Components for Highly Advanced time-Resolved fluorescence Microscopy based on Nonlinear Glass fibres

FP7 Call identifier: FP7-ICT-2011-7
Activity code: ICT-2011.3.5: Photonics
Project start date and duration: 01.09.2011 for 3.5 years
Contract type: STREP
Grant Agreement number: 288786
Date of the latest version of Annex 1 against which the assessment is made: 09/05/2014

Publishable summary

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Periodic Report: 2nd
Period Covered: from 03/2013 to 02/2015

Workpackage: WP 1
Est. person months: 0.1
Version : 3 (04/06/2015)

Dissemination level: PU
Nature: Report
Total number of pages : 5

Any work or result described in this report is in principle genuinely a result of this project. When some results from other work or projects are used, related sources are properly referenced in the text.

http://www.charming-project.eu

1. Publishable summary

1.1. Charming project in brief

Charming means “Components for Highly Advanced time-Resolved fluorescence Microscopy based on Nonlinear Glass fibres”. The aim of this project was then to develop new diagnosis tools providing high resolution in compact and rapid systems.

Super-resolution techniques like STED and photo-switching microscopy are just opening the doors to a truly new dimension in optical microscopy for bio-imaging. For the first time it is not only possible to really “see” the structure of cell organelles in much more detail but also to study for example the internal structure of bacteria, with a size typically far below the optical diffraction limit.

Fluorescence Lifetime Imaging Systems (FLIM) are applied in many biomedical fields. This technique can for instance, be applied in in vivo studies to localize tumors and detect their progression with adapted fluorophores.

The most promising direction in this field is for safe in-vivo skin and tissue characterisation to identify tumours and other specific defects based on a lifetime assisted auto-fluorescence imaging. In recent years, several research consortia started to develop instruments for medical diagnosis for epidermal skin imaging, ophthalmology and endoscopic imaging inside living specimen.

CHARMING contributed to this field of activity by providing new laser sources which are essential for the development of all these diagnostic tools that are more and more routinely used for screening pathologies at early stage.

1.2. Executive summary

Today semiconductor laser diodes are available from 350 to 500 nm. To cover the spectra between 515 nm and 630 nm frequency conversion technologies are employed. In order to guarantee a perfect Gaussian beam overlap between all wavelengths, free space frequency doubling and subsequent fibre coupling is used. This solution suffers from important drawbacks in terms of stability, compactness and cost.

Additionally given the emission band possibilities of currently used species in solid-state lasers and amplifiers like Neodym, Ytterbium and Erbium some wavelengths are difficult to reach even though frequency doubling. For instance 560 nm is a very interesting wavelength for fluorescence imaging as it corresponds to classical fluorophores but requires a laser source at 1120 nm. This is at the edge of Ytterbium emission band. As a consequence, this wavelength can be achieved with a specific Ytterbium doped fibre composition, the performances are degraded compared to classical 1064 or 1030 nm bands and fibre and amplifier designs require much more attention. If one considers now 590 nm that can have various applications, not limited to biomedical imaging, the challenge is even higher. Indeed, in order to reach 590 nm through second harmonic generation one needs to develop a fundamental laser source at 1180 nm which is out of reach of Neodym, Ytterbium or Erbium emission bands. The primary semiconductor sources at theses wavelengths (1120 and 1180 nm) are also not necessarily available with the required performances (polarization maintaining fibre output, narrow spectrum, good behaviour in pulsed mode). Finally we can add that 590 nm is in particular interesting for depletion in super-resolution microscopy, and to generate 295 nm radiation for Tryptophan imaging. This means that for both applications, in case the challenge was not high enough, a high average power (hundreds of milliwatts in tens of picoseconds regimes) is needed for the 590 nm radiation.

Two main options can be envisaged for tackling this difficulty. The first type of options is to consider amplification at 1180 nm through special fibres like Bismuth doped fibres or Raman gain fibres. The second type of options is to consider direct generation of 590 nm or 295 nm through non linear conversion schemes in crystals like Sum Frequency Generation but this is usual complicated and low efficient.

Then in the project CHARMING we decided to develop these different exotic laser sources using fibre based solutions for the amplification. We tested both Bismuth and Raman amplification for 1180 nm and used Ytterbium for 1120 nm. Regarding the frequency doubling step we also developed in the frame of the project fully fibre based solutions to avoid any free-space alignment in the process. This was of course another important part, the main one, of the challenge of the project CHARMING.

