The Application of PAT to Complex Molecule Synthesis

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C&BD CHEMISTRY AND BIOTECHNOLOGY DEVELOPMENT

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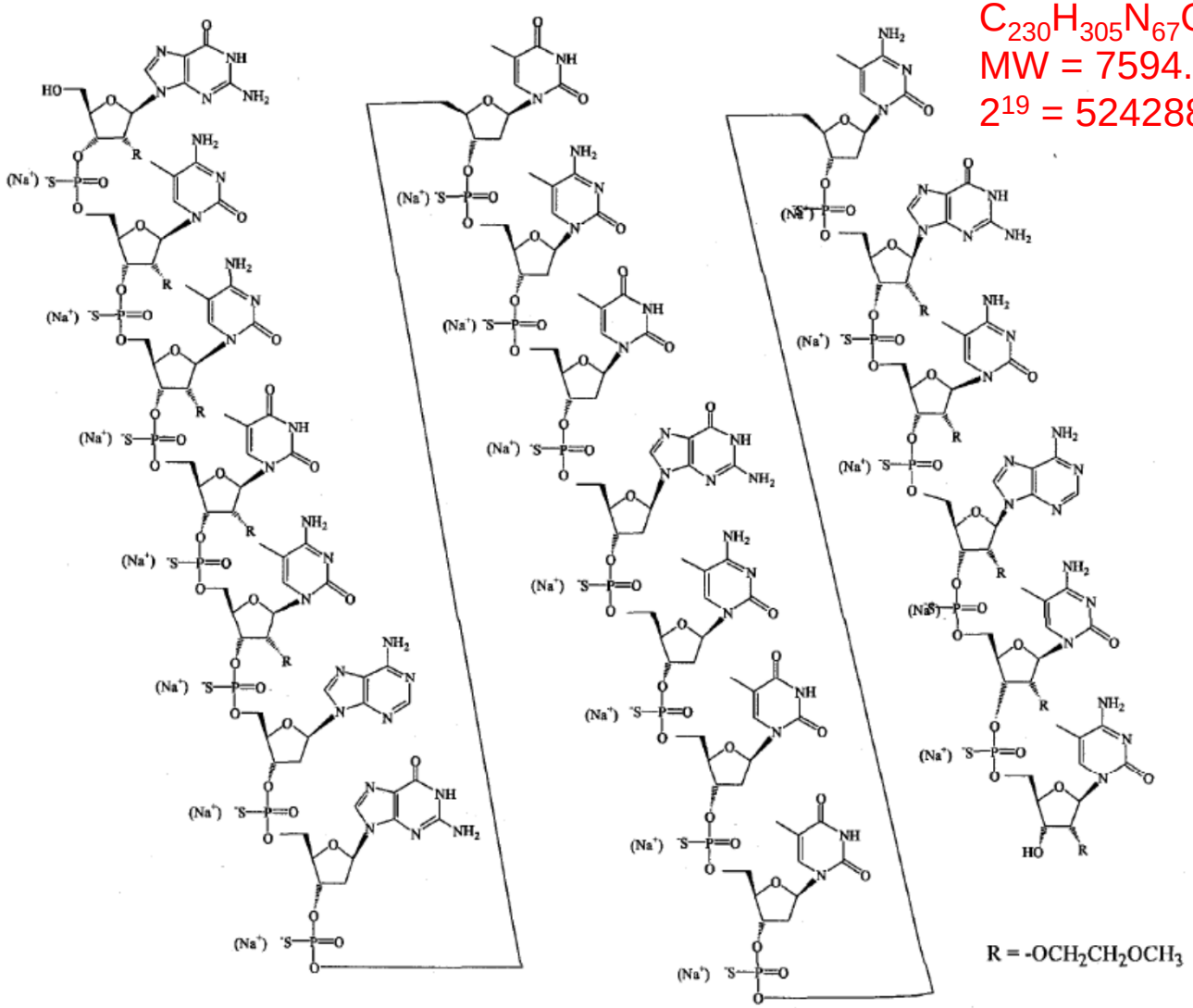
- **Case study: Oligonucleotides**
- **Oligonucleotides are large small molecules that are excluded from current ICH guidelines**
- **About 175 oligonucleotides are currently in clinical trials**
  - **1st generation antisense oligonucleotides**
  - **2nd generation antisense oligonucleotides**
  - **siRNA**
  - **miRNA**
  - **Aptamers**
  - **LNAs**

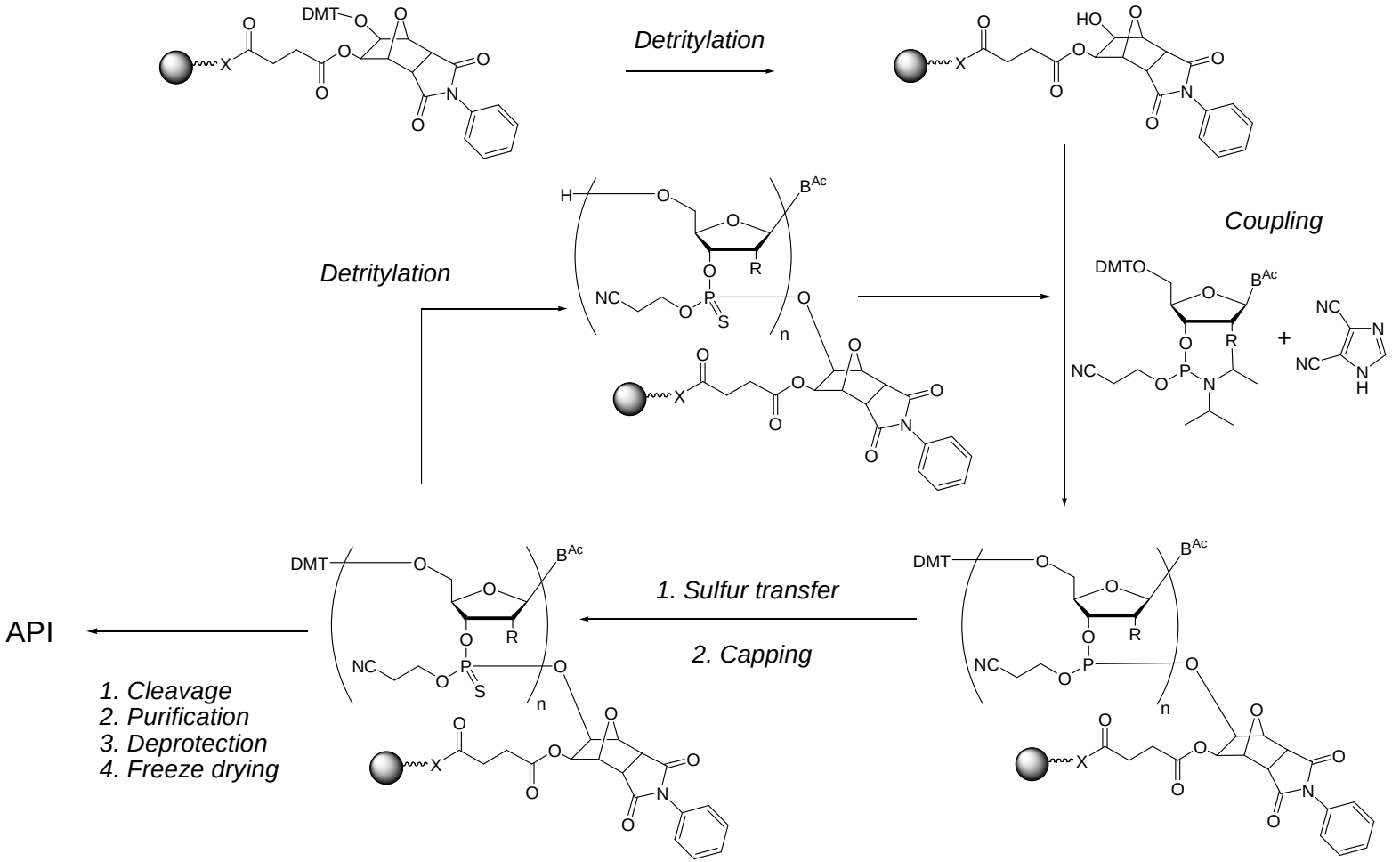
Mipomersen structure



$C_{230}H_{305}N_{67}O_{122}P_{19}S_{19}Na_{19}$
MW = 7594.9 Da
2^{19} = 524288 isomers at P

R = $-OCH_2CH_2OCH_3$

Mipomersen (antisense oligonucleotide)



QbD and PAT for oligonucleotides

Automated synthesis

- **Solid phase (*cf* peptides) synthesis**
  - **Excess reagents used to ensure completion of conversion at each step**
- **If PAT can be used to prove delivery of correct amidite (base) can this be used in lieu of final testing (at least for sequence)?**
  - **What about leaks, *etc*?**
    - prove that what enters the column was delivered
- **Can PAT control process?**
  - **Mipomersen is prepared according to “global optimum” for oligonucleotides.**
  - **No large scale manufacture of an oligonucleotide has been required before**
  - **Could reduce cost by reducing excess reagents, solvent use, recycle loops, *etc***

