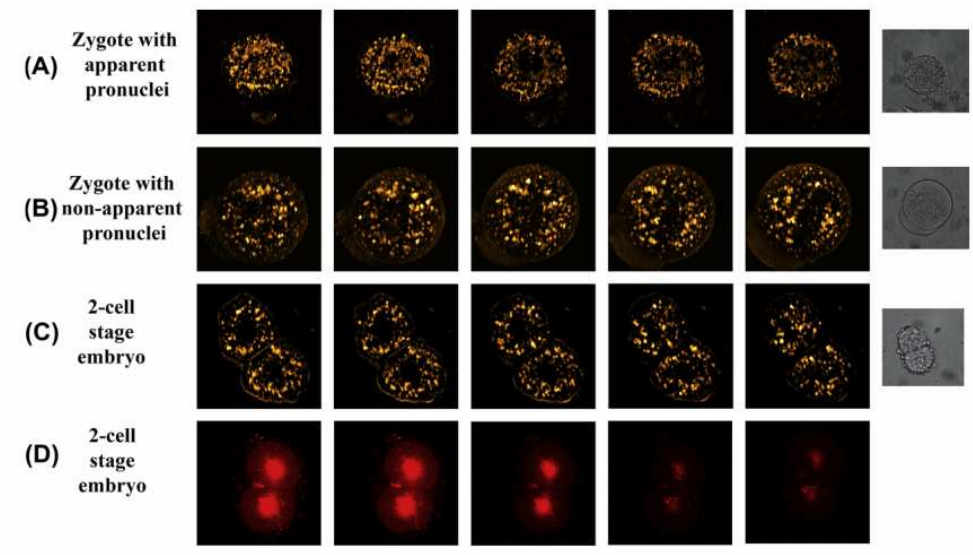
WP6 - Highlights

Biophotonics applications and prototypes

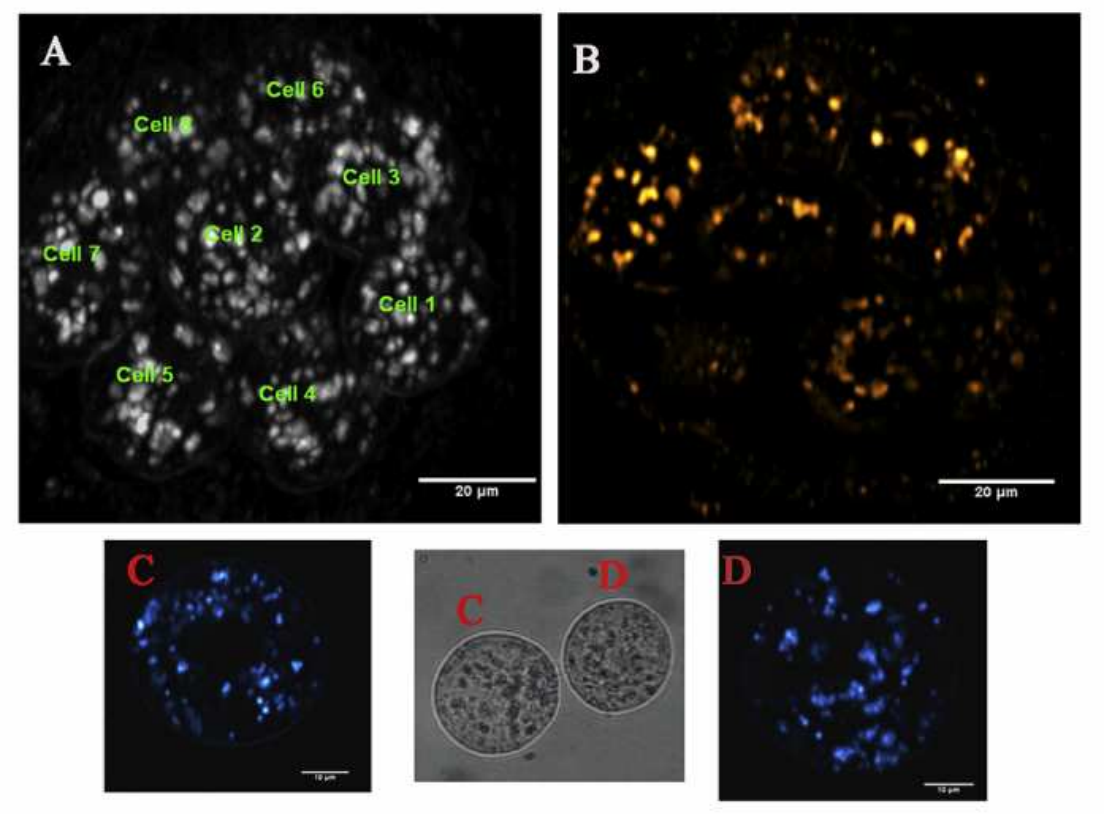
The FAST-DOT consortium has taken the new high performance quantum dot based materials and components and implemented them in a range of prototypes for validation and demonstration in bio-photonic applications where the novel properties offer advances in terms of cost and performance to a number of biological imaging and intervention techniques.

