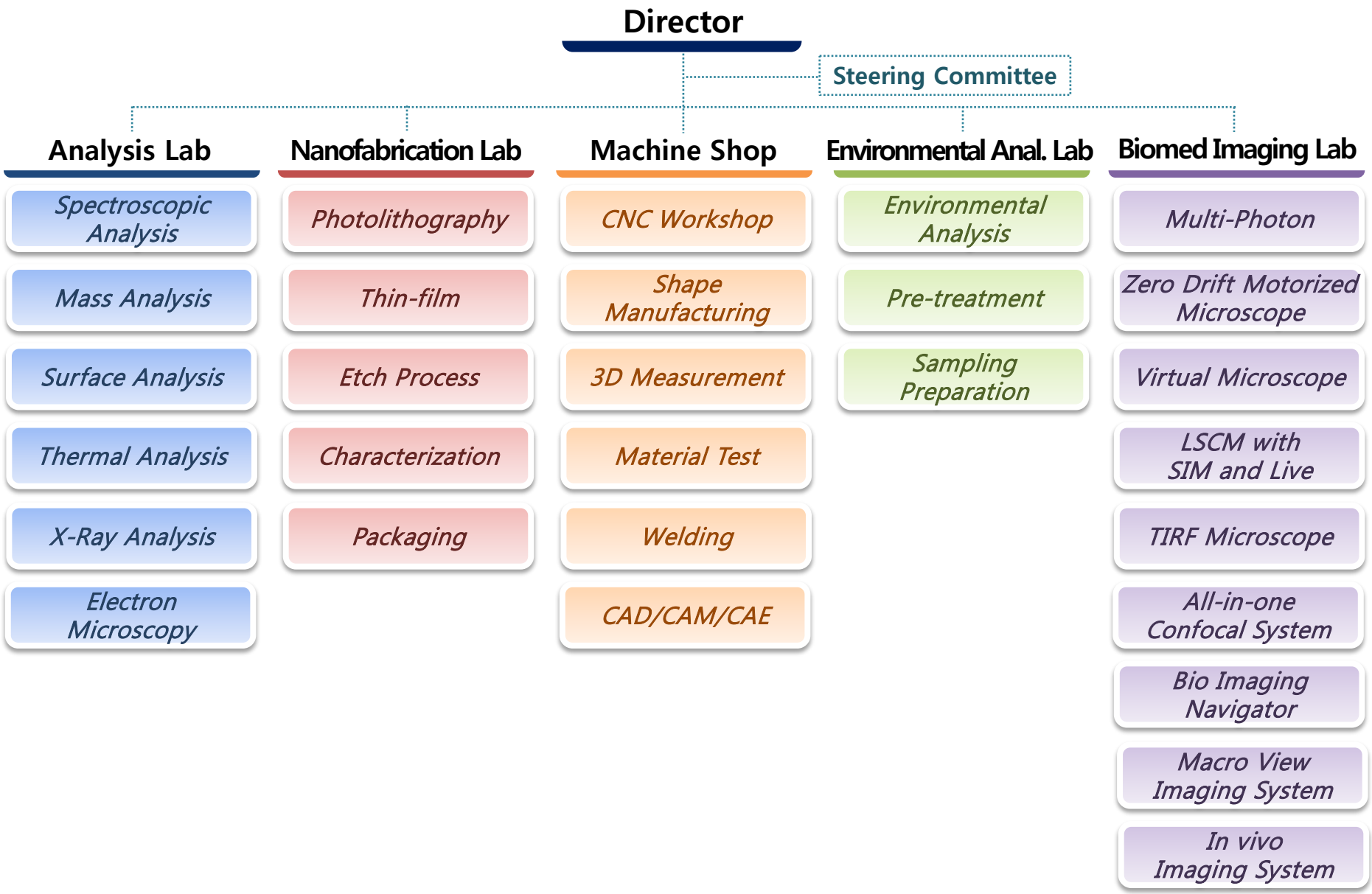
Micro-Raman self-user training

SEON HYE SON

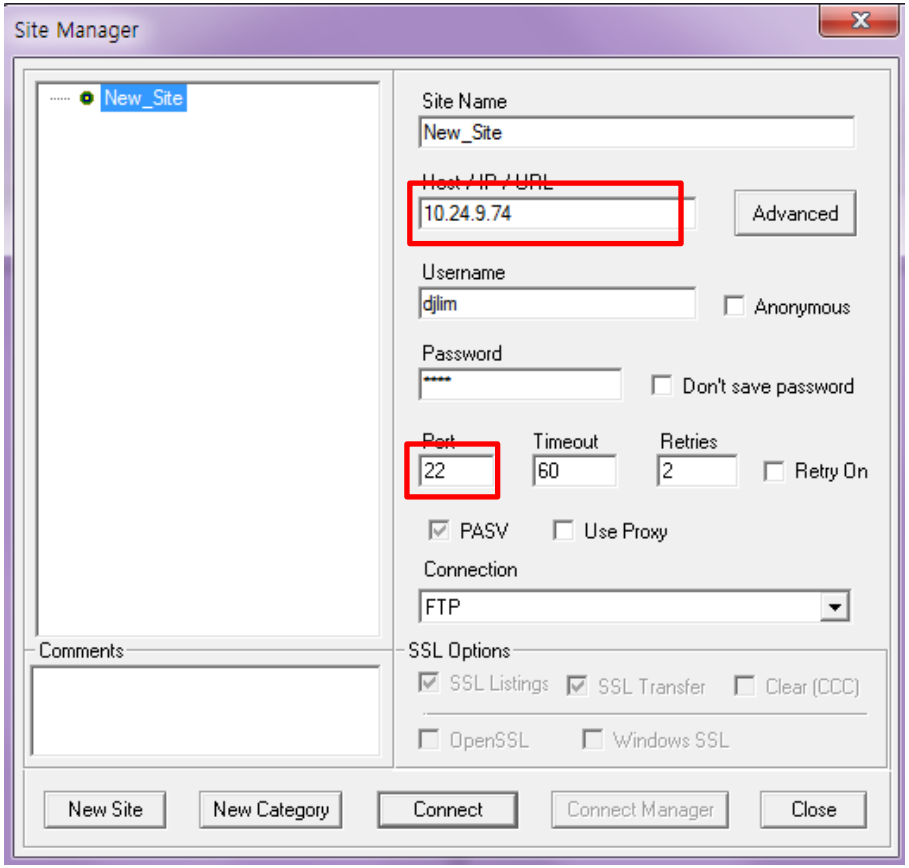
UNIST Central Research Facilities (UCRF)
Ulsan National Institute of Science and Technology (UNIST)

Organization

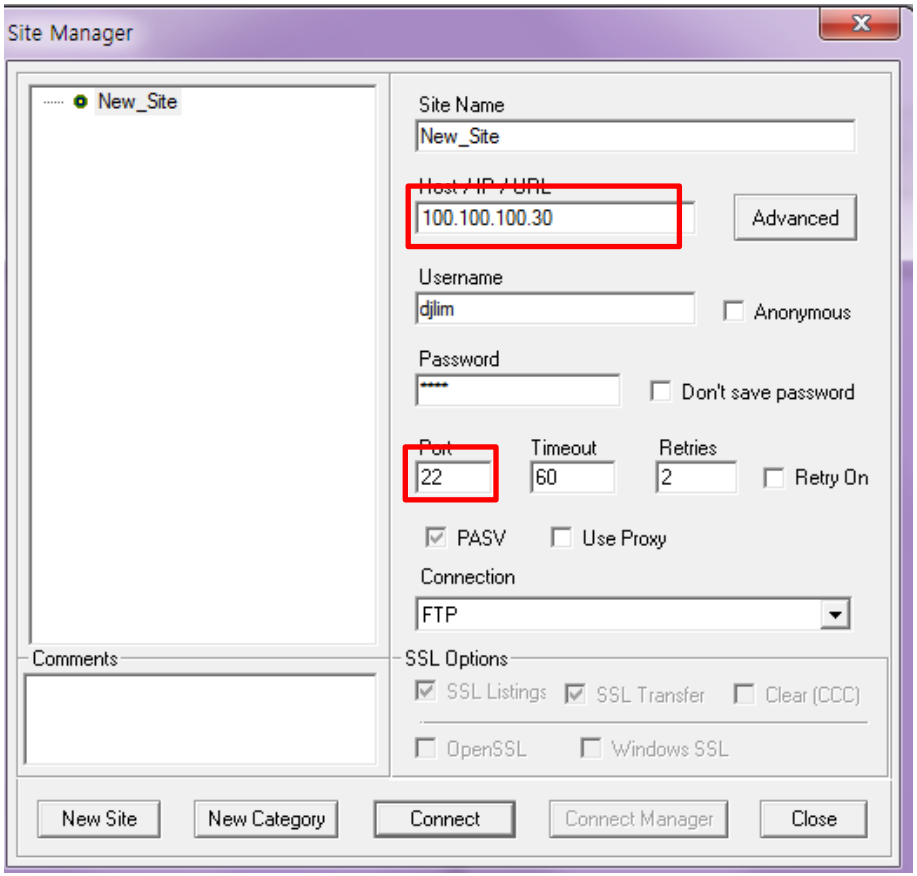


Log in System

Other place

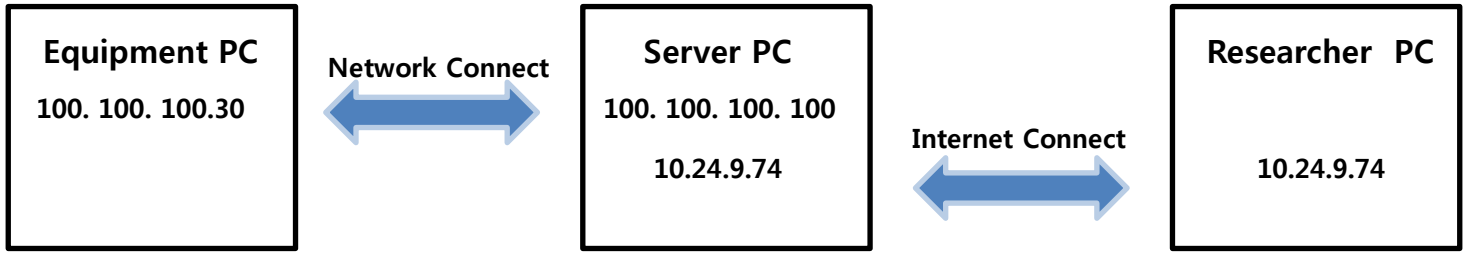


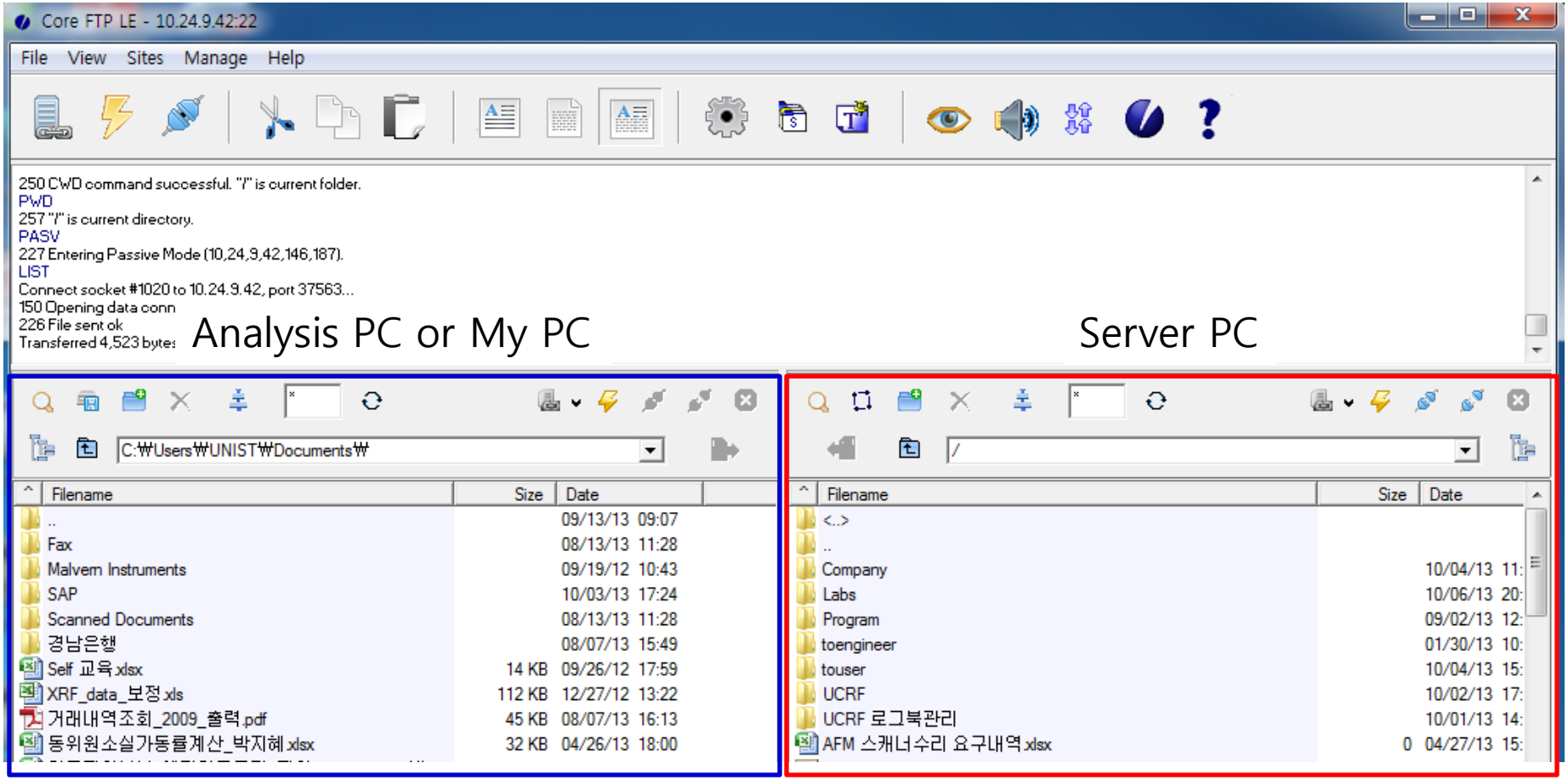
In UCRF



Common ID : djlim
Common PW : 0254

Log in System

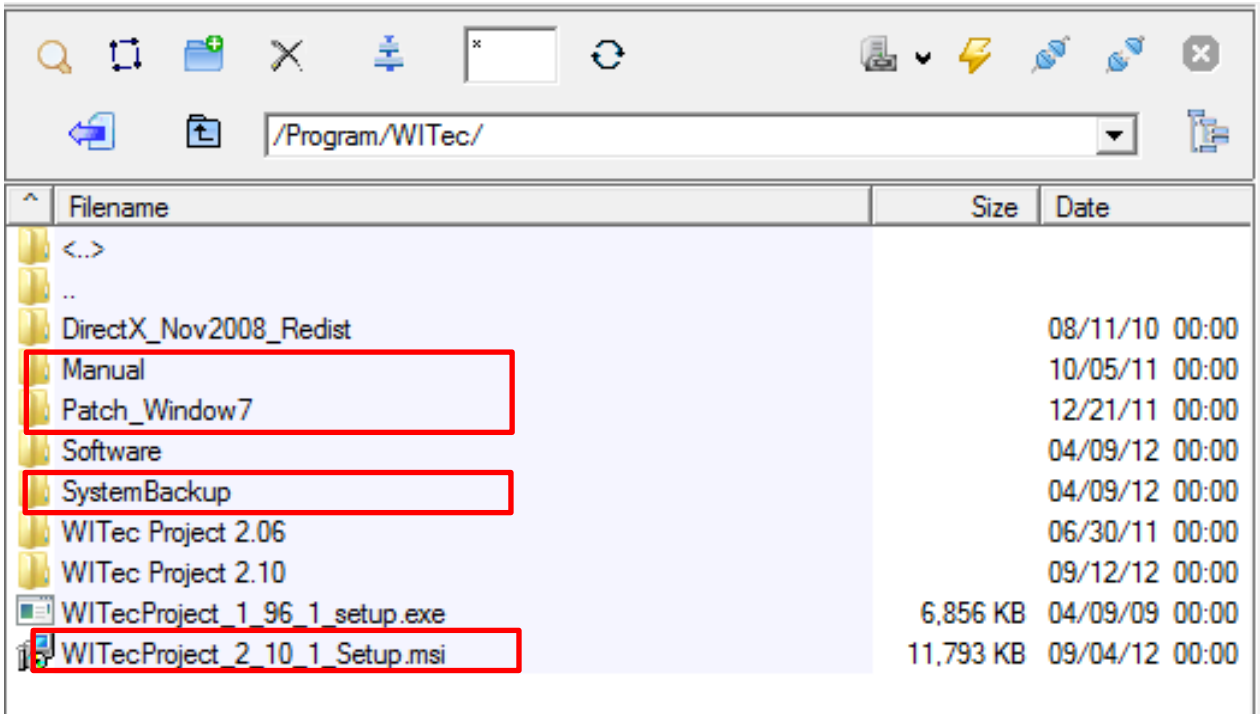




Raman Analysis Program

- Core FTP > Program

- Download :
  1) WITecProject 2_10_1 setup
  2) System Backup



Guideline for the Operation of the UCRF

Article 1 (Purpose)
This guideline is intended to provide detailed requirements for operating the Central Research Facilities at Ulsan National Institute of Science and Technology (UNIST) (hereinafter referred to as “UCRF”) in accordance with Article 10, Operational Regulations of Central Research Facilities at UNIST.

