# FemtoLux 3 laser for the rapid wide-field second harmonic generation microscopy

*Application notes*

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FemtoLux 3 laser was used as an illumination source in the wide-field second harmonic generation (SHG) microscope. Relatively high laser pulse energy at a medium pulse repetition frequency allowed for a faster single image acquisition compared to that using laser-scanning. It was also possible to acquire images of relatively large sample areas, which paved the way for the rapid imaging of macroscopic sample areas with microscopic resolution.



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## Considerations for the optical path for the high energy laser pulses

The optical scheme of the wide-field SHG microscope in presented in *Figure 1*. To illuminate the sample in the wide-field, the expanded, collimated laser beam is focused onto the back focal plane of the objective. To introduce the optical sectioning by exploiting temporal focusing [1)], the laser beam is additionally dispersed by a diffraction grating. This spreads the different spectral components at different angles, they are focused in the back focal plane of the objective into a segment rather than a point, and they also emerge from the objective at different angles. Therefore, the side effect of using the scheme of the temporal focusing is the possibility of employing high laser pulse energy and high average power without damaging the objectives used for the excitation and signal collection.

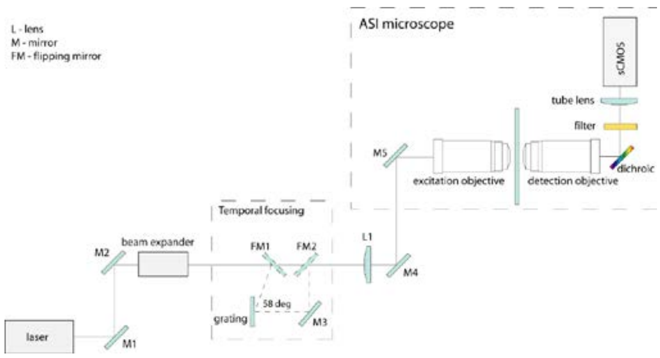


*Figure 1.* The optical scheme of the wide-field SHG microscopy setup.

## The utility of the high energy laser pulses for the wide-field imaging

The number of SH photons generated per unit time from a unit area can be expressed as:

$$N = k \cdot \chi^2 \left(\frac{E}{S \cdot \tau}\right)^2 \tau \cdot f \quad (1)$$

here, k is the proportionality coefficient, χ - second order effective surface susceptibility, E – laser pulse energy, S – illuminated area, τ – laser pulse duration, f – laser pulse repetition frequency [2)].

The pixel brightness (number of photons) is $B = N \cdot t \cdot S_p$, here t is the integration time, $S_p$ – pixel area. The image acquisition time in the wide-field is simply the integration time: $T_{wf} = t_{wf}$. For the laser-scanning it is $T_s = t_s \cdot S/S_p$. Assuming equal pixel size, it is straightforward to derive from (1) the ratio of the acquisition times of images of the same brightness in the wide-field and laser-scanning configurations:

$$\frac{T_s}{T_{wf}} = \frac{1}{n} \cdot \left(\frac{E_{wf}}{E_s}\right)^2 \cdot \frac{\tau_s}{\tau_{wf}} \cdot \frac{f_{wf}}{f_s} \quad (2)$$

here, s subscript denotes laser scanning, wf – wide-field, T is image acquisition time, E – laser pulse energy, τ – laser pulse duration, f – laser pulse repetition frequency, n – number of pixels in an image.

Laser-scanning imaging is customarily performed with a relatively low pulse energy at high pulse repetition frequency. Typical values are 0.1 nJ and 90 MHz, respectively [3)]. On the other hand, FemtoLux 3 laser employed in the wide-field setup features 3 μJ of pulse energy at 1 MHz pulse repetition frequency. Assuming for simplicity equal pulse durations, inserting these values together with a typical image size of 512×512 pixels yields ~40x advantage of the wide-field versus laser scanning in terms of speed of image collection.

Since the ratio of the acquisition times depends quadratically on the ratio of the pulse energies, high laser pulse energy is essential. The point can be further strengthened by computing the time ratio with the full pulse energy of the high repetition rate laser in place of $E_{wf}$. With 3 W average power the pulse energy is ~30 nJ. Inserting this into (2) yields $T_s/T_{wf} = 0.34$ – with low laser pulse energy the wide-field acquisition not only is not advantageous but is slower than that with the laser-scanning.

Since the SHG intensity depends quadratically on the excitation fluence, E/S, high laser pulse energy is also required in order to be able to reach sufficient fluence for SHG over a larger sample area, which is required in combination with a fast mechanical XY scanning for obtaining tiled images of arbitrarily large sample areas. The minimum time of such an acquisition is an optimum between the size of a single image determining the required integration time and the scanning time determined by the number of the scanning steps. The scanning time dependence on a single image size was measured, whereas the required single image integration time was calculated based on the integration time of an image of a chosen size and satisfactory intensity. For the minimum total acquisition time the image had to be 450×450 µm² large and acquired with 0.15 s integration time (*Figure 2*), which is an exposure time sufficiently long for a regular scientific CCD or CMOS camera. Overall, large sample areas could be imaged with a rate of 1 mm² in as little as 2 s on average.

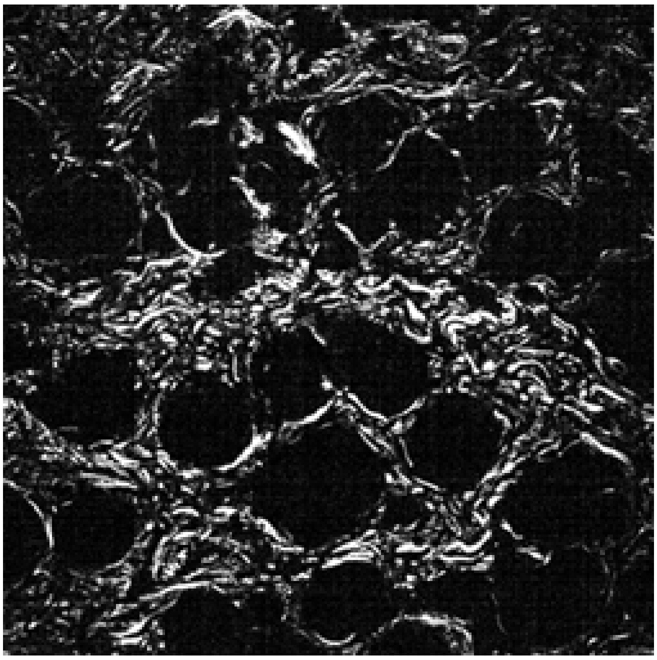

*Figure 2.* SHG image of colagen structures of 450×450 µm² area of a mouse skin sample. The integration time was 0.1 s

## References


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