# 18.

# Building a Haematological Database with LAFIA

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## Introduction

The aim of this presentation is to demonstrate the use LAFIA as a tool for building a haematological database. The hospital is located in the Walloon part of Belgium, more precisely in the area of Charleroi. It has 650 beds and has a large department of Haemato-oncology. The diagnosis and follow-up of haematological pathologies is based on the multi-disciplinary approach as outlined in the new World Health Organisation classification and requires information obtained from cytometry, cytogenetics, histology, immunohistochemistry, molecular biology and, of course, clinical data (**figure 1**). LAFIA (Laboratory Filing System for Image Analysis) can be used to support this multi-disciplinary approach, at least in part. From a functional point of view LAFIA consists of two main parts: (1) a work screen and (2) a data list.

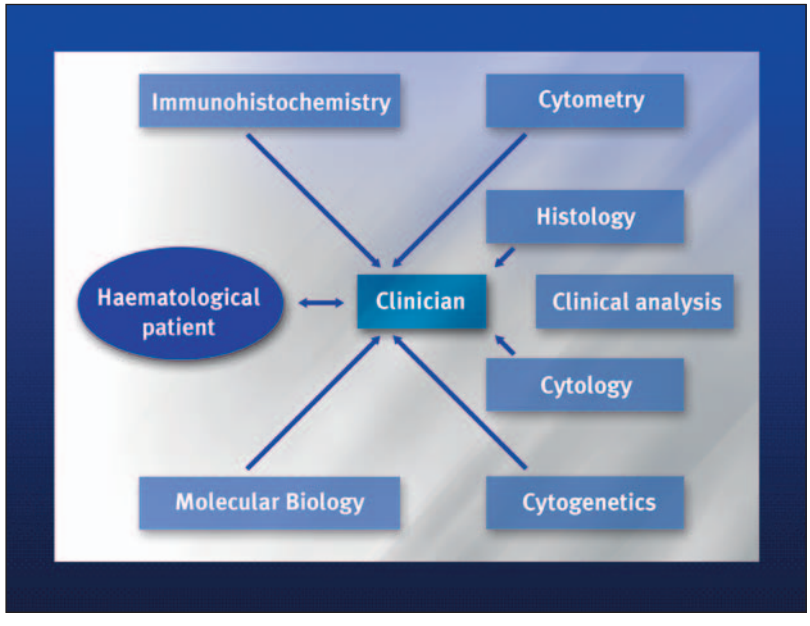


***Figure 1***
*The multidiscipline approach to the haematological pathologies as required by the new World Health Organization classifications.*

## LAFIA: The Work Screen

The work screen enables the collection of all information about a patient and his disease. LAFIA is in connection with the PC-DPS from which the patient's data is extracted. The process is illustrated in **figure 2**. Using the 'sample patient inquiry' icon, patient demographic data become available and then by selecting the 'access number' it is possible to call up the cell count and differential data from the XE-2100 via the PC-DPS (**figure 3a**). These data can be complemented with digital cytology images. By clicking on the 'capture' icon, the image is automatically added to the patient file (**figure 3b**). Each image can be identified by cell type (**figure 3c**).

