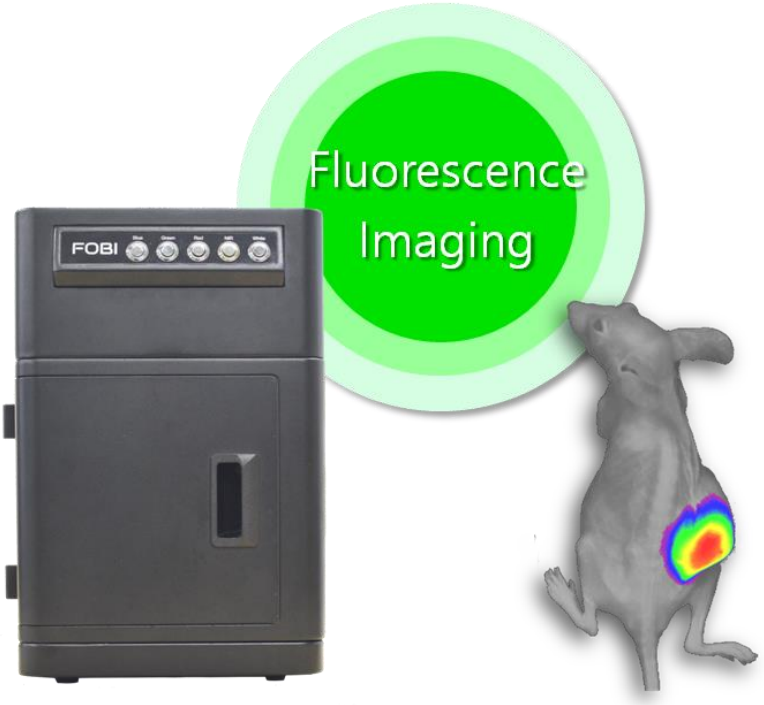
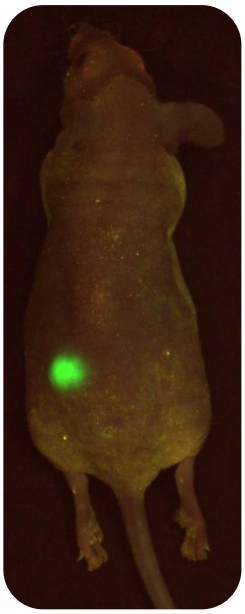
Applications of FOBI



CELLGENTEK Co., Ltd.

1. Tumorization



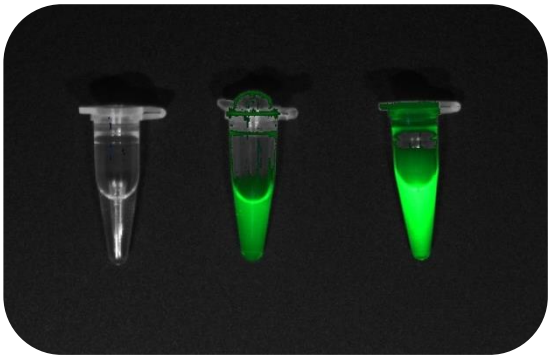
The first application of In Vivo Imaging is Tumorization. With traditional tumor sizing, weighing and measuring the tumor is very subjective, so the data may not be accurate.

Fluorescence imaging is an excellent method for objective research data. We can get quantitative data through tumorization imaging analysis from a fluorescent gene inserted in a stable cell line.

The best method to insert the fluorescent into the tumor cell is using the Lenti-virus. You can insert the fluorescent gene using plasmid or another virus, but the Lenti-virus method is the strongest. And the cell lines have to express GFP 100 percent. The degree of fluorescence is gradually reduced when non GFP cells are mixed in because non GFP cells proliferate faster than GFP cells.

In Vivo imaging is more difficult than In Vitro imaging because the skin. Fluorescence should be strong when conducting an In Vivo experiment. We recommend eGFP, cop-GFP and Turbo-GFP.

The cell line must be confirmed the fluorescent expression level by FOBI. GFP should be adequately expressed to get a fluorescent image in the suspended cells in the microtube.