Oligonucleotide PAT

- **It is possible despite**
  - **Dilute solutions onto column**
  - **Very dilute samples from column**
  - **Fluorescence of samples (problem with Raman)**
  - **Standard platform (GE Unicorn) supporting only 12 input signals (solved using 3rd party integrator)**
- **Just monitoring inputs and outputs is insufficient – PAT is not just about the use of sensors! QbD requires a much deeper understanding of processes**
  - **What happens inside synthesis column? Why are excess reagents required?**
  - **Why does scale up beyond a certain level fail? Why is solid phase peptide synthesis above 60 cm column size performed in stirred beds?**

Magnetic Resonance Imaging

- **All images recorded at Cavendish Laboratories, University of Cambridge and used with permission of Dr Mick Mantle**

Mipomersen MRIs.mov

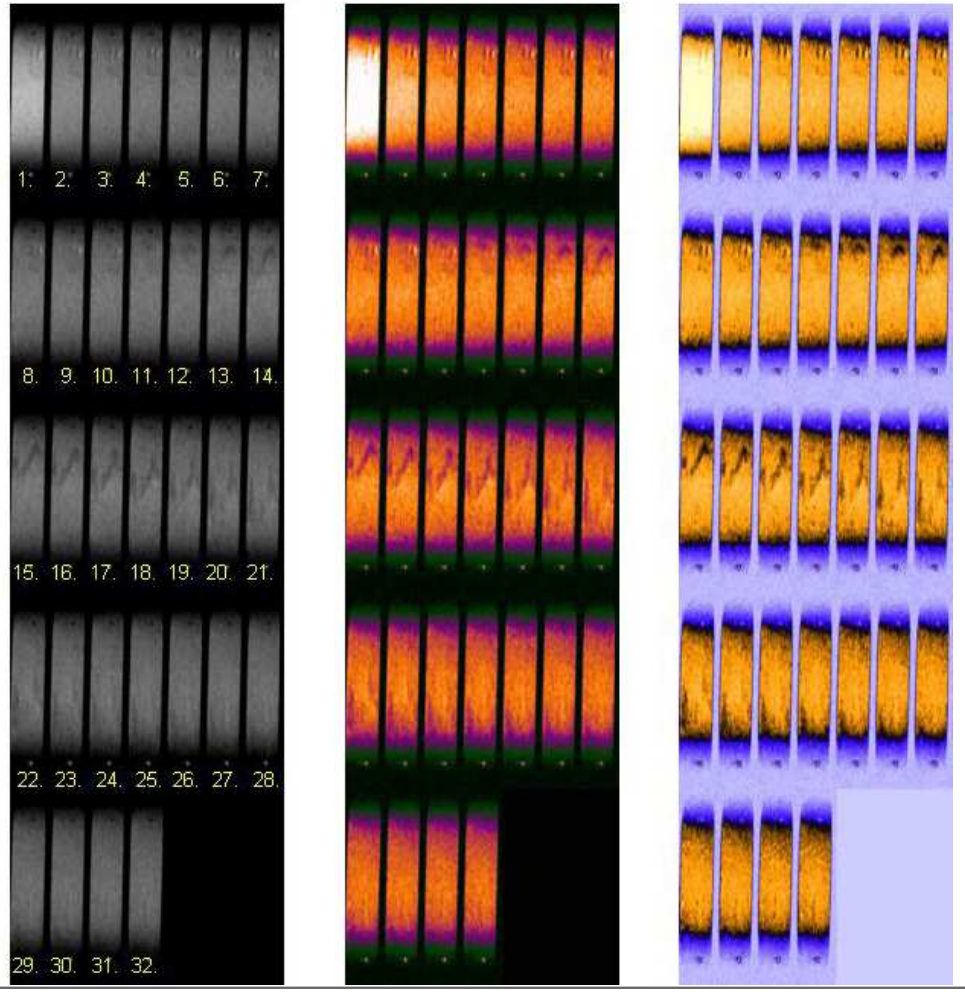
amidite addition MRI movie-PCWH-Jan 2011.avi

Magnetic resonance imaging

Each image is three seconds apart.

Detritylation reagent appears to cleave the trityl group at image 13.

The majority of the trityl group leaves the column by image 24 (*i.e.* 33 seconds)



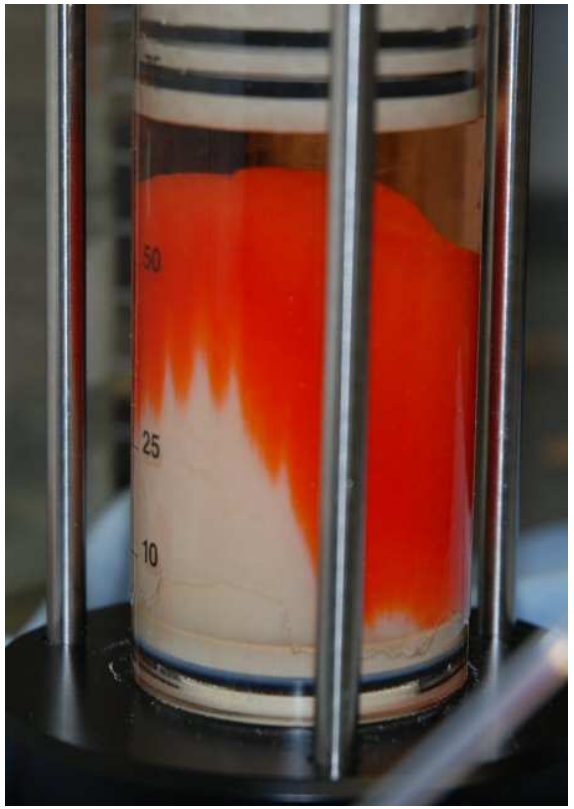
Challenges

- **“Linear” (one step at a time) synthesis**
- **99% yield at each step gives:**
- **0.99^80 < 50% overall yield**

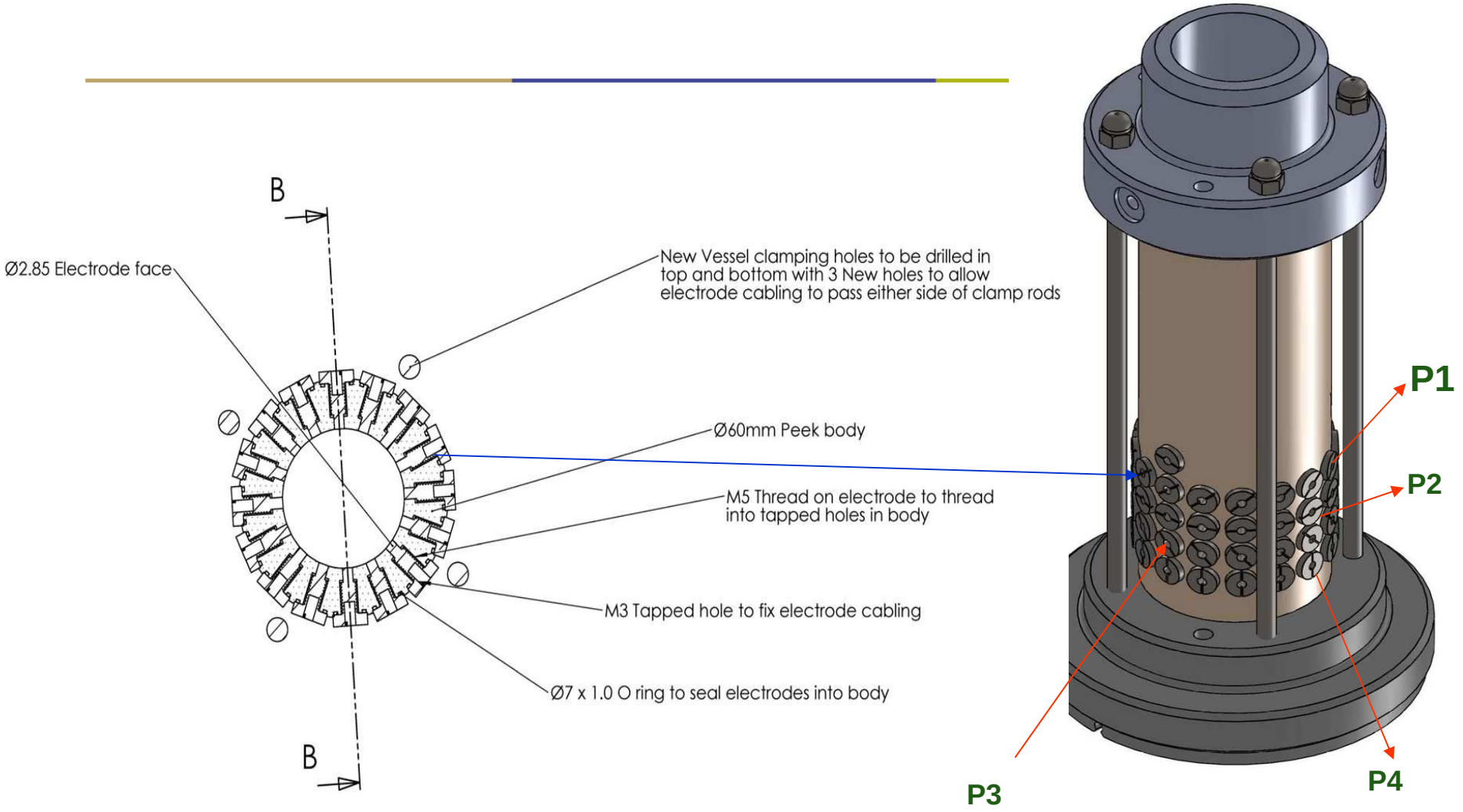
- **Yield depends critically on good distribution of reagents**

