3D Super-Resolution Fluorescence Microscopy

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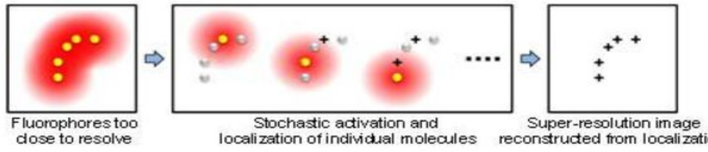
Background

- An estimated 15% of couples (48.5 million worldwide) struggle with infertility. 15-30% of these infertility cases are unexplained [1,2]
- In order to understand more about fertility, high resolution 3D images of sperm cells are of interest.
- **Problem Statement:** Current imaging methods do not have high enough axial resolution to observe the sperm cell structure and behavior and do not allow for sequential imaging of live cells, which allows the observation a cell's response to a stimulus
- In order view sperm cells in 3D, researchers use **Fluorescence Microscopy**
  - Stochastic Optical Reconstruction Microscopy **(STORM)** allows for super-resolution



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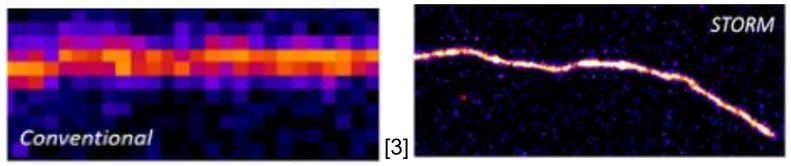

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Project Objectives

Our project aims to improve Krapf lab's current STORM system by:

1. Designing and implementing a new microscope stage to securely hold samples
2. Analyzing cells with **Correlative Imaging:** image a cell, manipulate the cell, then locate and image the same cell again
3. Increasing the axial range and axial resolution to image an entire sperm cell by investigating the use of a **Tetrapod Point Spread Function**
   a. Additionally explore the DeepSTORM 3D machine learning algorithm to create a tetrapod point spread function from our data

Constraints

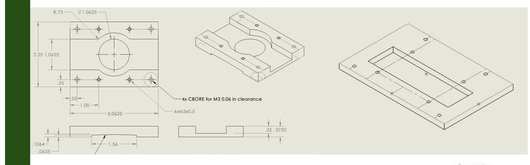
Correlative Imaging	Attach sperm heads but not tails
	Regularly relocate a specific sperm cell
	Allow for manipulation of sperm cell

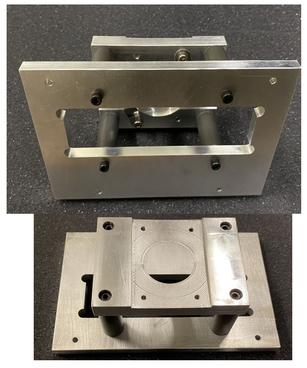
Tetrapod Localization	5 µm axial range
	25nm Lateral Resolution
	50nm Axial Resolution

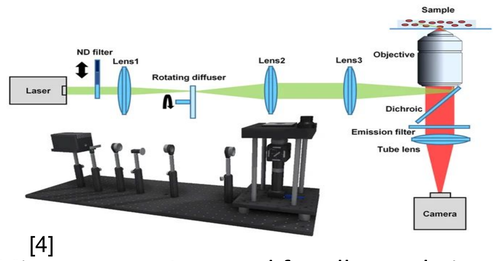
Note, all procedures must be carefully documented for future research

Design

Final Solidworks design used to manufacture the stage







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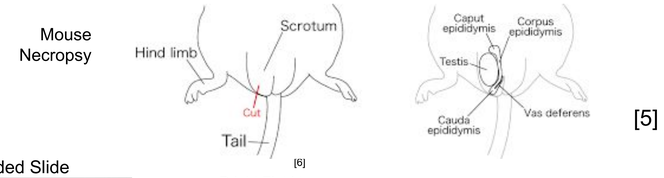
Microscope setup used for all sample imaging

