The design and implementation of a compact fluoresensor for medical diagnostics

Markus Andreasson & Ola Sandström

Masters thesis
Lund Reports on Atomic Physics, LRAP-239
Lund, December 1998

LUND
UNIVERSITY

Abstract

A compact fluoresensor with a user-friendly interface has been developed. The work includes market investigation, hardware specification, CAD, software development, documentation and the actual assemblage of the components. Evaluation of the system has been done in a clinical study at the Karolinska Hospital, Stockholm.

Contents

1. Introduction

Medical diagnostics have been carried out by scientists from Lund University Medical Laser Center for many years, both in laboratories and at medical clinics. Two optical multichannel analyzer (OMA) systems have been developed with the ability to do real-time measurements using laser-induced fluorescence. Research has increased the number of applications, leading to higher demand and geographic spreading. A need for a more compact, portable and user-friendly OMA system grew, thus this subject for a Master's thesis was announced.

1.1 Background

It is of certain diagnostic interest to be able to differentiate between normal and diseased tissue. Traditionally, this is performed either visually *in situ* or microscopically studying a biopsy. Using laser-induced fluorescence is another diagnostic approach, where the light from different fluorescent molecules is used for differentiation. The relative concentrations of these molecules vary with type of tissue, hence the type of tissue can be determined.

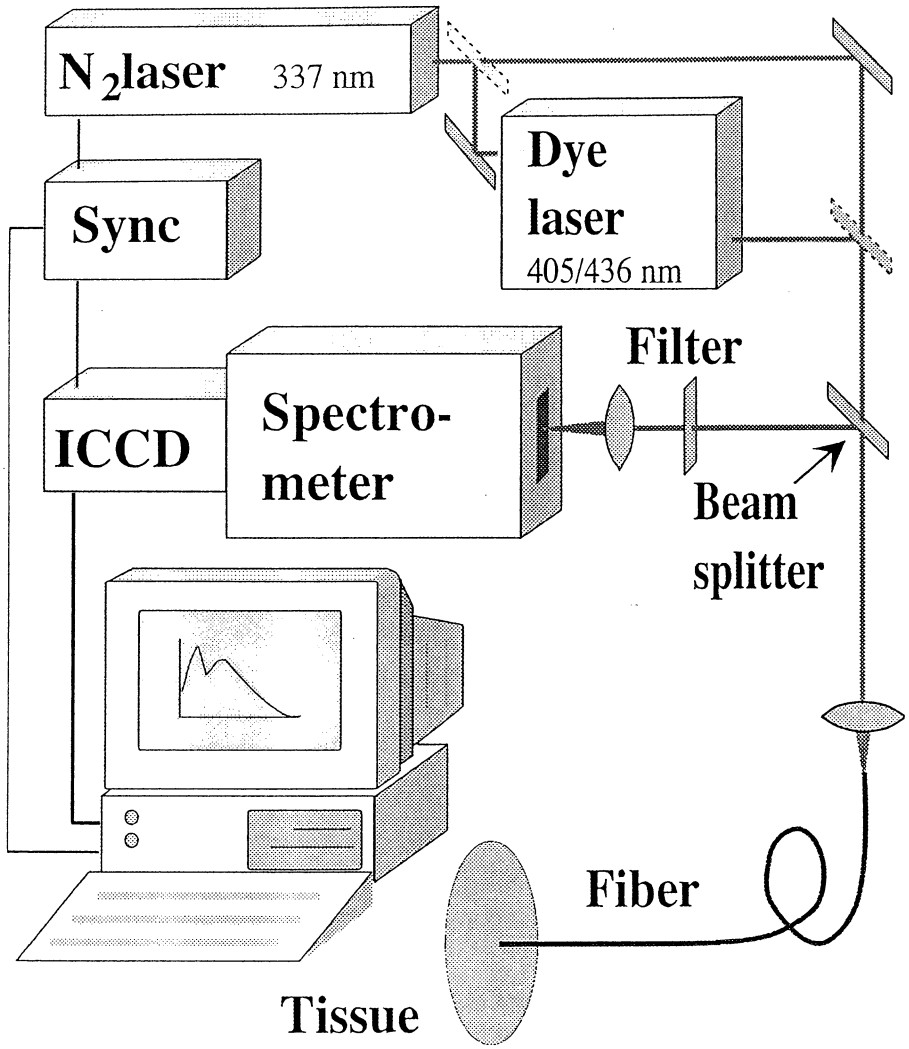


Figure 1-1: Schematic view of a fluoresensor. Different lasers induce fluorescent light, which is analyzed in the spectrometer and CCD. The results are displayed and stored on a computer.

The principal components of a fluoresensor capable of induce and collect fluorescence light can be seen in Figure 1-1. Different lasers are used to induce fluorescence, and the fluorescence light is collected and analyzed in a spectrometer and CCD. The transport of light to and from the sample is done in an optical fiber. The components are further described in section 3.

When the planning for the third OMA system started, two similar systems existed already, similar by means of functionality. The first, presented in 1991 [1], was constructed on a mobile trolley. It functioned guiding both the excitation light and fluorescence light through the same optical fiber. The size of the trolley was about 85×70 cm with a height of approximately 140 cm, see Figure 1-2a. The weight was more than one person could handle.

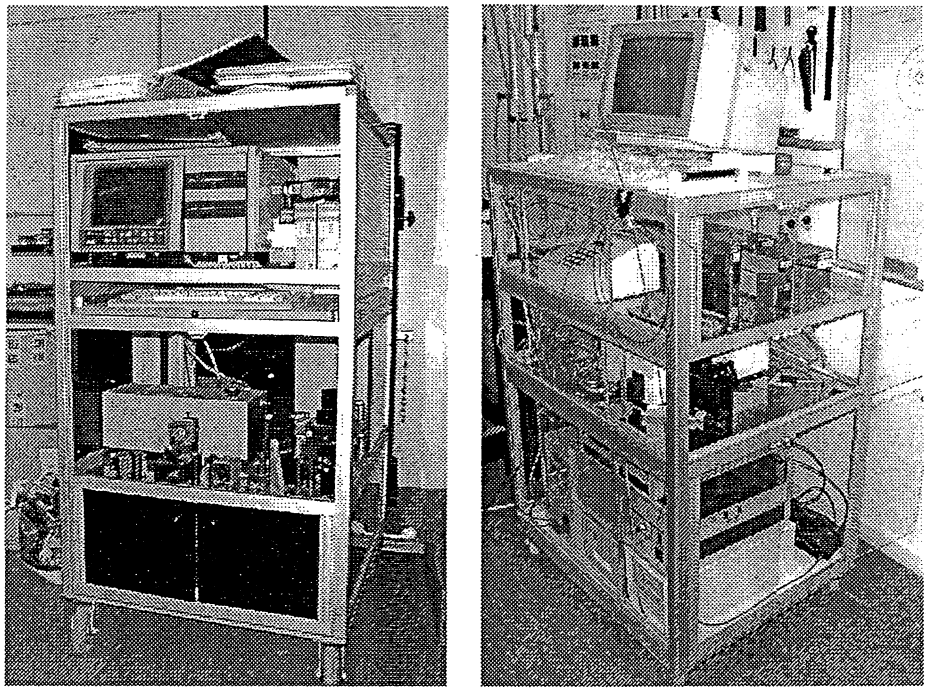


Figure 1-2: Previous systems. To the left (a)the first , to the right (b) the second.

The second system, presented in 1994 [2], is still in use and has more or less the same dimensions as the first, but technically improved components, see Figure 1-2b. Both systems are however difficult to use unless you are a technician, hard to move without a van and slow to work with since there are several manual steps involved in the acquisition process.

1.2 Portable and compact system

How small can this type of OMA system be? When the standards for a third system were set, computer dimensions and optical components size were considered. A notebook computer with a small docking station was present, however old and obsolete, with an approximate size of 30×40×6 cm (w×d×h). The first calculations of the optical components size aimed at adding only 10 cm to the height and giving a total weight of no more than 20 kg (see Figure 1-3). All parts mentioned should be bundled into a portable case, small enough to bring as a hand luggage at any public communication.

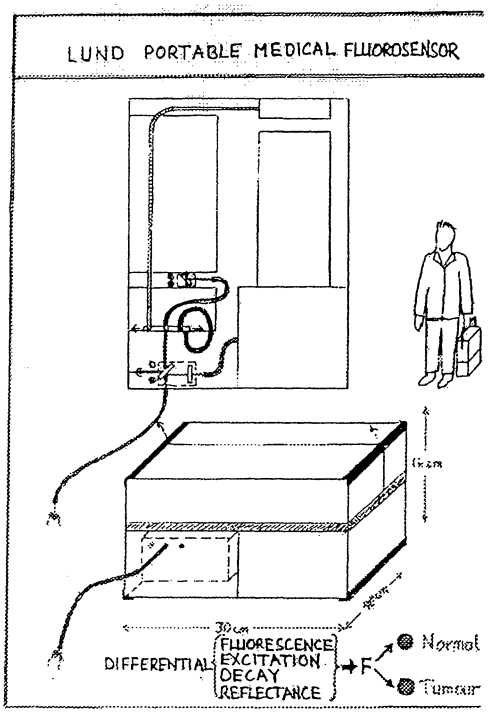


Figure 1-3: Initial vision of the compact system. Graphics: S Svanberg.

1.3 Intuitive and simple interface

The normal procedure, performing medical investigations with the fluoresensor in use today, involves at least three persons plus the patient in the room: the examining doctor, an assistant and a physicist running the equipment. A problem is the need for the physicist; advanced equipment like these often need advanced operators, especially when unpredictable events occur.

One goal with the third OMA system is to make it possible for the examining doctor, or the assistant, to handle the equipment. This demands automated procedures and easy interaction with the controlling computer. Using the system in its most simple mode, it could give audio directions, give simple indications on the characterization of the tissue, and be voice controlled.

The software used with the present systems are general, third-part applications, constructed only to acquire a spectrum without knowledge about the situation. A more custom-designed software is welcomed.

1.4 Time-resolved measurements

When specifying the system it was desired to be able to perform time-resolved measurements. This can be used when monitoring changes in fluorescence light life-times, which applies to tissue differentiation. Usually, the system gives intensity as a function of wavelength, but in a time-resolved measurement another dimension is added; time. The intensity of different wavelengths as a function of time is of interest. Today's technology should offer this feature, even in compact

systems. This option has not been disabled by any included component, though not yet implemented.

2. Laser-induced fluorescence in tissue

2.1 Introduction to fluorescence

Fluorescence is a process in matter, starting with absorption of photon energy [3], see Figure 2-1[4,5]. If an incoming photon has the same energy as the difference between two electronic states in an atom or molecule, it can be absorbed if the lower state is occupied. In the atom case, the excited atom returns to the initial state by emitting a photon, which therefore has the same energy as the absorbed photon. This is called *resonance radiation*. In the molecule case, the emitted photon energy may be less than the absorbed. This happens if the excited molecule is deexcited to an energy level higher than the original level, or if it relaxes before emission. This radiation is called *fluorescence*.

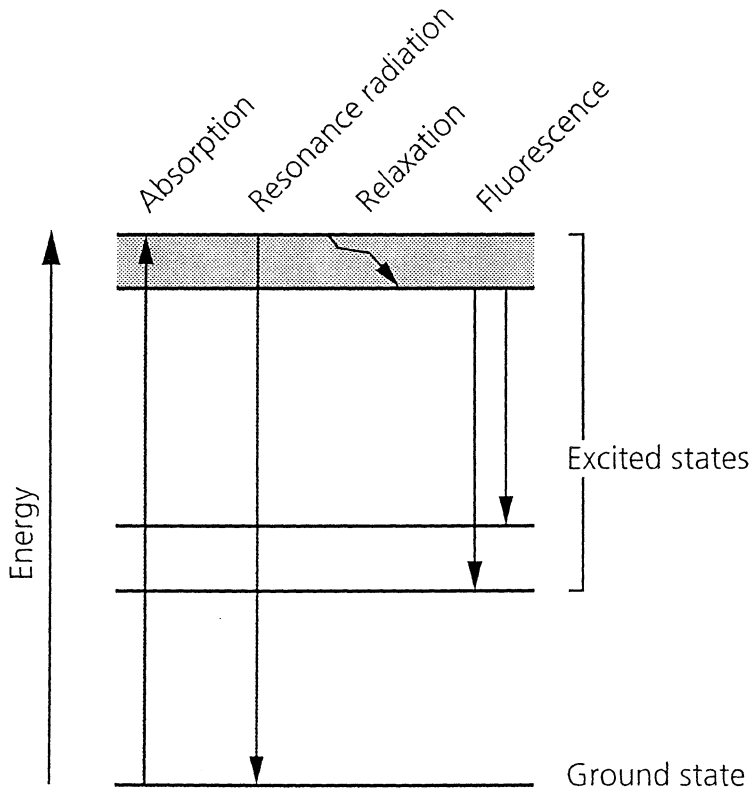


Figure 2-1: Energy level diagram and terminology for radiative processes.

Relaxation is a radiationless process, in which some of the excess molecular energy is released to the surroundings. The electronic energy states in a molecule consist of many levels of almost equal energies, forming an energy band. The many, in energy closely spaced, molecular configurations are due to differences in

molecular vibration and rotation. It is within these bands relaxation takes place. Typical relaxation energies within vibrational bands are of the order of 10^{-1} eV. This process is very fast compared with electronic deexcitation, and the energy is released as heat to the surrounding molecules.

When the electron has reached the lowest level within the energy band, it eventually is deexcited to a lower electron state. This state can form another band, in which another relaxation process can take place.

The relatively wide bands make the exciting wavelength non-critical within an interval of the order of 10-50 nm for visible light, and gives a continuos fluorescence distribution ranging over about 100 nm.

2.2 Laser-induced fluorescence

The quantum yield for the fluorescence process, *i.e.* the number of fluorescence photons emitted divided by the number absorbed, is low for most molecules. The fluorescence is often weak and broadly distributed, and light propagation in the medium of interest may be limited. It is thus essential to have a relatively high power excitation source when performing fluorescence measurements. Laser excitation is thus often preferable, since a laser delivers outstanding power within a narrow wavelength band.

Other advantages of lasers as excitation sources are that they are spectrally clean, and have a high brightness. A single wavelength is critical. The fluorescence excitation source should emit light at a wavelength absorbed by the molecules studied but nothing at the wavelength where the fluorescence is analyzed. Any light emitted by the source at this wavelength will disturb the fluorescence detection.

A high brightness is also essential to enable efficient guiding of the excitation light from the source to the sampling volume. Fiber delivering is often necessary in medical applications.

2.3 Spectrally- and time-resolved laser-induced fluorescence

In order to enable that fluorescence from different molecules can be distinguished in the analysis of complex samples, such as tissues containing several fluorescent molecules, the fluorescence is often resolved spectrally. This means that the fluorescence intensity is measured as a function of wavelength. This is how the fluorescence has been analyzed in the old systems at the Division of Atomic Physics, Lund.

The system described in this thesis utilize this means of distinguishing contributions from different molecules. However, the new fluorescence system also employs an additional tool to distinguish fluorescence from different molecules – it provides fluorescence *lifetime* information.

Lifetime is by definition the inverse of the probability of deexcitation for a given transition. The lifetime of fluorescent light can be determined by doing

time-resolved measurements, where studying the decay of the fluorescence intensity from a excited matter.

Normally, a detection time resolution of picoseconds is needed, since typical fluorescence light lifetimes vary down to nanoseconds or even picoseconds [3,6,7]. However, if achieving the actual lifetime is not necessary but only the ability to prove presence of a certain matter, nanosecond resolution of the detection system may be sufficient.

One way to do this is to compare late and early intensity integration of the fluorescent light. This affords a controlled gating of the detector in means of both width and delay.

2.4 Tissue fluorescence

Tissue fluorescence origin from numerous fluorophores. Native as well as added exogenous fluorophores can be utilized for tissue diagnostic purposes. The fluorophores of diagnostic interest often exhibit absorption in the violet region and fluorescence in the visible wavelength region.

A commonly used absorption band for tissue fluorescence is the *Soret band* of porphyrins, a very strong absorption band in the blue region of the optical absorption spectrum [5,8]. The corresponding excitation wavelength to the absorption is 405 nm, which can be obtained with a dye laser. The dye can be pumped with a nitrogen laser (337 nm) or a frequency-tripled Nd:YAG laser (355 nm).

The states between which absorption occurs are the ground singlet states S_0 and S_2. *Internal conversion* relaxation (see Figure 2-2) brings the electron to S_1, from which it deexcites, emitting a photon of 635 nm or 700 nm, to S_0. *Phosphorescence* may also occur, if *intersystem crossing* brings the electron from S_1 to a triplet T_1 state, and from there deexcites to S_0.

