COLIN GORDON & ASSOCIATES

Vibration Sensitivity of Optical Microscopes

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Visually Observable

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Overview

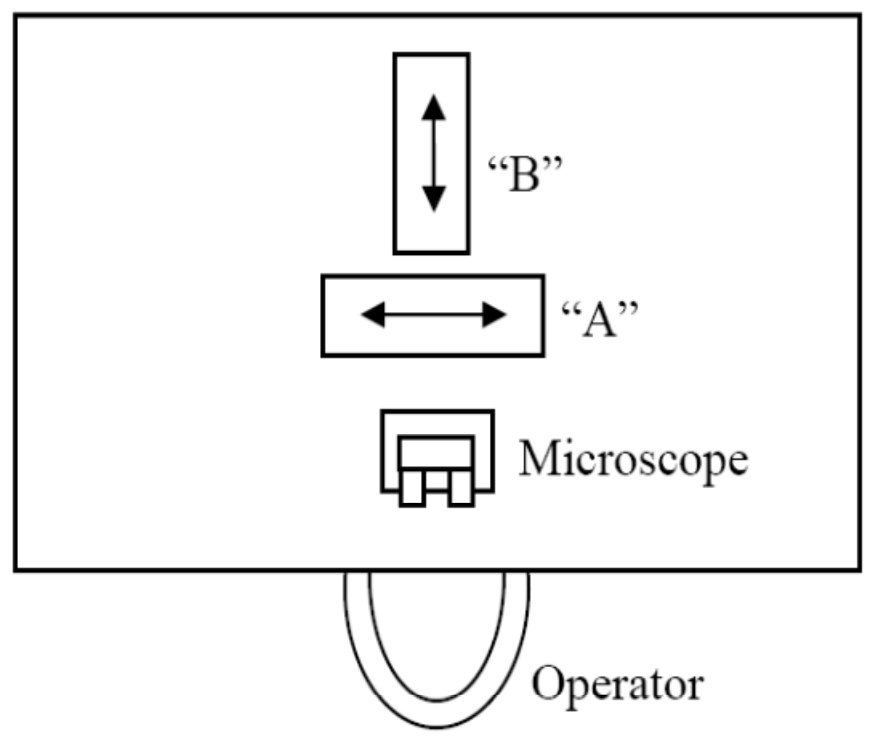
- Experimental Study by Amick & Stead
  - Experimental setup
  - Results are dependent on magnification
  - Headroom
  - Comparison with other criteria and benchmarks
- Thoughts Regarding Further Experiments
  - