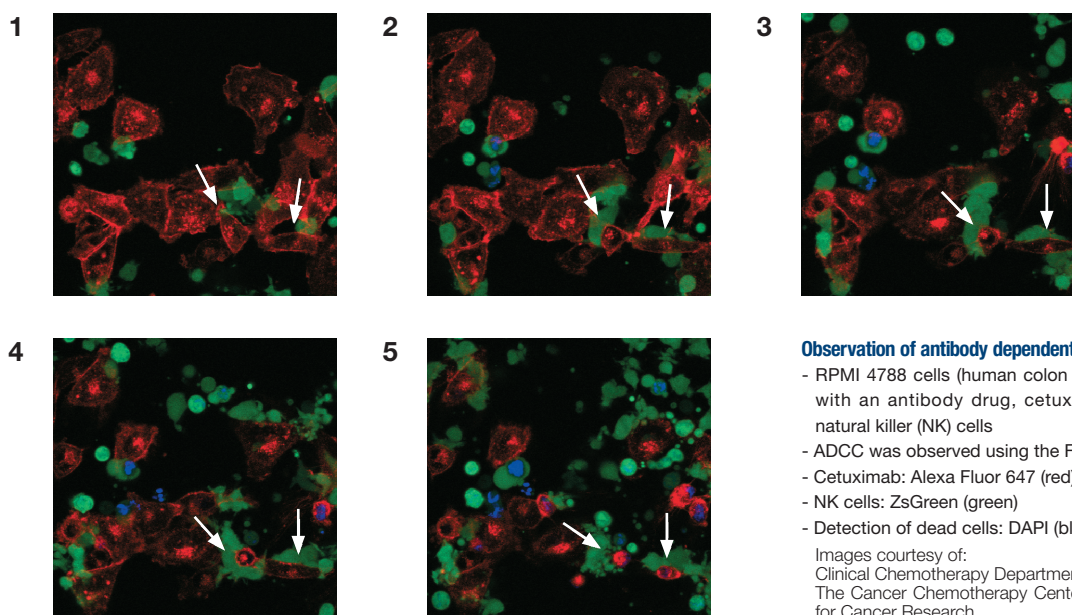


Observing live cell as time lapse experiment is an essential method to study the movement of molecules in cells and interactions between cells. Combines time-lapse experiments and confocal laser scanning microscopy has enabled three-dimensional observation on live cell. This allows researcher to investigate cell interaction and mechanism of drugs function.

The FV10i has made above sophisticated experiments in a single self contain box and not compromise on required function.



Observation of antibody dependent cellular cytotoxicity (ADCC)



Observation of antibody dependent cellular cytotoxicity (ADCC)

- RPMI 4788 cells (human colon cancer cell line) were treated with an antibody drug, cetuximab, and co-cultured with natural killer (NK) cells
- ADCC was observed using the FV10i after addition of NK cells
- Cetuximab: Alexa Fluor 647 (red)
- NK cells: ZsGreen (green)
- Detection of dead cells: DAPI (blue)

Images courtesy of:
Clinical Chemotherapy Department
The Cancer Chemotherapy Center of the Japanese Foundation
for Cancer Research

Antibodies have high affinity, binding specificity and stability in blood, which allow applications in the diagnosis, prevention, and treatment of different kinds of human diseases. Remarkable effects have been reported in some therapies using chimeric or humanized antibodies generated by recombinant DNA technology, and this has drawn attention to the research and development of antibody drugs using these antibodies in recent years.

Mechanisms of action of antibody drugs include growth inhibition and apoptosis induction, complement-dependent cytotoxicity (CDC), and antibody-dependent cellular cytotoxicity (ADCC). ADCC is a phenomenon in which effector cells (for example, NK cells and monocytes) destroy the antibody-bound target cells (for example, cancer cells) by attacking with perforin or granzyme B or phagocytosis.

Observation of ADCC using fluorescent imaging enables counting of living cells, dead cells, and effector cells, and so, it can be effectively used for evaluation of the treatment efficacy of antibody drugs.

