SUPPLEMENTAL MATERIAL: Blind Deconvolution of Widefield Fluorescence Microscopic Data by Regularization of the Optical Transfer Function (OTF)

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1. TV Regularization in the Frequency Domain

For widefield recordings, we know that the PSF is not smooth, such that a regularization of the kernel by imposing smoothness is not reasonable. In contrast, the OTF $\mathcal{F}(h)$ has a well defined support region and is smooth inside this region. To develop a regularizer that acts on the OTF, we decompose the OTF into its amplitude and phase:

$$\mathcal{F}(h) = \underbrace{|\mathcal{F}(h)|}_{\text{amplitude}} \cdot \underbrace{\frac{\mathcal{F}(h)}{|\mathcal{F}(h)|}}_{e^{\imath\phi}} \tag{1}$$

where $\imath$ is the imaginary unit, and penalize the variation of the amplitude:

$$P_h(h) = \int \big\|\nabla|\mathcal{F}(h)|(\xi)\big\| d\xi. \tag{2}$$

The optimum of this TV energy, a constant amplitude of the OTF, does not account for the fact that the support of the OTF is limited. We therefore enforce a limited support by shrinking all values outside the support region to zero [1]. To make sure that valid frequencies are not cut off, we compute the largest theoretically possible OTF support for a widefield microscope [2]. Values outside this support are set to zero in every iteration of the deconvolution update.
For minimizing the resulting functional with TV regularization of the kernel in the frequency domain (KFTV), we compute the gradient with the calculus of variations, resulting in

$$\begin{aligned}\left(\left(\tfrac{\partial}{\partial h}P_h\right)\left(\hat{h}_k\right)\right)(\mathbf{x}) = \\ -\mathcal{F}^{-1}\left(\operatorname{div}\left(\frac{\nabla|\mathcal{F}(\hat{h}_k)|}{\big\|\nabla|\mathcal{F}(\hat{h}_k)|\big\|}\right)\cdot\frac{\mathcal{F}(\hat{h}_k)}{|\mathcal{F}(\hat{h}_k)|}\right)(\mathbf{x}).\end{aligned} \tag{3}$$

This gradient can be used in the multiplicative update for the deconvolution kernel. Here, $\mathcal{F}(h)^*$ denotes the complex conjugate of $\mathcal{F}(h)$. The detailed derivation of the gradient is given in the following.

2. Deriving the TV regularization of the frequency space magnitudes

For the computation of the gradient of the TV regularization of the OTF magnitudes, we first show the following

Lemma 1. *Given two square-integrable functions $A : \mathbb{R} \to \mathbb{C} : \xi \mapsto A(\xi)$ and $B : \mathbb{R} \to \mathbb{C}$. Then*

$$\int_{\mathbb{R}} A \frac{d}{d\xi} B \, d\xi = -\int_{\mathbb{R}} \frac{d}{d\xi} AB \, d\xi. \tag{4}$$

Proof. We rewrite the functions as Fourier integrals:

$$\int_{\mathbb{R}} A\frac{d}{d\xi}B\,d\xi = \int_{\mathbb{R}}\int_{\mathbb{R}} a(x_1)e^{-i\xi x_1}dx_1 \cdot \frac{d}{d\xi}\int_{\mathbb{R}} b(x_2)e^{-i\xi x_2}dx_2\,d\xi \tag{5}$$

$$= \underbrace{\int_{\mathbb{R}}\int_{\mathbb{R}} a(x_1)e^{-i\xi x_1}dx_1 \cdot \int_{\mathbb{R}}(-ix_2)\cdot b(x_2)e^{-i\xi x_2}dx_2\,d\xi}_{\neq 0 \text{ iff } x_2=-x_1} \tag{6}$$

$$= -\int_{\mathbb{R}}\int_{\mathbb{R}}(-ix_1)a(x_1)e^{-i\xi x_1}dx_1 \cdot \int_{\mathbb{R}} b(x_2)e^{-i\xi x_2}dx_2\,d\xi \tag{7}$$

