

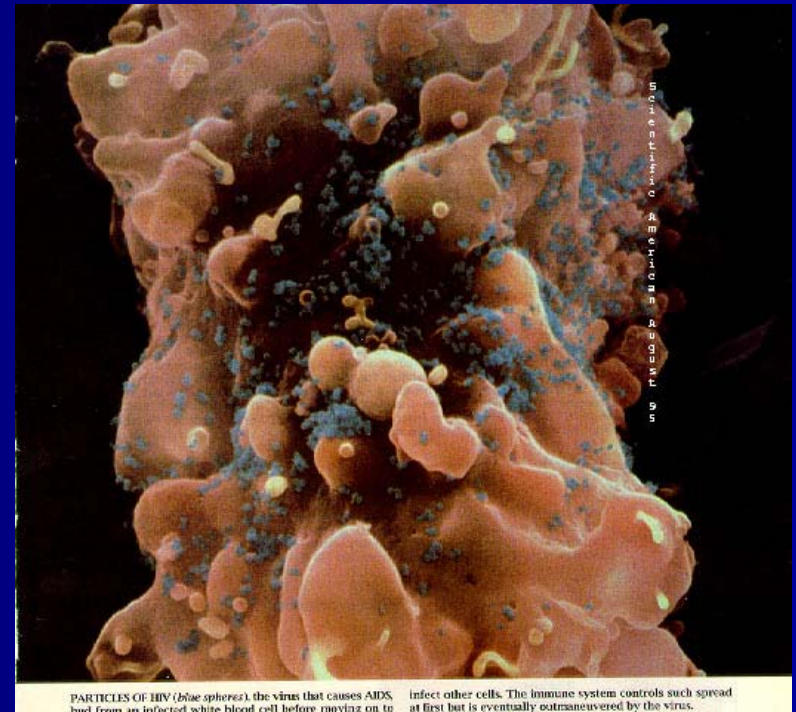
# Development of Optical Trapping Techniques in Preparation for Mechanistic UDG Studies

Joshua T.B. Williams, under the guidance of  
Dr. Daniel Burden

Wheaton College Chemistry Department,  
Wheaton, IL 60187

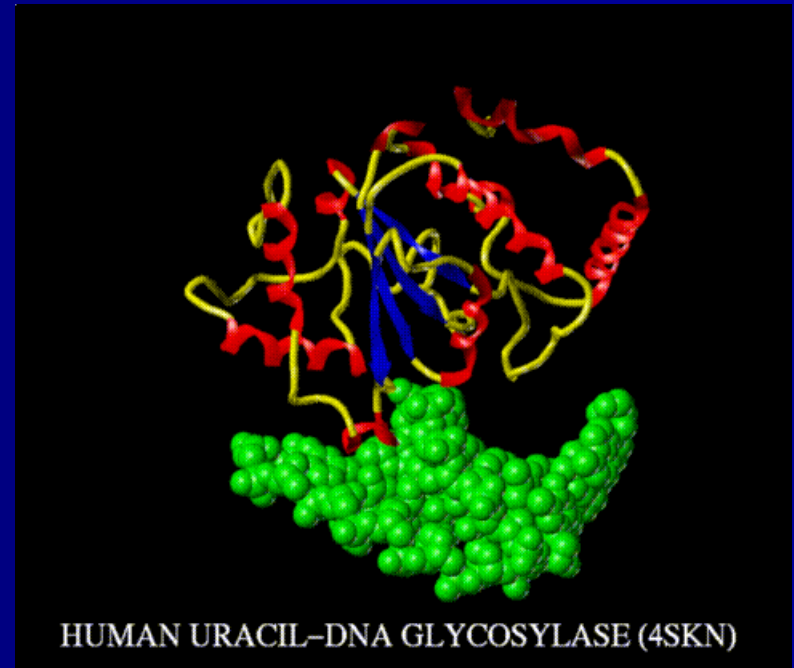
# UDG Significance

- Potential drug target against microbial pathogens
  - HIV
  - Tuberculosis
  - Chicken Pox
  - Herpes Simplex Viruses 1 and 2
- Cancer fighting capabilities



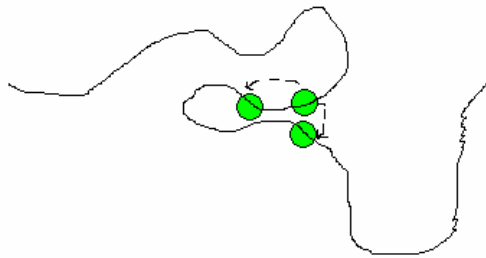
# Background

- Uracil DNA Glycosylase (UDG)
  - Role in DNA base excision repair pathway (BER)
  - Mechanisms for damaged base pair location
    - 1D sliding
    - 3D hopping or jumping



