CLAIMS

1. A method for the characterization of fluorescently labeled particles in solution by analyzing changes in the fluorescence intensity and spectral shifts and/or broadening or narrowing of the spectrum of the fluorescent label of the fluorescently labeled particles, comprising the steps:

    a) providing a sample of the fluorescently labeled particles in a solution under first conditions,

    b) exciting the fluorescently labeled particles at a first wavelength,

    c) detecting the fluorescence emission intensity of the fluorescently labeled particles at a second and a third wavelength,

    d) calculating the ratio between said fluorescence intensities at the second and the third wavelength,

    wherein said third wavelength is different from said second wavelength,

    e1) repeating steps b) to d) for said sample of the fluorescently labeled particles under second conditions, or

    e2) repeating the steps a) to d) for a second sample of the fluorescently labeled particles under second conditions,

    wherein said second conditions are different from said first conditions,

    f) characterizing the fluorescently labeled particles based on the calculated ratios obtained for the different conditions,

    wherein the second and third wavelength are detected simultaneously, and wherein the second wavelength is detected at an at least 2.5 nm shorter wavelength, and the third wavelength is detected at an at least 2.5 nm longer wavelength than an emission maximum of the fluorescence emission of the fluorescently labeled particles under the first conditions.

2. The method according to claim 1, wherein the sample volume containing the fluorescently labeled particles is less than 100 µl, preferably between 1 µl and 25 µl.

3. The method according to any one of claims 1 or 2, wherein the sample containing the fluorescently labeled particles is provided in a capillary.

4. The method according to any one of the preceding claims, wherein the fluorescently labeled particles are labeled with an environment sensitive label.

5. The method according to any one of the preceding claims, wherein the particles are selected from the group consisting of organic molecules, biomolecules, nanoparticles, microparticles, vesicles, biological cells or sub-cellular fragments, biological tissues, viral particles, viruses, cellular organelles, lipid nanoparticles (LNPs), and virus like particles.

6. The method according to claim 5, wherein said biomolecules are selected from the group consisting of amino acids, proteins, peptides, mono- and disaccharides, polysaccharides, lipids, glycolipids, fatty acids, sterols, vitamins, neurotransmitter, enzymes, nucleotides, metabolites, nucleic acids, and combinations thereof.

7. The method according to any one of the preceding claims, wherein the concentration of the fluorescently labeled particles in the solution is from 10 pM to 10 µM, preferably 50 pM to 500 nM.

8. The method according to any one of the preceding claims, wherein the changes in the detected fluorescence intensity of the fluorescently labeled particles result from spectral shifts or broadening of the spectrum or narrowing of the spectrum, or combinations thereof.

9. The method according to any one of the preceding claims, wherein the fluorescence intensity of the fluorescently labeled particles changes due to mechanisms selected from the group consisting of conformational changes of the fluorescently labeled particles, relocalization of the fluorescently labeled particles, interactions between the fluorescently labeled particles and one or more ligands, and combinations thereof.

10. The method according to any one of the preceding claims, wherein the calculated ratios obtained in step f) are used to determine the localization of the fluorescently labeled particles or parameters selected from the group consisting of dissociation constants, half maximal effective concentrations (ECso), equilibrium constants, binding kinetics,

enzymatic reaction kinetics, thermodynamic parameters, unfolding or refolding kinetics, opening and closing reactions, and combinations thereof.

11. The method according to any one of the preceding claims, wherein the second conditions of step e) are altered by adding a ligand and/or different concentrations of the ligand and the calculated ratios obtained in step f) are used to determine the dissociation constant of the fluorescently labeled particles and the ligand.

12. The method according to any one of the preceding claims, wherein the first and second conditions of the fluorescently labeled particles differ with regard to their temperature and/or chemical composition.

13. The method according to any of the preceding claims, wherein the fluorescence emission intensity of the fluorescently labeled particles at a second and a third wavelength of step c) is detected during a defined temperature perturbation.

14. A device for the characterization of fluorescently labeled particles in solution by analyzing changes in the fluorescence intensity and spectral shifts and/or broadening or narrowing of the spectrum of the fluorescent label of the fluorescently labeled particles, in particular adapted for performing the method according to any of the preceding claims, the device comprising:

a sample holder for holding a sample of fluorescently labeled particles in solution under a plurality of conditions;

means for exciting the fluorescently labeled particles at a first wavelength;

means for detecting the fluorescence emission intensity of the fluorescently labeled particles at a second and a third wavelength;

means for calculating a ratio between said fluorescence intensities at the second and the third wavelength, wherein said third wavelength is different from said second wavelength;

wherein the device is configured to consecutively excite fluorescently, detect fluorescence emissions and calculate the ratio for samples at different conditions;

means for characterizing the fluorescently labeled particles based on the calculated ratios obtained for the different conditions,

wherein device is configured to simultaneously detect the second and third wavelength, and wherein the second wavelength is detected at an at least 2.5 nm shorter wavelength and the third wavelength is detected at an at least 2.5 nm longer wavelength than an emission maximum of the fluorescence emission of the fluorescently labeled particles under a first condition of the different conditions.

15. The device according to claim 14, wherein the means for exciting is an excitation light source, preferably at least one light source from the group consisting of laser, laser fibre laser, diode-laser, LED, HXP, Halogen, LED-Array, HBO.

16. The device according to claim 14 or 15, wherein the means for detecting is light detector, preferably at least one detector from the group consisting of PMT, siPM, APD, CCD or CMOS camera.

17. A computer program comprising instructions which, when the program is executed by a computer, cause the computer to carry out the method according to any of claims 1 to 13.

18. A computer-readable data carrier comprising instructions which, when executed by a computer, cause the computer to carry out the method according to any of claims 1 to 13.

19. Use of a device according to any of claims 14 to 16 for the characterization of fluorescently labeled particles in solution, preferably in accordance with any of method claims 1 to 13.

20. Use of a capillary for the characterization of fluorescently labeled particles in solution by analyzing changes in the fluorescence intensity and spectral shifts and/or broadening or narrowing of the spectrum of the fluorescent label of the fluorescently labeled particles, wherein a sample of the fluorescently labeled particles in a solution is filled in the capillary and provided for analyzing, in particular according to the method of any of claims 1 to 13.