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N Sundari Devi
Department of Botany, Kakching Khunou College, Thoubal, Manipur, India

Thoudam Santosh Singh
Department of Agricultural Microbiology, Pandit Deen Dayal Upadhyay Institute of Agricultural Sciences, Utlou, Bishnupur, Manipur, India

Senjam Jinus S
Department of Horticulture, Pandit Deen Dayal Upadhyay Institute of Agricultural Sciences, Utlou, Bishnupur, Manipur, India

Sukham Joybi Singh
Department of Horticulture, Pandit Deen Dayal Upadhyay Institute of Agricultural Sciences, Utlou, Bishnupur, Manipur, India

Tracila Meinam
Department of Horticulture, Pandit Deen Dayal Upadhyay Institute of Agricultural Sciences, Utlou, Bishnupur, Manipur, India

Lourembam Sanajaoba Singh
Department of Plant Pathology, Pandit Deen Dayal Upadhyay Institute of Agricultural Sciences, Utlou, Bishnupur, Manipur, India

Mutum Seityavhani Devi
Department of Agricultural Microbiology, Pandit Deen Dayal Upadhyay Institute of Agricultural Sciences, Utlou, Bishnupur, Manipur, India

O Premila Chanu
Department of Zoology, Modern College, Imphal West, Manipur, India

RK Imotomba Singh
Krishi Vigyan Kendra, Utlou, Bishnupur, Manipur, India

Thiyam Jefferson Singh
ICAR, Research Complex for NEH Region, Manipur Centre Lamphelpat, Imphal, Manipur, India

Corresponding Author:
N Sundari Devi
Department of Botany, Kakching Khunou College, Thoubal, Manipur, India

Under the foldscope: Relatively inexpensive tool for understanding down-to-earth applications in plant sciences

N Sundari Devi, Thoudam Santosh Singh, Senjam Jinus S, Sukham Joybi Singh, Tracila Meinam, Lourembam Sanajaoba Singh, Mutum Seityavhani Devi, O Premila Chanu, RK Imotomba Singh and Thiyam Jefferson Singh

Abstract
The super-cheap easy-to-use paper-based foldscope has been travelling around the world since the past decades, thereby making them operational in lab-field-based applications. In this review, we have recognized the requirements for the use of this tool in the fields of plant science and especially where measurements must be rapid, cheap and easy to access wherever we go. This new microscope doesn't require any special labels and could help increase access to low-cost plant science diagnostic testing tool and would be beneficial in developing parts of the world where there is meagre access to agricultural science lab and few high-tech diagnostic facilities. Moreover, current challenges and future perspectives are discussed to provide recommendations for further research in plant sciences.

Keywords: easy-to-use microscope, Manu Prakash, traits of choice, crop improvement, abiotic and biotic stress

Introduction
A plant scientist without a microscope is like an astronomer without a telescope. So, every plant scientist needs a microscope to discover the hitherto mysterious microscopic world. To date, the microscopic unit's services in plant science are in high demand and greatly admired (Banerjee, 2018) [1]. Some unwanted features that render less acceptable of a regular microscope are: it is expensive, heavyweight, bulky, fragile and need to be operated by an expert only. The high maintenance cost makes limited access especially in developing parts of the world (Choudhary and Choudhary, 2017, Sameer, 2017) [6, 12]. Suppose, during a practical farm demonstration class, when you ask plant science students to mention essential farm tools, they might say a tractor, a greenhouse, perhaps an indigenous *"khurpi"* or garden shovel, but probably they wouldn't say handy microscope like *"foldscope"*, even some have never used and heard before (Cybulsk *et al*. 2014) [8]. So, we believe that microscope should become a key part of any farmer's and plant science's toolkit. In the adventurous beginning journey of farm science, you don't need a really expensive, high-tech piece of kit to get started adventurous basic jobs like sampling soil micro-flora, plant disease infection studies, plant's microscopic cells analysis etc. A simple handy inexpensive *"foldscope"* is sufficient to see the underlying groups of microscopic worlds, and will set you back around 500-900 INR. Furthermore, doing such analysis yourself is much cheaper in the long run, as it makes this tool viable to access outside of the regular class lab. Hence, with a relatively inexpensive *"foldscope"* and a yearning eye, we are able to observe bacteria, fungi, nematodes, insects, mites and parasites that threaten global food security with ease. Hence, it will offer an accessible microscope to help inspire students and researchers around the tiny globe to explore amateur microscopy and gain a hands-on approach to the plant science disciplines (Cox and Carpenter, 1989 [7]; Bredderman (1983) [4] and Cybulsk *et al*. (2014) [8]. Keeping in view the importance of "*foldscope"*, this review is aimed to produce a guide to good practice, and provide an introduction to and appreciation of some of the emerging new application of *"foldscope"* in plant science odyssey. Although we will provide a cursory overview and explanation of the principles of *"foldscope"*, we do not provide an in-depth review of the technique itself and the theory behind measurements and complete dogma, and we refer readers to some few excellent literatures covering this (see Cybulsk *et al.* 2014, Zev Leifer, 2018, Banerjee, 2018 and Buragohain, 2019) [8, 22, 1, 5].

