

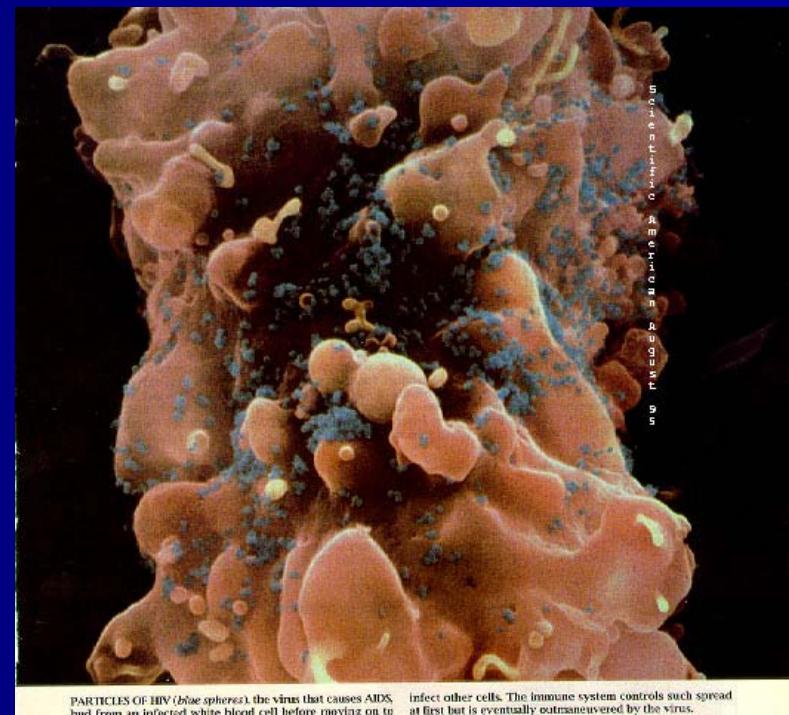
Development of Optical Trapping Techniques in Preparation for Mechanistic UDG Studies

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UDG Significance

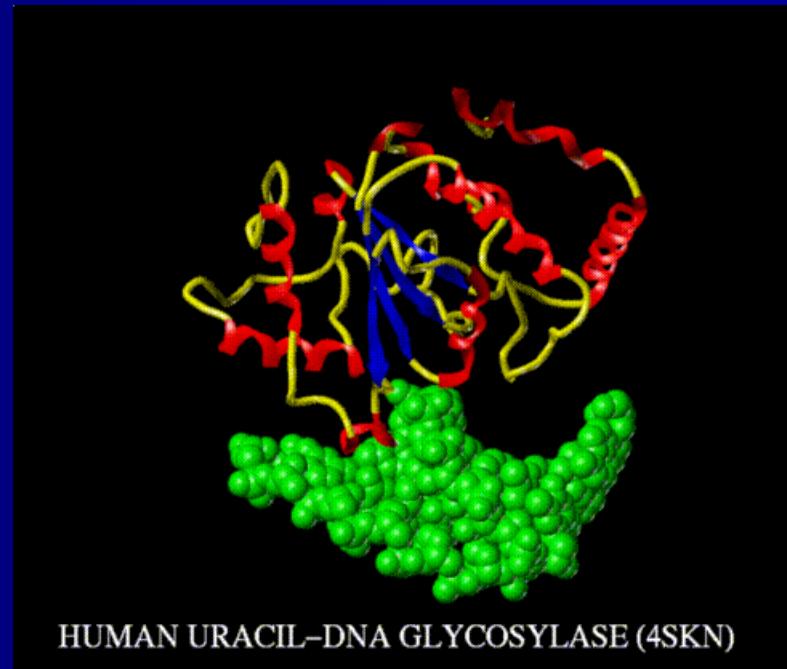
- Potential drug target against microbial pathogens
 - HIV
 - Tuberculosis
 - Chicken Pox
 - Herpes Simplex Viruses 1 and 2
- Cancer fighting capabilities



PARTICLES OF HIV (blue spheres), the virus that causes AIDS, bud from an infected white blood cell before moving on to infect other cells. The immune system controls such spread at first but is eventually outmaneuvered by the virus.