- **Ideally we would like plug flow – the reality is somewhat different!**

- **Swelling & shrinking of bed during synthesis results in poor bed packing**



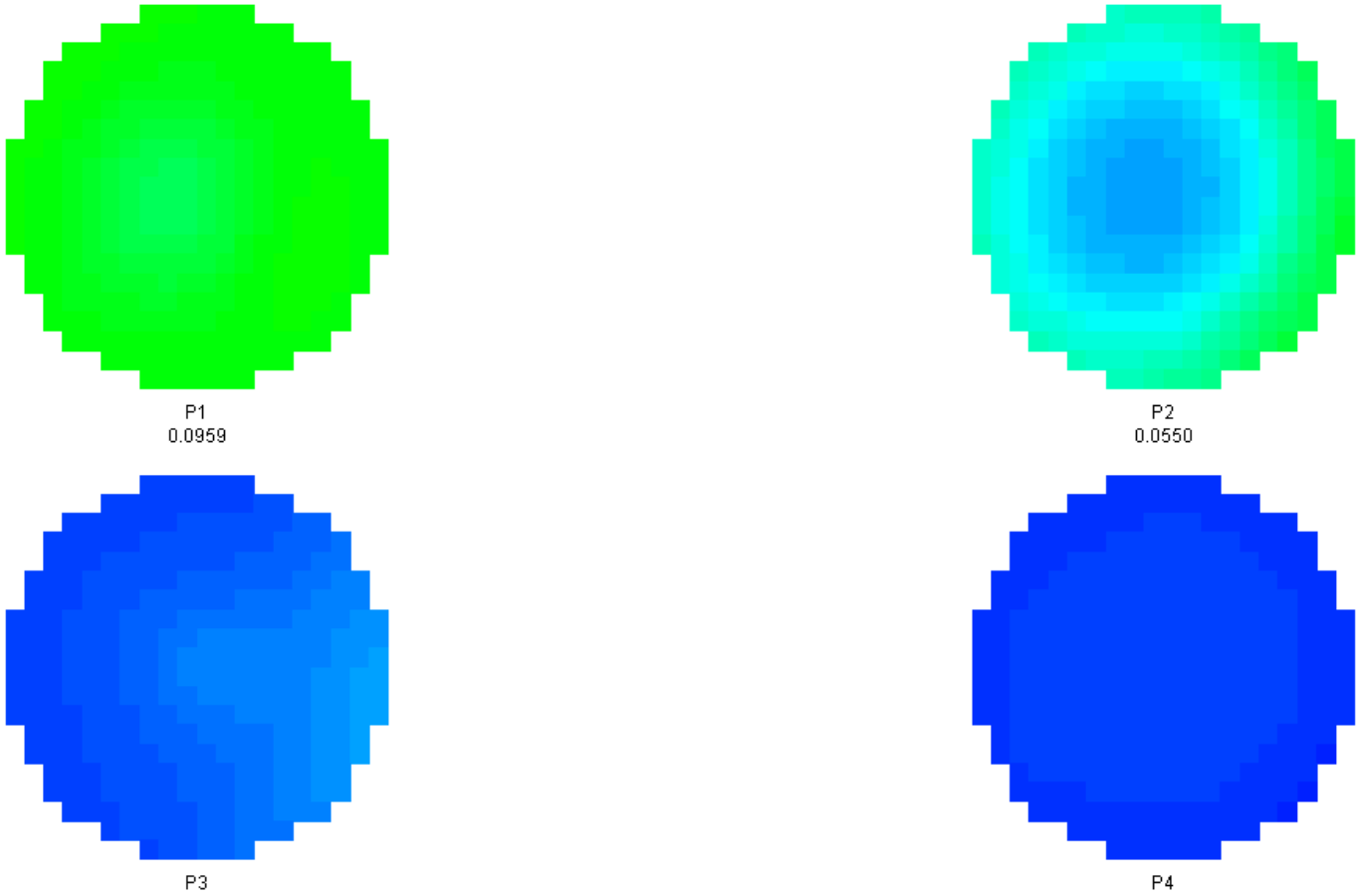
Electrical Resistance Tomography Column Design



Column Design

- **New column uses the architecture of the existing column as far as practical**

Time :- 1:54:29



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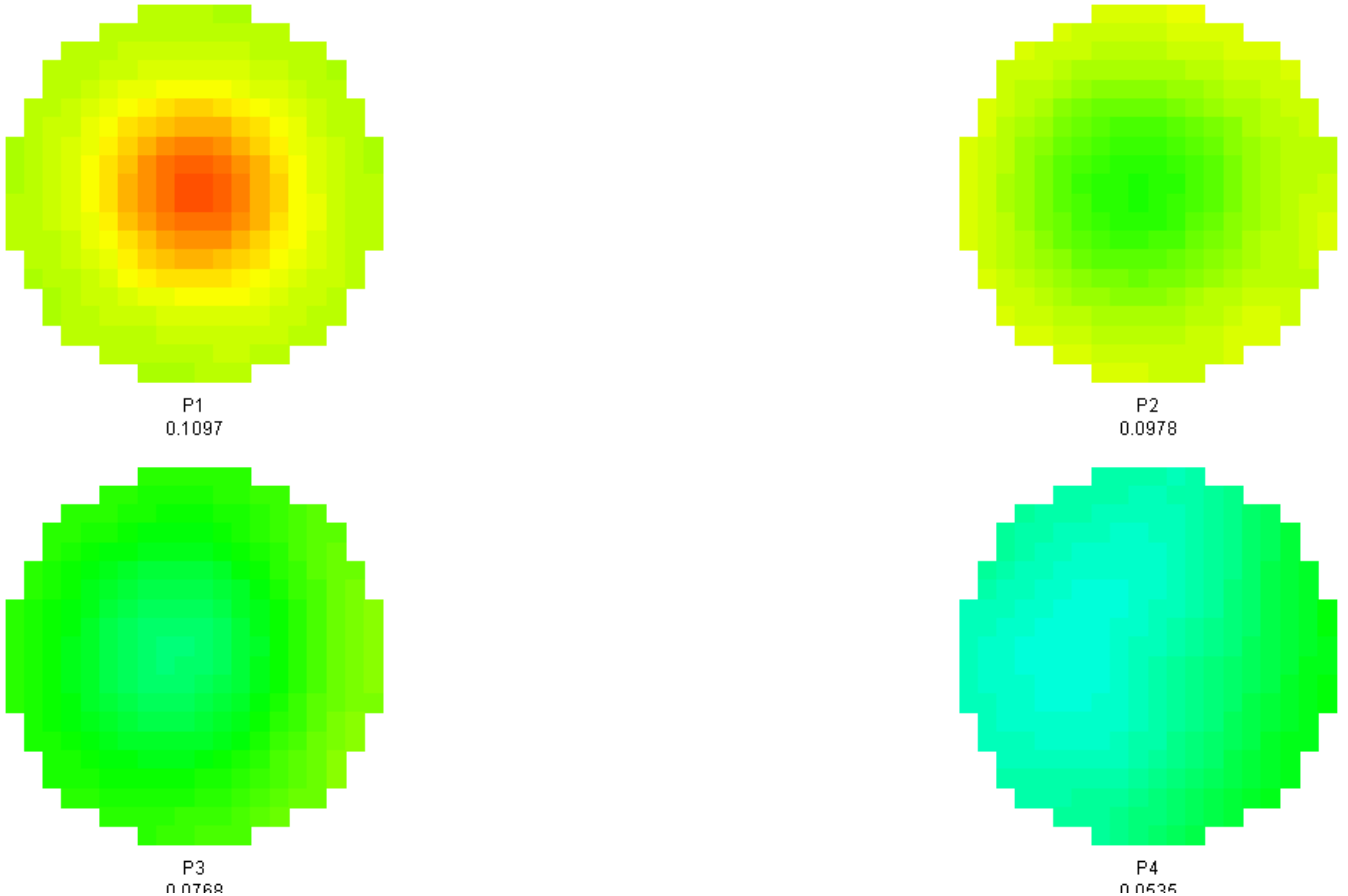

