

Foldscope: A smartphone based diagnostic tool for fungal keratitis

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Purpose: Smartphone-based microscopy tool like foldscope (FS) may serve the purpose of a low-cost diagnostic alternative to the compound light microscope especially in areas with limited resources. The purpose of this study was to detect fungal pathogens causing keratitis on direct smear by smartphone-mounted FS and to evaluate the efficacy of FS against routine compound light microscope (CLM). **Methods:** The prospective study was conducted at a tertiary eye care center from September 2019 to March 2020. The study included 60 smear examinations (Gram stain [GM] $n = 30$, Lactophenol Cotton Blue [LCB] $n = 30$) to detect fungal pathogens from corneal scraping material of clinically suspected fungal keratitis (FK) cases. The diagnostic utility of FS was compared with CLM for both GM and LCB wet mount. Data collected were used to quantify the agreement using Cohen's kappa between CLM and FS imaging. **Results:** Forty-six samples out of 60 were positive for fungi using CLM. GM stain and LCB showed 22/30 (73.33%) and 24/30 (80%) positive results with CLM, respectively. Moderate agreement (0.49) was observed between CLM and FS with the smartphone method. LCB mount showed high specificity of 1.00 over 0.87 of GM stain for FS with the smartphone. **Conclusion:** Direct smear can be an early and sensitive measure to diagnose FK other than clinical suspicion. The smartphone-mounted FS has limited sensitivity as an alternative to CLM, but excellent specificity in the present study for FK. The FS as a smartphone-based diagnostic tool is simple, portable, and inexpensive in resource-constrained rural or remote clinical and public health settings in the absence of CLM and other higher diagnostic modalities.

Key words: Foldscope, fungal keratitis, smartphone-based microscopy

Microbial keratitis (MK) is a major contributing pathology of corneal blindness especially in developing countries like India.^[1] Of the organisms that cause keratitis, fungi remain one of the most elusive and challenging organisms to diagnose and treat, especially in underserved areas due to limited access to diagnostic and treatment care facilities and economic constraints. This often results in late referrals to the higher centers with sight-threatening complications. Direct microscopy is an integral tool for rapid diagnosis of infectious keratitis; however, this basic technology is not routinely available in health centers of rural or underserved areas. Smartphone-based microscopes are being used as low-cost devices for evaluation or providing diagnostic support at the point of care.^[2-6] The foldscope (FS) is an origami-based optical microscope (no financial interest) that can be assembled from a flat sheet of paper in under 10 minutes.^[7,8]

To the best of the author's knowledge, the role of FS for the detection of fungal pathogens on direct smear for fungal keratitis (FK) has been explored but not published in the peer-reviewed literature in ophthalmology till the present date.^[9] Hence, the present study aims to detect fungal pathogens causing keratitis on direct smear with smartphone-mounted FS

and to evaluate the efficacy of FS in comparison to a routine compound light microscope (CLM)

Methods

Study design

The prospective observational and comparative study was conducted at a tertiary eye care center between September 2019 and March 2020 in accordance with the institutional ethics committee (ECR/72/Inst/GJ/2013/RR-2019) and the Declaration of Helsinki.

A total of 60 corneal scraping specimens were collected from clinically suspected patients of FK for diagnostic purposes. They were analyzed on direct smear using Gram stain (GM stain, $n = 30$) and lactophenol cotton blue (LCB, $n = 30$) to observe the presence of fungal filaments. The use of GM stain for the detection of fungal elements is well-known. In our study, LCB was preferred as a wet mount for easy identification of fungi as stained pathogens in smear over potassium hydroxide (KOH) as KOH does not stain structures of fungi.^[10-13] The FS has 2 μm resolution which is not sufficient to observe the organisms of less than 2 microns (e.g. Bacteria) hence detection of bacterial

