# Lecture 7: Networks in the Context of Neuroscience

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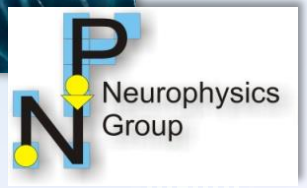




# 1. From yesterday… to today

■ Living neuronal systems as a complex network: designate first the **nodes** (voxels, neurons) and the **links** (functional connections, synapses).

■ The **network measures** reflect important features about the structure of the network, its integrity and 'modus operandi'.

■ To better understand the role of topological features one can use smaller networks, i.e. at a **mesoscopic** scale.

▫ Mesoscopic approaches combine models with a large but tractable number of elements, experiments, and the repertoire of resources from network theory and dynamical systems. It is an attractive approach in both Physics and Neuroscience.

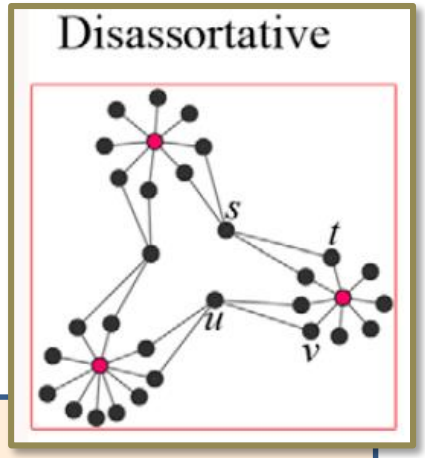
▫ 'In vitro' experiments in the form of neuronal cultures offer an excellent platform to investigate key aspects**: structural and functional connectivity, network structure, and resilience to damage**.

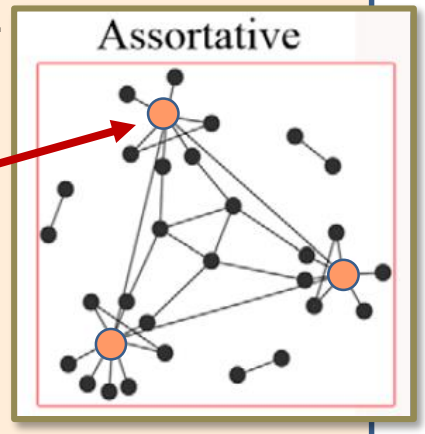
**This lecture** covers examples of the use of network measures in 'in vitro' preparations.
Lecture 8 will cover network modeling as a dynamical system.
Lectures 9 and 10 will treat how to gain further insight into network structure from Physics.

# 2. Resources from network theory

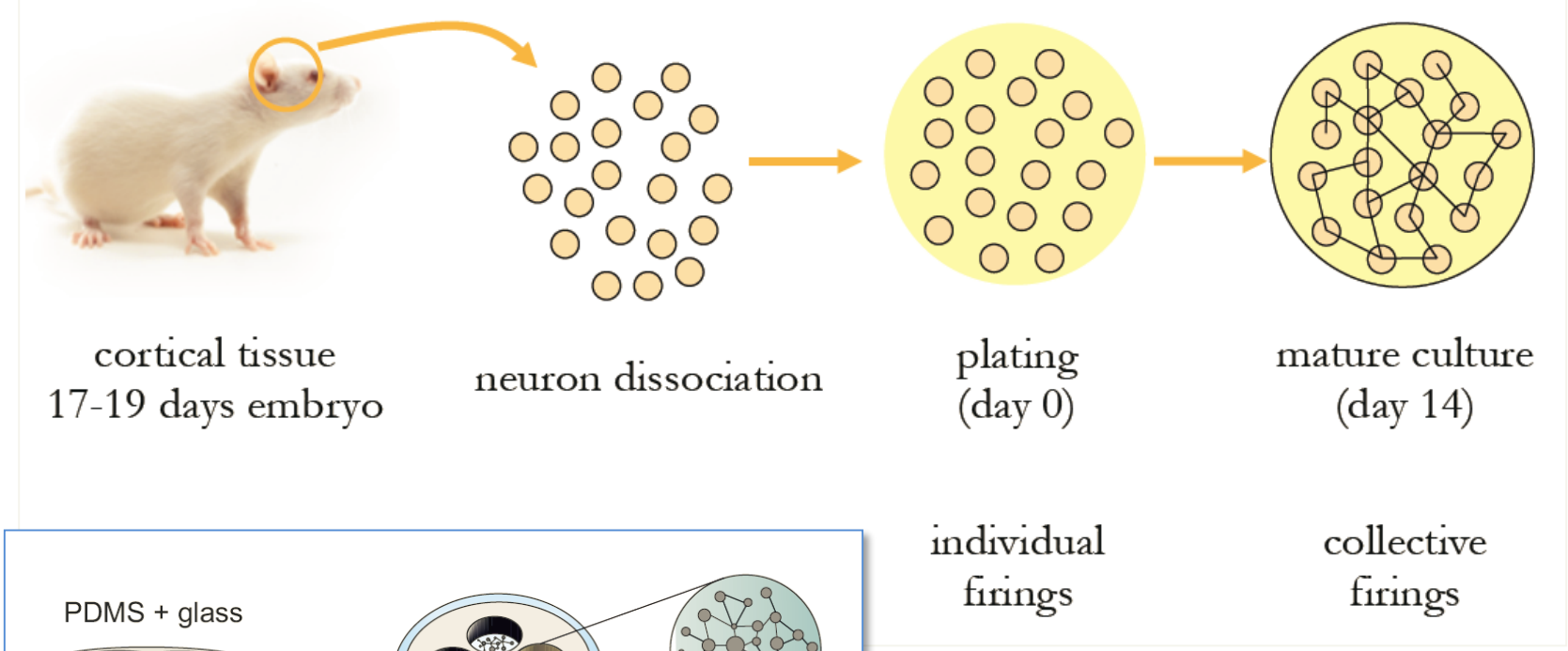
- These resources have been introduced by Jesús Gómez
  - Network description: nodes, links, distribution of connections.
  - Network measures: clustering, small-world phenomena…
  - Complex features: hierarchical organization, multiplex networks,…
  - Resilience aspects: hubs, node deletion, cascades of failure…

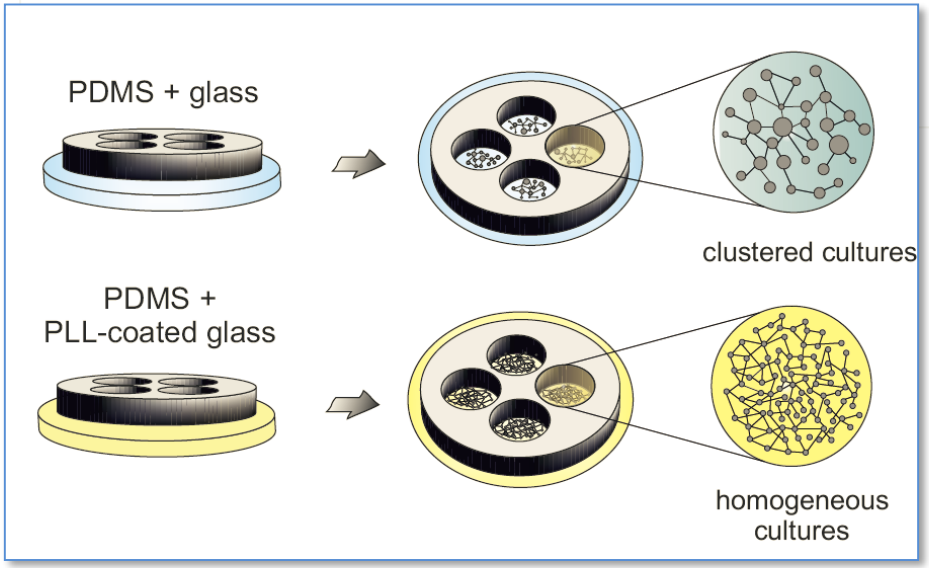
- Other brain-relevant measures and concepts include:
  - **Assortativity**, i.e. the tendency of nodes to connect with others of similar degree (high-high; low-low). **Dissassort**: low-high.
  - **Rich-club core**, i.e. the existence of a subset of nodes with high degree and mutual interconnectivity.
  - The identification of a node as a **hub**.
  - Resilience of living neuronal networks to **random or targeted** attacks (deletion of nodes).