Ideas of foldscope: In the year 2011, an assistant professor of Bioengineering, Manu Prakash first crystallised the ideas of *"foldscope"* while travelling in Thailand and he was astounded to see that several remote clinics had state-of-the-art microscopes, but never fingered it. The estimated costs of the tools were sky-high that everyone's scared of touching them. After years of tinkering, he came up with the *"foldscope"* (Ed Yong, 2015) [10]. The *"foldscope"*, developed by Manu Prakash and his student Jim Cybulski at Stanford University in 2014, provides over 2000X magnification with submicron resolution, weighs 8.8 grams, fits in a pocket ($70x20x2mm^3$), is battery-powered for up to 50 hours on a single button cell, and is rugged enough to withstand being dropped from three story building or being stomped on a crazy and curious plant scientist's boot. Best of all, it's an actual microscope that costs around 300INR (900-1000 for higher magnification), and can be assembled easily in under 10 minutes. (Cybulski *et al.* 2014) [8].

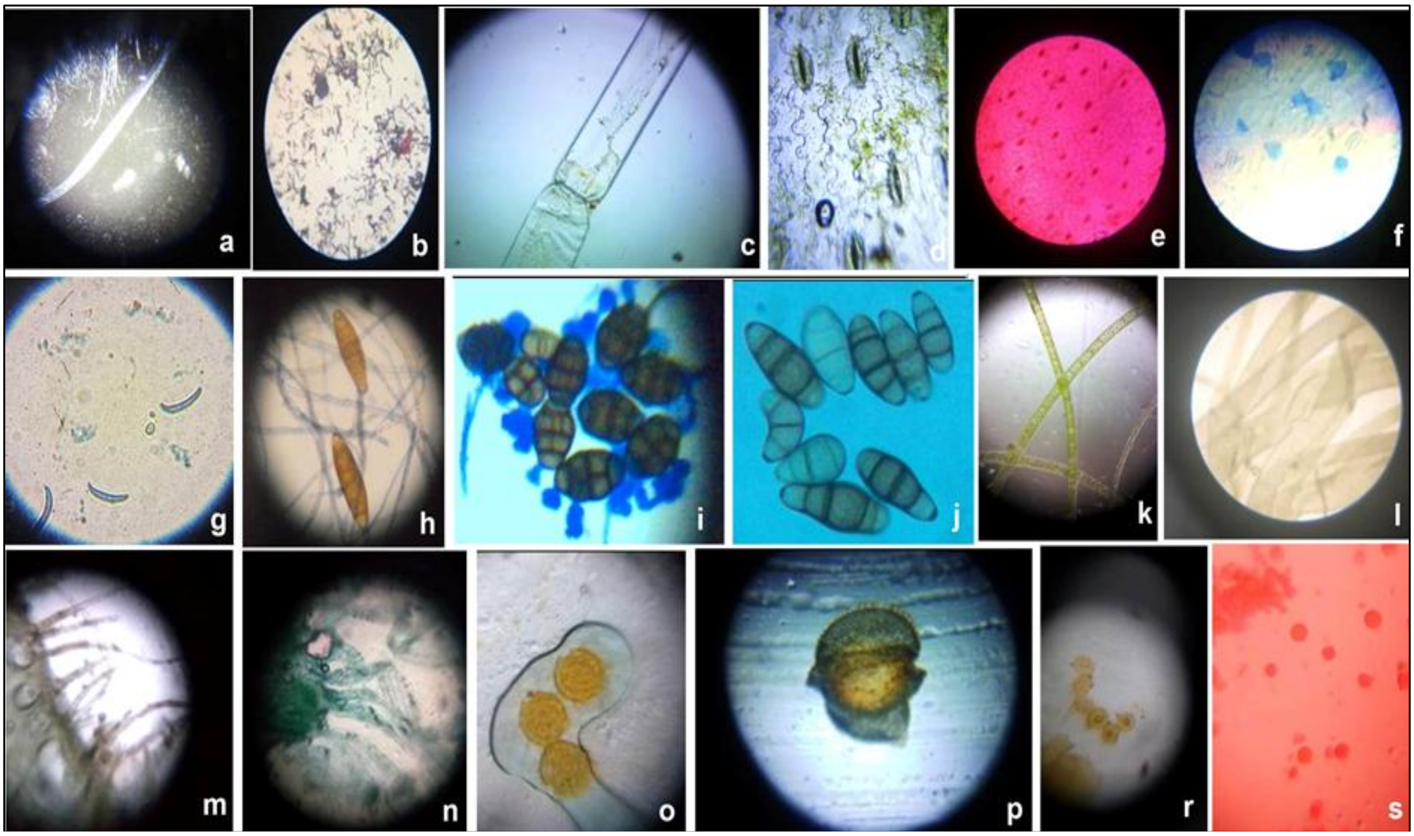

Fig 1: Motif of foldscope Images showing diverse application in Plant science

(a) Nematode (b) Gram positive bacterial rods (c) Trichome of cucurbits (d) Stomata of lily (e) Stomata of tobacco plant (f) Stomata of canna (g) Spores of *Colletotricum* causing Anthracnose of chilli (h) Spores of *Helminthosporium* (i) Spores of *Pithomyces* (j) Spores of *Curvularia* (k) Filaments of *Spirogyra* (l) Filaments of Nungsam (*Lemanea manipurensis*, Red algae) (m) Cross section of leaves of *Trapa natans* (n) Cross section of leaf stalk of *Trapa natans* (o) Pollen of *Hibiscus cannabinus* (p) Pollen of *Mimosa pudica* (r) Pollen of *Hibiscus sinensis* (s) Pollen of *Ageratum* **(Photos copyright:** Mrs N. Sundari Devi, Dr. Senjam Jinus S., Dr Thoudam Santosh Singh and Mr. Sukham Joybi Singh)

