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Review Article

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Frugal Compound Microscopy for Microbiological Analysis and Education

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Abstract
We report a very frugal microscope building method from easily realizable parts. We show a highly portable field ready compound type microscope with bright field, dark field and projection microscopy capabilities. It has good resolution and magnification for multiple aspects of education and diagnosis. We demonstrate that such a system can be built from simple lenses in laser pointers and camera modules with no specific 3d printed parts or costly lenses. We show all the parts of the system like stage, coarse and fine focussing system, microscope body, even slides can be built from commonly available materials like soda bottle and DVD disks. We list alternate, safer and easily available staining methods and chemicals. The microscope is developed with a target that it can be self-developed even in rural areas of the world with only resources at one's disposal with very little education. This features a stable focussing and panning system for comfortable viewing through eyes and also easy imaging with a smartphone. We show its utility for microorganism analysis and potential for clinical diagnosis.

Keywords: compound microscope, inexpensive microscopy, ultra-low-cost microscope

Introduction
The need for low cost microscopy is well known in education [1,7] and diagnosis [2- 9] and there has been a lot of focus recently on developing portable and affordable microscopy [1-3, 8, 9]. Small ball lenses or bead lenses have a long history as use of simple microscopes [1, 4, 6] whereas [12, 13] use custom lenses fabricated from polymers and elastomers. There have been attempts at mobilising compound microscope objectives and eyepieces to be used with cell phones with custom made attachments and use for portable microscopy [3]. Also an inverted cell phone lens which provides good resolution along with a good field of view to be used with cell phone magnification [2] has become quite popular in literature of low cost simple microscopy. For the focusing system and microscope body these use 3d printed parts [2, 3, 7, 8, 9] and foldscope uses paper parts [1]. The bead/ ball lenses are known to have a smaller field of view [4,6]. Access to 3d printers is still limited in the majority of the world. We tend to overcome some of these limitations by proposing a compound microscope where we have good resolution and higher magnification than other low cost microscopes and since it doesn't require 3d printed parts but uses lenses in getattable laser pen modules and/or cell phone lens modules.

The microscope is ultra compact (55x30mm) because for both the eyepiece and objective we use powerful lenses (instead of much weaker lenses for objective).

Our microscope satisfies the following criteria

1. The design of the body of the microscope should have a stable focuser and platform but should also be compact.
2. Use of comeattable items (but not limited only to items specified by us) and hence should also easily be mass producible or can be mass assembled.
3. Not to produce lenses from scratch but use lenses which are accessible in lasers, bead lenses, cell phone camera modules.
4. Should have good enough resolution and magnification, and have comfortable viewing/imaging.
5. It should not use any external lighting (not limited to) but should work with mobile phone flash or torch light for ease with field microscopy as everyone owns one in a cell phone flash.

Construction and Materials
We found glass bead lenses to be not suitable for our design be-

cause it has a lower field of view (Fig 2a) [2, 4, 6] which gets even lower when compounded. In our final design we ended up using a lens from an old phone module (or webcam lens module for objective lens) and a laser pointer lens module as eyepiece lenses. Using laser lens modules for both eyepiece and objective gives similar efficiency in results.

For construction the neck area of two soda bottles (polyethylene terephthalate or PET) is cut off including the cap. This gives us inbuilt helical focuser design with the platform. Our platform for the microscope hence is a rotating platform but the slide (which is inserted through a slit cut off across the cap) stays in place as it's held in place by holding elastic clips. The platform has a small condenser lens which is a 20mm diameter plastic magnifier lens but any other lens of similar size can be used. We used a small diffuser below the condenser inside the platform itself. An optional dark field filter can be used between the diffuser and the condenser lens.

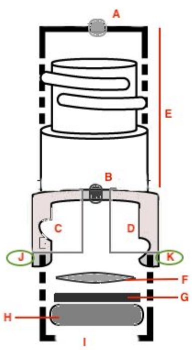

Figure 1. The cross section cartoon diagram of the microscope with following parts A. The eyepiece lens, B. The objective lens, C. and D. is slit through the focussing cap for the slide to be placed, J. and K. are elastic bands to be pulled over the slide and act as slide holder clips, F. Condenser lens, G. Optional dark field filter, H. Diffuser, I. Cavity to pass the led light through as the whole system is placed on cellphone flash or led torch. E. Body tube serving as minimal distance between eyepiece and objective and also fine focuser.

The Objective lens is mounted on the microscope cap such that the lens is inverted towards the sample with a slit in the cap itself. This increases the field of view keeping all the objects in the view area in focus [2]. A slide is inserted in the slit and is held in position by a single elastic band attached to the cap which acts analogously to conventional slide clips to keep the slide in place (Fig 1- J,K). This gives us the freedom to have the sample to place it ~0-10 mm distance below the objective.

