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Comparative evaluation of Foldscope versus conventional light microscopy for exfoliative cytology in OPMDs

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Abstract

In oral cancer screening camps, diagnosis relies on visual examination. Exfoliative cytology has proven useful, as cellular alterations exhibit distinct histological features even at the early stages. Early detection is crucial for improved outcomes, and chairside adjunct tools can enhance diagnostic accuracy while also serving as educational aids for patients. Foldscope, a low-cost, origami-based portable microscope with 2000× magnification, provides real-time cytological assessment. It serves as an effective tool for bridging diagnostic gaps and enhancing early detection particularly in resource-limited settings. Aims & Objectives of the study is to 1) assess the efficacy of Foldscope as a diagnostic tool for detecting dysplastic changes in exfoliative cytology and 2) to compare its diagnostic accuracy with that of a conventional light microscope in exfoliative cytology. The study population included 30 patients with clinically diagnosed oral potentially malignant disorders (OPMDs) such as leukoplakia, OSMF, Oral Lichen planus. Oral smears were collected and stained using the Rapid, Economic, Acetic acid Papanicolaou (REAP) stain. Exfoliative cytology smears were then examined under both Foldscope and conventional light microscope, followed by cytomorphometric analysis. Comparison of the Nuclear & Cytoplasmic area and N/C ratio between groups revealed no statistically significant differences (p value >0.05) between Conventional Light microscope and Foldscope. These findings suggest that Foldscope is equally effective in detecting dysplastic changes in exfoliative cytology and can serve reliable, low cost alternative, especially in resource constrained setting.

Keywords: Foldscope, light microscope, OPMDs, exfoliative cytology

Introduction

Oral squamous cell carcinoma (OSCC), originating in the oral mucosa, is one of the most prevalent malignancies affecting the head and neck region^[1]. According to the Global Cancer Observatory (GCO), an estimated 377,713 new cases of OSCC were reported globally in 2020, with the highest incidence observed in Asia^[2]. The disease predominantly affects males, particularly those in middle to older age groups. Oral cancer is often preceded by conditions collectively known as oral potentially malignant disorders (OPMDs), which include oral submucous fibrosis, erythroplakia, leukoplakia, oral lichen planus. In 2017, the World Health Organization (WHO) defined OPMDs as “clinical presentations that carry a risk of cancer development in the oral cavity, whether in a clinically normal mucosa or in a clinically definable precursor lesion.”^[3] The malignant transformation rates are approximately 9.5% for leukoplakia, 5.2% for oral submucous fibrosis (OSMF), and 1.39% for oral lichen planus (OLP)^[4-6].

Early detection of OPMDs plays a crucial role in preventing their malignant transformation, with special emphasis on identifying and evaluating epithelial dysplasia, which is regarded as the gold standard for predicting the risk of progression to oral cancer. OED as defined by WHO is a spectrum of architectural and cytological epithelial changes caused by accumulation of genetic changes, associated with an increased risk of progression to squamous cell carcinoma^[7]. Histopathological examination of invasive tissue biopsies remains the standard method for detecting dysplastic changes that precede oral cancer.

However, this approach has several limitations, including the need for technical expertise, reliance on incisional biopsy, tissue processing facilities and longer processing times.

Exfoliative cytology, a non-invasive method, which assist in detection of alterations occurring in cell morphology (cellular and nuclear) through microscope. In 2017, World Health Organization (WHO) proposed several criteria for the characterization of epithelial dysplasia, including abnormalities in nuclear and cell size, and an increased nuclear-to-cytoplasmic (N:C) ratio^[8]. But it is underutilized, mainly due to lack of necessary armamentarium, dependency on microscope in community based oral cancer screening and involves transportation of smear to a laboratory as most clinicians do not have a light microscope in a clinical setup.

Therefore, the development of a cost-effective, efficient, and user-friendly chairside tool is crucial for the early screening and detection of dysplastic lesions. The Foldscope a portable, paper-based microscope offers a promising solution. Functioning similarly to a conventional light microscope, it utilizes visible light and a system of lenses to magnify small objects. This innovative device is versatile, durable, lightweight, and affordable, capable of delivering magnifications ranging from $\times 140$ to $\times 2000$, making it a

valuable tool for point-of-care diagnostics.

