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DIAGNOSTICS

Evaluation of a lens-free imager to facilitate tuberculosis diagnostics in MODS

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SUMMARY

Tuberculosis (TB) control efforts are hampered by a mismatch in diagnostic technology. Lack of adequate early diagnostics and Multi-drug resistant (MDR) detection is a critical problem in control efforts. Alternate and novel diagnostic approaches are required, especially in low-resources settings where they are needed most.

The Microscopic Observation Drug Susceptibility (MODS) assay is a cost-effective, highly sensitive, and specific method based on the detection of characteristic cording growth patterns of *Mycobacterium tuberculosis* (MTB), in microscopic examination of a liquid culture under an inverted microscope. By adding antimicrobials to the wells, MODS also determines antimicrobial susceptibility in both MDR and Extreme Drug Resistant (XDR) tuberculosis.

The interpretation of a MODS culture performed in a 24 well plate, requires an extensive inspection over the entire surface to detect TB cords. This process requires significant time and effort from a trained microscopist.

We evaluated a lens-free imager system, able to render microscopic images of live specimens, for the proof of principle to be used for MODS culture interpretation. The lens-free imager system is able to digitalize a 24-mm^2 surface with approximately 40X magnification in a single capture.

The evaluation of the lens-free imager found that it produced microscopic images that were adequate for MODS interpretation by a human expert. Compared to the average time that takes a microscopist to completely examine a MODS culture sample, the lens free imager notably reduced the time of inspection.

Therefore, lens-free imager variants may constitute promising systems to aid in the diagnostics of tuberculosis, by simplifying and reducing the time of inspection and permitting automatization of MODS interpretation.

1. Introduction

Tuberculosis (TB) is a contagious airborne disease caused by the bacteria *Mycobacterium tuberculosis* (MTB) affecting the poorest population of the world. TB is widespread and causes an important number of deaths worldwide. An estimated of 8.7 million of new cases of TB and 1.4 million deaths occurs per year [1].

In recent years, drug-resistant TB has emerged, largely due to delays in treatment, gaps in treatment protocol, and delayed drug-susceptibility testing [2]. Multi-drug resistant tuberculosis (MDR-TB) is defined by the resistance of the bacillus to isoniazid and rifampin, while extensively drug-resistant TB (XDR-TB) is defined by the resistance to any fluoroquinolone and at least one of three injectable second-line drugs (amikacin, kanamycin or capreomycin) [3,4].

In 2011 the World Health Organization endorsed the Microscopic Observation of Drug Susceptibility (MODS) as a new

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phenotypic technique for TB diagnosis and drug susceptibility testing. MODS is based on the visual inspection of *M. tuberculosis* colonies and the identification of microscopic cording patterns in a sputum liquid culture. This characteristic phenotype is observed in an inverted microscope at magnifications between 40× and 100× [5,6]. MODS has proven to be highly sensitive (98% sensitivity), fast in average it requires 7 days to detect a TB positive sample, and cheap (about $3–4 per sample to diagnose TB and to determine MDR-TB) [6,8]. The incorporation of TB drugs into the culture medium allows for the determination of antimicrobial susceptibility. Besides detecting MDR-TB, MODS has recently been validated for the detection of Extreme Drug Resistant Tuberculosis (XDR-TB) [7].

The cording pattern of colonies observed between days 7–10 is specific of MTB and is the basis of MODS [6,8]. This characteristic cording pattern has led to the development of an automated pattern recognition algorithm to facilitate automatic MODS interpretation in microscopic digital images [9].

Despite the advantages of MODS, it requires an inverted microscope, which is a relatively costly piece of equipment and a trained technician to interpret MODS cultures. To examine one sample in a 24-well plate of a MODS culture at 40X magnification, a microscopist takes approximately 1–2 min reading at least 4 fields per well.

The lens-free imager systems are able to autonomously render microscopic images of live specimens in a wide field and in a single step. This technique leverages the broad availability of high-performance sensors to provide a simple, cost-effective and automated microscopy solution [10]. The basic functioning of a lens-free imager consists in placing the specimen directly on the surface of an image sensor that is illuminated by a point source of light from the top. In this configuration, the image of the shadow is generated on the surface of the sensor, which is captured without the need of any lens or focusing procedure. This image reproduces the detail of the specimen when observed in a standard optical microscope. Notably, this process takes a single capture step to digitalize the complete surface of the sensor.

