A quaternion model for single cell transcriptomics

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Abstract

We present an approach for modeling single cell RNA-sequencing (scRNA-seq) data using quaternions. Quaternions are four dimensional hypercomplex numbers that, along with real numbers, complex numbers and octonions, represent one of the four normed division algebras. Quaternions have been most widely employed to represent three-dimensional rotations in computer graphics with most biomedical applications focused on problems involving the structure and orientation of biomolecules, e.g., protein folding, chromatin conformation, etc. In this paper, we detail an approach for mapping the cells in a single cell transcriptomics data set to quaternions. According to this model, the quaternion associated with each cell represents a vector in $\mathbb{R}^3$ with vector length capturing sequencing depth and vector direction capturing the relative expression profile. Assuming that biologically interesting features of an scRNA-seq data set are preserved within a rank three reconstruction, this representation has several benefits for data analysis. First, it supports a novel approach for scRNA-seq data visualization that effectively captures cell state uncertainty. Second, the model implies that transformations between cell states can be viewed as three-dimensional rotations, which have a corresponding representation as rotation quaternions. The fact that these rotation quaternions can be interpreted as cells enables a novel approach for characterizing cell state transitions with specific relevance to the analysis of pseudo-temporal ordering trajectories. Finally, a quaternion representation supports the genome-wide spectral analysis of scRNA-seq data relative to a single variable, e.g., pseudo-time, or two variables, e.g., spatial coordinates, using a one or two-dimensional hypercomplex Fourier transform.

1 Background

1.1 Quaternions

Quaternions are four dimensional hypercomplex numbers that, along with real numbers, complex numbers and octonions, represent one of the four normed division algebras. Formally, hypercomplex numbers are elements of a finite-dimensional algebra over the real numbers that is unital but may not be associative or commutative [1] and have a general representation given by:

$$a_0 + \sum_{j=1}^{n} a_j i_j, i_j^2 \in \{-1, 0, 1\}$$

where a_0 and the a_j are real numbers and the i_j form an n-dimensional basis. Following this representation, $n = 0$ for real numbers, $n = 1$ for complex numbers, $n = 3$ for quaternions and $n = 7$ for octonions with $i_j^2 = -1$, i.e., real numbers, complex numbers, quaternions and octonions are the hypercomplex numbers with dimensions 1, 2, 4 and 8 that are each comprised by a real number and a vector in an n-dimensional imaginary space. While real and complex numbers are commonly employed in applied mathematics to model experimental data, quaternions, and especially ontonions, are infrequently used outside of a theoretical context.

Quaternions were first explored by Gauss in 1809, however, because that work was not published until 1900, the discovery of quaternions is usually credited to the Irish mathematician William Hamilton in 1843 [2]. Hamilton's discovery was motivated by attempts to extend complex numbers to three dimensions. In his work on three dimensional hypercomplex numbers, Hamilton struggled with multiplication/division, i.e., what is the quotient of two points in $\mathbb{R}^3$? Hamilton realized these problems could be solved by moving to four dimensional hypercomplex numbers and, when he had this realization, famously carved the formula for the quaternion imaginary basis vectors into the Brougham Bridge:

$$i^2 = j^2 = k^2 = ijk = -1$$

More specifically, a quaternion q is a four dimensional hypercomplex number defined by (1).

$$q = a + bi + cj + dk \tag{1}$$

where $\{a, b, c, d\} \in \mathbb{R}$ and i, j, and k form a three dimensional imaginary basis with:

$$\begin{aligned} i^2 = j^2 = k^2 = ijk = -1 \\ ij = k, ji = -k \\ jk = i, kj = -i \\ ki = j, ik = -j \end{aligned} \tag{2}$$

