Supplemental Document

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Multicolor two-photon light-patterning microscope exploiting the spatio-temporal properties of a fiber bundle: supplement

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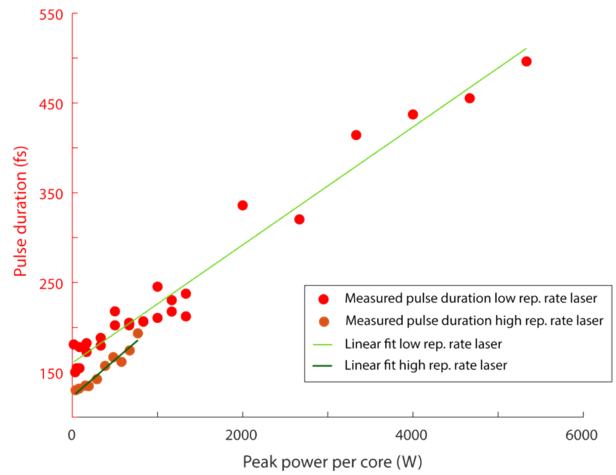
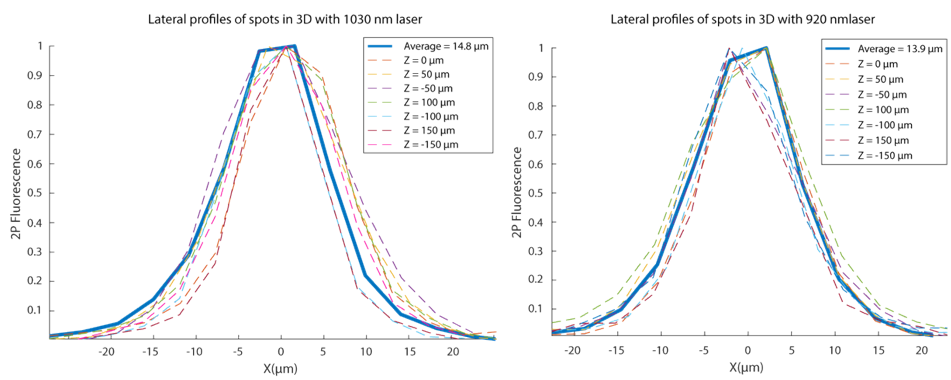
Supplementary Figure 1



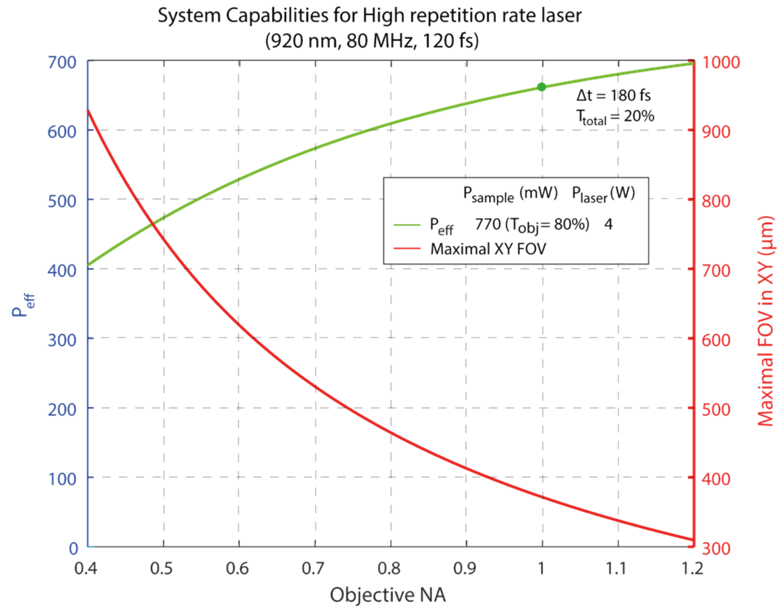
Supp. Fig. 1: Dependence of the pulse broadening when using high peak power per core. Measured pulse durations for both lasers (red dots for 1030 nm laser and orange dots for the 920 nm laser) when using different peak power per core (P_{peak}). Linear fit for the measurements of each laser ($\tau = m \cdot P_{peak} + \tau_0$) shows a similar dependence on the pulse broadening with the peak powers (m = 66 fs/kW for low repetition rate laser, light green curve and m = 81 fs/kW for high repetition rate laser, dark green curve). The use of a low repetition rate laser presents a higher peak power per core compared to the use of a high repetition rate laser.

