



JUN 19, 2023

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Protocol Citation: Bryan Cannon, Christine Camacho 2023. Commercial MIBI Walkthrough: Protocol & Instructions. [protocols.io](https://protocols.io/view/commercial-mibi-walkthrough-protocol-and-instructions-cttzwnp6)
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Protocol status: Working
We use this protocol and it's working

Created: May 05, 2023

Last Modified: Jun 19, 2023

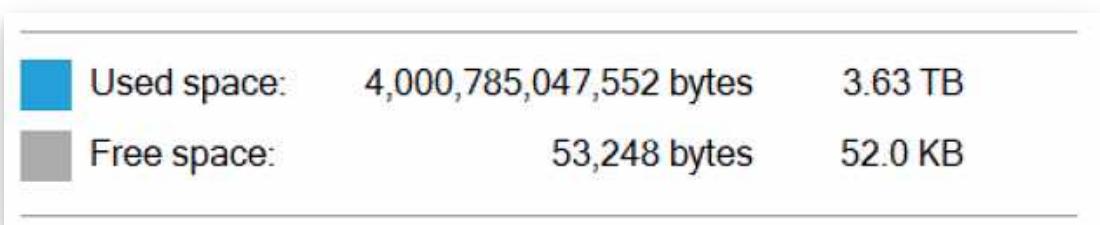
PROTOCOL integer ID:
81497

Pre-check

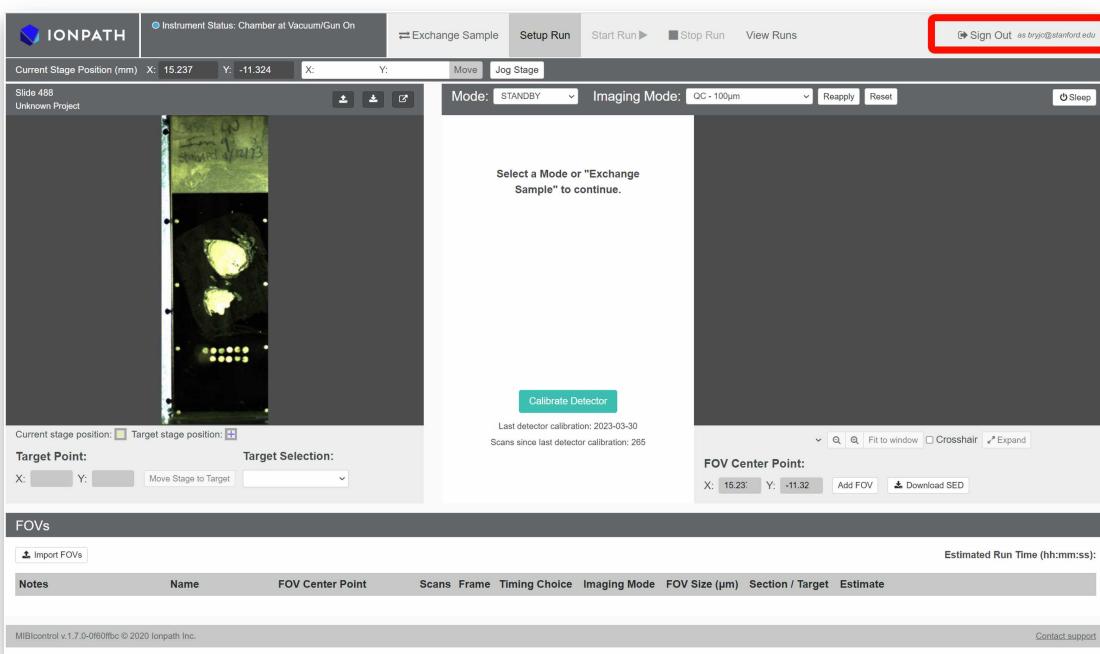
- 1 Ensure the machine you're working on (P9, M13, M16) is currently UP on the [machine status sheet](#) and slack channel #mibi_instrument_status.

MIBI Instruments Queue 2023					
File Edit View Insert Format Data Tools Extensions Help					
G13	A	B	C	D	E
1	MAY	Betty	Creed (P9)	Drago (M13)	Adriane (M16)
2	Status	Up	Down (ticket submitted)	Down (ticket submitted)	Up
3	Instructions:	Use Betty for test runs, titrations, and non-cohort related tests. Use commercials for dress rehearsals, cohorts, and runs that can't be completed on Betty.			
4	On days an instrument is down, please be sure to update Slack channel mibi_instrument_status. Please also indicate down days on this sheet. (Red background = DOWN)				

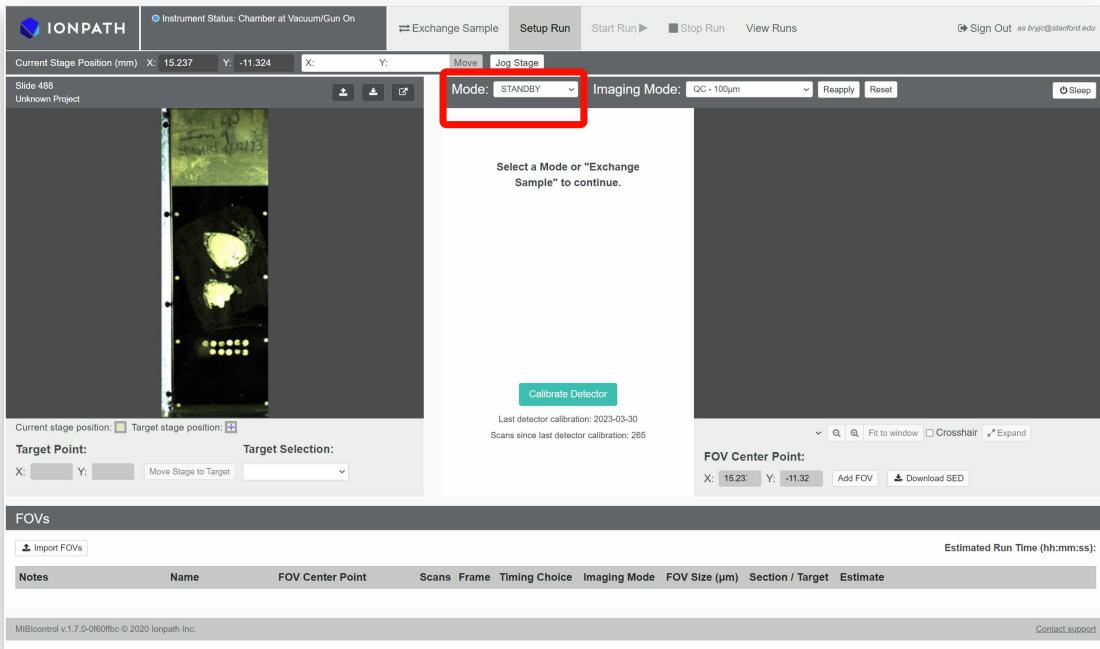
- 1.1** **NOTE:** If you encounter an issue with your run and need to send a ticket to Ionpath (support@ionpath.com), make sure to update the sheet with the machine as DOWN and cc Mako (makogold@stanford.edu) & Christine (christinecamacho@stanford.edu) so they can sign the machine back to UP status after resetting the appropriate imaging settings once Ionpath has finished.
- 2** Check available space on machine's D drive, named MIBIData (ensure there is at least 50GB on drive free for non-cohort runs)



- 3** Log into MIBI Control with your account (upper right hand corner of UI)



- 4** Unless actively navigating your tissue or slide, make sure to keep the MIBI Control in **STANDBY** mode (not SED or SPOT)



- 5** Make sure Lens 1 voltage where it should be in HV Control Chrome tab: ~505V.

