**ECE514/BioE516 – Advanced Biosensors**

**Instructor:**

Prof. Brian T. Cunningham

**Description:**

The course will teach the fundamental operating principles of technologies at the forefront of biosensor research that have not yet been developed into commercial products, but that hold promise for offering benefits in terms of sensitivity, selectivity, quantitation, multiplexing, and cost. In addition to signal transduction technologies that utilize engineering principles, the course will also discuss the challenges for detecting novel classes of biological analytes, and ultraselective molecular biology approaches for recognizing them with specificity. The course is organized through case studies, surrounding detection of classes of biological target materials. We will discuss the biological and clinical relevance of each target class, followed by discussion of the engineering and biochemical principles used to detect it. The target classes considered in class evolve from biomolecules, through more complex analytes such as extracellular vescicles, viruses, and cells.

**Prerequisite:**

ECE/BioE 416 – Biosensors

**Text:**

None. Assigned reading from scientific journal papers

**Outline:**

(29 class sessions. Class sessions for each section in parentheses)

1. Introduction to the class and overview (1)
2. Key principles extended from ECE416 (6)
   1. Principles of fluorescence
   2. Introduction to radiometry principles
   3. Principles of optical, acoustic, and electrochemical sensors
   4. Rapid review of surface functionalization
   5. Rapid re-introduction to diffusion and mass transport limitations with flow
3. Challenges at the forefront of biosensing (7)
   1. Digital resolution: Single cell, single virus, single molecule
   2. In vivo
   3. Multiplexing at large scale
   4. Getting out of the lab: Point of care
   5. Point of care biosensors and health disparities
   6. Wearables: Perspiration sensors and tattoo sensors
4. Advanced molecular biology tools for biosensing (4)
   1. Nucleic acid targets for liquid biopsy: miRNA, ctDNA
   2. Nucleic acid target detection options
   3. CRISPR/Cas technology: From gene editing to biodetection. SHERLOCK, DETECTOR, and ACC
   4. Nanostring
   5. 10x Genomics
   6. Proximity extension assays for protein detection
5. Advanced transducers (4)
   1. Circulating tumor DNA and magnetic cell sorting
   2. Plasmonic fluors
   3. Synthetic biology-based biosensors
   4. Biosensors and machine learning
6. Biosensor microscopy (4)
   1. Mass photometry (iScat) and label-free single molecule detection
   2. DNA Origami, self-assembled nanoantennas, and smartphone-based detection of individual fluorophores
   3. Photonic crystal enhanced fluorescence
   4. Photonic resonator interference scattering microscopy
7. Recorded lectures: Preparing the sections of an NIH R21 grant
8. In-class presentations of R21 proposals (1)
9. Conducting an in-class NIH review panel (2)

**Grading:**

* 40% Exams (3 – take home midterms)
* 40% Term Paper: Preparation of an NIH R21 grant proposal
* 20% Class Participation and Attendance

**Course Objectives:**

* Teach fundamental operating principles of technologies at the forefront of biosensor research that have not yet been developed into commercial products, but that hold promise.
* Discuss challenges for detecting novel classes of biological analytes and ultraselective molecular biology approaches for recognizing them with specificity
* Utilize case studies that discuss biological and clinical relevance along with engineering and biochemical principles
* Develop skills in literature research, creative problem solving, and articulating a novel research project in the form of a written funding proposal
* Develop critical thinking, writing, and speaking skills through participating in a mock proposal review panel

**Detailed Topic Description**

1. **Review of key concepts from ECE416**

*The course will begin with a short review of key concepts covered in the pre-requisite required class, as Advanced Biosensors will utilize previous lessons on the principles of fluorescence excitation/emission, optical resonators, and label-free biosensors using a variety of transducers (optical, acoustic, electrical). Advanced Biosensors also will rely upon previous knowledge of immobilization of nucleic acid and protein biomolecules to surfaces using bifunctional linker chemistry. The introductory lectures will provide brief reminders on topics that include noise sources, nonspecific binding, referencing, detection limits, dose-response characterization, and receiver operating characteristics.*

