DR. AL JOHNSON: So, I guess everybody can go drift off to sleep this afternoon now. I have the dead man's watch here. You know, lunch is just sort of settling down, and so I'm going to introduce myself. "Al, this is Al." Al, *thank* you. So, I am a physicist.

[CHUCKLING.]

DR. JOHNSON: I am a physicist at Duke Medical Center, and –

VOICE: We can't hear you. That's why no one clapped.

DR. JOHNSON: They can't hear me? [Chuckles.] And I'm going to talk a little bit about – when Ralph assigned me this task, I said, "Well, you know, we really haven't done much with repair. We do stuff with destruction, but repair" – but we've dug around and found a few things, so I'm going to try to cross the cultural divide between regenerative tissue people and engineering people and imaging people; and, hopefully, at the end of my talk, I will leave you nothing to hope for.

[CHUCKLING.]

DR. JOHNSON: So, what I want to do is spend a little bit of time on physics. I actually used to be a physicist, and I think it's kind of cool to see how the gizmos work. I think it's *remarkably* cool to have a laser that does that and that. I – I just – that's *cool*.

[CHUCKLING.]

DR. JOHNSON: I'm going to try to do a little bit of biology just because it helps demonstrate where the imaging can go. I'm going to focus on two organ systems that I think people at this meeting are interested in, heart and brain, and then I'm going to do what we were just told at the lunch meeting. I'm going to encourage collaboration.

Our lab is a NIBIB P-41 national resource. We get funded, we get paid, we get renewed, dependent upon the sort of collaborations we can establish; and there're a lot of big dogs in this audience that could help us in that renewal. So, if you see something

that interests you, look us up on the Web. I'm very anxious to talk to any of you.

As I've listened to the word "imaging" – "imaging" gets used so many different ways. I mean you can talk about imaging and pictures of your kids. You can talk about the cinema. You can talk about clinical imaging. You can talk about imaging at the microscopic level that I've seen some just humbling examples of during this meeting – imaging individual cells flowing through vessels. I think that just blows me away. And this gives us some sort of range over which we can talk about this.

In our domain, with MRI and CT, it's about the boxile[?], the size of the chunk of tissue that you're trying to image. So, when I talk about resolution, I talk about the chunk of tissue that we're looking at. Most people think of it only as a two-dimensional thing. I think of it as a three-dimensional thing, and if you use that volumetric scale here, you see that there are – I don't know – what? About 11, 12 orders of magnitude over which we could be imaging things.

So, let’s ask a simple question – what seems a simple question, the sort of thing we were just discussing. What’s the best way to look at the rodent heart? Well, what is it you want to see? Do you want to see whether it’s repairing? You want to see the ejection fraction? You want to find out something about the mechanical properties? You want to see whether there’s profusion? You want to see whether there’s molecular activity? It sort of depends on what you want to get out of it, so you need to think a little bit about – as we establish this conversation between imaging and regenerative medicine people – you need to help us out a little bit. What *is* it you’d like to see?

I’m always reminded of office space. Anybody reme- -- I have to children who – two boys who we let watch “Office Space.” And, you know, there’s a point in “Office Space” where they’re about to shut the whole operation down, and they bring in Bob and Bob, the two consultants; and everybody goes into the conference room with Bob and Bob: “Hi, I’m Bob.” “Hi, I’m Bob.” They sit down and say, “What is it exactly that you

do?” And everybody gets concerned, knowing, “Well, I can’t tell the boss what I do. He might not actually hire me.”

But that’s what we need to understand at the outset. What is it exactly that you *do*? What do you want to look at? And so let’s look at some examples. In computer tomography. The marker that we get – the physical marker – is simply the x-ray absorption coefficient. There are at least two flavors. You can look at the photoelectric effect, which gives you some sensitivity to the atomic number, or you can look at density. So, you can look at calcium, and you can look at just density with Compton, or you can look at some atomic markers. Iodine is one of the things we use as vascular markers, and CT is really not very sensitive. You have to have pretty – it’s not a molecular imaging gizmo, but you can look at structure and function.

Resolution is all over the place here. I saw some elegant images this morning down at 30, 40 microns. And our particular take on this is someplace between that elegant micro CT we saw this morning and the

clinical CT, because what we're interested in looking at is cardiac function.