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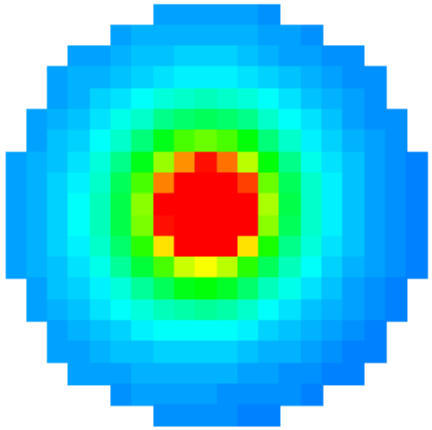
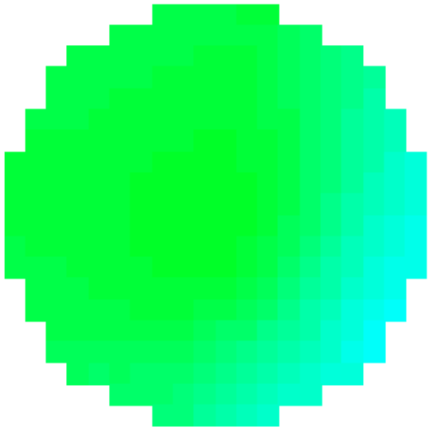


P1
0.0483



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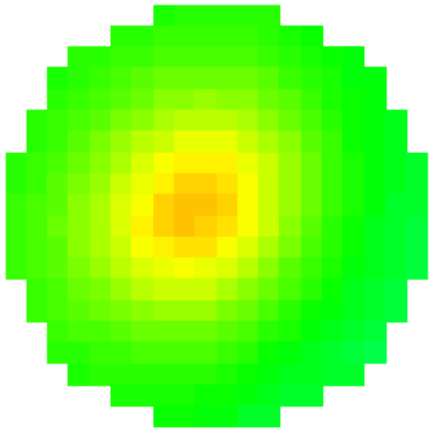

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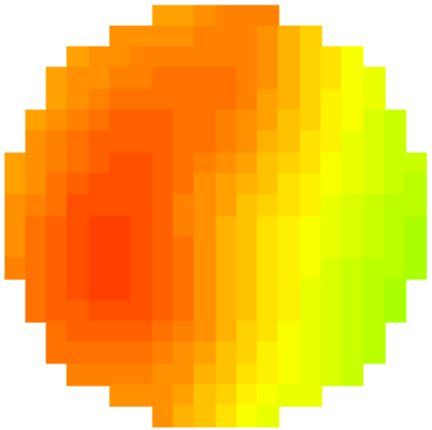
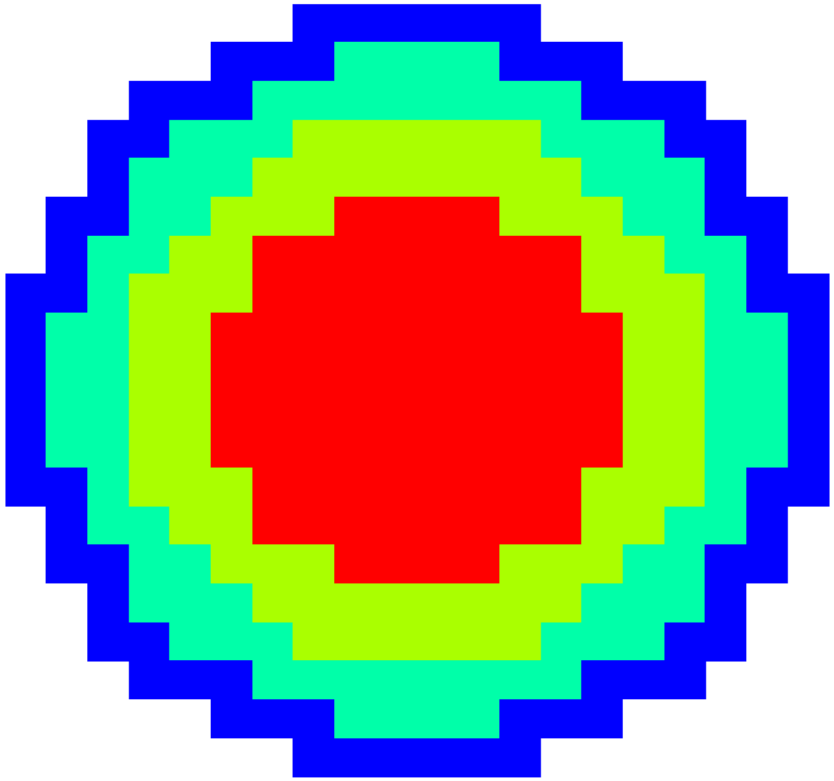


P4
0.1231

Zoned Averages



1 4

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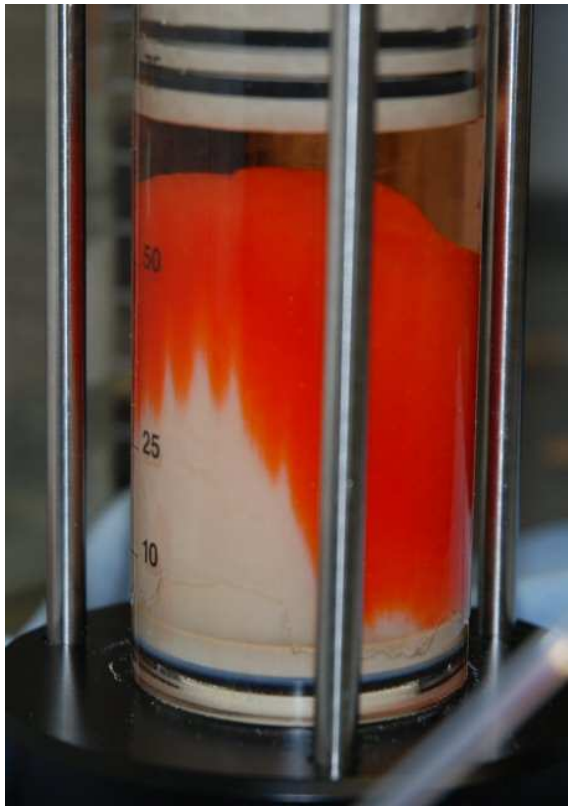

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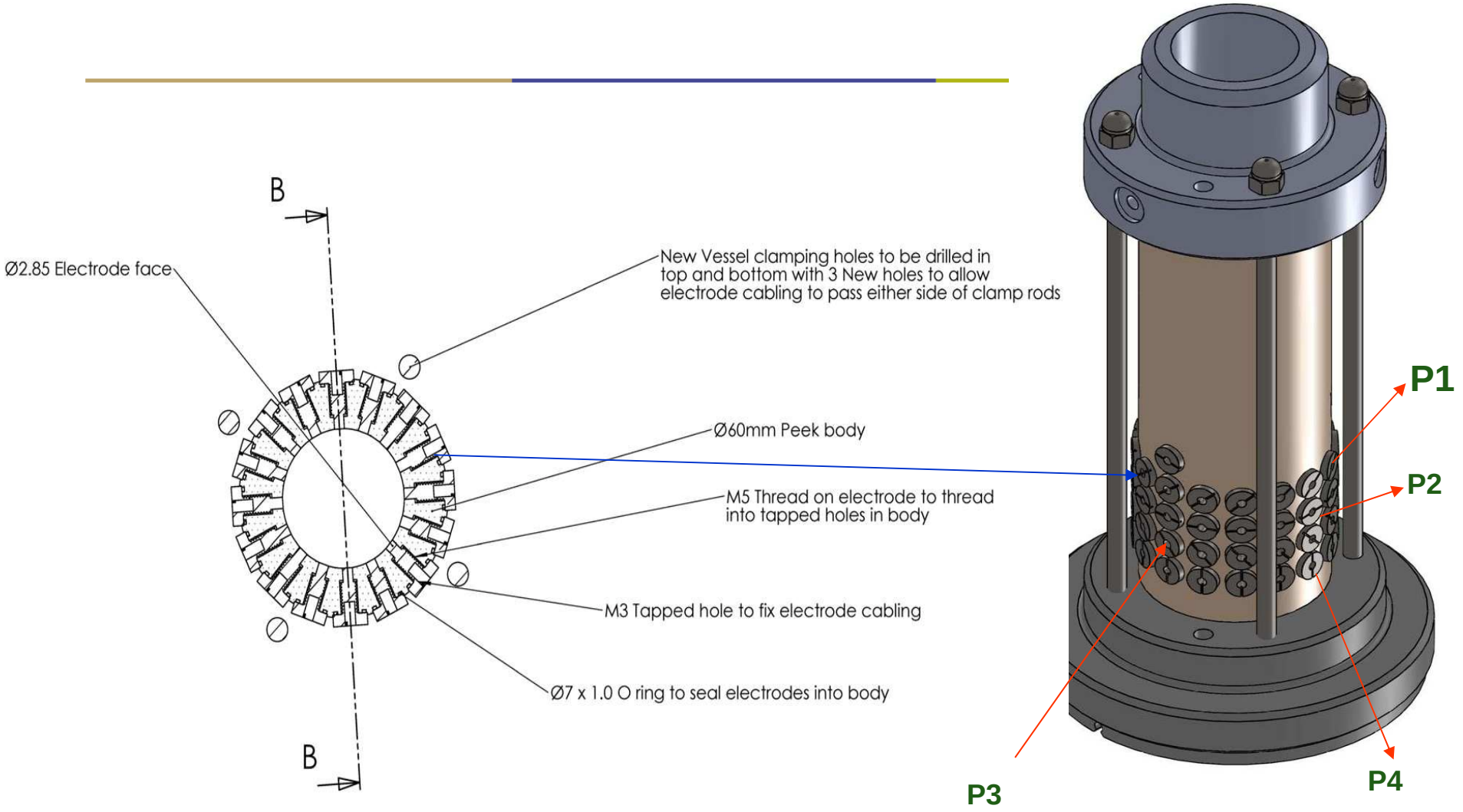