Addition and subtraction of quaternions is simply performed by adding and subtracting the associated terms. For example, if $q_1 = a_1 + b_1 i + c_1 j + d_1 k$ and $q_2 = a_2 + b_2 i + c_2 j + d_2 k$, then $q_1 + q_2 = a_1 + a_2 + (b_1 + b_2)i + (c_1 + c_2)j + (d_1 + d_2)k$. Multiplication of quaternions follows the distributative law and the rules for multiplication of the basis vectors given by (2). An important consequence of (2) is that multiplication of quaternions is not commutative, e.g., $ij \neq ji$. Division of quaternions is defined by the pre or post multiplication by the inverse of the denominator, which, given the noncommunicativity of multiplication, may be distinct. So, q_1/q_2 has two possible values: $q_1 q_2^{-1}$ or $q_2^{-1} q_1$. The inverse of a quaternion is defined as the ratio of the conjugate (q^*) and the square of the norm ($||q||$):

$$q^{-1} = \frac{q^*}{||q||^2} = \frac{a - bi - cj - dk}{a^2 + b^2 + c^2 + d^2} \tag{3}$$

It is often convienent to separate quaternions into scalar ($r = a$) and vector ($\vec{v} = bi + cj + dk$) parts:

$$q = a + bi + cj + dk = r + \vec{v} = (r, \vec{v}) \tag{4}$$

If $b = c = d = 0$, the quaternion is referred to as a scalar quaternion. If $a = 0$, the quaternion is referred to as a vector quaternion. Using the scalar/vector notation, multiplication of quaternions can be conveniently represented using vector dot and cross product notation:

$$q_1 q_2 = (r_1, \vec{v}_1)(r_2, \vec{v}_2) = (r_1 r_2 - \vec{v}_1 \cdot \vec{v}_2, r_1 \vec{v}_2 + r_2 \vec{v}_1 + \vec{v}_1 \times \vec{v}_2) \tag{5}$$

Interpretation of (5) can be aided by considering two special cases:

1. Multiplication by a scalar: If q_2 is a scalar quaternion, i.e., $\vec{v}_2 = 0$, then the multiplication simplifies to the scaling of q_1 by the scalar part of q_2:

$$\begin{aligned}
&(r_1 r_2 - \vec{v}_1 \cdot \vec{v}_2, r_1 \vec{v}_2 + r_2 \vec{v}_1 + \vec{v}_1 \times \vec{v}_2) \\
&(r_1 r_2 - \vec{v}_1 \cdot 0, r_1 0 + r_2 \vec{v}_1 + \vec{v}_1 \times 0) \\
&(r_1 r_2, r_2 \vec{v}_1)
\end{aligned}$$

2. Multiplication of two vector quaternions: If both q_1 and q_2 are vector quaternions, i.e., $r_1 = r_2 = 0$, then multiplication simplifies to just the dot and cross product terms:

$$(\vec{v}_1 \cdot \vec{v}_2, \vec{v}_1 \times \vec{v}_2)$$

If $\vec{v}_1 \perp \vec{v}_2$, then $q_1 q_2 = \vec{v}_1 \times \vec{v}_2$, which is a vector quaternion that is orthogonal to the plain defined by q_1 and q_2. If $\vec{v}_1 || \vec{v}_2$, then $q_1 q_2 = \vec{v}_1 \cdot \vec{v}_2$, which is a scalar quaternion.

Following their introduction by Hamilton, quaternions were widely used in geometry and physics until the development of vector analysis methods. While interest in quaternions is now primarily restricted to pure mathematics, they are still employed for the efficient computation of 3D rotations, as detailed in Section 1.2 below.

1.2 Quaternion representation of three-dimensional rotations

Any important feature of quaternions is their ability to efficiently represent rotations in $\mathbb{R}^3$ [3]. According to Euler's rotation theorem, any single rotation (or sequence of rotations) in $\mathbb{R}^3$ can be modeled as a rotation of angle θ about an axis (the Euler axis) that can be represented by a unit vector. Let the $\mathbb{R}^3$ vector $\vec{v}$ represent a vector from the origin to the point (v_x, v_y, v_z) and model $\vec{v}$ using the vector quaternion $q_v = v_x i + v_y j + v_z k$. The rotation of $\vec{v}$ by angle θ about axis defined by unit vector $\vec{u} = (u_x, u_y, u_z)$ can be represented by the rotation quaternion $q^r = cos(\theta/2) + sin(\theta/2)(u_x i + u_y j + u_z k)$ and computed mathematically as:

