

Johns Hopkins Engineering

Immunoengineering

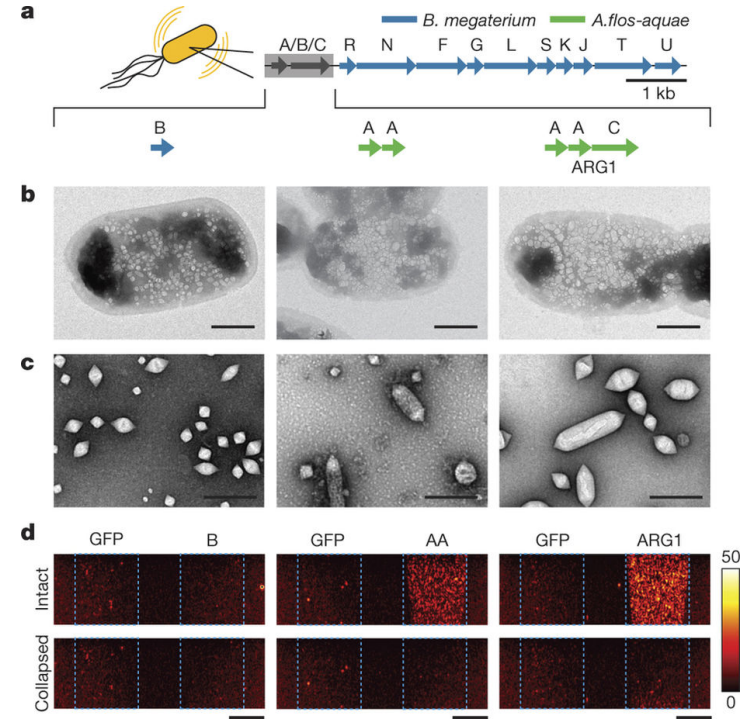
**Immunoengineering - Immunoprofiling
Imaging**

Limitations to Current Imaging Techniques

- Limited *in vivo* imaging modalities
 - Invasive/Toxic
 - Immunogenic
 - Expensive
 - Static
 - Penetration depth
- Limited *ex vivo* imaging modalities
 - Static
 - Complex *ex vivo* culturing environments
 - 2D

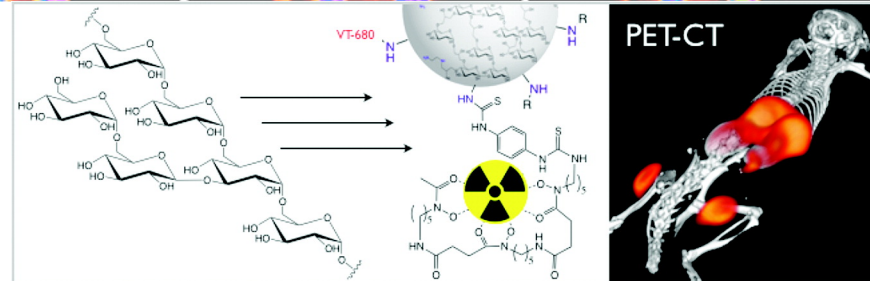
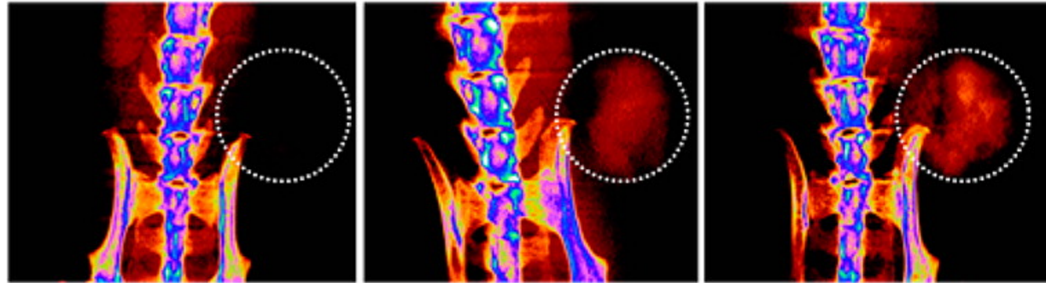
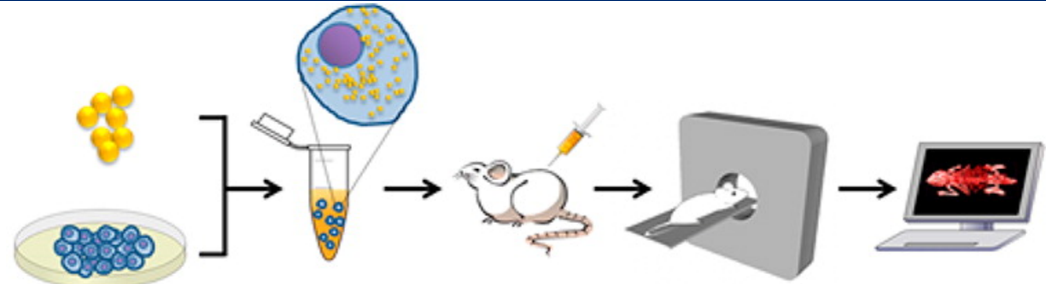
Designer Bacteria for Acoustic Reporting

