

# REVIEW OF DYNAMIC OPTICAL COHERENCE TOMOGRAPHY FOR INTRACELLULAR MOTILITY: SIGNALS, METRICS, AND THEIR APPLICATIONS: SUPPLEMENTAL DOCUMENT 1

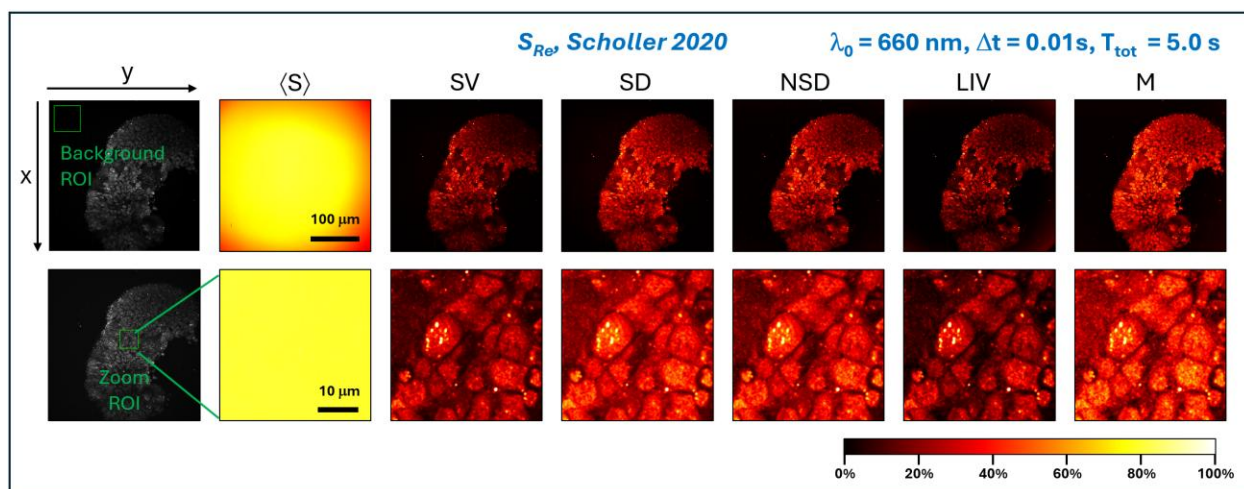
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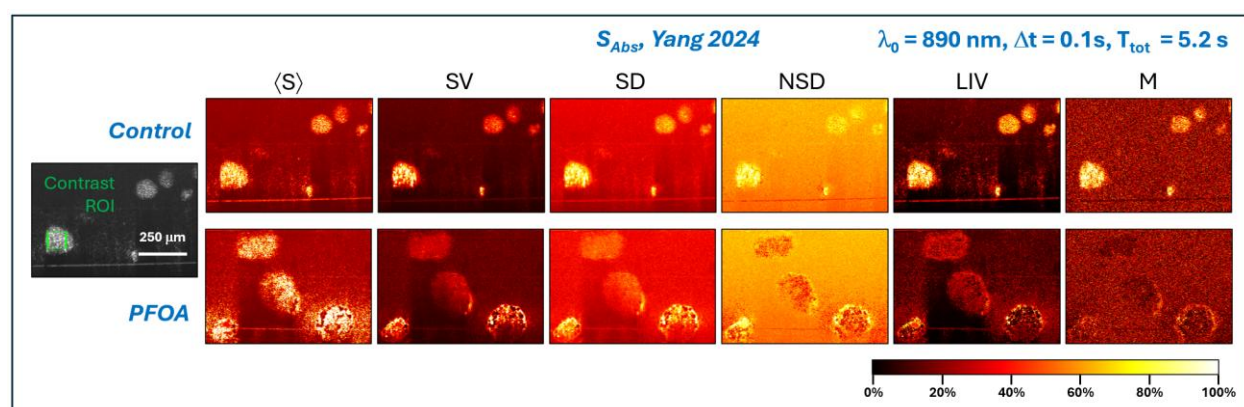
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## 1. Figures



**Fig. S1.** Comparison of background-subtracted dOCT metrics applied to 3D cell cultures via FF-OCT real-part signals  $S_{Re}$  (data from [1]). For each metric, the average within the background ROI depicted in the upper left was subtracted, then the contrast was scaled such that the average within the contrast ROI (shown in Fig. 2) was 80%. A zoomed view is shown in the bottom row of each panel.



