Ronald W. Davis, PhD | Biomarkers

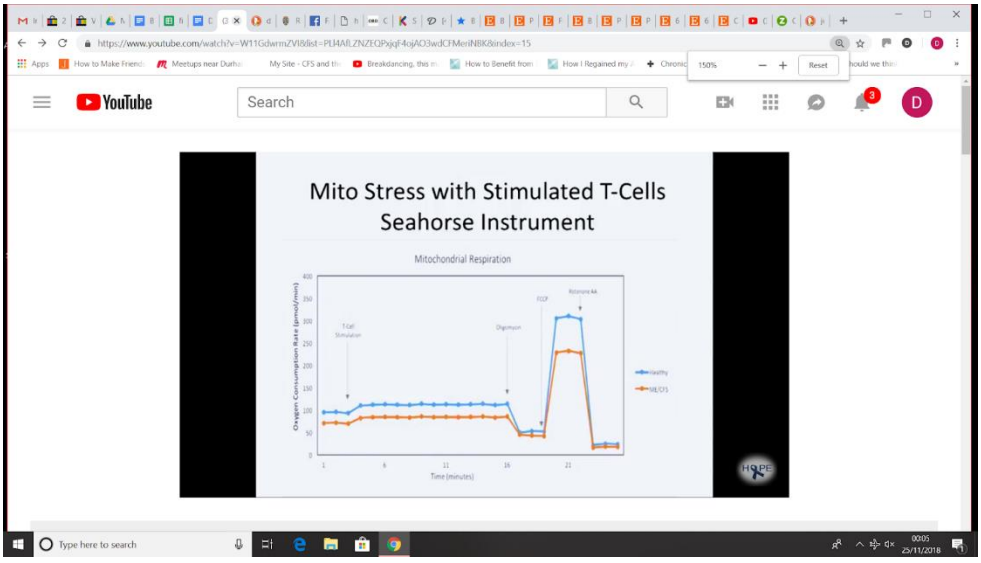
https://www.youtube.com/watch?v=W11GdwrmZVI&index=15&list=PLl4AfLZNZEQPxjqF4ojAO3wdCFMeriNBK

Dr. Raeka Aiyar: So our next speaker needs no introduction but he's going to get one anyways. Ron Davis is probably one of the greatest out-of-the-box thinkers in the field of genetics and genomics. It's been a pleasure for those of us who have gotten to work with him over these years. We were kind of playing a six degrees of separation thing the other day with some of my distant colleagues and I think almost everybody who's good in genetics and genomics is about 6 degrees separated from Ron Davis. They've either kind of come through his lab as a postdoc, a PhD, or they've collaborated with him. He's really transformed the field in that way. He is also credited with being one of the greatest living inventors on a list that included Elon Musk timely [Laughter] and Jeff Bezos. How about that, yes! So Ron, I actually introduced him from a personal standpoint, I just want to impress upon you, who are maybe outside the field of genetics, just how much he's actually changed that field. He's credited with helping to launch the recombinant DNA revolution that, you know before there was CRISPR and gene editing there was recombinant DNA. There was just as much excitement then and that had just as much of a transformative effect on what we're able to do as genetics researchers. The scientists who have gone through his lab are really some of the greatest in this field. And as you see Ron's approach is let's just try new things, let's break things, let's innovate. He's inspired generations of us with this. I mean I'm actually two generations removed from him, he's my PhD grandfather [laughter]. That's been a major influence on my scientific career. The idea that technology drives biology has come through I think in a lot of the talks that we've seen today and certainly in the discussions we had with the scientists. That is sort of Ron's motto, figure out how to do the technology and you'll just open up brand new possibilities in biology. So his approach to that and bringing together all these interdisciplinary people, with diverse talents, encouraging them to collaborate, they're legendary throughout the field, it's what it's what he's known for. The fact that he's channeled all of that sort of expertise and vision and perspective into this disease, is something that I think makes this community very lucky to have someone like him fighting for it. You know Ron typically avoids the spotlight, that's kind of been his way as a scientist, but he's stepping into it now with a little bit of encouragement, or a lot of encouragement from his family. But I think we're all really lucky to have him and to work with him and he's the reason that all of us are sitting here in this room today. So with that I'm going to welcome Ron Davis to the podium.

