Novel IR-LEGO system enables single-cell labeling and tracking in zebrafish embryos

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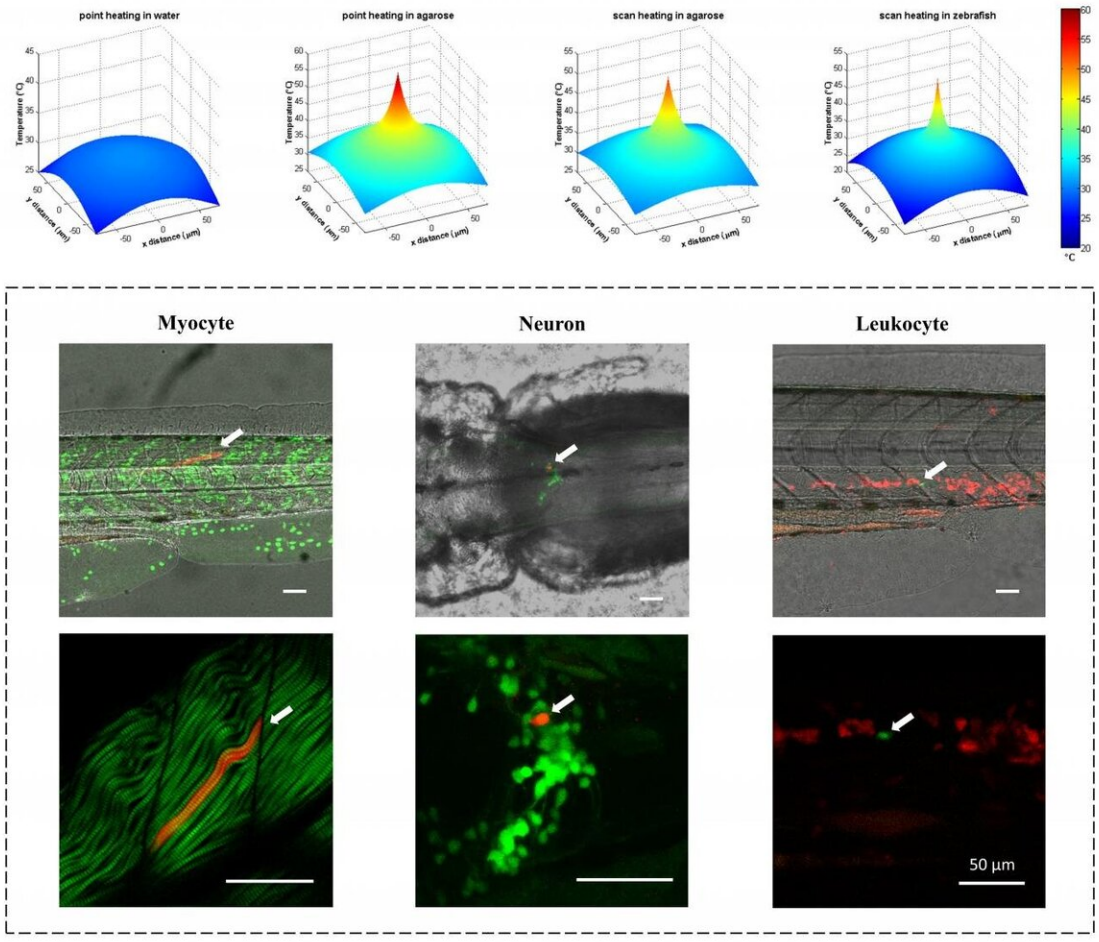

3-D temperature measurement by fluorescent thermometry and single-cell labeling in different tissues of zebrafish. Credit: HKUST

Heterogeneity broadly exists in various cell types both during development and at homeostasis. Investigating heterogeneity is crucial for understanding the complexity of ontogeny, dynamics, and function of specific cell types.

However, traditional bulk-labeling techniques are not competent to dissect heterogeneity within cell population, while the new single-cell lineage tracing methods invented in the last decade can hardly achieve high-fidelity single-cell labeling and long-term in-vivo observation simultaneously.

To tackle these problems, a team of scientists, led by Prof. Qu Jianan and Prof. Wen Zilong from the Hong Kong University of Science and Technology (HKUST), has developed a novel optical technique, which enables them to label a single hemogenic endothelium cell in a zebrafish embryo and track the cell and its descendants.

"We develop an infrared laser-evoked gene operator (IRLEGO) technology, in which a two-photon fluorescent thermometer is utilized to measure the temperature rise in-vivo to achieve precise single-cell labeling," said Prof. Qu, Professor at the Department of Electronic and Computer Engineering at HKUST. "The two-photon microscopy-based thermometry could perform in-vivo and non-invasive imaging of a temperature rise in the region around the Infrared laser focal point in the tissue with an accuracy of about 0.5°C. Using this state-of-the-art fluorescent thermometer, we successfully measured the temperature distributions in the region close to the infrared laser focal point in live zebrafish. The results show that the temperature can be raised to 40-50°C for heat-shock induction in a well-defined small 3-D volume (~15-20 μm), which is about the size of one or two cells."

In addition, the background noise—common issue among various single-cell lineage tracing technologies and namely the appearance of green

fluorescent protein signals in zebrafish without heat shock—creates a major challenge to single-cell lineage tracing. To minimize the effect of this background noise, the team developed a statistical algorithm to determine the lineage distribution derived from a single cell.

"Using this tool, we documented that the hemogenic endothelium (HE) cells in the posterior blood island of zebrafish are heterogeneous in terms of hematopoietic potential. Our study demonstrated that the high-precision single-cell IRLEGO technology has outstanding capacity to perform single-cell labeling and long-term in-vivo lineage tracing," Prof. Wen, Professor at the Division of Life Science at HKUST said.

The research team—in collaboration with Prof. Xu Jin of the Division of Cell, Developmental and Integrative Biology, School of Medicine, South China University of Technology—also revealed that there are at least two distinct populations of hemogenic endothelium cells. One of them can give rise to both lymphoid and myeloid cells, while the other can only give rise to myeloid cells.

"These findings shed light on the mechanisms of blood formation, and potentially could provide useful tools to study the development of diseases such as leukemia. Additionally, the single-cell labeling technology could be applied to study the development of other tissues and organs and to answer many important questions related to stem cell biology, including cancer stem cells," Prof. Wen added.

The research findings were recently published in the scientific journal *eLife*.

"In future, we aim to apply the single-cell labeling technology to answer fundamental biological questions and develop therapeutic strategy against a variety of diseases, such as developmental origin(s) of different types of tissues, tracing single cell movement in vivo, e.g. immune cell

responses to a variety of induction signals, monitoring cell competition in tissue homeostasis and cancer formation and etc.," Prof. Qu added.

More information: Sicong He et al, In vivo single-cell lineage tracing in zebrafish using high-resolution infrared laser-mediated gene induction microscopy, *eLife* (2020). DOI: 10.7554/eLife.52024

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