Automated Segmentation of Leukocytes using Marker-based Watershed Algorithm from Blood Smear Images

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Abstract

The aim of this paper is to perform segmentation of white blood cells (WBCs) using blood smear images with the help of image processing techniques. Traditionally, the process of morphological analysis of cells is performed by a medical expert. This process is quite tedious and time consuming. The equipment used to perform the experiments are very costly and might not be available in all hospitals. Further, the whole process is quite lengthy and prone to error easily because of the lack of standard set of procedure. Hence there is a need for innovative and efficient techniques. An automated image segmentation system can make the blood test process much easier and faster. Segmentation of a nucleus image is one of the most critical tasks in a leukemia diagnosis. In this work, we have investigated and implemented image processing algorithms to segment cells. The proposed model detects WBCs and converts cell images from RGB to HSV color space using Otsu thresholding. The resultant image is then processed with the morphological filter because the segmented image contains noise which affects the system performance. Lastly, the Marker-based watershed algorithm is implemented in which specific marker positions are defined. The proposed model is tested on publicly available ALL-IDB2 dataset. The system's performance was overall examined and resulted in 98.99% overall precision for WBC segmentation.

Keywords: White blood cells (WBCs), Image processing, Blood smear images, Otsu thresholding, Watershed algo, HSV

1 Introduction

Blood cells can broadly be divided into three categories: platelets, Red Blood Cells (RBCs) and White Blood Cells (WBCs). WBCs are a fundamental component of the body's immune response because they shield us from pathogens by destroying viruses, bacteria, parasites and fungi. Malfunctioning of WBCs can weaken the immune system of the body, leading to various other physical disorders. Leukemia, a type of blood cancer leads to massive production of immature and non-functioning leukocytes or white blood cells of various forms. Its early recognition is beneficial for the recovery of affected children and adults. Therefore, rapid and reliable diagnosis of leukemia is required for its effective treatment [1]. In this paper, we focus on particularly Acute Lymphoblastic Leukemia (ALL) which is found commonly in children under 5 years and old people above 50 years of age. It spreads at a fast rate to all the vital body organs and is found to be fatal. Thus, timely diagnosis and treatment of leukemia is required to lead a healthy lifestyle [2]. The conventional method of detecting ALL consists of manual analysis, which totally depends on the skills and experience of hematologist. Diagnosis of a large number of samples becomes a challenge for a pathologist because it is time-consuming, imprecise and cause uncertainty.

Blood smear image consists of non-uniform illumination so an automated system helps to aid pathologists in blood diagnosis. It's important to design a low-cost and reliable recognition system. Benefit of designing a device focused on the methods of medical image processing is that targeted diseases are extracted with greater precision and time and cost are minimized. To overcome inter-observer and intra-observer bias

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involved in manual analysis, many researchers [2] [3] [4] [5] have proposed automated techniques for detection of leukemia from blood smear images. Processing of medical images includes four steps: pre-processing, segmentation, feature extraction, and classification. Amongst these steps, leukocyte segmentation is an important pre-requisite step because a good segmentation algorithm further enhances detection accuracy. The whole process of WBCs segmentation involves extraction of cells from a complex background to utilize both intensity and shape information of the cell and make a representation of an image more meaningful. [1].

In this work, we have implemented image processing techniques to automate the blood cell segmentation process using standard acute lymphoblastic leukemia-image database (ALL- IDB) [6]. The proposed model includes certain steps: Pre- processing step to enhance image features for further processing, segmentation step to separate WBCs from background and image cleaning to remove noise from segmented image [7].

