Electronic Supplementary Material (ESI) for Nanoscale Advances.

Resolving the internal morphology of core-shell microgels with super-resolution fluorescence microscopy

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We investigate the internal morphology of smart core-shell microgels by super-resolution fluorescence microscopy exploiting a combination of 3D single molecule localization and structured illumination microscopy utilizing freely diffusing fluorescent dyes. This approach does not require any direct chemical labeling and does not perturb the network structure of these colloidal gels. Hence, it allows us to study the morphology of the particles with very high precision. We found that the structure of the core-forming seed particles is drastically changed by the second synthesis step necessary for making the shell, resulting in a core region with highly increased dye localization density. The present work shows that super-resolution microscopy has great potential with respect to the study of soft colloidal systems.

Electronic Supporting Information

S1 Atomic Force Microscopy

All measurements were performed on a DI Nanoscope IIIa (Digital Instruments, now Bruker, Germany) mounted on a Zeiss Axiovert 135 inverted microscope (Carl Zeiss Microscopy GmbH, Germany) in semi-contact mode using Budget Sensors (Innovative Solution Bulgaria Ltd., Bulgaria) Al-Reflex Tap300Al-G cantilevers with a tip radius of < 10 nm, a resonance frequency of about 300 kHz and a spring constant of 40 N/m at room temperature in the dried state. For the sample preparation a silicon wafer (Siegert Wafer GmbH, Germany) was spin-coated with a PEI solution (0.25 wt%, 0.1 mL) and of a diluted microgel suspension ($c = 0.1$ wt%). Prior to the PEI-coating the wafer was treated in a plasma cleaner (Zepto, Diener Electronics, Germany) and cleaned with ethanol (HPLC grade). The resulting images were treated with GWYDDION [1].

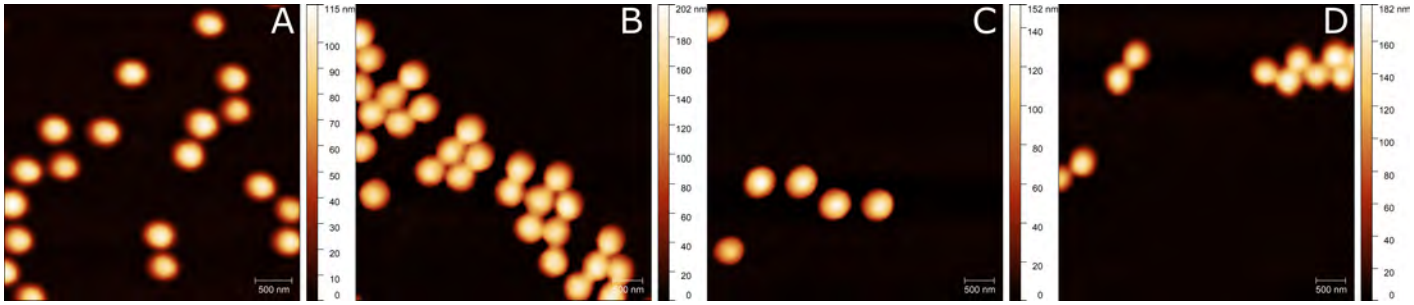

Fig. S1 Atomic force microscopy images of core microgels (A) and core-shell microgels with thin (B), intermediate (C) and thick (D) shells.

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S2 3D calibration and PSF shaping with ThunderSTORM and SMAP cspline implementation