**Fig. S2.** Comparison of dOCT metrics applied to 3D MEC spheroid cultures via SD-OCT absolute value signals ( $S_{Abs}$ ), (data from [2]). The top and bottom rows illustrate control and PFOA-treated MEC spheroids, respectively. Contrast is set by the control image for each group using the average value within the ROI (left), to aid in comparison.

## 2. Analytical OCT signals: definitions and statistical properties

Equations are provided below in support of the manuscript. Definitions of all variables can be found in the manuscript unless noted here.

### 2.1 Single scatterer

$$\begin{aligned} S(t) &\propto |E_r + E_s|^2 \\ &= \langle |E_r|^2 \rangle + 2 \langle |E_r| |E_s| \rangle \cos(\Delta\varphi(t)) + \langle |E_s|^2 \rangle \\ &\approx \langle |E_r|^2 \rangle + 2 \langle |E_r| |E_s| \rangle \cos(\Delta\varphi(t)) \end{aligned} \quad (S1)$$

$$I_{ref} = \langle |E_r|^2 \rangle \quad (S2)$$

$$S_{Re}(t) = I_{ref} + 2 \langle |E_r| |E_s| \rangle \cos(\Delta\varphi(t)) \quad (S3)$$

$$\tilde{S}_{CA}(t) = 2 \langle |E_r| |E_s| \rangle e^{i\Delta\varphi(t)} \quad (S4)$$

$$S_{Abs}(t) = |\tilde{S}_{CA}(t)| = 2 \langle |E_r| |E_s| \rangle \quad (S5)$$

### 2.2 Multiple scatterers

$$k = \frac{2\pi n}{\lambda_0} \quad (S6)$$

$$E_s = \sqrt{I_{ref}} \sum_{j=1}^{N_p} A_j e^{i2kz_j(t)} \quad (S7)$$

$$E_r = \sqrt{I_{ref}} e^{i\varphi_{ref}} \quad (S8)$$

In the above,  $\varphi_{ref}$  is the relative phase between  $E_r$  and  $E_s$  when  $z = 0$ .

$$S_{Re}(t) = I_{ref} \left( 1 + 2 \sum_{j=1}^{N_p} A_j \cos(\varphi_{ref} + 2kz_j(t)) \right) \quad (S9)$$

$$\tilde{S}_{CA}(t) = 2I_{ref} e^{i\varphi_{ref}} \sum_{j=1}^{N_p} A_j e^{i2kz_j(t)} \quad (S10)$$

$$S_{Abs}(t) = 2I_{ref} \left| \sum_{j=1}^{N_p} A_j e^{i2kz_j(t)} \right| \quad (S11)$$

### 2.3 Model of a single population of scatterers with diffusion and bulk flow

The axial motion of each particle is comprised of a diffusive part and bulk flow.

$$z_j(t) = z_{D,j}(t) + vt \quad (S12)$$

Identical particles have a common reflection coefficient:

$$A_j = A \quad (S13)$$

$$S_{Re}(t) = I_{ref} \left( 1 + 2A \sum_{j=1}^{N_p} \cos(\varphi_{ref} + 2kz_{D,j}(t) + 2kvt) \right) \quad (S14)$$

$$\tilde{S}_{CA}(t) = 2AI_{ref} e^{i(\varphi_{ref} - 2kvt)} \sum_{j=1}^{N_p} e^{-i2kz_{D,j}(t)} \quad (S15)$$

$$S_{Abs}(t) = 2AI_{ref} \left| \sum_{j=1}^{N_p} e^{-i2kz_{D,j}(t)} \right| \quad (S16)$$

