Development and design of advanced two-photon microscope used in neuroscience

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Abstract. This work represents the real steps to development and design advanced two-photon microscope by efforts of laboratory staff. Self-developed microscopy system provides possibility to service it and modify the structure of microscope depending on highly specialized experimental design and scientific goals. We are presenting here module-based microscopy system which provides an opportunity to looking for new applications of this setup depending on laboratories needs using with galvo and resonant scanners.

1. Introduction

Application of modern techniques offer high resolution properties for living systems research is significant key for neurobiology progress. Often researchers face the necessity both of enhancing of research techniques and using several techniques based on one system. Working with selected methods require both of high-technology equipment availability and knowledge and skills to applying non-trivial mathematical algorithms for control two-photon microscopy system [1] and for well-formed data analysis [2,3]. This work may be regarded as a quick source to laboratory staff who wish to develop and design their own microscope system for self-service it and modify the structure of microscope in order to study highly specialized tasks.

2. Optical scheme

Proposed type of microscope is module-based (Figure 1). Main module of microscope is body represented as metal base to which other modules are mounted. It could be chamber for fix the sample (stereotaxis in case of *in vivo* experiments), moving platform with fixed objectives nosepiece, scanners, optical elements designed for work of differential interference contrast (DIC) [4] and multi-photon mode. DIC produces contrast by visually displaying the refractive index gradients of different areas of a specimen. Galvo [5] and resonant [6] scanners are presented on Figure 1. The first one may be used for rectangular area (line-by-line) scanning, line scanning or point scanning and for uncaging some drug (e.g. glutamate, GABA), the second one – for fast frame rectangular area scanning. Maximal speed for galvo scanner is up to 2 fps (for 512x512 pixels frame), and for resonant scanner is up to 30 fps (for 512x512 pixels frame). IR femtosecond pulsed lasers (tuning wavelength range is 690–1080 nm) are using for fluorophore activation. Non-descanned detectors (NDDs) are using for detecting of fluorescence. Each NDD based on pair of bandpass filter to extract dye absorption wavelength and photomultiplier to detect fluorescent signal. NDDs are mounted exactly above the objectives aperture which is useful for low level fluorescence detecting in living tissues. Often researchers are using at list two detectors to split fluorescence produced by different dyes (e.g.,

morphology and calcium indicators). Modern microscopy systems allow to mount up to 4–6 detectors depending on researcher goals.

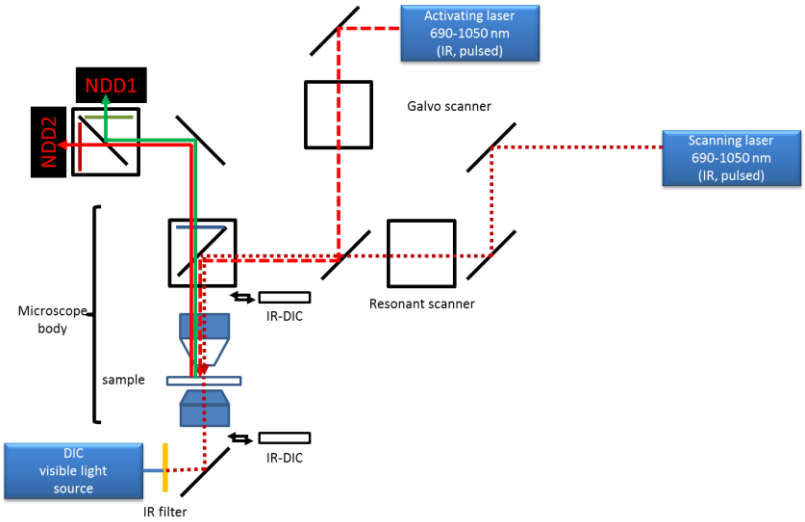

Figure 1. Optical scheme of two-photon microscope with galvo and resonant scanners.

