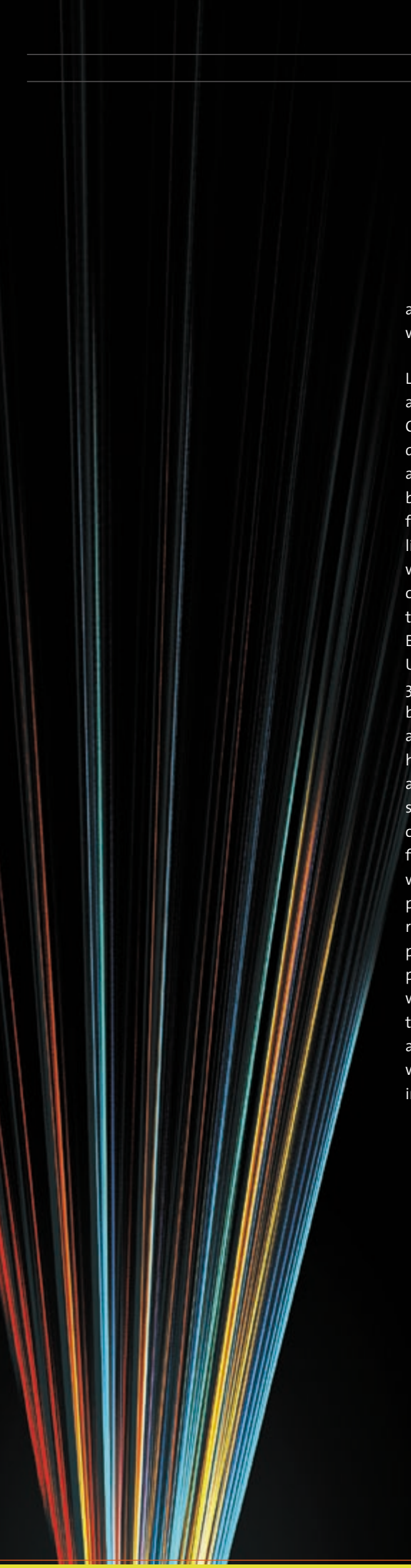
Signaling success

The Center for High Throughput Cell Biology, a brand new facility at Yale University, Connecticut, is combining automation with siRNA screening techniques to investigate signal transduction pathways. With the aim of 're-writing the book' the laboratory aims to use the high throughput capacity of its Freedom EVO® workstations to conduct genome-wide screens of primarily HUVEC cells to validate new and existing data.

Professor James E Rothman, recently returning to his alma mater as Chair of the Department of Cell biology at Yale University, has been instrumental in establishing the Center for High Throughput Cell Biology, a state-of-the-art facility situated at Yale's new research campus between West Haven and Orange, Connecticut. Professor Rothman is no stranger to high throughput screening programs, having previously been involved in the NIH Roadmap initiative as Director of the Genome Center of Columbia University in Manhattan, New York. The Center for High Throughput Cell Biology is a facility affiliated to the Department of Cell Biology, under the directorship of Dr Lars Branden – a former associate director of the Genome Center at Columbia – and is supporting its ambitious research program with provision of screening services for external laboratories.

Lars explained the aims of the Center's research: "The basic question we are trying to answer is 'How are signal transduction networks organized?' The intention is to identify the signal transduction network connections between the different signaling pathways, and the components within each pathway. Our approach differs from other projects in that we are primarily looking at one specific cell type, human umbilical vein endothelial cells (HUVEC) for our internal research. This is because many of these transduction pathways have been elucidated in universal terms, but the theoretical pathway often does not agree with experimental findings when you get down to specific cell types. We are using pooled HUVEC samples to ensure specific genetic variations do not skew our findings, and are hoping to take in all the information about signal transduction from existing databases and validate it for our experimental set-up."

"The primary technology we are using to identify the key regulatory entities within different signaling pathways is genome-wide screens with siRNAs. If we identify a particular gene we want to know more about, we can run it against all the signal transduction pathways we have established assays for. We have already developed over 150 high-content assays in-house and about 50 low-content assays. If we find a gene that perturbs a specific pathway, we then perform extensive secondary assays, 'biological profiling', to identify the more specific biological relevance of the gene in question. Combining these techniques to identify the key regulators of each pathway is our initial goal, however, this is really a stepping stone for trying to understand the connections between pathways. From there we can look at how this 'transcriptional network' varies between cell types. If you can understand these differences then this could help to explain why you get side effects from



a certain drug, or how paracrine signaling works in organogenesis."

Lars added: "A project of this scale requires a multi-disciplinary approach, so within the Center we have units responsible for assay development, high throughput screening and informatics. It is hard to separate the biological science from the technology for this project, as the two are intrinsically linked. The project would be impossible without automation, and Tecan really cares about what we are doing and listens to our needs. We brought one Freedom EVO platform with us from Columbia University, and this unit is dedicated to 384-well plate formats. Tecan is currently building three new platforms for us, two assay workstations and an immuno-histochemistry workstation, which will all be running 1,536-well plates. These systems will greatly improve our throughput compared with our previous set-up. When first designing our system four years ago, we integrated many functions into a single platform, and we have continued down that route with our new units. The Freedom EVO platforms are very flexible, offer amazing precision and, by using a modular approach, we can optimize the space available on the deck of the workstation. The plates are all in racks to simplify transfer between workstations; the whole point of automated integration is that it gives you total control and this cannot be achieved if the process requires too much human intervention."

"The new 1,536-format platforms should be able to run up to 80 plates in 24 hours and, if you consider that a genome-wide screen in quadruplicate only requires about 52 plates, this really demonstrates our potential capacity. Our new imaging workstation also has facilities to conduct both primary and secondary screening assays so, if we get a positive result, we can re-screen the well at high resolution and run secondary assays we have built into the system. This multiplex approach helps to minimize cost and increase efficiency, generating a lot of data very rapidly, and our biggest bottleneck will then most definitely be in data storage and data processing."

"Working closely with Tecan to design this system, we have realized that we would like to take our collaboration to the next level. Tecan has believed in this work from the beginning and, even before a proposal was put to us, there was a lot of joint work done to resolve potential problems. It has to be a two-way relationship and, from the very beginning, we have been developing some new ideas together. It's a lot easier to work with a company with whom you have established a good rapport and, with Tecan, we know that we will always get the support we need."