

WEBVTT

NOTE duration:"00:26:10.4560000" language:en-us

NOTE Confidence: 0.859801054000854

00:00:00.060 --> 00:00:11.630 Numa score actually an Habermann's presenting now on the flow cytometry shared Resources, which is one of our oldest and scope used and most impactful.

NOTE Confidence: 0.889191508293152

00:00:12.180 --> 00:00:29.400 Shared resources and is in the Immunology Department. She's in the cancer immunology research program an In addition to the work. She will be discussing about slow her research is also centered on really beautiful to photon image Ng.

NOTE Confidence: 0.88677316904068

00:00:30.350 --> 00:01:02.600 Your immune system in action and more and more thank you. Thank you that introduction like David mentioned on the director for the flow cytometry core, which is managed by expertly by Jeff Lion, who I believe is in the audience to so this is a fee per surface cost recovery facility that provides open access to all yell. Investigators many of you are already familiar with some of the equipment.

NOTE Confidence: 0.896137535572052

00:01:03.150 --> 00:01:25.600 And those who are may not be aware of some special features and capabilities like David was saying. It's challenging on this campus to advertise elements of your core facility and so time permitting. I'd really like to spend a little time describing to other core facilities that which you may not be aware of, but could be particularly impactful for cancer related research.

NOTE Confidence: 0.928967595100403

00:01:29.590 --> 00:01:42.390 OK, so as the name suggests flow cytometry. This technique involves the quantification of florescence of cells that are moving through fluidics and as the cells move through the interrogation point.

NOTE Confidence: 0.628762543201447

00:01:43.090 --> 00:01:44.740 Here we go.

NOTE Confidence: 0.88902485370636

00:01:47.460 --> 00:02:19.170 Pretty hard to point here as the cells are moving through the interrogation point. They are going past. A series of lasers and out off of each laser. The admission that emanates at that point in time is collected in dependently to cohort of detectors for his pass through a serious of optical filters. And this is where set amateurs can differ the most is in the nature of

the lasers that they're outfitted an in the way that they parse out the light that they collect.

NOTE Confidence: 0.361144632101059

00:02:19.860 --> 00:02:20.430 Laser.

NOTE Confidence: 0.896100163459778

00:02:23.300 --> 00:02:54.490 So, In addition to fluorescence detection. There's other parameters that can also be collected as the cells move through the cytometer that include something known as forward scatter where detectors just on the other side or at an angle to the laser detects what moves forward or off to the side, so in the case will forward scatter. It can be used as a circuit for cell size in terms of side scatter is a circuit for internal side as well.

NOTE Confidence: 0.880937814712524

00:02:54.900 --> 00:03:18.410 The so those 2 together, even in the absence of all fluorescence can be used to distinguish many different lookaside cell types, but then of course, fluorescence is quantified and distinguish over the course of many, many logs, although great sensitivity can be also discerned when they're very low levels of protein as well.

NOTE Confidence: 0.911756932735443

00:03:20.010 --> 00:03:43.900 So step wise of course, this begins with the acquisition of your sample, which needs to be prepared as a single cell suspension to pass through the flow cytometer for people who are doing human studies. This can often involve the cryo preservation of your samples so that later very large collection of samples can be analyzed simultaneously on the same theme cytometer.

NOTE Confidence: 0.921112537384033

00:03:45.810 --> 00:03:47.830 Then the cells are.

NOTE Confidence: 0.885296940803528

00:03:48.610 --> 00:03:57.120 The cells are stained with a recently tagged or reagent, which is very typically antibodies on specific before particular proteins.

NOTE Confidence: 0.898801445960999

00:03:57.810 --> 00:04:30.840 And then you hope to pass your samples through a well maintained well calibrated instrument with confidence for your data is acquired and then later. It is analyzed. And here is where we are in particular, most helpful and that we offer a large collection of instruments, which are differently configured ensure that when you arrive to the equipment that you can wear confidently acquire your samples without loss.

NOTE Confidence: 0.902566373348236

00:04:31.410 --> 00:04:47.130 To get you up and going with data analysis using the gold standard software for flow cytometry. That's flojo for this. We also oversee a site license at yell at very low cost to individuals.

NOTE Confidence: 0.942375242710114

00:04:50.070 --> 00:04:55.390 I'm going to return to the analytic equipment and in just a bit, but I want to start with.

NOTE Confidence: 0.887588620185852

00:04:56.070 --> 00:05:01.240 When cells sorting first let's see if this slide here.

