
Supplementary information

***ν*LUME: 3D virtual reality for single-molecule localization microscopy**

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vLUME: 3D Virtual Reality for Single-molecule Localization Microscopy

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Supplementary Information

Supplementary Table 1 identifies and compares a variety of tools for 3D VR, and a 2D SMLM tool, alongside vLUME. The key features described within this table show the unique abilities that vLUME presents and the first recorded 3D VR tool for SMLM.

Supplementary Table 1

	ViSP¹	Virtualitics²	syGlass³	InViewR⁴	ExMicroVR⁵	Genuage⁶	vLUME
VR Mode	No	Yes	Yes	Yes	Yes	Yes	Yes
SMLM Compatibility	Yes	No	No	No	No	Yes	Yes
Availability	Academic	Paid	Paid	Paid	Academic	Academic	Academic
Visualization	Point Cloud	Point Cloud	Volumetric	Volumetric	Volumetric	Point Cloud	Point Cloud
Data Type	SMLM	Statistics/Data Science	3D Bioimaging	3D Bioimaging	Confocal Imaging	SMLM	SMLM
VR Headset Compatibility	N/A	HTC Vive, HTC Vive Pro, Oculus Rift, Oculus Rift S	HTC Vive, Oculus Rift	Oculus Rift	HTC Vive, Oculus Rift, Microsoft Mixed Reality	HTC Vive, Oculus Rift, Oculus Rift S	HTC Vive, HTC Vive Pro, Oculus Rift, Oculus Rift S
Embedded Particle Tracking	No	No	Yes	Yes	No	Yes	No
Annotation	No	No	No	No	No	No	Yes
VR Data Manipulation	N/A	No	No	No	No	No	Yes
Bespoke Analysis Code	No	Yes	Yes	Yes	No	No	Yes

Supplementary Videos Captions

1. Overview of vLUME. Overview of the main GUI and functionality in vLUME. The video shows a variety of data sets and the ease of going from the micron scale to nanoscale regions.
2. Selecting and annotating data. Demonstration of a user isolating a single microtubule from a complex tangle in vLUME to be saved as isolated data.
3. Loading and filtering data. Demonstration of a user isolating a stalk from a pre-division stage of a *Caulobacter crescentus* in vLUME to be saved as isolated data.
4. Manipulation of data. Data manipulation features of vLUME on a single *Caulobacter crescentus*, showing the user actions simultaneously.
5. Selecting Data and Running Scripts. Maximum and Minimum distances and Nearest neighbor script application to a NPC dataset.
6. Outputting a video. Setting waypoints in the 3D space using vLUME and saving these points as a video.
7. Case Study. Exploring annotating and analyzing spectrin rings in plated neurons.

Supplementary References

1. El Beheiry, M. & Dahan, M. ViSP: representing single-particle localizations in three dimensions. *Nat. Methods* **10**, 689–690 (2013).
2. Product webpage: <https://www.virtualitics.com/>
3. Pidhorskyi, S., Morehead, M., Jones, Q., Spirou, G., Doretto, G. syGlass: Interactive Exploration of Multidimensional Images Using Virtual Reality Head-mounted Displays. Preprint at *arXiv:1804.08197* (2018).
4. Product webpage: <https://www.arivis.com/en/inviewr/>
5. Product webpage: <http://www.immsci.com/exmicrovr-license/>
6. Blanc, T., El Beheiry, M., Masson, J-B., Hajj, B. Genuage: visualizing and analyzing multidimensional point cloud data in virtual reality. Preprint at *bioRxiv* <https://doi.org/10.1101/2020.03.26.000448> (2020).