Aplikace fluorescence v in vivo zobrazovacích metodách

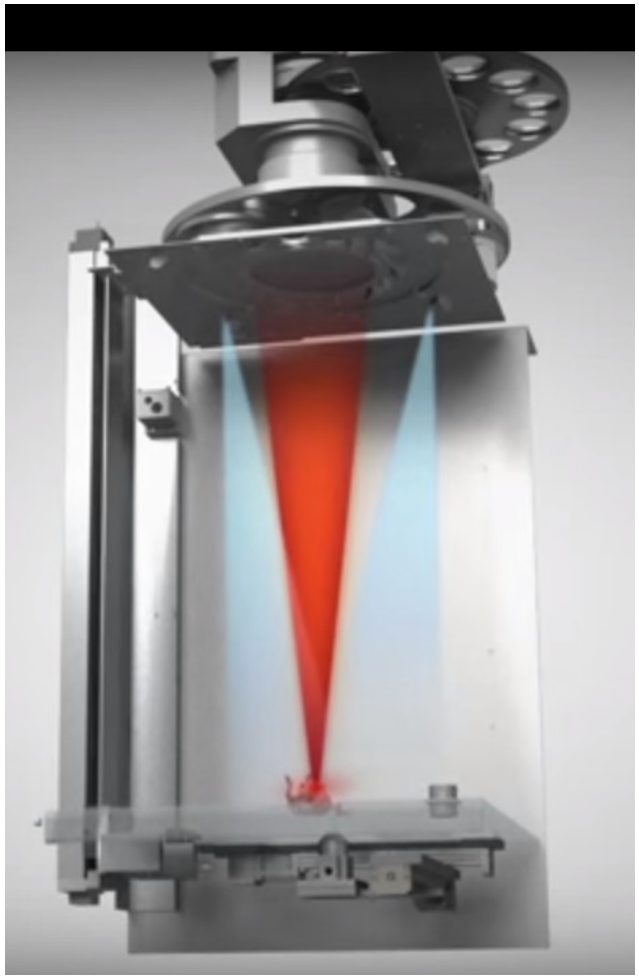
Sara Eliáš

In vivo fluorescence

- Využívá sensitivní kamery
- Rozptyl fotonů
- Pozitiva:
  - Práce na makroskopické úrovni → celé tělo malých zvířat
  - Pozorování tkání v neporušeném stavu, za přirozených fyziologických podmínek
  - Nízké fluorescenční pozadí
- Negativa:
  - Vlastní autofluorescence
  - Náročnost na kontrastní látky/zobrazovací sondy

