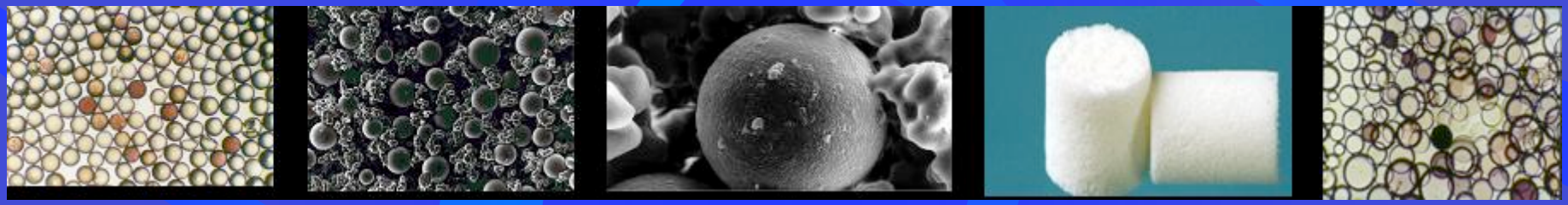
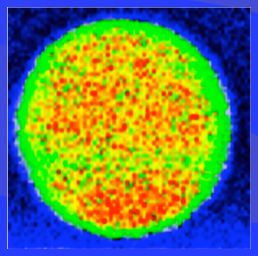
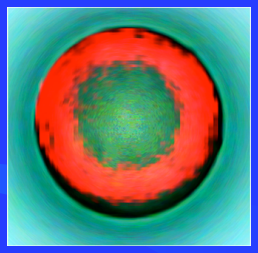
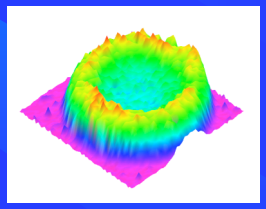
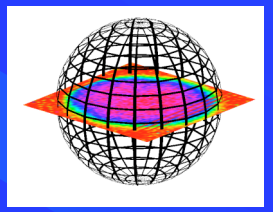
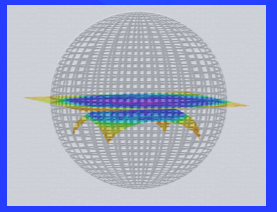
Looking inside solid supports

Confocal Raman Microscopy of polymer beads



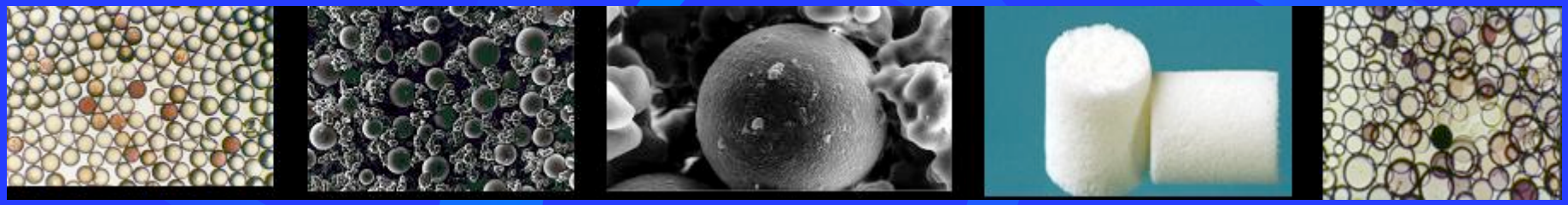
Talk Outline

- Solid Phase Supports
- Fluorescence studies
- Confocal Raman Microscopic Maps
- Diffusion vs. Reaction Kinetics
- Dry beads: Is what you see real?
- Edge effects
- Summary

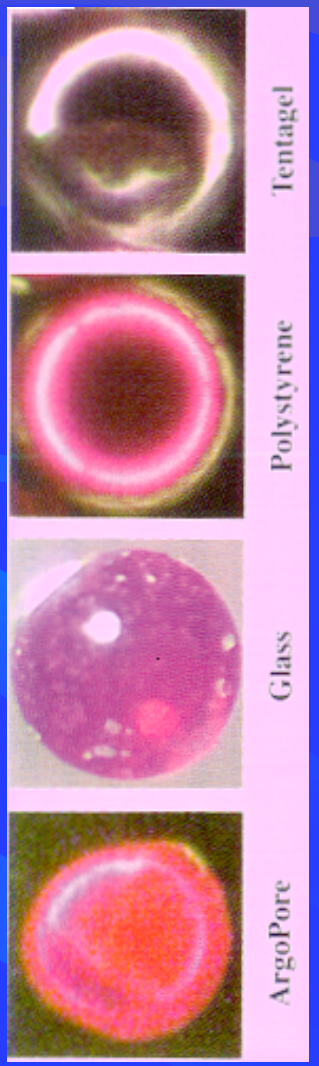
Resin Beads and Active Site Location

- Are sites on resin beads are uniformly distributed on PS, TentaGel and Glass beads?
- Which sites react first?
- Probe with Fluorescence & Raman



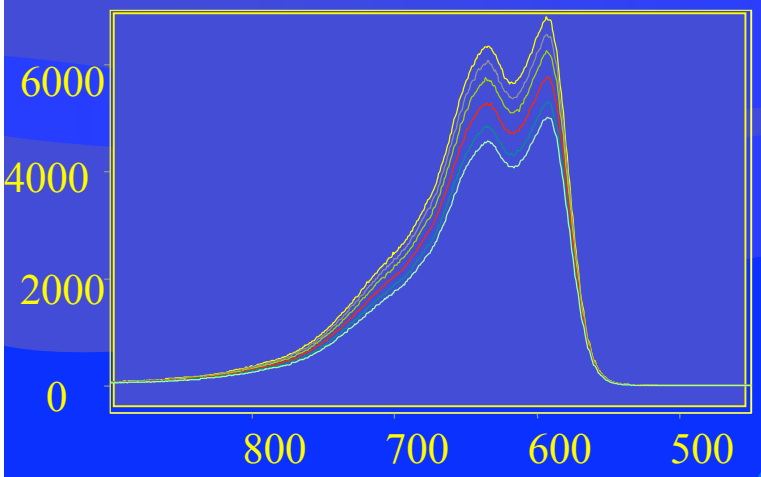
Issues with Fluorescence

- Dyes have large absorption cross-sections as well as high fluorescence quantum yields.
- No. of sites approx 2-400 pmols so the concentration in 100 μm diameter bead is *ca*. 0.3 mol dm^{-3}
- At even moderate loadings there will be significant absorption traversing the bead.