Aims & Objectives

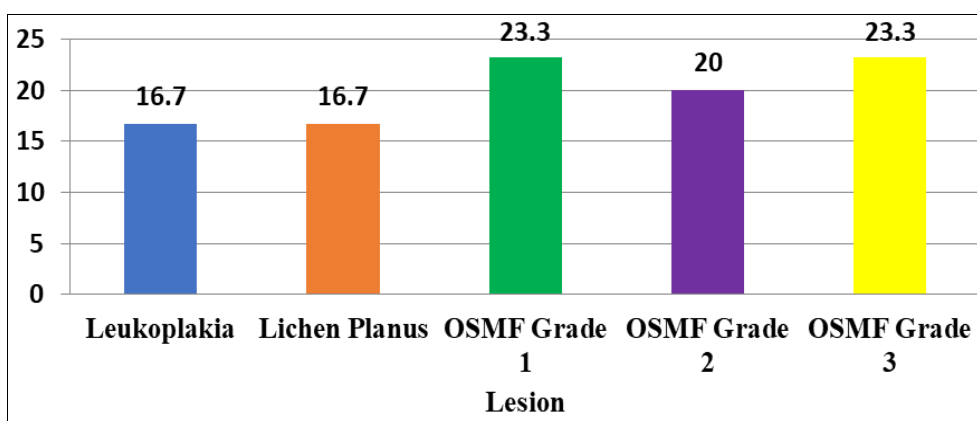
- To assess the efficacy of Foldscope as a diagnostic tool for detecting dysplastic changes in exfoliative cytology.
- To compare the diagnostic accuracy of Foldscope with that of a conventional light microscope in exfoliative cytology.

Methods & Materials

The study population had included 30 patients with clinically diagnosed oral potentially malignant disorders (OPMDs).

Table 1: Frequency distribution of the study participants according to type of lesion

Lesion	Frequency (n)	Percent (%)
Leukoplakia	5	16.7
Lichen Planus	5	16.7
OSMF Grade 1	7	23.3
OSMF Grade 2	6	20.0
OSMF Grade 3	7	23.3
Total	30	100.0



Graph 1: Percentage distribution of the study participants according to type of lesion

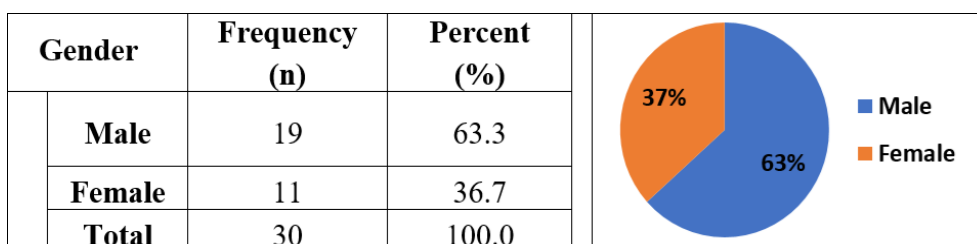


Fig 1: Frequency and percentage distribution of the study participants according to Gender



Fig 2: Leukoplakia involving tongue and buccal mucosa



Fig 3: Reticular Lichen Planus involving buccal mucosa



Fig 4: OSMF showing blanching of buccal mucosa and fibrotic bands with reduced mouth opening

Equipment used

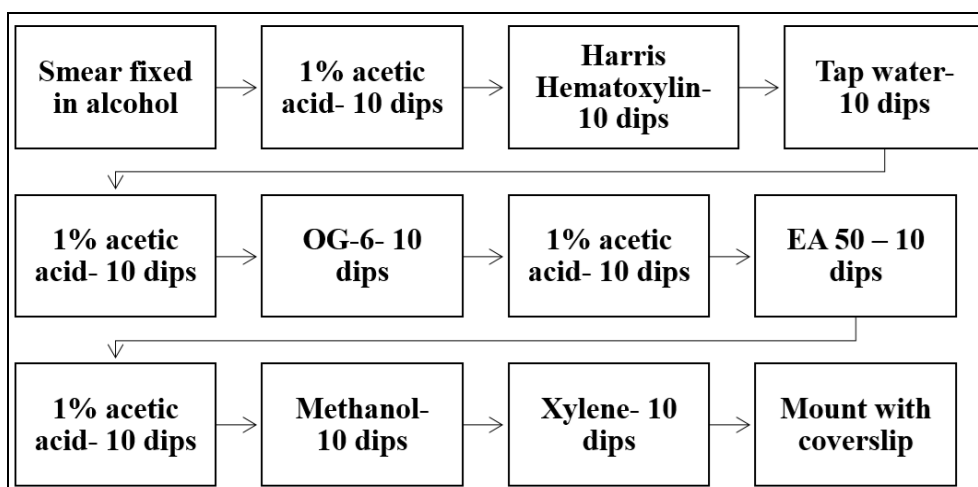
- Cytobrush
- Glass slides
- Spray Fixer (Cytology Fixative)
- Rapid, Economic, Acetic acid and Papanicolaou stain (REAP)
- Foldscope version 2.0
- Compound Light microscope
- ImageJ software version 1.47
- Digital camera - Resolution 48 megapixels.

Procedure

- The study included 30 patients, clinically diagnosed with oral premalignant lesions. At the beginning, the entire study protocol was explained in detail to each participant.

- Cytological smears were then obtained from the lesional areas using a Cytobrush. The brush head was first moistened with water and gently pressed against the mucosal surface. It was then rotated with mild pressure over the lesion for 10 complete turns, until pinpoint bleeding was observed, indicating adequate cell collection.
- The collected cellular material was transferred onto clean glass slides by rolling the Cytobrush in a single continuous motion from one end of the slide to the other.
- The smears were immediately fixed using a spray fixative and subsequently stained with a commercially available REAP staining kit.

