

# Design and installation of a multi-mode microscopy system

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## ABSTRACT

We describe design and installation of a multi-mode microscopy core facility in an environment of varied research activity in life-sciences.

The experimentators can select any combination of a) microscopes (upright, upright fixed-stage, inverted), b) microscopy modes (widefield, DIC, IRDIC, widefield epifluorescence, transmission LSM, reflection and fluorescence CLSM, MPLSM), c) imaging techniques (direct observation, video observation, photography, quantitative camera-recording, flying spot scanning), d) auxiliary systems (equipment for live specimen imaging, electrophysiology, time-coordinated laser-scanning and electrophysiology, patch-clamp).

The equipment is installed on one large vibration-isolating optical table (3m × 1.5m × 0.3m). Electronics, auxiliary equipment, and a fiber-coupled, remotely controlled Ar<sup>+</sup>-Kr<sup>+</sup> laser are mounted in a rack system fixed to the ceiling. The design of the shelves allows the head of the CSLM to be moved to any of the microscopes without increasing critical cable lengths. At the same time easy access to all the units is preserved.

The beam of a Titanium-Sapphire laser, controlled by means of an EOM and a prism GVD, is coupled directly to the microscopes. Three mirrors mounted on a single precision translation table are integrated into the beam steering system so that the beam can easily be redirected to any of the microscopes.

All the available instruments can be operated by the educated and trained user.

The system is popular among researchers in neuroanatomy, embryology, cell biology, molecular biology - including the study of protein interactions, e.g. by means of FRET, and electrophysiology. Its colocalization with an EM facility promises to provide considerable synergy effects.

## 1. INTRODUCTION

In the region of Oslo, Norway, a university and several private and autonomous state institutes involved in biomedical research are established. Electron microscopy and light microscopy as well as digital image analysis had, of course, been applied on a large scale for decades in this research environment. However, a facility for multi-photon laser microscopy (MPLM) had hitherto been lacking, and confocal scanning laser microscopy (CSLM) had not been broadly in use as routine experimental techniques. The geographical and logistical concentration of a diversity of biomedical research centres in the region of Oslo would be completed by the installation of a state-of-the-art laboratory for confocal and multi-photon laser scanning microscopy, techniques which are of increasing importance in many branches of the life-sciences. Individual setups for linear and non-linear laser scanning microscopy have been described before.<sup>1</sup> The aim of this publication is to review the complicated process of the establishment, installation, introduction, and popularisation of a large core facility (CF) for laser microscopy in the Institute Group of Basic Medical Sciences (IMBA) at the Univ. of Oslo (UiO).

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## 2. MATERIALS AND METHODS<sup>§</sup>

### 2.1. The Context

Biochemistry, Cell Biology, Embryology, Electrophysiology, Neuroanatomy as well as Neurophysiology are among the fields of research represented at IMBA. The new instrument would be integrated into an environment, where two electron microscopes and a laboratory for digital image analysis already were established.

### 2.2. The Demands

From the beginning of the planning it was clear that the equipment would have to be flexible enough to meet a broad variety of experimental demands. Standard fixed preparations on microscope slides should be imaged as well as living cells in a patch-clamp setup, cardiomyocytes demanding a temperature stabilisation with an accuracy of  $\pm 0.1^\circ \text{C}$  as well as chicken embryos in an opened egg, enamel, metal or ceramic surfaces as reflective odontological or surgical specimens as well as fluorescent cell biological preparations.

The potential users of the instruments would chiefly be experienced researchers who, however, would not have worked on a laser microscope before. The aim was from the beginning to educate potential users so that they could perform their own experiments without permanent support by a system-expert.

The financial frame for the acquisition and installation was in the order of 500,000.\$.

### 2.3. Basic Technical Considerations

The establishment of a core facility for standard and multi-photon laser microscopy demands a sufficiently large room which is provided with an appropriate infrastructure (gas, water, compressed air, 3-phase high power electric connectors, cooling water and so on). The diversity of experiments to be performed on the future facility required the installation of three different setups, an upright research microscope (for morphological records), an upright fixed-stage microscope (for combined and time co-ordinated patch-clamp and laser microscopic compound experiments in a setup enclosed by a sufficiently large Faraday cage), and an inverted microscope (in a setup for cell biology and electrophysiology combined with laser microscopy, even this microscope in a sufficiently large Faraday cage). External instrumentation for electrophysiology demanded one to two 19 inch racks. Computer desks for a Couple of large screens were likewise necessary. Modules for the Titanium-Sapphire Laser (Ti-Sap) as the light source for multi-photon excitation and a diversity of smaller instruments had to be housed in the laboratory.

Also, the future users demanded a sufficient supply with laboratory benches for preparative work. Cupboards and drawers for storing tools, optics, chemicals and so on had to be provided.

An independent server PC located in the laboratory, fitted with a diversity of stations for storing media was demanded as well.

Finally, and trivially enough, it should be possible to have at least two chairs around each setup and provide ample space for comfortable experimenting even over many hours. Every point in the laboratory should be easily and safely accessible even in the dark and during ongoing experiments. It should be possible to work simultaneously on the three setups, albeit not applying laser scanning microscopy (LSM) on more than one setup at a time.

A conservative estimation led to the conclusion that an area of at least  $6\text{m} \times 6\text{m}$  had to be available. In order to create such a comparatively large laboratory area, a wall between two former standard labs had to be removed and the infrastructure in the new room upgraded.

There were two alternatives on how to arrange the microscopic setups and the units for LSM in the laboratory:

One could install the three setups individually on small experimental tables, possibly vibration isolated and on casters and the Ti-Sap and the electronic control box, the PC and an  $\text{Ar}^+ \text{-Kr}^+$  laser as conventional laser for the CSLM on a fourth table. Thus, a considerable flexibility would have been achieved since the setups could have been moved around not only in this laboratory. However, it would have been unavoidable to use a fibre for coupling the beam of the Ti-Sap into the scanner head of the laser scanning microscope, with all the possible consequences involved.

