Stem cell (and general biological) applications for PLISH

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Relevant experimental features of PLISH

• “On-demand” gene selection
• Combinatorial marking
• Any RNA species
• Single cell resolution
• Quantitative
Relevant technical features of PLISH

• Any species

• Compatible with immunostaining

• Very high signal-to-noise

• No specialized expertise required

• No specialized equipment required

• Inexpensive and rapid to analyze hundreds to thousands of cells
Caveats of PLISH

• Hassle to validate specificity on a per-gene basis

• Ease of detection depends upon abundance of transcript

• Lack of up-front flexibility of gene combinations

• Limited number of genes in combination

• Apparently stochastic variability in probe efficiency

• Occasional inexplicable bright background (in multiple channels)
Basic applications of PLISH

• Co-localize GOI for which antibodies not available or incompatible

• Identify cellular source of secreted factors (matrix, ligands)

• Infer signaling centers for POI (ligands and receptors)

• Validate single cell RNA-seq results

• Search for rare cell types that might be missed by scRNA-seq

• Count cells from different classes
Advanced applications of PLISH

• Quantify heterogeneity between cells within a population (e.g., iPSC)

• Integrate with genetically-encoded epitopes (e.g., GFP, Cre...)

• Correlate mRNA and protein expression

• Interrogate molecular interactions within architecturally complex system

• Deep molecular characterization of a signaling niche (e.g., stem cell niche)

• Limited empiric screening for markers of interest (e.g., stem cell markers)
Identifying and confirming a Wnt5a niche \textit{in vivo}

Col1a2

Pdgfra

Wnt2b

Wnt5a

AT2 far from Wnt5a

AT2 near Wnt5a

%AT2 cells Axin2$^+$

Far Near Wnt5a

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\text{bar graph}

\text{column graph}
Summary

• PLISH can substitute for, complement, or expand applications currently performed by immunostaining

• PLISH can validate and/or provide spatial content information for transcriptional profiles obtained by tissue-dissociative techniques

• PLISH can substitute for scRNA-seq to dissect heterogeneity within a population of interest

• PLISH can enable new biology (e.g., spatial localization of non-coding RNAs)
Making *in situ* hybridization great again!

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