Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Super-Resolution Optical Microscopy resolves Network Morphology of Smart Colloidal Microgels

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We present a new method to resolve the network morphology of colloidal particles in an aqueous environment via super-resolution microscopy. By localization of freely diffusing fluorophores inside the particle network we can resolve the three dimensional structure of one species of colloidal particles (thermoresponsive microgels) without altering their chemical composition through copolymerization with fluorescent monomers. Our approach utilizes the interaction of the fluorescent dye rhodamine 6G with the polymer network to achieve an indirect labeling. We calculate the 3D structure from the 2D images and compare the structure to previously published models for the microgel morphology, e.g. the fuzzy sphere model. To describe the differences in the data an extension of this model is suggested. Our method enables the tailor-made fabrication of colloidal particles which are used in various applications, such as paints or cosmetics, and are promising candidates for drug delivery, smart surface coatings, and nanocatalysis. With the precise knowledge of the particle morphology an understanding of the underlying structure-property relationships for various colloidal systems is possible.

Electronic Supporting Information

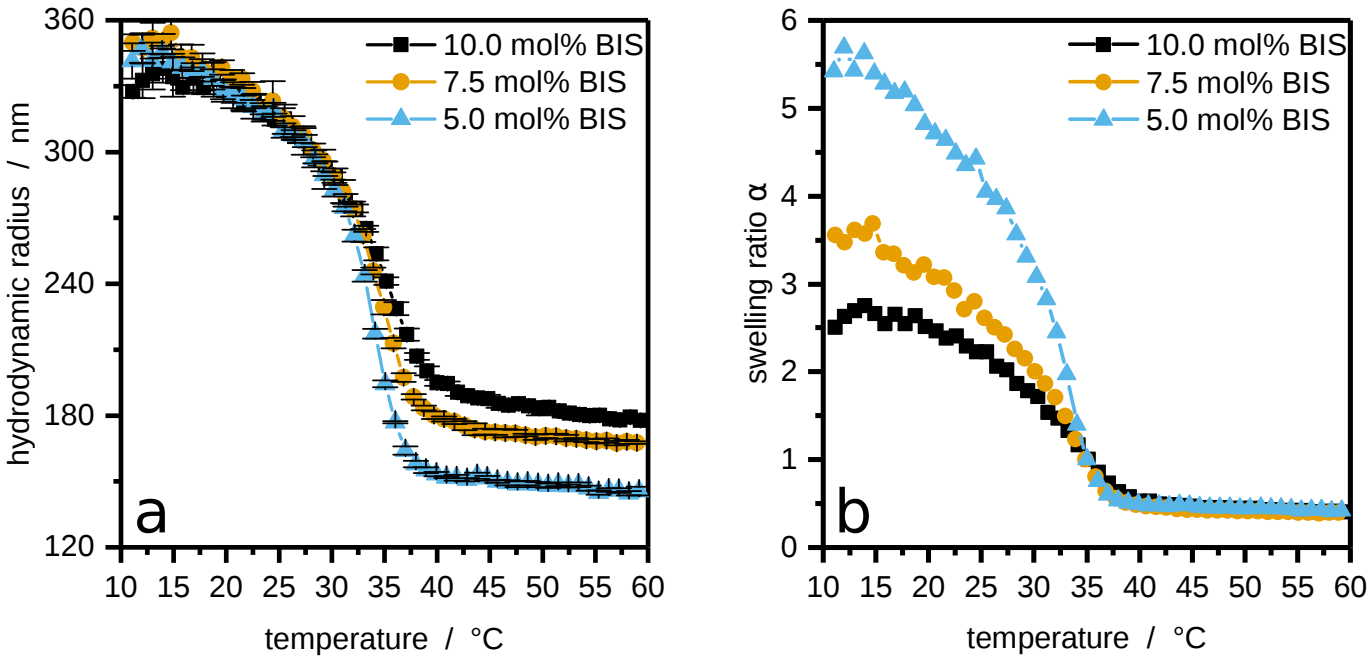

Fig. S1 Photon Correlation Spectroscopy experiments for microgels with different cross-linker content (5.0 (blue triangles), 7.5 (yellow circles) and 10 mol% (black squares). In a) the hydrodynamic radius in dependence of the temperature is shown, in b) the swelling ratio. The figures show the normal thermoresponsive behavior of NIPAM based microgels, which exhibit a reversible phase transition from a swollen state below the volume phase transition temperature (VPTT) to a collapsed state above. The volume phase transition temperature is slightly shifted towards higher temperatures for higher cross linker contents.