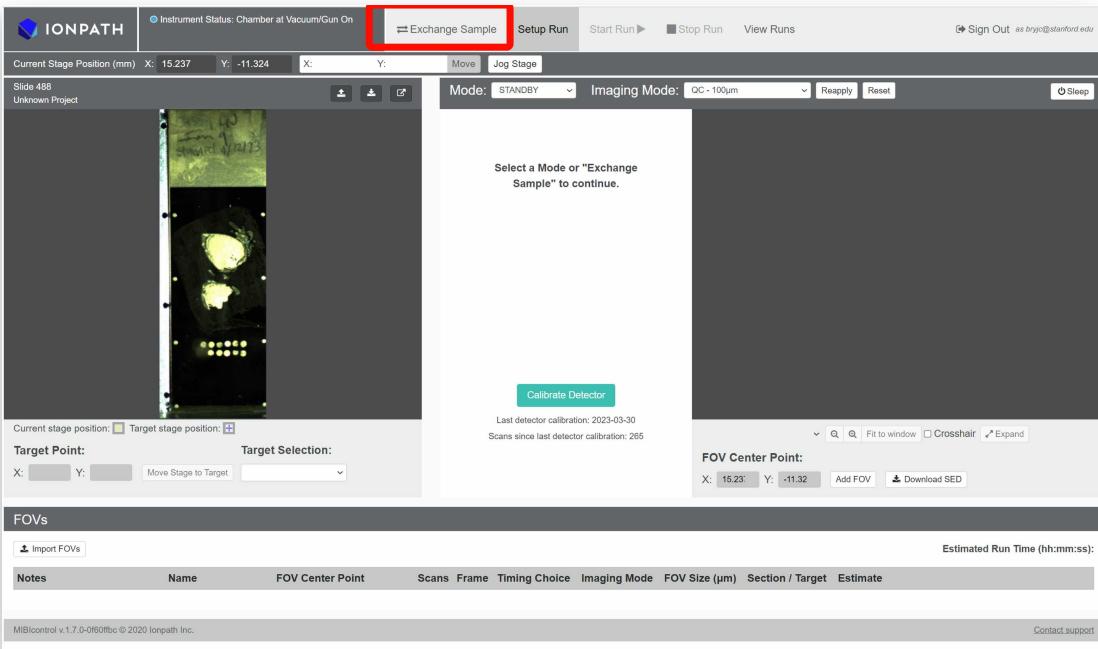


5.1 DO NOT Change - talk to Mike, Marc, Christine, or Mako if it shows a different value.

Sample Exchange

- 6** ■ One slide at a time - press Exchange Sample in MIBI Control, and confirm. Take the current

slide out of the slot further from you and put your slide into the slot closest to you.



6.1 Make sure the slide loads properly; the image should load in the left window. Assign slide ID as created in [Ionpath MIBtracker](#) under your account.

Note

The panel and slide ID must be entered and linked in the Ionpath MIBltracker prior to sample exchange.

6.2 Enter your run, date, and slide info into the log sheet

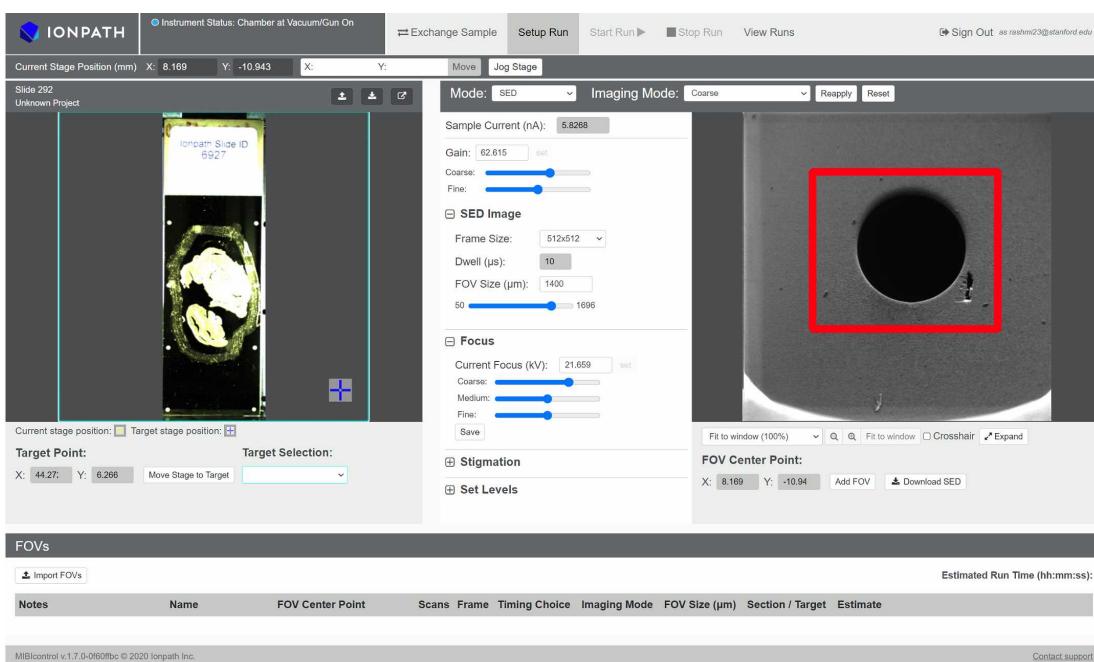
Quality Control: General

- 7
- **Current check:** confirm imaging mode current values match expected output.
 - **Detector Calibration:** To maintain a consistent ion response, or image brightness, the detector gain is calibrated by measuring Molybdenum Foil counts over a range of gain settings.
 - **Molybdenum Foil check:** confirm built-in, general counts acquired at a specific current.
 - **PMMA check:** confirm specific counts at mass ranges of interest at a specific current.
 - Ionpath [Quick start guide](#) outlines more specific details as needed.