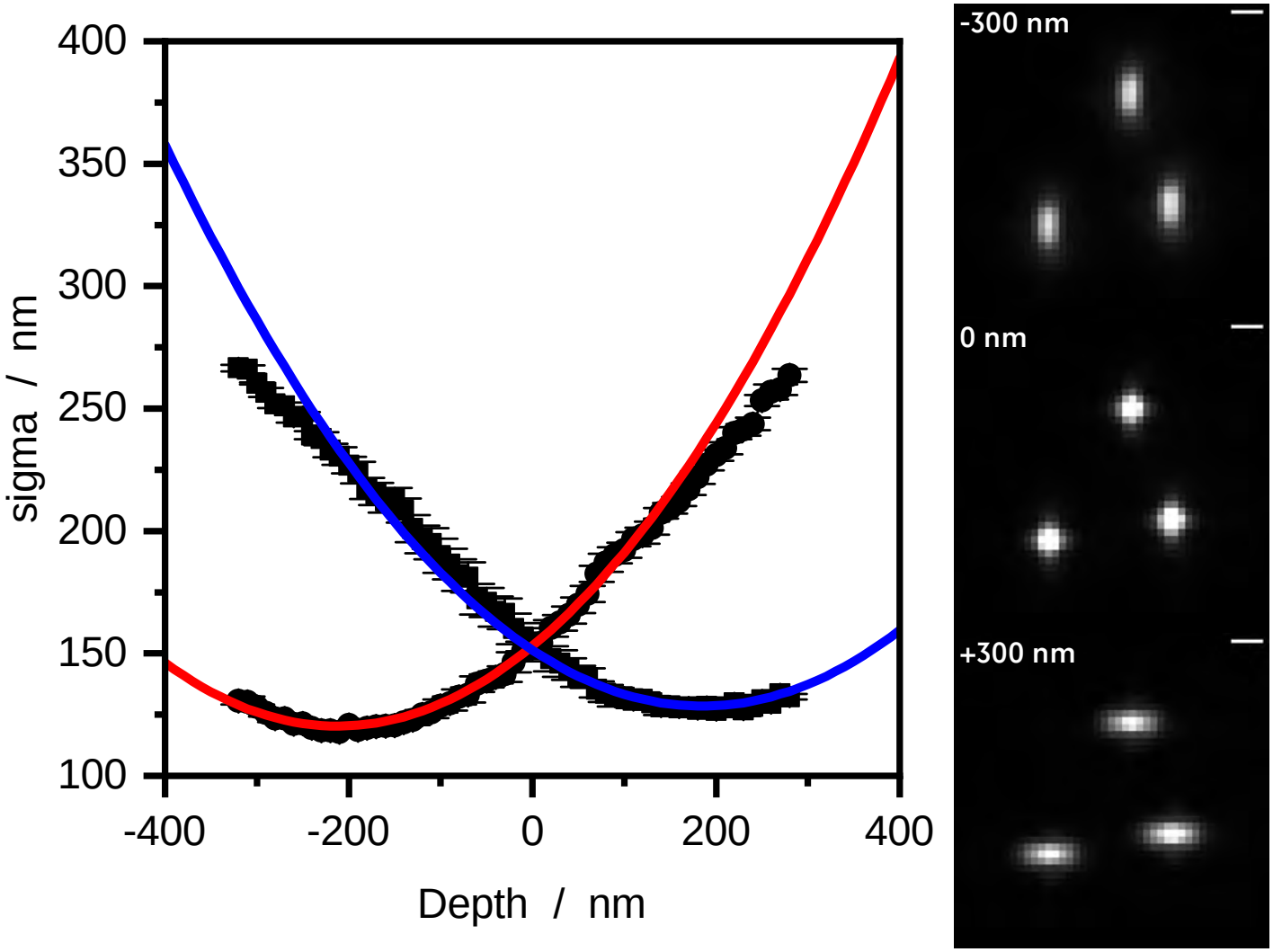

Fig. S2 3D calibration based on 200 nm TetraSpeck beads embedded in glycerol and analyzed with ThunderSTORM. The objective lens is moved by the PIFOC stage in 10 nm steps through the sample to obtain a height dependent PSF of the beads. The applied model (red and blue curve) deviates from the ThunderSTORM calibration. Therefore, a more sophisticated method, SMAP, is used for the 3D reconstruction (Fig. S3). Scale bar: 500 nm.

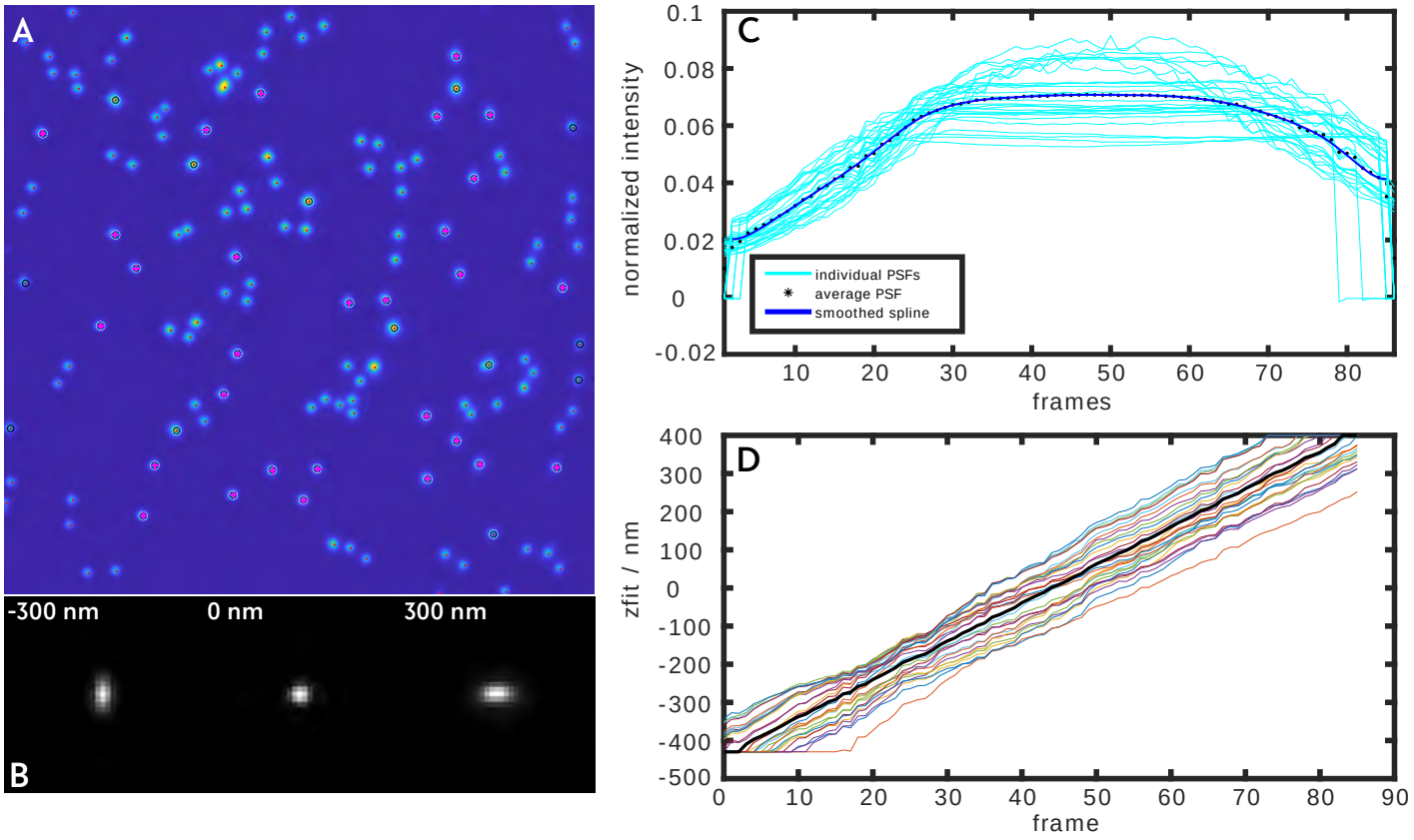

Fig. S3 To account for the deviation from the standard model for 3D imaging, the superresolution microscopy analysis platform (SMAP), a more advanced reconstruction software by Jonas Ries (EMBL), is used. The same data set is analyzed with this toolbox in combination with the fit3Dcspline algorithm[2]. In **A** the maximum z-projection of the single fluorescent beads is shown. From this image beads with a distance of 27 pixel to the next are selected for the 3D cspline calibration. From all matching fluorescent beads an averaged point spread function (PSF) is calculated (**B**) and a 3D calibration is generated by a cspline fit. Therefore, different views are generated. **C** visualizes the PSFs profile along the z-axis in the middle of the PSF at x=0 and y=0. The resulting height calibration from 37 single fluorescent beads is shown in **D**.

