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► **To cite this version:**

Simon Labouesse, Jérôme Idier, Anne Sentenac, Thomas Mangeat. Image Variance Based Random Illumination Microscopy. 2020. hal-02495824

**HAL Id: hal-02495824**

**<https://hal.science/hal-02495824>**

Preprint submitted on 2 Mar 2020

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# Image Variance Based Random Illumination Microscopy

March 2, 2020 - Simon Labouesse, Jérôme Idier, Anne Sentenac, and Thomas Mangeat.

## I. INTRODUCTION

Super-resolution fluorescence microscopy is an indispensable tool for studying the dynamics of macromolecules in cell biology. Presently, structured illumination microscopy (SIM) is the best compromise between high resolution and practical in-vivo imaging. It allows to improve the lateral resolution of widefield microscopes beyond the diffraction limit, up to a super-resolution (SR) factor of two in epi-illumination [1], [2]. SIM consists in recording several low-resolution images of the biological sample under different positions and orientations of a known periodic illumination. However, it requires a tight control of the illumination patterns, which makes it difficult to image over long period of time and limits its application to weakly scattering samples [3].

In order to release this major constraint, the principle of a microscope using totally uncontrolled speckle illuminations has been proposed [4], [5]. Random illumination microscopy (RIM) implementation is much simpler than SIM as it does not require the knowledge of the illuminations. Moreover, random speckles are intrinsically insensitive to aberrations and scattering.

In [6], it is shown that the theoretical SR capacity of RIM is identical to that of SIM. However, the latter study exploits the statistical *covariance matrix* of the recorded images, which is not a realistic scheme in terms of storage and of computing operations: manipulating images of  $1000 \times 1000$  pixels would generate a covariance matrix of size  $10^{12}$ , which represents 20To using a usual double precision floating scheme on a modern computer system.

Second-order statistics being quadratic functions of the unknown sample, the issue of retrieving spatial frequency components of the latter from the former belongs to the family of *quadratic inverse problems* (QIP) [7]. As a consequence, it is substantially more difficult to formally characterize the SR capacity of RIM, compared to SIM.

Some instances of QIP are intensively studied, such as the phase retrieval problem [8], [9]. In the field of fluorescence microscopy, characterizing the intrinsic resolution of SOFI is

The authors acknowledge partial financial support for this paper from the Agence Nationale de la Recherche under Grant ANR-12-BS03-0006 and the ITMO Cancer of the ‘Plan Cancer 2014-2019’.

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also a QIP [10]. However, the latter is a simple one, since it can be trivially transformed into a linear inverse problem in the squared density of fluorophores.

In this letter, we prove a new result about RIM that reconciles the theoretical SR capacity and an affordable computational burden. Indeed, under fairly realistic assumptions, we mathematically show that the statistical *variance* of the recorded images is sufficient to recover an image of the biological sample with the same SR factor as covariance-based RIM (and SIM). Let us remark that variance-based RIM has been already proposed, either for its sectioning properties [11], or in the context of super-resolution [12]. However, to our best knowledge, our contribution is the first one to mathematically characterize the super-resolution property of variance-based RIM.

In Section II, we introduce a mathematical description of the image model. For sake of self-consistency, we also recall known results for covariance-based RIM. Our novel result concerning variance-based RIM is presented in Section III, and the proof is postponed in Section III-B. Section IV contains elements of discussion and perspectives.

## II. MATHEMATICAL BACKGROUND AND KNOWN RESULTS

### A. Imaging model

For the sake of clarity, we mainly restrict ourselves to the case of two-dimensional biological samples, and we formulate the problem in a fully discrete setting, where both the recorded images and the biological sample are represented on fine grids, with a sampling rate common to both. RIM images can then be modelled by:

$$\mathbf{z}_m = \mathbf{y}_m + \boldsymbol{\epsilon}_m, \quad (1)$$

with

$$\mathbf{y}_m = \mathbf{H}(\boldsymbol{\rho} \circ \mathbf{I}_m), \quad (2)$$

where  $\boldsymbol{\epsilon}_m$  is a random variable modeling an additive noise,  $\mathbf{y}_m$  is a vectorized image corresponding to the  $m$ th illumination  $\mathbf{I}_m$ ,  $\mathbf{H}$  a convolution matrix corresponding to a convolution by the the Point Spread Function (PSF)  $\mathbf{h}$  of the microscope,  $\boldsymbol{\rho}$  the fluorescence density map to recover, and  $\circ$  the Hadamard (*i.e.*, entrywise, or Schur) matrix product [13, Chapter 5]. The speckle covariance  $\text{Cov}(\mathbf{I}_m) = \mathbf{C}$  as well as the noise covariance  $\text{Cov}(\boldsymbol{\epsilon}_m) = \boldsymbol{\Gamma}_\epsilon$  are supposed to be known statistics. The covariance matrix of each  $\mathbf{z}_m$  reads

