# The role of computer-assisted analysis in the evaluation of nuclear characteristics for the diagnosis of precancerous and cancerous lesions by contact laryngoscopy

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# ABSTRACT

**Purpose:** Contact endoscopy (CE), through the direct contact with the surface of the mucosa enables in vivo visualization of upper epithelial layers. There is a broad spectrum of laryngeal pathologies, as has been confirmed by earlier CE reports. The aim of the study was to resolve some of the limitations of CE through the application of computer-assisted image analysis. Quantitative and qualitative evaluation of nuclei was applied in the diagnosis of precancerous and cancerous lesions.

**Materials and Methods:** Fifty four patients with various laryngeal pathologies were included in the study. Paraffin section histopathology showed 15 benign lesions, 12 precancerous lesions (5 mild and 7 severe dysplasias) and 27 invasive squamous cell cancers (SCC). After staining the mucous with 1% methylen blue, examination with contact endoscope (Karl Storz, Germany) connected to the C-7070 Wide Zoom Olympus high-resolution camera was performed.

**Results:** The most discriminative parameters were revealed to be as follows: nucleus area (p<0.001), nuclei density index (p<0.001), elongation coefficient (p<0.05), nucleus area to equivalent area ratio (p<0.05). Computer-assisted image analysis composed with data mining techniques is presented for nuclei categorization.

**Conclusions:** We established that computer-aided image analysis can indicate, with a high level of reliability, cases of severe dysplasia and carcinoma. By implementing the technique described in this paper, we can substantially increase the sensitivity of CE.

Key words: contact endoscopy, computer-assisted diagnosis, endoscopic image interpretation, medical technologies applications

# **INTRODUCTION**

Early diagnosis of precancerous laryngeal lesions and locally advanced carcinoma is the most important factor for curative and function preserving therapy. Although classical microlaryngoscopy and white light endoscopy are still the gold standard, much effort has been made oven the years towards improving the pretreatment evaluation and providing intra-operative data on the type of laryngeal pathology.

Contact endoscopy (CE) has been known for almost 30 years[1,2] and has been mostly used in gyneacology. Through direct surface contact of the mucosa the clinician has the

possibility of seeing upper epithelial layers. Stained superficial layers reveal morphological changes, mainly cellular alteration reflecting the nature of the lesion. This technique is an aid in the assessment of in vivo laryngeal fold epithelium, during direct laryngoscopy, without taking biopsies. The characteristic of a broad spectrum of laryngeal pathologies obtained by CE has been well reported[3]. According to the published data, the clear advantage of the technique lies in the monitoring of preneoplastic changes and, moreover, determining the grade of dysplasia[4]. Contact endoscopy helps to highlight tumor margins, thus offering the possibility of more precise removal of laryngeal lesions. The method, which was adopted by Adrea and Dias for laryngological use in the early 90's, is not so widely exploited in this area[5]. It is probably due to the unclear reliability of this technique and the difficulty in data interpretation. Observations from earlier studies suggest that collaboration between endoscopist and pathologist is of the utmost importance, especially at the initial stages[3,4,6].

Progress in computer sciences and imaging techniques has made it possible to improve the contact endoscopy technique. The present study was undertaken to resolve some of contact endoscopy's limitations through the application of computer assisted image analysis.

To the best of our knowledge, this is the first study in which morphometry by image analysis, with quantitative and qualitative evaluation, was applied as a "second opinion" in contact endoscopy diagnosis. The concept of computeraided diagnosis proposed herein can be defined as a diagnosis of laryngeal pathologies supported by the output from a computerized analysis of CE images.

### MATERIALS AND METHODS

The study included 54 patients with various laryngeal pathologies. The patients were being treated or diagnosed in the Department of Otolaryngology at Wroclaw Medical University, Poland. Paraffin section histopathology diagnosed 15 benign lesions, 12 precancerous lesions and 27 invasive squamous cell cancers (SCC). Precancerous changes were evaluated according to the WHO classification, which revealed 5 cases of mild dysplasia (MD) and 7 severe dysplasias (SD). There were 22 men and 17 women in the group, with ages ranging from 47 to 69 years (mean age, 51.9 y). Informed consent was obtained in all cases.

