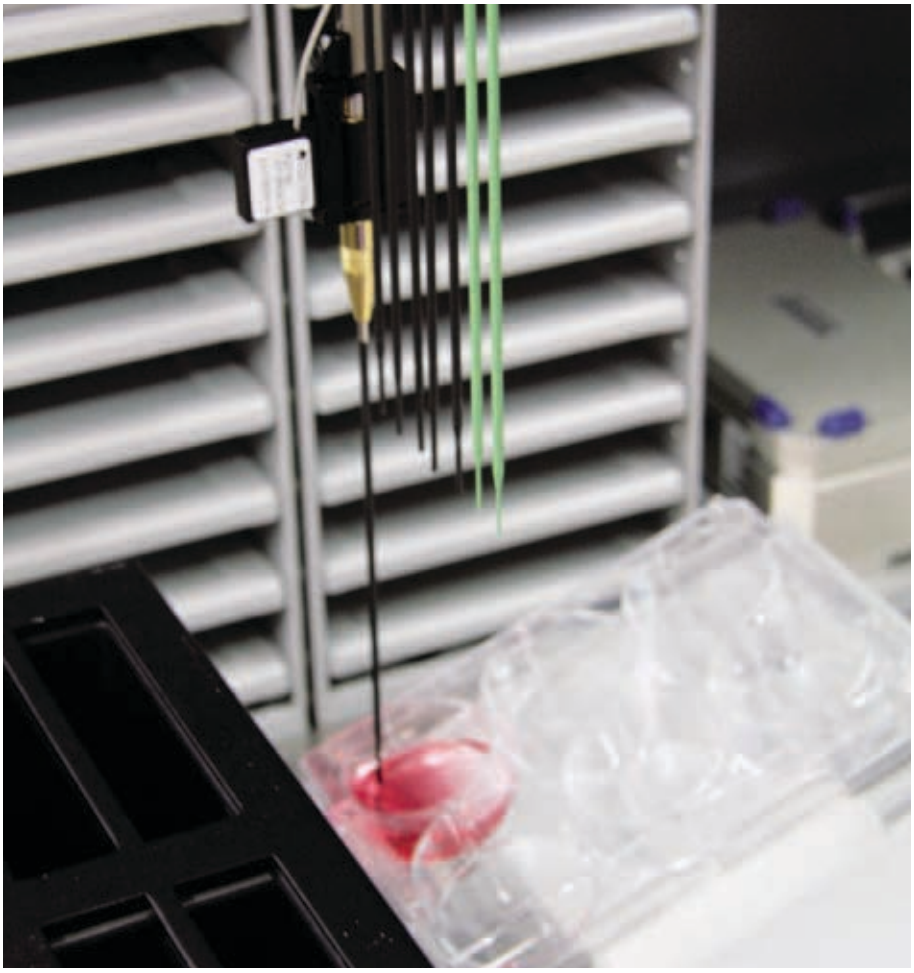
Culturing adult hippocampal precursor cells

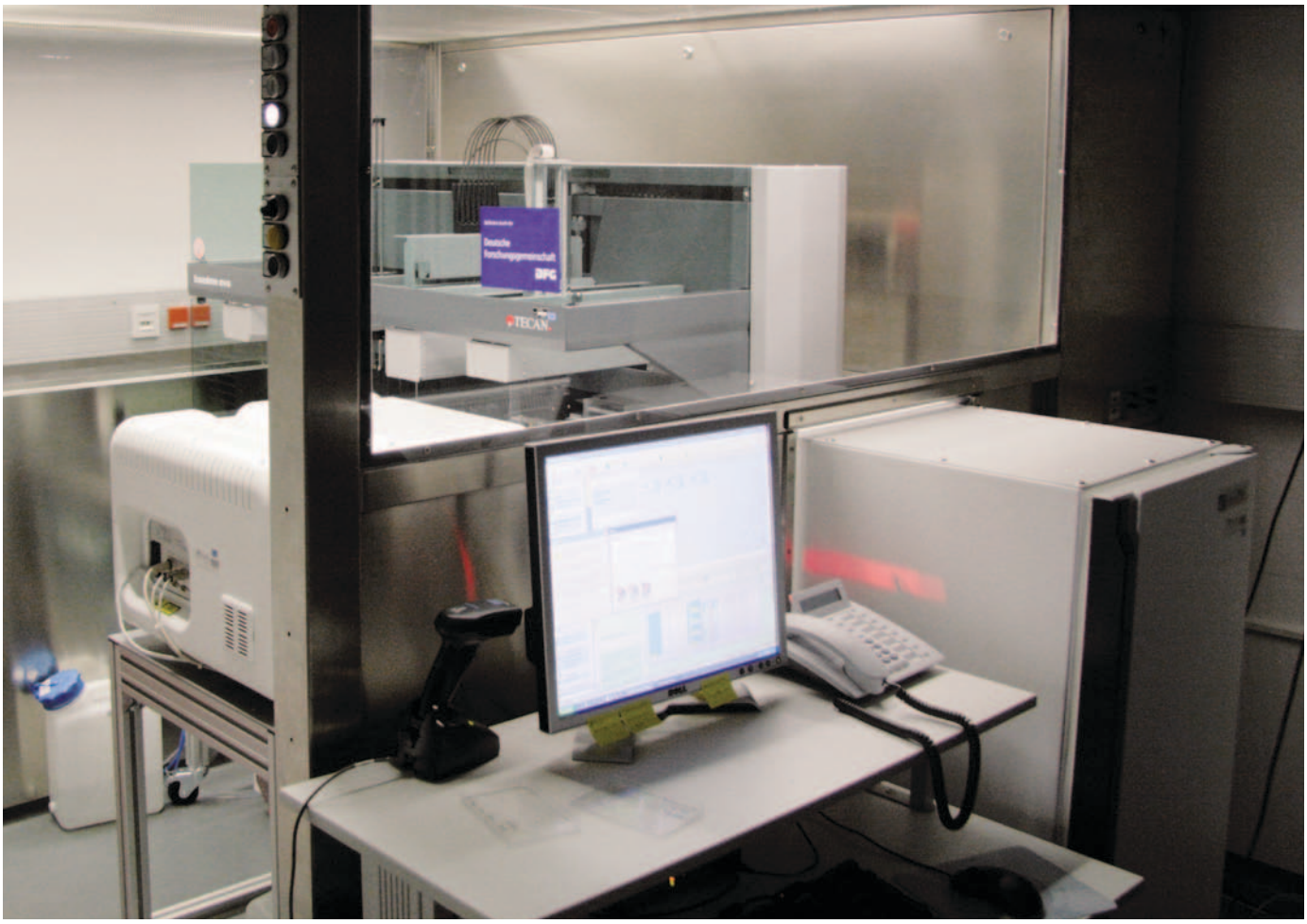
Scientists studying adult neurogenesis at the CRTD, the DFG Center for Regenerative Therapies Dresden, are using a Freedom EVO® liquid handling platform for cell culture maintenance.



