The Role of Machine Learning in Medical Data Analysis. A Case Study: Flow Cytometry

Paolo Rota[1], Florian Kleber[1], Michael Reiter[1], Stefanie Groeneveld-Krentz[2] and Martin Kampel[1]
[1]*Computer Vision Lab (CVL), TU Wien, Vienna, Austria*
[2]*Charité - Universitaetsmedizin, Berlin, Germany*

Keywords: Flow Cytometry, Leukemia (ALL), Deep Learning, Stacked Auto Encoders, GMM.

Abstract: In last years automated medical data analysis turned out to be one of the frontiers of Machine Learning. Medical operators are still reluctant to rely completely in automated solutions at diagnosis stage. However, Machine Learning researchers have focused their attention in this field, proposing valuable methods having often an outcome comparable to human evaluation. In this paper we give a brief overview on the role of Computer Vision and Machine Learning in solving medical problems in an automatic (supervised or unsupervised) fashion, we consider then a case study of Flow Cytometry data analysis for MRD assessment in Acute Lymphoblastic Leukemia. The clinical evaluation procedure of this type of data consists in a time taking manual labeling that can be performed only after an intensive training, however sometimes different experience may lead to different opinions. We are therefore proposing two different approaches: the first is generative semi-supervised Gaussian Mixture Model based approach, the latter is a discriminative semi-supervised Deep Learning based approach.

1 INTRODUCTION

One of the recurrent questions is how Computer Vision and Machine Learning techniques are actually making the difference in the daily routine. Learning based applications have been successfully employed for word and image search (Zheng et al., 2015), semantic retrieval (Hofmanninger and Langs, 2015; Ramanathan et al., 2015), object classification (Gonzalez-Garcia et al., 2015) etc. These outstanding results have contributed to increase the consciousness of the potential of Machine Learning, directing the researcher's attention on other topics, targeting different applications where the error is by far less tolerated. These area of interest span from Video Surveillance to Medical Applications, crossing Biometrics and Bioinformatics.

Medical data analysis is a topic where human supervision has still a central position in every phase of the process, from diagnosis to each stage of the treatment. However research groups have focused their attention on medical data analysis with the purpose of automating different stages of the medical process (Yoo et al., 2012).

Image based medical data analysis often relies on Magnetic Resonance Imaging (MRI) or Positron Emission Tomography (PET). In (Zhu et al., 2014) the authors propose a joint regression and classification for Alzheimer's disease and Mild Cognitive Impairment diagnosis, analyzing the features in a novel framework composed by similarity matrix and loss sparse function reaching accuracy close to 100%. In (Hofmanninger and Langs, 2015) they use medical imaging in order to find correspondences between image segmentation and radiology reports bridging semantics to medical data. However MRI and PET are not the only possible source of information for high standard medical data analysis. In (Qureshi et al., 2014; Staal et al., 2004) the authors uses 2D color images of the retina in order to detect diabetes. In (Zhou et al., 2014) the authors focus their attention on multispectral images observed by the microscope in order to perform cell classification. Alternative technologies are used in order to extract important information from cellular tissue, one of the most popular is the Flow Cytometry (FCM), since it is a fast and cheap methodology for cell analysis. FCM is a laser-based biophysical technology that measures physical (size and granularity) and biological (different cell types can be detected with different markers) characteristics of single cells in fluid stream passing through a laser beam. FCM is currently widely used by oncol-

Rota, P., Kleber, F., Reiter, M., Groeneveld-Krentz, S. and Kampel, M.
The Role of Machine Learning in Medical Data Analysis. A Case Study: Flow Cytometry.
DOI: 10.5220/0005675903030310
In *Proceedings of the 11th Joint Conference on Computer Vision, Imaging and Computer Graphics Theory and Applications (VISIGRAPP 2016) - Volume 3: VISAPP*, pages 305-312
ISBN: 978-989-758-175-5

