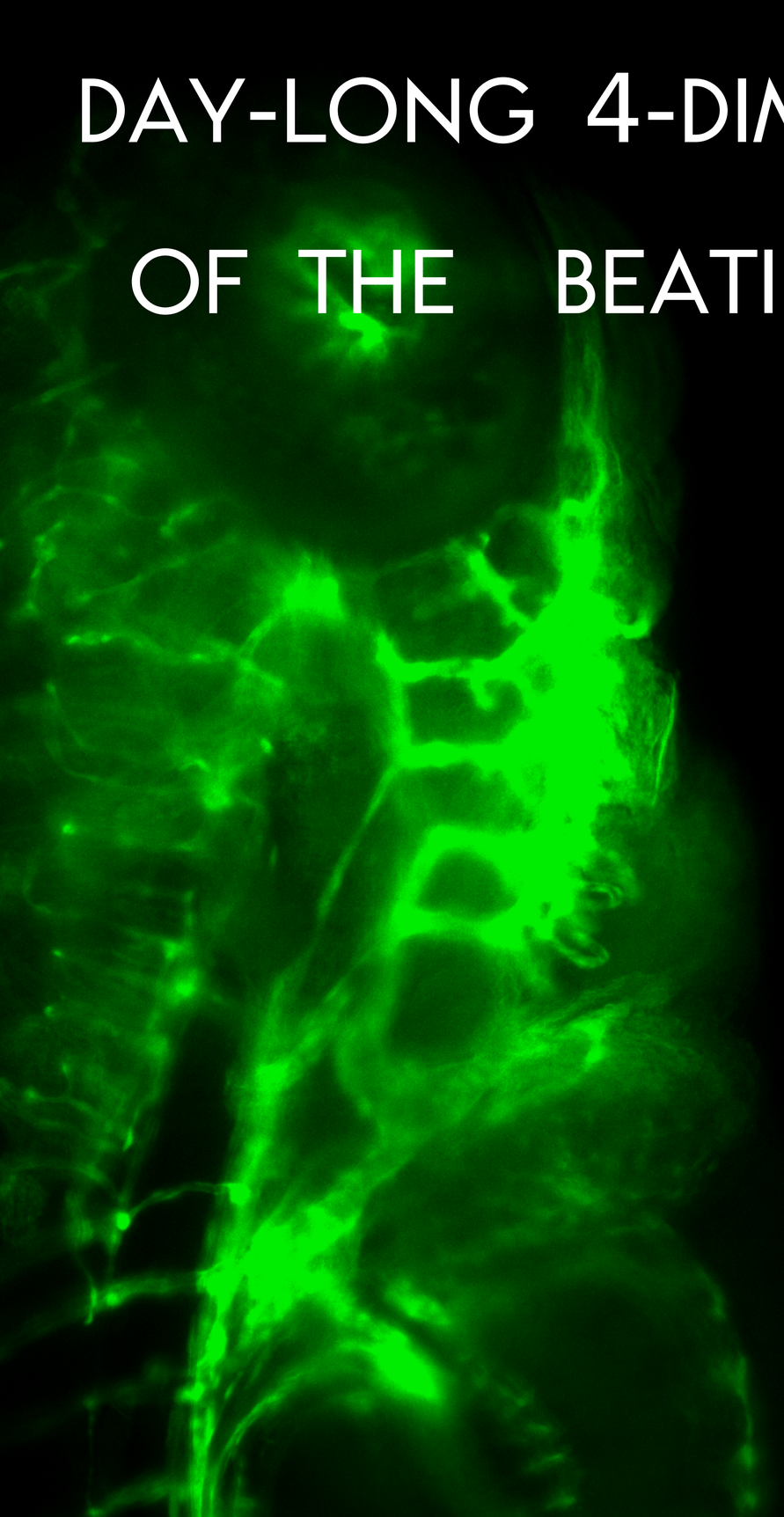
DAY-LONG 4-DIMENSIONAL TIME-LAPSE IMAGING OF THE BEATING HEART IN LIVING ZEBRAFISH



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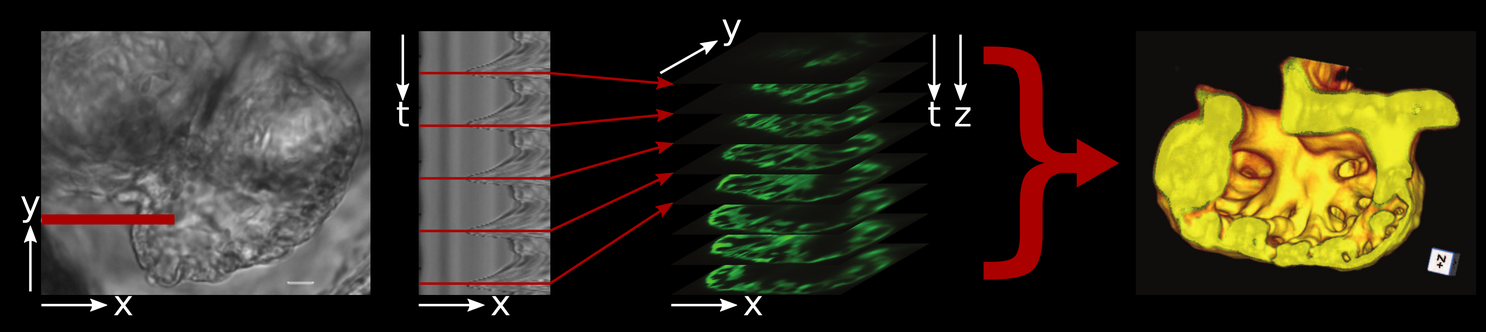
REAL-TIME PROSPECTIVE OPTICAL GATING ALLOWS THE CAPTURE OF 3D SNAPSHOTS OF THE 'COMPUTATIONALLY FROZEN' HEART

