Non-invasive continuous imaging of drug release from soy-based skin equivalent using wide-field interferometry

Haniel Gabai, Maya Baranes-Zeevi, Meital Zilberman, and Natan T. Shaked
Department of Biomedical Engineering, Tel Aviv University, Ramat Aviv 69978, Tel-Aviv, Israel

ABSTRACT

We propose an off-axis interferometric imaging system as a simple and unique modality for continuous, non-contact and non-invasive wide-field imaging and characterization of drug release from its polymeric device used in biomedicine. In contrast to the current gold-standard methods in this field, usually based on chromatographic and spectroscopic techniques, our method requires no user intervention during the experiment, and only one test-tube is prepared. We experimentally demonstrate imaging and characterization of drug release from soy-based protein matrix, used as skin equivalent for wound dressing with controlled anesthetic, Bupivacaine drug release. Our preliminary results demonstrate the high potential of our method as a simple and low-cost modality for wide-field imaging and characterization of drug release from drug delivery devices.

Keywords: Drug release, drug delivery devices, interferometry, holography.

1. INTRODUCTION

Monitoring the principal characteristics of drug release (i.e. release rate and duration) from its drug delivery device (DDD), *in-vivo* or *in-vitro*, is highly important for achieving safe and desirable local therapeutic effects. Predicting these desirable characteristics when designing a DDD is a challenging task, since the drug release mechanisms are influenced by a variety of factors such as drug and polymer composition, polymer shape, drug location within the polymer, type and pH of the immersion medium, and many other factors, as detailed in Ref. (1-2). Due to this complexity, a comprehensive set of *in-vitro* drug release monitoring experiments are needed to evaluate the drug release properties of the DDD. Next, extrapolation from the *in-vitro* experiments into *in-vivo* model is needed. However, performing such mathematical manipulations is highly difficult, due to the multiple parameters and computational complexity required for the modeling. Another approach is to perform direct *in-vivo* measurements. Existing methods includes X-ray computed tomography (CT)[3,4], positron emission tomography (PET)[5] and magnetic resonance imaging (MRI)[6]. However, PET and CT use ionizing radiation and all these three techniques are highly expensive and require well trained operators, making them less attractive and available for clinical use.

Better understanding of the *in-vitro* drug release can significantly improve the extrapolation process to the *in-vivo* device and may reduce the use of direct *in-vivo* experiments. Current *in-vitro* measurements are performed by the analysis of an indirect measurement of the concentration from the DDD immersion medium by high-performance liquid chromatography (HPLC) or infrared spectroscopy. Although these methods are popular and considered as the gold-standard for these tasks, they are expensive and suffer from several limitations. For instance, during the experiment, an extraction of the immersion medium is required, a process which is considered to be intrusive. Moreover, in order to achieve the release profiles over time, a test-tube is needed to be filled with immersion medium for each measurement in time. For example, for 24 hours analysis of a single DDD, where a test tube is taken every hour, 24 test-tubes are needed to be taken and analyzed by the clinician. In order to perform longer experiments, with better time resolution, tens of test-tubes are required. This makes these experiments highly expensive in terms of man hours and lab consumable instruments. Moreover, the large amount of test-tubes used and the routine labor, increase the chance for human errors to occur. Due to the mentioned above, time resolution, which is essential in determining the quality of the drug release profile, is very poor and sample measurements are usually taken every few hours.

In this work, we suggest using off-axis interferometry as a unique non-invasive continuous imaging modality for characterization and wide-field imaging of *in-vitro* drug release from DDD. Our underlying assumption is that drug release will result in an immediate local change in the refraction index of the immersion medium, and consequently a local change in the spatial quantitative phase profile. In contrast to the traditional modalities,by using interferometry only a single test-tube has to be prepared, no intervention from the clinician is needed during the experiment, and

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significantly higher time resolution is achievable, which is limited only by the frame rate of the camera. This is especially useful for fast-releasing DDDs. Moreover, off-axis interferometry provides whole-environment analysis (i.e. wide-field imaging) of the drug release without the need for scanning. The wide-field imaging information can be useful in the extrapolation process for the predicted *in-vivo* characteristics of the DDD.

For this propose, we have constructed a low-cost, noise reduced, wide-field interferometric imaging system[7]. The system is based on a single beam-splitter cube positioned at the output of an inverted microscope illuminated by a coherent or partially coherent light source. This simplified design significantly reduces the need for additional optical elements required for applying the Linnik's or other conventional and hard-to-align interferometric configurations. Moreover, no special optical expertise is needed for the construction and operation of the proposed inexpensive imaging system.

