Super-resolution photoacoustic fluctuation imaging with multiple speckle illumination: supplementary material

THOMAS CHAIGNE^{1,2,*†}, JÉRÔME GATEAU^{1,2,†}, MARC ALLAIN³, ORI KATZ^{1,2}, SYLVAIN GIGAN², ANNE SENTENAC³, AND EMMANUEL BOSSY¹

¹ESPCI ParisTech, PSL Research University, CNRS UMR 7587, INSERM U979, Institut Langevin, 1 rue Jussieu, 75005, Paris, France

²Laboratoire Kastler Brossel, Université Pierre et Marie Curie, Ecole Normale Supérieure, Collège de France, CNRS UMR 8552, 24 rue Lhomond, 75005 Paris, France

³Institut Fresnel (CNRS UMR 7249), Université Aix-Marseille, Campus de St Jérôme, 13013 Marseille, France,

*Corresponding author: thomas.chaigne@espci.fr

† These authors contributed equally to this work

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This document provides supplementary information to "Super-resolution photoacoustic fluctuation imaging with multiple speckle illumination," <http://dx.doi.org/10.1364/optica.3.000054>. The first section contains theoretical information: the conditions are given to model the photoacoustic images reconstruction as a convolution, the variance image is shown to result from the convolution of the squared PSF, and details on the deconvolution algorithm used are given. The second section provides more detailed information on the experimental methods, including samples preparation, measurements, the processing of photoacoustic signals, the backprojection algorithm used for the photoacoustic reconstruction, and some insight about the choice of the deconvolution parameters. The last section analyzes some properties of the photoacoustic point spread function. © 2016 Optical Society of America

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1. THEORY

A. Photoacoustic imaging as a convolution process

In its simplest form, the generation and propagation of photoacoustic (PA) pressure waves $p(\mathbf{r}, t)$, generated by a distribution $\mu_a(\mathbf{r})$ of optical absorption illuminated by a pulsed light with intensity $I(\mathbf{r}) \times f(t)$, may be described in a homogenous acoustic medium (with speed of sound c_s and Grüneisen coefficient Γ) by the following equation

$$\left[\frac{\partial^2}{\partial t^2} - c_s^2 \nabla^2 \right] p(\mathbf{r}, t) = \Gamma \mu_a(\mathbf{r}) I(\mathbf{r}) \frac{\partial f(t)}{\partial t} \quad (\text{S1})$$

Eq. (S1) shows that for a given temporal pulse shape $f(t)$, the pressure field is *linearly* related to the distribution $\mu_a(\mathbf{r}) \times I(\mathbf{r})$ of optical absorption times the optical intensity pattern. Various algorithms exist that aim at reconstructing $\mu_a(\mathbf{r}) \times I(\mathbf{r})$ from a set of PA signals measured at various points located on some bound-

ary around the sample to be imaged [1, 2]. In the context of our work, we consider a conventional back-projection algorithm based on summing values of the PA signals taken at appropriate retarded times [1]. As such, the reconstructed images remain *linearly* related to $\mu_a(\mathbf{r}) \times I(\mathbf{r})$. Moreover, if this linear reconstruction process may also be considered as translation-invariant, i.e. a spatial translation of the object simply translates the reconstructed object, then the reconstruction process may be written as a convolution as assumed by Eq. (1) of the main manuscript. Because the PA signals are measured on some boundary around the region to be imaged, the translation invariance cannot be strictly verified. However, for a field of view small enough such that every point that it contains is reconstructed with the same point spread function (PSF), it becomes possible to reasonably assume that the reconstruction process is translation-invariant. In our case, this assumption was validated by measuring PSF at four different locations in the field of view. The four PSF

appeared identical (see sec. B below), and the results shown in the manuscript were independent of the particular PSF that was used to perform the deconvolutions, thus validating our assumption that the reconstruction process may be written as a convolution.

