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Signal Processing Methods for Cell Localization and Activity Detection from Calcium Imaging Data

Pier Luigi Dragotti

Joint work with Stephanie Reynolds, Jon Onativia and Simon Schultz

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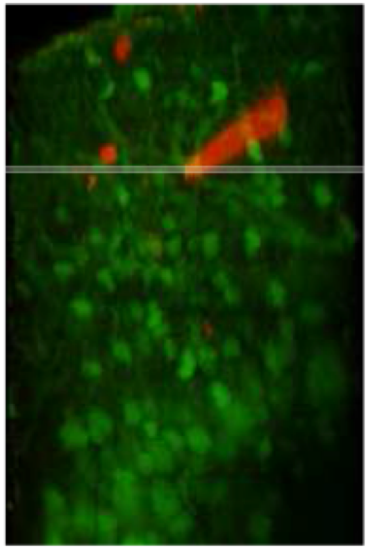
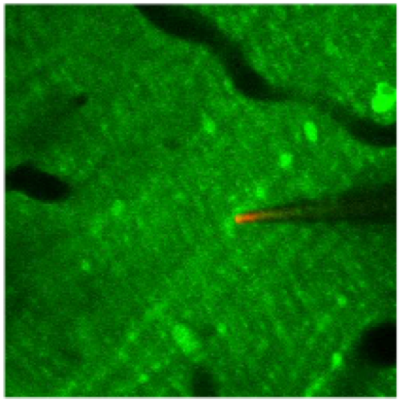
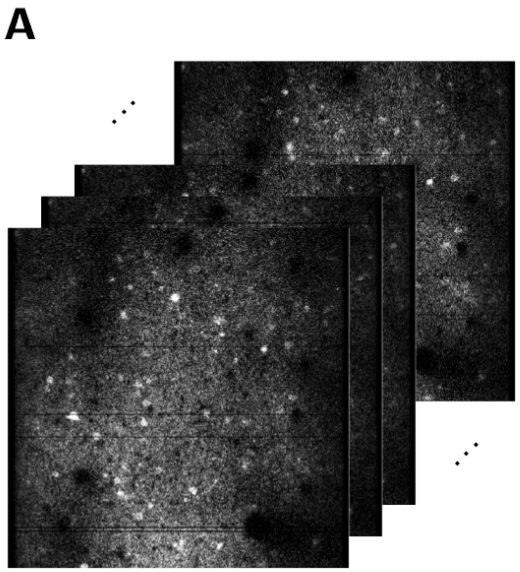
Motivation







- *Goal of Neuroscience: to study how information is processed in the brain*
- *Neurons communicate through pulses called Action Potentials (AP)*
- *Need to measure in-vivo the activity of large populations of neurons at cellular level resolution*
- *Two-photon microscopy combined with calcium indicators is the most promising technology to achieve that*

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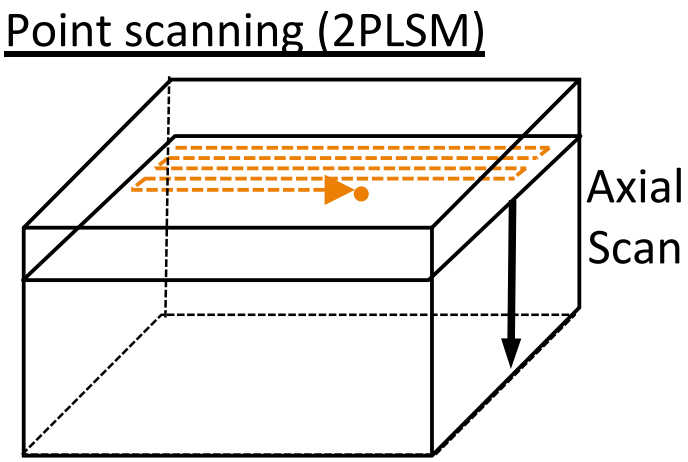
Two-Photon Microscopy

- Fluorescent sensors within tissues
- Highly localized laser excites fluorescence from sensors
- Photons emitted from tissue are collected
- Focal spot sequentially scanned across samples to form image
- Two-photon microscopes can go deeper in the tissue than single-photon microscope



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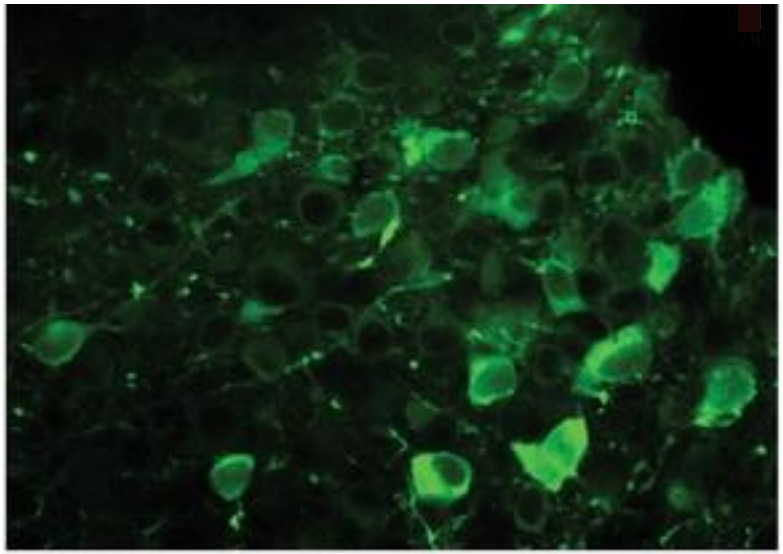
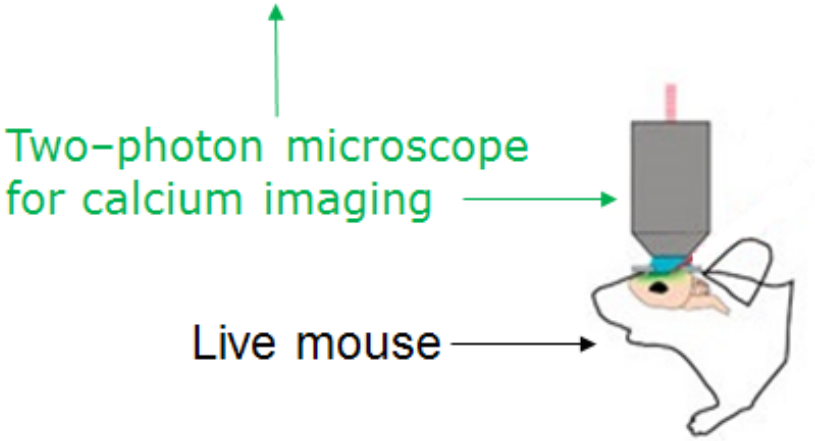
Calcium Imaging





- The calcium concentration of a cell is a reliable indicator of spiking activity.
- Calcium imaging uses fluorescent indicators whose fluorescence intensity reads out calcium concentration.
- Can monitor 100s of neurons **simultaneously.**
- Know the spatial relationships between neurons.

[1] Betley, J. Nicholas, et al. "Neurons for hunger and thirst transmit a negative-valence teaching signal." *Nature* 521.7551 (2015): 180-185.

Advantages of Calcium Imaging

- Can monitor activity of 100s - 1000s of neurons simultaneously, at single cell resolution.
- Can image *in vivo* in behaving animals.
- Can image same cell populations over multiple months.

BUT the datasets present challenging signal processing problems:

1. Low-time resolution
2. Need to segment automatically regions of interest

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Outline

- Motivation
- Sparse Sampling for calcium transient detection at high-temporal resolution
- Variation of Level-Set Method for Cell Localization and Segmentation
- Conclusions and Future Work

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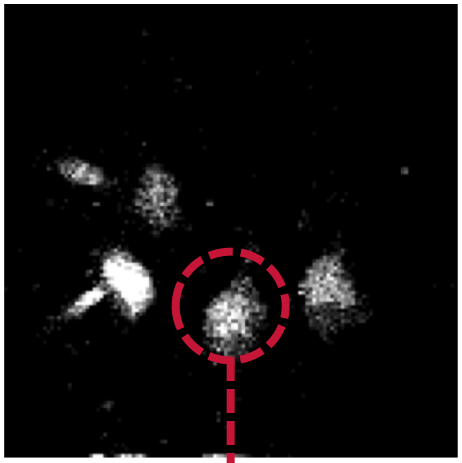
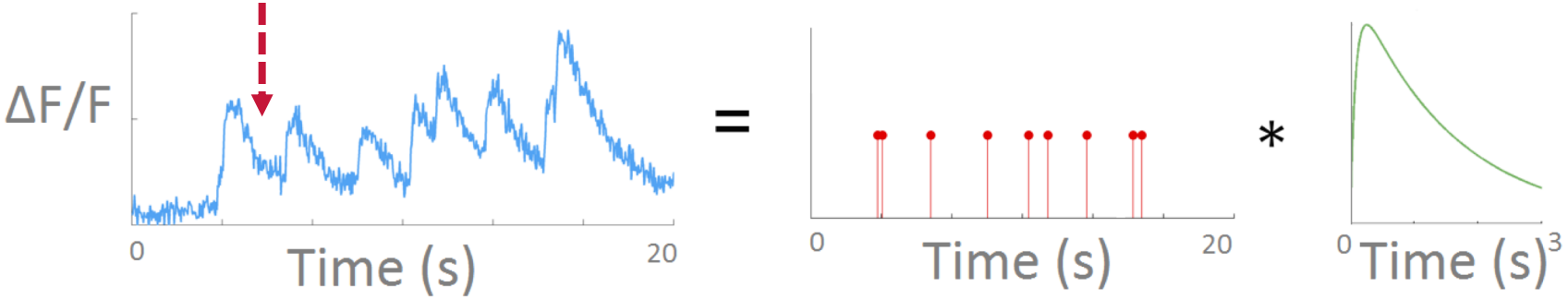
Calcium Transient Detection



