A Novel Method for ICSI: Rotationally Oscillating Drill, Design and Control

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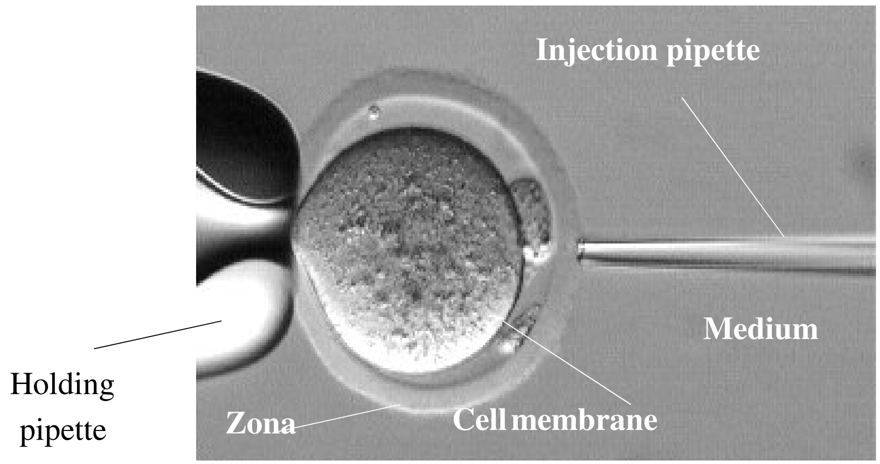
Problem Statement

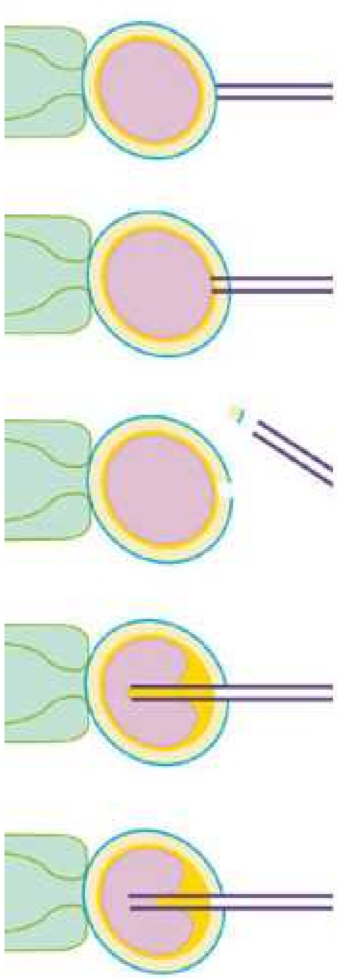


Micro Injection Experiment

Designing Minimally Invasive Mercury-Free μ–drill for Micro Injection

What is Micro-Injection?

Micro injection is a method widely used in cell biology.



Zona

Zona penetration

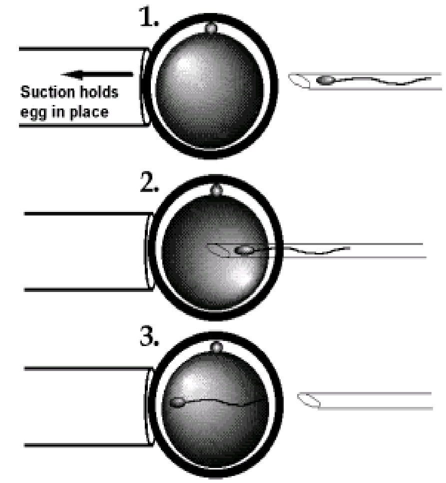
Taking out the dissected zona

Oolemma Penetration

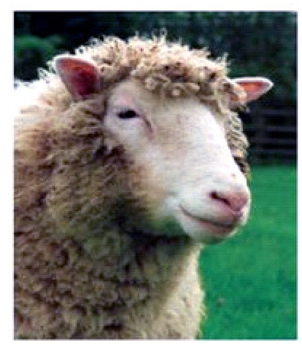
Injection

•ICSI

(Intracytoplasmic sperm injection).



•Nuclear Transfer for cloning.



Current Setup

(for mouse ICSI)

Manipulator drive signal

Current Feedback & Controller

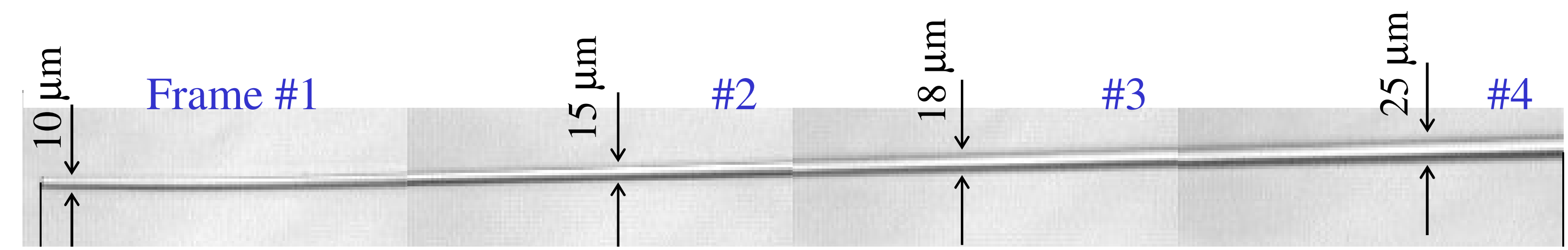
Disadvantages

- Piezo-ICSI needs to have highly toxic mercury to increase success rate.

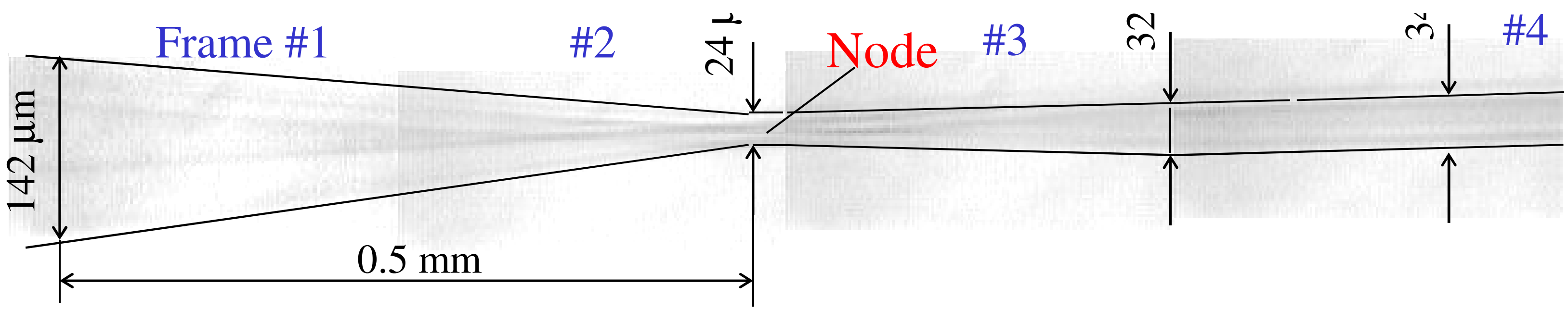
- The operation is highly dependent on human expertise.

- Piezo-ICSI devices are considerably expensive

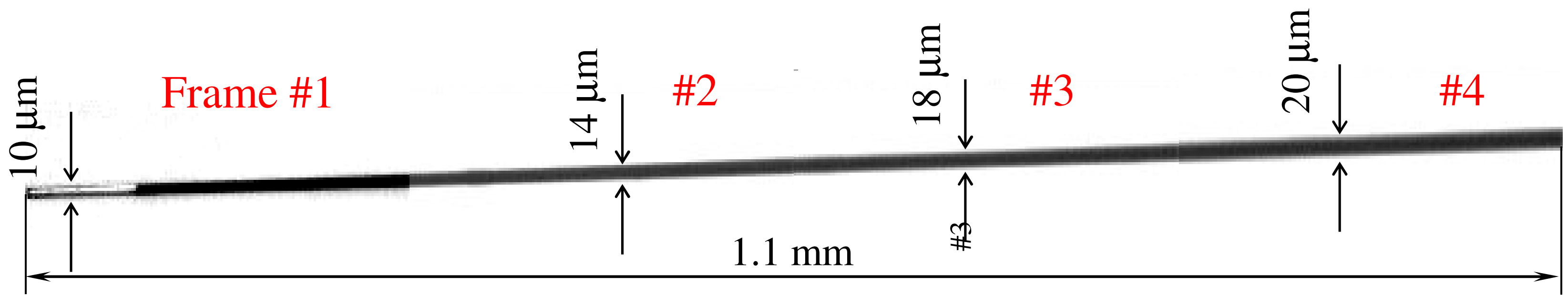
Amplitudes of the oscillations without mercury

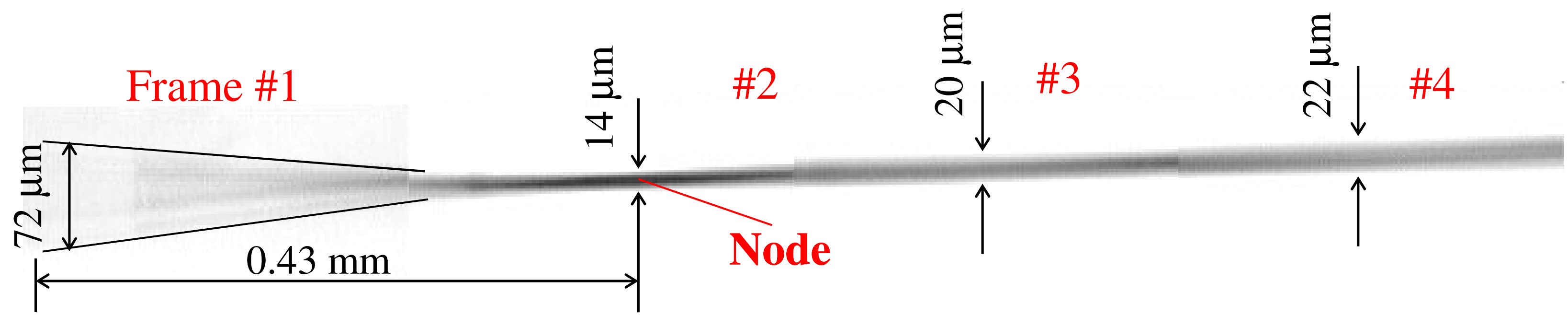


Why does mercury increase the success rate ?