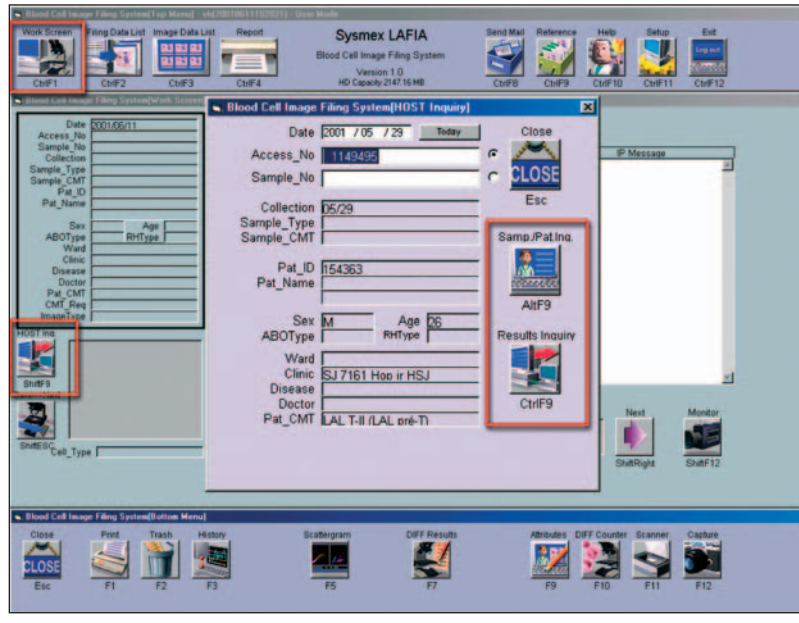


***Figure 2***
*Work screen – 'sample patient inquiry' icon for demographic data.*

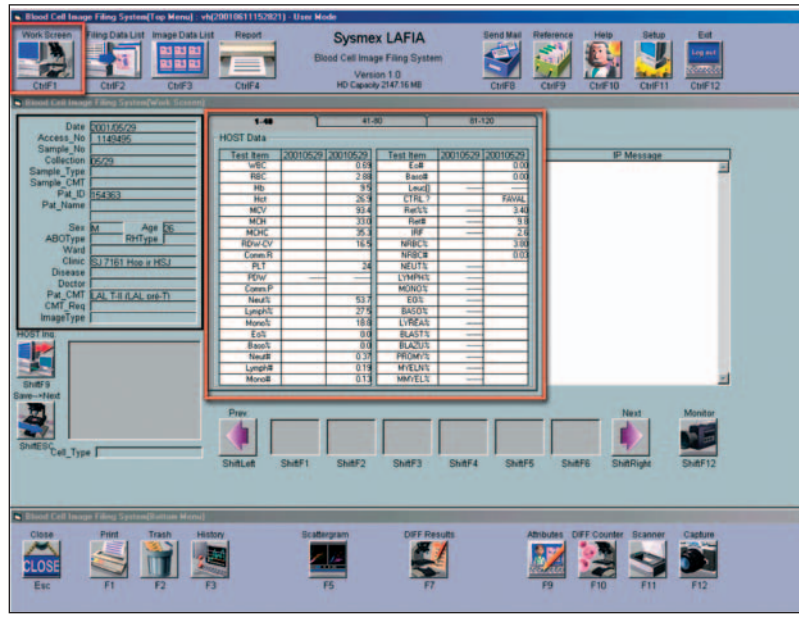


***Figure 3a***
*Work screen – Accessing count data.*

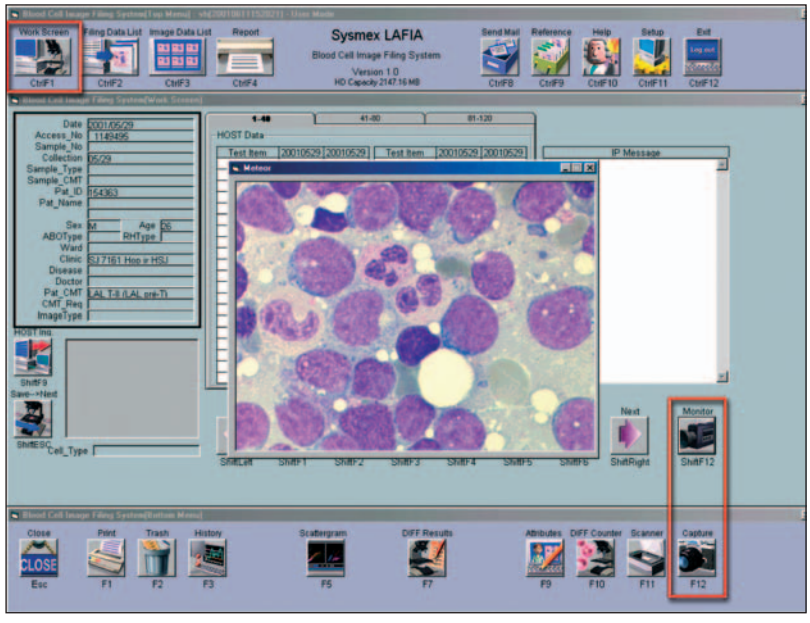


***Figure 3b***
*Work screen – Accessing digital cytology images.*

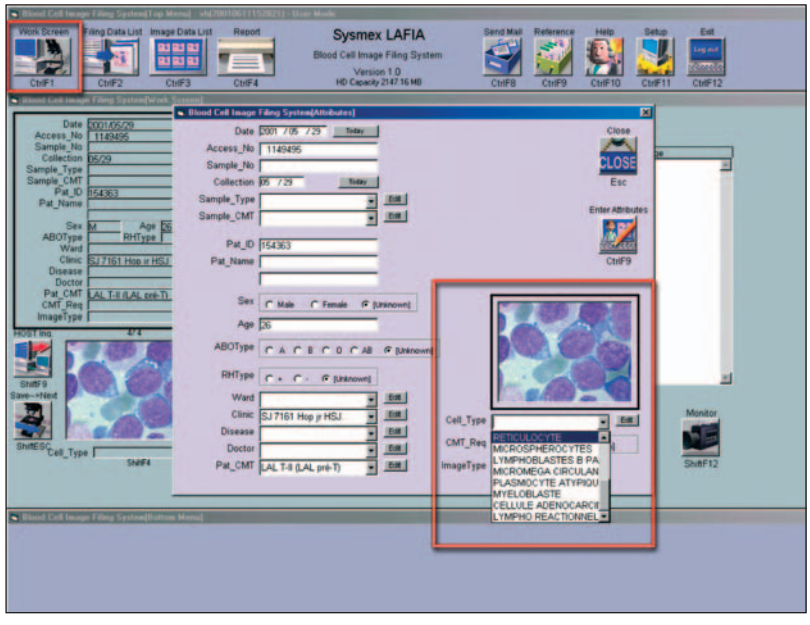


***Figure 3c***
*Work screen – Identification of the cell type.*

All this information including scattergrams and histograms, if necessary, is saved in the database, which is located on a hard disk or on the hospital network. The scanner provides a very useful function enabling the addition of, for example, the immuno-phenotype data which are then accessible through the same patient file (**figure 4**)