IVIS Spectrum Preclinical In Vivo Imaging System

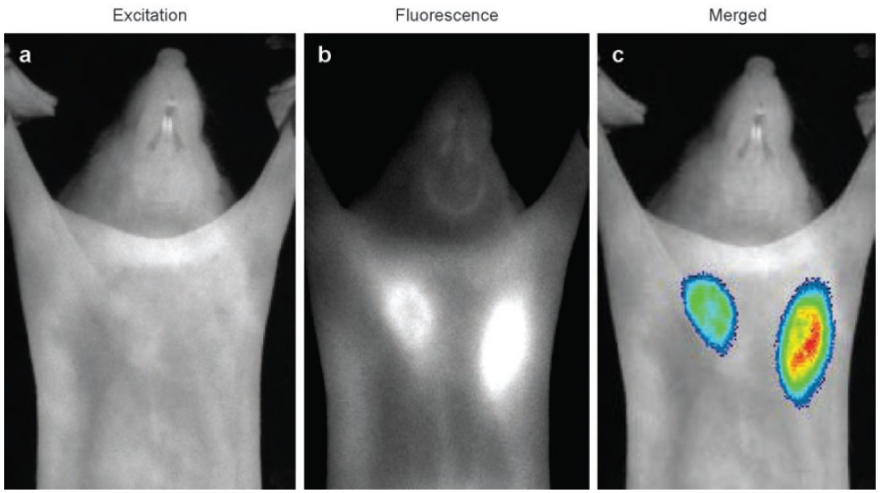
PerkinElmer
For the Better

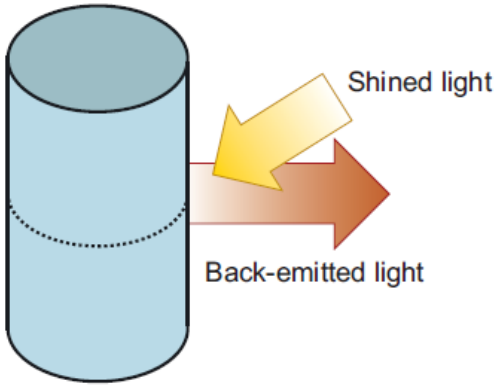


http://www.perkinelmer.com/catalog/product/id/ivisspe
https://www.youtube.com/watch?v=vKMva9XauBA
http://www.perkinelmer.com/

Epi-illumination

- Povrchová, podpovrchová fluorescence
- Světlo neprochází skrz
- Lineární závislost na koncentraci fluorochromu
- Nelineární závislost na hloubce a optických vlastností okolní tkáně

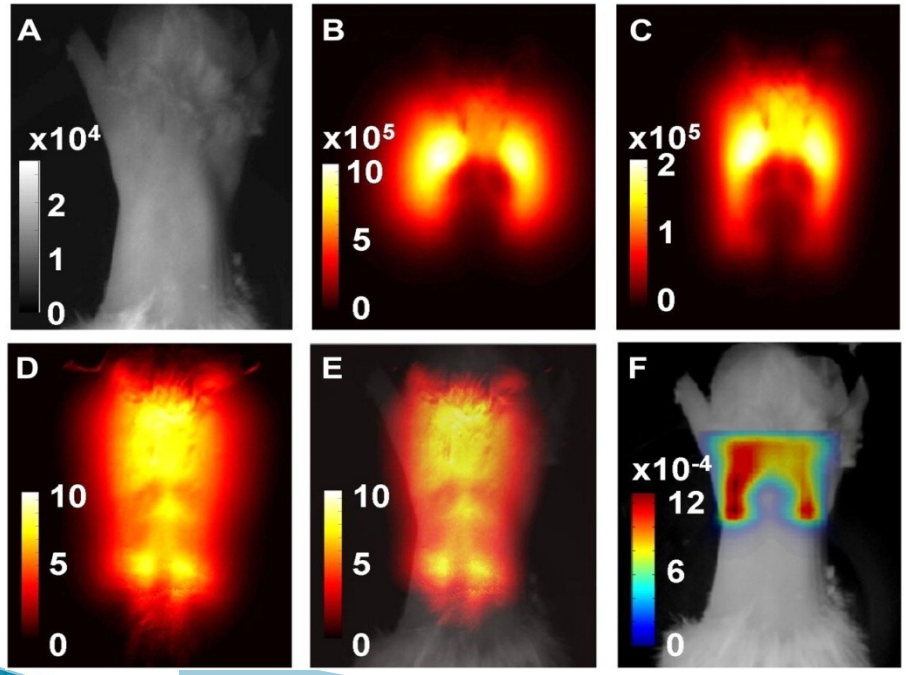




Ntziachristos V., Annu. Rev. Biomed. Eng., 8:1-33

Transillumination

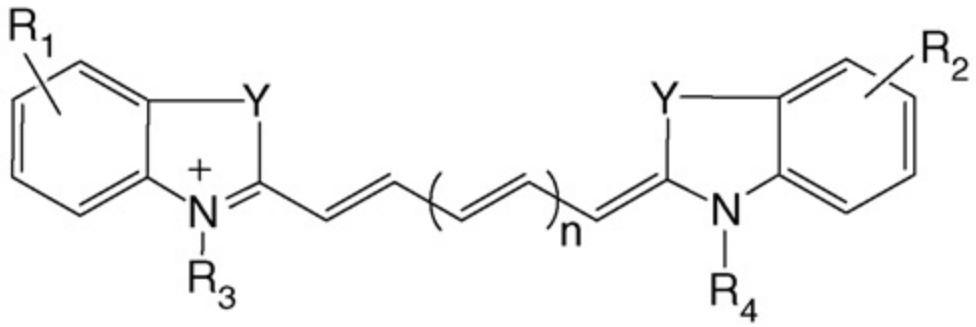
- Světlo svítí skrz tkáň
- Podobné nelineární závislost jako u epi – illumination
- Výsledný obraz je normalizován