Mean values:

$$\langle S_{Re} \rangle = I_{ref} \quad (S17)$$

$$\langle \tilde{S}_{CA} \rangle = 0 \quad (S18)$$

In limit of fully developed speckle ( $N_p$  large):

$$\langle S_{Abs} \rangle = \sqrt{\pi} AI_{ref} \sqrt{N_p} \quad (S19)$$

Mean squared values:

$$\langle S_{Re}^2 \rangle = I_{ref}^2 (1 + 2A^2 N_p) \quad (S20)$$

$$\langle \tilde{S}_{CA}^* \tilde{S}_{CA} \rangle = \langle |\tilde{S}_{CA}|^2 \rangle = 4A^2 I_{ref}^2 N_p \quad (S21)$$

$$\langle S_{Abs}^2 \rangle = \langle |\tilde{S}_{CA}|^2 \rangle = 4A^2 I_{ref}^2 N_p \quad (S22)$$

Variances:\*

For simplicity,  $S$  is presumed to be generally complex here and beyond.

$$\begin{aligned} \text{Var}(S) &= \langle S^* S \rangle - \langle S^* \rangle \langle S \rangle \\ &= \langle |S|^2 \rangle - |\langle S \rangle|^2 \end{aligned} \quad (S23)$$

$$\text{Var}(S_{Re}) = 2A^2 I_{ref}^2 N_p \quad (S24)$$

$$\text{Var}(S_{CA}) = 4A^2 I_{ref}^2 N_p \quad (S25)$$

$$\text{Var}(S_{Abs}) = (4 - \pi) A^2 I_{ref}^2 N_p \quad (S26)$$

\*Note that these expressions are equivalent but may offer different computational efficiencies:

$$\text{Var}(S) = \langle S^* S \rangle - \langle S^* \rangle \langle S \rangle = \langle (S^* - \langle S^* \rangle)(S - \langle S \rangle) \rangle \quad (S27)$$

Autocorrelations:

$$\Gamma(S; \tau) = \langle S^*(t) S(t - \tau) \rangle \quad (S28)$$

$$\Gamma_{Re} = \Gamma(S_{Re}; \tau) = I_{ref}^2 \left( 1 + 2A^2 N_p \exp(-4k^2 D\tau) \cos(2kv\tau) \right) \quad (S29)$$

$$\Gamma_{CA} = \Gamma(\tilde{S}_{CA}; \tau) = 4A^2 I_{ref}^2 N_p \exp(-4k^2 D\tau) \exp(i2kv\tau) \quad (S30)$$

$$\Gamma_{Abs} = \Gamma(S_{Abs}; \tau) = A^2 I_{ref}^2 N_p \left( \pi + (4 - \pi) \exp(-4k^2 D\tau) \right) \quad (S31)$$

Normalized autocorrelations:

$$g(S; \tau) = \frac{\Gamma(S; \tau)}{\Gamma(S; 0)} \quad (S32)$$

$$g_{Re} = g(S_{Re}; \tau) = \frac{1 + 2A^2 N_p \exp(-4k^2 D\tau) \cos(2kv\tau)}{1 + 2A^2 N_p} \quad (S33)$$

$$g_{CA} = g(\tilde{S}_{CA}; \tau) = \exp(-4k^2 D\tau) \exp(i2kv\tau) \quad (S34)$$

$$g_{Abs} = g(S_{Abs}; \tau) = \frac{1}{4} \left( \pi + (4 - \pi) \exp(-4k^2 D\tau) \right) \quad (S35)$$

Covariances:\*

$$\begin{aligned} \text{Cov}(S; \tau) &= \Gamma(S; \tau) - \langle S^* \rangle \langle S \rangle \\ &= \Gamma(S; \tau) - \langle S \rangle^2 \end{aligned} \quad (S36)$$

$$\text{Cov}_{Re} = \text{Cov}(S_{Re}; \tau) = 2A^2 I_{ref}^2 N_p \exp(-4k^2 D\tau) \cos(2kv\tau) \quad (S37)$$

$$\text{Cov}_{CA} = \text{Cov}(\tilde{S}_{CA}; \tau) = 4A^2 I_{ref}^2 N_p \exp(-4k^2 D\tau) \exp(i2kv\tau) \quad (S38)$$

$$\text{Cov}_{Abs} = \text{Cov}(S_{Abs}; \tau) = (4 - \pi) A^2 I_{ref}^2 N_p \exp(-4k^2 D\tau) \quad (S39)$$

