

# Effect of Mercury Column on the Microdynamics of the Piezo-Driven Pipettes

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*This study is on an interesting phenomenon concerning cellular microinjection procedures which are used for various biomedical applications, and in particular intracytoplasmic sperm injection. Recent years have brought considerable practical improvements in these operations. One of them suggests aspirating a very small quantity of mercury in the injection pipettes prior to piercing into cells. This process is proven to enhance the rate of success considerably. We present a unique study in determining the influence of mercury on the microdynamics of the pipette. The effort contains both numerical simulations and corresponding experimental verification. Ultimately we offer two critical results: (1) The mercury column increases the mass loading and expectedly decreases the natural frequencies of the pipette and (2) The lateral oscillations, which play a destructive role in piercing, are subdued in amplitude due to the mass loading of mercury. Simulation results are presented, which are also verified experimentally using high-speed digital imaging. As a consequence of these findings we also propose some alternative design directions for future microinjection devices. [DOI: 10.1115/1.1894368]*

## 1 Introduction

A widely utilized microinjection procedure, piercing with piezoactuated pipettes, is studied in this paper. It is an accepted methodology in most laboratories, which handle subcellular subjects [e.g., in nucleation and enucleation for cloning, or in vitro fertilization (IVF) with intracytoplasmic sperm injection (ICSI)]. It is successfully used on variety of species like mouse, rat, and cattle [1–6]. It is also a very effective method in treating male factor infertility [7–12]. In essence, it entails a two-step operation: opening a section in zona pellucida (the protective layer outside the membrane) and then piercing through the membrane with a glass pipette tip. The pipette carries out the injection (or aspiration) once the piercing is completed.

In the earlier deployment of ICSI a manual procedure was used, that is often called the Conventional ICSI [11,13,14]. In this technique the tip of the injection pipette is pushed gently about half-way through the oocyte. Then it is advanced forward swiftly until it penetrates the zona and the membrane. Afterward the sperm is injected into the ooplasm. Literature points the fact that this operation has large failure rates (higher than 90% for rats, mouse, and cattle [13,15]) which is measured by the abnormal growth of the cell in the subsequent developmental phases. The primary reason for this failure is recognized as the damage caused on the membrane by the ICSI pipette during the incision.

A clever process, so-called Piezoassisted ICSI is introduced in later years as a remedial methodology [2,11,12,14,16,17], which uses a series of controlled impulsive forces along the glass pipette.

This device is known as the Piezodrill. It is attached to the pipette holder, and the force is deployed as an impulse train with adjustable frequency, amplitude, and durations. All of these operating parameters are to be properly selected for the particular species involved. Even for the experimentally tuned ideal settings of these parameters, the piezoassisted ICSI still results in undesirably low rates of success [4,18]. The research presented here is primarily motivated by a more recent progress reported on this procedure, which suggests that a small amount of mercury is aspirated in the pipette before the piezoassisted ICSI operation. This procedure resulted in a substantial increase of the rate of success. There has been, however, no scientific consensus explaining the physics behind this progress. The present report is a second one in a sequel to address this question. In the first document [19] we looked at the effects of piezoimpulses on the transverse motion of the pipette tip, which expectedly caused damage on the membrane when there is no mercury within the pipette. In this study the focus is on the influence of the mercury column on the piercing of micropipettes into oocytes. We first create a realistic model for the microdynamics at hand, incorporating the mass loading of the mercury. The pipette is taken as a thin flexible beam under impulsive type excitation at a point very close to its base (i.e., where it is attached to an ultra precision micropositioner). The dynamic model for this infinite dimensional system is created using the Lagrangian method. The ensuing partial differential equation driven by a nonuniform excitation force is solved using the conventional Galerkin approximation. Time domain simulations are generated and the resulting pipette deformations are obtained. The highlight of the present text is in the experimental verification of these dynamics using high-speed digital imaging techniques. This exercise describes clearly how the mercury mass-loading assists the microdynamics of the pipette.

The text is formed as follows. Operating principles of the “piezodrill” is described in Sec. 2. A brief modeling study and ensuing numerical simulations are presented in Sec. 3. Section 4 covers the description of the microscopic imaging work and the experimental results. Section 5 offers comparative observations and comments on the piezoactuated pipettes. Finally in Sec. 6 we present the conclusions.

## 2 Working Principle of the Piezo Actuated Drill

The ICSI procedure using a piezodrill is described in many earlier reports [2–4,11,17]. The hardware mainly consists of two segments: ultra precise microrobot (manipulator) and piezoelectric transducer (PZT) assisted microinjector.

Figure 1 displays the composition of the piezostack (which creates the impulsive piezoforce), the pipette holder, and the injection pipette (which is attached to the pipette holder), and the micromanipulator, which positions and orients the pipette relative to the target oocyte. A typical geometric disposition of the thermally drawn glass pipette and the mercury column are given in Fig. 2. An operational depiction of the components is displayed in Fig. 3. One should take note of the extremely thin (thus flexible pipette tip) and its susceptibility against impulsive excitations originating from the pipette holder (Fig. 1).

As the piezoforce is implemented, micromanipulator attachment shows a three-dimensional (3D) motion (albeit at microscopic levels). In our earlier study we claim that this 3D motion was substantial in transverse direction to the pipette’s axial line, along which the force is deployed [19]. In that effort a conventional thin beam study is done to analytically verify this hypothesis utilizing a simplifying assumption for an initial deformation and velocity profile after the impulsive forcing. In the present study we advance the mathematical model further. Differently from the earlier study, we now start with quiescent initial conditions and deploy a pointwise impulsive force exerted in the transverse direction to replicate the piezo excitation to the pipette. The mathematical model evaluates the ensuing behavior. In the interest of space and unnecessary duplication of the standard methodolo-

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