

Application of Machine Learning for Automatic MRD Assessment in Paediatric Acute Myeloid Leukaemia

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Abstract: Acute Myeloid Leukaemia (AML) is a rare type of blood cancer in children. This disease originates from genetic alterations of hematopoietic progenitor cells, which are involved in the hematopoiesis process, and leads to the proliferation of undifferentiated (leukaemic) cells. Flow Cytometry (FCM) measurements enable the assessment of the Minimal Residual Disease (MRD), a value which clinicians use as powerful predictor for treatment response and diagnostic tool for planning patients' individual therapy. In this work we propose machine learning applications for the automatic MRD assessment in AML. Recent approaches focus on childhood Acute Lymphoblastic Leukaemia (ALL), more common in this population. We perform experiments regarding the performance of state-of-the-art algorithms and provide a novel GMM formulation to estimate leukaemic cell populations by learning background (non-cancer) populations only. Additionally, combination of backgrounds of different leukaemia types are evaluated regarding their ability to predict MRD in AML. The results suggest that background populations and combinations of these are suitable to assess MRD in AML.

1 INTRODUCTION

Acute Myeloid Leukaemia (AML) is the most common leukaemia type in adults, which incidence increases with age (Juliussen et al., 2009) and accounts for 20 percent of leukaemias in children (Creutzig et al., 2013b). The peaks of the AML prevalence in the United States lie in childhood between the age of 0 and 1 year at 18.4 per million, children ages 5 to 9 years 4.3 per million and at 7.7 per million for ages between 10 to 14 years (Puumala et al., 2013). Children at ages younger than 15 years at the time point of diagnosis have a five year survival rate of approximately 70 percent, dependent on the AML subtype (Creutzig et al., 2013b). It affects the blood generation caused by genetic lesions of myeloid progenitor cells and leads to a decrease of the number of mature blood cells and an increase of the number of malignant progenitor cells (Puumala et al., 2013).

1.1 MRD assessment in AML

For determining the clinical outcome and for the stratification according to risk for relapse, clinicians observe genetic features (Rubnitz and Inaba, 2012) to retrieve the Minimal Residual Disease (MRD). MRD is a prognostic value, which is used as an indicator for treatment response and to quantify the remaining leukaemic cells (blasts) at defined therapeutic time points (Brüggemann et al., 2010). It has been identified as a powerful predictor for treatment outcome

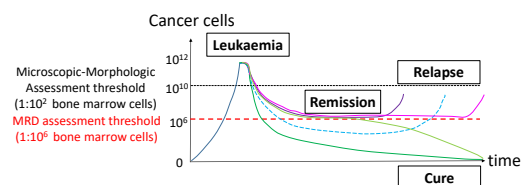


Figure 1: MRD assessment in different therapeutic stadia of AML.

and thus is used as guiding diagnostic tool for planning the intensity of treatment of an individual patient. It encodes the proportion of leukaemic blasts among the amount of normal cells observed. Figure 1 illustrates schematically the relations of MRD to different leukaemic cell stadia during treatment: Leukaemia, Remission, Relapse and Cure. The treatment of AML is divided into three phases (Löwenberg et al., 2003), (Rubnitz and Inaba, 2012), (Creutzig et al., 2013a):

- Induction therapy (day 1 - 33): Remission induction targets a Complete Remission (CR). CR is achieved if less than 5% of blasts are in cellular marrow, no blast in the circulation, no presence of extramedullary leukaemia and a regeneration of platelets and granulocytes, resulting in increased counts.
- Consolidation (day 33 - 78): The second phase aims at the removal of MRD after patients have recovered from the previous phase in a rest period (Löwenberg et al., 2003).
- Intensification: The third phase focuses on the treatment after remission, consisting of e.g. prolonged chemotherapy (1-2 years) or Stem Cell Transplantation (SCT). Two types of SCT exist (Löwenberg et al., 2003). In case of AML, autologous SCT is rarely recommended, but allogeneic Hematopoietic SCT (HSCT) is a reasonable option, for resistant or high risk cases in first remission. However, HSCT is strongly recommended for most children with AML after relapse (Rubnitz and Inaba, 2012).