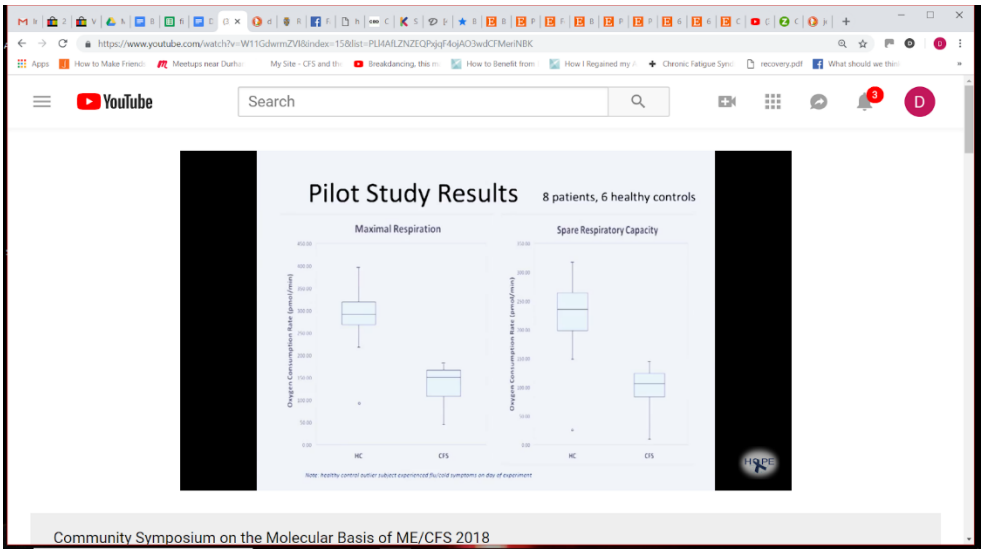
03:23 **Dr. Ron Davis**: So, when Ashley [Ashley Haugen, Event Organizer, Ron's daughter] tells you you have to do it you have to do it. So this first slide is just as a reminder that all the work that we've done has been supported by the Open Medicine Foundation, absolutely everything. Our first grant was the grant to do the T-cell activation but that just got started. If you have to skip a talk, don't skip the next one [Robert Phair, PhD | Metabolic Traps: A new way to think about ME/CFS https://www.youtube.com/watch?v=uej1LXzRbnY&index=16&list=PLl4AfLZNZEQPxjqF4ojAO3wdCFMeriNBK] because I think he has some really great ideas. So I'm going to

talk about something that is not that exciting but something that has to be done and that is we really needed a biomarker. So we've been working on that part and it's an extremely large need because of the fact that doctors say there's nothing wrong and even some doctors say well if you don't have a you know if you've don't have a diagnostic test, the disease doesn't exist. We don't have a real good diagnostic test so we have to find one. So we've been working on this fairly hard and just show you this, is just an update we're getting there. There is a lot of work outside our lab as well that's perfectly great. This is not a competition and what we want to do is to try to take the very best one out there and make sure it works. We have a lot of experience in this area. Also this is to remind everybody, because doctors don't know this either usually, that a lot of diagnostic tests have a high rate of false negative and false positives. It's not uncommon to have a diagnostic test have 10% false positive and 10% false negative. So it's a real problem in the community because doctors believe it's a hundred percent accurate, whatever it is. If it's negative then you don't have it, when you know for sure you do. So we have to solve that. So in fact the National Bill of Standards has established a lab at the genome center to really take on these kinds of problems. We've got to do better, we have to make it better for the diagnostic test.

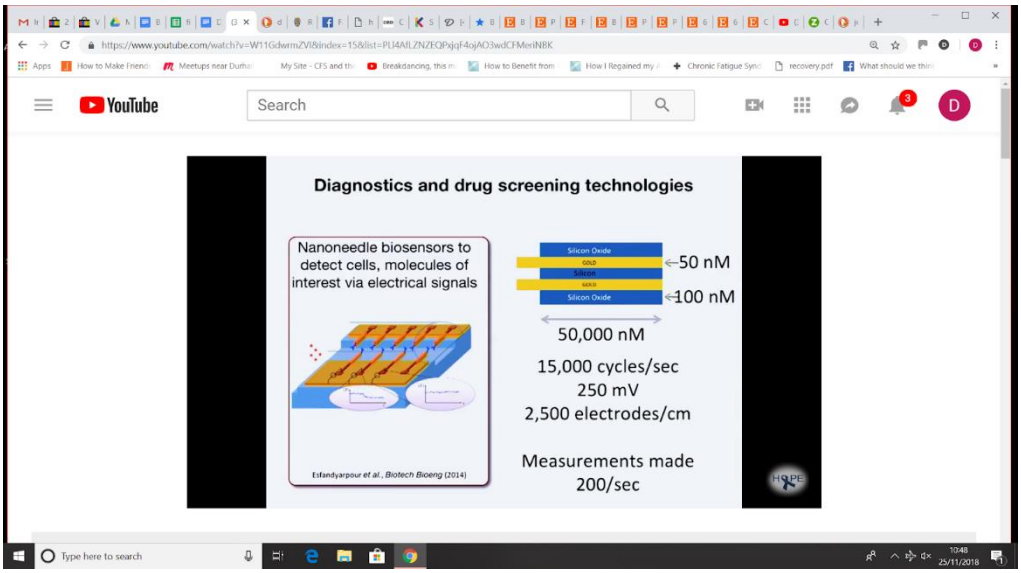
06:20 So the simplest form of this, and Maureen Hanson talked a little bit about this [Maureen Hanson, Metabolism and ME/CFS https://www.youtube.com/watch?v=hPAzInLeSx4&index=11&list=PLl4AfLZNZEQPxjqF4ojAO3wdCFMeriNBK&t=0s], is a commercial instrument called the Seahorse instrument and this has potential.