3. Development and design

Researchers should develop and design high-technology platform for realisation module-based microscope (e.g. using Kompas software). Microscope consists of high-technology alloy allowed both to follow requirements of structure rigidity and minimal mass of microscope. All the components and elements of microscope should be developed in such a way as to have possibilities for receiving all the performance goals on the one hand and provide to evolution and addition techniques which could be used in that unique microscopy system on the other. For example if researchers are planning to have an *in vivo* experiments than working space (Figure 2) and, thus, the body of microscope should be higher than systems without *in vivo* approaches. Moving platform located above DIC elements and working space is using for mounting of objective nosepiece, filter cube(s) and NDDs. Moving platform should be constructed as handy element in the context of ergonomics and functional. Moreover, developer should turn attention to minimal approach increment (stepper motor with at list 500 steps per rev should be used) of moving platform on the one hand, and to high-speed displacement and no slop (free motion) on the other hand. Other important aspect is accurate positioning of chamber for brain tissue (or alive animals) as well as micromanipulators for using electrophysiological and other (e.g., bolus loading) methods. U-shaping table can be adapted for specified type of microscope. It is possible to to set up this table independently from the microscope. For maintenance purposes it is possible to remove this table without disassembling the microscope. Motorized axes could be controlled by independent controller (control box and joystick or keyboard) or PC using especial software which could be integrated in mail control software for microscopy system.

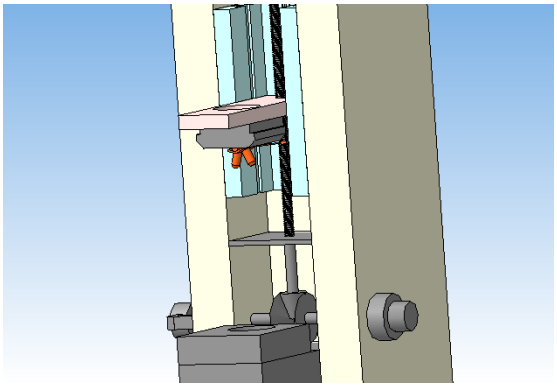

Figure 2. Working space of the microscope model developed using Kompas software.

4. Microscope system control

Control of system is realized either exactly by using DA/AD converter(s) or by using control element of microscope components (e.g. USB, COM connection). However development of cross functional and convenient software to control the system is very important object for researchers. For achieving these goals developers could use commercially available software or create their own software using Matlab, LabView, Python or others. In case of software development researcher could create few sub-programs for each of elements controlled by software (e.g. manipulators, focus, lasers, scanners, acousto-optic modulator (AOM), detectors and others). After this step sub-programs could be combine to one main program which will control all of them and synchronize their integrated work. Software also should implement algorithms to scan region(s) of interest. These algorithms will depend on scanner type. Galvo scanner could be used for rectangular area (line-by-line, few options are presented in Figure 3) scanning, line scanning or point scanning and for uncaging some drug (e.g. glutamate, GABA) in sequence of points, resonant scanner could be used for fast frame rectangular area scanning in relation to feature of construction. Working cooperatively, these scanners could fast scan rectangular area (resonant scanner) and activate caged drugs (galvo scanner) using different wavelength from different laser sources. Important aspect during any type of scanning is synchronizing between movement of laser beam by scanner and input signal from detector(s). Input signal received in one moment of time is detected fluorescent signal from the point where was directed laser beam by scanner at the same moment of time. Thus, software could construct fluorescent signal frames and simply analyze them (overlapping to each other, scale bar, construct 3D images and others). Moreover, researchers could realize more complicated data analysis allow represent data correctly. Additionally all the experimental data (include parameters of hardware such as laser intensity and wavelength, photomultipliers sensitivity and others) could be saved in especial file which could be opened both in that software to repeat experimental parameters and special software to represent data and analyze it (e.g. Matlab and others).

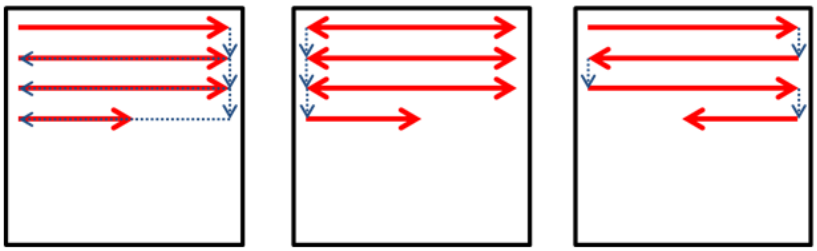

Figure 3. Few options of laser beam movement in case of rectangular area (line-by-line) scanning using by galvo scanner.

Acknowledgments
The work was performed at the Lobachevsky State University of Nizhny Novgorod with financial support of the government represented by the Ministry of Education and Science of the Russian Federation, the unique identifier of the project is RFMEFI57814X0079, the agreement on granting a subsidy №14.578.21.0079 dated by 28.11.2014.

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