Chronoprints: Identifying samples by visualizing how they change over space and time

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**Optimal lighting**

Our initial experiments with food oils revealed the importance of proper lighting in obtaining quality chronoprints. Overly-lit experiments caused saturated pixels in the resulting videos and poor contrast in the resulting chronoprints, which in turn affected how well our *feature tracing* MATLAB script could convert the chronoprint into curves; see Supplementary Figure 1 for an example. In later experiments we reduced the lighting on the chip to obtain results such as those shown in the main text.

![Supplementary Figure 1: Curves resulting from using the *feature tracing* analysis of chronoprints from from 100% olive oil (channels 1 and 4), 100% peanut oil (channels 2 and 5), and a 1:1 mixture of olive and peanut oils (channels 3 and 6). Excessive lighting during video recording saturated some pixels and adversely affected the quality of these results.](image-url)
Optimal experiment duration

We also explored the optimal duration for oil chronoprint experiments. Supplementary Figure 2 shows the results of using the feature tracing analysis of a chronoprint obtained from a 25-second-long video of the oil samples reacting to a dynamic temperature gradient. While the resulting curves are roughly grouped by their oil type, the separation between the oil types is not very pronounced. We attributed this to insufficient experiment time and ran subsequent experiments for longer durations, which resulted in greater separation between the different oil curves.

Supplementary Figure 2: Curves resulting from using the feature tracing analysis of chrono-prints from from a 1:1 mixture of olive and peanut oils (channels 1 and 4), 100% peanut oil (channels 2 and 5), and 100% olive oil (channels 3 and 6). The separation between the oil types is not very pronounced. We attribute this to insufficient experiment duration time. The curves from samples in channels 1 and 2 are slightly higher than the curve from the same samples in channels 4 and 5. We attribute this error to poor centering of the channels on this microfluidic thermometer chip, which exposed channels 1 and 2 to a faster-dropping temperature gradient than the other channels.
Thermometer chip edge effects

Occasionally, small variations in our chip milling process can yield thermometer chips with channels that are not perfectly centered on the chip. When this happens, the samples in the channels may experience slightly different changing temperature gradients during an experiment; the sample in the channel closer to one edge of the chip is more exposed to the liquid nitrogen bath and cools faster than the other channels. This results in a shifted curve for the sample, as shown for the oil samples in channels 1 and 2 of Supplementary Figure 2 and the sample in channel 1 of Supplementary Figure 3. Taking care to fabricate symmetric thermometer chips minimizes this source of error.

Replicate of experiment in main text Figure 5C

We performed several replicates of the oil experiment shown in Figure 5C of the main text and included one here (Supplementary Figure 3). In both experiments, chronoprints successfully distinguish these different oil types.
Supplementary Figure 3: Curves resulting from using the feature tracing analysis of chronoprints from from 100% peanut oil (channels 1 and 4), a 1:1 mixture of olive and peanut oils (channels 2 and 5), and 100% olive oil (channels 3 and 6). This is a replicate of the experiment shown in Figure 5C of the main text. Among the samples of the same oil types, the chronoprint traces had relatively small differences: the maximum sum-of-squared-differences of $8.47 \times 10^4$, $3.61 \times 10^5$, and $1.35 \times 10^5$ between the two 100% olive oil samples, the two 100% peanut oil samples, and the two 1:1 olive and peanut oil samples, respectively. However, between the different oil types, the chronoprint traces had greater differences: the maximum sum-of-squared-differences were $7.34 \times 10^6$ between the 100% olive oil and 100% peanut oil samples.
Comparisons of chronoprint image similarity algorithms

As explained in the main text, we used five different image similarity algorithms to quantify the similarity of pairs of chronoprints. Four of these algorithms used image hashing, which converts each chronoprint to a reduced-resolution, 64-bit binary “hash” of the original chronoprint. These algorithms differed in which pixel value we used as a threshold for converting the chronoprints into binary representations:

- **Local Mean**: the average pixel value in each chronoprint was used as the threshold.
- **Local Median**: the median pixel value in each chronoprint was used as the threshold.
- **Global Mean**: the average pixel value across all six chronoprints in an experiment was used as the threshold.
- **Global Median**: the median pixel value across all six chronoprints in an experiment was used as the threshold.

The fifth algorithm, image differences, converted each chronoprint to a reduced-resolution grayscale version, then calculated the sum of the pixel-by-pixel differences between two chronoprints to assess their similarity.