# Background

3D Hopping or Jumping

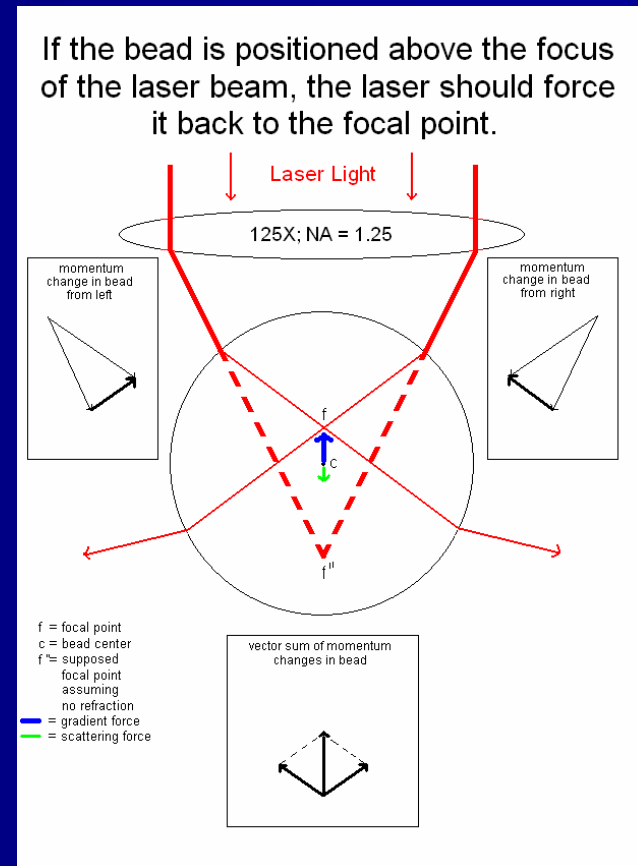


1D Sliding



# Background

- Optical "Tweezers"
  - Radiation Pressure
    - Scattering Force
    - Gradient Force or Restoring Force
  - Light Rays
    - Newton's 2nd
  - Requirements for stable trapping
    - Ability of gradient force to overcome scattering force imperative



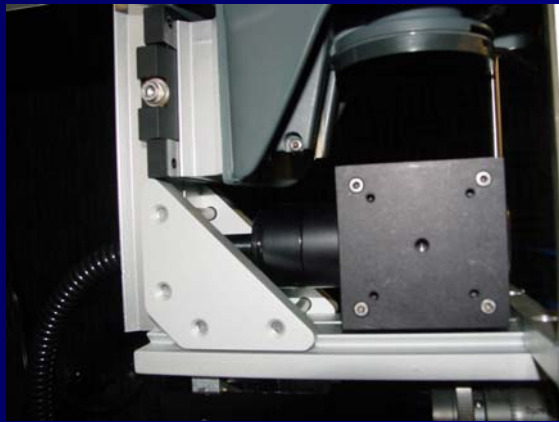
# Activities

## ■ Construction and Part Installation

- Addition of white light transilluminator to Confocal/Optical Trapping Microscope
- Customization of *Warner Instruments* bath chamber to allow for high magnification objective immersion and pipette tip insertion
- Installation of PM2000B pressure injector
- Addition of *Rainin* peristaltic pump to bath chamber
- Installation of P2000 micropipette puller
- Construction and installation of laser diode ( $\lambda = 690\text{nm}$ )

