A note on computing the DF/F

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1 A CNMF approach

The general model for representing the spatiotemporal fluorescence data is

$$Y = AC + bf + E, \tag{1}$$

where $Y \in \mathbb{R}_+^{d\times T}$ is the observed data, $A \in \mathbb{R}_+^{d\times K}$ is the set of spatial footprints of the identified components, $C \in \mathbb{R}_+^{k\times T}$ is the set of temporal components, $b \in \mathbb{R}_+^{d\times n_b}$, $f \in \mathbb{R}_+^{n_b\times T}$ are the spatial and temporal background respectively (n_b denotes the number of background components), and $E \in \mathbb{R}^{d\times T}$ is the observation noise. Here we assume that no preprocessing, e.g., drift removal, has been applied to the data before applying the CNMF algorithm. Moreover, each temporal trace $\boldsymbol{c_i}$ has also a baseline activity denoted by c_i^b. We can therefore rewrite C as the sum of the baseline for each component plus the varying parts.

$$C = \boldsymbol{c^b}\mathbf{1}_T^\top + \tilde{C}. \tag{2}$$

We consider the background for each component to come from two sources: i) the local baseline c_i^b, and ii) the global background bf averaged over the spatial extend $\boldsymbol{a_i}$. What is not included in the background is the activity of the component $\tilde{c}_i$, the contribution of overlapping components, and the effect of noise. Note that the noise under model assumptions should have zero mean.

The averaged data over the spatial extent of component i can be written as

$$\boldsymbol{a_i}^\top Y = \underbrace{\|\boldsymbol{a_i}\|_2^2\tilde{c}_i}_{\text{component}} + \underbrace{\|\boldsymbol{a_i}\|_2^2 c_i^b + (\boldsymbol{a_i}^\top b)f}_{\text{background}} + \underbrace{\sum_{j\neq i}(\boldsymbol{a_i}^\top \boldsymbol{a_j})c_j}_{\text{overlap}} + \underbrace{\boldsymbol{a_i}^\top E}_{\text{noise}}. \tag{3}$$

Based of the above decomposition, the slow varying background fluorescence for component i is given by

$$f_0^i = \mathcal{F}(\|\boldsymbol{a_i}\|_2^2 c_i^b + (\boldsymbol{a_i}^\top b)f), \tag{4}$$

where $\mathcal{F}(\cdot)$ is an appropriate filtering operator, e.g., median or percentile filtering of the whole trace or over a moving window. The DF/F ratio can then be computed as

$$c_i^{df} = \frac{\|\boldsymbol{a_i}\|_2^2\tilde{c}_i}{f_0^i}, \tag{5}$$

where f_0^{i} is the background fluorescence for each component.

In matrix notation, if we denote $AA = A^\top A$, $nA = \mathrm{diag}\{AA\}$, and $\overline{AA} = AA - \mathrm{diag}\{nA\}$, we can rewrite (3) as

$$A^\top Y = \underbrace{\mathrm{diag}\{nA\}\tilde{C}}_{\text{component}} + \underbrace{AA\boldsymbol{c^b}\mathbf{1}_T^\top + (A^\top b)f}_{\text{background}} + \underbrace{\overline{AA}C}_{\text{overlap}} + \underbrace{A^\top E}_{\text{noise}}, \tag{6}$$

and the DF/F signal can be given as

$$\begin{aligned} F_0 &= \mathcal{F}(AA\boldsymbol{c^b}\mathbf{1}_T^\top + (A^\top b)f) \\ C^{df} &= \frac{\operatorname{diag}\{nA\}\tilde{C}}{F_0}, \end{aligned} \tag{7}$$

where the division is element-wise.

A note on normalization: Since the DF/F measure is a ratio of the signal over the background, the value is invariant under normalization.