I'm just sharing that, it's not everything is invented in our lab. It is a possibility and we've gotten pretty good results with with this if we use stimulated T-cells. What you see from this, and this is just a trace that you get out from the instrument, is that ME/CFS sample shows less activity than a healthy control. It's pretty reproducible. The instrument is a little expensive although it can be a multiple sample that could be put into a clinic diagnostic lab. But we'd like to try to do something that's maybe simpler and cheaper.

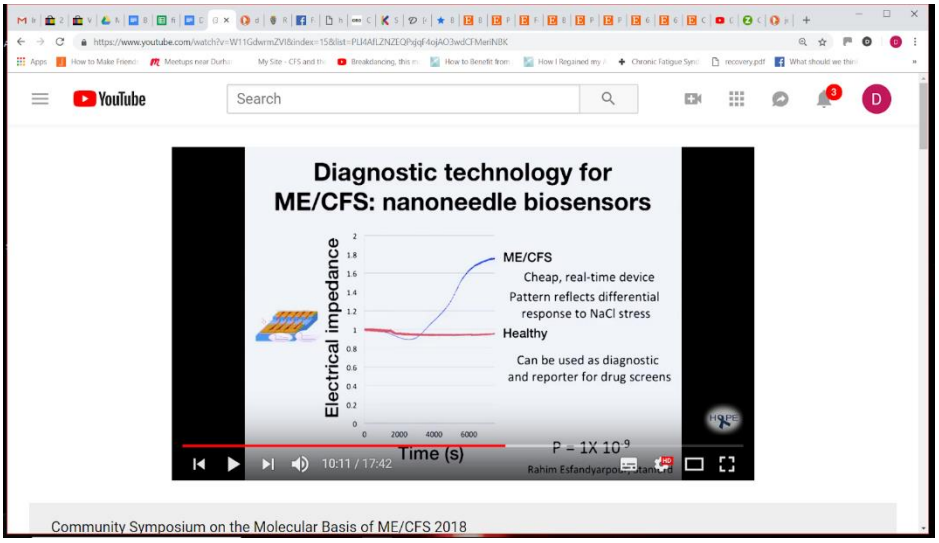


07:06 But this is a little pilot study to show that there is a clear difference between them, reproducible. That's a sufficient separation, probably make a diagnostic and so I'll talk about that a little later.



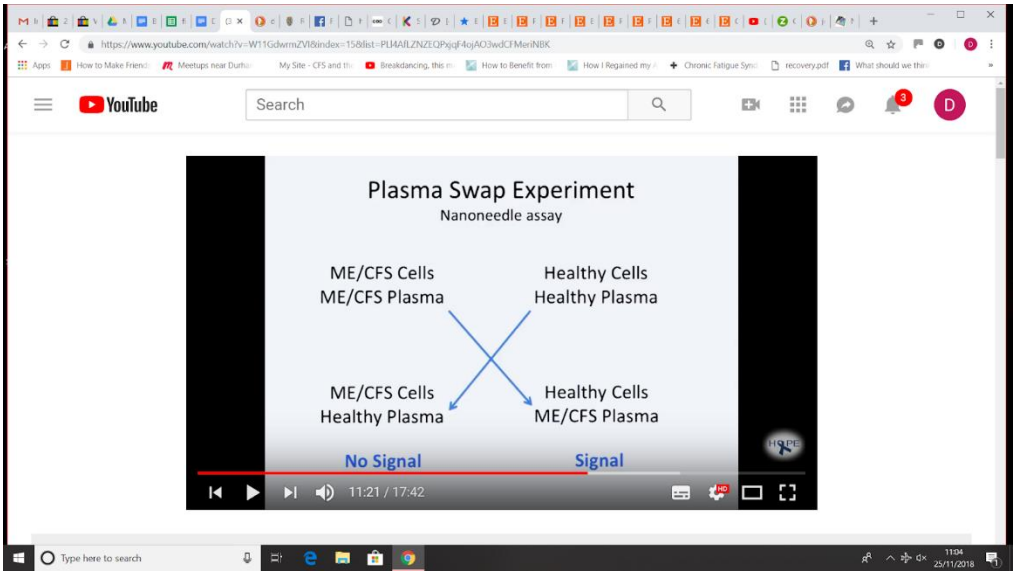
07:15 This is an instrument that I have talked about before that, for those who haven't seen it, it's something that's been nano-fabricated. You can see on the upper right the size of this. This is using same technology used to make computer chips. We have two gold electrodes that are very small and they're separated by silicon oxide, is basically sand, it's not a good conductor. So gold is a good electrical conductor. So this is just like a wire that you use to plug into something, except you can't see it because it's way too small. Then the other thing we do in trying to do better measurements is to take lots of measurements with the same, as a lot of technology takes one, and we take measurements from 2,500 of these electrons per centimeter and we sample them around

200 times a second, so you get an extremely large data set in one experiment. That guards against just a one false measurement. We use these multiple electrodes to measure electrical properties. Why we like electricity is that it's very cheap.



