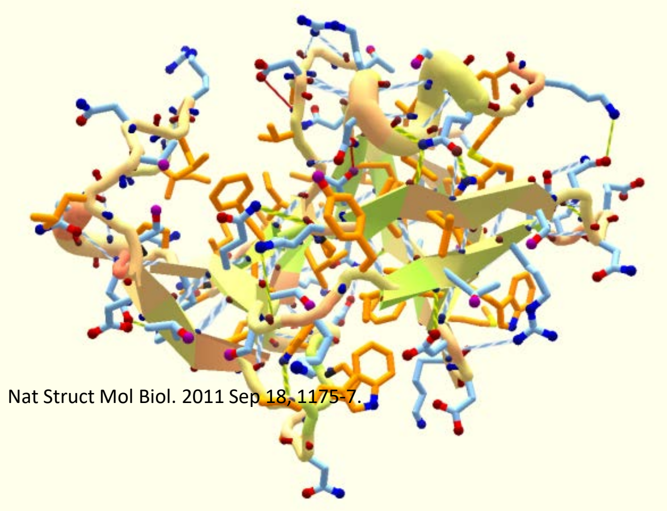
Future of fluorescent nanodiamonds (and phosphor) for bio-sensing

Philip Hemmer – Texas A&M University

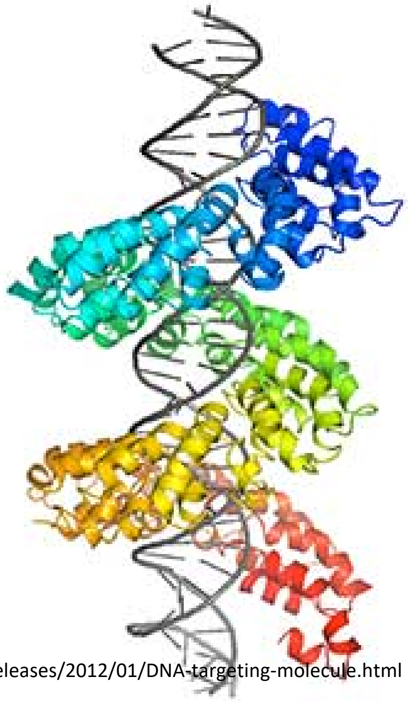
Technical Areas of Expertise/Interests: Bio-sensing applications of ultra-small non-bleaching fluorescent tags, especially diamond and phosphors. Novel diamond growth techniques. Solid materials for quantum optics, Sub-wavelength imaging, Single molecule imaging, Fluorescent color centers in diamond and oxide crystals. Nanoscale magnetometers based on nitrogen-vacancy diamond, Ultrasound modulated optical tomography, Nano-fabrication of surface plasmon structures. Quantum computing and storage in solid materials, Quantum communication and teleportation in with solid materials, Sensitive chemical/biological agent detection using quantum coherence, Ultraslow and stopped light in solids, Materials and techniques for resonant nonlinear optics, Phase-conjugate-based turbulence aberration compensation, Spectral holeburning materials and techniques, Holographic optical memory materials, Smart pixels devices, Optical correlators, Photorefractive applications, Laser trapping and cooling, Atomic clocks.

Long term goal

- Imaging of **single** bio-molecules **in live cells**
  - New level of understanding of life processes
  - Cure numerous diseases
    - Cancer, neural degeneration, ageing, pandemics
- Example -- Proteins
  - Structure determines function
  - Most structures not known
  - Known structures in purified form
  - **Not in live environment**



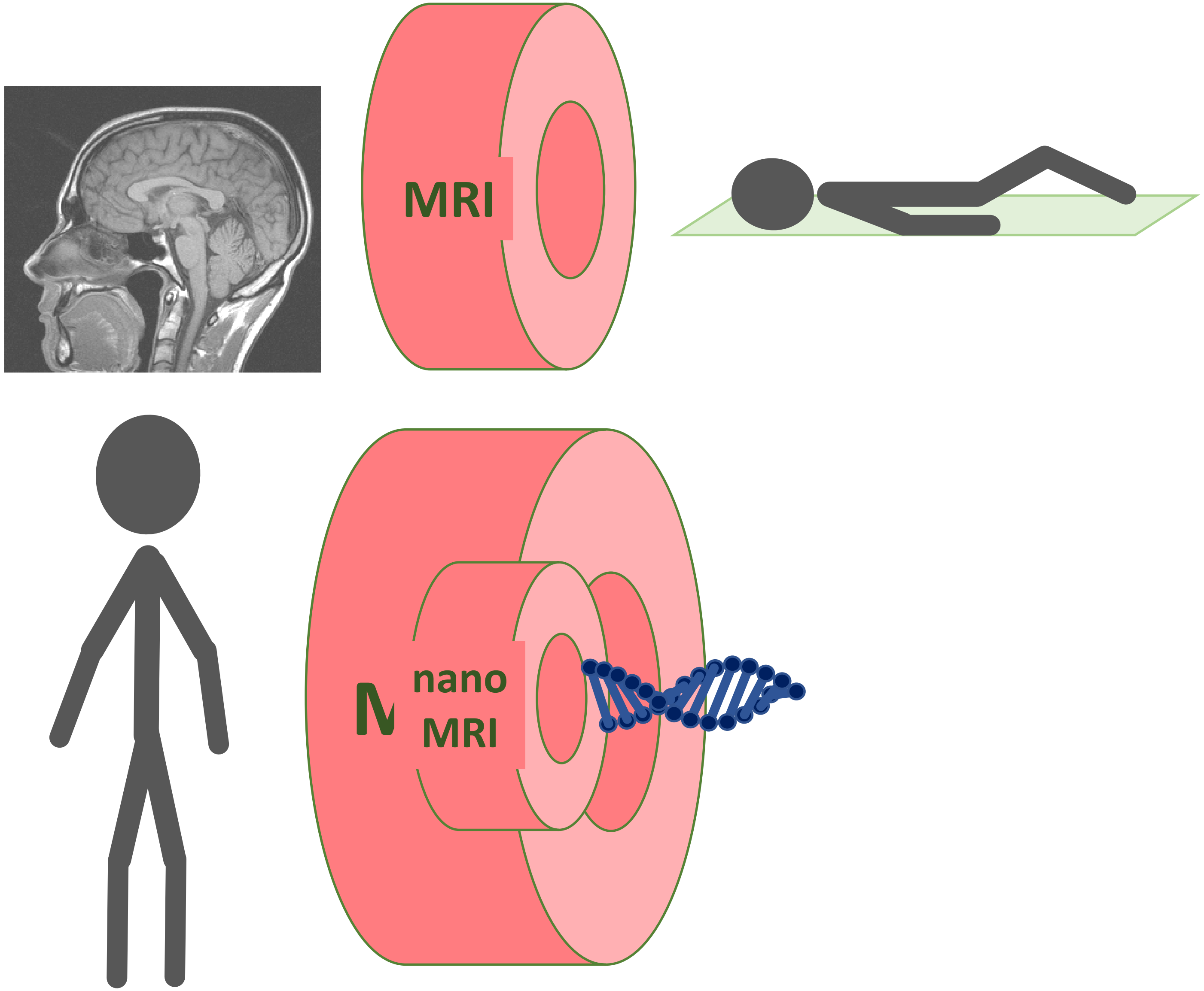
Nat Struct Mol Biol. 2011 Sep 18, 1175-7.



http://www.fhcrc.org/en/news/releases/2012/01/DNA-targeting-molecule.html

Adapt MRI to single molecules ?

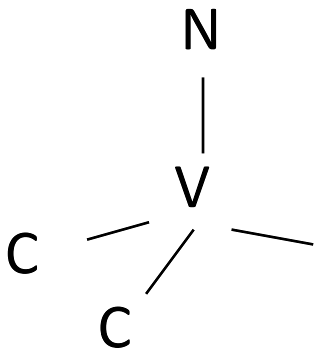
- MRI is ultimate super-resolution
  - All others only approximate
- Key ingredients to image (nuclear) spins
  - Polarize – use really big magnet
  - Distinguish – apply magnetic gradient
  - Detect – Spins radiate RF fields –
    - **Weak link**
- Look for optical detection alternative



First serious proposal in 2002:
"Proposed work
NMR-like imaging of single molecules"
"…A lower-risk choice for room temperature operation is to use single molecule microwave Raman NMR imaging. This is because spin lifetimes tend to depend only weakly on temperature, as mentioned earlier. Microwave Raman dark resonances have already been observed in NV diamond…"

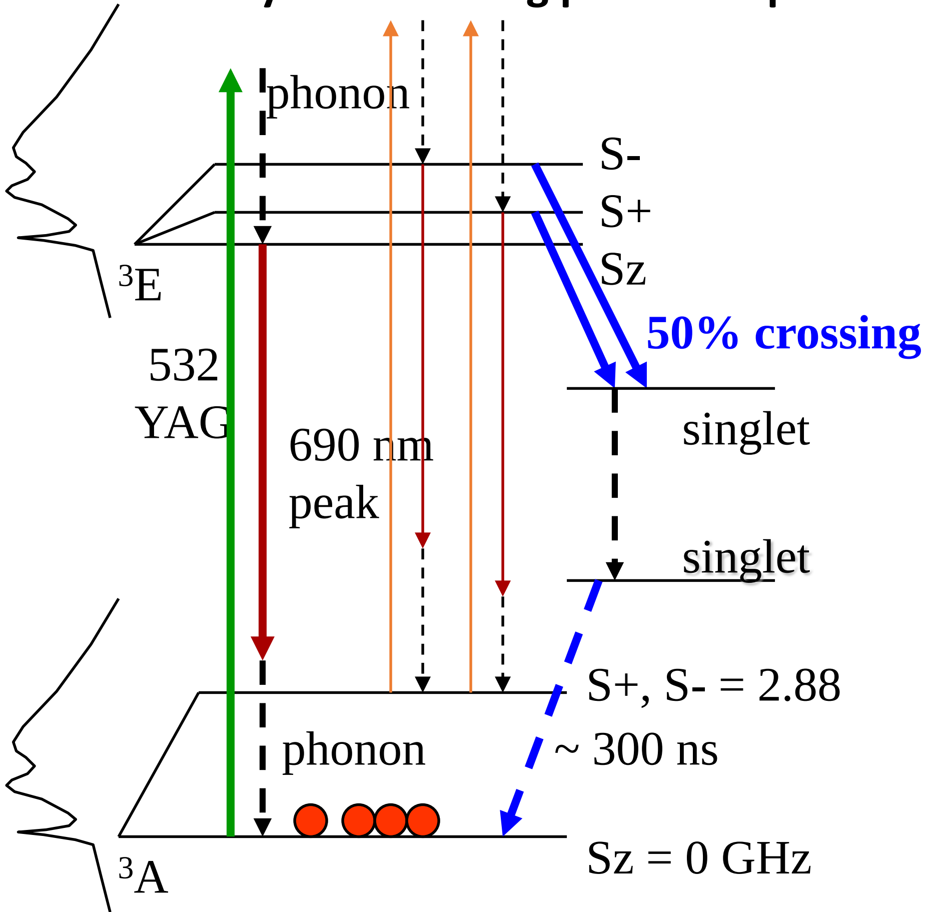
Nitrogen-vacancy (NV) diamond – a single spin you can see

- Shine flashlight on NV diamond at room temperature
  - 80% spin polarization
- Spin readout = 30% suppressed fluorescence for m=+/-1 states
  - Minimum you need



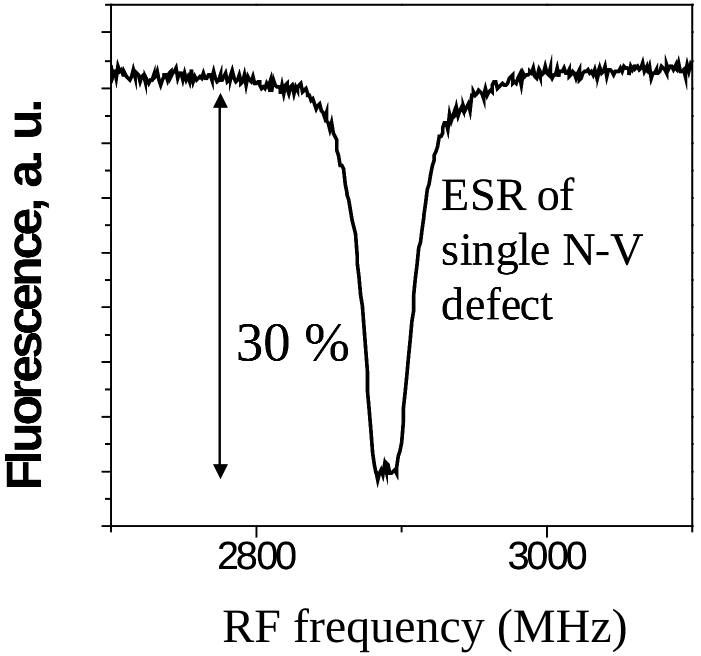
Nitrogen-vacancy (NV)

