The evolution of Phase Holographic Imaging from a research idea to publicly traded company

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ABSTRACT

Recognizing the value and unmet need for label-free kinetic cell analysis, Phase Holograhic Imaging defines its market segment as automated, easy to use and affordable time-lapse cytometry. The process of developing new technology, meeting customer expectations, sources of corporate funding and R&D adjustments prompted by field experience will be reviewed. Additionally, it is discussed how relevant biological information can be extracted from a sequence of quantitative phase images, with negligible user assistance and parameter tweaking, to simultaneously provide cell culture characteristics such as cell growth rate, viability, division rate, mitosis duration, phagocytosis rate, migration, motility and cell-cell adherence without requiring any artificial cell manipulation.

Keywords: Quantitative phase microscopy, label-free time-lapse cytometry, kinetic cell analysis, epithelial-mesenchymal transition.

1. INTRODUCTION

Advanced live cell imaging microscopes are available. But, the quantitative capabilities of these microscopes rely on that cells are either genetically modified or “labeled” with toxic fluorescent stains, affecting cell behavior. These and other practical limitations have resulted in that scientists at large study the dynamic behavior of living cells by interpreting a few snapshot images.[1]

We realize how difficult and misleading it can be to comprehend life from snapshots when we look at the few pictures our grandparents left behind. Obviously, our understanding of their lives would have been dramatically more true and complete, if we instead had the opportunity to watch a movie of their entire lives, secretly and non-invasively recorded to not affect their behavior. A similar leap of understanding is what label-free time-lapse cytometry offers to medical science.

This is what motivates us at Phase Holographic Imaging to commercialize time-lapse cytometry based on quantitative phase microscopy (QPM), which is increasingly being used to non-invasively study and quantify the dynamics of individual cells in a population of cultured cells.[2][3][4][5]

2. THE ROLE OF COMMERCIALIZATION

New technology will first have a significant scientific impact when it has developed into an established technology that is accessible, affordable, easy to use and well-known to most scientists who may benefit from the technology. The process of transforming a proof of concept prototype into a product with these qualities is the often unappreciated, but crucial, role of commercializing companies.

Commercializing innovative technology can be extremely rewarding. But, it demands hard work, patience and loads of persistence. We often perceive it as established technologies were instantly embraced when introduced. This is, however, an illusion. In most cases we first become aware of a new technology when it is becoming well-known, creating a false impression of immediate success. When in fact, the technology has matured in obscurity for years before that.

A related example is the predecessor of QPM, the phase contrast microscope. The first prototype of this revolutionizing microscope was in the early 1930s built and demonstrated to the leading microscopy company in the world, Carl Zeiss, by Frits Zernike. Zeiss immediately rejected the new technology as useless: “If this had any practical value, we would ourselves have invented it long ago”.[6] Zeiss did eventually commercialize the phase contrast microscope, ten years later in the 1940s. However, it took an additional ten years for phase contrast microscopy to become universally recognized, in the 1950s.[7] In 1953, Zernike was awarded the Nobel Prize for his invention.

Incidentally or not, the recognition of phase contrast microscopy occurred in parallel with the development of cell culturing techniques in the 1940s and 50s.[8] Since then, the phase contrast microscope has become an indispensable tool for cell biologists.

3. ADAPTING TO A HARSH ENVIRONMENT

Cells are cultured in a cell incubator over several days. To assess cell health before or during an experiment, the culture is still typically removed from the incubator once a day and visually inspected using a conventional phase contrast microscope. Obviously, this process, which was established when cell culturing was developed, could be made more efficient by placing the microscope inside the incubator. Furthermore, cell behavior could be monitored and quantified continuously during the course of the experiment by video recording the cells.

To achieve this the microscope must be adapted to the environment inside the incubator. Mammalian cells are cultured in nearly 100 % humidity and at 37° C. This environment not only maximizes cell growth; it also promotes corrosion and the growth of microorganisms on both the outside and the inside of the microscope. Moreover, heat dissipation from the microscope must be kept very low to not induce heat shock responses by raising the temperature of the cells significantly above 37° C. These circumstances make it very challenging to place a microscope inside a cell incubator,[1] especially when considering that the microscope is expected to operate flawlessly for at least a year without being serviced.

