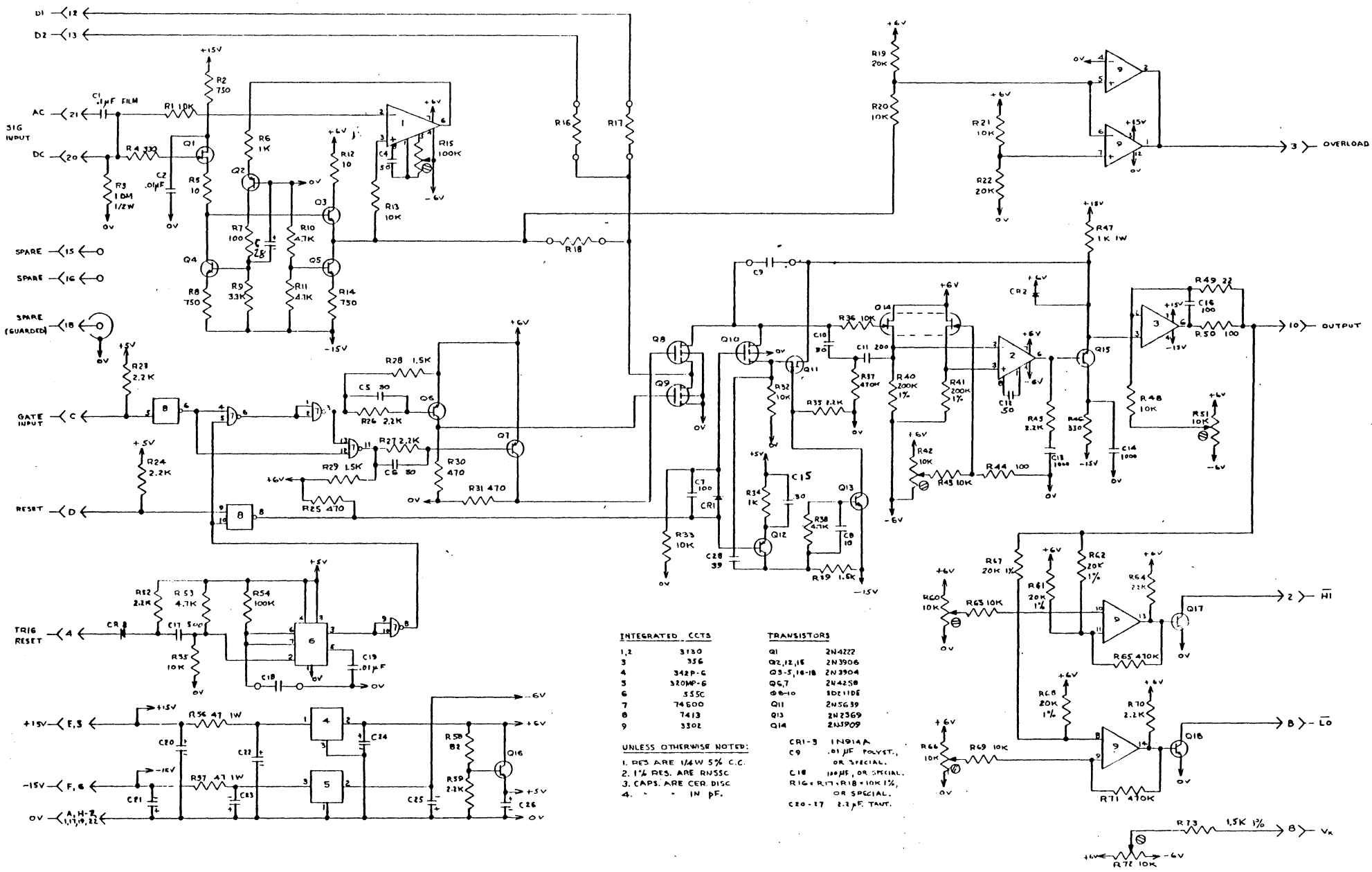

SCHEMATIC GATED INTEGRATOR MODEL 4130