Deep-learning method based on tri-frequency ultrasound images for high-resolution observation of live cells in culture

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1. Introduction

Live-cell imaging is very important for assessing cell morphology, structure, and cell state[1]. Various optical microscopy techniques have been employed to achieve this, and they require long periods of intense light exposure to obtain high-resolution images with good signal-to-noise ratio[2,3]. However, light irradiation causes oxidation of intracellular molecules and degrades cell functions, which prohibit us to observe the same cell for a long time with a high frame rate.

On the other hand, scanning acoustic microscopy (SAM) is expected to solve the above problem because it uses acoustic properties to produce images without light illumination, labeling, or physical contacts with the cell. However, due to the long wavelength of ultrasound, spatial resolution is considerably poorer than that of optical microscopy.

To solve these problems, we propose a deep-leaning (DL) method to generate high-resolution images from ultrasound images. DL approaches have been utilized to improve the resolution of the SAM image [5], but the resolution of the generated image is inferior to that of an optical microscope image. In this study, as input images, we construct RGB (three-layer) images consisting of spectroscopic acoustic images of three specific frequencies, and train them with their corresponding optical images. For this, we develop the original convolutional neural network (CNN). Then, we applied this DL method to observe the same cell for more than 24 hours using our original SAM system for live-cell imaging[6].

2. Methods

Longitudinal wave pulses with a center frequency of 180 MHz were focused on the culture surface by the acoustic lens to obtain spectroscopic images. Echoes from the culture surface were detected by the same acoustic lens to perform Fast Fourier transform (FFT) processing. and 8-bit spectroscopic images of various frequencies were constructed. Then, optical images of the same cells were obtained. **Figure 1** indicates this procedure.

109,824 sets of ultrasound and optical microscopy images (512 pixels square) of human mesenchymal stem cells (MSCs) were prepared. We trained them using the original CNN as shown in **Fig. 2**, which has more convolutional-ReLU (rectified linear unit) sequences than U-Net[7].

3. Results and discussion

Figure 3 compares, the optical image, the generated image, and the input image (RBG spectral acoustic images) for MSC. The resolution of the generated images is significantly higher than that of the original acoustic image indicating that the DL method developed in this study is very effective.

We investigated various inputs of acoustic images, including mono-frequency images and RGB images of various frequency combinations, and the following findings were obtained. When a mono-

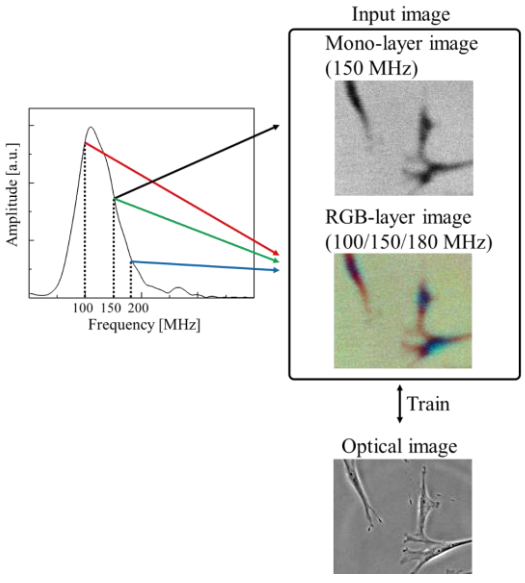

Fig 1. Construction of spectroscopic acoustic images for datasets.

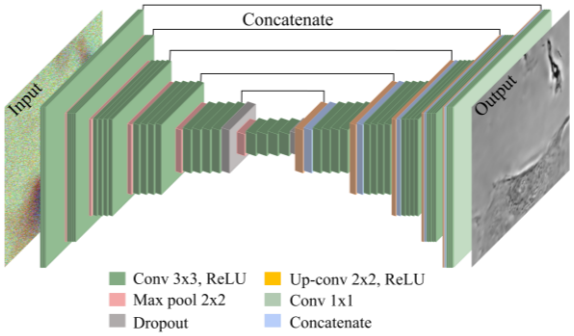

Fig 2. CNN developed in this study for generation of high-resolution acoustic images.

layer image of 180 MHz was used as the input, the generated image can reproduce the cell shape due to the short wavelength. However, it failed to reproduce the nuclei (not shown in the figure). On the other hand, an 80-MHz image favorably reproduced cell nuclei because the nucleus absorbs acoustic energy at 80 MHz, which attribute to the nucleus resonance.[6].

Following these results, we combine higher and lower frequencies in the three-layer input image (**Fig. 1**(a)), which succeeded in accurately generating both contour shapes and nuclei for various cell morphologies, as shown in **Fig. 3**. Thus, this RGB acoustic image can be apply to high-resolution live cell imaging without damaging the cells.

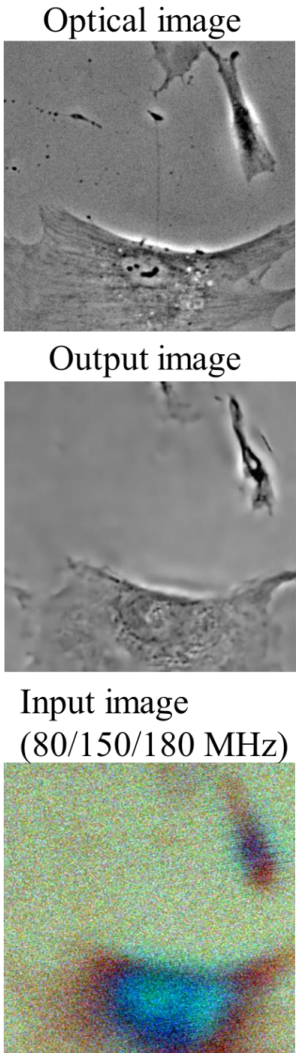

Fig. 3 Example of the high-resolution image generation using the DL method. The optical (answer) image (upper), output (generated) image (middle), and spectroscopic acoustic image as input (lower) of MSC.

4. Conclusion

We have developed a deep learning method for generating high-resolution images from a three-layer acoustic image. By training a dataset consisting of spectroscopic acoustic images and corresponding optical microscope images, the resolution of the generated images can be comparable to that of the optical microscope images.

Our method realizes long-time live cell observations of the same cell at high frame rates.

We plan to prepare the dataset of corresponding acoustic images and fluorescence images of cytoplasm and trained them to generate images of even higher resolution.

References

1) D. J. Stephens et al., Science **300**, 82 (2003).
2) J. R. Swedlow and M. Platani, Cell Struct. Func. **27**, 335 (2002).
3) M. M. Frigault et al., J. of Cell Sci. **122**, 753 (2009).
4) J. Rosner et al., J. Microsc. **264**, 215 (2016).
5) P. Banerjee et al., Sci. Tech. **5**, 015007 (2024).
6) N. Fujiwara et al., Jpn. J. Appl. Phys. **63**, 03SP65 (2024).
7) O. Ronneberger et al., MICCAI 2015, pp. 234–241.