Supplementary Figure 2



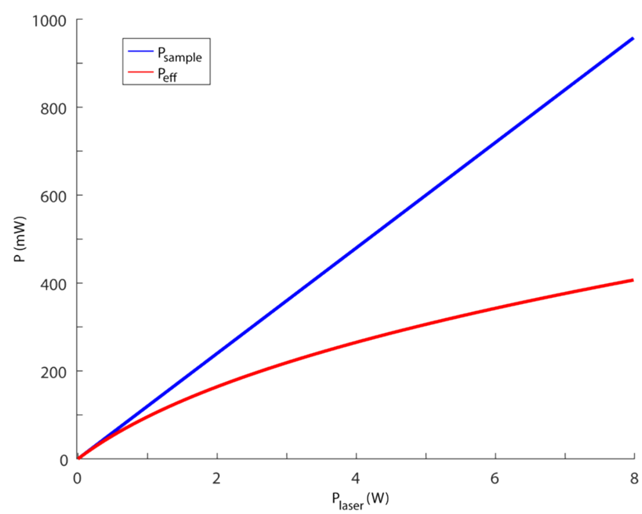
Supp. Fig. 2: Lateral profile of multiple spots multiplexed in a 3D volume with the SLM through a fiber bundle. Characterization of the lateral profiles for different spots created with the laser at 1030 nm (left) and the laser at 920 nm (right) at different depths (Z positions) throughout the entire FOV in Fig. 5. The average lateral profile for these spots is shown in solid blue line with FWHM = 14.8 µm (for the 1030 nm laser) and FWHM = 13.9 µm (for the 920 nm laser).

Supplementary Figure 3



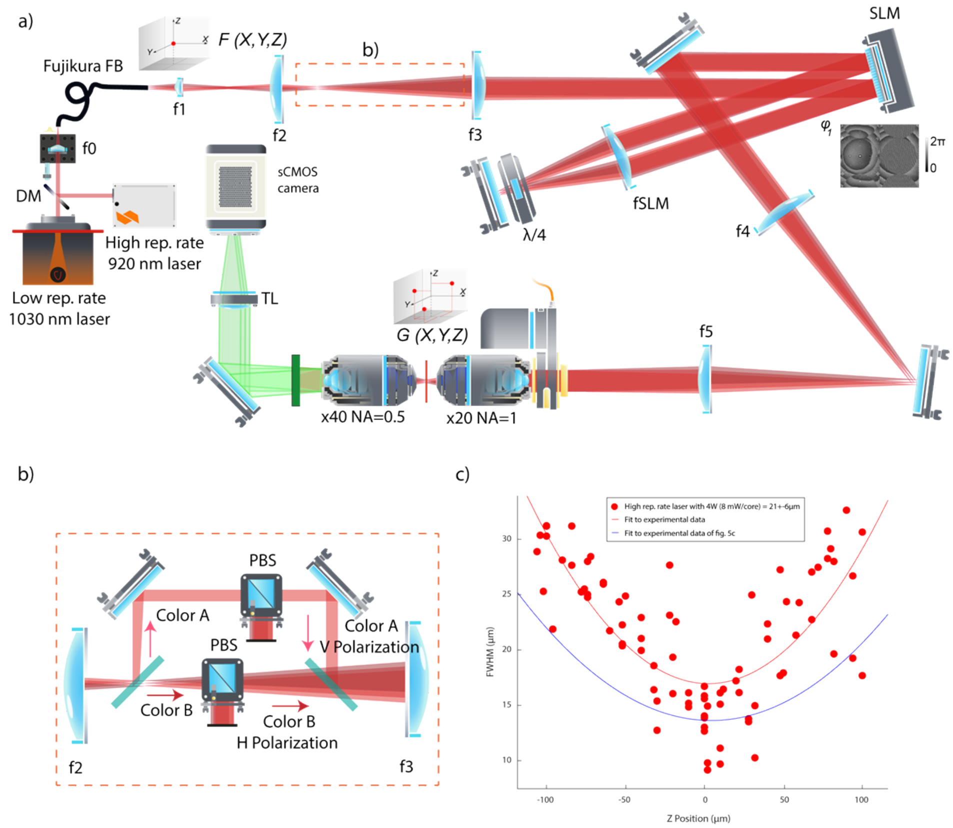
Supp. Fig. 3: Systems capabilities and comparisons for different objective NAs. Left vertical axis (green curve): Calculated P_{eff} at the sample plane considering an incident power of 4 W and an 80% transmission objective as a function of the effective objective NA, for an incident wavelength of 920 nm at a repetition rate of 80 MHz. Right vertical axis (red curve): calculated 2D FOV for different effective objective NAs using a SLM of 1272x1024 pixels.

