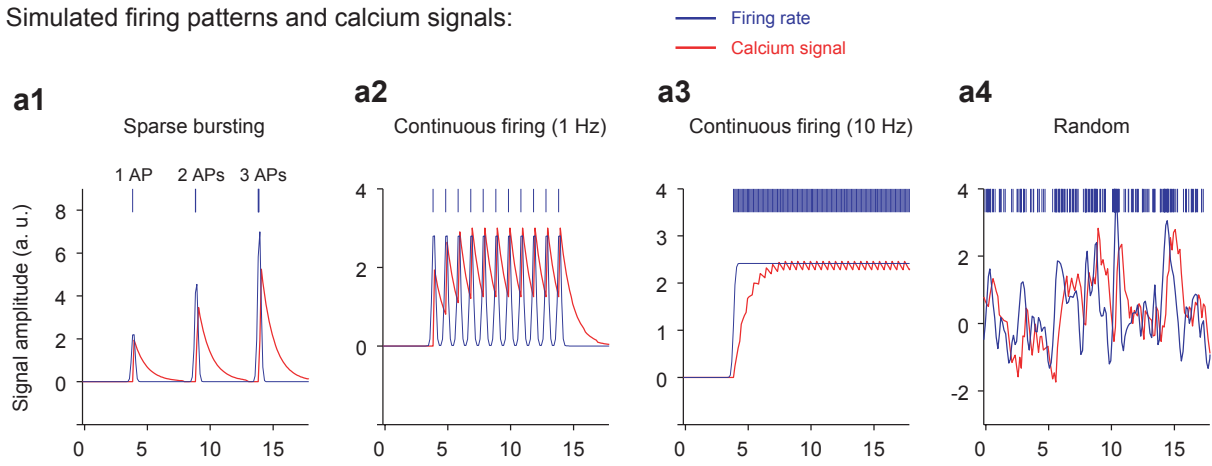
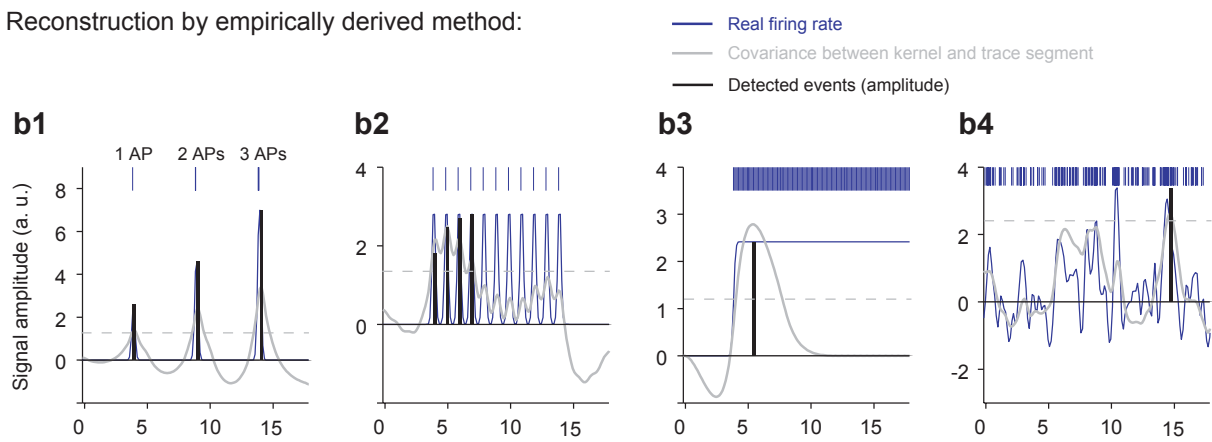


