

# 3D Super Resolution Microscopy



WALTER SCOTT, JR.  
COLLEGE OF ENGINEERING  
COLORADO STATE UNIVERSITY

Dr. Diego Krapf, Carsten Dietvorst, Elliot McCormick,  
and Audrey Banks

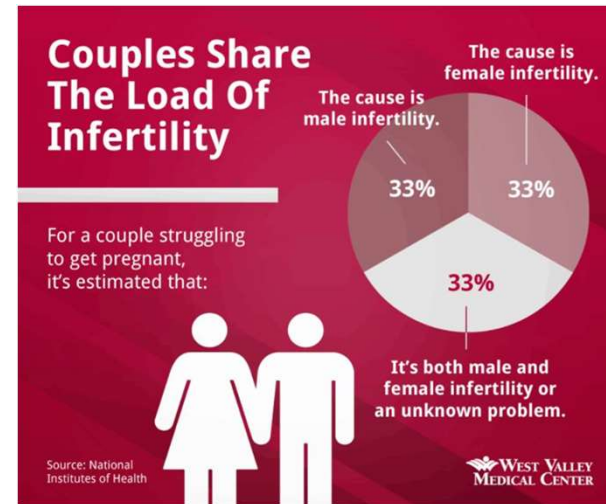
Department of Biomedical Engineering

Department of Electrical and Computer Engineering

Colorado State University, Fort Collins, CO, USA

# Motivation

**Goal:** Improve super-resolution imaging of sperm cells in order to better understand sperm physiology. With this knowledge, male infertility and contraceptive research can be expanded upon.



Male infertility is an equal contributor to infertility issues; however, little is known about male infertility. More needs to be understood about the causes of male infertility as well as the possibility of male contraceptives.

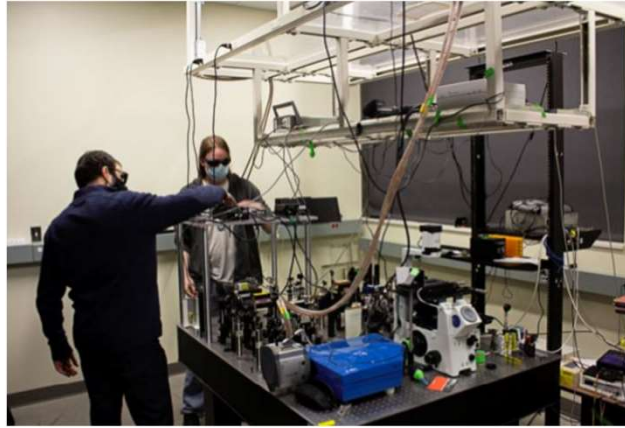
N. (2015, July 02). Couples share the Load of Infertility [Digital image]. Retrieved April 12, 2021, from <https://westvalleymedctr.com/blog/entry/truth-and-lies-about-male-fertility>



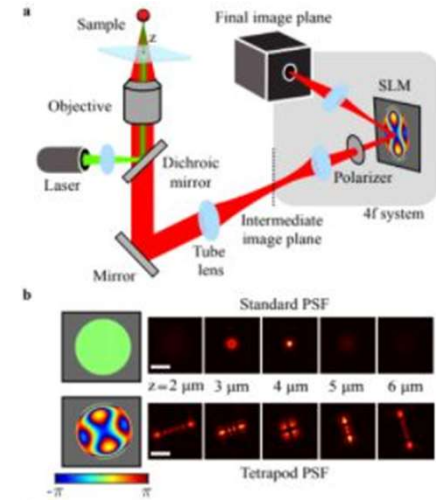
# Methods/Experimental Setup



**Figure 1.** Obtain sperm samples from adult mice stored at the CSU animal research facility, 1<sup>st</sup> step



**Figure 2.** Prepare samples for imaging in the microscope by introducing fluorophores that luminesce when impacted by a specific wavelength of light, 2<sup>nd</sup> step. (See microscope above.)



**Figure 3.** Image samples by causing individual fluorophores to luminesce. Then use a formable mirror (MICA0 3DSR) made by Imagine Optic to create tetrapod PSF, 3<sup>rd</sup> step.

**Figure 1.** Askham Voyles, A. (2021, January 11). Mouse Match [Digital image]. Retrieved April 12, 2021, from <https://www.spectrumnews.org/news/litter-effects-may-skew-findings-in-mouse-studies/>

**Figure 2.** Dickerson, R. (n.d.). [3D Microscopy SURE Team Spring 2021]. Retrieved April 12, 2021.

**Figure 3.** E. Nehme et. al, (2019, September 12). *DeepSTORM3D: Dense three dimensional localization microscopy and point spread function design by deep learning* [Scholarly project]. Retrieved April 12, 2021.



# Results

Full tetrapod point spread function (PSF) range – tetrapod imaging allows for an analysis of the 3-dimensional depth of a sample. The PSF changes shape as the light point moves further and closer to the aperture of the microscope which allows us to precisely localize the depth of the fluorophore.

