## Supporting data information for paper:

# Acoustic levitation of pollen to study hygroscopic behaviour 

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Two different species of pollen, Lilium orientalis (Oriental Lily) and Populus deltoides (Eastern Cottonwood), were levitated using a modified commercial acoustic levitator (tec5, Oberursel, Germany) with the setup described below. Relative humidity (RH) was measured by a DHT22 RH sensor ( $2-5 \%$ RH accuracy between $0-100 \% \mathrm{RH}$ ) controlled by an Arduino and Raspberry pi, connected to the air pump that delivered air, via wet and dry channels, into the chamber. A Leica MC190 HD macroscope with digital camera attached was used to take snapshot images at varied RH increments between ambient and maximum RH. At the end of this document in the Appendix, tables for each of the experiments reported in this work can be found which give the corresponding RH values for the raw image snapshots available within this supporting data.


A bespoke program using Python 3.9 was used to construct contours around the pollen grains in each image and determine the area within the contour by pixels, as well as the perpendicular diameters of an ellipse fitted on the contour. Datasets were outputted in each experiment case and aggregated and processed by the accompanying Python (Jupyter Notebook) script 'DataAggregation.ipynb'. The processing involved pairing the raw measurements of pixel areas and diameters with the corresponding RH value for each data point, averaging over the 5 image samples for each RH increment, and then area ratio and diameter ratio measurements were calculated to produce the
processed dataset 'AllDataProcessed.csv'. The table below describes each of the columns present in this dataset.

| Column name | Description | Units |
| :---: | :---: | :---: |
| Index | Numbers 0-185 for each row | - |
| Direction | '1Increasing' or '2Decreasing' denoting whether relative humidity ( RH ) was being increased or decreased respectively for given data point. | - |
| RH | Relative humidity | \% |
| Area | Area of pollen grain determined from contour fitted on image by Python program, averaged over 5 images. | Pixels |
| DiameterA | Shorter measured diameter from ellipse fitted on pollen grain image by Python program, averaged over 5 images. | Pixels |
| DiameterB | Longer measured diameter from ellipse fitted on pollen grain image by Python program, averaged over 5 images. | Pixels |
| Expt | Individual experiment identifier | - |
| ID | Experiment group identifier (e.g. 'Lily_stat' for stationary Lilius orientalis experiments and 'EC_lev' for levitated Populus deltoides pollen. | - |
| AreaRatio | Area as a ratio of initial area for given experiment, i.e. area/initial area. | - |
| DiameterRatio | Ratio of measured diameters, the shorter diameter over the longer, i.e. <br> DiameterA/DiameterB | - |

This aggregated dataset was then analysed and visualised by the Jupyter Notebook script 'AllExptAnalysis.ipynb' to produce the results presented in the paper.

## Individual experiment data

The following are tables for each of the experiments reported in this work (and some not reported) which give the experimental conditions and corresponding RH values for the raw image snapshots taken by the macroscope, which are also available within this supporting data. Each experiment run is in a separate folder named with its date, and within the folder can be found: a folder containing the raw images from the macroscope, a folder containing each image as output from the Python program with a fitted contour, Python scripts used to process the image and draw the contour, and the CSV file output from this script with image name, calculated area within the contour, and perpendicular diameters of the fitted ellipse for each image.

2020/12/03 (not used in paper)
Lilium orientalis (levitated)
Back-lit

| Image name | RH (\%) |
| :--- | :--- |
| AM100707 | 50 |
| AM100708 | 60 |
| AM100714-16 | 61 |
| AM100717-19 | 61 |
| AM100720-22 | 70 |
| AM100723-5 | 48 |
| AM100726-8 | 50 |
| AM100729-32 | 60 |
| AM100733-35 | 70 |
| AM100736-38 | 80 |
| AM100739-40 | 85 |
| AM100741-43 | 90 |
| AM100744-46 | 95 |
| AM100747-48 | 90 |
| AM100749-51 | 85 |
| AM100752-54 | 80 |
| AM100755-56 | 70 |
| AM100757-59 | 60 |
| AM100760-62 | 50 |

