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Cousins at work: How combining medical with optical imaging enhances *in vivo* cell tracking

Alessia Volpe, Ewelina Kurtys, Gilbert O. Fruhwirth*

Department of Imaging Chemistry and Biology, School of Biomedical Engineering and Imaging Sciences, King's College London, SE1 7EH, London, UK

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ABSTRACT

Microscopy and medical imaging are related in their exploitation of electromagnetic waves, but were developed to satisfy differing needs, namely to observe small objects or to look inside subjects/objects, respectively. Together, these techniques can help elucidate complex biological processes and better understand health and disease. A current major challenge is to delineate mechanisms governing cell migration and tissue invasion in organismal development, the immune system and in human diseases such as cancer where the spatiotemporal tracking of small cell numbers in live animal models is extremely challenging.

Multi-modal multi-scale *in vivo* cell tracking integrates medical and optical imaging. Fuelled by basic research in cancer biology and cell-based therapeutics, it has been enabled by technological advances providing enhanced resolution, sensitivity and multiplexing capabilities. Here, we review which imaging modalities have been successfully used for *in vivo* cell tracking and how this challenging task has benefitted from combining macroscopic with microscopic techniques.

1. Introduction

Two major discoveries, one enabling observation of smaller objects and the other allowing to look inside subjects/objects, significantly boosted biological/biomedical research. The first compound microscope was invented by Hans and Zaccharias Jansen in the late 16th century, which triggered later microscopy development that in turn enabled the direct observation of atoms, single molecules and single-/multi-cellular organisms including their dynamics. The second transformation was Wilhelm Roentgen's discovery of X-rays in 1895, which enabled investigations of inner subject/object structures in a non-invasive way (genetic effects of radiation were only recognized later) and founded medical imaging. Both microscopy and medical imaging rely on the interaction of biological matter with electromagnetic waves, but medical imaging employs a wider range than microscopy including α/β/γ-ray-emitting radioisotopes, X-rays, visible (VIS)/near-infrared (NIR) light, radio waves and ultrasound (Fig. 1). Medical imaging revolutionized the diagnosis and treatment of human disease by providing anatomical, physiological and molecular information (Mankoff, 2007). Imaging modalities differ in their capabilities and limitations (Fig. 1), hence combination technologies were introduced to exploit them best ('multi-modal imaging'). For example, positron emission tomography (PET) offers best-in-class sensitivity and absolute quantification but only at millimetre resolution and was combined with modalities providing higher resolution such as computed tomography (CT) (Basu et al., 2014) or magnetic resonance imaging (MRI) (Catana, 2017). How medical imaging can be used to develop biomarkers providing diagnostic, prognostic, predictive, and treatment monitoring information was recently standardized (O'Connor et al., 2017). Photoacoustic tomography (PAT) and Cerenkov luminescence imaging (CLI) are special in that they both rely on electromagnetic waves from different parts of the spectrum for imaging. PAT delivers NIR laser pulses into biological tissues with the latter absorbing and converting some of the laser pulse energy into heat, leading to transient thermoelastic

Abbreviations: BLI, bioluminescence imaging; CEST, chemical exchange saturation transfer; CLI, Cerenkov luminescence imaging; CM, confocal fluorescence microscopy; CT, X-ray computed tomography; FLI/FRI, fluorescence imaging/fluorescence reflectance imaging; FMT, fluorescence mediated tomography; HF, high-frequency; IVM, intravital microscopy; MRI, magnetic resonance imaging; MSOT, multispectral optoacoustic tomography; NIR, near-infrared spectrum; OCT, optical coherence tomography; OPT, optical projection tomography; PAT, photoacoustic tomography; RSOM, high-resolution raster scanning optoacoustic mesoscopy; PET, positron emission tomography; SPECT, single photon computed emission tomography; SRM, super-resolution microscopy (a group of various technologies including but not limited to photoactivated localisation microscopy (PALM), various stochastic optical reconstruction microscopy (STORM) techniques, stimulated emission depletion microscopy (STED), and ground state depletion individual molecule return (GSDIM)); TPM, tow-photon excitation microscopy; US, ultrasound imaging including Doppler and high-frequency ultrasound techniques; VIS, visible light spectrum

* Corresponding author at: Department of Imaging Chemistry and Biology, School of Biomedical Engineering and Imaging Sciences, King's College London, St. Thomas' Hospital, Lambeth Wing 4th floor, SE1 7EH, London, UK.

