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1 INTRODUCTION

1.1 Purpose

This SRS describes the software functional and non-functional requirements for release 1.0 through 7.0 of the Scanning Confocal LabVIEW Microscope (SCLM).

1.2 Project Scope and Product Features

SCLM will permit users of the Scanning Confocal Microscope (SCM) to perform several measurements on the fluorescence of single molecules. A detailed project description is available in the *SCLM Vision & Scope* document [1]. The section “Scope of Initial and Subsequent Releases” in that document lists the features that are scheduled for full or partial implementation in this release.

1.3 References

1. Moene, Martin. *SCLM Vision & Scope*.
2. Karl E. Wieggers. [Software Requirements](#). Microsoft Press, 2nd edition, 2003. ISBN-10 0735618798.

2 OVERALL DESCRIPTION

2.1 Product Perspective

SCLM is a LabVIEW application to perform several kinds of measurements on fluorescence of single molecules using the SCM setup. The context diagram in Figure 2-1 illustrates the external entities and system interfaces for release 1.0. The application will evolve over several releases, expanding on the kinds of measurements supported.

2.2 Product Features

- FE-1: Find optical focus in the sample
- FE-2: Acquire a fluorescence image
- FE-3: Select location-of-interest
- FE-4: Acquire binned time-trace
- FE-5: Perform time-tagged time-resolved spectroscopy (T3R, FCS)
- FE-6: Perform fluorescence lifetime imaging (FLIM)
- 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).
- FE-10: Combine time-tagged time-resolved spectroscopy with voltammetry.
- FE-11: Combine FLIM with voltammetry.

2.3 User Classes and Characteristics

User Class	Description
Scientist/User (favored)	One or two users perform the measurements at the location of the SCM setup. Measuring sessions may have a duration of several hours. There are probably no more than 5 different users.
PicoQuant SymPhoTime	Read and process T3R datafiles created by SCLM

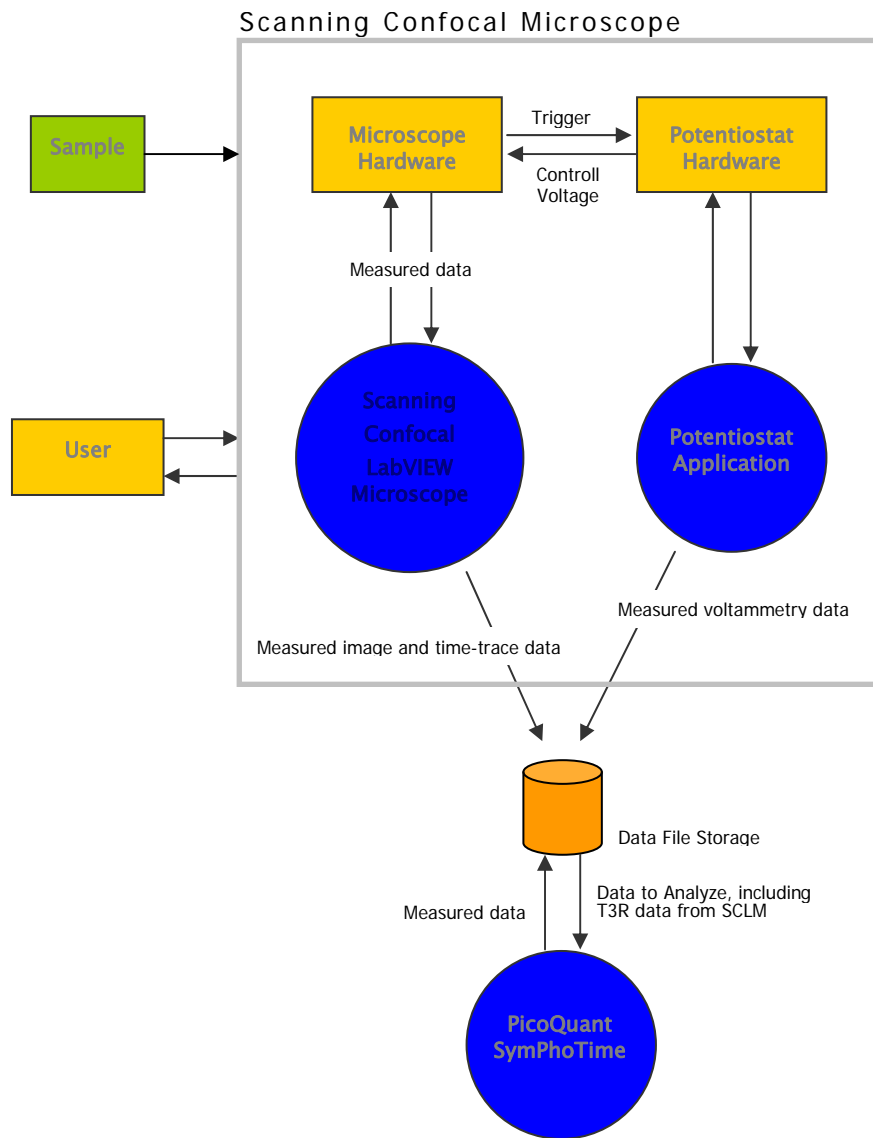


Figure 2-1: Context diagram of the Scanning Confocal LabVIEW Microscope application

2.4 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.

OE-1: SCLM shall operate on a PC with Windows XP

OE-2: SCLM shall use LabVIEW

OE-3: SCLM shall interface with dedicated photon counting boards located in the PC

OE-4: SCLM shall interface with a translation table through a dedicated GPIB board located in the PC

OE-5: SCLM shall interface with a National Instruments multifunction device through USB

OE-6: SCLM is located next to the optical table with the experiment

OE-7: SCLM is operated in a dark environment

2.5 Design and Implementation Constraints

CO-1: program shall be created with LabVIEW 8.2 or above

2.6 User Documentation

UD-1: Use documentation TBD

3 ASSUMPTIONS AND DEPENDENCIES

AS-1: Assumption TBD

DE-1: Dependency TBD

3.1 Experimental Setup

The figure below gives a simplified schematic of the scanning confocal microscope setup. See Figure 15-1: Scanning Confocal Microscope for a more detailed schematic of the SCM.

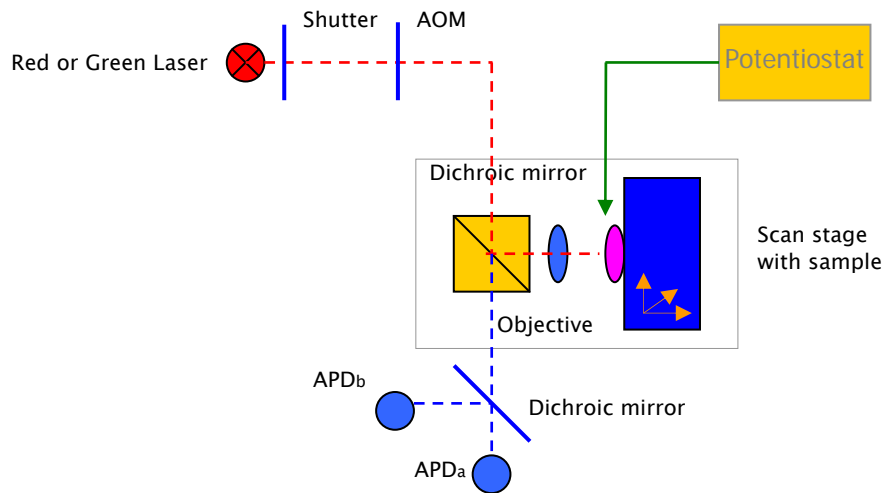


Figure 3-1: Simplified scanning confocal microscope setup showing laser, 3-axis scan stage with sample, potentiostat and APDs

The following parts comprise the microscope:

- Pulsed red laser with an AOM driven by a DAQ analog output and a shutter
- Green laser with an AOM driven by a DAQ analog output and a shutter
- Dichroic-mirror in the microscope to separate excitation light from emission light
- Dichroic-mirror to separate red detection (APDa) from green detection (APDb) in case of a 2-color experiment
- Potentiostat (CH Instruments CHI832b)
- 3-axis piezo translation stage (Physik Instrumente E710, GPIB)
- APDa, APDb
- Splitter for APDa signal, or
- 4-Channel SPAD Router for TimeHarp 200 (PicoQuant NRT 400)
- Photon counting card (Becker & Hickl PMS 300, in PC)
- Photon counting card (PicoQuant TimeHarp 200, in PC)
- Multifunction DAQ (USB-6259 BNC-terminated system) to
 - Control shutters
 - Modulate red laser via AOM
 - Modulate green laser via AOM
- GPIB interface (National Instruments, in PC)
- PC

See Table 14-1 for an overview of which parts of the experimental setup each measurement uses.