One cause of morbidity and mortality in AML beside the disease itself are complications induced by infections, haemorrhage or side effects caused by the highly haematotoxic and immunosuppressive therapy (Creutzig et al., 2013b). Thus, additionally prophylactic therapies are considered to reduce the incidence of bacterial or fungal infection as well as supportive therapy (Rubnitz and Inaba, 2012). Treatment is guided by treatment protocols, evaluated by performing international clinical trials over several years, to ensure quality and safety (Creutzig et al., 2013a).

1.2 Flow Cytometry

Flow Cytometry (FCM) enables a reliable MRD assessment, in a more cost- and time effective way than polymerase chain reaction (Gaipa et al., 2012) by detecting leukaemia specific immunophenotypes (Basso et al., 2009; Dworzak et al., 2002).

For this technique it is required to draw a blood or bone marrow sample of a patient in a first step

and subsequently, mark cellular antigens in a *staining* step with a combination of specific fluorescence-labelled antibodies. Dependent on the antigen expression of a single cell, different fluorescence signal patterns are detectable using FCM. Its biophysical technology is based on lasers of different wavelengths, which employment enables the measurement of physical (granularity, size) and biological characteristics of every single cell in a fluid stream and establishes the difference between normal blood, bone marrow or leukaemic cells (Rota et al., 2016). The challenges assessing MRD using FCM lie especially in the late phases of induction and consolidation therapy, where it is particularly important to detect small leukaemic cell populations, which compose about 0.1% of all observed cells, to be able to adapt therapy if a risk of relapse is determined. Additional challenges in FCM lie in the limited number of cells in the test tube and in the influencing factors for MRD assessment, as treatment- or age-related variances of the regeneration status of bone marrow precursors (Gaipa et al., 2012).

1.2.1 Manual Gating

Current FCM based MRD assessment is performed manually, where operators draw polygons (gates) around relevant cell populations in two-dimensional graphical representations (dot plots) (cf. Figure 2) of multi-dimensional FCM data. The scale of each gate's axis is of logarithmic scale and one dimension corresponds to a FCM measured feature. In Figure 2 every dot (*event*) represents a measured blood cell. In diagnostic laboratories a hierarchical gating procedure is manually executed to detect MRD. The identified events of interest of a gating step serve as input of the

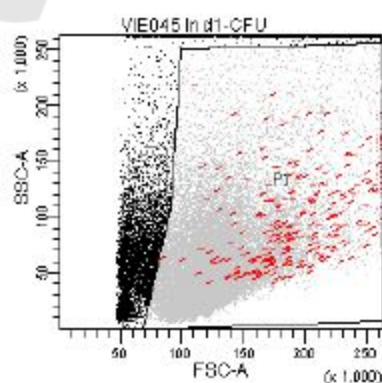


Figure 2: Illustration of a sample obtained by a flow cytometer and the manual drawn viable gate (polygon) composed by the features Side Scatter (SSC-A) and Forward Scatter (FSC-A). Leukaemic cells are illustrated in red, viable cells in gray, and non viable in black.

subsequent gate in the hierarchy. In a first step the gate is defined to identify nucleated cells in a *sample* (all cells of a patient's FCM measurement). For this gate the granularity measure Side SCatter-Area (*SSC-A*) and the size measure Front SCatter-Width (*FSC-W*) are observed. By observing the Side SCatter-Area (*SSC-A*) and the CD 45 feature (fluorescence marker) the relevant cells (leukocytes) are filtered. The next step (*CD34+ or progenitor gate*) excludes cells that are more mature and thus, CD 34 negative. For detecting leukaemic cells subsequently, CD 117 positive and CD 33 positive cells are observed to define a *blast gate*. The manual gating procedure introduced strongly relies on the operator's skills and expertise, is highly subjective and time-consuming.

