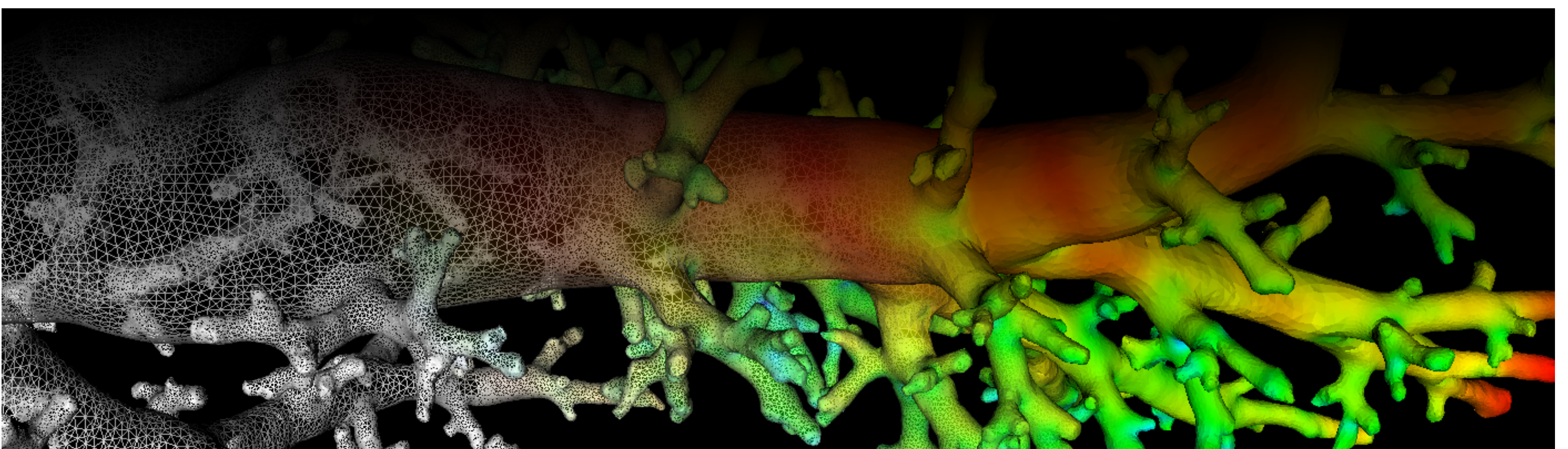
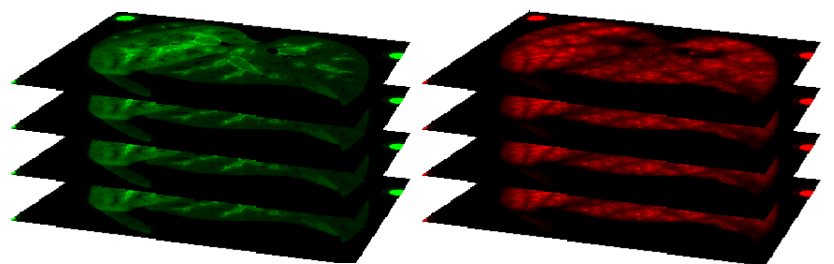
Lung Anatomy + Particle Deposition (lapd) Mouse Archive

for Modeling and Computational Toxicology



Folder *_RawCryomicrotomeData



Raw Cryomicrotome Data

Folder ***_RawCryomicrotomeData** contains the raw data from the imaging cryomicrotome. This includes all image slices for the aerosol deposition image channel (rd), the autofluorscent image channel (fl), and additional calibration and measurement files.

Starting with the **RawCryomicotomeData**, the individual autofluorescent channel image slices are converted, assembed into an image volume, artifacts are removed, and the volume is cropped to the field of view containing only the lung and trachea. The resulting images are available in [*_Aerosol.mha] and [*_Autofluorescent.mha]. Aerosol image data after deconvolution and normalization are available as [*_AerosolDeconv*.mha] and [*_AerosolNormalized*.mha], respectively.

The data in folder ***_RawCryomicrotomeData** is separated by color channel (`RR` = ol, fl, rd) and archived in archived in ***_RawCryoImages_RR.tar** files. Each of these comprises several smaller image sets:

- Slicing images: `RR####.nef` : Serial images taken for each channel (`RR`) starting with `RR0001.nef` .
- Bright field images: Generally taken in groups of 10, paired with the same filters as used for slicing (`white-RR##.nef`)
- Calibration layer: images to adjust for inter-channel shifts, taken in sets of 15 (`cal-RR##-##.nef`)
- Dark images: measure of noise in sensor taken with the lamp off after all slicing is completed (`dark-RR##.nef`)
- Scale: Pixel size is estimated for every data set by taking an image of graph paper with 1mm divisions (`scale.nef`)

Slicing Protocol

- **Imaging**: We acquire images by pairing excitation and emission filters to highlight specific parts of the sample. Each channel is given a two letter shorthand value (ol = outline; rd = red; fl = autofluorescence).

Channel	Reason	Excitation (cwl/fwhm)	Emission (cwl/fwhm)
ol	Lung extents	neutral density	470/30
fl	Airway walls	485/30	535/30
rd	Aerosol particles	560/30	635/30

- **Image Acquisition**: The main part of image acquisition starts with removing a thin slice from the frozen sample block. We take take a series of images of the block face: one image for each channel. The images are numbered sequentially with the slice number becoming part of the filename, e.g. `rd0001.nef`.
- Several smaller sets of data are needed to complete the sample:
  - **Calibration layer**: Differences in the manufacture of the emission filters can appear as a pixel shift between channels. The calibration layer is a set of 15 consecutive images in each channel taken of a still scene with lots of internal texture. We can remove the inter-channel shift by comparing the calibration layer.
  - **Bright fields**: The excitation light can be shaped by the excitation filters. To get the best estimate of the excitation light, we measure a separate bright field for each channel. We developed an OCT phantom that allows us to pair our standard excitation and emission filters used during slicing. We take a series of 10 images of this phantom, slicing it in between.
  - **Dark images**: The base current in the camera sensor may increase the noise of images, especially those with long acquisition times. At the end of imaging, we take a dark image (excitation light off, shutter open) to estimate the baseline noise in the camera.

Related Data Structures

* Autofluorescent.mha | * Aerosol.mha | * AerosolDeconv*.mha | * AerosolNormalized*.mha

Related Code Examples

(none)

Updated: 7/24/19
2018 r2b