Foldscope: Origami-Based Paper Microscope

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Abstract

Here we describe an ultra-low-cost origami-based approach for large-scale manufacturing of microscopes, specifically demonstrating brightfield, darkfield, and fluorescence microscopes. Merging principles of optical design with origami enables high-volume fabrication of microscopes from 2D media. Flexure mechanisms created via folding enable a flat compact design. Structural loops in folded paper provide kinematic constraints as a means for passive self-alignment. This light, rugged instrument can survive harsh field conditions while providing a diversity of imaging capabilities, thus serving wide-ranging applications for cost-effective, portable microscopes in science and education.


Introduction

Microscopes are ubiquitous tools in science, providing an essential, visual connection between the familiar macro-world and the remarkable underlying micro-world. Since the invention of the microscope, the field has evolved to provide numerous imaging modalities with resolution approaching 250 nm and smaller [1]. However, some applications demand non-conventional solutions due to contextual challenges and tradeoffs between cost and performance. For example, in situ examination of specimens in the field provides important opportunities for ecological studies, biological research, and medical screening. Further, ultra-low cost DIY microscopes provide means for hands-on science education in schools and universities. Finally, this platform could empower a worldwide community of amateur microscopists to capture and share images of a broad range of specimens.

Cost-effective and scalable manufacturing is an integral part of “frugal science and engineering” [2]. For example, manufacturing via folding has emerged as a powerful and general-purpose design strategy with applications from nanoscale self-assembly [3] to large-aperture space telescopes [4]. More recently, possibilities of folding completely functional robots have been explored [5–7], with actuators, sensors and flexures integrated in a seamless fashion. Modern micro-lens fabrication technology is another prime example of scalable manufacturing. Although the use of high-curvature miniature lenses traces back to Antony van Leeuwenhoek’s seminal discovery of microbial life forms [8], manufacturing micro-lenses in bulk was not possible until recently. Modern techniques such as micro-scale plastic molding and centerless ball-grinding have grown to serve numerous applications, including telecommunication fiber couplers, cell phone cameras, and medical endoscopes.

By combining principles of optical design with origami [9–11], here we present a novel platform for the fabrication of flat microscopes cheaply in bulk (figure 1). The Foldscope is an origami-based optical microscope that can be assembled from a flat sheet of paper in under 10 minutes (see video S1, figure S8). Although it costs less than a dollar in parts (see Bill of Materials in table 1), it can provide over 2,000× magnification with submicron resolution, weighs less than two nickels (8.8 g), is small enough to fit in a pocket (70×20×2 mm3), requires no external power, and can survive being dropped from a 3-story building or stepped on by a person (see figure 1G and video S2). Its minimalistic, scalable design is inherently application-specific instead of general-purpose, providing less functionality at dramatically reduced cost. Using this platform, we present our innovations for various imaging modalities (brightfield, darkfield, fluorescence, lens-array) and scalable manufacturing strategies (capillary encapsulation lens mounting, carrier tape lens mounting, self-alignment of micro-optics by folding, paper microscope slide).

The Foldscope is operated by inserting a sample mounted on a microscope slide (figure 1B), turning on the LED (figure 1C), and viewing the sample while panning and focusing with one’s thumbs. The sample is viewed by holding the Foldscope with both hands and placing one’s eye close enough to the micro-lens so one’s eyebrow is touching the paper (figure 1F). Panning is achieved by placing one’s thumbs on opposite ends of the top stage (colored yellow in figure 1A-C) and moving them in unison, thus translating both optics and illumination stages while keeping the stages aligned (figure 1B). Focusing is achieved using the same positioning of one’s thumbs, except the thumbs are pulled apart (or pushed together). This causes tension (or compression) along the optics stage, resulting in −Z (or +Z) deflection of the micro-lens due to flexure of the supporting structure of the sample-mounting.
stage (figure 1C). Unlike traditional microscopes, the Foldscope anchors the sample at a fixed location while the optics and illumination stages are moved in sync.

Results

Design Platform

Construction from flat media. The Foldscope is comprised of three stages cut from paper _ illumination, sample-mounting, and optics _ and assembled via folding (figure 1A–C, video S1). Other primary components include a spherical ball lens (or other micro-lenses), lens-holder apertures, an LED with diffuser or condenser lens, a battery, and an electrical switch (see figure 1D). The three stages are weaved together to form an assembled Foldscope (figure 1B,C,E) with the following features: fully-constrained X–Y panning over a 20×20 mm² region (figure 1B), flexure-based focusing via Z-travel of the optics stage relative to the sample-mounting stage (figure 1C), and a vernier scale for measuring travel distances across the sample slide with 0.5 mm resolution. The total optical path length from the light source to the last lens surface is about 2.7 mm (figure S1), only 1% that of a conventional microscope. Flat polymeric sheets and filters can also
Table 1. Bill of Materials.