$$\boldsymbol{\Gamma}_z(\boldsymbol{\rho}) = \boldsymbol{\Gamma}_y(\boldsymbol{\rho}) + \boldsymbol{\Gamma}_\epsilon, \quad (3)$$

with

$$\boldsymbol{\Gamma}_y(\boldsymbol{\rho}) = \mathbf{H}\text{Diag}(\boldsymbol{\rho})\mathbf{C}\text{Diag}(\boldsymbol{\rho})\mathbf{H}^t. \quad (4)$$

The variance identifies with the diagonal of the covariance matrix  $\mathbf{v}_z(\boldsymbol{\rho}) = \text{diag}(\boldsymbol{\Gamma}_z)$ . The noise covariance function  $\boldsymbol{\Gamma}_\epsilon$  is assumed to be known. The knowledge of  $\mathbf{v}_z$  is thus equivalent to that of

$$\mathbf{v}_y = \text{diag}(\boldsymbol{\Gamma}_y). \quad (5)$$

Hereafter, we refer to  $\mathbf{v}_y$  and  $\boldsymbol{\Gamma}_y$  as  $\mathbf{v}$  and  $\boldsymbol{\Gamma}$ , respectively. In this document, we adopt the standard assumption of a perfect circular lens. For 2D imaging at the focal plane, the PSF  $\mathbf{h}$  is a discretized Airy pattern [14, Sec.4.4.2], and the optical transfer function (OTF)  $\tilde{\mathbf{h}}$  has a frequency cut-off  $2\text{NA}/\lambda$ , with NA the numerical aperture of the microscope and  $\lambda$  the emission/excitation wavelength. We further assume that the illumination of the sample and the collection of the emitted light is performed through the same optical device. Ignoring the Stokes-shift, we will assume that  $\mathbf{H} = \mathbf{H}^t = \mathbf{C}$ .

Since our goal is to demonstrate a factor two in terms of super-resolution, the sampling rate of the object must be at least four times the cutoff frequency imposed by the PSF. In the rest of this document, we make use of the following notations:  $f_{\text{PSF}} \leq 1/4$  denotes the normalized cutoff frequency imposed by the PSF, and

$$\mathcal{G} = \{\boldsymbol{\nu} \in \mathbb{R}^d, \|\boldsymbol{\nu}\|_\infty < 1/2\} \cup \{\mathbf{n}/N, \mathbf{n} \in \mathbb{Z}^d\}$$

denotes the  $d$ -dimensional normalized frequency grid limited by the Nyquist frequency ( $d = 2$  for 2D imaging). Here, we assume that RIM acquisitions  $\mathbf{z}_m$  are made of  $N = n^d$  elements. Then each of them can be decomposed over the set of discrete frequencies  $\mathcal{D}_{\text{PSF}} = \mathcal{D}(f_{\text{PSF}})$ , where  $\mathcal{D}(f)$  is a generic notation for the ‘‘discrete interior’’ of a ball of radius  $f$ :

$$\mathcal{D}(f) = \{\boldsymbol{\nu} \in \mathcal{G}, \|\boldsymbol{\nu}\| < f\}.$$

### B. Covariance-based RIM

In the 2D case, [6] obtains that the knowledge of  $\boldsymbol{\Gamma}$  allows to retrieve the frequency components of  $\boldsymbol{\rho}$  within the ball  $\mathcal{D}_{\text{SR}} = \mathcal{D}(2f_{\text{spec}})$ , provided that the speckle illuminations have a cut-off frequency not larger than that of the PSF, *i.e.*,  $f_{\text{spec}} \leq f_{\text{PSF}}$ . When  $f_{\text{spec}} = f_{\text{PSF}}$ , we have  $\mathcal{D}_{\text{SR}} = \mathcal{D}(2f_{\text{PSF}})$ , which exactly corresponds to an SR factor equal to two.

