The World Health Organization predicts that one third of the earth’s population, approximately 2 billion people, has latent Tuberculosis (TB). Of these 2 billion people worldwide, about 10% of these cases will develop into active TB. Furthermore, there are about 9 million new cases of TB and over 1.8 million TB related deaths globally each year. Tuberculosis is second only to HIV in annual infectious disease deaths worldwide. It is also the leading killer of persons with HIV. As you can see, there is a need to greatly reduce the spread of TB, in underdeveloped nations and throughout the world.

There are already many forms of TB testing, however, current TB tests require resources that are not readily available to the developing world. In addition, these tests focus primarily on active pulmonary TB, not extra-pulmonary TB and take days for results. With the rising number of TB cases, there is a desperate need for an affordable, portable detection system.

Our goal is to develop a field-friendly immunological biosensor. This biosensor will utilize specific surface chemistry and laser-based fluorescence in order to reduce TB detection time down to under an hour, and to detect not only pulmonary but also extra pulmonary TB. Our detector is going to be portable, battery powered and able to be used with minimal training (see Figure 1 for schematic). This device holds much potential for detecting other infectious diseases as well.

In our preliminary experiments (using a larger table top setup) we observed fluorescently labeled antibodies as they were binding to a glass slide treated with biotinylated polyethylene glycol (bioPEG) in order to inhibit non-specific adsorption (as shown in Figure 2). Each sample was excited by a frequency modulated 640nm laser. The emission is collected and focused onto a Si photodiode. This is the setup used to produce Figure 3.

Figure 1: Antibody Sandwich Assay. Through this specific surface chemistry we are able to increase sensitivity and specificity for a desired target antigen.

Figure 2: Schematic of Detector Setup. Our sample is excited by a frequency modulated 640nm laser. The emission is collected and focused onto a Si photodiode. This is the setup used to produce Figure 3.
incubated with primary antibodies bound to the slide. Mixtures of fluorescently labeled secondary antibodies were incubated with different concentrations of TB antigens. The secondary antibody solutions were then flushed across the bioPEG/antibody slides, resulting in samples covering a gradient of antigen bound fluorescently labeled antibodies. The sample was excited by a chopper modulated HeNe laser. The emission produced by the excited, fluorescently labeled antibodies is then read with a Silicon (Si) photodiode connected to a lock-in amplifier the corresponding signal was plotted as seen in Figure 3. This illustrates the feasibility of this detector.

Our table top setup currently contains a lock-in amplifier, chopper, and HeNe laser. We would like to build and design our own electronics to replace these devices in order to decrease costs and reduce the size of our detector. Obviously, low cost and small size for mobility is a requirement for this project’s practicality. We will use a microcontroller and laser diode to replace the HeNe laser, chopper and lock-in amplifier. The HeNe laser can be replaced with a laser diode and modulated by the microcontroller’s internal clock. We can then read the signal from the Si photo detector and use the microcontroller to condition the signal to eliminate dark current noise, effectively replacing the lock-in amplifier. Currently we are working to incorporate a flow-cell so that we can start testing human serum. Serum contains many other variables that may cause noise and, of course, much testing must be done to characterize this noise. In order to do that, we will use non-infected serum and spike it with target antigens to develop a detection threshold. The next step would be to read infected and non infected samples at random to test accuracy.

With the proper funding, we believe that we will be able to complete our goal and develop a field-friendly immunological biosensor that utilizes specific surface chemistry and fluorescence microscopy in order to reduce TB detection time down to under an hour, and to detect not only pulmonary but also extra pulmonary TB. Allowing for more efficient and through screening.

Thus far, our project has been recognized by the American Physical Society 4 Corners Chapter and by the Colorado Photonics Industrial Association as an award winning presentation. This project was presented at the American Physical Society National Conference in Portland Organ.