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A Comparative Study of Efficacy and Acceptability of a new Paradigm Foldscopy and Conventional Microscopy for analysis of Goblet Cells in patients with Limbal Stem Cell Deficiency

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Abstract

Background

Assessment of conjunctival goblet cells by impression cytology is an established diagnostic approach for evaluating ocular surface disorders, particularly limbal stem cell deficiency (LSCD), dry eye disease (DED), and Stevens-Johnson syndrome (SJS). Conventional light microscopy remains the gold standard; however, its use is limited in resource-constrained settings because of high equipment costs and the requirement for trained personnel. Foldscope, an inexpensive origami-based paper microscope, has emerged as a promising point-of-care diagnostic device.

Purpose

To evaluate the efficacy and acceptability of Foldscope compared with conventional bright field microscopy for the assessment of conjunctival goblet cells in patients with ocular surface disorders.

Methods

Fourteen patients (28 eyes) diagnosed with ocular surface disease were prospectively enrolled. Conjunctival impression cytology specimens were collected and stained with haematoxylin and eosin (H&E). Slides were independently evaluated using Foldscope and a high-resolution Zeiss automated virtual bright field microscope (automated Digital virtual Slide Imaging System). Goblet cell density, epithelial morphology, and cytological alterations were graded according to Nelson's grading system.

Results

Among the 14 patients (28 eyes), four were diagnosed with Stevens–Johnson syndrome, while the remaining had dry eye disease. Females predominated (10/14). Reduced goblet cell density was observed in four eyes, while complete goblet cell loss was detected in eight eyes. Goblet cell density demonstrated an inverse association with disease severity. Foldscope successfully identified epithelial morphology and goblet cells in most specimens, although image quality and field of view were inferior to conventional microscopy.

Conclusions

Foldscope demonstrates considerable potential as a portable, low-cost screening tool for conjunctival goblet cell assessment in ocular surface diseases. Although it cannot currently replace conventional microscopy for definitive diagnosis or disease grading, it may provide a valuable alternative in low-resource settings. Strengths First comparative evaluation of Foldscope for conjunctival impression

cytology. Direct comparison with a Zeiss automated virtual microscope. Assessment of clinically relevant ocular surface disorders (DED, LSCD, and SJS). Demonstrates the potential of an affordable, portable imaging platform for point-of-care ophthalmic diagnostics.

Strengths

- First comparative evaluation of Foldscope for conjunctival impression cytology.
- Direct comparison with a Zeiss automated virtual microscope.
- Assessment of clinically relevant ocular surface disorders (DED, LSCD, and SJS).
- Demonstrates the potential of an affordable, portable imaging platform for point-of-care ophthalmic diagnostics.

Introduction

The ocular surface is composed of the cornea, conjunctiva, tear film, lacrimal glands, meibomian glands, and limbal stem cells, which function together to maintain optical clarity and epithelial homeostasis. Limbal stem cells, located at the corneoscleral junction, continuously regenerate the corneal epithelium and establish a functional barrier preventing conjunctival epithelial migration onto the corneal surface[1, 2]. Damage or depletion of these stem cells results in limbal stem cell deficiency (LSCD), characterized by conjunctivalization of the cornea, chronic inflammation, neovascularization, recurrent epithelial defects, and progressive visual impairment [1, 3].Conjunctival goblet cells play an essential role in maintaining tear film stability through secretion of gel-forming mucins, primarily MUC5AC. Goblet cell depletion is a hallmark of several ocular surface disorders, including dry eye disease, Stevens-Johnson syndrome [3–5]. Consequently, evaluation of goblet cell density has become an important diagnostic indicator of ocular surface health.Conjunctival impression cytology is a minimally invasive technique widely employed for assessing epithelial morphology, goblet cell density, and squamous metaplasia[6, 7]. Nelson's grading system remains one of the most widely accepted methods for interpreting impression cytology findings.

