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A Global Reproducibility Initiative in Light Microscopy

Community Standards for Instruments, Metadata, and Publications

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Reproducing published research results can be challenging. In the field of light microscopy, there is a global open community bridging the gap between academia and industry to improve the reproducibility of light microscopy experiments in the life and materials sciences. The community is working towards establishing standards for quality assessment and quality control (QA & QC) for imaging instrumentation, improving reporting of imaging experiments, and data quality regarding metadata contained within. The development of the initiative Quality Assessment and Reproducibility for instruments and images in Light Microscopy (QUAREP-LiMi) is presented, and the current topics and achievements of the 15 working groups are summarized. Several accessible online resources utilized by QUAREP-LiMi are introduced here, as well as the future goals, followed by an invitation to join and contribute to the success story of QUAREP-LiMi.

Introduction

The reproducibility crisis of research results has emerged as a major challenge in science [1], resulting in increased resource consumption due to additional experiments required. A lack of awareness, knowledge, and agreement on common standards and guidelines for quality assessment and reproducibility in the field of light microscopy and image data analysis is the main reason for the under-reporting of imaging methods. The community-driven initiative “Quality Assessment and Reproducibility for Instruments & Images in Light Microscopy (QUAREP-LiMi)” addresses this crisis by (1) defining robust and easy-to-implement Standard Operating Procedures (SOPs) characterizing relevant instrument parameters for data acquisition, (2) establishing a common metadata model for image data, including quality control data and associated analysis, (3) developing community agreed guidelines for publication of image data and its analysis, ensuring reproducibility and AI-compatible data mining. Ultimately, these efforts will lead to more robust results

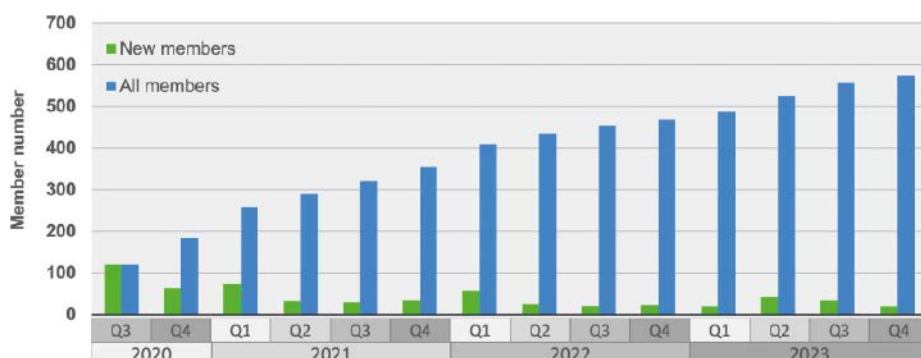


Fig. 1: Dynamic development of the QUAREP-LiMi memberships from the foundation in April 2020 until the end of 2023.

