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SMIAL - A Toolkit for Multidimensional Data Analysis and Classification

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INTRODUCTION

Motivation: Advances in multispectral imaging enable high-throughput, multidimensional data collection across diverse fields, including diagnostics, disease monitoring and drug development [1]. These data, combined with machine learning, can reveal complex patterns and novel biological insights.

Challenge: Despite advances in open-source image analysis, many existing tools are designed for fluorescently labelled data, offering limited customisation for machine learning workflows, or require substantial programming expertise.

Solution: We developed SMIAL [1], an open-source, user-friendly software for multi-dimensional data analysis and classification, available as a standalone .exe and MATLAB source code [2], with demonstrated applications in label-free cell classification [3].

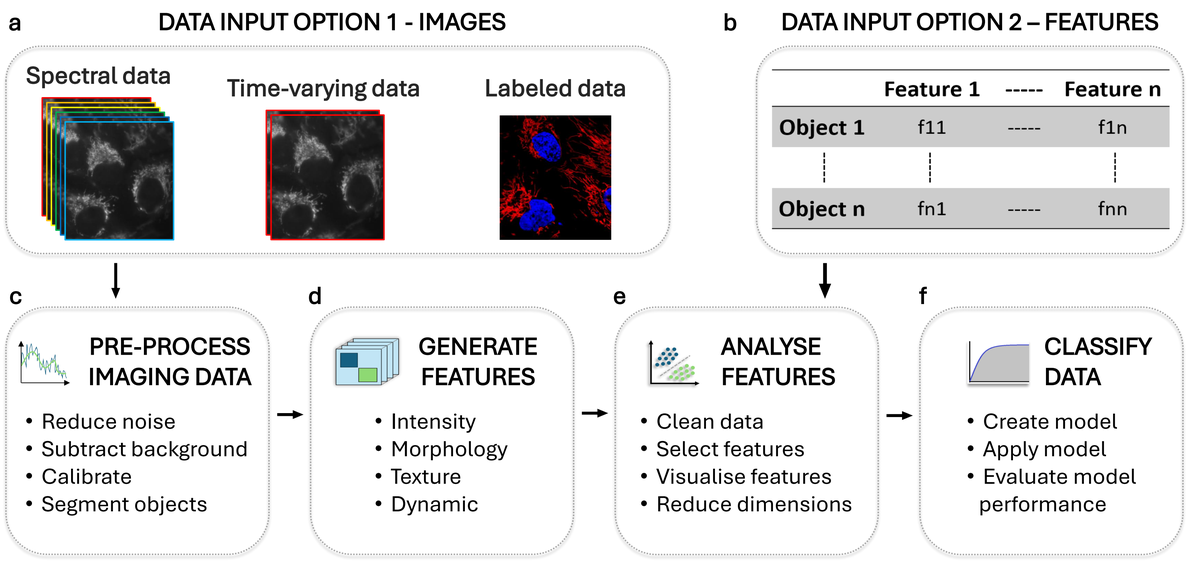


Figure 1. Overview of SMIAL's toolbox for multidimensional data analysis and classification. (a) SMIAL supports multidimensional optical data (labelled and label-free; across spatial, spectral and temporal domains) in .jpg, .png, .tif and .mat formats and **(b)** numerical feature tables in .xls(x) and .mat formats. **(c-f)** Its analytical workflow encompasses four main functions: **(c)** image pre-processing, **(d)** feature generation, **(e)** feature analysis, and **(f)** classification.