Haller J., et all., 2008, Journal of Applied Physiology

Zobrazovací sondy

- Molekuly absorbující v blízké infračervené oblasti (700 – 1000 nm)
- Polymethiny (aj. polymethines) – pentamethine, heptamethine cyanines



Rao J. et al., 2007, Current Opinion in Biotechnology, 18:17–25

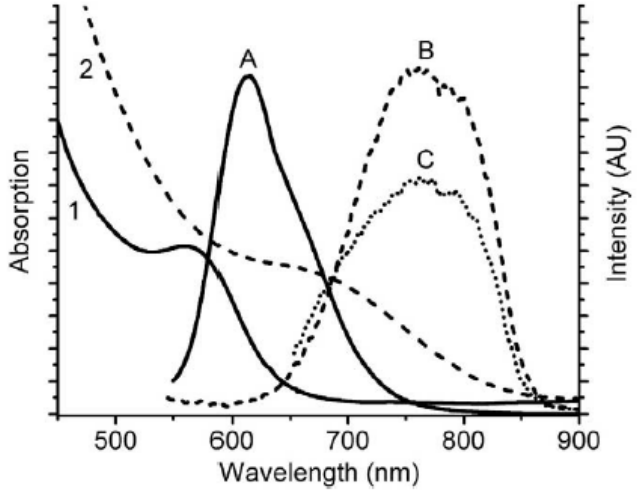
Necílené sondy

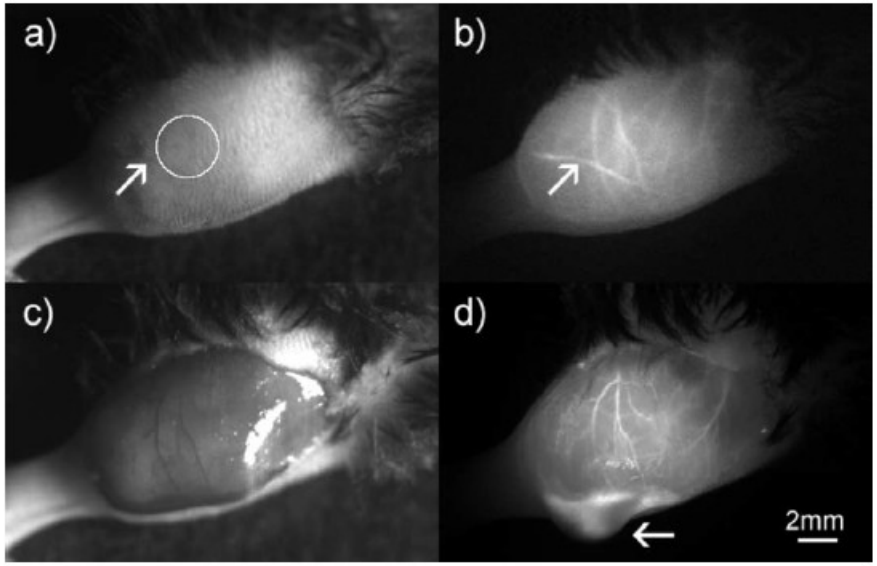
Real Time In Vivo Non-invasive Optical Imaging Using Near-infrared Fluorescent Quantum Dots[1]

Nicole Y. Morgan, PhD, Sean English, BA, Wei Chen, PhD, Victor Chernomordik, PhD, Angelo Russo, MD, PhD, Paul D. Smith, PhD, and Amir Gandjbakhche, PhD

Rationale and Objective. Deep-tissue optical imaging is of particular interest, as the equipment costs are lower than for competing technologies such as MRI. For this purpose, the development of novel contrast agents with near-infrared (NIR) fluorescence is especially important. We report on the use of NIR semiconductor nanocrystals in deep-tissue in vivo optical imaging.

