A Versatile, Cheap, and Easy Self-Made Cell Culture Dish

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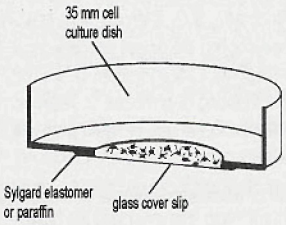
Anybody who has ever worked with cells or tissues in culture knows that the dish in which to grow and look at the specimen can be of crucial importance to the results obtained. With the growing importance of live cell microscopy, be it simple phase contrast or sophisticated multilabel confocal laser scanning microscopy (CLSM), people have been looking for dishes that combine optimal growth conditions with optimal optical properties. As follows, I will describe a solution that has worked for us with a multitude of different cells, tissues and applications. Apart from that, it is cheap and easily self-made.

The bottom of a standard 35 mm plastic cell culture dish has a hole of *ca.* 10 mm drilled into it and a coverslip glued to its downside to cover the hole. For a glue we usually use Sylgard, an inert silicone elastomer (Dow Corning) or melted paraffin if we intend to take off the coverslip again. Now this is very simple but at the same time gives a lot of advantages over a normal cell culture dish



While the dimensions of the dish remain the same and it still fits into most standard stages and climatized chambers of inverted microscopes, the optic properties have improved dramatically. Most of the microscope lenses are designed for use with coverslips and by choosing the appropriate coverslip thickness and material (*e.g.*, quartz glass for UV applications), the optical performance can be optimized. Refractive index mismatches can be minimized by choosing the right coverslip and, for example, oil immersion techniques. Another advantage is the thickness of the coverslip itself. Standard plastic dishes have a relatively thick bottom and problems may occur with lenses of small working distance when focusing deep into the specimen. Sufficient focusing depth becomes especially important in CSLM when stacks of optical slices are recorded. Also autofluorescence of the plastic, which may become a problem in low light level applications, can be eliminated when imaging through a glass coverslip instead.

While observing the specimen from below with an inverted microscope has advantages, for example in live cell observation or physiological setups where electrodes and superfusion pipettes can then easily be mounted without interfering with the objective, some applications may require an upright microscope. For this purpose, we used a dish to which the coverglass had been attached with paraffin. The cells were fixed and stained immunocytochemically. We then took off the coverslip with the cells attached to it by the use of forceps and placed it upside down with mounting medium (*e.g.*, Mowiol) onto a microscopic slide. This results in a durable preparation that can be kept for a long time.

Not only are the optical properties improved, but the glass bottom dish also offers a variety of cultivation conditions that should accommodate most culture preparations. The glass bottom can be coated with many different molecules such as poly-lysine, poly-ornithine, collagen, laminin and many more to serve as suitable substrates for adhesion of cells and outgrowth of *e.g.*, nerve cell processes. Also the volume of the culturing medium can be varied by filling the dish only over the glass coverslip (*ca.* 100 µL) to filling the whole dish (*ca.* 3 mL). Small medium volumes are needed, for example, if the effect of secreted factors are studied in a co-culture system. Large medium volumes may dilute the factor in this case too much to see its effect. On the other hand can larger medium volumes substantially increase the times between medium changes thus reducing stress for the cells and work for the scientist. We have used this dish with a variety of cells and applications. Among others we have successfully cultivated and imaged cell lines (3T3 fibroblasts, PC 12 phaeochromocytoma cells) as well as a variety of primary cells [frog dorsal root ganglion cells, frog spinal motoneurons (Neurosci. Lett. 176: 251-254), frog muscle fibers (J. Exp. Biol. 199:2359-2367), rat and chick dorsal root ganglion cells, rat oligodendrocytes and Schwann cell]. Most other cell types should, if provided with the appropriate substrate and medium also grow happily in this dish. In conclusion we have a cell and tissue culture dish that is optimized for optical and culture properties. We like them so much that we even clean them after use (the Sylgard type only, the paraffin type gets leaky), UV sterilize and reuse them for a couple of times.

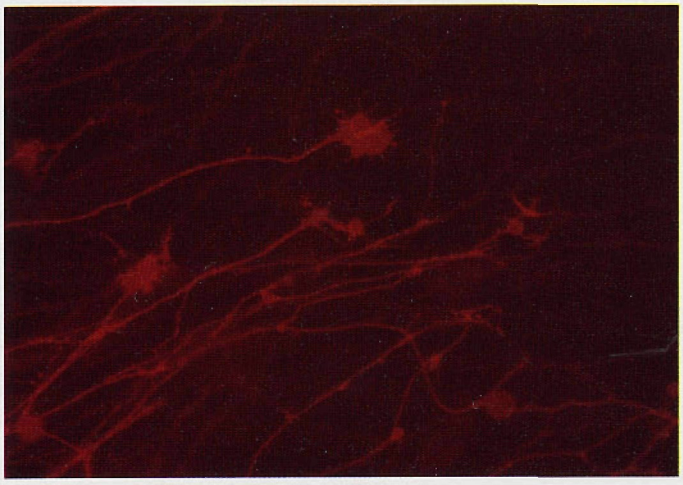


Figure 1: Nerve fibers growing from an explanted chick dorsal root ganglion in culture. The fan-like structure at the tip of the growing fiber, the growth cone, explores with its filopodia and lamellipodia the environment and guides the nerve fiber to its target, where a synaptic connection will be formed. Dorsal root ganglia were taken from embryonic day 13 chicks (8 days before hatching), explanted in a culture dish, and a crystal of the fluorescent lipophilic neuronal tracer DiI placed on top of it. The DiI distributes in the cell membranes and thus labels the outgrowing nerve fibers. The preparation was observed on an inverted Olympus microscope at a magnification of 400 x.

