Confocal Application Letter No.14

Two-Photon-Microscopy

Comparison of femtosecond and picosecond technology

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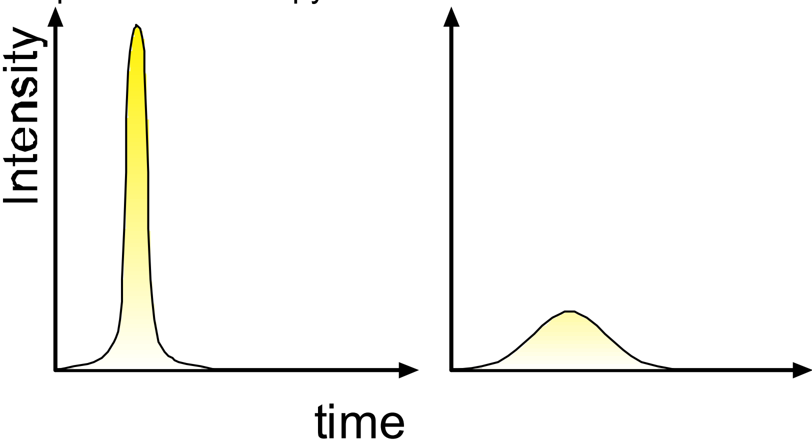
Femtosecond versus Picosecond Laser Pulses:

Picosecond pulses differ from femtosecond pulses in pulse duration and peak power.

The physical differences between femtosecond and picosecond laser pulses in Multiphoton microscopy can be visualized as in the following sketch.



The main differences betweeen femtosecond and picosecond pulses lie in pulse length and power considerations. It is important to consider both average and peak power. Typical values required to obtain equal excitation rates are listed in the table below. (Data relates to Ti-Sapphire systems with a repetition rate of 80 MHz, since all commercial two photon scanning laser microscopes are based on the Ti-Sapphire laser.) As the pulses are shortened, the peak power is increased, however the average power from the laser is also reduced.

Another important point to consider is that the optics in a microscope cause substantial pulse broadening in femtosecond pulses. The exact amount depends on the system as well as the objective used, however it is not unreasonable to expect to see a typical 100 fs pulse (with direct coupling) broaden to 350 fs, while a 1.2 ps pulse (with fiber coupling) might only broaden to 1.3ps.

	Picosecond	**Femtosecond**
Pulseduration	1 ps (=1000 fs)	100 fs
Peak Intensity	800 GW/cm²	2.5 TW/cm²
Average Power	130 mW	40mW

Data taken from Koester, et al.

Fluorescence intensity and cell damage:

Both picosecond and femtosecond pulses show the same fluorescence efficiency for non-damaging average power values

Recent studies have shown that the two photon fluorescence efficiency and cell damage follow the same relationship with respect to power and pulselength ($\sim P^2/\tau$). For example König et al. proved that both picosecond as well as femtosecond pulses provide approximately the same relative optical window for safe two-photon fluorescence microscopy. The table below shows the relationship between the onset of irreversible cell damage and average

power for various pulse lengths.

Similar results were found by Koester, et al. It was shown that for a given photodamage rate, femtosecond pulses involved a higher peak power, but a lower average power, while the picosecond pulses involved a lower peak power and a higher average power; thus (for low excitations) the photodamage is just proportional to the two-photon fluorescence rate.

Pulse duration (λ=780 nm)	**Maximum applicable average power without cell damage[1]**	**Achieved relative fluorescence intensity[2] using maximum average power**
170 fs	3 mW ± 0.5 mW	68% … 136%
242 fs	4.3 mW ± 0.5 mW	112% … 178%
568 fs	6.6 mW ± 0.7 mW	115% … 177%
1.4 ps	9 mW ± 1mW	86% … 135%
2.2 ps	12 mW ± 1.2 mW	100% … 150%

Data taken from: K.König et al; Pulse length dependence of cellular response to intense near-infrared laser pulses in multiphoton microsopces, Optics letters, January 15, 1999 / Vol. 24, No.2

Penetration depth:

The depth at which the optical intensity drops to 1/e or about 1/3 of its initial value. It is equal to $1/\alpha$ (α = absorption coefficient).

The penetration depth is a material characteristic that is related to the wavelength of light and the material in question, and is not in any way influenced by the pulse length or peak power. (Recall however that very high peak powers can cause optical breakdown, causing irreversible cell damage!)

A more interesting question is the depth at which sufficient fluorescence is observed to produce an image of good quality. Test are underway to experimentally compare the depths at which good quality images can be obtained for femtosecond and picosecond pulses. It is also important to note that the laser output from the optical fiber in Leica systems is 500-600 mW, while in Zeiss sytems it is approximately 50mW.

[1] Measured cell damage parameter: cloning efficiency of CHO cells reduced to 50%.
[2] Reference intensity is the fluorescence intensity at 170 ps and 3mW average power

Resolution power:

The resolution power depends only upon the wavelength and numerical aperture.

In two photon microscopy, as in standard one photon confocal microscopy, the resolution depends only on the wavelength and the numerical aperture! Thus multiphoton microscopy produces no improvement in resolution. The table below lists the lateral resolution for typical wavelengths in the UV, visible, and IR range.

Wavelength	Lateral resolution
350 nm	150 nm
600 nm	257 nm
850 nm	364 nm

Data calculated using the formula: respower = 0.6*Wavelength/numerical aperture. NA = 1.4.

Main field of application

Live cell imaging, signal transduction studies, Ion concentration measurements, thick tissue imaging.