Despite its diagnostic utility, conventional bright field microscopy requires sophisticated laboratory infrastructure and trained personnel, limiting its application in rural healthcare settings. Foldscope is an ultra-low-cost paper microscope developed using origami engineering principles and is capable of achieving approximately 140X magnification with nearly 2 µmoptical resolution(Fig. 1). Because of its portability, affordability, and smartphone compatibility, Foldscope has emerged as a promising tool for point-of-care diagnostics in low-resource environments[8, 9].The present study aimed to compare the performance of Foldscope with conventional high-resolution bright field microscopy in evaluating conjunctival goblet cells and epithelial morphology in patients with ocular surface disorders.

Materials and Methods

Study Design

This prospective observational study was conducted to compare the diagnostic efficacy of Foldscope with conventional bright field microscopy for the evaluation of conjunctival goblet cells in patients with ocular surface disorders. The study enrolled 14 consecutive patients (28 eyes) diagnosed with ocular surface diseases, including dry eye disease (DED), and Stevens-Johnson syndrome (SJS). The participants ranged in age from 18 to 65 years (mean age: 28 years), with a female-to-male ratio of 10:4. Informed consent was obtained from all participants prior to enrolment, and the study adhered to the principles of the Declaration of Helsinki.

Clinical Ophthalmic Evaluation

All participants underwent a comprehensive ophthalmic examination. Best-corrected visual acuity (BCVA) was measured using a Snellen visual acuity chart. Slit-lamp bio-microscopy was performed to assess the ocular surface, conjunctiva, cornea, and eyelid abnormalities. Tear film evaluation included Schirmer's test without topical anaesthesia to assess aqueous tear production and tear break-up time (TBUT) to evaluate tear film stability according to the TFOS DEWS II recommendations. Meibomian gland morphology was assessed using infrared meibography, and lipid layer thickness was measured using a non-invasive tear film interferometer.

Conjunctival Impression Cytology

Conjunctival impression cytology specimens were collected from the bulbar conjunctiva using sterile cellulose acetate membrane filters following standard procedures. Briefly, topical anaesthetic was instilled before gently applying the membrane filter onto the superior or temporal bulbar conjunctiva for a few seconds with mild pressure. The membrane was carefully removed, immediately fixed in 95% ethanol, and processed for histological examination.

Histological Staining

The collected impression cytology specimens were stained using the haematoxylin and eosin (H&E) staining protocol following standard histopathological procedures. Stained slides were air-dried, mounted using DPX mounting medium, and prepared for microscopic evaluation.

Foldscope Imaging

The stained impression cytology slides were examined using a Foldscope (Foldscope Instruments Inc., USA), an origami-based paper microscope equipped with a high-resolution spherical lens providing approximately 140× optical magnification and a spatial resolution of approximately 2 µm. The Foldscope

was coupled with a smartphone camera for image acquisition. Multiple representative microscopic fields were manually focused and digitally captured for subsequent evaluation. Particular attention was paid to epithelial cell morphology and goblet cell visualization.

Conventional Bright-field Microscopy

The same stained slides were independently examined using a Zeiss automated virtual bright field microscope. Whole-slide digital scanning was performed to obtain high-resolution virtual images with automated focusing, enabling detailed visualization of epithelial morphology and goblet cell distribution across the entire specimen.

Cytological Evaluation

All slides were independently evaluated using both imaging platforms. Conjunctival epithelial morphology, goblet cell density, nuclear-to-cytoplasmic ratio, cellular enlargement, and squamous metaplasia were assessed according to Nelson's grading system. Goblet cell density was qualitatively categorized as normal, decreased, or absent, and the cytological findings were correlated with the clinical severity of ocular surface disease.

Statistical Analysis

Descriptive statistics were used to summarize demographic characteristics, clinical findings, and cytological observations. Continuous variables are presented as mean ± standard deviation or median (range), whereas categorical variables are expressed as frequencies and percentages. The findings obtained using Foldscope were qualitatively compared with those obtained using the Zeiss automated bright field microscope.