B. The variance image as a convolution with $h^2(\mathbf{r})$

From $A(\mathbf{r}) = [\mu_a(\mathbf{r}) \times I(\mathbf{r})] * h(\mathbf{r})$ (valid only for objects lying in a small enough field of view, see sec. A above), the variance image $\sigma^2[A](\mathbf{r})$ reads

$$\sigma^2[A](\mathbf{r}) = \iint C(\mathbf{r}', \mathbf{r}'') \mu_a(\mathbf{r}') \mu_a(\mathbf{r}'') h(\mathbf{r} - \mathbf{r}') h(\mathbf{r} - \mathbf{r}'') d\mathbf{r}' d\mathbf{r}''$$

where

$$C(\mathbf{r}', \mathbf{r}'') = \langle I(\mathbf{r}') \cdot I(\mathbf{r}'') \rangle - \langle I(\mathbf{r}') \rangle \cdot \langle I(\mathbf{r}'') \rangle$$

is the autocovariance of the intensity fluctuation in the speckle patterns. Under the common assumption that the speckle is wide-sense stationary [3, p.67], the autocovariance function C is a function of only one variable $\mathbf{r} = \mathbf{r}' - \mathbf{r}''$. This function $C(\mathbf{r})$ is sharply peaked around its center, and its characteristic length is by definition the speckle grain size [3, p.72]. If the speckle grains are small enough compared to the PSF, $C(\mathbf{r}' - \mathbf{r}'')$ may be considered in the double integral above proportional to a delta function $\delta(\mathbf{r}' - \mathbf{r}'')$, yielding the following convolution expression:

$$\sigma^2[A](\mathbf{r}) \propto \int \mu_a^2(\mathbf{r}') h^2(\mathbf{r} - \mathbf{r}') d\mathbf{r}' = \mu_a^2(\mathbf{r}) * h^2(\mathbf{r})$$

This expression is strictly equivalent to that found for the second-order analysis in SOFI [4], apart from the origin of the fluctuations.

C. Principle of the deconvolution algorithm: FISTA

As introduced in the main manuscript, deconvolution of the variance image was performed by minimizing the following constrained least-square functional:

$$J(x) := \|h^2 * x - \widehat{\sigma^2[A]}\|^2 + \alpha \|x\|^2 \quad \text{subject to} \quad x \geq 0 \quad (\text{S2})$$

From a practical viewpoint, an iterative minimization algorithm is required for the numerical evaluation of $\hat{x}_\alpha$. Since J is a strictly convex functional if $\alpha > 0$, its global minimizer $\hat{x}_\alpha$ is asymptotically reached by any locally convergent iteration, whatever the initial-guess of the algorithm. For this constrained optimization task, a natural candidate is the standard projected-gradient (PG) since its computational burden is very low. The PG is however rather slow to converge and the FISTA iteration [5] that achieves a faster convergence is presently considered as a popular alternative and was used in our work. In comparison to the standard projected-gradient algorithm [6], the FISTA method [5] only requires the additional storage of an auxiliary component $y^{(k)}$. Let $x^{(0)}$ be an initial-guess and $y^{(0)} = x^{(0)}$, the FISTA update for $k = 0, 1, \dots$ is:

$$\begin{cases} x^{(k+1)} &= \mathcal{P}_+ \left(y^{(k)} - \theta g(y^{(k)}) \right) \\ y^{(k+1)} &= x^{(k+1)} + \frac{k-1}{k+2} \left(x^{(k+1)} - x^{(k)} \right) \end{cases} \quad (\text{S3})$$

with $\theta > 0$, $\mathcal{P}_+$ the projection operator over $\mathbb{R}_+$, and g the gradient of the penalized least-square functional above that reads

$$g(x) := 2 \left(h^2 \right)^\dagger * \left(h^2 * x - \widehat{\sigma^2[A]} \right) + \alpha x \quad (\text{S4})$$

where $h^\dagger$ stands for the adjoint of the operator h . It is worth noting that all the convolution operations that appear above can be computed efficiently in the Fourier domain *via* the fast Fourier transform (FFT) and appropriate boundary conditions [7]. Finally, the global convergence of Eq. (S3) is granted provided that the constant step-size θ is adjusted within the interval $(0, \bar{\theta})$ with

$$\bar{\theta} \equiv 1 / \max_{\omega} (|\tilde{h}(\omega)|^2 + \mu)$$

where $\tilde{h}(\omega)$ stands for the Fourier transform of h .

