Rapid Raman Microspectroscopy and Imaging: The Role of the Electron-Multiplied CCD (EMCCD)

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Andor Technology

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Agenda

- Sensitivity: What and Why?
- Current Competing Technologies
- Andor Newton EMCCD Camera
- EMCCD Technology
- Effect of EM on CCD performance
- Overview

Sensitivity: What and Why?

WHAT

- System Noise – defines detection limit to overcome

  lower noise = fewer photons needed for detection

- Quantum Efficiency – measure of the camera's ability to capture photons

  Higher QE = fewer photons reqd to overcome noise

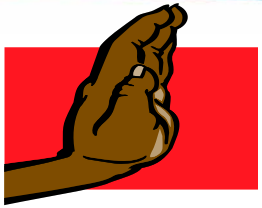
  = higher SNR

WHY

- Low Concentrations
- Short Exposures
- High Photon Loss
- Lower excitation powers
- Single photon sensitivity
- Raman and other weak signals
- Rapid data acquisition and processing

For the highest SNR – attain maximum sensitivity

Current Technologies

		
CCD	• High and broad QE • High pixel resolution	• Noise floor (readout restriction)
ICCD	• Single photon sensitive	• Restricted QE • Cross talk • High Multiplicative noise • Artifacts
EBCCD	• Single Photon Sensitive	• High Spurious Charge • Restricted QE • Artifacts
APD/PMT	• Best Single Photon Sensitivity	• Point detection only

Andor Newton EMCCD Camera

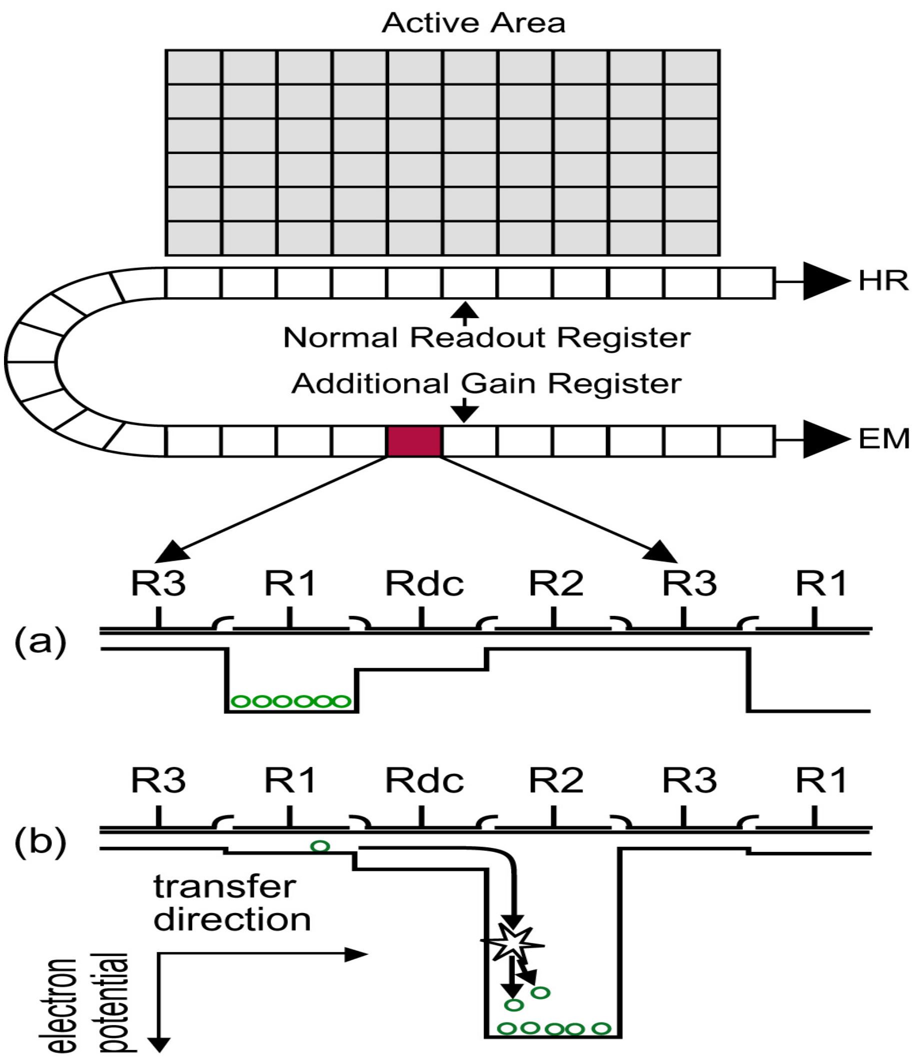
World's first EMCCD camera for spectroscopy

- Sensitivity – single photon sensitive, with 1000X gain!
- Spectral Rate: in Vertical Binning mode up to 600 spectra/sec
- Sensor: 25.6 x 6.4 & 25.6 x 3.2 sq.mm –a standard spectroscopy chip
- Pixel Size: 16um FOR 1600x400/1600x200 – Unique to Spectroscopy
- Dynamic Range: 16 bits
- Wavelength Range = 200 – 1050nm
- Quantum Efficiency – upto 97% for the BI
- Cooling – TE cooler down to -75C with air, -100C with water
- Noise – down to <1 elec, with minimal dark charge and spurious charge

Ease of use: Replace, Plug and Play!

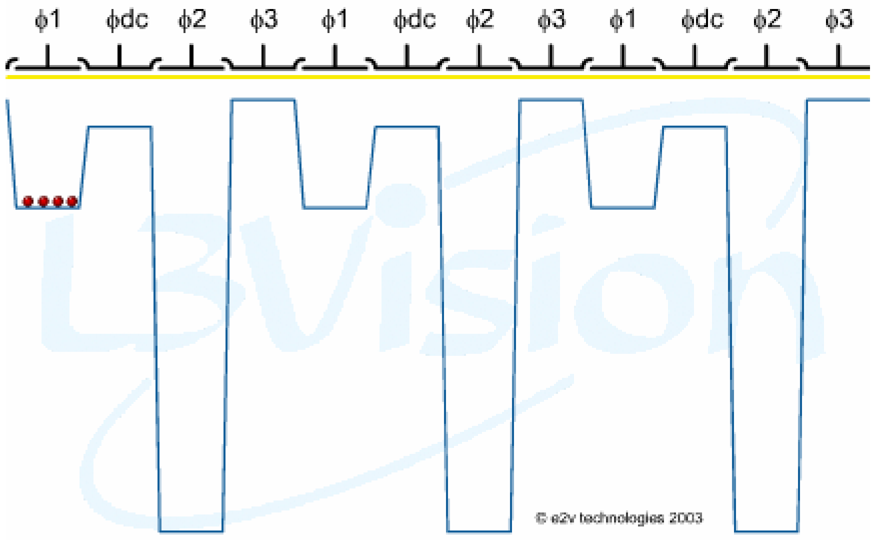
USB 2.0 Connectivity!