**Proposition 1.** *Let  $\boldsymbol{\rho}$  be any entrywise nonnegative vector of size  $N$ . For any entrywise nonnegative solution  $\mathbf{q}$  to the quadratic system  $\boldsymbol{\Gamma}(\mathbf{q}) = \boldsymbol{\Gamma}(\boldsymbol{\rho})$ , the frequency components of  $\mathbf{q}$  coincide with that of  $\boldsymbol{\rho}$  in  $\mathcal{D}_{\text{SR}}$ .*

*Proof:* The fact that all frequency components in  $\mathcal{D}_{\text{SR}}$  can be retrieved is based on a unicity argument for the factorization

$$\boldsymbol{\Gamma}(\boldsymbol{\rho}) = \mathbf{H} \text{Diag}(\boldsymbol{\rho}) \mathbf{H} \text{Diag}(\boldsymbol{\rho}) \mathbf{H} \quad (6)$$

$$= \sqrt{\mathbf{H}} \left( \sqrt{\mathbf{H}} \text{Diag}(\boldsymbol{\rho}) \sqrt{\mathbf{H}} \right)^2 \sqrt{\mathbf{H}} \quad (7)$$

and from the fact that the knowledge of  $\sqrt{\mathbf{H}} \text{Diag}(\boldsymbol{\rho}) \sqrt{\mathbf{H}}$  is equivalent to that of  $\mathbf{H} \text{Diag}(\boldsymbol{\rho}) \mathbf{H}$ , which uniquely determines the spectral components of  $\boldsymbol{\rho}$  in  $\mathcal{D}_{\text{SR}}$ . ■

Moreover, such a result is tight, since the frequency components of  $\mathbf{q}$  outside  $\mathcal{D}_{\text{SR}}$  are not identifiable, according to the following proposition.

**Proposition 2.** *Let  $\boldsymbol{\rho}$  be any vector of size  $N$ . Then  $\boldsymbol{\Gamma}(\boldsymbol{\rho} + \boldsymbol{\delta}) = \boldsymbol{\Gamma}(\boldsymbol{\rho})$  for any vector  $\boldsymbol{\delta}$  with no components in  $\mathcal{D}_{\text{SR}}$ .*

*Proof:* For any vector  $\boldsymbol{\delta}$  with no components in  $\mathcal{D}_{\text{SR}}$ , each column of matrix  $\text{Diag}(\boldsymbol{\delta})\mathbf{H}$  has no frequency components in  $\mathcal{D}_{\text{PSF}}$ , so  $\mathbf{H} \text{Diag}(\boldsymbol{\delta})\mathbf{H} = \mathbf{0}$ , and therefore,  $\boldsymbol{\Gamma}(\boldsymbol{\delta}) = \mathbf{0}$ . As a consequence,

$$\begin{aligned} \boldsymbol{\Gamma}(\boldsymbol{\rho} + \boldsymbol{\delta}) &= \mathbf{H} \text{Diag}(\boldsymbol{\rho} + \boldsymbol{\delta}) \mathbf{H} \text{Diag}(\boldsymbol{\rho} + \boldsymbol{\delta}) \mathbf{H} \\ &= \boldsymbol{\Gamma}(\boldsymbol{\rho}) + \boldsymbol{\Gamma}(\boldsymbol{\delta}) + \mathbf{H} \text{Diag}(\boldsymbol{\rho}) \mathbf{H} \text{Diag}(\boldsymbol{\delta}) \mathbf{H} \\ &\quad + \mathbf{H} \text{Diag}(\boldsymbol{\delta}) \mathbf{H} \text{Diag}(\boldsymbol{\rho}) \mathbf{H} \\ &= \boldsymbol{\Gamma}(\boldsymbol{\rho}). \end{aligned}$$

■

## III. VARIANCE-BASED RIM

### A. Super-resolution from variance equations

The quadratic system of Proposition 1 is made of  $\frac{1}{2}N(N+1)$  real equations, for only  $M$  free real-valued variables, where  $M$  stand for the cardinality of  $\mathcal{D}_{\text{SR}}$ . Since  $M \leq \frac{\pi}{4}N$  in 2D (and  $M \leq N$  is 1D), there is room left for a refined identifiability result, using a smaller number of equations. In this vein, Theorem 1 states that the  $N$  variance equations are sufficient to uniquely determine the  $M$  frequency components in  $\mathcal{D}_{\text{SR}}$ , provided that  $\boldsymbol{\rho}$  is an entrywise positive vector.