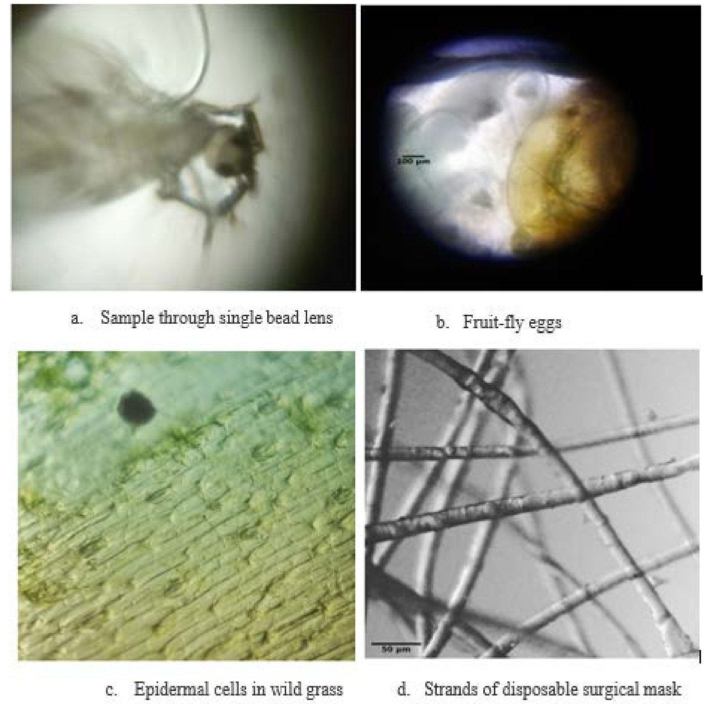

Figure 2. Samples through a simple microscope variant; a. A sample through a bead lens shows shallow depth of field and focus. b. sample through single lens module but non-inverted showing smaller field of view. c. and d. Samples through a single lens module inverted showing improved field of view.

This design explained above is sufficient for a simple microscope which has ~ 56x magnification (Fig 2- c, d, 4c). It is imperative that we invert the lens such that the flatter meniscus is facing towards the sample; Application of non-inverted lens results in a smaller field of view as shown in Fig-2b. We extend this to a compounded microscope by attaching another bottle cap at a distance of ~ 27 mm and using a laser lens as eyepiece. For this we use thread and the cap of the neck area of another soda bottle which also works as fine focus by increasing the variable distance upto ~ 35mm.

In this manner we have two helical focusers. The one attached to the objective works as a coarse focuser and one with eyepiece embedded in it works as a fine focuser (Fig 1). Fig 3 shows some examples from compounded assembly under brightfield setting view. Compounded view is capable of ~ 220x magnification at its optimum view with resolution <= 5 microns.

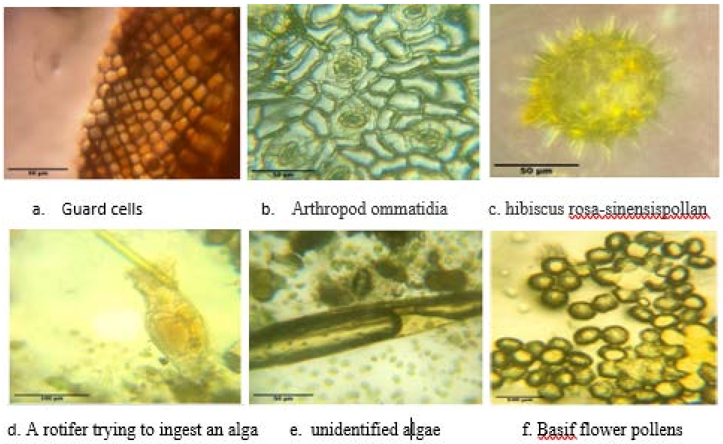

Figure 3. Samples through our compound microscope in bright-field. a. Guard cells and stoma from *Murraya koenigii,* b. Compound eye of housefly, c. Single pollen of hibiscus flower, d. A rotifer with its stomach, vitellarium and other parts, e. An unidentified alga among other algae, f. Alcohol dried pollen of *Ocimum basilicum* revealing surface details and hexagonal shapes

Slides and Staining

To work with this microscope we use custom slides which do not rely on supply of standard slides though it's easily possible to use standard glass slides with a variant of 'capscope' made from a bigger diameter helical focussing system. For making custom slides we use thin long strips of plastic sheets cut from the soda bottle itself, these are not completely flat but curved longitudinally. We can use clear acrylic sheets as an alternative or clear and separable layer of easily available DVD disks. The cutouts from the same are used as cover slips. We find these as acceptable slides where no heating is required for staining.