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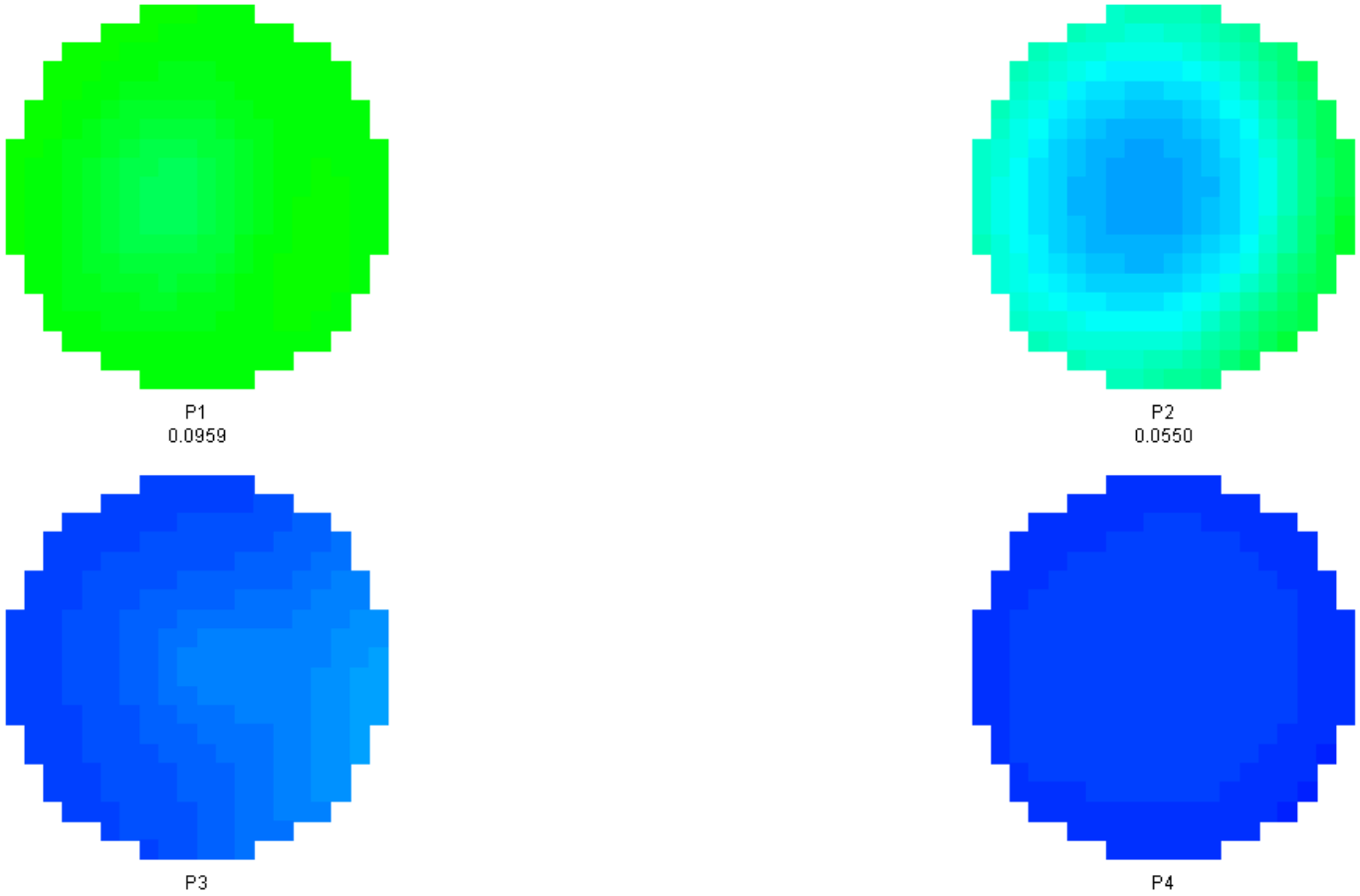

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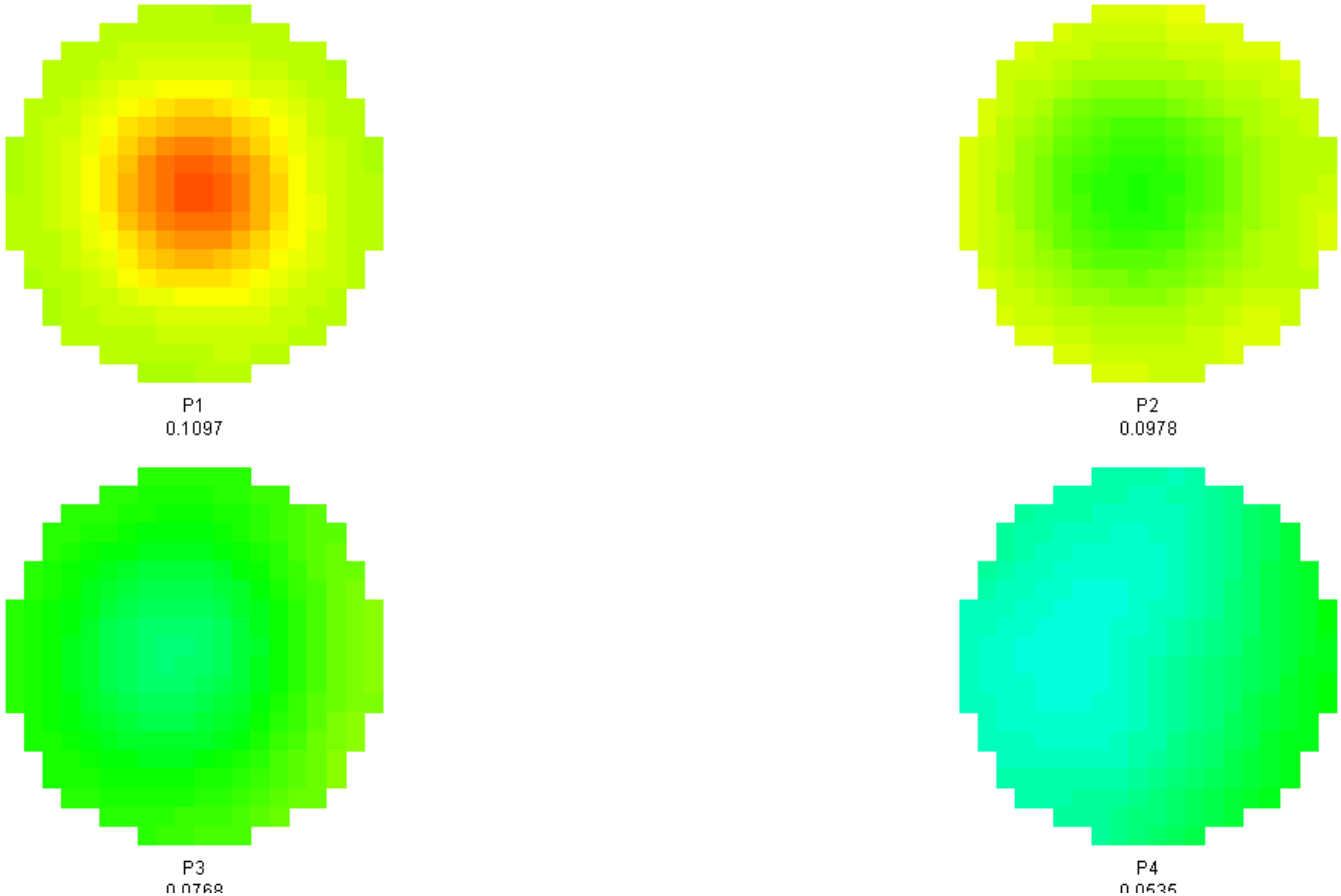

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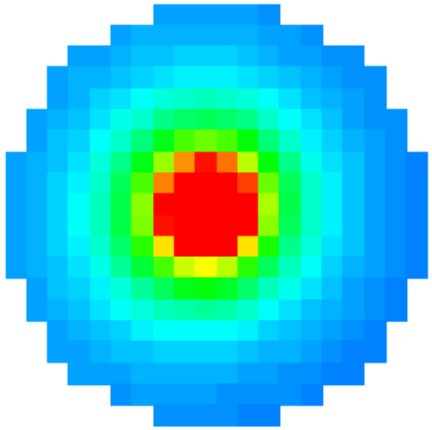