E-mail address: gilbert.fruhwirth@kcl.ac.uk (G.O. Fruhwirth).

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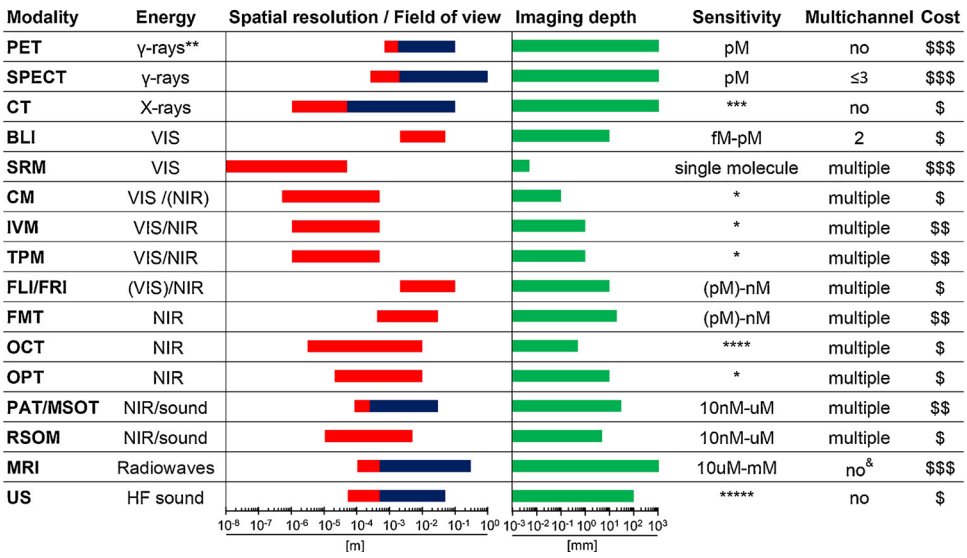

Fig. 1. Macroscopic and microscopic imaging modalities. Imaging modalities are ordered according to the electromagnetic spectrum they exploit for imaging (top: high energy; bottom: low energy). Routinely achievable spatial resolution (left end) and fields of view (right end) are shown in red. Where bars are blue they overlap red bars and indicate the same parameters but achievable with instruments used routinely in the clinic. Imaging depth is shown in green alongside sensitivity ranges. Instrument cost estimations are classified as ($) < 125,000 $, ($$) 125–300,000 $ and ($$$) > 300,000 $. * Fluorophore detection can suffer from photobleaching by excitation light. ** Generated by positron annihilation (511 keV). *** Contrast agents sometimes used to obtain different anatomical/functional information. **** In 'emission mode' comparable to other fluorescence modalities (~nM). ***** Highly dependent on contrast agent. & Multichannel MRI imaging has been shown to be feasible (Zabow et al., 2008). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

expansion and thus wideband ultrasonic emission (Ntziachristos et al., 2005; Wang and Yao, 2016). CLI relies on the collection of light produced by charged particles traversing through biological tissue with a velocity greater than the phase velocity of light in that medium (Ciarrocchi and Belcari, 2017). Brightfield microscopy and, less frequently, fluorescence microscopy are routine techniques providing confirmatory pathology information obtained from biopsied tissues. Recently, automated multiplex fluorescence histopathology (Mansfield et al., 2008; Stack et al., 2014) has enabled rigorous tissue profiling, *e.g.* immune infiltration in tumour tissues (Galon et al., 2014).