Now, this is a standard geometry for a CT scanner. You got an x-tray tube. You got a detector. They rotate around your patients. That's probably not the best way to do a mouse. The problem with a mouse is up here at the top, you have a source of x-rays that's coming from some finite amount of x-ray 2, and it is really the rate limiter on what your resolution is. If you take a – conventional geometry. The focal spot in a clinical CT is on the order of a millimeter or so, and if you try to image, with that x-ray tube, a mouse at 100 microns, your focal spot's bigger than what you're looking at. So, the geometry doesn't help you at all there.

So, what we've done is a little bit different geometry. We back the animal way off, and we spin the animal. And the reason we do that is that we can make this distance between here and here small, put the detector back here, make this distance as large as we need to, so this resolution – this geometrically defined resolution – becomes what we want it to be.

And we can match it to – I get to use the clever buzzwords of engineers. We can match it to the niqua [phonetic] same of our detector. We have a detector down here that has pixels that say – maybe our pixels are 20 microns. Maybe they're 40 microns – something like that. But when we do that, we end up getting bucket loads more of x-ray photons, because the rate limiter, if you're looking to do a[n] image of the heart, is – oh, it's *moving*. And if you want to get something fast enough while it's moving, you['ve] got to push a lot of photons through there.

So, this is a schematic that shows that, as we change the focal spot size, but we maximize things for this geometry for a resolution of 25 microns, 50 microns, maybe 100 microns, you can see that we get, even on a log* scale, 3, 400 times more x-ray photons than a commercial CT with a stationary micro focal anode.

So, this is the thing that we have built. It's got what I'd call a real man's x-ray tube here. This is the sort of tube that we use in the hospital for "whale-o-grams." We get a 300-pound patient in for angiography. You need a lot of x-rays to go through

them, so this is like a 50-kilowatt x-ray tube. It's a *big* x-ray tube.

And another one here. And then these are two very sensitive detectors that are matched for the energies that we choose to use. The animal sits right here on a little mechanical stage, and we spin the animal, and we control the biology the whole time. We have that animal instrumented very well. We have a rectal thermistor right here. The chairman of the department's been thinking about using those for some of the faculty member[s] just to see whether we're coming to work. But –

[CHUCKLING.]

DR. JOHNSON: -- we can intubate the animals, if we want. We have a little tube – a little pillow right here that measures where they are in the respiratory cycle. All of this is integrated through a lab view[?] module, so that we pulse the x-rays exactly when we want to pulse them.

So, when we do that, we can get this sort of four-dimensional dataset. This is about ten phases of the cardiac cycle. The x-ray exposures are on the order of 5 to 10 milliseconds, and during those 5- to 10-millisecond exposures, at each of about 300 different angles as we step the animal around, takes about five minutes to do the scan. Then we do some pretty fancy math, where we take the information from those two different detectors and map them into a similar space, push them into a pretty big computer that has some very clever stuff written by some very clever faculty and graduate students; and you can get this sort of image.

This shows you the four-dimensional nature a little bit. Watch right here. You're going to see the coronary artery. I heard somebody this morning asking could we see the coronary artery. Yeah, we can *do* that – but you have to own both the x-ray – ah! There it is. Which artery is that? Which coronary artery is it? [Inaudible response to his question.] It's what? Is there a cardiologist in the house?

[CHUCKLING.]

DR. JOHNSON: Matthias, you must know. Which one is it?

DR. NAHRENDORF: The right one.

DR. JOHNSON: It's the right one. See? I *knew* it was.

[CHUCKLING.]

DR. JOHNSON: Thank you. And what we're doing is we're just gradually slicing down through the stack. The stack is about 600 images. It's about – I think it's about 600 by 600 by maybe 800 images here, so each dataset – each package – is a 600 by 600 by 800 array times ten. Somebody – in fact, several people have suggested that the image arrays are large. You *bet* – and many, many, *many* dimensions. You'll see that as we get further into it.

You can take that and re-slice it, do all the fancy volume rendering. In this case, we're actually using a clinical package, I think, to just unwrap the coronary, so this is actually a curved surface that has been formed here. It's just a little bit of eye candy, but it can be useful if you're trying to look

for stenosis. So, if you're looking for a stenotic lesion, if you're trying to do vascular repair, this sort of thing can work.

