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Super-resolution capacity of variance-based stochastic fluorescence microscopy

Simon Labouesse (1), Jérôme Idier (2), Marc Allain (3), Guillaume Giroussens (3), Thomas Mangeat (1) and Anne Sentenac (3)*

(1) *LITC Core Facility, Centre de Biologie Intégrative, Université de Toulouse, CNRS, UPS, 31062, Toulouse, France.*

(2) *Nantes Université, École Centrale Nantes, LS2N, CNRS, Nantes, France. and*

(3) *Aix Marseille Univ, CNRS, Centrale Marseille, Institut Fresnel, Marseille, France.*

Improving the resolution of fluorescence microscopy beyond the diffraction limit can be achieved by acquiring and processing multiple images of the sample under different illumination conditions. One of the simplest techniques, Random Illumination Microscopy (RIM), forms the super-resolved image from the variance of images obtained with random speckled illuminations. However, the validity of this process has not been fully theorized. In this work, we characterize mathematically the sample information contained in the variance of diffraction-limited speckled images as a function of the statistical properties of the illuminations. We show that an unambiguous two-fold resolution gain is obtained when the speckle correlation length coincides with the width of the observation point spread function. Last, we analyze the difference between the variance-based techniques using random speckled illuminations (as in RIM) and those obtained using random fluorophore activation (as in Super-resolution Optical Fluctuation Imaging, SOFI).

The light intensity recorded by the camera of a fluorescence microscope cannot exhibit spatial frequencies above $2/\lambda$ where λ is the wavelength of the emitted light. This low-pass filtering, due to the loss of the evanescent waves at the detector plane, cannot be circumvented. Therefore the challenge of super-resolution imaging is to recover spatial frequencies of the sample fluorescence density beyond $2/\lambda$ from data that are frequency limited to $2/\lambda$. A widespread solution consists in processing multiple images obtained by changing the illumination, like translating focused spots [1–4] or rotating and translating periodic light patterns [5, 6]. The data processing of most techniques using structured illuminations requires the knowledge of the illumination patterns, either explicitly as in Structured Illumination Microscopy [5, 6] or implicitly as in confocal or Image Scanning microscopy [1]. In this context, Random Illumination Microscopy [7–9] stands out as an exception as it does not require the knowledge of the illumination patterns: the super-resolved image is formed from the variance of multiple diffraction-limited images recorded under different random speckled illuminations. While attractive because of its simplicity and significant image improvement [7], RIM variance-based processing lacks a rigorous analysis of its resolution potential, the non-linearity of the variance being a significant obstacle to its derivation. In this work, we study the sample information that can be extracted from the variance of speckled images as a function of the statistical properties of the random illumination and we derive the condition under which the variance can provide a resolution gain.

To model the data provided by a fluorescence microscope under an inhomogeneous illumination, we introduce the point spread function of the microscope h and the illumination intensity function E . Importantly, these two functions are defined at a macroscopic scale inside the sample, through the averaging over regions large

enough to contain thousands of atoms (typically of the order of a thousand nm^3), to wash out the microscopic fluctuations. In this context, we define the macroscopic fluorescence density ρ such that $V\rho(\mathbf{r})E(\mathbf{r})$ is the energy (detected by the camera) of the fluorescent light emitted by a macroscopic volume V centered about \mathbf{r} . Hereafter we neglect the Poisson noise. The fluorescence density depends on the fluorophore concentration and the molecular brightness.

With these definitions, the microscope image can be written as,

$$I(\mathbf{r}) = \int \rho(\mathbf{r}')E(\mathbf{r}')h(\mathbf{r} - \mathbf{r}')d\mathbf{r}' \quad (1)$$

where \mathbf{r} indicates a position in the image domain that is conjugated to a point in the object domain. This model can be applied to two- or three-dimensional imaging configurations. In the Fourier space, Eq. (1) reads,

$$\tilde{I}(\boldsymbol{\nu}) = \tilde{h}(\boldsymbol{\nu}) \int \tilde{\rho}(\boldsymbol{\nu} - \boldsymbol{\nu}')\tilde{E}(\boldsymbol{\nu}')d\boldsymbol{\nu}' \quad (2)$$

where $\tilde{f}(\boldsymbol{\nu}) = \int f(\mathbf{r})e^{-i2\pi\boldsymbol{\nu}\cdot\mathbf{r}}d\mathbf{r}$ stands for the Fourier transform of f .