EMCCD Technology



EMCCD Technology

- Gain register amplifies signal before readout to ensure signal is not readout noise limited.
- The high voltages in the special gain register allow a small probability of electrons acquiring sufficient energy to create additional electrons – hence Electron Multiplication.
- The gain occurs over many stages to enable the gain to have a minimum additional noise. (Low noise factor)



Noise Sources in EMCCD vs. CCD

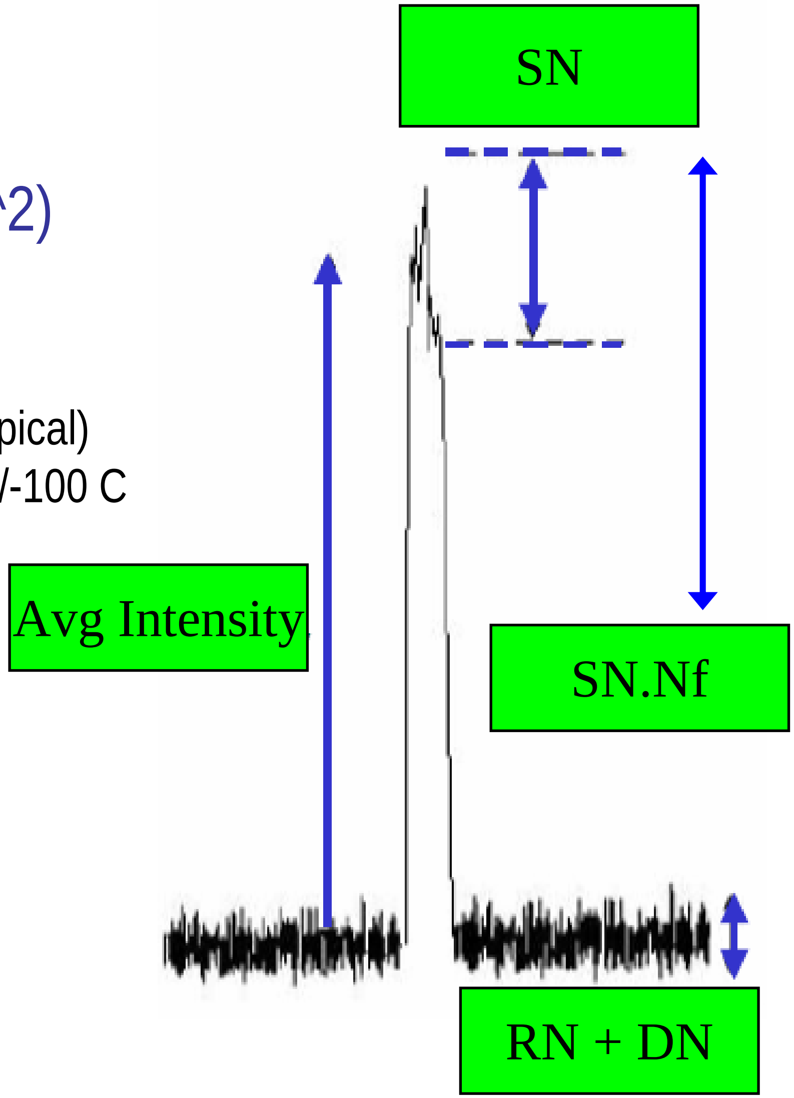
Shot Noise = SN = √Signal = √S
DN = Dark Noise

CCD R.M.S. Noise = √(RN^2 + DN^2 + SN^2)
EMCCD R.M.S. Noise:
= √((RN/Gain)^2 + Nf^2·(DN^2 + S))

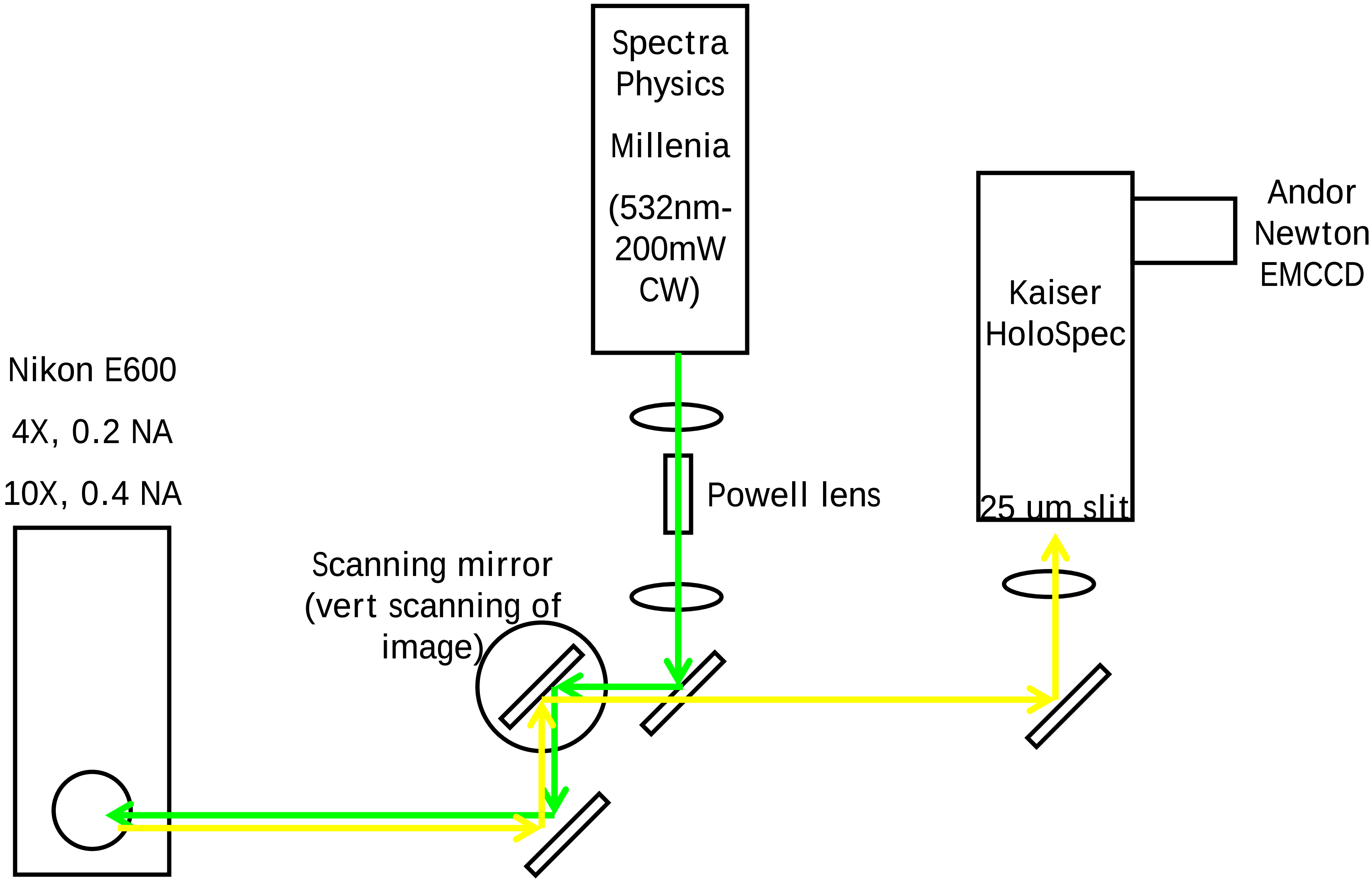
- Where Nf = Multiplicative Noise Factor = 1.4 (typical)
- DN is negligible at very low temperatures of -70/-100 C
- RN/Gain ≈ negligible at high gain values