Amplitudes of the oscillations with mercury





Euler method

E : Young's Modulus

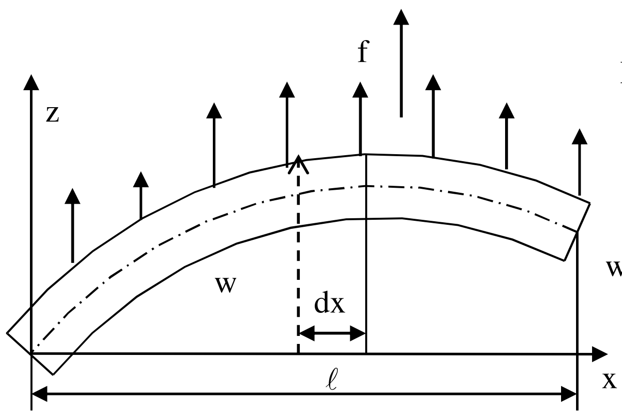
I : Bending moment of inertia

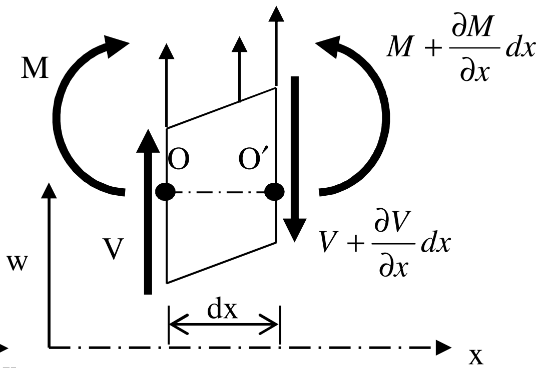
ρ : Mass per unit length

V : Shear force

M : Moment

w : Deformation function





Forcing in transverse direction

$$\frac{\partial V}{\partial x} = -\rho \frac{\partial^2 w}{\partial t^2} + f$$

Thin beam theory

$$M(x,t) = EI \frac{\partial^2 w}{\partial x^2}(x,t)$$

Moment around O

$$\frac{\partial M}{\partial x} = V$$

Euler-Bernoulli beam equation

$$EI \frac{\partial^4 w}{\partial x^4}(x,t) + \rho \frac{\partial^2 w}{\partial t^2}(x,t) = f(x,t)$$

Euler method

Separation of variables

$$w(x,t) = T(t)U(x)$$

$$\underbrace{\frac{EI}{\rho}\frac{U''''}{U}}_{\text{Eigenvalues problem}} = \frac{\ddot{T}}{T} = \omega^2$$

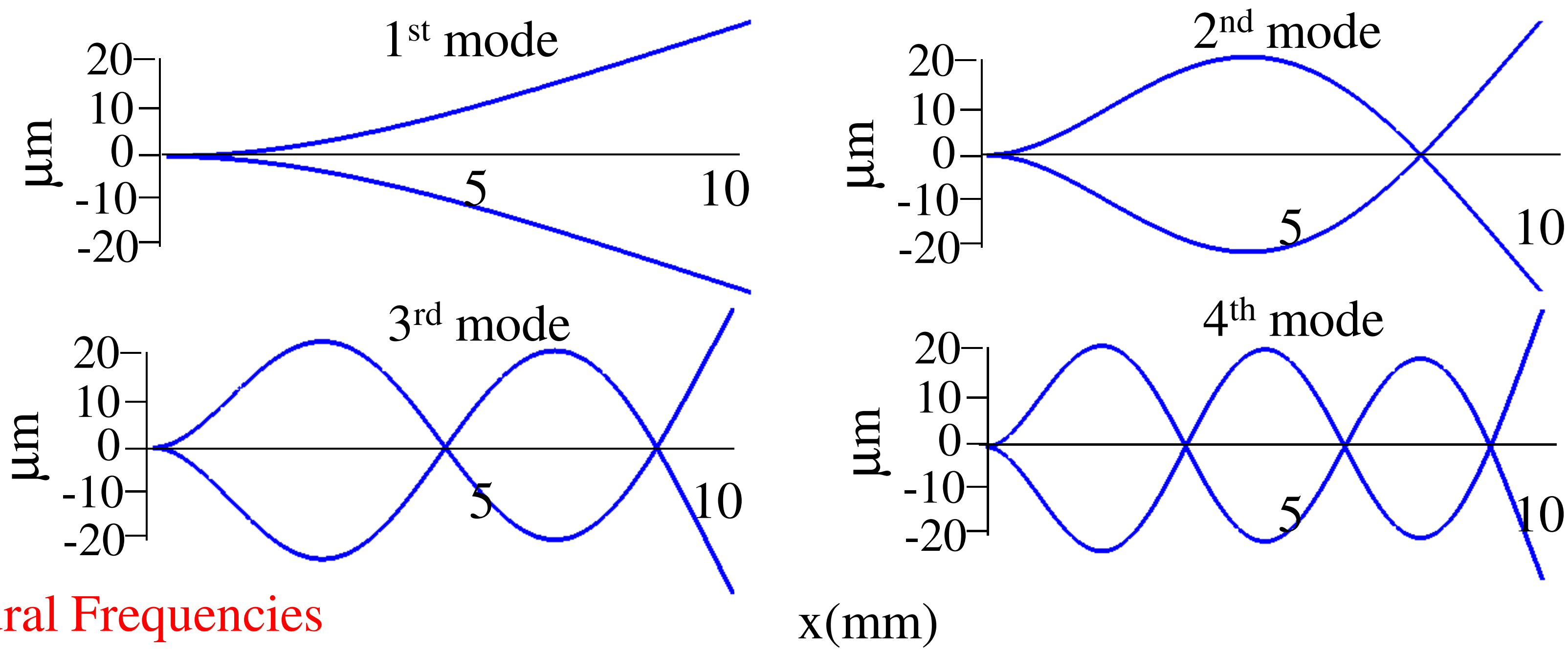
$$U(x) = C_i(\text{Cosh}(\beta_i x) - \text{Cos}(\beta_i x) + \alpha_i(\text{Sin}(\beta_i x) - \text{Sinh}(\beta_i x))$$

$$\alpha_i = \frac{Cos(\beta_i L) + Cosh(\beta_i L)}{Sin(\beta_i L) + Sinh(\beta_i L)}$$

Deformation function

$$w(x,t) = \sum_{i=1}^{\infty} \{C_i(\text{Cosh}(\beta_i x) - \text{Cos}(\beta_i x) + \alpha_i(\text{Sin}(\beta_i x) - \text{Sinh}(\beta_i x))(\text{Sin}(\omega_i t))\}$$

Mode shapes



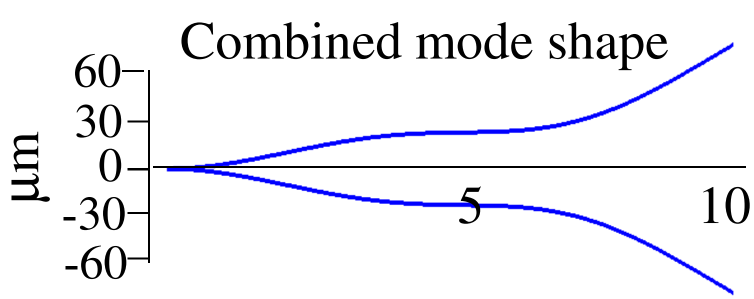
Natural Frequencies

ω_1 = 10 Hz

ω_2 = 63 Hz

ω_3 = 175 Hz

ω_4 = 341 Hz



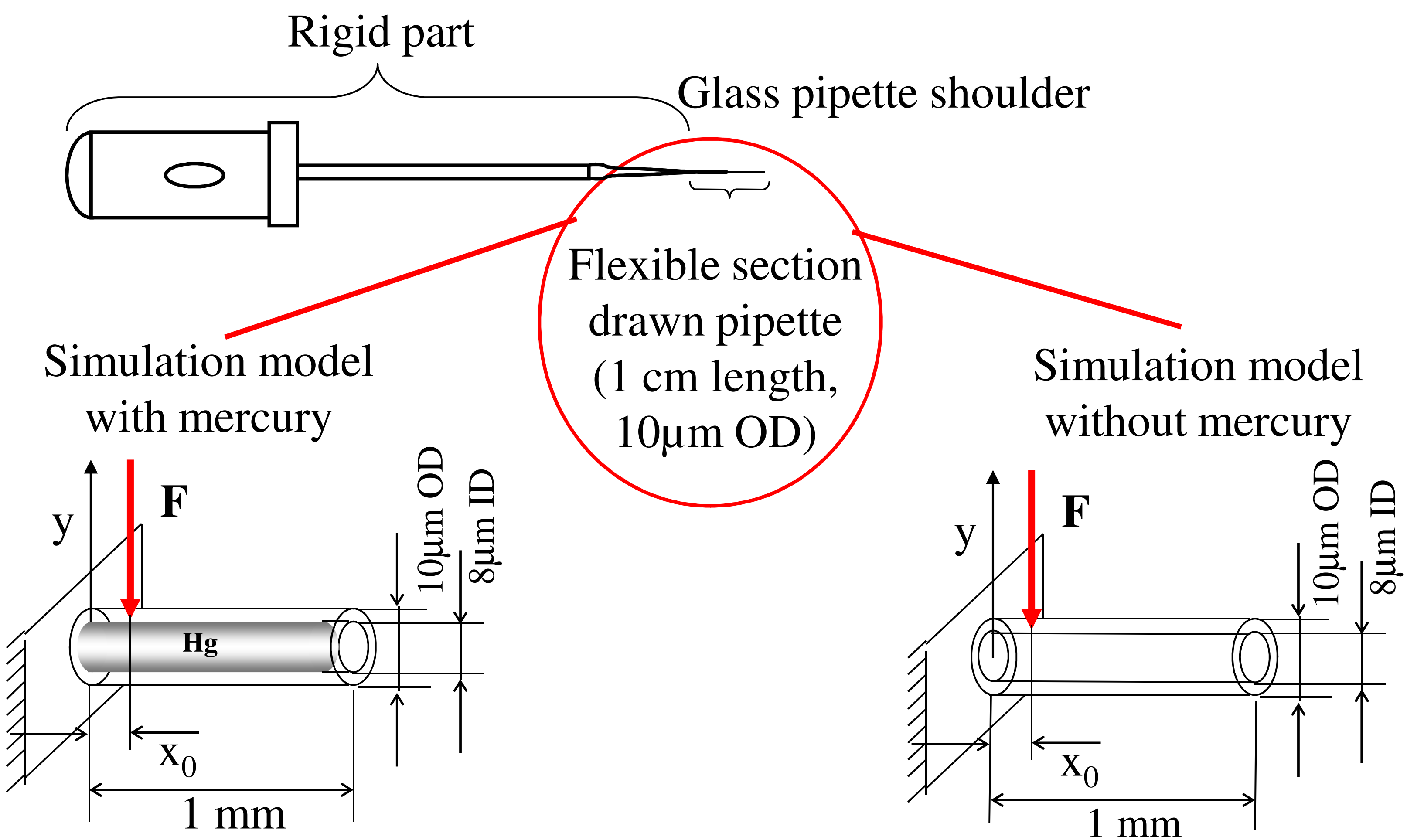
Galerkin method

- In this method two different models are analytically studied. The only difference between them is the existence of the mercury.

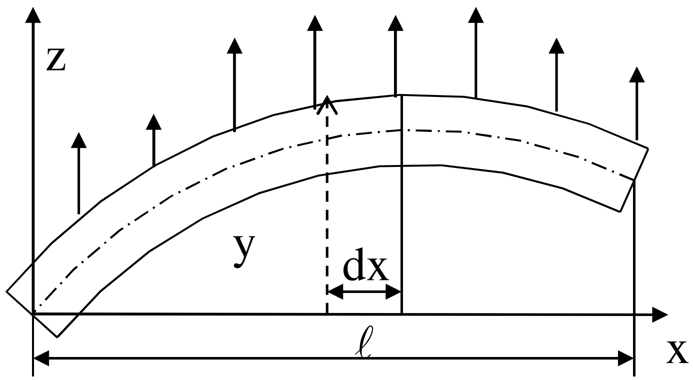
- The aim of this analysis is to simulate the difference between the transverse micro-dynamics of the drawn sections with and without the mercury.

- In these simulations, different from the Euler method an impulse force is applied very close to the base of the drawn section.

Simulation models



Galerkin Method



Kinetic energy

$$T = \frac{1}{2}\rho\int_0^L \left(\frac{\partial y}{\partial t}\right)^2 dx$$

Potential energy

$$U = \frac{1}{2}EI\int_0^L \left(\frac{\partial^2 y}{\partial x^2}\right)^2 dx$$

Deformation function

$$y(x,t) = \sum_{i=1}^{n} \phi_i(x) q_i(t)$$

Ortogonality conditions between the mode shapes

$$\int_0^L \rho\phi_i(x)\phi_j(x)dx = N_i\delta_{ij} \qquad \int_0^L EI\phi_i^{``}(x)\phi_j^{``}(x)dx = S_i\delta_{ij}$$

$$T = \frac{1}{2}\sum_{i=1}^{n} N_i\dot{q}_i^2 \qquad U = \frac{1}{2}\sum_{i=1}^{n} S_i q_i^2$$

E : Young's Modulus

I : Bending moment of inertia

ρ : Mass per unit length

$\phi_i(x)$: Mode shape for the i_{th} mode of vibration.

$q_i(t)$: Time dependent generalized coordinate for the i_{th} mode

Galerkin Method

Lagrange equation

$$\frac{\partial}{\partial t}\left(\frac{\partial L}{\partial \dot{q}_i}\right) - \frac{\partial L}{\partial q_i} = Q \qquad Q = F(t)\frac{\partial}{\partial q_i}\left(\sum_{i=1}^{n}\phi_i(x_0)q_i\right)$$

$$N_i\ddot{q}_i + S_i q_i = F(t)\phi_i(x_0) \qquad \text{For } i = 1..3$$

Laplace form

$$(N_i s^2 + S_i)Q_i(s) = F(s)\phi_i(x_0) \qquad \text{For } i = 1..3$$

Internal damping

$$S_i = S_i + cs$$

Galerkin method

Matrix form of the equations of motion for 3 modes

$$\begin{bmatrix} s^2N_1+S_1+cs & 0 & 0 \\ 0 & s^2N_2+S_2+cs & 0 \\ 0 & 0 & s^2N_3+S_3+cs \end{bmatrix} \begin{bmatrix} Q_1 \\ Q_2 \\ Q_3 \end{bmatrix} = \begin{bmatrix} F(s)\phi_1(x_0) \\ F(s)\phi_2(x_0) \\ F(s)\phi_3(x_0) \end{bmatrix}$$

$$\phi_i(x) = H_i[Sin(\kappa_i x) - Sinh(\kappa_i x) - \alpha_i(Cos(\kappa_i x) - Cosh(\kappa_i x))]$$

$$\alpha_i = \frac{Sin(\kappa_i L) + Sinh(\kappa_i L)}{Cos(\kappa_i L) + Cosh(\kappa_i L)}$$

H_i is defined by normalizing

$$\rho\int_0^L \phi_i(x)\phi_j(x)dx = \rho$$

Deformation function

$$y(x,t) = \sum_{i=1}^{n} \phi_i(x)q_i(t)$$

Motion of the Micro-Pipette

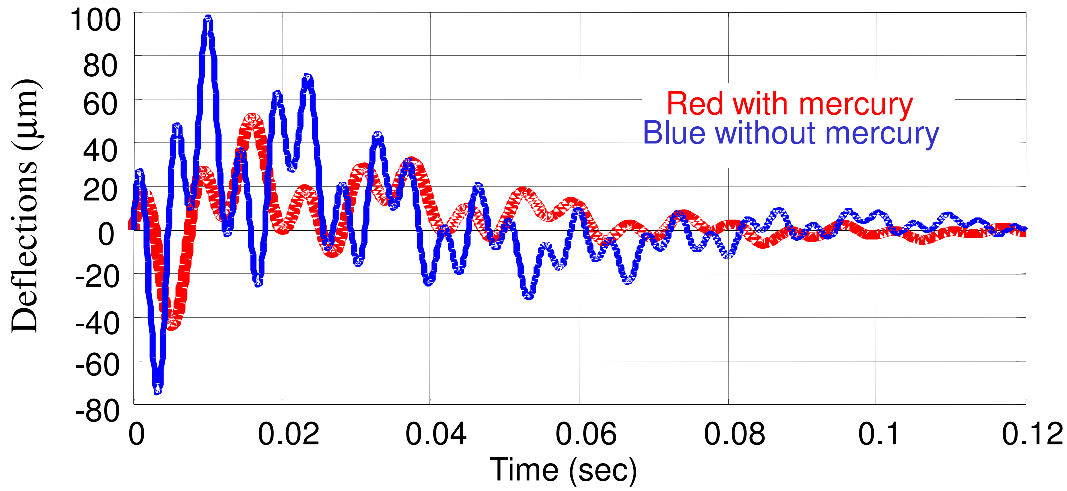
- The flexible pipette shows extensive lateral oscillations [1,2].

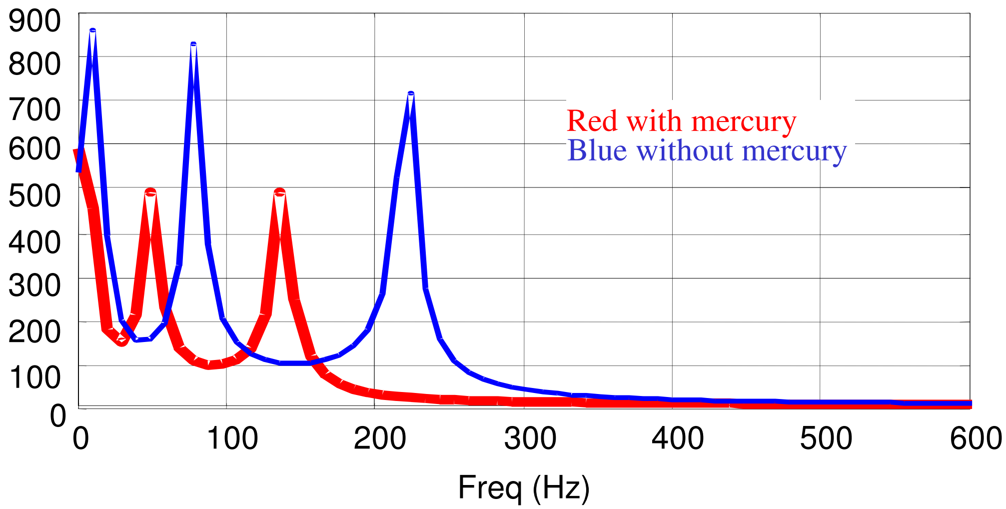
- The mercury increases the mass of the drawn section. Amplitudes of the lateral oscillations of the pipettes without the mercury are significantly higher than with the mercury [1,2].

- The natural frequencies of the pipette filled with the mercury are lower than the natural frequencies of the pipette without the mercury [1,2].

