WG5 3rd Meeting 12/11/20, Minutes

**Agenda:**

1. Welcoming new participants
2. Agreement on the minutes from last meeting
3. Agreement on recording the meeting
4. Summary of the contacts with beads companies - partnerships - samples to send
5. Iso Manual V2: edit the PSF acquisition
6. Summary of Laurent bead slide acquisitions
7. PSF check update by Alex Corbett
8. Other resolution measurements
9. Other topics

**Attendees:**
Glyn Nelson, Orestis Faklaris, Laurent Gelman, Mathieu Fallet, Claire Mitchell, Alex Corbett, Dan Metcalf, Hella Hartmann, Ioannis Alexopoulos, Stan Schwarz, Laure Plantard, Mike Shaw.

**Minutes:**
1. Laure introduced herself as working with Laurent, previously having worked in Dresden and Copenhagen. Dan introduced himself as a confocalist, then worked at NPL on super-res systems then Nikon as an application specialist before moving to Scientifica. Mathieu works in Metrology mostly on PSF objectives in CIML facility in Marseilles.
2. Minutes were agreed upon.
3. Recording agreed upon.

Other business- Mission statement for the white paper. Orestis introduced the current statement and asked for comments/suggestions. GN said does it cover the whole system, or just objectives. CM, said yes, it should. MS- resolution or FWHM. Resolution is required for comparing in biological samples rather than FWHM. GN asked if it is possible to correlate the two for a given objective NA and wavelengths? LG said he had done theoretical comparisons and thinks it is possible. MS- hard to do directly. Further discussions led to small changes made to wording based on discussion.
**Action Point- OF to submit to WG8 for white paper.**

4. GN- thermofisher have sent samples for 175nm PS-Speck beads and 110nm green beads plus mountant. Has sent beads to OF and LG. Requested further volunteers to prepare samples from the same lots. None given.
OF- MS had suggested Streambio as a source of 70nm 'beads'. OF, GN and MS discussed with Streambio, and they are delivering two colours of nanoparticles, blue and green/yellow. These three plus LG will receive samples to test. MS will determine best seeding method, then pass method to others to replicate and test samples.
**Action Point: MS to develop seeding and mounting method and pass to others.**
OF- contacted Gattaquant. They have 23nm particles. They were enthusiastic, but need to arrange a meeting.
OF- contacted Spherotech, but no reply.

5 and 6. OF- would be nice to have PSFs once for all objectives on a system and then regular tests of the most used/ highest objectives. Need to decide on a pixel size for lower NA lenses and adjust the method.
DM- temperature is important too, plus any NA collar needs to be set to maximum.

GN- should we record the temperature at the objective in the spreadsheet? DM- yes, ideally.
**Action Point- add column for Temperature in spreadsheet (GN) and all to record (All).**
OF- noise needs accounting for too since it effect the floor for the baseline and therefore resolution limit. DM- need to use whole dynamic range. IA- should avoid averaging, bidirectional, no offset and use dynamic range.
LG- image 1 bead with these different parameters to see if they affect FWHM and S/N. OF- has stydied FWHM variation with pixel size.
**Action Point- OF to share data pixel size – FWHM parameters.**

LP- has compared beads with different mount- Prolong Gold, Glass and the PS-Speck mounting medium. All similar, except Prolong Glass gave better zed FWHM. All data in spreadsheet. DM- commented RI varies with setting time. IA- bleaching is important to consider with the different mountant, and has found that with PS-Speck beads Prolong Diamond is better than the PS-Speck mountant for bead bleaching.

Action Point- GN to edit method for acquisition based on these discussions and pass round for people to comment on.

Re. software for analysis. Should state the software used. So far we have PSFj, metroloj, LG macro and Huygens distiller. LP will compare data on server with the LG macro. Next meeting we should go over the softwares used to compare:
**Action Point: prepare 10 min presentations for next meeting: LG macro- LP. PSF distiller (Huygens)- GN, PSFj, CM and Metroloj- OF.**
**Action Point- add column to spreadsheet to state software used (GN).**

7. PSFCheck. AC introduced PSFcheck as a piece of hardware (slide), and software for automated PSF analysis. Hardware is being redesigned to give an array of subdiffraction spots- still in progress. Once manufactured, will share slides with WG to test. Introduced PyCalibrate- an onlinePSF analysis method that works with any bioformats compatible image file containing a single 3D image (can be multichannel though). User creates a project and uploads data files, then runs analysis- uses some autofinding criteria and uses a Gaussian fit for FWHM estimates from beads in image. Creates a pdf and csv that can be downloaded and are stored with the original files. 100Gb data limit each user. Invited WG member to try out over the next 2 months.
**Action Point: test Pycalibrate at psfcheck.com with samples (All).**

8. OF pointed out that he, IA and GN had taken some mirror slide images. Will discuss at next meeting.

Next meeting agreed as 10th December 2020.
**Action Point- organise next meeting (GN).**

**Action Points from meeting:**
Action Point- OF to submit to WG8 for white paper.
Action Point: MS to develop seeding and mounting method and pass to others.
Action Point- add column for Temperature in spreadsheet (GN) and all to record (All).
Action Point- OF to share data on bead pixel size acquisition parameters.
Action Point- GN to edit method for acquisition based on these discussions and pass round for people to comment on.
Action Point: prepare presentations for next meeting: LG macro- LP. PSF distiller (Huygens)- GN, PSFj, CM and Metroloj- OF.
Action Point- add column to spreadsheet to state software used (GN).
Action Point: test Pycalibrate at psfcheck.com with samples (All).

Action Point- organise next meeting (GN).