# Supporting Bioscience

## Multi-mode Imaging Inverted Microscope TE2000

In recent years, with the dramatic development of peripheral equipment (lasers, sensors, cameras, personal computers, and scanners, etc.) for optical microscopes, inverted microscopes for research in particular have been playing an increasingly important role in bioscience. Nikon has been producing inverted microscopes for research since 1964 when the model MD was developed under the leadership of the late Professor Hajimu Katsuta of the Institute of Medical Science, the University of Tokyo. In 1980, Nikon released the model TMD incorporating the basic development concept of the Shinya-scope produced by Professor Shinya Inoue of the Marine Biology Laboratory in Woods Hole, Massachusetts, U.S.A., and which enjoyed a high reputation by many researchers. This was followed by the model TMD300, which benefited from a large number of improvements, and then the model TE300, which incorporated infinity optics. In 2001, Nikon introduced the TE2000 multi-mode imaging inverted microscope in response to requests from many researchers working at the cutting-edge of bioscience. Here, we would like to describe our aims in developing this microscope and its features.

Configuration with Nikon C1 confocal imaging system, Epi-Fl and TIRF imaging systems

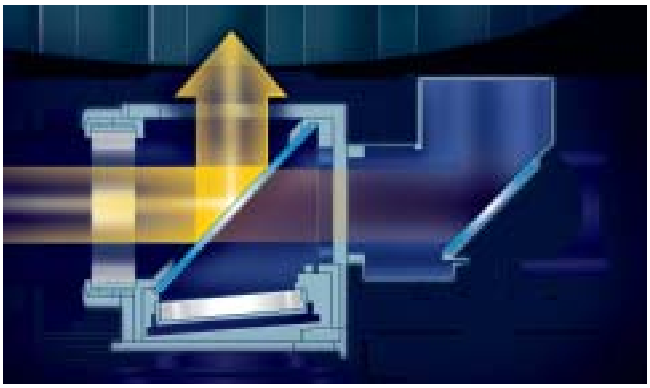
### Aims of the Development

1 Considering that optical microscopes are the core input devices in whole research systems, it is primarily essential to maximize their basic performance. In particular, the image quality under the epi-fluorescence, confocal, and TIRF microscopy using evanescent wave illumination must be enhanced. It is also vital to create a microscope that hardly changes the focal position due to vibrations and heat.

2 The microscope must enable multi-mode imaging and must be flexibly expanded according to applications by simultaneously accommodating various attachments and accessories.

# Major Features

With the TE2000, contrast in fluorescence images is dramatically improved, enabling the visualization of single molecular mechanisms in living cells with an ordinary epi-fluorescence method. This has been mainly achieved by adopting an original Noise Terminator mechanism and zoom illumination optics for epi-fluorescence illumination coupled with major improvements in their optical devices such as objectives and filters that are optimized for fluorescence applications.



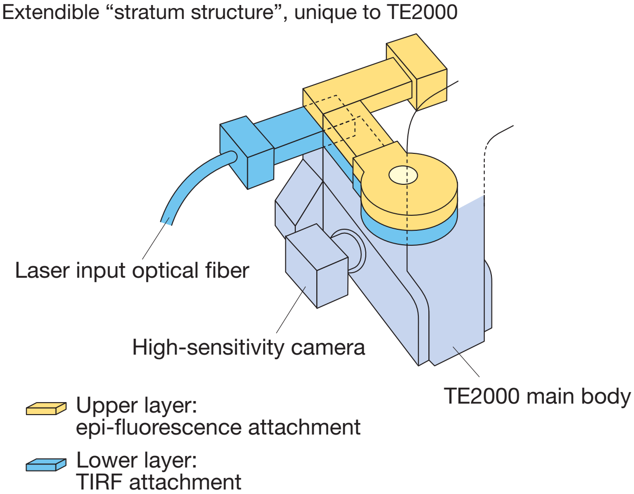
Noise Terminator mechanism incorporated in filter blocks provides unparalleled S/N ratio.

We have created two types of dedicated objectives for TIRF microscopy: CFI Plan Apo TIRF 100X/1.45 and CFI Plan Apo TIRF 60X/1.45. Standard coverglass and immersion oil are both usable with these objectives. The CFI Plan Apo TIRF 60X/1.45 objective, in particular, is the world's first oil-immersion-type objective to incorporate a correction ring for temperature changes and coverglass thickness. By rotating its correction collar, researchers can easily counteract influence to the image quality from temperature-induced changes in the refractive index of the immersion oil within the range from 23°C (room temperature) to 37°C (physical temperature) and influence from variation in the coverglass thickness. This mechanism ensures the optimum image regardless of temperature and coverglass thickness. Furthermore, this objective provides the optimum image not only under TIRF microscopy but also under Nomarski DIC, epi-fluorescence, multiphoton confocal, and deconvolution imaging, while providing a strong trapping power during applications using laser tweezers. Thus, this objective has been highly optimized for multi-mode imaging microscopes.