In all the cases, standard microlaryngoscopy under general anesthesia with the Kleinsasser tube was used to examine the entire larynx. The area of interest was stained with 1% methylene blue using a cotton-ball applicator. The methylene blue staining is an *in vivo* staining procedure coloring the cytoplasm and nuclei of the upper epithelial layer. Staining was repeated if longer observation was necessary. The contact endoscope (Karl Storz, Tuttlingen, Germany, 8715BA) was positioned on the epithelial surface. Illumination was provided by a 300W xenon light source (Karl Storz, Tuttlingen, Germany).

The image detector of the system was the C-7070 Wide Zoom Olympus high-resolution camera (7 Megapixels) connected with the endoscope by the converter specially constructed for our measurements. The camera was computercontrolled by USB 2.0 connection to avoid the artifacts caused by camera movements during image capture and in order to adjust the exposure parameters by a computer operator. The preview of the real endoscopic view was available for the clinician to see on the LCD screen of the camera as the image signal was being transmitted to a remote controlled computer imaging system. All the captured images were saved on a 4 GB Compact Flash camera card and can be easily downloaded for further computer-assisted analysis.

In cases of the precancerous or cancerous lesions, after the completion of endoscopic examination, biopsies or excised lesions were taken at the exact contact sites for histological examination. Tissue samples were fixed in 10% formaldehyde, embedded in paraffin and stained with hematoxylin-eosin.

In our study, the computer-assisted system of image analysis was tailor-designed and implemented in order to realize the following: 1) image acquisition and correction (noise removal, image sharpening and contrast modification) 3) nuclei detection algorithm dealing with problems associated with blurred and poorly visible nuclei envelopes[7] 4) nuclear cell parameter calculation 5) nuclei categorization, and finally 6) quantitative and qualitative evaluation of the categorization results[8,9]. The final computer-assisted evaluation of the CE images were compared with those obtained in histopathological analysis. The contact endoscopic images and tissue specimens were independently evaluated by a single pathologist who was unaware of the histological diagnoses and patient data.

According to the suggestions given, previous CE might not be sufficient for precise differentiation between similar grades of dysplasias[10]. In correlation with the histopathological findings, contact endoscopic images were classified by the pathologist into the following types: cancers (SCC), severe dysplasias (SD), and mild dysplasias (MD). The control group (normal epithelium, NE) included the images captured on unchanged epithelium from the opposite side of unilateral benign laryngeal lesion (i.e. polyp and Reinke'a oedema).

In our examinations, we captured all the endoscopic images using 150:1 magnification with a constant image resolution  $3072 \times 2304$  pixels and constant zoom settings.

In every group of the endoscopic images, a semiautomatic methodofcellnucleidetection[7]wasperformed. Morphometric analysis was carried out on 26 260 cell nuclei in total. With the help of special software designed and implemented for the aim of this study, 13 morphometric parameters were calculated for each nucleus:

- area and perimeter
- area to convex area ratio and perimeter to convex perimeter ratio
- length, width and length to width ratio (aspect ratio)
- elongation coefficient[11]
- feret-shape coefficient[12]
- blair-bliss shape coefficient[12]
- perimeter to equivalent perimeter ratio, where the equivalent perimeter is related to the perimeter of a circle having the same area as the measured nuclei
- nucleus area to equivalent area ratio, where the equivalent area is related to the area of a circle having the same area as the measured nuclei
- nuclei density index the idea defined for this index is based on the partition of the image space using multiple

Image type	Histopathologic results	Description of the contact endoscopy image characteristics
SCC	Squamous cell cancer (Fig. 1 a)	Disordered, crowded cells, enlarged nuclei, high variable nuclear morphol- ogy, increased nucleus to cytoplasm ratio
SD	Severe dysplasia (Fig. 1 b)	Enlarged nuclei, crowded cells with moderate variable nuclear morphol- ogy, increased nucleus to cytoplasm ratio
MD	Mild dysplasia (Fig. 1 c)	Moderate changes in the size of the selected nuclei, nuclei spatial relation- ships slightly abnormal, not uniform shape of the nuclei
NE	Normal epithe- lium (Fig. 1 d)	Regular cell formation, uniform round nuclei with a slight size variation

*Table 1.* Description of CE images in the four analyzed groups of findings.

