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# Application News

# No. S43

**Scanning Probe Microscope**

# Observation and Measurement of Cells with SPM (II): Measurement of Cell Hardness

## Outline

Progress in regenerative medicine using iPS cells has been remarkable in recent years, and clinical research has also been reported. Among the result of research on iPS cells, it has been reported that the colony shape, proliferation rate, and other features differ depending on the cell line derivation and culture method (Fig. 1), and depending on the case, iPS cells may form cancer cells. Since it can be inferred that the differences in iPS cells referred to as "individuality" are one factor that controls differentiation into diverse types of cells, elucidation of iPS cell individuality may be a key to large advances in regenerative medicine. However, the origins of individuality are still largely unknown, and this has become an obstacle to the use of iPS cells.

Therefore, in Application News No. S38 [1], iPS cells and HeLa cells were observed with a scanning probe microscope (SPM), and as a result, it was found that the intercellular adhesion between iPS cells has a network structure.

Focusing on the mechanical properties of the cells, hardness was analyzed in the following report. The results suggested that the hardness of HeLa cells is substantially uniform, as the difference between hard and soft parts is slight, whereas the intercellular adhesion area of iPS cells is hard, and the cell body itself is soft.

A. Kogure, T. Maruo, K. Hoshino,
K. Yamasaki, T. Fujii, H. Nakajima

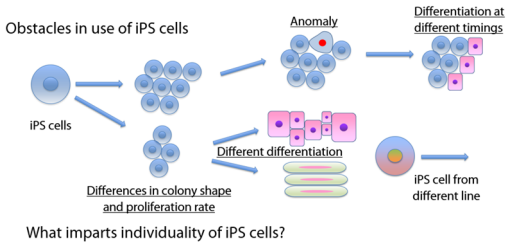


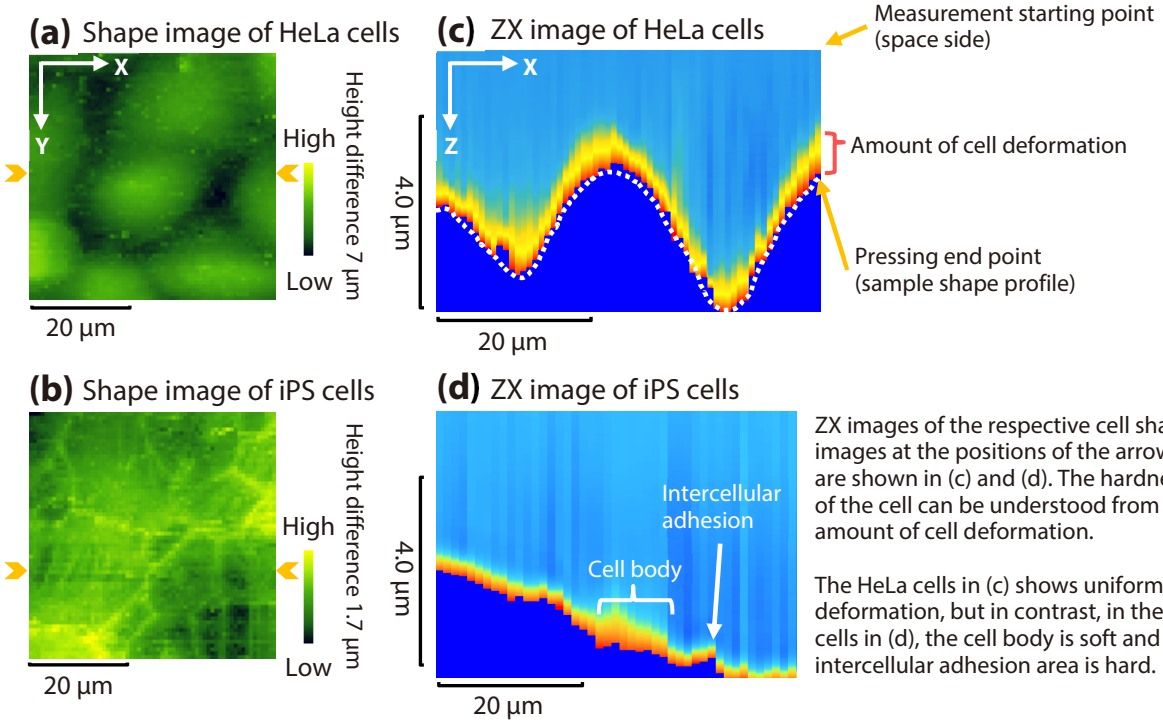

**Fig. 1 Obstacles in Use of iPS Cells**

## Measurement of Cell Hardness

Fig. 2 (a) and (b) show images of the cell shapes of HeLa and iPS cells observed by SPM, and (c) and (d) show the corresponding ZX images. Here, (c) and (d) are images of the force applied to the probe at the X line position indicated by the arrows in (a) and (b), as seen from the sample cross-sectional direction. The top of the figures is the measurement starting point (space side), and the white dotted line in the lower part of the figures shows the pressing end point, and thus represents the cross-sectional shape profile of the sample.

In the ZX images, the positions where force was detected after the probe made contact with the sample are shown in colors from yellow to red. Because this indicates deformation of the cell by the probe, it can be understood that larger amounts of cell deformation show softer parts of the cell.

The analysis results suggested that HeLa cells have substantially uniform hardness, as there is little difference between the soft parts and hard parts, but in contrast, the iPS cell body is soft and intercellular adhesion areas are hard.




