

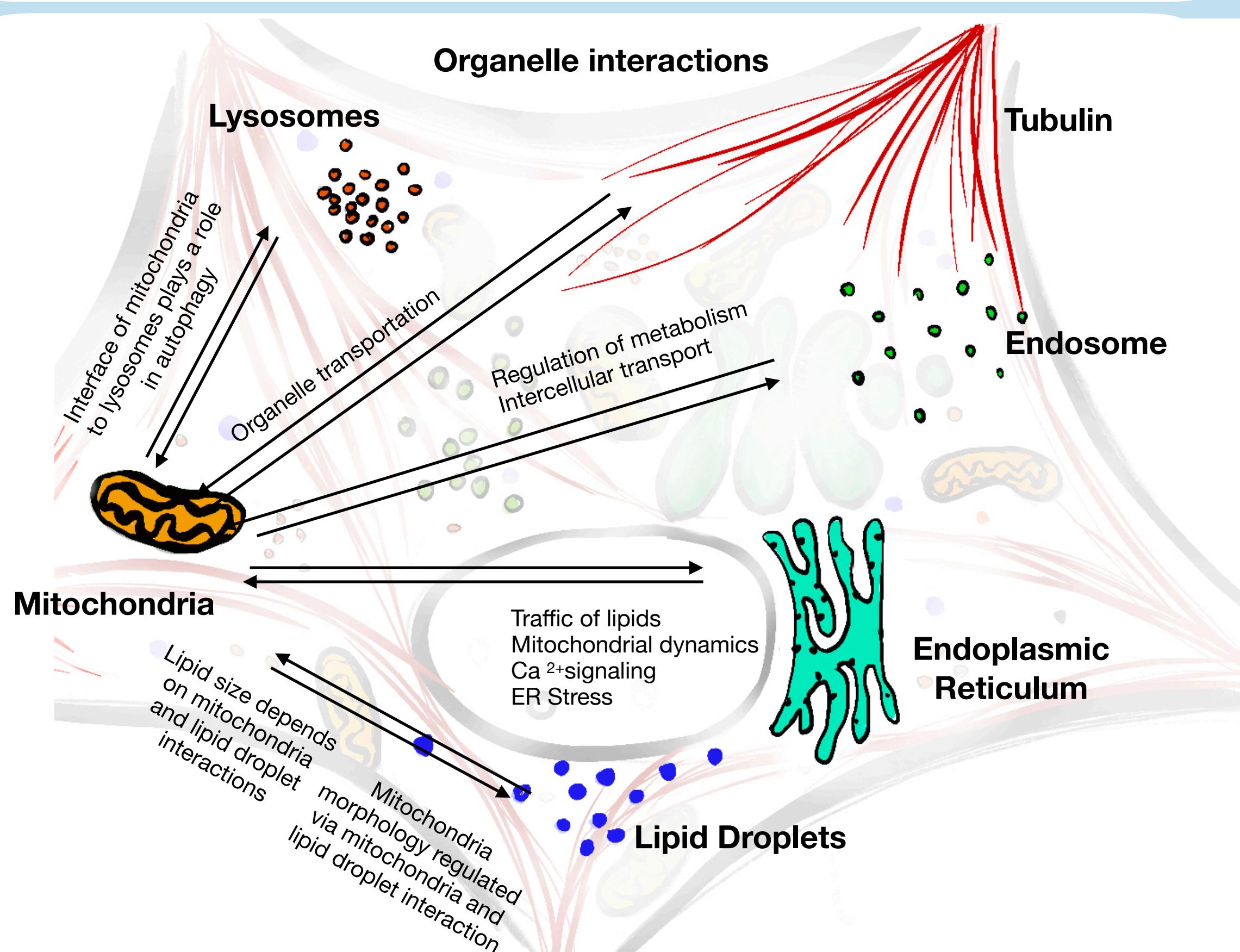


Phasor Unmixing to Reveal Organelle Organization and