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Multistage histopathological image segmentation of Iba1-stained murine microglias in a focal ischemia model: Methodological workflow and expert validation

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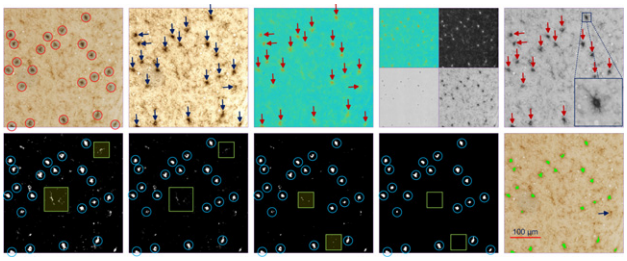
HIGHLIGHTS

- Microglias are cellular contributors to neurological outcome of ischemic brain damage.
- Automated cell counts are useful for quantifying brain's response to post-ischemia.
- Multistage microglia segmentation workflow is developed for histopathological images.
- Purpose is to deliver rapid and statistically accurate microglia cell counts.
- Workflow counts were validated with ground-truth data acquired from neuropathologist.

GRAPHICAL ABSTRACT



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ABSTRACT

A multistage workflow was developed for segmenting and counting murine microglias from histopathological brightfield images, in a permanent focal cerebral ischemia model. Automated counts are useful, since for the assessment of inflammatory mechanisms in ischemic stroke there is a need to quantify the brain's responses to post-ischemia, which primarily is the rapid activation of microglial cells. Permanent middle cerebral artery occlusion was induced in murine brain tissue samples. Positive cells were quantified by immunohistochemistry for the ionized calcium-binding adaptor molecule-1 (Iba1) as the microglia marker. Microglia cells were segmented in seven sequential steps: (i) contrast boosting using quaternion operations, (ii) intensity outlier normalization, (iii) nonlocal total variation denoising, (iv) histogram specification and contrast stretching, (v) homomorphic filtering, (vi) global thresholding, and (vii) morphological filtering. Workflow counts were validated on an image subset, with ground-truth data acquired from manual counts conducted by a neuropathologist. Automated workflow matched ground-truth counts pretty well; 80–90% accuracy was achieved, as regards to time after pMCAO and correspondence to ischemic/non-ischemic tissue.

1. Introduction

Despite considerable advances in the understanding of the pathophysiology of cerebral ischemia, therapeutic options for acute stroke are still limited (Eltzschig and Eckle, 2011). Inflammatory mechanisms represent a key target of current

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