2020/12/04_A (not used in paper)
Lilium orientalis (levitated)
Back-lit

| Image name | RH (\%) |
| :--- | :--- |
| AM100766-8 | 45 |
| AM100769-74 | 50 |
| AM100775-79 | 60 |
| AM100780-84 | 70 |
| AM100785-88 | 75 |


| AM100789-92 | 80 |
| :--- | :--- |
| AM100793-96 | 85 |
| AM100797-803 | 90 |
| AM100804-11 | 95 |

## 2020/12/04_B (not used in paper)

Lilium orientalis (levitated)
Back-lit

| Image name | RH (\%) |
| :--- | :--- |
| AM100812-17 | 50 |
| AM100818-20 | 60 |
| AM100821-24 | 70 |
| AM100825 | 75 |
| AM100826-29 | 80 |
| AM100830 | 85 |
| AM100831-34 | 90 |
| AM100835-38 | 95 |
| AM100839 | 80 |
| AM100840 | 70 |
| AM100841 | 60 |
| AM100842-44 | 50 |

## 2020/12/04_C (not used in paper)

Lilium orientalis (levitated)
Back-lit

| Image name | RH (\%) |
| :--- | :--- |
| AM100848-52 | 50 |
| AM100853 | 55 |
| AM100854-55 | 75 |
| AM100856057 | 85 |
| AM100858 | 90 |
| AM100859-67 | 95 |

## 2021/03/09_A (used in paper)

Lilium orientalis (levitated)
Back-lit

| Image name | RH (\%) |
| :--- | :--- |
| AM100878-882 | 45 |
| AM100883-887 | 50 |
| AM100888-892 | 60 |
| AM100893-897 | 70 |
| AM100898-902 | 80 |


| AM100903-907 | 85 |
| :--- | :--- |
| AM100908-912 | 90 |
| AM100913-917 | 95 |
| AM100918-922 | 99 |
| AM100923-927 | 95 |
| AM100928-932 | 90 |
| AM100933-937 | 85 |
| AM100938-942 | 80 |
| AM100943-947 | 70 |
| AM100948-952 | 60 |
| AM100953-957 | 50 |

## 2021/03/09_B (used in paper)

Same conditions as above.

| Image name | RH (\%) |
| :--- | :--- |
| AM100958-962 | 45 |
| AM100963-967 | 50 |
| AM100968-972 | 60 |
| AM100973-977 | 70 |
| AM100978-982 | 80 |
| AM100983-987 | 85 |
| AM100988-992 | 90 |
| AM100993-997 | 95 |
| AM100998-1002 | 99 |
| AM101003-1007 | 95 |
| AM101008-1012 | 90 |
| AM101013-1017 | 85 |
| AM101018-1022 | 80 |
| AM101023-1027 | 70 |
| AM101028-1032 | 60 |
| AM101033-1037 | 50 |

## 2021/03/09_C (used in paper)

Same conditions as above.

| Image name | RH (\%) |
| :--- | :--- |
| AM101038-042 | 42 |
| AM101043-047 | 50 |
| AM101048-052 | 60 |
| AM101053-057 | 70 |
| AM101058-062 | 80 |
| AM101063-067 | 85 |
| AM101068-072 | 90 |
| AM101073-077 | 95 |
| AM101078-082 | 99 |
| AM101083-087 | 95 |
| AM101088-092 | 90 |


| AM101093-097 | 85 |
| :--- | :--- |
| AM101098-102 | 80 |
| AM101103-107 | 70 |
| AM101108-112 | 52 |
| AM101113-117 | 50 |

2021-03-20_A (used in paper)
Lilium orientalis (levitated)
Back-lit

| Image name | RH (\%) |
| :--- | :--- |
| AM101119-23 | 50 |
| AM101124-28 | 60 |
| AM101129-33 | 70 |
| AM101134-38 | 80 |
| AM101139-43 | 85 |
| AM101144-48 | 90 |
| AM101149-53 | 95 |
| AM101154-58 | 99 |
| AM101159-63 | 93 |
| AM101164-68 | 90 |
| AM101169-73 | 85 |
| AM101174-78 | 80 |
| AM101179-83 | 70 |
| AM101184-88 | 56 |
| AM101189-93 | 50 |