If E is a constant, as in a standard fluorescence microscope, the recorded image depends only on the sample spatial frequencies belonging to the support of the Optical Transfer Function (OTF) \tilde{h} , noted W_h , which is at best a disk of radius $2/\lambda$ (in the 2D case) or exhibits a torus-like shape in the 3D case [10].

On the other hand, if E is not a constant, and noting W_E the support of \tilde{E} , the recorded image depends on the sample spatial frequencies in the domain

$$W_{hE} = \{\boldsymbol{\nu} - \boldsymbol{\mu} \mid \boldsymbol{\nu} \in W_h, \boldsymbol{\mu} \in W_E\}, \quad (3)$$

which is no more limited by $2/\lambda$ and corresponds to the Fourier support of hE .

Now, sensitivity to spatial frequencies of the sample outside W_h is a necessary but not a sufficient condition for being able to form a super-resolved image. One also needs a technique for extracting the high spatial frequencies of the sample from the diffraction-limited images I_n obtained for various illumination intensities E_n . For example, in an ideal confocal microscope, the n^{th} illumination is a focal spot centered about \mathbf{r}_n and the measured data is $I_n(\mathbf{r}_n) = \int \rho(\mathbf{r}')E_n(\mathbf{r}')h(\mathbf{r}_n - \mathbf{r}')d\mathbf{r}'$. If one neglects the Stokes shift and forms the illumination through the same optical path as the collection, one gets (using the reciprocity theorem [10]) $E_n(\mathbf{r}) = h(\mathbf{r}_n - \mathbf{r})$ and

$$I_n(\mathbf{r}_n) = \int \rho(\mathbf{r}')h^2(\mathbf{r}_n - \mathbf{r}')d\mathbf{r}' \quad (4)$$

which permits to recover the fluorescence density over $W_{hE} = W_{h^2}$ with $W_{h^2} = \{\boldsymbol{\nu} - \boldsymbol{\nu}' \mid \boldsymbol{\nu} \in W_h, \boldsymbol{\nu}' \in W_h\}$. Note however that the super resolution capacity of an ideal confocal microscope requires the use of an infinitely small pinhole which is impossible in practice. Generally, when the different illumination intensities are known, the sample frequencies in W_{hE} can be obtained explicitly from a linear combination of the diffraction-limited images (see [6] for exemple).

On the other hand, when the illuminations are unknown speckles as in Random Illumination Microscopy, there is no simple way for recovering the sample frequencies from the diffraction-limited images. While a link between the (numerically intractable) full covariance of the images and the sample was derived in [11], there is currently no rigorous result concerning the sample information that can be extracted from the sole variance.

Let us consider an experiment in which multiple images of a sample are recorded under different speckled illuminations E_n that are considered as different realizations of the same random process E . The variance of these diffraction-limited images, $V_\rho(\mathbf{r}) = \mathbb{E}[I^2(\mathbf{r})] - \mathbb{E}[I(\mathbf{r})]^2$, where \mathbb{E} stands for the ensemble average over the random illuminations, can be expressed as a function of the speckle autocovariance $C(\mathbf{r}, \mathbf{r}') = \mathbb{E}[E(\mathbf{r})E(\mathbf{r}')] - \mathbb{E}[E(\mathbf{r})]\mathbb{E}[E(\mathbf{r}')]$, as [11],

$$V_\rho(\mathbf{r}) = \int d\mathbf{r}_1 d\mathbf{r}_2 \quad (5) \\ h(\mathbf{r} - \mathbf{r}_1)\rho(\mathbf{r}_1)C(\mathbf{r}_1, \mathbf{r}_2)\rho(\mathbf{r}_2)h(\mathbf{r} - \mathbf{r}_2).$$

In the following, we assume that E is a second-order stationary random process so that $C(\mathbf{r}_1, \mathbf{r}_2) = C(\mathbf{r}_1 - \mathbf{r}_2)$ and the Fourier support of C is equal to the Fourier support of E , W_E [12].

