Quantum Dot applications in Fluorescence Imaging – for Calibration and Molecular Imaging

Introduction

In this application note, we will discuss the application of quantum dots in fluorescence imaging, both as a calibration material or building block of molecular probes.

A fluorescence imaging system is considerably more complicated than a conventional imaging system that merely measures light emissions. The performance of a fluorescence image system depends on many factors besides the image sensor itself; these are excitation light source, emission filters excitation filters, and the optics. Considerations have to be taken to minimize direct absorption of the emission light by the detector.

There is often a need to test and calibrate a fluorescent imaging system as it is being designed and implemented. There are several methods to accomplish this:

1. Using calibration glasses slides. These are solid materials that will auto-fluorescence.
2. Using biological calibration solutions. These are biological samples (DNA/RNA, Protein, tissues etc.) that are tagged with fluorescent dye,
3. Using fluorescence emitting quantum dots. For example, quantum dots particles that are soluble in liquid such as water.

Figure 1, fluorescence calibration slides

Figure 2. CdTe Water soluble fluorescence emitting quantum dots

The advantage of using glass slides is that there is no need to handle fluid. Also they are typically shaped in the same way as glass slides for microscopes. For this reason, this method is optimal for calibrating microscopes. However, for other applications where the target analyte is in fluid form, this method has limited applicability.

Using biological samples is potentially messy and requires proper handling to avoid contamination (some companies go as far as providing HIV virus based samples for fluorescence calibration). It can be difficult and expensive to obtain suitable samples. Moreover, fluorescence from biological samples is usually unstable. The magnitude of fluorescence signal will attenuate in a short time window, making this method problematic for calibration purposes.

Here we introduce the method of using water soluble quantum dots material to calibrate the fluorescence imaging system. Quantum dots materials are inexpensive, easy to handle and have long stable signal life, as well as shelve life. Water soluble quantum dots are particularly useful for calibrating systems whose analytes are also water-based.

Materials and methods:

Quantum dots

We have sourced water soluble quantum dots from “CHINA BEIJING BEIDA JUBANG SCIENCE & TECHNOLOGY Co., Ltd.” (http://www.pkuchemqd.com/). These are CdTe quantum dots with COOH groups on the surface. In liquid form, this type of material has over 1 year of shelve life in room temperature. And in solid form (as powder), they can be stored for more than two years. The only handling requirement is to avoid direct sun light.

Below is the excitation and emission spectrum of this type of quantum dots. As can be seen, there are quite a few different materials to choose from for calibration of fluorescence system in different portion of the typical fluorescence spectrum.

We have chosen a particular quantum dots material below to calibrate the FAM fluorescence channel of our system, the CFI 324 system.

Vendor：CHINA BEIJING BEIDA JUBANG SCIENCE & TECHNOLOGY Co., Ltd.
Part Number：W23-000-510
Name：Water Soluble CdTe type Quantum Dots（COOH）
Concentration：5μmol/L
Particle size：2-10nm
Excitation wavelength：470nm
Emission wavelength：510nm
Solution：Water

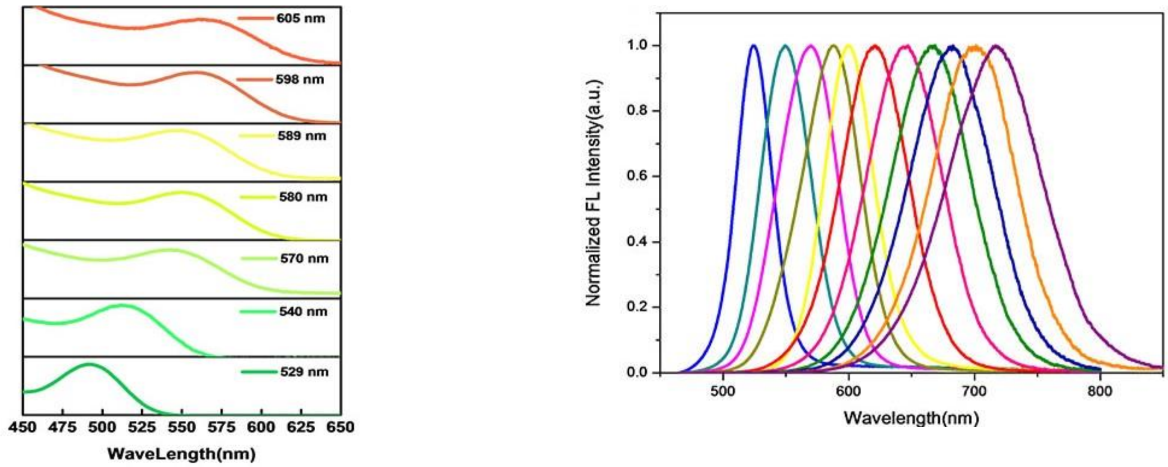


Figure 3 CdTe type quantum dots excitation and emission spectrum

Fluorescence imaging system

A set of Anitoa CFI x24 system is used for this experiment. This is a turn-key fluorescence imaging system that includes the light source, the image capture module, and software.

