"HISTOPATHOLOGICAL ASSESSMENT OF SURGICAL MARGINS IN ORAL SQUAMOUS CELL CARCINOMA BY MANUAL METHOD AND BY USING IMAGEJ SOFTWARE – A COMPARATIVE STUDY"

By

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LIST OF ABBREVATIONS

SR. NO.	ABBREVATIONS	FULL FORM
1.	%	Percentage
2.	i.e.,	That is
3.	No.	Number
4.	OSCC	Oral squamous cell carcinoma
5.	OED	Oral epithelial dysplasia
6.	WHO	World Health Organization
7.	NIH	National Institute of Health
8.	OPMDs	Oral Potentially Malignant Disorders
9.	GBS	Gingivobuccal sulcus
10.	UV rays	Ultra-Violet rays
11.	DNA	Deoxyribonucleic acid
12.	RNA	Ribonucleic acid
13.	TNM	Tumor(T), nodes(N), and metastases(M)
14.	POI	Pattern of invasion
15.	LRR	Locoregional recurrence
16.	TIC	Touch imprint cytology
17.	OCT	Optical coherence tomography
18.	SM	Surgical margins
19.	CAP	College of American Pathologists

20.	NCCN	National Comprehensive Cancer Network
21.	p-value	Probability
22.	HNMs	Head and Neck malignancies
23.	DFS	Disease free survival
24.	CIS	Carcinoma in-situ
25.	IAOO	International Association of Oral Oncology
26.	JRE	Java runtime environment
27.	H & E	Hematoxylin and eosin
28.	RND	Radical Neck Dissection
29.	IntDen	Integrated density
30.	WDSCC	Well differentiated squamous cell carcinoma
31.	MDSCC	Moderately differentiated squamous cell carcinoma
32.	PDSCC	Poorly differentiated squamous cell carcinoma
33.	RT	Radiation therapy
34.	ENE	Extra-nodal extension
35.	NM	Normal mucosa
36.	MD	Mild dysplasia
37.	MOD	Moderate dysplasia
38.	SD	Severe dysplasia
39.	BM	Buccal mucosa

ABSTRACT

Introduction:

Despite the fact that there are over 2,70,000 new cases of oral cancer detected each year and that the prognosis is not good, it is not a widely publicized health care problem. Whether adjuvant therapy is used or not, surgery is the major standard of care during the early stages. Obtaining full tumour extirpation with disease-free margins is still a need for successful surgical care of oral squamous cell carcinoma (OSCC). The existence of mild to severe oral epithelial dysplasia around excised oscc surgical margins presents a high risk for the formation of local recurrence. Clear margins exhibit good prognostic values with a low or no recurrence rate, but near and involved margins may exhibit recurrence and exhibit poor prognosis. Because it determines the prognosis, survival, recurrence, and postoperative therapy for patients with head and neck cancer, microscopic examination of the margins continues to be a crucial part of oncologic care. The classification of mucosal dysplasia at the margins of resection is another point of variation among studies and a possible source of debate because the rate of progression to invasive carcinoma may differ depending on the degree of the dysplasia. Dysplasia grades are based on architectural and cytological abnormalities, therefore subjective impressions can lead to significant intra and interobservational variability. This subjectivity will make it more difficult to accurately diagnose marginal status. As computer power has become more accessible and affordable in recent years, there has been a fascinating increase in automation and enhancing histopathological diagnosis through image analysis and pattern recognition. The development of a novel, repeatable, and objective method for grading dysplasia may benefit from the new quantitative techniques.

Aim of the study:

Histopathological assessment of surgical margins in oral squamous cell carcinoma by manual method and its comparison by using ImageJ software.

Objectives:

1. To assess the epithelial architecture and cytological features of normal oral mucosa, dysplasia and surgical margins of OSCC by manual method.

2. To analyse the epithelial architecture and cytological features of normal oral mucosa, dysplasia and surgical margins of OSCC using ImageJ software.

3. To compare both methods for histological assessment of surgical margins of OSCC.

Material and methodology:

A total of 120 samples were taken for this study. 40 each from surface of normal oral mucosa, dysplasia (mild, moderate and severe dysplasia) and surgical margins in oral squamous cell carcinoma. Total of 726 photomicrographs were captured using Olympus camera and magvision software under 40x magnification. Architectural and cellular analysis of the images was done in ImageJ software using different plugins. Morphometrical analysis of the segmented images were performed in the software for cellular segmentation and nuclei detection.

Results:

Microscopic evaluation of normal mucosa, different grades of dysplasia were performed. Assessment of anterior, posterior, medial and lateral surgical margins showed negative status except for two cases with dysplasia and one with positive status. Comparison of morphometric analysis of normal mucosa and different grades

of dysplasia was done using one-way ANOVA tests and the results showed statistically significant difference in mean values of the parameters analyzed for both cellular segmentation and nuclei detection. Further, the corelation of normal mucosa and different grades of dysplasia with all four surgical margins was performed. The morphometrical analysis of surgical margins using ImageJ, corelating the mean values with normal mucosa and different grades of dysplasia showed that the mean values of parameters obtained in surgical margins were in close relation to mean values obtained for different grades of dysplasia also along with mean values of normal mucosa. Also, the results obtained from both manual method and image analysis showed that there was difference in diagnosing dysplasia at surgical margins.

Conclusion:

The results show that there are statistical differences on the morphometrical parameters analysed across the diagnostic groups. These can be reliably used for discrimination purposes. This objective analysis can help quantitatively to distinguish the feature between normal and dysplastic features. Also, can be helpful in assessing the surgical margins and minimize the subjective variation occurring during catergeroizing the grades of dysplasia.

Keywords: oral squamous cell carcinoma, recurrence, radical neck dissection, surgical margins, oral epithelial dysplasia, ImageJ

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INTRODUCTION

The term cancer describes uncontrolled and abnormal proliferation of cells that have the potential to invade or spread to other areas of the body and harm nearby tissue.[1] A small, unidentified growth or soreness in the lips, cheeks, sinuses, tongue, hard and soft palate, oropharynx, or base of the mouth can be the first sign of oral cancer.[1]

Globally oral cancers rank sixth among all types. It ranks as the 15th most common cause of mortality globally and the 16th most prevalent malignancy. The incidence of oral cancer is four cases per 10,000 persons worldwide, with large regional variations based on gender, age groups, nations, racial and ethnic groupings, and socioeconomic circumstances.[1-3] Unquestionably, the major disparities between the developed and developing world stem from different population habits, life expectancies, preventive education, and medical record quality in various nations like poverty, illiteracy, advanced stage at presentation, lack of access to health care, and inadequate treatment infrastructure.[1,4,5] A quarter of all cases worldwide—roughly 77,000 new cases and 52,000 deaths—are reported each year in India.[6] As one of the most prevalent types of cancer affecting a significant population, oral cancer has emerged as a significant public health concern in India. India accounts for one-third of the world's mouth cancer cases, making it the country with the highest incidence.[6-8]

OSCC commonly results from potentially malignant lesions or normal epithelium linings.[6,9] Oral Potentially malignant disorders (OPMDS) such as inflammatory oral submucous fibrosis, erythroplakia, leukoplakia, candidal

leukoplakia, dyskeratosis congenital, and lichen planus are indicators of the preclinical phase of oral cancer.[6,9] Some of the risk factors for oral cancer include tobacco use, especially smokeless tobacco , chewing betel nut, excessive alcohol use, poor dental hygiene, a diet low in nutrients, and persistent viral infections, such as the human papillomavirus.[6,7]

However, if detected in the early stages (I and II), survival rates can surpass 80%. Oral cancer has a terrible prognosis, with an overall 5-year survival rate as low as 40%. [10-12] As most patients are not symptomatic in the early stages and do not seek medical attention until they display apparent signs like pain, bleeding, or a mass in the mouth or neck if the lymphatic dissemination is already present. 50% of oral cancers are detected at an advanced stage (stage III and IV). [10,13]

The clinical and pathological stage at diagnosis remains the most crucial factor in influencing prognosis. Because oral cancer has a high death rate, early detection of the condition and anticipation of a diagnosis improves prognosis and survival rates while lowering treatment-related morbidity.

Whether adjuvant therapy is used or not, surgery is the major standard of care during the early stages. Radiation therapy, chemotherapy, and systemic therapy may be used alone or in combination after surgery in more advanced stages. Also, the stages and histological gradings influence the choice of treatment.[16-21]

Locoregional control becomes the most important component in determining treatment success and future recurrence prevention if surgical excision is chosen as the primary treatment strategy.[22,23] Complete tumour extirpation with disease-free margins must still be attained for surgical management of OSCC to be successful.[18]

Even after margin-free surgical resection, the locoregional recurrence(LRR) rate range between 16 to 20%, suggesting that the issue of margins is still considered the most determinant prognostic factor in survival.[22,23] Margin status is used to advise the use of adjuvant therapy, such as radiation therapy, systemic chemotherapy, or revision surgery, as well as to determine the prognosis of the patient.[24] Additionally, numerous studies have demonstrated that achieving negative resection margins is beneficial for local recurrence and overall survival.[25] Studies have indicated that because dysplasia appears before OSCC, having mild to severe epithelial dysplasia at the margins of excised OSCC entails a high risk for the development of local recurrence.[25,26]

The present clinical approach relies on employing frozen sections intraoperatively followed by traditional histopathological investigation postoperatively to determine the status of the surgical margin(SM).[18,27] A precise evaluation of the pathologic surgical margin is required to optimize patient management since attaining a clean margin has a significant impact.[24] Margin status is reported as 'Positive or Negative', 'Involved or clear' and close when assessed histopathologically. Additionally, what distance is an 'Adequate or Clear' margin for patient care continues to be debated.[28] Most studies that specifically define margin distance use a somewhat arbitrary definition of > or = to 5mm to define the safest margin.[29] Therefore, a key component of managing oral cancer is to complete removal of tumour and obtain clear surgical margins.

Due to the intricate three-dimensional architecture of the subsites of the oral cavity, the handling of the specimen from resection to interpretation, and tissue shrinkage just after surgery and after fixation, OSCC surgical margins are

challenging to interpret.[24,29,30] As a result, a dedicated team of head and neck specialists, including surgeons and pathologists, are essential to the functioning multidisciplinary team treating osccs.[24] In addition to variation at the macroscopic level, there is disagreement on how to define the microscopic margin of the tumour specimen. The true margin may include any or all of the following: the border at which there is no invasive cancer, the border at which there is no carcinoma-in situ or the border where there is no dysplasia.[24,30,31] According to studies, there is a high chance of local recurrence when there is mild to moderate epithelial dysplasia at the margins.[32]

When it comes to diagnosing dysplastic features present in the epithelium of margins, there are variations in the pathological interpretation and classification of dysplasia.[33] Dysplasia features are graded into three categories: mild, moderate and severe.[29,32,33] Despite the existence of many architectural and cellular alterations and WHO-defined criteria for evaluating epithelial dysplasia, these criteria are typically subjective and inconsistent.[29,32,33] In the agreement of the gradings, numerous investigations have discovered a significant amount of variation.[29,32] There is poor to moderate inter-observer agreement among pathologists, according to recent studies on head and neck pathology.[24,29,30] According to Pindborg et al., there is a 1–78% discrepancy in the dysplasia diagnosis made by different pathologists. Abbey et al., discovered a 42–62% discordance rate between the dysplasia gradings.[24,32,34] Additionally, there is intra-observer variability, which means that the same pathologist could have two distinct perspectives on whether dysplasia is present and also in categorizing them. Other studies have supported these findings of poor to moderate agreement in detecting dysplasia with a large deal of subjectivity.[29] This

subjectivity will make it more difficult to accurately diagnose marginal status, which may further influence treatment options, prognosis, and survival without disease.

Also, with an improved understanding of tumour biology and technological advances, new techniques have emerged to analyze margins at the molecular level. Such molecular margins analysis interrogates tissue for genetic, epigenetic or proteomic changes that may detect the tumour presence or other aggressive features which are not captured by standard histopathologic techniques. All these methods are technique sensitive, cost affective and need sophisticated equipment.

As computer power has become increasingly ubiquitous and less expensive, there has been an increase in interest for automation and enhancing histopathological diagnosis, particularly those that depend on subjectivity in visual perception, through the use of quantitative analysis of images and by pattern recognition.[35] The new quantitative methods might be helpful in establishing a new, reproducible and objective approach in assessing dysplasia grades.[35] Many studies have attempted to analyse the histopathologic images and automated quantitatively using many software like ImageJ, Qu path, Fiji, Cmarker, Cell profile etc. Automating and quantifying the trivial problem in categorizing the dysplasia can allow more diagnostic accuracy in confirming the margin status of OSCC surgical specimens.

Since traditional histological descriptions include meta information like topological placement, physiological and pathological mechanisms in tissues, the clinical aspects of lesions, or potential causal agents in addition to directly quantifiable features like cell geometry, shape, and form received from the picture, it is challenging to describe histopathology data statistically. The majority of imaging methods used to diagnose issues measure the geometric properties of components

present in tissues like cell and nuclear number, shape, staining intensity and texture. The characteristics that can be summed up in terms of the models and image-derived features now used in histopathology are anticipated to become quantitative markers. It is conceivable that characteristics that may be summed up in terms of geometrical models already in existence will become quantitative markers.[35]

Gabriel Landini et al., have analyzed the architecture of normal epithelium, dysplasia & oral cancer by means of automation and quantification using ImageJ software to see the accuracy of the diagnosis, that aids in better prognosis. In order to describe the local spatial arrangement of cells in tissues, the study came up with a quantitative and objective method.[35]

Other advantage of ImageJ software is that it is an open access software by NIH, USA, and has shown potential results for quantitative assessment of dysplastic features. Hence, the present study aims to quantitatively assess the surgical margins of OSCC using ImageJ and compare it with histopathological assessment.

AIM OF THE STUDY:

Histopathological assessment of surgical margins in oral squamous cell carcinoma by manual method and its comparison by using ImageJ software.

OBJECTIVES:

1. To assess the epithelial architecture and cytological features of normal oral mucosa, dysplasia and surgical margins of OSCC by manual method.

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REVIEW OF LITERATURE

Oral Cancer

A type of tumours commonly known as oral cancer can develop anywhere in the mouth cavity, pharynx, or salivary glands. In contrast, oral squamous cell carcinoma, the most common oral tumour, is frequently used interchangeably with this phrase. According to estimates, OSCC make up more than 90% of all oral neoplasm.[36,37]

The fact that this neoplasm can emerge and go undetected in the beginning is one of its main risks. Despite having easy access to the oral cavity for clinical inspection, OSCC is typically identified in advanced stages. The most frequent causes are an incorrect initial diagnosis and patient or attending physician ignorance.[36] Usually, the early stages are asymptomatic, but as it progresses, a burning feeling or discomfort may appear. The tongue, lips, and mouth's floor are typical locations for OSCC to grow. Some oscc develop in mucosa that appears to be normal, while others are preceded by premalignant lesions that are clinically visible, particularly erythroplakia and leukoplakia. OSCC typically manifests as an ulcer with elevated exophytic edges or fissuring.[36]

The percentages of OSCC morbidity and mortality have not changed considerably during the past 30 years, despite advances in therapeutic methods.[36,38] In Asia, India has the highest rate of oral cavity cancer. Their regional variations in disease incidence are probably caused by the various social customs. The most common cause is the habit of chewing tobacco (smokeless tobacco), which is combined with betel nuts and unidentified compounds for flavor and colour.[39] Due to

the positioning and retention of the tobacco-lime mixture in the area, the gingivobuccal sulcus of the mandible is the site of classic Indian cancer. Pan masala has gained popularity over the past three decades as a result of its accessibility in pre-mixed packaging. This can be eaten or retained in the mouth, i.e., GBS, causing the contents to continuously act on the oral mucosa.

However, Oral cancer is believed to be primarily brought on by the practice of chewing betel nut leaves that have been rolled with lime and tobacco and called "pan." This practice causes prolonged contact of the carcinogens with the buccal mucosa.[40] 74% of the population's attributable risk is attributed to alcohol consumption and tobacco use.[41] The higher incidence of cancer in the younger population is explained by viral infections, particularly the Human Papillomavirus.[42,43] Genetic vulnerability resulting from variations in DNA repair pathways and carcinogen metabolizing enzymes, which typically happen in diets deficient in micronutrients, is another factor connected to oral carcinogenesis [44-46] Excessive sun radiation/UV light, Sulphur dioxide, pesticides, mists from strong inorganic acids, and the use of fossil fuels are other lesser-known risk factors.[47,48]

Recurrence

In recent years, there has been a lot of research done on the variables that affect the recurrence of OSCC.[49] Numerous investigations revealed that age, sex, the presence of alcohol or tobacco use, the tumour site, TNM stage, surgical margins, and other clinicopathological factors could all have an impact on local recurrence. Additionally, some research showed that clinical and demographic factors, such as depth of invasion and POI, might provide reliable parameters for local recurrence.[23]

Over the past few decades, improvements in surgery, chemotherapy, radiotherapy, and more recent treatments including molecularly targeted medicines and immunotherapy have greatly decreased morbidity and increased survival.[50] Despite these advancements, cancer continues to be the second greatest cause of mortality in the US, behind cardiovascular disease.[50] A long-term cure is rarely attainable at the stage of metastatic disease, which is where the majority of contemporary emphasis in antineoplastic therapy is directed. Control on a local level is crucial for reliably curing cancer. However, LRR is a clinically significant, predominate pattern of failure in many cancers in the in terms of poor survival and quality of life for individuals with these tumours is local recurrence following surgical resection.[50,51]

Certain original tumour characteristics, such as the T-stage, N-stage, and histological differentiation; patient variables, such as age, alcohol use, and smoking status; and treatment considerations, such as positive resection margins, all affect the probability of locoregional recurrence.[23,52,53,54,55] Despite the development of multimodality therapy and advances in our knowledge of prognostic factors and carcinogenesis, there is still about one oral cancer-related death for every two new diagnoses. Inoperable primary tumours, treatment-related problems, second primary tumours resulting from field cancerization, locoregional recurrence, and distant metastases are the most common causes of disease-related death.[11,56,57] Recurrence risk and relapse-free survival are often used to evaluate treatment response in oral cancer management.[56]

Recurrence was described as:

(1) Local recurrence—recurrence at the same anatomic site within 5 years after primary treatment
(2) Regional recurrence—lymph node metastases of the neck within 5 years after primary treatment.
(3) Distant metastases—metastases elsewhere in the body, e.g., the lungs
(4) Second cancer of the oral cavity—carcinomas elsewhere in the aerodigestive tract within 5 years after primary treatment or recurrence at the same anatomical site 5 years after primary treatment.[58]

Tumor Recurrence and Follow-Up intervals in Oral Squamous Cell Carcinoma was a study done by Sebastian Blatt et al.[58] The purpose of this study was to evaluate the recurrence pattern, detection technique, and related characteristics for potential risk categorization. 24 percent of recurrences were found during the first 12 months. Advanced histological grading, lymph node metastasis, and tumour recurrence all substantially linked with one another.[58]

According to a number of studies, pathological variables (such as grade, stage, and nodal status) and treatment methods may have an impact on the recurrence rate but are not necessarily independent factors for local OSCC recurrence.[49,59,60]

In 275 patients that had been documented with OSCC, Bo Wang et al., noted recurrence and survival. The authors discovered that the 5-year survival rate was 54.5% and the recurrence rate was 32.7% with a time range of 2 to 96 months. T stage, degree of differentiation, pN stage, application of a flap, resection margin, and lymphovascular invasion were variables of recurrence, according to a univariate

analysis ($P<0.05$). The T-stage, level of differentiation, and pN stage were identified as independent recurrence factors by multivariate analysis.[55]

In order to determine the time to recurrence and patient survival in recurrent OSCC, Annelies Weckx et al., undertook a study. The duration to recurrence, along with other independent criteria, is crucial in determining the prognosis and survival probability after recurrence, according to the authors. A worse patient prognosis is indicated by a shorter time to recurrence. They also came to the conclusion that there is a strong correlation between the lymph node ratio, the margin status, and the main tumour grade and the time of recurrence.[61]

A study done by T. C. Chen, et al., looking at the relationship between oral dysplasia and recurrence found that patients with a known history of oral epithelial dysplasia may benefit from an intensified post treatment follow-up to screen for recurrent disease. A history of oral epithelial dysplasia was in significant association with a higher risk of locoregional recurrence of early-stage oral squamous cell carcinoma.[62]

A meta-analysis was performed by Caroline Racheal Anderson et al., to determine whether surgical margins were related to the OSCC recurrence rate. This systematic analysis of studies found that when recurrence rates were pooled, there was a 21% absolute risk reduction in local recurrence with margins clear by more than 5 mm (95% confidence interval, 12-30%; $p = 0.00001$). In patients with margins clean by more than 5 mm, the unweighted pooled recurrence rates were 20%. According to their research, the lowest permissible margin size in OSCC is a 5 mm pathological margin.[63]

Surgical margins

With cervical nodal dissection or en-bloc removal of the original tumour depending on the presence or occult risk of regional metastases, surgery is the mainstay of treatment for OSCC.[68,69] The borders of the resection specimen that the surgeon excises are known as the surgical margins or resection margins.[70] The condition of these removed surgical margins is a crucial and useful indicator of how well the treatment will work.[66]

Hinni et al., defined a resection margin as "any tissue plane where the surgeon's knife meets the patient."[68]

To achieve full excision of the tumour during surgery, the palpable and visible tumour is resected with a perimeter of healthy tissue.[67,68]

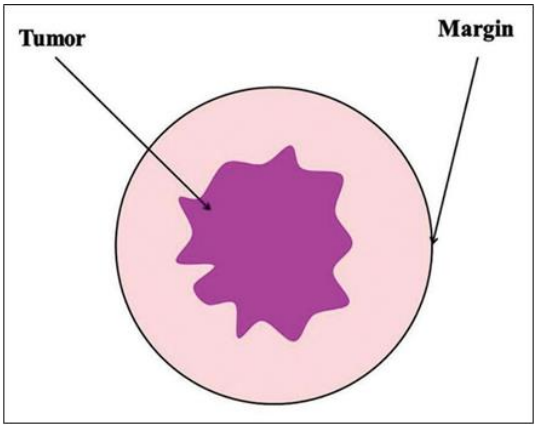


Figure 1: A conceptual illustration of the resection Margin

During surgery for oral cancer, the operating surgeon evaluates the surgical margins clinically through visual inspection and palpation. When performing final histopathology, the surgeon typically attempts to achieve a 1-2 cm gross visible margin to ensure appropriate microscopic tumor-free clearance.[69]

Inking of surgical margin

The histological examination includes the anatomical orientation of the excised OSCC specimen accurately and the identification of all surgical margins. Using dye or pigments, sectioning techniques, and attaching clips and sutures are few ways to distinguish surgical margins before and after processing; the former is the most accurate. The inking of margins has recently become popular due to its simplicity of usage and distinct demarcation of borders. Traditionally, the surgically resected margins have been marked with Indian ink. India ink is made of proteins, and cross-linked fixatives firmly fix it to the tissue. Consequently, the employment of multicolour inking is growing which is used for

1. Examination of multiple surfaces/margins
2. Postoperative comparison of tissue planes
3. Benefit of post-grossing three-dimensional reconstruction
4. Reduce in identification error when multiple sampling is required from the same tissue or when obtaining similar specimens from different patients.[66,70,71,72]

Types of tumour margin

The margins of the tumors are categorized as follows:

- Clinical margins: They are margins in tumour on clinical observation and palpation, which are included during the resection of tumour tissue.[66,73]
- Surgical Margins: The term "resection margin" refers to any tissue plane in patient, where the surgical instrument comes in contact. It also comprises the

submucosal and deeper connective tissues around the tumour, in addition to the surface mucosa (at the tumour's margin).[66,68,74]

There are two different types of resection margins, each determined by the anatomical nature of the tissue:

1. Mucosal margins: a mucosal margin is a border of mucosa that surrounds the tumour.

2. Soft-tissue or deep margins: To achieve the total removal of tumour tissue, a three-dimensional resection is necessary. This avoids the excision of the tumour with a cuff of normal or tumor-free soft tissues around and underneath the tumour, i.e., from beyond the deepest invasive focus. This is what the resection's "soft-tissue margin" or "soft-tissue base" entails. Deep resection margins are associated with recurrences more commonly, according to studies. All connective tissue components, such as skeletal muscle, adipose tissue, and neurovascular bundles, are included in the deep or soft tissue margins.[67,75]

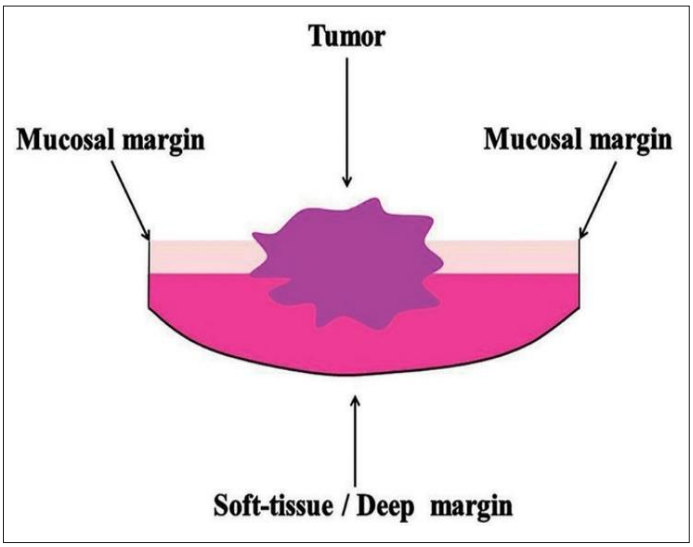


Figure 2: An illustration of the "mucosal" and "soft-tissue" borders

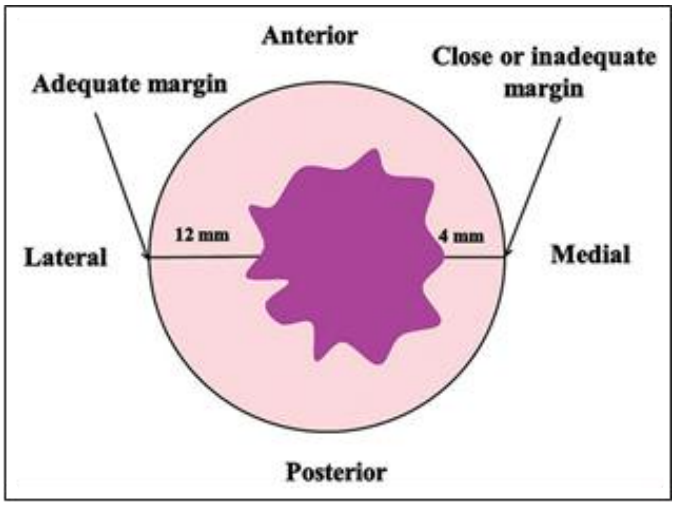


Figure 3: Resection margins that are "sufficient" and "inadequate or near," respectively are depicted in a diagram.

Microscopically, surgical margins are categorized into histological and molecular margins.[66]

Histological margins: Pathologists examine the specimen's borders and margins for signs of tumour cells.

Negative or clear margin: surgical margins are histologically separated from the invasive carcinoma by more than 5 mm.

- Close margin: Histological separation between the invasive cancer and surgical margins of 1 to 5 mm
- Involved/positive margin: Histological separation of the invasive carcinoma from surgical margins of less than 1 mm.[30,66,76,77,78,79]

Molecular margins: The histologically normal margins may harbour genetic changes. Thus, various molecular markers have been recently employed to detect these fields of genetically altered cells.[66,80]

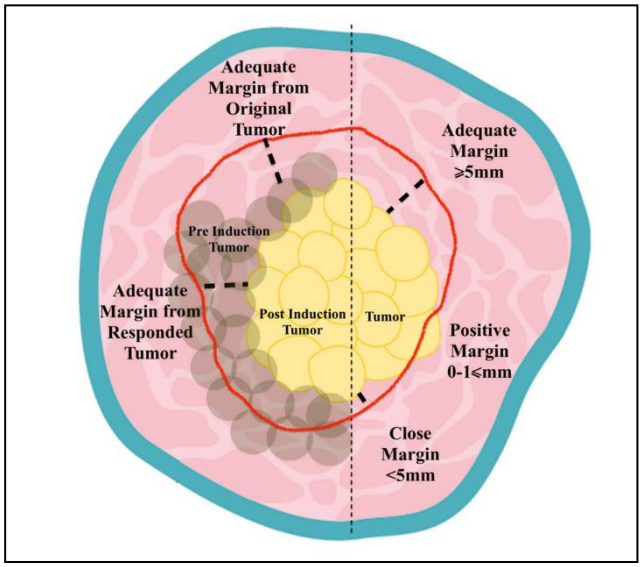


Figure 4: Another image demonstrating proper and improper margins

In order to ensure full excision of the tumour, the area of resection must be increased if there is a tumour or moderate- severe epithelial dysplasia at the resection margin, or if the margin is insufficient. This is accomplished by removing more tissue fringes up until a "tumor-free" or "negative" margin is reached.[67,81] "Revised," "reinforced," or "supplemental" margins are the names for these additional margins. Therefore, the mucosal site just next to the previous resection margins is where the updated margins are taken from. Similarly, the edges of the surgical defect left after the resection, or sections from the mucosal tissues outside the resection perimeter, comprise genuine margins. In situ residual disease is either present or absent depending on the actual margin's condition.[67]

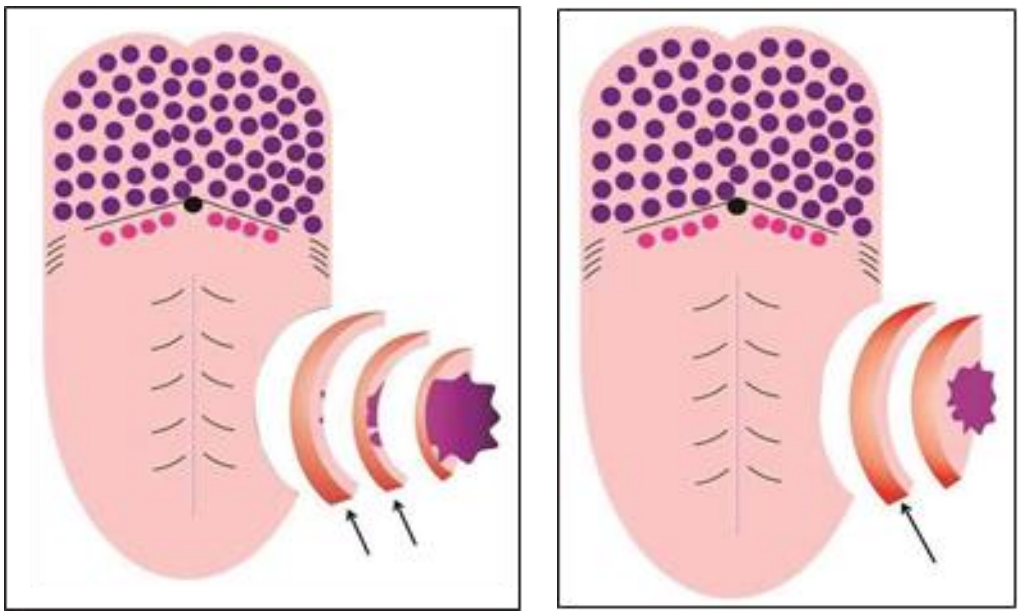


Figure 5: A pictorial illustration in tongue tumour resection is shown, along with an explanation of "actual" resection margins and "revised" margins (both shown by black arrows

Assessment of surgical margins

The pathological evaluation of the resection margins is crucial. The prognostic and therapeutic consequences of SM status (clean/close/positive) are significant. Different techniques have been developed to accurately evaluate surgical margins and, consequently, the effectiveness of tumour removal. A practical classification of techniques for rating SM based to time of usage relative to the time of surgery was proposed by Ravi and Annavajjula (Table 1). It is advised that the surgeon and/or pathologists use the most appropriate approaches from the variety of available ways to ensure the removal of the tumour.[66,82,83]

Table 1: Evaluation of surgical margins		
Preoperative Assessment	**Intraoperative assessment**	**Postoperative assessment**
Vital staining fluorescent visualization	Thickness of resected margins Frozen section analysis TIC Micro endoscope OCT	Mohs microsurgery Frozen technique (Intraoperative assessment) Formalin technique (postoperative assessment) Ultrasonography Molecular assessment Gene signature
TIC=Touch imprint cytology, OCT=Optical coherence tomography		

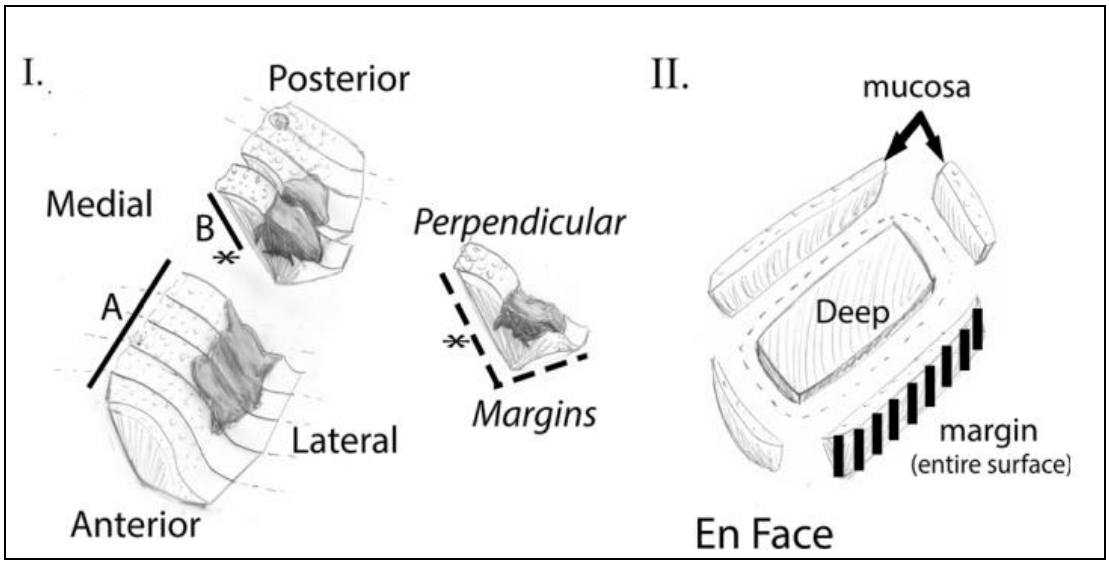


Figure 6: Methods of assessment of surgical margins. I- specimen assessment and serial sectioning versus II- surgeon directed tissue sampling from the tumor defect.

Factors affecting the assessment of surgical margin

Surgeon measures surgical margins (which appear appropriate before and during surgery) and the margins examined by pathologists after resection frequently differ from one other. This discrepancy has been attributed to a number of causes, including:

- Tissue shrinking following excision and pathologic processing.[66,84,85]
- A reduction in the size of the tissue under strain after being surgically released from the tissue it was attached to.[66,86]

Tissue shrinkage –

One of the significant aspects that affects how preoperative and postoperative margins are measured is tissue shrinkage.[66,76] There are a number of internal and extrinsic variables that affect this tissue shrinking. Different tissue compositions, the location and stage of the tumour, the cohesiveness of the tumour cells, the degree of keratinization, the level of inflammation, and the tumour site are intrinsic factors. Additionally, it has been noted that shrinkage differs at various specimen edges taken from the same source. [66,84,85]

Only three studies have examined the amount of shrinking in relation to the tumour's anatomical location; Table 2 presents their results. There is evidence that tissue shrinking is more rapid at margins which are without bone support.[66,87,88,89]

Table 2: Degree of shrinkage of surgical margins obtained from different anatomical sites

Authors (year)	Sample size	Site	Shrinkage (%)
Mistry et al., 2005[24]	27	Buccal mucosa	21.2
		Tongue	23.5
Cheng et al., 2008[25]	41	Buccal mucosa, mandibular alveolar ridge, retromolar trigone	71.9
		Maxillary alveolar ridge and palate	53.33
		Tongue	42.14
El-Fol et al., 2015[26]	61	Buccal mucosa	66.7
		Tongue	35
		Floor of mouth	33.3
		Retromolar trigone	16.7
		Mandibular alveolus	15.4

Mucosal elasticity: In the case of buccal cancer, mucosal elasticity is hypothesized to affect the tumour dimension and surgical margins. Recently, Tsai et al., indicated that this elasticity should be taken into account while computing sufficient surgical margins for transoral resection of the buccal mucosa after discovering a 32.35% magnification of buccal mucosa elasticity due to stretching during maximal mouth opening.[66,86]

Optimal resection margin: An important factor in ensuring local control and determining adjuvant radiotherapy is the optimal resection margin, or SM. The location of the tumor, anatomical constraints, biological characteristics, and the scope of the operation all affect how effectively the tumour is removed. Factors associated

with the rate of involved margins according to various anatomical sites are depicted in table 3.[66,90,91]

Table 3: Effect of tumor location on the margin status

Site of tumor	Margin status	Comment
Tongue	Involved margins are less common in this Site	This is related to the tongue's architecture, which allowed for the design and modification of hemiglossectomy procedures that were sufficient for obtaining clear margins.
Floor of mouth and margins retromolar areas	Frequently show involved margins	Anatomical restrictions and poor access in cases of floor of mouth the presence of loose areolar tissue around the sublingual gland and deep to it, the location of muscular bulk deep within the body, and the invasion of the lingual nerve and sublingual ganglion can all be possible sources of tumour infiltration and spread.
Buccal mucosa	Top-ranked site for involved margins	A split-thickness cheek excision has inherent laxity that causes an excessive amount of specimen shrinking.
Bone	Involved bone margins are rarely encountered and are usually seen in association with involved mucosal or deep soft tissue margin	Invasion at the periosteum under the cortical plates or inside the cancellous bone may be noticed when bone is implicated.

Histologically tumor-free margins: Even at the clinically normal margins of the tumour, changes take place at the histological and molecular levels as a result of the oral mucosa as a whole being exposed to carcinogens. On routine histopathologic examination, tumour-free margins might show features of chronic mucosal irritation,

cellular atypia, and mild epithelial dysplasia and thus reported as head and neck malignancies(HNMs).[66,92]

Molecular margins: Molecular boundaries in accordance with the literature, 10% to 30% of OSCC patients with HNMs experience local recurrence. The reasons put forth include (a) minimum residual cancer, which is cancer that cannot be diagnosed by standard histology, and (b) a non-resected "field of genetically changed cells" that is macroscopically invisible. These areas act as breeding grounds for the development of invasive malignancy and potentially harmful lesions.[66,93]

According to histological analysis, a margin of at least 5.0 mm constitutes a full excision. A margin of 1.0-4.9 mm is regarded as "near," while one of 1.0 mm is regarded as "involved."[94] Most respondents to a survey among American Head Neck Society members believed there was a clear margin when the size was higher than 5 mm.[94]

In addition, less than one-third of respondents thought that dysplasia at the margin was implicated and not all respondents thought that margins with cancer in-situ were positive.[94,95] The College of American Pathologists (CAP) comments that values between 3 mm and 7 mm have been effectively employed and regards moderate/severe dysplasia at resection margins as a favorable margin, which only serves to further the confusion.[94]

A clear margin is defined by the most recent National Comprehensive Cancer Network (NCCN) guidelines as an invasive tumour that is at least 5 mm away from the resected margin. An invasive tumour with a near margin is one that is 1 to 4.9 mm away from the margin of the resection. A positive margin is one with an invasive

tumour less than 1 mm from the resection margin. Re-resection is recommended if the final histology shows a positive or near margin.[69,95,96] This shows that different head and neck clinicians have different standards for what constitutes a sufficient margin status.[94]

Surgery margins in head and neck squamous cell carcinoma were examined, evaluated, and related to the clinical outcome by Arjun Singh et al. The review came to the conclusion that the definition of an adequate margin varies depending on the site in the head and neck region, with a margin of 5 mm at final histopathology being the most widely accepted standard.[97]

The surgical excision margins of OSCC that has already advanced frequently contain epithelial dysplasia.[98] Epithelial dysplasia at the margins of excision is one of the clinical and histological markers that have been discovered as indications of prognosis and is a crucial parameter that raises the probability of recurrence.[98] It's possible to locate OED at the mucosal edges after the OSCC is removed. It is important to include this in the histopathological report.[98] The 2017 WHO categorization of oral epithelial dysplasia allows for the inclusion of the dysplasia's grade, which can be mild, moderate, or severe. The existence and grade of OED in OSCC excision margins must be taken into consideration, according to more recent studies evaluating the components that affect the surgical margin.[98,99]

Patients with OSCC were observed by Tseng Cheng Chen et al., to be impacted by dysplastic surgical margins. The study showed that after surgery, about 10% of individuals with oral cancer would have margins that were dysplastic. Dysplastic margin was substantially related with T1/T2 disease or tumour thickness

10 mm. According to the results, the dysplastic margin had much better disease management than the positive margin.[62]

In a study, Jasjit K. Dillon et al., investigated the relationship between the surgical excision margins of OSCC patients and results of disease-free and overall survival. According to the findings, having a small surgical margin (1 to 5 mm) carries a similar risk factor as having an involved margin and is thus linked to a worse chance of remaining disease-free and overall survival.[100]

A study by Yamada et al., estimating the width of the free margin with a substantial impact on local recurrence in surgical excision of oral squamous cell carcinoma revealed that whether a free margin of within 1, 2, or 4 mm was regarded a close margin, the probability of local recurrence was significantly different in clear, close and involved surgical margins of the patients. The difference between clear and close margin did not reach statistical significance when close margin was defined as being within 5 mm of the resection margin. According to the study's findings, the benchmark for oncological surgery should be a 5 mm clearance at the surgical resection margin.[101]

In a study done by Y. Pu et al., local recurrence rates and various margin status were compared clinically in patients who are undergoing primary surgical therapy for oral squamous cell carcinoma. Recurrence was seen in some individuals with dysplasia at the first margin in the authors' clinical practice. The findings showed that low disease-free survival (DFS) and higher local recurrence were independent indicators of mild dysplasia at the first margin without re-excision. In contrast to the group without re-excision, modest dysplasia with re-excision was linked to better control in local control and disease-free survival.[102]

Epithelial dysplasia

Over 60 years ago, oral pathology developed the idea of epithelial dysplasia after realizing potentiality of some oral lesions to develop into oral cancer, which are today known as oral potentially malignant disorders (OPMDS).[103] Epithelial dysplasia which means abnormal growth, is the preferred word for the histological alterations in the oral epithelium that suggest a malignant transformation risk.[103] There is no clinical or morphological counterpart for epithelial dysplasia; it simply describes histological changes.[103]

'Dysplasia (dys abnormal/bad; plasia = growth) was a concept introduced by Reagon in 1958, describing the cells exfoliated from uterine cervix lesions. In a broader sense, it means the abnormal, atypical proliferation encountered principally in the epithelium. Epithelial dysplasia, epithelial atypia and dyskeratosis, in the past, were used synonymously.

