

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

- | | | |
|-------------------------------------|-------------------------------------|--|
| ☐ | ☒ | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☐ | ☒ | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☒ | ☐ | The statistical test(s) used AND whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☐ | ☒ | A description of all covariates tested |
| ☐ | ☒ | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐ | ☒ | A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☒ | ☐ | For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☒ | ☐ | Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated |

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection Cell Vision-v1.4.0 (Coolight, Beijing)

Data analysis ImageJ-v1.4.3.67, MATLAB R2022b, Origin 2021 (OriginLab Corporation)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

Demonstration datasets and MATLAB source codes for fragments simulation, deep learning network training and classification are provided at <https://drive.google.com/drive/folders/1nS6RoRF6hYfIY5vCtnQGsudkrbxMEt32?usp=sharing>.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

This study does not involve data related to sex or gender.

Reporting on race, ethnicity, or other socially relevant groupings

This study does not involve data related to race, ethnicity, or other socially relevant groupings.

Population characteristics

This study does not involve human research.

Recruitment

No participants were recruited.

Ethics oversight

This study does not involve human research, so no approval is needed.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences

☐ Behavioural & social sciences

☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

1. The training dataset consists of about 1,000,000 simulated fragments, and the validation dataset consists of about 20,000 simulated fragments.
2. The benchmark of DEBRIS applied five datasets: the ribosome contains 1920 traces, the $\beta 2$ adrenergic receptor #1 ($\beta 2AR$ #1) contains 1586 traces, the $\beta 2AR$ #2 contains 1591 traces, the DNA Holliday Junction in low salt buffer contains 1221 traces, and the DNA Holliday Junction in high salt buffer contains 1563 traces.
3. For application of DEBRIS in two-color traces of steady signals, $\beta 2AR$ (148C-378C) in varying concentration of GsGDP, from low to high, contains 3838, 5355, 3576, 4135, 2794, 5970, 4044, 3441, 3013 traces.
4. For application of DEBRIS in two-color traces of dynamically emerging signals, 5 nM AsCas12a-WT on 0 MM contains 2075 traces, 5 nM AsCas12a-WT on 3 MM contains 2297 traces, 5 nM AsCas12a-WT on 6 MM contains 2041 traces, 5 nM AsCas12a-WT on 7 MM contains 1621 traces, 10 nM LbCas12a-WT on 0 MM contains 2845 traces, and 20 nM FnCas12a-WT on 0 MM contains 2160 traces.
5. For application of DEBRIS in one-color traces of steady signals, Cy3-PCB contains 1325 traces, Cy3-POD contains 1864 traces, Cy3-FBP contains 1553 traces.
6. For application of DEBRIS in one-color traces of dynamically emerging signals, each group contains three repeats (a total of about ~5000 traces).

Data exclusions

No traces data was excluded for training, benchmark and application test of DEBRIS.

Replication

For application, testing datasets were obtained based on more than 3 movies .

Randomization

In this study, the term "experimental group" refers to specific biological samples; therefore, there was no collection of samples followed by random group allocation in this paper. Fragments for training were randomly simulated, and traces for benchmark and application were randomly collected from TIRF-based movies.

Blinding

There is no group allocation and blinding in this study.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involvement in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involvement in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Plants

Seed stocks

This study does not involve seed stocks.

Novel plant genotypes

This study does not involve novel plant genotypes.

Authentication

This study does not involve authentication procedures for seed stocks used or novel plant genotypes.