$$q'_v = q^r q_v (q^r)^{-1} \tag{6}$$

The coeffients of the generated vector quaternion q'_v capture the coordinates of the rotated vector $\vec{v}$ in $\mathbb{R}^3$. The product of multiple rotation quaternions generates a quaternion whose orientation is produced by the corresponding sequence of rotations. There are several important benefits of the quaternion model for computing rotations relative to the vector analysis approach that employs a 3×3 rotation matrix:

- Rotation quaternions provide a more compact representation (4 numbers vs 9 for a rotation matrix)
- Rotation quaternions are a more interpretable representation (i.e., easy to translate between desired rotation and rotation quaternion)
- Rotation quaternions support efficient calculation of smooth rotations by robustly dividing a single large rotation into a sequence of small rotations.

These benefits have led to the frequent use of quaternions in computer graphics.

1.3 Analysis of biomedical data using quaternions

Given the advantages of quaternions for modeling rotations, most biological applications of quaternions have involved the structural analysis of biomolecules including problems involving protein folding [4], chromatin structure [5], and molecular dynamics [6]. Outside of these structural analysis problems, use of quaternions for the analysis of biological data has been very limited with the only notable developments involving the discovery of repetitive elements within either one-dimensional sequences (e.g., DNA) or two-dimensional arrays (e.g., images). For example, Brodzik explored the use of quaternions for the detection of tandem repeats in DNA using a periodicity transformation on a novel mapping of DNA bases to quaternions [7], and, in the space of image analysis, Ell and Sangwine [8] explored the mapping of RGB pixel values into quaternions to support the analysis of color images using a two-dimensional hypercomplex Fourier transform.

1.4 Single cell transcriptomics

Although multicellular tissues are comprised by a complex set of cell types and states, high-throughput genome-wide profiling has historically been limited to bulk tissue assays. The genomic values captured by these techniques reflect the average across all cell types and states present in the sample and, when significant heterogeneity exists in the tissue sample, provide only a rough approximation of the true biological state. To address the limitations of bulk assays, researchers have recently developed a range of methods that support the single cell measurement of RNA expression (e.g., single cell RNA sequencing or scRNA-seq [9]), DNA sequence (e.g., single nucleus exome sequencing [10]), chromatin accessibility (e.g., single cell ATAC sequencing (scATAC-seq) [11]), epigenetic modifications (e.g., single cell bisulfite sequencing [12]) and protein abundance (e.g., Cellular Indexing of Transcriptomes and Epitopes by Sequencing (CITE-seq) [13]. Among these techniques, scRNA-seq has seen the most rapid development with state-of-the-art systems (e.g., 10x Genomics Chromium System [14]) now able to cost-effectively profile tens-of-thousands of cells isolated from a single tissue sample. More recent technical advances have enabled the joint profiling of multiple omics modalities for dissociated cells (e.g., joint scRNA-seq/scATAC-seq and joint scRNA-seq/CITE-seq) and the spatial transcriptomics analysis of whole tissue samples at the resolution of single cells (e.g., MERFISH [15]) or small cell groups (e.g., 10x Genomics Visium technology [16]). Important applications of single cell transcriptomics include cataloging the cell types in a tissue [17, 18], discovering novel cell subtypes [19], reconstructing dynamic processes via pseudo-temporal ordering [20], and analyzing cell-cell signaling [21].

