UCSF Helen Diller Family Comprehensive Cancer Center

Update from the HDFCCC Preclinical Therapeutics Core Facility, September 2021

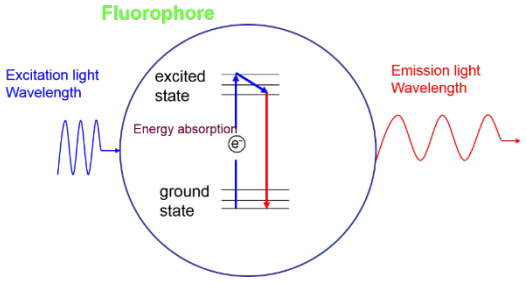
THERE'S LIFE BEYOND THE FIREFLIES

Optical imaging is a bidimensional imaging technique based on the analysis of light. The quantification of this light is made counting photons, and they can be generated in three ways:
- **Bioluminescence (BLI),** where an oxidative reaction between a protein (luciferin) and an enzyme (luciferase) results in emission of lights - this reaction requires O2 and ATP;
- **Cherenkov**, based on the analysis of photons created after the annihilation of a positron and an electron from a radioactive source;
- **Fluorescence (FLI),** where a compound called fluorophore absorbs light at a particular wavelength to subsequently emits light of a longer wavelength.

The most frequently used methods are BLI and FLI. Cherenkov imaging is a complex, dangerous technique, and there is no advantage in making a 2D image of a radioactive source when you can have a nice 3D study using the PET. Because BLI is more frequently used and it is easier to control (despite sublte aspects to take into account for a correct BLI imaging), we are going to focus our September's newsletter on FLI. Please join us in this trip to discover this powerful technique. You will find that it is not complex and has numerous and attractive possibilities in research.

How does FLI works?

Fluorophores are structures with the capability to emit a specific filtered light (not complete light, just in a specific wavelength) called **emission light**, after it is exposed to another filtered light, called the **excitation light**. Both are fixed for each fluorophore. There are a lot of natural fluorophores, like chlorophyl, collagen, hemoglobin and similar. They produce what is called as autofluorescence.



How to use FLI for my research?

In the same way that you can correlate the light emitted in a BLI reaction to the number of cells (because this reaction is exclusively intracellular), similarly you can make a correlation between the photons emitted in a FLI process and the number of fluorophores involved in this reaction. Knowing this, it is as easy as linking a fluorophore to the target you need to analyze or create a fluorescent probe conjugated with an antibody specific for any biological structure (Fig.1), or antigen in primary cell line (notoriously hard to transfer) - the possibilities are almost infinite.

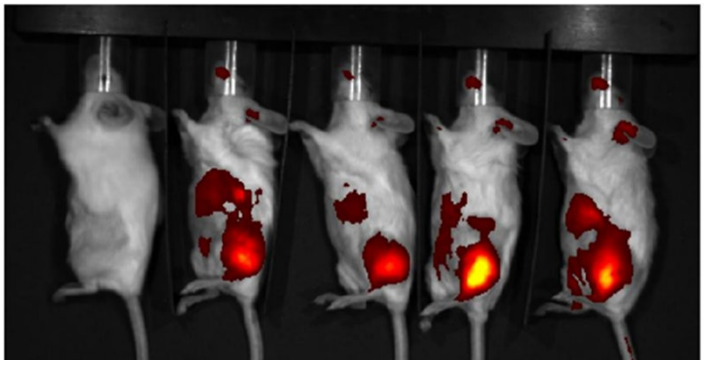


Figure 1: I*n vivo* FLI study using a fluorophore linked to a specific tracker of procaryotic metabolism, allows to find bacterial aggregates

How to perform a FLI experiment?

Before starting injecting mice with any commercially available dye (Perkin Elmer or Sigma), there are some *tips and tricks* you need to take into consideration:

- *Forget about using GFP for imaging live animals.* You cannot use the GFP dyes you use in confocal studies - sorry. GFP works in the green wavelength and it is a weak light, it gets almost completely absorbed. Every

macroscopic structure will absorb the GFP. For *in vivo* FLI, you have to shift as far as possible in the light wavelength range, *i.e.*using a red dye (tomato, Dsred, M-Cherry or similar). If possible, go to the infrared. These dyes are almost “immune” to tissue absorption.

- *Before doing in vivo FLI study, spend some time making an in vitro FLI study.* Sometimes the wavelengths (excitation and emission) change slightly between manufacturers and this can affect the pair of filters used during the acquisition.

- *Always use a negative control animal when doing in vivo FLI studies.* Because of tissue autofluorescence, you will always have a small amount of filtered light in your animals, so a control animal is needed to correct the background in your study. Every time you acquire a FLI image, there must be a control animal in the picture (Fig.2).

- *Perform an ex vivo study of the target organs at endpoint.* You could find differences in the signals comparing *in vivo* and ex vivo images (Fig.2). The last day of the experiment, spend some time in making an *ex vivo* study of the organs you are interested in. Tissue absorbance can mask your fluorescent signal, while you could be able detect if examining organs alone.

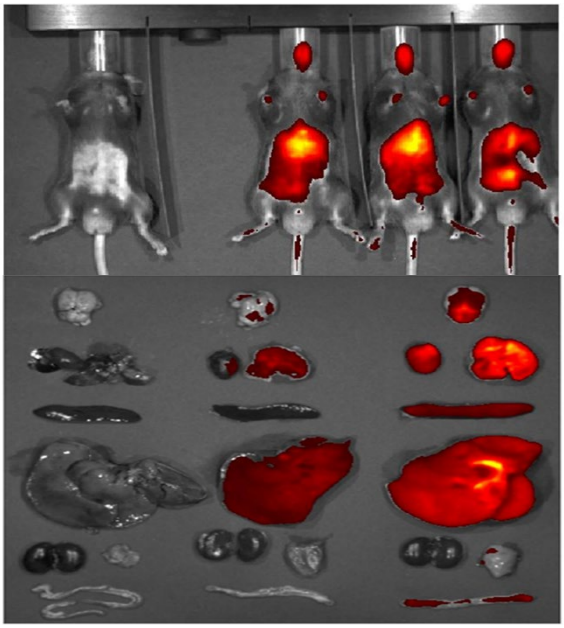


Figure 2*: In vivo* FLI study using a fluorophore targeting specific targeting for liver tissue (top); *Ex* vivo image in a biodistribution study of a novel drug (bottom). Control animals on the left (top and bottom).

Who said that FLI is complicated?

With this information and a few tricks, you will be able to obtain impressive images and, importantly, you will be able to perform your research without modifying your cells. Just remember: **no GFP, include control animals, go to the far-red,** and **make ex vivo studies.**

And ask me if you have any question or doubt. You can email me at JuanAntonio.Camaraserrano@ucsf.edu