1st Phase

PROGRESS REPORT (01/09/2019 to 02/12/2020)

1. Project title: "Design of smartphone platform fluorescence microscopic system for resource poor regions"
2. PI (name & address): Prof. Pabitra Nath, Dept. of Physics, Tezpur University, Napaam, Sonitpur, Assam-784028
3. Co-PI (name & address): Nil
4. Date of start: 31-07-2019
5. Duration: 2 Years
6. Objectives of the proposal:
   - To design and develop a low cost, compact opto-mechanical fluorescence microscopic set-up which can be attached to the rear camera of the smartphone so that it will work as a field-portable, easy-to-use fluorescence microscopic system.
   - To develop an application for android platform so that the analysis and further processing of the captured images could be done within the smartphone itself.
   - To determine the field applicability of the proposed smartphone fluorescence microscope, the device would be assessed for diagnosis of tuberculosis in particularly.
7. Methodology:

Figure 1 below shows the schematic of the proposed smartphone fluorescence microscope. The proposed set-up will employ an epi-fluorescence illumination system. As shown in the figure, a collimated light beam from an LED or diode laser propagate through an excitation filter to fall on the dichroic mirror. The dichroic mirror is kept at 45^0 angle and will reflect the light coming from the excitation filter to the specimen slide. Before hitting the specimen, the normally incident light beam is focused by an objective lens (magnifying lens) so that a well collimated beam of light is incident on the specimen. Since the specimen will be stained with fluorescent dye (such as Alexa Fluor® dyes), the portion of the specimen which retains the fluorescent dye will absorb the incident excitation light and will emit florescent light at longer wavelength. The emitted fluorescent light will then reflect back and incident on the emission filter after propagating through the dichroic mirror. The emission filter will allow passing only the emitted fluorescent light to the camera sensor of the smartphone. For illumination of the

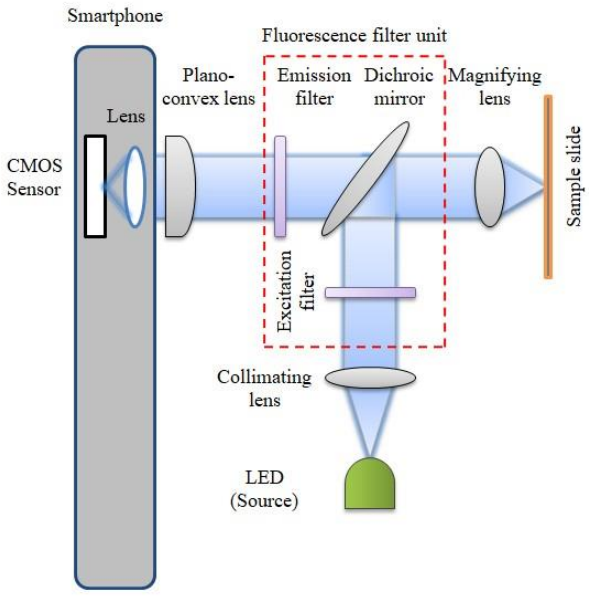

Figure 1: Schematic of the proposed smartphone fluorescence microscope.

specimen an external light source or the inbuilt flash lamp of the smartphone would be used.

8. Summary on progress (during the period of report):

In this period of the project work, the designing and fabrication of the smartphone bright-field (BF) microscopic system as well as its performance has been evaluated. The detail of the work done during this period has been summarized below-

A schematic of the BF smartphone microscope and the photo image of the developed microscope are shown in figure 2 below. The 3D-printed optical set-up which