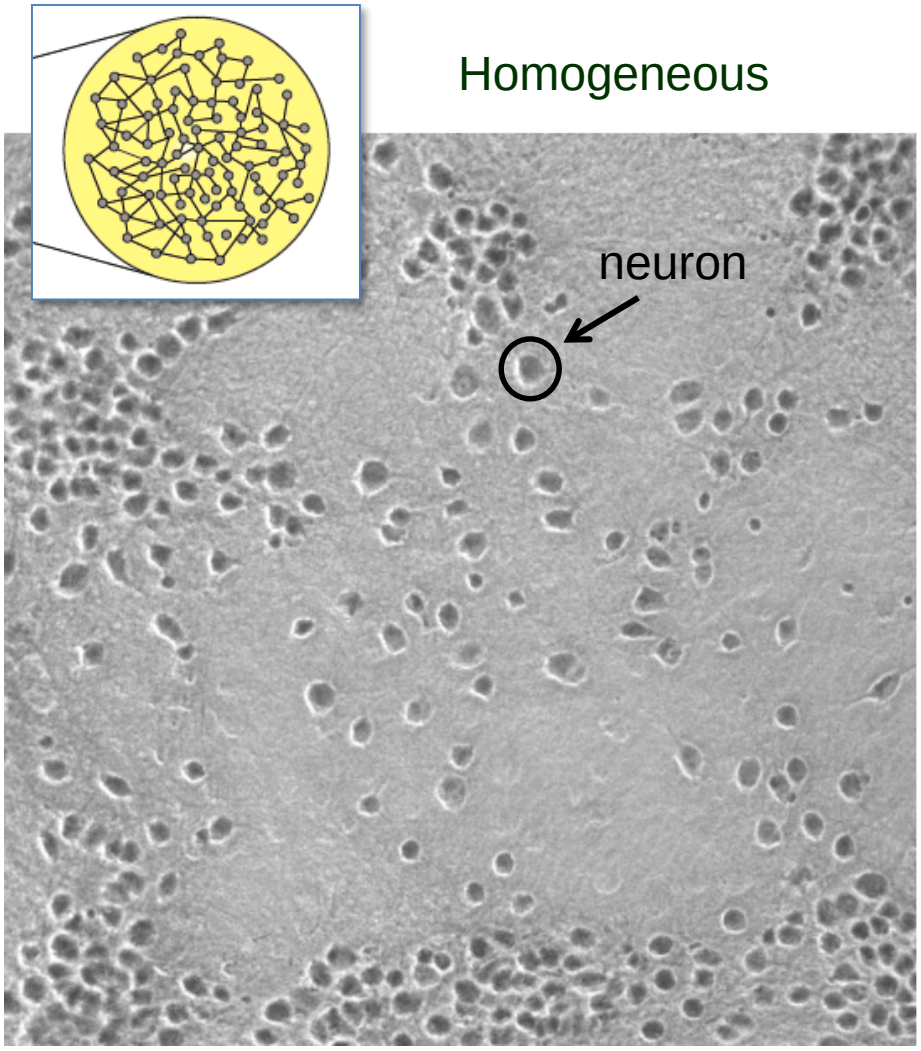



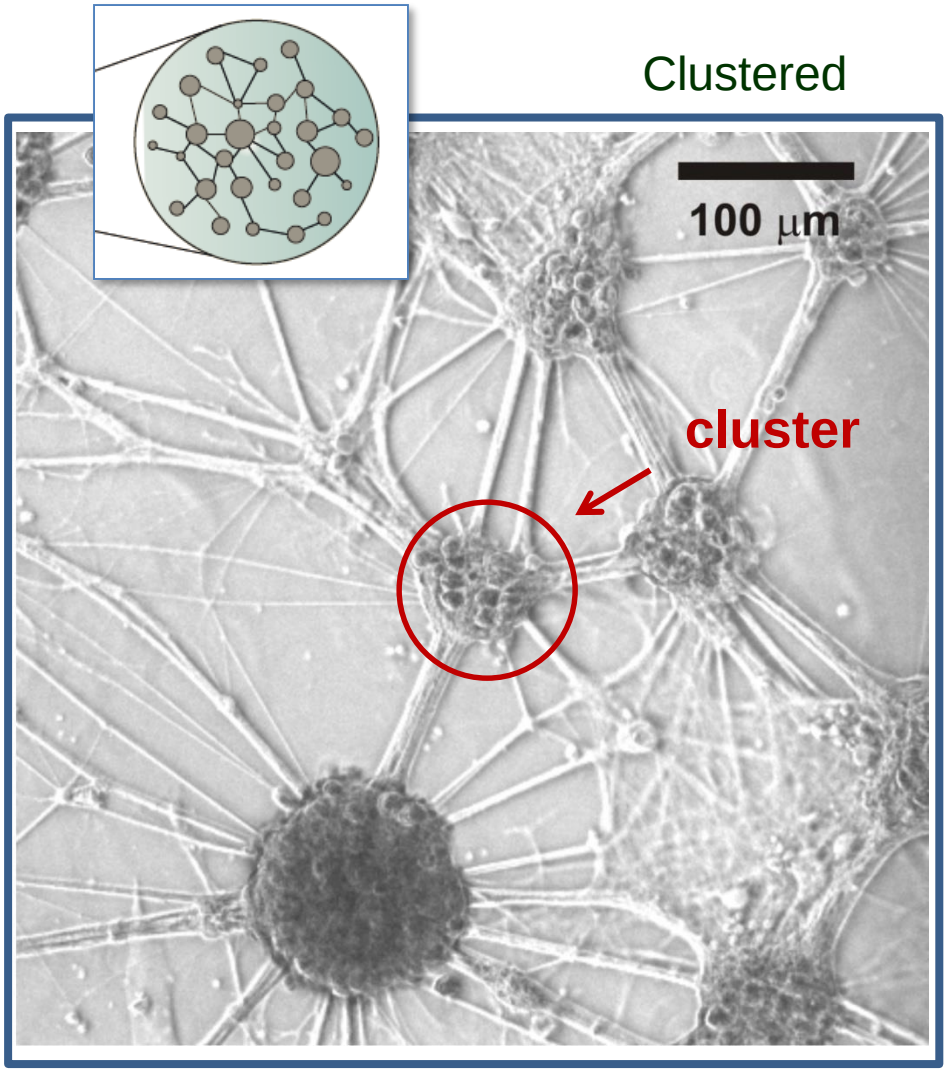
# 3. Introducing neuronal cultures



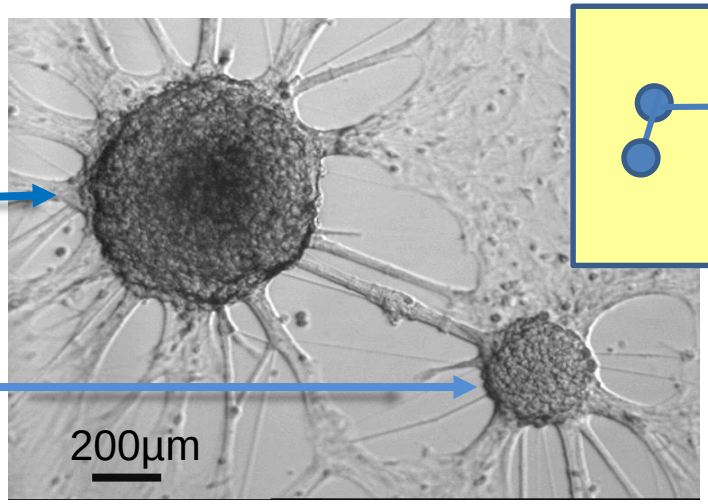
# 3. Introducing neuronal cultures

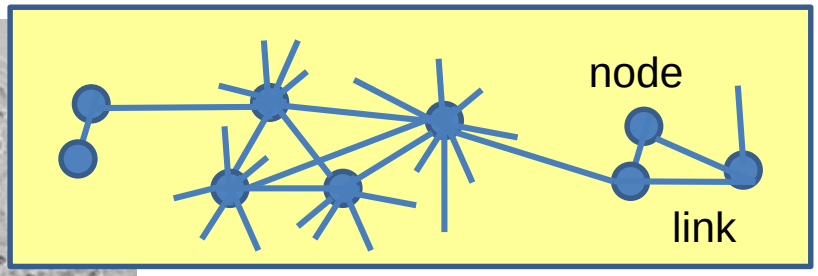


# 4. Clustered neuronal networks

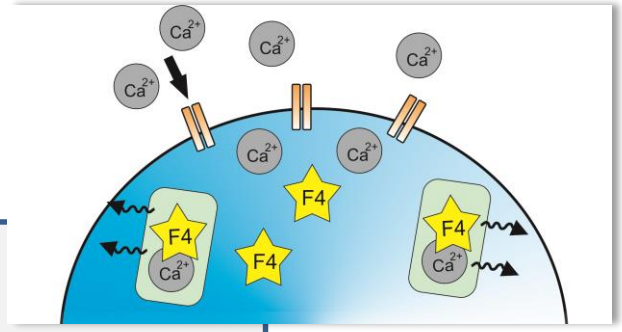
■ Offer higher control, since nodes are well visible and their number is small (around 50 in the culture). Some connections can be resolved.

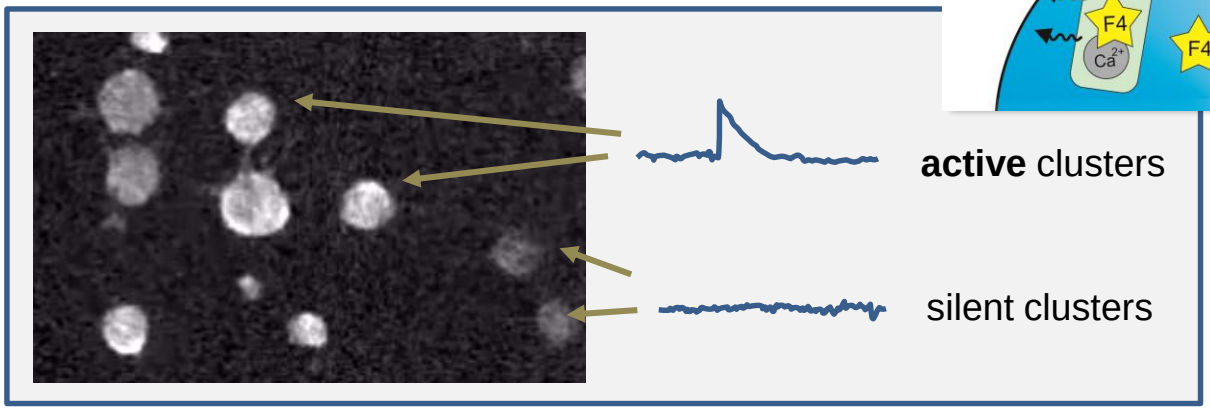


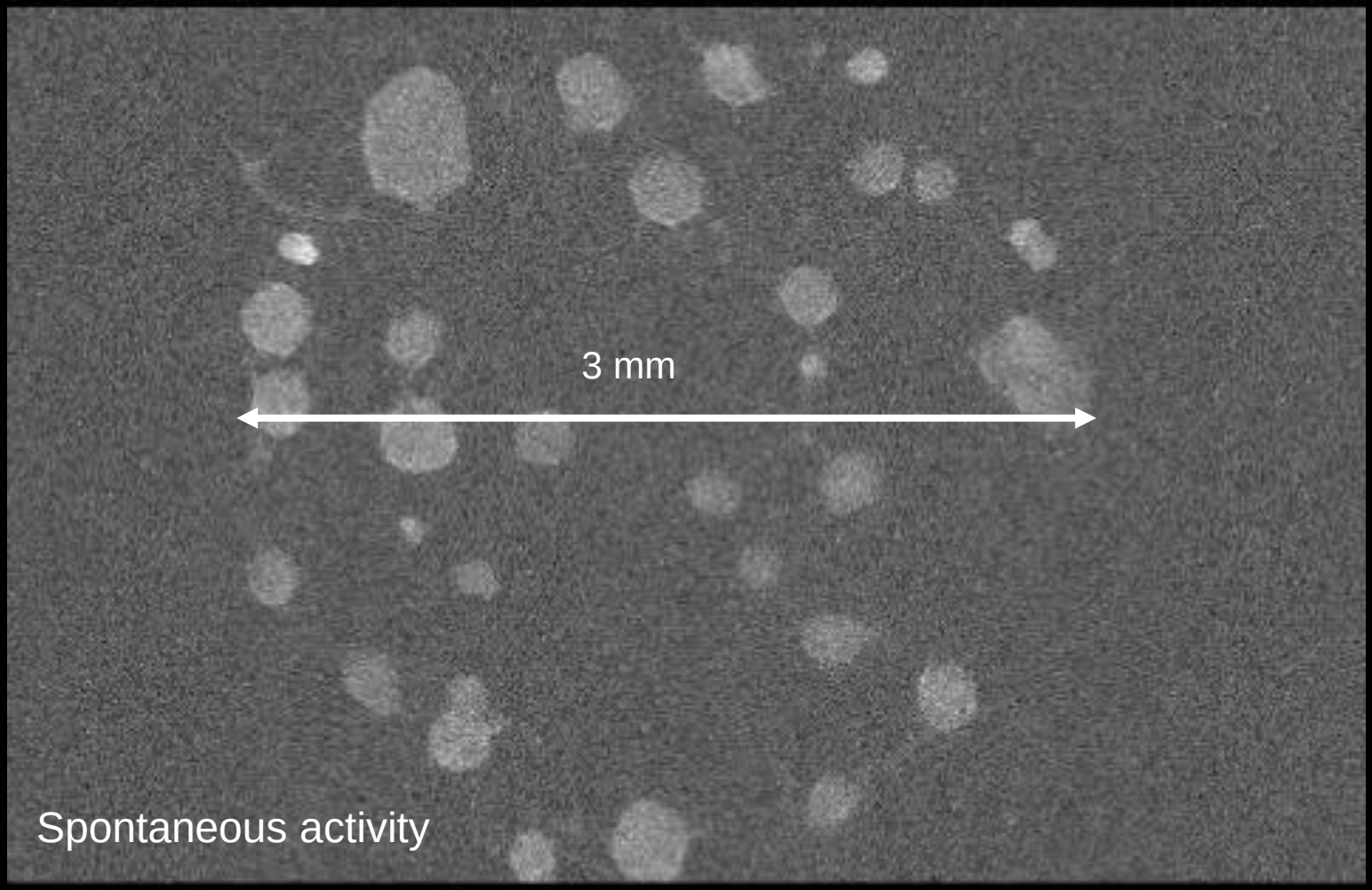




■ How do I measure?