Pindborg (1977) defined oral epithelial dysplasia as "A lesion in which part of the thickness of the epithelium is replaced by the cells showing varying degrees of cellular atypia".

Burckhardt and Maerker (1981) claimed epithelial dysplasia "As a measure of tissue and cellular deviation from the normal."

Kumar et al., (1992) defined it "as a disturbance in the maturational sequence of the stratified squamous epithelium and disturbance in cell kinetics of the proliferative compartment with cytological changes."

It is the microscopic transformation of healthy cells or tissue into malignant cells (cellular atypia). Few of the abnormalities may be visible in the benign and innocuous epithelium, such as a regenerating epithelium on the edge of an ulcer or lesion, and these dysplastic cells can be found in pre-malignancy and malignancy. The first microscopic sign that a premalignant lesion may eventually grow into a carcinoma is typically dysplastic alterations.[104,105]

Oral pre-cancer lesions are altered epithelial lesions that are more likely to develop into squamous cell carcinoma. They are most frequently seen as leukoplakia and erythroplakia. At a professional workshop held in London in 2005, it was suggested that the distinction between potentially malignant lesions and conditions should be abandoned in favor of a shared terminology of Oral Potentially Malignant Disorders after reviewing the terminology, natural course, and prognostic value of this group of lesions (OPMDs).[106,107]

This nomenclature acknowledges the possibility of malignancy developing in patients elsewhere as a result of field change, even in lesions like leukoplakia, which is typically a limited lesion. The Working group also recommended a new definition for leukoplakia which suggested that "The term leukoplakia should be used to identify white plaques of questionable risk having omitted (other) identified diseases or conditions that carry no increased risk for cancer". [106,107]

The predictive significance of the currently available molecular markers has not been established, and they have not yet been thoroughly examined in prospective investigations. Oral epithelial dysplasia (OED) is the accepted histological name in oral and maxillofacial pathology for a lesion that can be precancerous.[33,108]

The severity of the histological grade is typically correlated with the risk of progression. Accurately forecasting which lesions will develop into cancer and at what rate is still a challenge. Epithelial dysplasia is graded according to cytological and architectural alterations. Despite being used widely by oral pathologists, it is nonetheless hampered by the existence of inter- and intra-examiner variation in its assessment.[31]

OED lesions can be regarded of as morphological variations of various phases in the development of normal tissue into malignant tissue. According to the architectural features based on the thickness of the dysplastic layers compared with the aggregate of the epithelial height, which was proposed in the classification of tumours of the head and neck given by WHO in 2005, epithelial dysplasia was divided into hyperplasia, mild, moderate, severe, and carcinoma in situ.[109,116]

Numerous dysplastic traits have been graded in various combinations. Assessment and standardization of the various degrees of epithelial dysplasia have proven challenging. To standardize the severity of the dysplastic characteristics, systems of grading epithelial dysplasia were devised. Only if the grading system can be replicated by different observers will it be clinically useful. Additionally, the variables taken into account during the histological assessment should have biological significance and represent the lesion's risk for malignancy.[109]

The various grading systems put forth by different authors are as follows[109]:

A. Smith and Pindborg's photographic method (1969)
B. Mehta et al. (1971)
C. Bancozy and Csiba (1976)

D. WHO (1978)
E. Kramer (1980)
F. Burkhardt and Maerkar (1981)
G. Shafer (1983)
H. Lumermann H et al. (1995)
I. Neville et al. (1995)
J. Speight PM et al. (1996)
K. Kuffer and Lombardi (2002)
L. Ljubljana (2003)
M. Brothwell DJ (2003)
N. WHO system (2005)
O. Binary system (2005)

WHO Dysplasia Classification (2005):[110]

The WHO dysplasia system includes the following categories.

Hyperplasia with Increased Number of Cells: There may be an increased number of cells in the spinous layer (acanthosis) or the cell layers of the basal and parabasal region (basal cell hyperplasia).There is no cellular atypia, and the architecture of epithelium is preserved.

Grades of Dysplasia

1. Mild Dysplasia: The limitation of the architectural disturbance is to the lower third of the epithelium and is accompanied by cytological atypia.
2. Moderate Dysplasia: The initial criterion for recognizing this category of dysplasia is the presence of disturbance of architecture extending almost to the

middle third of the epithelium. However, based on the degree of cytological atypia, upgradation to severe dysplasia may be required.

3. Severe Dysplasia: Architectural disturbance with associated cytological atypia is greater than two-thirds of the epithelium. However, as noted in moderate dysplasia, architectural disturbance limited to the lower and middle third of the epithelium but with sufficient cytological atypia may be included in this category.
4. Carcinoma In Situ: Carcinoma in situ (CIS) is represented by full or nearly full-thickness architectural abnormalities noted in the viable cellular layers along with pronounced cytological atypia. The presence of atypical mitotic figures and abnormal superficial mitoses is relatively common

Binary Grading System (2005):[111]

Two class classification (no/questionable mild suggestive of low risk; moderate or severe - suggestive of high risk) of dysplasia to reduce the ambiguity among pathologists was introduced for reducing inter-examiner, and intra-examiner variation as most pathologists diagnose oral epithelial dysplasia based on the architectural and cytological changes."

The binary grading system was perfected by the WHO Classification 2005 and proved beneficial in making clinical decisions, mainly in cases of moderate dysplasia, which are troublesome to diagnose.

The cut-off point for a "high-risk" lesion (with potential susceptibility for malignant transformation) was established by observing the least possible of four architectural changes and around five cytological changes. However, the cutoff point

for a "low-risk" lesion (does not have the potential susceptibility for malignant transformation) is associated with observation of architectural changes of a minimum of four in number or cytological changes of less than five in number."

Table 4: Architectural and cytological features of oral epithelial dysplasia from the 2017 WHO classification

Architectural features	Cytological features
1. Irregular epithelial stratification	1. Abnormal variation in nuclear size
2. Loss of polarity of basal cells	2. Abnormal variation in nuclear shape
3. Drop-shaped rete ridges	3. Abnormal variation in cell size
4. Increased number of mitotic figures	4. Abnormal variation in cell shape
5. Abnormally superficial mitoses	5. Increased nuclear-cytoplasmic ratio
6.Premature keratinization in single cells (dyskeratosis)	6. Atypical mitotic figures
7. Keratin pearls within rete ridges	7. Increased number and size of nucleoli
8. Loss of epithelial cell cohesion	8. Hyperchromasia

Correlation between oral epithelial dysplasia and malignant transformation:

The association between dysplastic alterations in the oral mucosal lining and its eventual development of OSCC has been known for a long time. The period between diagnosing the presence of cancer developing from a dysplastic lesion lasts around two to five years from the moment of the diagnosis of the dysplastic lesion. Some cases may also be much longer. The risk of malignant transformation is between 6.6% and 36.4%. However, a recent meta-analysis indicated a rate of 12.1% as per a study done by M. W. Ho et al. It is also possible that different risk factors are operative in dysplasia and carcinoma in situ than are responsible for the development of frank malignancy. The lack of data from comparable well-defined patient groups

and the lack of a consensus on the grading and reproducibility of dysplasia are additional factors that negatively impact its predictive value, as exemplified by two recent studies.[112,113]

Since the first thorough description by Kramer et al., the diagnostic criteria for epithelial dysplasia have changed over a number of decades. The 2005 WHO dysplasia grading system, which has five diagnostic categories, has undergone a number of revisions that have been recorded. More recently, the WHO's 4th edition (2017) included a three-tiered system for grading dysplasia, with mild, moderate, and severe categories; severe dysplasia and cancer in situ are seen as interchangeable. Reibel et al., also took into account the high-risk and low-risk classification scheme for reporting dysplasia that was first put forth by Kujan et al.[114,115] Nankivell et al., confirmed the binary grading method, and new enhancements were suggested.[114,116]

Inter and Intraobserver variability

Inter-observer variation is the difference in findings from two or more observers looking at the same object. When an observer views the same object more than once, there is an amount of variation known as intra-observer variation.

Histopathological analysis is currently the gold standard in the diagnosis of OPMDs. Higher grade dysplastic lesions are thought to be more susceptible to transformation. The determination of dysplasia is subjective and not reproducible. Due to the subjective nature of its assessment, there can be significant inter- and intraobserver variability when grading dysplasia based on architectural and cytological changes.[116,117]

When reporting epithelial dysplasia using the WHO grading system (2005), as assessed by Warnakulasuriya et al., intra-observer and inter-observer discrepancies were noted. Pindborg et al., were the first to draw attention to inter-observer difference in reporting on a range of disorders from dysplasia to oral cancer.[33,34,121] Later, only a few authors have made an effort to quantify or statistically analyse the changes that were discovered. The majority of authors noted low or average agreement between various observers.[118,119,120,121,122,123,124] The results they obtained using various statistical techniques ranged from weak to significant agreement.[29]

At a pre-symposium held in May 2017 in Bengaluru, India, in conjunction with the 6th World Congress of the International Association of Oral Oncology (IAOO), a group of Indian oral and general pathologists reviewed the difficulties associated with dysplasia scoring. Those who published the proceedings were Warnakulasuriya et al., in a multi-center study among a few top academic oral pathology centers in India. There was general agreement among the participants to evaluate the observer variability in reporting epithelial dysplasia.[121,125]

A review of English-language publications revealed the need for a consistent approach to reporting OED. This would add yet more variable into the classification of OED lesions. Despite the fact that the 2005 WHO categorization described each category and provided guidance on how to evaluate OED lesions, it lacked definitions or model photomicrographs that might highlight the distinct architectural and cytological aspects.[29]

Lakshmi Krishnan et al., conducted a study to examine the diversity in three grading systems for oral epithelial dysplasia, found that there was fair to good intraobserver agreement as well as minimal to poor inter-observer agreement. Author

suggested that the variability in dysplasia diagnosis could be reduced by defining the individual characteristics more precisely, establishing the grading systems in a repeatable manner, and training pathologists.[124]

Subhashini A. R. et al., carried out a cross-sectional investigation to determine how differently the two pathologists graded the dysplasia in the same patients. The diagnosis of oral potentially malignant illnesses can be quite subjective because the grading of dysplasia is dependent on architectural and cytological alterations. The study's findings provided proof that there is subjectivity in the grading and diagnosis of dysplastic characteristics.[117]

In order to examine intra-observer and inter-observer variability in two grading systems for oral epithelial dysplasia, Ranganathan et al., undertook a multicentric study in India. The WHO 2005 criteria and the binary system served as the two grading scales. The results showed that the lead investigator and other observers had low to fair levels of inter- and intra-observer agreement.[121]

In one study that evaluated inter-observer agreement in histopathological grading of OED, Dost et al., concluded that OED grading has a such poor predictive value that it should not be used as a treatment guide. Edwards (2014) responded in a follow-up editorial to this result by arguing that, despite its drawbacks, OED grading affords a pathologist the best chance to communicate the total risk of malignancy to the clinician. The authors also concurred that molecular indicators are required to aid the pathologist and may ultimately result in a more precise OED risk categorization.[126]

When a suitable gold standard is not available, inter- and intra-observer agreement levels can be used to determine the validity of grading systems. There is a critical need for a trustworthy approach that derives the highest level of agreement from the current flawed gold standard given the poor levels of inter- and intraobserver agreement on histopathology OED grading described in various literature reviews.[138] An odds ratio of 2.4 (99% CI 1.5-3.8) in a recent meta-analysis found that moderate/severe OED was linked to a higher probability of malignant transformation than mild OED.[139] Patients with higher OED grades reportedly have increased rates of malignant transformation, according to several studies.[129,130,131,132]

To determine the morphometric alterations in the suprabasal cell layer present in various degrees of oral epithelial dysplasia, Viswanathan Prema et al., performed a computer-assisted microscopic study on the suprabasal cell layer. Comparison of histological grading and grouping based on nuclear area, nuclear perimeter, N/C ratio, and nuclear volume density between grades of dysplasia and normal mucosa showed association between the two was discovered. The potential of a cell's biology is reflected in its nuclear shape. As a result, the combination of numerous nuclear data reveals the aggressiveness of the tumour behavior. The morphometric technique offers the chance to measure the nuclear alterations and creates an impartial framework for grading dysplasia using a process supported by computers and a variety of parameters. As it provides a precise objective assessment for the grading of dysplasia, computer-assisted morphometric analysis may help to lower interobserver variability.[133]

ImageJ Software

For decades, biologists have used software to view and analyse imaging data. Imaging software needs to evolve as acquisition methods get more complex, producing greater multidimensional information.[134] With the help of its active and helpful user and development communities, ImageJ is an open-source image analysis software platform that has benefited academics with a variety of image analysis applications.[134]

ImageJ is open-source software that has developed into a crucial lab tool. It is written in Java and can run on Linux, Mac OS X, and Windows in 32-bit and 64-bit modes. In addition to its impressive functionality, this cutting-edge image-processing tool.[146]

The main developer of ImageJ, Wayne Rasband bravely began over with ImageJ using the Java programming language after ten years of working on the Macintosh-based National Institutes of Health (NIH) Image. Rasband freed the software from a certain operating system by switching to Java. To run ImageJ on a specific system, all that is required is the Java runtime environment for that operating system. Java runtime environments (JRE) are freely downloadable from Sun or come pre-installed on different platforms with ImageJ (rsb.info.nih.gov/ij). The majority of operating systems support JRE.[135,136]

While Rasband is the creator of the core programme, a large team of external developers have built and made accessible a growing collection of quick add-on applications to supplement the basic program's capability. The plugins for ImageJ are written in Java, while the additional files are written in ImageJ's macro language (macros). These features can be accessible via menu commands just like any other

core feature once they have been saved to the ImageJ plugins folder and are loaded upon startup.[135]

After being set up, it is simple to update the main ImageJ application (the IJ.JAR file) by downloading a new version from the NIH website.[135] A popular open-source programme called ImageJ enables users to display, examine, quantify, and validate scientific image data.[134,137] Imaging-based techniques have advanced significantly over the past few decades and play a critical role in the life sciences. The foundation of image analysis is having repeatable and reliable ways to interpret biological images when new imaging modalities and datasets arise.[145,148] Numerous plugins are installed with ImageJ. This extensive list of plugins illustrates the widespread use of ImageJ in science and engineering, including microscopy, the material sciences, and medical imaging.[134,137]

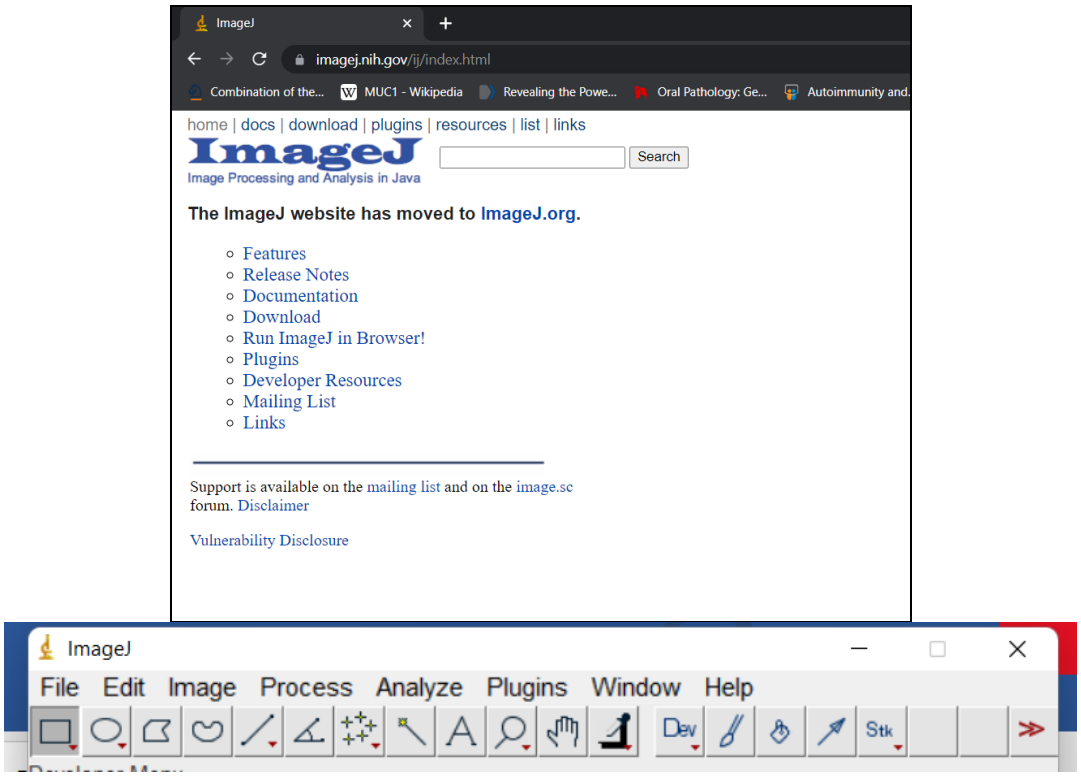


Figure 7: Picture of ImageJ website in browser and installed ImageJ software tool.

Over the past few decades, numerous enormous biological improvements have been made possible by novel imaging modalities that offer improved resolution, specificity, and coverage. Thus, effective and reliable techniques for manipulating, interpreting, and visualizing such sophisticated, multidimensional imaging data are necessary in modern research. This is critical for a variety of biological studies, such as estimating the closeness of fluorescently labelled proteins, monitoring the fate of cells over time, automating cell counting, observing cancer cells that have invaded healthy cells, gathering whole-slide data, estimating and characterizing brain cells like microglia, or registering multiview light sheet fluorescence microscopy datasets to study development. Additionally, image analysis plays a significant biological function in the interpretation of diagnoses. The ability to take measurements manually becomes impractically time-consuming when the incidence of large multidimensional datasets keeps rising. Doing so can severely limit its sensitivity, accuracy, objectivity, and reproducibility.[134]

ImageJ allows users to segment 62 track particles,63 and register64 their datasets. Biologists will be able to computationally separate certain regions in their photographs using segmentation, or object recognition and delineation, such as the total area occupied by cells in brightfield microscopy images.[134]

Plugins used in the study:

MorphoLibJ.[138]

It is a set of ImageJ plugins and mathematical morphological approaches developed at the INRA-IJPB Modeling and Digital Imaging lab.

The library adds a number of features that were either not included in ImageJ or only partially addressed by previous plugins. Namely:

- Morphological filtering for 2D/3D and binary or grey level images: erosion & dilation, closing & opening, morphological gradient & Laplacian, top-hat…
- Morphological reconstruction for 2D/3D and binary or grey level images, allowing fast detection of regional or extended extrema, removing of borders, hole filling, attribute filtering
- Watershed segmentation + GUI, making it possible to segment 2D/3D images of (for instance) cell tissues.
- 2D/3D measurements: photometric (intensity) and morphometric measurements such as volume, surface area, inertia ellipse/ellipsoid
- Binary/label image utilities for removing or keeping the largest connected component, performing size opening, fill holes, kill borders
- Plugins adopted from MorphoLibJ in the present study.[138]

Regional minima

They are defined as connected regions of elements (pixels or voxels) with the same value and whose neighboring elements all have values greater than that of the region. Similarly, regional maxima are regions of connected pixels or voxels with the same value, whose neighbors all have a smaller value.

One problem arising with regional minima or maxima is that they are very sensitive to noise. It is often more convenient to use the so-called extended extrema. The principle is to define a tolerance value for filtering the extrema. For example, extended maxima are defined as a connected region containing elements such that the

difference of the value of each element within the region with the maximal value within the region is lower than the tolerance and such that the neighbors of the regions all have values smaller than the maximum within the region minus the tolerance. This definition allows the identification of larger extrema that better takes into account the noise within the image. The extended minima are defined in a similar way and are efficiently used as a pre-processing step for watershed segmentation.[138]

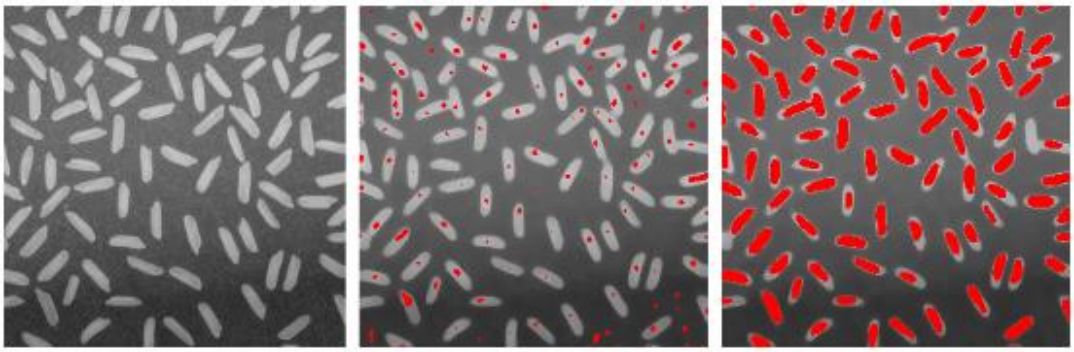


Figure 8: depicting regional and extended maxima on grey-level image. Left: original image. Middle: result of regional maxima. Right: result of extended maxima

Watershed segmentation

The watershed algorithm assimilates the grey level image to a digital elevation model and aims at detecting the different catchment basins. In the grey-level image, the catchment basins correspond to dark regions surrounded by bright structures (the "crests"). It is a prevalent technique especially used to segment touching objects. The MorphoLibJ suite contains several implementations of the algorithm and plugins that make use of it, out of which we have implemented

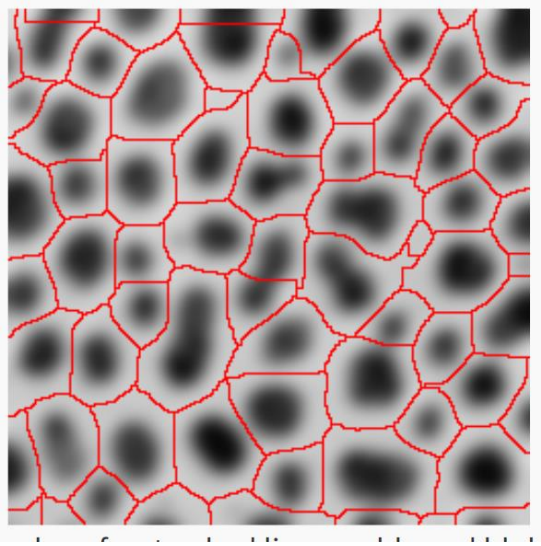


Figure 9: Picture showing overlay of watershed lines on blurred domes.

Marker-controlled Watershed, a plugin to perform watershed in 2D/3D images by flooding from specific seed points or markers by Meyer and Beucher.[138]

Fiji software (Fiji Is Just ImageJ) upgrades ImageJ's underlying architecture while freeing academics to concentrate on creating ground-breaking, novel approaches to biological image analysis. Powerful software libraries are introduced by Fiji to enable quick conversion of algorithmic discoveries into useful image analysis tools. Fiji's core algorithms can be used with a variety of scripting languages that are well-known to bioinformaticians, which makes it even easier to prototype novel bioimage solutions. Last but not least, Fiji offers a reliable distribution system that makes certain new algorithms are made available to its large user base as soon as feasible, starting an iterative refining process based on dialogue between programmers and consumers. In summary, Fiji is designed to serve as a software engineering ecosystem where computer science and biology research communities can collaborate to turn algorithms into usable programs for solving biological research questions.[139]

The plugin used for nuclei detection

StarDist Plugin- is the ImageJ/Fiji plugin for StarDist, a cell/nuclei detection method for microscopy images with star-convex shape priors (typically for Dapi-like staining of nuclei). The plugin can be used to apply already-trained models to new images.[140]

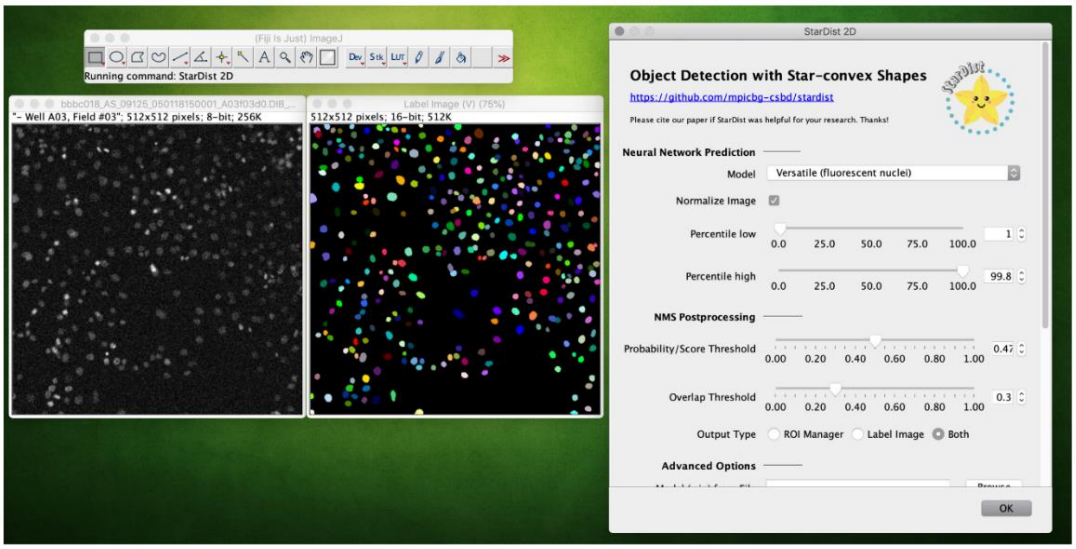


Figure 10: Depicting the StarDist plugin in ImageJ/Fiji software.

When reviewing the literature, it was discovered that many studies used ImageJ software to quantify their data and get accurate results.

In order to determine the accuracy of the diagnosis, which would undoubtedly aid in prognosis, Gabriel Landini et al., automated and quantified the architecture of normal epithelium, dysplasia, and oral cancer. The research established a quantitative and objective method to describe the regional spatial organization of cells in tissues.[35]

According to Gabriel Landini et al., in the research on quantitative analysis of the epithelial lining architecture in radicular cysts & odontogenic cysts. While the majority of epithelial cysts are thought to grow passively as a result of the hydrostatic pressure created by the cyst fluid inside the lumen, other varieties of odontogenic

cysts are thought to have specific epithelial linings and behave differently. Whereas some cysts actively proliferate their cells. Therefore, the study was conducted to statistically examine the histomorphological differences between the various cysts for a more accurate diagnosis using ImageJ software. The study found that radicular cysts and keratocysts have very different epithelial architecture.[141]

In their paper, "Oral Epithelial Dysplasia: Can Quantifiable Morphological Features Help in the Grading Dilemma?", Rasha Abu Eid and Gabriel Landini explored this question. In this work, histological images of sections of epithelial dysplasia with varying degrees of severity were subjected to quantitative, repeatable procedures using ImageJ analysis. Results obtained showed statistical differences in the morphological analysis between the grades of dysplasia.[142]

METHODOLOGY

Case selection

The present study comprised of histopathological sections that included 40 each of histopathologically confirmed cases of oral squamous cell carcinoma, dysplasia cases and normal mucosa.

STUDY DESIGN: Observational study

SOURCE OF DATA:

- Archived tissue blocks (H&E sections) of normal oral mucosa, dysplasia and surgical margin sections of OSCC cases were retrieved from Department of Oral and Maxillofacial Pathology and Oral Microbiology, KLE VK Institute of Dental Sciences, Belagavi.

SELECTION OF SUBJECTS: confirmed H&E sections of normal oral mucosa (adjacent to 3rd molar and gingival flap surgery), dysplasia and surgical margin sections of OSCC cases

INCLUSION CRITERIA:

Study group (OSCC- treated RND cases):

- 40x H and E images of surgical margins in OSCC treated with radical neck dissection following clinical and histopathological confirmation

Control group:

- 40x H and E images of normal mucosa and Oral epithelial dysplasia (Mild, moderate & severe grades)

EXCLUSION CRITERIA:

Study group (OSCC- treated RND cases):

Surgical margins diagnosed with presence of invasive carcinoma and connective tissue only were excluded from morphometric analysis using ImageJ.

SAMPLE SIZE ESTIMATION:

Based on Gabriel Landini[35]

SD of length in I group – $S_1 = 3.18$

II group - $S_2 = 3.63$

Mean difference = margin of error = $d = x_1 - x_2 = 2.25$

$N = 2S^2(z_\alpha + z_\beta)^2 / d^2 = 36$ in each group whereas, $z_\alpha = 1.96$ at 5% α error

$Z_\beta = 0.842$ at 20% β error

$S = S_1 + S_2 / 2$

N = 36 in each group to achieve 80% power and 95% confidence

The sample size was rounded off to 40 in each group

TOTAL SAMPLE SIZE IS-

120 archival tissue blocks (H&E sections) of - 40 normal oral mucosa

40 dysplasia cases

40 cases of OSCC (surgical margins)

MATERIALS AND ARMAMENTARIUM:

1. Retrieved archival tissues (H&E sections) of normal mucosa, dysplasia and surgical margin sections of OSCC cases.

2. Photomicrographs of normal mucosa, dysplasia and surgical margins (Olympus WHB10X-H/20 & Magvision software) of 40x magnification

3. ImageJ software of version 4.6: downloaded from imagej.nih.gov/ij/plugins/index.html

Equipment required to conduct the research were utilized from Department of Oral and Maxillofacial Pathology and Oral Microbiology, KLE VKIDS, Belagavi.

Methodology

- Formalin fixed paraffin embedded tissue blocks of 40 normal mucosa, 40 dysplasia and 40 surgical margin sections of OSCC cases were retrieved from the archives of the Department of Oral and Maxillofacial Pathology and Oral Microbiology, KLE VKIDS.

- The blocks were sectioned in to 4µm thickness and stained using the standard method of H&E staining technique.

A. Normal Mucosa

40 cases of normal mucosa were retrieved from the Department archives and reconfirmed. Total of 80 images under 40x magnification were clicked in Magnus software and subjected to Image analysis.

B. Oral Epithelial Dysplasia

40 cases of epithelial dysplasia, 10 each of Mild, Moderate and Severe groups were retrieved and reconfirmed. These sections were reviewed by 3 oral pathologists and diagnosed. Where ever there were differences with opinion, the common consenses was arrived at after discussions. Total of 140 images under 40x magnification were clicked in Magnus software and subjected to Image analysis.

C. Surgical Margins (Anterior, Posterior, Medial, Lateral) of OSCC

40 cases of OSCC treated with Radical Neck Dissection were selected, assessment of surgical margins of the same were done for Positive, Negative and Dysplasia at the margin levels. For surgical margins which consisted of epithelial architecture, the total of 106 images under 40x magnification were clicked in Magnus software and subjected to Image analysis. Surgical margins with presence of only connective tissue and invasive carcinoma were not analyzed.

- Photomicrographs were captured using Olympus camera and Magvision software under 40x magnification. These photomicrographs were uploaded into ImageJ software.

- Subsequent imaging procedures were performed using ImageJ version 1.34 (a multiplatform, free and open-source imaging program written by W. Rasband at the NIH, USA). The analytical procedures were either written in ImageJ's internal macro scripting language or as "plugin" modules for ImageJ written in the Java computer language (Sun Microsystems Inc., Santa Clara, USA)

D. Image analysis

Cell profile segmentation

The boundaries between neighbouring epithelial cells cannot be consistently determined under microscopic examination on H&E-stained sections. Hence, a space division method was used to approximation theoretical cell profile extents in the software. Two stages were taken to complete the segmentation:

1) Nuclear localization based on the optical density of the histological stain.

2) A spatial partition of the epithelial compartment into exclusive areas of influence of each nucleus profile.

- By isolating the haematoxylin-stained regions using the colour deconvolution technique created by Ruifrok & Johnston, the nuclear localization was determined. The "deconvolved" image only retains the spatial localization of nucleic acids, making it simple to recover the nuclear positions. The entire epithelial compartment can also be segregated using optical intensity

thresholding as epithelial cells have rich RNA component and maintain some (although less intense) haematoxylin staining in their cytoplasm (therefore segmented from the underlying connective tissue and the empty lumen).

- The spatial partition (step 2) uses an image processing plugin called MorphoLibJ plugin that has water segmentation, assimilates the grey level image to a digital elevation model, and aims to detect the various catchment basins to divide the epithelial compartment into exclusive "areas of influence" or "catchment basins" relative to each nucleus (so each area is associated with only one nucleus).

Nuclei detection

Nuclei detection was done using StarDist plugin in Fiji software which is based on ImageJ. The epithelium compartment was separated from the connective tissue using crop tool and images were subjected to StarDist plugin.

Cell profile segmentation using MorphoLibJ Plugin

1. Colour deconvolution of the image to retain optical density of the Haematoxylin as an 8-bit greyscale image.
2. Extraction of the epithelial tissue extent by histogram equalization, followed by greyscale thresholding. (Image 1)
3. Smoothing of Image1 with a 5 × 5 average filter to preserve only large detail and prevent over-partitioning by the watershed transform. (Image 2)
4. Smoothened image is treated with regional extended maxima to obtain the domes

5. Greyscale reconstruction with dome extraction extracts the bright domes up to a predefined grey level
6. Greyscale inversion to restore the domes as dark spots, now of comparable grey level, which overcomes differences in staining intensity of the nuclei. (Image3)
7. Image1, image 2 and image 3 subjected to marked level segmentation using MorphoLibJ plugin to get the watershed segmentation of the cells present in the epithelium.
8. Segmented image is analyzed using measuring tool (analyse regions) available in MorphoLibJ plugin.

Nuclei detection using StarDist Plugin

1. Uploaded image in Fiji, ImageJ based software is cropped to separate epithelial and connective tissue components.
2. The epithelial component obtained is subjected to StarDist plugin to detect the nucleus of the cells.
3. The detected nuclei particles are analyzed using analyse particles tool present in software.

Morphometrical analysis

Analysis of the resulted images were done using analyse particles plugin in MorphoLibJ for cellular profile segmentation and analyse particles tool available in ImageJ for nuclei detection using StarDist plugin.

Table 5: For cellular profile segmentation and nuclei detection, the parameters analysed were (in Pixels):[138,140]

For cellular segmentation	For nuclei detection
Area	Total Area
Perimeter	Average Size
Circularity	%Area
EulerNumber	Perim.
Centroid.X	Major
Centroid.Y	Minor
Ellipse.Orientation	Angle
Ellipse.Elongation	Circ.
ConvexArea	Solidity
MaxFeretDiam	Feret
MaxFeretDiamAngle	FeretX
InscrDisc.Radius	FeretY
	FeretAngle
	MinFeret
	IntDen
	Skew
	Kurt

Total Area- The square-pixel size of the selected area. If Analyze>Set Scale was used to spatially calibrate the image, area is displayed in calibrated units, such as square millimeters. “The area inside the polygon defined by the perimeter”

Min & Max Gray Level - Minimum and maximum gray values within the selection.

Centroid: The selection's central point. The x and y coordinates of every pixel in the image or selection are averaged out to produce this value. Uses the table titles for the X and Y Results.

Perimeter - The length of the outside boundary of the selection. Perimeter calculated from the centers of the boundary pixels. The general idea is to count the number of crossings between a collection of lines with different orientations and the region(s) of interest. The estimate is impartial since it averages over all potential directions.

Fit Ellipse: Fit an ellipse around the chosen area. Utilizes the terms Major, Minor, and Angle. The primary and secondary axes of the best-fitting ellipse are Major and Minor. Angle (0–180 degrees) is the angle made by the main axis and a line that runs perpendicular to the image's x-axis. If Centroid is selected, the ellipse's Centre's coordinates (X, Y) are shown.

Circularity: 4π*area/perimeter^2. A perfect circle has a value of 1.0. The value suggests an increasingly elongated shape as it gets closer to zero. For very small particles, values might not be applicable.

Solidity: area/convex area.

Feret- The maximum calliper, also known as Feret's Diameter, is the "largest distance between any two locations along the selection boundary". The angle formed by the diameter of the Feret and a line perpendicular to the image's x-axis is known as the FeretAngle (0–180 degrees). The smallest calliper diameter is called MinFeret. The FeretX and FeretY initial coordinates for the diameter are also shown.

Integrated Density - Calculates and displays two values: "IntDen" (the product of Area and Mean Gray Value) and "RawIntDen" (the sum of the values of the pixels in the image or selection). "RawIntDen" is only available in ImageJ 1.44c or later. "IntDen" and "RawIntDen" values are the same for uncalibrated image.

Skewness - The third order moment about the mean.

Kurtosis - The fourth order moment about the mean.

Euler Number- The connectivity chosen affects how the Euler number is measured. The 4-connectivity, which corresponds to the orthogonal neighbours, and the 8-connectivity, which also takes into account the diagonal neighbours, are common choices for planar images.[138,140].