**Theorem 1.** *Let  $\boldsymbol{\rho}$  be any entrywise positive vector of size  $N$ . For any entrywise nonnegative solution  $\mathbf{q}$  to the quadratic system of  $N$  equations  $\mathbf{v}(\mathbf{q}) = \mathbf{v}(\boldsymbol{\rho})$ , the frequency components of  $\mathbf{q}$  coincide with that of  $\boldsymbol{\rho}$  in  $\mathcal{D}_{\text{SR}}$ , while the frequency components of  $\mathbf{q}$  outside  $\mathcal{D}_{\text{SR}}$  remain arbitrary (up to the nonnegativity constraint on the entries of  $\mathbf{q}$ ).*

### B. Proof of Theorem 1

Let us define the bilinear vector-valued function:

$$\mathbf{f}(\mathbf{x}, \mathbf{y}) = \text{diag}(\mathbf{H} \text{Diag}(\mathbf{x}) \mathbf{H} \text{Diag}(\mathbf{y}) \mathbf{H}), \quad (8)$$

so that  $\mathbf{v}(\boldsymbol{\rho}) = \mathbf{f}(\boldsymbol{\rho}, \boldsymbol{\rho})$ . Each component of  $\mathbf{f}$  is a symmetric form, since  $\mathbf{f}(\mathbf{x}, \mathbf{y}) = \mathbf{f}(\mathbf{y}, \mathbf{x})$ . Let us define

$$\begin{aligned} \mathbf{M}_x &= \mathbf{H} \text{Diag}(\mathbf{x}) \mathbf{H}, \\ \mathbf{B}_x &= \mathbf{H} \circ \mathbf{M}_x, \end{aligned}$$

so that

$$\mathbf{f}(\mathbf{x}, \mathbf{y}) = \mathbf{B}_x \mathbf{y} = \mathbf{B}_y \mathbf{x} \quad (9)$$

according to the matrix identity [15]

$$\text{diag}(\mathbf{A} \text{Diag}(\mathbf{v}) \mathbf{B}^t) = (\mathbf{A} \circ \mathbf{B}) \mathbf{v} = (\mathbf{B} \circ \mathbf{A}) \mathbf{v}.$$

In particular, for a given object  $\boldsymbol{\rho}$ , the (noiseless) data variance vector (5) is given by  $\mathbf{f}(\boldsymbol{\rho}, \boldsymbol{\rho}) = \mathbf{B}_\rho \boldsymbol{\rho}$ .

**Proposition 3.** *For any two real solutions  $\boldsymbol{\rho}$  and  $\mathbf{q}$  to Eq. (5), we have  $\boldsymbol{\rho} - \mathbf{q} \in \text{Ker}(\mathbf{B}_{\boldsymbol{\rho}+\mathbf{q}})$  and  $\boldsymbol{\rho} + \mathbf{q} \in \text{Ker}(\mathbf{B}_{\boldsymbol{\rho}-\mathbf{q}})$ .*

*Proof:* Indeed,

$$\begin{aligned} \mathbf{f}(\boldsymbol{\rho} + \mathbf{q}, \boldsymbol{\rho} - \mathbf{q}) &= \mathbf{f}(\boldsymbol{\rho}, \boldsymbol{\rho}) - \mathbf{f}(\mathbf{q}, \mathbf{q}) + \mathbf{f}(\mathbf{q}, \boldsymbol{\rho}) - \mathbf{f}(\boldsymbol{\rho}, \mathbf{q}) \\ &= \mathbf{v} - \mathbf{v} + \mathbf{f}(\mathbf{q}, \boldsymbol{\rho}) - \mathbf{f}(\mathbf{q}, \boldsymbol{\rho}) \\ &= \mathbf{0} \end{aligned} \quad (10)$$

Combining Equations (9) and (10), we obtain

$$\mathbf{B}_{\rho+q}(\rho - q) = \mathbf{B}_{\rho-q}(\rho + q) = \mathbf{0},$$

which proves the assertion. ■

**Proposition 4.** *For any vector  $\mathbf{x}$  with positive entries,  $\text{Ker}(\mathbf{B}_{\mathbf{x}})$  is the linear span of frequency components outside  $\mathcal{D}_{\text{SR}}$ .*