We use relatively thinner slides, with ~1cm breadth and 5- 7cm length as this is adequate and helps for full viewing and panning end to end breadth of the slide in such a compact system.

From the same clear separable layer of DVD disks dark field filter is created by darkening in the center with paint with two thirds of the area. Fig 4 shows some examples under dark-field setting

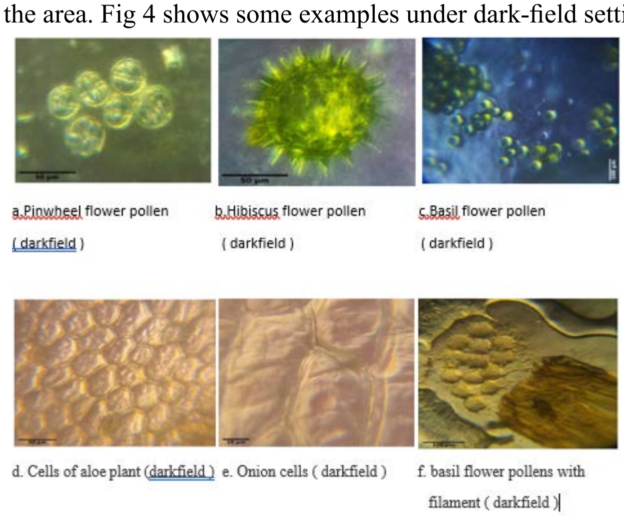

Figure 4. Images from compound microscope with darkfield illumination. a. Pollens of Tabernaemontana divaricata flower, b. Single pollen from Hibiscus rosa-sinensis, c. Pollen from Ocimum basilicum, d. Aloe plant cell, e. Onion cells and nucleus unstained under darkfield, f. Pollens of Ocimum basilicum along with the filament detached showing some surface details (undried).

A lot of literature exists around staining methods and without use of harmful chemicals which are easily available [10,11] . We didn't use differential staining methods (e.g. gram's method); Rather we went with simpler staining methods with alternate dyes. We used Alta(dye from lac) (fig 5b), a much safer natural option for pigmentation of nucleus of plant cells and blue fountain ink (mostly triarylmethane dye) to stain animal cells (fig 5-a, c). We used a mixture of 50% Alta with 50% synthetic vinegar v/v to make the solution more acidic (fig 5b). The staining required washing with water afterwards. We also tried food coloring (ins-534, ins-110, ins122) but didn't get satisfactory results. It showed inconvenient impurity with staining.

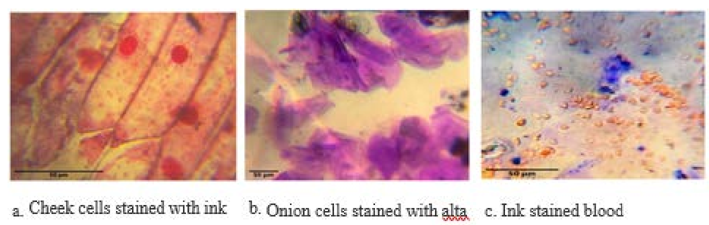

Figure 5. Stained samples with our microscope. a. Epithelial cells stained with ink showing nucleus and also bacteria, b. Onion cells stained with equal volume solution with acetic acid showing nucleus, c. Ink stained blood clearing showing disc shape of red blood cells.

Our simple microscope design is capable of ~56x magnification and 10mm2 field of view and compounded design is capable of ~220x. The reported resolution is <= 5 microns. Compounding only increases the magnification.

Conclusion

Plastic bottles are the most common substances on earth now. Also small laser pointers are pretty common toys even in the most underdeveloped countries. Smartphones are now in every corner of the world and so is smartphone junk. We developed and demonstrated a method using these pretty common items we can create a powerful enough compound microscope for education and microbiological analysis. It cost us <2$ to assemble this in India.

We also showed/listed methods for creating and working with alternative slides and staining methods which are safer if considered for education.

We believe it's easily possible to use this for medical diagnosis or check the quality of material and water in rural areas where people can build this kind of microscope with little education and inexpensive items or 3D printed parts. Microscopes with similar magnification and resolution have shown potential utility for quick portable and low cost diagnosis [4, 5, 6, 8,].

The microscope can be easily mass produced and/or assembled given the high availability/production of the parts that goes into making it. The versatility and compactness of the assembly makes it a very usable pocket microscope and since for light it uses any cell phone's led flash makes it a useful companion field microscope. This can also be used as a projection microscope if used with a stronger light source like that of a led torch.

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