<table>
<thead>
<tr>
<th>Component</th>
<th>Unit cost (10,000 pcs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paper (400 cm²)</td>
<td>$0.06</td>
</tr>
<tr>
<td>Ball Lens (low mag/high mag)</td>
<td>$0.17/$0.56</td>
</tr>
<tr>
<td>3V button battery (CR2016)</td>
<td>$0.06</td>
</tr>
<tr>
<td>LED</td>
<td>$0.21</td>
</tr>
<tr>
<td>Switch</td>
<td>$0.05</td>
</tr>
<tr>
<td>Copper Tape (5 cm²)</td>
<td>$0.03</td>
</tr>
<tr>
<td>Foldscope</td>
<td>$0.58/$0.97</td>
</tr>
</tbody>
</table>

Summary of unit costs for Foldscope components in volumes of 10,000 units, not including assembly costs. This assumes a Foldscope in brightfield constructed from the following: polypropylene sheets (Press Sense 10mil Durapro); a 140X low-mag lens (Winsted Precision Ball 2.4 mm borosilicate ball, P/N 3200940F1ZZ00A0, from www.mcmaster.com, P/N 8996K21) or a 2,180X high-mag lens (Swiss Jewel Co. 0.2 mm sapphire ball lens); a 3V CR2016 button cell (Sanyo CR2016-TT18 #8565 from Batteriesandbutter.com); a white LED (Avago ASMT CW40 from Mouser.com); an electrical slider switch (“Off/On MINI SMD Switch” from AliExpress.com); and copper tape (Sparkfun P/N 76555A648).}
Table 2. Foldscope Analytical Model Parameter Summary Table.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Functional Form in Optimized System</th>
<th>Parameter Values at Select Combinations of r, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAG</td>
<td>[ MAG = \frac{2.5e5\mu m}{EFL} = (5e5\mu m) \frac{n-1}{n} ]</td>
<td>r = 1200  r = 500  r = 400  r = 150  r = 150  r = 100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 1.517  n = 1.517  n = 1.517  n = 1.517  n = 1.77  n = 1.77</td>
</tr>
<tr>
<td>BFL</td>
<td>[ BFL = EFL - r = \frac{1}{2} r \frac{(2-n)}{n-1} ]</td>
<td>140  340  430  1140  1450  2180</td>
</tr>
<tr>
<td>RES</td>
<td>[ RES = k_2 f \left( \frac{\lambda}{n} \right) \frac{1}{4} = k_2 \left( \frac{r^3(2n+2n(n-1))}{128(n-1)^3} \right)^{1/4} ]</td>
<td>561  234  187  70  22  15</td>
</tr>
<tr>
<td>nOAR</td>
<td>[ nOAR = \frac{a}{n} - \frac{k_1}{r} \left( \frac{\lambda}{n} \right) \frac{1}{4} = k_1 \left( \frac{8i\lambda^3 n}{r(n-1) + 2n(2n-1)} \right)^{1/4} ]</td>
<td>1.90  1.52  1.44  1.13  0.86  0.77</td>
</tr>
<tr>
<td>OAR</td>
<td>[ OAR = nOAR - k_1 \left( \frac{\lambda}{n} \right) \frac{1}{4} = k_1 \left( \frac{8i\lambda n^3 r^3}{(n-1) + 2n(2n-1)} \right)^{1/4} ]</td>
<td>353  183  155  74  77  56</td>
</tr>
<tr>
<td>EFL</td>
<td>[ EFL = \frac{1}{2} n \frac{r(n-1)}{(n-1)} ]</td>
<td>1761  734  587  220  172  115</td>
</tr>
<tr>
<td>NA</td>
<td>[ NA = \frac{2a(n-1)}{r n} = k_1 \left( \frac{128\lambda(n-1)^3}{r(n-1) + 2n(2n-1)} \right)^{1/4} ]</td>
<td>0.294  0.366  0.387  0.495  0.510  0.565</td>
</tr>
<tr>
<td>FOV</td>
<td>[ FOV = \frac{a(n-1)}{2(n-1)^3} = k_1 \left( \frac{\lambda^3 n^7}{2(n-1)^3 + 2n(2n-1)} \right)^{1/4} ]</td>
<td>0.200  0.249  0.264  0.337  0.444  0.491</td>
</tr>
<tr>
<td>DOF</td>
<td>[ DOF = \frac{\lambda}{NA} = \frac{1}{k_1} \left( \frac{\lambda^3 r n(n+2n(n-1))}{128(n-1)^3} \right)^{1/4} ]</td>
<td>518  268  227  109  88  65</td>
</tr>
<tr>
<td>SR</td>
<td>[ SR = e^{-1/3} ]</td>
<td>13.7  8.8  7.9  4.8  2.8  2.3</td>
</tr>
</tbody>
</table>

