Case Study

The transformative impact of the ZEISS Celldiscoverer 7

London School of Hygiene & Tropical Medicine, UK

ZEISS

Seeing beyond

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The Mostowy Lab is a biosafety level 2 facility nestled inside the London School of Hygiene & Tropical Medicine. It is led by Professor Serge Mostowy, who was trained in the cell biology of infection at the Pasteur Institute in Paris. The lab is level 2 because its pioneering research involves the highly invasive bacteria *Shigella*, a pathogen closely related to *E. coli*. Work here focuses on how the cytoskeleton – a network of filaments and tubules within cells that helps them keep their shape and internal organization – responds to *Shigella* infection. *Shigella* is not only an important human infection, but also a paradigm in the field of cellular microbiology, so studying its infection process allows us to better understand host cell responses more widely.

Serge Mostowy, Professor of Cellular Microbiology

While the lab works with a variety of *Shigella* infection models, a particular focus is on tissue culture cells. During infection, *Shigella* is known to produce actin tails in the cytosol of cells in a bid to escape host cell immunity, says Mostowy. "Our research has discovered a different cytoskeleton component in the host cell, called the septin cytoskeleton, which forms cage-like structures to recognize and entrap the *Shigella* that are trying to produce actin tails. These septin cages prevent actin tail formation and target *Shigella* bacteria for destruction. It's a brand new way to think about host cell immunity."

While investigating this phenomenon at the bacterial level, Mostowy's team also wants to better understand the whole-animal consequences of *Shigella* infection. "For that we have chosen the zebrafish larva infection model, because its innate immune system is so close to that of humans – and it really lends itself to gorgeous microscopy."

That is fortuitous, because in 2019 the Mostowy Lab took delivery of a flagship ZEISS product, the Celldiscoverer 7 (CD7). An automated microscope designed for live cell imaging, the CD7 combines the easy-to-use automation of a boxed microscope with the image quality and flexibility of a top-of-the-line inverted research microscope.

Productivity boost

The level of smart automation of the CD7 system quickly had a profound effect not only on how science is done in the lab, but also on productivity, says Mostowy. Straight away, so much mundane work is dispensed with – the microscope automatically works out what sample carrier is being used and its dimensions, sets the correction collars on the objectives and adjusts the lens group positioning, so that the images are immediately the best possible quality.

So far, so useful, but it is the CD7's more advanced automation that is revolutionizing how science is done in the Mostowy Lab. An example is provided by Dr Margarida Gomes, a postdoc in the lab who uses the zebrafish larvae model to investigate the innate immune responses to *Shigella* infection, and whose work relies on imaging. "My experiments typically have three different conditions, and I need to image those fish at different time points quite close together – every two hours or so. Previously, I had to limit the number of fish per condition to five so that I could squeeze in all the stereo-microscope imaging I needed to do within that timeframe. Now, with the CD7, I have 12 to 24 fish per condition and the imaging is carried out automatically. A single experiment using the CD7 saves me weeks of work. It's amazing."

Mostowy tells a similar story: "Historically, we would put one to three zebrafish larvae under a canonical confocal microscope and, depending on the level of resolution, watch how leukocytes, namely macrophages and neutrophils, would respond to the bacterial infection process. But now, with the CD7's ability to process 96-well plates, we have the capacity to visualize the infection process in as many as 96 different individuals in real-time – that's a real luxury."