NOTE Confidence: 0.844809293746948

00:05:06.980 --> 00:05:13.610 Brand new computer my last one just fried last weekend series that was one of his touch bars that agonized.

NOTE Confidence: 0.895298898220062

00:05:14.180 --> 00:05:44.190 Not used here OK, so with cell sorting it. In contrast to the analytic equipment. Whereas the cells go past the interrogation point. They just go straight into waste and those can be recovered will still sorting and stud samples can be sorted an collected based on their fluorescent futures by a process of deflection largely usually this involves some electrostatic charge, but does not always case. Sometimes it's through disposable chips that use.

NOTE Confidence: 0.912942826747894

00:05:45.310 --> 00:06:16.920 But regardless of the means they can be collected in 2 temperature controlled containers. Many people are not aware that in addition to a chilled chamber. You can also have warmed chambers or for those who need immediate application to something that must be at a physiologic temperature. In addition to these systems can also deposit your sight your samples on to slides directly another very classical future.

NOTE Confidence: 0.897702813148499

00:06:17.710 --> 00:06:50.620 Deposit in your samples into plates, either, as individual cells or you know truly whatever number of cells. You want per well. A future that most people are unaware of is indexed plates sorting and that is where the information in each individual event that goes into a particular well is recorded and retrospectively you can then go back to 2 again analyze the typical features of the cells that were in that well. This is handy. Let's say if you're doing an in vitro assay and you want to look at the.

NOTE Confidence: 0.846295952796936

00:06:51.310 --> 00:07:00.760 Are the failures in some aspects and then to look backwards to see? What level of PD one. You know say for example, you may may have on that sample.

NOTE Confidence: 0.899449050426483

00:07:03.200 --> 00:07:34.330 Here just a quick moment to talk about some of the features that distinguish these the BD Facs Arios. Uh we have 5 reviews on campus. There they are considered the gold standard or they once were the gold standard for cell sorting in part because of the very high speed. Two of these are inside biosafety cabinets, so for people who are working with human cells. We have a paired set of sorters that are identically configured inside bio safety cabinets, one inside the Amistad Building, an another.

NOTE Confidence: 0.890815734863281

00:07:34.800 --> 00:07:40.650 PH inside the bio safety Level 3 facility for people who are working with infectious samples.

NOTE Confidence: 0.879511296749115

00:07:43.290 --> 00:08:10.860 In addition, we've held on to Beckman Coulter, both low, although it doesn't have as quite as many of the bells and whistles, but it does have in the way of features is a lower pressure an ability to employ larger natural nozzle sizes and this is best for very large cell types. If you can imagine a dip aside so or other more fragile cell types? Do very well on this cell sorter.

NOTE Confidence: 0.897190868854523

00:08:13.560 --> 00:08:43.060 And the Sony SH 100. We have only one of the examples of this at the moment, yeah, it's in the attack building. It has particularly intuitive software very easy to learn and for this reason, we've made it available for a self sorting, including after hours, so for physician scientist clinicians who acquire samples late in the day without much advance notice would have likely ready access to this instrument after 5.

NOTE Confidence: 0.89675772190094

00:08:44.450 --> 00:09:12.130 In this case has for laser lines and contrast to some of them. The BD areas, which are often configured with five losers and this instrument is best for people who are only requiring 2 populations. An no more than 6 colors at this point in time. We are hopeful that we may soon acquire funding for an upgrade on this for for populations multiple entire interrogation points that will allow for more colors.

NOTE Confidence: 0.890432775020599

00:09:13.800 --> 00:09:33.470 Like I mentioned our instruments are spread out on campus, including a cell sorter. Anna and Elsa are 2 Analyzer and 300, George St Samad Amistad. I mentioned the biosafety Level 3 cell sorter, but the majority of equipment is in the tax building.

NOTE Confidence: 0.870585083961487

00:09:35.210 --> 00:10:05.270 Returning now to the analyzers. Most people are probably most familiar with the BDLS R2. Those who are doing a flow cytometry. Those are currently configured with 4:00 and 5:00 lasers. The BD vex calibers are aging instruments with only 2 lasers. The strata times, however, are more mid tier in their capacity. One of these is outfitted with a plate loader offer people who need to assess in vitro assays or.

NOTE Confidence: 0.896186530590057

00:10:05.320 --> 00:10:37.050 To survey a large number of independent samples, but the one I'd like to focus on today is the Amna's image stream image. Ng cytometer, so this instrument is combines the sensitivity of flow cytometry with the detailed imagery and functional insights of microscopy so as cells flow would pass the interrogation point. It's collecting that just the mean fluorescence in every channel, but also obtaining an image of every single event that is blowing past that interrogation point.

