



Software Firms Tap Max Planck Lab to Aid in Development of Cell-Imaging Tools

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By Vivien Marx

Given the research trend toward translational medicine, two German image analysis software companies, Definiens and Transinsight, are seeking proximity to academic labs and publicly funded research ventures to expand their product capabilities and their potential circle of users.

In particular, biochemist Marino Zerial, director at the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden, is currently working with both firms on different cell analysis software projects: Transinsight recently began marketing a software package based on technology developed in Zerial's lab, while Definiens is collaborating with him in a systems biology research consortium called EndoSys.

Zerial studies the mechanisms of a cellular process called endocytosis — the uptake, transport, sorting, and processing of particles by cells. In particular, he studies endocytosis signaling pathways in liver cells. To do so he is also developing a range of tools, including high-content image analysis software.

"We have developed software in order to quantify the performance of the ability of the cells to take up nutrients or to process signaling information," Zerial told *BioInform* in an interview. "The software allows us to make predictions on the performance of the hepatocyte, just by looking at images."

Hepatocytes are of interest to cell biologists, said Zerial, because of the central functions they fulfill in the body to support the metabolism of proteins, lipids, and carbohydrates. They also play a role in diseases such as cirrhosis and liver cancer, and are of great significance to drug developers for whom drug metabolism is a crucial part of success or failure of a drug candidate, he said.

Zerial leads a project funded by the German Federal Ministry of Education and Research called EndoSys, which falls under a broader large-scale systems biology project focusing on hepatocytes called HepatoSys. The goal of HepatoSys is to develop quantitative models for a number of cellular processes in hepatocytes, while EndoSys is focused on creating a mathematical model to accurately describe the cellular mechanism of endocytosis and signaling in liver cells.

Experiments and Math Hand in Hand

EndoSys involves several of Zerial's colleagues at MPI-CBG as well as at research institutions in Hamburg, Leipzig, Heidelberg, and Bochum along with two industry partners: Definiens and Evotec Technologies.

The venture progresses in iterative steps: high-content image analysis from cell-based assays generates data that can help to refine the mathematical models of endocytosis and to help study signaling and its derailment in disease, which in turn can shine a light on pharmacokinetics.

Ultimately, linking cellular metabolism and clinical phenotypes could help to develop novel strategies to combat disease, according to Zerial. "Images tell us a lot. We have to educate ourselves to learn how to look at them, how to analyze them, how to extract the data from them," he said.

Zerial uses PerkinElmer's Opera high-content screening instruments, which can capture 100,000 images a day. To extract complex information from these images, he has been using various platforms, including software from Definiens, but said he felt the need to add to the toolbox with software he developed especially for the analysis of hepatocytes.

The live-cell imaging software, which he said was “completely programmed by us in Dresden,” is being commercialized by Transinsight. The company quietly launched the software in February under the name Kalaimoscope motiontracker Version 7.5.

While Definiens has “fantastic software,” there is a role for other platforms, too, Zerial said. In particular, Zerial and his colleagues aim to develop an in silico version of a hepatocyte that delivers spatial temporal information, telling scientists where reactions are taking place, when, and how fast they are occurring.

“You really want to see how the cell performs in four dimensions — that is really the vision,” said Zerial. “Of course, that will take a while.”

In the meantime, experimental scientists and programmers in his lab have been working together for the past five years or so to create the current version of the software, which can analyze around 60 parameters.

Yannis Kalaidzidis, the primary software developer in the lab, began collaborating with Zerial when he was a scientist at Moscow University and in 2004 he moved to a full-time researcher position at MPI-CBG.

Although there were some available open source tools for image analysis, Kalaidzidis said those tools would not be the right choice to deliver the quality he sought and they also would not be able to cope with high-content screening.

Endosomes are tricky to image, Kalaidzidis said, because they engulf particles, but they also act as sorting stations by “picking up cargo from the membrane, from the outside, then deciding to deliver the different cargo” to different places in the cell.

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“If you follow it for a few seconds it looks like random movement, without any order, [but] in reality if you average it over a longer time you will find out it is ... quite organized,” he said. What the software does, he said, is “fit intensity distribution, what we see in the image, with an analytical function.”

In addition, he noted that fluorescence microscopy images are far from perfect. “They are quite dirty in terms of being very noisy,” he said. This is “unfortunately inevitable” because increasing the laser intensity to make the image clearer bleaches the fluorescent marker and can damage cells under observation.

Changes from one image frame to the next also posed a challenge, even in faster microscopes when there is less time between two sequential frames. “Analyzing one frame to the next turned out to be difficult ... Because even by eye you can’t recognize which endosome is which, or which object is which object because they move too much,” he said.

Kalaidzidis said he found he could improve the quality by analyzing “four to five frames at once.” The dynamic-programming algorithm he developed essentially takes all the found objects in four sequential frames and then tries to find the best “who-is-who” assignment across those frames based on criteria such as fluorescence, size, position, speed, and directionality.

“A statistical model takes an estimation of what is the most probable connection between [the objects],” Kalaidzidis said.

In a recent genome-wide screening project, the software churned through 2.5 million 8-megabyte images and required on average 20,000 CPU hours per day. “We used two PC farms — one with 120 CPUs, another with 2,500 CPUs — plus a local cluster with 60 CPUs,” said Kalaidzidis. The project required 10 months of imaging and “roughly speaking the same time of calculation,” he said.

In order to better exploit parallel computing, Kalaidzidis said that he is developing a new version of the software that will run on Germany’s scientific computing grid, called D-Grid. That capability will be available in an upcoming release of the Kalaimoscope motiontracker software scheduled for September, he said.