IMAGES- CELLULAR SEGMENTATION

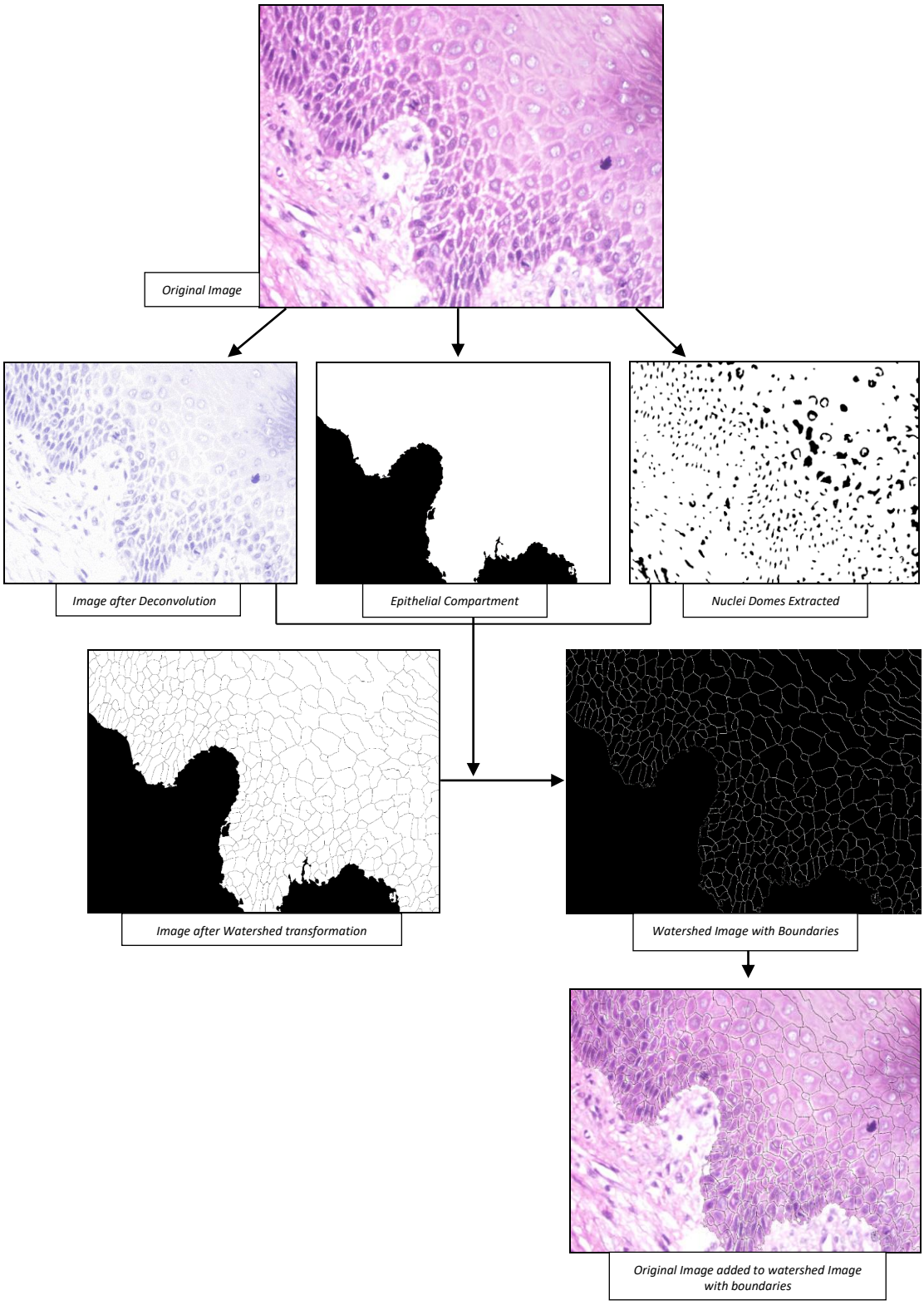


Figure 11: schematic representation of the steps involved in cellular segmentation

IMAGES- NUCLEI DETECTION

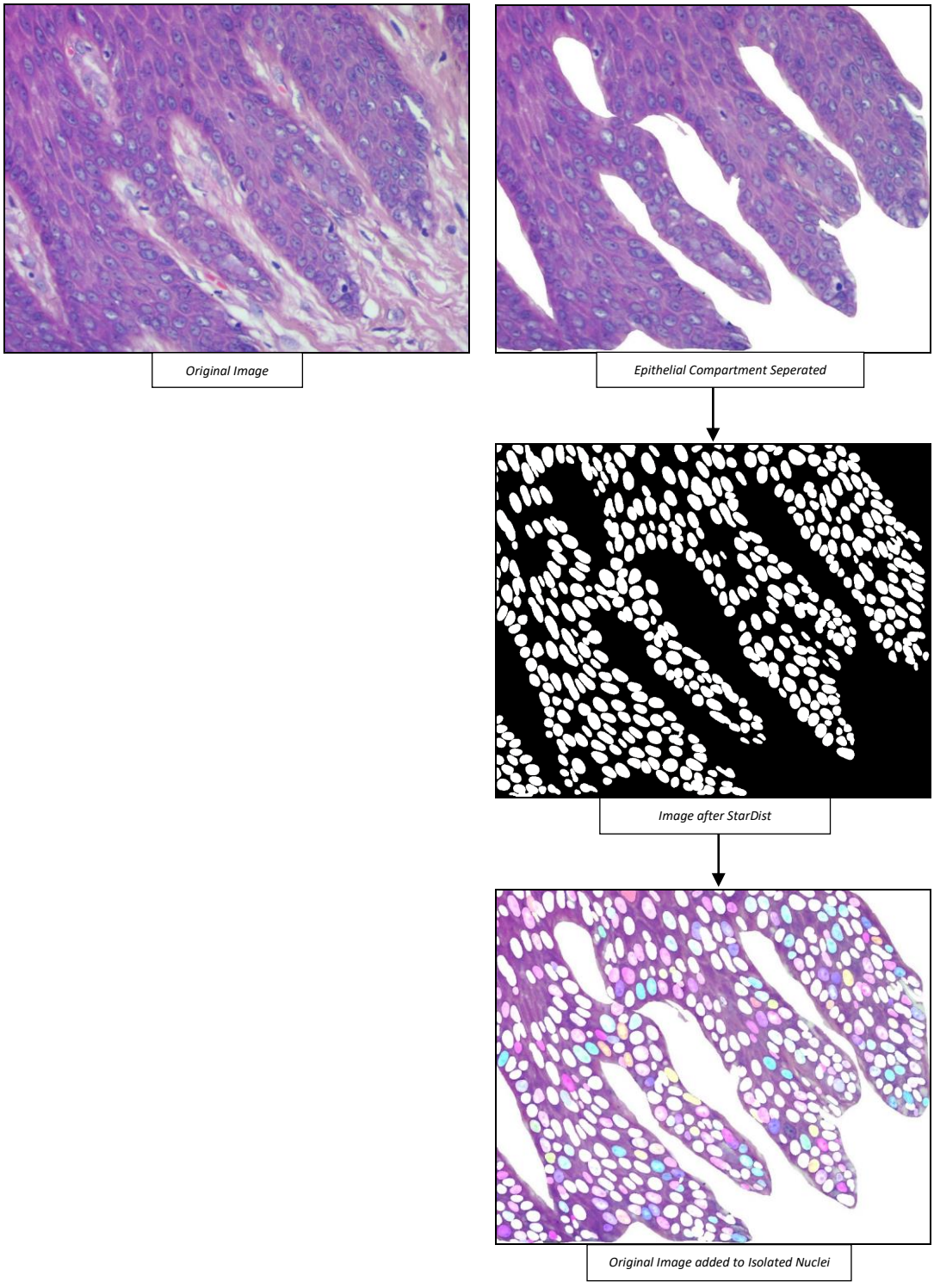


Figure 12: schematic representation of the steps involved in nuclei detection

RESULTS

A. Routine microscopic evaluation:

- Confirmed the diagnosis of normal oral mucosa and dysplasia (Mild, moderate, severe) on evaluating H and E-stained sections of 40 cases each.
- Confirmed the diagnosis of surgical margins of 40 resected OSCC cases on evaluating H and E-stained sections.
- The results of evaluation of surgical margins (Anterior, Posterior, Medial, Lateral) were as follows:

Table 6: Evaluation of surgical margins by manual method

Case No.	Clinical Diagnosis	Histopathological Diagnosis	Anterior Margin	Posterior Margin	Medial Margin	Lateral Margin
1	Carcinoma of Tongue	WDSCC	Negative	Negative	Negative	Negative
2	Carcinoma of Lower Alveolus	MDSCC	Negative	Negative	Negative	Negative
3	Carcinoma of Left Border of Tongue	MDSCC	Negative	Negative	Negative	Negative
4	Carcinoma of Mandible	WDSCC	Negative	Negative	Negative	Negative
5	Carcinoma GBS	MDSCC	Negative	Negative	Negative	Negative
6	Carcinoma of Tongue	MDSCC	Negative	Negative	Negative	Negative
7	Squamous Cell Carcinoma	WDSCC	Negative	Negative	Negative	Negative
8	Papillary Squamous Cell Carcinoma	Papillary Squamous Cell Carcinoma	Negative	Negative	Negative	Negative
9	Carcinoma of Left GBS	MDSCC	Negative	Negative	Negative	Negative
10	Carcinoma Right Buccal Mucosa	MDSCC	Negative	Negative	Negative	Negative

Case No.	Clinical Diagnosis	Histopathological Diagnosis	Anterior Margin	Posterior Margin	Medial Margin	Lateral Margin
11	Squamous Cell Carcinoma	MDSCC	Negative	Negative	Negative	Negative
12	Squamous Cell Carcinoma	WDSCC	Negative	Negative	Negative	Negative
13	Carcinoma of Mandible	PDSCC	Negative	Negative	Negative	Negative
14	Squamous Cell Carcinoma	MDSCC	Negative	Negative	Negative	Negative
15	Squamous Cell Carcinoma	MDSCC	Negative	Negative	Negative	Negative
16	Squamous Cell Carcinoma	PDSCC	Negative	Negative	Negative	Negative
17	Carcinoma of Linguogingival Sulcus	WDSCC	Negative	Negative	Negative	Negative
18	Carcinoma of Right BM	WDSCC	Negative	Negative	Negative	Negative
19	Carcinoma of Right BM	MDSCC	Negative	Negative	Negative	Negative
20	Squamous Cell Carcinoma	WDSCC	Negative	Negative	Negative	Negative
21	WDSCC	WDSCC	Negative	Negative	Negative	Negative
22	Carcinoma	PDSCC	Negative	Negative	Negative	Negative
23	Carcinoma of Hard Palate	WDSCC	Mild To Moderate	Mild To Moderate	Negative	Negative
24	Carcinoma of Lower Lip	WDSCC	Negative	Severe Dysplasia	Negative	Negative
25	Carcinoma of Right BM	PDSCC	Negative	Negative	Negative	Negative
26	Carcinoma of Left BM	WDSCC	Negative	Negative	Negative	Negative
27	Carcinoma of Left BM	MDSCC	Negative	Negative	Negative	Negative
28	Carcinoma	WDSCC	Negative	Negative	Negative	Negative
29	Carcinoma Left BM	WDSCC	Negative	Negative	Negative	Negative
30	WDSCC	MDSCC	Negative	Negative	Negative	Negative
31	Carcinoma of Left BM	MDSCC	Negative	Negative	Negative	Negative
32	Carcinoma of Left BM	Early Invasive SCC	Negative	Negative	Negative	Negative

Case No.	Clinical Diagnosis	Histopathological Diagnosis	Anterior Margin	Posterior Margin	Medial Margin	Lateral Margin
33	Carcinoma of Left BM	WDSCC	Positive	Negative	Negative	Positive
34	Primary SCC Right BM	MDSCC	Negative	Negative	Negative	Negative
35	Carcinoma of Tongue	MDSCC	Negative	Negative	Negative	Negative
36	Carcinoma	WDSCC	Negative	Negative	Negative	Negative
37	Carcinoma of Tongue	WDSCC	Negative	Negative	Negative	Negative
38	Oral Squamous Cell Carcinoma	WDSCC	Negative	Negative	Negative	Negative
39	Carcinoma of Alveolus	MDSCC	Negative	Negative	Negative	Negative
40	Carcinoma	WDSCC	Negative	Negative	Negative	Negative

Inference: Out of 40 cases of OSCC treated with surgical excision, one case was histopathologically diagnosed as papillary squamous cell carcinoma, one case as early invasive squamous cell carcinoma18 cases were diagnosed as WDSCC, 16 were diagnosed as MDSCC and 4 as PDSCC. Among the surgical margins analysed for all 40 cases, one case showed positive anterior and posterior margins, one case showed mild to moderate dysplasia in anterior and posterior margin and one case showed severe dysplasia in posterior margin. Remaining 37 cases were diagnosed with all four negative margins.

Morphometric analysis in ImageJ- Cellular Profile Segmentation

Table 7: Comparison of four groups with cellular segmentation scores by one way ANOVA test.

Variables	Normal Mucosa		Mild Dysplasia		Moderate Dysplasia		Severe Dysplasia		F-value	P-value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Area	172054.95	37122.79	174964.86	33331.73	174566.38	27131.42	205241.21	62022.90	8.6256	0.0001*
Perimeter	178160.71	27998.49	185004.38	32636.40	181882.59	24228.87	204260.69	32482.15	9.6513	0.0001*
Circularity	0.000070	0.000020	0.000066	0.000011	0.000067	0.000009	0.000064	0.000021	1.9040	0.1294
EulerNumber	17913.33	4120.40	20062.03	4950.77	19522.26	4208.66	21257.59	5533.39	5.9569	0.0006*
Centroid.X	1263.68	208.03	1197.62	195.50	1303.48	163.12	1190.66	167.54	5.0234	0.0021*
Centroid.Y	948.93	109.73	979.53	133.75	971.85	72.47	987.27	101.61	1.6724	0.1735
Ellipse.Orientation	-5.00	31.30	-7.92	28.84	2.19	29.51	4.50	25.85	2.4475	0.0643
Ellipse.Elong	1.45	0.24	1.41	0.21	1.41	0.29	1.40	0.22	0.6234	0.6005
ConvexArea	4063434.29	591969.25	3944113.6	544214.00	4084490.1	470217.55	4224893.4	403991.49	2.9247	0.0345*
MaxFeretDiam	2924.34	262.43	2905.58	226.18	2998.53	193.62	3011.41	193.54	3.3813	0.0189*
MaxFeretDiamAngle	103.83	52.28	89.14	55.26	96.08	52.63	92.27	53.45	0.9808	0.4024
InscrDisc.Radius	2.88	0.58	2.80	0.41	2.74	0.44	2.93	0.53	1.7437	0.1586

Inference: Among the 40 normal mucosae analysed morphometrically by using Image J, for cellular profile segmentation, the mean area seen in normal mucosa was 172054.95. The values increased as the grade of dysplasia shifted from mild to severe (174964.86, 174566.38, 205241.21) and this was found to be statistically significant($P<0.05$). The mean perimeter was 178160.71 for normal mucosa, 185004.38 for mild dysplasia, 181882.59 for moderate dysplasia and 204260.69 for sever dysplasia. The perimeter value gradually

increased from normal to dysplasia except for moderate dysplasia. However. The values were also statistically significant in all 4 groups($P<0.05$). Mean of circularity seen in normal mucosa was 0.000070 and the values were seen to decrease as the grades of dysplasia increased. However, the mean values were not statistically significant. For Centroid X variable, mean value of normal mucosa was 1263.68, mild dysplasia was 1197.62, moderate dysplasia was 1303.48, severe dysplasia was 1190.66 and the values were found to be statistically significant($P<0.05$). For centroid Y parameter the mean value of normal mucosa was 948.93, mild dysplasia was 979.53, moderate dysplasia was 971.85 and severe dysplasia was 987.27. But the results were statistically insignificant. Mean values of normal mucosa, mild, moderate and severe dysplasia for Ellipse orientation and elongation variables was found to be statistically insignificant. Convex area parameter showed mean value of 4063434.29 in normal mucosa, 3944113.6 in mild dysplasia, 4084490.1 in moderate dysplasia, 4224893.4 in severe dysplasia and the results were found to be statistically significant($P<0.05$). Mean value for maximum Feret diameter in normal mucosa was 2924.34, 2905.58 in mild dysplasia, 2998.53 moderate dysplasia, 3011.41 in severe dysplasia and results were statistically significant($P<0.05$). The mean values obtained in all four groups for max Feret diameter angle and InscrDisc.Radius were found to be statistically insignificant.

Table 8: Pair wise comparison of four groups(NM,MD,MOD,SD) with cellular segmentation scores by Tukeys multiple posthoc procedures

Variables	Normal Mucosa vs Mild Dysplasia	Normal Mucosa vs Moderate Dysplasia	Normal Mucosa vs Severe Dysplasia	Mild Dysplasia vs Moderate Dysplasia	Mild Dysplasia vs Severe Dysplasia	Moderate Dysplasia vs Severe Dysplasia
Area	P=0.9780	P=0.9860	P=0.0001*	P=1.0000	P=0.0010*	P=0.0010*
Perimeter	P=0.5340	P=0.8850	P=0.0001*	P=0.9400	P=0.0030*	P=0.0001*
Circularity	P=0.4180	P=0.7670	P=0.1650	P=0.9540	P=0.9560	P=0.7320
EulerNumber	P=0.0420*	P=0.1990	P=0.0001*	P=0.9250	P=0.5140	P=0.1940
Centroid.X	P=0.1720	P=0.6080	P=0.1110	P=0.0130	P=0.9970	P=0.0070*
Centroid.Y	P=0.3490	P=0.6050	P=0.1670	P=0.9800	P=0.9800	P=0.8650
Ellipse.Orientation	P=0.8650	P=0.9370	P=0.4860	P=0.9370	P=0.2410	P=0.2410
Ellipse.Elong	P=0.9740	P=0.6780	P=0.7770	P=0.6780	P=0.9990	P=0.9990
ConvexArea	P=0.5370	P=0.9950	P=0.2720	P=0.4550	P=0.0180*	P=0.4590
MaxFeretDiam	P=0.9620	P=0.2280	P=0.1160	P=0.1150	P=0.0550	P=0.9900
MaxFeretDiamAngle	P=0.3820	P=0.8360	P=0.5950	P=0.8950	P=0.9890	P=0.9800
InscrDisc.Radius	P=0.7380	P=0.3520	P=0.9500	P=0.9330	P=0.4680	P=0.1760

*p<0.05

Inference: Pair wise comparison of the parameters in all four groups for cellular profile segmentation was performed using Tukeys multiple posthoc tests. The results obtained for Euler number variable between normal v/s mild dysplasia and normal mucosa v/s severe dysplasia were statistically significant($P<0.05$). Also, normal mucosa v/s severe dysplasia, mild dysplasia v/s severe dysplasia and moderate dysplasia v/s severe dysplasia showed statistically significant difference for area and perimeter($P<0.05$). Moderate dysplasia v/s severe dysplasia showed statistically significant difference in centroid X parameter. Convex area variable for Mild dysplasia v/s severe dysplasia showed statistically significant difference($P<0.05$). Other variables in pair wise comparison between all four groups showed differences but the results were not statistically significant.

Table 9: Comparison of cellular segmentation of surgical margins in OSSCC scores

Variables	Anterior Margin		Lateral Margin		Medial Margin		Posterior Margin		Overall	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Area	166405.55	36201.11	163976.15	38064.69	174256.96	38145.33	170520.55	39214.38	168704.05	37599.27
Perimeter	178844.36	38175.92	176548.60	39038.37	186523.22	39700.26	183914.85	40973.35	181406.07	39159.69
Circularity	0.00007	0.00002	0.00007	0.00002	0.00007	0.00002	0.00007	0.00002	0.00007	0.00002
EulerNumber	20731.34	4933.01	19975.31	5453.90	20504.25	5067.51	20639.71	5266.84	20477.27	5119.73
Centroid.X	1250.53	156.43	1266.87	245.75	1252.03	122.58	1277.00	163.73	1262.18	175.54
Centroid.Y	974.49	61.12	963.83	77.50	964.52	47.98	961.55	66.81	966.15	63.86
Ellipse.Orientation	-6.30	37.13	11.91	36.10	2.58	33.30	-6.64	35.90	-0.15	36.06
Ellipse.Elong	1.45	0.28	1.50	0.36	1.43	0.22	1.58	0.71	1.50	0.45
ConvexArea	3995250.55	659283.48	3966839.00	861241.02	4188456.21	714992.71	3992593.06	875131.12	4029940.13	779508.32
MaxFeretDiam	2944.81	239.37	2903.99	306.76	3023.43	184.44	2933.36	259.16	2949.09	252.47
MaxFeretDiamAngle	103.11	50.52	106.90	52.15	99.81	52.61	98.49	53.12	101.99	51.48
InscrDisc.Radius	2.90	0.31	2.96	0.20	2.88	0.34	2.87	0.34	2.90	0.30

Inference: Table shows mean and SD of all parameters analyzed in all four surgical margins (Anterior, Posterior, Medial, Lateral). Overall mean and SD of each parameter in surgical margins were also calculated and tabulated. The parameters were further compared with mean values of normal, mild, moderate and severe dysplasia.

Table 9a-Comparision of anterior margin with normal mucosa, mild, moderate and severe dysplasia for cellular profile segmentation.

Variables	Anterior Margin		Normal Mucosa		Mild Dysplasia		Moderate Dysplasia		Severe Dysplasia	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Area	166406	36201.1	172055	37122.8	174965	33331.7	174566	27131.4	205241	62022.9
Perimeter	178844	38175.9	178161	27998.5	185004	32636.4	181883	24228.9	204261	32482.2
Circularity	0.00007	0.00002	0.00007	0.00002	0.00006	0.00001	0.000067	0.000009	0.000064	0.000021
EulerNumber	20731.3	4933.01	17913.3	4120.4	20062	4950.77	19522.3	4208.66	21257.6	5533.39
Centroid.X	1250.53	156.43	1263.68	208.03	1197.62	195.5	1303.48	163.12	1190.66	167.54
Centroid.Y	974.49	61.12	948.93	109.73	979.53	133.75	971.85	72.47	987.27	101.61
Ellipse.Orientation	-6.3	37.13	-5	31.3	-7.92	28.84	2.19	29.51	4.5	25.85
Ellipse.Elong	1.45	0.28	1.45	0.24	1.41	0.21	1.41	0.29	1.4	0.22
ConvexArea	3995251	659283	4063434	591969	3944114	544214	4084490	470218	4224893	403991
MaxFeretDiam	2944.81	239.37	2924.34	262.43	2905.58	226.18	2998.53	193.62	3011.41	193.54
MaxFeretDiamAngle	103.11	50.52	103.83	52.28	89.14	55.26	96.08	52.63	92.27	53.45
InscrDisc.Radius	2.9	0.31	2.88	0.58	2.8	0.41	2.74	0.44	2.93	0.53

Inference- In the anterior margin analysis, Parameters such as Area, Perimeter, Circularity Centroid number, ellipse elongation, convex area and Maximum Feret Diameter Angle were found to be in close proximation with the analysis of normal Mucosa. Centroid Y and Maximum Feret diameter were close to analysis of moderate dysplasia. Euler Number and InscrDisc.Radius parameters were close to analysis of severe dysplasia and Ellipse orientation was found to be in close relation to analysis of mild dysplasia. The summary

Summary of variables in anterior margin analysis (In proximity with)	
Area	Normal Mucosa
Perimeter	Normal Mucosa
Circularity	Normal Mucosa
EulerNumber	Severe Dysplasia
Centroid.X	Normal Mucosa
Centroid.Y	Moderate dysplasia
Ellipse.Orientation	Mild dysplasia
Ellipse.Elong	Normal Mucosa
ConvexArea	Normal Mucosa
MaxFeretDiam	Moderate dysplasia
MaxFeretDiamAngle	Normal Mucosa
InscrDisc.Radius	Severe Dysplasia

Table 9b: Comparison of posterior margin with normal mucosa, mild, moderate and severe dysplasia for cellular profile segmentation.

Variables	Posterior Margin		Normal Mucosa		Mild Dysplasia		Moderate Dysplasia		Severe Dysplasia	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Area	170521	39214.4	172055	37122.8	174965	33331.7	174566	27131.4	205241	62022.9
Perimeter	183915	40973.4	178161	27998.5	185004	32636.4	181883	24228.9	204261	32482.2
Circularity	0.00007	0.00002	0.00007	0.00002	0.00006	0.00001	0.000067	0.000009	0.000064	0.000021
EulerNumber	20639.7	5266.84	17913.3	4120.4	20062	4950.77	19522.3	4208.66	21257.6	5533.39
Centroid.X	1277	163.73	1263.68	208.03	1197.62	195.5	1303.48	163.12	1190.66	167.54
Centroid.Y	961.55	66.81	948.93	109.73	979.53	133.75	971.85	72.47	987.27	101.61
Ellipse.Orientation	-6.64	35.9	-5	31.3	-7.92	28.84	2.19	29.51	4.5	25.85
Ellipse.Elong	1.58	0.71	1.45	0.24	1.41	0.21	1.41	0.29	1.4	0.22
ConvexArea	3992593	875131	4063434	591969	3944114	544214	4084490	470218	4224893	403991
MaxFeretDiam	2933.36	259.16	2924.34	262.43	2905.58	226.18	2998.53	193.62	3011.41	193.54
MaxFeretDiamAngle	98.49	53.12	103.83	52.28	89.14	55.26	96.08	52.63	92.27	53.45
InscrDisc.Radius	2.87	0.34	2.88	0.58	2.8	0.41	2.74	0.44	2.93	0.53

Inference: In the posterior margin analysis, parameters such as Area, Circularity, ellipse elongation, convex area, Maximum Feret Diameter Angle and InscrDisc.Radius were found to be in close proximation with the analysis of normal Mucosa. Centroid X, centroid Y, and Maximum Feret diameter were close to analysis of moderate dysplasia. Euler Number parameter was close to analysis of severe dysplasia. Perimeter and Ellipse Orientation was found to be in close relation to analysis of mild dysplasia.

Summary of variables in posterior margin analysis (In proximity with)	
Area	Normal Mucosa
Perimeter	Mild dysplasia
Circularity	Normal Mucosa
EulerNumber	Severe dysplasia
Centroid.X	Moderate dysplasia
Centroid.Y	Moderate dysplasia
Ellipse.Orientation	Mild dysplasia
Ellipse.Elong	Normal Mucosa
ConvexArea	Normal Mucosa
MaxFeretDiam	Moderate dysplasia
MaxFeretDiamAngle	Normal Mucosa
InscrDisc.Radius	Normal Mucosa

Table 9c: Comparison of medial margin with normal mucosa, mild, moderate and severe dysplasia for cellular profile segmentation.

Variables	Medial Margin		Normal Mucosa		Mild Dysplasia		Moderate Dysplasia		Severe Dysplasia	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Area	174257	38145.3	172055	37122.8	174965	33331.7	174566	27131.4	205241	62022.9
Perimeter	186523	39700.3	178161	27998.5	185004	32636.4	181883	24228.9	204261	32482.2
Circularity	0.00007	0.00002	0.00007	0.00002	0.00006	0.00001	0.000067	0.000009	0.000064	0.000021
EulerNumber	20504.3	5067.51	17913.3	4120.4	20062	4950.77	19522.3	4208.66	21257.6	5533.39
Centroid.X	1252.03	122.58	1263.68	208.03	1197.62	195.5	1303.48	163.12	1190.66	167.54
Centroid.Y	964.52	47.98	948.93	109.73	979.53	133.75	971.85	72.47	987.27	101.61
Ellipse.Orientation	2.58	33.3	-5	31.3	-7.92	28.84	2.19	29.51	4.5	25.85
Ellipse.Elong	1.43	0.22	1.45	0.24	1.41	0.21	1.41	0.29	1.4	0.22
ConvexArea	4188456	714993	4063434	591969	3944114	544214	4084490	470218	4224893	403991
MaxFeretDiam	3023.43	184.44	2924.34	262.43	2905.58	226.18	2998.53	193.62	3011.41	193.54
MaxFeretDiamAngle	99.81	52.61	103.83	52.28	89.14	55.26	96.08	52.63	92.27	53.45
InscrDisc.Radius	2.88	0.34	2.88	0.58	2.8	0.41	2.74	0.44	2.93	0.53

Inference: In the medial margin analysis, Parameters such as Circularity, Centroid X, ellipse elongation, Maximum Feret Diameter Angle and InscrDisc.Radius were found to be in close proximation with the analysis of normal Mucosa. Perimeter and Euler Number were found to be in close relation to analysis of mild dysplasia. Area, centroid Y, and Maximum Feret diameter were close to analysis of moderate dysplasia. Convex area and Ellipse orientation parameters were close to the analysis of severe dysplasia.

Summary of variables medial margin analysis (In proximity with)	
Area	Moderate dysplasia
Perimeter	Mild dysplasia
Circularity	Normal Mucosa
EulerNumber	Mild dysplasia
Centroid.X	Normal Mucosa
Centroid.Y	Moderate dysplasia
Ellipse.Orientation	Severe dysplasia
Ellipse.Elong	Normal Mucosa
ConvexArea	Severe dysplasia
MaxFeretDiam	Moderate dysplasia
MaxFeretDiamAngle	Normal Mucosa
InscrDisc.Radius	Normal Mucosa

Table 9d: Comparison of lateral margin with normal mucosa, mild, moderate and severe dysplasia for cellular profile segmentation.

Variables	Lateral Margin		Normal Mucosa		Mild Dysplasia		Moderate Dysplasia		Severe Dysplasia	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Area	163976.15	38064.69	172054.95	37122.79	174964.86	33331.73	174566.38	27131.42	205241.21	62022.90
Perimeter	176548.60	39038.37	178160.71	27998.49	185004.38	32636.40	181882.59	24228.87	204260.69	32482.15
Circularity	0.00007	0.00002	0.000070	0.000020	0.000066	0.000011	0.000067	0.000009	0.000064	0.000021
EulerNumber	19975.31	5453.90	17913.33	4120.40	20062.03	4950.77	19522.26	4208.66	21257.59	5533.39
Centroid.X	1266.87	245.75	1263.68	208.03	1197.62	195.50	1303.48	163.12	1190.66	167.54
Centroid.Y	963.83	77.50	948.93	109.73	979.53	133.75	971.85	72.47	987.27	101.61
Ellipse.Orientation	11.91	36.10	-5.00	31.30	-7.92	28.84	2.19	29.51	4.50	25.85
Ellipse.Elong	1.50	0.36	1.45	0.24	1.41	0.21	1.41	0.29	1.40	0.22
ConvexArea	3966839.00	861241.02	4063434.29	591969.25	3944113.6	544214.00	4084490.1	470217.55	4224893.4	403991.49
MaxFeretDiam	2903.99	306.76	2924.34	262.43	2905.58	226.18	2998.53	193.62	3011.41	193.54
MaxFeretDiamAngle	106.90	52.15	103.83	52.28	89.14	55.26	96.08	52.63	92.27	53.45
InscrDisc.Radius	2.96	0.20	2.88	0.58	2.80	0.41	2.74	0.44	2.93	0.53

Inference: In the lateral margin analysis, Parameters such as Area, Perimeter, Circularity, Centroid X, ellipse elongation, Maximum Feret Diameter Angle and Convex area were found to be in close proximation with the analysis of normal Mucosa. Maximum Feret diameter and Euler Number were found to be in close relation to analysis of mild dysplasia. Centroid Y was close to analysis of moderate dysplasia. InscrDisc.Radius and Ellipse Orientation parameters were close to the analysis of severe dysplasia.

Summary of variables in lateral margin analysis (In proximity with)	
Area	Normal Mucosa
Perimeter	Normal Mucosa
Circularity	Normal Mucosa
EulerNumber	Mild dysplasia
Centroid.X	Normal Mucosa
Centroid.Y	Moderate dysplasia
Ellipse.Orientation	Severe dysplasia
Ellipse.Elong	Normal Mucosa
ConvexArea	Normal Mucosa
MaxFeretDiam	Mild dysplasia
MaxFeretDiamAngle	Normal Mucosa
InscrDisc.Radius	Severe dysplasia

Table 10: Comparison of four groups with nuclei detection scores by one way ANOVA

Variables	Normal Mucosa		Mild Dysplasia		Moderate Dysplasia		Severe Dysplasia		F-value	P-value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Total Area	182226.00	58952.53	232841.1	81776.71	183363.3	65537.29	196116.3	74831.18	7.1156	0.0001*
Average Size	557.74	183.96	616.14	171.48	640.18	161.97	576.06	159.95	2.8718	0.0370*
%Area	15.90	4.99	21.01	6.78	15.63	5.74	17.23	6.77	10.2481	0.0001*
Perim.	91.29	16.47	97.99	14.84	98.17	11.96	92.32	14.77	3.5597	0.0149*
Major	32.99	5.30	35.50	5.19	35.97	3.97	33.26	5.05	5.3824	0.0013*
Minor	19.71	3.73	20.12	3.37	20.81	3.31	19.95	2.87	1.0248	0.3822
Angle	93.28	19.18	89.28	17.28	88.77	19.35	92.46	15.88	0.9492	0.4174
Circ.	0.80	0.03	0.77	0.05	0.78	0.03	0.80	0.03	8.6552	0.0001*
Solidity	0.93	0.01	0.92	0.02	0.93	0.01	0.92	0.01	7.2022	0.0001*
Feret	33.94	5.44	36.60	5.39	36.87	4.04	34.27	5.14	5.2464	0.0016*
FeretX	590.25	99.73	588.36	82.12	600.19	101.30	582.21	78.37	0.2484	0.8624
FeretY	480.16	49.99	476.05	44.96	473.60	55.98	476.86	42.10	0.2194	0.8829
FeretAngle	93.67	19.26	89.26	17.21	88.71	20.11	93.66	16.04	1.2159	0.3045
MinFeret	20.80	3.82	21.46	3.42	21.97	3.33	21.09	3.00	1.2215	0.3024
IntDen	142197.14	46887.42	157089.4	43714.75	163229.7	41297.79	146874.8	40769.04	2.8764	0.0367*
Skew	-23.99	5.38	-26.53	4.34	-25.67	4.64	-25.87	4.77	3.8832	0.0097*
Kurt	663.58	297.60	799.61	255.65	736.52	274.74	761.33	294.72	3.2191	0.0234*

*$p<0.05$

Inference: Among the 40 normal mucosae analysed morphometrically for nucleus detection, the mean total area seen in normal mucosa was 182226.00. The values were seen to increase with grades of dysplasia when compared to normal mucosa. This was found to be

statistically significant(P<0.05). The mean value of average size in normal mucosa was 557.74, in mild dysplasia it was 640.18, in moderate dysplasia it was 640.18, and in severe dysplasia it was 576.06. The results were statistically significant(P<0.05). The mean values of % area in mild dysplasia was found to be more (21.01) as compared to normal mucosa (15.90), moderate dysplasia (15.63), and in severe dysplasia (17.23). However, the results were statistically significant(P<0.05). The mean perimeter was 91.29 for normal mucosa, 97.99 for mild dysplasia, 98.17 for moderate dysplasia, 92.32 for sever dysplasia and found to be statistically significant in all 4 groups(P<0.05). Higher perimeter value was in moderate dysplasia followed by mild dysplasia, severe dysplasia and normal mucosa. The mean of major diameter parameter was 32.99 for normal mucosa35.50 for mild dysplasia, 35.97for moderate dysplasia, 33.26 for sever dysplasia and results were found to be statistically significant in all 4 groups(P<0.05). The results obtained for mean values of minor diameter and angle variables of all four groups were statistically insignificant. Mean of circularity parameter was 0.80 in normal mucosa, 0.77 in mild dysplasia, 0.78 in moderate dysplasia, 0.80i in severe dysplasia and the values were found to be statistically significant(P<0.05). The mean values of solidity variable in normal mucosa, mild dysplasia, moderate dysplasia and sever dysplasia were 0.93, 0.92, 0.93 and 0.92. The results were found to be statistically significant(P<0.05). The mean vales of Feret variable in normal mucosa, mild dysplasia, moderate dysplasia and sever dysplasia were 33.94, 36.60, 36.87, 34.27 and results were statistically significant(P<0.05). The mean values of parameters such as FeretX, FeretY, Feret angle and MinFeret showed statistically insignificant results. Also, the results of variables such as skewness, kurtosis and IntDen were found to be statistically significant(P<0.05).

Table 11: Pair wise comparison of four groups(NM,MD, MOD, SD) with nuclei detection scores by Tukeys multiple posthoc procedures

Variables	Normal Mucosa vs Mild Dysplasia	Normal Mucosa vs Moderate Dysplasia	Normal Mucosa vs Severe Dysplasia	Mild Dysplasia vs Moderate Dysplasia	Mild Dysplasia vs Severe Dysplasia	Moderate Dysplasia vs Severe Dysplasia
Total Area	P=0.0001*	P=1.0000	P=0.6760	P=0.0040*	P=0.0550	P=0.8380
Average Size	P=0.1920	P=0.0520	P=0.9410	P=0.9180	P=0.7060	P=0.3690
%Area	P=0.0001*	P=0.9940	P=0.5910	P=0.0001*	P=0.0130*	P=0.6130
Perim.	P=0.0460*	P=0.0700	P=0.9830	P=1.0000	P=0.3070	P=0.3320
Major	P=0.0170*	P=0.0080*	P=0.9910	P=0.9720	P=0.1650	P=0.0860
Minor	P=0.8920	P=0.3100	P=0.9810	P=0.7900	P=0.9960	P=0.6960
Angle	P=0.6960	P=0.5630	P=0.5400	P=0.5630	P=0.9990	P=0.9990
Circ.	P=0.8120	P=0.0001*	P=0.1060	P=0.0001*	P=0.2720	P=0.2720
Solidity	P=0.0001*	P=0.9990	P=0.7910	P=0.0040*	P=0.0330*	P=0.9170
Feret	P=0.0130*	P=0.0130*	P=0.9860	P=0.9950	P=0.1560	P=0.1240
FeretX	P=0.9990	P=0.9390	P=0.9660	P=0.9350	P=0.9900	P=0.8320
FeretY	P=0.9580	P=0.8840	P=0.9830	P=0.9950	P=1.0000	P=0.9910
FeretAngle	P=0.4640	P=1.0000	P=0.4870	P=0.6830	P=0.4640	P=1.0000
MinFeret	P=0.6840	P=0.2810	P=0.9720	P=0.9090	P=0.9610	P=0.6930
IntDen	P=0.1920	P=0.0520	P=0.9400	P=0.9170	P=0.7060	P=0.3690
Skew	P=0.0140*	P=0.2600	P=0.1730	P=0.8520	P=0.9250	P=0.9980
Kurt	P=0.0250*	P=0.5060	P=0.2460	P=0.7300	P=0.9230	P=0.9810

*p<0.05

Inference: Pair wise comparison of the parameters in all four groups for nucleus detection was performed using Tukeys multiple posthoc tests. Variables such as area, %area, perimeter, major solidity and Feret diameter were found to have statistically significant difference in normal mucosa v/s mild dysplasia comparison($P<0.05$). Parameters such as major and circularity showed difference in normal mucosa v/s moderate dysplasia comparison and it was statistically significant($P<0.05$). Normal mucosa v/s severe dysplasia pair showed statistically insignificant results. Mild dysplasia v/s moderate dysplasia showed statistically significant difference in total area, %area, circularity, and solidity variables($P<0.05$). Mild dysplasia v/s severe dysplasia comparison showed statistically significant difference in % area and solidity parameters($P<0.05$). Moderate dysplasia v/s severe dysplasia showed statistically insignificant results for all the parameters.

Table 12: Comparison of nuclei detection of surgical margins in OSCC.

Variables	Anterior Margin		Lateral Margin		Medial Margin		Posterior Margin		Overall	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total Area	161482.07	75065.01	169650.96	66193.06	188445.00	55323.48	185331.42	90571.30	175942.64	74118.80
Average Size	577.93	214.49	552.06	167.45	564.25	138.88	611.03	245.67	578.39	199.00
%Area	14.89	6.01	15.60	5.59	16.16	3.75	16.53	6.86	15.79	5.73
Perim.	94.74	18.86	93.18	15.55	93.68	12.12	98.72	19.89	95.27	17.11
Major	34.29	6.15	33.97	5.48	34.11	4.37	35.36	6.51	34.48	5.72
Minor	19.39	4.33	18.85	3.30	19.68	2.68	19.87	4.34	19.47	3.77
Angle	87.67	18.31	85.24	16.57	85.89	12.97	86.44	17.89	86.38	16.56
Circ.	0.77	0.05	0.77	0.04	0.78	0.04	0.77	0.07	0.77	0.05
Solidity	0.92	0.02	0.91	0.02	0.92	0.01	0.91	0.02	0.92	0.02
Feret	35.31	6.29	35.01	5.64	35.05	4.43	36.57	6.78	35.54	5.90
FeretX	563.95	137.91	582.57	119.71	599.36	77.23	565.32	134.97	576.29	121.20
FeretY	466.98	63.53	466.14	68.78	470.65	37.30	463.99	69.73	466.75	61.26
FeretAngle	88.04	18.99	86.06	16.42	86.40	13.53	86.56	17.91	86.82	16.82
MinFeret	20.69	4.48	20.13	3.38	20.83	2.63	21.27	4.47	20.76	3.87
IntDen	147342.90	54675.91	140746.58	42688.22	143867.43	35410.26	155778.19	62636.15	147462.67	50733.06
Skew	-25.53	6.16	-25.53	5.89	-25.04	4.13	-24.20	6.05	-25.05	5.63
Kurt	771.77	359.35	755.90	323.92	704.61	236.17	688.98	370.61	730.18	329.07

Inference: Table shows mean and SD of the all parameters analyzed in all four surgical margins (Anterior, Posterior, Medial, Lateral) overall mean and SD of each parameter in surgical margins.

The parameters were further compared with mean values of normal, mild, moderate and severe dysplasia.

Table 12a: Comparison of anterior margin with normal mucosa, mild dysplasia, moderate dysplasia and severe dysplasia for nuclei detection

Variables	Anterior Margin		Normal mucosa		Mild dysplasia		Moderate dysplasia		Severe dysplasia	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total Area	161482	75065	182226	58952.5	232841	81776.7	183363	65537.3	196116	74831.2
Average Size	577.93	214.49	557.74	183.96	616.14	171.48	640.18	161.97	576.06	159.95
%Area	14.89	6.01	15.9	4.99	21.01	6.78	15.63	5.74	17.23	6.77
Perim.	94.74	18.86	91.29	16.47	97.99	14.84	98.17	11.96	92.32	14.77
Major	34.29	6.15	32.99	5.3	35.5	5.19	35.97	3.97	33.26	5.05
Minor	19.39	4.33	19.71	3.73	20.12	3.37	20.81	3.31	19.95	2.87
Angle	87.67	18.31	93.28	19.18	89.28	17.28	88.77	19.35	92.46	15.88
Circ.	0.77	0.05	0.8	0.03	0.77	0.05	0.78	0.03	0.8	0.03
Solidity	0.92	0.02	0.93	0.01	0.92	0.02	0.93	0.01	0.92	0.01
Feret	35.31	6.29	33.94	5.44	36.6	5.39	36.87	4.04	34.27	5.14
FeretX	563.95	137.91	590.25	99.73	588.36	82.12	600.19	101.3	582.21	78.37
FeretY	466.98	63.53	480.16	49.99	476.05	44.96	473.6	55.98	476.86	42.1
FeretAngle	88.04	18.99	93.67	19.26	89.26	17.21	88.71	20.11	93.66	16.04
MinFeret	20.69	4.48	20.8	3.82	21.46	3.42	21.97	3.33	21.09	3
IntDen	147343	54675.9	142197	46887.4	157089	43714.8	163230	41297.8	146875	40769
Skew	-25.53	6.16	-23.99	5.38	-26.53	4.34	-25.67	4.64	-25.87	4.77
Kurt	771.77	359.35	663.58	297.6	799.61	255.65	736.52	274.74	761.33	294.72

Inference: In the anterior margin analysis, Parameters such as total Area, and Minor and minimum Feret were found to be in close proximity with analysis of normal mucosa. Parameters such as average size, perimeter, major, circularity, solidity, Feret, IntDen and kurtosis were close to analysis of mild dysplasia. %Area, angle, Feret Y, Feret angle, skewness were in close proximity to the analysis of moderate dysplasia. Solidity and Feret X were close to analysis of severe dysplasia.