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§ SoMaCoFit downloadable from: `https://publications.rwth-aachen.de/record/670555/`

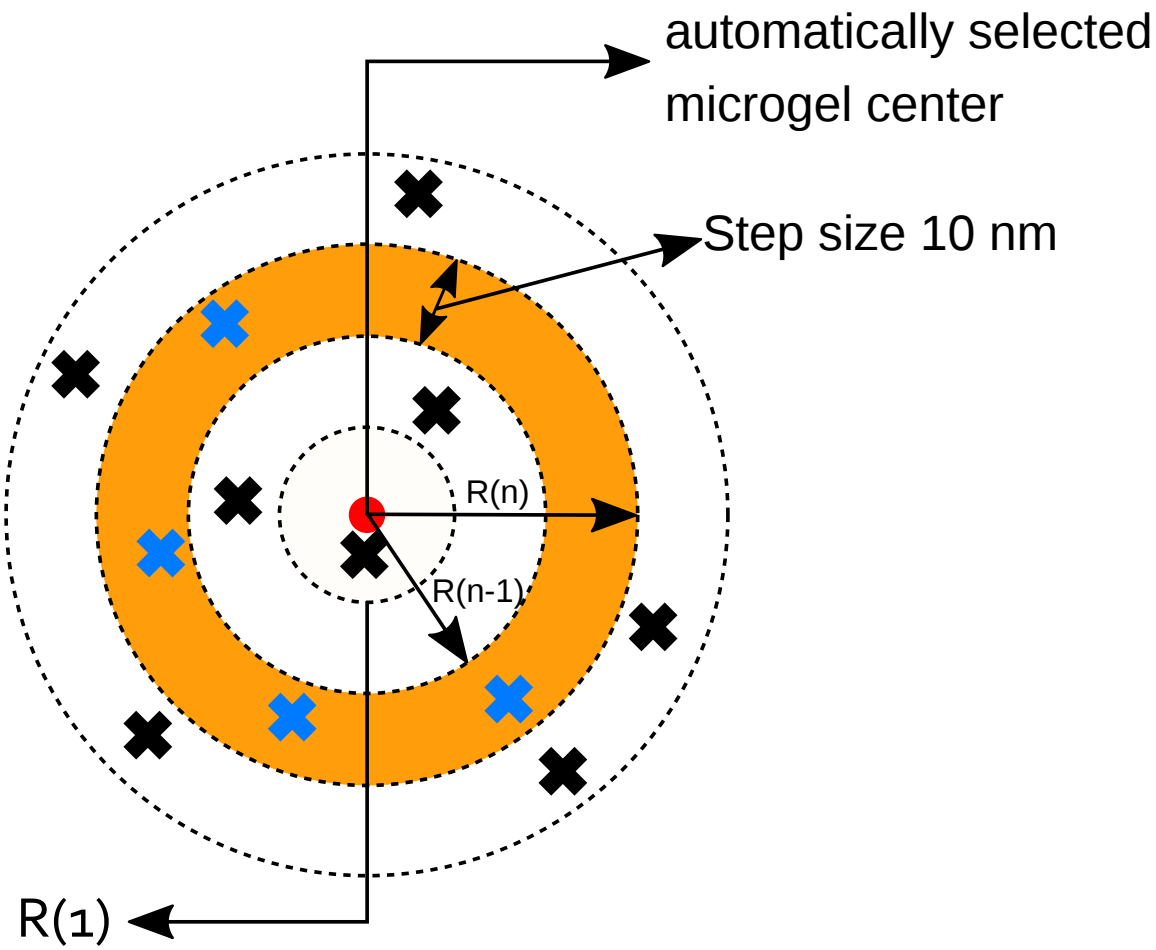

Fig. S2 Schematic illustration of the localization density calculation. Via the imaging tool box the microgels are recognized and the center is determined. From the center consecutive rings with a width of 10 nm are drawn and the localizations within these rings are counted. For the final 2D density calculation the total number of localizations in each ring is divided by the ring area.