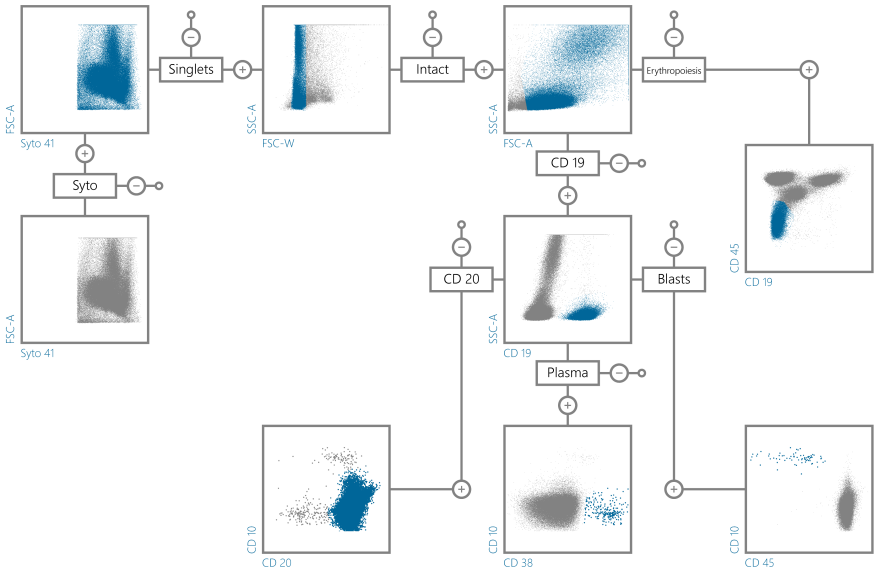


Figure 1: Example of gating hierarchy used for manual minimal residual disease assessment in B-ALL.

ogists to manually detect remaining leukemic cells in the bone marrow sample of a patient. Up to now analysis of FCM data is done manually.

In this paper we give an overview of the FCM technique, describing the data and the acquisition process, with particular interest to the Acute Lymphoblastic Leukaemia of type B (B-ALL) data. We focus on the criticalities of the FCM data analysis, examining the medical process highlighting the problems connected to the application of Machine Learning techniques on this type of data. The manual labeling strategy consists in a hierarchical procedure named *gating* that strongly relies on the skills and expertise of the FCM operator. To overcome this subjectivity issue we propose two automated, efficient and objective approaches to evaluate the FCM data; the first is supervised, based on deep learning and a second generative semi-supervised based on Gaussian Mixture Model (GMM). Minimal Residual Disease (MRD)is the number of remaining leukemic cells at certain time points during the treatment, allowing the doctors to tailor therapy intensity according to the response of each patient. The challenge of this methodology is that often certain cell populations are very small compared to the overall sample size (sometimes less than 0.1%).

The paper is structured as follows: in Sec. 2 is described the FCM data acquisition procedure and the way the assessment is performed by clinicians. In Sec. 3 we outline the two proposed methods to assess MRD in patients affected by B-ALL. In Sec. 4.1 we give a brief description of the dataset used for the evaluation stage which is described in Sec. 4. In Sec. 5 the results are presented and conclusions are drawn.

2 FLOW CYTOMETRY

In this section we describe briefly the FCM data from the acquisition to the MRD assessment in B-ALL. In order to perform the acquisition, the bone marrow sample must be prepared, this procedure is called *staining* and consists in adding a proper panel of conjugates (fluorochrome/antibodies combination) to the sample. The antibodies are ideally specifics to the protein expression of a certain cell type. The fluorophores attached to the antibodies, are excited by the laser beam of the Flow Cytometer. The stained cells inside the sample are pushed through in a single flow and measured by lasers with different wavelengths. Due to the possibility of noise, each measurement is called event. The fluorophores excitement is captured by an electronic sensor. The device will also produce physical measurement; the Forward Scatter (FSC, for size measurement) and the Side Scatter (SSC, for granularity measurement). A data value compensation is necessary due to the partial overlapping of the fluorescence spectra of the different fluorochromes, it is called *spillover compensation* and contributes in building the statistical independence of the data. The described procedure produces multiparameter readings for each event present in the sample.

In order to ease the comprehension to not familiar readers we propose a short description of the data generated by the flow cytometer:

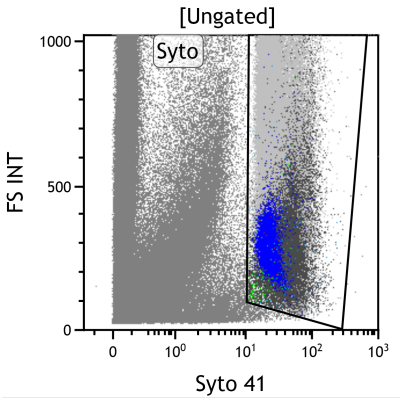


Figure 2: A sample as it is generated by the flow cytometer. The figure is representing the *Syto* gate on the entire set of cells in the 2D dimension composed by *Syto 41* and *Forward Scatter* parameters.