2 Connection to more traditional approaches

Consider the binary matrix $M = (A > 0)$ which creates the binary mask for each spatial component. A more traditional approach for computing the DF/F ratio for component i would be to first average over the spatial extent of each ROI:

$$f^i = \boldsymbol{m_i}^\top Y. \tag{8}$$

Again normalization here is not necessary, since we'll be taking the ratio of two quantities. Then the slowly varying background fluorescence is computed as

$$f_b^i = \mathcal{F}(f^i), \tag{9}$$

and the DF/F ratio is computed as

$$\hat{c}_i^{df} = \frac{f^i - f_b^i}{f_b^i}. \tag{10}$$

Although not immediately clear, this measure is closely related with the measure derived in (5), with some differences:

1. The CNMF approach performs a weighted average of the data with $\boldsymbol{a_i}$, whereas the traditional approach performs a plain average with $\boldsymbol{m_i}$. The two approaches are largely similar, although it is expected the CNMF approach to have slightly better SNR because it weighs the important pixels more.
2. The CNMF approach excludes the effects of overlap, noise, and spiking transients from the calculation of the background. This is desirable since these quantities are not related to the background fluorescence.

Other than this exclusion, the two calculations are largely similar. We'll exhibit how the DF/F measure for the CNMF of (5) can be mapped to the more traditional measure of (10) under a few simplifying assumptions. Note that using (3), equation (4) can be written as

$$f_0^i = \mathcal{F}(\boldsymbol{a_i}^\top Y - \|\boldsymbol{a_i}\|_2^2 \tilde{c}_i - \sum_{j \neq i} (\boldsymbol{a_i}^\top \boldsymbol{a_j}) c_j - \boldsymbol{a_i}^\top E). \tag{11}$$

By assuming i) no overlap and ii) zero mean noise, the equation becomes

$$f_0^i \simeq \mathcal{F}(\boldsymbol{a_i}^\top Y - \|\boldsymbol{a_i}\|_2^2 \tilde{c}_i). \tag{12}$$

Define also

$$\tilde{f}_0^i = \mathcal{F}(\boldsymbol{a_i}^\top Y) \tag{13}$$

Our assumption here is that since the filtering operator $\mathcal{F}(\cdot)$ captures only slow background variations of the data, $\tilde{f}_0^i$ will be close to f_0^i. (This assumption is further justified in the case where the neuron response is sparse so the background computation in terms of percentile is not affected by it.) Now using again (3), and ignoring the effects of background and noise, equation (5) can be written as

$$c_i^{df} = \frac{\boldsymbol{a_i}^\top Y - (\|\boldsymbol{a_i}\|_2^2 c_i^b + (\boldsymbol{a_i}^\top b)f)}{f_0^i}. \tag{14}$$

Now from (3) the term $\|\boldsymbol{a_i}\|_2^2 c_i^b + (\boldsymbol{a_i}^\top b)f$ corresponds to the background fluorescence for $\boldsymbol{a_i}^\top Y$, therefore

$$c_i^{df} = \frac{\boldsymbol{a_i}^\top Y - \mathcal{F}(\boldsymbol{a_i}^\top Y)}{f_0^i} \overset{(13)}{\simeq} \frac{\boldsymbol{a_i}^\top Y - \mathcal{F}(\boldsymbol{a_i}^\top Y)}{\mathcal{F}(\boldsymbol{a_i}^\top Y)}. \tag{15}$$

Up to the different averaging procedure, (15) and (10) become the same.

Dealing with classified components: After classifying the components, we tend to discard the bad components. This can lead to biases in the DF/F estimation, since the bad components may contribute background signals or signals from other sources. This bias will be more prominent in the case where bad components overlap with good components. We can think of three ways for dealing with this issues:

1. After discarding the bad components, update the spatial and temporal components once again to refine their estimates in lieu of the new data.
2. Subtract the bad components from the data, i.e., $Y \leftarrow Y - A_{\text{bad}} C_{\text{bad}}$, before applying DF/F.
3. Perform the DF/F calculation on all the components, then discard the bad ones.

In general, the first option should be the most principled, although I don't expect big variations among the three options.