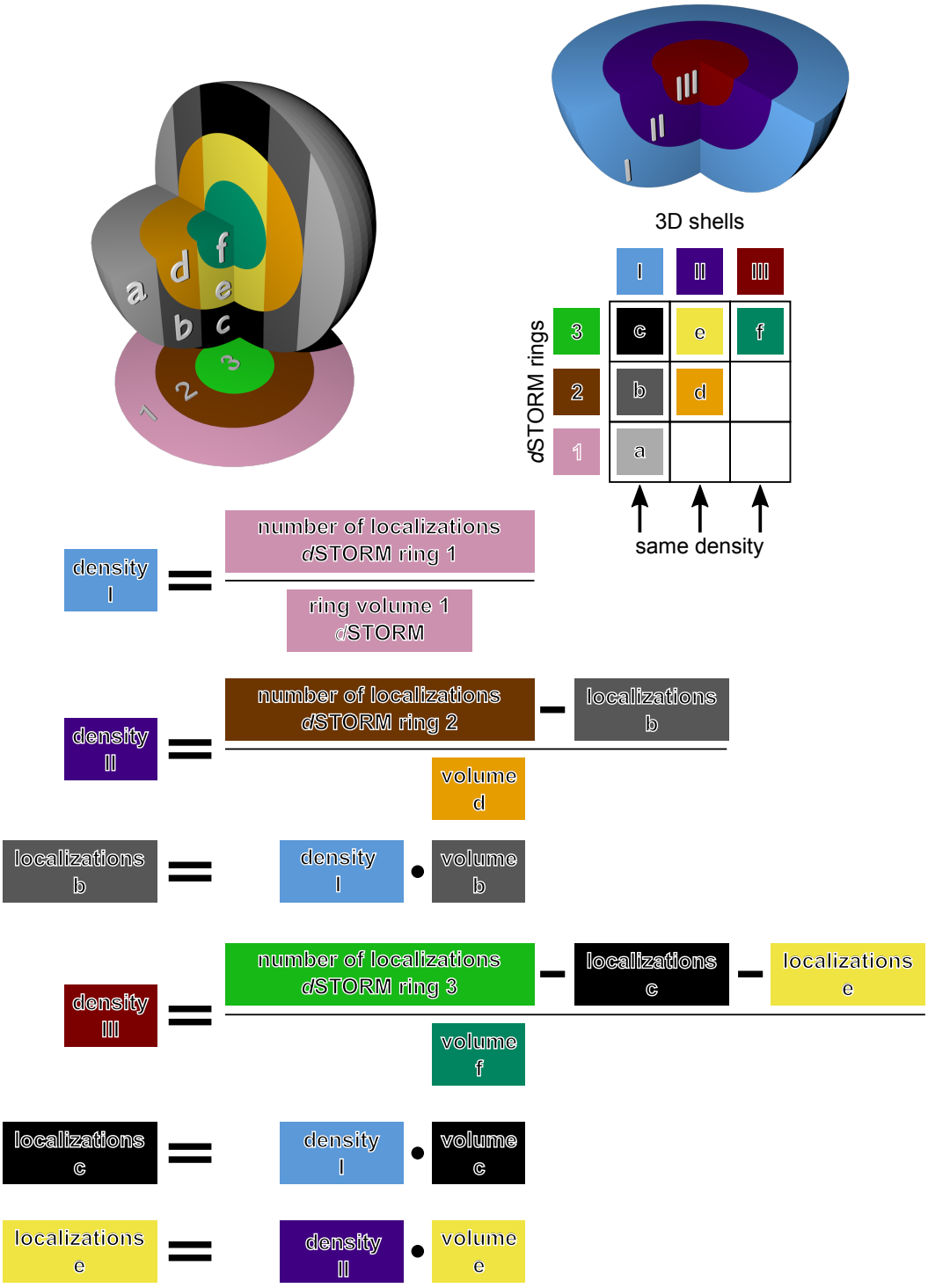

Fig. S3 Schematic illustration of the conversion of 2D *d*STORM data to 3D density profiles. The matrix (upper right corner) explains the elements needed for the calculation and their respective density. The exemplary equations show the calculation for a microgel with 3 equally wide *d*STORM ring/shells.

