Supplemental Document

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Wide-field intensity fluctuation imaging: supplement

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Wide-field Intensity Fluctuation Imaging: supplemental document

This supplemental document details the theory of within-exposure modulation and particularly, the interpretation of 2-pulse modulation from the statistics perspective of view. It also provides supporting material on the impact of non-zero residual illumination between the 2 pulses and that of pulse duration on the accuracy of estimating the relative and absolute values of $g_2(\tau)$, respectively, with the 2-pulse modulation method.

1. RELATING $K^2(T)$ AND $g_2(\tau)$ IN ARBITRARY MODULATION

Define the AOM modulation function as $m(t)$, the intact speckle signal as $I(t)$, and the modulated speckle signal as $I_m(t)$ such that

$$I_m(t) = I(t)m(t) \tag{S1}$$

Then the intensity of pixel i on the camera sensor within intensity-modulated exposure time T would be

$$S_{i,T} = \int_0^T I_i(t')m(t')dt' \tag{S2}$$

where $I_i(t)$ is the intact speckle signal of pixel i and $m(t)$ is the modulation function on the illumination intensity.The second moment of modulated pixel intensity would be

$$\langle S_T^2 \rangle = \frac{1}{N}\sum_{i=1}^{N}(S_{i,T})^2 \tag{S3}$$

where $\langle\ \rangle$ denotes averaging and N is the number of averaged pixels. The definition of intensity autocorrelation function $g_2(\tau)$ is given by

$$g_2(t' - t'') = \frac{\langle I_i(t')I_i(t'')\rangle}{\langle I \rangle^2} \tag{S4}$$

where $\langle I \rangle$ is the average intensity of the intact speckle signal. Note that the autocorrelation function is an even function.

Based on Eq. S1 to S4, we can derive the expression of the second moment of modulated pixel intensity with respect to the intensity modulation function $m(t)$ and the intensity autocorrelation function $g_2(\tau)$ of the intact signal as follows:

$$\langle S_T^2 \rangle = \langle (S_{i,T})^2 \rangle$$

$\langle\ \rangle$ denotes averaging over independent instances

$$= \langle (\int_0^T I_i(t')m(t')dt')^2 \rangle$$

$$= \langle (\int_0^T I_i(t')m(t')dt')(\int_0^T I_i(t'')m(t'')dt'') \rangle$$

$$= \langle \int_0^T \int_0^T I_i(t')I_i(t'')m(t')m(t'')dt'dt'' \rangle$$

Since $m(t)$ is independent of i, we can write

$$= \int_0^T \int_0^T \langle I_i(t')I_i(t'')\rangle m(t')m(t'')dt'dt''$$

Considering Eq. S4, we have

$$= \langle I \rangle^2 \int_0^T \int_0^T g_2(t' - t'')m(t')m(t'')dt'dt''$$

Considering the symmetry of t' and t'' and that $g_2(\tau)$ is an even function, we can write

$$= 2\langle I \rangle^2 \int_0^T \int_0^{t'} g_2(t' - t'')m(t')m(t'')dt''dt'$$

Let $t' - t'' = \tau$, then $t'' = t' - \tau$, $dt'' = -d\tau$

$= 2\langle I\rangle^2 \int_0^T \int_0^{t'} g_2(\tau)m(t')m(t'-\tau)d\tau dt'$

By changing the order of the integral, we have

$= 2\langle I\rangle^2 \int_0^T \int_\tau^T g_2(\tau)m(t')m(t'-\tau)dt'd\tau$

$= 2\langle I\rangle^2 \int_0^T g_2(\tau)(\int_\tau^T m(t')m(t'-\tau)dt')d\tau$

Let $t = t' - \tau$, then $t' = t + \tau$, $dt' = dt$

$= 2\langle I\rangle^2 \int_0^T g_2(\tau)(\int_0^{T-\tau} m(t)m(t+\tau)dt)d\tau$

Define

$$M(\tau) = \int_0^{T-\tau} m(t)m(t+\tau)d\tau \tag{S5}$$

then

$$\langle S_T^2\rangle = 2\langle I\rangle^2 \int_0^T g_2(\tau)M(\tau)d\tau \tag{S6}$$

