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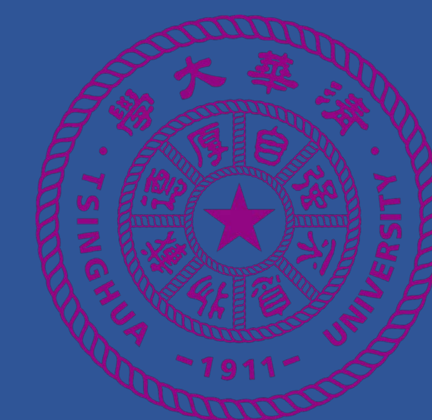
Simultaneous fluorescence and quantitative phase microscopy with single-pixel detectors

Yang Liu, Jinli Suo, Yuanlong Zhang, and Qionghai Dai
Department of Automation, Tsinghua University, Beijing 100084, China.



Abstract

We propose a simultaneous fluorescence and quantitative phase microscopy approach with single-pixel detectors. By exploiting

- reference-free interferometry,
- single-pixel detection, and
- structured illumination,

we demonstrate the **first** multimodal microscopy method for fluorescence and quantitative phase imaging **with single-pixel detectors** at the same time, to the best of our knowledge.

Introduction

Multimodal microscopy offers high flexibilities for biomedical observation and diagnosis. Conventional multimodal approaches either use multiple cameras or a single camera spatially multiplexing different modes.[1,2] The former needs expertise demanding alignment and the latter suffers from limited spatial resolution. However, single-pixel imaging offers an alternative solution for alignment-free, full-resolution multimodal imaging, as shown in Tab. 1.

	Multi-camera	Single-camera	Single-pixel cameras
Alignment-free	☆☆☆☆☆	★★★★☆	★★★★★
Full-resolution	★★★★★	★★☆☆☆	★★★★★
Cost-efficient	☆☆☆☆☆	★★★★☆	★★★★★

Table 1. Comparison of multimodal microscopy modalities.

Conventional single-pixel cameras[3] lacks the ability of phase imaging because of incoherent illumination and total-intensity measurements, as shown in Fig. 1.