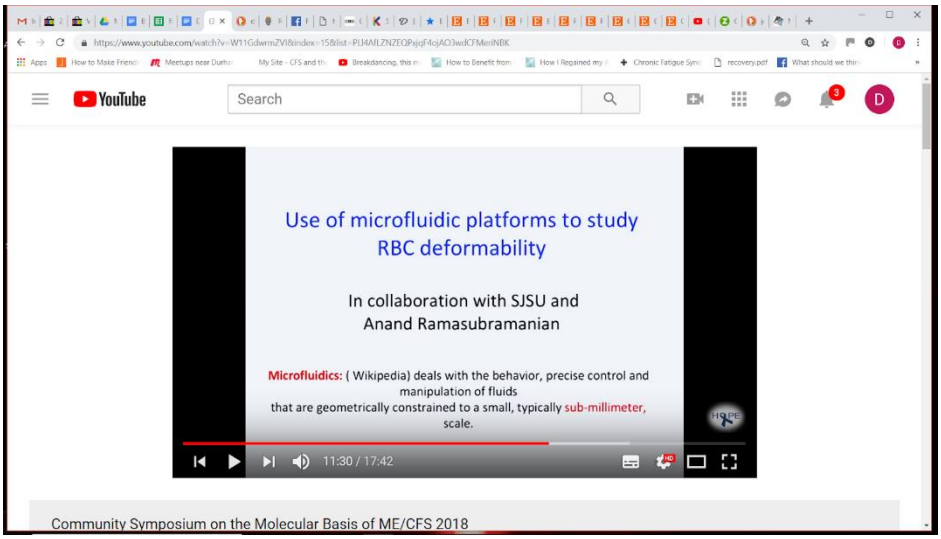
08:53 So if you do a test with blood in this device, healthy control just as unaffected. We put some salt into the blood, we don't know why that works, but we see after some time that there's a big increase in electrical impedance. So this is a little bit weird. It came about from studying some other things with cancer cells and so forth, which I won't get into. But the surprise was in fact it's very reproducible and it's also very cheap. So it's a response, the ME/CFS cells somehow respond differently to salt. We got into this because we thought the cells had less capacity to make energy and if you put in salt the cells have to pump it out and that takes energy. So, they'll have less capacity to do that than a healthy cell. That may be what's happening but we're not sure. We also can maybe use this as a drug screen. We have now started doing some drug screens. We have found one drug, that's used for other purposes, that seems to have a big effect on this assay. We have to explore that a lot more before we talk about it.

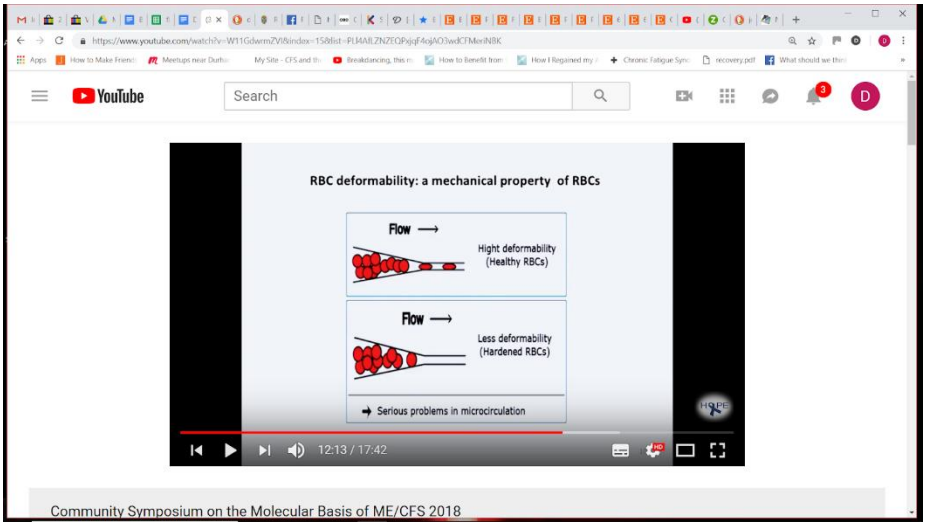
10:25 Now the probability . . .Let me just show you one thing, we've done 20 samples of what patients the red is the patients and the green [correction: blue] are the healthy controls. They're completely separated and the probability that could occur by accident is 10 to the minus 9 [0.000000001] so that's better than the lottery. So the chances that this is incorrect is very, very, very low and so people often do the percentage of the likelihood to work, that's a hundred percent.



