SAND2012-9877P

Hyperspectral Imaging of Fixed and Live Fungi in collaboration with Aaron Neumann and Matt Graus

Sample Preparation: 10-23-2012 (Matt)

Candida albicans, strain SC5314

Candida parapsilosis, strain NRRL Y-12969

Saccharomyces cerevisiae, strain S288C

Imaging: 10-23-2012 (Howland)

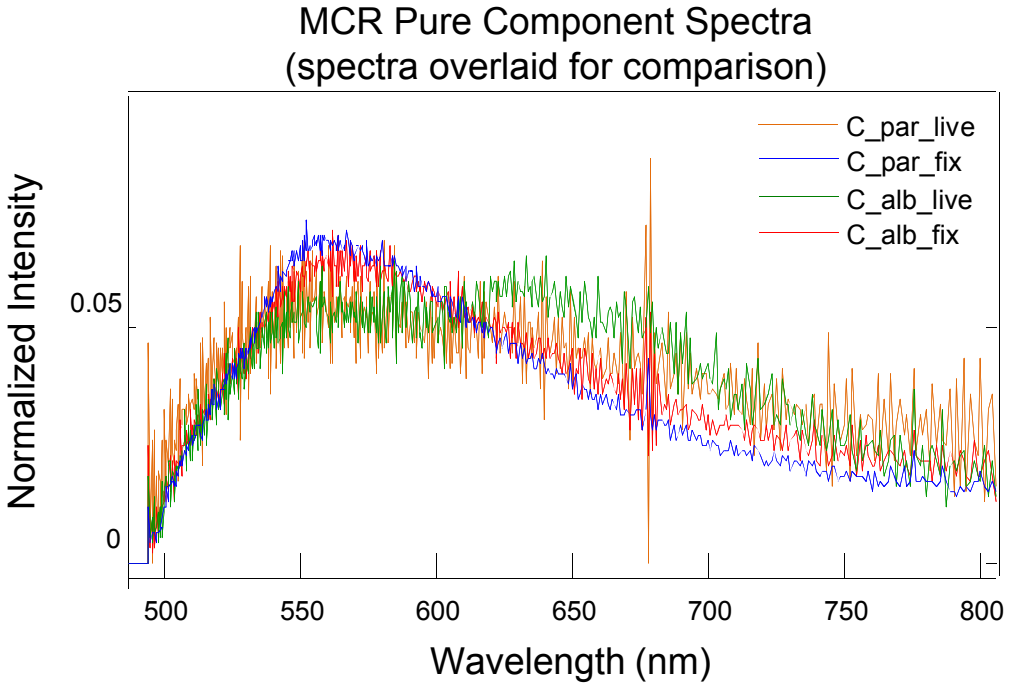
Analysis: 10-29-2012 (Jeri)

Sample prep: either live or fixed fungi were placed on slide, covered with #1.5 coverslip, and sealed with nail polish.