\*Note that these expressions are equivalent but may offer different computational efficiencies:

$$\begin{aligned} \text{Cov}(S; \tau) &= \langle S^*(t - \tau) S(t) \rangle - \langle S^* \rangle \langle S \rangle \\ &= \langle (S^*(t - \tau) - \langle S^* \rangle) (S(t) - \langle S \rangle) \rangle \end{aligned} \quad (S40)$$

Correlation coefficients:

$$\rho(S; \tau) = \frac{\text{Cov}(S; \tau)}{\text{Var}(S)} \quad (S41)$$

$$\rho_{Re} = \rho(S_{Re}; \tau) = \exp(-4k^2 D\tau) \cos(2kv\tau) \quad (S42)$$

$$\rho_{CA} = \rho(\tilde{S}_{CA}; \tau) = \exp(-4k^2 D\tau) \exp(i2kv\tau) \quad (S43)$$

$$\rho_{Abs} = \rho(S_{Abs}; \tau) = \exp(-4k^2 D \tau) \quad (S44)$$

Power spectral densities:

$$PSD(S; \omega) = \mathfrak{F}\{\Gamma(S; \tau)\}_{\omega}, \quad (S45)$$

where  $\mathfrak{F}$  is the Fourier transform operator.

$$\begin{aligned} PSD_{Re} &= PSD(S_{Re}; \omega) \\ &= 2\pi I_{ref}^2 \delta(\omega - 2kv) + \frac{A^2 I_{ref}^2 N_p}{k^2 D} \left( 1 + \left( \frac{\omega - 2kv}{4k^2 D} \right)^2 \right)^{-1} \end{aligned} \quad (S46)$$

$$\begin{aligned} PSD_{CA} &= PSD(S_{CA}; \omega) \\ &= \frac{2A^2 I_{ref}^2 N_p}{k^2 D} \left( 1 + \left( \frac{\omega - 2kv}{4k^2 D} \right)^2 \right)^{-1} \end{aligned} \quad (S47)$$

$$\begin{aligned} PSD_{Abs} &= PSD(S_{Abs}; \omega) \\ &= 2\pi^2 A^2 I_{ref}^2 N_p \delta(\omega) + \frac{(4 - \pi)}{4k^2 D} \left( 1 + \left( \frac{\omega}{8k^2 D} \right)^2 \right)^{-1} \end{aligned} \quad (S48)$$

Normalized power spectral densities:

Note: delta functions are omitted in this normalization step as they would normally be excluded in analysis of discrete data.

$$NPSD(S; \omega) = \frac{PSD(S; \omega)}{\int_{-\infty}^{\infty} PSD(S; \omega) d\omega} \quad (S49)$$

$$NPSD_{Re} = NPSD(S_{Re}; \omega) = \frac{1}{\pi 4k^2 D} \left( 1 + \left( \frac{\omega - 2kv}{4k^2 D} \right)^2 \right)^{-1} \quad (S50)$$

$$NPSD_{CA} = NPSD(S_{CA}; \omega) = \frac{1}{\pi 4k^2 D} \left( 1 + \left( \frac{\omega - 2kv}{4k^2 D} \right)^2 \right)^{-1} \quad (S51)$$

$$NPSD_{Abs} = NPSD(S_{Abs}; \omega) = \frac{1}{\pi 8k^2 D} \left( 1 + \left( \frac{\omega}{8k^2 D} \right)^2 \right)^{-1} \quad (S52)$$

### 3. Expressions for discretely sampled signals

See manuscript for definitions.