- Challenging to see optically inside the body because of tissue light scattering
- Tracking of bacterial communities
- Incorporate acoustic reporting genes – gas vesicles– use ultrasound
- Resolution of 100 μm at low densities
- Different gas densities to label different populations



Nanoparticles for Tracking Cells

- Ex vivo label CAR T cells by gold nanoparticles to enhance CT imaging signal
- Radionuclide conjugated nanoparticles enhance macrophage tracking
- Challenge in verifying cells transferred in are tracked (gold standard)

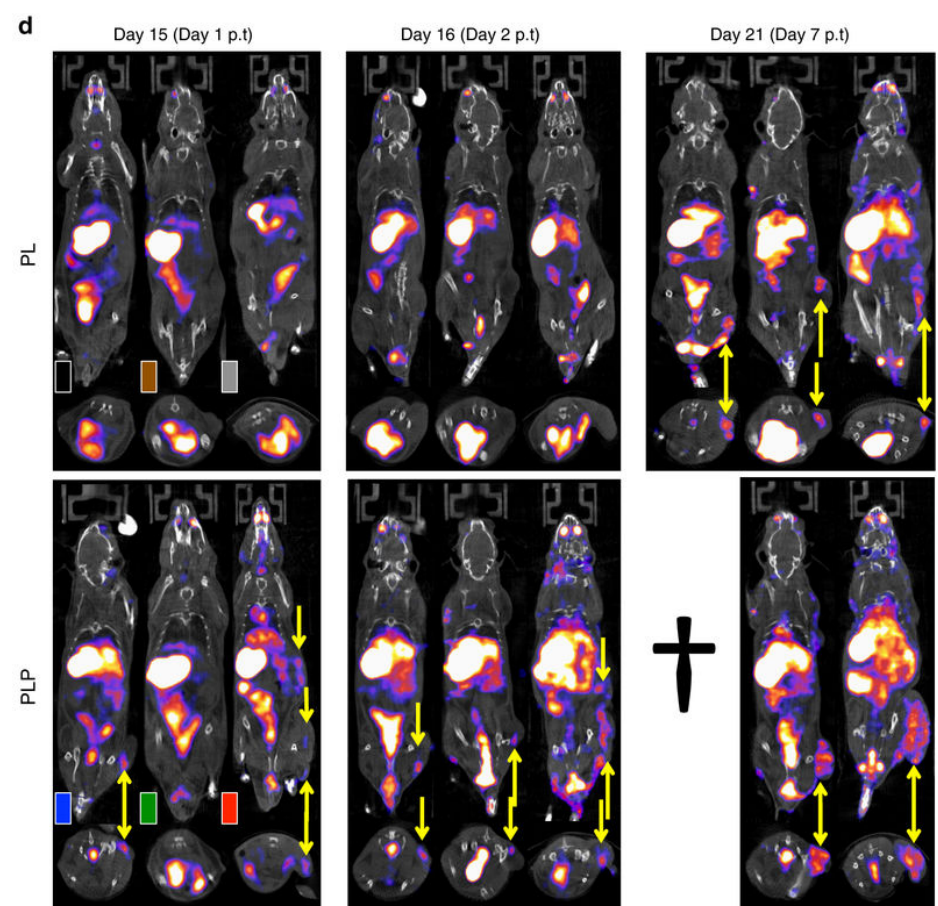


Meir, Rinat, et al. "Nanomedicine for cancer immunotherapy: tracking cancer-specific T-cells in vivo with gold nanoparticles and CT imaging." *Acs Nano* 9.6 (2015): 6363-6372.

Keliiher, Edmund J., et al. "⁸⁹Zr-labeled dextran nanoparticles allow in vivo macrophage imaging." *Bioconjugate chemistry* 22.12 (2011): 2383-2389.