Fig 2: Foldscope components and Lab demonstration with students

1. Unassembled foldscope sheet (b) Lens stage (c) Panning guide (d) Sample stage (e) Magnetic coupler (1) front (2) backside (f) LED magnifier, 3X/10X (g) Lens stage (back side) (h1 and h2) Users demonstrating the technique for using the Foldscope in the plant science lab

Components of foldscope: The *"Foldscope"* is a completely down-to-earth microscope with all kinds of advanced microscopy, built purely by folding paper. Its kit contains pre-assembled *"foldscope"* for ready use (fig. 2), prepared glass slide, standard paper slides, microwell paper slide, extra magnetic coupler, ring stickers, cover slip stickers and cotton swab. Components included in pre-assembled foldscope kit (fig. 2) are enough to observer various specimens around us. The deluxe individual kit is made to allow any curious explorer to perform microscopy experiments anywhere at any time. It came packed with handy accessories, it includes LED magnifier, scissors, tweezers, well plates, petridish, eppendorf tubes, pipettes, strainers, clear tape roll, microscope slide set, reusable slide and coverslips, circle dot stickers, diffuser stickers, ziplock sample bags, filter sheets, notesbook and pencil. Once assembled, the *"foldscope"* is operated by inserting a standard microscope glass slide, turning on the LED, and viewing while panning and focusing with one's thumbs. The microscope components are versatile and each can be designed to execute a single microscope technique, such as brightfield, darkfield, fluoroscopy, or lens-array. Cheap manufacturing costs could allow for labs around the world, especially in developing countries, to have plant science students' lab drawers full of magical wand *"foldscopes"*, each for carrying out a unique diagnostic test of microscopic world (Cybulski *et al*. 2014) [8].