Intersystem crossing polarizes spins



Key is high spin contrast on readout

Single spins at room temp

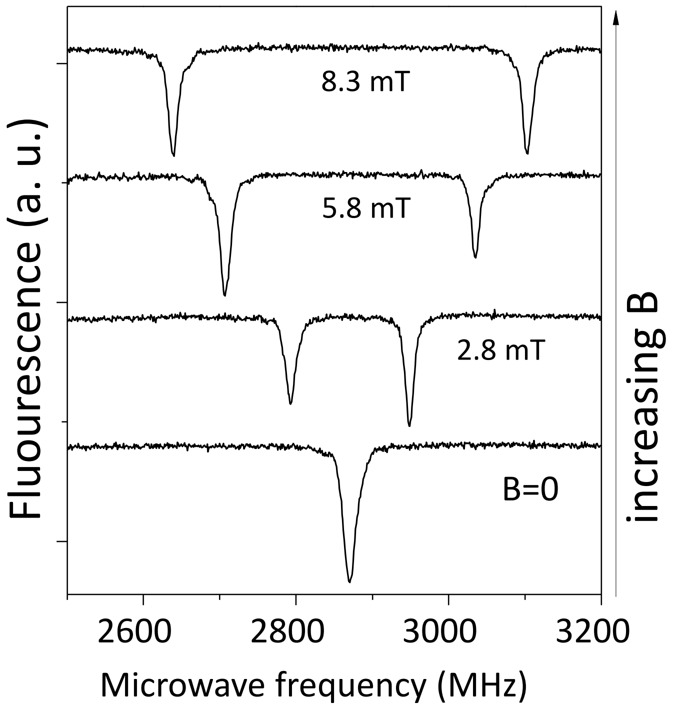


Nizovtsev et al, Optics and Spectrosocopy, 94, 848, (2003)

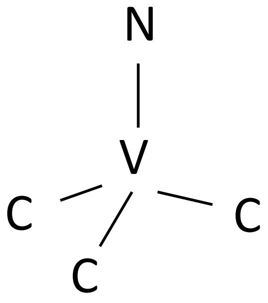
NV diamond nano-magnetometry

- Zeeman shift is similar to atomic vapor
  - Narrow room temperature spin transition
    - kHz to MHz depending on purity
  - doi:10.1038/nature07278, doi:10.1038/nphys1075

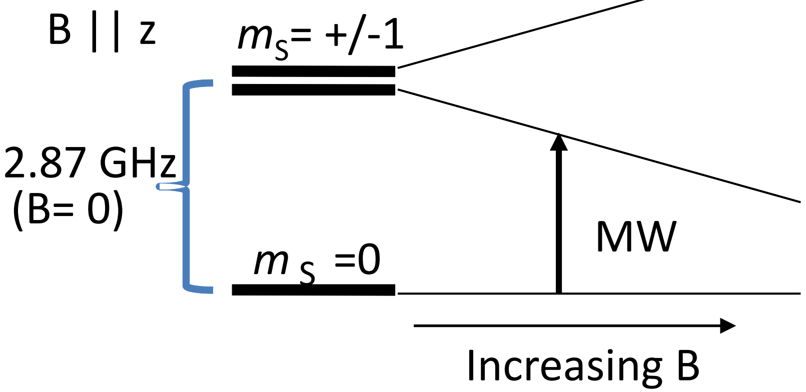
Magnetometry via CW ESR



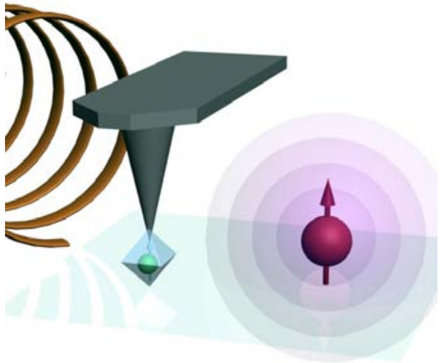
Nitrogen-vacancy (NV)



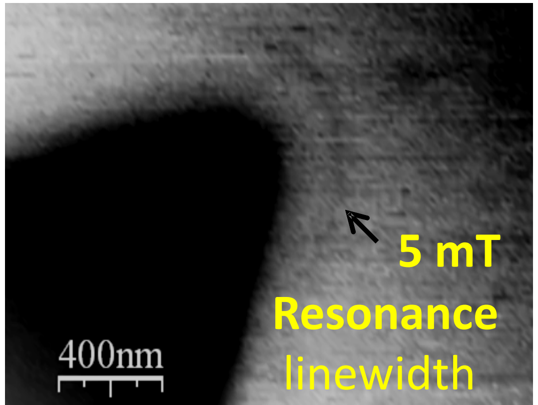
NV magnetic Zeeman shifts

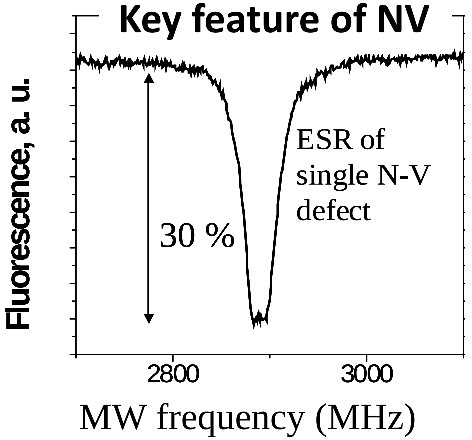


Scanning probe NV magnetometer



Field of nickel micromagnet





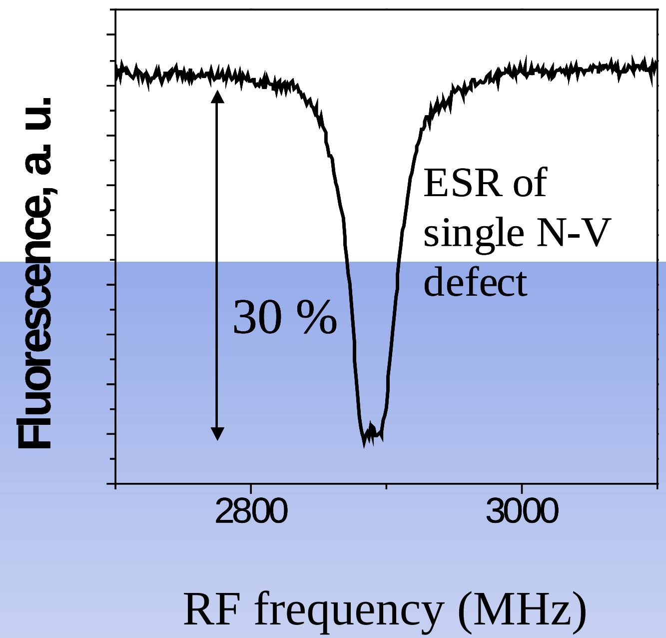
Nizovtsev et al, Optics and Spectroscopy, 94, 848, (2003)

Probing single dark spins via backaction on NV

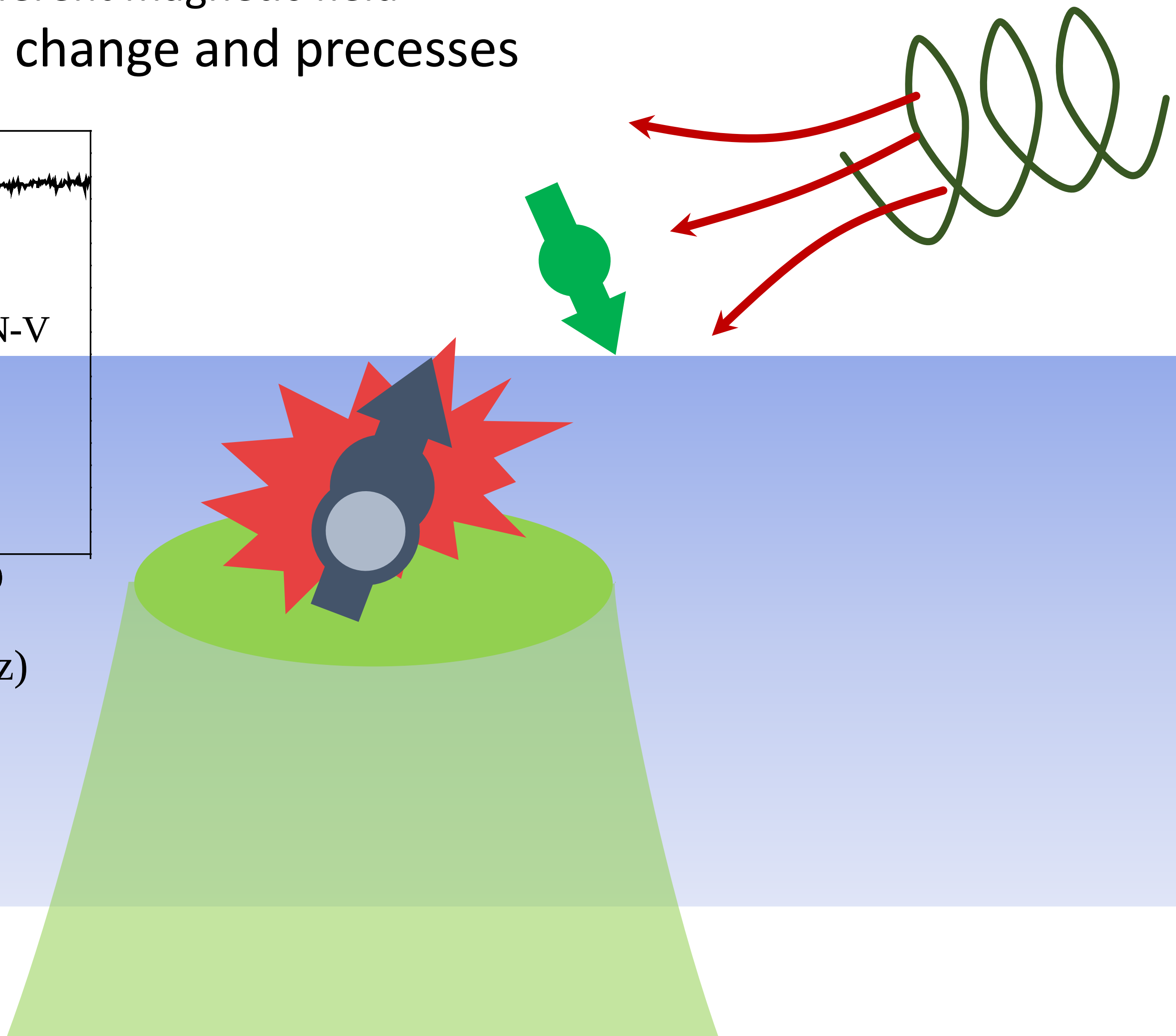
Dark spin is flipped by resonant MW or RF

- Generates different magnetic field

NV senses field change and precesses



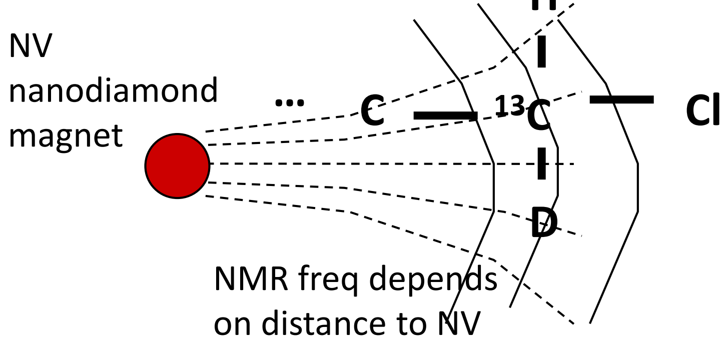
Nizovtsev et al, Optics and Spectrosocopy, 94, 848, (2003)