EMCCD R.M.S. Noise = 1.4·√S

SNR(CCD) = S/√(RN^2 + S)
SNR(EM) = S/1.4·√S
= √S/1.4
SNR(CCD) is Read Noise LIMITED
SNR(EM) is Shot Noise LIMITED



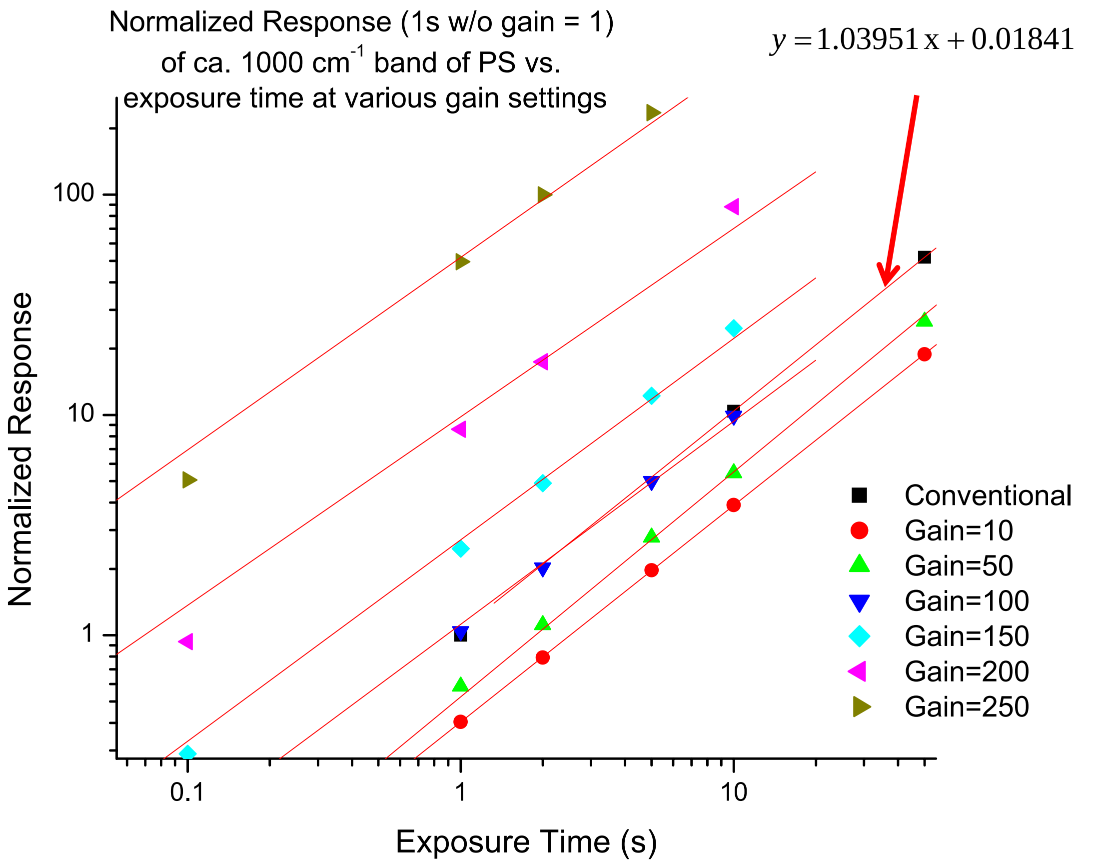
Instrumentation



Improved Performance

Polystyrene Sample Examination

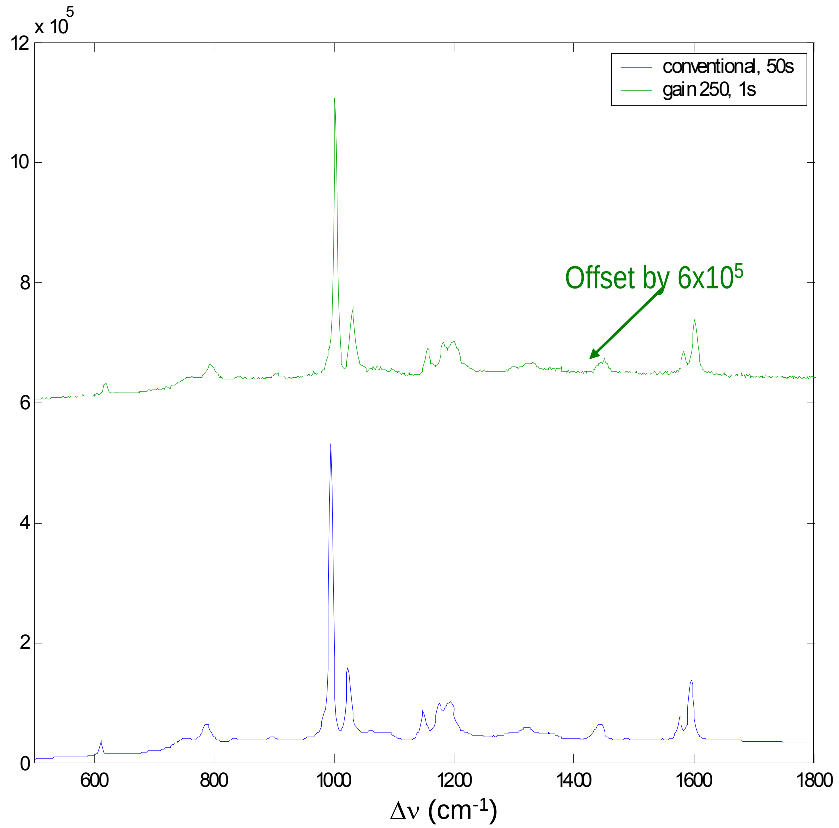
- Linear response from 0.01s to 50s over full range of gain
- Factor of 50 increase in signal over conventional CCD (for a gain of 250 over no gain)
- Gain of 100 is equivalent to Conventional CCD for same exposure