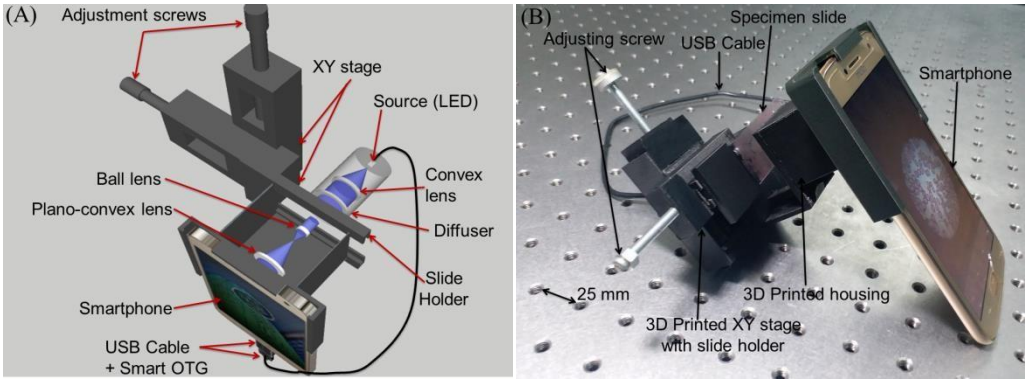

Figure 2: *Smartphone based microscope: (A) the optical layout, (B) the 3D printed prototype installed on an android smartphone device.*

has been coupled to the rear camera of the phone houses all the required optical components including the sample holder along with XY stage. In the next step, we evaluate the performance of the designed microscopic system by capturing images from standard 1951 USAF resolution test target. As can be seen in figure 4, the highest resolving group of the target element (indicated in red square) can be easily resolved with our proposed microscopic system. This infers that the optical resolution of tool is as good as ~2 µm. Upon capturing the image of the test target, the region of the highest resolving group element has been cropped and sends to the cloud server for processing. Figure 4 (C) represents the post-processed image of the same region obtained from the cloud. Clearly, it can be seen that as compared to the original image the post-processed image has been significantly improved in terms of resolution and contrast. Figure 4 (D) represents the intensity profile of the unprocessed and processed image of the same line element indicated in red square in figure 4 (B) and (C).

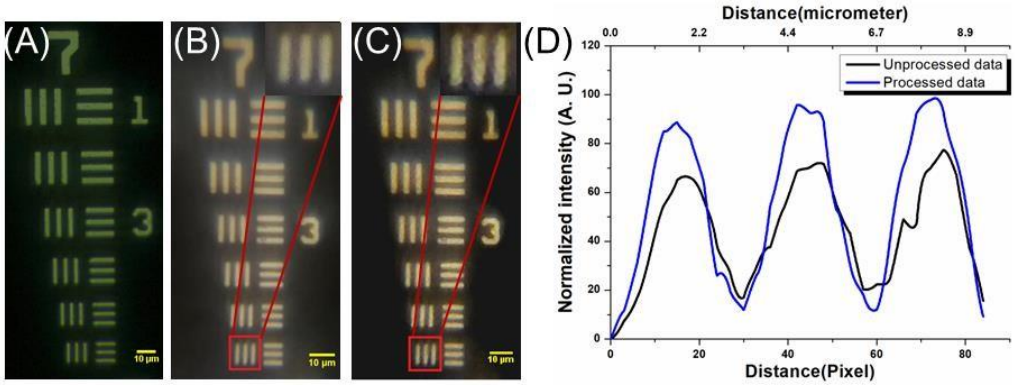

Figure 4: *Images of USAF Resolution test target (A) obtained from standard optical microscope (Carl Zeiss's Primo Star), (B) with the designed smartphone based optical microscope, (C) its corresponding processed image and (D) intensity profile of the selected region. Scale bars are 10 µm.*

In the next step, imaging of blood sample using the designed microscope has been performed. Stained blood specimens have been acquired from the Tezpur University Health Centre. Figure 5 (A) represents the full field of view (FOV) of the blood sample captured by our smartphone microscope and figure 5 (B) represents the cropped region indicated in square in the figure 5(A). Again, figure 5(C) shows the post-processed image of the cropped region as obtained from the cloud. Figure 5(D) and (E) are the images of same the blood specimen captured by the laboratory-grade microscope (Carl Zeiss's Primo Star).