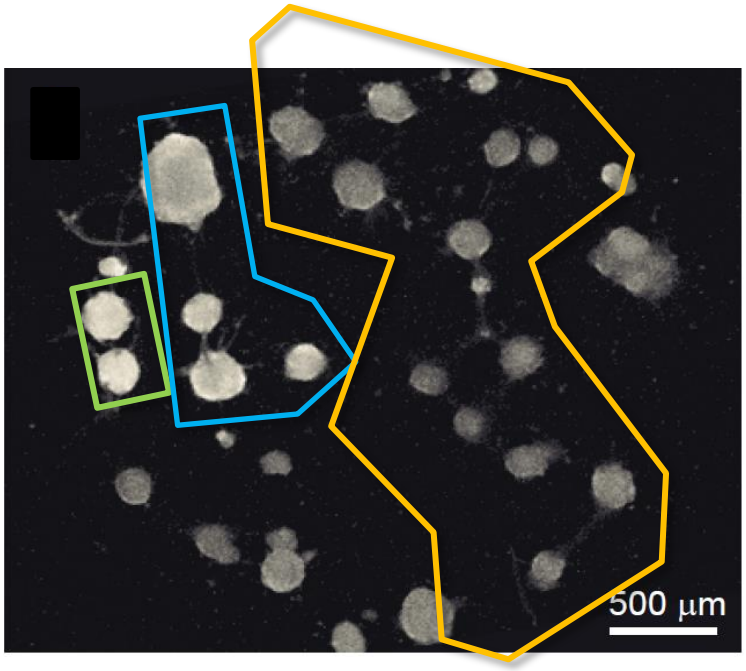
Calcium imaging: fluorescent calcium probe + **camera.**



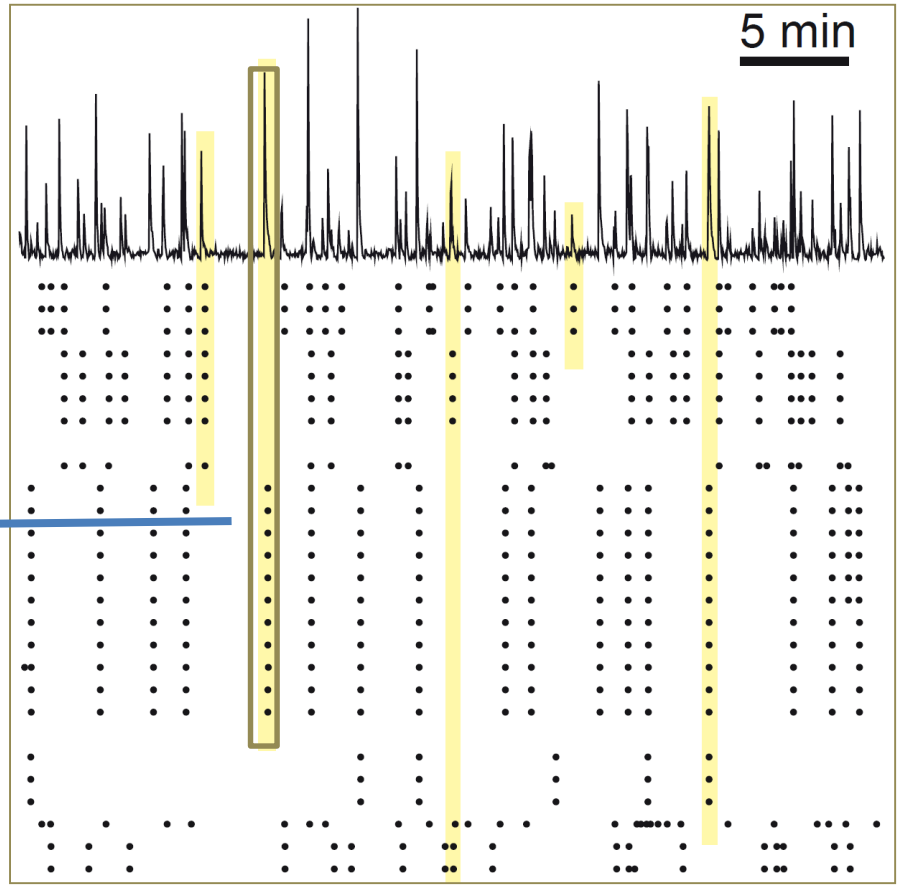




3 mm
Spontaneous activity

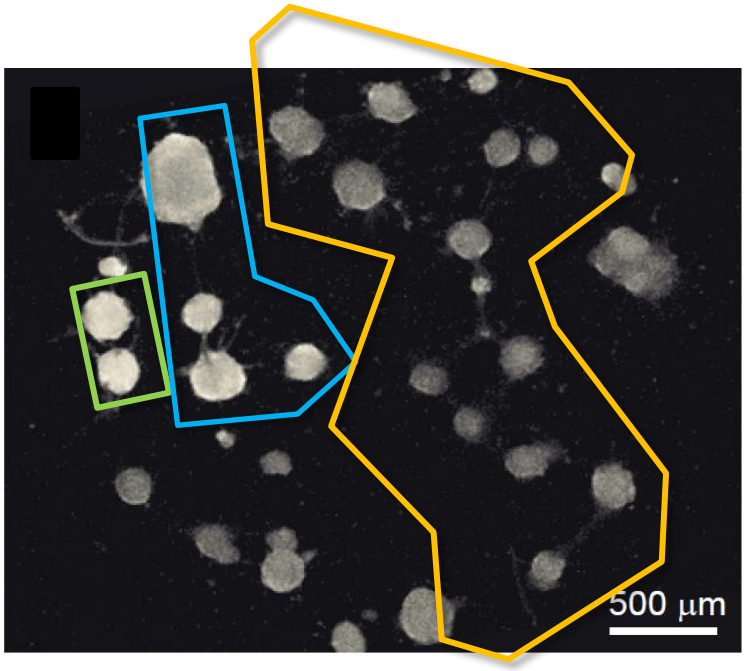
# 5. Characteristic dynamics of clustered networks

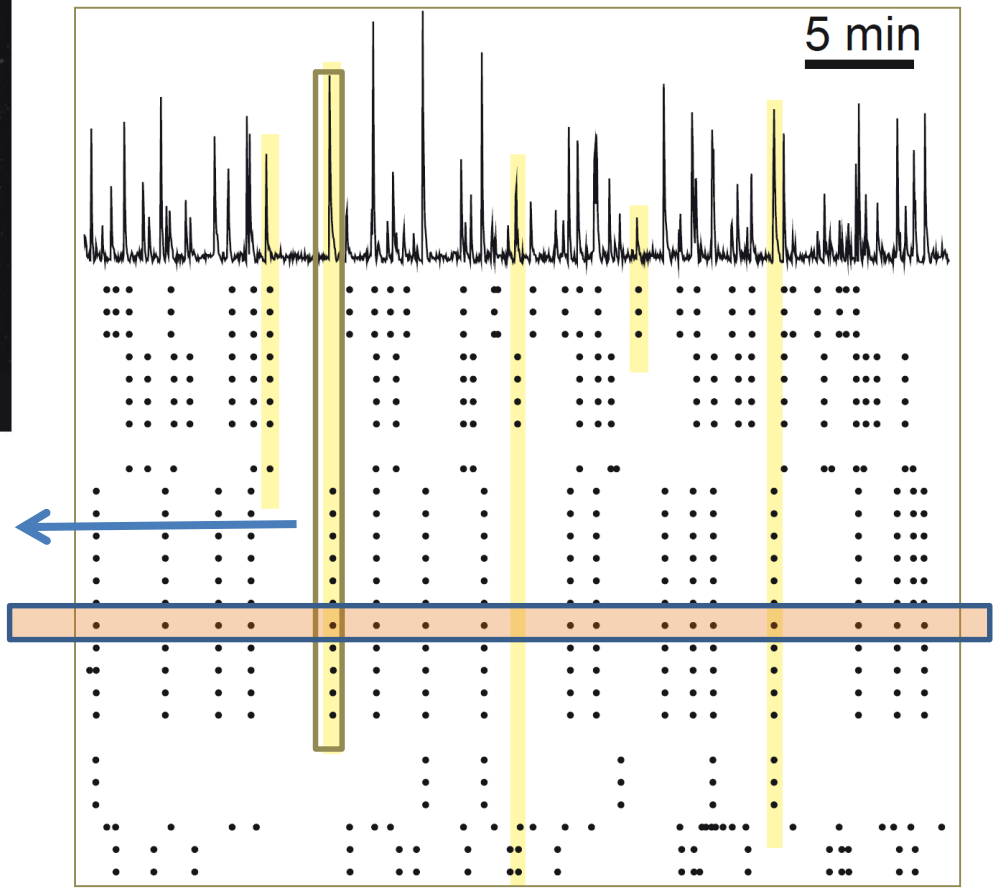



firing sequence

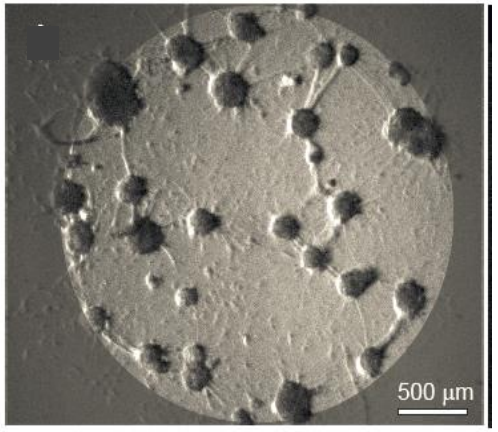


# 5. Characteristic dynamics of clustered networks

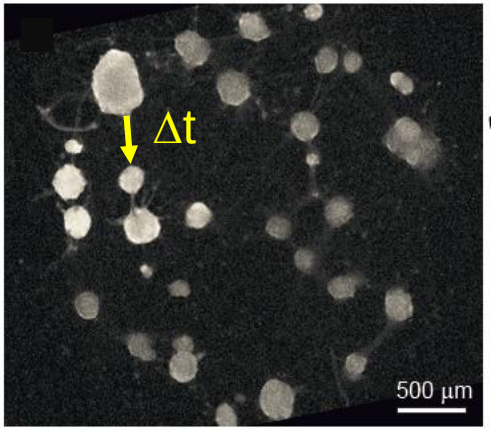


■ **Assignation of links**: we compute the likelihood that any two clusters are connected by looking at the time delays in activation Δt. The link weight *w* will go as 1/Δt.