Since the variance depends on the square of raw images that are frequency limited to W_h , its Fourier support corresponds to W_{h^2} .

On the other hand, each raw image is sensitive to the spatial frequencies of the sample in W_{hE} , so we expect the variance to be also sensitive to the spatial frequencies of the sample in W_{hE} (we show on a specific example in appendix B, that this is indeed the case). These

preliminary remarks bring out the main question posed by variance-based super-resolved microscopy approaches: what sample information in W_{hE} can we extract from an image that is frequency limited to W_{h^2} ?

We first consider RIM configuration in which the illumination is performed through the same objective as the observation and the Stokes shift can be neglected. In this case, the speckle autocovariance C is equal to the point spread function h of the microscope [7, 13] and W_E is similar to W_h . Thus, the domain of sample spatial frequencies acting on RIM raw images, W_{hE} , matches the Fourier support of the variance, W_{h^2} . In the following, we demonstrate that, indeed, if $h = C$, there is a bijection between the RIM variance and the sample frequencies within W_{h^2} .

We start by noting that the Fourier transform of C , \tilde{C} , is positive as C is an autocovariance function. We define h_E such that $\tilde{h}_E = \sqrt{\tilde{C}}$. One easily shows that $\int h_E(\mathbf{r}_1 - \mathbf{x})h_E(\mathbf{r}_2 - \mathbf{x})d\mathbf{x} = C(\mathbf{r}_1 - \mathbf{r}_2)$. Using this decomposition of the speckle covariance, V_ρ^{RIM} can be cast as,

$$V_\rho^{\text{RIM}}(\mathbf{r}) = B_{\rho, \rho}(\mathbf{r}) \quad (6)$$

where

$$B_{U, V}(\mathbf{r}) = \int M_U(\mathbf{r}, \mathbf{x})M_V(\mathbf{r}, \mathbf{x})d\mathbf{x} \quad (7)$$

with

$$M_V(\mathbf{r}, \mathbf{x}) = \int h(\mathbf{r} - \mathbf{r}_1)V(\mathbf{r}_1)h_E(\mathbf{r}_1 - \mathbf{x})d\mathbf{r}_1 \quad (8)$$

and U and V are integrable real functions. To pursue the demonstration, we need to point out several properties of $B_{U, V}$ and M_V . First, it is easily seen that $B_{U, V}$ is symmetric with respect to (U, V) , $B_{U, V} = B_{V, U}$ and bilinear, $B_{U+U', V+V'} = B_{U, V} + B_{U', V'} + B_{U, V'} + B_{U', V}$. Second, the Fourier transform of M_V with respect to (\mathbf{r}, \mathbf{x}) ,

$$\tilde{M}_V(\boldsymbol{\nu}, \boldsymbol{\mu}) = \tilde{h}(\boldsymbol{\nu})\tilde{V}(\boldsymbol{\mu} + \boldsymbol{\nu})\sqrt{\tilde{C}(\boldsymbol{\mu})}, \quad (9)$$

is bounded, so M_V is an analytic function. Third, we show in appendix A that, if $h = C$,

$$\int B_{U, V}(\mathbf{r})V(\mathbf{r})d\mathbf{r} = \int |M_V(\mathbf{r}, \mathbf{x})|^2 U(\mathbf{r})d\mathbf{r}d\mathbf{x}. \quad (10)$$