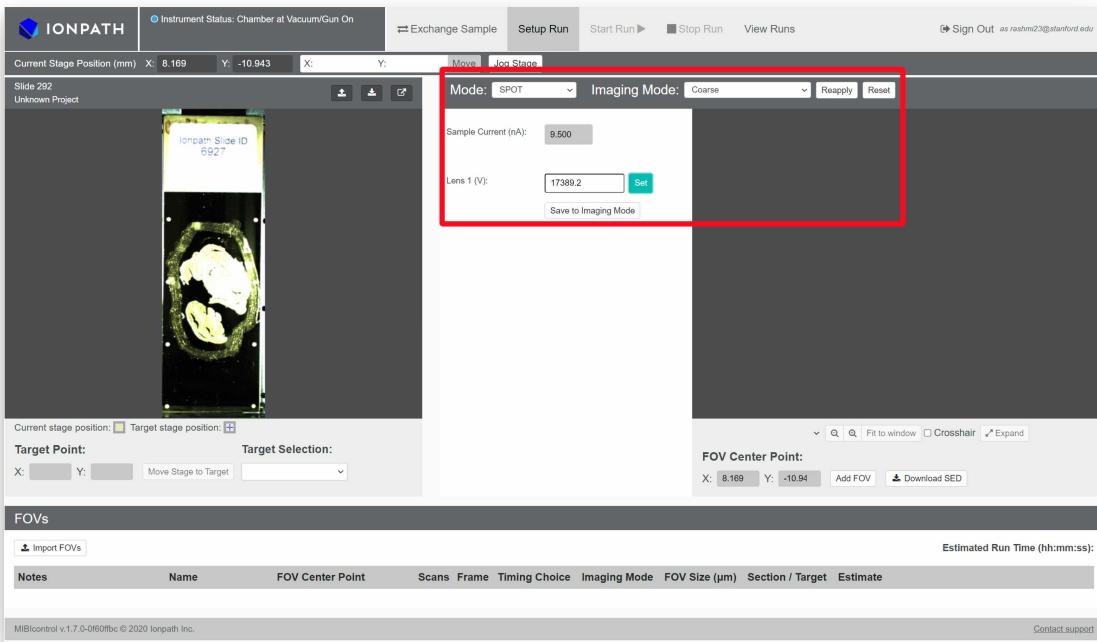
Quality Control: Current

- 8 Check current values for consistency and accuracy.

- Change 'Target Selection' to select Faraday cup.
- Switch Mode to SED.
- Alter Detector Gain to see image.
- Turn on image crosshairs and use jog to center cross to the middle of the cup.



- 8.1
- If acquiring in **Coarse** mode: Change Mode to SPOT, Imaging mode to Coarse.
 - Sample current - want around 9.5.
 - Change Lens 1(V).
 - Record values in log sheet.
 - **SAVE** to Imaging Mode.

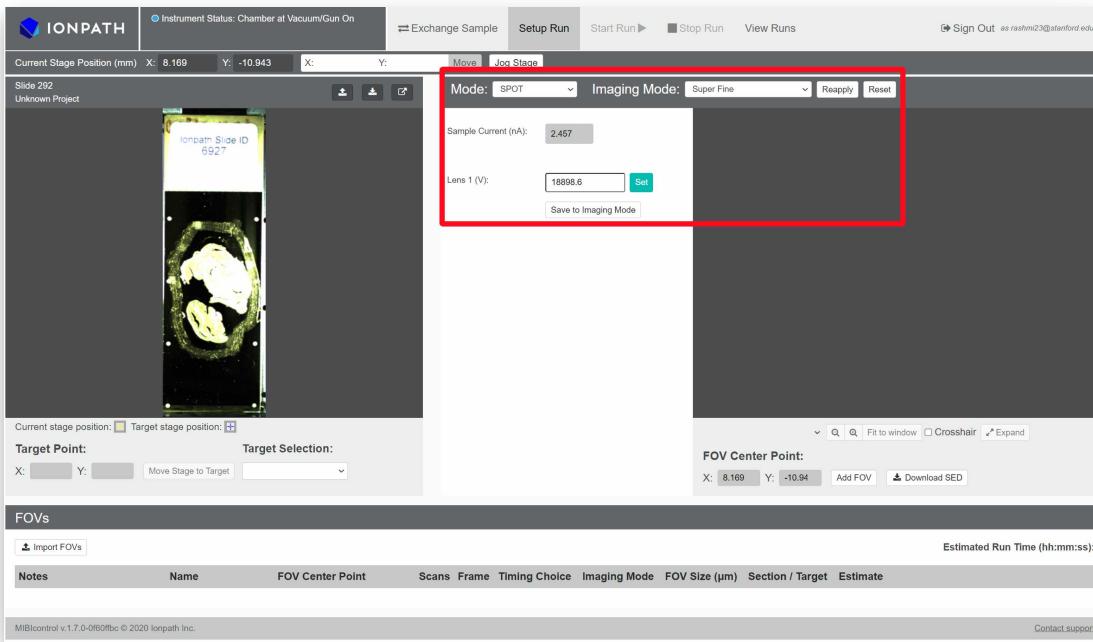


Note

The sample current (nA) and lens 1 (V) have an inverse relationship. To decrease the sample current, increase the lens voltage incrementally.

8.2

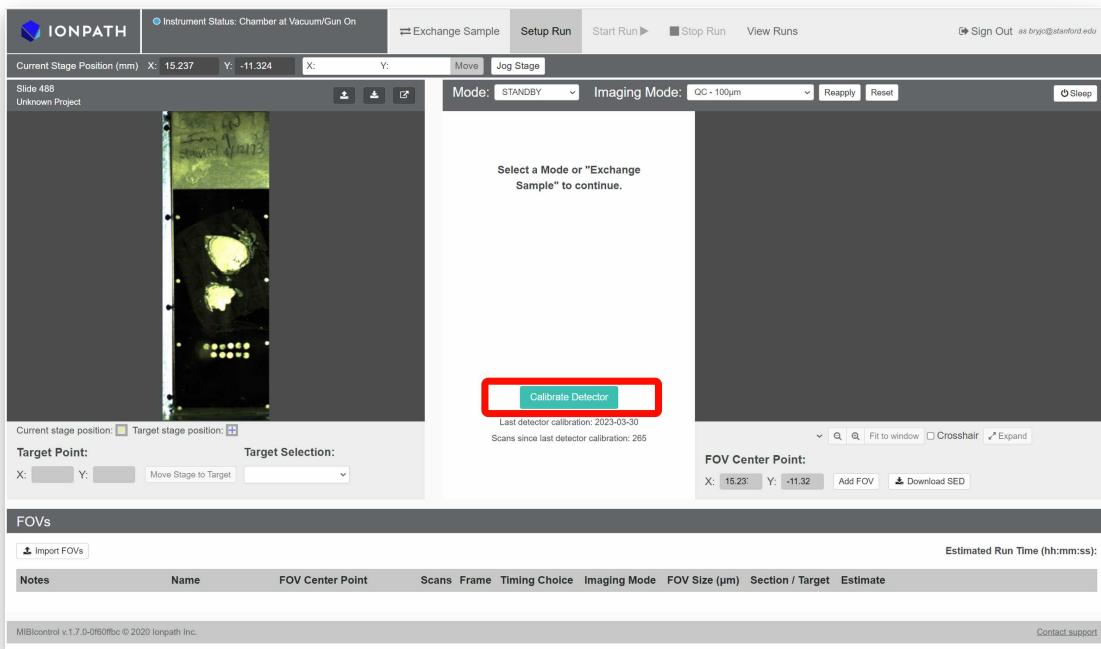
- If acquiring in **Super Fine** mode: Change Mode to SPOT, Imaging mode to Super Fine Imaging Mode.
- Sample current - want around 2.5
- Change Lens 1(V).
- Record value in log sheet.
- **SAVE** to Imaging Mode