In this study, we evaluate a lens-free imager system based on a 24 mm^2 CMOS sensor for digitalizing and interpreting MODS cultures.

2. Materials and methods

2.1. Samples

Ten sputum samples were collected anonymously from the Mycobacterium Laboratory of the Faculty of Science, at the Universidad Peruana Cayetano Heredia in Lima–Peru during 2014. Five TB positive (smear positive 2+) and five TB negative sputum samples used were remnants of routine processes.

2.2. MODS culture

MODS culture was performed following the protocol previously described [5,6,8]. Briefly, 2 mL of sample after its decontamination and concentration by the standard NaOH–N-acetyl-L-cysteine methodology was inoculated with 5 mL of Middlebrook 7H9 broth-OADC 10% and 100 μL of the antimicrobial supplement PANTA. One ml of the mixture was distributed in 4 wells of a 24-well plate. Plates with their lids were sealed in Ziploc bags and incubated at 37 °C. Plates were examined every two days starting at day five of culture, using an inverted light microscope at 40× and 100× total magnification to observe characteristic growing cords indicating MTB culture positivity. A technician expert in the MODS assay and interpretation of TB cords evaluated all the samples. All the procedures were performed in a level-3 biosafety facility.

2.3. Implementation of the lens-free imager system

The lens-free imager system evaluated in this study was donated by Dr. Changhuei Yang from the Biophotonics Laboratory at the California Institute of Technology. It is a custom miniature microscopy prototype system that comprises a CMOS imaging sensor, an aluminum clamp, a socket board, a stainless steel stand and a plastic dish cover, while the controlling software resides on a computer (Figure 1). The hardware upper part is composed by the sensor and the clamp. It uses the commercial Aptina (MT9P031) CMOS image sensor with 5 Mpixels (pixel size: 2.2 × 2.2 μm) resolution and a total imaging area of a 6 mm × 4 mm [11]. The sensor was glued to bottom of the clamp using poly dimethylsiloxane (PDMS), by mixing 1:10 with base and curing agent, which is resistant to sterilizing-autoclave temperatures of 120 °C. Under the clamp there is a socket board that hosts the sensor and serves as an intermediate platform for electrical connection. This upper part is screwed on top of a custom stainless steel stand; everything weights 235 g and has 30 mm × 30 mm × 40 mm of size. A plastic dish cover is used for preventing the sample from evaporation and contamination. A white LED is placed on the top of the dish cover being approximately right above the center of the CMOS sensor. The intensity of light was controlled by limiting the current using a potentiometer. To control the aperture of the light source, an aluminum foil with a perforated hole of a variable diameter was placed in the dish cover below the LED. The image sensor circuitry is connected to a computer via USB and it is controlled by software (IC Capture) that allows user-controlled imaging. For autonomous imaging, a custom USB switch box that receives commands from a Matlab script, and switches connection between the computer and up to four lens-free imager devices was available. The specimens to be observed are deposited in liquid solution over the CMOS sensor. When the specimens deposit on the surface of the sensor, the light from the LED projects a shadow over the sensor, which is digitally captured and produces an image similar to the observed under an inverted optical microscope.

2.4. Evaluation

To evaluate the lens-free imager system we completed a series of three tests. The first two were used to determine optimal parameters for illumination and mechanical-equilibrium of the specimens. The third test evaluated the acquisition and interpretation of MODS images, under optimal parameters.

2.4.1. Test 1: determination of the optimal aperture of the LED light source in the dish cover

The quality of the acquired images, in terms of contrast and border definition depends on the size of the light source as determined by the aperture of the LED. The LED placed on top of the plastic dish cover was covered with an aluminum foil with a small hole perforated under the center of the plastic cover to control light aperture. We tested different diameter sizes for the hole, including 0.5 mm, 1 mm, 1.5 mm, 2 mm, and a test without the aluminum foil. In each case the technician manually selected the most appropriate light intensity from the LED.

All the sputum samples were cultured in MODS. After the seventh day of culture and after confirming the presence of cords in the TB positive samples, the bottom content of the MODS well (800 μL) was gently transferred to the lens-free imager using a micropipette. The transferred sample rested for 16 min to stabilize. To prevent overheating of the sample with the CMOS sensor, the lens-free imager was turned on every 3 min and captured five digital images of each sample during a 16 min period.

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