REAP staining procedure-



Assembly of the Foldscope

The Foldscope kit included essential components such as paper-based structural elements, a ball lens, button cell battery, surface-mounted LED, switch, copper tape, and

polymeric filters. The Foldscope was assembled according to the step-by-step instructions provided in the user manual, ensuring proper alignment and functionality of all components.

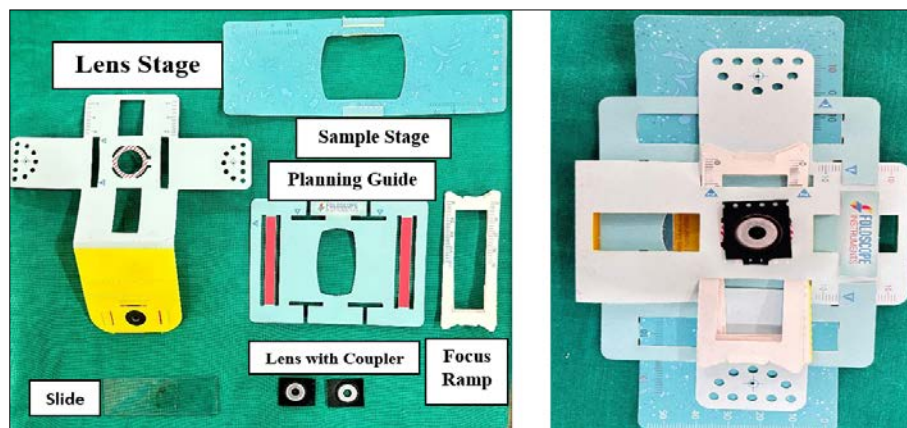


Fig 5: Parts of Foldscope

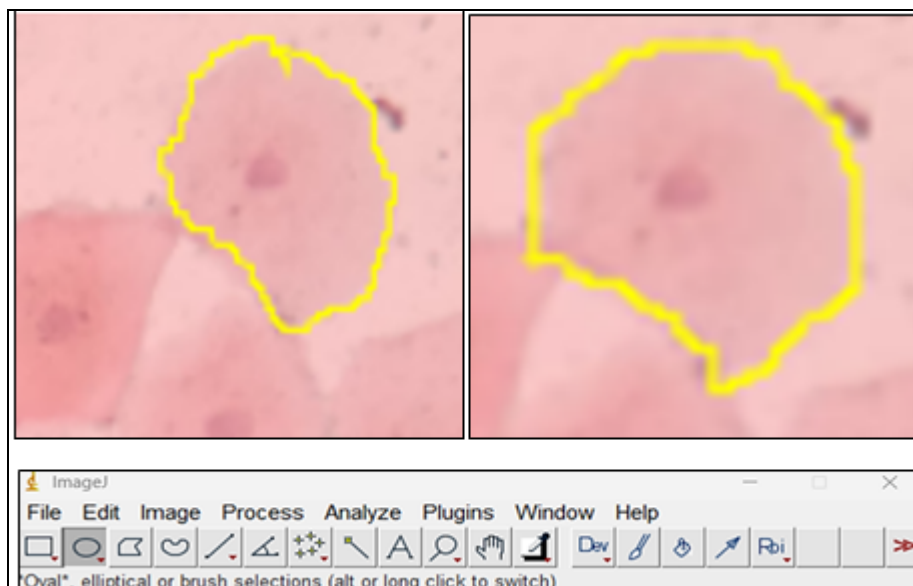


Fig 6: Tracing of cell outline using ImageJ software

Subsequently, the stained smears were examined using both the Foldscope and a conventional light microscope. Photomicrograph of the smears were captured from both devices. These images were then subjected to cytomorphometric analysis to assess parameters like cellular area, and nuclear area and nuclear-cytoplasmic (N/C) ratio using ImageJ software (version 1.54). Digital images were recalled on the monitor, and all measurements were performed using the integrated measuring tools of the image-analysis software. Twenty-five cells with well-defined borders were randomly selected from each section, beginning with the first representative field on the left and sequentially advancing the stage to encompass five fields per section. Stage coordinates were recorded to allow reassessment. Only clearly delineated cells were included, while clumped, folded, or morphologically distorted cells and nuclei were excluded. Cell outlines were traced manually using the mouse pointer, and the nuclear area (NA) and cytoplasmic area (CA) were automatically computed by the software. The nuclear-to-cytoplasmic area ratio (NA/CA) was calculated using the formula:

$$\text{NA/CA ratio} = \text{Nuclear Area} \div \text{Cytoplasmic Area.}$$

All data were tabulated and subjected to appropriate statistical analysis. Statistical analysis was performed using Statistical package for social sciences (SPSS) software (IBM Corp) (v.21.0). Frequency and percentage statistics was performed

of the different parameters in the study. Independent samples t-test proportion was used to assess significant differences between 2 groups. A p value of less than 0.05 was considered as statistically significant at 95% confidence intervals in the study.