The signal from one neuron can be modelled as that neurons spike train convolved with a characteristic pulse shape:

$$f(t) = x(t) * p(t)$$
$$= \sum_{k=1}^{K} a_k \delta(t - t_k) \; * \; (e^{-\alpha t} - e^{-\gamma t}) 1_{t \geq 0} .$$



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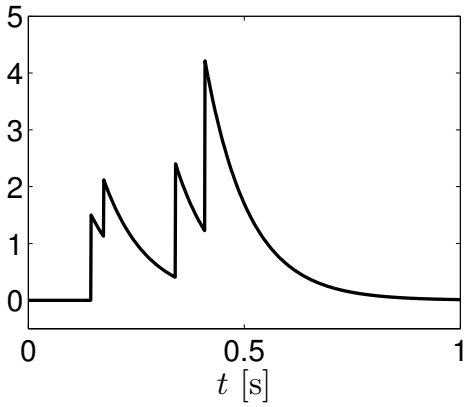
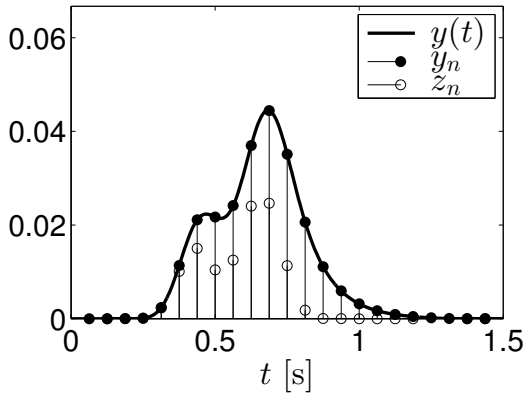
Calcium Transient Detection and Sparse Sampling

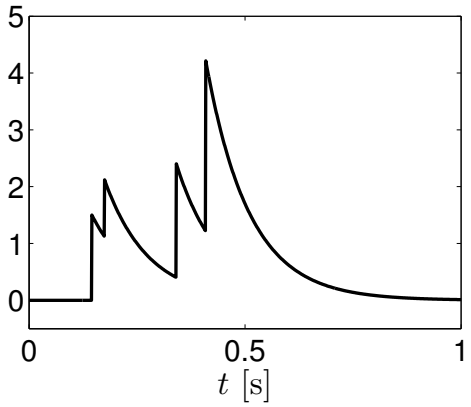
- *Signal Model: Stream of decaying exponentials*
- *This type of signal is well understood in the context of sparse sampling theory (Vetterli-Dragotti-Blu), where reconstruction is possible at very low sampling rate*



(a) Input signal, $x(t)$



(b) Filtered and sampled signal



(c) Reconstructed signal

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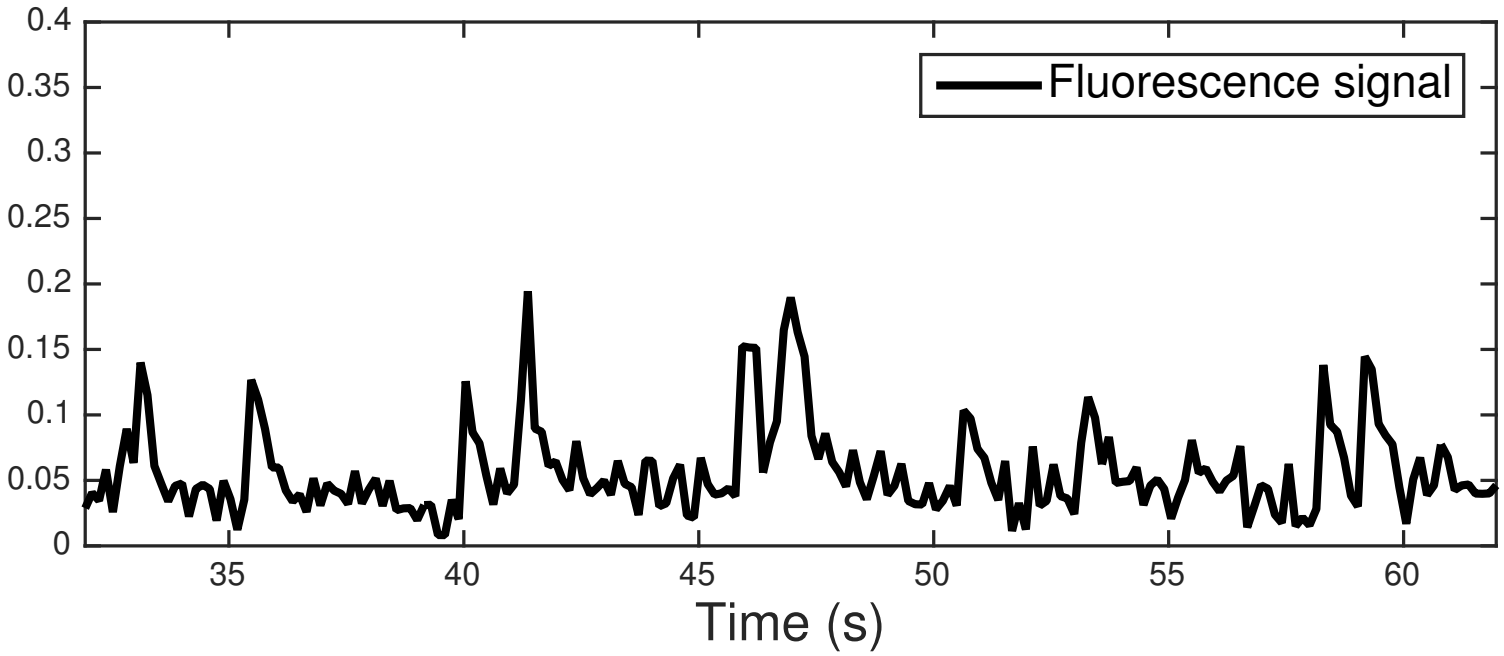
Calcium Transient Detection and Sparse Sampling



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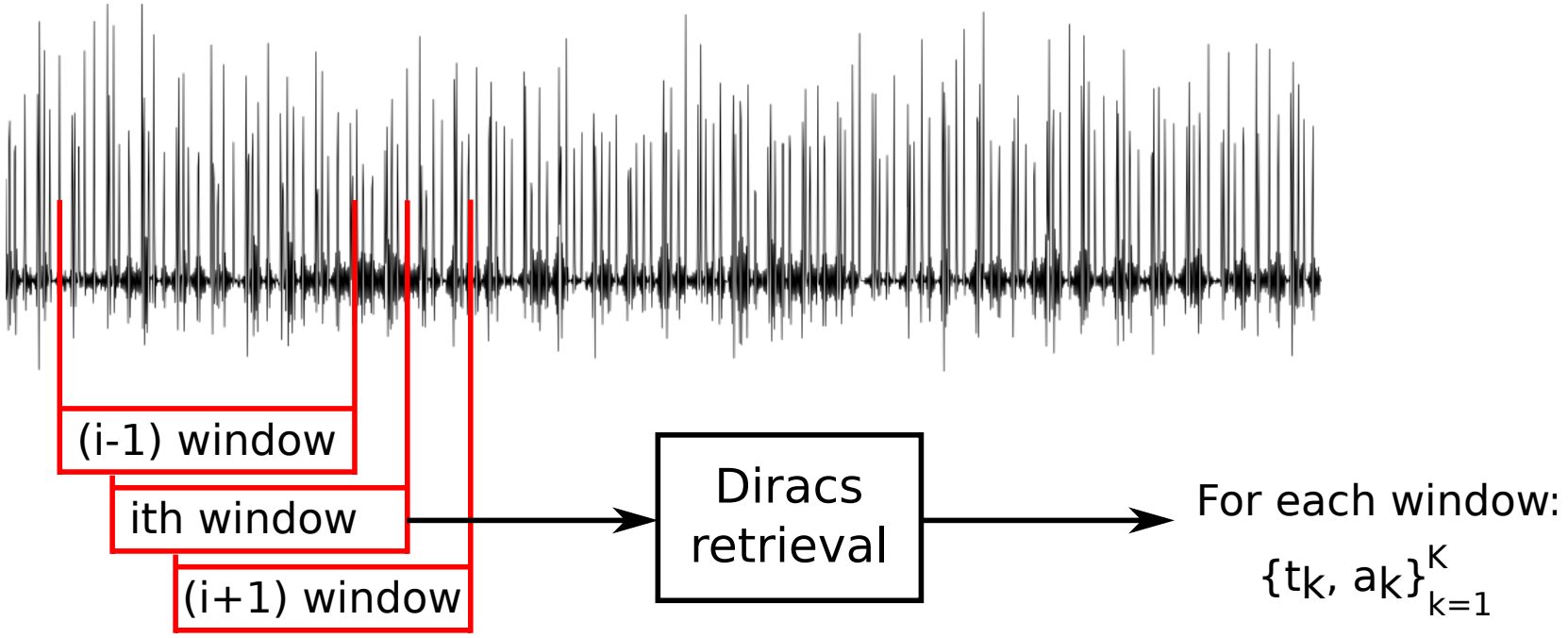
Calcium Transient Detection and Sparse Sampling



