Moving towards personalized medicine with fluorescence imaging

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The successful demonstration of near-IR contrast agents brings non-nuclear diagnosis closer to clinical use.

One of the promises of personalized medicine is the use of highly specific molecular therapies tailored to the genetic and molecular basis of an individual’s disease. While many conditions are diagnosed by a tissue biopsy, this is not always possible, and disease markers can vary over time or between biopsy locations, as is often the case for cancer.

Compared to invasive biopsies, molecular imaging involves the non-invasive administration of a contrast agent that targets specific disease markers in all tissues in order to track their expression during disease progression and therapy. Because disease markers can be seen below picomolar tissue concentrations, extremely sensitive imaging techniques are required for personalized diagnoses.

Current nuclear-imaging techniques, such as gamma imaging, single-photon-emission computed tomography and positron-emission tomography, provide the sensitivity necessary for such molecular imaging diagnoses. Imaging depends upon labeling a targeting moiety (a molecule or part of such as an antibody or peptide) with a radionuclide which, upon decay, results in a high-energy photon emission that travels through tissues with minimal scatter. Each radionuclide relaxes only once, causing one photon event that is collected to generate an image that occurs over several minutes.

However, radionuclides have finite half-lives of only a few hours. In addition, these nuclear contrast agents require preparation immediately before administration, and they need a pharmacokinetic design that enables clearance from non-diseased tissues for maximum image contrast and optimal disease-marker detection.

Figure 1. Non-invasive NIR fluorescent images of human lymph nodes following micro dose administrations of ICG. Dotted yellow lines indicate body outline. (a) axillary. (b) cubital orbital. (c) popliteal. (d) inguinal.

While not used clinically at present, near-IR (NIR) fluorescence can provide the sensitivity required for diagnostic molecular imaging, but without some of the problems associated with nuclear contrast agents. The approach consists of administering a targeting moiety labeled with a NIR (>750nm) excitable fluorophore, illuminating tissue surfaces with dim NIR light, and then collecting the remitted fluorescence for

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imaging. Because the fluorophore is repeatedly activated by tissue-penetrating NIR light, as many as $10^8$ photons per fluorophore per second can be generated.

NIR fluorophores are not radioactive and have no finite half-life, thereby reducing the limitations of agent storage, preparation, and pharmacokinetic design. As a result, their use opens new opportunities for image-guided molecular surgery. In addition, because there is no fluorescence within the body at NIR wavelengths, there is no background signal noise, resulting in high sensitivity and image contrast.

However, in comparison to the nuclear techniques, NIR photons are low-energy, and they are more easily absorbed and scattered by tissues, which complicates imaging and diagnosis. Nonetheless, the large photon budget available for collection justifies the development of new imaging devices, contrast agents, and advanced algorithms to evaluate tomography.

Our interdisciplinary research team has successfully demonstrated the sensitivity of NIR-fluorescence imaging in humans by using micro doses (<100µg) of indocyanine green (ICG) off-label. ICG is approved for intravenous administration (up to 25mg) for hepatic clearance and ophthalmologic applications, and while not used for its fluorescent properties, ICG is dimly fluorescent when excited at NIR wavelengths. Although ICG also does not have a functional group for conjugation to targeting moieties, it does provide non-specific molecular contrast for evaluation purposes.

We are designing these agents for non-invasive investigation of the nodal stages associated with breast, prostate, bladder, and melanoma cancers. Figure 1 shows that using as little as 100µg of ICG administered intradermally, axillary lymph nodes (LNs), the cubital orbital LN, the poplital LN, and the inguinal LNs can be readily imaged with our NIR-imaging device. We have also found that as little as 10µg of ICG enabled non-invasive detection of the lymphatic vessels and axillary lymphatic basins in breast cancer patients. In these individuals, excised LNs were found to be fluorescent, demonstrating the successful image-guided removal of cancer positive LNs.

Due to the high photon count rate, we were able to collect fluorescent signals from our imaging devices between 100 to 400ms to create ‘movies’ that showed rapid and dynamic lymphatic trafficking through lymphatic vessels, never before non-invasively visualized in humans.

These results provide the first glimpses of lymphatic function in humans. The lymphatic system represents an understudied, but important component of the circulatory system that is implicated in a number of human diseases, which can now be studied using dynamic NIR-fluorescence imaging.

Our work using micro doses of ICG not only demonstrates feasibility, but also suggests that low amounts of contrast agent are required which can help to minimize the risk of adverse events. Moreover, while our early studies did not allow us to investigate penetration depth, the surgical removal of fluorescent LNs from breast cancer subjects suggest that tissue depths of 3 to 5cm may be possible. We are now seeking to enhance tissue penetration by using brighter fluorophores and integrating measurements over longer times (>400ms).

Using the unprecedented photon count rates that enable successful planar imaging in humans, we are now integrating time-dependent measurements for 3D tomographic reconstructions to assess cancerous LNs. Furthermore, our emerging contrast agents are dual-labeled for nuclear imaging to enable validation against conventional imaging approaches, an advantage that promises to advance personalized medicine.

The work described is made possible by the generous support of the Wilson Foundation, the Longaberger Foundation through the American Cancer Society (RSG-06-213-01-LR), and the NHLBI (R01 HL092923).

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References