Article 2 (Scope)
This guideline shall apply to faculty, graduate students, undergraduate students and researchers at UNIST, as well as external clients, who request services from UCRF, and equipment managers.

Article 3 (Definitions)
Terms used in this guideline shall be defined as follows:
“Autonomous use” means that UNIST faculty members or students use UCRF's equipment without any help from the equipment manager.
“Autonomous user” refers to users who have qualifications for the “autonomous use” of the equipment in paragraph 1 above, according to procedures set by UCRF.
“Request for analysis and processing” is a request to the equipment manager to perform a series of analyses and processes, so autonomous users can use UCRF's common equipment to obtain the results of a test analysis or process.

Article 4 (Access Management)
① If any personnel want authorized access to laboratories with restricted access, they must fill out an application form and receive approval from the supervising professor and Center manager to register their ID.
② If any personnel needs to access laboratories for equipment maintenance and repair, they must be accompanied by a competent manager or have the manager's approval to gain access to the labs.
③ For laboratories that require safety training for personnel with access, approval for access will be withheld until they complete prior training, as specified for each laboratory.

Article 5 (Requests for Analysis and Processing)
① If a client requests for analysis and processing that can be supported by UCRF, the client should discuss with the equipment manager beforehand.
② A client who requests analysis and processing shall cooperate with the equipment manager in identifying the necessary information needed to maintain the normal operations and safety of equipment or facilities.
③ Analysis and processing services will be available to clients on a first-come-first-serve basis. In any special circumstances such as equipment inspection and repair is needed, requests for such services may be reserved or cancelled at the equipment manager's discretion.

Guideline for the Operation of the UCRF

④ If there are no special requests from the client, each manager may discard any specimens that are seven days or older after the results-notice date, and may also discard the outcome or results data produced by the analysis and processing service three months from the day of said notice or later.

Article 6 (Qualifications for Autonomous Use)

① Authorized persons who qualify for autonomous use shall be limited to graduate students, researchers, professors and authorized undergraduate students (with the supervising professor's approval) at UNIST.
② Qualifications for autonomous use shall be granted to any persons who satisfy the requirements specified by each laboratory (e.g. safety training, equipment user training, evaluation, etc.).
③ A list of autonomous users shall be updated every 6 months and shall be published on the UCRF homepage.
④ An autonomous user's qualifications may be cancelled if the equipment manager deems it necessary, or if the user does not frequently use the equipment (less than the minimum limit of 10 times in the last 6 months). In such cases, users may discuss with the manager and go through a re-orientation process to be qualified for autonomous use again.

Article 7 (Responsibility of Autonomous Users)

① Autonomous users should follow the instructions for using the equipment as they learned during the orientation. If there is something significant to report, they must discuss with a competent manager and help operate and maintain the safety of the research equipment facilities.
② Autonomous users will be liable for any accidents, equipment damage, failure and loss incurred as a result of their negligence when using the equipment.
③ Equipment reservations should be made a day (24 hours) prior to when they need to use the equipment, and may be cancelled no later than 12 hours before the booked start time. If a user wants to cancel their reservation, they must inform the equipment manager via phone or e-mail during regular work hours (weekdays: 09:00 - 18:00) or via e-mail during off-hours.
④ Any reservations that are made less than 24 hours in advance may be cancelled before the booked start time. If users want to cancel their reservation, they must inform the equipment manager via phone or e-mail during regular work hours (weekdays: 09:00 - 18:00) or via e-mail during off-hours.
⑤ After using the equipment at night or during the equipment manager's off-hours, authorized users should make sure the laboratory is put back in order, the lights are turned OFF, and the entrance door is properly locked before leaving.

Guideline for the Operation of the UCRF

Article 8 (Restrictions for Autonomous Use)

① For the convenience of other users, a comfortable research environment, and to promote proper use of the equipment, UCRF may sanction users.

② Sanction criteria from the above paragraph 1 shall follow "Table 1. Penalty Points and Sanction Criteria for Users of Common Equipment."

Article 9 (Billing for Test Analysis Fees)

① Clients or autonomous users will receive bills for test analysis fees in the following month after the analysis and processing has ended, and may only pay for these bills to UCRF's bank accounts.

② Clients or autonomous users shall follow the specified procedures to pay bills charged under the standards of test analysis fees in accordance with Article 8, "Operational Regulations of Central Research Facilities at UNIST."

③ The standards of test analysis fees, as stipulated in Article 7, Operational Regulations of Central Research Facilities at UNIST, may be provided to clients or users before request or use.

④ If this is their first request or first time using the equipment, clients and users should submit copies of their business license and their bank book to UCRF's administrative offices.

⑤ When there is any change to the business license, they shall inform the administrative manager of the change and send a copy of the new business license to the manager.

⑥ Bills for test analysis fees shall be issued by UCRF's administrative office, and clients or users shall pay the bill to UCRF no later than 1 month after the bill is sent to them. If the payment is overdue, UCRF may stop supporting services for users and laboratories in arrears.

⑦ If more time is required for analysis and process due to negligence on the part of clients, additional test analysis fees may be charged.

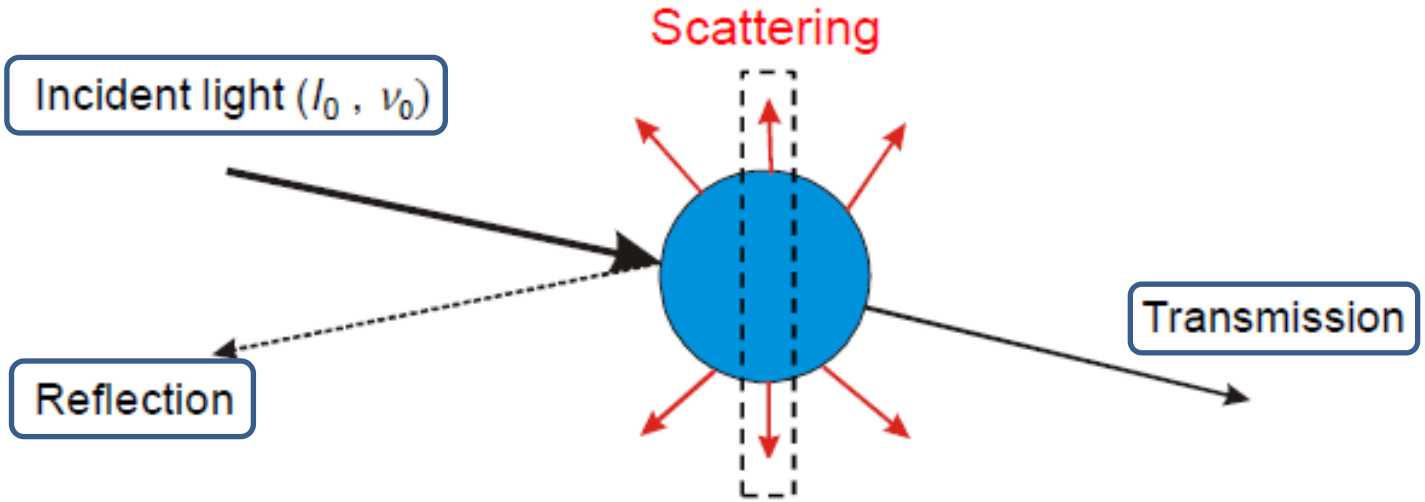
Penalty Points and Sanction Criteria

	Behaviors subject to penalty points	Penalty pts
	[Eligibility to use equipment]	
1	Unauthorized use of equipment without permission	5
2	Use of equipment without a reservation	3
3	Someone other than the equipment lessee used the equipment	3
	[Reservations for using equipment]	
4	Reserved and used equipment outside of permitted hours	1
5	Use of equipment beyond the time reserved without making another reservation beforehand for extra time	1
6	Failed to use the equipment durng the reserved time and did not cancel reservation in advance	3
7	Cancelling reservations for equipment after the cancellation deadline, under Article 7, Guideline for the Operation of the UNIST Central Research Facilities (UCRF)	1
8	Use of any equipment without giving a prior notice to the equipment manager, after making a reservation	1
	[Careless behaviors]	
9	Using functions on the equipment that are not permitted	3
10	Failure to promptly notify the manager of any errors or failures detected during use	3
11	Negligence that resulted in damages or failure to the equipment	5
12	Negligence that resulted in loss or damage to an equipment component or part	5
13	Failure to record in the equipment usage log after using any equipment, or misrepresentation or partial representation of the facts	1
14	Failure to provide specimen information required by the equipment manager to ensure normal operations and safety of equipment or facilities, thus resulting in damage or failure to the equipment	3
15	[Careless behaviors]	3
16	Using functions on the equipment that are not permitted	5
17	Failure to promptly notify the manager of any errors or failures detected during use	5

Penalty Points and Sanction Criteria

Classification	Penalty pts.	Follow-up actions
(Individual users of equipment)		
Sum up penalty points imposed to individuals	≥ 5 points	Equipment manager will notify user(s) and their supervising professor by email of their penalty points total, and shall post the details of their penalty points on the bulletin board of the equipment room. Users with penalty points 8 points or higher may not use the relevant equipment for 3 months.
	≥ 8 points	Equipment manager will notify user(s) and their supervising professor by email that the user(s) may not use the relevant equipment for 3 months until they complete the re-orientation course; will also forward an official notice to their supervising professor; and will post details of their penalty points on the bulletin board of the equipment room.
(User's laboratory)		
Sum up penalty points imposed on the students in the laboratory for the same equipment in the same laboratory	≥ 12 points	Equipment manager will notify the user(s) and their supervising professor by email that user(s) with penalty points 15 points or higher may not use the relevant equipment in the laboratory for 3 months.
	≥ 15 points	Equipment manager will email the supervising professor to inform that the user(s) may not use the relevant equipment in the laboratory for 3 months; will also forward an official notice to their supervising professor; and will post the details of their penalty points on the bulletin board of the equipment room.
Sum up penalty points imposed on the students in the laboratory for all UCRF equipment in the same laboratory	≥ 20 points	UCRF will notify students and their supervising professor by email that the user(s) with 25 penalty points or higher may not use any UCRF equipment in the laboratory for 1 month.
	≥ 25 points	UCRF will notify students and their supervising professor by email that user(s) may not use any UCRF equipment in the laboratory for 1 month; will also forward official notice to their supervising professor; and will post details of their penalty points on the bulletin board of UCRF.