Manifold application in plant sciences: The *"foldscope"* has a wide range of application in plant science research purposes. With a *"foldscope"* microscope using the required magnification levels, we can view the microscopic cells of plants in good magnification with relatively good image quality. Thereby we can quickly examine under the lens. The relative important traits for crop improvements such as: stomata density counting, diseases identification, algae's structural studies, pollen morphology analysis, plant's pollen sterile line determination, trichomes variation studies and soil microbe detection as a diagnostic test making it very easy to identify foreign microbial communities, pathogen or fungal diseases in your plant and soil samples. To decipher few instances, recently the in-tissue activity analysis was reported to detect various antioxidant (including glutathione, NADPH oxidase, SOD, POD, MDAR etc) and secondary metabolites (including lignin, lipid, cellulose and hemicelluloses, etc.) in pollens and stomata. Moreover, some of the relative important traits and observation, which have been mentioned above and, given in the fig. (1), as a part of preliminary research was conducted with a team collaboration of some colleges and institutions (fig. 2, h1&2) located at Manipur, and those *"traits of choice"* (fig.1) can be studied and used further in various crop improvement programmes in plant science. Briefly deciphering, some supported literatures of various co-workers on which the *"traits of choice"* mentioned for crop improvement programme which are viable to access under the foldscope are discussed. As we observed the *"traits of choice"* say the motifs of stomata here (fig1. d,e&f) , many co-workers in abiotic stress study deciphered that the majority of water loss from plants occurs *via* transpiration through epidermal pores stomata making these cellular structures an attractive target in the battle to prevent water loss. Recent several laboratory studies have demonstrated that it is possible to improve drought tolerance and Water Use Efficiency (WUE) by reducing the frequency of stomata on leaves, by using genetic manipulation, to reduce stomatal density, improved WUE has been achieved across several model dicot species including *Arabidopsis* (Franks *et al*. 2015) [11], *Nicotiana tabacum* (Yu *et al*. 2008) [27] and *Hordeum vulgare* (Jon *et al*. 2017) [15]. Another special structures and physiological functions of trichome (fig.1c) such as reflectance, energy balance (Bickford, 2016) [3]; ultraviolet protection (Karabourniotis *et al*. 1992) [16], drought resistance (Hong-Qin and Ping, 2010) [14], gas exchange, insect resistance (Levin, 1973, Handley *et al*. 2005) [17, 13] and disease resistance (see Xiao *et al*. 2017) [26]. Recent study also indicated that fresh water algae (fig.1. k&1) biomass can be used to develop high capacity biosorbent materials for the removal and recovery of toxic heavy metal ions from dilute industrial wastewater streams (Rezaee *et al*. 2006) [23] and even we examined the beautiful hairy clear pattern of *spirogyra* and *Lemanea manipurensis* (fig.1, k&l) under the foldscope too**.** According to an estimate, plant parasitic nematodes (fig.1a) are causing much more damage annually compared to insect pests. A crop yield loss due to these tiny unseen pests in various countries is enormous (see Singh and Singh, 2015) [24]. Some of the most damaging nematode is shown in the fig.1(a) through the lens of *"foldscope"*. Meanwhile, rice brown spot (BS), caused by *Cochliobolus miyabeanus* which is seen in the image (Fig. 1h) is a chronic disease that affects millions of hectares of rice every growing season, grown by some of the most resource-poor farmers. Reported yield losses in relative terms vary widely from 4 to 52% (Barnwal *et al.* 2013) [2]. Of the 5000 and above recognised bacterial species, over 100 species are the causal agents of plant diseases. They constitute a very important factor limiting growth and cropping of cultivated plants. Identification of such aggressive species from the base level with the help of *"foldscope"* by studying its phenotypic characters will also ease the pain of many plant pathologists' dilemma (Sobiczewski, 2008) [25]. *Colletotricum* which is shown in the fig. 1(g), the most frequently isolated species and causes anthracnose on numerous plants worldwide (Damm *et al.* 2012) [9]. Leaf spot disease of cabbage, *pithomyces* which is shown in the fig. 1(i), is affecting 80% of plants growing in greenhouse and field. Identification of such isolate from the basic level under the lens of *"foldscope"* helps to determine its morphological characters and can also be used for student's lab analysis purpose (Naschi *et al.* 2014) [19]. Over and above, pollen biology has direct apropos in agriculture, horticulture (vegetable science and pomology), plant pathology, plant breeding (vegetable breeding), agricultural microbiology and biotechnology (Margariet *et al*. 2000, Noor *et al*. 2004) [18, 20]. Taking into account, the applied form of palynology was found desirable to carry out palynogical work (fig.1. o,p,r&s) dealing with the fundamental palynology of the plant, cultivated in Manipur. Wherefore, the epitome dossier provided here could prove worthwhile for the identification of many microbial flora of plants and soil, as well as for diagnostic screening through the lens of cheapest paper based *"foldscope"* in the field of plant science.