Materials and Methods. Semiconductor nanocrystals of CdMnTe/Hg were grown in aqueous solution and then coated with bovine serum albumin (BSA). The nanocrystals were approximately 5 nm in diameter and have a broad fluorescence peak in the NIR (770 nm). Nanocrystals were injected either subcutaneously or intravenously into athymic NCR NU/NU and C3H/HENCR MTV mice and then excited with a spatially broad 633 nm source; the resulting fluorescence was captured with a sensitive CCD camera.



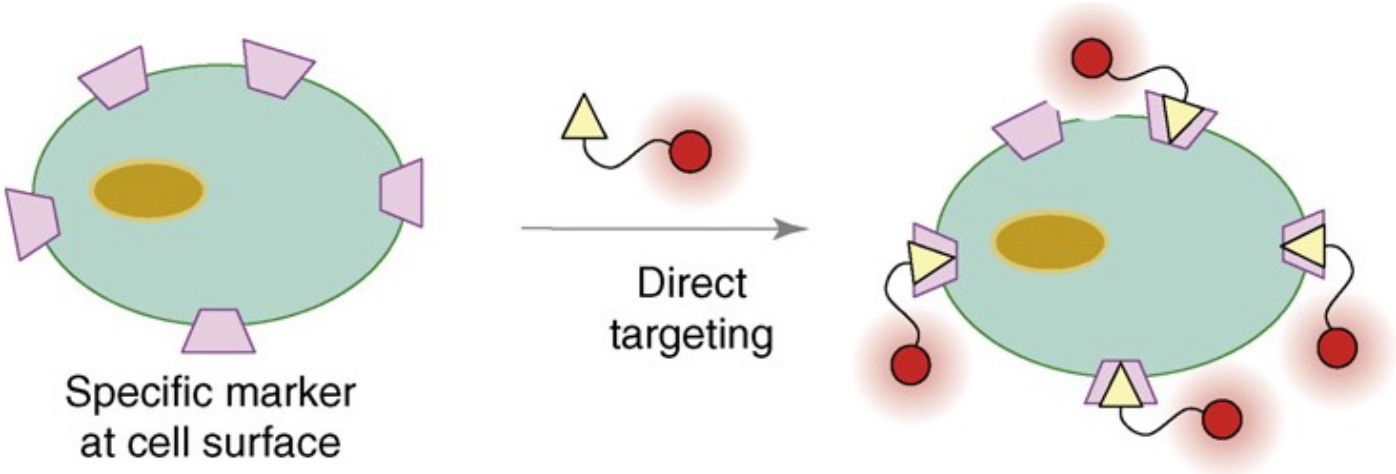


CdMnTeHg/BSA

Morgan N. Y., et all., 2005, Acad Radiol, 2:313–323.

Aktivované sondy

- Váže se na konkrétní cíl
- Složeny ze dvou částí – fluorochromu a ligandu
- Ligand – malé molekuly, proteiny, peptidy, protilátky