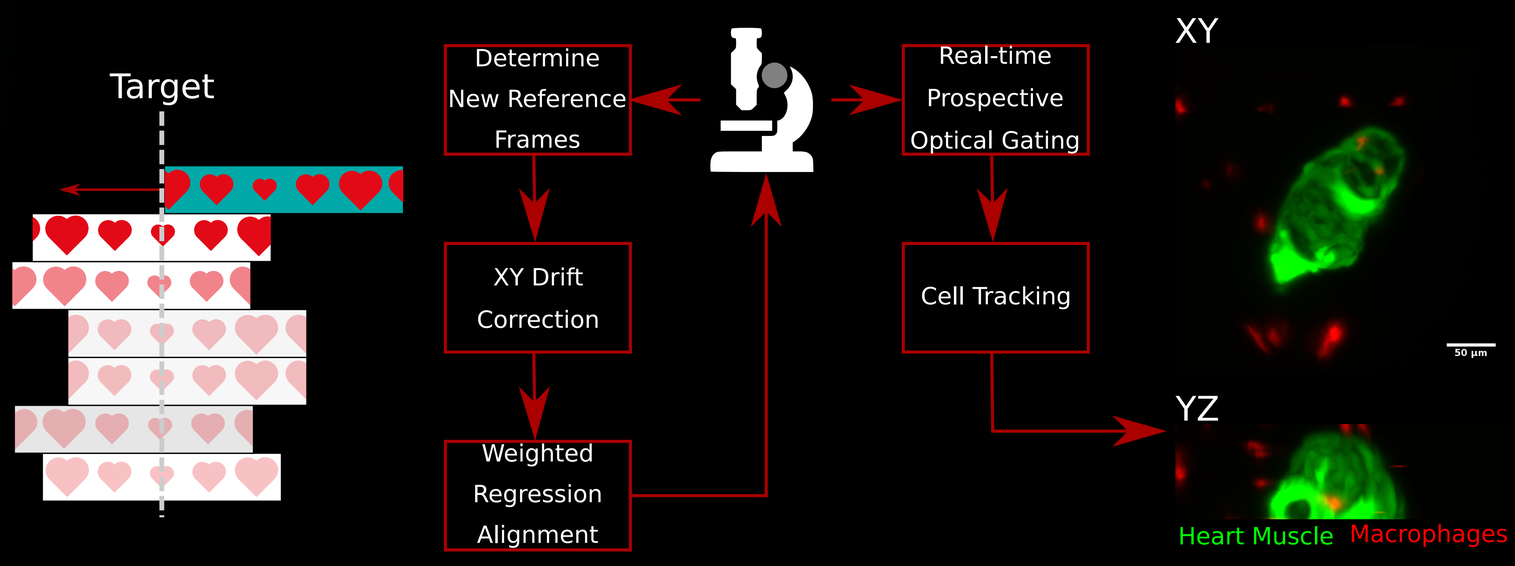


Challenge: The zebrafish heart is constantly moving making 3D imaging difficult

- Prospective optical gating uses **real-time image analysis** to target fluorescence acquisition at a precise *phase* within the heartbeat
- This is accomplished with a constant, infrared brightfield (left) and a reference heartbeat (video A)
- Using this we trigger one fluorescence capture per heartbeat (centre)
- And build a **computationally frozen** 3D volume (right; video B)
- Achieved *without* invasive use of chemicals or electrical pacing

Outcome: In the same way that light sheet microscopy eliminates redundant excitation of fluorescence in the spatial domain, our prospective gating approach eliminates redundant excitation in the time domain

ADAPTIVE PROSPECTIVE OPTICAL GATING ALLOWS THE CAPTURE OF 4D TIMELAPSES OF THE LIVING, BEATING HEART



Challenge: Over large timescales the heart changes shape and size (video C) - this makes the reference heartbeat ineffective for triggering fluorescence

- We *combine* retrospective optical gating with prospective optical gating
- **Prospective optical gating** allows us to collect 3D stacks (right and above)
- **Adaptive Prospective optical gating** applied to brightfield reference frames between stacks allows us to phase-lock over tens of hours (left; video D)
- We have successfully maintained phase lock for **over 48 hours** and imaged heart development (video E) and injury response (video F)

Outcome: Without hybrid optical gating it would be impossible to a) collect data at the exact same phase over tens of hours and b) collect high-quality timelapse images of the heart without photodamage or invasive techniques

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