To test our hypothesis, we have used a soy-based protein matrix. The DDD was monitored for two hours with sampling rate of a frame per 3 minutes. Our preliminary results demonstrate the high potential of interferometry, and quantitative phase imaging, as a unique, continuous, simple, and low-cost modality for wide-field imaging and characterization of *in-vitro* drug release from DDDs.

2. METHODS

2.1 Materials

The DDD used in our experiments is a soy-based protein matrix, utilized as skin equivalent for wound dressing with anesthetic, Bupivacaine anesthetic[8]. The DDD contains 3% of Bupivacaine. Comprehensive description about the DDD preparation procedures and properties are fully described in Ref. (8).

2.2 Sample preparation

The 5 mm × 3 mm DDD was placed on a cover-slip covered by a chamber-like silicon sticker (32 mm × 17 mm CoverWell perfusion chamber). The DDD was not presented in field of view (FOV), and was positioned at the periphery of the chamber as demonstrated in Fig. 1.

In our system, half of the beam serves as the interferometric reference beam, and the second one serves as the interferometric sample beam, and therefore, there is a need to divide the total volume of the chamber into two sections by a thin silicon bar (see Fig. 1). This division is necessary in order to ensure that no leakage of drug will diffuse into the reference-beam section.

Each section was slowly filled with 225 μl of phosphate buffered saline (PBS) using the chamber ports, which gradually start the drug eluting process. No enzymes were introduced to the PBS in order to avoid polymer degradation. Then, the filling ports were sealed by a sticker, which prevents leakage of the medium outside of the chamber and wave-like motion inside the sections during the drug release process.

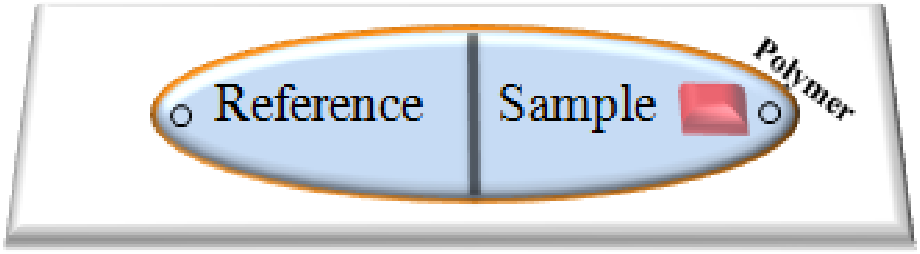

Figure 1. Illustration of the experimental chamber. Cover-slip covered by a chamber-like silicon sticker, divided by a thin silicon bar into two sections. Right section contains the DDD (red square) and PBS, and serves as the interferometric sample. Left section contains PBS only and serves as the interferometric reference.

2.3 System configuration

The proposed system is illustrated in Fig. 2. A tunable, temporally low-coherence supercontinuum fiber laser source (SC-400, Fianium, coupled to acusto-optical tunable filter, with FWHM spectral bandwidth of ~6 nm) is spatially filtered by a 25 μm pinhole, while expended by two 4f lens systems containing four achromatic lenses (L_{0-3}) with focal lengths of 35, 50 25, and 150 mm, respectively, creating an expended, collimated beam with a diameter of 1". Half of the illuminating beam interacts with the sample (DDD immersion medium) and serves as the interferometric sample beam. The second half of the beam, which does not interact with the sample (no drug eluting occurs in this section), serves as the interferometric reference beam. A third achromatic 4f lens system (L_{4-5}) delivers the amplitude and the phase of the sample to the right half of the beamsplitter (BS), and un-diffracted plane waves from the left reference section, to the left half of the BS. The BS diagonal plane is slightly tilted from the optical axial. This way, the BS serves as a common-path interferometer and creates two 180º phase-shifted interferograms on the camera plane in parallel. For the experiments presented, we used only one of these two interferograms in order to increase the FOV. The camera used is the DCC1545M CMOS camera from Thorlabs with 1280 × 1024 square pixels of 5.2 μm each. For each interferogram, a wedge-shaped optical path delay is created between the transmitted and reflected light beams within the BS, enabling the off-axis interference pattern. The off-axis spatial frequency of the interference pattern is determined by the angle θ between the optical axis and the diagonal semi-transparent surface of the BS. When increasing the angle θ by titling the BS the interference frequency increases accordingly. Both camera and light source are controlled and synchronized via LabView code in order to enable multiple acquisitions of drug release over the experiment period.