Although scRNA-seq data has the potential to more accurately characterize the biological state of complex tissues, statistical analysis of this data is very challenging. Single cell transcriptomics is high-dimensional (transcript abundance is typically measured on $10,000$ or more genes), extremely sparse (90% or more gene counts are typically 0), and noisy (due to significant ampliplification bias and other technical factors). Single cell assays typically output a $n \times p$ matrix of transcript counts for n cells and p protein-coding genes. The use of unique molecular identifiers (UMIs) means that these counts correspond to the number of unique mRNA molecules whose sequence aligns to a specific gene. Statistically, scRNA-seq data is typically modeled using a Poisson or negative binomial distribution, which is sometimes extended to support zero-inflation. To enable the comparison of cells with different library sizes (i.e., the total number of UMIs captured for a given cell), the raw scRNA-seq count data is usually normalized to relative abundance values. One simply normalization approach (refered to as "log-normalization" in the Seurat framework [22]) that adjusts for library size takes the log of a pseudo count of 1 plus the ratio of the scaled counts for a specific gene to the total number of counts for the cell or $log(10000x_{i,j}/\sum_{k=1}^{p} x_{i,k} + 1)$, where

$x_{i,j}$ holds the UMI counts for gene j in cell i. More complex normalization methods that adjust for library size include the SCTransform method [23], which uses the residuals from a regularized negative binomial regression model of the raw counts on library size. Following normalization, some form of dimensionality reduction is typically employed (e.g., principal component analysis (PCA), uniform manifold approximation and projection (UMAP) [24], etc.) to make subsequent statistical analyses more tractable. Visualization of scRNA-seq data is usually done using a projection onto the first two reduced dimensions.

2 Quaternion model for scRNA-seq data

Our approach for mapping single cell RNA-sequencing (scRNA-seq) data to quaternions is detailed in Algorithm 1 below. Although the primarily motivation of this model is scRNA-seq data, the same approach can be applied to other forms of count-based genomic data, e.g., bulk RNA-seq or spatial transcriptomics.

Algorithm 1 Quaternion model for scRNA-seq data

Input: An $n \times p$ matrix $\mathbf{X}$ that holds that unnormalized UMI counts for p genes measured in n cells via scRNA-seq.

Output: Length n vector $\mathbf{q}$ of quaternions that represent the projection of the n cells onto a rank 3 subspace of $\mathbf{X}$.

Notation: Let q_a, q_b, q_c, and q_d represent the real-valued coefficients for quaternion $q = a + bi + cj + dk$ with real component a and vector component $\{b, c, d\}$.

1: $\mathbf{X} = \mathbf{U}\mathbf{D}\mathbf{V}^T$ ▷ Compute the singular value decomposition (SVD) of $\mathbf{X}$.

2: $\mathbf{P} = \mathbf{U}_3\mathbf{D}_3$ ▷ Project $\mathbf{X}$ onto the first three singular vectors of $\mathbf{X}$. $\mathbf{U}_3$ is a $n \times 3$ matrix that holds the first three columns of $\mathbf{U}$ and $\mathbf{D}_3$ is a 3×3 matrix that holds the first three diagonal elements of $\mathbf{D}$.

3: $\forall_{i \in 1 \ldots n} q_{i,a} = ||p_i||_2 = \sqrt{p_{i,1}^2 + p_{i,2}^2 + p_{i,2}^2}$ ▷ Set the real component of the quaternion for cell i to the Euclidean norm of the rank 3 projection.

4: $\forall_{i \in 1 \ldots n} q_{i,b} = p_{i,1}/||p_i||_2, q_{i,c} = p_{i,2}/||p_i||_2, q_{i,d} = p_{i,3}/||p_i||_2$ ▷ Set the complex coefficients for the quaternion for cell i to the coordinates of the rank 3 projection normalized to unit length.

return q

To improve the computational performance of Algorithm 1 on large scRNA-seq data sets, step 1 can be replaced by a randomized SVD algorithm (e.g., the randomized SVD implementation in the *rsvd* R package [25]) with only the first three singular vectors computed.

2.1 Properties of quaternion model

The quaternion scRNA-seq model outlined in Algorithm 1 projects the unnormalized count data for each cell onto the first three uncentered principal components, i.e., each cell becomes a vector in $\mathbb{R}^3$. The magnitude of the vector for each cell (i.e., the real part of the corresponding quaternion) represents library size and the direction of the vector (i.e., the vector part of the quaternion) captures the cell's relative expression profile, i.e., all cells with the same relative expression profile will be scaled versions of the same vector.