Left: CFI Plan Apo TIRF 60X/1.45, right: CFI Plan Apo TIRF 100X/1.45

The merits of the unique CFI60 infinity optical system (with tube lens focal length of 200mm and parfocal distance of 60mm) have been tapped to the maximum so that the distance between the microscope's main unit and the objective could be lengthened. This made it possible to incorporate our unique extendible "stratum structure" into the TE2000. This stratum structure provides a space in which two sets of turrets each having a capacity of six filter blocks can be stacked simultaneously. This design negates the need to modify the microscope body to introduce laser light, enabling the simultaneous observations under epi-fluorescence and evanescent field illuminations, along with laser tweezers, or a laser photolysis. In addition, supporting fluorescence resonance energy transfer (FRET), the image obtained by the objective can be exported to two different external units, being divided into dual wavelengths by a dichroic mirror. Thus the stratum structure enables simultaneous mounting of import and export devices of laser illumination, or two laser illumination systems on a single microscope.
Furthermore, the images can be directed to 5 different ports for a CCTV camera or a photomultiplier or other detectors using a 5-stage optical path switching dial on the microscope.
Therefore, this multi-mode imaging microscope allows researchers to easily use all types of methods, such as brightfield, phase contrast, polarizing, Nomarski DIC, epi-fluorescence, total internal reflection fluorescence (TIRF), as well as confocal, with a single microscope to observe the reactions of live specimens by applying laser light or electrical or chemical stimulus using a micromanipulator.

Extendible "stratum structure", unique to TE2000



# Examples of System Configurations

## 1. Epi-Fl + TIRF illuminations

Because both epi-fl (top position) and TIRF (bottom position) illumination systems can be mounted simultaneously, utilizing the stratum structure, both an epi-fl image and a TIRF image can be obtained in different wavelengths and simultaneously observed. In addition, because the system now provides a high S/N ratio—the result of the adoption of a new Noise Terminator mechanism—it is possible to use the SRIC (Surface Reflective Interference Contrast) method without the use of dedicated objectives and other accessories. The SRIC method can be easily operated with aperture and field diaphragms on the epi-fl system, allowing researchers to focus or check whether or not the specimen is visible by TIRF before fluorescence excitation. Switching to TIRF observation is a snap.

Images courtesy of Dr. Gregg G. Gundersen, Columbia University

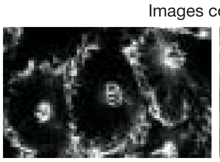


Image under TIRF observation

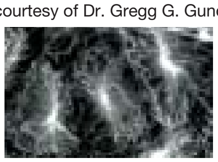
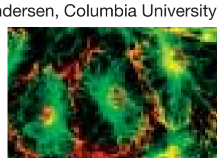
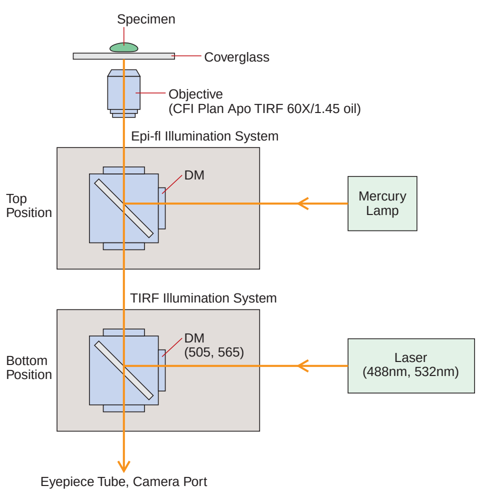


Image under epi-fluorescence observation



TIRF/epi-fluorescence image overlay (pseudo-color)