Cellular Response

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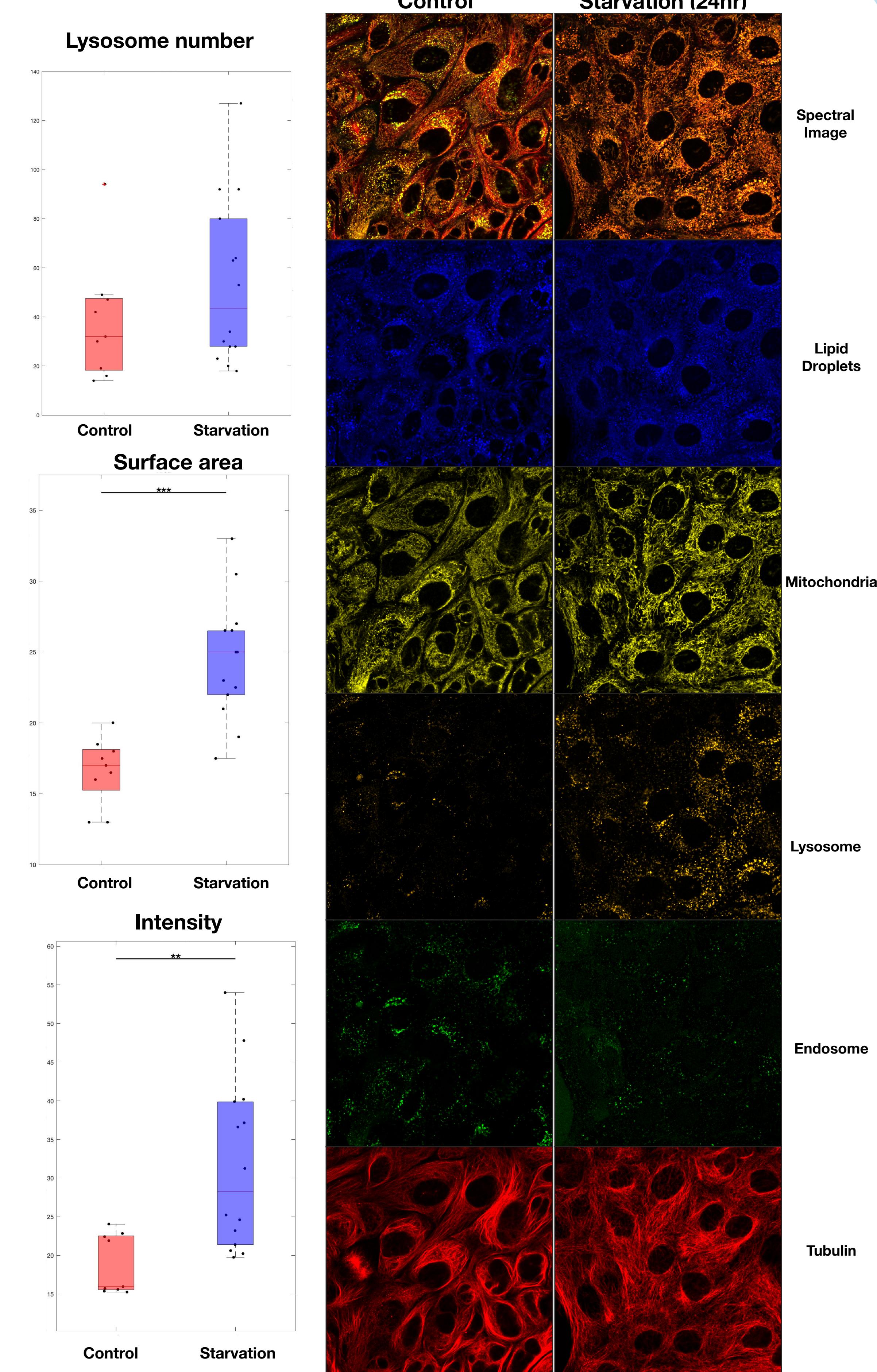