ZX images of the respective cell shape images at the positions of the arrows are shown in (c) and (d). The hardness of the cell can be understood from the amount of cell deformation.

The HeLa cells in (c) shows uniform deformation, but in contrast, in the iPS cells in (d), the cell body is soft and the intercellular adhesion area is hard.

**Fig. 2 Shape Images and ZX Images of HeLa Cells and iPS Cells**

## Observation and Measurement by SPM

Fig. 3 shows the configuration of the SPM, and Fig. 4 shows the configuration for observation in a solution. Although the SPM is different from optical microscopes and electron microscopes in that a beam and lens are not used in observation, its resolution is comparable to that of the transmission electron microscope (TEM). In the SPM, a shape image can be acquired by tracing the sample with a fine needle called a probe and detecting the minute force acting between the probe and the sample as deflection of the cantilever. In the contact mode and dynamic mode, which are the general observation methods with an SPM, the sample surface is scanned in the horizontal direction, but in the case of soft samples with large surface irregularities, like cells, it was difficult to obtain normal shape images because the cell surface was scratched by the probe. Force curve measurement was used as a solution to this problem.

Fig. 5 shows the principle of force curve measurement [2]. In this measurement method, force is plotted while changing the scanner Z [3][4][5]. Because horizontal scanning is not used, cells are not scratched and observation and measurement of soft, large irregularities is also possible. In this experiment, the pressing force (repulsive force) to the cells was 2.5 nN [1]. It is possible to determine the hardness of the sample by comparing the distance from the position where the probe comes into contact with the sample until the force of 2.5 nN is achieved.

After mapping measurement of the measurement area at 64 × 64 points, a shape image and ZX image were formed from the obtained ZXY information and the force curve of the points. A cantilever with a spring constant of 0.15 N/m was used, and the measurements were conducted with the cells in a live condition in a culture solution.

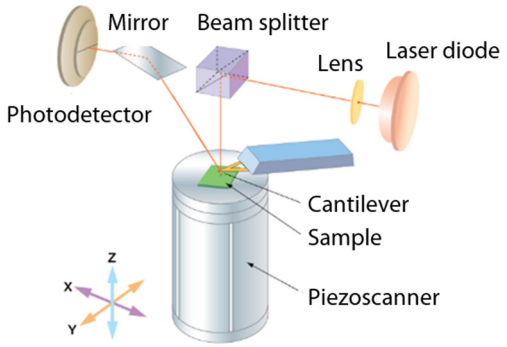



**Fig. 3 Configuration of SPM**

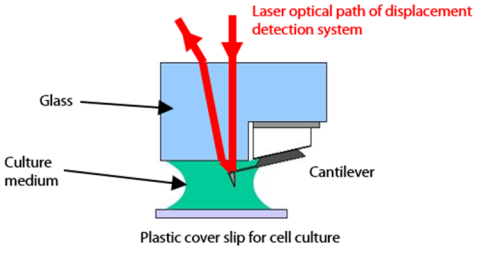



**Fig. 4 Configuration for Observation in Solution**
**Observation of Specimens in a Solution with This Configuration.**

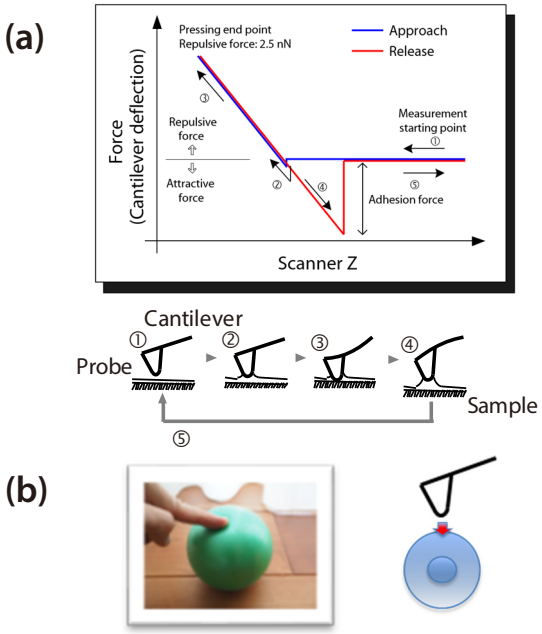



**Fig. 5 Explanation of Force Curve Measurement**

(a) Force applied to the probe is measured while changing the scanner Z. Pressing (Approach) is stopped when the force reaches 2.5 nN, and the probe is then retracted (Release). A shape profile image is formed by mapping the Z-positions where 2.5 nN was achieved at each measurement point.
The distance from the position where the probe and sample come into contact ② until the specified repulsion force is achieved ③ is defined as the amount of sample deformation, and the hardness of the sample can be measured by comparing this amount at each point.
(b) Feeling the shape of a ball by pressing it with a finger is an easy-to-understand image.

## Conclusion

The hardness of HeLa cells and iPS cells in the live condition was measured with SPM. It was suggested that the HeLa cells have substantially uniform hardness, whereas the iPS cells display high hardness in areas of intercellular adhesion. It is considered possible that this hard intercellular adhesion has some kind of influence on maintenance of the undifferentiated state and the pluripotency of iPS cells. Measurement of cell hardness by SPM is expected to provide new perspectives in research on regenerative medicine.

References

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Specimens were provided by Prof. Hirotaka James Okano and Chikako Hara, Assistant Professor, The Jikei University School of Medicine, Division of Regenerative Medicine.

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