# CONSTRUCTION OF A FLUORESCENCE BRONCHOSCHOPE FOR DETECTION OF EARLY LUNG CANCER

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# I LUMENS DALAR

Ack du skrämmande djuplila ljus
om blott du ville excitera
I lysande fiber ett stilla sus
när strålar av ljus penetrera

I skimrande grönt och glödande rött
vävnaden tindrar och blänker
Detta flämtande ljus varför är det så sprött
kan undra vad strålarna tänker

Luminiscens!

Från dunkla kamrar, svartaste svart
de smyger så stillsamt, förunderligt lätt
Vart ska de hän man frågar sig vart?
där fåtal blir mångfald och glest det blir tätt

I visdomens mångbenta bräckliga tempel
förtäljes om ljuset som glittrande tändes
Budbärare sätt nu raskt Eder stämpel
Och du klocka ljud! Och det hördes och kändes

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ABSTRACT

The aim of our work was to construct a fluorescence bronchoscope for in vivo detection of small amounts of Hematoporphyrin derivative (HPD). HPD gathers selectively in tumors after intravenous injection.

# PART 1

# THEORY

## 1. FLUORESCENCE IN MOLECULES

Luminescence is the common name of all light emitting processes, and photoluminescence is the name of light emission caused by absorption of light. Photoluminescence is traditionally divided into phosphorescence and fluorescence.

In a molecule there are more available energy states for the electrons than in an atom, due to the vibrational and rotational movements. As these energy splittings are of lower magnitude than the electronic ones, the vibrational states are given by splitting the electronic states, and the rotational states by splitting the vibrational states. For molecules in solution the rotational levels are broadened and merge together into one continuous band for each vibrational level. This can also be true for vibrational states in complex organic molecules, like Hematoporphyrin, and one obtains energy bands rather than levels. This makes the fluorescence spectrum continuous instead of discrete. Fig. 1.

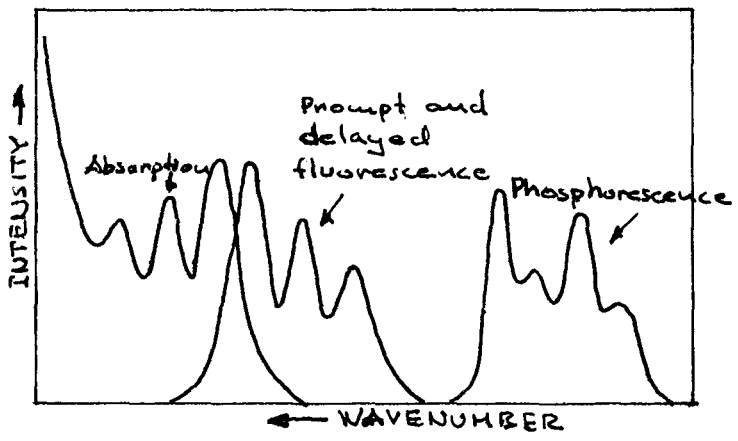



Fig. 1

Multiplicity

A sample of molecules under normal conditions is subject to the Boltzmann distribution:

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

Here k is the Boltzmann constant, T the temperature, E the energy and N the number of particles. As the energy splitting between vibrational levels is of the order of 0.1 eV and kT at room temperature is about 0.025 eV almost all molecules are in the vibrational ground state.

Nearly all molecules have an even number of electrons, except the free radicals, hence, the resultant spin quantum number must be zero in the ground state, due to the Pauli exclusion principle. If the spin is zero the state is called a singlet state. If an electron is excited to an upper state by interaction with a photon, 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. The spin vector may have three values -1, 0, 1 when the molecule is situated in an external magnetic field. This gives rise to a small splitting of the energy the excited electron.

Radiative transitions between singlets and triplets are theoretically forbidden in the first order approximation, but the weak spin-orbit coupling gives a small transition propability. 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 long life time. Fig. 2

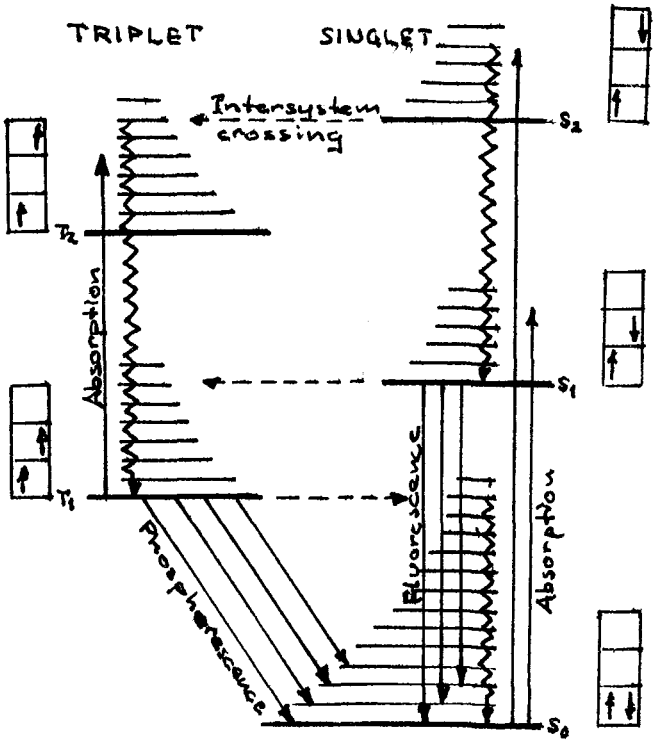



Fig. 2

Different transitions

A photon is able to interact with an electron if there is an energy interval which corresponds to the energy of the photon. It is known that when an electron is excited to an electronic state higher than the first excited one, the molecule undergoes internal conversions and the molecule goes from a low vibrational level to a high vibrational level in a lower electronic state, fig 2. The molecule will fast lose its vibrational energy by collisions with solvent molecules, Fig. 2. Transitions caused by internal conversions and collisions are radiationless. Some substances may undergo photochemical reactions when raised to upper excited states, for example in the case of photosynthesis.

Conclusion: Most electrons come to the first excited electronic level, where they gather due to a relatively long life time. In that state the molecule can either undergo a chemical reaction or decay to any of the levels in the ground state with light emission. The fluorescence light spectral distribution will depend on the vibrational level distribution. This explains why absorbed photons of different energy often give rise to similar fluorescence.

We now deal with the problem why not all transitions from excited electronic states fall to the lowest vibrational level in the state below. The energy levels of a molecule changes during the vibration. In Fig. 3 is the energy plotted as a function of the distance between the atoms in a diatomic molecule.

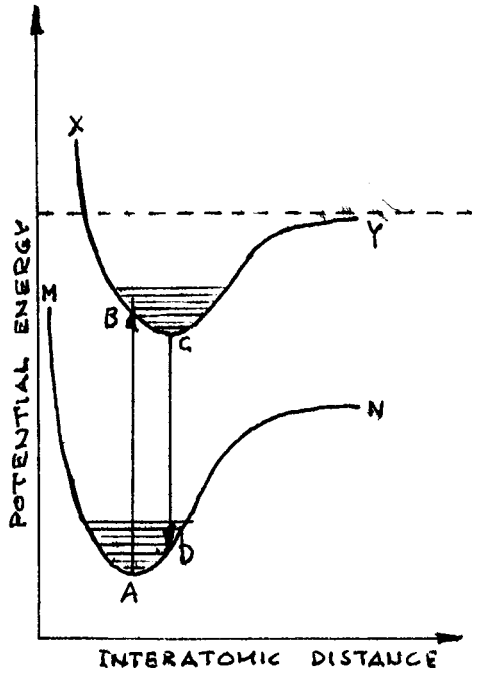



Fig. 3

The Frank-Condon principle is stated as follows. The interatomic distance does not change during a light emission or absorption, i.e. a transition is vertical in the diagram above. If the molecule absorbs a photon when the atoms are at a distance $r_1$ the electron makes a transition from A → B. After some collisions the electron will be

sited in the lowest level in the excited state, C. During these radiationless transitions the interatomic distance has changed to $r_2$ and correspondingly the energy of the lowest level in the ground state has increased, i.e. the emitted photons have 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.