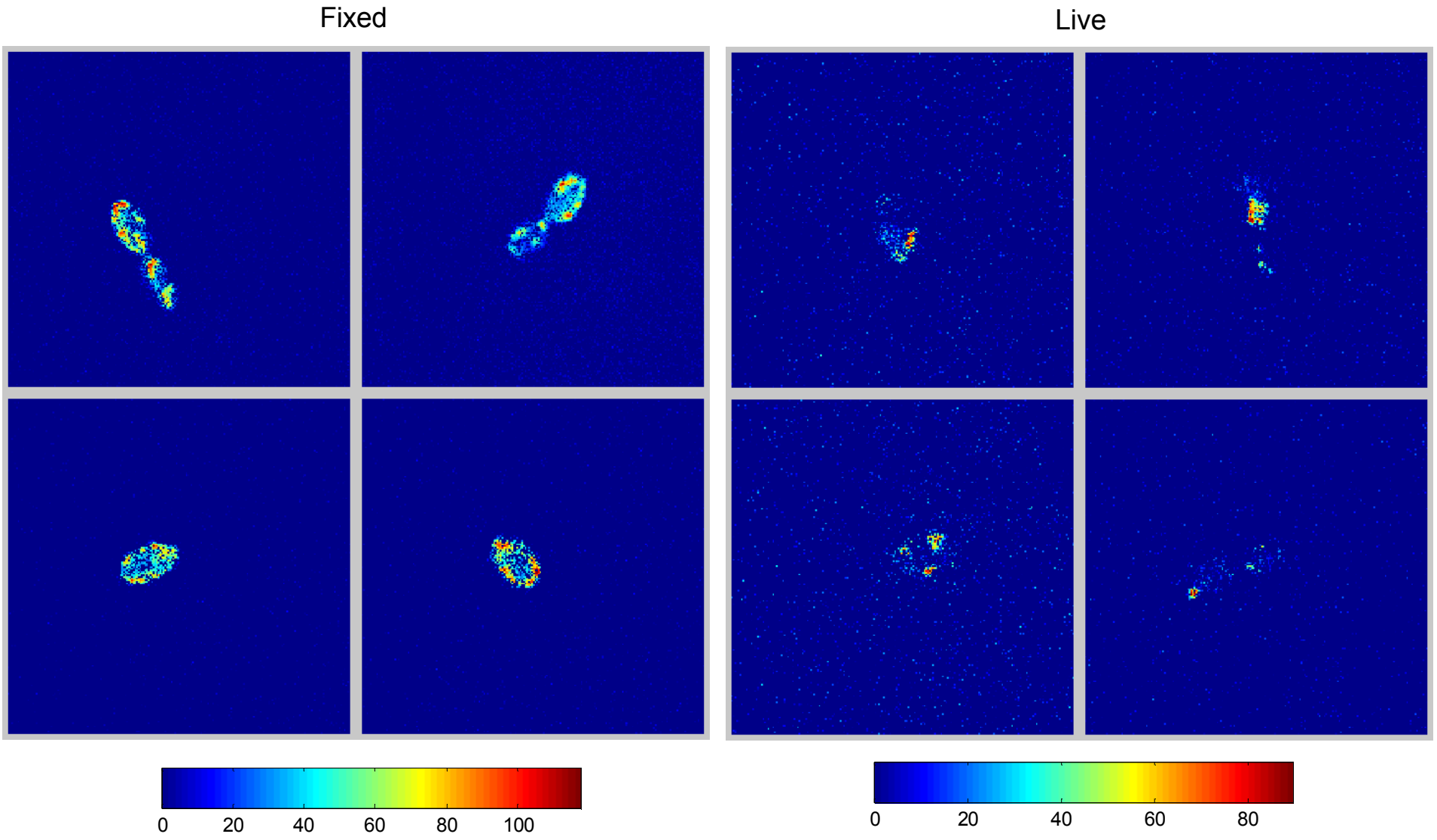
Acquisition parameters: 488 nm excitation, 60x oil objective, 25 µm x 25 µm field of view, 0.24 msec/pixel, gain = 210, OD=0.

Hyperspectral Imaging Results

- Overall the autofluorescence intensity was extremely weak from the samples (~ 2-8 cts). *C. albicans* and *C. parapsilosis* had sufficient autofluorescence to quantify, but S*. cerevisiae* did not.
- Differential autofluorescence was observed between live and fixed cells
  - Spectral differences – live cell spectra are bimodal vs. unimodal for fixed cells
  - Spatial localization – fixed cells characterized by multiple punctate spots of AF around the outside of the cells vs. live cells which showed one (sometimes two) punctate spots per cell
  - Abundance – fixed cells had ~ 2x brighter autofluorescence intensity
- No significant difference in AF spectral shape was observed between different species, however *C. parapsilosis* was ~1.5x brighter than *C. albicans*.



C. parapsilosis Autofluorescence



C. albicans Autofluorescence