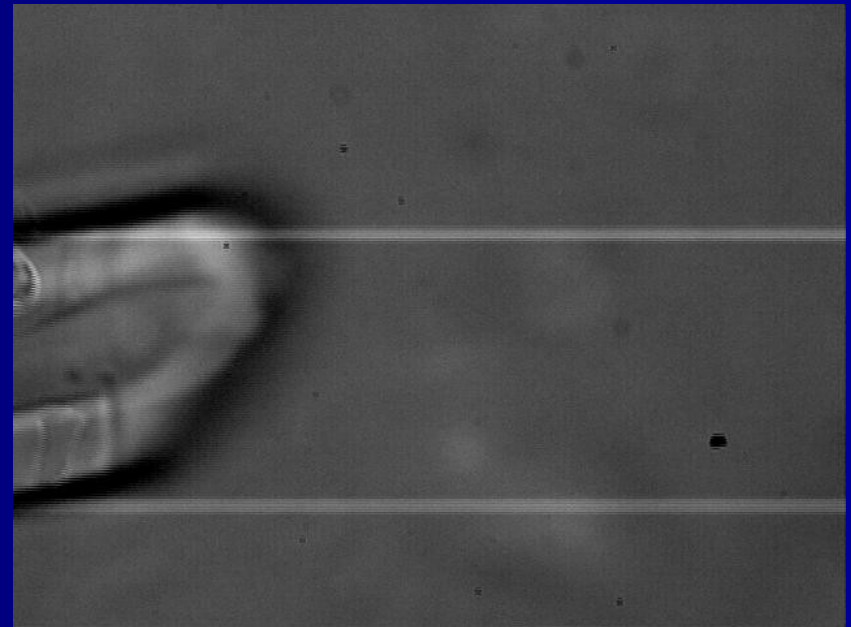
# White Light Transilluminator

- Determination of lack of parfocality in high mag and low mag objectives
  - Air Force test target



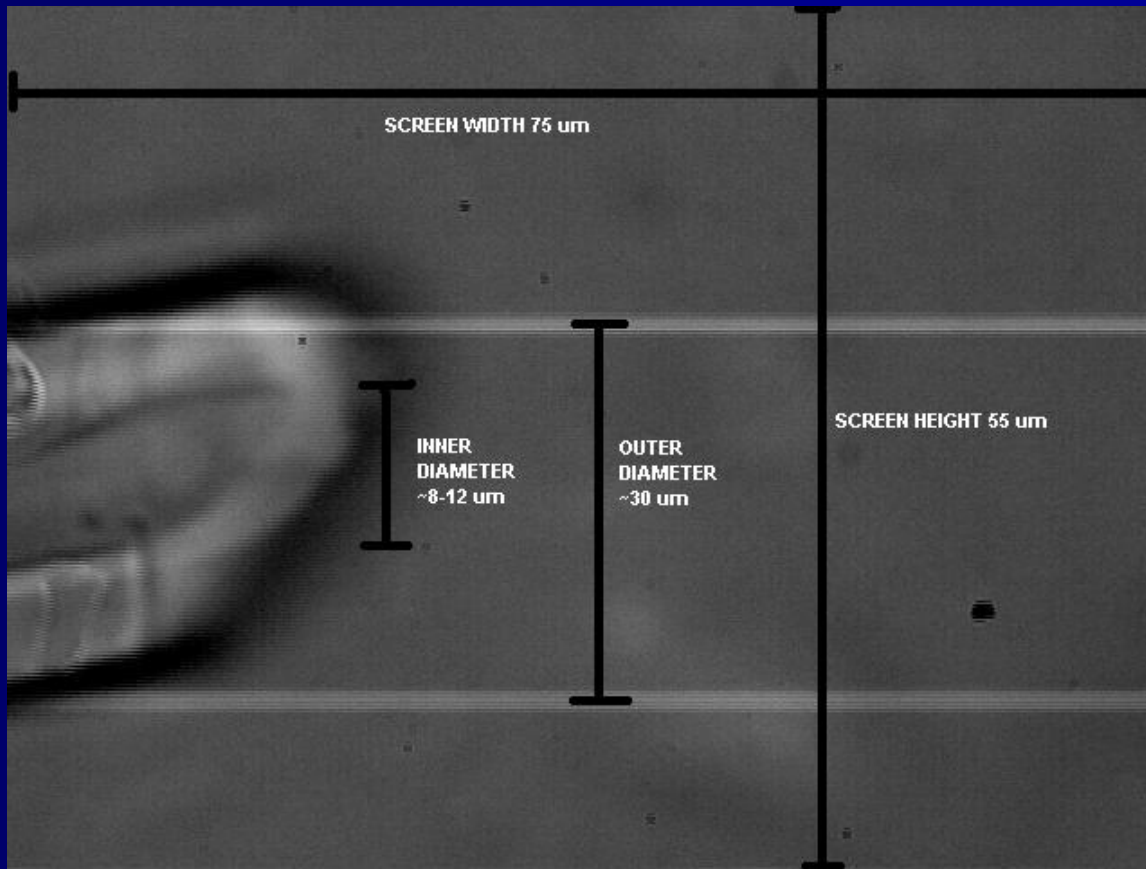
# Pipette Tip Visualization

- Visualization of empty micropipette tips unattached to pressure injector
  - Use of HeNe laser to guide visualization
- Determination of CCD camera FOV size
  - Estimation of pipette tip inner & outer diameters