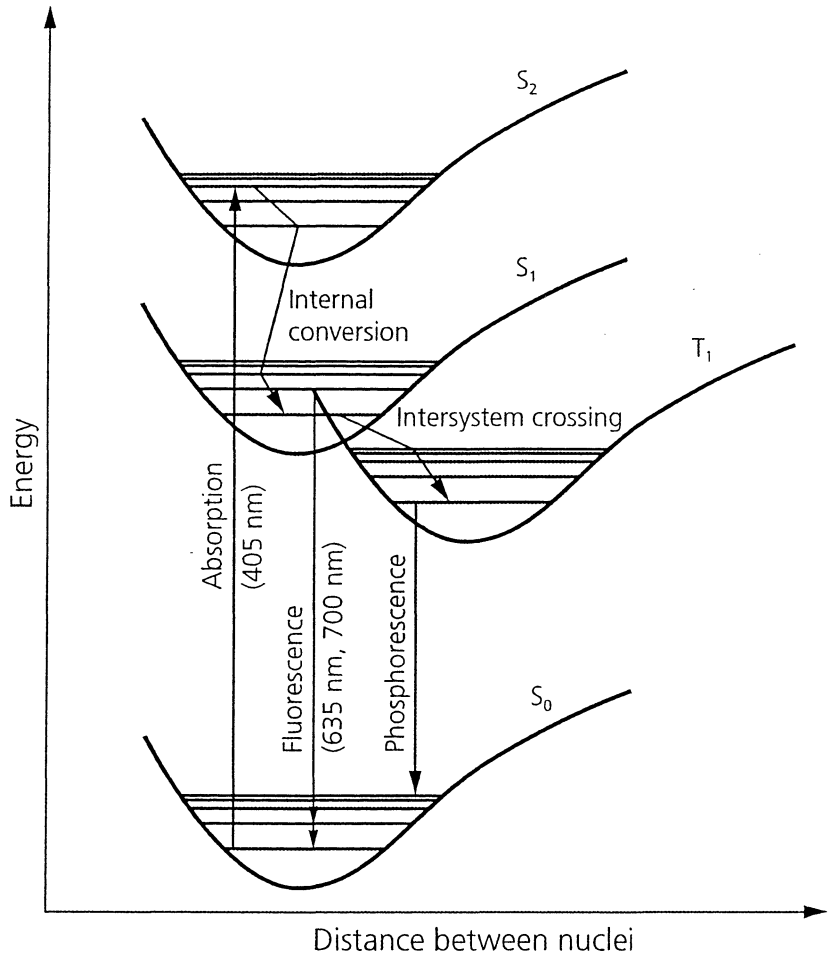


Figure 2-2: Illustration of absorption, internal conversion, intersystem crossing and phosphorescence in a Pp IX molecule.

2.4.1 Autofluorescence

The fluorescence in tissue depends on many factors, *e.g.* the type of cells, pH and the kind of fluorescent substances/molecules - fluorophores. The absorption spectra of some important tissue molecules can be seen in Figure 2-3. Examples of tissue fluorophores are collagen, carotene, elastin and NADH [5]. When using UV light as an excitation source, NADH fluoresces in a bluish color, peaking at 470 nm, while carotene fluoresces around 530 nm. Collagen and elastin have slightly separated peaks around 400 nm.

The balance between NADH and the less fluorescent NAD^+ depends on pH, thus a low pH gives less fluorescence. The natural fluorophores contributes to the native tissue fluorescence – tissue autofluorescence.

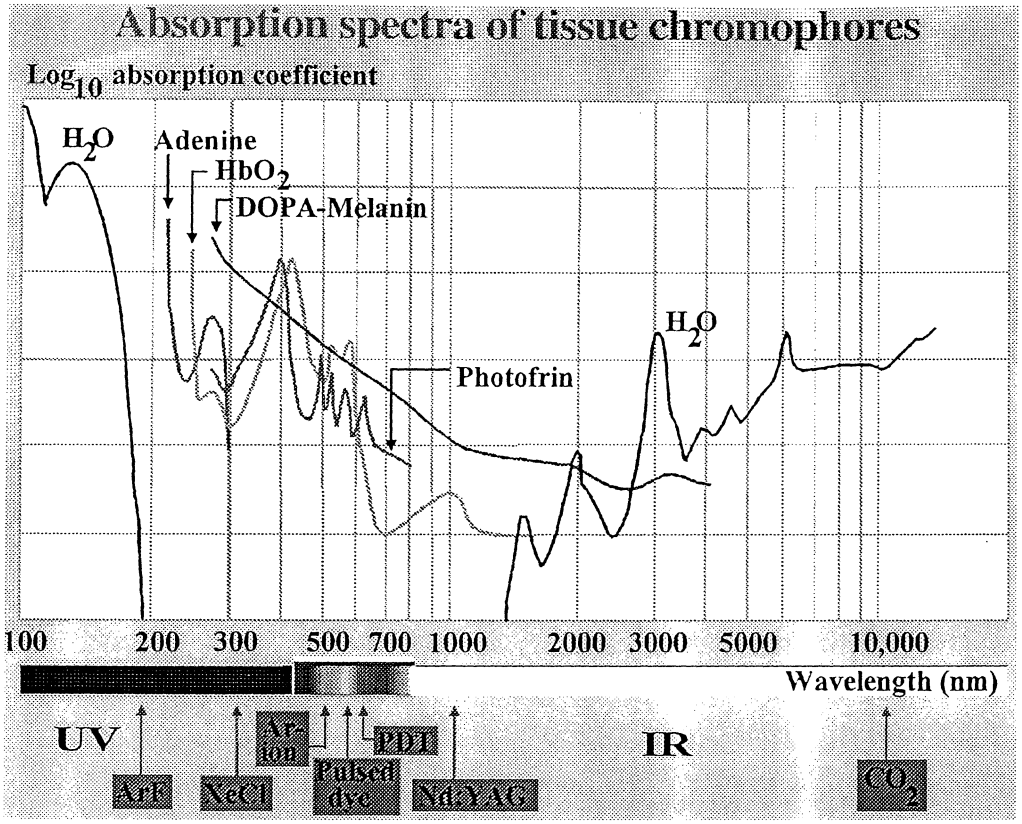


Figure 2-3: Absorption spectra of tissue chromophores (Graphics: Boulnois).

2.4.2 Fluorescent tumor markers

To perform laser-induced fluorescence (LIF) measurements, one can use exogenous fluorescent tumor markers. These substances have characteristic fluorescence emission, and can therefore easily be detected. One important fluorescent tumor marker is HpD, a hematoporphyrin derivative. Hematoporphyrins exist naturally in the body, and HpD is tumor-seeking thus useful for cancer diagnostics. A probable reason for the tumor selectivity is that tumors have weaker barriers between blood circulation and cells, which HpD normally is too large to enter. One problem with HpD is its instability and its ability to aggregates [5].

A newer substance among fluorescent tumor markers is ALA, δ-amino levulinic acid. This is a precursor in the heme cycle of the body, and transforms into protoporphyrin IX, Pp IX. ALA is easy to give to the patient intravenously, orally or topically and Pp IX has similar fluorescence characteristics as HpD. An advantage is that ALA itself is not fluorescent, making the given dose less critical. When used for LIF, the most commonly used excitation wavelength is 405 nm. The major fluorescence peak is at 635 nm, and a minor at 700 nm, see Figure 2-4 (right).

2.5 Medical diagnostics

Since certain fluorescent tumor markers accumulate in tumors, laser-induced fluorescence can be used to detect cancer [5]. The pH in tumors is often lower than in normal tissue, thus autofluorescence due to NADH is decreased. Comparing the peak intensity of the specific fluorescence from the sensitizer with the bluish autofluorescence makes it possible to distinguish tumors from normal tissue (see Figure 2-4).

It is also possible to characterize tissue without adding a fluorescent tumor marker. UV excitation light has shown to give good contrast in the bluish autofluorescence distribution between tumor and normal surrounding tissue [1].

Using dimensionless scalars, *e.g.* the ratio between the Pp IX peak intensity and autofluorescence intensity, gives many advantages compared to using absolute values. Excitation power dependence, spatial dependence, detection angle dependence and other factors, are eliminated.

Laser-induced characterization have shown high correlation with biopsy results in for example malignant brain tumors [9], bladder malignancies [10], oral and oropharyngeal tumors [1], colon cancer [11-13] and atherosclerotic plaque [1,14,15].

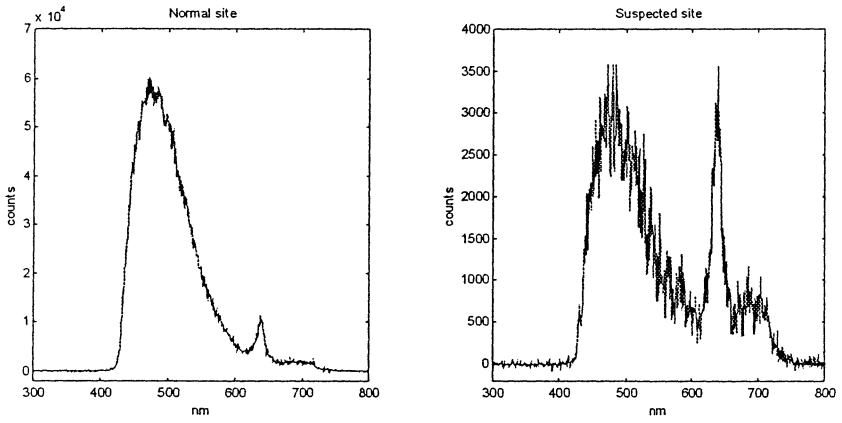


Figure 2-4: Typical spectra from a patient given ALA. The left graph shows a spectrum from a normal site, a large autofluorescence peak at about 480 nm and a small Pp IX peak at 635 nm is shown. The right graph shows a spectrum from what is considered a suspected site. A significantly smaller autofluorescence peak at 480 nm, and relatively large Pp IX peak at 635 nm. The Pp IX peak at 700 can be dimly seen. (Backgrounds are subtracted from both graphs.)

A non-LIF method is using a white light source to get an elastic scatter classification tool. One example is classifying bladder cancer, where elastic scatter measurements have shown good results [16].

It has been shown that fluorescence light from porphyrins have a longer effective lifetime than autofluorescence light from tissue at 635 nm [17,18], thus time-resolved measurements can be used as a tool for tumor differentiation. The time-resolved method can also be used for detection of atherosclerotic plaque [19].

3. Hardware

3.1 Introduction

An OMA system for fluorescence measurements has two basic functions: to provide excitation light, and to detect fluorescence light. In this case the common substance in which fluorescence appears is tissue and the information in the fluorescence light can be used to characterize the tissue. Tissue diagnostics demands motivate the use of three light sources: light for protoporphyrin excitation, light for autofluorescence excitation and broadband light for reflectance measurements (see section 0). The collected fluorescence light is conveniently analyzed in a computer, thus a suitable detector is a computer-controlled CCD (charge coupled device) camera attached to a spectrometer.

An optical fiber is a good transportation medium for excitation and fluorescence light, and simultaneously an effective probe. Using a fiber raise small restrictions to accessibility to the area of interest. Using only one fiber for both delivery of excitation light and collection of fluorescence light is convenient and provides a reproducible detection geometry, but requires a beamsplitter to separate outgoing and incoming beam paths (see Figure 3-1).

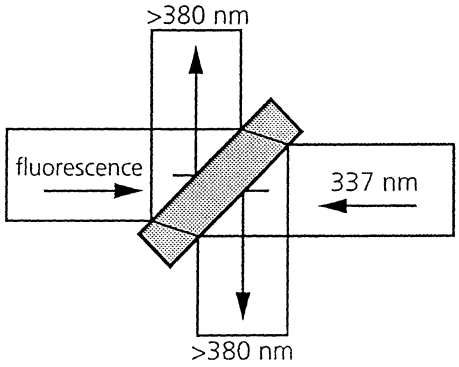


Figure 3-1: The principles of a dichroic beamsplitter. Excitation light, short wavelength, enters from the right (337 nm) and is mostly transmitted. The fluorescent light, long wavelength, returns from the left, and is reflected.

The use of a pulsed light source and a gated detector makes it possible to efficiently suppress ambient background light and thus allows use in normal daylight. Gating means that the CCD is exposed only when light of interest is expected, thus shielded from unwanted light.

A major part of this project was to gather information about and order the different components in the OMA system. Some of the important components, such as lasers and detector, were already ordered when we entered the project.

3.2 Light sources

3.2.1 UV laser

UV light at 337 nm has shown to give good contrast in the bluish autofluorescence distribution between tumor and normal surrounding tissue (see 2.5). A nitrogen (N_2) laser is thus suitable as an excitation light source, and such a laser (VSL-337, Laser Science) already existed from a previous system at our disposal.

This is a pulsed laser and currently can be operated at 1-20 Hz, with a pulse duration and energy of 3 ns and 100 µJ, respectively. The physical dimensions were originally 117×260×53 mm (w×d×h), but have been slightly modified to fit the system.

The laser runs on 12 V DC, conveniently fed from the power adapter (see 3.7.2).

3.2.2 Dye laser

A wavelength efficiently matching the Soret band of porphyrins is 405 nm, see (2.4). This light is produced by pumping a dye laser (DLM220, Laser Science) with the above mentioned UV laser. The dye laser uses the dye DPS (4,4'-Diphenylstilbene), which is fluorescent at 394-416 nm with the peak at 404 nm. The dye laser tolerates a maximum average power of 0.5 mW, but our setup produces a pulse energy of 5-10 µJ at 20 Hz.

The dye laser also existed in previous systems, thus present when we entered the project. We had to redirect its beam path (see Figure 3-2) to achieve the compactness of the system, and remove some excess material.

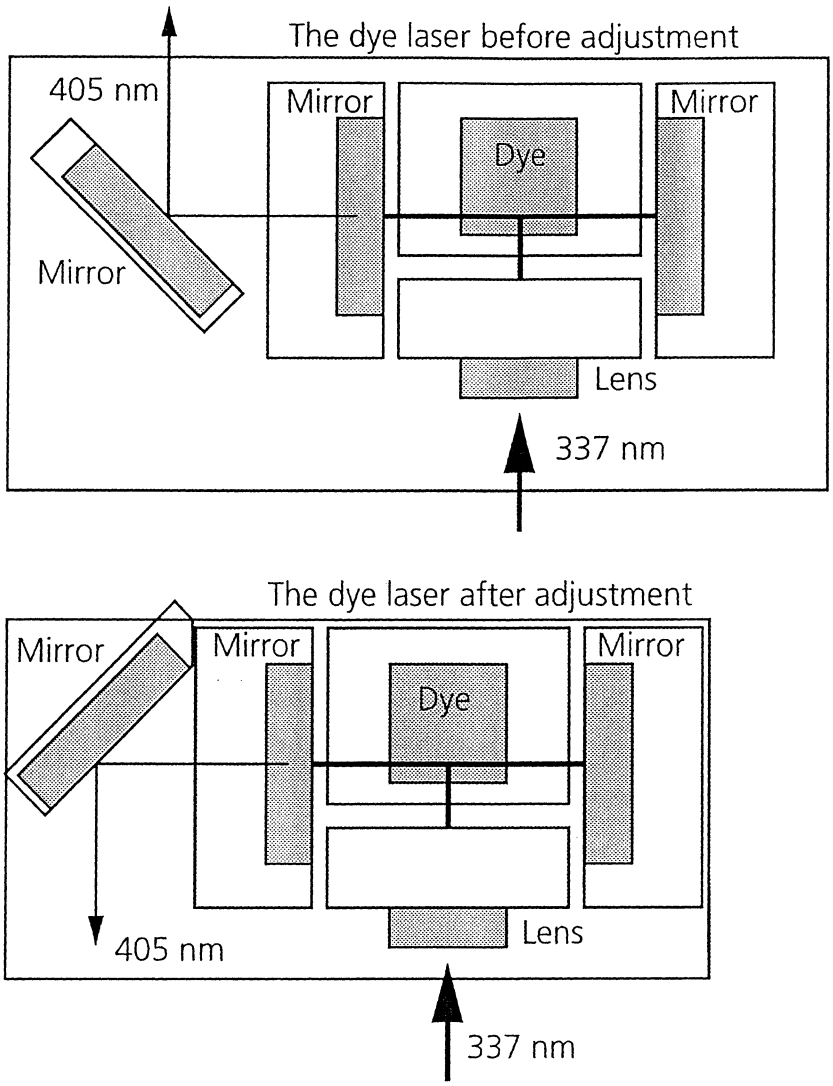


Figure 3-2: Geometry of the dye laser before and after modification.

