Versatile and powerful 3D imager for GFP research!

Fluotracker

Integrated confocal laser microscope for genetically labeled fluorescent markers;

Fluorescent lifetime imaging
Fluorescence intensity confocal image
FRET (Fluorescence Resonance Energy Transfer)
FLIP (Fluorescence Loss in Photobleaching)
FRAP (Fluorescence Recovery after Photobleaching)
Photo-pulse labeling by photoconversion
(Chloride imaging in cells
Detection of microenvironmental change with lifetime)

3D imaging including fluorescence intensity and life time image
Fluorescence decay time point measurement
Simultaneous of fluorescent intensity and lifetime imaging
Long time - lapse measurement

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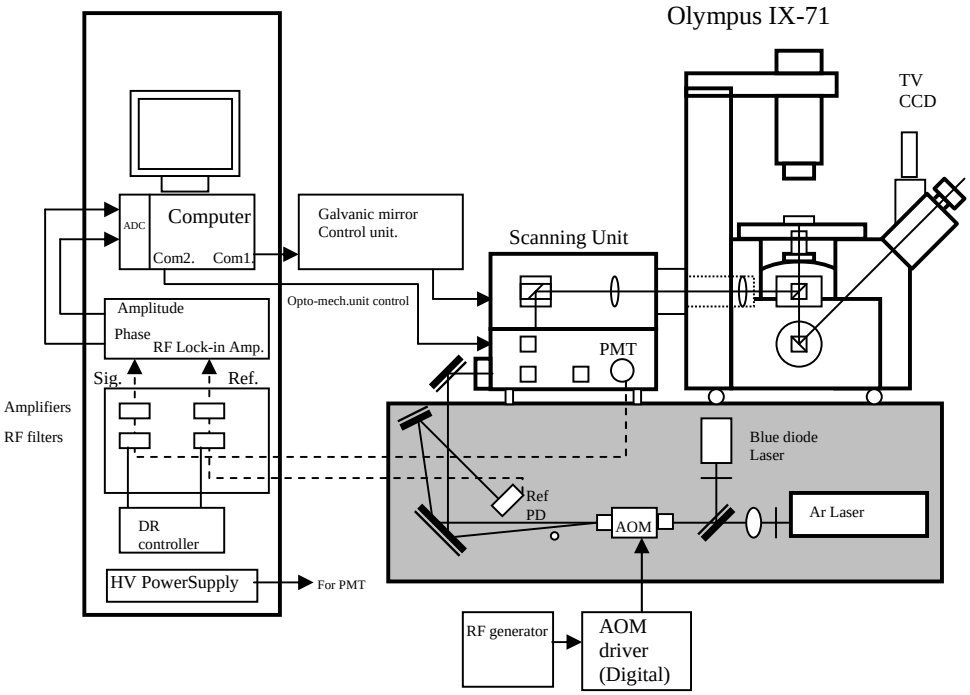
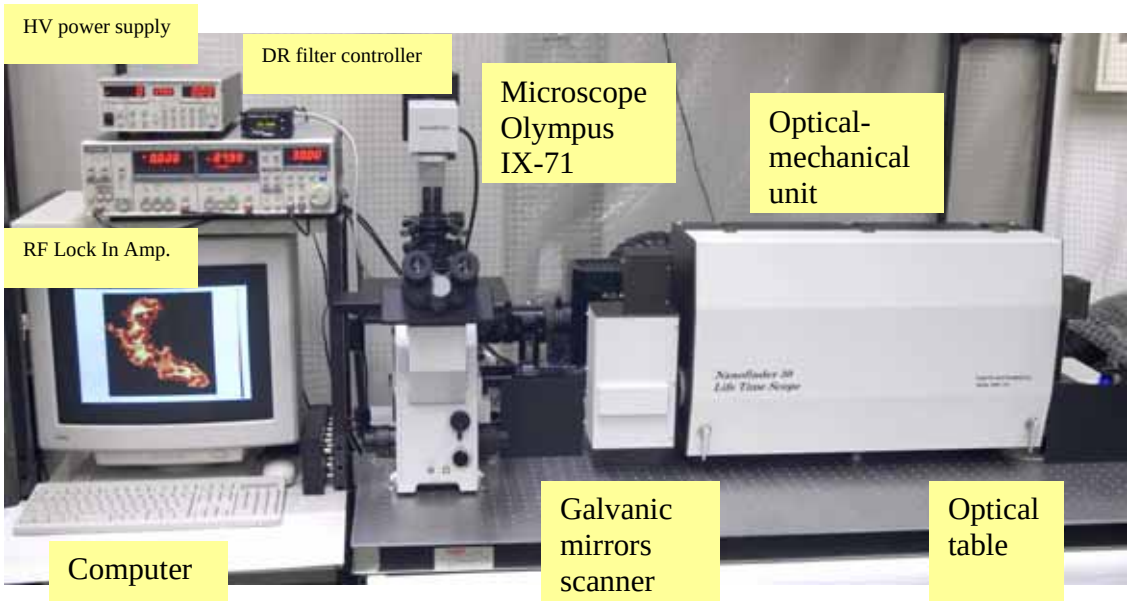
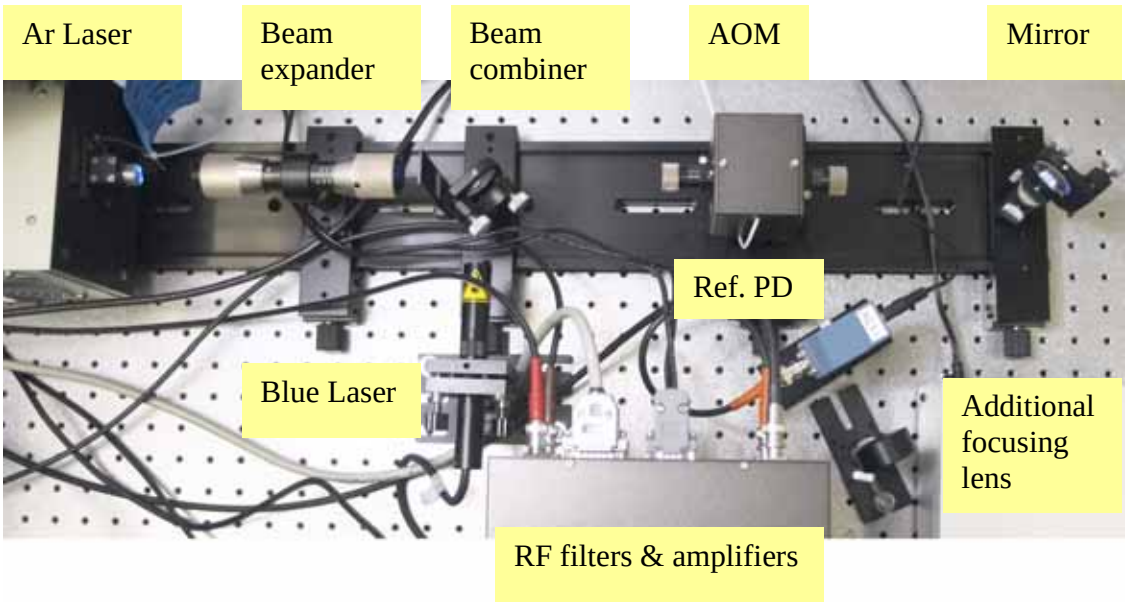
System diagram.



