1. BACKGROUND, RESEARCH OPPORTUNITY, AND USER NEEDS

Scientists of several physics and chemistry research groups of Leiden University—subsequently indicated by 'we'—investigate biological relevant processes in specific molecules. One aspect is to do fluorescence imaging measurements and time-resolved spectroscopy measurements on single-molecules in our samples. The measurements are performed with the Scanning Confocal Microscope (SCM) setup that is located at the LION Biological Physics group. The kind of microscopy used, is also known as Confocal Laser Scanning Microscopy (CLSM, or LSCM).

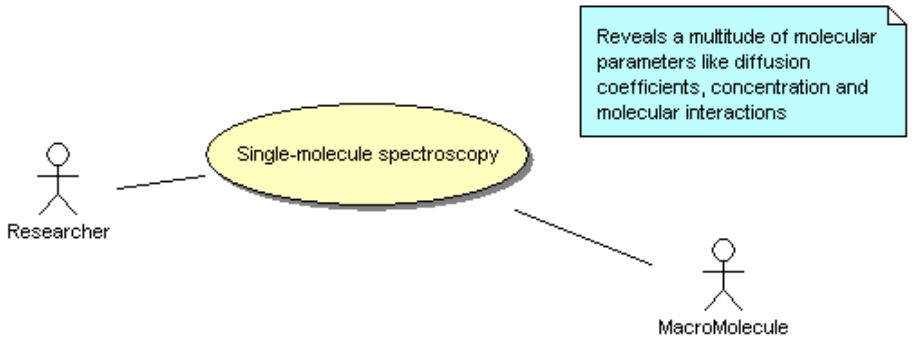

Figure 1-1: research relevant parameters on single-molecules

Previously a LabVIEW program written by Gerhard Blab was used with named setup. However, using the program was unsatisfactory and adapting it to our needs showed to be very difficult, leading to the initiation of the SCLM project. Since then the setup also changed substantially—and at the time of writing still is changing.

Several of the envisioned measurements, such as fluorescence imaging and measuring time-resolved spectra already can be done with the PicoQuant SymPhoTime commercial application. However, this application is unable to control all relevant elements of the setup. Parts it cannot control are shutters and acousto-optic modulators (AOM) that enable and modulate the laser beams.

Furthermore, we want to determine the time relation between changes in the sample's potential induced by a potentiostat and the imaging and spectroscopy measurements.

Because not all relevant parts of the setup are under control of the PicoQuant SymPhoTime commercial application and since we want to control the measurements from a single application, we decide to develop such an application ourselves.

2. RESEARCH OBJECTIVES AND SUCCESS CRITERIA

RO-1: Study biological relevant processes in specific molecules through fluorescence spectroscopy optionally combined with (cyclic) voltammetry

SC-1: Achieve and publish excellent scientific results earlier than other groups

SC-2: Achieve, determine ....

2.1 Business Risks

RI-1: Other research groups may succeed earlier.

3. VISION OF THE SOLUTION

For scientists of several Leiden University physics and chemistry research groups, the Scanning Confocal LabVIEW Microscope (SCLM) application is a measurement and control application to perform spectroscopic investigations on fluorescence of single molecules using the Scanning Confocal Microscope setup. Unlike the PicoQuant SymPhoTime commercial application, SCLM enables the control of all relevant parts of the setup, such as acousto-optic modulators (AOMs) and enables to combine voltammetry measurements with the spectroscopic measurements.

3.1 Major features

FE-1: Find optical focus in the sample.
FE-2: Acquire a fluorescence image of a specified rectangular area of the sample.
FE-3: Select a location-of-interest in the sample from the image.

Then at the location-of-interest, the application enables us to:
FE-4: Acquire a fluorescence time-trace (binned).
FE-5: Perform time-tagged time-resolved spectroscopy (T3R, FCS).
FE-6: Perform fluorescence lifetime imaging (FLIM, time-tagged).
FE-7: Perform FCS and FLIM with AOM excitation control.
FE-8: Perform two-color spectroscopy (FCCS).
FE-9: Perform alternating-laser excitation spectroscopy (ALEX, FCCS).