Quality Control: Detector

9 Detector Calibration

- Press button, and MIBI Control will begin a detector sweep.
- MAKE SURE NO FOVS ARE LOADED AT THIS POINT OR CHECK WILL OVERWRITE.



If the calibration passes, record detector value from HV Control (top image) in log sheet

9.1 (bottom image)



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	Q	
i	Run name	Date	Slide	Fc coarse	L1 coarse	Fc Superfine (Angelo Custom)	L1 Superfine (Angelo Custom)	Moly 92 counts	Moly 98 counts	98MPH from previous run	92Mo (MPH)	Target Mo98 (MPH)	L2	Stig deg	Stig Amp	Detector V	Acquisition time
193	2023-03-29:ATC_TestGAP	2023-03-29	03002	0.954	17433.1						21.677	166	7	2270		24min	
194	2023-03-29: ATC_TestGC	2023-03-29	03002	0.954	17433.1						21.677	166	7	2270		24min	
195	2023-03-29:T99CountCC	2023-03-29	03099	0.938	17096.4						21.677	166	7	2270		24min	
196	230405 Test	2023-04-05		0.951	17398.8	2.495	18918.8	1681393	548052								
197	230405_MBIIButGold_Slide Control	2023-04-05	03063	0.951	17395	2.521	18917.2	8992	4911								
198	230405_MBIIButGold_Slide Control 2	2023-04-05	03063	0.951	17395	2.521	18917.2	46149	1092431								
199	230405_MBIIButGold_Ad_Resistance Edge Test	2023-04-05	03097	0.954	17395	2.546	18917.2	289961	1092431				21.677	167.1	7.5	2330	
200	230508_ISH_parameterOptimization	2023-04-06						546174	1222257							2312	
201	230405_HV test5	2023-04-06	Slide 401	0.976	17394			651874	1301066				21.667	165	7.5	869min	
202	230408_eyo_eyo	2023-04-08	Slide 402	0.976	17398.2			701298	1301064				21.667	165	8	2308	
203	230408_eyo_eyo	2023-04-08	Slide 403	0.951	17683			544272	1218548				21.665	165	8	101min	
204	230409_DNA891 test	2023-04-09	Slides 404-406					455419	1046798				21.665	165	8	101min	
205	230411_HV test5_rerun	2023-04-11	Slide 401	0.954	17408.3			451555	1109846							2350	
206	2023-04-13T14-20-22_GFAP AD Test	2023-04-13	Slide 402	0.951	17411			451539	1298482							2377	
207	230411_HV test5_rerun	2023-04-11	Slide 401	0.954	17408.3			583735	1298482				21.524	160	7.5	2412	
208	2023-04-13T14-20-22_GFAP AD Test	2023-04-13	Slide 402	0.951	17411			509750	1298482				21.663	165	8	2412	
209	2023-04-14_DNA891_hexap Test	2023-04-14	Slide 430-432					2002161	1278114							2450	
210	2023-04-14_BannerTest	2023-04-14	Slide 433	0.926	17419			620259	1376546							2468	
211	2023-04-14_eyo_eyo	2023-04-17	Slide 434	0.954	17417.9			575469	1324031				21.665	164	8	2495	
212	2023-04-16_DNA891_TA899	2023-04-16	Slide 435	0.956	17417.9			588569	1326010				21.66	165	8	102min	
213	2023-04-16_DNA891_TA899	2023-04-16	Slide 436	0.956	17419			471244	1285469				21.66	166	8	2529	
214	2023-04-21_ISH_Beta	2023-04-21	Slide 404	0.95	17414.7			500791	1106273				21.667	166	7	2630	
215	2023-04-21_ISH_Beta_ test_2	2023-04-21	Slide 404	0.95	17414.7			465916	1143993				21.667	166	7	2844	
216	2023-04-23_DNA891_Beta_ 2023test	2023-04-23	Slide 450	0.951	17415			489546	1085176				21.667	166	7	3015	
217	2023-04-23_DNA891_hexap test	2023-04-23	Slide 450	0.951	17415			112601	1054756				21.667	166	7	3015	
218																	
219																	

9.2 If the calibration fails, up Detector voltage in HV control (bottom of page) and run again. If subsequent calibrations fail, then talk to Mike, Marc, Christine, or Mako.

Quality Control: PMMA Counts

10 If running a PMMA, you can use the instructions below with the PMMA slide assigned to each machine.

- Load PMMA slide and move stage to a position close to previously imaged PMMA FoV.
- Switch Mode to SED.
- Alter Detector Gain to see image.
- MAKE SURE NO FOVS ARE LOADED AT THIS POINT OR CHECK WILL OVERWRITE.
- Set up FOV - 512 x 512, 200um, 0.5 ms, 1 scan, Imaging mode: Coarse, Target: PMMA slide.

Add FOV

Confirm FOV to continue. At least one FOV is required to Start a Run.