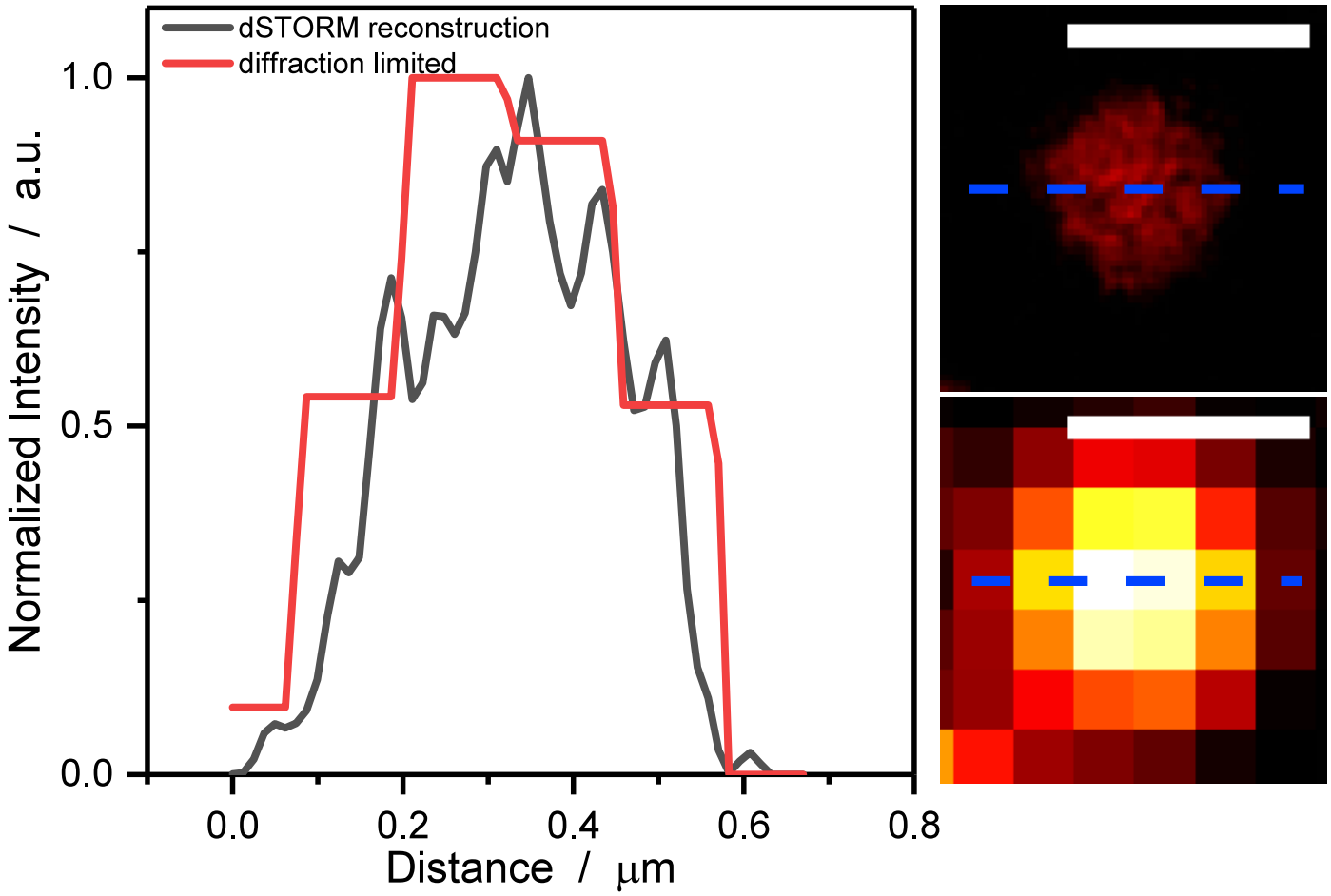

Fig. S4 An example to demonstrate that localization microscopy is capable of gaining a deeper insight into the microgel. Here, the diffraction limited fluorescence image and the reconstructed image of a microgel with 5.0 mol% is shown. A line plot through the same microgel indicates the higher information content which can be seen in the graph. The information gain for the diffraction limited image is limited by the number of photons collected per camera pixel. In our setup 1 pixel matches 124 nm of our sample. Scale bar 500 nm

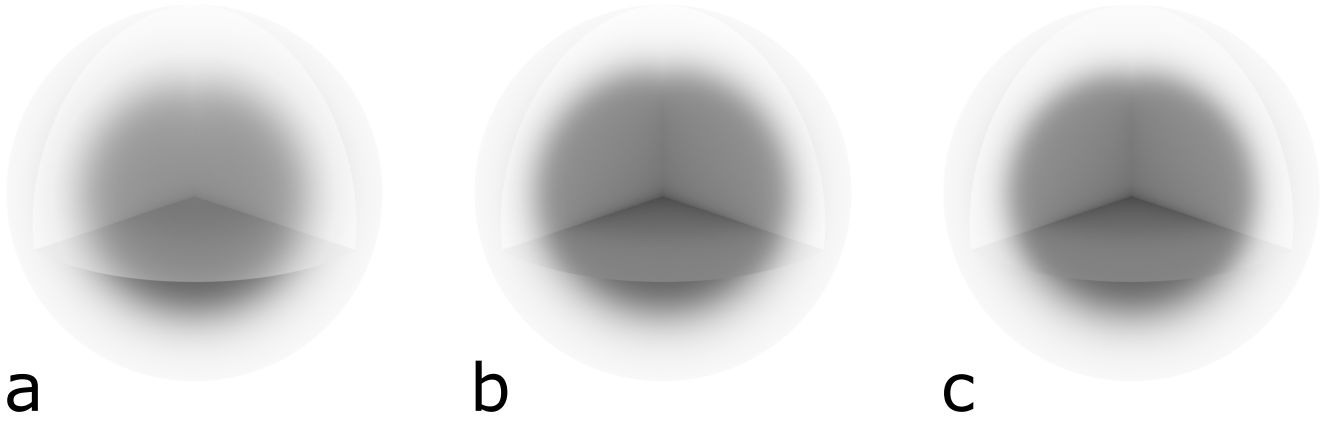

Fig. S5 Illustrations of the reported morphology. The image was created using "blender" with shaders representing the localization density. The particles shown represent the microgel with a) 5 mol% , b) 7.5 mol% and c) 10 mol% crosslinker.

