We also observed the ear skin tissue of a three-week old mouse sedated by intraperitoneal anesthesia. Figure 6 shows reflected light images of mouse skin obtained at three different acquisition rates. The stratum corneum and hairs are visible in all images. An anesthetized mouse shows a small body movement due to the pumping of blood by the heart. Although small, the continuous and repetitive movement of the mouse in the depth direction causes the stratum corneum to periodically appear focused and defocused. The close-up images of the skin feature in two consecutive frames clearly demonstrate our microscope's confocal sensitivity. For three different acquisition speed settings, we compared sequential image frames to measure the period for focus/de-focus time for the same feature. We have empirically found that 2, 4, and 7 frames are needed to observe the repetition in the acquired images for 30 fps, 100 fps, and 200 fps, respectively. Therefore, at a higher scanning rate more frames can be captured in a given time period revealing shorter time scale changes.

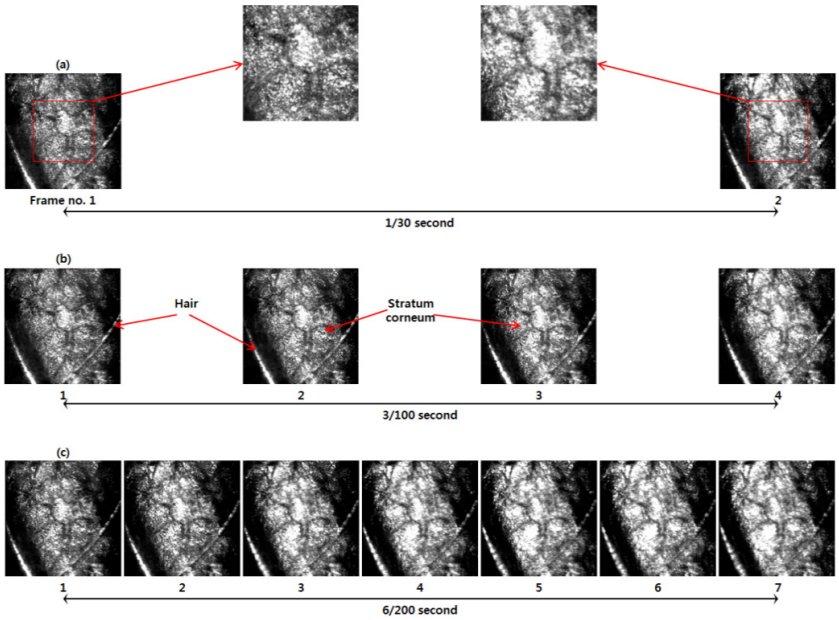

Fig. 6. *in-vivo* mouse skin showing structures and movement. (a) 30 fps, (b) 100 fps, (c) 200 fps.

4. Conclusion

In conclusion, we developed a high-speed laser scanning confocal microscope utilizing a fast polygonal scanning mirror and a bi-directionally operated galvanometer mirror scanner. Necessary high speed analog electronics and imaging software have been designed and developed as well. Confocal images of 512 x 512 pixels with a high spatial resolution can be routinely acquired at a rate up to 200 fps. The frame rate analysis was carried out using a moving resolution target demonstrating minimal distortions in the obtained images for moving samples, and the constructed confocal microscope can be applied to observe appropriately fast dynamic phenomena in clinical and biological applications.

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