Variable blood flow regimes influence a range of cellular properties ranging from cell orientation, shape, and permeability, which are dependent on endothelial cell-cell junctions. In fact, cell-cell junctions have shown to be an integral part of vascular homeostasis through the endothelium by allowing intercellular signaling and passage control through tight junctions (TJs), adherens junctions (AJs) and gap junctions (GJs). It was our objective to determine the structural response of both AJs and TJs under steady and oscillatory flow. Human brain microvascular endothelial cells (HBMECs) were cultured in a parallel plate flow chamber and exposed to separate trials of steady and oscillatory fluid shear stress for 24 hours. Steady flow regimes consisted of a low laminar flow (LLF) of 1 dyne/cm², a high laminar flow (HLF) of 10 dyne/cm² and oscillatory flow regimes consisted of low oscillatory flow (LOF) +/- 1 dyne/cm² and high oscillatory flow (HLF) of +/- 10 dyne/cm². We then imaged the TJs ZO-1 & Claudin-5 and AJs JAM-A & VE-Cadherin and subsequently analyzed their structural response as a function of pixel intensity. Our findings revealed an increase in pixel intensity between LLF and LOF along the boundary of the cells in both TJs ZO-1 & Claudin 5. Therefore, our results demonstrate the variable response of different cell-cell junctions under fluid shear and for the first time observes the difference in cell-cell junctional structure and reorganization among steady and oscillatory flow regimes.