Summary of variables in anterior margin analysis (In proximity with)	
Total Area	Normal Mucosa
Average Size	Mild Dysplasia
%Area	Moderate Dysplasia
Perim.	Mild Dysplasia
Major	Mild Dysplasia
Minor	Normal Mucosa
Angle	Moderate Dysplasia
Circ.	Mild Dysplasia
Solidity	Mild and severe Dysplasia
Feret	Mild Dysplasia
FeretX	Severe dysplasia
FeretY	Moderate Dysplasia
FeretAngle	Moderate Dysplasia
MinFeret	Normal Mucosa
IntDen	Mild Dysplasia
Skew	Moderate Dysplasia
Kurt	Mild Dysplasia

Table 12b: Comparison of posterior margin with normal mucosa, mild dysplasia, moderate dysplasia and severe dysplasia for nuclei detection

Variables	Posterior M		Normal Mucosa		Mild Dysplasia		Moderate Dysplasia		Severe Dysplasia	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total Area	185331.42	90571.30	182226.00	58952.53	232841.1	81776.71	183363.3	65537.29	196116.3	74831.18
Average Size	611.03	245.67	557.74	183.96	616.14	171.48	640.18	161.97	576.06	159.95
%Area	16.53	6.86	15.90	4.99	21.01	6.78	15.63	5.74	17.23	6.77
Perim.	98.72	19.89	91.29	16.47	97.99	14.84	98.17	11.96	92.32	14.77
Major	35.36	6.51	32.99	5.30	35.50	5.19	35.97	3.97	33.26	5.05
Minor	19.87	4.34	19.71	3.73	20.12	3.37	20.81	3.31	19.95	2.87
Angle	86.44	17.89	93.28	19.18	89.28	17.28	88.77	19.35	92.46	15.88
Circ.	0.77	0.07	0.80	0.03	0.77	0.05	0.78	0.03	0.80	0.03
Solidity	0.91	0.02	0.93	0.01	0.92	0.02	0.93	0.01	0.92	0.01
Feret	36.57	6.78	33.94	5.44	36.60	5.39	36.87	4.04	34.27	5.14
FeretX	565.32	134.97	590.25	99.73	588.36	82.12	600.19	101.30	582.21	78.37
FeretY	463.99	69.73	480.16	49.99	476.05	44.96	473.60	55.98	476.86	42.10
FeretAngle	86.56	17.91	93.67	19.26	89.26	17.21	88.71	20.11	93.66	16.04
MinFeret	21.27	4.47	20.80	3.82	21.46	3.42	21.97	3.33	21.09	3.00
IntDen	155778.19	62636.15	142197.14	46887.42	157089.4	43714.75	163229.7	41297.79	146874.8	40769.04
Skew	-24.20	6.05	-23.99	5.38	-26.53	4.34	-25.67	4.64	-25.87	4.77
Kurt	688.98	370.61	663.58	297.60	799.61	255.65	736.52	274.74	761.33	294.72

Inference: In the posterior margin analysis, Parameters such as average size, %Area, major, circularity, solidity, Feret, minimum Feret and IntDen were close to analysis of mild dysplasia. Perimeter, angle, Feret Y, Feret angle, skewness and kurtosis were in close proximity to the analysis of moderate dysplasia. Solidity total Area, Minor and Feret X were close to analysis of severe dysplasia.

Summary of variables in posterior margin analysis (In proximity with)	
Total Area	Severe dysplasia
Average Size	Mild Dysplasia
%Area	Mild Dysplasia
Perim.	Moderate Dysplasia
Major	Mild Dysplasia
Minor	Severe dysplasia
Angle	Moderate Dysplasia
Circ.	Mild Dysplasia
Solidity	Severe dysplasia
Feret	Mild Dysplasia
FeretX	Severe dysplasia
FeretY	Moderate Dysplasia
FeretAngle	Moderate Dysplasia
MinFeret	Mild Dysplasia
IntDen	Mild Dysplasia
Skew	Moderate Dysplasia
Kurt	Moderate Dysplasia

Table 12c: Comparison of medial margin with normal mucosa, mild dysplasia, moderate dysplasia and severe dysplasia for nuclei detection

Variables	Medial Margin		Normal Mucosa		Mild Dysplasia		Moderate Dysplasia		Severe Dysplasia	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total Area	188445.00	55323.48	182226.00	58952.53	232841.1	81776.71	183363.3	65537.29	196116.3	74831.18
Average Size	564.25	138.88	557.74	183.96	616.14	171.48	640.18	161.97	576.06	159.95
%Area	16.16	3.75	15.90	4.99	21.01	6.78	15.63	5.74	17.23	6.77
Perim.	93.68	12.12	91.29	16.47	97.99	14.84	98.17	11.96	92.32	14.77
Major	34.11	4.37	32.99	5.30	35.50	5.19	35.97	3.97	33.26	5.05
Minor	19.68	2.68	19.71	3.73	20.12	3.37	20.81	3.31	19.95	2.87
Angle	85.89	12.97	93.28	19.18	89.28	17.28	88.77	19.35	92.46	15.88
Circ.	0.78	0.04	0.80	0.03	0.77	0.05	0.78	0.03	0.80	0.03
Solidity	0.92	0.01	0.93	0.01	0.92	0.02	0.93	0.01	0.92	0.01
Feret	35.05	4.43	33.94	5.44	36.60	5.39	36.87	4.04	34.27	5.14
FeretX	599.36	77.23	590.25	99.73	588.36	82.12	600.19	101.30	582.21	78.37
FeretY	470.65	37.30	480.16	49.99	476.05	44.96	473.60	55.98	476.86	42.10
FeretAngle	86.40	13.53	93.67	19.26	89.26	17.21	88.71	20.11	93.66	16.04
MinFeret	20.83	2.63	20.80	3.82	21.46	3.42	21.97	3.33	21.09	3.00
IntDen	143867.43	35410.26	142197.14	46887.42	157089.4	43714.75	163229.7	41297.79	146874.8	40769.04
Skew	-25.04	4.13	-23.99	5.38	-26.53	4.34	-25.67	4.64	-25.87	4.77
Kurt	704.61	236.17	663.58	297.60	799.61	255.65	736.52	274.74	761.33	294.72

Inference: In the medial margin analysis, Parameters such as Minor was found to be in close proximity with analysis of normal mucosa. Parameters such as, perimeter, major and solidity were close to analysis of mild dysplasia. Angle, circularity, Feret X, Feret Y, Feret, skewness and kurtosis were in close proximity to the analysis of moderate dysplasia. Total Area, %Area, average size, solidity, Feret angle, minimum Feret and IntDen were close to analysis of severe dysplasia.

Summary of Variables in medial margin analysis (In proximity with)	
Total Area	Severe dysplasia
Average Size	Severe dysplasia
%Area	Severe dysplasia
Perim.	Mild Dysplasia
Major	Mild Dysplasia
Minor	Normal Mucosa
Angle	Moderate Dysplasia
Circ.	Moderate Dysplasia
Solidity	Mild Dysplasia and Severe dysplasia
Feret	Mild Dysplasia
FeretX	Moderate Dysplasia
FeretY	Moderate Dysplasia
FeretAngle	Moderate Dysplasia
MinFeret	Severe dysplasia
IntDen	Severe dysplasia
Skew	Moderate Dysplasia
Kurt	Moderate Dysplasia

Table 12d: Comparison of lateral margin with normal mucosa, mild dysplasia, moderate dysplasia and severe dysplasia for nuclei detection

Variables	Lateral M		Normal Mucosa		Mild Dysplasia		Moderate Dysplasia		Severe Dysplasia	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total Area	169651	66193.1	182226	58952.5	232841	81776.7	183363	65537.3	196116	74831.2
Average Size	552.06	167.45	557.74	183.96	616.14	171.48	640.18	161.97	576.06	159.95
%Area	15.6	5.59	15.9	4.99	21.01	6.78	15.63	5.74	17.23	6.77
Perim.	93.18	15.55	91.29	16.47	97.99	14.84	98.17	11.96	92.32	14.77
Major	33.97	5.48	32.99	5.3	35.5	5.19	35.97	3.97	33.26	5.05
Minor	18.85	3.3	19.71	3.73	20.12	3.37	20.81	3.31	19.95	2.87
Angle	85.24	16.57	93.28	19.18	89.28	17.28	88.77	19.35	92.46	15.88
Circ.	0.77	0.04	0.8	0.03	0.77	0.05	0.78	0.03	0.8	0.03
Solidity	0.91	0.02	0.93	0.01	0.92	0.02	0.93	0.01	0.92	0.01
Feret	35.01	5.64	33.94	5.44	36.6	5.39	36.87	4.04	34.27	5.14
FeretX	582.57	119.71	590.25	99.73	588.36	82.12	600.19	101.3	582.21	78.37
FeretY	466.14	68.78	480.16	49.99	476.05	44.96	473.6	55.98	476.86	42.1
FeretAngle	86.06	16.42	93.67	19.26	89.26	17.21	88.71	20.11	93.66	16.04
MinFeret	20.13	3.38	20.8	3.82	21.46	3.42	21.97	3.33	21.09	3
IntDen	140747	42688.2	142197	46887.4	157089	43714.8	163230	41297.8	146875	40769
Skew	-25.53	5.89	-23.99	5.38	-26.53	4.34	-25.67	4.64	-25.87	4.77
Kurt	755.9	323.92	663.58	297.6	799.61	255.65	736.52	274.74	761.33	294.72

Inference: In the lateral margin analysis, Parameters such as Total Area, average size, Minor, minimum Feret and IntDen was found to be in close proximity with analysis of normal mucosa. Parameters such as, perimeter, major and solidity, circularity, Feret and Feret X, were close to analysis of mild dysplasia. %Area, Angle, Feret Y, Feret angle and skewness were in close proximity to the analysis of moderate dysplasia. Kurtosis and solidity were close to analysis of severe dysplasia.

Summary of variables in lateral margin analysis (In proximity with)	
Total Area	Normal Mucosa
Average Size	Normal Mucosa
%Area	Moderate Dysplasia
Perim.	Mild Dysplasia
Major	Mild Dysplasia
Minor	Normal Mucosa
Angle	Moderate Dysplasia
Circ.	Mild Dysplasia
Solidity	Mild Dysplasia and severe dysplasia
Feret	Mild Dysplasia
FeretX	Mild Dysplasia
FeretY	Moderate Dysplasia
FeretAngle	Moderate Dysplasia
MinFeret	Normal Mucosa
IntDen	Normal Mucosa
Skew	Moderate Dysplasia
Kurt	Severe dysplasia

Cellular Profile Segmentation Using MorphoLibJ- Example 1

Image 1: Original Image under 40x magnification

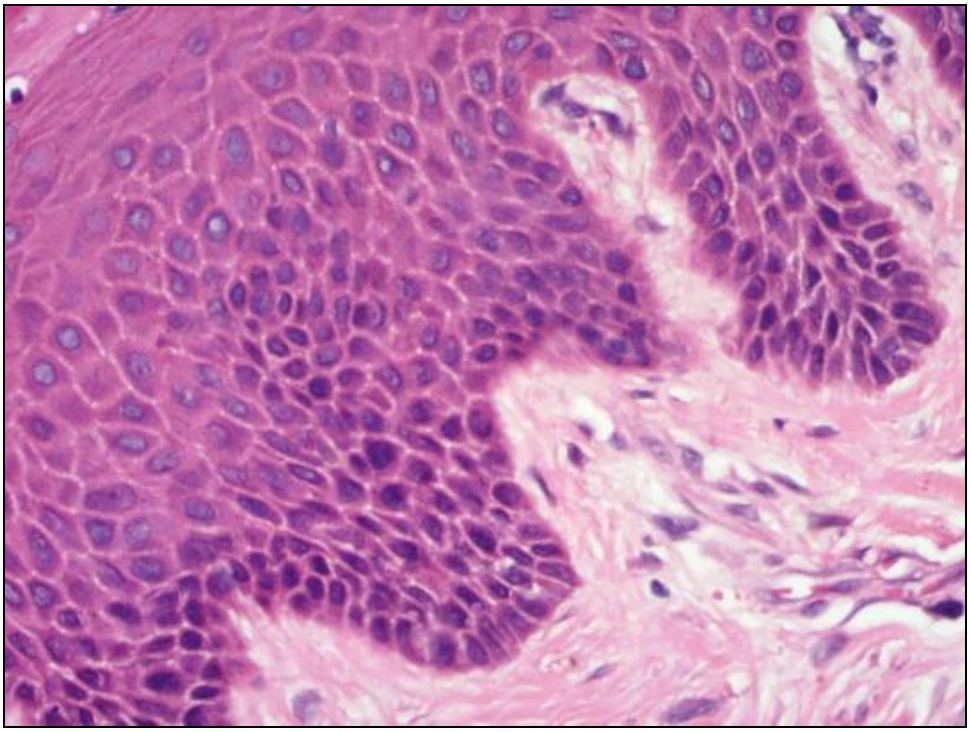


Image 2: Image resulted after Deconvolution (Hematoxylin component)

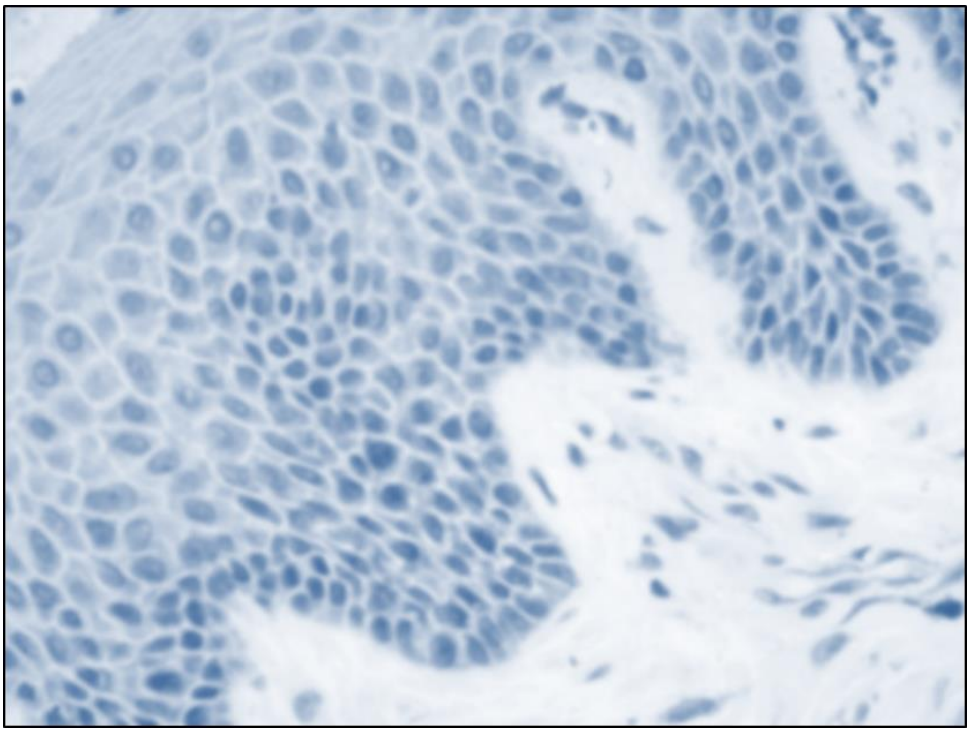


Image 3: Smoothened version of Image 2 after binary conversion

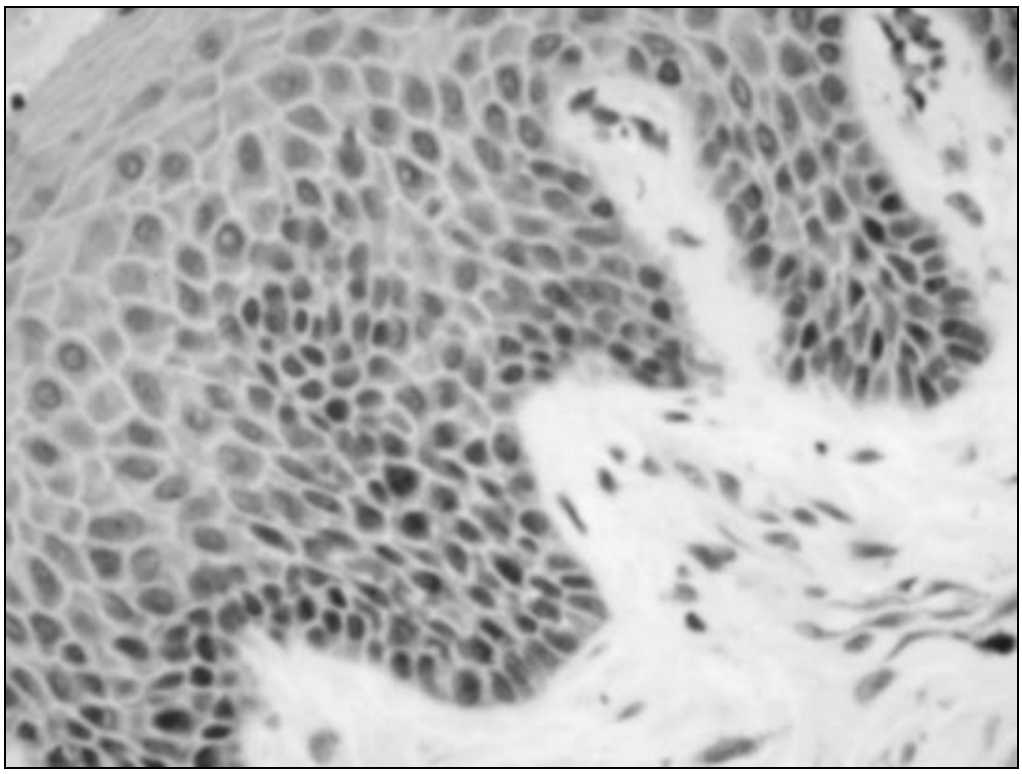


Image 4: Masked version of Original Image 1(only epithelial compartment)

Image 5: Domes of Image 3 after subjecting to regional extended maxima and minima

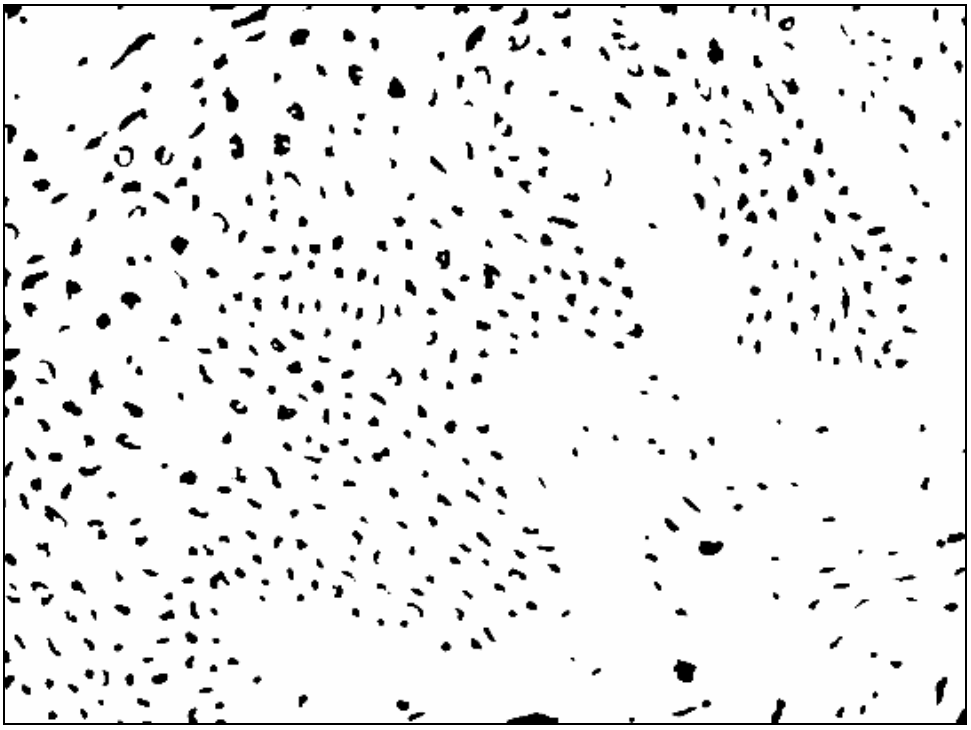


Image 6: Watershed after subjecting Image 3, image 4 and image 5 to marker-controlled segmentation

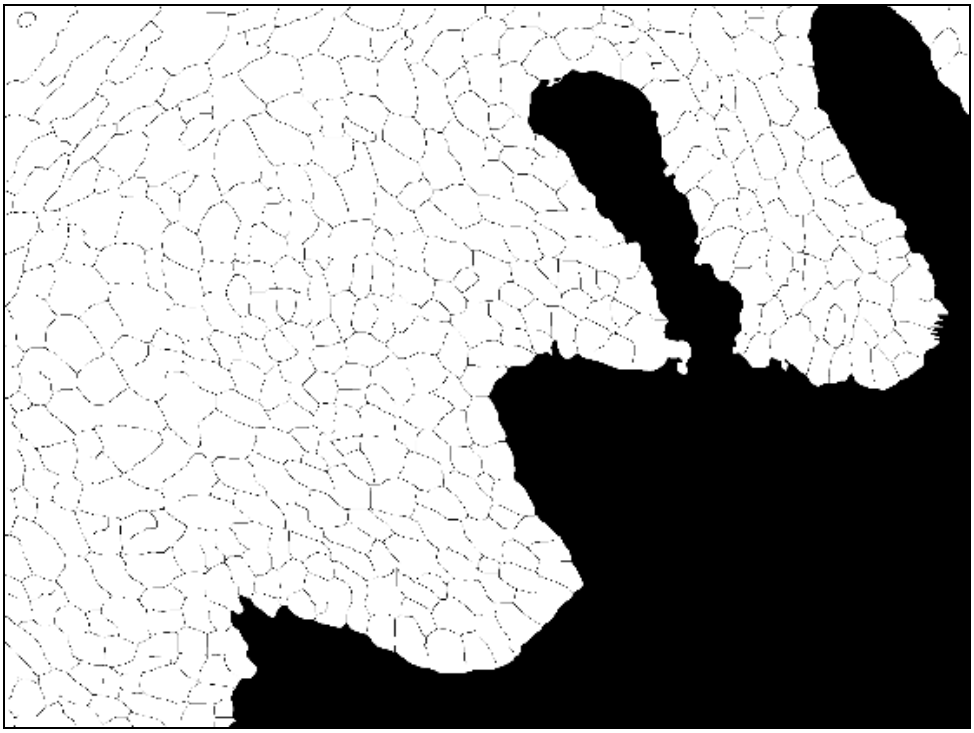


Image 7: Labelled boundaries of Image 6

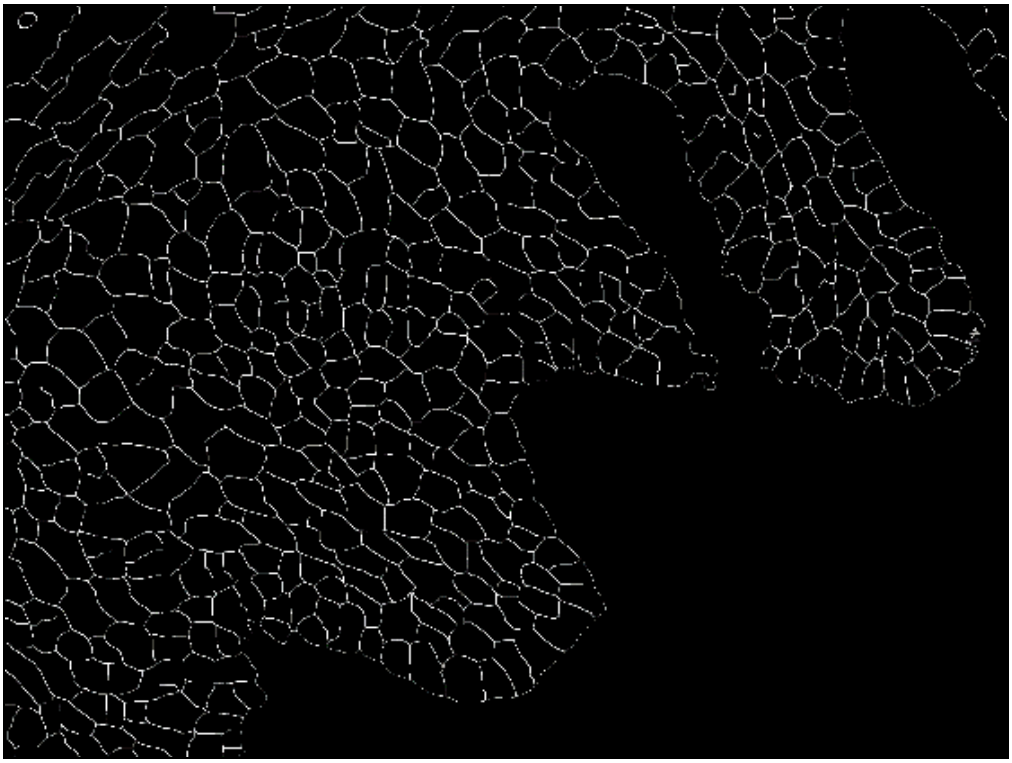
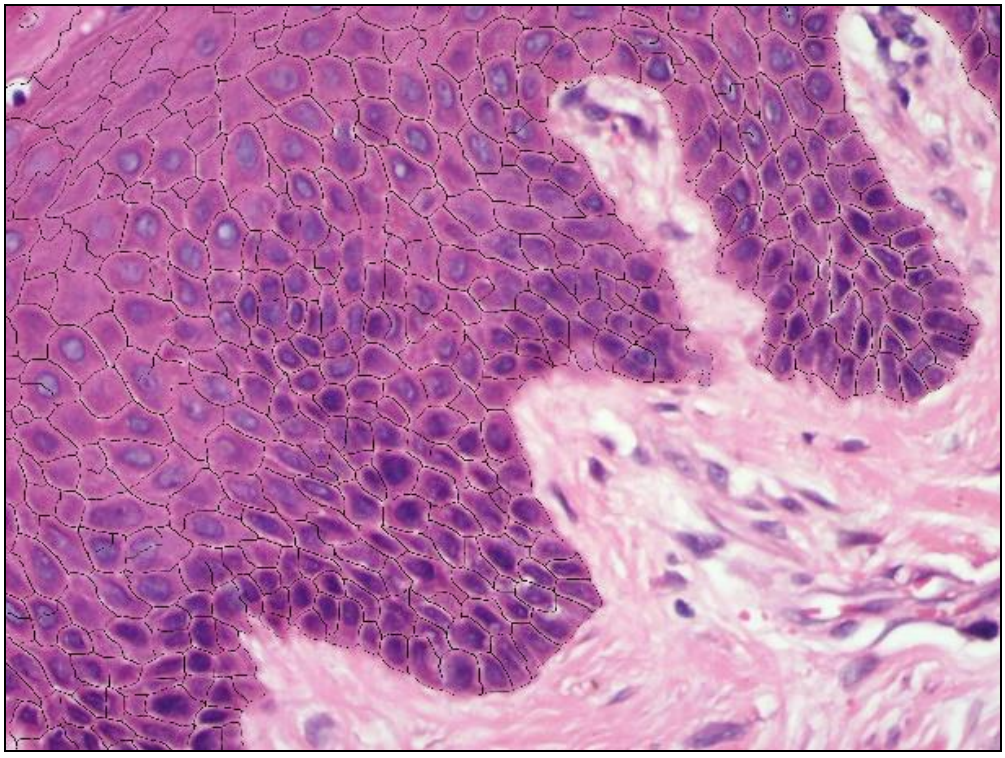


Image 8: Adding of Original image 1 to the Watershed Image with boundaries (Image7) by using Image calculator tool in the software.



Cellular profile segmentation using MorphoLibJ- Example 2

Image 1: Original Image under 40x magnification

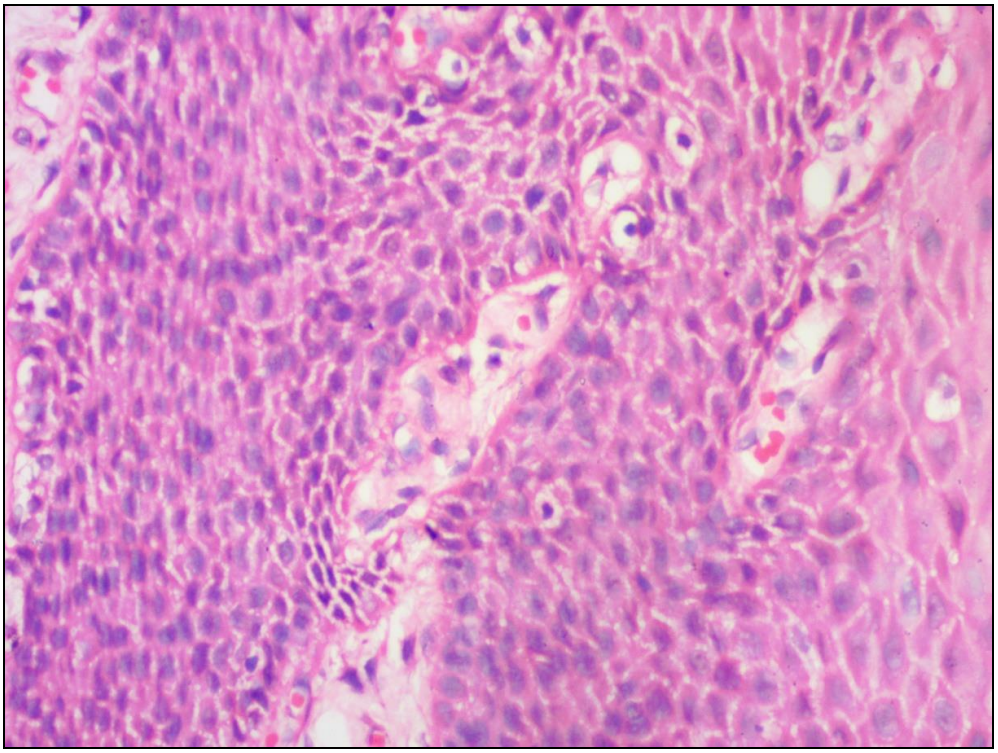


Image 2: Image 2 after Deconvolution (Hematoxylin component)

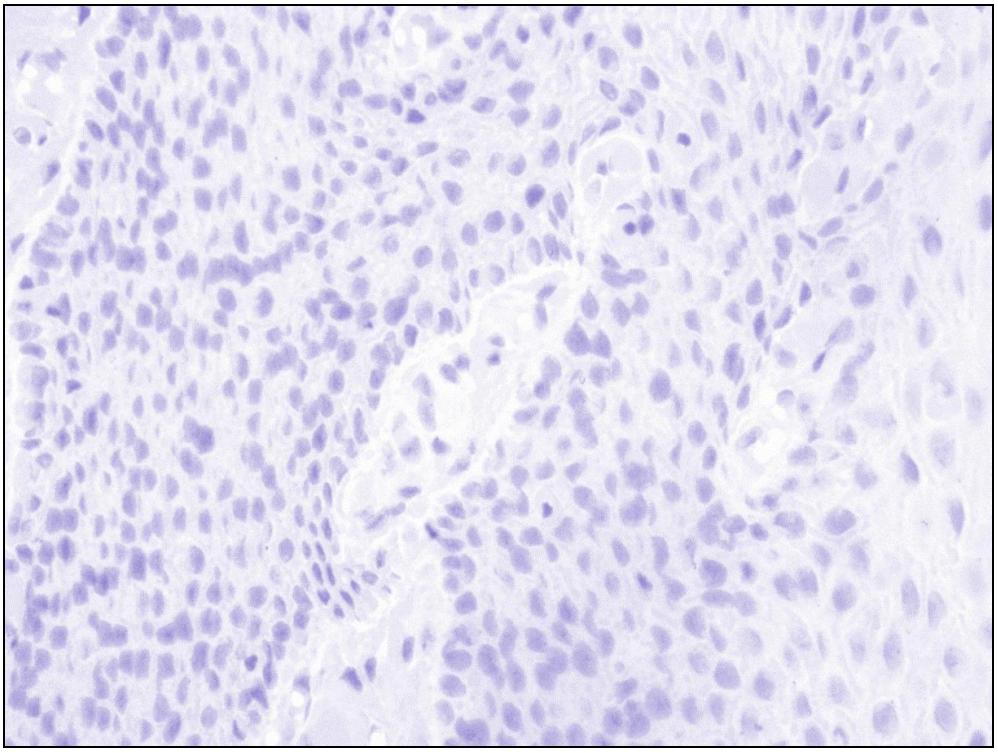


Image 3: smoothened version of image 2 after binary conversion

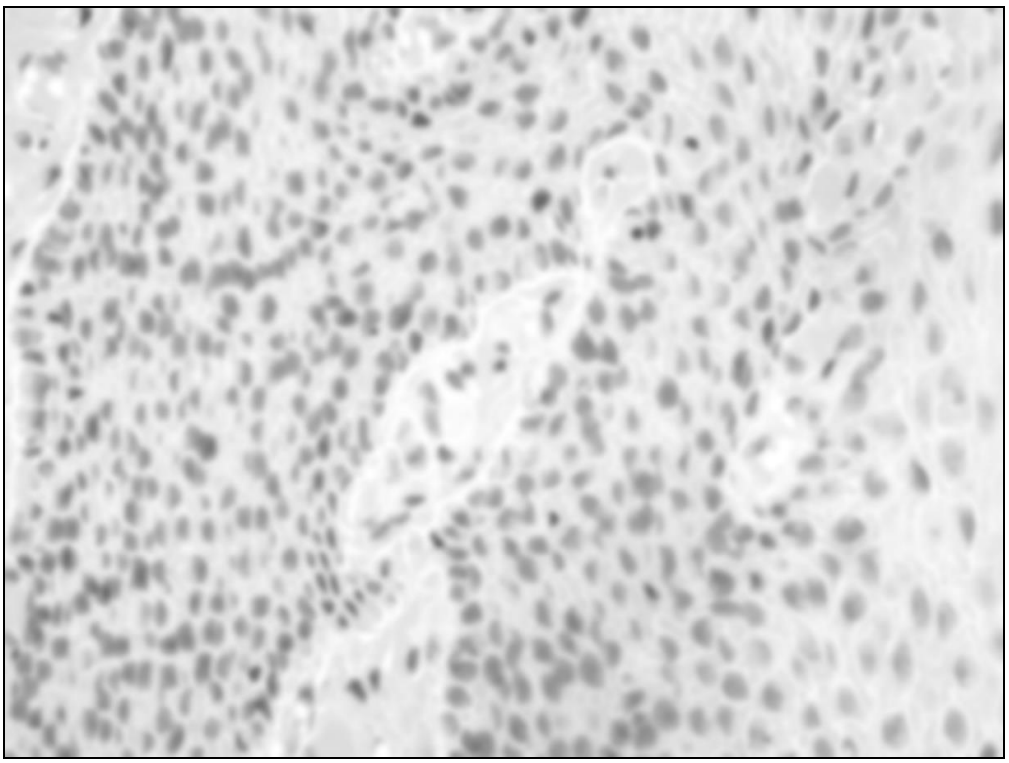


Image 4: masked version of Original Image 1 (only epithelial compartment)

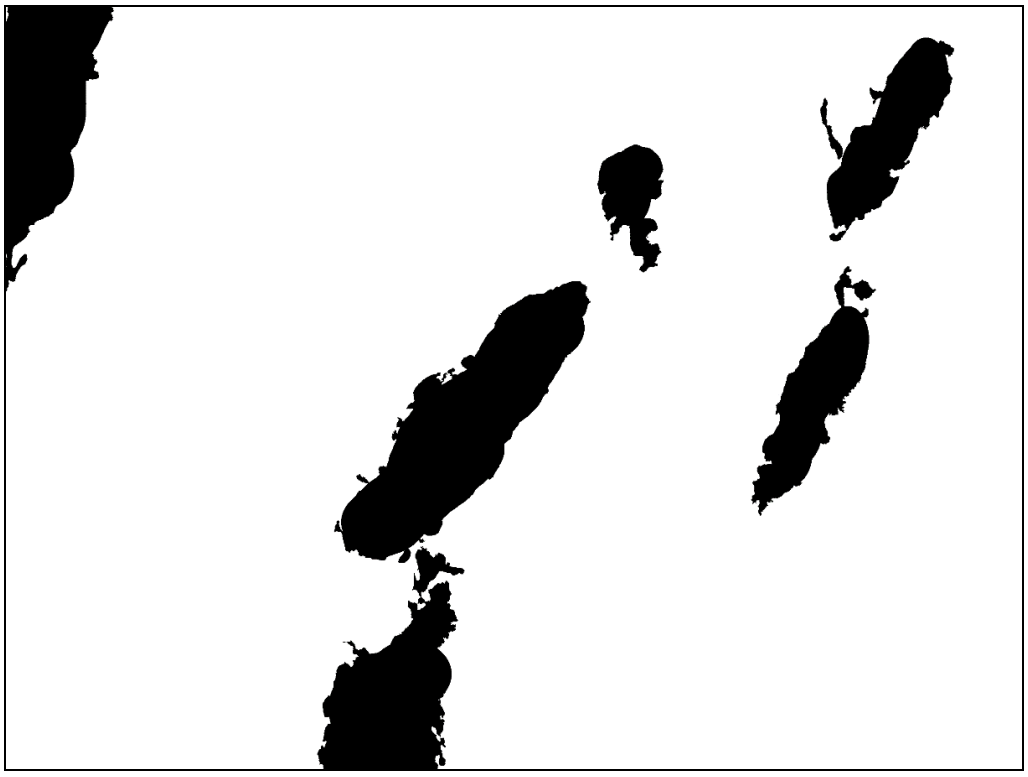


Image 5: domes of image 3 after subjecting to regional extended maxima and minima

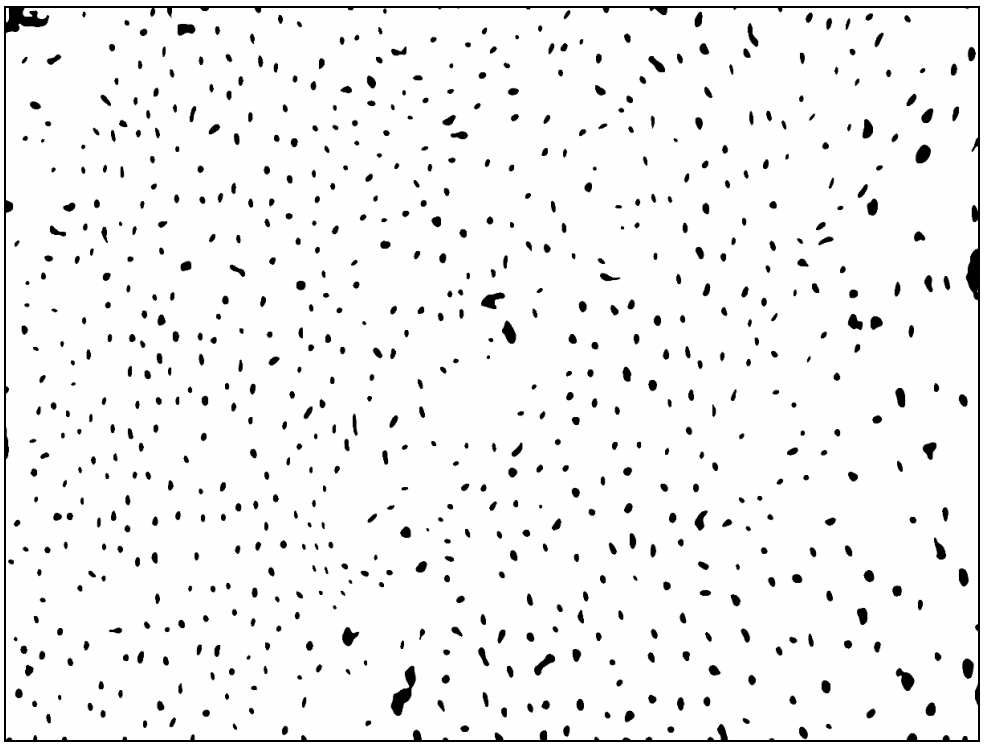


Image 6: watershed after subjecting Image 3, image 4 and image 5 to marker-controlled segmentation

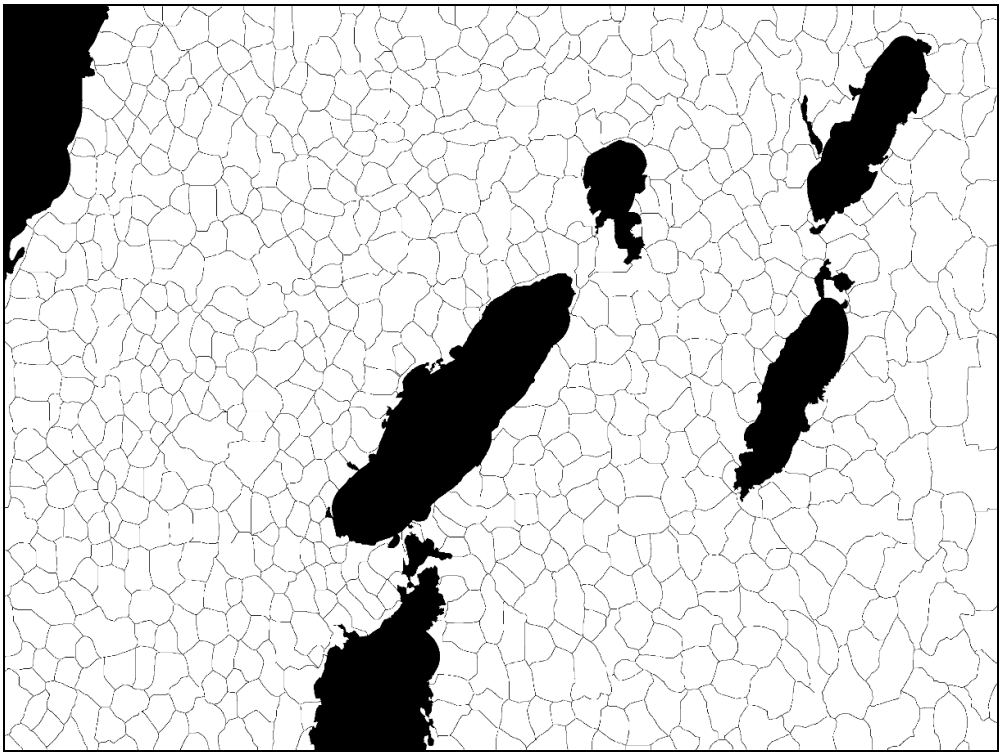


Image 7: after subjecting image 6 to labelling the boundaries

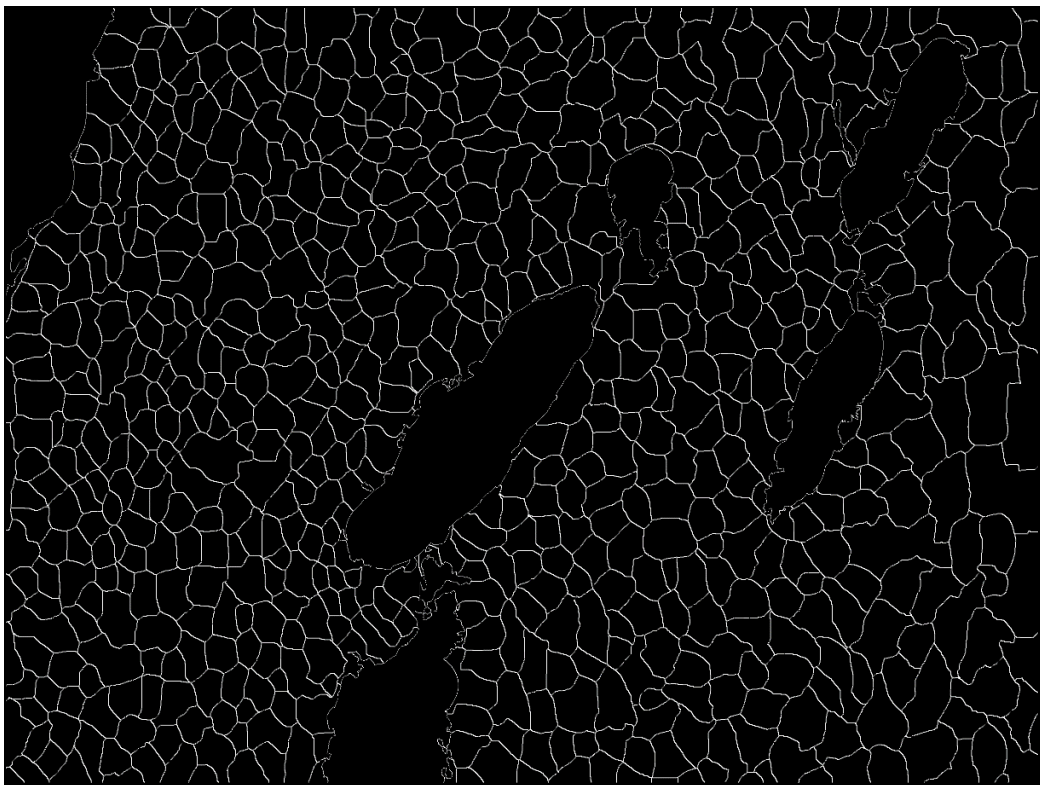
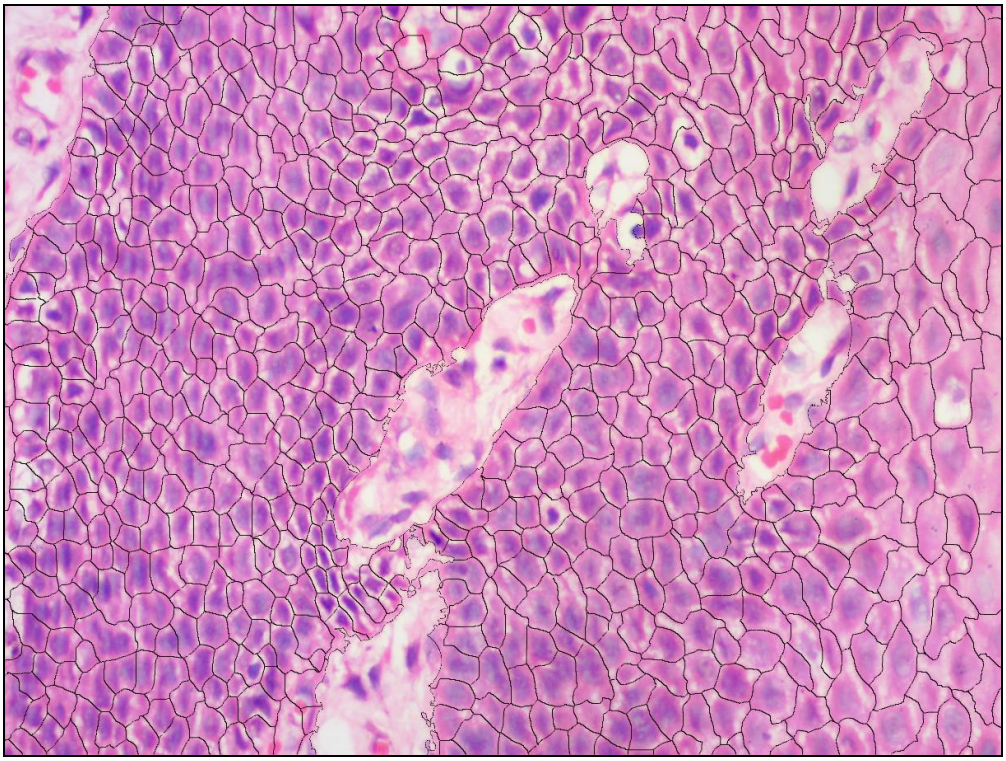


Image 8: Adding of Original image 1 to the Watershed Image with boundaries (Image7) by using Image calculator tool in the software.