Functional form and select numerical values for the following dependent parameters: Magnification (MAG), Back Focal Length (BFL), Resolution (RES), nOAR (Normalized Optimal Aperture Radius), OAR (Optimal Aperture Radius), Effective Focal Length (EFL), Numerical Aperture (NA), Field of View (FOV), Depth of Field (DOF), Stehl Ratio (SR). These are calculated for infinte “object” distance per analytical model RM2, with aperture radius

\[ a = OAR - k_1 \left( \frac{\lambda}{n} \right) \frac{1}{4}, \quad k_1 \geq 0.9321, \quad k_2 \geq 0.7415 \]

and with aberration coefficient \( s = -\frac{n}{n+2n(n-1)(2n-1)} \sqrt{2n^2} \). All calculations assume an incident wavelength of \( \lambda = 0.55 \mu m \), and all specified distances are in units of \( \mu m \).

The indices of refraction \( n = 1.517 \) and \( n = 1.77 \) correspond to borosilicate glass and sapphire, respectively.

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used to image 2 μm diameter red poly-fluorescent polystyrene beads as shown in figure 2B. For fluorescent imaging requiring higher contrast, small pieces (3 mm square or smaller) of interference filters can be used in place of the polymeric sheets at reasonable cost due to the small size.

**Lens-array and Multi-modality.** Since a micro-lens has a very small footprint, multiple optical paths can be independently configured in a single Foldscope (figure 2C, G). Such a lens array may be comprised of identical lenses or of different lenses with different magnifications and/or back focal lengths. This provides for the design of a lens-array Foldscope with several key features. For non-contiguous samples such as blood smears, a larger field of view can be obtained by overlapping a number of small fields of view. Alternatively, an optimal array pitch will give tangential non-

Figure 2. Foldscope imaging modalities. (A) Brightfield Foldscope image of a monolayer of 1 μm polystyrene microspheres (Polysciences 07310-15) using a 1,450X lens. (B) Fluorescent Foldscope image of 2 μm polyfluorescent microspheres (Polysciences 19508-2) using a 1,140X lens with Roscolux gel filters #19 and #80. (C) 2X2 lens-array Brightfield Foldscope image of Giemsa-stained thin blood smear using 1,450X lenses. (D) 140X Darkfield Foldscope images of 6 μm polystyrene microspheres (Polysciences 15714-5), using a 140X lens for the darkfield condenser. Darkfield condenser aperture shown in inset has 1.5 mm inner diameter and 4.0 mm outer diameter. (E–H) Schematic cross-sections of Brightfield, Fluorescence, Lens-Array, and Darkfield Foldscope configurations, showing the respective arrangements of ball lenses, filters, and LEDs. See table 2 for ball lenses used for specific magnifications.

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Figure 3. Manufacturing innovations for lens- and specimen-mounting. (A) Fabrication, mounting, and characterization of capillary-encapsulation process for lens-mounted apertures. X and Y error bars for all measurements are 2.5 μm. (B) Reel of polystyrene carrier tape with custom pockets and punched holes for mounting over 2,000 ball lenses with optimal apertures. The first ten pockets include mounted ball lenses. Inset shows sectioned view from CAD model of carrier tape mounted lenses. Note the aperture is the punched hole shown on the bottom side of the ball lens. This tape is 16 mm wide and is designed for 2.4 mm ball lenses (aperture diameter is 0.7 mm). (C) Top: Paper microscope slide shown next to standard glass slide with coverslip, both with wet mount algae specimens. Bottom: Schematic of paper microscope slide, showing specimen containment cavity formed between upper tape and lower tape in middle of slide.

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overlapping fields of view (as seen in figure 2C), thus reducing the
time required to scan a slide for a feature such as a parasite. Since
individual lenses have independent optical paths, the novel
capability of building multi-modality lens-array microscopes arises.
One such combination is a two-by-one array of brightfield and
fluorescence modalities, which could be used to scan a sample for
the presence of fluorescence markers and then identify the non-
fluorescent surrounding structures.

**Darkfield.** The Darkfield Foldscope configuration, shown in
figure 2H, requires a diffuser, a darkfield condenser aperture (inset
in figure 2D), and a condenser lens. The diffuser helps to evenly
distribute light from the small LED over the aperture area, while
the condenser focuses a hollow cone of light onto the specimen.
Since the specimen must be placed at the focal point of the
condenser, the slide thickness has to match the back focal length
of the condenser plus the spacing from the condenser to the slide.
A 140X Darkfield Foldscope was used to image 6 μm polystyrene
microspheres as shown in figure 2D.

