Fluorescence imaging assay for chloride flux:

Materials:
50mM SPQ dissolved in water
Bioptechs delta T dish coated with Fn or other suitable substrate
Cell media
Buffers (ie, chloride, nitrate, iodide)

1. Add cells to Fn-coated dish and incubate to allow time for cells to attach firmly (seed cells at low density to aid in image analysis)

2. Load cells with probe (ie, 10 mM SPQ):
Add 10 mM SPQ in 50% hypotonic solution (may use PBS, cell media, or iodide substituted buffer) for 15 min at 37ºC. Wash dish several times with buffer (PBS, cell media, iodide buffer).

3. Place dish on heated stage (do not use dish cover). Use 10 or 20X objective and UV filter set (appropriate for SPQ spectra). Acquire images at 30 sec-intervals for several minutes to establish baseline fluorescence

4. At this point dish component can be exchanged with test compound/drug/buffer, as images are being acquired. To exchange dish buffer carefully use vacuum system with glass pipette **(avoid spills and bumping microscope stage**). Be sure to note time/image frame when exchanges are made.

5. Image analysis (Metamorph):
Assemble the acquired images into a single stack. Use threshold feature or manually draw region around cells. Sample the background fluorescence signal. Log fluorescence intensity values to excel.