

WG5 meeting 01-10-2020

Attendees :

Glyn Nelson, Orestis Faklaris, Baptitste Monterrosso, Hella Hartmann, Ioannis Alexopoulos, Jeremie Teillon, Laure Plantard, Laurent Gelman, Marcel Kirchner, Stan Schwarz, Tobias Muller, Aurelien Dauphin, Ian Dobbie, Roland Nitschke, Uros Krzic, Mike Shaw, Claire mitchell, Alex corbett

Points from the Agenda

1. How often do we want to meet when and for how long?
 - Once a month ? yes
 - 2nd Thursday in a month 4:30 – 6pm CET : yes
2. What do we want to deliver? Definition of our WG5 mission statement.
 - Validate ISO protocol with fluorescent beads
 - Explore other alternatives for measuring the resolution of a microscope – explore the methods that the microscope constructors use

Laurent said he had posted bead slides to all that requested. Said the preparation method was difficult to get right and took some time.

GN proposed the above options for the WG mission statement. No disagreement. Discussed later under point 3.

3. Which intermediate steps do we define?
 - ISO protocols : use beads – prepare slides – send slides to labs – do acquisitions – analyse – share data.
 - Validate sample preparation-acquisition-analysis protocol for beads

Ioannis beads first to test reproducibility from Laurent's samples first and purchased premounted beads would be good. No supplier known- OF : ThermoFisher provides such – Ioannis : they are mounted on slide, not coverslip.

Protocols :

Sample preparation : LG did : Sonication, mounting media, sealing, clean coverslips (incubate 30min in HCl 1M) – bead dilution nicely spread. He altered the sample prep method considerably to get a good even dispersion, and easily mountable. Discussed mounting options, as non setting mountants were difficult to use. LG will share his protocol. GN will emulate in Newcastle and send samples out too. Will also create samples using different

mountants to see if there is any difference- none of us think it makes a difference if imaging beads at coverslip, but should have the data so we can say in protocol.

Image acquisition : Agreed ISO 10x oversampling is too great (as per last meeting). Discussed various sizes to capture. Ratio of 3 should be fine. Agreed to propose two scales : 40x 40 x100 nm or 70 x 70 x 200 nm (xyz) depending on whether scanning for validating microscope performance or routine quality check. For the purpose of our tests of the protocols, the smaller is more important, but we should run some tests on the larger pixel sizes to ensure it is satisfactory for routine tests. Other parameters discussed : image area needs to be large enough to ensure capturing whole Airy disks. For 40nm x100nm voxels, a set of 256x256 pixels (10um x 10um) should be large enough in xy and 50 z planes (5 um). SD systems must capture at the best performance possible (eg 100x best NA every 100nm in z). Use 2x lens if search for optimal performance, but be aware of additional aberrations. Only image beads that are on the coverslip. A minimum of 5 beads per microscope. Images must have a minimum metadata set of Airy unit, NA, mag, pixel size, and excitation and emission lambdas, and users should also record the number of beads imaged. Images must be analysed by the user with their preferred software. Results and raw data should be shared via bwsyncandshare website, and state method used for results.

Data analysis: everyone gives his PSF results with his way of analysis and next meeting discuss on analysis methods.

Data: put all data on server, GN will arrange the folder, OF will create the excel list with the necessary info to put in.

- [contact bead companies to share products \(beads, mounting medium\)](#)

GN : agreed with ThermoFisher for samples to test. Will be sent Prolong Gold, Prolong Diamond, and Prolong Glass mountants and PS-Speck beads, 100nm green Fluospheres and TetraSpeck microspheres. Will use to produce samples.

Mike Shaw suggested streambio for beads, and said he had a connection there- would be good to test too. OF will contact MS to arrange samples if possible.

Gattaquant also sell beads- 23nm. OF will contact them.

- [Test other methods and tools \(gattaquant beads, Argolight, mirror, origamis, ...\) for resolution measurements than PSF – how to measure resolution with them - get feedback of companies methods of measuring resolution](#)

Contact manufacturer about tools they use to test. Stan : brakenhoff measure uses 10x sampling in zed and most manufacturers use a mirror slide. Confocal ISO mostly written by leica engineers and some zeiss. Would have to ask. Uros (zeiss) on line asked for comments. No reply. Mirror slide is fastest- all mfrs use it, but always gives better values than beads. Could compare mirror with beads directly, one after the other. DIN and ISO avoided argolight slide as method- since it cannot be defined and built/ purchased elsewhere. Have 3 or 4 groups compare mirrors. Laurent- prefers beads to argolight and mirror as you get all data (not just z resolution) and it is more similar to biological samples. Aurelien- cross in 3D on argolight, but not diffraction limited.

Orestis proposes to compare the data from psfcheck, argolight, mirrors, other beads. Laurent and glyn to do psfcheck slides and upload data. Glyn, Orestis and Ioannis for mirrors. Roland said he had an Abberior slide with 40nm multicolour beads. DNA origami

gattaquant : Orestis will ask them, also for 23nm beads. No one has any. Many commented they don't last. No other manufacturer known for DNA origami without making ourselves.