System diagram description.

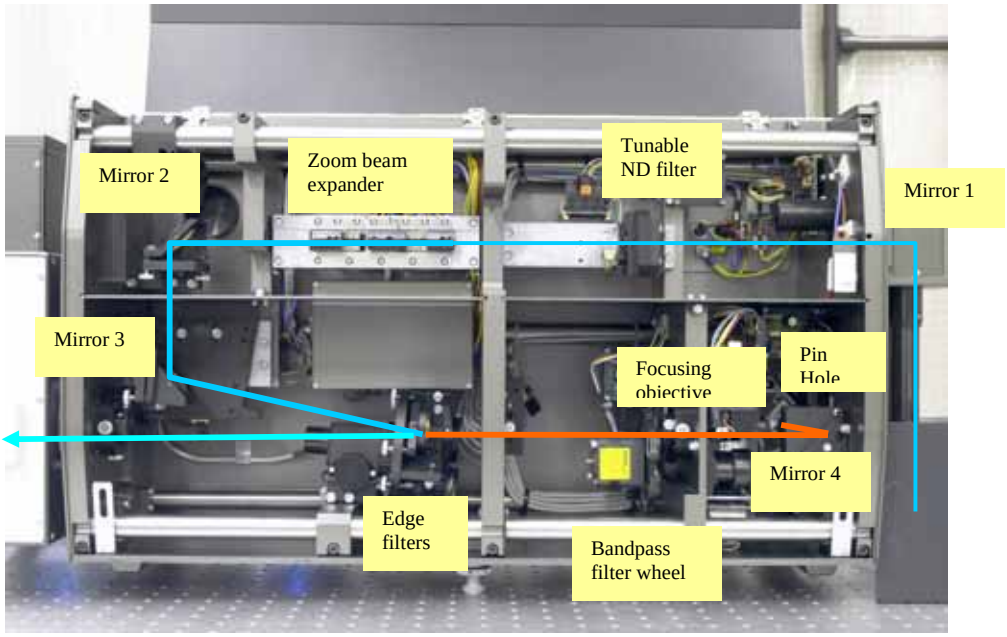
Laser beams from Ar and blue diode lasers are modulated at frequences 30-50 MHz by Acousto-Optical Modulator (AOM). Modulation frequency is settled by RF generator knobs. Not diffracted beam is used for taking optical signal by reference photodiode (Ref PD). Signal from Ref PD is connected to Ref. Input of RF Lock-In Amplifier after passing through RF filter and amplifier.
Diffracted beam enters to Optical mechanical unit “Nanofinder 30 FLTI”. Galvanic scanner of Optical Mechanical unit insures connection to Olympus IX-71 microscope and confocal laser beam scanning on the sample.
Fluorescent signal from sample is detected by PMT and after filtering and amplification is connected to Sig. Input of RF Lock In Amplifier.
Central frequency of RF filters for both Sig. and Ref. Channels is controlled by DR RF filter controller and should be equal to frequency of RF generator AOM.
Electrical signals, proportional to amplitude and phase of fluorescent signal are taken from analogue exits of RF Lock-In Amplifier and directed to analogue-digital converter board (ADC).


HV power supply
DR filter controller
Microscope Olympus IX-71
Optical-mechanical unit
RF Lock In Amp.
Computer
Galvanic mirrors scanner
Optical table


Ar Laser
Beam expander
Beam combiner
AOM
Mirror
Ref. PD
Blue Laser
Additional focusing lens
RF filters & amplifiers

Optical-mechanical unit diagram.

Laser beam pass and control optics are shown in optical-mechanical unit photo.



Laser beam, reflected by mirror 1 passes through motorized ND filter, expanded by zoom beam expander to appropriate diameter and collimated condition. After reflections from mirrors 2 and 3 laser beam falls onto edge filer. Reflected from edge filter laser beam is directed into Galvanic mirror scanner and enter into right side microscope port. After reflection from the right side port mirror beam focused by microscope objective lens onto the sample.

Fluorescence from the sample, excited by focused laser beam is collected by the same objective lens and goes back through the same optical path. Edge filter reflect the scattered laser light, but transmit fluorescent signal in detection channel. Bandpass filters, installed in motorized filter wheel transmit to detector only specified wavelength range. Focusing objective, installed on motorized XYZ stage focuses the fluorescence signal onto size changeable pinhole: motorized crossed slits. Relay lens transfer pinhole image onto photo cathode of PMT.

Main system specifications.