This type of projection has the useful property that all cells with identical relative gene expression profiles are projected onto a line that intersects the origin with distance along the line

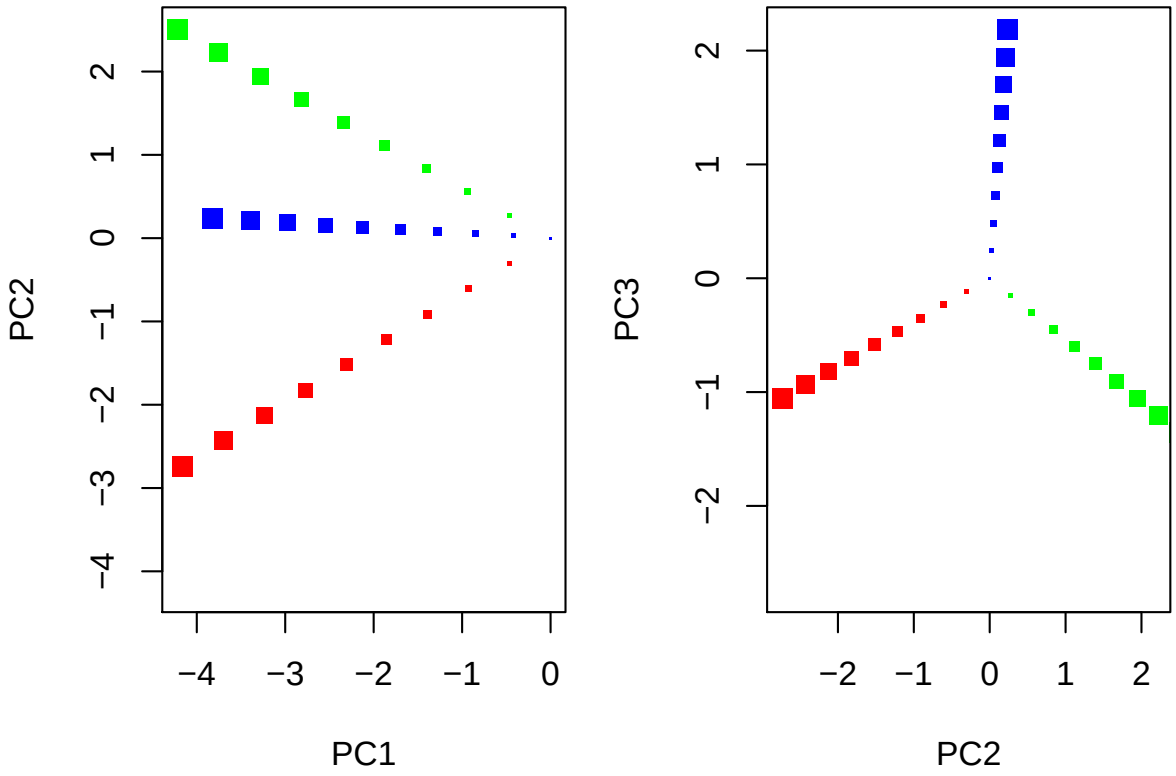

Figure 1: Projection of simulated scRNA-seq relative abundance data onto first three uncentered principal components. Points with the same color represent cells with the same relative abundance profile. Point size reflects the sum of all non-relative counts for the simulated cell.

representing library size. Figure 1 below illustrates this phenomenon for simulated scRNA-seq relative abundance data for 5 genes and 30 cells split into three different populations. The first 10 cells were simulated with relative abundance values of $\{0.5, 0.2, 0.1, 0.1, 0.1\}$, the next 10 with relative abundance values of $\{0.1, 0.1, 0.1, 0.2, 0.5\}$, and the last 10 with relative abundance values of $\{0.1, 0.3, 0.3, 0.2, 0.1\}$. Within each population, the relative library size varied from 1 to 10. When these cells are projected onto the first three uncentered principal components, all cells sharing the same relative expression profile lie along a single line with distance from the origin proportional to library size.