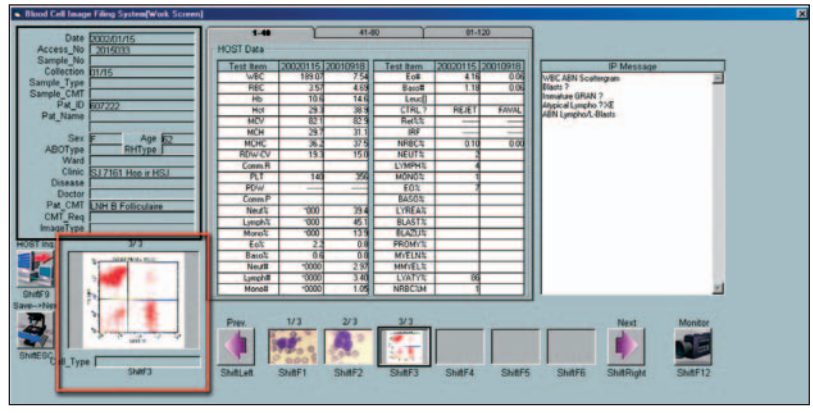

***Figure 4***
*Work screen – Addition of data to the system using a scanner. In this case immunophenotyping data has been added.*

## LAFIA: The Data List

All the collected data can be accessed through the filing data list or the image data list. Using the Filing Data list, LAFIA provides a wide spectrum of search criteria either by individual patient ID or by disease (**figure 5**). Access by patient ID, provides detail of the history, sample types, numerical data and images. By disease, it is possible to review all cases, e.g. of mantle cell lymphoma stored in the database. It is also possible to select and e-mail data and/or images (**figure 6**). The selected mailings will be exported automatically from the mailbox where they are located as attached files. An example is shown in **figure 7**. The Image Data list provides a different presentation of information (**figure 8**). There are 6 images per screen with some associated brief information.