- **Sample.** A sample is the outcome of the measurement of a stained bone marrow sample, at a specific time point (an entire sample is shown in Fig. 2).
- **Event/Cell.** Every measurement sensed by the flow cytomenter during the analysis of a sample. Generally, an event refers to a cell. In Fig. 2 each point is an event.
- **Gate.** The labeling phase is performed manually by experts, it consists in drawing polygons on 2D scatter plots (see Fig. 2) following a hierarchical procedure (an example of gating hierarchy used for MRD assessment in ALL is shown in Fig. 1.The main criticality of the gating procedure that can affect the results is the operator subjectivity, which is shown in clinical ring trials and present deviations in MRD values (Dworzak et al., 2008).
- **Blasts.** The name blasts is referred to all the cells that have been considered leukemic by expert. The medical assessment is therefore performed counting the blasts events in relation to the whole test sample (MRD value).

2.1 Related Works

In last few years there has been an increasing number of approaches aiming at automating the FCM analysis process (Bashashati and Brinkman, 2009; Aghaeepour et al., 2013). The main objectives of these algorithms is to automatically assign each event to a specific biologically meaningful population, sometimes relatively small (i.e. ten events out of two millions). Unlike the manual gating, the automated methods perform the event clustering considering the whole multidimensional space at once. The outcome can be used in the clinical routine or for further automatic interpretation of the data. Most of the existing approaches are unsupervised clustering methods adapted to be very sensitive for small populations i.e. (Naim et al., 2014). In this paper the authors propose a revised GMM integrated with a splitting and merging procedure that is particularly suitable to outline small biologically meaningful populations. (Pyne et al., 2009) is an EM-based multivariate finite mixture model algorithm. The authors observed that the data clusters are often skew and heavily-tailed, for this reason they proposed a method that employs skew-t distributions. In (Finak et al., 2009) the authors use an adapted version of flowClust (Lo et al., 2008) to identify cell subpopulations, allowing the user to define the number of distinct cell populations.

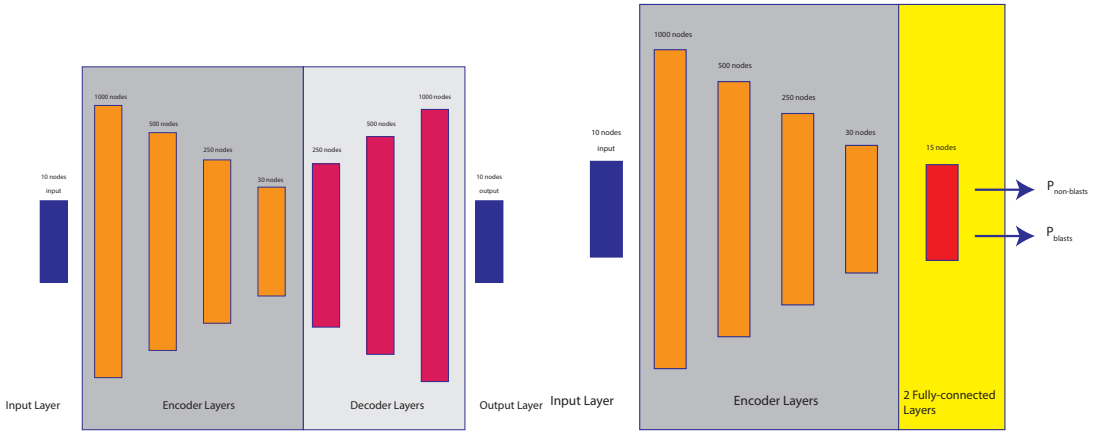
Regarding he automatic leukemic cell detection, in the work proposed by Costa et al. (Costa et al., 2010), the authors propose a supervised approach where new events are classified using a nearest neighbor classification in the 2D-principal subspace, obtained by principal component analysis of a labeled training set. In (Toedling et al., 2006), the authors propose a Support Vector Machines based framework to automate leukemic cell detection in cytometry where conventionally diagnosed data are used to train the classifiers. As in (Toedling et al., 2006), our interest is in classifying each event not only for discriminating different populations but also to identify that subset of events that corresponds to the blast population.