4. A MICROSCOPE BECOMES A TIME-LAPSE CYTOMETER

When we began development in 2000 at Lund University in Sweden, we knew nothing about cells or cell culturing, and hardly anything about phase contrast microscopy. Our initial goal was to design a lens-less holographic microscope for particle analysis in the pharmaceutical industry[9], attracted by the fact that a holographic microscope can be built inexpensively from optical and semi-conductor components found in consumer products.

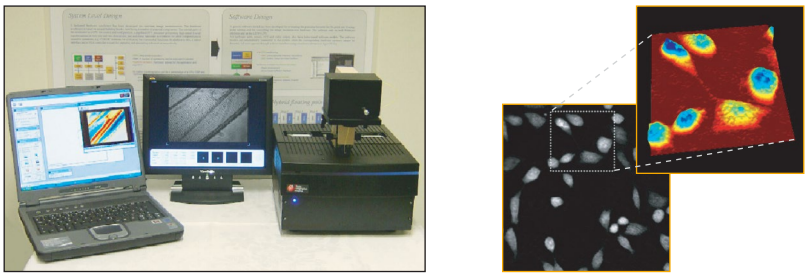


Figure 1: HoloMonitor M1 (left). Mouse fibroblast cells imaged by HoloMonitor M1 (right).

4.1 Proof of concept – HoloMonitor M1

It was not until around 2005, when introduced to cell biology, that we truly began to appreciate the ability of holographic microscopy to create quantitative phase images, and the benefits this new imaging modality brings to medical science in particular. This gave us a clear business idea that allowed us to receive the funding to build our proof of concept prototype HoloMonitor M1 (Figure 1) and establish Phase Holographic Imaging as a company. With the M1 we showed that is was possible to image and analyze unstained cultured cells. But, as so many before us have realized when commercializing new technology, coming up with the idea and building the first proof of concept prototype is the easy part.

4.2 The challenge

When a technology is established, customer and supplier expectations match. A car customer knows what to expect of a car; he knows it cannot fly like an airplane, needs to be repeatedly filled with costly fuel and that a license is required to use it. Reversely, a car supplier expects a customer not to expect that a car can fly, run on air and that it can be driven legally on public roads without a license. However, when technology is introduced, it is not yet obvious how the new technology is best used and what the technology requires of the user. This initial lack of mutual understanding between customer and provider results in a significant difference between customer expectations and what the new technology provider expects and perceives the customer expectations to be, or not to be (Figure 2). The challenge of commercializing new technology is to reach sufficient sales by eliminating the difference in expectations, before available funding is exhausted.

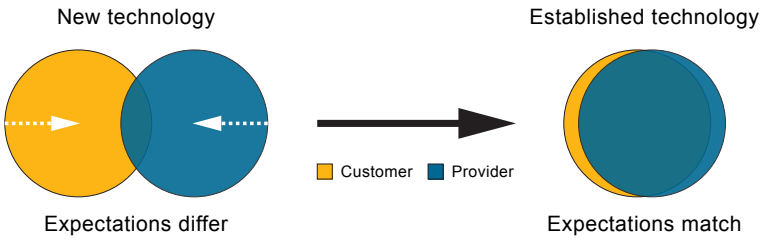


Figure 2: Initially, customers and the new technology provider have different expectations. As the technology is gradually adapted to customer expectations and as customers adjust their expectations with improved knowledge of the capabilities and limitations of the technology, the difference in expectations is reduced to eventually match when the technology is established.

4.3 Evaluation – HoloMonitor M2

After receiving additional funding in 2007, we began developing our evaluation version. HoloMonitor M2 was a modified Nikon phase contrast microscope with a holographic microscopy module attached to it, making it possible to both acquire quantitative phase images and conventional phase contrast images. Six M2 units where installed at Lund and Malmö University for evaluation.

4.4 Market launch – HoloMonitor M3

From these evaluation units, we learned that customers certainly appreciated the new cell analysis capabilities. However, the software and the mechanics were much too complicated to use. After successfully attracting a third venture capital investor, we set out to completely redesign both the hardware and the software to improve usability. HoloMonitor M3 was launched internationally in 2011.