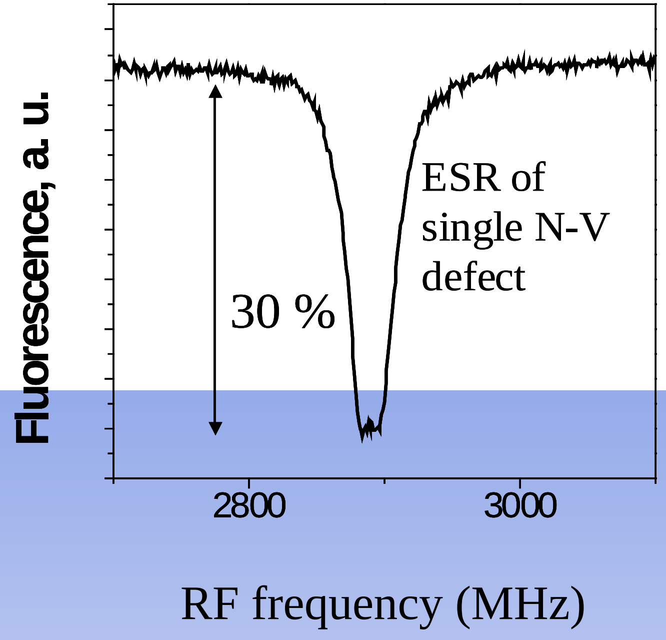


NV diamond as complete nano-MRI

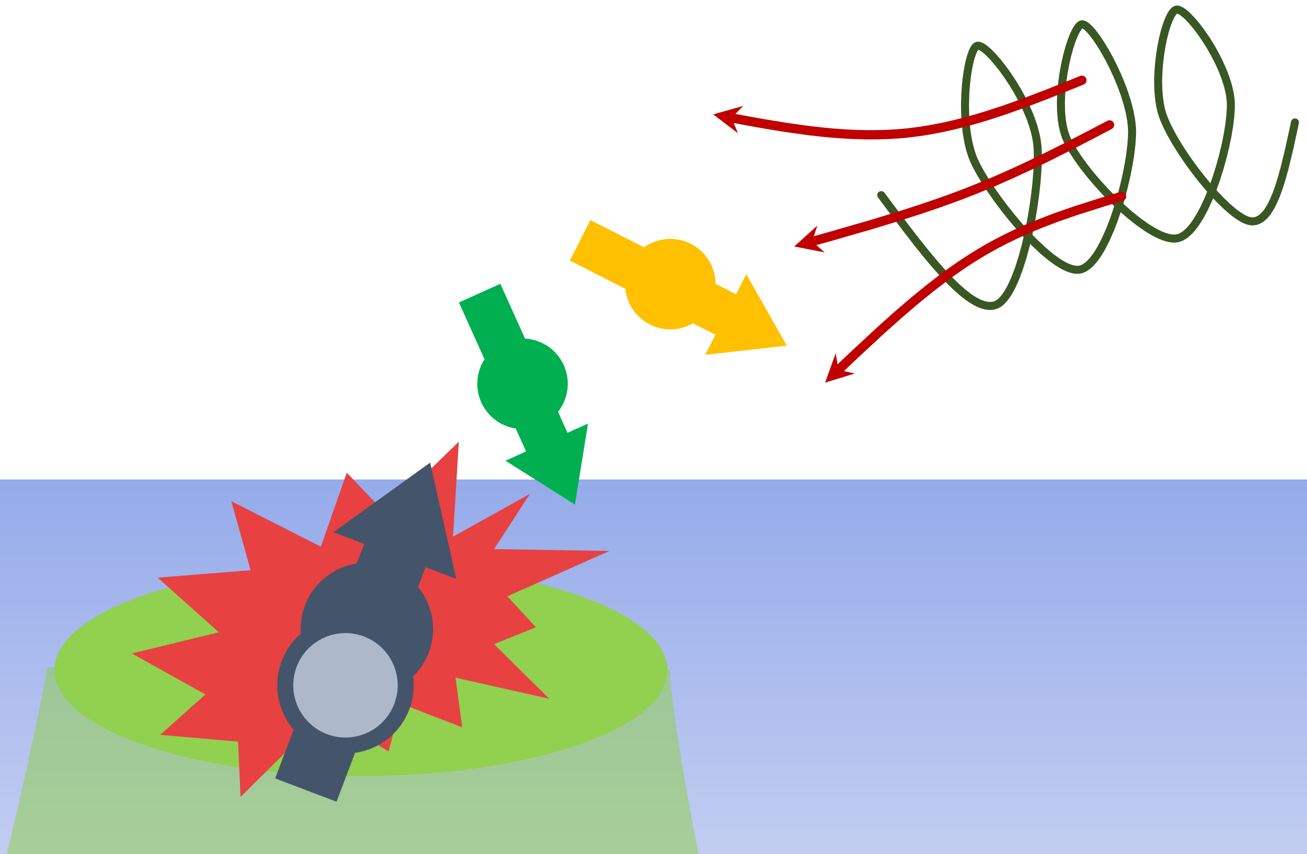
- Smallest magnet is single spin
  - NV has advantage -- can polarize target spin
  - Readout via backaction → hyperfine interaction
- Perform MRI of nearby nuclei in NV gradient

NV magnetic for gradient





Nizovtsev et al, Optics and Spectrosocopy, 94, 848, (2003)



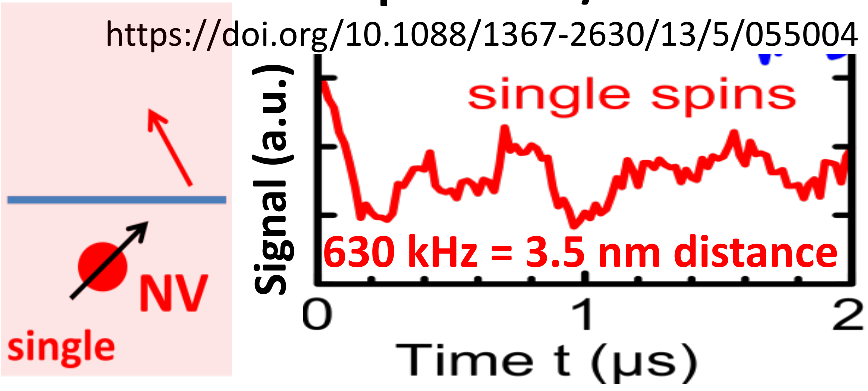
Protein imaging ?

Toward Molecular-Scale MRI, Feb 1, 2013, SCIENCE 339 (6119), 529-530
Single proteins under a diamond spotlight, MAR 6 2015, SCIENCE 347, 1071-1072

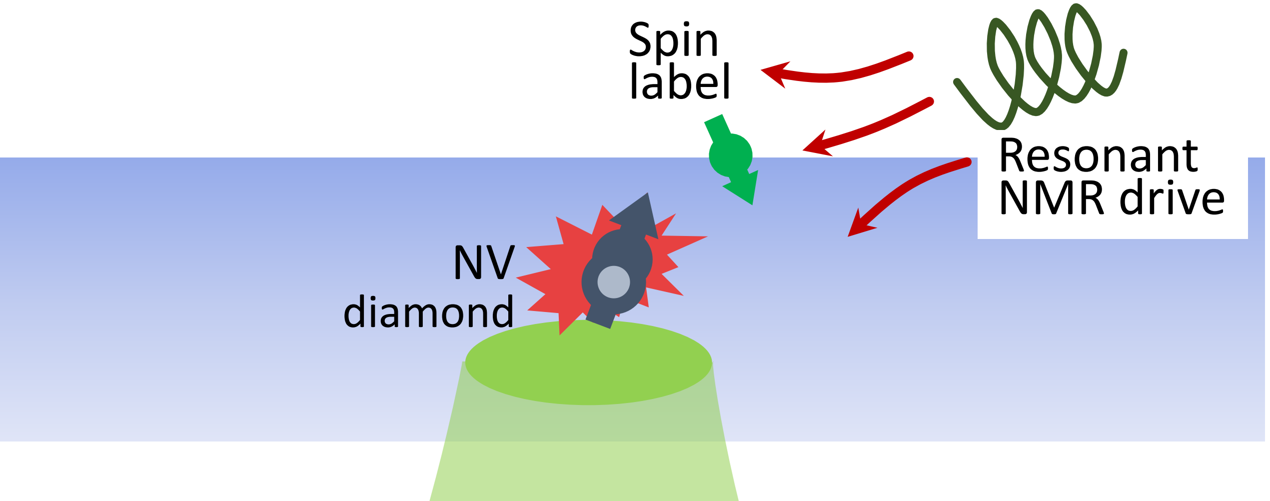
- Imaging of **single** bio-molecules **in live cells**
  - New level of understanding of life processes
  - Cure numerous diseases
    - Cancer, ageing, pandemics

First experiment/result

https://doi.org/10.1088/1367-2630/13/5/055004



Drive spin labels & detect w/ NV

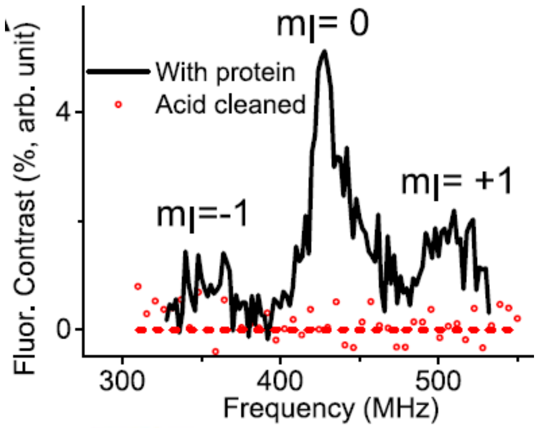


Recent experiment in Hefei

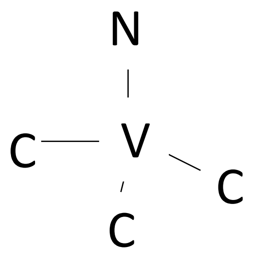
Univ. Science & Tech. China, Hefei /Stuttgart

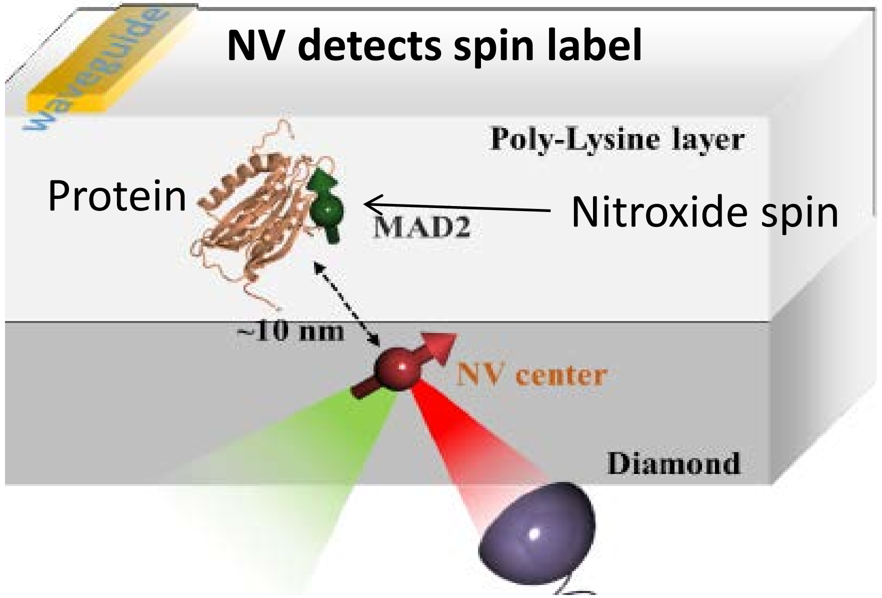
DEER signal

Protein conformations modify spectral sideband



NV diamond

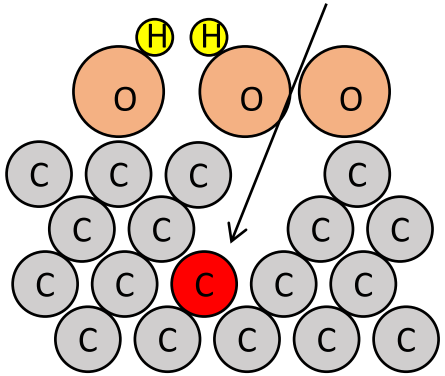




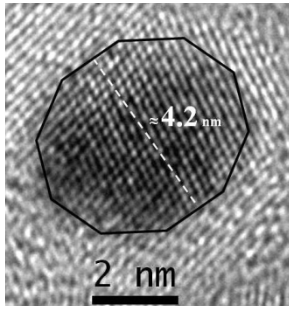
Diamond color center wish list

- Precise placement of atoms
- No spin or charge traps
  - No bulk defects except desired color center
- Charge compensation
  - P or N type, independent of color center
- Arbitrarily complex color center (NV, SiV, N3, NE8)

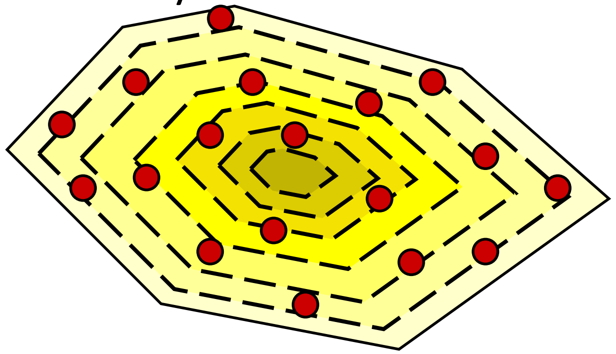
Sub-surface radical



- Ultrasmall nanodiamonds 1- 8 nm
  - Cell membranes 3-5nm
  - Kidneys of mammals <7nm
- Minimum one color center per diamond
  - No matter how small

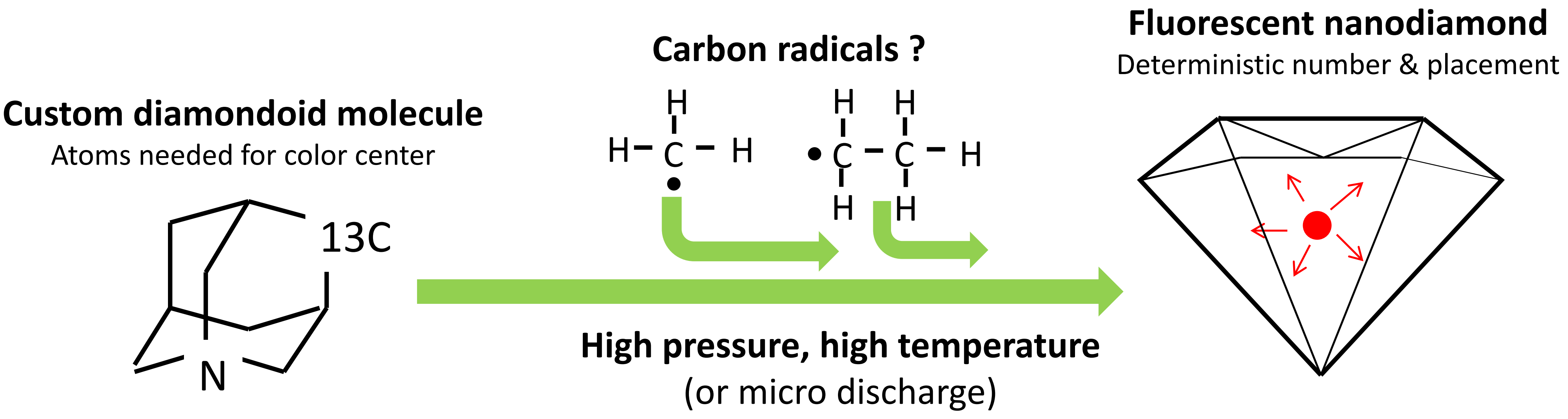


Probability of color center vs size



Designing diamond color centers

- Revolutionary bottom-up fabrication approach
  - Use diamond-like molecules for seeds
  - Cross between crystal growth and chemical synthesis