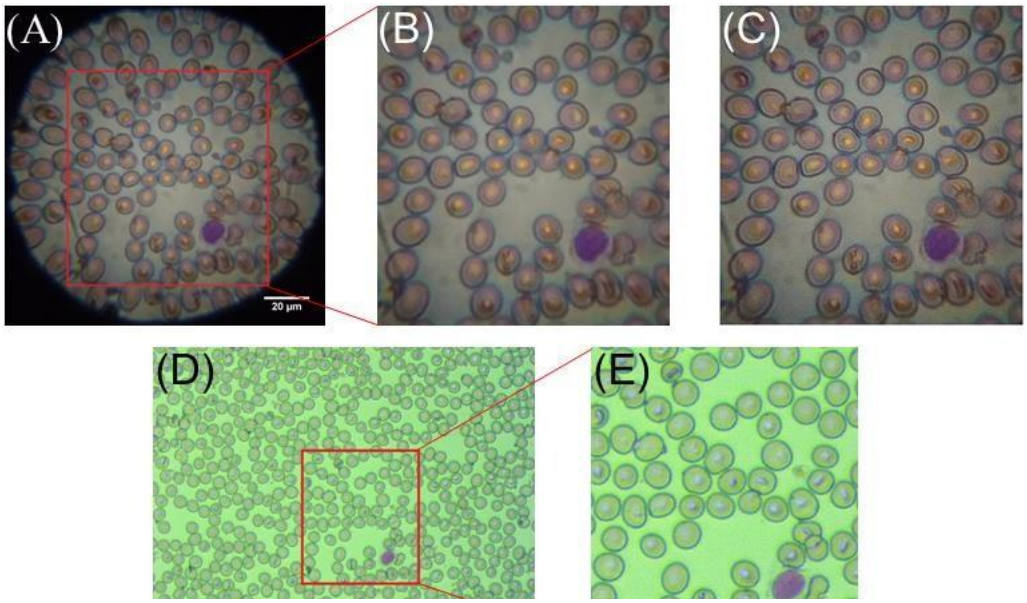

Figure 5: *Processed smartphone microscope image of thin blood smear. (A) Smartphone microscope image, (B) corresponding image of a cropped region, (C) corresponding processed image of the cropped region (D) a 40X/0.65 NA traditional microscope image of the blood sample and (E) zoomed version of the same region of interest (ROI) of the traditional microscope. Scale bars are 20 µm.*

9. Research work which remains to be done under the project:

In the final year of the project, mainly the following tasks will be performed:

- ❖ We will convert the developed BF microscopic set-up into fluorescence microscopic device by simply modifying the 3D-printed cradle.
- ❖ The developed device will be evaluated for imaging of tuberculosis diagnosis.

10. Any publications: Nil

11. Any patents applied for: Nil

12. If additional budget or staff is required for the remaining part of the research work, please give justifications and details: No

PROGRESS REPORT (10/12/2020 to 31/12/2022)

1. Project title : "Design of smartphone platform fluorescence microscopic system for resource poor regions"

2. PI (name & address): Prof. Pabitra Nath, Dept. of Physics, Tezpur University, Napaam, Sonitpur, Assam-784028

3. Co-PI (name & address): Nil

4. Date of start: 31-07-2019

5. Duration: 2 Years (Extended till 31/12/2022)

6. Objectives of the proposal:

   - To design and develop a low cost, compact opto-mechanical fluorescence microscopic set-up which can be attached to the rear camera of the smartphone so that it will work as a field-portable, easy-to-use fluorescence microscopic system.
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7. Methodology:

Figure 1 below shows the schematic of the proposed smartphone fluorescence microscope. The proposed set-up will employ an epi-fluorescence illumination system. As shown in the figure, a collimated light beam from an LED or diode laser propagates through an excitation filter to fall on the dichroic mirror. The dichroic mirror is kept at 450 angle and will reflect the light coming from the excitation filter to the specimen slide. Before hitting the specimen, the normally incident light beam is focused by an objective lens (magnifying lens) so that a well collimated beam of light is incident on the specimen. Since the specimen will be stained with fluorescent dye (such as Alexa Fluor® dyes), the portion of the specimen which retains the fluorescent dye will absorb the incident excitation light and will emit florescent light at longer wavelength. The emitted fluorescent light will then reflect back and incident on the emission filter after propagating through the dichroic mirror. The emission filter will allow passing only the emitted fluorescent light to the camera sensor of the smartphone. For illumination of the specimen an external light source or the inbuilt flash lamp of the smartphone would be used.

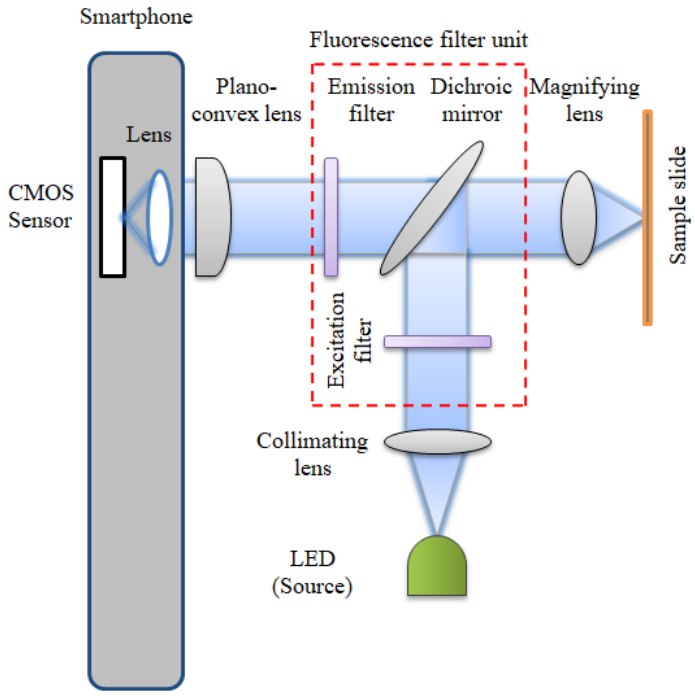

Figure 1: Schematic of the proposed smartphone fluorescence microscope.

8. Summary on progress (during the period of report):

In this period of the project work, a flexible smartphone-based multimodal imaging system that operates in Bright Field and fluorescence modes has been designed and fabricated. The detailed overview of the work performed during the mentioned period has been summarized below:

The developed setup utilizes the inbuilt primary camera of the smartphone, along with some optical components for the multimodal imaging platform. A schematic of the designed multimodal microscopic platform and the image of the fabricated setup is shown in Fig.2. In this work, 4f imaging system has been adapted for the development of the microscopic platform. The optical elements involved in this setup includes an achromatic doublet lens (diameter, D = 4 mm, FL = 6 mm, NA = 0.33) as an objective lens, plano-convex lens (D = 6 mm, FL = 11 mm) as a tube lens, iPhone 5s rear camera lens (FL = 4.12 mm, NA = 0.23, f/2.2) as a relay lens, and bandpass optical filter (centre wavelength, CWL = 520 nm, 67–016) for imaging in the fluorescent mode. The Bright Field imaging can be obtained in the same setup simply by removing the emission filter from the optical path. In the illumination panel, a white LED has been used for BF imaging, while two other LEDs that emit blue and green light with peak emission wavelengths 490 nm and 525 nm respectively have been used for fluorescence-based imaging purposes. The blue and the green LEDs were kept at an oblique angle of 32° to enhance the SNR in the final fluorescence image. The overall microscopic module has been designed with computer-aided design software and fabricated using a 3D-printer. A 3D-printed z-stage has been used for focusing the specimen in the slide holder.

