Investigating the signal origins in dOCT and translating a new functional contrast to fluorescence imaging

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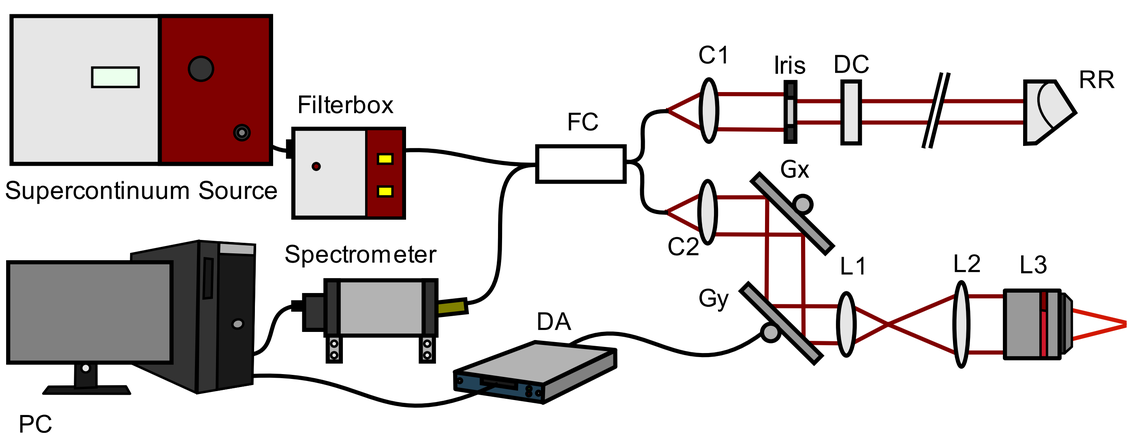
Introduction

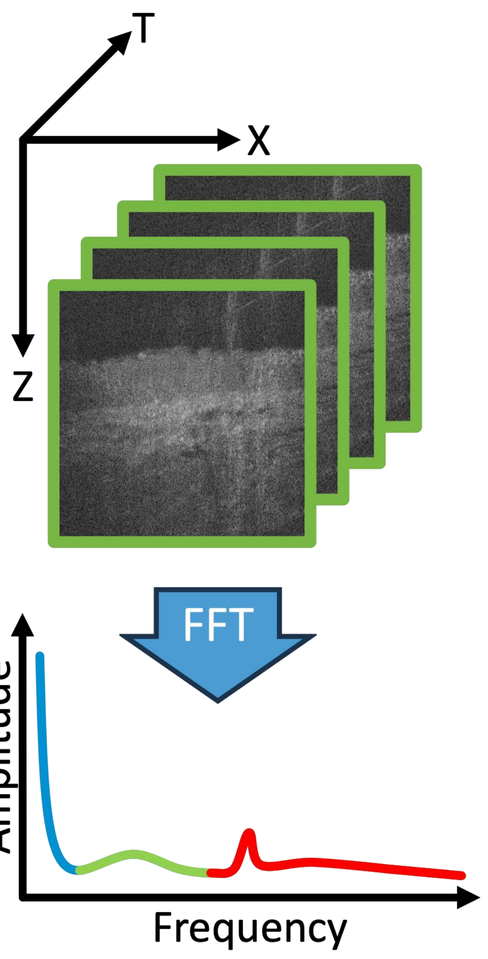
- Optical coherence tomography (OCT) provides tomographic images with µm resolution.
- Dynamic evaluation of time series data creates functional contrast
- Influence of individual organelles isn‘t yet understood