1.3 Contribution

Recent automated machine learning approaches applied on childhood leukaemia datasets focus on modelling leukaemic and non-leukaemic cells for Acute Lymphoblastic Leukaemia (ALL) (Licandro et al., 2016), (Naim et al., 2014), (Zare et al., 2010), (Aghaeepour et al., 2013), (Bashashati and Brinkman, 2009), (Reiter et al., 2016) and have as main goal the automatic assignment of a biologically meaningful population label to every observed cell. Instead of using a 2D feature representation, the multidimensional space is included at once in the automatic gating procedure. In contrast to AML, ALL is caused by genetic lesions of lymphoid blood-progenitor cells differentiating to T-cells (T-ALL) or B-cells (B-ALL), which consequently leads to the proliferation of aberrant (leukaemic) cells. The peaks of ALL prevalence are higher compared to AML and lie between the age of 2 and 5 years for B-ALL and at the age of 10 years for T-ALL's (Pui et al., 2008), (Inaba et al., 2013).

The contribution of the work proposed is three fold: First we want to demonstrate the applicability of state of the art machine learning algorithms on flow cytometry childhood AML data. Second we propose a novel background formulation for Gaussian Mixture Model based classification to assess MRD distributions in AML of small size. Finally, we combine background (non-cancer cells) of ALL and AML samples and evaluate if background outlier distributions can identify leukaemic cells more efficiently compared to simple backgrounds.

We hypothesize that blasts form outlier populations when background populations are observed in the multi-dimensional feature space and that a combination of background cells of different leukaemia types can be used to enable the enhancement of non

blast representations and robust modelling of background distributions for blast identification.

This paper gives an overview of methodologies and the dataset used in Section 2. The evaluation results are presented in Section 3 and the conclusion of this work and possibilities for future work are summarized in Section 4.

2 METHODOLOGY

This section introduces the formulation of Background Gaussian Mixture Model classification for leukaemic cell detection and summarizes the experimental setup of the additional machine learning approaches (Random Forest and Support Vector Machine) applied for automatic cell classification. For every approach the MRD assessment performance is evaluated by computing the ratio between predicted leukaemic cells N_{blasts} and normal cells $N_{normal-cells}$ as expressed in Equation 1.

$$MRD = \frac{N_{blasts}}{N_{normal-cells}} \quad (1)$$

Thus, for every approach the solving of a binary classification problem (blast, non-blast) for every measured cell in a sample and the estimation of cell counts for a class, are required to assess the MRD. In contrast to manual gating, the machine learning techniques evaluated within this work, observe the multidimensional feature space. The 13 features measured in our case correspond to the expression of ten different types of antibodies on the cell surface and three physical FCM measures (cf. Section 3.1 for details regarding the datasets used). Dependent on the condition of the patient, approximately $10^5 - 10^6$ cells are measured per subject. Additionally, manual annotations of blast and non blast cells are provided by medical experts.

2.1 Background Gaussian Mixture Model

As the first approach a Gaussian Mixture Model (GMM) based formulation is used to cluster and automatically classify cells into leukaemic and normal cells. GMMs are widely used, and known to be flexible in the analysis of FCM data and are less computational demanding compared to kernel model estimation based approaches (Naim et al., 2014), (Bishop, 2006). This generative approach is able to fit point cloud distributions, while keeping the model based description and using a restricted amount of parameters. We decided to model the distribution of non-blast populations (background) only, since more

background data without blasts are available. In an initial step a GMM model for non-blasts is learned by using an adapted Expectation Maximization (EM) algorithm and 2 Gaussian distributions. The trained GMM is used to detect and furthermore analyse cells lying outside the learned probability density function. A cell is classified as non-blast if the log probability is greater than 0 and as outlier if it is smaller. In a subsequent step the outliers are modelled using an additional GMM with 1 component. A cell in the outlier population is classified as blast if the log probability is greater than 3 and as non-blast if it is smaller. The number of Gaussian distributions and the log probability were estimated based on the results of preliminary experiments, where different parametrisations were tested.