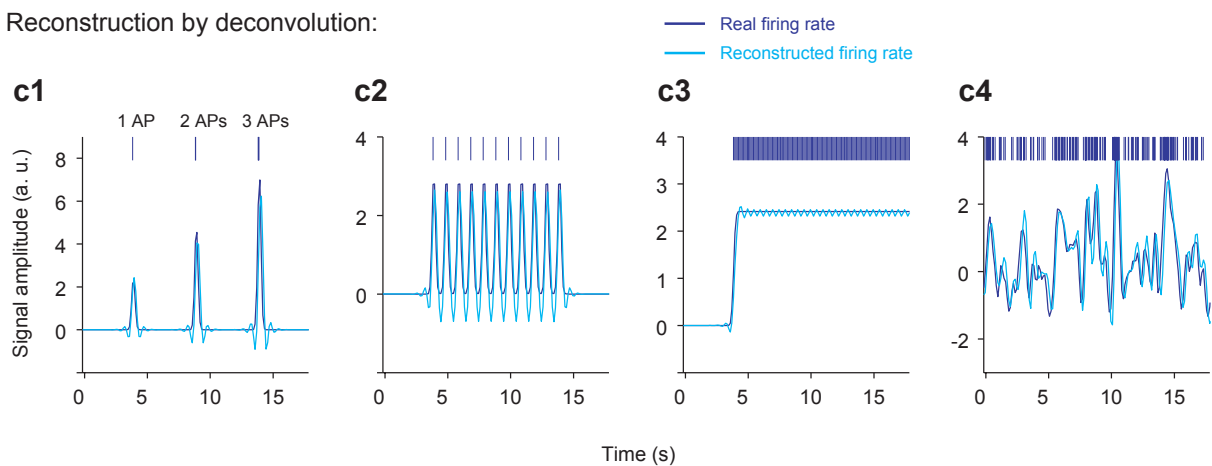
Simulated firing patterns and calcium signals:



Reconstruction by empirically derived method:



Reconstruction by deconvolution:



Supplementary Figure 5 | Comparison of firing rate reconstruction by empirically derived methods and temporal deconvolution. (a) Four different simulated firing rate patterns (blue) and associated calcium signals (red), generated by convolving each AP (ticks) with an exponential kernel. The time constant, $\tau = 1$ s, is similar to that of Ca^{2+} transients in the neocortex of anaesthetized rats¹. No noise was added. Data were originally simulated at a

temporal resolution of 1 ms. Subsequently, temporal resolution was reduced to 125 ms by omitting the intervening data points, thereby simulating the data acquisition process in scanning microscopy. **a1**: three bursts containing 1 - 3 APs, separated by 5 s. **a2**: continuous firing at low frequency (1 Hz). **a3**: continuous firing at a higher rate (10 Hz). **a4**: random firing at an average rate of 10 Hz. **(b)** Reconstruction of firing patterns by the method of Kerr et al.¹. This procedure is based on the covariance of the low-pass filtered Ca^{2+} signal and an exponentially decaying kernel using a sliding window (gray trace). Discrete events (black) are detected when the covariance exceeds the SD of the covariance by an arbitrary factor (here, 1.5; dashed line). The time and amplitude of each event is determined from the nearest peak in the raw Ca^{2+} signal trace. This procedure has been designed empirically to detect sparse bursts (**a1**). For such firing patterns, the covariance peaks at each burst, and the event amplitude reflects the number of APs within the burst (**b1**). However, the method fails when events (APs) are spaced more closely in time because it cannot separate summing Ca^{2+} transients. As shown in **b2**, the covariance does not faithfully follow AP firing when the inter-AP-interval equals the decay time constant of the calcium transient, and only the first few events in a simple firing pattern are detected. These reconstruction problems become even more pronounced at higher firing rates: a prolonged train of APs at 10 Hz is detected as a single transient event (**b3**), and a complex (random) firing pattern cannot be reconstructed (**b4**). Similar results would be obtained using a method based on the temporal derivative of the Ca^{2+} signal^{2,3}. Hence, these procedures primarily perform event detection and are suited only for the reconstruction of temporally sparse firing patterns (approximately for event frequencies < 1 Hz, depending on the decay time constant of Ca^{2+} transients). Reconstruction of other firing patterns results in severe artifacts. **(c)**, Reconstruction of firing patterns by deconvolution of the low-pass filtered Ca^{2+} signal. All firing patterns are reconstructed with only small deviations that result from low-pass filtering and intermittent sampling (scanning). Because temporal deconvolution is based on the generic relationship between APs and Ca^{2+} transients, it permits the reconstruction of virtually any firing pattern and is constrained only by experimental limitations such as the signal-to-noise ratio or the frame rate.

1. Kerr, J. N., Greenberg, D. & Helmchen, F. Imaging input and output of neocortical networks in vivo. *Proc Natl Acad Sci U S A* **102**, 14063-14068 (2005).
2. Ikegaya, Y. et al. Synfire chains and cortical songs: temporal modules of cortical activity. *Science* **304**, 559-64 (2004).
3. MacLean, J. N., Watson, B. O., Aaron, G. B. & Yuste, R. Internal dynamics determine the cortical response to thalamic stimulation. *Neuron* **48**, 811-23 (2005).