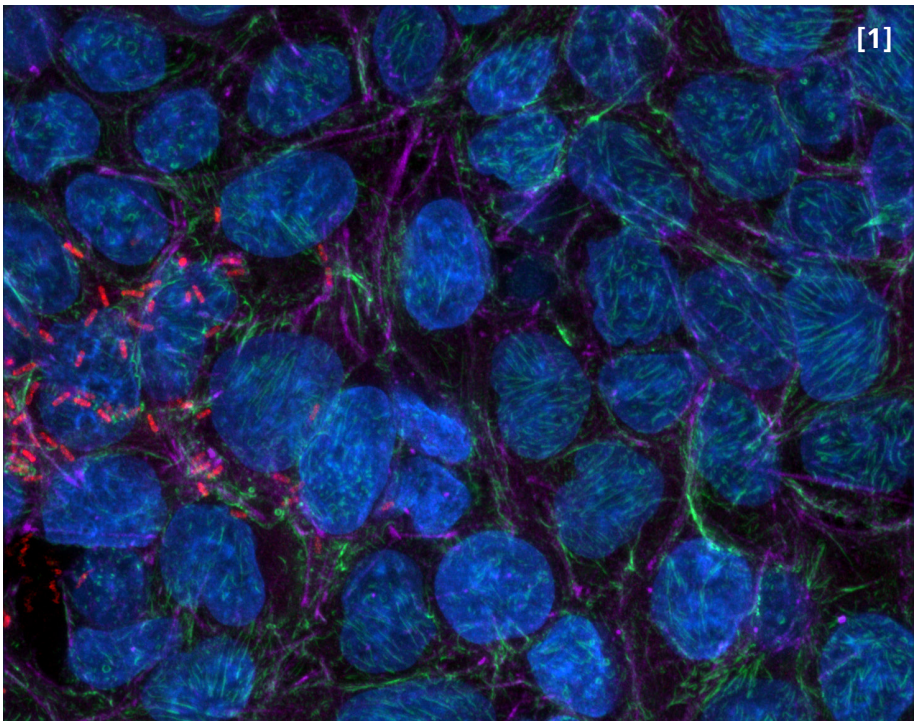
Another luxury is that throughput does not come at the cost of imaging power. "With plate-based imagers, there is always a compromise between speed of image acquisition – or the possibility of automated acquisition – and imaging power," says Dr Ana Teresa López Jiménez, a biochemist by training whose research at the Mostowy Lab aims to uncover new host and bacterial factors that are important for the formation of septin cages. "The CD7 doesn't compromise on that at all. The optical power is huge, particularly from its water-immersed lens. The capability to do super-resolution really pushes our work forward because many of these cellular structures have sub-micrometre volumes that we would not be able to resolve with any other type of plate imager."

Remote access

The automation enabled by the CD7 and its flexible software means that even a newcomer to the lab can put their plate in the machine, press a button, and get the answer they need. "Some scientists love microscopy, and some people just want an answer, and this machine caters to both crowds," says Mostowy. Another benefit of automation, he says, is that "you can start an experiment on a Friday afternoon and go home for the weekend knowing that by Monday morning the work will be completed". Then again, you would not have to take this on trust. Users can log in from home to check in real-time what is going on in the machine, make changes to their protocols and even analyse data on the fly. "It really is different to how science used to be," says Mostowy. When you are running such experiments with live organisms, potentially over several days, your samples need an optimal environment, says Gomes. "With the zebrafish, we do our *Shigella* experiments at 28.5°C – that is enough for us to see pathogenicity. By having a chamber with precise environmental and temperature control, the CD7 allows us to leave the plate in for longer without affecting the proper development of the embryo."

The easy access to a range of resolutions, known as multimodal imaging, is a key aspect of the CD7's exceptional functionality: it can switch from wide-field, to confocal and then employ Airyscan technology for super-resolution imaging, all at the "click of a button" within the software. This flexibility is particularly useful when dealing with entire organisms. "Zebrafish are relatively big animals, compared with a host cell, so it takes more time to image, but if we want to go from well to well, or even region to region within an animal, we don't necessarily need that information at the highest resolution. So as a first step we can screen with wide-field imaging," says Mostowy. "Then, upon recognition of an event of interest, we switch resolution to the confocal or the Airyscan to capture the details."

[1] HeLa cells infected with Shigella flexneri expressing mCherry at 3 hours post infection to investigate cytoskeleton rearrangements during intracellular infection eg. formation of septin cages, actin tails. The sample was stained with DAPI (blue, nuclei), anti-SEPT7 antibodies (green, septin filaments), and phalloidin (magenta, F-actin). Maximum Intensity Projection acquired using the Celldiscoverer 7 using the 50x water object and the 0.5x magnification optics. Sample courtesy of A. López Jiménez, London School of Hygiene and Tropical Medicine, UK.



Guided acquisition

But it is when the CD7's multimodal imaging functionality is paired with its guided acquisition workflow capability that the magic really starts to happen. "Guided-acquisition was a key reason we purchased this machine," says Mostowy. "We can simply put in the plate and, based on the parameters that we set, the microscope can automatically look for regions and events of interest – the formation of septin cages, for example."

The CD7 is paired with ZEN software, as all ZEISS microscopes are. Once the ZEN software has identified an event of interest, the microscope can dial up to its maximum resolution and acquire the best possible image of that infection event. "That ability to focus in on single events is something that CD7 uniquely offers, and then it can follow those events for days to help us understand the whole animal consequence," says Mostowy.

