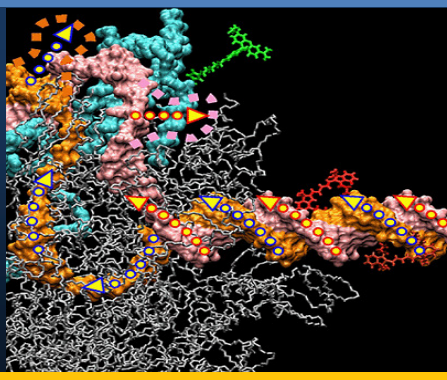
Two-color (2c) ALEX Single Molecule Reader Prototype

ALEX (Alternating Laser EXcitation) single-molecule fluorescence spectroscopy allows simultaneous stoichiometry- and FRET-based analysis of molecular complexes and supramolecular assemblies, giving you the edge for your next breakthrough discovery. It can visualize molecular dynamics and provide detailed views of molecular machines at work, e.g. as demonstrated for RNA polymerase performing "DNA-scrunching" during initial bacterial transcription.

Benefits of 2c-ALEX:

- ***Easy to use and low-cost instrument***
- ***Simultaneous stoichiometry- and FRET-based analysis of molecular interactions***
- ***Customizable laser wavelengths for your application***
- ***Short processing and analysis time***
- ***Ultra-sensitive target detection in minute sample volumes***
- ***Highly accurate target quantification***



Alternating Laser EXcitation (ALEX) Single Molecule Fluorescence Spectroscopy

DNA-Scrunching During Transcription*

Excitation sources (any combination of 2 lasers):
Green, 532 nm, average power 30 mW, CW laser
Green, 543 nm average power 30 mW, CW laser
Red, 635 nm average power 10 mW, CW laser
Blue, 477 nm, average power 30 mW, CW laser
Blue, 488 nm, average power 30 mW, CW laser
(wavelength and power at laser output)

Laser alternation method:
AOM, 4 channel PCAOM crystal and driver
PCI-6602 (NI) with 3 DMA channels, 8 counters

Pinhole:
Oriel pinhole mount with appropriate flanges and connectors and flexible beam enclosures

Microscope:
Water immersion objective 60 x NA 1.2
CCD camera
TV monitor

Detector unit:
APD, power supply and mechanical switches

Dichroic mirrors:
555dcxr
635dcxr

Analysis software:
Operating system: Windows XP or later
Photon histograms (alternation times of excitation wavelength)
Time traces for calculation of leakage and direct excitation
E vs. *S* histogram for burst count as well as FRET and Stoichiometry
All-photon-burst-search
Dual-channel-burst-search
Fluorescence Correlation Spectroscopy (FCS) in 2c-ALEX and single wavelength excitation modes

Computer:
Desktop computer with a Peripheral Component Interconnect (PCI) slot
RAM size: >3 GB
Processor speed: >3 GHz
Hard drive size: >500 GB

Input power:
120/220 volts AC 50/60 Hz

Dimension:
Width x depth x height: 4 feet x 2.5 feet x 1.5 feet

*** References:**

- "DNA-scrunching" by bacterial RNA polymerase during initial transcription. (ASBMB Today, Jan 2007, cover image)
- Kapanidis *et al.* (2006) Initial transcription by RNA polymerase proceeds through a DNA-scrunching mechanism. Science 314, 1144-1147.
- Kapanidis *et al.* (2005) Retention of Transcription Initiation Factor sigma(70) in Transcription Elongation: Single-Molecule Analysis. Mol Cell 20, 347-356.

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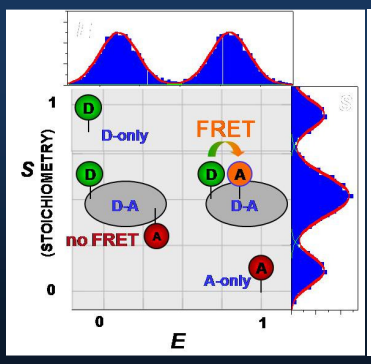
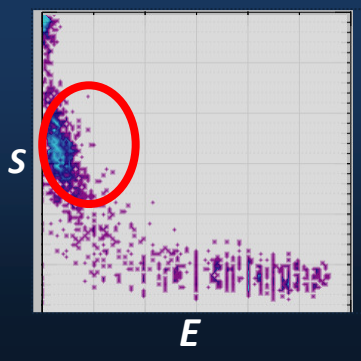
Two-color (2c) ALEX Single Molecule Reader Prototype

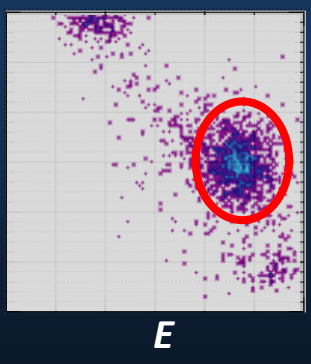
In 2c-ALEX, employing two alternating lasers to study molecular interactions (through probe stoichiometry ratio *S* and/or FRET efficiency *E*) and intramolecular distances for analysis of conformation and mechanism (through *E*), molecules are sorted in a two-dimensional histogram of *S* and *E*.



Sorting single molecules using FAMS. *E-S* histogram for D-only, A-only, and D–A species with different R_{D-A}. *E* sorts species according to FRET and R_{D-A}, reporting on structure; *S* sorts species according to D–A stoichiometry, reporting on interactions. Sorting is also possible by using 1D *E* or *S* histograms (in blue; red line, sum of Gaussian fits).



2c-ALEX with different excitation wavelengths: The *E-S* histogram (left) indicates that the target species (circled) can be detected using the blue/red dye conjugated probes with no FRET between the probes. The histogram (right) shows that presence of a different species can be detected with high FRET green/red dye conjugated probes used.



Fluorescence Activated Molecule Sorting | **Blue-Red No FRET** | **Green-Red High FRET**

Environmental operation conditions:
Temperature: room temperature
Ambient light: instrument should not be exposed to light during operation, no windows

Typical measurement time:
10 minutes

Typical sample size:
Minimum: 10 nL (with microfluidics, e.g. microfluidic formulator*)
Maximum: 70 µL (with coverslip)

Maintenance/Alignments:
Detection Unit: every 6 months (~30')
Excitation unit: weekly (~1 '), monthly (~30')

Vibration isolation (optional):
Vibration isolation plate from Thorlabs, PTT900600- Iso-plate passive isolation system (35.4 inches x 23.6 inches x 3.5 inches)

Instrument resolution:
1 nanometer

Average number of bursts:
CV for normalized burst count for targeted subpopulation is 7% for 10 minute acquisition

Coefficient of variation:
CV (E) of 0.24%
CV (S) of 2.26%
Test condition: High FRET Cy3B/Atto647N-labeled DNA samples

Laser 1 wavelength (nm)	Laser 2 wavelength (nm)	Filter set	Dye
532	635	580/60 and 665LP	Cy3B for green and Atto647N for red
543	635	580/60 and 665LP	Cy3B for green and Atto647N for red
488	543	520/35 and HQ640/100m-2p	Alexa488 for blue and Cy3B for green
488	635	HQ530/60m-2p and 665LP	Alexa488 for blue and Atto647N for red

* Kim *et al.* (2011) High-throughput single-molecule optofluidic analysis. Nat Methods 8, 242-5.

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