DETECTION OF U-SERRATED PATTERNS IN DIRECT IMMUNOFLUORESCENCE IMAGES OF AUTOIMMUNE BULLOUS DISEASES BY INHIBITION-AUGMENTED COSFIRE FILTERS

CHENYU SHI*, JOOST M. MEIJER*, JIAPAN GUO*, GEORGE AZZOPARDIad, GILLES F.H. DIERCKSb, ENNO SCHMIDT*, DETLEF ZILLIKENS*, MARCEL F. JONKMANb AND NICOLAI PETKOVA

*Johann Bernoulli Institute for Mathematics and Computer Science, University of Groningen, Groningen, The Netherlands
bCenter for Blistering Diseases, Department of Dermatology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
cCenter for Medical Imaging, Faculty of Medical Sciences, University Medical Center Groningen, University of Groningen, The Netherlands
dIntelligent Computer Systems, University of Malta, Malta
eDepartment of Dermatology, University of Lübeck, Lübeck, Germany

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ABSTRACT

Direct immunofluorescence (DIF) microscopy of a skin biopsy is used by physicians and pathologists to diagnose autoimmune bullous dermatoses (AIBD). This technique is the reference standard for diagnosis of AIBD, which is used worldwide in medical laboratories. For diagnosis of subepidermal AIBD (sAIBD), two different types of serrated pattern of immunodepositions can be recognized from DIF images, namely n- and u-serrated patterns. The n-serrated pattern is typically found in the most common sAIBD bullous pemphigoid. Presence of the u-serrated pattern indicates the sAIBD subtype epidermolysis bullosa acquisita (EBA), which has a different prognosis and requires a different treatment. The manual identification of these serrated patterns is learnable but challenging. We propose an automatic technique that is able to localize u-serrated patterns for automated computer-assisted diagnosis of EBA. The distinctive feature of u-serrated patterns as compared to n-serrated patterns is the presence of ridge-endings. We introduce a novel ridge-ending detector which uses inhibition-augmented trainable COSFIRE filters. Then, we apply a hierarchical clustering approach to detect the suspicious u-serrated patterns from the detected ridge-endings. For each detected u-serrated pattern we provide a score that indicates the reliability of its detection. In order to evaluate the proposed approach, we created a data set with 180 DIF images for serration pattern analysis. This data set consists of seven subsets which were obtained from various biopsy samples under different conditions. We achieve an average recognition rate of 82.2% of the u-serrated pattern on these 180 DIF images, which is comparable to the recognition rate achieved by experienced medical doctors and pathologists.
Subepidermal autoimmune bullous diseases (sAIBD) are among the most life-threatening inflammatory diseases of the skin. These bullous skin diseases can be differentiated in various subtypes, including the most common sAIBD bullous pemphigoid and epidermolysis bullosa acquisita (EBA). These variants of sAIBD share similar clinical symptoms (Buijsrogge et al., 2011; Schmidt and Zillikens, 2013), however, they differ substantially in treatment and prognosis. Therefore, it is vital to accurately diagnose different subtypes of sAIBD. All subtypes of sAIBD are characterized by immunodepositions along the epidermal basement membrane zone (BMZ). The BMZ is a region between the epidermis and the dermis, which is marked by the green dashed boundaries in Fig. 1. Due to different locations of target antigens in the BMZ, different shapes of serration immunodeposition patterns are formed in the subtypes of sAIDB. Direct immunofluorescence (DIF) microscopy, which provides the visualization of tissue-bound autoantibodies in skin sections from a biopsy, is considered as the reference standard for diagnosis of sAIBD (Vorobyev et al., 2017). Additionally, automated processing of DIF biopsies has been developed recently to improve efficiency and diagnostic accuracy (Lemcke et al., 2015). Along with immune serological tests, serration pattern analysis of DIF microscopy images becomes reference standard in the diagnostics of sAIBD (Vodegel et al., 2004).

Serration pattern analysis in DIF images concerns two types of patterns, which are referred to as n-serrated and u-serrated. Fig. 2(a,b) show examples of DIF images with these two types of serration patterns. Ridges undulate rounded n-shapes in n-serrated patterns while u-serrated patterns consist of finger-like ridges. DIF images that only contain n-serrated patterns indicate the pemphigoid group, while the presence of u-serrated patterns is confirmative for EBA (Vodegel et al., 2004; Terra et al., 2013). Manual serration pattern analysis is shown to be learnable, irrespective of expertise (Terra et al., 2013). In clinical practice, however, the use of manual serration pattern analysis is limited. This is mainly due to technical difficulties and lack of technical analysts (Terra et al., 2013). Standard DIF staining procedures are routinely performed in diagnostic laboratories worldwide, which allow performing serration pattern analysis without extra cost. In order to contribute in a sustainable system with feedback, it is necessary to provide information of the location of the possible u-serrated patterns which are minitial patterns in DIF images. Thus, dermatologists could focus on the final diagnosis of EBA and the subsequent treatment.

