

Optical Resolution and MTF of a Low-Cost Fourier Ptychography Microscope using a Raspberry Pi Computer

Haechan J. Yang, William Wasswa,¹ Raymond Li, Brandon Pautler, Aiden Fry, Rajan Leung, David W. Holdsworth and Ian A. Cunningham

Imaging Research Laboratories, Robarts Research Institute,
Western University, 1151 Richmond St N, London, Ontario, Canada, and
¹Mbarara University of Science and Technology, Mbarara Uganda

ABSTRACT

Introduction: Low-cost optical microscopes are under development by several groups to bring point-of-care malaria diagnosis services to under-served communities in low-resource settings. We describe a Fourier ptychography microscope (FPM) system based on an open-source design and a method of measuring spatial resolution in terms of the optical modulation transfer function (MTF) using images of the 1951 USAF resolution test device.

Methods: The FPM system uses a Raspberry Pi computer and 196-LED matrix light source. FPM images were reconstructed using in-house Python code. The MTF was determined from bar-patterns using a least-squares analysis to fit a square wave, including odd-harmonic terms, to image profiles through the bars. These were normalized to larger uniform regions of the pattern and combined to generate the MTF.

Results: Total component cost of the FPM was less than \$200. The theoretical diffraction limit imposed by the pupil function of this system was 280 cycles/mm, slightly less than the measured MTF 10% frequency of 300 cycles/mm. The 10% frequency in FPM images was 550 cycles/mm.

Conclusions: The USAF test pattern provides a practical method for assessing FPM performance in terms of the achieved MTF of the \$200 Raspberry Pi based Fourier ptychography microscope. The limiting frequency in FPM images, 550 cycles/mm, was slightly less than the Nyquist sampling cut-off frequency of 670 cycles/mm imposed by pixel spacing.

1. INTRODUCTION

Malaria is a blood-borne parasitic disease with an estimated 247 million cases and 619,000 deaths worldwide in 2021, with the highest prevalence being among children under the age of five in sub-Saharan Africa.¹ The World Health Organization (WHO) recommends treatments be initiated within 24–48 hours of initial symptoms to be effective,² but the non-specific flu-like symptoms (including chills, headaches and nausea) make this a challenge in rural regions where blood tests are not available. Treatments often exist, but are only used for positive cases due to limited availability, cost and risk of developing antimalarial resistance.³ Two vaccines are now recommended by the WHO,^{1,4} but the need for point-of-care diagnoses continues.

An affordable and accurate point-of-care diagnostic method could help achieve early diagnoses.^{5–7} Light microscopy is recommended by the WHO, requiring a thin blood smear. A Giemsa stain highlights DNA material and sufficient resolution (at least 1- μ m resolution is considered acceptable²) and field of view such that a trained technician can identify the fraction of infected red blood cells. A low-cost, portable microscope with sufficient performance could be a suitable candidate for point-of-care diagnoses.

Numerous studies have focused on developing low-cost, portable microscopes.⁸ For example, Breslauer *et al.*⁹ designed a microscope lens attachment to a cellphone camera to produce a portable diagnostic device. Switz *et al.*¹⁰ used an external mobile phone lens in a reversed configuration to achieve high resolution and large field of view. A low-cost ball lens was used by Agbana *et al.*¹¹ to reduce costs. Ganesan *et al.*¹² describe an interesting paper origami-based microscope, although the lack of device rigidity and difficulty of calibration limit its practical use. Although these methods improve portability and lower costs compared to laboratory-grade microscopes, the

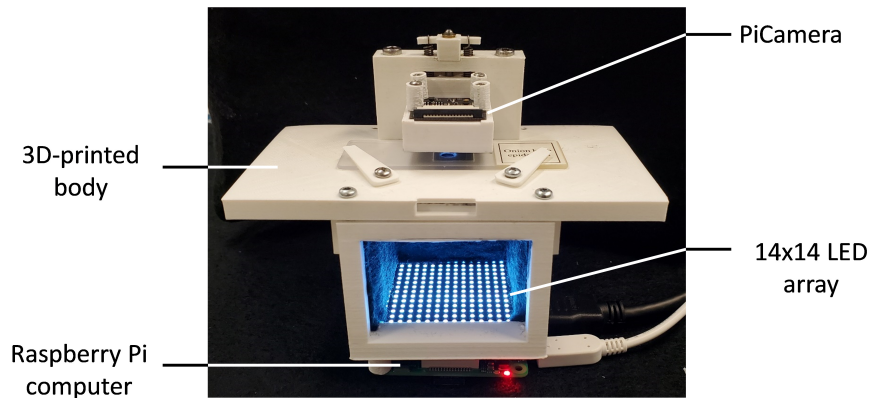


Figure 1. Prototype FPM device based on a design described by Aidukas.¹⁵

requirement of a mobile phone still limits their affordability. In addition, to view a wide area of the specimen, these devices generally require a slide-scanning mechanism to achieve large field of view.