3 AUTOMATIC CELL CLASSIFICATION

In this section we give a detailed description of the two approaches proposed to estimate the MRD in FCS data. The first in Section 3.1 is fully discriminative and based on deep architecture of Neural Networks, the second is a generative approach based on the Gaussian Mixture Model and it is proposed in Section 3.2.

3.1 Stacked Auto-encoders Approach

Recently, in the computer vision community, the Convolutional Neural Networks (CNN) have shown success in many important tasks such as object recognition (Zhang et al., 2015; Krizhevsky et al., 2012; He et al., 2014), image segmentation (Cimpoi et al., 2015; Hariharan et al., 2014), head pose estimation (Conigliaro et al., 2015), to name a few. Although this architectures are successfully used on images and



(a) (b)

Figure 3: Scheme of the model adopted for the Stacked Auto-Encoders. On upper part (a) the unsupervised phase where the first 4 layer are learned directly from the data. In the sketch at the bottom (b) the supervised part of the network with two extra fully connected layers in cascade to the encoders is shown.

videos, in FCM data this approach is not yet established. In images, neighboring pixels are highly related to each other, this spacial property is not present in FCM data. Parameters, in this domain, are indeed standalone features concatenated without specific and predefined order in an one-dimensional array. The combination of those features lead to the detection of meaningful populations that are not always positioned in the same location of the feature space.

In order to give a deep description of FCM data we propose an approach based on a deep network based on a Stacked layout of Auto-Encoders (SAE) (Bengio, 2009; Vincent et al., 2010). This network, unlike the CNN is more general and easily applicable to different type of data. There are two major reasons for our choice: Firstly, this neural network, unlike CNN, has more general purpose and it is easy to apply on different types of data. The second reason is that this type of neural network is extremely useful in discovering interesting structure in the data (Bengio, 2009). The proposed SAE architecture is composed by the input layer of size 10x1, that is the number of parameters used to describe an event, this means that tis approach is *event oriented*, and we are trying to find a multi-dimensional hyperplane capable to separate the two final classes (details on the structure are noted in Fig. 3). The training phase of the network consists of two steps: firstly an unsupervised approach, in which the network is forced to learn the data structure from the training proposing a new interpretation of the input features. The second is the supervised step, in which the output of the net is forced to the labels in order to adapt the weights to produce the final inference.

3.2 Gaussian Mixture Model Approach

In our particular case, the cardinality of the dataset, would make too computational demanding an approach based on kernel model estimation. A viable solution is to estimate the model approximating the training set with a distribution generated by a parametrized distribution. The Gaussian Mixture Model (GMM) is widely used approach to fulfill this task [1]. Because of its flexibility in FCM data analysis, in particular for population clustering (Naim et al., 2014), the GMM leads to very good results. This generative approach has the ability of fitting point cloud distributions reducing the number of parameters comparing to a kernel based method.

In ALL data, as mentioned in Sec. 1, the blasts population is often very small with respect to the whole set of events. In order to avoid losing information on the small populations and to semantically give meaning to those distributions, we learned two different models for blasts and non-blasts separately. The estimation of such model is performed by a modified Expectation Maximization (EM) algorithm similar to (Naim et al., 2014). The two distributions are generated by 10 and 2 gaussians for blasts and non-blasts populations respectively. These values are the empirical results of tests as an acceptable compromise between accuracy and computation burden. The final model is the result of merging the two distributions by averaging their components. The resulting distribution is shown in Fig. 4. Unlike (Naim et al., 2014) our interest is not only in discriminating dif-

[1] We remand to (Bishop, 2006) for the theoretical description of the model.