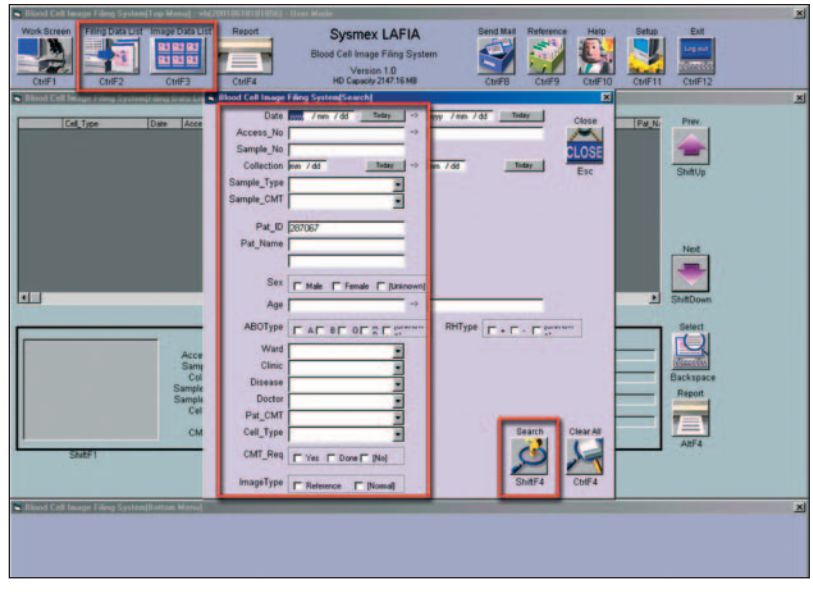


***Figure 5***
*Database – Filing data list. Accessing via patient ID or disease.*

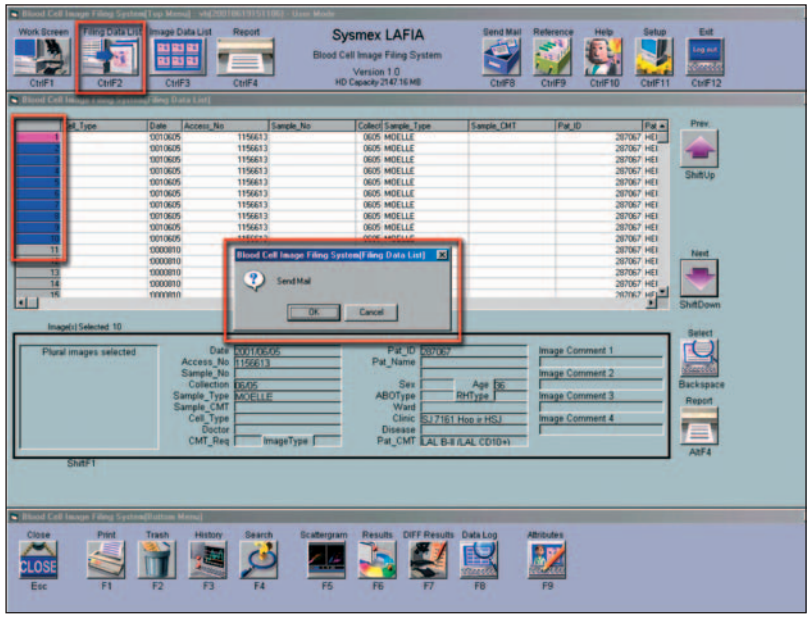


***Figure 6***
*Database – E-mailing data and/or digital images.*

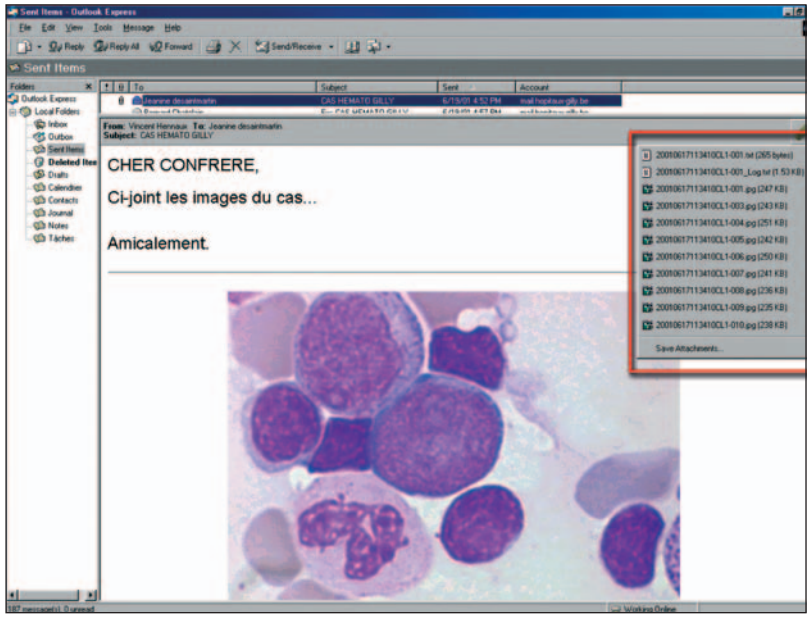


***Figure 7***
*Database – Sending mail to a colleague. Example of digital image e-mail.*

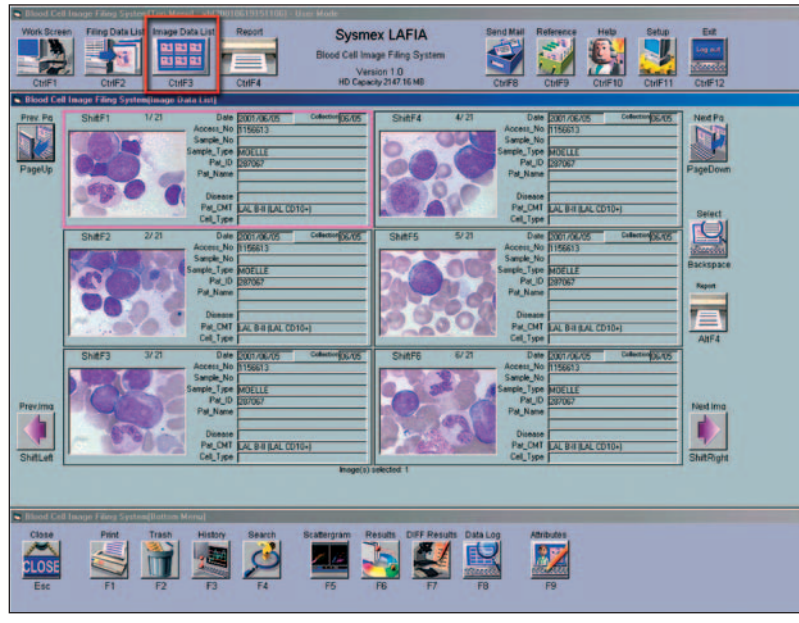


***Figure 8***
*Database – As many as 6 images per screen can be mailed with associated information.*

Data annexure has already been demonstrated in the Work screen. A further example is shown in **figure 9** in which a morphology image and scattergrams are integrated. In the DIFF scattergram some high fluorescence cells are present. Examination of a buffy coat preparation then revealed the presence of plasma cells. Likewise the immature cells present in the IMI scattergram were confirmed as myelocytes in the buffy coat.

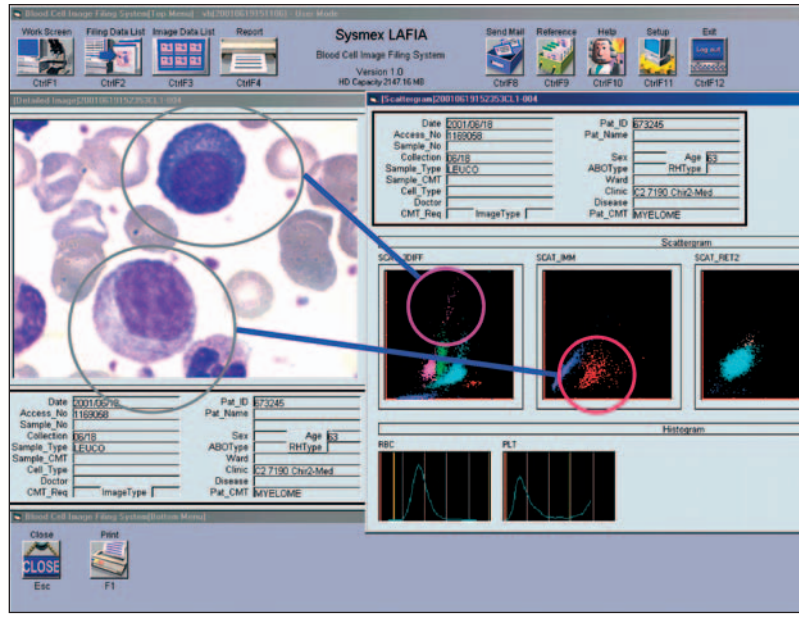


***Figure 9***
*Database – Integration of morphology images and scattergrams.*

## Clinical Cases

Following this general description of the database construction and use, the system will now be illustrated by some clinical cases.