[1] Kerem Ediz, Nejat Olgac, "Micro-dynamics of the piezo-driven pipettes in ICSI", Biomedical Engineering, IEEE Transactions on ,Volume:51
[2] K. Ediz, N. Olgac, "Effect of Mercury Column on the Microdynamics of the Piezo-Driven Pipettes", ASME Journal of Biomechanical Engineering, Vol. 127, pp. 531-535, June 2005)

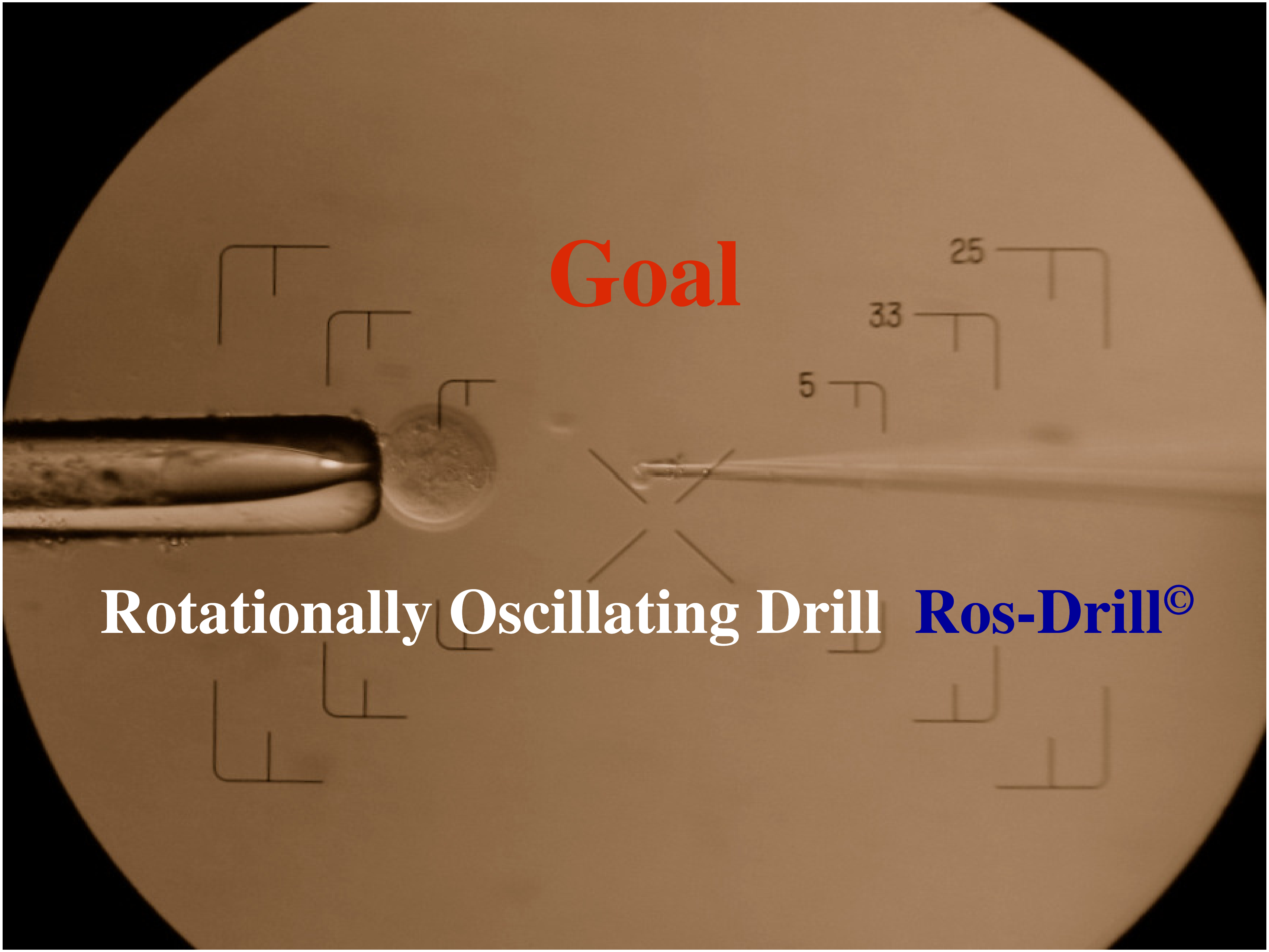
Natural Frequencies





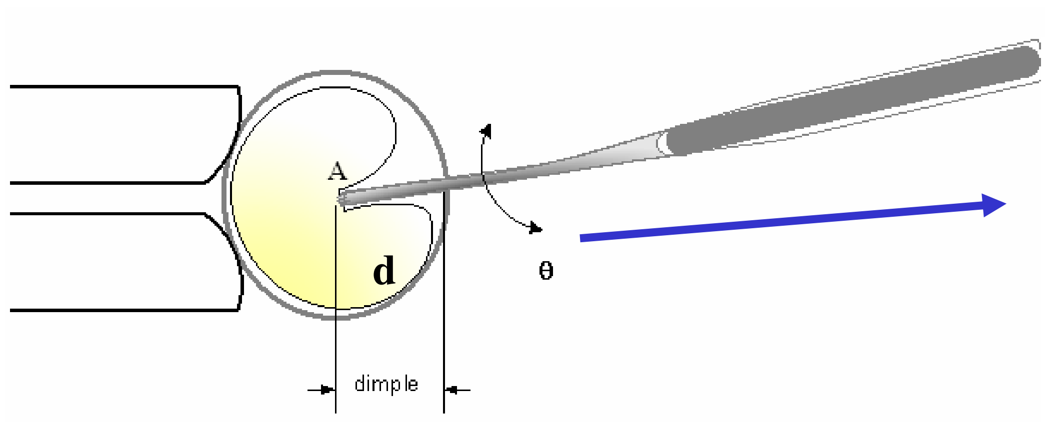
Damped natural Frequencies	
With mercury	Without mercury
7 Hz	12 Hz
50 Hz	79 Hz
139 Hz	221 Hz

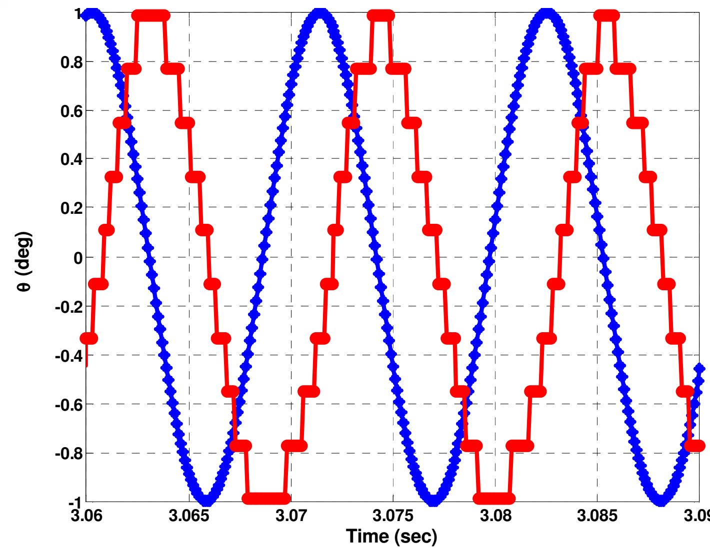
Goal



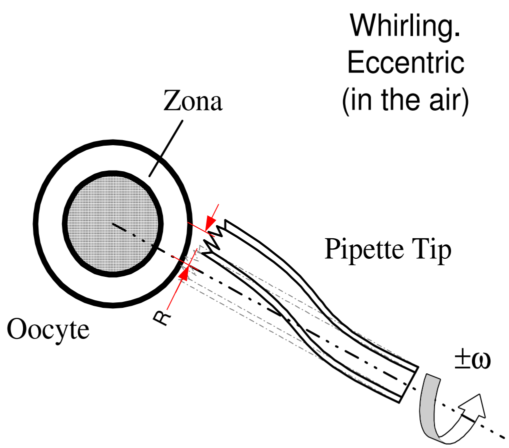
Rotationally Oscillating Drill Ros-Drill©

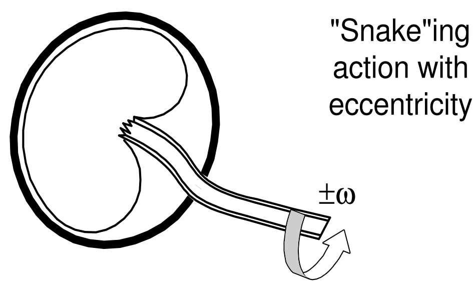
Rotationally Oscillating Drill Ros-Drill©



