**Title:** Single molecule fluorescent in situ hybridization (smFISH): a method for mRNA quantification in the auditory system

**Authors:** Charlie Nishanth Nelson ([cnelson@neomed.edu)](mailto:cnelson@neomed.edu)), Pezhman Salehi, Jianxin Bao

**Institution:** Department of Anatomy and Neurobiology, Northeast Ohio Medical University, Rootstown, Ohio

**Category:** Basic Research

**Objective:** The aim of this study was to develop a method to achieve robust visualization and quantification of single mRNA transcripts for biomarkers of the auditory system in cochlear tissue.

**Abstract:**

**Background:** Quantitative analysis of gene expression is essential to discovering biomarkers for diseases of the auditory system including noise-induced hearing loss and tinnitus. Real-time quantitative polymerase chain reaction (RT-qPCR) has been used to accurately gauge mRNA transcript levels in murine cochlear tissue, but it has not allowed for subcellular visualization and quantification of individual mRNAs. Single-molecule fluorescent *in situ* hybridization (smFISH) performs this by using fluorescent probes to bind to single mRNAs under high-resolution confocal microscopy, but its application in cochlear tissue has been hampered by the rigorous sample preparation steps that degrade target mRNAs and significantly increase background artifacts in images. In this study, we developed a method to achieve robust visualization and quantification of single mRNA transcripts for biomarkers of the auditory system in cochlear tissue.

**Methods:** Cochleae were harvested from CBA/J mice and perfused, decalcified, and dehydrated. Pre-chilled OCT cochlear molds were cryosectioned to 12 μm thickness, and smFISH was performed using an enhanced RNAscope® probe hybridization design for the α1H subunit of the voltage-dependent, T-type calcium channel (*Cav 3.2*). Following high-resolution confocal microscopy, we tailored an ImageJ-based transcript analysis pipeline for 2D manual segmentation of spiral ganglion neuron (SGN) immunohistochemistry in tandem to 3D quantification of *Cav 3.2* mRNA transcripts post-background artifact removal in image z-scans.

**Results:** Our protocol not only allowed us to process and quantify individual *Cav 3.2* mRNA transcripts in 3D but also ascribe these transcripts to the nuclei and cytoplasms of specific SGNs and the inner and outer hair cells of the organ of Corti (OC), the key morphological structures of the auditory system.

**Conclusions:** We successfully demonstrated and developed a quantitative image analysis protocol for smFISH of the auditory system via a freely available transcript analysis pipeline.