Medical Image Analysis 21 (2015) 1–14

Contents lists available at ScienceDirect

Medical Image Analysis

journal homepage: www.elsevier.com/locate/media

Bridging the gap in connectomic studies: A particle filtering framework for estimating structural connectivity at network scale

Simona Ullo [a,*], Vittorio Murino [a], Alessandro Maccione [b], Luca Berdondini [b], Diego Sona [a]

[a] Pattern Analysis and Computer Vision (PAVIS), Istituto Italiano di Tecnologia, Via Morego 30, 16163 Genoa, Italy
[b] Neuroscience and Brain Technologies (NBT), Istituto Italiano di Tecnologia, Via Morego 30, 16163 Genoa, Italy

ARTICLE INFO

Article history:
Received 17 January 2014
Received in revised form 24 November 2014
Accepted 26 November 2014
Available online 24 December 2014

Keywords:
Structural connectivity
Connectomics
Neuronal networks
Micro Electrode Arrays
Particle filtering

ABSTRACT

The ultimate goal of neuroscience is understanding the brain at a functional level. This requires the investigation of the structural connectivity at multiple scales: from the single-neuron *micro-connectomics* to the brain-region *macro-connectomics*. In this work, we address the study of connectomics at the intermediate *mesoscale*, introducing a probabilistic approach capable of reconstructing complex topologies of large neuronal networks. Suitable directional features are designed to model the local neuritic architecture and a feature-based particle filtering framework is proposed which allows the spatial tracking of neurites on microscopy images. The experimental results on cultures of increasing complexity, grown on High-Density Micro Electrode Arrays, show good stability and performance as compared to ground truth annotations drawn by domain experts. We also show how the method can be used to dissect the structural connectivity of inhibitory and excitatory subnetworks opening new perspectives towards the investigation of functional interactions among multiple cellular populations.

1. Introduction

Despite the complexity of the human brain and its multiple levels of organization, it is well proved that high-level cognitive functions rely on low-level structural connections. Therefore, the reconstruction of neural connectivity is crucial to the understanding of the basic mechanisms underlying brain processing and its correlation to neurological disorders (Yizhar et al., 2011; Gutierrez et al., 2009; Vecchia and Pietrobon, 2012).

In the last few decades, there has been a growing interest in the neuroscientific community towards the investigation of the so-called *connectomics*, i.e. the complete reconstruction of the neural connectivity at different scales (Sporns et al., 2005; Leergaard et al., 2012). Efforts in this direction range from the macroscopic analysis of functional and structural connections at the brain-region scale (*macroscale* connectomics, Sporns, 2013) to the microscopic study of soma, axons, dendrites and synapses at the neuron scale (*microscale* connectomics, e.g. Lu, 2011). Although the state of the art shows an increasing amount of works addressing the estimation of structural connectivity at both scales, a large gap emerges in between. Indeed, macro-connectomics deals with brain regions involving billions of neurons making any fine-grained analysis unfeasible. On the other hand, micro-connectomics usually focuses on single cells or very small assemblies of few neurons. A substantial amount of knowledge lies in this gap (DaCosta and Martin, 2013).

Brain information processing relies on stratified mechanisms involving the transmission of electrical and chemical stimuli. This occurs at different spatio-temporal scales and in a non-linear, parallelized way. Since many of such mechanisms can be particularly witnessed in neuronal networks, it is fundamental to capture how neurons collectively behave to accomplish the basic tasks on top of which high-level functions – such as learning and memory – build (Jimbo et al., 1999; Marom and Eytan, 2005; Raichman and Ben-Jacob, 2008).

Recent developments in data acquisition technologies provide valuable tools for investigating connectomics at an intermediate *mesoscale* (involving large assemblies with thousands of neurons, Sporns (2012)) achieving single-neuron resolution. In particular, the High-Density Micro Electrode Array (HD-MEA) technology introduced by Berdondini et al. (2009) allows to record the electrical activity of *in vitro* neuronal networks from thousands of electrodes at sub-millisecond resolution and down to single-cell scale. The capability of combining such a high-resolution functional data with fluorescence microscopy imaging enables the mapping of both activity and structure of neural assemblies at unprecedented resolution. Indeed, relatively sparse neuronal cultures – developed by

* Corresponding author.
E-mail address: simona.ullo@iit.it (S. Ullo).

http://dx.doi.org/10.1016/j.media.2014.11.011
1361-8415/

seeding few thousands of cells – allow to acquire in detail the morphological distribution of the network with respect to the electrode array position, thus providing in principle the possibility to correlate functional activity with neuronal structure over a large area. Although 2D *in vitro* models are an extreme simplification of the situation *in vivo*, they represent a starting point for developing adequate methodologies for estimating functional and structural connectivity at the mesoscale. In fact, 2D models allow a simpler access to electrophysiological recordings and imaging at cell resolution, thus providing the ideal framework to study the relationship among topological structure and functional organization, a hot topic of modern neuroscience. In this setting, highly-complex multimodal datasets are obtained posing new computational challenges and requiring innovative tools for their analysis.

In this paper, we propose a framework for the study of structural connectomics in large dissociated neuronal assemblies cultured on HD-MEAs. Our final goal is to provide a description of the network topology in terms of structural connections with respect to the electrodes. In fact, this offers a natural way of relating the network anatomy to the functional signal recorded by the HD-MEA.

