Noninvasive technique reveals how cells' gene expression changes over time

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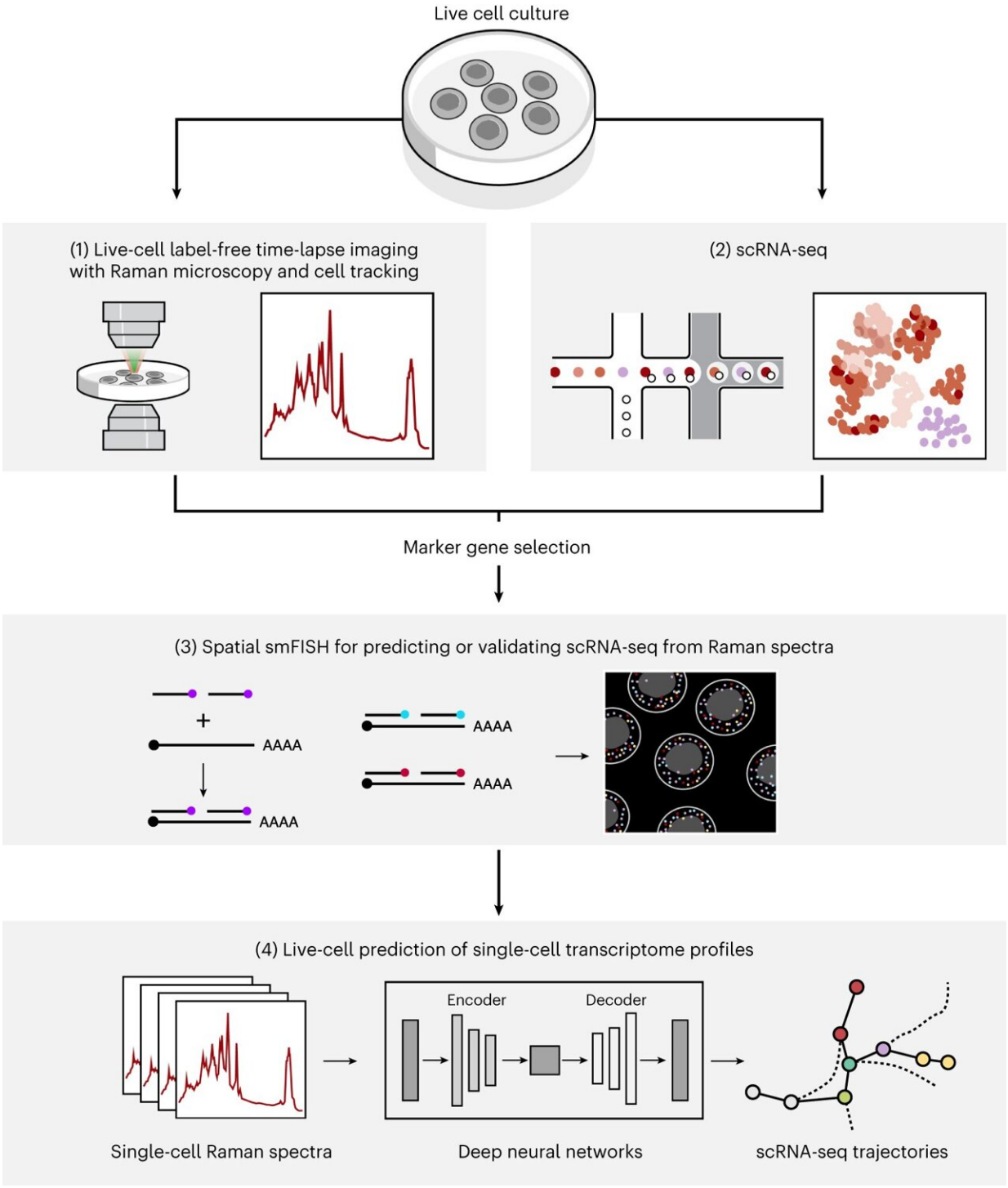

R2R. Credit: *Nature Biotechnology* (2024). DOI: 10.1038/s41587-023-02082-2

Sequencing all of the RNA in a cell can reveal a great deal of information about that cell's function and what it is doing at a given point in time. However, the sequencing process destroys the cell, making it difficult to study ongoing changes in gene expression.

An alternative approach developed at MIT could enable researchers to track such changes over extended periods of time. The new method, which is based on a noninvasive imaging technique known as Raman spectroscopy, doesn't harm cells and can be performed repeatedly.