Here, we review which imaging modalities have been successfully used for *in vivo* cell tracking and how this challenging task benefitted from combining macroscopic with microscopic techniques. For detailed information on the instrumentation of individual imaging technologies and their use, we provide references to recent specialist literature.

2. The need for *in vivo* cell tracking in cancer research

A major challenge in cancer research is to better understand the mechanisms governing cell migration and tissue invasion. A plethora of different models including animal tumour models are used for this purpose. It remains extremely challenging to reliably quantify the *in vivo* distribution, relocalisation, and viability of cancer cells in animal tumour models, which are sufficiently large to be optically opaque. For example, the spatiotemporal quantification of cancer cell spread in mouse models of metastasis is a needle-in-a-haystack task. Traditionally, in preclinical cancer research one target organ of metastasis was chosen, large animal cohorts were sacrificed at different time points to overcome inter-animal variability and these approaches were paired with microscopic or flow cytometric analyses in target tissues as read-outs. Whole-body imaging can (i) inform on *in vivo* cell distribution, for example, visualize unexpected metastatic sites; (ii) provide quantitative data, *e.g.* live tumour volumes/metastatic burden and extent of cell therapy on-site residence over time; (iii) provide cell viability data; (iv) reduce inter-subject variability as serial imaging of the same subjects provides statistically better paired data; and (v) can minimize animal usage during preclinical development. Similarly, when developing anti-cancer drugs, it is important to establish targeting efficiency, pharmacokinetics and pharmacodynamics, whether there is spatial heterogeneity to the delivery, and if drug presence is related to therapeutic efficacy. Again, this can be achieved by combining preclinical whole-body cancer cell tracking with conventional molecular imaging of drugs, for example, by image-based quantification of the extent a labelled drug reaches *in vivo* traceable cancer cells and whether the drug is delivered to all primary/secondary lesions.

Another area where *in vivo* cell tracking is an emerging valuable tool is the development and clinical translation of cell-based therapies. Unlike conventional chemotherapeutics or targeted therapies, they cannot be considered as 'fire-and-forget' weapons in the battle against cancer as they are live cell products, but little is known about their *in vivo* distribution and fate both preclinically and clinically. In 2017, the FDA approved the first clinical products, tisagenlecleucel and axicabtagene ciloleucel, which are autologous CD19b-targeted chimeric antigen receptor T-cell (CAR-T) immunotherapies for the treatment of certain blood cancers (B-cell lymphomas; (USFood&DrugAdministration, 2017a,b)). CAR-T immunotherapies have the potential to be curative, but not all patients respond and sometimes the effects are only temporary (Maude et al., 2018; Neelapu et al., 2017; Schuster et al., 2017). CAR-T are also associated with severe/life-threatening side-effects and fatalities during trials (Linette et al., 2013; Saudemont et al., 2018). Moreover, cellular immunotherapeutics for treating solid tumours are at the clinical trial stages but not yet routinely available to patients. Traditional approaches in preclinical cell therapy development rely on dose escalation with toxicity evaluation, tumorigenicity tests, and qPCR-based persistence determination. However, clinical trials are still performed without knowledge about the *in vivo* distribution and fate of the administered therapeutic cells, making it impossible to adequately monitor and assess their safety. Major unresolved questions in cell therapy development and use both preclinically and clinically are: (i) the whole-body distribution of therapeutic cells; (ii) their potential for re-location during treatment and the kinetics of this process; (iii) whether on-target off-site toxicities occur; (iv) how long the administered cells survive; and (v) which biomarkers are best suited to predict and monitor cell therapy efficacy. Whole-body imaging-based *in vivo* cell tracking can inform on many of these aspects in a truly non-invasive manner.

3. Rendering cells traceable *in vivo*

In vivo cell tracking exploits molecular imaging mechanisms but differs from conventional molecular imaging as contrast agents or contrast-forming features are added to the cells before their administration into subjects. On some occasions, features that can be exploited for generating contrast are intrinsic, for example, when cancer cells