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Feature-based Differentiation of Malignant Melanomas, Lesions and Healthy Skin in Multiphoton Tomography Skin Images

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Abstract: Malignant melanoma is a very aggressive tumour with the ability to metastasize at an early stage. Therefore, early detection is of great importance. Multiphoton tomography is a new non-invasive examination method in the clinical diagnosis of skin alterations that can be used for such early diagnosis.

In this paper, a method for automated evaluation of multiphoton images of the skin is presented.

The following features at the cellular and subcellular level were extracted to differentiate between malignant melanomas, lesions, and healthy skin: cell symmetry, cell distance, cell density, cell and nucleus contrast, nucleus cell ratio, and homogeneity of cytoplasm. The extracted features formed the basis for the subsequent classification. Two feature sets were used. The first feature set included all the above-mentioned features, while the second feature set included the significantly different features between the three classes resulting from a multivariate analysis of variance. The classification was performed by a Support Vector Machine, the k-Nearest Neighbour algorithm, and Ensemble Learning.

The best classification results were obtained with the Support Vector Machine using the first feature set with an accuracy of 52 % and 79.6 % for malignant melanoma and healthy skin, respectively.

Despite the small number of subjects investigated our results indicate that the proposed automatic method can differentiate malignant melanoma, lesions, and healthy skin. For future clinical application, an extended study with more multiphoton images is needed.

Keywords: skin neoplasm, nevus, skin cancer diagnosis, multiphoton fluorescence microscopy, biomedical image processing, machine learning

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1 Introduction

Malignant melanoma is a highly aggressive tumour metastasising at an early stage and potentially leading to death. For this reason, early detection of malignant melanoma and differentiation from other skin lesions is of great importance for treatment and prognosis. The gold standard for the diagnosis of malignant melanoma is a visual inspection of the whole body, which requires the appropriate experience of the physician. Abnormalities are further investigated by dermoscopy and clarified with a biopsy with subsequent histopathological analysis of the removed tissue [1].

Multiphoton tomography is a new non-invasive examination method in the clinical diagnosis of skin lesions based on the specific excitation of endogenous fluorescent

molecules in the skin and enables high-resolution examination of human skin at a subcellular level without damaging the surrounding tissue [2-5]. It is thus also called an optical biopsy. The manual interpretation of the multiphoton data is tedious because extensive data must be evaluated.

Therefore, we propose a method for automated evaluation of multiphoton images to support medical staff.

2 Methods

2.1 Data

The autofluorescence datasets of six subjects were used, two subjects with healthy skin, two subjects with lesions, and two subjects with malignant melanoma. Images were recorded using the multiphoton tomograph MPT flex ™ (JenLab GmbH, Berlin, Germany) [6].

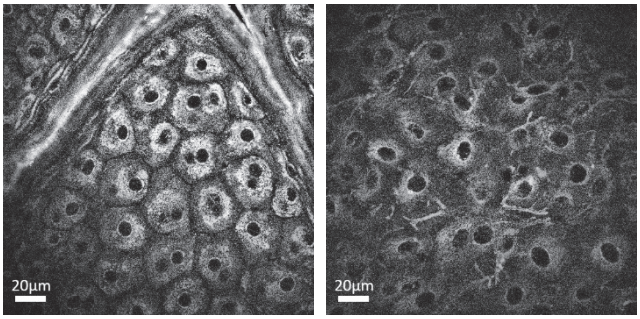

Figure 1: Multiphoton images of healthy skin (left) and malignant melanoma (right).

To ensure a balanced distribution of the three classes, seven autofluorescence images were selected from malignant melanoma, lesions, and healthy skin. Reliably recognizable cell structures in different layers of the epidermis were used. Subsequently, the contours of the cell cytoplasm and nuclei were manually annotated. Due to the different tissue characteristics, there were finally 348 annotated cells of malignant melanoma, 476 cells of lesions, and 707 cells of healthy skin.