Fourier ptychographic microscopy (FPM) is considered a good candidate for a low-cost, high-performance device.^{13–17} FPM consists of replacing the single light source of a conventional light microscope with an array of light-emitting diodes (LEDs) that provide off-axis illumination while attempting to satisfy the Fraunhofer condition. Each image corresponds to a sub-section of the Fourier domain that depends on illumination angle and computational methods are used to generate the final image. The major advantage of FPM is an improvement in resolution while displaying a wide field of view (FOV). Thus, a low-cost lens can be used to acquire images and ptychography reconstruction used to increase resolution while maintaining field of view.^{14,16}

While several groups have demonstrated the advantages of FPM, we describe a method of measuring the optical modulation transfer function (MTF) using the 1951 USAF resolution test device to quantify spatial resolution and evaluate improvements achieved with ptychography for this application. We do this in three steps:

1. Validate method of measuring MTF using idealized USAF test pattern.
2. Validate theoretically-expected improvement in MTF using Fourier ptychography.
3. Demonstrate improvement and evaluate MTF using Fourier ptychography microscope.

2. METHODS

Our microscope was constructed based on a design described by Aidukas *et al.*^{15,17} as illustrated in Fig. 1. OpenSCAD three-dimensional (3D) modeling software was used to construct mechanical components using polylactic acid (PLA) filaments on a consumer-grade Dremel 3045 printer. Electronic components included a Raspberry Pi 3B computer board, Pimoroni Unicorn HAT HD 14×14 LED array (3.3-mm spacing), and Raspberry Pi V2 NOIR camera module (1.12-μm sensor pixel size). The LED array was located 60.0 mm below the sample stage. The lens in the Raspberry Pi camera module has a focal length of 3.0 mm and is mounted approximately 5 mm from the sensor in this design, yielding a measured magnification of 2.6 x. Image acquisition and LED control was programmed in Python 3.10 to capture dark-frame images with no illumination, followed by images at each of 196 illumination angles using green (530 nm) light. Images were reconstructed using in-house Python code adapted from Tien *et al* and Rogalski *et al.*^{18,19} Figure 2 illustrates Fourier ptychography reconstruction. The three images on the left are simulations^{18,20} representing the image that would be acquired with on-axis illumination plus two off-axis illuminations. The Fourier transform of each is stitched into the composite Fourier domain. Since the camera captures only the magnitude of the complex raw images, phase components must be calculated.¹³ The magnitude of the inverse Fourier transform of the composite domain gives the reconstructed image. In this illustration, raw images are calculated in a simulation based on the 1951 USAF optical resolution test pattern.

