Supplementary Information

Parallel micro-Raman spectroscopy of multiple cells in a single acquisition using hierarchical sparsity

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The file includes:

(1) Algorithm of the hierarchical sparsity model.
(2) Algorithm of the $L_1$-norm regularization.
(3) Reconstructed Raman spectra of a 4-foci tweezers array occupied by four trapped cells from the same population.
(4) Reconstructed Raman spectra of a 4-foci tweezers array occupied by two trapped cells from one population while the other two foci being vacant.
Algorithm 1: hierarchical sparsity model

Goal: \( x^* = \min_x \phi(x) = \frac{1}{2} \|Ax - y\|_2^2 + \lambda_1 \sum_{g \in G} \|x_g\|_2 + \lambda_2 \|x\|_h \)

Set \( t := 0 \);
Choose \( \eta = 1.5, c = 0.01, \alpha_{\text{min}} = 0.001, \alpha_{\text{max}} = 5000 \);
Choose an initial \( x^0 = [1,1,\ldots,1] \);
while \( |\phi(x^t) - \phi(x^{t-1})|/\phi(x^{t-1}) > 10^{-5} \) do

Choose \( \alpha^t = \alpha_{\text{min}} \);
Set \( u^t \leftarrow x^t - \frac{1}{\alpha^t} \nabla \phi(x^t) \);

while \( \phi(x^{t+1}) > \max_{i=\text{max}(t,0),..,1} \phi(x^i) - \frac{1}{2} \|x^{t+1} - x^i\|_2^2 \cdot 10^{-3} \) do

for \( g = 1 \) to \( |G| \) do

Set \( r := 0 \);
Choose \( p^0 = [1,1,\ldots,1], b^0 = [1/2,1/2,\ldots,1/2], \beta^0 = [1/2,1/2,\ldots,1/2] \);
while \( \|b^{r+1} - b^r\|_2 / \|b^r\|_2 > 10^{-5} \) do

\[
\begin{align*}
\beta^{r+1} & = \frac{1}{c+1} S(u^r + c\beta^r - p^r, \lambda_1 / \alpha^t); \\
p^{r+1} & = p^r + c(b^{r+1} - \beta^{r+1}); \\
r & \leftarrow r + 1
\end{align*}
\]

Set \( x^t_g := b^{r+1} \);

end

Set \( \alpha^t \leftarrow \eta \alpha^t \);
end
Set \( t \leftarrow t + 1 \);
end

Notes:

1. \( f(x) = \frac{1}{2} \|Ax - y\|_2^2 \)
2. \( \lambda_1 = 1, \lambda_2 = 2 \)
3. Soft-thresholding operator \( S(a,c) = \text{sgn}(a) \max\{0,|a|-c\} \)
4. Vector shrinkage operator \( S_e(b,\lambda) = \left[1 - \frac{\lambda}{\|b\|_2}\right]_+ b \)
Algorithm 2: L₁-norm regularization

Goal: $x^* = \min_x \phi(x) = \frac{1}{2} \|Ax - y\|_2^2 + \lambda \|x\|_1$

Set $t := 0$;
Choose $\eta = 1.5$, $\alpha_{\min} = 0.001$, $\alpha_{\max} = 5000$;
Choose an initial $x^0 = [1,1,\ldots,1]$;

while $|\phi(x') - \phi(x^{t-1})| / \phi(x^{t-1}) > 10^{-5}$ do

Choose $\alpha = \alpha_{\min}$;
Set $u' \leftarrow x' - \frac{1}{\alpha} \nabla \phi(x')$;

while $\phi(x^{t-1}) > \max_{i = \max(t - 3, 0), \ldots, t} \phi(x') - \frac{1}{2} \|x^{t-1} - x'\|_2^2 \cdot 10^{-3}$ do

$x^{t-1} = S(u', \lambda / \alpha')$;
Set $\alpha' \leftarrow \eta \alpha'$;

end
Set $t \leftarrow t + 1$;

end
Supplementary Figure S1 | Demultiplexing of the superimposed Raman spectrum acquired by a 4-foci tweezer array that traps four cells from the population 1. (a-d) Reconstructed spectra of the individual cells using the hierarchical sparsity model (red) as well as their ground truths (black). A 10-fold variation in the componential contributions (e.g. the peak at 1742 cm$^{-1}$) was simulated, and noises were also added to the superimposed spectra, which results in a SNR of 40.
Supplementary Figure S2 | Demultiplexing of the superimposed Raman spectrum acquired by a 4-foci tweezers array loaded with only two cells. Two cells from the population 1 are trapped while the other two traps being not occupied. (a-d) Reconstructed spectra using the hierarchical sparsity model (red) as well as their ground truths (black). Noises were also added to the superimposed spectra, which results in a SNR of 40.