Automated Haematopoietic Colony Forming Cell (CFC) Assay

In collaboration with Nikon, the Centre for Commercialization of Regenerative Medicine ("CCRM") has developed a **functional assay to quantify haematopoietic progenitor cells based on their clonal ability to form haematopoietic colonies,** which is amenable for use in **both research and clinical settings** and may be of interest to industry members in the **haematopoiesis field**.

1 | Market Need

This assay was developed using the Nikon BioStation CT® for the automated enumeration and identification of haematopoietic colony forming cells (CFCs). Applications for such an assay include:

- Ex vivo haematopoietic cell expansion and differentiation research
- Supportive diagnostics for myeloproliferative diseases and leukemia
- Haematopoietic progenitor cell enumeration for cell assessment during haematopoietic stem cell transplantation
- Haematopoietic toxicity and dose evaluation of drug candidates

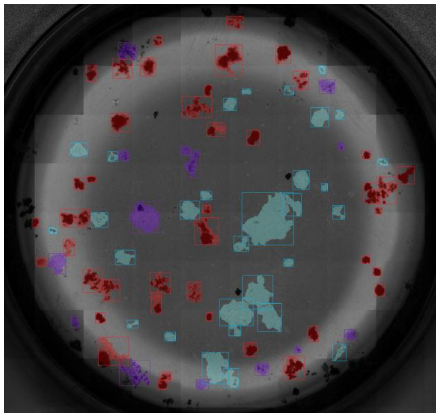
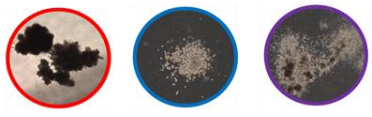


Figure 1. Example image from the Nikon BioStation CT® with colony identification algorithm applied.

This functional assay, which quantifies haematopoietic progenitor cells based on their clonal ability to form haematopoietic colonies, can be performed with fresh or frozen bone marrow, peripheral blood, UCB, or pluripotent-derived blood progenitors. The assay allows for identification of multiple colony types, distinguishing between multipotential and lineage-restricted progenitors of the erythroid, granulocytic, and macrophage lineages. The use of the Nikon BioStation CT® enables automated colony enumeration and kinetic data of colony formation from time-lapse images. Comparable products currently on the market include STEMCELL Technologies' STEMvision™ automated imaging instrument, which is currently marketed for automated colony identification.



Colony Identification:

- **CFU-E/BFU-E** - erythrocyte progenitors (red/dark colonies)
- **CFU-M/CFU-G** - monocyte/granulocyte progenitors (white colonies)
- **CFU-GEMM** - primitive multipotent progenitors (Mixed colonies containing both of above types)

2 | Products

- Automated haematopoietic colony forming cell (CFC) assay

3 | Competitive Advantages

Initial studies indicate that the Nikon BioStation CT® can perform similarly to manual colony counting. Advantages include:

- Standardized algorithm for colony identification minimizes operator-dependent variance
- Automated handling of multiple plates enables rapid analysis of multiple samples
- High resolution images of full wells recorded for further analysis and documentation
- Time-lapse imaging provides an option for earlier identification of progenitor potential and analysis of colony growth kinetics

4 | Technology

4.1 Technology Description

The Nikon BioStation CT® is a compact cell incubator and monitoring system that allows users to conduct live cell imaging and facilities a broad array of long-term time-lapse experiments, including studies of cell growth, morphology and protein expression. It provides consistent environmental control of temperature, humidity and gas concentration in combination with phase and fluorescence imaging.

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4.2 Proof of Principle

The assay was performed as follows:

1. Haematopoietic cells sourced (cryopreserved UCB cells) and $CD34^+$ enrichment performed;
2. $CD34^+$ cells plated at low density (500 cells/well) in semi-solid cytokine-supplemented media and incubated for 14 days;
3. End-point images taken on BioStation CT® at 2X magnification with image acquisition and processing of well performed within 10 minutes;
4. Colony enumeration and identification in less than 5 minutes (algorithm developed in CL-Quant software in collaboration with Nikon);
5. Tabulated report of colony enumeration exported;
6. Data from automated image analysis compared to manual colony scoring (n=10).