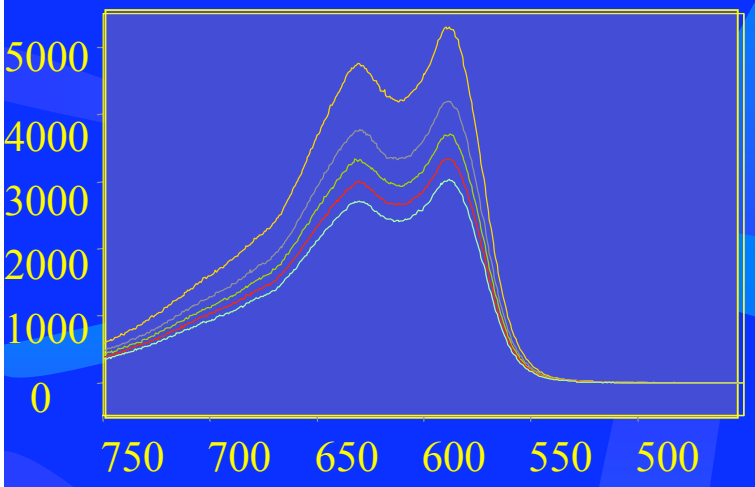


S. R. McAlpine, S. L. Schreiber, *Chem. Eur. J.* **1999**, *5*, 3528

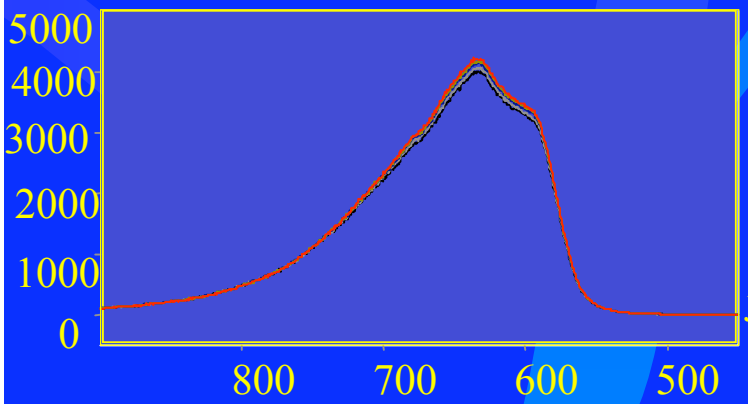
Fluorescent Bleaching and Broadening



PEGA resin, swollen in water, loading 2%, laser power 10%

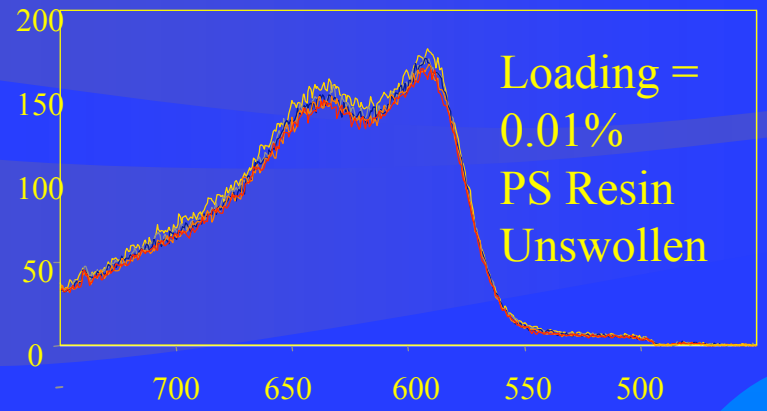


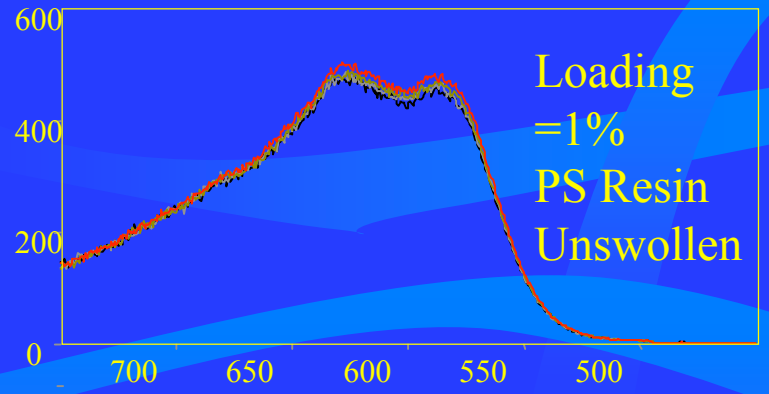
TG resin, swollen in water, loading 1%, laser power 10%

