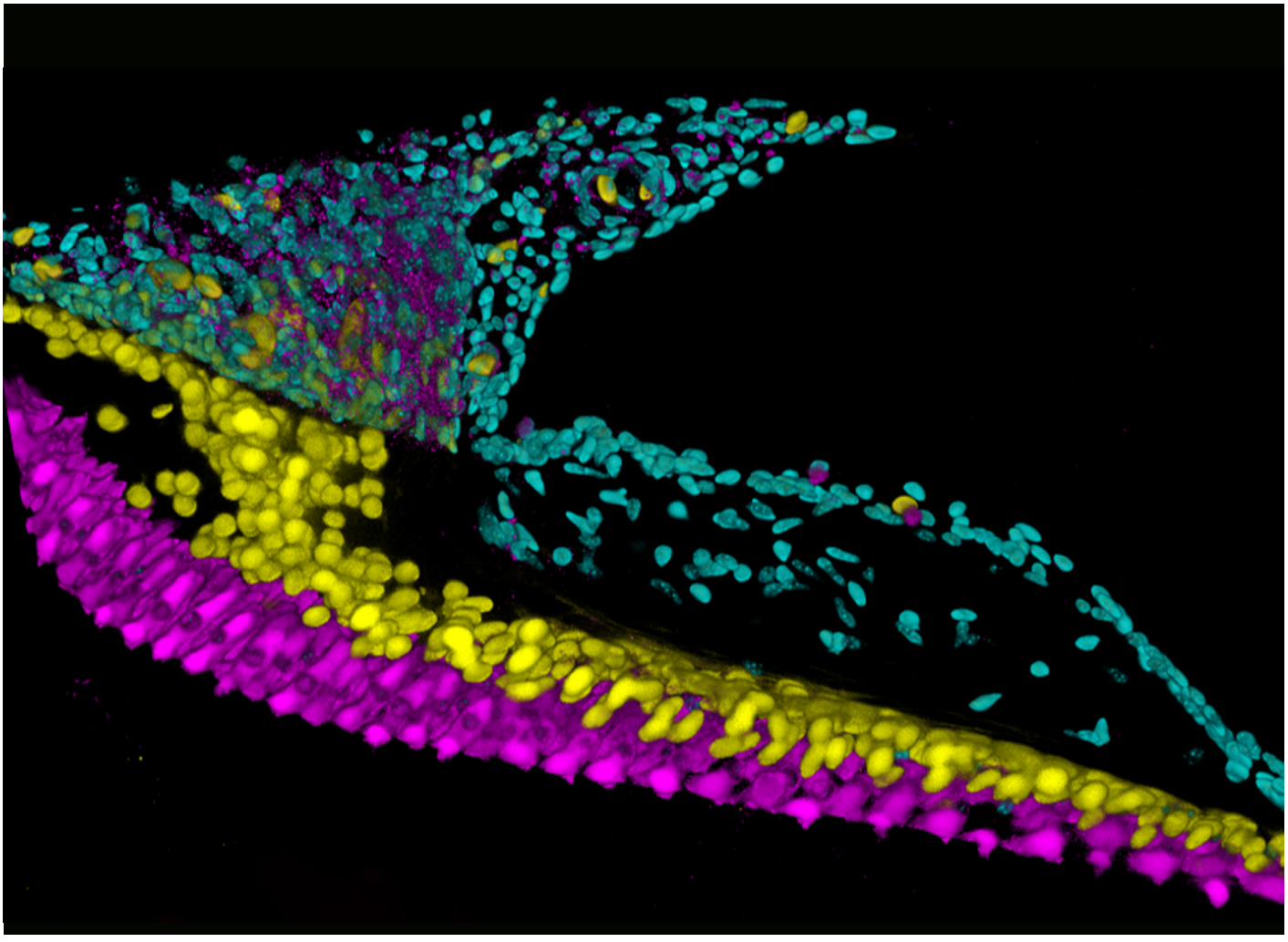
From Eye to Insight

Leica MICROSYSTEMS

DEVELOPMENT AND RECOVERY OF THE INNER EAR

Sensory hair cell regeneration in chicken embryo



Authors

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Abstract

This article discusses how thick cochlear tissues of chicken embryos can be sharply imaged for studying inner-ear hair cell regeneration. Sensory hair cells perform important functions for hearing and balance. The mechanisms by which they regenerate are still not well known. Avian hearing organs can recover from hearing loss, so chicken ear tissues are often used to study sensory hair cell regeneration. Crisp 2D and 3D images of the tissue clearly showing hair cells were taken with a THUNDER Imager. Haze and out-of-focus blur was eliminated with Computational Clearing.