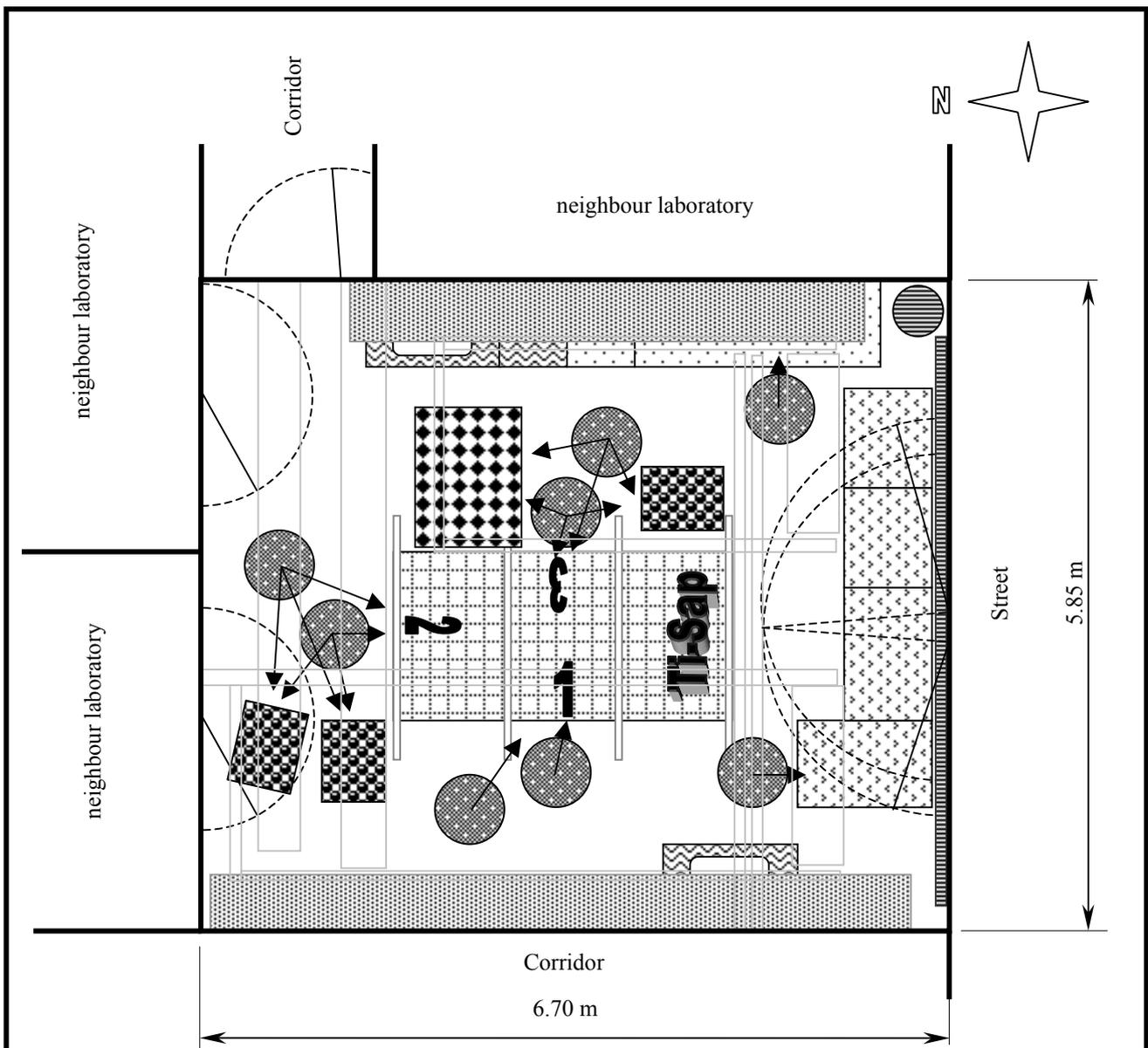
Alternatively, one could install everything on one large, fixed, optical table, thus saving space and money as well as avoiding the necessity to use fibre coupling of the Ti-Sap beam.

It was finally decided to mount all the three setups onto one large optical table. This alternative was more economic and solid, and less space demanding and complicated in operation.

In the laboratory, the optical table was placed centrally thus providing ample space between the table itself and the walls resp. the laboratory benches (see Fig. 1).

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<sup>§</sup> Also see the Materiallist in the Appendix.