3.2.3 White Halogen Tungsten lamp

Often abnormalities in the fluorescence spectra indicate that something is incorrect with the acquisition, *e.g.* blood on the fiber tip. The use of a broadband light source may help troubleshooting by showing low reflectance where hemoglobin absorbs. Another reason to include a white light source is to get an elastic scatter classification tool (see 2.5).

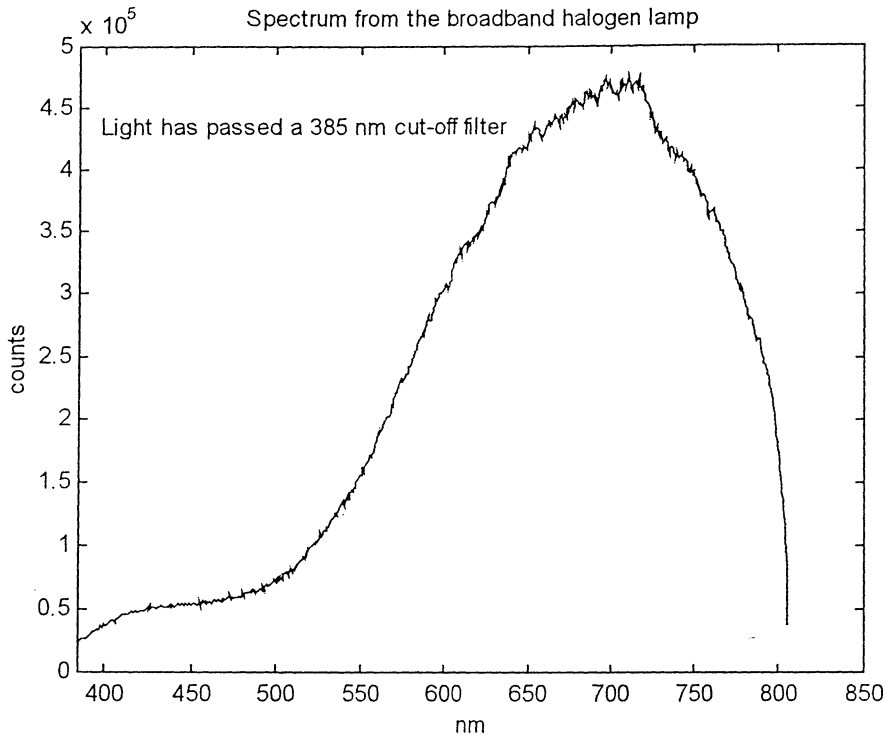


Figure 3-3: Spectrum from the broadband tungsten halogen lamp, as recorded by the system. The light has passed a 385 nm cut-off filter because of beta-version reasons.

A small broadband light source was needed (see Figure 3-3), and after investigating the market we decided to get a tungsten halogen lamp (LS-1, Ocean Optics). This lamp gets hot, but is small enough to be placed where heat production effects are minimized. The lamp is powered by 12 V DC.

3.3 Guiding the light

Minimizing the number of components and the path length were the main objectives when designing the different beam paths, see Figure 3-4 and Figure 3-6. A large number of optical components increases the complexity of the system and optical errors. Moreover the light exchange decreases with each surface passed. The path length is crucial as the beams actually are slightly divergent.

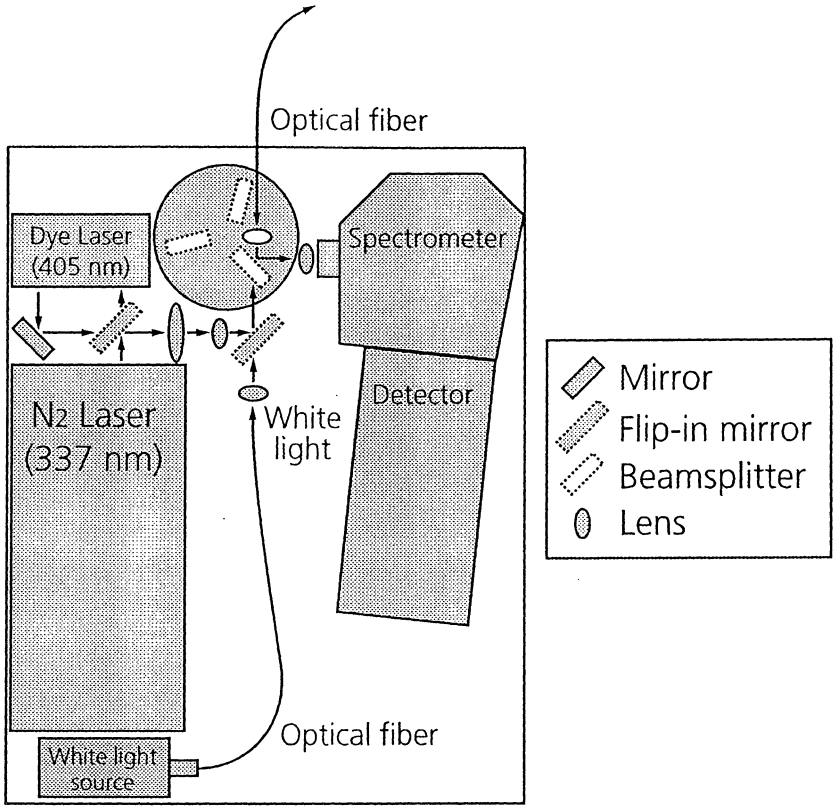


Figure 3-4: Schematic view of the different beam paths.

3.3.1 Laser light selector

Switching automatically between N_2 and dye laser is done by a flip-in mirror (8891M motorized Flipper™, New Focus). It provides fast switching and supports *TTL* control from a computer. Good repeatability is another important feature along with accurate tilt-and-trim adjustment (see Figure 3-5). The mirror is coated for maximum reflection at 351 nm, as 337 nm was not available.

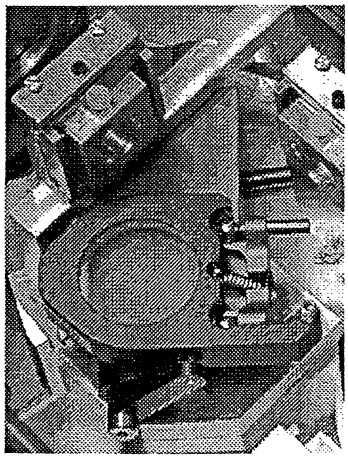


Figure 3-5: Detail of the New Focus motorized Flipper. Tilt-and-trim adjustment is offered by the allen-keys. This figure shows the flipper that selects between laser and white light.

The flipper came with a control handpad, which had to be modified for two reasons. We wanted to arrange with internal power supply instead of battery, and stripping the handpad cover and button decreased its size.

Guiding the light from the dye laser in an identical beam direction as light from the N_2 laser, *i.e.* hitting the same spot from the same incident angle, is arranged with a mirror, see Figure 3-6. The mirror is coated for maximum reflection at 364 nm (405 nm not available), and mounted in a holder with tilt-and-trim option (9771M Econo-Mount™, New Focus).

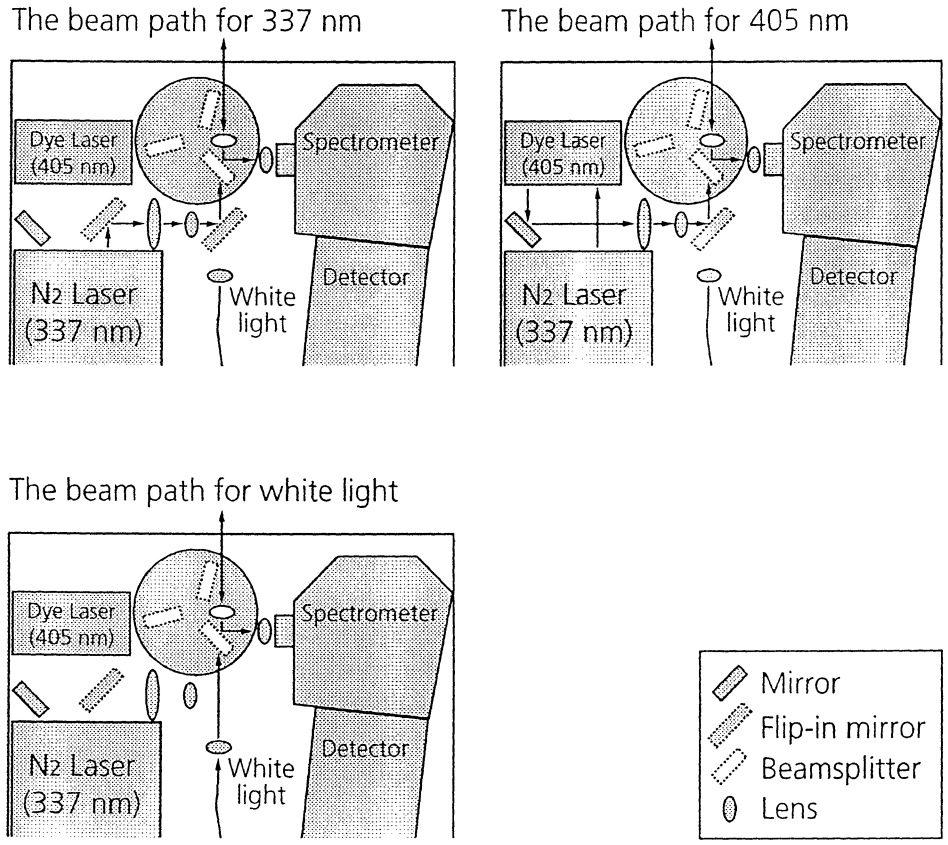


Figure 3-6: Detail view of each beam path. When a flip-in mirror is down, it is not shown.

3.3.2 Laser beam compression

The beam from the N_2 laser is too wide to fit the optics, thus compressing the beam is the next step along the way. Two positive *fused silica* lenses do the job (see Figure 3-7). Anti-reflective coatings help to keep the intensity up. The mounts were fabricated by the workshop at the Department of Physics, Lund University.

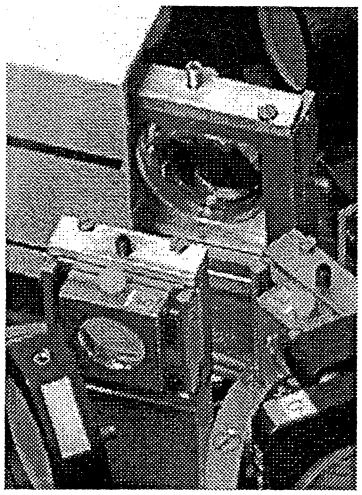


Figure 3-7: Detail of the laser beam compressor.

3.3.3 White light optical fiber

The white light lamp can be placed at a from heat aspects ideal position, since its light is guided in an optical fiber. The fiber is connected to a fiber port (FiberPort, OFR) with a collimating lens. The fiber port allows for adjustment in three dimensions plus tilt and trim. However, the light from the fiber port is slightly divergent, and since we assume parallel light at next component (see Figure 3-4), another positive lens collimates the light. This lens is mounted in a workshop-made lens holder, see (0).

3.3.4 Laser versus white light selector

Switching between laser light and white light is, just as laser light selection (see 3.3.1), done automatically by a flip-in mirror, see Figure 3-6 and Figure 3-5. The mirror is coated for maximum reflection at 364 nm, working well at 337 and 405 nm.

3.3.5 Rotating stage

The main objective of a rotating stage is to match the current light with a correct dichroic or broadband beamsplitter and cut-off filter. Since a lens adjusting the beam for the outgoing fiber port has to be on the stage, there has to be three of them. They have to be on the stage because numerical aperture and beam width sets the focal length. A minor benefit is that the lenses can have optimized coatings compared to having one lens for all cases.

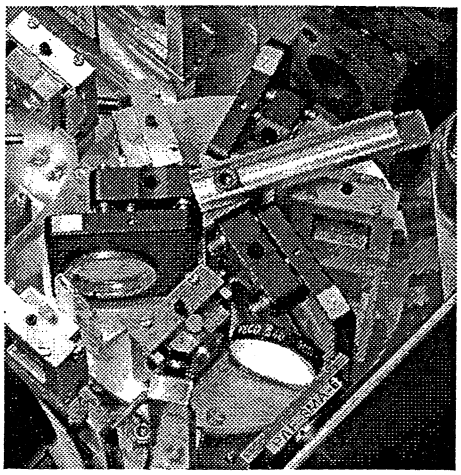


Figure 3-8: Detail of the rotating stage.

The beamsplitters vary in the three different light cases. For the UV 337 nm light, a short wave pass dichroic beamsplitter with a cut-off wavelength at 380 nm is used. The corresponding cut-off wavelength for the 405 nm dye light is 450 nm. The substrate is fused silica. The white light beamsplitter is a broadband 50-50% beamsplitter, working in the interval 488-694 nm, coated on a *BK7* substrate. All above mentioned beamsplitters are 1 inch in diameter and mounted in tilt-and-trim holders (9884MK, New Focus).

The lenses between the beamsplitters and the outgoing fiber port collect the light to fit the numerical aperture of the fiber port. The anti-reflection coatings are optimized for the ranges 337-600 nm (UV light), 400-700 nm (405 nm light) and 400-700 nm (white light). The lenses are mounted in workshop-made lens holders (see 0) since the available space is very limited.

When the fluorescence light enters the system, it is reflected in the dichroic beamsplitters. To make sure that all excitation light is discriminated, long pass cut-off filters are glued on the stage. For UV excitation light, the cut-off wavelength is 385 nm (BG 385, Schott) and for 405 nm dye light the cut-off wavelength is 435 nm (BG 435, Schott).

To improve the detected signal, plasma lines from the UV laser is not allowed to exit the system. A laser line interference filter is glued on the rotating stage, in front of the dichroic beamsplitter used for the 337 nm excitation light.

A close up picture of the optics on the rotating stage can be seen in Figure 3-8.

3.3.6 Optical fiber probe

The light to and from the external fiber is assembled at an OFR fiber port, identical to the one in section (3.3.3). The fiber port makes it easy to attach a sterilized optical fiber. The distal end of the fiber is positioned in contact with the tissue to be investigated. This fiber is made in quartz with a core diameter of 600 µm.

3.3.7 Spectrometer lens

Fluorescence light reflected by the beamsplitter is adjusted to be parallel, using the lenses on the rotating stage. To focus this light to the entrance slit of the spectrometer and to match its numerical aperture, a positive quartz lens is placed in front of the slit. The substrate is fused silica to minimize fluorescence in the lens itself.

Again, a high light throughput is important. The lens is thus mounted in a workshop-made lens holder, which in turn is placed on a translation stage. This gives adjustment possibilities in all three dimensions.

3.4 Spectrometer and detector

When we entered the project two different spectrometers were ordered. One Oriel Instruments MS 125 Spectrograph, and one ISA spectrograph model CP-140. The advantages with the MS 125 are its physical dimensions and its external geometry, *i.e.* the entrance slit is conveniently placed.

The CP-140 is larger and more difficult than the MS 125 to fit into the system, but has a better numerical aperture and a fixed grating.

The MS 125 arrived first, and we could quickly state its performance as satisfying. Since we wanted a compact system, a decision to use it was taken. As we are writing this thesis, the CP-140 has still not arrived, so we think we did the right thing.

3.4.1 Oriel MS 125 spectrometer

The Oriel Instruments MS 125 spectrograph is a crossed Czerny-Turner spectrograph with a F/number of 3.7 (see Figure 3-9). We are using it with a 100 µm slit and a 400 lines/mm ruled grating, blazed for 500 nm. This gives a working spectral range of 300-1200 nm and a resolution of 1 nm.

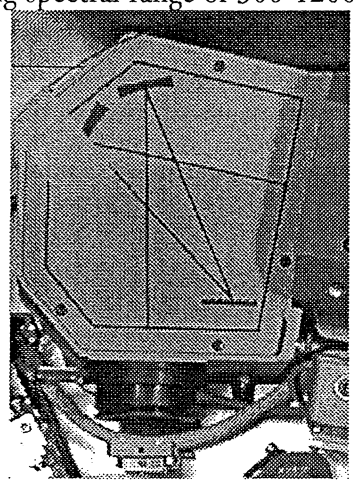


Figure 3-9: Detail of the spectrometer. The thick cable passes safely below the beam path.

3.4.2 Andor InstaSpec V ICCD detector

Connected to the spectrometer is the detector (InstaSpec V ICCD, Andor Technology). It consists of a few different components: a detector head with an image intensified CCD array (see Figure 3-10), a PCI card for the computer and a multi-IO box, used to send trigger and gating signals.

