

Although MPE-based light microscopy for intra-vital deep penetration light microscopy is not a particularly new technology, recent improvements in laser technology have enhanced the use. In addition, increasing demand in the scientific community have lead to further refinement and a wider range of added functionality/applications for MPE-microscopy.

This break-out session started with a brief overview of the technology and how it is implemented in a range of LM facilities in the UK.

Lasers - Pulsed Ti:Sapph as essential basis for all MPE-based systems to this date. Technology and reliability have improved and increasing demand have pushed the production of these lasers close to a mass product, helping to decrease prices as a welcome consequence. More reliable and technically improved **optical parametric oscillators (OPO)** have significantly improved the versatility, extending the suitable wavelength ranges and providing an independent second laser line. This allows the increasing use of red fluorophores and simultaneous excitation of multiple fluorophores. Applications and potential new developments were discussed, including excitation line mixing for simultaneous use for multi-colour imaging and for vibrational recordings (e.g. SHG, THG, etc)(see also Mahou et al., Nat Meth 2012).

The **potential future directions**, laser manufacturers could choose to design new products were stated, and here the three following options were discussed: (1) high power tunable lasers and OPOs, similar to what is used in most systems at the moment, (2) single wavelength, pulsed (fs) diode lasers providing high output power at reduced costs, and (3) smaller, downscaled and more affordable tunable, pulsed lasers.

Implementation of additional functionality – as for other LM platforms, microscopists have started implementing and combining MPE-microscopy with other advanced applications and a few technologies were discussed. The use of **adaptive optics** seems to be increasing. The available hardware has improved and is more reliable, but the programming of the mirrors for the wave front correction still seems to prove challenging and is subject of ongoing research. It was mentioned that this approach might significantly improve the contrast at deeper imaging depths, as has been shown by the initial literature. **Photo-manipulation** (photo-activation, -ablation, -switching, uncaging etc) has become increasingly popular, in particular on systems that now provide OPOs. **Fluorescence lifetime imaging** and **fluorescence correlation spectroscopy** were also discussed as a more often used additional recording dimension and how to control central imaging

parameters were briefly mentioned, e.g. the control of the pulse repetition rate using pulse pickers etc.

Location of MPE-microscope equipment – The discussion surrounding the question where this equipment could be located best, took a large part of the time. However, for a range of reasons the discussion points cannot be given here.