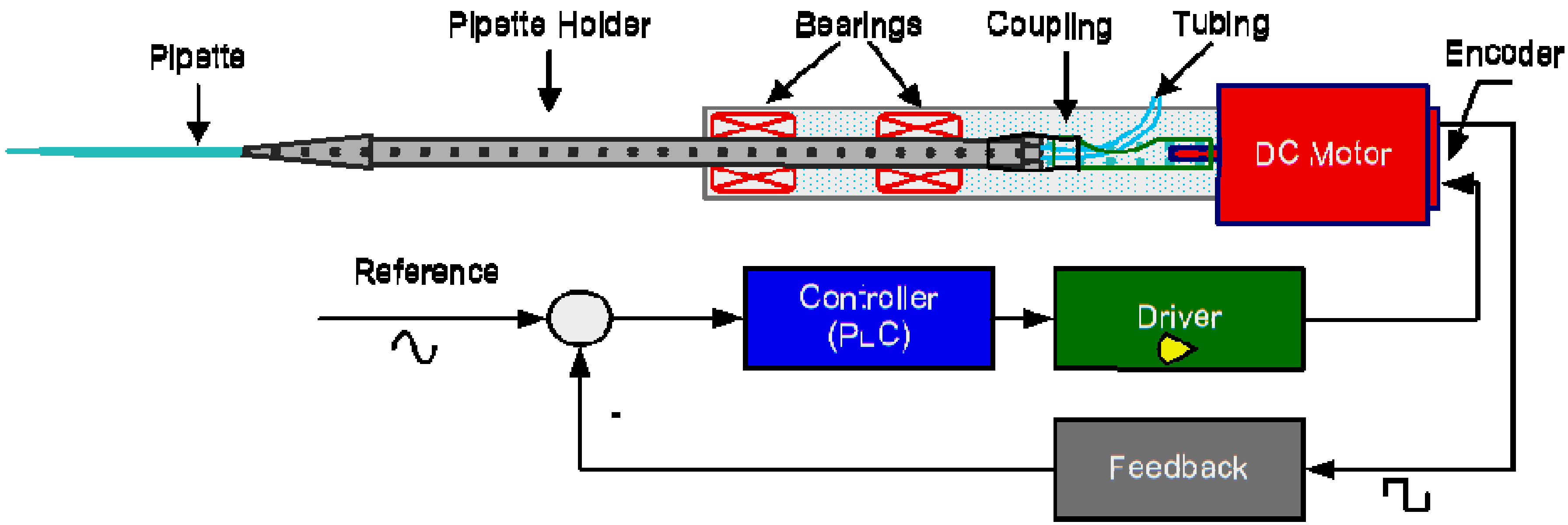


Rotation Profile

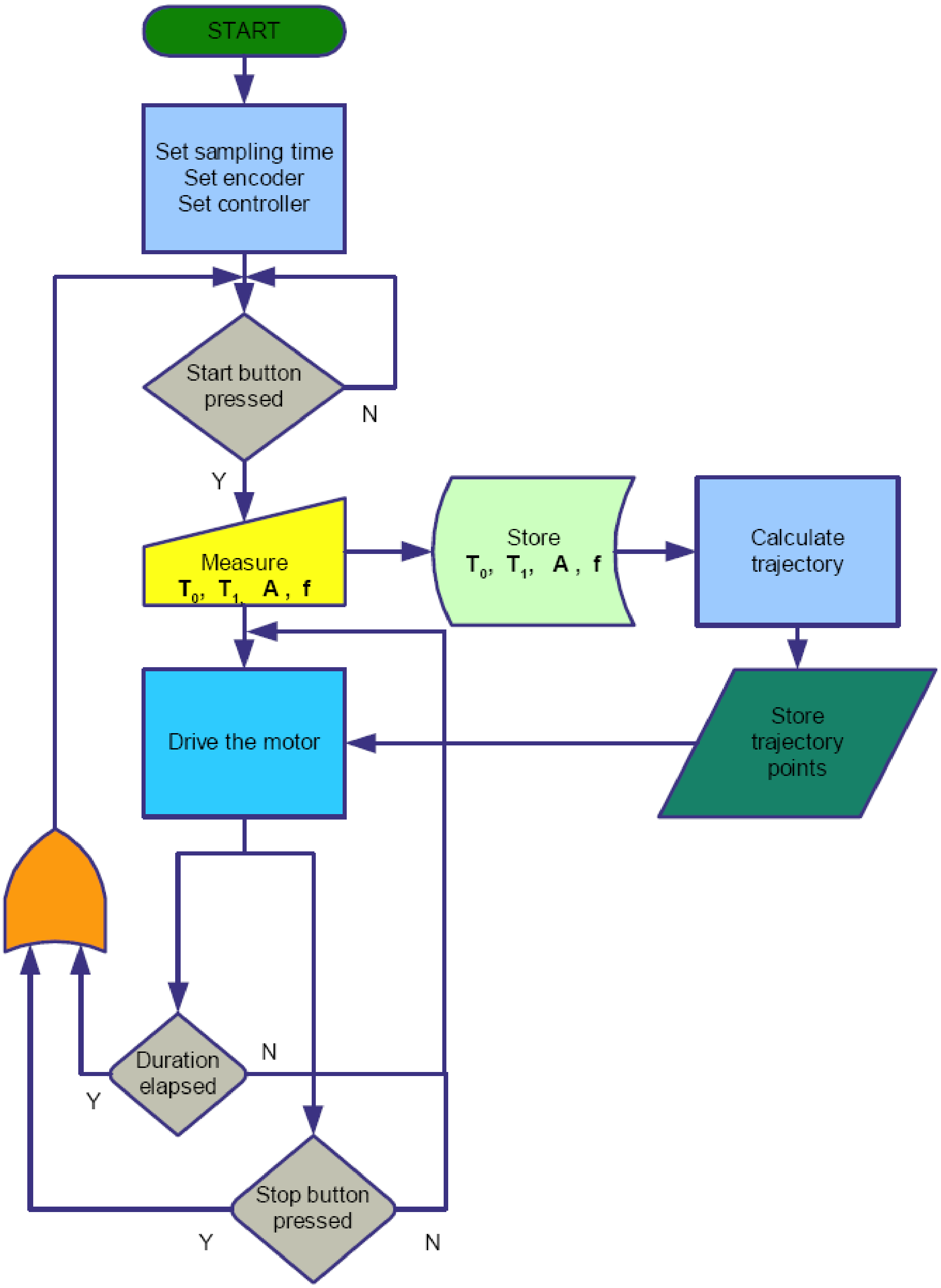




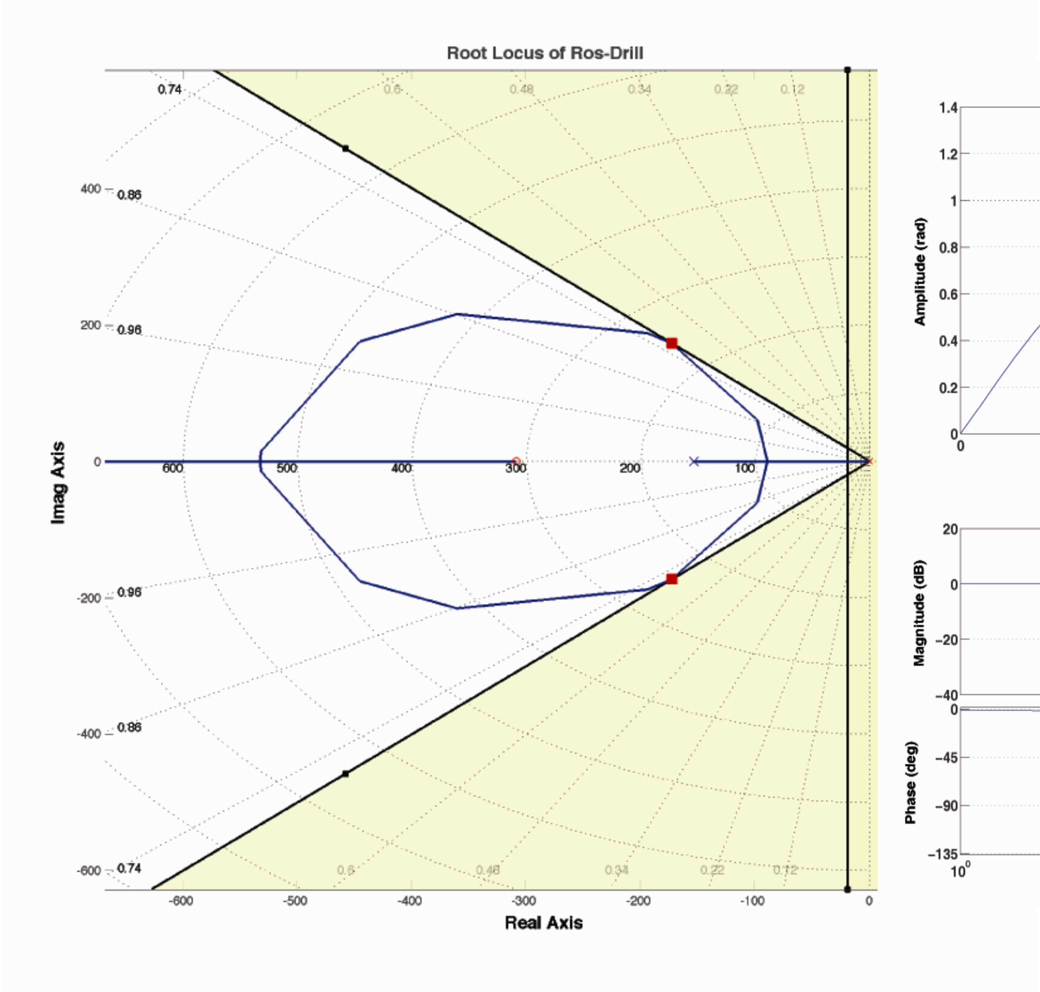
Ros-Drill© Assembly and Controller

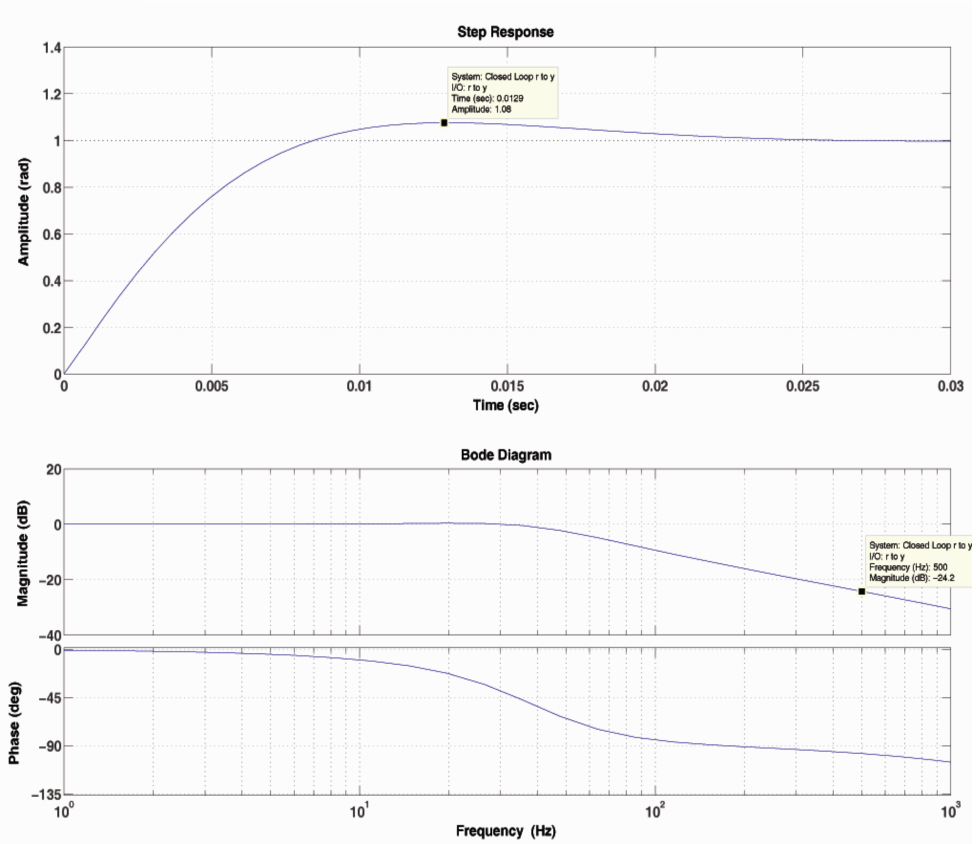


Flowchart of the controller program



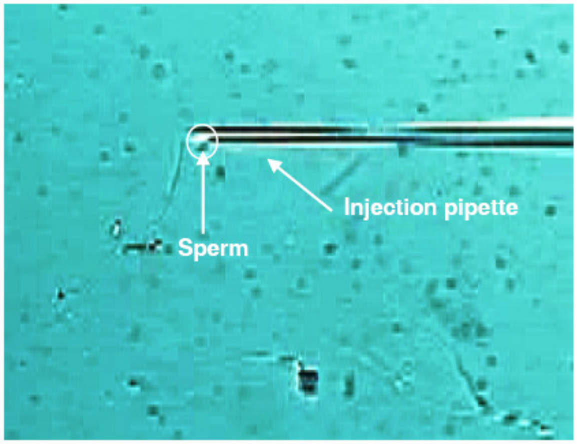
Controller Design





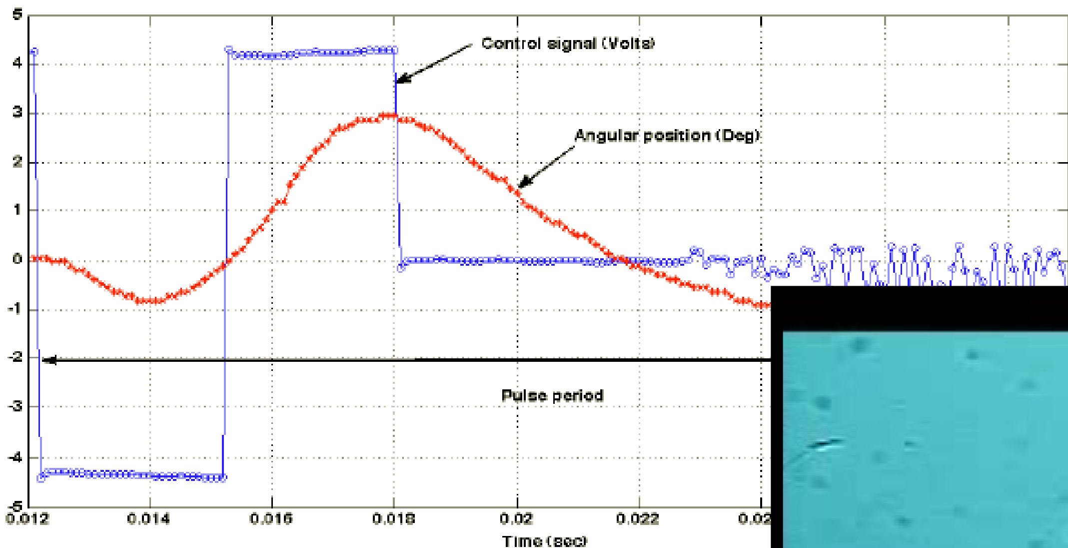