**Capillary Encapsulation Lens Mounting.** The process of
precisely mounting micro-optics to an aperture crucially governs
lens performance. Therefore, a capillary encapsulation process
was developed to automatically mount a ball lens while forming a
circular aperture of precisely tunable diameter (see figure 3A). By
partially encasing ball lenses in an opaque polymer held between
two glass substrates coated with flat nonstick PDMS (see top left of
figure 3A), a precise aperture is self-assembled around the ball-lens
(see Materials and Methods section for details). Pressure applied
between the substrates is used to precisely tune aperture size, with
greater pressure providing a larger aperture. The epoxy encaps-
sulated lens is then adhesively mounted to a paper aperture and
inserted in the Foldscope (see bottom right of figure 3A).

**Carrier Tape Lens Mounting.** Black polystyrene carrier
tape is commonly used for low-cost reel-to-reel packaging of
electronic components. This pre-existing infrastructure was
leveraged to create low-cost mounting structures for ball lenses
with optimized apertures. As depicted in figure 3B, the custom
thermoformed pocket holds the lens in place with a press fit, and a
punched hole in the bottom of the pocket precisely defines the
aperture. A single lens is cut from the carrier tape and adhesively
attached to a paper aperture which is then inserted into the optics
stage of the Foldscope, analogous to that shown for the epoxy
encapsulated lens in figure 3A.

**Paper microscope slide.** A low-cost microscope slide
(similar to that in [18]) was constructed out of 18mil polystyrene
synthetic paper and transparent tape as shown in figure 3C. If tape
is placed on only one side (either upper or lower), specimens are
conveniently mounted on the exposed sticky surface of the tape.
Using both upper and lower pieces of transparent tape creates a
cavity for mounting wet specimens such as live algae suspended in
water. Since the paper microscope slide is less than half the
thickness of a standard glass slide, a spacer is required to elevate
the sample closer to the lens. This is achieved by inserting the
specimen slide together with two blank paper slides beneath it.
Once the specimen has been viewed, the transparent tape can be
removed from the synthetic paper and replaced so that the slide

**Modeling and Characterization**

**Theory and analysis.** For a brightfield Foldscope, basic
measures of optical performance can be described in terms of the
ball radius (r), index of refraction (n), aperture radius (a), and
incident wavelength (λ; see text S1). Assuming the paraxial
approximation, these include effective focal length (EFL), back
focal length (BFL), and magnification (MAG). For a 300 μm
sapphire ball lens: EFL = 172 μm, BFL = 22 μm, and
MAG = 1,450X. Thus substantial magnification can be obtained,
but the sample must be separated from the lens by only a fraction
of the thickness of a human hair. Three additional optical
performance metrics include field of view radius (FOV), numerical
aperture (NA), and depth of field (DOF). These depend on
aperture radius (a), the optimization of which is discussed below.
For the previous example, the normalized optimal aperture radius
is nOAR = a/r = 0.51, giving: FOV = 88 μm, NA = 0.44,
DOF = 2.8 μm (see table 2).

The aperture radius controls the balance between diffraction
effects from the edges of the aperture with spherical aberration
effects from the lens. Therefore, a complete analytical model was
created to predict the normalized optimal aperture radius (nOAR)
and optimal resolution (RES), as well as the aberration coefficient
(s) for a ball lens (see Supplementary Materials and table 2), yielding:

\[ nOAR = \left( \frac{k_1}{r} \right) \left( \frac{a}{|x|} \right)^{1/4} \]
\[ k_1 = \left( 3 \sqrt{\frac{10}{4 \pi}} \right)^{1/4} \approx 0.9321 \]
\[ RES = k_2 \left( \frac{a}{|x|} \right)^{1/4} \]
\[ k_2 = 1.22 \left( \frac{\pi}{12} \right)^{1/4} \left( \frac{e}{10} \right)^{1/3} \approx 0.7415 \]
\[ s = - \left( n - 1 \right) \left( n + (2 - n)(2n - 1) \right) / (2m)^3 \]

The expressions for nOAR and RES are depicted as 2D design
plots as a function of desired MAG in figure 4A,B and figure 4C.

to be near 0.5 μm, based on the range of parameters identified for this figure.