In the main text, we presented results from only a single image similarity algorithm for each cold medicine experiment. Here, we present results from all five algorithms for each cold medicine experiment in the main text, as well as analysis of replicates of some of the experiments in the main text. Supplementary Figure 4 compares all five chronoprint similarity algorithms for the experiment in Figure 6A of the main text (comparing six identical cold medicine samples taken from the same bottle). Supplementary Figure 5 compares all five algorithms for the experiment in Figure 6B of the main paper (comparing six samples of cold medicine from six different manufacturer’s lots). Supplementary Figure 6 provides a replicate of the experiment in Figure 6A of the main text (comparing six identical cold medicine samples taken from the same bottle); in this case the thawing of the samples was
also recorded and included in the chronoprints. Supplementary Figure 7 provides a replicate of the experiment in Figure 6B of the main paper (comparing six samples of cold medicine from six different manufacturer’s lots). Supplementary Figure 8 compares all five different chronoprint similarity algorithms for the experiment in Figure 7A of the main text (comparing 50%, 75%, and 100% v/v dilutions of cough medicine in water). Supplementary Figures 9 and 10 provide replicates of the experiment in Figure 7A of the main text (comparing 50%, 75%, and 100% v/v dilutions of cough medicine in water). Supplementary Figure 11 compares all five chronoprint similarity algorithms for the experiment in Figure 7B of the main paper (90%, 95%, and 100% v/v dilutions of cold medicine in water). Finally, Supplementary Figures 12 and 13 provide replicates of the experiment in Figure 7B of the main paper (90%, 95%, and 100% v/v dilutions of cold medicine in water).
Supplementary Figure 4: Comparisons of different chronoprint similarity algorithms for six cold medicine samples from one bottle (the experiment shown in Figure 6A of the main text).
Supplementary Figure 5: Comparisons of different chronoprint similarity algorithms for six cold medicine samples from six different manufacturing lots (the experiment shown in Figure 6B of the main text).
Supplementary Figure 6: Replicate of comparing six cold medicine samples from one bottle (replicate of the experiment shown in Figure 6A of the main text. All sample chronoprints are very similar, with a minimum pixel difference of 2 and maximum pixel difference of 12 out of 64 pixels total between different sample chronoprints. The local mean image hashing analysis performed the best for this experiment.
Supplementary Figure 7: Replicate of comparing six cold medicine samples from six different manufacturing lots (replicate of the experiment shown in Figure 6B of the main text). All sample chronoprints are almost identical, with a minimum pixel difference of 0 and maximum pixel difference of 5 out of 64 pixels total between different sample chronoprints. The local mean image hashing analysis performed the best for this experiment.
Supplementary Figure 8: Comparisons of different chronoprint similarity algorithms for 50%, 75%, and 100% (v/v) dilutions of cold medicine in water (the experiment shown in Figure 7A of the main text).
Supplementary Figure 9: Replicate of the analysis of 50%, 75%, and 100% cold medicine samples (replicate of the experiment shown in Figure 7A of the main text). All three dilutions types are distinguishable. The global median image hashing analysis performed best for this experiment, with a maximum pixel difference of 28 of 64 pixels between the 75% and 100% sample chronoprints.
Supplementary Figure 10: Replicate of the analysis of 50%, 75%, and 100% cold medicine samples (replicate of the experiment shown in Figure 7A of the main text). All three sample types are distinguishable. The *local median image hashing* analysis performed the best for this experiment, with a maximum pixel difference of 28 out of 64 pixels between the 75% and 100% sample chronoprints.
Supplementary Figure 11: Comparisons of different chronoprint similarity algorithms for 90%, 95%, and 100% (v/v) dilutions of cold medicine in water (the experiment shown in Figure 7B of the main text).
Supplementary Figure 12: Replicate of the analysis of 90%, 95%, and 100% cold medicine samples (replicate of the experiment shown in Figure 7B of the main text). The 90% cold medicine samples are distinguishable from the others, but the 95% and 100% samples were not distinguishable. The local median image hashing analysis performed the best for this experiment, with a maximum pixel difference of 26 out of 64 pixels between the 90% and 100% sample chronprints.
Supplementary Figure 13: Replicate of the analysis of 90%, 95%, and 100% cold medicine samples (replicate of the experiment shown in Figure 7B of the main text). The 90% cough medicine sample is distinguishable from the others, but the 95% and 100% samples were not distinguishable. The *image differences* analysis performed the best for this experiment, with a maximum sum of pixel difference of 3134 out of 16320 between the 90% and 100% sample chronoprints.