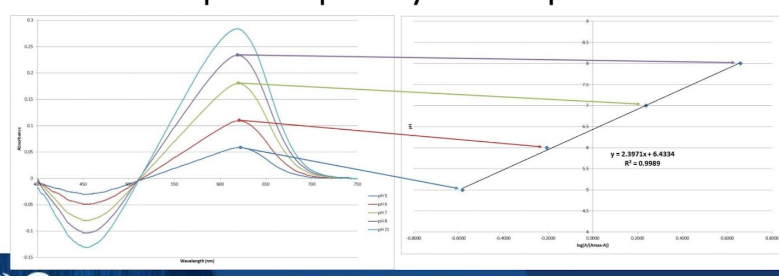
Progress Report

Week of 9/24/18

Alex Goodman
Work/Research Accomplished:

- Focused primarily on understanding some of the underlying physics around optical fibers and understanding the analysis which some people have gone through with optics
- The mathematical conversions from absorbance to pH make sense.
    1. Use a fiber optic as one light source and another as a receiver
    2. Subject the sample (known pH) to a monochromatic light source
    3. Obtain an absorbance reading
    4. Change the pH of the sample, and obtain another absorbance reading
    5. Do this many times, with the same monochromatic light. As the sample changes color, the monochromatic light will absorb more/less along with the change of color.
    6. Chart the known pH with calculated absorbance readings and the probe is calibrated.
    7. Theory of Operation

        - $pH = pK + Slope * log\left(\frac{Abs_{Sample}}{Abs_{pH11} - Abs_{Sample}}\right)$
        - With this y = mx + b format, known pH and absorbance values yield a linear plot with a defined slope and pK as y-intercept

- Problems arise when I considered what absorbance actually means
    - Defined as Intensity(revieced)/Intensity(projected)
    - Many varieties of Intensity: Measurement of either radiance or irradiance
    - Building a circuit using our own configuration would require heavy theoretical work and advanced equipment
- There exists Optical time-domain Reflectometry (OTDR) that is used to measure attenuation of optical fibers
    - Paper: http://iopscience.iop.org/article/10.1088/1742-6596/15/1/011/pdf
    - Item used: https://www.luciol.com/uploads/1/0/2/0/102052502/spec_votdr.pdf

    - Researchers calibrated this machine to measure the attenuation of Plastic Optical Fibers at a monochromatic wavelength to receive Attenuation vs. Distance readings
    - After removing the cladding, placed sensor tip at interface of color-changing pH dye. Proved that various pH's (colored dye) demonstrated different attenuations (due to fresnel reflection)
    - Also claimed they couldn't get a direct attenuation -> pH conversion (confused as to why)

Problems:

- Without proper exposure to optical fiber set-up's it's tough to know exactly what we may be capable of given the scope of this project - reading about current optical-fiber configurations seem complex and sensitive. The OTDR method may alleviate those problems
- Which definition of intensity is used to characterized absorbance? I.e. radiance or irradiance? What's the physical light quantity we're measuring

Will Bacon
Work/Research Accomplished:

- Last semester the group working on this project selected chlorophenol red for their pH indicator due to its high sensitivity within the pH range of 5 - 7
- Unfortunately chlorophenol red is known to be toxic
    - Would need to ensure that it doesn't leak into patient by adhering it to a biomaterial such as a hydrogel
- Additionally, papers using chlorophenol red for physiological experiments were difficult to find leading me to believe it isn't FDA approved for use in humans, which could lead to issues down the road
- To bypass these issues, I explored alternative dyes that could be used
- Most promising dye found was 2',7'-Bis-(2-Carboxyethyl)-5-(and-6)-Carboxyfluorescein, Acetoxymethyl Ester, more commonly known as BCECF
- Advantages to using BCECF include:
    - Many known studies using BCECF for measuring pH in both humans and other animals are documented
    - BCECF has a pKa of 6.98 making it sensitive in the physiological range of pH
    - BCECF is non-toxic
- BCECF relies on fluorescence spectroscopy which would potentially work as follows:
    1. Optical fiber shines light onto BCECF molecules at constant wavelength
    2. BCECF molecules absorb and emit a portion of the emitted light at a higher wavelength
    3. Optical fiber reads in emitted light and sends to circuit for analysis
- Depending on the pH, the intensity of the emitted light will vary thus allowing for quantifiable pH measurements to be made.