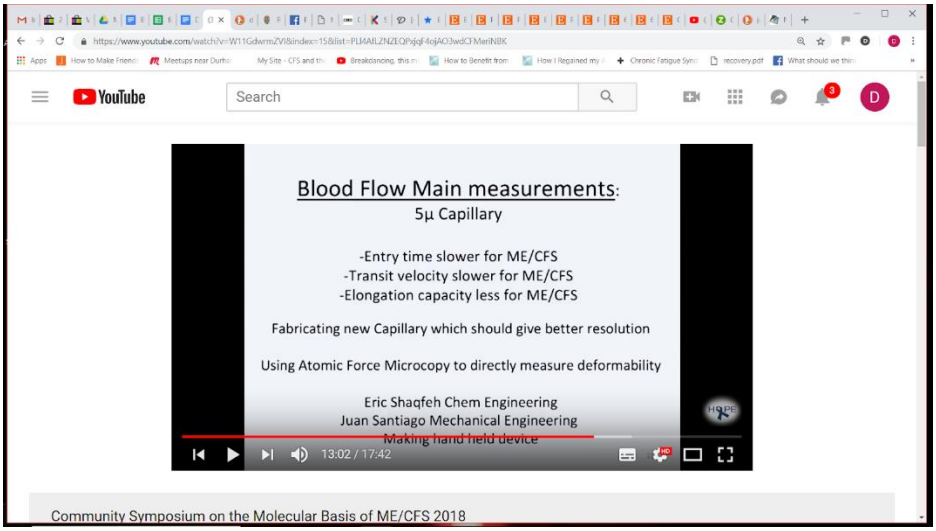
11:00 We've done a little bit of trying to understand where the signal comes from. We've done this plasma swap experiment and most of the signal seems to be tracking with the plasma, which is something in the plasma that's creating the signal. Now, that's encouraging because maybe we can figure out. We originally thought it was the cells, so we don't know what that is. We've heard another talk about

these small micro RNAs, that's a candidate, that those small micro RNAs are causing this signal (Alain Moreau, PhD | Deciphering MicroRNAs code in ME/CFS Pathogenesis https://www.youtube.com/watch?v=RzlKezyJVQc&index=10&list=PLl4AfLZNZEQPxjqF4ojAO3wdCFMeriNBK&t=0s).