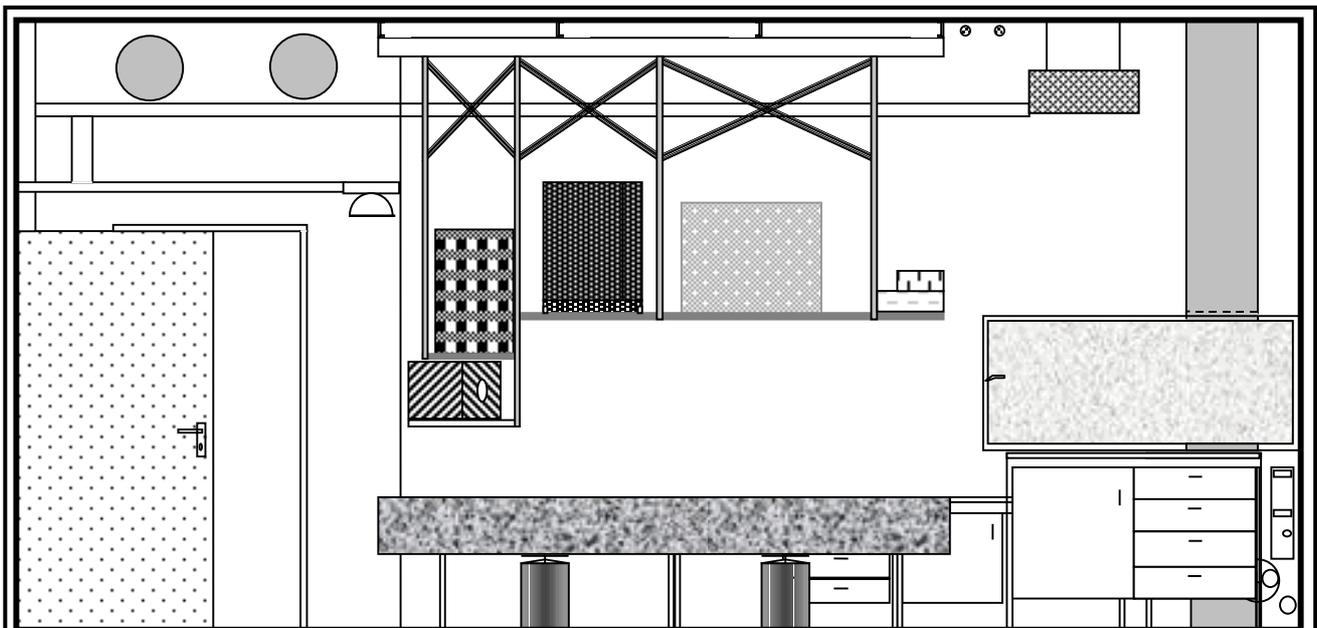
**Fig. 1: The arrangement of the laboratory**

The laboratory is 5.85m  $\times$  6.70m in size. The two neighbour laboratories on the lefthand side contain an electron microscope and a large conventional fluorescence microscope. The doors to these rooms are normally locked and are not needed for traffic, they can however be opened if necessary. The laboratory is accessed from the corridor via the third door. The windows on the righthand side can be opened without any problems.

The thin grey lines indicate infrastructure which is installed under the ceiling.

The 3m  $\times$  1.5m  $\times$  0.30m optical table is placed centrally in the laboratory. Four [ -shaped profiles, indicated in the figure as long, narrow rectangels on top of the table, are used to fix the system of shelves described in the text to the ceiling. On the table, the three setups and the Ti-Sap system are placed as indicated. At each setup, two persons can sit and work simultaneously; the chairs are indicated as circles in the drawing; the arrows indicate the operational directions to which the users have to orientate during an experiment. Left of the users at setup "2", two 19" racks containing electronic equipment can be placed. To the right of the users at setup "3", the table for the two 21"-screens for the CSLM is indicated, to the left a further 19" rack. Along the walls, cupboards, sinks and desks are provided.

The mounting of the Ar<sup>+</sup>-Kr<sup>+</sup> laser, the electronics unit and the PC for the CSLM as well as the control unit for the Ti-Sap and several other smaller components, represented a considerable technical problem. All the individual components should be comfortably accessible for potential service or repair operations. Safety considerations demanded that any horizontal cabling or hose was to be mounted at least 2m above the floor. It was thus decided to place all those components which would not require daily access by the user onto a large system of shelves fixed to the ceiling of the laboratory above the optical table. The construction of these shelves was, unfortunately, a major effort since a considerable number of constraints and boundary conditions had to be met simultaneously. The financial situation did not allow the acquisition of three complete scanning systems, one for each of the future microscopes. Instead, one CSLM system should be acquired. A pre-requisite was that the beam of the external laser beam could be coupled directly into the scanner head of the CSLM and that the scanner head easily could be moved from one microscope to another. The manufacturer of the CSLM was not prepared to provide cables between the control electronics box and the scanner head longer than in the standard configuration. Nevertheless, the aim was to assure that the scanning head of the CSLM could be moved easily between the three microscopes without having to move heavy and voluminous boxes while at the same time saving as much space as possible. Also, the rolling table carrying the two 21" screens, mouse and keyboard should be movable from setup to setup without the necessity to unplug and replug a lot of cables. In other words: There should be an easy, short, and comfortable routine to move the laser scanning components between the setups. The important main switches, e.g. for the PC or the Ar<sup>+</sup>-Kr<sup>+</sup> laser should be accessible without climbing a chair or ladder. The shelves should be located high enough to avoid problems with the installation on the table. Finally, the maximally allowed charge of the ceiling was 350kg per square meter. Besides that, the shelves would have to be solid enough and should not oscillate like a pendulum in spite of the fact that the ceiling was as high as 3.20m and thus the profiles bearing the construction as long as 1.50m. The arrangement which was finally designed is shown (see Fig. 2 and Fig. 3).



**Fig. 2: The arrangement of the shelves (compare Fig. 1)**

This view shows the laboratory towards east (see the compass in Fig. 1). Only the most important objects are shown. The system of shelves is constructed from Aluminium profiles and wooden plates. On the shelves, there are placed the monitor for IR DIC microscopy, , on the setup for patch-clamp experiments, the PC computer controlling the CSLM, , the box containing the electronics for the CSLM, , and the Box containing the Ar<sup>+</sup>-Kr<sup>+</sup> laser, . It is possible to connect the head of the CSLM to any of the three microscopes (not shown in this image) on the table without having to move anything else besides the monitors for controlling the CSLM. Neither the components mounted on the optical table, nor the Faraday cages are shown in this image, nor are chairs, racks, nor is any further movable equipment. The windows to the right hand side are drawn in the opened state, so is the door to the left.

