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Title: TD-DFT Guided Advanced E-Eye Sensing Technique for On-site Quantification of Fe, Cr, F, and As in the Environmental, Biological, and Food Samples

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Author Questionnaire

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No.**

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, done.**

Current Protocol Length

Number of Steps: 25
Number of Shots: 52 (19 SC)

Introduction

Note: The script was filmed using the draft and their edits, the interview answers can be trimmed based on the answers here

1.1. Tapas Mandal:

The scope is to develop an indigenous portable device, named as E-Eye, for estimating of the toxicants in a multi-matrix sample.

What are the current experimental challenges?

1.4 Tapas Mandal**:**

The major experimental challenges with the POCT devices:

- Getting the selectivity of a targeted toxicant within the complex sample matrix, such as industrial effluents
- Detection of very low concentration
- The simultaneous detection of multiple analytes.

What significant findings have you established in your field?

1.5 Tapas Mandal**:**

The indigenously developed multiplexed 'E-Eye' device mimics the portable UV-Vis spectrophotometer. ~~Here, E-Eye stands for “Optical sensor”.~~

This user-friendly Point-Of-Care-Testing (POCT) device comprises the followings:

- Working principle of a chemical-based colorimetric sensor with high selectivity
- Working principle of an optical sensor with high precision
- Fundamentals of both the principles;- TD-DFT approach for chemical sensor

What new scientific questions have your results paved the way for?

1.9, Tapas Mandal**:** Our results answer the following fundamental science questions:

- The pathway from computational exercise to translation technology for precise detection of the toxicants
- Sensing mechanism

- Selectivity
- Range of detection and

What research questions will your laboratory focus on in the future?

1.10, Tapas Mandal: In the future, we will focus on computationally guided advanced POCT device design employing infrared spectroscopic principles. It will cover the following areas:

- Water quality management for the community services
- Detection of various noxious elements/compounds/biomarkers including the VOCs in:

- Agricultural applications
- Environmental applications
- Healthcare applications.

2. Computing and Validation of the Reaction's Sensing Mechanism

Demonstrator: Shrikant Kashyap

2.1. To begin, select a lock-and-key reaction that specifically recognizes the targeted analyte based on its fundamental chemistry **[1]**.

2.1.1. WIDE: Talent taking a seat at the computer station.

2.2. Launch the Gaussian 09 program to perform all the calculations using the Becke, 3-parameter, Lee-Yang-Parr (B3LYP) *(B3-L-Y-P)* hybrid method, and the Los Alamos National Laboratory 2 Double ζ *(zee)* basis set to obtain more accurate results in electronic computation involving the targeted analyte **[1-TXT]**.

2.2.1. SCREEN: 2.2.1.revised.mp4 00:00:39 to 00:00:59. **TXT: Analyte chosen here: Iron**

Authors: We need only a 20-second clip for each shot showing the calculations

~~2.3. Activate the self-consistent reaction field using the polarizable continuum model to include solvent effects and set the solvent parameter to water **[1]**.~~

2.3.1. ~~SCREEN: 2.3.1.SCREEN: 2.3.1 &2.4.1.mp4 00:20-00:40~~ .

2.4. In the Gaussian input, add SCRF=PCM and choose Water and confirm the setting and show the input containing geom=connectivity and bonded atom list in the connectivity block. Click Submit **[1]**. Compute the ground-state energy required to compute the optimized structure of the molecule **[2]**.

2.4.1. SCREEN: 2.3.1 &2.4.1.mp4 00:30-00:55.

2.4.2. SCREEN: 2.4.2 00:10-00:20.

2.5. Obtain excited-state electronic transitions using the time-dependent self-consistent field method, requesting a number of states equal to 60 **[1]**. With the same method and solvent model, compute and export the predicted ultraviolet absorption spectrum **[2]**.

2.5.1. SCREEN: 2.5.1 00:30-00:50.

2.5.2. SCREEN: 2.5.2. 00:20-00:40.

2.6. Perform a ground-state density functional theory geometry optimization using the B3LYP functional with the LanL2DZ *(lan-L-2-D-zee)* basis set to refine the molecular structure **[1]**. Export the optimized structures of 1,10-phenanthroline *(1-ten-phen-anthroline)* and the ferrous ion for subsequent studies **[1-TXT]**.