We now consider two fluorescence densities, $\rho_1(\mathbf{r}) \geq 0$ and $\rho_2(\mathbf{r}) \geq 0$ which have the same RIM variance, $B_{\rho_1, \rho_1}(\mathbf{r}) = B_{\rho_2, \rho_2}(\mathbf{r})$. Using the bilinearity and symmetry of $B_{U, V}$, we can show that $B_{\rho_1, \rho_1} - B_{\rho_2, \rho_2} = B_{\rho_1 + \rho_2, \rho_1 - \rho_2} = 0$. Thus, one obtains

$$\int B_{\rho_1 + \rho_2, \rho_1 - \rho_2}(\mathbf{r})[\rho_1(\mathbf{r}) - \rho_2(\mathbf{r})]d\mathbf{r} = 0 \quad (11)$$

which, using Eq. (10), implies,

$$\int |M_{\rho_1 - \rho_2}(\mathbf{r}, \mathbf{x})|^2 [\rho_1(\mathbf{r}) + \rho_2(\mathbf{r})]d\mathbf{r}d\mathbf{x} = 0. \quad (12)$$

We now assume that $\rho_1 + \rho_2$ stays strictly positive in a non-empty open set Ω . In this case, Eq. (12) is satisfied if and only if $M_{\rho_1-\rho_2}(\mathbf{r}, \mathbf{x}) = 0$ for $\mathbf{r} \in \Omega$ and for all \mathbf{x} . Since $M_{\rho_1-\rho_2}$ is analytic, $M_{\rho_1-\rho_2}(\mathbf{r}, \mathbf{x}) = 0$ for all \mathbf{x} and for $\mathbf{r} \in \Omega$ implies that $M_{\rho_1-\rho_2}(\mathbf{r}, \mathbf{x}) = 0$ for all \mathbf{x} and all \mathbf{r} , thus $\tilde{M}_{\rho_1-\rho_2}(\boldsymbol{\nu}, \boldsymbol{\mu}) = 0$ for all $\boldsymbol{\nu}$ and $\boldsymbol{\mu}$. From Eq. (9), the nullity of $\tilde{M}_{\rho_1-\rho_2}$ is obtained only if $\tilde{\rho}_1(\boldsymbol{\eta}) - \tilde{\rho}_2(\boldsymbol{\eta}) = 0$ for $\boldsymbol{\eta} \in W_{h^2}$. Hence, if ρ_1 and ρ_2 have the same RIM variance, they have the same spatial frequencies in W_{h^2} . Conversely, if two samples ρ_1 and ρ_2 have the same frequency content in W_{h^2} , they generate the same variance image. We have thus demonstrated that there is a one-to-one correspondence between the spatial frequencies of the variance of diffraction-limited speckled images and the spatial frequencies of the sample fluorescence density in the super-resolved Fourier domain W_{h^2} provided the speckle autocovariance function C be similar to the observation point spread function h .

Now, what happens if the speckle autocovariance is different from the point spread function? If the speckle correlation length is larger than the width of the point spread function, *i.e.* $W_E \subset W_h$, it is always possible to filter the recorded images so that $h = C$. In this case, the variance of the modified images gives access to the sample spatial frequencies in W_{E^2} at least.

On the other hand, if the speckle correlation length is smaller than the width of the point spread function, $W_h \subset W_E$, we show in appendix B, that, while the variance is sensitive to the sample frequencies in W_{hE} , it does not necessarily allow their recovery, even on the restricted domain W_{h^2} . This remark applies in particular to configurations where the observation point spread function and the fluorescence density vary slowly over the speckle grain size. This is the case in fluctuation imaging techniques using near-field speckles and far-field detection [14] or optical speckles and acoustic detection [15]. In these techniques, hereafter called speckle-SOFI, the expression of the variance simplifies to,

$$V_{\text{speckle-SOFI}}(\mathbf{r}) \approx C_0 \int h^2(\mathbf{r} - \mathbf{r}') \rho^2(\mathbf{r}') d\mathbf{r}'. \quad (13)$$

where $C_0 = \int C(\mathbf{r}) d\mathbf{r}$. We observe that the variance is now linearly linked to the *square* of the sought parameter (optical absorption or fluorescence density) that is filtered over W_{h^2} . Now, knowing the Fourier transform of ρ^2 in W_{h^2} does not mean that $\tilde{\rho}$ can be retrieved over W_{h^2} . Actually, when the speckled illumination is assumed to be spatially uncorrelated, it is possible to find samples with different fluorescence density spectra in W_{h^2} that have the same variance image, see appendix B.