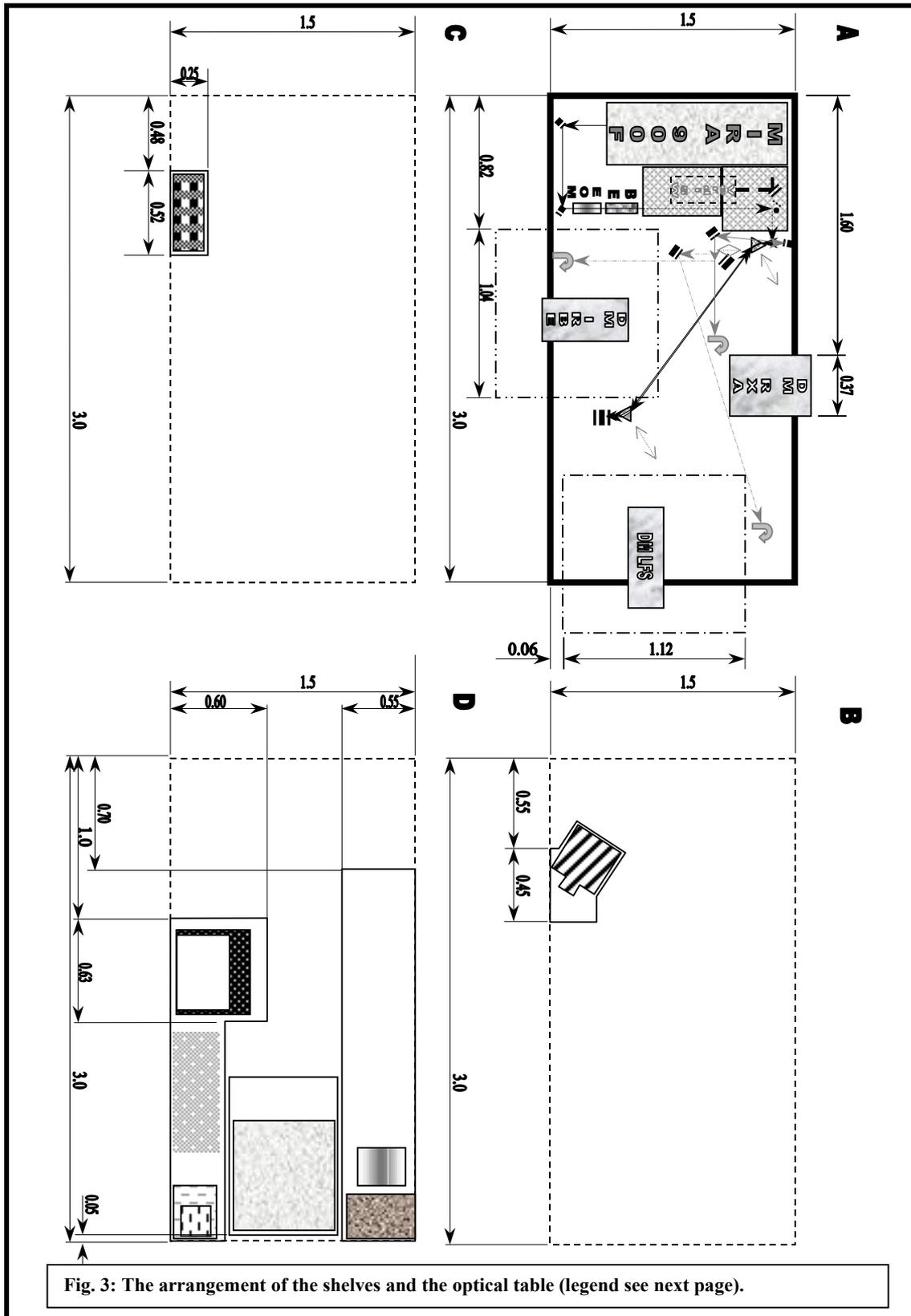


Fig. 3: The arrangement of the shelves and the optical table (legend see next page).

Fig. 3 (see above) shows the distribution of the large components of the setup onto the optical table and the shelves. All the indicated lengths are given in meters. Level B is 40cm above Level A, Level C 35cm above Level B, Level D 20cm above Level C. This arrangement was necessary in order to be able to move the scanner head from one microscope to another without moving any of the heavy equipment and without exceeding any limits for critical cable lengths. A detailed view of Level A is shown in Fig. 4. The equipment shown on Levels B, C, and D are identical to those shown in Fig. 2, however, on level D the control unit of the Ti-Sap, , as well as some other minor equipment (oscilloscope, pulse amplifier for an Electro Optic Modulator (EOM) (see text), controller for the wave-meter, multi-purpose DC power supply) are shown.

#### 2.4. The arrangement of the experimental setups and the optics

The natural choice for the arrangement of the three microscopes and the Ti-Sap laser on the table was to place each on one side and squeeze the space consuming Group-Wave-Dispersion-Compensator (GDC) (see below) centrally between two setups. The mounting of further components like an Electro-Optic-Modulator (EOM) (see below) and Beam Expander (BE) (see below) did not represent a considerable problem (see Fig. 4).

As light source for conventional single photon scanning laser microscopy, an Ar<sup>+</sup>-Kr<sup>+</sup> laser providing lines at 476nm, 488nm, 568nm, and 647nm was selected. These lines together give the impression of a white laser-beam, they are suited to excite virtually any of the presently available dye-substances in use in the life-sciences except those that have to be excited in the UV range.

As Ti-Sap system, a passively modelocked system initially pumped by an Argon ion laser which was later replaced by a solid-state laser was selected. The laser unit can emit wavelengths in the range between 690nm and 1000nm at a repetition rate of 76 MHz and pulse lengths in the 100fs-200fs domain.

