ECE 416 Homework 2  
Due: Friday, February 8.

“Biosensor Statistics”

In this homework, we will investigate several parameters that test a biosensor’s usefulness. We have provided an excel file on the ECE 416 website (‘HW2data\_1.xlsx’) that is to be used with this assignment. The file contains actual data taken from a new type of laser biosensor that you will learn about later in the semester. The sensor outputs a laser wavelength, whose value shifts color when it senses biomolecules, virus particles, or cells. The system is able to lase at two wavelengths simultaneously, where represents the ‘active’ sensor, while represents a reference.

1. Noise

Here, we will characterize the noise of the laser biosensor, and the ability of the reference to correct external sources of noise. “Noise” is defined as a shift in the laser wavelength that is not caused by attachment of biomolecules. To characterize noise, a sequence of laser wavelength measurements are gathered with the active and reference sensors exposed to pure water.

**Q1: Use the given data to calculate: MEAN LASER WAVELENGTH, STANDARD DEVIATION, and COEFFICIENT OF VARIATION.**

Now subtract the reference sensor values from the active sensor values on a point by point basis.

**Q2: Plot both the uncorrected and the reference corrected data on the same plot (you may want to introduce some small offset to the data so that you can directly compare finer details. How has the data changed?**

**Q3: Now calculate the MEAN LASER WAVELENGTH, STANDARD DEVIATION, and COEFFICIENT OF VARIATION for the corrected data**.

1. Z-factor and ROC Curves

A protein microarray was used to detect a biomarker in a serum sample. The microarray is comprised of a large number of individual small spots (200µm diameter) where each spot is used to perform an independent test for the biomarker.

The array contains a large number of “negative control” spots, where no antibody is printed. For our biomarker, we have applied an equally large number of “positive control” spots, where the capture antibody to our biomarker is printed. For the purposes of this homework, we will limit the test to 10 spots each. Real tests utilize many times this amount.

The negative control and positive control spots are all exposed simultaneously to the first test sample containing a HIGH biomarker concentration of 100 ng/mL, and then subsequently a sample with a LOW biomarker concentration of 10 ng/ml on a separate, second test. The microarray spots are read out using a laser scanner to measure their fluorescent output, where the fluorescent intensity of each spot is shown in the second file given on the website (‘HW2data\_2.xlsx’).

**Q4: Calculate the MEAN and STANDARD DEVIATION of both the positive and negative controls for the HIGH and LOW concentration samples. Calculate the Z-FACTOR for both the HIGH and the LOW concentration samples (10 values in total). Are these assays useful?**

**Q5: Create a frequency histogram for both cases (as shown on slide 21 in Lecture 3) “Fluorescent Counts” on the x-axis and “Number of spots” on the y-axis.**

graphs saved in directory – change the axis titles though, r script is also in directory

**Q6: Calculate the True Positive Fraction (TPF) and False Positive fraction (FPF) for each x-axis value and generate a ROC curve for the LOW and High concentration biomarker experiments.**

High: tpf: 1, fpf: 0

Low: figure out cutoff place, then calc;

Count num fp, num fn, then divide by total (of what?)

One item overlap: so

1. Amino Acids

**Q7: How many unique sequences of amino acids 15 peptides long can you make using only:**

1. **Hydrophobic amino acids with a benzene ring**
   1. **3^15**
2. **Hydrophilic acidic amino acids**
   1. **2^15**

**Q8: Which of the amino acids can form covalent bonds with glutaraldehyde molecules?**

**Lys, arg, asn, gln or tail­­**

**Q9: A single-stranded DNA molecule is immobilized to a surface with its 3’ end closest to the surface. The nucleotide sequence is: 3’:AATCGGCTCCTC:5’. List every possible complementary strand of DNA that can bind with the immobilized strand that is exactly 4 nucleotides in length.**

**Q10: For each of these proteins: Ribosomes, ligand-gated ion channels, DNA polymerase, G-couled protein receptors**

**Describe:**

**A: It’s approximate molecular weight,**

**B: The number of amino acids in the protein**

**C: Whether its highest level of structure is tertiary or quaternary**

**D: The relationship between the protein’s structure and its biological function, (i.e. do *α*-helices and *β*-sheets form structures that give the protein a desired shape?)**

**E: The biological importance of the protein.**

1. Microfluidics

Consider a “nano-scale” fluid channel (Height = 200 nm, Width = 50 nm, Length = 1 mm) where a biosensor comprises the bottom surface.

The biosensor is used to characterize the binding interaction between two different proteins, P1 and P2. P1 is flown in solution through the fluid channel, and P2 is immobilized on the sensor surface. For P1, D= 5x10-11 m2/sec. P2 is immobilized with a density of RT = 3x10-7 moles/m2.

Using the biosensor output (R), the following measurements were gathered.

**Table 1**

|  |  |
| --- | --- |
| **Concentration of P1 (M)** | **R (AU)** |
| 1.0E-13 | 128 |
| 1.0E-12 | 128 |
| 1.0E-11 | 142 |
| 1.0E-10 | 180 |
| 8.5E-10 | 268 |
| 1.0E-09 | 350 |
| 1.0E-08 | 490 |
| 1.0E-07 | 530 |
| 1.0E-06 | 535 |
| 1.0E-05 | 535 |

**Table2**

|  |  |
| --- | --- |
| **(AU/s)** | **R(AU)** |
| 0.1 | 150 |
| 0.08 | 250 |
| 0.05 | 380 |
| 0.02 | 500 |

**Q11: Find ka**

**Q12: Calculate the flow rate required to give a diffusion layer depth equal to the height of the flow cell.**

δ = where h = height of the channel, b = width of the channel, l = length of the channel

**Q13: What would the flow rate need to be in order to have approximately equal rates for mass transfer and chemical reaction on the sensor surface?**