Results

Among the fourteen enrolled patients, four (28.6%) were diagnosed with Stevens-Johnson syndrome, while ten (71.4%) had dry eye disease. Female predominance was observed among patients with dry eye disease.

Schirmer's test demonstrated aqueous tear deficiency in 23 eyes, including ten eyes with values below 10 mm/5 min and thirteen eyes with values below 5 mm/5 min. Tear film instability was evident in 17 eyes with TBUT below 5 seconds and in an additional nine eyes with TBUT below 10 seconds, indicating significant ocular surface dysfunction.

Meibomian gland dysfunction was identified in eighteen eyes. Complete gland dilatation was observed in eight eyes, gland dropout in three eyes, and gland truncation in seven eyes. Lipid layer thickness ranged from 26–100 nm, with abnormal values (< 60 nm) observed in seventeen eyes.

Impression cytology revealed decreased goblet cell density in four eyes and complete goblet cell absence in eight eyes. Goblet cell density decreased progressively with increasing disease severity, consistent with previous observations in dry eye disease and limbal stem cell deficiency.

Foldscope successfully visualized conjunctival epithelial cells and goblet cells in the majority of stained preparations (Fig. 2A-F). However, compared with the Zeiss automated microscope (Fig. 3A-D), Foldscope exhibited lower image resolution, a narrower field of view, inconsistent focusing across the specimen, and greater dependence on manual alignment.

Table 1
Nelson's Grading System for Conjunctival Impression Cytology.

Grade	Goblet Cell Characteristics	Cytological Features
Grade 0 (Normal)	Goblet cells are abundant (plentiful), plump, and oval in shape.	Normal conjunctival epithelium with healthy goblet cell population.
Grade 1 (Mild)	Goblet cells decrease in number but maintain their plump, oval morphology.	Mild reduction in goblet cell density without significant morphological alteration.
Grade 2 (Moderate)	Goblet cells are further decreased in number, appear smaller, and exhibit well-defined cellular borders.	Moderate squamous metaplasia with reduced goblet cell size and density.
Grade 3 (Severe)	Goblet cells are completely absent.	Severe squamous metaplasia with total loss of goblet cells.

Discussion

The present study evaluated the feasibility and diagnostic utility of Foldscope, an ultra-low-cost origami-based microscope, for the assessment of conjunctival impression cytology in patients with ocular surface disorders (Table 1). To our knowledge, this is among the first studies to investigate the application of Foldscope for visualization of conjunctival epithelial morphology and goblet cells in patients with dry eye disease (DED), Stevens–Johnson syndrome (SJS), and limbal stem cell deficiency (LSCD). Our findings demonstrate that Foldscope is capable of identifying conjunctival epithelial cells and goblet cells from stained impression cytology specimens, indicating its potential as a portable screening tool for ocular surface diseases. However, conventional high-resolution brightfield microscopy remains superior for detailed cytological evaluation, disease grading, and definitive diagnosis[3, 8, 9].

The integrity of the ocular surface depends on a functional tear film, healthy conjunctival epithelium, and an adequate population of goblet cells. Goblet cells synthesize and secrete the gel-forming mucin MUC5AC, which contributes to tear film stability, lubrication, and protection against environmental insults and microbial pathogens [4]. A reduction in goblet cell density is a well-recognized pathological hallmark of dry eye disease, LSCD, Stevens–Johnson syndrome, ocular cicatricial pemphigoid, and other chronic inflammatory ocular surface disorders [3, 5, 9, 10]. In agreement with these observations, our study demonstrated a progressive decline in goblet cell density with increasing disease severity,

including complete goblet cell loss in eight eyes. These findings further support the established role of impression cytology as an objective method for evaluating ocular surface damage and monitoring disease progression. Impression cytology has been extensively validated as a minimally invasive technique for assessing conjunctival epithelial morphology, goblet cell density, squamous metaplasia, and inflammatory alterations[5–7].