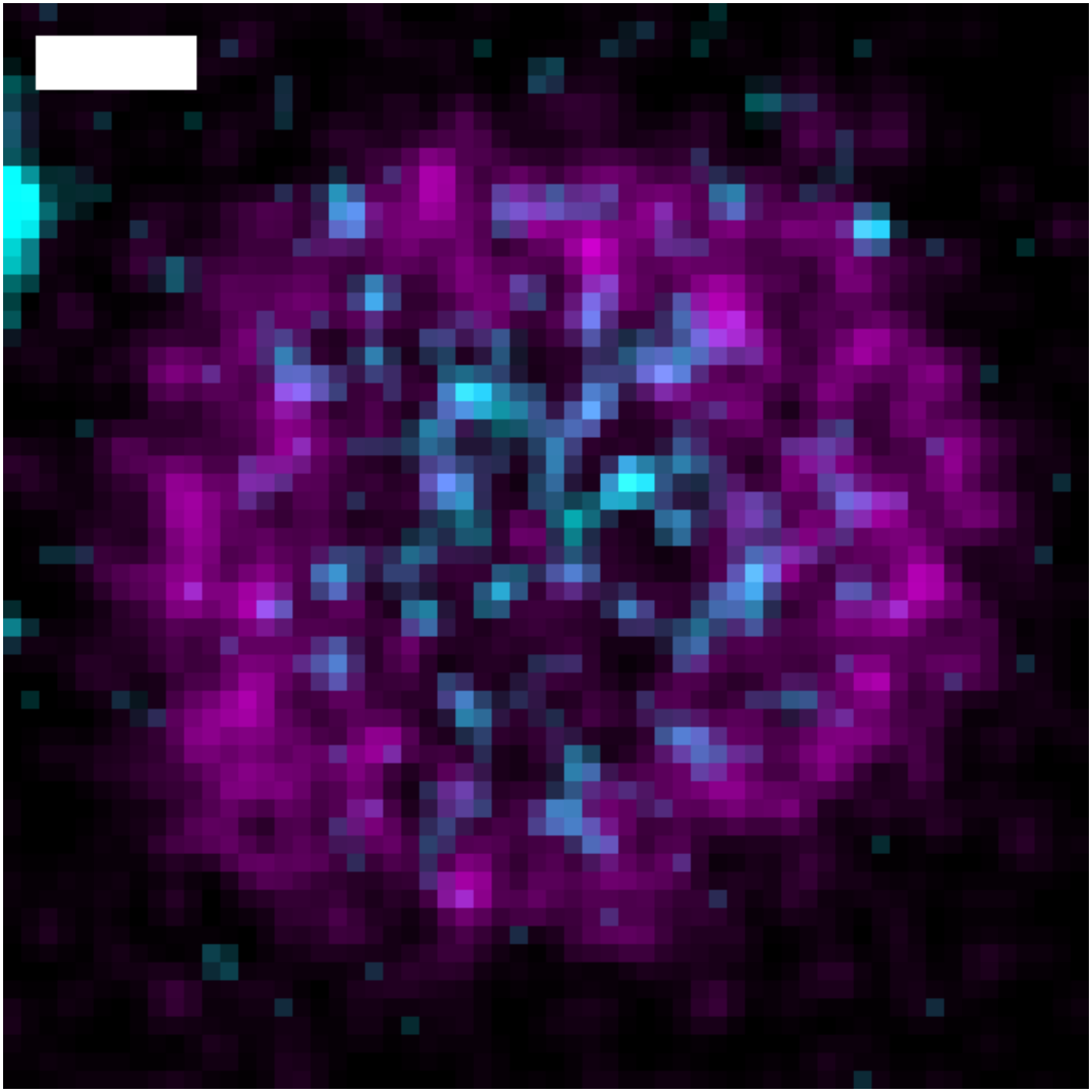

Fig. S4 Pseudo two color 2D representation of the core-shell microgel. Here, the localizations from a core microgel are represented with the cyan lookup table and the localizations from the core-shell microgel with the magenta lookup table. To obtain this image two single microgels (one core and one core-shell image) were cut out and placed matching over each other. This image is only for visualization purposes. Scale bar: 100 nm.

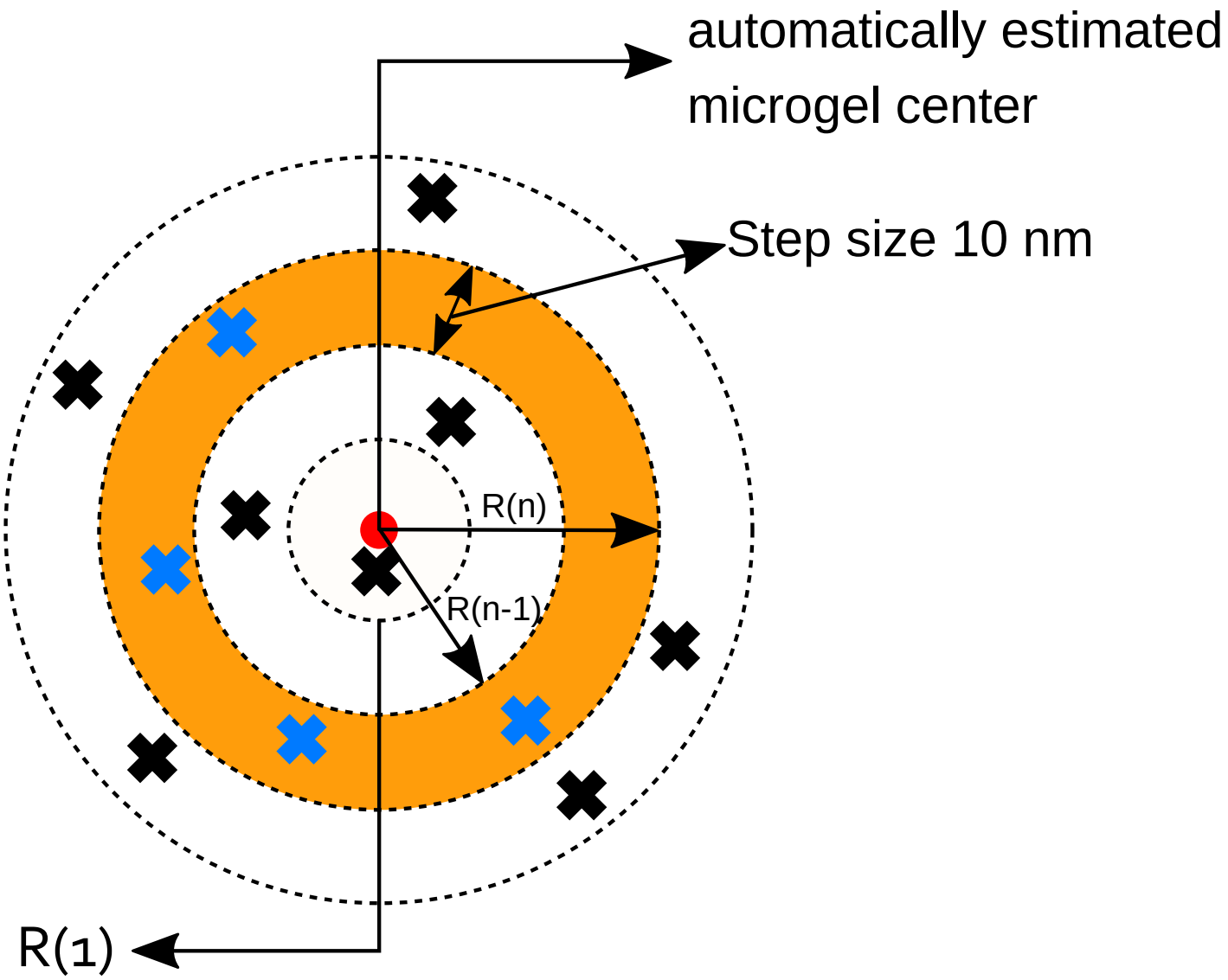

Fig. S5 Schematic drawing of estimating the localization density via counting the localizations within different rings with a thickness of 10 nm from single microgels.The microgel center is estimated with the imfindcircle function in Matlab which gives the center position and the radius of the detected circle. This function is applied to the superresolved image and the localization counting is done based on the obtained localization table.

