## Adding compounds to study their effect on ECIS measurements

The ECIS instrument is extremely sensitive; even small responses of the cells to temperature, shear stress, etc. are detectable. Care must be taken when adding compounds to cells to minimize unrelated responses and ensure that only the biological effects of the compounds being added are observed.

The following is a suggested protocol:

An hour before the compound is added, change the medium over the cells using the medium that will be present when the compound is added (400 microliters final volume). This gives the cells time to adjust and equilibrate to experimental conditions. The equilibration process can be followed via ECIS to be certain enough time has elapsed to give a steady impedance signal.

The compound to be tested should be prepared at twice the final concentration in the same solution as that in the wells and equilibrated to incubator temperature. In the doses to be tested, include a control without the compound.

With 400 microliters in the well, withdraw 200 microliters and then slowly add 200 microliters of the 2X solution to achieve the final 1X concentration. The addition should be done slowly, so the cells on the active electrodes are not exposed to shear stress from the fluid flow.

If the array is removed from the incubator for this addition, be sure to pause the measurement and work quickly to minimize temperature drops. Once the array is returned to the incubator, perform an electrode check to ensure contact has been reestablished and resume data collection.

If the compound addition is carried out without removing the array from its holder, pausing the measurement during this addition is recommended to avoid any unwanted impedance spikes. Once the compound is added to the wells, resume data collection.