Neuron-level analysis of the laminar structure of the human brain

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Introduction:
The structure of the cerebral cortex is defined by organization and distribution of neurons in the tissue. Today, most investigations in brain cytoarchitectonics are not automatized and require significant involvement of the researcher, which is very time consuming and may introduce significant bias. This type of analysis may not be accurate enough or reveal enough information about the structure of the tissue. Brain tissue forms complex structures whose organization and distribution may be reliably revealed only using systematic computational approach. The goal of this research is the development of novel computational methods for automatic and objective analysis of histological images of human brain suitable for advanced and fast analysis of brain cytoarchitectonics and laminar structure. The methods were developed aiming for a completely data-driven approach, with as little assumptions about the data as possible.

Methods:
Initial research in parcellation of the cortex using neuron density and cell body size presented how the two features, unevenly distributed among the neurons in different layers, reveal laminar structure of the cortex. They alone were not enough for precise delineation of the layers, or for more detailed investigations. We developed methods for computing neuron features beyond area and local density that consider more complex aspects of local patterns in the tissue. Two portions of histological preparations of human prefrontal cortex were given to three human experts who manually delineated borders between the layers in the cortex which contained over 50 000 neurons which were automatically segmented from the high resolution (0.45um/px) images of the tissue. Machine learning methods based on tree ensembles were used to predict neurons' layer within the cortex. Density-based clustering methods were used to identifying the radius of 100-300μm around the neuron for measuring characteristics of local patterns in neuron's neighborhood which is significant for estimation of its features. It usually included 200-600 nearest neurons.

Results:
More than 300 features of neurons were developed in several feature classes. The most successful were those derived from density and area of the neurons by computing various statistics and performing oriented measurement taken in different directions from a neuron. Through performance measurements it was shown that the machine learning model's output agrees with manual labels in the same amount as they agree with each other, thus creating results indistinguishable from that of human experts. The model was able to generalize well on a new set of data containing over 500 000 neurons and the segmentation of cortical layers on whole histological sections was obtained. Importance of developed features on both global model level and individual prediction level were estimated using SHAP analysis. Features of the highest level had largest importance, as they integrate low level features with variations in wider area around neurons. Features based on oriented measurements, also had considerable feature importance, identifying neurons on the border of layers.

Conclusions:
We have shown that through development of complex neuron features it is possible to learn the distribution of neurons within the cortex and descriptors of each cortical layer. Through analysis and comparison of human experts' performance we have addressed the question of correctness and existence of true baseline in such tasks. Machine learning model was able to create precise segmentation of cortical layers. Quantitative analysis of neuronal distribution and exploration of underlying principles of variation that facilitate parcellation of cortical layers resulted in novel framework and methodology for cellular-level investigations of brain tissue both in 2D and 3D, applicable on various histological samples such as the BigBrain.