1. **Digital resolution biosensing**

*This lecture will review the state of the art in biosensing, as technologies have evolved from detecting the aggregate properties of many analytes towards the capability of analyzing individual biomolecules, biological nano-objects (viruses and extracellular vesicles), cells, and bacteria. This initial lecture will provide an overview of the digital resolution field, while specific selected technologies will be explored in greater detail later in the semester. Reading: [1]*

1. **In vivo biosensors**

*Biodetection using a device that resides inside the body represents one of the most challenging frontiers in the field. Recently, the ability to provide power to implanted devices through wireless inductive charging of batteries, combined with miniature wireless data interfaces enable long-term operation of devices inside the body. However, challenges remain for preserving the function of biomolecular components of in vivo biosensors and prevention of fouling. In vivo biosensors often are integrated with micro/nanofabricated sampling devices such as needles that can penetrate tissue and withdraw material. The frontiers of in vivo biodetection currently include research applications in neuroscience, gene expression analysis, and synthetic biology. Reading: [2, 3]*

1. **Large scale multiplexing**

*A frontier of biodetection is simultaneous analysis of many different analytes at the same time. While previous technology generations, such as microarrays, addressed the multiplexing challenge through large arrays of printed capture spots, new approaches utilize nanoparticle and biomolecular tags to provide “bar code” systems that integrate multiplexing capability into detection workflows in a more elegant fashion. In ECE416, we discussed micron-scale particle bar code systems (Luminex) that can provide 100x multiplexing for detection of protein biomarkers in clinical samples. Large scale multiplexing for thousands of distinct nucleic acid molecules is at the heart of next-generation DNA sequencing technologies. This lecture will focus upon RNA-seq, an approach in which high throughout DNA sequencing technology is adapted toward transcriptome profiling (the complete set of transcripts in a cell, and their relative quantities at a specific stage of development). Reading: [4, 5]*

1. **Point of care diagnostics**

*Yet another frontier of the biodetection field is the development of sensors, sample preparation cartridges, and instruments that can be used at the point of care (POC). Point of care analysis systems must meet a challenging set of requirements in terms of low cost, robustness, assay simplicity, small size, and rapid time to deliver results. Point of care systems must also carefully consider the use-cases in which a result is required urgently or in resource-limited situations in which laboratory-based diagnostics are not available. This lecture will review and discuss POC systems deployed and developed in response to the COVID-19 pandemic, with emphasis upon rapid detection of virus-specific nucleic acid sequences (PCR and LAMP) and issues surrounding POC sample processing of saliva or nasalpharengeal swab extract. Reading: [6, 7]*

1. **Wearables**

*The last frontier area of biosensor technology development that will be covered in this set of lectures are sensors that analyze molecular biomarkers in the body while being worn outside the body. We will focus specifically on transdermal sampling methods that pierce through the skin or detect material in perspiration that is generated on the skin. Reading: [8, 9]*

1. **Nucleic acid target molecules for liquid biopsy**

*The idea of performing a biopsy through analysis of the biomolecule contents of a non-invasively obtained sample of blood, rather than inspection of tissue gathered with a conventional needle biopsy is gaining momentum for making clinical decisions, with a great deal of attention in cancer early diagnosis, therapy effectiveness monitoring, and remission detection. Identification of the molecules that have therapeutic value is being assisted through genomic studies, resulting in classes of molecules including circulating free DNA (cfDNA), circulating tumor DNA (ctDNA), exosomal micro RNA (miRNA), and long noncoding RNA (lncRNA) providing information about tumor size, cancer proliferation mechanism, optimal therapy selection, drug resistance, and emergence of new mutations. This lecture will discuss how liquid biopsy target biomarker molecules are identified and clinically validated, and discuss how each type of biomarker provides distinct information about disease processes. This lecture will also discuss the challenges for detecting nucleic acid biomarkers with biosensor technologies. Reading: [10-13]*.