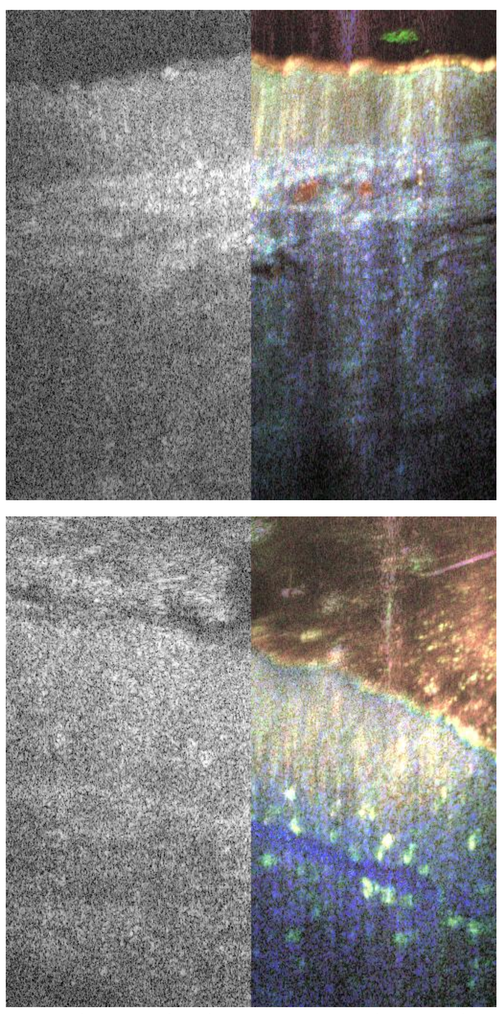
dOCT Imaging & Processing



Custom build OCT setup with microscopical resolution. FC: Fiber-coupler, C: Collimator, DC: Dispersion Correction, RR: Reference Reflector, G: Gyroscopic mirror, L: Lens, DA: Digital-Analog-converter

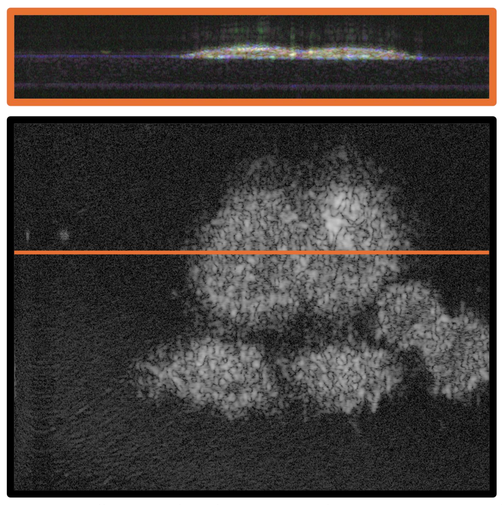
- Per sample 75 frames (B-Scans) are acquired using a framerate of up to 110 Hz
- Pixelwise Fourier transformation over the time yields the frequencies of the signal fluctuations in each pixel
- These spectra are binned into 3 channels to form an RGB image
- Binning is automated using the Neural Gas algorithm





The pixel wise Fourier transformation yields the signal fluctuations (left). Binning these greatly enhances contrast as shown on the right for a trachea (top) and bronchus (bottom).

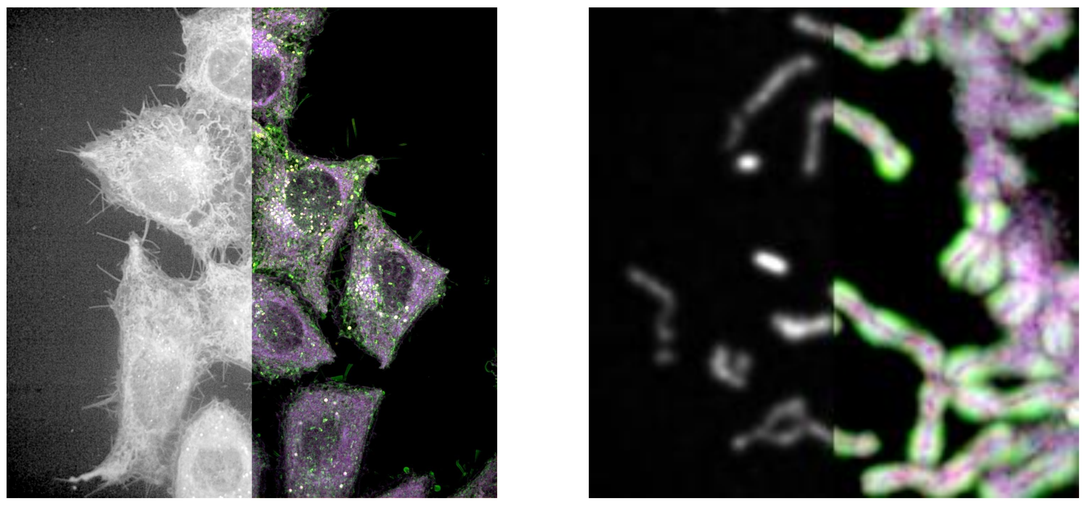
- Subcellular structure cannot be resolved
- Dynamic signals are generated by subcellular fluctuations
- Confocal fluorescence can resolve these
- Correlative measurements could evaluate dOCT signal origins



HeLa Kyoto cells imaged with OCT. Top: dOCT B-Scan. Organelles cannot be resolved. Bottom: OCT en-face plane annotated in orange with the position of the above B-Scan.