Nuclei Detection Using StarDist-Example 1

Image 1: Original Image under 40x magnification

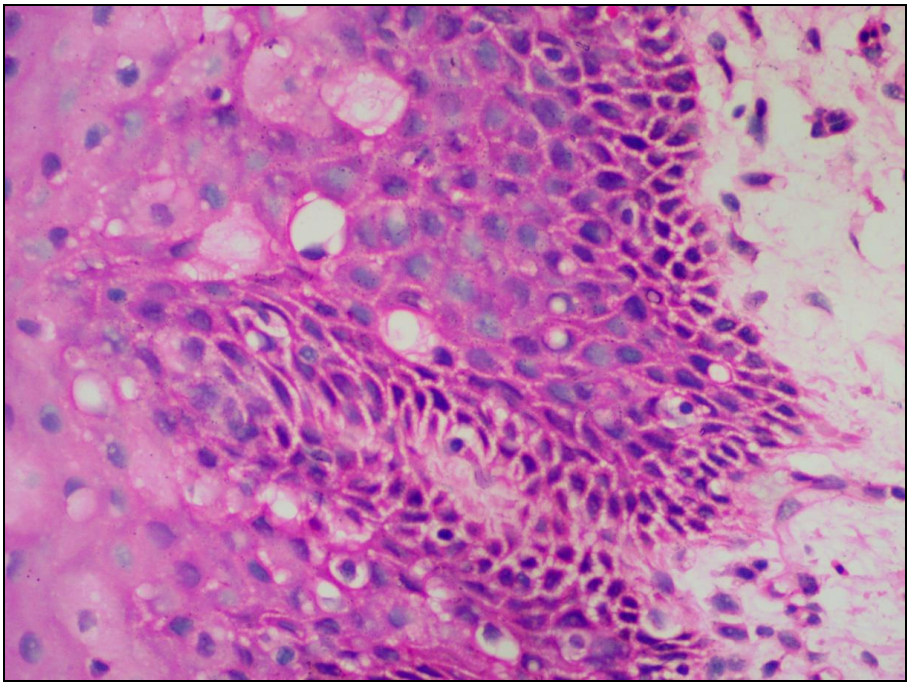


Image 2: only epithelial compartment separated from connective tissue, Image 2

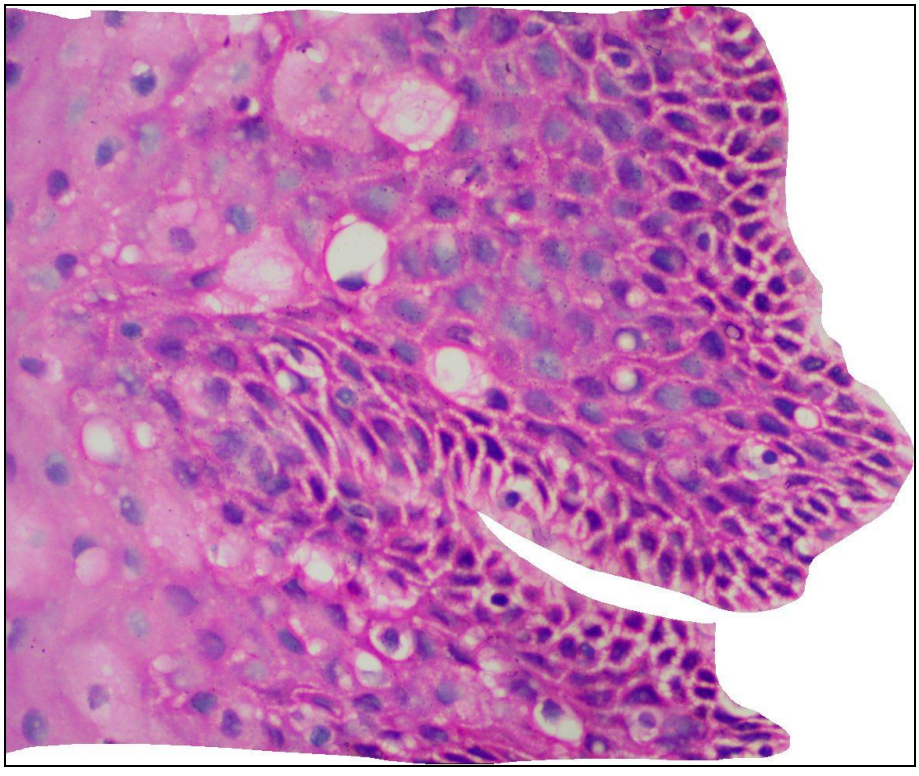


Image 3: Image after subjecting the Image 2 to StarDist Plugin, Image 3

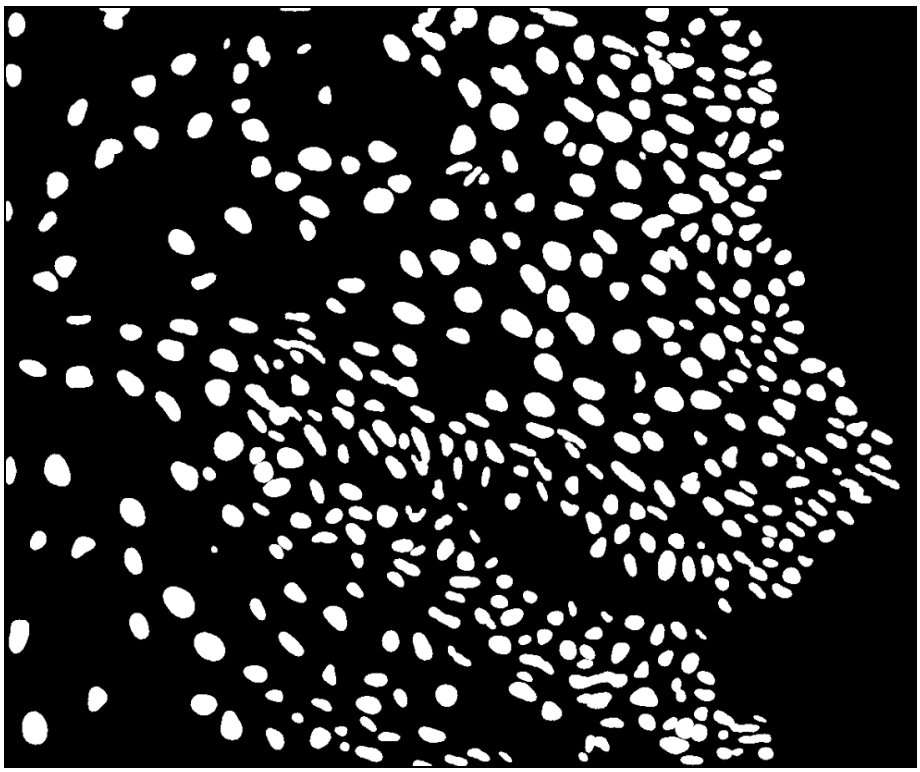
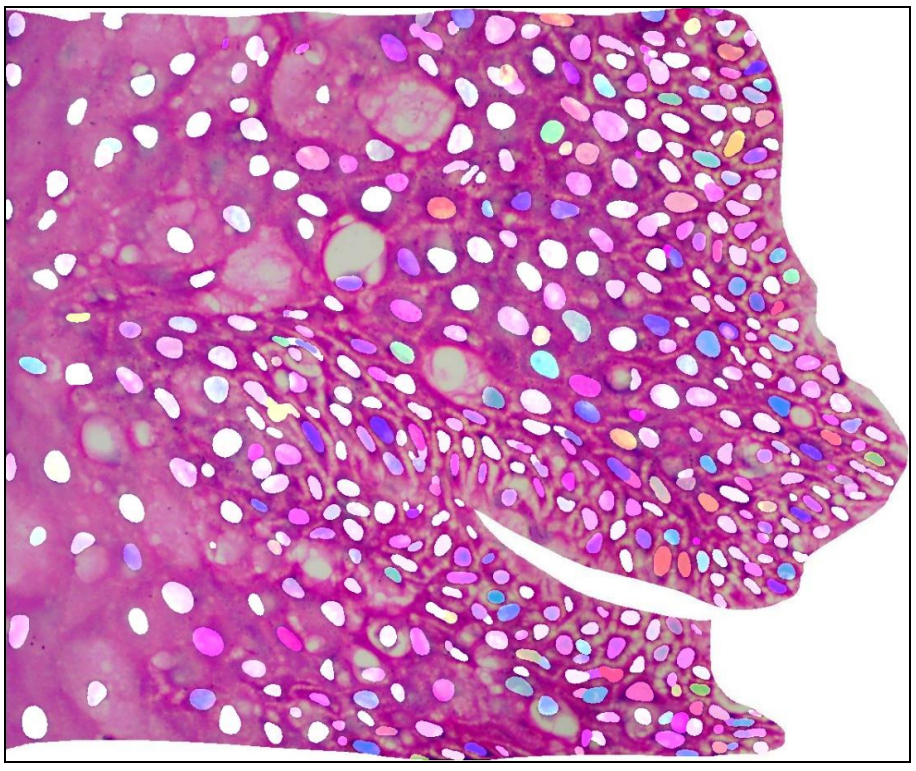


Image 4: Image after adding Image 2 and Image 3



Nuclei Detection Using StarDist- Example 2

Image 1: Original Image under 40x magnification

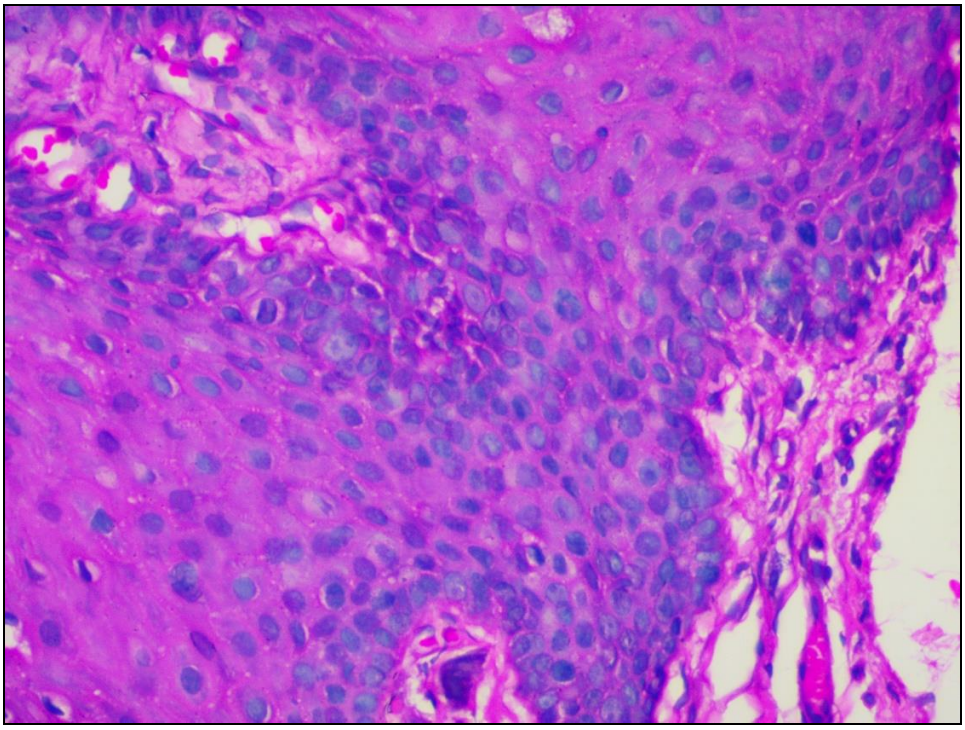


Image 2: only epithelial compartment separated from connective tissue, Image 2

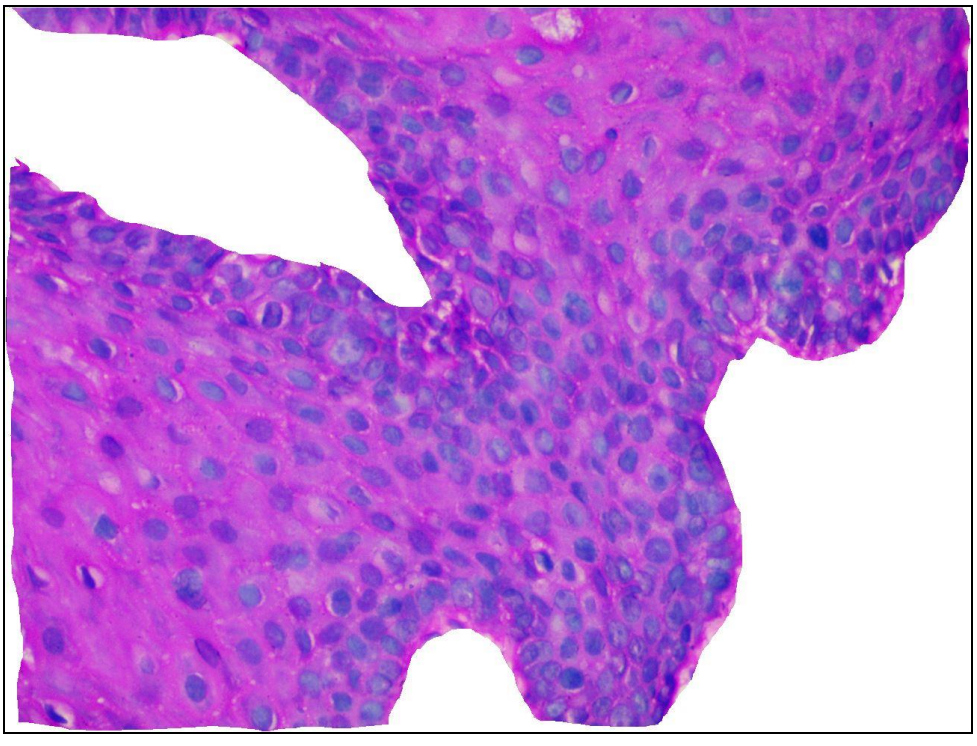


Image 3: Image after subjecting the Image 2 to StarDist Plugin, Image 3

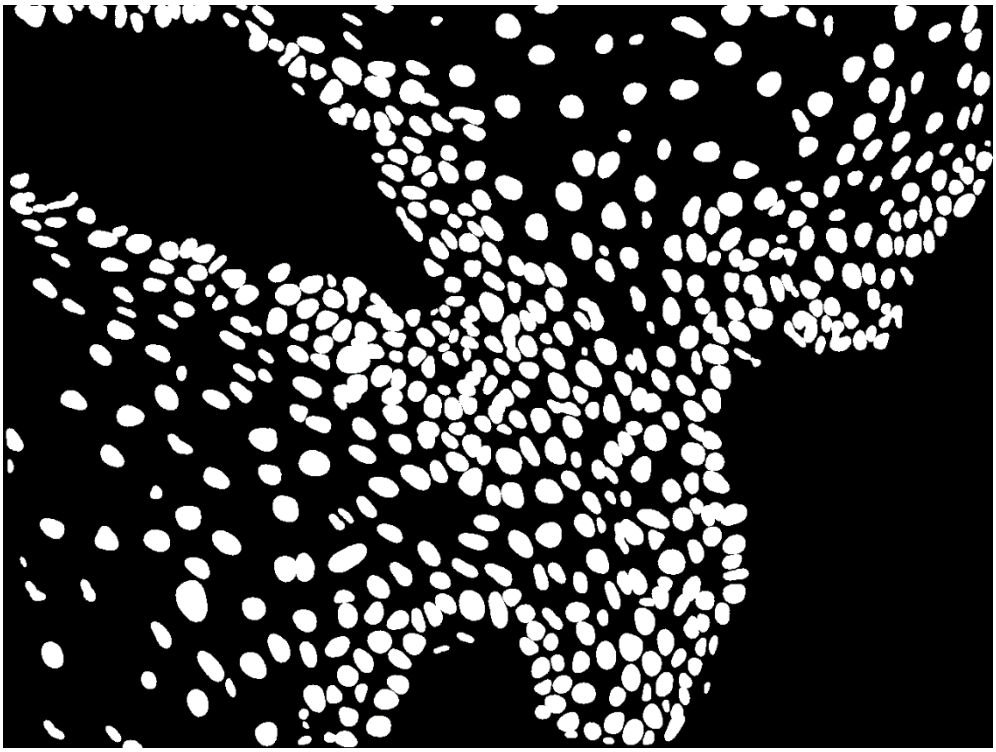
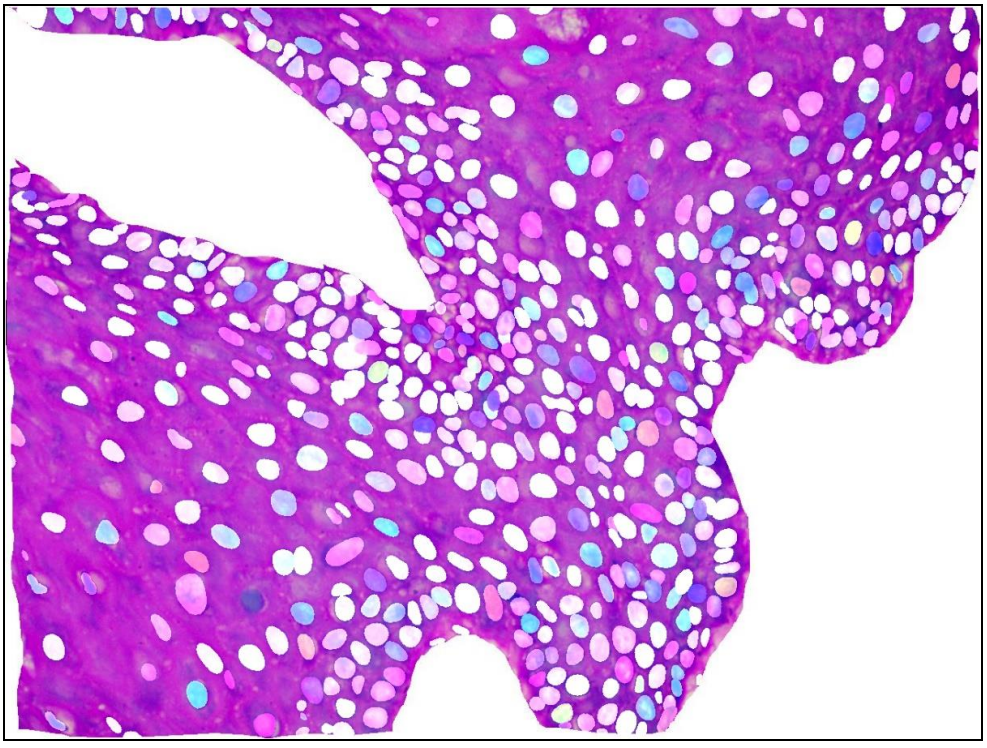


Image 4: Image after adding Image 2 and Image 3



DISCUSSION

It has been extensively researched how OSCC patients are affected by positive and close margins and presence of these margins are indication for adjuvant radiotherapy.[62,143-147] On the other hand, it's unknown how dysplastic margins would affect patients with OSCC. The existence of a dysplastic margin is usually unpredictable to a surgeon. The presence of a dysplastic margin could be attributed to the peculiar pattern of oral cancer formation.[62,148]

Due to close proximity to cancer, mucosal surgical margins are considered as biologically similar to the transferred cancer tissue. Molecular alteration observed at surgical margins correlates with the molecular alterations in the primary tumor cells.[149,150]

Even in a wide surgical field, the dysplastic lesion that surrounds the primary tumour is generally broad and difficult to see, making it impossible to entirely remove. A skilled surgeon can avoid a positive margin or narrow margin during surgery by performing a more comprehensive excision of the primary OSCC.[62,150,151] Excision of the primary tumour with macroscopically sufficient margins should always be the primary objective of curative OSCC surgery for safety.[62]

Meanwhile, whether the epithelium at the surgical margin is normal, with dysplasia (mild, moderate, or severe), or positive can be observed through frozen section during the operation, and the results can be used as a valuable guide for the surgeon and histopathological evaluation post-surgery which will become an important factor in deciding the further treatment with adjuvant therapy or re excision.[102,153]

The impact of dysplastic margins in OSCC remains unclear and controversial. Indeed, a previous study found that the relative risk of local recurrence in patients with a dysplastic epithelium at the surgical margin was five times higher than in those with a negative surgical margin.[102]

Results from earlier research indicated that margin dysplasia would either considerably affect the survival and local control of OSCC patients or would not.[56] The malignant transformation rates of mild or moderate margin dysplasia found in OSCC patients should be comparable to those of non-OSCC patients who have the same degree of dysplasia. Adjuvant RT should be suggested without hesitation if a dysplastic margin is present together with additional significant risk factors like ENE or positive neck nodal metastases.

However, repetitiveness poses a serious problem while assessing the presence or absence of epithelial dysplasia in radical dissections. Therefore, some of the local recurrences observed in many studies may have been due to insufficiently wide surgical margins and not to the presence of epithelial dysplasia in the mucosal margins. At the same time, local recurrences in patients, in the absence of epithelial dysplasia in the mucosal margins may perhaps be explained by the presence of epithelial dysplasia that was not included in the histological sections studied.

Furthermore, there is some degree of subjectiveness in the assessment and grading of epithelial dysplasia. Over the years many grading systems have been put forward in an attempt to obtain objectivity in grading oral epithelial dysplasia. However, despite these efforts, variability remains unresolved.[154] Epithelial dysplasia has been a subject of much debate owing to increased intra- and inter-observer

variability in its grading. Many researchers have found out that there is moderate to poor disagreement in diagnosing the different grades.

Apart from routine H&E staining, immunohistochemistry might serve as very useful adjunct in grading epithelial dysplasia with considerable inter-observer agreement. However, cost and time consumption and technique sensitivity are still its limitations.[155]

Morphological descriptors and computer aided analyses can be proposed as one of the alternative or adjuvant techniques for evaluation. As consequence of computer power becoming ubiquitous and more affordable in recent years, there has been increasing interest in automating and improving histopathological diagnosis by means of quantitative image analysis and pattern recognition.[35,142] Automation and quantification could allow not only more diagnostic accuracy and reproducibility but also examination of more histological material than current standards

The introduction of more objective methods such as the use of genomic and proliferation markers associated with different histopathological parameters, the study acknowledges that much work is still to be done before such techniques are available and generalized for histopathological diagnosis.[35]

Numerous uses of quantitative microscopy have been made to characterize morphological structures such as the shape and size of cell nuclei in diseased tissues. But little has been done to characterize the interactions between the various tissue constituents. To accurately define the spatial relationships of cells in a tissue, such hierarchical architectural qualities are required. A more accurate description and compartmentalization of morphological data, that is, not only how morphometrical

parameters vary but also where those variations occur within a tissue, would be made possible by the precise quantification of architectural organisation.[35]

The study aimed at developing quantitative objective methods for characterizing morphological features of normal epithelium, oral epithelial dysplasia and surgical margins with the aim of minimizing the subjectivity in some aspects of such histopathological grading.

The present study consisted of 40 cases in each group of normal mucosae, epithelial dysplasia (10 each of mild, moderate and severe grades) and surgical margins of oral squamous cell carcinoma (Anterior, Posterior, Medial and Lateral margins). Microscopic evaluation of all the groups were performed manually and the diagnosis was tabulated. H and E images of the same were captured using Olympus camera and Magvision software under 40x magnification and analysed in ImageJ software using different plugins like color deconvolution, MorphoLibJ, watershed transformation for cellular profile segmentation and StarDist plugin for nuclear detection. Morphometric analysis of transformed images was performed.

Cellular profile segmentation

Total of 363 40x H and E images of normal mucosa, dysplasia and 4 surgical margins of OSCC were subjected to ImageJ analysis using Deconvolution and MorphoLibJ plugins for cellular profile segmentation.

The images analysed in-between the groups (normal mucosa, mild dysplasia, moderate dysplasia and sever dysplasia) using one way ANOVA analysis revealed that the mean values of the morphological parameters such as area, perimeter Euler number, centroid X, convex area and Maximum Feret Diameter were statistically

different between normal mucosa and dysplasia Groups. (Table 7) It was found that there is difference in the geometrical and morphometrical features of normal and abnormal cells. Also, the Tukeys multiple posthoc tests performed for pair wise comparison also shows statistically significant results. (Table 8)

The mean values of parameter like area, perimeter, Euler number, ellipse orientation, convex area, maximum Feret diameter were seen to increase from normal to dysplasia cases. The abnormal changes listed in WHO criteria like Increased nuclear-cytoplasmic ratio, abnormal variation in cell size, abnormal variation in cell shape may play a role for the difference seen in the parameters.

Similar study was conducted by Rasha Abu Eid and Gabriel Landini with the applying objective and quantitative image analysis techniques to one problematic area in histopathological diagnosis: the grading of the severity of epithelial dysplasia. Following parameters: cell perimeter, area, radius of the inscribed circle centered at the center of mass, radius of the enclosing circle centered at the center of mass, largest axis length (Feret), breadth, convex Hull, area of the convex Hull polygon, radius of the minimal bounding circle, aspect ratio, roundness, area equivalent diameter, perimeter equivalent diameter, equivalent ellipse area, compactness, solidity, concavity, convexity, shape, Rfactor, modification ratio, sphericity, Feret breadth, rectangularity, and greyscale statistics (integrated density, minimum, maximum, modal, median, average, average deviation, standard deviation, variance, skewness, kurtosis and entropy values in the particle) were assessed. With the exception of circularity, solidity, concavity, convexity, Rfactor, rectangularity, grey value mode, grey average deviation, grey standard deviation and kurtosis, all the parameters were

statistically different between moderate and severe dysplasia. All the parameters were statistically different between mild and moderate dysplasia except for convexity.[142]

Bosman also emphasized the importance of developing newer and better morphological definitions for grading epithelial dysplasia based on research into the pathogenesis of premalignancy. He suggested that only through correlation between changes at the molecular level and the clinical outcome, new criteria can be accepted to replace the "gold standard" for grading epithelial dysplasia.[142]

Rasha Abu Eid and Gabriel Landini also studied in normal mucosa, epithelial dysplasia and pseudoepitheliomatous hyperplasia using the same method of algorithmically segmentation of cells. Study proved useful in statistically distinguishing other oral epithelial lesions including normality, epithelial dysplasia (regardless of its grade) and pseudo-epitheliomatous hyperplasia.[142]

In present study, correlation was done between the morphometrical analysis of normal mucosa and dysplasia with surgical margins, the mean values of all parameters analysed in surgical margins. (Table 4) The values which were in close relation to either the normal or dysplasia (mild, moderate and sever) mean values were corelated.

The tables (Table 9a,9b,9c,9d) represent correlation between mean values of each margin with normal mucosa and different grades of dysplasia. The results showed that there is difference in the diagnosis of marginal status by manual method and by ImageJ. Most of the margins except for 2 cases showed negative status in routine/manual evaluation.

However, in the morphometric analysis, for the anterior margins, parameters such as area, perimeter, circularity, centroid number, ellipse elongation, convex area and

Maximum Feret Diameter Angle were found to be in close proximation with the analysis of normal Mucosa. Centroid Y and Maximum Feret diameter were close to analysis of moderate dysplasia. Euler Number and InscrDisc.Radius parameters were close to analysis of severe dysplasia and ellipse orientation was found to be in close relation to analysis of mild dysplasia.

For posterior margins, parameters such as area, circularity, ellipse elongation, convex area, Maximum Feret Diameter Angle and InscrDisc.Radius were found to be in close proximation with the analysis of normal mucosa. Centroid X, centroid Y, and Maximum Feret diameter were close to analysis of moderate dysplasia. Euler Number parameter was close to analysis of severe dysplasia. Perimeter and ellipse orientation was found to be in close relation to analysis of mild dysplasia.

For medial margins, parameters such as circularity, centroid X, ellipse elongation, Maximum Feret Diameter Angle and InscrDisc.Radius were found to be in close proximation with the analysis of normal mucosa. Perimeter and Euler Number were found to be in close relation to analysis of mild dysplasia. Area, centroid Y, and Maximum Feret diameter were close to analysis of moderate dysplasia. Convex area and ellipse orientation parameters were close to the analysis of severe dysplasia.

For lateral margins, parameters such as Area, Perimeter, Circularity, Centroid X, ellipse elongation, Maximum Feret Diameter Angle and Convex area were found to be in close proximation with the analysis of normal Mucosa. Maximum Feret diameter and Euler Number were found to be in close relation to analysis of mild dysplasia. Centroid Y was close to analysis of moderate dysplasia. InscrDisc.Radius and ellipse orientation parameters were close to the analysis of severe dysplasia.

The results show that there are differences in assessing surgical margins by routine microscopic evaluation and through ImageJ analysis.

Nuclei detection

Total of 363 40x H and E images of normal mucosa, dysplasia and 4 surgical margins of OSCC were subjected to ImageJ analysis using StarDist plugin for Nuclei detection.

The images analysed in between the groups (normal mucosa, mild dysplasia, moderate dysplasia and sever dysplasia) using one way ANOVA analysis revealed that the mean values of the morphological parameters such as Total area, average size, % area, perimeter, major, circularity, solidity, Feret, IntDen, skewness and kurtosis showed statistically significant difference between normal mucosa and different grades of dysplasia.(Table 10) Also, the Tukeys multiple posthoc tests performed for pair wise comparison also shows statistically significant results. (Table 11)

The increase in proliferation of epithelial cells, abnormal variation in nuclear size, abnormal variation in nuclear shape, increased nuclear-cytoplasmic ratio, basal cell clustering might be the reasons for the increase in total area, average size, %area, perimeter, Feret, skewness, kurtosis, minimum Feret, IntDen parameters in dysplasia compared to normal mucosa.

To corelate the morphometrical analysis of normal mucosa and dysplasia with surgical margins, the mean values of all parameters analysed in surgical margins and the values which were in close relation to the either of the normal or dysplasia (mild, moderate and sever) mean values were taken.

The tables (12a,12b,12c,12d) represent correlation between mean values of each margin with normal mucosa and different grades of dysplasia. The results showed that there is difference in the diagnosis of marginal status by manual method and by ImageJ. Most of the margins except for 2 cases showed negative status in routine/manual evaluation. But in the morphometric analysis,

For the anterior margins, parameters such as total area, and minor and minimum Feret were found to be in close proximity with analysis of normal mucosa. parameters such as average size, perimeter, major, circularity, solidity, Feret, IntDen and kurtosis were close to analysis of mild dysplasia. %Area, angle, Feret Y, Feret angle, skewness were in close proximity to the analysis of moderate dysplasia. Solidity and Feret X were close to analysis of severe dysplasia.

For posterior margins, parameters such as average size, %Area, major, circularity, solidity, Feret, minimum Feret and IntDen were close to analysis of mild dysplasia. Perimeter, angle, Feret Y, Feret angle, skewness and kurtosis were in close proximity to the analysis of moderate dysplasia. Solidity, total area, Minor and Feret X were close to analysis of severe dysplasia.

For medial margins, parameters such as Minor was found to be in close proximity with analysis of normal mucosa. Parameters such as, perimeter, major and solidity were close to analysis of mild dysplasia. Angle, circularity, Feret X, Feret Y, Feret, skewness and kurtosis were in close proximity to the analysis of moderate dysplasia. Total Area, %Area, average size, solidity, Feret angle, minimum Feret and IntDen were close to analysis of severe dysplasia.

For lateral margins, parameters such as Total Area, average size, Minor, minimum Feret and IntDen was found to be in close proximity with analysis of normal mucosa. Parameters such as, perimeter, major and solidity, circularity, Feret and Feret X, were close to analysis of mild dysplasia. %Area, Angle, Feret Y, Feret angle and skewness were in close proximity to the analysis of moderate dysplasia. Kurtosis and solidity were close to analysis of severe dysplasia.

The results show that there are differences in assessing surgical margins with dysplastic features in manual and ImageJ methods.

The comparative evaluation of both cellular and nuclear architecture morphometrically showed that there is difference in manual and ImageJ methods in diagnosing the surgical margins and this signifies that there is subjective variation occurring in categorizing the margin status when epithelial dysplasia is present at the margins.

The objective evaluation signifies the importance of subjective variability and it can be one of the reliable quantitative and unbiased approach to the description of the local spatial arrangement characteristics of cells in a tissue.

The results show that there are statistical differences in the morphometrical parameters analysed across the diagnostic classes, these can be reliably used for discrimination purposes. This objective analysis can help quantitatively to distinguish the feature between normal and dysplastic features. Also, can be helpful in assessing the surgical margins and minimize the subjective variation occurring during catergeroizing the grades of dysplasia.

SUMMARY AND CONCLUSION:

- Aim of the present research was to evaluate histopathological assessment of surgical margins in oral squamous cell carcinoma by manual method and compare it with computerized method by using ImageJ software.
- Total of 120 cases comprised of histopathological sections that included 40 each of histopathologically confirmed cases of oral squamous cell carcinoma, dysplasia cases and normal mucosa.
- Microscopic evaluation of histopathologic sections by manual method and morphometrical analysis of the H and E images using different plugins available in ImageJ software were done.
- The manual microscopic evaluation of surgical margins was of negative status except for 3 cases.
- Total of 726 H and E photomicrographs under 40x magnification from all 120 cases were transferred to ImageJ software and analysis of images for cellular segmentation and nuclei detection using different plugins (color deconvolution, MorphoLibJ, StarDist) was performed.
- The morphometric analysis of H and E images of normal mucosa and different grades of dysplasia showed difference in the parameters analyzed. The results were statistically significant in few parameters.
- The mean values of surgical margins correlated with normal mucosa and different grades of dysplasia by morphometrical analysis of using ImageJ.
- There was a statical difference in analysis of surgical margins by manual method and analysis by the software in diagnosing dysplasia.

- The present study indicates that the subjective errors can be minimized when quantitative methods are applied while assessing for presence or absence of epithelial dysplasia in surgical margins of OSCC that will assist surgeon in deciding adjuvant therapy or further treatment.

LIMITATIONS:

- Not all cellular and architectural features of cell are quantifiable. The dysplastic features like irregular epithelial stratification, drop-shaped rete ridges, loss of polarity of basal cells, dyskeratosis, keratin pearls within rete ridges, loss of epithelial cell cohesion and hyperchromasia could not be reproduced in ImageJ for quantification.

- The sample size could have been more and more RND cases with dysplastic surgical margins could have helped the study to derive a quantified results.
- The photomicrographs were analyzed in higher magnifications. The analysis of images with full epithelial thickness can give better morphometric picture of the architectural changes.

FUTURE SCOPE:

The study contained less sample size and most of the cases were with negative margins. Multicentric studies with larger sample size and with epithelial dysplasia at surgical margins will help in validating the study. Also, studies including clinical correlation with the margin status assessed and the recurrence data might help in concluding the significance of surgical margin assessment in OSCC.

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ANNEXURE-I

WAIVER FORM

Department of Oral and Maxillofacial Pathology and Oral Microbiology, KAHER VK Institute of Dental Sciences, Nehru Nagar, Belagavi.

"Histopathological Assessment of Surgical Margins in Oral Squamous Cell Carcinoma by Manual Method and by Using ImageJ Software – A Comparative Study".

Waiver of informed consent form

It is not feasible to obtain individual informed consent of donors of specimens used in this study. However, I assure that confidentiality of the participant information will be ensured and no identifying information related to the study participants will be disclosed in any report/ publication arising from the study.