1. **Nucleic acid toehold probe technology**

*One of the limitations of conventional detection of specific DNA or RNA nucleic acid sequences is Watson-Crick base pair binding interactions, where a small number of nucleic acid mis-matches can lead to nonspecific detection of non-target sequences that are present in a sample with the target sequence. “Toehold probes” represent a departure in which thermodynamic principles are used to strategically engineer probe molecules that can perform unique functions when they encounter the target sequence. Toehold probes can be designed with relatively simple (and broadly available) software tools, and the design molecules can be ordered from commercial vendors. This lecture will introduce the toehold probe technology and describe several examples of how they can be used to achieve highly sensitive and selective biosensors. Reading: [14]*

1. **DNA origami probes**

*DNA can be engineered to detect biological targets besides biomolecules. This lecture will describe how “DNA origami” can be designed to form star-like shapes whose vertices are an exact mechanical match for the array of spike proteins displayed on the outside of a virus. The Designer DNA Nanostructures (DDNs) can be used as high affinity and high selectively capture probes for viruses in conjunction with several types of biosensor transducers. We will also show how complex and large DNA origami structures can be used to link with other nanomaterials, such as gold nanoparticles, to enable positioning with nanometer-scale precision for creating electromagnetic hot spots for amplification of fluorescence and detection with a smartphone-based microscope. Reading: [15, 16]*

1. **Biosensors based upon CRISPR/Cas systems**

*Gene editing technology using the CRISPR/Cas system was recognized with the Nobel Prize in 2020. The biomolecule family used in CRISPR/Cas is quite versatile, and has been effectively applied for high sensitivity detection of nucleic acid molecules for rapid and portable diagnostics. This lecture will discuss the SHERLOCK (Specific High Sensitivity Enzymatic Reporter unlocking) and DETECTR (DNA endonuclease-targeted CRISPR trans reporter) technologies, their advantages relative to PCR, and the remaining challenges.. Reading: [17-19]*

1. **NanoString’s nCounter platform**

*The upcoming two lectures will feature selected biosensor technology platforms that have recently been developed into commercial products and are undergoing broad adoption in life science research. The NanoString nCounter technology utilizes a combination of high resolution fluorescence microscopy, fluorescent molecular barcodes, and novel biochemistry for analysis of molecules that such as mRNA for measuring gene expression. Reading: [20]*

1. **10x Genomics and massively parallel single cell gene expression analysis**

*Studying the genomic characteristics of tissues or aggregates of thousands of cells will miss important details, especially when cells with rare properties are among large populations of other cells. This lecture will discuss the 10x Genomics approach for single cell gene expression analysis, enabling thousands of individual cells to be analyzed at once, with the help of a next-generation sequencing system, such as those from Illumina. The class should review the Illumina lecture from ECE/BioE416. Reading: [21]*

1. **Circulating Tumor Cells (CTC) and Magnetic Cell Sorting**

*Circulating tumor cells represent an informative but challenging type of cancer biomarker that requires sorting a small number of cells with specific characteristics from a much larger number of red and white blood cells. This lecture will discuss the characteristics of CTCs and one specific novel technology for labeling cell features with magnetic nanoparticles that when flowed through a microfluidic device with precisely engineered flow velocity features, can partition CTCs into sub-populations. Reading: [22]*

1. **Synthetic biology biosensors**

*Recently, the proteinproduction and gene regulation machinery of living cells has been adapted toward functioning with the cell, for purposes of selectively recognizing and detecting target molecules. This lecture will describe how transcription factors and the ribosome can be designed for detection of pathogens and other analytes through generation of visually observable products, essentially re-creating the environment of cells in microfluidic devices, paper, and fabric. The lecture will summarize several synthetic biology biosensor strategies and recent examples of implementation for detection of small molecule organic compounds, antibiotics, and metal ions. Reading: [23]*

1. **iScat – label free single biomolecule analysis by mass photometry**

*The next several lectures describe biosensor-enabled microscopies with the capability to detect individual (or very low numbers of) analytes over a field of view. Optical metamaterial surfaces can be designed to provide resonant modes that can interact with surface-attached biomolecules, and by illuminating the device with a tunable laser, shifts in the resonant spectra can be measured on a pixelated basis to create images of the attached material.Interferometric scattering (iScat) microscopy has emerged as a label-free imaging approach that can measure the presence and kinetic motion trajectories of individual biomolecules. It is becoming a useful tool for measuring biomolecular interaction affinity constants, observing the formation of macromolecules from their smaller components, virus detection, and exosome detection. This lecture will discuss the physics principles underlying iScat, the design of iScat instrumentation, and several of the initial applications. Reading: [24]*