Introduction

Because the mechanisms that trigger, sustain, and terminate the development and regeneration of the inner ear are largely unknown, much research is dedicated to better understand them. Chicken embryo has been used as a model organism for more than 30 years to study sensory hair cell regeneration in the ear, specifically because of the ability of chickens to naturally recover from hearing loss within weeks. This same phenomenon does not occur in mammals. The inner-ear sensory hair cells are sensitive to displacement of the fluid that surrounds them and serve a mechanosensitive purpose where they mediate hearing, balance, and head rotation. For this reason, scientists are studying the mechanisms concerning sensory hair cell regeneration.

Challenges

Typically, thick sections tissue are challenging specimens for widefield fluorescence microscopes, due to the haze or out-of-focus blur produced by light scattering inherent to thicker specimens. The haze can obscure structures of interest deep inside the specimen.

Methods

Thick vibratome sections of post-hatch 7-day chicken cochlear tissue were used to visualize the sensory hair cells in the inner ear. A 43-µm thick vibratome section of a post-hatch day 7 chicken cochlea was stained with a nuclear DAPI stain (cyan), antibodies for Myosin 7a labeling sensory hair cells (magenta), and Sox2 labeling supporting cells (yellow). The section was imaged with a THUNDER Imager Tissue using a 40x oil-immersion objective having a numerical aperture (NA) of 1.3. Both a 10-position 2D tilescan image (refer to figure 1) as well as a 3D image via a 43 µm z-stack in 159 z-steps (refer to figure 2) were acquired. The 3-channel 10-position tilescan took under a minute to acquire and process with Instant Computational Clearing (ICC) [4,5]. Because ICC is a 2D method and does not require z-stacks, it could be applied to this single plane tilescan. The haze and background was cleared from the raw widefield images using ICC (refer to figure 1) or Large Volume Computational Clearing (LVCC) (refer to figure 2), making them suitable for observation of individual sensory hair cells (magenta) and supporting cells (yellow) of the inner ear.

Results

The images of the chicken cochlear tissue section acquired with the THUNDER Imager Tissue are shown below in figures 1 and 2.

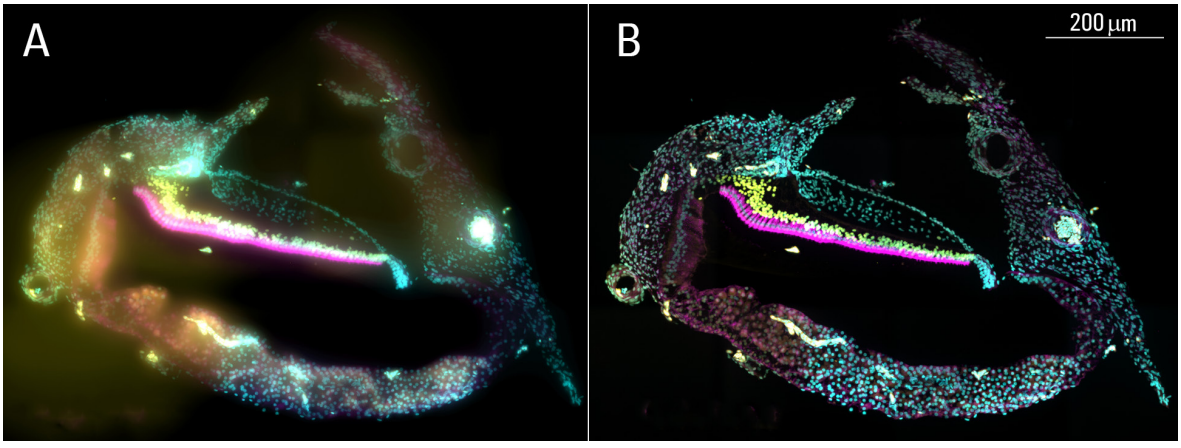

Figure 1: Tilescan 2D image of chicken cochlea taken with a THUNDER Imager Tissue. A) Raw epifluorescence image and B) the result after ICC. Image courtesy of Dr. Amanda Janesick, California, USA.

