UCLA
UCLA Previously Published Works

Title
Assembling the μs-ALEX Setup.

Permalink
https://escholarship.org/uc/item/55b4m6bn

Journal
Cold Spring Harbor Protocols, 2015(11)

ISSN
1940-3402

Authors
Kapanidis, Achillefs
Majumdar, Devdoot
Heilemann, Mike
et al.

Publication Date
2015-11-01

DOI
10.1101/pdb.prot086413

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

Downloaded from http://cshprotocols.cshlp.org/ at UCLA Library on November 7, 2015 - Published by
Cold Spring Harbor Laboratory Press

Protocol

Assembling the μs-ALEX Setup

Achillefs Kapanidis, Devdoot Majumdar, Mike Heilemann, Eyal Nir, and Shimon Weiss

This protocol describes the construction of a microsecond-alternating laser excitation (μs-ALEX) using two lasers, a green 532-nm acousto-optically modulated laser and a red 635-nm directly modulated laser.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

Cold Spring Harbor Protocols
www.cshprotocols.org
CSH PROTOCOLS

Equipment

Avalanche photodiodes (APDs)
Bayonet Neill Concelman (BNC) cables
Desktop PC
Dichroic mirror (DM)
Emission beam splitter (650DRLP [dichroic reflector long pass])
Fiber coupler
Lasers (green 532-nm acousto-optically modulated and red 635-nm directly modulated)

Extreme caution is required when using lasers. In most cases, lasers used for ALEX applications belong to the class IIIB (underlying the International Laser Safety Standard IEC 60825). It is important to follow laser safety instructions, which include general safety rules and special institutional rules. A laser safety officer should be contacted before any ALEX system is set up and should help with risk assessment and safety guidelines.

Microscope with appropriate objectives

See Table 1 for a listing of suppliers of parts for constructing ALEX microscope modules.

Mirrors
Modulator
Optical fiber (single-mode)
Optical table
Paper (white)
Polarizers (λ/4, λ/2)
Slide (glass) (optional; see Step 5)
Spectral filters (585DF70 for the green channel, 650LP for the red channel)

Adapted from *Imaging: A Laboratory Manual* (ed. Yuste). CSHL Press, Cold Spring Harbor, NY, USA, 2011.

© 2015 Cold Spring Harbor Laboratory Press
Cite this protocol as *Cold Spring Harb Protoc*; doi:10.1101/pdb.prot086413

Downloaded from http://cshprotocols.cshlp.org/ at UCLA Library on November 7, 2015 - Published by Cold Spring Harbor Laboratory Press

TABLE 1. Suppliers of parts used for constructing ALEX microscope modules

Components	Types	Provider
Excitation module		
Lasers	Argon ion	Melles Griot, Newport
	Nd:YAG	Cobolt, Laser2000
	Diode laser	Coherent, Picoquant
	HeNe	Melles Griot
Modulation	Electro-optical modulator	Conoptics, Linos
	AOM (acousto-optical modulator)	Isomet
	Acousto-optical tunable filter	AA-Optoelectronics
Optics	Filters, dichroics	Semrock, Omega, Chroma
	Lenses, mirrors	Linos, Thorlabs
	Polarizer, λ/4, λ/2	Newport
	Prism	Melles Griot
Fiber optics	Optical fiber	Thorlabs
	Fiber coupler	Thorlabs, Newport
	Multiplexer	AA-Optoelectronics, Linos
Optomechanics	Posts, mounts, kinematics	Thorlabs, Comar, Linos
	Translation stages	Linos, Newport, New Focus, Thorlabs
Sample holder module		
Microscope	I × 71 Inverted microscope	Olympus
	Axiovert 100, 200, 200M	Zeiss
	Leica DM IL	Leica
Objective	PLAPON 60×O/TIRFM 1.45 (oil immersion)	Olympus
	UPLSAPO 60×/1.2 W (water immersion)	Olympus
	Alpha Plan-Fluar 100×/1.45 (oil immersion)	Zeiss
Temperature control	Objective heating collar	Biosciencetools
	Objective cooling collar	Intracel
	Microscope temperature control	Olympus, Zeiss, Leica
Emission module		
Detectors	Avalanche photodiodes	PerkinElmer (SPCM-AQR-14 or 15), Picoquant (PDM 20/50/100CT)
	EMCCD camera	Roper Scientific, Andor
Optomechanics	Translation stages for detectors	New Focus
	Kinematic mounts, filter holders, posts, bases, translation stage for pinholes	Thorlabs, Comar
Optics	Filters, dichroics	Omega, Chroma, Semrock
	Lenses, pinholes	Linos, Comar

The above suppliers are used in the authors' laboratories; many other suppliers are also available, especially for components of the excitation module.

METHOD

1. Arrange lasers, the modulator, and the fiber coupler on an ∼50-cm × 50-cm area on an optical table. Verify that the polarization of the lasers is both linear and vertical. If this is not the case, linearize the polarization using a polarizer, and convert to vertical orientation either by adjusting the position of the laser or by using a combination of λ/2 and λ/4 plates. Use two mirrors to direct the green laser beam into the AOM aperture.
2. Overlap both laser beams using a mirror (M1, Fig. 1) and a DM (DM1, 560DRLP) for the green laser and two mirrors (M2, M3) for the red laser, and couple the combined beam into a single-mode optical fiber.
3. Mount the output of the optical fiber on the microscope breadboard, followed by a 10×–20× collimating objective mounted on an *x*–*y*–*z*-positioning stage.

   The choice of the collimating objective depends on the diameter of the back aperture of the focusing objective, which should be fully illuminated. With common single-mode optical fibers and a 10× collimating objective, beam diameters of 5–10 mm are achieved.

