A COMPUTER-CONTROLLED FLUORESCENCE-MONITORING SYSTEM

Diploma Paper
by

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INTRODUCTION

The aim of this work was to construct a computer-controlled fluorescence-monitoring system. The system is primarily intended to be used as a fluorescence bronchoscope for the detection of early-stage lung cancer. This does not mean that the instrument can only be used for this purpose. On the contrary, it is designed as a multi-task instrument which can be used in a wide field of applications also outside the medical area.

The technique used in the case of the fluorescence bronchoscope is based upon the fact that a substance called HPD (Hematophorphyrin Derivative) concentrates in tumour cells when injected into the body, and fluoresces in the red when excited with light of certain wavelengths.

In 1978 a bronchoscope using this technique was developed by Kinsey, Cortese and Sanderson (Ref.1). This instrument only detected the strength of the red fluorescence form HPD and was therefore very sensitive to variations in distance between the fibre tip and the cancer cells.

In 1985 a first generation fluorescence bronchoscope was constructed here at the Department of Physics (Ref. 3, 4). Here the detection technique used the fact that the tissue itself fluoresces strongly in the blue. In tumour cells however, this blue fluorescence decreases while the red fluorescence from HPD increases. Measuring the blue and the red fluorescence and taking the ratio between them, will give a value that is independent of variations in distance. This ratio will then give a good contrast between tumour cells and normal cells.

The new instrument, designed according to the principles given in Ref.4 and described in the present paper, has even more facilities. It uses 3 wavelengths for detection, the blue, the red and one in between to be able to "lift off" the red HPD emission from the red tissue background. The instrument also measures the background light level for each filter. This is then subtracted from the fluorescence value for this filter.

While the computer-controlled instrument searches for tumour cells, the examining doctor must be able to examine the lungs with his own eyes. This is achieved by letting white light from a lamp pass through the fibre, unfiltered, down into the lungs.

When, on the other hand, the computer searches for tumour cells, no white light must come down to the lungs, since that would give us too much light to be able to detect the weak fluorescence signal.

This problem has been solved by using a rotating chopper wheel. The chopper wheel lets white light or filtered excitation light down into the lungs and it also makes sure that no detection is made during the white light illumination. The chopper wheel has the advantage of allowing the use of 2 excitation wavelengths and 3 detection wavelengths to give maximum tissue discrimination.

The detection signal is taken care of by the amplifying and controlling card. The amplifier is specially designed to handle very small signals. It is controlled by the computer to ensure that maximum resolution is achieved and to compensate quickly for variations in distance between fibre and lung cells.

The detection signals are then sampled by the computer, 32 samples per excitation filter plus 32 background samples per detection filter, to give reliable mean values. The data are carefully examined and the result of the calculations will decide whether there are tumour cells or not. Finally, a tone of varying pitch, the higher the nearer the tumour centre, is heard.

In PART 1 the theory of fluorescence and some facts about HPD are briefly described.

PART 2 consists of a very thorough description of the instrument, especially the computer and electronics which have been my main task.

PART 1
THEORY

MOLECULES AND FLUORESCENCE

In molecules the energy level structure can be divided into three parts. These are rotational structure, vibrational structure and electronically excited states. In liquids or solids the vibrational and rotational structure cannot be resolved. The order of separation between rotational levels is 10^{-3} eV and for vibrational levels 10^{-1} eV. For the electronically excited states the order of separation is 1 eV.

The number of molecules in a certain excited or non-excited state is given by the Boltzmann distribution. The Boltzmann distribution states that in a sample of N molecules at temperature T, the number of molecules with energy E is given by

$$N\ E) = N*g*\exp(-E/kT)$$

where g is the degeneracy of the level and k is the Boltzmann constant. At room temperature kT is about 1/40 eV, but the first electronically excited state is usually of the order of 1 eV above the ground state. This means that at room temperature the electronic absorption spectrum is normally due entirely to transitions originating from the ground electronic state.

The unresolved vibrational structure gives the molecules very wide absorption bands, according to the Franck-Condon principle. The Franck-Condon principle states that because the nucleus is so much more massive than the electrons an electronic transition takes place faster than the nucleus can respond. This is illustrated in Fig. 1.

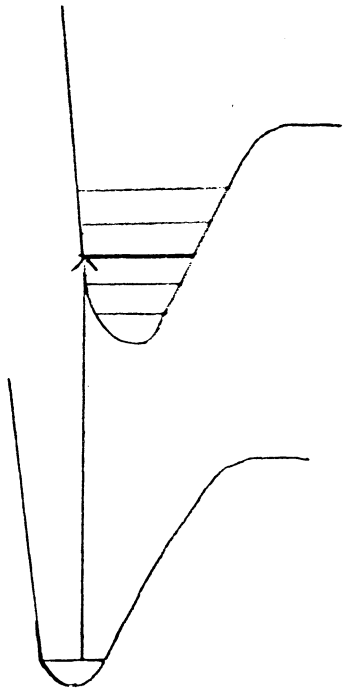


Fig. 1 The Franck-Condon principle

Before absorption the molecule is in the ground vibrational state of its ground electronic state. When absorption takes place the molecule is excited to the upper state. According to the Franck-Condon principle the nucleus maintains its position during the transition and therefore the transition can be represented by a vertical line. This vertical transition denotes that an electronic transition has taken place without any change in nucleus geometry. The vertical transition passes several vibrational levels in the upper state. The most probable one is the one marked in the figure. However, this is not the only possible transition since several nearby vibrational levels have a high probability for transitions to take place. This phenomenon, together with the fact that in liquids the vibrational levels cannot be resolved, gives the molecule its wide absorption band.

When a molecule has been transferred to an electronically excited state, it can loose its energy in several ways. The only interesting way, from our point of view, is radiative decay in which the molecule discards its excitation energy as a photon.

In Fig. 2 the process leading to fluorescence can be seen.

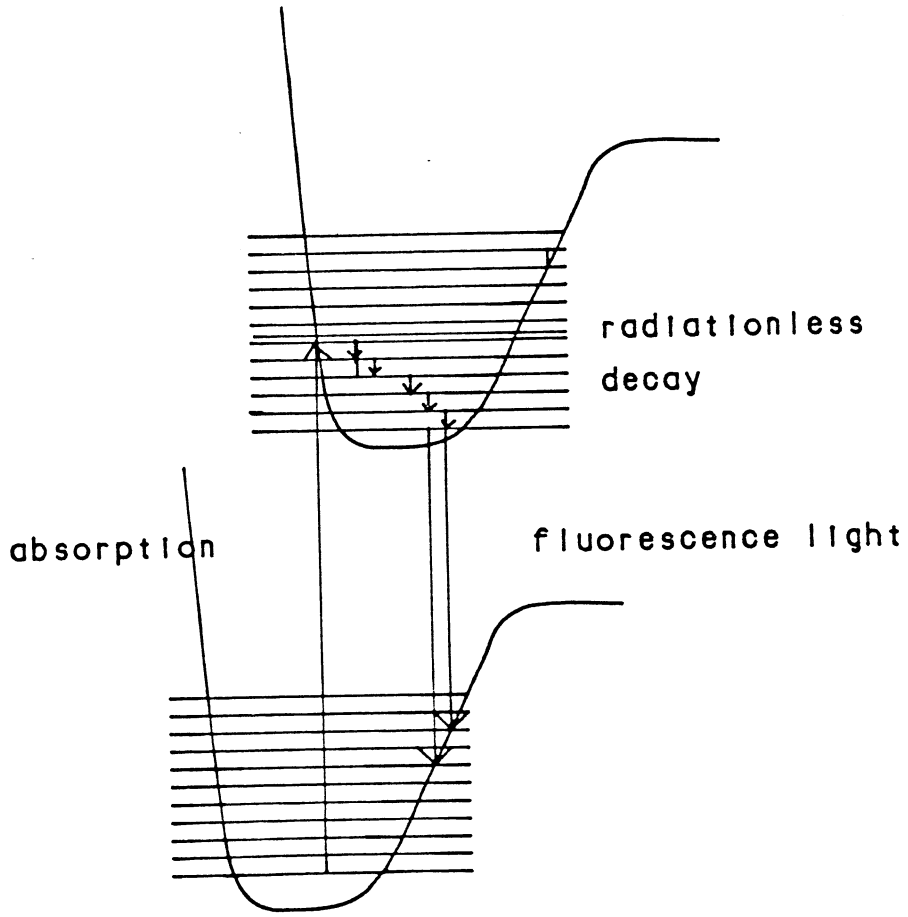


Fig. 2 Fluorescence light generation

Absorption takes the molecule to an excited electronic state. In the excited state the molecule undergoes collisions with the surrounding molecules and decays down through the vibrational levels to the bottom of the excited state. The electronic transition, giving the fluorescent light, is vertical according to the Franck-Condon principle. As can be seen from Fig. 2 the fluorescent light will have a lower photon energy, which means that the light is shifted towards red, compared with the exciting light. This is called the Stokes shift.

Another radiation phenomenon that may occur is phosphorescence. This can be studied in Fig. 3.

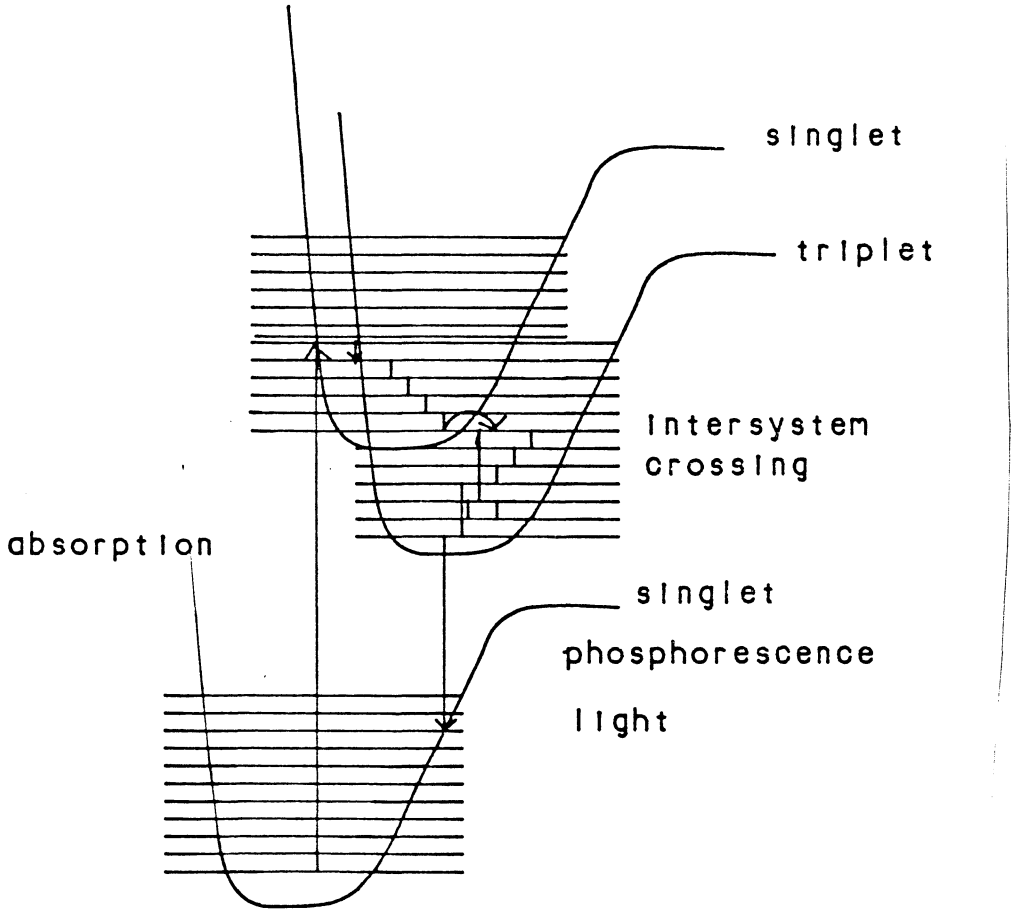


Fig. 3 Phosphorescence light generation

The first steps are the same as in fluorescence, but the presence of a triplet state plays an important role. The excited molecule can, for instance via collisions, make an intersystem crossing, i.e. be transferred to a lower level in a state with different multiplicity (triplets). The molecule then decays down through the vibrational levels of the triplet, to the lowest vibrational level. At this ground level, however, it is trapped. The molecule cannot radiate its energy because the transition to the ground state is a triplet-singlet transition which is forbidden. Therefore molecules may be collected here for some time. However, the transition is not totally forbidden and the molecules are able to emit weakly. This emission may continue long after the original excited state is formed.

Phosphorescent light always has a longer wavelength than fluorescent light from the same molecule.

In this work only the fluorescence light is of interest.

HPD

HPD stands for Hematoporphyrin Derivative and is a mixture of several porphyrin compounds with a chemical structure as shown in Fig. 4.