Further, the application enables us to:
FE-10: Combine time-tagged time-resolved spectroscopy with voltammetry.
FE-11: Combine FLIM with voltammetry.
Note that voltammetry measurements are the responsibility of a potentiostat with its own application.

3.2 Assumptions and Dependencies

AS-1: *Assumptions TBD*
DE-1: *Dependencies TBD*

4. SCOPE AND LIMITATIONS

4.1 Scope of Initial and Subsequent Releases

The requested functionality will be delivered in increments. The table below shows the major features and the planned release in which they will be implemented.

Feature \ Release	1	2	3	4	5	6	7
1–Find optical focus in the sample	√	√	√	√	√	√	√
2–Acquire a fluorescence image	√	√	√	√	√	√	√
3–Select location–of–interest	√	√	√	√	√	√	√
4–Acquire binned time–trace	√	√	√	√	√	√	√
5– Perform time–tagged time–resolved spectroscopy (FCS)	—	√	√	√	√	√	√
6–Perform fluorescence lifetime imaging (FLIM)	—	—	—	√	√	√	√
7– Perform FCS and FLIM with AOM excitation control	—	—	—	—	√	√	√

Feature \ Release	1	2	3	4	5	6	7
8- Perform two-color spectroscopy (FCCS)	—	—	—	—	—	√	√
9-Perform alternating-laser excitation spectroscopy (ALEX)	—	—	—	—	—	—	√
10-Combine T3R (FCS) with voltammetry	—	—	√	√	√	√	√
11-Combine FLIM with voltammetry	—	—	—	√	√	√	√

4.2 Limitations and Exclusions

LI-1: Application will not combine SCLM data with Potentiostat data

Scope		
In	Out	Item
√		Fluorescence imaging and spectroscopy
√		Initiate voltammetry acquisition and record time-relation to T3R spectroscopy
	√	Process measured SCLM data and measured Potentiostat data to combine the two

5. BUSINESS CONTEXT

5.1 Stakeholder Profiles

Stakeholder	Major Value	Attitude	Major Interest	Constraints
Group Leader				
User				

5.2 Project Priorities

Dimension	Driver	Constraint	Degree of Freedom
Schedule			
Features			
Quality			
Staff			
Cost			

5.3 Business Rules

ID	Definition	Type of Rule	Static or Dynamic	Source
BR-1	Implement measurement applications in LabVIEW	Constraint	Static	Management

5.4 Operating Environment

The Scanning Confocal LabVIEW Microscope application is a LabVIEW application that runs on a dedicated PC with Windows XP. The computer is located next to the optical table with the experiment. The computer contains electronic boards to perform photon counting and to control other devices through GPIB, such as a 3-axis translation stage (scan table). Further, through USB, the PC connects to an external National Instruments multifunction device that may issue triggers signals and control shutters and AOMs and do data acquisition.

Figure 5-1 below shows the context in which the Scanning Confocal LabVIEW Microscope application operates.

