# 3 - 40 Viability Determination of *Spirulina Maxima* by Double Staining with Fluorescein Diacetate and Propidium Iodide

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*Spirulina* (*Arthrospira*) is a kind of filamentous cyanobacteria with multicellular cylindrical trichomes. This microalgae has many properties such as high protein content, unique composition of fatty acids and vitamins, and high carbohydrate content. And it is one of the most potent sources of natural nutrition, including all the essential amino acids, natural pigments, such as chlorophyll and betacarotene, and other natural phytochemicals. Spirulinais also rich in γ-linolenic acid (GLA), which has enormous pharmaceutical potential for reducing infammation and alleviating the symptoms of premenstrual syndrome, heart diseases, Parkinson's disease and multiple sclerosis.

Many kinds of fluoresceins have been used to detect the enzyme activities and cell membrane integrity, among which the most frequently used were fluorescein diacetate (FDA) and propidium iodide (PI). FDA is a non-polar, hydrophobic, non-fluorescent esterified compound with molecular weight of 416 Da. PI is a fully cell-membrane impermeable fluorescent dye which can only combine with DNA in cells that are dead or that have broken membranes, and thus has been used to indicatedead cells for a wide range of microorganisms. Cells with an intact cell membrane are stained bright green by FDA and cells with a damaged cell membrane are stained bright orange with PI.

*Spirulina Maxima* was chosed to do FDA-PI dyeing experiment, which was supplied by Kai-yuan Biotechnology Center, Hexi University. The culture condition of *Spirulina Maxima* was grown in Zarrouk'smedium at 25 °C with a cycle of 12 h light/12 h dark for the culture, under white fluorescent lamp set light intensity of 49∼57 μmol·m$^{-2}$·s$^{-1}$. And the microalgae suspension was diluted 5 times to be observe by Laser-scanning confocal microscopy, Carl Zeiss LSM 700. The FDA working solution was 100 μg/mL, and the PI working solution was 60 μg/mL. The fluorescein converted from FDA was detected in the FL1 detector (515∼545 nm), and fluorescence of PI was detected in the FL2 detector (564∼606 nm). As shown in Fig. 1, the cytomembrane of *Spirulina Maxima* conchocelis was dyed green by FDA and the thallus of algae was dyed red by PI, which entered the cell binding DNA or RNA of broken cell or died cell. We could also find that a part of conchocelis was light green, and it was because FDA could readily permeate the cell membrane of living cells and was hydrolyzed by non-specific esterases producing a fluorescein.

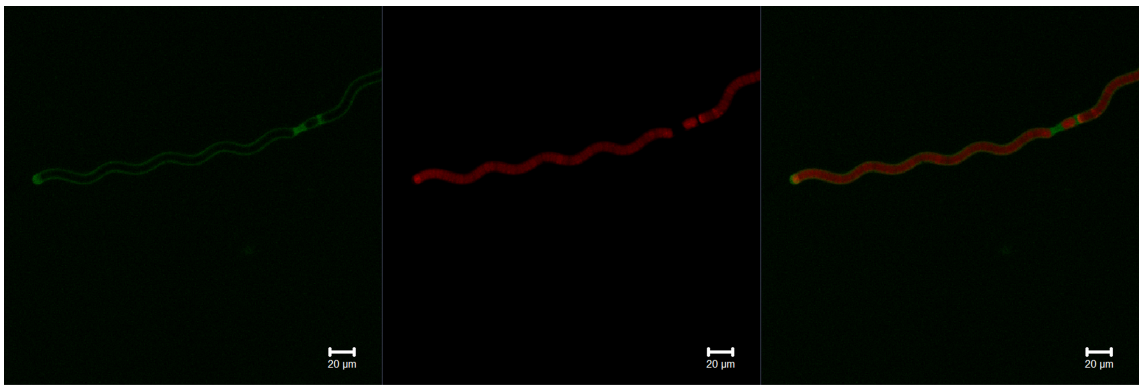


Fig. 1 (color online) Epifluorescence micrographs of *Spirulina Maxima* by FDA-PI double fluorescent dyes.

From the test results, it is known that the FDA-PI was effective to observe the irradiated cells, and it is a promising method by combining Flow cytometry analysis to collect living cells from irradiation algae or bacterium solution. Compared to traditional plate screening method, this way could be more helpful and high-efficiency to mutant isolation.