

Automated Time-Lapse Imaging and Multipurpose-Fluidics for Cellular Niche Exploration *In Vitro*

Alfred Bahnson¹, Doug Koebler¹, Yifang Song², Julie Goff², Tao Cheng², Ray Houck¹, and Lex Cowser¹

¹Automated Cell, Inc., Pittsburgh, PA

²University of Pittsburgh Cancer Institute, Pittsburgh, PA

Specialized tools are required to resolve the time- and space-dependent interactions in multi-cellular niches that are thought to control the multiplication and differentiation of stem cells. Time-lapse imaging is one such tool that has come of age through automation of position and focus (x, y, and z) and control of the stage environment so that cultures can be monitored intermittently or continuously for weeks or more. Multi-well plates permit simultaneous examination of effects from hundreds of independent variables such as matrix and/or soluble factors. Storage of the digital images provides a non-exhaustible wealth of raw data that we are learning to mine with automated image analysis techniques for time-resolved quantitation of motility and growth (Bahnson, et al., 2005) and that offer potential for measurement of parameters of specific relevance to the dynamics of interaction between heterogeneous cell types that constitute a niche. For example, when different phenotypes are resolvable, "proximity analysis" can quantify the prevalence of the contacts and, at the individual cell level, contacts can be correlated with phenotypic features. Such analysis is enhanced by fluorescent protein expression in the stem cells. We are further developing and incorporating micro-fluidics hardware and proprietary methods that will allow us to 1) add or modify soluble factors in the medium and 2) stain with fluorescent markers for surface antigen phenotype analysis without disruption of cell positions and without interruption in the incubated environment or in the monitoring of multi-well experiments. For this workshop, we will demonstrate examples of how this system can be applied to hematopoietic stem cell-stromal cell interactions in real time for exploring effects of known stem cell regulators such as Notch1.

References

1. Bahnson A, Athanassiou C, Koebler D, Qian L, Shun T, Shields D, Yu H, Wang H, Goff J, Cheng T, Houck R, Cowser L. Automated measurement of cell motility and proliferation. *BMC Cell Biology* 2005;6:19

[**ACKNOWLEDGMENT:** This work was supported by NIH grants RO1EB001051 (to R.H), RO1 HL70561 and KO8DK02761 (to T. C.)]