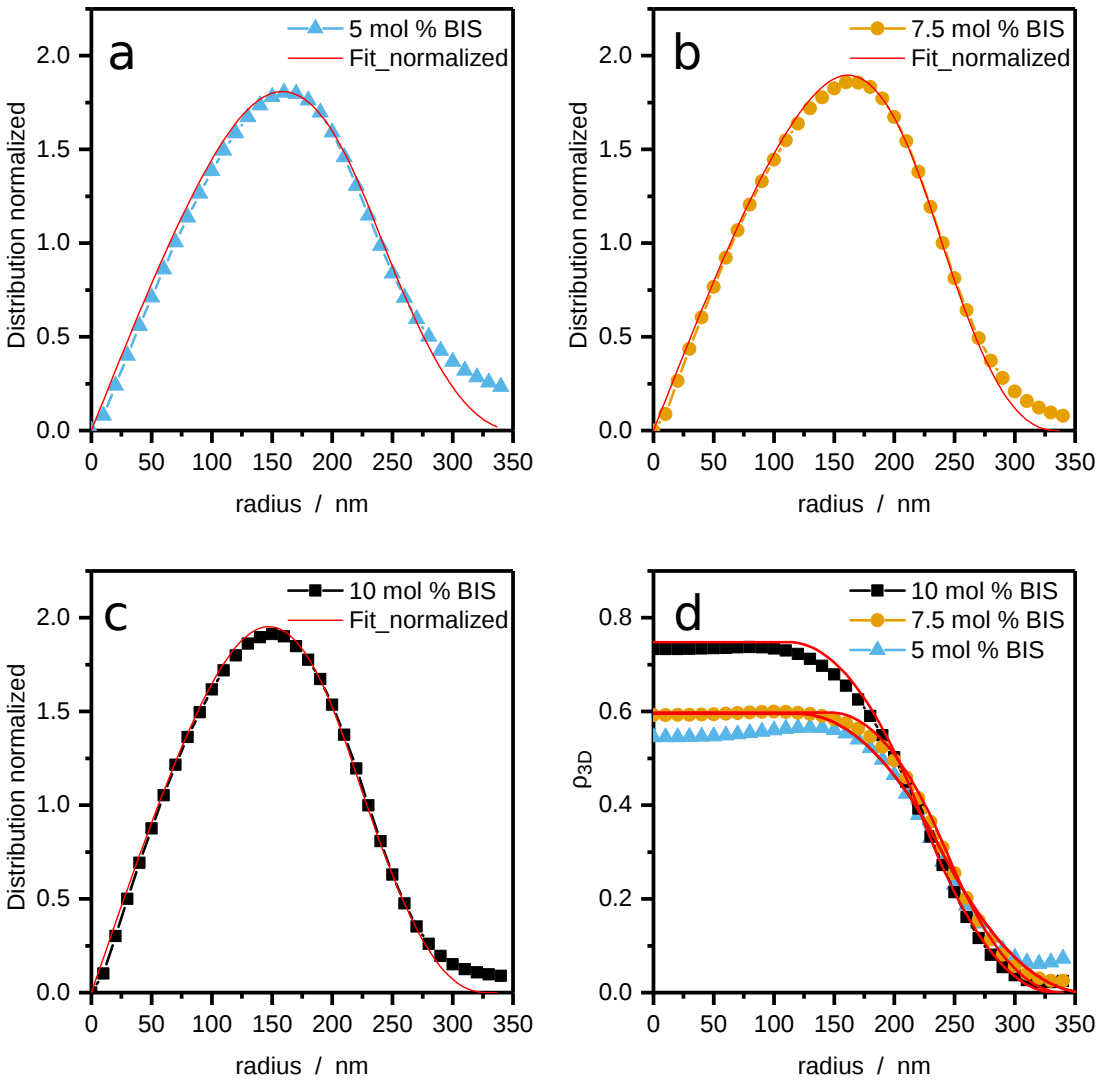

Fig. S6 2D density of microgels with 5.0 a), 7.5 b) and 10.0 c) mol% BIS after regularization with $\lambda = 3.8614$. In d) the calculated 3D density distribution after the regularization is shown for 5.0 (blue triangles), 7.5 (orange circles) and 10.0 (black squares) mol% BIS. The solid red lines is showing the fuzzy sphere model fitted to this data.