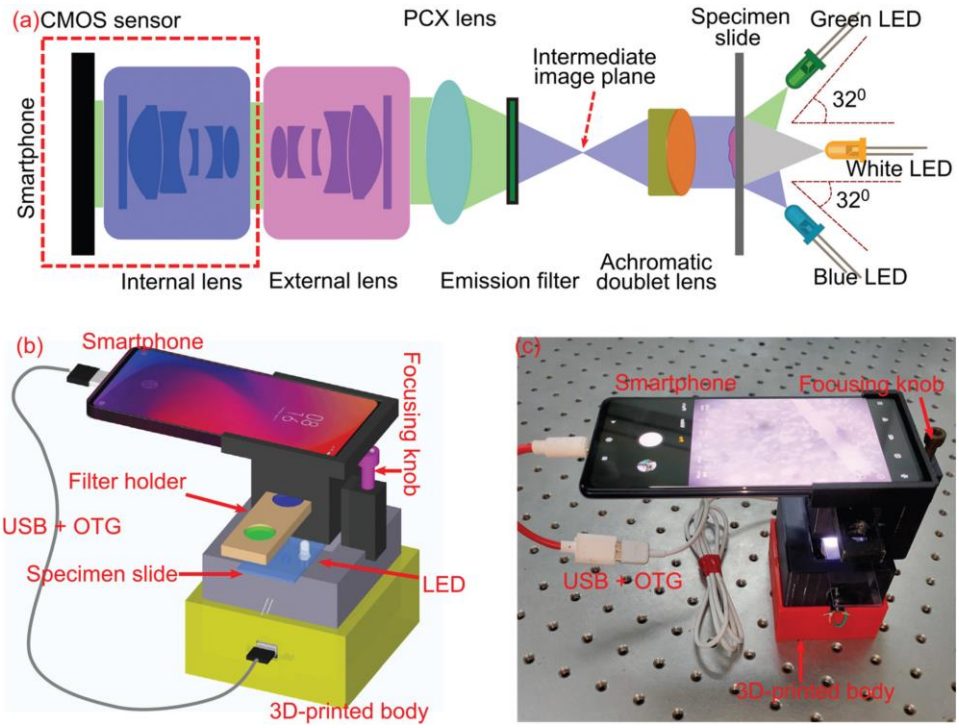

Fig.2 Design of the proposed Bright Field and fluorescence microscopy device. (a) Optical layout diagram of the system; (b) 3D design of the system; (c) fully assembled device

In the next step, the performance of the designed microscopic system was evaluated by capturing images from standard 1951 USAF resolution test target. As can be seen from the figure below, the highest group element bars were resolved (Group 7 element 6).