Doing a mouse is a little bit harder. It turns out that a mouse is not just a small rat. Their heart beats faster. A 25-gram mouse is about 3,000 times smaller than a human. And, oh, incidentally, their heart R to R interval is about 100 milliseconds, and their end title[?] volume is – what – 2, 300 microliters – something like that. So, a whole bunch of things that you have to worry about – that the biologists have to worry about.

So, what's the best study that we have done at Duke? Matthias' study. Matthias did some work with Christian Badilla [phonetic] a couple of years ago that was pretty successful in which they were looking at a delayed hyper enhancement. Animals – you did the surgery in Boston. Sent them down South, and they made it all the way. And then they were imaged at five days – control, five days, 35 days; and one could look at both the development of the infarct and then the loss of function, reduction in the ejection

fraction and changes in the volumes and end systolic and end diastolic volume.

To do this, you need a little bit of physics, a little bit of biology, a little bit of engineering, a little bit of computer science; and you[’ve] got to tie them all together. *Several* people have alluded to the fact that the image analysis gets to be the bottleneck, so the solution that we have undertaken for that is we offload all of the computational processes to dedicated computers. Computers are cheap, so I have six MRI systems. An MRI system – a state-of-the-art MRI system – the fanciest one I’ve got right now – is about $3 million – 3.2. And someplace along the line, you ask it to do a foyer* transform. Well, you know, I don’t need a graduate student tying up my $3.2 million machine while he’s doing a foyer* transform. And if I wait for Brucker[?], or Agilent, or GE to do what I want to do, you know, they won’t get there. It’ll be a little while. So, we offload all of our computational processing to dedicated workstations along the way.

One set of dedicated workstations are what we call "image processing pipelines." So, we take pearl[?] scrips[?], mat[?] lab, VTK, ANTS[?] software for registration; and you just stream them together. Now, it sounds simple, and it sounds like it's – somebody – we were talking about semi-automated segmentation. When somebody tells you they've got an automated segmentation, don't buy any land from them. It's not –

[CHUCKLING.]

DR. JOHNSON: -- something that you want to do.

So, in this case, what we've done, though, is a pipeline where we segment one; and we might segment every tenth slice at one phase. But then you can do some reasonably clever things to propagate that through, and it gives you as an output the cardiac phase. It'll give you the volumes of about three of the chambers of the heart. The fourth one is kind of a little dicey. You have to do a little bit more. It'll get you stress and strain maps and wall

thickness. There's an awful lot that you can do with it. So, that's CT.

We have a new toy that has just recently arrived. I had a PET scanner for many, many years, and it was sort of like a boat. You know, you're *ecstatic* when you get the boat. I was ecstatic when I got my micro PET system, and then I just poured money into it, and I poured money into it, and I poured money into it. And I was happy when I sold it.

[CHUCKLING.]

DR. JOHNSON: The problem that I had with PET is if we wanted to do FDG, we could do FDG. And then on alternative days, we could do FDG. And if you were really interested, you could do FDG. So, getting PET radionuclides takes an infrastructure that is *enormous*, but in the case of SPECT[?], we have New Jersey. New Jersey has about a dozen radioactive chemistry labs. I don't know why they're in New Jersey; but that's where they are, and so you just call up. These are long-life – long half-lives. The other thing that's interesting for me as a small

animal imager [is] there's a lot of politics about why PET makes it into the hospital. PET makes it into the hospital because GE would like it to be there. Siemen's would like it to be there. And there's some underlying biology. Carbon, nitrogen – those things that they can make PET-active are – you know, they're pretty biological. But the problem is they're all short-lived radionuclides, and the infrastructure necessary to put those together is huge.

On the other hand, these are long enough half-lives that you can just call one of these places up in New Jersey, and they'll ship them to you. No radiochemistry, no licensing. It's like – when I built my house many, many years ago, I went over to Raleigh, and I bought a bunch of dynamite because I had to clear out part of my woods. It's harder to do that these days, but you can still get the –

[LAUGHTER.]