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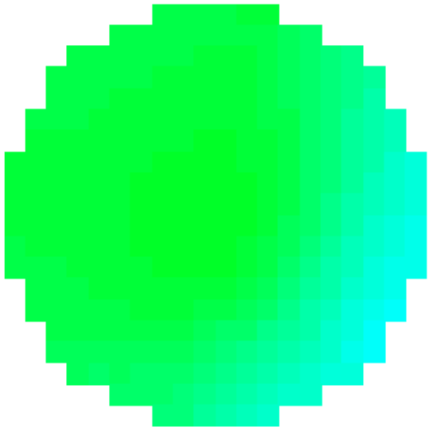

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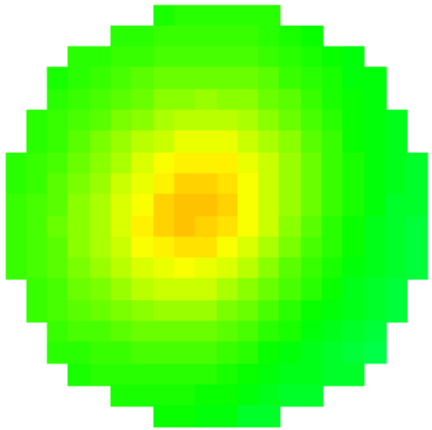
0.0606



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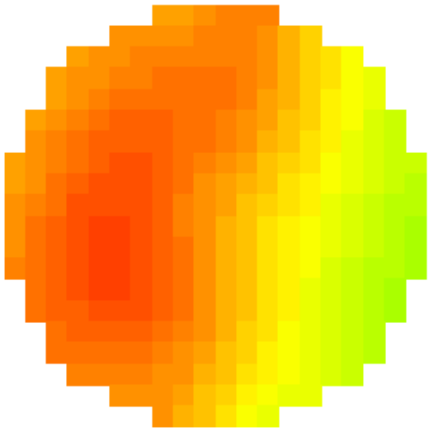

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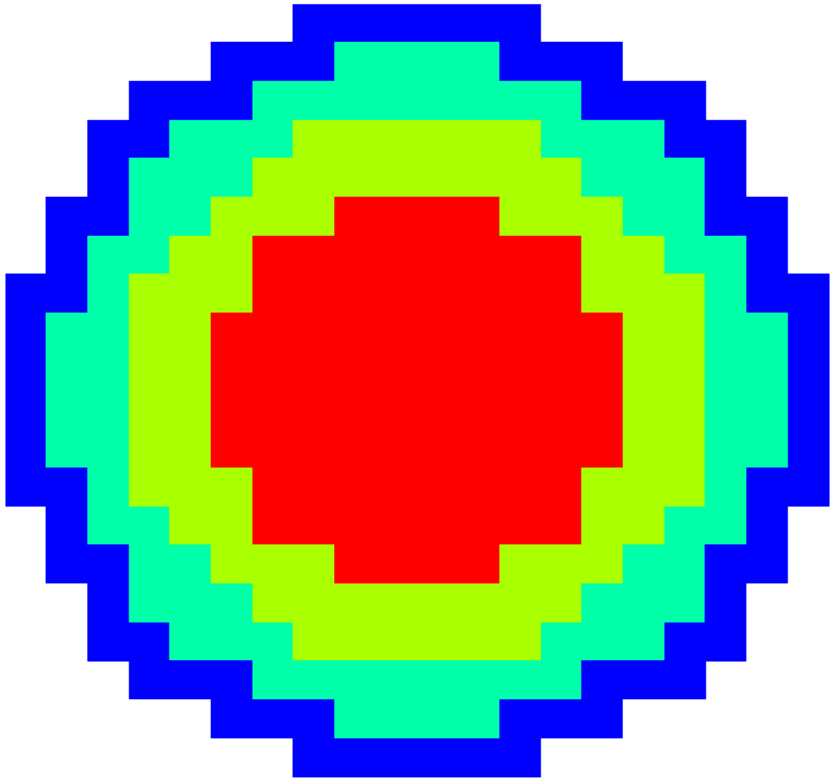
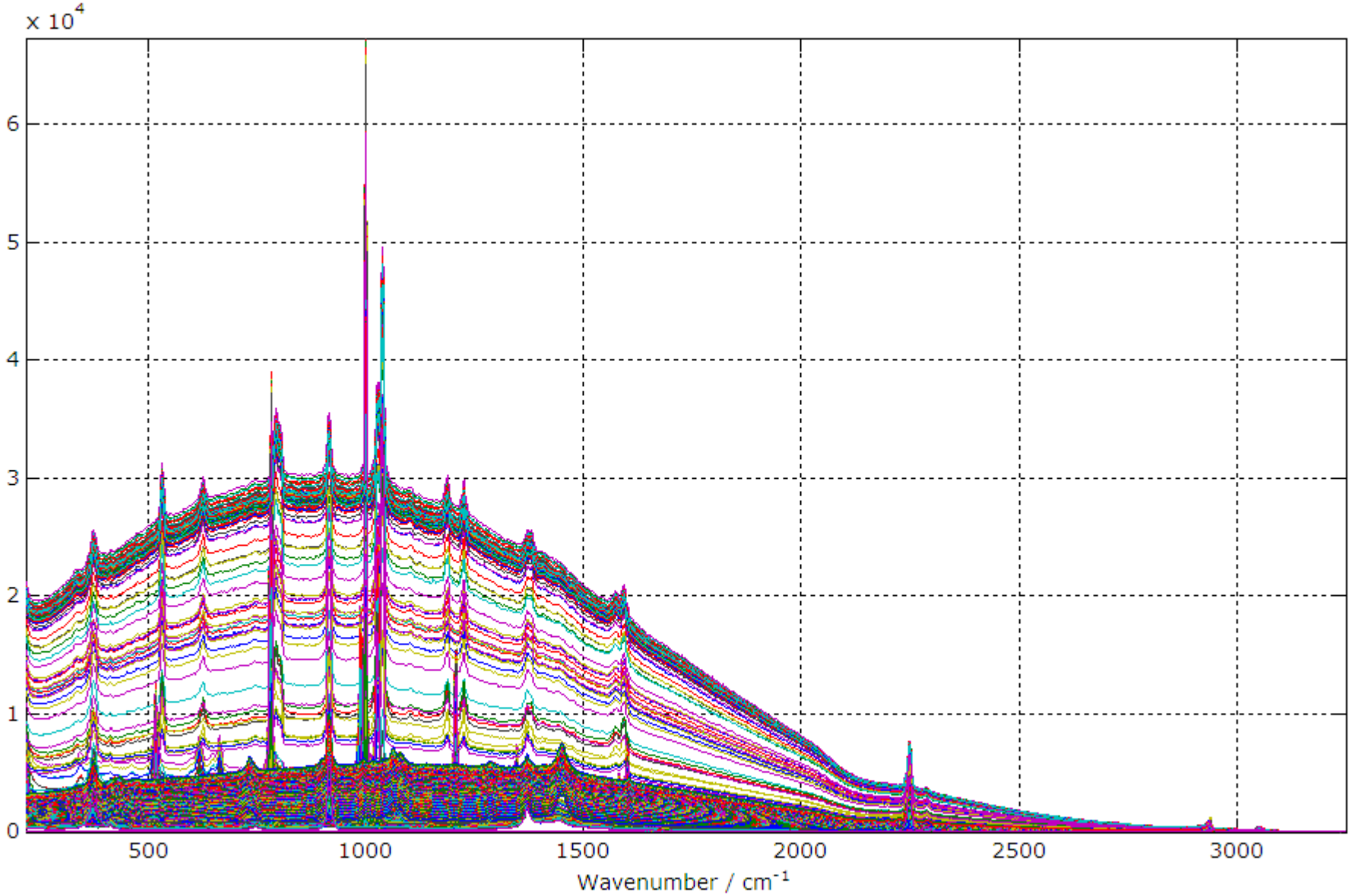
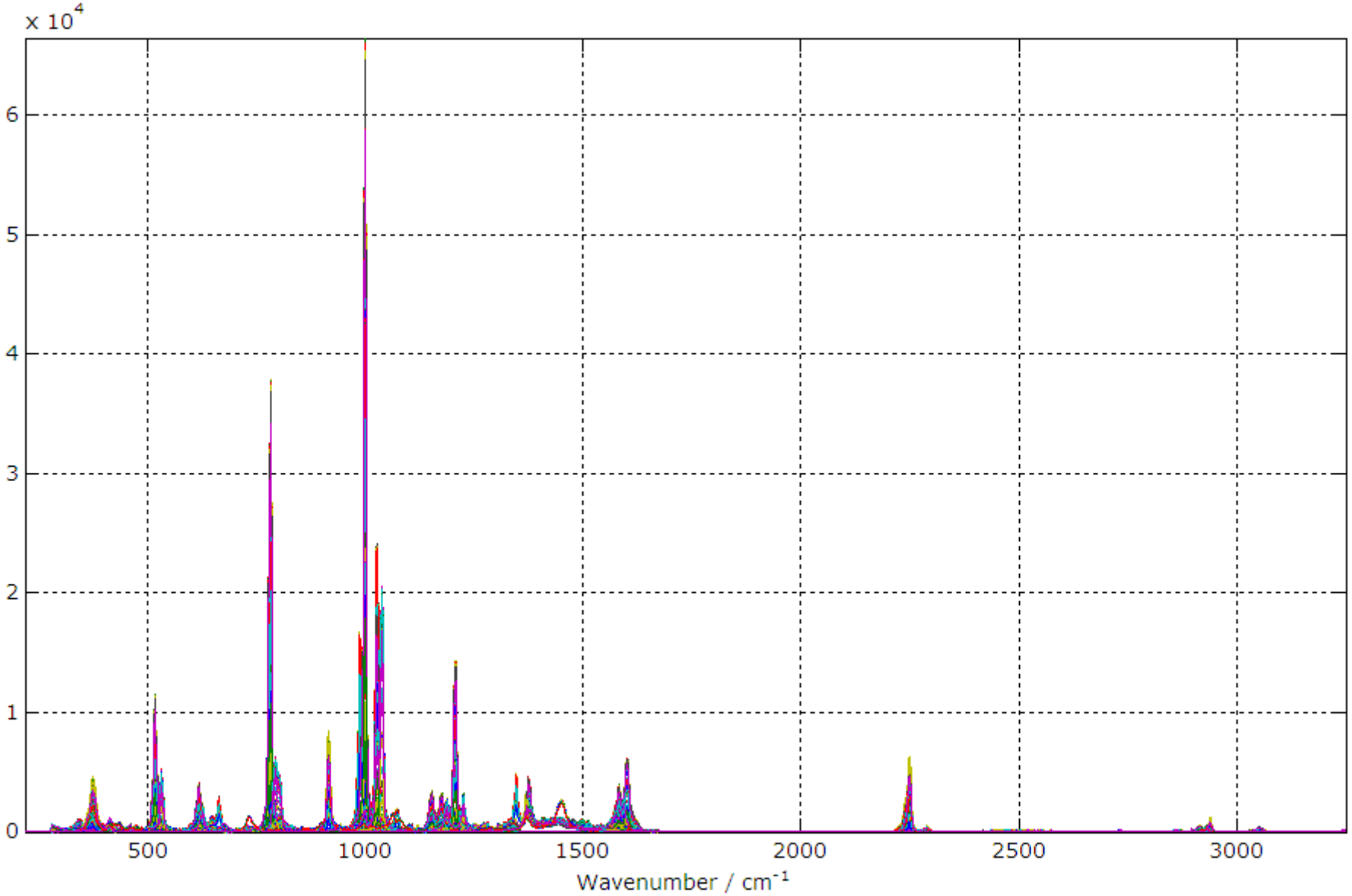