Results

This study aimed to assess the effectiveness of the Foldscope as a potential chairside diagnostic tool for identifying cytological changes in potentially malignant oral lesions, in comparison to the conventional light microscope. The primary objective was to evaluate whether the Foldscope could reliably visualize cellular features essential for the early detection of dysplasia.

Observation

Examination of the cytological smears revealed that images captured through the Foldscope demonstrated slightly reduced clarity, lower contrast, and diminished resolution compared to those captured using a standard light microscope. Despite these limitations, the Foldscope provided sufficient detail for visualization of key cellular features. Notably, nuclear morphology including shape and size along with the cytoplasmic boundaries, remained discernible and interpretable. These features are critical for assessing epithelial dysplasia and for determining the risk of malignant transformation.

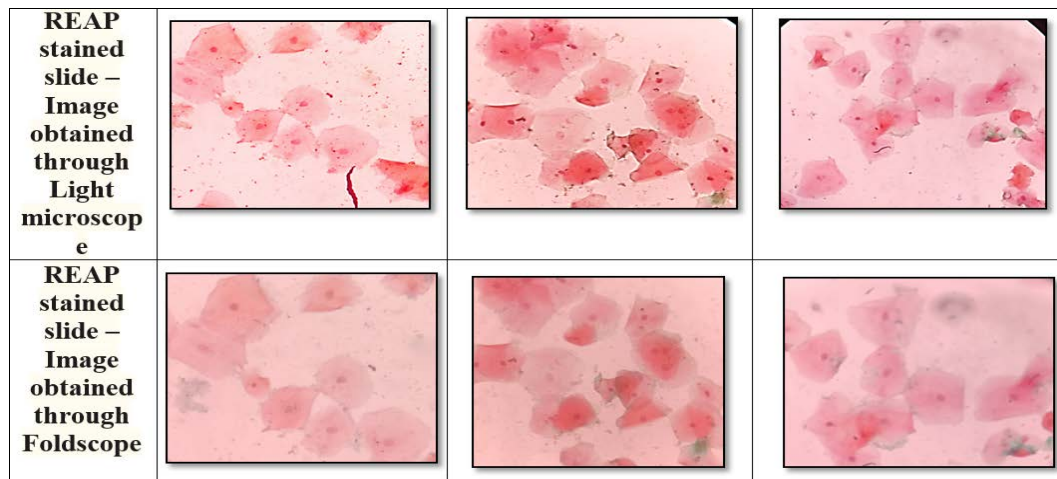


Fig 7: images showing smear of Leukoplakia cases stained by REAP {shows blurring, low contrast and Less resolution}

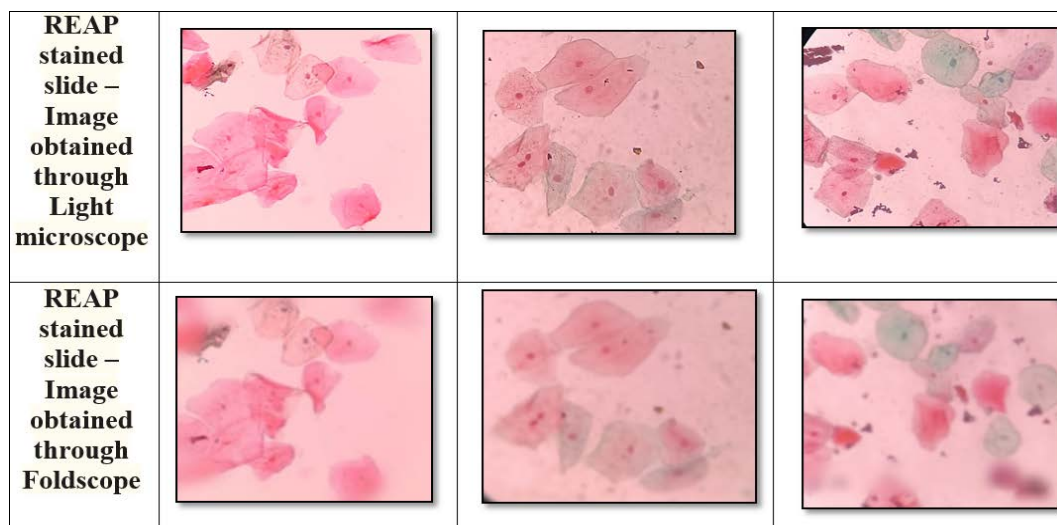


Fig 8: Images showing smear of Oral Lichen Planus cases stained by REAP {shows blurring, low contrast and less resolution}