**Numerical Modeling.** A ray-tracing numerical model was developed for the Foldscope using Zemax software to confirm the results of the analytical model and to evaluate points across the field of view (see Materials and Methods section for details). The results for nOAR and RES show very good agreement, with correlation coefficients of $R^2 = 0.985$ for nOAR and $R^2 = 0.998$ for RES (see figure 4C). The numerical modeling results across the field of view are shown in figure S4A, where the optimal field of view used for calculating the MTF (Modulus of the Optical Transfer Function) is defined. The MTF for this system is plotted in figure 4D for a field point on the optical axis and another at the edge of the optimal field of view. The field point at the optical axis shows near-diffraction-limited response, and the tangential and sagittal curves for the edge of the field drop to half of their low-frequency value at a spatial frequency of about 300 cycles/mm.

**Empirical Characterization of Resolution.** The resolution of the 430X and 140X lenses were empirically characterized using a standard USAF 1951 resolution target and a GUPPY Pro 503C scientific camera (with bare sensor) as shown in figure S7. Images taken with these lenses are shown in figure 4E,G, and the relevant intensity profiles are shown in figure 4F,H. The formula for the resolution (in line pairs/mm) of a given group and element of the USAF 1951 target is given by $RES_{LP} = \frac{\text{Group} \times \text{Element} - 1}{6}$, and the center-to-center distance between the lines in microns is given by $RES_{CC} = 1000/RES_{LP}$. From table 2, we see the theoretical values for the resolution of the lenses are 1.44 μm and 1.90 μm, respectively. Based on the critically resolved intensity profiles in figure 4F,H, the empirical values for $RES_{CC}$ are found to be 1.38 μm and 2.19 μm.

For the 430X lens, note that the empirical value is 4.2% smaller than the theoretical value. A less conservative theoretical model (see text S1) predicts values 11.5% smaller than those in table 2, indicating the empirical value is reasonable and near the limits predicted by theory. For the 140X lens, note that the empirical value is 15.3% larger than the theoretical value. This difference is attributed to the fact that this particular lens was selected for its low cost at the expense of larger tolerances on diameter and sphericity (grade 40) compared to the 430X lens (grade 10). For example, diameter tolerance per ball sphericity is 0.25 μm for grade 10 and scales linearly with grade number.

Since the USAF 1951 resolution target has a glass covering with thickness comparable to a standard coverslip (140 μm), ball lenses with magnification higher than 430X could not be assessed with this resolution target due to short back focal length. Instead, a monolayer of 1 μm polystyrene beads was imaged using a 1450X ball lens (figure 2A) to demonstrate sub-micron resolution for this lens (theoretical value is 0.86 μm).

**Discussion and Conclusions**

By removing cost barriers, Foldscope provides new opportunities for a vast user base in both science education and field work for science and medicine. Many children around the world have never used a microscope, even in developed countries like the United States. A universal program providing “a microscope for every child” could foster deep interest in science at an early age. While people have known for decades that hands-on examination and inquiry is crucial in STEM (Science, Technology, Engineering, and Mathematics) education [19–20], the challenge posed by J. M. Bower to engage “all teachers and all children” [21] requires

![Figure 5. Mosaic of Foldscope Images.](http://example.com/figure5)

Bright field images of (A) *Giardia lamblia* (2,180X), (B) *Leishmania donovani* (1,450X), (C) *Trypanosoma cruzi* (1,450X), (D) gram-negative *Escherichia coli* (1,450X), (E) gram-positive *Bacillus cereus* (1,450X), (F) *Schistosoma haematobium* (140X), and (G) *Dirofilaria immitis* (140X). Unstained (H) leg muscles and (I) tarsi of an unidentified ladybug (genus *Coccinella*). (J) Unstained leg muscles (fixed in formaldehyde) of an unidentified red ant (genus *Solenopsis*). An LED diffuser (Roscolux #111) was added for (A) and an LED condenser (2.4 mm borosilicate ball lens) was used for (C). Images (H–J) were taken by novice user using a self-made Foldscope (140X). See table 2 for ball lenses used for specific magnifications. White scale bar: 5 μm; black scale bar: 100 μm.

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large-scale adoption of practices and broad availability of tools that were previously cost-prohibitive [22]. Moreover, the opportunity to make microscopes both approachable and accessible can inspire children to examine the rich bio-diversity on our planet as amateur microscopists and to make discoveries of their own, as already seen in the field of amateur astronomy ([23]; see images taken by novice user with self-made Foldscope in figure 5H–J).

Disease-specific Foldscope designs are an important vision for future development [24–25]. Figure 5 depicts early bench-test data, including high-magnification brightfield images of *Gardaia lambita*, *Leishmania donovani*, *Trypanosoma cruzi* (Chagas parasite), *Escherichia coli*, and *Bazillus cereus* (figure 5A–E), and low-magnification brightfield images of *Schistosoma haematobium* and *Dirofilaria immitis* (figure 5F–G). Note that these include magnifications ranging from 140X to 2,100X, none of which require immersion oil. In the future, darkfield and fluorescence Foldscopes will also be adapted for diagnostics, and sensitivity and specificity will be measured for various disease-specific Foldscopes in the field as clinical validations against existing diagnostic standards.