The proposed quaternion model also has the useful property that transitions between different relative expression profiles are represented by a rotation in $\mathbb{R}^3$, which is equivalent to the use of cosine similarity to capture the distance. Cosine similarity works well as a distance measure when the focus is on relative vs. absolute values (i.e., angle will be 0 for scaled versions of the same vector so is equivalent to comparing relative data) and is therefore appropriate for scRNA-seq that is typically normalized by library size to generate relative values. Section 3 details additional features of this model in the context of four different applications: visualization of cell state uncertainty, interpretation of cell state transitions, and spectral analysis.

3 Quaternion model applications

3.1 Visualization of cell state uncertainty

Single cell transcriptomics data is typically visualized by projecting normalized cells onto a two-dimensional subspace with the first two principal components or first two UMAP dimensions common options. Although library size-based normalization does enable the comparative evaluation of cells that may contain different amounts of mRNA or have different capture efficiencies/sequencing depths, it introduces an important limitation for standard visualization techniques (and other statistical analyses). Specifically, the uncertainty in the relative abundance values generated by normalization is proportional to the library size for the cell, however, this information regarding cell state uncertainty is lost when visualizing normalized data. Our approach for representing cells as vectors in $\mathbb{R}^3$ enables a visualization that captures both the data characteristics seen in a two-dimensional projection of normalized scRNA-seq data as well as uncertainty in the relative expression profile. Specifically, all cells that share a similar relative expression profile will be distributed along a line that intersects the origin with the distance from the origin capturing relative library size. Cells far from the origin therefore have a much more accurately inferred cell state than cells close to the origin. This property can be used informally by users to guide the interpretation of the visualized single cell data or formally to generate confidence regions for each cell according to a specific statistical model for the unnormalized UMI counts.

3.2 Interpretation of cell state transitions

According to the quaternion model detailed in Section 2, the transition between cell states is represented by a rotation in $\mathbb{R}^3$. As detailed in Section 1.2, this rotation can be represented by a rotation quaternion. An important benefit of this model is that the rotation axis defined by the rotation quaternion corresponds to a cell state and thus provides a biological interpretation for cell state transitions. More specifically, the rotation axis represents the cell state that is invariant to the associated transition, i.e., a cell in that state would be unaffected by whatever biological perturbation triggers a transition between those cell states. Although viewing cell state transitions in terms of an invariant or orthogonal state may not always be intuitive, it does provide a unique biological perspective on cell state differences.

An important application of this cell state transition model involves the intepretation of trajectories generated by pseudo-temporal ordering methods such as Monocle [20]. Specifically, the transitions between cells lying on a computed trajectory correspond to rotations and therefore have an interpretion via the associated invariant cell state. The quaternion rotation model also makes it possible to efficiently compute over the set of potential paths that connect cells in distal parts of the trajectory, e.g, cell at the root and cells at the leaves, which can be utilized to estimate the likelihood of the observed trajectory relative to the space of possible trajectories.

3.3 Spectral analysis using hypercomplex Fourier transformation

The genome-wide spectral analysis of scRNA-seq data is challenging. For exploring the spectra of single cell gene expression in one dimension (e.g., pseudotime) or two dimensions (e.g., spatial transcriptomics) a one or two dimensional Fourier analysis is typically performed on the expression values for each gene separately. Alternatively, the projection of each cell onto a reduced dimension (e.g., principal component or UMAP dimension) could be done but exploring the joint spectral characteristics of multiple gene expression variables is challenging. Ell and Sangwine [8] used a mapping from RGB color values to quaternions to support the application of a two-dimensional hypercomplex Fourier transform to color image data. The proposed scRNA-seq quaternion model

enables the spectral analysis scRNA-seq data relative to a single variable (e.g., pseudo-time) or two variables (e.g., spatial location) to be performed on a genome-wide basis by used a one or two-dimensional hypercomplex Fourier transformation applied to the quaternion representations of each cell's gene expression data.