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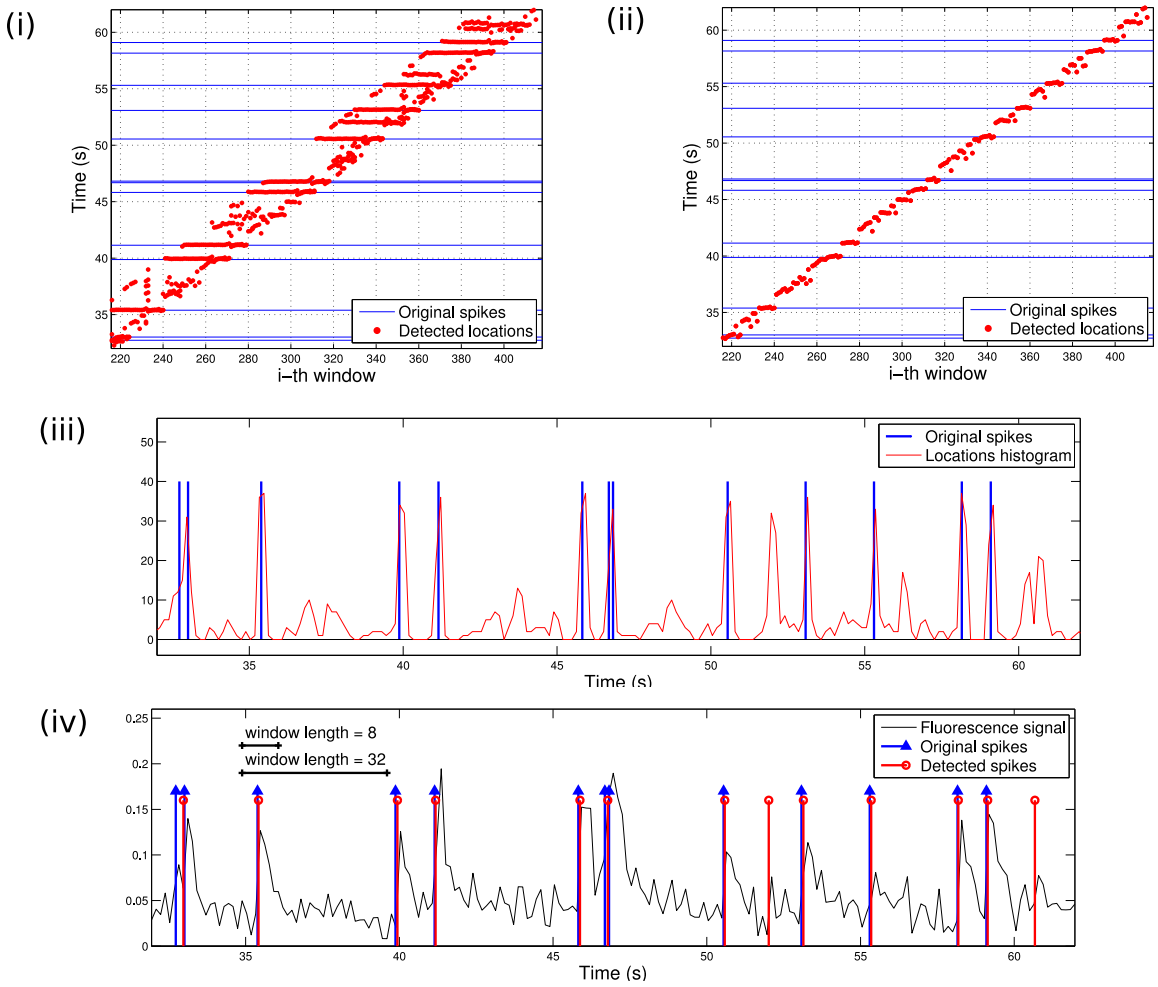
Calcium Transient Detection and Sparse Sampling



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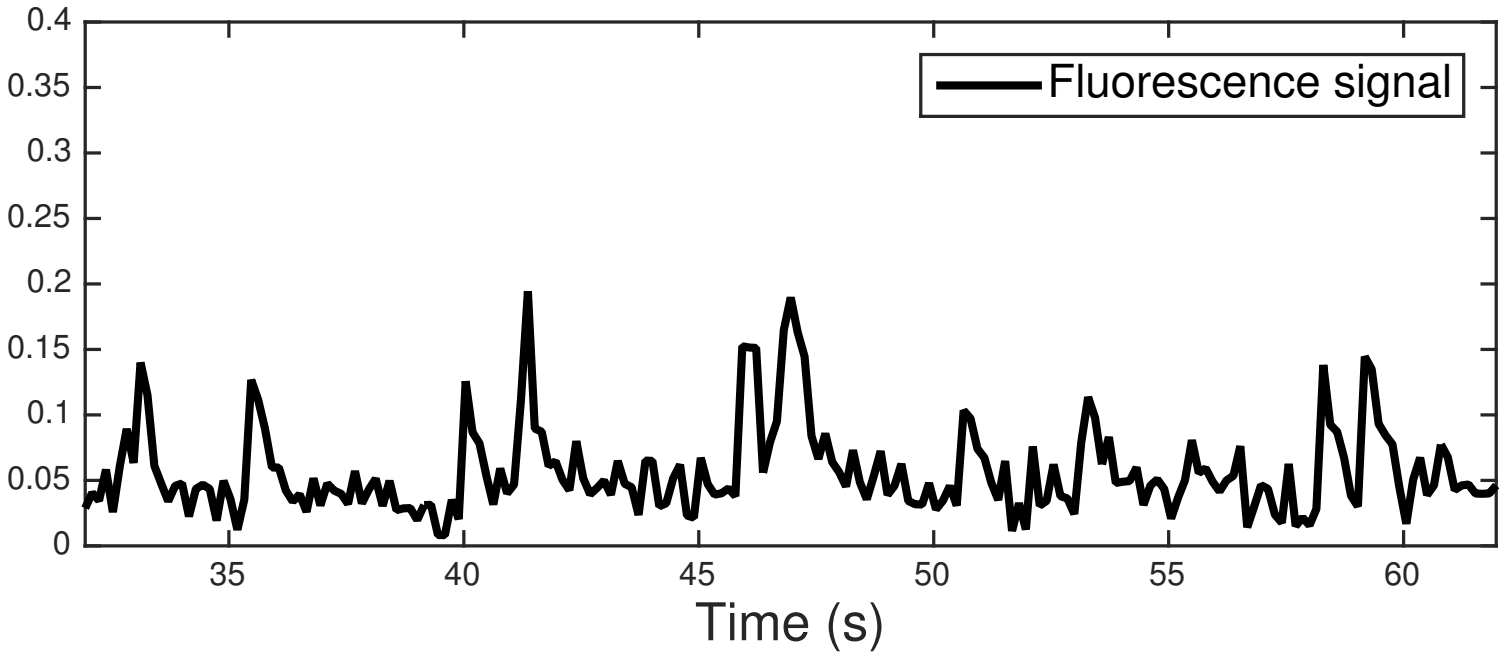
Calcium Transient Detection and Sparse Sampling



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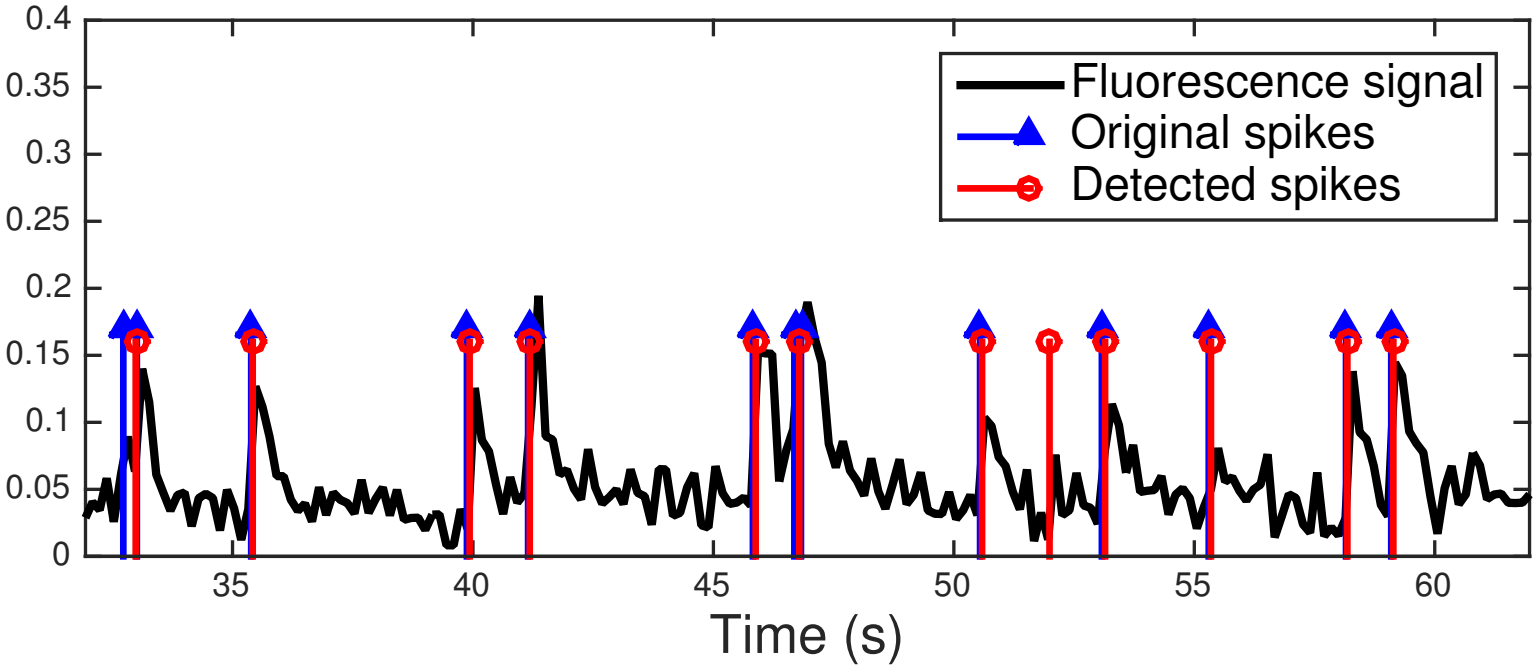
Calcium Transient Detection and Sparse Sampling