Nelson's grading system remains one of the most widely accepted methods for cytological evaluation of ocular surface disorders and has been adopted in numerous clinical studies [6]. In the present investigation, the same grading criteria were applied to images obtained using both Foldscope and conventional microscopy, allowing direct comparison between the two imaging platforms. The ability of Foldscope to detect clinically relevant cytological features suggests that this low-cost device can provide meaningful diagnostic information despite its optical limitations.

Foldscope was originally developed by Cybulski and colleagues as an inexpensive paper microscope intended to democratize microscopy by making it accessible in resource-limited environments [8]. Since its introduction, Foldscope has been successfully applied in the diagnosis of parasitic infections, microbiological investigations, environmental monitoring, histopathology, and science education. Several studies have highlighted its usefulness for point-of-care diagnostics owing to its portability, ease of assembly, low manufacturing cost, and compatibility with smartphone-based imaging systems [8, 9]. Nevertheless, its application in ophthalmology remains limited. The present study extends the potential clinical utility of Foldscope by demonstrating its ability to visualize conjunctival goblet cells and epithelial morphology in impression cytology specimens, thereby expanding its application to ocular surface diagnostics.

One of the principal advantages observed in this study was the portability and affordability of Foldscope. Unlike conventional laboratory microscopes, which require expensive optical systems, dedicated laboratory infrastructure, regular maintenance, and trained personnel, Foldscope can be assembled within minutes, transported easily, and integrated with a smartphone for digital image acquisition [9]. These characteristics make it particularly suitable for community-based eye screening programmes, tele ophthalmology initiatives, primary healthcare centres, and outreach services in rural and resource-constrained settings. Furthermore, smartphone-enabled image acquisition facilitates remote consultation and image sharing with ophthalmologists, thereby improving access to specialistcare in underserved populations[11, 12].

Our findings indicate that Foldscope successfully identified goblet cells and epithelial morphology in the majority of stained specimens. However, several technical limitations were encountered during image acquisition. Compared with the Zeiss automated virtual microscope, Foldscope produced images with lower optical resolution, a substantially narrower field of view, and reduced image uniformity. Manual focusing frequently resulted in portions of the specimen remaining out of focus, limiting comprehensive evaluation of the entire impression cytology membrane. In addition, stabilization of the specimen slide and maintenance of precise alignment between the Foldscope optics and smartphone camera proved

technically challenging, often resulting in asymmetrical focusing and inconsistent image acquisition. Similar optical and mechanical limitations have been reported in previous evaluations of Foldscope for biological and clinical applications [13].

Conventional automated brightfield microscopy demonstrated clear advantages over Foldscope in all aspects of image quality. Whole-slide digital scanning enabled rapid examination of the complete impression cytology specimen, while automated focusing and high numerical aperture optics generated consistently sharp images across the entire slide. These characteristics greatly facilitate accurate goblet cell quantification, assessment of epithelial morphology, and identification of subtle cytological abnormalities. Consequently, although Foldscope may provide sufficient image quality for preliminary screening, conventional microscopy remains indispensable for definitive diagnosis, disease grading, and research applications [6, 14].

An important observation in the present study was the inverse relationship between goblet cell density and disease severity. Goblet cell depletion has been consistently associated with tear film instability, increased ocular surface inflammation, epithelial squamous metaplasia, and progressive ocular surface damage in patients with dry eye disease and limbal stem cell deficiency[3–5, 10]. Therefore, the ability of Foldscope to identify goblet cell loss may provide clinically relevant information during initial patient assessment, particularly where conventional microscopy is unavailable.

The present study also highlights several opportunities for future technological development. Incorporation of higher-quality optical lenses, improved illumination systems, enhanced specimen stabilization mechanisms, and dedicated smartphone adapters may substantially improve Foldscope performance. Recent advances in computational imaging and artificial intelligence (AI)-based image enhancement offer additional opportunities to overcome current optical limitations[15, 16]. Smartphone-acquired Foldscope images could potentially be enhanced using deep learning-based super-resolution algorithms or automated segmentation techniques for goblet cell detection and quantification. Integration with cloud-based tele-ophthalmology platforms may further facilitate remote diagnosis and expand access to ophthalmic care in underserved populations [8, 9, 13].