   For some applications, it may be desirable to underfill the back aperture of the objective to increase the volume of the confocal spot. This in turn increases the diffusion time of molecules and the photons emitted per diffusing molecule.

Cold Spring Harbor Protocols www.cshprotocols.org CSH PROTOCOLS

Downloaded from http://cshprotocols.cshlp.org/ at UCLA Library on November 7, 2015 - Published by
Cold Spring Harbor Laboratory Press

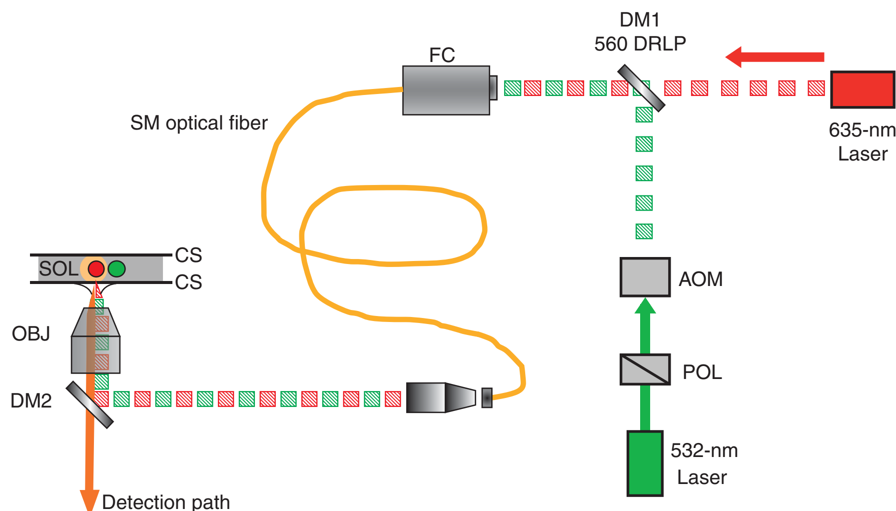

FIGURE 1. Excitation module for ALEX: 635-nm laser (directly modulated and linearly polarized) and 532-nm laser. POL, polarizer; AOM, acousto-optical modulator. Laser beams are overlaid with three mirrors (M1, M2, and M3) and a dichroic beam splitter (DM1; 560DRLP) into a fiber coupler (FC) to which a single-mode (SM) optical fiber is connected; 10× objective serves as fiber output unit; dual-band dichroic beam splitter (DM2) directs light through oil immersion objective (NA 1.45, 60× or 100×).

Cold Spring Harbor Protocols
www.cshprotocols.org
CSH PROTOCOLS

4. Direct the laser beam to the side port of the microscope. The beam should be centered in the back aperture of the focusing objective as well as in the field of view. Use the *x–y–z*-positioning stage to align the beam position and collimate the beam.
5. Align the detection path by reflecting scattered excitation light through the objective. (Place a glass slide and focus onto the upper side of the slide, or place a mirror on the objective.) The light will pass the excitation dichroic and can be visualized along the detection path using a small piece of white paper. Place the pinhole at the focal point of the microscope lens, onto an *x–y* or *x–y–z* positioner, followed by a second lens to collimate the light.

   The parallel light is split by the emission beam splitter on two detectors, split by a dichroic mirror, which is followed by further spectral filters along each beam path. A 20-mm lens focuses the light on the active area of each APD, mounted onto micrometer-precision x–y–z-positioning stages.

6. Mount each detector on an *x–y–z*-positioning stage and connect to a counting board in a desktop PC using BNC cables.

RELATED INFORMATION

See **Alternating Laser Excitation for Solution-Based Single-Molecule FRET** (Kapanidis et al. 2015a), **Aligning the μs-ALEX Setup** (Kapanidis et al. 2015b), and **Sample Preparation and Data Acquisition for μs-ALEX** (Kapanidis et al. 2015c).

REFERENCES

Kapanidis A, Majumdar D, Heilemann M, Nir E, Weiss S. 2015a. Alternating laser excitation for solution-based single-molecule FRET. *Cold Spring Harb Protoc* doi: 10.1101/pdb.top086405.

Kapanidis A, Majumdar D, Heilemann M, Nir E, Weiss S. 2015b. Aligning the μs-ALEX setup. *Cold Spring Harb Protoc* doi: 10.1101/pdb .prot086421.

Kapanidis A, Majumdar D, Heilemann M, Nir E, Weiss S. 2015c. Sample preparation and data acquisition for μs-ALEX. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot086439.

Downloaded from http://cshprotocols.cshlp.org/ at UCLA Library on November 7, 2015 - Published by
Cold Spring Harbor Laboratory Press

Cold Spring Harbor Protocols

Assembling the μs-ALEX Setup

Achillefs Kapanidis, Devdoot Majumdar, Mike Heilemann, Eyal Nir and Shimon Weiss

Cold Spring Harb Protoc; doi: 10.1101/pdb.prot086413

Email Alerting Service	Receive free email alerts when new articles cite this article - **click here.**
Subject Categories	Browse articles on similar topics from *Cold Spring Harbor Protocols.* Characterization of Proteins (183 articles) Fluorescence (460 articles) Fluorescence, general (307 articles) Imaging/Microscopy, general (555 articles) Labeling for Imaging (314 articles) Multi-Photon Microscopy (100 articles) Photoactivation (36 articles) Photobleaching (12 articles) Protein Identification and Analysis (170 articles) Structural Analysis (16 articles)

To subscribe to *Cold Spring Harbor Protocols* go to:
http://cshprotocols.cshlp.org/subscriptions

© 2015 Cold Spring Harbor Laboratory Press