Computer-aided diagnosis has been implemented in radiology images, immunofluorescence assays and stained tissue sections for pathology to improve diagnostic accuracy, such as discrimination of benign and malignant melanomas (Wiltgen et al., 2003; Kostopoulos et al., 2017), detection of antinuclear antibodies in serum (Bizzaro et al., 2014) or lymph node metastasis in breast cancer (Song et al., 2016; Bejnordi et al., 2017) or bacteria in microscope images (Song et al., 2017). So far, there are only a few automatic techniques for serration pattern analysis in DIF images. The work proposed in (Shi et al., 2015a) attempted to use as a feature vector the histogram of absolute orientations of ridges to automatically differentiate between n- and u-serrated DIF images. Later, the authors improved the method by considering the local orientation of the BMZ and constructed histograms of relative orientations of ridges with respect to the BMZ (Shi et al., 2015b). Both methods, however, are unable to provide location information of u-serrated patterns in DIF images. In order to extract such information, we seek to locate ridge-endings that are used to characterize the u-serrated patterns. There have been a large body of work that focuses on line or ridge detection (von Gioi et al., 2010; Cho et al., 2017; Prabhakar et al., 2003; McLean and Kotturi, 1995; Berlemont and Olivo-Marin, 2010; Chung et al., 2010) or texture descriptors (Barata et al., 2015; Riaz et al., 2017; Shi et al., 2017).
Fig. 1 Example of a pathology picture (magnication ×400) showing a sectional view of the normal skin - the epidermis, the dermis and the basement membrane zone (BMZ).

Fig. 2 Examples of (a) an n-serrated and (b) a u-serrated DIF images. The areas marked by the red dashed lines indicate the BMZ (400× original magnification). (c,d) Enlargements of the enframed n- and u-serrated patterns in (a,b), respectively. The white dashed lines indicate ridges that have the characteristic shapes of the n- and u-serration patterns.
Most of the approaches aim at either the detection of minutae features in fingerprints (Prabhakar et al., 2003; Alaei et al., 2011; Ram et al., 2010; Chang and Fan, 2001; Abutaleb and Kamel, 1999) or the detection of parallel lines in three-dimensional space to estimate their vanishing points in the 2D view (McLean and Kotturi, 1995; Almansa et al., 2003). To the best of our knowledge there is, however, no work on the exclusive detection of line- or ridge- endings.

In this work, we propose an automatic approach to identify and localize the u-serrated patterns in DIF images. We first apply inhibition-augmented COSFIRE (COmbination of Shifted FIlter REsponses) filters that are only selective for ridge-endings. Then we apply a hierarchical clustering approach to detect suspicious u-serrated patterns from the candidate ridge-endings. Finally, we provide a score for each detected u-serrated pattern that indicates the reliability of the detection.

The rest of the paper is organized as follows. In Section 2, we elaborate on the proposed method for ridge-ending detection. In Section 3, we experiment on a data set that we created with a variety of samples from different labs and report the achieved results. Finally, we discuss some aspects of the proposed method in Section 4 and draw conclusions in Section 5.

2. Ridge-ending detector

In this section, we explain how to construct a detector that responds exclusively to ridge-endings, such as the pattern in Fig. 3a, but that does not respond to complete lines nor to patterns with curvatures, such as those in Fig. 3(b-h). The proposed ridge-ending detector is based on the trainable bar-selective COSFIRE (B-COSFIRE) filter approach (Azzopardi et al., 2015). We augment the B-COSFIRE filters by adding a brain-inspired inhibition mechanism, which increases the selectivity of such filters without compromising generalization (Guo et al., 2016, 2017). We configure such an inhibition-augmented B-COSFIRE filter by using two different types of prototype, a positive and a set of negative patterns. We use as a positive prototype pattern a ridge-ending (Fig. 3a) while the negative prototypes are a group of seven patterns that contain the ridge-ending pattern of the positive prototype along with some additional lines, Fig. 3(b-h). In the following, we explain in detail how we configure and apply the proposed ridge-ending selective filter.

