

Human cell response to has been long studied as cells are responsible for the homeostatic operation of the body. For example, research has detailed that the gradient of rigidity of substrates can lead to cell movement bias, where cells exhibit preference towards the direction of softer, smoother regions. To further understand cell sensing of its mechanical environment, this research investigates the formation of microscale stiffness patterns by patterning two polymers of different stiffnesses. To isolate the effects of stiffness patterns from the potential effects of topography cues, we undertake the challenge of fabricating a flat substrate . To accomplish excellent optical microscopy of the cell response, our substrates need to be optically clear and have a homogenous index of refraction. Finally, substrates must also have high refractive index to allow the technique of total internal reflection microscopy. Topography was verified with fluorescent microscopy, surface homogeneity of the surface with scanning electron microscopy, and stiffness gradients via force mapping. In the future, flat dual substrates could assist in the development of the field of cell motility, allowing for characterization of mechanical cell responses based solely on stiffness patterns. Additionally, protocols developed during this research could allow for future augmentation of the pattern dual substrates used for other purposes.