Fixed tip pipetting from a six-well cell culture plate on the tilting rack

Researchers at the DFG Center for Regenerative Therapies, based in Dresden, Germany, are investigating adult neurogenesis – the production of new neurons in the adult brain. Dr Gerd Kempermann, Professor for Genomics of Regeneration at the CRTD, explained: "Small scale generation of new neurons from a population of resident stem cells in the adult brain occurs exclusively in two areas, the hippocampus and the olfactory bulb. We are investigating why these new neurons are in the hippocampus and how they contribute to hippocampal function. An intriguing aspect of this study is that adult neurogenesis is regulated by both physical and cognitive activity, but how does something as broad as physical exercise or cognitive stimuli affect hippocampal stem cells and stimulate the production of a new neuron? Activity-dependent control requires the convergence of very many regulatory factors, and is extremely complicated. To understand this complex regulation, the impact of the genetic background, and how different aspects of regulation target different aspects of development from the precursor cells, a variety of *in vitro* studies are necessary. Neurogenesis is a highly polygenic trait, and so we screen for specific genetic parameters in isolated precursor cells. This requires performing repeated experiments on precursor cells from the hippocampus with a standardized, reliable system for cell culturing, and this is where we take advantage of the Freedom EVO's liquid handling capabilities."

The CRTD is working with a Freedom EVO 150 workstation optimized for cell culturing, which is equipped with liquid handling (LiHa), robotic manipulator (RoMa) and Pick and Place (PnP) arms, two thermo carriers and a tilting carrier. The tilting carrier is an ideal add-on when working with different multiwell plates for cultivating cells, enabling a complete medium exchange. A StoreX™ STX 110-IC incubator, a Rotanta 46 RSC centrifuge and a Cellavista™ Analyzer are also integrated onto the system. Dr Kempermann's group is looking at adherent primary cell cultures,



The Freedom EVO workstation at the DFG Center for Regenerative Therapies Dresden

where cells grow on the surface of a culture plate. The advantage of adherent precursor cells is the homogeneity of the culture, allowing access to individual cells, rather than small clusters of cells. These clusters, known as neurospheres, tend to be very heterogeneous, so the cells have a variable access to growth factors depending on the size of the agglomerate and the cell's position within that structure. However, adherent cultures are more difficult to handle and require a lot of attention, since passaging takes quite some time. In order to grow enough cells for the assays, the cultivation of primary cells from the adult hippocampus requires an extremely consistent, standardized method that allows production of a large number of cells, in numerous wells, and achieves reliable, reproducible results.
Dr Kempermann commented: "This is hard to do manually, and may be error-prone, and this is where the Freedom EVO is really beneficial to our work".

He continued: "We have had a very good relationship with Tecan from the beginning, and the interaction with their development specialists has been extremely helpful. Our workstation is based on an off-the-shelf platform, but has been customized to meet our needs. One of the reasons for choosing the Freedom EVO was the integration of a Cellavista Analyzer. The Cellavista can assess the confluency in individual wells – cells are passaged at a confluency of about 80 % – and then decide whether or not that particular cell culture needs passaging, which is a major advantage."

Harvesting is especially crucial when cultivating neuronal cells. It is important to obtain literally all cells when trypsinization takes place, and this tedious procedure can only really be managed successfully by a well trained and experienced person. By automating the process, the Freedom EVO takes on the role of the skilled operator, eliminating the human errors that can arise with manual protocols. This frees the researcher to perform other tasks, and results to date are comparable with those achieved manually.

Dr Kempermann concluded: "The system is still evolving, and the protocols will be further fine-tuned. At the moment, this is small scale and not high throughput; it is really a preparatory stage to allow us to establish how this could work and how large we could actually grow using this kind of system. This automated method will make it easy to expand our work in the future, allowing us to scale up to whatever conditions and number of cells we require."

To find out more on Tecan's cell culture solutions, visit
www.tecan.com/cellbiology

To find out more about the DFG Center for Regenerative Therapies Dresden, visit
www.crt-dresden.de/research/crtd-core-groups/kempermann