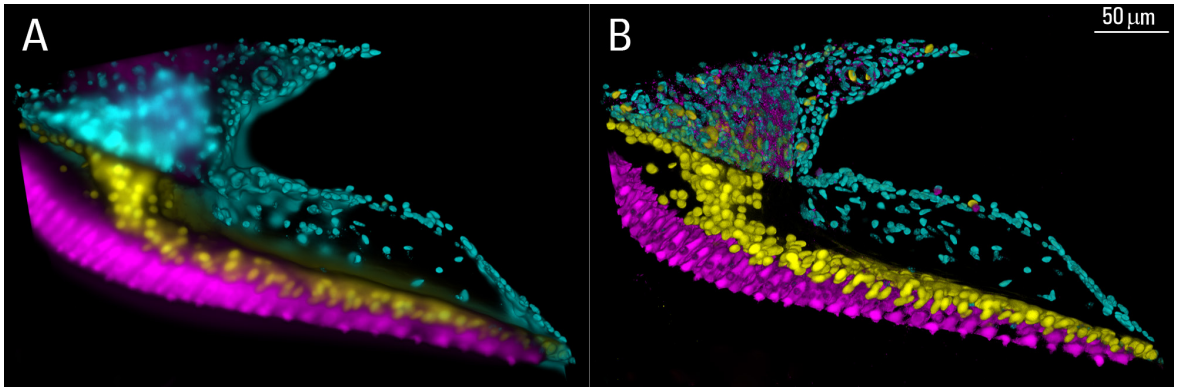

Figure 2: 3D image of a chicken cochlea tissue section taken with a THUNDER Imager Tissue shown before (A) and after LVCC (B) . Image courtesy of Dr. Amanda Janesick, California, USA.

Conclusions

The results presented here show that THUNDER images of a thick (43 µm vibratome) section of chicken cochlea tissue enable the observation of individual sensory hair cells and supporting cells of the inner ear. Both fast processing and rendering of crisp 2D tilescan images using Instant Computational Clearing (ICC) and acquisition of sharp 3D images using Large Volume Computational Clearing (LVCC) were achieved. Computational clearing helped to remove haze and out-of-focus blur in the images.

References

> Janesick, A.S., Scheibinger, M., Benkafadar, N., Kirti, S., Heller, S., Avian auditory hair cell regeneration is accompanied by JAK/STAT-dependent expression of immune-related genes in supporting cells, Development (2022) vol. 149. iss. 8, dev200113, DOI: 10.1242/dev.200113.

> Scheibinger M., Janesick A.S., Diaz G.H., Heller S., Immunohistochemistry and In Situ mRNA Detection Using Inner Ear Vibratome Sections. In: Groves A.K. (eds) Developmental, Physiological, and Functional Neurobiology of the Inner Ear. Neuromethods, vol 176. (Humana, 2022, New York, NY, USA), pp. 41-58, DOI: 10.1007/978-1-0716-2022-9_3.

> Janesick A.S., Scheibinger M., Heller S., Molecular Tools to Study Regeneration of the Avian Cochlea and Utricle. In: Groves A.K. (eds) Developmental, Physiological, and Functional Neurobiology of the Inner Ear. Neuromethods, vol 176. (Humana, 2022, New York, NY, USA), pp. 77-97, DOI: 10.1007/978-1-0716-2022-9_5.

> J. Schumacher, L. Bertrand, THUNDER Technology Note: THUNDER Imagers: How Do They Really Work? Science Lab (2019) Leica Microsystems.

> L. Felts, V. Kohli, J.M. Marr, J. Schumacher, O. Schlicker, An Introduction to Computational Clearing: A New Method to Remove Out-of-Focus Blur, Science Lab (2020) Leica Microsystems.

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