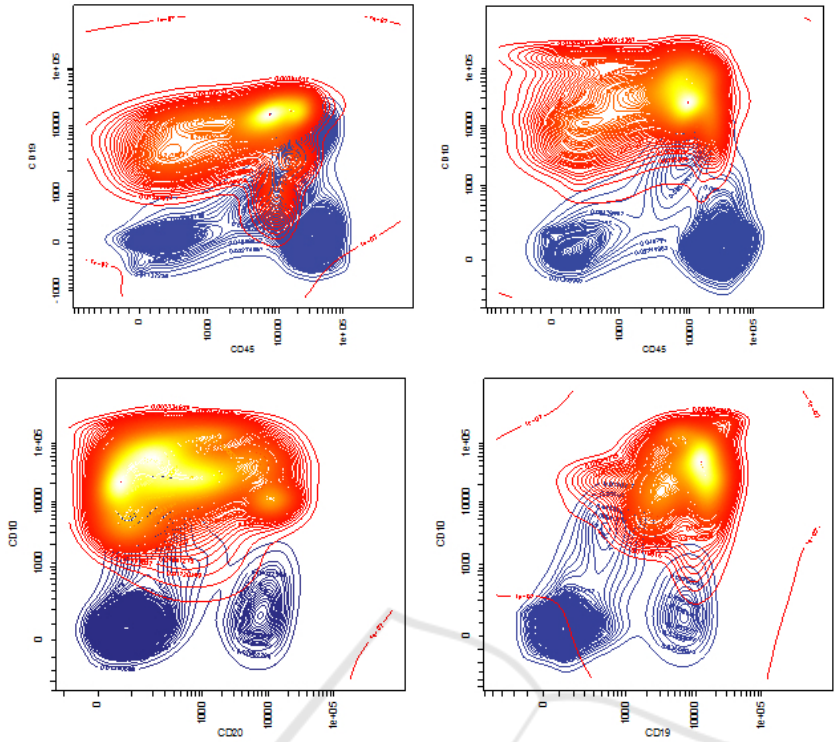


Figure 4: The result of the modeling performed by the GMM, we can distinguish in red the blasts population and in blue the non-blasts.

ferent populations but also in the blasts identification, therefore the classification of observations of a new sample is done by a Bayes decision using the posteriors obtained from the GMM components, where the priors are set according to the average relative frequencies of leukemic events in the training set. In Fig. 5 we can see a 2D projection of the samples with respective gates drawn (A), and a GMM approximation of the point distribution (B), the model of non blasts (C) and blasts (D) respectively highlighted.

4 EVALUATION

MRD is the relative frequency between the blasts cells and the overall number of events in a sample as it is stated in Eq. (1)

$$MRD^{(i)} = N^{(i)}_{blasts}/N^{(i)}_{events} \quad (1)$$

where i refers to the i-th sample.

4.1 Dataset Description

In order to evaluate the performance of the algorithms, we collected FCM-MRD measurements from 200 ALL patients treated according to the AIEOP-BFM 2009 protocol. MRD was measured in bone marrow samples of treatment day 15. Sample preparation and MRD assessment was performed following the current international standard operating procedure for 6color FCM-MRD detection. All FCM datasets were gated manually by experienced operators using a uniform gating procedure that is depicted in Fig. 1, however, the parameters on which the blasts gate is defined may change among samples according to the appearance of the sample and operator experience.

The FCM output provides for each individual cell 10 different parameters (three optical [FSC-A, FSC-W, SSC-A] and seven fluorescence based parameters [CD20, CD10, CD45, CD34, SYTO 41, CD19, CD38]). Each cell parameter becomes/represents a dimension in the multidimensional data space.

For the evaluation the dataset has been divided in two groups, training and test composed by 184 and 16 samples respectively. The division has been performed randomly pooling the whole set of data. Validation for parameter tuning has been performed on a random 30% of the training set. This operation has been performed for 11 times in order to enlarge the test set, resulting in a final test set composed by 176 samples.

