Evanescent field (EF) based fluorescence microscopy is a powerful technique to study molecular events occurring at the interface between biology (e.g. proteins, cells) and substrates. These microscopy techniques rely on the confinement of light, which provides localized fluorescence excitation in a thin film above a solid substrate, where the specimen is located. The confined EF illumination results in a selective excitation of fluorophores and enhances the contrast of the images. The inhomogeneity in the illumination source results in a perfect resolution normal to the surface (z-axis) in the range of few nanometers, in a high sensitivity to movements along the z-axis and in a very low background. These important features, in addition to the necessity for imaging the interface between a cell and a solid substrate, an important region in cell signaling, have made evanescent microscopy methods valuable imaging tools in cell biology.

Two evanescent field based fluorescence microscopes: Total internal reflection fluorescence (TIRF) and waveguide evanescent field fluorescence (WEFF) have been implemented to study 3D intracellular vesicle mobility and release, cell- adhesion, cell volume, interactions of vesicles with the cytoskeleton, cellular motility, individual molecule dynamics, cytosolic calcium concentration dynamics, mapping cell substrate topography and investigate the structural organization of interphase 3T3 fibroblasts.

WEFF microscope offers some advantages over TIRF microscope. The first application of WEFF microscope was carried out on cell-substrate contacts. However, in recent years, it has been applied to image Langmuir- Blodgett films on a waveguide surface, measure cell-substrate distances at the contacts and solid thin film thicknesses, study kinetic cell behaviour on a waveguide, visualize the solubilization process of the plasma membrane of a living cell, study the influence of thin polymer coatings on the quality of cell-substrate contacts for implant applications, dye distance mapping enabling to see the membrane bending between cell adhesions and identify independent lipid raft domains in living cells.

In this presentation, the basic principles of Waveguide Evanescent Field Fluorescence Microscope and its Applications in Cell Biology will be discussed.