Michael Alvers, CEO of Transinsight, said that approximately 15 groups have bought the software, including academic labs and biotech and pharmaceutical companies, though there is “more use in academia.” The company recently licensed Kalaimoscope motiontracker to the European Molecular Biology Laboratory for an undisclosed sum.

Transinsight also markets GoPubMed, an ontology-based literature search engine. One of its clients is Unilever, who is using GoPubMed for internal data-mining purposes, said Alvers.

GoPubMed sorts and filters PubMed results according to gene ontology and MeSH terms. The idea, Alvers said, is “to

market it to companies as an intranet search engine.” The firm collaborates closely with the Technical University of Dresden on the development of GoPubMed.

Text and Images Don’t Mesh

Transinsight actually markets the Kalaimoscope software through a separate business unit that is also called Kalaimoscope.

Alvers, who was formerly director of product marketing at Definiens before founding Transinsight in 2002, said the firm found that “it was impossible to clarify to investors or clients that a company could work in [both] text and images.”

“That is why I separated the two, for corporate identity reasons,” he said. If Kalaimoscope takes off, the plan is to form a company around the product but for now it is a business unit within Transinsight.

Transinsight and Definiens occupy partially the same market space, but Alvers said he doesn’t view Definiens as a direct competitor. “Our strength lies in sub-resolution particle tracking; we do not deal with large objects such as the cell or mitochondria ... which is why we don’t view ourselves as competitors.”

Not Pixels but Objects

Rene Hermes, vice president of marketing at Definiens, agreed that the two firms have carved out separate niches within the image-analysis software market. The main difference between Kalaimoscope and Definiens’ recently launched XD platform, said Hermes, is specialization. “We have a platform technology to develop applications and they have an application for one specific research field,” he said.

Definiens launched XD in June. The platform allows scientists to extract volumetric measurements in an automated fashion and compare images from different modalities such as *in vivo* and *in vitro* imaging, 2D and 3D images, and confocal microscopy images taken over time.

In another departure from Transinsight, the intended customers for the Definiens software are in biotech and pharmaceutical companies, as well as clinical and research organizations, said Hermes, adding that the company’s technology is deployed in 16 of the top 20 pharma companies.

Customers are capturing a wider variety of images, said Hermes, including 2D or 3D images that are acquired over time, leading to a “significant uptake in image acquisition.” but image analysis technologies fall short in analyzing those more complex structures, which is where XD comes in.

Like Transinsight, Definiens also partners with Zerial’s lab. As an image analysis partner in the EndoSys project, “we have developed our technologies to be able to analyze results from simulations, meaning alphanumeric data ... and with the same tool we are analyzing experimental images,” said Maria Athelgou, senior scientist at Definiens.

Participating in such research projects not only lays the groundwork for future products, said Hermes, but “gets us inside the latest and greatest in science [and shows us] how we can support that from a technology point of view.”

The parameters gained in these image analyses feed back into the research, and are used to fine-tune the project, Athelgou added. “Our software is a bridge [between] modeling, simulations, and experiments,” she said.

Jonathan Sutcliffe, director of product marketing at Definiens and co-developer of XD, explained that the software was designed “to make it easy to get image data into the system from any platform, to be able to include our automation into a processing workflow, and be able to take the results generated — whether they are tabular data, image data, or visualizations — into whatever analytics or reporting workflow there might be at the end of the process.”

At the platform’s core is Definiens Developer, he said, which “allows you to create a high-level description of the image analysis process using what the company calls “cognition network language.”

The company’s proprietary Cognition Network Technology is an object-based image analysis approach developed by physics Nobel Laureate and Definiens founder Gerd Binnig. It is not model- or pixel-based but rather “relates an object to its environment” said Hermes.

One feature that has been added to XD, said Athelgou, is a “performance booster” that combines object- and pixel-based analysis in order to accelerate image analysis.

The software is able to extract cells and components such as the nuclei or cytoplasm from images. “The problem with other software [products] is that they can find [an] agglomeration of pixels based on position and color intensity, but they cannot create objects,” said Athelgou. This ability, she said, enables the Definiens software to identify and extract cellular components such as nuclei and then quantify their morphology in relation to other objects in the cell.

XD is an important milestone for the company, explained Hermes, since it bridges many areas in translational medicine and biomedical research, such as high-content screening and cell based assays, tissue-based diagnostics, tissue-based drug discovery, and medical imaging, where the need for image analysis of complex images is intensifying. "Now we are rounding the edges, making sure the platform is open and can connect to all acquisition devices [in this space]."

Large pharmaceutical companies, he said, are working with many different image analysis systems and need integration. "There is no way to compare the results of one system with another," said Hermes. "We are able to cope with that proliferated landscape of point solutions by having a solution that companies can use in all situations in which images play a role."

XD allows image analysis results to be output in different ways. For example, it might be a 3D visualization, it might be several fused images from different modalities, or it can be a table of morphological details. "A radiologist might like to have a volumetric assessment of a lymph node ... [but] a cell biologist will want to have many more morphological parameters," Hermes said.

XD is based on a software modules that can be configured in different ways to build different solutions, said Sutcliffe. For example, "it has been designed to enable high-throughput, high content analysis [and to] correctly classify and quantify 3D objects," he said. "Because we create a 3D model it is very easy to measure the volume, to compare the change in volume over two different time points."

The software can be run on a laptop or can scale to a cluster as needed, Sutcliffe said.

Definiens believes its technology takes researchers deep into their images, which enables them to analyze complex goings-on in the appropriate biological context, such as relative morphology changes and movement. "How are the nuclei behaving, [or] particles or clusters of particles? [How is] the cytoplasm [behaving] in relation to the membrane or other components? All that is possible to analyze with our technology," Athelou said.

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