Uniqueness of the Random Illumination Microscopy Variance Equation

Simon Labouesse¹, Jérôme Idier², Anne Sentenac¹ and Thomas Mangeat³.

¹Aix Marseille University, CNRS, Centrale Marseille, Institut Fresnel, F-13013 Marseille, France.
²Laboratoire des Sciences du Numérique de Nantes, Centrale Nantes, F-44321 Nantes, France.
³Centre for Integrative Biology, Université de Toulouse, CNRS, UPS, F-31002 Toulouse.

Abstract—Recently, it has been shown theoretically that fluorescence microscopy using random illuminations (RIM) yields a doubled lateral resolution and an improved optical sectioning. Moreover, an algorithm called algoRIM, based on variance matching, has been successfully validated on numerous biological applications. Here, we propose a proof of uniqueness of the RIM variance equation, which corresponds to a first theoretical validation of algoRIM.

1 Introduction

Fluorescence microscopy is inherently limited by diffraction to a resolution of ≈200nm. Structured illumination microscopy (SIM) breaks this resolution limit by exciting the fluorophores inside the object to recover with multiple harmonic illuminations [3][2]. From multiple SIM images and a precise knowledge of the illuminations, the resolution of an epifluorescence microscope can be doubled. However, the effective super-resolution (SR) capacity of SIM is often hampered by grid distortions due to light scattering and optical aberrations.

Random illumination microscopy (RIM) is an evolution of SIM which is based on speckle illuminations. Whereas SIM relies on the tight control of the illumination grids, RIM only relies on the knowledge of the speckle statistics [5][6], with a potential of increased robustness compared to SIM. In [5], 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.

Here, we demonstrate that the knowledge of the statistical variance is actually sufficient to recover an image of the biological sample with the same SR factor as covariance-based RIM and SIM. Our novel result concerning variance-based RIM is Proposition 2 in Section 3.1 and the proof is postponed in Section 3.2.

2 Imaging model

For the sake of clarity, we mainly restrict ourselves to the case of 2D 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. We assume a linear invariant response of the microscope to the incoherent fluorescence light and a linear response of the fluorophores to the intensity of the excitation coherent light. RIM images can then be modeled by:

\[ z_m = y_m + \epsilon_m, \]
with
\[ y_m = H (\rho \circ I_m), \]
where \( \epsilon_m \) is a random variable modeling an additive noise, \( y_m \) is a vectorized image corresponding to the \( m \)th illumination \( I_m \), \( H \) a convolution matrix corresponding to a convolution by the PSF \( h \) of the microscope, \( \rho \) the fluorescence density map.

The variance identifies with the diagonal of the covariance matrix, which corresponds to a first theoretical validation of algoRIM.

3 Variance-based RIM

3.1 SR from variance equations

In the 2D case, [5] obtains that the knowledge of \( \Gamma \) allows to retrieve the frequency components of \( \rho \) within the ball \( D_{SR} = \)

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

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

\[ D(f) = \{ \nu \in \mathcal{G}, \|\nu\| < f \}. \]
\[ D(2f_{\text{spec}}), \] provided that the speckle illuminations have a cut-off frequency not larger than that of the PSF, i.e., \( f_{\text{spec}} \leq f_{\text{PSF}} \).

Proposition 1. Let \( \rho \) be any entrywise nonnegative vector of size \( N \). For any entrywise nonnegative solution \( q \) to the quadratic system \( \Gamma(q) = \Gamma(\rho) \), the frequency components of \( q \) coincide with that of \( \rho \) in \( D_{SR} \).

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 \( M \) stands for the cardinality of \( D_{SR} \). Since \( M \leq \frac{1}{2} N \) 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, Proposition 2 states that the \( N \) variance equations are sufficient to uniquely determine the \( M \) frequency components in \( D_{SR} \), provided that \( \rho \) is an entrywise positive vector.

Proposition 2. Let \( \rho \) be any entrywise positive vector of size \( N \). For any entrywise nonnegative solution \( q \) to the quadratic system of \( N \) equations \( v(q) = v(\rho) \), the frequency components of \( q \) coincide with that of \( \rho \) in \( D_{SR} \), while the frequency components of \( q \) outside \( D_{SR} \) remain arbitrary (up to the non-negativity constraint on the entries of \( q \)).

3.2 Proof of Proposition 2

Let us define the bilinear vector-valued function:

\[
 f(x, y) = \text{diag}(H \text{Diag}(x)H \text{Diag}(y)H),
\]

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

\[
 M_x = H \text{Diag}(x)H,
 B_x = H \circ M_x,
\]

so that

\[
 f(x, y) = B_x y = B_y x
\]

according to the matrix identity [7]

\[
 \text{diag}(A \text{Diag}(v)B) = (A \circ B) v = (B \circ A) v.
\]

In particular, for a given object \( \rho \), the (noiseless) data variance vector is given by \( f(\rho, \rho) = B_{\rho} \rho \).