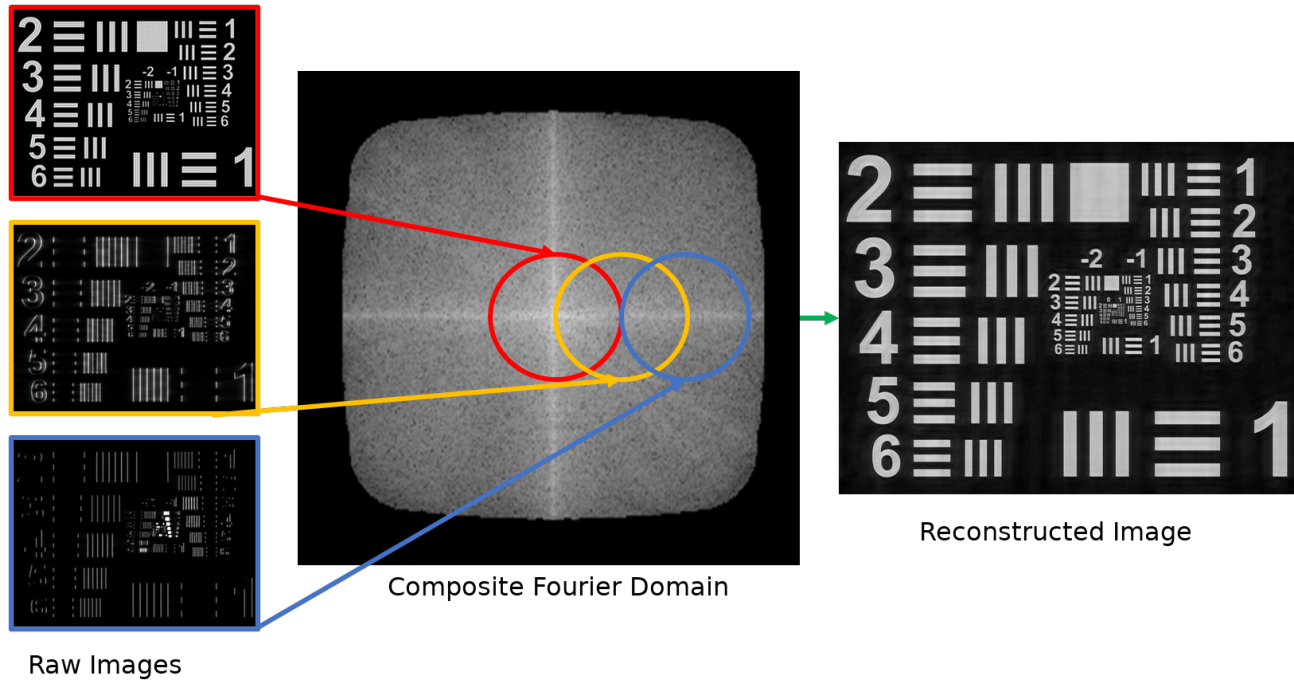


Figure 2. Schematic illustration of Fourier ptychography reconstruction.

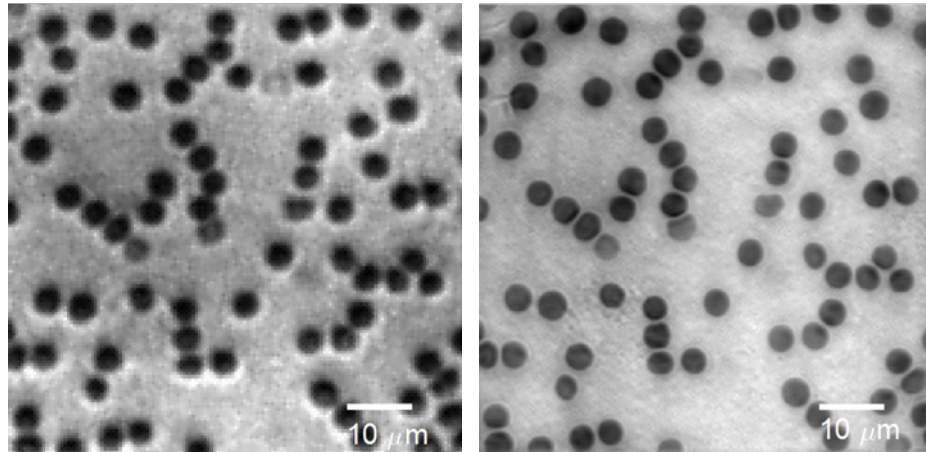


Figure 3. Comparison of a “conventional” image obtained with on-axis illumination (left, single LED illumination at angle closest to optical path) and reconstructed FPM image (right) of Wright’s-stained anemic red blood cells (not infected with malaria parasite) obtained using the Raspberry Pi FPM device.

A typical image obtained with our device is illustrated in Fig. 3, showing Wright’s stained anemic red blood cells (not infected with malaria parasite). On the left is the “conventional” image obtained with the Raspberry Pi microscope, corresponding to the illumination LED closest to the central optical path. Evidence of misalignment is visible. On the right is the computed FPM image calculated using 196 illumination angles.