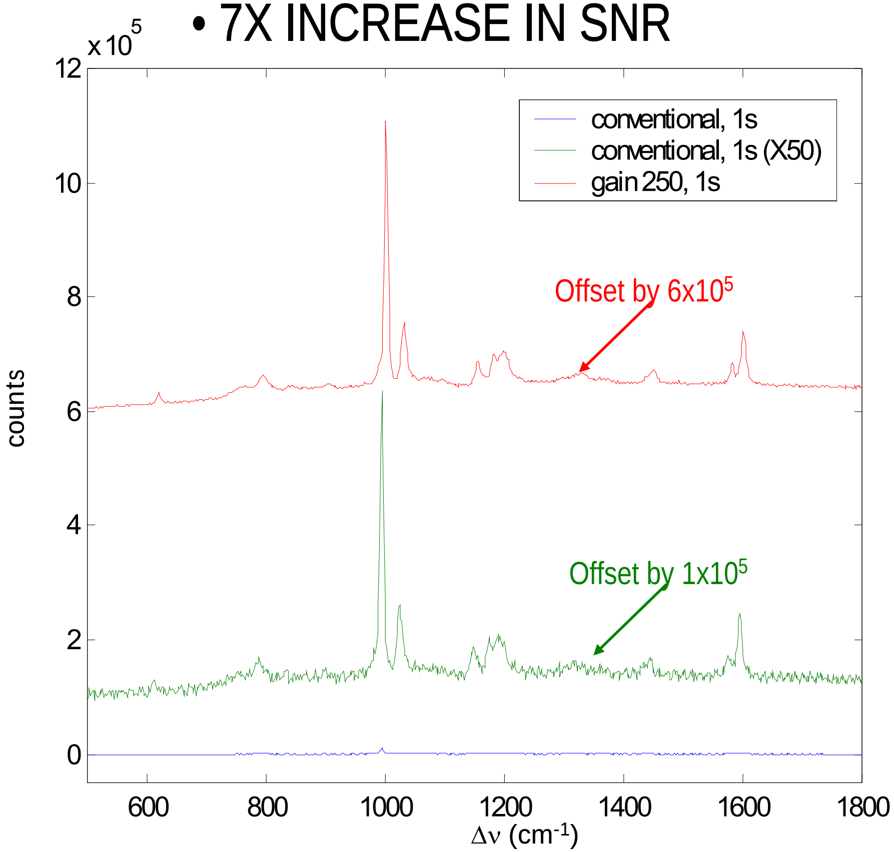
Effect of EM on Exposure Time

50s and 1s exposure, with and without gain

- 50s exp in Conv = 1s exp with EM

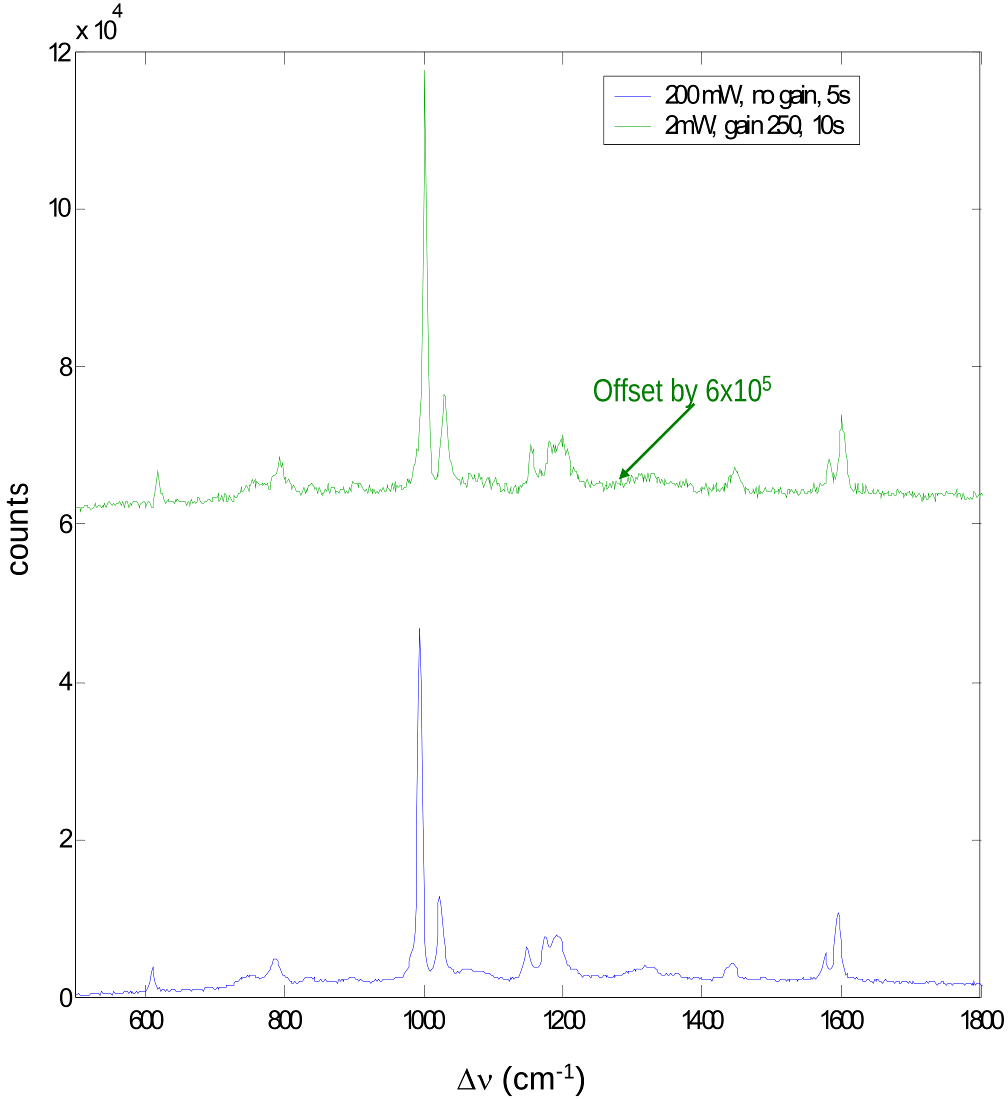


- 50 X increase in signal
- 7X INCREASE IN SNR



Reduce exposure time by 50X and increase SNR by 7X!