DR. JOHNSON: -- you can still get these radioactive compounds. And there's a clever company in Holland from whom we bought this. It's called MI Labs. It's

a clever group, where they've taken just off-the-shelf gamma cameras. So, they've got a gamma camera here, here, here. It's no sophisticated technology in the sense that it's just sodium iodide detectors and PMT – stuff that has been around for generations. One here, here and here; but the only moving part is this thing. In a traditional gamma camera in the clinical domain, they're really *awful* resolution. They have collimators like this that sort of – you have parallel, whole collimators.

These guys use a pinhole. And if you have a pinhole right here, you can project the image onto a detector, and so you have a magnified image. The problem is if you have a pinhole, you're losing most of the radiation, so SPECT[?] is already less sensitive than PET. So, you know, you run into a lack of photons again. So, what they've done to counter that is they have this clever, little technology where they have a whole bunch of pinholes. So, they have a fancy machine shop. It's really quite cool to watch the machine CAD things that they do – where they've got a whole bunch of individual pinholes, all projecting 75 different images into different parts of these

multiple detectors. And then they do a bunch of computer mumbo-jumbo to put it all together.

What they have is a sweet spot, so this is a[n] optical image – three different projections of the mouse – and this is the sweet spot, and you define how much of the animal that you would like to scan. And so it goes through an algorithm and just sort of moves the animal around, collects a bunch of views here and here, here, here and here. So, with this, they can get to spatial resolution that's about 350 microns. That's about 60 times higher resolution than we were able to get with the PET, so that rings my bell. And this what we're looking at for a mouse image with just a standard technetium tetrofosmin. Getting this is so simple. I simply walk up to the hospital to the nuclear pharmacy lab and say – [whispers] – "Hey, can I have some of that stuff?" They said, "Sure. Take it away."

I don't even get *charged* for it. That doesn't happen frequently at Duke.

But you can see we're even picking up papillary muscles there. A different level. So, we've only been playing with this about three weeks now, but we're pretty pumped about it. And you can merge them.

So, this is a myocardial infarct in a mouse, and you can see delayed enhancement there and some profusion deficit there. So, we're hoping we'll get better.

I'm reluctant to put up anything about optical imaging. Every time I see what you guys do with real microscopes, it just – it's really astounding. I just think it's *cool* to, in real time, look at all those vessels and all those cells going around. So, I'm a little bit of cautious of putting [it] up, but I'll put it up anyway. What you get is the possibility of single molecular sensitivity. Single-molecule sensitivity – that's a big deal. Resolution? Well, I'm – [chuckles] – out of date. What did we see yesterday? Resolution in the order of 10, 20 *nanometers*? Wow. I should go home. And you can look at structure, and you can look at all sorts of clever molecular pathways.

So, there is a very clever fellow in the group named P.J. Nichols who has been working on a specific mouse. I had the good fortune of going drinking with Roger Chen some years ago, and I said, “Roger, you know, making things fluorescent – what happens when it’s dead?” He says, “Well, you know, if you fix it, and you don’t change the pH” – stuff that you guys know well – “you can maintain the fluorescence.”

So, we modified a Zeiss confocal microscope, and we’ve taken – there was a paper called “Scale” in “Nature Methods” about two years ago. It’s an advanced – people have been doing tissue clearing and index matching for some time, but this is one of PJ’s hearts from an animal that he’s constructed, that up-regulates CAS[?] phase[?] 3 whenever there’s apoptosis. And as a physicist, I just love that. So, that array is huge. It’s 10,000 by 10,000 by 100. And we’ve got *ten* of them. So, you can zoom in on any one point.

[I’m] going to finish with MRI. The embryo. So, this is work we’ve been doing for years with Kathy Sulick [phonetic]. This is a 3-D array of a mouse embryo.

This is MR at about 20 microns. You can slice through at multiple levels. And you can look at many different contrasts. Diffusion is one of the most widely exciting ones right now, where you're really putting a bunch of gradients on so that you can map the microscopic motion.

Given the time I've wasted, you get a tenser at every point from about eight or nine different image arrays. You do a bunch of computations; and this, then, is what you can see. This is an animal. This is a fixed tissue. This a control, and this is from an animal that has been exposed to alcohol, and you can see – the color coding is telling you about the direction of mobility of the spins. And you can track it. So, you can put a seed here and say, "Find what's connected." So, you can see loss of connectivity in the alcohol-exposed animal.