Study of the pre-implantation embryo patterning based on Third Harmonic Generation (THG) using a femtosecond t-pulse laser

Embryo patterning is subject to intense investigation. So far only large, microscopically obvious structures like polar body, cleavage furrow, pro-nucleus shape can be evaluated in the intact embryo. Using non-linear microscopic techniques, FAST-DOT partner FORTH describes new methodologies to evaluate pre-implantation mouse embryo patterning. Third Harmonic Generation (THG) imaging, by detecting mitochondrial/lipid body structures, could provide valuable and complementary information as to the energetic status of pre-implantation embryos, time evolution of different developmental stages, embryo polarization prior to mitotic division and blastomere equivalence. Quantification of THG imaging detected highest signalling in the 2-cell stage embryos, while evaluating a 12–18% difference between blastomeres at the 8-cell stage embryos. Such a methodology provides novel, non-intrusive imaging assays to follow up intracellular structural patterning associated with the energetic status of a developing embryo, which could be successfully used for embryo selection during the in vitro fertilization process.



Serial sections from a zygote with apparent pronuclei at 18 h post-hCG (A), a zygote with no apparent pronuclei at 21 h 30 min post-hCG (B), a 2-cell stage embryo at 45 h 30 min post-hCG (C) visualized by THG imaging and a 2-cell stage embryo stained with TOPRO-3 for the detection of nuclei (D). Mouse embryos were generated from BALB/c mice upon in vivo fertilization procedures and submitted to THG imaging without any further manipulation.



THG signal of blastomeres in 8-cell stage embryos with their zona pellucida (A: 3D reconstruction, B: central slice image) and in isolated blastomeres from 4-cell stage embryos (C and D). Signal quantification in blastomeres C and D (calculated as described in Section 3) reveals a 16% difference.

Use of semiconductor disk lasers (SDLs) for nonlinear imaging applications

FAST-DOT partners ICFO, FORTH, M2, TBWP, ETHZ developed a portable ultrafast Semiconductor Disk Laser (SDL) (or vertical extended cavity surface emitting laser—VECSELs), to be used for nonlinear microscopy The SDL is mode-locked using a quantum-dot semiconductor saturable absorber mirror (SESAM), delivering an average output power of 287 mW, with 1.5 ps pulses at 500 MHz and a central wavelength of 965 nm. Specifically, despite the fact of having long pulses and high repetition rates, they demonstrated the potential of this laser for Two-Photon Excited Fluorescence (TPEF) imaging of in vivo Caenorhabditis elegans (C. elegans) expressing Green Fluorescent Protein (GFP) in a set of neuronal processes and cell bodies. Efficient TPEF imaging is achieved due to the fact that this wavelength matches the peak of the two-photon action cross section of this widely used fluorescent protein. The SDL extended versatility is shown by presenting Second Harmonic Generation images of pharynx, uterus, body wall muscles and its potential to be used to excite other different commercial dyes. Importantly this non-expensive, turn-key, compact laser system could be used as a platform to develop portable nonlinear bio-imaging devices.