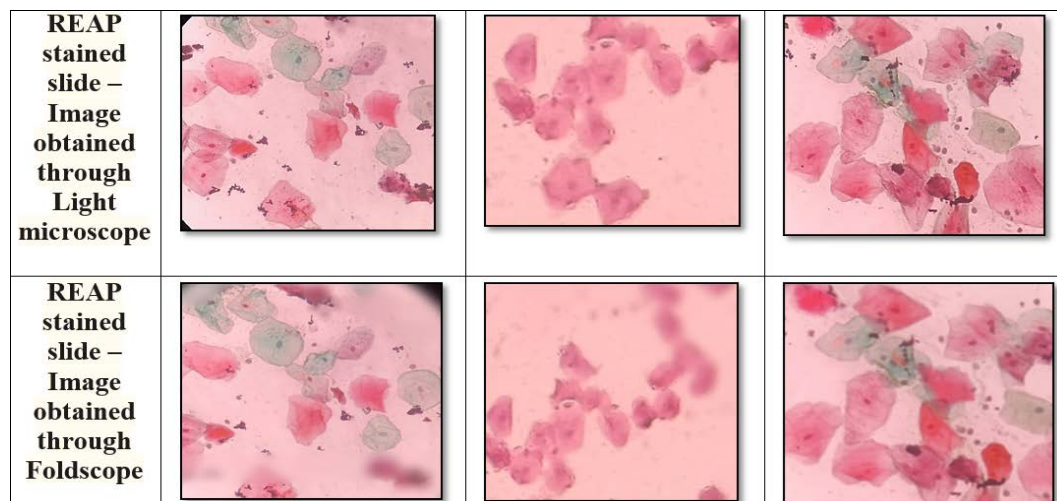
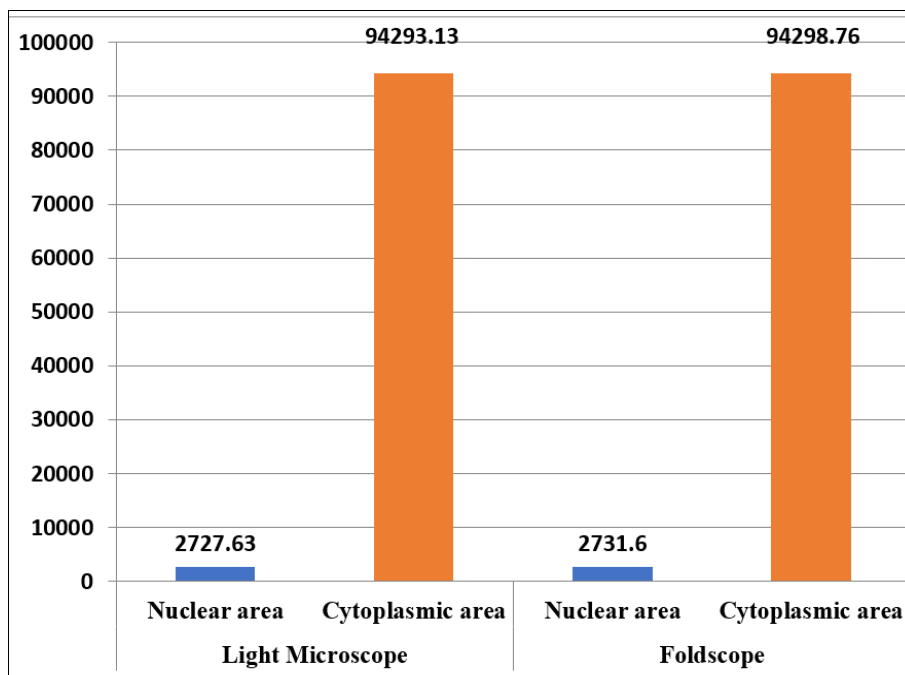


Fig 9: images showing smear of OSMF cases stained by REAP {shows blurring, low contrast and less resolution}

Table 2: Intergroup comparison of the Nuclear area and Cytoplasmic area between Light Microscope and Foldscope

Parameter	Groups	N	Mean	Std. Deviation	p value
Nuclear area	Light Microscope	30	2727.6333um2	1180.45270	.990
	Foldscope	30	2731.6000um2	1180.77600	
Cytoplasmic area	Light Microscope	30	94293.1333um2	21361.11787	1.000
	Foldscope	30	94298.7667um2	21360.26081	

(*p value <0.05 statistically significant, <0.01 highly significant, <0.001 very highly significant)