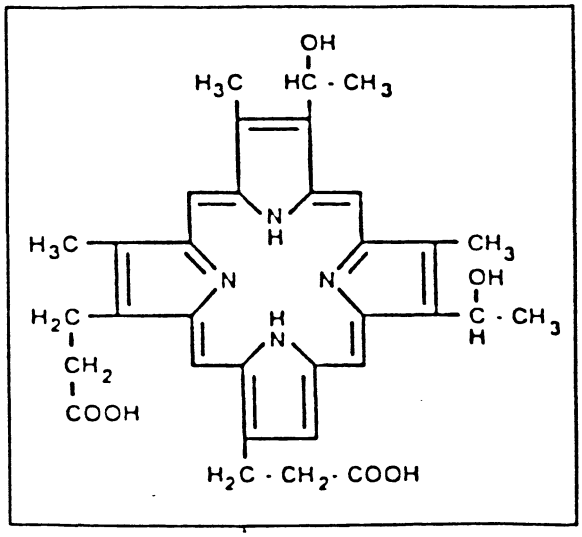


Fig. 4 Hematophorphyrin Derivative: chemical structure (Ref.3 Fig. 6a)

HPD is known to localize at a higher level in malignant tumour tissue than in normal tissue. In 1960 HPD was used for tumour localization for the first time by Lipson. The use of HPD for the treatment of tumours by photodynamic theraphy was started by Dougherty in 1978. An investigation monitored by the Food and Drug Administration in the USA, is in progress all over the world to assess advantages and possible disadvantages of HPD for cancer treatment. The results of this investigation should be available in the Summer of 1988.

Recently, a new form of HPD, dihematophorphyrin ether, DHE, has become available. This is a purified form of HPD, which should give better results in treatment.

HPD has the advantage of emitting a characteristic red flourescence (2 peaks) when excited in the Soret band at 405 nm (Fig. 5). This, together with the fact that it localizes at a much higher level in tumour tissue, makes HPD suitable for the early detection of tumours, especially in endoscopic investigations.

One to four days after the HPD has been injected, it is excited with UV light at 405 nm and a fluorescence spectrum will show a good contrast between tumour and normal tissue (Fig. 5).

HPD can also be used for photodynamic therapy. In this process, red light of 630 nm is used. After the HPD has absorbed the light a transfer of energy from the HPD molecule to oxygen molecules in the cells takes place (Fig. 6).

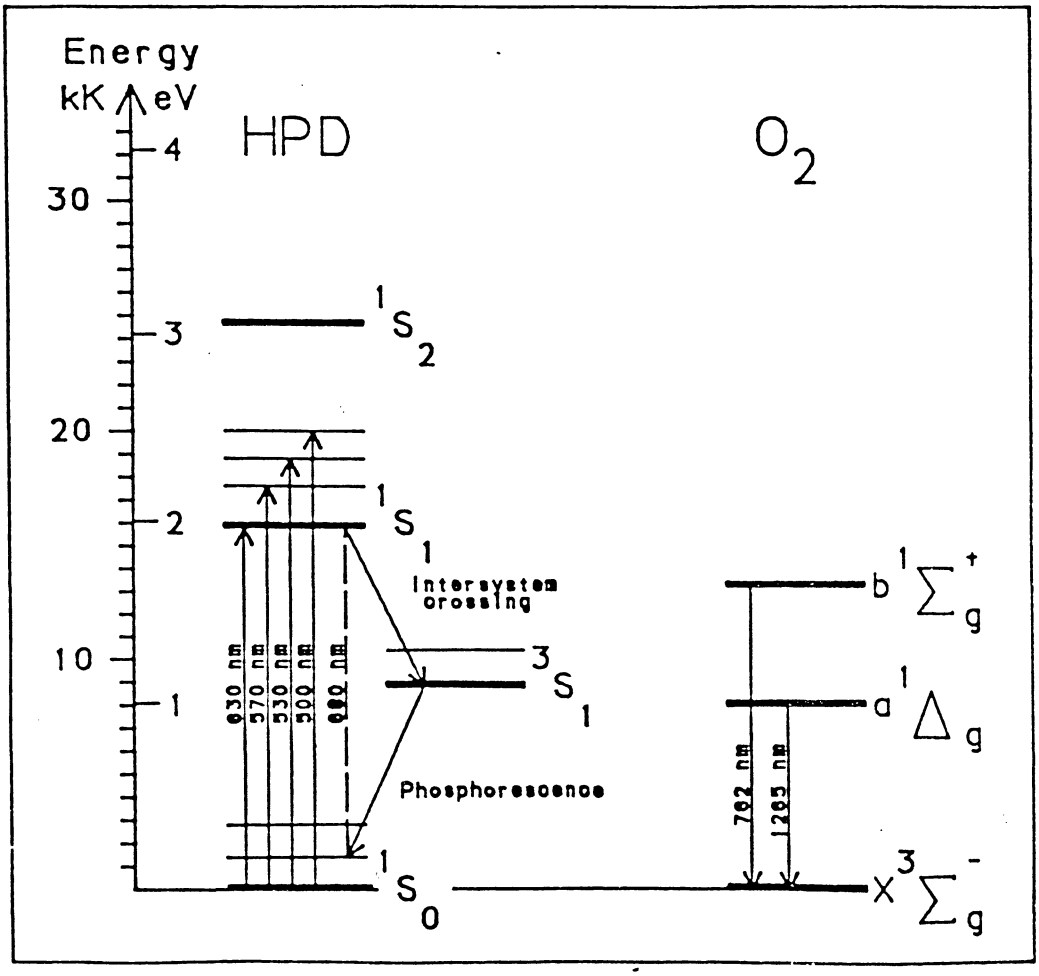


Fig. 5 Energy level diagram of HPD and molecular oxygen (Ref.3 Fig.5)

This energy transfer converts the normal oxygen, which is in a triplet state, to singlet oxygen which is very toxic to tissue. Irradiated HPD-containing tissue, i.e. tumour tissue, will be destroyed by the toxic singlet oxygen.

The fluorescence bronchoscope uses light of 405 nm (and/or 365nm). The measured fluorescence intensities are those marked A,B,D in Fig. 5. B is from the tissue itself and A from the HPD. D is measured to be able to "lift off" A from the background.

The ratio (A-D)/B is calculated and its variations show very clearly whether there is tumour tissue or not.

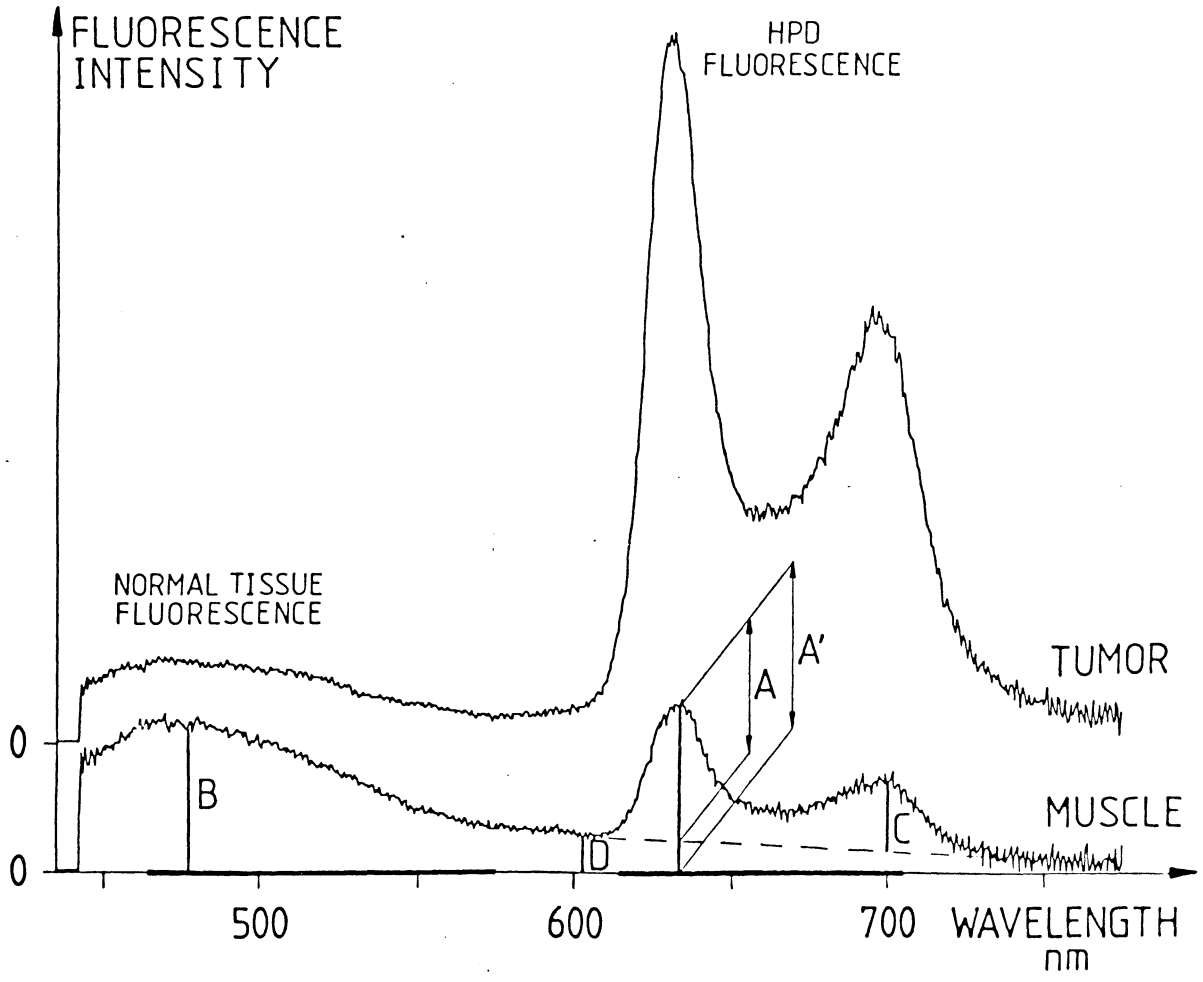


Fig. 6 Fluorescence spectra from normal and tumour tissue

PART 2

INSTRUMENT DESCRIPTION

INTRODUCTION

A general layout of the fluorescence-monitoring system is given in Fig. 7.

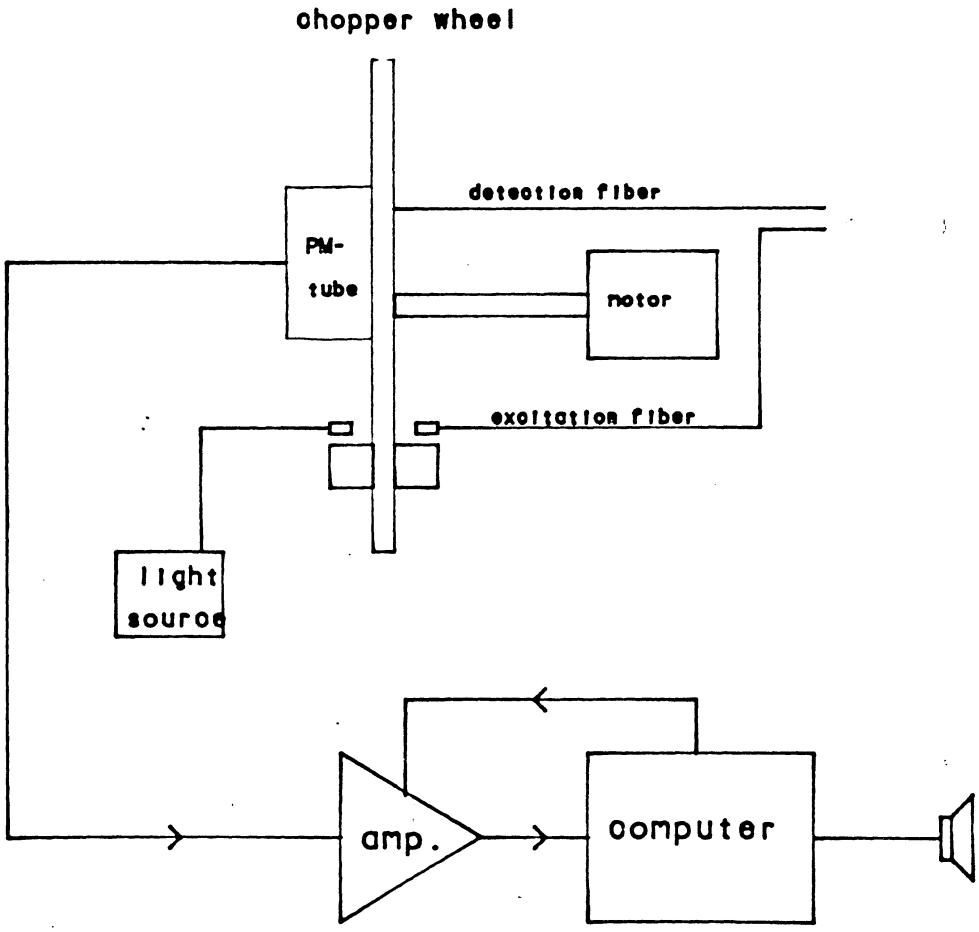


Fig. 7 Fluorescence-monitoring system (principal scheme)

THE CHOPPER WHEEL AND LIGHT SOURCE

The chopper wheel has been designed by Stefan Andersson at the Dept. of Physics. The main features are shown in Fig. 8.

The outer circle is for illumination, either with white light (the two big sector holes), with 405 nm (3 holes) or 365 nm (3 holes). The possibility of two excitation wavelengths is of importance since the response of the HPD fluorescence is different at 405 and 365 nm. In the first tests, however, we used only 405 nm.