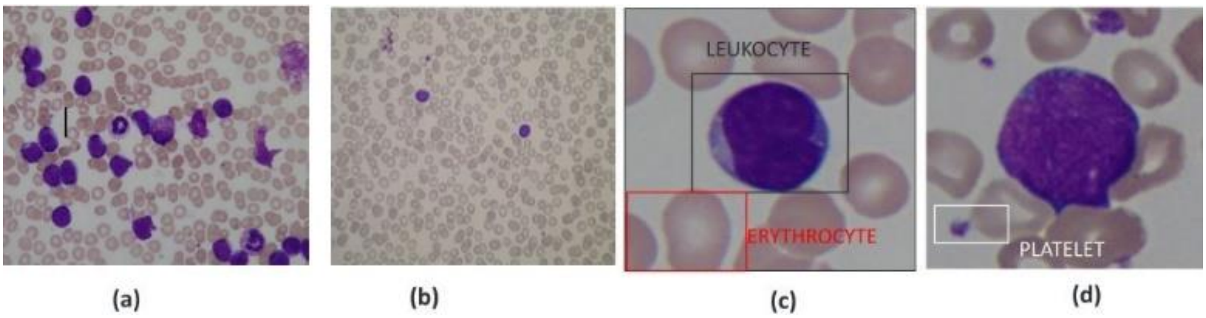

Fig. 1. Blood smear images, extracted from (a) and (b) ALL IDB1. (c) and (d) ALL IDB2. (c) and (d) is cropped from (a) ALL-IDB1 dataset. The red box is example of RBC; the black box shows a WBC; while the white one is a platelet.

2 Related Work

The proposed method presents several mechanisms for the study of leukocytes, which face problems such as segmentation, identification or classification, to differentiate between the different forms and check if a cell is leukemic or not [8]. There are various methods to perform segmentation of nucleus from the whole cells such as watershed algorithm, active contours and thresholding algorithms. However, thresholding methods are generally preferred due to their high performance and reliability [1]. Different methods and algorithms have been developed that can be grouped into three types: methods based on thresholds, methods based on pattern recognition, and methods based on deformable models. Since there is no general solution for the segmentation problem so combination of Otsu's method (region growing procedure) and watershed method is used in order to solve the problem [9]. Zheng et al. [8] implemented k- means clustering algorithm, a semi-automatic algorithm to segment WBCs from background. However, presence of noise and variation in image quality lead to outliers and hence, degraded segmentation accuracy. Hegde et al. [10] developed a method to initially enhance the image in order to remove noise factor. Later, TissueQuant was used as a tool for color segmentation to detect nuclei in the images. Liu et al. [3] proposed adaptive threshold techniques for segmenting leukocytes. Kumar et al. [11] presented detection of leukemia by color-based image segmentation using k-means clustering and comparison of CIELAB and CMYK color space. Sudha et. al. [12] employed an edge strength-based Grabcut method and GCHT technique.

Putzu et. al. [13] proposed the threshold value based on the triangle method or Zack algorithm. Otsu's thresholding is used in the majority of the works. Begum et. al. [14] used Otsu's global threshold method for segmenting the white blood cell. Watershed segmentation is used in variety of experiments related to blood smear image isolation and analysis to separate overlapping cells or are touching each other. Ghosh et. al. [4]

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proposed a region segmentation procedure that involves background scaling and redundant region elimination from the region set. After segmentation, by using gradient-based region growing with neighborhood effect, the more reliable region boundary is restored. A method based on morphology of cells was proposed by Wang et al. [5]. This method lessens the probability of identifying non-WBCs in blood smear image.

Li et.al. [9] proposed a dual threshold method where RGB and HSV color space-based single-threshold methods are combined for improving the conventional single-threshold approaches. The majority of the paper is structured as follows: In the next part, the suggested approach is discussed. In the experimental data and results section, experimental data and the outcomes of the proposed methods are explained. Lastly, in the conclusion section, the paper is concluded.

3 Materials and Methods

3.1 Overview of the Approach

The purpose of the research is to precisely preserve the maximum useful information while segmenting WBCs from the background. This will make other phases (feature extraction and classification) easier helping clinician in identifying diseases in a faster way. The proposed method consists of three phases: HSV color conversion, threshold segmentation and postprocessing. Fig.2 presents a block diagram of the proposed approach. HSV (Hue Saturation Value) is a model of cylindrical color that transforms the major RGB colors into dimensions. In the RGB color circle, Hue determines the angle of the color, the amount of color used is regulated by saturation and value influences the color's light. Transforming the image into an HSV color model is important to correctly obtain the color details of the picture. The exact range of HSV can be calculated programmatically using OpenCV (Open-Source Computer Vision Library) for an object to be detected or tracked. The suggested methodology focuses on the segmentation of WBC from other components (RBCs and background). The entire process can be outlined as follows:

Step 1. Given an input image, convert it into HSV color Model as it helps to differentiate intensity from color.