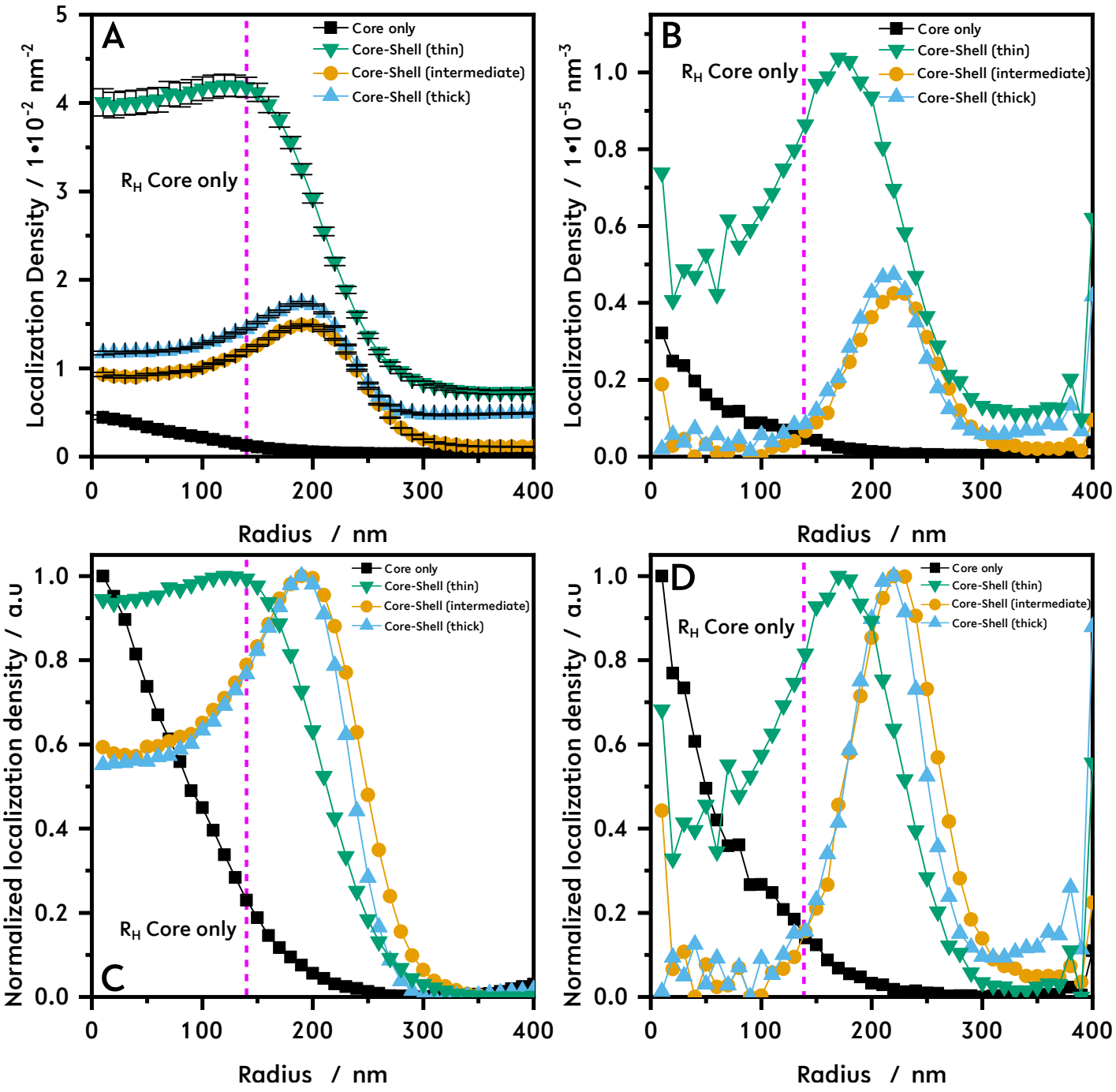

Fig. S6 In addition to the 2- and 3D localization densities shown in Fig. 4 **A** and **B** the localization density is visualized here by normalizing the values to values between zero and one.