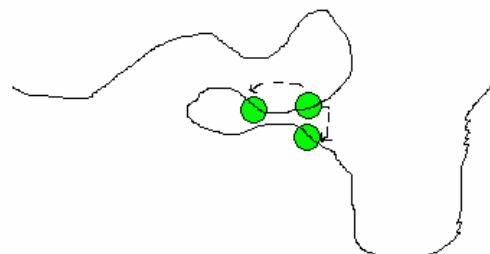
Background

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

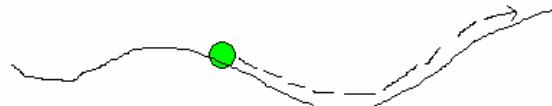


Background

3D Hopping or Jumping

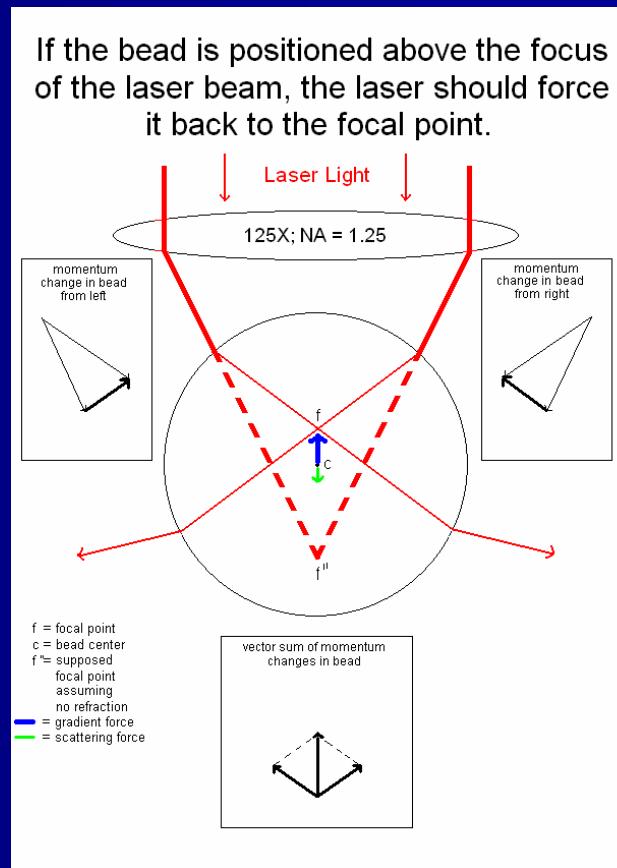


1D Sliding



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

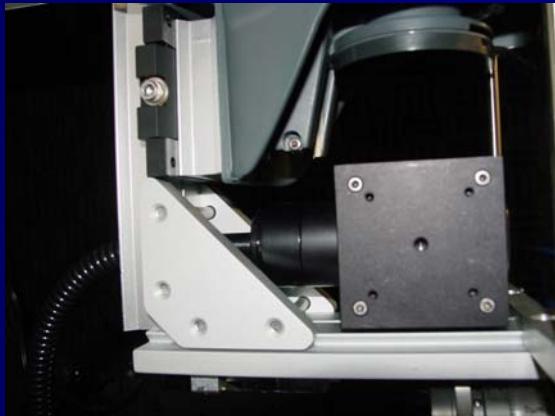


Activities

- 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

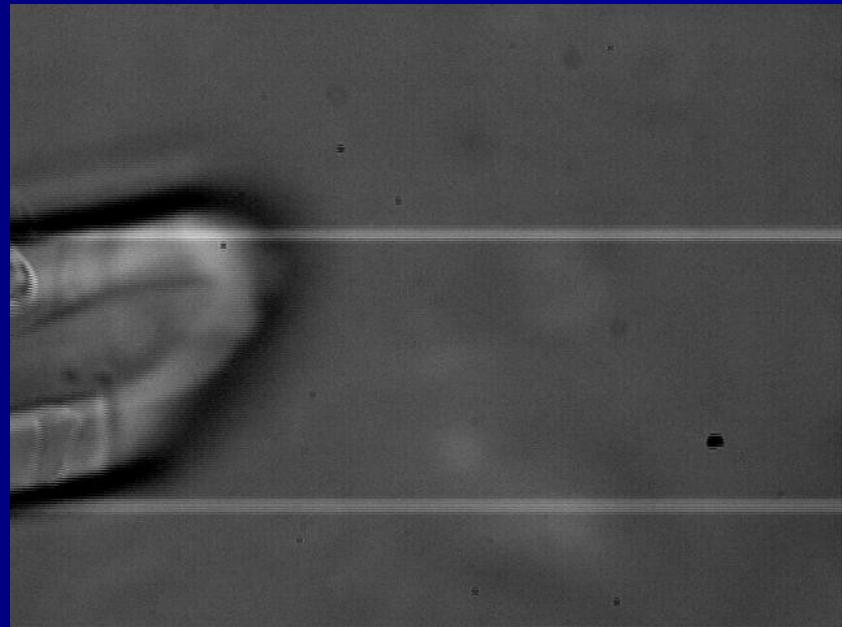
- Determination of lack of parfocality in high mag and low mag objectives
 - Air Force test target



