19.

Computerized Pattern Recognition Systems in Routine Haematology

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Introduction

Success in laboratory medicine requires that the correct components can be brought together. These are

1. clinical relations
2. biotechnology
3. information technology.

Traditionally haematology has been very strong in clinical relations. Biotechnology is developing apace as is information technology. The real and important challenge is to link all three arms of the triangle together optimally.

Haematology will be shaped very much by the same forces as all other sections of laboratory medicine. These forces include science and technology both of which are advancing very rapidly. Each laboratory discipline has its own particular economic restraints, however, the 'need to do more with less' is common to all disciplines. Quality demands, both technical and functional, are increasing, as is the need for their demonstrable assurance. The number and complexity of regulations are increasing too and, what is very apparent from a practical sense, in the day to day running of the laboratory, is a perceived increasing lack of competence with an old generation moving out and a new one coming in.

These challenges are, of course, being faced by many new techniques and automated instruments that utilise IT in a comprehensive and positive way. Automated systems and work cells abound in all branches of laboratory medicine. Information Technology is involved inter alia in electronic test requesting, in process control, in autovalidation, in reflex testing and in expert systems. It is integral to hospital and laboratory information systems. Flow cytometry is developing very strongly with its integration into routine cell counters, with the development of new software, new markers, and the emergence of new clinical indications not only in haematology, but also outside this field. Molecular biology is also a rapidly developing tool for the future with genotyping, expression profiling and pharmacogenetics emerging as major diagnostic areas.

Coming from Clinical Chemistry, that bastion of automated and robotic measurement, the question must be asked – 'does the microscope still have a place in the future?'
Will not flow cytometry, molecular biology, etc replace microscopy? These new technologies will not totally replace microscopy. There is a certain strength in morphology that is very hard to bypass. Although, actually a drawing by Salvador Dali from 1942 when he was writing his book *"The Secret Life of Salvador Dali"* this could be a cell in a sense (**figure 1**). There is a nucleus and some granules floating around. This is a very interesting case of morphology and pattern recognition. Salvador Dali can be recognised very much from the shape of the drawing

and his name is even in the right lower segment. Unfortunately, cells do not specify their identity in writing. What is very clearly specific in the figure is a pattern with an enormous amount of information. This is something, which in art, an art expert (or a haematologist) will recognise very easily. But the problem now, in laboratory medicine, is how to get this visual, intellectual, artistic impression into modern laboratory systems and how to use it.

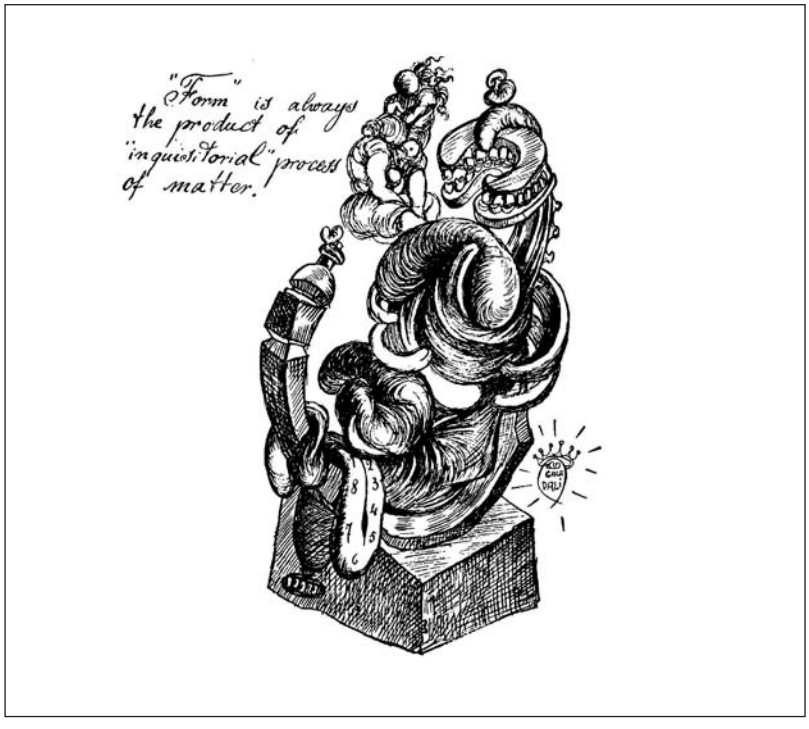

Figure 1
The Strength of Morphology – a drawing by Salvador Dali from his book 'The Secret Life of Salvador Dali'. Reproduced with permission
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Figure 2 is a general view of how the future logistics of haematology may develop. Samples and requests arrive by whatever means; there is some form of automatic sample processing; a cell counter does the major work; reflex testing is performed when required; and finally generation of a single report. This process will be supported strongly by different IT solutions, as has already been demonstrated in this symposium. Molecular biology, flow cytometry and also microscopy will have their place in the shape of digital technology as the further diagnostic tools.