4.5 Profitability – HoloMonitor M4

Eager to prove that they have made a solid investment, investors push to rapidly achieve the first sales. As the technology is new, engineering experience of the technology is limited, and many unknowns remain. The result of this conflicting situation is that a less risky, but more expensive, product design is selected. The resulting profitability issue is conveniently solved for the moment by raising the price of the product. The M3 was no exception to this. To solve the profitability issue properly, we began developing HoloMonitor M4 to dramatically reduce the manufacturing cost, but also to further meet customer expectations. By the time we had completed the first prototype of HoloMonitor M4, early stage venture capitalist funding had more or less ceased to exist in Sweden. The only viable option for us was to become publicly traded in 2014 on one of the two so called trading platforms that very successfully specialize in providing capital to startup companies. At first we were skeptical, but in all aspects it has proved to be a much better option than conventional venture capitalist funding, which in Sweden still has not recovered from the financial crisis in 2008.

4.6 Label-free time-lapse cytometer

Our efforts to meet customer expectations by improving usability, reliability and affordability, while securing profitability, have resulted in that HoloMonitor has evolved from a holographic microscope to a holographic time-lapse cytometer (Figure 3), with the following key attributes:

- Non-invasively records multiple time-lapse videos of cell cultures inside a cell incubator
- Provides software to visualize, quantify and analyze the long-term kinetic behavior of individual cells, without requiring any artificial cell manipulation
- Affordable to any cell laboratory

Figure 3: HoloMonitor® M4 time-lapse cytometer with a motorized stage operating inside a cell incubator. To avoid condensation and surface vibrations a special lid was developed, which is partly immersed into the cell culture media (insert). Approximately 100 HoloMonitor units are currently in operation in cell laboratories around the world.

5. THE BENEFIT OF QPM

The full adoption of image processing methods for cell analysis is in general held back by the fact that users are required to optimize multiple processing parameters to ensure high-quality output.[10][11] It is difficult to understand and optimize these often unintuitive parameters, which can be as many as half a dozen or more. The lack of understanding results in a considerable amount of parameter tweaking by the user and an unfortunate perception that the obtained measurements are less reliable. In many cases, this perception is, however, unfounded as the image processing errors are random and generally cancel out.

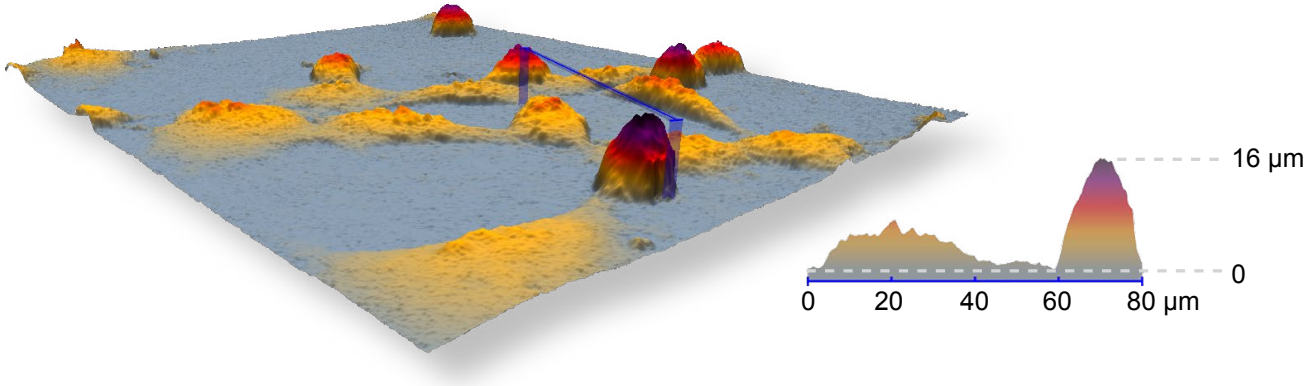


Figure 4: Pseudo colored QPM image of cultured cells, created by HoloMonitor M4.

Nevertheless, QPM images of mono-layered cells can be meaningfully interpreted as topography maps (Figure 4), where the peaks identify the position and the maximum optical thickness of each cell in the image. By reducing the problem to identifying local maxima peaks, we have successfully been able to limit the number of imaging processing parameters to a couple of intuitive processing parameters, such as minimum cell size and thickness, allowing image processing with negligible user assistance and parameter tweaking.[12]

A surprising amount of relevant biological information can be obtained from just the number of cells, cell positions in space and time and cell thickness. Characteristics like cell growth rate, viability, division rate, mitosis duration, phagocytosis rate, migration, motility and cell-cell adherence can all be simultaneously quantified using these simple parameters. These characteristics can be combined to characterize more complex characteristics, for example the aggressiveness of cancer cells.

