Subject: Re: BARseq instrument and acquisition .json files for metadata
Date: Monday, February 9, 2026 at 11:44:45 PM Pacific Standard Time
From: Aixin Zhang
To: Doug Ollerenshaw, Saskia de Vries, Xiaoyin Chen, Polina Kosillo
CC: Yoh Isogai, Carolyn Eng, Daniel Birman

Hello Doug,
I can answer some of these questions:

1. Pixel size is 0.33
2. Dimensions of each tile before max projection is 3200x3200x100 in geneseq01 and bcseq01,3200x3200x120 in any hyb cycle, 3200x3200x80 in any geneseq02+ and bcseq02+. The dimension is in pixel space. We don't save the raw images only save the max projection images. The software will automatically delete the raw image stacks if the number of max projection matched with the required tile number.

   Acquisition channel order:
   Geneseq01 and bcseq01: G T A C Hyb-DAPI
   Geneseq02+ and bc seq02+: G T A C
   Hyb: Hyb-GFP Hyb-YFP Hyb-TxRed Hyb-Cy5 Hyb-DAPI DIC

3. Xiaoyin may look up for you.
4. exposure time is: G 60, T 30, A 20, C 40, DIC 20, Hyb-GFP 100, Hyb-YFP 30, Hyb-TxRed 30, Hyb-Cy5 20, Hyb-DAPI 20.
5. I think now image core will help us collect data.

   Let me know if you have any questions! Thank you!

Best
Aixin

Get Outlook for iOS

From: Doug Ollerenshaw <doug.ollerenshaw@alleninstitute.org>
Sent: Monday, February 9, 2026 3:03:59 PM
To: Saskia de Vries <saskiad@alleninstitute.org>; Xiaoyin Chen <xiaoyin.chen@alleninstitute.org>; Polina Kosillo <polina.kosillo@alleninstitute.org>; Aixin Zhang <aixin.zhang@alleninstitute.org>
Cc: Yoh Isogai <yoh.isogai@alleninstitute.org>; Carolyn Eng <carolyn.eng@alleninstitute.org>; Daniel Birman <daniel.birman@alleninstitute.org>
Subject: Re: BARseq instrument and acquisition .json files for metadata

Hi all, following up here and adding Dan to the thread.

Can anyone point me to the location of the raw data assets so I can try to infer approximate timestamps?

I also have some additional questions (probably more will follow).

1. What is the pixels-to-microns scale for the scope?
2. For the metadata, we need to know the position and acquisition order of every image that gets saved to disk. These are the tiles in x/y/z space that are then used to create the max projection. This can be in pixel space or real space, whichever is easiest.
3. What are the excitation and emission wavelengths?
4. What are the exposure times?
5. Who actually collected the data.

If any of that can be pulled automatically from the raw data path, let me know and I can dig in and see what I can extract once I have the paths to that raw data.

Doug

From: Doug Ollerenshaw <doug.ollerenshaw@alleninstitute.org>
Date: Friday, February 6, 2026 at 10:47 AM
To: Saskia de Vries <saskiad@alleninstitute.org>, Xiaoyin Chen <xiaoyin.chen@alleninstitute.org>, Polina Kosillo <polina.kosillo@alleninstitute.org>, Aixin Zhang <aixin.zhang@alleninstitute.org>
Cc: Yoh Isogai <yoh.isogai@alleninstitute.org>, Carolyn Eng <carolyn.eng@alleninstitute.org>
Subject: Re: BARseq instrument and acquisition .json files for metadata

Hi all,

I'm back to working on building out the acquisition.jsons. Given Saskia's feedback, there'll be three of them, corresponding to three distinct

raw assets, one for each round of data acquisition.

When we last met (Saskia, Caroline, You and Polina), I believe the plan was for me to use the max projection files as a source for the date time fields that are needed the acquisition file. Can someone point me to those files for subjects 780345 and 780346?