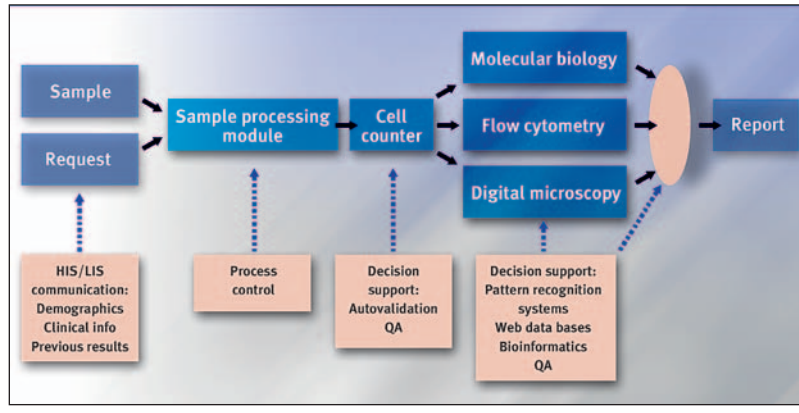

Figure 2
Future logistics in haematology.

Differential leukocyte counting

The informatics of differential leukocyte count (DLC) is very complicated compared to that of many clinical chemistry data sets currently handled. The DLC represents a great deal of biological complexity. The interesting thing is, that the DLC can be performed by pattern recognition systems and developments in information and communication technologies can now facilitate its interpretation and reporting.

In recent years the way microscopy differentials are handled and analysed in the laboratory has changed very much. Automated cell counters have revolutionised the work. In our laboratory only about 10% of differentials are counted microscopically, the remaining 90% being analysed with high quality and great reproducibility in modern cell counters. That means, of course, that we have only the difficult samples left for microscopy. One disadvantage of this is that there is loss of microscopy expertise particularly when combined with one generation leaving the stage and another very different generation coming into the laboratory.

Back to Reality

So let us return to laboratory management and laboratory reality and view two hypothetical scenarios. This is today. We have in the clinical ward Doctor NN who is soon to retire. He is the senior expert. He follows his patients to the bitter end and he prefers a typewriter. He is the real morphologist – the real art expert. Young Doctor MM will soon replace him. He is a team player working with different groups and he is a consultant in a network of hospitals. He is a scientist and he would not do anything or go anywhere without his laptop computer.

This fundamental change is also happening at the technologist level. Today we have a number of technicians above the age of 50 years and they know everything about microscopy. They can recognise all these Salvador Dali-like patterns at first glance. For them, the microscope is a symbol of pride and laboratory competen-

ce and they like the pencil because it is good for writing on glass slides. On the other hand, the young technologists have studied molecular biology for three years and are planning to write their theses in the future in some fancy new area like proteomics. They have actually tried to use a microscope a few times and they think it is a very interesting instrument but they will not countenance the use of pencils. In this scenario the morphology tradition will definitely change.

Is there a solution?

Can the situation be rectified? Over the past few years we have been collaborating with a young start-up company called CellaVision based in Lund, southern Sweden. Some six or seven years ago this company approached me saying – 'we want to try to use artificial neural networks to approach the differential leukocyte count'. The result is DIFFMASTER™ Octavia (**figure 3**), a software product on a hardware platform consisting of an automated microscope, a very high quality CCD camera and a computer, which automates the differential leukocyte count. DIFFMASTER consists of a microscope that automatically locates cells on a blood smear. The automated slide holder holds eight slides at a time, which are processed and scanned automatically.

Figure 3
The DIFFMASTER system consisting of a microscope, a CCD camera and a computer.

For every blood cell that DIFFMASTER finds, an image is taken that is then analyzed and pre-classified by means of advanced image processing. The results are presented clearly on a computer monitor, and all cells of the same class can be studied at the same time (**figure 4**). Different classes can be displayed simultaneously, or an individual cell can be studied more closely using the virtual microscope (**figure 5**). The operator always visually verifies the classification suggested by the system.

Figure 4
Presentation of cytology images on the DIFFMASTER monitor for validation.