ICFO participated as end users to assess the use of ultrashort pulsed lasers constructed in Fast-Dot for nonlinear microscopy applications. Such demonstration completely closes the

chain of efforts starting from theoretical calculations and simulations, wafer design, integration, packing and testing. In some cases, our demonstrations represented the required final test (target application) step that allowed companies a direct commercialization. In particular, ICFO achieved two important milestones:

1. First demonstration of the use of a Semiconductor disk laser (SDL) for nonlinear imaging applications. It was shown that the SDL can be used for TPEF imaging of samples labeled with GFP, one of the most widely used fluorescent markers in biology. Currently the tested laser is under commercialization where the IP has been protected with a patent.

2. First proof of concept demonstration of the use of an edge emitting semiconductor lasers for nonlinear imaging applications. It was shown for the first time, TPEF images of selected samples using these matchbox sized devices.

Details and demonstration results of these two milestones are in what follows:

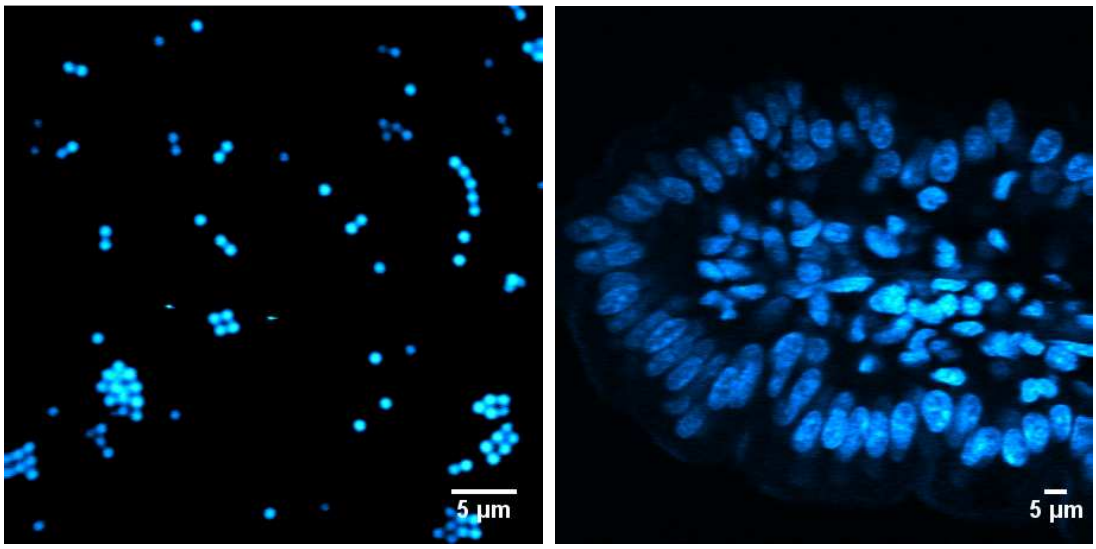
First demonstration of the use of a semiconductor disk laser (SDL) for nonlinear imaging applications

A compact SDL (240x140x70 mm) for non-linear bio-imaging applications was used in collaboration with Msquared lasers. Its center wavelength (965 nm) enables a very efficient excitation of GFP at low peak powers. Demonstration of high performance of the SDL for:

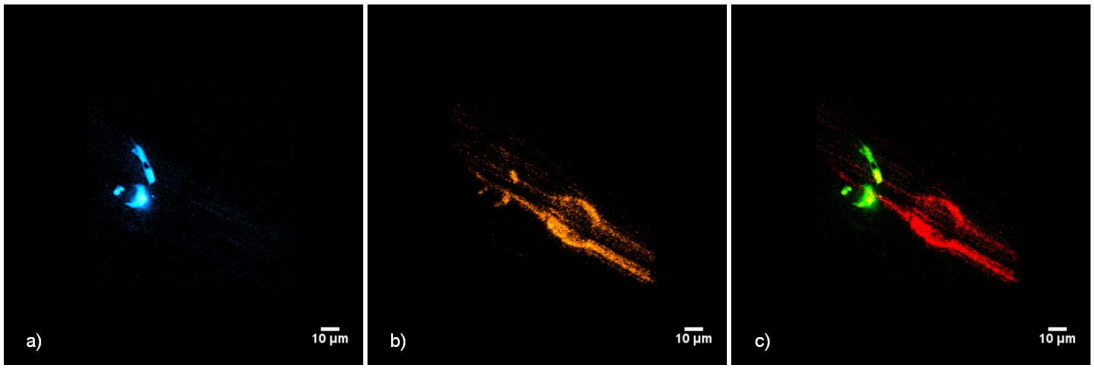
- Deep imaging (with high NA)
- Time lapse studies

The Imaging performance of the SDL was compared vs. a Ti:sapphire where it was found that:

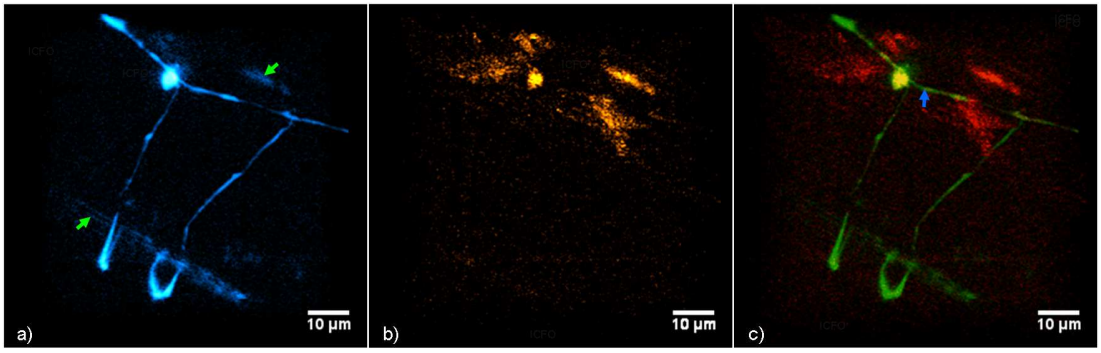
- It provides similar performance in TPEF imaging if high NAs are used
- It produces less auto fluorescence
- For higher SHG signal Higher peak powers are needed



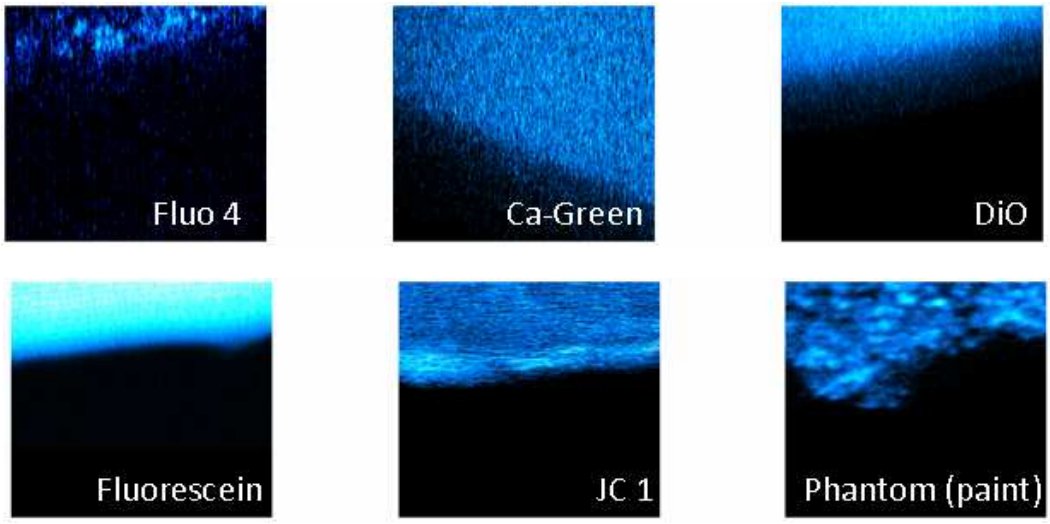
TPEF images from green fluorescent beads (left) and (right) mouse intestine section labeled with Alexa Fluor 350 WGA (mucus of goblet cells), Alexa Fluor 568 phalloidin (filamentous actin prevalent in the brush border), and SYTOX Green nucleic acid stain (nuclei of goblet cells). All the images are 500x500 pixels.