FOV Center Point: *X: 8.169 *Y: -10.943

*Scans: 1 *Frame: 512x512 *FOV Size (μm): 200 μm

*Dwell Time: 0.5 ms

Notes: Remaining characters: 80

FOV Name: PMMA-Check

*Imaging Mode: Coarse

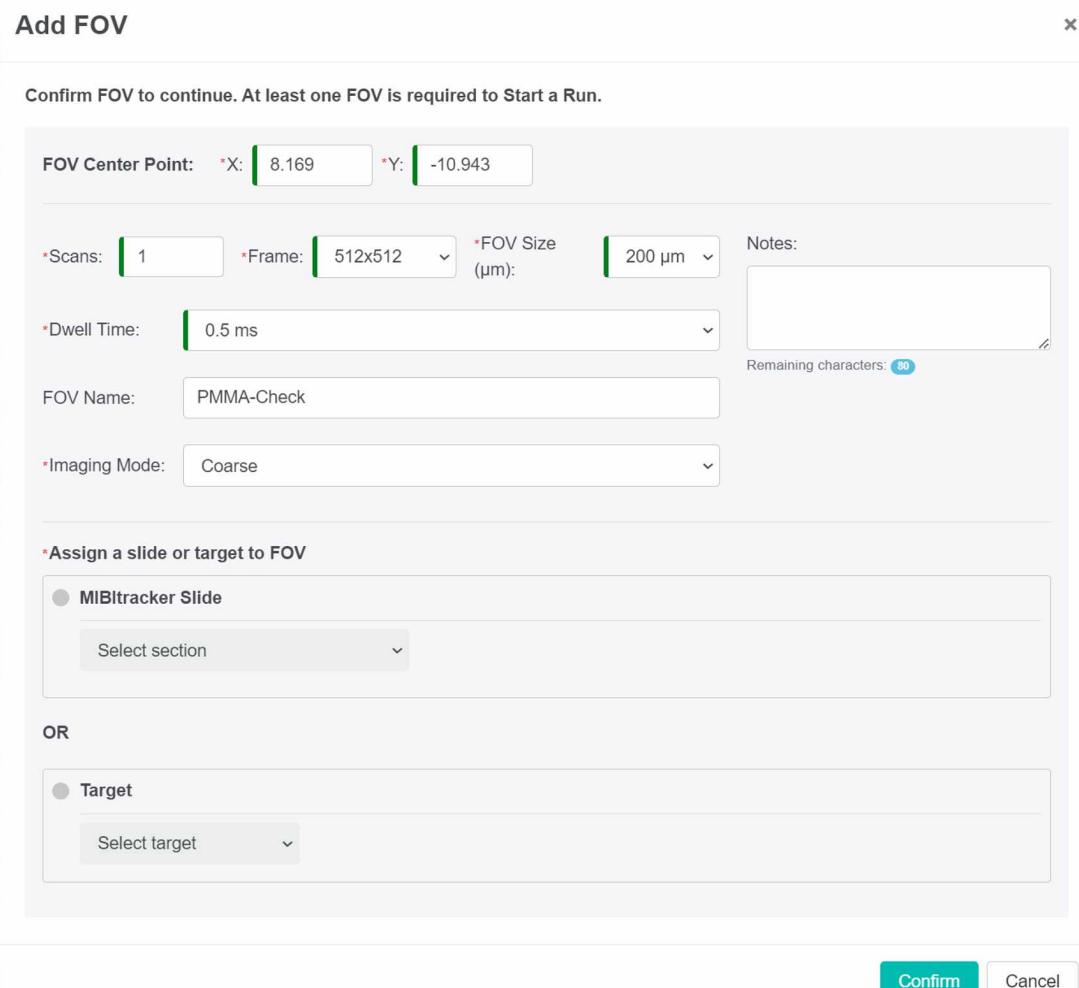
*Assign a slide or target to FOV

MIBtracker Slide
Select section

OR

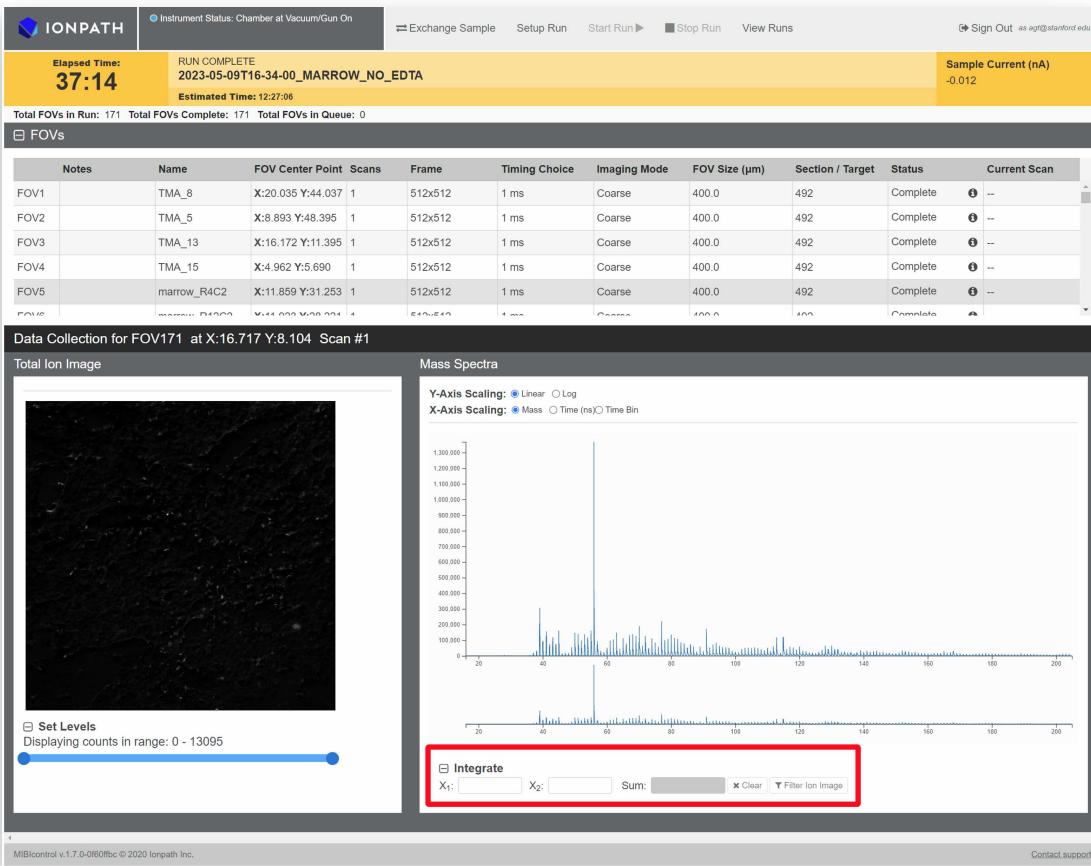
Target
Select target

Confirm **Cancel**



10.1 Start Run.

- Integrate values for each PMMA peak (89, 113, 142, 145, 150, 159, 176).
- Use the GUI to enter in the integration values from -0.3 to 0 (e.g. 88.7-89).



10.2

Record values in log sheet under the "PMMA" tab.

The screenshot shows a Microsoft Excel spreadsheet titled 'Drago_M13_Run_Log'. The spreadsheet has a header row with columns A through K. Below the header, there is a table with data. A red box highlights the 'PMMA' tab at the bottom of the sheet.