Supplementary Figure 4



Supp. Fig. 4: Dependence between P_{eff} and P_{sample} with P_{laser}. Due to pulse broadening when using high power per core, the 2PE becomes less efficient so that a different factor of dependence is obtained between P_{sample} and P_{laser} (curve in blue) compared to P_{eff} and P_{laser} (curve in red). Even though, it is shown that is advantageous to use the maximal laser power to maximize P_{eff}.

Supplementary Figure 5



Supp. Fig. 5: Second approach of experimental setup for a multicolour 3D light-patterning 2P microscope. a) The lasers illuminate the fiber bundle as in Fig. 1. In this configuration, a telescope is used to match the beam with one half (right side) of the SLM (f2 = 300 mm and f3 = 600 mm). We use a 4f 1:1 system (two times fSLM = 500 mm) with a quarter-wave plate λ/4 (AQWP10M-1030, Thorlabs) to rotate 90 degrees the light polarization and illuminate the other half of the SLM (left side). Finally, another telescope is used to match the size of the objective pupil (x20 NA = 1) with lenses (f4 = 400 mm and f5 = 1000 mm). **b)** Inset of a) to perform dual color 3D light targeting simultaneously by separating the two colors (color A and B) to polarized them with vertical (V) and horizontal (H) linear polarizations and recombining them to illuminate the SLM, which displays a single-phase mask. **c)** FWHM of the axial profiles of the distributed in a 3D FOV of 210x210x200 μm^3 in function of the depth position (z) for spots in random (x, y) positions when using 4W to illuminate the fiber bundle (8 mW/core). Red: quadratic fit of the measured resolutions and in blue of the measured FWHMs in Fig. 5c.

Supplementary Table 1

(1) Opsins/ indicators	(2) Laser (f_{REF},τ_{REF},λ_{REF}) reference	(3) Power per cell (P_{cell}^{REF}), reference	(4) Laser (f, τ , λ) current system	(5) Power per cell current system (P_{cell})	(6) Number of cells (N^{cell})
ChRmine[31]	2 MHz, 350 fs, 1030 nm	10 mW	10 MHz, 150 fs, 1030 nm	14.6 mW	31
ChroMe[31]	2 MHz, 350 fs, 1030 nm	25 mW	10 MHz, 150 fs, 1030 nm	36.5 mW	12
WicHR[63]	0.5 MHz, 300 fs, 1030 nm	7-8 mW	10 MHz, 150 fs, 1030 nm	22-25 mW	18-20
JEDI-2P[35]	0.25 MHz, 100 fs, 940 nm	2.5-5 mW	10 MHz, 150 fs, 1030 nm	19-38 mW	12-24
GCaMP6[33,34]	80 MHZ, 140 fs, 920 nm	5-30 mW	80 MHz, 120 fs, 920 nm	4.6-27.8 mW	24-143

Supp. table 1. Estimation of the number of cells that could be targeted with the current system, based on previously published works. Column (1) lists the opsins and indicators considered, together with the reference to the corresponding works. Column (2) gives the properties of the lasers used in the reference works, particularly the pulse duration τ_{REF}, the repetition rate f_{REF} and the central wavelength λ_{REF}. Column (3) indicates the power per cell at the sample plane used in the reference works (P_{cell}^{REF}). Column (4) lists the parameters of the lasers used in the current manuscript. To match the excitation wavelengths of the reference works we have considered to use the 1030 nm laser with effective pulse duration of $\tau = 150$fs, and repetition rate $f = 10$ MHz for the first 4 lines in the table, and the 920 nm laser with effective pulse duration of $\tau = 120$fs, and repetition rate $f = 80$ MHz for the last line. In column (5) we estimate the power per cell that we would need with our 1030 nm laser for the first 4 lines and 920 nm laser for the last line to produce the same 2P excitation as with the powers per cell and lasers parameters listed in column (2) and (3). This can be derived as follows: $P_{cell} = P_{cell}^{REF} \cdot \sqrt{\frac{\tau \cdot f}{\tau_{REF} \cdot f_{REF}}}$. In column (6) we give the total number of cells N^{cell} that could be simultaneously targeted with our system when considering an 80% transmission excitation objective (NA = 0.8) and using a total effective power at the sample plane $P_{eff} \approx$ 450 mW in the case of the first 4 lines (related to Fig. 6) and $P_{eff} \approx$ 660 mW in the case of the last line (related to Supp. Fig. 3). This number can be derived as follows:

$$N^{cell} = \frac{P_{eff}}{P_{cell}} = \frac{P_{eff}}{P_{cell}^{REF}} \sqrt{\frac{\tau_{REF}\, f_{REF}}{\tau\, f}}$$