Step 2. Define the upper and lower limit of the blue to be detected (creating a mask).

Step 3. Apply Otsu thresholding along with morphological operators (erosion and dilation) on the resultant image from previous step to fill small holes and remove noise in the image.

Step 4. Marker-based watershed algorithm is employed for segmentation of WBCs.

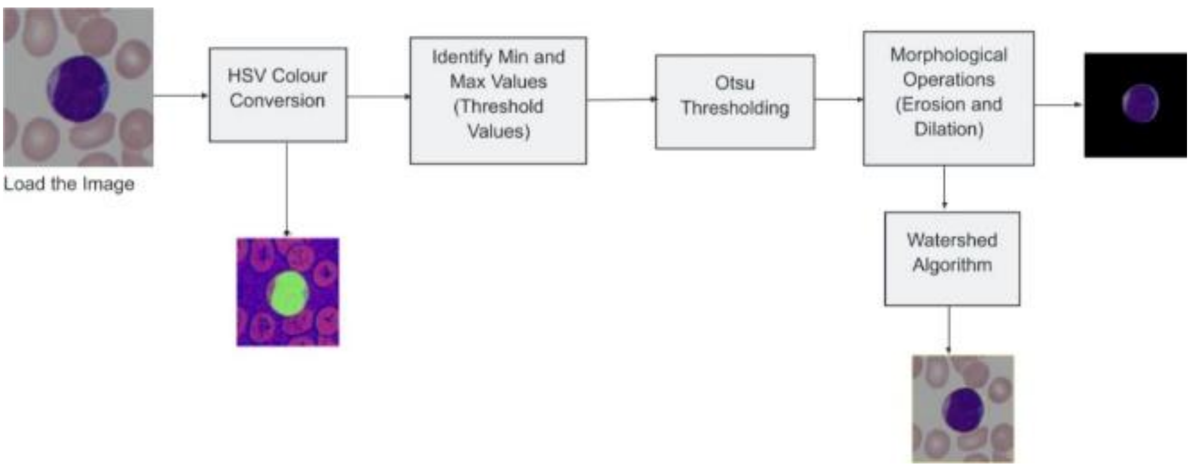

Fig. 2. Block Diagram of the proposed methodology.

3.2 Color-Conversion

HSV (Hue, Saturation, Value) is a color space which interpret the image similar in a way human perceive and understand colors. HSV color space is known for its better performance in comparison with RGB at different intensity values. Thus, HSV is better suited for human cells' image segmentation. Thresholding and masking operations are initially performed using HSV color space [1]. The upper and lower limit of the blue to be detected is defined which is also called mask. To find the limit the range-detector script can be used in the imutils library. The mask simply represents a specific part of the image. The Hue is within the range of 0-179 whereas Saturation and Value is within the range of 0-255.By adjusting min and max, optimum values for H, S, and V are found so that the background is masked and only the objects of interest remain.

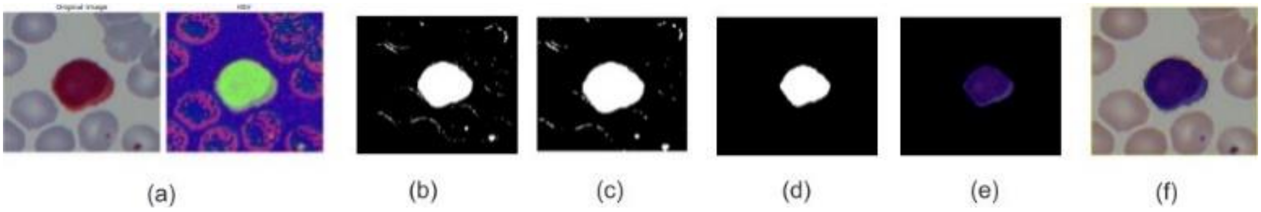

Fig. 3. The result of Nucleus Segmentation (a) Input Image and HSV Image (b) Image after mask is applied by adjusting min and max values (c) and (d) Image cleaning after applying Otsu thresholding and morphological operations (e) Bitwise operator to apply mask on image (f) Marker-based Watershed algorithm

3.3 Segmentation

From figure 3 (b), it can be shown that noise in final segmented image is mainly present in background, red blood cells and platelets [9]. In the proposed method, the segmentation step is performed using two different techniques which are the Otsu threshold and the Marker-based Watershed algorithm

3.4 OTSU Threshold

In the field of medical image seg- mentation, thresholding has great potential to outperform traditional image segmentation procedures. For ALL detection, one of the most widely used thresholding technique is Otsu method. It helps to determine the optimum value of threshold such that intra-variance is minimum for leukocyte class. Otsu's thresholding, a global thresholding algorithm gives optimal results. Since it obscures local edges found in WBCs, various edge-preserving filters are utilised to retain various edges [15].