1. **Photonic resonator interferometric scattering microscopy**

*Using a metamaterial that supports formation of optical resonances at the same wavelength of an illuminating laser, scattering of light from surface-attached nano-objects (proteins, nanoparticles, viruses, and exosomes) can be magnified, and the out-scattered light will follow pathways that are dictated by the photonic band diagram of the metamaterial surface. These principles have been applied to enable an amplified form of iScat, called Photonic Resonator Interferometric Scattering Microscopy (PRISM) that enables objects to be detected with greater signal-to-noise ratio, instruments that do not require liquid-coupled microscope objectives, using lower power lasers. Reading: [25]*

1. **Plasmonic Fluors**

*This lecture will introduce a technology that features gold nanoparticles that serve as nanoantennas for selectively capturing specific wavelengths of light, and enhancing their electromagnetic fields for the purpose of boosting the photon output of fluorescent dye molecules. The Plasmonic Fluors (PF) are thousands-fold brighter than individual fluorophores, and can be used as tags for a variety of bio-assays. PFs have been used in lateral flow test strips, in cell media to detect protein expression, and in biomarker assays with ultralow detection limits. Reading: [26]*

1. **Photonic crystal enhanced fluorescence microscopy**

*Using enhanced intensity electromagnetic field excitation, Purcell enhancement of the fluorescence lifetime, and directional out-coupling of emitted photons, individual quantum dots on a photonic crystal metamaterial surface can be dynamically imaged and their motion trajectories can be tracked. Importantly, a spatial light modulator can be placed in the path of the emitted photons to select specific wavelengths, offering a novel method for multiplexed and digital resolution ultrasensitive diagnostic assays. This lecture brings together many of the concepts discussed throughout the course. Reading: [27, 28]*

1. **Proximity extension assays for protein detection**

*This lecture will discuss a newly-developed technology for highly multiplexed and quantitative detection of protein biomarkers in complex media that leverages the detection capabilities of next-generation DNA sequencing. Reading: [29]*

1. **Biosensors and machine learning**

*Artificial intelligence (AI) and machine learning (ML) are pervading all parts of engineering and science, with the field of biosensors being no exception. This lecture will explore how ML-based approaches can be applied toward interpreting biosensor microscopy images, complex Raman spectra, and interpreting multi-sensor and multi-modality data to generate “classifiers” that can inform physicians.*

**Literature Reading Assignments**

1. Huang, Q., et al., *Critical Review: digital resolution biomolecular sensing for diagnostics and life science research.* Lab Chip, 2020. **20**(16): p. 2816-2840.

2. Shi, S., E.L. Ang, and H. Zhao, *In vivo biosensors: mechanisms, development, and applications.* J Ind Microbiol Biotechnol, 2018. **45**(7): p. 491-516.

3. Zhang, Y., et al., *Picoliter Droplet Generation for Fast Monitoring the Brain Chemistry with Scaled Silicon Nanodyalisis Probe.* 2019 20th International Conference on Solid-State Sensors, Actuators and Microsystems & Eurosensors Xxxiii (Transducers & Eurosensors Xxxiii), 2019: p. 209-212.

4. Wang, Z., M. Gerstein, and M. Snyder, *RNA-Seq: a revolutionary tool for transcriptomics.* Nat Rev Genet, 2009. **10**(1): p. 57-63.

5. Stark, R., M. Grzelak, and J. Hadfield, *RNA sequencing: the teenage years.* Nat Rev Genet, 2019. **20**(11): p. 631-656.

6. Ganguli, A., et al., *Rapid isothermal amplification and portable detection system for SARS-CoV-2.* Proc Natl Acad Sci U S A, 2020. **117**(37): p. 22727-22735.

7. Gibani, M.M., et al., *Assessing a novel, lab-free, point-of-care test for SARS-CoV-2 (CovidNudge): a diagnostic accuracy study.* Lancet Microbe, 2020. **1**(7): p. e300-e307.