2. EXPERIMENTAL METHODS

A. Samples preparation

A collection of black polyethylene microspheres (Cospheric, 50 μm and 100 μm in diameter) was used to fabricate phantoms with isotropic emitters. Estimates of the PSF $h(\mathbf{r})$ were measured using 50 μm diameter microspheres, while patterns to be imaged were formed using 100 μm diameter microspheres. For the two samples with precisely positioned beads, samples 2 and 3, melted gel was first poured on a mold drilled with micrometer precision (Mini Mill, Minitex), and the beads were then manually placed in the molded holes of the solidified gel.

B. Measurements and signal processing

The intensity of the laser pulses was monitored with a photodiode to compensate for the laser pulse-to-pulse energy fluctuations. In addition, for each speckle illumination (i.e. each diffuser position), the PA signals were averaged over 25 laser pulses to improve the signal-to-noise ratio. The signals were then filtered between 1 MHz and 8 MHz (3rd order butterworth filter) for noise removal outside of the array bandwidth.

For sake of clarity, Eq. (3) in the main manuscript was written for a noise free situation. In practice, variance between images is also induced by the residual measurement noise and possible triggering-induced temporal jitters, even after the compensation for pulse-to-pulse fluctuations as described above. However, because the deterministic fluctuations from multiple speckle illumination and these other sources of fluctuations are independent, the total variance is given by the sum of the variance of interest (from multiple speckle variations) and the "unwanted" variance. Under this assumption, the total measured variance may be written as

$$\sigma^2[A](\mathbf{r}) \propto \mu_a^2(\mathbf{r}) * h^2(\mathbf{r}) + \epsilon \quad (\text{S5})$$

The "unwanted" variance term ϵ was estimated from 100 measurements obtained with a single speckle realization. To apply our approach with multiple speckle illumination, series of photoacoustic images was reconstructed for 100 uncorrelated speckle patterns. The mean and variance images of this data set were then computed on a per-pixel basis. The "unwanted" variance previously estimated was subtracted to obtain an estimate of the variance due solely to multiple speckle illumination. Of course, because only statistical estimation may be produced from a finite set of images, a residual fluctuation necessarily remains present, which can be considered as noise on the variance image.

C. Backprojection algorithm

In the context of our work, we considered a conventional back-projection algorithm based on summing values of the PA signals taken at appropriate retarded times. More specifically, the back-projection algorithm described in [8, Eq. (20)] was implemented while keeping only the first time-derivative of the processed signal, assuming point-like detectors located in the centers of the elements of the ultrasound array.

D. Parameters used for the deconvolution algorithm.

According to standard inversion theory [9], the regularization parameter was adjusted so that a trade-off is reached between two opposite behaviors: whereas an over-estimated parameter α restricts too much the spectral bandwidth of the inverted data, leading to an over-smoothed reconstruction, an under-estimated parameter α inverts some spectral components dominated by noise, leading to unstable reconstructions. In this article, this trade-off was obtained visually from reconstructions obtained with various values of the parameter α . In practice, this tuning-step is indeed rather simple as the solution changes smoothly with α (this is a direct consequence of the strict convexity of the criterion). Let us finally note that this “optimal” tuning is expected to be driven by the square of the PSF $h^2(\mathbf{r})$ and the noise level in the data to be inverted. We would like to stress that the same strategy was adopted for the tuning of the regularization parameter in the deconvolution of the mean image. Doing so, the mean and variance images were processed with equivalent care, such that each image exhibits the best accessible resolution. In practice, the parameters used to produce the images shown in the main manuscript were the following:

- for fig 4 (sample 2), $\alpha = 10^5$, $N_{\text{iterations}} = 800$.
- for fig 5 (sample 3), $\alpha = 10^6$, $N_{\text{iterations}} = 600$.

