Minutes from the WG1 Workshop, Florence 2018; Palazzo Congressi, 4th July

Participants: Katja Graumann (WG1 Leader), Christophe Tatout (Chair), Myriam Charpentier (UK), David Evans (UK MC alternate), Hank Bass (USA), Gwenaelle Detourne (F), Frederic Pontvianne (F) and Nathalie Picault (F)

1 Introduction to WG1

KG introduced the COST Action to those present who were not familiar with the details of the Action. CT reminded delegates of the recent *JCS congress report* describing the Action. KG described the labs participating in WG1 ranging from advanced microscopy techniques to imaging processing and analysis. She then outlined the aims of WG1.
Deliverables of Grant period 2 include: a review on the next challenges in Plant microscopy and an inventory of searchable resources for all members of WG.
CT explained the COST did not fund research, but did fund integrative actions; also that WG4 is integrating image data and – omics; and that this is at an early stage.

2 Opinion paper (Review) This will be Challenges in "next challenges in plant microscopy" not a review of methods. For details to date see Appendix 1.

KG reported that this is being written by Tao Dumur, Susan Duncan, Celia Baroux and Christophe Tatout and Katja Grauman with KG as editor. The review will have two components on techniques and on image analysis. This will be published in the *Nucleus* Special Issue associated with the SRB meeting, to be submitted in November 2018. We discussed inclusion of techniques in preparation of fixed and live cell samples. This will be identifying the requirements we have for development.

HB asked whether this would include probes needed for future development eg photoactivatable this could be included in the searchable inventory. CT suggested probes/approaches for single locus 3D FISH. DE antibodies and techniques for EM level. KG noted issues of fixation, resins etc between LM and EM. Possibly use of quantum dots. Cryo EM would be a possibility. This could also be in a repository to inform COST action partners abour=t the tools and techniques available in the community. KG asked who is putting together the inventories? Aline Probst and Geraint Parry have made a more general survey but with with less details.

Actions

- **KG** will share the Google Doc of content of the Review paper for the Nucleus special issue with the group present
- **Action all** further inclusion of suggestions in the Nucleus review paper.
- **Action KG**: will liaise with AP & GP (to avoid overlapping) to set up an inventory of WG1 facilities, devices, tools hold by WG1 participants.

3 Training School 1 – 3 D FISH Clermont Ferrand

Introduced by CT. Details provided to the group. Want people to attend with lap top and they will load image analysis software to lap-tops and people will then be able to practice on own images. Possibly also include someone who is an ImageJ specialist to assist. HB asked about bringing maize probes and this will be included. He asked whether people would bring their own samples. CT not sure whether this would be feasible. HB can set up Omero accounts for delegates (see details hereafter).

Registration site open, already have 10 attendees (12 expected). These include people from ITC and Near Neighbour Countries. The applicants have sent CV and letter of motivation for selection.

4 Training School 2 – Oxford Brookes. High resolution and electron microscopy. Suggested June 2019.

Possible content- AiryScan, INM-ONM separation including infiltration; transient expression, tomography and 3 D reconstruction; (Imaris), Harwell and FRET- FLIM; possibly optical tweezers; light sheet. Harwell- would be a visit and lecture.
Getting speakers may be challenging as we need to separate this from the RMS (Royal Society) meeting in April 2019

Suggested speakers- International speaker: Iris Meier (USA); tomography: Martin Goldberg? Local speakers: Sue Vaughan, Louise Hughes, Meike Kittelman, Stan Botchway. CT recalls that partners from International partner Country (IPC) (Iris Meier) can be funded as trainer in training school within the budget.

Actions:

- **Action CT** to inform of budget available
- **Action KG/ DE** to plan training and to advertise from January 2019.

5 Image metadata and image repository

a. Image repository.