Modes of Operation

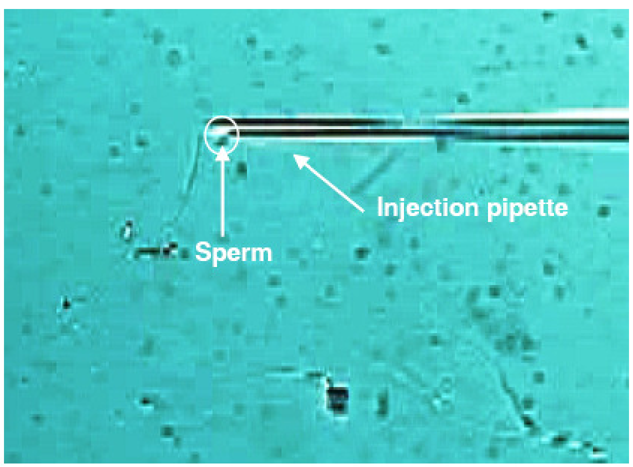
1. Sperm head isolation



2. Oocyte membrane piercing

1. Sperm Head-Tail Separation

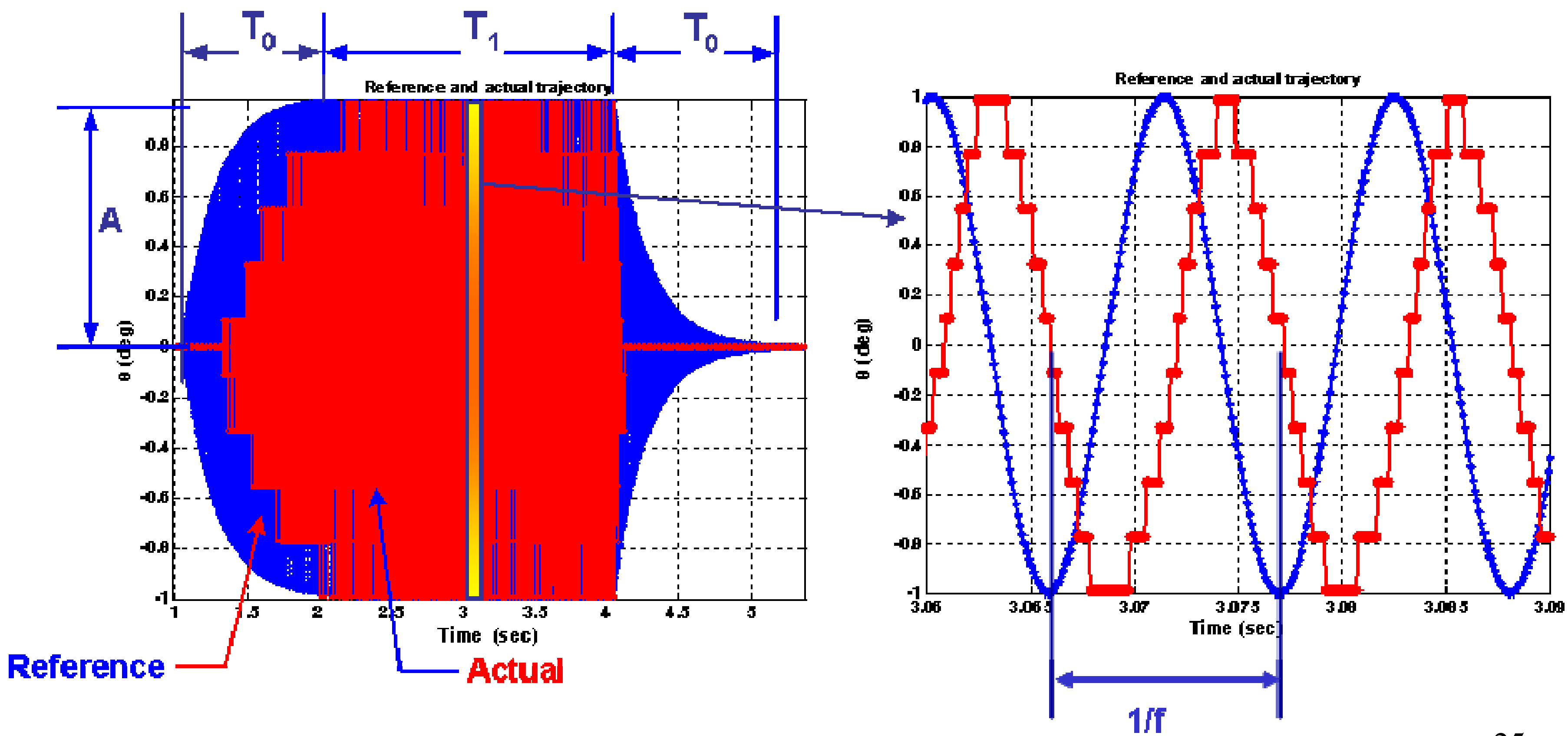




Sperm heads isolated using bidirectional rotational pulse

2. Oocyte Membrane Piercing

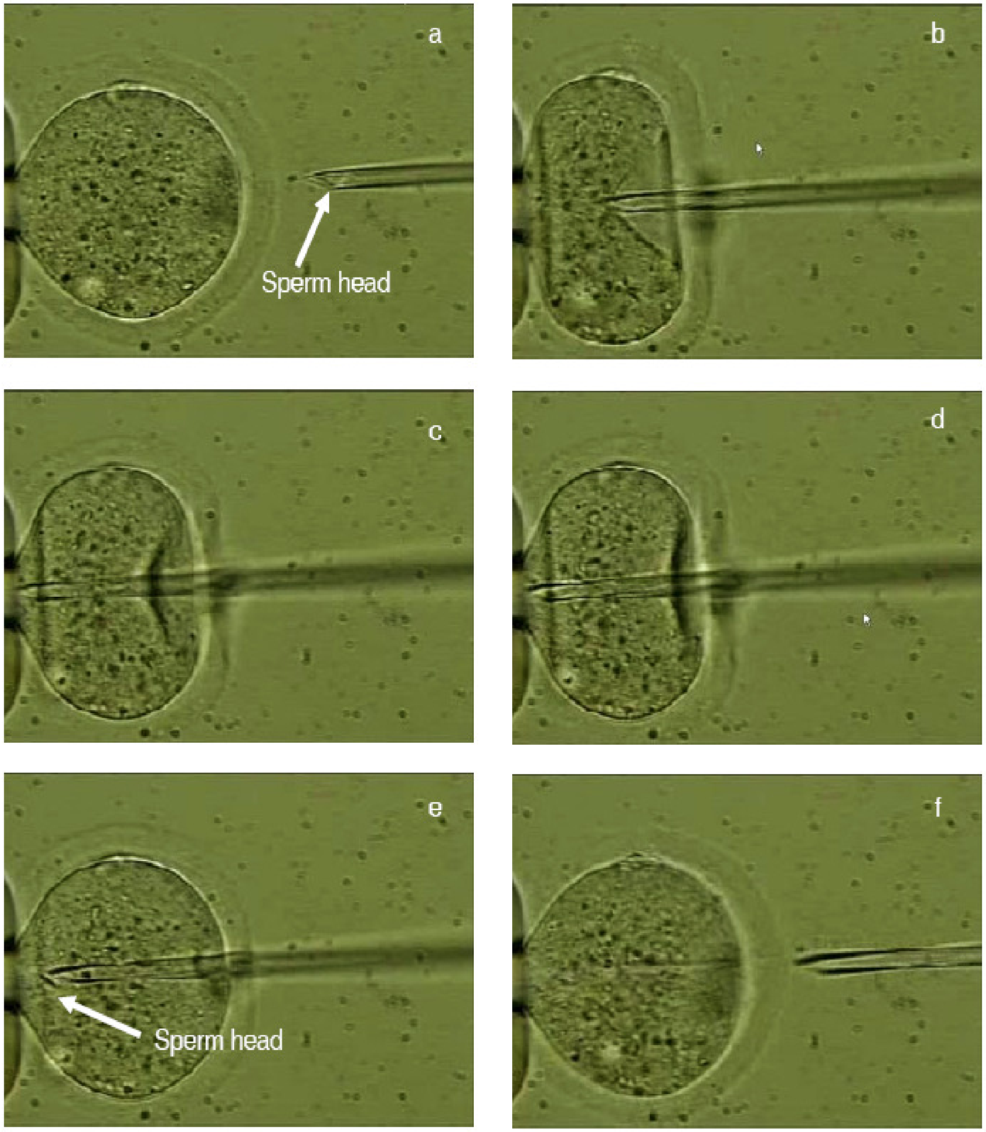
Reference and Actual Rotational Oscillatory Trajectories