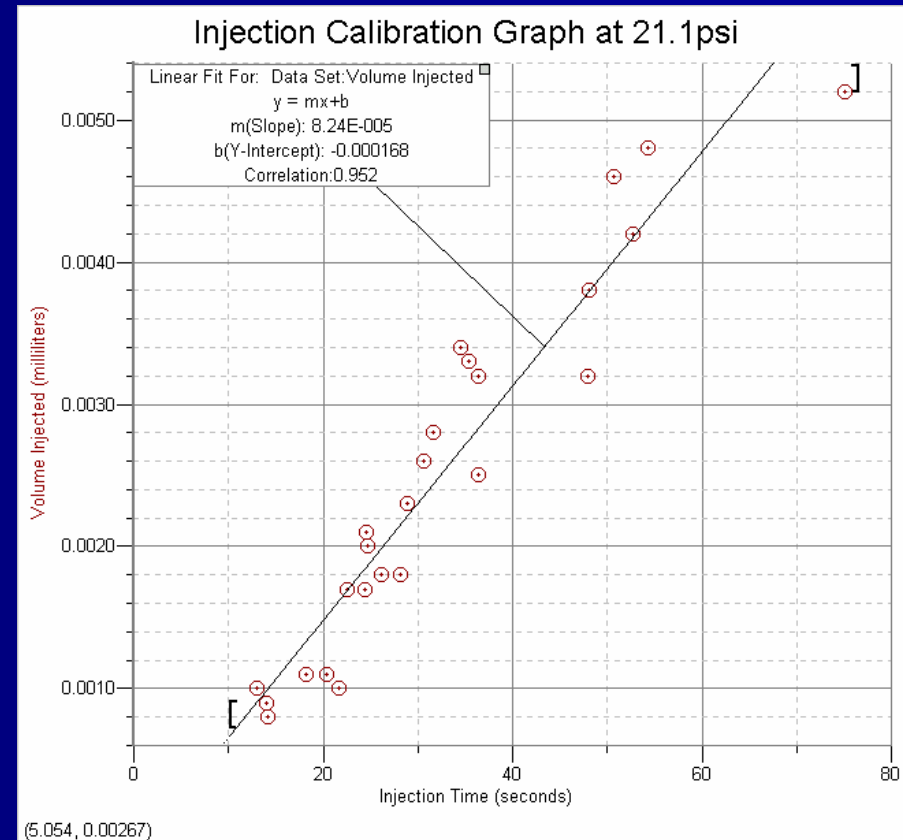
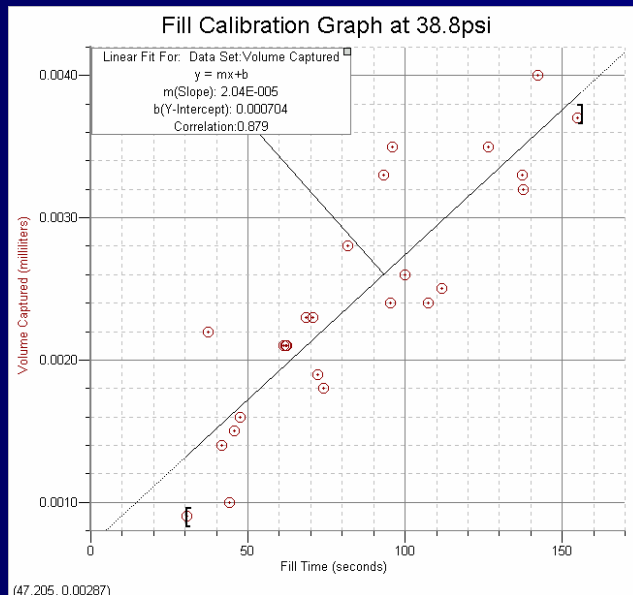