1 4

Superimposed spectra of raw Raman data from in line flow cell

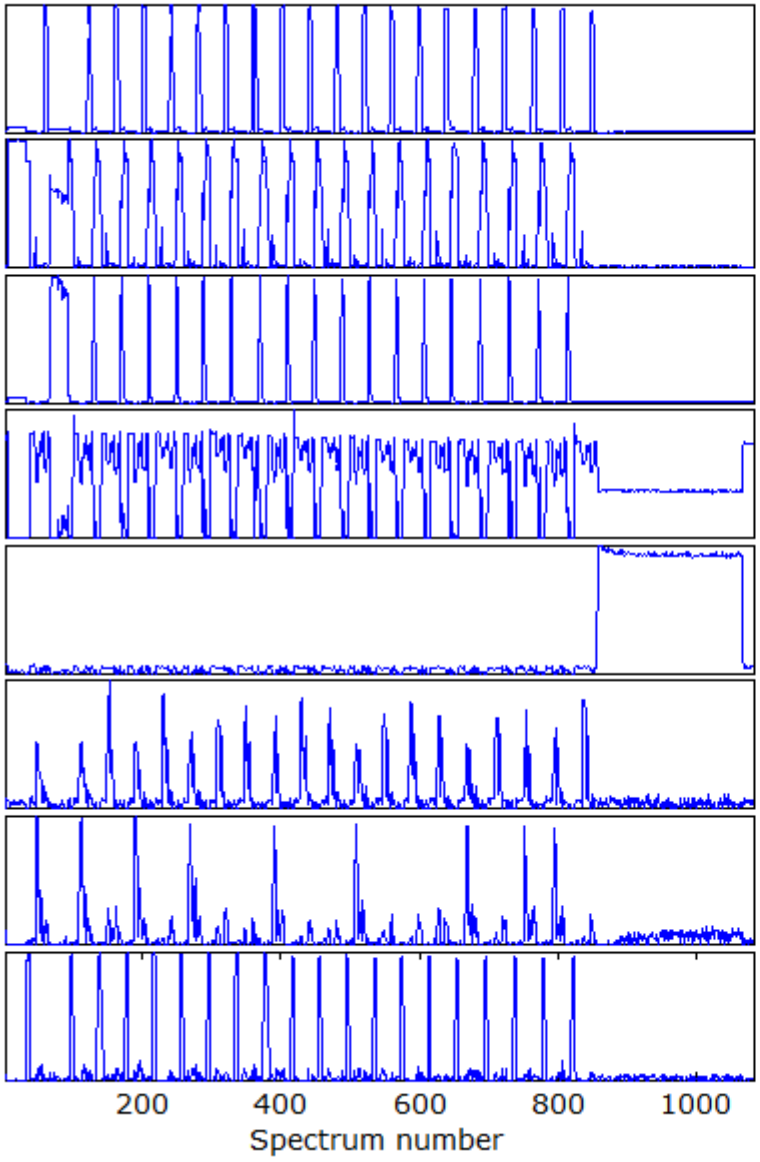


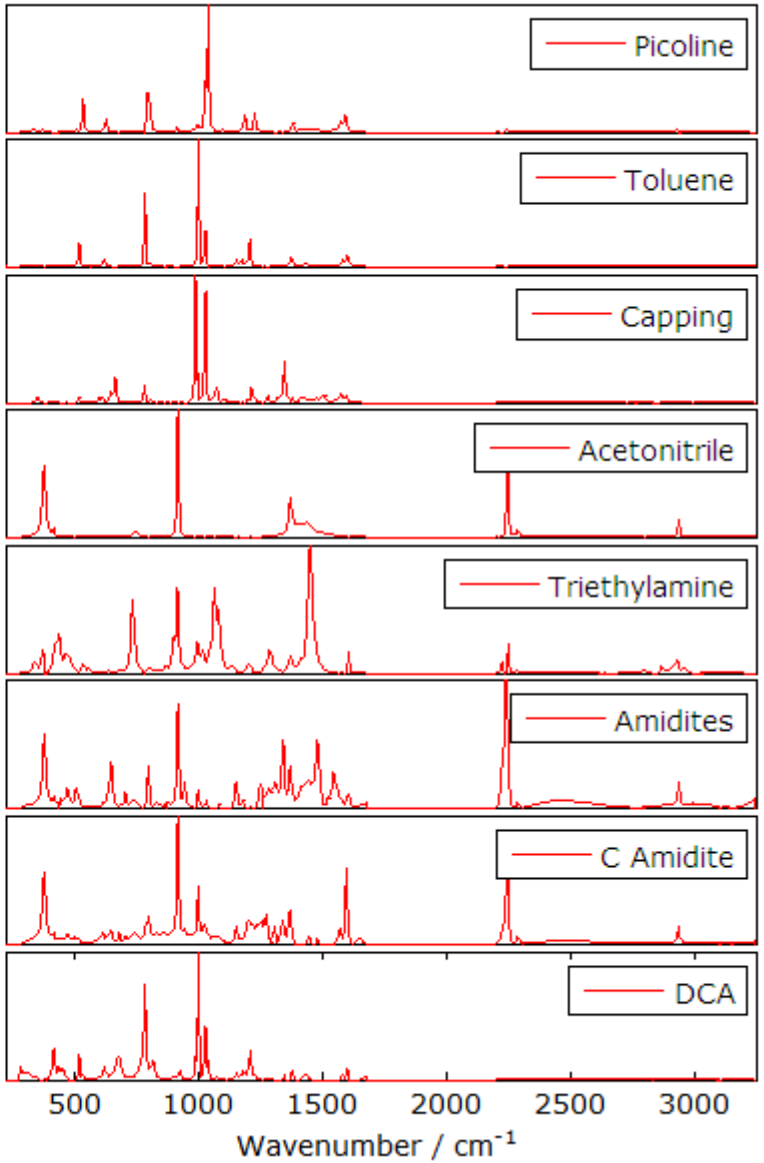
Superimposed spectra of raw data after removal of fluorescence