Since

$$K^2(T) = \frac{\mathrm{Var}\,(S_T)}{\langle S_T\rangle^2} = \frac{\langle S_T^2\rangle - \langle S_T\rangle^2}{\langle S_T\rangle^2} \tag{S7}$$

where $\langle S_T\rangle$ is the mean pixel intensity of modulated speckle signal within exposure time T and $\langle S_T\rangle = T\langle I_m\rangle$ where $\langle I_m\rangle$ is the mean intensity of the modulated speckle signal, we arrive at the expression of speckle contrast of the within-exposure modulated speckle signal (Eq. S8).

$$K^2 = \frac{2\langle I\rangle^2}{T^2\langle I_m\rangle^2} \int_0^T g_2(\tau)M(\tau)d\tau - 1 \tag{S8}$$

Notice that when the modulation function $m(t)$ is a constant 1, we have $M = T - \tau$ and Eq. S8 reduces to the classic expression of speckle contrast that is commonly seen. In other words, the classic expression of speckle contrast we use is a particular case of Eq. S8 when the illumination intensity is held constant. Finally, we would like to introduce one important observation about $M(\tau)$ (Lemma 1.1).

Lemma 1.1 (Integral property of $M(\tau)$)**.** *: In case of a continuous-time signal I(t) which fluctuates around the expectation value over time, i.e.* $\forall t > 0$, $\mathrm{E}\,[I(t)] = \langle I\rangle$, *(in other words, if* $I(t) = \langle I\rangle + \delta I(t)$ *where* $\delta I(t)$ *is the fluctuation part, then* $\mathrm{E}\,[\delta I(t)] = 0$*), and that* $\langle I_m\rangle$ *is the expectation of the average modulated signal* $I_m(t)$ *over the modulation period* T*, i.e.* $\langle I_m\rangle = \mathrm{E}\,[\frac{1}{T}\int_0^T I_m(t)dt]$*, then the integral of* $M(\tau)$ *satisfies* $\int_0^T M(\tau)d\tau = \frac{T^2\langle I_m\rangle^2}{2\langle I\rangle^2}$.

Proof. Because $I_m(t) = I(t)m(t)$ and $I(t) = \langle I\rangle + \delta I(t)$, we have

$$\begin{aligned}\langle I_m\rangle &= \mathrm{E}\,[\frac{1}{T}\int_0^T I_m(t)dt] \\ &= \mathrm{E}\,[\frac{1}{T}\int_0^T I(t)m(t)dt] \\ &= \mathrm{E}\,[\frac{1}{T}\int_0^T (\langle I\rangle + \delta I(t))m(t)dt] \\ &= \frac{\langle I\rangle}{T}\int_0^T m(t)dt + \mathrm{E}\,[\frac{1}{T}\int_0^T \delta I(t)m(t)dt]\end{aligned} \tag{S9}$$

Consider one example of $\delta I(t)$, i.e. $\delta_\omega I(t)$, $\omega \in \Omega$. If we define the probability density of $\delta_\omega I(t)$, $\omega \in \Omega$ as $p(\omega)$, then

$$\begin{aligned}\mathrm{E}\,[\frac{1}{T}\int_0^T \delta I(t)m(t)dt] &= \int_\Omega \frac{1}{T}\int_0^T \delta_\omega I(t)m(t)p(\omega)dtd\omega \\ &= \frac{1}{T}\int_0^T m(t)\int_\Omega \delta_\omega I(t)p(\omega)d\omega dt \\ &= \frac{1}{T}\int_0^T m(t)\,\mathrm{E}\,[\delta I(t)]dt\end{aligned} \tag{S10}$$