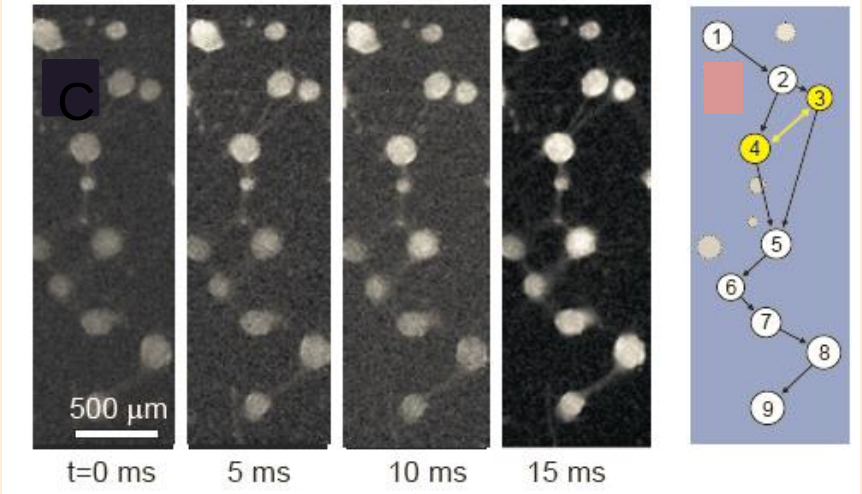


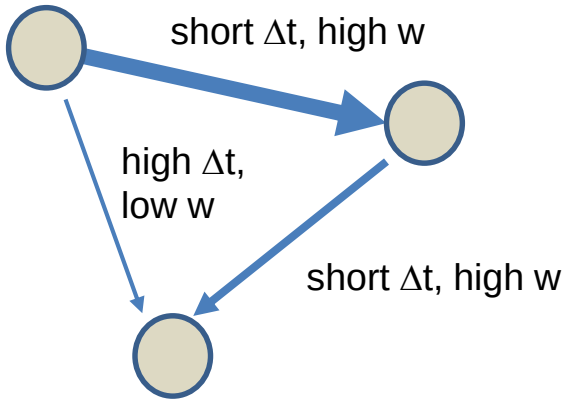

real network




fluorescence

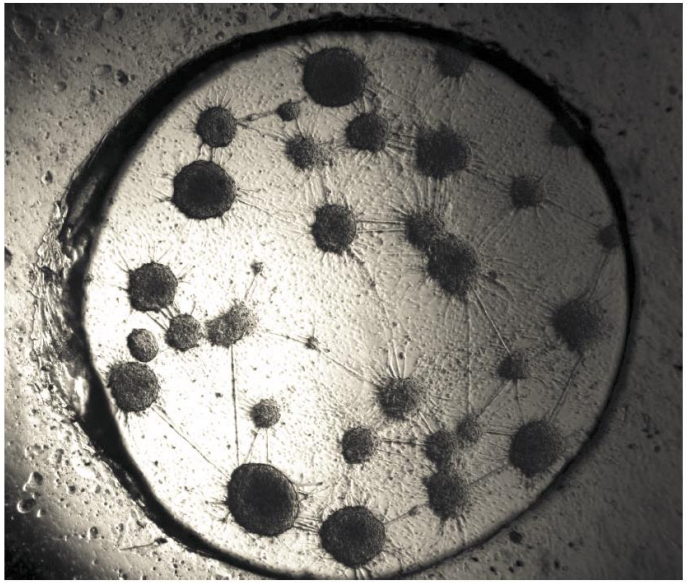
example of activation sequence



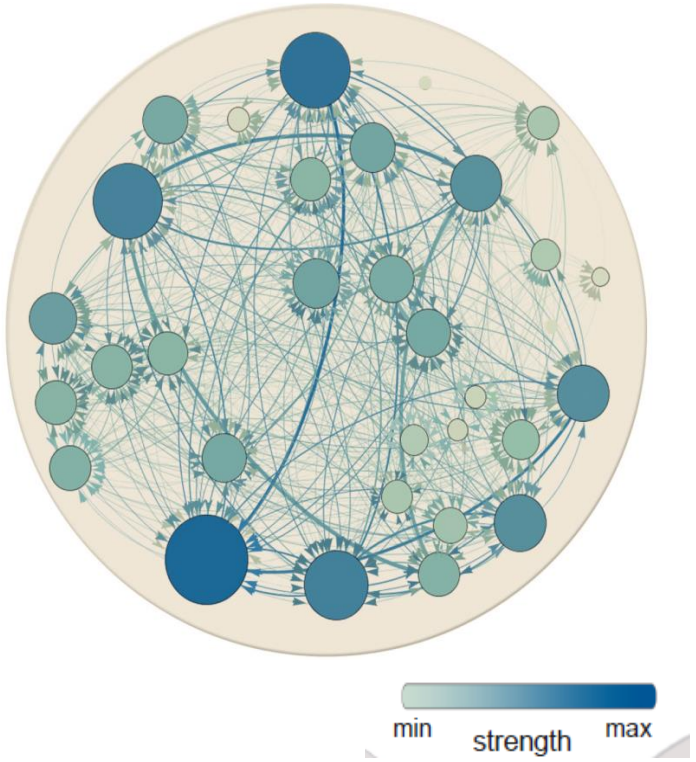



# 6. Extracting functional organization

■ The computation of all the weights between clusters (i,j), averaged over all the observations, shapes the **effective network**, which is directed and weighted.



'structural' network

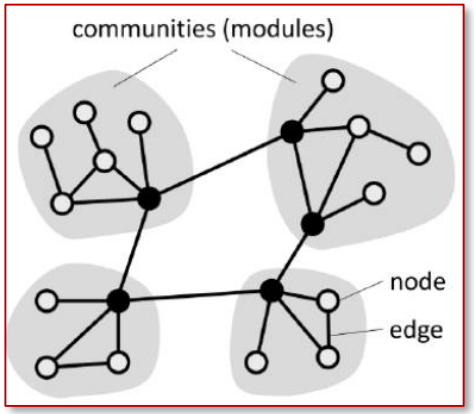


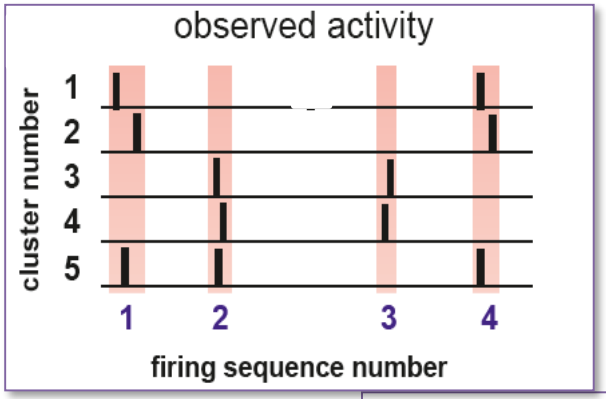

effective network (inferred from dynamics, directed & weighted)

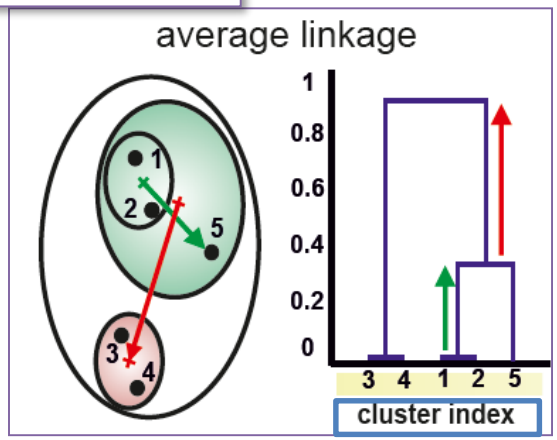
# 6. Extracting functional organization

■ The next step is extracting the supra-organization of the network, i.e. **its most representative modules**. Clusters within a module are more connected between them than between clusters in other modules.

▫ Those clusters that persistently fired together form a module.



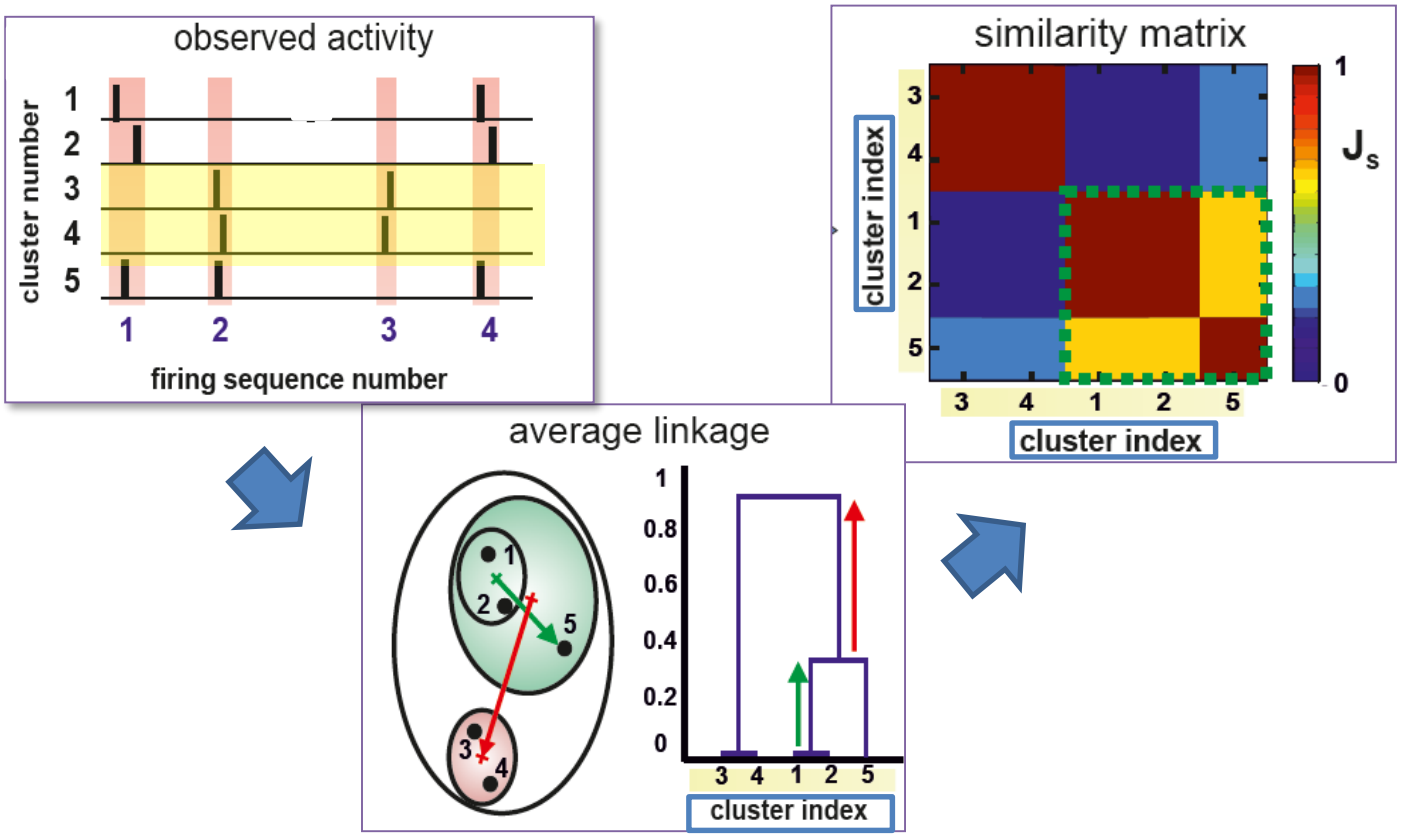



# 6. Extracting functional organization

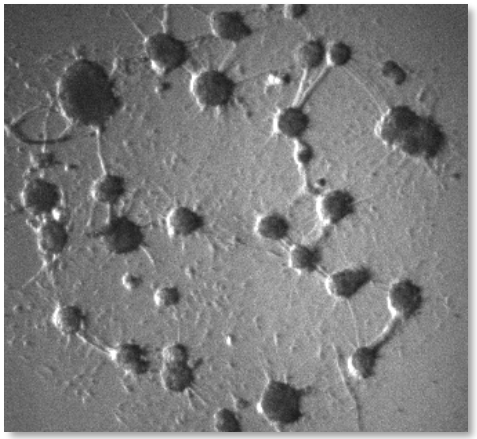
■ The next step is extracting the supra-organization of the network, i.e. **its most representative modules**. Clusters within a module are more connected between them than between clusters in other modules.

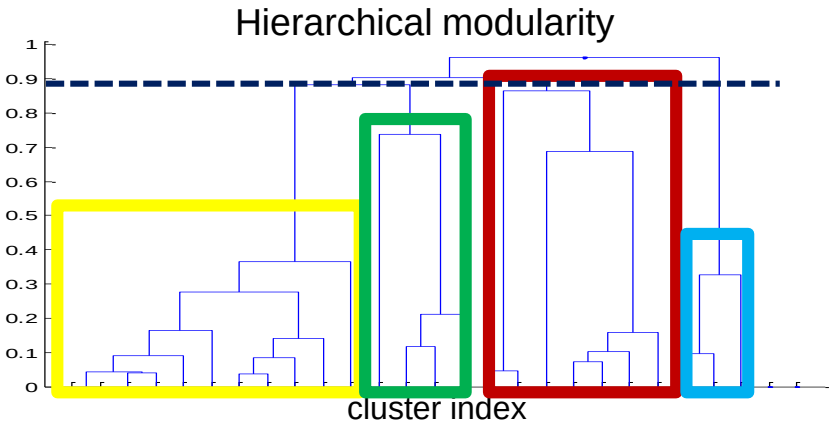
▫ Those clusters that persistently fired together form a module.
For instance, clusters #3 and #4 (and #1 and #2) shared similar behavior.