NOTE Confidence: 0.926025629043579

00:10:37.720 --> 00:10:46.990 Just this then gives you the great ability to have large statistics associated with an image that can be otherwise very hard to acquire by microscopy.

NOTE Confidence: 0.912049293518066

00:10:47.720 --> 00:11:07.740 Have a lot of staff support for both the acquisition and the analysis. Although the image analysis of the data. Afterwards is pretty Intuitive. However, we can. Most certainly help you get up an on your way with that. Alternatively, we can perform that analysis for you, or in each of these cases perform the service entirely for you.

NOTE Confidence: 0.871537983417511

00:11:08.560 --> 00:11:11.040 Except for this all standing that you have to do.

NOTE Confidence: 0.906955480575562

00:11:13.330 --> 00:11:43.440 Alright so there are many different applications. Let me just go over a couple the data in many ways looks like traditional flow cytometry. You can get on it, so and then look at individual images every event that falls within that gate so in this case for a cell cycle measurement here on the X axis is drag 5 for an assessment of DNA and of those that are higher denic content. Here, you can see, there are.

NOTE Confidence: 0.908645868301392

00:11:43.470 --> 00:12:13.060 Many veteran anaphase telophase you can then also use this analysis to quantify other morphologic features aspect ratio total cell size. Anything that is punctate. You can quantify even the number of individual spots that are within a cell. It really if you think about it anything that you could mentally articulate is something that you would want to measure

as something that you can then define as a parameter for any pipeline in your analysis.

NOTE Confidence: 0.908254265785217

00:12:14.560 --> 00:12:36.400 Here's another an example for a pop ptosis in Ducks in which you're assessing the size of the nucleus at the extent of condensation. But here on the X axis is a parameter for the extent of fragmentation so this more clearly highlights a pop ptosis without any additional markers needed.

NOTE Confidence: 0.900183260440826

00:12:38.260 --> 00:13:13.010 But you could easily imagine any a variety of applications for example, internalization or Association with the plasma membrane or the side is all cell cycle DNA damage. Probably the most heavily employed for those purposes quantification of movement of fluorescently tagged anything between the side as well, and the nucleus and relating that as a ratio for success in cell signaling. It's also been used for defining immunological synapse is an features that are within or excluded from that.

NOTE Confidence: 0.910600662231445

00:13:15.510 --> 00:13:45.960 So the staff for this core is very can do an very approachable and the idea is that there should be a feeling that assistance is there for you. On this campus that if there's something that you want to achieve relating to flow cytometry. We want to make that possible for you and certainly when it comes to panel design choosing your flow cytometer or choosing your floor forest, which can for many people, the same blue bewildering, especially as more heavily outfitted equipment comes about.

NOTE Confidence: 0.808824717998505

00:13:46.710 --> 00:13:49.600 With them a technical resource as well.

NOTE Confidence: 0.911290347576141

00:13:50.510 --> 00:14:18.590 So as I mentioned many, many fluorescent compounds out there, many, many different fluorescent proteins. And here on the right hand side is just a series of I've just only some of the guys that are available off of the Violet laser can be excited with the Violet loser and the goal really is to choose floor force that have minimal spectral overlap and we're here to help you make those choices and to help you choose. The cytometer that's right for your particular application.

NOTE Confidence: 0.909760653972626

00:14:19.490 --> 00:14:50.340 Our website has a number of helpful resources here, you can find information on how to join the Flojos Light license. We also have a couple of hallway workstations for the analysis in order to minimize the congestion on the cytometer's we also have a way to remotely. Access your data, an on line scheduling system so that you need and contact any individual to adjust your plans.

NOTE Confidence: 0.921977341175079

00:14:51.790 --> 00:15:22.410 We're very excited about the possibility of acquiring a BD Symphony, which we think likely will occur soon. Although the funding yet for this has not been yet completely nailed down the Cancer Center has very kindly offered to pay for various substantial portion of this instrument, which is which is extremely powerful. It's designed for very high dimensional studies in a way that is future proof and I find that.

NOTE Confidence: 0.892397522926331

00:15:22.990 --> 00:15:54.740 Exciting but it has the ability to install up to 10 lasers, including very unique laser lines on such as deep UV312 nanometer there. BD is about to release a whole series of tandem dies off of the deep UV which will instantly add 3 colors to anybody who wishes without any spectral overlap as well. Currently, there is a 785 and a 980 nanometer laser that you could install the 785 there's already 2 existing.

