‘Designer’ PDMS Cover Slips for Registration of Second Harmonic Generation and Atomic Force Microscopy Images.

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Statement of Purpose: The integration of functional and standard imaging in biomaterials has been utilized to not only characterize the macroscopic behavior of the subject but to also break down the behavior of its meso- and microscopic composition. This project has been focused on combining the nanomechanical analysis from Atomic Force Microscopy (AFM) with Confocal Second Harmonic Generation Microscopy (SHG). The high resolution of these modalities make it difficult to manually register the images. Fixing the samples for staining has been known to shift the mechanical behavior [1] and stitching together images adds significant time to the scan as the sample undergoes necrosis and dehydration. There is a need for a methodology that allows for faster image alignment that can be recognized in both AFM and SHG images. The authors looked to Polydimethylsiloxane (PDMS) Sylgard 184 as a material to create a custom cover slip that could allow quick alignment between AFM and SHG. The authors were able to create a thin PDMS filament using a technique developed by Kim at al by floating PDMS on water to control the thickness [2]. This thin sheet is then cut with a low power laser to create small perforations that act as wells for the AFM and SHG to conduct their analysis in traceable holes. These holes then provide a custom gridded overlay that is both visible by the SHG and can be measured topographically by the AFM. This novel overlay system can then allow for the consistent and convenient registration of non-hybrid multimodal imaging studies. Methods: PDMS is mixed at room temperature and then vacuum treated for 30 minutes. A single 10 µg drop is then floated onto room temperature water where it sits for 24 hours while it cures. The PDMS sheet is then self-adhered to a cover slip which acts as a transfer material for the PDMS to then attach to the myocardium. The PDMS is cut by a CO_2 laser which perforates 100 µm wells at regular 100 um intervals. The PDMS is then transferred to the myocardium for imaging analysis. SHG images are acquired at 435 nm with a resolution of 80 µm. AFM analysis is completed with a pyramid silicone coated tip with a rip diameter of 7 nm. Contact mode force maps are created at 90 µm with a sample rate of 3 pixels per micron. Results: AFM results are more locally specific in comparison to similar studies and now in combination with the high level ultrastructure detail from the SHG provide morphological context to the mechanical behavior of the tissue. The PDMS sheets act as a short cut for the tissue analysis as it can be prepared ahead of time and eliminates the need for staining or a large number of images to be collected saving both time and tissue waste. PDMS under a CO_2 laser is sufficient for hole creation at higher laser powers but it is believed to be ablating the PDMS and cover slip to the point of bubbling and significantly deforming the perimeter. It appears that a more moderate power level is more effective at providing consistent perforation sizes as well reducing complex well shapes. The AFM tip is sensitive to high adhesion materials and requires careful placement within the holes to reduce damage to the tip. Further steps will require the liquid immersion of the PDMS grid to remove the risk of dehydration of the sample during long term scanning.

References: [1] Calò, A. Scientific Reports, 2020; 10:1-12. [2] Kim, D. Polymers, 2019, 11(8).