3.2 Measurements

This section gives an overview of the measurements that SCLM shall support.

3.2.1 Imaging (binned)

An intensity image of photon counts is made through a raster scanning movement in the x- and y-direction of the 3-axis translation table. See Figure 3-2 below.

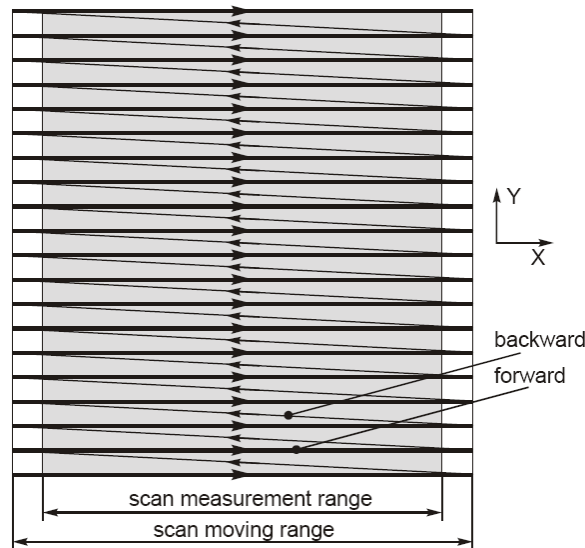


Figure 3-2: Unidirectional scanning pattern, showing zone where external measurement is enabled.

The scan movement is specified in width, height, resolution and bin-time. This yields a certain number of points in horizontal direction and in vertical direction. Each point represents a bin that contains the number of photons (intensity) collected during that point's bin time.

The translation table provides two trigger outputs. These shall be configured to 1) issue a positive on output Trigger 1 at the start of each point and 2) issue a positive trigger on output Trigger 2 at the start of each scan line. See Figure 2-1 below. Trigger2 is required to enable the free running collection of a line of bins using the PMS300 photon counting card.

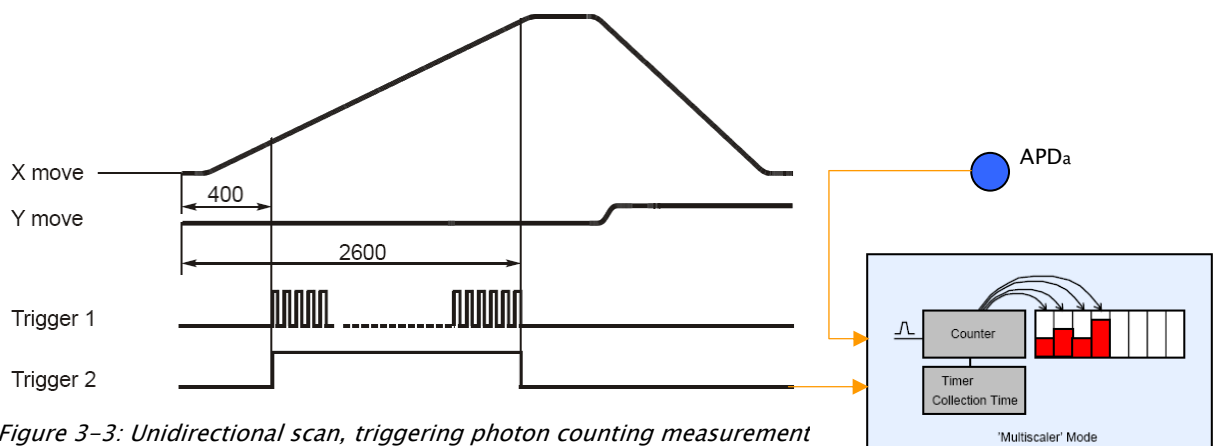


Figure 3-3: Unidirectional scan, triggering photon counting measurement during constant velocity phase (Trigger 2 starts a line-collection)

See Figure 15-1: Scanning Confocal Microscope: imaging and binned time-trace with PMS 300.

3.2.2 Imaging with TimeHarp 200

TBD

Bin via T3R acquisition?

SymPhoTime uses line trigger from E710 scanner (Mohsen)

3.2.3 Time trace (binned)

In a binned time-trace measurement, a list of photon counts is collected at a specific location-of-interest in the sample and during a certain collection time in the order of minutes. The measurement time is divided in bins with a time in the range of tens of ms. During the collection time of a bin, its count is incremented when a new photon is detected.

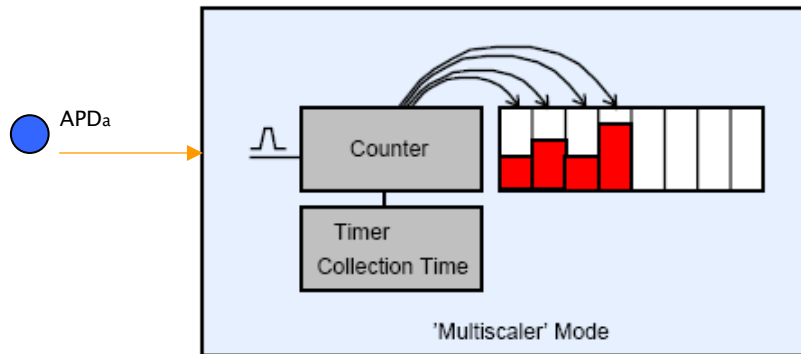


Figure 3-4: Collecting photon counts in the bins of a time-trace.

Thus, a histogram of photon counts is collected over the duration of the measurement.

See Figure 15-1: Scanning Confocal Microscope: imaging and binned time-trace with PMS 300.

3.2.4 Time-tagged time trace (FCS, T3R)

In a Time-tagged time-trace measurement, the arrival time of photons is recorded for certain a duration. In this case the sync input of the TimeHarp 200 photon counting card is externally connected to its 10 MHz clock signal. Note that we're not interested in the lifetime information (see below) here.

See Figure 15-2: Scanning Confocal Microscope: imaging and T3R (FCS) with TimeHarp 200.

Implementation note: Raymond Koehler has created cabling for Wiepke Koopmans to feed the TimeHarp's 10 MHz clock both to its sync input and to the NI DAQ device to create the AOM control signals. Wiepke measured the AOM control voltage—transmission curve and uses this to control the laser power in his alternating colour experiment.

3.2.5 Fluorescence lifetime imaging (FLIM)

In addition to the macroscopic photon arrival time as measured in time-tagged time-trace measurements, *fluorescence lifetime* experiments measure the time between the laser excitation pulse and the arrival of a photon. See Figure 3-5 below. Here, the clock that controls the laser to pulse is fed into the sync input of the TimeHarp 200 photon counting card.