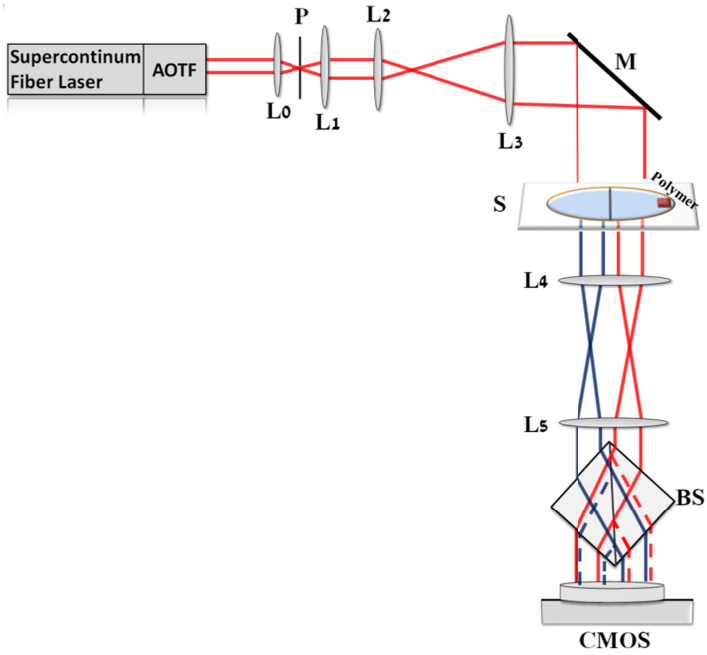

Figure 2. Low-coherence, dual-channel common-path interferometric imaging system used for drug release imaging. Light source – tunable, low-coherence light source. L0-5 – achromatic lenses, P – 25 μm pinhole, M – mirror, S – sample, where the red square indicates on the polymer location, BS – beam splitter. Blue lines – reference arm, red lines – sample arm. Solid lines – transmitted light, dashed lines – reflected light. CMOS – CMOS digital camera, which is synchronized with the light source by a computer.

2.4 Quantitative phase measurement of drug release

The off-axis interference enables us to capture the entire FOV in single camera exposure (i.e. wide-field imaging). This property makes it possible to image and monitor dynamic samples with sub-nanometer thickness resolution[10]. Transverse resolution is determined by the numerical aperture of the objective lens[11].

Drug release from the DDD is a dynamic process, as the drug leaves the DDD and spreads within the surrounding medium. The presence of the drug within the media results in a local change in its refractive index, and thus it can serve as a drug concentration indicator. The quantitative phase measured by interferometry is defined as[10]:

$$\phi(x,y)=\frac{2\pi}{\lambda}\cdot\left[\int_0^{h_c}\left[n_{total}(x,y,z)\cdot dh\right]\right] \quad (1)$$

where λ is the illumination wavelength, h_c is the height of the silicone chamber, n_{total} is the spatially varying refractive index within the immersion medium. For simplicity, if we consider n_{total} as a superposition of two refractive indexes (i.e. drug and immersion medium), then for each infinitesimally small volumetric unit, the refractive index can be related to drug or immersion medium only.

When examining Eq. (1), it can be seen that the phase value in each volumetric point in the FOV is determined by the quantity of the drug within that point. Moreover, since the quantitative phase in each lateral position is a weighted integral value proportional to the quantity of the drug in each point, we hypothesize that it can serve as an equivalent value to accumulative volumetric drug concentration, which is one of the most important parameters when characterizing drug release from DDD[1], where a fitting process is required in order to turn the phase profile into drug concentration profile. Note than since the reference beam passes through the chamber with PBS only, in time zero we get zero drug concentration. Thus, the system is already unbiased according to the zero concentration point.

The DDD was positioned, as described in Section 2.2, at the periphery of the silicone chamber in order to avoid diffraction and multiple scatterings artifacts, and therefore the drug release was monitored over time by capturing the immersion medium plus drug only.

When the PBS was injected to the chamber, only a few minutes of initial preparations were needed in order to start the imaging process. In this short period of time, we assume that a negligible amount of drug was released. The first frame captured served as a reference interferogram, which was used to eliminate wavefront curvatures and inherent noise factors from the spatial phase profile[9]. Next, we immediately acquired additional interferograms in a sample rate of one interferogram per three minutes, for duration of two hours. From all the interferograms acquired, the quantitative phase profile of the FOV was extracted using a digital off-axis interferometric process[10].