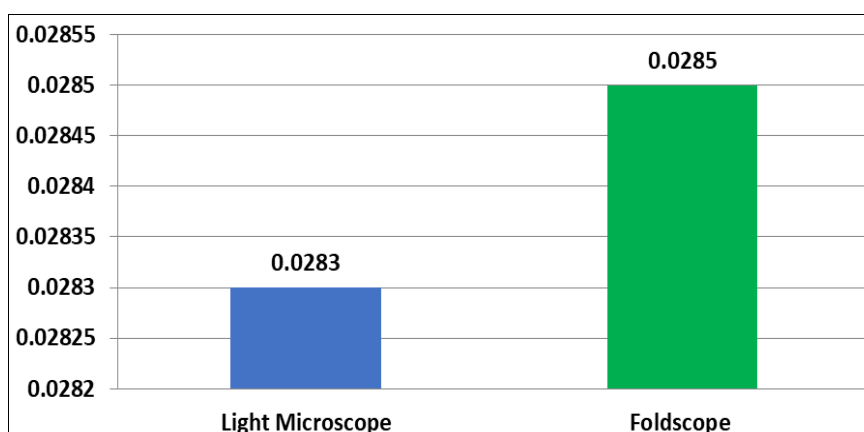


Graph 2: Mean values of Nuclear area and Cytoplasmic area as evaluated by Light Microscope and Foldscope

Table 3: Intergroup comparison of the Nuclear /Cytoplasmic ratio between Light Microscope and Foldscope

Groups	N	Mean	Std. Deviation	Mean difference	t value	p value
Light Microscope	30	.02830	.0122648	-.0002000	-.061	0.951
Foldscope	30	.028500	.0129847			

*p value <0.05 statistically significant, <0.01 highly significant, <0.001 very highly significant



Graph 3: Mean values of Nuclear/Cytoplasmic ratio as evaluated by Light Microscope and Foldscope

Interpretation

In this study, Intergroup comparison of the Nuclear/Cytoplasmic ratio between Light Microscope and Foldscope was conducted using the Independent samples t-test. The analysis did not reveal any statistically significant differences (p value >0.05) between light microscope and foldscope. This suggests that the Foldscope is comparable to conventional light microscope in its ability to detect dysplastic changes in exfoliative cytology.

Discussion

The global incidence of oral cancer has been steadily increasing in recent years. Despite advancements in diagnostic techniques and treatment modalities, the 5-year survival rate for oral cancer has remained relatively unchanged over the past three decades, hovering around 50% [9]. A significant proportion of oral squamous cell carcinomas (OSCC) are preceded by clinically identifiable

oral potentially malignant disorders (OPMDs). The etiological factors contributing to the development of OPMDs and oral cancer are well-established, with tobacco use, alcohol consumption, and areca nut (betel quid) chewing recognized as major risk factors. Early detection of oral premalignant lesions is crucial for reducing morbidity and mortality, as it allows timely clinical intervention before malignant transformation occurs. However, conventional oral examinations have several limitations, including a high rate of false-positive findings. These may lead to psychological distress, overdiagnosis, unnecessary use of healthcare resources, and challenges in accurately identifying the diverse clinical manifestations of premalignant lesions.

The oral mucosa undergoes rapid cellular turnover, and the exfoliated cells provide valuable insights for the early diagnosis of potentially malignant disorders. Oral exfoliative cytology (OEC) involves the microscopic examination of cells shed from the epithelial surface. It is a simple, non-invasive, and cost-effective diagnostic technique that serves

as a useful adjunct to biopsy, particularly in cases where biopsy is not feasible¹⁰. While exfoliative cytology is a valuable diagnostic tool, it does have certain limitations. The procedure necessitates specific armamentarium, access to a microscope, and most importantly, the expertise of a trained oral pathologist for accurate interpretation of smears. These limitations have driven interest in developing diagnostic methods that are simpler, non-invasive, cost-effective, time-efficient, and require minimal equipment at the point of care. In this context, the Foldscope, an origami-style paper microscope emerges as a promising chairside diagnostic tool that may facilitate routine cytological assessment in resource-limited settings.

The Foldscope is an origami-based optical microscope that can be assembled from a flat sheet of paper in under 10 minutes. It is specifically designed for simplicity, portability, and affordability. The device is accompanied by an accessory toolkit containing essential components such as paper slides, plastic coverslips, micro-lenses, and micro-magnets, enabling efficient sample preparation and observation. The Foldscope was invented by Indian-origin researcher Dr. Manu Prakash and his student Dr. Jim Cybulski at the Prakash Lab, Stanford University, USA^[11]. The Foldscope incorporates 140x spherical lens capable of producing high quality magnified images^[12]. Foldscope works on the principle of light magnification using micro-lenses made of boro-silicate or sapphire to produce a virtual, enlarged image of object and viewed directly or through digital device. When used in conjunction with smart phone camera, it can reach magnification of over 2000x, making it suitable for examining cellular structures^[11].

