

# Quantification of Human Neural Stem Cell Fate Using Volocity® 3D Image Analysis Software

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## Introduction

The promise of stem cell therapy offers hope for the treatment of many human diseases and injuries [1]. Spinal cord and traumatic brain injuries can cause the loss of neurons and glial cells. These lost cells may be partially replaced by transplanting multipotent human neural stem cells (hNSCs), which can differentiate into specialized neural cell types [2, 3]. The potential for these remarkable cells to promote recovery of neural function has made them a strong focus of researchers in this field.

Accurate and efficient quantification of human stem cell lineage and fate is essential for this research. In many laboratories, stem cells cultured and differentiated *in vitro* are routinely classified and quantified from 2D or 3D images. Recognition of hNSC progeny in 2D is challenging because of their heterogeneous cell morphology, long overlapping cell processes, and high cell density, which make cell fate quantification highly error prone [4]. In addition, manual methods are frequently used for hNSC fate analysis, which can be time-consuming, subjective, and inconsistent [5]. The ability to visualize and analyze image data in 3D can overcome many of these classification errors and provide a deeper understanding of cellular samples [6]. The development of an automated method for cell fate analysis would also improve the efficiency of stem cell research.

Human NSCs have the ability to differentiate into (1) neurons, (2) astrocytes, which largely function to provide nutritional and structural support to neurons, and (3) oligodendrocytes, specialized cells that form the myelin sheath around axons and insulate neural signals [3]. These three specialized neural cell types can be identified using specific nuclear and cytoplasmic cell fate markers [7]. In a recent study published in *Stem Cell Research*, B. J. Cummings and colleagues from the Sue & Bill Gross Stem Cell Center at the University of California set out to create

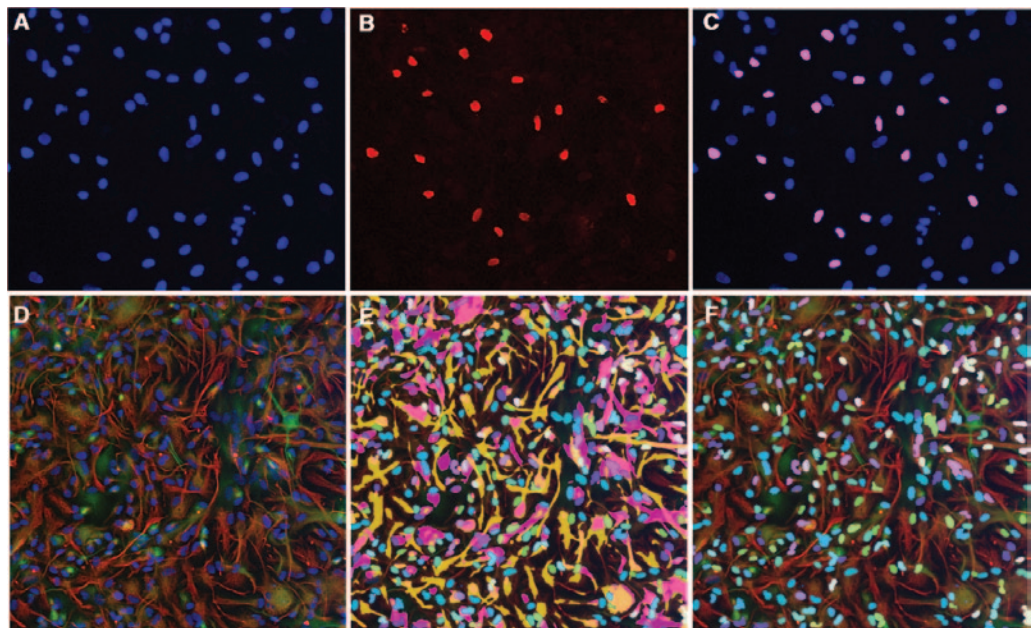
and optimize two image analysis protocols to quantify these cell fate markers using PerkinElmer's Volocity® 3D Image Analysis Software [8].