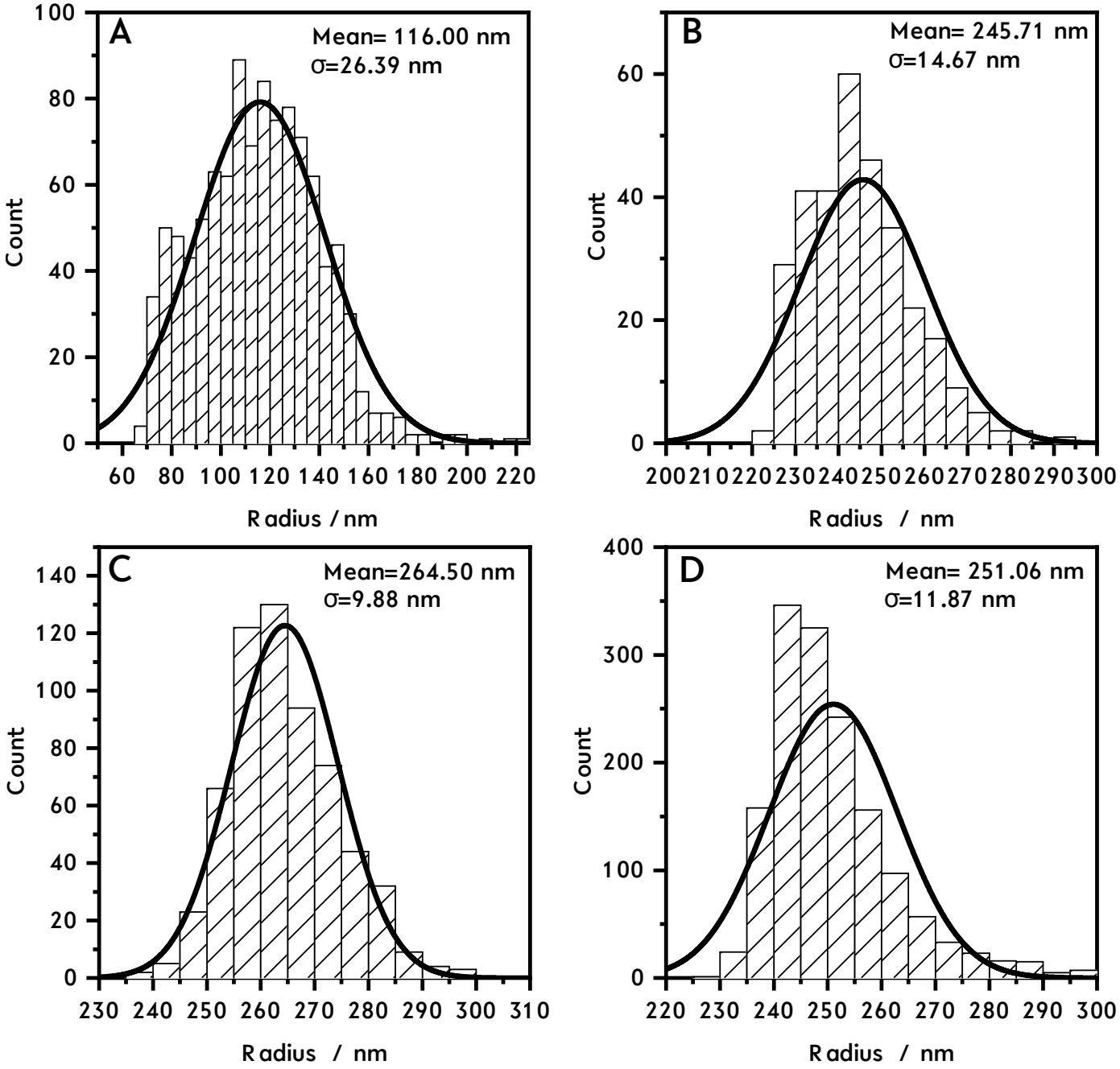

Fig. S7 The histogrammed microgel radii for core microgels (**A**) deviate strongly from the measured hydrodynamic radius obtained by PCS around 16 °C (see. Fig. 1 **A**) and show a radius half of the measured hydrodynamic radius $R_H \approx 240\,\text{nm}$. The total number of localized dye molecules is compared to the core shell microgels lower and the reconstructed image is less well defined. The imfindcircle function has therefore problems to recognize the radius properly. By adding the shell to the core the estimated radii of the different core-shell microgels is reaching the range of the hydrodynamic radii (see Fig. 3 **A**,**D** and **G**), but remains too small. With the fluorescent dye molecules we can only probe the microgel network where it is dense enough to hold the dye inside the microgel during the washing steps and the imaging with the imaging buffer. Moreover, in PCS also the hydration shell contributes to the "seem" R_H.

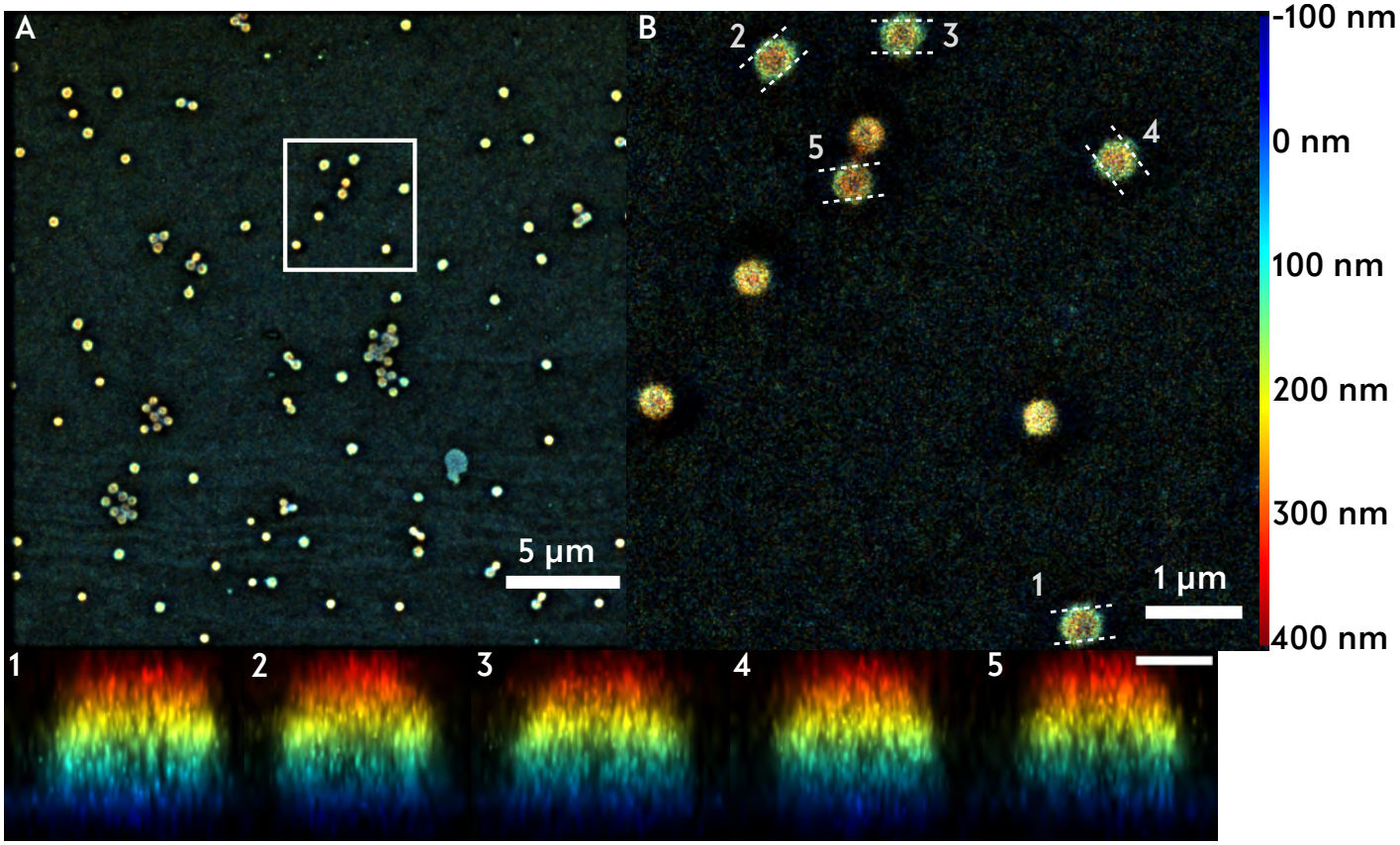

Fig. S8 One of the data sets contributing to the 2D density distribution is analyzed with SMAP. **A** displays the core-shell microgels with thin shell. The height is color coded from -100 nm (dark blue) to 400 nm (dark red). For a better insight the white marked area is shown with a zoom-in in **B**. A 300 nm thick line plot, indicated with the white dashed lines, from **B** is displayed via xz-projection for the numbered microgels one to five. Scale bar 200 nm.