In order to control and regulate the beam of the Ti-Sap, several units were purchased. Laser Power control via the PC and the software for the CSLM is possible by means of an Electro Optic Modulator (EOM), a four crystal Sodium-di-Deuterium-Phosphate (KD\*P)-unit driven by a comparatively cheap standard pulse amplifier (i.e. a Couple of transistor switches in push pull configuration). This device providing the possibility to perform line blanking and frame blanking of the laser beam while scanning, and exposing the specimen to short trains of light pulses, e.g. to release caged compounds, is sufficient for this purpose; a more advanced, real "amplifier" required e.g. when applying the IMS method<sup>2, 3</sup>, is not necessary here. According to results of measurements of the effect of an EOM of this type on the pulse durations of the beam of a Ti-Sap laser performed at the Univ. of Innsbruck, Austria, the pulse duration does not increase considerably.<sup>4</sup>

In order to compensate for group wave dispersion effects, a prism GDC was built, partly from commercially available and partly from home-made optomechanic components. The equilateral prisms are of SF10 glass. The race path between the prisms was selected at 1.20m length in order to be able to compensate for the dispersive effect of thick lenses. At present, the prisms are adjusted empirically by determining the optimal position for two-photon excitation on a test preparation. The acquisition of a Michelson Interferometer as auto-correlator for measuring the length of the pulses of the Ti-Sap is being considered.

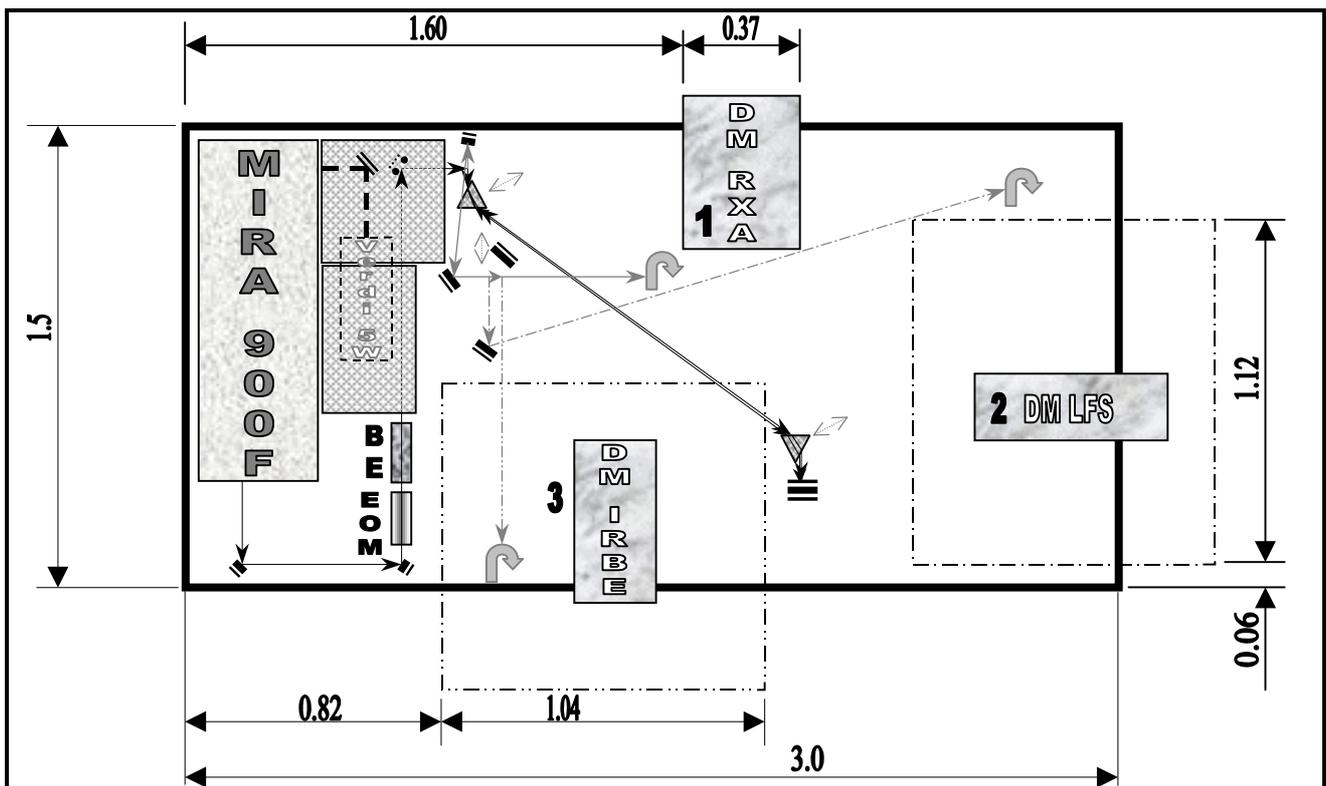
In order to avoid uncontrollable beam divergence effects, a 2.5× beam expander was assembled and combined with a spatial filter.

An electronic wavemeter catching a parasite beam allows to measure the wavelength of the light emitted by the Ti-Sap.

For alignment purposes, the IR-laser beam can be viewed by means of a standard video camera fitted with a wide field objective and standard IR longpass filters.

A conventional power meter is used to measure the power of the beam.

Beam steering is done by means of optical mirrors (∅ 25mm) coated with Yttrium-oxide protected Gold, providing sufficiently high reflectivity over the entire emission range of the Ti-Sap and at the same time reducing dispersion effects which hardly can be avoided when applying dielectrically coated mirrors. Two of the periscopes which are necessary to couple the beam of the Ti-Sap into the scanning unit were built in the local mechanical workshop, the third was assembled from commercially available rails and profiles.



