Exploring Perturbation Effects on Transcriptional Dynamics with ContrastiveBiVI

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The Problem

- CRISPR-based single-cell screens (Perturb-seq) let us measure how genetic perturbations change gene expression at scale, linking genotype to phenotype.
- Most analyses compare mature (spliced) mRNA abundance across perturbation groups, treating expression as a static readout.
- Those counts are the result of underlying dynamics; transcriptional bursting, splicing, and degradation, which are not directly modelled.
- As a result, different regulatory mechanisms can produce similar observed expression changes, making interpretation ambiguous.
- At the same time, perturbation effects are mixed with shared background variation across cells (e.g., cell cycle), so it is often unclear what signal is actually driven by the perturbation.
- Biophysical models of transcription address the first issue, and contrastive models address the second, but there's no unified approach that does both.

Biophysical Modelling of Transcription

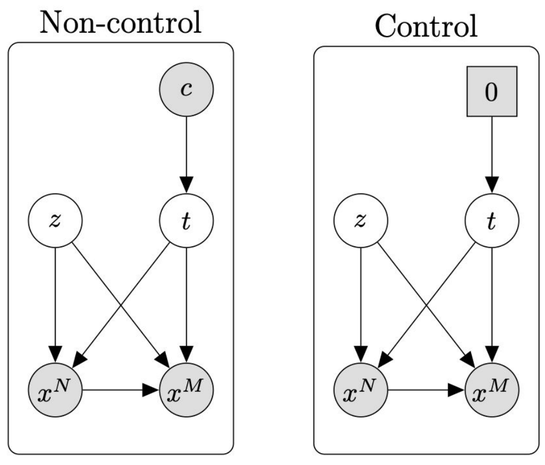
- Experimental evidence [1, 2] shows mRNA is transcribed in instantaneous bursts, processed, and degraded, so observed counts reflect underlying transcriptional dynamics rather than a static signal.

$$\varnothing \xrightarrow{k} B \times \mathcal{N} \xrightarrow{\beta} \mathcal{M} \xrightarrow{\gamma} \varnothing$$

- These dynamics can be modelled as a two-species reaction system, where bursts with rate k generate nascent mRNA ($\mathcal{N}$) that is spliced at rate β into mature mRNA ($\mathcal{M}$) and eventually degraded with rate γ.
- The resulting chemical master equation (CME) admits a steady-state solution that defines a joint distribution over nascent and mature counts, enabling inference of kinetic parameters such as burst size, splicing rate, and degradation.

Our Solution: ContrastiveBiVI

- We introduce ContrastiveBiVI, a generative model for single-cell perturbation data that combines the contrastive latent structure of ContrastiveVI [3] with the biophysical modelling framework of biVI [4].
- ContrastiveVI learns salient variables t capturing perturbation-specific effects and background variables z shared across cells, but treats expression as a static readout; biVI jointly models x^N and x^M using a CME-derived generative process, but has no mechanism to isolate perturbation signal.