5.1 Epithelial–mesenchymal transition

Over 80 % of all cancers originate from epithelial cells.[13] Epithelial cells are skin cells and other cells that cover or line organs. Epithelial cancer cells create metastases by penetrating and spreading through the circulatory system. To become invasive, the normally non-invasive epithelial cancer cells transform through a process known as epithelial-mesenchymal transition (EMT). After passing through the circulatory system, the invasive cancer cells revert to their initial non-invasive state through the reverse process, mesenchymal-epithelial transition (MET), to form metastatic tumors (Figure 5).

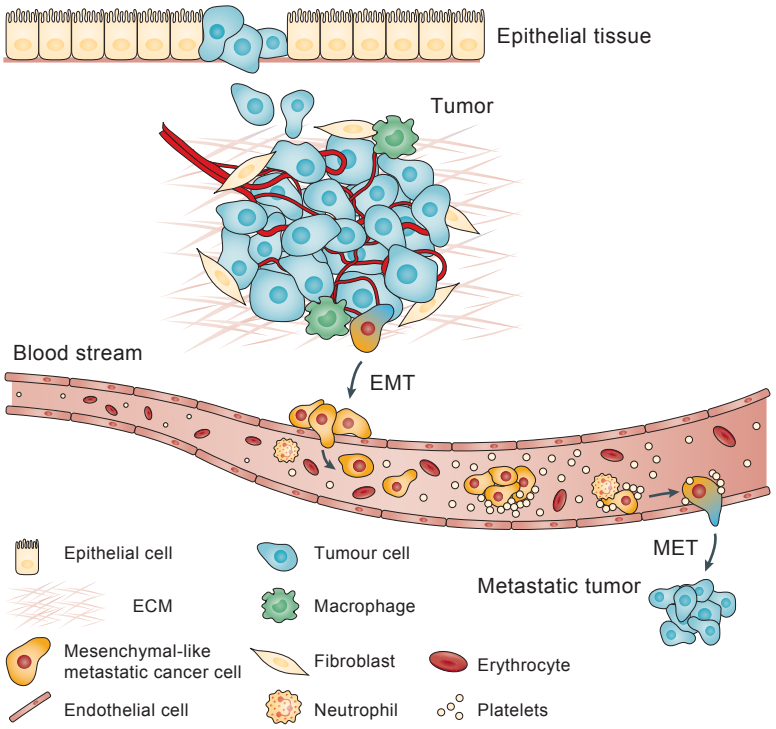
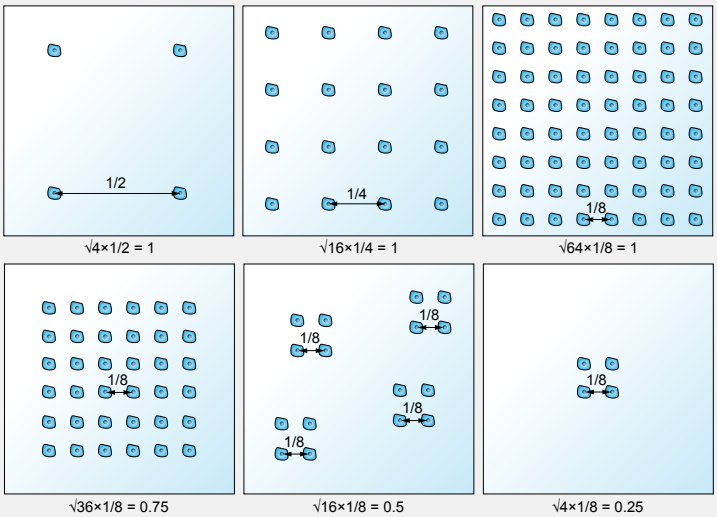


Figure 5: Epithelial–mesenchymal transition (EMT) and its reverse, mesenchymal-epithelial transition (MET), are the processes that allow cancer cells to spread through the circulatory system to form metastases.