#### 3.1 Basic expressions

$$S_j = S(t_j); \quad t_j = j\Delta t; \quad j = 0 \dots N_t - 1 \quad (\text{S53})$$

$$\langle S_j \rangle = \frac{1}{N_t} \sum_{j=0}^{N_t-1} S_j \quad (\text{S54})$$

$$\text{Var}(S_j) = \frac{1}{N_t} \sum_{j=0}^{N_t-1} (S_j^* - \langle S_j^* \rangle) (S_j - \langle S_j \rangle) \quad (\text{S55})$$

Autocorrelation (unbiased):

$$\Gamma(S_j; t_k) = \frac{1}{N_t - k} \sum_{j=0}^{N_t-1-k} S_j^* S_{j+k} \quad (\text{S56})$$

Normalized autocorrelation:

$$g(S_j; t_k) = \frac{\Gamma(S_j; t_k)}{\Gamma(S_j; 0)} \quad (\text{S57})$$

Alternative methods of normalization that avoid noise at  $\tau=0$  include the following two approaches. First, one may normalize by the first sampling point  $t_1=\Delta t$ , which assumes that  $\tau_{1/e} \gg \Delta t$ :

$$g_{\Delta}(S_j; t_k) = \frac{\Gamma(S_j; t_k)}{\Gamma(S_j; \Delta t)} = \frac{\Gamma(S_j; t_k)}{\Gamma(S_j; t_1)} \quad (\text{S58})$$

Second, one may normalize by a projected value of  $\Gamma(0)$ , which assumes that early decay of  $\Gamma$  is dominated by a single exponential:

$$\frac{\Gamma(2\Delta t)}{\Gamma(\Delta t)} \approx \frac{\Gamma(0)\exp(-2\Delta t / \tau_{1/e})}{\Gamma(0)\exp(-\Delta t / \tau_{1/e})} = \exp(-\Delta t / \tau_{1/e}) \quad (\text{S59})$$

$$\Gamma(0) \approx \Gamma_{\text{projected}}(0) = \Gamma(\Delta t) / \left( \frac{\Gamma(2\Delta t)}{\Gamma(\Delta t)} \right) = \frac{\Gamma(\Delta t)^2}{\Gamma(2\Delta t)}$$

$$g_{\text{projected}}(S_j; t_k) = \begin{cases} \Gamma(S_j; \tau) / \Gamma_{\text{projected}}(S_j; 0), & \tau \neq 0 \\ 1, & \tau = 0 \end{cases} \quad (\text{S60})$$

Covariance:

Note that it is important to match the data points over which the mean is computed before subtraction. A clipped mean is thus defined as:

$$\langle S_j \rangle_{a,b} = \frac{1}{b-a+1} \sum_{j=a}^b S_j \quad (\text{S61})$$

Then the covariance is computed by subtracting the data-matched means to each term:

$$\text{Cov}(S_j; t_k) = \frac{1}{N_t - k} \sum_{j=0}^{N_t-1-k} (S_j^* - \langle S_j^* \rangle_{0, N_t-1-k}) (S_{j+k} - \langle S_j \rangle_{k, N_t-1}) \quad (\text{S62})$$

Correlation coefficient:

Note that while the analytical expression for correlation coefficient has a single variance in the denominator, here we divide by the square root of two variances computed over a subset of the data matched to the subsets used in the covariance calculation. A clipped variance is first defined as:

$$\text{Var}(S_j)_{a,b} = \frac{1}{N_t} \sum_{j=a}^b (S_j^* - \langle S_j^* \rangle_{a,b}) (S_j - \langle S_j \rangle_{a,b}) \quad (\text{S63})$$

Then the correlation coefficient uses data-matched variances in the denominator:

$$\rho(S_j; t_k) = \frac{\text{Cov}(S_j; t_k)}{\sqrt{\text{Var}(S_j)_{0, N_t-1-k}} \sqrt{\text{Var}(S_j)_{k, N_t-1}}} \quad (\text{S64})$$