*Figure 1.* Input CE images of specified laryngeal lesions: squamous cell cancer (a), severe dysplasia (b), mild dysplasia (c) and normal epithelium (d) with corresponding results of image analysis (right column) (see text for detailed description).



grids of different size, also called multi-resolution grids. The value of this index is defined as the combination of interpolated and weighted superposition of multi-

resolution values of the nuclei density function. It is calculated for every nucleus (for a detailed description of this density function – see ref[8]). This index takes decimal values from zero to one and describes local nuclei distribution and density in the analyzed image.

The area and perimeter values are expressed in image pixels. All the remaining parameters, except for the nuclei density index, are shape parameters. All of them are nondimensional quantities and take decimal values in the range 0-3.

In our investigations, we also used data mining techniques: 1) the fuzzy clustering approach proposed by Gustaffson and Kessel[13](the detailed description of using it in CE imaging is presented in ref[8]), and 2) a "learning by examples"[14] method for final rules generation. These rules are subsequently used for evaluation of nuclear morphometry, as applied to CE images. The resultant rule bank can be saved in the computer system memory for the evaluation of other CE images.

The statistical analysis of our results consisted of parameter descriptive analysis and Liliefors testing to ascertain how the data correlated with normal probability distributions. Variation analysis (ANOVA), using the Friedman test, was used for the evaluation of the dependence of all numerical parameters from the findings in the control group and histopathology. The spearman correlation test was used to evaluate the correlations between nucleus parameters.

#### RESULTS

In our study, the CE findings were similar to those presented in previous publications [3,4,10]. These results relating to the four specified histological evaluations are summarized in *Tab. 1* and selected images from the each group are presented in *Fig. 1*.

Variation analysis selected the four best discriminative parameters for specified histology evaluations. They are as follows:

- nucleus area (p < 0.001)
- nuclei density index (p < 0.001)
- elongation coefficient (p < 0.05)
- nucleus area to equivalent area ratio (p < 0.05)

The *Fig. 2* shows the median values with 1<sup>st</sup> quartile spread of the selected nuclei parameters in relation to specified histological evaluations.

We used the Spearman correlation test to estimate the statistical correlations between nuclei density and other parameters. The results showed no significant correlation of nuclei density to shape the coefficient and high correlation with nucleus area parameter.

Based upon the measurements derived from all analyzed images (26 260 cell nuclei), the cluster analysis found three classes of nuclei and a rule bank for nuclei categorization was created. The hypothetical results of nuclei categorization for the input images shown in *Fig. 1* in the left column are



*Figure 2.* Median with 1<sup>st</sup> quartile spread of the selected parameters in relation to specified histological evaluations (SCC - squamous cell cancer; SD - severe dysplasia; MD - mild dysplasia; NE - normal epithelium).

presented in the same figure in the right column. The interpretation of the resultant categorization was linguistically described by the pathologist from abnormal (pathological, class 1 shown in red in Fig. 1) to normal nuclei (class 3 - shown in green). Whereas classes 1 and 3 are easily distinguishable, class 2 (blue color in Fig. 1) shall be considered an intermediate nuclei category. However, class 2 should be considered an abnormal category, and as such, is it more similar to class 1 (i.e. pathological cases) than to class 3 (i.e. normal). The number of nuclei belonging to class 1 on the images of normal epithelium was in range 0 - 3, and the amount of area occupied by this category of nuclei was never more than 1% of the total nuclei area and is not statistically significant. For this reason nuclei from class 1 found on the NE images can be treated as the outliers and therefore can be excluded from further analysis. Fig. 3 and 4 shows the results of the categorization by a rule-based system described as the mean values of each nuclear parameter for categorized nuclei in relation to specified histological evaluations.

The last step of computer-assisted analysis was used for the verification of the hypothesis that the amount of area occupied by nuclei from every class is characteristic for each type of laryngeal lesion. The results of variation analysis (p<0,05) showed that the input parameters related to the amount of area occupied by every category of nuclei on the analyzed CE image originate from different distributions. Mean values of the area occupied by every category of nuclei in relation to specified histological evaluations are presented in *Fig. 5.* 

*Figure 3.* Mean values of nuclei area and density for classified nuclei in relation to specified histological evaluations (SCC - squamous cell cancer; SD - severe dysplasia; MD - mild dysplasia; NE - normal epithelium).