For the drug release analysis, several evaluations were taken. First, the average phase value in the entire FOV in each interferogram was calculated, as an indicator for the total drug release within the FOV. Second, by taking into advantage the wide-field imaging property of our system, we had the ability to examine the phase changes in particular spatial point as an indicator for local concentration changes due to drug release. To evaluate the drug release in different distances from the polymer, we examined the phase values in several spatial points along the entire measurement. This approach enables the clinician to accurately learn about the spatial distribution of the drug over time. Such information can be highly useful in the extrapolation process of the *in-vitro* results into characterization of an *in-vivo* device.

3. RESULTS

We examined the system ability to image and monitor drug release from DDD. As it can be seen from Fig. 3, significant temporal and spatial change in the quantitative spatial phase profile is observed over time. As demonstrated in this figure, in areas which are in close proximity to the DDD location, the most significant change in the phase values are observed, whereas in areas which are distant from the polymer, the phase changes are more moderate.

To extract the drug release profile within the FOV, we calculated the mean value of the quantitative phase profile in each frame, as an indicator of the total drug concentration. Then, we used the wide-field imaging property to examine the temporal phase changes in specific spatial points as an indicator for local drug concentration changes (Figs. 3 and 4).

We have examined three points in different locations, as demonstrated in Figs. 3 and 4. As can be seen from Fig. 4, at point P3, the closest point to the DDD location, the most significant temporal phase change is observed, where the phase values increase most rapidly and have the highest values along the entire measurement in comparison to the other two points examined. At point P2, located at the center of the FOV, the temporal phase behavior is similar to the averaged phase profile. In both points P3 and P2, moderate temporal phase changes are observed at the last 50 minutes of the measurement, which is a typical behavior after the drug-release burst effect that occurred after approximately one hour in this case. For point P1, located far from the DDD, a little change is observed, where after one hour (after the burst effect is over), only a negligible temporal change is observed.

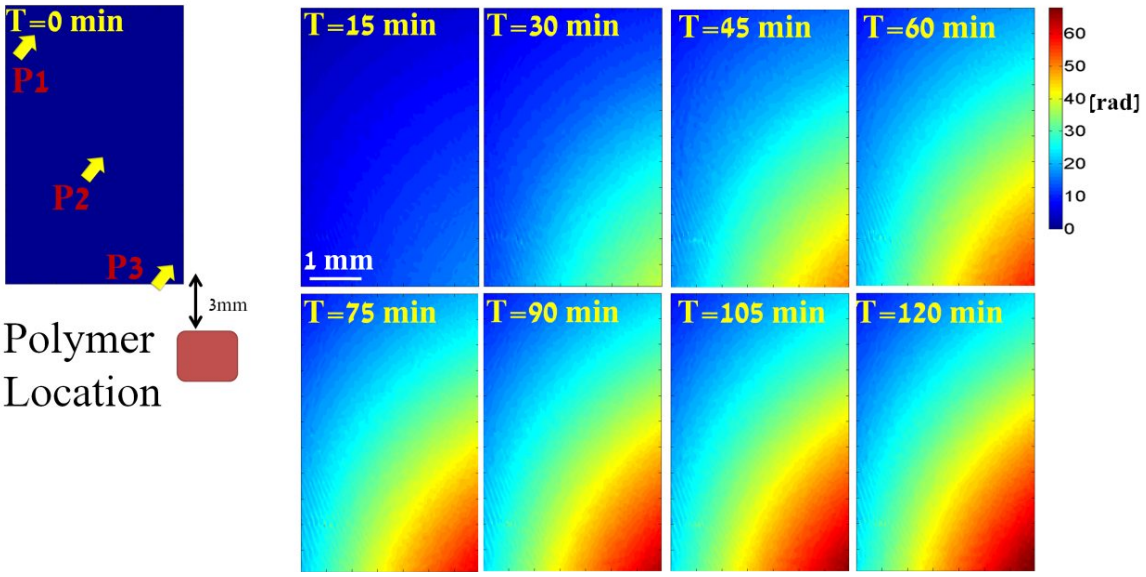

Figure 3. Wide-field imaging of the spatial phase distribution resulted from drug release, during two hours of continuous measurements. P1-P3, selected spatial points indicated by yellow arrows. The red square near the image of T=0 indicates on the location of the DDD relatively to the FOV.