Constructing instruments from 2D media provides other unique advantages and opportunities. Embedding flat rare-earth magnets in paper provides means for magnetic self-alignment, allowing the Foldscope to be reversibly coupled to a conventional smartphone for image capture, for smartphone-based diagnostics, or for telediagnosis [26–29]. By printing text and images on the paper, this platform provides an efficient information-delivery method for specific staining protocols, pathogen identification guides, or language-free folding instructions (figure S6). Some applications in highly infectious diseases may benefit from a disposable microscope _ or “use-and-throw” microscopy _ where the entire microscope can be incinerated. Also, in place of a glass slide, the 2D media also allows direct attachment of the sample to a paper-based micro-fluidic assay [30] for automated staining and/or pathogen-concentration, thus yielding an independent fully-functional diagnostic system.

Future work will build upon the key features of this platform. Roll-to-roll processing of flat components and automated “print-and-fold” assembly make yearly outputs of a billion units attainable. Ongoing work with advanced micro-optics and illumination design _ including spherical GRIN lenses [31–32], aspheric multi-lens optics, and condenser lens provisions for Kohler illumination _ is expected to improve both resolution and field of view at low cost. International field-work in both diagnostics and education will provide vital inputs for further improvements. Our long-term vision is to universalize frugal science, using this platform to bring microscopy to the masses.

**Materials and Methods**

**Ball Lenses**

The ball lenses used in constructing Foldscopes included material types borosilicate, BK7 borosilicate, sapphire, ruby, and S-LAH79. The vendors included Swiss Jewel Co, Edmund Optics, and Winsted Precision Ball. Part numbers for some select lenses include: 300 µm sapphire lens from Swiss Jewel Co. (Model B0.30S), 200 µm sapphire lenses from Swiss Jewel Co. (Model B0.20S), 2.4 mm borosilicate lenses from Winsted Precision Ball (P/N 3200940F1Z1000A0), 300 µm BK7 borosilicate lenses from Swiss Jewel Co. (Model BK7-0.30S), and 1.0 mm BK7 borosilicate lenses from Swiss Jewel Co. (Model BK7-1.00). Note that half-ball lenses from both Edmund Optics and Swiss Jewel Co. were also tested for use as condenser lenses for the LEDs.

**2D Media and Filters**

The 2D media used in constructing Foldscopes included black 105 lb card stock (ColorMates Smooth & Silky Black Ice Dust Card Stock, purchased from thePapermillstore.com), polypropylene (PressSense Durapro CC 10mil), and others. Foldscope parts were cut from 2D media using a CO₂ laser (Epilog Elite, Mini24). Copper tape was used for providing connectivity (by soldering) between the LED, battery, and switch. The filters used in constructing Foldscopes included Roscolux colored gel filters (including Primary Blue #80 and Fire Red #19, which approximate an Acidine Orange filter set), Roscolux diffuser filters (Tough Rolex #111), and polymeric linear polarizers (Edmund Optics P/N 86181). Each type of filter is assembled to the Foldscope by cutting out a 3–5 mm square piece and adhesively attaching it to the appropriate stage with single-sided or double-stick Scotch tape. Paper microscope slides were constructed from polypropylene sheets (PressSense Durapro CC 18mil) and transparent scotch tape.

**LEDs, Switches and Power Sources**

The LEDs used in constructing Foldscopes included the Avago HSMW-CL25 (now replaced by P/N Avago ASMT CW40) white LED for brightfield Foldscopes, the Kingbright APTD1608QBC/D blue LED for fluorescence Foldscopes. The electrical slider switch was purchased from AliExpress.com (“Off/On MINI SMD Switch”, Product ID: 665019103). The power sources included Duracell 3V CR2032 button cells, Sanyo 3V CR2016 button cells (Sanyo CR2016-TT1B #8563 from Batteriesandbatter.com), and a GW Instek DC power supply (Model GPD-3303D). Button cells were used with no resistors for Foldscopes.

