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Report on workstations for advanced microscopy with extended temporal and spatial resolution

Lead Beneficiary: VUA-LLAMS

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Deliverable Nature	
R = Report, P = Prototype, D = Demonstrator, O = Other	R
Dissemination Level	
PU = Public PP = Restricted to other programme participants (incl. the Commission Services) RE = Restricted to a group specified by the consortium (incl. the Commission Services) CO = Confidential, only for members of the consortium (incl. the Commission Services)	PU

A. Abstract / Executive Summary

LaserLab Amsterdam has now two workstations for SHG and THG (and 2- and 3-photon excited fluorescence) microscopy available, plus two devices for handheld SHG/THG imaging. One of the workstations has been optimized for long-duration experiments and speed.

B. Deliverable Report

1 Introduction

Third harmonic generation microscopy is a label free technique that can be applied to live tissue, up to a depth of ~0.5 mm, yielding intrinsic contrast, with a resolution of ~0.5 μm. Augmented with second harmonic and/or 2-and 3-photon excited fluorescence signals an almost full morphological image of tissue can be obtained [1-5].

2 Objectives

The objective was to extend the temporal and spatial resolution. We have extended this objective to duplication of the workstation and to the implementation of a long needle-like objective to prepare for the development of handheld devices for *in-vivo* measurements. Duplication was necessary because due to its success, the many users left not enough time for improvements and innovations to the workstation.

3 Work performed / results / description

A THG microscope was home-built using a Multi-Photon Microscopy KIT (Thorlabs), featuring a resonant and a galvo scanner, scan and tube lens, 2 photomultipliers and a 25×/1.10 (Nikon APO LWD) water-dipping objective. Tunable laser pulses were provided by a custom optical parametric oscillator (Mira-OPO, APE) pumped at 810 nm by Ti-sapphire laser (Coherent Chameleon Ultra II), yielding a tunability from 760-1600 nm. THG spatial resolution was 0.7 μm in transverse and 1.8 μm in longitudinal direction. THG image collection was efficient, and we demonstrated a tissue ‚inspection mode' where in 60 seconds a piece of brain tissue 5x3 mm was scanned for cell density. Also, high stability and reproduceability enabled tracking of one cell in deep tissue over multiple days.

For the needle-like objective we have tested a commercial high-NA multi-element hemisphere-GRIN lens micro-objective (GT-MO-080-018-810, GRINTECH) composed of a plano-convex lens and two GRaded INdex (GRIN) lens with aberration compensation, object NA = 0.80 and object working distance 200 μm (in water), image NA = 0.18 and image working distance 200 μm (in air), magnification ×4.8 and field of view diameter of 200 μm. The GRIN lenses and a plano-convex lens were mounted in a water-proof stainless steel housing with outer diameter of 1.4 mm and total length of 7.5 mm. Originally designed for wavelength range of 800–900 nm this GRIN objective provided good focusing of 1200 nm femtosecond pulses and collection of back-scattered harmonic and fluorescence photons.Lateral and axial 3P FWHM values can be theoretically evaluated for λ = 1200 nm, n = 1.33 (immersion liquid – water) and objective NA = 0.8: $FWHM_{x,3PF}$ = 0.6 μm, $FWHM_{z,3PF}$ = 2.8 μm. Measured values are, and $FWHM_{x,3PF}$ = 0.7 μm, $FWHM_{z,3PF}$ = 3.9 μm for the GRIN assembly, with measurement error ±0.1 μm. The experimental and theoretical values of the PSF FWHM values are very close indicating that also the GRIN objective performs very well, while it was designed for 800 nm.

These results mean that we can handle even more users to our THG workstation. Currently, we are running projects on brain tumors, brain epilepsy, breast tumors, skin morphology, fat cell viability (for fat transplantation procedures), cartilage tissue regeneration and bacterial infection of mouse mucosa cells, with researchers from VUmc, AMC and the Burn Center in

Beverwijk. The neurobiologists of the VU run cognition and addiction research lines. The success of the needle objective means that we can in the future perform *in-vivo* experiments.

Because we have given priority to the above described work, the increase of the spatial resolution of each of the workstations will be performed in a later stage.

4 Conclusions

The number of workstations has been doubled, the second workstation has an extended wavelength range, a fast scanning mode for tissue has been demonstrated. A good spatial resolution for a long needle-objective has been demonstrated. The spatial resolution of the workstations will be improved in a later stage.

5 References

1. S. Witte, A. Negrean, J. C. Lodder, C. P. J. de Kock, G. T. Silva, H. D. Mansvelder, M. L. Groot, Label-free live brain imaging and targeted patching with third-harmonic generation microscopy. *P Natl Acad Sci USA* **108**, 5970-5975 (2011)

2 S.-w. Chu, S.-p. Tai, C.-l. Ho, C.-h. Lin, C.-k. Sun, High-Resolution Simultaneous Three-Photon Fluorescence and Third-Harmonic-Generation Microscopy. **197**, 193-197 (2005).

3. D. Debarre, N. Olivier, E. Beaurepaire, Signal epidetection in third-harmonic generation microscopy of turbid media. *Opt Express* **15**, 8913-8924 (2007).

4. D. Debarre *et al.*, Imaging lipid bodies in cells and tissues using third-harmonic generation microscopy. *Nat Methods* **3**, 47-53 (2006).

5. P. Mahou *et al.*, Combined third-harmonic generation and four-wave mixing microscopy of tissues and embryos. *Biomed Opt Express* **2**, 2837-2849 (2011).

6 Publications

(Publications resulting from the JRA need to indicate DOI and whether open access will be/is granted (yes/no). Remember the obligation to acknowledge EC support through Laserlab-Europe, EC-GA 284464)

Third harmonic generation imaging enables fast, label-free pathology of human brain tumors, N.V. Kuzmin, P. Wesseling, P.C. de Witt Hamer, D.P. Noske, G.D. Galgano, H.D. Mansvelder, J.C. Baayen, M.L. Groot, under review.

Novel 3D printed cage construct for ear cartilage tissue engineering, D.O. Visscher, E.J. Bos, N.V. Kuzmin, D. Iannuzzi, M.L.Groot, P. van Zuijlen, in preparation