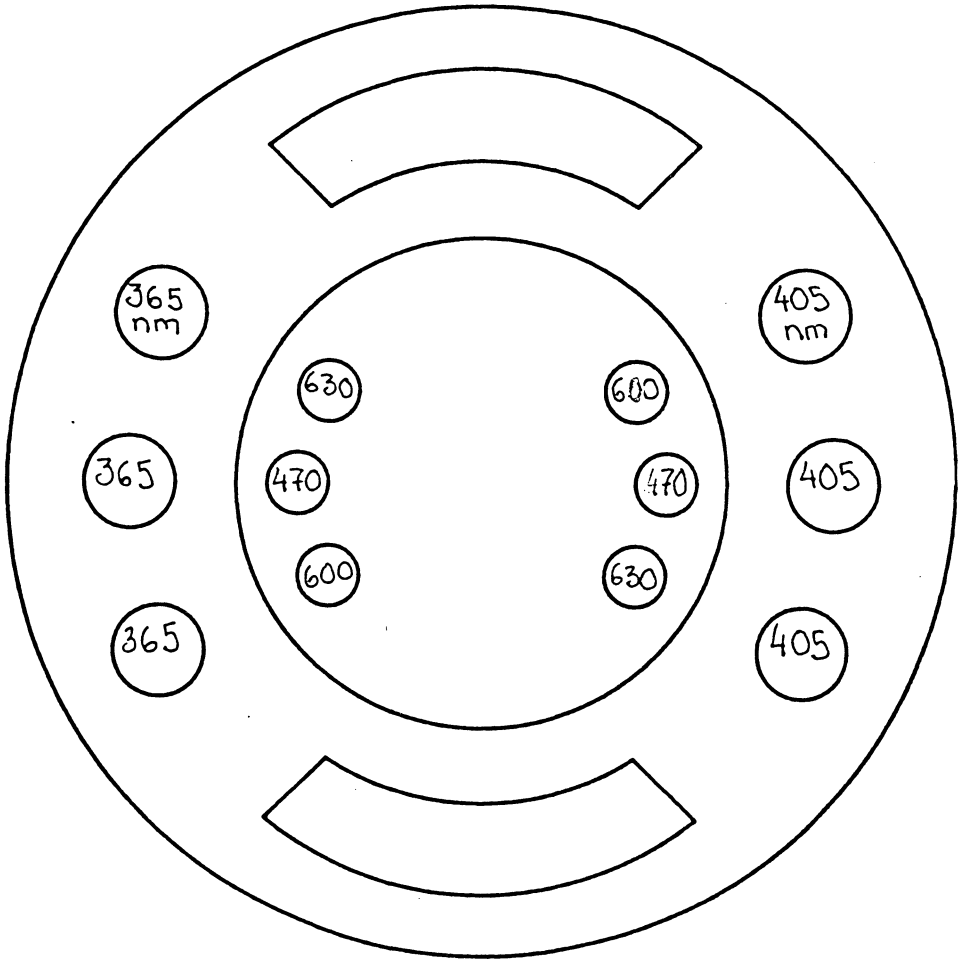


Fig. 8 The chopper wheel

The inner circle is for detection of the fluorescent light. The detection is made with 3 different filters, as described in the theory section. When white light is transmitted through the outer circle, no detection is made. Before each illumination the background light is measured. The background is then subtracted from the fluorescence light. The chopper wheel is driven by a motor which rotates at 16 2/3 Hz. This means that one turn takes 60 ms.

Each filter is read for 0.6 ms and each background also for 0.6 ms. The white light illuminates twice per revolution, 15 ms each, which should be enough for the doctor who is looking through the bronchoscope.

To give the computer a starting signal before the first filter, a photodiode and a phototransistor are used (Fig.9). They are situated in a specially designed hole in the wheelhouse, facing the rotating wheel. The edge of the wheel is black except for one place where it is metallic. Here the light from the diode is reflected onto the phototransistor. The signal from the phototransistor is then connected to the computer. The signal becomes high when the reflecting part on the wheel passes the phototransistor, otherwise it is low.

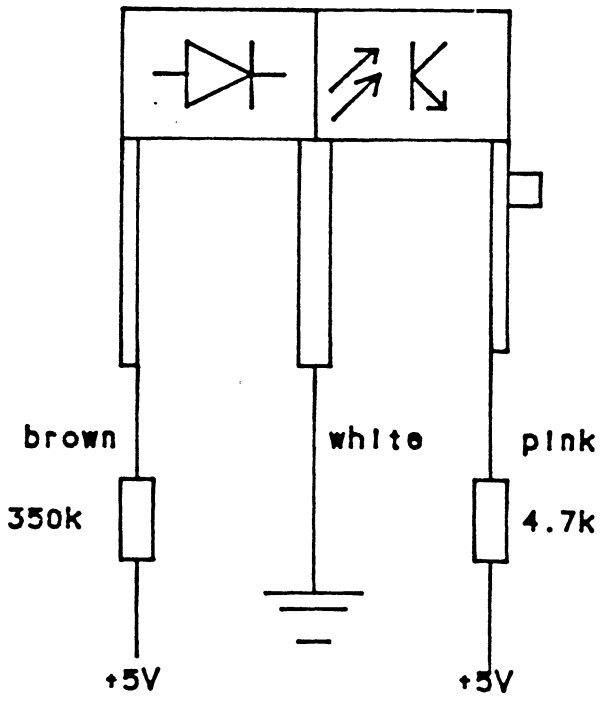


Fig. 9 The photodiode and phototransistor SFH900

The light for illumination comes from a high-pressure mercury lamp via a fibre (Fig.10). The light then goes, via a lens through a filter or as white light via another lens down into the bronchoscope.

From the bronchoscope another fibre, the detection fibre, goes back to the wheel. The detected light passes through the detection filters and out of the other side of the wheel house through a small hole to the PMT, which gives the computer the detection signal.

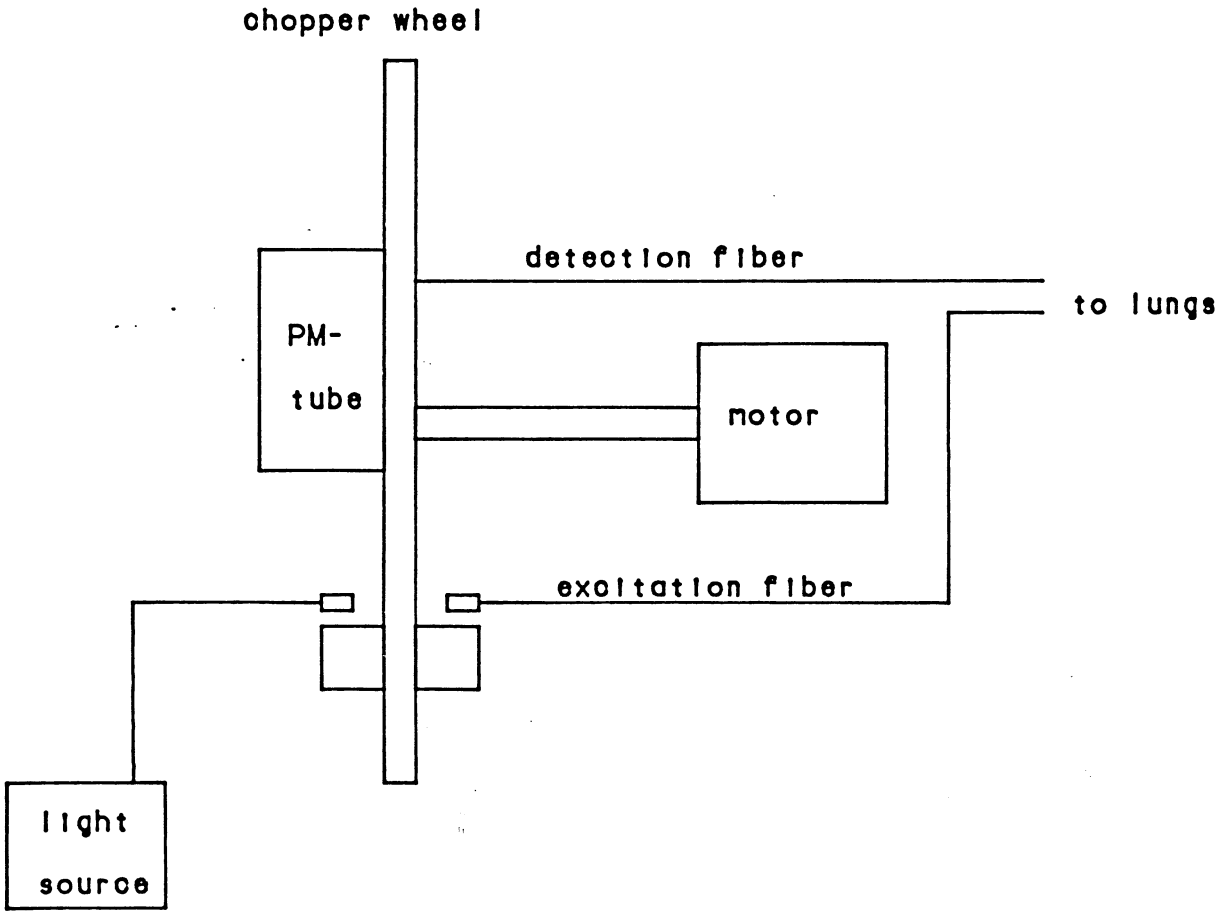


Fig. 10 The chopper wheel and its fibers

THE COMPUTER

To achieve flexibility at low cost a small mini computer, Sinclair Spectrum, was chosen. The Sinclair Spectrum has 48k memory, which is more than adequate, and is easily equipped with an input-output control unit, via the 52-pin user port. It also has a sufficiently high clock frequency to ensure high computational speed if assembly routines are used.

The computer is controlled by a Z-80 processor for which assembly language is used. Fast assembly routines are used so that as many measurements as possible can be made during a short time. The program is stored on tape, using a normal tape recorder, as part of a BASIC program, which is loaded and run as usual with the LOAD and RUN commands.

Another advantage of the mini computer is that it has a sound processor of its own. This can be programmed to give any tone at any length and other sounds. Fortunately, it is possible to call the sound subroutine from an assembly program, in BASIC it would simply take too much time. Due to the possibility of setting the length of the tone, the sound can be adjusted to the amount of time given.

For testing and programming purposes, a normal TV can be used as a monitor. This is then connected to the computer via the TV output of the computer.

Under working conditions no TV is needed (nor wanted because of the time loss it causes) since the sound is all that is required to present the result. (A printer may be connected to present the ratio).

The sound can be transmitted from a small loud-speaker in the computer, via the 'mic' output to headphones or through the loud-speaker in the tape recorder. In Fig. 11, the 52-pin user port and its connections are shown.

The computer is connected to the measurement and control card via the user port and a flat cable. The flat cable is kept rather short in order to minimize the influences from noise picked up from the surroundings. Due to the small currents passing through the cable it is very sensitive to such noise.

For further information about the computer, see Ref.8.

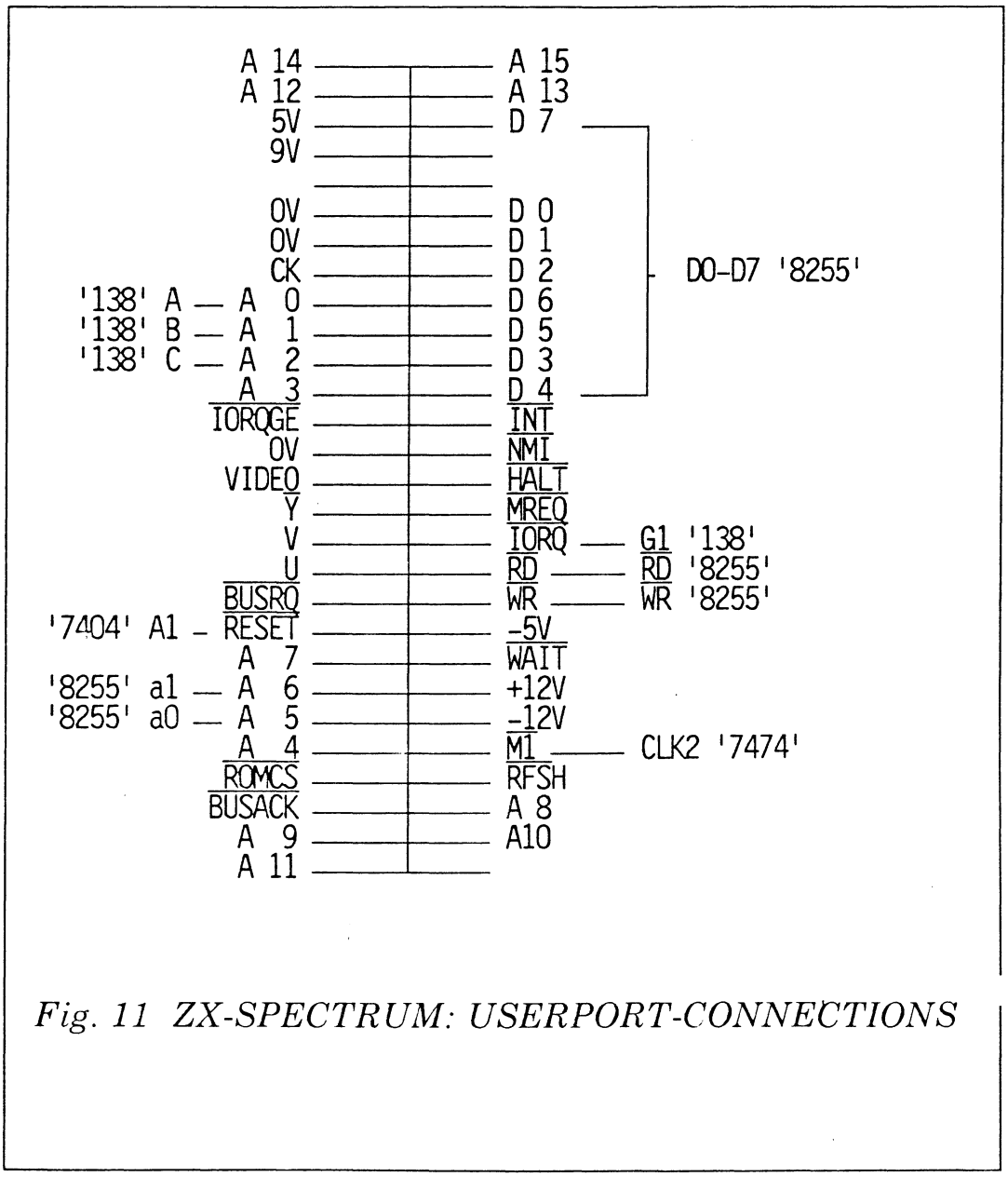


Fig. 11 ZX-SPECTRUM: USERPORT-CONNECTIONS

THE AMPLIFIER

The amplifier can be divided into three main parts.