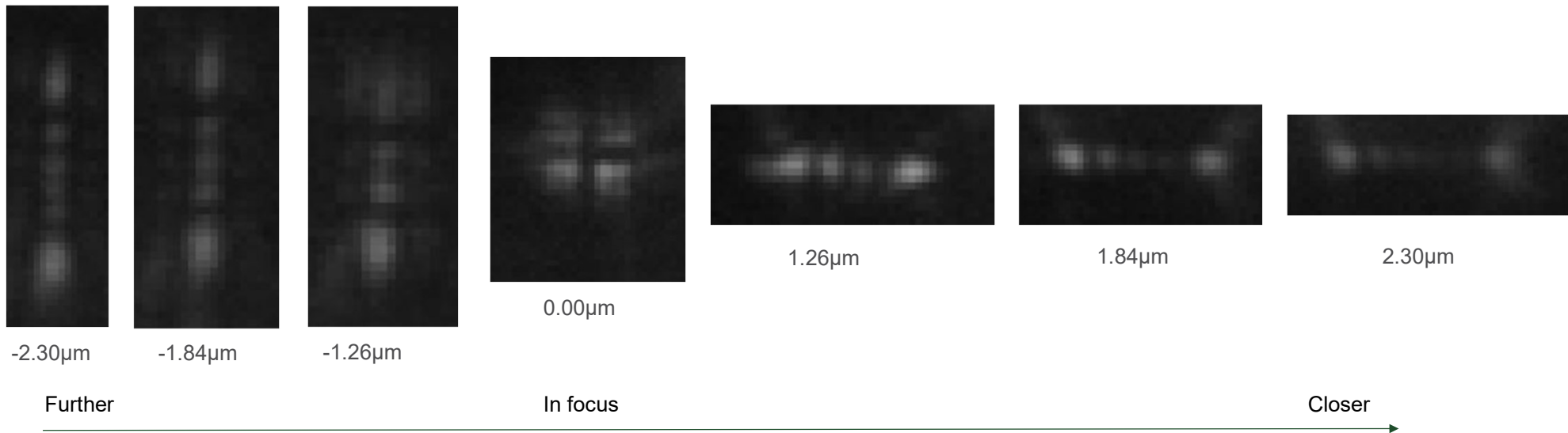


Image specs: 1/1000 dilution. ND 1.0 filter. No scope. 0.200 astigmatism at 0. -0.140 5<sup>th</sup> order astigmatism at 0. 200ms exposure. 90 gain. -5µm to 5µm range at 0.02µm steps (501 steps).



## Discussion/Next Steps

The next step for this project is to use a machine learning algorithm to separate each PSF from an image containing multiple PSFs and to reconstruct a 3D image of the structure.

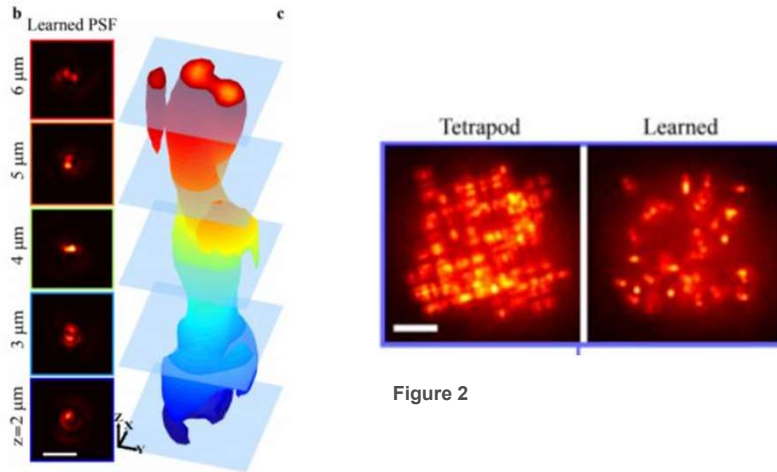


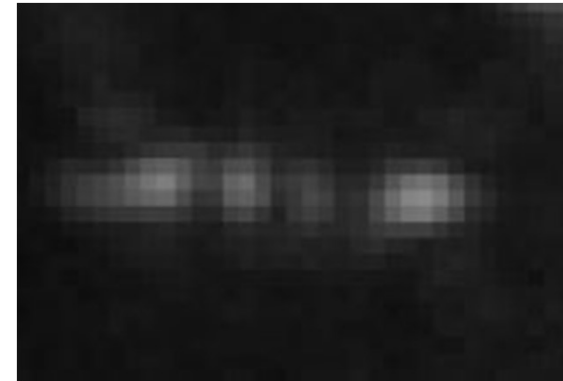
Figure 1

Figure 2

Figure 1 and 2. E. Nehme et. al, (2019, September 12). *DeepSTORM3D: Dense three dimensional localization microscopy and point spread function design by deep learning* [Scholarly project]. Retrieved April 12, 2021.

## Conclusions

3D super resolution imaging is a tool that continues to be improved upon in order to image microscopic materials with greater and greater accuracy. Imaging at this level will someday allow greater understanding of the materials within our bodies to improve healthcare and medicine.



## What benefits did you get from you SURE experience?

Audrey: From this experience I gained an understanding of basic research techniques and procedures. Additionally, I learned the value of communication and collaboration in research. From this project I was also able to witness the challenges and roadblocks researchers face as well as the problem solving and ingenuity it requires to overcome these challenges. Moving forward, the research techniques and skills learned through this experience will serve me well as I pursue future opportunities.

Elliot: I learned a good deal about microscopy, machine learning, and real life research from this project. Working as a team and with other researchers from across the world was a valuable lesson in collaboration and clear communication. I was given an opportunity to continue to grow problem solving skills and to deal with adversity. Research is solving a lot of little, unexpected problems to achieve a big end goal, but it is interesting and I'm grateful to have been a part of taking steps towards a brighter future.

## References & Acknowledgements

Nehme, E., Weiss, L. E., Michaeli, T., & Shechtman, Y. (2018). Deep-STORM: Super-resolution single-molecule microscopy by deep learning. *Optica*, 5(4), 458-464. Retrieved April 12, 2021, from Optical Society of America

Ferdman, Boris, et al. "VIPR: Vectorial Implementation of Phase Retrieval for Fast and Accurate Microscopic Pixel-Wise Pupil Estimation." 2020, doi:10.1101/2020.01.02.892661.

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# Thank you



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