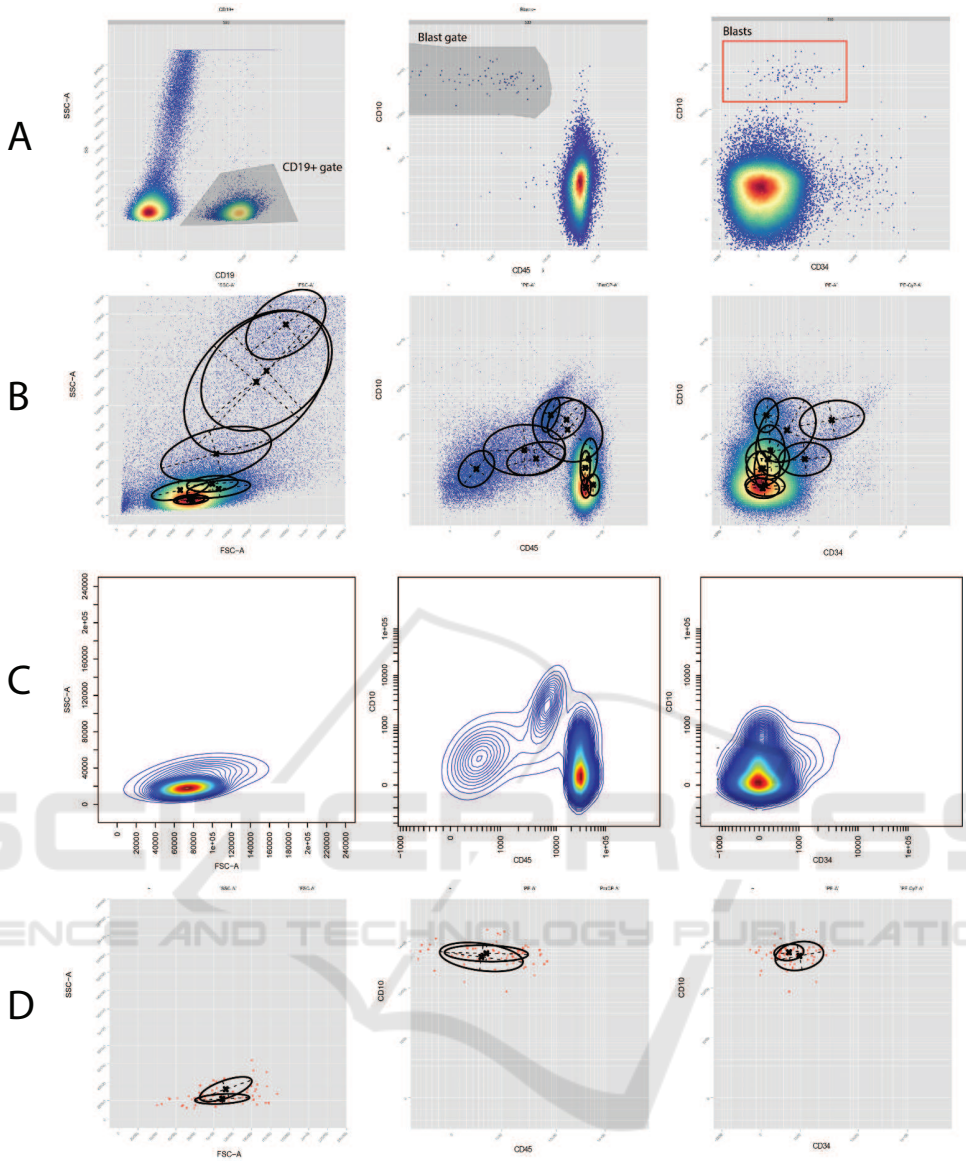


Figure 5: In row (A) is shown the gating as it has been performed manually by medical experts. In row (B) the GMM components are sketched as they are trained by the EM process. In row (C) a representation of the PDF related to the non-blasts population while in row (D) the model for blasts is shown along with the classified events.

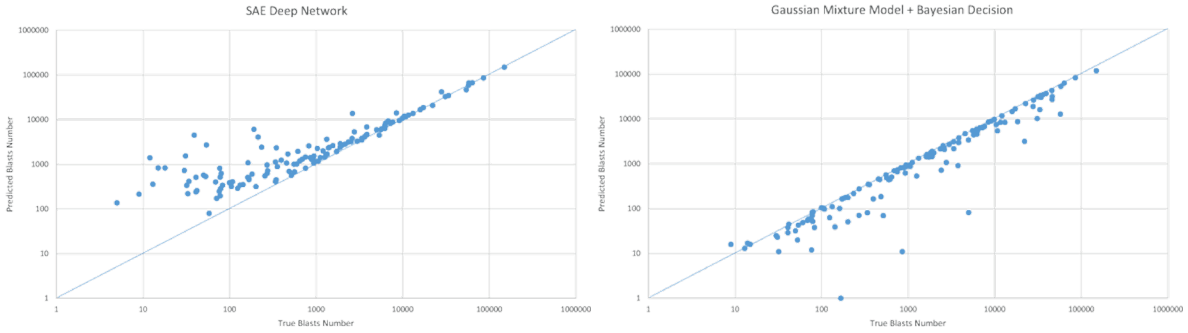
4.2 Results

In order to fairly compare the approaches presented in this paper, we show the results in two different forms: graphical and numerical. The results proposed in graphical form are scatter plots, where each point represents one sample. The 2D coordinates of each point are the values of true blasts in relation with the predicted quantity. An ideal algorithm will produce a scatter plot with samples disposed along the line $y = x$ (see Fig. 6).