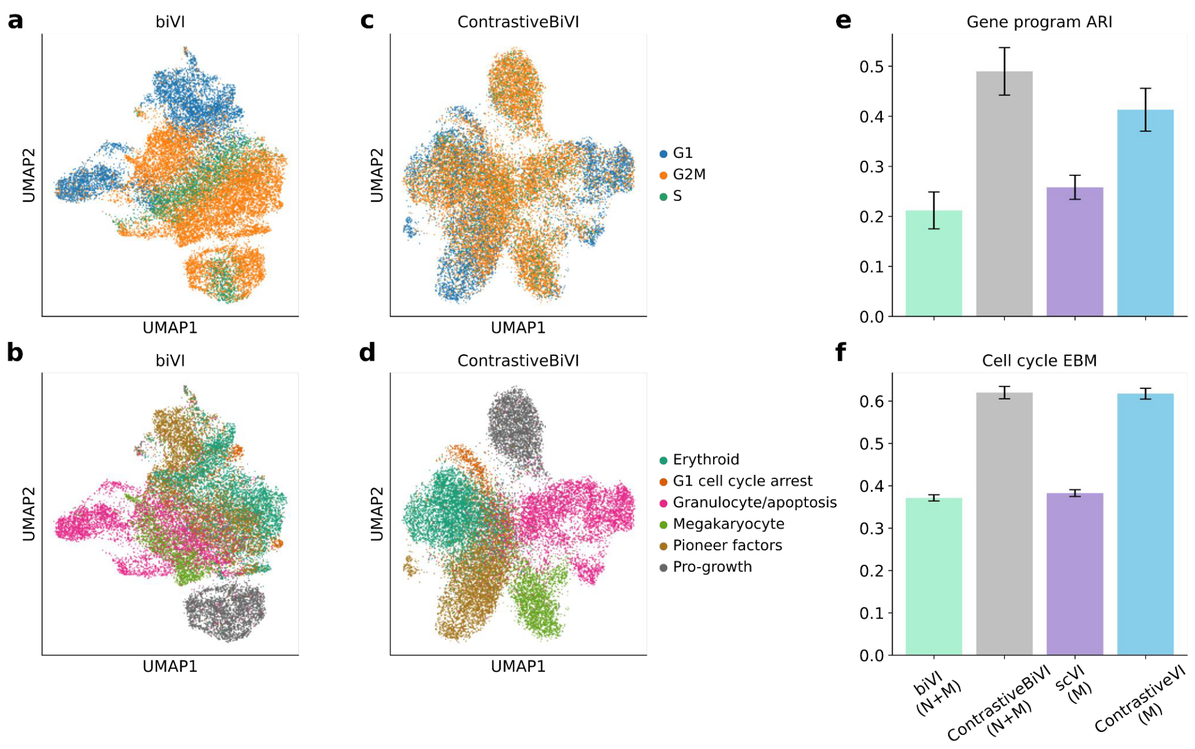


- For perturbed cells $t_i \mid c_i \sim \mathcal{N}(\mu_c, I_q)$; for control cells $t_i = 0$, forcing shared variation to be explained by z instead.
- A neural decoder ($f_\eta(z_i, t_i)$) outputs gene-wise nascent and mature expression frequencies (ρ_i^N, ρ_i^M), jointly normalized via softmax and scaled by library size (ℓ_i) to obtain gene-specific mean parameters.
- Nascent counts (x_{ig}^N) are modelled with a negative binomial marginal; mature counts (x_{ig}^M) are modelled conditionally on x_{ig}^N through the two-species bursty transcription CME.
- The CME is approximated with a pre-trained neural solver [5], from which burst size (b) and relative degradation rate (γ/k) can be recovered per perturbation.

$$
\begin{aligned}
z_i &\sim \mathcal{N}(0, I_p) && \leftarrow \text{background variation} \\
t_i \mid c_i &\sim \mathcal{N}(\mu_c, I_q), \qquad t_i = 0 \text{ for control cells} && \leftarrow \text{salient perturbation state} \\
(\rho_i^N, \rho_i^M) &= \mathrm{softmax}(f_\eta(z_i, t_i)) && \leftarrow \text{normalized expression} \\
x_{ig}^N &\sim \mathrm{NB}(\ell_i \rho_{ig}^N, \alpha_g) && \leftarrow \text{nascent counts} \\
x_{ig}^M \mid x_{ig}^N &\sim P_{\text{Bursty}}\big(m \mid n = x_{ig}^N;\ \ell_i \rho_{ig}^N, \ell_i \rho_{ig}^M, \alpha_g\big) && \leftarrow \text{mature counts}
\end{aligned}
$$