Effect of EM on Excitation Power



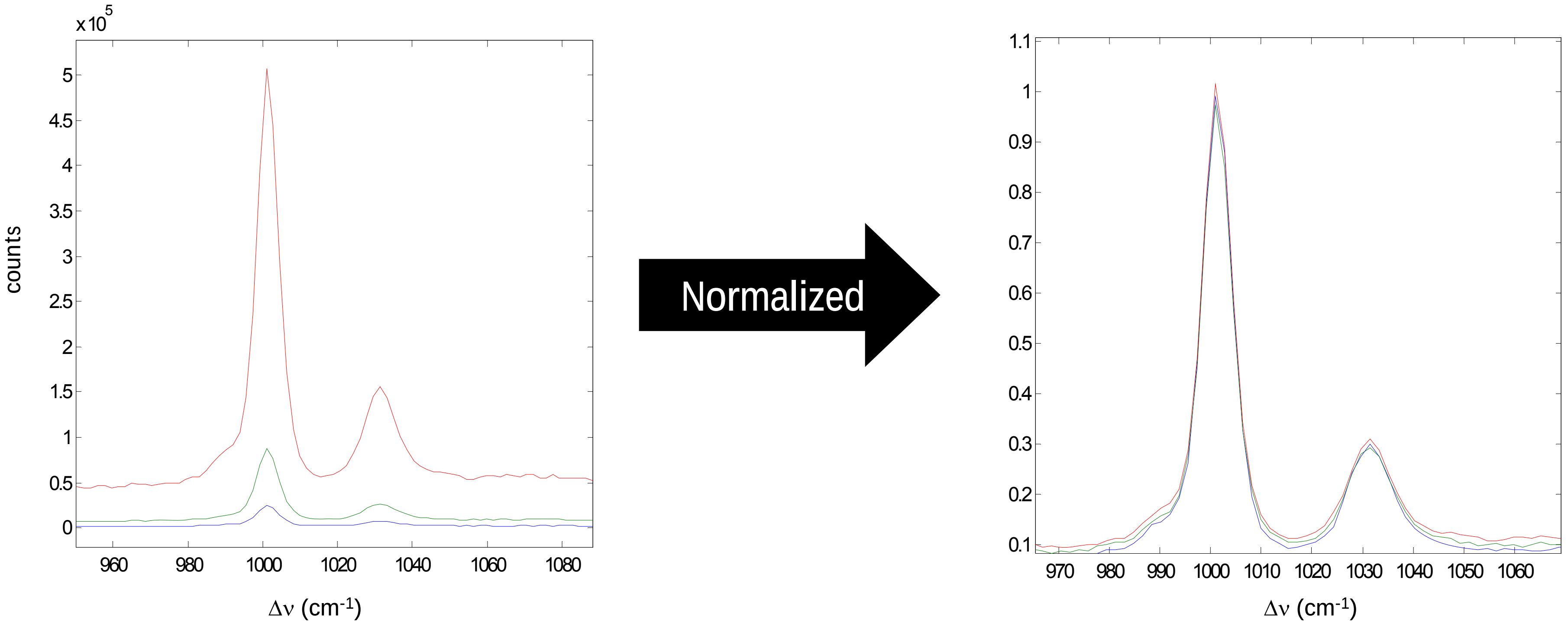
- Reduce laser excitation power, maintaining equivalent signals
- Advantage: Power Resolved Studies
- Advantage: Maximize sample life

Linearity of Response

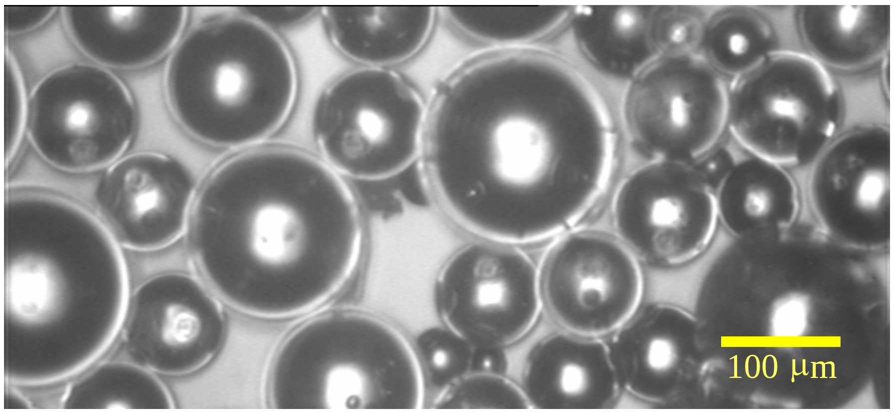
Question: Does gain alter band area ratios?

