Chapter 25

CCD Based Fiber-Optic Spectrometer Detection

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Summary

Highly sensitive and cost effective measurement tools are required in biotechnology research and applications. Fluorescence provides very simple, cost effective, and sensitive methods in most of the biosensor techniques. Spectrometer is an essential tool for any kind of spectroscopic measurements. A charged coupled device (CCD)-based fiber optic spectrometer is highly compact, light weight, and an extremely easy to use tool. In this chapter, we have described the use of CCD-based fiber-optic spectrometers in detection of fluorescence signal from a fiber-optic-based sensor. The method can easily be extended to fluorescence detection in any other application.

Key words: Spectrometer, CCD, Charge coupled device, Fiber-optic, Fluorescence, Fiber-optic sensor.

1. Introduction

Biotechnology research and applications require highly sensitive and cost effective measurement tools, thus fluorescence provides very simple, cost effective, and sensitive methods in most of the biosensor techniques. These biosensors can be used to detect various kinds of bioactive compounds like an enzyme, an antibody, or a nucleic acid *(1)*. The fluorescence is generated either by the attached fluorescence molecules to the analytes *(2, 3)* or it could be autofluorescence generated by the analyte.

To achieve better results and faster measurements, new technologies are emerging. A charged coupled device (CCD)-based fiber-optic spectrometer is one such device. These spectrometers are highly compact, light weight, and extremely easy to use. Here we have described the use of CCD-based fiber-optic spectrometers

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in detection of fluorescence. To illustrate various aspects of its use we have chosen its application in a fiber-optic-based sensor *(4)*.

2. Materials

2.1. CCD-Based Fiber-Optic Spectrometer

1. High-resolution Spectrometer (HR2000+, Ocean Optics, Inc., Dunedin, FL).
2. HR Grating 400–800 nm range; Installed (H9, Ocean Optics, Inc., Dunedin, FL).
3. Order-sorting detector filter; Installed (OFLV-400800, Ocean Optics, Inc., Dunedin, FL).
4. Detector Collection lens; Installed (L2, Ocean Optics, Inc., Dunedin, FL).
5. Optical bench entrance aperture, 200 μm width; Installed (SLIT-200, Ocean Optics, Inc., Dunedin, FL).
6. Laptop computer with USB2 port and Window XP operating system (Latitude D620, Dell, Inc.).
7. 600-μm core silica clad, collection fiber with SMA 905 terminators on both ends (P6002-VIS/NIR, Ocean Optics, Inc., Dunedin, FL).

2.2. Laser Diode

1. Laser diode 405 nm and 4 mW Power (LDM405, Thorlabs, Inc., Newton NJ).
2. 405-nm narrow band filter (NT43104, Edmund Optics, Inc, Barrington, NJ).
3. Lens tube (SM1L05, Thorlabs, Inc., Newton NJ).
4. Lens tube Spanner Wrench (SPW602, Thorlabs, Inc., Newton NJ).

2.3. Collection/ Excitation Chamber

1. 30-mm Cage Cube (C4W, Thorlabs, Inc., Newton NJ).
2. Two packaged collimation lenses of f = 11.0 mm and NA = 0.25 (F220SMA-A, Thorlabs, Inc., Newton NJ).
3. Two collimation lens Mounting Adapters (AD11F, Thorlabs, Inc., Newton NJ).
4. Kinematic Mounting Plate (B4C, Thorlabs, Inc., Newton NJ).
5. Cage Cover Plate (B1C, Thorlabs, Inc., Newton NJ).
6. Short-pass filter (SP500R/25, Maier Photonics, Inc., Manchester Center, VT).
7. 1″ Optic Mount for mounting Beam splitter (B5C, Thorlabs, Inc., Newton, NJ).
8. 1″ End cap (SM1CP1, Thorlabs, Inc., Newton NJ).

9. Laser Line Long-pass Filter 457.9NM (NT47501, Edmund Optics, Inc, Barrington, NJ).
10. Lens tube (SM1L10, Thorlabs, Inc., Newton NJ).
11. Fiber adapter (SM1SMA, Thorlabs, Inc., Newton NJ).

