Biosensors and Bioelectronics 99 (2018) 513–518

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

A low cost mobile phone dark-field microscope for nanoparticle-based quantitative studies

Dali Sun, Tony Y. Hu*

School of Biological and Health Systems Engineering, Virginia G. Piper Biodesign Center for Personalized Diagnostics, The Biodesign Institute, Arizona State University, 727 E. Tyler St. B 130-B, Tempe, AZ 85287, United States

ARTICLE INFO

Keywords:
Dark field microscope
Mobile phone
Nanoparticle
ImageJ
Immunoassay

ABSTRACT

Dark-field microscope (DFM) analysis of nanoparticle binding signal is highly useful for a variety of research and biomedical applications, but current applications for nanoparticle quantification rely on expensive DFM systems. The cost, size, limited robustness of these DFMs limits their utility for non-laboratory settings. Most nanoparticle analyses use high-magnification DFM images, which are labor intensive to acquire and subject to operator bias. Low-magnification DFM image capture is faster, but is subject to background from surface artifacts and debris, although image processing can partially compensate for background signal. We thus mated an LED light source, a dark-field condenser and a 20× objective lens with a mobile phone camera to create an inexpensive, portable and robust DFM system suitable for use in non-laboratory conditions. This proof-of-concept mobile DFM device weighs less than 400 g and costs less than $2000, but analysis of images captured with this device reveal similar nanoparticle quantitation results to those acquired with a much larger and more expensive desktop DFMM system. Our results suggest that similar devices may be useful for quantification of stable, nanoparticle-based activity and quantitation assays in resource-limited areas where conventional assay approaches are not practical.

1. Introduction

Nanoparticles probes are useful in point-of-care assays, including lateral-flow chromatographic immunoassays, since antibody-labeled nanoparticles can be dried and stored under ambient conditions until use, unlike enzyme-linked antibodies employed by conventional immunoassays. These point-of-care devices are not quantitative, however, and require extensive development and validation (Chao et al., 2012; Sajid et al., 2015). Gold nanoparticles are used to quantify different targets (Hu et al., 2003; Rajendran, 2013; Wagner et al., 2014) in cell imaging, (Hu et al., 2009; Li et al., 2016) biomolecular quantification, (Li et al., 2016; Li et al., 2016; Yuan et al., 2016) and interaction studies (Jin et al., 2015; McFarland and Van Duyne, 2003), but these assays rely on complex and relatively non-robust equipment that limits their utility outside controlled laboratory settings. Portable spectrometers can quantify nanoparticles, but suffer from low throughput (Heider et al., 2012; Verma et al., 2016; Zuber et al., 2016) or have low sensitivity and require complex setup prior to their use for quantitation (Wang et al., 2017). DFM image analysis used to detect and quantify nanoparticle assays predominantly utilize high-magnification, since low-magnification (far-field) DFM images are sensitive to surface artifacts and debris that can mask nanoparticle signal. The size, cost and delicacy of these DFM systems limit their utility in field hospitals and other settings where these factors represent barriers. Attempts to develop more portable DFM approaches date back to at least 1958, (Goldman and Sawyer, 1958) when dermatologists still relied on DFM analysis to identify pathogens, including syphilis, responsible for certain skin lesions, (Brown and Frank, 2003) but these devices fell out of use upon development of more specific assays, effectively ending portable DFM development. Recent technology advances have spurred the use of mobile phone cameras for medical applications, including portable microscopy for numerous point-of-care diagnostics (Table S5), but none of these devices use DFM to quantify nanoparticle-based assays.

We now report the development of a mobile phone-based DFM (MDFM) system that can quantify nanoparticle signal for a variety of research and medical applications. We analyzed the potential of this approach to quantify methods that form the basis of most clinical assays, binding kinetics and biomarker quantification, as well as a novel nanoparticle-based serum diagnostic assay for tuberculosis.

Abbreviations: DFM, Dark field microscope; DSM, DarkScatterMaster; MDFM, Mobile dark field microscope; DDFM, Desktop dark field microscope; AuNR, gold nanorod; FFLM, Far-field low magnification; CV, coefficient of variation

* Corresponding author.
E-mail address: tyhu@asu.edu (T.Y. Hu).

http://dx.doi.org/10.1016/j.bios.2017.08.025
Received 18 May 2017; Received in revised form 7 August 2017; Accepted 9 August 2017
Available online 16 August 2017
0956-5663/

MDFM analysis of these assays yielded robust results that were similar to, albeit less sensitive than, those obtained with a much more expensive and cumbersome desktop DFM system. These results suggest that a lightweight, portable MDFM device allows simple and rapid assay quantitation, and may thus serve as a valuable platform for biomarker quantitation in resource-limited settings, where simplicity and robustness are more important than absolute assay sensitivity.

2. Material and methods

2.1. Image capture and processing

Solidworks 2013 CAD software (Dassault Systemes SolidWorks Corporation) was used to design the MDFM case, which was fabricated with black acrylonitrile-butadiene-styrene (ABS) using a 3D printing service (3D hubs). DDFM images were acquired under consistent lighting and magnification using an Olympus IX81 microscope equipped with a dark-field condenser, a 4× or 10× objective lens, and an Olympus DP71 digital camera, using a 1/45 s exposure time. MDFM images were acquired using the Motorola Moto G2 camera to capture images from a slide holder case containing a dark-field condenser and a 10× or 20× objective lens and illuminated with a constant triple-LED white light source (Modgy, Inc.). Table S1 lists the components of these systems. All images were processed and quantified using our previously reported "DarkScatterMaster" (DSM) DFM algorithm (Sun et al., 2016) using the following software input parameters: contour threshold (Ct) = 253.020, center scale (S) = 0.8, type = Red, Low (Lt)/High (Ht) quantification limit: 0/62. Motorola Moto G2 (XT1068) images were captured with a 1/15 s exposure time with Open Camera (Version 1.32.1)(Harman, 2017) using an ISO 5000 configuration and allowing autofocus and 4× digital zoom. Magnification (M) was defined as the sample image height (h_i) divided by the height of the sample object (h_o), where h_o was the target well diameter (1.5 mm) and h_i was the diameter of this image in pixels multiplied by the resolution of the sensor chip (72 vs. 432 pixels/inch for MDFM and DDFM, respectively).