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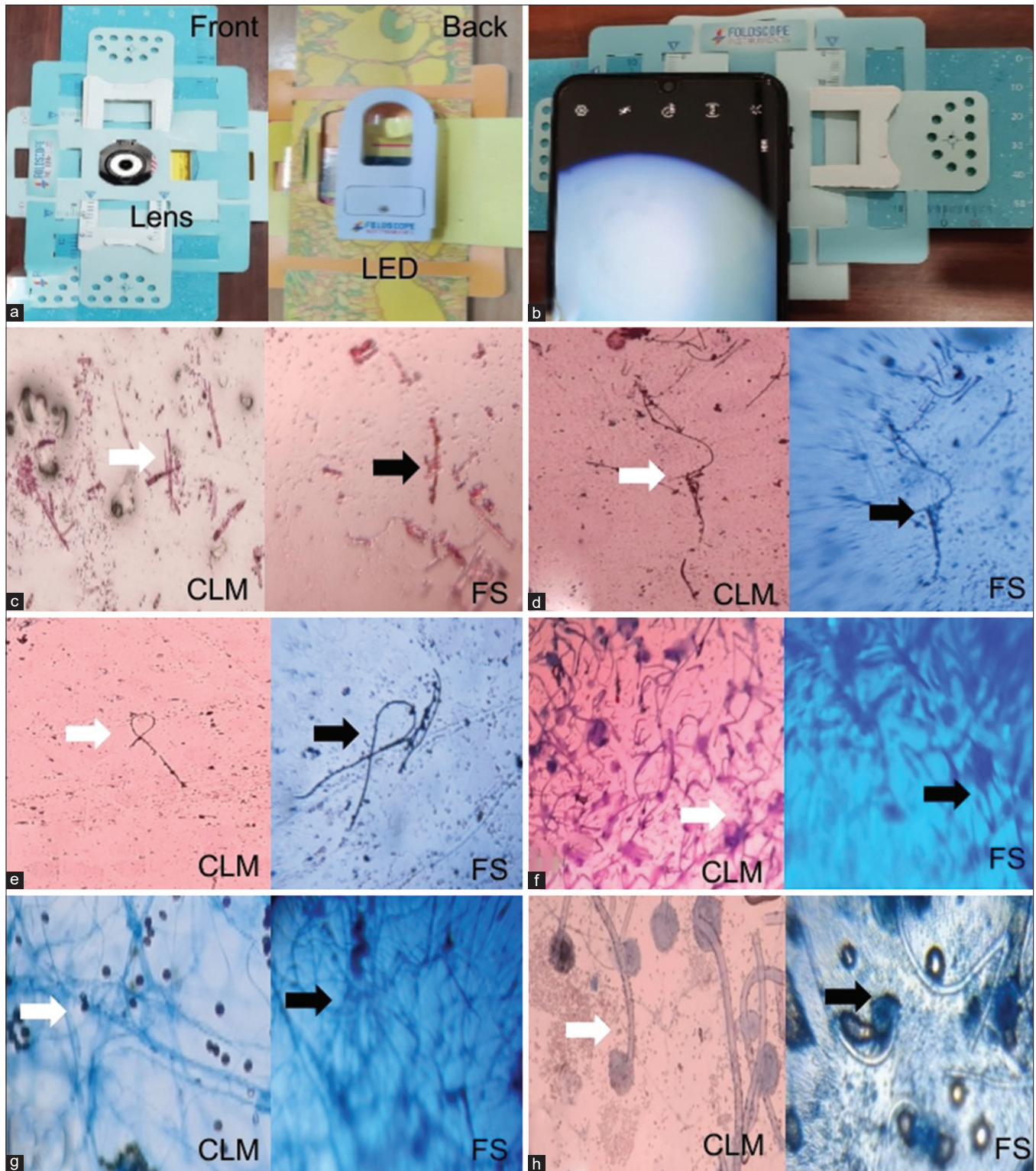


Figure 1: (a–h) Foldscope (FS) and Compound light microscope (CLM) images: Assembled foldscope with front and back surface view (a), foldscope with smartphone camera (b), GM stain images showing CLM (white arrow) and FS (black arrow) view of each smear (c, d, e), LCB wet mount images showing CLM (white arrow) and FS (black arrow) view of each smear (f, g, h)

organisms was not considered and observed in the present study.^[7]

After obtaining informed and written consent of the patient with clinically suspected FK, corneal scraping was performed

under aseptic condition by a trained ophthalmologist using a sterile 15 number blade on slit lamp (58 patients) or operating microscope (for ensuring adequate co-operation while procedure,two patients required supine position under operating microscope) under topical anesthesia (Proparacaine

0.5% eye drops) from the area of interest (ulcer base and margin). After obtaining an adequate sample, the scraped material was smeared on clear glass slides as a thin layer and labeled with marking of the sample area.

For GM staining, the heat fixated smear was covered with the primary stain—crystal violet (2% w/v) and was gently rinsed off with water. After that GM's iodine (3% w/v) was applied on smear and left for 1 minute. Excess of GM's iodine was poured off with water followed by application of GM's decolorizer (50 mL acetone + 50 mL ethanol) over the smear until the solution appeared clear. Gentle rinsing of the smear with water was followed by covering the smear with the Safranin-O stain (0.5% w/v), the counterstain. Gentle rinsing of the stain again with water and blot drying of the sample with bibulous paper were performed before the examination.^[14]

For the preparation of the LCB mounts, a drop of LCB was placed on a clean and dry microscopic slide. The corneal scraping material was allowed to immerse in the drop of LCB carefully. The stained area was covered with coverslip avoiding trapping of air bubbles under the coverslip.^[15]

Microbiological assessment for growth and identification of fungal organisms included inoculation of scraped material on Sabouraud dextrose agar (SDA). However, details of the method have been excluded from the description.