2.4. Fiber Probe

1. 600-μm core silica clad fiber (Fiber-600-VIS/NIR, Ocean Optics, Inc., Dunedin, FL).
2. Bare Fiber Terminator (BFTU, Thorlabs, Inc., Newton NJ).
3. SMA 905 Fiber Connector (10640A, Thorlabs, Inc., Newton NJ).
4. Diamond Wedge Scribe (S90W, Thorlabs, Inc., Newton NJ).
5. Bunsen Burner (17928027, VWR Scientific Products).
6. Ultrasonic Cleaner (1533722C, Fisher Scientific International).
7. Centrifuge (13100510, Fisher Scientific International).
8. Hydrofluoric acid 50% (A1461LB, Fisher Scientific International).
9. PBS (BP24384, Fisher Scientific International).
10. Dry Acetone (AC32680, Fisher Scientific International).
11. Sodium Hydrochloride (Fisher Scientific International).
12. Sodium Bicarbonate (Fisher Scientific International).
13. Aminosilane Reagent (3-Aminopropyltriethoxysilane) (80370, Pierce Biotechnology, Inc., Rockford, IL).
14. Cysteamine hydrochloride (MEA) (30078, Sigma-Aldrich Co., St. Louis, MO).
15. EDTA (17892, Pierce Biotechnology, Inc., Rockford, IL).
16. Desalting Column (CS-800, Princeton Separations, Adelphia NJ).
17. Antibodies to be immobilized.
18. Sulfo-SMCC cross linker (22322, Pierce Biotechnology, Inc., Rockford, IL).

3. Methods

3.1. Selection of Spectrometer and Its Accessories

1. For the biosensor applications it is assumed that real time signal monitoring is the preferred method. This requires that the spectrometer should have a high speed of spectra recording. HR2000+ from Ocean Optics has the capability of transferring spectra continuously at a rate of 1 ms per spectra. Such a high speed is ideal for real time recording of the signal.

2. Grating is the main component of any spectrometer. Choice of grating depends on the required spectral range. For most of biosensor experiments, the spectral range is generally from 400 to 800 nm *(4)*. For this kind of spectral range, a preinstalled H9 grating should be ordered with the spectrometer.
3. Most of the time higher order spectra interfere with required first order spectrum therefore it is always safe to get a spectrometer with preinstalled long-pass filter. HR2000+ should be obtained with a preinstalled OFLV (Variable Long-pass Order-sorting Filters) filter to the detector's window to eliminate second- and third-order effects. This will give a clean first-order spectrum.
4. Another parameter to decide while getting a spectrometer is the slit width as most of the CCD-based miniature spectrometers come with a preinstalled slits. The width of the slit determines the amount of light entering the spectrometer. Higher the width more light will enter the instrument but smaller slit width gives better optical resolution. If no slit is used, the diameter of the fiber connected to the spectrometer determines the size of the entrance aperture. In most of the biosensor experiments, the recorded spectra are either from a molecular dye or from the autofluorescence of the sample. The spectral width of these spectra is typically from 30 to 100 nm *(4)*. A highest available slit width of 200 µm is reasonable to get maximum light collection efficiency and optimum resolution of 5 nm.
5. Generally slit height is larger than the CCD array height, to utilize maximum input intensity a cylindrical lens is needed to focus the light from the tall slit onto the shorter detector elements. The spectrometer should be ordered with a preinstalled L4 lens. This cylindrical lens is fixed to the detector's window and it increases light-collection efficiency and reduces stray light. It is also useful in a configuration with a large-diameter fiber for low light-level applications.

3.2. Assembling Collection/Excitation Chamber

A photograph of assembled collection chamber is shown in **Fig. 1**:

1. Laser diodes produce significant emission in the red spectral range besides lasing at the laser wavelength. This red light can interfere with the fluorescence signal. To avoid this unnecessary emission (*see* **Note 1**) a laser line filter NT43104 (Edmund Optics, Inc.) should be installed in front of the Laser diode LDM405 (Thorlabs, Inc.). Filter should be first mounted into the lens tube SM1L05 (Thorlabs, Inc.) as this lens tube fits well on the laser diode head.
2. Mount the laser assembly (*see* **Note 2**) on one of the four side holes of the cage cube C4W (Thorlabs, Inc.).