2.2. Binding affinity assay

Carboxyl-functionalized AuNRs (C12-25–650-TC-50, Nanopartz) were activated to covalently bond amine groups by mixing 40 µL of AuNR (4.22×10^{12}/mL) with 20 µL of EDC/NHS-sulfo PBS (2 mg/mL of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride and 1 mg/mL of N-hydroxysulfosuccinimide, Sigma-Aldrich) for 10 min at 25 °C. These amine-reactive AuNRs were then PBS-washed and 1 µL of indicated AuNR concentrations were applied to replicate wells on 192-well amine-functionalized slides (2×10^{12} group/mm^2, Arrayit), which were sonicated (Q500 Sonicator, Qsonica) for 8 min at 80% amplitude using a 5 s on/off cycle to accelerate hybridization. Slides were then washed for 10 min at 25 °C with 0.01% Tween-20 in PBS (PBST, pH 7.0), and deionized water, and then air-dried for DFM imagery. Binding affinity was calculated by nonlinear curve fitting with Origin 2015 software (OrginLab Corporation).

2.3. Protein quantification assay

Protein A/G-modified 192-well slides (Arrayit) were blocked with 1 µL/well Pierce Protein-Free Blocking Buffer (Thermo Scientific) for 1 h at 25 °C, then incubated with the indicated amounts of biotinylated CD9 antibody (NB110-81616, Novus) for 1 h at 25 °C, and PBS-washed for 10 min at 25 °C before hybridization with AuNR. Neutravidin-functionalized AuNR (Nanopartz C12-25–650-TN-50, 7×10^{-9} M) were PBS-diluted (40 µL AuNR to 200 µL PBS) after which 1 µL/well of AuNR was applied to replicate wells, which were sonicated (Q500 Sonicator, Qsonica) for 8 min at 80% amplitude using a 5 s on/off cycle to accelerate hybridization. After hybridization, slides were washed for 10 min at 25 °C with 0.01% Tween-20 in PBS (PBST, pH 7.0), and deionized water, and then air-dried for DFM imagery.

2.4. Data analysis

Limits of detection (LOD) and quantification (LOQ) were defined as 3× and 10× the standard deviation of the assay blank, respectively. Assay precision was determined with five replicates of three samples analyzed in a single assay (intra-assay) or in three assays analyzed on three different days (inter-assay). Graphs were generated with Origin 2015 and Microsoft Excel.

3. Results and discussion

3.1. Optical design and characterization

To generate an inexpensive, lightweight, and portable device capable of sensitive far-field DFM image analysis for nanoparticle quantitation, we wrapped a triple-LED light source, a standard dark-field condenser and a 20× or a 10× objective lens in a 3D-printed case that mates these components to a mobile phone camera (Fig. 1). We then compared the nanoparticle quantitation properties of this MDFM

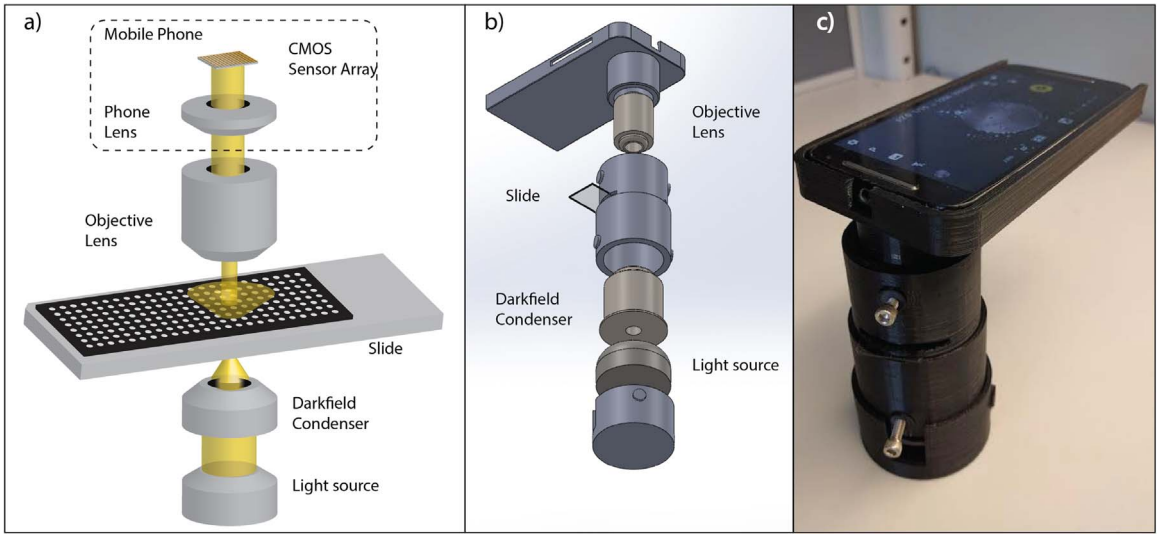

Fig. 1. MDFM system **a)** layout schematic, **b)** assembly and **c)** working prototype.