2.1 MTF Calculation using Idealized Bar-Patterns

Our method of determining the optical MTF was validated by calculating the MTF using 12 selected bar-pattern ROIs in an idealized 1951 USAF optical resolution test pattern (groups -3 and -4), illustrated in Fig. 4 (0.129- μm pixels).^{21,22} Columns in each ROI were summed to generate a series of 1-D profiles. Each profile was fit to

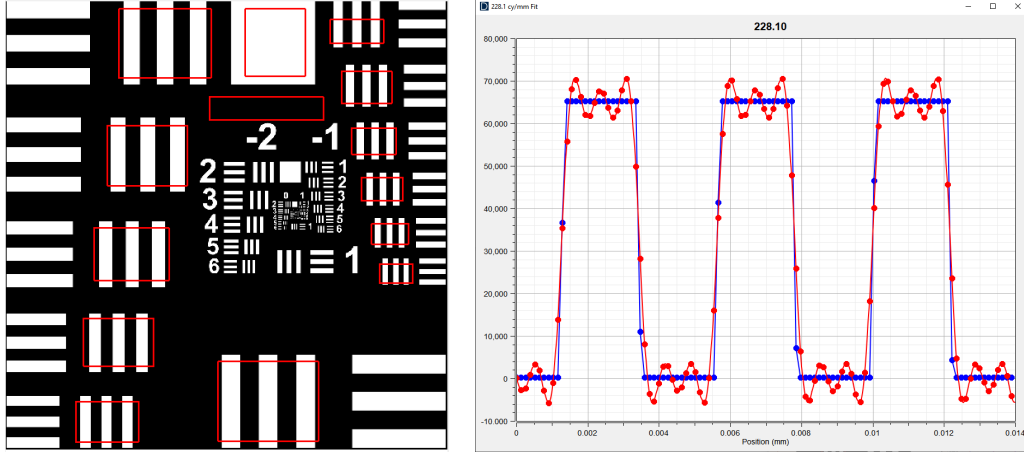


Figure 4. Left: Schematic representation of an idealized 1951 USAF optical resolution test device. Right: The blue line shows the profile created from the lower-left bar pattern. The red line shows a least-squares fit of a fundamental plus two odd harmonic frequencies. For both, dots indicated sample points connected by straight line segments. If the fit line is not sufficiently over-sampled to adequately capture the harmonics, errors will result in the χ^2 term between the two lines.

$s(x)$ in Eq. (1) which includes the fundamental frequency u_o plus N odd-harmonic terms, phase ϕ , and a sloped baseline $mx + b$:

$$s(x) = \sum_{k=1}^N \frac{A_k}{2k-1} \sin(2\pi((2k-1)u_o x + \phi)) + mx + b. \quad (1)$$

For each ROI, the least-squares analysis was used to solve for A_k (for fundamental and specified number of odd-harmonic terms), u_o , ϕ , m and b . Each A_k term corresponds to the MTF amplitude at frequency $u_k = (2k-1)u_o$. Combining all such terms from all 12 ROIs and normalizing to large near-zero-frequency regions gives the MTF curve:

$$T(u) = \frac{A(u_k)}{A(0)}. \quad (2)$$

There are three primary limitations to this approach.

1. If the bars are not well aligned with columns in the image, the profile will be blurred by an amount determined by a combination of misalignment and camera pixel size. In the idealized pattern in Fig. 4, we do not know details of how it was generated, but resolution would also be limited by a sampling aperture width if included in that calculation. This seems likely as a single sample point is seen on the profile across each bar edge in Fig. 4.
2. If high-frequency harmonic components in the profiles are not sufficiently over-sampled, an error results in the calculated sum of squared differences (χ^2 value) between theory and measured profiles in the least-squares analysis, resulting in errors in the fit amplitudes of other terms (see right side of Fig. 4). This is a form of over fitting and puts an upper limit on the terms that can be included in the analysis relative to the profiles' Nyquist sampling cut-off frequency given by $u_c = 1/(2x_o)$ where x_o is the image pixel size.
3. Trying to solve for more harmonic terms than are supported by the data (ie. terms with frequencies at or above the MTF limit) places an additional limit on the number of harmonic terms that can be included in the analysis. This is an additional form of over-fitting. It is therefore necessary to manually review results and confirm that the minimum number of harmonic terms consistent with the bar-pattern profile is used for each ROI.

In addition, off-axis illumination may result in distortions that affect the fit. The MTF calculated using the idealized USAF pattern in Fig. 4 should have a value near unity for all frequencies, although it may be reduced if the pattern is generated using a finite aperture width in the spatial-sampling process.