CT introduced OMERO and BISQUE platforms

OMERO: https://www.openmicroscopy.org/

The Open Microscopy Environment produces open tools to support data management for microscopy. Designed to interact with existing commercial software, all OME formats and software are free, and all OME source code is available under the GNU General public license or more permissive open source licenses. Analysis with Omero is limited but some ImageJ plugin can be linked to the Omero platform; Omero is a very useful and widely used repository. We do not have OMERO developers within the current INDEPTH group- this would be a weakness. CT introduced potential OMERO experts- CT and KG Have approached Andrew French (University of Nottingham UK), but with limited success. Others possible. Prof Jason Swedlow Division of Computational Biology, School of Life Sciences, University of Dundee; Prof Richard Baldock MRC Human Genetics Unit, Edinburgh; Prof Rafael Carazo Salas University of Cambridge; Dr Alvis Brazma European Bioinformatics Institute, Cambridge; Dr Eduouard Bertrand Montpellier ROI Imaging Montpellier; Prof Gaudenz Danuser UT Southwestern; Dr Ilan Davis University of Oxford; Mr Kevin Eliceiri University of Wisconsin-Madison: Prof Paul French Imperial College London; Dr Robert Murphy Carnegie Mellon University; Dr Spencer Shorte Institut Pasteur; Dr Gianluigi Zanetti CRS4, Sardinia

BISQUE https://bioimage.ucsb.edu/bisque

Bisque (Bio-Image Semantic Query User Environment) : **Store, visualize, organize and analyze images** in the cloud. Bisque was developed for the exchange and exploration of biological images. The Bisque system supports several areas useful for imaging researchers from image capture to image analysis and querying. The bisque system is centered around a

database of images and metadata. Search and comparison of datasets by image data and content is supported. Novel semantic analyses are integrated into the system allowing high level semantic queries and comparison of image content

One of the key issues is the space and cost for long term storage of image datasets ….plus human resource for administrating and keep the platform updated (updating versions etc).

HB has experience with OMERO. He said Swedlow (leader of the OMERO consortium) had advised that it was possible for IT people with basic set up using instructions on web page. He says they will just direct us to the web site and tell us to get on with it. There is a major problem that there is no place to deposit the images associated with publications, unlike for sequencing. OMERO takes all kinds of file types including EM.

HB has access to Florida State University (FSU) repository – space is not really an issue- he has 5 TB of storage and has uploaded the last 5 years of images using only 1/3 of the available space. He would make this available. If used in classroom, HB would be able to justify use for educational purposes. We could base this on INDEPTH log in and folders and then add to it progressively.

HB recall one of his recent paper: (**2017**) Savadel SD and Bass HW. "Review: Take a Look at Plant DNA Replication: Recent Insights and New Questions." ***Plant Signaling & Behavior***, 12:4, e1311437 (https://doi.org/10.1080/15592324.2017.1311437).
The 3D data on OMERO can be viewed. "3D Data DNA Rep, ZmRootNuclei," https://goo.gl/CTI06F is available using the login "Public" and the password "omero"

Actions:

- **HB to advise on how this is done** HB would be happy to work with anyone who has published images to upload their image data to FSU server
- **DE to discuss with GP/AP about web site links and possible use of RADAR:** HB advised that we can have a web link via a URL or DR Code to the data. We can therefore look at links (perhaps through the IT service from Oxford Brookes called RADAR which is hosting the INDEPTH website) to this data. Can also have a link for unpublished data or to have data for submitted publication.
- **KG (CT) to inform Björn Grüning WG4 of our approach.** DE pointed out need to coordinate this with WG4. The proposition is that WG1 should pilot this and keep WG4 informed of what we are doing.

b. Image Metadata

CT - **Start to think about the relevant Metadata**. Björn Grüning (WG4 leader) started to propose a first list …to be completed by WG1 members? This is available at:- https://docs.google.com/spreadsheets/d/1LT8wFYYyyRc5awYpkuRLtP4bSRgU4HrkBaIBdoXMzWg/edit?usp=sharing Storage facilities: list the possibilities within WG1 members?

