# Supplementary Material

April 22, 2022

## 1 Introduction

This supplementary note contains details of the delta method calculations (Section 2) and justification for the optimal cone half-angle (Section 3).

## 2 Delta Method Calculations

For the reader's convenience, we begin by recalling some terminology and definitions.

### 2.1 Definitions

Suppose $X$ and $Y$ are Fourier transforms of two independent (gold standard) reconstructions of the same particle. In the Fourier domain, let $S(\omega)$ be a shell of radius $\omega$ and thickness $\Delta\omega$, let $d$ be a direction (a half-infinite ray from the origin), and let $C_d$ be a solid cone with half angle $\Omega$ with $d$ as its axis (figure 1a, main manuscript).

Set $D = S(\omega) \cap C_d$, then the *directional FSC* in $S(\omega)$, along the direction $d$ is

$$dFSC(\omega, d) \quad = \quad \frac{\int_D w(u)\mathrm{Re}\left(X^*(u)Y(u)\right)du}{\sqrt{\int_D w(u)X^*(u)X(u)du}\sqrt{\int_D w(u)Y^*(u)Y(u)du}}, \tag{1}$$

where Re() is the real part of the complex number enclosed in the bracket, the superscript $^*$ is the complex conjugate, and $w(u)$ is a real-valued "weight"function $w : D \to R$ satisfying:

1. $w$ is non-negative, continuous, and bounded.
2. $\int_D w(u)du = 1$.

For numerical calculation, the integrals in equation (1) are approximated by Riemann sums. To do this, assume that $\{u\}$ are a set of $N$ points contained in $D$ and uniformly spaced in a spherical grid. Let $\Delta u$ be the volume associated with each grid unit,

$$\Delta u = \frac{\mathrm{vol}(D)}{N},$$

where $\mathrm{vol}(D)$ is the volume of $D$. Then,

$$dFSC(\omega, d) \quad \simeq \quad \frac{\sum_u w(u)\mathrm{Re}\left(X^*(u)Y(u)\Delta u\right)}{\sqrt{\sum_u w(u)X^*(u)X(u)\Delta u}\sqrt{\sum_u w(u)Y^*(u)Y(u)\Delta u}}.$$

To simplify the notation, let $j = 1, \cdots, N$ be an index for the $u's$, and let $x_j$ and $y_j$ denote the values of the Fourier transforms $X$ and $Y$ at $u_j$. Similarly let $w_j$ denote the value of of $w(u)\Delta u$ at $u_j$. Then,

$$dFSC(\omega, d) = \frac{\sum_{j=1}^{N} w_j \text{Re}\left(x_j^* y_j\right)}{\sqrt{\sum_{j=1}^{N} w_j x_j^* x_j}\sqrt{\sum_{j=1}^{N} w_j y_j^* y_j}}. \tag{2}$$

Because the Fourier transforms $X, Y$ are noisy, $dFSC(\omega, d)$ is a random variable. We want to understand the probability of the event $dFSC(w, d) > c$, where $c$ is equal to the global FSC in the shell $S(\omega)$. An exact analysis of this is complicated. Instead we provide a good approximation via asymptotic analysis as $N$, the number of $u$'s in the Riemann sum, becomes large. The asymptotic analysis is carried out using the Central Limit Theorem and the Delta Method.

Here is a list of all the assumptions we make:

1. As mentioned above, $N$, the number of $u$'s in the Riemann sum, is large.
2. The half angle of the cone is small enough so that the signal in the Fourier transform is approximately constant for all $u \in S(\omega) \cap C_d$.
3. The noise is white and normal $N(0, \sigma^2)$.
4. The function $w(u)$ is uniform, so that $w_j = \frac{1}{N}$, This assumption along with assumptions 2 and 3 above simplify the calculations. This assumption can be relaxed to allow for a more general weighting function $w$, and essentially the same result can be derived. However, the calculations are more complex, and do not provide any additional insight.