At this point, it is interesting to differentiate fluctuation imaging using quasi-uncorrelated speckled illuminations from Super-resolution Optical Fluctuation Imaging (SOFI). In SOFI, the intensity fluctuations observed in the recorded images come from the random activation of the fluorophores and not from the illumination (which is kept homogeneous and equal to E_0 during the whole experiment). To account for this phenomenon, one needs

to explicit further the characteristics of the fluorescence density ρ which is related to the fluorophore concentration and the molecular brightness. We define the fluorophore concentration g at the macroscopic scale such that, $Vg(\mathbf{r})$ is the number of fluorophores contained in a macroscopic volume V centered about \mathbf{r} . Next, we introduce the mean molecular brightness b which accounts for the fluorophores' quantum yield and for the environment-dependent ability of the incident/emitted photons to reach the fluorophore/detector. If all the fluorophores are activated in V , the mean brightness b is defined such that $Vg(\mathbf{r})b(\mathbf{r})E_0$ is the energy measured by the camera of the photons emitted from V . In other terms, if all the fluorophores are activated, the fluorescence density is the product of the fluorophore concentration times the mean brightness, $\rho = g \times b$.

In SOFI, only a few fluorophores of V are activated during the image recording and they change at each novel image. Let us assume that they follow a Poisson point process of intensity proportional to the total number of fluorophores in V . Then, the number of activated fluorophores in V observed when recording one image becomes a Poisson variable of parameter $Vg(\mathbf{r})p(\mathbf{r})$ where p is the mean percentage of activation. Under this assumption, we show in appendix D that the variance of SOFI images reads,

$$V_{\text{SOFI}}(\mathbf{r}) = E_0^2 \int h^2(\mathbf{r} - \mathbf{r}') b^2(\mathbf{r}') g(\mathbf{r}') p(\mathbf{r}') d\mathbf{r}'. \quad (14)$$

While RIM is able to recover the fluorescence density $\rho = g \times b$ over W_{h^2} , SOFI has a similar super-resolution capacity, but the latter applies to a distinct density $g \times b^2 \times p = \rho \times b \times p$.

It is worth noting that, if the mean brightness b is homogeneous, RIM and SOFI are able to restore the fluorophore concentration g over W_{h^2} , (provided the mean activation percentage p in SOFI is also homogeneous). On the contrary, even if b is homogeneous, fluctuation imaging using quasi-uncorrelated speckled illuminations (speckle-SOFI) can only restore the square of the fluorophore concentration, g^2 , over W_{h^2} . Thus, SOFI and speckle-SOFI yield *a priori* different results and their umbrella denomination as 'fluctuation imaging' can be misleading.

In conclusion, we have shown that the variance of images obtained under random speckled illuminations gives the same fluorescence density as an ideal confocal microscope, *provided the speckle correlation length matches the width of the observation point spread function* (which is the case for RIM). Our demonstration provides a solid theoretical ground to the two-fold resolution gain, the optical sectioning and the linearity to fluorescence observed in RIM [7]. Also, we have pointed out the difference between the variance of SOFI-images obtained using the random activation of fluorophores and the variance of images obtained with quasi-uncorrelated speckled illuminations (speckle-SOFI). If the fluorophores brightness is homogeneous, SOFI variance is linearly linked to the

fluorophore concentration while speckle-SOFI variance is quadratically linked to the fluorophore concentration.

Appendix A: Proof of Identity

Introducing Eqs. (7,8) in the left-hand term of Eq. (10) yields,

$$A = \int B_{U,V}(\mathbf{r})V(\mathbf{r})d\mathbf{r}$$

where

$$B_{U,V}(\mathbf{r}) = \int d\mathbf{r}_1 d\mathbf{r}_2 d\mathbf{x} \\ h(\mathbf{r} - \mathbf{r}_1)U(\mathbf{r}_1)h_E(\mathbf{r}_1 - \mathbf{x})h(\mathbf{r} - \mathbf{r}_2)V(\mathbf{r}_2)h_E(\mathbf{r}_2 - \mathbf{x}).$$

