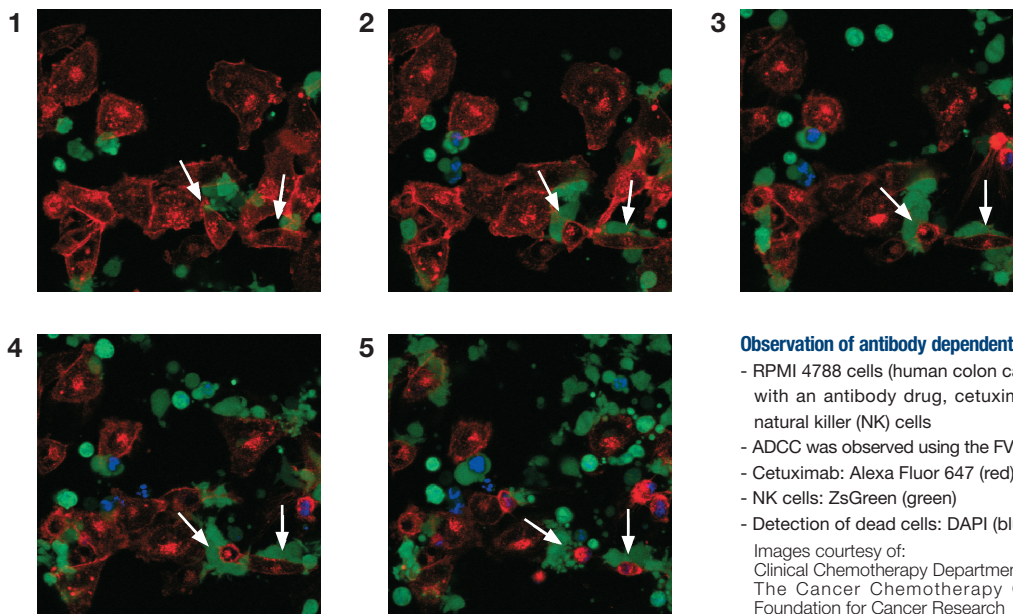


Observing live cells as time lapse experiment is an essential method in studying the movement of molecules within cells and the interactions between cells. Combining time-lapse experiments and confocal laser scanning microscopy has enabled three-dimensional observation of live cells. This allows the researcher to investigate cell interaction and the mechanism of drugs function. The FV10i has achieved this sophisticated combination in a single self contain box without compromising any required functionality.



## 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 in recent years to the research and development of antibody drugs using these antibodies.

Mechanisms of action of these 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, granzyme B or phagocytosis.

Observation of ADCC using fluorescent imaging enables the counting of living cells, dead cells, and effector cells, and so 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- $\gamma$  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 an indicator.

ADCC observation was done by culturing the cells on the built-in culture stage of the FV10i-LIV confocal laser scanning microscope at 37°C under 5% CO<sub>2</sub> 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 dead RPMI 4788 cells were observed (panels 4 and 5). Effector cells were also observed with actively changed morphology and attacking the target cells over time. Some cells were observed with DAPI nuclei stained as dead cells (panels 4 and 5).

## FV10i-LIV

The FV10i-LIV is the world's first self-contained confocal laser scanning microscope.

The FV10i-LIV supports multi-area and multi-color imaging, enabling efficient and easy data acquisition.

The FV10i-LIV has a newly developed automated water dispensing system enabling 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, 512 × 512, 1024 × 1024<br>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<br>60× objectives: 1 × – 10 × in 0.1 × increments   |
| Focus              | Objectives                | Exclusively designed 10× phase contrast objective / NA 0.4(equivalent to UPLSAPO 10×)<br>Exclusively designed 60× phase contrast waterimmersionobjective / NA 1.2 (equivalent to UPLSAPO 60× W) / with motorized correction collar and motorized nosepiece with remote switching by software switching from software by electric revolver |
|                    | Automatic focus (AF)      | Automatic detection of interface between specimen and cover glass by laser reflected light detection<br>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<br>Minimum increment: 0.3μm   |
|                    | Specimen holder           | Only the dedicated specimen holder can be mounted<br>For three glass bottom dishes with 35mm diameter<br>For a glass slide, For one set of cover glass chamber (8 wells type)<br>For Well slide (8 wells type), Culture pod(for a glass bottom dish with 35mm diameter)   |
| Incubator          | Operating specifications: | Temperature: 37±0.1°C, 0.5°C (can be switched off)<br>Humidity: more than 90%<br>CO <sub>2</sub> concentration: 5% (recommended), 1 – joint fitting (ø2mm) for exterior CO <sub>2</sub> adjustor  |
| Control device     | Controller                | OS: Windows Vista Business, 32 bit (English version),<br>RAM: 2GB × 2, HDD: 500GB × 2,  |



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