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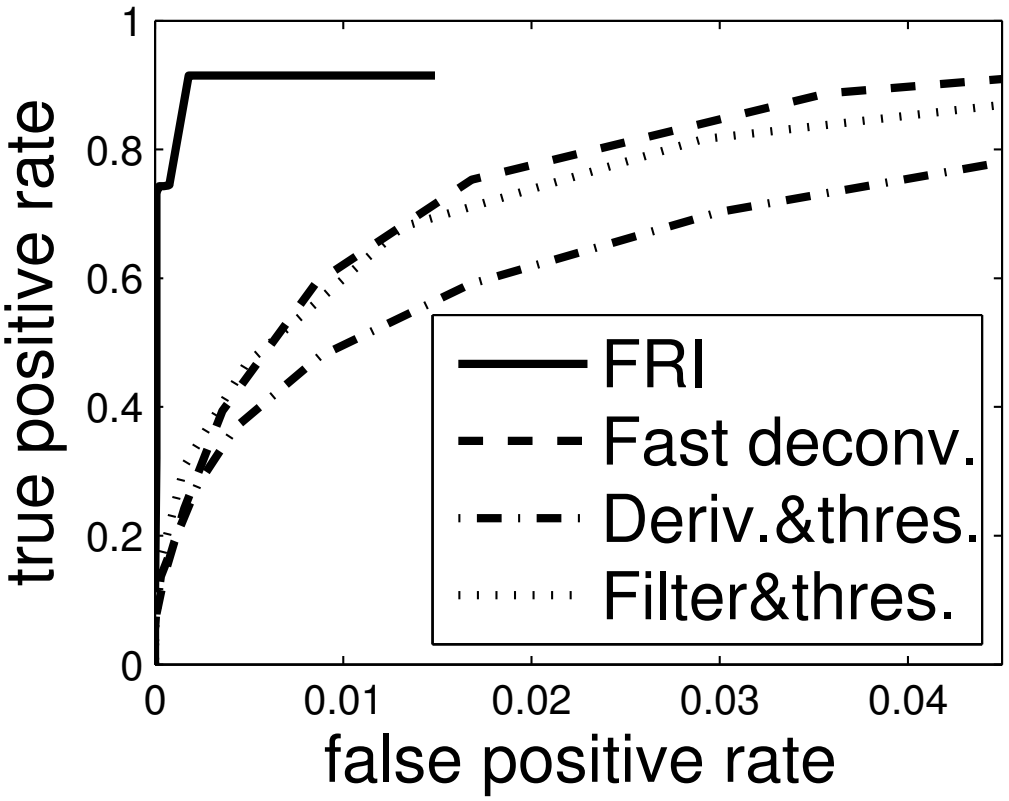
Sparse Sampling and Neuroscience



- *The algorithm **outperforms** state-of-the art methods*
- *Can operate in **real-time** simultaneously on 80 streams*
- *Increase in **resolution** by factor 3*

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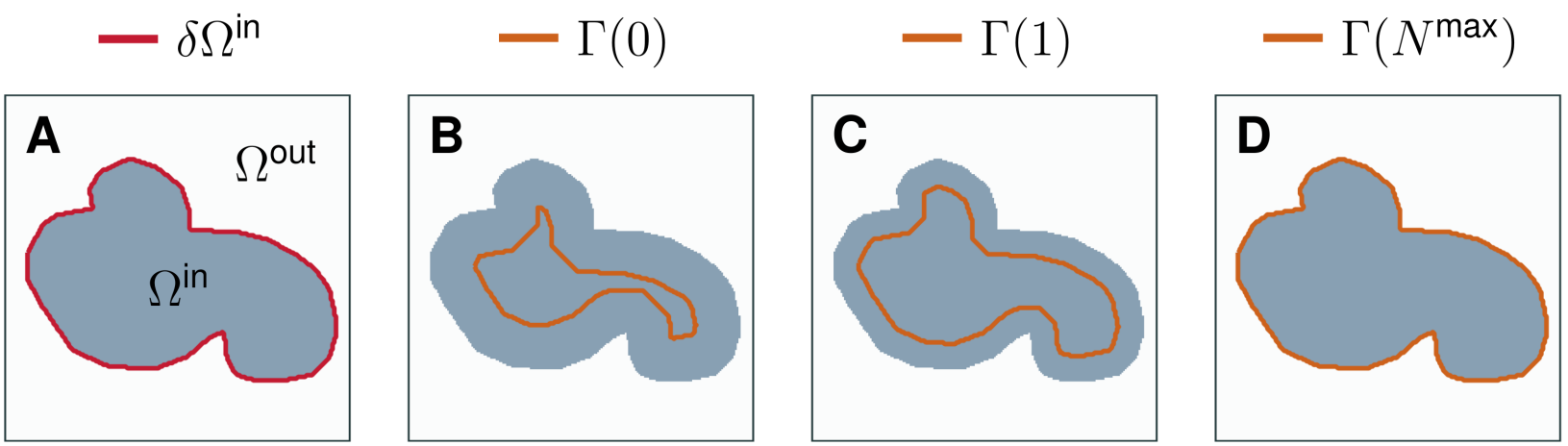
Segmentation by energy minimisation: 2D example

We search for the partition of Ω which minimises this energy:

$$E(\Omega^+, \Omega^-) = \int_{\Omega^+} |V(\boldsymbol{x}) - c^+|^2 d\boldsymbol{x} + \int_{\Omega^-} |V(\mathbf{x}) - c^-|^2 d\mathbf{x}\,.$$

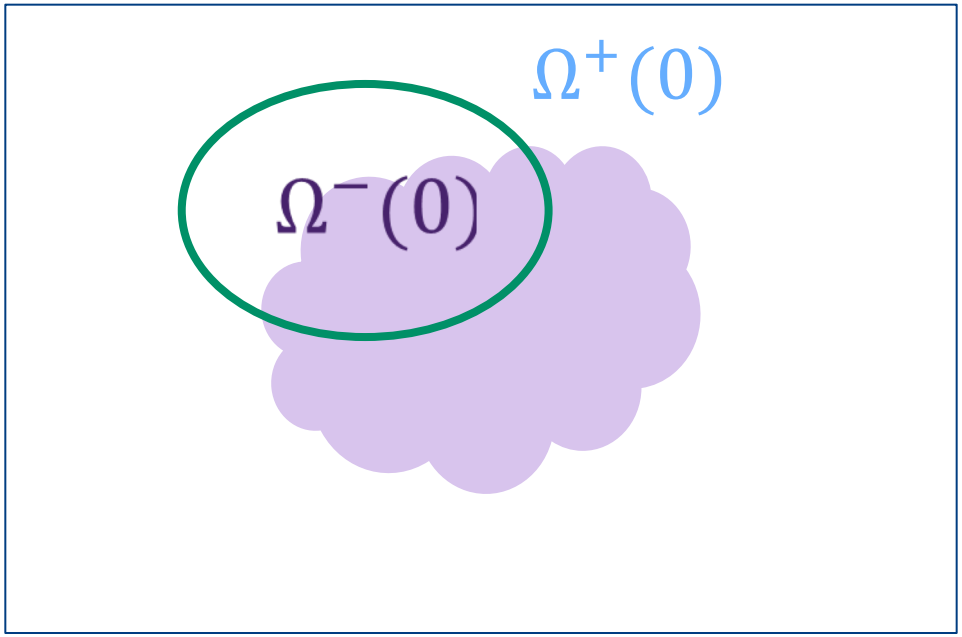
We update $\Omega^+(\tau)$ and $\Omega^-(\tau)$ at each iteration τ and calculate $c^+(\tau)$ and $c^-(\tau)$ as the average within each region, respectively.