*Case 1:* The first case is a two-year follow-up of B cell acute lymphoblastic leukaemia (B-ALL). This patient's cytology is shown in **figure 10**. On the right, the bone marrow cytology at the time of diagnosis in August 2000 is shown. On the left is the cytology found in June 2001 following allogeneic bone marrow transplant is shown. The problem was the presence of 20% of the cells illustrated on the left a few months after transplant. The question was: do we have a relapse or an immune reaction? The cell morphology is clearly different from that at the time of original diagnosis. This is in favour of an immune reconstruction, rather than a relapse. Cytogenetic analysis provided the answer in that the abnormal karyotype originally present had disappeared. However, a further year later the original cytology had re-emerged indicating relapse. Serial cytology and immunophenotypic analysis with CD19 and CD10 is shown in **figure 11**.

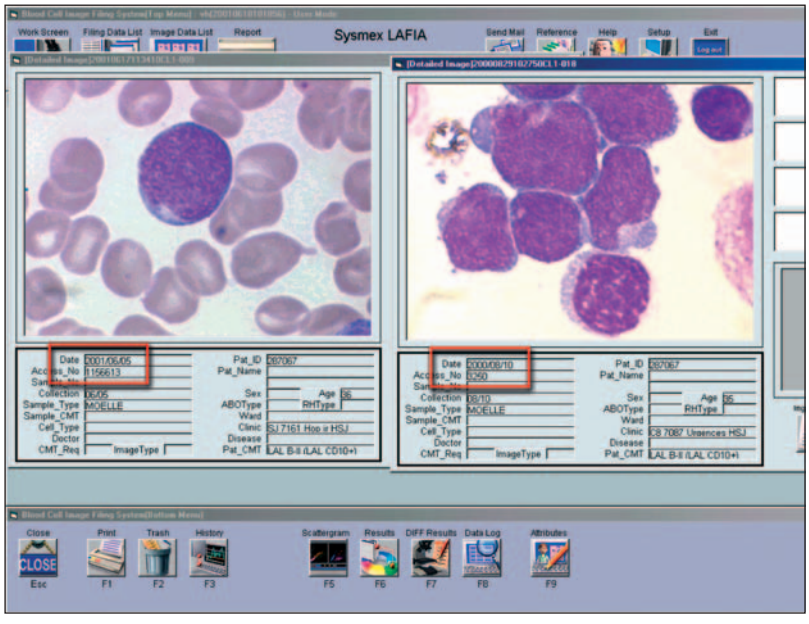


***Figure 10***
*Database –*
*Case 1 – the digital image on the right illustrates the cytology at the time of diagnosis. The image on the left was obtained following allogeneic bone marrow transplantation.*

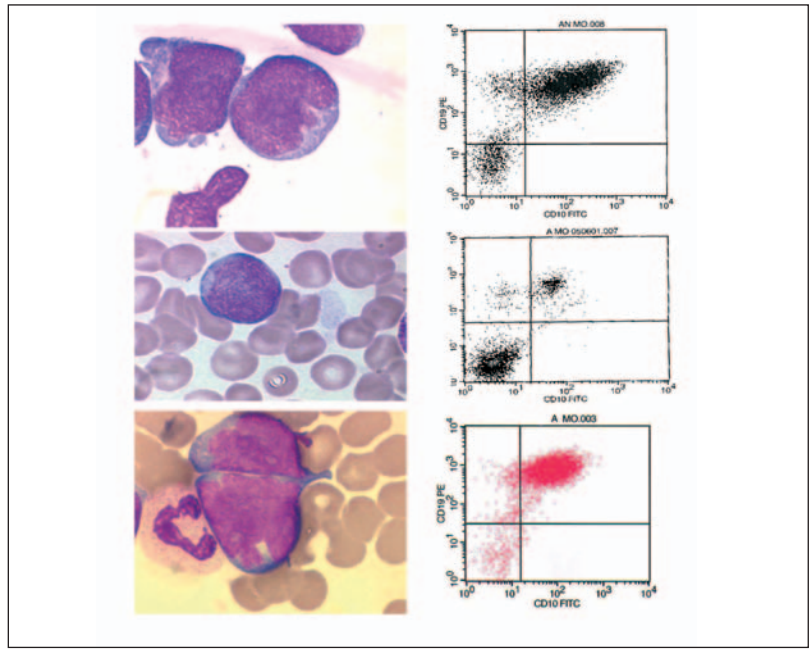


***Figure 11***
*Database –*
*Case 1 – upper combination of digital cytology image and immunophenotyping demonstrates original status in August 2000; middle combination shows disappearance of abnormal karyotype following transplantation and the lowest combination indicates relapse.*

*Case 2:* The second case is an acute promyelocytic leukaemia treated with all-transretinoic acid (ATRA) a differentiating agent. At the time of diagnosis the CBC count revealed severe pancytopenia and the blood film showed some abnormal promyelocytes (**figure 12**). The IMI scattergram showed a few immature cells. ATRA therapy was commenced. The white blood cell count increased and abnormal cells appeared on the scattergram. By day 6, progressive maturation of promyelocytes was clearly observed in the change of the scattergrams (**figure 13**). By day 9, the differentiation of cells continued to occur and the scattergrams became more normal. During treatment, the patient developed septicaemia and bacteria were observed within the cytoplasm of the developing granulocytic cells, indicating that they were recovering their ability for phagocytosis (**figure 14**). The patient was entering remission.

