# An optimized method for in vivo lung microscopy reveals intimate cell collaborations during infection.

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**Résumé**

**Background**: Dynamic microscopy is critical to revealing the spatiotemporal interactions of cells within the tissue environment following infection. In the lung in vivo microscopy is particularly challenging because of tissue motion arising from the beating heart and breathing. As a result, infected lung tissue has never been imaged in vivo at a sub-micrometric scale thus far, and little is known about the kinetics of the mucosal immune response at the cellular level.
**Methods**: We have developed an optimized post-processing strategy to overcome tissue motion, based upon two-photon and second harmonic generation (SHG) microscopy. Most of our strategy is based on an oversampled multimodal acquisition (collagen from SHG and fluorescence by two-photon excitation fluorescence). We used CX3CR1+/gfp mice that express the green fluorescent protein (GFP) in CD11b dendritic cells (DCs) and inflammatory monocytes, and Flk1+/gfp that express GFP in the endothelial capillaries of the lung.

**Results**: Using Flk1+/gfp we have shown that our technique allows imaging the lung over a period of time of 1 hour without any mechanical stabilization of the parenchyma and without inducing any leak. This would indicate that our strategy was not invasive and did not alter lung physiology. Next, we infected CX3CR1+/gfp mice by intra-tracheal route with stained spores of Bacillus anthracis, the agent of anthrax. We observed striking connections between DCs and spores engulfed by alveolar cells. To determine what cells were connected to DCs, we instilled intra-nasally rhodamine-dextran in CX3CR1+/gfp mice, and infected them either by intra-tracheal or intranasal route. We demonstrated that alveolar macrophages phagocytize spores in the first line and then establish contacts with DCs.
**Conclusions**: Contacts between alveolar macrophages and DCs are present at the homeostasis, but increase dramatically after infection. These interactions may participate in a better coordinate immune response. Our results not only demonstrate the phagocytizing task of lung DCs but also infer a cooperative role of alveolar macrophages and DCs after infection.

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