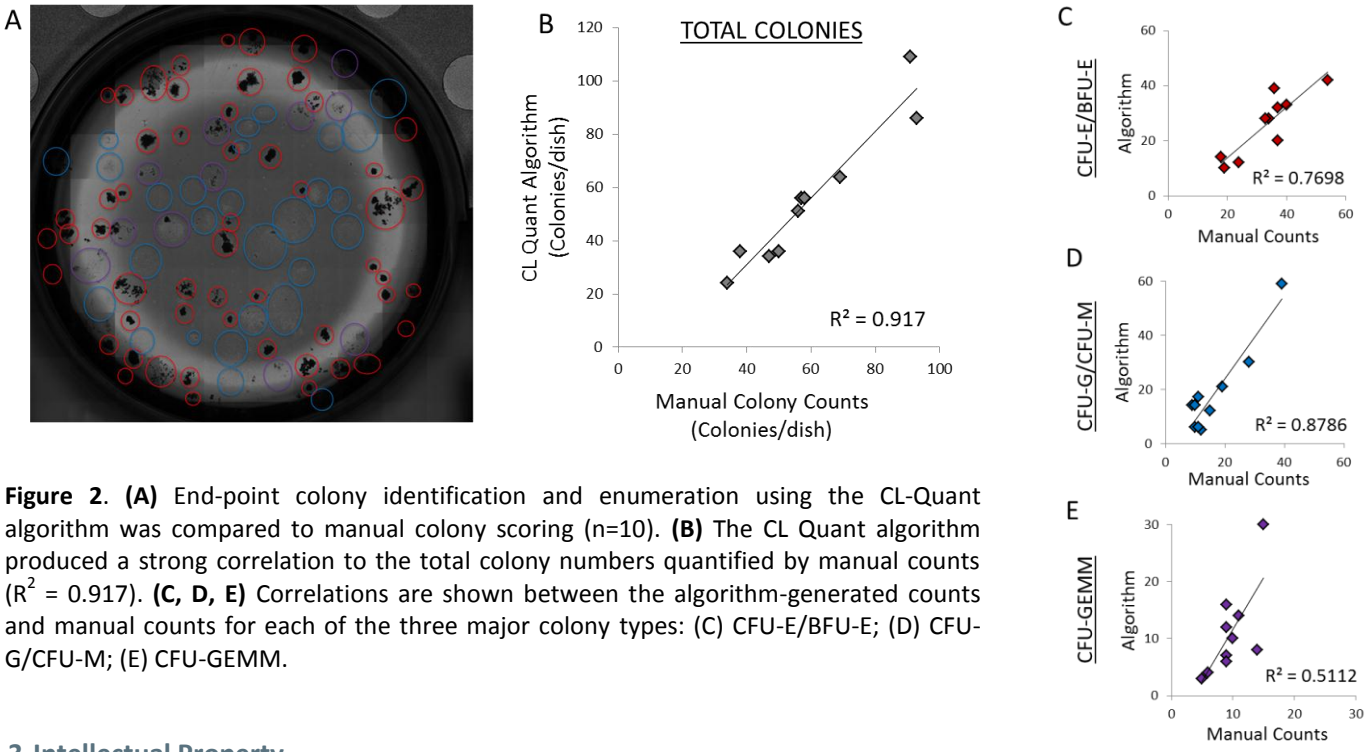

Figure 2. **(A)** End-point colony identification and enumeration using the CL-Quant algorithm was compared to manual colony scoring (n=10). **(B)** The CL Quant algorithm produced a strong correlation to the total colony numbers quantified by manual counts (R^2 = 0.917). **(C, D, E)** Correlations are shown between the algorithm-generated counts and manual counts for each of the three major colony types: (C) CFU-E/BFU-E; (D) CFU-G/CFU-M; (E) CFU-GEMM.

4.3 Intellectual Property

- No IP has been filed for this assay. Nikon owns the rights to the BioStation CT®.

4.4 Lead Inventor(s)

- Dr. Elizabeth Csaszar at the Centre for Commercialization of Regenerative Medicine, Toronto, Ontario
- Nikon

4.5 Relevant Reference

Pereira C, *et al.* (2007) Hematopoietic colony-forming cell assays. *Methods Mol Biol,* 407, 177-208.

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