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Introduction: Cellular reaction to traumatic brain injury is complex and involves considerable interactions between cells and reactivity to foreign bodies. Our objective was to assess neurons, microglia, astrocytes, and intracellular Ca^{2+} signaling by creating a novel confocal microscopy technique involving an air immersed lens that does not sacrifice resolution and limits signal attenuation. This study aimed to create a consistent dynamic methodology to observe the cortical cellular response using real-time intravital imaging as trauma is being induced.

Methods: Once surgical plane was achieved, rodent cortices were exposed via craniotomy and blunt insertion with a silicone shunt catheter into the lateral ventricle was performed at a controlled rate. Neurons, microglia, astrocytes, and intracellular Ca^{+2} signaling were fluorescently tagged with DiD (4-chlorobenzenesulfonate), tomato lectin from lycopersicon esculentum, sulforhodamine B, and Cal 520 AM, respectively. Activity tracking of fluorescently tagged markers 700 microns from blunt insertion TBI was performed using upright resonant scanning confocal microscopy.

Results: Neurons, microglia, astrocytes, and Ca^{2+} signaling were identified at a depth of 100 microns from the meninges. Gross movement of cells was visualized during shunting by identifying specific cells and tracking movement over time. Preliminary data shows that astrocytes are in closest proximity to the inserted shunt catheter.

Conclusion: This novel method identified cell types and tracked gross movement. Although preliminary data and other post-hoc studies indicate primarily astrocytic involvement, it shows that we can successfully record immediate cell involvement around the shunt catheter for the first time. Future studies will improve cellular tracking and imaging resolution.