	A	B	C	D	E	F	G	H	I	J	K
1	Parameters: 200um, 512x512, 0.5ms dwell, coarse. Measure counts from -0.3 to 0 (e.g. 144.7 to 145). Make sure to go adjacent to the previous x/y coordinate for consistency										
2	Date	PMMA slide	x coord	y coord	89	113	142	145	150	159	176
3	04/13/2023	New PMMA 1	7.068	47.042	5869716	8041638	10702071	6267036	11181491	6927039	4835326
4	04/13/2023	New PMMA 1	7.288	47.042	5079393	7154761	9975988	5683741	10560171	6267630	4281046
5	04/17/2023	New PMMA 1	7.508	47.042	4332813	6265795	8765916	4903042	9535279	5729064	3877111
6	04/17/2023	New PMMA 1	7.748	47.042	5475721	7427717	10511284	5959424	11152889	6680488	4577479
7	04/21/2023	New PMMA 1	7.988	47.042	5923294	7833587	11006818	6239923	11385180	6757428	4583484
8	4/25/2023	New PMMA 1	8.228	47.042	6194999	8312722	11999379	7087073	12491570	7884852	5523068
9	04/27/2023	New PMMA 1	8.508	47.042	6054014	8338634	12145098	7015802	12569083	7836356	5321355
10	05/01/2023	New PMMA 1	8.748	47.042	5015597	7627085	11233655	6481298	12047206	7639874	5226718
11	05/04/2023	New PMMA 1	9.008	47.042	6521876	9179748	11049899	6256228	11226814	6690971	4308824
12	05/04/2023	New PMMA 1	9.248	47.042	7859067	13222620	14218462	9169385	14133622	9410402	6756523
13											
14											
15											

10.3

- If values are NOT massively different from previously recorded values (i.e. 20% difference okay, ~log difference not okay) then pass.
- If values are problematic, ask other lab members and/or Mike.

10.4

NOTE: PMMA slide is used for checking counts distribution from channels we will actually

extract from.

- Ideal to run before loading a slide during cohort runs, ensuring signal distribution is normal.
- Do NOT do this between separate runs on the same slide, e.g. if setting up multiple runs on a single slide with a large TMA.

Quality Control: Molybdenum Foil Counts

11 Run a single moly FoV to check general counts, should do this before every run.

- Change 'Target Selection' to select Molybdenum Foil.
- Switch Mode to SED.
- Alter Detector Gain to see image.
- MAKE SURE NO FOVS ARE LOADED AT THIS POINT OR CHECK WILL OVERWRITE.
- Set up FOV - 128 x 128, 200um, 1 ms, 3 scans, Imaging mode: QC 100um, Target: Molybdenum Foil.

Add FOV

Confirm FOV to continue. At least one FOV is required to Start a Run.

FOV Center Point: *X: 15.237 *Y: -11.324

*Scans: 3 *Frame: 128x128 *FOV Size (μm): 200 μm Notes:

*Dwell Time: 1 ms Remaining characters: 80

FOV Name: moly

*Imaging Mode: QC - 100μm

*Assign a slide or target to FOV

MIBltracker Slide 492 (marrow 2023-05-04 no EDTA)

Select section

OR

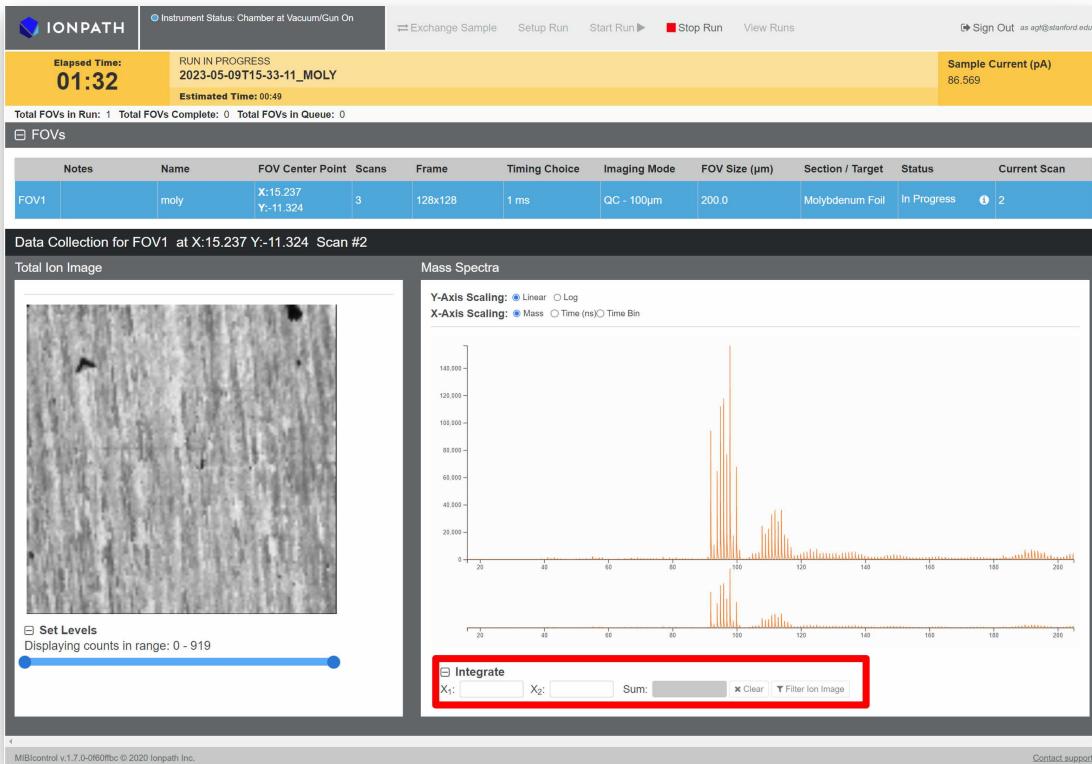
Target

Molybdenum Foil

Confirm **Cancel**

11.1 Start Run.

- Integrate Moly 98 and Moly 92 counts for the 3rd scan.
- Use the GUI to enter in the integration values (97.7-98.3, 91.7-92.3, respectively)