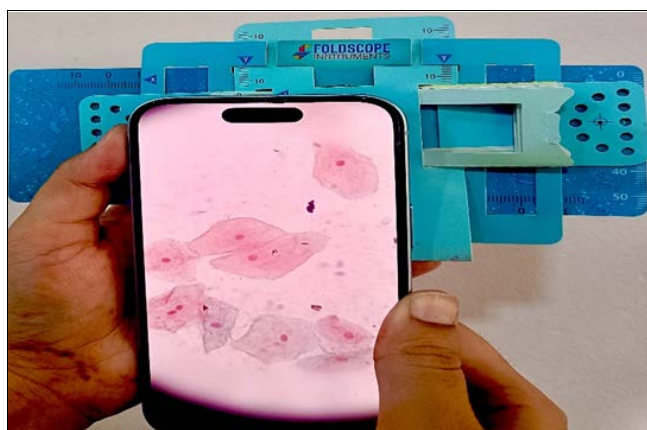


Fig 10: Foldscope attached to smart phone camera

The Foldscope demonstrates promising capabilities when compared to conventional light microscopes, particularly for use in low-resource or chairside diagnostic settings. In terms of numerical aperture (NA), the Foldscope provides a range of 0.25-0.55, which is narrower but comparable to the 0.10-1.40 range of a standard light microscope. The resolution of the Foldscope is approximately 0.5 μm , slightly lower than the 0.25 μm resolution of a traditional light microscope¹¹. However, the Foldscope compensates with a remarkably high magnification range of 140x to 2000x, surpassing the 40x to 1000x range typical of standard microscopes.

The focal length of the Foldscope is fixed at 172 μm , whereas light microscopes have a variable focal length between 2-40 mm. Both devices produce a magnified, virtual, and erect image. In terms of depth of field, the Foldscope has a depth of 2.8 μm , which falls within the lower range of the 0.25-25 μm depth of field offered by light microscopes^[11]. Notably, the Foldscope has a significantly shorter optical path length (2.7 mm) compared to the 160 mm of a light microscope,

enhancing its portability and compact design^[11]. Unlike traditional light microscopes which employ two convex lenses, the Foldscope employs a spherical ball lens or other micro-lenses, which simplifies its structure and allows for easy assembly and use in remote or resource-constrained environments.

This study was undertaken to assess the efficacy of cytomorphometric analysis using Foldscope. Based on subjective evaluation, images obtained with the Foldscope demonstrated certain limitations compared to conventional light microscopy. Specifically, there was a noticeable degree of peripheral blurring, reduced image contrast, and lower overall resolution. These arise primarily from spherical aberration, wherein peripheral rays fail to converge at the same focal point as central rays, and from chromatic aberration, which introduces color fringing and reduces sharpness. Additional contributions from field curvature and coma distort off-axis regions, producing a clear central image but a blurred periphery. The small numerical aperture of the lens further limits light collection, thereby lowering image contrast. Consequently, while sufficient for basic magnification, the Foldscope remains constrained by the inherent aberrations of its simple ball-lens design. These optical constraints directly affect cytological evaluation: peripheral blurring and diminished contrast hinder accurate delineation of nuclear and cytoplasmic borders, while the reduced resolution may obscure fine nuclear details such as chromatin pattern, nucleolar prominence, and subtle membrane irregularities—features essential for detecting dysplastic or malignant changes. Chromatic aberration can also create false color fringes along cellular margins, potentially affecting interpretation of staining intensity. Despite these limitations, the essential diagnostic features, such as nuclear morphology, cellular arrangement, and cytoplasmic boundaries remained sufficiently discernible. Walliulah *et al.* (2018). conducted a study utilizing the Foldscope to compare the morphological features of nonhuman histopathological samples. Their findings revealed that the images obtained using the Foldscope were comparable in morphological detail to those captured through conventional light microscopy¹². These observations highlight the potential of the Foldscope in identifying and grading of epithelial dysplasia in potentially malignant oral lesions. In the present study, Leukoplakia samples demonstrated enlarged nuclei with irregular borders (Fig 10) which are hallmarks of epithelial dysplasia. Notably, these dysplastic changes were clearly appreciable under the Foldscope.

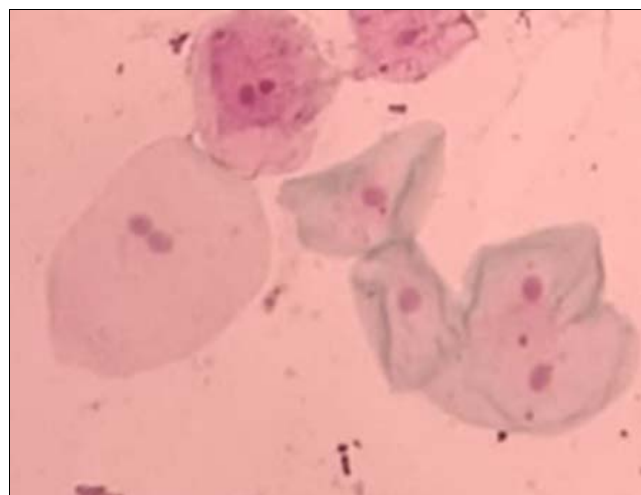


Fig 11: Leukoplakia case showing dysplastic features under Foldscope

Cytomorphometric analysis of images was conducted using ImageJ software. The parameters assessed included Nuclear area, cytoplasmic area and calculating Nuclear/Cytoplasmic ratio, a key parameter in detecting dysplastic transformation. The study findings revealed no statistically significant difference in the measurement obtained using Foldscope and a conventional light microscope (p value - .990, 1.000, 0.951). These findings are consistent with earlier studies conducted by Bruno M. *et al.* ^[14], who evaluated cervical cytological samples and found that the images obtained via the Foldscope closely resembled those captured through traditional optical microscopy, with comparable cytological detail ^[14]. From public health perspective, the Foldscope offers immense value, it weighs less than 8.8g, inexpensive, requires no external power and reduces time consumed for transportation of the slide for microscopic examination. It enables real time on-site cytology assessments, potentially reducing diagnostic delays and improving accessibility in resource limited settings.

