

Label-Free Drug Screening Platform: Development of Force Nanosensor Arrays

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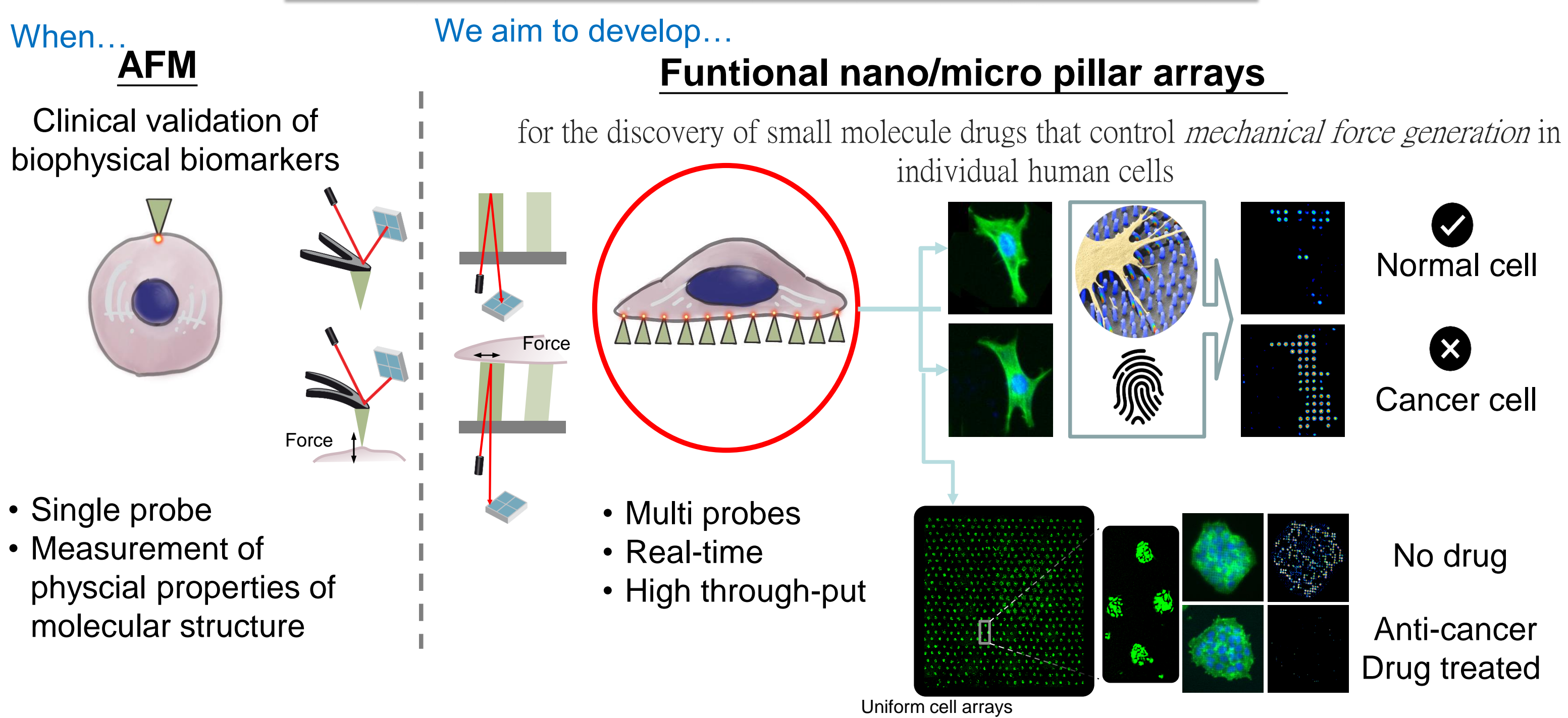
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Abstract

