From Eye to Insight

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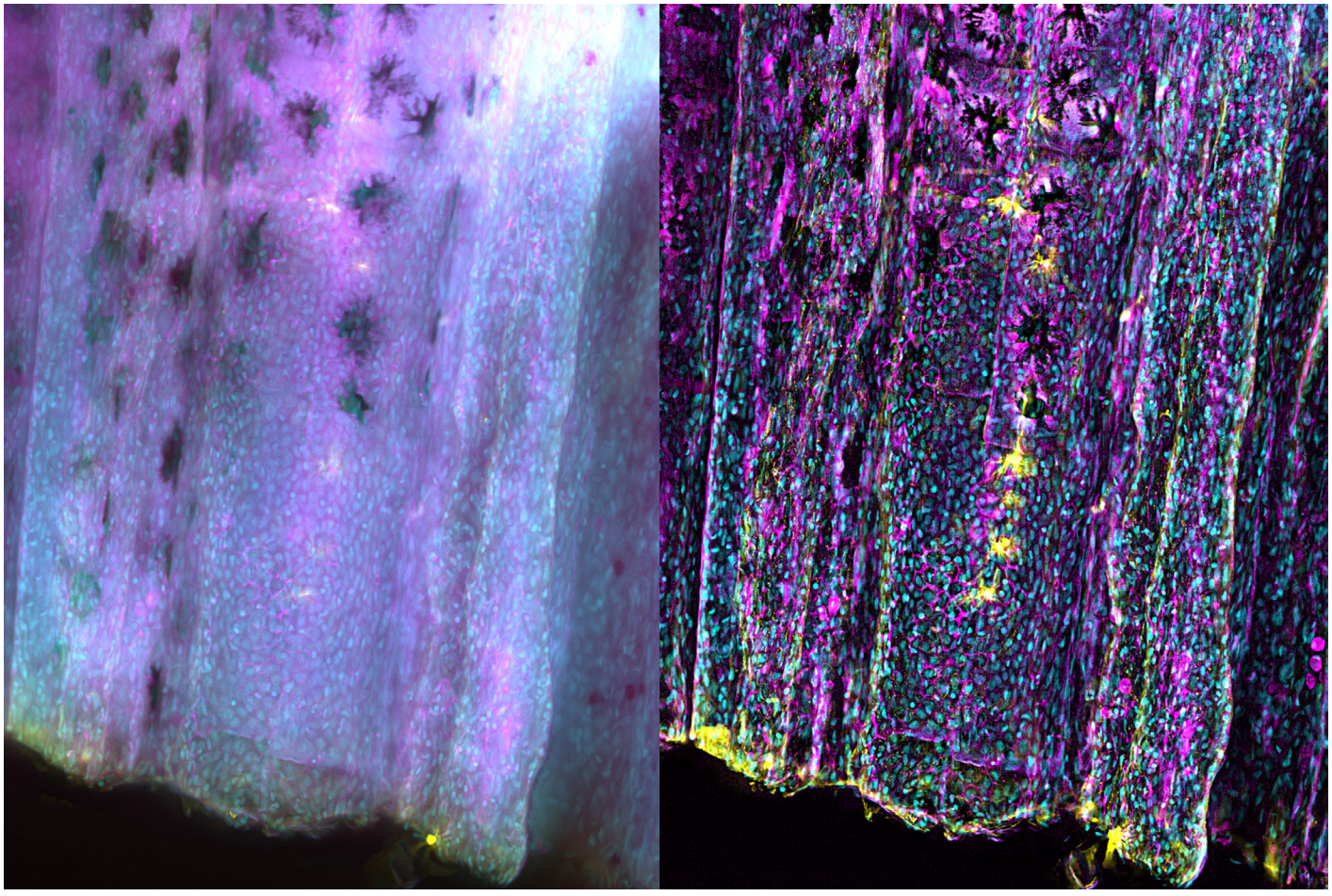
DISEASES LINKED TO SCAFFOLD PROTEINS AND SIGNALING

Studying mutations in Daple scaffold proteins that regulate crosstalk between G proteins during signaling and other growth promoting pathways



Authors

James DeRose , Ph.D.[1], Cristina Rohena , PhD.[1]
Leica Microsystems

Abstract

How diseases related to scaffold proteins, like Daple, can be studied efficiently in zebrafish models with a THUNDER Imager is discussed in this article. Scaffold proteins regulate crosstalk in G-protein signaling and other growth promoting pathways. Mutations in these proteins can lead to diseases and abnormalities like hydrocephalus and cancer. Scientists doing research in the field can gain insights rapidly concerning scaffold regulation of G-protein signaling in zebrafish with THUNDER and computational clearing.

Investigating scaffold protein mutations and disease

There is a significant amount of research focused on diseases which are caused by mutations in scaffold proteins, like Daple, that regulate crosstalk between G protein signaling and other growth promoting pathways. Mutations in these proteins have been linked to abnormalities like hydrocephalus and several cancers. Many cancer researchers investigate protein signaling in model organisms like zebrafish using genetically modified fluorescent strains with the hopes of gaining a better mechanistic understanding of how faulty signaling causes disease.

