Supplement 2 A 7-step flowchart of implementation for acquisition of tears Raman spectra

1. Power on
2. Open software
3. Raman shift calibration
4. Set experimental conditions
5. Set spectral post-processing algorithm
6. Collect pre-normalized spectrum and correct its noise for each sampling
7. Normalize spectrum for each sampling

- Open central control computer system, selected emitting laser, illumination and motorized stage of confocal microscopy.
- Open OMNIC® for Dispersive Raman.
- After laser warm-up, perform internal calibration of instrument and external registration by silicon wafer at Raman shift 520 cm⁻¹.
- Set background exposure time, sample exposure time, collection time and accumulation times, collecting range of Raman shifts, etc.
- Correct the background noise by subtracting pre-collected background signal during warm-up. Correct autofluorescence by 3rd order polynomial of each spectrum.
- Load dried teardrops –Ti/Al slide. Collect a spectrum, remove the spectrum with a low S/N ratio or interfered by a cosmic ray, and correct noise by subtracting root mean square of 1800–2000 cm⁻¹.
- Find maximal peak of 1000–1004 cm⁻¹ of a spectrum. After minus mean spectral intensity from 700–1799 cm⁻¹, and divided by peak intensity, a normalized spectrum was acquired.