Project Summary

Microinjection is a widely used transgenic engineering technique that underpins genetic manipulation, experimentation, and knowledge discovery across virtually all areas of biomedical science. It consists of using an industry standard, transparent, clear glass microneedle (ISN) to manually inject foreign materials (e.g. RNA and DNA), into biological cells. This thirty-year-old technology has driven advances in the fundamental research of stem-cell gene manipulation, created the intra-cytoplasmic sperm injection method as part of in-vitro fertilization cycle therapy, and allowed extensive human disease prevention modeling via pathophysiological investigations.

Three design-induced constraints – often attributed to poor user technique - affect the technique's efficacy and experimental rigor and reproducibility:
(1) the transparent needle tip creates low contrast visibility in vitro, leaving the user unable to view and track the penetrating needle tip.
(2) cytoplasmic material (e.g. embryo yolk) can clog the needle tip, diminishing volume injected over time.
(3) manual creation of each needle via industry standard pipette pullers introduces calibration variability with every new needle used.

Although other foreign-material-introduction methods exist, such as electroporation, chemical membrane permeabilization, and automated, robotic systems, none have proven responsive to addressing these key design constraints.

The objective of this proposal, prototyping a colored-tip, microcapillary needle with anti-clogging properties as a minimal viable product (MVP), bridges an important need in the biomedical industry.
The working hypothesis is that the patented, SeeTrue MVP will simply and powerfully increase the accuracy of the microinjection technique as compared to ISNs by a) improving the efficacy of delivering material to the embryo, and b) reducing variability in the volume injected.

The MVP will be tested, and its efficacy compared to ISN's in the Zebrafish embryo biological system by both experienced and inexperienced Zebrafish embryo microinjectors.
The MVP fabrication and testing will be achieved in the following two aims:
Aim 1: Fabricate a nanoparticle-coated, colored glass MVP with anti-clogging capabilities.
Aim 2: Test the colored and anti-clogging SeeTrue MPV using Zebrafish embryos.
The MVP's ability to increase an injectors' accuracy will be quantified by measuring the amount of material delivered to the embryo and by the degree of variability in the amount of material delivered to embryos following multiple microinjections. In addition, a comparative user satisfaction survey will be administered to all testers. The proposed SeeTrue MVP introduces the simple, elegant, design innovations of enhanced visibility and anti-clogging properties to an established, fundamental technique. It has the potential to correct known rigor and reproducibility (technique) deficits and increase the efficacy of microinjection biomedical transgenic research. This will, in turn positively impact foundational human-health related research and medical applications.

This proposed research – a prototype for a colored, anticlogging microinjection needle - is relevant to public health research because it represents a new and substantive departure from the status quo. This prototype has the potential to increase the rigor, reproducibility, and efficacy of a broad range of research that uses the manual microinjection technique by introducing two design-improvements that will improve the accuracy of the technique. This is relevant to the part of NIH's mission that pertains to both basic human-health transgenic research and medical applications.