MULTISPECTRAL IMAGING APPLICATIONS

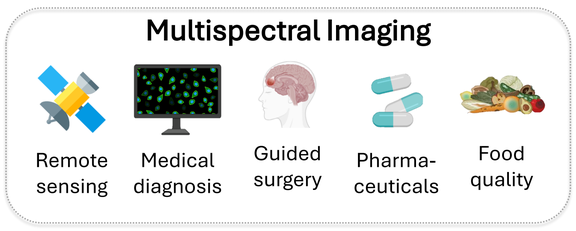
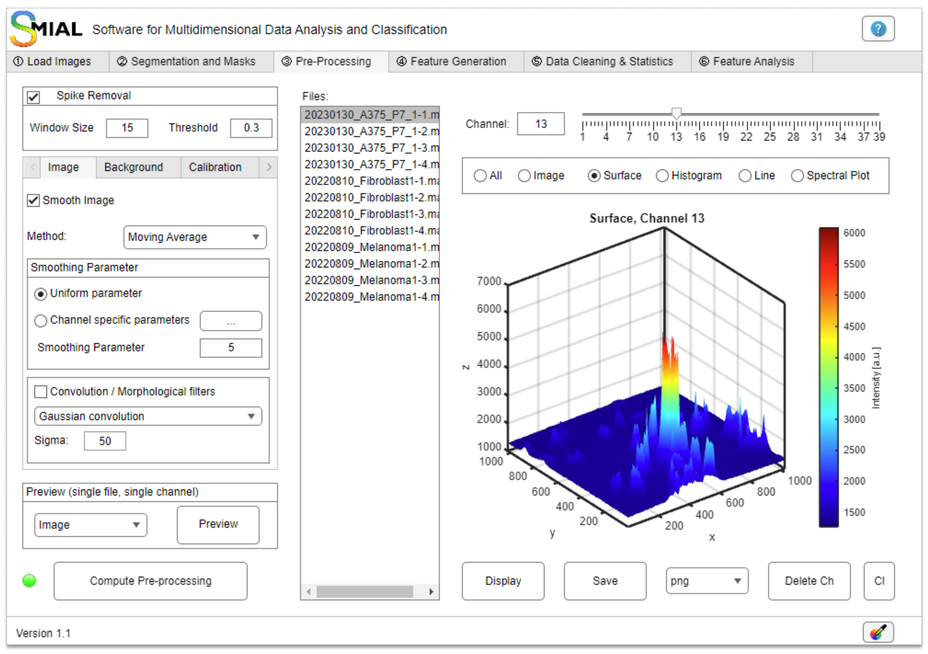


Figure 2. Multidisciplinary applications of multispectral imaging. Imaging across multiple wavelength bands provides rich spectral information beyond conventional imaging, spanning environmental, bio-medical, pharmaceutical and food sectors.

METHODOLOGY



1. Load data.
2. Segment image.
3. Generate features.
4. Pre-process image.
5. Clean data.
6. Feature analysis.

Figure 3. SMIAL software for multidimensional data analysis and classification, available at [2]. Six modules support data loading, image segmentation and region-of-interest selection, pre-processing, feature generation (intensity, morphology, texture, temporal features), data cleaning and statistical analysis, and feature analysis including feature selection, classification, and performance evaluation. Integrated visualisation, performance metrics and parameter saving support data exploration, model evaluation and reproducible workflows.