Segmentation by energy minimisation: 2D example

Iteration $\tau = 0$:

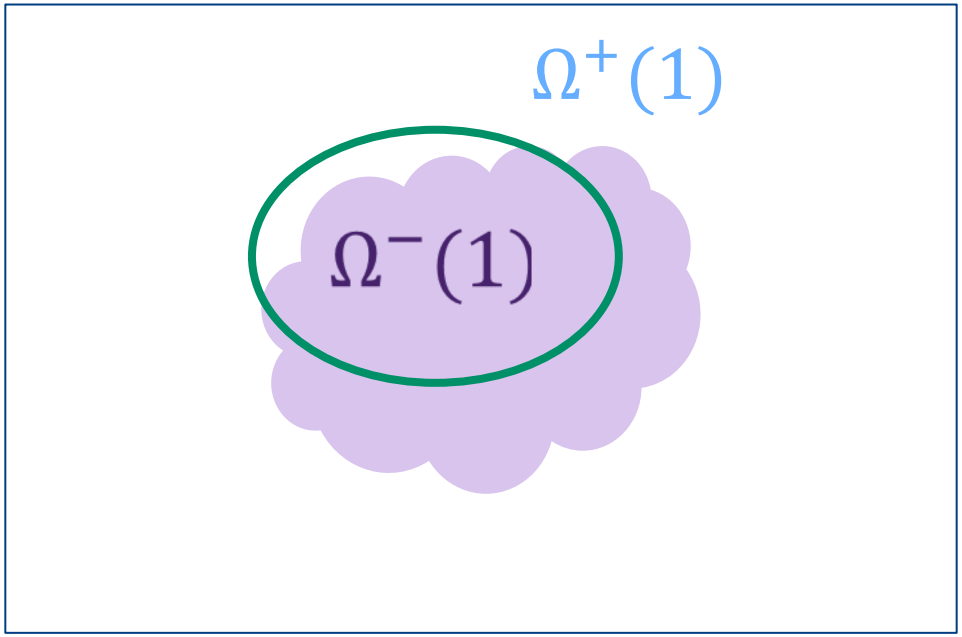
$$E(\Omega^+(0), \Omega^-(0)) = \int_{\Omega^+(0)} |V(\boldsymbol{x}) - c^+|^2 d\boldsymbol{x} + \int_{\Omega^-(0)} |V(\mathbf{x}) - c^-|^2 d\mathbf{x}$$



Segmentation by energy minimisation: 2D example

Iteration $\tau = 1$:

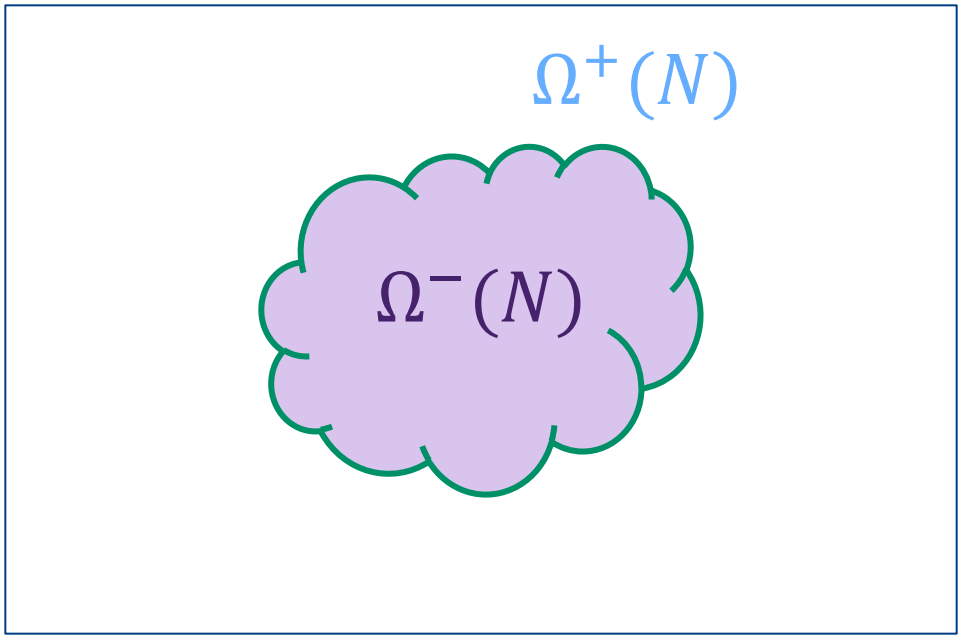
$$E(\Omega^+(1), \Omega^-(1)) = \int_{\Omega^+(1)} |V(\boldsymbol{x}) - c^+|^2 d\boldsymbol{x} + \int_{\Omega^-(1)} |V(\mathbf{x}) - c^-|^2 d\mathbf{x}$$



Segmentation by energy minimisation: 2D example

Final iteration, $\tau = N$:

$$E(\Omega^+(N), \Omega^-(N)) = \int_{\Omega^+(N)} |V(\boldsymbol{x}) - c^+|^2 d\boldsymbol{x} + \int_{\Omega^-(N)} |V(\mathbf{x}) - c^-|^2 d\mathbf{x} = \mathbf{0}$$



Segmentation using Active Contours

- This approach based on evolving the curve is known as active contour
- The contour can be modelled parametrically or implicitly
- The level-set method models the contour implicitly
- Several advantages in using the level-set method:
  - Easier to evolve
  - Allows changes of topology (split or merge)
  - No prior on the shape of the region to be segmented
  - Naturally scale to higher dimensions

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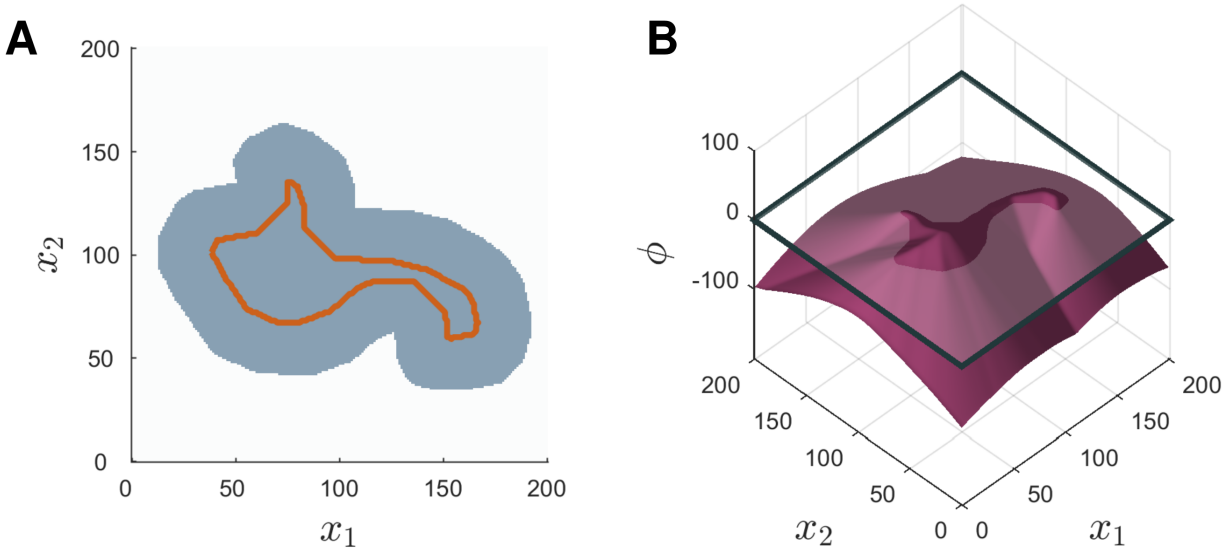
Simplification: define regions by single function ϕ



Define $\phi: \Omega \rightarrow \mathbb{R}$, such that

$$\phi(\boldsymbol{x}) < 0 \quad \leftrightarrow \quad \boldsymbol{x} \in \Omega^-$$

$$\phi(\boldsymbol{x}) > 0 \quad \leftrightarrow \quad \boldsymbol{x} \in \Omega^+$$

$$\phi(\boldsymbol{x}) = 0 \quad \leftrightarrow \quad \boldsymbol{x} \in \delta\Omega^-$$

Segmentation by energy minimisation

Write the minimisation in terms of ϕ

$$\phi^* = \text{argmin}_\phi\{E(\phi)\},$$

and evolve ϕ by gradient descent to minimise $E(\phi)$

$$\frac{\partial \phi}{\partial \tau} = -\frac{\partial E}{\partial \phi} + \mu \frac{\partial R}{\partial \phi}.$$

We add a smoothing term to keep ϕ well conditioned.

We then solve this PDE numerically:

$$\frac{\phi^{\tau+1} - \phi^\tau}{\Delta \tau} = -\frac{\partial E(\tau)}{\partial \phi} + \mu \frac{\partial R(\tau)}{\partial \phi}.$$

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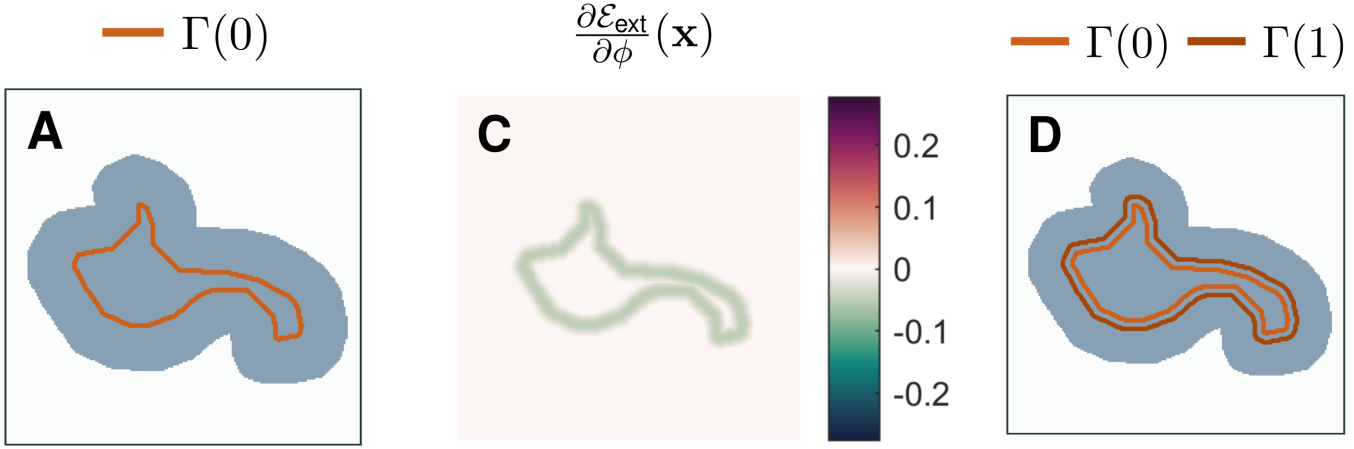
Segmentation by energy minimisation

