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Data Article

A comprehensive dataset of image sequences covering 20 fluorescent protein labels and 12 imaging conditions for use in super-resolution imaging



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ABSTRACT

Super-resolution fluorescence microscopy techniques allow imaging fluorescently labelled structures with a resolution that surpasses the diffraction limit of light (approx. 200nm). The quality and, thus, reliability of each of these techniques is strongly dependent on (1) the quality of the optics, (2) the fitness of the specific fluorescent label for the given technique and (3) the algorithms being used. Of these, the fitness of the labels is most subjective, as fitness metrics are scarce, and generating samples with different labels and imaging them is laborious. This prevents rigorous fitness assessment of fluorescent labels.

We have developed a mathematical framework for assessing the quality of SOFI data [1], [2], which we used to assess the fitness of 20 different fluorescent protein labels for SOFI imaging. Here, we report this dataset of 2240 image sequences, representing 10 fields of view each of transfected Cos7 cells expressing each of the 20 different fluorescent proteins under 4–12 imaging conditions. The labels span the visible spectrum and include non-photo-transforming and photo-transforming fluorescent proteins. The imaging conditions consist of 4 different excitation powers, each with three different powers of 405 nm light added (except for the blue labels that are excited with 405 nm light).

Though this data was in essence generated to assess which labels are best suited for SOFI imaging, it can be used as a benchmark for further development of the SOFI algorithm, or for the development

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of other super-resolution imaging modalities that benefit from similar input data.

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Specifications Table

Subject	Spectroscopy
Specific subject area	Super-resolution fluorescence microscopy
Type of data	Image Table
How data were acquired	Data was acquired using a Nikon Ti2 wide-field fluorescence microscope equipped with a TIRF illuminator, 100 × TIRF objective, Oxnius laser box containing a 405, 445, 488 and 561 nm laser, and an Andor iXon 897 EMCCD camera.
Data format	Raw, non-analyzed tiff files. Table representing the 12 imaging conditions.
Parameters for data collection	We varied the excitation intensity of the laser by setting the illumination at 3, 10, 30 and 100% of the laser output. Additionally, for the cyan, green and red fluorescent protein-expressing samples, we added 405-nm laser light at 0 (no light), 1 or 10% of the laser output. The corresponding illumination densities in epi mode are given in the Data Table (Table 1) included in this dataset.
Description of data collection	We seeded Cos7 cells on glass-bottom dishes which were then transfected with membrane-tagged fluorescent protein-expressing plasmids. The next day, the medium was changed to HBSS. For each dish, we selected 10 fields of view which were imaged, first with the lowest illumination intensity, working up to the highest intensity. For the cyan, green and red labels, a separate set of 10 fields of view were selected for each of the 3 different 405-nm light irradiation settings, resulting in 30 fields of view.
Data source location	Department of Chemistry University of Leuven Celestijnenlaan 200G 3001 Heverlee Belgium
Data accessibility	With the article: Data Table (Table 1) representing the illumination power densities associated with the 12 imaging conditions. In a public repository: Repository name: FigShare Data identification number: https://doi.org/10.6084/m9.figshare.c.4732631.v1 Direct URL to data: https://figshare.com/collections/SOFI_images_Data_Collection/4732631/1
Related research article	Moeyaert, Benjamin., Vandenberg, Wim. & Dedecker, Peter. SOFlevaluator: a strategy for the quantitative quality assessment of SOFI data. Biomed. Opt. Express 11, 636–648 (2019). DOI 10.1364/BOE.382278

Value of the Data

- The currently presented dataset provides, to the best of our knowledge, the first publicly available head-to-head comparison on a large set of fluorescent proteins in regard to their performance in super-resolution microscopy, e.g. pcSOFI [1].
- Our extensive dataset can be used by researchers developing novel super-resolution microscopy techniques, or try to improve upon the existing ones, without having to go through the trouble of (re)measuring large amounts of data.
- The dataset can furthermore be used to assess the dynamic behaviour of the different fluorescent proteins under a wide range of illumination settings.

1. Data description

The dataset which we describe here consists of sets of image sequences, 500 frames long, of fluorescent-protein expressing cells. Some of these labels are reported to be reversibly

photoswitchable (PS), others are irreversibly photoconvertible (PC). In the latter case, the green form was imaged. In the blue region, we include EBFP2 [3] and mTagBFP [4]. In the cyan wavelength region, we measured data on mCerulean [5], mTurquoise2 [6] and mTFP0.7 [7] (PS). The green region was sampled with wQ [8] (PS), rsGreen1 [9] (PS), EGFP, Dendra2 [10] (PC), SkylanS [11] (PS), ffDronpa [12] (PS), mNeonGreen [13], mEos3.2 [14] (PC) and EYFP. Finally, red fluorescent labels were mOrange2 [15], mKO2 [16], rsTagRFP [17] (PS), mScarlet1 [18], rsFusionRed3 [19] (PS) and mCherry.

We imaged 10 cell each under 4 (blue labels) or 12 (cyan, green and red labels) different illumination conditions. In the Table 1 below, we report the specific illumination densities for each of the 12 conditions. The contents of this Table 1 has previously been published [2], but is reproduced here as it is integral to the dataset.

The data contains the raw TIFF files and has not been processed in any way except for re-naming for clarity.

Table 1
Illumination density settings for all imaging conditions.

405 nm (W/cm ²)						
cyan labels	green and red labels					
3.3	3.3	8	9	10	11	
0.003	0.030	4	5	6	7	
0.000	0.000	0	1	2	3	
		0.67	2.9	9.7	35	405 nm (W/cm²)
		0.91	3.6	15	43	445 nm (W/cm²)
		3.3	12	39	146	488 nm (W/cm²)
		1.6	5.1	15	43	561 nm (W/cm²)

2. Experimental design, materials, and methods

We used a Nikon Ti2 microscope equipped with a 100 × 1.49 NA CFI apo TIRF objective (Nikon) and an Oxxius laser box equipped with a 405, 445, 488 and 561 nm laser (LBX-405-100-CSB, LBX-445-100-CSB, LBX-488-200-CSB and LCX-561S-100-CSB, Oxxius). For blue, green and red label, we used a Chroma ZT405/488/561/640rpcv2 dichroic mirror and a Chroma ZET405/488/561/640 m emission filter. For cyan labels, we used a Chroma 440/488/561/635rpc dichroic mirror and a Chroma ET480/40 m emission filter. Fluorescence images were collected at 33 Hz with an Andor iXon 897 EMCCD camera cooled to −60 °C with an EM gain of 60. The resulting pixel size of this setup was 118 nm.

Cos7 cells were seeded at a density of 300 000 cells onto a glass-bottom 35 mm dish (P35G-1.5-14-C, MatTek) and transfected with a plasmid encoding the fluorescent protein fused to a membrane anchor [9] using FuGene6 (Promega) according to the manufacturer's protocol. The next day, cells were washed with warm (37 °C) HBSS buffer once and subsequently imaged in HBSS buffer.

The data which we report here are the raw TIFF files which can be read with standard software such as the ImageJ/Fiji [20].

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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