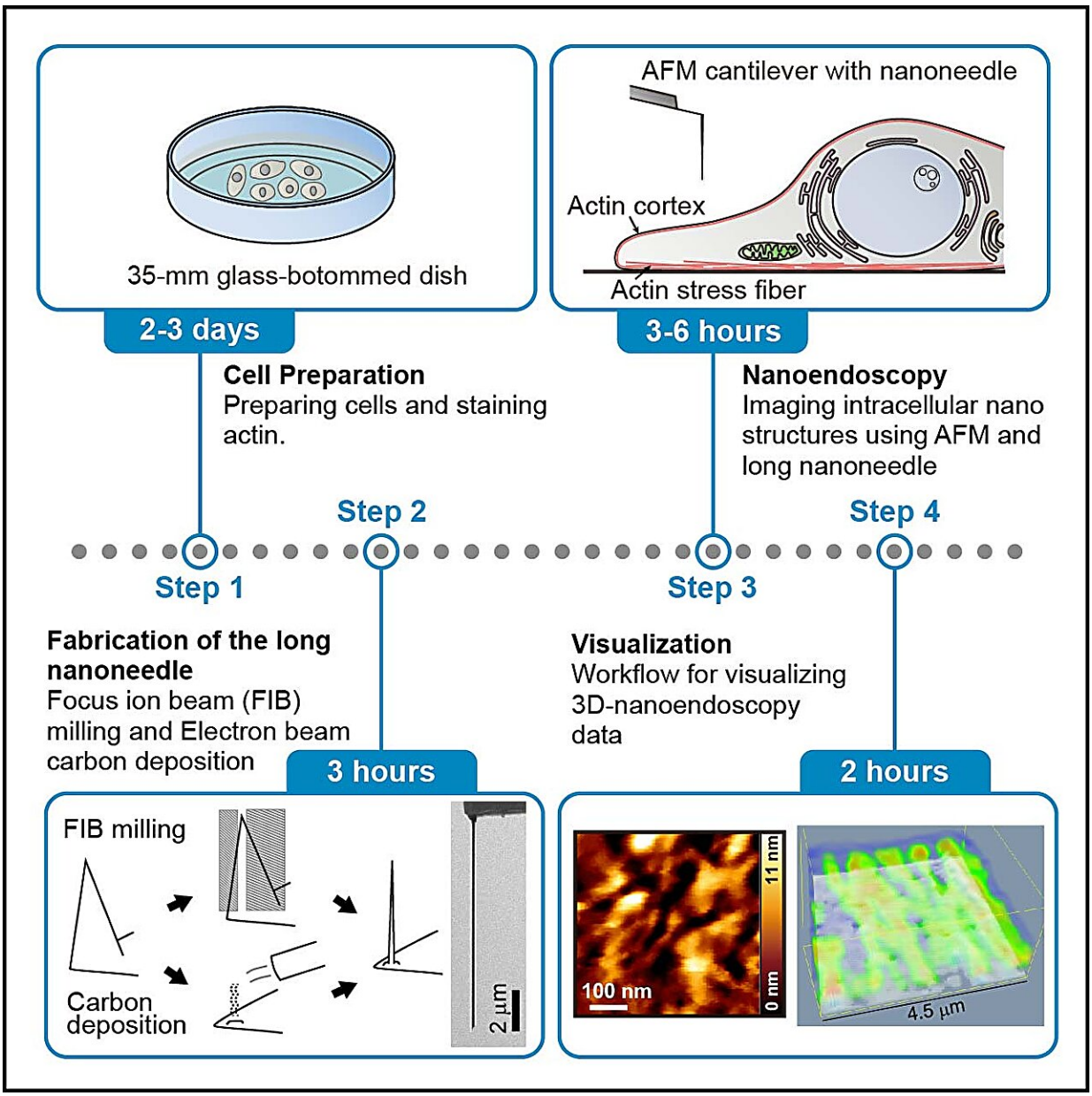
Researchers define protocol for high-resolution imaging of living cells using atomic force microscopy

September 5 2023



Overview of the method for observing actin fibers in living cells using nanoendoscopy-AFM. Credit: *STAR Protocols* (2023). DOI: 10.1016/j.xpro.2023.102468

Images of nanoscale structures inside living cells are in increasing demand for the insights into cellular structure and function that they can reveal. So far, the tools for capturing such images have been limited, but researchers led by Takeshi Fukuma and Takehiko Ichikawa at Kanazawa University have now devised and reported a full protocol for using atomic force microscopy (AFM) to image inside living cells. The research is published in the journal *STAR Protocols*.

AFM was first developed in the 1980s and uses the changes in the forces between a sample surface and a nanoscale tip attached to a cantilever to identify surfaces and produce images of the topography with nanoscale resolution. The technique has grown increasingly sophisticated for extracting information about samples at speeds sufficient for the tool to capture moving images of dynamics at the nanoscale. However, it has so far been limited to surfaces.

Other techniques exist that can provide a view of the inside of a cell, but they have limitations. For instance, there is electron microscopy, which is capable of resolving details at the nanoscale and smaller, but the required operating conditions are not compatible with living cells. Alternatively, fluorescence microscopy is regularly used on living cells, but while fluorescence techniques exist to increase resolution, there are practical challenges that inhibit fluorescence imaging at the nanoscale.

AFM suffers from neither limitation, and by embellishing the tool with a

nanoneedle to penetrate cells, Fukuma, Ichikawa and their collaborators have recently demonstrated its ability to image inside cells at the nanoscale, which they describe as nanoendoscopy-AFM.

In their protocol, the researchers break down the method for nanoendoscopy-AFM into four stages. The first few steps involve cell preparation and staining with a fluorescent dye and checking the fluorescence, which is used to identify the imaging area quickly. Next is the fabrication of the nanoneedles themselves, for which there are two options—either etching away a nanoneedle structure with a focused ion beam or building one up with electron beam deposition.

Then comes the nanoendoscopy stage itself, and in the report, the researchers describe the approach for both 2D and 3D nanoendoscopy. There are even details outlined to describe the best way to clean up after the nanoendoscopy images are captured before finally outlining the data processing needed to visualize the measured data. The method is replete with tips for successfully accomplishing each stage as well as a guide for troubleshooting when things are not quite working out.

This technique should be suitable for the observation of intact intracellular structures, including mitochondria, focal adhesions, endoplasmic reticulum, lysosomes, Golgi apparatus, organelle connections, and liquid–liquid phase-separated structures. They conclude, "This protocol can be expected to become a standard tool for studying nanoscale structures."

More information: Takehiko Ichikawa et al, Protocol for live imaging of intracellular nanoscale structures using atomic force microscopy with nanoneedle probes, *STAR Protocols* (2023). DOI: 10.1016/j.xpro.2023.102468

Provided by Kanazawa University