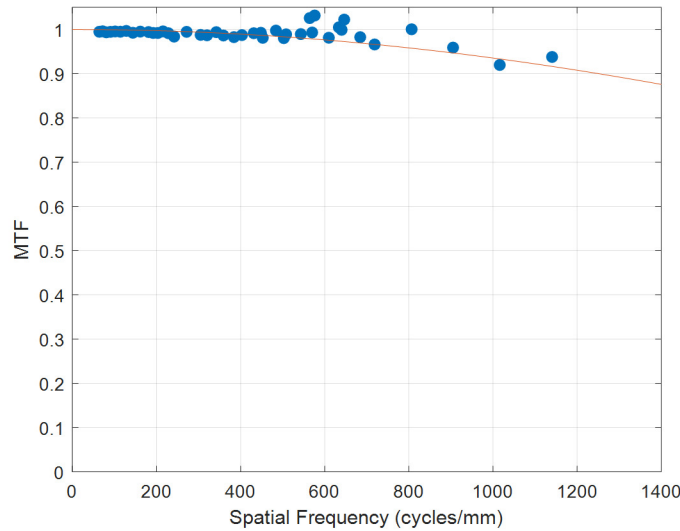


Figure 5. MTF calculated from 12 ROIs in the idealized USAF test pattern in Fig. 4. Errors due to under-sampling of harmonic terms increase with increasing frequency. In this calculation the Nyquist sampling cut-off frequency was $u_c = 3875$ cycles/mm and the number of harmonic terms in the least-squares analysis was chosen to keep the maximum frequency below $\frac{1}{3}u_c \approx 1300$ cycles/mm.

2.2 MTF using Simulated Fourier Ptychography

Images corresponding to a range in illumination angles were simulated using open-source code.^{18,20} Three such images are shown in Fig. 2. The MTF was calculated using a selection of 12 ROIs in the USAF pattern. The “conventional” MTF was determined using a single image corresponding to illumination on the central optical axis. The FPM MTF was determined using the image computed from 196 simulated illumination angles.

2.3 MTF using Experimental Fourier Ptychography

Experimental MTF curves were determined from images acquired of the USAF pattern using groups 6 and 7. The “conventional” MTF was determined using the illumination LED closest to the optical axis and was compared with the theoretical diffraction limit imposed by the pupil function, u_p , given by

$$u_p = \frac{N_A}{\lambda} \quad (3)$$

in cycles/mm, where $N_A = 0.15$ is the numerical aperture of the microscope and $\lambda = 530$ nm is the wavelength of (green) light used, giving $u_p = 280$ cycles/mm. The FPM MTF was determined using all 196 illumination angles.

3. RESULTS

3.1 MTF using Idealised Bar Patterns

The MTF determined from the idealized USAF optical test pattern is shown in Fig. 5. Pseudo-random errors caused by harmonic under-sampling were limited to $\pm 5\%$ when the number of harmonic terms in the least-squares analyses was chosen to keep the maximum harmonic frequency below $\frac{1}{3}u_c \approx 1300$ cycles/mm where $u_c = 3875$ cycles/mm is the Nyquist sampling cut-off frequency in this calculation. This guideline was followed in all results presented here. Pixel size in the idealized pattern is not the same as simulated or experimental images.

The MTF should ideally be unity for all frequencies in this calculation. The MTF in Fig. 5 is near-unity up to approximately 1000 cycles/mm, with an overall decreasing trend with increasing frequency. It is assumed

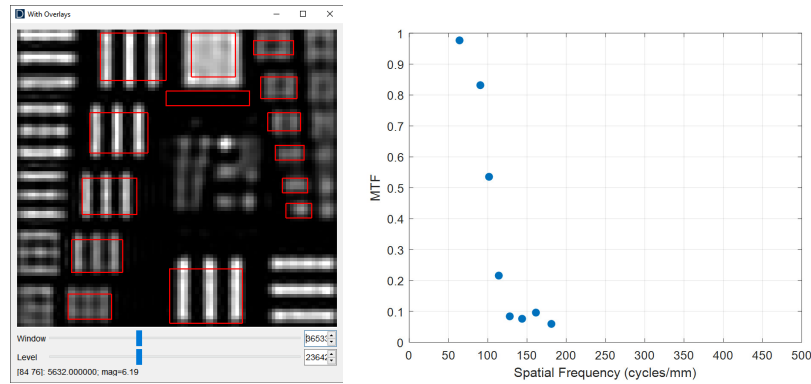


Figure 6. Simulated bar patterns and calculated MTF corresponding to a “conventional” system by using only on-axis illumination. The MTF shows a steep cut-off at 100 cycles/mm, corresponding to the pupil function pass-band in the simulation. Values from only the first eight bar patterns shown on the left were used.