Integrating over \mathbf{x} and using

$$\int h_E(\mathbf{r}_1 - \mathbf{x})h_E(\mathbf{r}_2 - \mathbf{x})d\mathbf{x} = h(\mathbf{r}_1 - \mathbf{r}_2)$$

(where we have assumed that $C = h$) yields,

$$B_{U,V}(\mathbf{r}) = \int d\mathbf{r}_1 d\mathbf{r}_2 h(\mathbf{r} - \mathbf{r}_1)U(\mathbf{r}_1)h(\mathbf{r}_1 - \mathbf{r}_2)h(\mathbf{r} - \mathbf{r}_2)V(\mathbf{r}_2).$$

Then, decomposing $h(\mathbf{r} - \mathbf{r}_2) = \int h_E(\mathbf{r} - \mathbf{x})h_E(\mathbf{r}_2 - \mathbf{x})d\mathbf{x}$, one obtains

$$B_{U,V}(\mathbf{r}) = \int d\mathbf{r}_1 d\mathbf{r}_2 d\mathbf{x} \\ h(\mathbf{r} - \mathbf{r}_1)h_E(\mathbf{r} - \mathbf{x})h(\mathbf{r}_1 - \mathbf{r}_2)V(\mathbf{r}_2)h_E(\mathbf{r}_2 - \mathbf{x})U(\mathbf{r}_1).$$

Finally, using the expression of M_V in Eq. (8) and the symmetry of h and h_E , we get

$$A = \int |M_V(\mathbf{r}_1, \mathbf{x})|^2 U(\mathbf{r}_1) d\mathbf{x} d\mathbf{r}_1.$$

Appendix B: What happens when $W_h \subset W_E$?

We have shown that provided $h = C$ (which can be obtained with an appropriate filtering of the raw images as soon as $W_E \subset W_h$), there is a one to one correspondence between the spatial frequencies of the variance of the speckled diffraction-limited images and that of the sample in W_{h^2} . We now study a case where $h \neq C$ and $W_h \subset W_E$. To simplify the discussion, we assume that (W_h, W_E) are centered plain disks with frequency cut-offs ν_h and ν_E respectively, with $\nu_h < \nu_E$. We further assume that the point spread function h is symmetric so that \tilde{h} is a real positive symmetric function, like \tilde{C} . We consider a sample whose Fourier spectrum is restricted to the null frequency and a high frequency $\pm\mathbf{k}$, $\rho(\mathbf{r}) = A + B \cos(2\pi\mathbf{k}\cdot\mathbf{r} + \varphi)$ with (A, B) real positive such

that ρ is real positive. The variance of the raw images, given by Eq. (5), obtained with such sample reads,

$$V_\rho^{\text{RIM}}(\mathbf{r}) = A^2\alpha + B^2\beta(\mathbf{k}) + 2AB\gamma(\mathbf{k}) \cos(2\pi\mathbf{k}\cdot\mathbf{r} + \varphi) \\ + B^2\eta(\mathbf{k}) \cos(4\pi\mathbf{k}\cdot\mathbf{r} + 2\varphi), \quad (\text{B1})$$

with

$$\alpha = \int |\tilde{h}|^2(\boldsymbol{\nu})\tilde{C}(\boldsymbol{\nu})d\boldsymbol{\nu}, \\ \beta(\mathbf{k}) = \int |\tilde{h}|^2(\boldsymbol{\nu} + \mathbf{k})\tilde{C}(\boldsymbol{\nu})d\boldsymbol{\nu}, \\ \gamma(\mathbf{k}) = \int \tilde{h}(\mathbf{k} - \boldsymbol{\nu})\tilde{h}(\boldsymbol{\nu})\tilde{C}(\boldsymbol{\nu})d\boldsymbol{\nu}, \\ \eta(\mathbf{k}) = \int \tilde{h}(\boldsymbol{\nu})\tilde{h}(2\mathbf{k} - \boldsymbol{\nu})\tilde{C}(\mathbf{k} - \boldsymbol{\nu})d\boldsymbol{\nu}.$$

