Supplemental Document

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Moxifloxacin based axially swept wide-field fluorescence microscopy for high-speed imaging of conjunctival goblet cells: supplement

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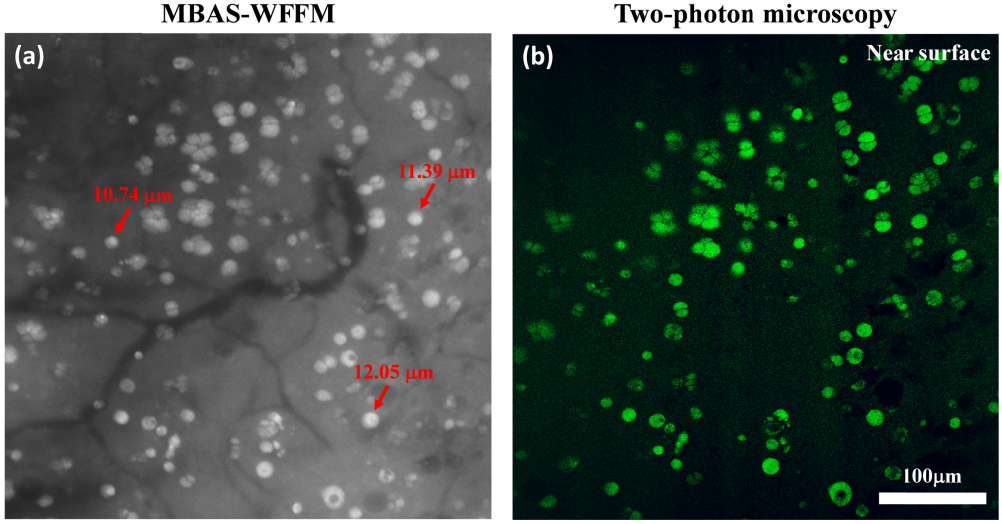

Fig. S1. Moxifloxacin based axially swept wide-field fluorescence microscopy (MBAS-WFFM) and two-photon microscopy (TPM) images of the normal mouse conjunctiva, *ex vivo*. (a-b) a magnified MBAS-WFFM image and a corresponding TPM image near the surface. The red arrows in (a) mark representative isolated GCs. Approximately 10 isolated GCs were analyzed, and the size was 12 ± 4 μm.

Moxifloxacin based axially swept wide-field fluorescence microscopy (MBAS-WFFM) was compared with two-photon microscopy (TPM) in the imaging of normal mouse conjunctiva, *ex vivo*. Freshly excised mouse conjunctiva specimens were imaged by both MBAS-WFFM and TPM sequentially after labeling with moxifloxacin ophthalmic solution. MBAS-WFFM and TPM images of the *ex-vivo* mouse conjunctiva are shown in Fig. S1. Both the MBAS-WFFM and TPM images were acquired in the same conjunctival region with the field of view (FOV) of 450 μm × 450 μm. The TPM image was a single plane image near the surface. Both the MBAS-WFFM and TPM images showed conjunctival GCs in high contrasts, although the MBAS-WFFM image had some background signal. The MBAS-WFFM image additionally showed vasculature in the conjunctiva, and the vasculature could be used for image registration with bright-field images. Isolated GCs and GCs in the clusters could be resolved in both images, although the TPM image showed the higher depth sectioning than the MBAS-WFFM image. The size of GCs in the mouse conjunctiva was measured by analyzing isolated GCs. Approximately 10 isolated GCs were analyzed, and the size was 12 ± 4 μm in diameter on average. The measurement result was consistent with the previous report which showed the GC size to be 11 μm in diameter on average [1].

A custom TPM system was used in the experiment, and the optical configuration of TPM system was explained in a previous report [2]. Briefly, the TPM used a resonant scanner running at 8 kHz for high-speed scanning. A Ti-Sapphire laser (Chameleon II, 140fs, Coherent) as the light source. The laser beam from the source first passed through a power control unit, consisting of a motorized half wave plate (HWP, 10RP52-4, Newport) and a polarizer (PL, GL5-B, Thorlabs). After the power control unit, the laser beam was expanded

by a lens pair (f = 50mm & 100mm) in 4f configuration. The transmitted laser beam went to the combination of a resonance scanner and a galvanometer scanner for the 2-axis scanning. After the scanner combination, the laser beam was expanded by the combination of a scan lens (f=50mm) and a tube lens (f=200mm). The excitation beam was transmitted through a long-pass dichroic mirror (700LP, Chroma) and went to an objective lens (XLUMPLFN 20x, 1NA, 2mm WD, water immersion, Olympus Inc.). The objective lens focused the excitation laser into the sample. The excitation focus scanned the sample in the transverse plane with the galvano & resonant scanner combination. Axial scanning was performed with a piezoelectric objective translator (209SRG&P-725-4CD, PI). Emission light from the sample was collected by the objective lens, reflected on the dichroic mirror, passed through a barrier filter (680SP, Chroma), spectrally split by a dichroic mirror (450SP, Chroma) and emission filters (390 nm – 410 nm, Thorlabs), and then collected at two photomultiplier tubes (PMTs, H10770A-40, Hamamatsu). Excitation wavelength was 790 nm and the image resolution of 0.7 μm and 3.0 μm in the lateral and axial direction respectively. Imaging speed was 31 frames/s and the FOV was 450 μm × 450 μm consisting of 1024 × 1024 pixels.

One 12-week-old SKH1-Hrhr male mouse was used for the MBAS-WFFM and TPM imaging of the *ex-vivo* mouse conjunctiva. The mouse was euthanized via cervical dislocation, and mouse eyes were enucleated using the transpalpebral method ensuring that the periocular tissue was excised along with the eyeball. The cornea and conjunctiva tissues were then separated from the eyeballs circumferentially with scissors. The region of interest (ROI) in the conjunctiva was determined by using the eyelid and corneal borders as landmarks. The excised tissue specimens were labeled with drops of moxifloxacin ophthalmic solution in the same way as the *in-vivo* experiments, and then spread on the slide glasses and covered with coverslips.

References

1. M. J. Doughty and J. P. Bergmanson, "Heterogeneity in the ultrastructure of the mucous (goblet) cells of the rabbit palpebral conjunctiva," Clin. Exp. Optom. **87**(6), 377-385 (2004).
2. B. Kim, H. Le, B. H. Oh, and K. H. Kim, “High-speed combined reflectance confocal and moxifloxacin based two-photon microscopy,” Biomed. Opt. Express **11**(3), 1555-1567 (2020).