At last the fluorescence quantum efficiency function $\Phi_{\lambda_{exc}}(\lambda)$ is defined

$$\Phi_{\lambda_{exc}}(\lambda) = \frac{\text{photons emitted at wavelength } \lambda}{\text{photons absorbed at exc. wavelength } \lambda_{exc}}$$

hence, the fluorescence quantum efficiancy, $\Psi$.

$$\Psi_{\lambda_{exc}}(\lambda) = \int_{\lambda_{exc}}^{\infty} d\lambda \cdot \Phi_{\lambda_{exc}}(\lambda)$$

Ref. 1.

## 2. HEMATOPORPHYRIN DERIVATIVE, HPD

Porphyrins are naturally occuring dyes which consists of chained pyrol rings, Fig. 4. Porphyrins are able to bind small molecules like Oxygen, Nitrogen, Carbon dioxid, Nitrogen monoxide etc. They are important in biological systems and are among other things included in enzymes, chlorophyll and haemoglobin. Porphyrins are often photo-dynamically active in vitro.

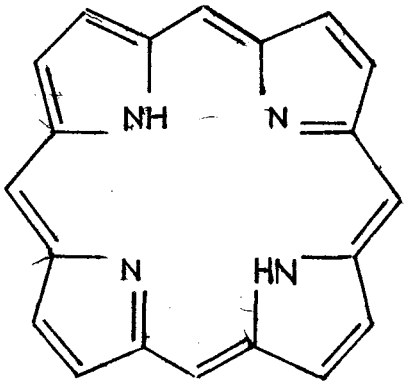



Fig. 4

Hematoporphyrin (HP) is a metabolic product from haemoglobin. Its empirical formula is $C_{34}H_{38}O_6N_4$ and its molecular weight is 598.7, Fig. 5.

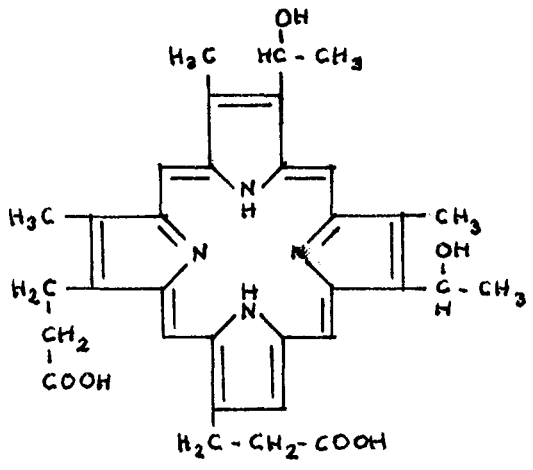



Fig. 5

By a special treatment of HP with acetic acid one obtains Hematoporphyrin derivative (HPD). This agent has proved to be much more photodynamically efficient than HP, in vivo. Pure HP is probably photodynamically inactive in vivo.

HPD is not a pure chemical substance. Its components have been investigated, but not all are indentified. In vitro Hematoporphyrin-diacetate is the photodynamically most active component. When injecting HPD in humans, the substance is solved in a solvent in which acetates do not survive. Thus in vivo there must be other, still unknown, active components. Recent investigations indicate that dihematoporphyrin ether is the most active component.

HPD has three important properties:

It

1) accumulates in malignant tissue
2) causes necrosis of the tissue when irradiated with light at a proper wavelength
3) Fluoresces characteristically when excited.

1) HPD has a different excretion in malignant vs normal tissue. After the HPD is injected intravenously it will be taken up by all tissue. The normal tissue excrete the HPD faster than malignant tissue†. The origin of the selective retention has been suggested to be due to the high vascular permeability together with the lack of adequate lymphatic drainage of tumors. After 2-3 days the ratio between the HPD concentration in tumor vs in normal tissue is about 10 or even higher. It takes about one month until the HPD has been totally excreted. (During this period the patient must stay out of sunlight).

† According to several papers there is also a difference in the uptake of HPD for normal vs malignant tissue.

HPD is also accumulated in areas of moderate and marked squamos cellular atypia. Thus diagnostic methods using HPD are fully reliable only if combined with biopsy and subsequent pathological investigation.

2) When light is absorbed in a HPD-molecule it is excited to a higher energy state ($^1HPD^*$). The excited HPD reacts with oxygen in the tissue. This reaction produces aggressive singlet oxygen, which causes necrosis, Eq. 1 and Fig. 6.

| | |
|---|---|
| * $HPD + h\nu \rightarrow {}^1HPD^*$ | abs. → exc. molecule |
| * $^1HPD^* \rightarrow {}^3HPD^*$ | singlet → triplet transition (radiationless) |
| * $^3HPD^* + {}^3O_2 \rightarrow {}^1O_2 + HPD$ | exchange reaction with "normal" oxygen |
| * $^1O_2$ + substrate → oxidation | the singlet oxygen affects the mitochondria and the cell membranes. |

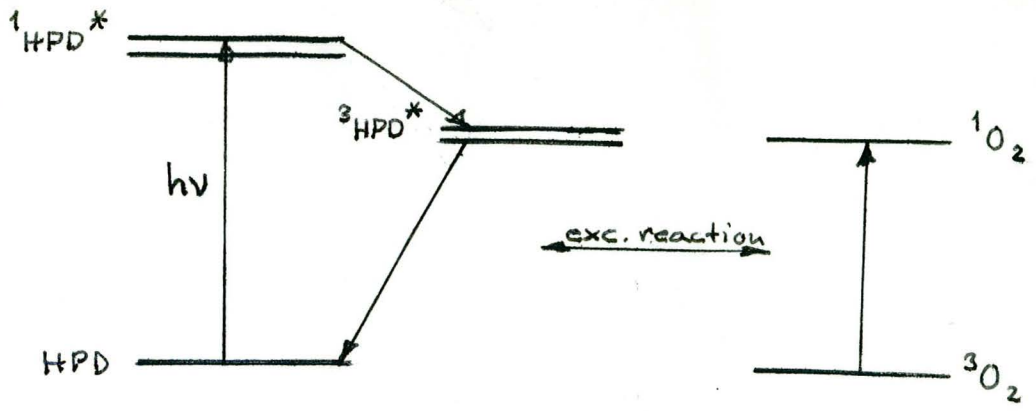



Fig. 6

3) 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. 7. The fluorescence quantum efficiancy, Ψ, in HPD is Ψ = 0.01 (defined on page 10).

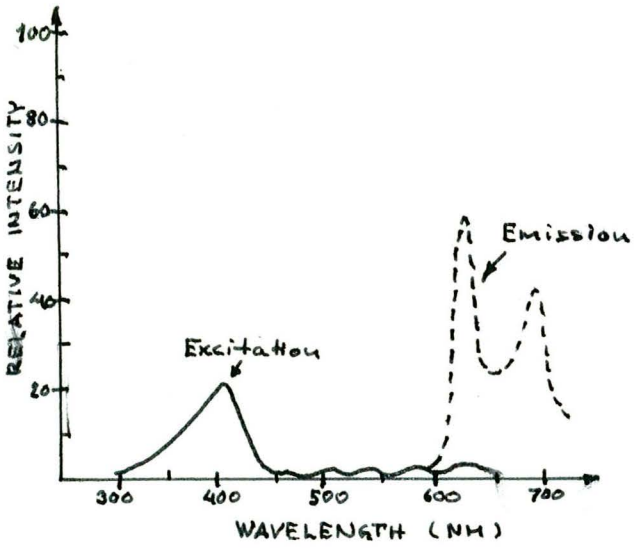



Fig. 7a (Ref. 2)

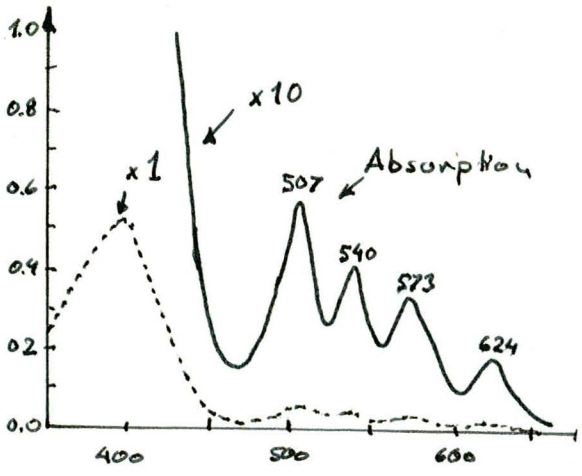



Fig. 7b (Ref. 3)

The fluorescence component in HPD is not the same as the one which

causes necrosis. It would be a great advantage if one could isolate them from each other. Then it would be possible to increase the diagnostic sensitivity, using more light. At present the diagnostic light dose has to be kept far below the treatment dose which is 10-20 $J/cm^3$.