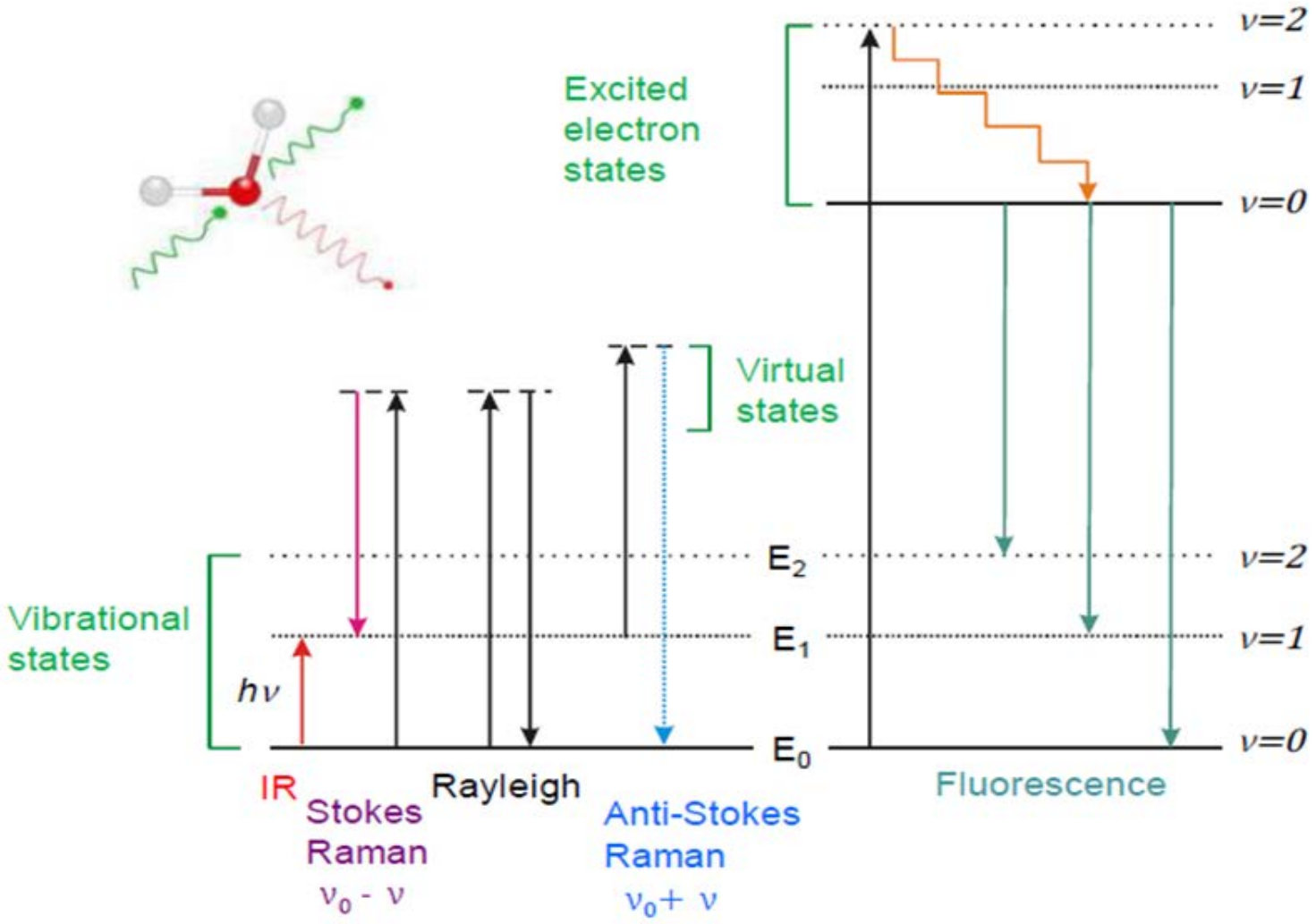
Raman Spectroscopy



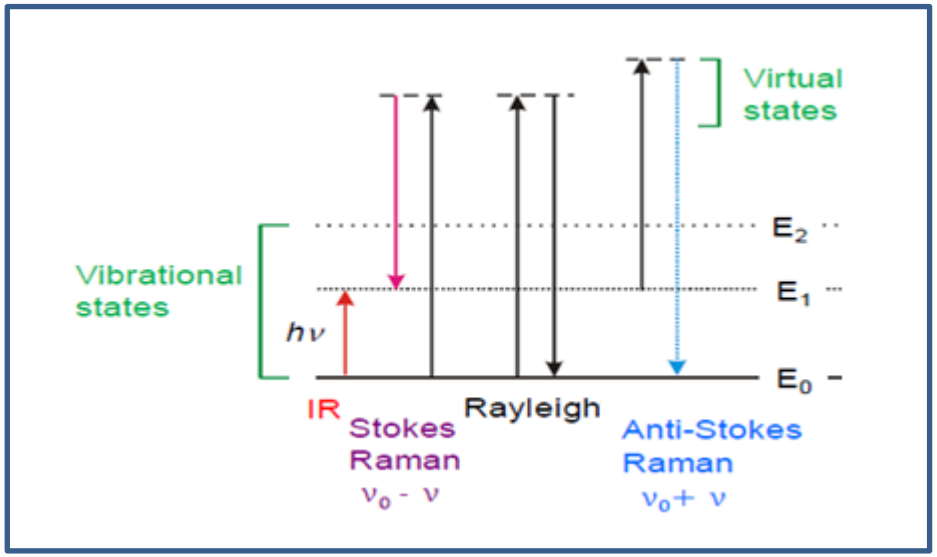
Scattering processes

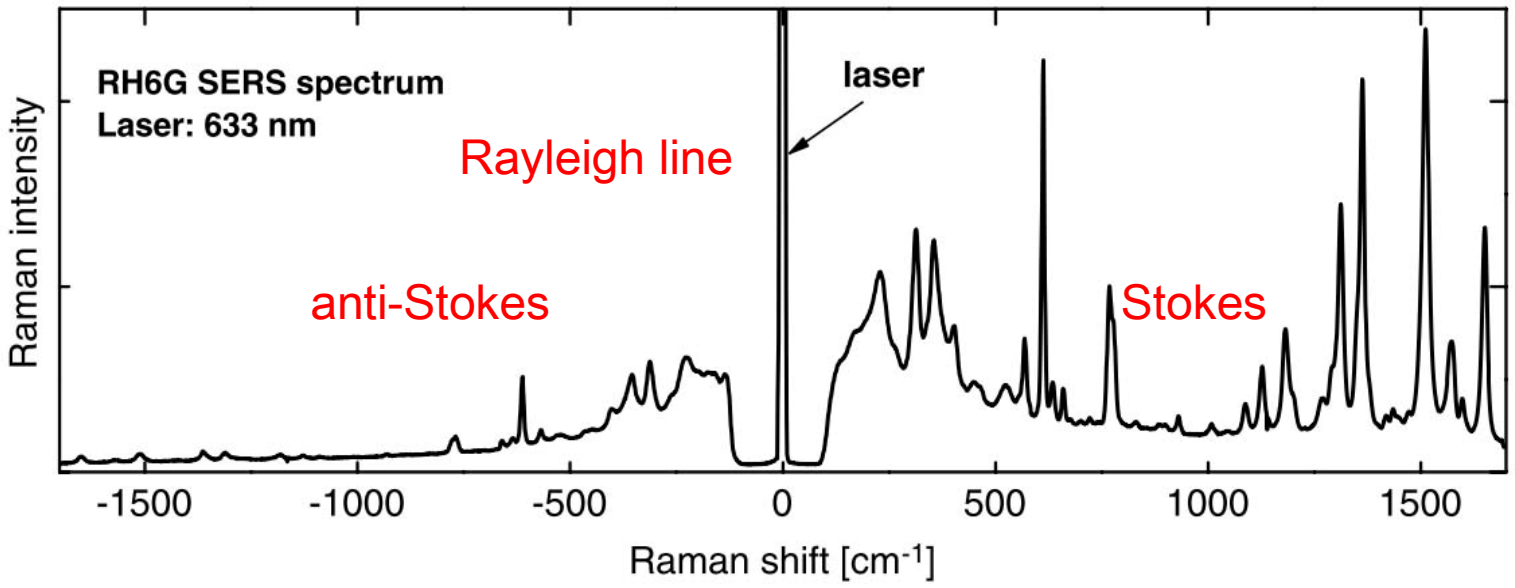
Rayleigh	Raman
ν_0	$\nu_0 \pm \nu$
particles	molecular vibrations

Raman Spectroscopy



Stokes and anti-Stokes



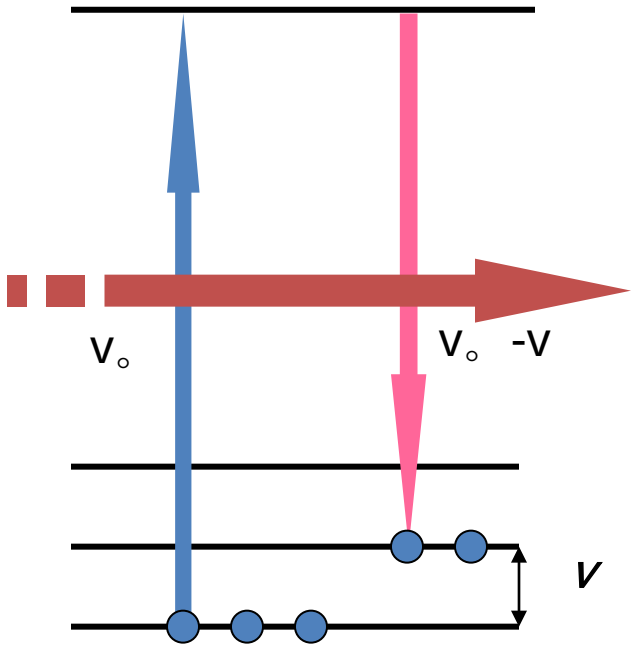


Raman and IR

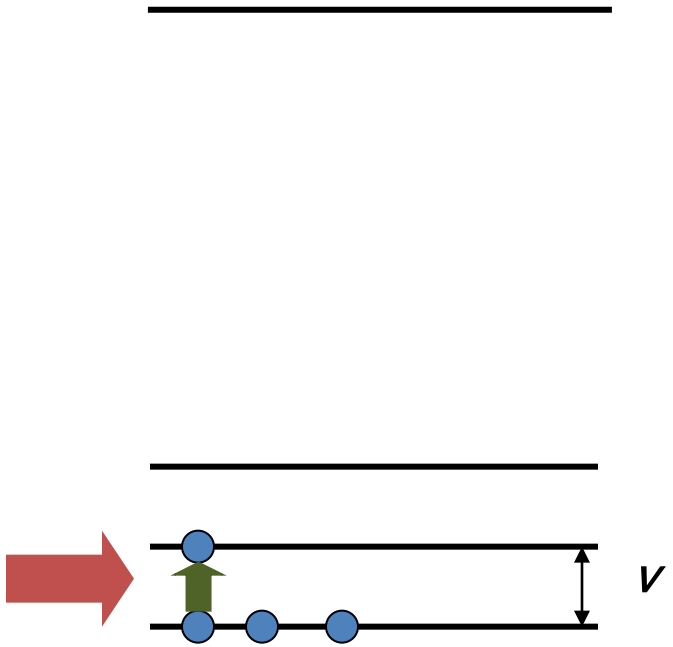
IR and Raman spectroscopy - **Vibrational spectroscopy** :

⇨ probing well-defined vibrations of atoms within a molecule

	Raman	IR
Spectroscopy	Scattering	Absorption (Transmission)
Selection rules	Polarizability	Dipole moment



Raman scattering (stokes)

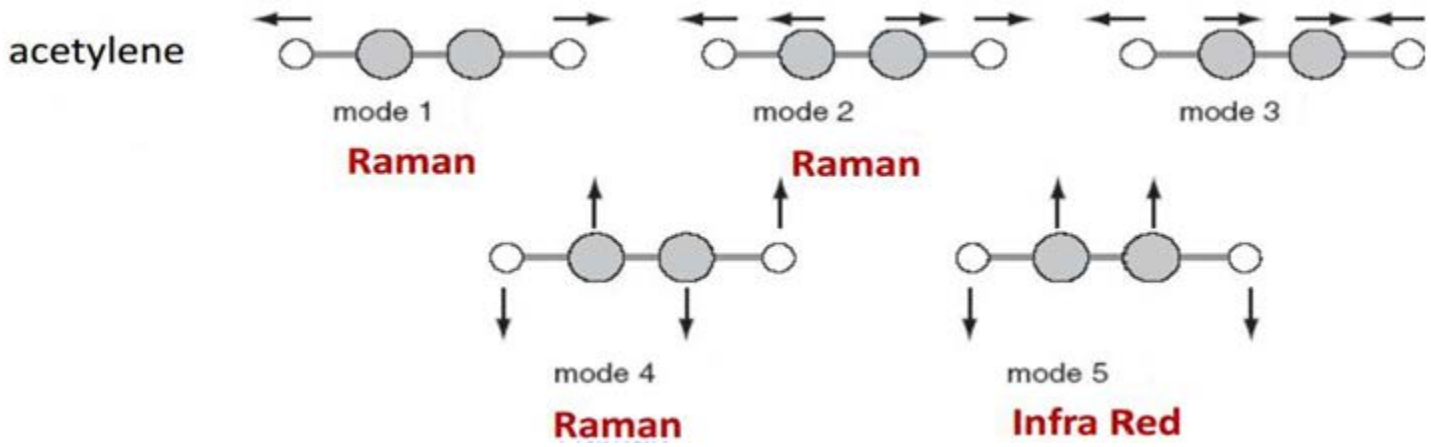


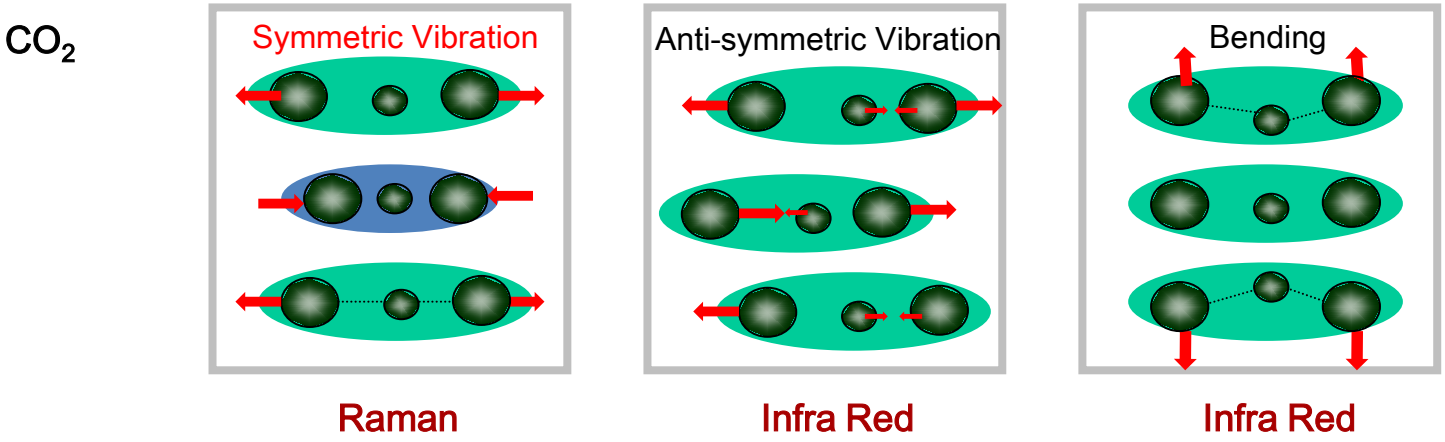
IR absorption

Characteristics of Raman

Selection rules

- The polarizability must change during the vibration (Symmetric vibration)
- In a centrosymmetric molecule (i.e.., one with a center of inversion symmetry) a vibration mode may be either IR active or Raman active, but not both.



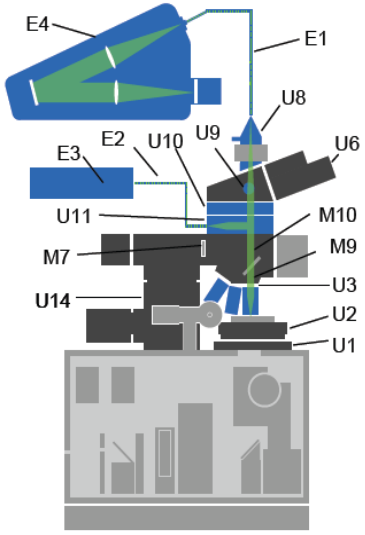


Micro-Raman

Model : Alpha300R (WITec, Germany)

Specifications

- Only Raman mode
  - Single Spectrum
  - Line Spectrum
  - Image Scan
- Scan area : 50 ㎛ x 50 ㎛
  line by point : 512 x 512
- Laser wavelength
  - 532 nm
- Objectives : x50, x10
- Heating cryostat
  - temperature range : -96 ~ 600°C
  - Up to 130°C/min heating
  - Temperature stability <0.1°C
- Freezing cryostat
  - temperature range : 77 ~ 300K
  - temperature stability : ± 0.1 K (over 10 min.)
  - hold time at 77K : 15 hrs



Confocal Raman mode

◆ Objective lens

Type	N.A.	WD
x50	0.8	0.54 mm
x10	0.25	15.34 mm

AFM-Raman

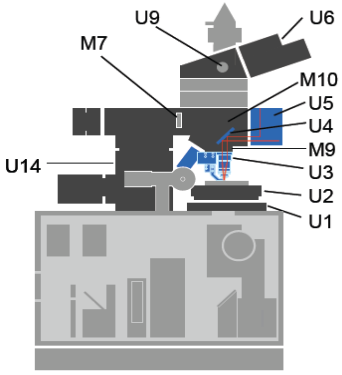
Model : Alpha300S (WITec, Germany)

Specifications

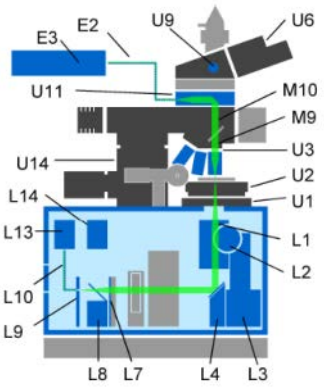
- Modes
  - SNOM (Scanning near-field optical microscope)
  - AFM (Acoustic AC, contact mode)
  - Confocal Raman (single, line, and image scan)
- Laser wavelength
  - 532nm/ 633nm/ 785nm
- Objectives : x100, x50, x20
- Inverted microscope system : x60 objective