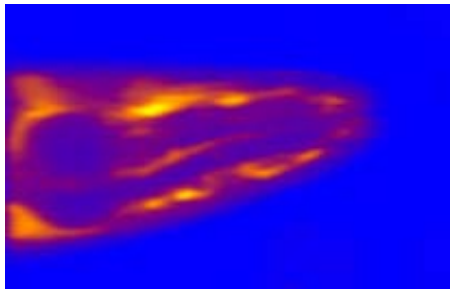
-spatial resolution 0.2×0.2 μm (objective 100X, oil-immersion);
-scanning range > 200×200 μm (with objective 40X),
-within fluorescence intensity nonuniformity <30%;
fluorescence life time nonuniformity < 200 ps (modulation frequency 30 MHz, time constant Lock-In 10 ms, point measurement 30 ms).
-life time measurement range up to 20 ns (phase shift 75 degree at 30 MHz modulation frequency);
-fluorescence life time resolution: equivalent noise 10 ps (with modulation frequency 30 MHz, Lock-In time constant 300 ms, laser power on the sample 1 mW, sample – fluorescein water solution).
-Phase/amplitude intercross influence.
when signal amplitude changes 30 times fluorescence life tme is within fluctuation ±50 ps (with modulation frequency 30 MHz, Lock-In time constant 300 ms, laser power on the sample variable, sample – fluorescein water solution)
-modulation frequency range 30-50 MHz;
-fluorescence excitation laser power on the sample: 488 nm 1mW
Blue diode (405±10 nm 250μW).
-detection wavelength range: 450-900 nm;
493-900 nm.
-bandpass filters in detection channel up to 6 filters,
for example , central wavelength 480 nm, bandwidth 30 nm (CFP);
central wavelength 535 nm, bandwidth 26 nm (YFP).
-detection channel throughput (from sample to PMT) >25%.

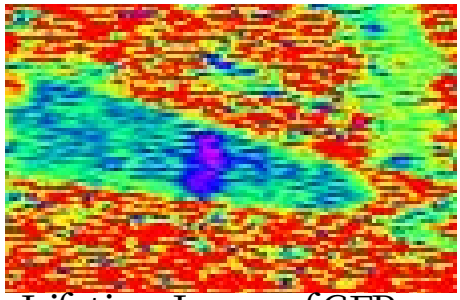
Principle of life-time imaging.

Fluorescence intensity and life time detection principle is based on the phase modulation and lock-in detection technique. System measures the amplitude and the phase shift of the fluorescence signal. Fluorescence decay time is calculated by formula:

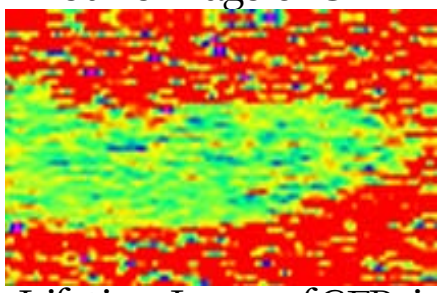
$\tau = \tan(\phi)/(2\pi f)$, where ϕ is a fluorescence phase shift and f , laser modulation frequency.

The both values are taken in a single point of the sample. Image is produced by confocal point by point mapping along the sample.





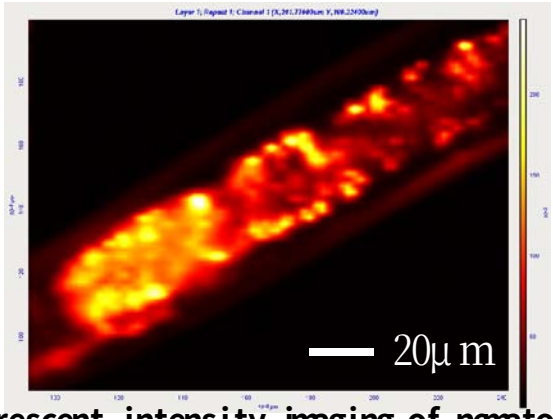
Lifetime Image of CFP



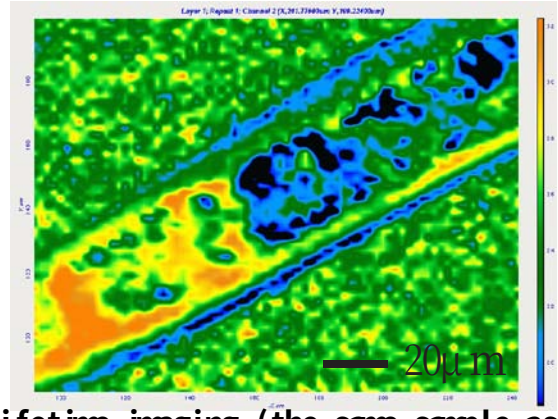
Lifetime Image of CFP in the presence of YFP