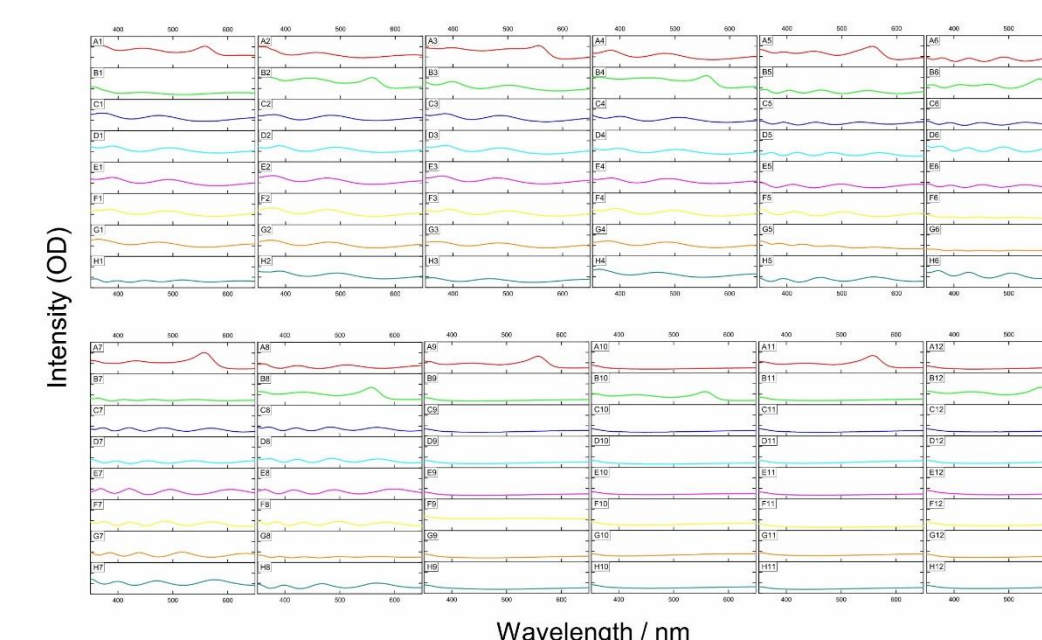
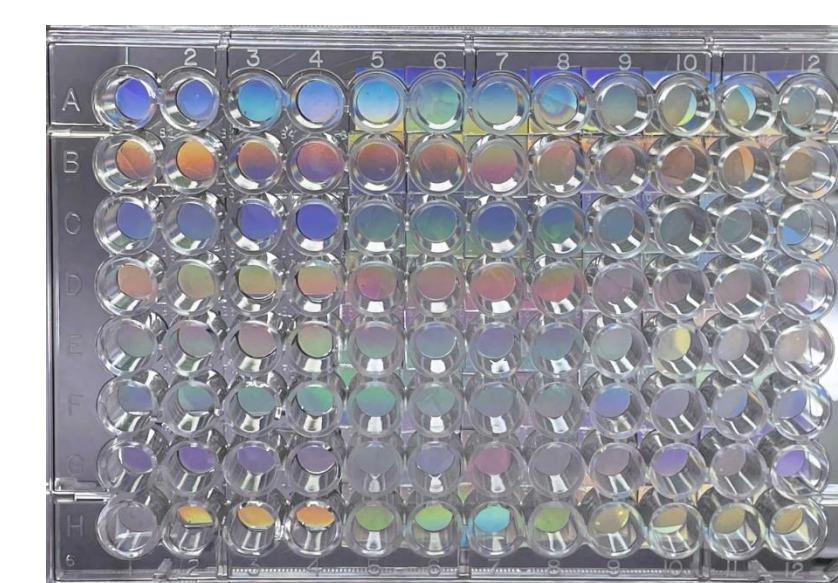
Mechanical force plays an important role in regulating the proliferation, differentiation, and death of cells. Cells can sense the mechanical cues in their microenvironment and respond to environmental changes through mechanotransduction pathways. When cells adhere to surfaces, as in the case of cell culture, the focal adhesions are formed on the surfaces where cells exert forces to the extracellular matrix elements on the surfaces through these anchorage points. It is known that when cells undergo division, differentiation, migration, apoptosis, morphological changes, and cell-cell interaction, the forces exerted by cells, also known as traction force, will change accordingly. Therefore, the forces generated by cells can be used as an indication of the status of the cells. In this project, we propose to develop a label-free drug sensing system based on the measurement of the cellular traction force. Our idea is to use the cells as reactors, which interact with different drugs. When any functions or signaling pathways of cells are altered by the drugs, the traction force will change, which can be detected by traction force nanosensors located underneath the cells. In this project, we will develop the traction force nanosensor using polymeric nanopillar arrays. When cells exert force on the polymeric nanopillars, the forces applied to each nanopillar can be calculated by the displacement of individual nanopillars using the corresponding Young's modulus. Therefore, the status of the cells can be monitored by the alterations in the traction force. Our system offers the following advantages over traditional sensing schemes for drug screening: 1) Label-free; the changes in cellular function induced by drugs can be monitored through variations in the traction force, which can be directly measured by the traction force nanosensors. The control experiment eliminates any cellular dependences on the chemical and physical properties of the traction force nanosensors. 2) Improved sensitivity in force sensing; the traction force is measured through the reflection of the incident light, whereas the conventional traction force is calculated by the fluorescence signals from the tips of nanopillars. The signal-to-noise ratio is greatly improved. 3) High throughput; the force nanosensor is compatible with traditional drug screening systems such as 96- or 384- well plate at a cell density of 1,700 cell/mm². We will build a high-speed reader in the project to read out the signals from individual cells at a speed of 17,000 cell/s for high throughput drug screening applications. 4) Single-cell resolution; with the cell patterning technique, we can measure the traction forces of cell arrays where the individual cellular responses can be recorded.

