# Fluorescent amplification of individual mRNAs in single cells

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***Short Abstract* — We present a new method for the fluorescent detection of RNA that combines the specificity of oligonucleotides and a new chemical ligation strategy in order to achieve highly specific and high-gain signal amplification. Using this method, we have achieved >100-fold signal amplification of individual transcripts while maintaining minimal off-target binding. The signal intensity is so strong that it can detect and separate cells based on RNA signal using flow cytometry and low-magnification microscopy, enabling the analysis and separation of large numbers of cells.**

***Keywords* — RNA FISH, RNA amplification, single cell analysis.**

RNA signatures can be used to define cell types, cell states, and disease phenotypes. However, RNA expression within a given cell population is heterogeneous and these differences can be important for cellular function. The ability to separate cells based on their RNA signatures would provide sufficient material to perform biochemical assays such as mass spectrometry and ChIP that will help to tease out the functional differences between cells. However, the current state of RNA imaging technology (i.e. single-molecule FISH[1]) provides signal that is too weak for current separation methods such as flow cytometry. We present a new method for the fluorescent detection of RNA that combines the specificity of oligonucleotides and a new chemical ligation strategy in order to achieve highly specific and high-gain signal amplification. Using this method, we have achieved >100-fold signal amplification of individual transcripts while maintaining minimal off-target binding. The signal intensity is so strong that it can detect and separate cells based on RNA signal using flow cytometry and low-magnification microscopy, enabling the analysis and separation of large numbers of cells. This method circumvents enzyme-based amplification schemes that suffer from poor cell penetration and exceeds the reported amplification of nucleic acid based amplification methods. Perhaps most importantly, the chemical ligation step enables stringent wash conditions to reduce background. Our results demonstrate the power of RNA amplification in situ and open up the single-cell and RNA fields for mechanistic studies using high-throughput, analytical methods.

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## REFERENCES

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