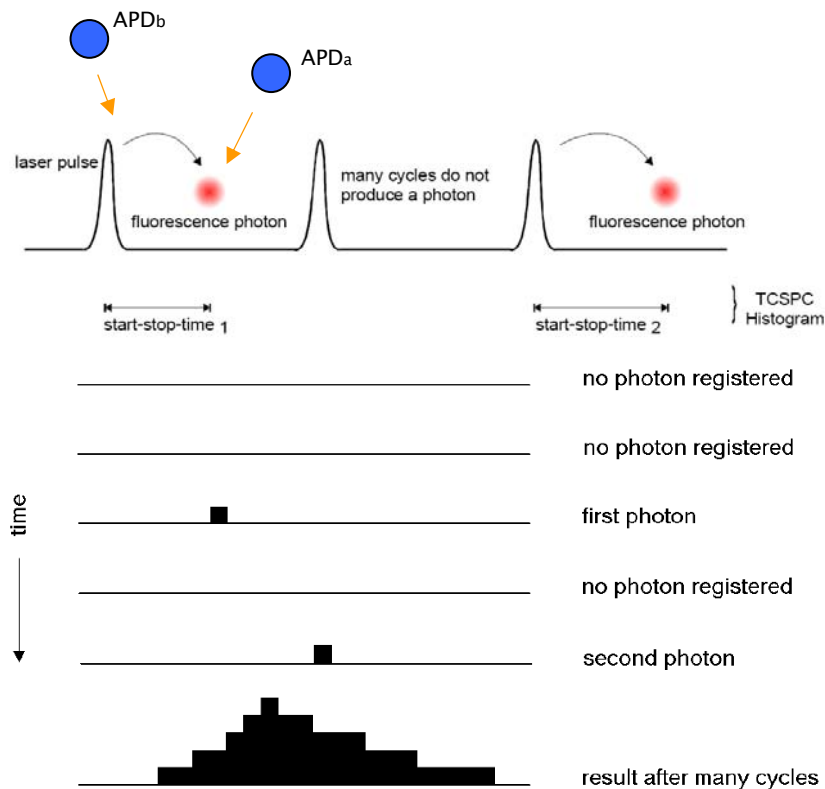


Figure 3-5: Fluorescence lifetime measurement

A whole series of laser pulse--photon arrival times is measured in the experiment and then collected in a histogram as shown in Figure 3-5 above.

See Figure 15-3: Scanning Confocal Microscope: T3R and FLIM (FLIM with red laser).

3.2.6 Fluorescence cross-correlation spectroscopy (FCCS)

Fluorescence cross-correlation spectroscopy analyses the detection of fluorescent photons at two colors. Excitation may be done at a single color or by using two colors as in ALEX experiments (see below). The two APDs, one for each color, are connected to a router that supplies the arrival event with the source (color channel) of the detected photons to the TimeHarp photon counting card. Thus the color information of a photon arrival event can be determined from the channel information present in the T3R data.

See Figure 15-5: Scanning Confocal Microscope: T3R using AOMs and TimeHarp 200 (FCCS).

3.2.7 Alternating-laser excitation (ALEX, FCCS)

In an alternating-laser excitation experiment both the detection and the excitation are performed with two colors. Here the excitation colors alternate in rapid succession, for example with a frequency of 1 MHz. See Figure 3-6 below.

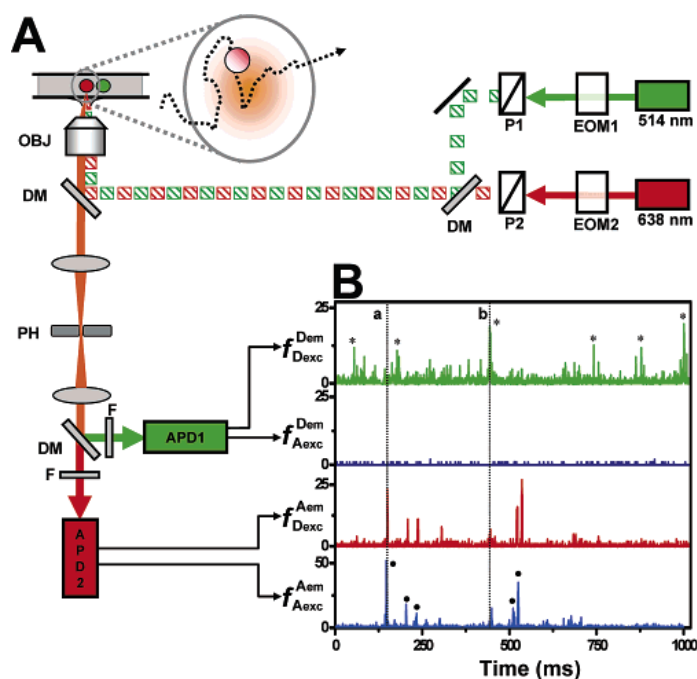


Figure 3-6: from Kapanidis 2005.

See Figure 15-6: Scanning Confocal Microscope: T3R with 2-color alternation using AOMs and TimeHarp 200 (ALEX, FCCS)

3.2.8 T3R + voltammetry

Here a T3R (FCS) measurement is combined with a cyclic voltammetry experiment. The program initiates a voltammetry experiment (it triggers the potentiostat via the NI DAQ device) and performs a T3R (FCS) measurement. The program records both the T3R data and the potentiostat's control voltage (via an analog input of the NI DAQ device). See Figure 15-6.

3.2.9 FLIM + voltammetry

Here a FLIM measurement is combined with a cyclic voltammetry experiment. The program initiates a voltammetry experiment (it triggers the potentiostat via the NI DAQ device) and performs a FLIM measurement. The program records both the FLIM data and the potentiostat's control voltage (via an analog input of the NI DAQ device). See Figure 15-6.

4 USE CASES



Figure 4-1: SCLM's major features

Use Case ID	UC-1
Use Case Name	Perform fluorescence spectroscopy (Summary)
Created By	Martin Moene
	Last Updated By
Date Created	2008-Sep-16
	Date Last Updated
Actor	User
Description	User performs measurement and obtains data
Preconditions	<ol style="list-style-type: none"> 1. Usable sample is in place 2. Setup works, 3. System is idle
Postconditions	<ol style="list-style-type: none"> 1. Measurement data saved in file 2. System is idle
Normal Course	<ol style="list-style-type: none"> 1.0 Perform fluorescence spectroscopy <ol style="list-style-type: none"> 1. include <i>Find Optical Focus</i> 2. include <i>Acquire Intensity Image</i> 3. include <i>Select Location of Interest</i> 4. include <i>Acquire Time-Trace</i>
Extensions	Repeat steps 3 and 4
Alternative Courses	
Exceptions	
Includes	<i>Find Optical Focus, Acquire Intensity Image, Select Location of Interest, Acquire Time-Trace</i>
Priority	
Frequency of Use	
Business Rules	
Special Requirements	
Assumptions	
Notes and Issues	

Use Case ID	UC-2	
Use Case Name	Find Optical Focus	
Created By	Martin Moene	Last Updated By
Date Created	2008-Sep-16	Date Last Updated
Actor	User	
Description	Perform an xz-scan to determine the z-value with optical focus. This z-value is obtained from the photon count peaks in the measured intensity image	
Preconditions	<ol style="list-style-type: none"> 1. Setup works 2. Usable sample is in place 3. User mechanically focused sample 4. System is idle 	
Postconditions	<ol style="list-style-type: none"> 1. Optical focus established 2. System is idle 	
Normal Course	<p>2.0 Find Optical Focus</p> <ol style="list-style-type: none"> 1. User selects xz-scan type 2. System sets or restores xz-measurement settings 3. Include <i>Scan Area</i> 4. include <i>Select Location of Interest</i> 	
Alternative Courses		
Exceptions		
Includes	<i>Select Location of Interest</i>	
Priority		
Frequency of Use		
Business Rules		
Special Requirements		
Assumptions		
Notes and Issues		