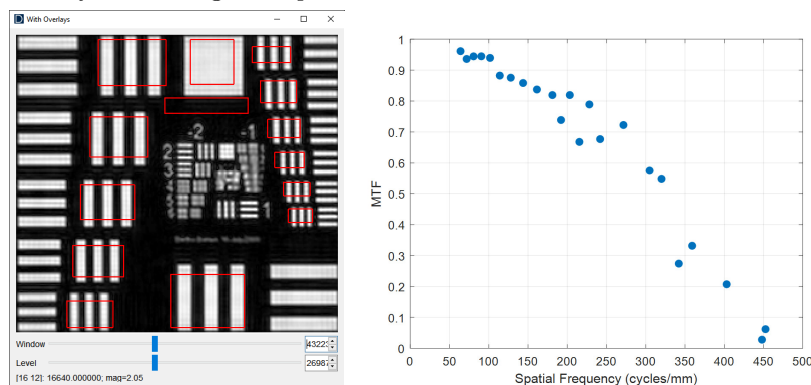


Figure 7. Simulated bar patterns and calculated MTF corresponding to the FPM system using all illumination angles. The MTF has a 10% value at approximately 430 cycles/mm.

this overall decrease corresponds to a sinc-function-shaped MTF (or similar) resulting from the aperture used to create the pattern, but this is not confirmed. The dashed line shows a sinc-function corresponding to a 2- μ m aperture for reference.

3.2 MTF using Simulated Fourier Ptychography

The MTF determined from simulated bar patterns corresponding to on-axis illumination is shown in Fig. 6. Only the first eight patterns identified were used as there was insufficient modulation at higher frequencies. No harmonic terms were used in the fits. The MTF shows a steep cut-off at 100 cycles/mm, corresponding to the passband of the pupil function used in the simulation. It is not the same as the experimental pupil function. The MTF 10% value is approximately 120 cycles/mm.

The MTF of the simulated FPM system has a 10% value at approximately 430 cycles/mm, corresponding to just over $3\times$ improvement in limiting frequency, consistent with theoretical predictions and illustration in Fig. 2.

3.3 MTF using Experimental Fourier Ptychography

The MTF of the FPM microscope was evaluated in the same way. Using the image corresponding to illumination closest to the optical path, the conventional image and MTF are shown in Fig. 8. Artifacts from slight off-axis illumination were observed which likely contribute to uncertainty in the MTF. The MTF drops relatively quickly but not as steeply as in the simulated images (Fig. 6) with an MTF 10% value of approximately 300 cycles/mm. This value is slightly greater than (but consistent with) the diffraction limit imposed by the pupil function of $u_p = 280$ cycles/mm given by Eq. (3).

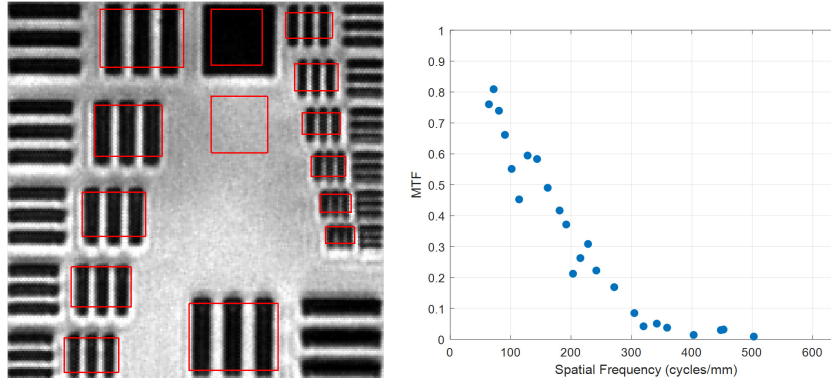


Figure 8. Left: Image acquired using only the single LED closest to the central optical path. Right: The corresponding MTF determined from this image has a 10% frequency of approximately 300 cycles/mm. The theoretical limiting frequency determined by Eq. (3) is 280 cycles/mm.