Assumptions 2 and 3 above imply that

$$x_j = \theta + \epsilon_j, \; y_j = \theta + \delta_j \text{ for } j = 1, \cdots, n, \tag{3}$$

where $\theta$ is the complex signal and $\epsilon_j$ and $\delta_j$ are complex random variables representing noise, each of whose real and imaginary parts are iid with distribution $N(0, \sigma^2)$. Note that the signal-to-noise (SNR) is $\mid \theta \mid^2 / 2\sigma^2$. If the particle is isotropic, then this is also the global signal-to-noise ratio. The global FSC is $\mid \theta \mid^2 / (\mid \theta \mid^2 + 2\sigma^2)$.

**Main result:** The main result we wish to establish is that, under the above assumptions,

$$\text{Prob. of } dFSC(\omega, d) \geq c \sim \begin{cases} 0 & \text{if } c > \frac{|\theta|^2}{|\theta|^2 + 2\sigma^2}, \\ 0.5 & \text{if } c = \frac{|\theta|^2}{|\theta|^2 + 2\sigma^2}, \\ 1 & \text{if } c < \frac{|\theta|^2}{|\theta|^2 + 2\sigma^2}. \end{cases} \tag{4}$$

In other words, the probability of $dFSC(\omega, d) \geq c$ looks approximately like a step-function of $c$, with the step occuring when $c$ equals the global FSC of the shell $S(\omega)$.

## 2.2 Background

To establish the above result, we need some background.

### 2.2.1 Normal Distributions

We need the following elementary facts about normal distributions:

1. Every central moment of a normal distribution is finite.
2. All odd central moments of a normal distribution are zero.

### 2.2.2 The Central Limit Theorem

We need the multivariate version of the Central Limit Theorem.

**Central Limit Theorem (CLT):** Let $\{x_j,\ j = 1, \cdots, n\}$ be a sequence of identical independent random variables with finite mean $\mu$ and a covariance matrix $\Sigma$. Let $\bar{x}_n = \frac{1}{n}\sum_{i=1}^{n} x_i$, then

$$\sqrt{n}(\bar{x}_n - \mu) \rightsquigarrow N(0, \Sigma).$$

The symbol $\rightsquigarrow$ means "convergence in distribution", i.e. the random variable on the left hand side of the symbol has a probability distribution which converges to the probability distribution on the right hand side of the symbol as $n \to \infty$.

We will find it useful to write the CLT in a modified form:

$$\bar{x}_n \rightsquigarrow N(\mu, \frac{1}{n}\Sigma).$$

### 2.2.3 The Multivariate Delta Method

We also need the multivariate delta method.

**Multivariate Delta Method:** Suppose $x_n,\ n = 1, \cdots$ is a sequence of $k-$ dim vector random variables such that

$$\sqrt{n}(x_n - \mu) \rightsquigarrow N(0, \Sigma).$$

Also suppose that $g : R^k \to R^m$, $m \leq k$ is a continuously differentiable function. Then, according to the Delta Method

$$\sqrt{n}(g(x_n) - g(\mu)) \rightsquigarrow N(0, \nabla^T g(\mu)\Sigma\nabla g(\mu)),$$

where $\nabla g(\mu)$ is the $k \times m$ matrix of partial derivatives $\left[\frac{\partial g_j}{\partial x_i}\right]_{ij}$ evaluated at $\mu$.

As we did for the CLT, we will use an alternate form

$$g(x_n) \sim N(g(\mu), \frac{1}{n}\nabla^T g(\mu)\Sigma\nabla g(\mu)).$$

## 2.3 Establishing the result

To establish the main result, we begin by considering terms that occur in the sums of the numerator and denominator of the dFSC. Let :

$$\begin{aligned}
a_j &= \mathrm{Re}(x_j^* y_j), \\
b_j &= x_j^* x_j, \\
c_j &= y_j^* y_j, \\
x_j &= \begin{bmatrix} a_j \\ b_j \\ c_j \end{bmatrix}.
\end{aligned}$$