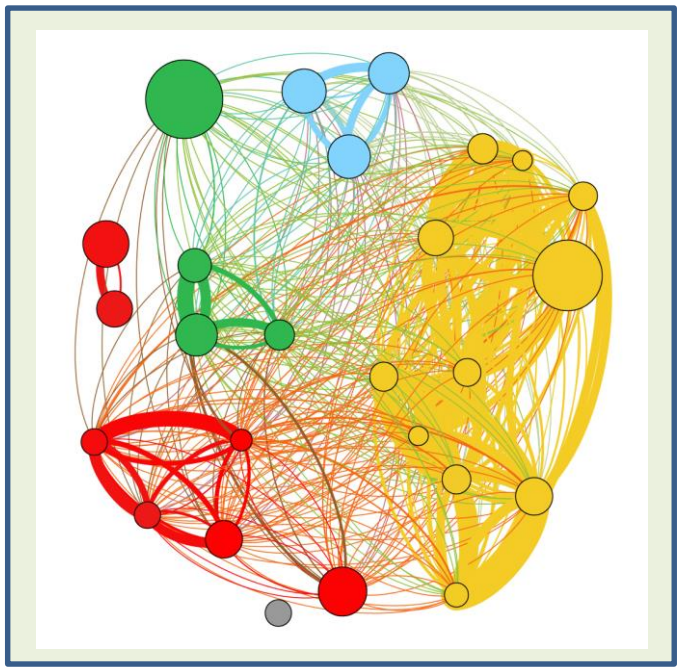


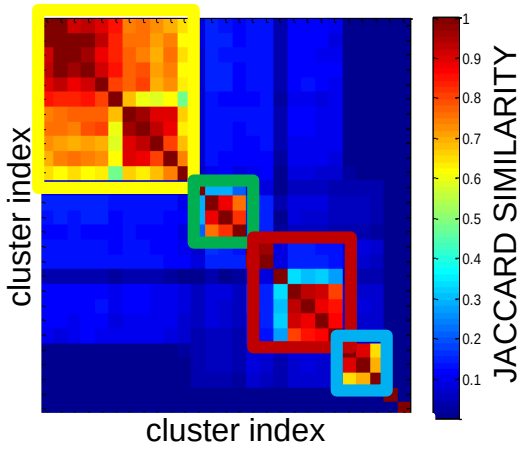
# 6. Extracting functional organization

- Extended to all clusters, one obtains for a representative experiment:

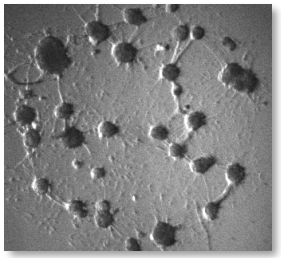




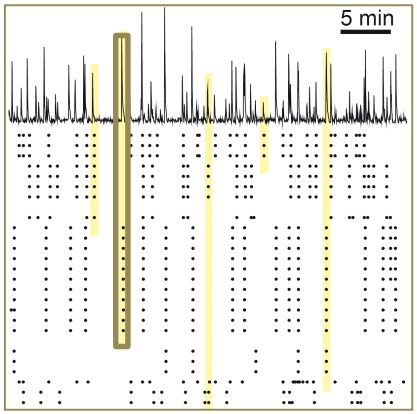


# 6. Extracting functional organization



■ Summarizing:

▫ Prepare a simple network in the lab and measure spontaneous activity.
▫ Analyze the data to get the activation times of the clusters.
▫ Compute time delays within concurrent activations and assign weights of interaction. Get effective network.
▫ Analyze for the existence of modules, i.e. local organization.




The overall analysis shapes the **functional organization**

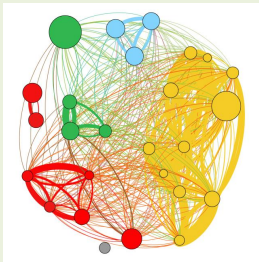
**Is it enough?**

No, I have to verify that the inferred connectivity
is not biased by random events.

**Clusters could randomly fire together.**

Data must be reshuffled (500 realizations) and reanalyzed.

Surrogates construction!

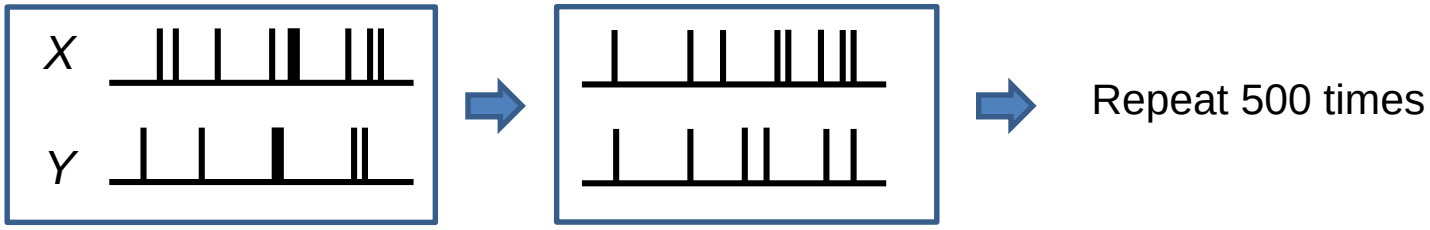


surrogates checked!

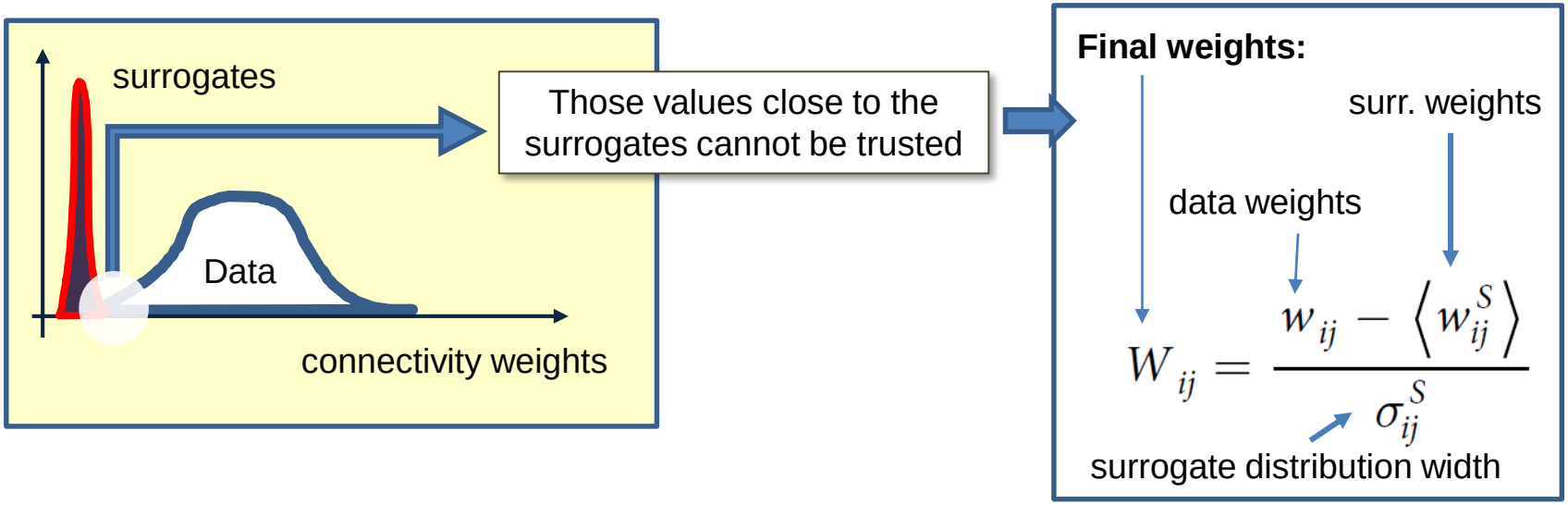
# 7. The importance of surrogates

■ Surrogates show which connections among clusters are meaningful, and excludes all those interactions that may occur by chance.

■ Construction:



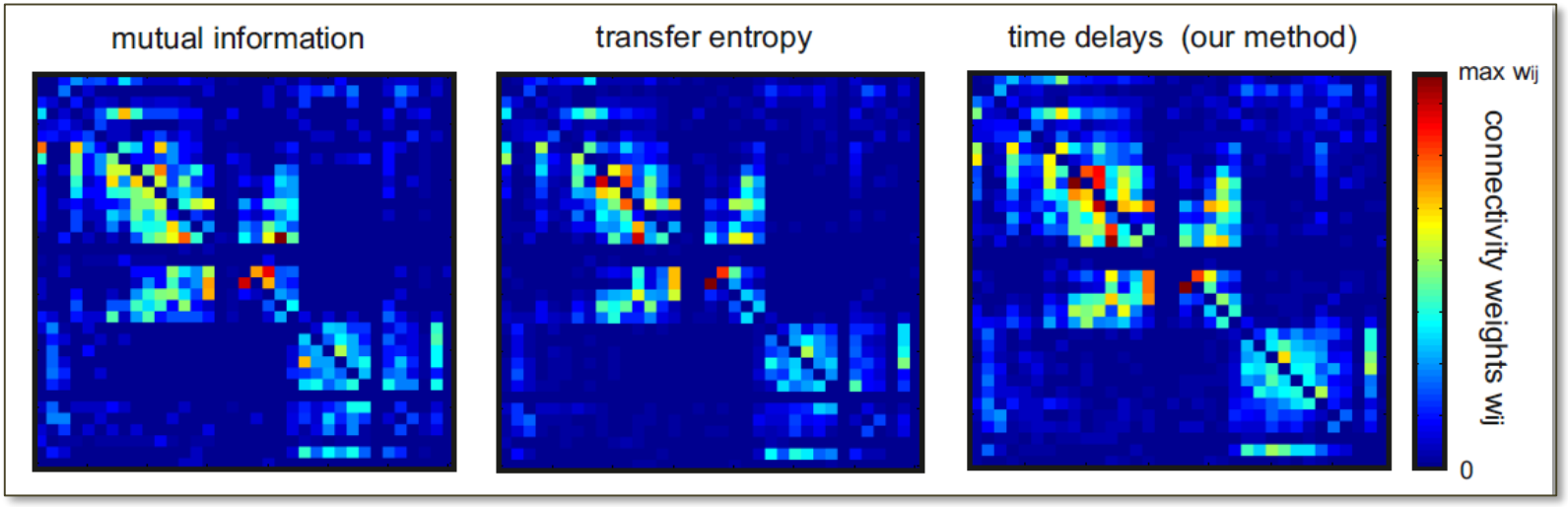

■ If the data is meaningful, one should get a histogram like this:



# 8. Other methods for inferring network connectivity

■ It is always good to test different connectivity-inference methods among clusters.

■ We used time delays, but we could have also tested information-theoretic measures such as Mutual Information or Transfer Entropy. The latter naturally infers causal relationships among nodes (lecture 10).




■ 'In vitro' networks are very attractive platforms to study different methods.

# 9. Fine tuning the analysis: hubs

■ One can also analyze other characteristics of the network in order to identify **hubs** or other interesting nodes.

Hubs are important to hold the modules together

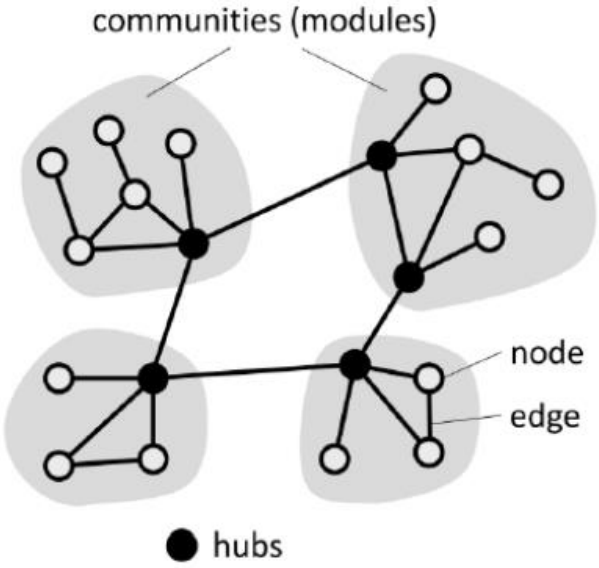
■ Hubs are identified as those nodes that score the best in the next 4 categories (*we note that this scoring concept is not widely accepted; it must be taken as an idea*):

▫ **Strength**: Sum of weighted links of the candidate node.

