Using Surface plasmon resonance coupled emission (SPCE) and metal enhanced fluorescence (MEF) to detect conformational changes at the single molecule level.

The motivation for this work is the detection of conformational changes in ion channels as they evolve from closed to the open states at the single molecule level. Single molecule recording allows the description of structural changes with a high level of resolution and is able to detect possible subunits interactions that can be missed in the ensemble. We have succeeded in recording the movement of individual subunits in single KcsA channels reconstituted in supported bilayers (Blunck et al, 2008). The object of the present investigation is to develop techniques that will improve on the signal to noise ratio of these measurements and to extend the duration of the recordings by decreasing the bleaching of the fluorophores. Our approach has been to take advantage of plasmon resonance and metal enhanced fluorescence. A new microscope was built to implement these techniques. Results have, so far, been published in abstract form. The microscope is shown in Fig. 1 in its SPCE configuration. It measures conformational changes in single molecules, namely, ionic channels that are nanometers in size and are shown as small brown dots in the membrane.

- *Blunck, R., H. McGuire, H. C. Hyde and F. Bezanilla. (2008) Fluorescence detection of the movement of single KcsA subunits reveals cooperativity. PNAS 105:20263-20268*

- *Hyde, H.C, M. Rosasco, F. Bezanilla (2009) Nanoplasmonic Fluorescence Enhancement Applied to Study of Ion Channels. Biophys. Soc Abstracts.*

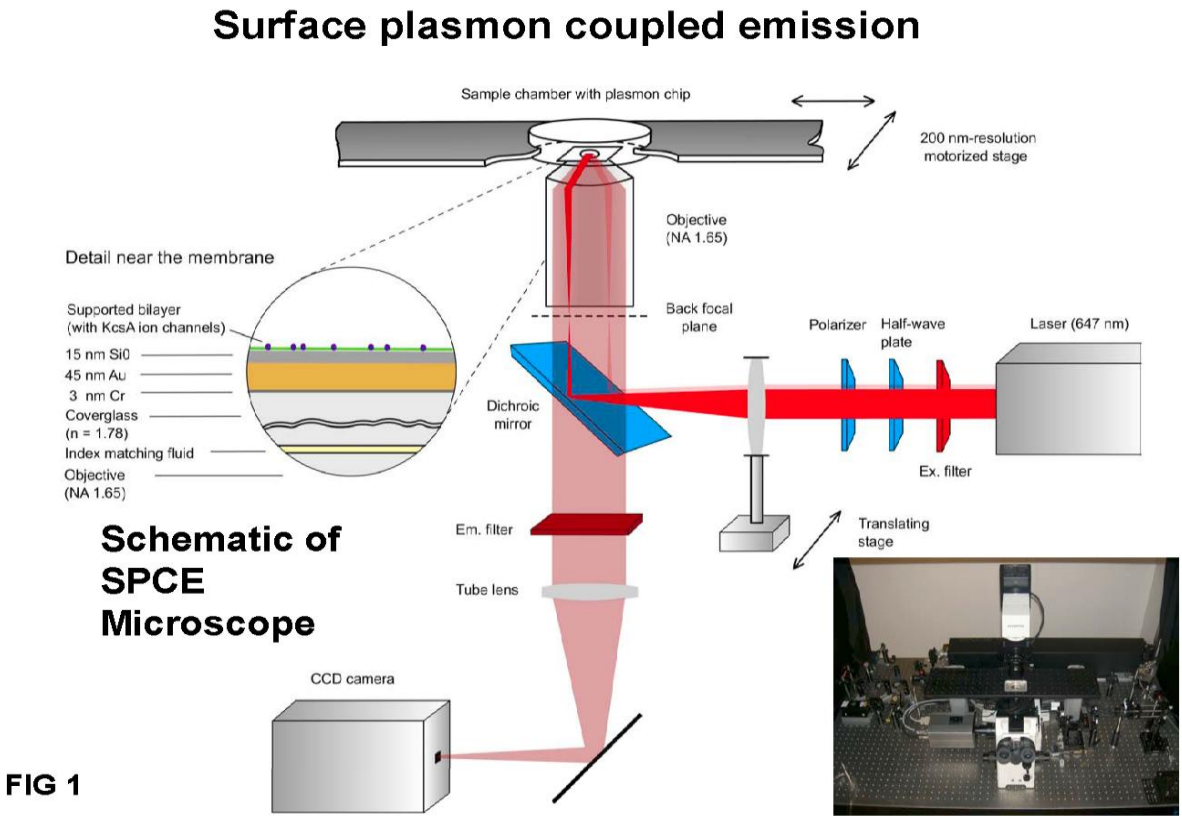

FIG 1