Then,

$$
\begin{aligned}
E[x_i] &= \begin{bmatrix} E[a_j] \\ E[b_j] \\ E[c_j] \end{bmatrix} = \begin{bmatrix} \mid \theta \mid^2 \\ \mid \theta \mid^2 + 2\sigma^2 \\ \mid \theta \mid^2 + 2\sigma^2 \end{bmatrix} = \mu, \\
V[x_i] &= \text{Cov. of } x_i = \Sigma \\
&= \begin{bmatrix} 2 \mid \theta \mid^2 \sigma^2 + 2\sigma^4 & 2 \mid \theta \mid^2 \sigma^2 & 2 \mid \theta \mid^2 \sigma^2 \\ 2 \mid \theta \mid^2 \sigma^2 & 4 \mid \theta \mid^2 \sigma^4 + 4\sigma^4 & 0 \\ 2 \mid \theta \mid^2 \sigma^2 & 0 & 4 \mid \theta \mid^2 \sigma^4 + 4\sigma^4 \end{bmatrix}.
\end{aligned}
$$

Section 2.4 below derives these formulae.

Note that the mean and covariance given above are independent of $i$. In fact, $x_1, \cdots, x_N$ are i.i.d. Applying CLT to $x_1, \cdots, x_N$ we get

$$\sqrt{n}(\bar{x}_N - \mu) \rightsquigarrow N(0, \Sigma),$$

where,

$$\bar{x}_N = \frac{1}{N} \sum_{j=1}^{N} x_j = \begin{bmatrix} \frac{1}{N} \sum_{j=1}^{N} \mathrm{Re}(x_j^* y_j) \\ \frac{1}{N} \sum_{j=1}^{N} x_j^* x_j \\ \frac{1}{N} \sum_{j=1}^{N} y_j^* y_j \end{bmatrix}.$$

Next, we use the Delta Method. For this, define $g : R^3 \to R^2$ by $g(a, b, c) = (a, \sqrt{bc})^T$. Then

$$\nabla g(a, b, c) = \begin{bmatrix} 1 & 0 \\ 0 & \frac{1}{2} \frac{c}{\sqrt{bc}} \\ 0 & \frac{1}{2} \frac{b}{\sqrt{bc}} \end{bmatrix},$$

which is finite as long as neither $b$ nor $c$ is zero.

Applying the Delta Method to $\bar{x}_N$, we get

$$g(\bar{x}_N) \sim N(g(\mu), \frac{1}{N} \nabla^T g(\mu) \Sigma \nabla g(\mu)), \tag{5}$$

where, $\Sigma_g = \frac{1}{N} \nabla^T g(\mu) \Sigma \nabla g(\mu)$, and

$$
\begin{aligned}
g(\mu) &= (\mid \theta \mid^2, \mid \theta \mid^2 + 2\sigma^2)^T, \\
\Sigma_g &= \frac{1}{N} \nabla^T g(\mu) \Sigma \nabla g(\mu) \\
&= \frac{1}{N} \begin{pmatrix} 2 \mid \theta \mid^2 \sigma^2 + 4\sigma^4 & 2 \mid \theta \mid^2 \sigma^2 \\ 2 \mid \theta \mid^2 \sigma^2 & 2 \mid \theta \mid^2 \sigma^2 + 4\sigma^4 \end{pmatrix} \\
&= \frac{2\sigma^2}{N} (\mid \theta \mid^2 + 2\sigma^2) \begin{pmatrix} 1 & \frac{|\theta|^2}{|\theta|^2 + 2\sigma^2} \\ \frac{|\theta|^2}{|\theta|^2 + 2\sigma^2} & 1 \end{pmatrix}.
\end{aligned}
$$

The covariance matrix has eigenvalues

$$\frac{2\sigma^2}{N} (\mid \theta \mid^2 + 2\sigma^2) \left( 1 \pm \frac{\mid \theta \mid^2}{\mid \theta \mid^2 + 2\sigma^2} \right)$$

and eigenvectors $(1, 1)^T$ and $(1, -1)^T$. Note that as $N \to \infty$ the eigenvalues approach 0. That is, the 2-dim distribution of $g(\bar{x}_N)$ becomes highly concentrated at its mean.

The situation is illustrated in Figure 1 which shows the normal approximation of the distribution of $g(\hat{x}_N)$. For further discussion, it will be useful to explicitly refer to the first and second components of $g(\hat{x}_N)$ as $g_1$ and $g_2$.

