Patent Publication No.2004-138735

Total Internal Reflection Fluorescence Microscopy and its Illumination Optics

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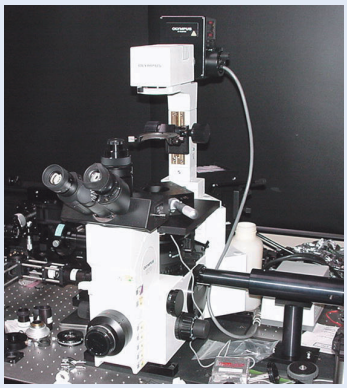

Fluorescence Microscope (image)

Outline of technology

A total internal reflection fluorescence microscope is a microscope with which the user observes a sample by exciting a fluorescent dye that produces light of a wavelength longer than that of the illumination light. The produced light is irradiated onto a sample plane to generate total internal reflection; the user then observes the corresponding fluorescence. The conventional total internal reflection microscope generates an evanescent field by irradiating laser light onto the sample plane from one direction. However, if the dye features an oscillation moment in a given direction, it may be impossible with this unidirectional method to excite the dye and cause it to fluoresce, and to observe the sample, depending on the orientation of the sample. This invention enables the user to observe the sample and check for the presence of a target protein or the like regardless of the direction of the target that contains the fluorescent dye; the observer is thus able to count the number of molecules of the fluorescent dye in a given location. Moreover, unlike NMR and X-ray analysis after crystallization, this invention enables analysis of changes in three-dimensional biomolecular structures, such as the behavior of activated protein in water solution, in addition to DNA and RNA observation.

With this invention, light from a laser light source is diverged and formed into a conical shape by passing through a diffractive diffuser; the resultant light is then converged by a series of lenses. Since this configuration produces light containing polarization components in all directions, the dye is excited uniformly, as discussed above (Fig.1).

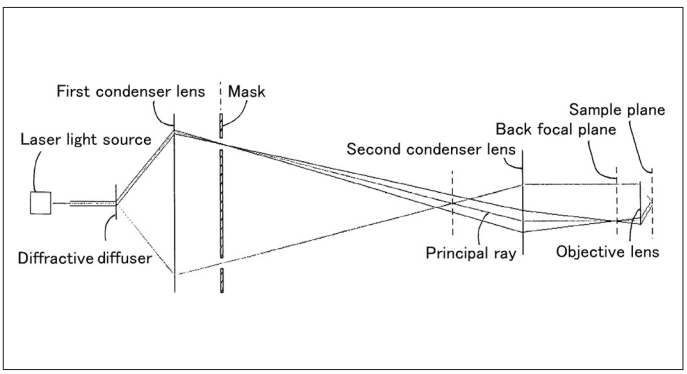

Fig.1 Illumination optics of total internal reflection fluorescence microscope

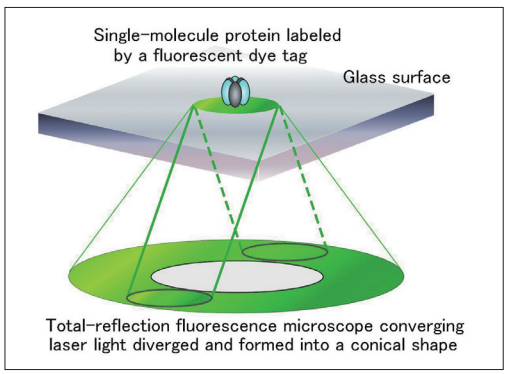

Fig.2 Configuration in which the dye is excited uniformly

Background to development of prototype

Organisms are made up of proteins forming minute components as small as 10 nm. Investigation of the functions of these components on a molecular level leads to the clarification of a range of biological phenomena. In the most advanced research in this field, development of single-molecule imaging is now underway, in which one molecule of a protein is observed without damage to its functions. In this process, fluorescent dyes play an active role. A fluorescent dye tag is attached to a protein that is too small to observe; the protein is then irradiated with intense light and the dye fluoresces. Thus, this procedure can allow observation of a protein's motion in water, for example, or dynamic observation of the gathering and dispersal of two or more molecules of a protein. Our Protein Biophysics Group began this research approximately ten years ago, and has succeeded in observing a single molecule of a fluorescent dye using special light (referred to as "evanescent light") using an instrument known as a total internal reflection microscope. Recently, various manufacturers have marketed an increasing number of these microscopes in easy-to-use formats.

However, conventional total internal reflection microscopes featured a drawback: the polarized light was extremely biased. Since the dye molecules have an asymmetric shape, when observing a single molecule of the dye with polarized light, the molecular signal will be weaker if the dye and the polarized light are not aligned. Depending on the sample, the resultant signal may be impossible to quantify accurately.

This invention has successfully eliminated this drawback, improving the microscope greatly. Although the signal from the single molecule is originally very weak, the assessment of this signal is rendered more reliable. Moreover, this microscope is provided with a device that avoids the interference pattern peculiar to lasers; in addition, the lens is capable of withstanding laser light 100 times more intense than the previously assumed limit. It is expected that with the spread of this instrument, movements of proteins and various elusive biological phenomena will begin to be clarified at numerous universities and research institutions.

Commercialization

This technology is scheduled to be brought to the commercial stage and sold as a product by SIGMA KOKI Co., Ltd. This new optical system, which addresses the problem of polarization, will be commercialized in a structure that is easily attached to biological microscopes currently on the market. It is expected that with the spread of this instrument, movements of proteins and various elusive biological phenomena will begin to be clarified at numerous universities and research institutions.