

A NEW LIGHT SOURCE FOR MULTIPHOTON IMAGING INCLUDING CARS

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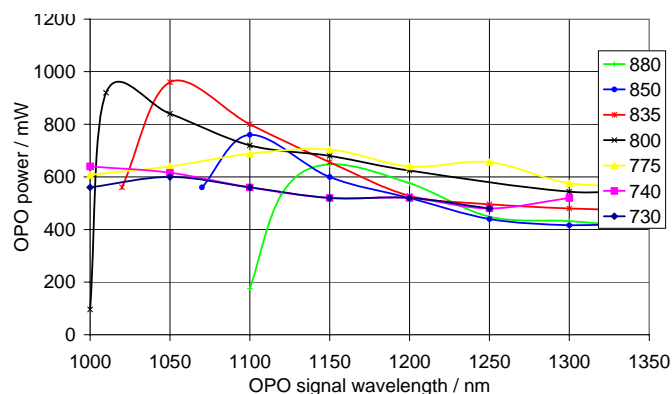
1. INTRODUCTION

Extending the wavelength range for Multi Photon Excitation (MPE) microscopy from the Ti:Sapphire wavelength range into the Infrared with synchronously pumped OPOs pumped by Ti:Sapphire lasers has been applied successfully in the past resulting in deeper penetration depths and efficient excitation of red fluorescent dyes. But current OPOs designs require specific pump wavelength. In contrast imaging two different dyes simultaneously with Ti:Sapphire and OPO is only possible when dyes such as GFP also have reasonable excitation cross section at the OPO pump wavelength. Thus flexibility in pump- and OPO wavelengths to optimally excite both dyes in question is highly desirable.

CARS and SRS microscopy would benefit from such a flexible system too. One intrinsic feature of synchronously pumped OPOs is generating light pulses, which are fixed in time to their pump pulses without any timing jitter, an important feature for CARS light sources. Flexibility in the energy distance between the two wavelengths is required.

Uncaging and drug release microscopy via MPE is possible due to the lowest OPO pump wavelength of <730 nm allowing for uncaging the compounds with the Ti:Sapphire. The activated molecule can be monitored via two photon excitation with the OPO, opening multi photon microscopy for uncaging experiments.

2. THE FAN POLED OPO AS A MICROSCOPY LIGHT SOURCE



The nonlinear crystal in the OPO is a periodically poled crystal with a linear fan pattern. By linear translation of the crystal the poling period is continuously changed and adapted to the desired pump- and output wavelength. Thus the OPO generates from 1000 to >1350 nm with pump wavelength varying from 730 to >880 nm, see fig, with well maintained beam pointing. Typical pulse lengths of the

OPO are ~200 fs for 140 fs input pulses with a time-bandwidth product of 0.6 and maximum power levels close to 1 W pumped by a Chameleon Ultra II (Coherent Inc.). APE has, together with Coherent Inc, recently introduced a compact OPO including the above features, completely hands free and computer controlled, optimized for the needs of MPE imaging.

3. APPLICATION EXAMPLE

We did employ this colocalized excitation of Ti:Sapphire at 850 nm and OPO at 1110 nm to simultaneously excite EGFP and tdRFP expressed in different cells of the central nervous or of the immune system. The aim of these experiments is to monitor in a genuine environment, i.e. in the living organism, the 3D-morphology of tissue in health and disease, the dynamics of cell-cell interactions and typical cellular motility pattern in order to better understand autoimmune reactions as they occur, for instance, in the multiple sclerosis (MS).