We evolve ϕ as follows:

$$\phi(\tau + \Delta\tau) = \phi(\tau) - \Delta\tau(\lambda \frac{\partial E}{\partial \phi} + \mu \frac{\partial R}{\partial \phi})$$

with

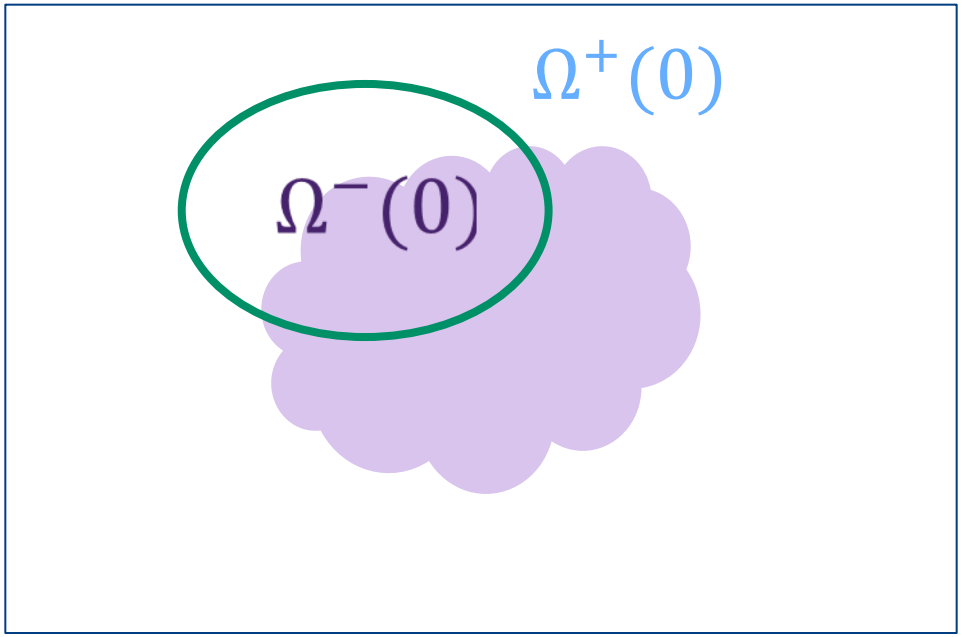
$$\frac{\partial E}{\partial \phi}(x) = \delta_\varepsilon(\phi(x))\{|V(x) - c^+|^2 - |V(x) - c^-|^2\}$$



Segmentation by energy minimisation: 2D example

Iteration $\tau = 0$:

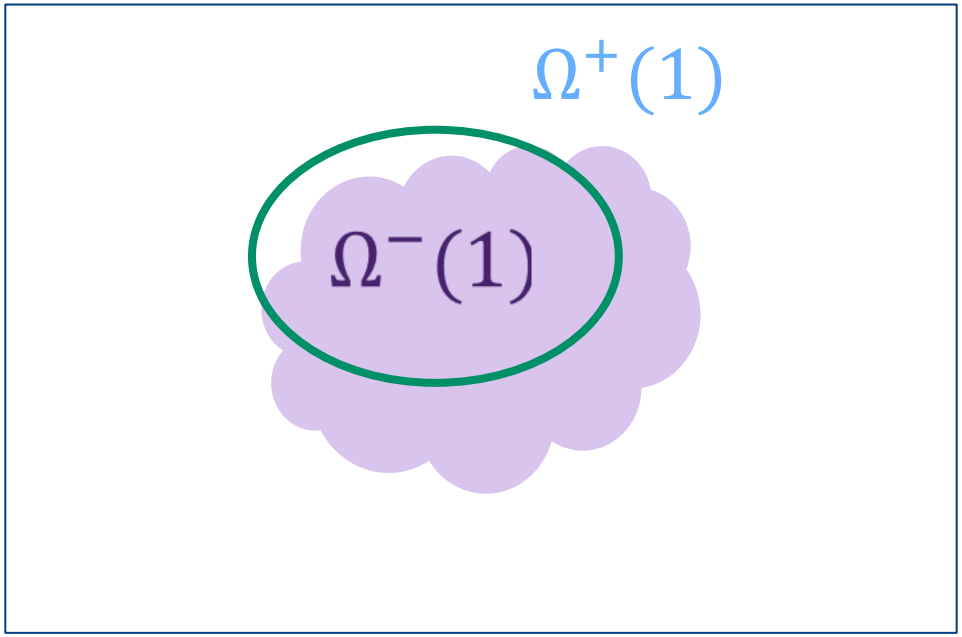
$$E(\Omega^+(0), \Omega^-(0)) = \int_{\Omega^+(0)} |V(\boldsymbol{x}) - c^+|^2 d\boldsymbol{x} + \int_{\Omega^-(0)} |V(\mathbf{x}) - c^-|^2 d\mathbf{x}$$



Segmentation by energy minimisation: 2D example

Iteration $\tau = 1$:

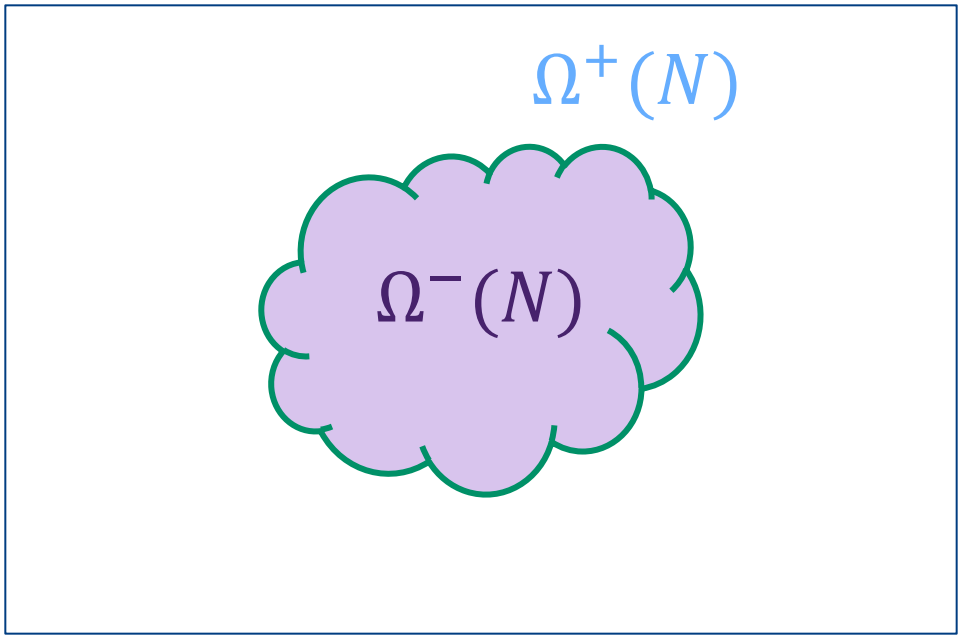
$$E(\Omega^+(1), \Omega^-(1)) = \int_{\Omega^+(1)} |V(\boldsymbol{x}) - c^+|^2 d\boldsymbol{x} + \int_{\Omega^-(1)} |V(\mathbf{x}) - c^-|^2 d\mathbf{x}$$



Segmentation by energy minimisation: 2D example

Final iteration, $\tau = N$:

$$E(\Omega^+(N), \Omega^-(N)) = \int_{\Omega^+(N)} |V(\boldsymbol{x}) - c^+|^2 d\boldsymbol{x} + \int_{\Omega^-(N)} |V(\mathbf{x}) - c^-|^2 d\mathbf{x} = \mathbf{0}$$



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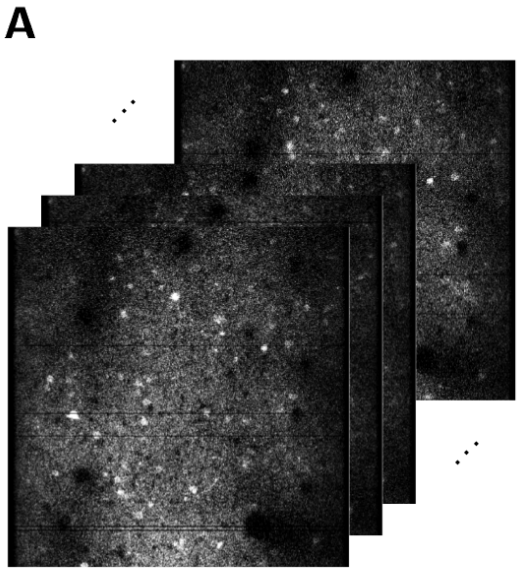
Level-Set Method for Calcium Imaging Data