Imaging of CFP and YFP in nematode, *C.elegance*.
Uppermost: intensity imaging
Middle: lifetime imaging of CFP
Lower: Lifetime imaging of CFP
in the presence of YFP
The lifetime in the middle decreased from 3.5 ns (blue) to 3.0 ns (yellow green) by the fluorescence energy transfer.

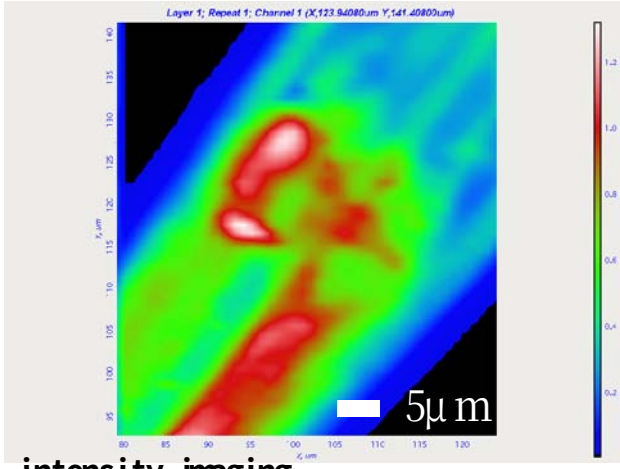
(Courtesy of Prof. Ogura of Kumamoto Univ.)



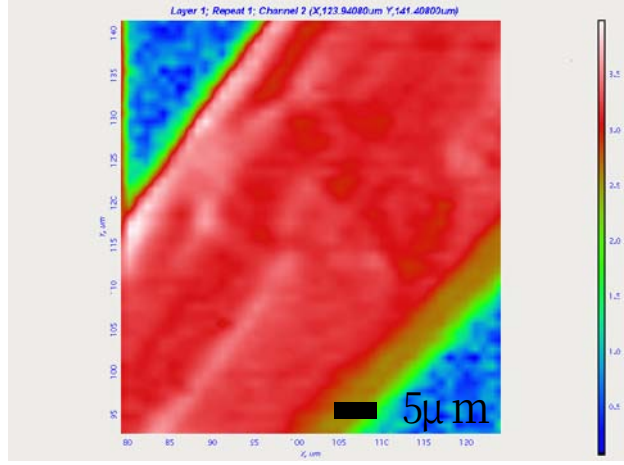
fluorescent intensity imaging of nematoda



lifetime imaging (the same sample as right)

