RALPH WEISSLEDER: So good morning and welcome to the second of the overview talks here. This one will focus on imaging technologies from the mouse up to humans.

My apologies to the imaging folks in the audience. This will be rather more basic and didactic. So you can tune off, go to your iPhones. I won't be offended.

I would like to thank Mark and Gordana for organizing this meeting and bringing these apparently disparate communities together all in the hopes that something good will come out of this.

So what I decided to do over the next 40 minutes or so is cover four different topics. Number one is I will review some of the underlying imaging or some of the principles underlying different imaging modalities, then spend about 20 minutes talking about current state-of-the-art capabilities of cellular resolution or microscopic imaging, then talk about macroscopic imaging at the tissue and organ level and finish up with some thoughts on our needs, both from an imaging perspective and from a biology perspective.

So let’s start with the overview. So as you all know, all imagining technologies use energy in forms of waves at different wavelengths to interrogate tissues, cells, humans, experimental animals, and these range in frequencies from the megahertz, gigahertz, terahertz, petahertz, edahertz [phonetic], all the way up to zettahertz.

So, for example, down here, the megahertz level we have MRI. We have optical-imaging technologies here, and the higher energy X-rays and gamma-ray imaging technologies over here.

Now, all of these, because we use energy that we pump into our tissue, have biological effects, either inducing currents, heating up our tissue, exciting molecules, doing some sort of photo chemistry, all the way to DNA damage.

Now, the amount of these biological tissues obviously depends on how much energy we put in. At the diagnostic end, we put in very little energy. At the therapeutic end, for example, in radio-frequency oblation, in radiation therapy, we put in much more energy.

But the point I'd like to make is that this is a sliding scale, and it's important to remember because some of the issues is if we image even with light something rather innocuous, you know, with a lot of energy, we can induce damages in cells.

So the other point I want to make is that imaging is a continuum. Imaging is different things to different people, but there are common principles all the way from single-molecule imaging, cellular imaging, tissue imaging, medical imaging all the way up to astronomy.

And the principles being that we need two things. We need a detector and we need a source of contrast, and the source of contrast can be fluorescent antibodies, stains, contrast agents, natural contrast and so on.

The other point I'd like to make is that imaging has played an unbelievably important and revolutionary role in understanding biology, in understanding space, in understanding many, many things that we take for granted today.

So we spoke about three things on how to break down the imaging space, the energy wavelengths, use radiation effects and resolution.

There are other ways of breaking down the imaging space. One is by information content. It was already mentioned. One can image at the molecular level, at the cellular level, at the physiologic level, at the anatomic level, at all of them combined and so on.

By the probes used one can use imaging technologies that are label free, use genetic reporters' injectable imaging agents, by dimensions, line scans, two-dimensional pictures, three-dimensional tomographies, longitudinal imaging. And then there's 5-D imaging, 6-D imaging and so on or the application, clinical versus just experimental imaging. So these are some of the things that one needs to think about before actually choosing an imaging modality.

So, as I said before, imaging has undergone tremendous changes over the last 20 or 30 years. Most of the clinical-imaging technology that we use in hospitals today, be it X-ray, CT, be it ultrasound, be it MRI or be it PET imaging or other nuclear-imaging technologies, while they have been developed, two, three, four, five decades ago, or even a century ago with X-ray, each of them have undergone tremendous changes over the last 20 years.

For example, for PET imaging, the initial acquisition of Planar image, all the way to fusion of PET MRI, MRI just from plain T1, T2 imaging to diffusion imaging, Connectom imaging and so on, ultrasound from 2-D imaging to 3-D ultrasounds, X-ray and CT to screening. So all of these modalities have undergone incremental changes.

Similar things have also happened in the optical world with microscopic imaging technologies down here, with tomographic imaging modalities and with some reflectance imaging technologies which we will review in more detail in other talks.

So one of the things that one needs to keep in mind is imaging time and dataset size. So as we had heard from Gordana on the wish list is we want to image single-cell resolution in the entire body over time.

Now, unfortunately, that’s not quite possible. And it’s not quite possible because of this here, because in order to do this, we would be imaging for the next several decades. So the imaging time or the time it takes to acquire images is a function of the count rate, how many photons do I get back, how often do I do an experiment, the spatial resolution and the coverage or the scale of how big is my field of view.