3.5 Watershed-Marker Controlled

Images can be effectively segmented using the marker-based watershed transformation which is a regional based approach. Direct application to gradient image can lead to over segmentation of image so to avoid this concept of markers is used. Marker-controlled watershed segmentation is faster and more accurate method for segmentation. It takes in input a binary image which is composition of either large marker areas or single marker point. These markers are preferably generated automatically to produce efficient results and discourage the use of human interference [16].

3.6 Image Cleaning

Morphology or mathematical morphology is a collection of theoretical-based methods which are used to extract information regarding shapes of various components in an image. Two processes namely, erosion and dilation are used to understand the morphology of human cells. While, the erosion is used to contract

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the foreground objects, dilation performs the opposite and fill any gaps left behind [15]. To improve the final result, image cleaning is used to remove leukocytes on the border and impurities and platelets.

4 Experimental Results

4.1 Dataset

A publicly accessible data collection is used for performance measurement and comparison. All images in the datasets are captured with a PowerShot G5 camera. These images are available in .jpg format with 24-bit color depth and a resolution of 2592 × 1944. ALL-IDB has two independent variants (ALL-IDB1 and ALL-IDB2) used for segmentation and classification. This dataset is provided by Department of Information Technology at University degliStudi di Milano. These samples are collected by medical experts which includes affected(leukemic) and non-affected (normal cells) samples. The ALL-IDB1 dataset consists of total 108 images and is used for testing the segmentation and classification functionality of various algorithms [6]. ALL IDB2 contains cropped images (normal and blast cells) from the ALL-IDB1 and is used to cross-check the performance of our proposal. It has a total of 260 images in which half are of ALL patients and other half are of non-ALL patients (normal WBCs) [9]. Figure 4 displays two samples from ALL IDB1 and ALL IDB2 database in which sub image(cd) contains cropped WBC extracted from several white blood cells image using a proper method [9]. developed rapidly so that further steps such as classification can be improved by using the segmentation method. The efficiency of the suggested methodology is more beneficial than the various techniques present in the literature indicated in table1. The experimental result shows that the average precision is 98.99 by marking foreground and background. Image processing has

TABLE I: ANALYSIS RESULTS OF THE PROPOSED SEGMENTATION APPROACHES ALL-IDB DATASET

Approaches	**Precision** (%)	**Recall** (%)
[17]	79.12	94.59
[18]	96.00	88.70
[19]	85.20	90.68
[20]	75.45	81.82
[21]	83.50	68.57
[22]	82.61	89.77
[23]	82.80	89.45
[24]	80.09	89.27
[25]	75.63	85.17
Proposed Methodology	98.99	92.08

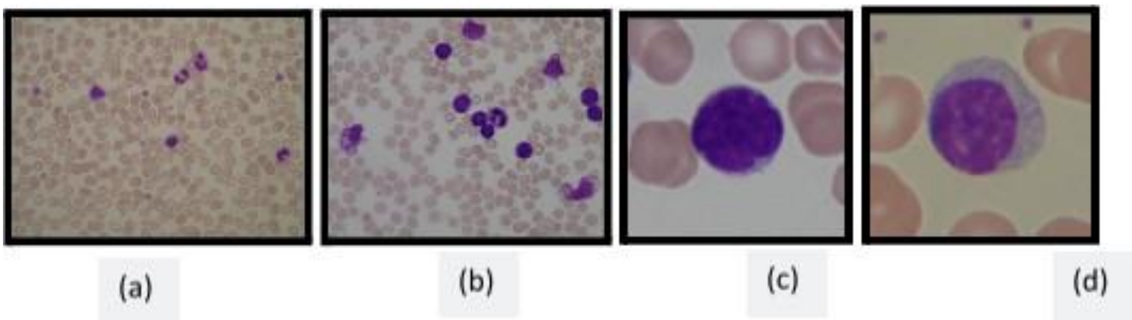

Fig. 4. Samples from ALL-IDB1 (a&b) and ALLIDB2 (c&d). a and d are healthy cells(non-affected) from non-ALL patients. (b) and (c) are probable lymphoblast from ALL-patients

