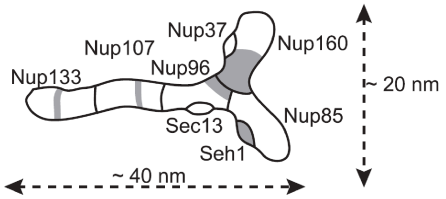
IMAGING DATA STANDARDS: ESTABLISH STANDARDS FOR DATA FORMATS AND QUALITY CONTROL
Co-Chairs: Mark Ellisman, Xavier Darzacq

Discussion thread Common Samples: calibration standards for superresolution microscopy
Joan Ritland, Julia Roberti (on behalf of Jan Ellenberg), summary from June 7 2016

From the discussions of the teleconference, we agreed on defining in detail the use of nuclear pore complex proteins to calibrate and test the performance of photon-based systems.
Jan Ellenberg's lab has extensive experience in stochastic localization microscopy applied to the elucidation of the nuclear pore complex (NPC) structure (Szymborska et al., Science 2013). The original work was carried out by immunofluorescence labelling of different nucleoporins of the Nup 107-160 subcomplex, the largest building block of the NPC. Later on, proteins tagged with EGFP were targeted using anti-GFP nanobodies coupled to fluorophores. The NPC scaffold structure was resolved with a precision well below 1 nm.



Schematic representation of the current model of the human Nup107-160 complex based on homology to the yeast Nup84 subcomplex. The dimensions of the yeast complex are indicated. From Szymborska et al., Science 2013.

For calibration purposes, a genomic-edited cell line is highly desirable, to ensure endogenous and complete tagging of the protein of interest (POI) at known copy numbers. Regarding the POI, a core nucleoporin is a sensible choice. Further decisions to be made include:
* POI location in the NPC: proteins located in the periphery would allow a larger separation of individual subcomplexes.
* Copy number: e.g 16/NPC, 32/NPC.
* Fluorescence label / tagging system: photoactivatable fluorescent protein (e.g. mEos2, mEos3, mMaple), EGFP / nanobodies-organic fluorophores, SNAP/Halo-tag / organic fluorophores, …

From the discussion, it was clear that a decision on these points *a priori* would not necessarily constitute the best choice in the end. Therefore, the consensus was to take the best available cell line and use it as a proof of principle, and then refine the requirements listed above.

As a starting point, Ellenberg lab is willing to share the following U2OS cell lines:
* U2OS ZFN SNAPf-Nup107: homozygously tagged (checked by Southern blot)
* U2OS CRISPR SNAPf-Nup133: homozygously tagged (checked by Southern blot and PCR)
U2OS cells are flat, nice for imaging purposes, and suitable for genome editing. Karyotype stability has not been addressed though.

The conditions of use are restricted to standardization purposes within the 4DN consortium. Requests must be addressed directly to J. Ellenberg by the PI. Cell vials will be delivered on a case-by-case basis, along with an MTA from EMBL; in this way, the cells can be tested as the MTA is evaluated and signed by the requesting lab. Publications involving the use of the cell lines will be done under collaboration terms, i.e. with co-authorship of Ellenberg's lab, until their nuclear pore biology paper with these cell lines has been published.

If the proof of principle is satisfactory, Ellenberg's lab is willing to produce a cell line with a PAFP-labeled nucleoporin, or with the tagging system of choice.