So the imaging time, obviously, has to be physiologically compatible.

So the size of the datasets that come out of these imaging studies primarily depends on the spatial resolution and the coverage. So this was recently summarized by one of my colleagues in this article.

Oops. Let me just go back. Sorry.

Just to make a long story short, so chest X-rays typically have megabyte sizes, a typical CT MRI somewhere in the gigabyte-size range. The visible human here is somewhere in the terabyte range or a Connectom, somewhere, a single-brain thing, in the petabyte range. So these are enormous computational and data-storage requirements.

This technology, of course, will advance over time, but it does exist. So we need to be at least cognizant of this.

Which brings me to some thoughts on IT infrastructure underlying all imaging modalities. So data storage is clearly an issue. In our operation at Harvard or MGH where I work, the storage requirement keeps going up.

It used to be gigabytes, then it’s terabytes and we’re soon going to go to petabyte storage.

Beyond that, another issue is how does one disseminate this data because the imaging equipment is frequently now so expensive that no single researcher can afford a machine, and so all of these things or many of these things will be shared; for example, mass spec imaging. So software to disseminate the data is becoming very important.

Tools for quantitative analysis, currently, primarily through GPU performance enhancements, MATLAB imaging and cell profiler are there in rudimentary fashion, but, clearly, there is a need to build on these, particularly for this group.

And, finally, there is quite an opportunity to further improve how we actually visualize the data and display the data. There is some software packages out there that you’re all familiar with -- Osirix, Amira, Emaris and so on -- but there is clearly a need for other and newer ways of displaying this increasingly complex amount of data. And it gets more complex as one tries to integrate data from different imaging modalities.

Anyways, so molecular contrast is another topic that we should briefly touch on. There's two ways of getting to molecular contrast. I'm not talking about tissue contrast. I'm talking about molecular contrast. One is through genetic reporters. The other one is through injectable molecules.

Genetic reporters is primarily limited to experimental applications. So examples are the fluorescent proteins. The limitation here, of course, depth penetration, bleaching, not quite clinical.

The injectable molecules, there are some in the clinic. Of course, their limitations are that they are pharmokinetically dependent and there's only a limited number with limited specificities.

However, this group of agents to interrogate specific molecular targets has expanded over the past, so it now ranges from small molecules, active-site binders, site -- protein tags, environmental probes, all the way to nano materials, some of which have been both developed and tested here at NIST, all with the idea of interrogating biological function.

So let me just switch to the second topic and that is high-resolution imaging, in-vivo imaging to define where we are and what is required to do this.

It seems, in my travels in the imaging world, people are always awed by the ability to watch cells in vivo in orthotopic environments, how they live and how they interact with each other.

So in order to do this type of high-resolution, single-cell resolution in-vivo imaging, one needs three things. One needs microscopes, very good, objective mouse models and support systems to monitor these critters that live under the microscope.

Now, another way of dealing with this is building microscopes, and there is some very interesting work going on in different groups in the country miniaturizing microscopes so they can be implanted into animals. So that's sort of the next frontier.

Beyond these obvious three requirements, there is a number of different things that one needs to consider, and I won't go into details. It has just been published in Cell, and other speakers will talk about this, Charles Lin and so on.

But for in-vivo imaging, the support infrastructure is really, really critical, particularly, I think, one of the biggest impediments to in-vivo imaging at the microscopic resolution is this mouse control in immobilizing the mouse. Just imagine if the mouse breathes ever so slightly, the cell that was just in focus is going to be out of focus. So really critical.

So several years ago, when we did in-vivo imaging, we were very excited that we were, for the first time, able to actually see how host cells and cancer cells were trailing each other and eating each other up and doing nasty things to each other.

And so this whole field has then evolved more recently to much higher spatial-resolution type of imaging where we now not only see individual cells in vivo, but can actually see intracellular detail, such as this dividing cell here, through the use of genetic reporters. And so this is slowly where the whole field is going.