# Experimentation



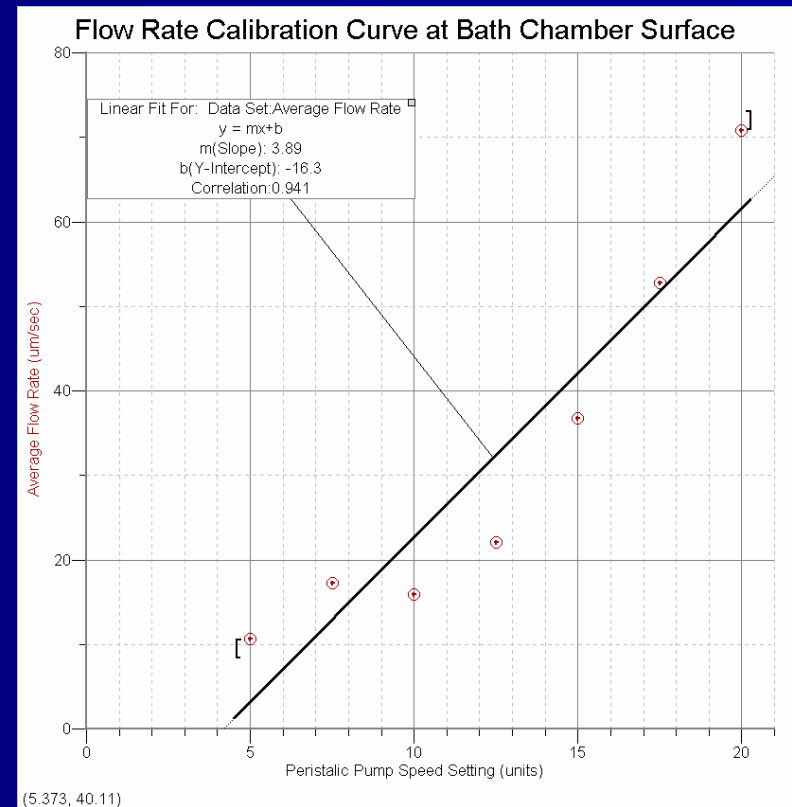
# Experimentation

- Installation and Calibration of PM2000B pressure injector
  - Fill, Inject, Balance Pressure features



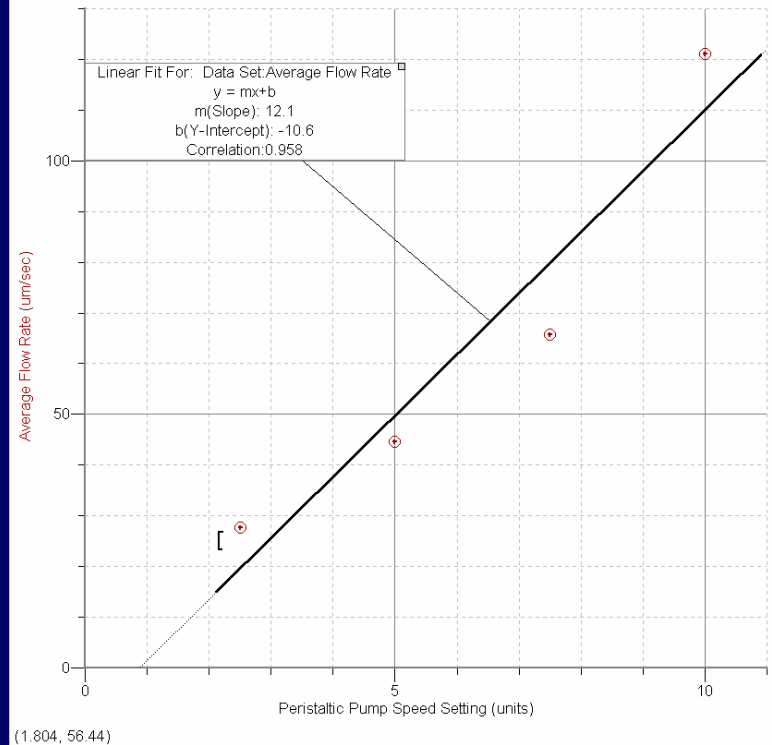
# Experimentation

- Installation and Calibration of *Rainin* peristaltic pump
  - Flow profile near surface
  - Flow calibration curves downstream from the pipette tip
  - Flow calibration curves orthogonal to the pipette tip