2.1 Bar-selective COSFIRE filter

A B-COSFIRE filter1 is a brain-inspired computational model for the detection of bar-like shapes, and has been shown to be effective for blood vessel delineation (Azzopardi et al., 2015; Strisciuglio et al., 2015a,b). It is based on the COSFIRE approach (Azzopardi and Petkov, 2013) and the CORF (Combination of Receptive Fields) computational model (Azzopardi and Petkov, 2012). Such a filter takes as input the responses of a Difference-of-Gaussians (DoG) filter at certain positions, and combines them by (weighted) geometric mean. The positions where the DoG responses are taken are determined in an automatic configuration step, which we explain below.

1Matlab scripts: http://tinyurl.com/y8rplkwsj
Fig. 3 Synthetic input images (of size 50×50 pixels) that are considered as (a) positive and (b-h) negative prototype patterns. The width of each line is 5 pixels.

Fig. 4 (a) Synthetic image (of size 50×50 pixels) of a vertical ridge-ending (of width 5 pixels). (b) Response map of a center-on DoG filter (here $\sigma = 2.5$) to the ridge-ending image in (a). The white cross indicates the center position of the pattern of interest. We consider the dashed circles of given radii (here $\rho \in \{0, 6, 12\}$) around the center of the pattern of interest. The black dots indicate the positions of the local maximum DoG responses along these circles. (c) Structure of the resulting B-COSFIRE filter $P_f$. The black cross indicates the center of the B-COSFIRE filter and the pairs of concentric circles represent the support areas of the involved DoG filters.
Let us consider the synthetic ridge-ending in Fig. 4a and use it as a positive prototype. Fig. 4b shows the corresponding response image of a center-on DoG filter, whose outer Gaussian function has a standard deviation $\sigma = 2.5$ (the width of the line in Fig. 4a is 5 pixels). For the configuration of a B-COSFIRE filter, we consider the DoG responses along three concentric circles with radii $\rho \in \{0, 6, 12\}$ pixels around the center point. We choose the local maximum responses along these circles and form one 3-tuple for each such response. The elements of each tuple $i$ include the standard deviation $\sigma_i$ of a DoG function and the polar coordinates $(\rho_i, \phi_i)$ of the preferred position with respect to the support center of the concerned B-COSFIRE filter. For the pattern shown in Fig. 4a this procedure results in a set of the following three tuples:

$$P_f = \left\{ \begin{array}{l}
(\sigma_1 = 2.5, \rho_1 = 0, \phi_1 = 0), \\
(\sigma_2 = 2.5, \rho_2 = 6, \phi_2 = 3\pi/2), \\
(\sigma_3 = 2.5, \rho_3 = 12, \phi_3 = 3\pi/2)
\end{array} \right\}$$

Fig. 4c shows the structure of the resulting B-COSFIRE filter $P_f$. The pairs of concentric circles represent the support of the concerned DoG functions.

We compute the response $r_{P_f}(x, y)$ of the B-COSFIRE filter as the geometric mean of all the DoG responses that correspond to the tuples described by $P_f$:

$$r_{P_f}(x, y) = \left( \prod_{i=1}^{\left|P_f\right|} s_{\sigma_i, \rho_i, \phi_i}(x, y) \right)^{1/\left|P_f\right|}$$

where the intermediate representation $s_{\sigma_i, \rho_i, \phi_i}(x, y)$ is the blurred and shifted DoG response that corresponds to the tuple $i$. We blur the DoG responses in order to allow for some tolerance with respect to the preferred locations. We then shift the blurred DoG responses by a vector $\Delta x_i, \Delta y_i$ so that all responses in the locations specified by the polar coordinates in the set $P_f$ meet at the support center of the filter. For further technical details about the blurring and shifting operations we refer to (Azzopardi and Petkov, 2013) and (Azzopardi et al., 2015).

Fig. 5(a-h) show the corresponding response maps of the B-COSFIRE filter to every image in Fig. 3. The B-COSFIRE filter configured by a ridge-ending in Fig. 3a gives strong responses also to the patterns in Fig. 3(b-h). This is because the line segment of the ridge-ending is also present in the other patterns in Fig. 3(b-h). The additional line parts do not have influence on the response of the filter. In the following, we explain how we use inhibition-augmented B-COSFIRE filters in order to exclusively detect ridge-endings.

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For $\rho = 0$ we only consider the center point

The standard deviation of the inner Gaussian function is 0.5 $\sigma$.