In the experiment above, the antibody drug cetuximab was labeled with Alexa Fluor 647 following the protocol for the Molecular Probes Alexa Fluor 647 Protein Labeling Kit (Invitrogen).

RPMI 4788 cells (human colon cancer cell line) were used as the target cells for the antibody drug. Therefore, the surface of the target cells exhibited red fluorescence from Alexa Fluor 647.

KHYG-1 cells (a cell line derived from NK cell leukemia) transfected with the Fc- γ receptor IIIa genes (158V or 158F) were used as effector cells. These cells exhibited green fluorescence from ZsGreen.

DAPI was added to distinguish between living and dead cells. The blue staining of the nuclei in dead cells was used as indicator.

ADCC observation was done with culturing the cells on the built-in culture stage of the FV10i-LIV confocal laser scanning microscope at 37°C under 5% CO₂ for 4 hours. For cell culture, a glass bottom dish (Greiner #627965, CELLview(glass bottom dish, 35 × 10 mm, Advanced TC(treated) was set on the stage incubator.

At the beginning, the surface of the RPMI 4788 cells exhibited a clear boundary visualized by the cetuximab labeled with Alexa 647 (red, indicated by arrows in panel 1 in the figure). Following this, the effector cells (green) that targeted the cetuximab antibodies covered and entered into the RPMI 4788 cells (panels 2 and 3), deformed and death RPMI 4788 cells were observed (panels 4 and 5). Effector cells were also observed with actively changed their morphology and attacked the target cells over time. Some cells were observed with DAPI nuclei stained as died cell (panels 4 and 5).

FV10i-LIV

The FV10i-LIV is the world's first self-contained box-type confocal laser scanning microscope. The FV10i-LIV supports multi-area and multi-color, enabling efficient and easy data acquisition. The FV10i-LIV is equipped with a water-immersion objective lens, the optimum lens for time-lapse, which has a newly developed automated water dispensing system that enables long-term time-lapse imaging. In addition, the FV10i-LIV is provided with a simplified built-in incubator and a culture pod with recirculation ability, making it the most ideal system for live cell imaging.



FV10i-LIV

Specifications		
Laser light source	LD lasers:	405nm(17.1mW), 473nm(11.9mW), 559nm(15mW), 635nm(9.5mW)
Scanning	Scanning mode	Pixel size: 256 × 256 1024 × 1024 Scanning speed: 1.1 s / frame (for pixel size 512 × 512, High Speed scanning mode)
Detection	Detector module	Fluorescence: 2 channels, Phase Contrast: 1 channel
	Field number	18
	Optical zoom	10× objectives: 1 × – 6 × in 0.1 × increments 60× objectives: 1 × – 10 × in 0.1 × increments
Focus	Objectives	Exclusively designed 10× phase contrast objective / NA 0.4(equivalent to UPLSAPO 10×) Exclusively designed 60× phase contrast waterimmersionobjective / NA 1.2 (equivalent to UPLSAPO 60× W) / with motorized correction collar Remote switching from software by electric revolver
	Automatic focus (AF)	Automatic detection of interface between specimen and cover glass by laser reflection light detection Automatic detection of cover glass thickness and automatic setting of motorized correction collar
	Water supply	Automatic water supply and air cleaning mechanism for 60× Water-immersion objective
XY stage	XY driving method	Motorized XY stage module by stepping motor Minimum increment: 0.3μm
	Specimen holder	Only the dedicated specimen holder can be mounted For three glass bottom dishes with 35mm diameter For a glass slide, For one set of cover glass chamber (8 wells type) For Well slide (8 wells type), Culture pod(for a glass bottom dish with 35mm diameter)
Incubator	Room environment:	Temperature: 37±0.1°C, 0.5°C (can be switched off) Humidity: more than 90% CO ₂ concentration: 5% (recommended), 1 – joint fitting (ø2mm) for exterior CO ₂ adjustor
Control device	Controller	OS: Windows Vista Business, 32 bit (English version), RAM: 2GB × 2, HDD: 500GB × 2,



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