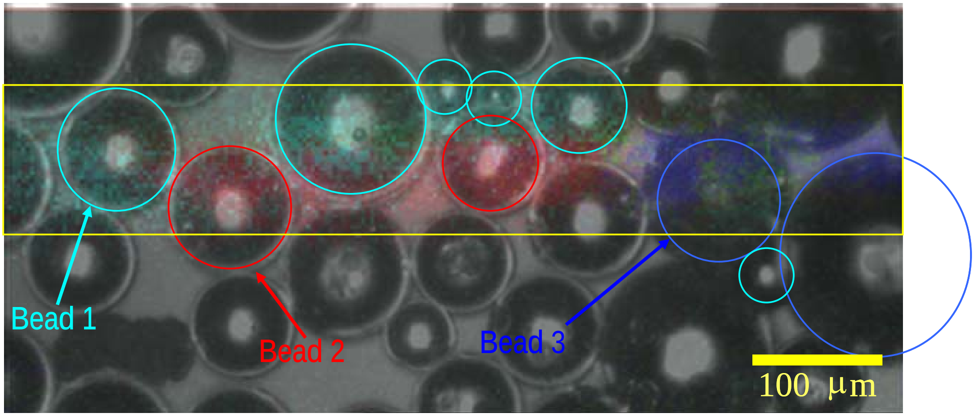
Answer: NO! This is good!

1s Polystyrene with gains of: 150, 200, 250



Rapid Particle Identification/detection

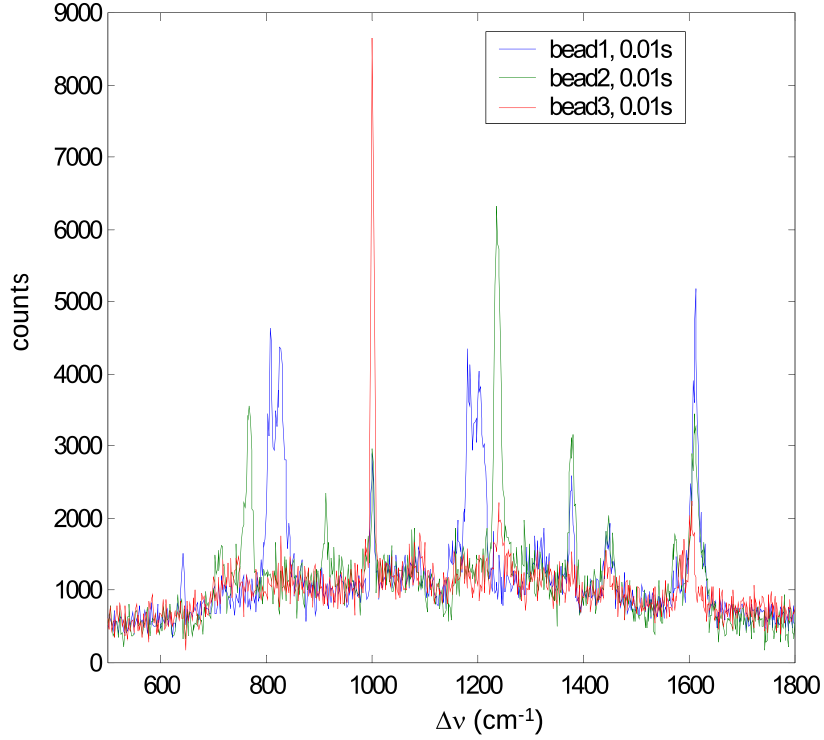




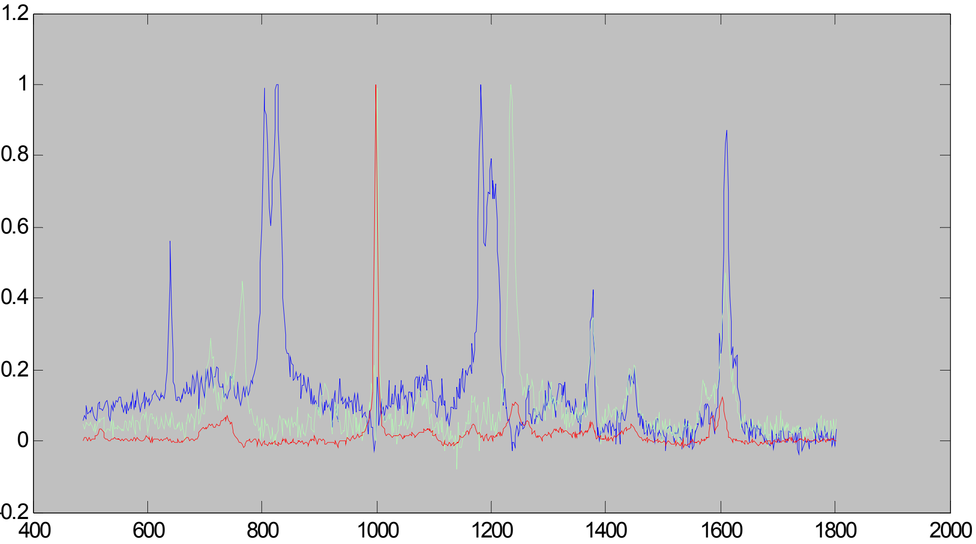
- Chemical Raman Imaging done on 3 different types of polystyrene beads
- Exp 1 – Rapid Bead Spectra: Each of the three types examined individually to show quality high speed spectra per bead
- Exp 2 – High Speed Raman Imaging: the mixture of the three types examined at once, to show reduction in time for scanning

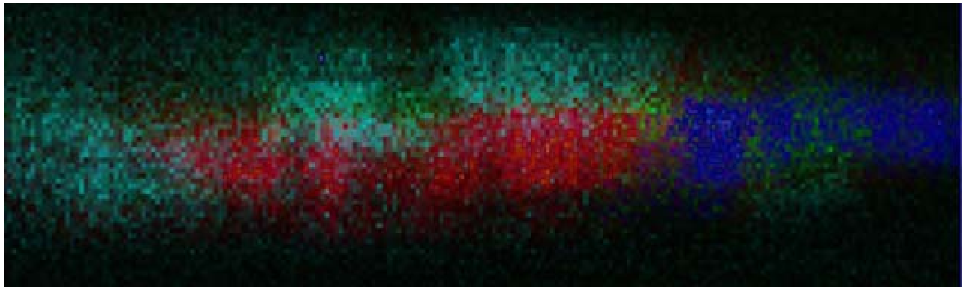
Rapid Bead Spectra

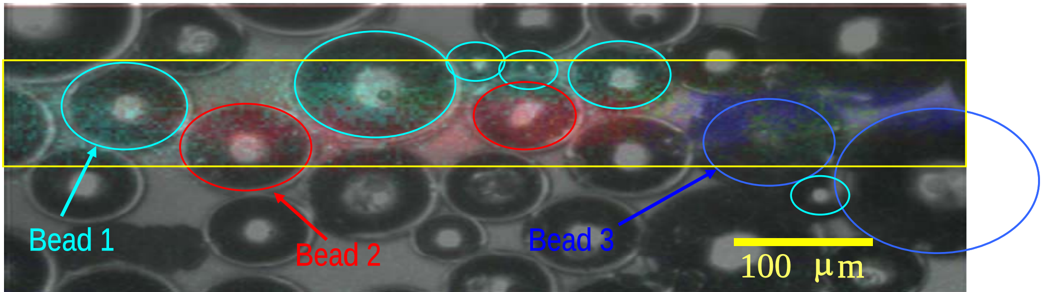
- Sample consists of 3 types of PS beads
- 0.01s Exp time with EM Gain of 250 gives quality spectra
- Acquisition of this spectra would normally take 50 times more time
- Enables acquisition of high quality spectra at shorter exposures/faster frame rates