◆ Objective lens

Type	N.A.	WD
x100	0.9	1.0 mm
x50	0.5	10.6 mm
x20	0.4	3.8 mm

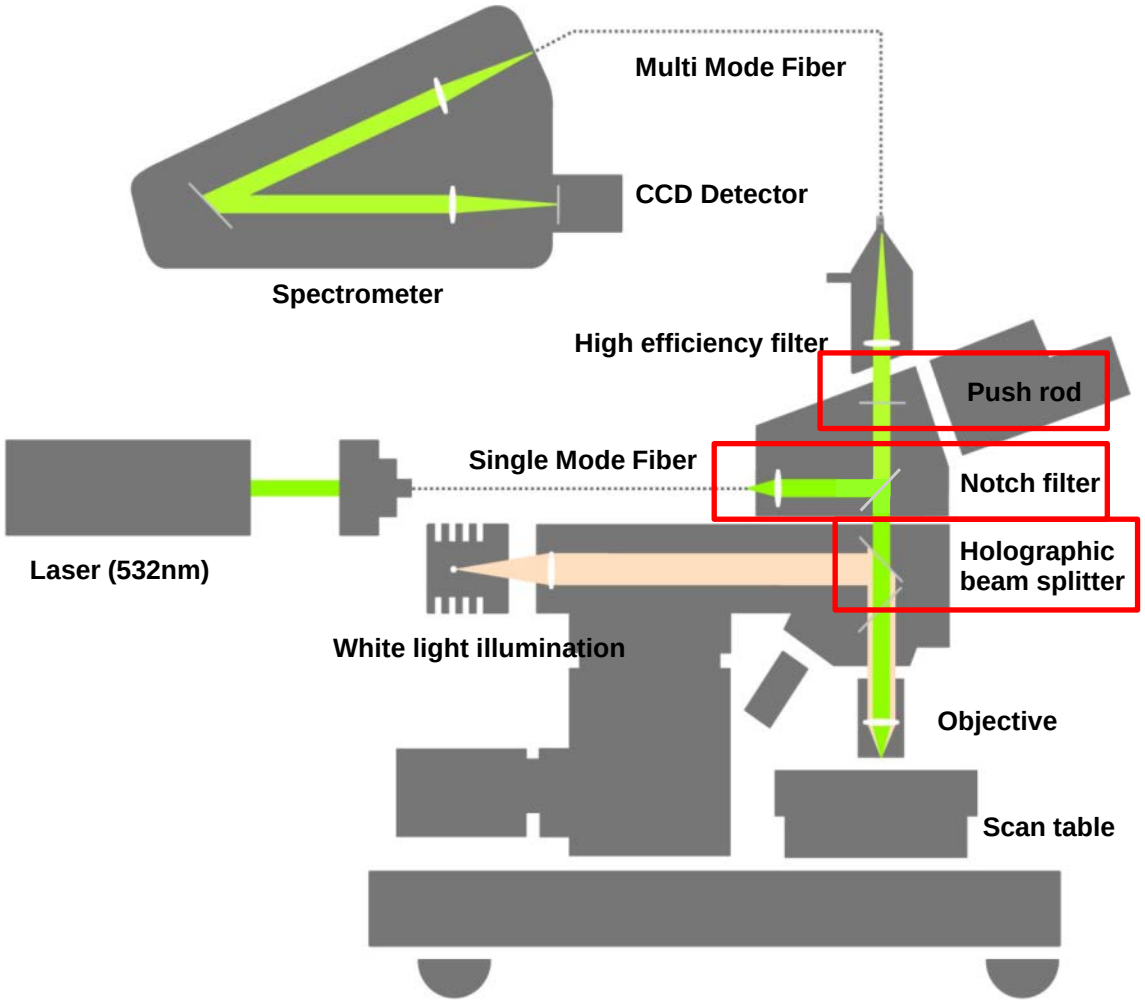


AFM mode



SNOM in transmission mode
(Inverted microscope)

Raman Mode



	Beam Splitter	Notch Filter	Push Rod
Image Mode	BF	IN	IN
Laser Mode	DF	OUT	IN
Raman Mode	DF	IN	OUT

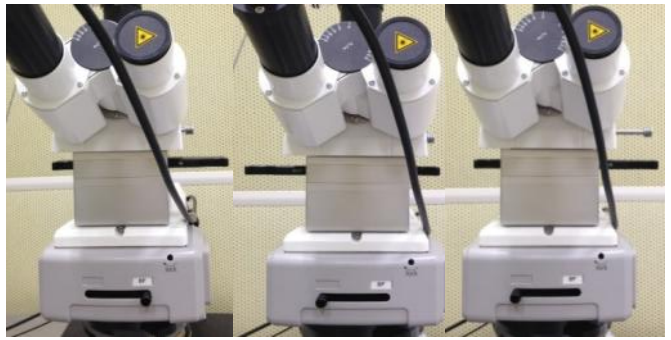
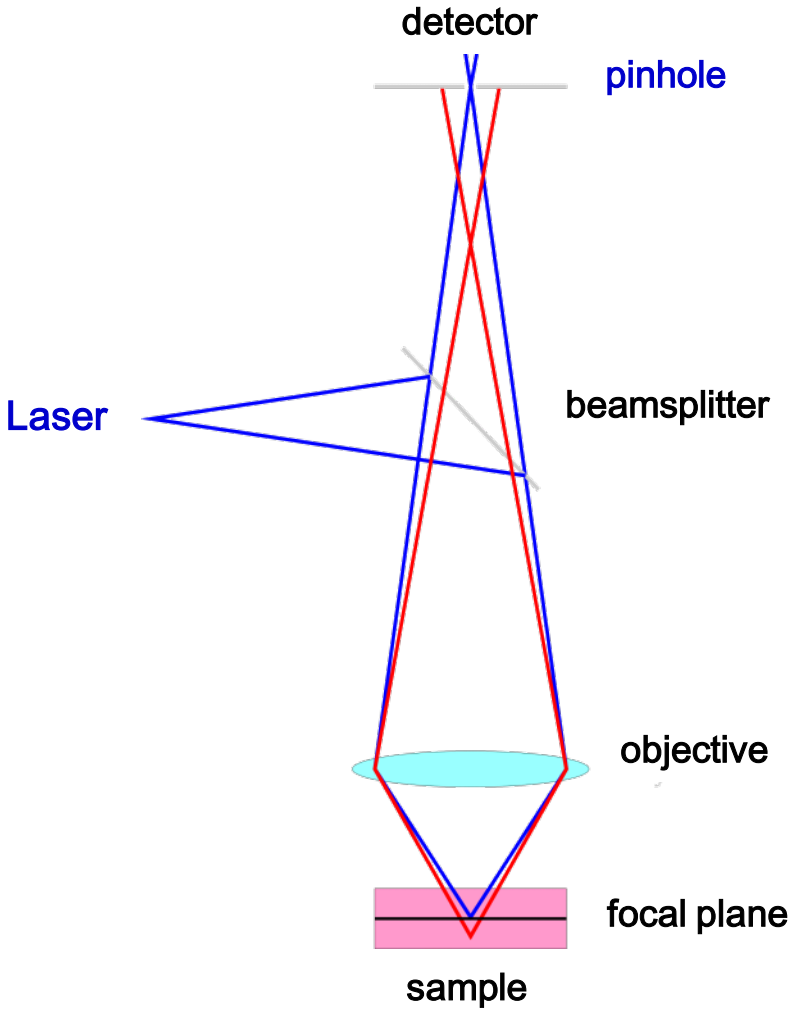


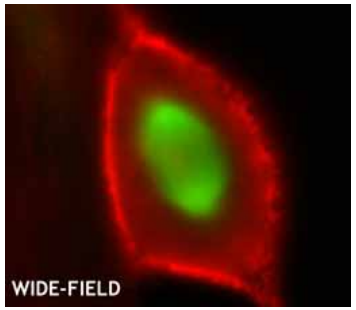
Image Mode Laser Mode Raman Mode

Confocal Microscopy

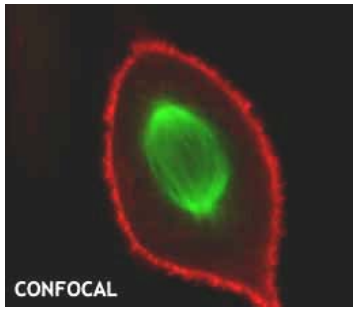


Confocal Microscopy

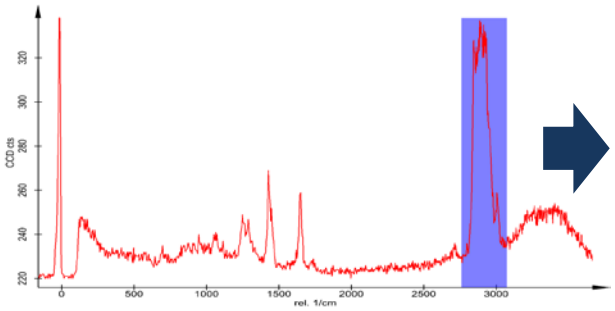
- much smaller background
- 3-D information
- slightly higher resolution

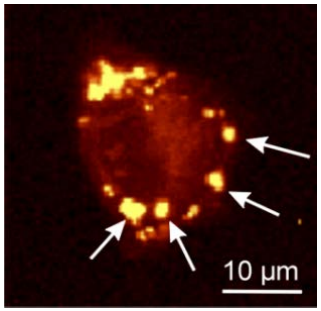


Without pinhole



With pinhole

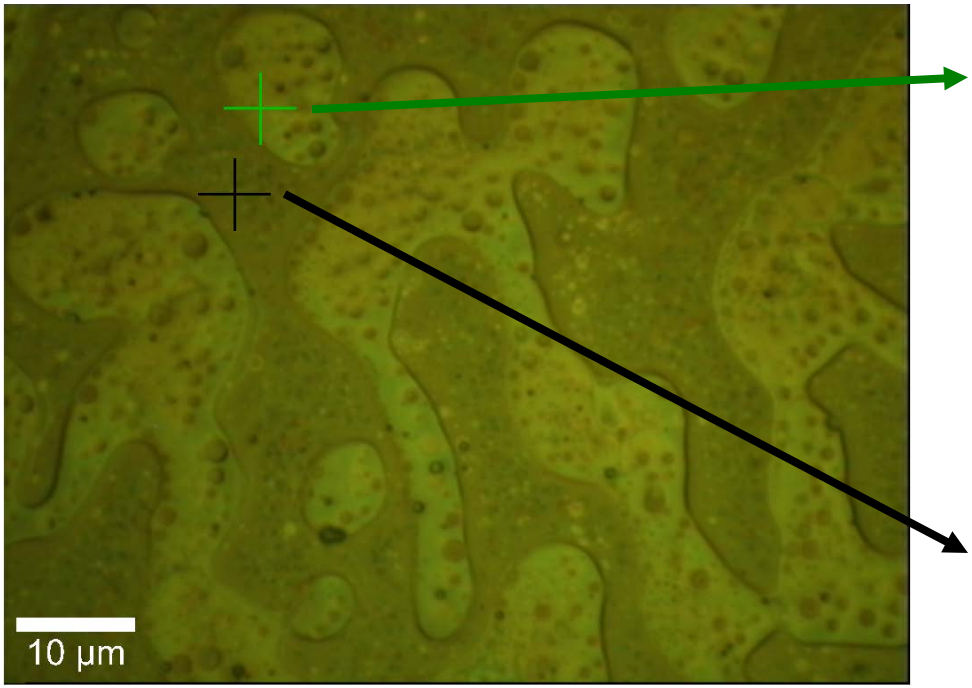




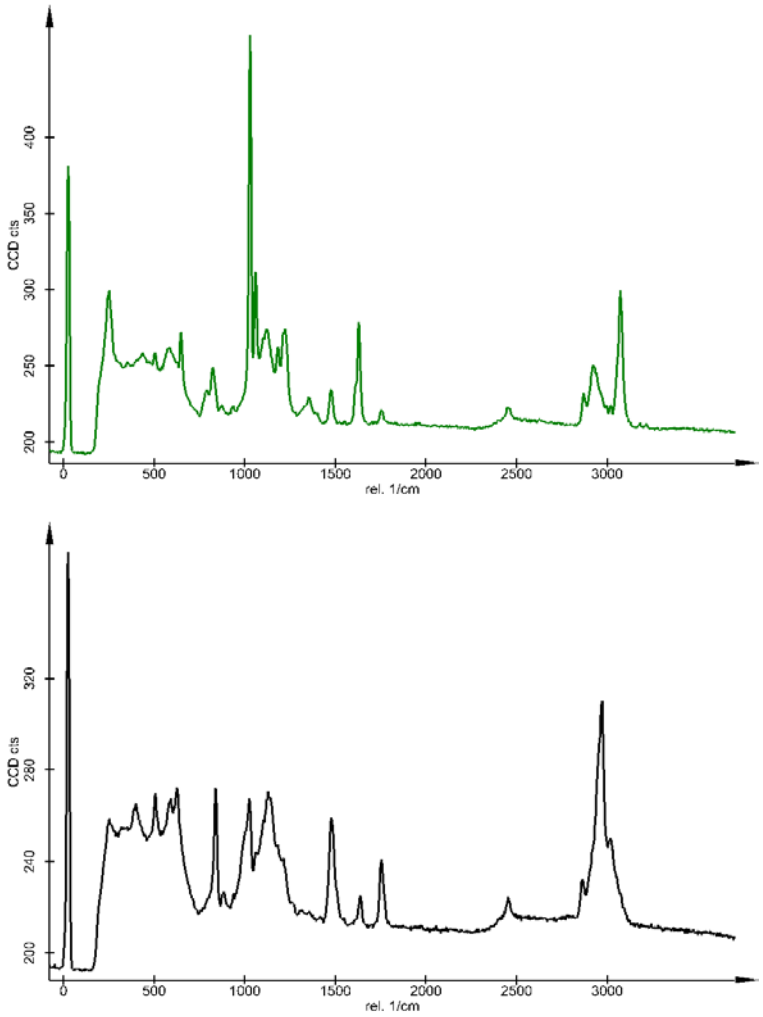
Confocal Microscopy + Raman Spectroscopy → 3-D Imaging with chemical sensitivity

Single Spectra

Sample: PS-PMMA on Glass



White light image



Single spectra of selected positions

Spectral Imaging (Mapping)

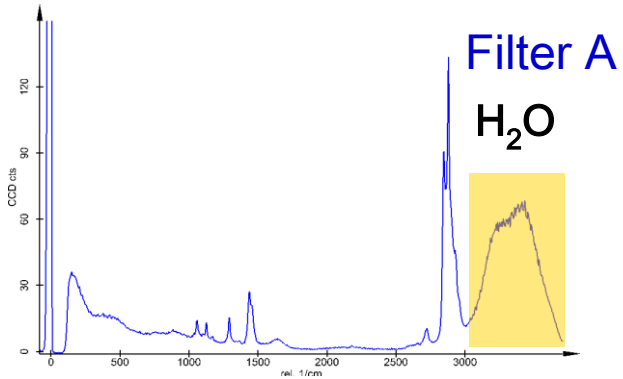
Sample: Oil/Water Emulsion

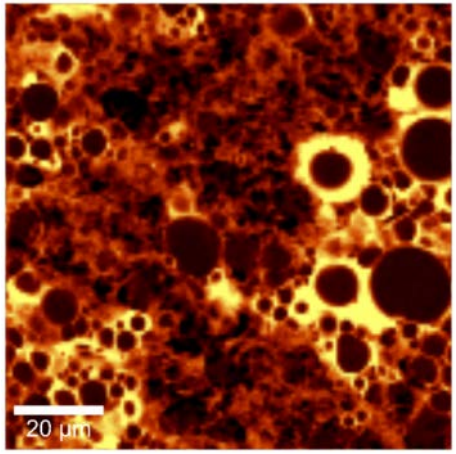
Excitation: 532nm, 2 mW

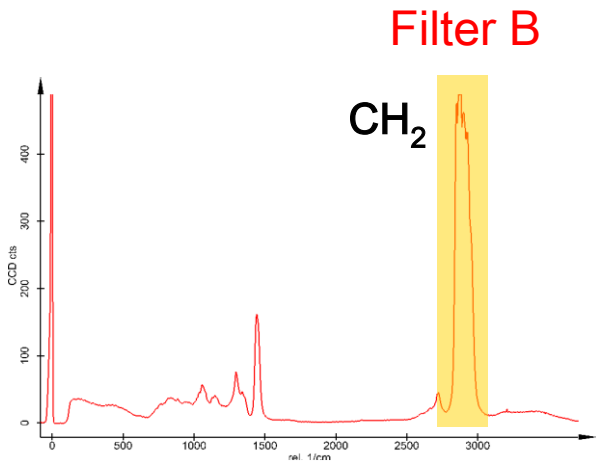
Scan Range: 100 μm^2

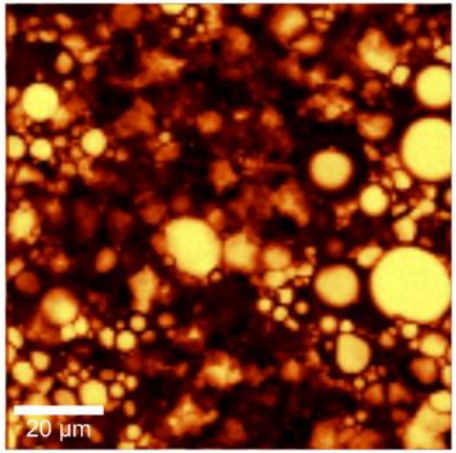
Resolution: 180 x 180 point

40ms/spectrum, 22 min









Optical Terminology

Working Distance(W.D.)