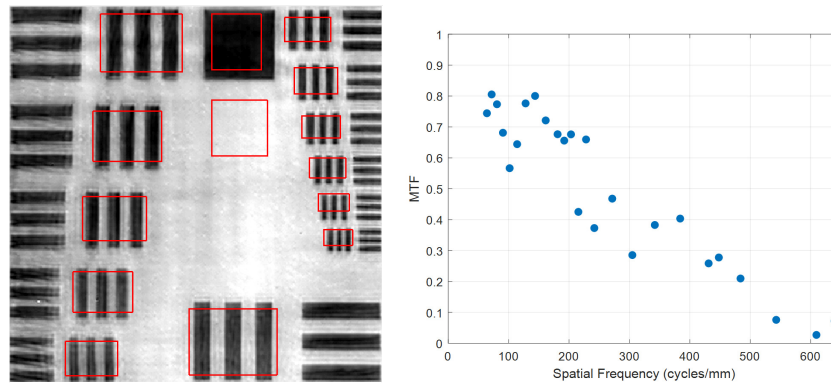


Figure 9. Left: Image computed from all 196 illumination angles shows a substantial improvement in image quality. Right: The corresponding MTF determined from this image has a 10% frequency of approximately 550 cycles/mm.

The computed FPM image in Fig. 9 shows a significant improvement in resolution. The MTF quantifies this improvement, but it is not as substantial as the improvement observed with simulated images. The MTF drops to a 10% value at approximately 550 cycles/mm, corresponding to an increase by $1.8\times$ relative to the image obtained with near on-axis illumination, and slightly less than the Nyquist sampling cut-off frequency of 667 cycles/mm (pixel size is $0.75\text{ }\mu\text{m}$).

4. CONCLUSIONS

A method of measuring the optical MTF using bar patterns from the 1951 USAF resolution test pattern is described. The method uses a least-squares fit of a square wave, including odd harmonic terms, to measured profiles. It is shown that FPM reconstruction using an array of off-axis LED illuminations increases spatial resolution of a low-cost microscope design using a Raspberry Pi camera and electronics and increases the MTF 10% value to 550 cycles/mm, an increase by $1.8\times$ compared to a single image obtained using near on-axis illumination of the same device.

The following specific conclusions are made.

1. An accurate MTF is determined from an idealized 1951 USAF optical resolution test pattern by restricting harmonic frequencies in a least-squares analysis of bar-pattern profiles to $1/3$ of the Nyquist cut-off frequency and manually restricting the number of harmonic terms to prevent over fitting.
2. Using simulated images of the USAF pattern corresponding to illumination angles of the FPM microscope, the MTF 10% value was increased by a factor of just over $3\times$, consistent with theoretical expectations.

3. Using experimental images of the USAF pattern, the MTF 10% value of the FPM microscope when using only on-axis illumination was 300 cycles/mm. This was slightly better than the expected limit imposed by the pupil function of 280 cycles/mm. In computed FPM images, the 10% frequency was 550 cycles/mm, an increase of approximately $1.8\times$. While this improvement is less than anticipated, it represents a substantial increase in spatial resolution compared to images acquired with only on-axis illumination.

ACKNOWLEDGMENTS

The authors acknowledge funding from the Western University Frugal Biomedical Innovation program and are grateful to Dr. Paul Picot for advice. Dr. Wasswa acknowledges support from Academics Without Borders' Strengthening Engineering Research program.