Rao J. et al., 2007, Current Opinion in Biotechnology, 18:17–25

Aktivované sondy

In vivo tumor imaging in mice with near-infrared labeled endostatin

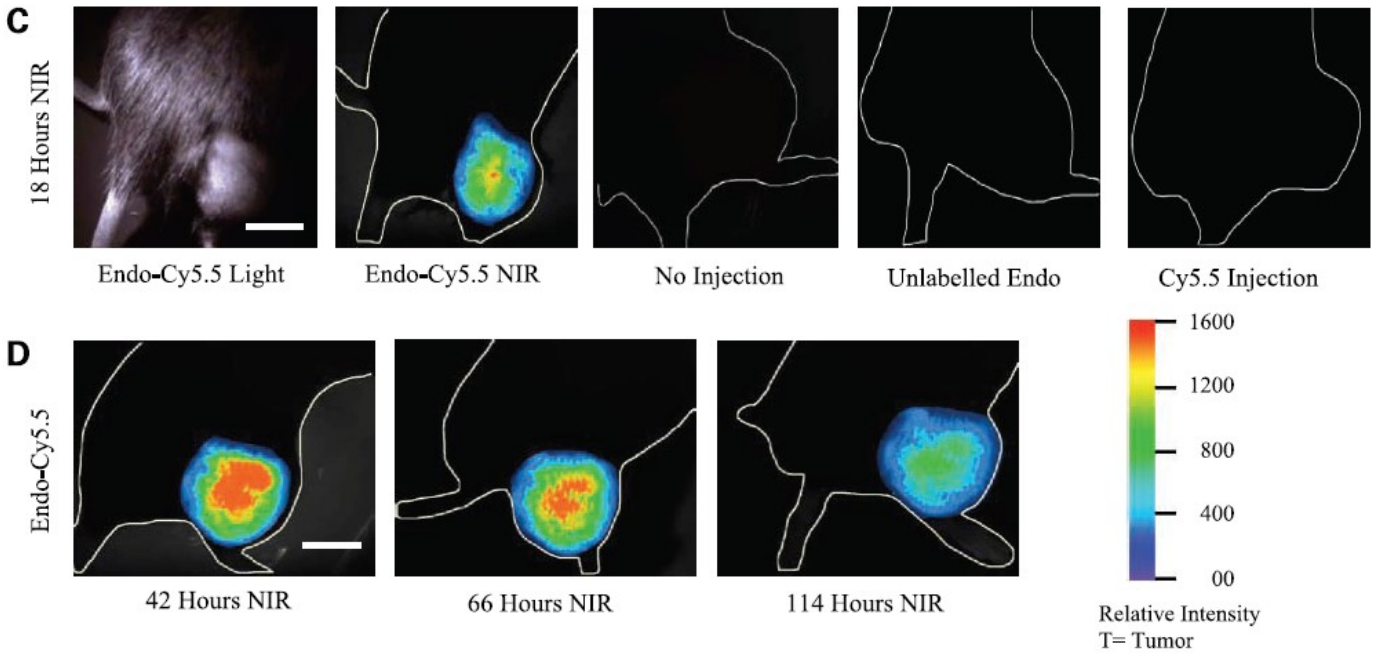
Deborah Citrin,[1] Andrew K. Lee,[4] Tamalee Scott,[1] Mary Sproull,[1] Cynthia Ménard,[1] Philip J. Tofilon,[3] and Kevin Camphausen[1,2]

[1]Imaging and Molecular Therapeutics Section, Radiation Oncology Branch, [2]Vascular Biology Faculty, and [3]Molecular Radiation Therapeutics Branch, National Cancer Institute, Bethesda, MD and [4]Department of Radiation Oncology, University of Texas MD Anderson Cancer Center, Houston, TX

Introduction

The development of novel cancer treatment strategies is critically dependent on the use of tumors grown in experimental animal models, usually rodents. The inhibition of tumor growth is often an essential parameter in predicting the clinical potential of a given treatment strategy and thus its further development. However, evaluating tumor treatment response typically relies on the gross examination of tumor size through physical examination. This approach severely limits the use of clinically relevant orthotopic tumor models

endostatin-Cy5.5



Citrin D., et all., 2004, Molecular Cancer Therapeutics, 3(4):481-8.