At the *micro-connectomics* scale, traditional methods in the literature mainly address the *neuronal tracing* problem, i.e. the morphological characterization of single or a few cell's neurites from microscopy images (Meijering, 2010; Lu, 2011). Such methods can be roughly classified into local and global approaches. The former iteratively reconstruct neurites starting from seed points by optimizing a local cost function (Xie et al., 2010; Schmitz et al., 2011). The latter usually process the image as a whole, deriving a graph-based representation (Meijering, 2010). Hybrid approaches have also been introduced combining local appearance with global graph optimization (Chothani et al., 2011; Peng et al., 2011; Zhao et al., 2011). Although – in principle – a generalization of the neural tracing techniques to the segmentation of larger assemblies would be possible, they would hardly face the connectivity complexity imposed by large-scale neuronal networks. In particular, our target application is characterized by hundreds to thousands of neurons interconnected by intricate crossing filaments.

Fig. 1 shows two examples of typical fluorescence images acquired on HD-MEAs depicting dissociated neuronal cultures. It is worth noting that the imaging capabilities only allow to partially resolve the structural connectivity due to cellular density, size of neurites and limitations of the adopted microscopy techniques. Even in cases of particularly low cell density (Fig. 1a), the image renders the complexity of the data when targeting the estimation of the connectivity. As our main goal is to quantify the structural connectivity for the electrodes contributing to the functional signal, it is preferable to compute a probabilistic connectivity *electrode-wise* rather than relying on a deterministic pixel-wise segmentation. Moreover, probabilistic methods intrinsically offer a quantitative measure for the reliability of their results, naturally dealing with data uncertainty. This information can be reinterpreted as a measure of the connection strength when applied to the analysis of structural connectivity.

In this paper, we introduce a probabilistic approach that takes advantage of a Sequential Monte Carlo method – called *particle filtering* (Cappé et al., 2007). This probabilistic framework is reformulated to address the spatial tracking of neurites on microscopy images based on prior biological knowledge.

A particle filter (PF) is typically used for the temporal tracking in time-lapse datasets where a time-varying process has to be characterized. The great value of this technique lies in the capability to model both system dynamics and uncertainty in data acquisition within a Bayesian framework (Cappé et al., 2007; Doucet and Johansen, 2009).

Particle filtering approaches have been recently applied to various bioimage analysis problems, both for temporal tracking in videos (e.g. the analysis of the motion patterns of growing axons proposed by Yang et al. (2012), or the neurofilament tracking proposed by Yuan et al. (2012)) and spatial tracking in images, where they are particularly suited for following filament-like structures (Myatt et al., 2006) or to solve tractography problems in diffusion MRI data (Pontabry and Rousseau, 2011; Yap et al., 2011).

In this paper, we draw inspiration from brain tractography where the intrinsically directional diffusion data are used to track brain fibers. To address the reconstruction of the structural connectivity at network scale from fluorescence images, we introduce a novel data encoding in which directional features are designed to capture the neuritic structure in terms of main local orientations. A new instance of particle filter is then defined to reconstruct the structural connectivity based on the extracted features. As opposed to standard PF implementations for spatial tracking, that propagate particles on the image using low-level pixel information, in this setting we introduce an original abstraction of the spatial domain to a feature space. This provides a more flexible granularity for the particle evolution and allows to describe the network structure in terms of connections between electrodes. Moreover, we propose a novel formulation for the particle filter which takes advantage of prior biological knowledge on neuronal morphology. This allows to define new mechanisms for particle evolution and resampling which better capture the task specificity.

The experimental validation of our probabilistic approach is carried out on two datasets with different complexity. More specifically, we compare the proposed method to our previous determin-

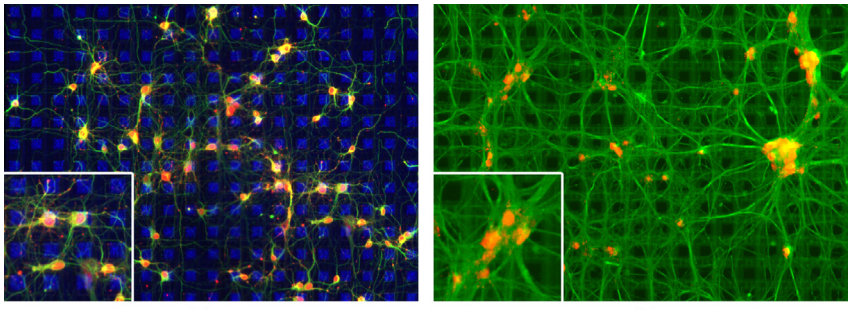

Fig. 1. Two examples of fluorescence microscopy images of *in vitro* neuronal networks acquired on a high-density Multi-Electrode Array (HD-MEA). Dissociated hippocampal cultures are seeded on the MEA and labeled with two specific antibodies: β3-tubulin for neurites (green) and NeuN for neuronal nuclei (red). The images show two cultures with different cellular density. (a) A dataset with about 100 neurons. A bright field image of the electrode array is separately acquired and shown as an additional image channel (blue channel), (b) Another example of dataset with about 1000 cells. In this case, the electrode array is partially visible in the background (green channel) when not occluded by neurons or cell processes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)