3D projections of a) TPEF signal from neurons forming the nerve ring expressing GFP (blue) and b) SHG signal from the pharyngeal region (orange) of the C. elegans nematode. c) Merged TPEF (Green) and SHG (red) images of both structures.

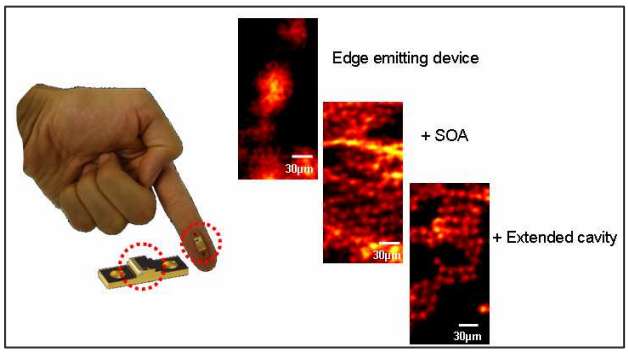


3D projections of a) TPEF (blue) of a set of motoneurons expressing GFP and b) SHG (orange) signal of the muscles in the vulval region in a C. elegans mid body region. c) Merged TPEF (Green) and SHG (red) images.



TPEF images from different dyes in solution.

First proof of concept demonstration of the use of an edge emitting semiconductor laser for nonlinear imaging applications

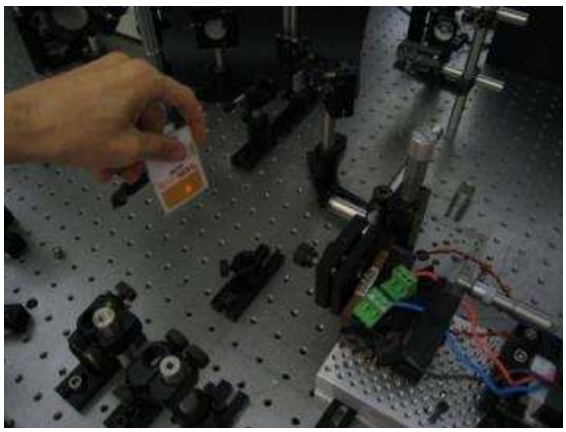


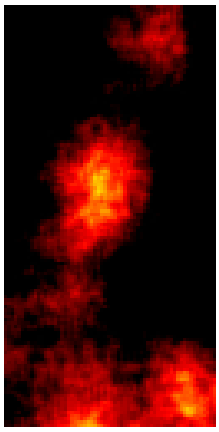
Demonstration of the first nonlinear imaging application using WP2 Edge-emitting prototypes in collaboration with the University of Dundee. The partners presented the first demonstration of TPEF imaging obtained with 1.26-m wavelength chip-scale semiconductor ultrashort pulsed edge emitting devices and SOAs. This was achieved using different configurations to increase the output peak power.

The first device had pulse duration of 1.0 ps, an output average power of 100 mW, and a repetition rate of 10 GHz, corresponding to ~10-W peak power. In this case the peak power of the laser system has been up scaled considerably (compared with the previous devices), thus being able to deliver a couple of watts into the sample plane. Fluorescent bead samples (suitable for NL excitation at 1260 nm) were imaged.

The second system to be tested consisted on an edge emitting device and a SOA where the pulse duration was 3-4 ps, the repetition rate was 10 GHz and the output average power from the system ranged between 290-370 mW. The system output peak power has been considerably increased up to ~12 W by employing an amplification stage consisting of a QD based SOA. In this case more power directly enables to efficiently obtain a nonlinear image.