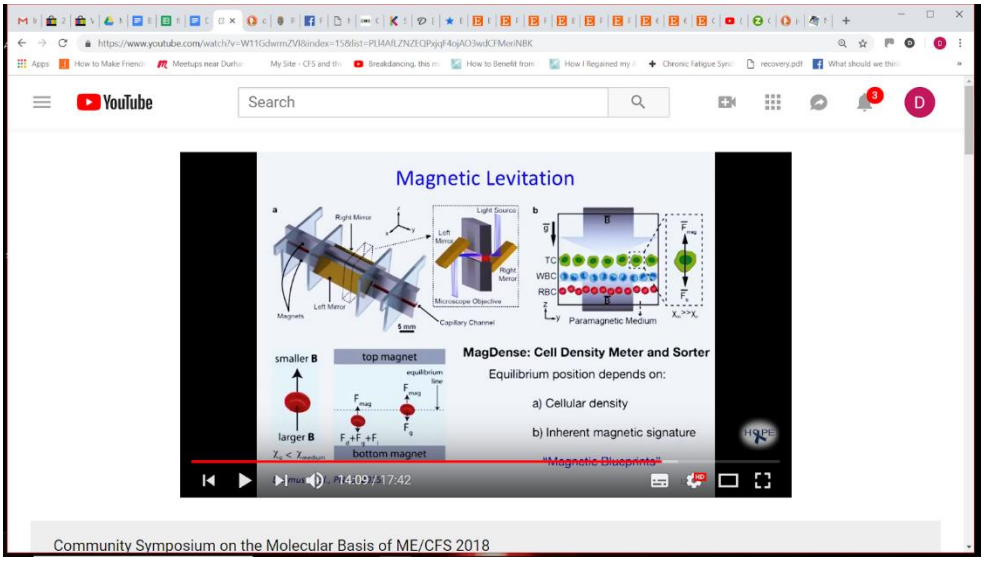




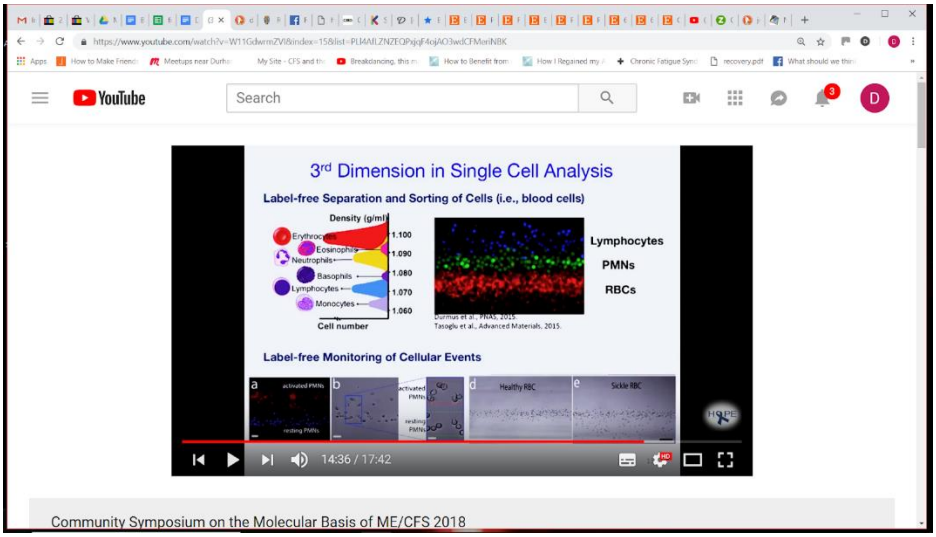
11:30 Now we've also never done a collaboration with San Jose State [University, SJSU] and Anand [Anand Ramasubramanian] is a engineer. He has built a microfluidic device that can look at blood. This was an idea that patients have something wrong with their blood, so we looked at what's called the deformability of red blood cells. You put them through a tiny little capillary and the red cells have to deform. This is what happens in your body, they have to deform in your body to go through the capillaries but red cells from CFS patients don't deform as well. They have a hard time entering in this little capillary and that's pretty reproducible. So what we've done now is looked at lots of different properties.



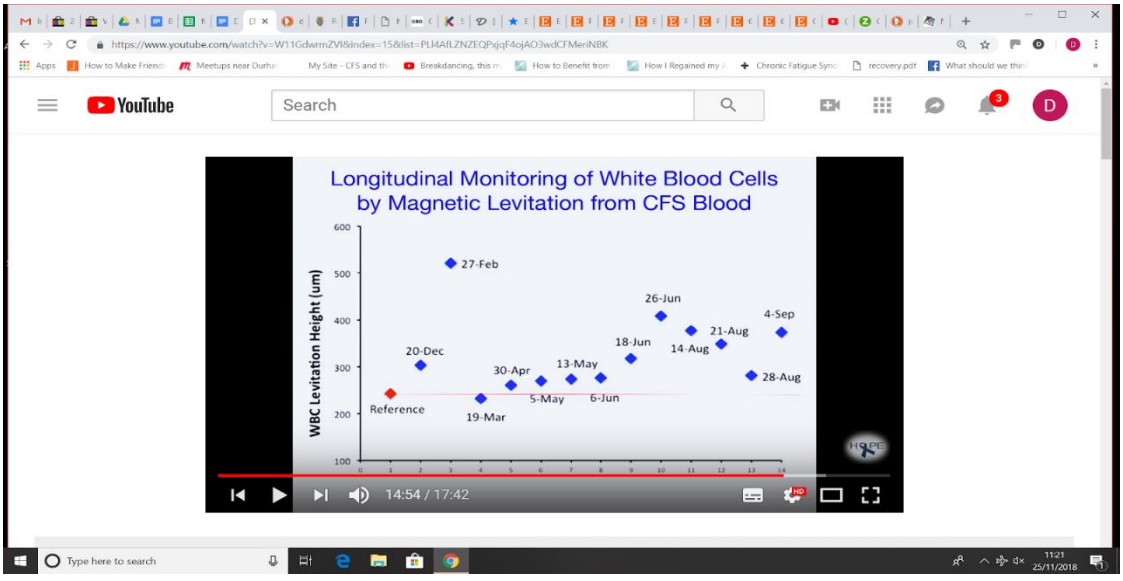
12:30 There is a difference between CSF patients and healthy controls, it's not good enough to do as a biomarker because it's not completely separated. So we're building a new device that we think will give this complete separation. It'll be again a microfabricated little device. Then since that all got started we've gotten other individuals, might even be here I think, Juan Santiago is here and also Eric [Eric Shaqfeh] from chemical engineering. These are very high level engineers, they do a lot of work for the Defense Department and so forth. Derek has access to Defense Department computers so he's doing a lot of the modeling. The plan is to build a different device to try out, which will be a handheld device, that can measure this rigidity. One reason for having such a device in measuring it is that we could try, if it is a problem for the patients, we have an assay to try a diet change to see how we can improve that. Probably a lot of that rigidity is coming from the membrane and it probably has to do with the oils that people are eating. So we can try out lots of different oils and see if we can improve it. So this could be another diagnostic instrument. It's also possible that we can combine them because these measure red cells and the other device measures white cells.



14:07 We have another device that we can look at red or white and it measures the magnetic levitation. That's simply putting the cells, that aren't magnetic, but if you put them in a fluid that is magnetic then you're going to actually separate them by their density and white cells and red cells will separate. We've been using it to separate out certain tumor cells that are in the blood supply and here's just a picture where you can see you can separate different cells in this device.