2021-03-30_B (used in paper)
Same conditions as above.

| Image name | RH (\%) |
| :--- | :--- |
| AM101194-198 | 50 |
| AM101199-203 | 60 |
| AM101204-208 | 70 |
| AM101209-213 | 80 |
| AM101214-218 | 85 |
| AM101219-223 | 90 |
| AM101224-228 | 95 |
| AM101229-233 | 99 |
| AM101234-238 | 95 |
| AM101239-243 | 90 |
| AM101244-48 | 83 |
| AM101249-253 | 80 |
| AM101254-258 | 68 |
| AM101259-263 | 60 |
| AM101264-268 | 50 |

## 2021-03-31_C (used in paper)

Same conditions as above.

| Image name | RH (\%) |
| :--- | :--- |
| AM101269-273 | 48 |
| AM101274-278 | 60 |
| AM101279-283 | 70 |
| AM101284-288 | 81 |
| AM101289-293 | 86 |
| AM101294-298 | 90 |
| AM101299-303 | 95 |
| AM101304-308 | 99 |
| AM101309-313 | 95 |
| AM101314-318 | 90 |
| AM101319-323 | 85 |
| AM101324-328 | 80 |
| AM101329-333 | 70 |
| AM101334-338 | 59 |
| AM101339-343 | 50 |

## 2021/07/06_A (used in paper)

Lilium orientalis (stationary) $\times 2$ pollen grains
Back-lit

| Image name | RH (\%) |
| :--- | :--- |
| AM101345-349 | 70 |
| AM101350-354 | 75 |
| AM101355-359 | 80 |
| AM101360-364 | 85 |
| AM101365-369 | 90 |
| AM101370-374 | 95 |
| AM101375-379 | 96 |
| AM101380-384 | 95 |
| AM101385-389 | 90 |
| AM101390-394 | 85 |
| AM101395-399 | 80 |
| AM101400-404 | 75 |
| AM101405-409 | 70 |

2021/07/06_B (used in paper)
Lilium orientalis (stationary) $\times 2$ pollen grains
Front-lit

| Image name | RH (\%) |
| :--- | :--- |
| AM101410-414 | 70 |
| AM101415-419 | 75 |


| AM101420-424 | 83 |
| :--- | :--- |
| AM101425-429 | 85 |
| AM101430-434 | 90 |
| AM101435-439 | 95 |
| AM101440-444 | 96 |
| AM101445-449 | 95 |
| AM101450-454 | 90 |
| AM101455-459 | 85 |
| AM101460-464 | 80 |
| AM101465-469 | 75 |
| AM101470-474 | 65 |

2021/07/06_C (used in paper)
Lilium orientalis (stationary) x1 grain
Back-lit

| Image name | RH (\%) |
| :--- | :--- |
| AM101475-479 | 60 |
| AM101480-484 | 70 |
| AM101485-489 | 75 |
| AM101490-494 | 80 |
| AM101495-499 | 85 |
| AM101500-504 | 90 |
| AM101505-509 | 95 |
| AM101510-514 | 95 |
| AM101515-519 | 90 |
| AM101520-524 | 85 |
| AM101525-529 | 80 |
| AM101530-534 | 75 |
| AM101535-539 | 60 |

2021/08/11 (used in paper)
Populus deltoides (stationary)
3 grains chosen out of multiple visible grains.
Back-lit

| Image name | RH (\%) |
| :--- | :--- |
| AM101540-544 | 60 |
| AM101545-549 | 65 |
| AM101550-554 | 70 |
| AM101555-559 | 75 |
| AM101560-564 | 80 |
| AM101565-569 | 85 |
| AM101570-574 | 90 |
| AM101575-579 | 94 |
| AM101580-584 | 94 |

2021/08/26 (used in paper)
Populus deltoides (levitated)
Back-lit

| Image name | RH (\%) |
| :--- | :--- |
| AM101614-624 | 48 |
| AM101625-635 | 95 |
| AM101636-640 | 90 |
| AM101641-645 | 50 |

