Summary description of project context and objectives.

Brain computations are distributed over large distances which makes it difficult to study the neuronal circuits involved in any particular task. One example is the visual system, where the image that enters the eye is processed in the retina (located in the eye), the lateral geniculate nucleus (LGN) (located centrally in the brain), and the visual cortex (close to the surface of the brain). Understanding such distributed brain computations requires methods that can simultaneously report neuronal activity in functionally connected but distant regions. Despite the large distances between connected brain regions, within each site the computation is performed by small neuronal microcircuits, or even the neuronal compartments of a single neuron, such as dendrites or axons. Therefore we not only need to record activity at a sub-micron spatial resolution in a given region, but also perform such recordings simultaneously in different regions, perhaps separated by millimetres or centimetres.

We engineer and test a novel three-dimensional two-photon laser scanning microscope which can simultaneously image three different brain regions in the three spatial dimensions (***3x3D system***), where each volume scanned could exceed cubic millimeters. Such a ***3x3D system*** is essential in understanding distributed brain computations, since it will allow the simultaneous recording of neuronal activity in multiple distant brain volumes that are functionally connected. As each site is to be scanned with sub-millisecond temporal resolution in several hundred locations, the connectivity of distant neuronal microcircuits or even such neuronal compartments as dendrites or axons will be followed.

Participants of the project:

- Femtonics Ltd with third party joint units such as IEM-HAS and PPCU, Budapest (Project Coordinator: Balázs Rózsa MD, PhD)
- Friedrich Miescher Institute for Biomedical Research (FMI), Basel, (Prof. Botond Roska)
- Max Delbrück Center for Molecular Medicine (MDC), Berlin (Dr. James Poulet)
- University of Szeged, Szeged, (Prof. Károly Osvay)
- Budapest University of Technology and Economics (BME), Budapest (Dr. Pal Maák)

Description of the work performed since the beginning of the project and the main results achieved so far.

During the first year of the 3x3D imaging project, we planned and then realized the key components. During the kick-off meeting in Budapest, on the 30th of July, 2013; the project was initiated and since then it has been running smoothly according to plan. Some of the work packages related to the biological testing were started earlier than predicted.

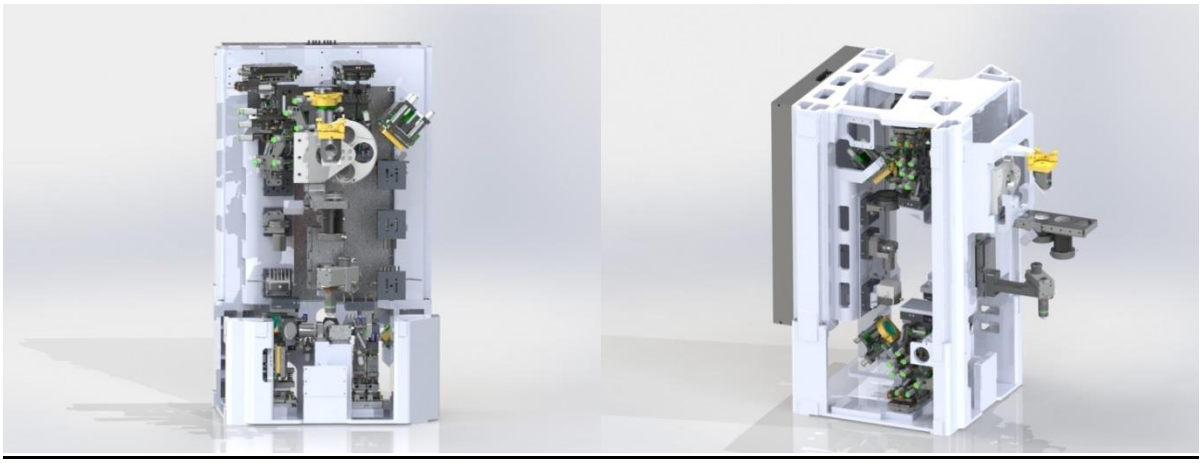
WP1 Development of new acousto-optical (AO) deflectors for the 3x3D system

We started to construct new AO deflectors using more traditional manufacturing technologies; meanwhile we are also experimenting with the optimization of new bonding approaches. Besides the optimization of the bonding of the crystals, we also optimized the size and the

orientation of the crystals in order to fulfill the optical requirements of the 3x3D microscope. Finally, optical modelling of the new AO deflectors was improved and new parameters were introduced in order to handle new crystal orientation described in the previous tasks. Besides optical modelling, hardware design of the scan-head was also successfully carried out.