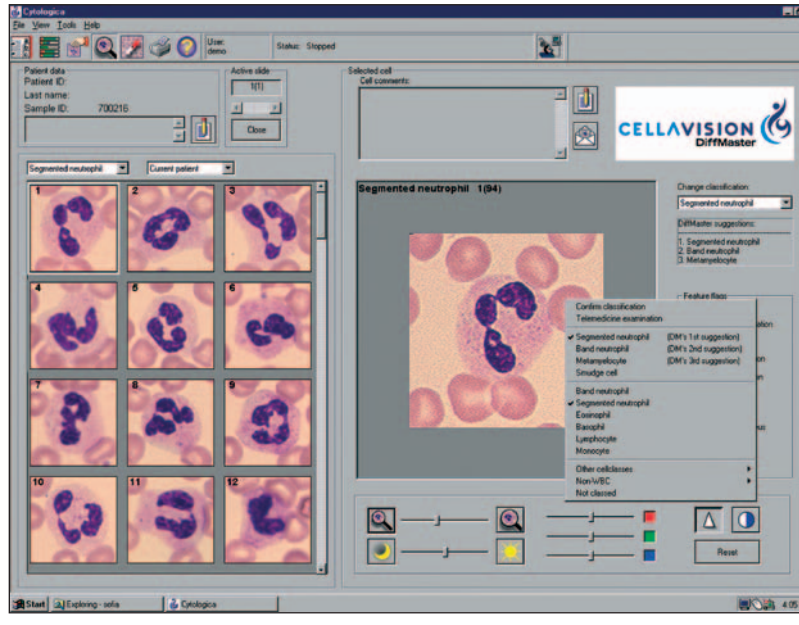

Figure 5
Producing image enhancement using the virtual microscope.

The images of the cells are saved in a standard format, so that they may be e-mailed within the organization or to colleagues for comment and assessment. The digital format offers new possibilities, such as following how a patient responds to a particular treatment.

The software is not algorithm based but operates using a series of artificial neural networks. This is a system for pattern recognition that has been in use increasingly over the last years. The training set contains some 35,000 cells classified by an expert panel. Each picture of each cell is broken down into more than 100 different features and these features can then be analysed by the computer and processed through an artificial neural network and a classification is suggested. Following a number of iterations the system learns to identify the cells.

Evaluation of DIFFMASTER™

The system supplies results comparable to those that a trained operator can achieve at the microscope, in terms of accuracy (**figure 6**), reproducibility, and speed. The image quality is as high as with traditional microscopes. Cell images are illustrated in figures 4 and 5 with their pre-classification. The technologist now reviews this and agrees or disagrees with the classification. Reclassification is possible by the validating technologist.

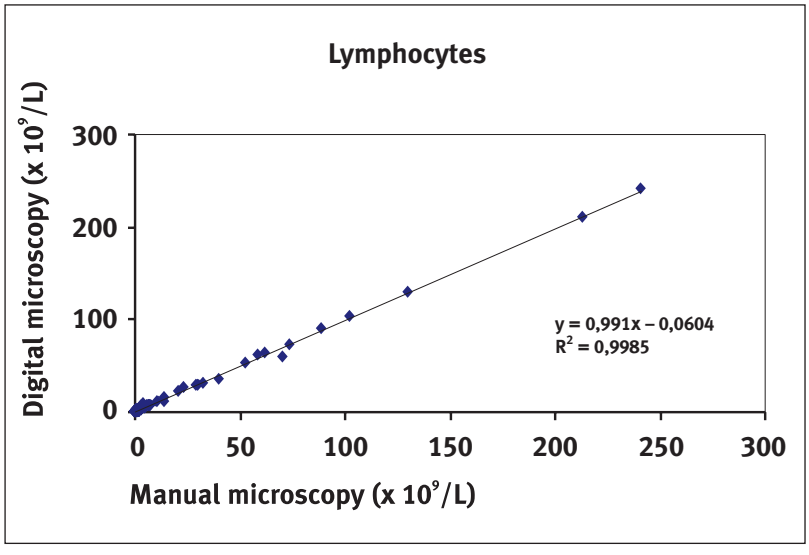

Figure 6
Illustration of the comparability between digital and manual microscopy for physiologically occurring cell types.

There are, therefore three stages in the process: the first is cell location; the second is cell pre-classification; and the third is visual validation. In about 10% of the cases, reclassification by the technologist is necessary.

The system has been evaluated and improved over time and the correlation of physiologically occurring cell types is excellent as is shown for lymphocytes in **figure 6**. Many pathological cases have also been observed and generally the correlation is also good between manual and digital microscopy (**figure 7**).

