Single cells or large populations?

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History

It is evident that research and development on cells and single cell analysis on chip has increased dramatically over the past few years. Fig. 1 shows the increase of publications in this area over the last seven years. In 2000 we saw the first few publications with single cells on a chip and in 2006 there were as many as 116. The most cited publications with respect to cells on chip deal mostly with soft lithography for cells.[1–3] At the last MicroTAS conference about 75 extended abstracts (out of a total of about 550) had 'cells' in the keywords, and 21 had 'single cells'. In addition, there have been a few recent additions to the literature reviewing single cells.[4–6] In the commercial sector also, a few single cell analysis tools were recently introduced.[7] One example is a slide-based tool for real-time study of individual, living cells, adhering and non-adhering, within heterogeneous populations. This technology was developed at Bar Ilan University in Israel.[8] There are also several single cell conferences and workshops available (for example the 4th Münster Conference on Single Cell and Molecule Technologies 2007 and the 2nd International Workshop on Approaches to Single-Cell Analysis, Tokyo, Japan).

Why is single cell analysis of interest?

Today life-science researchers often perform bulk techniques because they are simple, available and well established. However, cells under seemingly identical environmental conditions often display a distribution of heterogeneous behavior due to, for example, lack of synchronization among cells, which can not be detected with bulk techniques. An example of how bulk experiments on protein levels among cells could be misleading has been illustrated in ref. 5. Nevertheless, unlike conventional methods, single cell analysis avoids the loss of information associated with ensemble averaging. High throughput single cell analysis offers the possibility of analyzing a large quantity of individual cells (providing some statistical information) and detecting the distribution of responses. Of course, this is very attractive to the cell biologist!

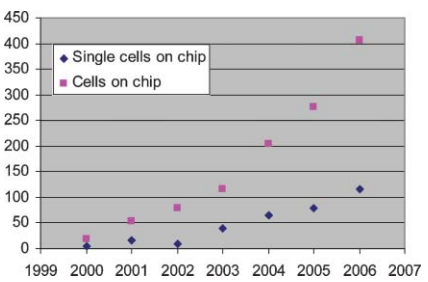

Fig. 1 Graph showing the increase of publications on cells and single cell analysis on chip over the past seven years.

Large quantity…

So what is a *large* quantity of cells? This is an important measure to put numbers on, since it is difficult to enable single cell analysis on chip for millions of individual cells. Today, millions of cells are normally used in bulk methods and most often this is because the cells are readily available: there is no reason to use less. When using primary cells there are normally fewer cells available but we can still be talking about several hundred thousands of cells. But if the biologist really had to use fewer cells in exchange for more detailed data what would be the result, and what numbers would we be talking about? The general consensus among clinicians and biologists does seem to be that, for a proper answer, they would need to have single cell techniques available, because today nobody knows how heterogeneous different cell populations are, and what new techniques could offer. This leads us to a "chicken and egg" problem: as long as biologists don't have the appropriate tools they cannot tell whether these could be useful. It also depends on what kind of cell sample you are looking at: a cancer cell population is much more heterogeneous than a "normal" cell population. In order to have statistical relevance, typically at least a thousand cells are needed, but this also depends on the type of analysis required. Another advantage of looking at individual cells is that cell–cell and cell–surface interactions can be studied in detail. The con is that most cells need interactions with surfaces and neighboring cells to function properly, and will exhibit deviating behavior when these interactions are lacking. Nevertheless the general conclusion is still that additional single cell analysis methods would be very welcome

So why have cell biologists avoided single cells until now?

Well this is not entirely true—there are more than 80 000 publications (on PubMed) on flow cytometry where indeed it is the single cell that is analyzed in large quantities. However, cytometry gives us "instant" information about the average properties of cells, revealing how the group of cells changes, but does not provide time-dependent information about single cells. Also, cytometry offers no options on handling and manipulation of single cells. The detection level, although strongly dependent on the parameter to be measured, is in the order of about 1000 molecules per cell, which is another limitation when studying low abundant (copy number) molecules. Unfortunately, apart from flow cytometry, there are practically no other tools available today for cell biologists to perform single cell handling or analysis. Nevertheless, people in the cell biology field are interested in gaining more information about the single cell (in large quantities) to study for example, the cell cycle, heterogeneous populations, cell–cell communication, drug screening, differentiation, electroporation *etc*. The general consensus appears to be that you must be able to look at a large population of cells to get statistically relevant data and avoid interpreting individual, cell-specific properties as generic features of the cell population.

What has changed recently to make single cell analysis on chip "emerging"?

Lately, unprecedented possibilities of performing single cell analysis have been presented by using micro- and nano-technologies and microfluidics. A long list can be made of general microfluidics techniques that are applicable for single cell handling and analysis. Several new, sensitive techniques have been developed, for example, counting low copy number proteins,[9] micro-PCR that enables DNA analysis of single cells,[10] monitoring protein expression,[11,12] many different single cell manipulation techniques,[4] electroporation platforms,[13,14] mRNA analysis of single cells as shown in Fig. 2,[15] single cell culture (an example is shown in Fig. 3),[16,17] chips enabling gradient studies[18,19] and patch clamp systems.[20] In addition, imaging techniques have improved enabling time-lapse imaging to visualize non-synchronized processes in cell development, including the use of non-bleaching quantum dot labeling.[21–23] Another new technique that is very promising for even more detailed resolution of multiple intracellular phenomena is SERS nanoparticles (that contain a variety of "identifying" organic molecules each having their own spectrum, enabling a practically unlimited number of markers to be used simultaneously).[24]

