**WG5 First Meeting. 27th July 2020.**

## Agenda:

1. Brief round presentation and experience on resolution measurements (20min)
2. Agree Chair and co-chair (5min)
3. Define what we want to obtain as measurements (ISO based and/or else) (20min)
4. Define a preliminary list of which samples to test and split the work between us (20min)
5. Decide timelines for reporting back these data to the group (15min)
6. Any other business/ things we've missed that are raised (10min or more)

## Attendees: (WG leader in bold)

Ioannis Alexopoulos
Steve Bagley
**Orestis Faklaris**
Laurent Gelman
Peter Hemmerich
Marcel Kirchner
Claire Mitchell
Baptiste Monterroso
Tobias Müller
**Glyn Nelson**
Stanley Schwartz

**1.** Attendees introduced themselves and their experiences measuring system resolution. Several questions were raised during this regarding our remit:

1. Frequency of testing
2. Which objectives to test
3. Airy units for pinhole
4. Human impact on measurement accuracy
5. time taken-how to speed up
6. which wavelengths to cover
7. Co-registration as part of the test?
8. Automation
9. What threshold should be set for highlighting problems.

All discussed in point 3.

**2.** Volunteers were requested for anyone that wished to lead the WG. OF and GN were asked if they wanted to continue. Both said they were happy to if no one else wanted the role. Since no other volunteers were offered, OF and GN will continue to run WG5.

**3.** LG: measurements should fit confocal ISO. GN pointed out confocal ISO demands very small pixels and very small beads (~<80 nm beads and ~16nm pixels for NA1.4 objectives at 500nm). Discussion around bead sizes and difficulty of imaging and gathering robust data due to low SNR. It was agreed that the confocal ISO needn't be followed, and could itself be altered to fit the demands of the field.

Discussion around alternative options instead of beads.

i) PSF Check: similar to Argolight slide (excitation in UV emission in green orange). Not possible for pulsed laser measurements. Stability up to a year, no more. Price: around 350 pounds.

ii) Argolight: pattern of different z for z calibration. Can fit for a fast 2D objective check.

iii) Zeiss objective: it has some lines for resolution measurement. 5 times out of 10 it gives different results. Objective more reliable for scanner calibration but not reliable for resolution measurements. Even Zeiss technicians not (know how to) use it.

iv) DNA origami 6nm cubes: very small, ideal for point source - but not stable in time.

v) Mirror for axial resolution. More specific on excitation characteristics. Used by manufacturers.

TM comments that for his 20 systems he prefers doing regularly a fast 2D check to see if objectives are damaged. The whole 3D check of PSF takes time and thus its frequency is lower.

Beads seem to be the best tool for PSF measurements. Stanley Schwartz notes that microscope manufacturers do not use beads for PSF because the result is sample preparation dependent and also system aberration dependent. LG adds that sometimes due to bad PSF he found out that the z motor was not working, or camera vibrations or other issues.

IA pointed out that samples should be available to the vast majority of users and easily accessible. Agreement on beads as providing xy and z resolution, easy to acquire, relatively cheap and very long lasting if bought as a solution. Agreed that we need to define a sample preparation method that is as robust as possible. Discussion on creating robust samples by users following the protocol. Coverslip thickness 1.5H required. Mountants shown to make little difference when beads are attached to glass (LG and others).

** Discussion on ISO:

SS commented that ISO norms are here to follow the same system performances over time. Not compare systems.

LG opinion is that the standardization precision is here to compare systems too.

Frequency of testing discussions: LG tests weekly, several others said monthly or quarterly, all dependent upon system usage and objectives. LG discussed advantages of comparing systems over time- gradual reduction of system performance noticed, plus other faults. Highlighted cleaning regimes- we need to advocate regular cleaning of objectives as part of the protocol.

Airy unit size- agreed that for variable pinhole systems these should be wide open for measurement.

Objectives to measure. Discussion on which ones showed that higher performing objectives were those that should be tested. Agreed that objectives >= NA 0.8 should be classed as those to check.

Wavelengths to measure. GN highlighted that you could capture co-registration data with multicolour beads at the same time as capturing xyz resolution. Other comments described problems of imaging these at blue and red ends of spectrum. OF said that systems should be tested at all wavelengths when new, but for routine QC, one wavelength was enough. From his experience some plugins, like the MetroloJ work better if bead size is big enough (1-4um). Agreed that 488ex 525/50 em was sufficient for QC, since all faults seen are seen at all wavelengths.

Speed of testing. TM said ideally we would have one quick method and 1 higher specificity test to perform if faults were seen with the quick test. Based on everyone's experience of bead tests, agreement was reached to use larger 175nm beads for quick tests and 100nm beads for high specificity tests. We would try both to see if there is enough resolution in the larger beads to highlight problems, since they save time by being easier to find and brighter. Agreed to identify suppliers for beads to test.

** We measure PSF either for finding the system performance or/and for following the performance over time. Different beads can be used for each purpose.

Automation. CM said she had an analysis pipeline that could be implemented in OMERO with ImageJ. She offered to provide this as a means of storing the imaging data form our tests. GN said it would be nice to fully automate this- will discuss with the wider community. Everyone else was implementing manual analysis methods using opensource (ImageJ plugins) or commercial software (Huygens).

Sampling:  Number of beads per test ranged from 3 to a couple of dozen- largely dependent upon capture time and analysis method. It was agreed that we would compare variance over number of samples to determine a recommended number of beads for analysis and a recommended standard deviation.

Immersion medium where needed. It was agreed that they manufacturers recommended immersion medium would be used for each objective.

Room temperature for acquisition: Room temperature (20-21°C) was used by everyone, and would be used for tests. SS pointed out that manufacturers optimise for 24°C.

Microscope type: Agreed to focus on confocals and spinning disk samples for the first round of tests and introduce widefields later if time and resource allowed.

**4**. Based on discussion in point 3, the following were agreed upon:

test 100nm and 175 nm beads dried onto coverslips mounted in prolong gold.

Compare samples prepared by one lab (LG volunteered to produce 1 sample per WG member) with a sample produced by yourself using the same aliquot of beads.

Each WG member will run tests of their sample plus the LG provided one on at least one microscope and at least one objective over time- ideally once a week for 4 weeks minimum, capturing multiple beads.

**5.** Next meeting would be end of September, w/c 21st or 28th, giving time to pass out samples and run some tests before then.

**Actions:**

Create a spreadsheet on bwsyncandshare to gather bead info and shipping addresses (**GN**).

Determine bead suppliers (**ALL**) and order (**OF** and/or **LG**).

Mount beads and pass out mounted samples and suspensions to everyone. Also provide mounting protocol for everyone to follow (**LG**).

Provide shipping addresses for LG on bwsyncandshare (**ALL**).

Run tests of 175 and 100nm samples on at least one microscope and at least one objective over time- ideally once a week for 4 weeks minimum, capturing multiple beads (at least 5) - ideal case: capture for at least one time the PSF of every objective to build a data base of the group (**ALL**).

Investigate running OMERO for networked use outside Warwick to allow WG members to upload images (**CM**).