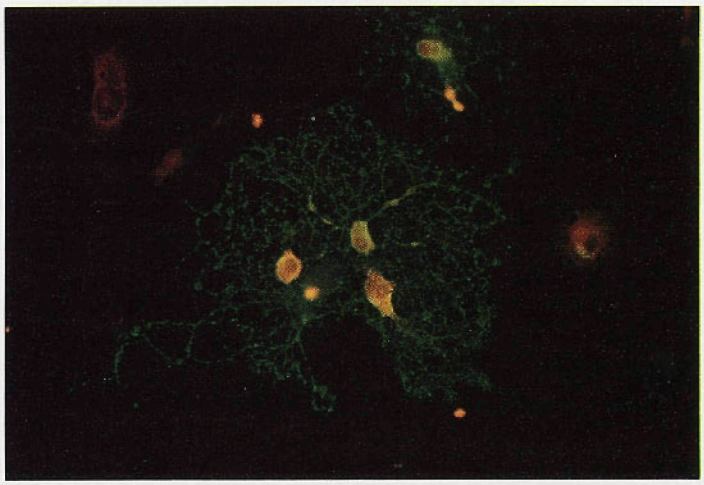
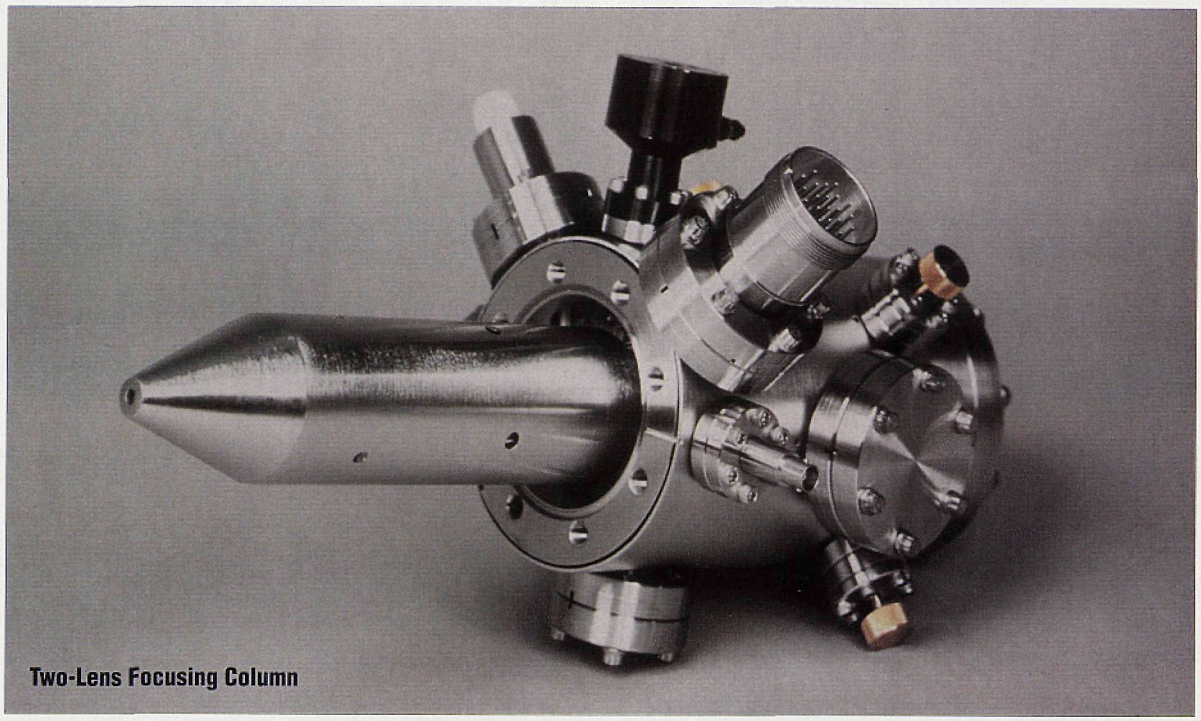


Figure 2: Oligodendrocytes, the cells that provide nerve fibers in the central nervous system with their insulating sheath (myelin). Cells were isolated from brain of newborn rat and differentiated in culture in presence of certain factors to their typical wheel-like mature form. Cells have been immunolabelled by an antibody to galactocerebroside, a surface marker of mature oligodendrocytes (green channel), a second antibody against a protein of the cell body (red channel), and viewed on an inverted Olympus microscope at a magnification of 400 x.



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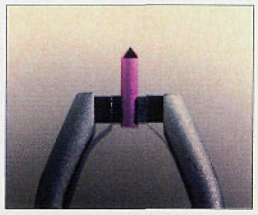

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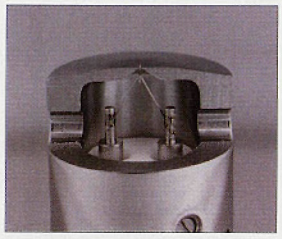

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