Use Case ID	UC-3	
Use Case Name	Acquire Intensity Image	
Created By	Martin Moene	Last Updated By
Date Created	2008-Sep-16	Date Last Updated
Actor	User	
Description	Perform an xy-scan to determine the location(s)-of-interest from the measured intensity image	
Preconditions	<ol style="list-style-type: none"> 1. Sample is focused, 2. System is idle 	
Postconditions	<ol style="list-style-type: none"> 1. xy intensity image displayed 2. Image data saved to file 3. System is idle 	
Normal Course	<ol style="list-style-type: none"> 3.0 Acquire Intensity Image <ol style="list-style-type: none"> 1. User selects xy-scan type 2. System sets or restores xy-measurement settings 3. include <i>Scan Area</i> 	
Alternative Courses		
Exceptions		
Includes	<i>Scan Area</i>	
Priority		
Frequency of Use		
Business Rules		
Special Requirements		
Assumptions		
Notes and Issues		

Use Case ID	UC-4	
Use Case Name	Scan Area	
Created By	Martin Moene	Last Updated By
Date Created	2008-Sep-16	Date Last Updated
Actor	User	
Description	Perform an area scan and present photon counts as intensity image	
Preconditions	<ol style="list-style-type: none"> 1. Sample is focused, 2. System is idle 	
Postconditions	<ol style="list-style-type: none"> 1. Image displayed on screen 2. Image data saved 3. System is idle 	
Normal Course	<p>4.0 Scan Area</p> <ol style="list-style-type: none"> 1. User selects xy scan mode 2. User changes scan parameters (size, bin-time) 3. System records values for later use 4. User starts image acquisition 5. System scans area, acquires photon counts and updates image 6. System presents generated filename for image data file 7. User accepts or changes filename and path and saves data 	
Alternative Courses	<ol style="list-style-type: none"> 4.1 User selects xz scan mode (replaces step 1) 4.2 User discards data (branch at step 6) 	
Exceptions		
Includes		
Priority		
Frequency of Use		
Business Rules		
Special Requirements		
Assumptions		
Notes and Issues		

Use Case ID	UC-5		
Use Case Name	Select Location of Interest		
Created By	Martin Moene	Last Updated By	Martin Moene
Date Created	2008-Sep-16	Date Last Updated	2008-Nov-21
Actor	User		
Description	User selects point of focus, or user selects location for time-trace acquisition		
Preconditions	<ol style="list-style-type: none"> 1. Intensity image displayed, 2. xy-mode selected, 3. System is idle 		
Postconditions	<ol style="list-style-type: none"> 1. Program and scan table use selected location, 2. System is idle 		
Normal Course	5.0	Select Location of Interest	
		<ol style="list-style-type: none"> 1. User points to location of interest in intensity image (key-mouse combination) 2. System updates xy scan centre and brings scan table to selected xy-position 	
Alternative Courses	5.1	Select z position of Focus (xz-scan mode selected precondition)	
		<ol style="list-style-type: none"> 1. User points to location of interest in intensity image (key-mouse combination) 2. System updates z scan parameter and brings scan table to selected z-position 	
Exceptions			
Includes			
Priority			
Frequency of Use			
Business Rules			
Special Requirements			
Assumptions			
Notes and Issues			

Use Case ID	UC-6		
Use Case Name	Acquire Time-Trace		
Created By	Martin Moene	Last Updated By	Martin Moene
Date Created	2008-Sep-16	Date Last Updated	2008-Nov-21
Actor	User		
Description			
Preconditions	1. System is idle		
Postconditions	1. System is idle		
Normal Course	6.0 Acquire Time-Trace		
	1. <i>Acquire Binned Time-Trace</i>		
Extensions	1. Combine with voltammetry		
Alternative Courses	6.1 <i>Acquire Time-Tagged Time-Trace</i>		
Exceptions			
Includes			
Priority			
Frequency of Use			
Business Rules			
Special Requirements			
Assumptions			
Notes and Issues			

Use Case ID	UC-	
Use Case Name	<i>Use Case Template</i>	
Created By	Martin Moene	Last Updated By
Date Created	2008-Sep-16	Date Last Updated
Actor	User	
Description		
Preconditions		
Postconditions		
Normal Course		
Alternative Courses		
Exceptions		
Includes		
Priority		
Frequency of Use		
Business Rules		
Special Requirements		
Assumptions		
Notes and Issues		

Table 4-1: Requirement Tracability Matrix

User Requirement	Functional Requirement	Design Element	Code Module	Test Case

5 SYSTEM FEATURES

5.1 Find Optical Focus

5.1.1 Description and Priority

5.1.2 Stimulus/Response Sequences

5.1.3 Functional Requirements

Focus.

Focus.

5.2 Acquire a fluorescence image

5.2.1 Description and Priority

5.2.2 Stimulus/Response Sequences

5.2.3 Functional Requirements

AcquireImage.

5.3 Select Location-of-Interest

5.3.1 Description and Priority

5.3.2 Stimulus/Response Sequences

5.3.3 Functional Requirements

SelectLocation.

5.4 Acquire binned time-trace

5.4.1 Description and Priority

5.4.2 Stimulus/Response Sequences

5.4.3 Functional Requirements

AcquireBinnedTimeTrace.

6 EXTERNAL INTERFACE REQUIREMENTS

6.1 User Interfaces

Chapter 1 TBD

6.2 Hardware Interfaces

HI-1: TBD

6.3 Software Interfaces

SI-1: TBD

6.4 Communications Interfaces

CI-1: TBD

7 OTHER NONFUNCTIONAL REQUIREMENTS

7.1 Performance Requirements

PE-1: program shall support the measurement of time-traces as long as several minutes (5 min?).

7.2 Safety Requirements

No safety requirements have been identified.

7.3 Security Requirements

No security requirements have been identified.

7.4 Software Quality Attributes

Availability, robustness ...

8 OTHER REQUIREMENTS

8.1 Verification

Note : verification versus validation.

9 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

9.1 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

10 DATA DICTIONARY AND DATA MODEL

scan area = scan width
 + scan height
 scan height * height of scan area in [nm] *
 scan width * width of scan area in [nm] *
 sample time * photon count bin time for each point of scan area in [ms] *

11 ISSUES LIST

No issues have been identified.