The distance between the front edge of the objective lens and the specimen surface (with the surface of the cover glass in case of the cover glass objective lens) when the Specimen is focused.

Numerical Aperture (N.A.)

The numerical aperture is a key factor to the performance of objective lens (resolving power, focal depth and brightness). The N.A. is determined by the following formula :

$N.A. = n \times \sin\theta$

n = refaction rate of the medium between specimen and objective lenses. (air : n=1, oil : n=1.515)

θ = angle which is made by the optical axis and refaction of the light farthest from the center of lens.

Resolving power

The resolving power of an objective lens is measured by its ability to differentiate two lines or points in an object. The larger the N.A., the higher the resolving power.

$$\varepsilon = 0.61 \times \frac{\lambda}{N.A.}$$ (Reyleigh formula)

λ : wavelength or radiation in use

N.A. : objective lens N.A.

Focal depth of Microscope

The focal depth refers to the depth of the specimen layer which is in sharp focus at the same time, even if the distance between the objective lens and the specimen plane is changed when observing and shooting the specimen plane by microscope.

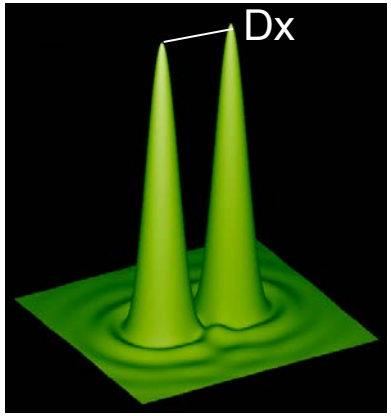
$$\pm D.O.F. = \frac{\omega \times 250{,}000}{N.A. \times M} + \frac{\lambda}{2(N.A.)^2} \quad (\mu m)$$

ω : resolving power

N.A. : objective lens N.A.

Resolution

♣ **Resolving power :** Capacity of an instrument to resolve two points which are close together.



$$\triangle x = \frac{0.61 \times \lambda}{N.A.}$$

Magnification	N.A.	Λ [nm]	△x [nm]
20x	0.4	532	811
		633	965
		785	1197
50x	0.8	532	405
		633	482
		785	598
100x	0.9	532	360
		633	429
		785	532

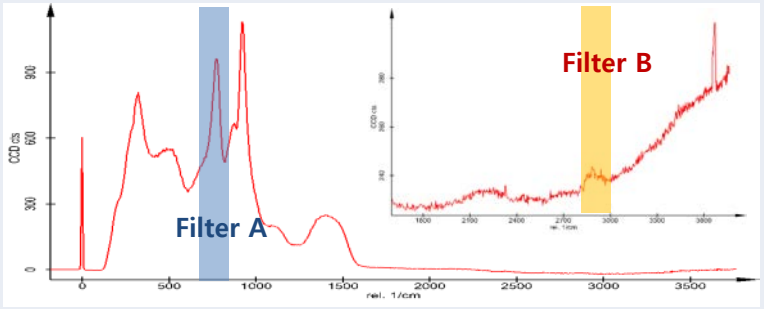
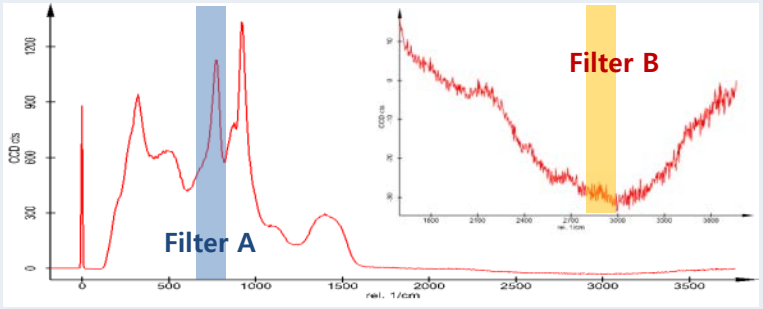
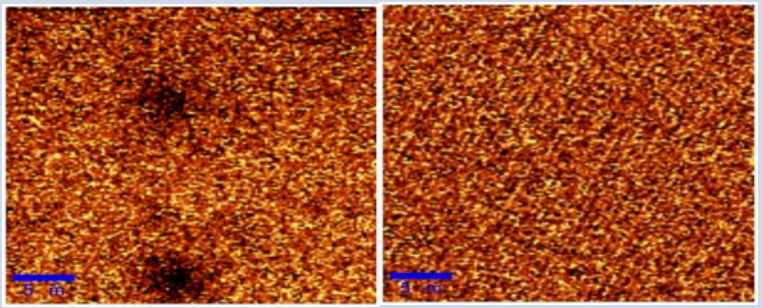
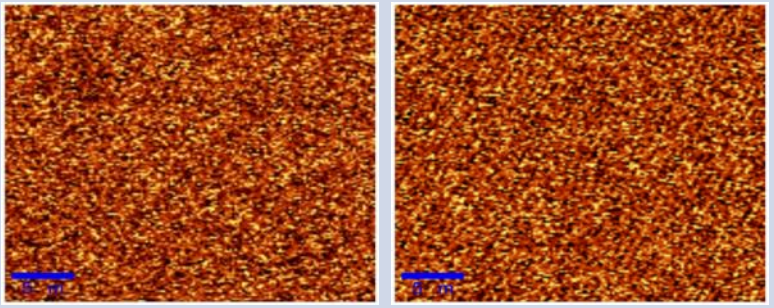
For example. Image scan : 30x30 ㎛ with Raman 532nm, 100x objective

$$\text{Point/Line (Line/Image)} = \frac{\text{Geometry Width (Height)}}{\text{Resolution } (\triangle x)} = \frac{30{,}000 \text{ nm}}{360 \text{ nm}} = 83 \text{ Point}$$

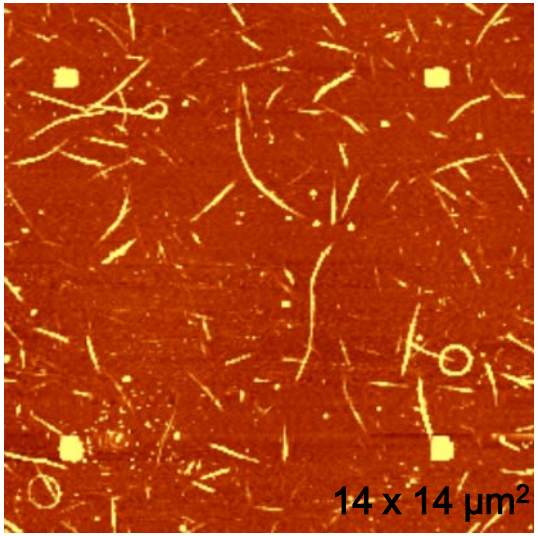
Image scan (Point/Line & Line/Image) will be 83 point and it is enough good.
To improve image scan, Image scan can be multiple three times by 83 points.
Why three times? That's a kind of statics.
The more you do image scan double, triple and four times, the more you get the better image.
However, measurement time is increasing. Triple is enough.

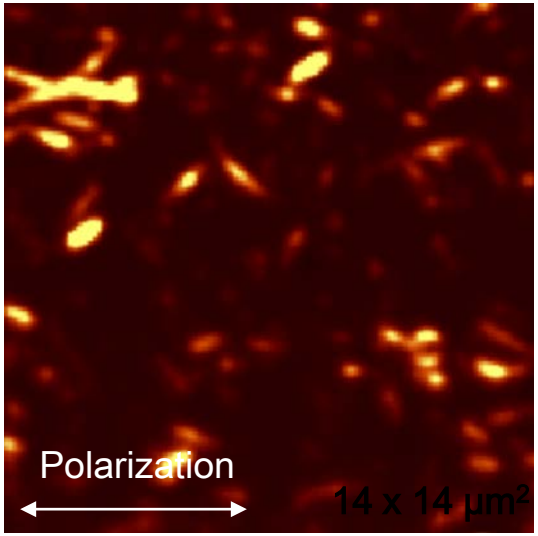
- **Optical Resolution: 200 nm/ laterally, 500 nm/ vertically**
- **Spectral Resolution: 0.02 wavenumbers**

Spectral Imaging

Name	A	B
Confocal Image		
Single Spectrum		
Scan Image		

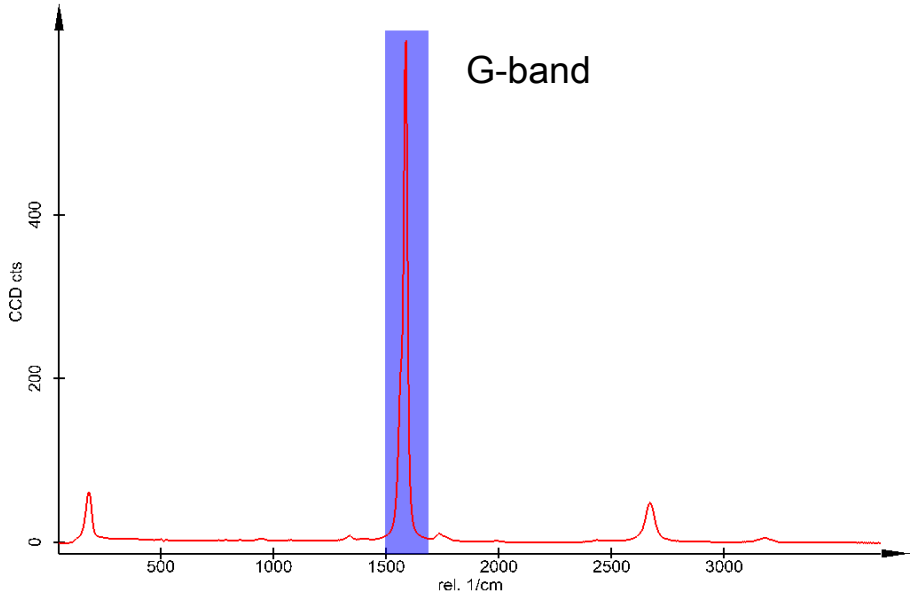
AFM & Raman





AFM AC Mode
topography image of nanotubes spin coated on Si substrate.

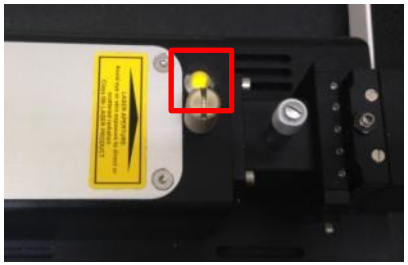
Raman Spectral Imaging
Nikon air objective, @ 532 nm
600 g/mm grating, BLZ 500 nm
150x150 spectra (pixel);
integration time: 50 ms/spectrum (total acquisition time = 20 min)



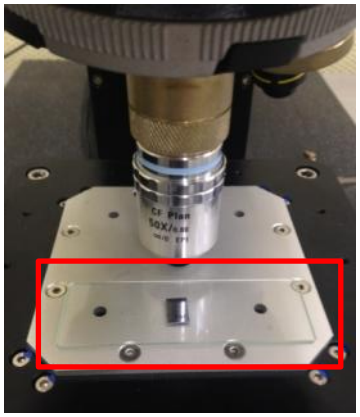
=> intensity of G-band depends on the polarization of the laser

Manual - Calibration

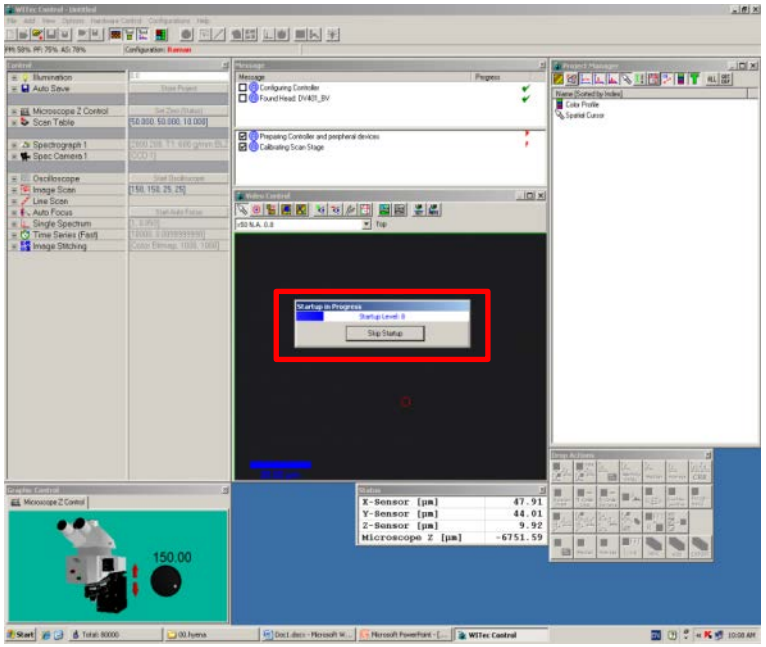
1. Laser on→ warm up (10 min)