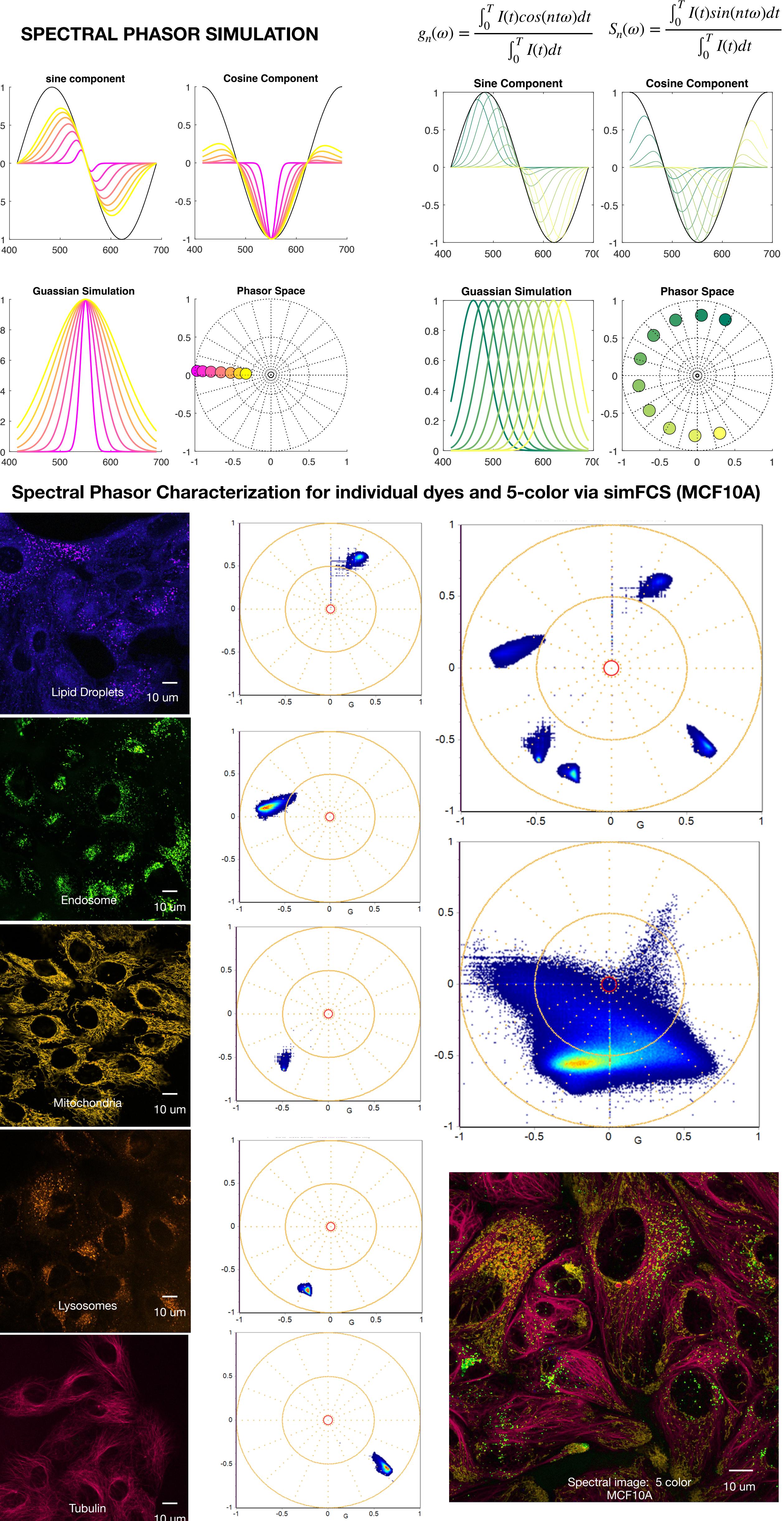
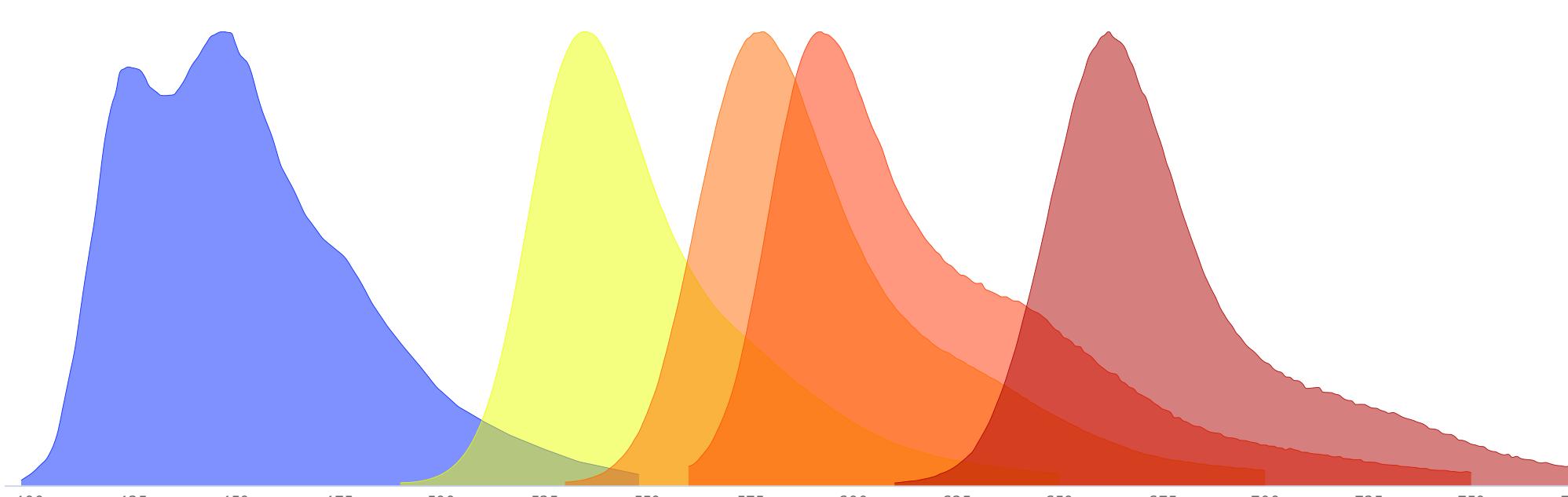
ABSTRACT