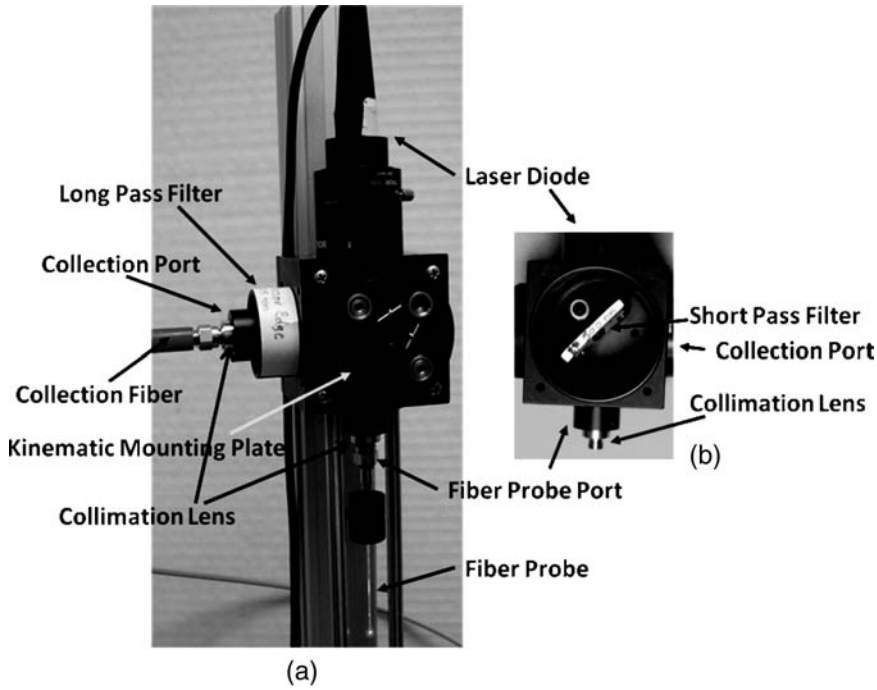

Fig. 1. **a** Photograph of the collection chamber along with a fiber probe. **b** Photograph showing interior of the collection chamber.

3. Intensity of small amount of scattered or back-reflected laser light is generally comparable with the fluorescence signal and thus can interfere with its detection. To avoid this interference, direct entry of the scattered and back-reflected laser light should be prevented from entering the spectrometer. This can be achieved by using laser line long-pass filter NT47501 (Edmund Optics, Inc.). Transmission of this filter for fluorescence signal wavelengths (457–630 nm) is around 98% but for laser wavelength (405 nm) its transmission is only 10^{-6}%. First mount the filter NT47-501 (*see* **Note 3**) in a SM1L10 lens tube and then mount one end of this tube on one of the side holes (right angle to the laser mounted hole) of the cage cube C4W. On the other end of the lens tube, mount the collimation lens F220SMA-A (Thorlabs, Inc.) with the help of lens Mounting Adapters AD11F (Thorlabs, Inc.). Collimation lens should face toward the cage cube. This is the fluorescence collection port (**Fig. 1**) of our experimental setup. Connect one end of a 2-m fiber P600-2-VIS/NIR (Ocean Optics, Inc.) to this port (SMA connector side of the collimation lens assembly) and other end to the spectrometer input port.
4. Third hole of the cage cube opposite (180°) to the laser mounted hole is used as the output port or fiber probe port.

Second collimation lens F220SMA-A (Thorlabs, Inc.) should be mounted with the help of lens Mounting Adapters AD11F (Thorlabs, Inc.) on this hole. Collimation lens should face toward the cage cube.

5. Block the fourth hole with the help of 1″ end cap SM1CP1 (Thorlabs, Inc.).
6. Short-pass filter SP500R/25 (Maier Photonics, Inc.) should be mounted on a 1″ Optic Mount B5C (Thorlabs, Inc). This filter has about 98% reflectivity from 530 to 689 nm and about 90% transmission for 405 nm. The mounted short-pass filter should further be mounted on the kinematic mounting plate B4C (Thorlabs, Inc.).
7. Kinematic mounting plate with short-pass filter SP500R/25 should be fitted on bottom of the cage cube in such a way (*see* **Note 4**) that filter is inside the cage and makes 45° angle with the incoming laser light (**Fig. 1**). The angle should be such that the reflected fraction of the laser should hit the blind port with end cap SM1CP1. For optimum alignment of the short-pass filter replace the SM1CP1 end cap (*see* **Note 5**) with fiber adapter SM1SMA (Thorlabs, Inc.). Connect one end of the P600-025-VIS/NIR fiber to the fiber adapter and point other end toward a white paper. Turn the laser switch on and make sure the shutter in front of laser is on. Now slowly rotate kinematic mounting plate for maximum laser output intensity through the P600-025-VIS/NIR fiber. At the maximum output intensity position tighten the four screws of the kinematic mounting plate. Once the alignment is over, replace the fiber adapter with end cap.
8. Now the setup is ready to record fluorescence signal. A photograph of the experimental setup is shown in **Fig. 2** and a schematic of the setup is shown in **Fig. 3**.

