Attack biological problems using a state-of-the-art microscope.

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Microscope has been used for biological research for centuries. The earliest microscope is said to be developed by Zacharias Janssen in the 16th century, and first observations of living organisms with microscope was done by Antonie van Leeuwenhoek in the 17th century. At the same time, Robert Hooke discovered cell using his original microscope. Since then, many medical or biological researchers started to use them. In the 20th century, there was a big innovation. New microscope called 'phase contrast microscope' was invented by Frits Zernike, which visualizes the shape of transparent living organisms. The development of microscope is continuing now, leading the discovery of biology.

All living things consist of molecules. Mainly, there are four biomolecules in the cell: lipid, nucleic acid, carbohydrate, and protein. Understanding the structures of them is very important because their structures and their functions are tightly related. For example, James Watson and Francis Crick discovered the structure of DNA, which revealed the mechanism of heredity. Thus, researchers want to get the information of such molecules. The microscopes which can visualize the molecular bonds are very important. A lot of methods have been developed so far.

One of the most successful technologies is 'fluorescent microscope'. Fluorescence is the phenomenon of light radiation when injected another color light, which is called excitation light. In the fluorescent microscope, the excitation light is illuminated to the object, and the fluorescent light is observed. Fluorescent light radiation occurs only on specific molecular bonds, so the positions of such molecular bonds are observed. Therefore, we can visualize the target biomolecules by chemically attaching fluorescent tag to them.

However, there are some limitations on the fluorescent microscope. For example, sometimes it is difficult to find the fluorescent tag to the target molecules. In some cases, fluorescent chemicals effect on the behavior of the observed biomolecules. Therefore, other molecular bond sensitive microscope without labelling, which is called label-free, needs to be developed.

Recently, a new label-free technology is being developed. It is called photothermal microscope [1]. Photothermal effect is the phenomenon of temperature rise when the matter

absorbs light. As in fluorescent microscope, the light absorption occurs only on certain chemical bonds with certain wavelengths of the light. The temperature changes appear on the changes of refractive index, which is the property of bending the direction of light. It can be detected by some microscopes like phase contrast microscope. Unlike fluorescent microscope, there is no need to label the molecules. Therefore, photothermal microscope can observe cells without the influence on them.

It has one more advantage to previous methods. A microscope has its own spatial resolution, which indicates how small structure it can see. The spatial resolution is about the half of its wavelength. For example, the wavelength of visible light is about 500 nm, so its spatial resolution is about 250 nm. Typically, light absorption by biomolecules occurs on wavelengths about few micrometers, which is called infrared light. There are some ways to get the information of biomolecules, but they simply use infrared light, limiting its spatial resolution to few micrometers. However, photothermal microscope uses visible light, so its spatial resolution is few hundred nanometers. Thus, it can get the information of biomolecules, with high spatial resolution.

Photothermal microscope is hoped to be used in new biological region. Recently, the phenomenon called liquid-liquid phase separation (LLPS) is getting known in biology. When LLPS happens, the solution is separated into droplets and its surroundings, and the droplets contains different substances from surrounding environment. In some neurodegenerative diseases like Alzheimer disease or Parkinson disease, certain proteins which is related to them undergo LLPS. Therefore, LLPS is said to be the cause of such neurodegenerative diseases, so understanding the mechanism of them will help us to find the treatment. Recently, one research group observed the protein structure changes in such diseases by certain label-free technology [2]. The observation had not been realized by previous techniques. Photothermal microscope will be strong tool in such research too.

Photothermal microscope can be applied to other regions. For example, it can be used in the failure analysis of manufacturing semiconductor devices. Sometimes, contamination of organic staff occurs within semiconductor devices. It can be detected by photothermal microscope, and the material is distinguished. So, it implies where the contamination happens. The analysis of cultural heritage like painting is another example. Understanding the mechanism of corrosion in cultural heritage is very important to its preservation. Also, it can be one of the

tools to distinguish whether it is a genuine article or an imitation. In these examples, the samples need to be observed noninvasively in nanometer scale, where photothermal microscope has the advantages to other technologies.

Microscope has been playing a vital role in science, especially in biology. The technology is evolving even now. Photothermal microscope is one of them, which enables the observations of cells without labelling. It will discover some cellular dynamics which hasn't be observed by previous microscope, opening the door to the new biological region.

I used online English dictionary like weblio.

[1] Zhang et al. Light: Science & Applications (2019) 8:116. https://doi.org/10.1038/s41377-019-0224-0

[2] Matsuura et al. Sci Rep 13 6389 (2023). https://doi.org/10.1038/s41598-023-33268-y