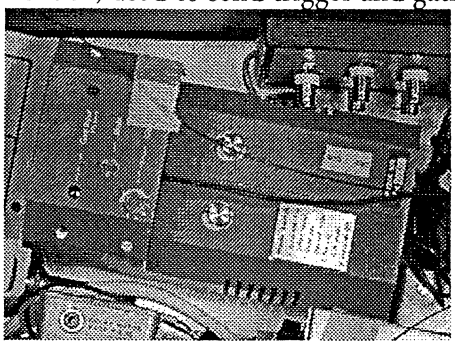


Figure 3-10: The Andor InstaSpec V ICCD.

The CCD array has the resolution of 1024x128 pixels, where the second dimension's 128 pixels usually are summed together, binned. The horizontal 1024 pixels give a resolution well matching that of the spectrometer.

To be able to do time-resolved measurements (see 1.4), the image intensifier can be gated with gating times down to 3 ns. This requires that external adjustable delay electronics is connected. Normally larger gate times will be used. An external adjustable delay generator, necessary for short gating times, is bulky and would considerably increase the size of the system. It is for that reason excluded in this first compact version of the fluoresensor.

To minimize the acquisition of background light, the multi-IO box (see Figure 3-11) is custom-designed with a built-in delay function trigged with a diode, giving a 0-100 ns delay followed by a 20-100 ns gate signal. The trigger diode is fed from an optical fiber, picking up stray light from the output mirror of the UV laser. The delay function is used to compensate for the time difference between the trigger signal and the fluorescence to reach the detector. To catch the incoming fluorescence we have chosen the delay and gate width to 40 and 100 ns, respectively.

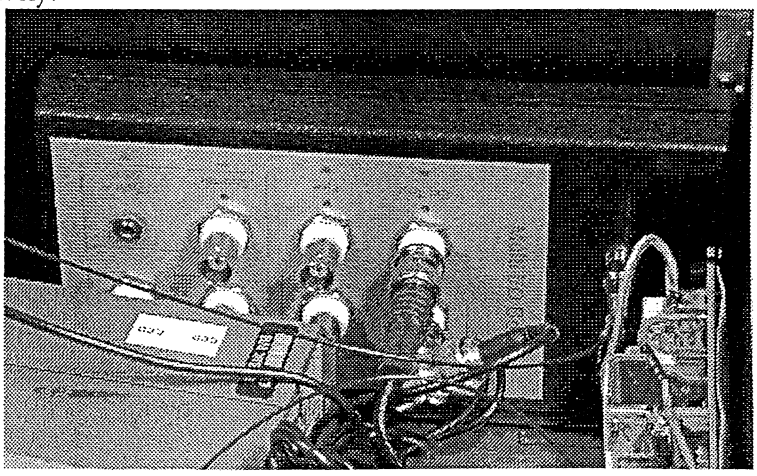


Figure 3-11: The modified multi-IO box. The optical trigger diode connector is in the black area to the right.

The detector has a gain of maximum 4500 counts per *photoelectron*. In most cases this is too much, resulting in saturation of the CCD. Useful values of gain have shown to be 30-120 counts per photoelectron.

Cooling the CCD decreases the dark current signal, and this is done in up to three stages [20]. Our system only use single stage cooling, which requires additional power supply to the PCI card. This power, 5V DC 5 A, is supposed to be delivered from the power supply of the computer but the used computer does not support this feature, so a 5V DC 5A power adapter has been added to the system (see 3.7.2).

3.5 Computer-controlled components

Switching between different light sources takes a long time in present systems, especially from a patient's point of view. The components associated with each light source are dichroic beamsplitters, cut-off filters, mirrors and lenses with different coatings. All these components had to be replaced, manually one by one. The system developed now is designed to switch these components automatically, when possible in parallel, in the new system.

3.5.1 Light source control

The UV laser fires when receiving a trigger pulse from the PCI card via the multi-IO box. If an acquisition without laser excitation light is desired, the software makes sure that no trigger pulse is generated. The same procedure is valid in the dye laser case.

The white light lamp is activated and inactivated by turning on and off the current through the lamp. A computer-controlled relay does this job.

3.5.2 Light selecting flip-in mirrors

The flipper controllers accept TTL signals. They can thus be software controlled via the parallel port on the computer. Controlling the parallel port on Windows NT turned out to be quite tricky. Windows NT does not allow direct access to the port itself, as MS-DOS and Windows 95 do. After consulting several "USENET consultants" we found a library to download, including several functions we could use.

3.5.3 Rotating stage

The rotating stage is motorized via a cog driving belt. The 12 V DC motor is controlled by the computer, using two functions. Each time we want to turn the stage one step (120°), a pulse is given on one parallel port pin. The control circuit of the rotating stage has an optical indicator answering with "high" when the current position is at a reference position. This indicator is connected to the parallel port as well.

Each time the system starts, or more precisely, when the software starts, it rotates the stage until the reference position is reached. From there, the software remembers in which position the stage is, *i.e.* which set of optics is used.

A springed steel ball ensures that the stage is stopped accurately in exact positions. Mechanical precision is needed, since the accuracy of the positions where the motor stops are not sufficient for our purposes.

3.5.4 Detector

Controlling the detector is the most sophisticated task. A wide range of parameters can be set, all by calls to library functions provided by the detector manufacturer. Examples of parameters are *exposure time* and *number of accumulations*, see Figure 3-12. Commands to the detector are sent in the same way, by calling functions. Examples of commands are *start acquisition*, *get data* and *get status*.

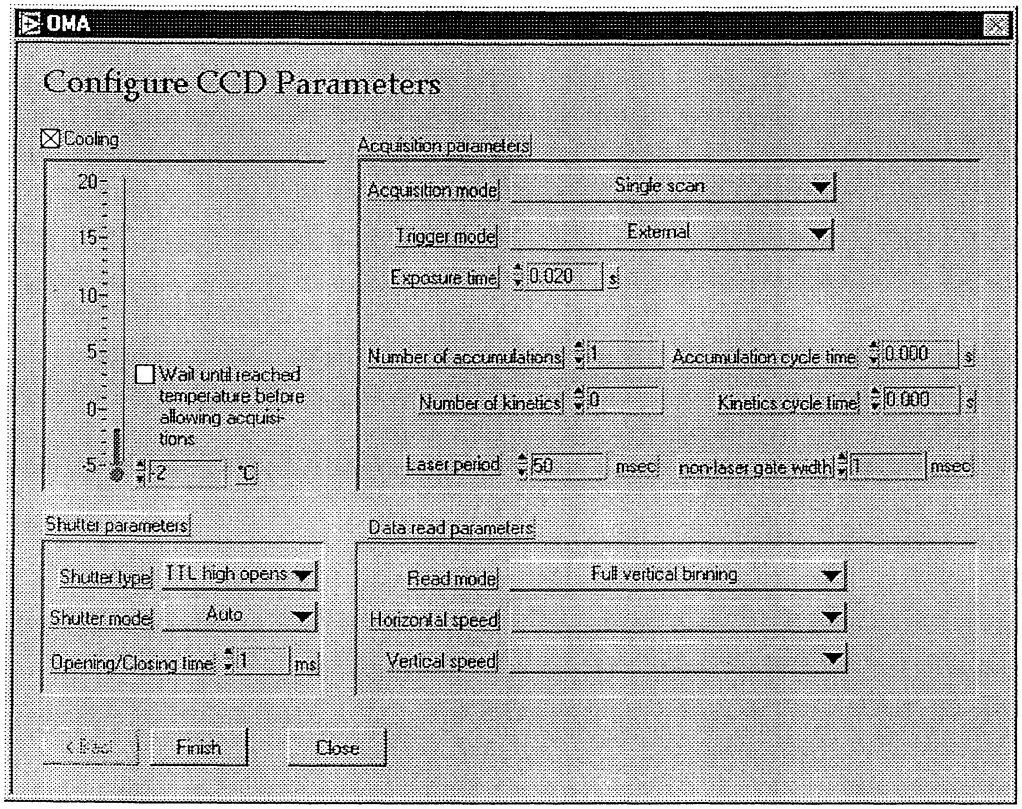


Figure 3-12: CCD Setup module. Most available detector parameters can be set. The values are stored in the CcdProps.txt file in the library folder.

3.6 Computer

Several demands on the system computer were quite hard to fulfill; it should be small and light, yet fast and expandable with a PCI card. Most notebook computers only support PC-card (PCMCIA card), or PCI cards in an external docking station, often huge and heavy.

After a lot of research, we found a company in California, *Dolch*, who manufactures a portable computer named FieldPAC [21]. It fulfilled the demands mentioned above, though quite heavy being a portable, 7.8 kg. However, it is built into a rugged aluminum attaché case, operating in demanding conditions.

It has a nice large 14.1" color display, and standard sound features. Thus it can provide easy communication with its operator, even at a few meters distance.

3.7 Miscellaneous hardware

3.7.1 Base plate

A small yet rigid base for all components in the system is necessary. We came to the conclusion to use a strainless plate of aluminum, 10 mm thick. It is now perforated with mounting holes and completely covered with components.

3.7.2 Power adapter

Two power adapters are needed, one for transformation of 220 V AC to 12 and 9 V DC, and one 220 V AC to 5 V DC. The first serve all mechanics such as rotating stage motor, flippers, laser and halogen lamp. The second was thrown in at a late stage to provide power to cool the CCD. This power was expected to be taken from a more conventional computer, but not supported by the FieldPAC.

4. Software

4.1 Introduction

Since the OMA system should allow to be maneuvered by a non-specialist, *e.g.* a physician, a simple graphical user interface is required. Additionally, the software must be possible to upgrade and further develop, thus understandable and structured. The commercial applications of today has a physicist's approach, which makes knowledge about parameters and procedures necessary. These applications give a good detail control, a mandatory part even in our application, but some parts can be greatly simplified.

The Division of Atomic Physics expressed a wish that we should develop the software in LabVIEW, a graphical development environment from National Instruments, made for instrument control and data acquisitions. This would itself provide for maintainability and a low step-in level when learning to modify the application.

4.2 Control the acquisition process

To make a correct acquisition, one must follow some necessary steps in a certain order. The detector parameters must be set, the light sources should be triggered and then the detected information can be collected and stored.

Andor distributed the detector system with software drivers, including functions controlling all aspects of the camera, and examples using these functions in a few different programming languages. Andor also provided a programmer's guide [22], explaining the functions and suggested order of usage (see Figure 4-1).

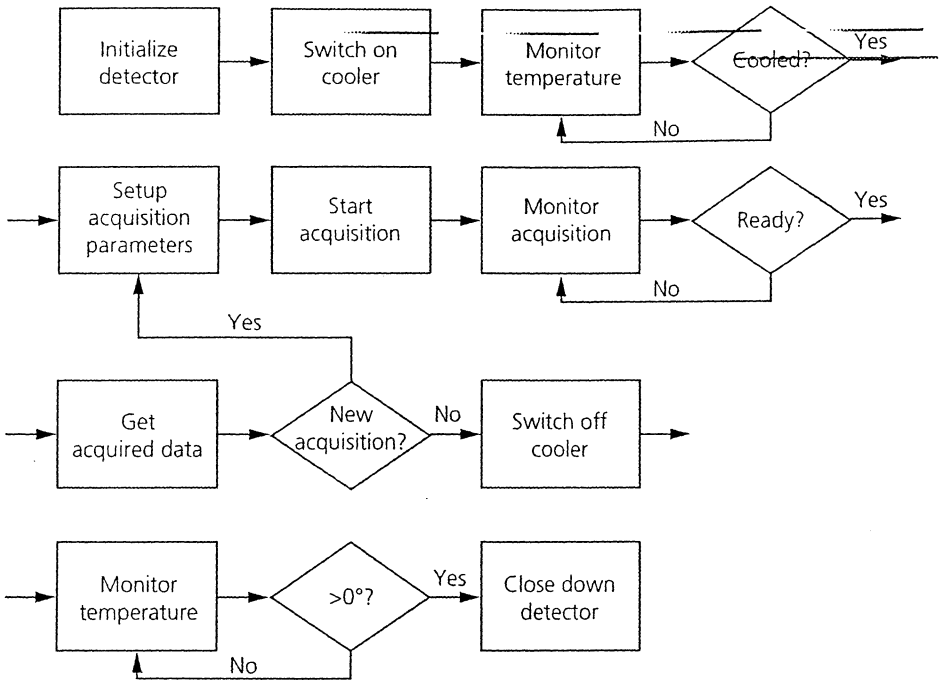


Figure 4-1: Block diagram of the acquisition process. An acquisition should not be performed before the temperature has stabilized at desired level, and the system should not be shut down before the temperature is above 0°C.

We implemented these functions and rules into what we call "Andor Boxes", *e.g.* SetupAcquisitionParameters, StartAcquisition and GetData. Some simple functions were implemented too, for instance SwitchCoolerOff, for reasons of consistency. An example of the use of these boxes can be seen in Figure 4-2.

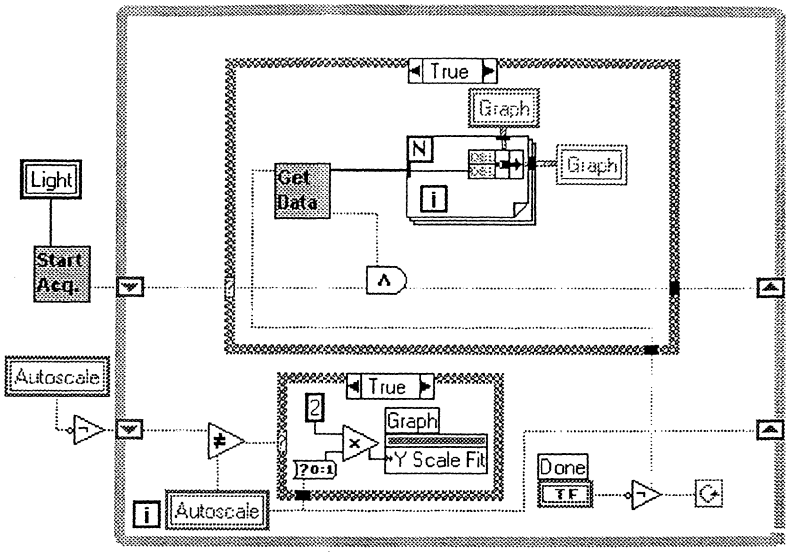


Figure 4-2: This diagram shows the use of some Andor boxes. A typical real-time acquisition is done, initialized with the Start Acq.-box. Get Data reads the spectrum, and if not Done is pressed, the succeeding acquisition starts. While the succeeding acquisition is exposed, the previous is displayed.

Selecting the excitation light source is divided in different layers. The top layer is a *VI* (Virtual Instrument) called SetLight, which simply requires a parameter 0-2, indicating which source to use. Inside this VI, there is an abstract component layer, which consists of VIs for each motorized component. Depending on the desired light source, the flippers and the rotating stage must be given signals. These VIs also return estimated values of the time required to change state. SetLight itself returns the largest of these values, see Figure 4-3.

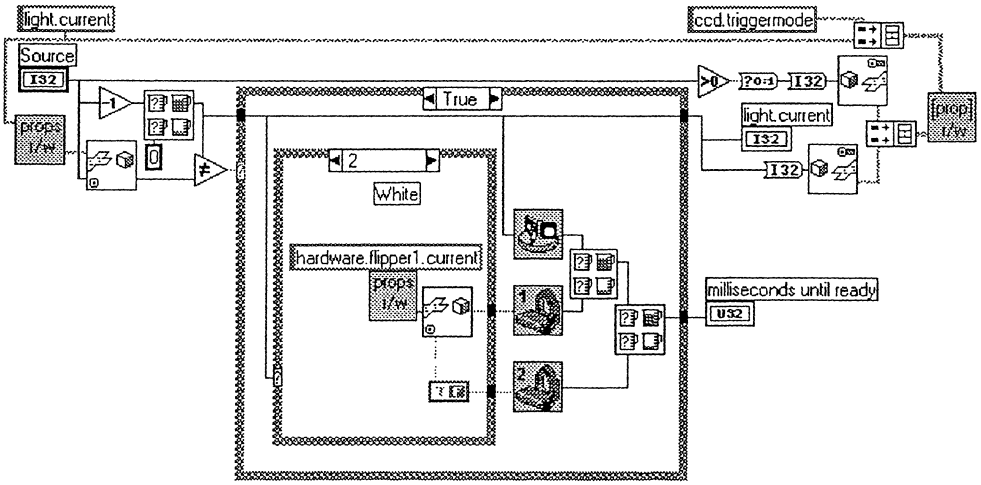


Figure 4-3: A view from SetLight.vi's diagram. The frame shows what is done when selecting white light as an excitation source. Flipper number one is not changed, while flipper number two is down (false). The rotating stage is in position 2 (0-2). Each component returns an estimated time needed to fulfill the request, and the maximum time is considered.