Thanks,
Doug

From: Saskia de Vries <saskiad@alleninstitute.org>
Date: Tuesday, January 20, 2026 at 9:59 AM
To: Doug Ollerenshaw <doug.ollerenshaw@alleninstitute.org>, Xiaoyin Chen <xiaoyin.chen@alleninstitute.org>, Polina Kosillo <polina.kosillo@alleninstitute.org>, Aixin Zhang <aixin.zhang@alleninstitute.org>
Cc: Yoh Isogai <yoh.isogai@alleninstitute.org>, Carolyn Eng <carolyn.eng@alleninstitute.org>
Subject: Re: BARseq instrument and acquisition .json files for metadata

No, I don't agree. Each cycle has a folder of images. That is one asset with one acquisition. These different assets then get processed together to create a single derived asset.

Saskia de Vries
Associate Director, Data and Outreach
Allen Institute for Neural Dynamics
(she/her)

Book time with Saskia de Vries

From: Doug Ollerenshaw <doug.ollerenshaw@alleninstitute.org>
Date: Tuesday, January 20, 2026 at 7:36 AM
To: Xiaoyin Chen <xiaoyin.chen@alleninstitute.org>, Polina Kosillo <polina.kosillo@alleninstitute.org>, Aixin Zhang <aixin.zhang@alleninstitute.org>
Cc: Yoh Isogai <yoh.isogai@alleninstitute.org>, Carolyn Eng <carolyn.eng@alleninstitute.org>, Saskia de Vries <saskiad@alleninstitute.org>
Subject: Re: BARseq instrument and acquisition .json files for metadata

Thanks Xiaoyin. It sounds like treating a dataset as a single asset described by a single acquisition.json makes the most sense. Saskia, do you agree?

Doug

From: Xiaoyin Chen <xiaoyin.chen@alleninstitute.org>
Date: Friday, January 16, 2026 at 6:00 PM
To: Doug Ollerenshaw <doug.ollerenshaw@alleninstitute.org>, Polina Kosillo <polina.kosillo@alleninstitute.org>, Aixin Zhang <aixin.zhang@alleninstitute.org>
Cc: Yoh Isogai <yoh.isogai@alleninstitute.org>, Carolyn Eng <carolyn.eng@alleninstitute.org>, Saskia de Vries <saskiad@alleninstitute.org>
Subject: Re: BARseq instrument and acquisition .json files for metadata

Hi Doug,
In each experiment, each dataset will have a combination of those three types of acquisitions. Different experiments can have different combinations (the common ones include one with only genes + hyb and ones with all three). For each acquisition cycle, you get a folder of images that are technically separated from the others, but they are largely useless unless they are used together.
So if you want one dataset associated with one json, then what you are doing now seems more appropriate. If you can have multiple json files associated with each dataset, and have some way of describing their relationships, then having multiple jsons is fine.

Xiaoyin

From: Doug Ollerenshaw <doug.ollerenshaw@alleninstitute.org>
Sent: Friday, January 16, 2026 5:01 PM
To: Xiaoyin Chen <xiaoyin.chen@alleninstitute.org>; Polina Kosillo <polina.kosillo@alleninstitute.org>; Aixin Zhang <aixin.zhang@alleninstitute.org>
Cc: Yoh Isogai <yoh.isogai@alleninstitute.org>; Carolyn Eng <carolyn.eng@alleninstitute.org>; Saskia de Vries <saskiad@alleninstitute.org>
Subject: Re: BARseq instrument and acquisition .json files for metadata

Hi Xiaoyin, I have a rough draft of an acquisition.json that describes the BarSeq process as I understand it so far. I'll share it for sanity checking after it's a little bit more complete. But Saskia flagged on key question that I'm hoping you can help resolve before I go any further.

I currently have a single acquisition.json with three distinct data streams (gene sequencing, barcode sequencing, and hybridization) run sequentially. But Saskia is asking whether in practice these are actually three distinct acquisitions, each with their own data assets, in which case we'd actually want to have three separate acquisition.json files describing each.

What do you think makes more sense?