Final manufactured stage

Methods



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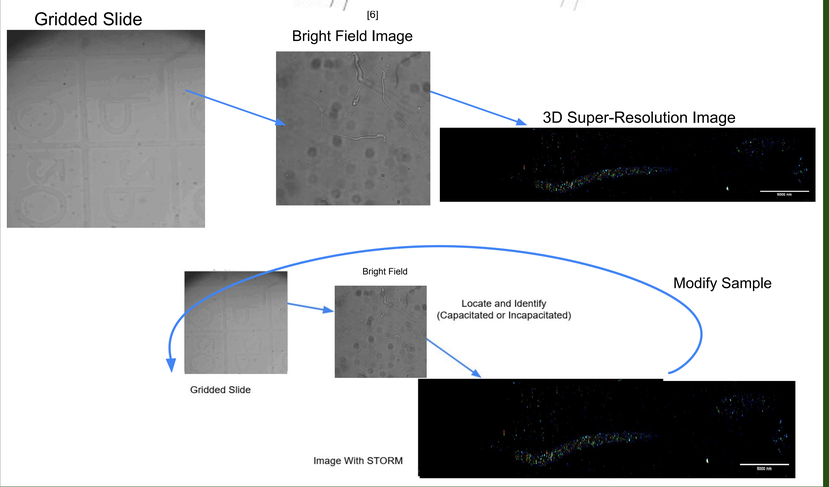
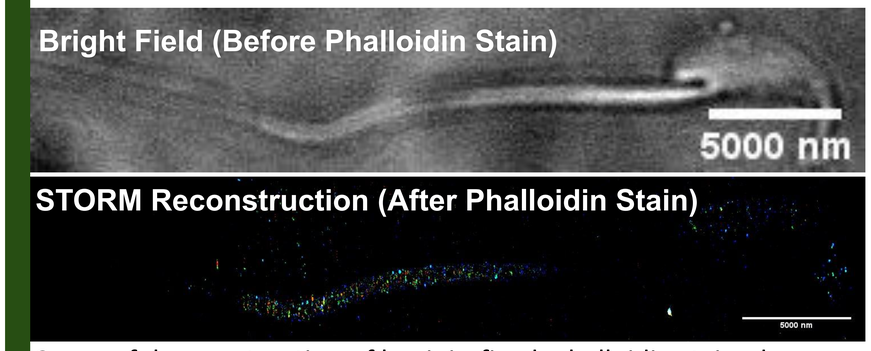


Ethics

•All team members involved with mice have **mouse handling training**, which includes humane sacrificing and necropsy procedures.
•Experiments involving mouse sperm are carefully planned and prepared for, so that all samples collected from mice are used effectively.
•All procedures are done with the intention of contributing to fertility treatment, and ultimately creating life.

Results



Successful reconstruction of laminin-fixed, phalloidin stained sperm cell on a gridded dish.

Constraint	Analysis
Attach sperm heads but not tails	Success
Regularly relocate a specific sperm cell	Success
Allow for manipulation of sperm cell	Success

- The protocol was carefully documented for future use in Dr. Krapf's lab, and to allow for the results to be replicated.
- Results have the potential to demonstrate how proteins and structures within the sperm cell change under different conditions (capacitated vs uncapacitated)

Conclusion & Implications

- Having established a correlative imaging protocol with phalloidin staining, this allows for future experiments:
  - 2-color imaging
  - Protein movement in capacitated cells
- Tetrapod Reconstruction + Correlative Imaging procedures can apply to imaging different types of cells
- Better 3D images of sperm cells leads to increased understanding of fertility
  - Structural patterns of healthy sperm cells
  - Intra-cell changes in healthy sperm cells

References

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[2] Quaas, A., & Dokras, A. (2008). *Diagnosis and treatment of unexplained infertility*. Reviews in obstetrics & gynecology. Retrieved April 17, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2505167/

[3] "Super-resolution microscopy | Cherry Biotech." https://www.cherrybiotech.com/scientific-note/microscopy/super-resolution-microscopy (accessed Nov. 30, 2021).

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[5] "The Manual for Lab Mouse." http://card.medic.kumamoto-u.ac.jp/card/english/sigen/manual/cetransp.html (accessed Apr. 14, 2022).