Concept



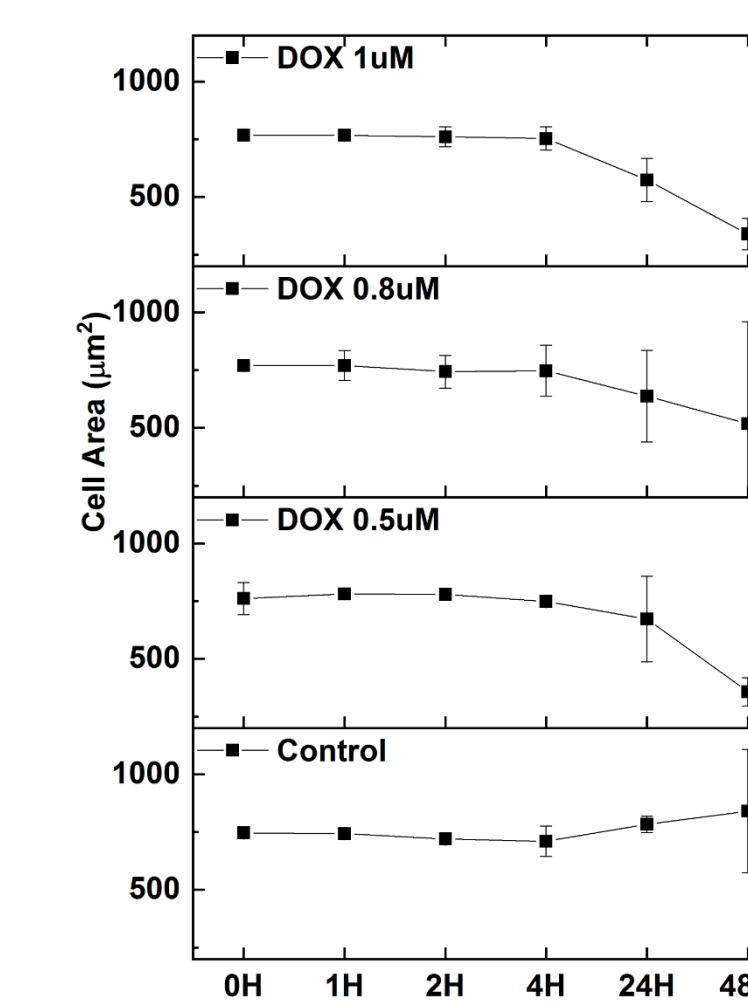
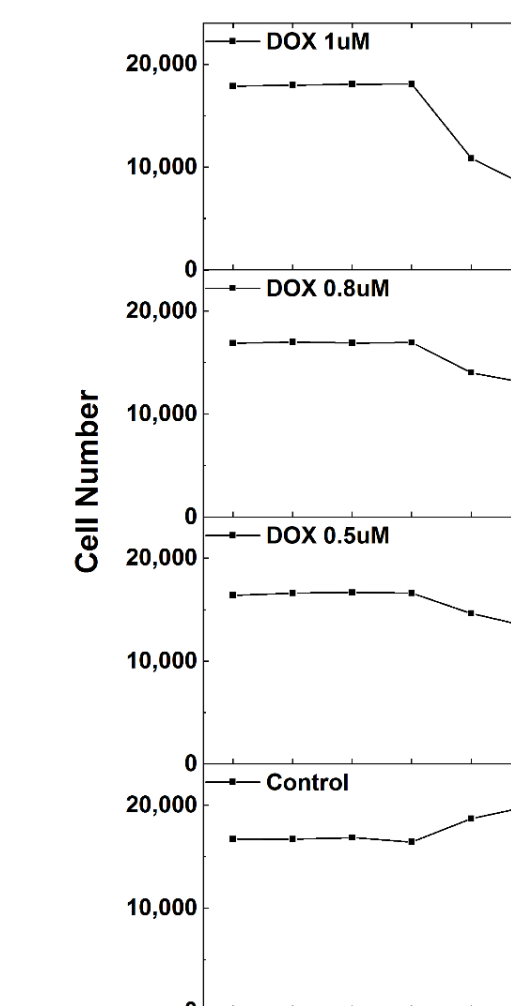
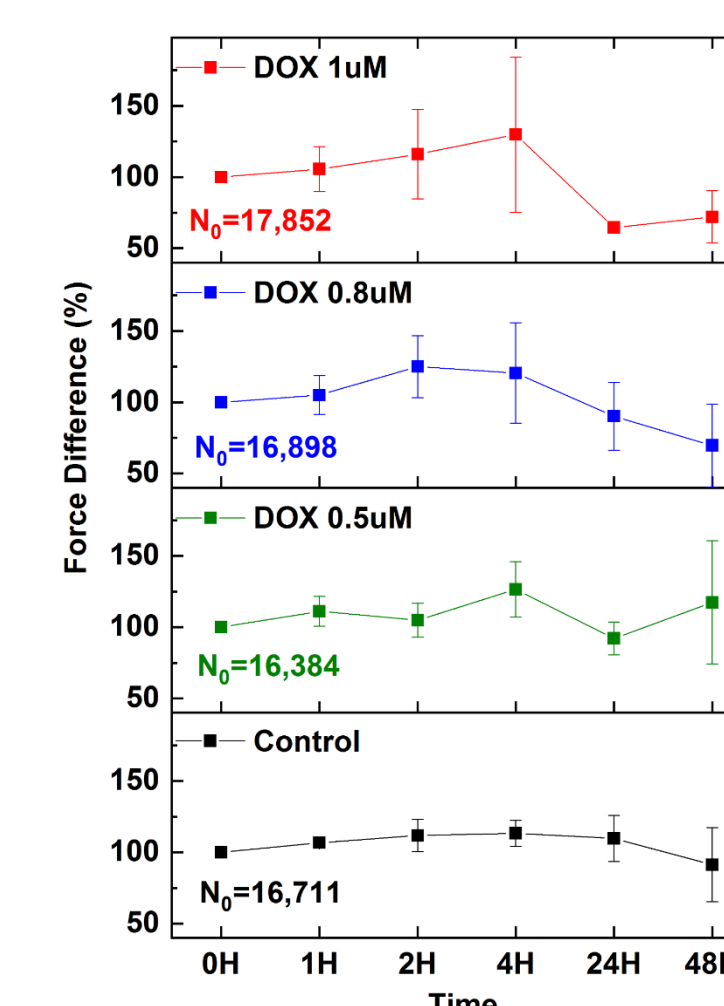
Drug Screening