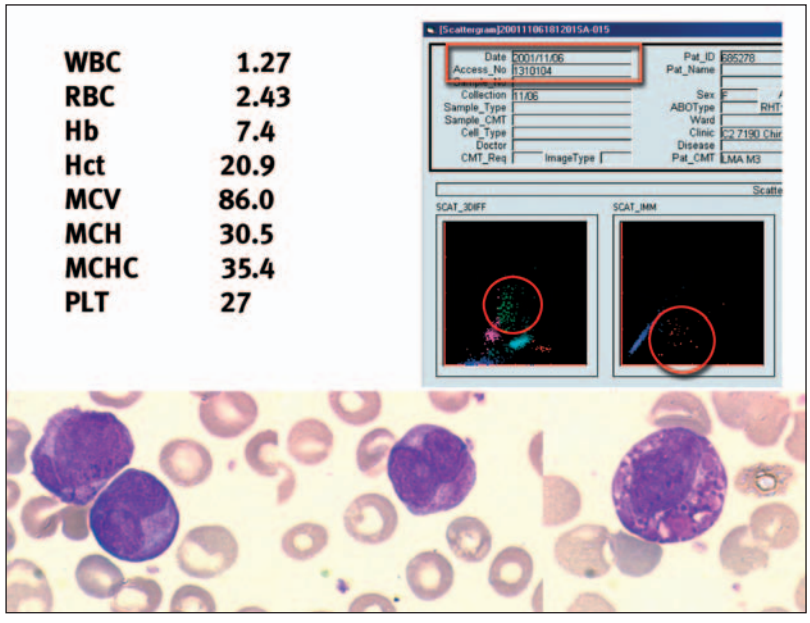


***Figure 12***
*Database –*
*Case 2 – acute promyelocytic leukaemia at the time of diagnosis (blood counts, scattergrams and cytology.*

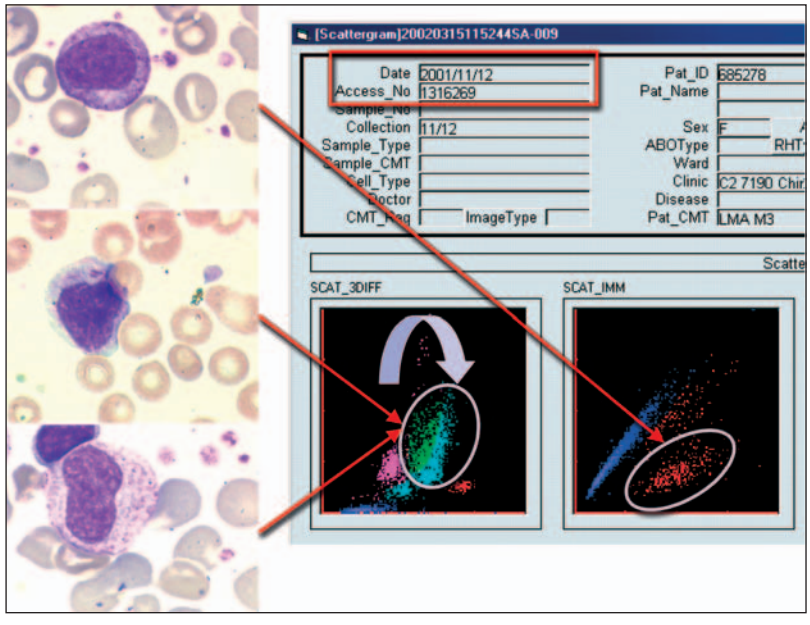


***Figure 13***
*Database –*
*Case 2 – six days after starting ATRA. Note the altered morphology and the change in the IMI scattergram indicating a degree of granulocytic maturation.*

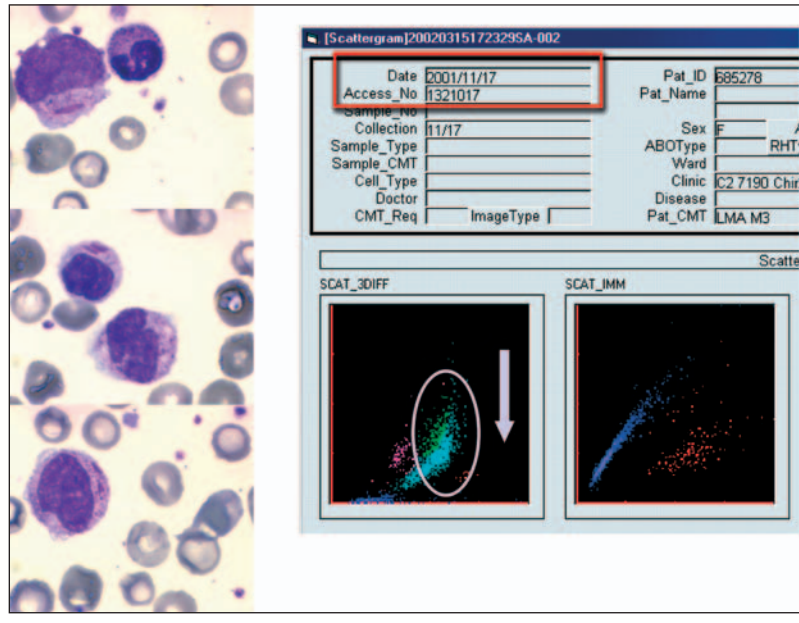


***Figure 14***
*Database – Case 2 – patient develops septicaemia. Note phagocytosed bacteria in developing granulocytic cells. Patient is entering remission.*

