Why enable 64 simultaneous scanning laser beams higher frame rates than a single one?

For most 2-photon applications the maximum frame rate is limited by the amount of photons emitted by the excited molecules – in other words: if the dwell time for each pixel is very short the image will be too dark.

To overcome this problem the averaged laser power E_{av} may be increased as the measured averaged photon flux $F(t)_{av,2\text{-}photon}$ *[Xu and Webb, 1996]*

$$F(t)_{av,2-photon} \approx \frac{E(t)_{av}^2}{\lambda} \frac{g_{laser}}{f_{laser}\tau_{laser}}$$

λ is the laser wave length
τ_{laser} is the pulse-length of the laser
f_{laser} is the laser repetition rate
g_{laser} is pulse shape of laser

is proportional to the square of the averaged power E_{av} – on the other hand the non-linear photo damage $N_{damage,2\text{-}photon}$ depends stronger on the laser power *[Hopt and Neher, 2001]*.

$$N_{damage,2-Photon} \approx E_{av}^{2.5}$$

As the induced damage rises faster with increasing laser power than the number of excited molecules, it does not make sense to increase the power over a critical level. General experience shows that in 2-photon microscopy the damaging/bleaching rate is almost negligible and much lower than in single photon microscopy if the laser power is kept below the critical level. This level depends strongly on the sample and ranges typically between 2 and 10 mW laser power in the microscope focus. This behaviour seems to be quite non linear *[Denk et al., 1990; Williams et al., 1994]*.

At typical wavelengths for 2- and 3-photon processes, the main reason for cell damage *[Koester et al., 1999]* is the excitation process itself and not the generation of heat that is caused by single photon absorption *[Schönle and Hell, 1998; König et al., 1996]*.

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The only way to increase the number of fluorescence photons per time unit without raising photo damage is to parallelize the excitation process. Thereby the overall excitation laser power is increased without increasing the power within a single focus. Simultaneous excitation with N foci results in N times more excited molecules. Thus the sample emits more fluorescence light in the same time interval - even with low laser power in each focus. For this reason LaVision BioTec developed the ***TriM Scope*** that scans the specimen with up to 64 laser beams simultaneously.

This unique technique delivers up to 64 times more light in the same time unit compared to single beam scanning devices! As a consequence the TriM Scope enables high frame rates in combination with a low laser power in each focus.

⇒ *TriM Scope* ≡ Highest frame rates in combination with low photo-damage!

Literature

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