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References

[1] I. L. Kantor and A. S. Solodovnikov. *Hypercomplex Numbers: An Elementary Introduction to Algebras*. Springer, 1989.

[2] Sir William Rowan Hamilton LL.D. P.R.I.A. F.R.A.S. Hon. M.R.Soc. Ed. and Dub. Lxxviii. on quaternions; or on a new system of imaginaries in algebra. *The London, Edinburgh, and Dublin Philosophical Magazine and Journal of Science*, 25(169):489–495, 1844.

[3] Jack B. Kuipers. *Quaternions and rotation sequences : a primer with applications to orbits, aerospace, and virtual reality*. Princeton Univ. Press, Princeton, NJ, 1999.

[4] Andrew J Hanson and Sidharth Thakur. Quaternion maps of global protein structure. *J Mol Graph Model*, 38:256–78, Sep 2012.

[5] Claudia Caudai, Emanuele Salerno, Monica Zoppè, and Anna Tonazzini. Inferring 3d chromatin structure using a multiscale approach based on quaternions. *BMC Bioinformatics*, 16:234, Jul 2015.

[6] T. F. Miller, M. Eleftheriou, P. Pattnaik, A. Ndirango, D. Newns, and G. J. Martyna. Symplectic quaternion scheme for biophysical molecular dynamics. *The Journal of Chemical Physics*, 116(20):8649–8659, 2002.

[7] Andrzej K Brodzik. Quaternionic periodicity transform: an algebraic solution to the tandem repeat detection problem. *Bioinformatics*, 23(6):694–700, Mar 2007.

[8] Todd A. Ell and Stephen J. Sangwine. Hypercomplex fourier transforms of color images. *IEEE Transactions on Image Processing*, 16(1):22–35, 2007.

[9] Evan Z Macosko, Anindita Basu, Rahul Satija, James Nemesh, Karthik Shekhar, Melissa Goldman, Itay Tirosh, Allison R Bialas, Nolan Kamitaki, Emily M Martersteck, John J Trombetta, David A Weitz, Joshua R Sanes, Alex K Shalek, Aviv Regev, and Steven A McCarroll. Highly parallel genome-wide expression profiling of individual cells using nanoliter droplets. *Cell*, 161(5):1202–1214, May 2015.

[10] Marco L Leung, Yong Wang, Jill Waters, and Nicholas E Navin. Snes: single nucleus exome sequencing. *Genome Biol*, 16:55, Mar 2015.

[11] Jason D Buenrostro, Beijing Wu, Ulrike M Litzenburger, Dave Ruff, Michael L Gonzales, Michael P Snyder, Howard Y Chang, and William J Greenleaf. Single-cell chromatin accessibility reveals principles of regulatory variation. *Nature*, 523(7561):486–90, Jul 2015.

[12] Sébastien A Smallwood, Heather J Lee, Christof Angermueller, Felix Krueger, Heba Saadeh, Julian Peat, Simon R Andrews, Oliver Stegle, Wolf Reik, and Gavin Kelsey. Single-cell genome-wide bisulfite sequencing for assessing epigenetic heterogeneity. *Nat Methods*, 11(8):817–820, Aug 2014.

[13] Marlon Stoeckius, Christoph Hafemeister, William Stephenson, Brian Houck-Loomis, Pratip K Chattopadhyay, Harold Swerdlow, Rahul Satija, and Peter Smibert. Simultaneous epitope and transcriptome measurement in single cells. *Nat Methods*, 14(9):865–868, Sep 2017.

[14] Grace X Y Zheng, Jessica M Terry, Phillip Belgrader, Paul Ryvkin, Zachary W Bent, Ryan Wilson, Solongo B Ziraldo, Tobias D Wheeler, Geoff P McDermott, Junjie Zhu, Mark T Gregory, Joe Shuga, Luz Montesclaros, Jason G Underwood, Donald A Masquelier, Stefanie Y Nishimura, Michael Schnall-Levin, Paul W Wyatt, Christopher M Hindson, Rajiv Bharadwaj, Alexander Wong, Kevin D Ness, Lan W Beppu, H Joachim Deeg, Christopher McFarland, Keith R Loeb, William J Valente, Nolan G Ericson, Emily A Stevens, Jerald P Radich, Tarjei S Mikkelsen, Benjamin J Hindson, and Jason H Bielas. Massively parallel digital transcriptional profiling of single cells. *Nat Commun*, 8:14049, 01 2017.