The original B-COSFIRE method computes the output as the weighted geometric mean of the involved DoG responses. In this work we give equal weights to all tuples.
2.2 Configuration of an inhibition-augmented B-COSFIRE filter

Let us consider the two patterns in Fig. 3(a-b), which we refer to as a positive and a negative pattern, respectively. Fig. 6b is the response map of the same center-on DoG filter ($\sigma=2.5$) to the negative pattern in Fig. 3b, which is replicated in Fig. 6. We consider the DoG responses along the same concentric circles with radii $\rho \in \{0, 6, 12\}$ pixels around the center point, and denote by $N_{f1} = \{(\sigma_j, \rho_j, \varphi_j) | j \in 1...n_2\}$ the B-COSFIRE filter that is configured with the negative pattern:

$$N_{f1} = \{ (\sigma_1 = 2.5, \rho_1 = 0, \varphi_1 = 0 ),$$
$$ (\sigma_2 = 2.5, \rho_2 = 6, \varphi_2 = \pi/2 ),$$
$$ (\sigma_3 = 2.5, \rho_3 = 6, \varphi_3 = 3\pi/2 ),$$
$$ (\sigma_4 = 2.5, \rho_4 = 12, \varphi_4 = \pi/2 ),$$
$$ (\sigma_5 = 2.5, \rho_5 = 12, \varphi_5 = 3\pi/2 ) \}.$$

Fig. 6c shows the structure of the resulting B-COSFIRE filter $N_{f1}$.

Then, we form a new set $S_f$ by selecting tuples from the two sets, $P_f$ and $N_{f1}$, as follows. We include all tuples from $P_f$ into the new set $S_f$ and mark them with a tag $\delta = +1$ to indicate that the corresponding DoG responses provide excitatory input to the resulting filter. Then we compute the minimum distance $d(N_{f1}, P_f)$ between the spatial coordinate of the $j$-th tuple from $N_{f1}$ and the spatial coordinates of all tuples from $P_f$:

$$d(N_{f1}, P_f) = \min_{i \in \{1,...,|P_f|\}} \left( \sqrt{X^2 + Y^2} \right)$$

where

$$X = \rho_i \cos \varphi_i - \rho_j \cos \varphi_j$$
$$Y = \rho_i \sin \varphi_i - \rho_j \sin \varphi_j$$

Fig. 5 (a-h) Response maps of the B-COSFIRE filter $P_f$ to the corresponding images in Fig. 3(a-h).
If the distance \( d(N_f, P_f) \) is larger than a certain distance threshold, we include the tuple \( N_f \) into the set \( S_f \) and mark it with a tag \( \delta = -1 \) indicating that the corresponding DoG response provides inhibitory input. Here we use \( \eta \) equals to 5 pixels which is twice the standard deviation \( \sigma \) of the outer Gaussian function. For further comments on the choice of the value of \( \eta \) we refer the reader to (Guo et al., 2016). We repeat this procedure for each tuple in the set \( N_f \). In this way we obtain a new set \( S_f = \{(\sigma_k, \rho_k, \varphi_k, \delta_k) | k \in 1...n_3\} \) of labeled excitatory and inhibitory tuples. The parameter \( n_3 \) denotes the number of tuples in the set \( S_f \). For the considered example, the above procedure results in the following set \( S_f \):

\[
S_f = \{ (\sigma_1 = 2.5, \rho_1 = 0, \varphi_1 = 0, \delta_1 = +1), \\
(\sigma_2 = 2.5, \rho_2 = 6, \varphi_2 = 3\pi/2, \delta_2 = +1), \\
(\sigma_3 = 2.5, \rho_3 = 12, \varphi_3 = 3\pi/2, \delta_3 = +1), \\
(\sigma_4 = 2.5, \rho_4 = 6, \varphi_4 = \pi/2, \delta_4 = -1), \\
(\sigma_5 = 2.5, \rho_5 = 12, \varphi_5 = \pi/2, \delta_5 = -1) \}
\]

Fig. 7a shows the structure of the resulting inhibition-augmented B-COSFIRE filter, which we denote by \( S_f \). The white and black pairs of concentric circles indicate DoG filters whose responses provide excitatory and inhibitory inputs, respectively.