Cell organelles are a set of subcellular structures that perform various functions inside of eukaryotic cells, working coherently to maintain cell function. Dynamic organelle interaction controls cell behavior, subtract transport and organelle-organelle communication, etc. Alterations in such intra organelle activity directly affect cellular physiology and are associated with pathology. However, most studies typically consider one organelle at a time which limits our understanding of organelle-organelle interaction, organelle-cytoskeleton interaction and cooperative behavior. Here, we propose to develop a method for simultaneous fluorescent labeling of up to seven organelles. We then unmixed their spectral emissions using the phasor analysis to quantify functional and morphological changes in such organelles upon different conditions.



Fluorescent Dyes	Organelle/cytoskeletal structure	Color	Cell function
Lipi Blue	Lipid droplets	Blue	- Energy storage - Protection against oxidative stress
pHrodo	Endosome	Green	- Endocytosis - Proteins and lipids trafficking
TMRM	Mitochondria	Yellow	- Cellular respiration Energy production
Lyso Red	Lysosome	Orange	- Macromolecule digestion Autophagy
Tubulin DR	Tubulin	Red	- Intracellular transport Mitosis

• Lipi- Blue • pHrodo
• Green Dextran 10,000 • Tetramethylrhodamine, methyl ester • LysoTracker Red DND-99 • Tubulin Deep Red



CONCLUSION

We unmixed and quantified five organelles simultaneously.
We measured the effect of serum starvation on several pathways

PERSPECTIVE

Increase the number of organelle structures unmixed
Quantify different kinds of stress response and diseased conditions

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