Tracking CAR T Cells

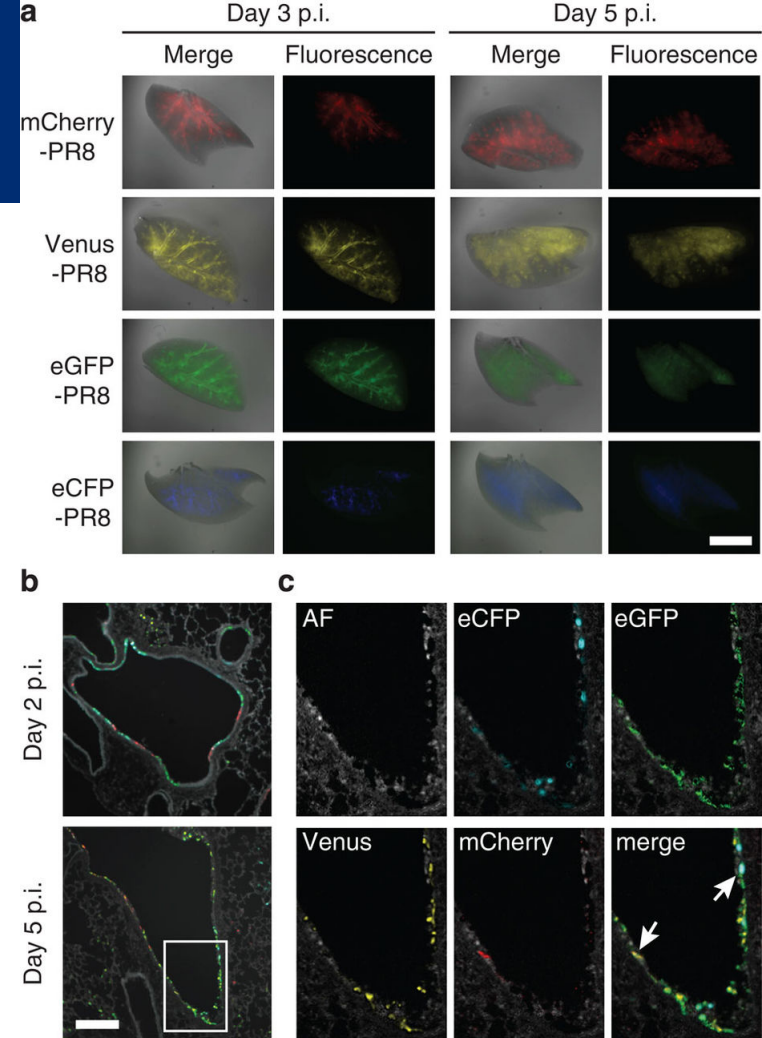
- Understanding both efficacy, long-term survival (design), and off-target effects of CAR T cells
- Human sodium iodide symporter
 - Available radioisotopes
 - Also expressed in other tissue like the stomach
 - Use of SPECT/CT



Flu Viral Strains in vivo Labeling

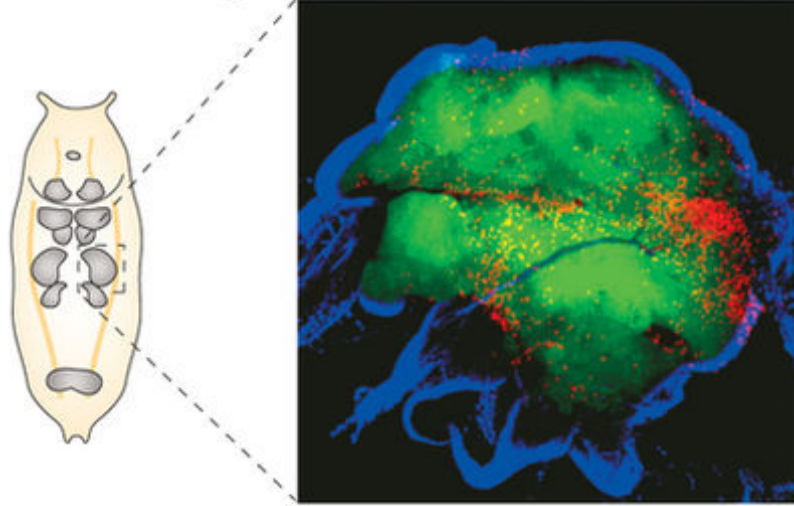
- Understanding variants of influenza virus infection
 - Look at organ and cellular level distribution
- Genetically encode fluorescent markers into virus
 - Not attenuated

Fukuyama, Satoshi, et al. "Multi-spectral fluorescent reporter influenza viruses (Color-flu) as powerful tools for in vivo studies." *Nature communications* 6 (2015): 6600.