Doug

From: Xiaoyin Chen <xiaoyin.chen@alleninstitute.org>
Date: Friday, December 12, 2025 at 4:54 PM
To: Doug Ollerenshaw <doug.ollerenshaw@alleninstitute.org>, Polina Kosillo <polina.kosillo@alleninstitute.org>, Aixin Zhang <aixin.zhang@alleninstitute.org>
Cc: Yoh Isogai <yoh.isogai@alleninstitute.org>, Carolyn Eng <carolyn.eng@alleninstitute.org>, Saskia de Vries <saskiad@alleninstitute.org>
Subject: Re: BARseq instrument and acquisition .json files for metadata

Hi Doug,
Here are the filters (not all of them are used in our current configuration):

Dicrhoic		
D1	FF421/491/567/659/776-Di01	DAPI/GFP/RFP/Cy5/Cy7
D2	ZT405/514/635rpc	DAPI/YFP/Rs Cy5
D3	FF421/491/572-Di01-25x36	DAPI/GFP/TxRed

emission			Dichroic	laser
E1	FF01-441/511/593/684/817	DAPI/GFP/Red/Cy5/Cy7	D1	405
E2	FF01-565/24	YFP	D2	520
E3	FF01-585/11	RFP	D1	546
E4	FF01-676/29	FarRed	D1	638
E5	FF01-775/140	RS Cy5	D2	638
E6	FF01-391/477/549/639/741-25	YFP/Rs Cy5		
E7	69401m	DAPI/GFP/TxRed		
E8	ZET532/640m	Alexa532/Cy5(wide)		

There are no excitation and we don't currently use a camera splitter.

the micromanager configuration file and the microscope config file is attached.

For the images, in cycles geneseq01 and bcseq01, the channels are G/T/A/C/DAPI. In other geneseq or bcseq cycles, the channels are GTAC. In hyb cycles, the channels are GFP/G/TxRed/Cy5/DAPI/DIC.

Let me know if you need additional information.
Xiaoyin

From: Doug Ollerenshaw <doug.ollerenshaw@alleninstitute.org>
Sent: Friday, December 12, 2025 3:18 PM
To: Xiaoyin Chen <xiaoyin.chen@alleninstitute.org>; Polina Kosillo <polina.kosillo@alleninstitute.org>; Aixin Zhang <aixin.zhang@alleninstitute.org>
Cc: Yoh Isogai <yoh.isogai@alleninstitute.org>; Carolyn Eng <carolyn.eng@alleninstitute.org>; Saskia de Vries <saskiad@alleninstitute.org>
Subject: Re: BARseq instrument and acquisition .json files for metadata

Hi all, here are notes I took when Dan, Polina, Xiaoyin and I just met at the rig:
2025.12.12 - barseq instrument meeting.docx

Next steps, assuming I followed everything:

- Xiaoyin to share:
    - Micromanager files
    - Filter files
    - Calibration files
- Dan to start generating a list of components that will go into the instrument from the above, then request further info as needed.

Is that accurate?

Doug

From: Xiaoyin Chen <xiaoyin.chen@alleninstitute.org>
Date: Thursday, December 11, 2025 at 10:29 PM
To: Polina Kosillo <polina.kosillo@alleninstitute.org>, Aixin Zhang <aixin.zhang@alleninstitute.org>, Doug Ollerenshaw <doug.ollerenshaw@alleninstitute.org>
Cc: Yoh Isogai <yoh.isogai@alleninstitute.org>, Carolyn Eng <carolyn.eng@alleninstitute.org>
Subject: Re: BARseq instrument and acquisition .json files for metadata

Hi Polina,
I'm happy to provide information on the imaging systems, but someone at AIND should generate the actual file since we are not familiar with your requirements. If you are doing this, perhaps we can meet up tomorrow in the lab and figure out the list of components you need to have listed, and we can send you the exact list later tomorrow.

Xiaoyin

From: Polina Kosillo <polina.kosillo@alleninstitute.org>
Sent: Thursday, December 11, 2025 1:58 PM
To: Aixin Zhang <aixin.zhang@alleninstitute.org>; Xiaoyin Chen <xiaoyin.chen@alleninstitute.org>; Doug Ollerenshaw <doug.ollerenshaw@alleninstitute.org>
Cc: Yoh Isogai <yoh.isogai@alleninstitute.org>; Carolyn Eng <carolyn.eng@alleninstitute.org>
Subject: Re: BARseq instrument and acquisition .json files for metadata

@Doug Ollerenshaw would you be able to help with Aixin's questions - perhaps a touch base in person?

