LASERLAB-EUROPE

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"Report on workstations and methodologies for in vivo, deep tissue, high resolution imaging"

Lead Beneficiary: STFC

Due date: Month 42

Date of delivery: Month 42

Project webpage: www.laserlab-europe.eu

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

We have implemented a unique method of simplifying the requirements of laser light sources for multiple microscopic techniques. To achieve this, we have implemented OCTOPUS - optics clustered to output unique solutions is a microscopy platform that combines single molecule and ensemble imaging methodologies. A novel aspect of OCTOPUS is its laser excitation system, which consists of a central core of interlocked continuous wave and pulsed laser sources, launched into optical fibres and linked via laser combiners. Fibres are plugged into wall-mounted patch panels that reach microscopy end-stations in adjacent rooms. This allows multiple tailor-made combinations of laser colours and time characteristics to be shared by different end-stations minimising the need for laser duplications. This setup brings significant benefits in terms of cost effectiveness, ease of operation, and user safety. We have interlinked multi-photon/multicolour confocal fluorescence lifetime imaging for several modalities of fluorescence resonance energy transfer (FRET) and time-resolved anisotropy, total internal reflection fluorescence (TIRF-M), single molecule imaging of single pair FRET, single molecule fluorescence polarisation, particle tracking, and optical tweezers. This has allowed us to develop several combinational microscopic techniques including a 5-colour single molecule workstation together with super high resolution (TIRF-FLIM).

B. Deliverable Report

1 Objectives

OBJ2, Advanced microscopy will lead to significant improvement in capacities for advanced imaging beyond what is commercially available and to the development of novel methodologies for the investigation of living cells and animals. STFC-CLF will develop a 5-colour single molecule workstation together with super high resolution (TIRF-FLIM), in which multiple light sources are linked to multiple imaging stations, allowing a combination of techniques.

2 Work performed / results / description

STFC-CLF has developed several single molecule multi-colour TIRF-based microscopes. A number are combined with fluorescence lifetime to allow both surface and deeper tissue imaging. This is now finding applications in both mammalian and plant studies and is used by several independent research groups. The initial pump-prime studies using these instruments have led to significant national funding (~2million Euros) for the purchase of several super resolution techniques (STORM/PALM, SIM, STED) to increase our capabilities and to be made available to users. Outside our task that is now completed and moving beyond, we are working with Vutara Instruments to develop new real-time fast STORM technique for live cell super resolution. When completed, this would provide a 3D, 20x20x20nm resolution at video rate speeds.

Users are mainly UK based but the next facility access call will include EU users (funded through H2020).

3 Conclusions

STFC-CLF has developed several single molecule multi-colour TIRF-based microscopes and combined with other advanced microscopic based techniques such as multiphoton confocal, FLIM and optical tweezers.

4 References

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5 Publications

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