Integration of Nanosensors Sensor with 96 Well

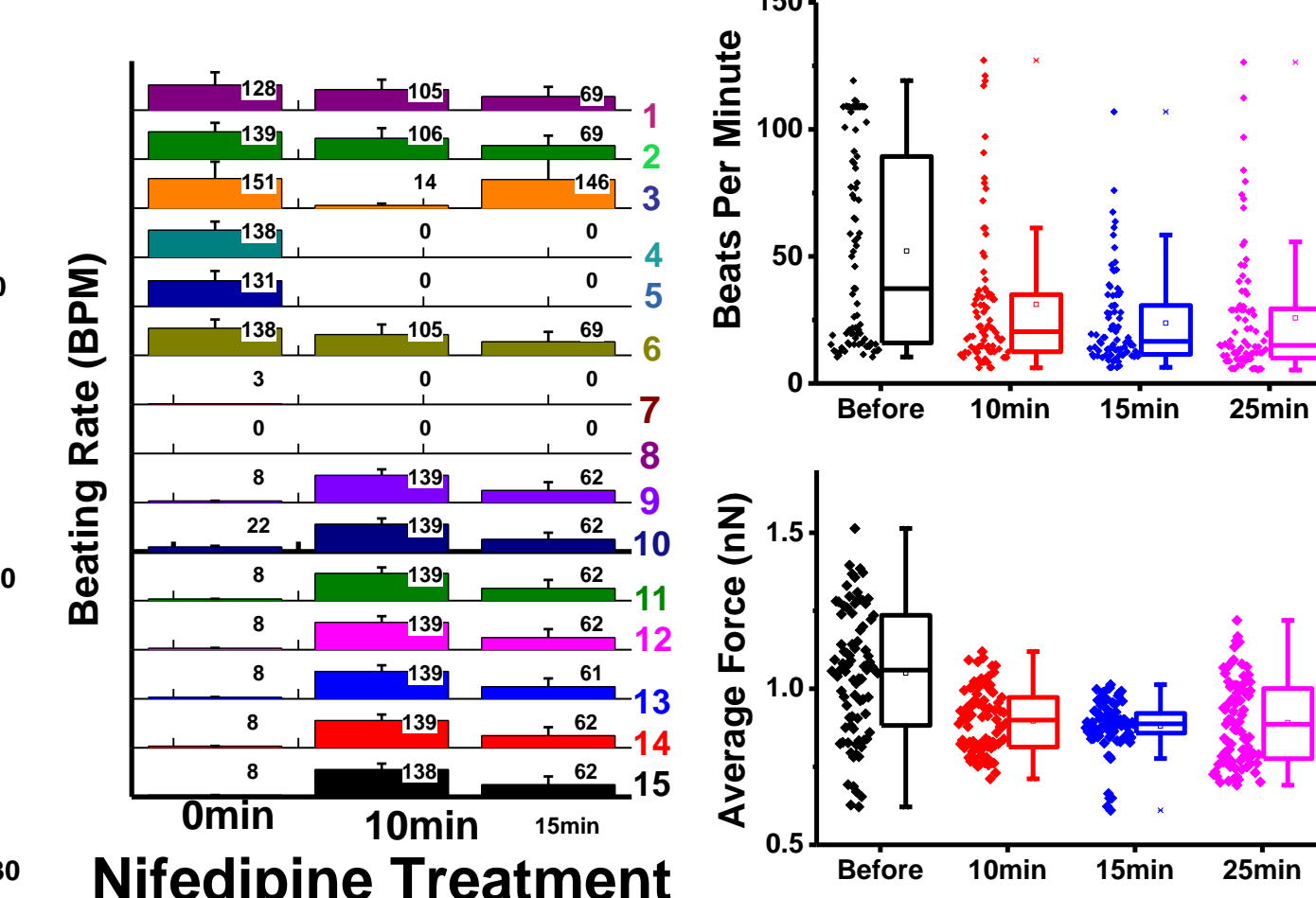