The abstract component VIs change the states of the corresponding parallel port pins, or as in the rotating stage case, generate a short pulse on a pin. As mentioned in section (3.5.2), controlling the parallel port in Windows NT is not implemented in LabVIEW, but Scientific Software Tools provided a *DLL* (dynamic link library) we could download. This library has functions we could call from LabVIEW, changing the state of the parallel port.

4.3 Analysis of acquired information

The purpose of the OMA system is to provide data for classification. Signal processing is therefore an important issue, just as it is important to save the raw data for later external analysis.

4.3.1 Wavelength calibration of the detector system

To be able to relate a pixel number (channel) on the CCD to a specific wavelength, a calibration must be performed, at least once. Since the grating of the spectrometer is locked in position the wavelength calibration should be fixed, but new calibration spectra will probably be acquired at each measurement session.

To achieve precision in the calibration, light sources with sharp and well determined lines should be used. Fortunately, an ordinary fluorescent light tube fulfills these requirements, since its mercury lines are apparent and known with a resolution far better than most of the system.

To make this process easy, even automatic, we have implemented a library, in which known calibration sources can be stored. Once and for all, the peaks of a source can be defined with desired precision. When given a sample, the system picks the most similar library spectrum, and relates the peak wavelengths from the library with the detected peak channels in the sample. The method used to pick the most similar library spectrum is cross-correlation [23]. Optionally, the system gives an audio confirmation of the calibration.

The result of the calibration is a linear function determined by a least squares fit, giving slope and intercept values. These values are stored and used in future acquisitions.

4.3.2 Intensity calibration of the detector system

The sensitivity of the detector system is not uniform at all wavelengths, thus an acquisition of a light source with a well-known intensity distribution must be performed. All following acquisitions can be compensated for the hereby known irregularities.

4.3.3 Processing

Some data processing is done automatically or optionally. A background acquisition can be subtracted to remove fiber fluorescence, surrounding light and

dark current effects (see 3.4.2), all to display a more true result on the screen as well as to provide better data for analysis.

4.3.4 Data storage

All acquired spectra are saved to disk. A text-based file format containing most parameters is used, *e.g.* if a background is subtracted, a note about which background subtracted is attached. It is necessary to save all data for a proper statistical evaluation.

4.3.5 Fast analysis

It is possible to design a criterion which can be used in real-time to do a fast analysis of a spectrum. A parameter in the criterion could be the intensity over a certain wavelength interval, and ratios can be formed for different intervals. Figure 4-4 shows the design of a criterion, and Figure 4-6 the use of the same criterion in an examination situation.

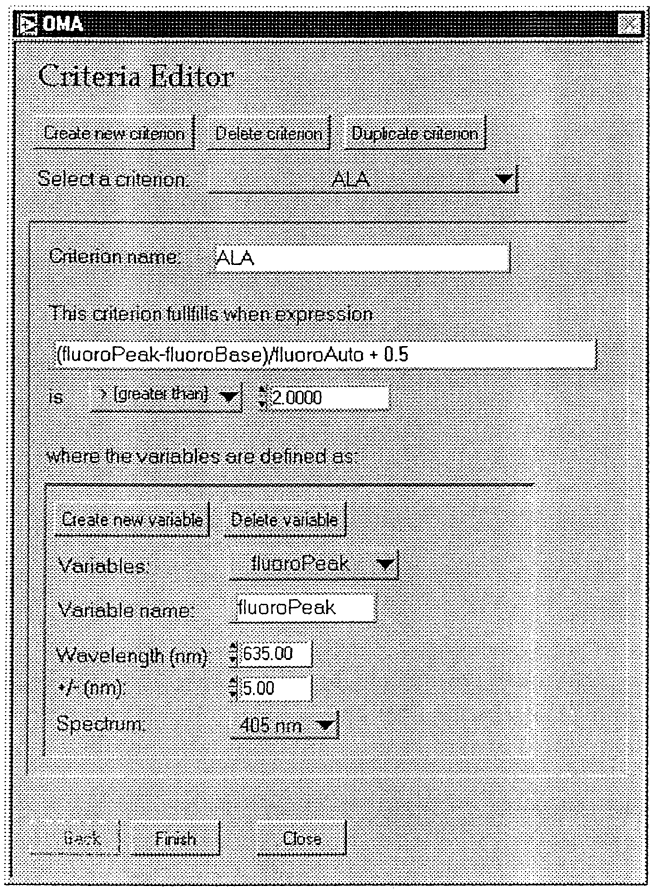


Figure 4-4: The Criteria Editor module. Here a criterion called "ALA" is shown. The expression uses three variables, and below the expression the definition of the variable "fluoroPeak" can be seen.

A simple cancer criterion could be

$$\frac{fluoroPeak - fluoroBase}{fluoroAuto} + 0.5 > 2$$

$$fluoroPeak = I(635)$$

$$fluoroBase = I(600)$$

$$fluoroAuto = I(500)$$

which compares the intensity of the protoporphyrin fluorescence with the auto-fluorescence intensity and can be used to classify skin tumors as described in section (2.5). One way to present the result is a red and green lamp, where red corresponds to a fulfilled criterion, see Figure 4-6.

4.4 Technical structure of the software

One important issue when we designed the structure of the software was to make it easy to add new functions and interfaces. For instance, if large-scale examinations are planned, a tailor-made sequence of functions and interfaces may be preferred.

4.4.1 Modular implementation

We have striven for making it easy to combine elementary functions into larger tasks or sequences. The concept *module* developed, which is a VI that can be called from a menu, put in a sequence or put in a loop structure. A module must take certain parameters, and return some specified values. Examples of modules are *Calibrate*, *CCD setup* and, actually, *Advanced menu* which is an application of the *Select* module.

Inter-module communication is supported via so called *properties*, which are variants of global variables. This is necessary, since the parameters to and from a module are fixed.

4.4.2 Client-server design

After a few weeks of the software development process, we wanted to separate hardware communication from user interaction. This separation made it possible to simulate the hardware, which was essential since the hardware has been absent during most of the development period. The separation also led to that we could develop the two parts independently, and sometimes in parallel on different computers. An intellectual advantage can also be motivated with a more distinct layer design, making it easier to grasp a certain function.

When structuring the new design, we found no reason against making it a client-server application. This means that the user interacts with a client, communicating in some way with a server, controlling the hardware. The

communication between client and server can be made general, and as in most cases, over the Internet if the client and server are run on different computers.

Running the client and server on different machines rise new interesting possibilities. The user can be an expert anywhere, not necessarily in the same room or country as where the examination takes place. If the acquisition is made in an environment not suitable for humans, the control staff can be at a friendlier location. In the medical case, this is known as telemedicine.

Multiple clients is another feature of the client-server design, where one client probably should act as master client, and the others as observers.

4.5 User interface

The target groups for this application vary a lot in what they want to achieve and what they know about the different issues involved in the OMA system. A physician probably wants to use the system as a tool for diagnostics, while a physicist wants to use it for acquiring pure spectral information.

We have approached this problem by developing multiple sets of modules, primarily performing the same tasks, but with different interfaces showing either all available or only essential information. The standard, or simple, modules can be reached directly from the Main Menu, while the more advanced modules can be found under the Advanced Menu, see Figure 4-5. No difficult steps must be afforded to reach standard modules, thus no Standard Menu.

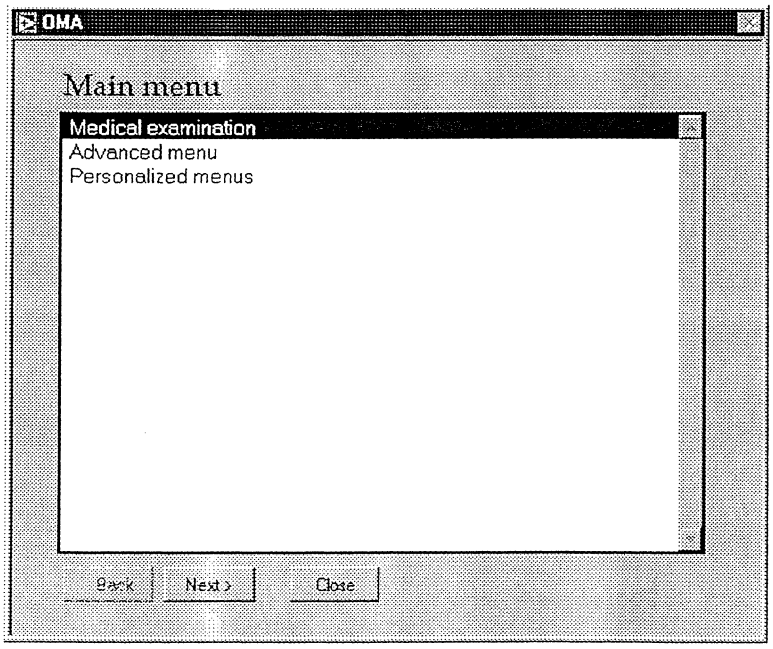


Figure 4-5: The Main menu. Standard modules or sequences can be run from here, while advanced or personalized modules are found under respective sub menu.

4.5.1 Standard mode

With standard mode, we refer to using the modules with only essential interface components. These modules are designed to hide all technical and most physical aspects, and act as automatically as possible. Examples of standard mode modules are Calibrate and RedGreen.

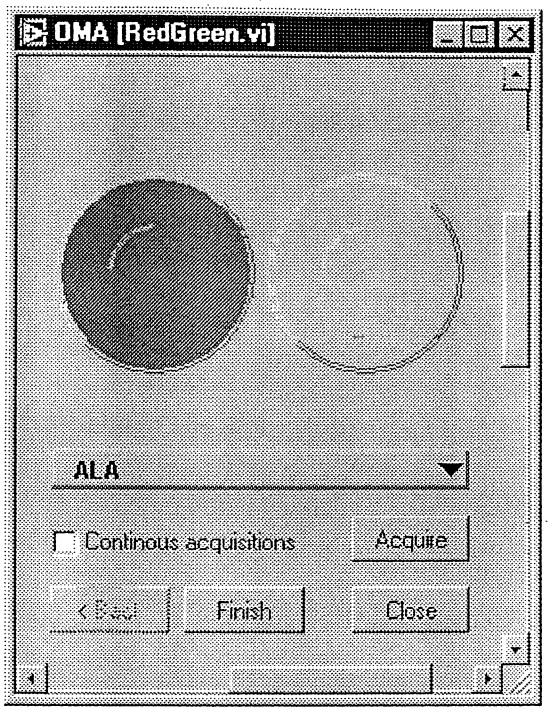


Figure 4-6: View from the standard module RedGreen.

The module Calibrate performs a calibration (see 4.3.1) by first asking the user to aim the fiber at an available calibration light source, and then repeatedly makes acquisitions and compares each one with the library spectra until a match is found. The module simply terminates by declaring, in text and audio, which library spectrum it used for the calibration.

RedGreen is a module using "fast analysis" (see 4.3.5) to do a preliminary diagnose using a selected criterion. The user can only select between continuous and single acquisition, and select which criterion to be used, see Figure 4-6. The result is simply displayed with a red or green lamp combined with an associated sound, for effective communication if the user is alone or visually occupied.

4.5.2 Advanced mode

In the advanced mode modules we try to give the user maximum control and feedback, so that the desired task can be carried out. All settings that can be adjusted are available, and original terms are used such as *Full vertical binning* and *Kinetic cycle time* which are terms about detector settings.

Advanced mode also covers modules used to configure the system and the application itself. Examples are the *Edit Initialization File* module, in which menus and sequences can be designed (see Figure 4-7), and *CCD setup*, which is

used to configure the detector. More information about the application customization can be found in Appendix A.

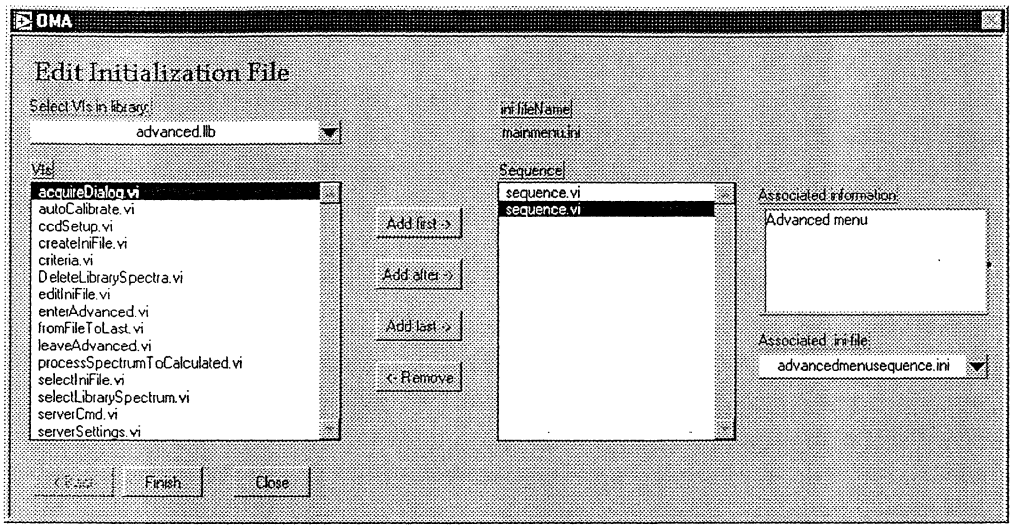


Figure 4-7: Edit Initialization File module. This module is used to build menus and sequences. The list box to the left shows the modules in the advanced library, from which modules can be selected and inserted into the Sequence list box. For each entry in the Sequence list box there is Associated information, *e.g.* the menu item displayed.

4.5.3 Personalization

Embedded modules for changing the appearance of menus and sequences provide for customization. A user can define a personal set of modules and list them in a new, separate menu. Tailor-made modes can be developed, where personal opinions about user interface and interaction can be considered, or where task-specific modules can be bundled. An example of personal menus can be seen in Figure 4-8.

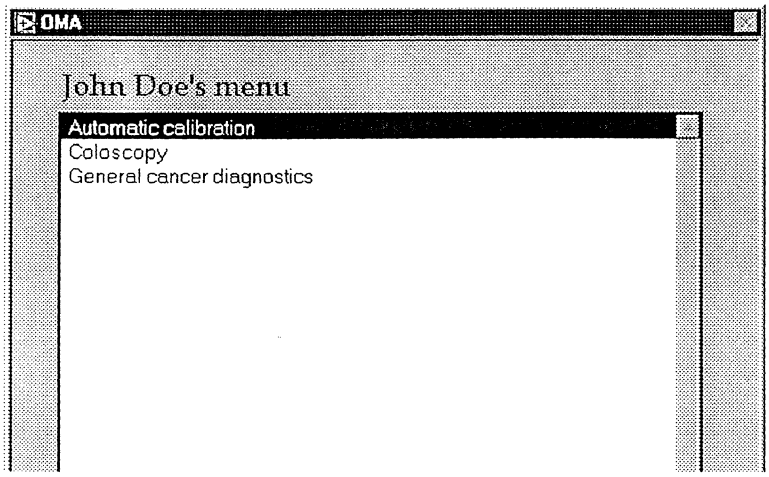


Figure 4-8: Example of a personalized menu.

5. Evaluation

The OMA system was tested in a campaign of *in vivo* measurements of the human colon at the Karolinska Hospital in Stockholm. In the campaign LIF spectra was collected from normal colonic and rectal tissue and various tissue polyps which may occur in the colon or the rectum.

Polyps in the colon are typically 1-10 mm in diameter and have approximately the same light-red color as the normal colonic tissue, and can roughly be divided into *adenomatous* and *hyperplastic polyps*. The adenomatous polyps are considered precancerous, while the hyperplastic polyps are considered harmless. To prevent future cancer, polyps are removed from the colon during colonoscopy. The decision which polyps to remove is made by the endoscopist from visual impression since adenomatous polyps generally are darker red than the hyperplastic polyps, but this method is inaccurate even for experienced endoscopists. Studies have shown considerable difference between the polyp classifications of an endoscopist and a pathologist [24]. Therefore biopsies are taken from all polyps found during colonoscopy. Picture of a polyp can be seen in Figure 5-1.

