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"Parallel fluorescence spectroscopy tools for micro and nano-analytical applications down to single biomolecules (PARAFLUO)": the successful results of the first year activities

Bolzano, 01 June 2010 – The PARAFLUO project funded by the European Commission under the Seventh Framework Programme for Research and Technological Development has successfully concluded its first year of activities.

The aim of the FP7 PARAFLUO is an innovative instrumentation system that will enhance and extend the FLIM usefulness, enabling to obtain simultaneously spectrally resolved FLIM data for the various spectral components of the emission. There is widespread consensus that this technique (sFLIM) will support a better understanding of the biological processes involved, which may be paramount for the (patho)physiology of tissues and organisms and provide a better insight in key medical issues, as the origin and growth of tumors. The instrumentation developed will be useful also in other applications, e.g. multi-spectral profiling of objects by laser detection and ranging (LADAR).

Being a project specifically designed for undertaking research for the benefit of the Small and Medium Enterprises (SME), PARAFLUO includes 3 SME's with consolidated know-how and active in the scientific-technical market, 5 RTD performers with high international standard and collaboration experience with SMEs and 1 Other enterprise experienced in professional management. The project is structured according to the main new developments, i.e:

1. a photon-counting linear 8×1 array detector based on the silicon single-photon avalanche diode (SPAD) technology (WP2 and WP3);
2. a new micro-lens system for focusing light onto the array detector (WP4);
3. a new multichannel time correlated single photon counting (TCSPC) system based on fast application-specific integrated circuits (ASIC) in advanced technology (WP5) and integrated with an optoelectronic setup in a confocal microscope (WP6).

In the first year very satisfactory progress has been achieved towards all the objectives.

In WP2 and WP3: (a) the 8×1 SPAD array with pixels of wide diameter (50-80µm) was designed and fabricated. A monolithic circuit is associated to each pixel for fast current pick-up, with custom-designed on-chip MOS transistors; (b) the chip with 8 integrated Active Quenching Circuits (iAQC) was designed and fabricated in high-voltage 0.35µm CMOS technology and satisfactory operation with 45 ns dead-time was checked in preliminary tests; (c) the package of the SPAD array detector was designed and fabricated, as well as the auxiliary electronic circuits: programmable voltage source; microcontroller-based driver for the Peltier cooler; external fast comparators for relaying the photon-timing information to the TCSPC.

In WP4 the diffractive microlens array designed for the integration with the 8×1 SPAD array was fabricated. The design based on optimised quantisation of the lens phase profile is predicted to give an increase of >100% in the light falling onto each individual SPAD pixel, compared to that without the lens array. The lenses produce near diffraction limited spots with very little noise outside the focal spot region. Initial trials of integration of the microlens set with the SPAD array showed highly encouraging results.

In WP5 the key objective attained was the design of an 8-channel TDC (Time-to-Digital Converter) ASIC capable of processing high photon counting rates (> 15Mc/s) and providing photon-timing jitter better than 20ps FWHM. The internal interface circuitry was designed to match the requirements of the PicoQuant HydraHarp hardware and software, looking to integrate the new 8-channel module in this system.

In WP6 a spectrograph was implemented into the imaging confocal microscope for being ready to integrate the 8×1 array detector. Fast efficient algorithms were developed for reducing the huge quantity of spectrally-resolved data that must be handled, stored and processed for fluorescence decay analysis. An approach based on moment-analysis of fluorescence decay curves was devised, tested and compared to previous approaches. New software was developed for visualizing the data in compressed form, displaying data in easy-to-understand two-dimensional correlation plots.

Management and dissemination activities have been performed to sustain the project research results above described.