$$= -\int_{\mathbb{R}}\frac{d}{d\xi}\int_{\mathbb{R}} a(x_1)e^{-i\xi x_1}dx_1 \cdot \int_{\mathbb{R}} b(x_2)e^{-i\xi x_2}dx_2\,d\xi \tag{8}$$

$$= -\int_{\mathbb{R}}\frac{d}{d\xi}AB\,d\xi\,. \tag{9}$$

□

Lemma 1 generalizes to n-dimensional functions, as

$$\int_{\mathbb{R}^n} A\nabla B\,d\xi = -\int_{\mathbb{R}^n}\nabla AB\,d\xi\,. \tag{10}$$

The energy formulated in section 1 for the frequency regularized blind deconvolution is

$$J_{\text{KFTV}}(s,h) = J_{\text{MLEM}}(s,h) + \lambda_h\int_{\mathbb{R}^3}\big\|\nabla|\mathcal{F}(h)|(\boldsymbol{\xi})\big\|d\boldsymbol{\xi}. \tag{11}$$

The minimization of this functional is done using the calculus of variations. Since the Fourier transform is linear, a variation in the spatial domain is equivalent to a variation in the Fourier domain.

$$\int_{\mathbb{R}^3}\big\|\nabla|\mathcal{F}(h+\epsilon g)|(\boldsymbol{\xi})\big\|d\boldsymbol{\xi} = \int_{\mathbb{R}^3}\big\|\nabla|\mathcal{F}(h)+\epsilon\mathcal{F}(g)|(\boldsymbol{\xi})\big\|d\boldsymbol{\xi} \tag{12}$$

The gradient of the kernel regularization term $\int_{\mathbb{R}^3}\big\|\nabla|\mathcal{F}(h)|(\boldsymbol{\xi})\big\|d\boldsymbol{\xi}$ can thus be computed as

$$\frac{\partial}{\partial\epsilon}\bigg|_{\epsilon\to 0}\int_{\mathbb{R}^3}\big\|\nabla|\mathcal{F}(h)+\epsilon\mathcal{F}(g)|(\boldsymbol{\xi})\big\|d\boldsymbol{\xi}. \tag{13}$$

$$\begin{aligned}&\frac{\partial}{\partial\epsilon}\int_{\mathbb{R}^3}\big\|\nabla|\mathcal{F}(h)+\epsilon\mathcal{F}(g)|\big\|d\boldsymbol{\xi}\\ &\quad=\frac{\partial}{\partial\epsilon}\int_{\mathbb{R}^3}\sqrt{\left(\nabla|\mathcal{F}(h)+\epsilon\mathcal{F}(g)|\right)^2}d\boldsymbol{\xi}\\ &\quad=\int_{\mathbb{R}^3}\frac{1}{2}\frac{1}{\sqrt{\left(\nabla|\mathcal{F}(h)+\epsilon\mathcal{F}(g)|\right)^2}}\cdot\nabla|\mathcal{F}(h)+\epsilon\mathcal{F}(g)|\cdot\nabla\left(\frac{\mathcal{F}(h)\mathcal{F}(g)^*+\mathcal{F}(h)^*\mathcal{F}(g)+\epsilon\mathcal{F}(g)\mathcal{F}(g)^*}{|\mathcal{F}(h)+\epsilon\mathcal{F}(g)|}\right)d\boldsymbol{\xi}\end{aligned} \tag{14}$$