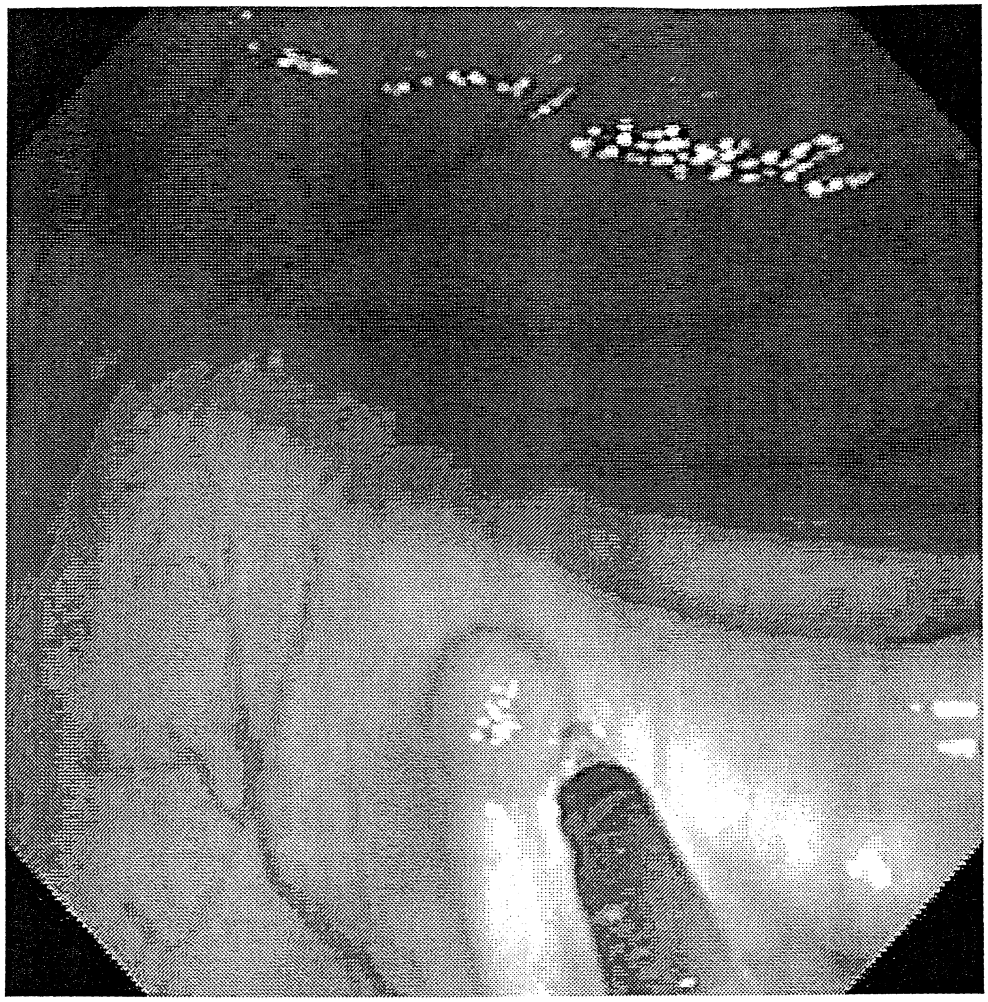


Figure 5-1: A polyp seen from the endoscope. The tip of the fiber is approximately 0.6 mm in diameter.

Laser-induced fluorescence is a promising approach to a tool for the differentiation between adenomatous and hyperplastic polyps. Previous investigations indicate that autofluorescence from adenomatous polyps has similarities to autofluorescence from malignant tumors [11,12]. It has also been shown that ALA can be used to distinguish adenomatous polyps from normal tissue [13]. The purpose of the campaign at the Karolinska Hospital was to collect enough LIF spectra from adenomatous and hyperplastic polyps to evaluate the possibilities to differentiate between these.

LIF spectra was collected during colonoscopy, in which an endoscope is used to view the inside of the colon. The endoscope is up to 170 cm long to reach the entire colon, and the tip of the endoscope is maneuverable from outside to make it possible to guide the endoscope up through the colon and to make navigation easier. The tip of the endoscope is also equipped with lighting, a video camera and a system for cleaning the camera lens. The video signal is transported to an external screen which can be viewed by the examining endoscopist. The endoscope is also equipped with a channel for accessory instruments, such as

biopsy tongs. The fiber probe of the OMA system was inserted into an open channel, and lead through this channel to the area of interest. Pictures of the fiber probe in the colon can be seen in Figure 5-2.

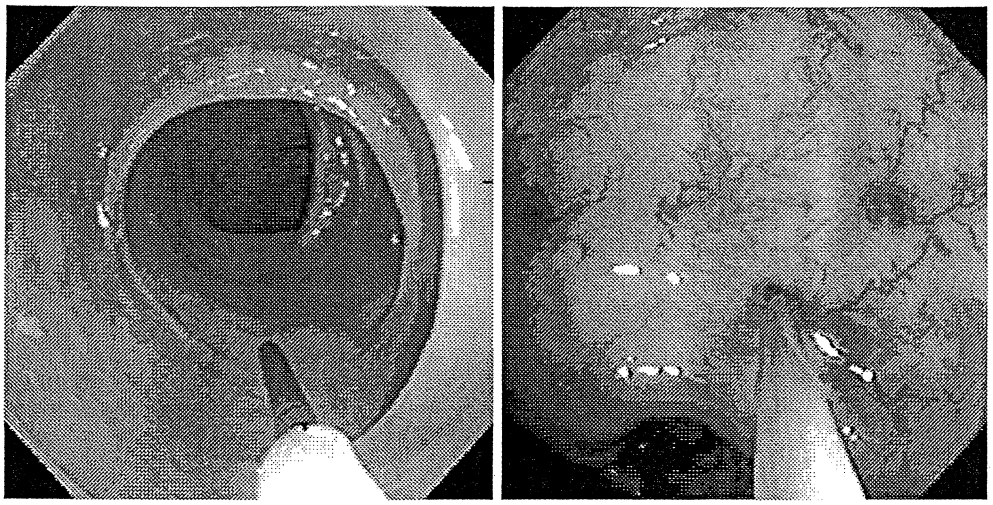


Figure 5-2: Acquisition from lumen and from a normal site.

Fluorescence measurements were made with the two supported laser wavelengths 337 nm and 405 nm. All polyps found during the examination and some normal area of the colon was investigated with LIF. Measurements were made in contact with the tissue to optimize the collection of fluorescence and to obtain a reproducible measurement geometry. Each acquisition consisted of 60 excitation pulses fired at 15 Hz.

Since fluorescence and stray light from the detection system itself and the background light in the colon should be subtracted from collected data, background acquisitions for each excitation wavelength were taken in the free space within the colon *lumen*. New background acquisitions were taken for each new measurement area.

Six endoscopists examined 79 patients in three investigation rooms and about half of the patients had been given ALA orally at least two hours before the examination. One hundred and seven (107) polyps and *** normal sites were measured. Typical spectra from the campaign can be viewed in Figure 5-3 to Figure 5-7.

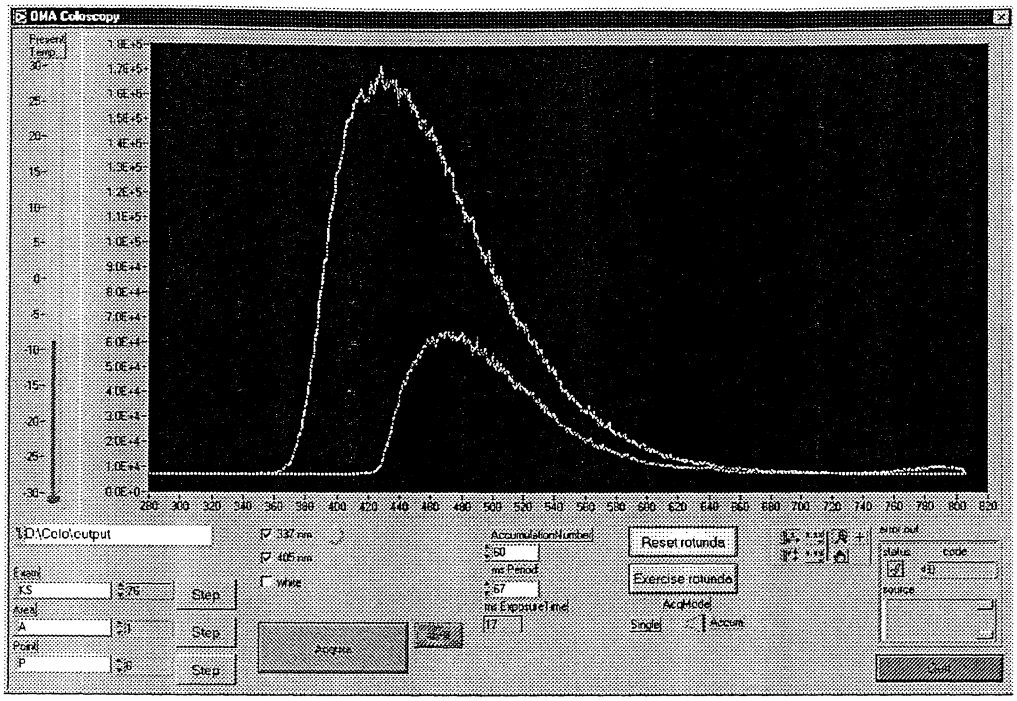


Figure 5-3: Acquisition from a normal site. Only autofluorescence is detected. This is a view from the program used at the study at the Karolinska Hospital, Stockholm.

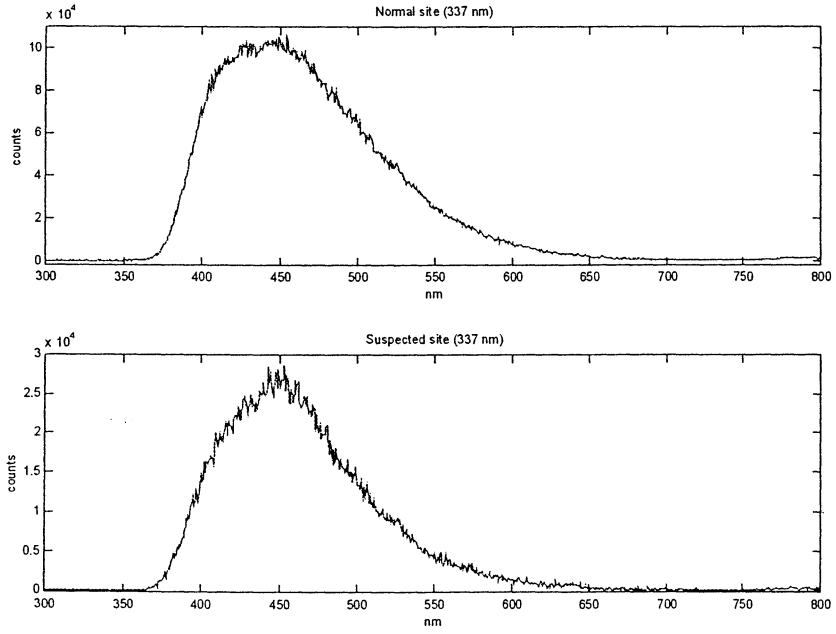


Figure 5-4: 337 nm spectra from a non-ALA patient. The upper spectrum is from a normal site, and the lower is from a suspected adenomatous polyp. Note the slope difference at about 400 nm, which is steeper in the normal case.

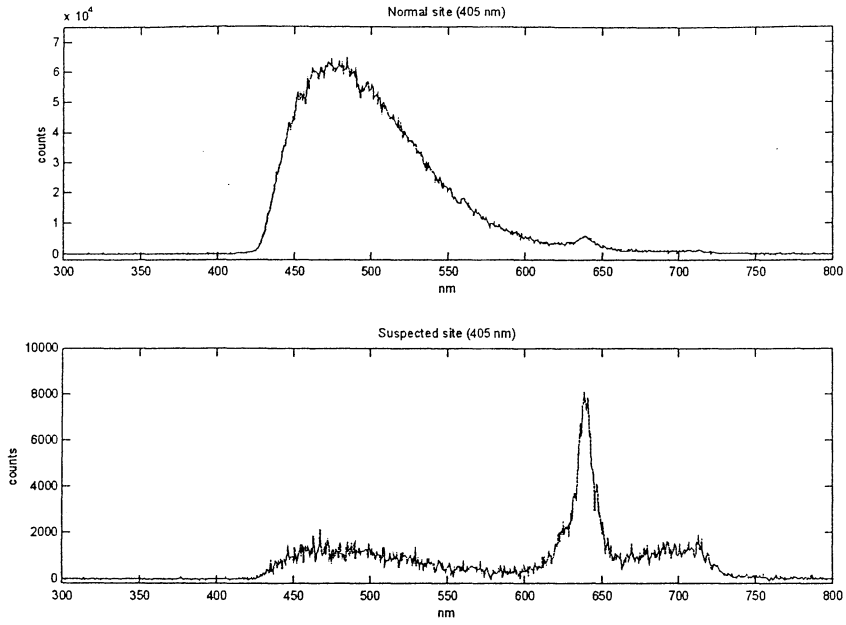


Figure 5-5: 405 nm. The upper spectrum is from a normal site, and the lower is from a suspected adenomatous polyp (same as in Figure 5-5). The peak at 635 nm is significant.

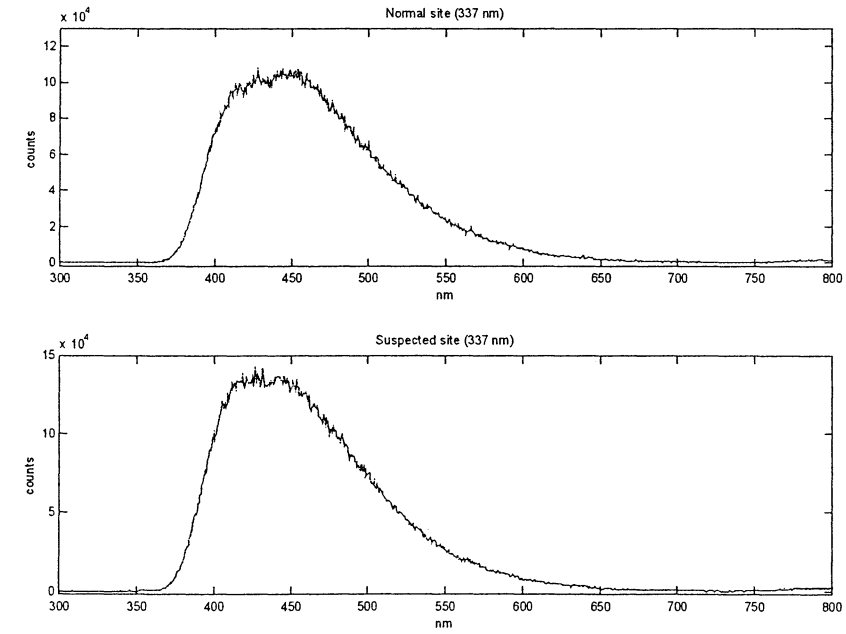


Figure 5-6: 337 nm spectra from a non-ALA patient. The upper spectrum is from a normal site, and the lower is from a suspected hyperplastic polyp. Note that no significant slope can be detected at 400 nm, compared to Figure 5-4.

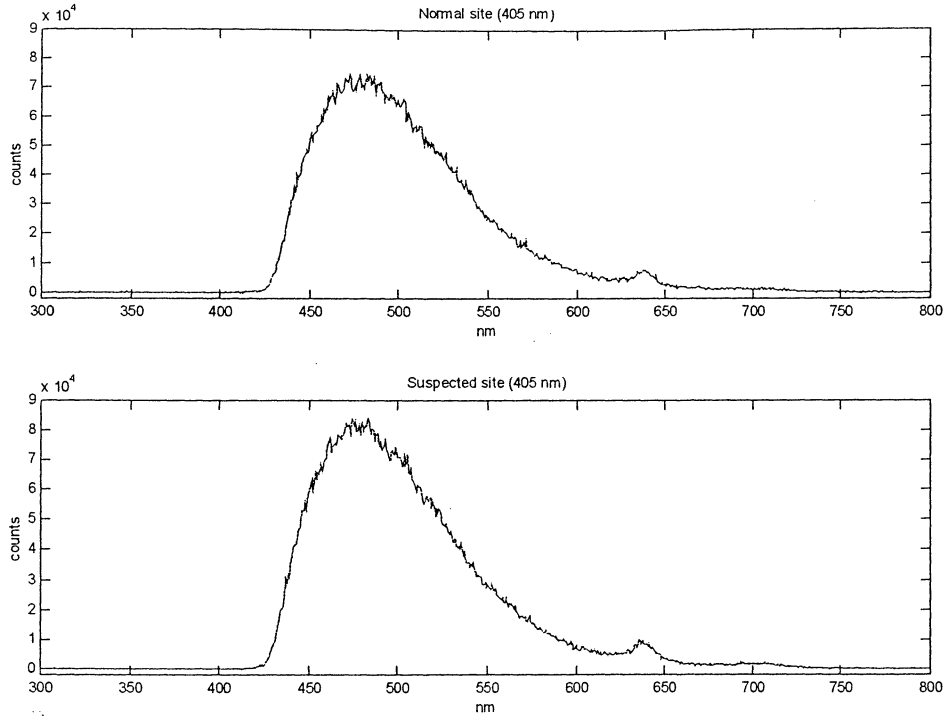


Figure 5-7: 405 nm spectra from a non-ALA patient. The upper spectrum is from a normal site, and the lower is from a suspected hyperplastic polyp (same as in Figure 5-6). No significant peak at 635 nm can be detected.