1 SoMaCoFit

Before fitting our localization data with SoMaCoFit[1§], we have normalized the data to the sum of 1. This was recommended in the SoMaCoFit manual to obtain comparable results in the 3D density ρ_{3D} between different measurements. For the fitting of the 2D data the fuzzy sphere model was chosen and all fitting parameters were free. After obtaining the 2D fit the data is regularized and inverted. To find the optimal regularization parameter λ the minimum in the provided generalized cross validation is chosen. For our data $\lambda = 3.8614$ showed the minimal value. For small regularization values ρ_{3D} deviates strongly from the expected distribution in the microgels (figures S7-S9).

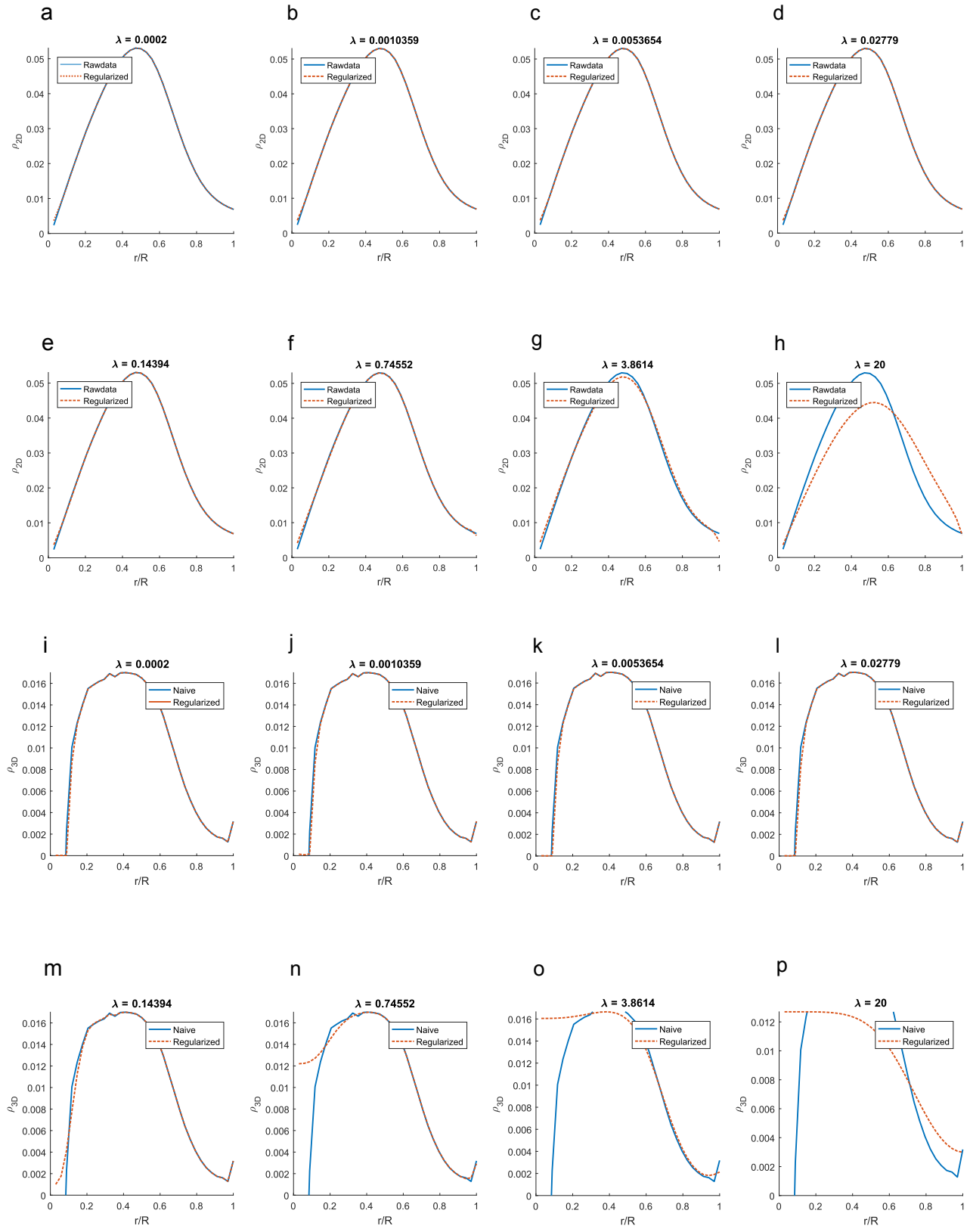

Fig. S7 SoMaCoFit is used to calculate the 3D profile density from our data of 5.0 mol% BIS microgels . As a result the software calculates the normalized 2D (figure S7 a to h) and 3D density (figure S7 i to p) for different regularizations λ of the given data. For a stronger regularization ($\lambda > 0.74552$) the data was smoothed to a high extend and especially close to the microgel centers the model differs strongly from the measured data.