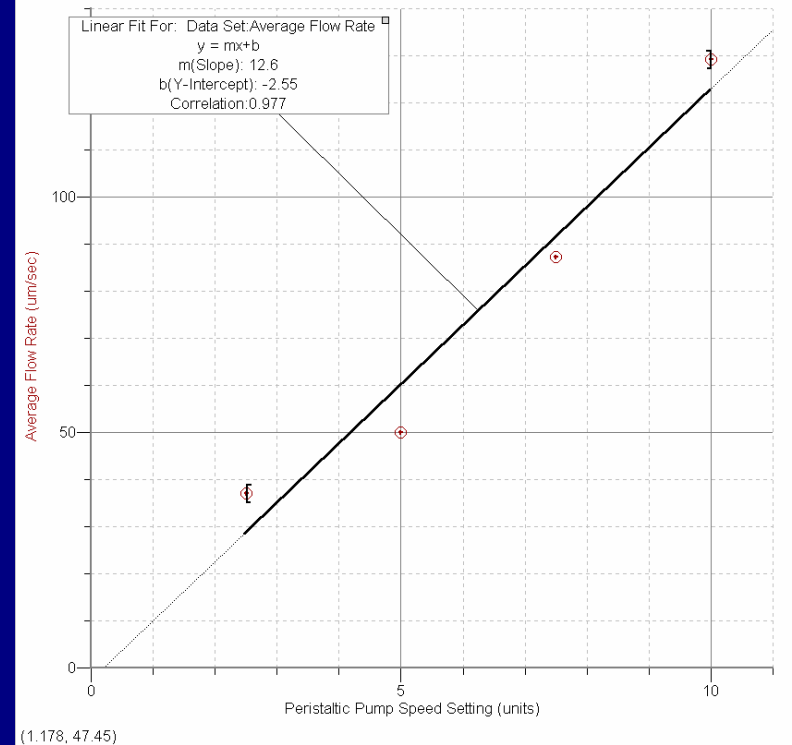


# Experimentation

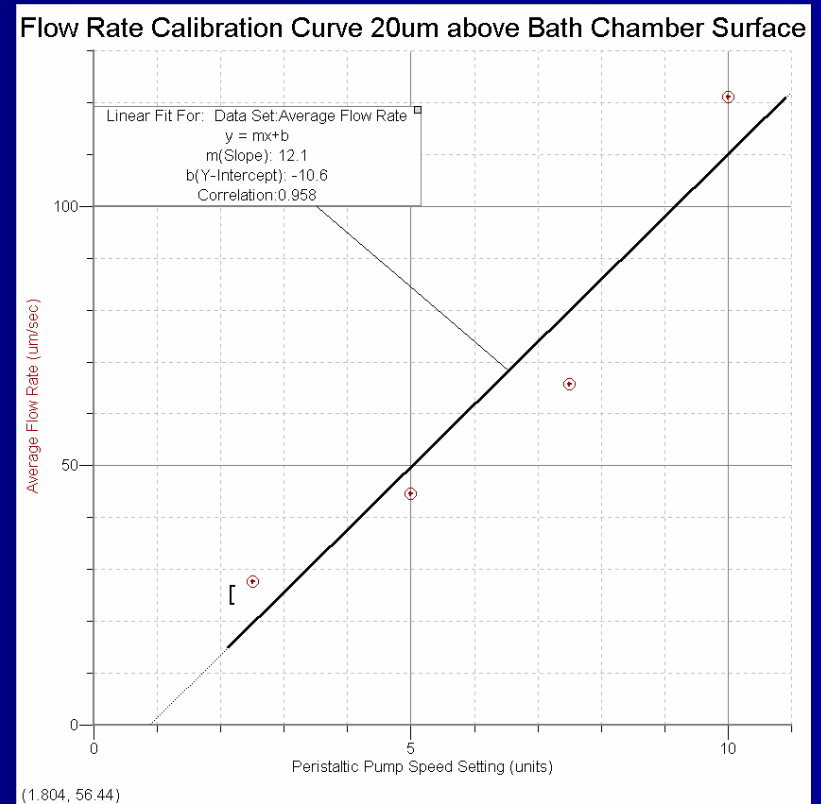
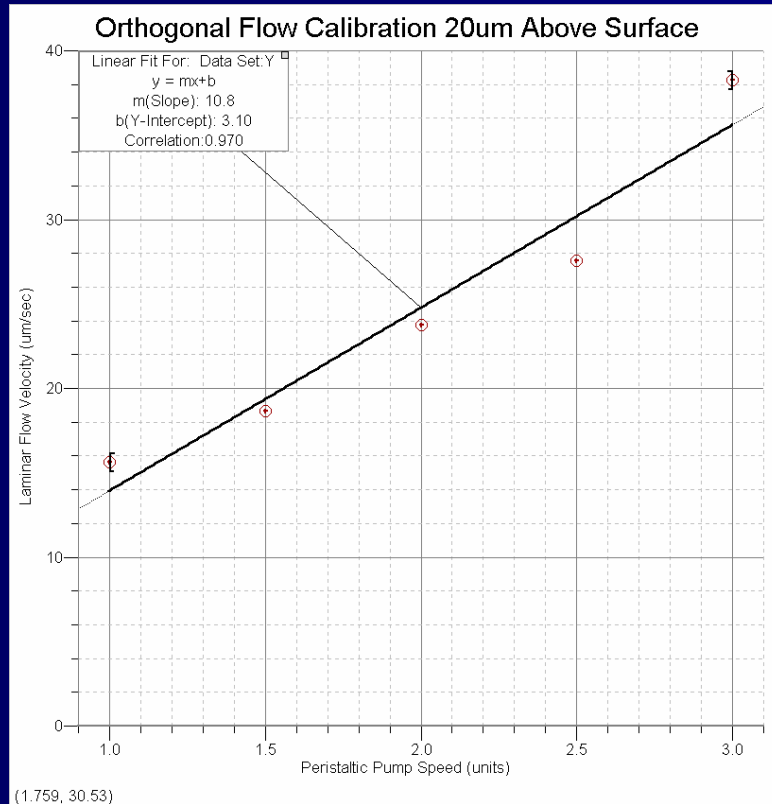
Flow Rate Calibration Curve 20um above Bath Chamber Surface



