rhodamine phalloidin in methanolic stock solution. After 30 min at room temperature, the substrate was quickly washed with PBS three times, and then the cells were dried with $N_2$. The following image process is similar to the fluorescent nanoparticles experiment, in which the green laser successively hits the different gratings, and the resulting evanescent waves illuminate the cell attached on top of the $TiO_2$ layer. Figure 4(a) shows the original green LED (M530L2 Thorlabs) image in reflection mode through a dichroic beam splitter (FF562-Di03-25x36, Semrock), exciting band pass filter (FF01-543/22-25, Semrock) and emission band pass filter (FF01-593/40-25, Semrock).

Unlike Fig. 4(a), which displays most of the fluorescent info throughout the cell, Figs. 4(b)–4(d) selectively excites a TIRF image at various layers with penetration depths at 19 nm, 25 nm and 38 nm. In Fig. 4(b), there are very few visible structures illuminated by the short decay length. The inset figure with highlight clearly shows some individual fluorescent dye particles close to the surface. In Fig. 4(c), some of the fluorescent dye suspended in the cell as well as the blurred edges of the cell becomes visible when the decay length increases to 25 nm. For emphasis, we highlight some clusters with higher intensities (inset figure). By contrast, in Fig. 4(d) both the edge of the cell and the F-actins in the cell can be easily distinguished, as they are farther away from the sample surface. It is important to note that the purpose of these images was to demonstrate depth control in our system, so we did not attempt to identify the visible structures. These results demonstrate that the ability to control decay length can be used to discern structures with very high resolution in the z direction.

## 3. Conclusions

In summary, by using multiple diffraction gratings coupled to a high-index $TiO_2$ layer, we were able to achieve a tunable decay length between 20 and 40 nanometers in an internally reflected waveguide mode. Fluorescent nano-beads were illuminated with the various grating couplers, and their fluorescence profiles were compared to simulated profiles in order to estimate their decay lengths. Finally, we demonstrated the application of our TIRF technique in an F-actin labeled HeLa cell, and showed that the variation of decay length makes it possible to distinguish unique structures at various distances from the surface. By contrast, conventional TIRF microscopy is limited to a single decay length, and is not able to access this additional z-direction information. Therefore, the capabilities of our compact TIRF technique will be valuable for various systems and lab-on-a-chip applications, such as stretched DNA and protein detection, where information exists in multiple depth planes close to the surface.

## Acknowledgment

This research is partially funded by NSF-ECCS under Grant 0969405.

#208130 - $15.00 USD Received 13 Mar 2014; revised 17 Apr 2014; accepted 17 Apr 2014; published 25 Apr 2014