12 BUSINESS RULES

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

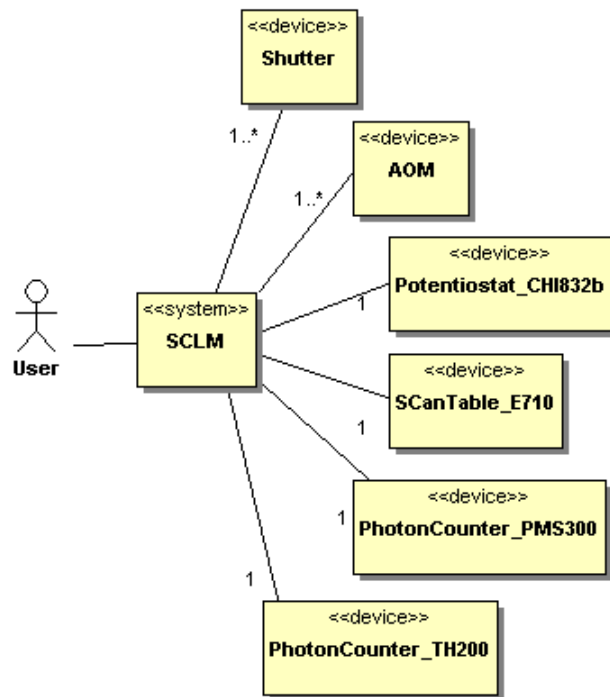


Figure 13-1: SCLM context class diagram

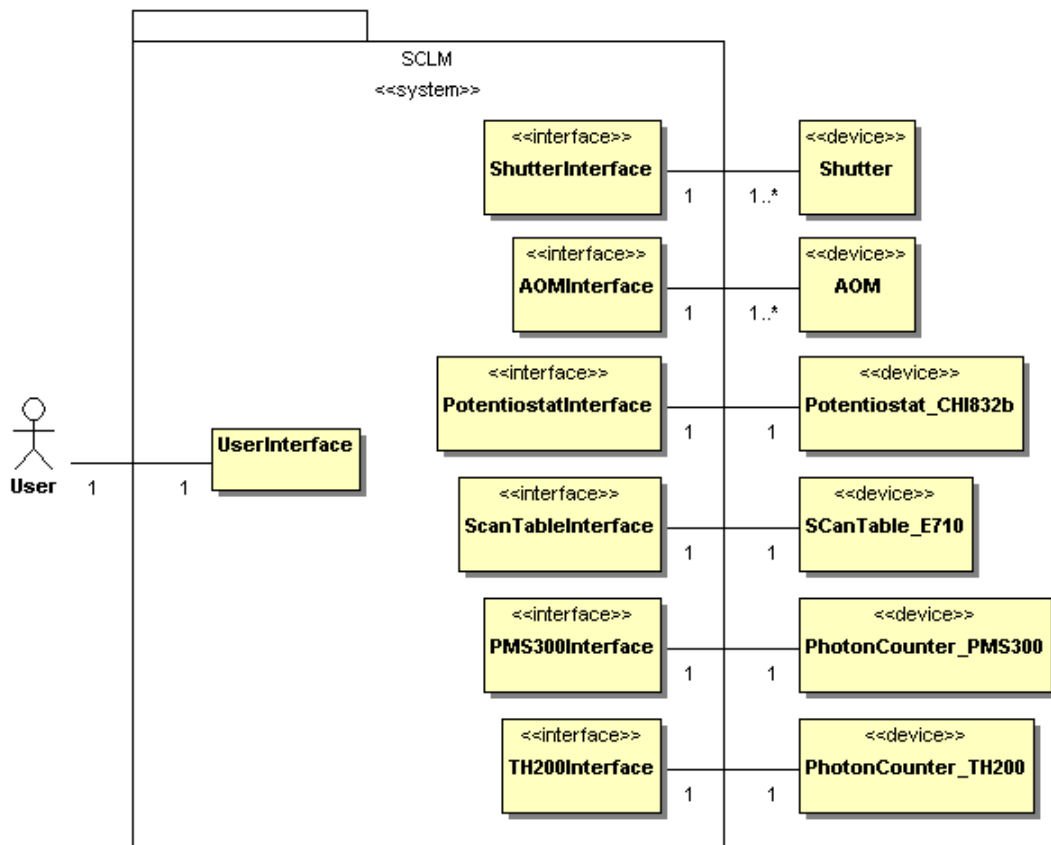


Figure 13-2: SCLM external classes and interface classes diagram

14 SETUP: USAGE OF PARTS

Measurement	Part																	
		L _r	L _g	L _{argon}	S _r	S _g	AOM _r	AOM _g	AOM _{argon}	E710	APD _a	APD _b	Splitter	Router	800-b	PMS300	TH200	CHI832b
1-Find optical focus		•	○		•	○			✓	✓		✓			✓			
2-Acquire image		•	○		•	○			✓	✓		✓			✓			
3-Select location-of-interest									✓									
4-Acquire binned time-trace		•	○		•	○				✓		✓			✓			
5-Perform time-tagged time-resolved spectroscopy (T3R, FCS)		•	○		•	○				✓			✓				✓	✓
6-Perform fluorescence lifetime imaging (FLIM)		✓			✓					✓				✓			✓	✓
7-FCS and FLIM with AOM excitation control		•	○		•	○	•	○		✓				✓			✓	✓
8-Perform cross-correlation spectroscopy (FCCS)		✓	✓		✓	✓	✓	✓		✓	✓		✓				✓	
9-Perform alternating-laser excitation spectroscopy (ALEX)		✓	✓		✓	✓	✓	✓		✓	✓		✓				✓	
10- Combine T3R (FCS) with voltammetry		•	○		•	○	•	○		✓							✓	✓
11-Combine FLIM with voltammetry		•	○		•	○	•	○		✓							✓	✓

Table 14-1: Which measurements uses what; ✓ indicates used, • and ○ indicate one, other or both used.

15 SCHEMATICS

Schematics are on the following pages.

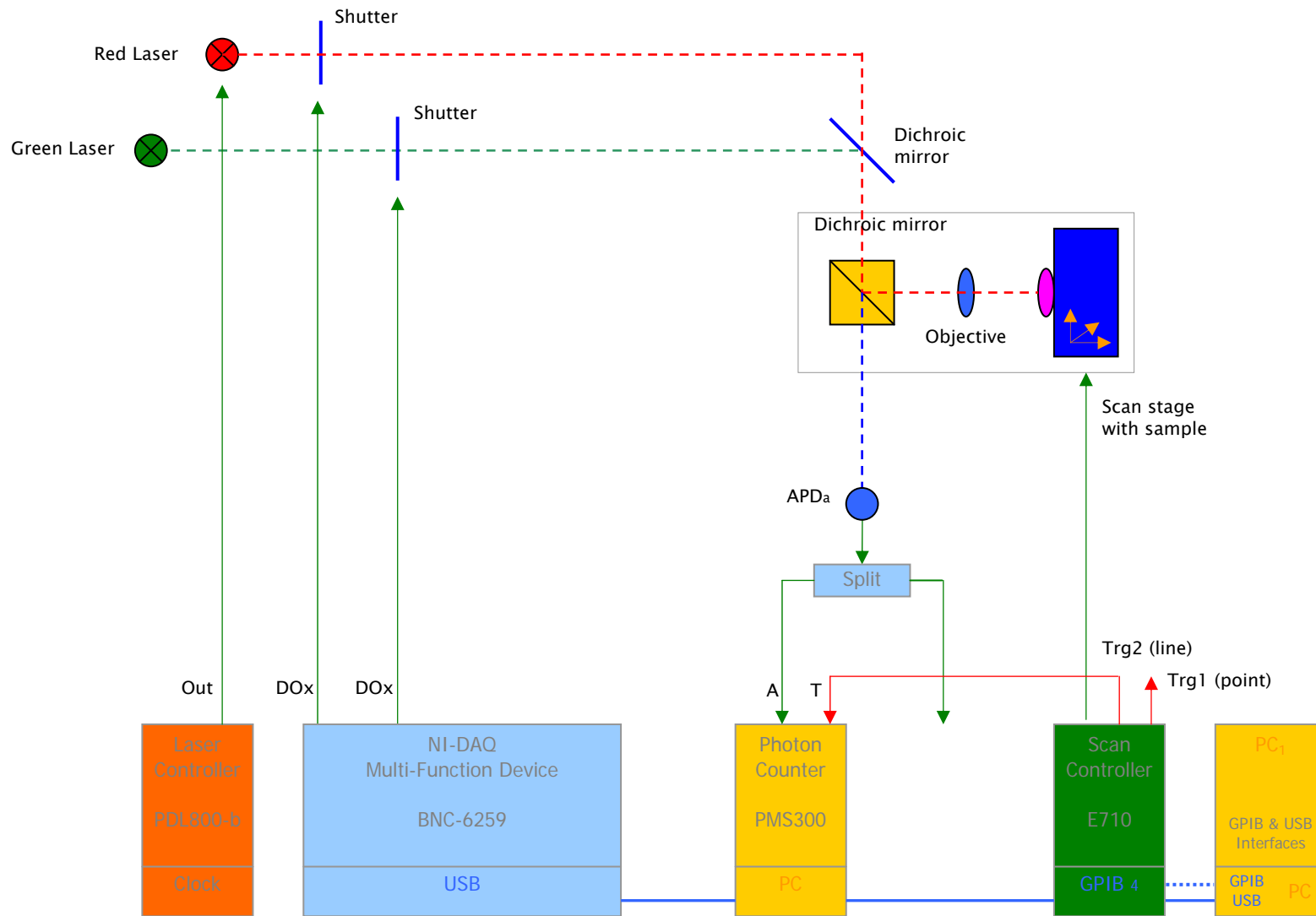


Figure 15-1: Scanning Confocal Microscope: imaging and binned time-trace with PMS 300