Fluorescentní proteiny v blízké infračervené oblasti

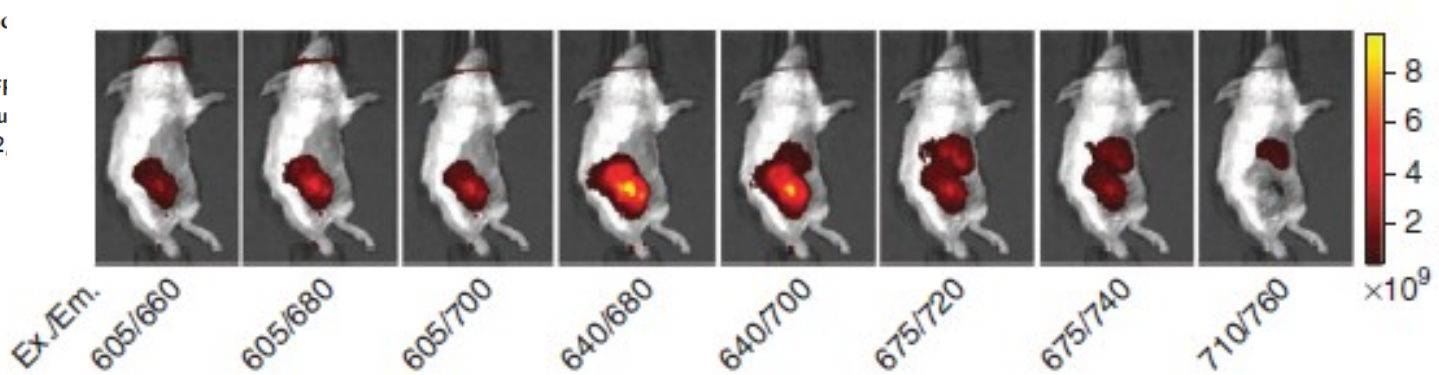
- Transparentnost savčích tkání je dána hemoglobinem a melaninem (absorpce v 650 nm) a vodou (absorpce 900 nm)
- Vyšší poměr signálu k pozadí → vyšší rozlišení

Near-infrared fluorescent proteins for multicolor *in vivo* imaging

Daria M Shcherbakova[1,2] & Vlad

Near-infrared fluorescent proteins (FP
for *in vivo* imaging. We developed fou
near-infrared FPs—iRFP670, iRFP682,

iRFP670 (dole v levo)
iRFP713 (noho v pravo)



Shcherbakova D. M., et all., 2013, Nature Methods 10, 751–754.

Zdroje

- **Rao J.** et al., 2007, Fluorescence imaging in vivo: recent advances, Current Opinion in Biotechnology, 18:17–25
- **Haller J.,** et all., 2008, Visualization of pulmonary inflammation using noninvasive fluorescence molecular imagine, Journal of Applied Physiology, 795–802.
- **Morgan N. Y.,** et all., 2005, Real Time In Vivo Non-invasive Optical Imaging Using Near-infrared Fluorescent Quantum Dots, Acad Radiol, 2:313–323.
- **Citrin D.,** et all., 2004, In vivo tumor imaging in mice with near-infrared labeled endostatin, Molecular Cancer Therapeutics, 3(4):481–8.
- **Ntziachristos V.,** 2006, Fluorescence Molecular Imaging, Annu. Rev. Biomed. Eng., 8:1–33
- **Shcherbakova D. M.,** et all., 2013, Near-infrared fluorescent proteins for multicolor in vivo imaging, Nature Methods 10, 751–754.
- http://blog.addgene.org/in-living-color-the-skinny-on-in-vivo-imaging-tools
- http://www.perkinelmer.com/catalog/product/id/ivisspe
- https://www.youtube.com/watch?v=vKMva9XauBA

Zdroje obrázků

- **Haller J.,** et all., 2008, Visualization of pulmonary inflammation using noninvasive fluorescence molecular imagine, Journal of Applied Physiology, 795-802.
- **Morgan N. Y.,** et all., 2005, Real Time In Vivo Non-invasive Optical Imaging Using Near-infrared Fluorescent Quantum Dots, Acad Radiol, 2:313-323.
- **Citrin D.,** et all., 2004, In vivo tumor imaging in mice with near-infrared labeled endostatin, Molecular Cancer Therapeutics, 3(4):481-8.
- **Shcherbakova D. M.,** et all., 2013, Near-infrared fluorescent proteins for multicolor in vivo imaging, Nature Methods 10, 751-754.
- **Ntziachristos V.,** 2006, Fluorescence Molecular Imaging, Annu. Rev. Biomed. Eng., 8:1-33
- http://www.perkinelmer.com/catalog/product/id/ivisspe
- https://www.youtube.com/watch?v=vKMva9XauBA
- http://www.perkinelmer.com/

Děkuji za pozornost

Sara Eliáš
409310@mail.muni.cz