Epi-Mode Quantitative Phase Imaging to Characterize Cell Structure and Dynamics with a Flexible Snake Robot

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Abstract (no more than 35-words): We develop an epi-mode quantitative phase imaging device to characterize cell structure and dynamic activity. It is composed of an optical imager using quantitative oblique back-illumination microscopy and a hyper redundant, high degree-of-freedom snake robot. © 2023 The Author(s).

1. Introduction

Quantitative phase imaging (QPI) measures the optical path length when light travels through various biological samples, which reflects the refractive index distribution among different histological structures. As a label-free, quantitative imaging modality, QPI has shown significant advantages of being non-destructive to samples (as compared to phototoxicity or photobleaching, induced by applying extraneous labels or stains and by exciting them with intensive light) and presenting highly accurate nanometer-level sensitivity. So far, QPI has been widely adopted to study biophysical properties and metabolic activities of many biological systems [1]. Even though QPI offers tremendous advantages to access important histological structures and biophysical properties with great details, it often requires thin samples to work with in a conventional transmission optical geometry. However, in many cases, epi-mode illumination and imaging are needed, especially when samples stay in an opaque or highly scattering environment, and when samples are not feasible to be made into thin slices.

Quantitative oblique back-illumination microscopy (qOBM) uses epi-mode illumination to realize thick scattering sample imaging [2-6]. Specifically, light propagation inside thick samples is modelled by Monte Carlo simulations, and with such knowledge, optical transfer function can be constructed using the ensemble photon profile after multiple scattering (forming the so-called “back-illumination”). With multiple sources of oblique illumination, full quantitative phase information can be retrieved by deconvolutions with the optical transfer function. Moreover, qOBM enables 3D [3], wide-field high-resolution imaging of complex structures, without requiring expensive instrumentation.

2. Method

A prototype fiber-based qOBM system [4-6] is shown in Fig. 1(a)-(c) that uses an imaging fiber bundle with 30,000 fiber elements, where each individual element is 4 μm in diameter. The bundle is attached to a gradient index (GRIN) objective lens (GRIN Tech; 1.4-mm diam.) with a 0.5 NA. The focal length is fixed to 60 μm and the magnification is 2.6X, yielding a resolution of 1.5 μm, given by the projection of the fiber elements onto the sample. Illumination is in epi-mode, with light delivered sequentially through fibers around the GRIN lens (Fig. 1(a)). With this configuration, the distal end of the probe (side closest to sample, Fig. 1(b)), is 4 mm in diameter, low weight, and flexible. On the proximal end (i.e., side of probe closest to the camera), the image relayed through the fiber bundle is detected using a conventional micro-scope (Fig. 1(c)). Imaging rate for a fully processed qOBM image is 20Hz (video-rate).

The snake robot is a highly maneuverable and customizable robotic arm that can be easily inserted in a bioreactor for imaging cells and support materials in situ. The snake robot is hyper redundant, using a series of motors and tendons to provide targeted tension to its flexible body, resulting in predictable bending profile. In this manner, the system can be controlled repeatedly, and the end effector can reach a large range of its workspace. A CAD model of a design is shown in Fig. 1(d), and the current prototype is shown in Figs. 1(e) and 1(f).

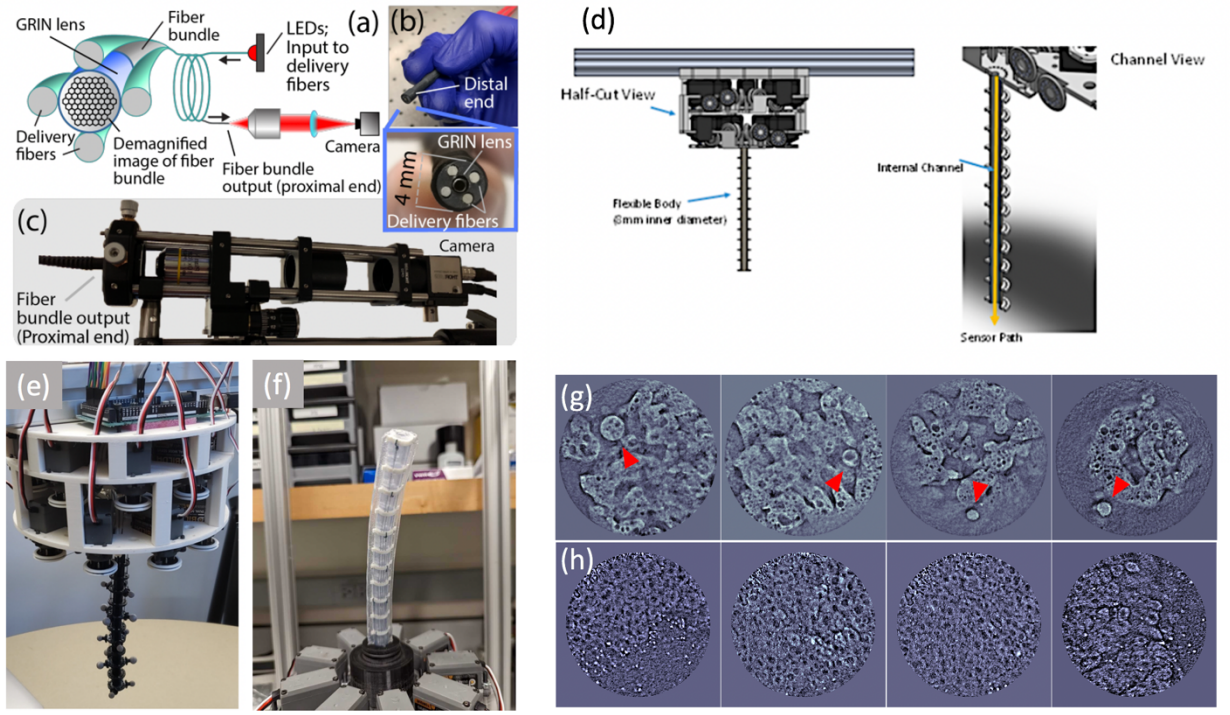

Fig. 1. (a) Optical fiber based qOBM schematic. (b) Photo of distal end, showing the GRIN lens and delivery fibers. (c) Photo of proximal end with detection module. (d) CAD design of snake arm system. (e) Current snake robot prototype. (f) Robotic housing of a previous design. (g) Representative images of microcarriers with cells (indicated by red arrows). (h) Representative images of freshly excised rat liver cells. Part of the figure is adapted from Ref. [4] with permission.

3. Results

Fig. 1(g) shows data acquired with our free-space qOBM system that shows MSC cells attached to porous microcarriers. Cell-like structures are also visible, as indicated by the red arrows. A time-series of qOBM images can be applied to isolate cells based on their dynamic behavior which correlates with metabolic activity. Fig. 1(h) shows images of freshly excised rat liver tissues, where cellular and subcellular structures are clearly visible. Specifically, the hepatocytes have a distinct “dark” nucleus resulting from the lower refractive index of the subcellular organelle, providing clear nuclear contrast. The snake robot is able to navigate to different locations while simultaneously imaging.

4. References

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