NOTE Confidence: 0.88075190782547

00:15:54.800 --> 00:16:26.910 Flora force that could be likewise instantly added to Europe. Anna this instrument has a Max of 48 detectors. But you have the option of quitting as many as 10 detectors half of any single laser and with this means for us. Even immediately is that the additional detectors prevents permits the easier discrimination of dies off of each laser with smaller number of detectors. The band passes before them need to be broader and that makes it a little more challenging to avoid spectral overlap.

NOTE Confidence: 0.917131960391998

00:16:27.600 --> 00:16:29.120 It helps to minimize that.

NOTE Confidence: 0.905468702316284

00:16:30.190 --> 00:16:41.640 So even with the addition of the 785 nanometer laser this very comfortably would allow for the separation of over 24 four is even even today.

NOTE Confidence: 0.884663462638855

00:16:44.250 --> 00:17:15.060 Alright so that's all I was going to say about that core facility and I'd like to move on to a couple of others. One of which could be extremely helpful for clinicians and position. Scientists even those who don't have laboratories, so this. This core known as the immune monitoring core facility is servicing by Leslie Divine and together her staff can together her staff can process blood an perform cryopreservation.

NOTE Confidence: 0.890766501426697

00:17:15.090 --> 00:17:45.860 They can stay in those samples for you perform all the flow cytometry for you, or allow you to take many of these steps yourself independently. There are also capable of performing all the data analysis for

you, including the possibility for more complex intracellular side kind staining they can also do self sorting for your populations anhave bio. Plex, 200, which is the equipment needed for a luminex tile sample analysis.

NOTE Confidence: 0.877347052097321

00:17:46.290 --> 00:18:08.870 To perform defined Lisa's with your a theorem if you so wish so this is a gun for Howard higher for clinicians who say for example, may wanna do correlative studies, but they themselves don't currently have a lab or staff to do this, or for the busy physician scientist who needs to maximize output with minimal staff.

NOTE Confidence: 0.892592370510101

00:18:10.400 --> 00:18:24.990 So I think I don't know if Leslie is here, but she had mentioned that she might be a bit odd. She's easily contactable here and could answer any of your questions. I think of those particular can do you bring her the samples and the rest can be done for you?

NOTE Confidence: 0.90506249666214

00:18:26.430 --> 00:18:56.660 Now moving on to something completely different this is like David was mentioning is really very hard to advertise facility is on this campus and so it's very nice to have a venue in order to be able to do that. And this one's particularly boutique. But for the right sets of questions extremely powerful. I also receive this core facility together with a highly talented facility manager an operator David Gonzalas, The This is.

NOTE Confidence: 0.889640986919403

00:18:56.690 --> 00:19:28.920 Also, a fee per service facility designed for the preparation of mice for the physiological time result imaging but it's also very ideal for image analysis of 3D reconstructions, so fundamentally this core facility is at its most simple level. A single microscope. It's a laser scanning microscope. That's up right? That's outfitted with a 2 photon laser and I'll describe in a minute have as an advantage.

NOTE Confidence: 0.903682291507721

00:19:29.490 --> 00:19:53.680 Is very superb optical sectioning at much greater depths in tissue than confocal microscope say for example in this case has been customized to accommodate live a Nesta ties mites in order to also In addition, offer the possibility for effectively time lapse image. Ng of cells that are performing their daily functions inside tissue physiologically.

NOTE Confidence: 0.925031363964081

00:19:54.560 --> 00:20:11.630 So we can perform all that work for you, or if you prefer we can train you to do any element of the animal preparation of microscopy where the image analysis. But we highly suggest that you get in touch with this person we can advise you an experimental design.

NOTE Confidence: 0.901081740856171

00:20:11.660 --> 00:20:42.690 The solution the Florissant solution inside this cuvette is clearly being excited. All the way down to the focal point, and beyond whereas with the the 2:00 photon laser coming through this bottom. But objective you can probably just barely discern that excitation is only occurring at the pulses of photons. It's not continuous wave their short pulses of much longer wavelengths and therefore of Lower Enerji and because multiple photons can be absorbed.

NOTE Confidence: 0.902847230434418

00:20:43.260 --> 00:21:01.180 CIV the same amount of excitation. This is a large and a vantage. But in the case with multiphoton. It has to be absorbed essentially a simultaneously when that happens, then the emission that occurs afterwards is characteristically the same whether it came from a single photon excitation or or 2.