Despite these promising findings, several limitations should be acknowledged. First, the sample size was relatively small, consisting of only fourteen patients(28 eyes), thereby limiting the statistical power and generalizability of the results. Second, the study primarily assessed the qualitative capability of Foldscope to visualize goblet cells and epithelial morphology without performing quantitative agreement analysis between Foldscope and conventional microscopy. Future investigations should include statistical methods such as Cohen's kappa coefficient, intraclass correlation coefficient, Bland-Altman analysis, and receiver operating characteristic (ROC) curve analysis to determine diagnostic agreement, reproducibility, sensitivity, and specificity[17, 18]. Third, only haematoxylin and eosin-stained specimens were evaluated. Future studies employing Periodic Acid-Schiff (PAS) staining, Papanicolaou staining, or immuno-cytochemical markers including MUC5AC, cytokeratin 3, and cytokeratin 19 may provide more comprehensive validation of Foldscope for ocular surface diagnostics [5–7].

Despite these limitations, our study provides important preliminary evidence supporting the clinical feasibility of Foldscope for ocular impression cytology. Very few published studies have specifically investigated the application of Foldscope for conjunctival goblet cell assessment in ocular surface disorders. Consequently, our findings contribute to the growing body of literature on frugal diagnostic technologies and demonstrate a novel ophthalmic application of Foldscope. Considering the increasing global burden of dry eye disease and ocular surface disorders, particularly in low- and middle-income countries where access to conventional microscopy remains limited, affordable diagnostic technologies such as Foldscope may substantially improve community-based eye care services [4, 8, 10].

Overall, Foldscope should not currently be regarded as a replacement for conventional laboratory microscopy. Rather, it should be considered a complementary diagnostic platform that offers substantial advantages in portability, affordability, simplicity, and accessibility[19, 20]. With continued technological refinement and validation in larger multicentre studies, Foldscope has the potential to become an effective point-of-care imaging device for the evaluation of ocular surface diseases and impression cytology specimens [8, 9].

Conclusion

Foldscope is a portable, inexpensive, and user-friendly microscopy platform capable of detecting conjunctival epithelial cells and goblet cells in patients with ocular surface disease. Its low cost, minimal infrastructure requirements, and rapid image acquisition make it particularly suitable for preliminary screening in low-resource settings.

Although Foldscope demonstrated good capability for identifying goblet cells, it was inferior to conventional automated microscopy in terms of image resolution, field of view, and overall diagnostic accuracy. Therefore, Foldscope should currently be considered an adjunctive screening tool rather than a replacement for conventional microscopy. Further technological refinement and validation in larger multicentre studies are required before routine clinical implementation.

Declarations

Human Ethics approval: Human Ethical approval for the study was obtained from the Institutional Ethics Committee (IEC) All India Institute of Medical Sciences (AIIMS) New Delhi, with reference number IEC-421/03.08.2018.

Consent to participate: Written informed consent was obtained from all the Human participants prior to their enrolment and participation in the study.

Clinical Trial Number: NA

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Conflict of Interest

The authors have acknowledged no competing financial or non-financial interests.

Consent for publication: Not Applicable

Availability of data and materials

All the analysed data during the current study are available and can be provided from the corresponding author upon reasonable request.

Authors' contributions

Sah R, Tittiyal JS, N Sharma, S Sen, Singh DK, K Kant and N Chourasia reviewed and approved the final version of the case report and accept responsibility for its content

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Figures



Figure 1

Foldscope-based imaging device for microscopic visualization.