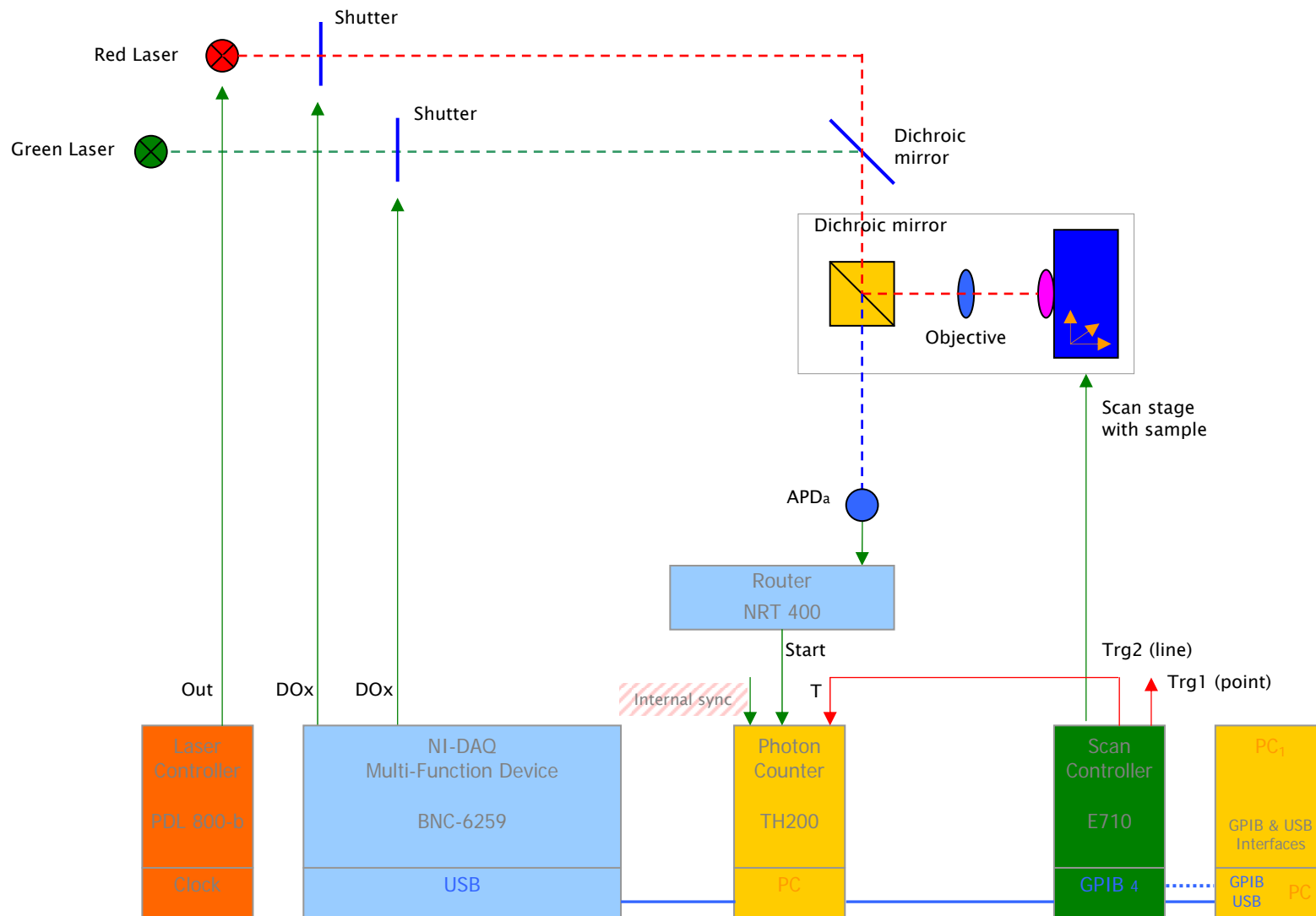


Figure 15-2: Scanning Confocal Microscope: imaging and T3R (FCS) with TimeHarp 200

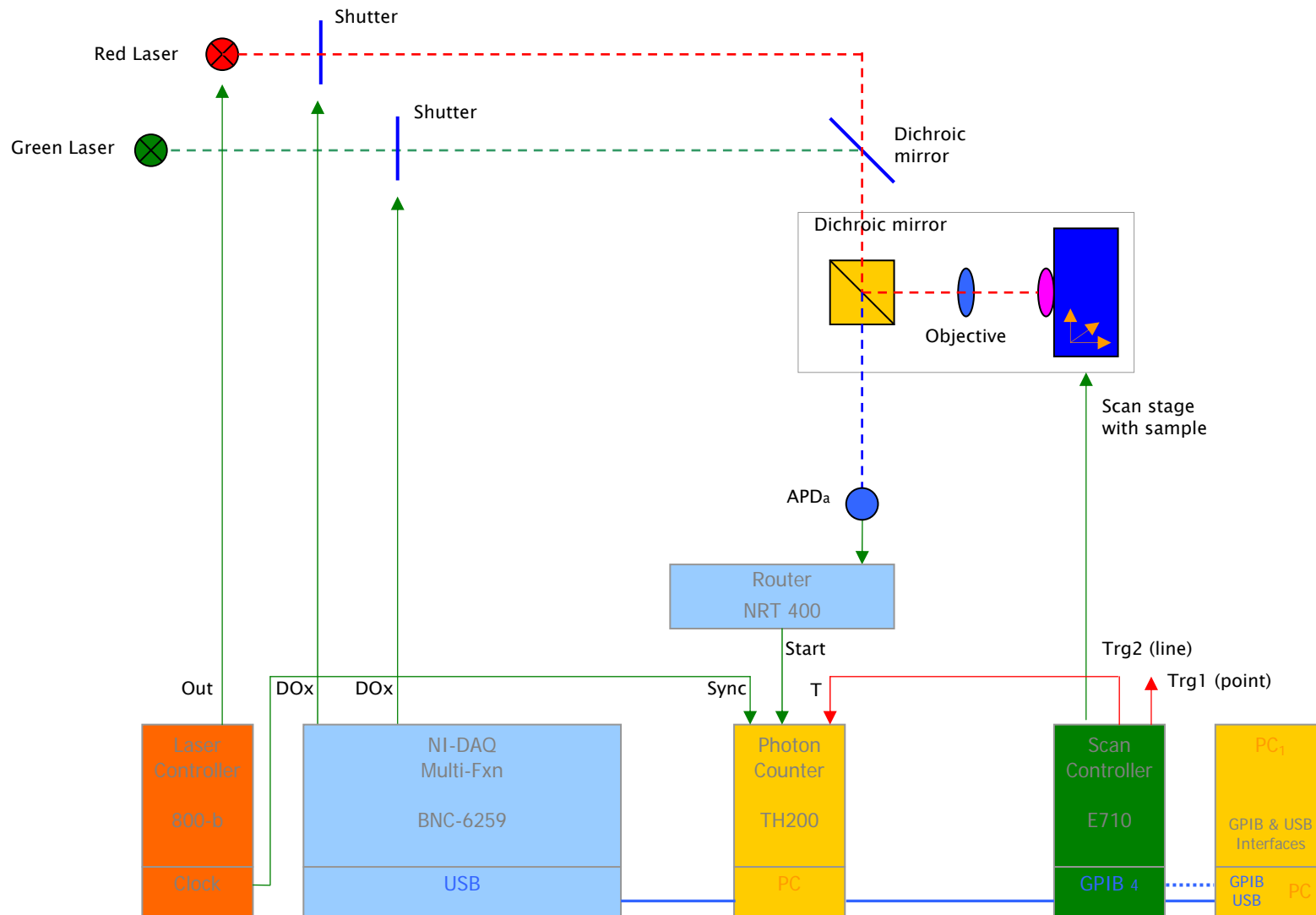


Figure 15-3: Scanning Confocal Microscope: T3R and FLIM (FLIM with red laser)