Since $\mathrm{E}\left[\delta I(t)\right] = 0$, we have $\mathrm{E}\left[\frac{1}{T}\int_0^T \delta I(t)m(t)dt\right] = 0$. Therefore, $\langle I_m \rangle = \frac{\langle I \rangle}{T}\int_0^T m(t)dt$, namely, $\int_0^T m(t)dt = \frac{T\langle I_m \rangle}{\langle I \rangle}$. Hence,

$$
\begin{aligned}
\int_0^T M(\tau)d\tau &= \int_0^T \int_0^{T-\tau} m(t)m(t+\tau)d\tau dt \\
&= \int_0^T m(t)\int_0^{T-t} m(t+\tau)d\tau dt \\
&\text{Let } t' = t+\tau \text{, then } dt' = d\tau \\
&= \int_0^T m(t)\int_t^T m(t')dt'dt \\
&= \int_0^T \int_t^T m(t)m(t')dt'dt \\
&\text{Considering the symmetry of } m(t) \text{ and } m(t')\text{, we have} \\
&= \frac{1}{2}\int_0^T \int_0^T m(t)m(t'')dt'dt \\
&= \frac{1}{2}(\int_0^T m(t))^2 \\
&\text{Since } \int_0^T m(t)dt = \frac{T\langle I_m \rangle}{\langle I \rangle}\text{, we have} \\
&= \frac{T^2\langle I_m \rangle^2}{2\langle I \rangle^2}
\end{aligned}
\tag{S11}
$$

The proof is over. □

Interpretation of Lemma 1.1: The integral of $M(\tau)$ is only dependent on $m(t)$. However, the integral of $M(\tau)$ can become equal to $\frac{T^2\langle I_m \rangle^2}{2\langle I \rangle^2}$ in cases of certain intensity signal where the continuous-time signal I(t) fluctuates around the expectation value over time, i.e. $\forall t > 0$, $\mathrm{E}\left[I(t)\right] = \langle I \rangle$.

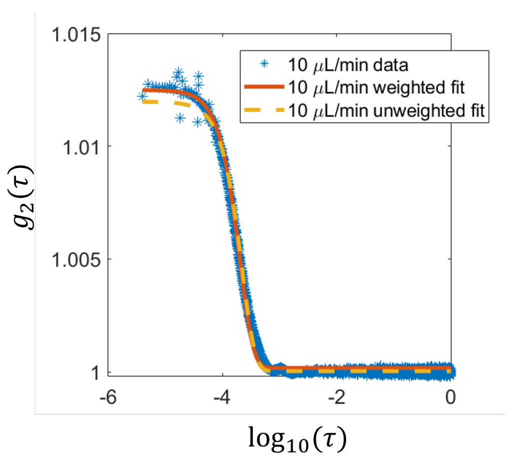

Fig. S1. Comparison of the performance of weighted fitting vs. unweighted fitting. The weighted fitting by $1/\tau$ improves the fitting performance in the head of $g_2(\tau)$ curve compared with unweighted fitting.

2. STATISTICAL INTERPRETATION OF 2-PULSE MODULATION: $K_{2P}^2(T) = \frac{1}{2}g_2(0) + \frac{1}{2}g_2(T) - 1$ IF $m(t) = \delta(0) + \delta(T)$

Proof. Denote $I(t)$ as I and $I(t+\tau)$ as I_τ, then according to $g_2(\tau) = \frac{\langle I(t)I(t+\tau)\rangle}{\langle I \rangle^2}$ we have

$$g_2(0) = \frac{\langle I^2 \rangle}{\langle I \rangle^2} \tag{S12}$$

and

$$g_2(\tau) = \frac{\langle I \cdot I_\tau \rangle}{\langle I \rangle^2} \tag{S13}$$

Since $\mathrm{Var}\,(I) = \langle I^2 \rangle - \langle I \rangle^2$ and $\mathrm{Cov}\,(I, I_\tau) = \langle I \cdot I_\tau \rangle - \langle I \rangle^2$ where $\mathrm{Var}\,(X)$ and $\mathrm{Cov}\,(X, Y)$ denote the variance of X, and the covariance between X and Y, we have

$$\begin{aligned} \frac{1}{2}g_2(0) + \frac{1}{2}g_2(\tau) - 1 &= \frac{1}{2}(\frac{\langle I^2 \rangle}{\langle I \rangle^2} - 1) + \frac{1}{2}(\frac{\langle I \cdot I_\tau \rangle}{\langle I \rangle^2} - 1) \\ &= \frac{\mathrm{Var}\,(I) + \mathrm{Cov}\,(I, I_\tau)}{2\langle I \rangle^2} \end{aligned} \tag{S14}$$

