Single-cell Automated Imaging and Nano-injection Tool (S.A.I.N.T.)

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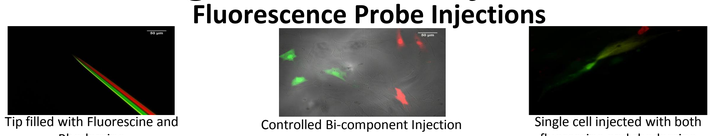
Abstract

Single-cell analysis via nano-injections using nanopipette technology is limited by how fast users operate the Biostinger, a single cell nano-manipulation platform, as cells have limited tolerance outside the incubator. We developed a point-click software interface that can increase the precision and throughput of injections (ie. toxin into cells) with patterns. A future extension of the system is to link this software to a cell-recognition platform and a computerized microscope to create an autonomous system.

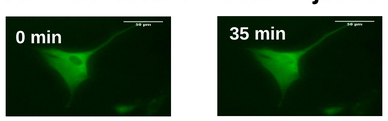
Single-Cell Nano-Injection

Fluorescence Probe Injections

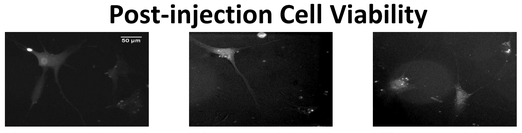


Tip filled with Fluorescine and Rhodamine — Controlled Bi-component Injection — Single cell injected with both fluorescine and rhodamine

Green Fluorescent Protein Injections

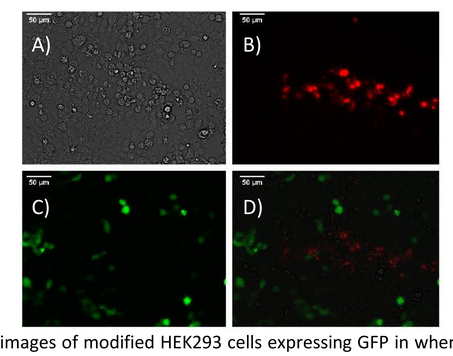


Post-injection Cell Viability



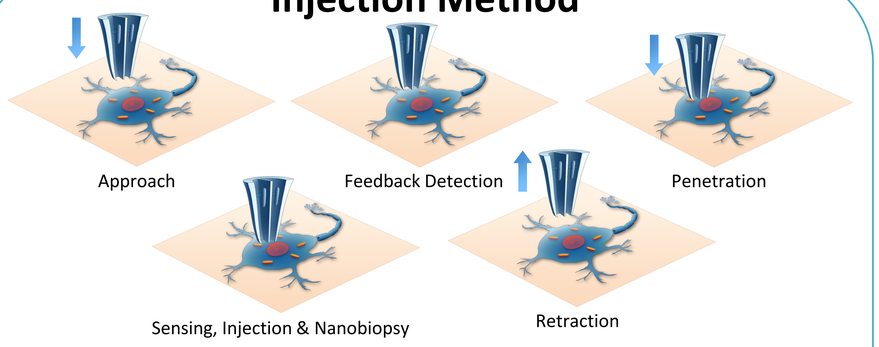
0 hours post-injection — 19 hours post-injection — 27 hours post-injection

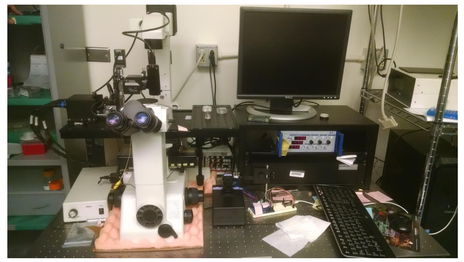
Automated Single-Cell Nano-Injection



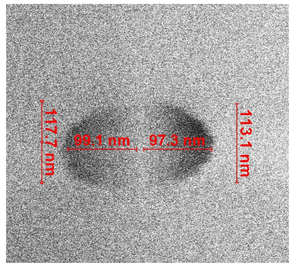
False color images of modified HEK293 cells expressing GFP in when induced by TNFα injected with Dexamethasone-Sulforhodamine 101 solution (inhibitor of TNFα pathway) after overnight TNFα exposure. Cells injected with automatic single-cell nano-injection software. A) Bright-field Image of injected cells, B) Red False color image of Dexamethasone-Sulforhodamine 101 solution, C) Green False color image of GFP expression after TNFα exposure, D) Merge of bright-field and false-color images.

Injection Method





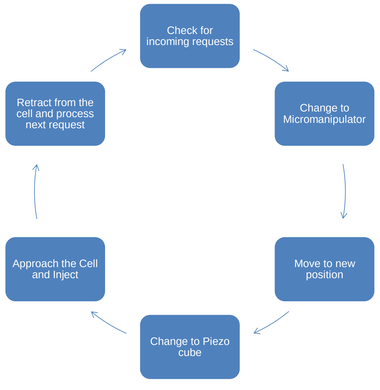
BioStinger Single-Cell Nanomanipulation setup



Pore of Double Barrel Injection Nanopipette

Automation

Single Cell Nano-injection is automated by emulating the mechanical process using a software abstraction called Hierarchical State Machine (HSM). The nano-injection method is broken into 5 unique states.

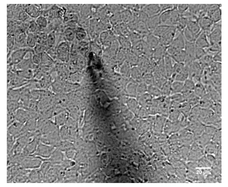


References

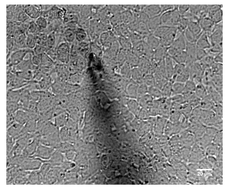
1. R. Seger, P. Actis, C. Penfold, M. Maalouf, B. Vilozny and N. Pourmand; Voltage controlled nano-injection system for single-cell surgery. *Nanoscale (2012), Vol 4.*
2. Actis, P., Maalouf, M.M., Kim, H.J., Lohith, A., Vilozny, B., Seger, R.A., Pourmand, N.; Compartmental Genomics in Living Cells Revealed by Single-cell Nanobiopsy. *ACS Nano (2013), Vol 8.*

Computer Vision

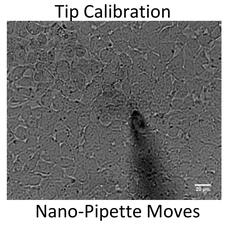
The point-click interface was designed with the physical pixel distance quantification. A reference scale is imaged and used to count the number of pixels seen in each objective. The user registers the nanopipette tip's position with a click of the image, and subsequent clicks generate a delta pixel position, which is used to move the nanopipette tip accurately. The computer vision code is written in OpenCV and communicates to the BioStinger Firmware through TCP.



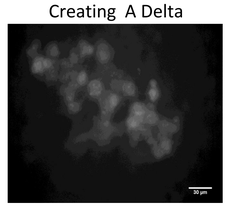
Tip Calibration



Creating A Delta



Nano-Pipette Moves



End Result Post Injections

Future Work

- Automate a closed system to study single cells over time
  - Incubate, relocate, identify and interrogate cells by nano-injection
    - Incorporate robust cell identification software to interrogate the same cell over time
    - Maintain cells at incubator environment during interrogation
    - Relocate cells from incubator to interrogation stage
- Increase throughput of cells interrogated
- Study the progression of disease models such as Alzheimer's and Breast cancer

Acknowledgements

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