We observe that, as long as $k < \nu_h + \nu_E$, $\beta(\mathbf{k}) \neq 0$ and the variance depends on the high spatial frequency of the sample, B . This result confirms the sensitivity of the variance to sample spatial frequencies in W_{hE} . However, if $2\nu_h < k \leq \nu_h + \nu_E$, $\gamma(\mathbf{k}) = \eta(\mathbf{k}) = 0$ so that $V_\rho^{\text{RIM}}(\mathbf{r}) = \alpha A^2 + \beta(\mathbf{k})B^2$. In this case, the variance is sensitive to the amplitudes of the null and high frequencies of the sample, (A, B) , but it has lost the information about the phase of the high frequency, φ . Worse, this example shows that a uniform sample defined by $\rho_1(\mathbf{r}) = (A^2 + \beta(\mathbf{k})B^2)^{\frac{1}{2}}/\alpha$ will have the same variance as the inhomogeneous sample defined by $\rho(\mathbf{r}) = A + B \cos(2\pi\mathbf{k}\cdot\mathbf{r} + \varphi)$. Thus, when $\nu_h < \nu_E$, the identifiability of the sample spatial frequencies from the variance is lost, even for frequencies belonging to W_{h^2} . This assertion is particularly counter-intuitive as it shows that decreasing the size of the speckle grains below the width of the observation point spread function is *a priori* detrimental to the sample reconstruction.

Appendix C: Imaging with spatially quasi-uncorrelated speckles, speckle-SOFI

When the point spread function and fluorescence density are slowly varying over the width of the speckle autocovariance function, the variance of the diffraction-limited images is linearly linked to the square of ρ filtered over W_{h^2} . In this section, we provide an example of two positive functions with different Fourier contents in the super-resolved domain W_{h^2} which, when squared, have exactly the same Fourier content in W_{h^2} .

We consider g the sum of a constant and a one-dimensional cosine along the x-axis with a frequency k laying in W_{h^2} but not in W_h , and f the sum of a constant and two cosines with period k and $2k$. Note that $2k$ lays outside W_{h^2} . We adapt the constant and the cosine amplitudes so that f and g are positive and f^2 and g^2 are equal in W_{h^2} . A possible solution is,

$$f(x) = 6 + \sqrt{2} \cos(2\pi kx) + \cos(4\pi kx) \\ g(x) = \frac{\sqrt{101} + 7}{2\sqrt{2}} + \frac{\sqrt{101} - 7}{2} \cos(2\pi kx) \quad (\text{C1})$$

Noting \mathcal{F} the low pass filter operator that removes all the frequencies outside W_{h^2} , we find,

$$\mathcal{F}[f^2](x) = \mathcal{F}[g^2](x) = 75 + 13\sqrt{2}\cos(2\pi kx).$$

Appendix D: Modeling SOFI at the macroscopic scale

Generally, SOFI data are modeled with a discrete sum that depends on the fluorophore positions [8]. However, it is clearly impossible to recover the fluorophores positions from SOFI (second-order) image, except if a constraint of sparsity is assumed. It may thus be interesting to relate SOFI images to sample characteristics that are defined at a macroscopic scale, such as the fluorophore concentration.

In structured illumination microscopy, one assumes that all the fluorophores are activated. The fluorescence density ρ is written as the product of the fluorophore concentration g with a mean brightness b . The intensity recorded by the camera is modeled as,

$$I(\mathbf{r}) = \int h(\mathbf{r} - \mathbf{r}')E(\mathbf{r}')g(\mathbf{r}')b(\mathbf{r}')d\mathbf{r}', \quad (\text{D1})$$

where E is the inhomogeneous illumination intensity and h the microscope point spread function.