If $m(t) = \delta(0) + \delta(\tau)$, the pixel intensity S would be $S = I + I_\tau$ and $K^2_{2P}(\tau)$ would be

$$K^2_{2P}(\tau) = \frac{\mathrm{Var}\,(I + I_\tau)}{\langle I + I_\tau \rangle} = \frac{\mathrm{Var}\,(I + I_\tau)}{4\langle I \rangle^2} \tag{S15}$$

Therefore, to prove that $K^2_{2P}(\tau) = \frac{1}{2}g_2(0) + \frac{1}{2}g_2(\tau) - 1$, based on Eq. S14 and S15, one only needs to prove that $\mathrm{Var}\,(I + I_\tau) = 2\,\mathrm{Var}\,(I) + 2\,\mathrm{Cov}\,(I, I_\tau)$, which is true since $\mathrm{Var}\,(I + I_\tau) = \mathrm{Var}\,(I) + \mathrm{Var}\,(I_\tau) + 2\,\mathrm{Cov}\,(I, I_\tau)$ and $\mathrm{Var}\,(I) = \mathrm{Var}\,(I_\tau)$. The proof is over.

□

3. THE IMPACT OF NON-ZERO RESIDUAL ILLUMINATION

We can model the non-zero residual illumination in 2-pulse modulation as

$$m'(t) = (1 - r)m(t) + r \tag{S16}$$

where r is the relative amplitude of residual illumination during the off state and ranges from 0 to 1. $m(t)$ here is the ideal 2-pulse modulation with zero residual illumination, and ranges between 0 and 1. Then the modulation autocorrelation function would be

$$\begin{aligned} M'(\tau) &= \int_0^{T-\tau} m'(t)m'(t+\tau)dt \\ &\approx (1-r)^2 M(\tau) + (T-\tau)[2r(1-r)d + r^2] \end{aligned} \tag{S17}$$

where $M(\tau) = \int_0^{T-\tau} m(t)m(t+\tau)dt$ and d is the duty cycle of $m(t)$ or the pseudo duty cycle of $m'(t)$. Fig. S2 shows an example of how $M(\tau)$ would be skewed in presence of a non-zero residual illumination (r=0.1). The square of speckle contrast corresponding to $m'(t)$ would then become

$$\begin{aligned} \widetilde{K}^2(T) &= \frac{2\langle I \rangle^2}{T^2 \langle I_{m'} \rangle^2} \int_0^T g_2(\tau)M'(\tau)d\tau - 1 \\ &= \frac{2}{T^2[d + (1-d)r]^2} \int_0^T g_2(\tau)M'(\tau)d\tau - 1 \\ &= \frac{2}{T^2[d + (1-d)r]^2} \int_0^T g_2(\tau)[(1-r)^2 M(\tau) + (T-\tau)(2r(1-r)d + r^2)]d\tau - 1 \end{aligned} \tag{S18}$$

Simplify Eq. S18, we get

$$\widetilde{K}^2(T) = p\,K^2_m + (1-p)K^2_0 \tag{S19}$$

where $K^2_m = \frac{2\langle I \rangle^2}{T^2 \langle I_m \rangle^2} \int_0^T g_2(\tau)M(\tau)d\tau - 1$, $K^2_0 = \frac{2}{T^2}\int_0^T (T-\tau)g_2(\tau)d\tau - 1$, and $p = \frac{d^2(1-r)^2}{[r + d(1-r)]^2}$. Therefore, the square of speckle contrast, K^2 in presence of a non-zero residual illumination in 2-pulse modulation would be the weighted sum of that of an ideal 2-pulse modulation plus that of no modulation on intensity. p indicates the proportion of the contribution by the ideal 2-pulse modulation. It is noticed that when r increases, p drops and that when d increases, p rises. Fig. S3b and 5b shows examples of how an AOM with limited OD when gating the light would affect the tail of $K^2_{2P}(T)$ curves when T is large. One important observation is that when T changes, the parameter p changes as the duty cycle of each modulation waveform changes, which induces the downtick of $K^2_{2P}(T)$ curves in the tail. By equalizing the camera exposure time across exposures, the duty cycle is kept the same and therefore, p remains invariant and $(1-p)K^2_0$ becomes a constant across exposures, which flattens the tail.