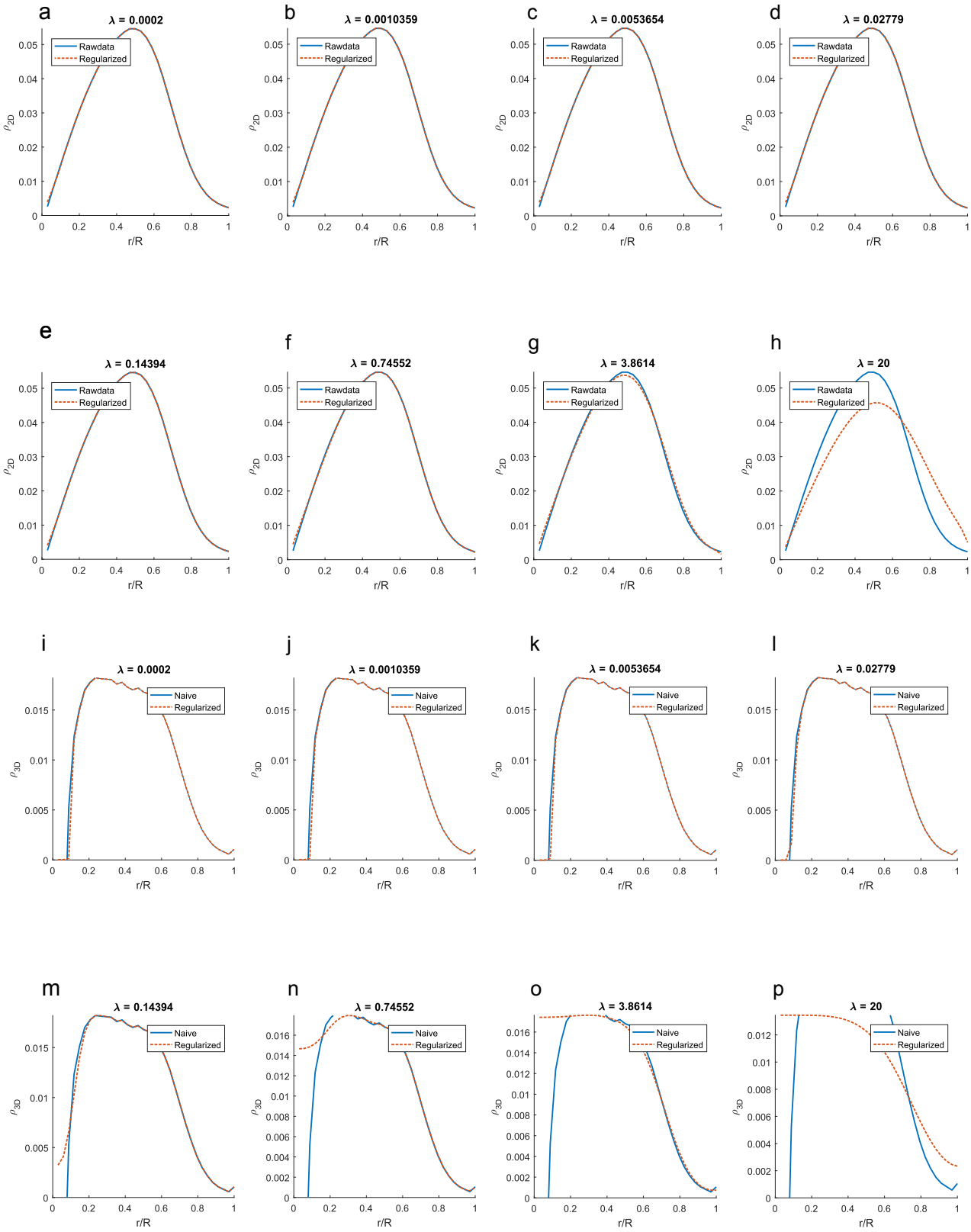

Fig. S8 SoMaCoFit is used to calculate the 3D profile density from our data of 7.5 mol% BIS microgels . As a result the software calculates the normalized 2D (figure S8 a to h) and 3D density (figure S8 i to p) for different regularizations λ of the given data. For a stronger regularization ($\lambda > 0.74552$) the data was smoothed to a high extend and especially close to the microgel centers the model differs strongly from the measured data.