Finally, we apply the above procedure to the remaining negative patterns in Fig. 3(c-h) and determine the inhibitory tuples from each of the sets \( N_{fm} \) \((m \in \{2,3,...,7\})\). For each set of the inhibitory tuples from the same pattern, we give it a unique tag \( \delta = -m \). For instance, the inhibitory tuples determined from the set \( N_f \) are each assigned the tag \( \delta = -1 \), the inhibitory tuples determined from the set \( N_{f2} \) are assigned the tag \( \delta = -2 \), and so forth. Fig. 7b shows the structure of the inhibition-augmented B-COSFIRE filter that takes into account the negative patterns in both Fig. 3b and Fig. 3c. In Fig. 7c, we illustrate the resulting structure of the filter configured by all negative patterns, in which the different coloured pairs of concentric circles represent the tuples from different negative patterns.
In the original B-COSFIRE approach, the DoG responses are blurred to allow for some tolerances. Here we do not blur the DoG responses and set $G_\sigma' = 1$.

### 2.3. Response of an Inhibition-Augmented B-COSFIRE Filter

For each group of tuples that share the same value in the set $S_j$, we first compute their combined responses by geometric mean as defined in Eq. 1$^5$. We denote by $r_{S_j'}(x, y)$ the output of the group of tuples with the tag $\delta = +1$. Similarly, we denote by $r_{S_j''}(x, y)$ the output of the group of tuples with the tag $\delta = -m$ that provides inhibitory input. Finally, we compute the output $r_{S_j}(x, y)$ of the inhibition-augmented B-COSFIRE filter by subtracting the maximum value of the outputs of all groups of tuples that provide inhibitory input from the output of the group of tuples that provide the excitatory input:

$$r_{S_j}(x, y) \overset{\text{def}}{=} r_{S_j'}(x, y) - \max_{j \neq j'} \{r_{S_{j'}}(x, y)\} \quad (3)$$

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$^5$In the original B-COSFIRE approach, the DoG responses are blurred to allow for some tolerances. Here we do not blur the DoG responses and set $G_\sigma = 1$.

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Fig. 7 (a-c) The structures of the inhibition-augmented B-COSFIRE filters configured by the positive pattern in Fig. 3a and one negative prototype pattern (Fig. 3b), two negative prototypes (Fig. 3b and Fig. 3c) and seven negative prototypes shown in Fig. 3(b-h), respectively. The pairs of concentric circles in different colors represent the tuples determined from different prototypes. The white pairs of concentric circles indicate the tuples of which the corresponding DoG responses provide excitatory input to the filter while the colored ones indicate those that provide inhibitory input. (d) The structure of the filter in (c) rotated anticlockwise by 45 degree.
where $S_{f}^{*} = \{(σ_i, ρ_i, φ_i) | ∀ (σ_i, ρ_i, φ_i, δ_i) ∈ S_f, δ_i = +1\}$,
$S_{f}^{-} = \{(σ_i, ρ_i, φ_i) | ∀ (σ_i, ρ_i, φ_i, δ_i) ∈ S_f, δ_i = -1\}, n = \max |δ_i|$. We denote by $r_{S_f}$ and $r_{S_f}^{-}$ the geometric mean of all shifted DoG responses that correspond to the tuples in the set $S_{f}^{*}$ and $S_{f}^{-}$.

Fig. 8(a-h) shows the response maps of the inhibition-augmented B-COSFIRE filter in Fig. 7c to all images in Fig. 3. The resulting filter responds exclusively to the ridge ending in Fig. 3a.

2.4. Achieving rotation tolerance

The proposed B-COSFIRE filters with inhibition achieve tolerance to rotation by manipulating the values of the parameter $φ$. We refer the reader to (Azzopardi and Petkov, 2013) for a thorough explanation. Fig. 7d shows the structure of an example of the inhibition-augmented B-COSFIRE filter that is rotated anticlockwise by 45 degrees.

3. Experimental results

3.1. Data set

We experiment on a data set named NversusU2017 with 180 DIF images of samples provided by the Immunodermatology Laboratory of the University Medical Center Groningen (UMCG) and the laboratory of the University Hospital Schleswig-Holstein, University of Lübeck (UL). All images were taken with the same microscope (Leica DMRA, Leica, Wetzlar, Germany) in the most commonly used standard settings, which are the 40x magnification dry objective
Table I. Eleven subsets in the N versus U2017 dataset