▫ **Participation coefficient**: Number of links of the candidate that are present in the different modules. A high value indicates that the node is important everywhere.

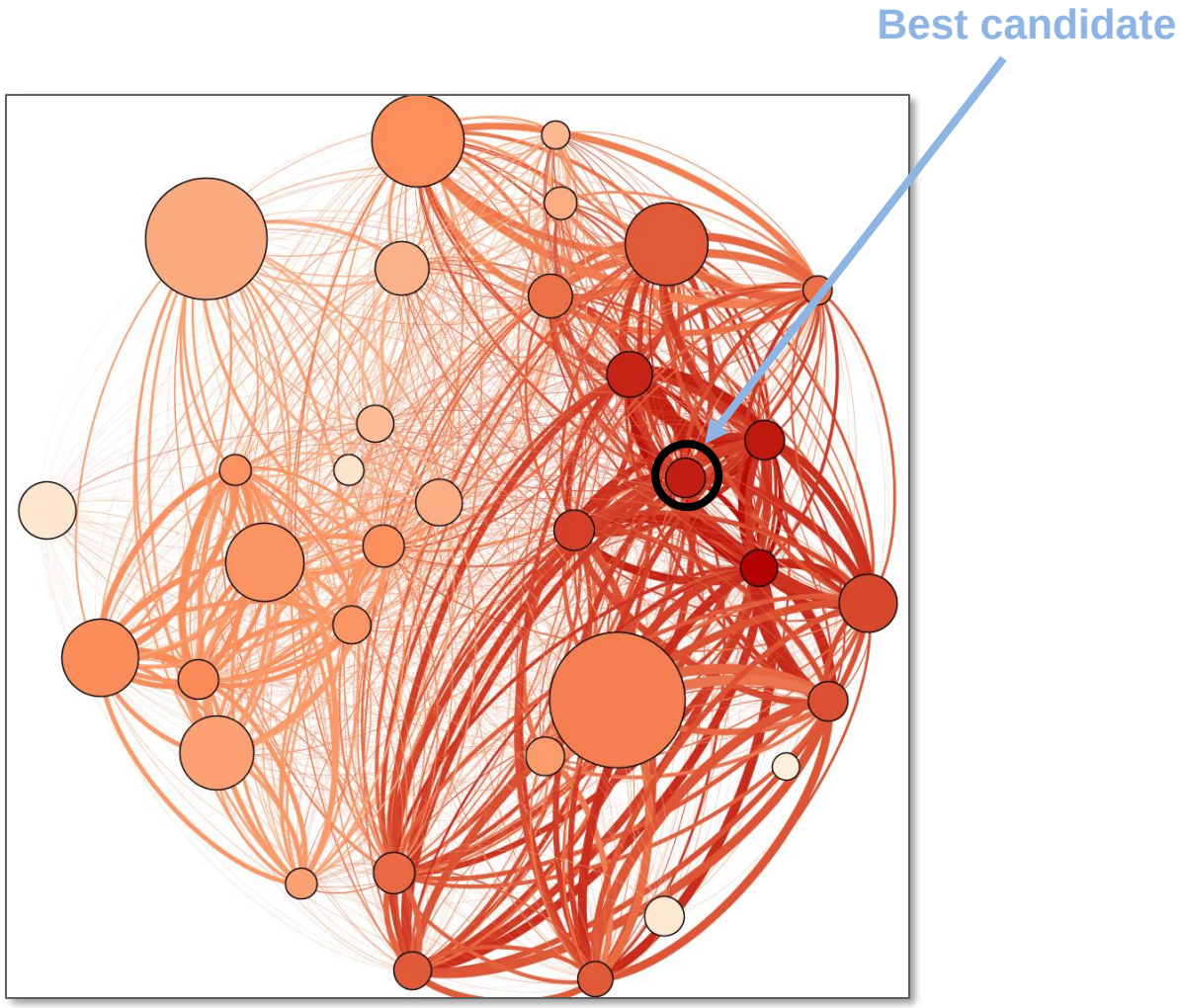
▫ **Betweenness:** The number of shortest paths that go through the candidate.

▫ **Local efficiency**: Measures the likelihood that the candidate's neighbors are interconnected.