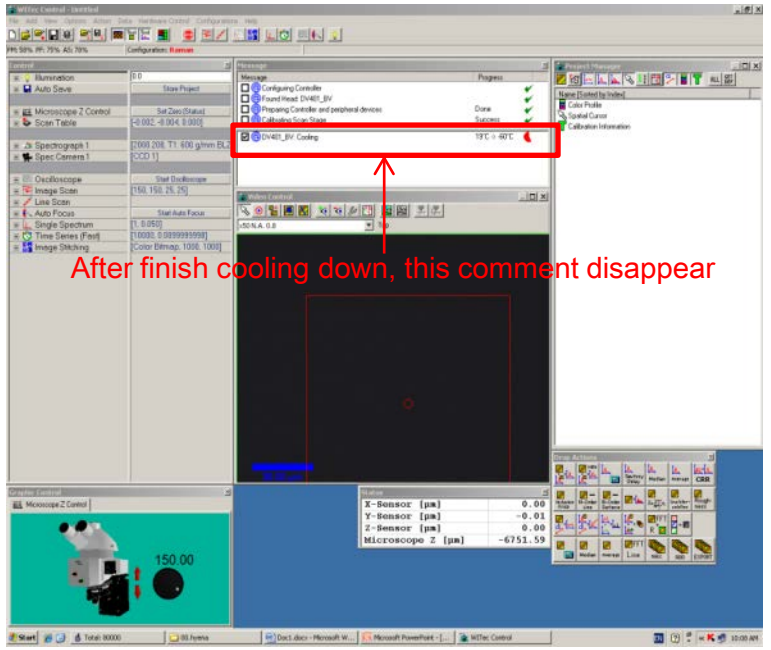


3. Put down a Si substrate(reference) on a slide glass



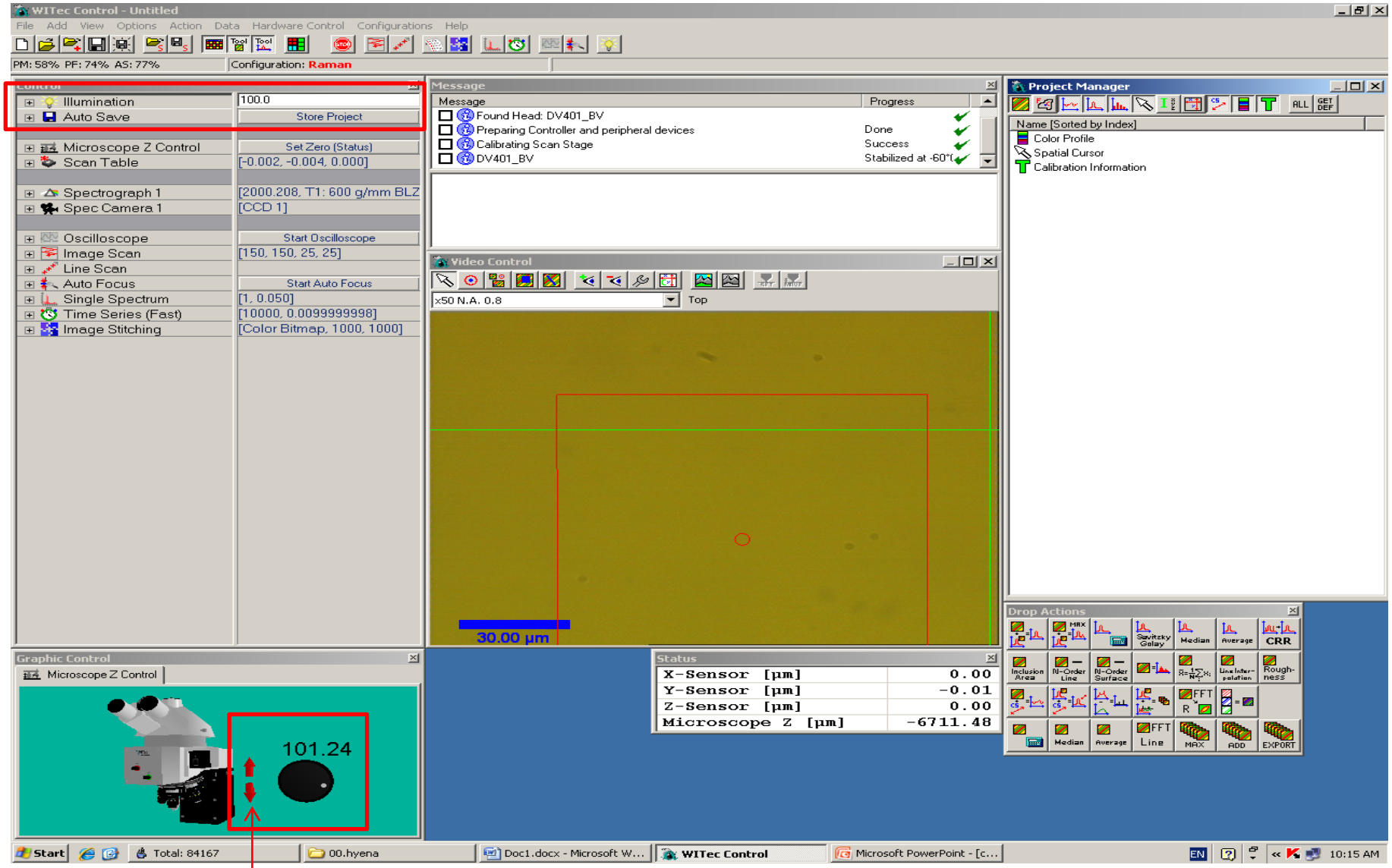
2. WITec Control Pro. START→ wait cooling down (10 min)





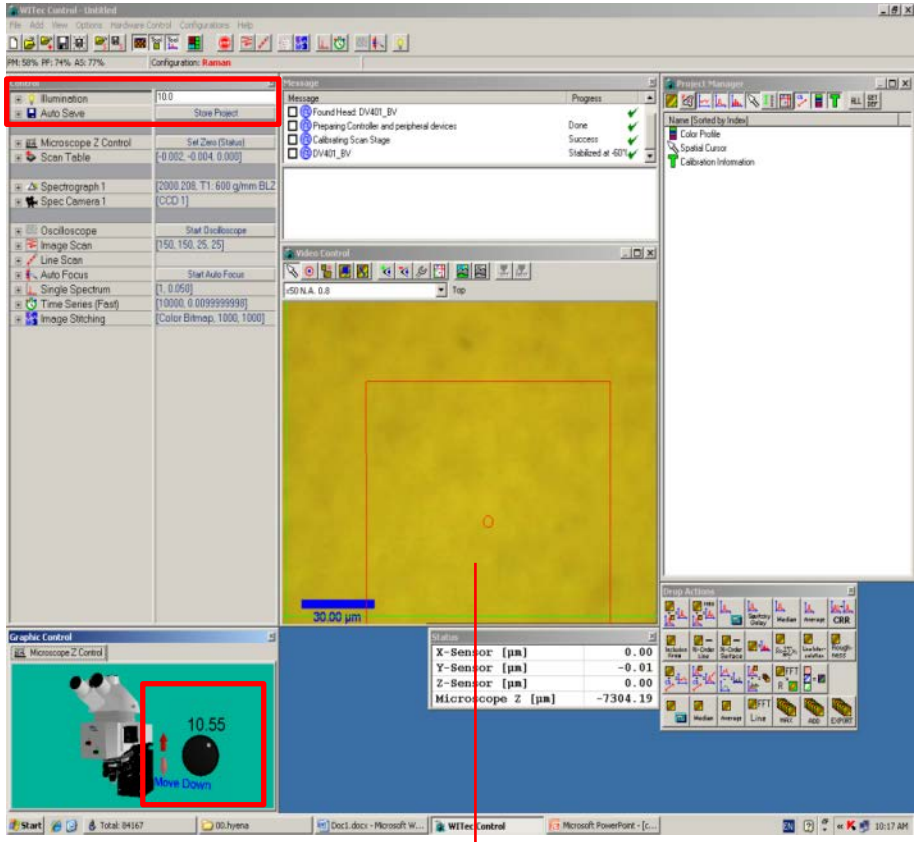
Manual - Calibration

4. Image Mode→ Control→ Illumination = 100, Speed 100→ down

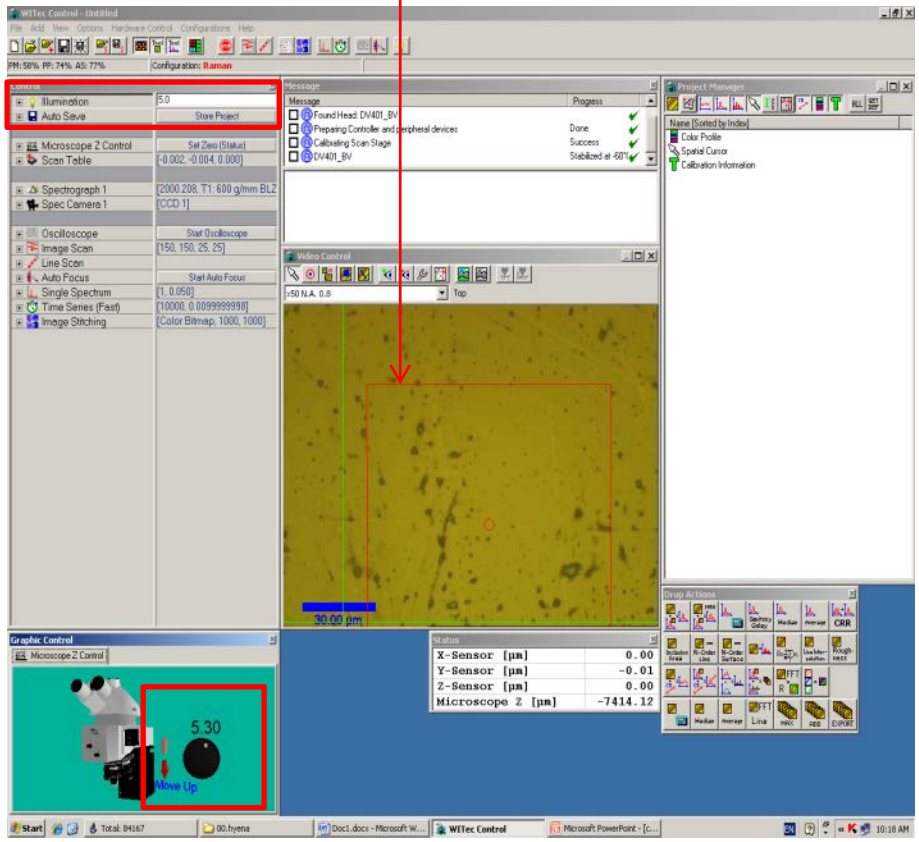


Manual - Calibration

5. Illumination = 50, Speed 50→ down→ Illumination = 30, Speed 30 → down→ Illumination = 10, Speed 10 → down→ Illumination = 5, Speed 5 → down (take a focus)

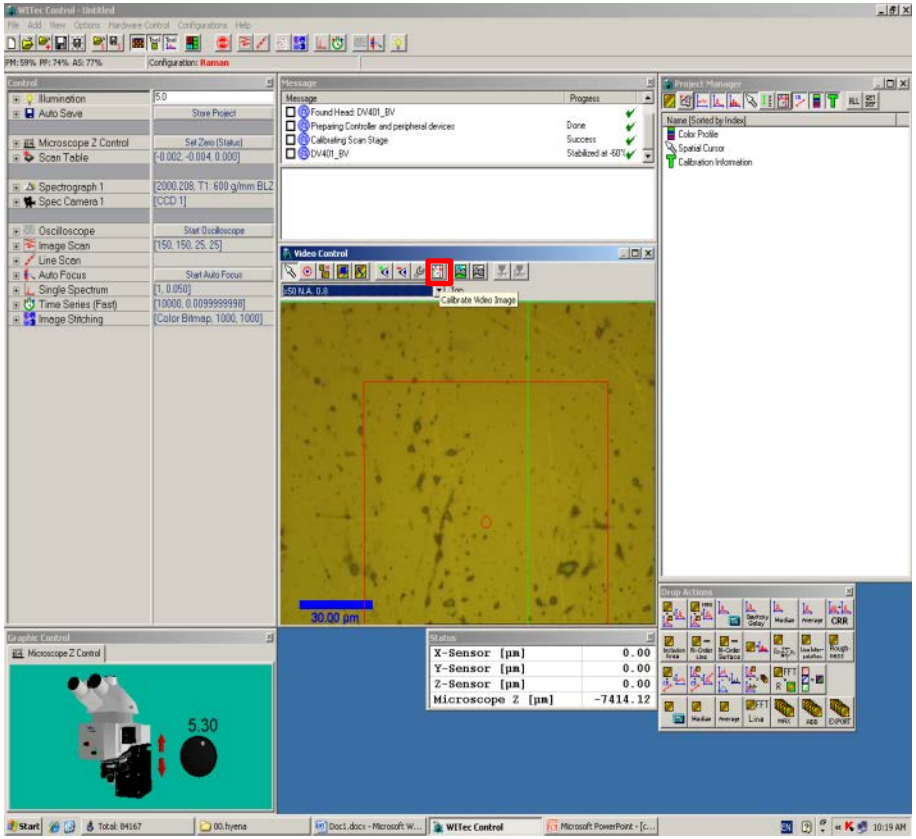


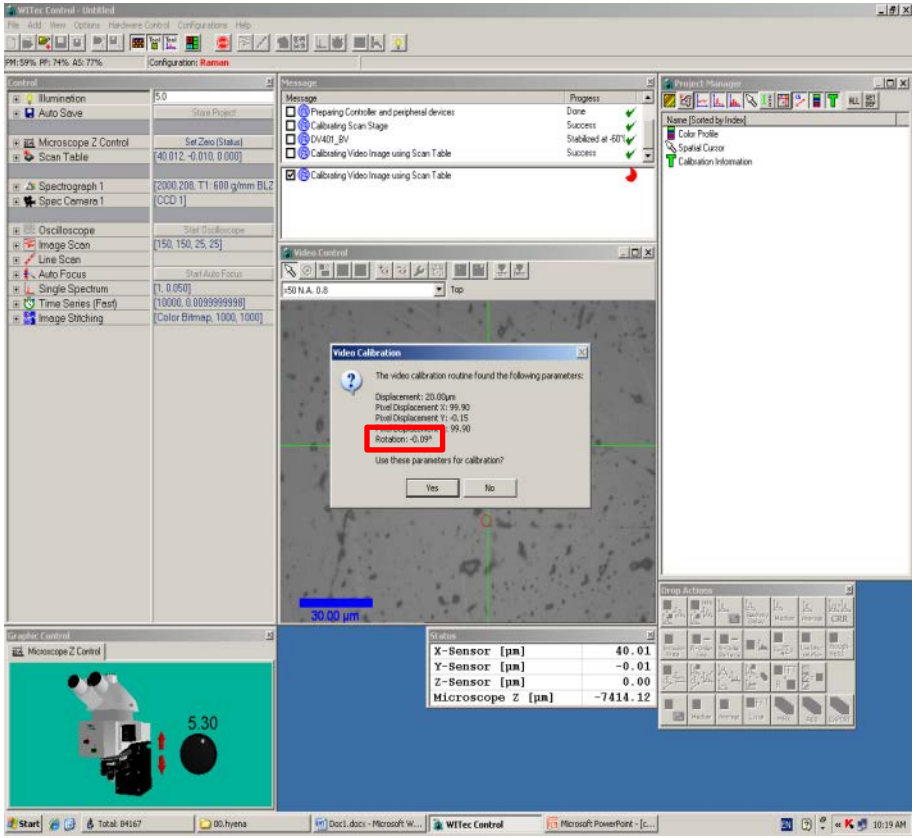
Diffuse layer



Manual - Calibration

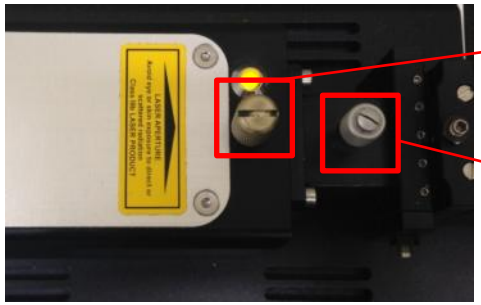
6. Video Control→ Calibrate Video Image click→ Rotation <±1