Wanting to find the characteristic red fluorescence from HPD in vivo one must separate it from the fluorescence of the tissue. Fig. 8 shows a typical spectrum from tissue, excited with a $N_2$-laser (337 nm).

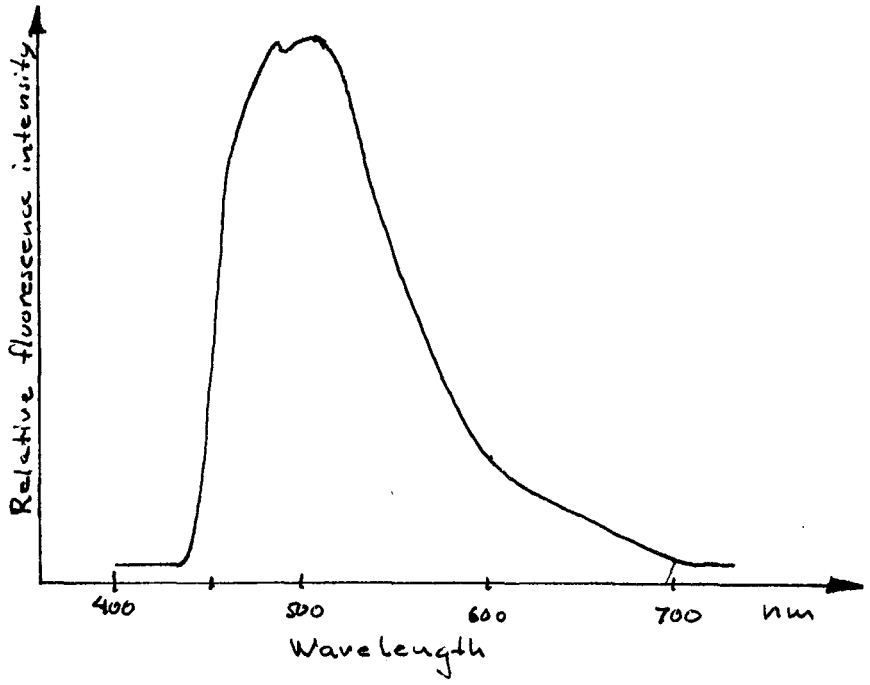



Fig. 8

Malignant tissue fluoresces less than normal, except in the red region, Ref. 4.

# PART 2

# THE APPARATUS

## 3. THE OPTICAL SYSTEM

When we began designing the equipment, which was to be used for the localization of occult lung tumours, we had as a starting point the apparatus built of Kinsey, Cortese and Sanderson, Ref. 5. In the following sections we discuss the choice of every important part in the equipment.

1) Choice of function of measure.

2) Light source.

3) Light transportation.

### 1) Choice of function of measure

We wanted to locate the two most significant wavelengths in the fluorescence spectrum, Fig. 9.

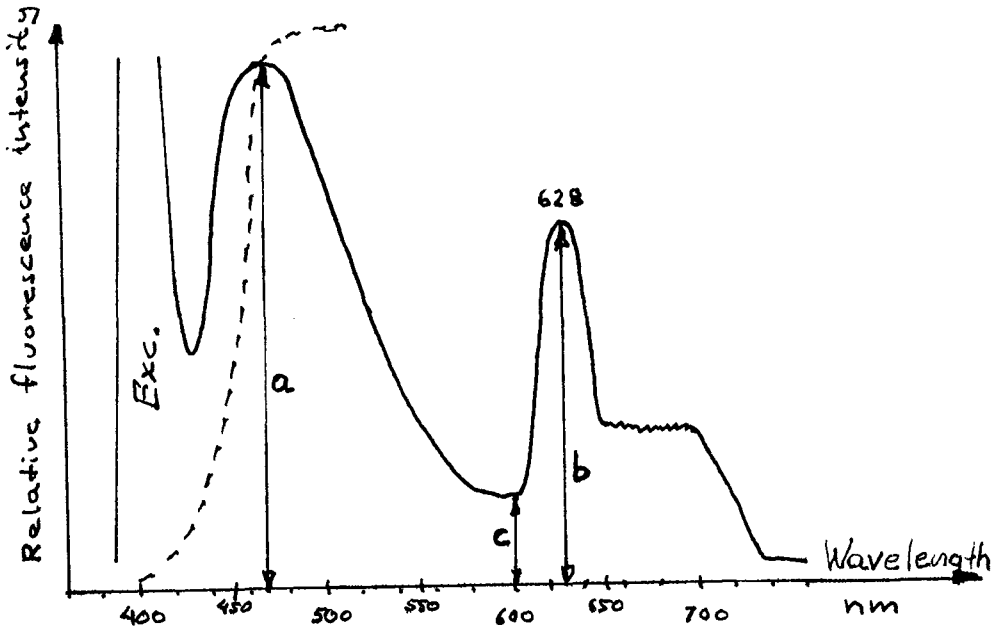



Fig. 9

Kinsey et.al. measured the red fluorescence.

We considered two alternatives:

* The fluorescence at b and c is measured using two narrrow interference filters, calculate b-c.

* The fluorescence around a and b respectively is measured, using two broadband filters, calculate b/a

We chose the latter alternative because b/a is independent of the distance between the bronchoscope and the searched area in the lung. A third alternative, and possibly an improvement, would be to choose (b-c)/a as the measured quantity.

2) Light source

We need a light source with the following properties:

* High intensity at $\lambda$=400 nm for excitation

* A small emitting area so we could focus it on an optical fiber bundle

* Emitting white light, for the ocular inspection through the bronchoscope.

Although the HPD excitation spectrum has some peaks at wavelenghts longer than 400 nm, we decided to use the latter absorption peak because it is stronger than the others and we have no use for the larger penetration depth of the longwave light since lung tumors are

normally situated at the surface of the bronchius.

We investigated the following light sources: Halogen lamps, lasers, high pressure lamps, light emitting diodes.

A halogen lamp will not be suitable as an excitation source, but may be used for illumination.

Some lasers are excellent as excitation source but they are very expensive.

A light emitting diode has many advantages. It is cheap, easy to pulse and only demands a low voltage source. After a slight market research we realized that light emitting diodes at 400 nm do not exist. We also tried to find a diode which fitted with some of the lower peaks in Fig. 7, but none of them had the neccessary emittance (light power/-unit area).

We investigated the possibility of focusing the light from many diodes. After a theoretical consideration we realized that this was not possible for fundamental physical reasons as the area of the P-N transition in the diode is of the same size as the fiberbundle. One soon reaches the limit of the number of emitting areas which can be superimposed. So, we had to abandon the idea of using light emitting diodes for the excitation. The diode we searched for will maybe exist in a couple of years.

High pressure lamps comply with our demands. We decided to use a 200 W Mercury lamp. This lamp has the following benefits:

* Plenty of light in the vicinity of 400 nm, due to a strong line in the Hg-spectrum. Fig. 10.

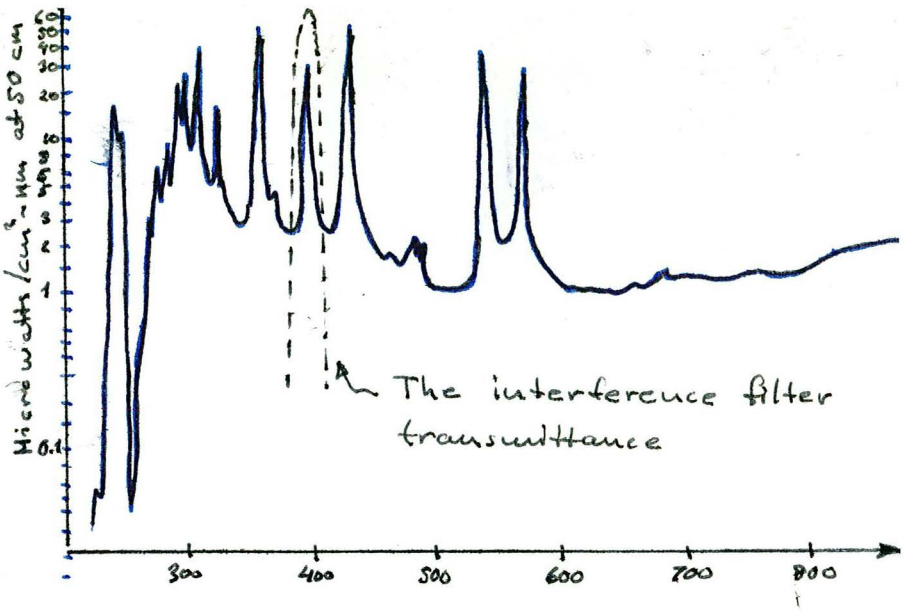



Fig. 10

* Due to pressure broadening it has a quasi-continuous spectral distribution. This quasicontinuum is our white light utilized in the ocular inspection phase.

