Dr Eva M Sevick, Professor and Cullen Chair of Molecular Medicine at UTHSC’s Brown Foundation Institute of Molecular Medicine, explains her work on near-infrared imaging and lymphedema

Can you describe some of the imaging devices that you have designed to overcome the lack of naturally-occurring molecules that can be excited in the NIR range? What advantages do these devices afford?

Firstly, this is a combinational drug and device product, so optimal imaging is achieved by both the device and the drug. The first step in overcoming fluorescence from naturally-occurring substances is to illuminate tissues using well-defined light sources with wavelengths above 780 nm, which produces virtually no fluorescence from naturally-occurring fluorophores. Secondly, we need to very efficiently collect the weak fluorescence generated, using the night-vision goggle technology (ie. NIR sensitive image intensifiers). Coupled with efficient optics, this enables us to collect the weak signal and reject all other light.

What are the limitations of the indocyanine green (ICG) dye that has been utilised to date? Are there any other dyes that would afford similarly advantageous qualities?

Because these devices are so sensitive to NIR fluorescence, we are able to use a very poor NIR dye – indocyanine green (ICG). ICG is a dark green-coloured dye which has been used safely in humans for the past 50 years for hepatic clearance and ophthalmic applications, and it has worked well with our highly-sensitive instrumentation. However, ICG is a very dim and unstable NIRF dye which has no functional group for conjugation to attach targeting imaging agents to.

There are several emerging NIRF dyes which are brighter and more stable. Recently we have developed new agents that fluoresce brightly in the near-infrared wavelength region, promising even better lymphatic imaging. Yet more agents promise to detect cancer cells in lymph node basins drained by tumour lymphatics.

What are the medical upshots of chemically-conjugating NIRF dyes to target peptides, antibodies and sugars?

At present, only the nuclear imaging techniques (ie. those that employ a radioactive label) are clinically-suitable for molecular imaging. By conjugating a NIRF dye onto targeting moieties, we can perform clinical molecular imaging without radioactivity, and much more easily than using radioactive agents. Since radio isotopes have half-lives, it is truly phenomenal but difficult work to design agents for nuclear imaging that have the correct pharmacokinetics and target-to-background ratio within the timeframe of the radio isotope half-life. In addition, chemical preparation of these agents must be done immediately prior to administration. NIRF dyes have no half-life and therefore enable medicinal chemists to relax the pharmacokinetic requirements in designing new agents. The development of NIRF imaging will enable clinicians to implement a simple reconstitution of a molecular imaging agent prior to administration, without the need for chemical conjugation and purification in a radiochemistry facility. The upshot is more efficient, economical clinical use and enhanced opportunities to develop diagnostic imaging approaches impacting a greater number of patients.

What are the potential applications of this technology for those at risk of developing lymphatic diseases, such as cancer patients? Could this technique provide better screening opportunities?

In our studies of cancer patients, we have observed striking lymphatic abnormalities before clinical symptoms of lymphedema occur. This is important, because it is thought that early treatment can ameliorate or even prevent the disease. If we can detect the
Could mean for long-term recovery? Can you elaborate on your work to develop treatments for those who are diagnosed with the disease. Can you elaborate on your work to develop highly specific NIR imaging agents for intraoperative guidance to remove only cancer-positive lymph nodes and what this could mean for long-term recovery? Because we are able to see lymph nodes non-invasively using the NIRF and the dim ICG, we believe we can conjugate a brighter NIRF dye onto a molecule that targets cancer cells and use this to image only cancer-positive lymph nodes. So instead of removing all the draining lymph nodes, surgeons could remove only those that are cancer-positive. With the advent of better therapeutics, one could image whether malignant lymph nodes respond to systemic drug treatments simply by imaging their cancer status. This would obviate the need to remove the nodes, and possibly ameliorate lymphedema.

Considering the prevalence of lymphedema in developing nations, have you actively tried to reduce costs so that these imaging devices may be used in poorer nations where they are sorely needed?

Yes. Our device is currently an ‘at point of care’ technology and therefore amendable for use in developing nations. We have recently begun a commercialisation effort that is economising the manufacture of the systems.
The method can be used to display images immediately at point of care within the clinic, so patients and clinicians can see the lymphatics directly, before, during, and after treatment. The technique has also been used to detect improved lymphatic pumping action after massage therapy. Previously, the stimulation of pumping action had been a hypothesised but unconfirmed therapeutic mechanism of manual lymphatic drainage techniques used to treat lymphedema subjects; near-infrared imaging shows that manual lymphatic drainage does indeed stimulate lymphatic pumping in some subjects.

**Better in 3D**

The team’s mathematical models use measurements of fluorescence and light excitation conducted at the tissue surface to reconstruct three-dimensional images of deep fluorescent tissues, and it is currently working to validate the NIRF tomography by labelling targeting agents with both a radioactive and NIRF dye. A collaboration with Siemens Medical Systems has resulted in the incorporation of the minimised NIRF system into the gantry of a small animal hybrid PET/CT system, and it is now imaging animals and phantoms. The imaging agents being dual-labelled should enable NIRF tomography to be displayed along with PET images, facilitating a comparison of the accuracy of NIRF relative to the clinical standard of PET.

The researchers are currently using ICG in FDA-approved investigational studies and Sevick hopes it will be soon approved for NIRF lymphatic imaging. “Because our imaging devices enable us to use microdose administrations of ICG, a very dim NIRF dye, we believe that new ‘first-in-humans’ imaging agents will likewise be enabled to be given in microdose administrations,” she explains. These new agents would require a comparable or smaller dose – less than 100 micrograms – and the imaging studies suggest they will enable detection of deeper structures within the tissues, as well as faster image acquisition than the existing rate of 200 milliseconds.

This coupling assists in translation, providing a means by which researchers can compare optical imaging results, and as each of the centres translates its optical technologies, it is developing consensus tools that others will use. The result is a set of tools and recommendations developed in the process of translation which will help other researchers accelerate the implementation of these technologies in standard-of-care practices. NIRF lymphatic imaging is showing significant clinical utility in diagnosing lymphedema, and directing and evaluating treatment. The initial stages of commercialisation are underway so that systems can be made available nationwide as well as internationally.

**Future Applications**

Since lymphatics play a critical role in the function of vascular circulatory system, NIRF lymphatic imaging may shed light on some blood vascular disorders. Sevick’s team is working with Dr Philip King at the University of Michigan, who has developed an animal model of a human genetic condition known in humans to impact the blood vasculature, but in animals has been shown to impact dramatically the lymphatic vasculature. This suggests there may be exciting new applications of NIRF lymphatic imaging which, in tandem with the genetic research into primary lymphedema also being conducted by Sevick and her collaborators, are for the first time in almost a century creating the exciting possibility of significantly raising the bar in the standard of care for lymphedema sufferers.