3.3. Preparation of Fluorescence Fiber Probe

3.3.1. Fabrication of Silica Fiber Probe

1. Use a diamond wedge scribe S90W (Thorlabs, Inc.) to cut a 10-cm long fiber piece from Fiber-600-VIS/NIR (Ocean Optics, Inc.)
2. With the help of a Bunsen Burner, burn off 2 cm buffer coating from one end of the 10-cm fiber probe.
3. Rinse the probe with distilled water in ultrasonic cleaner for 4 min.
4. Prepare a 15% Hydrofluoric acid solution by mixing about 2.2 parts of distilled water with one part of 50% hydrofluoric acid.
5. Dip buffer less part (2 cm) of probe in to the 15% hydrofluoric acid until etch to the desired diameter of 270 µm (for 15% HF, the etching rate is about 18.5 µm/h) (*see* **Note 6**).
6. Again rinse the etched probe with distilled water in ultrasonic cleaner for 4 min.

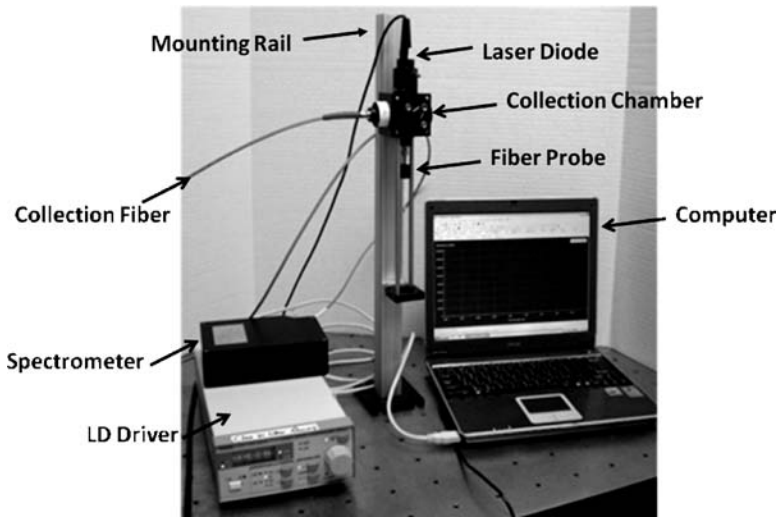

Fig. 2. Photograph of the experimental setup. The laser used in this setup has a separate laser diode driver but LDM405 from Thorlabs, Inc. has a built-in driver.

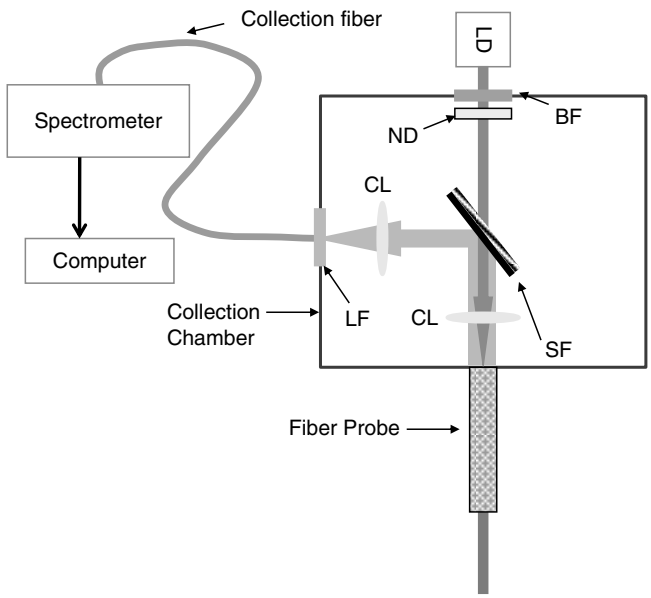

Fig. 3. Schematic of evanescent wave excited fluorescence-based fiber-optic sensor. LD Laser diode, CL Collimation lens, BF Band-pass filter, ND Neutral density filter, LF Long-pass filter, and SF Short-pass filter.