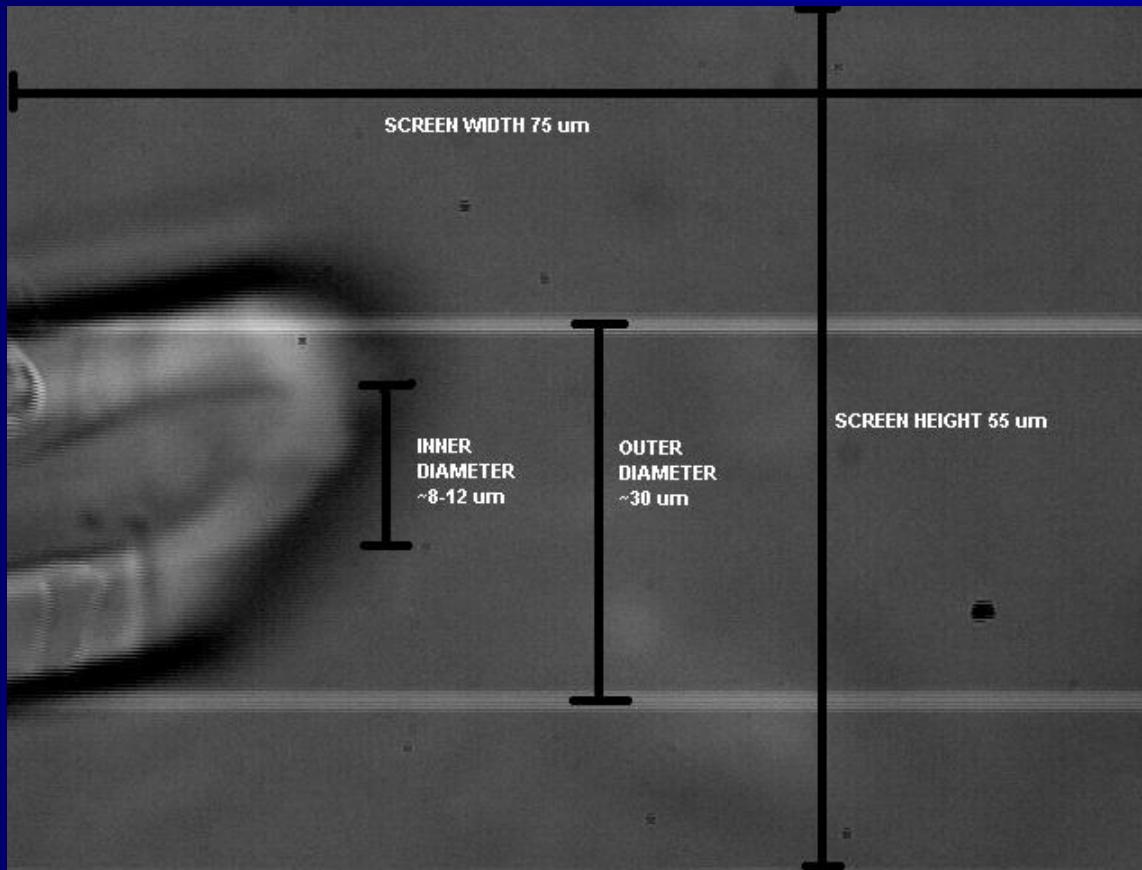
Pipette Tip Visualization

- 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