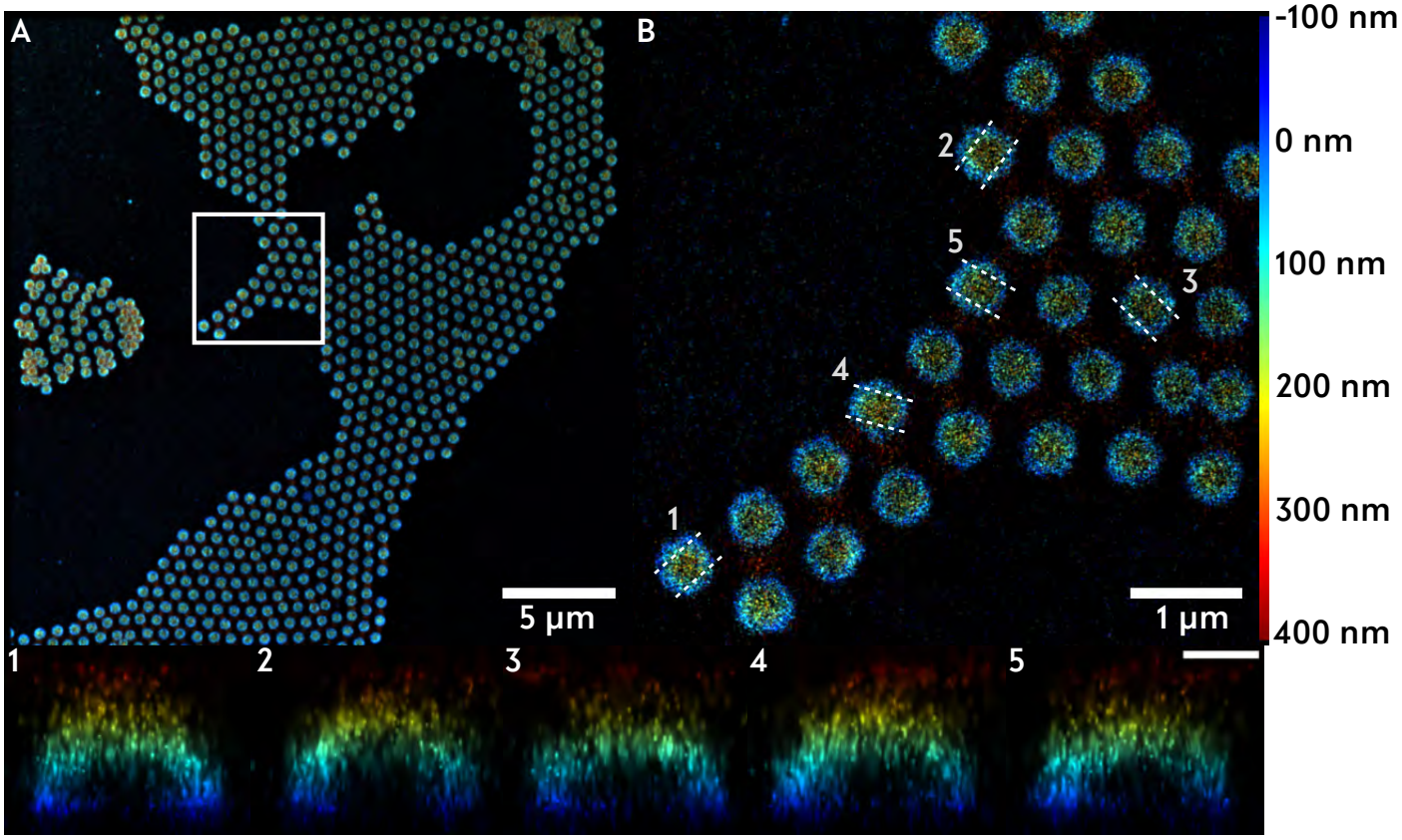

Fig. S9 The same data of core-shell microgels with a intermediate shell like in Fig. 3 **E** was also analyzed with the generated 3D calibration in SMAP. In **A** the 3D information is color coded from -100 nm (dark blue) to 400 nm (dark red). To obtain a more detailed view on single microgels a zoom-in of the white marked area is shown in **B**. Line plots with a thickness of 300 nm, indicated by the white dashed line, are displayed below the areal view and show the nearly unlabeled core by a xz-projection (numbered microgels one to five). Scale bar 200 nm.