Note that equation (5) is only an approximation (the support of the normal distribution on the right hand side of equation (5) is all of $R^2$, whereas we know that $g_2$ cannot be negative).

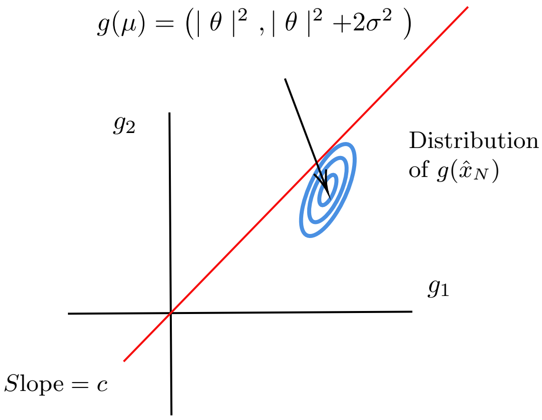


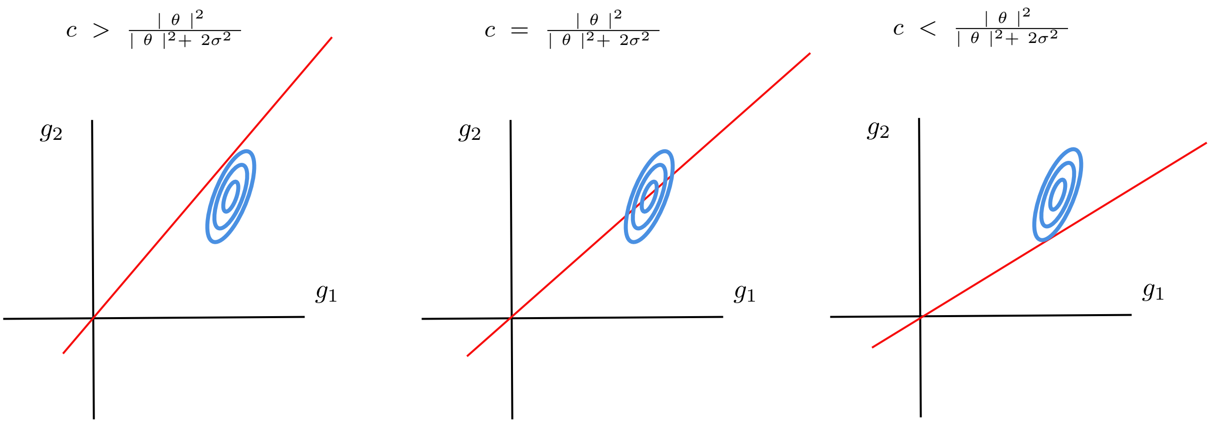

Figure 1: The distribution of $g(\hat{x}_N)$ using the delta method.

Now, $dFSC(\omega, d) = g_1/g_2$, and the condition $dFSC(\omega, d) \geq c$ $(c > 0)$ is equivalent to $g_1 \geq cg_2$. Figure 1 shows a line (in red) with slope $c$. The line divides $R^2$ in two half-planes. When $g_2 > 0$ and the distribution of $g(\hat{x}_N)$ is highly concentrated near the mean, the probability of $FSC(\omega, d) \geq c$ is closely approximated by the probability that $g(\hat{x}_N)$ is in the lower half-plane of the division of $R^2$ by the line.

Thus we are led to three situations (see Figure 2). These three situations arise after noting that the line through the origin passing through the mean of the normal approximation has a slope $\frac{|\theta|^2}{|\theta|^2+2\sigma^2}$. Thus: (1) The slope $c > \frac{|\theta|^2}{|\theta|^2+2\sigma^2}$. Then the distribution of $g(\hat{x}_N)$ is almost completely contained in the lower-half plane (Figure 2a) and the probability of $FSC(\omega, d) \geq c$ is approximately 1. (2) The slope $c = \frac{|\theta|^2}{|\theta|^2+2\sigma^2}$. Then the symmetry of the normal distribution implies that exactly half of the probability mass is above the line (Figure 2b), and half is below the line. Thus the probability of $FSC(\omega, d) \geq c$ is 0.5. (3) The slope $c < \frac{|\theta|^2}{|\theta|^2+2\sigma^2}$. Then the distribution of $g(\hat{x}_N)$ is almost completely contained in the upper-half plane (Figure 2c) and the probability of $FSC(\omega, d) \geq c$ is approximately 0.