Drilling Protocol

1. Hold the egg with holding pipette
2. Set the parameters
   i) Dimple depth (δ) - 90% of oocyte size
   ii) Rotation angle amplitude (θ) - ~0.6-1.2 deg (pp)
   iii) Rotation Frequency (f)- 100-500 Hz
   iv) Rotation acceleration time (T_0)- 0.6 sec
   v) Duration (T_1)- up to 3 sec
3. Create a dimple
4. Push the button and apply rotational oscillation with some negative suction until successful piercing

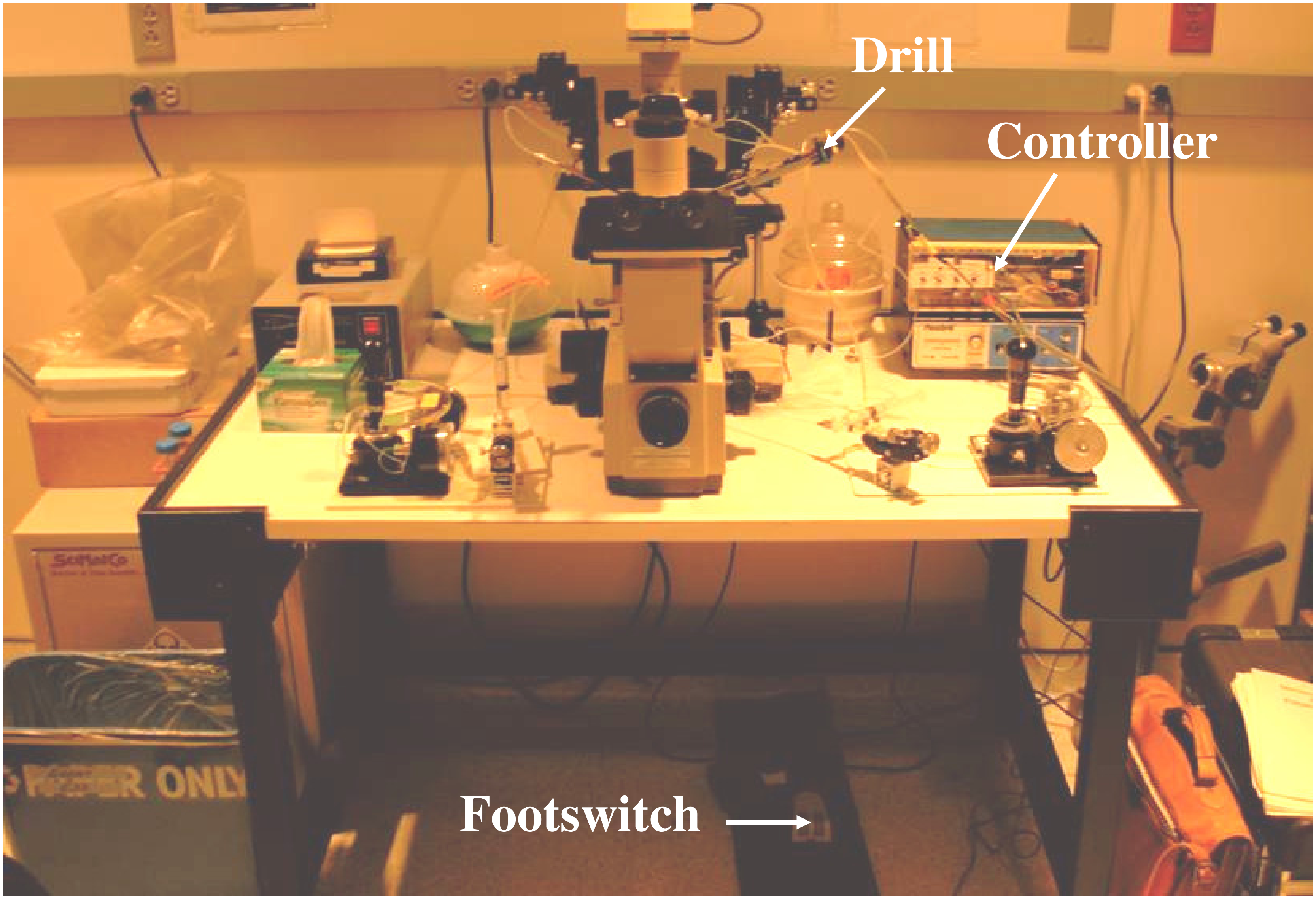
Drilling Protocol cont'd



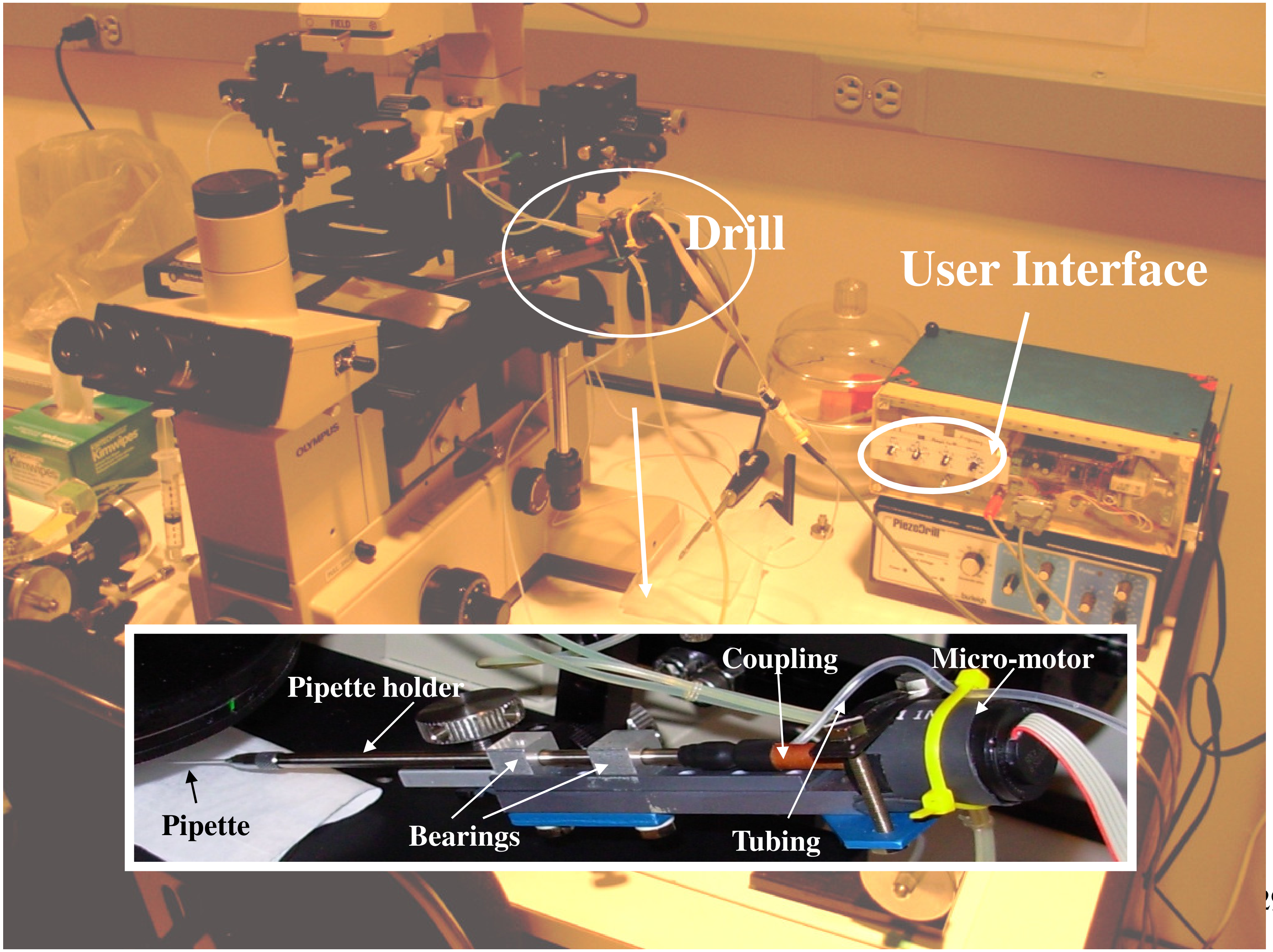
Oolemma pierced

rotational oscillations

Prototype and Experimental Setup



Prototype cont'd

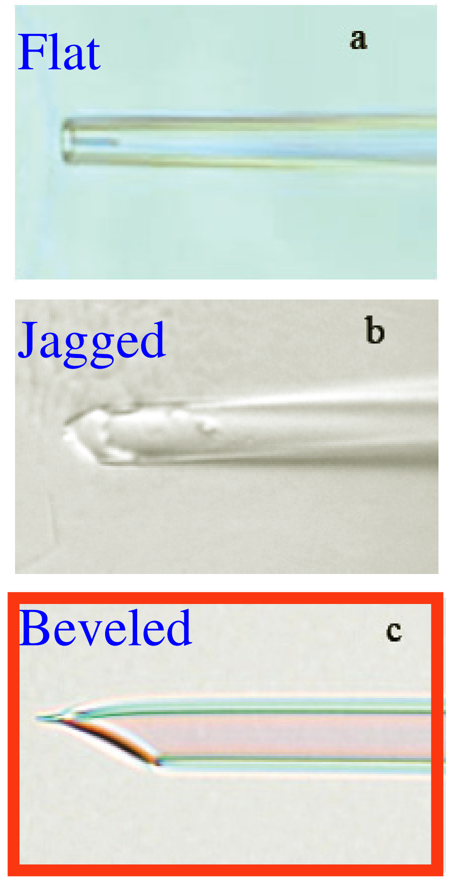


Preliminary Experiments

- Different pipette geometries

- Operational parameters.

$$(A, f, T_0, T_1)$$



Conducted in Center for Regenerative Biology, Department of Animal Science, UCONN and Shriners Hospital for Children, Boston.