Based upon the data mining technique described above, the final 4-decision set of rules was generated (in relation

*Figure 4.* Mean values of elongation and ratio: area to equivalent area for classified nuclei in relation to specified histological evaluations (SCC - squamous cell cancer; SD - severe dysplasia; MD - mild dysplasia; NE - normal epithelium).



to the four histological evaluations), taking into account the area's participation of nuclei from every of the three nuclei categories. The computer-assisted analysis of all CE images showed a sensitivity of 91% and specificity of 81% (p<0.05). All malignant lesions and severe dysplasias diagnosed by histopathology were confirmed by the CE. All false positive and false negative cases were positioned in the MD and normal epithelium. Thus, the specificity and sensitivity of the method increases up to 100% only in relation to SCC and severe dysplasia cases.

# DISCUSSION

Most of the widely ranging diagnostic methods, such as microlaryngoscopy, depend mainly on the personal experiences of the clinician which are often insufficient to define the character of the epithelial lesion. To date, the most reliable way to demonstrate the progress from preneoplastic changes to malignancy was multiple biopsies with a histological examination. Therefore any technique which reduces the need for more biopsies is obviously worth deeper investigation. Computer-assisted CE may reliably indicate the lesion for subsequent biopsying. Endoscopists will therefore have to be trained to recognize the atypical patterns of tumor cells.

The contact laryngoscopy ability to determine the magnitude of dysplastic changes is still not fully clear [4,10,15]. According to our results, it is not possible to discriminate with full certainty the grade of epithelial dysplasia. We revealed that computer aided image analysis, with a high reliability,

*Figure 5.* Mean values of area occupied by every category of nuclei in relation to the histological diagnosis (SCC - squamous cell cancer; SD - sever dysplasia; MD - mild dysplasia; NE - normal epithelium).



indicates cases of severe dysplasia and carcinoma. It is difficult to differentiate between mild dysplasias (MD) and the control group (NE). This is attributable to limited (or lack of) nuclei discrimination between these two groups. It was also reported in[11,16], that dysplasia and normal epithelium in histopathological image data cannot be correctly differentiated using nuclear morphometry analysis. The same is also true for contact endoscopic image data. Slight differences in nuclei shape in MD and NE cases may not be noticed or it can be distorted by image blurring, insufficient staining, or presence of secretion.

Regarding the diagnostic accuracy of CE, the authors in[15] obtained the following results: a sensitivity of within 75 to 88%; a specificity of 100%, but they only investigated malignant lesions. Our specificity results were comparable to theirs, however our sensitivity results are a clear improvement on theirs (100%). Furthermore, unlike others, we considered a mixture of malignant and mild lesions (for these we received: a sensitivity of 91% and a specificity of 81%).

Similarly to the previous reports, during the investigation, even with high speed camera settings and image correction methods, we were forced to repeat the examination to obtain a more representative image. This was caused by non-constant parameters in the endoscopic optical system related to changes of tangential axis to the examined tissue. From the practical point of view in computer-assisted analysis, high-resolution (at least 5 MPixels) image acquisition is required. Moreover, on the basis of our experiences the best solution would be a small and light, high-resolution and high-speed color digital video-camera equipped with an on-line preview on an LCD screen mounted with the endoscopic device. The received video signal would be analyzed in the computer system for selecting and analyzing the frames of the best quality.

In most of the earlier publications, researchers mainly referred to the images at a magnification of 60:1 due to insufficient image quality at 150:1[17,18,19]. In our opinion, a satisfactory morphometric analysis is practically impossible to obtain using the lower magnification (60:1), even with high resolution camera settings. Moreover, a magnification of 150:1 and image capture with high-resolution allows for more

detailed examination of a single layer of epithelial cells[20]. Thereby the clinician may more precisely determine the cellular appearance (alterations), which is the most informative hint from CE.