* Long life-time

* The light-arc between the electrodes has the neccessary high emittance.

Drawbacks:

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

* Due to the radiation below 200 nm, it produces ozone, which is toxic.

* Rather expensive as it demands housing and a high voltage support.

In order not to obscure the weak fluorescence light the excitaiton line is separated from the remaining lamp spectrum with an interference filter, Fig. 10.

3) Light transportation

The Lens tube.

The chosen light source radiates isotropically and we want to focus as much as possible of the light into the acceptance cone of the fibre optic bundle. We chose a positive lens having a short focal length and fixed it on the lamp housing with the light arc in the focal plane. A fused silica lens of aspherical type was selected because spherical abberation would be substantial for a spherical lens when operating at such low F-numbers. The collected solid angle is doubled, by placing a spherical mirror on the opposing side of the arc with this set-up the resulting collection efficiency is estimated to be 29%. For practical reasons the light beam is compressed down to a diameter of less than one inch, using two additional lenses. The lens system is placed in a metal cylinder. A schematic outline is shown in Fig. 11.

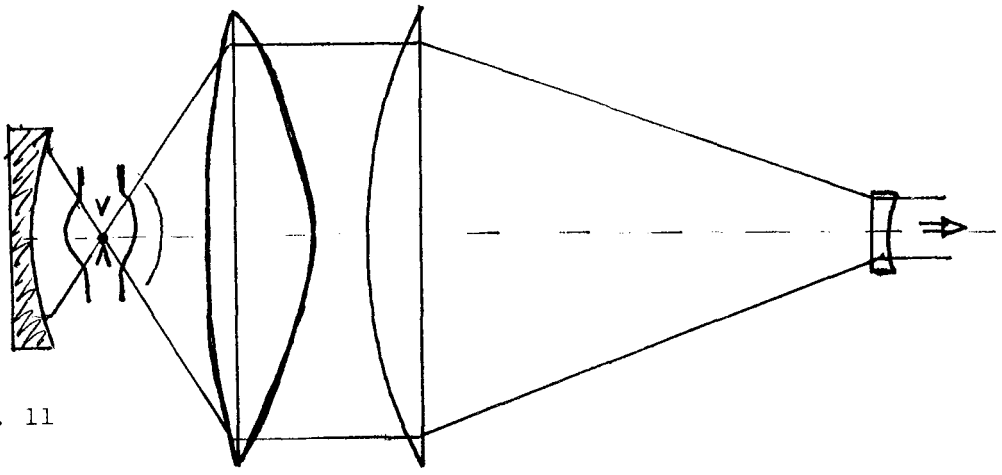


Fig. 11

Combination of illumination and fluorescence measurement

Our aim is to use the white light from the lens tube partly to excite and partly for illumination. As we cannot measure the faint fluorescence and at the same time illuminate with the white light, we have to use a chopping technique. Following Ref. 4 a chopper wheel was used. The chopper wheel was driven by a strong one pole asynchronous electrical motor. As we wanted a rotating frequency which was not a multiple of the mains system, we added a gear and got a rotating frequency at 16 2/3 Hz. The wheel was mounted in a housing. The chosen frequancy was expected to be high enough not to cause visual flicker. Unfortunately ones see a slight flicker. After passing the wheel, the incoming light is focused on the entrance of the fiber optic probe.

Travelling in the opposite direction, the collected fluorescence light is transformed to parallel light. On the other side of the chopper wheel a photomultiplayer tube (PMT) is mounted, transforming the fluorescence light into an electric current.

Since we want to measure the red and the green fluorescence while simultaneously exciting with the violet light as well as illuminating with white light at alternate cycles the chopper wheel was configured as shown in Fig. 12. The filter are fastened on the wheel.

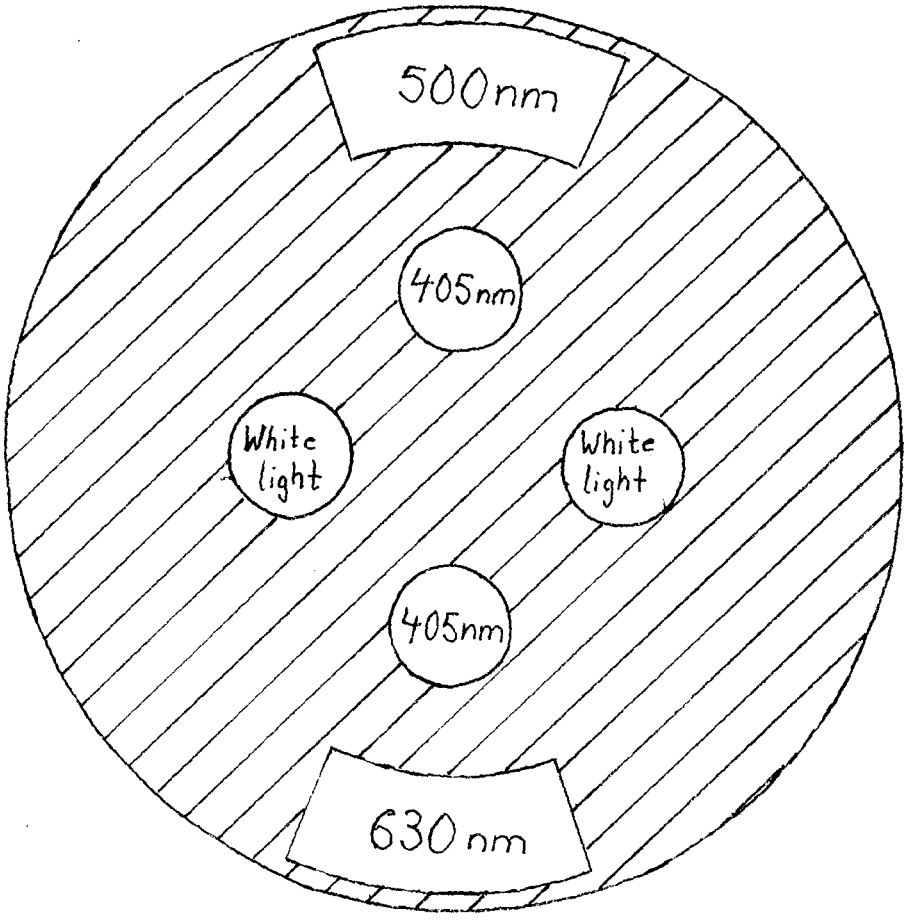



Fig. 12

As the white* light path from the lens tube to the fiber optic probe is opened the fluorescence light path to the PMT is blocked. This is the visual inspection phase. When the wheel has rotated a quarter of a revolution the excitation light path opens and simultaneously the fluorescence is transmitted to the PMT through the red or the green filter.

* The white light passes a cut-off filter blocking radiation below 435 nm, in order to minimize the damage of the examined tissue. Also, the eye's sensitivity is low at these wavelenghths.

A PMT was used instead of a photodiode, since the former yields a superior signal to noise ratio for low light levels. A photodiode has an about twice as high responsive quantum efficiency as a PMT but in the latter, the signal is amplified about a million times without much deterioration of the S/N in the incoming light signal. In a photodiode the noise mechanisms significantly degrades the S/N already before amplification. A red (and green)-sensitive PM tube type Hamamatsu R 928 was finally chosen.

The used fibre-optic cable consists of two fibre-bundles merging into one. It is enclosed in an opaque metal envelope, and has the neccessary high transmission at 400 nm and for our purpose does not give any significant fluorescence.

The PMT signal is fed to the micro-computerized signal conditioning unit.

## 4. ELECTRONIC SIGNAL CONDITIONING

The PMT delivers current pulses from the alternating red or green signals. The desired function is the ratio between the amplitudes the "red" and "green" pulse.

This function can be obtained by using sample-and-hold circuits and an analogue divider but we considered this to be a too restricted solution. For greater flexibility we chose to solve the problem with a digital system. For this purpose an 8-bit EPROM one-chip computer (MC 68705R3) equipped with an A/D-converter was procured. By using a computer many changes and improvements in the signal conditioning are turned into a software problem.

