1. ABSTRACT
We have developed a depth resolved wide field spectral imaging system for high throughput imaging cytometry. The primary goal of this system is to perform 3D spectroscopic imaging of the adherent cells at 1000 cells per second. This corresponds to about 20min data acquisition time for one million cells, which would allow image based cytometry with similar statistical significance with flow cytometry. The high speed imaging capability was realized by combining several key components. First, the depth resolved widefield imaging is implemented with HiLo microscopy which is a type of structured light illumination where infocus low frequency contents are extracted from the structured light illumination and the infocus high frequency contents are extracted from the uniform illumination[1]. This method requires only two images for generating the depth-resolved image and is much advantageous in terms of image acquisition speed compared to other structured light illumination methods or point scanning based methods. Second, the high speed depth scanning is implemented using the remote depth scanning scheme first proposed by Tony Wilson group[2]. Conventional depth resolved imaging by scanning the objective lens is limited in its bandwidth due to the heavy weight of the objective lens. However, in this technique, the depth scanning is performed by moving small mirror, which substantially increase the speed of the depth scanning. In addition, the large field of view, high NA objective lens and the recently introduced large pixelation, high frame rate sCMOS camera enables high resolution images of a large population of cells at the same time. For the widefield spectroscopic imaging, we implemented the imaging spectrometer based on Sagnac interferometer[3]. This system has the potential to combine the function of the flow cytometer for statistical study of the large cell population and the confocal microscope for optically sectioned structure imaging, which currently are being performed separately.

2. Reference