Supplementary Information

Morphology characterization of RITC and Cy7-containing microcapsules

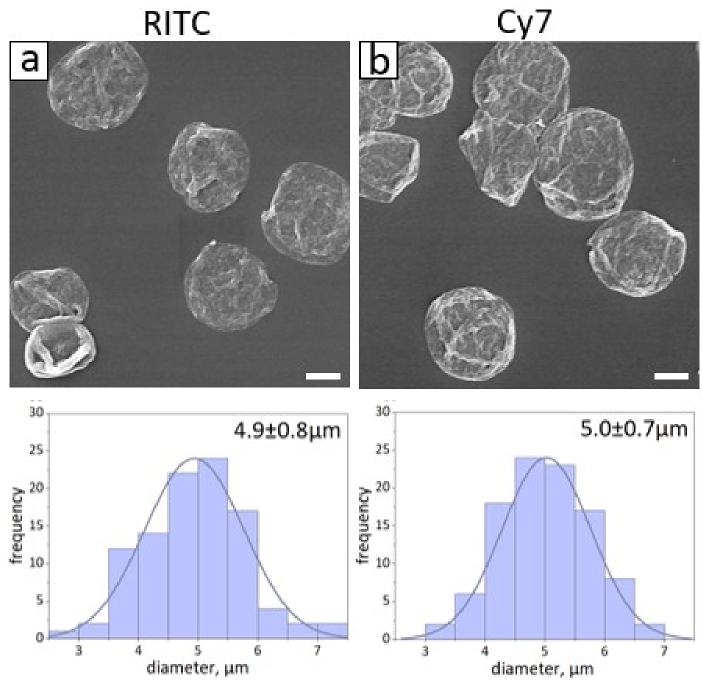

Fig. S1. SEM images and histograms of the size distribution of microcapsules with (a) RITC and (b) Cy7. The scale bar is 2 µm.

Absorbance spectra changes of microcapsules upon ultraviolet irradiation

Absorbance of microcapsules after photoconversion cannot be detected with confocal microscope. Therefore, to analyze the changes in capsules absorption, the photoconversion procedure was imitated. For this, suspension of microcapsules was irradiated with ultraviolet lamp for 30 and 60 min (Fig. S2)

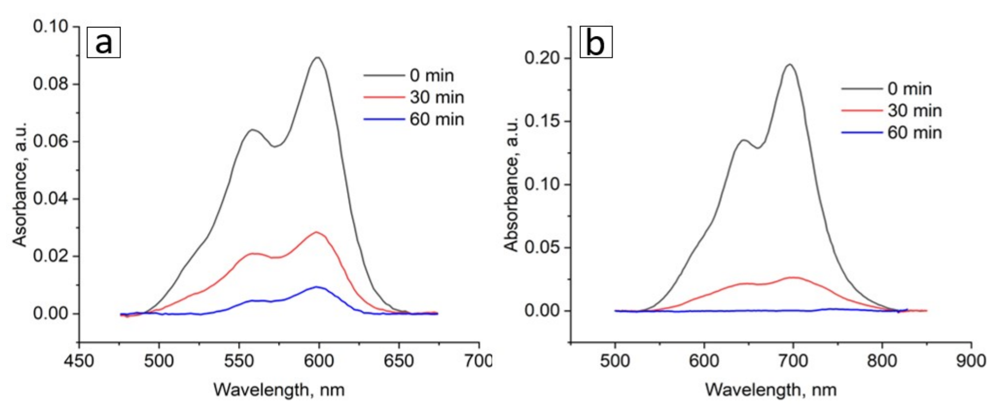

Fig. S2. Absorbance spectra changes of microcapsules containing (a) Cy3.5 and (b) Cy5.5.

It can be noted that the intensity of fluorescence reduces and peak shape changes, indicating the changes in chemical structure of the sample.

Photoconversion of encapsulated Cy7

CLSM images of microcapsules with encapsulated Cy7 before and after various combinations of LDPs and the duration of one pixel irradiation are presented in Figure S3a. Encapsulated Cy7 was excited with a 671 nm laser and the emission range was 686–790 nm. Photoconversion was performed with a 671 nm laser. The fluorescence signal after photoconversion was excited with the laser 488 nm and the emission range was 505–540 nm.

From the CLSM images, the photoconversion effect of microcapsules containing Cy7 was not as profound as in the case of Cy3.5, Cy5.5, or RITC. Indeed, the calculation of the microcapsule's fluorescence intensity revealed low values of the signal (less than 5 a.u. in comparison to 20-30 a.u. for Cy3.5 and Cy5.5) (Figure 2e-g and 3e-g). Nevertheless, the graph revealed a small trend of intensity increase upon irradiation. The decline of the fluorescence intensity at 0.25 ms indicates the bleaching of the dye due to high LPD and longer one pixel irradiation duration. The intensity of microcapsules in the NIR channel is reduced, most probably due to both photobleaching and photoconversion (Figure S3c). A similar effect of simultaneous Cy7 bleaching and photoconversion was observed earlier [1]. Cells were irradiated with a 640 nm laser at a low LPD of 0.1 kW/cm^2. That led to the appearance of the photoconverted signal in the red channel. Since in the current study, the signal is absent in the red channel and was observed only in the green channel, the removal of two C_2H_2 groups happened, forming the Cy3 product [1].