which is, by setting $\epsilon = 0$ and applying the product rule

$$\begin{aligned}&\overset{\epsilon=0}{\equiv}\int_{\mathbb{R}^3}\frac{1}{2}\frac{\nabla|\mathcal{F}(h)|}{\big\|\nabla|\mathcal{F}(h)|\big\|}\cdot\left(\nabla\frac{\mathcal{F}(h)^*}{|\mathcal{F}(h)|}\cdot\mathcal{F}(g)+\frac{\mathcal{F}(h)^*}{|\mathcal{F}(h)|}\cdot\nabla\mathcal{F}(g)+\nabla\frac{\mathcal{F}(h)}{|\mathcal{F}(h)|}\cdot\mathcal{F}(g)^*+\frac{\mathcal{F}(h)}{|\mathcal{F}(h)|}\cdot\nabla\mathcal{F}(g)^*\right)d\boldsymbol{\xi}\\ &=\frac{1}{2}\left(\underbrace{\int_{\mathbb{R}^3}\frac{\nabla|\mathcal{F}(h)|}{\big\|\nabla|\mathcal{F}(h)|\big\|}\cdot\nabla\frac{\mathcal{F}(h)^*}{|\mathcal{F}(h)|}\cdot\mathcal{F}(g)d\boldsymbol{\xi}+\int_{\mathbb{R}^3}\frac{\nabla|\mathcal{F}(h)|}{\big\|\nabla|\mathcal{F}(h)|\big\|}\cdot\frac{\mathcal{F}(h)^*}{|\mathcal{F}(h)|}\cdot\nabla\mathcal{F}(g)d\boldsymbol{\xi}}_{(A)}\right.\\ &\quad\left.+\underbrace{\int_{\mathbb{R}^3}\frac{\nabla|\mathcal{F}(h)|}{\big\|\nabla|\mathcal{F}(h)|\big\|}\cdot\nabla\frac{\mathcal{F}(h)}{|\mathcal{F}(h)|}\cdot\mathcal{F}(g)^*d\boldsymbol{\xi}+\int_{\mathbb{R}^3}\frac{\nabla|\mathcal{F}(h)|}{\big\|\nabla|\mathcal{F}(h)|\big\|}\cdot\frac{\mathcal{F}(h)}{|\mathcal{F}(h)|}\cdot\nabla\mathcal{F}(g)^*d\boldsymbol{\xi}}_{(B)}\right).\end{aligned} \tag{15}$$

Using lemma 1, (A) equals

$$\begin{aligned}
&\int_{\mathbb{R}^3} \frac{\nabla|\mathcal{F}(h)|}{\|\nabla|\mathcal{F}(h)|\|} \cdot \nabla \frac{\mathcal{F}(h)^*}{|\mathcal{F}(h)|} \cdot \mathcal{F}(g) d\boldsymbol{\xi} - \int_{\mathbb{R}^3} \operatorname{div}\left(\frac{\nabla|\mathcal{F}(h)|}{\|\nabla|\mathcal{F}(h)|\|} \cdot \frac{\mathcal{F}(h)^*}{|\mathcal{F}(h)|}\right) \cdot \mathcal{F}(g) d\boldsymbol{\xi} \\
&= \int_{\mathbb{R}^3} \frac{\nabla|\mathcal{F}(h)|}{\|\nabla|\mathcal{F}(h)|\|} \cdot \nabla \frac{\mathcal{F}(h)^*}{|\mathcal{F}(h)|} \cdot \mathcal{F}(g) d\boldsymbol{\xi} \\
&\quad - \int_{\mathbb{R}^3} \left(\operatorname{div}\left(\frac{\nabla|\mathcal{F}(h)|}{\|\nabla|\mathcal{F}(h)|\|}\right) \cdot \frac{\mathcal{F}(h)^*}{|\mathcal{F}(h)|} + \frac{\nabla|\mathcal{F}(h)|}{\|\nabla|\mathcal{F}(h)|\|} \cdot \nabla \frac{\mathcal{F}(h)^*}{|\mathcal{F}(h)|}\right) \cdot \mathcal{F}(g) d\boldsymbol{\xi} \\
&= -\int_{\mathbb{R}^3} \operatorname{div}\left(\frac{\nabla|\mathcal{F}(h)|}{\|\nabla|\mathcal{F}(h)|\|}\right) \cdot \frac{\mathcal{F}(h)^*}{|\mathcal{F}(h)|} \cdot \mathcal{F}(g) d\boldsymbol{\xi},
\end{aligned} \tag{16}$$

where $\operatorname{div}(\mathbf{x})$ denotes the divergence of vector $\mathbf{x}$. Now, we can use Parseval's theorem and get