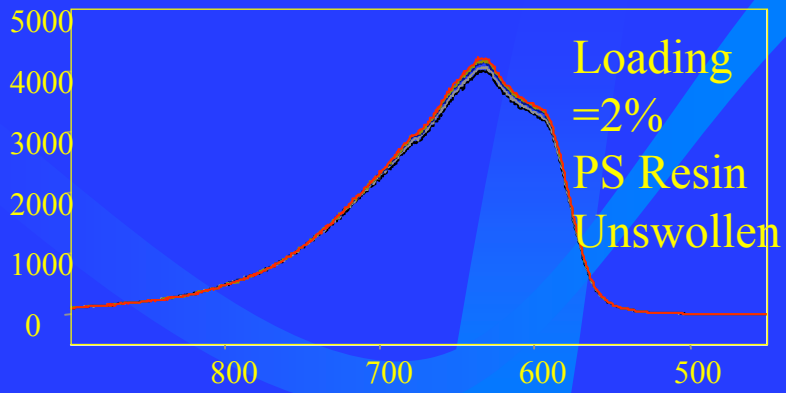


Loading =2% PS
Resin Unswollen

Fluorescence and Line Shape on Resin Beads

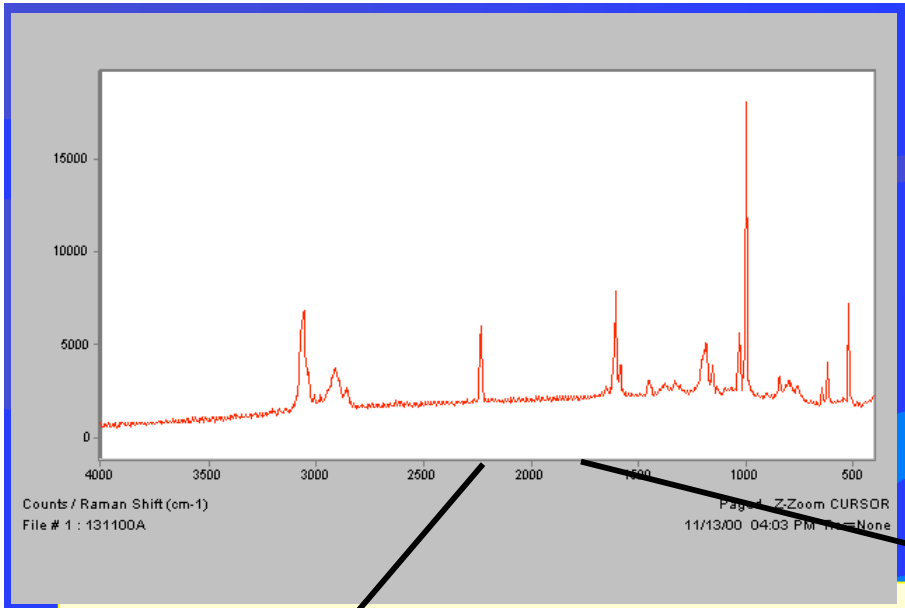




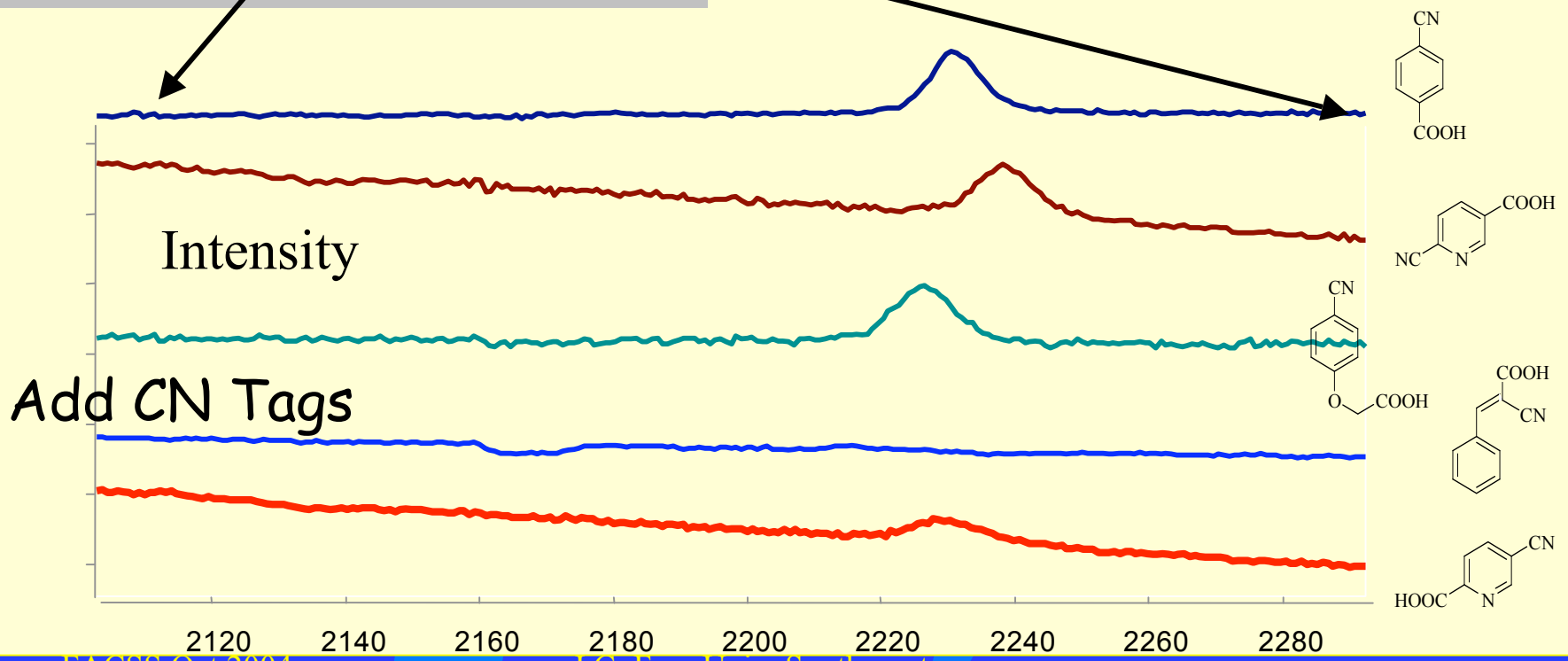


Interactions between dye molecules can be significant

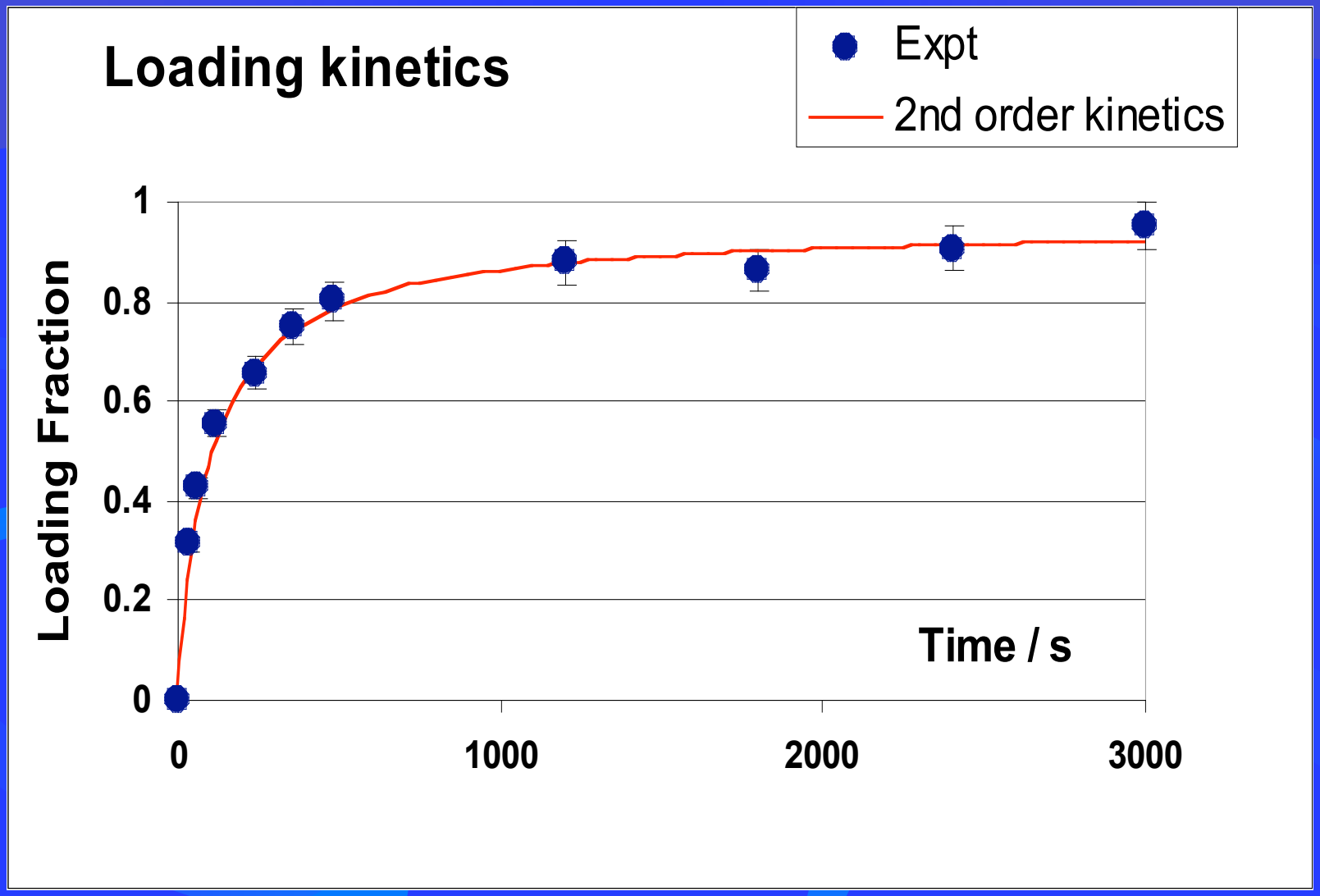
Raman Spectrum of PS Resin



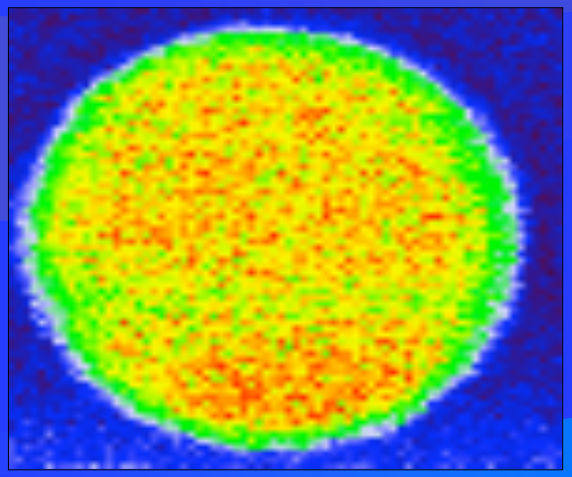
Exact peak position does depend on structure and environment but shifts small

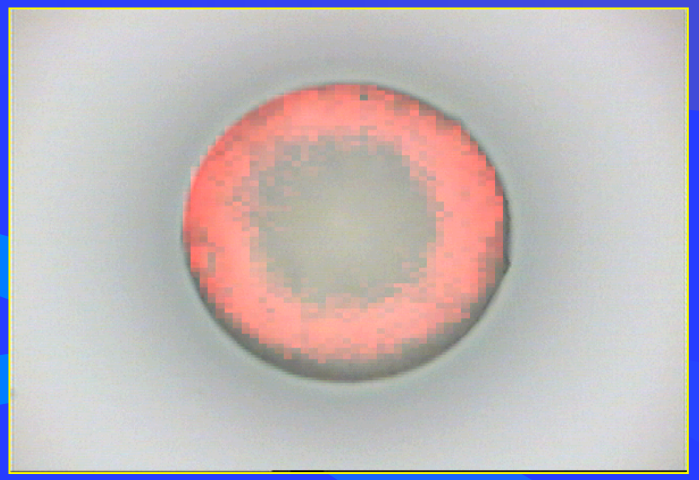


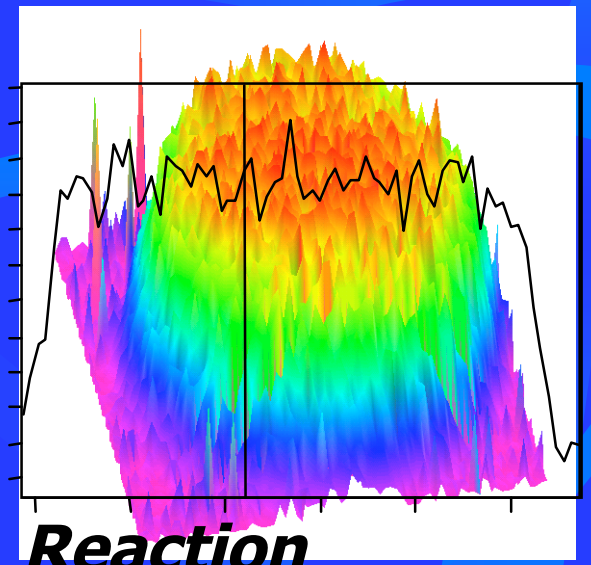
Bead-Site + R-CN → TAG



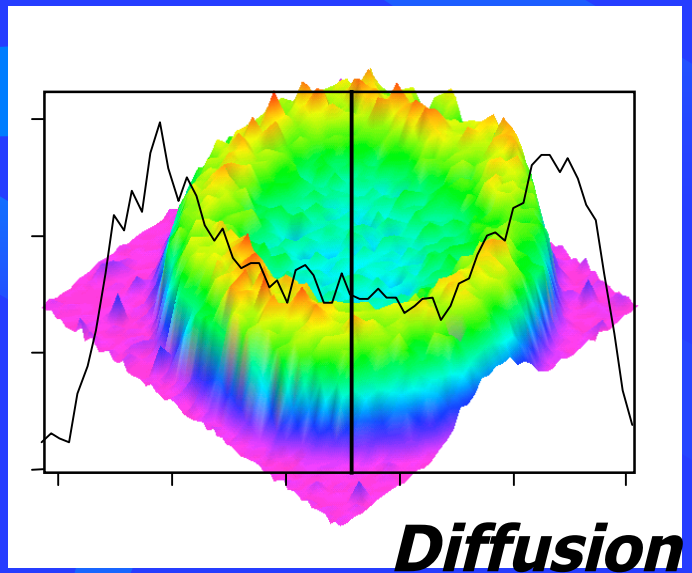
PS Dioxane - 4-cyanobenzoic acid - TentaGel Dioxane





