STUDY OF INDIVIDUAL TRANSMEMBRANE PROTEINS USING A COMBINED CONFOCAL/NEAR FIELD OPTICAL MICROSCOPE.

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Cells are complex structures in which a wide range of processes occur. By looking only at the cell membrane we already find a large number of proteins with different functions: receptor proteins that allow cell-to-cell communication, transport proteins to carry matter in-out of the cell, and marker proteins for cell identification. We focus particularly on the transmembrane receptor protein LFA-1. This protein plays an important role in the immune process regulating the adhesion strength of the cell via the formation of clusters. We study both individual LFA-1 molecules as well as their clustering. The LFA-1 molecule is fused to the Green Fluorescent Protein (S-65T mutant GFP) and expressed on L-cells. We have combined a confocal (CM) and a near field scanning optical microscope (NSOM) to visualise individual LFA-1 molecules at the cell membrane. We are also investigating the packing density of the clusters, the distance between individual components in a given cluster and cluster spacing distances. Our setup allows a spatial resolution of 35 nm combined with single molecule detection sensitivity. In this contribution we discuss instrumentational issues regarding the CM/NSOM setup and show most recent results.