*Proof:* Let  $K_{\min} = \min(\mathbf{x})$ , so that  $\mathbf{x}_{\min} = \mathbf{x} - K_{\min}$  is entrywise nonnegative. We have  $\mathbf{B}_{\mathbf{x}} = K_{\min} \mathbf{G} + \mathbf{B}_{\mathbf{x}_{\min}}$ , with  $\mathbf{G} = \mathbf{H}^2 \circ \mathbf{H}$ . Matrix  $\mathbf{G}$  is circulant. It can be seen as a convolution matrix with a filter  $\mathbf{g} = (\mathbf{h} \star \mathbf{h}) \circ \mathbf{h}$ , with  $\tilde{\mathbf{g}} = (\tilde{\mathbf{h}} \circ \tilde{\mathbf{h}}) \star \tilde{\mathbf{h}}$  and  $\star$  the discrete convolution. Vector  $\tilde{\mathbf{g}}$  has nonzero components for all spatial frequencies belonging to  $\mathcal{D}_{\text{SR}}$ . Moreover, matrix  $\mathbf{G}$  is obviously nonnegative definite. Matrix  $\mathbf{B}_{\mathbf{x}_{\min}}$  is also nonnegative definite according to the Schur product Theorem, as the Hadamard product between two nonnegative definite matrices [13, Theorem 5.2.1]. Therefore, we have  $\text{Ker}(\mathbf{B}_{\mathbf{x}}) = \text{Ker}(\mathbf{G}) \cap \text{Ker}(\mathbf{B}_{\mathbf{x}_{\min}}) \subset \text{Ker}(\mathbf{G})$ .

Similarly, let  $K_{\max} = \max(\mathbf{x})$ , so that  $\mathbf{x}_{\max} = K_{\max} - \mathbf{x}$  is entrywise nonnegative. We have  $\mathbf{B}_{\mathbf{x}} = K_{\max} \mathbf{G} - \mathbf{B}_{\mathbf{x}_{\max}}$ , and  $\mathbf{B}_{\mathbf{x}_{\max}}$  and  $\mathbf{B}_{\mathbf{x}}$  are both nonnegative definite. For all  $\mathbf{z} \in \text{Ker}(\mathbf{G})$ ,  $\mathbf{z}^\dagger \mathbf{B}_{\mathbf{x}} \mathbf{z} = -\mathbf{z}^\dagger \mathbf{B}_{\mathbf{x}_{\max}} \mathbf{z}$ , where the lhs and the rhs are nonnegative and nonpositive, respectively. We conclude that  $\mathbf{z}^\dagger \mathbf{B}_{\mathbf{x}} \mathbf{z} = 0$ , so  $\text{Ker}(\mathbf{G}) \subset \text{Ker}(\mathbf{B}_{\mathbf{x}})$ , and finally that  $\text{Ker}(\mathbf{B}_{\mathbf{x}}) = \text{Ker}(\mathbf{G})$ . ■

According to Proposition 3, we have  $\rho - q \in \text{Ker}(\mathbf{B}_{\rho+q})$ , where  $\rho + q$  is entrywise positive. Therefore, we can conclude that  $\rho - q \in \text{Ker}(\mathbf{G})$ , *i.e.*, that the frequency components of  $q$  coincide with that of  $\rho$  in  $\mathcal{D}_{\text{SR}}$ . Moreover, we know from Proposition 2 that the frequency components of  $q$  outside  $\mathcal{D}_{\text{SR}}$  have no impact on the data covariance, and hence on its diagonal.

#### IV. CONCLUSION AND PERSPECTIVES

This paper provides a mathematical proof that the super-resolution capacity of random illumination microscopy still holds when only the statistical variance of collected images is considered instead of the full covariance. Such a theoretical result meets practical evidences recently obtained concerning 2D variance-based imaging applied to various types of biological samples [16].

Several comments can be made about the novel variance-based result, compared to its covariance-based counterpart:

- Whereas the covariance-based result of Proposition 1 holds if  $f_{\text{PSF}} = f_{\text{spec}}$ , the proof of Theorem 1 is based on the fact that matrices  $\mathbf{H}$  and  $\mathbf{C}$  identify, which is more stringent. Indeed, we have a small size counterexample proving that Theorem 1 is no more valid when  $\mathbf{H} \neq \mathbf{C}$ , even if  $f_{\text{PSF}} = f_{\text{spec}}$ . A perspective could be to consider short-range correlations in addition to the variance equations.
- Another difference concerns the fact that strict positivity of the sample is needed in Theorem 1. However, we have strong elements showing that this condition could be relaxed. However, the maximal number of zero entries still compatible with provable super-resolution is currently indeterminate.

- Although we have restricted ourselves to the 2D case, a formal extension to 3D is straightforward, with the benefit of an axial super-resolution effect, on top of the lateral one obtained in 2D. Real-world applications would consist in considering several focalization depths (either successively, or jointly using a multifocus system [17]). For each depth, several speckle illuminations must be recorded, so that a 3D map of variance can be constructed, and a 3D map of fluorescence can be retrieved on this basis.

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