In order to assess numerically the performance of the algorithms we propose a comparison in terms of mean square error (MSE) of the blast cells found in each sample:

$$\frac{1}{N_e}\sum_{N_e}(Blasts_t - Blasts_p)^2 \qquad (2)$$

where N_e is the overall number of test samples, B_t and B_p are the number of true and predicted blasts in the single sample. Numerical results are proposed in Tab. 1



(a) (b)

Figure 6: Resulting graphs for the three approaches: (a) SAE and (c) the GMM based approach.

Table 1: The numerical results of the baseline compared to the proposed method. The mean and variance are referred to the absolute value of the difference between the automated MRD and the true MRD.

Method	*MSE*	*MSE Variance*
SVM	0.01467	0.000637
SAE Deep Network	0.00508	0.000121
GMM + Bayes Decision	0.00891	0.000739

5 DISCUSSION

Performing an accurate MRD estimation in FCM data with an automatic algorithm turns out to be a hard undertaking, either using discriminative or generative approaches. In the latter method we notice an important tendency to underestimate the number of blasts in the sample. This might be caused by the criticality in finding a unique value for the component priors of the model.

A critical drawback of the methods employed in this work is that they construct fix decision regions, no adaptation is provided for new unseen and complicated cases. Medical experts, use interpretation, based on their expertise in order to draw the correct gating around the blasts events, as result of several consideration about the whole sample. Because of this fact, the blasts gate can be drawn in a totally different position with respect to a similar sample. This leads to a non negligible error from an automatic classifier based on fix decision. This last observation stands in favor of the generative approach since it is, unlike the Deep Network, sample oriented, while the other two are event oriented. In this work the Deep network are considering all training events as part of an unique huge sample, this becomes a drawback during the test phase since no sample structure is learned. In all three cases however, the resolution of the algorithms, in terms of accuracy at low MRD levels (below 1000 events) is not sufficient for the clinical routine.

In conclusion, as future work, we will consider an extension of these approaches that, using the trained model as a starting point, it will adapt to the new sample refining the inference also in the most critical zone of the graph.

ACKNOWLEDGEMENT

AutoFLOW project is supported by the European Commission FP7-PEOPLE-2013-IAPP 610872. We also gratefully acknowledge NVIDIA Corporation for the donation of the Titan X GPU used for this research.

REFERENCES

Aghaeepour, N., Finak, G., Hoos, H., Mosmann, T. R., Brinkman, R., Gottardo, R., Scheuermann, R. H., Consortium, F., Consortium, D., et al. (2013). Critical assessment of automated flow cytometry data analysis techniques. *Nature methods*, 10(3):228–238.

Bashashati, A. and Brinkman, R. R. (2009). A survey of flow cytometry data analysis methods. *Advances in bioinformatics*, 2009:584603–584603.

Bengio, Y. (2009). Learning deep architectures for ai. *Machine Learning*, 2(1):1–127.

Bishop, C. M. (2006). *Pattern recognition and machine learning*. springer.

Cimpoi, M., Maji, S., and Vedaldi, A. (2015). Deep filter banks for texture recognition and segmentation. In *Conference on Computer Vision and Pattern Recognition*. IEEE.

Conigliaro, D., Rota, P., Setti, F., Bassetti, C., Conci, N., Sebe, N., and Cristani, M. (2015). The s-hock dataset: Analyzing crowds at the stadium. In *Conference on Computer Vision and Pattern Recognition*. IEEE.

Costa, E., Pedreira, C. E., Barrena, S., Lecrevisse, Q., Flores, J., Quijano, S., Almeida, J., del Carmen Garcia-Macias, M., Bottcher, S., Van Dongen, J., et al. (2010). Automated pattern-guided principal component analysis vs expert-based immunophenotypic classification of b-cell chronic lymphoproliferative disorders: a step forward in the standardization of clinical immunophenotyping. *Leukemia*, 24(11):1927–1933.

Dworzak, M. N., Gaipa, G., Ratei, R., Veltroni, M., Schumich, A., Maglia, O., Karawajew, L., Benetello, A., Pötschger, U., Husak, Z., et al. (2008). Standardization of flow cytometric minimal residual disease evaluation in acute lymphoblastic leukemia: Multicentric assessment is feasible. *Cytometry Part B: Clinical Cytometry*, 74(6):331–340.

Finak, G., Bashashati, A., Brinkman, R., and Gottardo, R. (2009). Merging mixture components for cell population identification in flow cytometry. *Advances in Bioinformatics*, 2009.