As CLM was considered as the gold standard confirmatory method in the study, the prepared slides were initially observed under FS and then under CLM with blinding for sequences of slides for both the staining methods, thus avoiding interpretation bias. Results were confirmed by a single experienced microbiologist for both staining methods. The prepared slides were examined under an FS lens with an LED illuminator as a light source attached on the back of the assembled FS to rule out the presence or absence of fungal hyphae [Fig. 1a]. A magnetic coupler over the lens of a smartphone camera or a tape was used to mount the smartphone to the FS.^[7] With manual

adjustment of the slides for centration and focusing, fungal elements were viewed on the screen of the smartphone [Fig. 1b]. The images were captured with the smartphone camera using the pinch-to-zoom function (Samsung M30s, Main camera 48 MP; F 2.0, no financial interest). In the case of CLM (Olympus model C × 21FS1 with scanning view of 40X magnification, Japan, no financial interest) same slides were initially focused under 10X magnification and later visualized under 40X magnification for detailed examination. Manual adjustment of mobile camera lens across eyepiece of CLM was required for obtaining focused images. The images were captured by an experienced ophthalmologist (cornea consultant) using the pinch-to-zoom function for both the staining methods [Fig. 1c–h]. Using CLM as the gold standard, positive and negative results were noted for the presence and absence of fungal elements, respectively.

Data collected were used to quantify the agreement using Cohen's kappa between conventional microscopy and FS imaging. According to Cohen's kappa statistics range of value from -1 to 0 shows disagreement, 0.0 shows poor, 0.0–0.20 shows slight, 0.21–0.40 fair, 0.41–0.60 moderate, 0.61–0.80 substantial, and 0.81–1.00 almost perfect strength of agreement (reproducibility).

Results

Out of the 60 slides examined using CLM as the gold standard, 46 (76.66%) slides showed the presence of fungal hyphae with both staining methods. Comparison of results for CLM and FS with smartphone method is as per Table 1.

Twenty-two out of 30 (73.33%) with GM stain and 24/30 (80%) with LCB wet mount were positive for fungal hyphae. Comparison of results for CLM and FS with smartphone method for GM stain and LCB wet mount are as per Table 2.

While moderate agreement (0.49) was seen between CLM and FS methods, the sensitivity and specificity were higher with LCB mount compared to GM stain with foldscope examination [Tables 3 and 4].

Forty-one samples out of 46 (89.13%) with a positive smear and four samples out of 14 (28.57%) with a negative smear on CLM subsequently showed fungal growth on SDA media. Fungal organisms isolated on SDA media were as per Table 5. Management and outcome details of the cases are beyond the scope of this study description.

Discussion

FK is more common in farmers and in rural areas where access to fully equipped diagnostic support is not widely available.^[16,17] Corneal scraping may not yield conclusive results on direct smear especially in cases with a late presentation due to ongoing multiple antimicrobial therapy.^[18,19] Though conventional culture methods are the gold standard for FK,

Table 1: Sensitivity, specificity, and kappa comparing compound light microscopy with smartphone-mounted foldscope for diagnosis of fungal pathogen in corneal scraping

	Smartphone-mounted foldscope			Total
	Results	Negative	Positive	
Compound light microscope	Negative	13	01	14 (23.33%)
	Positive	13	33	46 (76.66%)
	Total	26 (43.33%)	34 (56.66%)	60
kappa	0.49 (moderate agreement)			
Sensitivity	0.72			
Specificity	0.93			

Table 2: Comparison of the data visibility from foldscope and compound light microscope by different staining methods (Gram stain and lactophenol cotton blue wet mount)

Staining techniques performed	Fungal pathogen visible in both CLM and FS	Fungal pathogen not visible in FS and visible in CLM
Gram staining: positive 22/30 (73.33%)	15 (68.18%)	07 (31.81%)
Lactophenol cotton blue wet mount: positive 24/30 (80%)	18 (75%)	06 (25%)

Table 3: Sensitivity, specificity, and kappa comparing compound light microscopy with smartphone-mounted foldscope of Gram staining for diagnosis of fungal pathogen in corneal scraping

Gram staining	Smartphone-mounted foldscope			
	Results	Negative	Positive	Total
Compound light microscope	Negative	07	01	08 (26.66%)
	Positive	07	15	22 (73.33%)
	Total	14 (46.66%)	16 (53.33%)	30
kappa	0.45 (moderate agreement)			
Sensitivity	0.68			
Specificity	0.87			