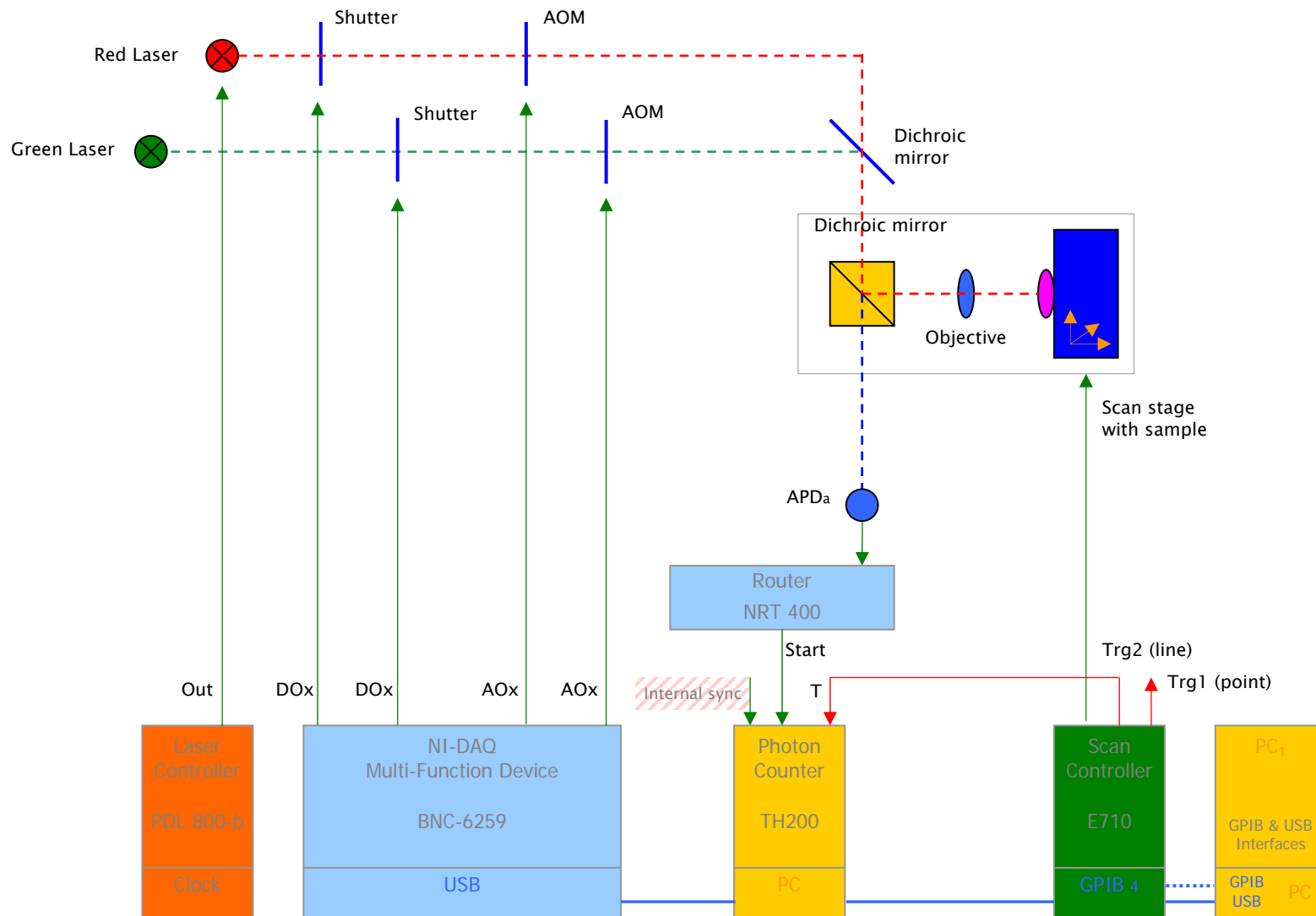


Figure 15-4: Scanning Confocal Microscope: imaging and T3R with TimeHarp 200 and AOM excitation control

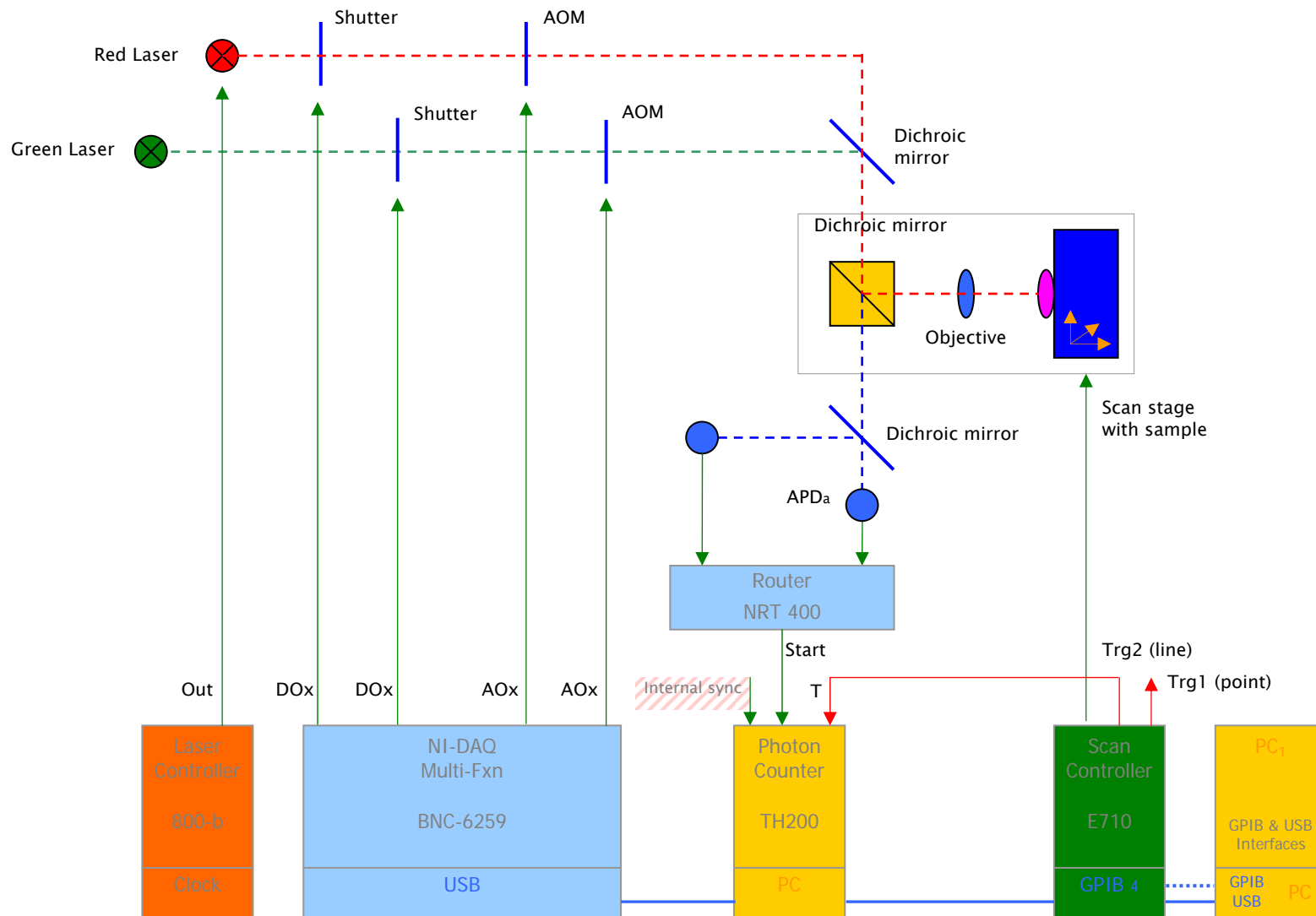


Figure 15-5: Scanning Confocal Microscope: T3R using AOMs and TimeHarp 200 (FCCS)

