Review of: "Characterization of Quantum Dots with Hyperspectral Fluorescence Microscopy for Multiplexed Optical Imaging of Biomolecules"

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Potential competing interests: The author(s) declared that no potential competing interests exist.

In this manuscript, the authors developed a customized wide-field hyperspectral fluorescence microscopy system for multiplexed fluorescence imaging with quantum dots. However, after reading this manuscript, I found that several issues need to be addressed. Some obvious examples are listed below.

1. The authors have described in the part of Abstract:" The optical properties of quantum dots were extensively characterized using a hyperspectral fluorescence microscopy system." However, the title "Characterization of Quantum Dots with Hyperspectral Fluorescence Microscopy for Multiplexed Optical Imaging of Biomolecules" can not reflect the novelty of this manuscript and should be modified.
2. Hyperspectral fluorescence microscopy can achieve multiplexed fluorescence imaging and detect the emission spectra of quantum dots. However, the information that hyperspectral fluorescence microscopy can provide is limited. Therefore, the characterization of quantum dots should be supplemented such as TEM images.
3. In the part of Introduction in Line 3-6, "a physical phenomenon in which a substance is excited by photons of a shorter wavelength and subsequently releases photons of a longer wavelength as it relaxes back into its ground state" was described. However, this is the phenomena in the most cases. There are some quantum dots that can undergo up-conversion luminescent, that is, quantum dots can be excited by photons of a longer wavelength and release photons of a shorter wavelength.
4. The size of figure note is in the same with the text part and should be shrank.
5. The fluorescence spectra of six quantum dots should be provided using a conventional fluorescence spectrophotometer.
6. Why the samples were tested when wet and dry? The authors should explain it.
7. In Figure S3, the linear fit on peak intensity values against concentration values of Qdot 605 exists a comparatively big deviation, which can not achieve the quantification of Qdot 605.
8. For Figure 6, The imaging comparison before and after multiplexed staining with three different quantum dot-antibody conjugates should be provided.