

Preface

Single cell analysis (SCA) and systems nanobiology (SNB) are nowadays puzzling keywords in a scientific community that is more and more interested in tackling complex and transdisciplinary problems in biology – the science of life. However, why is the analysis of single cells so fascinating and important that biologist, chemists, physicists, engineers, biomathematicians and bioinformaticians contribute from different perspectives to achieve these goals? Is it the fascination of the smallest organizational system that – by definition – represents life and its related consciousness that such a small systems maintains a highly complex and hierarchical architecture of interconnected molecular networks? Is it the hope that understanding such a complex system will help us to understand the functioning of complete organs and will give us new approaches to cure diseases and to develop personalized medicine within the framework of systems biology? Or is it a means to identify the relevant mechanisms of complex processes in cells which have developed over millions of years with a limited set of molecules and which are responsible for their self-maintaining, efficient, robust and evolutionary properties? The answer to all these questions is yes. But what is the difference from the established biotechnological procedures and approaches that were based on investigating cellular ensembles and which were developed over recent decades, proving their validity and effectiveness in bringing breathtaking insights of cells and bringing their molecular blueprint to light?

This book gives you a number of possible answers to these questions. Of course it is only a snapshot of a rapidly growing field. Here, scientists of many origins share with you their results and view of a field which is at present in its infancy but is ready to develop into an equivalent and complementary partner to the existing approaches. A field that strives to extract quantitative information on cellular properties and information at different cell organizational levels (genome, proteome, metabolome, etc.) which are not ensemble-averaged. This is certainly the most obvious paradigm shift when you rationalize that, to date, proteome analyses have been based on 10^3 – 10^6 cells and rely on the fact that weak (but important) cellular signals and responses can be hidden within an unspecific cellular background of cells that do not behave in an identical way – they are not identical and are characterized by a heterogeneous

response. Moreover, novel single cell techniques will hopefully allow the investigation of individual cell cycle dependent effects, will give access to cellular variabilities during cell proliferation, will give access to cellular subpopulations and differentiation states and will allow an insight into the different and inhomogeneous cellular responses to external stimuli.

It will be highly interesting to see how parameters like robustness of a cell against external stimuli is a global property of the cellular ensemble and how it relates to the property of an individual cell.

A similar shift in paradigm happened in biophysics when, over the past 15 years, single molecule biophysics opened a new field of activity that allowed investigation of biomolecular processes and identification of physical mechanisms, with complementary approaches in mechanics (forces), optics (photonics), electrodynamics (charge transport) and thermodynamics (energies). Their novel insights into specific interactions, structure–function related binding mechanisms, molecular dynamics and the description within novel statistical mechanics models allowed the extraction of information on metastable transition states, molecular subpopulations and thermodynamically driven heterogeneous variabilities that are beyond the statistically averaged information provided by molecular ensembles in their initial and final state.

Single cell analysis will profit from such expertise in the sense that, beyond pragmatic benefits like saving material, time and resources (no cell cultivation and amplification needed), the novel highly parallelized and microchip-based single cell analysis approaches will allow new screening concepts and applications where only small amounts of cells are available (e.g. stem cell research).

In that sense, this book aims to cover some of the very prominent fields of activities that contribute to the area of single cell and subcellular analysis. General and more specialized readers will find a mixture of very recent results that are embedded in review and trend articles, where renowned researchers give their view of the state of the art of emerging and innovative analytical technologies as well as their motivations and visions of this lively area.

This book is structured in three main areas:

- Part I Single Cell Analysis: Imaging
- Part II Single Cell Analysis: Technologies
- Part III Single Cell Analysis: Applications

The three parts are preceded by an introductory feature article from Ruedi Aebersold, where the general topic of single cell analysis is put into the framework of molecular systems biology.

In Part One, where the book's focus is on SCA imaging, exemplarily the three major microscopy techniques – optical, electron, scanning probe microscopy – are highlighted with their emphasis to analyze individual cells. Namely, Ulrich Kubitschek reports on the investigation of intracellular protein dynamics with fluorescence microscopy at the single molecule level, Robert Ros and Nicole Hansmeier describe their microscopy efforts to map protein structures on bacteria and how single molecule force spectroscopy can nowadays be used to quantitatively

affinity rank DNA–protein interactions from bacterial transcription regulation networks and Jürgen Plitzko gives an overview of the fascinating capabilities of 3D single cell electron tomography.

In Part Two, novel single cell analytical technologies are illustrated. Norman Dovichi and coworkers present their developments in single cell capillary electrophoresis (CE) and applications in cell development, oncology and neuroscience. Alexandra Ros and Dominik Greif demonstrate their achievements towards a label-free protein fingerprint of a single cell in a microfluidic chip format and Jonathan Sweedler and coworkers review current mass spectrometric approaches for examining single cells and highlight recent progress. Microfluidic cell isolation array structures for quantitative analysis of toxin pore formation and cell cycle dynamics in single cells are investigated by Luke Lee and Dino di Carlo, while Josef Käs and coworkers describe their optical stretcher technology that allows cancer diagnosis and stem cell characterization at the single cell level.

In Part Three, more application-driven examples are highlighted. Ulrich Walter and Jan Buer review how single cell analysis with various technologies contributed to a modern understanding of the immune system. Rare single tumor cells can nowadays be characterized by a variety of staining, detection, sorting and data analysis techniques. The chapter by James F. Leary gives an impressive overview about it and its consequences. Cellular variabilities and single cell heterogeneity is the topic of the contribution of Edgar A. Arriaga, where he investigates the diverse variations of isogenic cells, for example insulin-producing pancreas cells. Last but not least, Christoph Klein and coworkers review how single tumor cells can be analyzed at a genomic and transcriptomic level.

A number of different aspects of single cell analysis are gathered and described in this book. They are not complete, since this young and promising area of research has just left its infancy and started a very dynamic development. Although quite a number of possible applications and merits already can be identified, it will be interesting to see how the field develops when it gains momentum. Based on the pioneering and exciting work presented in this book (and others too, see e.g. the special issue on single cell analysis in *Analytical and Bioanalytical Chemistry* 387 (1), 2007) a more complete understanding of complex cellular processes will not only bridge different scientific disciplines in a transdisciplinary way from an analytical point of view, it will furthermore connect different perspectives from a reductionistic molecular view to a more holistic organism-oriented viewpoint. I am convinced that we can expect a wealth of new insights into the hidden mysteries of cells and their complex mechanisms in the future.

Let's get excited and inspired!

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