## Methods and Materials

The first image analysis protocol was designed to quantify hNSC progeny expressing the nuclear fate marker oligodendrocyte lineage transcription factor 2 (Olig2). The second protocol was designed to quantify progeny expressing the cytoplasmic fate markers  $\beta$ -Tubulin III ( $\beta$ -TubIII), which is neuron-specific, and glial fibrillary acidic protein (GFAP), which is expressed by mature astroglial cells and undifferentiated hNSCs. Fetal-derived hNSCs were differentiated for 14 days *in vitro* and then fixed, permeabilized, and stained with the nuclear counter stain Hoechst 33342 and antibodies against  $\beta$ -TubIII, GFAP, and/or Olig2.

## Application of the Protocols

Two-dimensional and 3D (confocal) images were acquired and imported into Volocity® software to develop the protocols for quantifying the proportions of cells positive for each cell fate

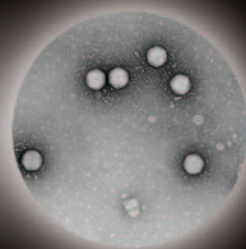
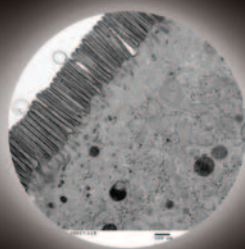
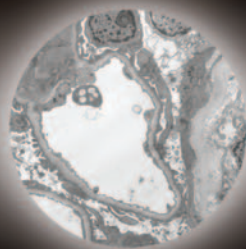
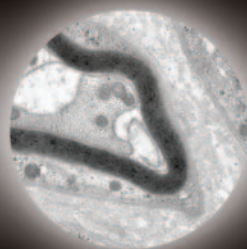
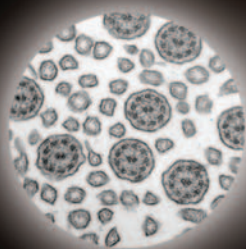


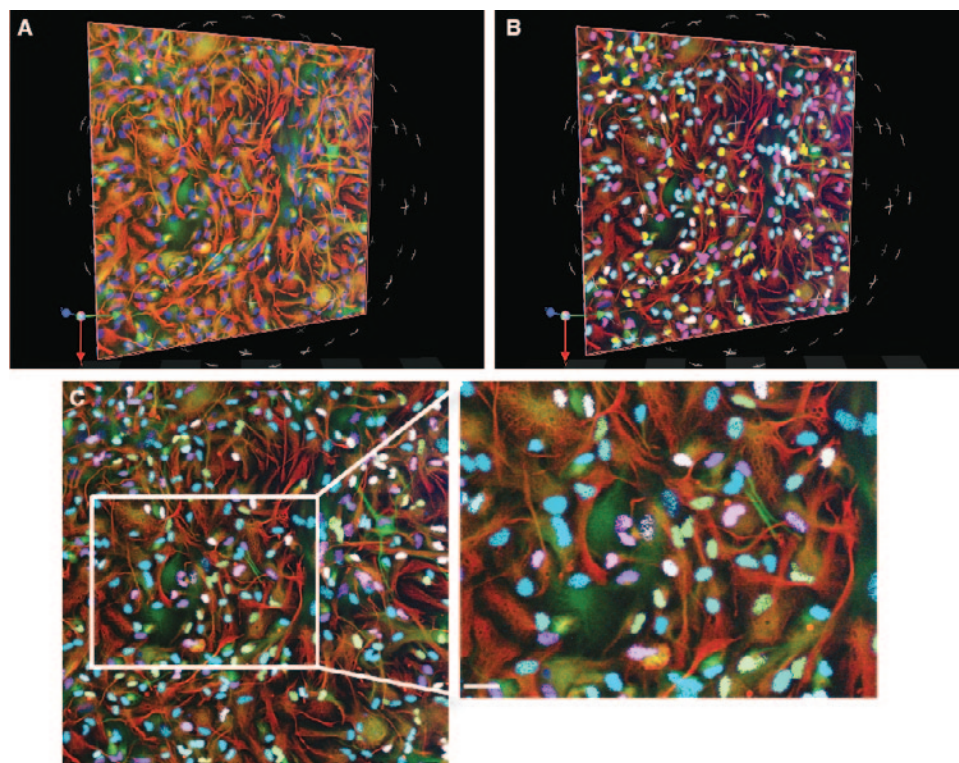
**Figure 1:** Classification of neuronal, astroglial, or oligodendroglial cell types after hNSC differentiation *in vitro*. Cells were stained with (A) the nuclear counter stain Hoechst 33342 and (B) an antibody against the oligodendroglial marker Olig2. (C) The nuclei of Volocity® software-recognized Olig2<sup>+</sup>/Hoechst<sup>+</sup> cells are shown (pink). (D) Volocity® software 3D-rendered image of cells stained with the nuclear counter stain Hoechst 33342 (blue), and antibodies against the cytoplasmic markers  $\beta$ -TubIII (green) and GFAP (red). (E) Volocity® software image analysis showing objects classified as  $\beta$ -TubIII<sup>+</sup>, GFAP<sup>+</sup>,  $\beta$ -TubIII<sup>+</sup>/GFAP<sup>+</sup>,  $\beta$ -TubIII<sup>+</sup>/Hoechst<sup>+</sup>, GFAP<sup>+</sup>/Hoechst<sup>+</sup>,  $\beta$ -TubIII<sup>+</sup>/GFAP<sup>+</sup>/Hoechst<sup>+</sup>, or Hoechst<sup>+</sup>. (F) The quantification of cell type-specific markers required the exclusion of cytoplasmic areas (objects that do not touch Hoechst). The resulting image after Volocity® software quantification is shown. Analyzed nuclei are shown as overlapping purple, yellow, white, and blue.