**Fig. 4: The arrangement of the setups and the instrumentation on the optical table (all lengths in meters).**

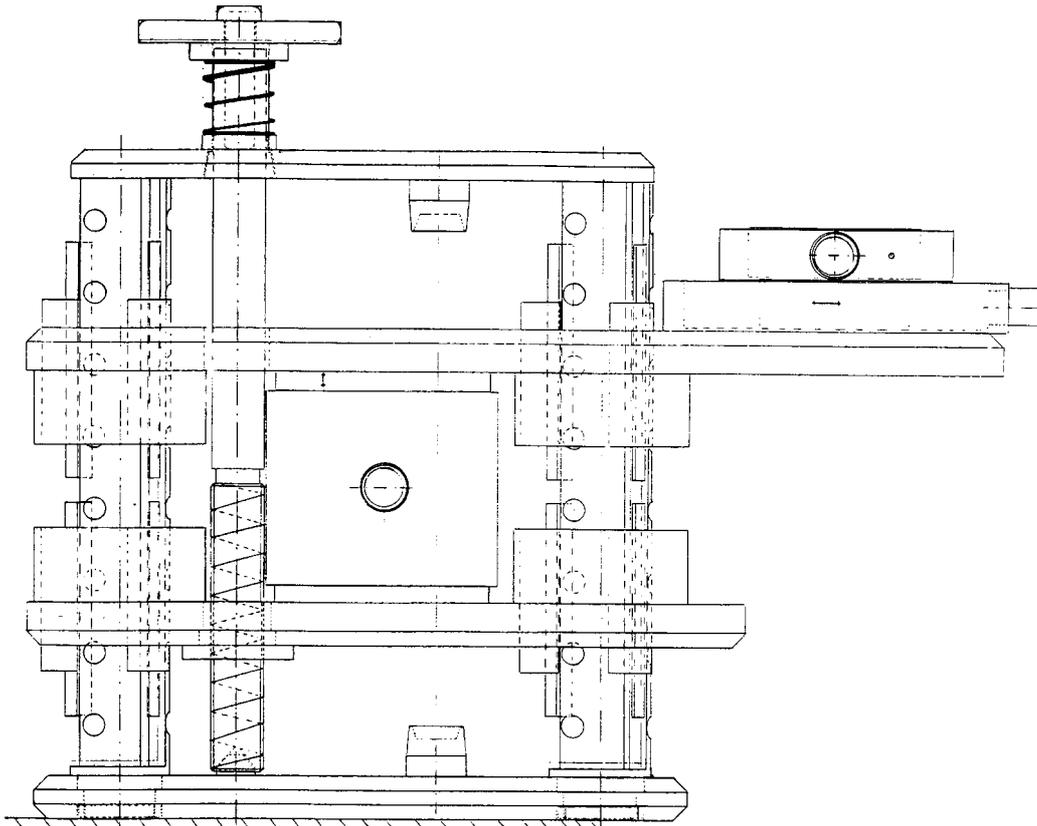
The three setups, the first with an upright microscope for morphological research, the second with an upright fixed-stage microscope for patch clamp experiments, the third with an inverted microscope for cell biology and electrophysiology (see Appendix for technical details), are arranged as shown in the figure. Setups nr. 2 and 3 are surrounded by Faraday cages. These cages are fixed to the system of shelves (see Fig. 2 and Fig. 3) and thus do not mechanically contact the optical table.

The laser beam of the Ti-Sap is guided on the indicated ray path. An EOM and a Beam Expander (BE) are used to control the power of this beam and compensate for increases in beam divergence in the EOM and the Group Wave Dispersion Compensator (GDC). To the right of the laser, two minor tables are mounted with the monitor for the video camera for the IR-laser beam and the controller for the Ti-Sap. The pumplaser is mounted under those and the Ti-Sap beam passes under those likewise. The GDC stretches diagonally over the table. The beam, passing the two prisms at a level of 120mm over the table top is lifted with a retroreflective angular mirror to a level of 140mm and travels back parallelly. The distribution of the beam to that one of the three setups, where the head of the CSLM actually is installed, can easily be performed by means of a mirror mounted on a precision translation stage. The ray paths are indicated in the same dot-dashed line style as the resp. Faraday cages. The "↻" symbolises the periscopes on each setup. The Faraday cage on setup "3" is cut so that the beam can pass as indicated. The prisms of the GDC are mounted on prism tables and translation stages and can thus be adjusted as indicated by the double arrows.

## 2.5. The experimental setups

### 2.5.1. The setup for patch clamp experiments

Compound experiments, which aim at the simultaneous and time co-ordinated registration of electrophysiological data, for example of patch-clamp experiments, and fluorescence optical data, e.g. a  $\text{Ca}^{2+}$  diffusion wave in a dendrite or axon, are a rich source of information for neuroscience studies.<sup>5, 6, 7</sup> Therefore, considerable effort was invested into the design and construction of an appropriate experimental stand. In a large Faraday cage, a state-of-the-art fixed stage microscope is the centre of the equipment. The diversity of potential preparations demanded the construction of a special microscope stage. This stage should provide three axis manipulation at micrometer precision. At the same time it was demanded that the height of the stage could be adapted for preparations in the range of brain-slice to hen's egg. The stage which was finally constructed by one of the authors (PJH) and built in the local mechanical workshop is shown and described in Fig. 5.



**Fig. 5: A multipurpose microscope stage for a fixed stage upright microscope.**

This stage consists of a base plate and two vertical bolts to which two plates are fixed via crossed roller bearings. The lower plate can be moved coarsely by means of a coarse screw. The upper stage is coupled to the lower stage via a precision vertical translation table. Two horizontal precision translation stages are fixed to the upper stage, mutually orthogonal. Any microscope stage plate can be fixed to the upper, horizontal translation stage. The uppermost plate is used to stabilize the entire frame and does not have any further function.

Using this arrangement, the stage can be adapted within less than a minute to quite different preparations. Its dimensions allow to do microscopy on standard fixed preparations, living brain slices in a bath chamber or an opened hen's egg. Nevertheless, the stage is mechanically extremely rigid.

A hydraulic micro manipulator is used to perform patching. The electronic equipment of the setup is described in detail in the Appendix.

A hydraulic micro manipulator carries the head stage of the amplifier used for patch-clamp recordings. The electronic equipment of the setup is described in detail in the Appendix.