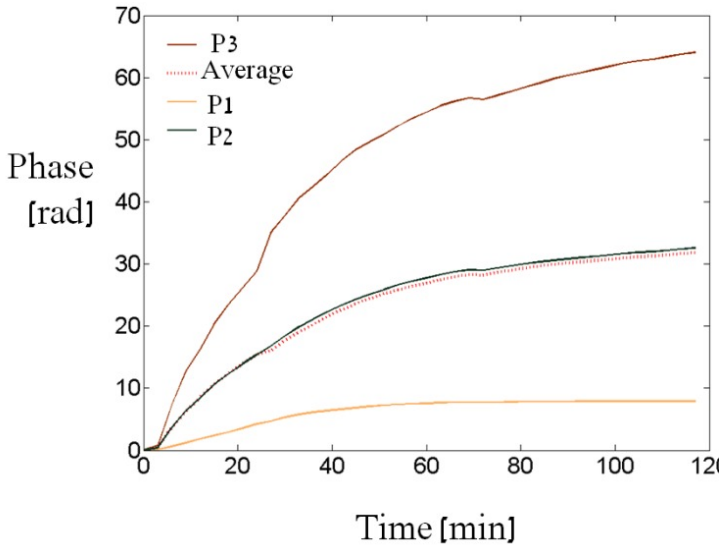

Figure 4. Temporal phase profiles of selected spatial points (P1-P3) and averaged phase value over time.

4. CONCLUSIONS AND FUTURE WORK

We introduced a unique method for *in-vitro* wide-field imaging of drug release from DDD. In contrast to traditional *in-vitro* drug release monitoring modalities, our system is low cost and requires only single sample preparation for the whole experiments. Moreover, precious time is saved since no intervention of clinician is needed during the experiment, and therefore the chances for human errors are significantly reduced.

The measurement is non-invasive since no extraction of the immersion medium is performed during the measurement period. In addition, the measurement is continuous, and time resolution is determined only by the frame rate of the camera. High temporal resolution can be helpful when trying to accurately characterize specific periods in the drug release profile, such as the burst effect, or for characterization of rapid drug release events. In order to convert the quantitative phase profile to the actual accumulative drug concentration profile, a standard curve-fitting process is needed with an initial drug release profile measured by a gold-standard modality, similar to the initial calibration required in HPLC.

Our preliminary results demonstrate the high potential of interferometry in wide-field imaging and characterization of drug release from DDDs. In addition to drug release monitoring, off-axis interferometry has the potential of providing direct imaging of polymer degradation[7], which is a crucial factor in the drug release mechanism and in tissue engineering applications.

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REFERENCES

[1] Shargel, L., Andrew, B. C., Wu-Pong, S., [Applied Biopharmaceutics and Pharmacokinetics], Appleton & Lange Reviews/McGraw-Hill, Chapter 1 (2005).

[2] Griffith, L.G. and Smith, A. S., "Polymeric Biomaterials," Acta Biomater. 961(1), 83-95 (2000).

[3] Szymanski-Exner, A., Stowe, N. T., Salem, K., Lazebnik, R., Haaga, J. R., Wilson, D. L., and Gao, J., "Noninvasive monitoring of local drug release using X-ray computed tomography: Optimization and in vitro/in vivo validation," J. Pharm. Sci. , 92(2), 289-296 (2002).

[4] Bhatnagar, A., Hustinx, R., and Alavi, A., "Nuclear imaging methods for non-invasive drug monitoring," Advanced drug delivery reviews, 41(1), 41-54 (2000).

[5] Szymanski-Exner, A., Stowe, N. T., Lazebnik, R. S., Salem, K., Wilson, D. L., Haaga, J. R., and Gao, J. , "Noninvasive monitoring of local drug release in a rabbit radiofrequency (RF) ablation model using X-ray computed tomography," J. Controlled Release, 83(3), 415-425 (2002).

[6] Taylor, J. S., and Reddick, W. E. ,"Evolution from empirical dynamic contrast-enhanced magnetic resonance imaging to pharmacokinetic," Adv. Drug Delivery Rev, 41(1), 91-110 (2000).

[7] Gabai, H., and Shaked, N. T., "Dual channel low-coherence interferometry and its application to quantitative phase imaging of fingerprints," Opt. Express 20(24), 26906-26912 (2012).

[8] Peles, Z., and Zilberman, M., "Novel soy protein wound dressings with controlled antibiotic release: mechanical and physical properties," Acta Biomater. 8(1), 209-217(2012).

[9] Ferraro, P., De Nicola, S., Finizio, A., Coppola, G., Grilli, S., Magro, C., and Pierattini, G., "Compensation of the inherent wave front curvature in digital holographic coherent microscopy for quantitative phase-contrast imaging," Appl. Opt., 42(11), 1938-1946 (2003).

[10] Shaked, N. T., Satterwhite, L. L., Rinehart, M. T., and Wax, A., [Holography, Research and Technologies], J. Rosen (Ed.), InTech, 219-236 (2011).

[11] Gustafsson, M. G., "Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy," J. Microsc. 198(2), 82-87 (2001).