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**Figure 2:** Visualization of neuronal and astroglial cell lineages. (A, B) Velocity® software 3D renderings of neuronal (green) and astroglial (red) cell lineages, with nuclei shown in blue, before (A) and after (B) identification of  $\beta$ -TubIII+Hoechst+, GFAP+Hoechst+,  $\beta$ -TubIII+GFAP+Hoechst+, and Hoechst+ objects using Velocity software. (C) Identified objects are shown in the XY plane and enlarged area.

After optimizing the two protocols, researchers then evaluated the time-efficiency and validity of using this software-based image analysis approach compared to manual analysis. Study results demonstrated that software-based quantification of hNSC progeny was more accurate than manual methods and was at least two-fold faster, particularly in samples of high cell density [8].

These two image analysis protocols could also be applied to the quantification of other human stem cell lineages, with only minor modifications such as adjustments in object size, color intensities, and z-stack intervals. These protocols can be downloaded [<http://www.sciencedirect.com/science/article/pii/S1873506111000663>] from the supplementary material associated with Piltti et al. [8] and tested or adapted in Velocity® software. A complimentary demo version can be found at <http://cellularimaging.perkinelmer.com/downloads/>.

marker. In Velocity® software, protocols are built up through the addition of a series of measurement tasks that perform functions such as finding, relating, thresholding, filtering, etc. These allow the user to measure biological structures and the relationships between them.

To quantify the proportions of cells positive for each of the three cell fate markers, the protocols were started with a thresholding measurement task to identify objects of interest in the appropriate channels. Velocity® software includes a range of flexible thresholding options that do not rely on absolute intensity values, meaning that the same protocols may be applied to multiple data sets and time-resolved experiments. Further steps in the protocols filtered and excluded objects based on their color and size, removed background noise, and excluded cytoplasmic areas (objects that do not touch Hoechst). Finally, measurements were performed to identify cells as neuronal, astroglial, or oligodendroglial.

## Results

Figure 1 shows the identification and quantification of neuronal, astroglial, and oligodendroglial cell types in Velocity® software after hNSC differentiation *in vitro*. The measurement feedback is shown as an overlay on the image data (Figure 1), and this was used for validation by manual inspection. In Velocity® software, objects can be viewed in different ways using a variety of image view modes. Figure 2 shows images taken from both the “XY Plane” view mode and the “3D Slice” view mode, the latter of which allows image data to be re-sliced in a different orientation, post-acquisition, to display a more informative visualization.