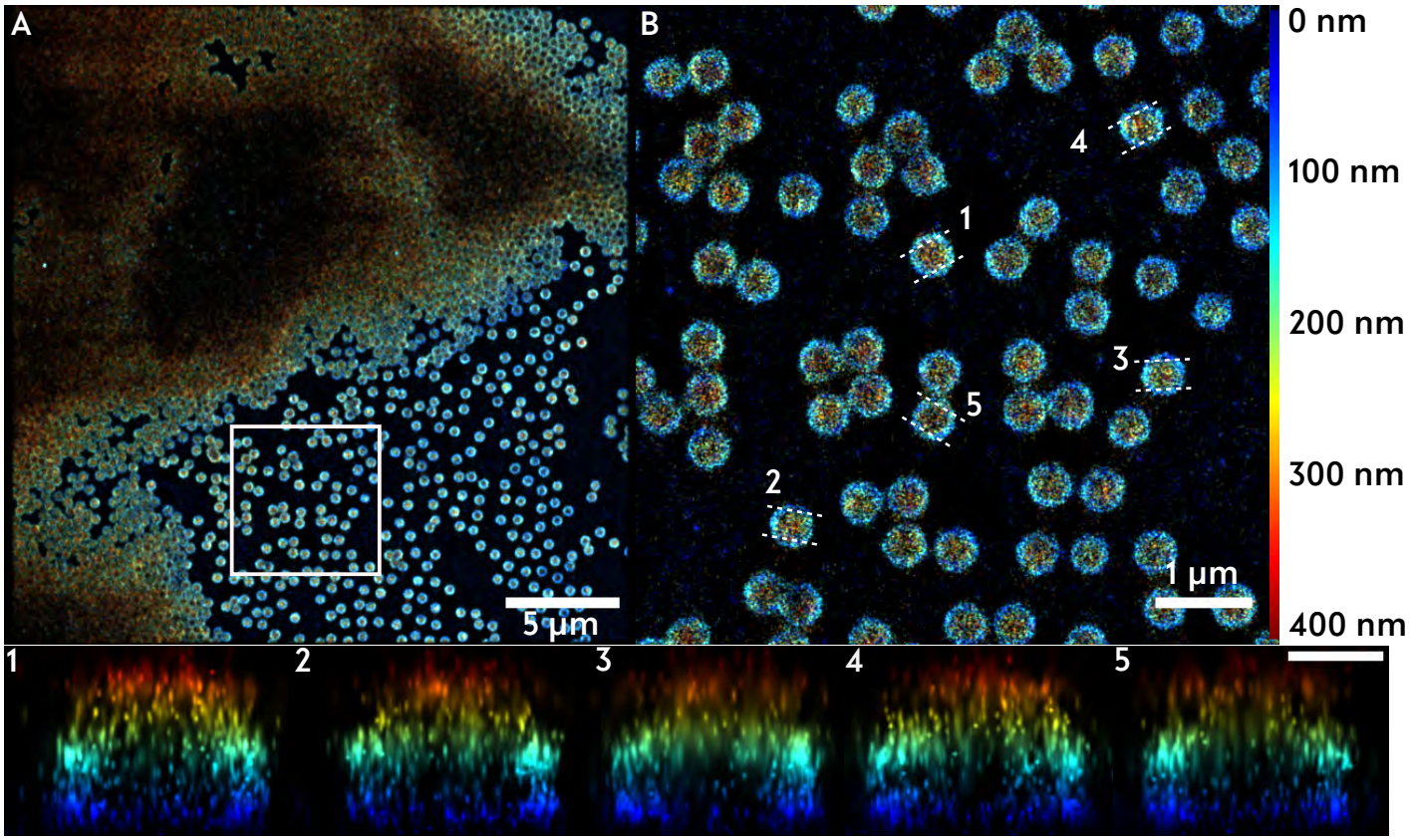

Fig. S10 One of the data sets contributing to the 2D density distribution is analyzed with SMAP. **A** displays the core-shell microgels with thick shell. The height is color coded from -100 nm (dark blue) to 400 nm (dark red). For a better insight the white marked area is shown with a zoom-in in **B**. A 300 nm thick line plot, indicated with the white dashed lines, from **B** is displayed via xz-projection for the numbered microgels one to five. Scale bar 200 nm.

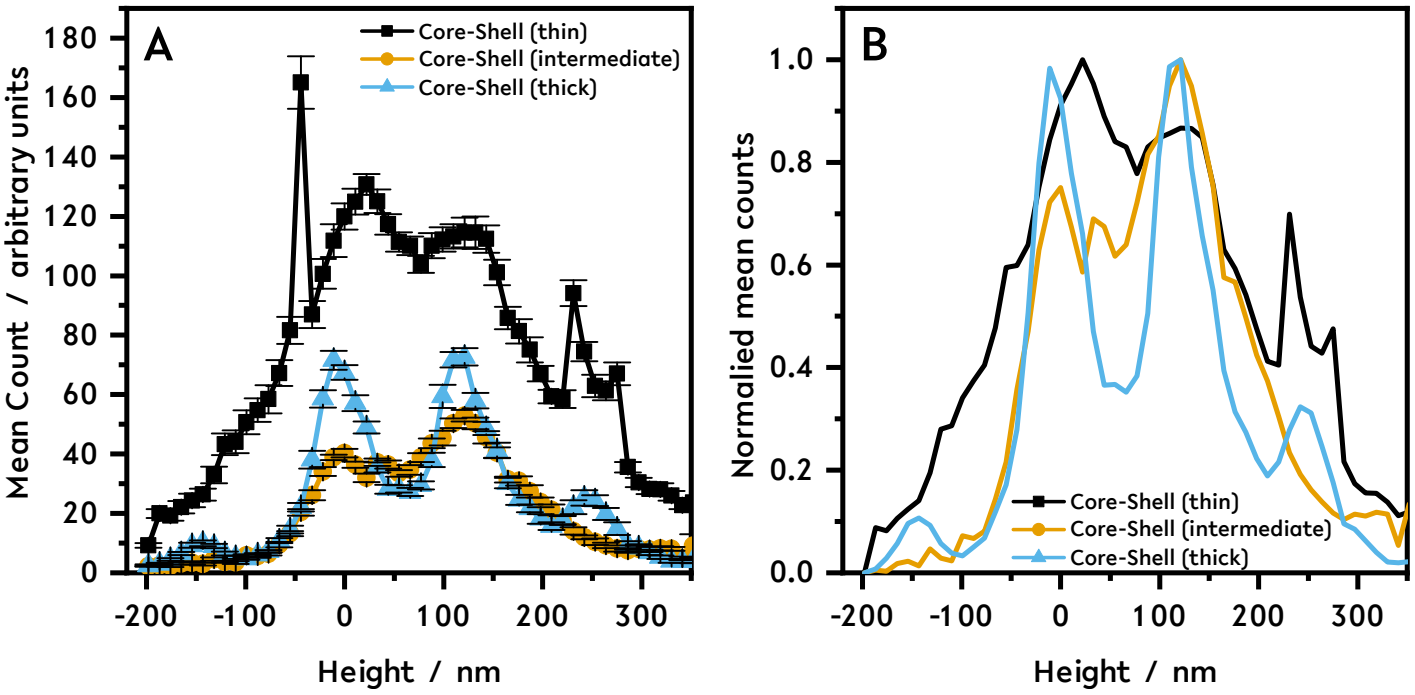

Fig. S11 Mean counts vs height of 20 microgels (black = thin shell, yellow = intermediate shell and blue = thick shell). A 300 nm thick line plot was used to get an averaged count through the whole microgel. The error bars representing the error of the mean. In **B** the graph is normalized to values between zero and one. The data point at -44 nm was excluded for the normalization.