Using this technique, the researchers showed that they could monitor embryonic stem cells as they differentiated into several other cell types over several days. This technique could enable studies of long-term cellular processes such as cancer progression or embryonic development, and one day might be used for diagnostics for cancer and other diseases.

"With Raman imaging you can measure many more time points, which may be important for studying cancer biology, developmental biology, and a number of degenerative diseases," says Peter So, a professor of biological and mechanical engineering at MIT, director of MIT's Laser Biomedical Research Center, and one of the authors of the paper.

Koseki Kobayashi-Kirschvink, a postdoc at MIT and the Broad Institute of Harvard and MIT, is the lead author of the study, which appears in *Nature Biotechnology.* The paper's senior authors are Tommaso Biancalani, a former Broad Institute scientist; Jian Shu, an assistant professor at Harvard Medical School and an associate member of the Broad Institute; and Aviv Regev, executive vice president at Genentech Research and Early Development, who is on leave from faculty positions at the Broad Institute and MIT's Department of Biology.

Imaging gene expression

Raman spectroscopy is a noninvasive technique that reveals the chemical composition of tissues or cells by shining near-infrared or visible light on them. MIT's Laser Biomedical Research Center has been working on biomedical Raman spectroscopy since 1985, and recently, So and others in the center have developed Raman spectroscopy-based techniques that could be used to diagnose breast cancer or measure blood glucose.

However, Raman spectroscopy on its own is not sensitive enough to detect signals as small as changes in the levels of individual RNA molecules. To measure RNA levels, scientists typically use a technique called single-cell RNA sequencing, which can reveal the genes that are active within different types of cells in a tissue sample.

In this project, the MIT team sought to combine the advantages of single-cell RNA sequencing and Raman spectroscopy by training a computational model to translate Raman signals into RNA expression states.

"RNA sequencing gives you extremely detailed information, but it's destructive. Raman is noninvasive, but it doesn't tell you anything about RNA. So, the idea of this project was to use machine learning to combine the strength of both modalities, thereby allowing you to understand the dynamics of gene expression profiles at the single cell level over time," Kobayashi-Kirschvink says.

To generate data to train their model, the researchers treated mouse fibroblast cells, a type of skin cell, with factors that reprogram the cells to become pluripotent stem cells. During this process, cells can also transition into several other cell types, including neural and epithelial cells.

Using Raman spectroscopy, the researchers imaged the cells at 36 time points over 18 days as they differentiated. After each image was taken,

the researchers analyzed each cell using single molecule fluorescence in situ hybridization (smFISH), which can be used to visualize specific RNA molecules within a cell. In this case, they looked for RNA molecules encoding nine different genes whose expression patterns vary between cell types.

This smFISH data can then act as a link between Raman imaging data and single-cell RNA sequencing data. To make that link, the researchers first trained a deep-learning model to predict the expression of those nine genes based on the Raman images obtained from those cells.

Then, they used a computational program called Tangram, previously developed at the Broad Institute, to link the smFISH gene expression patterns with entire genome profiles that they had obtained by performing single-cell RNA sequencing on the sample cells.

The researchers then combined those two computational models into one that they call Raman2RNA, which can predict individual cells' entire genomic profiles based on Raman images of the cells.

Tracking cell differentiation

The researchers tested their Raman2RNA algorithm by tracking mouse embryonic stem cells as they differentiated into different cell types. They took Raman images of the cells four times a day for three days, and used their computational model to predict the corresponding RNA expression profiles of each cell, which they confirmed by comparing it to RNA sequencing measurements.

Using this approach, the researchers were able to observe the transitions that occurred in individual cells as they differentiated from embryonic stem cells into more mature cell types. They also showed that they could track the genomic changes that occur as mouse fibroblasts are

reprogrammed into induced pluripotent stem cells, over a two-week period.

"It's a demonstration that optical imaging gives additional information that allows you to directly track the lineage of the cells and the evolution of their transcription," So says.

The researchers now plan to use this technique to study other types of cell populations that change over time, such as aging cells and cancerous cells. They are now working with cells grown in a lab dish, but in the future, they hope this approach could be developed as a potential diagnostic for use in patients.

"One of the biggest advantages of Raman is that it's a label-free method. It's a long way off, but there is potential for the human translation, which could not be done using the existing invasive techniques for measuring genomic profiles," says Jeon Woong Kang, an MIT research scientist who is also an author of the study.

More information: Prediction of single-cell RNA expression profiles in live cells by Raman microscopy with Raman2RNA, *Nature Biotechnology* (2024). DOI: 10.1038/s41587-023-02082-2. On *bioRxiv*: DOI: 10.1101/2021.11.30.470655

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