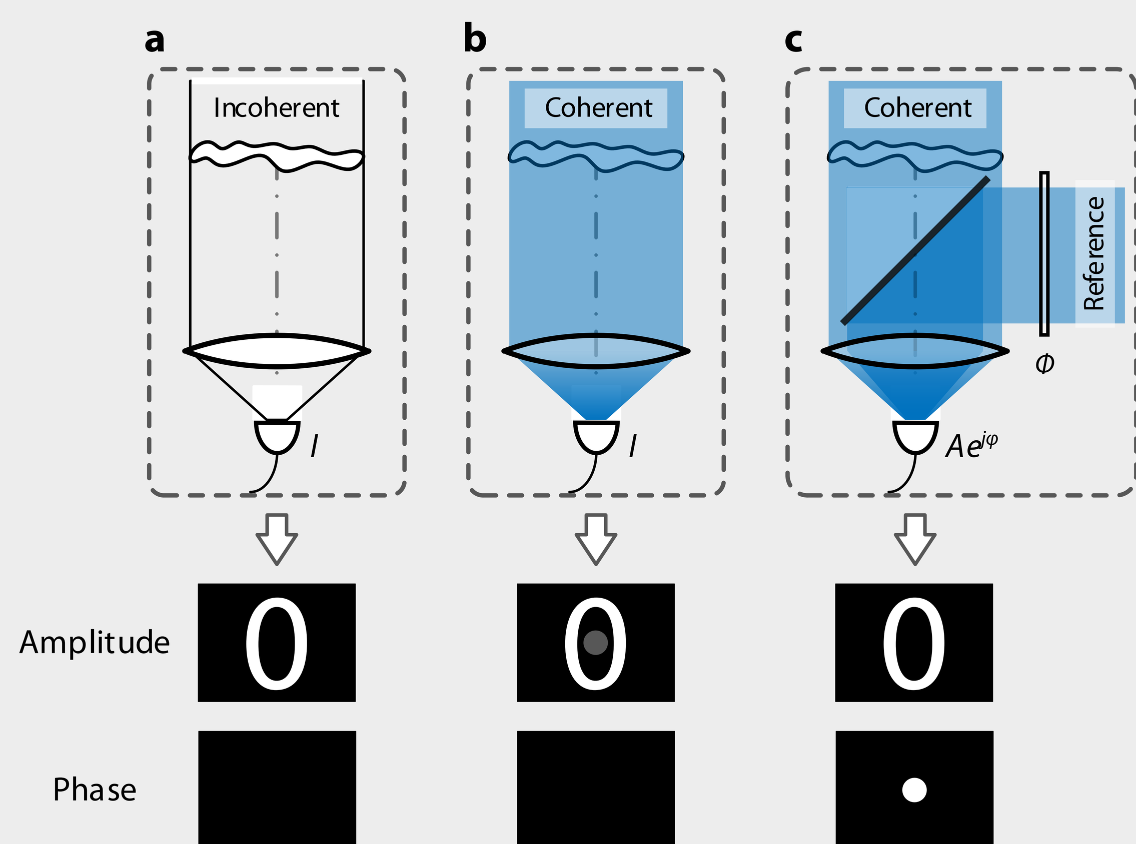


Figure 1. Comparison of illumination and detection in single-pixel cameras.

Previous single-pixel phase imaging method generally requires phase-mode spatial light modulation and two-beam interferometry.[4,5] Recently, a reference-free approach for single-pixel phase imaging has been demonstrated using the DMD for complex amplitude modulation to form a common-path reference beam with single-point detection.[6] However, it lacks the ability of simultaneous fluorescence imaging because of its passive imaging modality.

Methods

By combining reference-free interferometry with single-pixel detection, we encode the phase and fluorescence of the sample in two detection arms at the same time. We employ active imaging, *i.e.*, complex structured illumination[7] and add a bucket detector for fluorescence imaging to achieve simultaneous fluorescence and phase imaging.

Model of single-pixel imaging (coherent illumination)

$$I_k = \iint_\Omega |P_k(\vec{r}) \cdot S(\vec{r})|^2 \mathrm{d}^2\vec{r} = \iint_\Omega |P_k(\vec{r})|^2 \cdot |S(\vec{r})|^2 \mathrm{d}^2\vec{r}$$

where $S(\vec{r})$ is the complex field of the sample, $P_k(\vec{r})$ is the spatially modulated pattern, and I_k is the total intensity measured by the single–pixel detector."

Model of single-point detection

$$I_{k,\phi} = \left| \left(P_{k,\phi}(\vec{r}) \circ S(\vec{r}) \right)_{\vec{k}=0} \right|^2 = \left| \iint_\Omega P_{k,\phi}(\vec{r}) \cdot S(\vec{r})\, \mathrm{d}^2\vec{r} \right|^2$$

where $\vec{k} = 0$ represents the center point of the Fourier plane. In this way, the phases of both the sample and illuminations are reserved. By introducing a reference beam and phase shifts in the complex structured illumination, that is $P_{k,\phi} = (e^{j\phi} \cdot H_k + 1)/2$, where H_k is one basis of Walsh-Hadamard transform consisting of $\{+1, -1\}$ elements.

Model of single–pixel fluorescence imaging

$$I^F_{k,\phi} = \iint_\Omega |P_k(\vec{r})|^2 \cdot F(\vec{r})\, \mathrm{d}^2\vec{r}$$

where $F(\vec{r})$ denotes the fluorescent intensity of the sample.

Reconstruction of single-pixel imaging

$$\boldsymbol{I} = \boldsymbol{P} \cdot \boldsymbol{S} \Longrightarrow \boldsymbol{S} = \boldsymbol{P}^{-1} \cdot \boldsymbol{I}$$