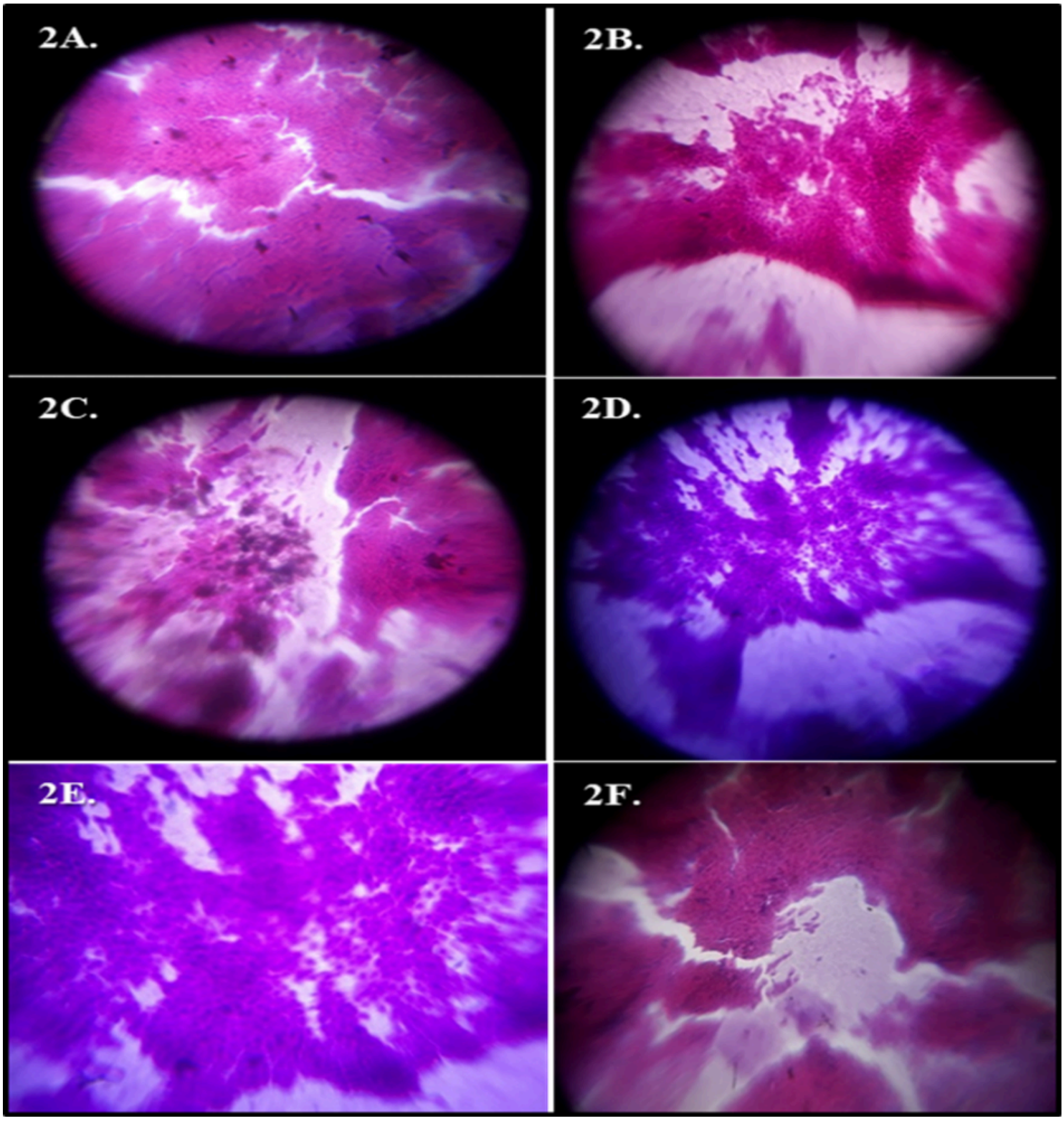


Figure 2

Representative Foldscope Images of Conjunctival Impression Cytology Specimens Showing Varying Degrees of Epithelial Morphology and Goblet Cell Distribution.