A four channel interface for the time co-ordination of the scanning electronics and the modules for electrophysiological measurements was built in the local electronics workshop. It provides a series of features (adaptation to positive and negative logic, manual or automatic setting of the length of TTL-pulses and wait periods, signal inversion a.s.o.), and allows both the CSLM electronics as well as the system for electrophysiology to be dominant in a master-slave configuration. However, due to restrictions in the time accuracy of the triggering of the CSLM system, the latter is preferably selected as the master.

A standard gas supply ( $O_2$ ,  $CO_2$ ) and perfusion system - operated either hydrostatically or by means of peristaltic pumps - is available.

### **2.5.2. The setup for cell biology and electrophysiology**

This setup is assembled around an inverted microscope. Considerable efforts were made to provide an optimal perfusion system allowing for rapid switches between five different solutions at minimum switch volumes, high quality temperature stabilisation, and the possibility to use a multitude of different dishes. An important maxim was that experimental dishes should be easily accessible and exchangeable in spite of a considerable number of mounted pipettes for supply and drain. While perfusion at a sufficient rate demands a certain minimum quantity of solution flowing through the dish, the flow itself must be laminar and any kind of turbulence has to be avoided in order to keep the cells at rest, a *conditio sine qua non* for quantitative digital microscopy in the time domain. There are some interesting, albeit expensive, systems available on the market which can be used to establish perfusion systems. However, all of these leave one or another problem unsolved. It was finally decided to construct and optimise an own system on an empirical basis. The basic features of this arrangement are shown in Fig. 5.

### **2.5.3. Computers and software**

The software and data management of the entire system is nearly completely organised by means of state of the art PCs with OS Windows NT, in spite of the fact that this OS does not provide the same operational possibilities as UNIX. The selection of the supplier of the CSLM system automatically induced this computer structure. The CSLM is controlled by a medium scale PC. Further work-stations are provided for the data administration and for the patch clamp setup.

## **3. RESULTS**

The only direct result, albeit an important one, of the installation of a CF is the respective system itself and its applicability and usefulness for a possibly large number of users. The considerable number of scientific projects (>10) which currently make use of this system is a clear proof of its usefulness. These projects cannot be reviewed here; the results of ongoing experiments will be published by the respective authors.

The popularisation of an apparently complicated instrument among users who are not experts in the field of instrumentalisation usually is a psychological problem, especially if a some knowledge is required in a field which is not the user's own field of research or education. Therefore, considerable efforts are made to educate long-term users so that they feel comfortable and can use the system without being assisted. It is, however, impossible to provide a short-term education for experimentalists who are not intending to become long-term users of the laboratory.

In this context, an important duty is to guarantee that the system is operationally safe so that even a totally un-experienced person will not be able to inflict harm on any user present in the laboratory. The industrial products acquired as components of this system can be regarded as operationally safe and are legally certified. That safety-relevant part of the instrument which is designed and built or assembled in the institute chiefly is the guiding, control, and coupling of the beam of the Ti-Sap. Protection of the users must be performed by keeping the beam physically covered. During alignment, no user is allowed to enter the laboratory. The laboratory itself is under the auspices of the officer for technical safety of the University. The institute is certified to operate the laser system by The Norwegian Radiation Protection Authority.

## **ACKNOWLEDGEMENTS**

The excellent help of the staffs of the Mechanical Workshop, the Electronic Workshop, and the IT-center of the Institute of Basic Medical Sciences, Univ. of Oslo, is gratefully acknowledged. The collaboration with the Dept. of Experimental Medicine at the Ullevål Hospital, Oslo, Norway, when designing the setup around the inverted microscope was very fruitful. Our colleagues, especially Dr. Marion J. Thomas and Prof. Jens-Gustav Iversen provided valuable help.

The multiphoton laser scan facilities and auxiliary infrastructure were financed jointly by the National Research Council, by the University of Oslo Advanced Research Equipment Program, Medical Faculty and Institute Group of Basic Medical Sciences, by grants from Professor Letten Fegersten Saugstad's Fund, the Jahre Foundation, and others awarded to the Laboratory of Molecular Neuroscience, and by a grant from the National Research Council to Professor Jens-Gustav Iversen.

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## APPENDIX

### List of the used modules and components

The large optical table is a model "RS4000", 3m × 1.5m × 0.3m, mounted on four model "I-2000" vibration isolators. This equipment is by Newport Micro-Contrôle, Évry, France.

Financial constraints and space problems enforced the restriction to one CSLM system. It was thus necessary to demand that the scanner head easily could be moved from one microscope to another. Another pre-requisite was that the beam of the external laser beam could be coupled directly into the scanner head of the CSLM. A further desire was that the manufacturer and deliverer of the CSLM system would educate the local staff so that even operations, which were slightly more complicated as usual, e.g. involving the opening of the housings of the CSLM-units as well as changing and adjustment of individual optical or electronic components inside the system, could be done locally without the help of service engineers. After intensive discussions with several manufacturers of CSLM systems, technological and practical considerations resulted in the decision to purchase the microscopes and the CSLM unit from Leica Microsystems GmbH, Heidelberg, FRG. The three microscopes are a DM RXA, a DM LFS, and a DM IRBE. The available standard objectives are N Plan 2.5× / 0.07, HC Pl Fluotar 5× / 0.15, HC Plan Apo 10× / 0.40, HC Plan Apo 10× / 0.40 IMM, HC Plan Apo 20× / 0.70, HCX Plan Apo 40× / 0.85 CORR, HCX Pl Apo 40× / 1.25-0.75 OIL, HCX Pl Apo 63× / 1.32 OIL, HCX Pl Apo 63× / 1.20 W CORR. Special objectives for IR DIC microscopy on the patch clamp setup are HCX Apo L U-V-I 10× / 0.30, HCX Apo L U-V-I 40× / 0.80, and HCX Apo L U-V-I 63× / 0.90. The microscopes are all fitted with DIC optics and epifluorescence illumination (HBO100 or Xe75 lamps) as well as standard fluorescence filter cubes. The DM LFS is equipped for infrared DIC microscopy (camera system and Monitor by T.I.L.L. Photonics, Planegg, FRG). All the microscopes are fitted with different C-mount adapters so that any standard CCD-, tube- or video-camera can be adapted.