<table>
<thead>
<tr>
<th>Data subset no.</th>
<th>Biopsies taken at</th>
<th>Processed at</th>
<th>Tissue section thickness</th>
<th>No. of images</th>
<th>No. of images with u-serrated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>UMCG</td>
<td>UMCG</td>
<td>6-µm</td>
<td>26</td>
<td>15 (58%)</td>
</tr>
<tr>
<td>2</td>
<td>UMCG</td>
<td>UMCG</td>
<td>6-µm</td>
<td>60</td>
<td>30 (50%)</td>
</tr>
<tr>
<td>3</td>
<td>UMCG</td>
<td>UMCG</td>
<td>6-µm</td>
<td>14</td>
<td>5 (36%)</td>
</tr>
<tr>
<td>4</td>
<td>UL</td>
<td>UL</td>
<td>6-µm</td>
<td>10</td>
<td>5 (50%)</td>
</tr>
<tr>
<td>5</td>
<td>UMCG</td>
<td>UL</td>
<td>6-µm</td>
<td>10</td>
<td>5 (50%)</td>
</tr>
<tr>
<td>6</td>
<td>UL</td>
<td>UMCG</td>
<td>4-µm</td>
<td>10</td>
<td>5 (50%)</td>
</tr>
<tr>
<td>7</td>
<td>UL</td>
<td>UMCG</td>
<td>6-µm</td>
<td>10</td>
<td>5 (50%)</td>
</tr>
<tr>
<td>8</td>
<td>UL</td>
<td>UMCG</td>
<td>8-µm</td>
<td>10</td>
<td>5 (50%)</td>
</tr>
<tr>
<td>9</td>
<td>UMCG</td>
<td>UMCG</td>
<td>4-µm</td>
<td>10</td>
<td>5 (50%)</td>
</tr>
<tr>
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<tr>
<td>11</td>
<td>UMCG</td>
<td>UMCG</td>
<td>8-µm</td>
<td>10</td>
<td>5 (50%)</td>
</tr>
</tbody>
</table>

Fig. 9 Examples of DIF images from different subsets. (a-c) Three n-serrated and (d-f) three u-serrated DIF images from subset 1, subset 2 and subset 3, respectively.
and the 10× magnification ocular. The NversusU2017 data set consists of eleven different subsets of DIF images (subset 1-11), listed in Table I. The DIF images in subsets 1-3 were obtained with routine processing techniques in the UMCG lab. Unlike these images, the DIF images in other subsets were captured under different conditions of DIF microscopy in order to represent daily practices and to determine the optimal conditions for serration pattern analysis. A number of biopsies obtained from the University of Lübeck were transported in a fixative named Michels medium while those from the UMCG were transported in saline (0.9% NaCl). To analyze influences of transport mediums and section thicknesses of the slides for optimal serration pattern analysis, samples from both laboratories were exchanged and processed in both laboratories. For instance, the DIF images in the subsets 4 and 5 were captured from biopsies that were collected from both laboratories and cut in the UL lab. Images in the subsets 6-11 were obtained from biopsies that were processed in the UMCG and cut in different section thicknesses of 4-µm, 6-µm and 8-µm. So far, subset 1 is publicly available as an online test while the other subsets are available upon request. Fig. 9(a-c) shows three examples of n-serrated DIF images and Fig. 9(d-f) are examples of u-serrated images from the subsets 1-3.

3.2. SEGMENTATION OF THE BMZ
As we see from the examples of DIF images shown in Fig. 9, serration patterns are located along the BMZ region. Here we explain how we detect the BMZ region before applying the proposed ridge-ending detectors. We use the algorithm proposed in (Shi et al., 2015a) to segment the BMZ from the green channel (Fig. 10b) of a RGB DIF image (of size 1392 × 1040 pixels). First, we use a disk-shaped structuring element (radius of 30 pixels) to perform morphological closing followed by a flood-ll operation, of which the result is shown in Fig. 10c. Then, we use the Canny edge detector (Canny, 1986) to extract the upper-most boundary of the largest connected component. In order to avoid the boundary effect, we do not consider the regions within 50 pixels from the borders of the image. Fig. 10d shows the resulting largest connected boundary. We then dilate the detected boundary using a disk-shaped structuring element (radius of 30 pixels) to obtain the BMZ mask (Fig. 10e). Finally, we extract the corresponding region in the green channel of a DIF image from the resulting BMZ mask, Fig. 10f.