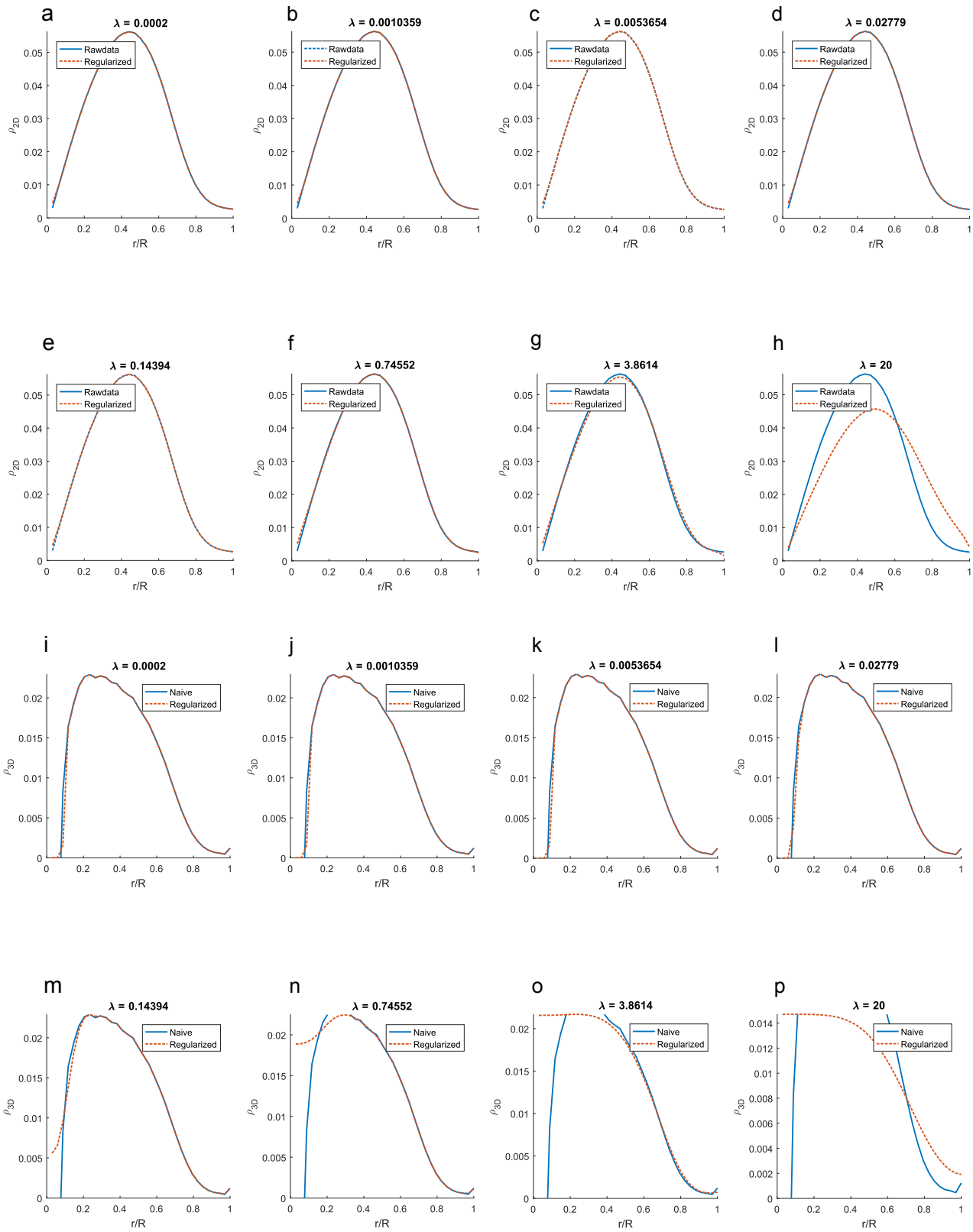

Fig. S9 SoMaCoFit is used to calculate the 3D profile density from our data of 10 mol% BIS microgels . As a result the software calculates the normalized 2D (figure S9 a to h) and 3D density (figure S9 i to p) for different regularizations λ of the given data. For a stronger regularization ($\lambda > 0.74552$) the data was smoothed to a high extend and especially close to the microgel centers the model differs strongly from the measured data.

References

1 A. P. H. Gelissen, A. Oppermann, T. Caumanns, P. Hebbeker, S. K. Turnhoff, R. Tiwari, S. Eisold, U. Simon, Y. Lu, J. Mayer, W. Richtering, A. Walther and D. Wöll, *Nanoletters*, 2016, **16**, 7295–7301.