NOTE Confidence: 0.895146250724792

00:21:03.030 --> 00:21:33.180 So the consequences for that, though, is huge so in this image on the right hand side. The laser coming through the top objective is a conventional is our single photon if you if you like you can see that the solution. The fluorescent solution inside. This cuvette is clearly being excited. All the way down to the focal point, and beyond whereas with the 2:00 photon laser coming through this bottom of objective you can.

NOTE Confidence: 0.907468497753143

00:21:33.210 --> 00:21:59.810 Probably just barely discern that excitation is only occurring at the focal point, so the advantage. There is that the light doesn't now have to be D scanned through a pinhole and instead all the light can be collected even deep from within tissue. Knowing exactly from one set derived and there's also minimizes. The photo damage and photo. Texas city that would happen above or below. That plan of focus, which allows you to image for much longer period of time.

NOTE Confidence: 0.921211898326874

00:22:01.360 --> 00:22:33.550 You can do this with pretty much any tissue that can be brought up to the objective physiologically with minimum disruption and hopefully minimum surgery. This works best if you're relevant regions of interest are within 350 microns of the surface of your tissue. But here there's many examples and even more tissues that are readily accessible on this way, including skin bone marrow within the calvarium.

NOTE Confidence: 0.865902125835419

00:22:34.160 --> 00:22:54.040 Inguinal or perhaps a chill on the mouse, subcutaneous tumors test and's lung even intimated minus if you can bring that issue to the lens and the mouse is happy, then it's something that you could observe

you just merely need to add fluorescence and I'll just give some examples of ways that you can do that.

NOTE Confidence: 0.913841784000397

00:22:55.240 --> 00:23:26.530 So here for inspiration. I just want to go over a few examples of parameters that can be addressed over overtime or in some cases, statically probably classically in the motivation for a lot of people in the beginning was just to assess rate, and direction of movement and timing and duration of cell contexts consequences. The behavior of these cells after events and so, if you've defined your tissue landmarks in many cases that's not even necessary.

NOTE Confidence: 0.916471123695374

00:23:27.240 --> 00:23:48.550 You can get a lot of insight into where cells are going after an event has happened in this particular movie? What's in green are activated B lymphocytes and red activated T lymphocytes and there is swimming in the context of non responding B lymphocytes that are expressing CFP here and this is in a popliteal lymph node.

NOTE Confidence: 0.908530533313751

00:23:49.490 --> 00:23:56.680 And what you can see up here are some examples of long lived in my logical glow synapses that are formed.

NOTE Confidence: 0.879854559898376

00:24:00.270 --> 00:24:30.740 All right, but you can also place this in the context of your tissue architecture or within a niche in this case. I my lap has used a boner a camera in which all the stromal cells are fluorescent and this. Kaymer received instead non fluorescent bone marrow and later the adaptive transfer of GFP and see if he expressing B cells immunized and now here we're seeing the behavior and their interaction with particular stromal cells.

NOTE Confidence: 0.904984951019287

00:24:31.470 --> 00:24:33.150 Stage of the immune response.

NOTE Confidence: 0.874089062213898

00:24:36.570 --> 00:25:07.900 So those examples prior involved adoptive transfers, but you can also take advantage of genetic modifications. Mice directly in this particular case that movie is an integrated mouse lung that of a mouse that was expressing Cree under the direction of vascular endothelium quickly expressing mirrors related TD tomato in the absence of creates though.

NOTE Confidence: 0.86863762140274

00:25:08.570 --> 00:25:37.160 What's GFP expressing our vascular endothelial if this were to receive other forms of fluorescents. You'd be able to take that in context, and then just one last example is Melanoma cells here expressing history in HDB. I'm cherry Fusion protein. You can see the active cell division

and the interactions with dendritic cells expressing YP under the direction of C11C.

NOTE Confidence: 0.918463349342346

00:25:38.380 --> 00:26:05.310 There's a wide variety of fluorescent tools that are available and to help you define context or features indicators for cell death. Fate mapping and we're certainly available for after any suggestions or inspiration in order to help you adjust your questions. The image analysis again. Anything that you could imagine that you want to quantify can be quantified and this can be quite powerful.

NOTE Confidence: 0.89460563659668

00:26:05.980 --> 00:26:07.920 And I will stop there.

NOTE Confidence: 0.807526290416718

00:26:08.580 --> 00:26:09.620 Any questions.