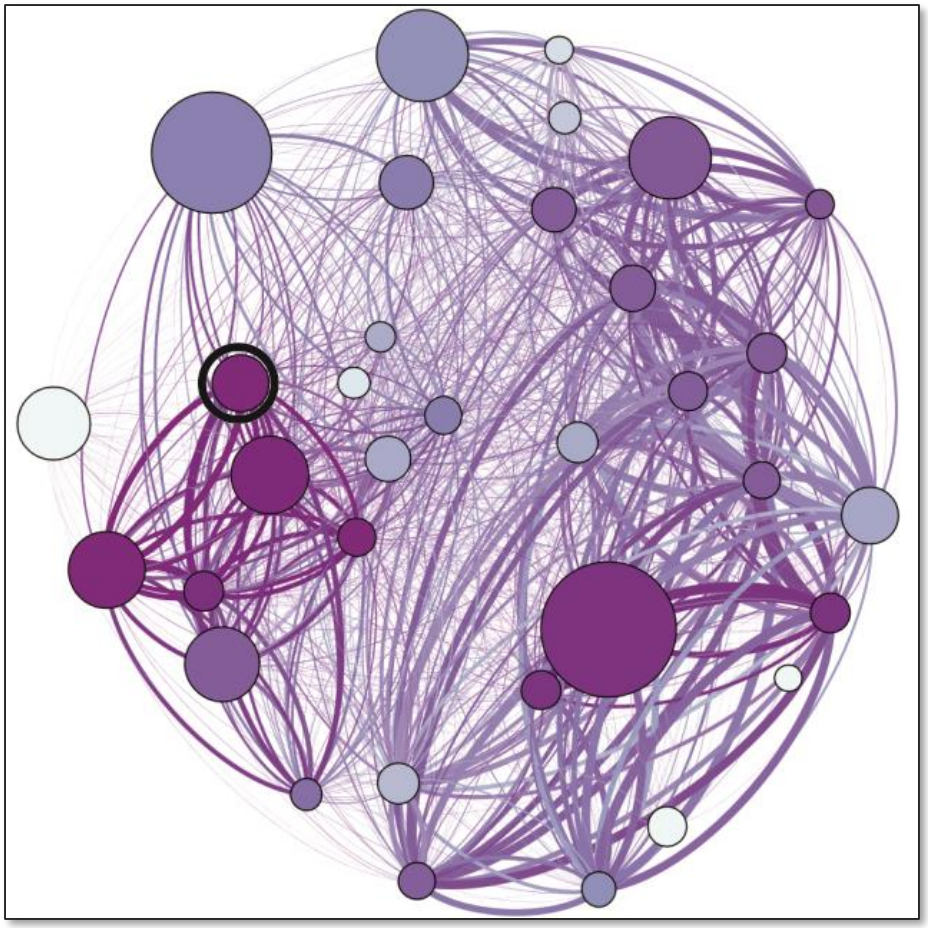
# 9. Fine tuning the analysis: hubs

TOTAL STRENGTH



# 9. Fine tuning the analysis: hubs

PARTICIPATION COEFFICIENT

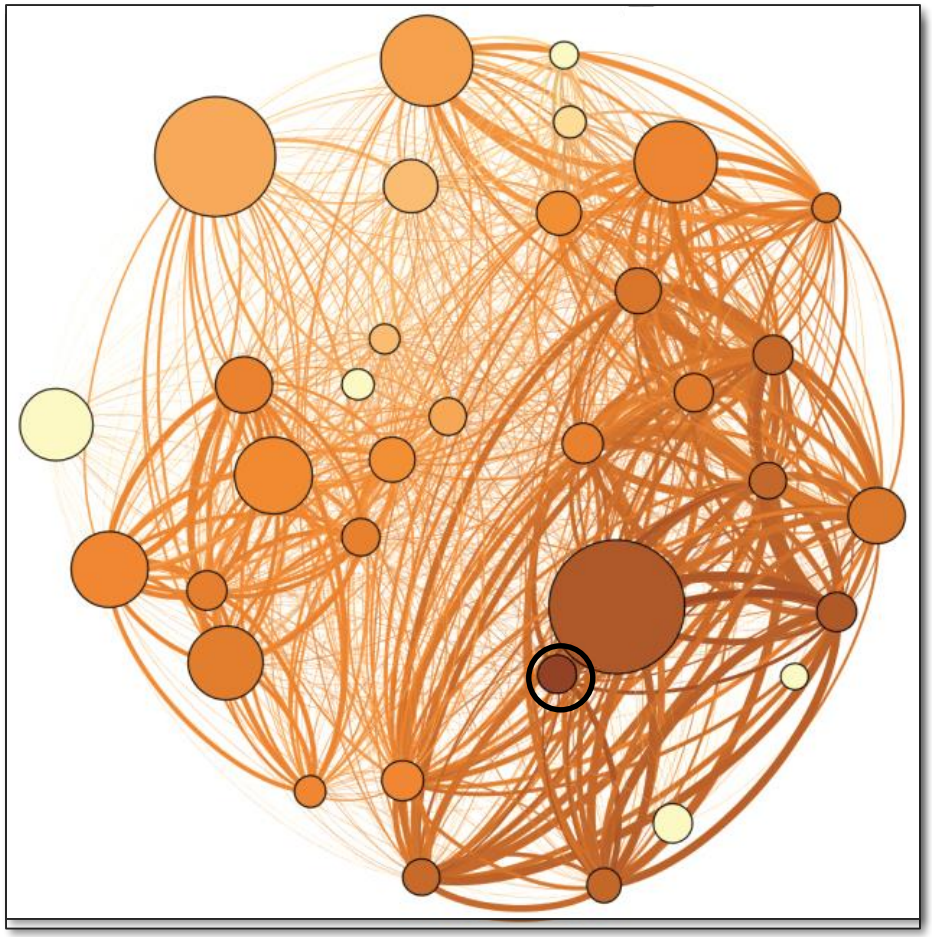


# 9. Fine tuning the analysis: hubs

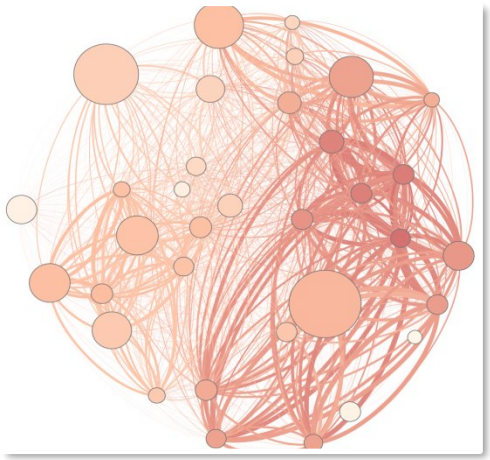
BETWEENNESS

# 9. Fine tuning the analysis: hubs

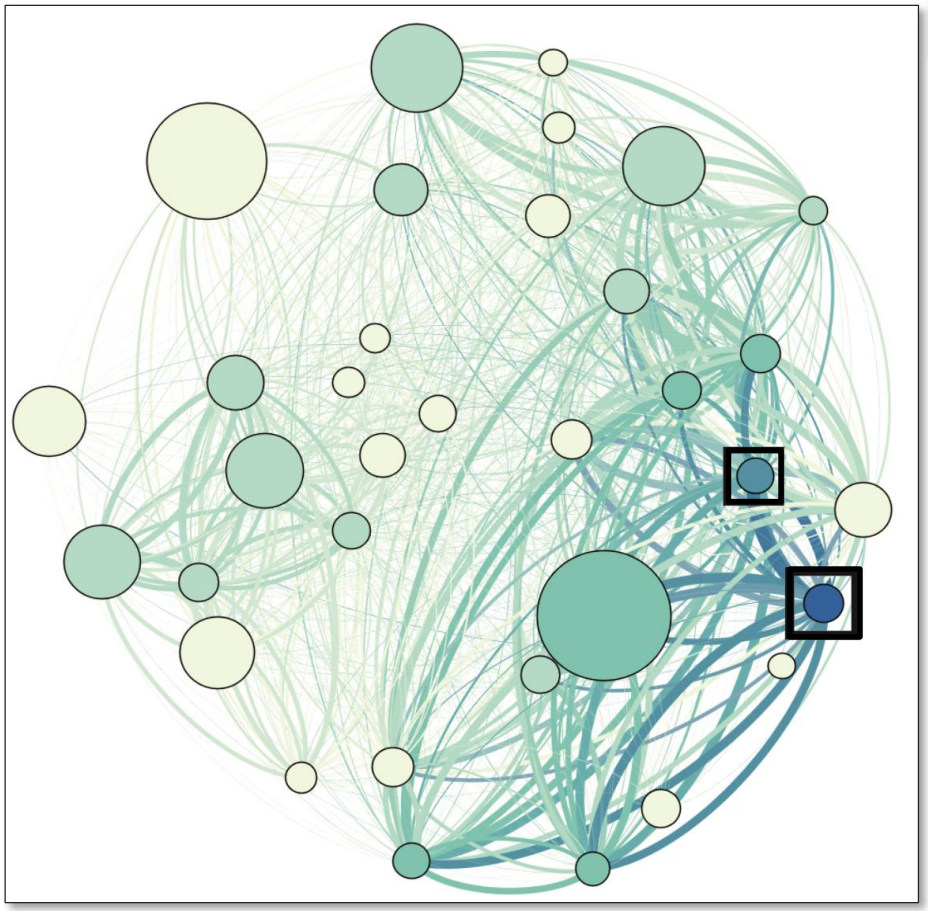
LOCAL EFFICIENCY

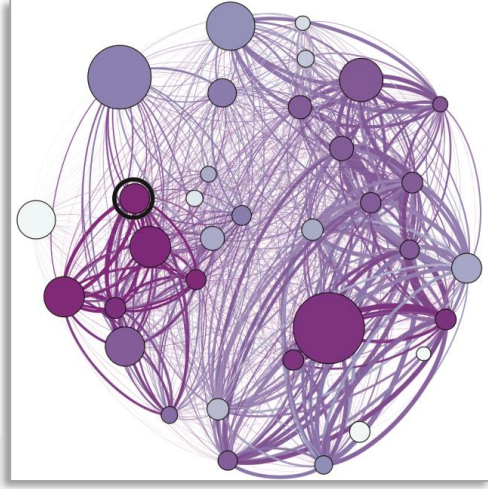


# TOP SCORING CLUSTERS



total strength

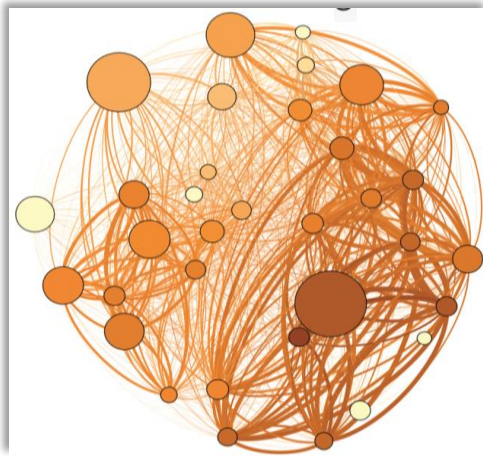




participation coefficient

betweenness

local efficiency

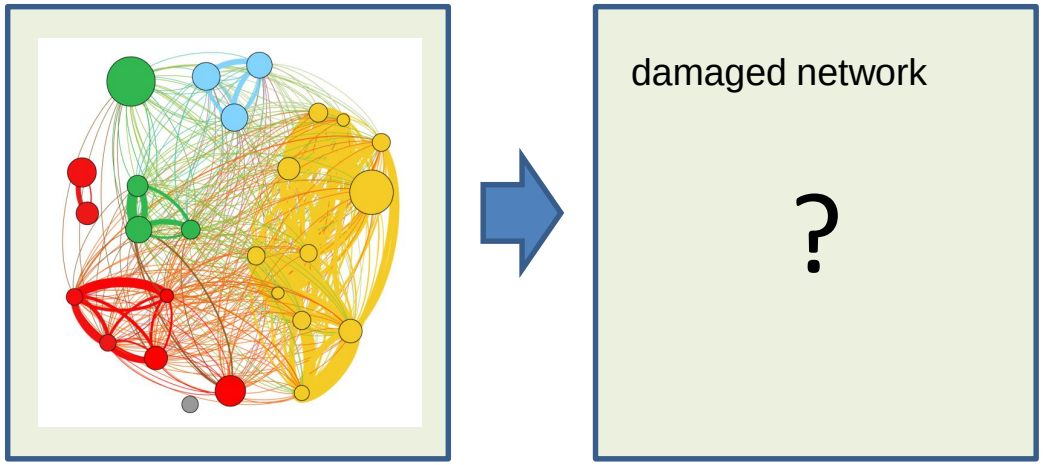


# 10. Importance and applicability of ‘in vitro’ networks

- What do I do with all the developed resources? Explore open problems!

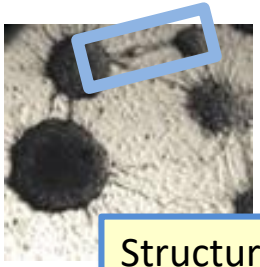
Small, *in vitro* system allow for a deep exploration of topics that cannot be tackled in a real brain, in particular the effect of aggressive perturbations

- Of interest:
  - Compare structural connectivity and functional one.
  - Study the response to damage by monitoring changes in the functional organization.
  - Study network resilience to damage by quantifying the role of hubs.



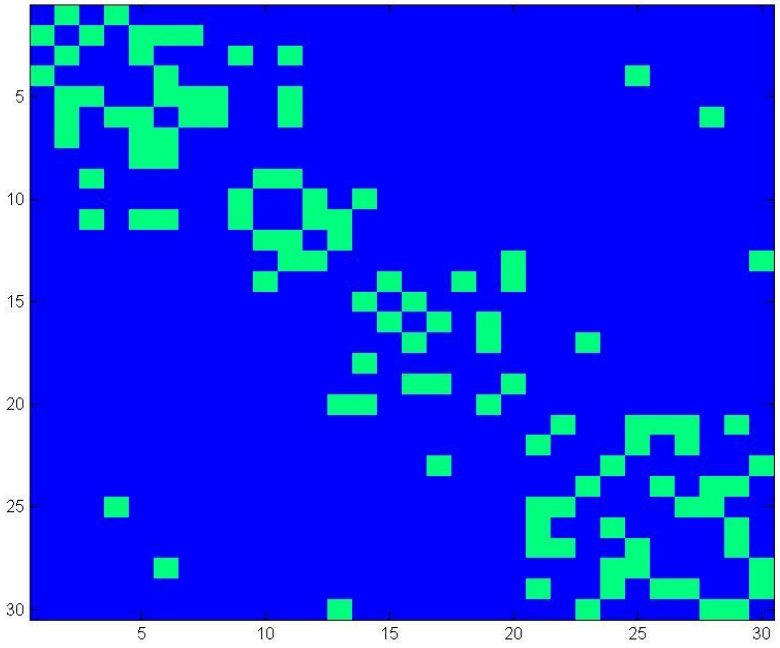
# 11. Study I. Comparing structure and function

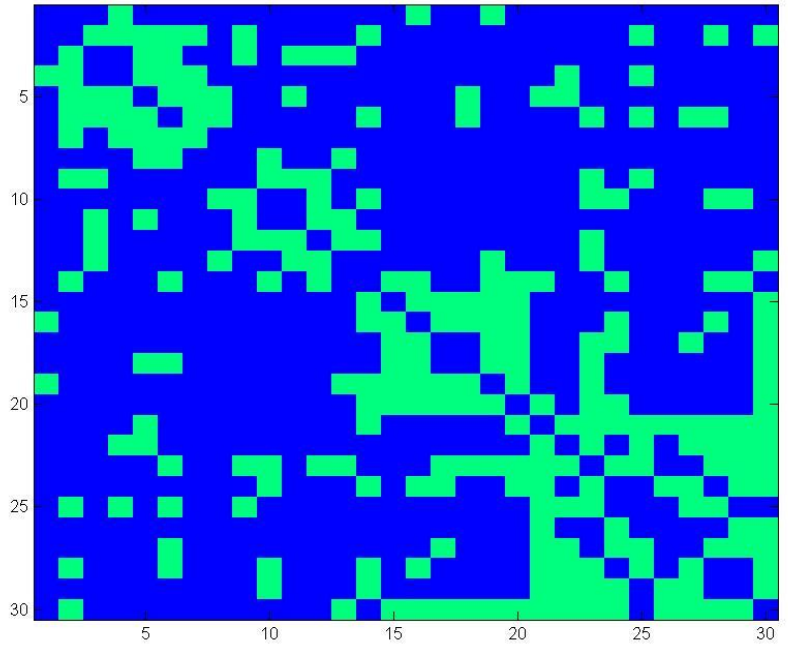
■ The structural network is obtained from the images of the culture upon measurement.



Structural (resolved by hand!)

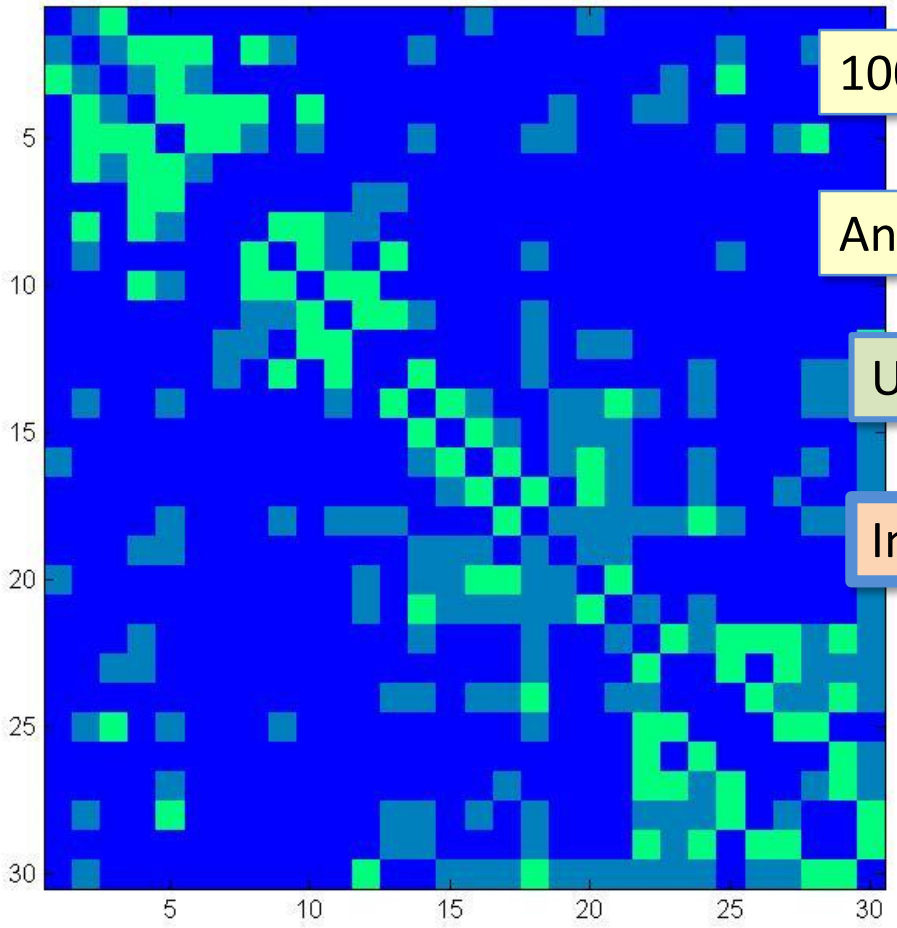
Functional (top links)



# 11. Study I. Comparing structure and function

■ The difference between the two matrices highlights common links (green) and non-common links (grey).