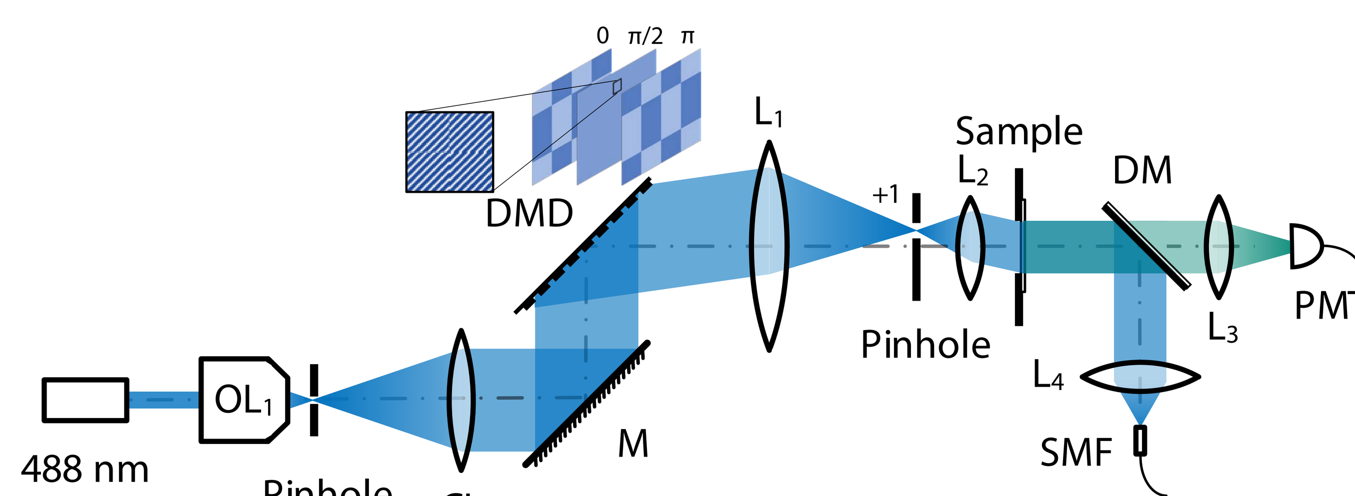


Figure 2. Experimental setup. Lee hologram[7] is applied for generating complex amplitude field at the sample plane, which provides both reference beam and phase shifts for interferometry.

Results

To validate the proposed method, we built a proof-of-concept setup, as shown in Fig. 2 for first imaging the phase of etched glass with the depth of a few hundred nanometers, as shown in Fig. 3 and then imaging the fluorescence and phase of the quantum dot drop, as shown in Fig. 4.