2.2 Feature Extraction

Based on previous publications, the following anatomical characteristics were used for differentiation: architectural order, variance of cell shape, size of intercellular distance, separability of cell and nucleus, size of nucleus, and homogeneity of cytoplasm [7-9]. Table 1 lists the established anatomical characteristics and the features that were calculated in the annotated fluorescence images using image processing.

Table 1: Anatomical characteristics and the extracted features used to distinguish malignant melanoma, lesions, and healthy skin.

Anatomical Characteristics	Extracted Features
Architectural order	Cell symmetry, Cell distance, Cell density
Variance of cell shape	Cell symmetry
Size of intercellular distance	Cell distance, Cell density
Cell separability	Cell contrast
Nucleus separability	Nucleus contrast
Nucleus size	Nucleus cell ratio
Homogeneity of cytoplasm	Haralick texture feature homogeneity

The extracted features were calculated on the multiphoton images as follows:

To describe the *cell symmetry*, the symmetry axes of the cell were determined by a principal component analysis. We defined a horizontal symmetry axis as the one which corresponds to the orientation of the eigenvector with the largest eigenvalue. A vertical symmetry axis is defined correspondingly orthogonal to it. To calculate the scalar symmetry values of a cell in the vertical and horizontal directions, the respective ratio of the two cell pixel subsets located laterally to the symmetry axis was calculated. The *cell distance* was calculated using Delaunay triangulation. The distance values were obtained from the Euclidean distances of the adjacent cell centres for the resulting triangulation grid. The *cell density* was calculated by the ratio of the pixels within the cell cytoplasm and nuclei and the total number of image pixels. The *cell and nucleus contrasts* were calculated using a mean gradient estimation along a border area of the cell respectively the nucleus. The *nucleus cell ratio* was described by the ratio of the pixels of the nucleus to the pixels of the cell cytoplasm. The *homogeneity of cytoplasm* was calculated by the Haralick texture feature of homogeneity on the cytoplasm of each cell.

2.3 Feature-based Classification

The classification of malignant melanoma, lesions, and healthy skin was based on the extracted features of the annotated fluorescence images. Three different classifiers established in image processing were used: Support Vector Machine (SVM), k-Nearest Neighbour (kNN) algorithm, and Ensemble Learning. We used the implementations in the Classification Learner App of Matlab 2021a (The Mathworks, Natick, USA).

Two different feature sets were employed as the learning sample for classification. The first feature set includes all the extracted features from Table 1. Feature set two includes a smaller number of features as it is limited to the features that show significant differences among the three classes. These significant different features were analysed with a multivariate analysis of variance (MANOVA) in IBM SPSS Statistics 27.0 (IBM, Armonk, USA). The performed post hoc test showed that there were significant differences in the features of cell distance, homogeneity of cytoplasm, cell contrast, and cell density between the three classes.

Due to the fact that the features were mainly calculated on the individual cells of the autofluorescence images, the classifier also made a class assignment for each cell. Since cell density was the only non-cell-specific feature, it had a special status. As a result, it was underrepresented in the learning sample, and thus this feature was not considered further in the classification. However, the global class assignment of the available image data was based on a physician's assessment of the overall image context.

The training process of the classifiers was based on five-fold cross validation, using six autofluorescence images from each of the three classes. The test of the trained classification models was then performed with the respective seventh autofluorescence images.

3 Results

Figure 2 shows the classification results with all features of the first feature set excluding cell density. The highest accuracy values were obtained for the SVM classifier, with the lesion class being the exception with the highest accuracy for the kNN classifier.

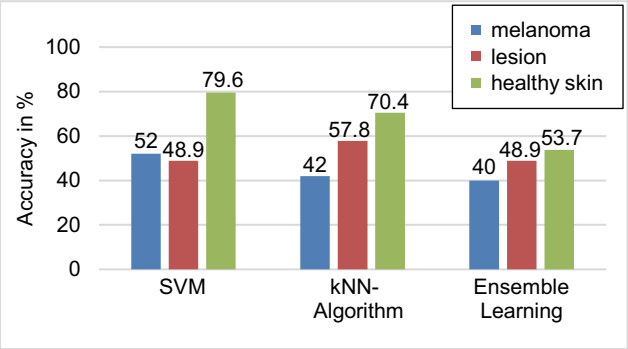

Figure 2: Accuracy of test images of malignant melanoma, lesion, and healthy skin for the trained classification models of SVM, kNN algorithm, and Ensemble Learning based on all features excluding cell density.