*Case 3:* The third case concerns a 59 year-old woman admitted with fatigue, pyrexia, night sweats, dyspnoea and splenomegaly › 20cm. No lymph node enlargement was present. The blood count data was as follows: Hb = 4.9 g/dL, WBC = 3.5 x $10^9$/L with 34% neutrophils and 55% lymphocytes. The blood smear revealed the presence of abnormal lymphoid cells (**figure 15**), which together with the immunophenotypic expression was considered to be consistent with a diagnosis of splenic marginal zone lymphoma without villous lymphocytes. The initial treatment consisted of splenectomy without chemotherapy and the patient entered remission. Unfortunately, one year later, the patient had a first relapse with similar cytology and phenotype. Since the cells continued to express CD20, treatment with Rituximab, a monoclonal antibody directed against CD20, was started. Two months later the patient was in remission but six months later, a second relapse occurred. The peripheral blood picture was unchanged but the CD20 expression was lost (**figure 16**). No other markers had changed. Bone marrow invasion and negativity for CD20 was confirmed by histology and immunohistochemistry (**figure 17**). So which mechanism explains this negativity for CD20? According to the literature, two hypotheses are possible: the first is a down-regulation of CD20 expression; the second is the selection of a CD20 negative clone. To identify the correct hypothesis, the electrophoretic profiles of PCR-IgH products of the bone marrow at the time of diagnosis with those 2 years later were compared (**figure 18**). The identity of the IgH (heavy chain) rearrangement peaks in the two samples confirms that the same clone exists. So the mechanism that explains the disappearance was a down-regulation of expression of CD20.