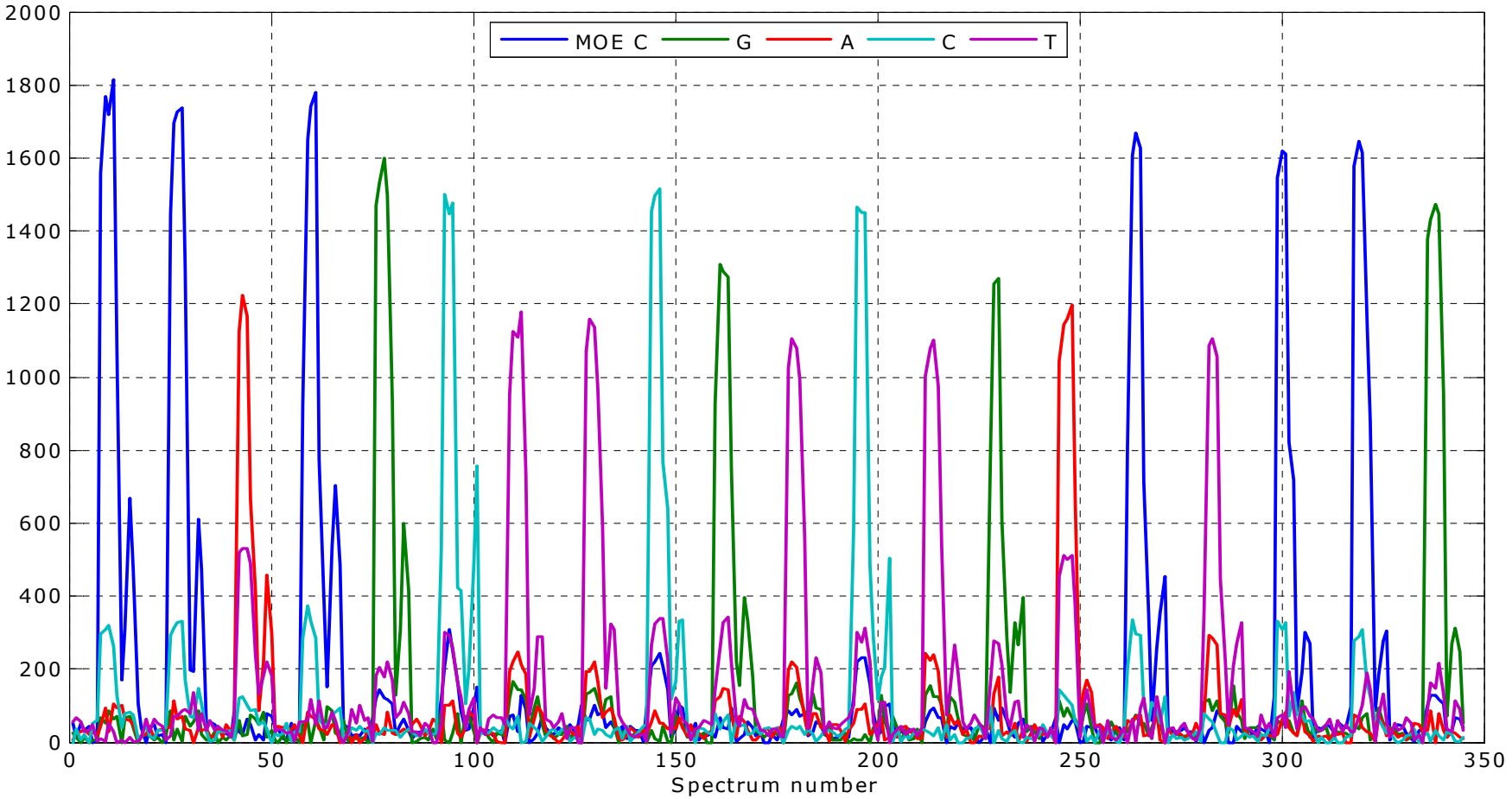
27_JUN_Spectra_baseline_corrected_2400_2800.wmv

Resolution of inlet Raman data – 8 components

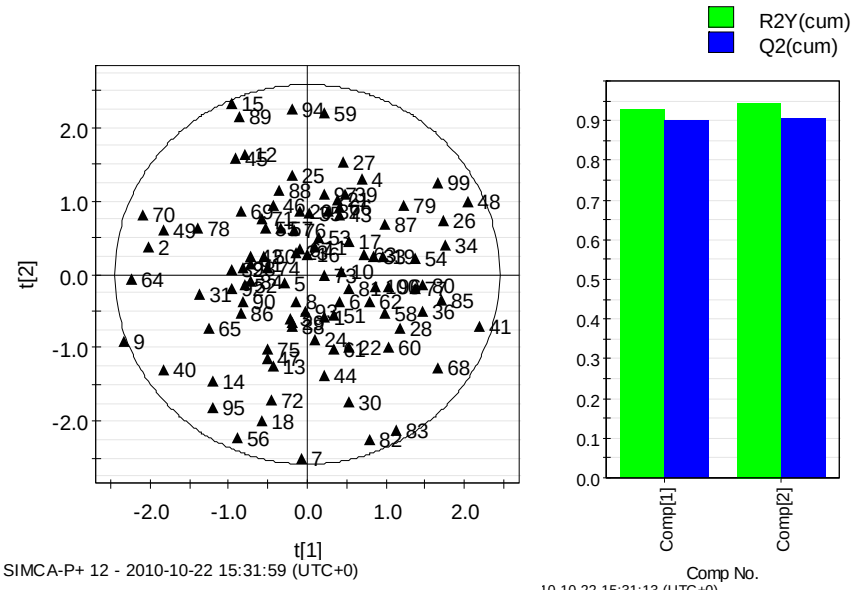


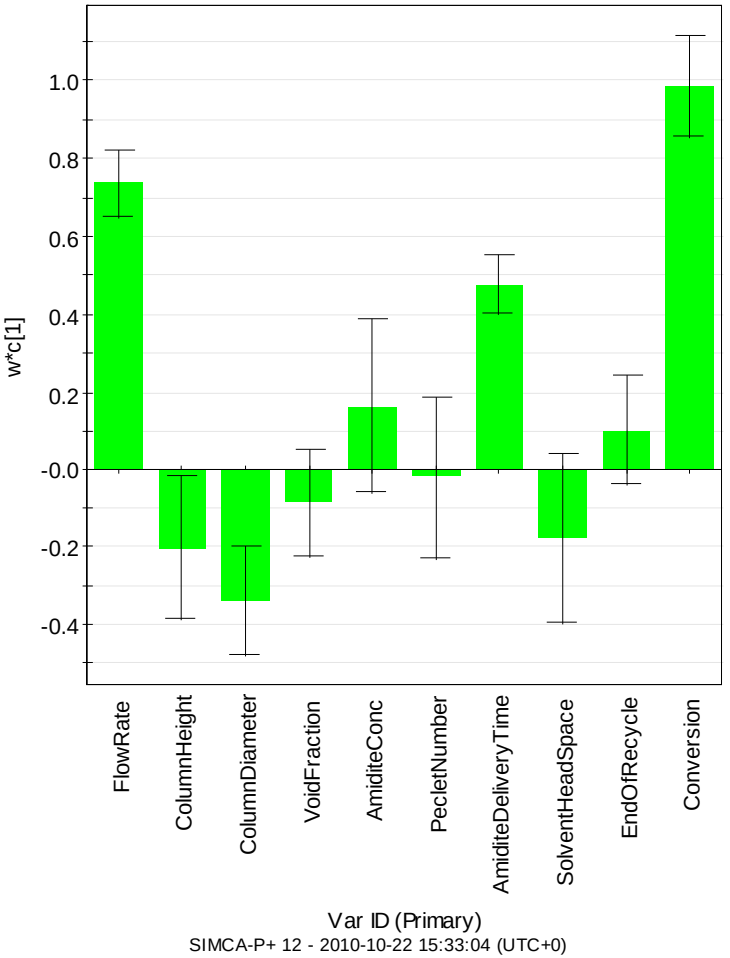


Multivariate Curve Resolution of Raman spectra able to see 5 components going on to column

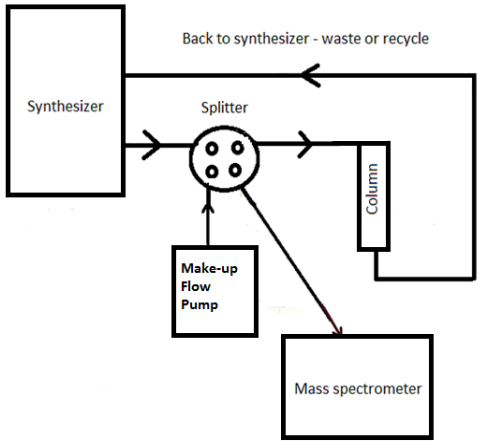


% Conversion of oligonucleotide





In-line miniature MS



Microsaic 3500 MD electrospray MS