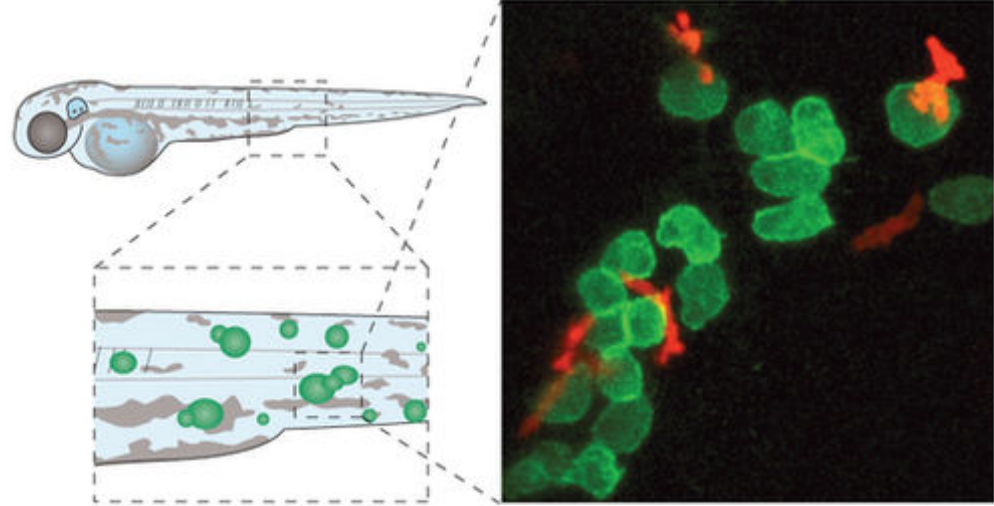


Translucent Animal Models of Immune-Cancer Cell Interactions

a *D. melanogaster*



b Zebrafish



Nature Reviews | **Cancer**

In vivo Imaging – Two Photon Intravital Imaging

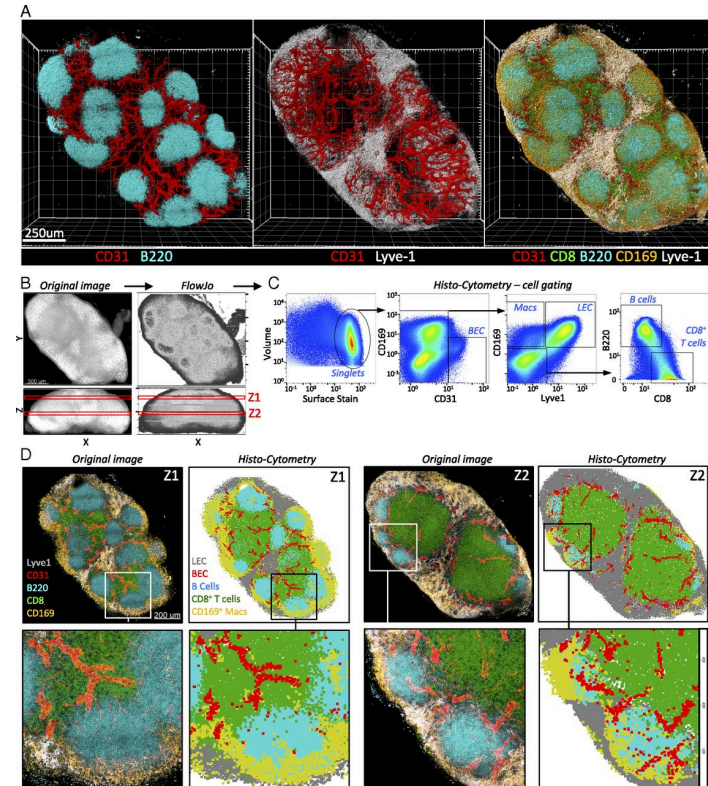
- Requires surgical removal of lymph node from inside body, but stay connected to animal
- See soluble antigen distribution and DC uptake

In vivo Dynamic Imaging – Two Photon Microscopy

- Two-photon fluorescent microscope
 - Optical-phase locked ultrasound lens for increased imaging speed
- Visualize neutrophil trafficking

Clearing-enhanced 3D Microscopy (C_e3D)

- Conventional location based imaging is 2D
- Cellular resolution with 3D requires dissociation
- Chemical screen to reduce light scattering in tissue, yet retain 3D structure, and allow multiplex cell-resolution fluorescent staining
 - N-methylacetamide (22% wt/vol) in Histodenz (86% wt/vol) with 0.1% Triton X-100





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