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Abstract

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Assessing in vivo neuronal reprogramming by automated, resonance-scanned confocal virtual tissue image acquisition and artificial intelligence-assisted stereology

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Direct in vivo reprogramming of non-neuronal cells in the mature CNS into phenotypically correct neurons can be achieved through forced expression of pioneering transcription factors, such as Ngn2, NeuroD1, and Ascl1, that normally act to direct neuronal fate specification during development. This process produces a variable population of induced neurons that can be identified through their expression of reporter genes tied to the induction process and expression of various neuronal phenotypic markers, requiring detection of multiple fluorescence labels with resolution by confocal microscopy. Following in vivo gene delivery of lineage instruction factors, the number of infected cells and their distribution present some challenges for accurate quantitation by design-based stereological sampling. Generally, too many cells are infected to completely count with accuracy across histological sections arguing for subsampling of the population by stereological principles. In addition, cell density varies widely from the site of injection to the most distant infected cells. This means that sampling frequency density must be high to reduce estimator variance to an acceptable level. Furthermore, traditional acquisition of confocal stacks is time consuming and inefficient. The recent availability of resonance scanning confocal microscopes permits the rapid generation of virtual section data sets. Efficient sampling design can now follow complete image acquisition of the histological material. The application of artificial intelligence to detecting cells with different label combinations within the virtual section data set makes it possible to automate cell counting if detection criteria can be achieved. However, cell detection must be combined with stereological sampling principles to account for sectioning and other artifacts and to accommodate fractionated sampling. These approaches are appropriate for other “rare” cell populations,

such as grafted cells, and could be extended to dense cell populations if shown to be efficient.

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