[15] Kok Hao Chen, Alistair N Boettiger, Jeffrey R Moffitt, Siyuan Wang, and Xiaowei Zhuang. Rna imaging. spatially resolved, highly multiplexed rna profiling in single cells. *Science*, 348(6233):aaa6090, Apr 2015.

[16] Patrik L Ståhl, Fredrik Salmén, Sanja Vickovic, Anna Lundmark, José Fernández Navarro, Jens Magnusson, Stefania Giacomello, Michaela Asp, Jakub O Westholm, Mikael Huss, Annelie Mollbrink, Sten Linnarsson, Simone Codeluppi, Åke Borg, Fredrik Pontén, Paul Igor Costea, Pelin Sahlén, Jan Mulder, Olaf Bergmann, Joakim Lundeberg, and Jonas Frisén. Visualization and analysis of gene expression in tissue sections by spatial transcriptomics. *Science*, 353(6294):78–82, Jul 2016.

[17] El-ad David Amir, Kara L Davis, Michelle D Tadmor, Erin F Simonds, Jacob H Levine, Sean C Bendall, Daniel K Shenfeld, Smita Krishnaswamy, Garry P Nolan, and Dana Pe'er. visne enables visualization of high dimensional single-cell data and reveals phenotypic heterogeneity of leukemia. *Nat Biotechnol*, 31(6):545–52, Jun 2013.

[18] Peter V Kharchenko, Lev Silberstein, and David T Scadden. Bayesian approach to single-cell differential expression analysis. *Nat Methods*, 11(7):740–2, Jul 2014.

[19] Stéphane Chevrier, Jacob Harrison Levine, Vito Riccardo Tomaso Zanotelli, Karina Silina, Daniel Schulz, Marina Bacac, Carola Hermine Ries, Laurie Ailles, Michael Alexander Spencer Jewett, Holger Moch, Maries van den Broek, Christian Beisel, Michael Beda Stadler, Craig Gedye, Bernhard Reis, Dana Pe'er, and Bernd Bodenmiller. An immune atlas of clear cell renal cell carcinoma. *Cell*, 169(4):736–749.e18, May 2017.

[20] Xiaojie Qiu, Qi Mao, Ying Tang, Li Wang, Raghav Chawla, Hannah A Pliner, and Cole Trapnell. Reversed graph embedding resolves complex single-cell trajectories. *Nat Methods*, Aug 2017.

[21] Simon Cabello-Aguilar, Mélissa Alame, Fabien Kon-Sun-Tack, Caroline Fau, Matthieu Lacroix, and Jacques Colinge. Singlecellsignalr: inference of intercellular networks from single-cell transcriptomics. *Nucleic Acids Res*, 48(10):e55, Jun 2020.

[22] Tim Stuart, Andrew Butler, Paul Hoffman, Christoph Hafemeister, Efthymia Papalexi, William M Mauck, 3rd, Yuhan Hao, Marlon Stoeckius, Peter Smibert, and Rahul Satija. Comprehensive integration of single-cell data. *Cell*, 177(7):1888–1902.e21, Jun 2019.

[23] Christoph Hafemeister and Rahul Satija. Normalization and variance stabilization of single-cell rna-seq data using regularized negative binomial regression. *Genome Biol*, 20(1):296, 12 2019.

[24] Leland McInnes, John Healy, and James Melville. Umap: Uniform manifold approximation and projection for dimension reduction, 2018.

[25] N. Benjamin Erichson, Sergey Voronin, Steven L. Brunton, and J. Nathan Kutz. Randomized matrix decompositions using r. *Journal of Statistical Software*, 89(11):1â 48, 2019.