PART 1

The first part consists of a negative current-to-voltage amplifier with the possibility of programming the gain factor suitably with four different resistors. The resistor chosen can easily be switched in manually, thus providing some flexibility as regard to different strength in the signal from the photomultiplier tube (PMT).

The strength of the signal is dependent on the fluorescent object, the PMT, the light source, the fibres etc. Thus it may be necessary to change the resistors depending on the instruments available, but when one of the resistors has been chosen it should not be necessary to change it again. The changes in signal due to the distance between fibre tip and object are handled by the computer-controlled second part.

The operational amplifier chosen for the first step is a TL081, which is a BIFET amplifier. It has the advantage of high output current , very high input impedance and low offset. A schematic diagram can be seen in Fig. 12.

The negative input is connected to the PMT. Between pin 1 and pin 5, a potentiometer (pot 1) is adjusted so that no offset gain will occur.

Between pin 2 and pin 6, one of the resistors is to be selected thus giving the desired gain.

For calibration the input can easily be connected to one of two resistors (R5,R6) connected to +5 Volt thus simplifying the calibration.

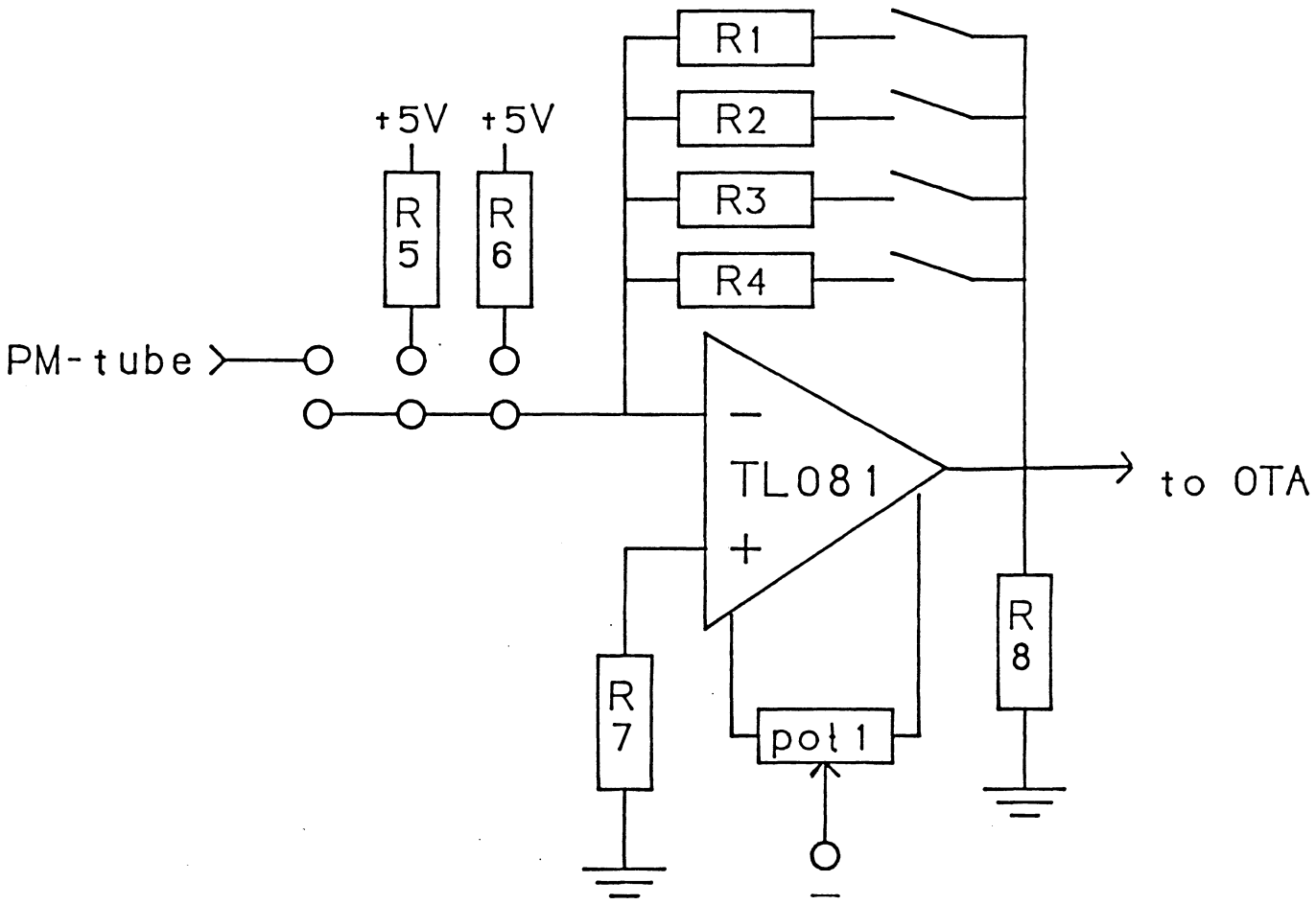


Fig. 12 The amplifier: part 1

R1 = 100 k

R2 = 56 k

R3 = 10 k

R4 = 1 k

R5 = 560 k

R6 = 56 k

R7 = 10 k

R8 = 1.2 k

POT1 = 10 k

PART 2

The second part of the amplifier is the computer-controlled OTA (CA3080). It can be studied in Fig.13.

The OTA, Operational Transconductance Amplifier, is an amplifier which can be gain-controlled over several decades with a programming current, the Amplifier Bias Current (Iabc).

In this case the amplifier operates in a gain range between 1 and 100.

This provides us with a powerful and easy way to adjust the gain, by software, so that the intensity fluctuations depending on the distance between the fibre tip and the object can be handled and to give maximum measurement accuracy by using the capacity of the A/D-converter at maximum.

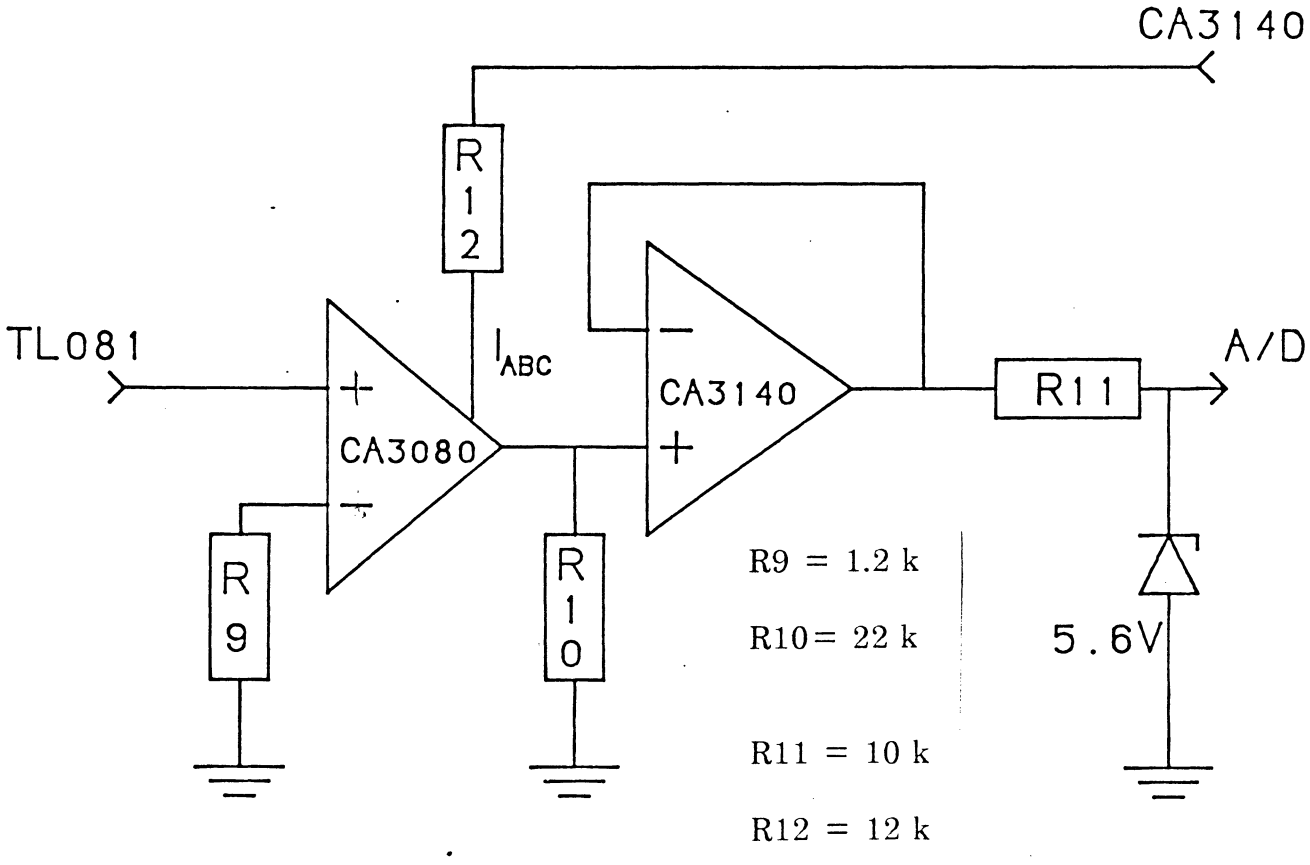


Fig. 13 The amplifier: part 2

For the OTA used as in Fig. 14 the formula

$$A_u = g_m x R_L$$

applies.

The g_m factor is taken from Fig. 15d, which shows gm as a function of Iabc. In this case Iabc varies between aproximately 5 and 500 uA, which gives a gain between 1 and 100. The resistor, R10, after the OTA has been chosen according to Fig.15b, which shows the peak output current vs. the amplifier current, to ensure that no high voltages which could damage the A/D-converter will occur.

To adjust the signal from the OTA to the CMOS-A/D-converter a buffer consisting of one operational amplifier (CA 3140) is needed. The signal then goes from the buffer to the A/D-converter via R11 to ensure that no high currents will flow. The 5.6V Zener diode is there to ensure that no high voltages accidently destroy the A/D-converter or the computer.

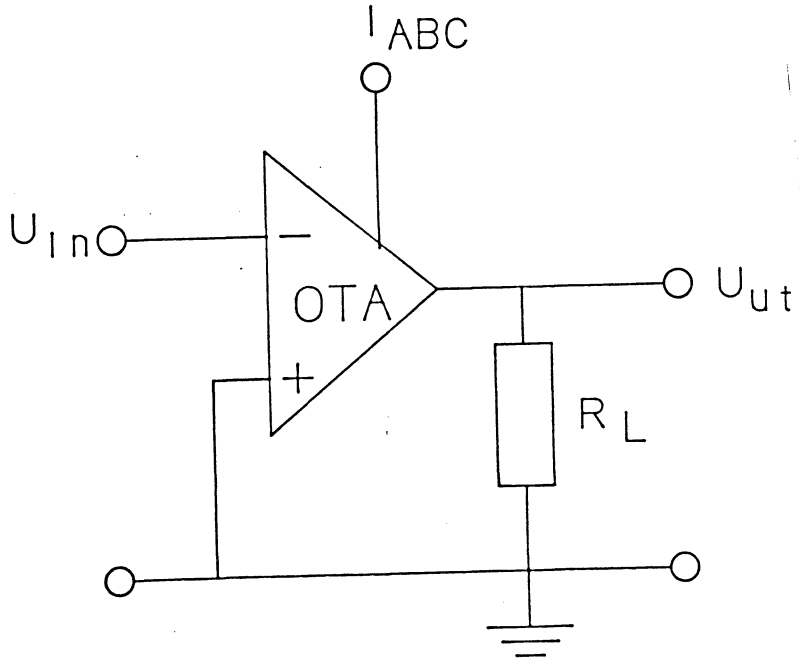
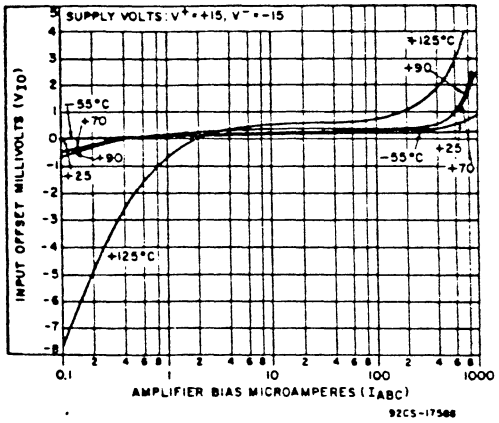
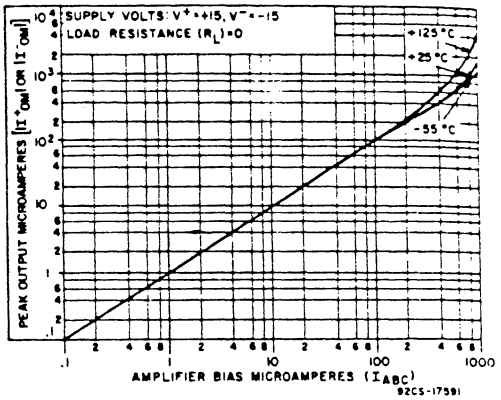


