Laser-Ablation Of Single Cells

(Source: J. Eisen from Zebrafish Book 5th Edition)

In principle, almost any cell in the zebrafish embryo that can be visualized (e.g. with Nomarski DIC optics) can be ablated by laserirradiation. The power output of the laser and the type of optical system used to focus the beam onto the cell will determine whether, in practice, a particular cell can be ablated. The following protocol has been used successfully to ablate single identified neurons in the spinal cord, individual floor plate, muscle, notochord, and neural crest cells, and entire ganglia.

Mount embryos either on microslides in 1.2% agar (see Agar Mounting, page 4.5) in Embryo Medium or between bridged coverslips (see Viewing Chambers, page 4.3) in Embryo Medium in such a way that the cell of interest is easily visualized with Nomarski DIC optics using a compound microscope. Use a water immersion lens with as high a numerical aperture as possible. A Zeiss 40xwater immersion lens with a 1.6 mm working distance is a good choice. A simple method for laser-irradiation is to focus the laser beam through the port of the vertical illuminator. This set up is described in Sulston and White. More detail about the design of such systems can be found in Berns.

There are currently at least two types of pulsed lasers available that work well for single cell ablations: dyepumped pulse lasers, such as those made by PhaseR and Candela, and pulsed nitrogen lasers with dye modules, such as those made by Laser Sciences. The advantage of the first type is that it contains a HeliumNeon spotting laser for relatively easy alignment. The advantage of the second type is that it is cheaper, smaller, and quieter. In both cases, you can use coumarin 450 (made by Exciton, Inc.) as the dye source; the dye is dissolved in high grade methanol (1 gram/4 liters for the dyepumped laser and 17.5 mg/20 ml for the nitrogen laser).

To ablate a cell, focus the laser microbeam onto the cell nucleus, or onto the cell membrane. Depending on the cell, the condition of the dye, and the type of laser, from 1100 pulses are needed to ablate a cell. Cells may die instantly after irradiation, or they may take up to 15 minutes. It is important to watch the cell die, or to have an independent measure, such as the loss of cellspecific antibody labeling. As cells die, their nuclei typically become granular in appearance and often swell.

Once the cell of interest has been ablated, the embryo should be removed from the agar or the bridged coverslip and placed in a dish of Embryo Medium to continue development.

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