**Aperture Manufacturing**

This method produces inexpensive apertures through polymer encapsulation of ball lenses while preserving the optical quality of the lens and allowing multiple lenses to be encapsulated at once. The experimental setup shown in the top left of figure 3A was used to encapsulate 300 µm sapphire ball lenses with aperture diameters ranging from 100 µm to 214 µm. The lens was sandwiched between parallel substrates (glass or silicon) coated with planar films of PDMS with thickness greater than 1 mm (formed from Dow Corning Sylgard 184 PDMS). A micrometer stage was used to precisely apply pressure between the substrates to adjust the diameter of the resulting elastic deformation of the PDMS film. This diameter was measured in situ using phase contrast microscopy to set the target value for the aperture. A fast-curing opaque polymer (Smooth-On Smooth-Cast Onyx Fast Polyurethane) was then injected into the cavity and allowed to cure. Reflected light microscopy was used to measure the dimensions of the final aperture formed. Once removed from the non-stick PDMS films, the encapsulated lens was attached to the underside of the optics stage of a Foldscope.

**Characterization of Self-Alignment by Folding**

Twenty independent microscopes were cut out of black 105 lb cardstock, each marked with a cross-hair in both the optics and illumination stages (see figure S2C). After folding, alignment was measured using a dissection microscope (Olympus upright, 30× magnification) via digitizing the cross-hair images, drawing lines through the center of each cross-hair (X and Y cross-hairs on both stages), and digitally measuring the X and Y displacements to characterize the alignment. Every Foldscope was iteratively folded, imaged to record X–Y alignment, and unfolded twenty times. The
Sample Preparation

Thin-blood smears of Plasmodium falciparum (ring stage), Trypanosoma cruzi, Giardia lamblia, Leishmania donovani, and Drosophilia immitis were freshly prepared from cultures provided by Center for Discovery and Innovation in Parasitic Diseases (CDIPD) at UCSF. The samples were fixed in methanol and stained in freshly prepared Giemsa solution (Sigma Aldrich, #48900-500ML-F) using standard protocols before imaging. Once fixed, the slides could be used for several weeks. Bacterial samples of Bacillus cereus and Escherichia coli were provided by KC Huang Lab at Stanford University. The samples were heat fixed onto glass slides using standard procedures and gram stained using standard protocols (Fisher Scientific Gram Stain Set, Catalog No. 23-255-959). Plasmodium-infected red blood cells were taken from cultures provided by the Center for Discovery and Innovation in Parasitic Diseases (CDIPD). Schistosoma haematobium and Escherichia coli were provided by the Michael Hsieh Lab at Stanford University. Insects used for imaging were caught on Stanford campus and imaged after fixing in formaldehyde without any stain. No human samples were utilized in the current work.

Image-Capture Protocol

Brightfield images were taken using a Canon EOS 5D Mark II with the Foldscope placed 3 cm away from the 100 mm focal length lens and using the following settings: F/3.2, 1/30 sec. exposure, ISO-2000. An initial image was first captured using automatic white-balance and then used as a reference white balance image during data collection. Fluorescence images were taken in a similar fashion to the brightfield images with typical camera settings: F/2.8, 15 sec. exposure, ISO-1000. Although not presented, images were also obtained by coupling the Foldscope to cell-phones including an iPhone using a custom magnetic coupler. USAF 1951 resolution target data was taken using GUPPY Pro 503C scientific camera, with 2592 x 1944 pixels and pixel size 2.2 x 2.2 μm², using the setup shown in Figure S7.

Numerical Model

Zemax software was used to model the Foldscope optics to assess optimal aperture radius and resolution. The basic model of the system consists of a ball lens, an aperture, an object at infinity, and an image plane (see figure S5A). This model requires two parameters to be independently optimized – lens-image distance and aperture radius. The analysis is carried out in four steps: 1) optimize lens-image distance in model by minimizing focusing metric (figure S5C); 2) determine search space for aperture radius as defined by empirically chosen limits on Strehl Ratio, 0.75 – 0.98; 3) optimize aperture radius using resolution metric (figure S5D); and 4) use Matlab surface-fitting tool to fit data for optical performance parameters as functions F(n,r, λ) and compare with analytical model.

Supporting Information

Figure S1 Foldscope Schematics. (A) Real image formation via projection. (B) Virtual image formation via direct observation with the eye. Note the drawings are not to scale. The indicated lengths are example values that show the versatility of this design as well as its extreme space efficiency. For example, the same system can be used for projecting or imaging simply by changing the object-lens distance by about 20 μm. Also, notice the total path length from the LED to the lens is almost an order of magnitude smaller than the size of the human eye.

Figure S2 Characterization of Self-Alignment by Folding. Twenty independent Foldscopes were constructed out of 350 μm thick black cardstock and manually folded and unfolded twenty times each, with alignment measured after each assembly. The data was used to produce plots of (A) assembly repeatability (distribution of all 400 values, adjusted to give zero mean for each Foldscope) and (B) assembly accuracy (distribution of 20 mean values calculated per Foldscope) using (C) cross-hair alignment features on the optics and illumination stages. Note that the span of the data in both plots is less than the thickness of the paper used to construct the Foldscopes. Based on the data shown in the plots, assembly repeatability was assessed as the mean value of twice the standard deviation for each Foldscope (65 μm in X and 25 μm in Y), while assembly accuracy was assessed as the mean value of all trials (59 μm in X and 67 μm in Y). A higher skew in X-axis repeatability results from structurally distinct constraints implemented for the X- and Y-axes, while the assembly accuracy errors in both directions are consequences of the design which can be compensated by feature shifts in future designs. Note that the X and Y error bars for all measurements are 8.4 μm.