Flow Rate Calibration Curve 40um above Bath Chamber Surface



# Experimentation



# Experimentation



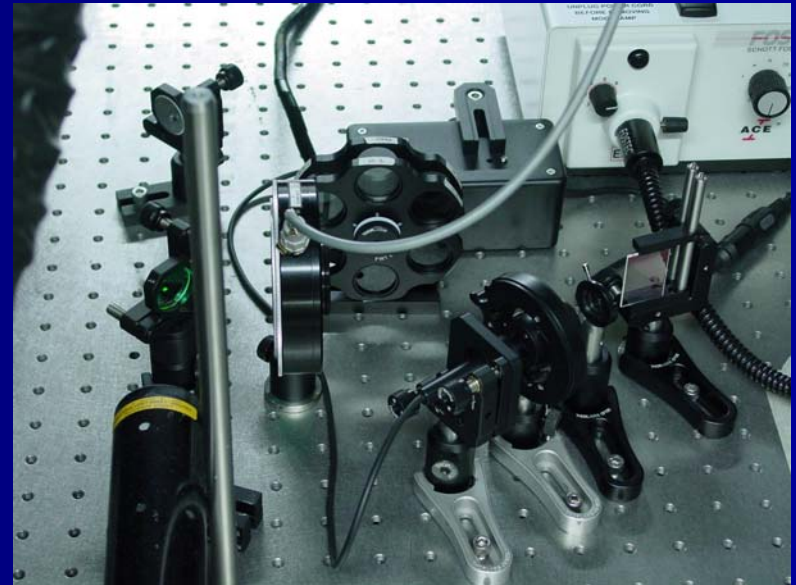
# Experimentation

- Installation and Calibration of micropipette puller
  - Basic layout
  - Heat, velocity, filter, delay, and pull components to program
  - Determination of an appropriate program for pulling single barrel pipettes with an ID of 20-40 $\mu$ m