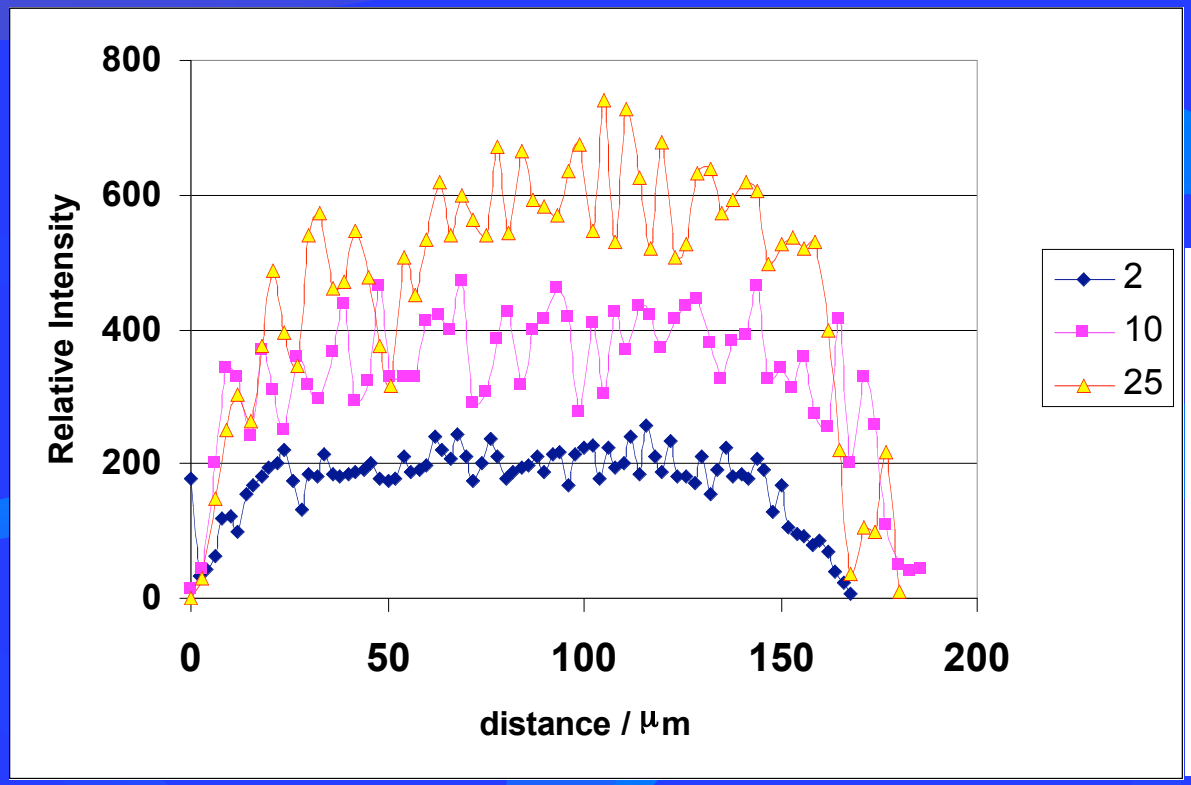


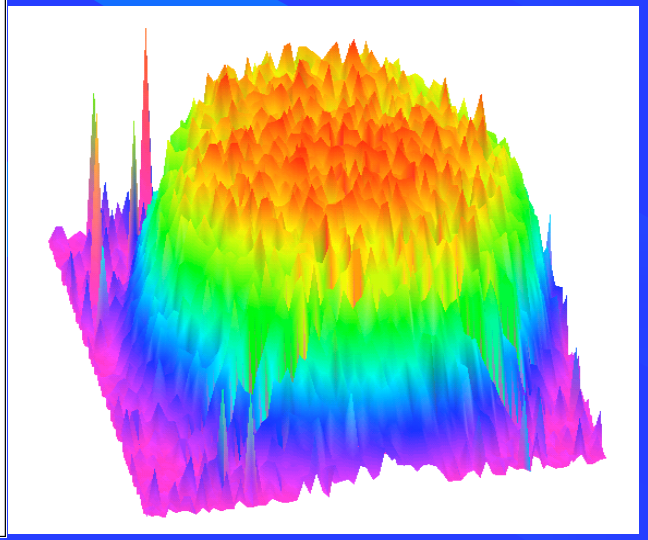
Reaction



Diffusion

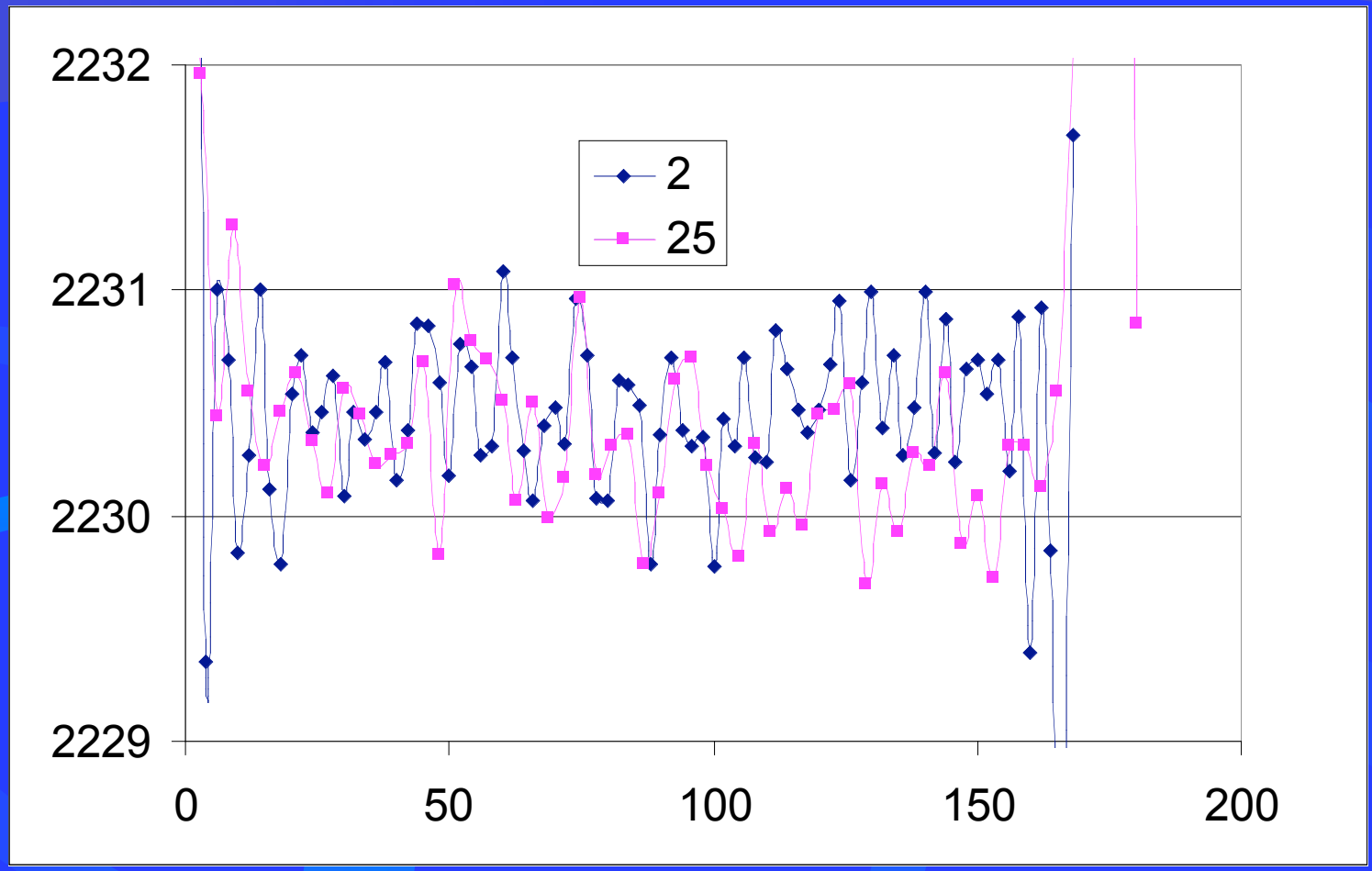
PS in Dioxane
Uniform Distribution - Reaction Slower Than Diffusion