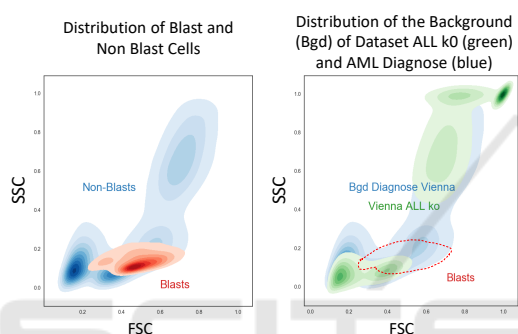


Figure 3: Visualisation of the distribution of non blast and blast blood cells population of the dataset *Diagnose* (left). On the right the difference between the background of the dataset *Diagnose* (blue) and *ALL k0* (green) are visualised and their relations to the blast population (red line) in the dataset *Diagnose*.

In Figure 3 on the left side the distribution of background cells (blue) of 13 samples of diagnosed AML cases are visualised, where blasts are visualised in red. On the right the same background (blue) is shown in relation to the background (green) extracted from 30 subjects diagnosed with ALL in the remission state where no blasts are present. First it is observable that the different background distributions have an overlying appearance in the feature space and second blast populations lie in regions of less density of the background's distribution.

2.2 Random Forest Classifier

As the second approach we evaluated the ensemble classifier Random Forest (RF) (Breiman, 2001). Its formulation is based on decision trees, where a random training subset of the FCM data is defined for each tree. For finding a maximum separation between leukaemic and non leukaemic cells, every node in the decision tree performs thresholding on the measured

features. By searching over a random subset of antibody features a new node in the decision tree is constructed (Langs et al., 2011) taking into account the decisions of the higher tree levels. In comparison to the GMM approach the RF is trained in a supervised way using the manual annotation labels of every cell. In the test phase one label for every cell of a new input sample (1 blast, 0 non-blast) is computed based on the RF trained. Details regarding the parametrisation of the RF classifier are given in Section 3.2.

2.3 Support Vector Machine

The Support Vector Machine (SVM) approach is used as a baseline to provide a comparison between its classification and those performed by RF and GMM. In the experiment proposed we use a RBF kernel based formulation of SVM. Sample classification is performed based on events, without including information about the neighbored events or the different populations observed. Also the SVM is trained in a supervised way. In the test phase one label for every cell of a new input sample (1 blast, 0 non-blast) is computed based on the SVM trained. Details regarding the parametrisation of the SVM classifier are given in Section 3.2.

3 RESULTS

In this section first the dataset and the preprocessing steps are introduced and second a description of the evaluation setup and results are presented.

3.1 Acute Leukaemia Datasets

The sample preparation and manual MRD assessment are performed at the national diagnostic reference center for paediatric AML according to the current international standard operating procedure for 10 color FCM-MRD detection. For each cell, thirteen parameters are obtained by the FCM measurement, consisting of three optical (FSC-A, FSC-W, SSC-A) and ten fluorescence based parameters which are tuned according to the leukaemia type. One feature represents a dimension in the multidimensional data space. Due to the partial overlapping of fluorescence spectra of different fluorochromes used, *spillover compensation* is applied to obtain statistical independence of the data by using a correction matrix. As last preprocessing step normalization of the parameter values is performed to obtain a range between 0 and 1. The dataset used in this work was generated in collaboration with experienced clinicians from the Children's Cancer Re-

search Institute in Vienna. All participants' guardians (parents) and patients were informed about the aim of the study and gave their written, informed consent prior to inclusion.

3.1.1 Dataset AML Diagnose

The dataset consists of FCM measurements of 13 AML patients whose therapy was guided according to the AML BFM 2004 treatment protocol (Creutzig et al., 2013b)¹. The fluorescence based parameters used are CD15, CD7CD19, CD34, CD117, CD33, CD13, CD11b, CD14, HLA-DR, CD45.

