THE AUTHOR FILE

Stefan Florian

Tracking cell cycles *in vivo*, and the temptation of science fiction.

Tumor cells are bad citizens. Instead of maintaining the tissue community around them, tumor cells grow quickly and uncontrollably. This disorderly conduct and the cells' deregulated cell cycle have long been thought to be tumor cells' vulnerability, which led to the idea that killing rapidly proliferating cells is a strategy to target tumors. But that approach might not be enough, says cell biologist Stefan Florian, a postdoctoral fellow in Tim Mitchison's lab in Harvard Medical School's systems biology department.

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Stefan Florian

These concepts about cancer cells come mainly from data collected from two-dimensional (2D) lab dishes, "where cells proliferate like crazy," says Florian. This motivated him to learn more about tumors *in vivo*. "I was blown away," he says, recalling the first time he heard about imaging tumors *in vivo*. "It sounded like science fiction."

Florian and his colleagues have developed a way to systematically and quantitatively image thousands of cells in a xenograft tumor growing in a live mouse. They can assess the cell-cycle state in each of these cells, follow a tumor's reaction to drugs and track other dynamic changes. The method need not be limited to imaging tumors. Some users, says Florian, might assess cell-cycle state in 3D *ex vivo* cell culture systems or quantify cell-cycle state through developmental stages in a model organism.

The team used fluorescent ubiquitination-based cell-cycle indicators (FUCCIs) to distinguish between four cell-cycle phases of imaged cells. They combined this system with a reporter protein to identify whether cells were dividing and then used an image analysis approach to distinguish the cell-cycle phases. Tumors in the anesthetized mice were imaged in sessions a few hours long, repeatedly, over a week.

Looking under the microscope, in one glance, scientists might see a tumor region with rapidly dividing cells, an area with cells taking a time-out from the cell cycle, and another with dying cells. Researchers can now profile this heterogeneity by precisely characterizing the cells' shape and location as well as the cells' stage in the cell cycle.

Counting cells that are arranged three-dimensionally on a 2D computer screen is tedious and error prone, says Florian. Cancer cells are not as neatly ellipsoid and regularly spaced as cells typically are, which slows down researchers trying to quantitatively characterize what they see. That is what motivated the team to develop a way to automatically classify cell states in the mouse xenograft.

One exciting moment occurred after the experiments were done and the software developed by Florian's Harvard colleague Deepak Chittajallu delivered a spreadsheet of 38,000 lines of results from tumors in seven mice: the effects of three drugs on those tumors, all of which were imaged in numerous sessions. "There are many such moments in a project," says Florian. "This is what I find fun about science."

The paper is the result of an intense collaboration with colleagues whom he now calls friends at Harvard, Massachusetts General Hospital, the University of Texas Southwestern Medical Center and the University of Colorado Boulder. The work is also multidisciplinary, drawing on the fields of computer vision, cell biology and translational medicine. "It was a lucky series of coincidences to meet the right people at the right time—that happens rarely," says Florian. Good collaborations take supportive principal investigators who also leave everyone the right degree of freedom. The paper produced a coherent result that is, he says, "truly more than the sum of what each of us could have produced individually."

"It sounded like science fiction."

Florian enjoys listening to music or exploring big cities, and he spends his free time with his wife and young son. He is originally from Romania and moved to Austria as a boy. He has an MD degree from the Medical University of Vienna and a PhD from the University of Konstanz in Germany.

His PhD advisor, cell biologist Thomas Mayer, was especially impressed that Florian wanted to do research instead of joining a hospital's clinical staff or starting a private medical practice. Florian enjoys science as an intellectual challenge, says Mayer, and thinks through experiments and their possible outcomes.

Mayer recalls speaking with Florian about why cell-state results might differ between experiments with fixed and live cells. Florian disappeared into the library for a few days and emerged with a mathematical formula to model this phenomenon. Only then did he do the experiments. He wants to get to the bottom of things, says Mayer. Florian has discovered that being a researcher is the right choice for him and says, "I enjoy the tinkering process along the way."

Vivien Marx

Chittajallu, D. *et al. In vivo* cell-cycle profiling in xenograft tumors by quantitative intravital microscopy. *Nat. Methods* **12**, 577–585 (2015).