In SOFI, the illumination E_0 is homogeneous but the fluorophores oscillate between an activated and non-activated state. Thus, only a subset of the fluorophores present in the sample contributes to the image intensity at a given time t . The activated fluorophores in the (macroscopic) volume V centered about \mathbf{r} can be seen as points popping up at random and independently of one another. This process is conveniently modeled with a Poisson point process of intensity proportional to the number of fluorophores in V . Under this assumption, the number of activated fluorophores in V at time t , written as $Vq(\mathbf{r}, t)$, where q is the activated fluorophore concentration, is a Poisson variable of parameter $Vg(\mathbf{r})p(\mathbf{r})$ with p the mean percentage of activation. With this definition, the intensity of the image recorded at t can be written as,

$$I(\mathbf{r}, t) = E_0 \int h(\mathbf{r} - \mathbf{r}')q(\mathbf{r}', t)b(\mathbf{r}')d\mathbf{r}'. \quad (\text{D2})$$

It is thus a filtered Poisson variable whose time variance reads [16, Chap. 5],

$$V_{\text{SOFI}}(\mathbf{r}) = E_0^2 \int h^2(\mathbf{r} - \mathbf{r}')b^2(\mathbf{r}')g(\mathbf{r}')p(\mathbf{r}')d\mathbf{r}'.$$

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- [1] C. Sheppard and C. Cogswell, Three-dimensional image formation in confocal microscopy, *Journal of microscopy* **159**, 179 (1990).
 - [2] T. A. Klar and S. W. Hell, Subdiffraction resolution in far-field fluorescence microscopy, *Optics letters* **24**, 954 (1999).
 - [3] C. R. Sheppard, Super-resolution in confocal imaging, *Optik (Stuttgart)* **80**, 53 (1988).
 - [4] C. B. Müller and J. Enderlein, Image scanning microscopy, *Physical review letters* **104**, 198101 (2010).
 - [5] R. Heintzmann and C. G. Cremer, Laterally modulated excitation microscopy: improvement of resolution by using a diffraction grating, in *Optical biopsies and microscopic techniques III*, Vol. 3568 (SPIE, 1999) pp. 185–196.
 - [6] M. G. Gustafsson, Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy, *Journal of microscopy* **198**, 82 (2000).
 - [7] T. Mangeat, S. Labouesse, M. Allain, A. Negash, E. Martin, A. Guérolé, R. Poincloux, C. Estibal, A. Bouissou, S. Cantaloube, E. Vega, T. Li, C. Rouvière, S. Alart, D. Keller, V. Debarnot, X. B. Wang, G. Michaux, M. Pinot, R. L. Borgne, S. Tournier, M. Suzanne, J. Idier, and A. Sentenac, Super-resolved live-cell imaging using random illumination microscopy, *Cell Reports Methods* **1**, 100009 (2021).
 - [8] T. Dertinger, R. Colyer, G. Iyer, S. Weiss, and J. Enderlein, Fast, background-free, 3d super-resolution optical fluctuation imaging (sofi), *Proceedings of the National Academy of Sciences* **106**, 22287 (2009).
 - [9] C. Ventalon and J. Mertz, Quasi-confocal fluorescence sectioning with dynamic speckle illumination, *Optics Letters* **30**, 3350 (2005).
 - [10] A. Sentenac and J. Mertz, Unified description of three-dimensional optical diffraction microscopy: from transmission microscopy to optical coherence tomography: tutorial, *JOSA A* **35**, 748 (2018).
 - [11] J. Idier, S. Labouesse, M. Allain, P. Liu, S. Bourguignon, and A. Sentenac, On the superresolution capacity of imagers using unknown speckle illuminations, *IEEE Transactions on Computational Imaging* **4**, 87 (2018).
 - [12] M. B. Priestley, *Spectral analysis and time series: probability and mathematical statistics*, 04; QA280, P7. (1981).
 - [13] J. W. Goodman, *Speckle phenomena in optics: theory and applications* (Roberts and Company Publishers, 2007).
 - [14] Y. Choi, M. Kim, C. Park, J. Park, Y. Park, and Y.-H. Cho, Wide-field super-resolution optical fluctuation imaging through dynamic near-field speckle illumination, *Nano letters* **22**, 2194 (2022).
 - [15] T. Chaigne, J. Gateau, M. Allain, O. Katz, S. Gigan, A. Sentenac, and E. Bossy, Super-resolution photoacoustic fluctuation imaging with multiple speckle illumination, *Optica* **3**, 54 (2016).
 - [16] D. L. Snyder and M. I. Miller, *Random Point Processes in Time and Space* (Springer New York, New York, NY, 1991).