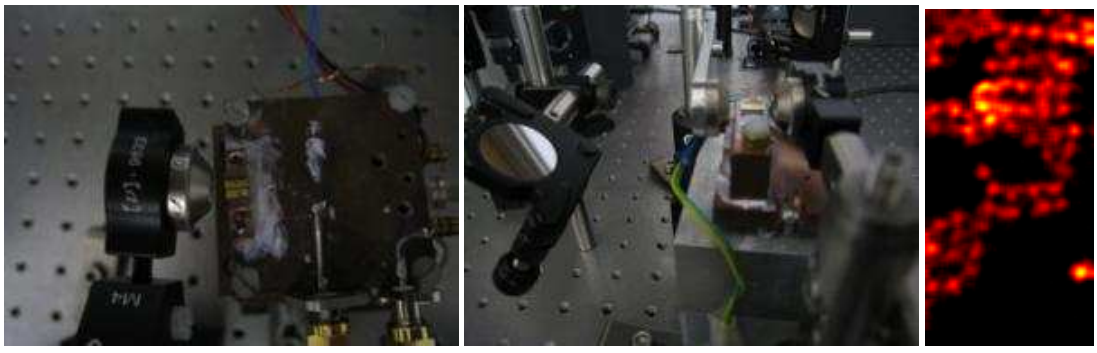
For further increasing the peak power, an external cavity configuration was employed. The repetition rate of QD-ECMLL was decreased to 648 MHz. The system had a pulse width of ~ 9 ps. As before, this configuration was amplified through a QD-SOA. In this experiment the highest peak power was ~30.3 W. The obtained TPEF image shows how the increase on peak power directly gives an improvement in the image quality.





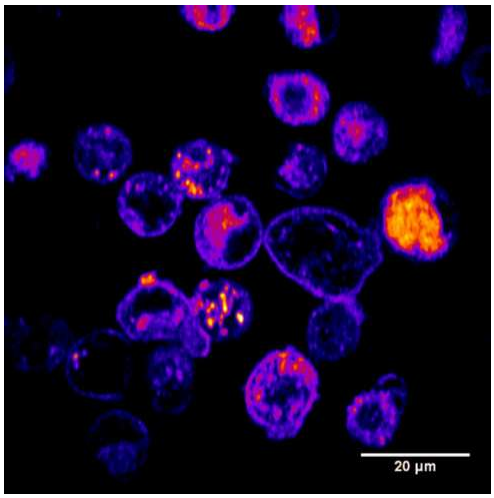
A ~10-W peak power tapered device was employed to obtain nonlinear images of fluorescent beads.

A tunable device and a SOA delivering ~12 W peak power enabled to achieve nonlinear imaging of fluorescent beads.



An external cavity configuration using a chirped Bragg grating (CBG) as an output coupler and a SOA was employed to increase the system peak power up to 30.3 W. This configuration was used to obtain two photon excited fluorescence from fluorescent beads.

Femtosecond laser nanosurgery of sub-cellular structures in HeLa cells by employing Third Harmonic Generation imaging modality as diagnostic tool



Femtosecond laser assisted nano-surgery of microscopic biological specimens is a relatively new technique which allows the selective disruption of sub-cellular structures without causing any undesirable damage to the surrounding regions. The targeted structures have to be stained in order to be clearly visualized for the nano-surgery procedure. However, the validation of the final nano-surgery result is difficult, since the targeted structure could be simply photobleached rather than selectively destroyed. This fact comprises a main drawback of this technique. FAST-DOT partners FORTH, Time-Bandwidth Products AG and collaborators have employed a multimodal system which integrates non-linear imaging modalities with nano-surgery capabilities, for the selective disruption of sub-cellular structures in HeLa cancer cells. Third Harmonic Generation (THG) imaging modality was used as a tool for the identification of structures that were subjected to nano-surgery experiments. No staining of the biological samples was required, since THG is an intrinsic property of matter. Furthermore, cells' viability after nano-surgery processing was verified via Two Photon Excitation Fluorescence (TPEF) measurements (fig. shows a THG image of HeLa cancer cells).