Vendor: Anitoa Systems, LLC (www.anitoa.com)
Part number: CFI x24 – 1ch
Name: Compact Fluorescence Imager
Number of channels: 1 to 4
Imager chip included: Anitoa ULS24 CMOS Ultra-low-light Image Sensor
Resolution: 24 x 24 pixels.
Excitation wave length: Channel 1: 470nm
Emission wavelength: Channel 1: 525nm
Excitation light source: LED, Supply voltage 4V, Current consumed 0.35A. Output level 1000 lux.
Software: Anitoa ULVision.exe running on Windows PC
Interface: USB HID

Figure 4. Anitoa CFI x24 Fluorescence Imager module

Method

The quantum dots material is obtained in liquid form. We first dilute the quantum dots with deionized water to make samples of volume 25ul. We created 6 different samples with different level of dilution. The result concentrations are 5μmol/L、4μmol/L、3μmol/L、2μmol/L、1μmol/L、0μmol/L（pure water）.

Put the mixtures in a centrifuge and spin at 5000 rpm for 15 seconds. This is to make sure that there are no bubbles in the solution.

Place the mixtures in the tube rack; we then apply excitation light from the top of the tube. The excitation light source signal strength is centered at around 470nm – channel 1 of CFI x24 light source module.

Capture image of the test tube use CFI x24 Imager Capture Module, Channel 1. The emission channel 1 is use, whose center wavelength is 525nm.

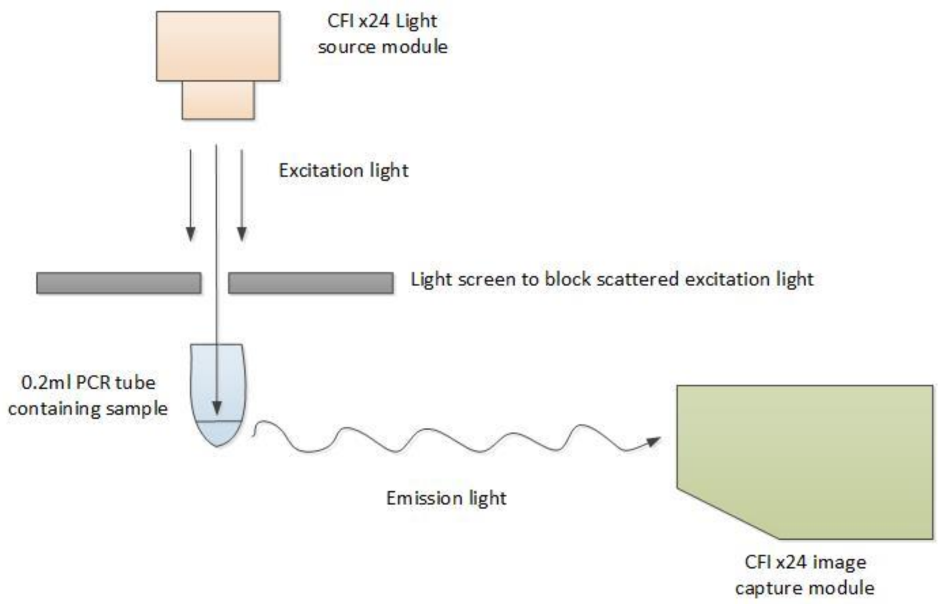


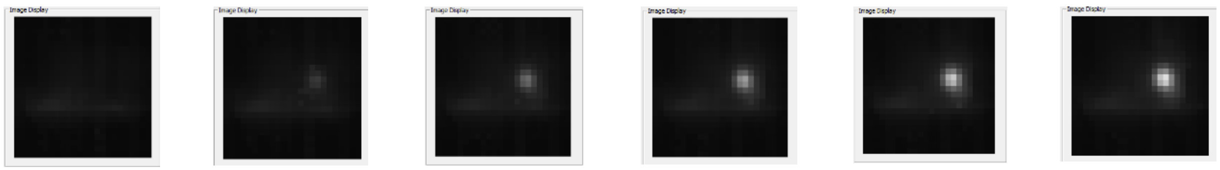
Figure 5. The test set up. The excitation and emission light paths are orthogonal

Results and analysis

Through ULVision.exe, we set the system in high gain mode. Integration time is set at 5ms. The ambient temperature is 30 ℃.

