loading antigen. As these cells mature, they downregulate CCR6, allowing them to leave the local environment. The subsequent upregulation of CCR7 causes maturing dendritic cells to home to T-cell areas in the tonsil, where MIP3β is expressed.

Immature dendritic cells have a high phagocytic capacity, but the bulk of their MHC class II molecules are intracellular, rendering them incapable of antigen presentation. Maturation, induced by factors such as LPS or TNFα, causes a change in the phenotype of the cells – they lose their phagocytic ability and shift MHC class II from internal stores to the plasma membrane. The targeting of class II molecules to the cell surface is regulated by the balance between cathepsin S and its specific inhibitor, calpstatin. In the ER, nascent class II molecules complex with a protein, the invariant chain (Ii), which both blocks the peptide-binding groove and targets the MHC complex to the endosomal compartment. Proteolytic processing of Ii by cathepsin S results in the targeting of class II to the MIIC compartment, where peptide is loaded, following which the complex traffics to the plasma membrane. Dendritic cell maturation is associated with decreased levels of calpstatin, resulting in increased cathepsin S activity and transport of class II to the cell surface [Ira Mellman (New Haven, USA), Ralph Steinman (New York, USA)].

Immature dendritic cells can internalize both necrotic and apoptotic cells. Mature dendritic cells can present these internalized antigens in the context of class II. This process might have significant implications for the maintenance of peripheral tolerance (Ralph Steinman). Interestingly, dendritic cells can also present antigens derived from apoptotic cells in the context of MHC class I. Such cross-priming, which drives a CTL response, might be important in immunity to viral infection, tumour immunity and also in autoimmune diseases. Macrophages phagocytose apoptotic cells more efficiently than dendritic cells but cannot cross-prime for a $CD8^+$ response (Nina Bhardwaj, New York, USA).

Concluding remarks

In the spirit of the Leiden Conferences, the first Keystone Meeting on Macrophage Biology brought together scientists from a wide range of disciplines. Precisely because macrophages perform such diverse functions, this meeting had an extraordinarily broad scope. While this was exhilarating to experience, it is impossible to report in full. We have highlighted, therefore, only a few themes and have had to omit many outstanding presentations that fell outside of these boundaries.

Automated analysis of patterns in fluorescence-microscope images

Michael V. Boland and Robert F. Murphy

The widespread proliferation of automated fluorescence-microscope systems has made the acquisition of digital images commonplace in cell-biology research. This has created a need for computer applications that automate the analysis of these images and an opportunity to develop new approaches to classical problems. Most software developed for analysing fluorescence images has focused on problems such as the quantitation of total cellular DNA content[1], determination of intracellular ion concentrations[2] or analysis of fluorescence *in situ* hybridization (FISH) experiments[3]. These applications of quantitative methods, in which the goal is measurement, can be distinguished from potential applications of pattern-analysis methods, in which the goal is the interpretation of images. There is an extensive literature describing the application of pattern recognition, pattern analysis and machine vision to images from many other disciplines, but little work has been done on applying pattern-analysis methods to fluorescence-microscope images. We describe here some recent work[4,5] aimed at making pattern-analysis tools available to cell biologists.

Selection of representative images

Investigators in many disciplines, but especially in cell biology, regularly need to choose a single image from a collection for presentation or publication. The unspoken implication of this choice is that the selected image is representative of the set. However, it is difficult to describe the biases, both conscious and unconscious, of the investigator making the selection. Furthermore, without making the entire set of images, and a set of explicit selection criteria, available to the research community, the degree to which the choice is representative cannot be verified by other investigators. We have developed, therefore, a system for automating the choice of representative microscope images and demonstrated that results consistent with biological knowledge can be obtained using it[5].

The starting point for the system is a set of digital images, all of which nominally contain the pattern of interest (for example, a set of images obtained via indirect immunofluorescence using a monoclonal antibody against a new protein). Ideally, the images will have been corrected for any artifacts or nonlinearities occurring during the image-acquisition process (i.e. out-of-focus fluorescence or nonlinear illumination), although such correction is not necessary. The system describes each image using a set of numerical 'features' and then uses these features to rank the images in order of their 'typicality'. The system has been implemented as a Web server (TypIC; for: 'typical image chooser'; see Box 1) so that biologists anywhere can upload a set of images and receive the rankings via e-mail.