The pulse amplitudes vary between 1 and 50 µA, due to the variations in distance between the optic probe and the examined area. To obtain high resolution in case of small signals and to be able to measure large signals as well, a preamplification-unit controlled by the micro-computer is necessary.

Since a voltage level on an output pin is hard to detect with the human senses, we decided to transform this output signal. According to the doctors concerned the best way to present the result is in form of an audible tone, the ears being of no use anyway during the examination.

Preamplification

The signal from the PMT is fed into a current-to-voltage converter, consisting of a resistor and a low-noise operational amplifier, followed by a non-inverting follower. Fig. 13.

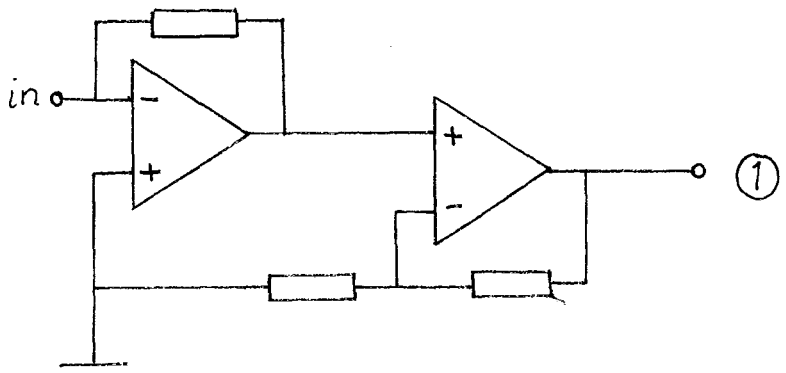



Fig. 13

The next step is an amplifier designed to amplify the red pulse only, this to make the quotient span between 0 and 1 and thereby obtain a sufficiently high resolution. The FET is functioning as a switch controlled by the microcomputer. The inverting amplifier is there only to provide the FET-gate with the necessary negative voltage. When the green pulse is measured the switch is open for unity gain. When the red pulse is put in, the switch is closed and a variable resistor determines the gain. With this resistor the quotient span is adjusted. This means that the maximum amplitude of the red pulse will be amplified to approximately the same level as the green pulse. Fig. 14.

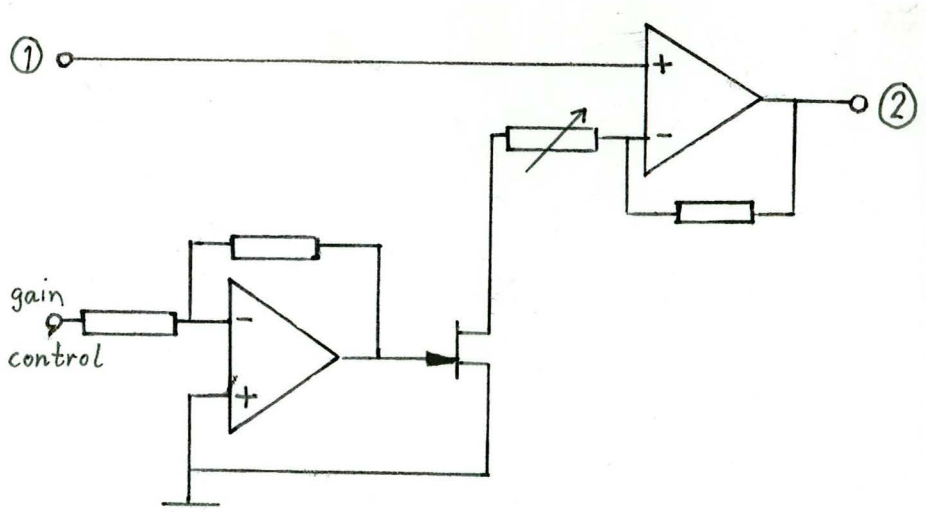



Fig. 14

Next step yields overall amplification, to optimize the use of the A/D-converter. The FET is here performing as a voltage-controlled resistor. When the gain is too high the gain-control output from the microcomputer goes high, and the capacitor is charged. This increases the FET-resistance, which, in turn decreases the gain until it becomes too low and the control output goes low and accordingly the gain increases. As a result the gain will slowly oscillate around an equilibrium. The zener-diode has a purely protective function.

Fig. 15.

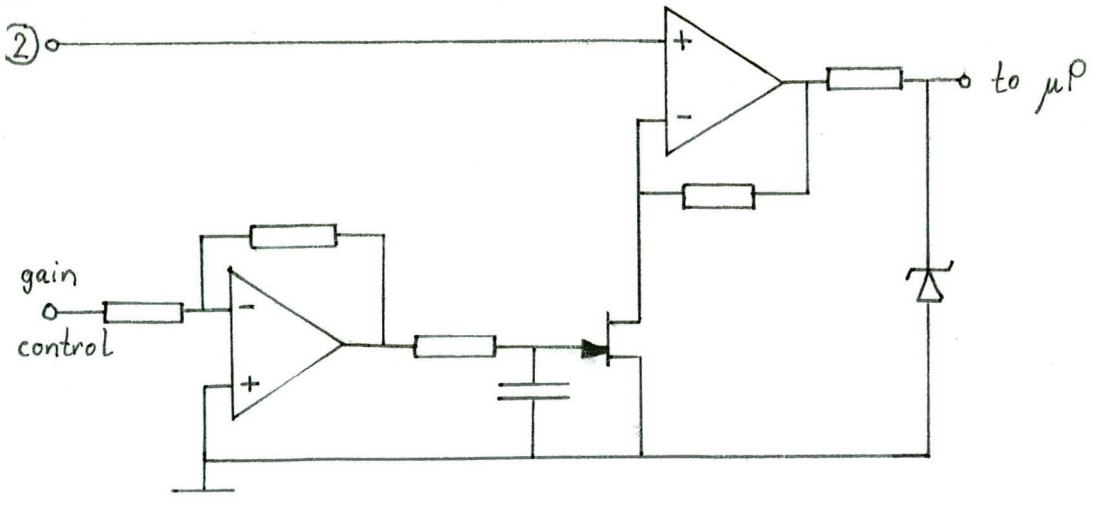



Fig. 15

Software

The main programme consists of two infinite loops which are interrupted in two different ways:

1. By a hardware interrupt input connected to the reference-signal, which is synchronous with the green signal and is derived from a reflective sensor inside the chopperwheel-housing.

2. By a software interrupt ( described on page 25) when the red pulse arrives at the input.

When the reference signal arrives the following functions are performed.

1. The software interrupt is adjusted to the actual chopper wheel frequency (see page 25).

2. The pulse is sampled 128 times during the duty cycle, the intervals between samples being obtained by software time loops. The mean value is calculated by add- and shift operations from these readings. This leads to low-pass filtering, unless the noise signal has the same frequency as the sample frequency or its overtones. In this way high-frequency noise is eliminated. Fig. 16.

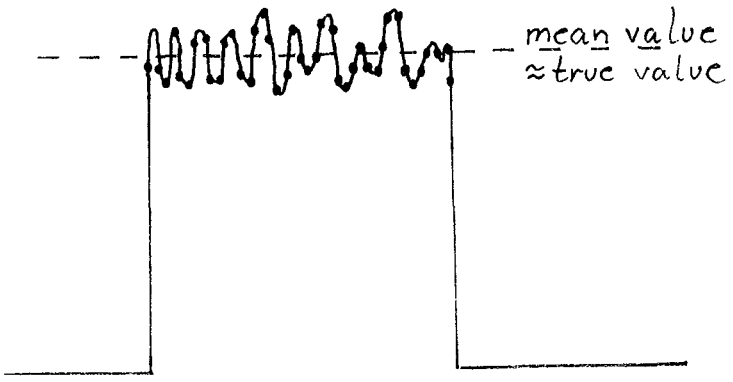
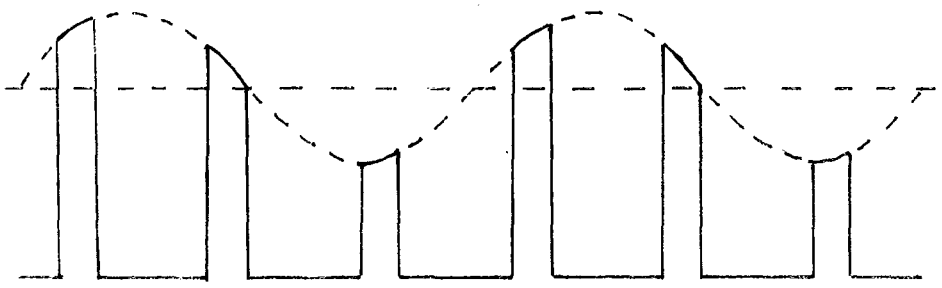



Fig. 16

3. The red pulse gain control pin is set since the next pulse will be a red one.

4. A mean value of previous green pulses is multiplied with seven and added to the new pulse. This sum is divided by eight by three shifts and stored as the new mean value. By doing this low-frequency noise components are surpressed. Fig. 17.