Figure 6 shows the obtained image from the 6 samples of different concentration.

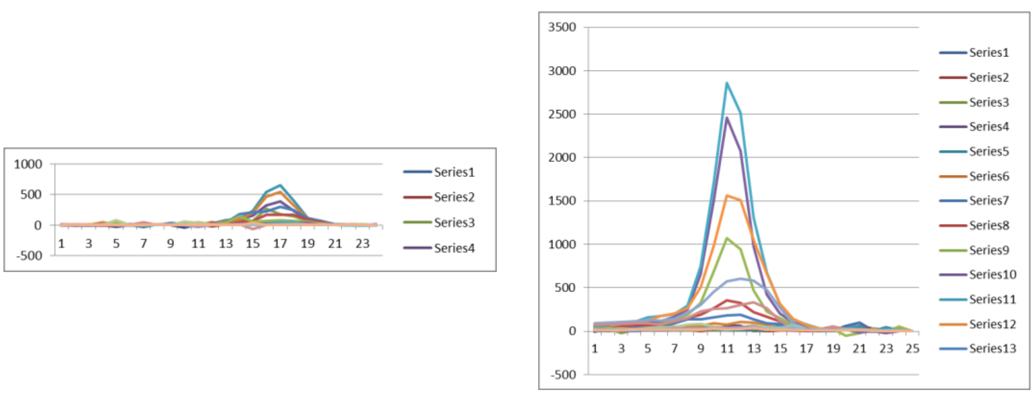
The signal strength is obtained by averaging the values from the pixels of a 4 x 4 area that corresponds to the peak of the signal area.



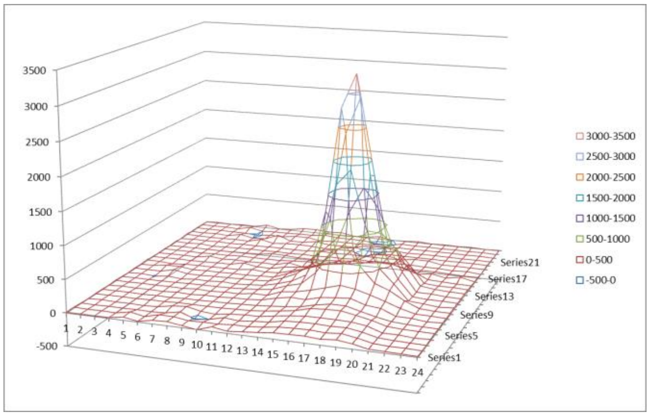
0 um/L 1um/L 2um/L 3um/L 4um/L 5um/L

Figure 6. Captured image of the 6 samples of different concentration.

Below (Figure 7) are the histograms of signals at 1um/L and 4um/L sample concentration from the images above. Dark correction is applied for these histograms.



a b



c

(Vertical axis: digital readout of the imager. Horizontal axis: row and column number of the imager.)

Figure 7. Histograms of images from a. 1 umol/L, b. 4umol/L and c. 5umol/L

The curve in Figure 8 shows the relation between sample concentration and fluorescent reading. We can see that there is a good linear correlation between sample concentration and signal strength.

