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Multicolor Imaging of Mouse Oocytes

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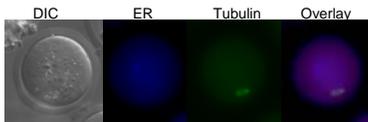


ABSTRACT

Oocyte (egg) morphology has been shown to correlate to viability. By observing the localization of subsurface organelles we hope to learn more about oocyte structure. Mouse oocytes were collected from superovulated female C57BL/6 mice using a hormone dosing regimen. Live oocytes were then stained using multiple organelle-specific fluorescent dyes and imaged on the Keck 3D Fusion Microscope (3DFM) to highlight different components of the eggs. The dyes used stained chromosomes, mitochondria, endoplasmic reticulum, membrane, tubulin, and lysosomes. Images were collected using epifluorescence and Differential Interference Contrast (DIC) microscopy and were compiled and overlaid using Metamorph software. These images serve as useful tools in exhibiting organization of developing oocytes.

SIGNIFICANCE

- Examining the characteristics of the oocyte will lead to a better understanding of their function in development
- Using Metamorph software allows the overlay of individual organelle images



- Overlaying these images leads to a clearer picture of the developing oocyte and highlights the localization of the organelles and their relation to one another

STATE OF THE ART

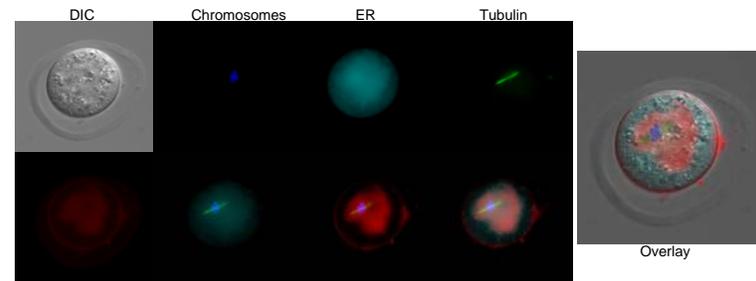
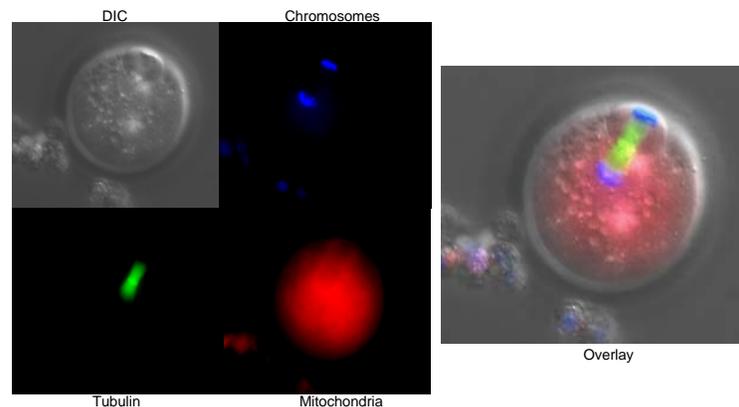
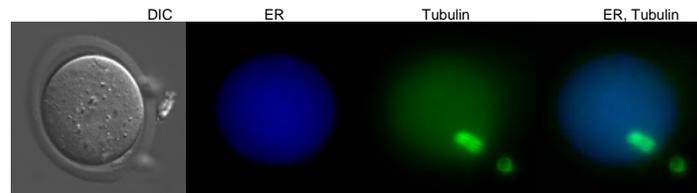
- The Keck 3DFM is a State-of-the-Art microscope with DIC, Confocal, and Two-Photon capabilities

TECHNICAL APPROACH

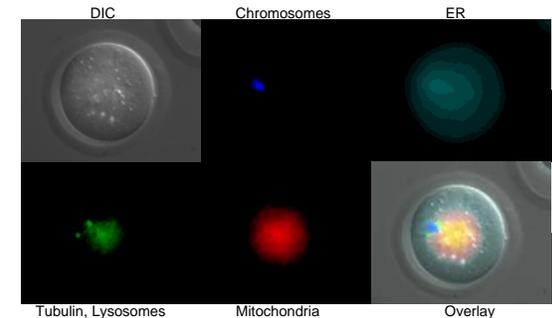
- Test a variety of organelle specific dyes to determine organization of developing oocyte

•Stain for:	•Dyes used:	•Fluorescent Color:
•Chromosomes	•Hoechst	•Blue
•Mitochondria	•MitoTracker Deep Red	•Red
•Endoplasmic Reticulum	•ER Tracker Blue-White	•Blue
•Membrane	•FM 1-43	•Red
•Tubulin	•TubulinTracker Green	•Green
•Lysosomes	•LysoTracker Yellow	•Green

OOCYTE CHARACTERISTICS



- Clearly visible spindle with chromosomes aligned in center
- Mitochondria co-localized with ER around metaphase plate
- Membrane dye apparent surrounding the cell



CONCLUSIONS

- Live oocytes can be stained with multiple fluorescent dyes
- Different colors allow organelles to be distinguished
- Overlay of images allows the relative location of the organelles to be determined

PLANS FOR THE FUTURE

- Apply this multiple stain technique to all stages of developing embryos
- Work has begun making 3D reconstruction models by using Z-stack imaging

TECHNOLOGY TRANSFER

- Understanding the structure of developing oocytes may lead to advancements in in vitro fertilization (IVF) therapy

REFERENCES

Wang, Qiang, Sun, Qing-Yuan, Evaluation of oocyte quality: morphological, cellular, and molecular predictors. *Reproduction, Fertility, and Development*, 2007, **19**, p.1-12.

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