7. After etching burn off the buffer coating from the whole 10 cm long probe on a Bunsen burner.
8. Rinse the probe with distilled water in ultrasonic cleaner for 4 min.

9. Rinse the probe with 1 N NaOH in ultrasonic cleaner for 4 min.
10. Rinse the probe with distilled water in ultrasonic cleaner for 10 min.
11. Rinse the probe with acetone in ultrasonic cleaner for 10 min.

3.3.2. Immobilization of Antibodies on Silica Fiber Probe

1. Thoroughly wash and dry the glass or quartz fiber probe surface to be coated (*see* **Note 7**).

Aminosilylate the Fiber Probe Surface

2. Prepare a 2% solution of the Aminosilane Reagent (3-Aminopropyltriethoxysilane) in acetone. For example, mix 1 part Aminosilane Reagent with 49 parts dry (i.e., water-free) acetone. Prepare a volume sufficient to immerse required probe length. (2 cm probe length will require 400 µl of solution in 1.0 mL without-cap plastic tube or 400 µl in 0.6 mL centrifuge tube.)
3. Immerse probes in the diluted reagent for 60 s.
4. Rinse surface with dry acetone.

Partially Reduce Antibody to Produce Sulfhydryls for Coupling

1. Prepare 0.5 M MEA stock solution by dissolving 6 mg Cysteamine hydrochloride (MEA) in 100 µl Coupling Buffer. (For future use, store this solution at 4°C).
2. Prepare 0.1 M EDTA stock solution by dissolving 3.72 g EDTA power in 100 mL PBS buffer. (This solution could be stored at room temperature.)
3. Prepare Reducing Agent by mixing 50 mM MEA and 10 mM EDTA into Antibody solution. (For total 20 µL of Antibody before dilution, mix 2.5 µL 0.5 M MEA, 2.5 µL 0.1 M EDTA, and 20 µL 0.5 mg/mL Antibody in a 0.2 mL tube.)
4. Incubate the Reducing Agent for 90 min at 37°C (*after this step start working on* **Subheading "Maleimide-Activate the Amino-Modified Surface,"** *after completing* **Subheading "Maleimide-Activate the Amino-Modified Surface,"** *come back to step 5* of this section).
5. Prepare a Desalting Column CS-800 (Princeton Separations) by adding 650 µL EDTA buffer in Desalting Column powder and incubate at least 30 min. Then spin it for 2 min (the maximum amount of reagent for one column could desalt is 100 µL).
6. Purify the reduced antibody from the Reducing Agent using the Desalting Column (*proceed to* **Subheading "Cross-Link Sulfhydryl-Containing Antibody to Activated Surface"** *as you must have already completed section 3*).

Maleimide-Activate the Amino-Modified Surface

1. Prepare a 4.2 mM Sulfo-SMCC cross linker solution in PBS. (For total 400 µL of cross linker solution, add 0.8 mg Sulfo-SMCC

powder to 400 µL PBS buffer). This solution must be used immediately to avoid hydrolysis (*see* **Notes 8** and **9**).

2. Incubate probes in cross linker solution for 1 h at room temperature.
3. Rinse the modified surface with Coupling Buffer (*see* **Note 10**).

Cross-Link Sulfhydryl-Containing Antibody to Activated Surface

1. Dilute the reduced antibody solution to about 5–10 µg/mL.
2. Submerge the maleimide-activated fiber probe with the antibody solution. The antibody solution may be diluted in Coupling Buffer to a volume sufficient to submerge the required probe length (generally 2 cm).
3. Incubate it for 4 h at room temperature.
4. Thoroughly rinse the surface with Coupling Buffer to ensure that only covalently attached antibody molecules remain.
5. The surface is now ready to use for detection assays and other applications. Depending on stability of the particular antibody, the surface material may be dried for storage or kept covered in buffer containing 0.02% sodium azide.

3.4. Recording Spectrum/Signal

When immobilized antibodies come in contact with the specific analyte, the analyte get attached to the probe. Now either the analyte itself produce some kind of autofluorescence, or fluorescence can be generated by attaching another labeled antibody to this analyte (sandwich method).

