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Application of shift-and-add algorithms for imaging objects within biological media

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ABSTRACT

The Shift-and-Add (SAA) technique is a simple mathematical operation developed to reconstruct, at high spatial resolution, atmospherically degraded solar images obtained from stellar speckle interferometry systems. This method shifts and assembles individual degraded short-exposure images into a single average image with significantly improved contrast and detail. Since the inhomogeneous refractive indices of biological tissue causes light scattering similar to that induced by optical turbulence in the atmospheric layers, we assume that SAA methods can be successfully implemented to reconstruct the image of an object within a scattering biological medium. To test this hypothesis, five SAA algorithms were evaluated for reconstructing images acquired from multiple viewpoints. After successfully retrieving the hidden object's shape, quantitative image quality metrics were derived, enabling comparison of imaging error across a spectrum of layer thicknesses, demonstrating the relative efficacy of each SAA algorithm for biological imaging.

1. Introduction

Over the past decades, stellar speckle imaging techniques have been developed to recover astronomical (celestial) images severely deteriorated by fluctuations in the terrestrial atmosphere's refractive index and by system (earthbound telescope) aberrations [1–3]. Among these techniques is the Shift-and-Add (SAA) method, in which a series of independent short-exposure speckle images are averaged to reconstruct a single high spatial-resolution image [4]. The quality of images reconstructed using SAA, as well as the speed at which they are produced, have been shown to depend on the algorithm by which the images are aligned, or shifted. Therefore, incorrect shifting of images can lead to numerous peaks in the reconstruction plane, significantly increasing background noise in the image. Since the SAA method overcomes the temporal variations of the atmospheric refractive index, it has been shown to improve the contrast of large mirror array imaging systems. A range of SAA algorithms has been developed, each with its unique benefits and limitations. For example, in the SAA method introduced by Bates and Cady [5], the maximum value of each speckle image (specklegram), I_i(x,y), is first obtained for a given position (x_o,y_o). Then, the image is shifted by (x_o,y_o) to the origin of coordinates and added. Thus, the resulting diffracted-limited image of the object, I_t(x,y), is the sum of all speckle images centered on the origin of coordinates at their respective maximum values. This operation can be represented mathematically in the following,

$$I_t(x, y) = \frac{1}{M}\sum_{i=1}^{M} I_i(x - x_o, y - y_o) \tag{1}$$

where M represent the number of speckle images and I_i(x,y) is given by:

$$I_i(x, y) = \left[I(x, y) * PSF_i(x, y)\right] + N_i(x, y) \tag{2}$$

$I(x,y)$ is the object function (object image), $PSF_i(x,y)$ and N_i(x,y) are the point-spread function and additive noise terms at the ith timepoint, respectively, and * denotes the two-dimensional convolution operation.

A variety of biomedical optical imaging techniques are used to visualize targets embedded in biological tissue [6] such as in the case of breast tumors imaged by optical mammography [7]. These techniques are relatively low in cost, noncontact, portable, easy-to-use, and are nonionizing. However, a key challenge of these modalities is to overcome the strong optical scattering of light resulting from the nonhomogeneous refractive indices of biological tissue [8]. Additionally, scattering restricts photon penetration depth in tissue, resulting in degraded images possessing reduced spatial resolution and contrast. Thus, biomedical optical images suffer from poor visibility. In order to reduce the effects of light scattering, a number of techniques have been developed, including

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time gating [9,10], spatial filtering, [11] angular filter array [12,13], polarization [14], interferometry [15], holography [16], phase conjugation [17], and wavefront shaping [18–20], each with their unique advantages and disadvantages. Nevertheless, these techniques are limited in their practical application since they are best suited for imaging close to the tissue surface, with the exception of multispectral optoacoustic tomography [21] and hybrid fluorescence molecular tomography [22,23] which can reach up to several centimeters below tissue surface, albeit at low resolution. Thus, current optical imaging techniques, still encounter difficulties retrieving high-resolution images from depths of more than a few millimeters below tissue surface, and are under ongoing development.

Thus, alongside optimization of system hardware performance, various computational reconstruction methods have been developed to improve signal-to-noise ratio and to enhance resolution of embedded objects. Since the nonhomogeneous refractive indices of biological tissue causes light scattering similar to that induced by optical turbulence in the atmospheric layers [24,25], we assume that the SAA methods can be successfully implemented for non-invasive biomedical optical imaging. The goal of this study was two-fold: 1) to provide proof-of-concept for the use of SAA for detection of hidden objects within a biological medium of variable thickness, and 2) to evaluate the performance of five different SAA algorithms for imaging within biological tissue. We arbitrarily selected five simple SAA methods described previously: central-of-mass [26], iterative weighted [27], self-deconvolution [28], continuous convolution [29], and cross-correlation [30], each of which will be briefly reviewed in the Methods section.