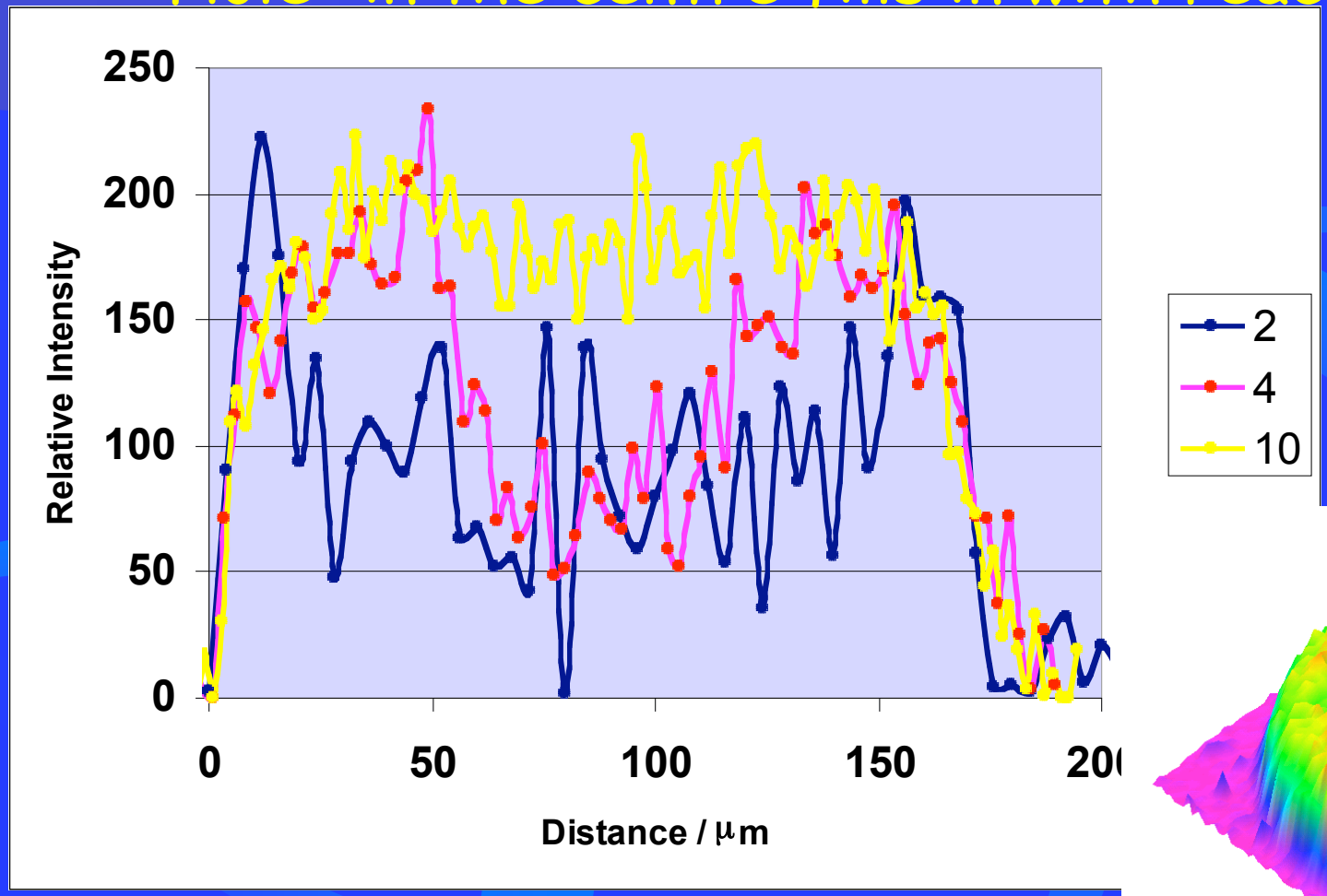
PS in Dioxane

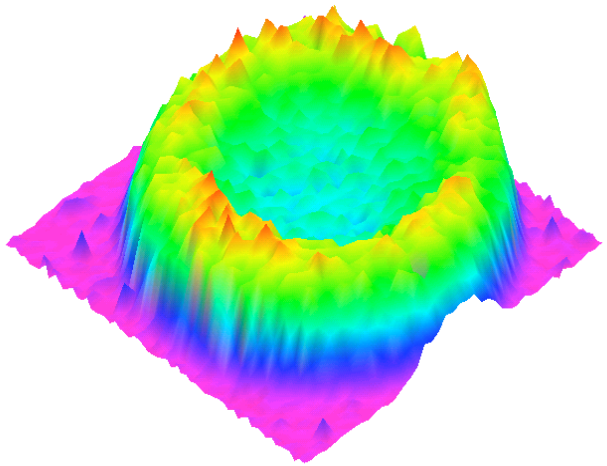
No trend in the CN peak position



PS Bead in DMF

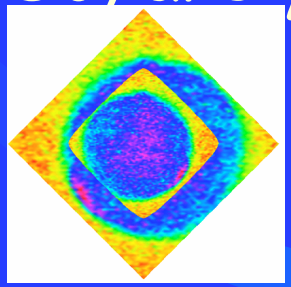
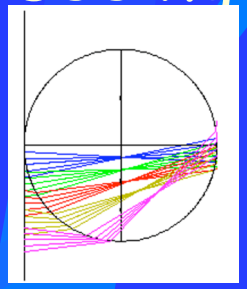
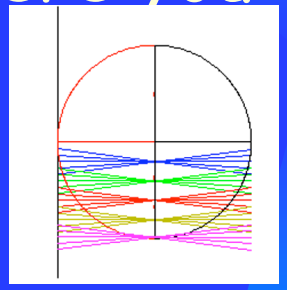
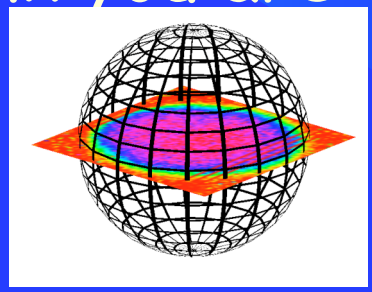
- "Hole" in the centre fills in with reaction time



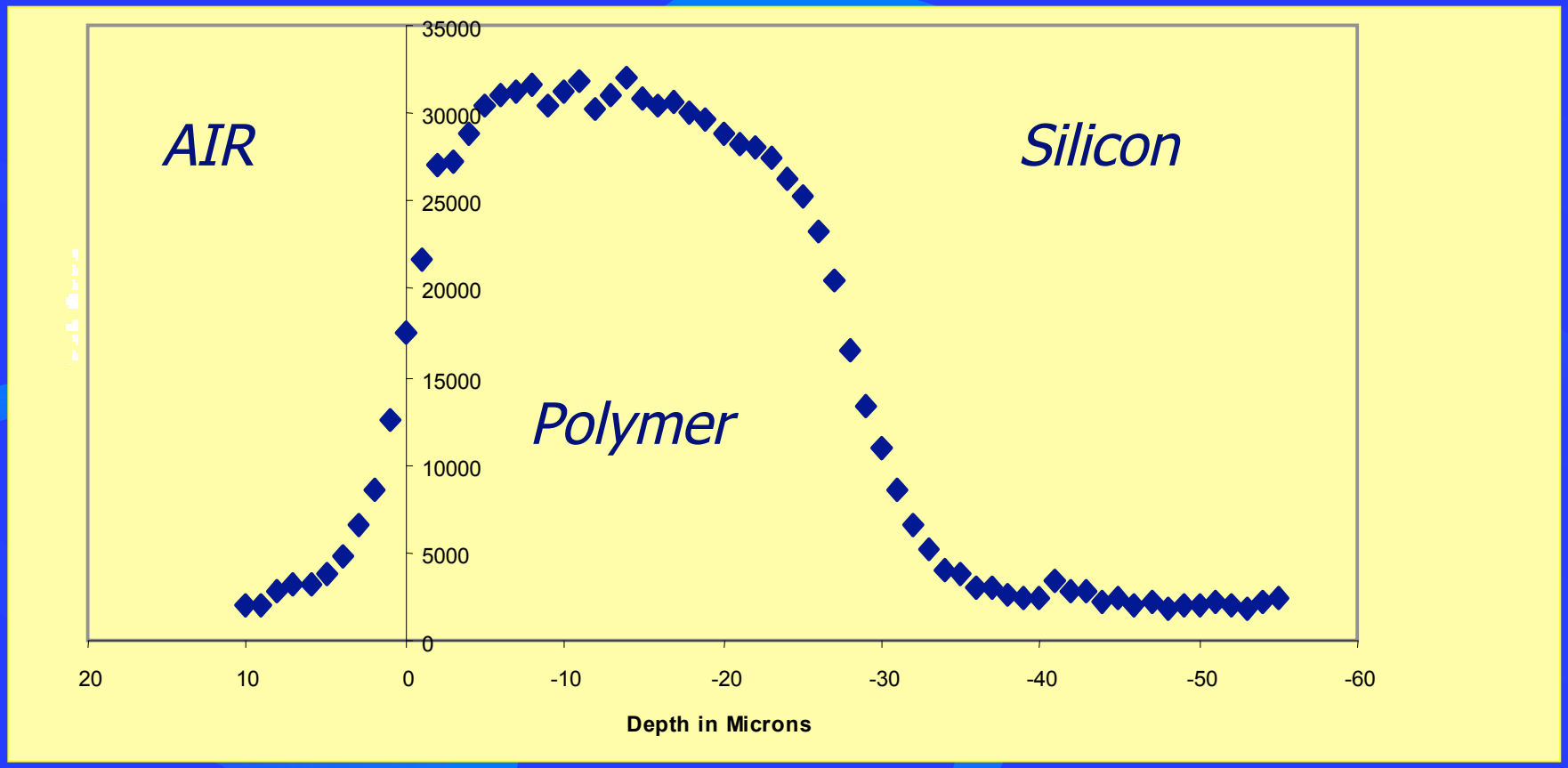


But is what you see what you get?

