Dynamics of Lipid Rafts in Living Cell Membranes Studied by Lightguide-based Total Internal Reflection Fluorescence Microscopy (lg-TIRFM)

Lipid rafts are dynamic structures in plasma membranes that play important role in several aspects of cell biology. Rafts are formed spontaneously and function as microdomains that partition membrane proteins promoting clustering of certain of them and excluding others. There is substantial body of evidence, which suggests that the same proteins function differently inside and outside the rafts. Lipid rafts are insoluble in non-ionic detergents at 4°C, a condition that yields detergent-resistant membranes (DRM). Isolating of DRM allows for studying the proteins present in DRM. However, DRM provides limited information about proteins in lipid rafts, since DRM contains isolated membrane fragments mixed with detergents. There is also a question about the dynamics of lipid rafts and proteins, which cannot be addressed by DRM studies. New methods are necessary that will enable studies of lipid rafts in intact cell membranes. The dynamics of association and dissociation and its dependence on the presence of additives and composition of the membrane are of interest for fundamental and applied studies, including cancer and infection diseases.

This Application Note shows that ightguide-based Total Internal Reflection Fluorescence Microscopy (lg-TIRFM) is an *in situ* real-time technique, which is capable of monitoring the dynamics of lipid rafts directly in living cells. lg-TIRFM probes only a shallow area near the bottom of the coverslip where cell membranes are located. Optical slicing in lg-TIRFM is as narrow as 50 nm - approximately one order of magnitude better than that in confocal microscopy. In contrast to through-objective TIRFM, the excitation light in lg-TIRFM does not interfere with the emission channel, thus providing superior signal-to-background ratio.

Beta-subunit of the cholera toxin (beta-ChTx) is a protein, which associates almost exclusively with lipid rafts. Figure 1 illustrates the dynamics of lipid rafts in intact living cells probed by fluorescently labeled beta-ChTx. lg-TIRFM allows to study the distribution of proteins in and out of lipid rafts. Figure 2 shows multicolor capabilities of lg-TIRFM that provide information about association and dissociation complexes within the rafts. In multicolor experiments lg-TIRFM monitors several fluorescently tagged proteins simultaneously. lg-TIRFM revealed that two proteins that have been found in DRMs, the low affinity neurotrophin receptor, p75, and the decay accelerating factor, DAF, are indeed present in different raft domains in the same cell. These proteins have been previously identified as constituents of lipid rafts. For more information about lg-TIRFM see articles: Cell Calcium, 45 (2009) 439; BBA,1801 (2010) 147; Sensors 2012, 12, 1800; Science Signaling, 2012, 5(219), ra29, J. Virology 87, (2013) 11894; Cell Calcium, 54, 3, (2013) 246.]

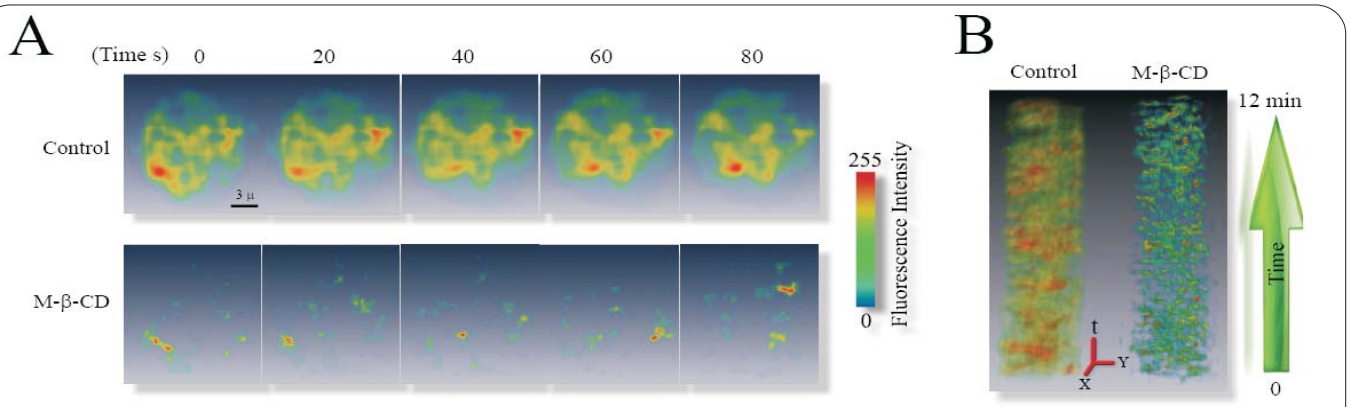

Fig. 1. A - time courses of the displacement of beta-ChTx-Alexa on the cell surface visualized with lg-TIRFM. Upper panel shows the cell under resting condition; lower panel- the same cell 3 minutes after the addition of 5 mM M-beta-CD. Fluorescence intensity is shown by pseudo-color mapping. B- time courses (12 minutes) illustrates as 3D plots, time moves upward, as indicated by the green arrow. X, Y and t are indicated in the center of the panel with the 3D axis in red. [Asanov A, Zepeda A, Vaca L. Biochim Biophys Acta, 1801 (2010) 147-155]

