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Holographic Imaging Phase

Profile Feature as seen in *Nature* 4th February 2016

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profile feature

Peter Egelberg, *co-founder and CEO of Phase Holographic Imaging, Lund, Sweden*

The English polymath Robert Hooke is thought to be the first person to have seen individual cells. In his 1665 book Micrographia he described using his early microscope and illumination system to observe cork cell walls. 350 years later, microscopy is still the most important tool available to many scientists. Cell biology and many branches of medical research would be impossible without it.

Yet for the countless fundamental advances it has enabled, microscopy has some major limitations. Scientists still have to manually count cells, and many still study cell changes by looking at a handful of snapshots taken hours apart. Methods used to make cells visible can leave them dead or dying, or introduce other changes that can make quantifying results difficult.

Frustrated by some of the drawbacks of traditional microscopy, Peter Egelberg developed a new imaging tool combining digital image sensors and holography to generate 3D images. Here he explains why he believes digital holographic cytometry is set to transform cell biology, cancer research and drug discovery.

Q: What first led you to develop a new form of microscopy?

Whilst developing computer vision technology to analyse particles in powdered pharmaceuticals in the 1990s, I encountered some of the problems associated with traditional microscopy: a lack of ability to monitor cells over time, the staining which may alter their function, and the frustratingly narrow focal depths involved. This got me thinking about holography. Traditionally it involves recording a hologram on a photographic plate, developing in a wet lab and illuminating it with a laser to produce a 3D image. This made it very impractical, so in 2000 I went to see my old physics professors at Lund University to ask whether the image sensors being used in digital cameras at that time were of high enough resolution for digital holographic microscopy. They said it may have been possible, so we began a feasibility study.

Q: How does a digital holographic imaging cytometry work?

Rather than using a lens to create the image, a digital holographic set up uses an image sensor to capture the information coming from an object, and a computer to turn the information into an image. When we're observing cells that are denser than the surrounding fluid, we can generate a representation based on the relative speed of the light that passes through them. We have one laser beam that passes through the cells, and another reference beam. The two are combined, which generates interference similar to what we see when wave fronts collide in the ocean. The hologram is this interference pattern – the computer uses it to generate a quantitative image which is then used to automatically analyse samples.

> "We're offering the ability to watch the game live."

Q: What are the technology's main advantages?

Many scientists are surprised when they first learn that the stains traditionally used to make cells visible under microscopes are toxic. This means scientists are frequently studying dead or dying cells, making their results potentially unreliable. Traditional phase contrast microscopy can remove the need for staining but it also alters the visible image in a way that makes automated analysis very difficult.

Holographic imaging cytometry analyses intact cells, automatically counts them, precisely measures cell thickness and volume, and tracks them over time. Many of the 120,000 labs performing cell-based assays worldwide are trying to get a picture of last night's game by looking at a few still images. We're offering cell biologists the ability to watch the game live.

Automation and quantification are fundamentally important features for setting up reproducible cell-based experiments. We enable quantification of cell death, migration, division and interaction effortlessly, to provide scientists with this reproducible data.

We're no longer limited by the very narrow focal depth of traditional microscopy, as the computer creates a stack of images and automatically selects the one in best focus. Our latest model can be installed inside cell incubators, meaning samples no longer have to be disturbed to make observations.

Q: Can you provide a specific example of the technology's benefits?

Scientists at Northeastern University in Boston, US, are using HoloMonitor to develop new methods of nano-scale drug delivery to target tumor cells without destroying non-tumor bystanders. This will have a direct effect on the medical industry – one of the problems in drug delivery research is the inability to accurately study how drugs interact with live cells. By using our technology, these researchers have shown they can do just that.

Q: What stage of development is your technology at?

The latest model – the HoloMonitor M4 – was launched in 2014 and is currently used by academic research institutions and pharmaceutical companies worldwide. We are also continuing our development of the HoloMonitor to carry out high content live-cell analysis in the pharmaceutical industry. Our focus will remain in label-free long-term quantitative cellular analysis, which will provide researchers with a wealth of data from live samples. However, researchers can complement our label-free cell analysis with fluorescently stained samples for improved molecular specificity.

Q: What is the ultimate potential of the technology?

Cancer research is currently the single greatest use but there are many additional applications in inflammatory and autoimmune disease, stem cell biology, gene therapy, regenerative medicine and toxicology.