Conclusion…

Given the sudden and rapid growth of new techniques for single cell experimentation we believe it is time for life-science researchers to extend their research paradigm of Petri-dishes and large cell-populations. Information about the behavior of individually treated cells, elucidation of (intra)cellular processes usually hidden in large populations, cell-cycle influences and study of cell communication as well as cell–surface interactions are all examples of phenomena that give additional information at individual cell level. No doubt the standard Petri-dish/culture flasks will remain the (major) research tool for the next decade(s); however, in addition to this, the single cell definitely deserves serious consideration, and we are convinced that single cell experimentation

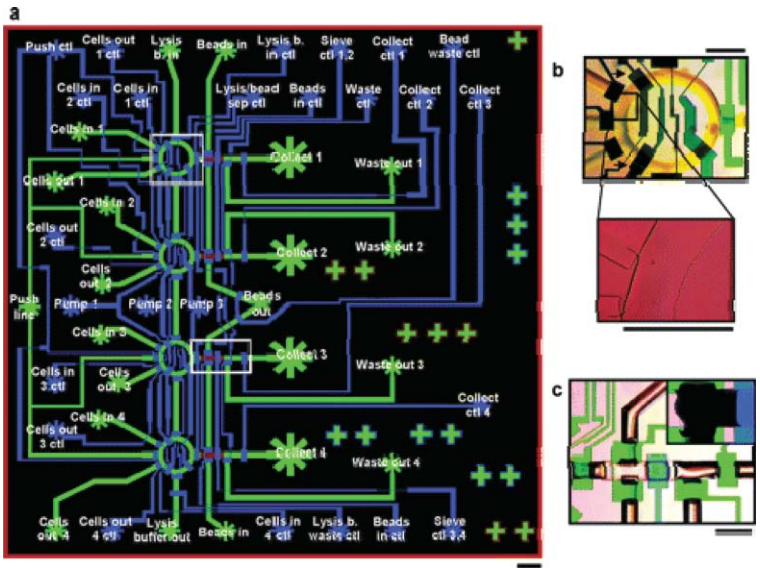

Fig. 2 4plex mRNA isolation/first strand synthesis device. (a) AutoCAD drawing of a device with inputs and outputs labeled according to function. Rounded flow channels are depicted in green and control channels are shown in blue. Unrounded (rectangular profile) flow channels for affinity column construction are shown in red. The portions of the drawing in white boxes are shown in (b) and (c), respectively. (b) Optical micrographs of the lysis ring and an NIH/3T3 cell captured in the ring. (c) Optical micrographs of the affinity column construction area and a stacked column. Scale bars are 400 μm.[15] Reprinted with permission from ref. 15. Copyright 2006 American Chemical Society.

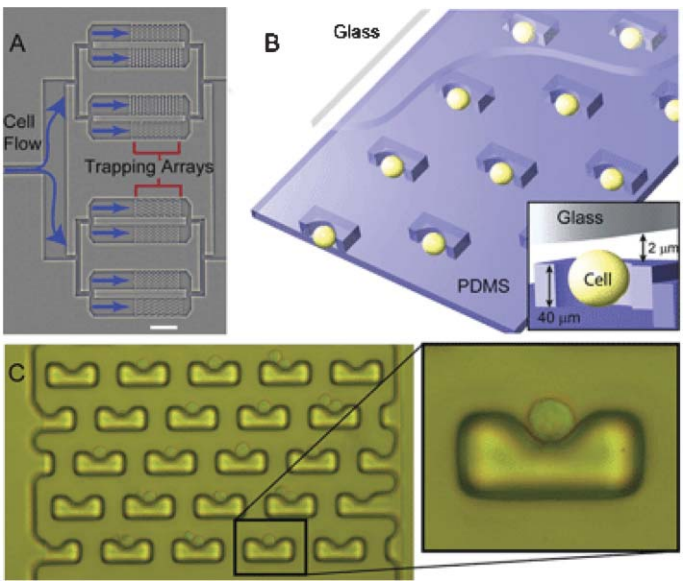

Fig. 3 Single cell trapping arrays. (A) A photograph of the cell trapping device is shown demonstrating the branching architecture and trapping chambers with arrays of traps. The scale bar is 500 μm. Cell and media flow enters from the left and enters the individual trapping chambers where it is distributed amongst the individual traps. (B) A diagram of the device and mechanism of trapping is presented. Traps are molded in PDMS and bonded to a glass substrate. Trap size biases trapping to predominantly one or two cells. The diagram is flipped from the actual device function for clarity; a functioning device is operated with the glass substrate facing down towards the earth. The inset shows the geometry of an individual trap. The device is not drawn to scale. (C) A high resolution brightfield micrograph of the trapping array with trapped cells is shown. In most cases cells rest at the identical potential minimum of the trap, while in some cases two cells are trapped in an identical manner amongst traps. The magnification shows the details of the trapped cell. Trapping is a gentle process and no cell deformation is observed for routinely applied pressures.[16]

will provide a welcome supplement to existing multi-cell techniques in the (near) future.

Helene Andersson Svahn

Royal Institute of Technology, AlbaNova University Center, Stockholm, Sweden and BIOS/Lab-on-a-Chip Group, MESA+ Research Institute, University of Twente, The Netherlands

Albert van den Berg

BIOS/Lab-on-a-Chip Group, MESA+ Research Institute, University of Twente, The Netherlands

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