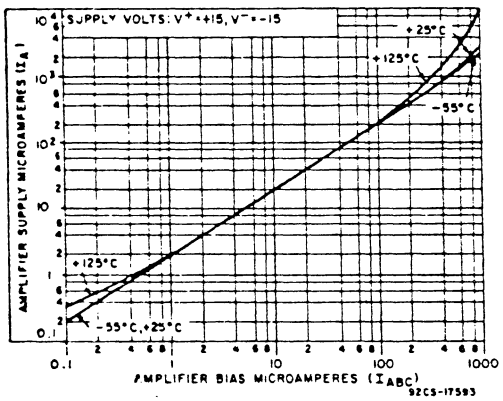
Fig. 14 The OTA used as an inverting amplifier



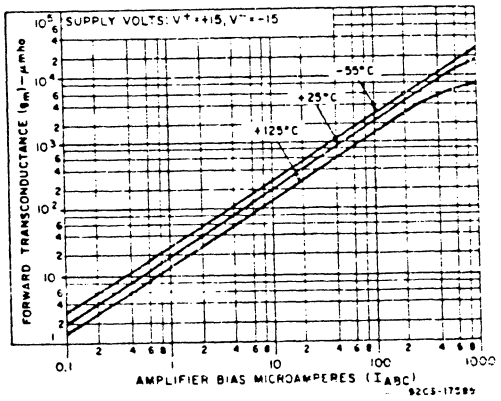
a *Input offset voltage vs. amplifier bias current.*



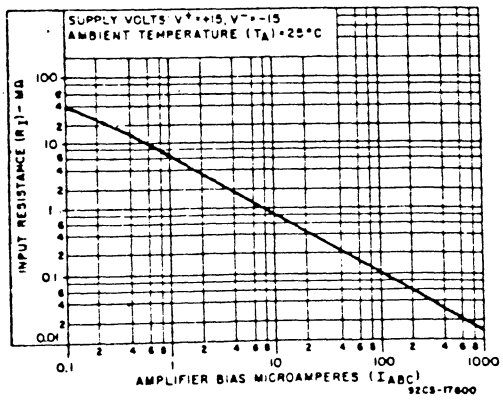
b *Peak output current vs. amplifier bias current.*



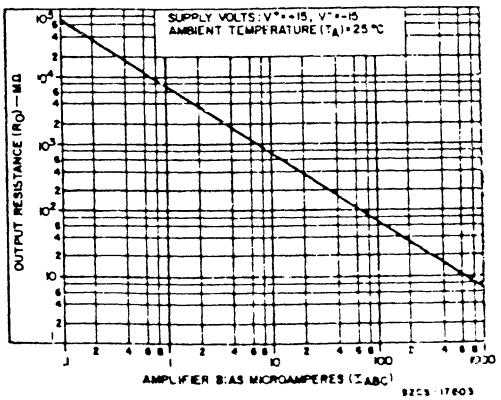
c *Amplifier supply current vs. amplifier bias current.*



d *Transconductance vs. amplifier bias current.*



e *Input resistance vs. amplifier bias current.*



f *Output resistance vs. amplifier bias current.*

Fig. 15 Data for the OTA CA3080

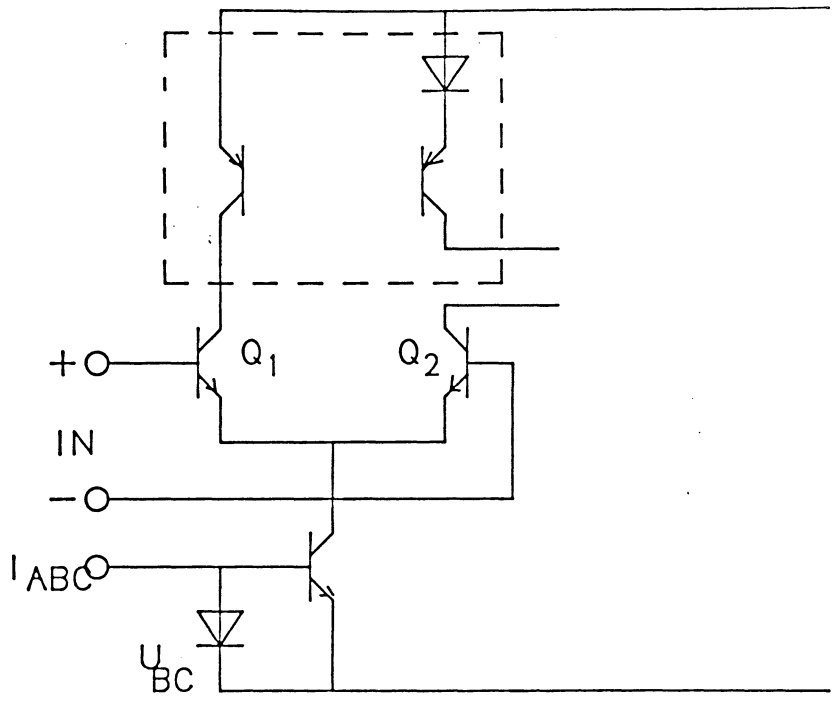


Fig. 16 The entrance step of the OTA

PART 3

The third part, shown in Fig. 17, is used for precise control of the OTA.

It is designed to make it possible to determine exactly how much one more bit from the computer should increase the gain in the OTA.

In this case it was chosen that one bit from the computer would give a 50% increase in gain of the original signal.

Thus, if we assume that the incoming signal has the value of 1V, then one bit from the computer would give the value 1.5V after the OTA and ten bits would give the value 5V. Another bit to these 5V would not give 7.5V, but 50%increase from the original signal, that is the 1V, which would give a sum of 5.5V.

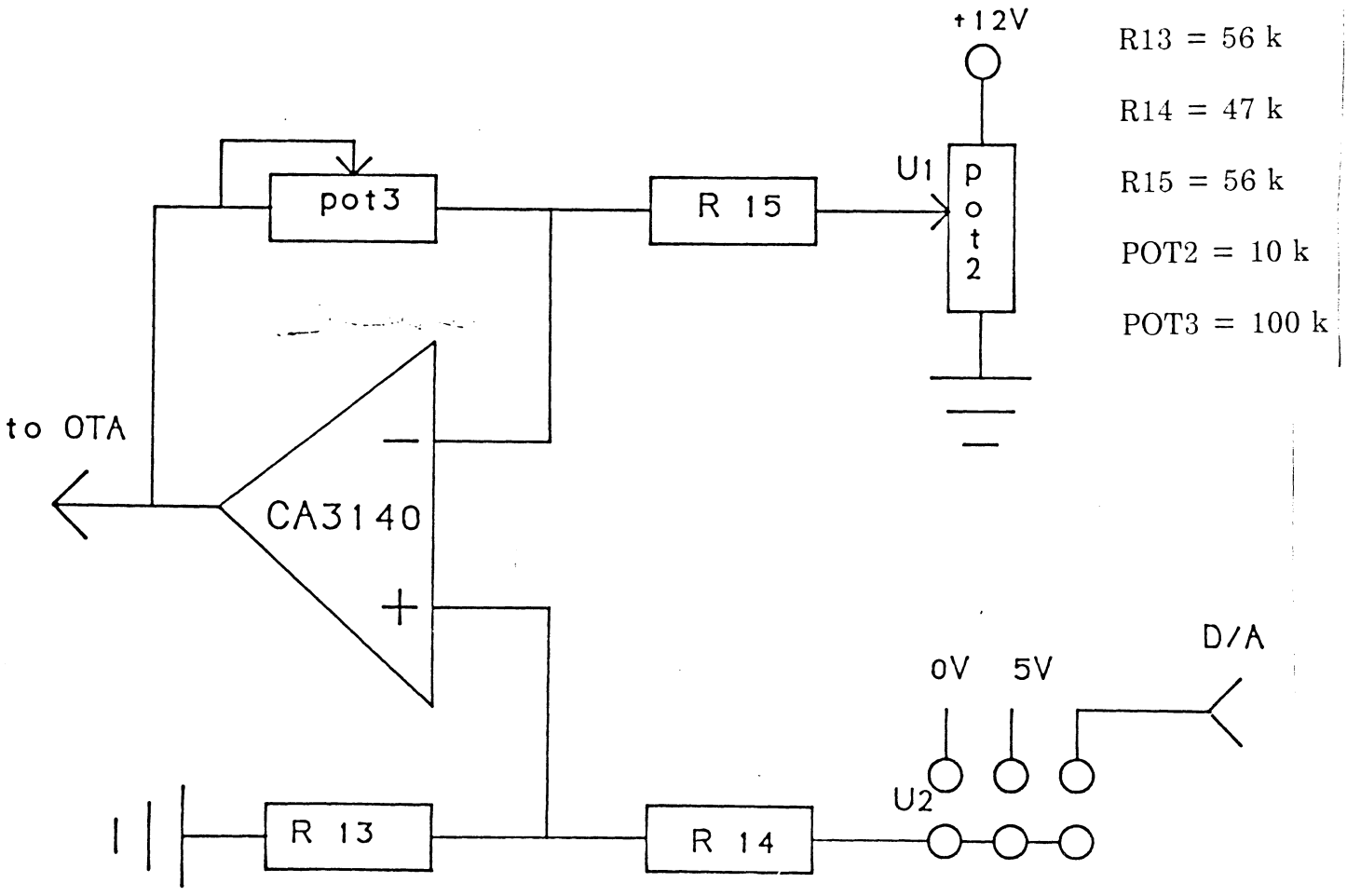


Fig. 17 The amplifier: part 3

The amplifying step used here is a so-called 'subtracting' amplifier (see Fig 18).

When An is not equal to Ap the equation giving Ua becomes rather complex.

Ua=(1+An)/(1+Ap)xApxU2-AnxU1 (Ref 9)

Fortunately, in our case An is approximately equal to Ap and a simplified equation

Ua=A(U2-U1) where A=An=Ap=total gain

can be used.

When U2=0V the amplifier bias current, Iabc, should be 5 uA (see Fig 15d) to give a gain of 1, i.e. the signal should not be amplified.

To calculate U1, which is set by the potentiometer pot2, and A which is set by pot3 one must also study the schematic diagram of the OTA, Fig. 16, and note that the voltage giving Iabc is over R12.

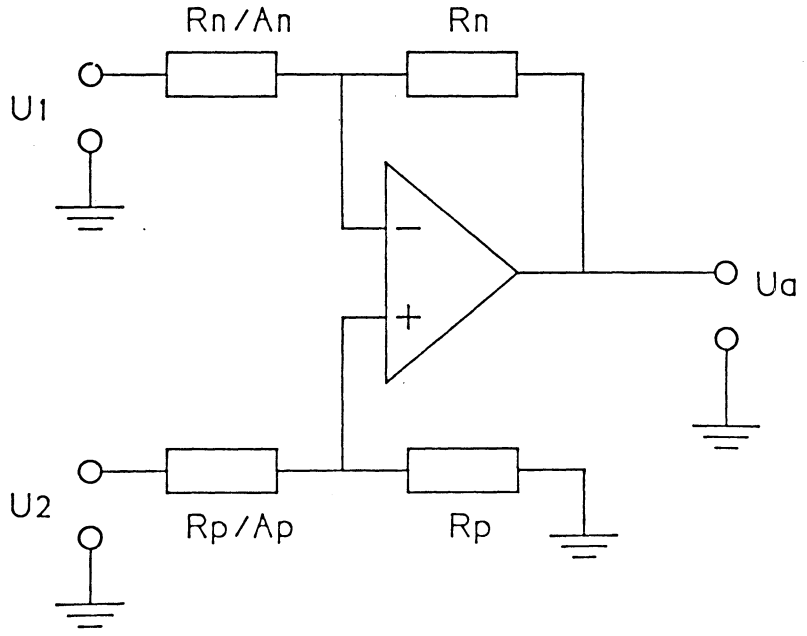


Fig. 18 A subtracting amplifier

The values given here are only theoretical ones and adjustments are always necessary in practice because of the wide tolerance of the components involved, for instance the resistors. Therefore a precise calibration following the scheme given below has to be made.

*Use the resistor R5 to give a signal to the first amplifier.

*Use the resistor R4 to set the minimum gain.

*Using the computer, set the gain of the OTA at 200 bits. This should give a gain of 100.

*Measure the signal before and after the OTA, and set the gain to 100 using pot2 (='gain').

*Using the computer, set the gain of the OTA at 0 bit. This should give a gain of one.

*Measure the signal before and after the OTA and adjust the gain to one using pot3 (='zero adj').

*This procedure has to be repeated until the desired values are achieved.

This calibration is only necessary after changing a component or other service work has been done. To examine the linearity of the amplifier the values given by the computer (by reading the A/D) were measured for different values of gain by the OTA. The result is shown in Fig.19. The linearity is very good except at very small voltages, which should be avoided.

