

Advantages and disadvantages of two photon excitation (TPE)

- The main advantage of two photon excitation is a deeper penetration and less photobleaching with point measurements (FCS) within confined volumes like cells, since only the confocal volume is prone to bleaching.
- As absorption spectra are broad, this can be an advantage for tissue profiling by autofluorescence, or for cross correlation measurements, as the the excitation volume is determined by the same excitation beam.

However, there are big disadvantages:

- The absorption is much less compared to one photon excitation. This leads very often to high intensities which can destroy the cell (boiling).
- The excitation is much broader so that many dyes are excited at the same time. This is an advantage when autofluorescence is investigated, but when specific excitation is intended, 2PE excitation can create strong background.
- The molecular brightness of the dyes is generally around one order of magnitude smaller: You will get just ten times less fluorescence photons. This leads to limitations for FCS and other single molecule applications.
- The photobleaching of the dyes is much more pronounced. This again limits the number of fluorescence photons you can achieve from your dyes. Bleaching only takes place in the excitation volume, but here it's more severe than with 1PE excitation.
- The repetition rate of the fs - lasers are 80 MHz, which in turn give you only an observation time of 12.5 ns in the life time of the dyes. This is too short for most of the dyes. With pulse pickers the repetition rate can be reduced. However, they work best in reducing the rep. rate with a factor of ~20 leading in turn to too low repetition rates.

Important for the Pulse picker is how efficient it can suppress adjacent pulses. Typically a suppression of far more than 2 orders of magnitude is needed.

Beware, that recording the [IRF](#) for TPE is not trivial.

Laser Safety

Lasers used for 2 Photon Excitation are usually laser class IV. Laser Class IV means: Hazardous to eye and skin from direct viewing AND diffuse reflection. The conditions under which these laser are used, the level of safety training of individuals using these lasers, and other environmental and personnel factors are important considerations in determining the full extent of laser safety control measures. These considerations require informed judgments to be made by a Laser Safety Officer (LSO). The LSO ensures that all employees who operate, maintain, or service laser products are properly trained.

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