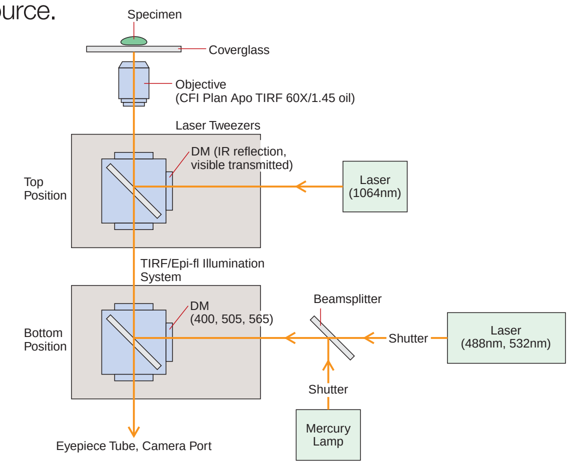



## 2. Confocal Imaging System (Nikon C1) + Epi-Fl + TIRF illuminations

Utilizing the stratum structure, Nikon's confocal imaging system (C1) can be combined with these two illumination systems. It is possible to provide both confocal and TIRF imaging utilizing the same laser light source because both the C1 and TIRF systems use an optical fiber to introduced the laser.

## 3. Laser Tweezers + Epi-Fl + TIRF illuminations

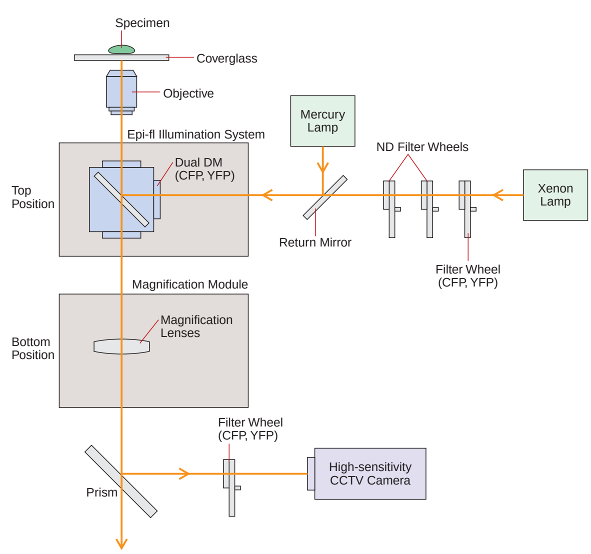
Utilizing the stratum structure, it is possible to trap the specimen with laser tweezers (mounted in the top position) and provide simultaneous observation with either TIRF or epi-fl illumination by simply switching the light source.




## 4. Epi-Fl illumination + Magnification Module + High-sensitivity CCTV Camera

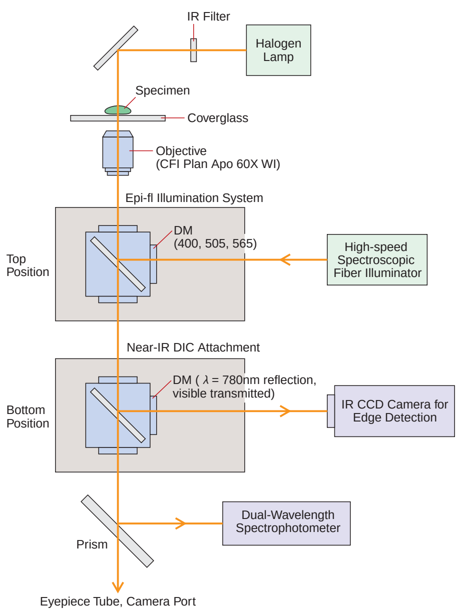
Utilizing the stratum structure, it is possible to mount an epi-fl illumination system at the top position and magnification lenses in the filter turret at the bottom position. This is optimal for observing weak fluorescence using a high-sensitivity CCTV camera because variable magnification lenses (0.66X, 1X, 1.5X, 2X) combined with 1X and 1.5X lenses inside the microscope body make it possible to achieve a wide magnification range from 0.66X to 3X.

To minimize influences from vibrations, it is possible to switch the mercury light source of the epi-fl illumination system to an external fiber light source.



## 5. Epi-Fl illumination +Near-IR-DIC attachment/FRET system + CCTV camera

Utilizing the stratum structure, it is possible to mount an epi-fl illumination system (top position) and a near-infrared DIC attachment (bottom position). The image can be observed under epi-fl illumination simultaneously with viewing of dynamics of living cells with near-IR DIC illumination as the image can be divided into two wavelengths by the dichroic mirror inside the filter turret of the near-IR DIC attachment.
In addition, FRET images can be captured with a spectrophotometer attachment.
Furthermore, simultaneous photometry of multiple fluorescence wavelengths with multi-photon excitation is possible.




## In Closing

We at Nikon are continuously engaged in the development and refinement of microscopes in response to the requests of all of you engaged in trail-blazing research. It is no exaggeration to say that Nikon microscopes are being brought to life through your efforts. We would be most pleased to continue receiving your ideas and feedback.

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