- We need to perform 2-D segmentation but the data is 3-D (2-D+t)
- Dissimilarity metric decided according to the type of dye
- Typical choice is the Euclidean distance



We evolve ϕ at each pixel $\boldsymbol{x}$ according to

$$\frac{\partial \phi}{\partial \tau}(\boldsymbol{x}) = -\frac{\partial E}{\partial \phi}(\boldsymbol{x}) = \delta_{\epsilon}\big(\phi(\boldsymbol{x})\big)\{|V(\boldsymbol{x},t) - c^{-}(t)|^2 - |V(\boldsymbol{x},t) - c^{+}(t)|^2\}.$$

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Level-Set Method for Calcium Imaging Data

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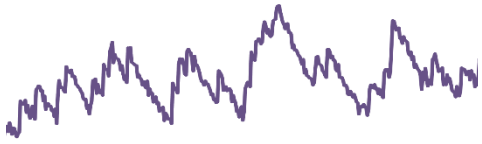
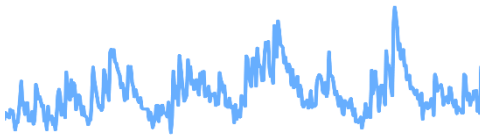
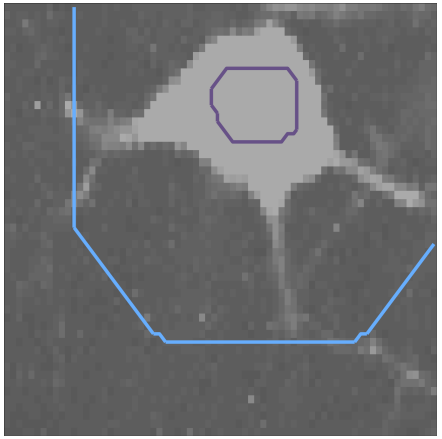
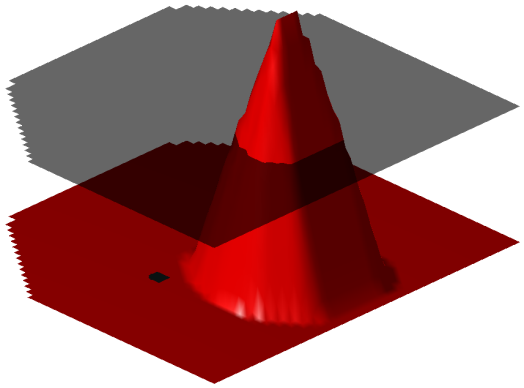
$$\frac{\partial \phi}{\partial \tau}(\boldsymbol{x}) = -\frac{\partial E}{\partial \phi}(\boldsymbol{x}) = \delta_\epsilon\big(\phi(\boldsymbol{x})\big)\{|V(\boldsymbol{x},t) - c^-(t)|^2 - |V(\boldsymbol{x},t) - c^+(t)|^2\}.$$

- Typical choice is the Euclidean distance

$c^-(t) =$ 

$c^+(t) =$ 





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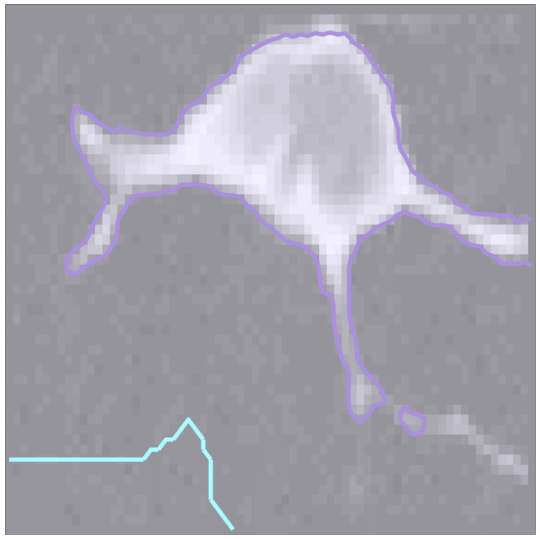
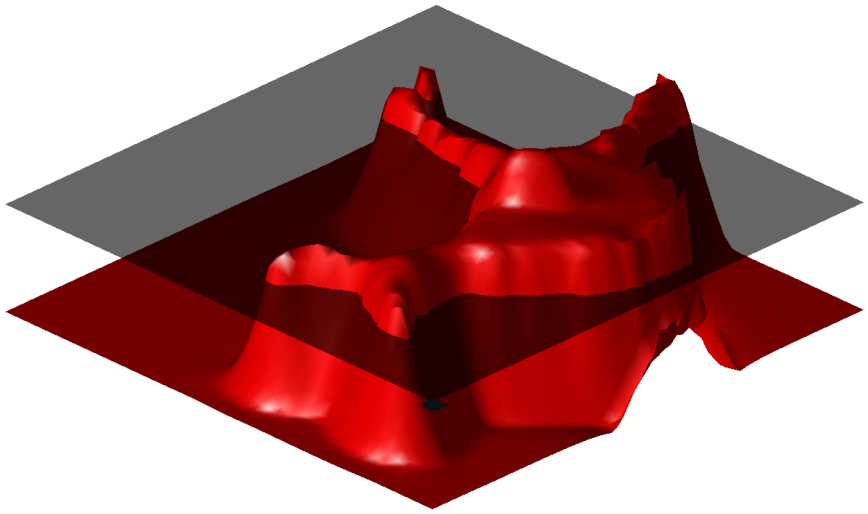
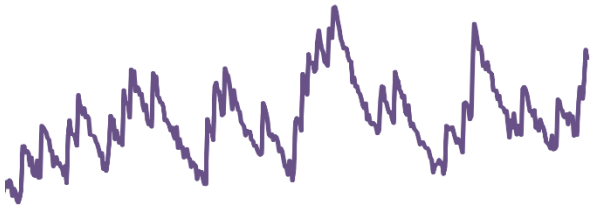
Segmentation result

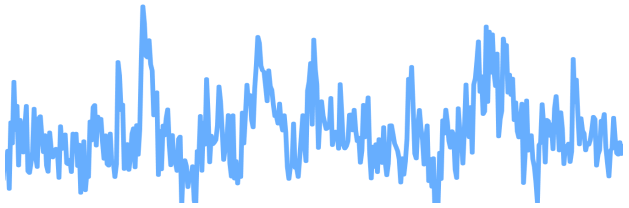
Zero level and narrowband



Final ϕ: contour that minimises energy



$c^-(t) =$ 

$c^+(t) =$ 

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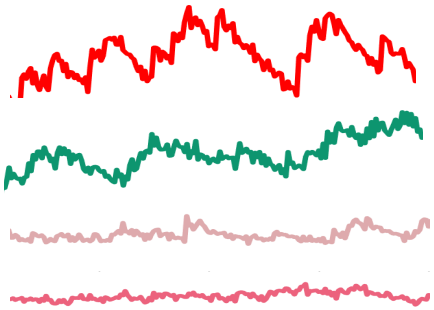
Extension to multiple regions

We evolve one function ϕ_i per region as the interior signals are distinct.



The ϕ_i evolve independently whilst there is no overlap.

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Coupling evolution in the case of overlap

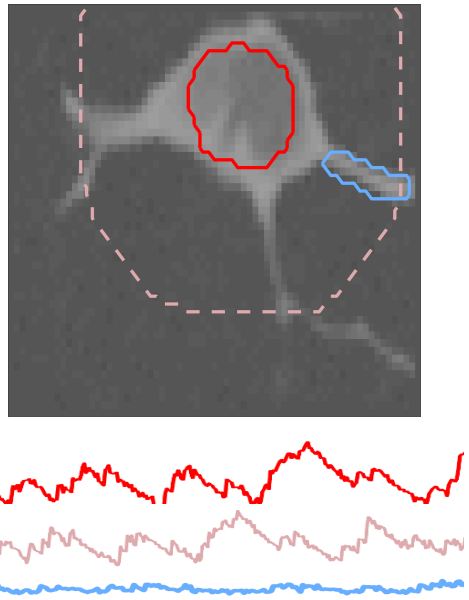
For pixels already in another cell we calculate the alternative velocity

$$\frac{\partial \phi}{\partial \tau}(\boldsymbol{x}) = \delta_\epsilon(\phi(\boldsymbol{x}))[V_{\text{out}} - V_{\text{in}}]$$