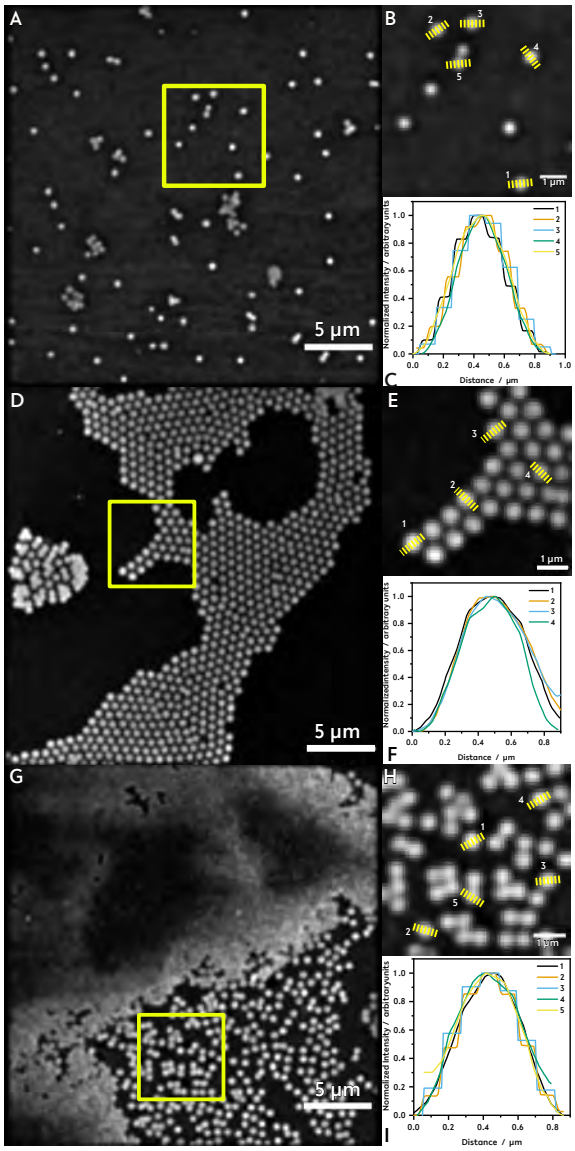

Fig. S12 A, **D** and **G** Diffraction limited fluorescence images of the core-shell microgels with the thin, intermediate and thick shell calculated from the single localizations. The images **B**, **E** and **H** displaying a zoom-in of the associated yellow boxes. In the zoomed-in areas single microgels are selected and 300 nm thick line plots are presented in the graphs **C**, **F** and **I**.

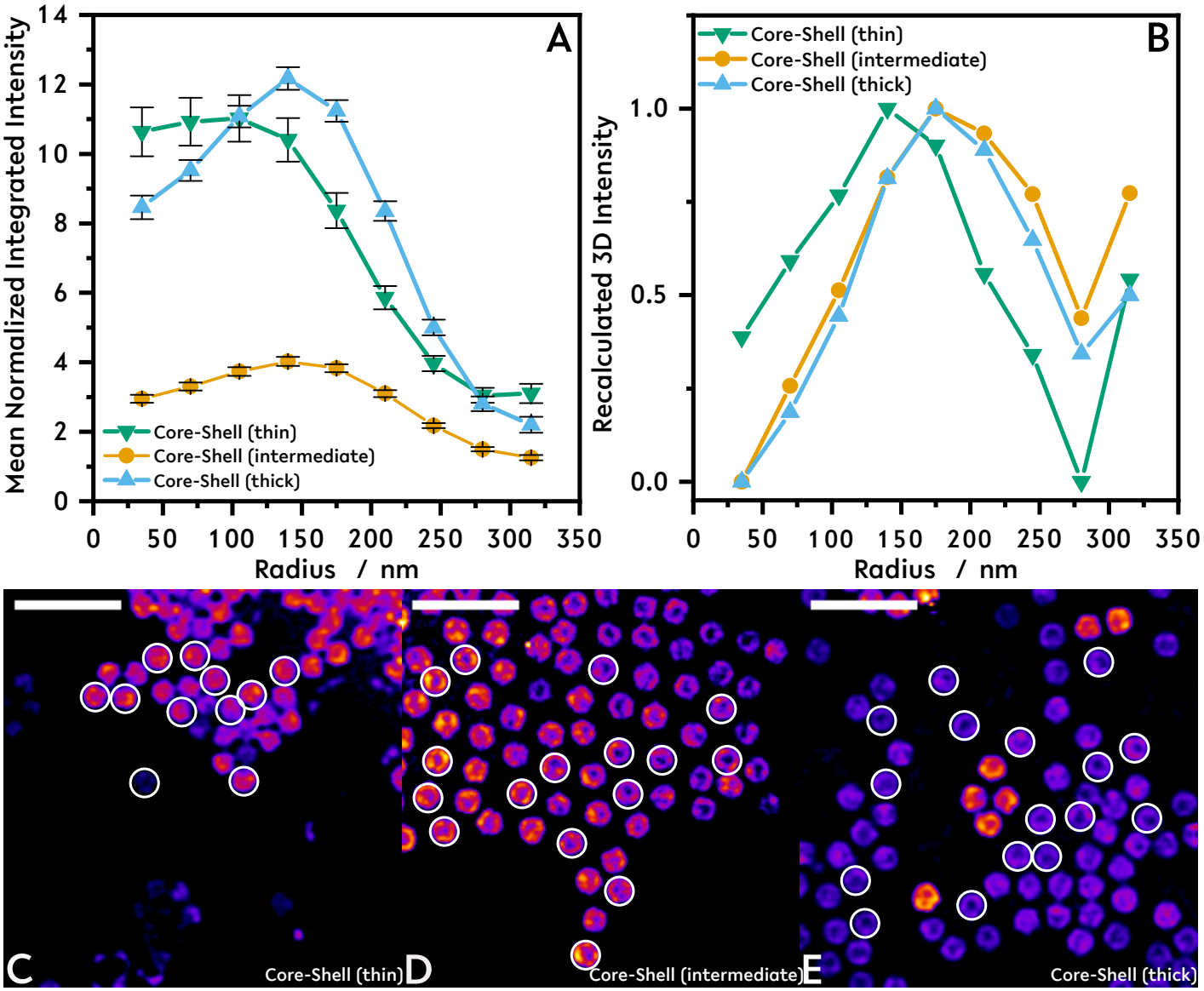

Fig. S13 To verify the results obtained by single molecule localization experiments, as shown in Fig. 4, structured illumination microscopy images of the same core-shell microgels as before were measured at a temperature of 17 °C and below to preserve the swollen state of the microgels. In **A** the mean normalized integrated intensity is plotted against the microgel radius. For this the white marked microgels in **C**, **D** and **E** were selected by hand. The results were also recalculated with the presented 3D recalculation scheme and the results are shown in **B**. In comparison, the microgels with intermediate and the thick shell are showing a slope with increasing signal intensity up to a radial distance of 150 nm, what can also be seen in the number of localizations in the SMLM experiments. Scale bar 2 μm in images **C**, **D** and **E**.

References

1 D. Nečas and P. Klapetek, *Open Physics*, 2012, **10**, 99.

2 Y. Li, M. Mund, P. Hoess, J. Deschamps, U. Matti, B. Nijmeijer, V. J. Sabinina, J. Ellenberg, I. Schoen and J. Ries, *Nature methods*, 2018, **15**, 367–369.