The other advance in the field of intra-vital imaging is the use and development of stick objectives. So these objectives are somewhat reminiscent of rigid endoscopes in that they can be stuck pretty much

anywhere, at least in the mouse -- in orthotopic organs, into the brain, into the lung, onto on the heart. They have a very, very small footprint here, and they result in spectacular or can result in spectacular resolution.

Now, each of these cancer-cell lines here have either a green label, a yellow label or some sort of red label in them.

Another exciting development is to go and be able not only to image one color or one cell type, but multiple cells. So there is this technology out there called Brainbow that was developed by Jeff Lichtman up at Harvard, and, more recently, there is another technology that has been propagated under the name of Lego [phonetic]. They are similar principles in that one uses three different fluorescent proteins and when they combine, one can make basically thousands of colors.

So this is very important for individual cellular labeling, so one can do tracing studies. For example, in the Connectome it’s important, because they want to see how one neuron connects and travels over space.

It’s important for clonal development of cells as they grow in cancer.

So I don’t want to go into too much detail since both of these have been published. This here, the Lego version, works by a vector-mediated introduction of these three different florescent proteins. This here, the original Brainbow method, uses a Cre-Lox approach in making these.

So applications to tissue engineering. Obviously, this has tremendous applications to stem-cell biology, material scaffolds, to very important applications. And this is how we use some of this technology to look at inflammation immunology in the heart, in other form or in other immunologic diseases to look at the efficacy of new therapies. Does my drug hit the target? What is the pharmacokinetics? And, ultimately, to do failure analyses of drugs. When do they fail and when do they actually work?

So let’s move on to macroscopic-type of imaging. So we’re now at the tissue organ type of scale. Again, there are some very, very exciting developments on the horizon. So these technologies, the macroscopic technologies are primarily used to do tissue organ

surveys. They are indispensable in cognitive neuroscience research, in cancer metastasis work, looking at pharmacokinetics, drugs and, as Gordana mentioned, for implantable devices, prosthesis.

They're also used when single-cell resolution is not necessary or not practical, and, of course, the vast majority of clinical-imaging modalities are in this bin, and this bin being MRIs, X-ray computed tomography, PET imaging, ultrasound and, more recently, development of some optical-imaging technologies at the horizon.

So let's start briefly with MRI. You all know the principle behind MRI, the big donut here, is that hydrogen or proton in a magnetic field aligns with the magnetic field. It can be pinged out of its orientation with a radio-frequency pulse, and when it gets released it emits an energy that is picked up.

So what we measure are recovery of longitudinal magnetization loss of face coherence and some other parameters. And, ultimately, this gets displayed into these maps.

It's very versatile technology. It's a mainstay of medical-imaging technology and a mainstay of

neurofunctional research, and, of course, it, at the spectroscopy level, also a mainstay of our analytical capabilities.

So this is all pretty much routine. You can go out and you can buy one of these and one of these and one of these and one of these. So where is the advancement here? So there's two very exciting developments on the horizon. One is in miniaturizing this technology.

And so here -- this is actually one of the world's smallest NMR systems here that we have built in our laboratory where all the RF generation happens in this little chip here called V2. And this is the magnet, by the way.

And so we developed this because we said, Well, MRI is a great technology because it allows us to look inside the body. Could we use the same technology to take cells out or take blood out or sputum and so on and look into cells at the cellular resolution?

So we miniaturized this and we built the entire electronics into this thing here. And so more and more this is being used for diagnostic purposes

because it circumvents the need for extensive purification. So very important technology.

Anyways, so another technology out there is PET imaging or PET CT imaging. As you know, PET uses radio tracers that are administered intravenously. These radio tracers emit positrons, and the positrons -- I don't know where it is. Here -- the red thing, interacts with electrons and it results in annihilation protons at 511keV in opposite directions. And these things are detected by the detectors in this ring back there.

There's a number of commercial systems now out there where PET imaging and CT scanning is integrated into the same machine. And why is this important? It's important for two reasons. Number one is because the functional information from the positron emitter that we're injecting intravenously is superimposed onto the anatomic reference points.

So, for example, in this patient here, there's one hot spot here, which is a cancerous lymph node. And if we just observed this without the reference, frequently it is just very difficult to analyze these images.

And, secondly, the CT part helps the PET imaging reconstructions.