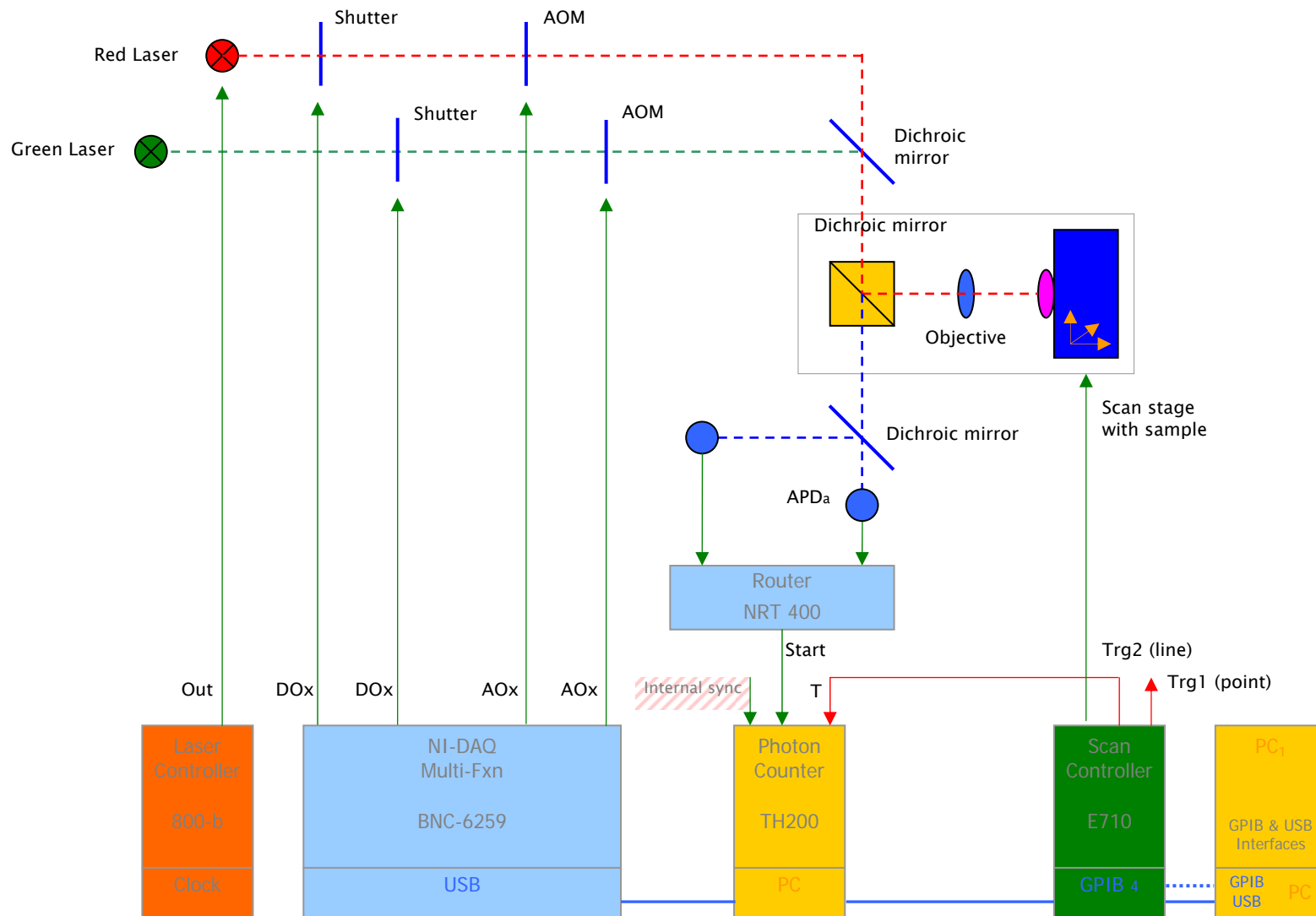


Figure 15-6: Scanning Confocal Microscope: T3R with 2-color alternation using AOMs and TimeHarp 200 (ALEX, FCCS)

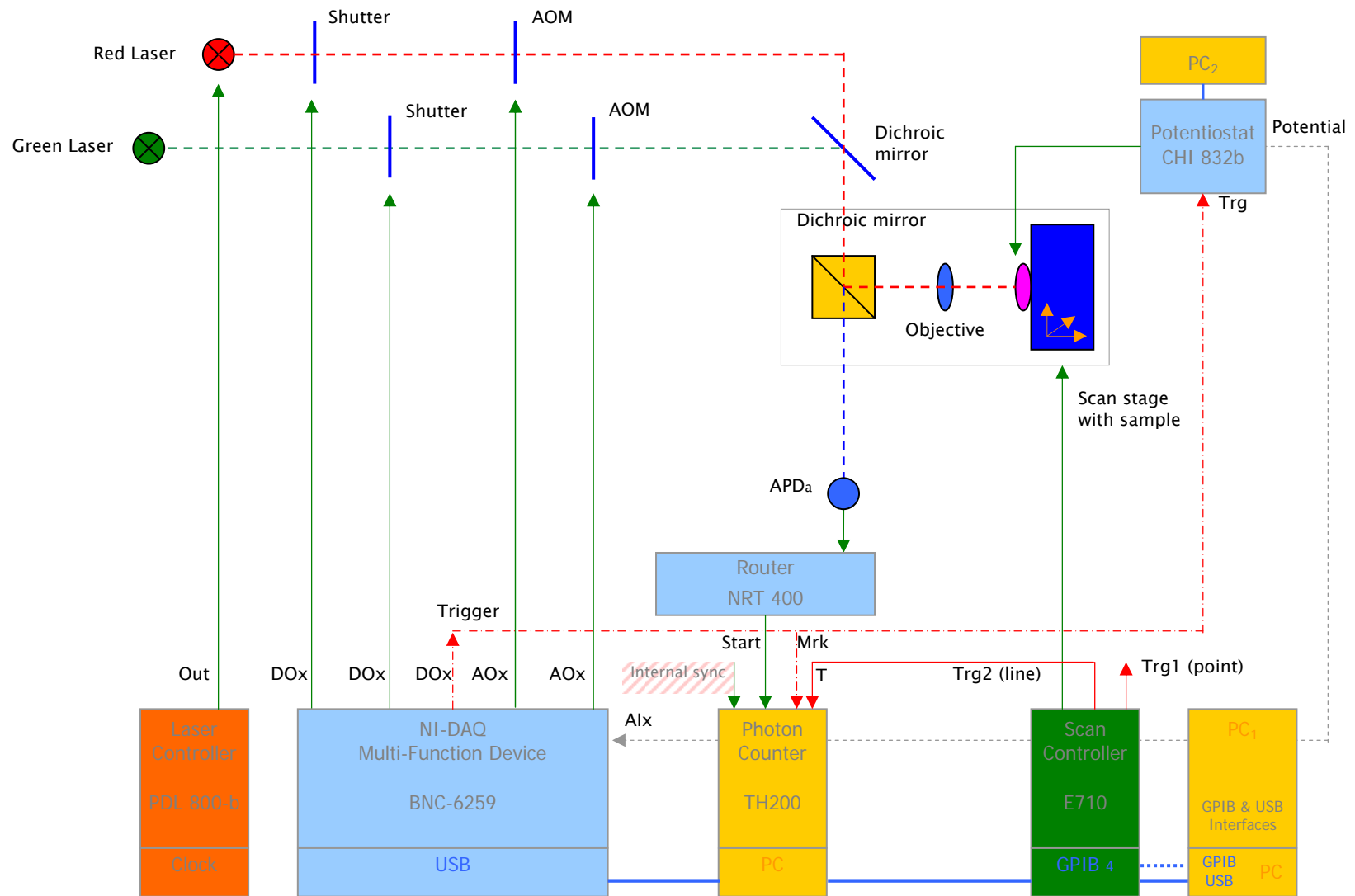


Figure 15-7: Scanning Confocal Microscope: imaging and T3R with cyclic voltammetry based on 1-color excitation scheme, (Figure 15-4)