100% match of structure!

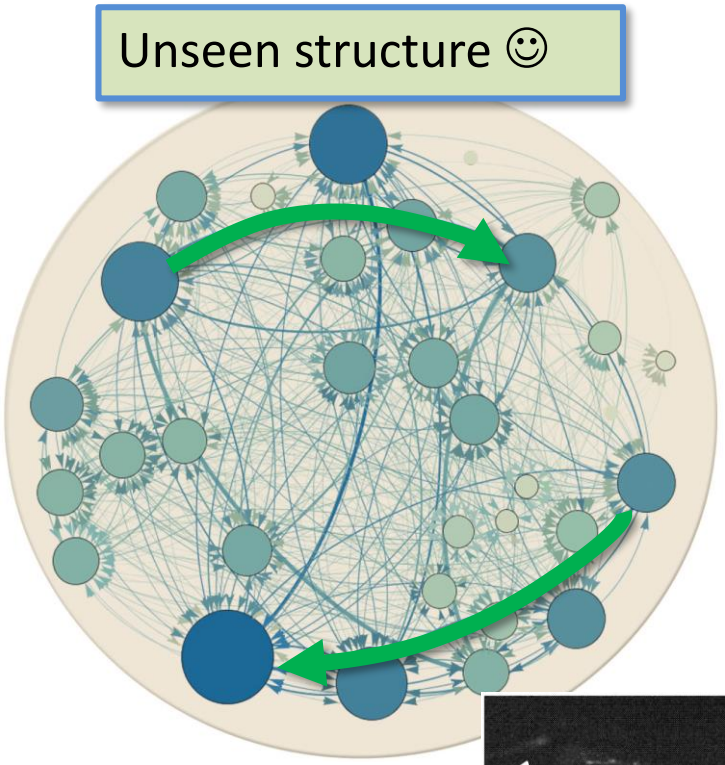
And the rest?

Unseen structure

Interesting features ?

# 11. Study I. Comparing structure and function

■ Ideally, those links that appear in ‘function’ but not in ‘structure’ are connections that cannot be well resolved from just the images.

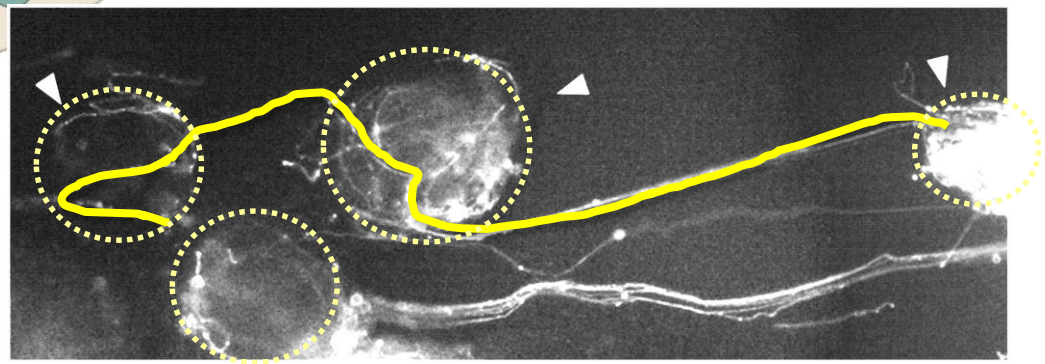



Some thin axons can travel long distances, but cannot be seen, explaining the long range connections.

■ Lack of correspondence may also indicate synchronization among distant clusters.

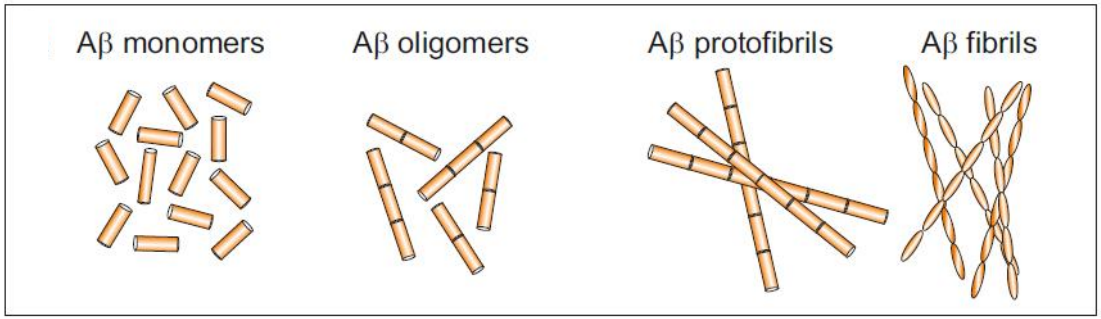
■ Links that appear in ‘structure’ but not in function may indicate preferred paths of activity.

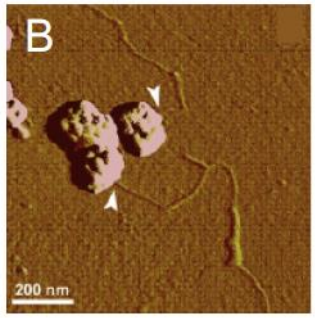
Connections resolved using axon staining (GFP)



# 12. Study II. Biochemical perturbation

■ In Alzheimer's, one of the working hypothesis is that **the accumulation of Amyloid-beta fibrils** causes extensive neuronal damage.



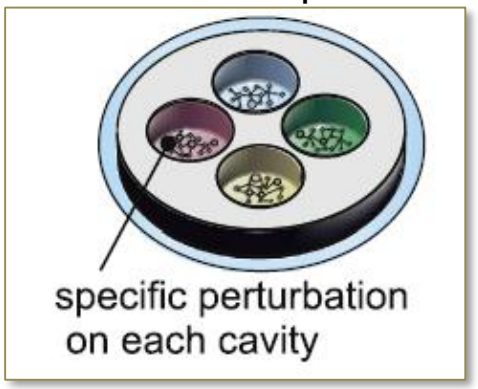



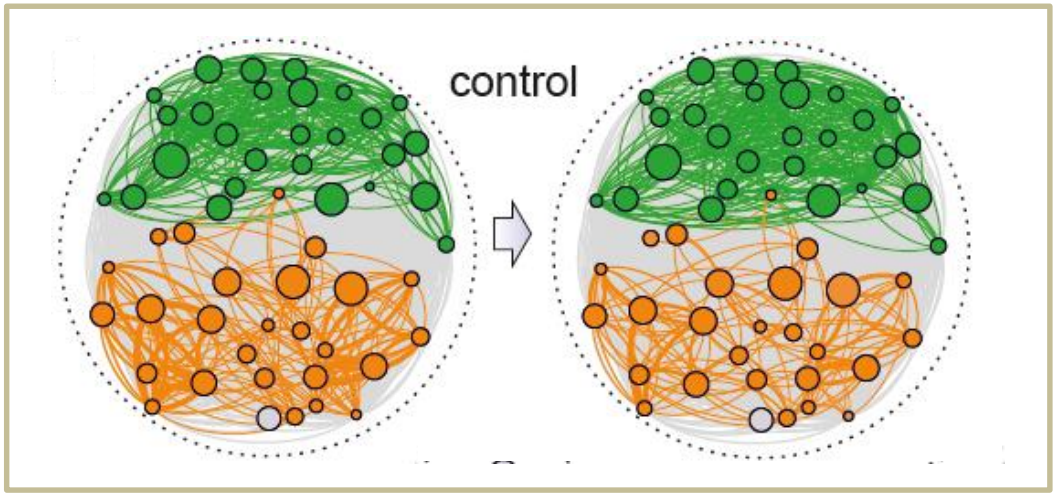

■ It has been suggested that magnetic nanoparticles help stabilizing Aβ fibrils in the brain by forming a M-Aβ complex.

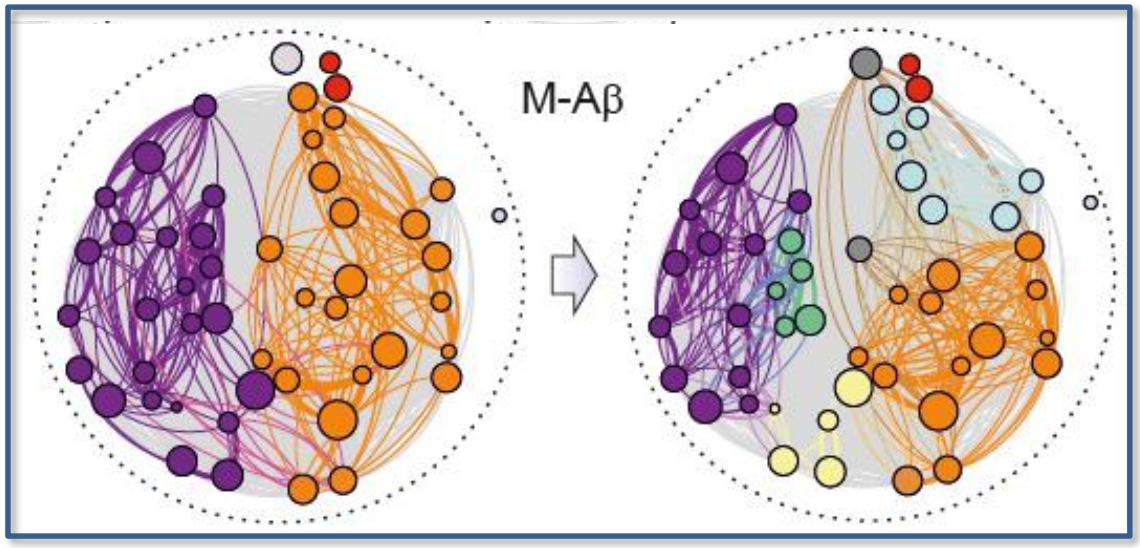
■ Experiments *in vitro* can help verifying this hypothesis by monitoring the degradation of functional organization upon M-Aβ application.

# 12. Study II. Biochemical perturbation

4-well setup




Modularity increases upon damage, indicating a deterioration of network integrity

End of lecture 7

**TAKE HOME MESSAGE:**

- Network theory offers a large number of resources to characterize the topological traits of living neuronal networks, in particular their effective connectivity and functional organization.

- Neuronal networks *in vitro* allow for studying and testing the goodness of network measures, the central role of hubs, and to investigate resilience aspects.

Questions and discussion aspects:

- What other mesoscopic living systems could one devise to investigate open problems in network science and the brain?

- How the studied experiments would change upon an external stimulation of the network, or upon noise?

- These networks are assortative and have a rich club core. What this information tells you?

## References


▫ D. Papo et al., “Complex network theory and the brain”, Philos Trans R Society B (2014).
▫ M. Rubinov and O. Sporns, “Complex network measures of brain connectivity: Uses and interpretations”, NeuroImage (2010).
▫ Karl J. Friston, “Functional and Effective Connectivity: A Review”, Brain Connectivity (2011).
▫ O. Sporns, “Structure and function of complex brain Networks”, Dialogues in Clinical Neuroscience (2013).
▫ S. Teller et al., “Emergence of Assortative Mixing between Clusters of Cultured Neurons”, PLoS Comput Biol (2013).
▫ S.Teller et al., “Magnetite-Amyloid-*β* deteriorates activity and functional organization in an *in vitro* model for Alzheimer’s disease”, Scientific Reports (2015).