Proposition 3. For any two real solutions \( \rho \) and \( q \) to Eq. (1), we have \( \rho - q \in \text{Ker}(B_{\rho+q}) \) and \( \rho + q \in \text{Ker}(B_{\rho-q}) \).

Proof. Indeed,

\[
 f(\rho + q, \rho - q) = f(\rho, \rho) - f(q, q) + f(q, \rho) - f(\rho, q) = v - v + f(q, \rho) - f(q, \rho) = 0
\]

Combining Equations 3 and 4, we obtain

\[
 B_{\rho+q}(\rho - q) = B_{\rho-q}(\rho + q) = 0,
\]

which proves the assertion.

Proposition 4. For any vector \( \chi \) with positive entries, \( \text{Ker}(B_\chi) \) is the linear span of frequency components outside \( D_{SR} \).

Proof. Let \( K_{\min} = \min(\chi) \), so that \( x_{\min} = x - K_{\min} \) is entrywise nonnegative. We have \( B_x = K_{\min} G + B_{x_{\min}} \), with \( G = H^2 \circ H \). Matrix \( G \) is circulant. It can be seen as a convolution matrix with a filter \( g = (h \ast h) \ast h \), with \( \hat{g} = (\hat{h} \ast \hat{h}) \ast \hat{h} \) and the discrete convolution. Vector \( \hat{g} \) has nonzero components for all spatial frequencies belonging to \( D_{SR} \). Moreover, matrix \( G \) is obviously nonnegative definite. Matrix \( B_{x_{\min}} \) is also nonnegative definite according to the Schur product Theorem, as the Hadamard product between two nonnegative definite matrices [4] Theorem 5.2.1]. Therefore, we have \( \text{Ker}(B_x) = \text{Ker}(G) \cap \text{Ker}(B_{x_{\min}}) \subset \text{Ker}(G) \).

Similarly, let \( K_{\max} = \max(\chi) \), so that \( x_{\max} = K_{\max} - x \) is entrywise nonnegative. We have \( B_x = K_{\max} G - B_{x_{\max}} \), and \( B_{x_{\max}} \) and \( B_x \) are both nonnegative definite. For all \( z \in \text{Ker}(G), z \ast B_x z = -z \ast B_{x_{\max}} z, \) where the lhs and the rhs are nonnegative and nonpositive, respectively. We conclude that \( z \ast B_x z = 0 \), so \( \text{Ker}(G) \subset \text{Ker}(B_x) \), and finally that \( \text{Ker}(B_x) = \text{Ker}(G) \).

\[ \square \]

According to Proposition 3, we have \( \rho - q \in \text{Ker}(B_{\rho+q}) \), where \( \rho + q \) is entrywise positive. Therefore, we can conclude that \( \rho - q \in \text{Ker}(G) \), i.e., that the frequency components of \( q \) coincide with that of \( \rho \) in \( D_{SR} \). Moreover, the frequency components of \( q \) outside \( D_{SR} \) have no impact on the data covariance, and hence on its diagonal [5].

4 Conclusion

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.

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 Proposition 2 is based on the fact that matrices \( H \) and \( C \) identify, which is more stringent. Indeed, we have a small size counter-example proving that Proposition 2 is no more valid when \( H \neq C \), even if \( f_{\text{PSF}} = f_{\text{spec}} \).

- Another difference concerns the fact that strict positivity of the sample is needed in Proposition 2. However, we have strong elements showing that this condition could be relaxed.

- 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. 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.
References

[1] J. W. Goodman. *Introduction to Fourier Optics*. McGraw-Hill Physical and Quantum Electronics Series, San Francisco, 2nd edition, 1996.

[2] M. G. L. Gustafsson, D. A. Agard, and J. W. Sedat. Doubling the lateral resolution of wide-field fluorescence microscopy using structured illumination. *Proc. SPIE*, 3919:141–150, 2000.

[3] R. Heintzmann and C. G. Cremer. Laterally modulated excitation microscopy: improvement of resolution by using a diffraction grating. *Proc. SPIE*, 3568:185–196, 1999.

[4] R. Horn and C. Johnson. *Matrix analysis*. Cambridge University Press, 1991.

[5] J. Idier, S. Labouesse, P. Liu, M. Allain, S. Bourguignon, and A. Sentenac. On the super-resolution capacity of imagers using unknown speckle illuminations. 4(1):87–98, Mar. 2018.

[6] T. Mangeat, S. Labouesse, M. Allain, R. Poincloux, A. Bouissou, S. Cantaloube, E. Courtais, E. Vega, T. Li, A. Grégoire, C. Rouvière, S. Allard, N. Campo, M. Suzanne, X. Wang, G. Michaux, M. Pinot, R. Le Borgne, S. Tournier, J. Idier, and A. Sentenac. Super-resolved live-cell imaging using Random Illumination Microscopy. *bioRxiv*, 2020.

[7] K. B. Petersen and M. S. Pedersen. *The Matrix Cookbook*. Technical University of Denmark, Nov. 2012.