Conclusion

Although the Foldscope is limited in certain optical parameters, its portability, affordability and accessibility make it an excellent tool for screening, training and outreach. The findings of this study suggest that the Foldscope holds significant promise as an effective device for mass screening and routine chairside diagnostic evaluation, particularly for the early detection of dysplastic changes in high-risk individuals of OPMDs.

Acknowledgement

Not available

Author's Contribution

Not available

Conflicts of Interest

"The authors declare no conflicts of interest"

Financial Support

Not available

Abbreviations

OPMDs-oral potentially malignant disorders
OSCC-oral squamous cell carcinomas
REAP -Rapid, Economic, Acetic acid Papanicolaou
WHO-World Health Organization
OSMF-oral submucous fibrosis
NA-nuclear area
CA-cytoplasmic area
NA/CA-nuclear-to-cytoplasmic area ratio

References

1. Vigneswaran N, Williams MD. Epidemiologic trends in head and neck cancer and aids in diagnosis. *Oral and Maxillofacial Surgery Clinics of North America*. 2014;26(2):123-141.
2. Romano A, Di Stasio D, Santella A, Gentile C, Serpico R, Lucchese A, *et al.* Noninvasive imaging methods to improve the diagnosis of oral carcinoma and its precursors: state of the art and proposal of a three-step diagnostic process. *Cancers*. 2021;13(11):2864.
3. Singh SP, Ibrahim O, Byrne HJ, Mikkonen JW, Koistinen AP, Kullaa AM, *et al.* Recent advances in optical diagnosis of oral cancers: review and future

perspectives. *Head and Neck*. 2016;38(Suppl 1):E2403-E2411.

4. Gates JC, Brown T, Nguyen S, Patel R, *et al.* Clinical management update of oral leukoplakia: a review from the American Head and Neck Society Cancer Prevention Service. *Head and Neck*. 2025;47(5):733-741.
5. Kujan O, Khattab A, Oliver RJ, Roberts SA, Thakker N, Sloan P. Malignant transformation of oral submucous fibrosis: a systematic review and meta-analysis. *Oral Diseases*. [Year not provided; please verify publication details].
6. Tsushima F, Sakurai J, Uesugi A, Ishikawa T, Ono Y, Ishibashi H, *et al.* Malignant transformation of oral lichen planus: a retrospective study of 565 Japanese patients. *BMC Oral Health*. 2021;21(1):298.
7. Janardhanan M, Radhakrishnan R, Mathew DG, Thomas G, Kumar GS. Epithelial dysplasia as a predictor of malignant transformation in oral potentially malignant disorders: the concepts, controversies, and challenges. *Journal of Head and Neck Physicians and Surgeons*. 2021;9(2):88-93.
8. El-Naggar AK, Chan JKC, Grandis JR, Takata T, Slootweg PJ, editors. *WHO Classification of Head and Neck Tumours*. 4th ed. Lyon: IARC Press; 2017.
9. Jemal A, Murray T, Samuels A, Ghafoor A, Ward E, Thun MJ. *Cancer statistics, 2004*. CA: A Cancer Journal for Clinicians. 2004;54(1):8-25.
10. Kumar S, Vezhavendhan N, Priya S. Role of oral exfoliative cytology in oral leukoplakia and squamous cell carcinoma. *International Journal of Clinical Dental Sciences*. 2011;2(1):93-97.
11. Cybulski JS, Clements J, Prakash M. Foldscope: origami-based paper microscope. *PLoS One*. 2014;9(6):e98781.
12. Waliullah AS. Application of mobile phone-based portable microscopy in clinical histopathology: a feasibility study. *International Journal of Clinical and Biomedical Research*. 2018;4(1):15-20.
13. Rameshbabu R, Priya AH, Muthukumar RS, Sivaraman K, Uthra D. Evaluation of efficacy of Foldscope—a paper microscope to be used as a chairside diagnostic tool in oral dysplastic lesions: a comparative study. *Contemporary Clinical Dentistry*. 2021;12(4):352-358.
14. Moya-Salazar J, Bruno M, Rojas-Zumaran V, Bhamla S, Prakash M. Implementation of the Foldscope as a cervical cancer diagnostic device: a verification and evaluation study. *Mexican Journal of Clinical Pathology and Laboratory Medicine*. 2016;63(3):141-147.

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