A special correlation coefficient of similar form is used for determination of OCT correlation decay speed (OCDS):

$$\rho_{\text{OCDS}}(S_j; t_k) = \frac{\text{Cov}(S_j; t_k)}{\text{Var}(S_j)_{0, N_t-1-k} \text{Var}(S_j)_{k, N_t-1}}, \quad (\text{S65})$$

and with spatial averaging (where  $\langle \rangle_{x,z}$  denotes averaging over a 2D window in  $x$  and  $z$ ):

$$\rho_{\text{OCDS}, x, z}(S_j; t_k) = \frac{\langle \text{Cov}(S_j; t_k) \rangle_{x, z}}{\langle \text{Var}(S_j)_{0, N_t-1-k} \rangle_{x, z} \langle \text{Var}(S_j)_{k, N_t-1} \rangle_{x, z}} \quad (\text{S66})$$

Reference-frame method with spatial averaging for correlation coefficient computation:

$$\rho_{\text{ref-frame}}(S_j; t_k) \approx \frac{\langle (S_0^* - \langle S_0^* \rangle_{x, z}) (S_k - \langle S_k \rangle_{x, z}) \rangle_{x, z}}{\sqrt{\langle (S_0^* - \langle S_0^* \rangle_{x, z})^2 (S_k - \langle S_k \rangle_{x, z})^2 \rangle_{x, z}}} \quad (\text{S67})$$

#### Power spectral density (PSD):

First we define the discrete Fourier transform (DFT) and its inverse as follows, noting that different conventions may be used for the normalization factors and sign in the exponent:

$$F_m = \text{DFT}\{f_j\} = \sum_{j=0}^{N_t-1} f_j \exp(-2\pi i j m / N_t) \quad (\text{S68})$$

$$f_j = \text{DFT}^{-1}\{F_m\} = \frac{1}{N_t} \sum_{m=0}^{N_t-1} F_m \exp(2\pi i j m / N_t)$$

Then we define the PSD as a power per unit frequency:

$$\text{PSD}_m = \text{PSD}(S_j; \omega_m) = \frac{\Delta t}{N_t} |\text{DFT}\{S_j\}|^2, \quad (\text{S69})$$

$$\omega_m = \frac{2\pi m}{T_{\text{tot}}}$$

where  $\omega_m$  is the angular frequency associated with the index  $m$  output from the DFT.

#### Normalized PSD (NPSD) and noise subtraction:

The PSD is converted into a probability density by dividing PSD by the value of its integral from some minimum frequency ( $f_{\text{min}} = m_{\text{min}} / T_{\text{tot}}$ , selected to omit low frequency noise), to the Nyquist frequency ( $f_{\text{Nyq}} = m_{\text{Nyq}} / T_{\text{tot}}$ ), as follows:

$$NPSD_m = \frac{PSD_m}{\sum_{m=m_{\min}}^{m_{Nyq}} PSD_m}, \quad (S70)$$

$$m_{Nyq} = \frac{N_t}{2}$$

Alternatively, a noise-subtracted and normalized PSD may be defined. A noise level is computed by averaging the PSD over a high frequency band from some  $f_{big} = m_{big} / T_{tot}$  to  $f_{Nyq}$  as follows:

$$n = \frac{1}{m_{Nyq} - m_{big} + 1} \sum_{m=m_{big}}^{m_{Nyq}} PSD_m, \quad (S71)$$

then the noise level is subtracted off the PSD:

$$\Delta PSD_m = PSD_m - n. \quad (S72)$$

Subsequently, a normalized and noise subtracted PSD may be defined:

$$\Delta NPSD_m = \frac{\Delta PSD_m}{\sum_{m=m_{\min}}^{m_{Nyq}} \Delta PSD_m}, \quad (S73)$$

### 3.2. Metrics

Here we define metrics computed directly from dOCT signals that do not require curve-fitting. Curve-fitting methods to extract  $D$ ,  $v$ , OCDS,  $\alpha_{IPL}$ , and other signals are described in the main text.