Fig. 17



5. The last pulse and the previous pulse is compared with the wanted level and the overall gain control pin is set or reset accordingly.

6. The last red pulse is divided with the new green one using a standard division routine.

7. Waits until the red pulse is expected to come.

8. Samples the red pulse and calculates the mean value.

9. The red pulse gain control pin is reset.

10. The red pulse is highpass filtered in the same way as the green one.

11. The overall amplification is checked and adjusted.

12. The new red pulse is divided with the last green one

13. Returns to the main loop and waits for the next reference signal.

See Appendix 1.

Frequency measurement and interrupt generation

Since the reference signal is used to "label" the green signal the programme is designed to calculate when to expect the red signal, in

case there are problems with the frequency stability. When the reference signal arrives the timer/counter is loaded with hexnumber FF and is counted down with a prescaling factor of F. A loop is entered in which the counter state is continously read and compared with a stored value. When the values are equal an interrupt is generated and the red signal is dealt with as described earlier. The timer continues to count down and does not stop until the next arrival of the reference signal. The readout is compared with an expected rest stored in the memory. If these values are not equal the frequency is not the expected one and therefore the interrupt value is changed to compensate for this error.

Error functions

An output pin on the computer is connected to a disabling input on the sound generating VCO. This output will become active in two cases:

1) If one of the pulses is greater than 5 volts. This can happen if the overall amplification is high, due to small signals and the probe is moved too fast to an area where the fluorescence is much stronger. This disabling function will also be activated if the signals are so small that the large gain makes the ratio inreliable.

Digital output-to sound-transformation

The digital output from the microcomputer is transformed by a D/A-converter. This analogue signal is allowed to control a voltage controlled oscillator, producing a square-wave signal.
This signal is amplified by a push-pull power amplifier of utmost simplicity, since we have no need for high fidelity. The oscillations

are transformed to sound by a small loudspeaker and a rather nasty little sound can be heard, due to the square wave. Fig. 18.

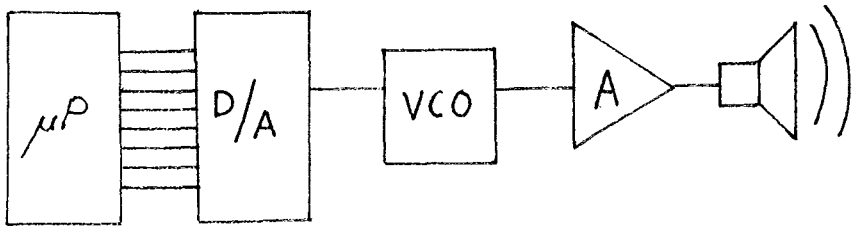
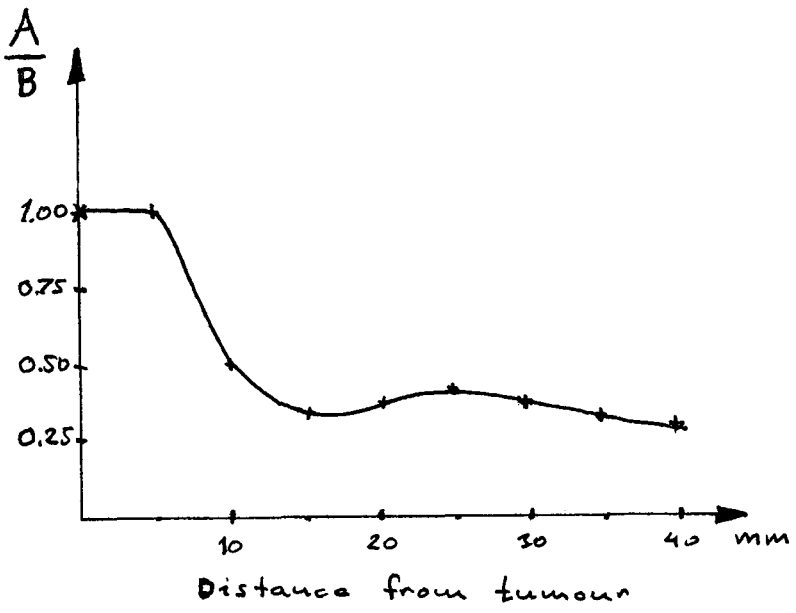
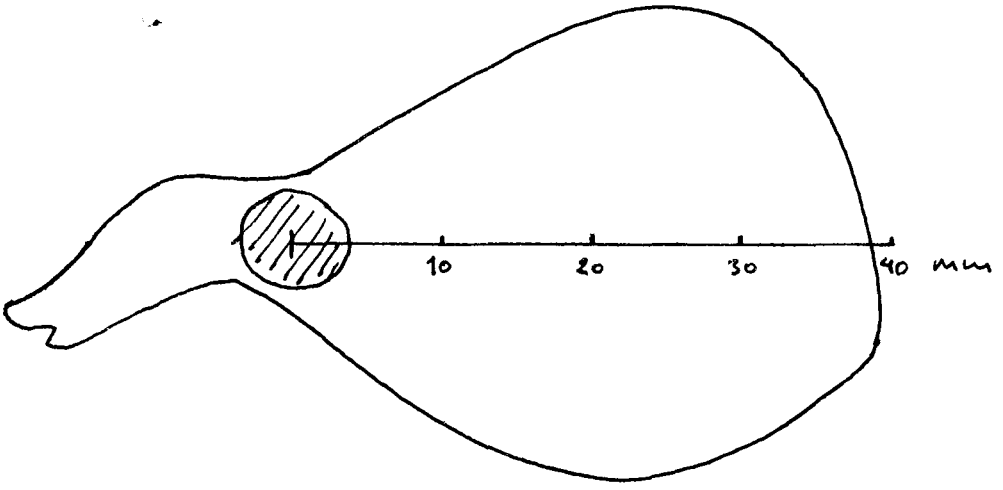