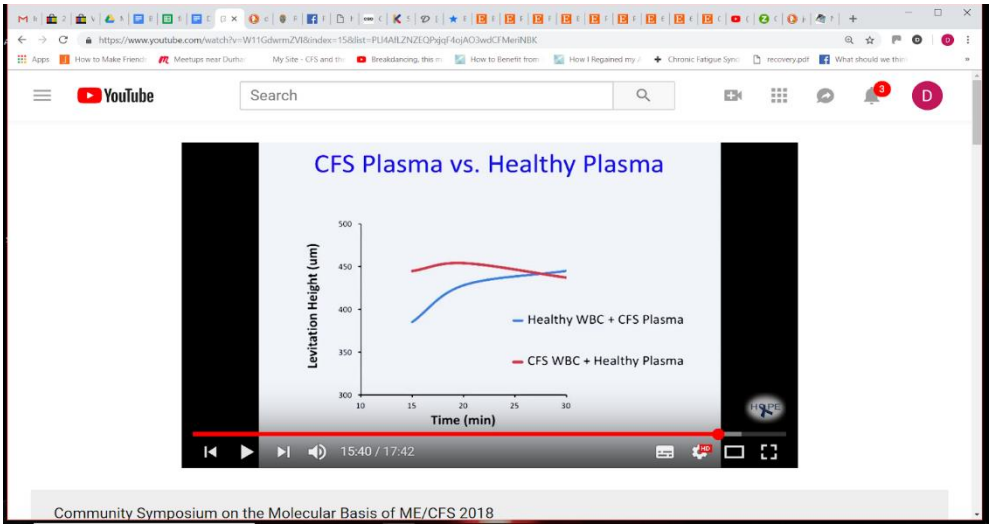


14:35 It's a little capillary, disposable, it cost five cents. Companies may not like that but it could be made into an instrument, because we see that the white cell population is generally the light and it separates out from healthy cells.

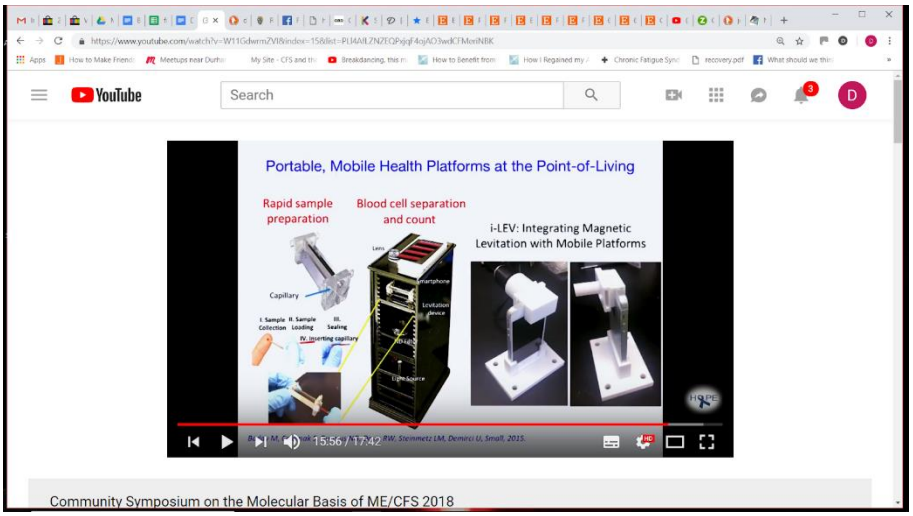


14:55 Healthy cells are on that reference line so we may combine all these into one instrument, that's a possibility. This instrument would separate the reds from the whites

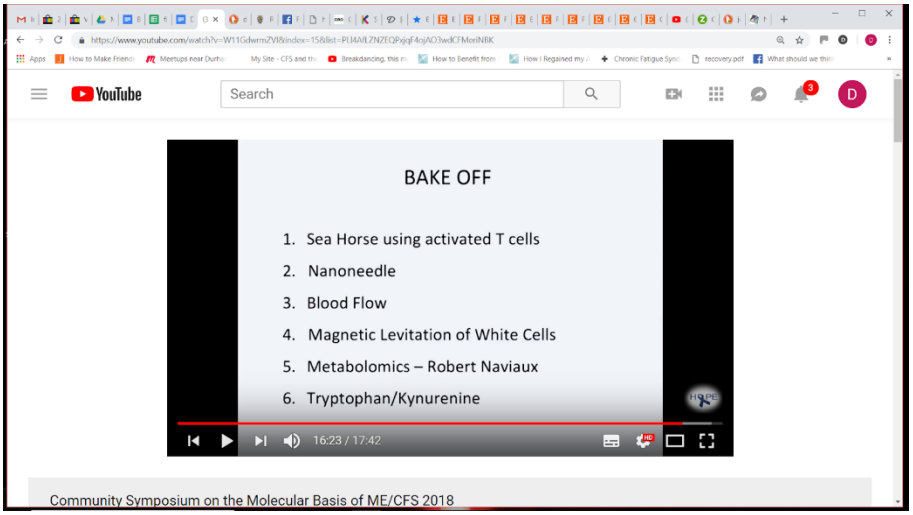
and then measure their density and then it goes to the next device which would measure the other properties. There are some interesting results here in the sense that there are a couple of samples that look weird and turns out they are happening right after a high fever. After that high fever the patient got a lot better, so for a short time, for a few months.* (supporting note at end of transcript)