Challenges when imaging model organisms

One of the limitations when imaging model organisms is the autofluorescence seen in some of the zebrafish tissues and the time it takes to image them. Conventionally, most of the imaging is done using confocal microscopy to achieve adequate resolution. This involves acquisition of z-stacks in multiple fluorescent channels and would take on average 10-15 minutes to acquire a confocal image.

Methods

Zebrafish fin tissue immunostained for GFP (yellow), actin (magenta), and nuclei (DAPI, cyan) was used for this study. Specimens were imaged with a THUNDER Imager Live Cell having a 20x, 0.8 NA (numerical aperture) objective. A z-stack of 162 slices every 0.5 µm (total of 80.25 µm) was obtained and processed using large volume (LVCC) or instant computational clearing (ICC). Images were acquired with speeds typical of widefield microscopy. Extended depth of field (EDoF) projections are shown.

Results

With the Thunder Imager Live Cell, the time to acquire a z-stack of 3 channels and 162 slices was just over 1 minute and 10 seconds, allowing a large increase in output compared to confocal microscopy. Additionally, by combining ICC and deconvolution, the background was greatly reduced and fine details more clearly revealed. THUNDER images with enhanced contrast and resolution are seen in comparison to raw widefield ones in figure 1 and 2.

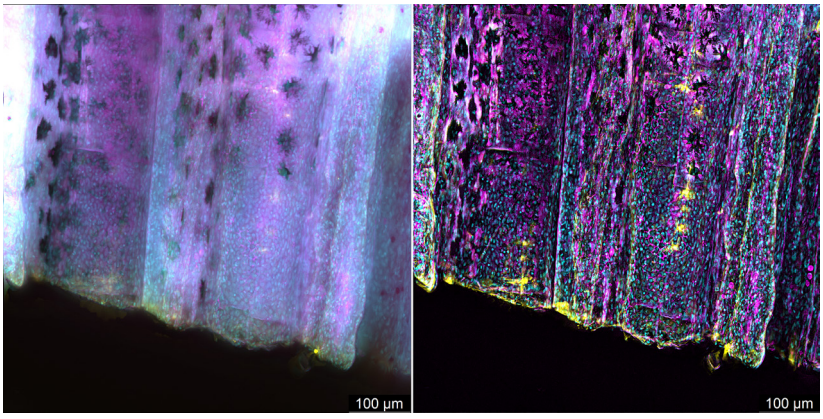


Fig. 1: EDoF projections of a GFP-tagged zebrafish fin: A) raw widefield and B) LVCC processed images. Images are courtesy of the Jason Ear lab at Cal Poly Pomona, California, USA.

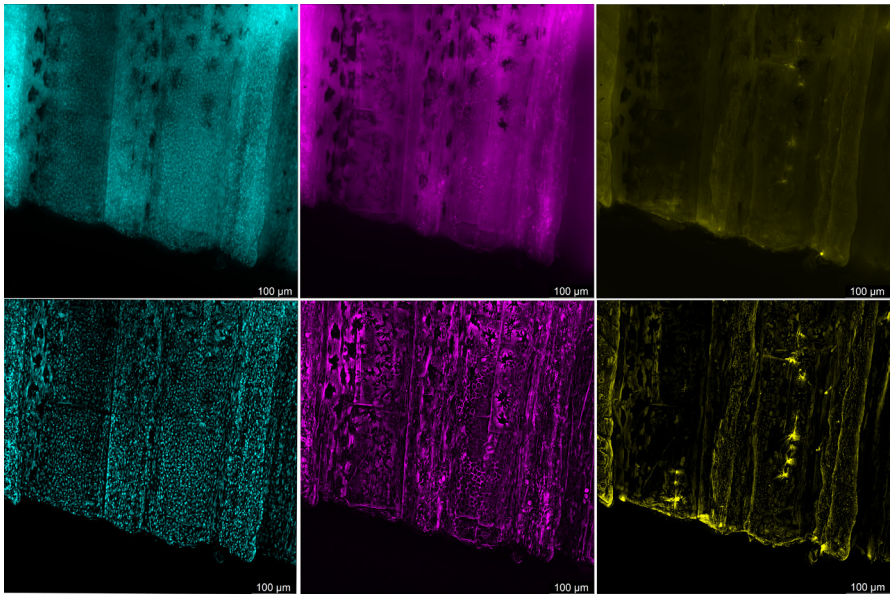


Fig. 2: Top row shows the raw widefield and bottom row the LVCC processed EDoF projection images of a GFP-tagged zebrafish fin. Images are courtesy of the Jason Ear lab at Cal Poly Pomona, California, USA.

Conclusions

THUNDER images of zebrafish fin, where large volume (LVCC) or instant computational clearing (ICC) were applied, revealed better fluorescence signals of tissue immunostained for GFP, actin, and nuclei compared to conventional widefield microscope images.

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Leica Microsystems CMS GmbH | Ernst-Leitz-Strasse 17–37 | D-35578 Wetzlar (Germany)
Tel. +49 (0) 6441 29-0 | F +49 (0) 6441 29-2599

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