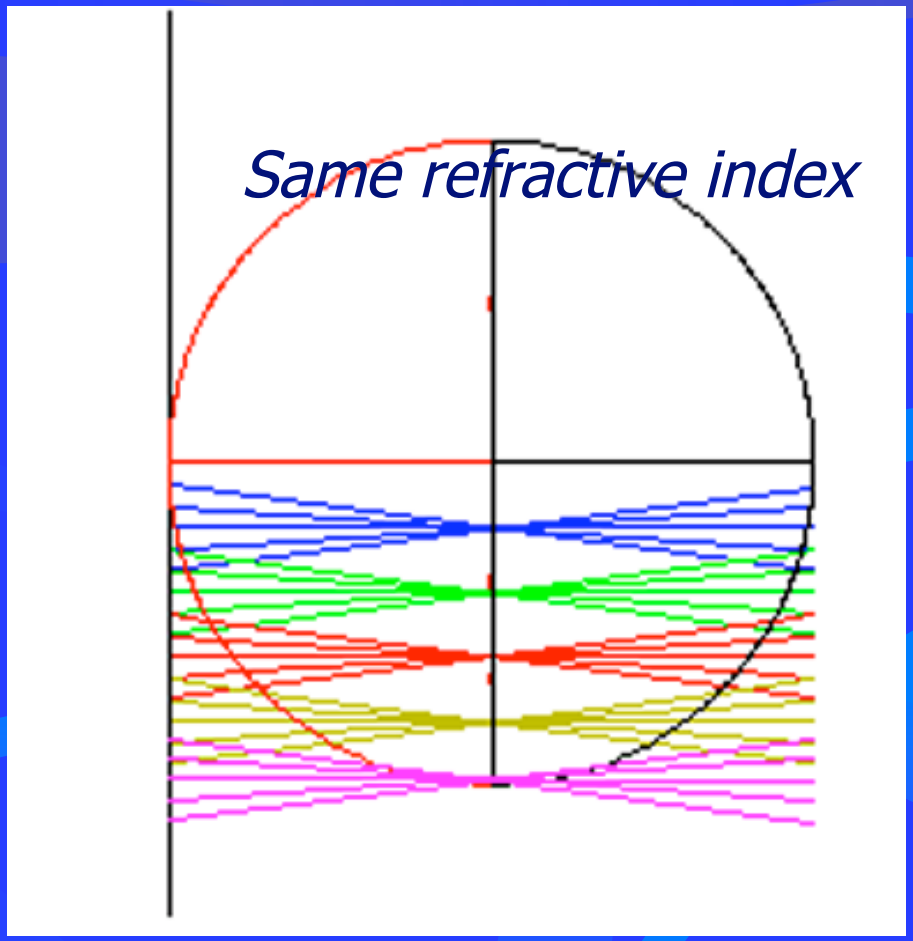
- The spherical surface of the beads act as a lens when the refractive index of the bead differs from the surroundings
- The focal plane moves away from the equatorial plane as the image is scanned across the bead.
- So, are you seeing where you think you are

