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Special Issue: Singularity Biology and Beyond

Commentary and Perspective (Invited)

Visualizing Singularity Phenomenon

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In a multicellular system, cells interact and communicate with the surrounding cells. A small number of cells that considerably impact the surrounding cells emerge, either by chance or necessity. When the cell-cell interaction centered in these minority cells triggers an avalanche of cell-cell interactions, the multicellular system irreversibly undergoes a phase transition with a collective state transition of the cells. In the case that the phase transition is essential or fatal for life phenomena, such as development, metamorphosis, and lesions, the minority cell is defined as a "singularity cell," and the phase transition is defined as a "singularity phenomenon." This definition is nominal. Systems in life constantly undergo phase transitions as physical phenomena, regardless of whether an observer is looking. Based on this definition, humans can explore mechanisms that only exist in life rather than complex combinations of physical principles.

In conventional biology, which is based on statistical quantification using average values, the emergence of singularity cells is recognized as an outlier and has not been experimentally predicted because of its extreme rarity. In recent years, owing to the development of single-cell omics technologies, experimental data in life sciences have become more complex and voluminous, enabling the detection of rare-emerged minority cells. However, they could not prove a causal relationship between minority cells and the system phase transition because of the lack of temporal information in the data. It is necessary to measure the dynamic parameters that can quantitatively represent the state of cells and the system rather than screening for parameters that express the cell state in more detail to discover singularity cells.

The basic procedure for experimentally identifying singularity cells is as follows: (1) macroscopically detect the phase transition of an entire system; (2) microscopically and retrogradely search all cells for candidates of the starting cell of the avalanche of cell state transition and (or) cell–cell interaction; and (3) theoretically or mathematically screen the candidate cells for the singularity cell causally related to the phase transition. Therefore, the first principle of visualization in singularity biology is to "see the forest for the trees." This means that the behaviors of all cells and cell–cell interactions comprise a multicellular system everywhere, with simultaneous observation of the dynamic behavior of the system. This poses physical dilemmas for measurement systems, for example, a wide field of view and high resolution are incompatible in an optical microscope. Innovations in elemental technology will gradually resolve these dilemmas; the above-mentioned incompatibility can be overcome by specially upsizing the apertures of the objective lens and detector [1,2]. On the other hand, in the era of singularity biology, not only specification improvements but also simplifying and lowering prices are needed, because various trials to discover singularity cells in the various biological events are what will establish singularity biology as a new discipline.

Research management team overcame that physical dilemma using only a combination of simple machinery. They adopted a machine vision camera and telecentric macro lens instead of an objective lens at the expense of three-

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dimensional resolution, maximally simplifying the system configuration and remarkably reducing costs [3]. The specification was achieved to 2.5 μm-resolution within a 14.6×10.1 mm^2 field. Many biological researchers still target and observe samples in two-dimensional dishes or glasses. This microscope enables them to observe one million cells in one field of view as they would use a current microscope daily. If desired, they can install it in their laboratories by themselves owning to its simple configuration. The research management team is being further developing the microscope for confocal three-dimensionalization and (or) multimodalization as the basis for a future imaging system named AMATERAS (A Multi-scale/modal Analytical Tool for Every Rare Activity in Singularity). In parallel, many biologists in A03 groups are exploring singularity cells in the biological phase transition of their interest using the present version without waiting for its final development completion.

Similar to multicellular systems, complicated interactions exist between lower-layered elements, such as proteins and genes, in a cell. Moreover, multicellular systems assemble to form an upper-layered system that enables more complex functions. Furthermore, "states" in the life sciences are ambiguous and can be defined by various parameters, including gene-expression profile, morphological features, and metabolic components. Given the recursive and fractal structure in life, the first principle of Singularity biology is ultimately going to be "see the forest, to the river, the tree, the fishes, the leaf, the weed, the leaf veins, and the symbiotic bacteria." The automated live imaging and cell picking system (ALPS), recently developed by A01-3 group, enables the investigation of the correlation between dynamic microscopic features and the transcriptome at a single-cell resolution [4]. The “transcriptome” is interchangeable with any biochemical investigation, such as the proteome or metabolome. The combination of AMATERAS and ALPS defines the singularity cells in many possible modalities across multiple layers. This fusion development has already begun.

Improvement in observation depth is essential for maintaining applicability to live specimens. A01-1 group (Table 1) developed an incubator-type dual-axis light-sheet microscope, which enables single-cell imaging with simultaneous tracking of the tissue formation process in a mouse embryo at embryonic day 5.5 stage for 12 h at 5-min intervals [5]. In the same developmental project, the pseudo confocal method with a global shutter in a camera was also invented to improve the optical sectioning in light-sheet microscopy [6]. The additional adoption of light over 1000 nm in wavelength, called the second optical window, promises to improve the observation depth and reduce photodamage to bio-samples [7]. The problem for the use of the second optical window is not optical, but rather biochemical: It is essential to develop effective dyes that have emission in this region and that do not affect biological phenomena, as well as to develop staining methods for these dyes. The spatial resolution of photoacoustic microscopes, which have a lower temporal resolution but allow more profound observations, approaches the subcellular resolution at a depth of 1 mm [8]. In the addition to resolution improvement, A01-1 group has empowered the photoacoustic microscopy with the ability to multimodality with single-photon emission computed tomography (SPECT) [9] or magnetic resonance imaging (MRI) [10] in combination with probe developments. Thus, the specifications of the elemental technologies have steadily improved. However, performing in-toto single-cell imaging across all layers using all modalities is still difficult. Instead, according to the AMATERAS concept, developers should tailor a unique system to investigate the singularity behavior of interest of each biologist by combining these advanced techniques. Along with the development of measurement technologies, digital data technologies, including transfer, storage, and mining, have been developed to manage the enlargement of the acquired image data by A02 groups.

Table 1 A01-1 group composition and collaborators in the Singularity Biology

A01-1 group	
Principal Investigator	Tomonobu M Watanabe (RIKEN BDR/Hiroshima University)
Co-Investigator (CI)	Tsuyochi Shiina (Shibaura Institute of Technology) Takeshi Namita (Shibaura Institute of Technology)
Collaborating Researcher (CR)	Go Shioi (RIKEN BDR) Junichi Kaneshiro (RIKEN BDR)
Collaborators in the Singularity Biology	
A02-1 PI	Shuichi Onami (RIKEN BDR)

Unlike simple materials such as water, states in biological phenomena are defined by repeated qualitative subdivisions. Scientists define cell states in advance using qualitative features such as morphology, habitats, and (or) functions. Subsequently, markers representing the states are discovered and used for identification and discrimination. Even for subpopulations discovered in omics data, their threshold and biological meaning must be determined by humans. Researchers now have developed a simplified understanding of complex phenomena by defining states using only a countable number of measurable parameters. With the development of AMATERAS and its advanced forms, the more the parameters become measurable, the more difficult it becomes to define states of cell. In the future, "states" in biology

will be defined by artificial intelligence besides definitions by humans. So, what is the role of humans in bioscience? What can we learn from living organisms? What does "clarify" or "elucidate" mean in biology? Singularity biology is a discipline that generates new biological questions by quantitatively elucidating biological phenomena that have thus far only been discussed qualitatively. Beyond that, biological scientists are faced with the philosophical question, "What is bioscience?".

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