REFERENCES

1. "World Malaria Report 2022," tech. rep., World Health Organization, 2022.
2. "WHO Guidelines for Malaria," tech. rep., World Health Organization, 2021.
3. J. Hanrahan, W. Amelo, and E. Makonnen, "Efforts made to eliminate drug-resistant malaria and its challenges," *BioMed Research International* **2021**, p. 5539544, 2021.
4. "Malaria vaccines," tech. rep., The World Health Organization, <https://www.who.int/news-room/questions-and-answers/item/q-a-on-rt-s-malaria-vaccine>, 2023.
5. C. A. Petti, C. R. Polage, T. C. Quinn, A. R. Ronald, and M. A. Sande, "Laboratory medicine in africa: a barrier to effective health care," *Clin Infect Dis* **42**(3), pp. 377–82, 2006.
6. D. Mabey and R. Peeling, "The optimal features of a rapid point-of-care diagnostic test," in *Revolutionizing tropical medicine: Point-of-care tests, new imaging technologies and digital health*, K. Atkinson and D. Mabey, eds., ch. 3, Wiley Online Library, 2019.
7. S. L. Rei Yan, F. Wakasuqui, and C. Wrenger, "Point-of-care tests for malaria: speeding up the diagnostics at the bedside and challenges in malaria cases detection," *Diagnostic Microbiology and Infectious Disease* **98**(3), p. 115122, 2020.
8. J. Salido, G. Bueno, J. Ruiz-Santaquiteria, and G. Cristobal, "A review on low-cost microscopes for open science," *Microscopy Research and Technique* **85**(10), pp. 3270–83, 2022.
9. D. N. Breslauer, R. N. Maamari, N. A. Switz, W. A. Lam, and D. A. Fletcher, "Mobile phone based clinical microscopy for global health applications," *PLOS ONE* **4**, pp. 1–7, 07 2009.
10. N. A. Switz, M. V. D'Ambrosio, and D. A. Fletcher, "Low-cost mobile phone microscopy with a reversed mobile phone camera lens," *PLoS One* **9**(5), p. e95330, 2014.
11. T. E. Agbana, J.-C. Diehl, F. van Pul, S. M. Khan, V. Patlan, M. Verhaegen, and G. Vdovin, "Imaging and identification of malaria parasites using cellphone microscope with a ball lens," *PLoS One* **13**(10), p. e0205020, 2018.
12. M. Ganesan, J. R. S. Selvan Christyraj, S. Venkatachalam, B. V. Yesudhasan, K. S. Chelladurai, M. Mohan, K. Kalimuthu, Y. B. Narkhede, and J. D. S. Christyraj, "Foldscope microscope, an inexpensive alternative tool to conventional microscopy-applications in research and education: A review.," *Microsc Res Tech* **85**(11), pp. 3484–94, 2022.
13. G. Zheng, R. Horstmeyer, and C. Yang, "Wide-field, high-resolution Fourier ptychographic microscopy," *Nature Photonics* **7**, pp. 739–745, jul 2013.
14. X. Ou, R. Horstmeyer, G. Zheng, and C. Yang, "High numerical aperture fourier ptychography: principle, implementation and characterization," *Opt. Express* **23**, pp. 3472–3491, Feb 2015.
15. T. Aidukas, R. Eckert, A. R. Harvey, L. Waller, and P. C. Konda, "Low-cost, sub-micron resolution, wide-field computational microscopy using opensource hardware," *Scientific Reports* **9**(1), p. 7457, 2019.
16. P. C. Konda, L. Loetgering, K. C. Zhou, S. Xu, A. R. Harvey, and R. Horstmeyer, "Fourier ptychography: current applications and future promises.," *Optics express* **28**, pp. 9603–9630, Mar. 2020.
17. T. Aidukas, *Next generation Fourier ptychographic microscopy: computational and experimental techniques*. PhD thesis, University of Glasgow, 2021.

18. M. Rogalski, P. Zdańkowski, and M. Trusiak, "FPM app: an open-source MATLAB application for simple and intuitive Fourier ptychographic reconstruction," *Bioinformatics* **37**, pp. 3695–3696, Apr. Rogalski2021.
19. L. Tian, X. Li, K. Ramchandran, and L. Waller, "Multiplexed coded illumination for fourier ptychography with an led array microscope," *Biomed. Opt. Express* **5**, pp. 2376–2389, Jul Tian2014.
20. G. Zheng, *Fourier ptychographic imaging: A MATLAB tutorial*, Morgan & Claypool, 2016.
21. D. N. J. Sitter, J. S. Goddard, and R. Ferrell, "Method for the measurement of the modulation transfer function of sampled imaging systems from bar-target patterns," *Appl Opt* **34**, pp. 746–751, 1995.
22. A. Gonzalez-Lopez, P. A. Campos-Morcillio, and J. D. Lago-Martin, "An oversampling procedure to calculate the mtf of an imaging system from a bar-pattern image," *Med Phys* **43**(10), pp. 5653–5658, 2016.