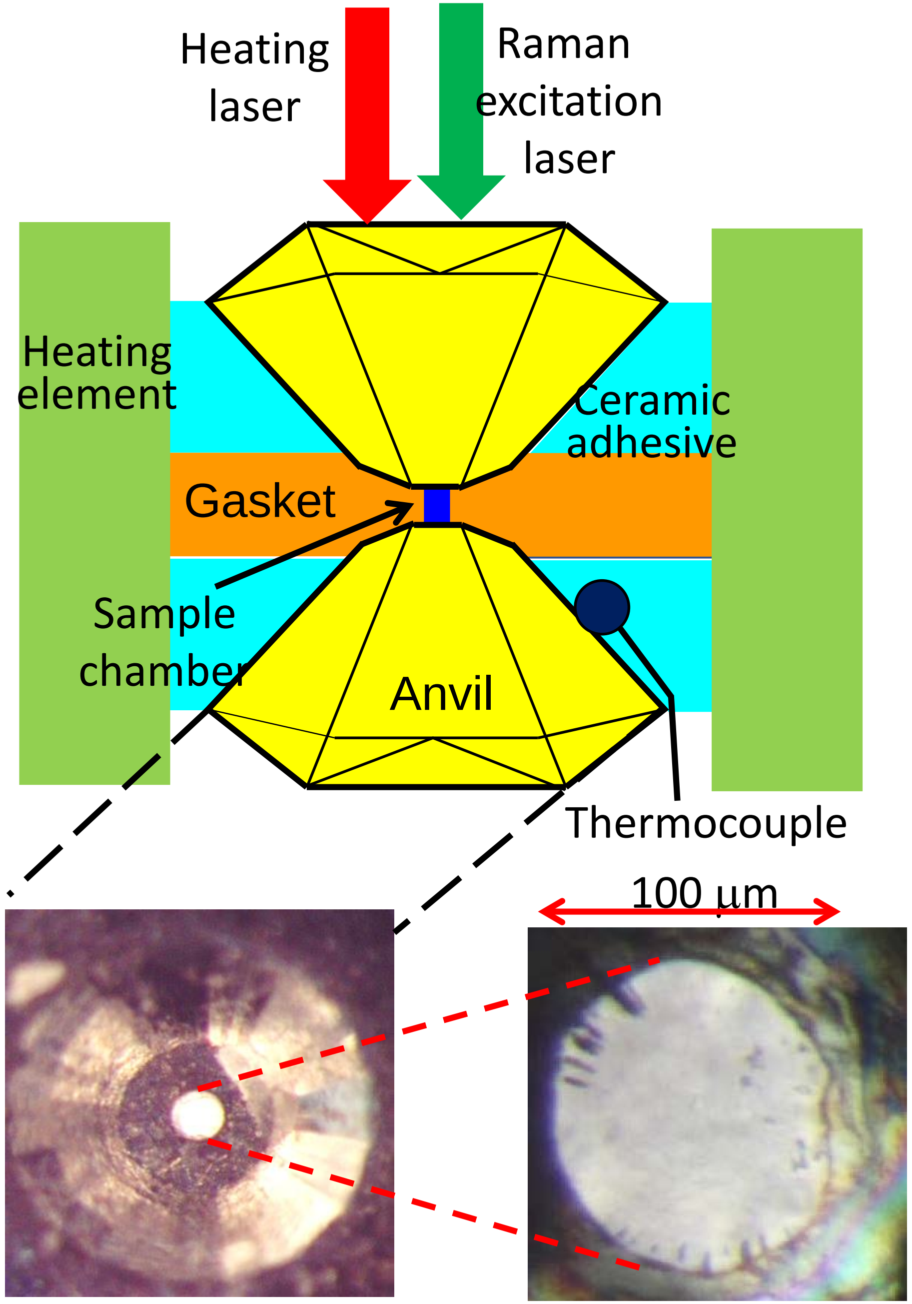
HPHT diamond growth

- Most of world’s diamonds made by HPHT
  - Perceived as difficult – need expensive facilities

Advantages of diamond anvil growth

- Ability to see growth in real time
  - Ultra-high pressure >60 GPa
  - Ultra-high temperature
  - Laser or resistive heating
  - Raman and X-ray diagnostics

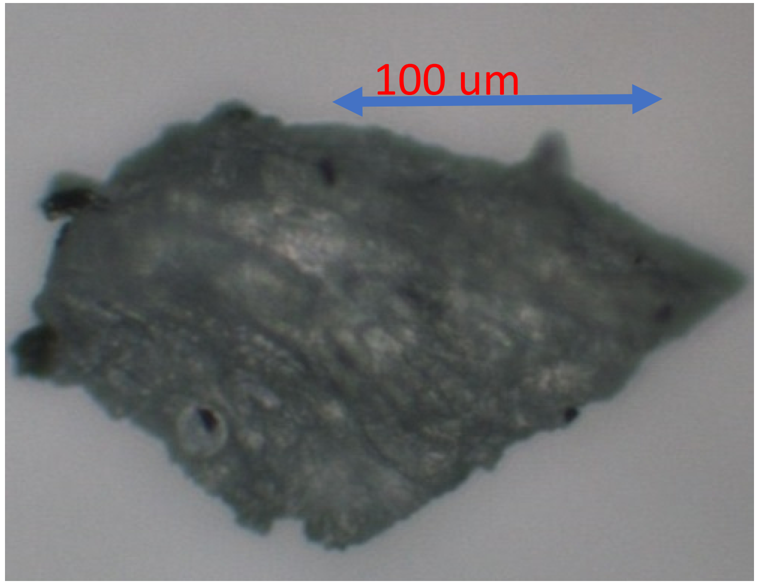
- Disadvantage
  - Small quantities
  - Can scale up w/ large presses



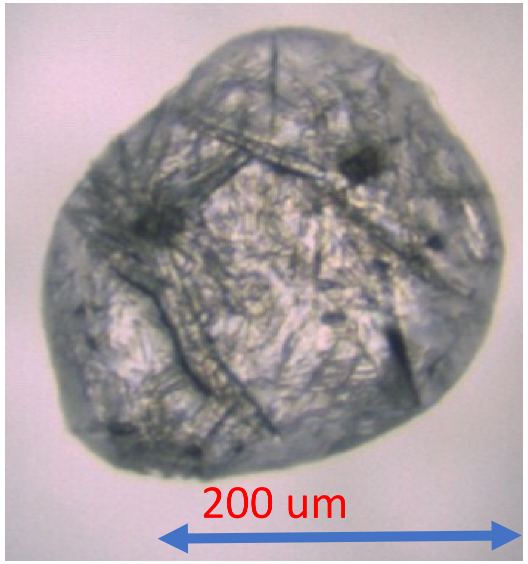
Typical low temperature growth products

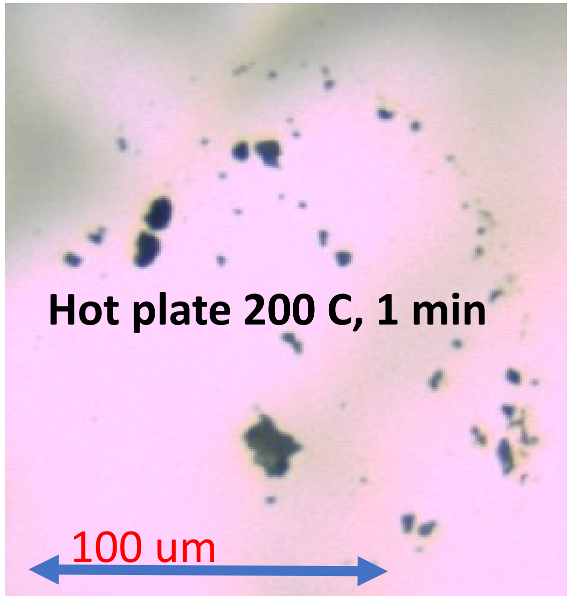
- Tetracosane and 1-adamantylamine, 8 Gpa, 500 C, 8 hours

Tetracosane residue w/ inclusions

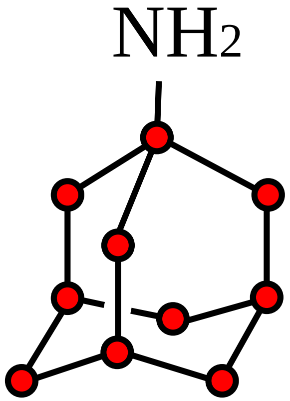


Few minutes on hot plate



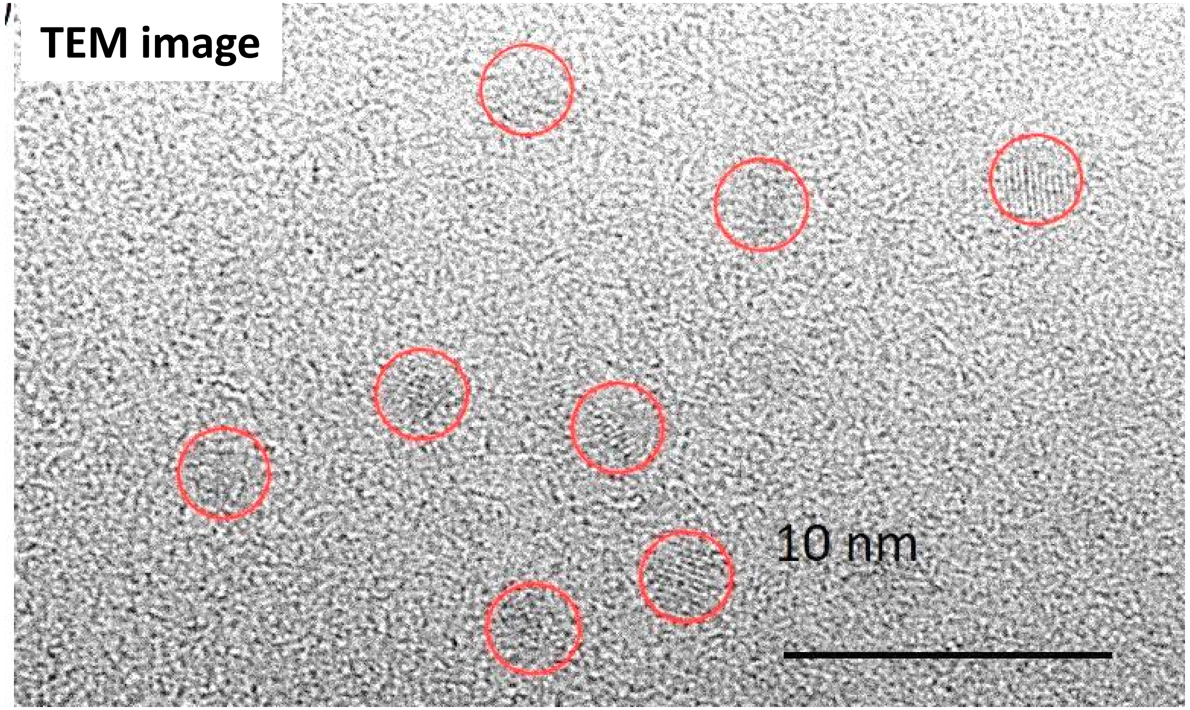


$CH_3(CH_2)_{22}CH_3$



First lower-pressure nanodiamond growth

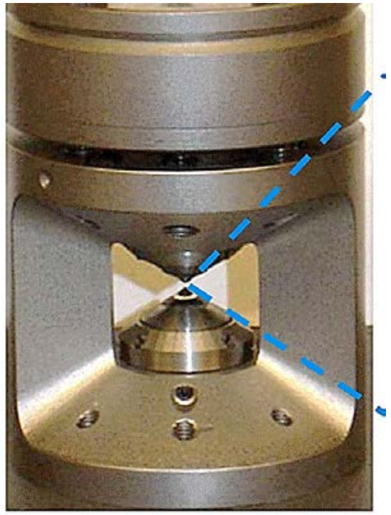
- Accidentally low pressure and temperature
  - Many small nanodiamonds
  - Low pressure 3 Gpa, Temperature 500 C
- Scalable to kilogram quantities