2.6.1. SCREEN: 2.3.1 &2.4.1.mp4 00:10-00:25

2.6.2. SCREEN: 2.5.2. 00:12-00:18. **TXT: Similarly, simulate the molecule structure of hexafluoroferrate (FeF6)**

3. Development of a Colorimetric Chemical Sensor

Demonstrator: Neha Majee

3.1. Transfer equal volumes of trisodium citrate dihydrate as masking agent, ascorbic acid as pH optimising agent, and 1,10-phenanthroline into a transparent glass culture tube **[1-TXT]**. Add 2 milliliters of stock solution to reach a total volume of 5 milliliters **[2]**

3.1.1. Talent pipetting equal volumes of each reagent. **TXT: Add 1 mL of each reactant into a 5 mL tube**

3.1.2. Talent adding 2 milliliters of the stock solution and gently mixing.

3.2. Place each sample and standard in a quartz cuvette with a 1-centimeter path length and keep all measurements at room temperature under standard laboratory conditions **[1]**. Measure iron in all standards and samples using UV–visible spectroscopy by scanning from 250 to 750 nanometers **[2 AND 3]**.

3.2.1. Talent filling quartz cuvettes with the sample.

3.2.2. Placing the sample in the UV spectrophotometer. **NOTE**: 3.2.2 AND 3.2.3 MAY HABE BEEN COMBINED

3.2.3. Talent adjusting the settings in the instrument system.

3.3. Configure the instrument settings to match the specified conditions by selecting

Scan speed as Medium **[1]**. Set Measurement type to Absorbance **[2]**, adjust Slit width to 5 nanometers **[3]** and set the Detectors to Direct **[4]**.

3.3.1. SCREEN: Open **Scan settings** and choose **Medium** under **Scan speed**. **NOTE**: All the screen captures in the section 3 are shot by the videographer, please use them

3.3.2. SCREEN: In **Measurement mode**, select **Absorbance**.

3.3.3. SCREEN: In **Optics**, set **Slit width** to **5.0 nanometers** and confirm.

3.3.4. SCREEN: In **Detector options**, select **Direct** and apply the configuration.

3.4. Perform baseline correction using a blank that contains the same water matrix as the samples **[1]**.

3.4.1. SCREEN: Select **Baseline Correction**, insert the matrix blank, and confirm **Apply baseline**.

3.5. After UV-absorbence is recorded, lambda max is observed at 510 nm which confirms the formation of the complex **[1]**.

3.5.1. Cuvette is placed in the UV-Vis spectrophotometer and showing 510 nm as peak.

4. Design and Fabrication of the E-Eye-Enable POCT Kit's Prototype

Demonstrator: Shrikant Kashyap

4.1. To begin, procure a LDR, a white LED, a LCD, a microcontroller or microprocessor board, and precision resistors **[1]**.

4.1.1. Talent laying out all components on an electrostatic discharge-safe mat and checking them against a bill of materials.

4.2. Using the three-dimensional printed box, align the LED and the light-dependent resistor directly opposite each other across the cuvette slot **[1]**.

4.2.1. Talent mounting the LED on one wall of the box and the light-dependent resistor on the opposite wall, facing each other.

4.3. Complete the circuit by installing a 3.3 kilo-ohm series resistor for the LED, routing clean wiring, and connecting the LCD model number 11497 **[1]**.

4.3.1. Talent soldering the 3.3 kiloohm resistor to the LED lead and connecting power and ground.

4.4. Interface the sensor with the microcontroller across a voltage divider that converts light-dependent resistor resistance changes into a measurable voltage **[1]**. ~~Using the Arduino Integrated Development Environment version 1.8.19 *(1 point 8 point 19)*, set up data acquisition to read analog inputs from analog pins A0 to A3 **[2]**~~.

4.4.1. Talent wiring the light-dependent resistor in a voltage divider configuration between the supply and an analog input, with the reference resistor to ground.

4.5. Install analog noise-suppression filters by adding a 10 kiloohm series or biasing resistor and a 1 microfarad capacitor to form a low-pass network at each analog input **[1]**. Place components close to the microcontroller pins and route short, twisted signal leads to improve stability **[2]**.

4.5.1. Talent soldering the 10 kiloohm resistor and the 1 microfarad capacitor to the analog input node. **NOTE**: 4.5.1 and 4.5.2 were combined

4.5.2. Close-up of the filtered analog lines being secured near the microcontroller headers.

4.6. Print the printed circuit board when the prototype schematic and layout are finalized and electrically verified **[1]**.