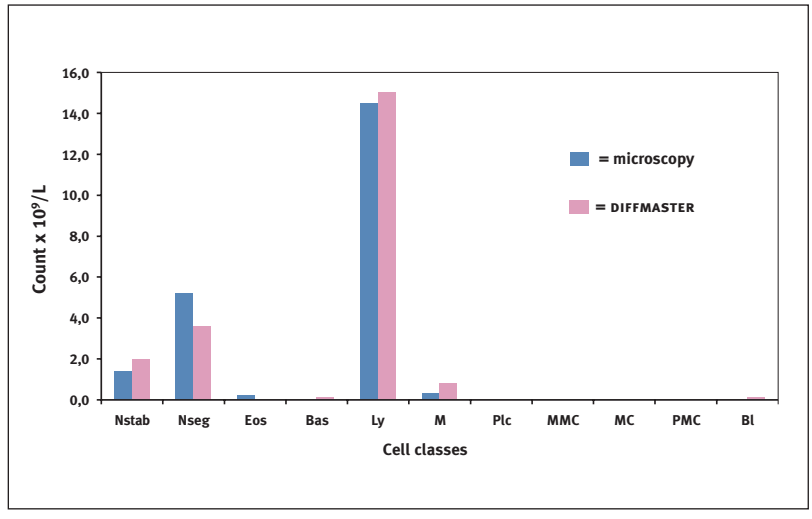

Figure 7
Example of comparability between digital and manual microscopy in pathological cases.

An extended evaluation has been performed at the Departments of Clinical Chemistry in Göteborg and Malmö, Sweden [**1**]. This follows the NCCLS-H20A protocol. Some 322 routine specimens were examined; half of them normal, half of them pathological. As defined in the NCCLS protocol, 400 cells were counted, 200 cells each by two different technologists on two different slides. For the automated limb, these slides were processed according to the standard instrument procedure. Instrument pre-classification was undertaken and then visual validation was performed. Generally the results show that there was good correlation between the two methods with concordance of about 89%. For the 6 physiological cell types and blasts cells, r^2 = 0.8–0.98. Concordance was higher for the physiological cell classes (92%) than for pathological cell classes (55%).

Since the differential leukocyte count is qualitative, it is desirable to know the sensitivity of the method. Does the digital differential count identify the samples that would be classified as pathological by visual microscopy? The correlation was also high. The diagnostic sensitivity was 98%. Only 2% (n = 4) of the pathological samples were not identified by the digital instrument. In two cases this was due to minor differences in distribution and in one case it was due to the presence of a single blast cell. In one case the distribution was markedly different but in this sample the results from the DIFFMASTER correlated closely with those from the cell counter. The diagnostic specificity was lower (82%). This was due to the

fact that the DIFFMASTER identified more immature cells than the manual reference method. Imprecision levels for the two methods were similar.

The throughput varies according to the counting requirements of the specimens being handled. As previously stated the automatic slide holder takes 8 slides. The number of cells counted on each slide can be adjusted; for 200 cell counts per slide the throughput is 12–15 slides per hour; for 400 cell counts per slide, it is 6–9 slides per hour. This includes time for red cell morphology.

Conclusions

The DIFFMASTER was introduced into the clinical routine about two years ago and for the past year digital microscopy has been used in the laboratory for › 90 % of the slides requiring microscopy review. The greatest advantage and what has made it popular, surprisingly even among these experts who are very used to the microscope, is that, using the zoom facility, it gives an improved view of the cells and makes it easier to classify them. Ergonomics are also improved. It is better to work in front of a screen than to locate cells on a microscope. Standardisation has improved in the laboratory. These digital images can be sent out to different laboratories as part of the National External Quality Assessment programme now available in Sweden. Training has also improved, both in the medical technician training and education programme. Clinical haematologists are interested in the technology from a training point of view. The system can be networked and be located in different hospitals. Centralised reviewing and case conferences are possible.

One special requirement is the need for high quality smears. These digital systems are not as robust in correcting for smearing mistakes and smearing errors as the human eye. A slide maker is an advantage but we have used a manual device successfully for making blood smears.

In conclusion, artificial neural networks can provide a decision support system which can help the morphologist to generate haematological reports of high quality in routine laboratory medicine. Automated cell location and pre-classification improves efficiency but the safeguard of manual validation remains. A common standard traceable to an expert cell atlas can be created in the future. Image storage and retrieval is simple and networking possibilities are considerable. In the future the laboratory will depend more and more on such systems.

References

[1] **Swolin B., Simonsson P., Backman S.** *et al:*
Differential counting of blood leukocytes using automated microscopy and a decision support system based on artificial neural networks – evaluation of DIFFMASTER TM OCTAVIA. Clin.Lab.Haem.2003, 25, 139–147.