same fish. The imaged section was chosen so as to avoid direct exposure of the eyes to the illumination beam. During 2P imaging, the flashes elicited acute neural responses in various regions of the brain (**Fig. 1b** and **Supplementary Video 1**) even at the lowest flash intensity (3.5 μW cm^{-2}). At an illumination intensity compatible with 3D imaging, the response during 1P imaging was either abolished or greatly attenuated (**Fig. 1b** and **Supplementary Video 2**). To quantify this observation, we extracted the maximum of the post-stimulus signal in the five most responsive regions in both experiments. In 2P imaging, all regions displayed a substantial response at the lowest stimulus intensity, which rapidly reached a plateau as the visual stimulation became more intense (**Supplementary Figs. 5** and **6,** and **Fig. 1c**). During 1P imaging, the visually evoked activity was mostly confined to the tectal neuropil, the region that receives direct projections from retinal ganglion cells. To elicit measurable signals in other brain areas, the stimulus intensity had to be increased up to 4,500 μW cm^{-2}, beyond physiologically relevant levels.

The exact mechanism by which 1P illumination leads to the observed reduction in visual sensitivity remains to be elucidated. The photoreceptors in the retina may receive excitation light scattered by the observation chamber or by the brain tissue, increasing the response threshold to subsequent visual stimuli. Alternatively, the visually evoked neural response may be attenuated owing to direct activation of light-sensitive cells throughout the brain. In any case, our results highlight the potential problems associated with visible illumination wavelengths and argue for the use of near-infrared wavelengths when studying visually driven processes.

2P light-sheet imaging combines the advantages of near-infrared illumination with the high speed of light-sheet microscopy. We produced 3D maps of flash-responsive neurons in a single experiment by sequentially moving the recorded plane across the brain and simultaneously recorded >36,000 individual neurons at 1 Hz (**Fig. 1d**,**e**, **Supplementary Fig. 7** and **Supplementary Video 3**), without compromising signal-to-noise ratio. Brain-wide parallel recording of multiple brain regions opens the possibility of identifying extended clusters of neurons by analyzing the structure of the correlation matrix (**Supplementary Methods** and **Supplementary Fig. 8**). Cluster dynamics can then provide a low-dimensional description of the post-stimulus time sequence of brain activity (**Fig. 1f**,**g** and **Supplementary Fig. 9**).

In conclusion, we demonstrated that near-infrared 2P light-sheet imaging is suitable for 3D brain-wide functional imaging in zebrafish larvae at cellular resolution. Notably, this imaging method eliminates the photostimulation associated with 1P functional imaging. Although the latter does not abolish robust visuomotor reflexes, we showed that it compromises visual perception in the blue domain. Therefore, 2P light-sheet imaging is a suitable alternative method for whole-brain network analysis of neural processes that require fine control over the visual stimuli or that are sensitive to the photic environment.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper (doi:10.1038/nmeth.3371).

ACKNOWLEDGMENTS

We thank F. Engert (Harvard University) for providing the Huc:GCaMP5G strain. The study was partly supported by Agence Nationale de la Recherche (contracts ANR-2010-JCJC-1510-01, ANR-11-EQPX-0029, ANR-10-INBS-04), Fondation Louis D. de l'Institut de France, European Union Seventh Framework Program (Marie Curie International Reintegration Grant no. 268379).

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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MiXCR: software for comprehensive adaptive immunity profiling

To the Editor: High-throughput sequencing is gaining importance in adaptive immunity studies, demanding efficient software solutions for immunoglobulin (IG) and T-cell receptor profiling[1]. Here we report MiXCR (available at http://mixcr.milaboratory.com/ and https://github.com/milaboratory/mixcr/), a universal framework that processes big immunome data from raw sequences to quantitated clonotypes. MiXCR efficiently handles paired- and single-end reads, considers sequence quality, corrects PCR errors and identifies germline hypermutations. The software supports both partial- and full-length profiling and employs all available RNA or DNA information, including sequences upstream of V and downstream of J gene segments (**Fig. 1**, **Supplementary Note 1** and **Supplementary Table 1**).

