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## MOTIVATION

1. Conventional methods of studying preclinical models of metastasis involve serial sacrifice of large cohorts and are cumbersome and expensive.
2. *In vivo* imaging methods of estimating tumor burden with efficacy testing are comparatively rapid and inherently more efficient.
3. *In vivo* high-resolution X-ray micro-CT can provide accurate visualization of deep tissue tumors in preclinical oncology.
4. X-ray micro-CT provides a methodology for characterizing preclinical lung tumor models which is clinically relevant.

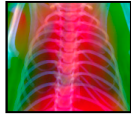
## AIMS

1. To use high resolution micro-CT to characterize the incidence, spread and growth of lung nodules in a murine IV model of lung metastasis.
2. To determine the feasibility of quantifying tumor burden in serial mouse lung micro-CT images.
3. To investigate the influence of breathing motion on tumor burden estimation in non-respiratory gated micro-CT.
4. To investigate ways of enhancing mouse lung/nodule visualization through direct volume rendering and multi-dimensional transfer functions.

## INTRODUCTION

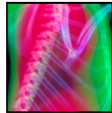
### Preclinical Models of Metastasis

- Conventional methods of detection generally involve serial sacrifice and laborious visual inspection or histopathology of large cohorts.
- Recent developments in *in vivo* optical imaging (bioluminescence and fluorescence) and micro-PET provide enhanced detection with high sensitivity, but low resolution and limited anatomical context.



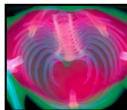
### In vivo Micro-CT

- Micro-CT is increasingly being used to provide unique insights in mouse models of cancer *in vivo*.
- Micro-CT provides high resolution (50-100  $\mu\text{m}$ , isotropic) images of normal and pathological mouse anatomy.



### Quantification and Visualization

- Identification and quantification of lung nodule incidence and tumor burden has been difficult, particularly in non-respiratory gated scanning.
- In the clinical setting, sophisticated computer-aided diagnostic algorithms exist which may be useful in preclinical applications.



## METHODS



Female C57/BL6 mice were implanted with  $1 \times 10^5$  tumor cells (B-16/BL6/F10 melanoma) by intravenous injection.



Beginning 7 days after B-16 injection, mice were scanned by micro-CT under injectible anesthesia (ketamine/xylazine).



The influence of respiratory motion was investigated by scanning mice freely breathing and immediately after euthanasia.



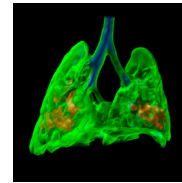
Lungs were harvested and fixed in formalin for *ex vivo* visual inspection of tumor incidence and size.



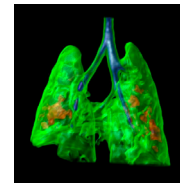
A cohort of animals was selected and euthanized at regular time intervals to monitor the spread and growth of lung tumors.

## RESULTS and CONCLUSIONS

### Volume Visualization of Mouse Lung Micro-CT Data



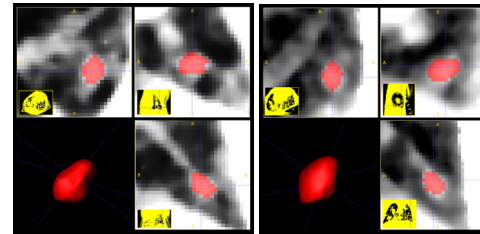
Ventral viewpoint



Dorsal viewpoint

- Representative data of a mouse with lung metastases. Two views of a volume rendering of lung micro-CT data are shown from ventral (left) and dorsal (right) viewpoints. Lung tissue is shown in green, major air passages in blue, and lung nodules in red.

### Influence of Respiration on Mouse Lung Micro-CT



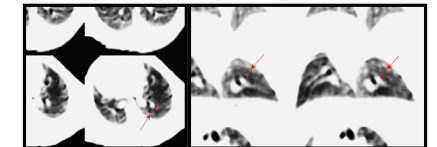
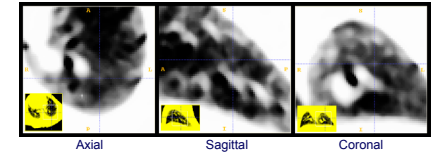
*Ex vivo*

*In vivo*

- Representative data from a mouse *in vivo* (right) and *ex vivo* (left). For each case, three orthogonal views are shown of the segmentation of a nodule, with a volume rendering of the nodule. The nodule shown is approximately 1 mm in diameter, and the apparent volume is 0.19  $\mu\text{L}$  (*ex vivo*) and 0.22  $\mu\text{L}$  (*in vivo*). *In vivo* tumor volume estimates are usually somewhat larger than *ex vivo*, with volume discrepancies typically between 10-20%.

### Estimating Lung Tumor Burden by Micro-CT

- Micro-CT data is pre-processed by Curvature Anisotropic Diffusion filtering, histogram equalization, and thresholding the intensity band for lung tissue.
- Apparent nodules are identified using a linked cursor in three orthogonal planes.



- Nodules are segmented in a tiled representation of the data which has been down-sampled by a factor of 2-3 in the slice direction.
- Nodule volume is determined by multiplying the number of voxels in the selection mask by the volume of a single voxel. This nodule volume is then averaged from a determination in at least two orthogonal directions.

### Conclusions

- Micro-CT was able to detect and delineate lung tumor metastases *in vivo*.
- Lung nodule volumes could be readily quantified by micro-CT.
- Although respiratory motion caused some blurring, nodules as small as 1 mm could be quantified with reasonable accuracy.
- Lung tissue and nodules were isolated in the micro-CT data and qualitatively visualized by volume rendering.