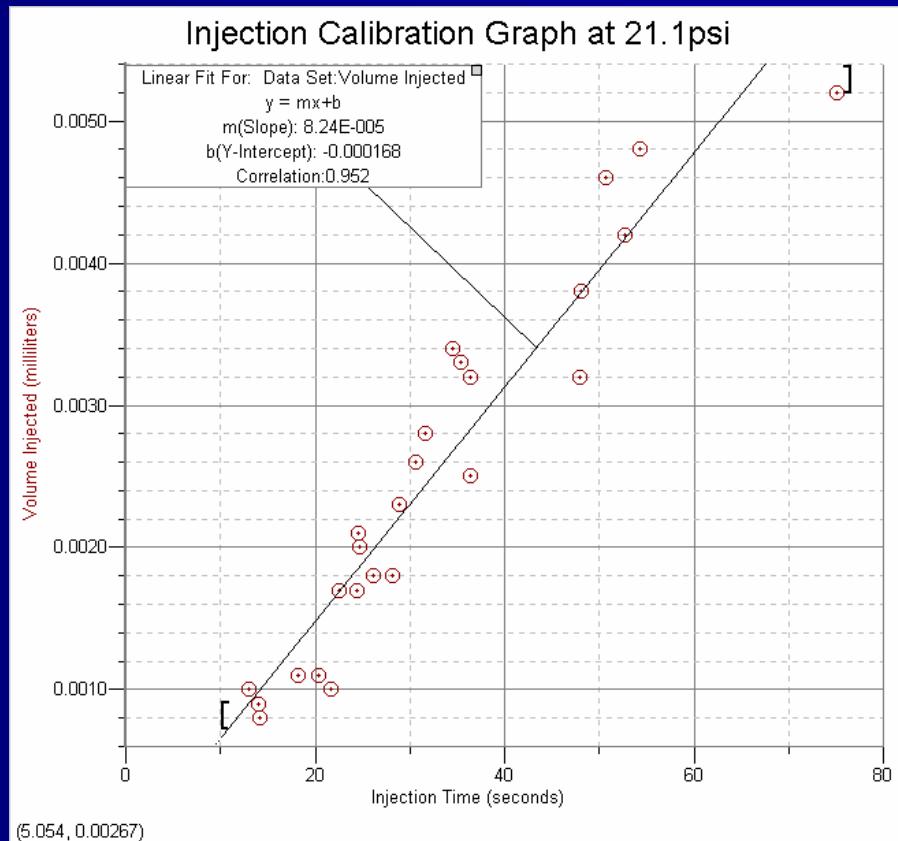
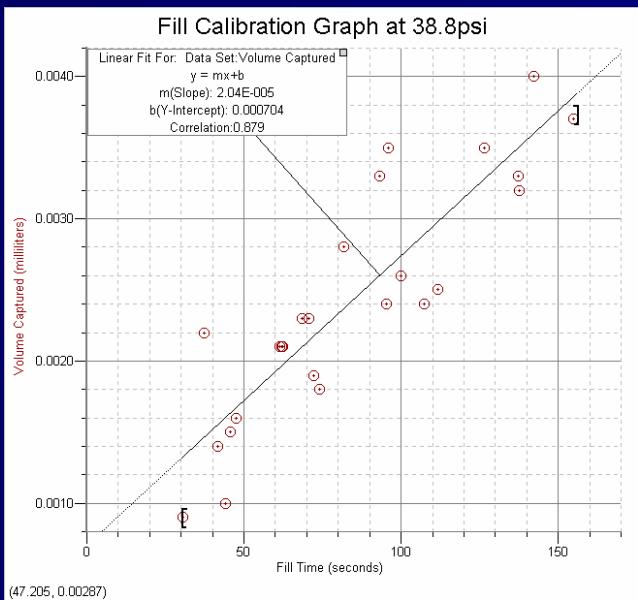


Experimentation



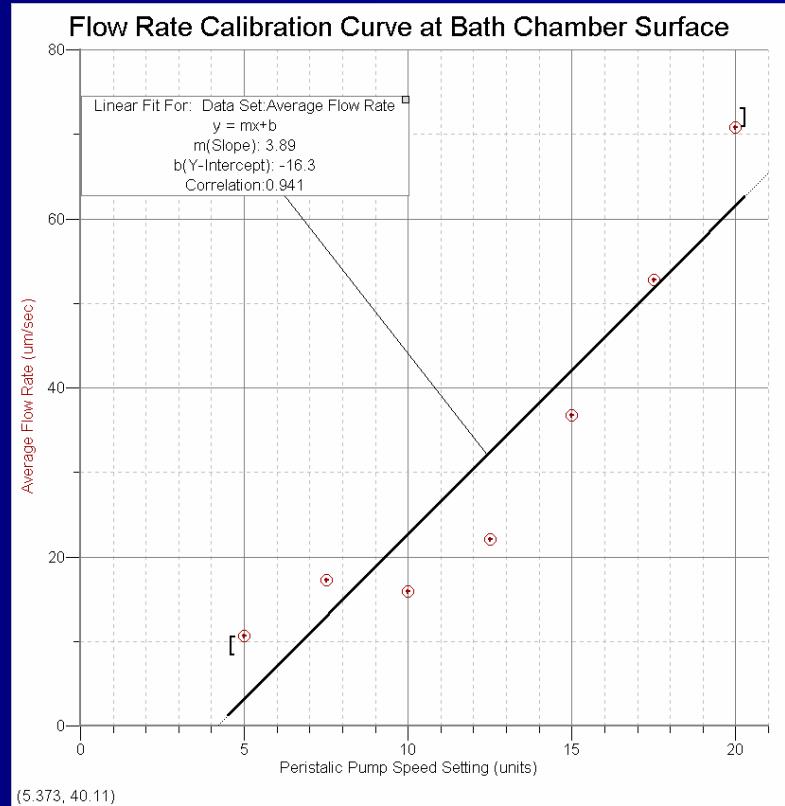
Experimentation

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

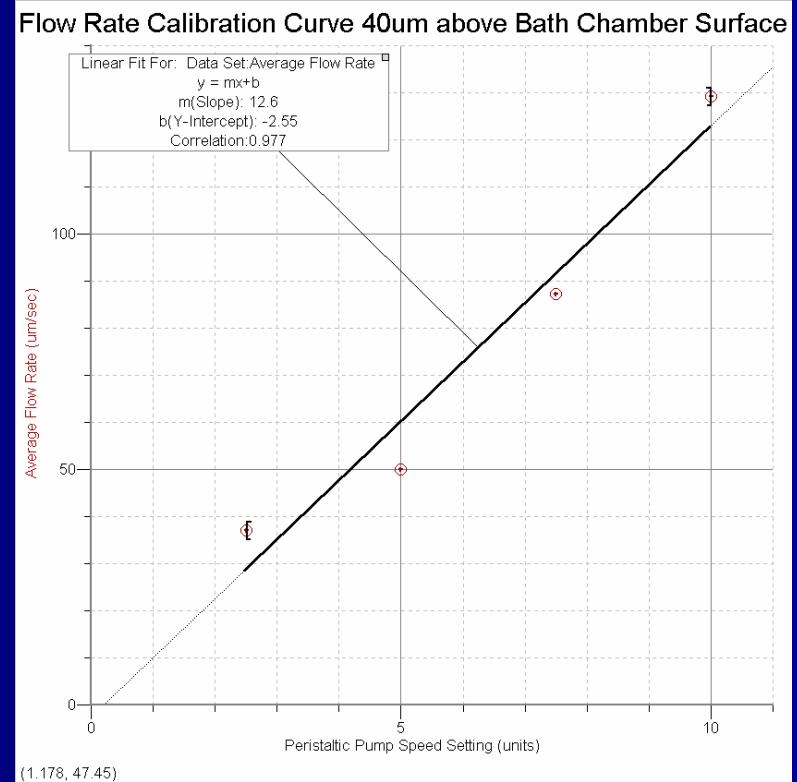
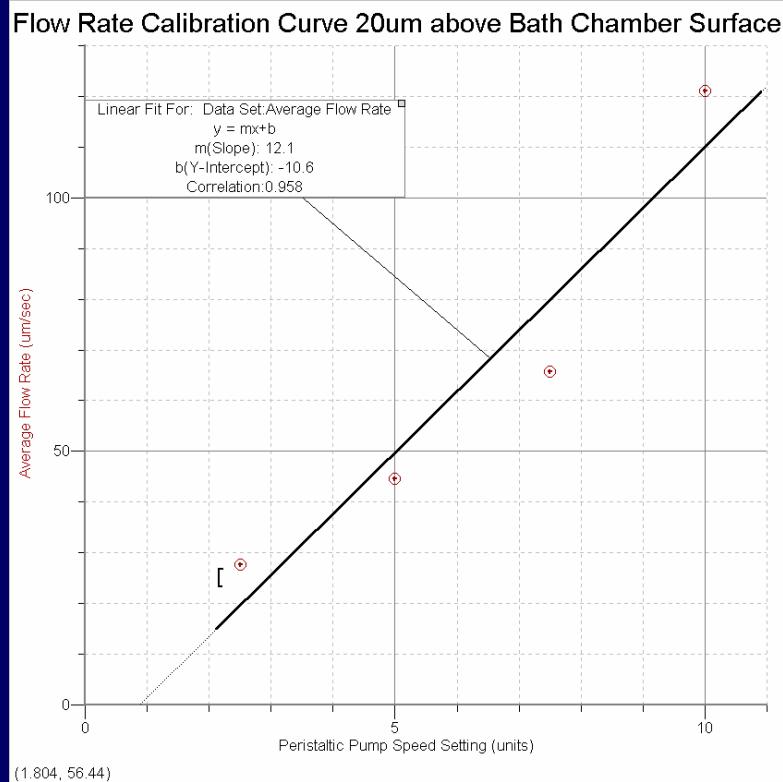


