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Supporting Material

Laser Photoablation of Guidance Microchannels into Hydrogels Directs Cell Growth in Three Dimensions

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SUPPLEMENTARY DATA

Focal Volume Dimensions Characterization

In the femtosecond system, the focal volume dimensions were assessed using Gaussian curve fitting to 3-D two-photon microscope fluorescence images of 0.051 μm fluorescent microsphere standards (TetraSpeck). The images were taken using the same setting used for photo-ablation. Two-photon fluorescence is proportional to the square of the irradiation intensity, and thus follows a Gaussian-Lorenzian distribution[1], which can in this case be approximated by an ellipsoid Gaussian. The fitted equation therefore takes the form: $Fluorescence\ Intensity \propto I^2(r,z) = I_0^2 e^{-4r^2/w_r^2} e^{-4z^2/w_z^2}$, where I_0 is the light intensity at the center of the beam (at the waist), r and z are the lateral and axial coordinates respectively, and w_r and w_z are the standard deviation values of the theoretical curve. The curve-fitting was done using MATLAB™ to minimize mean squared error. The focal volume dimensions were approximated as the full width at half maximum values (FWHM) of the fitted curves: $FWHM = \omega\sqrt{\ln 2}$, where ω is w_r or w_z, according to the relevant axis.

In the nanosecond system, for the lateral plane, line-scan fluorescence imaging of 0.5 μm fluorescent microsphere standards (TetraSpeck) was achieved by exposing the microspheres to the moving UV beam in an otherwise dark environment, taking a series of images of the light emitted by the microspheres. A data curve of fluorescence intensity values was built based on the matching stage location for each image. For the axial coordinate the same imaging method was used, positioning the stage in known heights to take data from a thin layer (~2.2 μm height) of Fluorescein-Sodium (Fluka). Since single-photon induced fluorescence is proportional to the light intensity, it follows a Gaussian distribution curve of the form $I(r,z) = I_0 e^{-2r^2/w_r^2} e^{-2z^2/w_z^2}$, where $FWHM = \omega\sqrt{2\ln 2}$ and ω is w_r or w_z, according to the relevant axis.

Peak Light Intensity Values

Peak power values were calculated from measurements of average power values at the exit from the respective system's objective, factoring in the laser system parameters. The peak light intensity value of a focused Gaussian laser beam can be derived from the peak power values (P_0) using $I_0 = \frac{2P_0}{\pi\omega^2}$, where ω is w_r of the respective system.

Difference of Gaussians (DOG) Curve Fitting

Specimens of Hydrogel B were inscribed with a set of lines using the specified energy levels, and imaged by phase contrast microscopy. The analysis of the phase-contrast image of each micro-channel was performed using MATLAB™, where for each micro-channel the intensity values were averaged in parallel to the channel for 5 segments (at different regions along the length), the intensity distributions on the paths perpendicular to the segments were recorded, and a DOG curve fit was performed on the average intensity distribution. The DOG curve describes the

[1] Brown, E. B., Webb, W. W. & Gerard, M. (1998) *Methods in Enzymology* **291,** 356-380

difference of two Gaussian equations (described above) with a common center. The final equation thus takes the form: $Intensity\ value = dc + a_1 e^{-(x-\mu)^2/2\sigma_1^2} - a_2 e^{-(x-\mu)^2/2\sigma_2^2}$, where dc denotes the general base-line in the picture, a_1 and a_2 are the Gaussian curves' amplitudes, x is the relevant coordinate, μ the shift of the Gaussian's center, and σ denotes the standard deviation of the Gaussian. The results yielded the averaged peak intensity value (± standard deviation). The ablation threshold values were determined from the results, as the energies leading to an intensity of more than 10% of the highest (saturation) value.