Also, here are some of the read the docs which explain what data schemas are looking for.

https://aind-data-schema.readthedocs.io/en/latest/instrument.html

https://aind-data-schema.readthedocs.io/en/latest/acquisition.html

Sincerely,
Dr. Polina Kosillo
(she/her)
Scientist II
Molecular Anatomy Group
Allen Institute for Neural Dynamics

From: Aixin Zhang <aixin.zhang@alleninstitute.org>
Sent: Thursday, December 11, 2025 1:54 PM
To: Polina Kosillo <polina.kosillo@alleninstitute.org>; Xiaoyin Chen <xiaoyin.chen@alleninstitute.org>
Cc: Yoh Isogai <yoh.isogai@alleninstitute.org>; Carolyn Eng <carolyn.eng@alleninstitute.org>; Doug Ollerenshaw <doug.ollerenshaw@alleninstitute.org>
Subject: Re: BARseq instrument and acquisition .json files for metadata

Would you like me to write a python code to generate json file for meta data or you need specific instrument like what laser we use, what scope we use? What is the format of these files? I don't think I can run that code to check the result. We can meet in person if you want.

From: Polina Kosillo <polina.kosillo@alleninstitute.org>
Sent: Thursday, December 11, 2025 1:46 PM
To: Aixin Zhang <aixin.zhang@alleninstitute.org>; Xiaoyin Chen <xiaoyin.chen@alleninstitute.org>
Cc: Yoh Isogai <yoh.isogai@alleninstitute.org>; Carolyn Eng <carolyn.eng@alleninstitute.org>; Doug Ollerenshaw <doug.ollerenshaw@alleninstitute.org>
Subject: Re: BARseq instrument and acquisition .json files for metadata

Hi Aixin,

Thank you for checking in!
We think that the required information would be similar to our ExA-SPIM system which is also a custom built imaging rig.

Here are examples of code to create the instrument and acquisition files for exaspim (these python scripts used to create the json files):

- instrument
- acquisition

Basically we want a record of specs for instrument and imaging sessions that we could use to create matadata.

Sincerely,

Dr. Polina Kosillo
(she/her)
Scientist II
Molecular Anatomy Group
Allen Institute for Neural Dynamics

From: Aixin Zhang <aixin.zhang@alleninstitute.org>
Sent: Thursday, December 11, 2025 1:41 PM
To: Polina Kosillo <polina.kosillo@alleninstitute.org>; Xiaoyin Chen <xiaoyin.chen@alleninstitute.org>
Cc: Yoh Isogai <yoh.isogai@alleninstitute.org>; Carolyn Eng <carolyn.eng@alleninstitute.org>
Subject: Re: BARseq instrument and acquisition .json files for metadata

Hi Polina,
What is instrument.json and **notes format for acquisition.json** ?

Aixin

From: Polina Kosillo <polina.kosillo@alleninstitute.org>
Sent: Thursday, December 11, 2025 6:30 AM
To: Xiaoyin Chen <xiaoyin.chen@alleninstitute.org>; Aixin Zhang <aixin.zhang@alleninstitute.org>
Cc: Yoh Isogai <yoh.isogai@alleninstitute.org>; Carolyn Eng <carolyn.eng@alleninstitute.org>
Subject: BARseq instrument and acquisition .json files for metadata

Hi Xiaoyin,
Hi Aixin,

Hope you both are doing well, and happy holidays!

Reaching out to you both to get help in procuring data for **instrument.json** and **notes format for acquisition.json** files for **LC-NE and thalamus BARseq** project systems.

This request is part of the AIND process for creating metadata necessary for migrating BARseq data generated for LC and thalamus projects to open bucket repositories on S3. Could you please let us know what would be the most effective way for getting those files and how we can assist with that?

Sincerely,
Dr. Polina Kosillo
(she/her)
Scientist II
Molecular Anatomy Group
Allen Institute for Neural Dynamics