$$-\int_{\mathbb{R}^3} \operatorname{div}\left(\frac{\nabla|\mathcal{F}(h)|}{\|\nabla|\mathcal{F}(h)|\|}\right) \cdot \frac{\mathcal{F}(h)^*}{|\mathcal{F}(h)|} \cdot \mathcal{F}(g) d\boldsymbol{\xi} = -\int_{\mathbb{R}^3} \mathcal{F}^{-1}\left(\operatorname{div}\left(\frac{\nabla|\mathcal{F}(h)|}{\|\nabla|\mathcal{F}(h)|\|}\right) \cdot \frac{\mathcal{F}(h)}{|\mathcal{F}(h)|}\right)^* \cdot g\, d\mathbf{x}, \tag{17}$$

which is, because $\operatorname{div}\left(\frac{\nabla|\mathcal{F}(h)|}{\|\nabla|\mathcal{F}(h)|\|}\right)$ is symmetric and $\mathcal{F}^{-1}\left(\operatorname{div}\left(\frac{\nabla|\mathcal{F}(h)|}{\|\nabla|\mathcal{F}(h)|\|}\right)\right)$ is real valued, equal to

$$-\int_{\mathbb{R}^3} \mathcal{F}^{-1}\left(\operatorname{div}\left(\frac{\nabla|\mathcal{F}(h)|}{\|\nabla|\mathcal{F}(h)|\|}\right) \cdot \frac{\mathcal{F}(h)}{|\mathcal{F}(h)|}\right) \cdot g\, d\mathbf{x}. \tag{18}$$

Now, we compute the same for (B). Because of lemma 1,

$$\begin{aligned}
(B) &= \int_{\mathbb{R}^3} \frac{\nabla|\mathcal{F}(h)|}{\|\nabla|\mathcal{F}(h)|\|} \cdot \nabla \frac{\mathcal{F}(h)}{|\mathcal{F}(h)|} \cdot \mathcal{F}(g)^* d\boldsymbol{\xi} - \int_{\mathbb{R}^3} \operatorname{div}\left(\frac{\nabla|\mathcal{F}(h)|}{\|\nabla|\mathcal{F}(h)|\|} \cdot \frac{\mathcal{F}(h)}{|\mathcal{F}(h)|}\right) \cdot \mathcal{F}(g)^* d\boldsymbol{\xi} \\
&= \int_{\mathbb{R}^3} \frac{\nabla|\mathcal{F}(h)|}{\|\nabla|\mathcal{F}(h)|\|} \cdot \nabla \frac{\mathcal{F}(h)}{|\mathcal{F}(h)|} \cdot \mathcal{F}(g)^* d\boldsymbol{\xi} \\
&\quad - \int_{\mathbb{R}^3} \left(\operatorname{div}\left(\frac{\nabla|\mathcal{F}(h)|}{\|\nabla|\mathcal{F}(h)|\|}\right) \cdot \frac{\mathcal{F}(h)}{|\mathcal{F}(h)|} + \frac{\nabla|\mathcal{F}(h)|}{\|\nabla|\mathcal{F}(h)|\|} \cdot \nabla \frac{\mathcal{F}(h)}{|\mathcal{F}(h)|}\right) \cdot \mathcal{F}(g)^* d\boldsymbol{\xi} \\
&= -\int_{\mathbb{R}^3} \operatorname{div}\left(\frac{\nabla|\mathcal{F}(h)|}{\|\nabla|\mathcal{F}(h)|\|}\right) \cdot \frac{\mathcal{F}(h)}{|\mathcal{F}(h)|} \cdot \mathcal{F}(g)^* d\boldsymbol{\xi}.
\end{aligned} \tag{19}$$