In all CE images, 13 parameters were calculated for each detected nucleus. All these parameters would be treated as the "numerical" description of characteristics presented in *Tab. 1*. There is one exception: nucleus to cytoplasm ratio. We found that correct detection of the cytoplasmic contours on the CE image is difficult to obtain. The computer method of cell border detection[7] for the calculation of the nucleus to cytoplasm ratio was drawn up, but their reliability was very low because of the highly blurred cell contours. Also, the statistical analysis proved its low reliability (p=0,68).

The presented results (*Fig. 3*) did not show statistically significant differences between normal and mild dysplastic nuclei, while both carcinoma and severe dysplasia parameters are significantly different; they are much more larger with high variances. The most discriminative (p<0.001) parameters are nuclei density index and area. The remaining parameters i.e. the elongation coefficient and the area to equivalent area ration are less discriminative (p<0.05).

The next step of computer-assisted analysis was related to the categorization of cell nuclei based upon the statistically selected features. The main problem with diagnostic interpretation of the CE results is due to the "subjective" description of the image attributes defined as: "highly deformed shape of the nuclei" and "the cells nuclei are grouped very closely". All these attributes defy precise description of the image objects such as: object size in pixels, value of the chosen shape coefficient etc., being the best modeled by fuzzy sets[21] . Thus, in our study this task was realized by means of the fuzzy clustering approach. Until now, there is no mention of nuclei categorization in published literature related to CE imaging. We wanted to propose a possible way of undertaking such a categorization. Due to the imprecise nature of pathological nuclei description, it seemed entirely appropriate to use the fuzzy clustering. This data mining technique found that the optimal number is three nuclei categories. The found clusters were further used for building the fuzzy rule-based system (a type of reasoning which is similar to human decision making). The resultant classification system is able to categorize nucleus into one of the three categories taking into account their nature. An analysis of the results presented in Fig. 3 and 4 showed that three of the four nucleus parameters (namely: area, elongation coefficient and area to equivalent area ratio) take similar values in each given nuclei category. This is independent of the degree of epithelial changes. The nuclei density shows statistically different values in both SCC and SD in comparison to values in MD and NE. These results are consistent with the "context dependent" interpretation of this parameter. That is to say, that this parameter is highly correlated with distances to all neighboring nuclei. We want to stress that this parameter would approximately describe the nucleus to cytoplasm ratio because shorter distances to neighboring nuclei

may suggest a bigger nucleus surrounded by a smaller amount of cytoplasm. Moreover, it would characterize the cytological description defined as "crowded cells". That's why it reaches high values in SCC and SD in comparison to MD and NE. All parameters achieve statistically different (p<0,05) values between elaborated classes of nuclei for all histological cases.

We assumed that the amount of nuclei from each category would be a good descriptor of nuclear characteristics and would be correlated to the amount of pathological changes. Statistical analysis of variances rejected the hypothesis that percentage amounts of the mean area of the categorized nuclei is not related to the findings in histopathology (p<0,05).

The most significant finding is the absence or very low number of nuclei from class 1 (most pathological) on the NE images. This finding increases the diagnostic accuracy between malignant lesion (SCC, SD) and precancerous or normal cases. Our results may also suggest that diagnosis of carcinoma and severe dysplasia can definitely be made when the nuclei in class 1 (i.e highly pathological) cover more than 5 % of the total nuclei area, and when nuclei in class 2 (i.e. moderately pathological) cover more that 40% of total nuclei area.

The observations from our research have direct implications. Specifically, we have confirmed that practical assessment of the nuclear morphometry for the diagnosis of laryngeal lesions by CE is not only possible but it can be improved by computerassisted analysis. Another advantage of the technique is that it can indicate the appropriate tissue area for biopsy. The method proposed herein may aid the clinician, especially in the initial phase of the learning curve as a valuable completion to the pathologist's advice and books[22].

# CONCLUSIONS

We conclude that contact laryngoscopy, with image analysis, facilitates *in vivo* diagnosis of laryngeal cancer and its precursor lesions. Diagnostic accuracy of CE can clearly be improved in carcinoma and severe dysplasia cases. Furthermore, having a "numerical" characterization of endoscopic pathological patterns, it is possible to make quantitatively more precise diagnoses. It should be noticed that this has not been possible hitherto, because of lack of such a numerical description. However, we want to emphasize that even the described modification cannot yet substitute diagnosis by histological examination, which still remains the gold standard.

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