Figure 3 provides the results of the classification based on the features that show significant differences between the three classes.

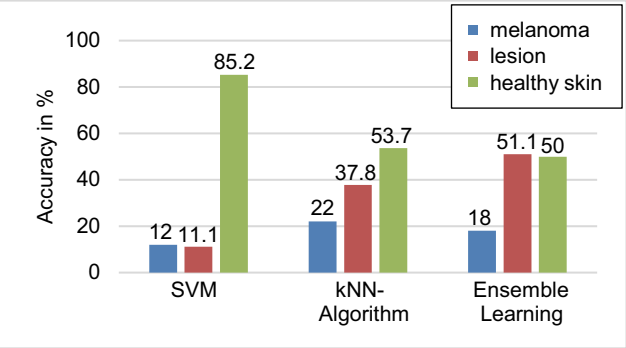

Figure 3: Accuracy of test images of malignant melanoma, lesion, and healthy skin for the trained classification models of SVM, kNN algorithm, and Ensemble Learning based on significantly different features excluding cell density.

Comparing the results of the two feature sets, the classification using all features showed the best results with higher accuracies for all three classifiers. The comparison of the classifiers showed that the SVM provides the best results. It achieved the highest single cell classification accuracy with 52 % and 79.6 % for malignant melanoma and healthy skin, respectively.

4 Discussion

The analysis of multiphoton images is a current research topic to gain deeper insights into disease patterns on a subcellular level. While the physician uses the image in its entirety to assign a diagnosis, we analyse the images at the individual cell level. In the fluorescence images of malignant melanoma, cells of a precursor lesion from which it may have developed and even healthy cells can also be found. Because analysis results strongly depend on which part of the skin, i.e. which part of the melanoma, is visible on the multiphoton image, current research focuses on obtaining a larger field of view. The stage of the disease also influences tissue alterations. A lesion can have characteristics of healthy skin as well as similarities with malignant melanoma. Depending on the type of lesion, healthy cells and even single cells closely resembling malignant melanoma may be present. Even in healthy skin, isolated abnormal cells may be seen. Due to the importance of early detection of malignant melanoma, even a comparatively small number of cells detected as cells of malignant melanoma or lesion, indicating disease of the skin, requires a more detailed evaluation by the dermatologist.

Better classification results were achieved using all features versus those features significantly different between all classes. Since all extracted features show significant differences between individual classes, this could be the reason why the inclusion of further features leads to better separation of individual classes. The inclusion of additional features could improve the classification results.

The main limitation of the present work is the small number of subjects. The multiphoton datasets of two subjects each with healthy skin, lesions, and malignant melanoma are available. From these, 21 multiphoton images with a total of 1531 cells are included in the classification. In addition to the small amount of data, the manual annotation of cells and nuclei is another source of uncertainty. Since the representation in multiphoton images is based on the distribution of endogenous fluorophores, an accurate reproduction of the morphology of anatomical structures is not possible. The contours of cells and nuclei can only be approximated in the images. Consequently, future work should include multimodal imaging.

5 Conclusion

The preliminary character of our study, which may have contributed in part to the low accuracy, does not allow for robust conclusions on future applications of the proposed methodology. Clearly, more data are needed. To address the problem of annotation of large datasets, a (semi-) automatic segmentation method can be used [10].

Possible extensions of our approach include the automatic differentiation of other skin diseases. It may be useful to assess the multiphoton images based on their overall context rather than on single cell level only. The transfer of the single cell-based features to a higher level of abstraction can be a first step to model the global context. This will contribute to the acceptance and wider use of multiphoton tomography in the clinical environment.

Author Statement

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