4.6.1. Shot of printing in progress.

4.7. Supply power by turning on the electronic switch and record the sensor response as resistance or converted voltage from each channel **[1]**. Observe the light path as the LED passes light through the reaction chamber and the light-dependent resistor senses transmitted intensity corresponding to ferroin formation **[2]**. Correlate the measured resistance with ferrous ion concentration to generate a calibration plot, and enter the resulting equation into the device algorithm for real-time reporting **[3]**.

4.7.1. Talent flipping the main power switch and confirming the power indicator.

4.7.2. Close-up of the illuminated LED shining through a cuvette containing the reaction mixture toward the light-dependent resistor.

4.7.3. SCREEN: 4.7.4.

4.8. Review the complete circuit drawing to confirm final wiring and channel assignments, then print and assemble the finished device enclosure **[1]**.

4.8.1. SCREEN: 4.8.1.

5. **Development of a Multiplexed Device**

5.1. Create four dedicated slots in the three-dimensional box to hold four cuvettes in fixed positions **[1]**. Isolate each detection slot both optically with opaque physical barriers and electronically with separate LED and light-dependent resistor pairs and individual voltage dividers to prevent signal overlap **[2]**.

5.1.1. Talent inserting opaque partitions between cuvette bays inside the enclosure.

5.1.2. Talent wiring four independent voltage divider circuits, one per channel, and confirming isolation with a multimeter.

5.2. Assign one slot to a single analyte and keep the assignment fixed **[1]**. Ensure the cuvette placed in each slot contains only the reagents specific to that analyte **[2]**.

5.2.1. Talent labeling each slot with the analyte name and affixing matching labels to the corresponding cuvettes.

5.2.2. Talent inserting analyte-specific cuvettes into their designated slots.

~~5.3. Repeat the interfacing, programming, filtering, printing, powering, and calibration steps for the four-channel configuration, and connect all four LED–light-dependent resistor pairs in parallel at the board level while maintaining separate voltage dividers per channel **[1]**. Verify that code reads all four analog channels and that wiring supports future expansion to additional slots if needed **[2]**.~~

~~5.3.1. Talent connecting four sensor channels to the board's analog inputs and tying power and ground busses in parallel.~~

~~5.3.2. SCREEN: Update the Arduino sketch to read analog pins A0 to A3, compute concentration with the stored calibration for each channel, and upload the program.~~

5.4. Turn on the main switch to supply power so that all four LEDs illuminate simultaneously **[1]**. Allow the light-dependent resistors to sense each analyte in parallel and display the results for all channels at once **[2]**.

5.4.1. Talent pressing the power switch and confirming all channel indicators illuminate.

5.4.2. Show the multi-channel readout with four concurrent measurements and corresponding calculated concentrations **NOTE**: 5.41. AND 5.4.2 WERE COMBINED

Results

6. Results

6.1. The multiplexed prototype accurately quantified total iron concentrations in peanut samples, with results closely matching the UV-Vis spectrophotometer **[1]** and remaining above the limit of detection in all cases **[2]**.

6.1.1. LAB MEDIA: Figure 7A. Video editor: Highlight the green bars.

6.1.2. LAB MEDIA: Figure 7A. Video editor: Highlight the red horizontal line.

6.2. The chromium and fluoride levels measured in hair samples using the multiplexed prototype were comparable to UV-Visible measurements**[1]**.

6.2.1. LAB MEDIA: Figure 7B and C. Video editor: Highlight the green bars.

6.3. Arsenic levels in Brahmaputra water samples increased progressively across sample numbers and were reliably detected by both the multiplexed prototype and UV-Vis system **[1]**.