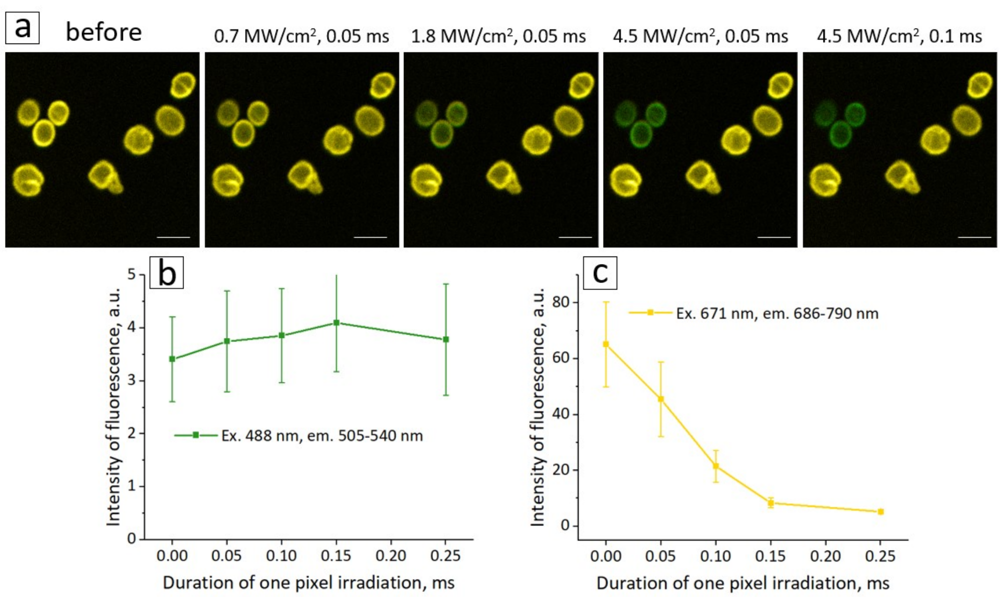

Fig. S3. (a) CLSM images of microcapsules with encapsulated Cy7 before and after photoconversion depending on the duration of one pixel irradiation at LPD of 0.7, 1.8, and 4.5 MW/cm^2. The scale bar is 5 µm. Changes in microcapsule fluorescence intensity depending on the duration of one pixel irradiation in the (b) green and (c) NIR channels. Each irradiation time point corresponds to its CLSM image and LPD of 0.7, 1.8, and 4.5 MW/cm^2.

Thermal treatment of Cy3.5, Cy5.5, and Cy7 conjugated with PAH

It is known that some dyes do not withstand thermal treatment, since high temperature might change their chemical structure [2]. To analyze if the chosen cyanine dyes could withstand thermal treatment, the solutions of the PAH conjugated with the dyes Cy3.5, Cy5.5, and Cy7 were thermally treated according to the procedure described earlier for photoconvertible microcapsules with RhB (3 h, 180 ∘C) [3]. Fluorescence intensities of the labeled PAH-dye solutions were recorded before and after the procedure (Figure S4).

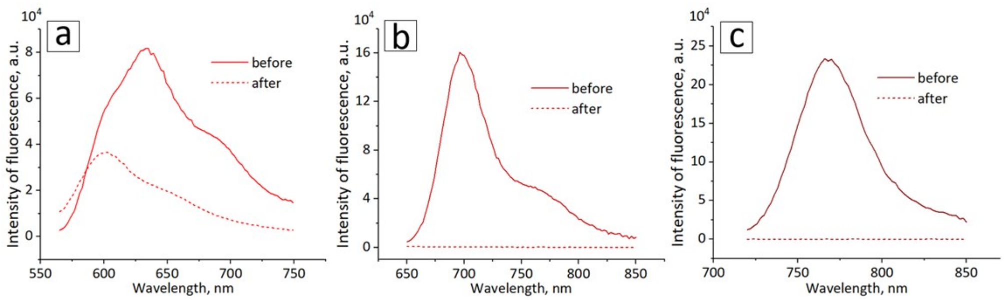

Fig. S4. Fluorescence intensities of the (a) PAH-Cy3.5, (b) PAH-Cy5.5, and (c) PAH-Cy7 aqueous solutions before and after thermal treatment.

According to the obtained spectra, fluorescence intensities of the PAH-Cy5.5 and PAH-Cy7 decreased dramatically to zero values after the thermal treatment. It means that the dyes did not withstand the high temperature and should be encapsulated without thermal treatment to preserve their fluorescent properties. As for PAH-Cy3.5, its intensity decreased around two times after thermal treatment and the peak of intensity shifted by 32 nm to the shorter wavelength. Such changes in the spectrum nature might be connected with changes in the chemical structure of the dye which could affect its ability to photoconversion [4]. Therefore, Cy3.5 dye should also be encapsulated without thermal treatment to preserve its fluorescence properties.

