Microspheres for Medical Diagnostics. An Investigation of the Whispering Gallery Mode Sensing Platform.

Jessica Mackinnon, Honours. Biosensors & Analytical Chemistry.

Supervisors: Dr. Ian Potter (Department of Chemistry, La Trobe University) & Dr. Karl Poetter (Genera Biosystems, Walter and Eliza Hall Institute for Medical Research)

LA TROBE UNIVERSITY
Department of Chemistry

A novel microsphere-Whispering Gallery Mode (WGM) based biosensor as a sensing platform to diagnose bacterial and viral agents using the biological analytes, Chlamydia trachomatis and the Human Papilloma Virus (HPV) was explored.

Silica microspheres were silanized with organo-silane derivatives to facilitate the covalent coordination of single stranded oligonucleotide probes specific for the target analytes. Experimental optimisation studies were conducted to enhance the conjugation efficiency of oligonucleotide probes to the silanized microsphere surface. Different coupling chemistries were investigated in the sensor fabrication, including thioether linkages and disulphide/thiol exchange reactions. Each oligonucleotide probe contained a thymine base modified with a primary amine to facilitate the effective coordination of fluorophores with N-succinimidyl ester functionality. Excitation of the fluorophore by a He-Ne laser during confocal microscopy studies resulted in the coupling of the emitted light into the microsphere resonator chamber and propagation by total internal reflection. Leakage of the propagating light from the resonator chamber generated a spectrum of sharp, period peaks, termed Whispering Gallery Modes.

Parameters affecting the Whispering Gallery Mode emission spectrum were investigated, including: resonator size, fluorophore distance and brightness, oligonucleotide orientation, microsphere uniformity and photo-bleaching. Interference studies were conducted to determine the extent of non-specific DNA binding to the microsphere surface, and the partial hybridization of non-specific oligonucleotides. The shift in the Whispering Gallery Mode emission spectrum upon hybridization of a transprobe to the complementary region of the DNA probe was investigated.

The potential of the WGM shift to confirm the presence of bacterial and viral agents was demonstrated. Further research is required to confirm the feasibility of a biosensor based on the microsphere-WGM sensing platform.

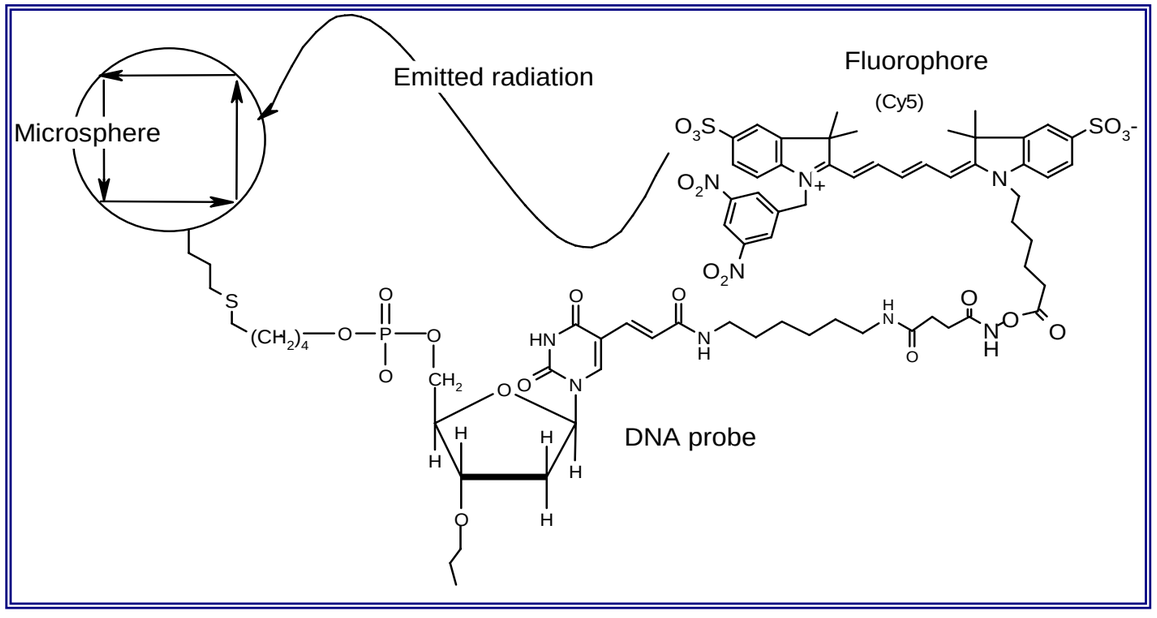

Figure 1. Fluorophore covalently coordinated to the oligonucleotide probe absorbs laser light. Subsequent excitation and relaxation results in emission of photons that are coupled into the microsphere internal cavity (resonator chamber) and confined by total internal reflection.

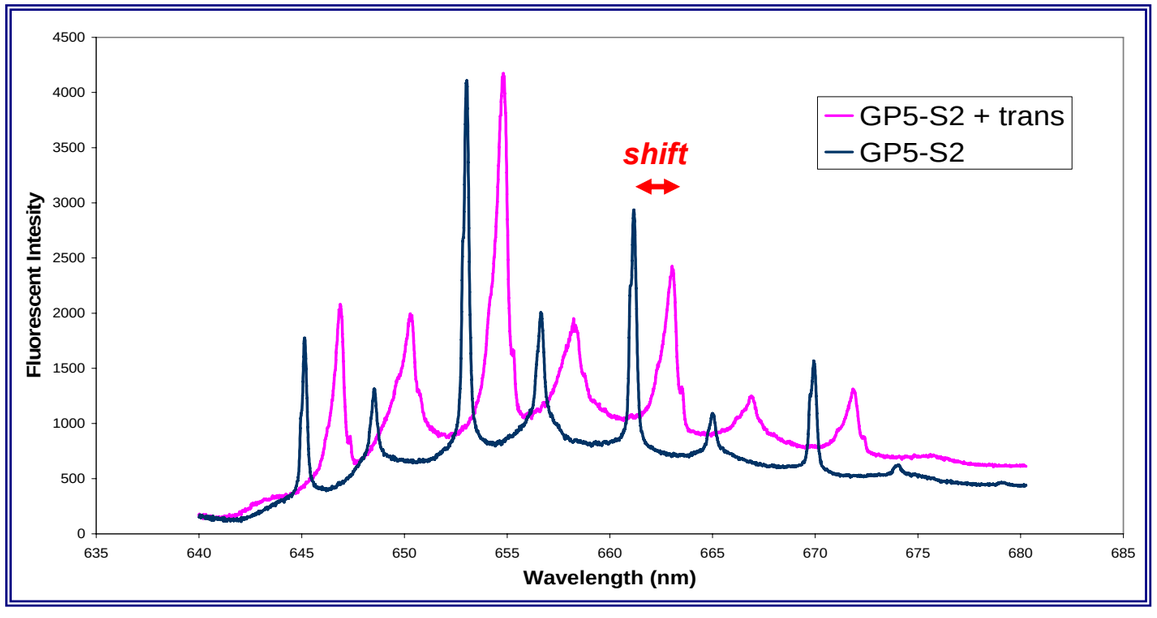

Figure 2. Confocal microscopy studies of 6.8 µm silanized microspheres with Cy5 GP5-S2 oligonucleotides. A red shift of 4-5 nm in the WGM emission spectra was observed following hybridization of the transprobe.