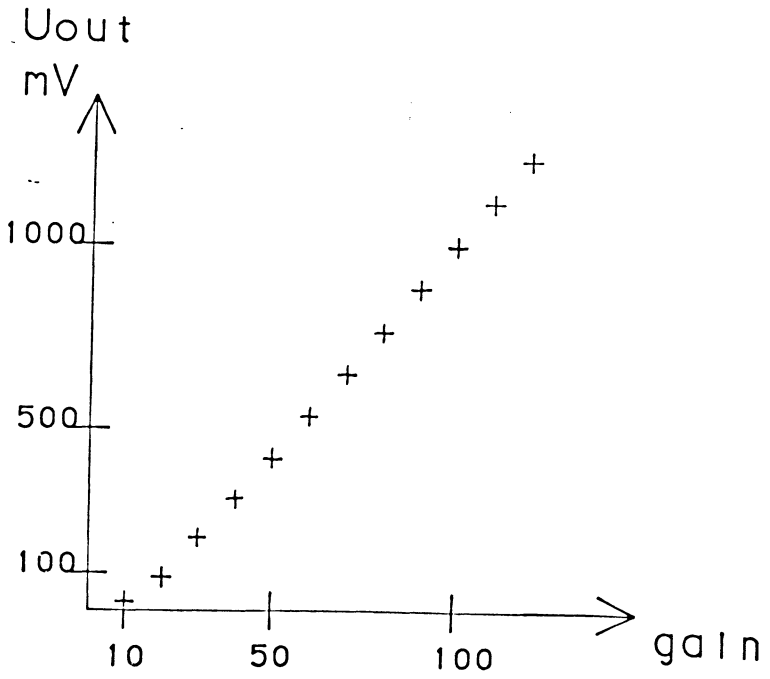


Fig.19 The linearity of the amplifier. Uin=10mV

THE 8255A

The device chosen for I/O control is the 8255A programmable peripheral interface. The 8255A is a general purpose progammable I/O device, designed for use with microprocessors. It has 3 different 8-bit ports, A, B and C, which can be used in input or output mode. Port C can be divided into 2 4-bit ports, C-low and C-high. The 8255A can be used in 3 different modes. Mode 0 is 'Basic input/output', mode 1 is 'strobed input/output' with "handshaking" signals, and mode 3 is used for communication with an 8-bit bus. It is here used in mode 0, with port A as an input, port B as an output and one part of port C as an input.

The pin configuration and connections can be studied in Fig.20.

PORT A (pin 1-4 + 37-40)

connected to the uPD7003 A/D-converter. The measured signal from the PMT is received here after suitable amplification.

PORT B (pin 19-25)

connected to the uPD7011 D/A-converter. This is used in output mode to control the OTA.

PORT C-low (pin 14-17)

The lower part of port C is used as an input port for the "start" signal from the chopper wheel. Port C1 is connected to the phototransistor and goes high when the "start" signal arrives. Port C2 and C0 are connected to ground. Port C3 is optionally connected to ground or used as one more input port (for instance as a "switching function" signal).

PORT C-high (pin 10-13)

not used and therefore free for further desired functions.

CS (pin 6)

connected to the 74138 decoder, which goes low to enable communication between the 8255A and the CPU.

A1, A0 (pin 8,9)

connected to the address pins A6 and A5 from the computer, and used to control the selection of the ports or the control word.

D0-D7 (pin 27-34)

connected to the computer's DATA-BUS.

RD, WR (pin 5, 36)

connected to the computer's RD and WR.

RESET (pin 35)

connected, via the 7404 inverter to the computer's RESET.

VCC (pin 26)

connected to +5V on the computer side.

GND (pin 7)

connected to the computer ground.

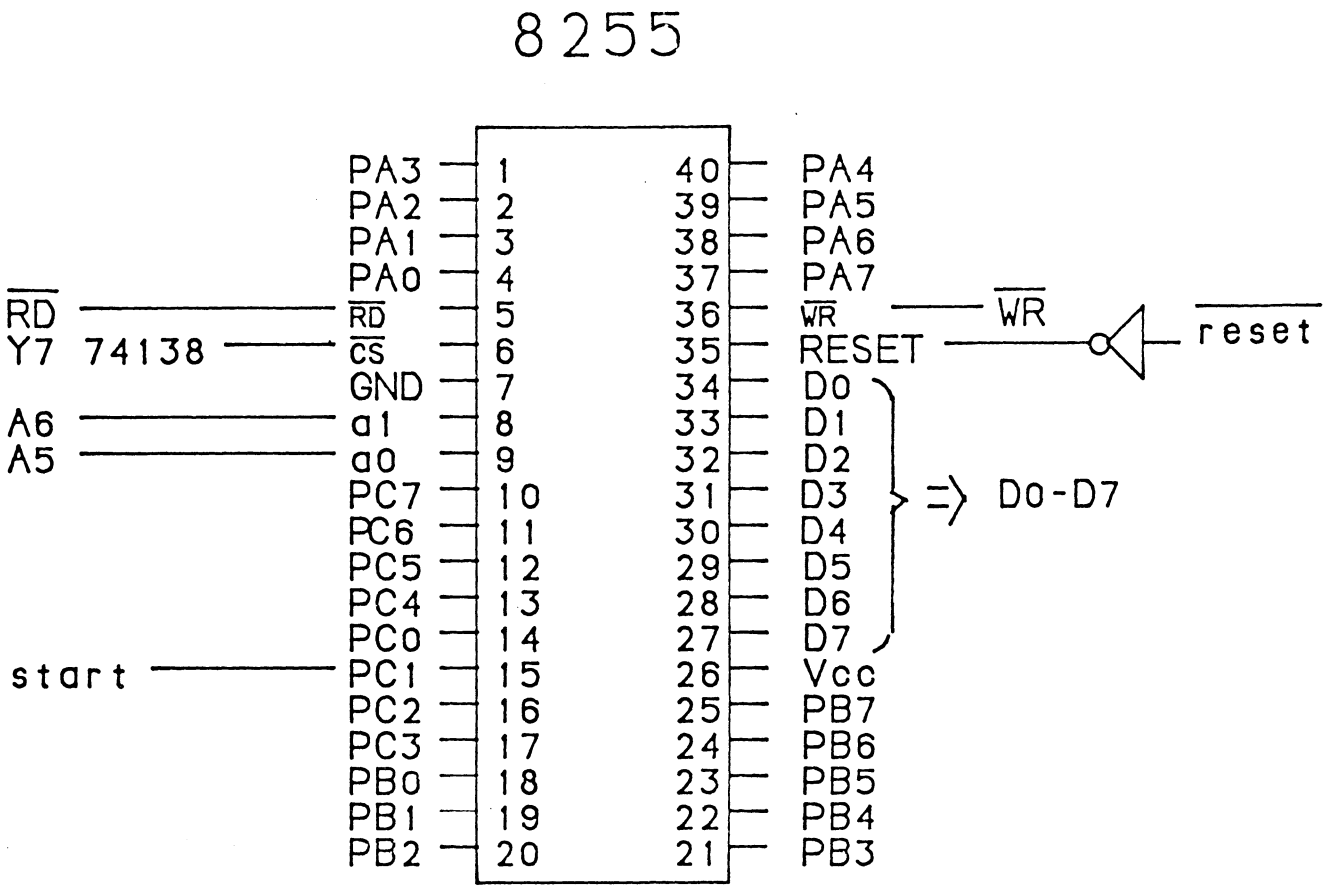

8255
PA3 1
PA2 2
PA1 3
PA0 4
RD 5
CS 6
GND 7
a1 8
a0 9
PC7 10
PC6 11
PC5 12
PC4 13
PC0 14
PC1 15
PC2 16
PC3 17
PB0 18
PB1 19
PB2 20
40 PA4
39 PA5
38 PA6
37 PA7
36 WR
35 RESET
34 D0
33 D1
32 D2
31 D3
30 D4
29 D5
28 D6
27 D7
26 Vcc
25 PB7
24 PB6
23 PB5
22 PB4
21 PB3
RD
Y7 74138
A6
A5
start
WR
reset
D0-D7

Fig. 20

The addresses used have been selected from the free addresses in the computer (Fig.21).

ADDRESSES

dec	bin (A0–A7)	hex	a1,a0 (=A6,A5
31	00011111	1F	00
63	00111111	3F	01
95	01011111	5F	10
127	01111111	7F	11

Fig. 21

The A1 A0 codes are shown in Fig.22, selected according to the data sheets.

A1	A0	INPUT OPERATION
0	0	port A - data bus
0	1	port B - data bus
1	0	port C - data bus
		OUTPUT OPERATION
0	0	data bus - port A
0	1	data bus - port B
1	0	data bus - port C
1	1	data bus - control

Fig. 22 Address-codes for input/output operations (8255A)

The control word is set from the scheme in Fig.23.

D0	port C (lower):	1 = input 0 = output
D1	port B	1 = input 0 = output
D2	mode selection	0 = mode 0 1 = mode 1
D3	port C (upper)	1 = input 0 = output
D4	port A	1 = input 0 = output
D5 D6	mode selection	00 = mode 0 01 = mode 1 1x = mode 2
D7	mode set flag	1 = active

Fig. 23 Controlword (8255A)

For further information consult the data sheets.

THE 74138 DECODER

The 74138 is a 3-to-8 line decoder which is used to give the 8255A a Chip-Select signal and thus enable communication between the CPU and the 8255A. Fig. 24 shows the pin configuration and connections.

A, B, C (pin 1,2,3)

The connections to A0, A1 and A2 are chosen from the addresses (Fig.12) to give the binary number 111 which selects output Y7.

G1 (pin 6)

connected via the inverter to IORQ on the computer. G1 must be high to enable the 74138.

G2A, G2B (pin 4,5)

connected via the inverter to address A0, which will ensure a low on these inputs when the required address is given.

When G1 is high and G2A, G2B are low the Y7 output will be low if the right address is given, thus enabling communication.

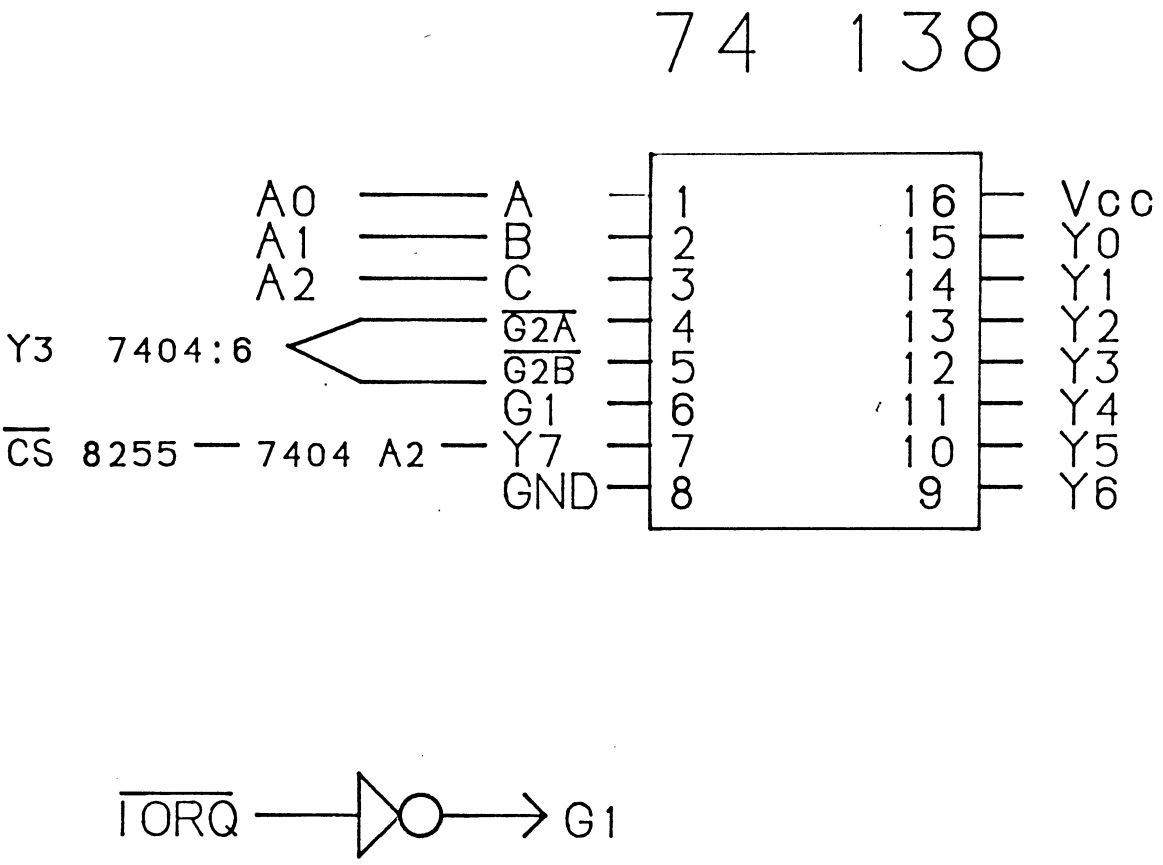

74 138
A0
A1
A2
Y3 7404:6
CS 8255
7404 A2
A
B
C
G2A
G2B
G1
Y7
GND
1
2
3
4
5
6
7
8
16
15
14
13
12
11
10
9
Vcc
Y0
Y1
Y2
Y3
Y4
Y5
Y6
IORQ
G1

Fig. 24

THE A/D CONVERTER

To convert the analogue amplified signal from the PMT, the A/D converter uPD 7003 is used. The uPD 7003 is a high-speed 8-bit analogue-to-digital converter, using a parallel conversion technique, with a resistor network and 256 voltage comparators. The 8-bit digital output is a 3-state parallell output which allows it to interface easily with microprocessors. The conversion time is 4 us and the non-linearity 1.25 LSB max. It uses pipe-line processing and therefore outputs the converted data per clock cycle.