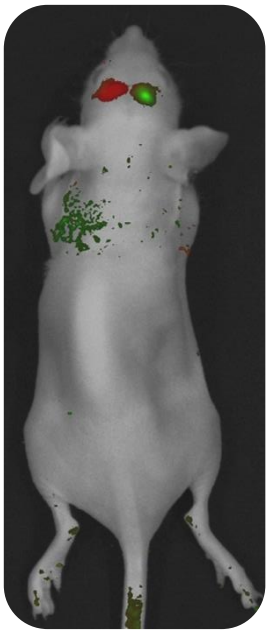


The first step of In Vivo is a subcutaneous experiment. Tumor target tissue is varied, but it is good to determine the anti-cancer effect subcutaneously first. There are many variables in the target tissue experiment. And there are many variables under the skin in optical terms. An experiment on real

target tissue after the subcutaneous experiment is very strong evidence for an anti-cancer effect.

Images obtained by FOBI can be quantified by read intensity. The quantitative value should use integrated density reflected in the area (pixel number) and intensity. (Integrated density = area x intensity)

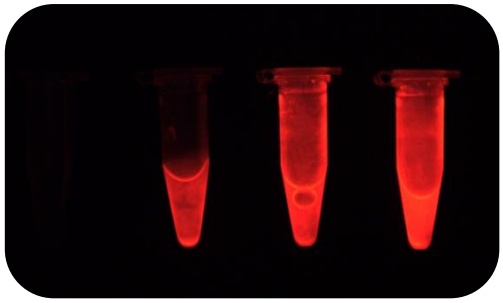
2. Stem cell tracking



In Vivo imaging experiments started in the tumor research field, but recently In Vivo imaging is in demand in the stem cell research field. It caused many people have a lot of interest in cell therapy.

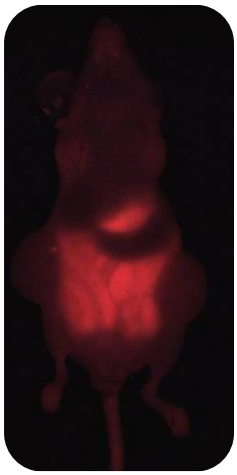
Unlike tumors, it is difficult to insert a fluorescent gene into the stem cell. Stem cell characteristics could change when a foreign gene is inserted. Stem cells have the ability to differentiate into various cell types, but by inserting a foreign gene, stem cells' differentiation ability may be lost. Therefore, stem cells are stained using a physicochemical method.

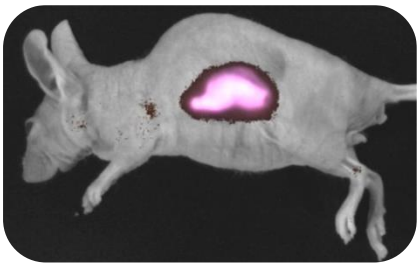
The physicochemical stain method presents problems also. Fluorescence particles may change stem cells' character, and fluorescent dye may have toxicity. But, it depends on the kind of stem cells, so we have to find the optimal staining method for the particular stem cells and then we can do the In Vivo imaging experiment.



Like the anti-tumor effect experiment, the staining level should be confirmed by FOBI. The fluorescence level should be checked before inject into the animal. The expression level could checked in a microtube.

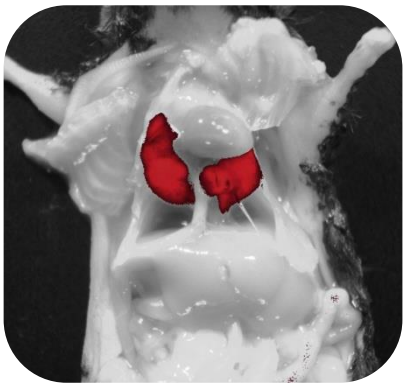
In stem cell In Vivo experiments, cell number is not enough, so efficiency is very important. Brightness, transmission of fluorescent dye and auto-fluorescence of the animal skin determines the efficiency. Animal skins have very strong auto-fluorescence in green and red. The auto-fluorescence can be removed after the fluorescence image is acquired. But it is better to use a wavelength that has a low auto-fluorescence level, so NIR (near infra-red) has attracted the attention of stem cell researchers. FOBI has two channels of NIR, "Red channel" and "NIR channel". The Red channel light source excites with 630nm and the emission filter is a 725nm long-pass filter. The NIR channel light source excites with 730nm and the emission filter is an 825nm long-pass filter.