Thus, we arrive at the main result:

$$\text{Prob. } dFSC(\omega, d) \geq c \sim \left\{ \begin{array}{cl} 1 & \text{if } c > \frac{|\theta|^2}{|\theta|^2+2\sigma^2}, \\ 0.5 & \text{if } c = \frac{|\theta|^2}{|\theta|^2+2\sigma^2}, \\ 0 & \text{if } c < \frac{|\theta|^2}{|\theta|^2+2\sigma^2}. \end{array} \right\}.$$




(a) (b) (c)

Figure 2: The three conditions for approximating Prob. $FSC(\omega, d)$.

## 2.4 Calculations

This section contains the details of all the calculations.

## 2.5 Moments of $a_j, b_j, c_j$

Our first task is to calculate moments of $a_j, b_j, c_j$. Terms such as $E[(\epsilon_j^r)^{k_1}, (\epsilon_j^i)^{k_2}(\delta_j^r)^{k_3}, (\delta_j^i)^{k_4}]$ will occur repeatedly in these calculation. Because $\epsilon_j^r, \epsilon_j^i, \delta_j^r, \delta_j^i$ are i.i.d. $N(0, \sigma^2)$, such terms are zero if any of $k_1, k_2, k_3, k_4$ is an odd integer. This simplifies the calculations considerably.

We start with $a_j$:

$$\begin{aligned} a_j &= \mathrm{Re}(x_j^* y_j) = \mathrm{Re}\left((\theta + \epsilon_j)^*(\theta + \delta_j)\right) \\ &= (\theta^r)^2 + \theta^r(\epsilon_j^r + \delta_j^r) + \epsilon_j^r \delta_j^r + (\theta^i)^2 + \theta^i(\epsilon_j^i + \delta_j^i) + \epsilon_j^i \delta_j^i \\ &= |\theta|^2 + \theta^r(\epsilon_j^r + \delta_j^r) + \theta^i(\epsilon_j^i + \delta_j^i) + \epsilon_j^r \delta_j^r + \epsilon_j^i \delta_j^i. \end{aligned}$$

Thus,

$$\begin{aligned} E[a_j] &= E\left[|\theta|^2 + \theta^r(\epsilon_j^r + \delta_j^r) + \theta^i(\epsilon_j^i + \delta_j^i) + \epsilon_j^r \delta_j^r + \epsilon_j^i \delta_j^i\right] = |\theta|^2, \\ V[a_j] &= E[(a_j - E[a_j])^2] \\ &= E\left[\left(\theta^r(\epsilon_j^r + \delta_j^r) + \theta^i(\epsilon_j^i + \delta_j^i) + \epsilon_j^r \delta_j^r + \epsilon_j^i \delta_j^i\right)^2\right] \\ &= E\left[\left(\theta^r(\epsilon_j^r + \delta_j^r) + \theta^i(\epsilon_j^i + \delta_j^i) + \epsilon_j^r \delta_j^r + \epsilon_j^i \delta_j^i\right) \times \left(\theta^r(\epsilon_j^r + \delta_j^r) + \theta^i(\epsilon_j^i + \delta_j^i) + \epsilon_j^r \delta_j^r + \epsilon_j^i \delta_j^i\right)\right] \\ &= E\left[(\theta^r)^2((\epsilon_j^r)^2 + (\delta_j^r)^2) + (\theta^i)^2((\epsilon_j^i)^2 + (\delta_j^i)^2) + (\epsilon_j^r \delta_j^r)^2 + (\epsilon_j^i \delta_j^i)^2\right] \text{ (drop odd powers)} \\ &= (\theta^r)^2(\sigma^2 + \sigma^2) + (\theta^i)^2(\sigma^2 + \sigma^2) + \sigma^4 + \sigma^4 \\ &= 2|\theta|^2 \sigma^2 + 2\sigma^4. \end{aligned}$$

Next we calculate the moments of $b_j$ (the moments of $c_j$ are identical):

$$\begin{aligned} b_j &= x_j^* x_j = (\theta^r + \epsilon_j^r)^2 + (\theta^i + \epsilon_j^i)^2 = (\theta^r)^2 + 2\theta^r \epsilon_j^r + (\epsilon_j^r)^2 + (\theta^i)^2 + 2\theta^i \epsilon_j^i + (\epsilon_j^i)^2 \\ &= |\theta|^2 + 2\theta^r \epsilon_j^r + 2\theta^i \epsilon_j^i + (\epsilon_j^r)^2 + (\epsilon_j^i)^2. \end{aligned}$$

Thus,

$$\begin{aligned}
E[b_j] &= E\left[\mid \theta \mid^2 + 2\theta^r\epsilon_j^r + 2\theta^i\epsilon_j^i + (\epsilon_j^r)^2 + (\epsilon_j^i)^2\right] = \mid \theta \mid^2 + 2\sigma^2. \\
V[b_j] &= E\left[(b_j - E[b_j])^2)^2\right] \\
&= E\left[\left(2\theta^r\epsilon_j^r + 2\theta^i\epsilon_j^i + (\epsilon_j^r)^2 + (\epsilon_j^i)^2 - 2\sigma^2\right) \times \left(2\theta^r\epsilon_j^r + 2\theta^i\epsilon_j^i + (\epsilon_j^r)^2 + (\epsilon_j^i)^2 - 2\sigma^2\right)\right] \\
&= E\left[4(\theta^r)^2(\epsilon_j^r)^2 + 4(\theta^i)^2(\epsilon_j^i)^2 + (\epsilon_j^r)^4 + (\epsilon_j^r)^2(\epsilon_j^i)^2 - 2\sigma^2(\epsilon_j^r)^2 + (\epsilon_j^i)^4 + (\epsilon_j^i)^2(\epsilon_j^r)^2 - 2\sigma^2(\epsilon_j^i)^2\right. \\
&\quad \left. -2\sigma^2(\epsilon_j^r)^2 - 2\sigma^2(\epsilon_j^i)^2 + 4\sigma^4\right] \\
&= 4(\theta^r)^2\sigma^2 + 4(\theta^i)^2\sigma^2 + 3\sigma^4 + \sigma^4 - 2\sigma^4 + 3\sigma^4 + \sigma^4 - 2\sigma^4 - 2\sigma^4 - 2\sigma^4 + 4\sigma^4 \\
&= 4 \mid \theta \mid^2 \sigma^4 + 4\sigma^4.
\end{aligned}$$

Finally, we calculate the correlation

$$\begin{aligned}
& E\left[(a_j - E[a_j])(b_j - E[b_j])\right] \\
=\ & E\left[\left(\theta^r(\epsilon_j^r + \delta_j^r) + \theta^i(\epsilon_j^i + \delta_j^i) + \epsilon_j^r\delta_j^r + \epsilon_j^i\delta_j^i\right) \times \left(2\theta^r\epsilon_j^r + 2\theta^i\epsilon_j^i + (\epsilon_j^r)^2 + (\epsilon_j^i)^2 - 2\sigma^2\right)\right] \\
=\ & E\left[2(\theta^r)^2(\epsilon_j^r)^2 + 2(\theta^i)^2(\epsilon_j^i)^2\right] \\
=\ & 2 \mid \theta \mid^2 \sigma^2, \text{ and}
\end{aligned}$$

$$E\left[(a_j - E[a_j])(b_j - E[b_j])\right] = 0 \text{ because } b_i \text{ and } c_i \text{ are independent.}$$

Therefore, if $x_i = [a_i\ b_i\ c_i]^T$, then

$$\begin{aligned}
E[x_i] &= \begin{bmatrix} \mid \theta \mid^2 \\ \mid \theta \mid^2 + 2\sigma^2 \\ \mid \theta \mid^2 + 2\sigma^2 \end{bmatrix}, \text{ and} \\
V[x_i] &= \text{Cov. of } x_i = \Sigma \\
&= \begin{bmatrix} 2 \mid \theta \mid^2 \sigma^2 + 2\sigma^4 & 2 \mid \theta \mid^2 \sigma^2 & 2 \mid \theta \mid^2 \sigma^2 \\ 2 \mid \theta \mid^2 \sigma^2 & 4 \mid \theta \mid^2 \sigma^4 + 4\sigma^4 & 0 \\ 2 \mid \theta \mid^2 \sigma^2 & 0 & 4 \mid \theta \mid^2 \sigma^4 + 4\sigma^4 \end{bmatrix}.
\end{aligned}$$

## 3 Optimal Cone Angle

We now turn to describing the analysis that results in finding an optimal cone angle. Recall that the cone angle $\Omega$ is measured from the cone axis. Also, recall that the weight function $w(u) = e^{\frac{-(\cos\theta - 1)^2}{\sigma^2}}$, where $\theta$ is the angle between $u$ and the cone axis $d$, and $\sigma$ is set to $\sigma = (1 - \cos\Omega)/4$ where $\Omega$ is the cone half-angle as defined above. For this value of $\sigma$ the weight function is essentially zero on the boundary of the cone.

Changing the cone angle $\Omega$ changes the weight function causing more, or less, averaging in the numerator and denominator terms of dFSC. To understand the effect of the cone angle, suppose we have two half volumes, each with a fringe pattern of frequency $\omega_0$ with additive independent white noise. Using a small cone angle will a focused dFSC along the $\omega_0$ direction, but dFSC in other directions will be non-negligible because of noise. Using a large cone angle will suppress the dFSC values from noise, but will spread the dFSC values caused by the fringe pattern to neighboring directions. This suggests that the optimal cone angle is one where the dispersion (width) of the dFSC response is as focused as possible around $\omega_0$.

To operationalize the above idea, define the dispersion of the dFSC around $\omega_0$ as

$$\sigma_{dFSC} = \int | \left(1 - \frac{u^\dagger \omega_0}{|u||\omega_0|}\right) dFSC(|\ \omega_o\ |, \omega_0/\ |\ \omega_0\ |)| du, \tag{6}$$

where, $u$ is a point in the shell $S(|\ \theta_o\ |)$ (which contains $\theta_0$) and $dFSC(|\ \omega_o\ |, \omega_0/\ |\ \omega_0\ |)$ is the directional FSC in the intersection of $S(|\ \theta_o\ |)$ and the cone with axis $\omega_0/\ |\ \omega_0\ |$. The the optimium cone angle is the one that minimizes $\sigma_{dFSC}$. Note that the function $\left(1 - \frac{u^\dagger \omega_0}{|u||\omega_0|}\right)$ takes a value of 0 on the cone axis and increases its value away from the axis.

To find the optimal cone angle, we simulated a fringe pattern with wavelength of 5 pixels in a box of $256 \times 256 \times 256$ pixels as signal in both half-volumes. White noise was added to both half-volumes. The standard deviation of the noise was set such that FSC=0.143 corresponded with the shell containing the peak amplitude of the signal. The numerically evaluated $\sigma_{dFSC}$ is plotted in Fig. 3(a). The minimum dispersion is achieved when the extension of the filter is $\theta_0 = 17^o$. To analyze the stability of this minimum, the experiment was repeated 100 times with different realizations of noise, thus the error bars in Fig. 3(a) were calculated and represent one standard deviation.

