Ahern Group Protocols

Whole mount imaging of TRPV1-lineage expression in mouse bladder

- The TRPV1-Cre transgenic mouse line (donated by Mark Hoon, NIH) was created using a BAC transgene containing the entire TRPV1 gene/promoter (~50 kbp of upstream DNA) and IRES-Cre-recombinase. TRPV1-Cre (hemizygous) are crossed with ai9 ROSA-stop-tdTomato mice (Jackson Labs).
- Mice are killed by CO_2/decapitation and bladders are removed, ventrally opened longitudinally from the bladder neck to the top of the dome and divided into hemisections.
- A bladder hemisection is placed (urothelial surface face down) in an imaging chamber containing standard physiological buffer: 140 mM NaCl, 4 mM KCl, 1 mM $MgCl_2$, 1.2 mM $CaCl_2$, 10 mM HEPES, 5 mM glucose, pH=7.3. The hemi-section is anchored by platinum-wire tissue harps.
- Low-resolution (10x) fluorescence microscopy is performed of the entire hemisection with an inverted epi-fluorescent microscope. tdTomato is excited at 540±12 nm and emitted florescence is passed through a 620±30 nm bandpass filter. Approximately 40-50 images are required to cover the hemisection of an adult bladder.
- The individual images are aligned using Adobe Indesign software (see Figure 1) to generate a montage that reconstructs the whole bladder hemi-section.

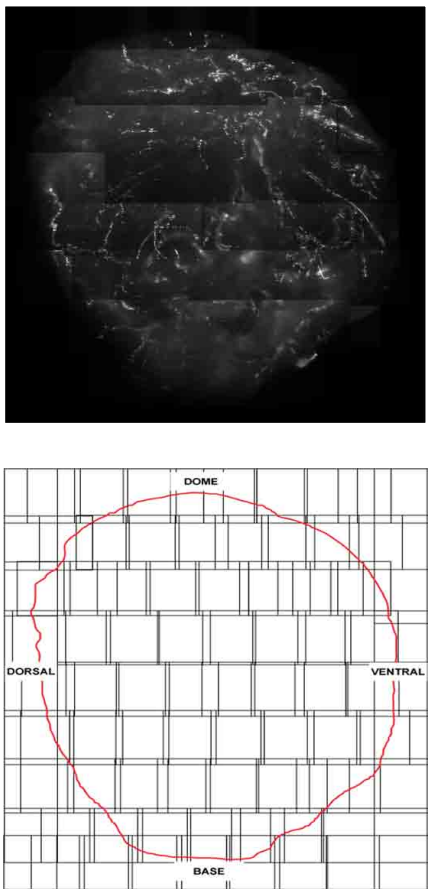

Fig. 1. Mapping TRPV1 lineage in bladder mounts. Bottom shows map of acquired overlapping images with bladder outlined in red. Top shows the assembled montage.