In contrast with previous software[2–5], MiXCR employs an advanced alignment algorithm that processes tens of millions of reads within minutes, with accurate alignment of gene segments even in a severely hypermutated context (**Supplementary Note 2** and **Supplementary Tables 2–6**). In paired-end sequencing analysis, MiXCR aligns both reads and aggregates information from both alignments to achieve high V and J gene assignment accuracy. It handles mismatches and indels and thus is suitable even for sequences with many errors and hypermutations. MiXCR employs a built-in library of reference germline V, D, J and C gene sequences for human and mouse based on corresponding loci from GenBank[6].

MiXCR further assembles identical and homologous reads into clonotypes, correcting for PCR and sequencing errors using a heuristic multilayer clustering. Additionally, it rescues low-quality reads by mapping them to previously assembled high-quality clonotypes[7] to preserve maximal quantitative information (**Supplementary Note 3**). The Illumina MiSeq platform currently allows for deep full-length IG repertoire profiling with ~20 million long paired-end reads. MiXCR captures all complementarity-determining regions (CDRs) and framework regions of immune genes and permits the assembly of full-length clonotypes. Flexibility to analyze partial-length data is also provided, allowing, for example, users to group reads into clonotypes

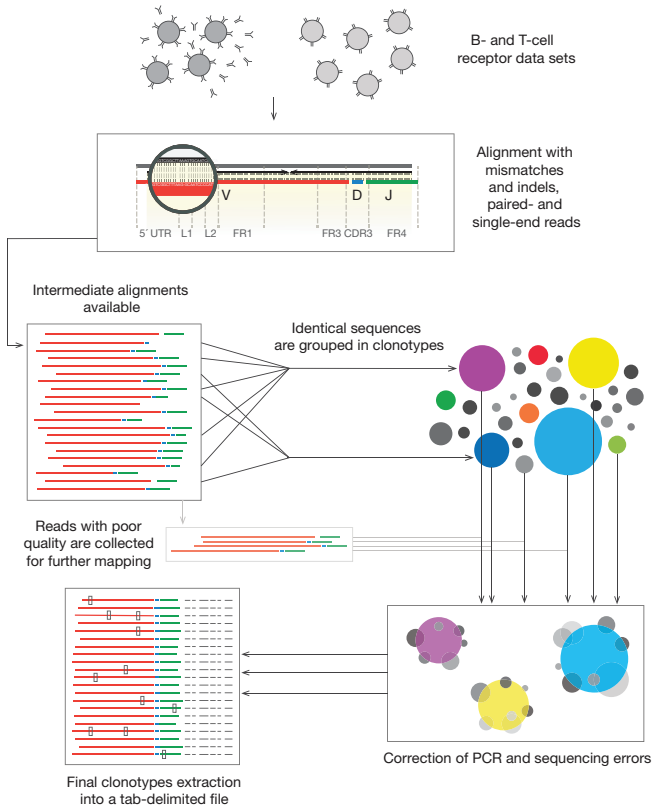

Figure 1 | MiXCR pipeline. The workflow from IG or T-cell receptor data sets to final clonotypes is shown.

on the basis of CDR1 and CDR3 or of CDR3 sequence only. MiXCR produces report files with overall run statistics. A clonotype list with detailed information as well as intermediate alignment results can be exported to tab-delimited text files.

MiXCR was tested against real data as well as synthetically generated data with allele frequencies, immunoglobulin somatic hypermutation, and PCR and sequencing error rates closely resembling those of real data. The extraction efficiency and accuracy were comparable to or better than those of existing tools in the field, and the execution on IG data was between two and four orders of magnitude faster (**Supplementary Tables 2–6** and **Supplementary Notes 4** and **5**). The software has a simple-to-use command-line interface, is easy to install using cross-platform binaries, works with different data formats and handles output from various sequencing platforms. MiXCR is free for scientific and nonprofit use.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper (doi:10.1038/nmeth.3364).

ACKNOWLEDGMENTS

This work was supported by the Russian Science Foundation (project no. 14-14-00533).

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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