Box 1: Quantifying cell motility and cell diffusion

- All cell positions (local maxima) are identified in a sequence of N+1 QPM images, separated in time with time t.
- t is chosen in such a way that the fastest moving cells do not move more than approximately a cell radius between subsequent images.
- N is empirically chosen in such a way that mesenchymal-like cells are expected to move a significant distance over the time-period (N+1) × t.
- M is the number of cell positions in images i=1 through N.
- m_i is the number of cell positions in image i.
- ***Cell motility*** is the average *instantaneous speed* of all M cell positions, where the *instantaneous speed* of a cell position in image i is the distance to the nearest cell position in image i+1, divided by t.
- ***Cell diffusion*** is the average *cell diffusion per image* of the N images, where *cell diffusion per image* is the square root of m_i multiplied with the average *nearest neighbor distance* of the m_i cell positions in the image, as exemplified. *Nearest neighbor distance* is the distance to the nearest neighboring cell position in the same image i.



Examples of cell diffusion per image values. Cell diffusion remains constant when the average nearest neighbor distance decreases due to a greater number of cells (top row). Cell diffusion decreases with the number of cells, when the average nearest neighbor distance remains constant (bottom row). The shown average nearest neighbor distance is expressed in fractions of the image width.

5.2 Quantifying EMT and MET

EMT and MET are a spectrum of biological changes and transitions. Quantifying the extent of EMT can be challenging, as the expression of markers often depend on the cell type and initiating signaling pathway.[14] However, a hallmark of EMT and the ability to form metastases is increased cell motility and reduced cell-cell adherence (Figure 6).[15][16][17]

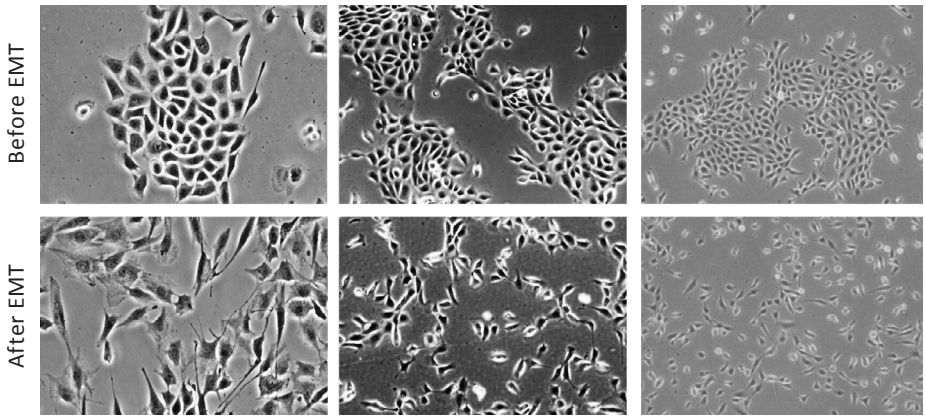


Figure 6: Images of cultured epithelial cells before and after EMT. Untransformed epithelial cells form cell clusters (top row). Transformed cells are solitary and motile, and do not form clusters (bottom row).[15]

An easy to use method for determining the extent of EMT based on quantifying cell motility and cell-cell adherence in a sequence of QPM images is proposed as outlined in Box 1. In a graph showing cell motility vs. cell diffusion, i.e. the opposite of cell-cell adherence, a sample of epithelial-like cells will travel from the lower left to the upper right corner, when an increasing number of cells complete the EMT process. A sample of mesenchymal-like cells will travel in the opposite direction, when cells complete the reverse MET process (Figure 7).

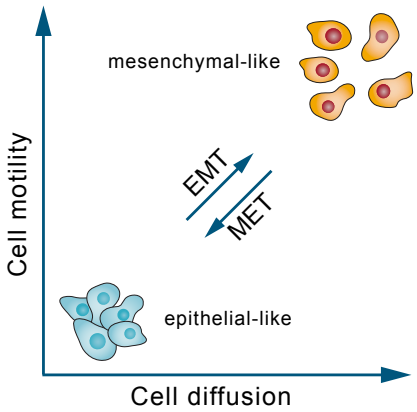


Figure 7: Illustration of how a sample of cells travels diagonally in a graph showing cell motility vs. cell diffusion, as an increasing number of cells complete the EMT/MET process.

As the method neither requires toxic labels nor genetic modification, it offers the novel ability to automatically monitor the progression of EMT and MET, when exploring ways to control and understand these complex processes that are responsible for the deaths of so many cancer patients.

Providing new affordable easy to use scientific tools that help cancer researches understand the enormous complexities of cancer is what Phase Holographic Imaging is all about. Without such new tools, it is unlikely that we will ever untangle the evolutionary mess nature has created over billions of years, to understand the causes of cancer.

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