Photoconversion of encapsulated RITC

CLSM images of microcapsules with RITC before and after photoconversion are presented in Figure S5a.

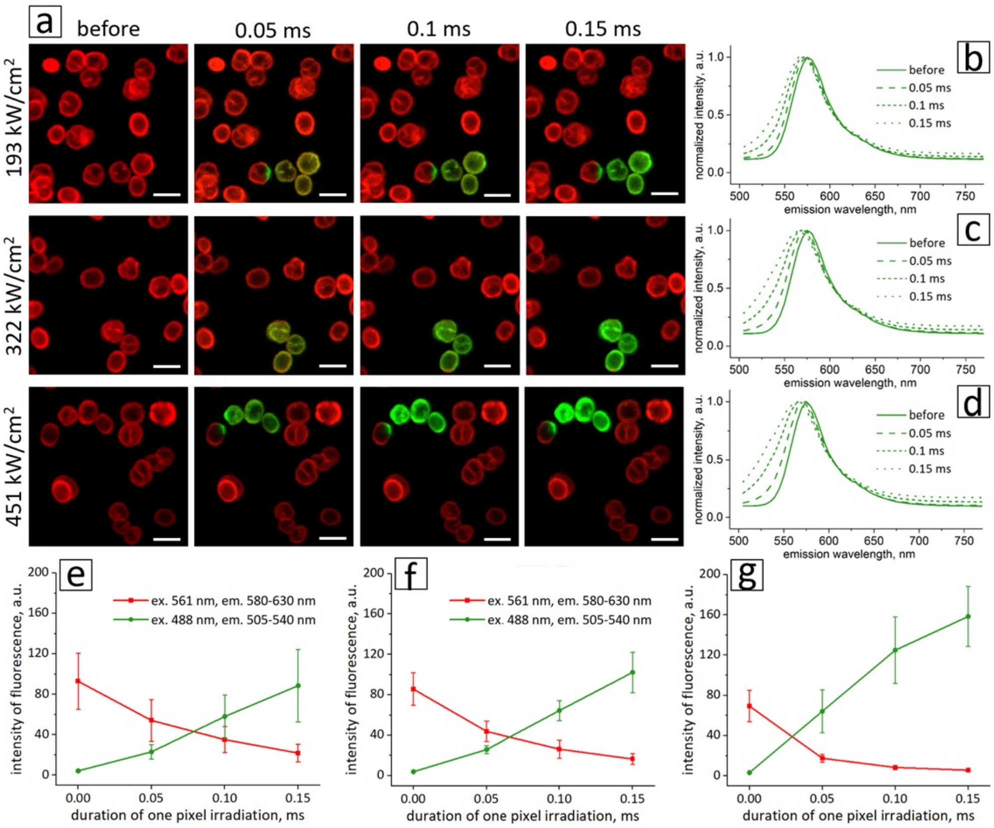

Fig. S5. (a) CLSM images of microcapsules with encapsulated RITC before and after photoconversion depending on the duration of one pixel irradiation at 193, 322, and 451 kW/cm^2 LPD. The scale bar is 5 μm. Lambda scans of encapsulated RITC before and after photoconversion depending on the duration of one pixel irradiation at (b) 193, (c) 322, and (d) 451 kW/cm^2 LPD. Corresponding changes of intensity with time under irradiation at (e) 193, (f) 322, and (g) 451 kW/cm^2 LPD.

Encapsulated RITC was excited with a 561 nm laser and the emission range was 580–630 nm. Photoconversion was performed with a 561 nm laser. The fluorescence signal after photoconversion was excited with the laser 488 nm and the emission range was 505–540 nm. To choose optimal photoconversion parameters, LPD and duration of one pixel irradiation were changed. The utilized LPDs were 193, 322, and 451 kW/cm^2. The duration of one pixel irradiation was changed from 0.05 to 0.15 ms. According to the obtained images, photoconversion of microcapsules containing RITC was achieved. Therefore, this effect is possible not only for free RhB dye, as was reported previously [5], but also for RITC chemically bound to the polymer.

Lambda scans of microcapsules are present in Figure S4b-d, demonstrating that the blue shift of the spectra increases with the duration of laser exposure. The rise of the spectra's left shoulder was also higher the bigger the utilized LPD. It indicates a greater impact of laser with higher intensity on photoconversion degree. Changes in fluorescence intensity of microcapsules in green and red channels are presented in Figure S5e-g. The pattern was similar in the case of 193 and 322 kW/cm^2 LPD: the continuous close-to-linear intensity rise in the green channel and decrease in the red channel with time was noticed. Notably, when 451 kW/cm^2 LPD was utilized, it gave almost two times higher increase in intensity in the green channel at 0.15 ms in comparison to other LPDs. Plato of minimal intensity in the red channel was reached at the 0.1 ms mark. Therefore, optimal photoconversion parameters for microcapsules containing RITC include 451 kW/cm^2 LPD, which was reported previously [3], and duration of one pixel irradiation 0.1 or 0.15 ms, depending on the desired brightness of the signal in the green channel.

References

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