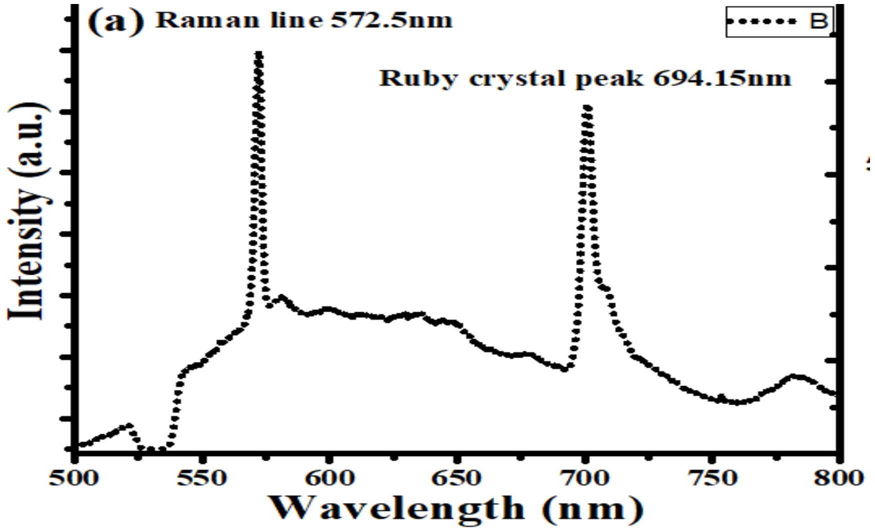
Recent seeded nano-diamond growth

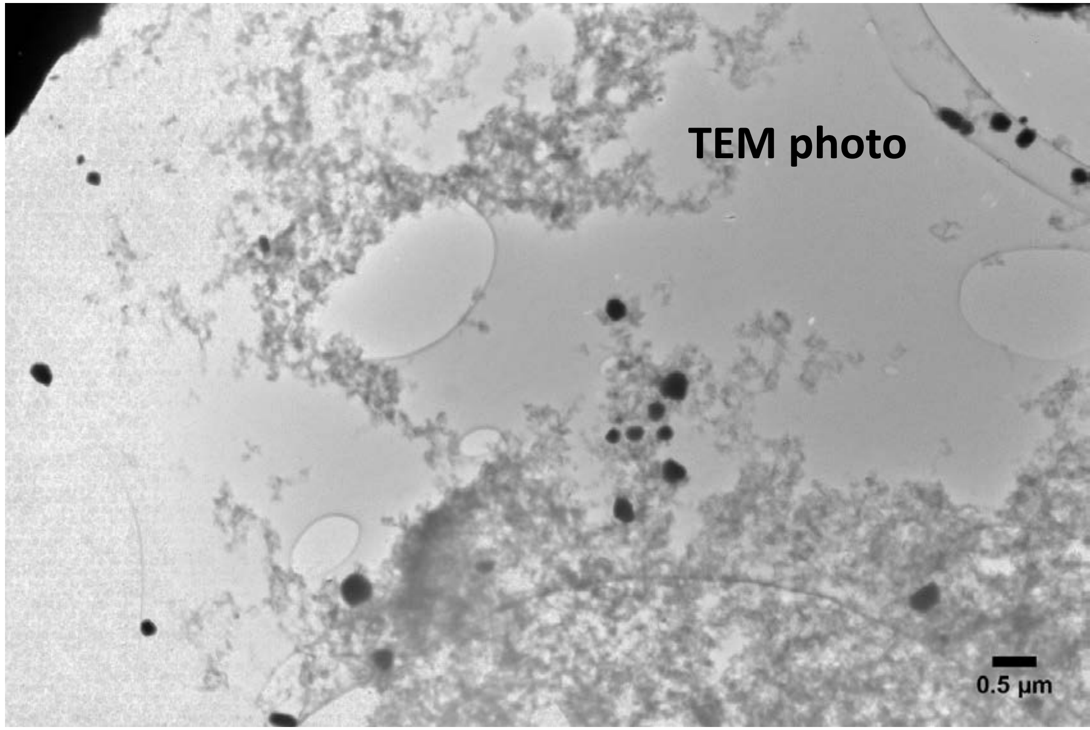
- Growth conditions: T=400C, P=9GPa, time 24h

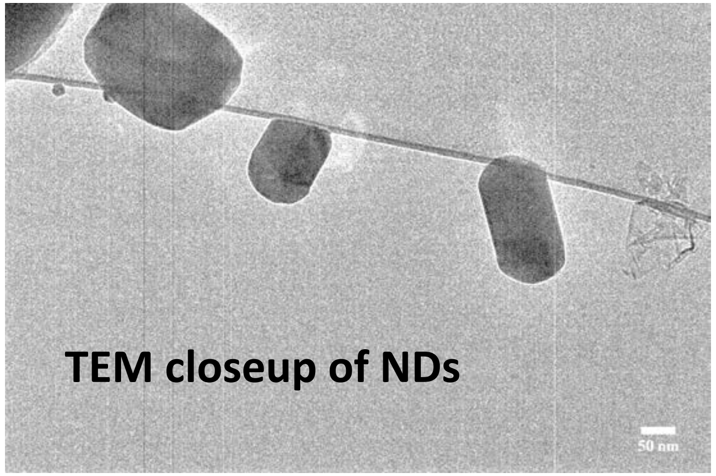
DAC photo



Optical spectrum (raw product)

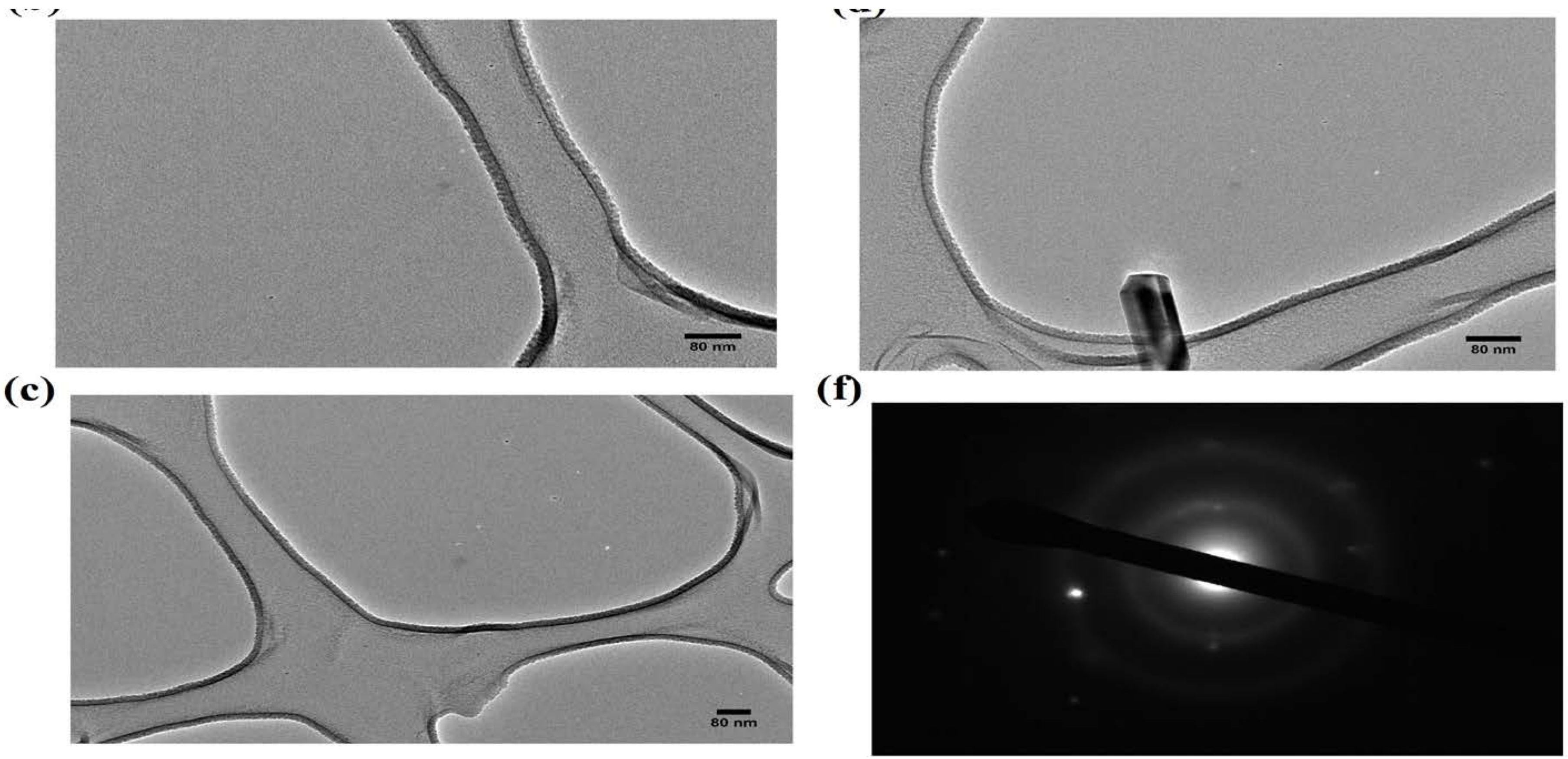






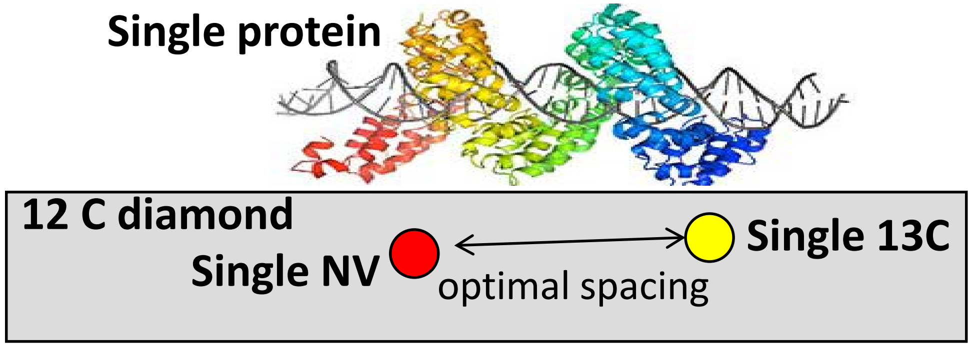
Control experiment – No seed molecules

- Otherwise same growth mix & conditions
- Only 1 diamond found on TEM grid vs. ~ 1 million with seed molecules



Quantum enhanced sensing

- Room temp quantum register (storage > 1 sec)
  - NV detects protein spins (via quantum coupling)
  - 13C stores for higher resolution
- Extend to nanodiamonds with engineered growth



Science AAAS

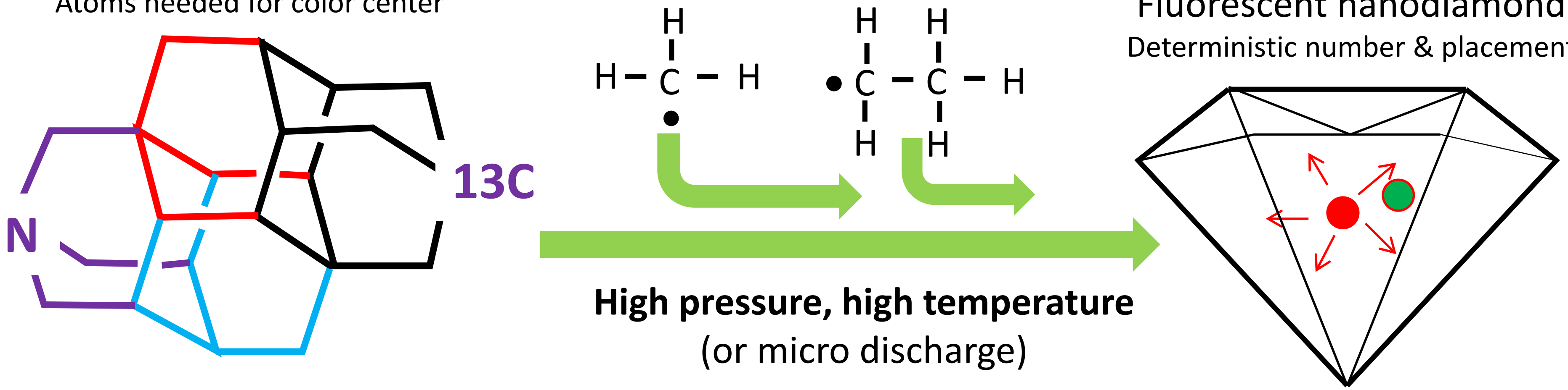
Room-Temperature Quantum Bit Memory Exceeding One Second
P. C. Maurer *et al.*
Science **336**, 1283 (2012);
DOI: 10.1126/science.1220513

Bottom-up growth of diamond quantum register

Custom diamondoid molecule
Atoms needed for color center

12C enriched carbon radicals

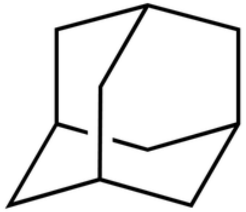
Ideal NV quantum register
Fluorescent nanodiamond
Deterministic number & placement