Fig. 18

## 5. THE FIRST TEST

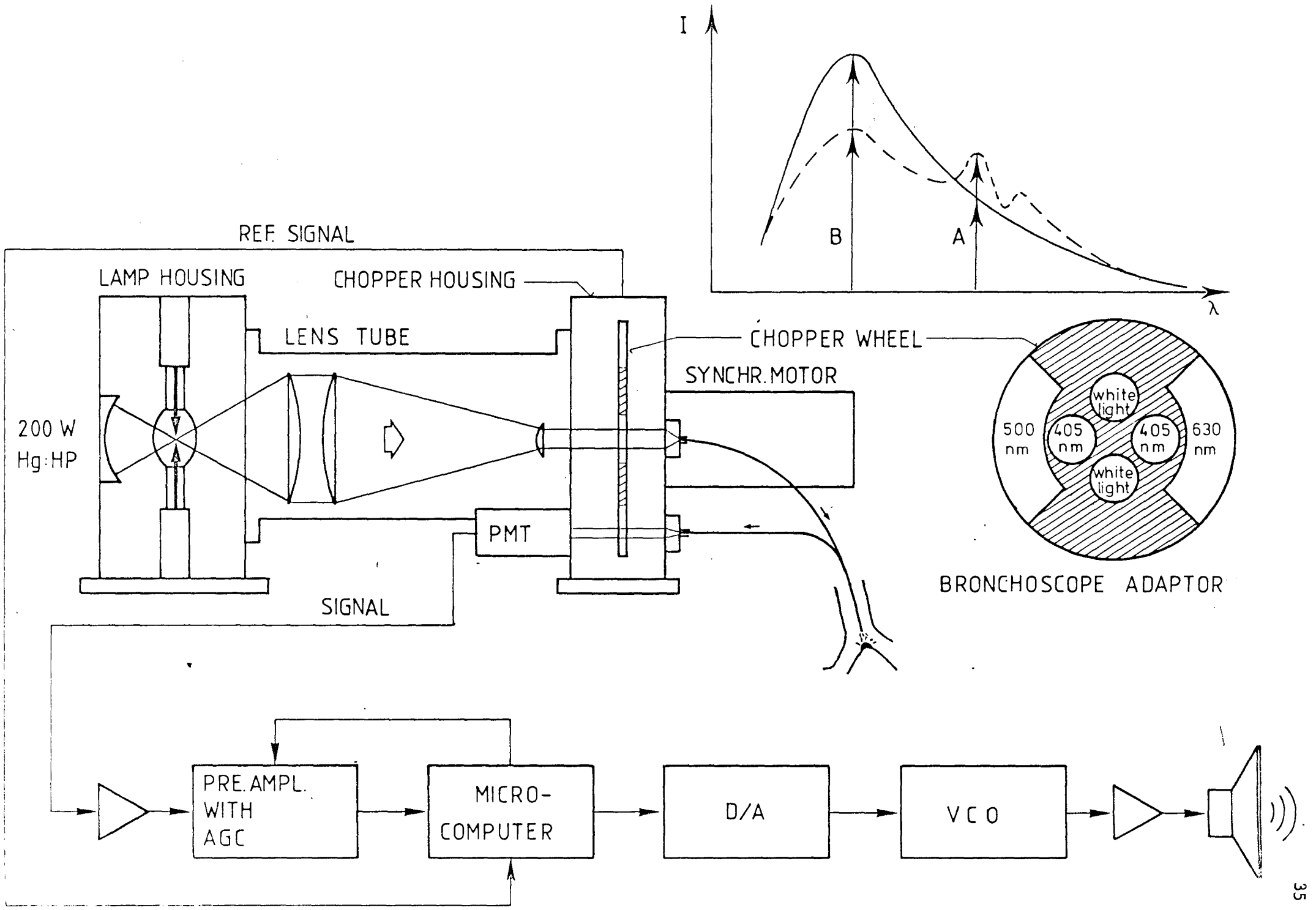
Due to initial problems with the electronic circuitry the optical system was tested by measuring the current pulses from the PMT directly with an oscilloscope. The object examined was a rat with a tumour situated in one of the legs. The rat was injected with a HPD solution (5 µg/ml) two days before the test. The skin was removed from the leg and it was scanned with the fibre-optic bundle. The measured signals were read, the quotients calculated and the result is presented in the diagram below.



Later the equipment was tested a second time on another rat with the microcomputer connected and the output from the D/A- converter connected to an X-Y plotter and a similar result was obtained.

Needless to say, the equipment has to be tested further to determine for example lower detection limits and reliability.


I
λ
B
A
REF. SIGNAL
LAMP HOUSING
CHOPPER HOUSING
LENS TUBE
CHOPPER WHEEL
SYNCHR. MOTOR
200 W
Hg:HP
PMT
SIGNAL
white light
500 nm
405 nm
405 nm
630 nm
white light
BRONCHOSCOPE ADAPTOR
PRE. AMPL. WITH AGC
MICRO-COMPUTER
D/A
VCO