1. Mount the prepared fiber probe on a bare fiber terminator BFTU (Thorlabs, Inc.) by inserting its unetched side into SMA 905 fiber connector attached to BFTU. A photograph of a mounted fiber is shown in **Fig. 4**.
2. Connect mounted fiber-optic sensor probe to the *output port* (*see* **Note 11**) of the collection chamber.
3. First install the spectrometer software (*see* **Note 12**) on the laptop Latitude D620 (Dell, Inc.) and then connect the

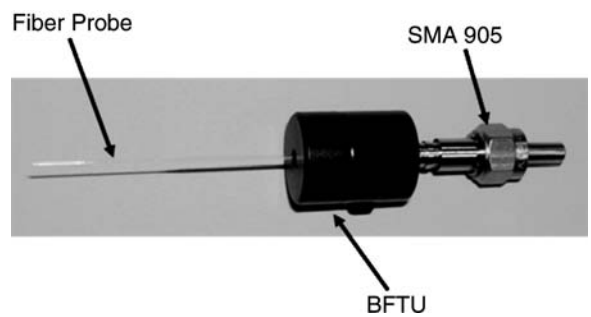

Fig. 4. A photograph of a mounted fiber probe.

spectrometer HR2000+ to the USB2 port of the laptop with the provided USB cable. Once the HR2000+ is installed, it must be configured by operating software's *Configure Spectrometer* options so that operating software (OOIBase32) recognizes the HR2000+ spectrometer. Details can be obtained from the operation manual of the software.

4. For power stabilization, it is always a good idea to start the laser few minutes before starting the experiment.
5. Start the spectrometer operating software and open the laser shutter. Record the fluorescence signal by clicking the appropriate button. Try to adjust the integration time of the spectrometer to a value, such that the maximum spectral amplitude is less than 90% of the full scale. This will ensure that recorded signal is not saturated.
6. For maximizing the signal adjust the three hex adjuster fine alignment screws (*see* **Note 13**) on the back (**Fig. 1**) of kinematic mounting plate B4C (Thorlabs, Inc.)
7. Once the maximum signal value is known and integration time is adjusted, close the laser shutter and record the dark back ground signal (*see* **Note 14**) and store it.
8. Save this signal and turn the background deduction button on. (Details about these various operations can be read in the software manual of the spectrometer).
9. Now record the spectrum of the collected (*see* **Note 15**) fluorescence and save it to the file.

4. Notes

1. Red tail emission from the laser diode can be further reduced if the laser is operated at much higher power than the required power and neutral density filters are placed in the laser path along with the band-pass filter to reduce the power level to the required value.
2. All the SM1 series lens tubes and cage cube (Thorlabs, Inc.) holes have compatible threading therefore mounting of these tubes on the cage cube is straightforward.
3. Make sure that the coated part of the laser line filter NT43-104 faces the laser side. Generally there is a mark on the ring of the filter which indicates the coated side.
4. Make sure that the coated part of short-pass filter faces the spectrometer port or collection port.

5. Replacement of end cap SM1CP1 with fiber adapter SM1SMA and its connection with the fiber P600-025-VIS/NIR is only done for alignment purpose. After completing the short-pass filter alignment, fiber adapter should be replaced back with end cap.
6. Make sure the probe stand vertically in the acid solution.
7. Perform **steps 2** and **3** in a fume hood.
8. The Reducing Agent (**Subheading "Partially Reduce Antibody to Produce Sulfhydryls for Coupling"**) should be made first because it needs 90 min incubating time.
9. The better reaction of NHS-ester is in the environment of pH = 8.3. However, maleimide group will slowly hydrolyze and loses its reaction specificity for sulfhydryls at pH values >7.5. For these reasons, conjugations with these cross linkers are usually performed at pH 7.2–7.5.
10. The maleimide-activated surface may be dried and stored desiccated at 4°C for later use.
11. This connector is for 630-μm outer diameter fiber. For other diameter fibers appropriate SMA 905 fiber connector should be used.
12. This port is called *output port* as the excitation laser comes out of this port although it also acts as an input port for collected fluorescence.
13. Never connect the spectrometer to the computer, without first installing the required software and drivers. This is a onetime step if you are using the same computer for future experiments.
14. This is one time adjustment for improving the signal to noise ratio.
15. Always keep the integration time of the back ground signal and actual signal same.

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