# Experimentation

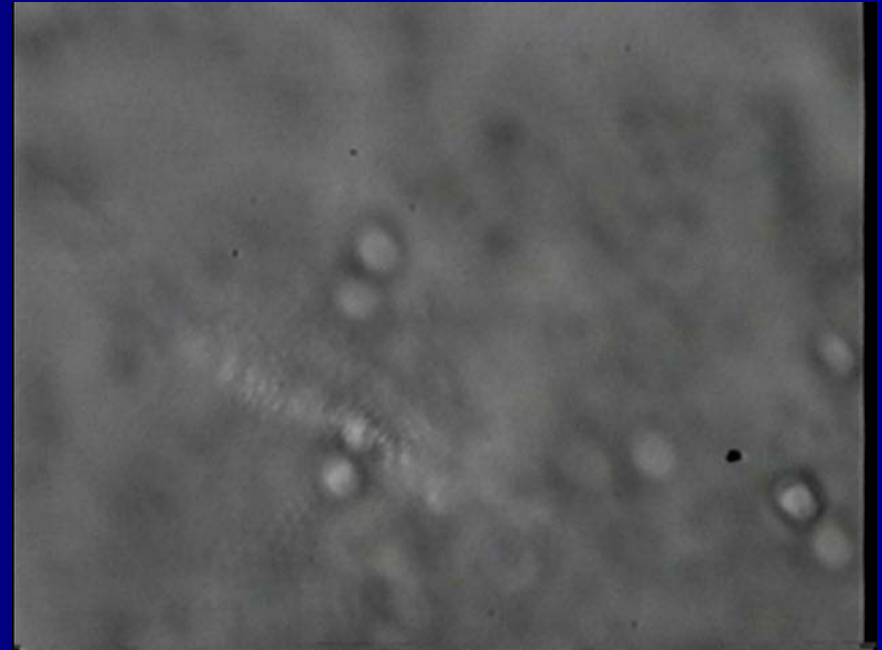
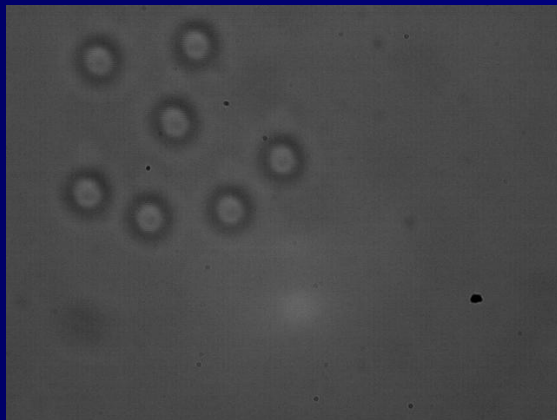
- Construction and Installation of laser diode ( $\lambda = 690\text{nm}$ ) to complement HeNe laser ( $\lambda = 534\text{nm}$ )
  - Laser diode driver, anamorphic prism, iris, dichroic mirror, and neutral density filter
  - Co-linear alignment with HeNe laser as guide





# Experimentation

- Demonstration of trapping capabilities in three dimensions with laser diode
  - Sensitivity to vibrations
  - Multiple potential wells?



← The finished product :o)

# Discussion

- So, what exactly did we do?
  - Instrumentation installation and customization
  - Calibration/Troubleshooting
  - Optical trapping in 3D
- Ok, what's left to do?
  - Everything
    - Assemble DNA-bead construct, let flowing stream stretch DNA to contour length, and mount construct on BSA-coated slide
    - Isolate method for drastically reducing vibration in z-axis stepper motor

# Conclusion

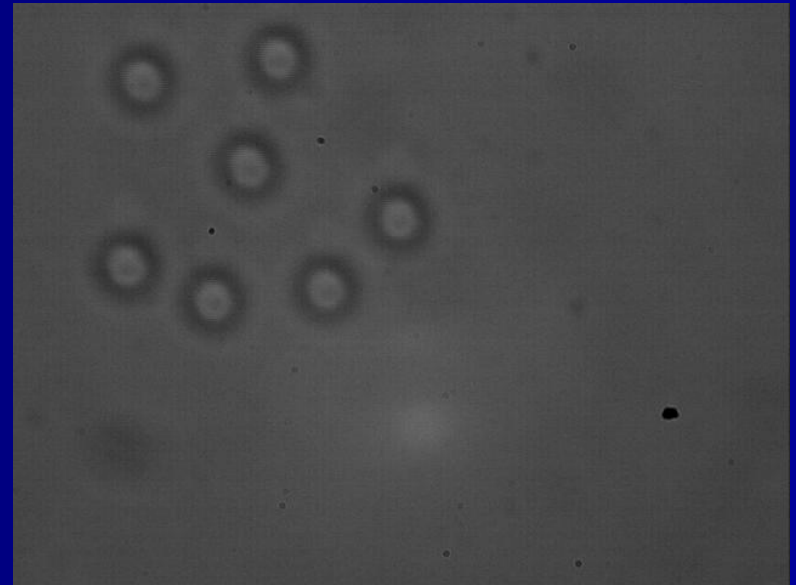
- Ability to successfully trap beads in three dimensions, when combined with SM analysis capabilities of confocal microscope, has provided a door to analyzing UDG's kinetics of DNA repair
- Further analysis of beam profile necessary to determine plausibility of multiple potential wells
  - Recently received spatial filter
- Further experimentation necessary to create a functional triple barrel micropipette
- In other words...

# Conclusion

**THE FUN HAS  
JUST BEGUN!!**

# Thanks go out to:

- Dr. Burden
- Wheaton College  
and the alumni  
association
- Dean Dorothy  
Chappell, Provost  
Stan Jones
- Physics Department
  - Professor DeSoto



# References

- K. Svoboda and S.M. Block, "Biological Applications of Optical Forces." *Annu. Rev. Biophys. Biomol. Struct.* **23**, 247-85 (1994).
- James T. Stivers at Johns Hopkins University