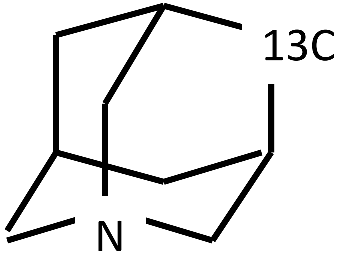
How to make a scalable room temperature quantum computer

- Advantage of superconductor qubits
  - Large size = deterministic placement, high yield fabrication, custom design
  - Disadvantage is low temperature
- Diamond color centers, like NV
  - Works at room temperature
  - How to control separation and properties ?
  - Implantation is probabilistic
  - straggle ~3 nm, ideal coupling distance ~3nm
- Solution is bottom-up approach
  - Synthesize molecule with NV, P1, etc precursors with desired locations
  - Grow diamond around it

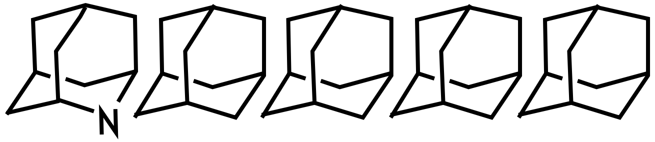
Adamantane



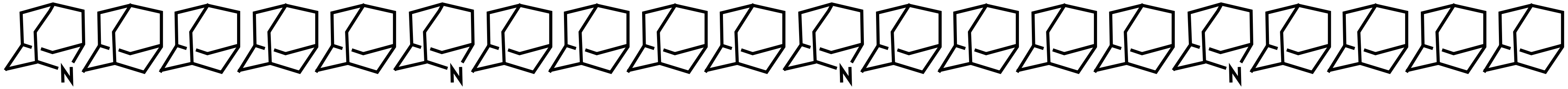
NV w/ 13C storage



Fabricate monomer



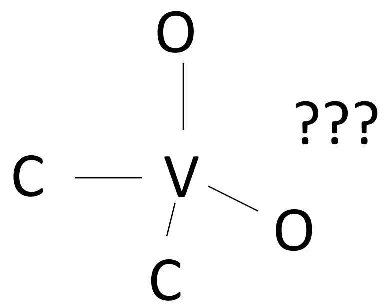
Make polymer

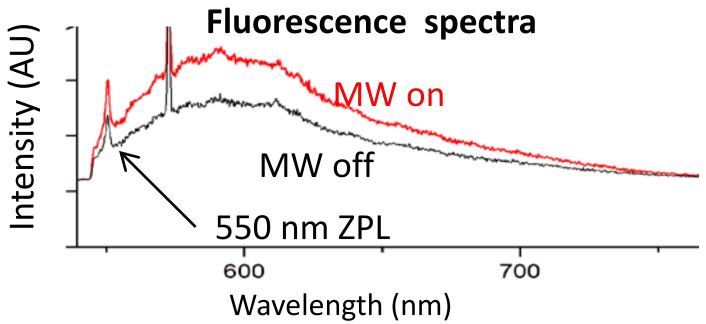


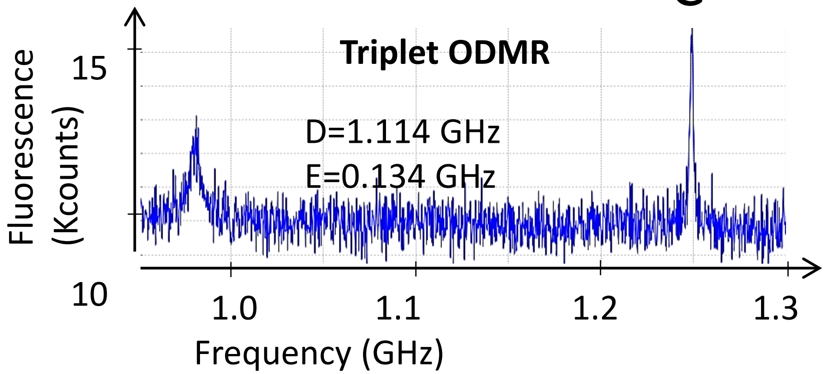
Use as diamond seed to get near-deterministic array of NVs

High contrast ODMR defects in diamond

- Four color centers so far
  - Triplet is metastable state
  - Symmetry suggests multiple atoms like O-V-O or O-O-V







Compare color centers	O-V-O	ST1	Ulm
Spin contrast (NV=30%)	??	50%	100%
Optical transition			
ZPL (nm)	440	550	560
Phonon sideband	500	600	630
Saturation power (mW)		20	2
Microwave transition (MHz)			
upper resonance	1122	1274	1340
lower	726	996	904

Identifying "super ODMR" centers

- Clues exist
  - Mass 27 implant
  - L2 = ST1
  - Tried Al, BO-, MgH2-
- Plan is to identify impurity atoms
  - ESR, Other optical centers, elemental analysis (TEM etc)

New J. Phys. 19 (2017) 053008 https://doi.org/10.1088/1367-2630/aa6d3f

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PAPER

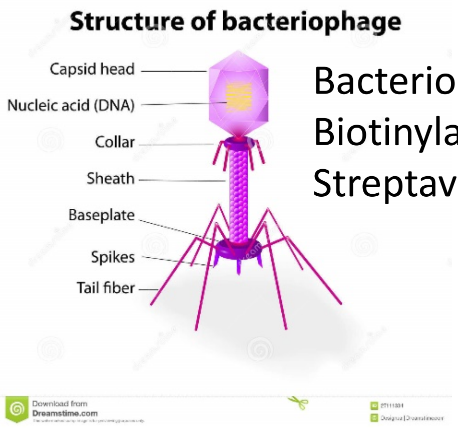
Bright optical centre in diamond with narrow, highly polarised and nearly phonon-free fluorescence at room temperature

Roger John, Jan Lehnert[1], Michael Mensing[1], Daniel Spemann[1], Sébastien Pezzagna and Jan Meijer

The artificial ODMR active L2 centre and a new L1 centre have been produced in (001) CVD grown diamond using ion beam implantation. The mass of the implanted ions was set around 27 amu, however the nature of the centres is up to now still unknown. The L2 centres' dipoles are oriented along the ⟨110⟩ directions whereas the dipoles of the L1 centres point in the ⟨111⟩ directions. The new L1 centre shows a bright, highly polarised and

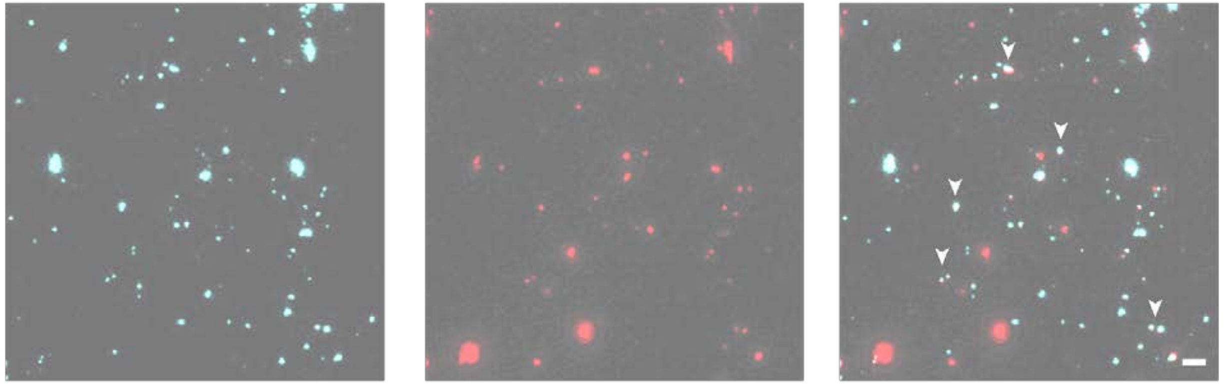
Nanodiamonds labeling of phages

- Project with **Ry Young, Lanying Zeng**, Jimmy Trinh
- Short term goal – Identify harmful bacteria by fluorescence
- Long term goal – Image phage infection process in real time
  - Needs super-resolution



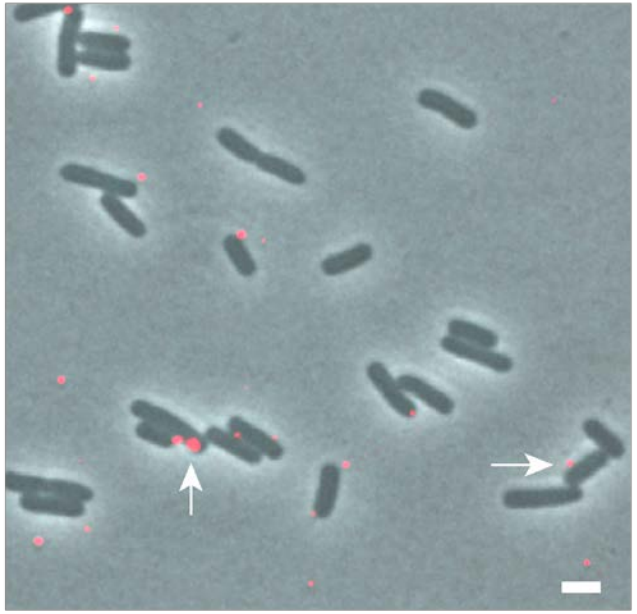
Bacteriophage lambda/*E. coli* system
Biotinylate a phage strain
Streptavidinated diamonds

Bacteriophage lambda/*E. coli* system

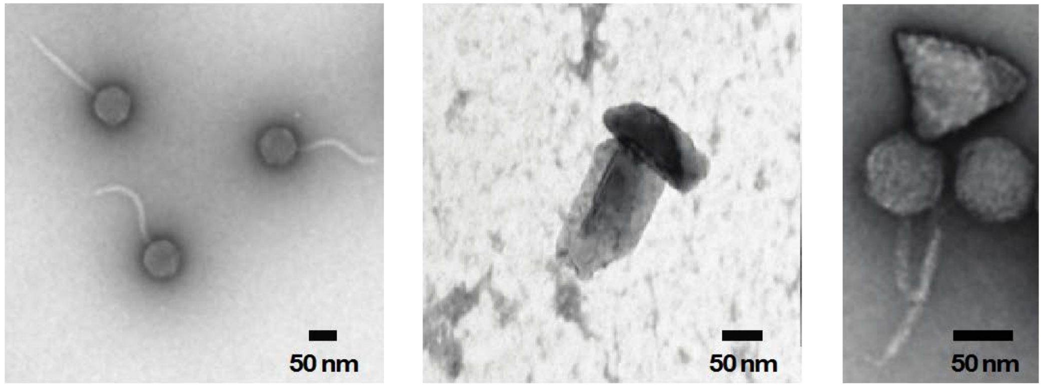


Phage (biotin) Diamond (streptavidin) Merged image

Tagged phages infect bacteria



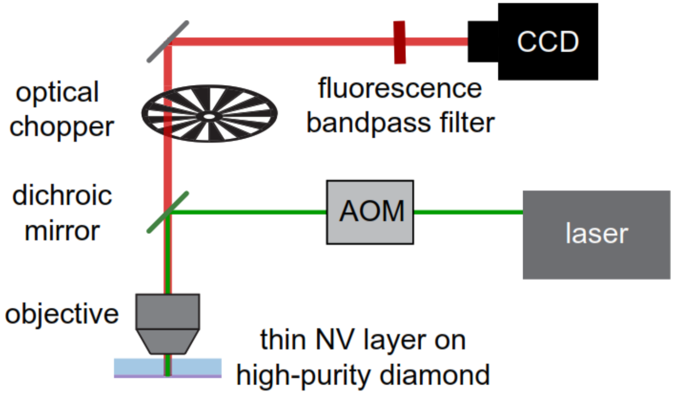
TEM images showing phage / diamond binding

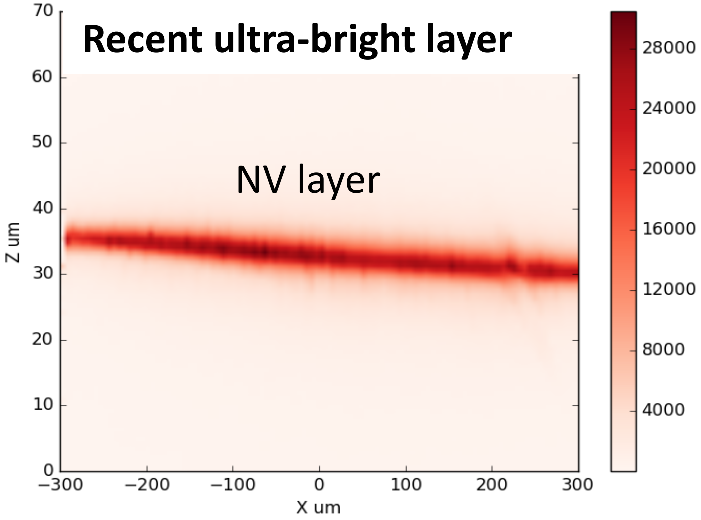


Wide-field NV magnetic imaging

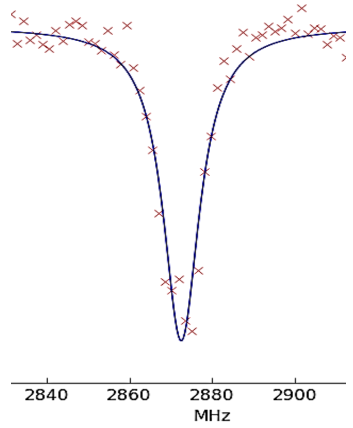
- Goal – NV magnetic imaging of neuron culture
- Stuttgart collaboration DOI: 10.1038/ncomms2588
- Harvard collaboration doi:10.1088/1367-2630/13/4/045021