We can again use Parseval's theorem and get

$$-\int_{\mathbb{R}^3} \operatorname{div}\left(\frac{\nabla|\mathcal{F}(h)|}{\|\nabla|\mathcal{F}(h)|\|}\right) \cdot \frac{\mathcal{F}(h)}{|\mathcal{F}(h)|} \cdot \mathcal{F}(g)^* d\boldsymbol{\xi} = -\int_{\mathbb{R}^3} \mathcal{F}^{-1}\left(\operatorname{div}\left(\frac{\nabla|\mathcal{F}(h)|}{\|\nabla|\mathcal{F}(h)|\|}\right) \cdot \frac{\mathcal{F}(h)}{|\mathcal{F}(h)|}\right) \cdot g\, d\mathbf{x}. \tag{20}$$

Now, we can put (A) and (B) together to

$$(15) = -\int_{\mathbb{R}^3} \mathcal{F}^{-1}\left(\operatorname{div}\left(\frac{\nabla|\mathcal{F}(h)|}{\|\nabla|\mathcal{F}(h)|\|}\right) \cdot \frac{\mathcal{F}(h)}{|\mathcal{F}(h)|}\right) \cdot g\, d\mathbf{x}. \tag{21}$$

According to the fundamental lemma of calculus of variations, the functional $J_{\text{KFTV}}(s, h)$ is minimized by the solution of the Euler Lagrange equation:

$$\int_{\mathbb{R}^3} s(\mathbf{y}) d\mathbf{y} - \left(s^m * \left(\frac{o}{(h * s)}\right)\right) - \lambda_h \mathcal{F}^{-1}\left(\operatorname{div}\left(\frac{\nabla|\mathcal{F}(h)|}{\|\nabla|\mathcal{F}(h)|\|}\right) \cdot \frac{\mathcal{F}(h)}{|\mathcal{F}(h)|}\right) = 0. \tag{22}$$

The resulting multiplicative update scheme for the deconvolution kernel is:

$$\hat{h}_{k+1} = \frac{\hat{h}_k \cdot \left(s^m * \frac{o}{(\hat{h}_k * s)}\right)}{\int_{\mathbb{R}^3} s(\mathbf{y}) d\mathbf{y} - \lambda_h \mathcal{F}^{-1}\left(\operatorname{div}\left(\frac{\nabla|\mathcal{F}(\hat{h}_k)|}{\|\nabla|\mathcal{F}(\hat{h}_k)|\|}\right) \cdot \frac{\mathcal{F}(h)}{|\mathcal{F}(h)|}\right)}. \tag{23}$$

3. Numerical Scheme for the Divergence Term

For the computation of the divergence term div $\left(\frac{\nabla|\mathcal{F}(h)|}{\left\|\nabla|\mathcal{F}(h)|\right\|}\right)$, we use the numerically stable scheme presented in [3]:

$$
\begin{aligned}
&\operatorname{div}\left(\frac{\nabla|\mathcal{F}(h)|}{\left\|\nabla|\mathcal{F}(h)|\right\|}\right)(\boldsymbol{\xi}) \\
&\quad= \frac{1}{d_{\xi_1}}\Delta_-^{\xi_1}\frac{\Delta_+^{\xi_1}|(\mathcal{F}(h))(\boldsymbol{\xi})|}{\sqrt{\epsilon+\left(\Delta_+^{\xi_1}|(\mathcal{F}(h))(\boldsymbol{\xi})|\right)^2+m\left(\Delta_+^{\xi_2}|(\mathcal{F}(h))(\boldsymbol{\xi})|,\Delta_-^{\xi_2}|(\mathcal{F}(h))(\boldsymbol{\xi})|\right)^2+m\left(\Delta_+^{\xi_3}|(\mathcal{F}(h))(\boldsymbol{\xi})|,\Delta_-^{\xi_3}|(\mathcal{F}(h))(\boldsymbol{\xi})|\right)^2}} \\
&\quad+ \frac{1}{d_{\xi_2}}\Delta_-^{\xi_2}\frac{\Delta_+^{\xi_2}|(\mathcal{F}(h))(\boldsymbol{\xi})|}{\sqrt{\epsilon+\left(\Delta_+^{\xi_2}|(\mathcal{F}(h))(\boldsymbol{\xi})|\right)^2+m\left(\Delta_+^{\xi_3}|(\mathcal{F}(h))(\boldsymbol{\xi})|,\Delta_-^{\xi_3}|(\mathcal{F}(h))(\boldsymbol{\xi})|\right)^2+m\left(\Delta_+^{\xi_1}|(\mathcal{F}(h))(\boldsymbol{\xi})|,\Delta_-^{\xi_1}|(\mathcal{F}(h))(\boldsymbol{\xi})|\right)^2}} \\
&\quad+ \frac{1}{d_{\xi_3}}\Delta_-^{\xi_3}\frac{\Delta_+^{\xi_3}|(\mathcal{F}(h))(\boldsymbol{\xi})|}{\sqrt{\epsilon+\left(\Delta_+^{\xi_3}|(\mathcal{F}(h))(\boldsymbol{\xi})|\right)^2+m\left(\Delta_+^{\xi_1}|(\mathcal{F}(h))(\boldsymbol{\xi})|,\Delta_-^{\xi_1}|(\mathcal{F}(h))(\boldsymbol{\xi})|\right)^2+m\left(\Delta_+^{\xi_2}|(\mathcal{F}(h))(\boldsymbol{\xi})|,\Delta_-^{\xi_2}|(\mathcal{F}(h))(\boldsymbol{\xi})|\right)^2}},
\end{aligned}
\tag{24}
$$