Post Graduate
REG NO: IH0220002
Department of Oral and Maxillofacial
Pathology and Oral Microbiology

Guide
Professor
Department of Oral and Maxillofacial
Pathology and Oral Microbiology

ANNEXURE II – ETHICAL CLEARENCE CERTIFICATE

Research and Ethics Committee
KLE V K INSTITUTE OF DENTAL SCIENCES
KLE University
Accredited 'A' Grade by NAAC Placed in Category 'A' by MHRD (GoI)
Nehru Nagar, Belagavi - 590 010, Karnataka State
☎: 0831-2470362 Web: http://www.kledental-bgm.edu.in
FAX: 0831-2470640 E-mail:principal@kledental-bgm.edu.in

Sl. No. : 1463

CERTIFICATE

This is to Certify that the synopsis titled

Histopathological assessment of surgical margins in oral Squamous Cell Carcinoma by manual method and using ImageJ software - A comparative study *Submitted by*

Dr. _ **REG. NO. IH0220002** _____ *P. G. Student / Staff, Guided by* _ *from Department of* Oral and Maxillofacial Pathology & Oral Microbiology *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

Date : 5/5/21

Member Secretary
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

ANNEXURE III – BIOSTATISTIC CLEARENCE CERTIFICATE

KLE V.K. Institute of Dental Sciences
(A Constituent unit of KLE Academy of Higher Education & Research
Deemed-to-be-University u/s 3 of the UGC Act, 1956)
Nehru Nagar, Belagavi-590 010 INDIA
Re-Accredited 'A' grade by NAAC (2nd Cycle) & Placed in Category 'A' by MHRD (GoI)

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Web: http://www.kledental-bgm.edu.in
E-mail: principal@kledental-bgm.edu.in

Biostatistics Clearance Certificate

This is to certify that the Biostatistics aspect of the Dissertation / Research work of **REG. NO. IH0220002** **Graduate Student**, under the guidance of **Professor**, **Department of Oral and Maxillofacial Pathology and Oral Microbiology**, entitled **"Histopathological Assessment of Surgical Margins in Oral Squamous Cell Carcinoma by Manual Method and by Using ImageJ Software- A Comparative study"** has been done under my guidance and considered satisfactory.

Place: Belagavi

Date: 13.12.2022

Name & Signature of Biostatistician

Dr. S. B. Javali
Sr. Asso. prof. in statistics
Dept. of com. medicine
USM KLE IMP, Belagavi

ANNEXURE- IV

CHART OF NORMAL ORAL MUCOSA CASES

Sl. No.	OP NO.	Sl. No.	OP NO.	Sl. No.	OP NO.	Sl. No.	OP NO.
1	3848	11	3496	21	6448	31	6257
2	3850	12	3262 A	22	3703	32	6331
3	1803	13	4228	23	3849	33	4472
4	6231	14	6234	24	6464	34	6305
5	6305	15	6276	25	6446	35	4470
6	3846	16	3262 B	26	6291	36	3703
7	6473	17	3861	27	6322	37	5383 A
8	6264	18	4509	28	3858	38	1766
9	1711	19	6466	29	4181 B	39	6236
10	6270	20	6258	30	6364	40	1715

CHART OF ORAL EPITHELIAL DYSPLASIA CASES

Sl. No	Mild Dysplasia	Moderate Dysplasia	Severe Dysplasia
1	OP 416	OP 2057	OP 1326
2	OP 520	OP 2418 E	OP 1747
3	OP 598	OP 2614 E	OP 2758
4	OP1190	OP 2974	OP 3163
5	OP1739	OP 3328	OP 3169
6	OP 1896	OP 3627	OP 3211
7	OP 2061	OP 3633	OP 3701
8	OP 2793	OP 4098	OP 4257
9	OP 3513	OP 4352	OP 4398
10	OP 3894	OP 4576	OP 4482
11	OP 4618	OP 5447	OP 5619
12	OP 5421	OP 6275	OP 5903
13	OP 5505		OP 6262
14	OP 5611		OP 6447

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ANNEXURE- V

CHART OF ORAL SQUAMOUS CELL CARCINOMA CASES TREATED WITH RADICAL NECK DISSECTION

SL N0.	OP NO.	CLINICAL DIAGNOSIS	HISTOPATHOLOGICAL DIAGNOSIS	Anterior Margin	Posterior Margin	Medial Margin	Lateral Margin
1	OP 3415/10	Carcinoma of Tongue	WDSCC	Negative	Negative	Negative	Negative
2	OP 3633/11	Carcinoma of Lower Alveolus	MDSCC	Negative	Negative	Negative	Negative
3	OP 3765/11	Carcinoma of Left Border of Tongue	MDSCC	Negative	Negative	Negative	Negative
4	OP 3814/12	Carcinoma of Mandible	Verrucous Carcinoma	Negative	Negative	Negative	Negative
5	OP 3840/12	Carcinoma GBS	MDSCC	Negative	Negative	Negative	Negative
6	OP 3986/12	Carcinoma of Tongue	MDSCC	Negative	Negative	Negative	Negative
7	OP 4010/12	Squamous Cell Carcinoma	WDSCC	Negative	Negative	Negative	Negative
8	OP 4077/12	Papillary Squamous Cell Carcinoma	Papillary Squamous Cell Carcinoma	Negative	Negative	Negative	Negative
9	OP 4152/13	Carcinoma of Left GBS	WDSCC With MDSCC	Negative	Negative	Negative	Negative
10	OP 4256/13	Carcinoma Right Buccal Mucosa	WDSCC With MDSCC	Negative	Negative	Negative	Negative
11	OP 4355/15	Squamous Cell Carcinoma	WDSCC With MDSCC	Negative	Negative	Negative	Negative
12	OP 4364/13	Squamous Cell Carcinoma	WDSCC	Negative	Negative	Negative	Negative
13	OP 4585/14	Carcinoma of Mandible	PDSCC	Negative	Negative	Negative	Negative
14	OP 4624/14	Squamous Cell Carcinoma	WDSCC With MDSCC	Negative	Negative	Negative	Negative
15	OP 3885/12	Squamous Cell Carcinoma	MDSCC	Negative	Negative	Negative	Negative
16	OP 4640/14	Squamous Cell Carcinoma	MDSCC With PDSCC	Negative	Negative	Negative	Negative
17	OP 4759/15	Carcinoma of Linguogingival Sulcus	WDSCC	Negative	Negative	Negative	Negative
18	OP 4771/15	Carcinoma of Right Buccal Mucosa	Verrucous Carcinoma	Negative	Negative	Negative	Negative
19	OP 4803/15	Carcinoma of Right Buccal Mucosa	WDSCC With MDSCC	Negative	Negative	Negative	Negative
20	OP 4814/15	Squamous Cell Carcinoma	WDSCC	Negative	Negative	Negative	Negative
21	OP 4820/15	WDSCC	WDSCC	Negative	Negative	Negative	Negative
22	OP 4864/15	Carcinoma	WDSCC With Pd Advancing Front	Negative	Negative	Negative	Negative
23	OP 5036/16	Carcinoma of Hard Palate	WDSCC	Mild to moderate	Mild to moderate	Negative	Negative
24	OP 5109/17	Carcinoma of Lower Lip	WDSCC	Negative	Severe dysplasia	Negative	Negative
25	OP 5250/17	Carcinoma of Right Bm	PDSCC	Negative	Negative	Negative	Negative
26	OP 5304/17	Carcinoma of Left Bm	WDSCC	Negative	Negative	Negative	Negative
27	OP 5375/17	Carcinoma of Left Bm	WDSCC With Advancing MDSCC Front	Negative	Negative	Negative	Negative
28	OP 5420/17	Carcinoma	WDSCC	Negative	Negative	Negative	Negative
29	OP 5440/17	Carcinoma Left Bm	WDSCC	Negative	Negative	Negative	Negative
30	OP 5601	WDSCC	WDSCC With Advancing MDSCC Front	Negative	Negative	Negative	Negative
31	OP 5696	Carcinoma of Left Bm	WDSCC With Advancing MDSCC Front	Negative	Negative	Negative	Negative
32	OP 5745/18	Carcinoma of Left Bm	Early Invasive SCC	Negative	Negative	Negative	Negative
33	op 5800/19	Carcinoma of Left Bm	WDSCC	positive	Negative	negative	positive
34	OP 5854/19	Primary SCC Right Bm	MDSCC	Negative	Negative	Negative	Negative
35	OP 5983/19	Carcinoma of Tongue	WDSCC Advancing Front MDSCC	Negative	Negative	Negative	Negative
36	OP4003/12	Carcinoma	WDSCC	Negative	Negative	Negative	Negative
37	OP 6138	Carcinoma of Tongue	WDSCC	Negative	Negative	Negative	Negative
38	OP 6213	Oral Squamous Cell Carcinoma	WDSCC	Negative	Negative	Negative	Negative
39	OP 6385	Carcinoma of Alveolus	MDSCC	Negative	Negative	Negative	Negative
40	OP 6084	Carcinoma	WDSCC	Negative	Negative	Negative	Negative

ANNEXURE- VI

MORPHOMETRIC ANALYSIS OF CELLULAR SEGMENTATION-NORMAL MUCOSA CASES

Sl No.	Area	Perimeter	Circularity	Euler Number	Centroid X	Centroid Y	Ellipse. Orientation	Ellipse Elongation	Convex Area	MaxFeretDiam	Max. Feret Diameter Angle	InscrDisc.Radius
1	187496	187028.3	0.00007	17743	1410.236	899.633	3.639	1.44	3646883	2684.93	159.324	4
2	247102	241066.5	0.00005	20811	1362.927	914.584	21.481	1.396	4443481	3222.622	37.08	4
3	174891	167696.1	0.00008	13942	1397.823	914.539	5.476	1.116	4385641	2951.684	38.687	4
4												
5	370184	150262.1	0.00021	-342	1394.46	965.007	16.468	1.183	4698208	3137.77	37.75	6
6	161691	176425.2	0.00007	19557	805.899	912.868	-72.447	1.348	2983939	2482.39	130.982	3
7	243514	246733.2	0.00005	23048	1301.17	959.973	-8.31	1.318	4857983	3166.336	144.915	3
8	168087	178666.1	0.00007	16691	1353.435	839.062	18.508	1.32	4166967	2931.804	32.378	2
9	186839	187131	0.00007	14780	1350.431	980.956	-23.116	1.378	4608920	3091.258	146.947	3
10	195651	202935	0.00006	21508	1480.098	944.567	14.87	1.196	3808604	2576.316	34.547	3
11	161450	163941.5	0.00008	14820	940.592	968.291	-80.704	1.237	3122869	2447.932	52.536	3
12	176518	180644.5	0.00007	18113	1466.961	1057.209	9.469	1.5	4123452	3000.913	149.701	3
13	81918	86093.74	0.00014	9134	716.272	1048.396	-77.207	2.373	1519079	2135.587	115.905	3
14	147965	153961.5	0.00008	15953	1013.216	963.819	-58.392	1.625	2871901	2640.537	135.706	3
15	158199	168367.4	0.00007	18694	1429.563	1053.487	8.853	1.783	3580403	2844.482	155.579	3
16	127061	145790.4	0.00008	20249	1092.124	1087.068	30.467	1.28	3543097	2726.657	45.357	3
17	171295	186119.7	0.00006	22184	1593.23	889.781	-2.282	1.229	3756845	2560.661	23.572	3
18	140423	148807.2	0.00008	17157	1101.764	1135.331	-69.626	1.449	2658443	2430.685	127.747	3
19	250938	264671.5	0.00005	20113	1189.655	875.926	-22.438	1.463	4236948	3171.797	142.223	2
20	187868	193118.1	0.00006	18273	1243.531	864.878	-12.747	1.555	4459173	3182.04	142.842	3
21	135332	141499.9	0.00008	13849	1164.464	729.208	-13.186	1.876	4178878	3215.832	142.874	3
22	202011	212509.1	0.00006	21356	1279.263	825.411	-4.088	1.617	4309468	2995.378	149.883	3
23	202011	212509.1	0.00006	21356	1279.263	825.411	-4.088	1.617	4309468	2995.378	149.883	3
24	142545	169285.3	0.00006	24094	1620.837	946.166	27.964	1.204	3915142	2640.28	40.823	2
25	136216	161615.5	0.00007	25074	1593.006	898.482	39.461	1.257	4413136	3160.026	34.922	3
26	114906	129354.3	0.00009	15057	1454.496	855.672	4.214	1.24	3207007	2310.914	144.742	2
27	124940	131927.5	0.00009	14268	890.667	921.624	84.423	1.139	3177212	2402.738	130.392	3
28	161499	183911.8	0.00006	24542	1301.471	833.611	-10.008	1.775	4200795	3075.639	145.84	3
29	161499	183911.8	0.00006	24542	1301.471	833.611	-10.008	1.775	4200795	3075.639	145.84	3
30	188829	202499.3	0.00006	23618	1456.653	852.061	21.147	1.535	4376071	3137.862	34.338	3
31	220111	234036.2	0.00005	26475	1459.079	877.263	22.11	1.455	4321310	3198.604	36.777	3
32	189036	190880.1	0.00007	16456	1118.88	870.756	-17.813	1.523	4361062	3050.472	148.144	2
33	208175	209279.5	0.00006	19591	1119.487	908.707	-9.864	1.06	4018812	2664.739	45.943	3
34	157192	155620.2	0.00008	14627	1280.687	986.25	10.58	1.175	4505902	2981.169	40.674	3
35	163815	164856	0.00008	16170	1227.874	915.633	-24.468	1.352	4230738	2864.665	148.213	3

36	139869	146393.9	0.00008	15331	1201.106	861.323	-28.809	1.569	4093877	2981.592	145.425	2
37	130457	152797.2	0.00007	21246	1157.562	1174.407	19.405	1.674	4138162	3013.603	30.709	2
38	140574	167519.5	0.00006	23039	1103.713	1075.754	18.891	1.438	5027068	3199.439	144.079	3
39	170793	187439.7	0.00006	23974	1086.041	1092.613	13.279	1.345	3846943	2644.678	24.84	2
40	162927	170553.2	0.00007	16414	1191.935	787.011	-12.42	1.669	4196234	3188.912	144.341	3
41	149294	156100.2	0.00008	15852	1084.473	767.506	-21.633	1.633	3930731	3124.387	146.025	3
42	166528	170065.1	0.00007	15845	1021.042	876.781	-26.773	1.542	4183615	3175.751	142.279	2
43	151533	156989.5	0.00008	15874	1113.281	774.795	-24.812	1.705	4736392	3203.551	143.978	3
44	192369	196266.8	0.00006	20257	1197.658	956.326	-21.561	1.252	4577465	2881.61	36.178	3
45	154696	162764.1	0.00007	15898	1140.062	978.412	-33.091	1.36	3689516	2622.318	135.958	3
46	151533	156989.5	0.00008	15874	1113.281	774.795	-24.812	1.705	4736392	3203.551	143.978	3
47	192369	196266.8	0.00006	20257	1197.658	956.326	-21.561	1.252	4577465	2881.61	36.178	3
48	154696	162764.1	0.00007	15898	1140.062	978.412	-33.091	1.36	3689516	2622.318	135.958	3
49	198629	195339.3	0.00007	18030	1569.513	966.176	-7.676	1.317	3810952	2753.702	157.225	3
50	191660	197104.5	0.00006	13741	1429.954	997.439	-5.097	1.232	4489421	2886.884	39.518	3
51	201841	218988.3	0.00005	14810	1398.502	996.149	-8.028	1.317	4654365	2974.72	37.941	3
52	140787	142078.5	0.00009	11766	1507.194	1147.926	0.151	1.561	3130947	2680.349	161.646	3
53	195926	200886	0.00006	18961	1321.032	988.572	6.192	1.378	4445229	2834.613	39.029	3
54	166674	170277.2	0.00007	16962	1457.308	1180.148	-16.713	1.813	3640661	3146.348	145.436	3
55	195862	192343.6	0.00007	17204	1396.594	1080.873	-16.817	1.604	4313017	3189.418	143.324	3
56	169250	167840.9	0.00008	13980	1095.565	1127.057	-5.356	1.268	4402970	3059.164	147.848	2
57	160545	159425	0.00008	12458	1363.108	1062.691	13.806	1.415	3915122	3061.504	37.342	3
58	136718	139495.6	0.00009	13151	1573.091	1041.212	-33.956	1.284	4094527	2741.46	134.867	3
59	142317	161356.1	0.00007	15462	1599.194	1112.924	-19.266	1.536	4325180	3194.167	144.21	3
60	140596	159792.6	0.00007	15638	1602.264	1100.377	-19.44	1.541	3974466	2803.627	157.542	3
61	182415	197033.2	0.00006	19610	1422.14	1119.733	-16.953	1.619	4334488	3196.67	143.51	3
62	167360	175241.1	0.00007	18985	1419.509	950.591	-29.977	1.327	4027669	2963.763	139.036	3
63	138161	155541.1	0.00007	19477	975.96	845.275	-31.781	1.491	3600756	2993.838	142.15	2
64	161714	177652.9	0.00006	23318	1200.408	871.679	-27.529	1.802	4832812	3192.833	143.392	3
65	172462	193017.4	0.00006	14867	1093.573	886.336	-18.101	1.307	4746834	3145.632	143.389	2
66	142814	159361.7	0.00007	18396	944.942	775.196	-33.071	1.355	3188576	2521.646	155.742	3
67	172462	193017.4	0.00006	14867	1093.573	886.336	-18.101	1.307	4746834	3145.632	143.389	2
68	187435	197444.6	0.00006	14551	1141.612	841.235	-14.558	1.468	4458342	3012.582	149.323	3
69	187643	191753	0.00006	16427	1164.576	846.311	-21.138	1.345	4104440	2975.508	150.549	3
70	157968	169840.7	0.00007	18912	1428.722	856.944	26.721	1.6	4191643	3195.206	36.755	2
71	155176	178052.7	0.00006	23281	1559.54	949.372	34.995	1.463	3839178	2732.937	43.725	2
72	186565	199392.5	0.00006	22993	1560.372	919.297	71.627	1.053	4172381	2869.866	42.585	3
73	170608	182623.8	0.00006	21870	1391.302	904.14	28.407	1.615	4498641	3139.82	36.191	3
74	179315	182181	0.00007	16964	1363.769	994.948	18.73	1.07	4392589	3071.733	37.713	3
75	220200	216784.9	0.00006	18351	1227.962	926.73	3.237	1.434	4747832	3026.359	145.664	3
76	163258	168766.7	0.00007	17885	1268.069	1001.979	18.422	1.255	4746365	3204.293	36.434	3
77	201275	207783.4	0.00006	18821	1326.929	995.913	-33.342	2.185	3394316	3202.963	143.992	3
78	157907	168118.3	0.00007	16414	846.08	976.779	63.683	1.392	3162399	2420.823	53.381	3

ANNEXURE- VII

MORPHOMETRIC ANALYSIS OF CELLULAR SEGMENTATION- MILD DYSPLASIA CASES

Sl No.	Area	Perimeter	Circularity	Euler Number	Centroid X	Centroid Y	Ellipse. Orientation	Ellipse Elongation	Convex Area	MaxFeretDiam	Max. Feret Diameter Angle	InscrDisc.Radius
1	154587	165136.3	0.00007	16629	1294.475	1219.772	11.332	1.489	3154814	2641.297	168.801	2
2	173473	169787.5	0.00008	14180	1277.06	1141.572	27.784	2.07	3176909	2968.772	32.566	3
3	167230	168386.5	0.00007	14961	1528.472	1089.701	27.391	1.422	3029551	2535.203	30.429	3
4	180069	195214.8	0.00006	22936	1504.901	1071.932	-17.469	1.377	4014831	3171.708	143.603	2
5	247554	268481.8	0.00004	31805	1398.709	954.367	1.2	1.251	4524931	2901.011	34.736	3
6	180859	199377.6	0.00006	24827	1435.045	922.198	7.868	1.189	4065940	2654.289	151.562	3
7	137582	159116.4	0.00007	21143	1331.787	860.518	24.167	1.268	4346031	2956.316	41.064	3
8	134482	158087.7	0.00007	23103	1141.046	873.571	-21.886	1.78	4150912	3055.762	147.985	2
9	215041	225446.9	0.00005	24035	1196.616	923.757	-14.476	1.367	4631104	3156.013	142.001	3
10	170621	181637.7	0.00006	16314	917.233	842.61	-37.879	1.357	3716540	2801.571	146.679	3
11	194571	203838.4	0.00006	21873	1021.638	850.259	-32.039	1.386	3952592	3153.031	142.876	3
12	165270	177405.4	0.00007	19615	1071.445	843.032	-27.807	1.509	5021125	3184.837	35.556	2
13	142721	155093.3	0.00007	17857	971.948	983.612	-62.479	1.227	3728007	2770.809	43.406	3
14	220284	233459.2	0.00005	24543	1234.752	905.862	3.663	1.544	4531677	3174.403	144.708	3
15	137053	151601.9	0.00007	16324	1043.699	871.542	-20.028	1.775	3885142	2979.309	146.486	3
16	191926	216064.1	0.00005	30585	1084.766	1043.986	15.751	1.365	4282518	2809.644	33.238	3
17	229340	232879.9	0.00005	22371	1190.372	1021.906	11.03	1.39	4289161	2923.8	36.839	3
18	237934	248894.4	0.00005	26085	1349.674	925.048	2.916	1.437	4677842	3137.59	35.164	3
19	175237	181578.6	0.00007	18072	1410.725	669.378	12.213	1.819	3616306	2858.011	25.713	3
20	222432	214635.2	0.00006	18234	1032.924	933.697	-25.739	1.196	4200731	3201.051	142.808	3
21	191552	180742.5	0.00007	13146	1069.545	762.769	-22.29	1.391	3680318	3073.034	141.262	3
22	237934	248894.4	0.00005	26085	1349.674	925.048	2.916	1.437	4677842	3137.59	35.164	3
23	175237	181578.6	0.00007	18072	1410.725	669.378	12.213	1.819	3616306	2858.011	25.713	3
24	222432	214635.2	0.00006	18234	1032.924	933.697	-25.739	1.196	4200731	3201.051	142.808	3
25	191552	180742.5	0.00007	13146	1069.545	762.769	-22.29	1.391	3680318	3073.034	141.262	3
26	157231	167511.9	0.00007	18126	1287.316	1292.59	-5.243	1.924	3724932	2947.424	151.531	3
27	159212	157362.1	0.00008	13668	942.549	1111.849	28.885	1.332	3991843	3001.56	37.977	2
28	223328	216604.5	0.00006	17904	1050.399	986.127	21.963	1.174	3926391	2779.044	29.111	3
29	194099	191692.7	0.00007	15974	986.629	1065.439	33.827	1.408	3694424	2842.617	42.762	3
30	166783	183828.8	0.00006	21705	1108.931	1088.926	14.462	1.482	4339208	2987.619	39.69	3
31	181542	189424.1	0.00006	19035	1260.349	1063.146	-1.43	1.524	4564147	3149.756	34.654	3
32	124667	133219.4	0.00009	11844	1315.541	1191.846	-5.859	1.401	4795640	3140.686	145.586	2
33	161251	166498.9	0.00007	16185	1707.711	1045.481	-46.285	1.404	3515394	2687.101	134.518	3
34	133288	140154.8	0.00009	13738	1213.15	1211.665	9.893	1.235	4005481	2941.722	151.736	2
35	161251	166498.9	0.00007	16185	1707.711	1045.481	-46.285	1.404	3515394	2687.101	134.518	3
36	167602	175049.2	0.00007	18987	1498.67	1140.907	-25.633	1.6	3912227	3191.253	143.46	2
37	231068	245929.3	0.00005	29285	1345.264	1040.585	-11.65	1.256	4552609	3124.913	141.601	3
38	185745	186896.2	0.00007	17807	1155.854	997.041	26.03	1.235	4247343	2940.649	39.564	2
39	140845	151485	0.00008	17537	1188.694	1034.958	14.461	1.371	4434141	2828.979	27.471	3
40	165958	172313.7	0.00007	19620	1139.941	975.722	-1.56	1.218	4349202	2755.795	28.667	3
41	140967	142399.6	0.00009	10948	1185.981	894.527	46.994	1.772	2998560	2828.184	41.23	2

42	150440	165203.2	0.00007	18707	1142.491	1237.043	14.943	1.576	3703662	2898.585	30.52	3
43	166194	189550.6	0.00006	26966	1060.585	932.481	-7.107	1.328	4947891	3155.907	35.352	3
44	181708	202328.9	0.00006	26010	1211.798	979.495	-16.151	1.446	4627006	3185.605	143.026	3
45	174291	172645.9	0.00007	14630	947.211	956.661	-63.927	1.113	3484714	2581.392	132.111	3
46	138198	140930.2	0.00009	11676	645.307	880.019	-71.086	1.463	2429053	2244.402	120.942	3
47	224259	239812.1	0.00005	24308	1256.894	982.815	-29.384	1.184	3845927	2772.205	142.15	3
48	175334	195023.8	0.00006	22566	1124.608	966.262	-66.245	1.29	3461851	2791.284	135.885	3
49	217535	235749.9	0.00005	25323	1174.169	908.892	-33.568	1.295	3938133	2922.273	139.316	3
50	144722	163516.6	0.00007	20301	1177.984	987.471	-71.632	1.446	2843569	2478.347	131.401	3
51	195820	211176	0.00006	26148	1549.513	1003.729	-38.861	1.331	4143746	3179.458	142.784	2
52	109836	119782.1	0.0001	12921	1238.808	1322.87	3.616	1.555	3045225	2791.931	21.87	2
53	128232	152369.2	0.00007	24056	1084.423	980.871	39.004	1.079	3897760	2734.389	45.133	3
54	156083	173739	0.00006	22757	1131.099	902.014	-25.271	1.384	3893375	2969.822	141.123	3
55	145864	159579.8	0.00007	20750	1001.874	895.158	3.368	1.168	3399072	2422.641	22.882	3
56	128232	152369.2	0.00007	24056	1084.423	980.871	39.004	1.079	3897760	2734.389	45.133	3
57	156083	173739	0.00006	22757	1131.099	902.014	-25.271	1.384	3893375	2969.822	141.123	3
58	145864	159579.8	0.00007	20750	1001.874	895.158	3.368	1.168	3399072	2422.641	22.882	3
59	192422	208577.6	0.00006	26255	1231.193	889.888	-5.226	1.419	4432400	2962.326	28.996	3

MORPHOMETRIC ANALYSIS OF CELLULAR SEGMENTATION- MODERATE DYSPLASIA CASES

Sl No.	Area	Perimeter	Circularity	Euler Number	Centroid X	Centroid Y	Ellipse. Orientation	Ellipse Elongation	Convex Area	MaxFeretDiam	Max. Feret Diameter Angle	InscrDisc.Radius
138	164670	176891	0.00007	22044	1370.75	1060.28	-24.26	1.613	4306798	3153.17	145.257	3
139	147318	162575	0.00007	18507	1208.21	1004.93	-38.048	1.693	3686645	3193.18	142.52	3
140	149085	162263	0.00007	20769	1436.36	1054.35	-28.173	1.557	4024736	3154.86	144.3	3
141	176901	191922	0.00006	24383	1319.19	942.035	0.537	1.377	4993598	3147.02	37.07	3
142	150755	159714	0.00007	19079	1119.26	924.993	-6.222	1.089	4131073	2893.44	137.816	3
143	128216	136216	0.00009	13633	1422.68	1028.08	82.656	1.221	3012589	2339.49	125.694	3
144	194468	197874	0.00006	21021	1493.68	1103.83	-19.38	1.575	3907630	2975.87	139.921	3
145	223077	226569	0.00005	23403	1387.2	1010.14	-26.317	1.41	4379104	3112.7	145.233	3
146	191954	187354	0.00007	16389	1466.75	1126.88	-20.46	1.449	3873856	2991.21	148.047	3
147	192118	187767	0.00007	16040	1404.91	1029.85	-20.579	1.334	4269228	2815.14	146.005	3
148	196435	197207	0.00006	17540	1380.28	1002.45	-13.665	1.297	4513593	3066.95	147.652	3
149	186532	187384	0.00007	17949	1484.31	972.894	-12.604	1.119	4079391	2766.05	43.271	3
150	206233	197169	0.00007	15311	1644.31	920.355	51.686	1.13	3802222	2797.63	42.61	2
151	174870	158463	0.00009	10496	1501.2	876.967	39.909	1.744	3455448	3094.92	38.888	3
152	197475	188344	0.00007	12839	1373.31	982.998	46.991	1.28	3883460	3187.6	36.812	3
153	227423	220021	0.00006	15195	1402.08	935.815	30.769	1.461	4083415	3050.76	39.56	3
154	180106	169676	0.00008	11563	1570.81	911.609	41.541	1.415	3589462	2997.06	38.239	3
155	195738	194548	0.00007	16508	1483.24	985.186	2.835	1.149	4336700	2842.69	43.118	3
156	193743	206483	0.00006	25626	1386.97	973.461	-17.067	1.203	4410352	3028.46	140.09	3
157	158337	167648	0.00007	20016	1580.73	1077.9	-55.811	1.112	3440849	2554.89	145.557	3
158	147103	153312	0.00008	15298	1652.31	1013.56	-11.362	1.043	3611930	2568.21	43.659	3
159	245367	244973	0.00005	23477	1382.63	993.456	-17.633	1.234	4594263	3139.39	144.411	3
160	190941	197593	0.00006	22482	1295.42	997.752	19.112	1.158	4475943	3015.69	39.2	2
161	192570	204044	0.00006	23482	1401.66	1006.14	-22.152	1.268	4132976	2872.97	141.359	3
162	192570	204044	0.00006	23482	1401.66	1006.14	-22.152	1.268	4132976	2872.97	141.359	3
163	143753	167173	0.00006	23735	1099.1	950.915	-43.586	1.641	3686728	3177.6	143.188	2
164	165427	186710	0.00006	26965	1143.35	943.653	-34.268	1.434	3865166	3148.11	142.096	3
165	148021	163399	0.00007	22022	1094.03	841.488	-28.371	1.631	3920884	3171.17	142.534	2
166	175191	193548	0.00006	27783	1196.6	933.412	-9.649	1.323	4611316	3168.28	142.721	3
167	170748	181153	0.00007	21609	1221.4	863.2	-17.473	1.32	4120142	2925.91	152.191	3
168	168813	179691	0.00007	19648	1253.01	1053.77	0.973	1.633	4227239	3002.93	30.365	3
169	169220	168992	0.00007	14447	1220.12	985.446	30.725	1.638	4124121	3175.24	36.574	2
170	143152	152168	0.00008	15944	1514.98	991.595	44.149	1.454	3634943	3099.58	38.582	2
171	131664	139189	0.00009	12183	1371.5	994.57	54.769	1.378	3256139	2779.14	43.527	2
172	176778	178600	0.00007	16644	1331.91	949.409	35.444	1.432	4312306	3170.36	35.189	3
173	177379	193729	0.00006	26176	1170.27	1068.79	20.491	1.473	4276491	3181.83	35.728	3
174	177524	185839	0.00006	20602	1151.24	1006.69	15.161	1.297	4456536	3193.55	37.43	3
175	186947	195310	0.00006	22464	1189.57	986.221	7.774	1.321	4613760	3219.44	36.589	2
176	150749	165658	0.00007	19766	1173.36	1116.54	16.321	1.609	4157692	3168.98	37.474	3
177	156640	168656	0.00007	22001	911.777	1015.07	66.145	1.109	3536069	2600.6	43.8	3
178	155625	165597	0.00007	21063	1172.03	998.604	19.701	1.223	4379179	2970.15	38.946	3

Sl No.	Area	Perimeter	Circularity	Euler Number	Centroid X	Centroid Y	Ellipse. Orientation	Ellipse Elongation	Convex Area	MaxFeretDiam	Max. Feret Diameter Angle	InscrDisc.Radius
179	168747	173952	0.00007	18678	1325.85	986.469	1.66	1.335	4907314	3195	143.04	2
180	188208	188823	0.00007	17777	1198.59	928.536	-26.912	1.471	4417570	3128.51	144.786	2
181	164100	164927	0.00008	14406	1147.78	972.778	-8.695	1.215	4267119	2805.24	138.685	3
182	155257	168968	0.00007	21336	1369.58	977.837	1.115	1.255	4725296	3062.34	142.056	3
183	139138	162659	0.00007	24013	1039.23	987.79	27.111	1.048	3942153	2850.36	140.266	3
184	169548	171553	0.00007	17077	1216.72	991.018	19.197	1.3	4114716	2793.2	41.313	3
185	197419	207778	0.00006	22511	1277.57	859.927	-0.188	1.549	4471766	3068.02	147.62	2
186	182749	190795	0.00006	21206	1235.95	861.284	-0.699	1.508	4413724	3042.06	148.4	2
187	175571	186959	0.00006	20675	1412	774.119	7.069	1.607	3931724	2942.2	28.281	2
188	127071	135362	0.00009	10771	1233.65	863.813	20.524	2.828	2898026	3090.3	34.065	2
189	201720	208362	0.00006	22998	1435.77	848.533	26.605	1.4	4393585	3206.61	36.759	3
190	236674	237289	0.00005	21039	1253.25	861.96	-10.779	1.656	4444105	3214.65	142.813	2
191	163033	183518	0.00006	22415	981.476	953.319	-5.182	1.155	4179962	2960.69	139.164	3
192	105499	115434	0.00010	12892	1052.71	885.072	-49.61	2.204	2541966	2804.98	137.167	3
193	206185	214011	0.00006	21829	1188.71	917.867	-0.247	1.367	4376729	2824.13	148.657	3
194	182058	193863	0.00006	19844	1099.56	1026.59	11.967	1.179	4238473	2938.15	41.191	3
195	160237	179472	0.00006	23240	1349.17	1029.21	-24.442	1.692	4329254	3202.96	143.992	3

MORPHOMETRIC ANALYSIS OF CELLULAR SEGMENTATION- SEVERE DYSPLASIA CASES

Sl No.	Area	Perimeter	Circularity	Euler Number	Centroid X	Centroid Y	Ellipse. Orientation	Ellipse Elongation	Convex Area	MaxFeretDiam	Max. Feret Diameter Angle	InscrDisc.Radius
1	557917	226990	0.00014	-696	1240.42	989.643	-9.092	1.208	4703169	3081.99	141.68	5
2	244181	244562	0.00005	22989	1245.8	973.124	0.639	1.188	4707503	3140.22	37.808	3
3	364018	157111	0.00019	-433	1124.79	908.339	17.294	1.346	4082023	2727.55	142.538	5
4	166549	173862	0.00007	18296	1029.6	1081.11	85.536	1.096	3551543	2541.68	49.58	3
5	158825	172040	0.00007	18788	926.074	1089.98	25.773	1.323	3809622	2755.54	36.292	3
6	131506	143254	0.00008	16324	929.569	992.999	28.228	1.384	3378513	2519.35	37.825	3
7	190283	204313	0.00006	22990	1039.51	882.952	-34.014	1.359	3959343	3060.02	140.583	3
8	237847	243952	0.00005	24180	1240.62	925.014	-11.119	1.305	4657979	3207.63	143.366	3
9	195252	210299	0.00006	21497	1217.53	924.11	9.464	1.285	4551860	3047.88	144.912	3
10	190029	199696	0.00006	21431	1145.36	819.72	-5.436	1.501	3624718	2855.52	145.631	3
11	174020	180651	0.00007	17968	1159.59	931.987	16.466	1.892	3767320	2820.97	36.248	3
12	206848	215661	0.00006	23630	1363.56	1156.2	-5.445	1.698	4265607	3121.88	145.506	3
13	258539	265120	0.00005	27188	1328.2	1048.18	-5.822	1.508	4729219	3157.74	145.137	3
14	275336	276764	0.00005	27063	1329.25	1039.1	-4.803	1.527	4684457	3134.48	145.752	3
15	249847	249235	0.00005	24081	1290.2	1136.79	1.857	1.617	4491835	3011.05	30.628	3
16	254232	251746	0.00005	21417	1238.91	1175.73	2.347	1.616	4045052	2958.46	28.861	3
17	241736	239642	0.00005	21051	1225.12	1118.2	7.558	1.48	4744517	3183.48	36.107	3
18	154880	171180	0.00007	24133	1079.33	862.58	-20.755	1.417	3831990	2818.47	153.99	3

Sl No.	Area	Perimeter	Circularity	Euler Number	Centroid X	Centroid Y	Ellipse. Orientation	Ellipse Elongation	Convex Area	MaxFeretDiam	Max. Feret Diameter Angle	InscrDisc.Radius
19	192579	206828	0.00006	26435	1095.68	879.648	-25.831	1.44	4054870	3146.69	142.697	3
20	214683	232979	0.00005	29346	1221.88	871.399	-2.871	1.421	4479947	3217.42	143.34	3
21	248016	248331	0.00005	25208	1210.25	944.552	-23.998	1.381	4482662	3217.13	143.647	3
22	158095	176307	0.00006	25931	1060.89	855.016	-30.096	1.453	3903523	2959.38	148.415	2
23	236326	246443	0.00005	22769	1078.56	914.206	-28.035	1.399	4160945	3191.83	144.268	2
24	182302	199317	0.00006	23782	1104.99	1028.61	22.279	1.433	4559487	3131.22	36.193	3
25	171444	185041	0.00006	22266	997.757	906.29	-24.941	1.219	3905158	2742.24	135.443	3
26	167402	173849	0.00007	20274	1178.13	1060.56	16.248	1.882	3951750	3160.74	37.932	3
27	176753	187022	0.00006	21049	1210.44	1084.65	19.246	1.801	4117689	3172.57	37.469	3
28	160068	166557	0.00007	16847	1134.44	1197.23	10.536	2.063	3681292	3028.07	32.635	2
29	127061	133679	0.00009	16471	1270.2	1111.55	6.446	1.965	3875708	3020.93	32.564	3
30	206321	208003	0.00006	21656	1084.75	968.747	-25.863	1.187	4175715	2801.13	143.682	3
31	171606	178970	0.00007	20260	1241	859.388	-23.419	1.402	4378426	3125.32	142.305	3
32	185177	198188	0.00006	23326	1307.6	1057.84	16.417	1.547	4352355	3034.36	34.256	3
33	174124	181972	0.00007	21709	1251.89	917.59	-27.476	1.47	4352168	3207.67	143.877	3
34	195153	207253	0.00006	25413	1257.54	828.275	-13.26	1.525	4444373	3044.6	140.344	3
35	207360	216228	0.00006	23998	1194.27	999.524	7.932	1.212	4832963	3195.11	144.156	3
36	208947	213209	0.00006	22180	1061.64	1058.49	16.957	1.223	4643845	3188.37	143.712	3
37	165324	177584	0.00007	21092	1514.88	1023.58	-2.064	1.094	4125594	2697.29	45.961	2
38	244773	260112	0.00005	31740	1184.24	1099.33	20.771	1.545	4442700	3219.5	36.411	3
39	229800	247621	0.00005	30353	1041.67	1048.67	24.846	1.26	4379828	3211.21	36.21	2
40	158910	179043	0.00006	23256	914.37	1116.18	37.242	1.443	3230527	2813.67	42.09	3
41	220987	231016	0.00005	24890	1197.93	994.211	26.152	1.376	4741809	3193	35.761	3
42	225019	238835	0.00005	26377	1238.24	1089.38	17.235	1.452	4476609	3163.46	35.011	3
43	223719	221670	0.00006	19832	1078.15	1007.77	28.799	1.14	4119516	2760.22	39.885	3
44	139687	147464	0.00008	16731	794.113	997.246	86.126	1.309	3219416	2560.11	49.372	2
45	185382	198070	0.00006	22091	1402.64	1016.8	4.027	1.113	4347879	2858.36	40.175	3
46	171320	187998	0.00006	20532	1630.41	891.934	26.491	1.306	4264183	3202.96	36.008	3
47	184311	203278	0.00006	22356	1645.23	956.991	32.245	1.296	4252972	3194.73	36.048	3
48	173875	178022	0.00007	17739	1583.82	1013.24	-23.818	1.126	3880013	2843.42	136.895	3
49	188580	187003	0.00007	16843	1518.86	1169.71	-20.412	1.56	3866667	2944.57	151.633	3
50	206798	214313	0.00006	19150	1163.42	922.638	-5.929	1.277	4832600	3181.29	142.356	3
51	207953	208539	0.00006	18173	1168.46	824.574	-12.964	1.417	4963133	3173.06	37.599	3
52	144062	152135	0.00008	11184	970.476	867.061	52.305	1.079	4233226	2851.42	141.321	3
53	241027	238765	0.00005	22924	1101.9	933.065	-20.525	1.198	4387894	2892.77	139.388	3
54	193679	198505	0.00006	22013	1097.13	796.997	-19.066	1.499	4007542	3059.29	141.223	3
55	183031	190656	0.00006	21278	1229.16	884.955	-15.513	1.527	4370009	3149.8	144.837	3
56	195037	202792	0.00006	22753	1184.51	911.759	-18.798	1.063	4092261	2797.75	136.014	2
57	194399	194942	0.00006	18403	1125.34	1053.67	26.832	1.329	4201889	3031.96	39.486	3
58	191055	202501	0.00006	22413	1158.1	1042.83	27.947	1.282	4668408	3133.21	38.326	2