Figure S3 Component Characterization. (A) LED voltage and intensity versus time for a white LED (Avago HSMW-CL25) powered by a Duracell CR2032 battery with no resistor. (B) Filter transmission spectra of three Roscolux filters – Tough Rolex Fire Red (#19), Primary Blue (#80) – measured with Ocean Optics Photo spectrometer USB4000. (C) Intensity profile of a white LED (Avago HSMW-CL25) as visualized in water with dissolved fluorescein. The left image is taken with the bare LED while the right image is taken with a condenser lens (2.4 mm borosilicate ball lens) placed adjacent to the LED in the optical path, demonstrating that a ball lens can be used to effectively collimate the light emitted by this LED.

Figure S4 Numerical Modeling Characterization of Optimal Field of View. (A) Plot of Wavefront Error over the full field of view defined by the aperture for a 300 μm Sapphire ball lens with a 147 μm aperture. With increasing field coordinate, the Wavefront Error becomes very large and the image will be out of focus. An “optimal field of view” is defined at the field coordinate of 21 μm, where the Wavefront Error is approximately 1/5 wave number. (B,C) Plots of Field Curvature and Distortion over the optimal field of view. (D) Plot of RMS spot size over the optimal field of view depicting four cases: optimized solution treated as reference with zero defocus (red line), defocus of 3 μm (green line), defocus of 3 μm (green line), diffraction limit (dashed black line). The reference solution provides the best achievable resolution at the center of the field of view (approximately equal to the diffraction limit for this choice of aperture), while other plots show that increasing defocus moves the region of best resolution radially out from the center in an annular ring. (E) Plot of RMS spot size over the optimal field of view depicting optimal aperture predicted by analytical model (red lines) and adjusted aperture giving uniform RMS spot size over the field of view (purple lines).

Figure S5 Diagrams and Plots for Numerical and Analytical Models. (A) Schematic of time-reversed Zemax model showing collimated light coming from an object at infinity, passing through aperture, and focused by the ball lens onto a focal point.
point in the image plane. (B) Schematic of time-reversed model showing key parameters used in some derivations for the analytical model. (C) Plot of Focusing Metric versus Lens-Image Distance for $l = 0.55 \mu m$, $r = 150 \mu m$, $n = 1.517$. This illustrates how focusing metrics FM1, FM2, and FM3 select different values for the optimal lens-image distance. (D) Plot of Resolution Metric versus Aperture Radius for $l = 0.55 \mu m$, $r = 150 \mu m$, $n = 1.517$. This illustrates how resolution metrics RM1 and RM2 select nearly the same camera, with 2592 images of the USAF 1951 resolution target viewed with the Radius for lens-image distance. (D) Plot of Resolution Metric versus Aperture Radius for $l = 0.55 \mu m$, $r = 150 \mu m$, $n = 1.517$. This illustrates how resolution metrics RM1 and RM2 select nearly the same camera, with 2592 images of the USAF 1951 resolution target viewed with the Radius for lens-image distance.

Figure S6 Artistic Layout of Foldscope Paper Components. Artistic version of Foldscope layout with integrated universal folding instructions based on color coding, where like colors are matched during the folding process to leave a single solid color in the final folded configuration.

Figure S7 Foldscope Image Capture Setup for Resolution Metric. Picture of the experimental setup used to capture images of the USAF 1951 resolution target viewed with the Foldscope. The data was taken using GUPPY Pro 503C scientific camera, with 2592 x 1944 pixels and pixel size 2.2 x 2.2 $\mu m^2$.

Figure S8 Quick Reference Guide for Foldscope Assembly. One-page handout to facilitate users in guided assembly of a Foldscope.

Video S1 Foldscope Assembly. A short video of 140X Brightfield Foldscope assembly process.

Video S2 Foldscope Drop Test and ruggedness. A short video of a three story drop test and ruggedness of Foldscope demonstrated by stomping under feet.

Text S1 Supplementary information. Analytical expressions and derivations related to optical performance metrics.

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Author Contributions

Conceived and designed the experiments: JSC MP. Performed the experiments: JSC JC MP. Analyzed the data: JSC JC MP. Wrote the paper: JSC MP.

References