with the function $m(a,b)$ defined as

$$
m(a,b) = \frac{\text{sign}a + \text{sign}b}{2}\min(|a|,|b|). \tag{25}
$$

The forward and backward differences are defined as

$$
\begin{aligned}
\Delta_+^{\xi_1}|\left(\mathcal{F}(h)\right)(u,v,w)| &= \tfrac{1}{d_{\xi_1}}\left(|\left(\mathcal{F}(h)\right)(u+1,v,w)| - |\left(\mathcal{F}(h)\right)(u,v,w)|\right)\\
\Delta_-^{\xi_1}|\left(\mathcal{F}(h)\right)(u,v,w)| &= \tfrac{1}{d_{\xi_1}}\left(|\left(\mathcal{F}(h)\right)(u,v,w)| - |\left(\mathcal{F}(h)\right)(u-1,v,w)|\right)\\
\Delta_+^{\xi_2}|\left(\mathcal{F}(h)\right)(u,v,w)| &= \tfrac{1}{d_{\xi_2}}\left(|\left(\mathcal{F}(h)\right)(u,v+1,w)| - |\left(\mathcal{F}(h)\right)(u,v,w)|\right)\\
\Delta_-^{\xi_2}|\left(\mathcal{F}(h)\right)(u,v,w)| &= \tfrac{1}{d_{\xi_2}}\left(|\left(\mathcal{F}(h)\right)(u,v,w)| - |\left(\mathcal{F}(h)\right)(u,v-1,w)|\right)\\
\Delta_+^{\xi_3}|\left(\mathcal{F}(h)\right)(u,v,w)| &= \tfrac{1}{d_{\xi_3}}\left(|\left(\mathcal{F}(h)\right)(u,v,w+1)| - |\left(\mathcal{F}(h)\right)(u,v,w)|\right)\\
\Delta_-^{\xi_3}|\left(\mathcal{F}(h)\right)(u,v,w)| &= \tfrac{1}{d_{\xi_3}}\left(|\left(\mathcal{F}(h)\right)(u,v,w)| - |\left(\mathcal{F}(h)\right)(u,v,w-1)|\right).
\end{aligned}
\tag{26}
$$

The added ϵ in the denominator of equation 24 renders the function robust as the gradient magnitude approaches zero. In our implementation, we have chosen $\epsilon = 0.1$. We assume mirrored boundary pixel values such that the derivatives evaluate to zero at the array boundaries.

4. Supplementary Qualitative Results on the Drosophila S2 Cell Recordings

In the main paper, we only report the resulting RMSE of our method on our new *Drosophila* S2 cell dataset (see figure 10 in the main paper). Here, we additionally show the central xy-sections of these deconvolution results in figure 1.