Guided acquisition would be of no use if the CD7 itself could not become an expert in what to look for. For that, Mostowy's team worked closely with the ZEISS Applications Specialists to train the microscope. "It had to understand how we recognize cells, infected cells, *Shigella* bacteria, actin tails and septin cages. Once the microscope could recognize these things, it could analyse and quantify our samples automatically," says Mostowy. "It took us time to fine-tune the system, but this customisation has transformed the way we do science across the entire lab because it's a much more robust, reproducible process. It standardizes quantifications across the whole team."

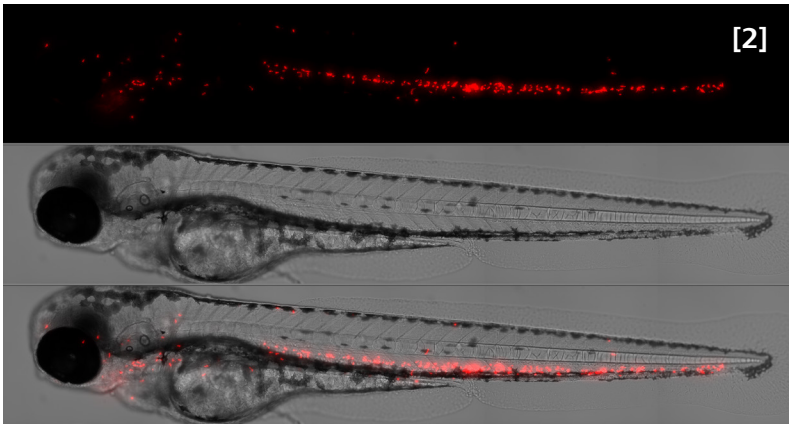
López Jiménez agrees: "Now, even a Master's student can just put a plate in and click 'Go' and they will acquire their data in an automated manner. It is a huge advantage."

Shopping around

Huge advantages in science generally require significant investment, particularly in relation to sophisticated pieces of hardware, so funders needed to be convinced, and Mostowy shopped around. "We did our due diligence. We knew we wanted a high-content imager and we tested a variety of different instruments with similar test tasks – for example, identifying the number of septin cages in a given sample," says Mostowy. "We tested instruments from some companies that were more relaxed on price but they really suffered in terms of resolution. It became rapidly clear that this was not good enough to capture the single bacterial events we were looking for."

Mostowy was initially attracted by the immediate results provided by some other machines. "It was tempting because I was getting answers on the day of the demo. But while it was satisfying that a number was coming out of these other machines, it actually wasn't at all what we knew to be the truth of our samples. You cannot advance science with those sorts of inaccuracies."

In the end, what tipped the scales for Mostowy towards ZEISS? "Look, we want to be five years ahead, to be the future of infection biology. In the end, if you care about image quality, there's really no choice to make. Also crucial was software flexibility – if you are willing to invest the time, you can discover a lot more with the ZEISS software."


[2]

The future of science

The CD7 had the unbeatable resolution Mostowy needed, and ZEISS Applications Specialists ready to work with him to develop algorithms and analysis software tailored to his lab's scientific questions. His trust in the machine came later. "When we first installed the CD7, we tested it with questions we knew the answers to. I had to build my faith in this machine, to be sure it was accurately quantifying things that I've been studying my whole life – and doing it reliably. After we built that trust, we dived into the discovery process," says Mostowy.

Indeed, using the CD7 has already led to significant advances. "We've discovered a new way to recognize a bacterial infection," says Mostowy. "Essentially, septins sense micron-scale curvature. For the first time, we now can envision that the host cell actually responds to biophysical properties of the invading bacterial cell: they can sense the curvature of an invading *Shigella* bacteria. Based on that shape of the cell, it recognizes it and targets it for destruction. That is a completely new way to think about host defense."

Ultimately, the discoveries made by focusing on septin-*Shigella* interactions could be applicable to a wide variety of broader infection processes. And if science can discover ways to encourage the formation of septin cages, it may even offer a new way to prevent bacterial infection.

"With the resolution and capacity of the CD7, we can now tackle questions that were impossible to answer even three or four years ago, because our scientific questions are so tightly linked to visualizing how bacteria are recognized," says Mostowy. "This microscope gives us so much more information than we have ever had access to, it is teaching us things we didn't even know we had questions about. It has transformed the way we do science in the lab."

[2] Zebrafish line Tg(lyz::dsRed). Observing emergency granulopoiesis in a 4 day post-fertilisation zebrafish embryo. The innate immune system induces this response to replenish the neutrophil (dsRed) population 48 hours post infection with a non-lethal dose of Shigella flexneri. Maximum Intensity Projection acquired using the Celldiscoverer 7 in widefield mode. Sample courtesy of M. Gomez, London School of Hygiene and Tropical Medicine, UK.

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