- [Measurements for confocal and spinning disk first – coming wide-field](#)

Mike Shaw also said SIM would be nice too. Group agreed. Sub-folder to make for high resolution (hardware) techniques (SIM, Airy scan)

[4. Define how we work – sub-groups-shared documents \(PSF results\) – shared data](#)

Claire Mitchell said she had looked into opening her OMERO database for the data storage and analysis via AutoQC. But access externally is not possible for her server. RN said that there should be enough space on the bwsync server, and he could get it enlarged if necessary. Alex C. psfcheck software on a server at exeter, using Bioformats. Test site available- can test (Glyn). Agreed we would use bwsyncshare website for now.

[5. Other points of discussion](#)

Allow choice of analysis method and state the one used when uploading data. ID : Make all images one bead to set up automated analysis easier.

How to measure. Comparing resolution measurements and FWHM depending on sample. Mike shaw- knowing a value for resolution is important to ascertain if the method used is good enough for the experimental sample. Laurent compared FWHM gaussians moving closer together with theoretical data. MS said he would look at that.

Orestis and Glyn to compile mission statement for next meeting.

Alternative VC software for next meeting due to poor quality connection. Can use Ioannis' zoom account or possibly GN.

[6. Agenda items for 3rd WG5 meeting](#)

Date : 12 November at 4:30pm CET

MESSAGES

Public Chat

NOTES

Shared Notes

USERS (2)

Gl

glyn nelson (You)

Or

Orestis

Public Chat

Welcome to **2nd WG5 meeting!**

For help on using BigBlueButton see these (short) [tutorial videos](#).

To join the audio bridge click the phone button. Use a headset to avoid causing background noise for others.

This server is running [BigBlueButton](#).

He

Hella - Germany(offline)

3:32 PM

I have no camera, sorry..

Or

Orestis(offline)

3:32 PM

ok

La

Laure(offline)

3:34 PM

the sound is horrible for me. Is it just me?

Ma

Marcel Kirchner(offline)

3:34 PM

same here

St

Stan Schwartz(offline)

3:34 PM

Bad sound for you Au

Aurelien Dauphin(offline)

3:37 PM

Hi ! I'am unable to connect to the sound :(Or

Orestis

3:38 PM

i come back without the camera He

Hella - Germany(offline)

3:38 PM

Please mute if you are not talking. Jé

Jérémie Teillon(offline)

3:38 PM

in general Ba

Baptiste Monterroso (Nice, FR)(offline)

3:39 PM

without camera for me also, bad connection... La

Laure(offline)

3:40 PM

better St

Stan Schwartz(offline)

3:41 PM

Over modulating the sound Ba

Baptiste Monterroso (Nice, FR)(offline)

3:42 PM

I think it's your microphone orestis that breathe (la)

Marcel Kirchner(offline)

3:42 PM

orestis can you please mute when you dont talk?

Orestis

3:42 PM

ok :)

La

Laure(offline)

3:48 PM

I can vouch that he spent a lot of time on the beads prep. :-)

Baptiste Monterroso (Nice, FR)(offline)

3:49 PM

your slides laurent are amazing, nice density and well mounted, thanks a lot !

Ian Dobbie(offline)

3:49 PM

You have to be careful with setting mountant as it can have quite variable refractive index depending on how set it is.

To

Tobias(offline)

3:59 PM

Sorry, successful connection only now.

To

Tobias(offline)

4:08 PM

Prolong Gold Data Sheet with RI over time: https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0002469_ProLongMSlowFade_Antifade_Mountants_PI.pdf

Mike Shaw (NPL)(offline)

4:10 PM

I have a contact at a UK company called StreamBio (<https://www.streambio.co.uk/>) who I'm sure would be happy to provide samples if of interest.

Ian Dobbie(offline)

4:11 PM

Good find Tobias I have not seen that curve before.

To

Tobias(offline)

4:12 PM

especially interesting air-dry vs. glass. Also: has anyone ever noticed hardset media getting a bit liquid again when stored in the fridge? Seems to occur at random.

Marcel Kirchner(offline)

4:14 PM

prolong glass is supposed to harden as well <https://www.thermofisher.com/order/catalog/product/P36980#/P36980>

Orestis

4:14 PM

yes Mike, we ll contact you for the streambio beads

Laurent Gelman(offline)

4:16 PM

back in a minute, sorry... la

Ian Dobbie(offline)

4:16 PM

We could make some, probably want to use Laurant's beads to ensure they are the same batch etc... Jé

Jérémie Teillon(offline)

4:16 PM

yes St

Stan Schwartz(offline)

4:17 PM

Several forums have discussed the media types and their properties. Microforum, microscopy twitter, Jennifer Waters Allison North, Christophe Leterrier

Laurent Gelman(offline)

4:18 PM

Stan, what is their conclusion? St

Stan Schwartz(offline)

4:20 PM

I forgot, but many like prolong Gold and prolong Glass. I remember seeing PFS of each mounting media collage

La

Laurent Gelman(offline)

4:20 PM

Tx

To

Tobias(offline)

4:24 PM

Very sorry, I have to leave already.