Conclusion remarks

The neoteric increased heed in the use of *"foldscope"* tool has been mainly due to viable research applications for the crop improvement tool and particularly for the screening of desirable *"traits of choice"* with responses to abiotic and biotic stress. However, several challenges remain, spotlighting differences compared to using a glass or digitized slide – *e.g.,* holding the *"foldscope"* up to a light source resulted in the liquid sample dripping on the observers face (Rebecca *et al*. 2018) [22]. The practicable strategies can be using semi-permanent slide with the help of transparent nail polish on the periphery of cover slips or methods can be developed to keep it flat, with attention to clamps to hold it and a light source appropriately placed. In addition, methods can be developed to take the images with *ipad* or android cell phone. Therefore, further development is needed as to methodology and as to validation given the requirement of increasing magnification in images analysis and further new advanced applications.

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References

1. Banerjee S. "Foldscope, the Frugal Innovation and its Application in Food Microscopy - A Review". Acta Scientific Nutritional Health. 2018; 2(6):53-54.
2. Barnwal MK, Kotasthane A, Magculia M, Mukherjee PK, Savary S, Sharma AK *et al*. A review on crop losses, epidemiology and disease management of rice brown spot to identify research priorities and knowledge gaps. Eur J Pathol. 2013; 136:443-457
3. Bickford CP. Ecophysiology of leaf trichome. Functional Plant Biology. 2016; 43(9):807-819
4. Bredderman T. The effects of activity-based science on student outcomes: A quantitative synthesis. Review of Education Research. 1983; 53:499-518.
5. Buragohain M, Kakoti N, Sarmah P, Pegu BK. Foldscopic and Microscopic view of Bacteria: A comparative study in water samples of Lakhimpur district Assam (India). International Journal of Advanced and Innovative Research. 2019; 8(3):2278-7844
6. Choudhary OP, Choudhary P. Scanning Electron Microscope: Advantages and Disadvantages in imaging components. International Journal of Current Microbiology and Applied Sciences. 2017; 6(5):1877-1882.
7. Cox CA, Carpenter JR. Improving attitudes toward teaching science and reducing science anxiety through increasing confidence in science ability in inservice elementary school teachers. Journal of Elementary Science Education. 1989; 1(2):14-34.
8. Cybulski JS, Clements J, Prakash M. Foldscope: Origami-Based Paper Microscope. PLoS ONE. 2014; 9(6): e98781. https://doi.org/10.1371/journal.pone.0098781
9. Damm U, Cannon PF, Woudenberg JHC, Crous PW. The Colletotrichum boninense species complex. Studies in Mycology. 2012; 73:1-36.
10. Ed Yong. The Atlantic. Sept 1, 2015 www.theatlantic.com
11. Franks PJ, Doheny-Adams TW, Britton –Harper ZJ, Gray JE. Increasing water-use efficiency directly through genetic manipulation of stomatal density. New Phytologist. 2015; 207:188-195.
12. Gajghate, Sameer. Introduction to microscopy. 2017; 10:13140/RG.2.2.24105.49768
13. Handley R, Ekbom B, Agren J. Variation in trichome density and resistance against a specialist insect herbivore in natural population of Arabidopsis thaliana. Ecol Entomol. 2005; 30:284-292
14. Hong-Qin S, Jian – Ping L. Comparison of drought resistance of pepper with different hairiness character under drought stress. J Nucl Agri Sci. 2010; 24:835-839.
15. Jon H, Christopher H, Chris D, Jessica A, Lee H, Jennifer S *et al*. Reducing stomatal density in barley improves