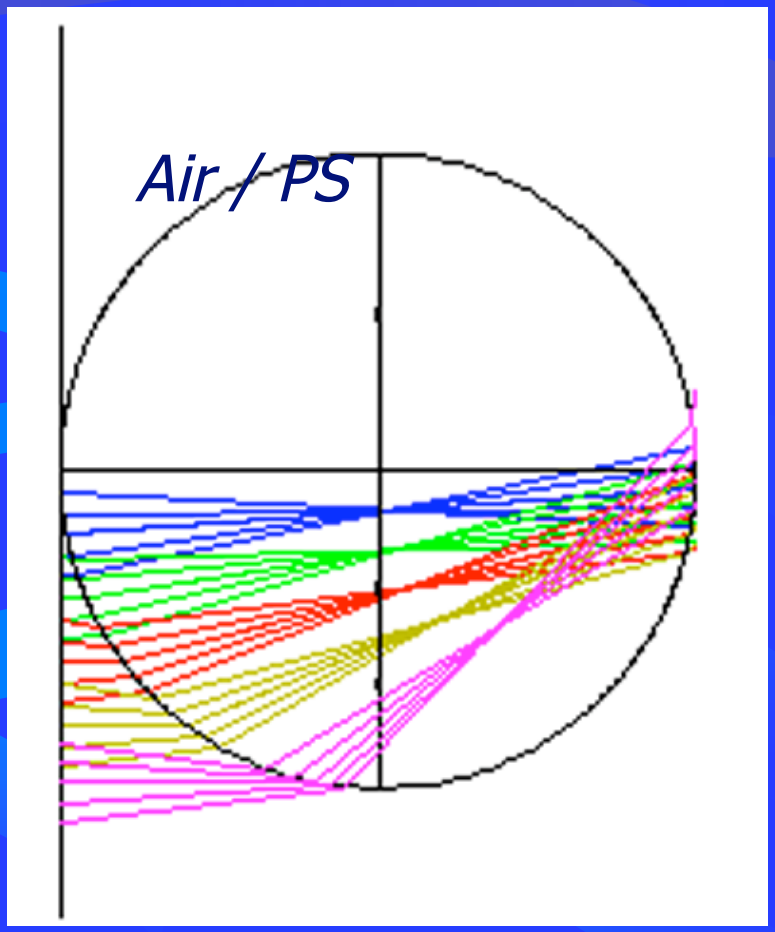





Depth resolution
ca 50 μm flat polymer on Si



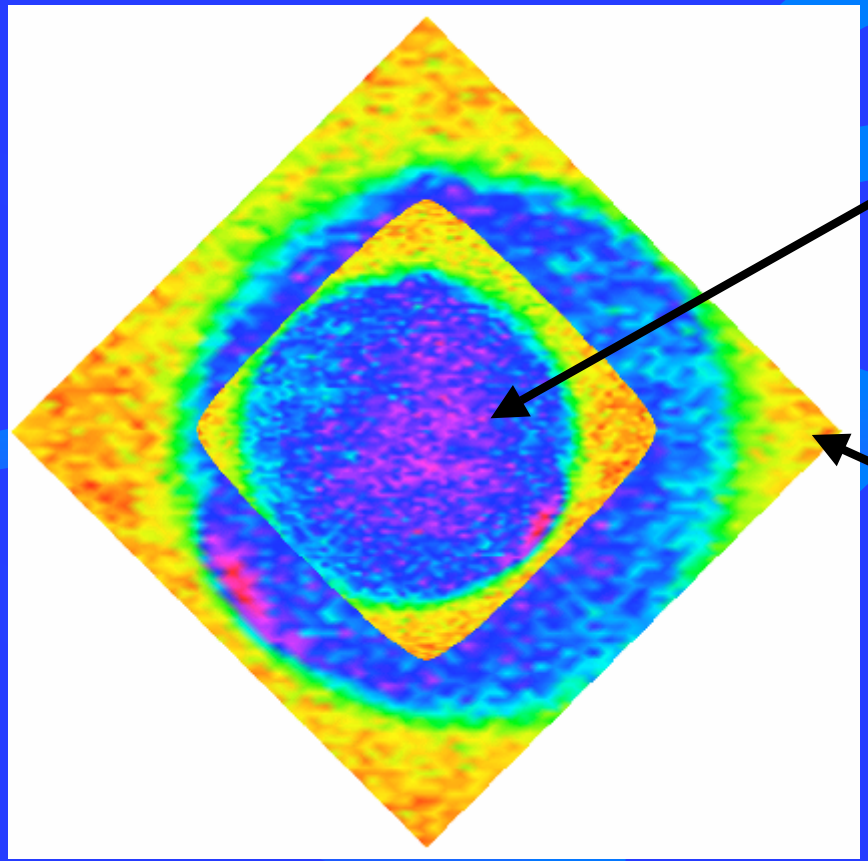
Lens effects

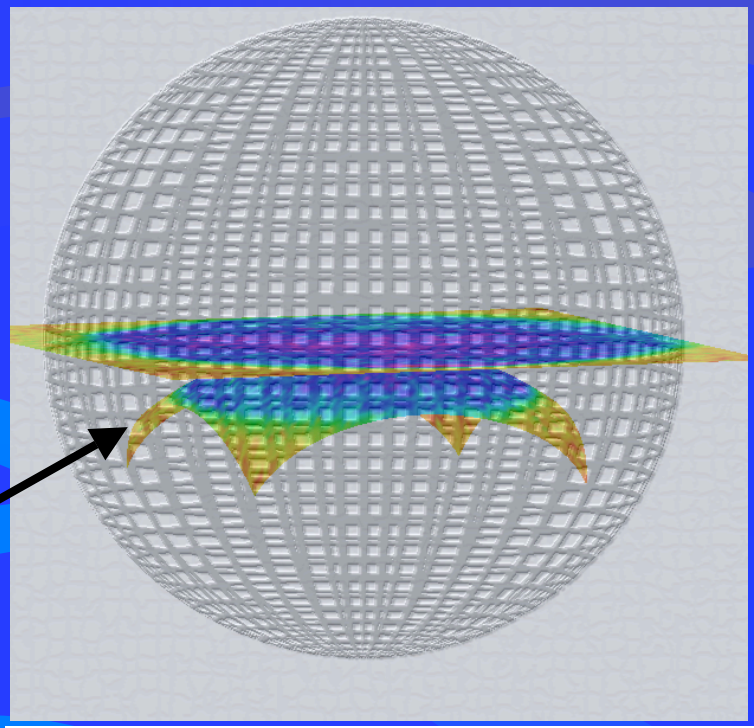


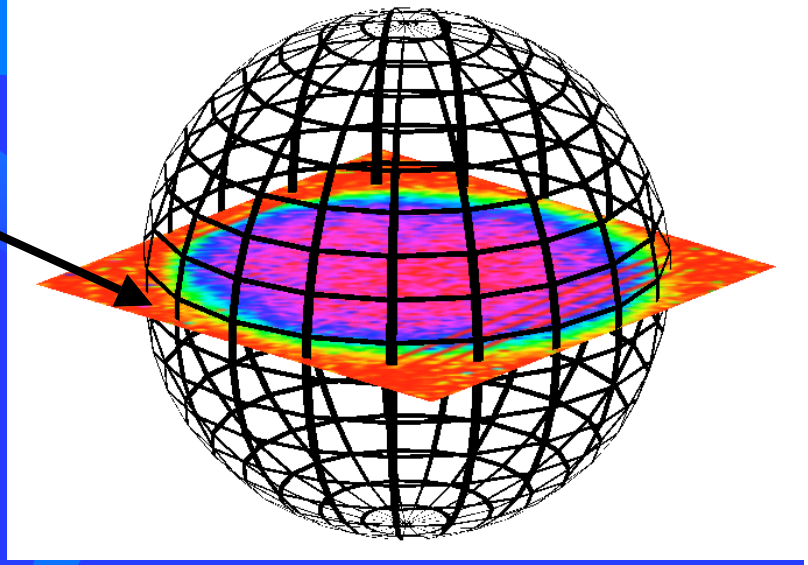


Ray Traces for Different Bead Offsets:
10,20,30,40, 49 μm

Looking from underneath shows how little of the bead is probed by the scan

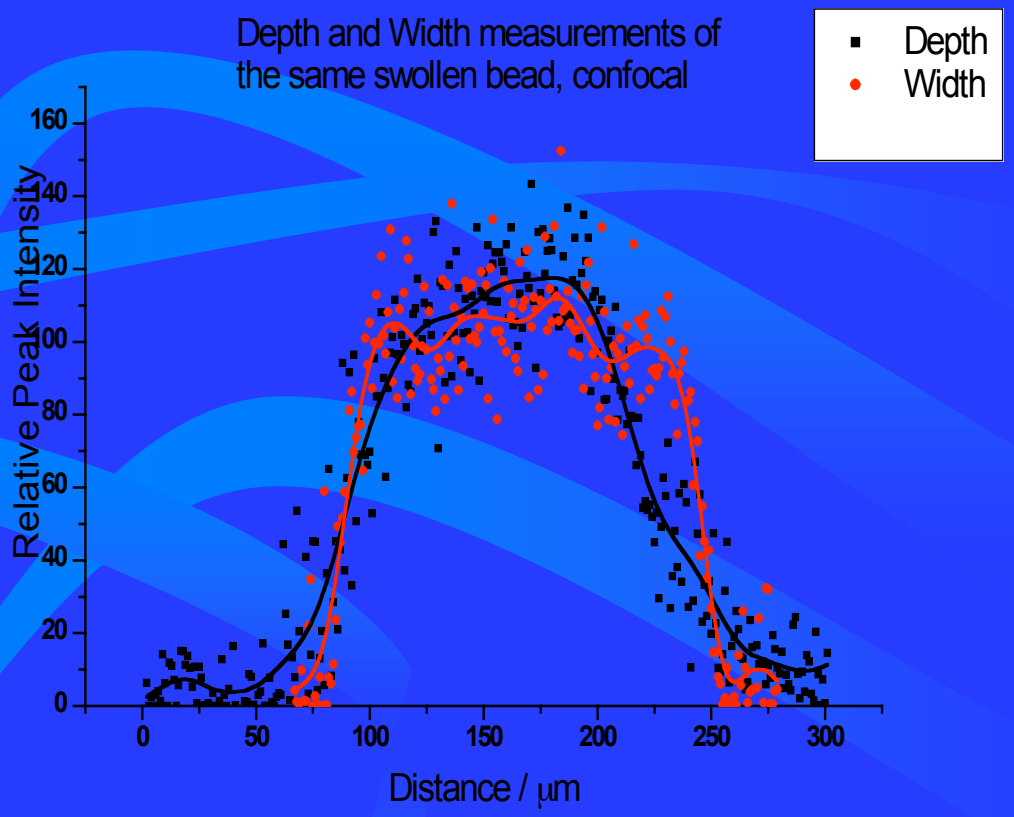




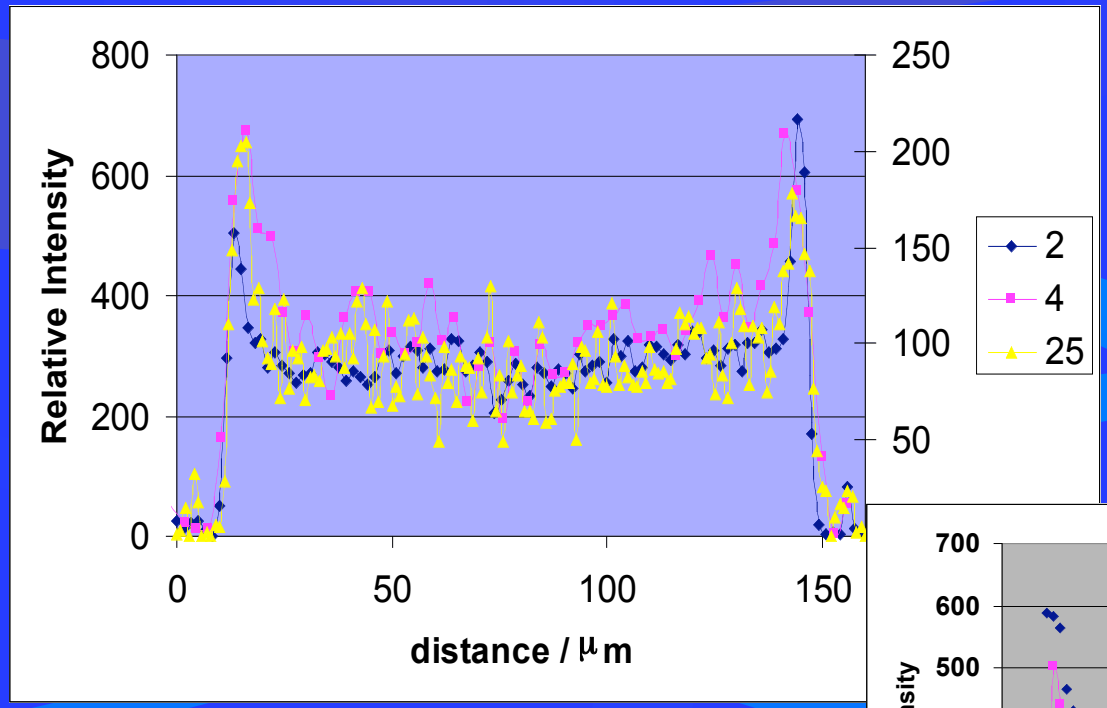


Are Swollen Beads OK?

