Sensors and Actuators A 279 (2018) 367–375

Contents lists available at ScienceDirect

Sensors and Actuators A: Physical

journal homepage: www.elsevier.com/locate/sna

A portable, optical scanning microsystem for large field of view, high resolution imaging of biological specimens

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ARTICLE INFO

Article history:
Received 13 January 2018
Received in revised form 3 June 2018
Accepted 17 June 2018
Available online 24 June 2018

Keywords:
Point-of-care
Microscopy
Large field of view
Optical imaging
Lens array

ABSTRACT

Adapting medical technology for use at the point of care, demands the development of portable, robust and accurate systems for the early diagnosis and monitoring of a wide range of diseases. Microscopy at the point of care, fueled by recent advances in micro-optics, micro-electronics and micro-electromechanical systems, is an emerging and promising field. However, imaging devices already developed remain rather sophisticated and bulky, mainly because of failure to address the most challenging technical limitation: to combine large field-of-view (FOV) with high resolution imaging of biological specimens. To address this need, we developed a portable, optical scanning microsystem that can image - with approximately 1 μm resolution- large areas (6 mm × 40 mm) from various biological samples. This is achieved through the use of a microfabricated - 2D lens array that scans a sample in 1 direction in few minutes. We demonstrated that our system can image blood smear and identify single white blood cells immobilized in a microfluidic chip.

1. Introduction

Early diagnosis and accurate continuous monitoring of the progress of a disease are both considered of critical importance. However, time consuming procedures and costly equipment required in many cases, act as serious obstacles in the early screening of a disease. Though, in the developed world advanced infrastructure and well-trained healthcare professionals are available, the cost of healthcare can still be prohibitive for patients to seek diagnosis. In low-resources settings, access to medical equipment may not even be available [1]. To address these issues, there is an emerging trend to adapt a patient-centered healthcare [2]. The use of point-of-care devices is part of this trend with excellent application in the field of microscopy imaging of biological specimens [3]. Recent technological advances in micro-optics, micro-electronics and micro-electromechanical systems (MEMS) have the potential to improve screening and detection of a wide range of diseases at the point-of-care in primary health care settings in both low and high-resource countries.

Microscopy imaging is the gold standard for the diagnosis of malaria, tuberculosis and sickle cell anemia, while it is also used for progress monitoring and treatment adjustment in the case of HIV-infected patients and patients undergoing chemotherapy for cancer treatment. Whole slide blood imaging and cell population counting represent irreplaceable steps of the diagnostic procedure in many cases. The conventional diagnostic process of Malaria, common infection in sub-Saharan region, is based on microscopic examination of stained peripheral blood smears. The standard process requires examination of the entire smear slide with light microscope to detect infected cells [4,5]. For HIV infected individuals, $CD4^+$T cell concentration is a common test to perform to define the response of the immune system to antiretroviral therapy. Cell count can be accurately extracted through microscopy imaging of a drop of patient's blood (1–10 μl), though flow cytometry remains the gold standard [6]. In tuberculosis-endemic countries, the low-cost, fast and accurate microscopy imaging of smears of non-concentrated sputum with Ziehl-Neelsen staining is the most common diagnostic test [7]. Other hematologic diseases, such as sickle cell anemia also require microscopy imaging for highly specific diagnosis. In both low and high-resource countries, frequent counting of white blood cells is required for patients undergoing chemotherapy or radiation therapy for cancer treatment as low white blood cell counts often represent an important side effect.

In general, in vitro biological specimen imaging such as blood film or sputum is considered as the most promising field of application for automated and miniaturized medical instrumentation that

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https://doi.org/10.1016/j.sna.2018.06.034
0924-4247/

could be used at the point of care [8]. High accuracy, in terms of specificity and sensitivity, low cost, portability, ease of use and low power consumption are the specifications imposed by the World Health Organization for devices used at the point-of-care in poor-resources settings [9]. Though a large number of devices have been developed in the field of automated portable imaging systems, unfortunately, so far, none of them has succeeded in complying with all criteria. Particularly, concerning the technical limitations and challenges met, major problem is the need to combine high resolution with large FOV imaging of the biological sample [8].

To address this need, two main approaches have been widely studied: (a) whole slide scanning [10–12] and (b) lens-free imaging techniques [13–16]. In particular, a certain number of evolved devices perform 2 or 3 direction scanning to cover an extended area of tissue or blood sample [11,12]. In these approaches, sophisticated motorized systems and complicated optical systems are employed, resulting in an increased cost of manufacturing. In cases where scanning components are excluded, medium or large format detectors have to be used instead [13], increasing furthermore the production cost. In case of non-scanning devices, the resolution is limited by the pixel size of the large format detector in use, much larger than desired resolution of at least a few micrometers. To compensate large pixel size of these sensors – approximately 10 μm – the existing approaches make use of complicated and bulk optical systems or sophisticated software algorithms for image information extraction. Consequently, time and computational power requirements radically increase. It is also a fact that the samples tested need to be rather sparse to reduce possible loss of information due to actual low resolution efficiency [13]. The same problems of augmented computational power demands and sparse sample requirements are encountered in approaches of lens-free imaging systems [13–16]. In the aforementioned platforms, image processing algorithms are used to detect particles in a sample, based on the shadow produced by the particles. Thus, provided accuracy may be highly affected by the sample density and result in a large number of lost particles in case of dense specimens. Preprocessing of blood is therefore a demand and a possible obstacle in areas where the necessary laboratory equipment is unavailable.