In our previous work, we combined speckle contrast projections with the use of optical clearing agents (OCAs) to image objects embedded within a biological tissue (scattering medium) [31]. Briefly, broken chicken leg or prostate tumor inserted within chicken breast tissue was illuminated using a coherent light source and speckled images from multiple projections (viewpoints) obtained by a lens array were averaged into a single image using SAA, to successfully reveal the shape of the hidden object. This was the initial stage of translation of the SAA technique from its use in astronomy to microscopy, for deep tissue imaging. We demonstrated that fusion of multiple speckle contrast projections improved resolution and contrast of reconstructed images, while OCAs markedly reduced photon scattering within tissue, improving both imaging depth and resolution. This method is non-invasive, noncontact, scan-free, does not require time-gated detection and benefits from a multiperspetive strategy. A lens array ensemble was used to produce a series of low resolved sub-images which were averaged into a single, higher resolution image, thus overcoming the limited spatial resolution of single imaging with larger numerical aperture (NA) objectives. Furthermore, the lens array increases the NA, broadening the total field of view and improving the signal to noise ratio (proportionally to the square root of the number of lenses used), alongside improved spatial resolution. The use of a lens array to assist in reconstruction of hidden objects within a scattering medium is unique [32], while lens arrays are widely used for aberration correction, [33,34] miniaturizing imaging systems [35,36], super resolution imaging [37], 3D integral imaging [38,39], and in holography [40,41]. Similar work using a lens array to visualize objects within a scattering medium differs in its technique, system configuration, and data processing was reported [42,43].

In this paper, we aimed to evaluate the use of SAA for imaging over a range of imaging depths, and to compare the performance of five selected SAA processing methods to resolve the shape of two hidden biological objects: one transparent (bone fracture) and the other opaque (tumor). To quantitatively assess the quality of images derived using each algorithm, we adopted four image quality metrics: signal-to-noise ratio (*SNR*), contrast-noise-ratio (*CNR*), relative root mean square error (*RRMSE*), and *visibility*, all commonly used in image processing.

The paper continues as follows: Section 2 provides a description of the experimental methods, instrumentation details, and a brief description of the SAA methods used. Section 3 presents the experimental results and their interpretation, while Section 4 ends the paper with conclusions.

2. Materials and methods

2.1. Sample preparation

Experiments were performed using one of two biological objects: broken chicken leg or prostate tumor. These objects were sandwiched between two layers of commercially available fresh chicken breast meat. Specifically, the broken chicken leg with thickness of 8 mm, at sizes itemized in Fig. 1(a) was embedded within chicken breast, yielding an overall thickness of 10 mm to 13 mm, while a 2 mm thick layer of prostate tumor at sizes specified in Fig. 1(b) was embedded between two 5.5 mm layers of

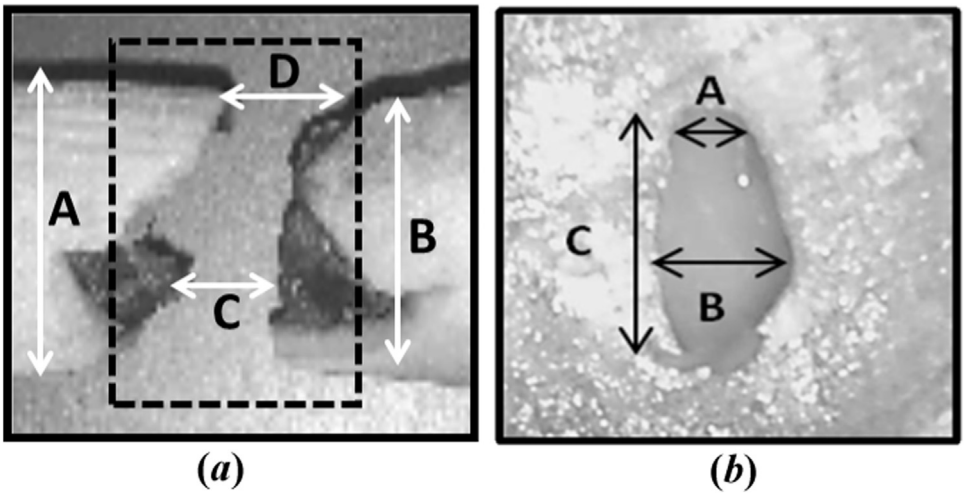

Fig. 1. Photographs of the biological samples used in this work. (a) Broken chicken leg with 8 mm thickness. A, B, C, and D are: 9 mm, 8.5 mm, 4 mm, and 4 mm, respectively. (b) Tumor of prostate cancer cells of 2 mm thickness. A, B, and C are: 2 mm, 4 mm, and 9 mm, respectively. These samples were sandwiched between variable chicken breast layers.