11.2 Record values in log sheet.

	A	B	C	D	E	F	G	H	I	ESI MPH from previous run	92Mo (MPH)	Target Mo98 (MPH)	L2	Stig deg	Stig Amp	Detector V	Acquisition time
1	Run name	Date	Slide	Fc coarse	L1 coarse	Fc Superfine (Anglo Custom)	L1 Superfine (Anglo Custom)	Moly 92 counts	Moly 98 counts				21.677	166	7	2330	
103	2023-03-29-HV_TestGAP	2023-03-29		9.564	17413.1								21.677	165	7	2270	24mins
104	2023-03-29-HV_TestCC	2023-03-29	(D392		9.564	17413.1							21.677	165	7	2270	
105	2023-03-29-HV_TestCC	2023-03-29	(D399		9.564	17408.9							21.677	165	7	2270	
106	2023-03-29-HV_TestCC	2023-03-29	(D399		9.564	17408.9							21.677	165	7	2270	
107	230405_MBiBtestGold_Slide_Control_1	2023-04-05	(D363		9.551	17395		2.495	18919.8	168193	540952						
108	230405_MBiBtestGold_Slide_Control_2	2023-04-05	(D363		9.564	17395.6		2.521	18917.2	8992	4511						
109	230405_MBiB test Resilience Edge Test	2023-04-05	(D397		9.564	17395		2.546	18917.2	452410	1467356						
110	230405_MBiB test Resilience Optimization	2023-04-06	(D397		9.564	17395		2.546	18917.2	2690101	1052431						
111	230406_HV_IonSIS	2023-04-06	(D361		9.576	17394				544174	127217		21.677	167	7.5	2330	
112	230406_HV_IonSIS	2023-04-06	(D361		9.576	17394				651874	1301066		21.667	166	7.5	2312	
113	230408_HV_IonSIS	2023-04-08	(D361		9.576	17395				701206	1349394		21.667	166	7.5	2328	
114	230408_HV_IonSIS	2023-04-08	(D361		9.576	17395				544272	1275548		21.667	165	7.5	2328	10mins
115	230409_DNA8H-test	2023-04-09	(Slide 404-408		9.551	17408.3				454148	1250523		21.667	165	7.5	2328	10mins
116										451555	1109848						2356
117	230411_HV_IonSIS_retest	2023-04-11	(Slide 401		9.564	17408.5				615138	1304679						2377
118	230411_HV_IonSIS_retest	2023-04-11	(Slide 401		9.564	17408.5				585735	1259482		21.524	165	7.5	2412	6hr24min
119	230414-13-26-22_GFAP AD Test	2023-04-14	(Slide 420		9.564	17411				508705	1289542		21.667	166	7.5	2452	
120	230414-14_DNA8H_Kapton Test	2023-04-14	(Slide 432-432		9.564	17411				2025151	4788914						2468
121	230414-17_BannerTest	2023-04-17	(Slide 413		9.565	17415				626259	1376546		21.667	165	7.5	2495	3hr 20mins
122	230414-14_opp_cc	2023-04-14	(Slide 434		9.564	17417.9				579489	1250521		21.667	165	7.5	2472	10mins
123	230414-16_DNA8H_Td8B9	2023-04-16	(Slide 435		9.566	17417.9				588568	1326210		21.667	166	7.5	2529	50mins
124	230421_mBiB test 1	2023-04-21	(D483		9.488	17419				471244	1156299		21.667	166	7	2614	8:09:20
125	230424_mBiB test 2	2023-04-24	(D484		9.55	17419				505797	1106273		21.667	166	7	2635	
126	230423_2023main	2023-04-23		9	17414.8				446981	1145625		21.667	166	7	2644		
127	230506_TA658_newborps test	2023-04-23	(Slide 450		9.51	17415				485540	1085176		21.667	166	7	3015	
128										1125817	1054798						
129																	

- ## 11.3 Record values in log sheet.
- If values are NOT massively different from previously recorded values (i.e. 20% difference okay, ~log difference not okay) then pass.
 - **If values are problematic, ask other lab members and/or Mike.**
 - If running for longer than 24 hours:
 - Monitor MPH, if below a certain value, detector gain will need to be boosted.
 - AutoGain will eventually handle this. **TBD**

Choosing Points

12 Tiling / TMA Tool: Highly recommended for longer runs

- [Tsai Lab tool for tiling on Optical or SED images](#)

MIBI Tile/SED/Array Interface 2023.03.08

[+] Optical Coregistration

[+] Files

[+] SED Tiler

[+] Label Map

[+] Slide Options

- Further notes of use are [here in the extended protocol.](#)

13 Individual Selection

- Switch Mode to SED, Imaging Mode to QC 300.
- Click on area of slide and push 'Move Stage to Target Position' button for large movements.
- Use 'Jog Stage' popout for larger movements.
- Single arrow = single increment denoted by number in window.
- Double arrow = 10x movement of number in window.
- Triple arrow = 100x movement of number in window.
- After finding a spot, click on 'Add FOV'.
- Enter parameters: resolution, FOV size, dwell time, imaging mode, etc.
- Give the point a name and assign it to a slide / section.
- Save.
- Move to next point and repeat until all points are selected.
- **Export FOVs as a json file (will need to import later).**

 Import FOVs

Export FOVs as:

fov-list

.json

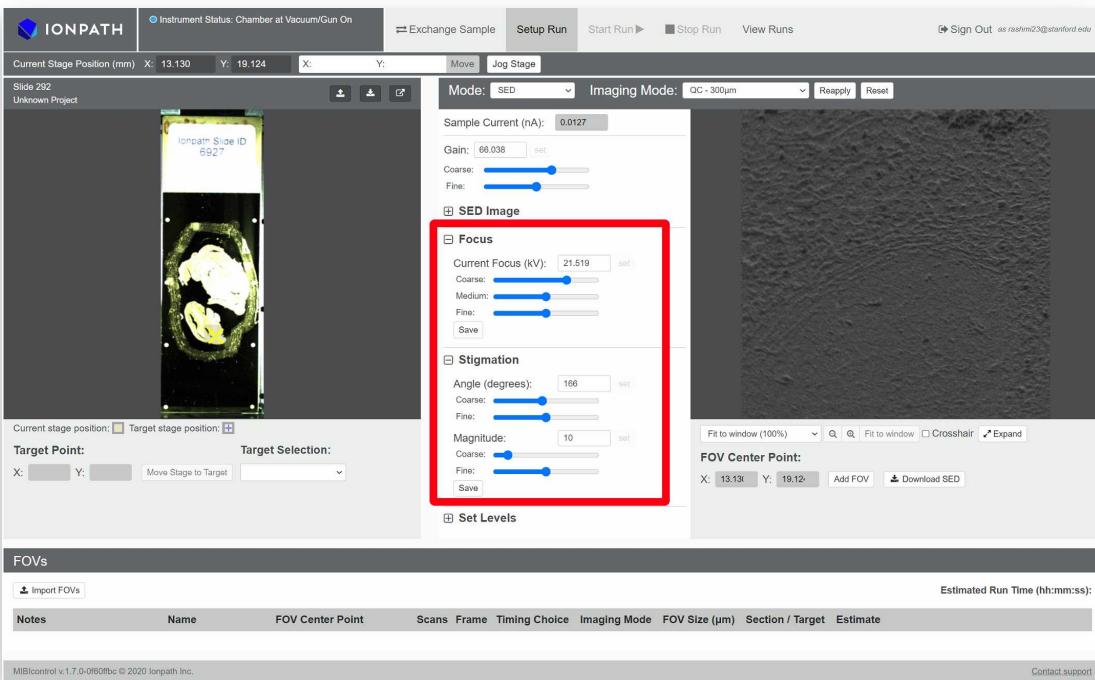


Focusing

14 You need to alter the focus and stigmation of your image before starting your run.

- Go to area of tissue not used for acquiring.
- Switch Mode to SED, Imaging Mode to whatever setting you set your points to.