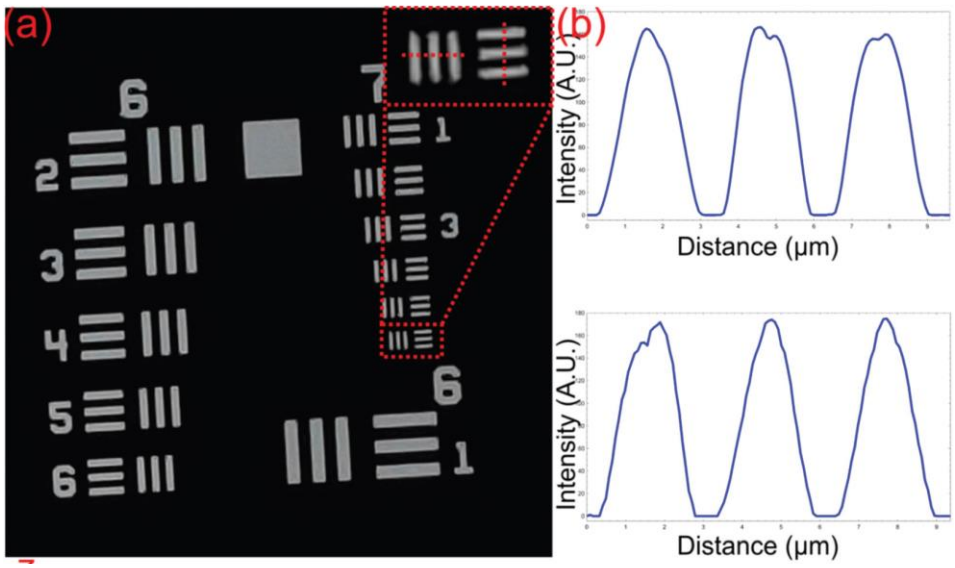

Fig.3 (a) Image of a USAF-1951 resolution test target acquired under BF illumination showing spatial resolution of the microscopic system up to Group 7 Element 6 (2.19 μm); (b) represents the intensity profile of the horizontal and vertical element bars of Group 7 indicated in the dotted red line in (a)

Next, the imaging of blood smear and standard microbeads were performed under Bright Field and Fluoresence modes of imaging with our developed setup. Fig. 4 shows the images captured by our microscopic platform. The lateral resolution of our system was found to be ~1.21 μm as compared to the theoretical value of 1.02 μm. The FoV was around 4530 μm and there are three different magnification levels supported (1.16×, 2.86× and 37.33×) by the present imaging platform.

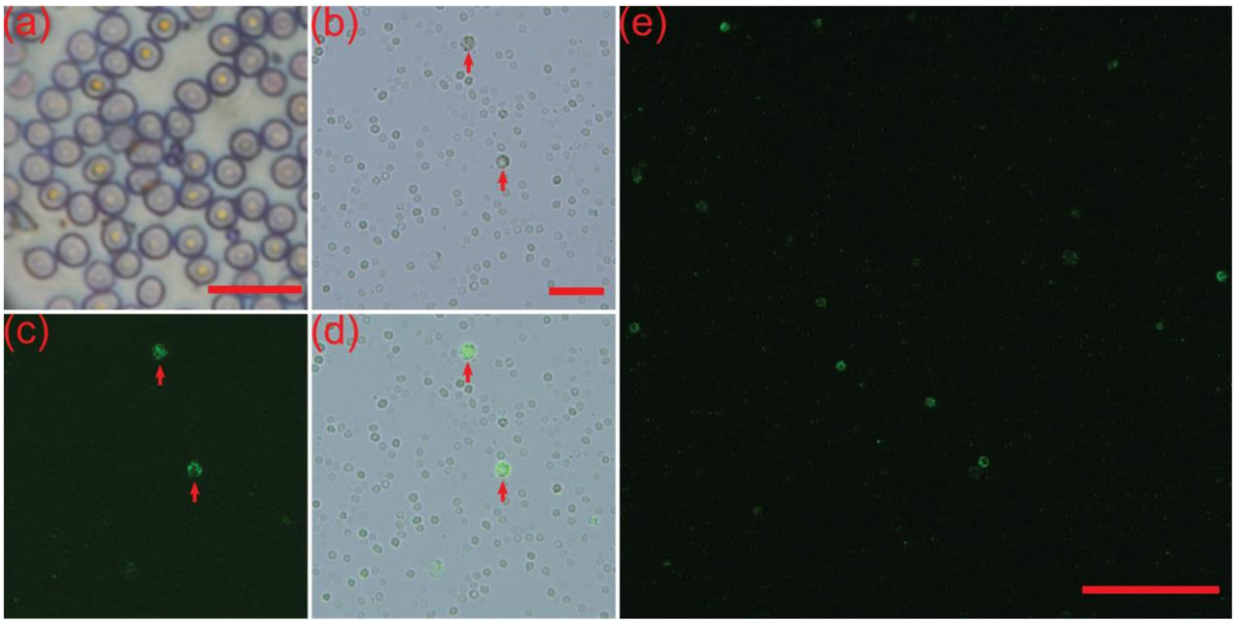

Fig.4 Applications of the microscopic device for the imaging of biological specimens. (a) Leishman stained blood smear image captured using the proposed device under BF illumination. Scale bar is 20 μm; (b) BF image of the Acridine orange stained blood; (c) fluorescence image of the same region in (a); (d) overlaid image of the BF and fluorescence image of blood shown in (b) and (c). Scale bars are 50 μm; and (e) a large FoV of the fluorescence image of whole blood. Scale bar is 200 μm.