Preliminary Experiments cont'd

Experiments	injected	survived	survival %	PN	cleaved	cleavage %
Month 1						
Exp 1	60	25	**42%**	25	22	**88%**
Exp 2	20	6	**30%**	4	4	**67%**
Exp 3	44	13	**30%**	9	9	**69%**
Month 2						
Exp 1	70	19	**27%**	15	15	**79%**
Exp 2	96	31	**32%**	25	22	**71%**
Exp 3	50	14	**28%**	13	11	**79%**
Exp 4	72	20	**28%**	19	17	**85%**

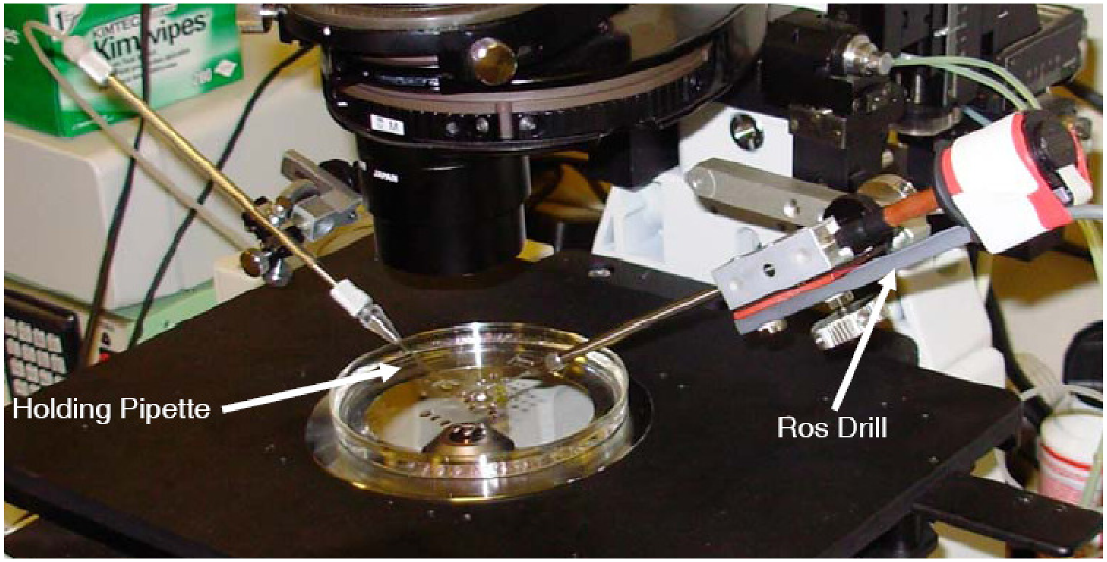
$A=0.6^{o}$, $f=100$ Hz, $T_0=0.5$ sec and $T_1=1$ sec

Oocytes were collected from female B6D2F-1 strain mice, 10 weeks old, superovulated with 7.5 IU PMSG and 7.5 IU hCG at 48-hour intervals, and sacrificed 14 hours after hCG injection. Embryo were cultured in CZB-G medium at 37°C in an atmosphere of 5% CO2.

Extensive Biological Experiments

Success Rate

- Verification
- Comparison



Conducted at the University of California, Davis by a biologist.

$A = 0.3^{o}$, $f = 500$ Hz, $T_0 = 0.5$ sec

Experimental Results

Method	Injected (%)	Survivors (%)	Developers (2-cells, %)	Blastocysts (%)
Ros-Drill ICSI	116 (100)	95 (81.9)	83 (87.4)	47 (56.6)
Piezo ICSI	111 (100)	106 (95.5)	103 (97.2)	76 (73.8)

Number of ova that survived, developed to 2-cell embryos and blastocyst after injection of sperm heads using either Ros-Drill-ICSI or Piezo-ICSI. The sperm heads were separated by freeze-thawing in Na-EGTA medium

Method	2-cell embryos (%)	3-4-cell embryos (%)	Non-compacted morulae (%)	Compacted morulae (%)	Blastocysts (%)	Total (%)
Ros-Drill ICSI	3 (3.6)	9 (10.8)	12 (14.5)	12 (14.5)	**47 (56.6)**	83 (100)
Piezo ICSI	7 (6.8)	8 (7.8)	7 (6.8)	5 (4.9)	**76 (73.8)**	103 (100)

Development of embryos (2-cell stage through blastocysts) after 96 hours of in vitro culture after injection of sperm heads using either Ros-Drill-ICSI or Piezo-ICSI.

Experimental Results cont'd

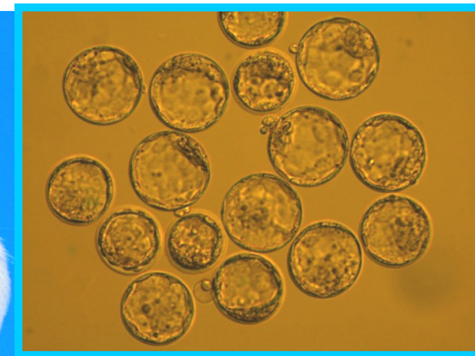
Method	2-cell embryos (%)	3-4-cell embryos (%)	Non-compacted morulae (%)	Compacted morulae (%)	Blastocysts (%)	Total embryos (%)
HCZB	3 (3.8)	7 (8.8)	11 (13.8)	21 (26.3)	**38 (47.5)**	80 (100)
Na-EGTA	3 (3.6)	9 (10.8)	12 (14.5)	12 (14.5)	**47 (56.6)**	83 (100)
Ros-Drill	2 (6.5)	1 (3.2)	3 (9.7)	5 (16.1)	**20 (64.5)**	31 (100)

Development of embryos (2-cell stage through blastocysts) after 96 hours of in vitro culture. Sperm heads were isolated by freeze-thaw in HCZB medium, Na-EGTA medium and Ros-Drill pulses.

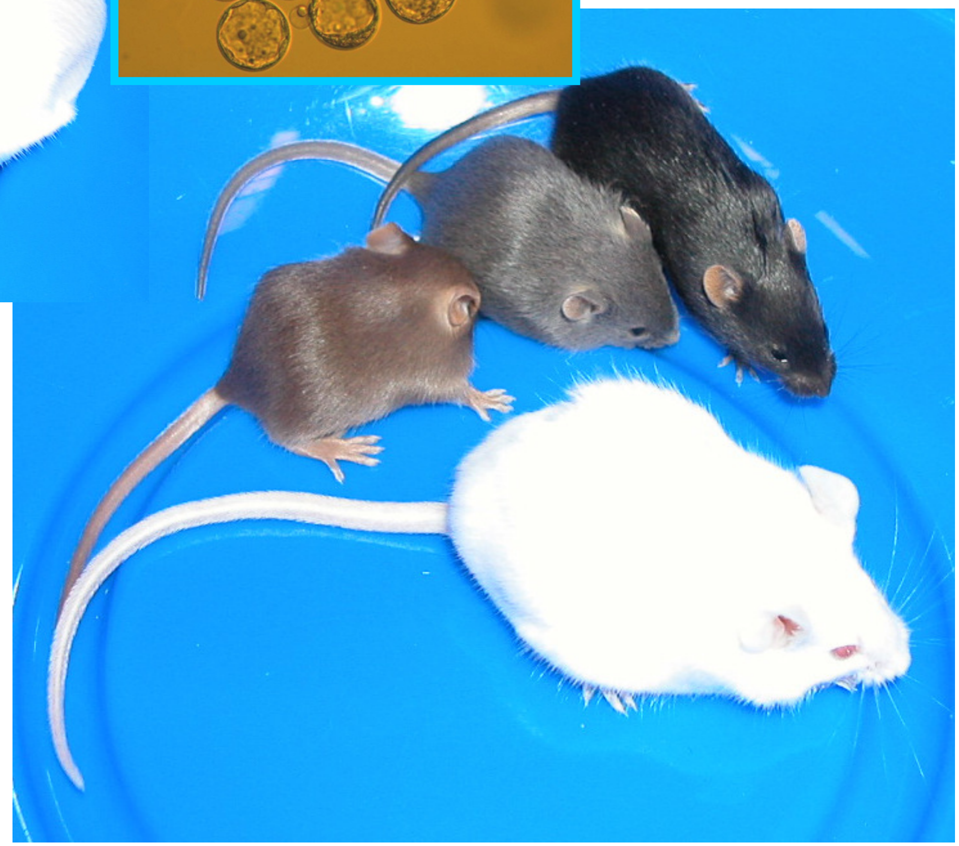
Sperm head preparation	No. blastocysts	No. pups born(%)	No. pups weaned(%)
Freeze-thaw in HCZB	36	**9 (25)**	9 (100)
Freeze-thaw in Na-EGTA	47	**20 (43)**	20 (100)
Ros-Drill	20	**6 (30)**	6 (100)

Number of pups born and weaned after embryo transfer of blastocysts ; sperm heads were isolated by freeze-thaw in HCZB medium, Na-EGTA medium and Ros-Drill pulses.

Experimental Results cont'd



8 days old pups and surrogate mother



13 days old pups and surrogate mother