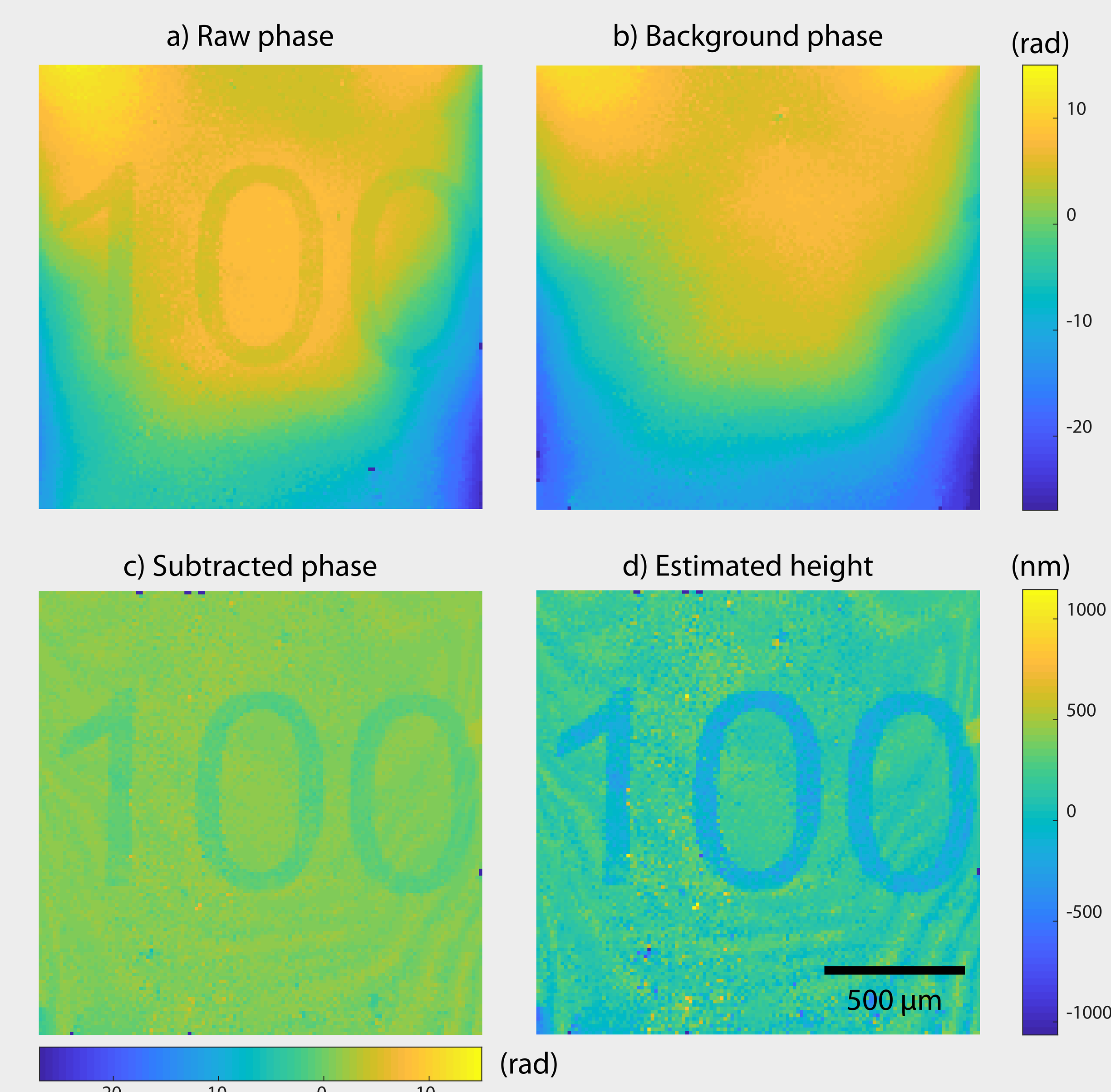


Figure 3. Phase imaging of the etched glass sample. Scale bar 500 µm.

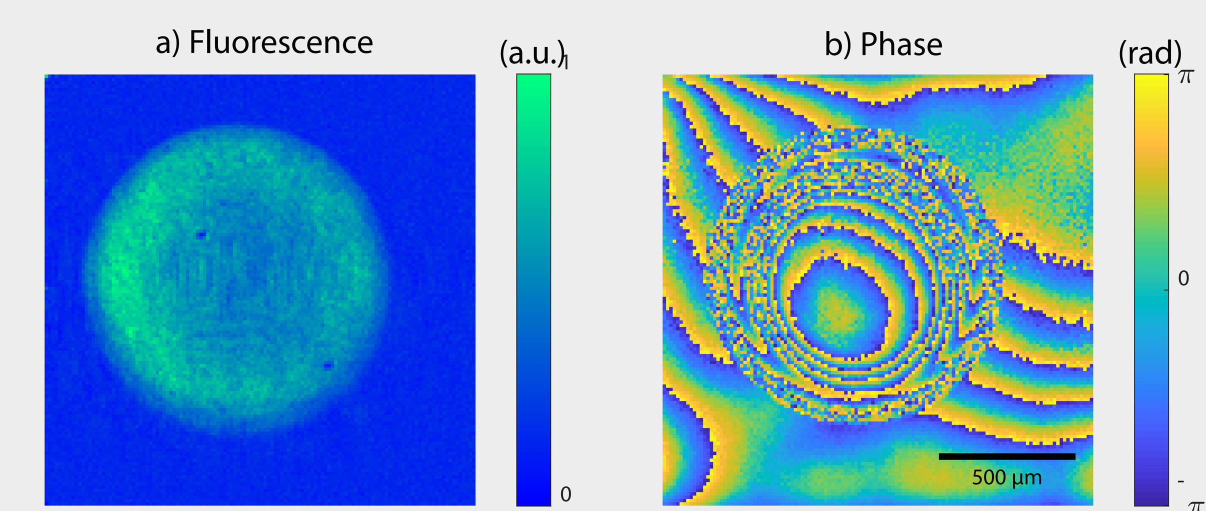


Figure 4. Simultaneous fluorescence and phase microscopy of the quantum dot sample. Scale bar 500 µm.

Conclusions

We propose a simultaneous fluorescence and quantitative phase microscopy approach with single-pixel detectors. Extensions of this method would include

- infrared imaging,
- Terahertz imaging,
- X-ray imaging,
- hyperspectral imaging,[8-10]
- high-speed imaging,[11] and
- applications in biomedical science and neuroscience

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Contact

Yang Liu
Department of Automation, Tsinghua University, Beijing 10084, China
email: y-liu16@mails.tsinghua.edu.cn
website: liuyang12.github.io