Furthermore, the designed imaging platform is equipped with an onboard cell recognition feature which has been obtained through the development of a smartphone application for automatic cell counting with high precision. Fig. 5 showcases the development of the cell recognition and counting algorithm and ability of the system.

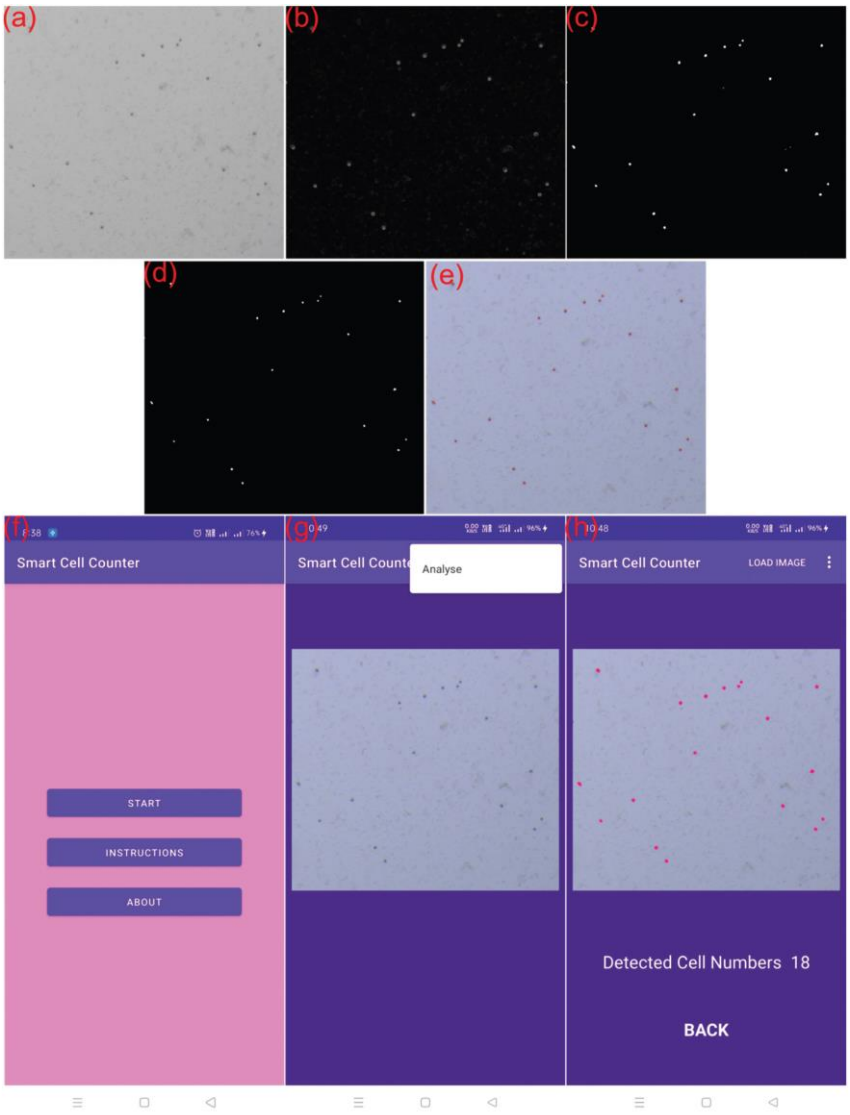

Fig.5 Development of the cell recognition and counting algorithm and the interface of the android application. (a) BF image of cells in a single FOV; (b) implementation of the Sobel operator to recognize the cell boundaries; (c) threshold image; (d) recognized cells after the removal of noisy artifacts; (e) cell number estimation using contour detection algorithm; (f) initial interface of the android app; (g) BF image read-in; (h) output results.