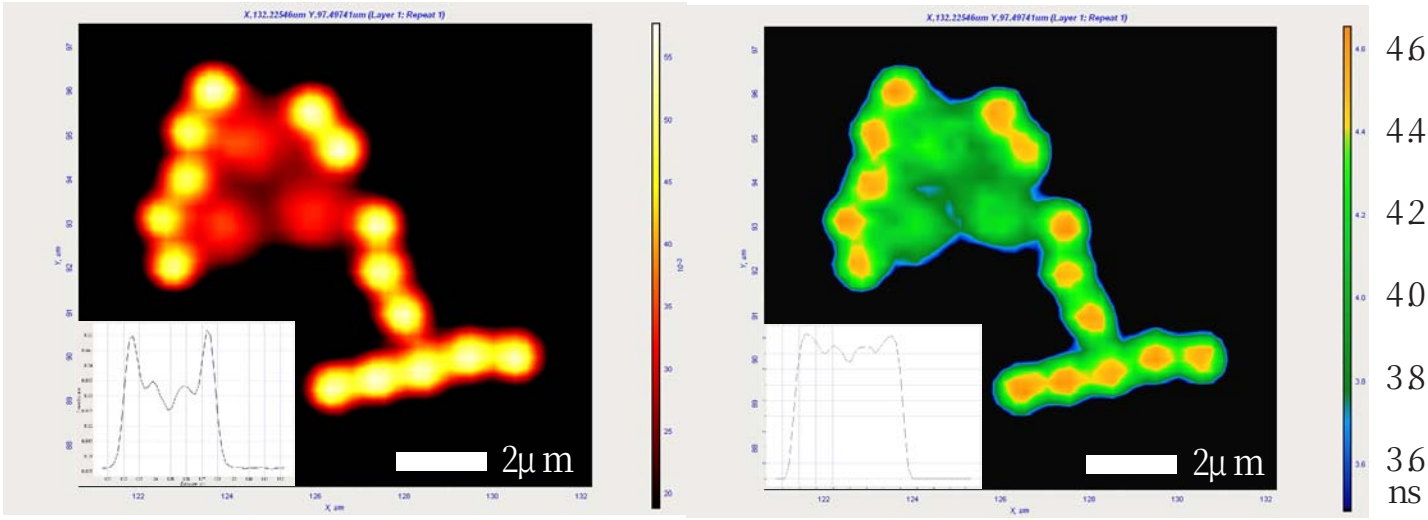


intensity imaging
(enlarged from the same sample above)

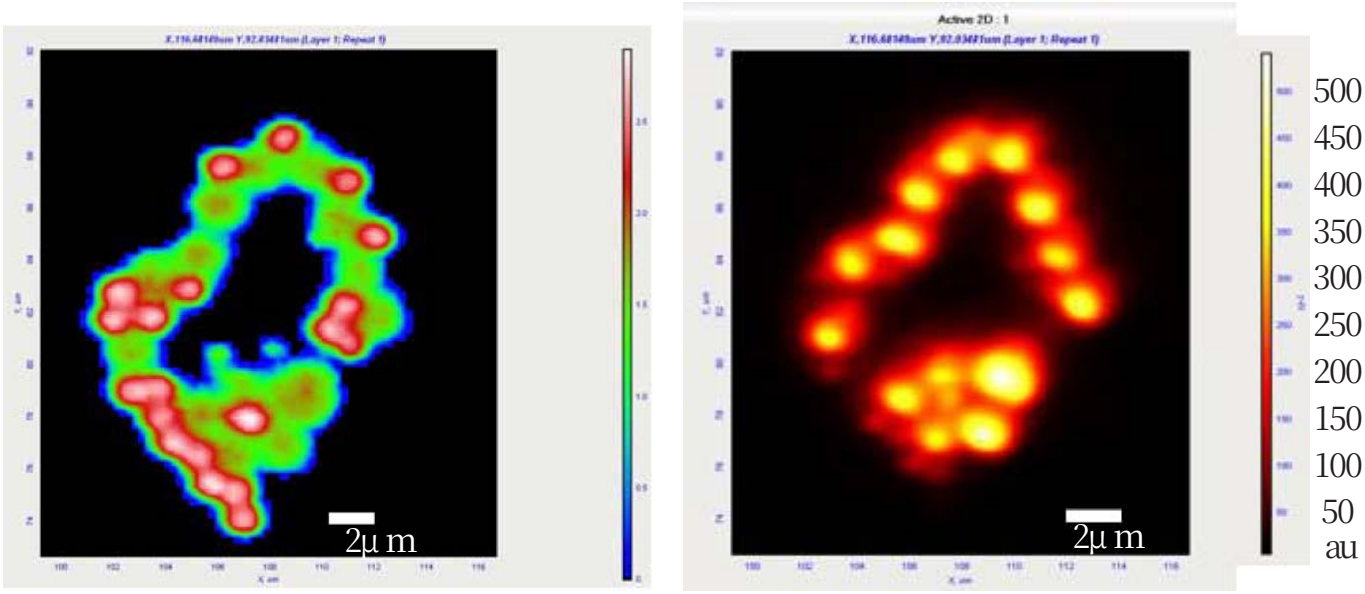


lifetime imaging
(enlarged from the same sample above)

excitation: 405nm , 25μw
detection: 465-495nm (480AF30)