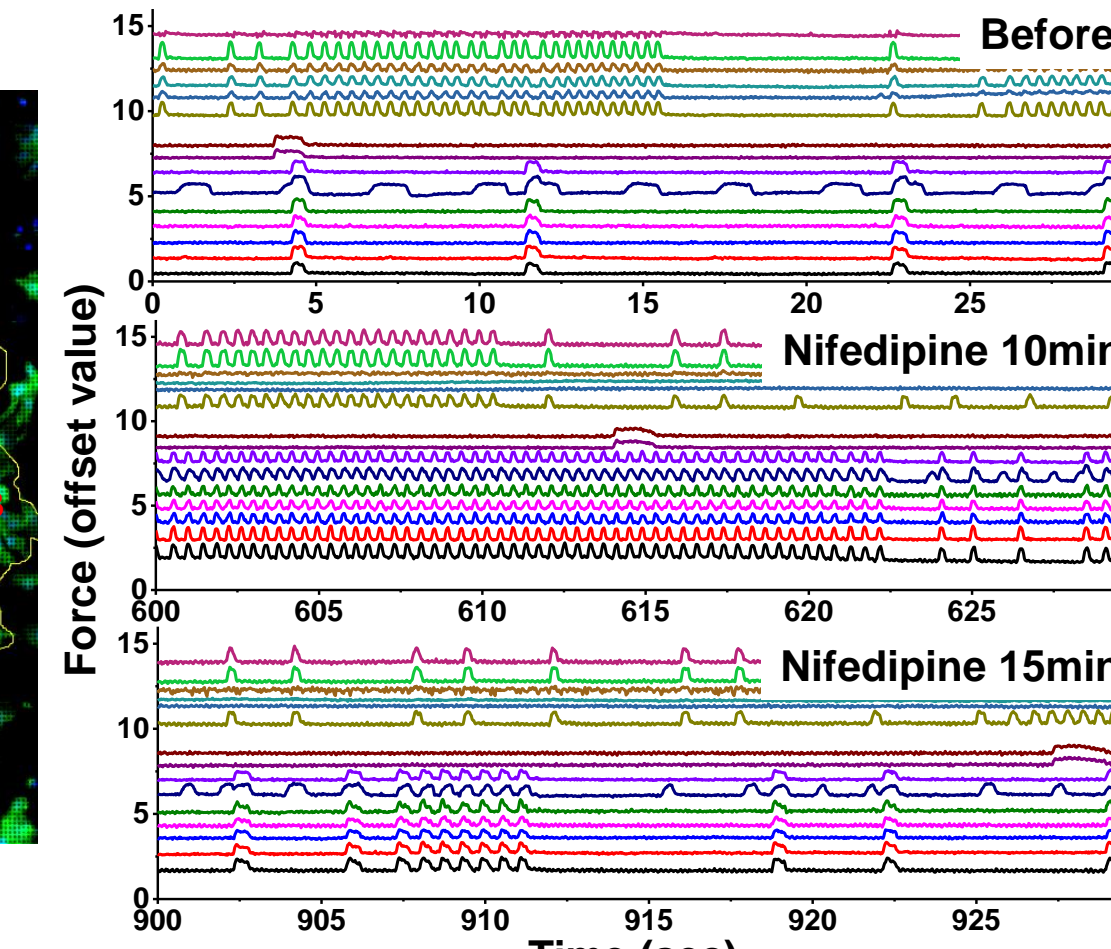
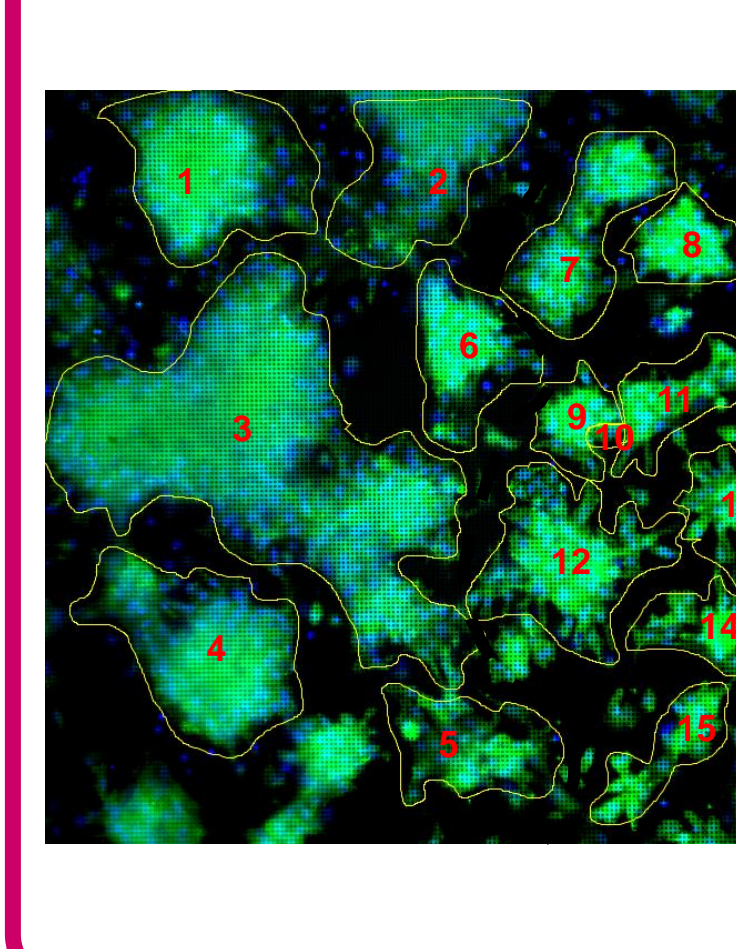