Wide field magnetic imaging setup





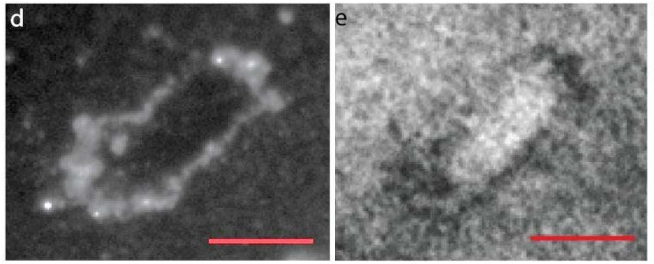
~7% ODMR contrast



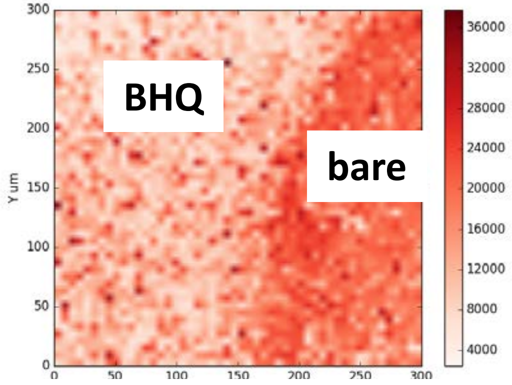
Early work: T1 image on cell membrane

[DOI: 10.1038/ncomms2588]

Fluorescence T1 (Gadolinium)

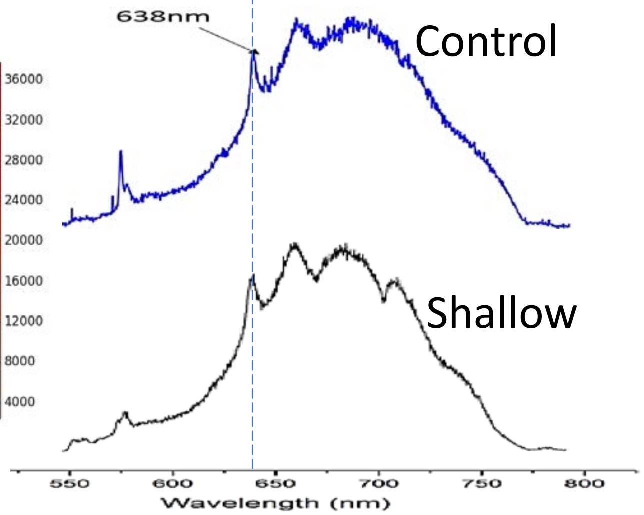


FRET depth estimate

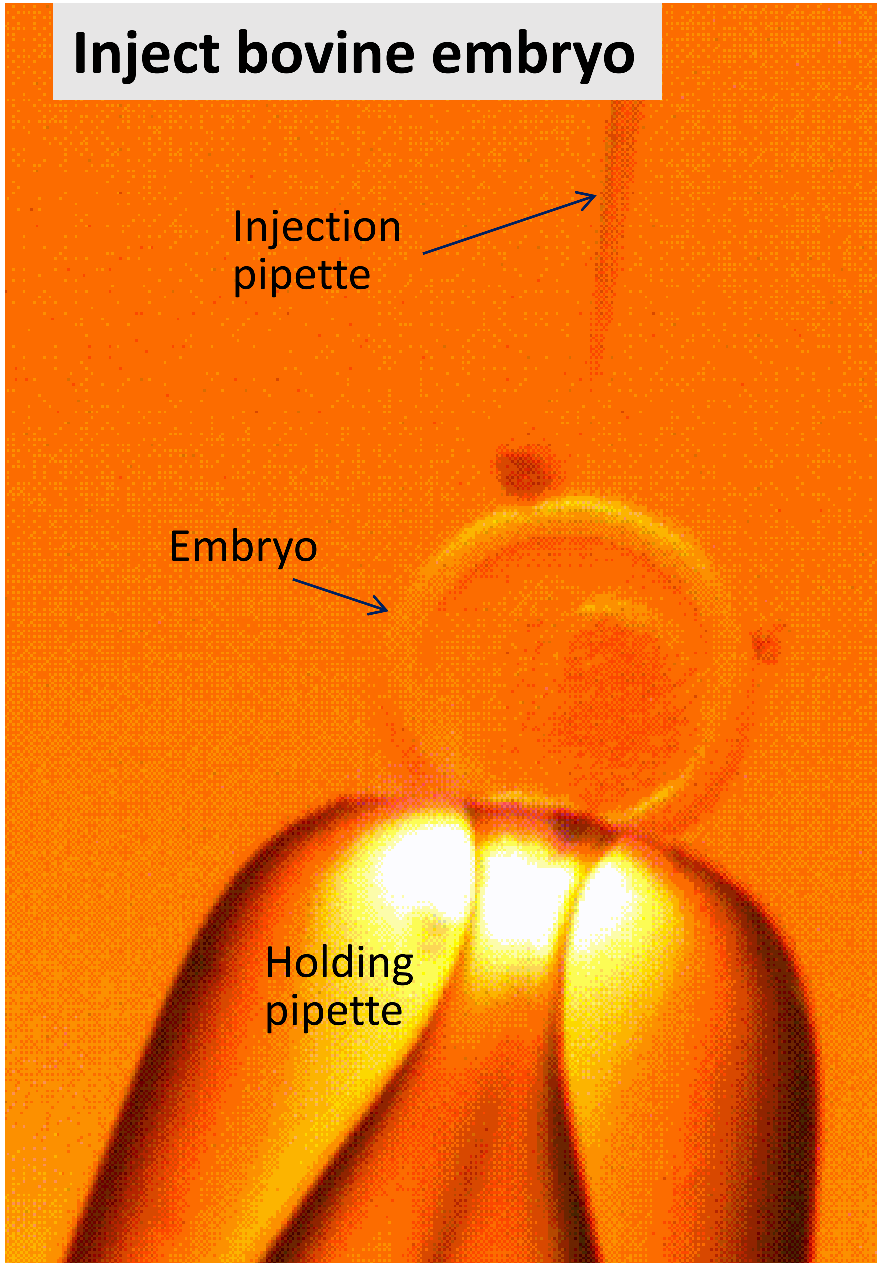


Depth estimate < 5nm

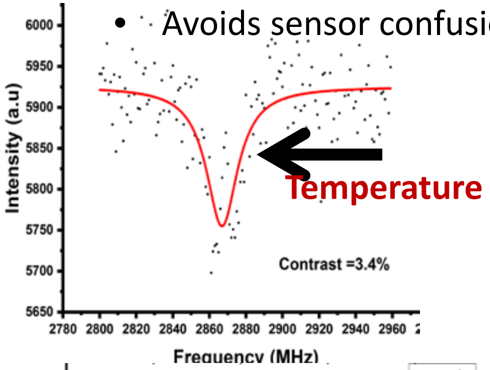
NV optical spectrum



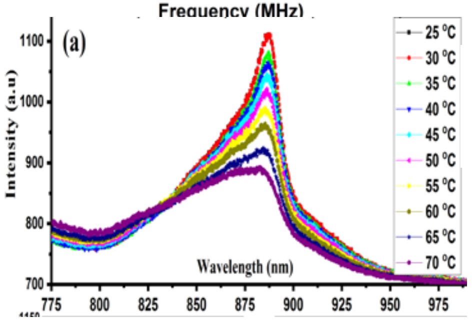
Temperature sensing in spherical cows



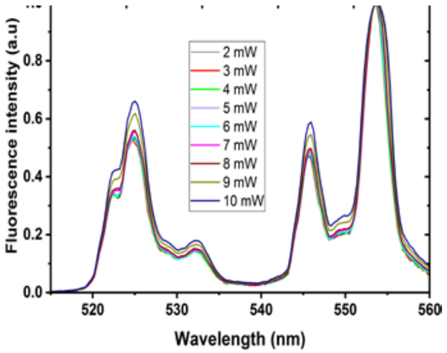
- *Local* temperature vs cow development
  - Up to 1 week continuously
  - Probe w/ non-bleaching particles
  - Heat locally w/ IR laser
  - Fertilize with fluorescence tagged sperm
- Multi-modality sensing
  - Avoids sensor confusion/artifacts



NV diamond sensing –ESR line shift



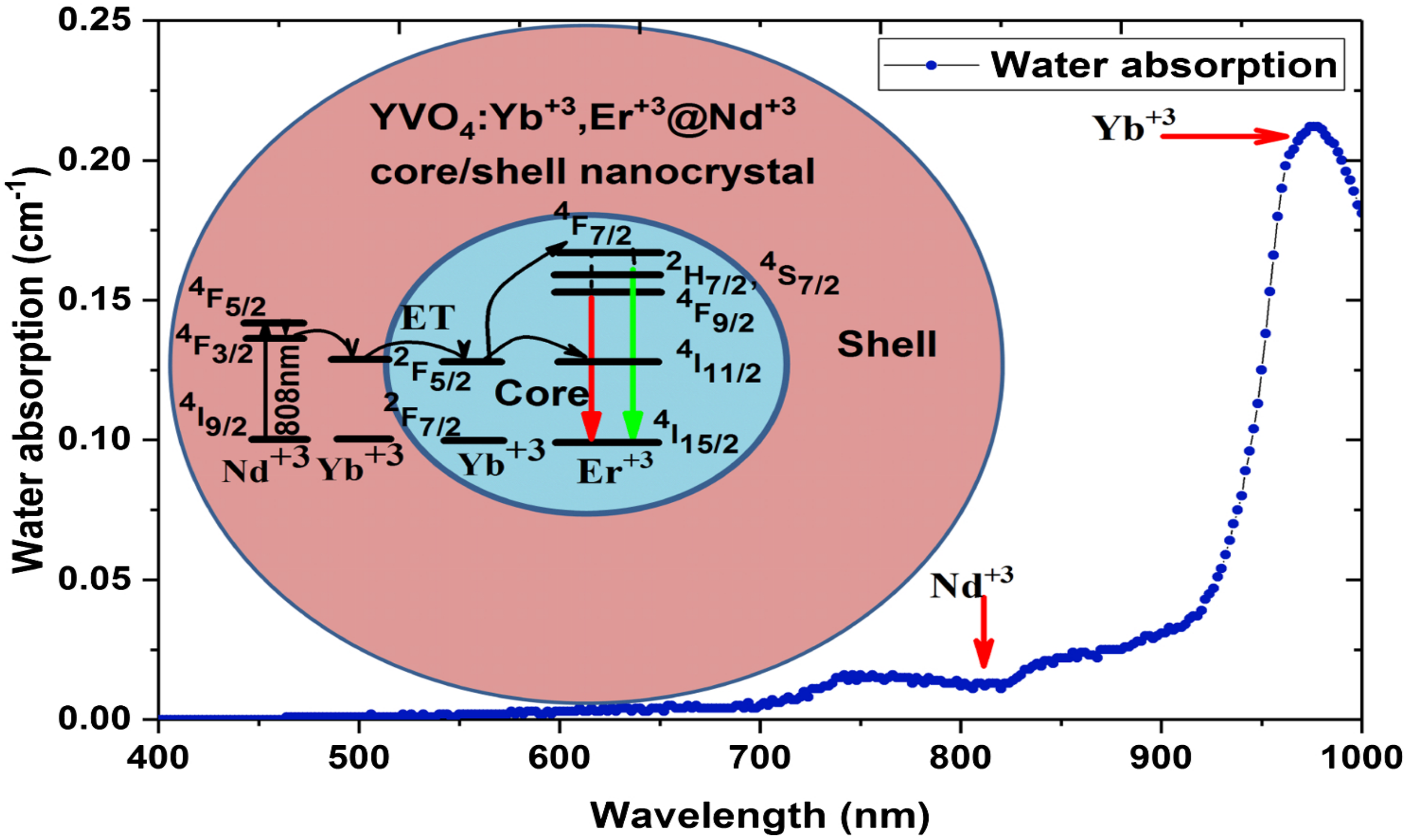
Ni diamond sensing – zero phonon height



Upconversion phosphor sensing – red/green ratio

Core-shell

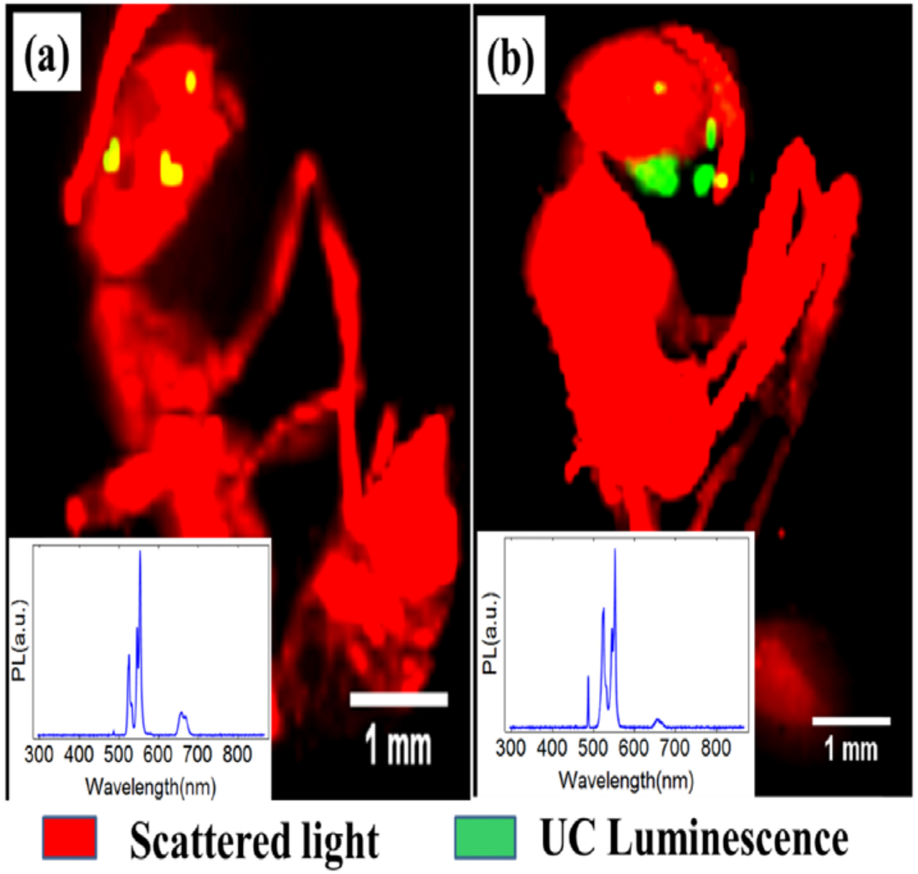
❖ Minimizing overheating problem in biological tissues induced by 980 nm laser