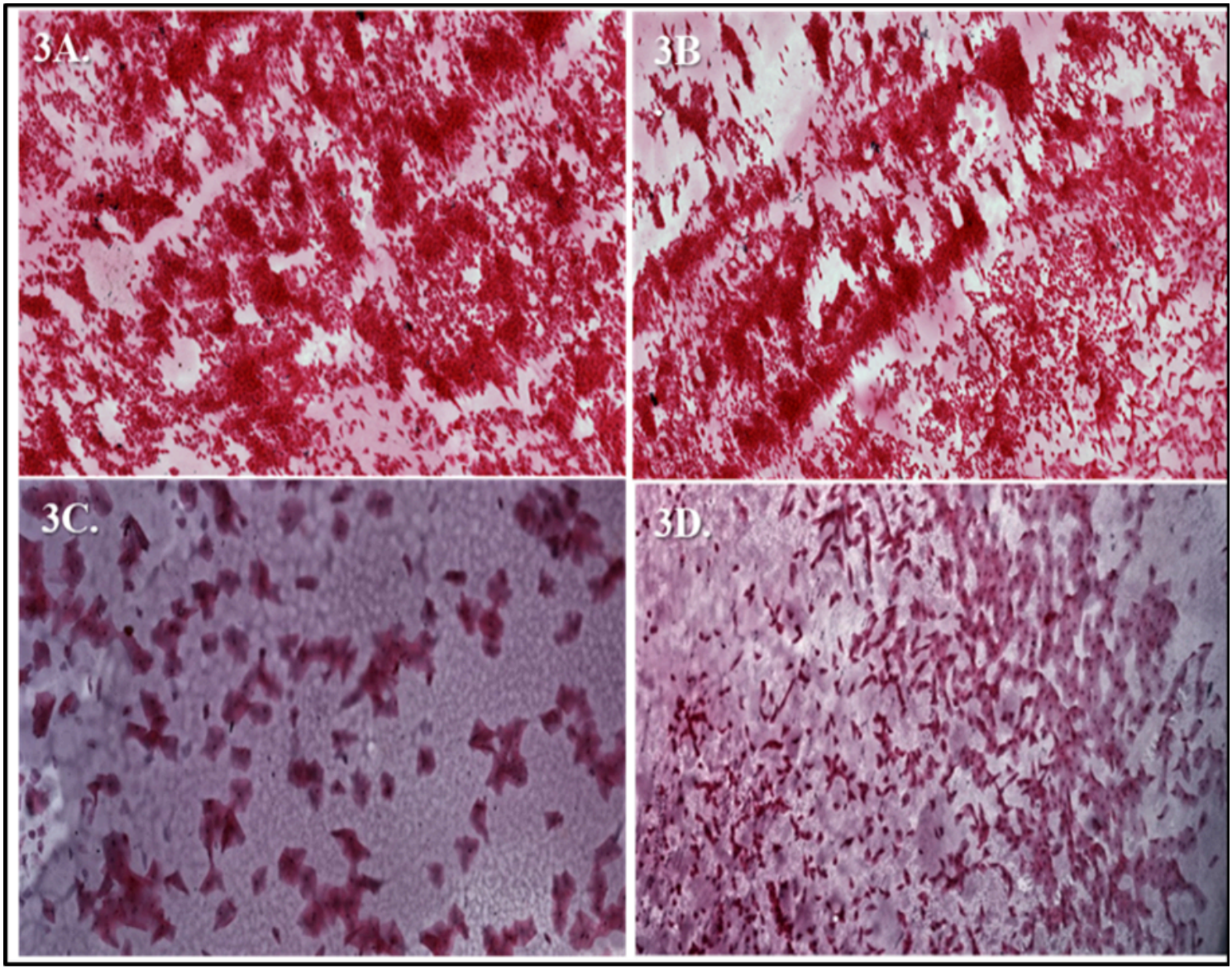


Figure 3

Representative Bright field Microscope Images of Haematoxylin and Eosin-Stained Conjunctival Impression Cytology Specimens