Advancing the ability to image single RNA molecules at the cellular level

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The Idea

Plant biology currently lags behind other fields in the study of cell-to-cell variation and subcellular localization of mRNA. During my PhD I helped to establish the first Single molecule Fluorescent In situ hybridization (smFISH) method for plants where each RNA molecule can be visualized as a single fluorescent dot in Arabidopsis thaliana root meristem tissue (Duncan et al., Plant methods, 2016 in press). This technique, in combination with an image analysis algorithm developed by colleagues in the Computational and Systems Biology department at JIC, was successfully used to quantify mRNA at the cellular level. It also revealed subcellular localisation of coding and non-coding RNA and provided data to enable the estimation of the frequency of transcriptional firing events. The high level of back ground autofluorescence emitted by many green plant tissues currently limits smFISH analysis to a single tissue type. With the support of OpenPlant we propose to promote and optimise this existing technique. In addition, we aim to adapt the methodology for use in other Arabidopsis tissues and to enable RNA imaging in the liverwort Marchantia polymorpha.

Who We Are

Dr Susan Duncan, Postdoctoral Researcher, Grieneisen Lab, Department of Computational and Systems Biology, John Innes Centre, Norwich.
Email: susan.duncan@jic.ac.uk.
I am a cell biologist researching Arabidopsis cell polarity. I have relevant smFISH expertise.

Dr Susana Sauret-Gueto, Research Associate and Lab Manager, Jim Haseloff group, Department of Plant Sciences, University of Cambridge.
Email: ss2359@cam.ac.uk.
I work on developing foundational technologies, protocols and workflows for the engineering of the model plant Marchantia polymorpha. I oversee instrumentation, including microscopes, in the OpenPlant Lab in Cambridge

Mr Christian R. Boehm, Doctoral Researcher, Department of Plant Sciences, University of Cambridge.
Email:crb59@cam.ac.uk.
I am a synthetic biologist working on the development of genetic circuits controlling transgene expression in Marchantia polymorpha. My expertise relevant to this proposal embraces maintenance, propagation and transformation of M. polymorpha, the development of circuits for transgene expression and quantitative confocal microscopy.

Implementation

We propose three specific aims:
1. Establish the existing smFISH technique in Cambridge and optimise imaging
Training scientists to use this technique in Cambridge will effectively promote its use across the plant science community. Also, observing similar A. thaliana samples using different microscopes, fluorescent filter sets and cameras available in Cambridge will test the robustness of the system and aid understanding of existing imaging limitations.

2. Development of two modified smFISH approaches

Two separate approaches will be used to detect amplified mVenus mRNA fluorescent signals in Arabidopsis root meristem cells. The first will use branched DNA probes together with a brighter dye (similar to Sinnamon and Czaplinski, Methods Mol. Biol 2015). The second will use multiple branched DNA probes together with a bright, stable dye to amplify the signals up to 100X (Battich et al, Cell2015).

3. Evalute the two smFISH approaches
The two smFISH approaches will be tested on Arabidopsis leaves and hypocotyls, in addition to Marchantia gemmae. Tissue clearing techniques can also be employed, if necessary, to observe RNA signals.

Outcome

This project aims to promote and advance the use of smFISH in studies of plant gene regulation. It will provide a clearer understanding of current imaging limitations together with the first co-ordinated attempt to expand the methodology into other plant tissues and species. The knowledge gained as part of this project will prove invaluable for ongoing work even if attempts to widen its applicability have limited success. It also has the potential to guide future plans to develop smFISH as a high throughput methodology.

Benefits and outcomes

Enhancing an existing smFISH protocol supports the OpenPlant remit as this open technology provides invaluable insights into plant gene regulation. As genetic engineering projects become more ambitious, such understanding will become increasing important and could play a key role in developing strategies to mitigate transgenic gene silencing issues. This project complements existing work in both laboratories. The smFISH technique is established in the Grieneisen lab at The John Innes Centre and both microscopy resources and Marchantia expertise are central to work carried out in the Haseloff laboratory in Cambridge.
We plan to publicly document outcomes from this project:
a. A YouTube video of the existing smFISH technique will be made freely available to promote this method more widely across the plant science community.
b. The images generated by different microscopes in Norwich and Cambridge will be made freely available online to help other researchers decide on the suitability of their equipment before embarking on smFISH experiments.
c. The project will lead to publication in a peer-reviewed journal if we achieve our aim of adapting smFISH for use in other Arabidopsis tissues and Marchantia gemmae.

Budget

Approach 1 Probes (IDT)	£ 700
Approach 2 Probes (Affymetrix)	£ 1000
Other consumable costs	£ 600
Microscopy expenses (JIC)	£ 960
Travel to Cambridge / Norwich	£ 140
Total	£ 4000