Experimentation

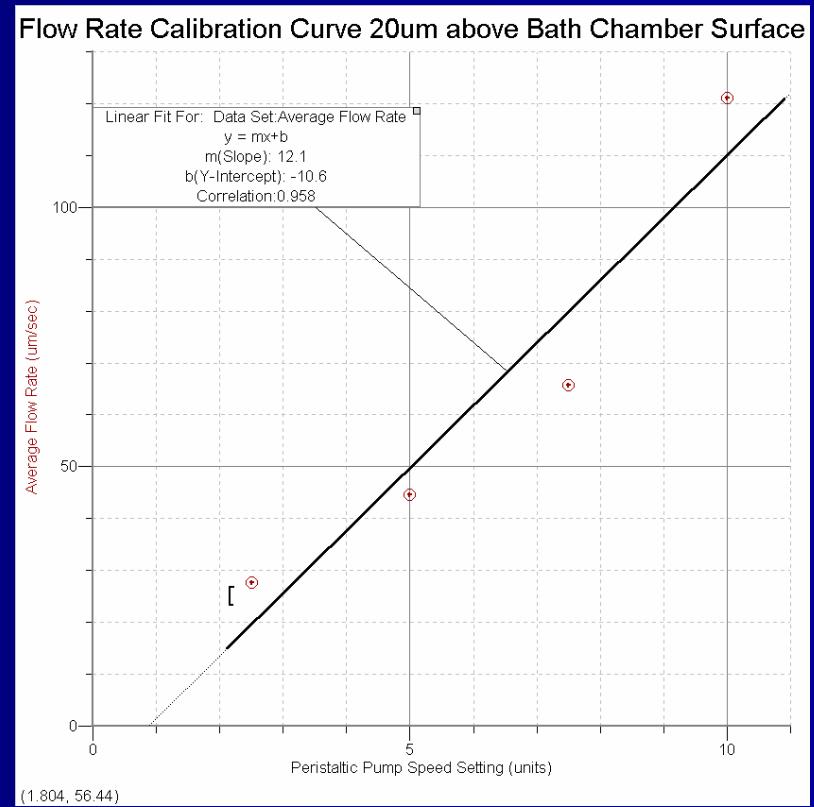
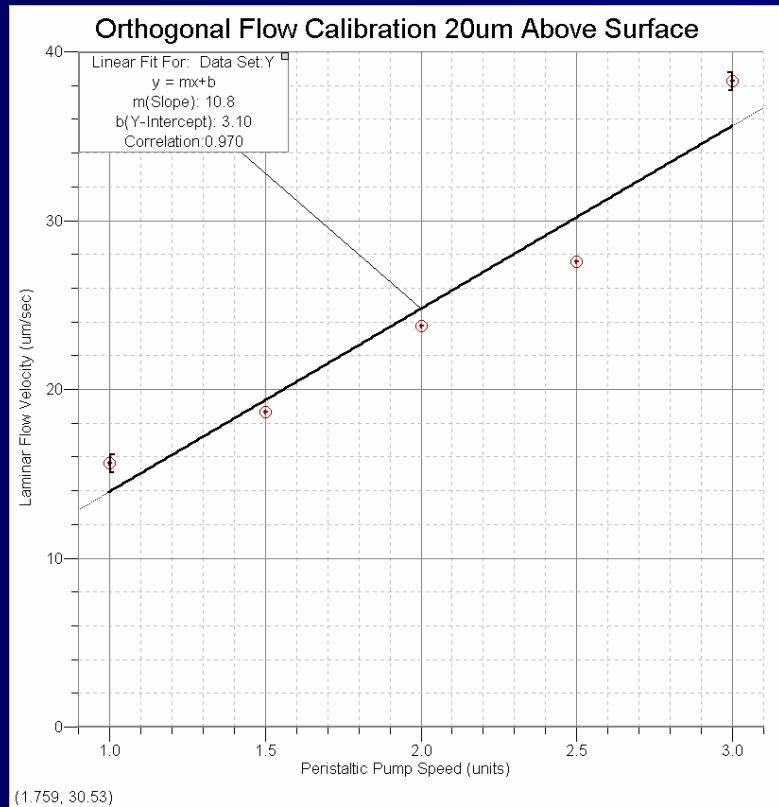
- Installation and Calibration of *Rainin* peristaltic pump
 - Flow profile near surface
 - Flow calibration curves downstream from the pipette tip
 - Flow calibration curves orthogonal to the pipette tip