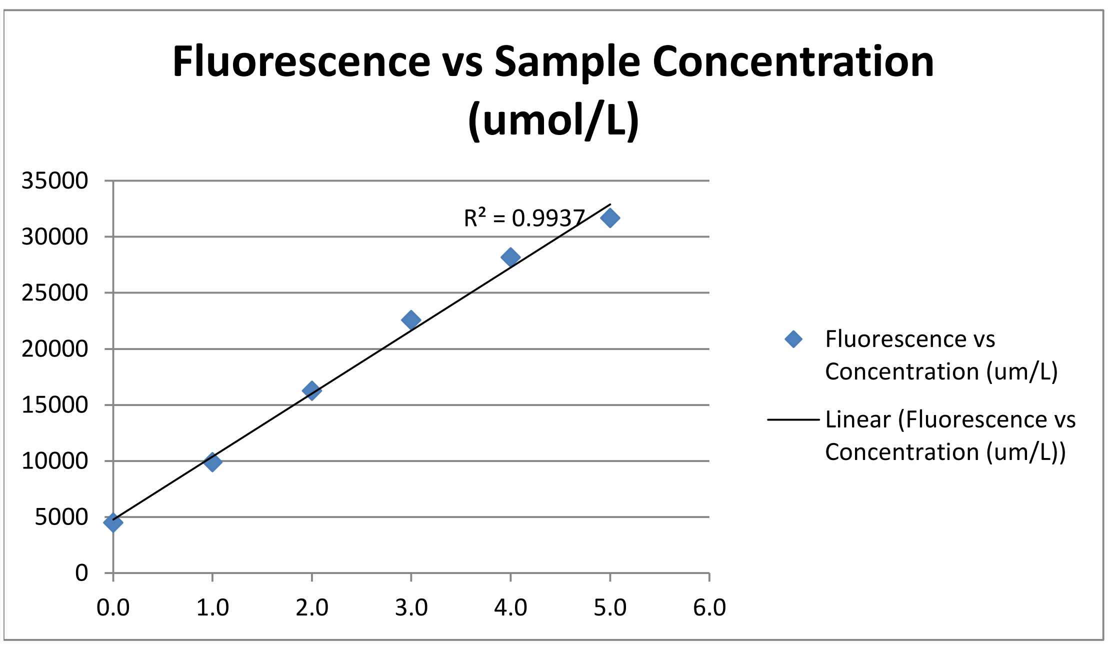


Figure 8. Concentration curve of quantum dot solution measurement

Discussion: Unique characteristics of Quantum Dots and its application as molecular probes

As shown in this study, quantum dots have a lot of desirable properties, such as its bright fluorescence signal level, high signal-to noise-ratio, and good stability. For this reason, quantum dots have been exploited in applications where quantum dots have been used as part of molecular probes.

Another very interesting property of quantum dots is the fact that they can be made to emit different wavelengths signals while sharing a similar excitation spectrum. This is best shown in Figure 3, where an array of different quantum dot material emit a range of nicely spaced signals while can be excited efficiently with a single signal in blue spectrum. This made quantum dots well suited for multiplex fluorescence probe design.

For example, MyQuVigen™ from Nvigen (www.nvigen.com) nanoparticles combine of SPIO and quantum dots providing high magnetic moment and bright stable fluorescence, ideal for controllable magnetic manipulation with extensive, multiplexed fluorescence imaging. MyQuVigen ™ magnetic nanoparticles are ideally used together with mouse antibody for isolation or labeling of cells (e.g. CTCs, stem cells) from a mixture of cell population obtained from tissues or organs.

In collaboration with Nvigen, Anitoa has developed a quantum dot camera that combine deep blue light source with multichannel ultra-low-light imaging module in an integrated unit (shown below Anitoa CFI 224QD). This camera is well suited for imaging of multiplex quantum dot-based assays (e.g. CTC assay) where low cost, tight space and possibly integration with microfluidics is part of the requirement.

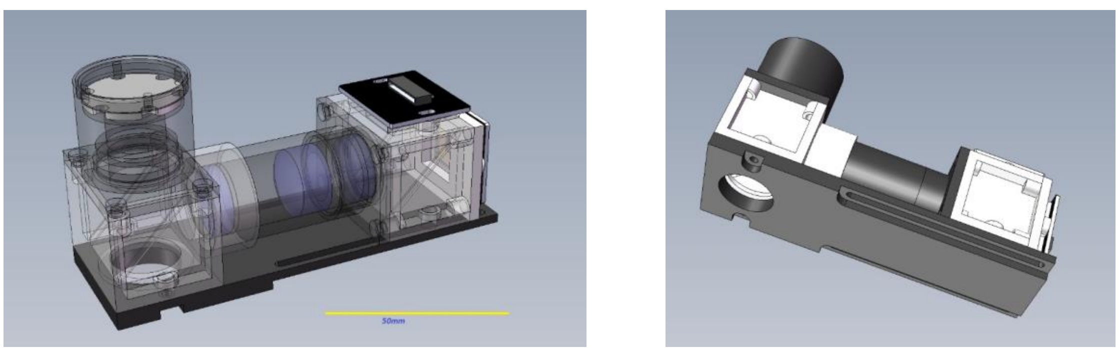


Figure 9. Anitoa's 2-channel integrated Quantum Dot camera (CFI 224QD)