3.1.2 Dataset ALLk0 Background

The dataset contains 24 FCM measurements of 30 ALL patients in the remission phase using the same fluorescence based parameters as the AML Diagnose dataset, where no blasts are present. The therapy was guided according to the AIEOP-BFM 2009 trial².

3.2 Evaluation Setup

According to the small amount of available annotated data we perform Leave One Out Cross Validation for every approach evaluated. The proposed GMM approach is trained using the background annotated cells only, while RF and SVM are trained on blast and non blast populations. The pipeline is implemented using the scikit-learn package for Python (Pedregosa et al., 2011). The SVM uses the following parametrisation: $C=1.0$, $cache_size=200$, $degree=3$, $gamma='auto'$, $kernel='rbf'$, $tol=0.001$. For the Random Forest classifier 1000 estimators and following additional parameters are used: $criterion='gini'$, $minimal_samples_split=2$, $min_samples_leaf=1$, $min_weight_fraction_leaf=0.0$, $min_impurity_split=1e-07$, $bootstrap=True$. For the GMM approach we use 2 Gaussian components for modelling non blasts and one component to model outliers (cf. Section for details 2.1), $covariance_type='full'$, $n_iter=10000$ and $n_init=1$. The parametrisation of every approach was defined based on the best performance achieved in

¹AML BFM 2004 is a conducted randomized clinical trial for children and adolescents with AML between age 0-18 years with 722 patients https://www.kinderkrebsinfo.de/health_professionals/clinical_trials/closed_trials/aml_bfm_2004/index_eng.html [accessed 2017-10-29]

²AIEOP-BFM 2009 is a conducted randomized clinical trial for ALL between age 1-18 years in 10 countries in- and outside Europe, with approximately 1000 patients observed per year (Dworzak, 2013) <http://www.bfm-international.org/> [accessed 2017-10-29]

preliminary experiments. Additionally, precision, recall and f-score are computed as quantitative score to compare approaches and labeling results of different datasets (Powers, 2011).

3.3 MRD Assessment of Paediatric Acute Myeloid Leukaemia

In a first step we analyse the performance of state-of-the-art algorithms regarding their classification accuracy of blast populations of childhood AML Diagnose data. Only the background of Diagnose cases in this dataset is used for training. Table 1 summarizes the evaluation results in the first three rows (RF, SVM and GMM). SVM shows the best performance. In

Table 1: MRD assessment performance of childhood AML.

Method	Precision	Recall	f-score
RF	0.76219	0.46249	0.57567
SVM	0.61986	0.58044	0.59951
GMM	0.44836	0.26391	0.33226
RFBgd	0.74169	0.39596	0.51629
SVMBgd	0.68014	0.53149	0.59669
GMMBgd	0.43861	0.26099	0.32725

a second step we analyse the performance of state-of-the-art algorithms regarding their classification accuracy of blast populations of childhood AML Diagnose data, but with a combined background. Therefore non blast cells from the dataset ALLk0 and AML Diagnose are merged and used for training. Table 1 summarizes the evaluation results in row 4 to 6 (RFBgd, SVMBgd and GMMBgd). In comparison to the simple background evaluation a decrease of performance of RF and GMM is observable and an increase of the SVM precision, when using the combination of backgrounds. In Figure 4 the MRD assessment accuracy of the evaluated algorithms for simple and combined background are visualised. A point corresponds to a sample for which the true and predicted MRD is plotted. Samples lying outside the accuracy threshold are drawn red, samples inside are visualised blue. The accuracy threshold was defined by clinicians. In case of GMM the failed predictions of MRD lies closer to the true MRD compared to RF and SVM failed cases, which underestimated the MRD in a wider range. In Figure 5 the classification results of the simple background (1st and 3rd row) and combined background (2nd and 4th row) analysis are qualitatively visualised for RF (1st column), SVM (2nd column) and GMM (3rd column) for two subjects. The corresponding manual annotations are shown in column 4. Additionally, the computed MRD values for every experiment are provided.