There is very low auto-fluorescence under the Red channel, but the stool has auto-fluorescence. The auto-fluorescence could be reduced by starvation. The NIR channel has lower auto-fluorescence than the Red channel, but the brightness and efficiency of the camera is lower. Therefore, researchers have to pretest for optimal fluorescence dye and channel. If you get an image with the Red channel of FOBI, the signal is shown in red. If you use the NIR channel of FOBI, the signal is shown in violet.

When observing the stained stem cells In Vivo, closer to the skin surface looks good. If there is no obstacle, such as the liver, intestine or brain, the fluorescence signal can be obtained easily. But, it is difficult to get a fluorescence signal of tissue away from the skin like the lungs and heart. There are many obstacles such as muscle, fat and



variable membranes. In this case the animal should be sacrificed at the end point of the experiment. Or an Ex Vivo experiment is good for this case.

3. DDS

DDS (drug delivery system) is attracting great interest as a stem cell. To determine whether a drug is moved to the desired target tissue is very important. Tracking the drug with fluorescence is helpful for DDS research.

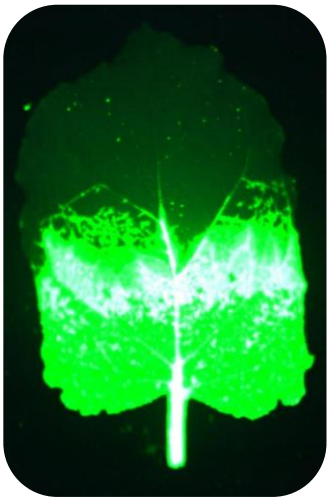
The fluorescent image of DDS has attracted attention from two perspectives. The first is, "How does an injected drug spread in the body?" and second, "How and where does the injected drug accumulate?"

The first image is that how much the drug spreads in the body, that is, how much fluorescence exists in the blood. In this case, the fluorescence tends to be mistaken as fluorescence from whole body. However, in practice, the fluorescence is from capillary and is placed on the skin. The fluorescent signal is not intended to take the light from the fluorescent source in the target tissue. The image shows the signal projecting light onto the skin surface after a number of optical phenomena (bend, diffraction, reflection ...). If the fluorescence spreads on the body, the light from the capillaries and skin will be strongest. So, fluorescence seen in the whole body in the initial time is from the skin surface.

Fluorescence gradually moves toward the target tissue over time, and appears increasingly strong in the target tissue. On the contrary, the fluorescence of the whole body is reduced because the exiting and accumulating fluorescence is increased.

As in a stem cell experiment fluorescence is strong closer to the skin, it is difficult to get an image from the signal in deeper located tissues such as the heart and lungs. In this case, to get the images, animals have to be sacrificed. Or Ex Vivo data is better.

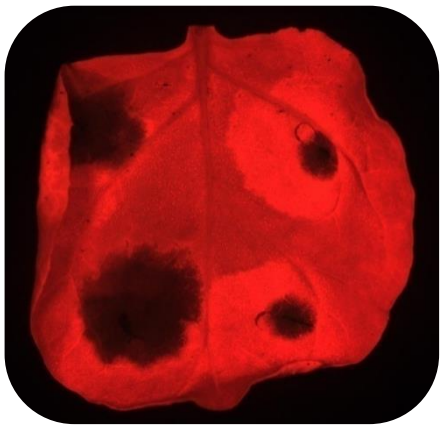
4. Plant



The fluorescent images can be obtained from plants such as GFP. GFP expression can be observed in tissue such as seeds, roots and leaves.

Auto-fluorescence is not a limiting factor in the seeds and roots because there is no chlorophyll. However, because seeds and roots have a white color there is the possibility that reflected excitation light could be detected. So it should be compared with a negative control.

Very strong auto-fluorescence comes from leaves' chlorophyll. The auto-fluorescence has a red color. But the red auto-fluorescence overwrites the GFP signal. In this case the GFP signal can be obtained clearly by removing the red wavelength using the cyan filter.



Chlorophyll is a problem when the GFP images obtained. But it is useful for other purposes. Chlorophyll is expressed in most of the leaves by presence of the chloroplasts. You can determine the physical condition by using the auto-fluorescence of chlorophyll. That is, if the expression levels are abnormal, the health status of the leaf can be considered abnormal.

just as it is...

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