Disentangling Perturbation Effects from Background

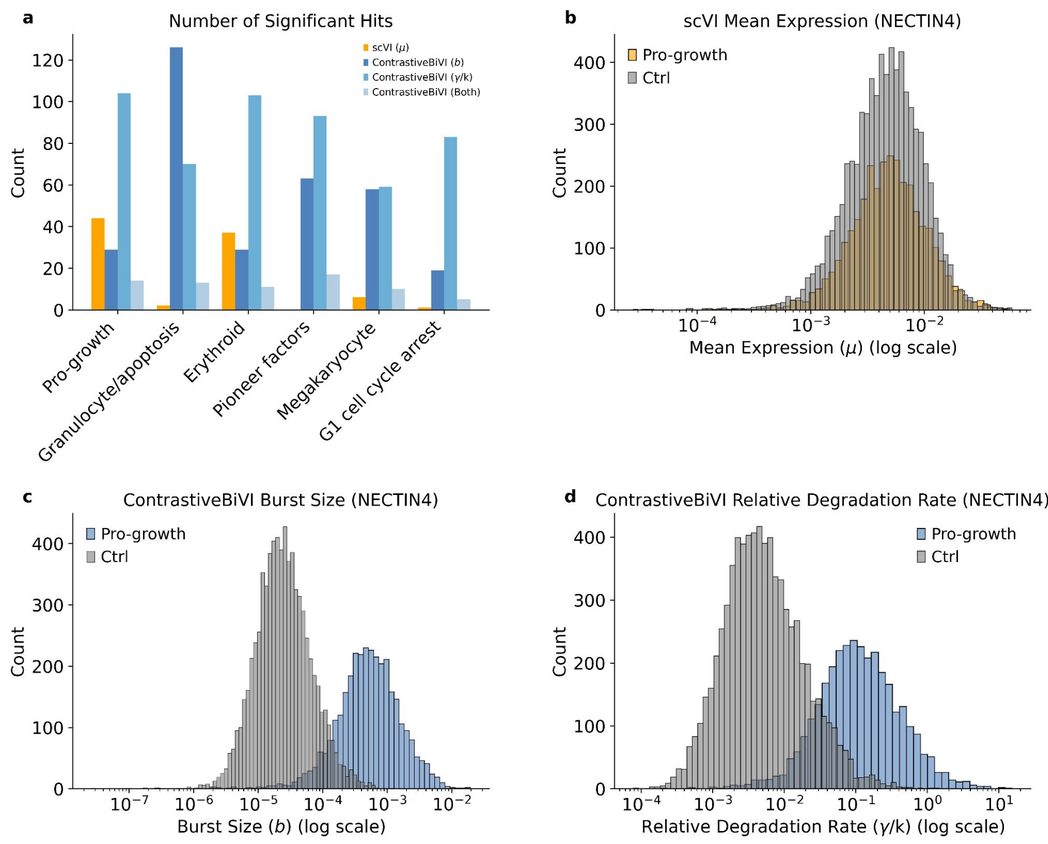
- We analyze a subset of the Norman et al. (2019) CRISPRa combinatorial Perturb-seq dataset [6] in K562 cells, comprising 46 unique genes and 52 gene-gene perturbation pairs associated with coherent gene programs.
- ContrastiveBiVI improves recovery of gene program structure over biVI, while maintaining cell cycle mixing comparable to ContrastiveVI (mature counts only).
- Consistent with these results, the salient latent space shows clearer separation by gene program and reduced cell cycle-related structure relative to biVI.



[a-d] UMAP visualizations of the biVI **[a-b]** and ContrastiveBiVI **[c-d]** representations of the Norman et al. (2019) dataset, colored by cell cycle phase **[a, c]** and gene program labels **[b, d]**. **[e]** Gene program recovery measured by ARI (higher = better). **[f]** Cell cycle mixing measured by EBM (higher = better).

Biophysical Decomposition Reveals Hidden Regulation

- Across gene programs, ContrastiveBiVI identifies more program-versus-control shifts in inferred biophysical parameters than are detected from mean spliced expression inferred by scVI.
- Many genes exhibit shifts in inferred kinetic parameters without corresponding changes in mean expression, suggesting regulatory differences not captured by mature mRNA-only analyses; a similar pattern is seen relative to ContrastiveVI (not shown).
- We see this with NECTIN4 in the Pro-growth program where despite little change in scVI-inferred mean spliced expression, ContrastiveBiVI infers compensatory shifts in burst size and relative degradation rate.



[a] Number of genes with strong evidence of program-versus-control shifts in scVI-inferred mean spliced mRNA expression and ContrastiveBiVI-inferred burst size, relative degradation rate, or both, across the six gene programs. **[b-d]** NECTIN4 parameter distributions in Pro-growth versus control cells: scVI mean expression **[b]**, ContrastiveBiVI burst size **[c]**, and ContrastiveBiVI relative degradation rate **[d]** (log scale).

References

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[3] Weinberger, E., et al., (2023). Isolating salient variations of interest in single-cell data with contrastiveVI. Nature Methods, 20(9), pp.1336-1345.
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[6] Norman, T.M., et. al., (2019). Exploring genetic interaction manifolds constructed from rich single-cell phenotypes. Science, 365(6455), pp.786-793.