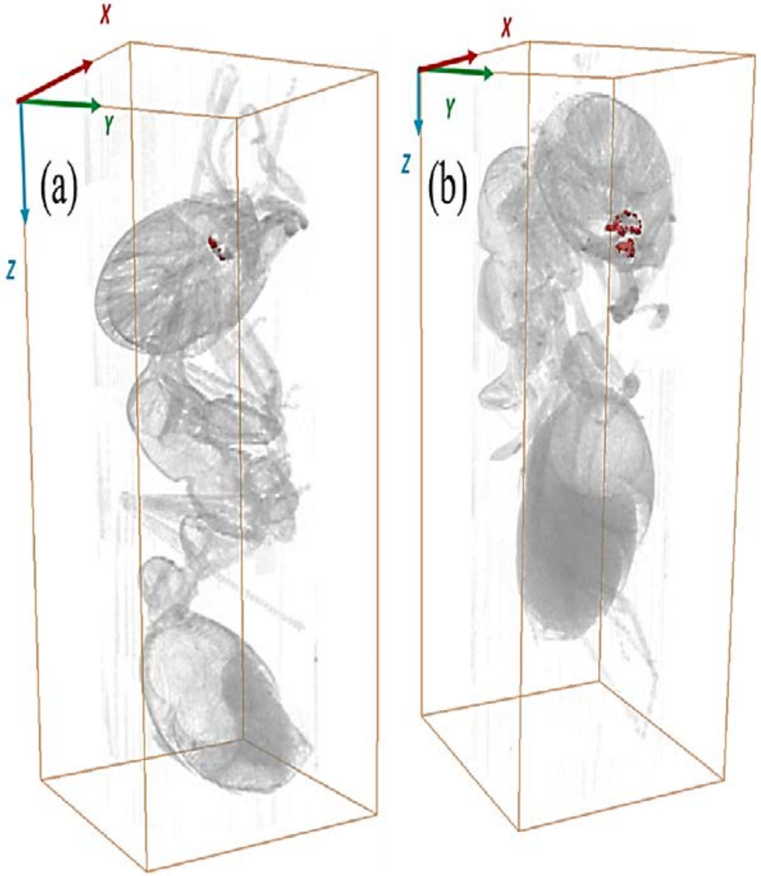
Alkahtani et al, Optics Letter Vol. 42, Issue 13, pp. 2451-2454 (2017)

❖Biological application of UCNPs (fire ants fed with UCNPs)

Optical images



X-ray tomography images



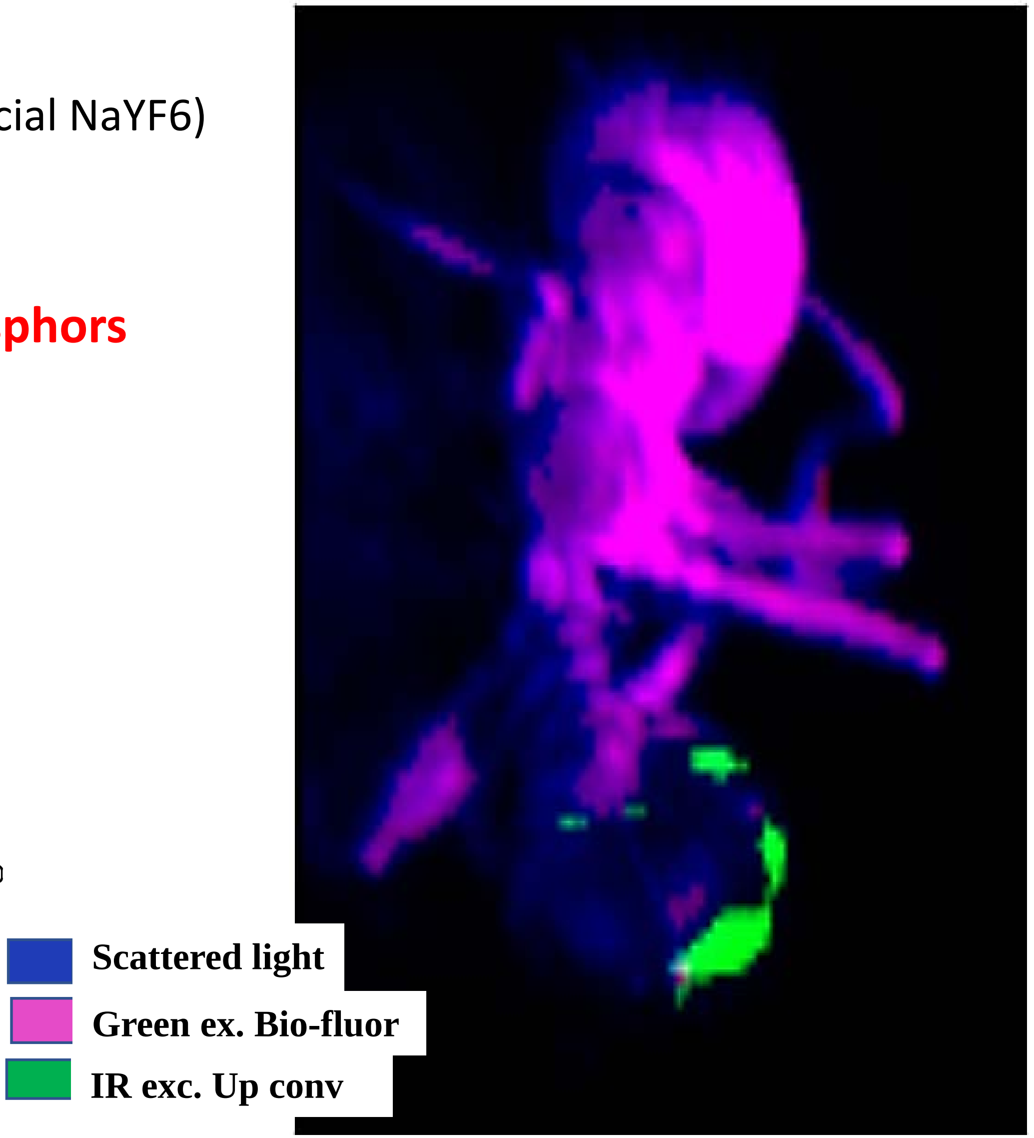
Alkahtani et al, **Optics Express** Vol. 25, **Issue 2**, pp. 1030-1039 (2017)

Up-conversion solves biofluorescence background

- Single emitters seen
  - Pr doped YAG
- Fire ant imaging
  - Er doped YVO4 (instead of commercial NaYF6)

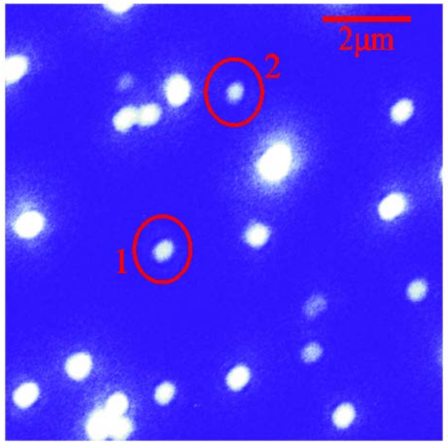
Upconversion image inside fire ant

Project w/ Hong (Helen) Liang MEEN



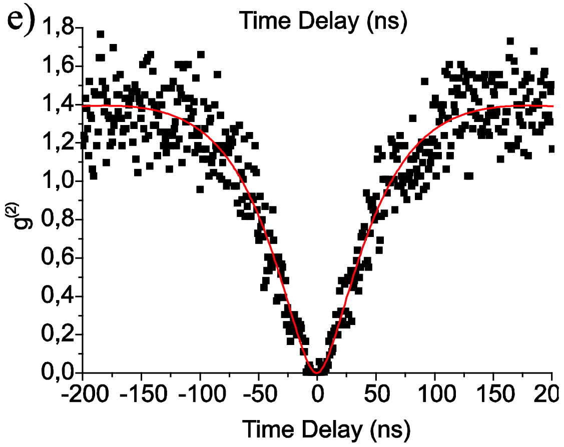
Future quantum bio-sensing w/ upconversion phosphors

Pr:YAG 2-photon UV fluor



Quantum correlations

100% anti-bunching



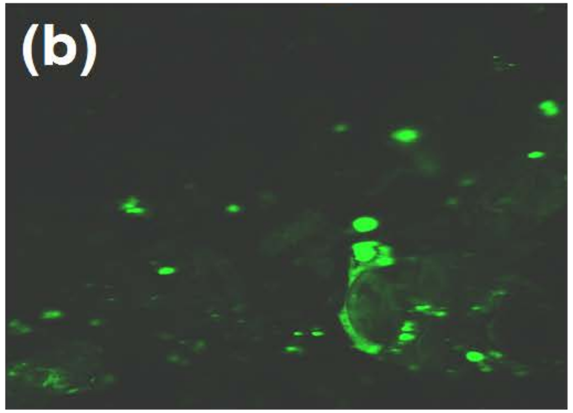
Optical detection of a single rare-earth ion in a crystal, NATURE COMMUNICATIONS 3, 1029

Upconversion particles in pumpkin seedlings

UCNPs were fed to plants(pumpkin seedlings) not injected

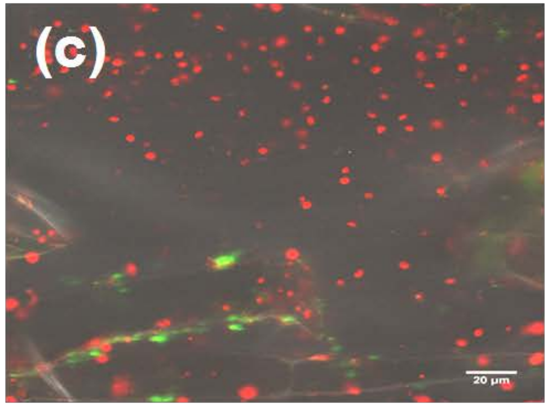
Confocal w/ NIR laser - Vet School

UCNPs feeding-seed "leaf"- 40X objective



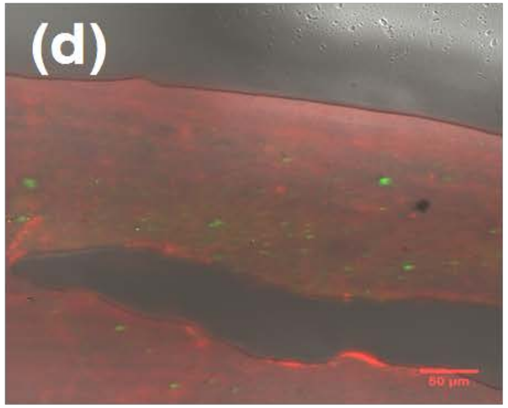
Laser 808 nm for excitation . Emission collected (500-600 nm)

UCNPs feeding- Upper Stem- 40X objective



Combine the bright field image with laser excitation 808 nm and 561 nm laser

UCNPs feeding- Root 20X objective



Combine the bright field image with lasers 808 nm excitation(green) & 561nm(red)

Summary

- Current state of art in diamond for bio-sensing
- Seeded growth demonstrated – **Brave new world of diamond growth**
    - Down to temperatures where virtually any seed molecule can survive
    - Preliminary NV properties – similar to bulk
- Bio-sensing applications demonstrated
    - Multi-modal temperature sensing in bovine embryos
    - Bacteriophage tagging
    - Ultra-shallow NV layer with red stability
- Nano phosphors also promising