Fluorescence spectroscopy and microscopy form today a set of widely used tools and techniques in various fields of science and technology. This is because of a spectacular sensitivity due to long-wavelength shift of the spectrum which allows filtration from the excitation light. Fluorescence was successfully used in genomic program and is a basic tool in cell and tissue imaging.
What are limitations of fluorescence applications?
First, most useful fluorophores have low or moderate efficiency of fluorescence (quantum yield) and sometimes a fluorescent background creates problems. Second, fluorophores exposed into excitation light degrade in time in a process called photo-bleaching or photo-degradation. While in excited state (either singlet or triplet) fluorophores are exposed to molecular oxygen and to chemical reactions. The photo-degradation occurs dominantly in the excited states of molecules. Most desired (for sensing and assays) but also most difficult fluorescence measurements are these on surfaces. From one site, the surface measurements are convenient and require minute amounts of samples, on another site, however, the fluorescence signals are low from very thin samples.
We realized that there is a way to overcome these limitations in fluorescence studies- a plasmonic approach. Similarly to surface enhanced Raman spectroscopy (SERS) fluorescence is also stronger in the presence of small silver particles deposited on the substrate surface. This is because of two simultaneously occurring effects - local electric field enhancement and radiative decay engineering (RDE). In the first effect, the interaction of impinging light with small noble metal particles creates localized plasmons (collective electron oscillations) which locally can significantly enhance the electric field. This will result in a higher excitation rate. Second effect is the interaction of excited molecules with small metallic particles. This effect results in accelerated fluorescence emission with shorter fluorescence lifetime, which is only possible with an increase of a radiative rate of molecule deactivation. The RDE effect also offers better photo-stability because fluorophores remain shorter time in the excited state. The effects of local field enhancement and RDE, contribute to the total fluorescence enhancement. Recently, we and others realized that deposition of metallic nanoparticles on metallic surface results in even stronger fluorescence enhancements due to traveling plasmons. Such substrates are called plasmonic platforms. The preparation of surface substrates for enhanced fluorescence becomes extremely important. Benefits of using plasmonic platforms include many-fold stronger fluorescence and much better photo-stability.
We propose to investigate three different approaches for substrates (plasmonic platforms) preparation:

1. Electrochemical deposition of silver fractals on glass.
2. Self-assembled silver colloids (SACS) on glass and metallic surfaces.
3. Deposition of periodic silver nanostructures through the masks.

We will test each method for fluorescence enhancements using selected fluorophores, optimize the preparation and write detailed protocols. For tests we will also use model immunoassays to evaluate practical applications. Finally, we will apply metal enhanced fluorescence for ultra-sensitive detection of metalloproteinase MMP-9, an enzyme overexpressed in many illnesses.
We believe that our proposal contains both, scientific and practical elements. The combination of plasmonic interactions with fluorescence is still under development and continuously new surface substrates are reported for enhanced fluorescence. Our study will provide better understanding and description of fluorophore-plasmon interactions. The practical aspect of our project will result in the protocols for the plasmonic platforms preparation. The goal is to develop protocols for reliable and reproducible plasmonic platforms which will enhance fluorescence at least two orders of magnitude, comparing to standard glass substrate. We will demonstrate enhanced fluorescence for surface assays on our plasmonic platforms. Finally, we will apply our platforms for detection of MMP-9 and its activity.