CT had liaised with EBI of Omics GI looking at making intercompatibility of data- who will be seeking to make the data formats interchangeable.

KG noted that there were issues of needing to re-enter data to a spreadsheet when this information was already available in the data stored. There are fields where this can be included by way of attachments and comments- so a meta-data spreadsheet could be added.

CT is working with a mathematician to identify where and how to store data- to make advances on the current confusion of non-compatible systems. We then need to agree on key words etc

to store. First step to address the range of images and information needed. This then needs to be shared with WG4.

Actions.

- **HB to make invitations:** Every WG member to upload ONE published image to an OMERO group called INDEPTH created by HB. Actions

- **CT and HB to set up login facility** HB to liaise with the person using OMERO in UCA (Fr!) to agree on use before the coming training school in October 2018.

- **DE/KG then all:** KG/DE to create a base Excel sheet with some of the key words (Ontology) and then ask people to add terms they think are important but are missing. To be done by Sept. Then to create a standard sheet to share (Google Docs?) with drop-downs for the ontology and then we all agree to work with it.

6 STSM and ITC conference applications

David introduced the STSM and ITC opportunities for the COST. The details are on the Action Web site and the details of the next opportunity will be released. CT pointed out that to get the final 50% of budget released, we have to spend 50% of the budget by the end of November 2018. We have six STSMs allocated – matches the budget, but we need to spend a bit more-so STSM applications are still open to end of October (**DE to chase up the two additional STSM enquiries).**
David also introduced ITC conference applications.

7 AoB

KG informed the group of the Prague meeting, where each group gets 8 speaker slots- invited suggestions of speakers. Also the SEB Symposium in Madrid- again speakers invited.

Appendix 1

Title: Probing the plant nucleus: Challenges and solutions for imaging nuclei

Tao Dumur, (Tristan Dubos?) Susan Duncan, Celia Baroux and Christophe Tatout, Katja Grauman

Draft by end of September

Introduction (Katja)

1. Preparing samples for light microscopy

Both fixed and live cell

Go from whole tissue to cell to nuclei to nucleolus…..

challenges in sample preparation

Possibly a **table/Box** summarising challenge/solutions/applications (Susan/Katja)

1.1 Challenges for live cell imaging (Tao and Katja -Celia):

Tissue and organelle suitable for live imaging

Stains and markers

Tracking technology

3D (X,Y,Z and X,Y, time), 4D (x,y,z and time)

1.2 Challenges for fixed tissue preparations (Susan and Christophe (whole mount):

Reducing complexity

Minimising autofluorescence

Tissue clearing

Bright field

Single cell isolation

Nuclei isolation

1.3 Perspectives (Celia): need of imaging solutions for more depth, objectives with better working distance,..adapt existing systems for plant tissues (currently plant scientists customize their own solution, nothing commercially available)

2. The need for automated image processing

2.1: What do we learn with image processing

Figure CB

-> quantification of nuclear signals: ratio of histone modifications/nuclei

-> nuclear morphology: shape extractions..

-> heterochromatin organisation

-> fine-scale chromatin organisation

-> gene position and transcriptional activity (mRNA signal positioning + level quantification)

->spatial distribution of discrete nuclear signals (TF, pol II)

->protein co-localisation /super resolution imaging

Suggestion to present workflows?

2.2 Current possibilities: Image processing of static 3D and 4D datasets

->Tao: storyboard of the “bad” workflow= manual, biais and limitations + describe customized workflow

->CT: explain existing processing software/approach to segment nuclei and extract shape/signal/position descriptors : **Figure CT/CB (NucleusJ/Imaris)**

2.3 Solution for plant microscopy

-> Tao: present his trial on the nuclei tracking in growing roots: general principle (workflow) + **Tao representative result : root growth movie + heat stress nuclear dynamics** // discuss technical requirements (microscopy and computational sides); opens perspective for future implementation

-> CT: dream solutions for image processing of 3D nuclei...