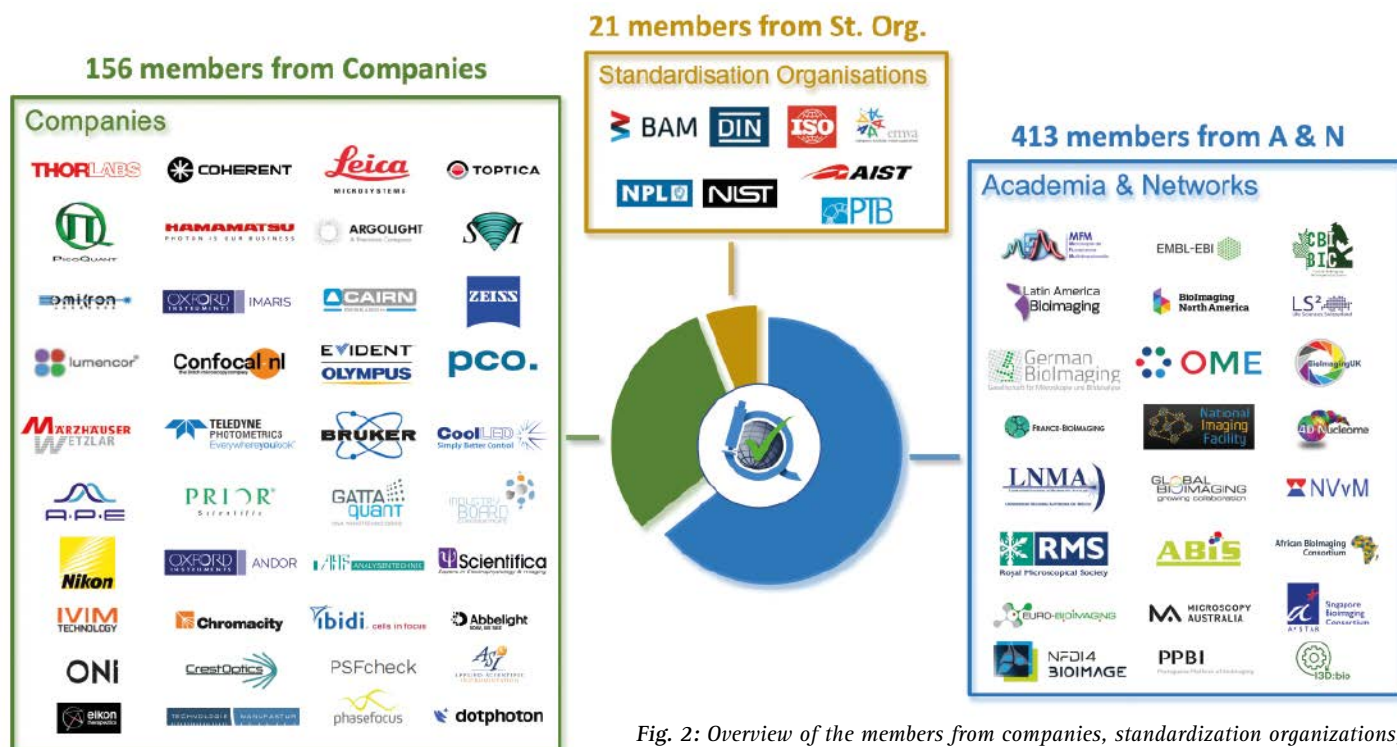


Fig. 2: Overview of the members from companies, standardization organizations, academia networks, and research groups working together in QUAREP-LiMi (due to space restrictions, not all member logos were included).

and a significant reduction in the use of resources. This will be achieved by providing better conditions for new research projects based on previous, more profound findings.

Groundwork and Organization

Initially, QUAREP-LiMi started as a grassroots collective within the German BioImaging Society and informal meetings of small groups of scientists at ELMI and other conferences. After an initial international online meeting with about 50 participants during the early Covid pandemic, it quickly gained traction and, since then, has continuously grown. Figure 1 summarizes the tremendous development of the QUAREP-LiMi membership numbers since 2020. Today, 590 people from 41 countries have joined QUAREP-LiMi, and 30% of the members are females. The majority of the 413 academic members work in light microscopy core facilities or research labs and have a strong focus on light microscopy techniques. The 156 members from equipment and software manufacturers are designated specialists in their field of expertise like optics engineers, specific product experts, and hardware or software developers. From those, 21 QUAREP-LiMi members are involved in national and international standardization organizations such as DIN or ISO or work as scientific editors for journals. Figure 2 provides an overview of the various organizations, networks, and industry partners working together in QUAREP-LiMi. All members participate voluntarily and without any financial contribu-

tion or benefit, ensuring complete independence for the best possible progress.

QUAREP-LiMi is organized in working groups (WGs) focusing on specific topics and tasks. Interested individuals can subscribe to specific QUAREP-LiMi WGs via online forms. About 250 members are actively contributing to 15 WGs, as listed in Table 1 with the main topics they are focusing on. WGs 1 to 6 are dedicated to technical parameters described in the ISO norm 21073:2019 - Optical data of fluorescence confocal microscopes for biological imaging. WG 7 - Microscopy data provenance and QC metadata and WG 10 - Image quality interact with most of the other WGs, as their topics are overarching. WG 8 - Education, training, and outreach. WG 14 - Environment, and WG 15 - FLIM have recently been established. WG 11 - Microscopy publication standards and WG 12 - Image visualization and analysis concentrate on establishing agreed-upon guidelines for extensive description and visualization of light microscopy data. WG 13 - Phototoxicity is raising awareness of the phototoxicity induced by microscopy and evaluating the best methods for assessing, minimizing, and reporting phototoxicity.

The work within each WG is coordinated and guided by at least two elected co-chairs in charge of all WG-related organizational tasks. Most WGs come together online monthly, and dates are published in an online calendar. All meetings are recorded, and meeting agendas and minutes, shared working documents, literature, recorded image data sets, and measurements are collected on a Nextcloud server that is openly accessible to all

QUAREP-LiMi members. QUAREP-LiMi is active on social media channels like LinkedIn and X, with about 800 followers. Further details and information are summarized on the initiative's extensive webpage.

Achievements and Work in Progress

The QUAREP-LiMi WGs 1-6 are working on or have already published detailed protocols for the assessment, control, and analysis of important imaging system parameters and properties:

- Illumination power WG 1
- Detection system performance WG2
- Uniformity of illumination field-flatness WG 3
- System chromatic aberration and co-registration WG 4
- Lateral and axial resolution WG 5
- Stage and focus precision WG 6

QUAREP-LiMi uses the platform protocols.io for the joint creation, management, and publication of the protocols. PDF versions of the protocols are also available for download from the QUAREP-LiMi protocol website. Moreover, WGs have developed macros for many protocols to automate image acquisition and instrument control such as microscopes and their devices or power meters. In addition, WGs created various data and image analysis program routines together with further tools for visualization of the measured data over time and their storage. All information is publicly available on the QUAREP-LiMi Github account. Working groups 1, 4,

and 5 also make use of Airtable, an online platform for gathering and presenting data. WG 1 collected hundreds of laser power measurements on confocal microscope systems, which are publicly available, and new measurements can be conveniently uploaded via a form or as bulk data. QUAREP-LiMi operates a YouTube channel that currently holds 20 videos. Each WG has published short videos summarizing their work and achievements, and additional videos explain protocols and measurement routines developed in different WGs. Steered by the newly founded WG 8 - Education, training, and outreach, more teaching and training videos will be put together in the future. In general, QUAREP-LiMi is putting a lot of effort into spreading our message within the light microscopy community by organizing sessions during the last three ELMI Core Facility days. Moreover, an ELMI community room has been established that serves as an informal meeting and discussion point and playground to see demos of protocols in use, and newly developed tools and devices.