The CSLM unit is of the TCS SP type providing frame-scanning, line-scanning, time-lapse and bleaching modes.

Its laser is a model 643 Ar<sup>+</sup>-Kr<sup>+</sup> laser (Omnichrome Melles-Griot, Carlsbad, California, U.S.A.).

The CSLM makes use of an internal prism to spectrally analyse the fluorescent light of a specimen and direct it to different detectors, two model R6357 and one model R 6358 photomultiplier tube (Hamamatsu Photonics K.K., Hamamatsu City, Japan). In front of each detector, an optical slit, mirrored at its outer edges, is located. The slits can be adjusted in position and width so that it is not any longer necessary to use any kind of chromatic filters - besides the main beamsplitter and the beam combiner for the beams of the two lasers - in this system. The control of the slits is performed by the user via the scanning software.

All the microscopes are fitted with a transmitted light detector model R 6357 (Hamamatsu Photonics K.K., Hamamatsu City, Japan) protected by a fixed shortpass filter blocking red and IR-light emitted by the Ti-Sap. Thus, it is possible to either scan simultaneously confocal or non-confocal fluorescence as well as morphological images of preparations illuminated by the Ar<sup>+</sup>-Kr<sup>+</sup> laser or use the transmitted light detector as fluorescence detector in case of multi-photon excitation and thus drastically increase resp. virtually double the solid angle for fluorescence photon detection.

The Ti-Sap, passively modelocked, is a model "Mira 900F" pumped by a model "Verdi 5W" Nd:YVO<sub>4</sub> - LBO solid state laser, both by Coherent Inc. Santa Clara, California, U.S.A. The Ti-Sap is fitted with three mirror sets which can alternatively be installed and cover the wavelength ranges from  $\approx 690\text{nm}$  to  $\approx 810\text{nm}$ ,  $\approx 790\text{nm}$  to  $\approx 900\text{nm}$ , and  $\approx 890\text{nm}$  to  $\approx 1000\text{nm}$ . The cavity of the Ti-Sap can be perfused by dry N<sub>2</sub> in order to avoid mode-locking problems at certain problematic wavelengths. Cooling water for the Ti-Sap crystal as well as the mounting plate of the pump laser is provided by means of a refrigerated bath circulator (model "RTE111" by Neslab Instruments, Inc., Portsmouth, NH, U.S.A)

Laser power is measured by an "LM10" power meter (Coherent Inc., Santa Clara, CA, U.S.A).

As wavemeter, a model "REES RE201" (Rees Instruments, Ltd., Surrey, UK) is used. A parasite beam as reflected by a coverslip is sufficient to measure the actual wavelength.

The EOM is a model "LM 0202 P 5 W IR", it is controlled by a model "LIV 8" pulse amplifier (both by Linos-Photonics / Gsaenger, Planegg, FRG).

In order to view the beam of the Ti-Sap for alignment purposes, a video surveillance camera (model "VCB-3512P" by Sanyo Electric Corp. Ltd., Osaka, Japan) fitted with an auto iris short distance objective (model computer "HG2616AFCS-3" by CBC/Chugai Boyeki Corp., Ltd., Tokyo, Japan) is used together with a standard B/W 15" monitor (model "SSM-121CE", Sony Corp. Japan). This camera can be mounted flexibly by means of a holder built in the local mechanical workshop.

The beam of the Ti-Sap is guided on the optical table top by means of a diversity of optomechanical components partly by Newport Micro-Contrôle, Évry, France, partly built in the local mechanical workshop. The mirrors used to redirect the beam are broad band gold mirrors ( $\varnothing$  25mm Duran50 substrate as well as one specially fabricated 90° angular mirror, all with an Yttrium Oxide protected Au coating, flatness  $\lambda/10$ ). The equilateral prisms used in the GDC are made from SF10 glass. Two sides are polished to a flatness of  $\lambda/10$ . The surfaces are un-coated since the beam of the Ti-Sap, TM-polarized, crosses these surfaces at an angle which is very close to the Brewster angle, so that losses by rest reflection are negligible. Mirrors and prisms are by Optische Werkstätten Bernhard Halle Nachfl., Berlin-Steglitz, FRG. The prisms and the retroreflective mirror are mounted on prism tables (model "M-PO46-50", Newport Micro-Contrôle, Évry, France).

The horizontal translation stages in the microscope stage (see Fig. 5) are model M-UMR8-51, the vertical translation stage is a model M-MVN80

In order to avoid a strong increase in beam divergence caused by the multiple optical surfaces, the Ti-Sap beam has to pass, a 2.5× beam expander fitted with a pinhole filter is used. It is assembled from Five-Axis Gimbal Optic Mounts and one Three-Axis Translator (models "M-LP-2B-XYZ", "M-LP-05B-XYZ", and "M-LP-1-XYZ" by Newport Micro-Contrôle, Évry, France), two achromatic lenses with 40mm and 100mm focal length (model "01LAL011/77" and "01LAL017/77" by Melles-Griot, Rochester, NY, U.S.A.), a  $\varnothing$  50 $\mu\text{m}$  laser pinhole (model "MLB 1.050", Optische Werkstätten Bernhard Halle Nachfl., Berlin-Steglitz, FRG). as well as mounting plates made in the local mechanical workshop.

The equipment for electrophysiological research comprises an "AxoPatch 1D" unit as well as the experimental control and data management software package PClamp 7 (Axon Instruments, Foster City, CA, U.S.A.), "SEC-05LX" and "GIA-05X" units (npi, electronic, Tamm, FRG), as well as "Clock Control" and "Quad Pulse" modules of the "HG100G" system (HiMed Instruments Ltd., Thatcham, Berkshire, UK).