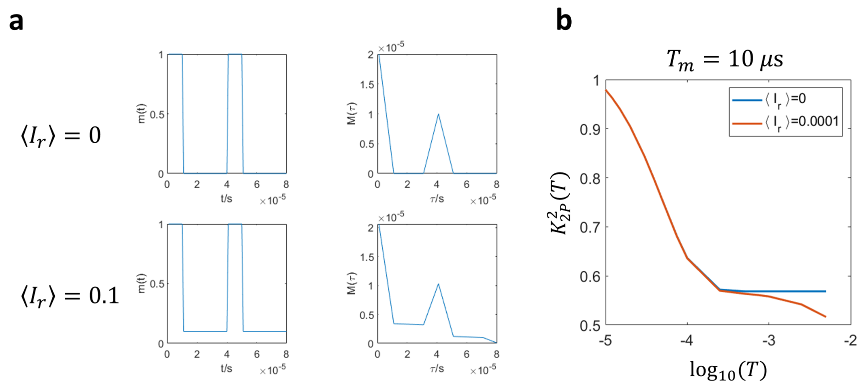

Fig. S2. The impact of non-zero residual illumination between two illumination pulses on $K^2_{2P}(T)$. **a** How the modulation autocorrelation function $M(\tau)$ would be skewed by a non-zero residual illumination (r=0.1). **b** The comparison of $K^2_{2P}(T)$ curves with and without residual illumination. An AOM with an OD of 4 when gating the light is simulated for the former case.

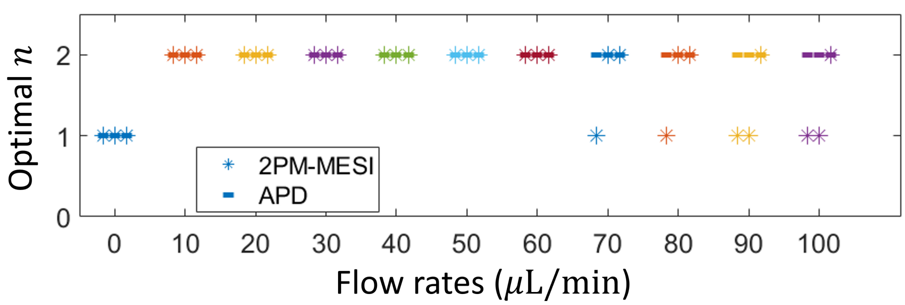

Fig. S3. The optimal n given by the fitting algorithm in various flow rates. Dashed line: APD. Asteroids: 2PM-MESI. For each flow rate, the experiment is repeated for five times. Three of the five repeats are shown here and grouped together by the same color in the plot. Different colors represent different flow rates. When the flow rate is zero, the optimal n is 1, which is true for both APD and 2PM-MESI fitting results. When the flow rate is not zero, the optimal n is 2 according to APD fitting results. 2PM-MESI identifies the same optimal n for small flow rates ($\leq$ 60 μL/min). But for higher flow rates, instability in estimating the optimal n is observed, which could be due to the downticking tail of the K^2_{2P} curve induced by the non-zero residual illumination between illumination pulses.

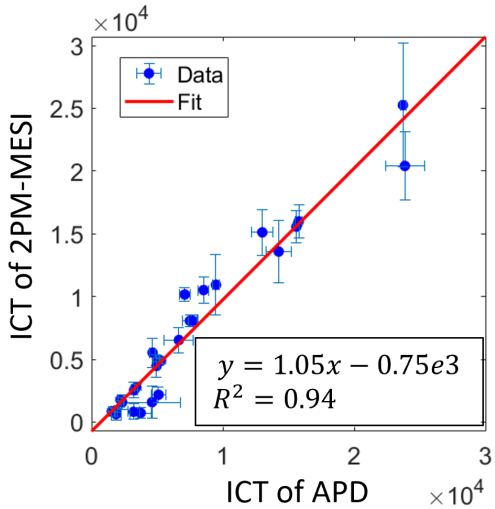

Fig. S4. Comparison of ICT values extracted from $g_2(\tau)$ and $K^2_{2P}(T)$ curves *in vivo* with unfixed n. 28 points from 4 mice.

