```
┌─────────┐ ┌─────────┐ ┌─────────┐   ┌─────────┐ ┌─────────┐ ┌─────────┐
│  CFP_o  │ │  YFP_o  │ │ FRET_o  │   │  CFP_t  │ │  YFP_t  │ │ FRET_t  │
└────┬────┘ └────┬────┘ └────┬────┘   └────┬────┘ └────┬────┘ └────┬────┘
      \         |         /                 \         |         /
       \        |        /                   \        |        /
        \       |       /                     \       |       /
      ┌──────────────────┐                  ┌──────────────────┐
      │    (FRET_c)_o     │                  │    (FRET_c)_t     │
      └────────┬─────────┘                  └────────┬─────────┘
      ┌──────────────────┐                  ┌──────────────────┐
      │     2DCWT_o       │                  │     2DCWT_t       │
      └────────┬─────────┘                  └────────┬─────────┘
                \                                   /
                 \                                 /
                  \                               /
                   ┌──────────────────────┐
                   │     Select scales     │
                   └───────────┬──────────┘
```

$$\text{Normalize matrices as:} \quad \left| \frac{(2DCWT_o - 2DCWT_t)^2}{\max (2DCWT_o - 2DCWT_t)^2} \right|$$

```
      ┌──────────────────────────────────────────────────┐
      │ Find the coordinates of potential domains in the image │
      └────────────────────────┬─────────────────────────┘
      ┌──────────────────────────────────────────────────┐
      │ The same coordinates are extracted from the control │
      │  and other time points of corrected FRET images     │
      └────────────────────────┬─────────────────────────┘
             ┌──────────────────────────────┐
             │    Significance by ANOVA test │
             └──────────────────────────────┘
```

Supplemental Figure Legend

Schematic of 2D-wavelet-based image processing. Corrected FRET values are calculated from three filter sets (CFP, YFP and FRET) for the control image $(FRET_c)0$ and images at each time point $(FRET_c)_t$ based on Eq. 1 in the text. The 2D-CWT is applied across multiple scales to the corrected FRET matrices. Scales for subsequent analysis are identified (scales = 2-5 as described in the text), and for each scale level, normalized difference matrices are computed according to the equation shown in the flowchart. Thresholding of these matrices is performed (threshold set at 0.5) and the coordinates of the potential domains of interest are identified. A one-way ANOVA is applied to the original corrected FRET values from images of control and other time points within these potential domains. Domains that show significant differences $(P < 0.05)$ are classified as positive domains.

Supplemental Table. Potential, positive, and false-positive domain discovery for synthetic images (Figure 1).

SNR levels	CWT Dimensions	Number of identified Potential Domains	Number of statistically significant domains out of identified potential domains	Number of real identified domains(out of 6 inserted real domains)	Number of False positive domains	Ratio of <Real found domains/Potential identified domains>
Level 1						
	1D-CWT	5	1	0	1	0/5
	2D-CWT	6	6	4	2	4/6
Level 2						
	1D-CWT	13	1	1	0	1/13
	2D-CWT	6	6	6	0	6/6
Level 3						
	1D-CWT	9	4	3	1	3/9
	2D-CWT	6	6	6	0	6/6
Level 4						
	1D-CWT	6	5	4	1	4/6
	2D-CWT	6	6	6	0	6/6