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BOX 1 – URLs FOR IMAGE SELECTION

`http://murphylab.web.cmu.edu/data/`
This page contains descriptions of and links to the image data that were used during the development of these methods.

`http://murphylab.web.cmu.edu/services/TypIC/`
The typical image chooser (TypIC) systematically ranks the images from a set in terms of their typicality. These rankings can then be used to select one or more representative images for the entire set.

`http://murphylab.web.cmu.edu/services/SLIC/`
The subcellular localization image classifier (SLIC) accepts a fluorescence image from the user and determines which of several known classes of subcellular localization it most closely resembles. Although this service is primarily for demonstrative purposes, the methods are applicable to a variety problems, particularly in the area of high-throughput screening.

The specific features used in such a system are crucial to the ultimate usefulness of the rankings it generates. This is an area of ongoing research, but the features used in TypIC describe typical immunofluorescence patterns well enough such that they can be used to classify those patterns (as discussed below). While it might appear that the features inappropriately bias the choice of typical images, the fact that the criteria used in selection are explicit and reproducible (unlike the case for many choices made by human observers) is a strength of the automated approach.

Automated selection of representative images could be useful for selecting images for publication, for summarizing the contents of an image database with a handful of images and for facilitating the analysis of experiments by providing the investigator with new insight into image data. TypIC currently has broad applicability, but it will be continually upgraded and improved based on user feedback.

Classification of protein-localization patterns

One of the most common applications of immunofluorescence microscopy is the identification of the organelle to which a newly identified protein localizes. As an initial demonstration of the feasibility of applying pattern-recognition methods to this problem, we developed a system that can classify the localization patterns characteristic of five cellular molecules (proteins and DNA) in Chinese hamster ovary (CHO) cells[4]. The database of images used in this study is available to others interested in developing and testing pattern-classification approaches. There is a Web server that will classify an uploaded image into one of the five classes (SLIC; for: 'the subcellular localization image classifier'; see Box 1). This server is not intended for practical use but, rather, to demonstrate the operation (and limitations) of the system. Current work is aimed at extending this system to more organelle classes and other cell types.

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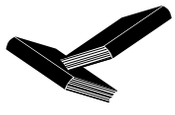

Do the locomotion...

Motion Analysis of Living Cells (Techniques in Modern Biomedical Microscopy)

edited by David Soll and Deborah Wessels, John Wiley and Sons, 1998. £115.00 (298 pages) ISBN 0 471 15915 8

One of the attractions of studying the fascinating and complex dynamic behaviour of migrating cells is that you can actually see it happening before you on the microscope stage, often in real time. This book is a collection of reviews by different authors describing how quantitative data can be obtained from such moving cells, and the insights these data can provide into the mechanisms responsible for motility. Early chapters discuss the acquisition and computer-aided analysis of motility data. A large amount of information can be obtained from moving cells, including such parameters as cell speed, directional change, acceleration, persistence and periodicity of movement, as well as a host of dynamic measures of cell shape. There are several computer programs for extracting and analysing data obtained by video microscopy of motile cells, and one, the Dynamic Image Analysis System (DIAS), is discussed in depth. Consideration of DIAS allows the authors of this chapter to present an accessible and helpful overview of the meaning and utility of a wide range of motility parameters. Use of some of these parameters to provide mechanistic insight is considered in several subsequent chapters. Detailed discussion of work on motility analysis of cytoskeletal mutants of *Dictyostelium* is presented. As pointed out, early qualitative analysis of some of these mutants failed to show motility defects and led to suggestions of considerable overlap in the functions of cytoskeletal proteins. Only with careful quantitative analysis of motility did it become clear that there were in fact gross motility defects in these cells in comparison with wild-type cells. This illustrates very clearly the importance of a quantitative approach to analysing motility, a theme also echoed in chapters on neural growth cone behaviour in *Drosophila* neurons and motility of *Listeria monocytogenes*. Just as with dynamics, it is becoming