ANNEXURE- VIII

MORPHOMETRIC ANALYSIS OF CELLULAR SEGMENTATION- SURGICAL MARGINS IN OSCC CASES

RND cases	Area	Perimeter	Circularity	EulerNumber	Centroid.X	Centroid.Y	Ellipse.Orientation	Ellipse.Elong	ConvexArea	MaxFeretDiam	MaxFeretDiamAngle	InscrDisc.Radius
OP3415												
Anterior M	209612	219957	5.44E-05	24091	1321.927	874.378	-2.932	1.299	4092530	2757.502	24.511	3
Lateral M	163258	168766.7	7.20E-05	17885	1268.069	1001.979	18.422	1.255	4746365	3204.293	36.434	3
OP3633												
Anterior M	172614	184572.5	6.37E-05	21801	1015.352	983.3	37.89	1.048	4204137	2927.943	139.127	3
Posterior M	201275	207783.4	5.86E-05	18821	1326.929	995.913	-33.342	2.185	3394316	3202.963	143.992	3
Lateral M	157907	168118.3	7.02E-05	16414	846.08	976.779	63.683	1.392	3162399	2420.823	53.381	3
OP3765												
Posterior M	199841	211642.4	5.61E-05	19947	1307.545	889.45	6.486	1.587	4249980	3145.781	34.549	3
Medial M	197102	204478.9	5.92E-05	22578	1441.12	1052.88	-27.594	1.425	4364898	3209.075	142.737	3
OP3814												
Medial M	192422	208577.6	5.56E-05	26255	1231.193	889.888	-5.226	1.419	4432400	2962.326	28.996	3
Lateral M	177537	182674.6	6.69E-05	21496	1360.137	960.894	-6.837	1.216	4445417	2809.026	142.885	3
OP3840												
Anterior M	193829	201090.4	6.02E-05	21008	1375.285	1025.219	5.747	1.165	4519121	2964.519	139.049	3
Posterior M	175139	186202.7	6.35E-05	18974	1578.227	1060.883	-66.832	1.122	3828092	2682.889	140.763	3
OP3986												
Anterior M	142624	157602.2	7.22E-05	17554	1331.475	985.625	38.526	1.894	3630063	3177.725	36.361	2
Posterior M	191069	207288.6	5.59E-05	23644	1288.257	975.099	0.495	1.259	4712591	3017.736	139.92	3
Medial M	205814	211415	5.79E-05	19874	1368.982	937.256	-14.509	1.458	4687010	3195.426	143.36	3
Lateral M	101599	111370	1.03E-04	10895	1220.943	715.416	2.472	2.39	2789356	2754.543	160.157	3
OP4010												
Anterior M	160319	181466.1	6.12E-05	21896	1416.27	1079.239	-23.467	1.456	4210734	3180.569	143.721	3
Posterior M	219792	241261.2	4.75E-05	29215	1405.166	1038.778	-11.843	1.401	4636217	3200.004	143.041	3
Medial M	222129	244617.6	4.67E-05	30648	1327.798	973.277	1.255	1.271	5020329	3173.378	142.245	3
OP4077												
Anterior M	222129	244617.6	4.67E-05	30648	1327.798	973.277	1.255	1.271	5020329	3173.378	142.245	3
Posterior M	159240	168141.6	7.08E-05	18963	1288.296	1019.492	8.458	1.167	3969498	2698.516	44.309	3
Medial M	150032	150573.5	8.32E-05	14326	1357.67	959.694	36.87	1.796	3971288	3183.006	36.762	3
Lateral M	161704	170444.5	7.00E-05	19502	1341.762	1047.075	-29.915	1.459	4215639	3155.803	142.48	2
OP4152												
Anterior M	164708	174544.7	6.79E-05	20555	1202.584	916.34	-36.513	1.852	3584685	3166.251	144.292	3
medial M	201250	207456.3	5.88E-05	19755	1319.092	862.232	-5.251	1.483	4489904	2960.873	151.054	3
Lateral M	168745	187524.1	6.03E-05	22754	1189.8	926.338	-6.316	1.295	4561057	2911.506	27.137	3
OP4256												
Anterior M	105111	115884.3	9.84E-05	11101	1420.974	958.803	-82.295	1.591	3191761	2599.408	48.306	3
Posterior M	138060	146663.3	8.07E-05	16118	1560.85	996.695	85.927	1.324	3255435	2471.434	132.573	3
Lateral M	138060	146663.3	8.07E-05	16118	1560.85	996.695	85.927	1.324	3255435	2471.434	132.573	3
OP4355												
Anterior M	96627	108047	1.04E-04	13680	1523.279	929.572	-70.205	2.06	3045298	2453.972	50.407	3

RND cases	Area	Perimeter	Circularity	EulerNumber	Centroid.X	Centroid.Y	Ellipse.Orientation	Ellipse.Elong	ConvexArea	MaxFeretDiam	MaxFeretDiamAngle	InscrDisc.Radius
Posterior M	93345	103516.1	1.10E-04	11762	780.486	992.341	-85.014	1.654	2406952	2261.905	120.794	2
Medial M	119811	128452.2	9.13E-05	12788	1178.905	942.7	55.456	1.726	2636286	2681.663	45.876	3
OP4364												
Anterior M	181658	185984.5	6.60E-05	21072	1233.889	1040.197	28.826	1.314	4232989	3179.888	37.641	3
Posterior M	184435	207672.6	5.37E-05	28518	1239.452	926.109	-22.29	1.525	4333296	3201.286	143.549	2
Medial M	233606	246319.9	4.84E-05	28346	1307.469	915.331	1.903	1.501	4860216	3167.896	143.575	3
Lateral M	245588	245579.1	5.12E-05	24290	1325.459	982.732	-10.265	1.381	4881927	3203.056	142.79	3
OP4585												
Medial M	99020	111813.9	9.95E-05	13275	1083.428	990.226	24.888	1.94	2738826	2752.746	41.333	3
Lateral M	212945	222724.2	5.39E-05	22962	1228.233	916.113	-7.392	1.434	4481697	2989.007	150.055	3
OP4624												
Anterior M	182735	202146.3	5.62E-05	26988	1204.123	997.103	-37.067	1.575	3950476	3125.905	143.599	3
Posterior M	218303	233650.4	5.03E-05	26371	1172.977	866.95	-29.399	1.707	3888498	3165.626	143.814	3
Medial M	179228	190390.8	6.21E-05	18822	1228.022	980.504	-49.974	1.754	3586160	3088.359	141.56	3
Lateral M	158550	179663.8	6.17E-05	24393	1140.589	960.037	-38.153	1.7	3377827	3155.225	141.99	3
OP4634												
Anterior M	188780	215570.7	5.11E-05	24795	1203.364	1049.7	-7.129	1.331	4912026	3191.171	142.538	3
Posterior M	206878	233833	4.76E-05	27748	1315.733	996.988	-5.61	1.402	5026806	3233.807	36.753	3
OP4640												
Anterior M	133557	141628.1	8.37E-05	18970	1312.439	1008.704	-27.274	1.784	3908381	3094.535	146.854	3
Posterior M	110882	124101.4	9.05E-05	11665	1008.786	924.611	83.011	1.353	2933298	2467.527	51.946	2
Lateral M	163673	186929.3	5.89E-05	20711	1300.655	1007.233	13.398	1.282	4852815	3080.396	147.259	3
OP4759												
Posterior M	138834	156640.7	7.11E-05	16290	1563.027	992.887	-73.243	1.345	2919920	2538.097	130.045	3
OP4771												
Posterior M	119959	131711.8	8.69E-05	13686	1211.702	961.671	-1.312	2.567	2683171	2741.223	160.944	3
Medial M	185992	202646.9	5.69E-05	21070	1187.372	917.267	-21.657	1.379	4838947	3149.469	143.509	2
Lateral M	179882	188586.2	6.36E-05	17043	1134.783	960.403	-5.779	1.229	4625697	3169.212	144.841	3
OP4803												
Anterior M	231340	248067.8	4.72E-05	25320	1249.462	987.488	13.568	1.365	4772416	3170.364	35.189	3
Posterior M	197647	213397.7	5.45E-05	21014	1433.67	1039.421	-6.263	1.238	4741854	3121.542	36.323	3
Medial M	171943	190234.6	5.97E-05	22811	1237.048	974.556	-16.489	1.464	4978555	3207.484	142.716	3
Lateral M	188314	202158.1	5.79E-05	20994	1217.214	1000.371	12.612	1.402	5015270	3174.124	36.364	3
OP4814												
Medial M	182955	191925.5	6.24E-05	20887	1334.053	969.521	-6.492	1.211	4451813	2930.791	147.91	3
OP4820												
Anterior M	230378	234187.4	5.28E-05	24067	1312.853	983.287	-7.839	1.267	4680540	2966.264	146.412	3
Posterior M	227738	237931.2	5.06E-05	25934	1234.324	964.888	-2.331	1.336	4936231	3106.164	144.222	3
Medial M	243766	255096.6	4.71E-05	28269	1237.114	975.608	-3.061	1.313	4941159	3196.508	144.152	3
Lateral M	214631	218181.2	5.67E-05	21891	1387.742	994.809	2.563	1.165	4749934	3100.852	141.955	3
OP4864												
Posterior M	155687	182268.2	5.89E-05	26591	1195.328	1032.202	-1.723	1.421	4946905	3161.532	37.921	3
Medial M	148472	177941.3	5.89E-05	22751	1210.916	955.262	-14.424	1.261	4982249	3198.266	35.892	3
OP5036												
Anterior M	182371	199139.3	5.78E-05	24621	1083.247	901.599	-30.958	1.532	3983704	2982.516	140.428	3

RND cases	Area	Perimeter	Circularity	EulerNumber	Centroid.X	Centroid.Y	Ellipse.Orientation	Ellipse.Elong	ConvexArea	MaxFeretDiam	MaxFeretDiamAngle	InscrDisc.Radius
Posterior M	197167	211519.6	5.54E-05	26330	1420.162	967.335	1.998	1.305	4881438	3099.05	38.212	3
Lateral M	149891	161690.7	7.21E-05	20010	1287.04	997.127	28.793	2.228	3616451	3166.91	35.1	3
OP5109												
Anterior M	142951	148843.9	8.11E-05	16007	920.091	924.913	-67.254	1.148	3543337	2491.526	132.088	3
Posterior M	138088	146662.3	8.07E-05	16404	1372.257	732.58	12.7	1.851	3606166	2941.249	28.247	3
Lateral M	102552	110107.7	1.06E-04	11268	700.816	916.026	75.298	1.21	2723883	2426.949	50.031	3
OP5250												
Anterior M	185985	188722.7	6.56E-05	19744	1561.463	899.832	48.642	1.328	3675600	2893.265	39.235	3
Posterior M	180053	202251.5	5.53E-05	22899	1311.027	961.273	-16.838	1.236	4589663	3136.841	144.288	3
OP5304												
Medial M	143969	153655.4	7.66E-05	14378	1262.379	994.33	62.456	1.103	3522029	2760.581	44.706	3
Lateral M	175018	185344.7	6.40E-05	17630	1422.206	962.119	8.695	1.142	4069059	2922.704	41.588	3
OP5375												
Posterior M	213991	222026.1	5.46E-05	24969	1291.552	952.736	-1.296	1.362	4858623	3091.343	145.544	3
Lateral M	123709	137079.5	8.27E-05	16019	1298.487	1044.77	-9.32	2.066	3090673	2745.165	160.707	3
OP5420												
Anterior M	168330	180360.4	6.50E-05	20197	1115.99	873.703	-29.177	1.543	4080346	3156.595	145.167	3
Posterior M	180376	186202.9	6.54E-05	18630	1199.515	1011.525	10.481	1.104	4178764	2722.095	41.903	3
Medial M	172097	193209.6	5.79E-05	22757	1164.028	1058.224	15.538	1.336	4312462	2873.665	39.918	3
Lateral M	193495	221874.3	4.94E-05	26355	1253.261	1010.824	7.501	1.374	4801241	3177.333	37.699	3
OP5440												
Anterior M	103354	107370.6	1.13E-04	10301	1301.205	1027.57	62.342	2.102	2002543	2486.756	51.383	3
Medial M	172552	189838.8	6.02E-05	22737	1083.02	919.431	-20.595	1.282	4536667	3190.079	144.312	3
OP5601												
Anterior M	201716	216351	5.42E-05	27367	1335.966	991.986	-3.926	1.351	4731625	2988.001	139.438	3
Posterior M	143087	152321.8	7.75E-05	19092	1001.696	889.468	-52.2	1.412	3300892	2837.335	138.286	3
OP5696												
Anterior M	141583	148936.7	8.02E-05	18561	1135.286	901.595	-33.853	1.773	3652940	3133.357	145.782	2
Posterior M	193915	203078.7	5.91E-05	21352	1432.148	1020.595	-25.861	1.369	4026283	2961.697	139.052	3
Medial M	230946	243570.3	4.89E-05	24679	1434.564	1053.284	-27.736	1.311	4499994	3190.801	142.487	3
Lateral M	204561	217721.4	5.42E-05	25054	1362.683	960.355	-12.444	1.258	4522222	2830.301	156.27	3
OP5745												
Anterior M	183713	197894.8	5.90E-05	23923	1246.197	911.111	-17.959	1.437	4465727	3119.367	146.162	3
Posterior M	155632	168300.8	6.91E-05	19702	1208.7	1013.176	38.354	2.108	3285201	3174.169	37.744	3
Lateral M	144865	164853.3	6.70E-05	25443	1318.143	1049.371	-27.12	1.874	3973797	3172.588	142.234	3
Op5800												
Anterior M	177537	182674.6	6.69E-05	21496	1360.137	960.894	-6.837	1.216	4445417	2809.026	142.885	3
Posterior M	192422	208577.6	5.56E-05	26255	1231.193	889.888	-5.226	1.419	4432400	2962.326	28.996	3
Lateral M	130857	143991.6	7.93E-05	16335	1349.851	761.452	-9.081	2.16	3114750	2630.328	157.608	3
OP5854												
Anterior M	173958	199713.8	5.48E-05	24670	1377.593	928.306	23.913	1.189	4308586	3086.366	39.016	3
Posterior M	157843	168835.8	6.96E-05	18244	1214.166	914.783	-13.055	1.256	4465140	2918.764	144.257	3
Lateral M	102558	110874.4	1.05E-04	12031	2048.467	894.635	80.911	1.79	2029166	2226.06	59.195	3
Op5983												
Anterior M	150181	168723.3	6.63E-05	20908	1173.671	1078.493	18.043	1.555	4380185	3042.058	31.6	3

RND cases	Area	Perimeter	Circularity	EulerNumber	Centroid.X	Centroid.Y	Ellipse.Orientation	Ellipse.Elong	ConvexArea	MaxFeretDiam	MaxFeretDiamAngle	InscrDisc.Radius
Medial M	169157	184862.9	6.22E-05	20460	1171.163	1005.596	24.292	1.762	4029249	3132.795	34.202	2
Lateral M	192400	214852.4	5.24E-05	30959	1264.472	1003.197	-16.067	1.466	4309671	3056.935	140.535	3
OP6048												
Anterior M	138137	142908	8.50E-05	15274	1232.885	985.621	11.867	1.166	4195981	2752.774	135.132	3
Posterior M	174201	193159.2	5.87E-05	22478	1231.551	982.8	10.7	1.248	4893590	3148.139	38.111	3
Medial M	170230	180035.8	6.60E-05	19326	1173.296	946.552	-1.292	1.235	4461877	2988.52	140.349	3
OP6138												
Posterior M	151968	159726.5	7.49E-05	18152	1331.36	985.056	1.84	1.399	4523565	2932.114	35.029	2
Medial M	134878	145469.2	8.01E-05	16345	1011.905	955.876	60.495	1.125	3822182	2791.165	41.514	2
OP6213												
Posterior M	206185	214011.2	5.66E-05	21829	1188.708	917.867	-0.247	1.367	4376729	2824.129	148.657	3
Medial M	126534	135481.8	8.66E-05	16451	1529.174	968.354	-60.309	1.432	3205898	2743.578	134.911	3
Lateral M	202945	226151.8	4.99E-05	29598	1281.728	997.071	4.284	1.254	4935614	3167.091	143.564	3
OP6385												
Anterior M	123521	133055.1	8.77E-05	12318	963.152	990.444	54.445	1.399	3047252	2649.886	47.095	3
Medial	128262	128492.8	9.76E-05	12514	1168.914	950.59	53.495	1.416	3152551	2833.771	41.481	3
Lateral M	108136	116338.4	1.00E-04	11308	829.225	1015.639	83.668	1.268	2790452	2382.044	54.655	3
OP6084												
Anterior M	136403	156425.5	7.01E-05	22276	1007.53	1091.968	-43.014	1.168	3393537	2678.628	134.486	2
Posterior M	63085	70980.16	1.57E-04	8234	1242.124	894.618	-12.5	4.969	1488871	2766.97	159.456	3

ANNEXURE- IX

MORPHOMETRIC ANALYSIS OF NUCLEI DETECTION-NORMAL MUCOSA CASES

sl.no	Total Area	Average Size	%Area	Perim.	Major	Minor	Angle	Circ.	Solidity	Feret	FeretX	FeretY	FeretAngle	MinFeret	IntDen	Skew	Kurt
1	217116	399.11	17.896	75.819	27.589	17.546	115.971	0.839	0.932	28.43	618.29	465.366	113.839	18.346	101765.6	-21.063	488.829
2	248807	428.978	20.809	78.829	28.969	17.883	125.199	0.833	0.932	29.76	610.417	446.648	126.078	18.675	109382.3	-16.792	296.979
3	228852	462.327	19.847	83.032	30.697	18.346	96.868	0.82	0.934	31.41	658.016	409.283	99.505	19.068	117882.1	-18.003	349.378
4	141747	359.764	11.449	71.788	26.113	16.611	92.245	0.846	0.932	26.904	631.832	515.967	97.531	17.221	91735.28	-28.768	866.501
5	244155	501.345	20.273	87.15	32.326	18.533	110.514	0.803	0.931	33.026	549.423	440.427	111.964	19.393	127824.1	-22.795	562.591
6	180959	310.393	14.797	66.369	24.222	15.236	115.117	0.841	0.923	25.03	617.58	471.333	115.389	16.02	79142.29	-20.743	471.034
7	104749	278.588	8.756	62.447	22.516	15.053	83.985	0.863	0.93	23.319	757.949	448.622	84.753	15.581	71036.49	-11.891	139.407
8	155430	507.941	13.392	90.269	31.581	18.992	76.509	0.791	0.918	32.799	689.095	502.608	77.41	20.223	129496.7	-25.551	701.181
9	170798	467.94	15.584	85.148	30.81	18.42	77.164	0.804	0.924	31.82	726.285	472.244	77.161	19.495	119314.8	-26.392	739.902
10	166719	580.902	14.574	94.673	34.151	20.312	92.802	0.797	0.928	35.148	552.028	469.101	95	21.393	148098.1	-22.869	560.237
11	243999	596.575	19.896	95.769	33.518	21.37	108.103	0.809	0.929	34.646	605.91	443.941	107.351	22.537	152104.1	-25.355	694.456
12	278156	609.991	22.88	95.79	34.321	21.438	106.766	0.819	0.935	35.254	658.68	487.072	106.953	22.458	155498.6	-22.221	549.874
13	246785	621.625	19.948	97.149	34.722	21.637	107.915	0.811	0.931	35.732	558.01	451.705	107.74	22.778	158504.7	-28.5	934.314
14	73258	554.985	6.204	89.079	28.558	17.383	90.849	0.796	0.904	30.102	611.295	415.833	92.988	19.032	141159.9	-19.245	484.602
15	75042	721.558	6.156	102.965	31.969	20.264	87.657	0.795	0.907	33.977	614.625	442.827	92.45	22.228	183958	-33.673	1199.134
16	197634	1029.344	16.033	129.502	41.144	26.33	85.044	0.786	0.92	43.143	763.781	512.036	87.833	28.452	262440.2	-37.382	1595.336
17	135012	594.767	11.144	96.528	33.579	19.173	103.915	0.766	0.909	35.044	663.203	469.546	106.345	20.743	151617.2	-29.192	1037.258
18	161297	916.46	13.296	127.342	41.136	23.544	105.071	0.748	0.902	43.45	553.665	434.04	110.465	26.025	233148.2	-29.884	1248.828
19	127257	748.571	10.958	109.433	35.458	20.907	92.826	0.774	0.905	37.513	513.453	506.447	96.239	23.023	190782	-20.357	455.061
20	74987	249.957	7.906	62.449	23.679	12.965	106.213	0.795	0.911	24.374	377.167	507.78	108.711	13.826	63735.55	-14.083	204.672
21	81137	234.5	6.866	59.681	22.926	12.354	108.963	0.792	0.911	23.504	488.607	380.977	112.999	13.045	59796.03	-13.802	188.505
22	74128	250.432	6.067	59.899	22.335	13.336	83.683	0.826	0.917	23.132	666.534	418.625	85.733	14.092	63845.63	-10.888	124.729
23	207867	633.741	16.742	99.562	35.406	21.538	95.306	0.806	0.931	36.467	730.338	457.662	95.153	22.646	161578.3	-24.46	688.733
24	252091	622.447	20.425	100.846	35.475	20.478	87.099	0.777	0.919	36.664	685.706	444.548	87.53	21.828	158699.4	-27.901	914.556
25	212011	571.458	20.134	91.779	31.923	21.321	64.103	0.837	0.934	33.164	546.264	456.348	63.291	22.421	145701.9	-27.016	930.934
26	136896	577.62	12.653	90.674	31.786	21.931	93.551	0.847	0.938	32.813	478.789	452.135	92.929	22.795	147283.5	-28.326	836.863
27	104347	511.505	12.637	86.711	30.823	20.055	93.451	0.831	0.932	31.685	339.848	518.064	98.62	20.907	130422.5	-22.265	566.025
28	173371	452.666	17.983	80.903	28.74	18.916	103.155	0.841	0.931	29.711	542.17	357.64	104.272	19.808	115424.5	-22.809	566.869
29	129651	611.561	11.691	101.14	35.202	19.927	73.806	0.758	0.908	36.716	578.382	431.392	74.424	21.62	155871.2	-25.136	764.203
30	116943	1044.134	26.92	142.507	44.863	25.941	74.636	0.725	0.906	47.416	232.393	485.152	80.527	28.454	265912.6	-19.337	434.671
31	164752	493.269	16.519	87.663	29.987	19.121	87.206	0.805	0.916	31.151	456.284	467.377	87.447	20.449	125756.2	-25.232	680.762
32	109087	441.648	20.534	80.288	28.85	17.819	91.402	0.819	0.927	29.696	239.753	508.814	92.062	18.654	112611.9	-29.483	908.626
33	162594	602.2	13.329	108.732	36.485	18.595	75.38	0.71	0.893	38.601	520.637	637.696	74.38	20.704	153152.1	-23.109	730.561
34	160089	452.229	13.34	85.322	31.455	17.309	97.683	0.776	0.92	32.371	493.655	421.684	101.34	18.358	115288.1	-18.018	368.943
35	91090	282.888	9.382	65.895	23.999	14.081	86.097	0.804	0.908	24.941	467.711	541.068	88.573	15.166	72129.36	-20.158	418.392
36	72313	241.043	7.081	62.5	23.594	12.222	72.382	0.77	0.896	24.425	537.7	540.383	72.246	13.382	61460.1	-16.878	290.42
37	102296	320.677	8.318	69.842	26.053	14.893	76.485	0.804	0.917	26.773	423.837	601.639	77.98	15.86	81765.47	-16.722	284.691
38	96090	289.428	8.024	67.034	25.408	13.848	89.593	0.79	0.91	26.125	513.855	569.87	89.107	14.823	73798.69	-21.149	457.294
39	85731	257.45	7.838	61.416	22.661	13.58	88.662	0.823	0.914	23.451	551.58	491.763	89.026	14.485	65642.97	-20.079	419.87
40	100733	204.327	8.948	54.431	20.545	11.923	105.224	0.824	0.911	21.265	658.189	495.391	104.828	12.676	52101.72	-13.663	184.672

sl.no	Total Area	Average Size	%Area	Perim.	Major	Minor	Angle	Circ.	Solidity	Feret	FeretX	FeretY	FeretAngle	MinFeret	IntDen	Skew	Kurt
41	219755	676.169	21.166	103.416	38.381	21.145	71.666	0.764	0.924	39.172	470.582	545.846	71.182	22.344	172406.7	-25.733	733.113
42	233610	732.32	18.859	108.515	39.776	22.462	59.692	0.777	0.929	40.839	704.433	487.727	62.495	23.814	186708	-25.278	721.389
43	245724	787.577	22.267	110.318	39.406	24.071	73.303	0.793	0.933	40.456	589.503	486.003	71.687	25.356	200810	-30.49	1015.299
44	164544	408.298	17.818	78.211	28.745	16.772	82.733	0.791	0.919	29.628	502.767	562.945	81.394	17.819	104102	-20.508	481.189
45	152102	349.66	12.643	77.939	30.089	14.029	96.037	0.715	0.899	30.82	579.287	474.586	96.497	15.214	89157.97	-25.132	711.614
46	72313	241.043	7.081	62.5	23.594	12.222	72.382	0.77	0.896	24.425	537.7	540.383	72.246	13.382	61460.1	-16.878	290.42
47	102296	320.677	8.318	69.842	26.053	14.893	76.485	0.804	0.917	26.773	423.837	601.639	77.98	15.86	81765.47	-16.722	284.691
48	96090	289.428	8.024	67.034	25.408	13.848	89.593	0.79	0.91	26.125	513.855	569.87	89.107	14.823	73798.69	-21.149	457.294
49	85731	257.45	7.838	61.416	22.661	13.58	88.662	0.823	0.914	23.451	551.58	491.763	89.026	14.485	65642.97	-20.079	419.87
50	100733	204.327	8.948	54.431	20.545	11.923	105.224	0.824	0.911	21.265	658.189	495.391	104.828	12.676	52101.72	-13.663	184.672
51	219755	676.169	21.166	103.416	38.381	21.145	71.666	0.764	0.924	39.172	470.582	545.846	71.182	22.344	172406.7	-25.733	733.113
52	233610	732.32	18.859	108.515	39.776	22.462	59.692	0.777	0.929	40.839	704.433	487.727	62.495	23.814	186708	-25.278	721.389
53	245724	787.577	22.267	110.318	39.406	24.071	73.303	0.793	0.933	40.456	589.503	486.003	71.687	25.356	200810	-30.49	1015.299
54	164544	408.298	17.818	78.211	28.745	16.772	82.733	0.791	0.919	29.628	502.767	562.945	81.394	17.819	104102	-20.508	481.189
55	152102	349.66	12.643	77.939	30.089	14.029	96.037	0.715	0.899	30.82	579.287	474.586	96.497	15.214	89157.97	-25.132	711.614
56	252921	558.325	20.129	92.109	33.271	20.447	103.521	0.805	0.929	34.189	607.232	484.625	100.889	21.525	142344	-19.192	405.213
57	249420	545.777	20.372	91.083	33.194	19.395	106.964	0.79	0.923	34.203	599.991	493.024	106.956	20.64	139150.2	-22.519	544.314
58	247227	531.671	20.157	91.7	33.371	19.084	73.382	0.775	0.92	34.319	581.551	525.48	73.463	20.303	135560.2	-24.339	623.065
59	322513	814.427	25.721	113.925	40.608	23.866	99.398	0.775	0.928	41.659	631.629	433.316	100.473	25.257	207634.4	-28.396	909.147
60	325939	1038.022	29.88	132	46.717	27.084	91.735	0.755	0.93	48.012	552.417	399.796	94.071	28.61	264638	-31.011	1059.316
61	339744	954.337	30.901	123.727	44.065	26.195	85.624	0.776	0.933	45.124	658.817	422.938	86.053	27.519	243318.7	-31.206	1098.695
62	99717	631.12	10.072	94.636	34.105	21.957	77.151	0.828	0.937	35.098	399.367	574.228	71.623	22.894	160929.2	-21.439	462.502
63	183281	557.085	17.206	96.644	36.991	18.358	50.315	0.732	0.912	37.957	541.699	490.857	49.57	19.831	142050.5	-28.045	883.602
64	157943	624.281	12.609	103.266	39.104	19.198	58.566	0.722	0.911	40.219	712.47	498.992	57.849	20.663	159172.4	-20.717	495.815
65	154109	583.746	16.771	100.942	38.297	18.746	80.094	0.726	0.915	39.014	504.155	496	79.904	20.022	148830.2	-22.201	530.884
66	161583	678.92	13.081	99.385	35.468	23.068	91.514	0.826	0.939	36.411	669.714	450.08	90.858	24.024	173106.4	-23.19	652.427
67	148192	590.406	12.724	91.344	32.218	22.341	95.265	0.845	0.94	33.152	565.466	496.486	95.83	23.143	150550.6	-20.125	403.002
68	155895	631.154	12.36	96.363	33.743	23.032	106.73	0.84	0.939	34.704	634.36	499.543	107.75	23.989	160933.9	-23.018	535.377
69	169517	667.39	14.799	97.982	35.398	23.013	98.76	0.833	0.943	36.225	738.075	468.161	100.511	23.806	170180.4	-29.11	940.668
70	174841	780.54	14.373	105.45	36.748	25.755	89.129	0.85	0.947	37.703	766.571	499.71	90.042	26.537	199024.1	-27.375	877.052
71	168652	887.642	13.808	114.651	40.006	27.298	76.644	0.838	0.947	40.866	822.942	427.742	74.572	28.165	226300.4	-23.236	606.71
72	185481	467.207	15.523	85.229	32.631	17.436	71.834	0.765	0.923	33.2	655.088	489.469	70.536	18.409	119136.4	-25.318	639.002
73	204960	420	16.598	80.655	30.163	16.758	79.135	0.775	0.918	30.945	727.129	540.469	77.823	17.718	107082.2	-20.156	456.998
74	237600	408.95	19.097	80.439	29.707	16.42	79.046	0.766	0.91	30.651	617.608	560.255	78.671	17.67	104278.8	-17.227	308.441
75	267831	503.442	22.215	88.73	32.567	18.396	85.44	0.762	0.916	33.489	622.662	520.769	85.664	19.545	128365.7	-22.372	570.316
76	195955	432.572	15.848	81.923	31.008	16.866	79.36	0.772	0.919	31.721	646.329	474.89	80.164	17.928	110303.5	-24.631	627.252
77	210559	548.331	16.893	90.426	33.21	19.659	79.036	0.79	0.926	34.121	560.893	497.758	78.533	20.664	139819.7	-24.69	615.46
78	278228	641.078	22.081	96.035	34.37	22.901	122.125	0.846	0.944	35.301	618.378	423.403	120.8	23.735	163466.2	-23.381	621.869
79	310845	557.07	27.296	88.206	30.942	22.144	100.35	0.87	0.945	31.868	548.572	486.337	100.391	22.801	142050.5	-31.503	1022.751
80	252839	515.998	21.462	84.522	29.693	21.19	99.551	0.869	0.944	30.622	714.655	406.088	98.387	21.849	131578.4	-35.013	1257.001
81	138529	669.222	12.221	96.754	34.32	23.747	111.501	0.852	0.945	35.2	747.652	502.957	112.267	24.514	170649.2	-24.3	588.502
82	210092	905.569	17.959	115.298	41.276	27.246	114.44	0.837	0.949	42.157	706.125	448.733	115.493	28.137	230915.7	-29.903	937.668
83	197720	941.524	16.56	116.745	41.058	28.285	100.608	0.849	0.95	42.071	671.167	425.995	99.281	29.012	240078.9	-32.656	1140.901
84	178045	361.88	15	76.136	29.665	15.122	62.52	0.765	0.92	30.243	582.081	471.829	62.83	16.107	92276.83	-15.92	252.504

sl.no	Total Area	Average Size	%Area	Perim.	Major	Minor	Angle	Circ.	Solidity	Feret	FeretX	FeretY	FeretAngle	MinFeret	IntDen	Skew	Kurt
85	124158	290.768	9.969	68.088	26.688	13.483	68.333	0.77	0.92	27.22	700.913	542.899	67.908	14.312	74139.91	-9.158	85.096
86	155277	502.515	12.416	87.964	33.393	18.397	57.577	0.778	0.927	34.044	607.239	532.761	58.752	19.432	128139.6	-31.208	1091.002
87	180847	565.147	14.81	92.576	34.304	20.376	64.462	0.803	0.936	35.063	559.263	512.737	65.358	21.27	144096.5	-28.277	938.627
88	232661	596.567	19.897	95.911	34.819	21.23	72.193	0.806	0.933	35.739	590.087	509.213	71.004	22.311	152119.3	-31.564	1018.715
89	193205	522.176	16.392	88.111	31.963	20.33	94.036	0.832	0.938	32.827	638.659	402.408	96.709	21.191	133150	-18.947	359.947
90	186402	651.755	16.311	98.237	35.409	22.739	103.237	0.828	0.94	36.254	575.038	418.105	104.366	23.707	166188.7	-27.189	779.696
91	222287	686.071	18.509	101.776	36.639	23.285	107.586	0.823	0.941	37.488	552.09	455.003	109.406	24.264	174939.4	-31.123	1076.126
92	252591	629.903	21.637	96.835	35.189	22.131	115.917	0.825	0.94	35.975	667.167	504.85	116.019	23.046	160620.7	-29.983	992.376
93	167438	615.581	13.385	95.954	35.38	21.559	114.821	0.818	0.939	36.16	616.614	412.504	115.237	22.461	156970.3	-29.436	864.501
94	169157	638.328	15.598	97.828	36.669	21.303	112.902	0.799	0.937	37.404	634.208	483.219	112.044	22.243	162766	-26.516	840.931
95	227790	439.749	19.118	82.043	30.563	17.458	112.106	0.792	0.923	31.451	694.506	451.523	111.968	18.537	112130.1	-26.579	737.983
96	199509	337.008	15.977	75.891	30.2	13.83	134.814	0.727	0.907	30.677	606.899	480.037	134.684	14.861	85931.99	-17.861	335.187
97	222766	583.157	19.333	96.555	36.625	19.579	134.272	0.766	0.928	37.34	579.851	478.183	136.231	20.714	148697	-26.033	715.196
98	232926	594.199	18.991	97.839	37.49	19.548	136.703	0.763	0.927	38.081	616.452	482.202	136.765	20.622	151514.9	-29.273	969.952
99	181867	770.623	17.362	108.725	40.066	23.589	133.773	0.804	0.939	40.819	630.657	401.983	133.549	24.66	196500.2	-29.758	951.768
100	254913	617.223	21.697	95.783	33.832	22.107	109.449	0.825	0.936	34.815	478.138	493.981	110.559	23.158	157367.7	-25.32	681.653
101	188282	557.047	15.912	90.907	32.382	20.914	98.319	0.827	0.934	33.439	704.497	487.086	98.504	21.913	142025.9	-23.124	573.768
102	226882	560.202	18.335	90.793	32.405	20.943	111.665	0.831	0.934	33.41	690.778	461.106	109.565	21.912	142840.3	-24.086	610.102
103	207648	570.462	18.851	92.838	33.608	20.747	110.728	0.818	0.933	34.472	533.247	492.835	111.94	21.853	145451.6	-25.538	690.31
104	161898	529.078	14.346	91.219	34.293	18.889	133.823	0.781	0.923	34.958	544.67	423.369	133.248	20.048	134909.2	-27.615	774.859
105	137328	579.443	11.693	92.265	33.334	21.187	116.418	0.818	0.933	34.235	599.118	511.705	118.078	22.091	147754.7	-27.973	787.001
106	203969	645.472	16.726	98.769	33.698	23.403	97.731	0.837	0.938	34.754	770.278	534.136	95.192	24.338	164559.7	-24.93	653.55
107	219133	656.087	17.658	96.803	34.175	23.601	108.107	0.855	0.946	35.098	635.545	546.982	106.005	24.351	167286.1	-22.599	540.871
108	196004	653.347	15.873	100.508	35.463	22.222	67	0.812	0.932	36.694	632.017	401.957	67.654	23.479	166593.2	-37.278	1862.18
109	173358	696.217	15.061	101.932	37.316	22.437	106.137	0.799	0.934	38.181	557.024	562.398	105.496	23.449	177519.9	-20.686	533.637
110	107758	686.357	15.726	100.358	36.324	22.437	87.278	0.801	0.93	37.21	300.503	498.331	88.593	23.467	175009.6	-25.545	661.418
111	233075	917.618	22.52	123.186	44.591	25.557	87.453	0.769	0.929	45.601	579.52	496.201	86.6	26.869	233973.5	-26.143	732.866
112	191475	719.831	16.2	103.704	36.311	23.954	89.159	0.82	0.937	37.368	579.305	426.447	90.137	25.043	183534.8	-29.002	1011.55
113	180336	712.791	14.664	106.044	38.987	22.496	80.069	0.771	0.927	39.925	563.719	433.731	80.674	23.698	181752.5	-22.526	528.477
114	201160	776.68	16.422	106.3	37.497	25.095	95.52	0.835	0.941	38.467	585.571	412.595	95.143	26.12	198044.4	-29.734	939.446
115	209523	445.794	16.624	83.049	30.991	17.607	127.852	0.791	0.922	31.785	617.094	463.44	126.242	18.728	113669.8	-20.061	462.183
116	154486	365.215	14.169	73.822	27.267	16.428	78.997	0.817	0.925	28.101	531.066	381.319	77.493	17.337	93123.83	-18.82	379.603
117	193564	488.798	15.441	83.848	30.269	19.632	85.162	0.837	0.935	31.194	581.949	468.035	84.31	20.506	124640.9	-19.912	394.503
118	201531	533.151	16.29	90.43	34.418	18.907	134.967	0.787	0.93	35.056	581.434	462.426	134.531	19.915	135946	-21.751	527.545
119	211474	530.01	16.905	92.07	35.805	18.237	138.245	0.767	0.93	36.335	589.559	433.211	136.269	19.214	135146.8	-20.956	454.603
120	162474	615.432	12.842	97.322	36.059	20.846	120.992	0.785	0.929	36.786	595.22	533.799	120.923	21.853	156920.6	-29.059	907.478
121	145546	539.059	11.68	90.857	33.636	19.629	109.927	0.79	0.928	34.334	654.267	450.278	110.574	20.588	137455.4	-30.937	970.626
122	197185	583.388	15.714	95.937	35.676	19.925	77.249	0.769	0.924	36.479	630.015	478.246	75.144	21	148750.3	-25.231	735.107
123	214704	606.508	19.347	96.192	34.941	20.468	66.904	0.773	0.92	36.051	557.26	508.723	67.266	21.793	154652.5	-15.255	234.317
124	205049	434.426	16.417	82.517	30.862	16.764	74.988	0.769	0.914	31.784	549.191	426.801	73.317	17.971	110771	-22.137	528.762
125	275827	569.89	23.535	92.616	33.443	20.401	85.419	0.8	0.927	34.44	605.021	449.593	86.569	21.542	145305.7	-23.684	645.473