In fact the situation is completely different for these two aspects of the project. Regarding the laser sources, a solution needed to be found where almost no suitable and competitive approach exists. For SHG, the aim was to develop a new approach for a problem that could already be addressed by non ideal but mature and competitive solutions in the market. Indeed SHG can already be addressed by crystal based conversion and use of free space optics to obtain a fibre coupled visible laser source. This results on a less practical solution since the light has to be coupled out of fibre before being converted and reinjected in another fibre with important losses, needs careful and precise alignment steps and is sensitive to external sources of perturbation like vibrations or temperature. Nevertheless SHG in crystals is a very mature technique that permits to achieve high conversion efficiencies allowing to compensate for the light coupling losses in this approach.

Then the situation regarding second harmonic generation in optical fibres was much less mature than crystal based solutions but it does not mean that the objectives were completely out of reach at the project beginning based on the pre-existing knowledge in the consortium. For instance 236 mW had already been generated at 775 nm in fully fibre configuration with a narrow linewidth semiconductor laser source, externally modulated and amplified. This was a record established by partners of the project before the start of CHARMING that has been far outreached within the project.

In this context the project objectives were clear and challenging: develop new visible laser sources in tens of picoseconds regime for different time-resolved fluorescence imaging applications and develop fibre based frequency conversion solutions to permit a higher degree of integration of these fibre laser sources in confocal imaging systems.

Regarding the wavelengths of interest, these are summarized in the following table.
Table 1: Lasers specifications for Time resolved fluorescence imaging:

Application		Wavelength	av. Power	prf	Pulse width	beam
Tryptophane fluorescence		**295 nm**	1 mW	10..20 MHz	100 ps	Collimated
Time-resolved Fluorescence Microscopy		**560 nm** **590 nm**	1..10 mW	10..80 MHz	100 ps	Single mode PM fibre
STED	Excitation	440 nm 510 nm 640 nm	1..10 mW	10..80MHz	100 ps	Single mode PM fibre
	Depletion	**590 nm** **770 nm**	**200..500 mW**	Sync. to excitation	200..500 ps	Single mode PM fibre
gSTED	Excitation	440 nm 510 nm 640 nm	1..10 mW	**160 MHz**	100 ps	Single mode PM fibre
	Depletion	cw at gSTED, not part of the project				

From this table our main targets were 295 nm/ 1 mW, 560 and 590 nm / 10 mW, 590 and 770 nm / 200 mW. Other wavelengths like 355 and 266 nm were added by PICOQUANT during the project based on their prospection and continuous update of the market expectations.

These main objectives implied a serie of different intermediate steps and targets to be fulfilled all along the project:

- As the preferred approach for reaching the visible domain was to use second harmonic generation, a first mandatory step was to develop the laser sources at corresponding fundamental wavelengths, i.e. 1120 and 1180 nm in particular. Two options were envisaged for that:
    - The first and preferred option was direct modulation of semiconductor diodes and subsequent amplification. This is the usual approach employed by PICOQUANT and then the most suitable and compatible solution with their system.
    - A backup solution envisaged in the project was to use mode-locking at a fundamental repetition rate of 80 MHz combined with in-fibre pulse picking to decrease the frequency of the laser source.
- Whatever the solution for the laser source subsequent amplification steps were required to achieve a suitable optical power for the application. In particular for depletion applications at 1180 nm, one order of magnitude higher power is needed compared to excitation at 1120 nm.
    - At 1120 nm, Ytterbium amplification was used with the aim to reach the hundreds of milliwatts regime.
    - At 1180 nm, both Bismuth and Raman amplification were envisaged with the aim to reach the Watt level.
- For frequency doubling both crystal and fibre solutions have been considered in function of the different targets. Of course the main goal is to cover as much as possible applications with the all-fibre configurations:
    - For conversion down to UV, crystals are the only suitable solutions.
    - For low power excitation applications, the aim of the project was to demonstrate the possibility to have an all in-fibre wavelength conversion.
    - For high power depletion applications, fibre based solutions are also experimented, in particular for 775 nm with the aim to demonstrate 50% conversion efficiency for 1 kW peak power signals.
    - An adapted solution for thermal stabilization of the fibre laser sources is also required for the devices integration. Ideally a passive solution would be preferred but both active and passive solutions are envisaged.

Finally all these developments have to be integrated into demonstrators compatible with FLIM or STED measurements.