drought tolerance without impacting on yield. Plant physiology, 2017. https://doi.org/10.1104/PP.16.01844
16. Karabourniotis G, Papadopoulos K, Papamarkou M, Manetas Y. Ultraviolet –B radiation absorbing capacity of leaf hairs. Physiologia plantarum. 1992; 86:414-418.
17. Levin DA. The role of trichome in plant defense. Quarterly Review Biology. 1973; 48:3-15.
18. Margariet H, Winston Mark L, Mukai A. Effect of pollen load size and source (self, outcross) on seed and fruit production in Highbush Blueberry CV. 'Blue Crop' (Vaccinium corymbosum; Ericaceae). American Journal of Botany. 2008; 87(11):1584-1591.
19. Naschi A, Bin Kadir J, Farnaz A, Mehdi E, Golkhandan E, Nyaka N. Identification of Pithomyces chartarum causing leaf spot of cabbae in Malayasia. Canadian Journal of Plant Pathology. 2014; 36:403-406.
20. Noor MJ, Ahmad M, Assghar R, Kanwal A, Pervaiz S. Polynological studies of cultivated plant species at University of Arid Agriculture, Rawal Pindi, Pakistan. Asian Journal of plant Sciences. 2004; 3(4):476-479.
21. Priya N, Arun DS. Foldscope based methods to detect in-situ antioxidant activity and secondary metabolites in pollen and stomata of Lantana camara. Research and Reviews in Biotechnology and Biosciences. 2018; 5(1):29-33.
22. Rebecca C, Daniel CM, Stevens, Zev Leifer. Preliminary studies in the use of the foldscope paper microscope for diagnostic analysis of crystals in urine. Issues in the analysis of liquid samples and potential applications in low budget/low tech regions of the world. 2018. https://www. Researchgate.net/publication/326493613
23. Rezaee A, Ramavandi B, Ganapati F, Ansari M, Solimanian A. Biosorption of mercury by biomass of filamentous algae Spirogyra species. J Biol. Sci. 2006; 6:695-700.
24. Singh S, Singh B, Singh AP. Nematodes: A threat to sustainability of Agriculture. Procedia Environmental Sciences. 2015; 29:215-216.
25. Sobiczewski P. Bacterial diseases of plants: Epidemiology, Diagnostics and Control. Zendirbyste-Agriculture. 2008; 95(2):151-157.
26. Xiao K, Mao X, Lin Y, Xu H, Zhu Y. Trichome, a functional diversity phenotype in plant. Mol. Biol. 2017; 6:183
27. Yu H, Chen X, Hong Y-Y, Wang Y, Xu P, Ke S-D *et al*. Activated expression of an Arabidopsis HD- START Protein confers drought tolerance with improved root system and reduced stomatal density. Plant cell. 2008; 20:1134-1151.
28. Zev Leifer. New York College of Podiatric Medicine FOOTPRINTS, 2018, 5(7). http://www.global-engage.com/event/digital-pathology-usa/.