## Conclusion

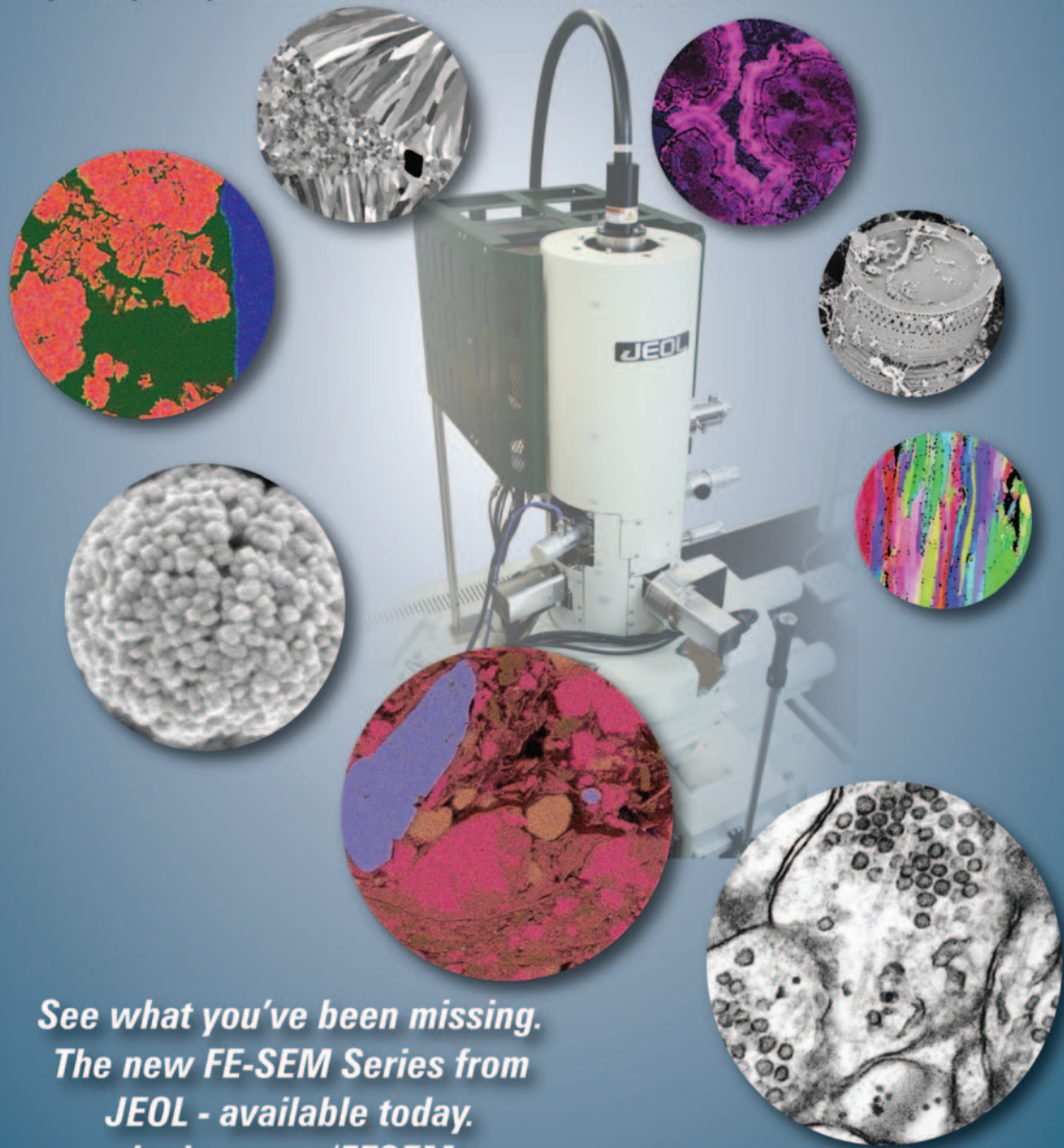
These results show that, compared to human-based manual 2D image analysis, Velocity® software-based 3D analysis allowed for two-fold faster analysis and more accurate analysis of cytoplasmic and nuclear cell fate markers after *in vitro* differentiation.

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