Table 4: Sensitivity, specificity, and kappa comparing compound light microscopy with smartphone-mounted foldscope for wet mount of lactophenol cotton blue for diagnosis of fungal pathogen in corneal scraping

Lactophenol cotton blue (wet mount)	Smartphone-mounted foldscope			
	Results	Negative	Positive	Total
Compound light microscope	Negative	06	00	06 (20%)
	Positive	06	18	24 (80%)
	Total	12 (40%)	18 (60%)	30
kappa	0.54 (moderate agreement)			
Sensitivity	0.75			
Specificity	1.00			

Table 5: Types of fungal organisms identified on SDA media

Fungal organisms identified on SDA media	Numbers (%)
Aspergillus:	
Aspergillus fumigatus (07)	23 (51.11%)
Aspergillus flavus (09)	
Aspergillus niger (07)	
Fusarium solani	16 (35.55%)
Curvularia	06 (13.33%)
Total	45 (100%)

it takes more time for sufficient growth and subsequent identification of the causative agent. Hence, early detection of fungi on direct smear is desirable to limit the infection in the initial stage. Also, it may be a supportive evidence at the time of referral to a higher center in non-responding cases of FK due to super-added microbial infection.

The FS has a weight of <10 gm, size of 70 mm × 20 mm × 2 mm, magnification of 140X, resolution of 2 microns, back focal length of 0.56 mm, depth of field of 0.013 mm, and field of view of 0.51 mm (diagonal radius).^[7,8] It is available from the online store as an assembled ready-to-use tool. With smartphone camera imaging, FS has been used as a cost-effective tool for cervical cytology with an accuracy of 80%.^[20,21] Its role has been explored for parasitic helminth infection diagnosis with a high specificity value of 93.3%.^[22,23] Other than a diagnostic tool, its effectiveness as an educational tool for motivating oral hygiene among school children has also been studied.^[24] The FS has been

used as a tool for the diagnosis of fungal infection by using wet mount examination in other fields.^[25,26] However, the authors of the present study did not find any published study describing the use of the FS in ophthalmology for the detection of fungal pathogens to compare the results.^[9]

The study has a limitation of a small sample size with comparison of only two staining methods for direct smear preparation. The main drawback using FS is fine adjustment of slides while focusing in small fields limiting its sensitivity. Compared to CLM (8–10 minutes), FS with a small field area of examination takes more time (13–15 minutes approximately) to evaluate one smear completely. Also, the limit of magnification and resolution restricts the use of FS for identifying bacterial organisms. However, the FS has better magnification (140X) to offer compared to a pocket microscope (100X) for detection of fungal hyphae on direct smear at the point of care.^[4,7] Also, the images obtained with the digital zoom of the smartphone camera are comparable to CLM and can be used as a tool for research and education. Image quality of a camera is affected by various features (sensor size, pixel size, camera aperture, etc.), the comparison of which is beyond the scope of the study. Authors in the present study used Samsung M30s (Main camera 48 MP; F 2.0, no financial interest), however, any smartphone with a main rear camera minimum of 12 MP with high pixels screen resolution may work for a decent quality of images. Although CLM is the gold standard for direct smear assessment, negative smear results do not rule out fungal infection, hence, culture methods are advisable for microbiological diagnosis of FK. The main benefit of FS as a diagnostic tool can be in an underserved rural area as a cost-effective approach (FS online price <500 INR, LED illuminator online price <200 INR, October 2020) in the absence of CLM. With an Internet-enabled transfer of direct smear images captured with smartphone-mounted FS, it may serve the purpose of teleophthalmology for FK management in remote health/eye care setup with travel and financial restrictions especially in times of the COVID-19 pandemic.

Since the present study has a small sample size and limited sensitivity, FS with the current specifications cannot be recommended as a primary alternative to standard microscopy for MK diagnosis. In the future, with higher power magnification of FS, the role of FS as a useful tool at the point of care can be further explored as an alternative to standard microscopy for other microbiological or pathological assessment methods in ophthalmology.

Conclusion

GM stain and wet mounts can be an early and sensitive measure to differentiate a fungal versus a bacterial cause for keratitis apart from clinical suspicion. The smartphone-mounted FS has limited sensitivity as an alternative to CLM, but high specificity in our study for the diagnosis of FK. The FS as a smartphone-based diagnostic tool is rapid, simple, portable and inexpensive in resource-constrained rural or remote clinical and public health settings. However, low sensitivity and current evidence limit its role as a primary alternative to standard microscopy for MK diagnosis.

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Conflicts of interest

There are no conflicts of interest.

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