So what's on the horizon there? Well, for one, it is the integration of PET imaging now with MRI and doing whole-body imaging in humans. So the ability to obtain metabolic information or information from radio-labeled small drugs and radio-labeled biologicals at the whole-body level is about there.

So the other group of macroscopic-imaging technologies that are sort of on the horizon or some of the optical technologies, we have already covered the microscopic ones that are being miniaturized into fiber-optic systems ultimately to be integrated into endoscopes, into intraoperative imaging systems with the ultimate goal of doing microscopy in the operating room during endoscopy, perhaps doing it during an eye exam or in dermatology.

At the more whole-body level, there are tomographic-imaging technologies that primarily depend on oxyd, oxyhemoglobin absorption difference to look at neurofunctional parameters.

And then later on we will be hearing about two other optical technologies, photoacoustic imaging. Lihong

Wang will be talking about this, and optical coherence tomography will be covered by Brent Bouma. So I won't go into any more detail.

So I've covered some of these imaging technologies out there. There's, of course, many, many others that I've not covered, such as SPECT imaging. There's bioluminescence imaging. I can't really read this from here, but there's a number of others. It's a complicated table. So they're all different in terms of resolution, depth, the time required, what the output -- what kind of target one can image. They're all very expensive. So you can just forget this column here. [Laughter].

And many of them are going from the animal into the human. So for those of you interested there was a recent review where this has been summarized here.

So I don't want to leave you with the impression that everything is apple pie in imaging. There are challenges in the field. Perhaps one of the biggest challenges is motion and motion compensation, particularly from microscopic imaging.

So if a cell moves one micrometer during the acquisition [phonetic] time, it's no longer in focus,

and that’s a big deal. And it can, of course, be out of focus for a number of reasons, a) because it wants to move or b) because some adjacent cell moves or contracts or blood vessel does something. So we frequently need to go to acquisition of Z-stacks, but much, much more work needs to be done here.

The whole discussion of resolution versus coverage. So we can’t have both, because we would be imaging forever. In an ideal scenario, what we would like to have is imaging modalities that allow us to cover very large areas or field of views, and then have the ability to zoom into areas of particular interest, but it’s a challenge.

Another challenge is the ability to do multiple or multichannel imaging of both targets, pathways, cells and how to integrate all of this data into model systems.

Another challenge is there’s clearly a need for many, many more validated reporters and probes, and, cynically, I write, which actually work. So many of them actually work great in vitro. Many of the reporters that you can buy -- monoclonal antibodies and so on -- they work great in cell culture. The minute you inject them in viva, they no longer work

because they bind nonspecifically to albumen or something happens to them or the macrophages like them and they stop working. So things that actually work in vivo are required.

Another big area in dire need is the whole issue of automation of image analysis. Twenty years ago, when we got 10 images per hour, it was okay to sit down and analyze them by hand. But now that we get thousands and thousands and thousands of images, it has become essential to develop image-analysis tools, automated image-analysis tools.

And then quantitation and modeling, of course, as I mentioned.

So, finally, the goals for this group, so I think importantly, so as a group, we should define our needs. From the tissue-engineering perspective, which molecular markers are really of importance to you? And so if you close your eyes and you think, Well, what are they? Is it really Kasbay's [phonetic] three or could it be Kasbay's seven or it could it be MOMP [phonetic] or could it be an Exin [phonetic] binding or could it be something else or could it be autophagy? So I think, for each of us, it's important to pin down what we really want to image.

Secondly, as Gordana mentioned, there is a need for permanent markers to trace cells, so things don't get biodegraded. There's a need for smart markers or smart reporter markers that actually -- just like the calcium channel dyes that change their properties when something happens.

The whole issues of human cells and human tissue in experimental model I think needs to be thought through much more. When do we use human cells and how do we best model their engraftment? And how do we deal with this? And can we really extrapolate this data to the human setting?

And then for the imaging folks in the audience, it's time for you to wake up again. What new imaging techniques and approaches are out there that could actually help with some of these questions up there, as well as integration of datasets?

So I'll stop here. I haven't used up all my time, but, hopefully, we can have a few questions. Thank you. [Applause].