Moving to the rat, this is 25 microns. These arrays are 800 by 800 by 1600. And we have it multiple times during the animal's evolution. We have it at each point. We have multiple specimens, and for each

specimen we had multiple different kinds of contrasts. So, this is the 25 micron.

This is a diffusion-weighted image, so you can see here – CA3 shows up here. It doesn't show up here. This is mapping essentially white matter. The color encoding here, particularly in the hippocampus in the molecular layer, tells you something about the directionality.

And this shows postnatal day 12 day. And there's 40 days. And I think I'm about out of time. Do I have one more slide? One more slide. So, this is work from Tennille Smith [phonetic]. Tennille is a graduate student working with Raphael Guzman at Stanford. As a national resource, we thrive on collaboration, so this has been one of the most wonderful ones so far. She's looking at response to their surgical model of stroke and recovery from stem cells. That's some of her histology, and here – this is with a saline treatment after – I think this is at ten days after, BND[?] 10. And this is with stem cell treatment, and what you're looking at is an increased

production of the connections that the stem cells have helped recover.

So, it probably doesn't surprise anybody here that – all of the work that's done by these good people – the dogs are there as part of heightened security effort we have at Duke.

[LAUGHTER.]

DR. JOHNSON: And with that, I will tell you there's our website, and I do most hope that I will have a chance to talk to some of you later. Thank you very much.

[APPLAUSE.]

MODERATOR: So, do we have time for questions?

Q: You mentioned strain imaging with CT. I haven't heard of that before. Can you explain how you do that?

DR. JOHNSON: I don't know how it's done. I really – [chuckles] – don't. I know that we're looking at changes over time. I think you can look at – clearly,

what Matthias is talking about in – the problem is, in MR you can tag an individual chunk of tissue and watch that individual chunk of tissue. And with CT, you don't tag the individual tissue. I think what people are doing is moment analysis. It is something that I'm not doing myself, and I'm embarrassed to say – well, I'm not terribly embarrassed to say – I don't understand how it works, but I think it is being done. In the clinical domain, I think they're doing it with clinical CT. Have you seen it done at all in clinical CT? [Pause.] You don't know. You caught me on that one. Other questions?

Q: Yes. I have a question about the diffusion test[?] you mentioned. Were those acquired in vivo with – [crosstalk] –

DR. JOHNSON: No, these were all fixed tissues.

Q: So, that's where my question is. You know, in terms of diffusion coefficient, the water transport – are there big differences in vivo versus – [crosstalk] –

DR. JOHNSON: When you fix tissue, the diffusion does change, but the anisotropy does not. And we do do in vivo studies as well – DTI in vivo. I wanted to show the highest resolution, so the highest resolution gets done on fixed tissues that we can stain for MR histology so that we can get higher signal. But, yes, all of the diffusion coefficients go down when you profusion-fix a tissue, but the anisotropy – the tissue structure still is maintained. So, you get something about anisotropy.

Q: -- so, how long? I mean how many hours after you sacrifice the animal that the – I mean where would you say the – [crosstalk] –

DR. JOHNSON: So, the diffusion tenser – I can take it today, next week, two weeks from now. As long as the tissue has been well fixed, it’s preserved. The anisotropy – the myelin bundles are what you’re really – you’re really looking at projections along the bundles of myelin, and that’s maintained once you fix the tissue. It’ll stay for months.

Q: -- so, there's no active water transport across membranes?

DR. JOHNSON: When you're looking at DTI in vivo, you are primarily looking at the morphology. You're not looking at active movement of water, you know. You're not – you know, you do this in kidneys. You're not going to pick up the effects of aquaporin and stuff like that. It's the macroscopic structure that dominates what you see in a DTI image. It's white matter.

Q: So, you casually mentioned some of the tools that you guys use for your image analysis, and I haven't heard Image J mentioned.

DR. JOHNSON: We use Image J –

Q: You *do* us it. Okay. All right.

DR. JOHNSON: -- all the time. Image J is a most powerful tool – yes.

MODERATOR: Okay. Well, let's thank our speaker again.

[APPLAUSE.]