8. Kim, J., et al., *Wearable biosensors for healthcare monitoring.* Nat Biotechnol, 2019. **37**(4): p. 389-406.

9. Tu, J.B., et al., *The Era of Digital Health: A Review of Portable and Wearable Affinity Biosensors.* Advanced Functional Materials, 2020. **30**(29).

10. Avanzini, S., *A mathematical model of ctDNA shedding predicts tumor detection size.* Science Advances, 2020. **6**: p. eabc4308.

11. Peng, Y. and C.M. Croce, *The role of MicroRNAs in human cancer.* Signal Transduction And Targeted Therapy, 2016. **1**: p. 15004.

12. Hayes, J., P.P. Peruzzi, and S. Lawler, *MicroRNAs in cancer: biomarkers, functions and therapy.* Trends in Molecular Medicine, 2014. **20**(8): p. 460-469.

13. Boukouris, S. and S. Mathivanan, *Exosomes in bodily fluids are a highly stable resource of disease biomarkers.* Proteomics Clin Appl, 2015. **9**(3-4): p. 358-67.

14. Chen, S.X. and G. Seelig, *An Engineered Kinetic Amplification Mechanism for Single Nucleotide Variant Discrimination by DNA Hybridization Probes.* Journal of the American Chemical Society, 2016. **138**(15): p. 5076-5086.

15. Kwon, P.S., et al., *Designer DNA architecture offers precise and multivalent spatial pattern-recognition for viral sensing and inhibition.* Nat Chem, 2020. **12**(1): p. 26-35.

16. Trofymchuk, K., et al., *Addressable nanoantennas with cleared hotspots for single-molecule detection on a portable smartphone microscope.* Nat Commun, 2021. **12**(1): p. 950.

17. Cecchetelli, A. *Finding nucleic acids with SHERLOCK and DETECTR*. Addgene Blog 2020; Available from: <https://blog.addgene.org/finding-nucleic-acids-with-sherlock-and-detectr>.

18. Gootenberg, J.S., et al., *Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6.* Science, 2018. **360**(6387): p. 439-444.

19. Chen, J.S., et al., *CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity.* Science, 2018. **360**(6387): p. 436-+.

20. Geiss, G.K., et al., *Direct multiplexed measurement of gene expression with color-coded probe pairs.* Nat Biotechnol, 2008. **26**(3): p. 317-25.

21. Zheng, G.X., et al., *Massively parallel digital transcriptional profiling of single cells.* Nat Commun, 2017. **8**: p. 14049.

22. Poudineh, M., et al., *Tracking the dynamics of circulating tumour cell phenotypes using nanoparticle-mediated magnetic ranking.* Nature Nanotechnology, 2017. **12**(3): p. 274-+.

23. Voyvodic, P.L. and J. Bonnet, *Cell-free biosensors for biomedical applications.* Current Opinion in Biomedical Engineering, 2020. **13**: p. 9-15.

24. Taylor, R.W. and V. Sandoghdar, *Interferometric Scattering Microscopy: Seeing Single Nanoparticles and Molecules via Rayleigh Scattering.* Nano Letters, 2019. **19**(8): p. 4827-4835.

25. Li, N., et al., *Photonic Resonator Interferometric Scattering Microscopy.* Nature Communications, 2021. **12**: p. 1744.

26. Luan, J., et al., *Ultrabright fluorescent nanoscale labels for the femtomolar detection of analytes with standard bioassays.* Nat Biomed Eng, 2020. **4**(5): p. 518-530.

27. Huang, C.S., et al., *Application of photonic crystal enhanced fluorescence to cancer biomarker microarrays.* Anal Chem, 2011. **83**(4): p. 1425-30.

28. Race, C.M., et al., *An Automated Microfluidic Assay for Photonic Crystal Enhanced Detection and Analysis of an Antiviral Antibody Cancer Biomarker in Serum.* IEEE Sens J, 2018. **18**(4): p. 1464-1473.

29. Assarsson, E., et al., *Homogenous 96-Plex PEA Immunoassay Exhibiting High Sensitivity, Specificity, and Excellent Scalability.* Plos One, 2014. **9**(4).