6.3.1. LAB MEDIA: Figure 7D. Video editor: Sequentially Highlight the green bars

- **analyte**

Pronunciation link: No confirmed link found
IPA: /ˈæn·əˌlaɪt/
Phonetic Spelling: AN-uh-lyt

- **hybrid** *(as in "hybrid method")*

Pronunciation link: https://www.merriam-webster.com/dictionary/hybrid
IPA: /ˈhaɪ·brɪd/
Phonetic Spelling: HY-brid

- **basis set**

Pronunciation link: No confirmed link found *(as a compound phrase; each word separately is common)*
IPA: /ˈbeɪsɪs ˌsɛt/
Phonetic Spelling: BAY-sis set

- **polarizable** *(as in "polarizable continuum model")*
Pronunciation link: No confirmed link found
IPA: /ˌpə·lər·əˈzaɪ·bəl/
Phonetic Spelling: puh-LER-uh-ZY-buhl

- **continuum**
Pronunciation link: https://www.merriam-webster.com/dictionary/continuum
IPA: /kənˈtɪn·ju·əm/
Phonetic Spelling: kun-TIN-you-um

- **geometry** *(in computational-chemistry context: molecular geometry)*
Pronunciation link: https://www.merriam-webster.com/dictionary/geometry
IPA: /dʒiˈɑ·mə·tri/
Phonetic Spelling: jee-AH-muh-tree

- **optimization**
Pronunciation link: https://www.merriam-webster.com/dictionary/optimization
IPA: /ˌɑp·təˌmɪˈzeɪ·ʃən/
Phonetic Spelling: op-tuh-mi-ZAY-shun

- **ferrous**
Pronunciation link: https://www.merriam-webster.com/dictionary/ferrous
IPA: /ˈfɛr·əs/
Phonetic Spelling: FERR-us

- **phenanthroline** *(1,10-phenanthroline)*
Pronunciation link: No confirmed link found
IPA (approximate): /ˌfɛn·ænˈθroʊˌliːn/
Phonetic Spelling: fen-an-THROH-leen

- **hexafluoroferrate** *(FeF_6 complex)*
Pronunciation link: No confirmed link found
IPA (approximate): /ˌhɛk·səˌflʊə·roʊˈfɛr·eɪt/
Phonetic Spelling: HEK-suh-fluh-roh-FERR-ate

- **trisodium** *(as in "trisodium citrate dihydrate")*
Pronunciation link: No confirmed link found
IPA: /traɪˈsoʊ·di·əm/
Phonetic Spelling: try-SOH-dee-um

- **citrate**
Pronunciation link: https://www.merriam-webster.com/dictionary/citrate
IPA: /ˈsɪtrɪt/ or /ˈsɪ·treɪt/
Phonetic Spelling: SIH-trit or SIH-trayt

- **dihydrate**

Pronunciation link: https://www.merriam-webster.com/dictionary/dihydrate
IPA: /ˌdaɪˈhaɪ·dreɪt/
Phonetic Spelling: dye-HY-drate

- **cuvette**

Pronunciation link: https://www.merriam-webster.com/dictionary/cuvette
IPA: /kjuˈvɛt/
Phonetic Spelling: kew-VET

- **nanometer** *(as in "nanometers" for spectral range)*

Pronunciation link: https://www.merriam-webster.com/dictionary/nanometer
IPA: /ˈnæ·noʊˌmi·tər/
Phonetic Spelling: NAN-oh-mee-ter

- **absorbance**

Pronunciation link: https://www.merriam-webster.com/dictionary/absorbance
IPA: /əbˈzɔr·bəns/ or /əbˈzɔːr·bəns/
Phonetic Spelling: ub-ZOR-buhns

- **resistor**

Pronunciation link: https://www.merriam-webster.com/dictionary/resistor
IPA: /rɪˈzɪs·tər/
Phonetic Spelling: ri-ZIS-ter

- **microcontroller**

Pronunciation link: No confirmed link found
IPA (approximate): /ˌmaɪ·kroʊ·kənˈtroʊ·lər/
Phonetic Spelling: MY-kroh-kun-TROH-ler

- **capacitor**

Pronunciation link: https://www.merriam-webster.com/dictionary/capacitor
IPA: /kəˈpæs·ɪ·tər/
Phonetic Spelling: kuh-PAS-ih-ter

- **multimeter**

Pronunciation link: https://www.merriam-webster.com/dictionary/multimeter
IPA: /ˌmʌl·taɪˈmi·tər/
Phonetic Spelling: mul-tye-MEE-ter

- **baseline** *(as in "baseline correction")*

Pronunciation link: https://www.merriam-webster.com/dictionary/baseline
IPA: /ˈbeɪsˌlaɪn/
Phonetic Spelling: BAY-s-line

- **lambda** *(as in "λ-max" / "lambda max")*

Pronunciation link: https://www.merriam-webster.com/dictionary/lambda
IPA: /ˈlæm·də/
Phonetic Spelling: LAM-duh