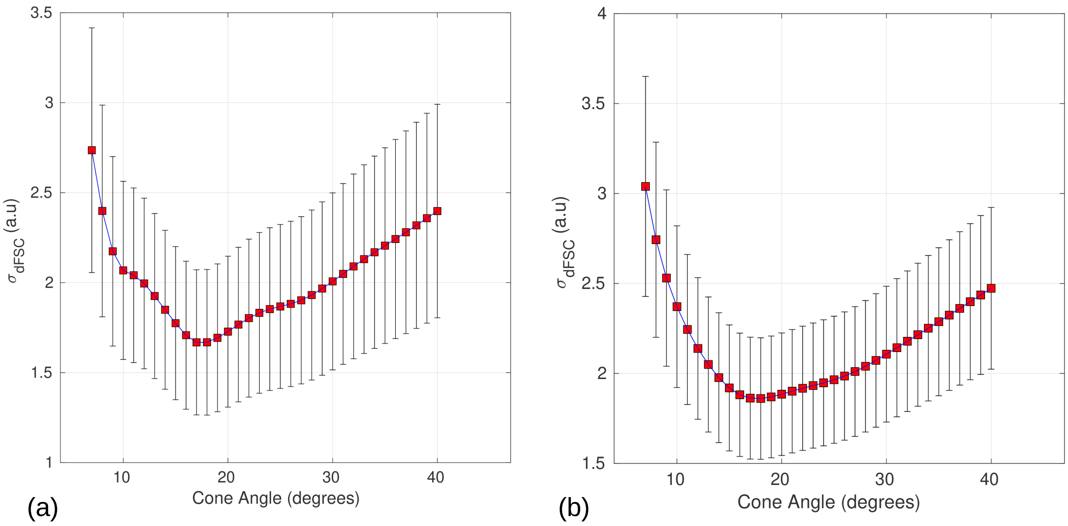



Figure 3: **Dispersion as a function of cone angle**. The dispersion function $\sigma_{dFSC}(\theta_0)$ is obtained by using two noisy fringe patterns such as the FSC=0.143 at 5Å. (a) $\sigma_{dFSC}$ obtained using fringe pattern, (b) $\sigma_{dFSC}$ obtained using the mask of the Human CMG bound to AND-1 (EMDB-10621).

To determine whether realistic protein shape had any effect on the result, the experiment was repeated applying masks to the 70S Ribosome (EMD-2275), Human CMG bound to AND-1 (EMDB-10621) and the T20S proteasome (EMD-6287). In all cases the results are similar and quite stable,all obtaining minimum dispersion around $17^o - 18^o$. Fig. 3(b) shows $\sigma_{dFSC}$ for the case of the Human CMG.

The ultimate use of dFSC is in calculating the FSO. Keeping this in mind, we evaluated the sensitivity of FSO to the cone angle. To analyze this sensitivity, we calclulated the FSO for different cone angles in the range 3-21 degrees in steps of 2 degrees. Fig. 4 shows the results of this experiment for the structure of human CMG bound to AND-1 (EMD-10621). Similar results were obtained for other structures that we experimented with. The results in Fig. 4 show that the FSO ($\chi = 0.143$) is relatively insensitive with the cone angle. This fact is demonstrated by the overlapping of the FSO curves in Fig. 4, with the exception of the FSO curves for angles lesser than $10^{\text{o}}$. Figure 4 also shows that the frequency at which FSO=0.5 is quite insensitive to the cone angle, further validating the delta method results.

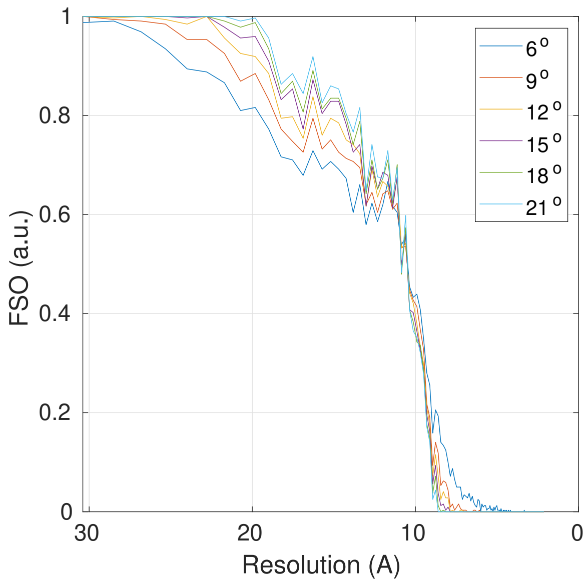



Figure 4: **Effect of the cone angle on the FSO**