4.2 Result

In this paper, experiment is performed on ALL-IDB2 dataset to segment nucleus from microscopic blood smear images. An experiment is conducted on sample of 25 images of microscopic white blood cell (WBC) and then compared with manually segmented images (ground-truth). Ground truth is generated with GNU Image Manipulation Program (GIMP) tool which is considered to be the correct segmentation result. Preprocessing and segmentation steps are performed on input images. RGB image is converted to HSV image and then thresholding-based algorithms are used. Generating HSV mask on image is important step as it enhances image which can improve segmentation. It can be seen from figure 2 that proposed methodology performs well in segmenting WBC from background (RBC and platelets). HSV mask (setting lower and upper range) applied in first step segmented the image better saving lot of time. The noise left is removed by applying dilation and erosion. Marker based watershed algorithm is then used to segment labeled regions

5 Conclusion

In this study, leukocyte segmentation from microscopic images using image processing techniques in medical field is presented. Automated system is used to overcome traditional methods which can lead to tiredness and manual errors. The methodology is mainly focused on segmentation step to im- prove accuracy of other steps which include feature extraction and classification. HSV color model is used to enhance the image which produced good segmentation performance. Otsu threshold and watershed marker-controlled algorithm are used in segmentation step. By generating a HSV mask and using it, segmentation results can be obtained more precise by most of the available works in the literature. Methods used in this paper consumes less time. Further steps can be devoted to study new features and increase overall accuracy. In future work, the results can be extended to extract features. Proposed segmentation techniques can be applied to other datasets for classification and segmentation purpose.

References

[1] M. Makem and A. Tiedeu, “An efficient algorithm for detection of white blood cell nuclei using adaptive three stage pca-based fusion,” *Informatics in Medicine Unlocked*, vol. 20, p. 100416, 2020.

[2] A. T. Sahlol, A. M. Abdeldaim, and A. E. Hassanien, “Automatic acute lymphoblastic leukemia classification model using social spider optimization algorithm,” *Soft Computing*, vol. 23, no. 15, pp. 6345– 6360, 2019.

[3]Z. Liu, J. Liu, X. Xiao, H. Yuan, X. Li, J. Chang, and C. Zheng, “Segmentation of white blood cells through nucleus mark watershed operations and mean shift clustering,” sensors, vol. 15, no. 9, pp. 22561– 22586, 2015.

[4] P. Ghosh, D. Bhattacharjee, and M. Nasipuri, “Blood smear analyzer for white blood cell counting: a hybrid microscopic image analyzing technique,” Applied Soft Computing, vol. 46, pp. 629–638, 2016.

[5] Q. Wang, L. Chang, M. Zhou, Q. Li, H. Liu, and F. Guo, “A spectral and morphologic method for white blood cell classification,” Optics & Laser Technology, vol. 84, pp. 144–148, 2016.

[6] R. D. Labati, V. Piuri, and F. Scotti, “All-idb: The acute lymphoblastic leukemia image database for image processing,” in 2011 18th IEEE International Conference on Image Processing, pp. 2045–2048, IEEE, 2011.

[7] Z. F. Mohammed and A. A. Abdulla, “Thresholding-based white blood cells segmentation from microscopic blood images,” UHD Journal of Science and Technology, vol. 4, no. 1, pp. 9–17, 2020.

[8] X. Zheng, Y. Wang, G. Wang, and J. Liu, “Fast and robust segmenta- tion of white blood cell images by self-supervised learning,” Micron, vol. 107, pp. 55–71, 2018.

[9] Y. Li, R. Zhu, L. Mi, Y. Cao, and D. Yao, “Segmentation of white blood cell from acute lymphoblastic leukemia images using dual-threshold method,” Computational and mathematical methods in medicine, vol. 2016, 2016.