lg-TIRFM for Lipid Rafts Studies

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Figure 2 show images obtained in multicolor lg-TIRFM experiment that reveal that both p75 and DAF co-localize with beta-ChTx. This finding was anticipated, since all three proteins have been isolated in DRM and have been shown to be present in lipid rafts. However, minimum co-localization between p75 and DAF was observed with lg-TIRFM, suggesting that these proteins are part of different raft domains (even though, beta-ChTx is present in both domains). Furthermore, the areas on the cell membrane where reduced co-localization between the two proteins (p75 and DAF) was observed to change dynamically with time, thus suggesting that these domains are dynamic in time and space. These results suggest a constant rearrangement of raft domains, and possibly, exchange of contents between different domains over time. The functional significance of these findings remains to be established. [Asanov A, Zepeda A, Vaca L. A novel form of Total Internal Reflection Fluorescence Microscopy (lg-TIRFM) reveals different and independent lipid raft domains in living cells. Biochim. Biophys. Acta, 1801 (2010) 147-155.]

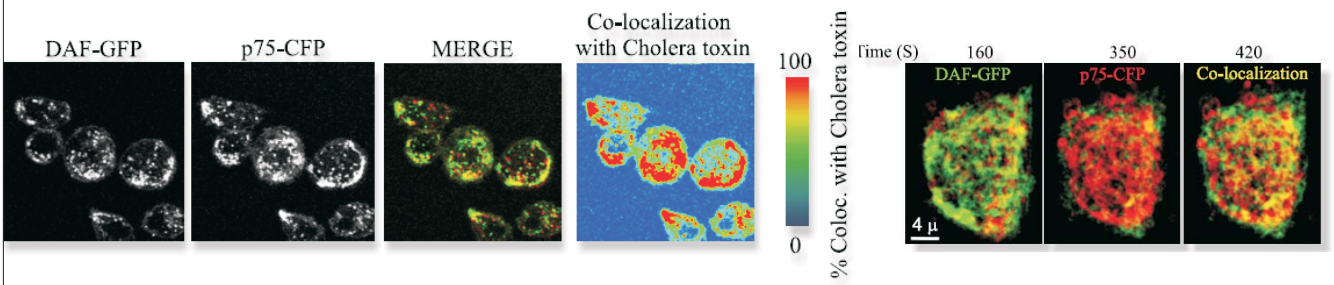

Figure 2. Multicolor lg-TIRFM experiments for simultaneous visualization of DAF-GFP and p75-CFP. To facilitate visualization, DAF-GFP was color mapped in green, and p75-CFP in red. Yellow illustrates the co-localization between both proteins overtime. The numbers above the panels show the times at which images were taken (160, 350 and 420 seconds). Notice the low amount of yellow in all three time points, indicating reduced co-localization between the two proteins overtime.

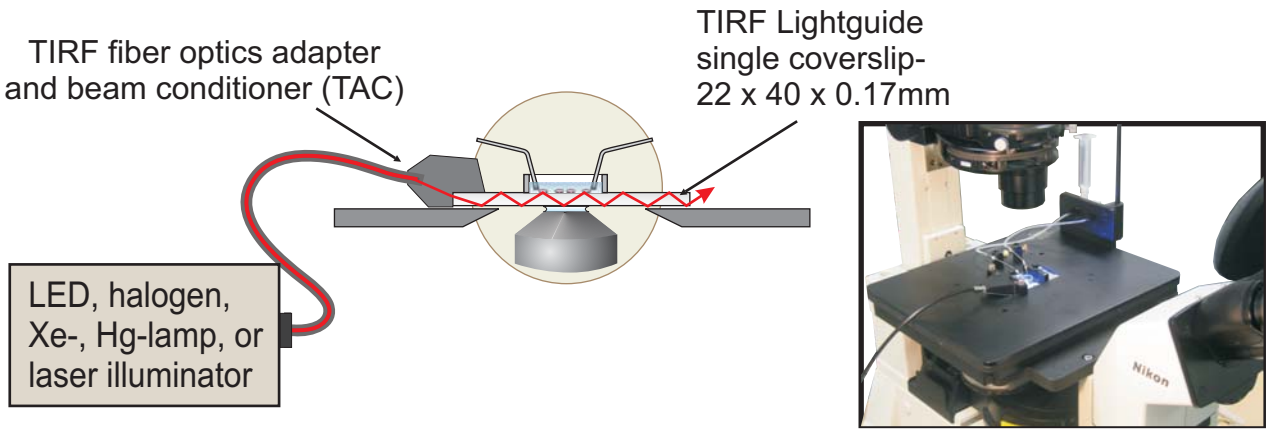

Fig. 3. Schematics and photograph of lg-TIRFM system installed as add-on accessory at the XY-translation stage of inverted microscope. The photo shows the model equipped with open perfusion chamber. Closed flow cells and heated chambers are available as options. The schematics and photo illustrate lg-TIRFM platform adapted to manual XY-translation stage of inverted Nikon Eclipse TE2000 microscope. Smaller and larger platforms are also available that fit into the windows that hold 96-well SBS plates, 110mm x 160mm frames, and are compatible with manual and motorized stages of Nikon, Olympus, and other microscopes.