- Many polymer beads swell in solvents and typically double in diameter
- 7/8 of the bead by volume is solvent
- $n_{swollen} \sim n_{solvent}$

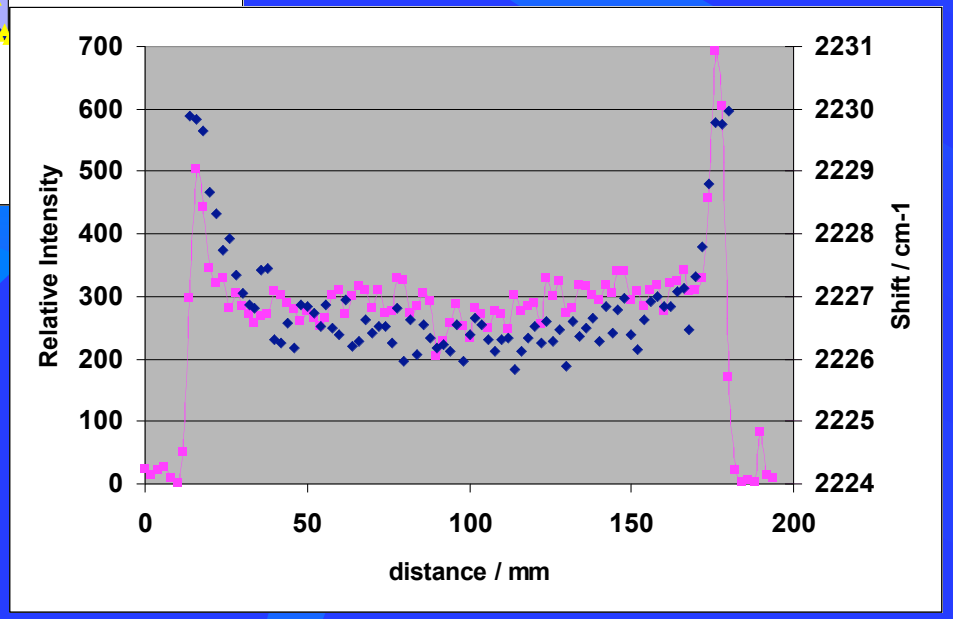


PS Bead in DMF



This suggests the bead environment at the very edge may be different from inside.

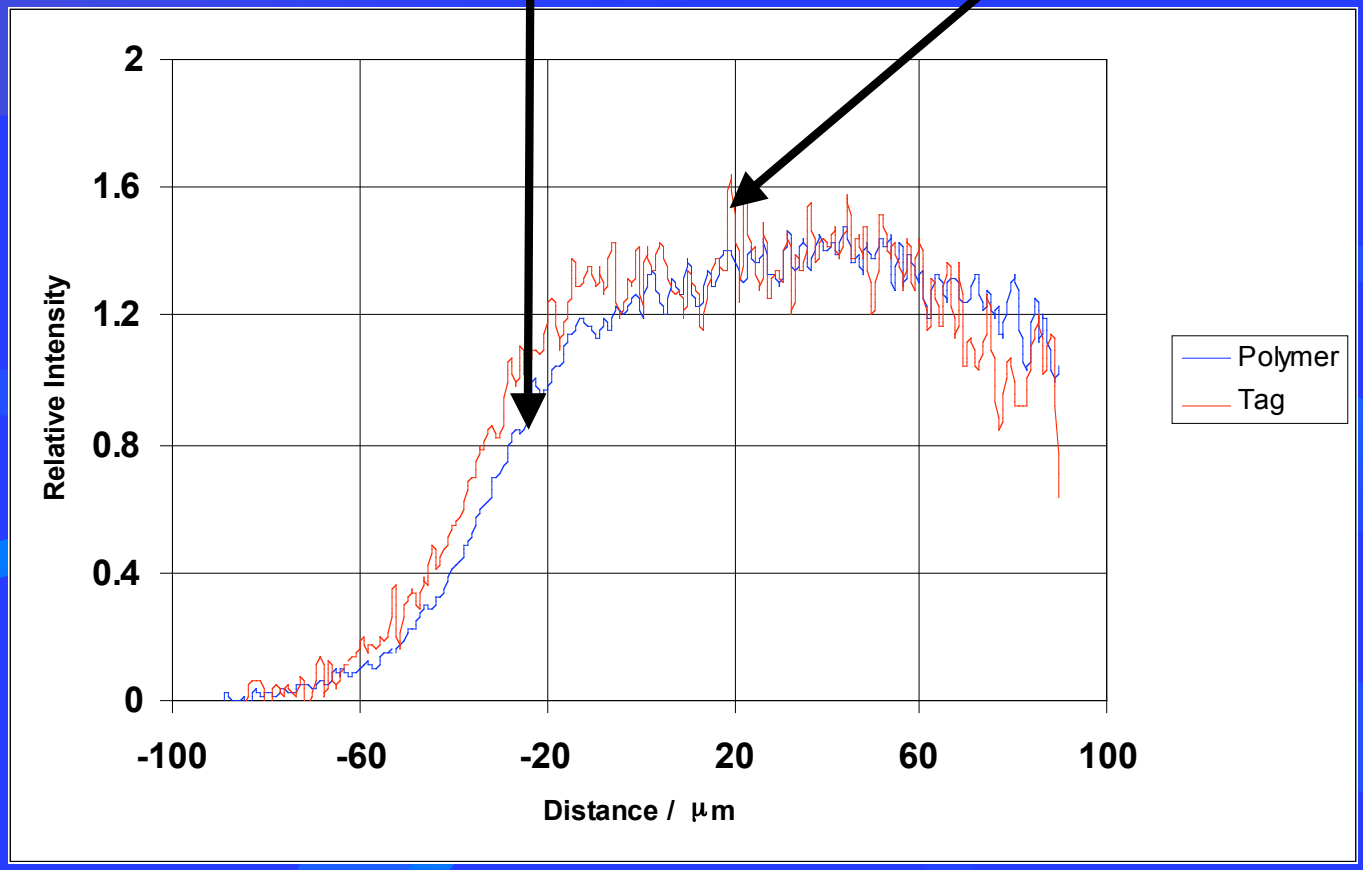
Time / min



Same pattern seen in the CN peak position. & intensity

PS Bead in dioxane

Polymer (blue) and CN Tag Raman (red) peaks



The CN tag seems to have a broader distribution than the polymer – is there a diffuse outer polymer layer?

Accessibility of solid supports

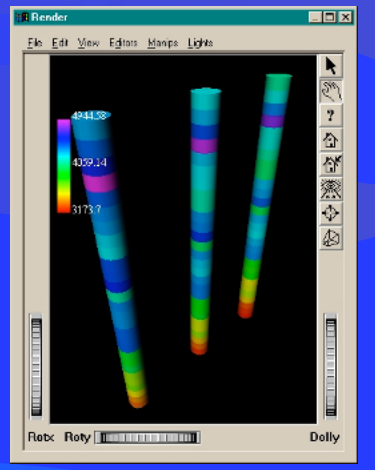
Beaded materials were loaded with the peptide 4-cyanobenzoic acid-Gly-Pro-Leu-Gly-Leu-Phe-Ala-Arg-OH, incubated with the enzyme and the CN Raman peak monitored

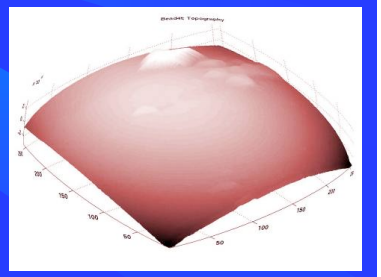
	MMP 12	Thermolysin	MMP 13	Clostridium Collagenase	NEP
kDa	22	35	42.5	68	90
TG	no cleavage	no cleavage	no cleavage	no cleavage	no cleavage
PEGA	cleavage	cleavage	no cleavage	no cleavage	no cleavage
CPG 155	cleavage	cleavage	cleavage	cleavage	cleavage

Raman Spectroscopy of Beads

- Raman spectroscopy provides a very useful probe of the spatial distribution of reactive sites within a polymer bead.
- The kinetics of reactions at these sites can be observed.
- The spectra are sensitive to the nature of the environment around these sites.

Beads





- Uniform distribution of reactive sites throughout the beads.
- The spatial distribution of reacted sites depends on the polymer type and solvent (and reaction time).
- Balance between reaction rate and diffusion rate.

Acknowledgements

- *Southampton*
  - Jurgen Kress, Riccardo Zanaletti, Abigail Rose, Mark Bradley, William Brocklesby, Helen Stanford, Lu Shin
- *GlaxoSmithKline*
  - Mark Ladlow, Geoff Mellor, Malcolm Skingle
- *Funding & Support*
  - *EPSRC, Univ. Southampton, CCE, Renishaw*