[10] R. B. Hegde, K. Prasad, H. Hebbar, and B. M. K. Singh, “Development of a robust algorithm for detection of nuclei and classification of white blood cells in peripheral blood smear images,” Journal of medical systems, vol. 42, no. 6, pp. 1–8, 2018.

[11] K. Anilkumar, V. Manoj, and T. Sagi, “Colour based image segmentation for automated detection of leukaemia: A comparison between cielab and cmyk colour spaces,” in 2018 International Conference on Circuits and Systems in Digital Enterprise Technology (ICCSDET), pp. 1–6, IEEE, 2018.

[12] K. Sudha and P. Geetha, “A novel approach for segmentation and counting of overlapped leukocytes in microscopic blood images,” Bio- cybernetics and Biomedical Engineering, vol. 40, no. 2, pp. 639–648, 2020.

[13] L. Putzu, G. Caocci, and C. Di Ruberto, "Leucocyte classification for leukaemia detection using image processing techniques," Artificial intelligence in medicine, vol. 62, no. 3, pp. 179–191, 2014.

[14] A. J. Begum and T. A. Razak, "Diagnosing leukemia from microscopic images using image analysis and processing techniques," in 2017 World Congress on Computing and Communication Technologies (WCCCT), pp. 227–230, IEEE, 2017.

[15] S. Shafique and S. Tehsin, "Computer-aided diagnosis of acute lym- phoblastic leukaemia," Computational and mathematical methods in medicine, vol. 2018, 2018.

[16] K. Parvati, P. Rao, and M. Mariya Das, "Image segmentation using gray- scale morphology and marker-controlled watershed transformation," Discrete Dynamics in Nature and Society, vol. 2008, 2008.

[17] H. Madhloom, S. Kareem, H. Ariffin, A. Zaidan, H. Alanazi, and B. Zaidan, "An automated white blood cell nucleus localization and segmentation using image arithmetic and automatic threshold," Journal of Applied Sciences, vol. 10, no. 11, pp. 959–966, 2010.

[18] A. A. Nasir, M. Mashor, and H. Rosline, "Unsupervised colour segmen- tation of white blood cell for acute leukaemia images," in 2011 IEEE International Conference on Imaging Systems and Techniques, pp. 142– 145, IEEE, 2011.

[19] S. Mohapatra, S. S. Samanta, D. Patra, and S. Satpathi, "Fuzzy based blood image segmentation for automated leukemia detection," in 2011 International Conference on Devices and Communications (ICDeCom), pp. 1–5, IEEE, 2011.

[20] M. Mohamed, B. Far, and A. Guaily, "An efficient technique for white blood cells nuclei automatic segmentation," in 2012 IEEE International Conference on Systems, Man, and Cybernetics (SMC), pp. 220–225, IEEE, 2012.

[21] M. Madhukar, S. Agaian, and A. T. Chronopoulos, "New decision support tool for acute lymphoblastic leukemia classification," in Image Processing: Algorithms and Systems X; and Parallel Processing for Imaging Applications II, vol. 8295, p. 829518, International Society for Optics and Photonics, 2012.

[22] E. A. Mohammed, M. M. Mohamed, C. Naugler, and B. H. Far, "Chronic lymphocytic leukemia cell segmentation from microscopic blood images using watershed algorithm and optimal thresholding," in 2013 26th IEEE Canadian Conference on Electrical and Computer Engineering (CCECE), pp. 1–5, IEEE, 2013.

[23] S. Arslan, E. Ozyurek, and C. Gunduz-Demir, "A color and shape based algorithm for segmentation of white blood cells in peripheral blood and bone marrow images," Cytometry Part A, vol. 85, no. 6, pp. 480–490, 2014.

[24] S. Nazlibilek, D. Karacor, T. Ercan, M. H. Sazli, O. Kalender, and Y. Ege, "Automatic segmentation, counting, size determination and classification of white blood cells," Measurement, vol. 55, pp. 58–65, 2014.

[25] I. Vincent, K.-R. Kwon, S.-H. Lee, and K.-S. Moon, "Acute lymphoid leukemia classification using two-step neural network classifier," in 2015 21st Korea-Japan Joint Workshop on Frontiers of Computer Vision (FCV), pp. 1–4, IEEE, 2015.