However, the clock from the computer is faster than the maximum clock frequency allowed for the uPD 7003, and so a special arrangement using the M1-signal has been made (Fig.25). The M1 signal is thus divided into four, using 2 D-flip-flops and then used as the clock signal for the uPD 7003. This ensures that no read errors will occur due to a too fast clock.

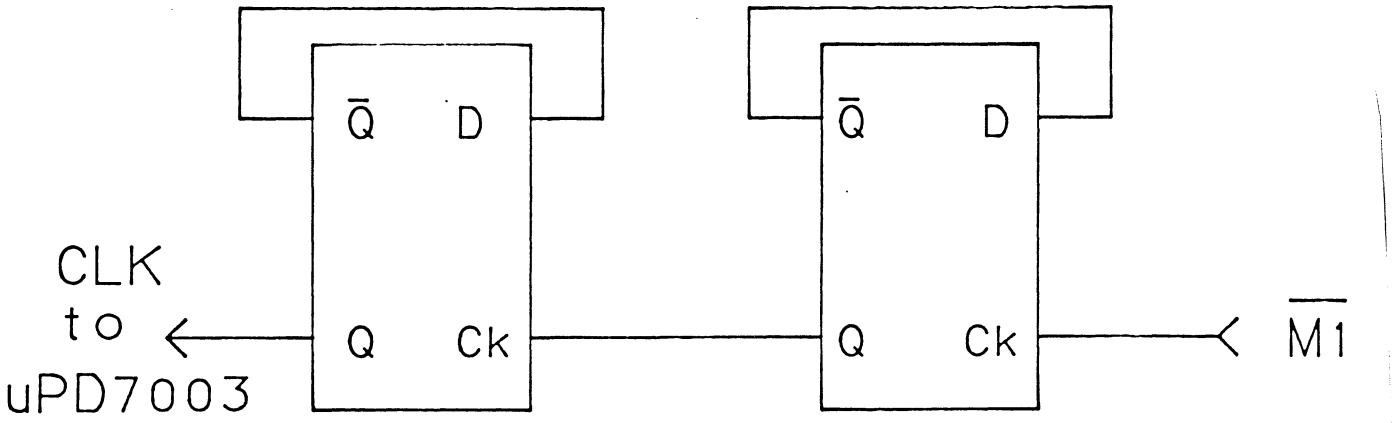


Fig. 25

In Fig. 26 the pin configurations and connections are shown.

D0-D7 (pin 24, 1-2, 4-8)

These data-outputs are connected to the 8255A port A.

MODE (pin 13)

Mode switch; according to data sheets connected to +5V for data refreshed with every clock pulse.

CS (pin 16)

connected to low for data read.

DACK (pin 17)

DMA-acknowledge; not used in this case and therefore connected to VDD.

VIN (pin 20)

Analogue input signal from the amplifier.

GND (pin 19,22)

Analogue ground is, in this case, the same as digital ground VSS.

VREFN (pin 21)

connected to ground since no external zero-adjustment is required.

For further information consult the data sheets.

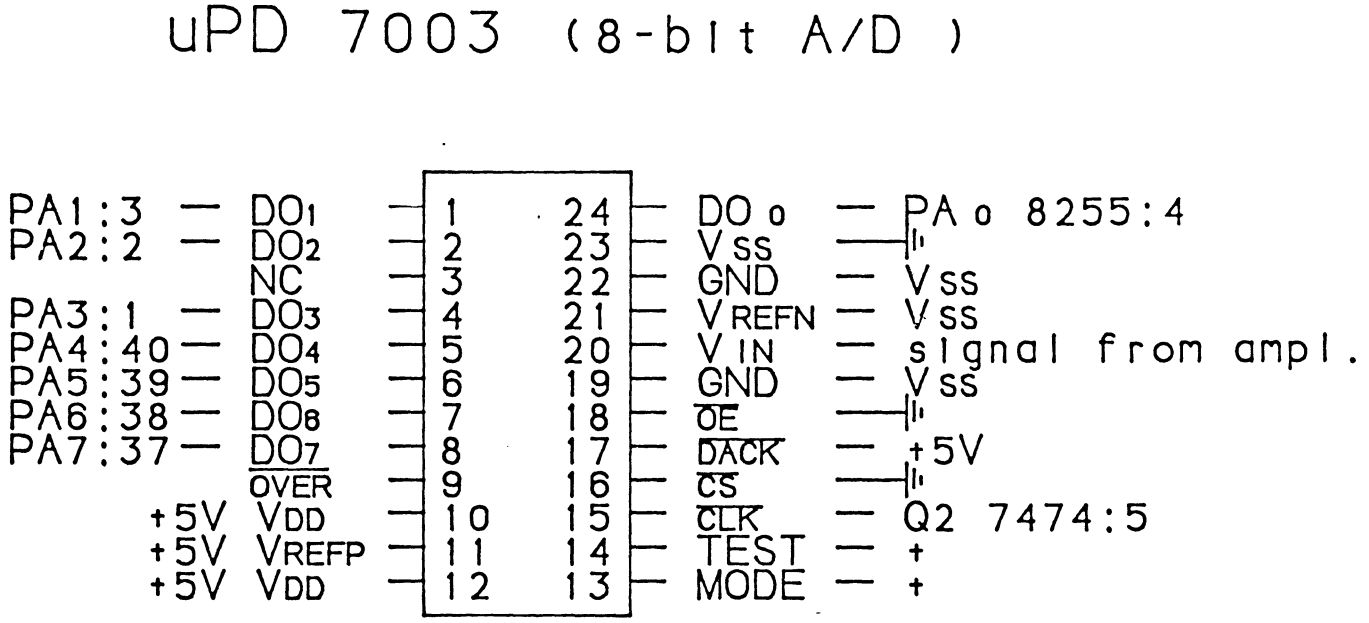

uPD 7003 (8-bit A/D)
PA1:3 — DO1 — 1
PA2:2 — DO2 — 2
NC — 3
PA3:1 — DO3 — 4
PA4:40 — DO4 — 5
PA5:39 — DO5 — 6
PA6:38 — DO6 — 7
PA7:37 — DO7 — 8
OVER — 9
+5V VDD — 10
+5V VREFP — 11
+5V VDD — 12
24 — DO o — PA o 8255:4
23 — Vss
22 — GND — Vss
21 — VREFN — Vss
20 — VIN — signal from ampl.
19 — GND — Vss
18 — OE
17 — DACK — +5V
16 — CS
15 — CLK — Q2 7474:5
14 — TEST — +
13 — MODE — +

Fig. 26

THE D/A CONVERTER

In order to be able to control the gain of the amplifier, a D/A converter is used to convert the digital signal from the computer to an analogue voltage which controls the OTA.

The uPD 7011 is an 8-bit D/A-converter with internal voltage reference. It can be used in either serial or parallel mode (here parallel mode is used). The non-linearity is 1.0 LSB max. The output is a complementary current output which, in this case, is converted to a complementary voltage output using two resistors (Fig. 27).

The pin configuration and connections can be seen in Fig.28.

D0-D7 (pin 10-17)

The digital DATA-inputs are connected to the 8255A port B.

WR (pin 2)

Connected to WR on the computer.

MODE (pin 3)

Low = parallel bus input mode
(high = serial input mode)

CS (pin 18)

Low to enable conversion.

Pins 4 to 8 are connected as in Fig. 27 to give a complementary voltage output according to the data sheets.

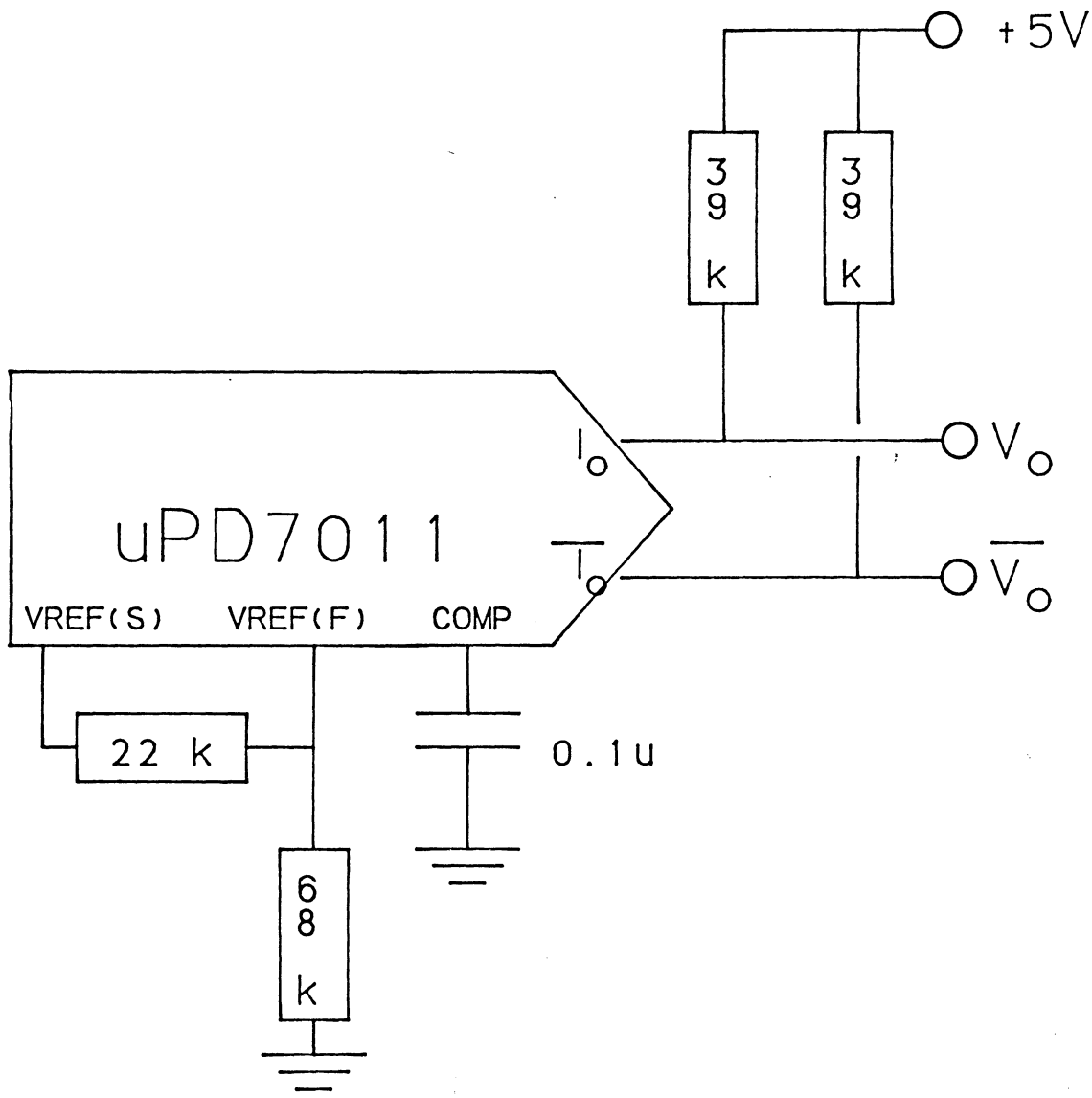

+5V
39 k
39 k
uPD7011
I_O
$\overline{I_O}$
V_O
$\overline{V_O}$
VREF(S)
VREF(F)
COMP
22 k
0.1u
68 k