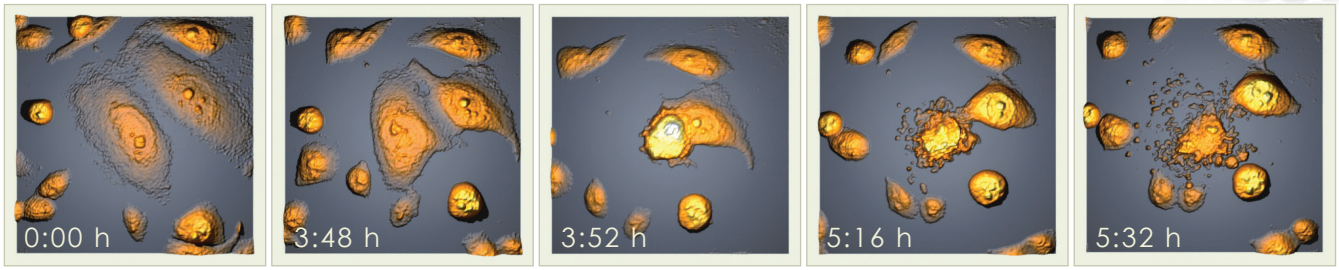
Scientists are always looking for new tools that provide better and more complete information to make decisive advances, and time-lapse holographic imaging cytometry gives researchers affordable and easy access to unprecedented quantitative data and analysis on cellular populations in their native environments.

The amount of quantitative information in a time-lapse movie of a developing cell culture is phenomenal. We have only just begun to create tools that make this kinetic information available to cell biologists. I believe these new tools will revolutionize cell biology and drug discovery.

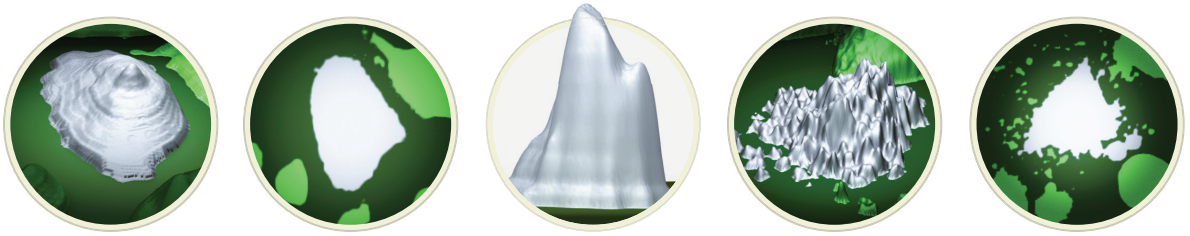
HoloMonitor® M4

HOLOGRAPHIC TIME-LAPSE IMAGING CYTOMETRY

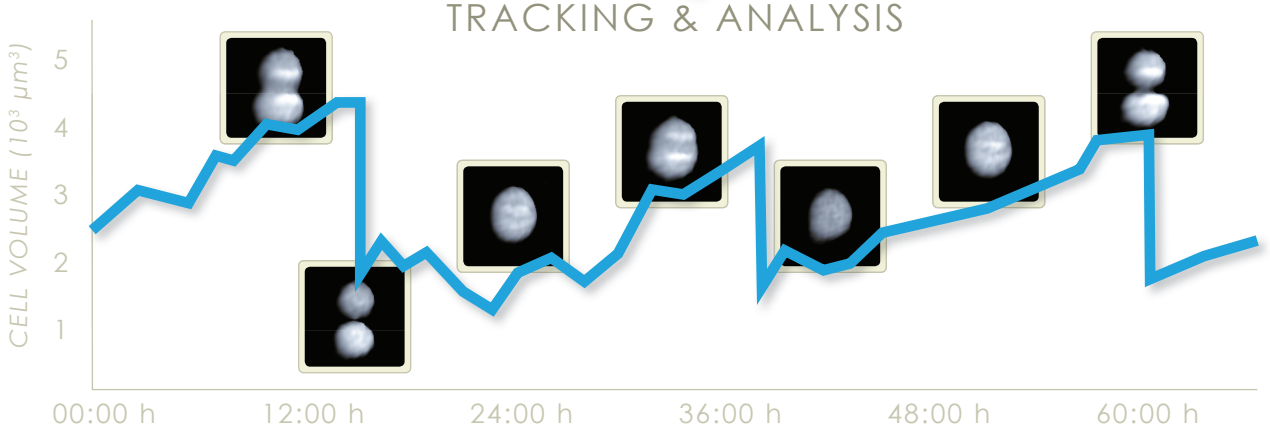
LONG-TERM LIVE-CELL IMAGING



LABEL-FREE QUANTIFICATION



TRACKING & ANALYSIS



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