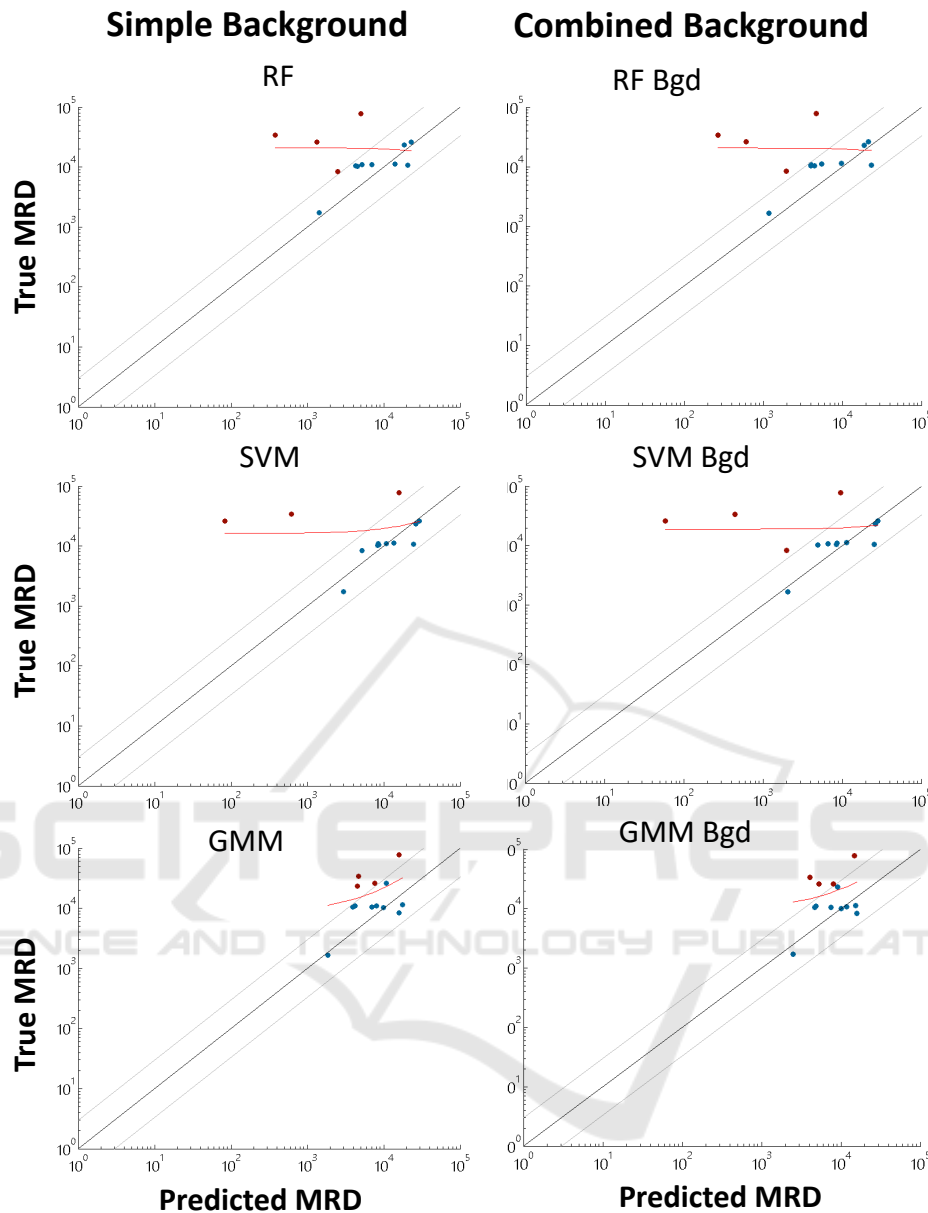


Figure 4: Visualisation of MRD assessment in AML using RF, SVM and Background GMM with simple background training (first column) and combined background training (second column).

4 CONCLUSIONS

In this work we demonstrate the applicability of machine learning to automatically assess MRD in childhood acute myeloid leukaemia. We evaluated three different approaches for AML routine data, where best results were achieved using Random Forests and Support Vector Machines. However these approaches show a higher variance in MRD estimations compared to GMM which underestimates MRD in a lower range.