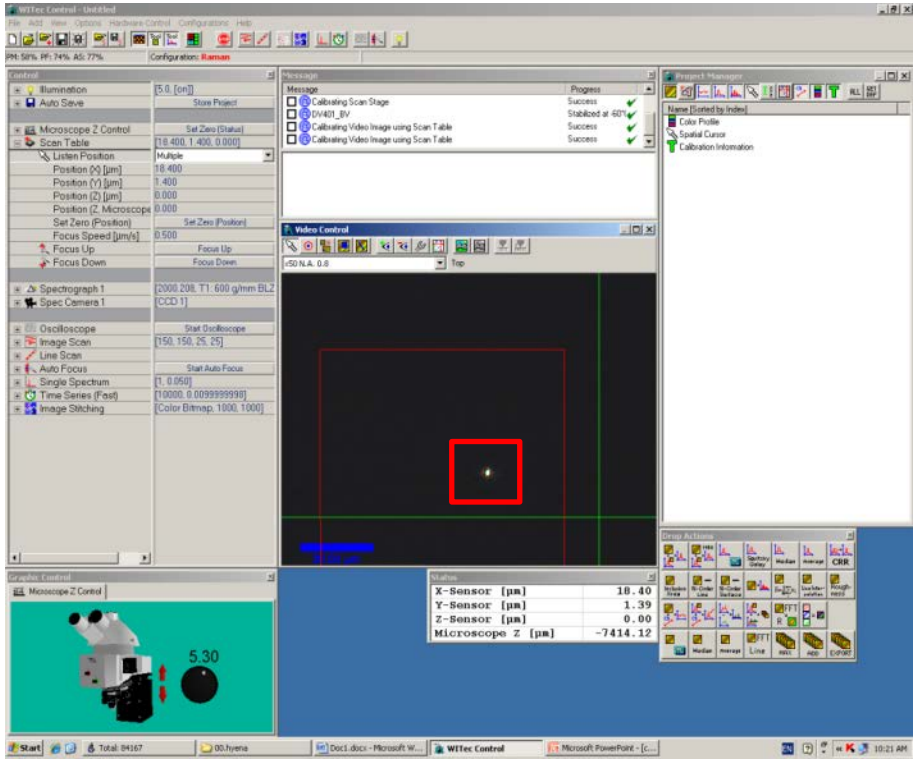


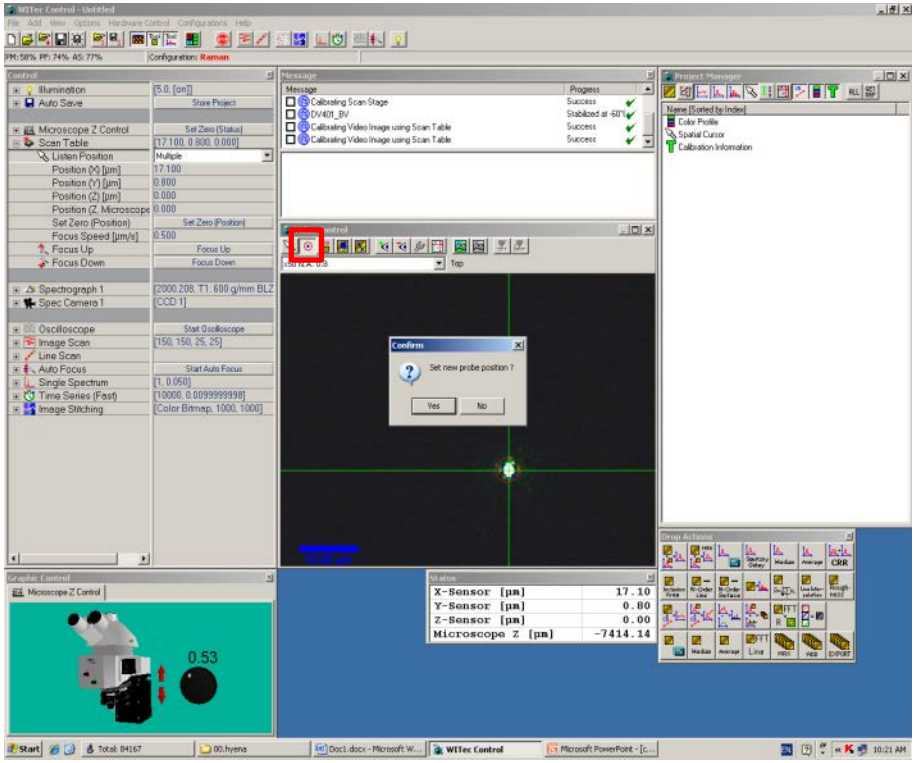


Manual - Calibration

7. Laser Mode→ Laser shutter on→ Slit decrease→ Laser position = red circle
(Video Control→ Set Video Origin click→ Laser position click)