Problems:

- One thing that isn't clear to me yet is how to calibrate the pH indicator dye probes. I know that it is regularly done for *in vivo* work, but I need to do more research on how exactly calibration is performed

Mark Austin

Work/Research Accomplished:

- Looked into alternative methods of taking measurements of O_2 saturation levels
    - Oftentimes done using NIRS (which we've been told to basically stay away from)
    - Other products exist, but are not said to be reliable and express a fair amount of caution against in vivo testing
- Began thinking about the circuitry that will be involved on the processing side of the probe
    - Photodiode circuit often includes some very high resistance resistors (sometimes around 1 GΩ) and some fairly low capacitance capacitors. I don't currently possess any resistors of this grade, but the capacitors should not be as hard to come by
    - Filtering of the signal will likely be best done by a bandpass filter by attenuating the signals of frequencies outside of the range of the signal we attempt to measure. This will include the use of resistors and capacitors in order to set corner frequencies around which we wish to attenuate signals
        - $f_c = 1 / (2\pi RC)$
        - As perfect matching of capacitors and resistors to attain a specific corner frequency is unlikely, we will have to combine multiple resistors in series and capacitors in parallel in order to obtain the desired R and C values
- Requested a quote for a similar pH microsensor product (largely used for pH testing in cell culture wells, etc.) and the probes were each said to cost around $116 and the transmitter costs about $7600.

Problems/Concerns:

- I am concerned that the filtering will be difficult on a patient-to-patient basis, as I assume the signal will vary significantly between two people, however I am not sure of this.
- Will we be needing to build our own transmitter? This would be a struggle to do on our own, and if we use someone else's, we will have to consider the inevitable integration into their system when designing our own circuitry to avoid future troubles.

Kelsey Murphy

Work/Research Accomplished

- Researched other methods of measuring pH, focusing on microelectrodes because they came up the most. Most literature after the 1980's, however, uses electrodes to measure pH in meat, and I didn't find any for human use, which leads me to believe that either the FDA doesn't approve or there are other contraindications.

- I read a bit about ppO2 for measuring pH, which is generally highly accurate. However, most established methods are either really expensive or use NIRS, neither of which the client wants.
- Will found that chlorophenol red is toxic, and we therefore can't use it in our design
- I focused on finding indicators that are already in use in physiological studies, and found a few that are used in cell studies. The most common of these, as Will also found, was BCECF, a fluorescent probe with a pKa of 6.98, which is right within the range we want.
    - Further research found that BCECF is nontoxic and doesn't affect the pH of its surroundings. However, I also found that its fluorescence increases with pH, meaning the signal would get dimmer as acidity increased (which it would with ACS). Would it still be possible to make a useful probe by looking for a *lack* of signal?
    - BCECF is also a dual-emission probe, which I don't know much about. If we decide that we can make a probe with decreasing intensity, I will need to look more into what dual-emission means and how to perform measurements with it.
- Another possible probe would be fluorescein or fluorescein diacetate. They have a pKa of ~6.4. Fluorescein is hydrophobic, which would be useful for our probe, and can be immobilized using dextran without large changes to its optical properties. I will need to do more research on this one.
- Another probe I came across was SNARF by Thermo Fisher. I haven't found as much literature about this one, but I will check a few more sources.

Problems

- From above: BCECF's intensity increases in the opposite direction we want it to, and I'm not sure we can make a probe based on a lack a signal.
- Many probes I've found are dual-emission, and I don't know what that means or what it entails for our measurement system. I'll need to find out soon for our design presentations next week.