WP2 Development of the 3x3D microscope

The whole optical pathway, including the scanning unit, the relay lens system, the objective and other passive optical elements were uploaded into the optical modelling software and calculations were performed showing that the system will be able to provide the necessary parameters. We also modelled the retinal adapter and showed that subcellular resolution is attainable with a relatively simple approach. We constructed a deep brain imaging adapter and by using an existing microscope prototype we were able to image GCaMP-expressing neurons in the mouse hippocampus. We started the optical and mechanical design of the adaptive optical system and the final microscope assembly.



WP3 Development of ultrafast lasers for deep imaging and efficient optogenetic activation

We have carefully examined three possible amplification schemes, and found that two alternatives exhibit similar successes, while the third would be less feasible to be implemented. In order to reduce the risk of this research and development work we have decided to proceed with both schemes, hence we designed them to work with the same pump laser. Their technical design and implementation have started. Calculations were made to optimize the number of passes, absorption coefficient and length of the Ti:S crystal, the pump energy and finally the beam diameters inside the amplifying medium. Implementation has been started with the acquisition of key components.

WP4 Development of control hardware and 3D software

Three new cards have been completed for the system, a digital IO module, an analogue IO module and an AO driver module. All three modules have an edge connector allowing them to be plugged in a motherboard replacing the currently used cable assemblies. This will enhance assembly, reliability and servicing. We have developed novel methods for the measurement and

analysis of neuronal network activity in multiple regions. The novel methods allow high-throughput data recording and analysis. In addition, transformation of the 3D volume data into 2D matrixes allows for the use of currently available well-tested 2D methods for motion artefact compensation.

WP5 Neurophysiological and pharmaceutical demonstration in vitro and in vivo

The consortium started to develop the technology which is necessary for imaging genetically encoded calcium indicators (GECIs) is neuronal cells. The laboratory, led by Prof. Botond Roska (FMI) had preliminary results with GCaMP3-and GCaMP5 expressing cells. FMI and Femtonics established a student-exchange program to share the technology between the participants. Meanwhile, the lab lead by Balázs Rózsa purchased viral plasmid containing GECI sequences. The technology was successfully transferred to IEM-HAS where the laboratory was converted to be viral-compatible and all the necessary licenses have been obtained.

WP6 Demonstration of simultaneous 3D measurements and photo-activation in vivo at depth

We have developed novel software and hardware tools for optogenetic activation in 2D. Preliminary *in vitro* (Femtonics) and *in vivo* (MDC) data were presented on subcellular two-photon optogenetic stimulation during the first annual meeting including some data. This dataset will now be completed with a plan to submit a collaborative publication in the coming months.

WP7 Development of the prototype of the product

This task is dependent on the planning of the system in WP2. The first prototype of the AOD crystal holders was manufactured. The prototype was tested on a mechanical stage to check the performance of the motorized parts built into the crystal holder.

WP8 Management

The consortium signed the Consortium Agreement and laid down the general terms and rules of Consortium Management. This included the election of General Assembly and Executive Board to control the scientific and financial progress of the project. A kick-off meeting was organized to discuss the project tasks and to facilitate the starting of the scientific work. The transferred EU pre-financing was distributed among the participants according to the previously agreed and signed decision.
Communication between the beneficiaries was coordinated by Femtonics (Patricia Varju and Balázs Rózsa) and the scientific progress towards the tasks in the WPs was controlled by Balázs Rózsa and Botond Roska.

WP9 Dissemination, Collaboration and Exploitation

During the first year of the project, the consortium partners published several high impact papers, participated in international conferences and organized workshops and summer schools.

Description of the expected final results and their potential impacts and use.

Our technology will cause long lasting changes to *Science,* since new principles about neuronal circuit function across distant brain regions will be discovered. To *Technology*, since the 3x3D system will enable large brain regions to be scanned in 3D at cellular resolution. To *Society*, since the effects of retinal vision restoration in animal models of blindness can be tested in higher visual centres at an unprecedented resolution. To *Theory,* since theories about distributed brain computations can now be directly tested across multiple brain regions.

After the completion of the project, the above list of impacts on Science, Technology, Society and Theory will be immediately realizable since the *3x3D* system will be made available for purchase from Femtonics Ltd.

The development and testing of the *3x3D* system requires a cross-European approach since there is no one country where all the required expertise for this project is present. It requires state-of-the-art knowledge and technologies in optics, scanning technologies, *in vivo* recordings, viral and genetic manipulations, physiology and computational neuroscience.

We are confident that the impact will be achieved since all the expertise required for the development of the 3x3D system is present in the three contributing groups and there is little dependence on external companies or external technologies.

More information: www.3x3dimaging.eu