With the advent of mobile phones, their extensive use and their fast technological development, there has been an integration of phone cameras in devices for cytological or histological specimen imaging. These devices, though assuring portability and autonomy, incorporate complicated and expensive optical systems to perform high resolution imaging by radically decreasing the detection area [17,18]. Though phone cameras provide high quality imaging, they are restricted for a short range of applications and are incapable of providing extended region imaging, unless combined with a scanning system.

Rapid progress in microfabrication technology resulted in an emerging trend towards the use of micro-lens arrays. The integration of high numerical aperture micro-lenses in optical systems, benefits of high resolving power, large FOV imaging and radically decreases cost of manufacturing. The inevitable problem of whole slide imaging related to the use of micro-lens arrays is caused by the existence of undetected areas of the imaged specimen due to the blind regions between neighbor lenses in an array. To mitigate this challenge, in some cases a multilayer lens-array assembly is employed, improving also provided resolution, while a sophisticated, 2 or 3 direction- scanning system can additionally be used [12]. Such an approach increases the manufacturing cost of the equipment, while it is rather questionable whether it meets the criteria of compactness and portability.

Despite the numerous approaches that already exist, the field still presents a challenge as there has been no approach that could overcome the aforementioned obstacles and provide a general purpose, portable, low-cost device for high resolution and wide FOV imaging. To be effective at the point-of-care, evolved technologies need to be simple [19]. Our approach proposes the use of a specially patterned mini-lens array combined with a simple, one direction (1D) scanning system for whole slide, high resolution imaging of a biological specimen. The large FOV (290 μm) and high numerical aperture (NA∼0.7) of the used sapphire ball lenses reduce the number of required images – approximately 400 images for a 4 cm long sample surface in 1X magnification. The simplicity of the proposed portable system – one direction scanning and one layer of mini-lenses (1 mm in diameter) – render it compact, inexpensive and with low power requirements. It is, therefore, ideal for use in poor resources areas.

2. Materials and methods

We developed an imaging, scanning platform (Fig. 1) that comprises of three components: (a) an optical, scanning head that images the specimen of interest (b) a motorized, translation stage that is responsible for moving the optical head along the specimen (1D scanning) and (c) an external white light LED array (Edmund Optics, #66-830) that provides homogeneous illumination of the entire specimen. The optical head is an assembly of a 10.7 Mpixel monochrome CMOS sensor (Imaging Source, DMM 27UJ003-ML) and of a custom-made mini-lens array. The optical head is attached to the translation stage that is connected to a computer-controlled, stepper motor through a lead screw. The specimen – typically blood sample inserted in a microfluidic chamber or sitting on a glass slide or coverslip – is placed at a short distance (∼0–500 μm) from the optical head and it is illuminated by the LED array light source. The CMOS/mini-lens array assembly moves in one direction and has a travelling distance of approximately 40 mm. This configuration creates a 40 mm x 6 mm image of the specimen. The distances between the mini-lens array, the CMOS sensor and the specimen are controlled by two step motors (20 μm step, 3 mm range) that allow us to obtain a sharp, focused image of the sample. The focusing procedure is performed prior to every scan.

2.1. The optical, scanning head

The optical, scanning head is responsible for image acquisition process. It comprises of a microfabricated lenslens array, a 10.7 Mpixel CMOS camera (of 1.67 μm square pixels) and two, computer controlled, stepper motors that control the distance between the sample, the lens array and the detector. The two stepper motors are used to achieve sharp in-focus images as well as to operate in different magnification modes – from 1X up to 4X magnification. The operational principle of the entire scanning system is based on the special design of the lens array. 36 sapphire ball lenses of high numerical aperture (NA∼0.7), refractive index of 1.67 and 1 mm in diameter (EDMUND OPTICS #43-638) are placed on top of a specially patterned silicon die to form the array of mini-lenses.

The mini-lens array is fabricated using standard microfabrication techniques (supplementary Fig. 1). A 400 μm thick, silicon wafer is Deep Reactive Ion Etched (DRIE) to create 960 μm in diameter, wafer-through holes [20]. After the etching process is completed, individual dies are glued with a UV curable optical adhesive (NORLAND 60, refractive index 1.62) on thin, glass coverslips of standard thickness (#1, 130–170 μm) to form the wells upon which the lenses are placed. The same adhesive is used to fill the wells and secure the lenses on top of the wells.

The design of the lens array is the key element of the operation of the entire system. The wells are patterned so that all mini-lenses in the array are equally spaced with an edge-to-edge distance of 50 μm. The lens array is placed with a tilt angle of $\varphi = 17^{\circ}$ with respect to the scanning direction. This tilted design assures that