Fig. 27

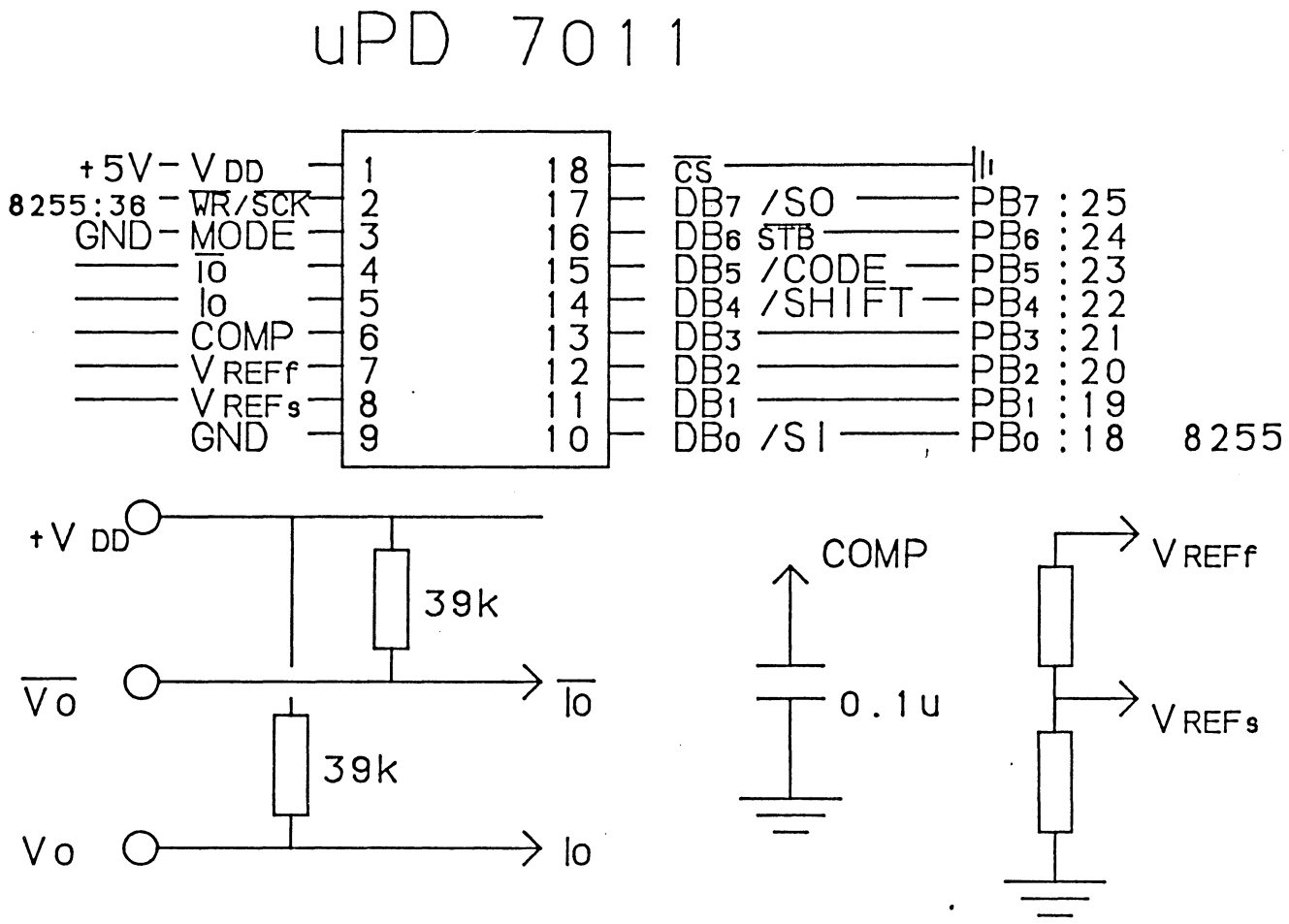

uPD 7011
+5V
VDD
8255:36
WR/SCK
GND
MODE
IO
Io
COMP
VREFf
VREFs
GND
1
2
3
4
5
6
7
8
9
18
17
16
15
14
13
12
11
10
CS
DB7 /SO
DB6 STB
DB5 /CODE
DB4 /SHIFT
DB3
DB2
DB1
DB0 /SI
PB7 : 25
PB6 : 24
PB5 : 23
PB4 : 22
PB3 : 21
PB2 : 20
PB1 : 19
PB0 : 18
8255
+V DD
39k
Vo
Io
39k
Vo
Io
COMP
0.1u
VREFf
VREFs

Fig. 28

THE INVERTER 7404

The inverter is necessary to invert some signals. The pin configurations and connections are indicated in Fig. 29.

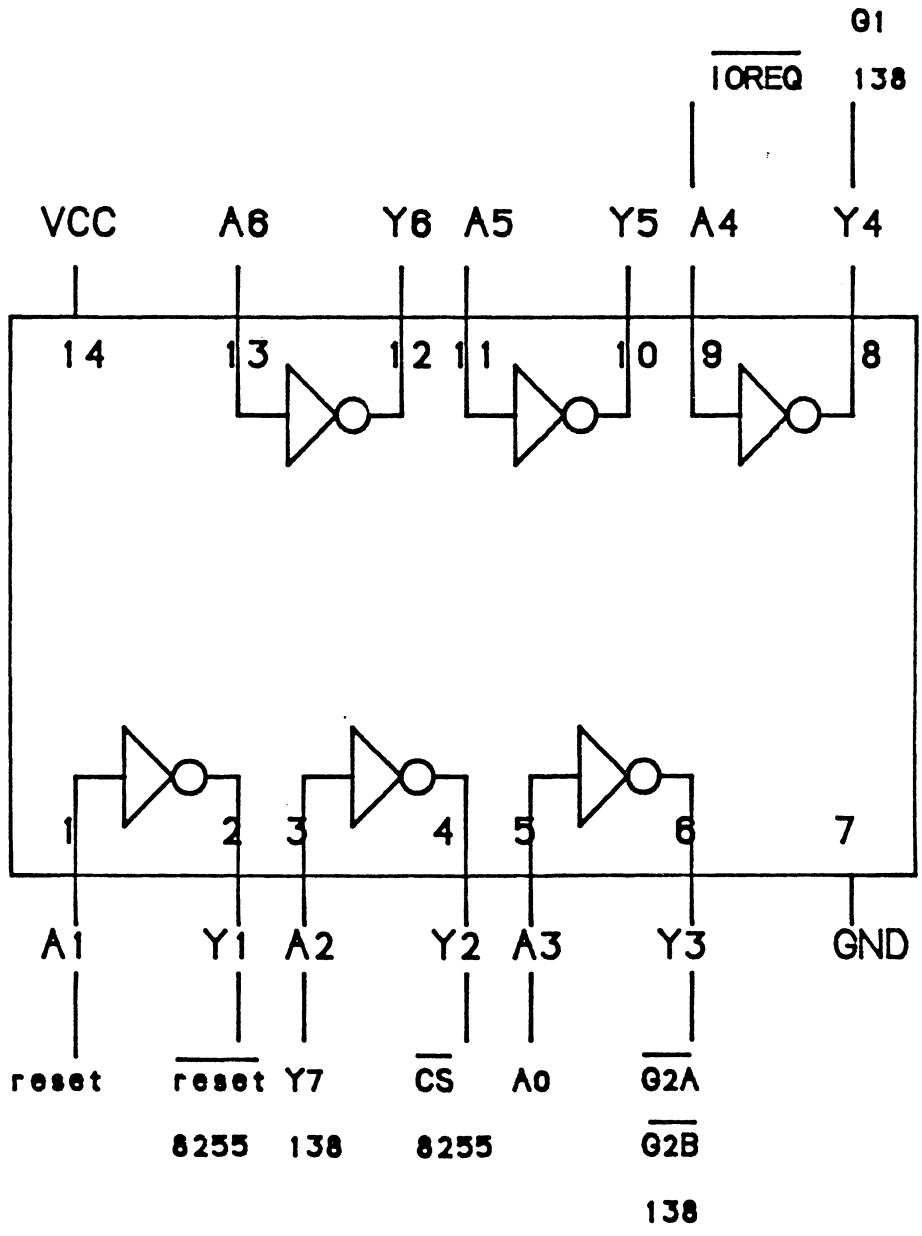


Fig. 29

THE DUAL D-FLIP-FLOP 7474

The 7474 is used, as described earlier, to divide the M1 signal by four, and thereby give an appropriate clock signal to the A/D-converter. The pin configurations and connections are shown in Fig. 30.

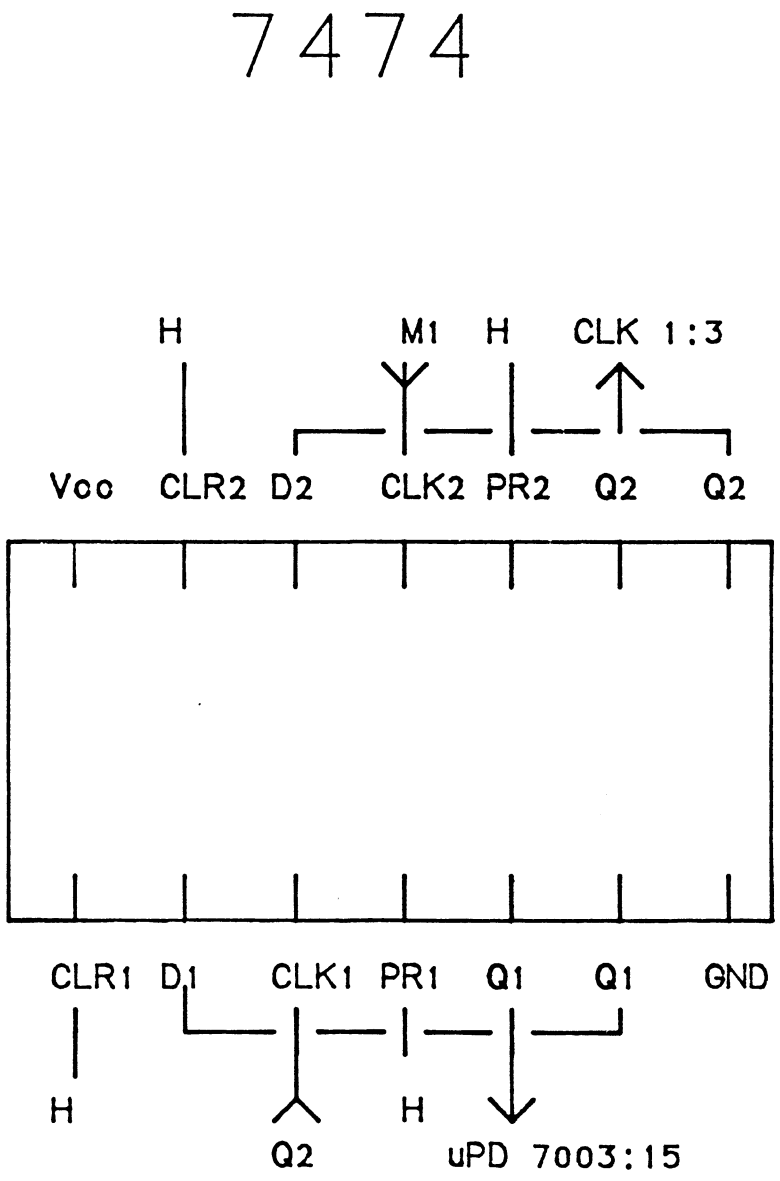


Fig. 30

THE VOLTAGE SUPPLY

The principle of the voltage supply can be studied in Fig. 31. The two big capacitors are to eliminate ripple, 50 Hz-variations and other undesired disturbances from the 220V mains. The smaller capacitors improve transient response for the voltage regulators. This voltage supply then supplies the socket for the PMT with +15V, the amplifying card with -8V and +12V and the photodiode and the phototransistor with +5V.

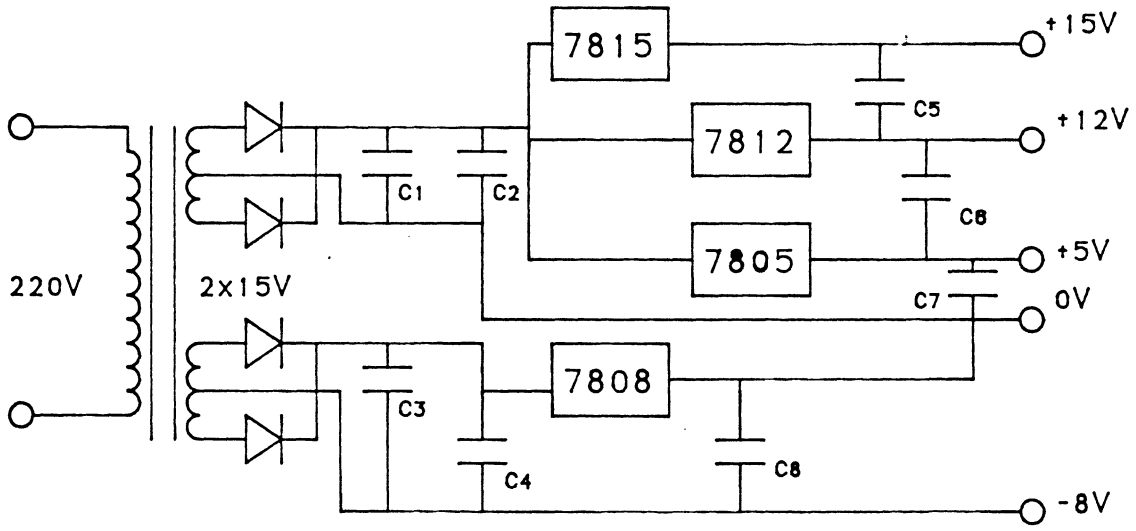


Fig. 31 The voltage supply

C1 = 2200 uF/25 V

C2 = 0.47 uF

C3 = 2200 uF/25 V

C4 = 0.33 uF

C5 = 0.1 uF

C6 = 0.1 uF

C7 = 0.1 uF

C8 = 0.1 uF

THE SOFTWARE

The main program can be divided into several shorter routines.

1. Define space and words

2. Initiate the 8255A. Here the control word, described in a previous chapter is set to make port A, and CL input and port B output.

3. Ready to start?
The computer waits for the starting signal from the photodiode.

4. Time delay. The program calls the time delaying loop TIME2 which takes 2 ms. This is the time it takes from the start signal to the commencement irst of the first background-reading.

5. Read background A.
The program calls the READ routine. This reads 32 values of the background takes the mean value and stores it in (BACKA). This takes about 0.6 ms.

6. Time delay: calls TIME1 which takes 1ms.

7. Reads filter A.
Calls the READ routine. This reads 32 values, takes the mean value and stores it in (RESA). This takes 0.6 ms.

8. Subtract background A.
Subtracts the background value from the fluorescence value and stores it in (RESA).

9. Gain control.
If the A signal is too low the computer calculates the increase in gain necessary to give a sufficiently high signal and sets the gain via the D/A-converter and OTA.

If, on the other hand, the signal is too high, the routine restores the gain.

10. The program then reads background and fluorescence values for filters B and D.

11. The value A-D is calculated.

12. A-D is divided by B which gives the resulting ratio.

This is performed by a subroutine (DIV) which divides the C register with the B register, rounds off the result and stores it in register C.

13. The sound subroutine

If the value of the quotient is less than or equal to 1 a constant low tone will be heard.

If not, a tone with frequency proportional to the quotient is heard. The higher the value of the quotient the higher the tone.

The computer's internal SOUND routine is available at address 03B5 through the command "call 03B5".

The frequency of the tone is set by the HL-register and the length by the DE register. The other registers must be saved on the stack before calling this subroutine.

14. Go back to 'ready to start?'.

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