# APPENDIX 1

```
                      1 "6805" LIST
                      2
                      3           DIRECT
                      4
          <0000>      5 PORTA     EQU         00H
          <0004>      6 DDRA      EQU         04H
                      7
          <0001>      8 PORTB     EQU         01H
                      9
                     10
                     11
          <0005>     12 DDRB      EQU         05H
                     13
          <0002>     14 PORTC     EQU         02H
          <0006>     15 DDRC      EQU         06H
                     16
          <0008>     17 TDR       EQU         08H
          <0009>     18 TCR       EQU         09H
          <000E>     19 ADCR      EQU         0EH
          <000F>     20 ADUT      EQU         0FH
                     21
          <007F>     22 DISINT    EQU         01111111B
          <003F>     23 ENINT     EQU         00111111B
                     24
                     25 ******** MASK OPTION REGISTER ****
                     26
                     27           ORG         0F38H
0F38 47              28 MOR       BINARY      01000111
                     29
                     30 ******** RESETVEKTOR *************
                     31
                     32           ORG         0FFEH
0FFE 07C0            33           HEX         07,C0
                     34
                     35 ******** RAM-AREA ****************
                     36
                     37           ORG         40H
                     38
0040 00              39 SLASK1    FCB         00H
0041 00              40 SLASK2    FCB         00H
0042 00              41 SLASK3    FCB         00H
0043 0000            42 SUM       FDB         0000H
0045 00              43 PULS      FCB         00H
0046 00              44 PULS1     FCB         00H
0047 00              45 UT1       FCB         00H
0048 0000            46 SUM1      FDB         0000H
004A 00              47 PULS2     FCB         00H
004B 00              48 UT2       FCB         00H
004C 0000            49 SUM2      FDB         0000H
004E 00              50 REST      FCB         00H
004F 00              51 KVOT      FCB         00H
0050 00              52 FLAG      FCB         00H
                     53
                     54 ****************************************
                     55 *                                      *
                     56 *    I N I T I E R I N G               *
                     57 *                                      *
                     58 ****************************************
                     59
                     60           ORG         7C0H
```

```
                        61
07C0 A6 FF              62              LDA         #11111111B
07C2 B7 04              63              STA         DDRA
07C4 A6 E0              64              LDA         #11100000B
07C6 B7 05              65              STA         DDRB
07C8 A6 00              66              LDA         #00000000B
07CA B7 06              67              STA         DDRC
07CC A6 00              68              LDA         #00000000B
07CE B7 0E              69              STA         ADCR
                        70
07D0 A6 7F              71  ONST        LDA         #DISINT
07D2 B7 09              72              STA         TCR
                        73
07D4 A6 00              74              LDA         #00000000B
07D6 B7 00              75              STA         PORTA
07D8 A6 DF              76              LDA         #11011111B
07DA B7 01              77              STA         PORTB
                        78
07DC A6 36              79              LDA         #54
07DE B7 4E              80              STA         REST
                        81
07E0 0102 FD            82  L1          BRCLR       0,PORTC,L1
07E3 0002 FD            83  L2          BRSET       0,PORTC,L2
                        84
07E6 AE 48              85              LDX         #72
07E8 A6 49              86  L4          LDA         #73
07EA 4A                 87  L3          DECA
07EB 26 FD              88              BNE         L3
07ED 5A                 89              DECX
07EE 26 F8              90              BNE         L4
                        91
                        92
07F0 0102 FD            93  LL3         BRCLR       0,PORTC,LL3
                        94
                        95  ****************************************
                        96  *                                      *
                        97  *     H U V U D P R O G R A M          *
                        98  *                                      *
                        99  ****************************************
                       100
07F3 CD 08ED           101  START       JSR         PULSIN,E
                       102
07F6 A6 FF             103  Z1          LDA         #0FFH
07F8 B7 08             104              STA         TDR
07FA A6 3F             105              LDA         #ENINT
07FC B7 09             106              STA         TCR
07FE 9A                107              CLI
                       108
07FF 1D 01             109              BCLR        6,PORTB
                       110
0801 B6 40             111              LDA         SLASK1
0803 B7 45             112              STA         PULS
0805 B7 46             113              STA         PULS1
0807 B6 48             114              LDA         SUM1
0809 B7 43             115              STA         SUM
080B B6 49             116              LDA         SUM1+1
080D B7 44             117              STA         SUM+1
                       118
080F AD 71             119              BSR         FILT
                       120
```

```
0811 B6 45          121 Z2      LDA         PULS
0813 B7 47          122         STA         UT1
0815 B6 43          123         LDA         SUM
0817 B7 48          124         STA         SUM1
0819 B6 44          125         LDA         SUM+1
081B B7 49          126         STA         SUM1+1
                    127
081D CD 0913        128         JSR         GAIN,E
                    129
0820 CD 0869        130 Z3      JSR         DIV,E
                    131
0823 B6 4F          132 Z4      LDA         KVOT
0825 B7 00          133         STA         PORTA
                    134
                    135 ******** PULS 2 **************************
                    136
0827 B6 08          137 L5      LDA         TDR
0829 B0 4E          138         SUB         REST
082B 26 FA          139         BNE         L5
                    140
082D A6 7F          141         LDA         #DISINT
082F B7 09          142         STA         TCR
                    143
0831 CD 08ED        144         JSR         PULSIN,E
                    145
0834 A6 FF          146 Z5      LDA         #0FFH
0836 B7 08          147         STA         TDR
0838 A6 3F          148         LDA         #ENINT
083A B7 09          149         STA         TCR
083C 9A             150         CLI
                    151
083D 1C 01          152         BSET        6,PORTB
                    153
083F B6 40          154         LDA         SLASK1
0841 B7 45          155         STA         PULS
0843 B7 4A          156         STA         PULS2
0845 B6 4C          157         LDA         SUM2
0847 B7 43          158         STA         SUM
0849 B6 4D          159         LDA         SUM2+1
084B B7 44          160         STA         SUM+1
                    161
084D AD 33          162         BSR         FILT
                    163
084F B6 45          164 Z6      LDA         PULS
0851 B7 4B          165         STA         UT2
0853 B6 43          166         LDA         SUM
0855 B7 4C          167         STA         SUM2
0857 B6 44          168         LDA         SUM+1
0859 B7 4D          169         STA         SUM2+1
                    170
085B CD 0913        171         JSR         GAIN,E
                    172
085E CD 0869        173 Z7      JSR         DIV,E
                    174
0861 B6 4F          175 Z8      LDA         KVOT
0863 B7 00          176         STA         PORTA
                    177
0865 0102 FD        178 L6      BRCLR       0,PORTC,L6
                    179
0868 A6 7F          180         LDA         #DISINT
```

```
086A B7 09          181             STA         TCR
                    182
086C B6 50          183             LDA         FLAG
086E 27 02          184             BEQ         L7
0870 1F 01          185             BCLR        7,PORTB
                    186
0872 B6 08          187 L7          LDA         TDR
0874 B0 4E          188             SUB         REST
0876 2B 05          189             BMI         LNER
0878 3C 4E          190             INC         REST
087A CC 07F3        191             JMP         START,E
087D 3A 4E          192 LNER        DEC         REST
087F CC 07F3        193             JMP         START,E
                    194
                    195 ****************************************
                    196 *                                      *
                    197 *         FILT, FILTRERING PULS        *
                    198 *         SUM=SUM-SUM/8+PULS           *
                    199 *         PULS=SUM/8                   *
                    200 *                                      *
                    201 *         SUM  EQU SLASK1              *
                    202 *         PULS EQU SLASK2              *
                    203 *                                      *
                    204 ****************************************
                    205
0882 B6 43          206 FILT        LDA         SUM
0884 B7 42          207             STA         SLASK3
0886 B6 44          208             LDA         SUM+1
0888 44             209             LSRA
0889 36 42          210             ROR         SLASK3
088B 44             211             LSRA
088C 36 42          212             ROR         SLASK3
088E 44             213             LSRA
088F 36 42          214             ROR         SLASK3
0891 B6 43          215             LDA         SUM
0893 B0 42          216             SUB         SLASK3
0895 B7 43          217             STA         SUM
0897 B6 44          218             LDA         SUM+1
0899 A2 00          219             SBC         #00H
089B B7 44          220             STA         SUM+1
089D B6 43          221             LDA         SUM
089F BB 45          222             ADD         PULS
08A1 B7 43          223             STA         SUM
08A3 B6 44          224             LDA         SUM+1
08A5 A9 00          225             ADC         #00H
08A7 B7 44          226             STA         SUM+1
08A9 B6 43          227             LDA         SUM
08AB B7 45          228             STA         PULS
08AD B6 44          229             LDA         SUM+1
08AF 44             230             LSRA
08B0 36 45          231             ROR         PULS
08B2 44             232             LSRA
08B3 36 45          233             ROR         PULS
08B5 44             234             LSRA
08B6 36 45          235             ROR         PULS
08B8 81             236             RTS
                    237
                    238 ****************************************
                    239 *                                      *
                    240 *     DIVISIONSRUTIN, DIV              *
```

```
                    241 *     dividerar SLASK1 med SLASK2        *
                    242 *     och lagger resultatet i SLASK3     *
                    243 *                                        *
                    244 ******************************************
                    245
08B9 3F 4F          246 DIV       CLR         KVOT
08BB B6 45          247           LDA         UT2
08BD B7 40          248           STA         SLASK1
08BF 3F 41          249           CLR         SLASK2
08C1 AE 08          250           LDX         #8
                    251
08C3 B6 40          252 DIV1      LDA         SLASK1
08C5 B0 47          253           SUB         UT1
08C7 B7 40          254           STA         SLASK1
08C9 B6 41          255           LDA         SLASK2
08CB A2 00          256           SBC         #0
08CD B7 41          257           STA         SLASK2
08CF 25 05          258           BCS         MINDRE
                    259
08D1 99             260           SEC
08D2 39 4F          261           ROL         KVOT
08D4 20 0F          262           BRA         DUT
                    263
08D6 B6 40          264 MINDRE    LDA         SLASK1
08D8 BB 47          265           ADD         UT1
08DA B7 40          266           STA         SLASK1
08DC B6 41          267           LDA         SLASK2
08DE A9 00          268           ADC         #0
08E0 B7 41          269           STA         SLASK2
08E2 98             270           CLC
08E3 39 4F          271           ROL         KVOT
                    272
08E5 38 40          273 DUT       LSL         SLASK1
08E7 39 41          274           ROL         SLASK2
08E9 5A             275           DECX
08EA 26 D7          276           BNE         DIV1
08EC 81             277           RTS
                    278
                    279 ******************************************
                    280 *                                        *
                    281 *         PULSIN                         *
                    282 *         MEDELVARDET AV INSIGNALEN      *
                    283 *         HAMTAS I SLASK1                *
                    284 *                                        *
                    285 ******************************************
                    286
08ED AE 09          287 PULSIN    LDX         #9
08EF 5A             288 P2        DECX
08F0 26 FD          289           BNE         P2
                    290
08F2 3F 40          291           CLR         SLASK1
08F4 3F 41          292           CLR         SLASK2
08F6 AE 80          293           LDX         #128
08F8 B6 0F          294 P1        LDA         ADUT
08FA B7 42          295           STA         SLASK3
08FC BB 41          296           ADD         SLASK2
08FE B7 41          297           STA         SLASK2
0900 B6 40          298           LDA         SLASK1
0902 A9 00          299           ADC         #00H
0904 B7 40          300           STA         SLASK1
```

```
0906 5A           301           DECX
0907 26 EF        302           BNE         P1
0909 38 41        303           LSL         SLASK2
0908 39 40        304           ROL         SLASK1
                  305
0900 AE 09        306           LDX         #9
090F 5A           307 P3        DECX
0910 26 FD        308           BNE         P3
                  309
0912 81           310           RTS
                  311
                  312 ****************************************
                  313 *                                      *
                  314 *         GAIN                         *
                  315 *         JUSTERAR FORSTARKNINGEN      *
                  316 *                                      *
                  317 ****************************************
                  318
                  319 ******** FORSTARKNINGSREGLERING ********
                  320
0913 B6 46        321 GAIN      LDA         PULS1
0915 B1 4A        322           CMP         PULS2
0917 24 02        323           BCC         G1
0919 B6 4A        324           LDA         PULS2
0918 B7 40        325 G1        STA         SLASK1
091D A0 C8        326           SUB         #200
091F 24 04        327           BCC         GNER
                  328
0921 1B 01        329 GUPP      BCLR        5,PORTB
0923 20 02        330           BRA         G2
                  331
0925 1A 01        332 GNER      BSET        5,PORTB
                  333
                  334 ******** GILTIGHETEN AV UTSIGNALEN ******
                  335
0927 B6 40        336 G2        LDA         SLASK1
0929 A0 FF        337           SUB         #0FFH
0928 27 0B        338           BEQ         GHOLD
                  339
092D B6 46        340           LDA         PULS1
092F A0 0A        341           SUB         #10
0931 25 05        342           BCS         GHOLD
                  343
0933 A6 01        344           LDA         #01H
0935 B7 50        345           STA         FLAG
0937 81           346           RTS
                  347
0938 1E 01        348 GHOLD     BSET        7,PORTB
093A A6 00        349           LDA         #00H
093C B7 50        350           STA         FLAG
093E 81           351           RTS
                  352
                  353 ******** INTERRUPTRUTIN ***************
                  354
093F 9C           355 INTERR    RSP
0940 1E 01        356           BSET        7,PORTB
0942 CC 07D0      357           JMP         ONST,E
                  358
                  359 ******** INTERRUPTVEKTOR **************
                  360
```

```
                    361             ORG         0FF8H
                    362
0FF8 093F           363             FDB         INTERR
                    364
                    365             END
```

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