For each figure, the same parameters were used for the deconvolution of the mean image by the PSF $h(\mathbf{r})$ and the variance image by the square of the PSF $h^2(\mathbf{r})$. The parameters are however different for the two samples, in particular because the PSF was recorded independently for each figure to be sure to obtain the experimental PSF in the vicinity of the object of interest.

3. PHOTOACOUSTIC POINT SPREAD FUNCTION

A. Measurements

The PSFs used to deconvolve images obtained with samples 2 and 3 were measured by concentrating light on a single 50- μm diameter bead located in the vicinity of the structured patterns with 100- μm diameter beads (see Fig. 2.a for sample 2). This ensured that the 50- μm diameter microsphere was the only PA source. The diffuser was removed from the light path during this step, to ensure a homogeneous illumination of the 50- μm diameter bead.

B. Shift-invariance of the point spread function across the field-of-view

PSFs were reconstructed for 4 different locations in the field of view of sample 2 to confirm that it can be considered shift invariant, as required to model the reconstruction by a convolution process. The corresponding results are summarized in Fig. S1. With our current ultrasonic detection and the back-projection algorithm, we showed that this approach was at least valid for a 3 mm x 3 mm field-of-view. On the deconvolved images, a slight difference in the PSF shapes would result in additional artefacts,

Fig. S1. a) Photograph of absorbing sample 2. The red arrows indicate the locations of the 50 μm diameter beads used to measure the PSF of the PA imaging system. b-e) PSF of the PA imaging setup, recorded at 4 different locations: (b) Top left, (c) Top right, (d) Bottom left, (e) Bottom right. f) Axial cross-sections of the 4 different PSFs (along z direction): top-left (blue), top-right (red), bottom-left (green), bottom-right (yellow). g) Transverse cross-sections of the 4 different PSFs (along x direction). Scale bars: 500 μm .

for instance some rebounds around the reconstructed objects that are located far from the place where the PSF is measured.

C. Analysis

Fig. S2 shows the PSF (Fig. S2.a) as estimated from one of the 50- μm diameter beads on sample 2 (top-right), its envelope (Fig. S2.b, computed using Hilbert transform), the squared PSF (Fig. S2.c) and the square of the PSF envelope (Fig. S2.d). The conventional resolution of the imaging system (when using uniform illumination) is given by the full width at half maximum (FWHM) of the envelope of the PSF, while the resolution of the variance image was obtained by the FWHM of the squared envelope of the PSF. The corresponding dimensions are 360/468 μm in the lateral/axial directions on the mean image and 261/338 μm on the variance image, resulting in a resolution enhancement of about 1.4, as was obtained with the same method from the signal of a single 100 μm diameter bead on sample 1.

The lateral resolution enhancement for the deconvolved images was found to be at least 1.8 from the mean to the variance image, estimated on the ability to separate beads, higher than the value of 1.4 derived directly from the FWHM of the PSF $h(\mathbf{r})$ and its square $h^2(\mathbf{r})$. While the resolution enhancement before deconvolution is linked only to the FWHM of the PSF $h(\mathbf{r})$ and its square $h^2(\mathbf{r})$, the resolution enhancement after deconvolution is not only strongly dependent on the shape of the spatial frequency spectrum of $h(\mathbf{r})$ and $h^2(\mathbf{r})$, but is also fundamentally related to the level at which each spectrum enters the noise level. Fig. S3 illustrates how the frequency spectrum of $h^2(\mathbf{r})$ extends further than that of the PSF alone (with a factor close to 2 in the axial dimension).

Fig. S2. a) Point spread function $h(\mathbf{r})$ measured from sample 2. b) Envelope of $h(\mathbf{r})$ (modulus of the Hilbert transform along the axial dimension). c) Squared PSF $h^2(\mathbf{r})$. d) Square of the PSF envelope. Scale bars: 200 μm .

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Fig. S3. a) Two-dimensional spatial Fourier Transform (magnitude) of the PSF. b) Two-dimensional spatial Fourier Transform (magnitude) of the squared PSF. c-d) Same as a-b) with logarithmic scale.