- Starting with a large FOV, alter focus and stigmation until center is clear, top/bottom are slightly blurry, and repeat for smaller and smaller FOV windows (e.g. 400 and 100 um) at your acquisition resolution.
- Each time you finalize an FOV's focus settings, make sure you hit SAVE.**



Note

The stigmatization (angle, magnitude) is uniform throughout the slide. The focus varies slightly along the y axis of the slide, i.e. the focus for a FOV towards the top of the slide will be slightly different than the focus for a FOV near the bottom of the slide. Therefore, focusing on tissue at the center of the slide is recommended for optimal image quality.

14.1 Write down all numbers in log sheet.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
	Run name	Date	Slide	Fc coarse	L1 coarse	Fc Superfine (Angelo Custom)	L1 Superfine (Angelo Custom)	Moly 92 counts	Moly 98 counts	98MPH from previous run	92Mo (MPH)	Target Mo88 (MPH)	L2	Stig deg	Stig Amp	Detector V	Acquisition time
1	Creed_P9_Run_Log	2023-03-29	Slide 401	9.576	17394								24.632	166	7.5	2312	2270
103	2023-03-29:HTC_TestGFAP	2023-03-29		9.576	17413.1								21.877	165	7	2270	24mins
104	2023-03-29 HV TestCC	2023-03-29	Slide 402	9.571	17413.1								21.877	165	7	2270	
105	2023-03-29:TestControlCC	2023-03-29	Slide 403	9.538	17396.4								21.877	165	7	2270	
106	200405_Test	2023-04-05	Slide 403	9.571	17398.8	2.495	18915.9	1681393	546952				21.877	165	7	2270	
107	200405_DNA�Grid_Slide Control_1	2023-04-05	Slide 403	9.571	17398.8	2.505	18917.2	1681393	546952				21.877	165	7	2270	
108	200405_MBRBlueCont_Slide Control_2	2023-04-05	Slide 403	9.571	17398.8	2.505	18917.2	1681393	546952				21.877	165	7	2270	
109	200405_MBR_Ad_Resistance Edge_Test	2023-04-05	Slide 407	9.564	17395.6	2.566	18917.2	289951	1092431				21.67	167.1	7.5	2330	
110	200506_ISH_parameterOptimization	2023-04-06											21.67	167.1	7.5	2330	
111	200406_HRV_Test	2023-04-06	Slide 401	9.576	17394								21.67	165	7.5	2312	2270
112													21.67	165	7.5	2312	2270
113	200408_gyo_control	2023-04-08	Slide 402	9.576	17398.2								21.665	165	8	1h10mins	
114	200408_gyo_gyo	2023-04-08	Slide 403	9.571	17403.3								21.665	165	8	1h10mins	
115	200409_DNA�H Test	2023-04-09	Slide 404-406										21.665	165	8	2300	
116													21.665	165	8	2300	
117	200411_HRV_Test_rerun	2023-04-11	Slide 401	9.564	17408.3								21.524	160	7.5	2412	6h24mins
118	2023-04-13TH-20-22_GFAP_AD_Test	2023-04-13	Slide 426	9.571	17411								21.663	165	8	2312	
119	2023-04-14_DNA�H_Repeat_Test	2023-04-14	Slide 430-432										21.663	165	8	2312	
120													21.663	165	8	2312	
121	2023-04-17_BennoTest	2023-04-17	Slide 433	9.526	17419								21.66	166	8	2495	3h 20mins
122	2023-04-14_gyo_gyo	2023-04-17	Slide 433	9.564	17417.9								21.66	165	8	2495	3h 20mins
123	2023-04-17_DNA�H_T4B9	2023-04-17	Slide 433	9.564	17417.9								21.66	165	8	2495	3h 20mins
124	200421_mLBG_But_1	2023-04-21	Slide 403	9.488	17419								21.667	166	7	2814	1h02:20
125	200421_mLBG_But_2	2023-04-22	Slide 404	9.5	17414.7								21.667	166	7	2830	
126	2023-04-22_GoatTest	2023-04-22		9.5	17414.6								21.667	166	7	2844	
127	2023-04-22_DNA�H_But2_2023test	2023-04-22	Slide 400	9.51	17415								21.667	166	7	2855	
128	200506_T4B9_reportsTest	2023-04-22	Slide 400	9.51	17415								21.667	166	7	2855	
129													21.667	166	7	2855	

14.2 When done, put SED back into standby mode so you don't ablate your tissue sample.

Acquiring Data & Monitoring

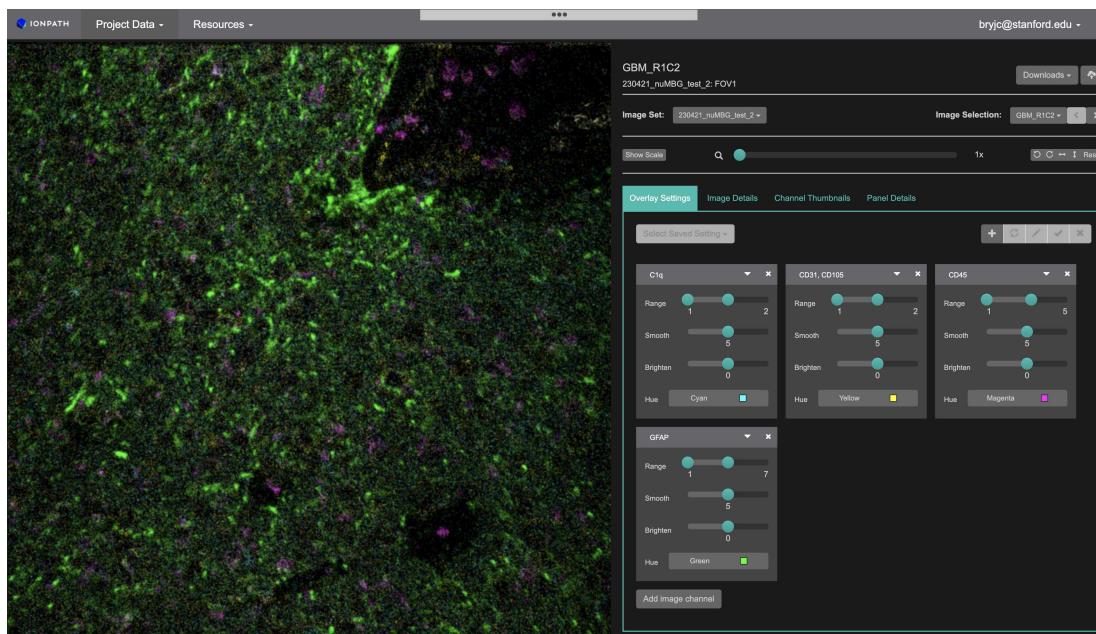
15 Load back FOVs from exported json file.



15.1 Start Run.

- Observe first few points in GUI for channel dropouts, low counts, any focus issues.

15.2 Look at your images in the Ionpath MIBIViewer.



Post-Run

- 16** Set the machine to STANDBY mode in MIBI Control.