The overall performance of the new OMA system was clearly adequate for measurements of the colon in a campaign of this extent. Especially the compactness and the speed of excitation light selections were appreciated by the endoscopists and the assisting staff. The measurement speed is essential since colonoscopy are sometimes very painful for the patient, and it can be very difficult to keep the fiber in contact with small polyps, due to bowel movements.

We found that the wavelength calibration of the system was steady throughout the campaign. New calibration spectra acquisitions will probably not be necessary to take on a daily basis.

Some weaknesses in the design of the system were observed. The trigger function of the white light source was not working properly so no white light spectra were acquired. Since the main purpose of the white light source is to detect abnormalities in the acquired fluorescence spectra this weakness was not crucial to the campaign. The system operator was responsible to discover abnormal acquisitions.

The dichroic beamsplitters were not functioning as expected and were replaced by neutral density beamsplitters. The light intensity loss in neutral density beamsplitters is 50% for each passage, which means that 75% (1-0.5×0.5) of the intensity was lost only in the beamsplitters. A dichroic beamsplitter is designed to preserve no less than 90% of the interesting intensity at each passage, which means that no more than 20% should be lost in the beamsplitters.

Correcting this problem should make the OMA system at least 3 times more sensitive to fluorescence light.

Another weakness in the system was that some periodic maintenance work had to be done. The dye laser beam path had to be realigned due to the transportation of the system between the three investigation rooms. This was done twice during the two weeks of the campaign. Also the rotating stage had to be maintained since the springed ball which ensures that the stage is stopped in exact positions was grinding brass chips from the stop heels on the stage. These chips periodically clog the ball's socket. The ball was cleaned and greased once during the campaign.

6. Discussion

6.1 Results

The system of today's date has several improvements to the previous ones. The total weight, including the 7.8 kg computer, is 26.8 kg, compared to the previous which could not be lifted by one man. The size, including an aluminum hood, is 47×40×21 cm (see Figure 6-1), significantly smaller than 85×70×140 cm.

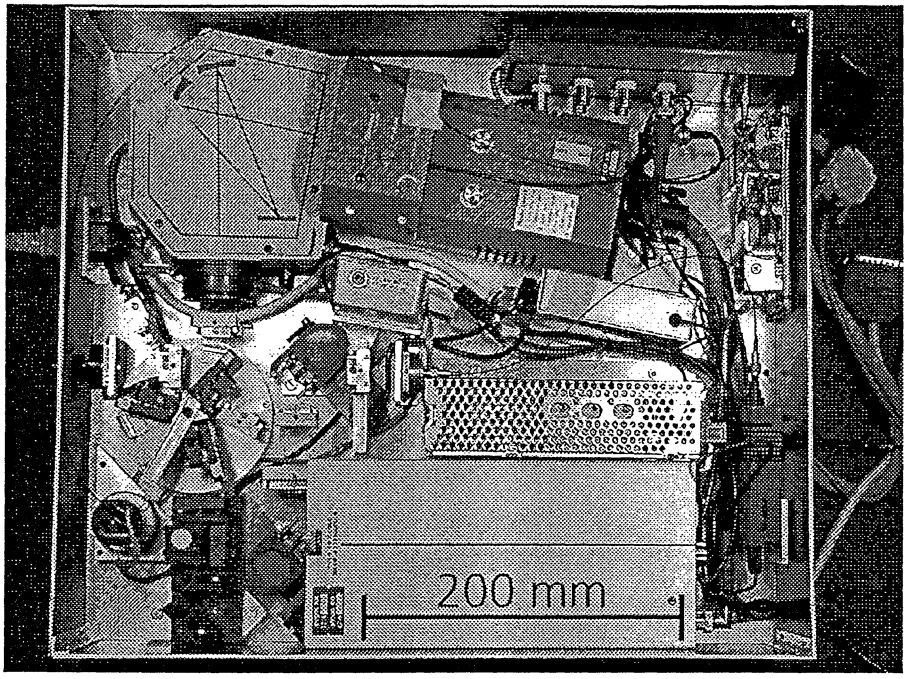


Figure 6-1: The optical side of the system seen from above.

Despite the fact of great intensity loss in the beamsplitters, the signal performance is still better than the older systems, according to senior physicists. The gain on the intensifier was set to 6 out of 9 when acquiring the colonoscopy spectra, corresponding to an amplification of 115 out of 4583 counts per photoelectron [20]. Reference measurements were acquired at gain 4 (37 counts/photoelectron).

Changing the optics for the different light sources takes about a second in the worst case. This is also much better than in previous systems, where manual changes took about 20 seconds. The fact that it is controlled automatically by the software makes the process even faster and is not forgotten by mistake. The accumulation frequency can be raised to a maximum of 20 Hz, where 10 Hz seems to be a comparable value in older systems.

The software, especially the tailor-made modules, improves the acquisition procedure by supporting file handling and structured storage of the data. The operator's effort is reduced to pressing a single button for each acquisition.

6.2 Future improvements of the system

The campaign at the Karolinska Hospital awakened many ideas to improve the system, and was essential to the evaluation process. The system was occasionally run by one person alone, and that indicated which tasks that should be scheduled to the computer.

6.2.1 Ensuring correct position of the rotating stage

After two weeks of intensive use, the rotating stage needed some adjustments. A small screw fixing the stage to the cog belt axis got loose, and needed to be tightened. Drilling a small slot for the screw in the axis may solve this problem.

Adding a third optical position indicator notifying that an exact position has been reached is also desired. If the computer cannot read this signal, it could simply order the stage to turn one additional revolution, and probably stop in a correct position.

Small brass chips from the stop heels could be found on the springed ball, and replacing the heels with ones made in hardened steel should prevent clogging.

6.2.2 A reliable mounting of the optical trigger fiber

The optical trigger fiber telling the multi-IO box when the excitation light actually is delivered, was mounted into the system at a very late stage. A temporary holder, made by tape and cardboard, gave a, though unreliable but working, fixation of the fiber.

A tiny fiber positioner (New Focus 9016M) mounted on a rigid pedestal is probably a solution.

6.2.3 Improving the performance of the beamsplitters

When testing the optical functionality of the components, the dichroic beamsplitters showed poor performance reflecting the long wave fluorescent light. The market was investigated once again for better beamsplitters without result. It is actually difficult to manufacture short pass beamsplitters with a broad reflection band.

The solution is changing the setup to long wave pass beam splitting, where suitable dichroic beamsplitters exist, for instance in previous systems. This probably means a new base plate, and perhaps some new optics such as lenses with different focal lengths.

6.2.4 A ventilation system

Several of the powered components produce heat, especially the halogen light source. Since the detector is cooled to produce less dark current, a low work

temperature is preferred. During the evaluation measurements, the temperature inside the system case raised to approximately 30° C, although the halogen lamp was not used. A ventilation system with a fan should be considered to lower the work temperature of the system.

6.2.5 Software improvements

Tailor-made modules for different applications than colonoscopy is desired. The system is planned to go to London in the beginning of 1999, where it is going to be used in heart surgery. A search-mode module with sound feedback is probably a user-friendly interface.

A monitoring client is not yet developed, useful when having an expert connected from a different location. The main stability of the client-server structure needs to be improved.

A general-purpose application, similar to AndorMCD, capable of firing the laser is a must. A simple modification of a small light source control program is enough if run in parallel with AndorMCD.

A criterion specification that can handle vector variables, allowing for multivariate linear regression would give instantaneous high-performance evaluation of spectra.

7. References

1. S. Andersson-Engels, Å. Elner, J. Johansson, S.-E. Karlsson, L.G. Salford, L.-G. Strömblad, K. Svanberg and S. Svanberg, Clinical recording of laser-induced fluorescence spectra for evaluation of tumour demarcation feasibility in selected clinical specialities, *Lasers Med. Sci.* 6, 415-424 (1991).

2. W. Alian, S. Andersson-Engels, K. Svanberg and S. Svanberg, Laser-induced fluorescence studies of *meso*-tetra(hydroxyphenyl)chlorin in malignant and normal tissues in rat, *Br. J. Cancer* 70, 880-885 (1994).

3. S. Svanberg, *Atomic and molecular spectroscopy*, (Springer Verlag, Heidelberg, Germany, 1992).

4. J. Johansson, Fluorescence spectroscopy for medical and environmental diagnostics, Dissertation thesis, Lund Institute of Technology, Lund, Sweden (1993).

5. R. Berg, Laser-based cancer diagnostics and therapy - Tissue optics considerations, Dissertation thesis, Lund Institute of Technology, Lund, Sweden (1995).

6. M. Yamashita, M. Nomura, S. Kobayashi, T. Sato and K. Aizawa, Picosecond time-resolved fluorescence spectroscopy of hematoporphyrin derivative, *IEEE J. Quant. Electr.* QE-20, 1363-1369 (1984).

7. H. Schneckenburger, K. König, K. Kunzi-Rapp, C. Westphal-Frösch and A. Ruck, Time-resolved in-vivo fluorescence of photosensitizing porphyrins, *J. Photochem. Photobiol. B* 21, 143-147 (1993).

8. S. Andersson-Engels, Laser-induced fluorescence for medical diagnostics, Dissertation thesis, Lund Institute of Technology, Lund, Sweden (1989).

9. S. Andersson-Engels, J. Johansson, U. Stenram, K. Svanberg and S. Svanberg, Malignant tumor and atherosclerotic plaque diagnosis using laser-induced fluorescence, *IEEE J. Quant. Electr.* 26, 2207-2217 (1990).

10. L. Baert, R. Berg, B. van Damme, M.A. D'Hallewin, J. Johansson, K. Svanberg and S. Svanberg, Clinical fluorescence diagnosis of human bladder carcinoma following low-dose Photofrin injection, *Urology* 41, 322-330 (1993).

11. K.T. Schomacker, J.K. Frisoli, C.C. Compton, T.J. Flotte, J.M. Richter, N.S. Nishioka and T.F. Deutsch, Ultraviolet laser-induced fluorescence of colonic tissue: Basic biology and diagnostic potential, *Lasers Surg. Med.* 12, 63-78 (1992).

12. R.M. Cothren, R. Richards-Kortum, M.V. Sivak, M. Fitzmaurice, R.P. Rava, G.A. Boyce, M. Doxtader, R. Blackman, T.B. Ivanc, G.B. Hayes, M.S. Feld and R.E. Petras, Gastrointestinal tissue diagnosis by laser-induced fluorescence spectroscopy at endoscopy, *Gastrointest. Endosc.* 36, 105-111 (1990).

13. C. Eker, S. Montán, E. Jaramillo, K. Koizumi, C. Rubio, S. Andersson-Engels, K. Svanberg, S. Svanberg and P. Slezak, Clinical spectral characterization of colonic

mucosal lesions using autofluorescence and δ-aminolevulinic acid sensitization, *Gut,* (1998). (In press).

14. P.S. Andersson, A. Gustafson, U. Stenram, K. Svanberg and S. Svanberg, Diagnosis of arterial atherosclerosis using laser-induced fluorescence, *Lasers Med. Sci.* 2, 261-266 (1987).

15. S. Andersson-Engels, A. Gustafson, J. Johansson, U. Stenram, K. Svanberg and S. Svanberg, Laser-induced fluorescence used in localizing atherosclerotic lesions, *Lasers Med. Sci.* 4, 171-181 (1989).

16. J.R. Mourant, I.J. Bigio, J. Boyer, R.L. Conn, T. Johnson and T. Shimada, Spectroscopic diagnosis of bladder cancer with elastic light scattering, *Lasers Surg. Med.* 17, 350-357 (1995).

17. S. Andersson-Engels, R. Berg, O. Jarlman, J. Johansson, K. Svanberg and S. Svanberg, Time-resolved spectroscopy for medical diagnostics, *CLEO'90,* Anaheim, California, 1990.

18. R. Cubeddu, P. Taroni and G. Valentini, Time-gated imaging system for tumor diagnosis, *Opt. Eng.* 32, 320-325 (1993).

19. S. Andersson-Engels, J. Johansson, K. Svanberg and S. Svanberg, Fluorescence diagnosis and photochemical treatment of diseased tissue using lasers: Part II, *Anal. Chem.* 62, 19-27 (1990). Invited paper.

20. Andor Technology, ICCD User's Guide, (1998).

21. Dolch Computer Systems Inc.. Dolch - FieldPAC Data Sheet. http://www.dolch.com/portables/fieldpacdata.html . 1998. Dolch Computer Systems Inc. 98.

22. Andor Technology, A Programmer's Guide to InstaSpec Drivers, Programmer's Guide, (1998).

23. R.C. Gonzalez and R.E. Woods, *Digital Image Processing,* (Addison-Wesley Publishing Company, 1992).

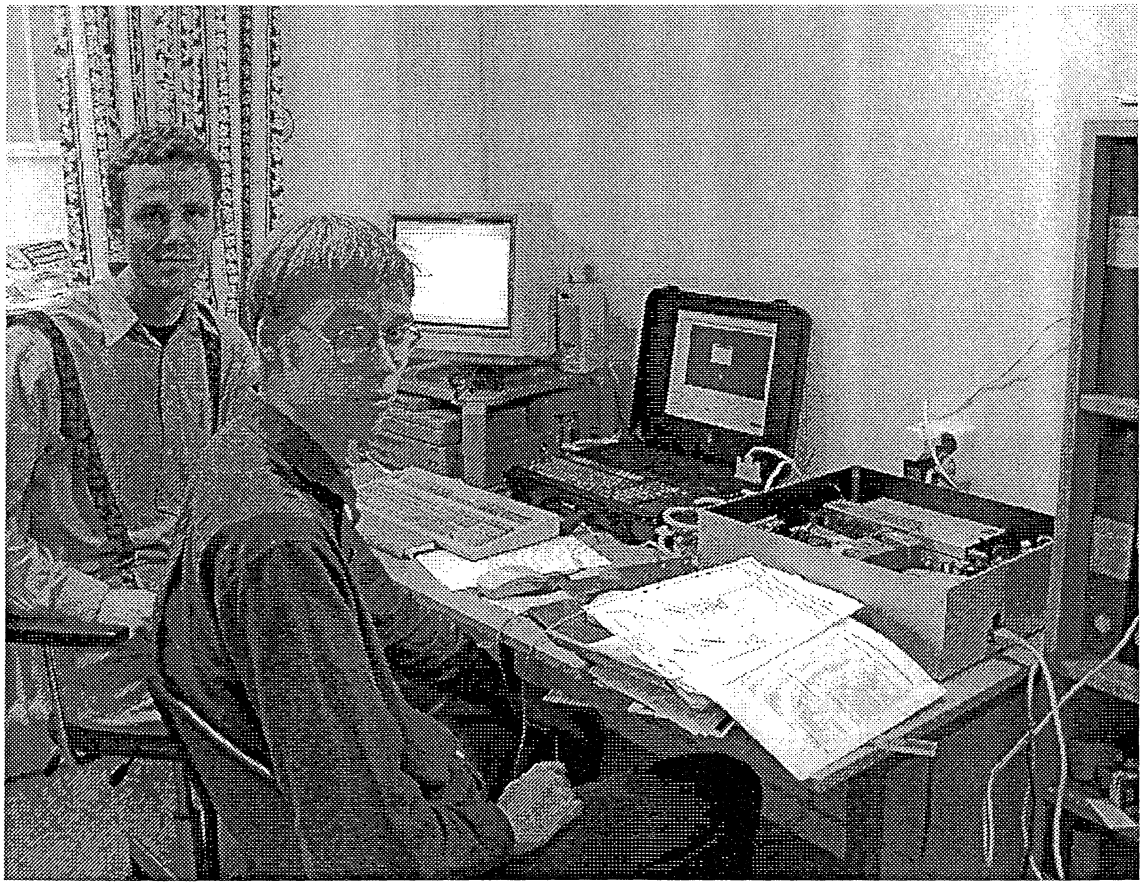
24. O. Kronborg and E. Hage, Hyperplasia or neoplasia. Macroscopic versus microscopic appearance of colorectal polyps, *Scand. J. Gastroenterol.* 20, 512-515 (1985).