As a result the project achieved various impressive results in terms of new laser sources, fundamental understanding of the physics of these non linear devices and state of the art performances like for instance:

- demonstration of high power SHG generation in fibre (>2W at 775 nm),
- all-fibre pulse picking at MHz regime,
- gain-switched sources at 1120 and 1180 nm,
- picoseconds pulses amplification at 1120 and 1180 nm,
- various all-fibre visible sources in pulsed regime, based on gain-switched and mode-locked lasers (521 nm, 560 nm, 590 nm).

From this project various results will be commercially exploited like:

- New designs for high power DFB semiconductor diodes,
- New PM coupled butterfly DFB sources at 1120 and 1180 nm.
- New gain-switched modules at 1120 and 1180 nm,
- High power amplifiers at 1120 and 1180 nm,
- Picosecond sources at 590/295 nm as a future product.
- Picosecond laser sources with high maturity level at different wavelengths on the visible (766, 355, 266, 561 nm) for fluorescence applications.
- Active and passive packaging units for long fibre devices that can be applied in telecom applications for long dispersion compensation filters for instance.

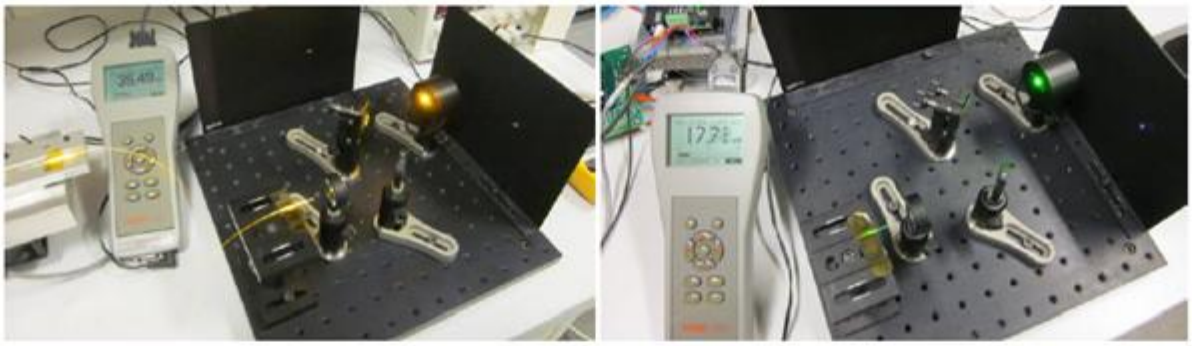

Figure 1: Picture of SHG experiments with gain switched laser diodes and fibre PPSF devices at 590 nm (left) and 560 nm (right)

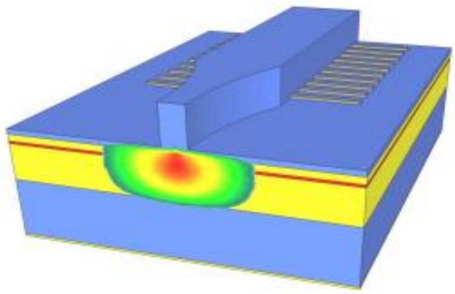

Figure 2: Picture of an all-in-fibre pulse picker (left) and a new high power DFB diode design (right)

The project CHARMING has also generated many scientific results that will impact the research in the domain of optical fibres poling in particular:

- Investigation and deep understanding of poling mechanisms in optical fibres,
- Development of a poling method with soft electrodes that can be injected and removed after poling from the optical fibre without degradation of the induced non linearities in the fibre.
- Validation of a new UV inscription wavelength for the periodic erasure of the poling along the optical fibre.
- Development of a complete periodic erasure set-up, permitting the precise and reproducible inscription of long devices in optical fibres.
- Complete understanding of the mechanisms of poling erasure by multi-photons absorption during laser operation.
- Development of a high speed and high extinction ratio electro-optical in-fibre device.

The results of this project will have short term and long term impacts in different application fields like:

- Life science: new diagnostic tools in medicine
- Telecommunications: quantum cryptography for future highly secured data transfer
- Lasers: Ultrashort sources for various domains like micromachining for instance.
- Sensors: High Voltage sensing

More information can be found in the CHARMING website (http://www.charming-project.eu/) or through the youtube project videos (http://www.charming-project.eu/videos.html) explaining all the steps from the optical fibres and semiconductors design to the biomedical imaging application.