References

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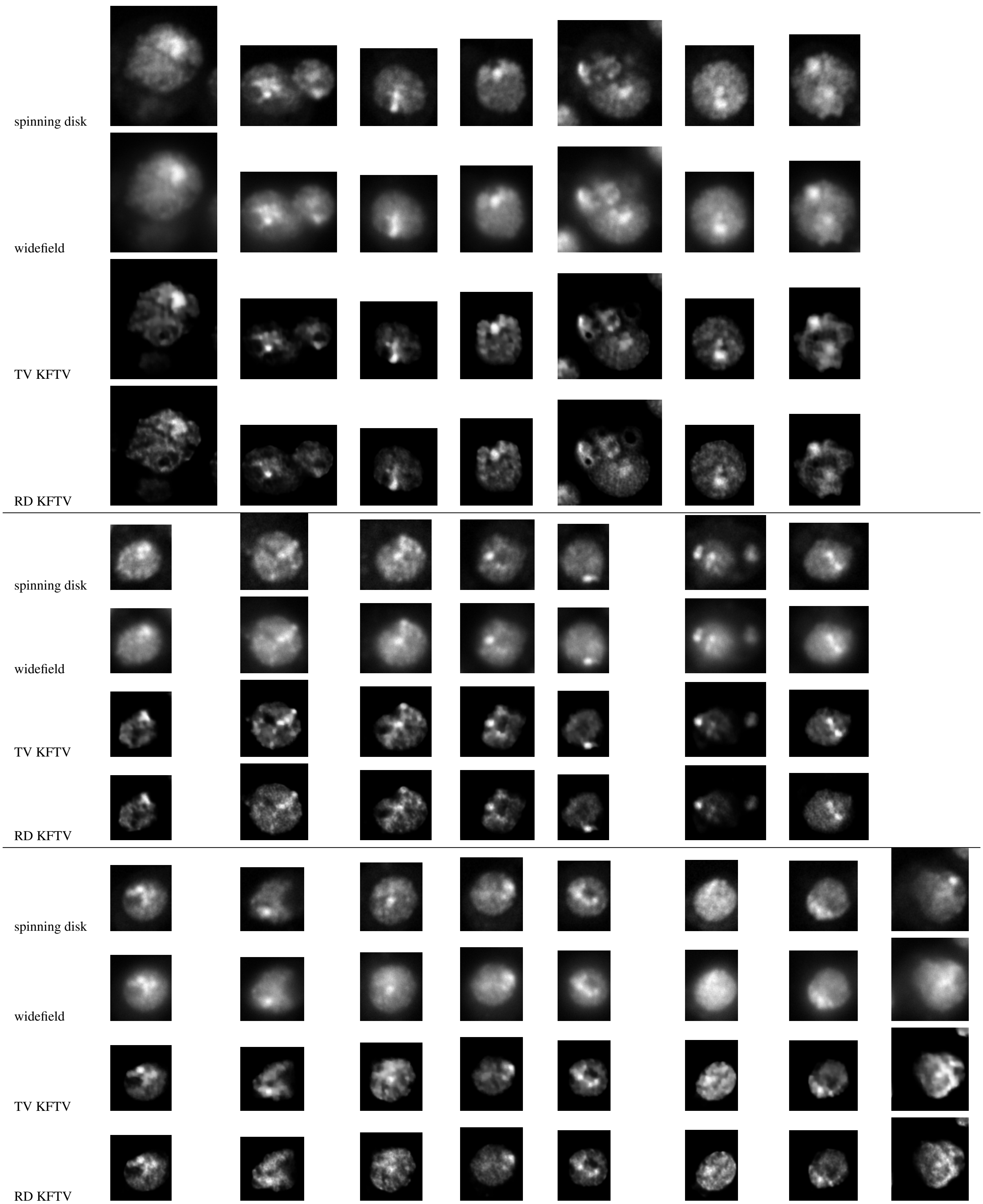

Figure 1. Central xy-sections of the *Drosophila* S2 cell nucleus recordings and the deconvolution results for $\lambda_h = 4$. These images correspond to the RMSE values given in figure 10 in the main paper.