The experimental findings suggest that the designed system has the ability to image and assess both the morphology of individual cells and the cell population statistics and it can be reliably used in clinical laboratories as an alternative to traditional microscopes.

9. Research work which remains to be done under the project: NA

10. Any publications: Rabha, D., Biswas, S., Hatiboruah, D., Das, P., Rather, M. A., Mandal, M., & Nath, P. (2022). An affordable, handheld multimodal microscopic system with onboard cell morphology and counting features on a mobile device. *Analyst*, *147*(12), 2859-2869.

11. Any patents applied for:

Patent Title: Methods and apparatus of multi-modal microscopic imaging on a smartphone and OLED display illumination - D. Rabha, and P. Nath, with the Indian Patent Application Number- 202231030989.

12. If additional budget or staff is required for the remaining part of the research work, please give justifications and details: No

STATEMENT OF EXPENDITURE (SOE)

FROM 01.09.2019 to 31.12.2022

In respect of the Project entitled **"Design of smartphone platform fluorescence microscopic system for resource poor regions" under the scheme, "Innovation, Technology Generation and Awareness" of Prof. Pabitra Nath, Tezpur University, Tezpur, Assam.**

1. Sanction letter No. : ASTEC/S&T/1614/8/2018-19/1149 dated: 31.07.19
2. Period of the Project : 31.07.2019 to 31.12.2022
3. Total Project Cost : Rs. 2,41,500.00
4. Sanction/Revised Project Cost (if applicable): NA
5. Statement of Expenditure:

Sl. No.	Sanctioned/ Heads	Funds Allocated		Expenditure Incurred		Balance as on 31.12.2022	Remarks
1	Recurring (Contingencies)	1st Year	2nd Year	1st Year	2nd Year	- Rs.7,209/-	
		Rs. 15,000/-	Rs.15,000/-	Rs. 32,545/-	Rs. 17,344/-		
2	Recurring (Consumables)	Rs. 15,000/-	Rs.15,000/-		Rs. 17,320/-		
3	Non-Recurring (Equipment)	Rs. 1,50,000/-		Rs. 1,35,404/-	Rs. 14,460/-	Rs. 136/-	
4	Travel	Rs. 10,000/-	Rs.10,000/-	-	-	Rs.20,000/-	Unspent
5	Overhead	Rs. 11,500/-		-	Rs. 4,268/-	Rs. 7,232/-	
	Total	Rs. 1,90,000/-	Rs.51,500/-	Rs. 1,67,949/-	Rs. 53,392/-	**Rs.20,159/-**	
		Total - **Rs. 2,41,500/-**		Total Expenditure - **Rs.2,21,341/-**			

23/1/23

Signature of the Principal Investigator
With date

Signature of Accounts Officer
With date

Finance Officer
Tezpur University

UTILISATION CERTIFICATE

(FROM 01.09.2019 to 31.12.2022)

Certified that out of **Rs. 2,41,500.00** of grants-in-aid sanctioned during the period **01.09.2019 to 31.12.2022** in favour of Prof. Pabitra Nath under ASTEC sanction letter no **ASTEC/S&T/1614/8/2018-19/1149 dated: 31.07.19**, a sum of **Rs. 2,21,341.00** has been utilized for the purpose of Equipment, Recurring head and Overhead expenditure for which it was sanctioned and that the balance of **Rs. 20,159.00** remain unutilized at the end of this period.

23/1/23

Signature of
Principal Investigator
With date

Signature of Registrar
of the Institute with date

Registrar
Tezpur University

Signature of Accounts Officer
of the Institute with date

Finance Officer
Tezpur University