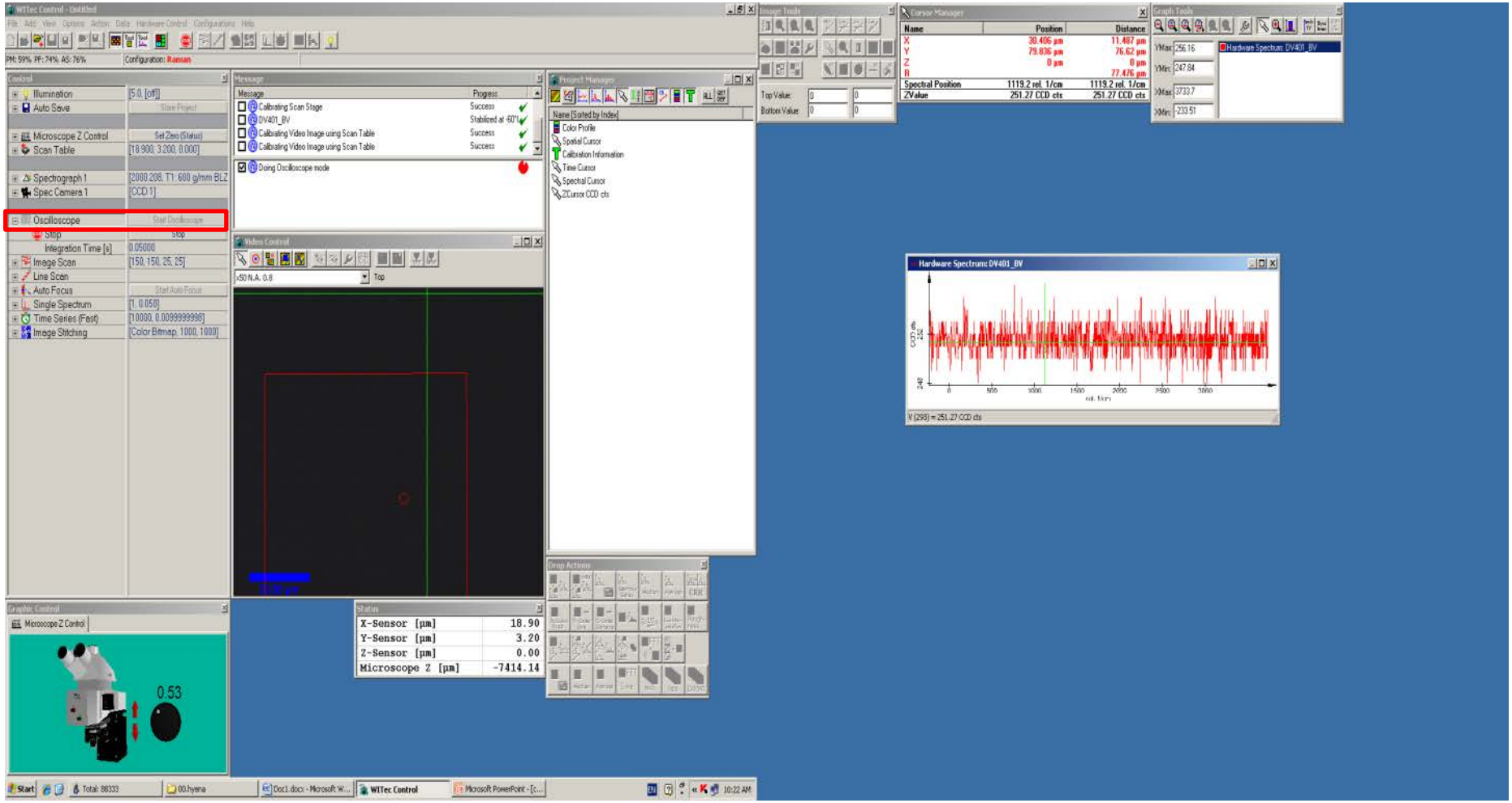






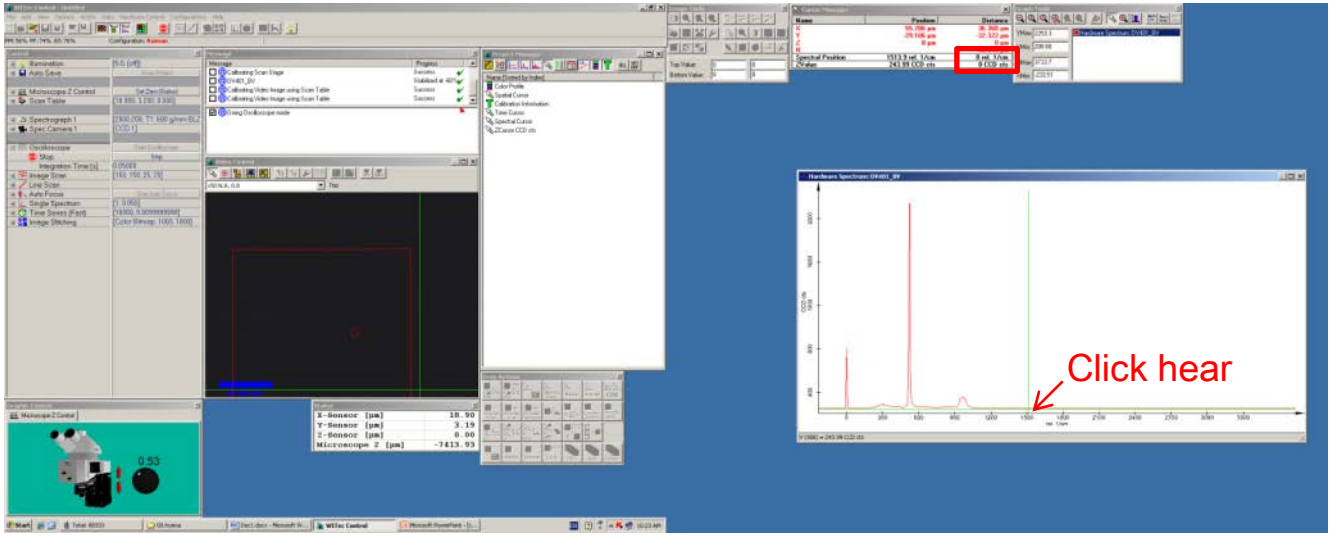
Manual - Calibration

8. Raman Mode→ Control→ Oscilloscope Start(Integration Time = 0.05 S)→ Slit increase

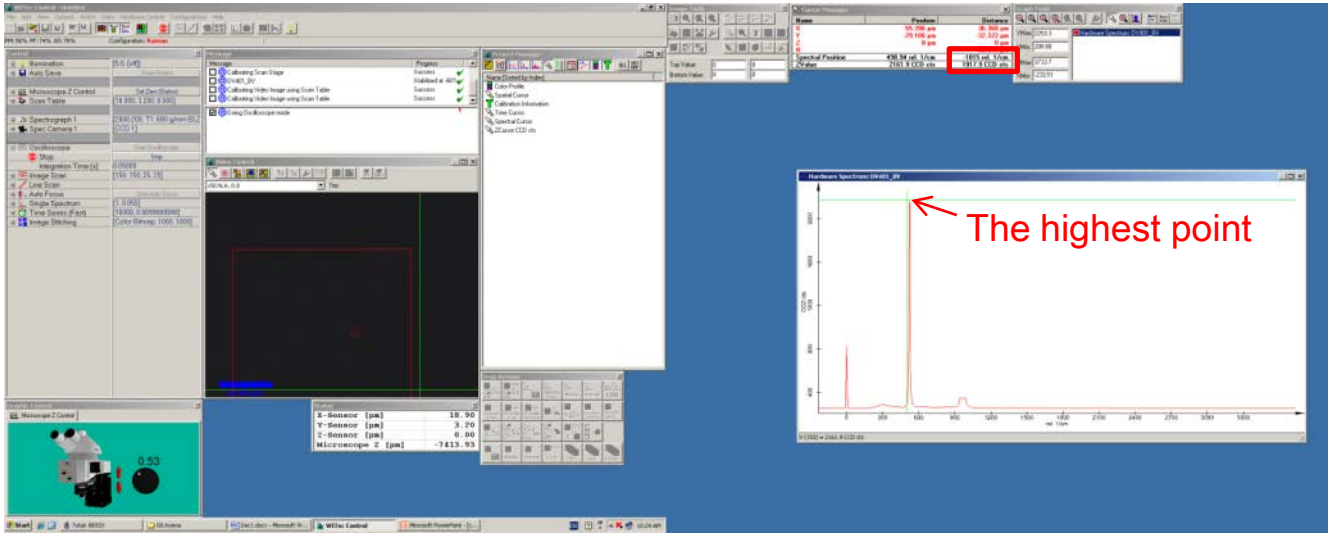


Manual - Calibration

9. Real Time Spectrum→ Lowest point click→ Cursor Manager→
Distance: Spectral Position = 0, Z Value =0

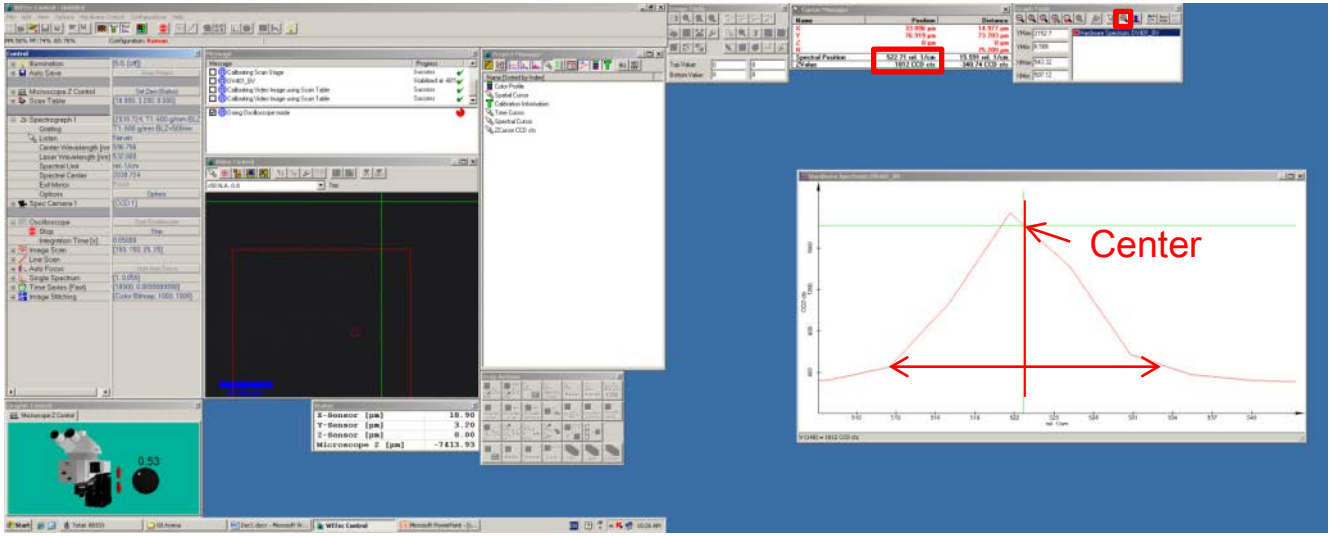


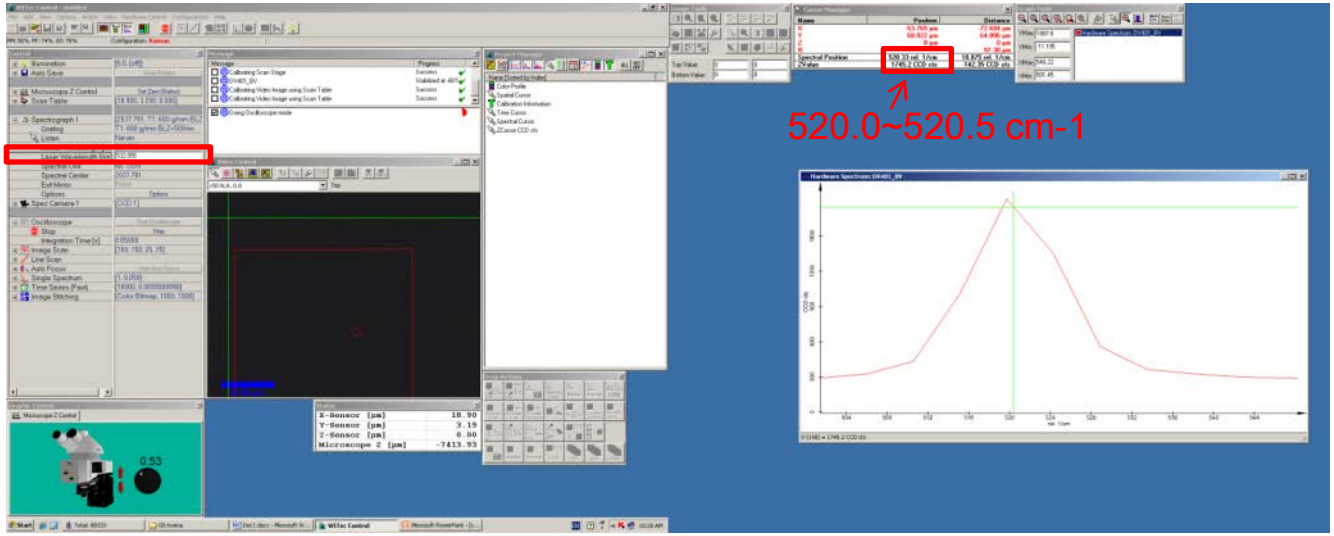
10. Real Time Spectrum→ Move a cursor to the highest point (520 cm-1 peak) →
Distance: Z Value ≥ 15000 CCD cts (If, Distance: Z Value < 15000 CCD cts→ Speed 0.5→ down or up)



Manual - Calibration

11. Graphic Tool→ Zoom click→ Zoom in 520 cm-1 peak→
Center point of 520 cm-1 peak = 520.0 ~520.5 cm-1(Position→ Spectral Position)→
Control→ Spectrograph→ Laser Wavelength change

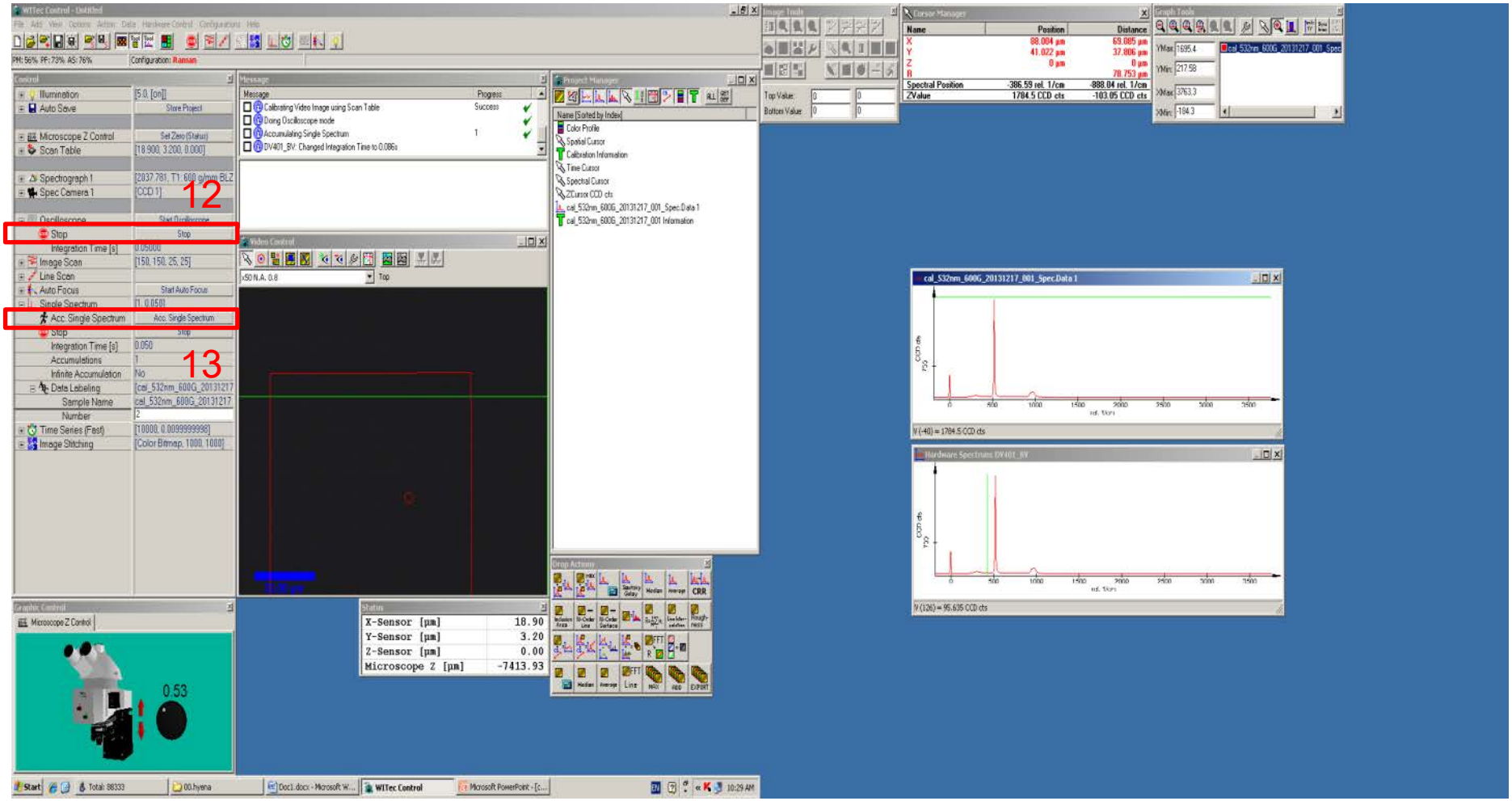




Manual - Calibration

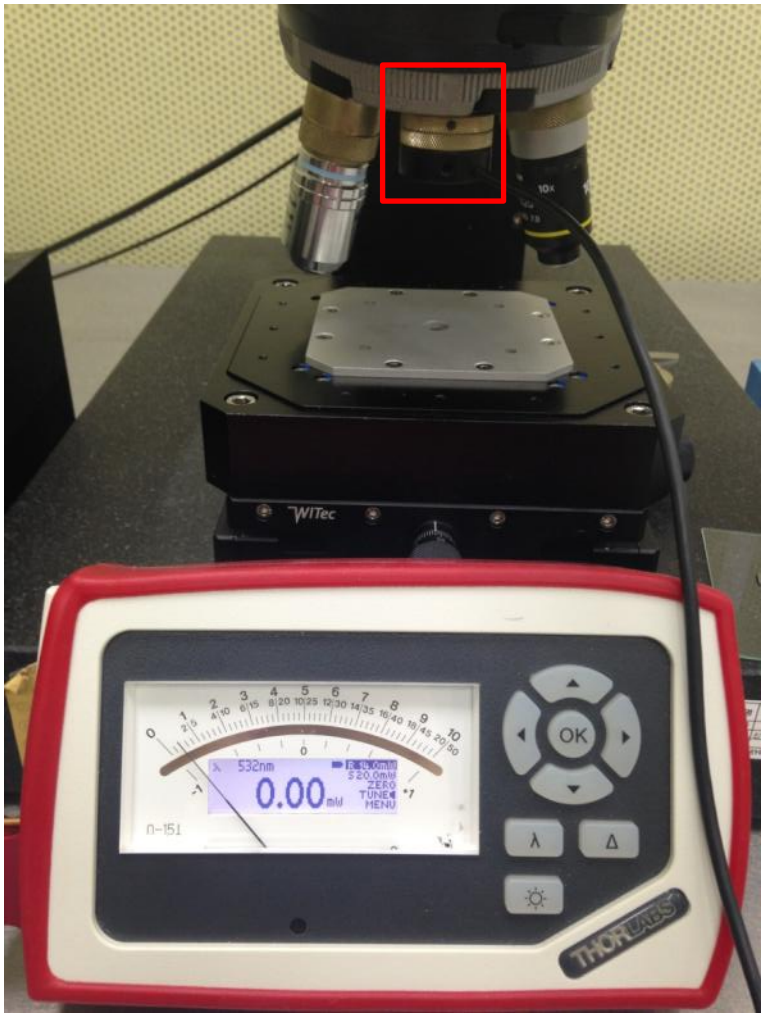
12. Oscilloscope Stop

13. Control→ Single Spectrum→ Acc. Single Spectrum click(Integration Time = 0.05 S)



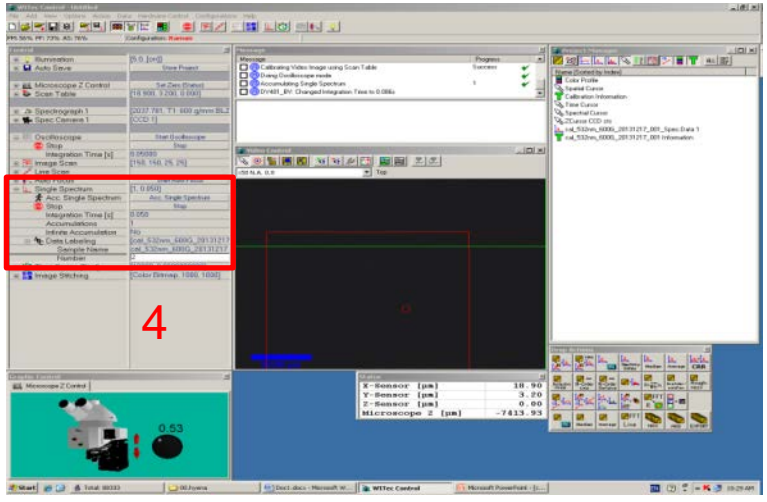
Manual - Calibration

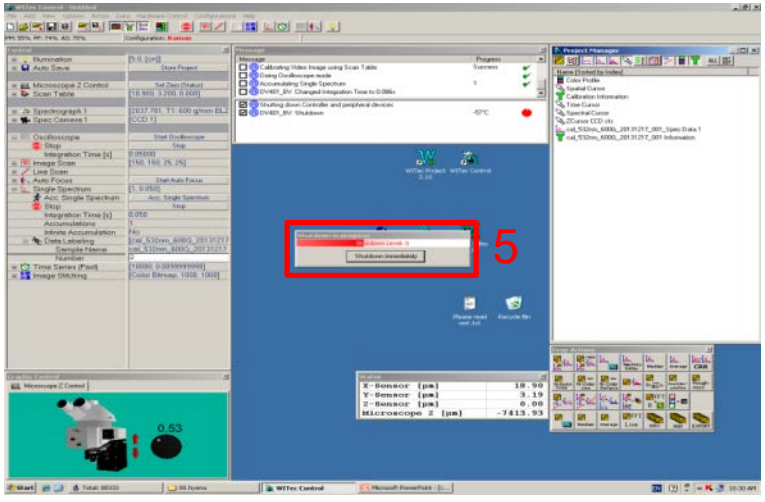
14. Laser Power Meter (power setting)- in Raman Mode



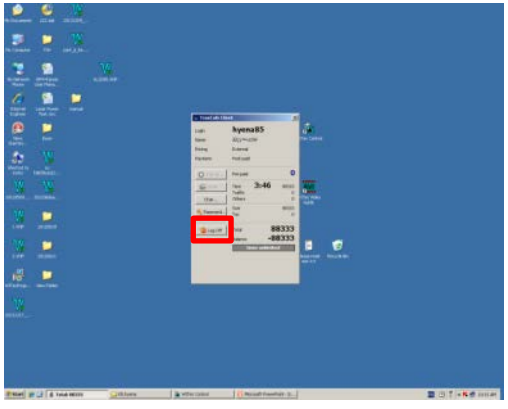
Manual - Calibration

1. After calibration, Image Mode→ Put down your sample on a slide glass
2. Take a focus (calibration 4,5 repeat)
3. Raman Mode→ Oscilloscope Start→ Increase main peak (Speed 0.5→ down or up)
4. Control→ Single Spectrum→ Parameter change → Acc. Single Spectrum click



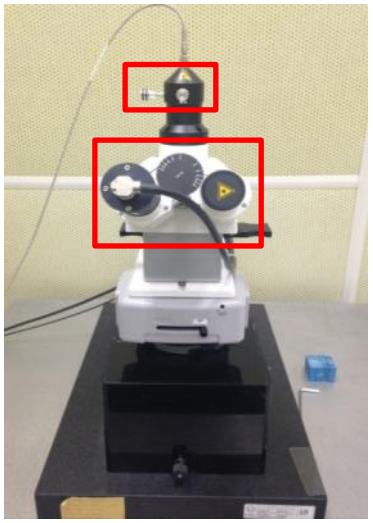


5. WITec Control Pro. CLOSE→ wait heating (10min)
6. Laser off
7. Log off True Cafe



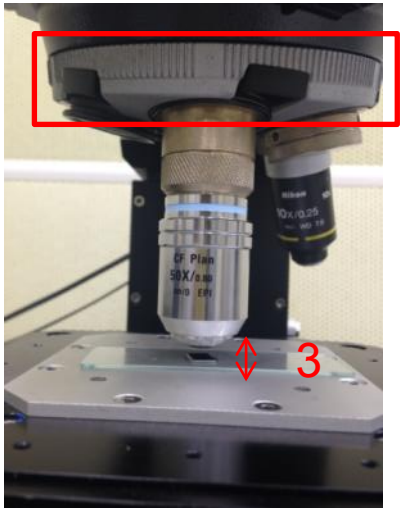
Cautions

1. Don't touch this part

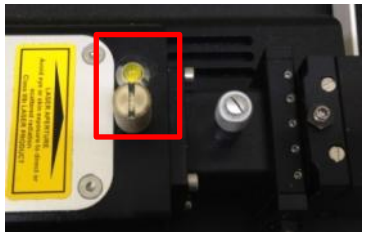


2. Hold this part, NOT lens

3. Lens up, whenever you change a sample



4. Laser off



5. Power Meter head is very sensitive

Charge

		Micro-Raman
UNIST students	Client(70%)	17,500/hr
	Self-user(50%)	12,500/hr

True Café using time	Charging time
0.1~0.7 hr	0.5 hr
0.8~1.2 hr	1.0 hr
1.3~1.7 hr	1.5 hr
1.8~2.0 hr	2.0 hr
…	…