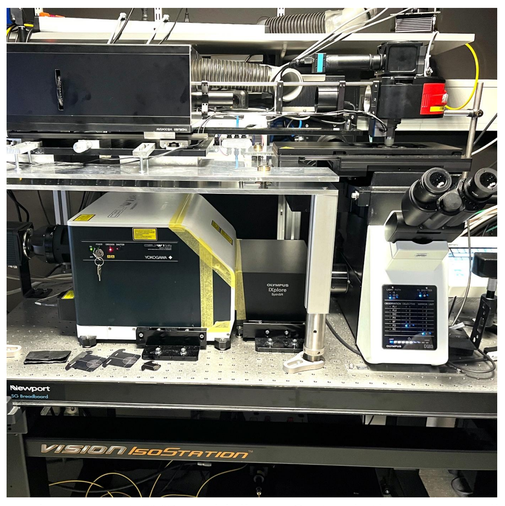
Translating the contrast to fluorescence

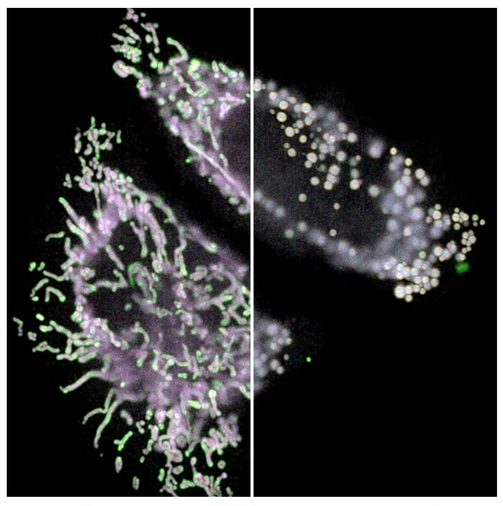
- Uncovering organelle signal contributions with spinning disk microscopy (SDM)
- Dynamic processing can be translated to other imaging domains
- Dynamic SDM yields organelle dependant contrast with Nile Red
- Global and intra-organelle signals separate with specific stains
- This allows for correlative measurements with OCT



Dynamic SDM contrast on Nile Red stained HeLa Kyoto cells (left). SDM with and without dynamic contrast of StayGold stained mitochondria (right).

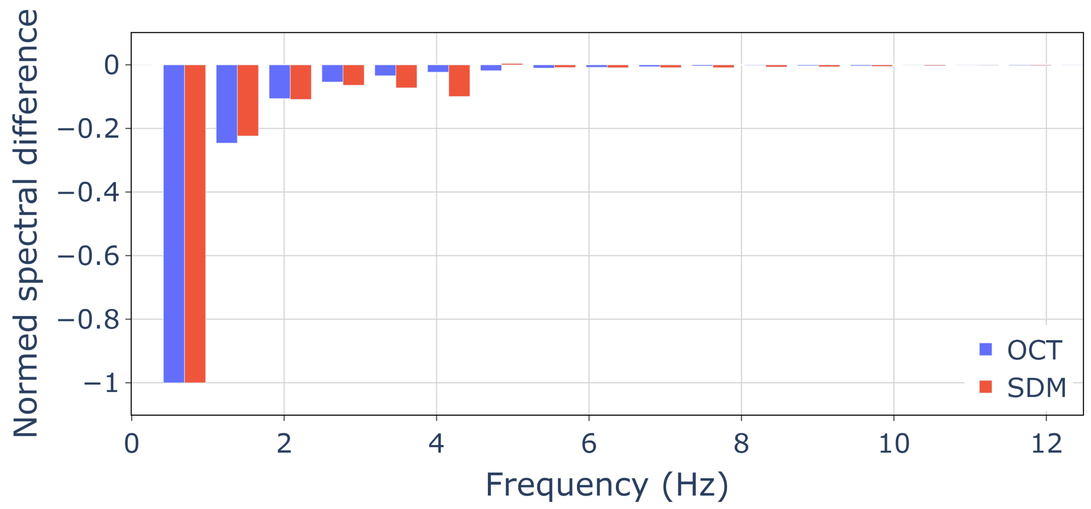
Investigating the signal origins





Merged systems at EMBL Heidelberg. On top is the custom build OCT whereas the Olympus iXplore SDM images from below. The sample is held within a micro incubator between the systems (left). Dynamic SDM of mitochondria before and after inhibition (right).

- Organelle inhibition leads to spectral changes
- SDM only captures the stained organelles and their changes
- OCT captures superposition of all changes
- Organelle changes can be found in dOCT difference nonetheless



Comparison of spectral difference of 10 cells where mitochondria were stained with StayGold and inhibited with ionomycin. Oct detects similar changes even though its signals are a superposition of all cellular changes.

Conclusion

Dynamic contrasting is translatable to other imaging modalities such as confocal fluorescence. Further, this can aid us in investigating dOCT signal origins.

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