ANNEXURE- X

MORPHOMETRIC ANALYSIS OF NUCLEI DETECTION- MILD DYSPLASIA CASES

Sl. No.	Total Area	Average Size	%Area	Perim.	Major	Minor	Angle	Circ.	Solidity	Feret	FeretX	FeretY	FeretAngle	MinFeret	IntDen	Skew	Kurt
1	284574	677.557	26.077	106.534	37.912	21.151	84.883	0.744	0.906	39.322	620.26	493.048	84.288	22.899	172759.5	-26.606	792.127
2	220765	673.064	19.115	110.857	40.889	19.713	122.708	0.685	0.888	42.446	550.317	478.61	120.965	21.743	171623.6	-26.812	755.802
3	271706	588.108	26.001	98.909	35.415	19.39	105.362	0.744	0.909	36.662	574.076	481.139	103.731	20.921	149874.9	-24.282	680.891
4	329084	571.326	26.382	99.034	36.754	18.511	69.995	0.718	0.903	37.954	678.608	556.625	70.924	20.024	145674.1	-29.769	975.811
5	361408	471.197	31.55	90.385	34.442	16.185	64.492	0.704	0.902	35.411	599.415	474.914	66.639	17.5	120108.3	-26.636	784.721
6	338264	972.023	26.701	128.357	46.919	23.591	70.288	0.713	0.906	48.78	592.687	495.638	67.393	25.637	247761.1	-26.667	967.978
7	207123	920.547	17.126	119.152	43.157	25.008	77.328	0.771	0.928	44.441	624.658	433.222	77.7	26.598	234728.1	-30.819	1036.251
8	366220	973.989	29.117	131.991	47.659	22.152	73.144	0.679	0.889	49.919	695.963	496.665	72.295	24.699	248239.1	-29.417	1052.693
9	259866	498.783	21.007	85.58	30.391	19.747	95.634	0.824	0.93	31.396	666.812	479.845	95.908	20.753	127177.9	-21.049	457.669
10	191572	504.137	15.485	84.521	30.113	19.905	109.912	0.838	0.932	31.003	569.632	407.155	110.077	20.792	128550.2	-25.684	745.086
11	266620	768.357	22.813	107.569	37.627	24.37	105.861	0.81	0.932	38.614	457.164	396.813	103.731	25.602	195904.7	-33.642	1280.071
12	191934	528.744	19.913	91.582	32.39	19.635	75.644	0.787	0.921	33.501	489.59	497.185	73.376	20.915	134809.3	-28.931	931.52
13	181286	372.251	15.048	76.488	28.372	15.802	102.303	0.783	0.914	29.268	607.778	514.049	101.123	16.934	94914.98	-22.851	595.327
14	282909	466.077	25.141	86.407	31.946	17.367	73.075	0.762	0.912	32.855	667.115	452.056	74.813	18.628	118838.4	-22.368	536.883
15	312366	758.17	26.239	111.188	41.275	21.933	50.749	0.752	0.923	42.381	594.131	533.755	50.137	23.434	193306.7	-30.998	1064.161
16	302702	572.216	24.88	92.277	33.558	20.089	96.823	0.797	0.926	34.507	645.14	503.781	95.939	21.209	145902.9	-23.932	623.766
17	56153	348.776	5.913	77.09	29.856	13.847	105.197	0.731	0.902	30.608	634.429	477.839	105.171	15.056	88931.65	-27.506	879.752
18	82748	344.783	8.75	76.225	28.904	14.244	95.641	0.738	0.9	29.719	547.892	513.383	97.308	15.381	87902.75	-18.313	364.397
19	177285	500.805	14.175	90.677	33.421	16.717	79.217	0.717	0.897	34.477	755.89	547.864	77.926	18.219	127678.6	-24.039	639.02
20	315153	669.115	31.827	104.874	35.922	21.043	94.497	0.761	0.912	37.339	652.461	443.1	94.1	22.631	170509.5	-26.917	860.689
21	291889	690.045	29.257	105.619	36.122	21.352	96.322	0.764	0.915	37.535	699.896	385.333	95.08	22.902	175873.4	-28.371	944.44
22	254462	542.563	22.018	93.318	34.064	19.189	96.154	0.771	0.92	35.001	514.452	419.341	96.503	20.432	138336.7	-23.351	608.267
23	277356	695.128	27.7	102.187	35.263	23.593	91.614	0.81	0.931	36.47	567.098	444.539	90.458	24.788	177235.2	-28.975	923.859
24	307757	866.921	28.39	114.875	38.93	26.961	103.322	0.821	0.938	40.166	589.465	502.642	103.492	28.174	221026.8	-28.825	922.433
25	276849	555.922	23.486	100.617	37.481	18.062	108.924	0.704	0.892	38.534	537.161	502.522	110.193	19.663	141710.9	-25.78	747.888
26	288960	813.972	23.382	111.13	38.665	25.467	88.04	0.818	0.936	39.825	611.231	543.332	87.878	26.657	207547	-31.832	1076.981
27	370539	847.915	30.454	114.773	39.364	25.709	97.19	0.805	0.932	40.511	578.268	432.446	97.817	27.105	216191.6	-29.968	990.64
28	236709	756.259	21.953	103.422	35.888	24.809	95.202	0.834	0.938	36.953	567.559	442.006	95.305	25.771	192833	-31.121	1077.568
29	216433	642.234	21.552	102.358	37.207	20.176	99.964	0.734	0.911	38.327	564.599	488.763	99.991	21.612	163758.4	-24.038	643.052
30	200225	725.453	16.101	108.013	39.526	21.351	98.969	0.74	0.915	40.754	728.496	495.192	98.333	22.774	184938.8	-24.532	741.59
31	239395	723.248	20.268	105.759	38.377	22.109	102.975	0.761	0.919	39.48	575.024	442.378	104.167	23.402	184414.3	-30.963	1042.138
32	306753	794.697	28.118	110.935	39.991	23.073	99.097	0.763	0.919	41.058	482.352	461.904	96.974	24.507	202620	-30.64	1051.107
33	188955	694.688	17.748	100.309	35.531	23.379	91.099	0.822	0.936	36.543	638.607	467.562	90.245	24.359	177134.1	-22.236	601.191
34	192824	531.196	19.198	91.202	32.221	18.824	88.304	0.784	0.911	33.352	548.672	491.306	88.562	20.264	135431	-26.546	780.839
35	236684	643.163	19.561	109.171	40.355	18.831	67.682	0.702	0.893	41.766	502.88	472.679	66.976	20.771	163992.7	-31.782	1104.002
36	242800	488.531	21.478	90.628	33.262	17.553	100.675	0.756	0.911	34.166	715.171	486.348	101.705	18.893	124554.9	-24.223	625.499

Sl. No.	Total Area	Average Size	%Area	Perim.	Major	Minor	Angle	Circ.	Solidity	Feret	FeretX	FeretY	FeretAngle	MinFeret	IntDen	Skew	Kurt
37	262857	529.954	23.488	96.738	36.411	17.687	93.828	0.721	0.905	37.258	670.782	491.266	94.378	19.057	135121.2	-23.838	615.803
38	365260	571.612	31.959	104.894	39.612	17.694	82.716	0.685	0.893	40.778	642.064	491.781	82.948	19.414	145744.7	-24.395	658.731
39	319375	549.699	34.015	105.767	39.812	16.561	73.939	0.665	0.884	41.324	531.69	499.565	73.94	18.446	140139.4	-25.432	796.386
40	136668	596.803	12.823	88.256	31.307	21.115	96.608	0.841	0.935	32.267	465.205	499.716	96.498	21.944	152183.8	-34.424	1183.001
41	107164	463.913	10.086	80.656	29.331	18.037	76.799	0.807	0.916	30.244	491.229	572.519	78.226	19.005	118295.7	-19.546	407.003
42	155727	351.528	13.571	72.022	26.391	15.623	71.933	0.8	0.915	27.317	631.526	434.734	70.998	16.613	89636.82	-20.368	451.128
43	104818	263.362	9.85	60.625	22.343	13.34	114.441	0.824	0.907	23.199	707.078	447.706	115.475	14.305	67154.7	-20.819	449.003
44	88814	394.729	13.355	75.909	28.168	16.549	95.397	0.808	0.922	28.929	400.493	430.427	99.44	17.43	100651.3	-16.295	271.504
45	108288	429.714	15.008	80.318	29.506	17.044	100.347	0.795	0.918	30.333	386.492	458.663	102.668	18.096	109569	-25.666	668.918
46	95857	450.033	10.35	79.841	29.615	17.675	117.635	0.814	0.925	30.338	624.357	330.563	117.635	18.56	114754.8	-20.688	426.502
47	176524	770.847	18.44	111.581	42.088	22.282	48.793	0.754	0.925	42.895	596.983	476.279	47.708	23.756	196559.3	-33.221	1203.901
48	182162	781.811	16.58	105.731	38.467	24.182	59.878	0.825	0.943	39.415	601.433	486.824	61.075	25.282	199358.6	-27.982	781.001
49	181938	866.371	17.427	112.905	40.807	25.863	53.768	0.825	0.943	41.774	553.905	495.152	57.076	27.001	220918.6	-36.354	1435.126
50	228278	594.474	19.387	90.214	31.963	21.546	99.592	0.835	0.935	32.949	470.026	532.49	97.74	22.432	151587.5	-26.891	726.335

MORPHOMETRIC ANALYSIS OF NUCLEI DETECTION- MODERATE DYSPLASIA CASES

Sl. No.	Total Area	Average Size	%Area	Perim.	Major	Minor	Angle	Circ.	Solidity	Feret	FeretX	FeretY	FeretAngle	MinFeret	IntDen	Skew	Kurt
1	120028	600.14	9.7	95.642	36.03	19.766	74.548	0.767	0.927	36.723	534.69	471.815	74.001	20.699	153029.3	-20.174	478.379
2	127903	761.327	10.153	111.803	43.312	21.105	74.543	0.747	0.925	43.731	499.637	530.512	74.36	22.392	194135.4	-33.736	1339.002
3	122481	658.5	9.965	102.275	38.948	20.507	76.431	0.767	0.929	39.511	567.876	522.844	77.121	21.565	167916.1	-35.426	1253.001
4	115480	508.722	9.281	87.11	33.03	18.175	72.021	0.777	0.923	33.612	569.564	366.211	71.409	19.139	129723.1	-24.393	593.002
5	172215	474.421	14.743	88.707	33.467	17.333	112.79	0.751	0.912	34.115	517.523	485.377	113.039	18.603	120968.3	-21.02	460.03
6	166269	463.145	13.357	85.164	32.263	17.37	108.283	0.772	0.921	32.908	595.384	481.504	110.111	18.412	118098.4	-27.799	775.876
7	134870	465.069	12.682	85.369	32.097	17.314	91.799	0.768	0.917	32.826	426.431	520.569	92.011	18.409	118581.2	-20.859	461.416
8	174956	627.082	13.9	96.553	36.077	20.686	117.588	0.799	0.932	36.769	626.971	472.247	121.167	21.723	159903.3	-22.904	583.003
9	137597	625.441	10.938	101.517	38.194	20.007	122.533	0.761	0.928	39.019	768.109	445.882	125.107	21.155	159478.2	-25.037	666.794
10	114488	612.235	9.154	96.25	35.223	20.965	119.83	0.799	0.93	36.144	693.652	523.166	118.597	22.107	156110.5	-17.094	290.42
11	202956	391.052	22.328	81.543	31.743	14.881	106.845	0.727	0.908	32.367	463.85	456.765	107.79	15.991	99715.81	-22.601	515.224
12	177197	501.975	14.305	91.182	34.336	17.595	118.929	0.749	0.91	35.295	548.3	463.224	119.86	18.982	127995.6	-27.849	817.439
13	165161	409.829	14.444	80.76	30.56	16.071	107.457	0.759	0.911	31.289	534.633	541.747	109.723	17.221	104497.5	-23.291	619.781
14	328882	1018.211	29.229	122.656	43.094	27.649	76.305	0.802	0.939	44.098	473.368	459.796	75.973	28.817	259619.2	-29.32	945.575
15	294402	903.074	26.581	115.161	40.537	25.835	79.561	0.798	0.934	41.683	466.212	504.936	77.398	27.071	230257.2	-29.103	947.569
16	197117	741.041	17.807	104.321	37.738	22.451	96.615	0.775	0.924	38.853	315.519	548.094	96.019	23.639	188958.8	-30.435	978.314
17	223083	791.074	21.064	108.406	39.114	23.388	76.327	0.782	0.929	40.156	404.355	420.135	77.354	24.601	201714.9	-37.931	1565.501
18	177600	655.351	15.06	101.866	39.207	19.965	67.365	0.755	0.928	39.728	627.701	520.413	66.728	21.029	167110.6	-22.371	500.752
19	217804	985.538	17.629	124.845	46.251	25.724	56.845	0.772	0.936	47.141	591.557	469	55.434	27.085	251290.4	-27.425	874.871
20	204355	880.841	16.306	112.155	41.217	24.765	58.463	0.798	0.935	42.115	644.302	510.672	57.897	25.893	224606.6	-28.755	879.085
21	209686	743.567	17.226	105.47	38.335	22.732	85.081	0.788	0.928	39.323	615.574	477.145	84.39	24.033	189596.1	-28.044	854.385
22	165065	687.771	13.097	99.02	35.489	23.461	97.671	0.841	0.942	36.393	691.379	460.775	98.175	24.377	175379.4	-28.289	799.001
23	182707	555.34	14.616	88.701	32.174	20.516	104.45	0.829	0.934	32.954	710.529	484.31	106.222	21.372	141607.9	-26.071	759.335
24	285633	723.122	22.698	106.512	37.69	22.582	67.396	0.778	0.923	39.074	585.456	524.896	65.689	24.119	184296.6	-27.449	928.45
25	239886	799.62	20.027	107.677	36.965	26.213	73.727	0.842	0.941	38.072	649.927	462.897	74.704	27.29	203882.7	-24.07	667.753
26	240494	780.825	19.618	105.664	36.461	25.931	77.74	0.846	0.942	37.506	681.143	453.169	76.734	26.956	199094.6	-28.82	922.905
27	155078	665.571	12.935	98.027	34.475	21.231	74.52	0.801	0.921	35.78	700.691	478.468	71.146	22.562	169707.4	-28.453	868.127
28	269997	617.842	21.544	99.945	34.678	21.361	105.061	0.786	0.923	35.825	658.819	474.911	103.933	22.763	157451.7	-24.8	677.689
29	209947	656.084	16.931	99.776	35.603	21.724	102.235	0.798	0.928	36.562	715.462	578.747	103.669	22.835	167273.6	-24.731	661.44
30	204026	775.764	19.606	113.826	40.508	22.083	57.744	0.748	0.918	42.092	586.555	546.053	56.58	23.76	197765.6	-27.013	918.153
31	227417	601.632	19.537	95.245	33.437	21.715	105.194	0.821	0.931	34.445	684.563	548.193	106.041	22.767	153393.3	-23.109	581.073
32	130752	416.408	12.875	82.293	31.229	15.411	115.648	0.74	0.901	32.183	626.713	392.162	114.759	16.732	106172.6	-21.948	515.845
33	239882	705.535	21.614	105.165	39.116	20.685	82.913	0.751	0.917	40.235	669.562	406.044	82.574	22.108	179897.3	-29.028	958.917
34	320319	509.251	25.961	91.03	33.552	18.264	103.647	0.76	0.913	34.516	678.005	427.844	105.035	19.585	129849.7	-26.198	710.689
35	66302	362.306	5.369	74.314	28.626	14.76	80.929	0.763	0.912	29.254	595.607	377.989	79.852	15.743	92385.25	-20.683	446.003
36	74425	387.63	6.364	76.3	28.954	15.711	87.437	0.778	0.915	29.604	703.885	348.891	87.109	16.696	98837.73	-20.215	430.003
37	77009	558.036	7.403	90.855	33.41	19.849	82.115	0.796	0.926	34.226	771.13	360.341	79.852	20.821	142295.5	-27.482	754.001
38	86121	652.432	7.777	94.984	33.956	22.367	85.165	0.832	0.936	34.876	632.197	445.811	82.055	23.222	166362.4	-15.125	226.754
39	191579	694.127	15.632	100.421	35.741	23.438	58.358	0.824	0.938	36.783	580.384	506.87	60.045	24.455	176999.6	-22.066	498.752

MORPHOMETRIC ANALYSIS OF NUCLEI DETECTION- SEVERE DYSPLASIA CASES

Sl. No.	Total Area	Average Size	%Area	Perim.	Major	Minor	Angle	Circ.	Solidity	Feret	FeretX	FeretY	FeretAngle	MinFeret	IntDen	Skew	Kurt
1	283206	460.498	24.963	87.597	33.753	16.414	120.925	0.733	0.914	34.469	609.257	498.836	119.518	17.541	117417.3	-20.414	448.597
2	325021	585.623	28.045	99.54	37.421	18.763	106.789	0.732	0.918	38.343	608.135	475.207	110.278	20.017	149317.9	-24.026	611.752
3	252220	696.74	24.985	102.854	36.502	22.741	84.615	0.806	0.932	37.649	565.069	521.135	85.975	23.888	177637.8	-29.823	1006.731
4	287280	563.294	24.431	92.702	33.504	19.606	93.966	0.772	0.917	34.633	578.443	460.329	94.741	20.882	143625.5	-27.248	859.269
5	303556	635.054	32.444	99.317	36.266	20.741	93.977	0.781	0.924	37.337	565.983	396.362	94.94	21.987	161925.5	-25.46	693.68
6	244146	626.015	23.328	98.253	35.506	20.829	94.285	0.785	0.925	36.459	596.938	467.521	95.539	22.065	159621.5	-27.188	805.359
7	198640	299.608	18.145	67.41	24.324	14.389	93.167	0.802	0.906	25.287	673.415	438.511	93.72	15.428	76391.54	-19.584	397.591
8	286576	341.569	22.691	71.123	26.093	15.961	103.15	0.827	0.924	26.917	654.665	501.775	106.578	16.813	87093.59	-19.338	395.785
9	271190	306.776	23.252	67.697	25.105	14.925	114.174	0.822	0.921	25.92	665.459	443.084	113.145	15.787	78222.69	-19.032	379.553
10	230217	605.834	18.639	94.092	33.517	21.507	62.306	0.815	0.932	34.595	617.587	498.839	62.454	22.624	154470.3	-21.316	527.125
11	205463	652.263	17.157	97.991	35.705	21.644	51.936	0.807	0.931	36.685	589.311	447.692	50.701	22.872	166319.1	-32.035	1125.302
12	247864	511.06	20.125	87.678	32.881	18.372	110.112	0.787	0.925	33.646	525.287	470.254	110.971	19.409	130314.5	-29.368	956.073
13	153656	467.04	12.315	86.433	30.973	18.31	95.026	0.785	0.921	32.001	618.815	508.617	94.493	19.38	119083.5	-25.58	735.521
14	130653	442.892	13.113	85.995	31.558	16.927	97.942	0.758	0.91	32.501	584.834	380.844	98.423	18.159	112911.4	-24.036	620.851
15	186125	590.873	17.453	94.598	33.717	20.683	98.226	0.8	0.921	34.736	520.81	507.822	99.577	21.899	150651.6	-22.333	543.742
16	139788	592.322	12.698	92.902	32.234	20.428	89.107	0.806	0.917	33.392	572.165	512.415	90.345	21.747	151020.5	-26.274	706.088
17	97791	423.338	8.243	76.925	27.056	17.771	88.578	0.831	0.919	28.177	635.216	514.87	91.621	18.795	107939	-33.893	1533.653
18	211487	602.527	17.199	97.09	34.964	19.781	79.77	0.769	0.915	36.241	739.456	411.228	81.99	21.221	153628.4	-24.176	634.469
19	142623	572.783	12.531	91.843	33.006	20.841	87.467	0.813	0.93	33.945	713.197	545.129	87.011	21.886	146057.7	-27.835	774.001
20	222439	563.137	18.935	93.145	33.873	19.295	91.805	0.779	0.917	34.93	553.97	543.77	91.798	20.606	143590.2	-30.919	1007.501
21	171475	680.456	15.742	101.44	37.957	20.797	63.761	0.776	0.929	38.667	537.917	546.79	66.636	21.802	173501.2	-28.453	1028.532
22	142388	745.487	12.261	104.344	37.386	22.941	103.358	0.805	0.933	38.326	508.806	520.618	105.202	24.003	190089.8	-27.556	797.002
23	127591	777.994	11.644	104.08	37.205	23.908	86.75	0.824	0.937	38.165	482.988	454.14	90.713	24.918	198383.8	-37.251	1510.751
24	63312	445.859	5.837	75.632	26.326	18.385	85.273	0.86	0.927	27.428	508.162	452.239	86.406	19.205	113681.5	-22.029	550.586
25	65247	459.486	5.532	74.459	26.282	18.095	81.771	0.86	0.925	27.267	474.901	508.415	84.782	18.86	117167.1	-36.592	1337.001
26	65439	532.024	6.096	80.774	28.403	19.442	94.182	0.843	0.926	29.457	342.049	450.154	95.657	20.284	135657.9	-27.023	730.001
27	174381	726.587	15.859	101.991	35.863	23.767	84.238	0.815	0.932	36.972	505.5	471.363	84.784	24.93	185196.9	-17.421	372.581
28	151631	659.265	12.772	96.805	33.737	22.317	78.073	0.812	0.922	34.918	666.291	483.57	77.077	23.519	168102.7	-32.101	1103.161
29	143303	675.958	12.07	96.894	33.881	22.682	74.36	0.821	0.925	35.05	668.524	487.519	75.712	23.778	172359.6	-28.153	904.96
30	366918	1019.217	31.972	135.187	47.969	25.665	120.566	0.736	0.922	49.267	564.933	439.986	121.638	27.617	259800.4	-30.273	1054.3
31	314405	998.111	27.006	131.507	45.064	26.478	112.332	0.758	0.924	46.59	566.257	405.076	115.4	28.17	254422	-27.721	853.761
32	252998	755.218	23.639	116.808	41.448	21.48	130.136	0.734	0.912	42.872	474.707	454.015	132.612	23.253	192487.7	-24.182	668.55
33	165444	641.256	13.447	97.373	35.265	21.785	92.427	0.813	0.933	36.136	664.24	513.124	93.013	22.761	163509.4	-24.904	677.515
34	149667	322.558	13.859	73.025	28.477	13.849	92.308	0.75	0.909	29.068	619.304	451.168	91.877	14.859	82251.24	-21.255	459.502
35	134101	435.393	11.514	79.69	28.954	17.528	84.989	0.794	0.915	29.952	679.627	527.805	86.785	18.65	111021	-23.962	603.558
36	134883	449.61	12.15	80.983	30.137	17.267	106.872	0.784	0.918	30.963	655.48	511.37	108.1	18.288	114642.9	-21.498	565.481
37	208147	546.318	16.777	89.963	32.617	20.499	88.152	0.824	0.935	33.538	554.903	483.013	88.747	21.462	139305	-25.5	712.502
38	189055	522.251	15.997	86.077	30.29	20.872	83.326	0.852	0.938	31.313	527.296	418.232	84.617	21.721	133172.7	-19.922	400.503
39	208212	533.877	17.083	88.307	31.892	20.394	85.741	0.831	0.936	32.738	516.297	474.831	89.181	21.243	136126.2	-23.083	598.8

ANNEXURE- XI

MORPHOMETRIC ANALYSIS OF NUCLEI DETECTION- SURGICAL MARGINS IN OSCC CASES

RND Cases	Total Area	Average Size	%Area	Perim.	Major	Minor	Angle	Circ.	Solidity	Feret	FeretX	FeretY	FeretAngle	MinFeret	IntDen	Skew	Kurt
OP3415																	
Anterior M	352466	821.599	28.997	119.635	39.528	23.538	86.917	0.757	0.911	41.251	584.289	434.217	84.821	25.573	209413.8	-31.183	1157.174
Lateral M	189125	685.236	17.998	102.256	36.215	22.03	82.054	0.796	0.927	37.449	665.685	476.848	83.865	23.281	174727.7	-39.238	1651.251
OP3633																	
Anterior M	242739	724.594	20.144	108.899	36.523	22.774	77.58	0.787	0.915	38.244	677.904	491.97	76.92	24.325	184509.6	-19.845	464.521
Posterior M	194470	633.453	20.583	112.568	35.707	18.05	81.865	0.701	0.876	38.427	631.694	401.003	79.271	20.635	161427.5	-27.742	968.559
Lateral M	171044	526.289	15.835	96.743	33.857	17.925	97.852	0.724	0.887	35.452	517.265	480.828	98.011	19.808	134035.8	-23.632	693.458
OP3765																	
Posterior M	151430	316.138	12.572	71.209	26.821	14.33	107.833	0.77	0.906	27.665	641.693	325.286	109.567	15.412	80610.34	-21.251	492.803
Medial M	311581	586.782	25.724	92.927	33.616	21.403	68.799	0.825	0.938	34.461	623.966	439.122	70.784	22.285	149621.1	-26.381	763.408
OP3814																	
Medial M	157783	695.079	13.935	99.623	36.024	22.969	75.179	0.825	0.941	36.774	630.044	434.683	80.016	23.699	177238.5	-25.269	712.669
Lateral M	209338	392.019	20.959	81.324	31.072	15.485	94.207	0.732	0.906	31.746	444.165	567.534	94.668	16.64	99957.61	-23.416	599.502
OP3840																	
Anterior M	171977	607.693	13.88	102.094	40.253	18.318	50.608	0.711	0.913	40.938	587.279	488.622	50.733	19.748	154955.3	-32.944	1163.126
Posterior M	263402	551.05	21.148	89	32.258	20.242	100.776	0.82	0.932	33.086	620.695	408.075	103.408	21.181	140508.7	-23.418	579.627
OP3986																	
Anterior M	157503	779.718	14.482	111.653	39.542	23.563	89.381	0.791	0.929	40.695	668.168	441.005	87.54	24.982	198781.3	-25.631	683.079
Posterior M	234464	800.218	18.962	108.691	38.107	25.086	79.247	0.827	0.937	39.113	656.362	519.939	79.021	26.206	204043.5	-30.743	1017.939
Medial M	172348	911.894	13.831	116.092	40.723	27.715	81.818	0.835	0.947	41.725	679.042	425.741	81.878	28.666	232515.5	-22.467	537.047
Lateral M	175849	726.649	20.795	108.973	38.822	22.56	102.121	0.781	0.927	40.289	596.24	309.851	101.432	23.867	185278.6	-31.198	1066.001
OP4010																	
Anterior M	285723	1035.228	23.291	126.787	42.836	28.533	86.487	0.794	0.929	44.097	628.993	512.018	84.409	30.116	263920.4	-29.1	952.558
Posterior M	379200	905.012	30.604	126.677	42.774	24.59	65.385	0.74	0.914	44.631	672.196	495.74	63.969	26.681	230689.8	-28.893	984.506
Medial M	241211	609.119	19.193	103.446	37.932	18.733	77.788	0.713	0.9	39.138	603.49	418.49	77.197	20.53	155309.2	-28.597	882.195
OP4077																	
Anterior M	56124	195.554	5.124	57.841	23.003	10.424	113.77	0.741	0.888	23.342	661.265	599.84	113.406	11.286	49862.72	-15.128	262.174
Posterior M	124790	513.539	9.979	94.343	35.703	17.713	99.834	0.737	0.91	36.478	562.852	502.551	99.832	19.127	130933.6	-22.466	517.617
Medial M	187792	495.493	15.047	87.15	31.668	18.898	78.999	0.8	0.922	32.617	537.359	491.681	78.554	20.024	126338	-22.884	575.761
Lateral M	156772	553.965	12.796	90.814	32.168	20.347	86.986	0.809	0.922	33.14	472.71	491.961	86.929	21.55	141249.3	-30.231	1010.863
OP4152																	
Anterior M	187839	890.232	21.97	119.061	39.922	26.366	96.528	0.8	0.929	41.226	464.536	464.379	97.876	27.773	226947.6	-23.9	638.783
Medial M	295697	564.307	23.341	95.851	35.75	19.18	108.483	0.761	0.921	36.653	613.876	471.412	109.611	20.438	143875	-22.059	540.17
Lateral M	109370	541.436	9.489	95.271	36.143	18.204	75.163	0.738	0.91	36.851	562.119	551.678	74.881	19.495	138015.6	-16.088	290.361
OP4256																	
Anterior M	160266	475.567	24.697	87.205	31.501	18.23	95.262	0.784	0.916	32.532	269.525	485.122	95.428	19.484	121253.6	-26.255	781.226
Posterior M	157661	473.456	18.663	87.795	31.334	18.153	103.674	0.77	0.912	32.487	320.069	510.697	102.399	19.465	120710	-24.053	680.683
Lateral M	165885	489.336	19.235	89.823	32.604	17.932	95.253	0.761	0.913	33.621	341.389	529.988	94.892	19.239	124761.2	-28.075	959.626
OP4355																	
Anterior M	54755	396.775	4.465	75.616	26.638	17.246	81.825	0.821	0.918	27.731	612.587	509.399	77.839	18.304	101170.3	-23.239	552.002

RND Cases	Total Area	Average Size	%Area	Perim.	Major	Minor	Angle	Circ.	Solidity	Feret	FeretX	FeretY	FeretAngle	MinFeret	IntDen	Skew	Kurt
Posterior M	250136	1488.905	25.84	155.284	52.976	32.809	116.448	0.794	0.932	54.386	480.47	474.887	117.132	34.921	379566	-37.803	1782.942
Medial M	109151	545.755	15.187	94.879	34.627	18.857	76.804	0.765	0.918	35.558	484.44	450.41	75.131	20.191	139148.4	-28.99	917.471
OP4364																	
Anterior M	296545	486.938	24.121	93.468	34.637	16.983	107.961	0.711	0.893	35.789	662.741	497.268	107.11	18.616	124148.2	-25.266	689.433
Posterior M	248913	477.76	19.99	92.226	33.524	16.815	99.538	0.712	0.892	34.75	566.726	475.34	99.255	18.492	121793.1	-21.675	523.433
Medial M	273686	544.107	23.152	97.947	35.216	18.278	98.618	0.731	0.901	36.475	665.781	479.913	99.241	20.013	138721	-25.293	730.369
Lateral M	296545	486.938	24.121	93.468	34.637	16.983	107.961	0.711	0.893	35.789	662.741	497.268	107.11	18.616	124148.2	-25.266	689.433
OP4585																	
Medial M	109598	458.569	11.195	85.35	30.25	18.164	69.977	0.793	0.917	31.398	542.109	446.322	68.953	19.376	116918	-23.83	646.455
Lateral M	68489	271.782	7.175	62.02	22.168	13.955	86.741	0.832	0.912	23.165	599.742	346.417	90.029	14.801	69302.32	-10.441	107.009
OP4624																	
Anterior M	131463	372.416	10.806	76.239	27.351	15.826	65.018	0.778	0.906	28.51	443.983	502.722	64.678	17.119	94949.58	-23.671	613.752
Posterior M	108706	464.556	11.975	95.396	36.715	15.202	90.446	0.676	0.889	37.432	322.06	461.987	90.486	16.684	118448.6	-22.245	534.869
Medial M	141965	436.815	11.755	85.346	31.139	16.716	61.748	0.757	0.905	32.279	674.046	548.48	61.072	18.185	111363.6	-23.095	632.687
Lateral M	64068	248.326	8.585	63.754	23.811	12.689	80.898	0.769	0.903	24.568	298.798	408.868	82.036	13.683	63301.28	-23.383	688.945
OP4634																	
Anterior M	203595	419.784	16.5	81.14	29.423	17.251	74.108	0.797	0.918	30.404	635.909	504.315	73.353	18.365	107031.1	-21.632	533.699
Posterior M	169701	345.623	13.883	72.823	26.941	15.731	72.83	0.805	0.92	27.787	669.063	461.208	72.494	16.708	88127.17	-24.087	627.967
OP4640																	
Anterior M	93058	465.29	8.024	86.748	31.314	17.15	109.507	0.756	0.9	32.478	665.035	506.56	111.453	18.669	118638.8	-32.132	1124.135
Posterior M	43100	391.818	7.57	80.885	29.6	14.968	84.365	0.749	0.905	30.712	428.791	506.182	84.814	15.936	99902.05	-13.357	176.406
Lateral M	42856	295.559	5.464	71.341	26.855	13.136	72.157	0.736	0.892	27.698	551.055	440.559	72.082	14.368	75365.69	-27.604	760.001
OP4759																	
Posterior M	379200	905.012	30.604	126.677	42.774	24.59	65.385	0.74	0.914	44.631	672.196	495.74	63.969	26.681	230689.8	-28.893	984.506
OP4771																	
Posterior M	81428	440.151	6.775	99.417	37.912	13.677	39.422	0.615	0.864	39.594	554.978	509.935	41.303	15.618	112195.9	-16.644	304.298
Medial M	176903	520.303	13.946	88.081	32.309	19.351	97.866	0.808	0.931	33.185	623.491	445.894	99.275	20.329	132672.8	-34.865	1338.626
Lateral M	160774	566.106	12.773	92.654	34.405	19.634	97.997	0.786	0.926	35.222	762.292	483.665	100.494	20.669	144355.1	-31.912	1092.001
OP4803																	
Anterior M	197618	477.338	16.54	84.107	30.136	19.11	102.729	0.823	0.929	31.052	681.957	428.758	104.6	20.065	121713.8	-29.123	895.251
Posterior M	137978	429.838	12.195	79.24	28.794	18.348	103.266	0.829	0.932	29.587	569.087	429.598	105.074	19.144	109605.5	-17.651	318.337
Medial M	161432	373.685	12.955	74.441	27.453	16.561	102.076	0.812	0.924	28.301	667.889	508.403	103.644	17.469	95279.69	-17.867	378.159
Lateral M	320739	837.439	26.282	119.344	41.911	22.104	68.567	0.714	0.9	43.749	724.141	506.373	68.098	24.154	213452.3	-31.537	1164.692
OP4814																	
Medial M	208147	546.318	16.777	89.963	32.617	20.499	88.152	0.824	0.935	33.538	554.903	483.013	88.747	21.462	139305	-25.5	712.502
OP4820																	
Anterior M	163770	433.254	13.387	81.498	30.497	16.924	103.097	0.788	0.92	31.265	633.37	478.704	103.945	17.973	110475	-16.908	285.154
Posterior M	244104	630.76	20.8	106.405	37.209	19.806	91.823	0.738	0.903	38.911	576.026	387.209	91.154	21.605	160787.1	-26.074	763.953
Medial M	214944	428.175	17.42	81.141	30.126	17.265	100.205	0.798	0.926	30.892	590.104	511	99.582	18.154	109180.6	-23.136	567.502
Lateral M	210581	475.352	17.905	86.108	31.585	18.036	103.318	0.789	0.924	32.327	557.928	505.953	103.366	19.068	121205	-22.611	531.565
OP4864																	
Posterior M	263804	909.669	21.153	136.39	50.296	21.626	59.865	0.656	0.893	52.185	603.941	595.183	59.303	23.881	231933.9	-38.455	1603.72
Medial M	253589	905.675	20.214	127.629	47.152	22.988	62.239	0.708	0.913	48.448	643.982	496.679	61.136	24.795	230911.6	-29.845	994.647

RND Cases	Total Area	Average Size	%Area	Perim.	Major	Minor	Angle	Circ.	Solidity	Feret	FeretX	FeretY	FeretAngle	MinFeret	IntDen	Skew	Kurt
OP5036																	
Anterior M	191655	517.986	16.688	103.268	39.263	15.509	68.684	0.632	0.866	41.142	560.108	530.597	68.121	17.735	132034.9	-23.714	771.834
Posterior M	347080	906.214	27.645	123.88	42.577	24.187	62.088	0.745	0.913	44.237	694.992	504.047	64.043	26.132	231046.6	-32.192	1149.224
Lateral M	218054	714.931	17.704	105.397	38.961	21.978	80.817	0.787	0.93	39.814	703.807	457.433	80.553	23.153	182298.2	-27.745	842.904
OP5109																	
Anterior M	132403	540.42	13.344	92.249	33.862	19.319	97.825	0.778	0.922	34.701	706.065	377.984	99.09	20.445	137799.9	-25.892	695.302
Posterior M	147738	489.199	13.41	103.146	40.587	14.399	75.919	0.607	0.858	42.034	486.417	459.911	77.925	16.486	124727.1	-15.541	256.967
Lateral M	215799	629.152	17.336	114.948	45.495	16.598	67.027	0.628	0.876	46.926	705.169	502.918	68.945	18.639	160429.9	-30.089	920.601
OP5250																	
Anterior M	113254	851.534	14.196	113.395	39.796	25.701	104.796	0.82	0.938	40.756	262.594	389.639	105.903	26.905	217125.8	-27.872	824.529
Posterior M	37960	446.588	4.976	79.504	28.102	19.328	105.102	0.858	0.936	29.013	223.541	507.718	103.213	20.112	113874	-18.868	354.003
OP5304																	
Anterior M	147673	593.064	14.14	104.698	39.231	18.209	87.961	0.683	0.899	40.054	379.855	464.831	90.145	19.702	151202.7	-25.593	722.092
Medial M	152020	586.95	15.832	105.479	39.522	17.771	81.958	0.674	0.898	40.271	345.942	497.008	80.935	19.226	149616.1	-26.215	786.333
OP5375																	
Posterior M	191051	563.572	15.786	89.944	32.082	20.663	87.314	0.823	0.928	33.113	611.723	399.929	85.879	21.593	143705.7	-21.434	495.003
Lateral M	156254	643.021	16.165	99.112	35.386	20.479	82.785	0.772	0.914	36.615	686.959	377.128	80.375	21.74	163875.8	-20.326	552.859
OP5420																	
Anterior M	138266	523.735	11.703	94.374	36.743	17.569	79.483	0.736	0.915	37.358	562.973	467.636	80.707	18.735	133550.5	-31.129	967.001
Posterior M	179939	497.069	17.262	86.34	31.492	19.001	115.753	0.806	0.926	32.461	586.215	541.856	114.999	20.111	126746.3	-19.4	413.379
Medial M	164321	370.928	13.757	76.3	28.387	15.789	84.195	0.781	0.914	29.231	618.962	495.587	83.436	16.939	94575.64	-19.798	435.878
Lateral M	118049	373.573	9.605	74.2	27.959	15.796	79.507	0.793	0.923	28.7	708.519	382.968	79.592	16.65	95258.64	-20.433	418.252
OP5440																	
Anterior M	87058	301.239	11.723	68.349	26.211	13.954	95.061	0.773	0.917	26.79	288.287	497.398	96.3	14.776	76812.35	-21.953	487.169
Medial M	166837	457.088	13.447	83.09	31.164	17.359	81.152	0.78	0.923	31.886	609.871	454.893	80.492	18.323	116551.1	-22.62	606.336
OP5601																	
Anterior M	302639	738.144	24.13	107.392	37.437	23.66	117.493	0.802	0.93	38.507	650.193	469.139	120.524	24.902	188198.1	-27.705	863.9
Posterior M	260675	538.585	20.946	90.483	32.662	20.165	75.421	0.816	0.932	33.479	653.184	506.698	76.636	21.122	137330.1	-24.617	629.835
OP5696																	
Anterior M	97064	557.839	8.119	89.706	33.18	19.764	91.109	0.805	0.932	34.026	631.954	477.103	95.633	20.618	142243.1	-28.58	921.002
Posterior M	241353	459.72	19.474	87.892	33.258	16.561	93.679	0.736	0.907	33.999	625.2	510.051	93.249	17.81	117224.2	-31.875	1086.43
Medial M	218469	514.045	17.45	87.633	32.171	19.581	89.345	0.815	0.932	32.953	628.722	465.828	92.552	20.479	131077.8	-23.018	548.835
Lateral M	265226	439.116	23.922	84.463	30.721	16.973	98.346	0.765	0.909	31.714	517.538	523.113	99.829	18.318	111959.4	-24.748	669.967
OP5745																	
Anterior M	153453	532.823	12.33	88.149	31.672	19.383	83.684	0.815	0.927	32.627	685.208	428.917	86.233	20.513	135847.7	-19.22	388.777
Posterior M	97271	361.602	18.586	73.248	26.325	15.804	77.134	0.802	0.917	27.266	221.193	430.454	76.487	16.791	91926.08	-18.883	471.794
Lateral M	156625	614.216	14.989	99.224	36.986	19.793	58.771	0.755	0.921	37.806	575.863	336.71	60.818	20.968	156598	-20.598	451.184
Op5800																	
Anterior M	50484	286.841	14.824	66.882	25.148	13.505	70	0.778	0.904	25.98	313.148	234.386	69.167	14.596	73124.15	-8.463	71.17
Posterior M	115659	540.463	9.73	87.579	31.662	19.107	96.186	0.8	0.92	32.661	487.981	493.827	95.136	20.174	137812	-17.531	305.42
Lateral M	150408	475.975	11.983	83.801	30.645	18.248	79.085	0.803	0.922	31.62	615.345	517.038	81.685	19.347	121366.3	-21.191	488.211
OP5854																	
Anterior M	171977	607.693	13.88	102.094	40.253	18.318	50.608	0.711	0.913	40.938	587.279	488.622	50.733	19.748	154955.3	-32.944	1163.126
Posterior M	166284	665.136	13.716	97.324	33.311	24.045	94.38	0.849	0.939	34.413	642.916	481.708	98.442	24.983	169591.3	-19.812	479.694

RND Cases	Total Area	Average Size	%Area	Perim.	Major	Minor	Angle	Circ.	Solidity	Feret	FeretX	FeretY	FeretAngle	MinFeret	IntDen	Skew	Kurt
Lateral M	135922	514.856	11.839	86.46	31.705	19.538	60.725	0.82	0.935	32.577	611.451	524.894	63.135	20.423	131285.4	-25.377	642.002
Op5983																	
Anterior M	91460	248.533	7.561	58.275	20.869	13.698	83.332	0.858	0.918	21.715	574.378	379.174	86.621	14.418	63366.11	-18.529	466.408
Medial M	132403	540.42	13.344	92.249	33.862	19.319	97.825	0.778	0.922	34.701	706.065	377.984	99.09	20.445	137799.9	-25.892	695.302
Lateral M	177797	639.558	14.584	99.134	34.75	22.107	53.889	0.818	0.933	35.907	717.824	465.755	56.048	23.226	163067.9	-27.821	895.06
OP6048																	
Anterior M	147399	982.66	13.937	124.372	40.792	26.602	92.032	0.777	0.917	42.544	735.173	502.48	92.417	28.446	250557.9	-37.974	1921.401
Posterior M	69064	874.228	6.787	114.258	40.82	25.665	75.989	0.811	0.935	42.027	667.861	293.468	76.164	26.97	222924.9	-25.788	663.002
Medial M	166719	580.902	14.574	94.673	34.151	20.312	92.802	0.797	0.928	35.148	552.028	469.101	95	21.393	148098.1	-22.869	560.237
OP6138																	
Posterior M	218851	580.507	18.32	92.237	32.295	21.264	105.537	0.824	0.93	33.339	680.682	527.748	105.433	22.313	148003.5	-26.76	788.444
Medial M	211829	520.464	17.07	87.278	30.182	20.259	90.558	0.819	0.925	31.243	678.44	507.243	89.431	21.248	132699	-22.233	517.164
OP6213																	
Posterior M	170798	467.94	15.584	85.148	30.81	18.42	77.164	0.804	0.924	31.82	726.285	472.244	77.161	19.495	119314.8	-26.392	739.902
Medial M	166719	580.902	14.574	94.673	34.151	20.312	92.802	0.797	0.928	35.148	552.028	469.101	95	21.393	148098.1	-22.869	560.237
Lateral M	187839	890.232	21.97	119.061	39.922	26.366	96.528	0.8	0.929	41.226	464.536	464.379	97.876	27.773	226947.6	-23.9	638.783
OP6385																	
Anterior M	110609	727.691	10.853	107.258	40.189	21.965	113.462	0.755	0.927	40.837	638.349	451.743	113.648	23.152	185542.7	-30.216	1037.952
Medial M	127536	768.289	14.109	106.966	38.395	23.971	101.887	0.799	0.938	39.123	558.066	507.614	102.928	24.932	195904.5	-35.31	1270.584
Lateral M	117866	770.366	10.601	109.773	40.521	22.347	122.197	0.768	0.925	41.33	501	503.484	124.774	23.722	196421.7	-31.344	1072.918
OP6084																	
Anterior M	153627	745.762	12.748	109.712	40.927	22.371	53.659	0.772	0.931	41.775	465.33	504.748	51.865	23.756	190154.5	-30.189	1055.268
Posterior M	69064	874.228	6.787	114.258	40.82	25.665	75.989	0.811	0.935	42.027	667.861	293.468	76.164	26.97	222924.9	-25.788	663.002