We provided a background formulation for GMM and showed that learned distributions of non cancer blood cells can be used to identify blast populations in AML data. Additionally, we showed that combinations of backgrounds of different leukaemia types lead to similar performance of the supervised and unsupervised approaches evaluated in detecting blasts in AML data. We demonstrated that MRD can be estimated on basis of non-blast observations only, which is a huge benefit in the case of rare diseases, where only a limited

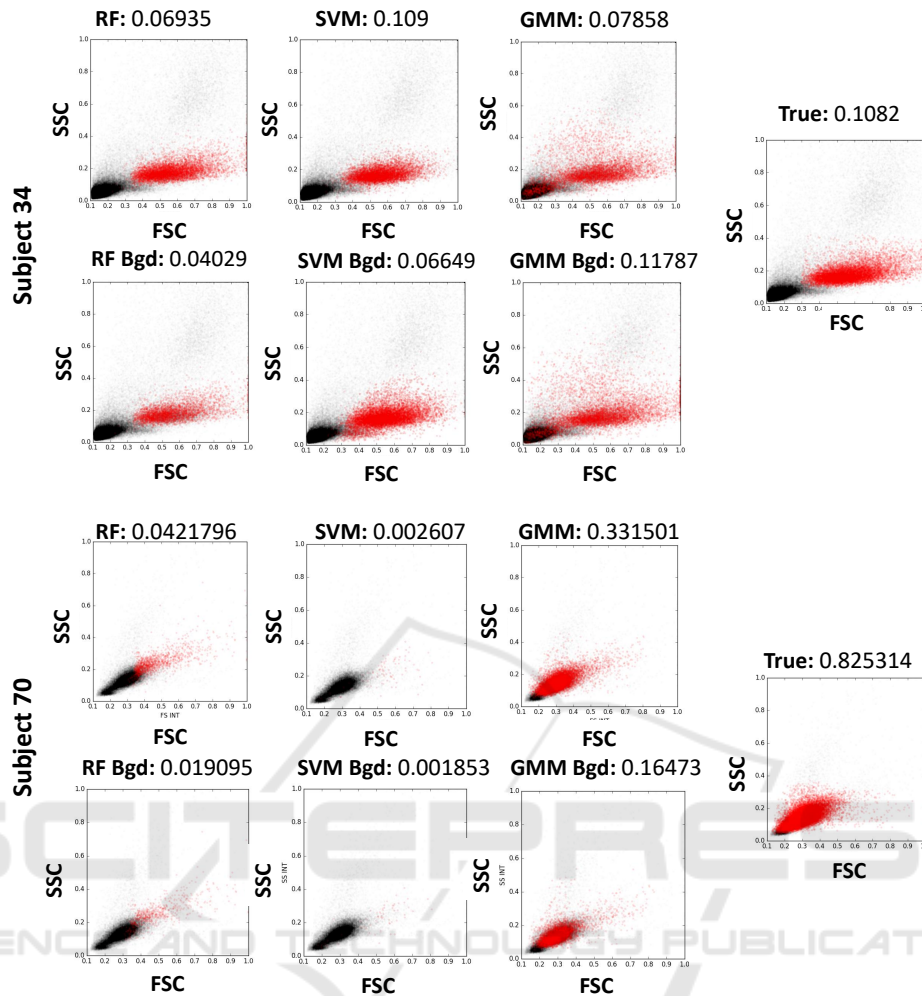


Figure 5: Visualisation of qualitative results and quantitative MRD estimation of two subjects by Random Forest (RF), Support Vector Machine (SVM) and Gaussian Mixture Model (GMM) using a simple and combined Background (Bgd) trainingset. The annotation results (True) are shown in column 4. Blasts are visualised in red and non-blasts in black.

number of data is available. The limit of our work lies in the small dataset available according to the rareness of the disease, thus for future work we aim to use data from different countries, machines and background samples.

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