3.3. IMPLEMENTATION AND EXPERIMENTAL RESULTS
In the following, we explain how we implement the proposed approach in order to detect ridge-endings and then localize the u-serrated patterns in DIF images. First, we configure an inhibition-augmented B-COSFIRE filter in the same way as proposed in Section 2. We apply the resulting filter in partial rotation-invariance mode that considers ridges with an orientation varying from $-\pi / 2$ to $\pi / 2$ in intervals of $\pi / 16$. We then take the locations of the local maximum responses in the output map to indicate the detected ridge-endings. We consider a circular neighbourhood of radius ($d_{\text{min}}$) 5 pixels when determining a local maximum response. Fig. 11(a-b) show the response maps of our ridge-ending detector to an n-serrated and a u-serrated DIF images, respectively. The red dots mark the locations where the ridge-ending detector responds strongly along the BMZ of the green channel images. Next we group together ridge-endings that are within a distance of ($r_w$) 40 pixels and ignore any ridge-endings that are isolated. In practice, we use agglomerative clustering with single
Fig. 10 (a) Example of an RGB DIF image (of size 1592 x 1040 pixels) and (b) its green channel image. (c) The resulting binary image after applying the morphological closing operation using a disk-shaped structuring element (radius of 30 pixels) to the green channel image. (d) The extracted boundary which is the largest connected edge between the black and white regions of the binary image in (c). We then dilate the extracted boundary to obtain (e) the binary mask of the BMZ region and use it to extract the corresponding region (f) in the green channel.

Fig. 11 Examples of the experimental results. (a-b) Output maps of a ridge-ending detector on an n-serrated and a u-serrated DIF image, respectively. The red dots indicate the locations of the detected ridge-endings on the green channel of the DIF images. (c-d) The output maps of the localization of u-serrated patterns. The dashed yellow rectangles indicate the presence of suspicious u-serrated patterns.
linkage with a cut-off criterion of a distance of 40 pixels. We also ignore clusters with less than four ridge-endings. For the remaining clusters we locate the corresponding u-serrated patterns. We then provide a reliability score that indicates the confidence of the presence of u-serrated patterns within each local region. We define such a reliability score \( \alpha \) by

\[
\alpha \overset{\text{def}}{=} \frac{\Omega_{\min}}{r_v}
\]

(4)

When \( \alpha \) is larger than 0.25 in the considered local region, we mark it as the u-serrated region. In Fig. 11d, the yellow bounding boxes indicate clusters with more than three ridge endings. Finally, we classify a DIF image as the u-serrated image if it has at least one region with \( \alpha >0.25 \).

For the two DIF images in Fig. 11, the one in the left column is classified as the n-serrated DIF image while the one in the right is the u-serrated DIF image.

We experiment on all 180 DIF images in the NversusU2017 set. Table II shows the recognition rates that we achieve on different subsets in the NversusU2017 data set. We achieve an average recognition rate of 82.2\% across all subsets. For subset 1, the recognition rate of u-serrated patterns is 84.6\%, which is higher than the average recognition rate of 78.6\% by trained medical doctors and pathologists (Terra et al., 2015) who perform the test on the same set. The proposed method performs significantly better than the other existing approach (Shi et al., 2015a).

<table>
<thead>
<tr>
<th>Data subset no.</th>
<th>Biopsies taken at</th>
<th>Processed at</th>
<th>Tissue section thickness</th>
<th>(Shi et al.,2015a)</th>
<th>(Shi et al.,2015b)</th>
<th>Proposed method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>UMCG</td>
<td>UMCG</td>
<td>6 ( \mu )m</td>
<td>84.6%</td>
<td>84.6%</td>
<td>84.6%</td>
</tr>
<tr>
<td>2</td>
<td>UMCG</td>
<td>UMCG</td>
<td>6 ( \mu )m</td>
<td>75.0%</td>
<td>81.7%</td>
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</tr>
<tr>
<td>3</td>
<td>UMCG</td>
<td>UMCG</td>
<td>6 ( \mu )m</td>
<td>64.3%</td>
<td>78.6%</td>
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</tr>
<tr>
<td>4</td>
<td>UL</td>
<td>UL</td>
<td>6 ( \mu )m</td>
<td>80.0%</td>
<td>90.0%</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>UMCG</td>
<td>UL</td>
<td>6 ( \mu )m</td>
<td>70.0%</td>
<td>90.0%</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>UL</td>
<td>UMCG</td>
<td>4 ( \mu )m</td>
<td>80.0%</td>
<td>70.0%</td>
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</tr>
<tr>
<td>7</td>
<td>UL</td>
<td>UMCG</td>
<td>6 ( \mu )m</td>
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<td>90.0%</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>UL</td>
<td>UMCG</td>
<td>8 ( \mu )m</td>
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</tr>
<tr>
<td>9</td>
<td>UMCG</td>
<td>UMCG</td>
<td>4 ( \mu )m</td>
<td>70.0%</td>
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</tr>
<tr>
<td>10</td>
<td>UMCG</td>
<td>UMCG</td>
<td>6 ( \mu )m</td>
<td>90.0%</td>
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<td></td>
</tr>
<tr>
<td>11</td>
<td>UMCG</td>
<td>UMCG</td>
<td>8 ( \mu )m</td>
<td>60.0%</td>
<td>100.0%</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td></td>
<td>72.2%</td>
<td>82.2%</td>
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</table>