25. Melles Griot, Melles Griot 1997-98, (1997).

26. O. Svelto, *Principles of Lasers,* (Plenum Publishing Corporation, New York, 1989).

8. Acknowledgments

The authors of this thesis would like to thank:
Stefan Andersson-Engels, our mentor, know-how and support.
Åke Bergquist, *always* professional and helpful.
Charlotta Eker, you trusted us when giving an opportunity to test-run the system in real life, and were a very nice company and boss at Karolinska.
Claes af Klinteberg, never far away with advice and help.
NUTEK, *Swedish National Board for Industrial and Technical Development*, for the financial support that made this project possible.
Göran Werner, you actually built this amazingly compact instrument.



9. Glossary

binning	when reading the CCD, different pixels (often all pixels in the same column) can be added and read as one value. This makes the array act as a one-dimensional vector. Less noise is another benefit, since a single readout adds less noise than multiple readouts [20].
BK7	A substrate used in common glass lenses [25].
CCD	Charge Coupled Device. Image area used in detector camera, commercial video cameras etc. [20]. Silicon-based semiconductor chip.
dark current	The CCD has always a dark current varying with temperature, resulting in noise [20].
DLL	Dynamic Link Library, a Windows function library format.
fused silica	A synthetic substrate used in lenses, which operates in the UV and visible range [25]. Has a low internal fluorescence.
internal conversion	A radiationless singlet-singlet transition where energy is transferred via collisions to a change in rotational or vibrational state.
intersystem crossing	A normally spin-forbidden singlet-triplet radiationless decay caused by collisions [26].
phosphorescence	Radiation emitted from an intersystem crossing decay. Significantly longer life time than in fluorescence [26].
photoelectron	The image intensifier of the CCD detector is based on the photo-electric effect. Each incident photon generates a photoelectron, which is accelerated and multiplied in a high voltage electric field.
TTL	Transistor-transistor logic. A digital circuit type for communication with 4-5 V signals.
VI	Virtual Instrument, a LabVIEW concept similar with subroutine

10. Index

Appendix A: User's manual

Table of Contents

1 Modularization

Module is a name we use on a VI that behaves in a certain way, good enough to be a menu entry.

Connectors on a module

A module must have four input parameters; *Input index*, *Sequence size*, *ini fileName* and *Info input*. There must be two output parameters, named *Output index* and *error out*.

Input index

The *Input index* indicates which item the module is in the current sequence. For example, if a sequence consists of the modules A, B and C, module B's Input index will be 2. This input helps to decide the appearance of the navigation buttons *Back*, *Next/Finish* and *Close*. For example, if input index is 1, *Back* will be disabled.

The LabVIEW data type is I32.

Sequence size

The *Sequence size* indicates the length of the current sequence. In the example mentioned in section Input index Sequence size would be 3. This input helps to decide the appearance of the navigation buttons *Back*, *Next/Finish* and *Close*. For example, if *Input index* equals *Sequence size*, *Next/Finish* is labeled *Finish*.

The LabVIEW data type is I32.

ini fileName

The *ini fileName* is used if the module will read an initialization file. If so, the file name will be passed here as a string, ABC.

Info input

The *info input* parameter is free to use as desired. The parameter is set in the *Additional info* string field in the *Edit Initialization File* module. Data type: ABC.

Output index

The *Output index* indicates how the user decided to end the module. If *Next/Finish* was pressed, *Output index* is *Input index* plus 1, and if *Back* was pressed, *Input index* minus 1. Zero indicates that *Close* was pressed. Data type: I32.

error out

If an error occurs in the module, it can be reported through the *error out* parameter.

Module storage

Since the application is constructed with different modes in mind, the localization of the modules is important. Three libraries are currently used to store modules; standard.llb, advanced.llb

2 Modules of certain interest

Colonoscopy module

This module is made especially to automate the acquisition process at a colonoscopy examination. It saves all spectra in a structured way, ordered by patient, area in the colon, and point at each area. It automatically acquires spectra for each checked wavelength.

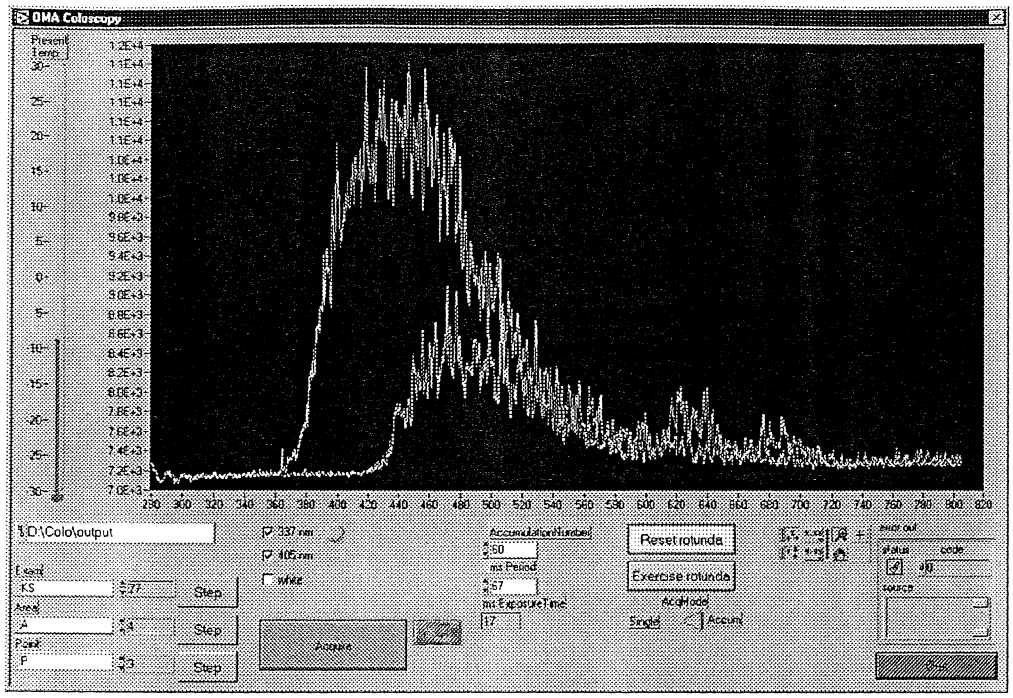
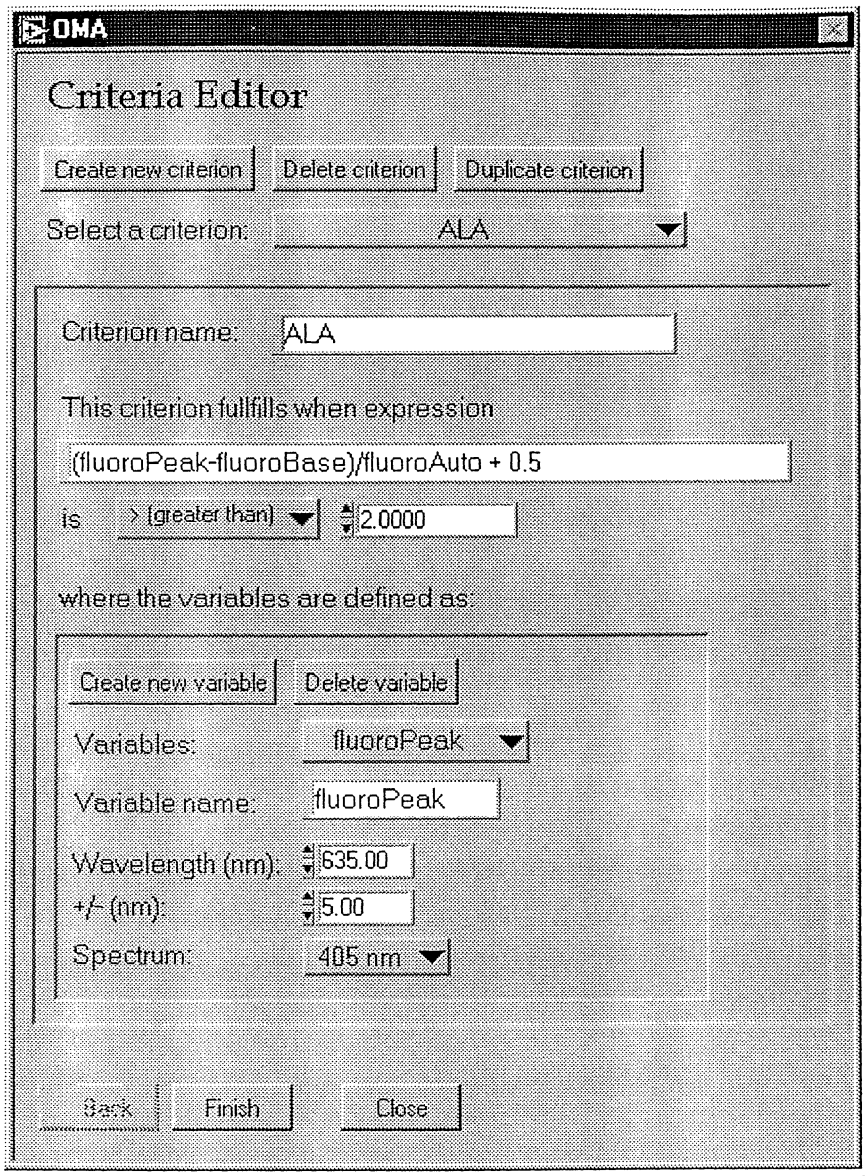


Figure 10-1: Colonoscopy module, seen with spectra from the lumen acquired. The bluish plot is fluorescence from 337 nm excitation light (mostly autofluorescence), and the more violet plot is from 405 nm excitation light. The two protoporphyrin peaks at approximately 635 and 700 nm can be seen.

The 337 and 405 nm spectra are shown simultaneously in the same graph with different plot colors. It seemed that the rotating stage could became a bit "cold", thus an "exercise" button was implemented.

Edit criteria library

You use this VI when you want to create a new criteria, or change an existing criteria. A criteria is used to determine if a certain condition is met or not, e.g. if a spectrum from skin might indicate abnormalities.



Existing criteria can be modified by first selecting them in the *Select a criteria* menu. Examples of expressions:

```
(fluoroPeak-fluoroBase)/fluoroAuto + 0.5
```

(365.32-248)*(3294.44+1.23e-3)

All variables used must be defined in the lower part of this dialog. The expression interpreter is case sensitive.

3 Strange things

Module calibrate.vi in Standard library can be allowed to ignore doing the calibration if the Description field passed along equals "ignore" and a calibration already is performed.

4 How to...

With this chapter we will try to answer questions about or explain the procedure to build the applications, *Client* and *Server*.

Add a calibration spectrum to library

It is also possible to associate a wave sound clip with a certain spectrum. Why not let the computer tell the user which library spectrum it used to do the calibration? Adding an appropriate sound clip to the *sound* folder, with the same name as the description of the spectrum, makes the thing. Example: The spectrum named "Lysrör i A105" is associated with the wave file "Lysrör i A105.wav".

Build the Client Application

All VIs used must be saved in the distribution in some way. Depending on whether the VIs are modules (see Modularization) or not, they should be placed in Advanced/Standard/Tools or General library.

All new or changed modules should be added to one of the libraries Standard or Advanced. Since the subVIs they use must be saved as well, *Save with Options* should be carried out. These subVIs are most likely not modules themselves, and should thus be placed in General library. *Save with Options* prompts you to enter this library, and if *Application Distribution* is selected, all VIs are saved without their diagrams.

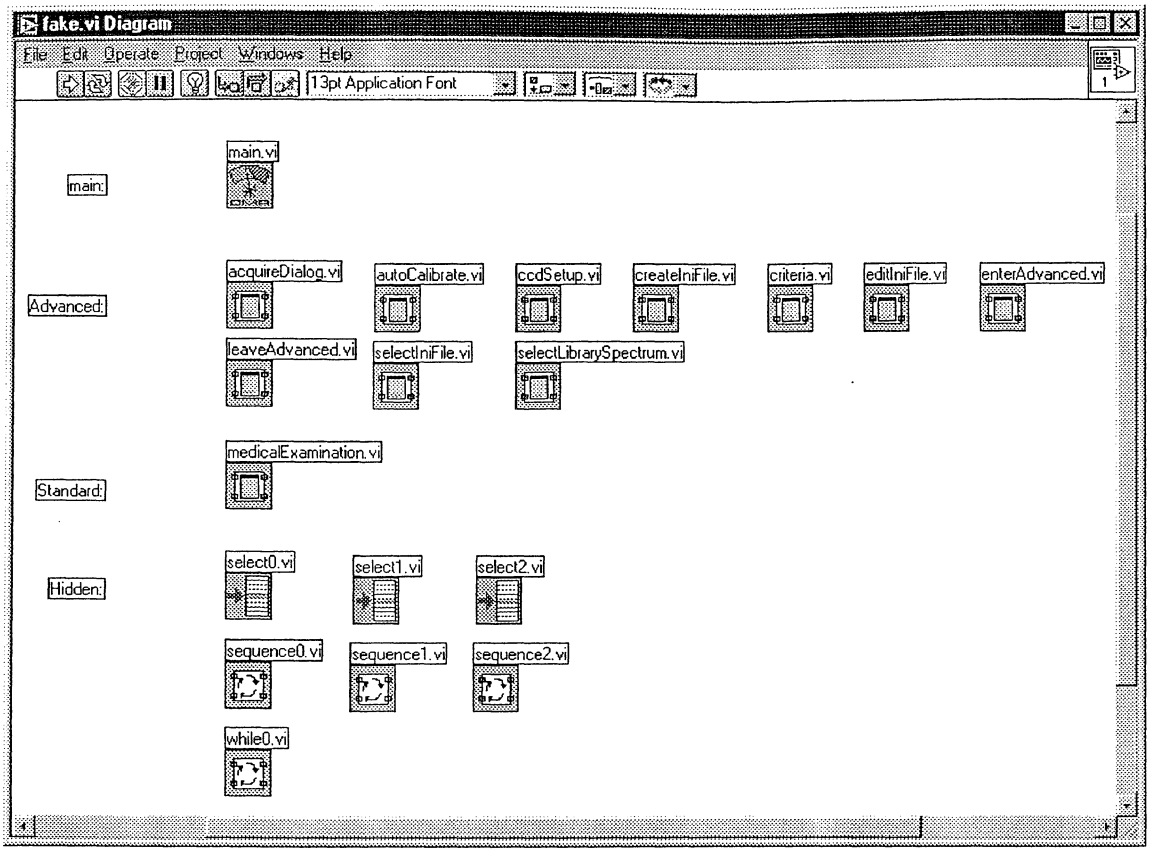


Figure 10-2: Example of a fake VI. This was from an early stage of the Client development.

Getting this job done for a large application can be a lot of work. It is therefore suggested that you place all modules used by the program in a fake VI. Now LabVIEW can build a tree of the statically used subVIs and save all of them in one single *Save with Options* to General. This means that the modules, and the fake VI, get saved in vain in General library, and if this bothers you, you can delete them manually, since the original copies are in Advanced/Standard/Tools.

Altogether, these are the steps:

1) When done with new/changed modules, save them into Advanced/Standard/Tools.

2) Update the fake VI so that it contains all modules and the main VI.

3) Save the fake VI with Options into General library, selecting the *Application Distribution* option.

4) If you wish, remove the modules and the fake VI from General library.

5) Close all VIs and open the main VI from General library.

6) Build application and select General.llb to be embedded into the executable. Make sure that the main VI is top-level. This is done in *Edit library* on the File menu.

Build the Server Application

Building the Server Application is a lot easier than the Client, since no VIs are called dynamically by the server. This means that all the subVIs can be saved with one single *Save with Options* from the Server.vi. These are the steps:

1) When done with changes, save the VIs as you normally do.
2) Close all VIs and open Server.vi.
3) Do a *Save with Options* into Server.llb.
4) Close Server.vi, but open it again from Server.llb library.
5) Build application and select Server.llb to be embedded into the executable. Make sure that the Server.vi is top-level.

Repair an Initialization file

If an essential initialization file gets damaged one way or another, it is possible to repair it running a small VI called *RepairInitializationFile.vi*. This VI does what the advanced menu item *Edit initialization file* normally does. This repair tool is needed, since the Client may not be runnable with damaged initialization files.

If the *Advanced menu* item is removed from *Main menu*, it is no longer possible to edit initialization files, so *RepairInitializationFile.vi* is needed in this case too.