Fluorescent intensity (left) and lifetime (right) of beads

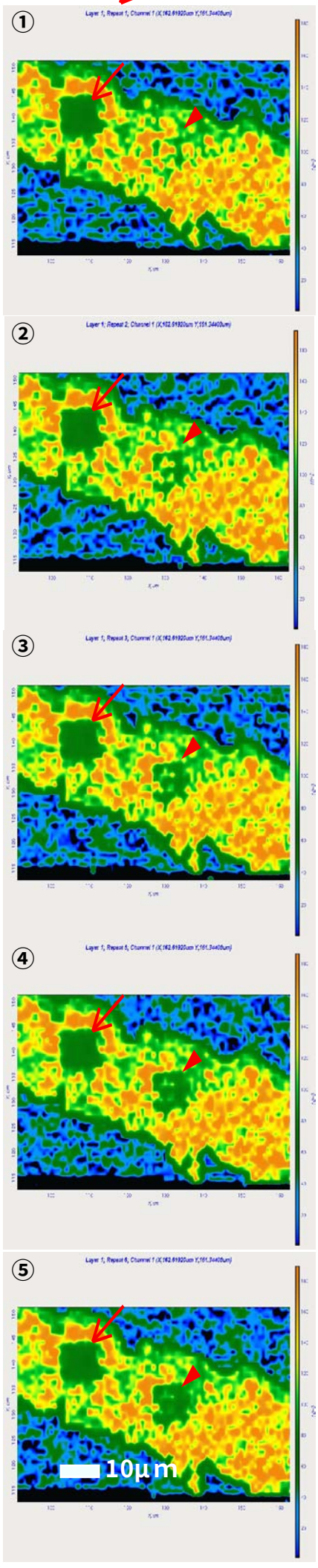


The same beads sample was excited with 2 different wavelengths.

The same sample was excited with 2 wavelengths, 405 nm (left) and 488 nm (right). Completely separate distributions of fluorescent intensity were observed.

Beads :
Fluospheres® **carboxylate-modified.**
mixture of red fluorescent (580/605) 1μm and yellow-green fluorescent (505/515) 2μm

FRAP (↙) and FLIP (▶) in model beads



FRET:

In order to observe FRET, 2 kinds of proteins are genetically coexpressed with 2 separate fluorescent markers. If 2 proteins are close enough and the emission spectrum of a donor marker overlaps with the absorption spectrum of the acceptor one, the absorbed energy in the donor transfers to the acceptor fluorescent marker. Then, we can observe the fluorescence from acceptor molecules. Such a FRET phenomenon tells us the geometrical relationship between 2 proteins. The above is conventional FRET measurement with intensity imaging. However, Fluotracker gives us the other way to observe FRET on top of the intensity imaging for the first time. That is, the observation of decrease in lifetime of fluorescence from donors due to the transfer of absorbed energy to acceptors. This method gives us more reliable results than the intensity imaging; for example, the lifetime imaging does not suffer from a bleaching problem during experiments.

FRAP:

FRAP:A certain area of a cell is bleached and the recovery of fluorescence there is observed.
This recovery comes from non-bleached molecules coming in the area. This phenomena gives us information of intracellular trafficking and biolgical metabolism.

FLIP:

FLIP:A certain area of a cell is bleached and the bleaching of the outside is observed.
This bleaching comes from bleached molecules going-out of the bleached area.
This phenomena suggesgt us how intracellular trafficking and biolgical metabolism are occurring.

Photo Pulse Labeling:

Some molecules become strongly fluorescent after irradiation followed by photoconversion.
Cells can be marked fluorescently by photoirradiation using such special genetically changed ones.
FRAP and FLIP above give us information of cellular metabolism on the bases of dynamics of fluorescently inactivated or bleached molecules. On the contrary, the photo pulse-labeling experiment make use of fluorescently activated molecules for the similar information.

FLIP :
Bleaching: 488nm
1mw
every30sec
Mapping ; 405nm
100μw

FRAP:
Bleaching:405nm
250μw
30sec
Mapping : 405nm
25μw