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論 文 内 容 の 要 旨

氏　名	（　李　泓翰　）

論文題名	Study of machine learning systems for cell biomechanics （細胞バイオメカニクスのための機械学習システムに関する研究）

論文内容の要旨

　The complexity of cellular mechanisms and their underlying structural determinants remain an area of intense study. Central to this goal is the understanding of cell morphology, cellular forces, and the pivotal role of actin stress fibers in various physiological processes. This doctoral thesis presents a series of interlinked studies that advance the understanding of cellular mechanics, specifically focusing on the relationship between cellular contractile forces, cell morphology, and the actin cytoskeleton. Employing advanced machine learning techniques, this research connected the computational analysis with biological experimentation in cellular biology.

　Initially, we introduce a novel method for evaluating cellular contractile forces using a machine learning model, the small-world U-Net (SW-UNet). We demonstrated that adjusting the topology of the neural network according to certain rules can lead to more accurate results with fewer dataset, and that by using this novel machine learning method, it is possible to infer changes in cellular contractility based on precisely segmented wrinkle patterns, and we have proved that the mutated KRAS oncogene inhibits cellular activity with this technique. Building on this foundational work, the thesis progresses to a more comprehensive approach with the development of Wrinkle Force Microscopy (WFM). This technique, which integrates traction force microscopy with generative adversarial networks (GAN) and SW-UNet, allows for the prediction of cellular force distributions from substrate wrinkles observed in microscope images with high throughput. WFM stands as a direct advancement from the initial study, moving from the quantification of cellular forces to a more detailed analysis of how these forces are physically manifested and can be visually interpreted. This method not only simplifies cellular force analysis but also enhances the ability to visualize and interpret the dynamic interplay between cells and their physical context.

　According to the understanding of cellular forces and their physical manifestations, we then further demonstrate the geometric principles that govern the relationship between cell morphology and the actin cytoskeleton (stress fibers). It investigates the relationship between cell morphology and the distribution of the stress fibers (SFs) using a diffusion model-based machine learning system. With the diffusion model, we can speculate on the possible locations of SFs inside the cell based on the shape of the cell only, and in this way, we find properties such as the area of the cell being proportional to the length of the SFs, and the aspect ratio of the cell being proportional to the order parameter of the SFs. Subsequently, based on this diffusion model, which incorporates the knowledge of the distribution of SFs from the cell shape, we can perform virtual micropatterning experiments for the relationship between the SFs geometrical properties and the cell topological characteristics, which are difficult to determine through experiments. With these "virtual experiments", we found that the local curvature of the cell outline is inversely proportional to the concentration of SFs, and directly proportional to the magnitude of the cellular contraction force.

　Furthermore, the final part of the thesis provides an in-depth analysis of actin stress fibers themselves. By developing a new machine learning method for quantifying individual attributes of these fibers within complex cellular networks, this research complements and deepens the understanding gained from the previous studies. We innovatively combine pixel features with angular features of SFs and confirm the effectiveness of this data labeling method with segmentation results. By separating SFs from cells individually, we demonstrate that for the process of cellular senescence, only the area feature is relevant among the geometric features of the cells, while the length, width, and number of SFs are strongly correlated with senescence.

　Overall, this thesis presents a continual study that starts from a macroscopic examination of cellular forces, advances through the study of their physical and morphological manifestations, and culminates in a detailed analysis of subcellular structures. This structured approach, supported by robust machine learning techniques, offers a comprehensive perspective on cellular mechanics, contributing significantly to the field of cellular biology.

論文審査の結果の要旨及び担当者

氏　　名	（　　李　泓　翰　　）		

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論文審査の結果の要旨

　細胞の力学的恒常性・適応現象と炎症性疾患の間には密接な関係があることが昨今の研究より明らかにされている。これらのメカニズムを理解するうえで細胞の力学的状態を計測する技術が必要であることから、これまでにさまざまな研究がなされてきた。その構造的・機能的な複雑さゆえに測定結果にばらつきの生じやすい細胞を対象とした生物学研究において有効な技術とするためには、計測の正確性に加えて、その技術が十分な測定効率を有することが重要である。Traction Force Microscopy（TFM）など細胞バイオメカニクス関連技術は一般に測定効率が限定されるために、その点において向上の余地があった。このような背景のもと、本論文では機械学習を用いてTFMを高い測定効率のもとで実施できる新しい技術を開発した。具体的に、Wrinkle Force Microscopy（WFM）と名付けた本技術ではTFM実験、および細胞の発生力の負荷に伴い微小なシワが生じるシリコーン基板を用いた実験を併せて実施し、両者で得られる画像パターンの関係性をGAN（Generative Adversarial Network）を用いたアルゴリズムにより学習し、両情報を相互に変換できるようにした。ここでシワ画像は位相差顕微鏡を用いて容易に取得できるために、本技術を介して当該シワ画像に相当するTFM画像、すなわち応力情報へと変換することが可能となった。これにより、高い測定効率でTFMに相当する実験データを取得できるようになった。本技術は学術論文として2022年にCommunications Biology誌に掲載され、現在に至るまで高く評価されている。その他にもdiffusion modelを駆使して細胞の輪郭情報から当該細胞内で力の発生に寄与するタンパク質複合体stress fibersの発生位置を予測する技術、また、実験画像より個々のstress fibersを抽出してその幾何学情報を定量化する技術など、機械学習に基づく多くの技術を開発している。これらの一連の新規技術は細胞バイオメカニクス研究を発展させるものであり、本論文が博士（工学）の学位論文として価値のあるものと認める。