Gonzalez-Garcia, A., Vezhnevets, A., and Ferrari, V. (2015). An active search strategy for efficient object class detection. In *Conference on Computer Vision and Pattern Recognition*. IEEE.

Hariharan, B., Arbeláez, P., Girshick, R., and Malik, J. (2014). Hypercolumns for object segmentation and fine-grained localization. *arXiv preprint arXiv:1411.5752*.

He, K., Zhang, X., Ren, S., and Sun, J. (2014). Spatial pyramid pooling in deep convolutional networks for visual recognition. In *European Conference of Computer Vision*. Springer.

Hofmanninger, J. and Langs, G. (2015). Mapping visual features to semantic profiles for retrieval in medical imaging. In *Conference on Computer Vision and Pattern Recognition*. IEEE.

Krizhevsky, A., Sutskever, I., and Hinton, G. E. (2012). Imagenet classification with deep convolutional neural networks. In *Advances in neural information processing systems*, pages 1097–1105.

Lo, K., Brinkman, R. R., and Gottardo, R. (2008). Automated gating of flow cytometry data via robust model-based clustering. *Cytometry Part A*, 73(4):321–332.

Naim, I., Datta, S., Rebhahn, J., Cavenaugh, J. S., Mosmann, T. R., and Sharma, G. (2014). Swift - scalable clustering for automated identification of rare cell populations in large, high-dimensional flow cytometry datasets, part 1: Algorithm design. *Cytometry Part A*, 85(5):408–421.

Pyne, S., Hu, X., Wang, K., Rossin, E., Lin, T.-I., Maier, L. M., Baecher-Allan, C., McLachlan, G. J., Tamayo, P., Hafler, D. A., et al. (2009). Automated high-dimensional flow cytometric data analysis. *Proceedings of the National Academy of Sciences*, 106(21):8519–8524.

Qureshi, T. A., Hunter, A., and Al-Diri, B. (2014). A bayesian framework for the local configuration of retinal junctions. In *Conference on Computer Vision and Pattern Recognition*. IEEE.

Ramanathan, V., Li, C., Deng, J., Han, W., Li, Z., Gu, K., Song, Y., Bengio, S., Rossenberg, C., and Fei-Fei, L. (2015). Learning semantic relationships for better action retrieval in images. In *Conference on Computer Vision and Pattern Recognition*. IEEE.

Staal, J., Abràmoff, M. D., Niemeijer, M., Viergever, M., Van Ginneken, B., et al. (2004). Ridge-based vessel segmentation in color images of the retina. *Medical Imaging, IEEE Transactions on*, 23(4):501–509.

Toedling, J., Rhein, P., Ratei, R., Karawajew, L., and Spang, R. (2006). Automated in-silico detection of cell populations in flow cytometry readouts and its application to leukemia disease monitoring. *BMC bioinformatics*, 7:282–282.

Vincent, P., Larochelle, H., Lajoie, I., Bengio, Y., and Manzagol, P.-A. (2010). Stacked denoising autoencoders: Learning useful representations in a deep network with a local denoising criterion. *The Journal of Machine Learning Research*, 11:3371–3408.

Yoo, I., Alafaireet, P., Marinov, M., Pena-Hernandez, K., Gopidi, R., Chang, J.-F., and Hua, L. (2012). Data mining in healthcare and biomedicine: a survey of the literature. *Journal of medical systems*, 36(4):2431–2448.

Zhang, Y., Sohn, K., Villegas, R., Pan, G., and Lee, H. (2015). Improving object detection with deep convolutional networks via bayesian optimization and structured prediction. In *Conference on Computer Vision and Pattern Recognition*. IEEE.

Zheng, L., Wang, S., Tian, L., He, F., Liu, Z., and Tian, Q. (2015). Query-adaptive late fusion for image search and person re-identification. In *Conference on Computer Vision and Pattern Recognition*. IEEE.

Zhou, Y., Chang, H., Barner, K., Spellman, P., and Parvin, B. (2014). Classification of histology sections via multispectral convolutional sparse coding. In *Conference on Computer Vision and Pattern Recognition*. IEEE.

Zhu, X., Suk, H.-I., and Shen, D. (2014). Matrix-similarity based loss function and feature selection for alzheimer's disease diagnosis. In *Conference on Computer Vision and Pattern Recognition*. IEEE.