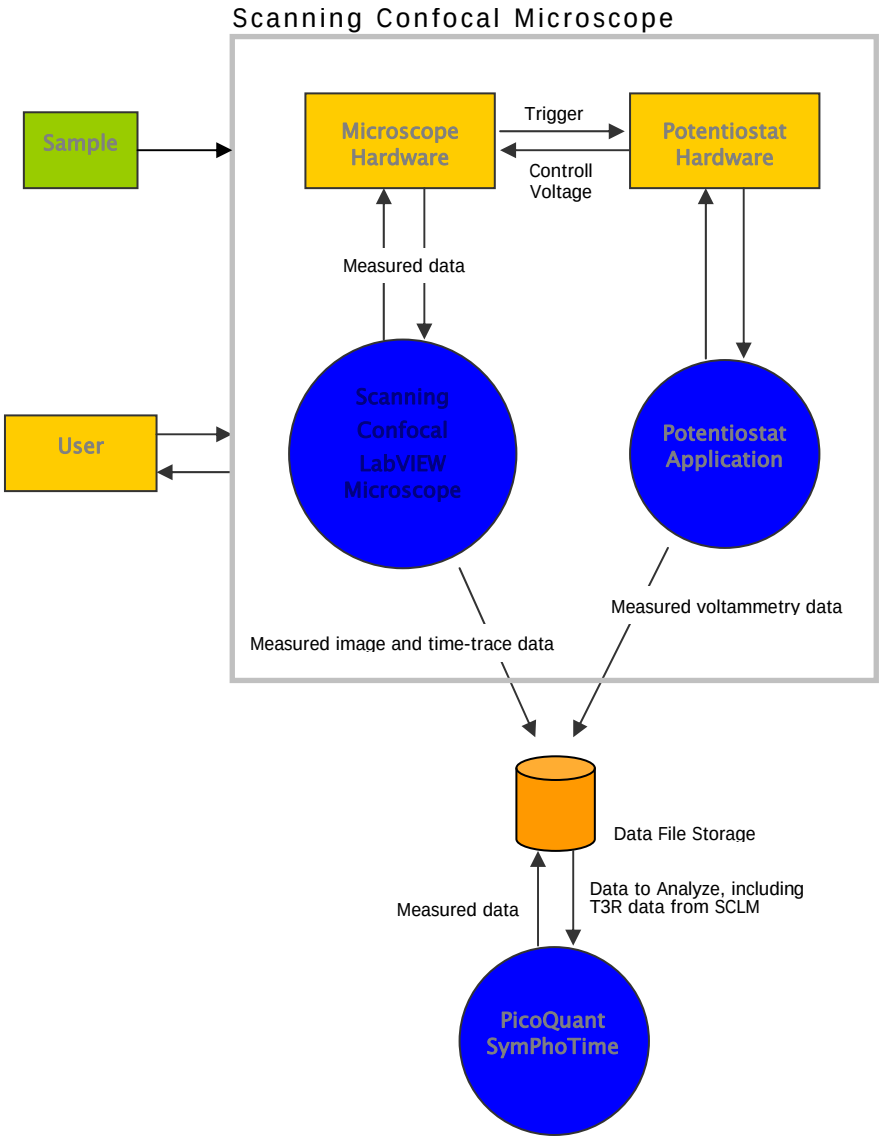

Figure 5-1: Context diagram of the Scanning Confocal LabVIEW Microscope application

6. REFERENCES

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2. http://en.wikipedia.org/wiki/Fluorescence_spectroscopy
3. http://en.wikipedia.org/wiki/Fluorescence_correlation_spectroscopy
4. http://en.wikipedia.org/wiki/Confocal_laser_scanning_microscopes
5. http://en.wikipedia.org/wiki/Two-photon_excitation_microscopy
6. http://en.wikipedia.org/wiki/Fluorescence_lifetime_imaging
7. PicoQuant SymPhoTime

7. GLOSSARY

ALEX	Alternating-Laser EXcitation
AOM	Acousto Optic Modulator
APD	Avalanche Photo Diode
FCCS	Fluorescence Cross-Correlation Spectroscopy
FCS	Fluorescence Correlation Spectroscopy
FLIM	Fluorescence Lifetime IMaging
TCSPC	Time-Correlated Single Photon Counting

7.1 Naming of Numbered Items

AS-n	Assumption
BO-n	Business Objective
BR-n	Business Rule
CO-n	Constraint
CI-n	Communication Interface
DE-n	Dependency
FE-n	Feature
HI-n	Hardware Interface
LI-n	Limitation
OE-n	Operating Environment
PE-n	Performance Requirement
RO-n	Research Objective
RI-n	Business Risk
SC-n	Success Criterion
SE-n	Security Requirement
SI-n	Software Interface
UC-n	Use Case
UD-n	User Documentation
UI-n	User Interface

Functional Requirement: Measure.Image.Area