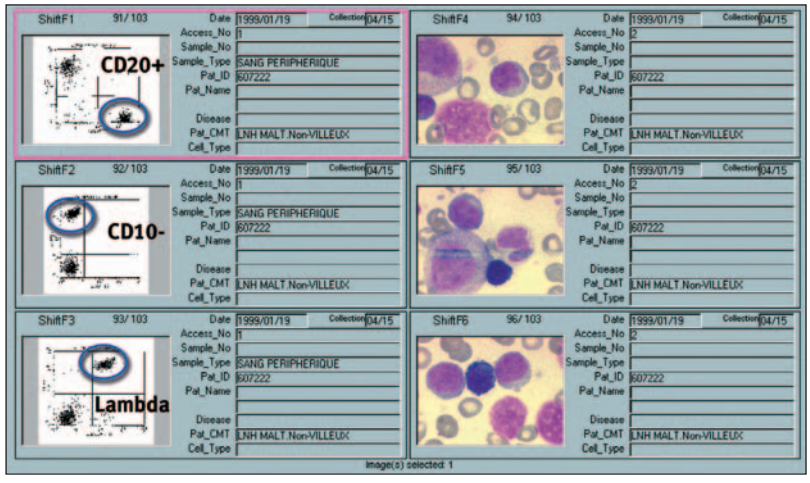


***Figure 15***
*Database –*
*Case 3 – cytology and immunophenotypic analysis consistent with marginal zone lymphoma.*

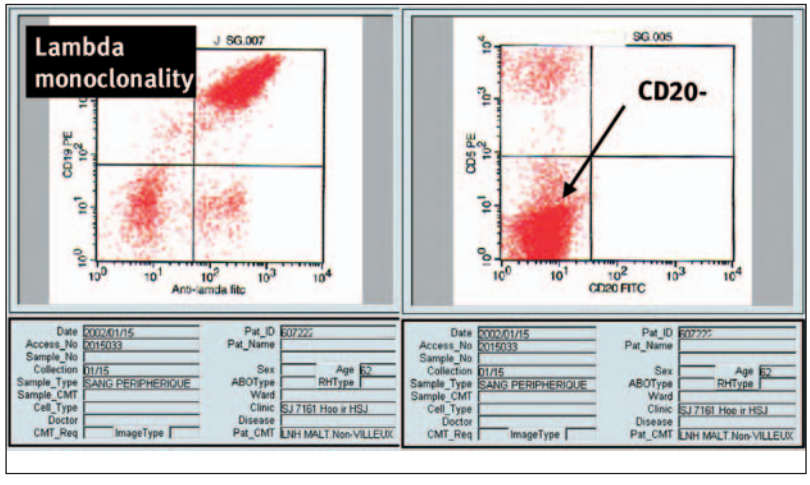


***Figure 16***
*Database –*
*Case 3 – second relapse.*

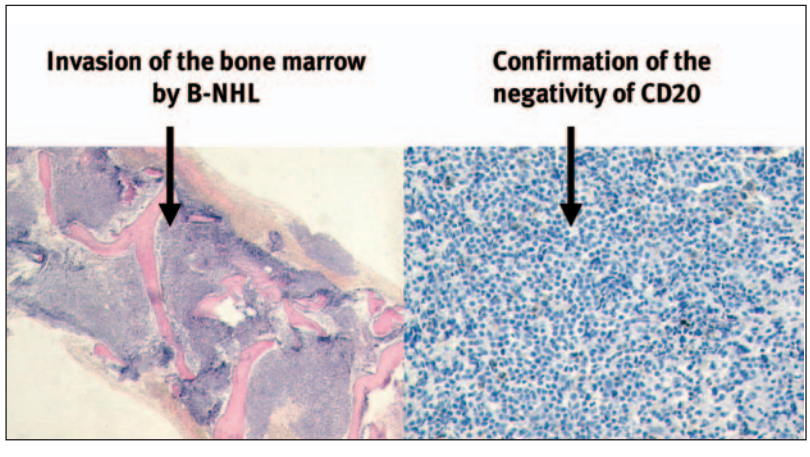


***Figure 17***
*Database –*
*Case 3 – bone marrow invasion and confirmation of the negativity for CD20.*

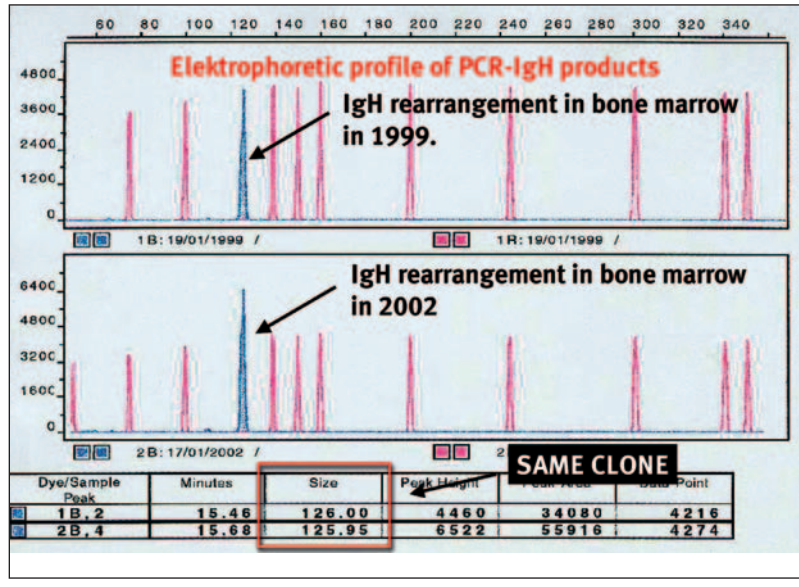


***Figure 18***
*Database –*
*Case 3 – electrophoretic profiles of PCR-IgH products of bone marrow.*

All the data and images described for these three cases reside within the LAFIA database and can be recalled, printed or transmitted at any time. LAFIA can also be used for non-haematology images as illustrated in **figure 19**. Finally image treatment is possible, either to alter colouring, or to add information to images.

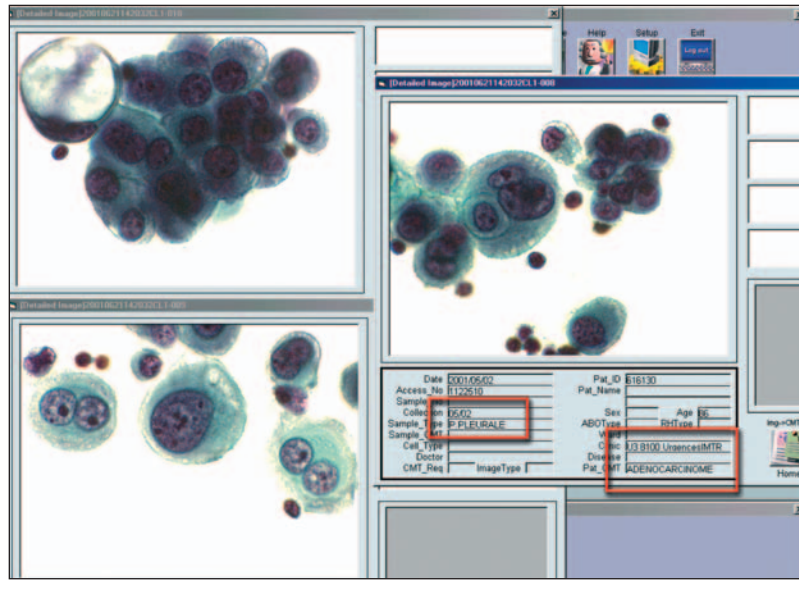


***Figure 19***
*Database – Example of storage of non-haematological images. In this case adenocarcinoma cells in pleural fluid.*

## Conclusions

While LAFIA is a very excellent tool in the haematology laboratory it could be improved even further. Dedicated locations within the database for cytogenetics and molecular biology would be valuable. Information about the staining used (e.g. May-Grünwald-Giemsa, special cytochemistry or Papanicolaou) and the magnification used would also be valuable. Finally, a diaporama (tape-slide presentation) facility or a gallery would be interesting for case presentations to multidisciplinary seminars.

In conclusion, in our hands, it has proved simple to build a database in haematology using LAFIA. The capture card and the Tri-CCD camera are of good quality. The ability to scan additional documents is very valuable. It is not time consuming since it is possible to capture images at the same time as reviewing smears, which is a very important point. The contacts between the cytologist and the pathologist are reinforced and excellent teaching material is produced.