Introduction
Disturbed flow has long been acknowledged to play important roles in atherosclerosis. Under this circumstance, the endothelial cells (ECs) are subjected to abnormally low or high fluid mechanical parameters, e.g. wall shear stress (WSS), spatial and temporal WSS gradients (T/SWSSG), and oscillatory WSS [Chiu, 2011]. However, it is difficult to differentiate their individual effects on the pathogenesis of the disease and their role in promoting endothelial dysfunction as these parameters are often co-localised in vivo. Understanding their individual effects could provide further insights on the mechanisms of atherogenesis.

Methods
HUVECs were cultured in either a parallel plate or a converging flow chamber (in-house design) and subjected to physiologically-relevant time-averaged WSS, TWSSG, and SWSSG, by either imposing a steady or physiologically-realistic coronary artery flow for 24 hrs, enabled by an in-house designed system. The WSS distribution was quantified computationally and mapped onto the morphology of HUVECs (alignment, aspect ratio), quantified using an in-house developed image processing programme (Figure 1). Static cultures were used as controls.

Results
HUVECs showed clear alignment (orientation) in the direction of the applied WSS for both a pulsatile flow that applied a combination of WSS, TWSSG and a small reversed WSS and a steady flow that applied the same average magnitude of WSS. The alignment and elongation (aspect ratio) appeared to be slightly stronger, albeit not significantly, for the steady than pulsatile WSS (Figure 2).

![Figure 1: HUVECs exposed to pulsatile flow (left to right), stained with DAPI-Phalloidin and segmented using our image processing routine.](image)

![Figure 2: Histogram showing orientation of HUVECs. n = 3 independent experiments.](image)

Discussion
There is evidence that EC morphology is indicative of the health of the endothelium. Cells which show elongated, aligned morphology, even in the absence of flow, have been shown to have lower inflammatory activation [Vartanian, 2010]. ECs aligning with the direction of flow and becoming more elongated are common features at locations which are resistant to atherosclerosis in vivo. Our results indicate that WSS magnitude exerts a stronger influence on endothelial morphological changes than WSSG and this is strongly dependent on the cell density. It will be interesting to further investigate if similar pattern is observed at the molecular level.

References
Chiu & Chien, Physiol Rev, 91:327-87, 2011
Vartanian et al, AJP: Cell, 298:C333-C341, 2010