NON-INVASIVE MELANOMA DIAGNOSTICS USING MULTISPECTRAL AUTOFLUORESCENCE IMAGING

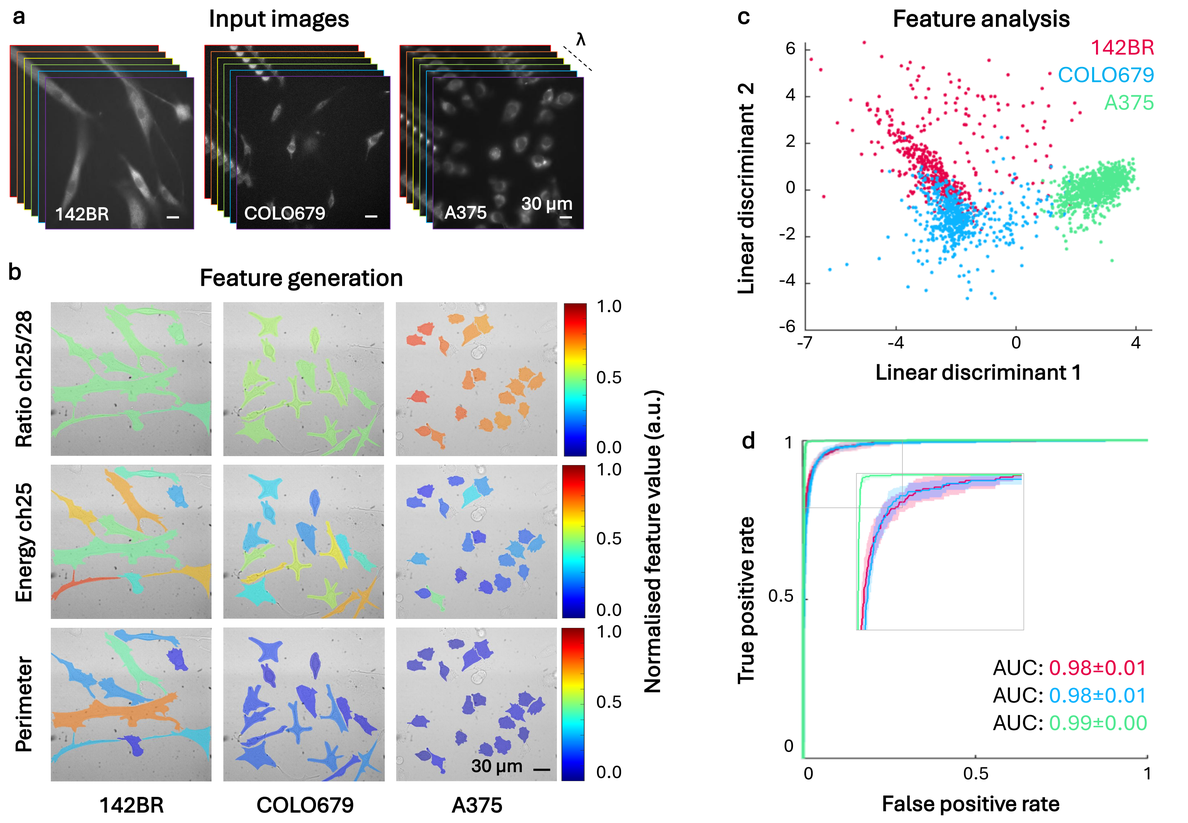


Figure 4. Visualising machine learning performance for melanoma classification using SMIAL. (a) Multispectral imaging of cellular autofluorescence, the natural fluorescence of molecules within cells, in two melanoma cell lines (A375, COLO679) and healthy fibro-blasts (142BR). **(b)** Feature maps compare selected cellular features across cell types. **(c)** Linear discriminant transformation showing cell clusters, with each point representing one cell. **(d)** Receiver operating characteristic (ROC) curves showing logistic regression classification performance, with area under the curve (AUC) reported for each cell type.

LABEL-FREE IMMUNE CELL PROFILING

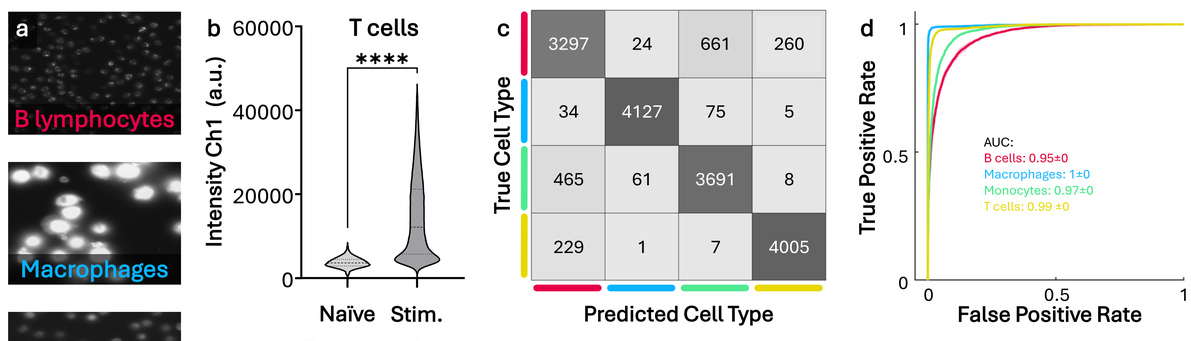


Figure 5. Identifying immune cell type and stimulation state using SMIAL. **(a)** Multispectral autofluorescence images of macrophages, monocytes, B and T lymphocytes across stimulation states. **(b)** Stimulated T cells (CD3 CD28) showed increased NAD(P)H-associated autofluorescence (accuracy: 0.90; AUC: 0.96; F1: 0.95). **(c)** Confusion matrix showing cell-type classification (accuracy: 88.94%). **(d)** ROC curves showing class-specific performance for B cells, macrophages, monocytes and T cells.

CONCLUSION

- SMIAL provides a user-friendly, open-access platform for reproducible, multi-dimensional data analysis and classification, integrating key analysis steps in a single graphical interface without requiring programming expertise.
- SMIAL enables a label-free cellular classification, demonstrated here for melanoma classification and immune cell profiling using multispectral autofluorescence imaging.
- SMIAL supports diverse biomedical applications, including kidney graft analysis, oocyte quality evaluation, stem cell characterisation, and retinal stress-response evaluation.

REFERENCES

[1] Knab, A., Handley, S., Xu, X. et al. (2026). Multidimensional data analysis and classification using SMIAL. Commun Biol 9, 650.
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[3] Campbell, J. M., et al. (2024). Single cell, Label free Characterisation of Human Mesenchymal Stromal cell Stemness and Future Growth Potential by Autofluorescence Multispectral Imaging. Stem cell reviews and reports, 20(8), 2283–2292.

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