High Speed Raman Imaging

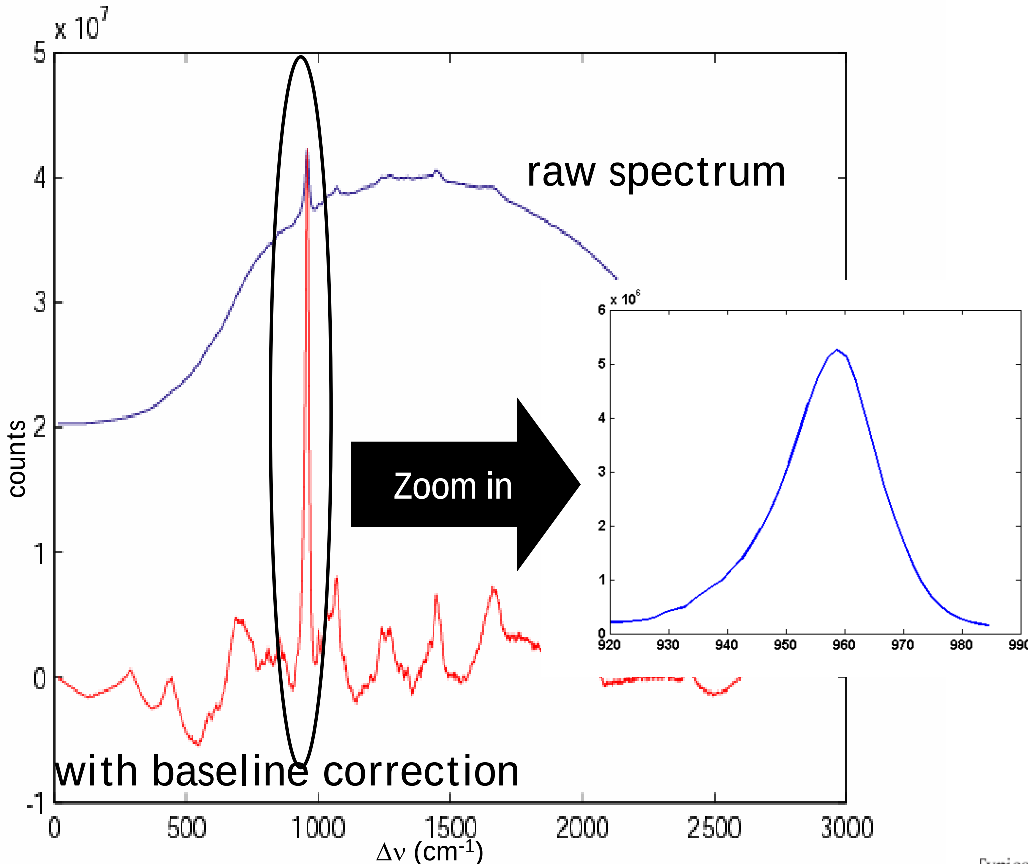


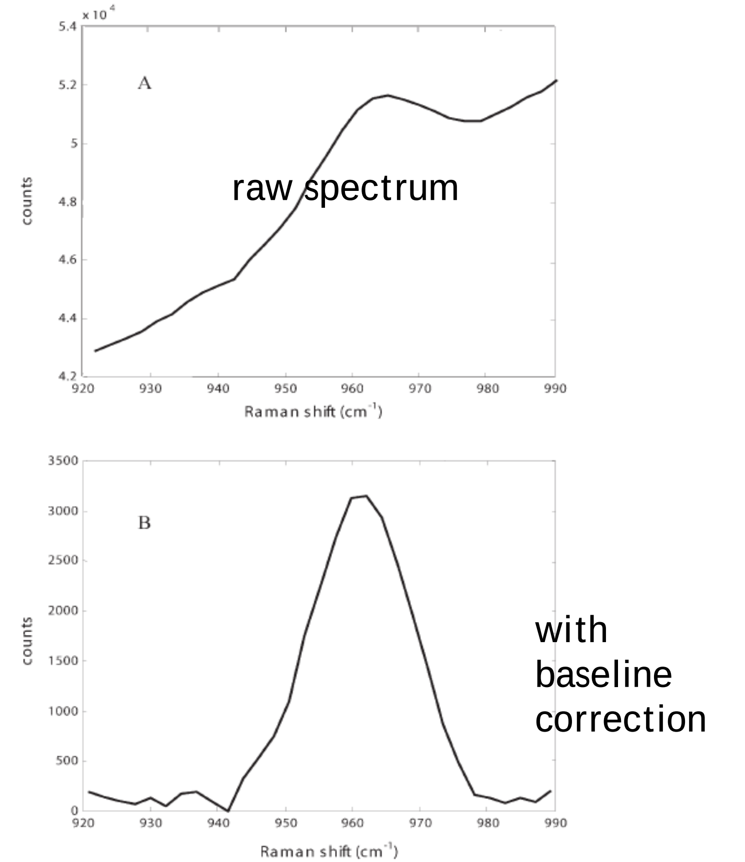




- Experiment performed on an area of 700x100 μm sq
- Exp Time = 1s, Gain = 250, 200 mW Power, digitization@2.5MHz
- Acquisition of this spectra w/o gain and with same exposure would have 50X smaller intensity
- EM reduces acquisition time by > 50X
- Conclusion: - Faster image scanning
  - follow time resolved processes
  - faster data acquisition

Bone Tissue Spectroscopy





Typical Raman spectrum of bone from ~900 cm−1 to ~1000 cm−1 collected on the 532 nm microprobe.
e baseline correction with a third order polynomial. (B) After baseline correction with a third order
al.