Experimentation



Experimentation



Experimentation



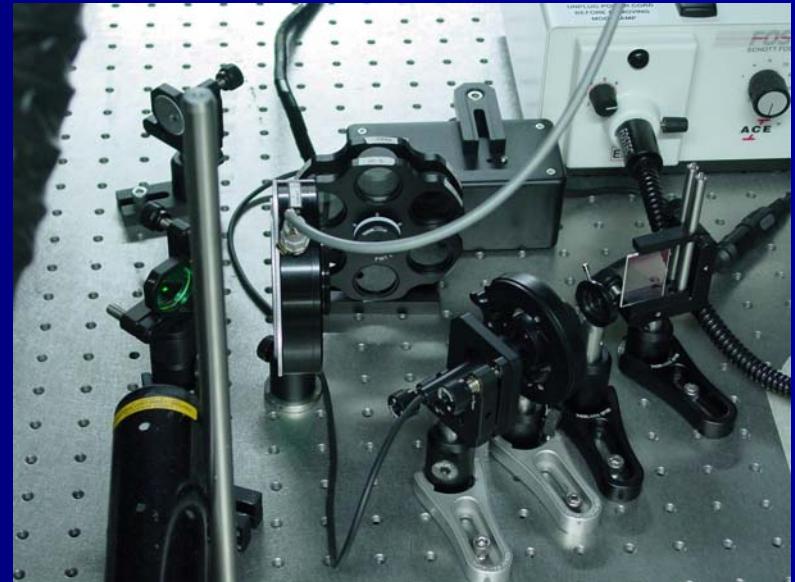
Experimentation

- 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 20-40um



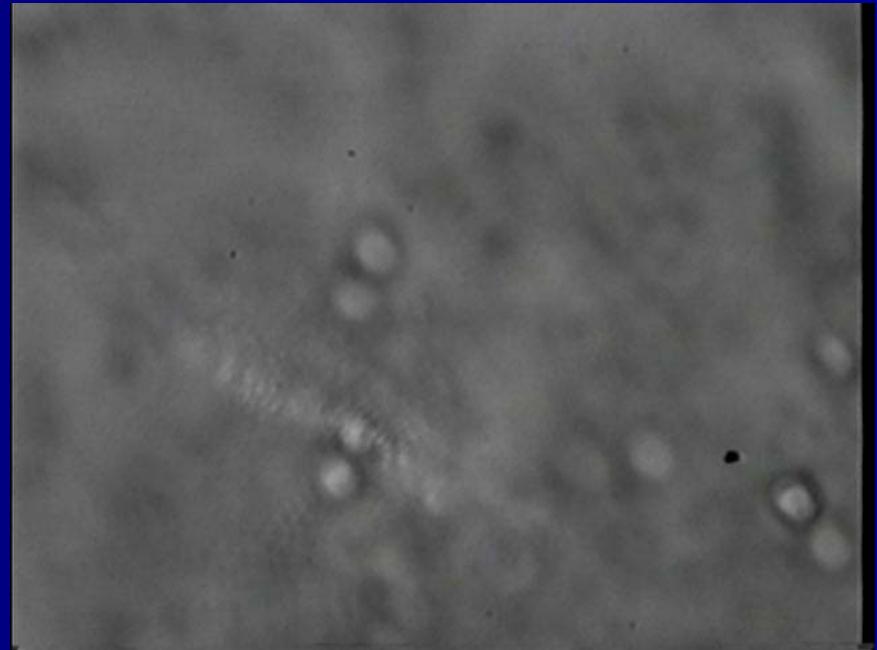
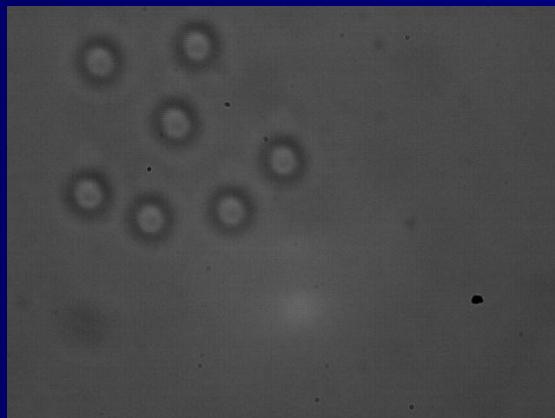
Experimentation

- 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

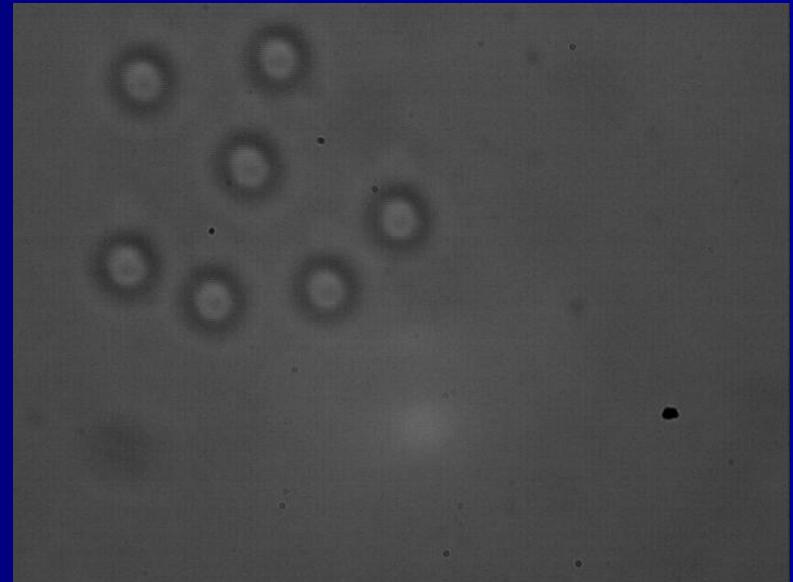
- Ability to successfully trap beads in three dimensions, when combined with SM analysis capabilities of confocal microscope, has provided a door to analyzing UDG's kinetics of DNA repair
- Further analysis of beam profile necessary to determine plausibility of multiple potential wells
 - Recently received 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
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- Dean Dorothy
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Stan Jones
- Physics Department
– Professor DeSoto



References

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- James T. Stivers at Johns Hopkins University