Single ion Channel Single Molecule Detection

Patch Clamp Technique Combined with Fluorescence Imaging Confined to the Tip of Patch Clamp Pipette

TIRF Labs introduces a novel single molecule technique, which combines the patch clamp method and fluorescence imaging of single molecules. We termed the technique Single ion Channel Single Molecule Detection (SC-SMD). SC-SMD allows for parallel electrophysiological study of a single ion channel and simultaneous fluorescence imaging of single molecules that comprise the same ion channel or are interacting with the channel. Figures below illustrate the principles of SC-SMD, show the schematics of the SC-SMD system on a microscope, and depict photos of the patch clamp pipette and its micron-diameter tip. In SC-SMD the tip serves as a micron-size probe, which delivers spatially confined excitation to the micron size area of the mouth of the pipette.

Using the SC-SMD technique we have studied the stoichiometry and molecular mechanisms of activation of Transient Receptor Potential Canonical (TRPC) channels. For more information on the TRPC study refer to SC-SMD Application Note.

SC-SMD system consists of a modified patch clamp pipette holder, and a fiber optics bundle as shown in the figures below. The holder serves a dual function: (i) as a mechano-electrical interface connecting the amplifier headstage to the pipette solution, as found in typical patch clamp systems, and (ii) as a mechano-optical enclosure to couple the excitation light from an illuminator into the patch pipette. In SC-SMD system, the patch clamp pipette functions also as a lightguide, which transmits the excitation light to the tip of the pipette, where the area of interest is located. Thus, the system allows simultaneously acquiring single channel electrophysiological recordings and detecting single molecule fluorescence within the same membrane patch. The SC-SMD system can be seamlessly integrated into existing patch clamp systems. The optical arrangement schematically shown below results in spatially-confined excitation of fluorescence, which is necessary for single molecule detection. The pipette efficiently concentrates the excitation light at the tip. The intensity of excitation light exiting the tip decays as the reverse square of the distance from the tip ~ 1/R². The spatial confinement of the excitation in SC-SMD system is, to some extent, similar to that in confocal microscopy and Near-field Scanning Optical Microscopy (NSOM). The pipette walls, the Ag/AgCl electrode, and the solution inside the pipette scatter certain portion of the excitation light. The stray light is captured and extinguished by miniature optical traps that are installed 5-10 mm away from the pipette tip. Intensity of the excitation light is one of the most important factors for single molecule detection experiments. Our measurements indicate that only about 8% of the optical power reaches the tip of the pipette. Nevertheless, because the optical power is concentrated in a very small (1-micron diameter) area, the intensity of the excitation light is sufficient for single molecule detection experiments.
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Patch clamp technique combined with fluorescence single molecule detection

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