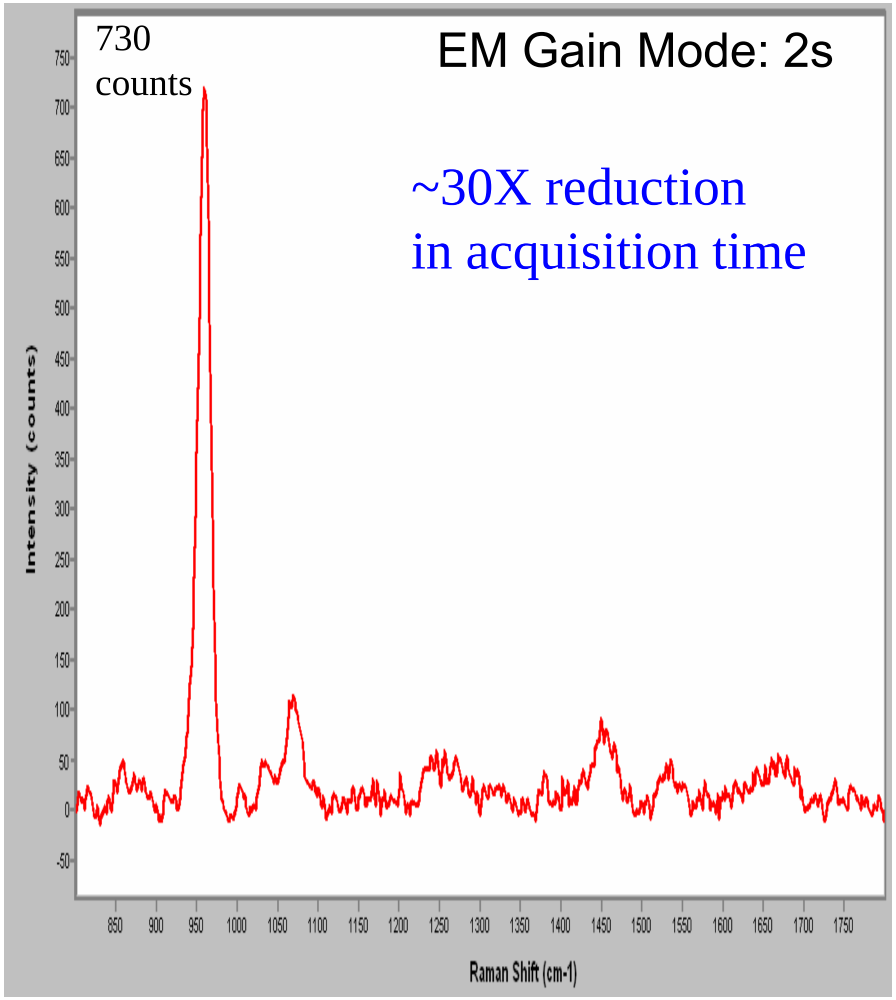
- 2s, line focus, 200mW
- 30X reduction in acquisition time
- Dramatic improvement in Signal

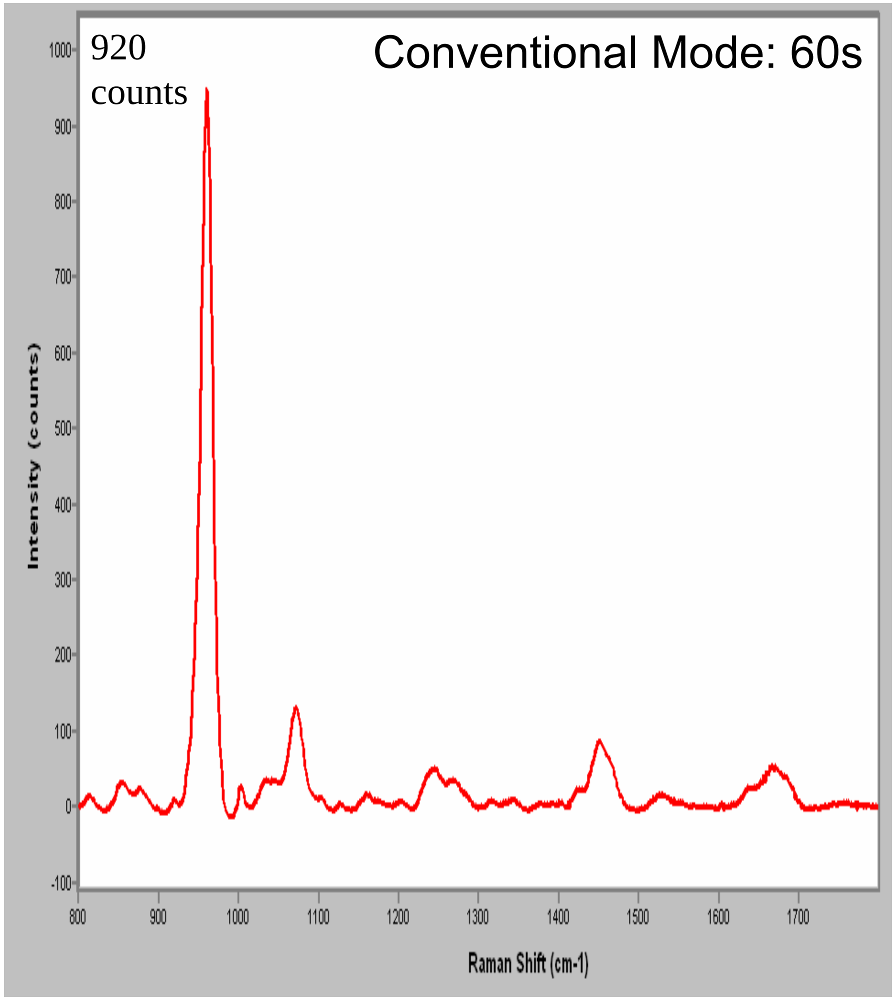
•60s, point focus, 200mW

(Finney et al. Faraday Discussion 126, 159-168, 2004)

EM Mode vs. Conv Mode

Line focus, 300mW, 532nm





Conclusions

- Dramatic improvement in SNR over CCD Operation
- Improvement in Signal intensity ~ 50X in polystyrene
- Experiment time down by a factor of >50X!
- Reduce laser excitation powers > 100X
- Reduce exposure times and hence faster frame rates
- Faster Raman/Image scanning and particle detection
- Reduce sample conc. – no need for strong signals
- Near zero noise floor!
- Single photon detection

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