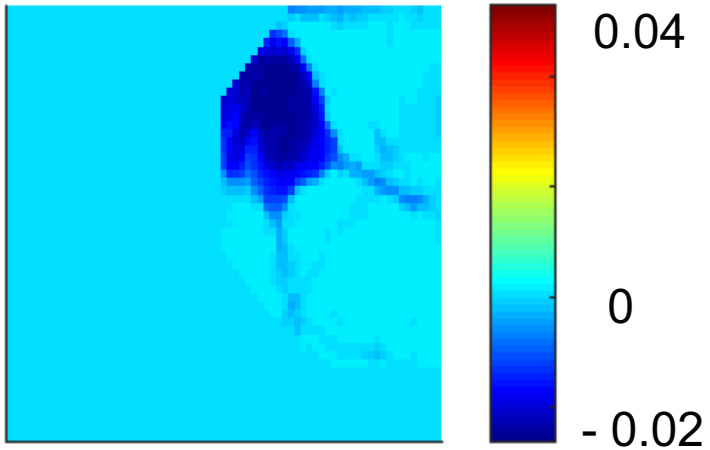
where

$$V_{out} = \min\{|V(x,t) - n|^2, |V(x,t) - b|^2\},$$

$$V_{in} = \min\{|V(x,t) - a|^2, |V(x,t) - a - b|^2\}.$$

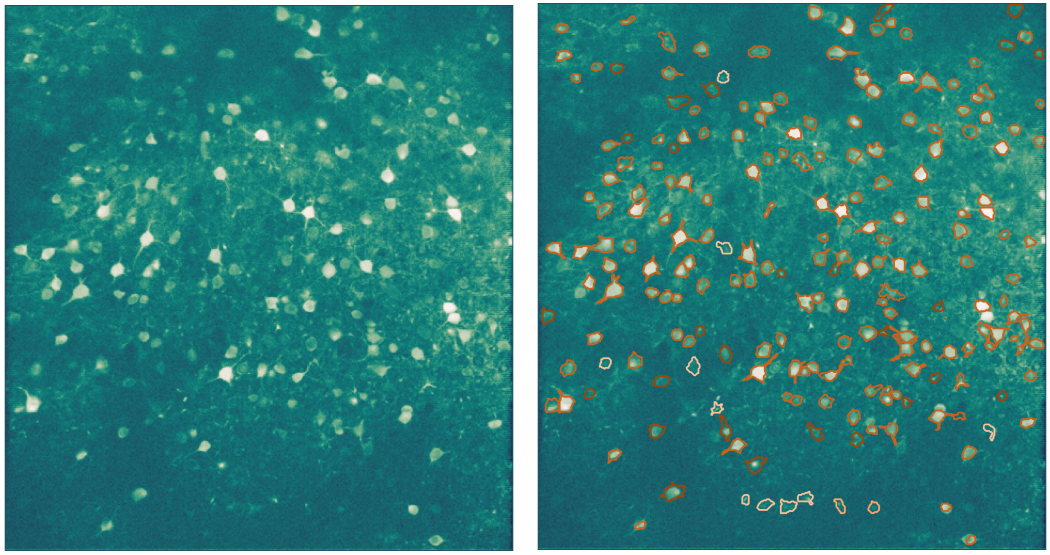


Velocity of smaller region:



Results...

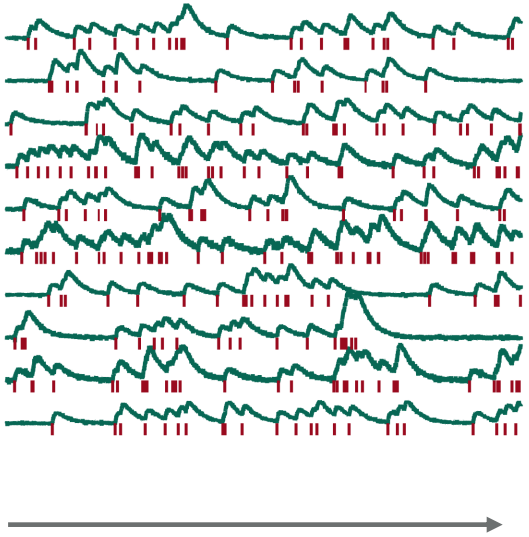
Detecting neuronal activity from calcium imaging data



DETECT CELL LOCATIONS

EXTRACT CELLULAR SIGNALS

DETECT SPIKES



Time (s)

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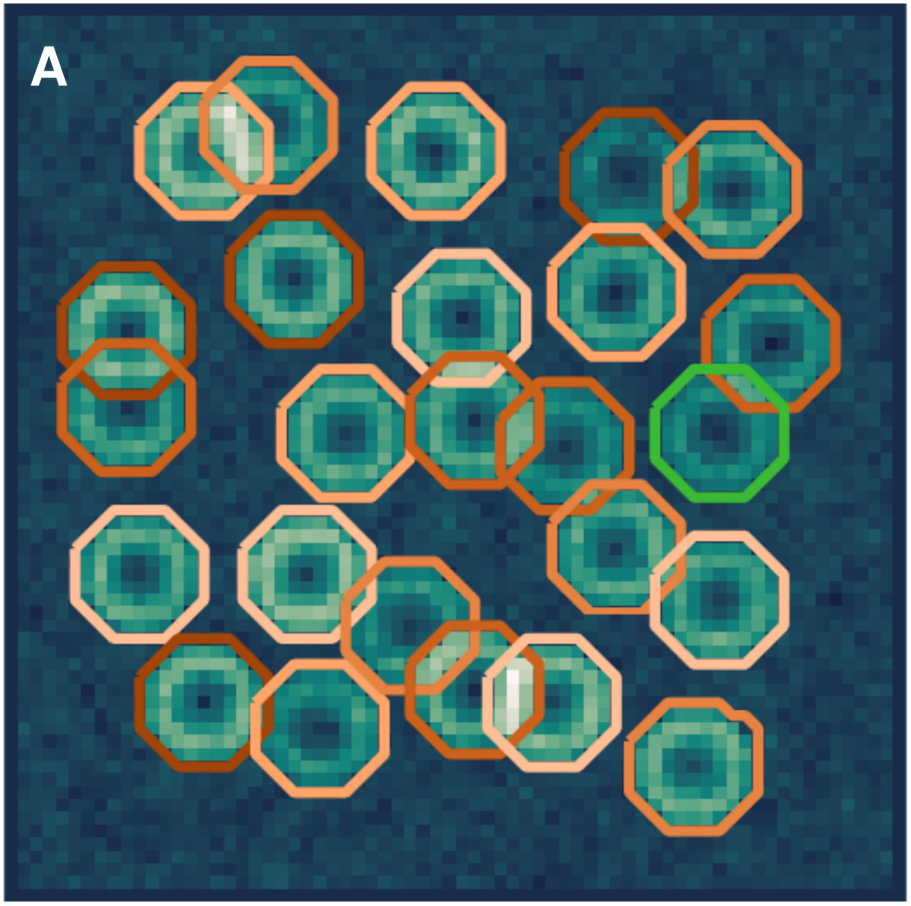
Segmentation of overlapping cells



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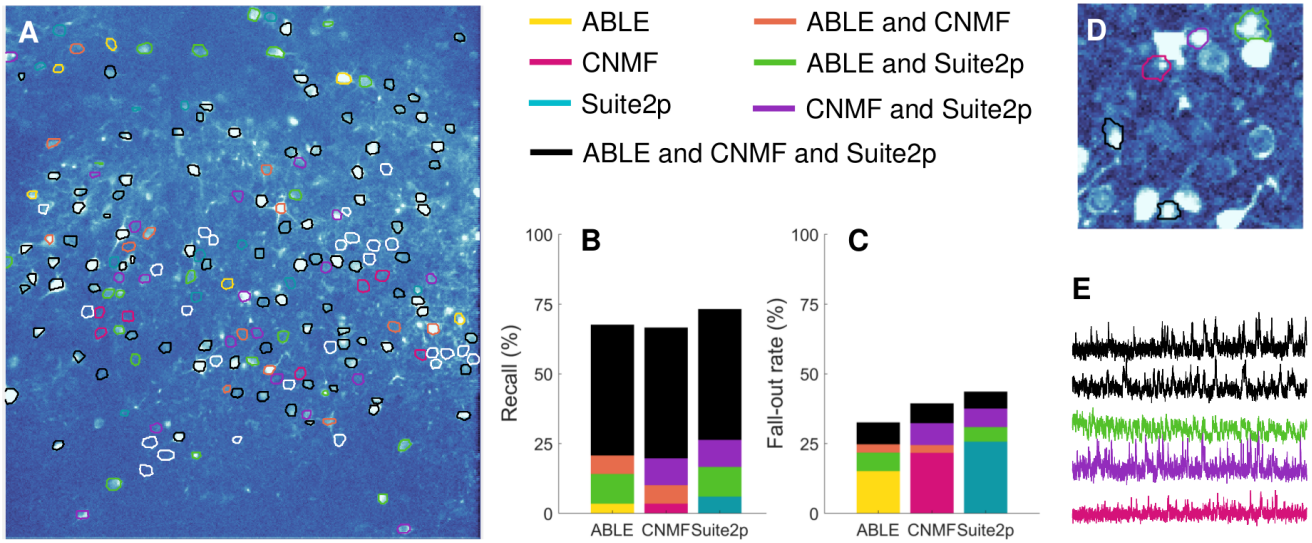
Comparisons



	Success rate (%)	Precision (%)	Recall (%)
ABLE	67.5	67.5	67.5
CNMF	63.4	60.7	66.5
Suite2p	63.7	56.5	73.1

Conclusions

- We have extended sparse sampling theory methods for calcium transient detection
- We have developed a segmentation algorithm for calcium imaging data based on the level set method.
- We have shown results on real data.

Future work

- Co-design of hardware and software to achieve fast scanning
- Inference of functional topology from large scale calcium imaging data (requires graph theory)

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Main References

- *S.Reynolds, Detecting Cells and Cellular Activity from Two-Photon Calcium Imaging Data, PhD thesis, Imperial College London 2018*
- *S.Reynolds et al. ABLE: An Activity-Based Level Set Segmentation Algorithm for Two-Photon Calcium Imaging Data, (open access), eNeuro, October 2017.*
- *Jon Onativia, Simon R. Schultz, and Pier Luigi Dragotti, A Finite Rate of Innovation algorithm for fast and accurate spike detection from two-photon calcium imaging, Journal of Neural Engineering, August 2013.*

Thanks for listening!