4. THE IMPACT OF PULSE DURATION ON THE ACCURACY OF MEASURING ABSOLUTE AND RELATIVE VALUES OF $g_2(\tau)$

In this section, we would like to answer the question of how to choose the pulse duration when doing 2-pulse modulated multiple exposure imaging. We demonstrated the validity of a 10 μs pulse duration in extracting correlation times as short as 30 μs (Fig. 3f). But it does not have to be always the case. The pulse duration can be longer when measuring $g_2(\tau)$ of slowly varying signals. We examined the optimal pulse duration selection through numerical simulation. For a given pulse duration T_m, we evaluated the discrepancy between $g_2(\tau)$ and its estimation by $K^2_{2P}(T)$ at various correlation times (Fig. S5). For a given pulse duration, the maximum percent discrepancy between $2[K^2_{2P}(\tau) - C]$ and the absolute value of $g_2(\tau)$ decreases as τ_c increases (Fig. S5a). When τ_c becomes larger than 10 times T_m, the percentage discrepancy drops below 0.2%. In other words, to recover the absolute value of $g_2(\tau)$ of the signal of interest within a maximum of 0.2% discrepancy threshold, the pulse duration T_m should be made shorter than 10% of the correlation time τ_c of the signal. On the other hand, if the correlation time is the only interest about $g_2(\tau)$, i.e., the relative value of $g_2(\tau)$ or $\tilde{g}_2(\tau)$ is of interest, then the pulse duration can be longer than 10% of τ_c (Fig. S5b). But considering that 2-pulse modulated multiple exposure imaging can only capture $g_2(\tau)$'s shape in the range of $\tau \geq T_m$, it is recommended that T_m not be longer than τ_c to ensure sufficient sampling of the exponential-decay region of $g_2(\tau)$ curve.

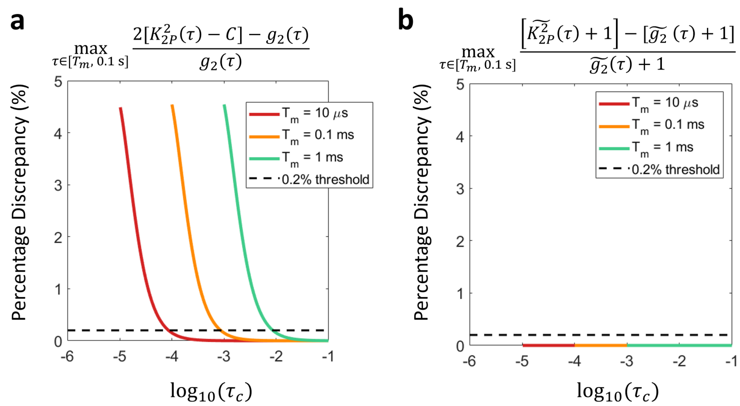

Fig. S5. The accuracy of estimating $g_2(\tau)$ and $\widetilde{g}_2(\tau)$ based on $K_{2P}^2(T)$ for signals of different correlation times. **a** The maximum percentage discrepancy between absolute $g_2(\tau)$ and that estimated by $K_{2P}^2(T)$. The y-axis is $\max_{\tau \in [T_m, 0.1\ s]} \frac{2[K_{2P}^2(\tau) - C] - g_2(\tau)}{g_2(\tau)}$ /%. **b** The maximum percentage discrepancy between normalized $g_2(\tau)$ and $K_{2P}^2(\tau)$. The y-axis is $\max_{\tau \in [T_m, 0.1\ s]} \frac{[\widetilde{K_{2P}^2}(\tau)+1] - [\widetilde{g}_2(\tau)+1]}{\widetilde{g}_2(\tau)+1}$ /%.