As a work in progress, a QUAREP-LiMi OMERO image database server has been set up. All image data currently stored on the Nextcloud will be transferred to OMERO and will be annotated according to FAIR data standards. In the future, the QUAREP-LiMi OMERO database will be open to the general imaging community and can serve as a repository for standard image data sets of QA & QC measurements.

The QUAREP-LiMi community initiative has accomplished an impressive number of 12 high-impact publications since 2020. The overall aims and the organization of QUAREP-LiMi were described in two whitepapers in the *Journal of Microscopy* [2] and *Nature Methods* [3] and are setting the frame for future QUAREP-LiMi developments. Most remarkably, *Nature Methods* highlighted in the December 2021 issue the importance of improving rigor and reproducibility in microscopy as well as proper reporting of meta-

WG	Working Group Topic	Members	Meetings until 01/2024
1	Illumination Power	133	30
2	Detection System Performance	126	38
3	Uniformity of Illumination Field – Flatness	98	28
4	System Chromatic Aberration and Co-Registration	90	33
5	Lateral and Axial Resolution	149	35
6	Stage and Focus – Precision and Other	75	27
7	Microscopy data provenance and QC metadata	139	25 + 12 sub
8	Training, Education and Outreach (before white paper WG)	54	1 10
9	Overall Planning and Funding	77	31
10	Image Quality	160	29
11	Microscopy Publication Standards	137	27
12	Image Visualization and Analysis	122	32
13	Phototoxicity	53	10
14	Environment (start announced for spring 2024)	21	Founding phase
15	FLIM (start announced for spring 2024)	28	Founding phase

Table 1: QUAREP-LiMi members work together in fifteen working groups (WGs), each dedicated to specific topics and tasks. Meetings of the WGs are usually every month with about 10-15% of the total WG members attending each time. Members are often part of more than one WG.

data in a collection of nine articles with significant contributions from QUAREP-LiMi members. Four publications described the efforts of finding and setting standards for open image data formats [4], recommended metadata standards for light microscopy [5,6], and the development of a next-generation file format to expand bioimage data-access strategies [7]. Another set of publications focused on tools for collecting, annotating, and publication of image data including rich metadata as specified by the community and that comply with FAIR image data principles [8–10]. That impressively demonstrates that the initiative evolved into a productive consortium uniting researchers, facility managers, as well as companies, standardization organization representatives, and publishers. In July 2022, two editorials in *Nature Methods* [11,12] highlighted the joint work of QUAREP-LiMi on developing the revision of the 4DN-BINA-OME-QUAREP (NBO-Q) Microscopy Metadata specifications data model related to cameras. This major task was achieved in more than 10 online meetings of camera specialists from academia and industry and shall be finalized by the end of 2024. Recently, the QUAREP-LiMi WG 12 - Image Visualization and Analysis published “Community-developed checklists for publishing images and image analyses” in *Nature*

Methods [13]. It was further highlighted in the *Journal of Cell Sciences* [14], resulting in several publishers expressing their strong interest in implementing the suggested guidelines. The *Journal of Histochemistry and Cell Biology* already implemented the guidelines [15].

Conclusions

The QUAREP-LiMi initiative has gained tremendous momentum in the light microscopy community in a relatively short period to advance the QA & QC issues that have been difficult to address for the last 20 years. The breakthrough seems to have been achieved by the very open and productive collaborative approach taken by all people involved, including academia, industry, standards organizations, and scientific publishers. The goal of improving the reproducibility and quality of research results based on imaging data by monitoring and documenting instrument quality parameters over time with scientifically accepted, harmonized SOPs, and thoroughly tested tools is coming closer.

QUAREP-LiMi aims to become a visible and essential actor in

the scientific landscape. It stands for sustainable use of imaging infrastructure, robust and reproducible results, and transparent reporting. High-quality FAIR image data, rich in metadata, will facilitate data reuse. It will also be a prerequisite for innovative, next-generation AI-supported data analysis methods.

Would you like to be part of this amazing new community? Participating in QUAREP-LiMi is easy and free – please complete the membership form. Contributing actively can be quite some work, but you will meet an open community and amazing colleagues along the way. Every expertise counts, and you could be part of the next success story

Acknowledgment

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More information:
<https://quarep.org>



References:
<https://bit.ly/IM-Nitschke>