Drug Screening by Force Nanosensors

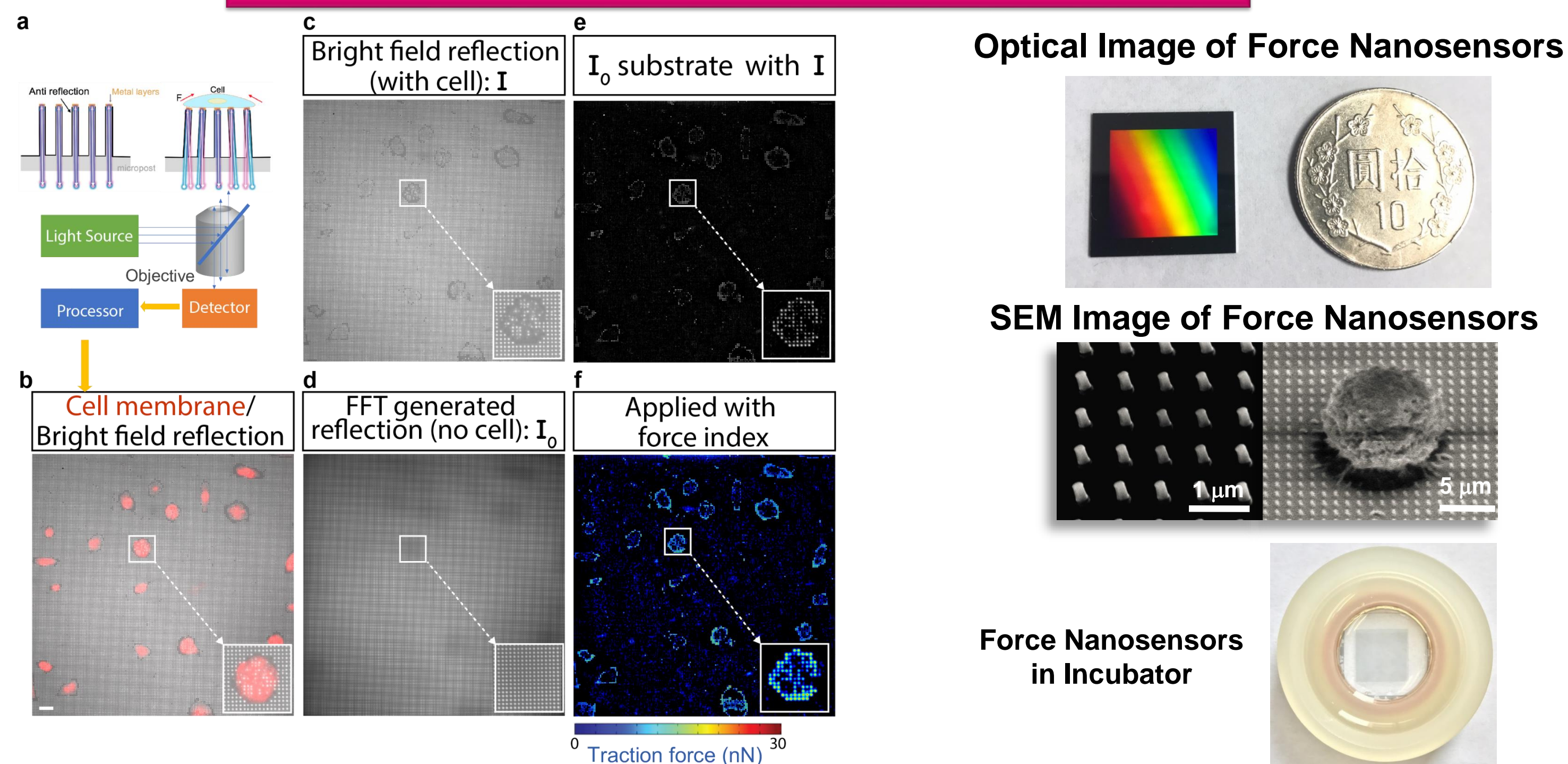
Drug Screening of DOX



Cardiotoxicity Test of Nifedipine

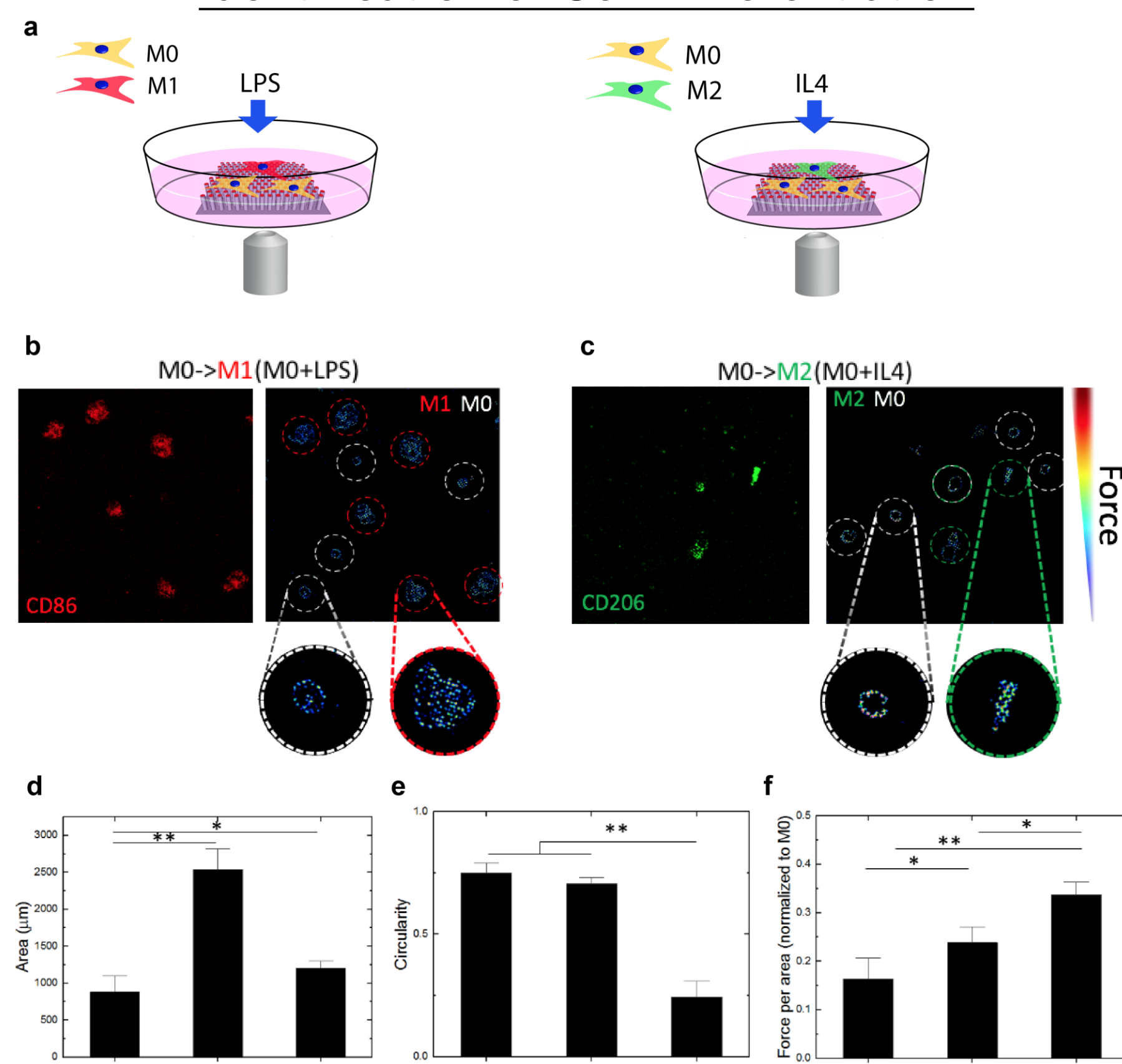


Devices

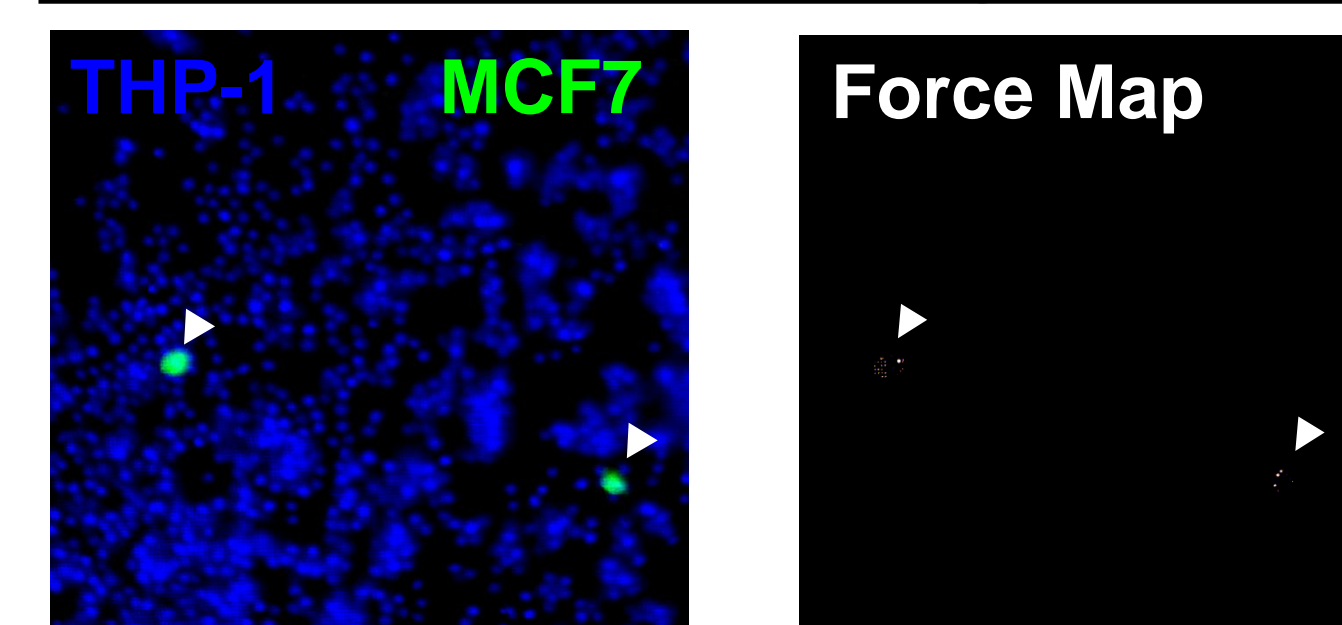


New Applications

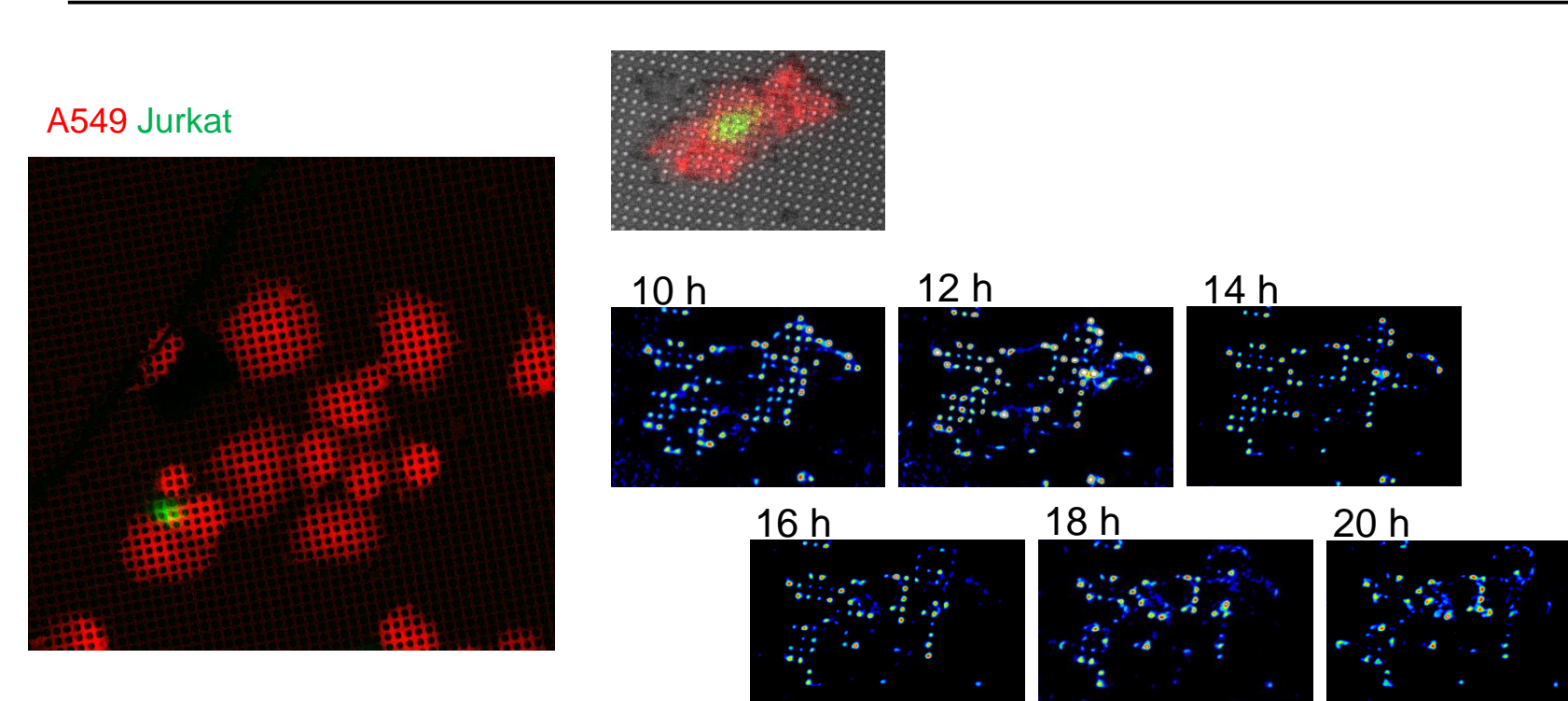
Identification of Cell Differentiation



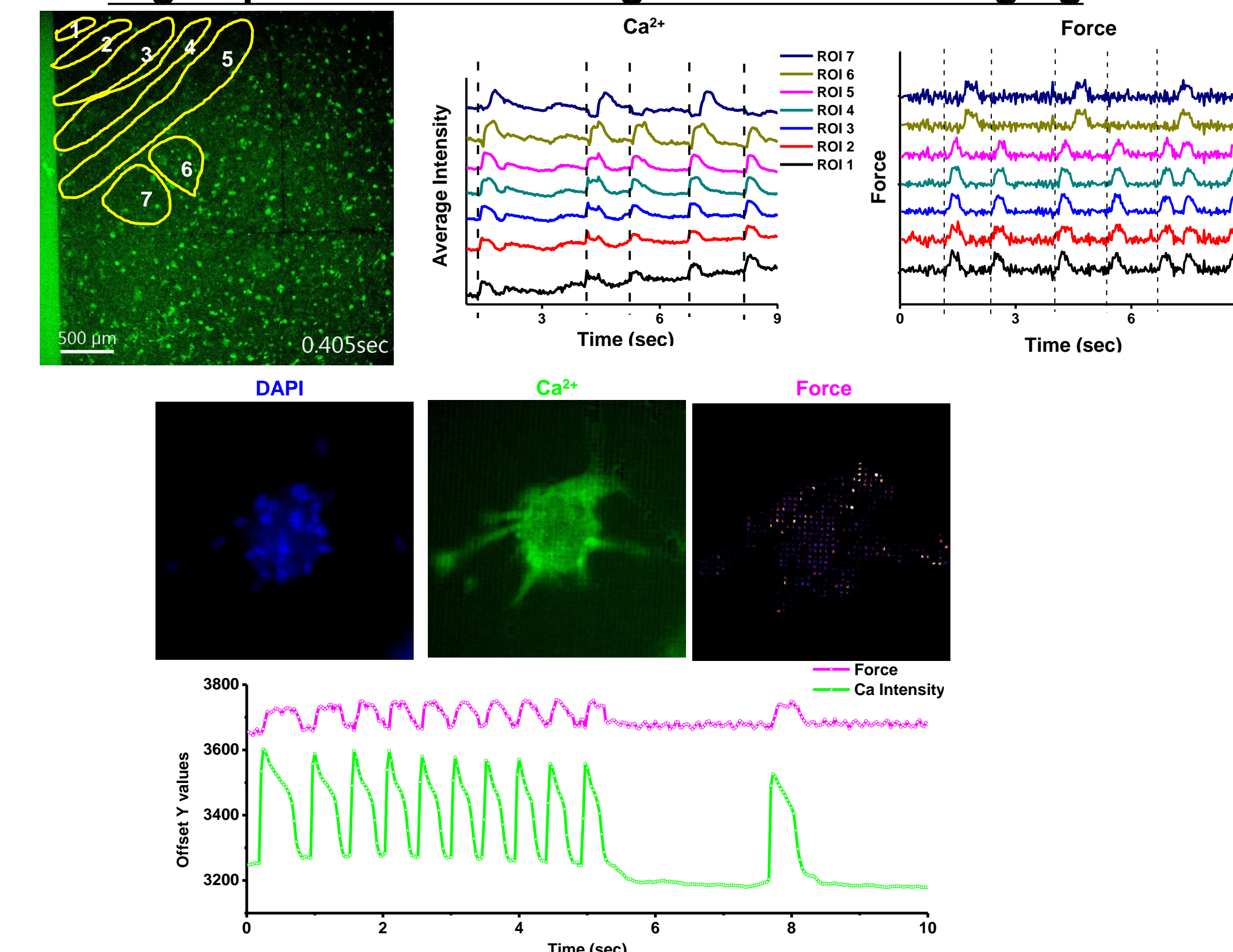
Identification of Circulating Tumor Cells



Interactions between cancer cells and immune cells



High Speed Force Sensing and Calcium Imaging



Conclusion

In the second year of this project, we improved the sensitivity of the force nanosensors by optimizing the nanopillars. We compared the performance of our system with the flow cytometry for drug toxicity tests. The results are very promising. During last year, we have established guidelines and protocols for drug screening using our platform. In addition, we have conducted some tests on several potential applications for force nanosensors. We have utilized the force nanosensors to distinguish the M0, M1 and M2 states of macrophage cells. We have also employed force nanosensors to identify different types of cancer cells and drug-resistant cells. In the last year, we have developed new technologies to directly integrate force nanosensors with 96-well plates using a microfluidic system to reduce the use of expensive biomedical reagents. At the same time, we also built a rapid reader for the force nanosensors with speed comparable to a plate reader for 96-well plates. We also conducted the cardiotoxicity experiments on the force sensors. Next year, we will explore the applications of force sensors for circulating tumor cell identification and immune cell interactions.