15:44 Then we also did the plasma swapping experiments and you can see a big effect there as well.

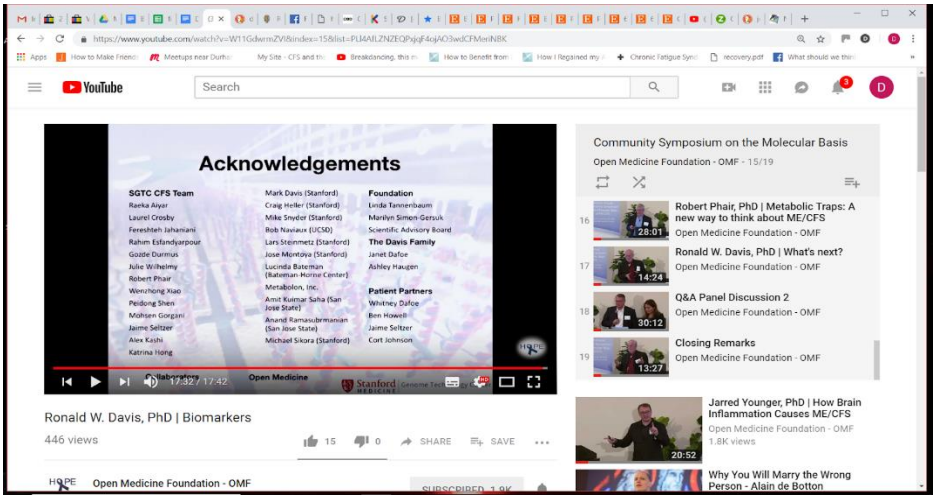


15:49 Then this is looking at the instruments, so the instrument basically is a capillary and all of the information is collected with a smartphone. So this is a new trend of using smartphones to build into the instruments because it's a very powerful computer and it's very cheap by comparison to putting in a real computer.



16:06 So we're now doing what's called a bake-off. That is we're taking blood samples and running them on all the instruments and looking to see how well they do. This is also to see if we get any inconsistencies and that will help us looking at false positive, false negatives. You're gonna hear a talk (reference to point 6) . . . also we're incorporating Rob Naviaux (reference to point 5), he came up with a pattern with the metabolomics that may be a diagnostic, we're gonna put that in the system as well, to see how well all of the different ones combined. Robert Phair has come up with another one which we we can express as the tryptophan/kynurenine ratio or the activity of IDO1 and that also might be a diagnostic. That's another thing that's easy to measure, so you have to hear his talk to understand that one (Robert Phair, PhD | Metabolic Traps: A new way to think about ME/CFS https://www.youtube.com/watch?v=uej1LXzRbnY&index=16&list=PLl4AfLZNZEQPxjqF4ojAO3wdCFMeriNBK) but that's going to go into the comparisons as well.

So the hope for this is we'll find some diagnostic tool that is very close to a hundred percent accurate. It has to be able to be implemented, it has to be simple enough that low skilled technicians can run it without making big mistakes. That's not easy, but I think we have all the right experience and expertise to do that.



17:32 So this is the team that we've had and also here at Stanford that helped us in all this. It is a big team effort, so thank you very much.

[Applause]

**Excerpt from 'Q&A Panel Discussion 2', https://www.youtube.com/watch?v=mSm8P5v8wCA*
11:14 ***Dr. Ron Davis:*** *So when you get a infection, not all infections, but many infections activate IDO1 and that is consistent with some of the patients saying, and we seen in our own son, you get better after an infection. In fact there are some cases that have been reported, that people actually get cured by an infection. They say I'm over it and you ask how'd you get over it, I had an infection and all the sudden I got better. Now don't go out there and eat dirt and try to find some microbe right, hoping to get a bad infection because it could kill you and it's not guaranteed to activate IDO1 (Robert Phair, PhD | Metabolic Traps: A new way to think about ME/CFS https://www.youtube.com/watch?v=uej1LXzRbnY&index=16&list=PLl4AfLZNZEQPxjqF4ojAO3wdCFMeriNBK). But you know there are some things here that have substance that we can figure, we can explain, can explore.*

Thank you to our wonderful volunteers for transcribing the Symposium.