4. DISCUSSION

Computer-assisted diagnosis is increasingly developed for detection of antibodies in autoimmune diseases, such as commercially available systems for analysis of anti-nuclear antibodies in a blood sample. Using digitalized images of immunofluorescence these systems can discrim-
inate between positive and negative samples, and classification of homogeneous, speckled or dotted patterns (Bizzaro et al., 2014). However, for autoimmune bullous diseases, the detection of autoantibodies in a skin biopsy is essential and requires pattern analysis for subtyping and final diagnosis. In a study by (Terra et al., 2013) it was found that the mean recognition rate of manual selection of u- and n- serrated DIF images by trained experts was 78.6% on an online test with 26 images from subset 1. They concluded that DIF images that contain u-serrated patterns were better recognized than the ones with n-serrated patterns. This is probably because u-serrated patterns have ridge-endings of finger-like structures which are more distinctive and better recognizable than n-serrated patterns. This is, in fact, similar to how our automatic method works - it uses ridge-endings as the most salient feature.

Since the presence of u-serrated immunodeposition patterns in DIF microscopy is a confirmation for EBA diagnosis, it is not necessary to use additional diagnostic techniques. Therefore, it helps with high accurate diagnosis and low medical expenses. This work proposes an automatic system for the localization of u-serrated patterns in DIF images which gives a visualization of the detected patterns together with a reliability score. In this way, we provide a computer-assisted diagnostic system to support the medical doctor or pathologist in the diagnosis process by indicating regions with suspicious signs.

Current studies also aim at defining the optimal technical conditions for serration pattern analysis, such as the way for transportation and storage of biopsies as well as the thickness of the biopsy sections. (Vodegel et al., 2004) concluded that transportation of biopsies in saline is superior to that in Michels medium for diagnosis of sAIBD in DIF microscopy. Transportation of biopsies in Michels medium may lead to false negatives or no detection of serration patterns, which is due to the relatively high undesired background fluorescence in biopsies. Despite such influence, the recognition rates of u-serrated pattern in subsets 4 (Michels medium) and 5 (saline) are the same and quite high (90%). Additionally, differences in section thicknesses of the skin biopsy do not have much influence on the automatic analysis of serration patterns, as the recognition rates of both 4-µm sections (subsets 6 and 9) and 6-µm sections (subsets 7 and 10) are similar. However, when sections are cut thicker at 8-µm, there is a significant difference in recognition rate between samples transported in saline (subset 11) and Michels medium (subset 8). The automatic approach of serration pattern recognition performs better on the DIF images obtained from biopsies transported and stored in saline. This might be explained by the increase of background fluorescence and the decrease of signal to noise ratio in thick sections stored in Michels medium biopsies, which brings negative influences on the detection of ridge-endings in u-serrated pattern DIF images.

The proposed ridge-endings detector can be considered as a general solution for many other applications, such as vessel-ending detection in retinal fundus images and minutiae detection in fingerprints, among others.

5. CONCLUSIONS

We propose a novel automatic system for serration pattern analysis in DIF images, which is used for computer-assisted diagnosis of autoimmune bullous skin diseases. The system uses inhibition-augmented B-COSFIRE filters for the exclusive detection of ridge-endings and localization of the u-serrated patterns based on the frequent and nearby occurrences of ridge-
endings. We demonstrated the effectiveness of our approach on the new NversusU2017 data set with 180 DIF images distributed in 11 subsets, with samples of various quality and laboratory processing resembling the clinical setting. This data set was collected from laboratories in both the University Medical Center Groningen and the University of Lübeck under different experimental conditions. For subset 1 we achieve a recognition rate of 84.6%, which is higher than that (78.6%) of the trained medical doctors and pathologists (Terra et al., 2013). For all 11 subsets we achieve an average recognition rate of 82.2%. The results also show that the most important technical conditions for optimal automatic serration pattern analysis are the sectionthickness of 4-µm or 6-µm, and transportation in saline for the best contrast ratio for pattern recognition.

6. ACKNOWLEDGEMENTS

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REFERENCES