Speckle Variance (SV):

$$SV(S_j) = Var(S_j) = \frac{1}{N_t} \sum_{j=0}^{N-1} (S_j^* - \langle S_j^* \rangle) (S_j - \langle S_j \rangle) \quad (S74)$$

Logarithmic Intensity Variance (LIV), (only applied to real-valued S):

$$S_{j,dB} = 10 \log_{10}(S_{j,Abs}) \quad (S75)$$

$$LIV(S_j) = Var(S_{j,dB})$$

Standard Deviation (SD):

$$SD(S_j) = \sqrt{Var(S_j)} \quad (S76)$$

Normalized Standard Deviation (NSD), (only applied to real-valued S):

$$NSD(S_j) = \frac{SD(S_j)}{\sqrt{\langle S_j \rangle}} \quad (S77)$$

Alternative Normalized Standard Deviation, (only applied to real-valued S):

$$NSD_{alt}(S_j) = \frac{SD(S_j)}{\langle S_j \rangle} \quad (S78)$$

Motility amplitude (M), (only applied to real-valued S):

$$M(S_j) = \frac{\sqrt{Cov(S_j; t_1)}}{\langle S_j \rangle} \quad (S79)$$

A minimum threshold of 0 is typically employed inside the square root to avoid complex values.



Mean frequency  $f_{mean}$ :

$$f_{mean} = \sum_{m=m_{min}}^{m_{Nsq}} f_m NPSD_m \quad (S80)$$

$$f_m = \frac{m}{T_{tot}}$$

Note that one could substitute NPSD with  $\Delta NPSD$  as defined in Eq. (S73) if noise subtraction is desired.

Bandwidth  $f_{BW}$ :

$$f_{BW} = \sqrt{\left( \sum_{m=m_{min}}^{m_{Nsq}} f_m^2 NPSD_m \right) - \left( \sum_{m=m_{min}}^{m_{Nsq}} f_m NPSD_m \right)^2} \quad (S81)$$

Note that one could substitute NPSD with  $\Delta NPSD$  as defined in Eq. (S73) if noise subtraction is desired. A minimum threshold of 0 is typically employed inside the square root to avoid complex values.

Median frequency  $f_{med}$ :

The median frequency index  $m_{med}$  is located on  $\Delta PSD$  (or  $\Delta NPSD$ , as the result will be identical), omitting the DC term ( $m = 0$ ), such that the equality is as close as possible:

$$\sum_{m=1}^{m_{med}} \Delta PSD_m \approx \frac{1}{2} \sum_{m=1}^{m_{big}} \Delta PSD_m, \quad (S82)$$

and the median frequency is simply:

$$f_{med} = \frac{m_{med}}{T_{tot}}. \quad (S83)$$

#### 4. Digital algorithm for computing autocorrelations via the DFT

The signal must first be padded with zeros, bringing its length from  $N_t$  to  $2N_t - 1$ :

$$S_{pad,j} = \begin{cases} S_j & j = 0 \dots N_t - 1 \\ 0 & j = N_t \dots 2N_t - 2 \end{cases} \quad (S84)$$

Using the definition of the DFT in Eq. (S68) above, which may be computed using an FFT algorithm, an unscaled power spectrum is computed via:

$$PSD_{unscaled,m} = PSD_{unscaled}(S_{pad,j}; \omega_m) = |\text{DFT}\{S_{pad,j}\}|^2 \quad (S85)$$

Then the biased autocorrelation, defined as

$$\Gamma_{biased}(t_k) = \sum_{j=0}^{N_t-1-k} S_j^* S_{j+k} \quad (S86)$$

is determined instead by

$$\Gamma_{biased}(t_k) = \text{Re}\{\text{DFT}^{-1}\{PSD_{unscaled,m}\}\} \quad (S87)$$

Computationally, Eq. (S86) and (S87) should produce the same result within machine precision. Usually the unbiased autocorrelation of Eq. (S56) is desired, which may then be computed by weighting each element of the result as follows:

$$\Gamma(t_k) = \frac{1}{N_t - k} \Gamma_{biased}(t_k) \quad (S88)$$

## References

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2. L. Yang, P. Ji, A. A. Miranda Buzetta, H. Li, M. R. Lockett, H. Zhou, and A. L. Oldenburg, "Longitudinal tracking of perfluorooctanoic acid exposure on mammary epithelial cell spheroids by dynamic optical coherence tomography," *Biomed Opt Express* **15**, 5115-5127 (2024).