GI

glyn nelson

4:25 PM

bye Tobias, thanks for coming.

Mi

Mike Shaw (NPL)(offline)

4:25 PM

Do we need to define a minimum set of metadata for each dataset?

Jérémie Teillon(offline)

4:26 PM

Sorry, have to leave. Thanks all for the meeting.

Ian Dobbie(offline)

4:28 PM

Mike, yes! objective, mag, NA, pixel size, wavelength of ex and em, anything else?

Mike Shaw (NPL)(offline)

4:28 PM

Pinhole diameter

Mi

Mike Shaw (NPL)(offline)

4:29 PM

Maybe others?

la

Ian Dobbie(offline)

4:29 PM

Pinhole size as what? fraction of airy disk? physical size? projected size in image?

Mike Shaw (NPL)(offline)

4:31 PM

Physical size or Airy units (at a specified wavelength)?

Ian Dobbie(offline)

4:34 PM

I would suggest airy units as physical size depends critically on internal mag of the microscope which might change with model or manufacturer

Claire Mitchell(offline)

4:34 PM

Hi all, sorry I got the timezone wrong! Or

Orestis

4:35 PM

so we keep for bead metadata: Airy unit, NA, mag, px size, and lambdas, and number of beads

beads St

Stan Schwartz(offline)

4:37 PM

Here is link about mounting media and PSF. <https://forum.microlist.org/t/hardening-mounting-media-that-doesnt-compress-sample/416>

Mike Shaw (NPL)(offline)

4:39 PM

Apologies if I missed this earlier, but have we specified #1.5H coverslips?

Stan Schwartz(offline)

4:39 PM

Also twitter Au

Aurelien Dauphin(offline)

4:40 PM

From Argolight web site, just for info :

Pattern F – Matrix of crosses. This pattern consists in a matrix of 6×6 crosses, having a length of $5 \mu\text{m}$.

The crosses are composed of vertical lines that are in the same plane, and by horizontal lines, going gradually deeper within the glass. The spacing between the vertical and horizontal lines gradually increases, from 0 to $3.5 \mu\text{m}$, with a step of 100 nm.

Aurelien Dauphin(offline)

4:40 PM

<http://argolight.com/products/argo-hm/> la

Ian Dobbie(offline)

4:40 PM

probably specify high spec 170um coverslip, so +/- 10um,

glyn nelson

4:40 PM

1.5H is what we want people to use, so yes. Au

Aurelien Dauphin(offline)

4:41 PM

Me too ! Cl

Claire Mitchell(offline)

4:41 PM

I have Laurent's beads too! La

Laure Plantard - FMI(offline)

4:44 PM

i think Gattaquant has beads too, but never tried them: <https://www.gattaquant.com/products/gatta-beads.html>

Roland(offline)

4:44 PM

Abberior <https://www.abberior.com/jtl-shop/Abberior-Alignment-Set-for-STED-775-nm>

Stan Schwartz(offline)

4:45 PM

<https://twitter.com/guijacquemet/status/1294259418092183552?s=21>

Ian Dobbie(offline)

4:58 PM

maybe we should have a data subfolder with subsubfolders for different people/institutions?

Mike Shaw (NPL)(offline)

4:59 PM

@Ian - agree. This would help keep the data organised.

Hella - Germany(offline)

4:59 PM

Maybe we also agree on a naming scheme for the files?

Orestis

4:59 PM

i am here

but an issue with microphone

perfect, we produce data Or

Orestis

5:01 PM

and not forget to produce the data and put them online. we ll try to keep data organised

Stan Schwartz(offline)

5:01 PM

For 2 D resolution i also like ronchi ruled coverslip and dusty deposition mirror coated coverslip. These are used by microscope companies for 2D resolution

Orestis

5:01 PM

we ll do a proposition of how to organise them

Baptiste Monterroso (Nice, FR)(offline)

5:02 PM

thanks for the meeting :) and again super thanks laurent for the slides!

Orestis

5:03 PM

ok, we find an alternative! Or

Orestis

5:03 PM

it is recorded Laurent... Ma

Marcel Kirchner(offline)

5:04 PM

I'd be fine with zoom as well. I do not have a premium account though

Orestis

5:04 PM

Stan, what do you mean with dusty deposition on mirror coated coverslip?

Laure Plantard - FMI(offline)

5:05 PM

Sorry, i need to run. Thanks a for the meeting. Have a nice evening.

Stan Schwartz(offline)

5:05 PM

Dust causing very small random pinholes in coverslip

Orestis

5:05 PM

thanks!! Au

Aurelien Dauphin(offline)

5:05 PM

Thank you !!! Ba

Baptiste Monterroso (Nice, FR)(offline)

5:05 PM

thanks !! bye !

GI
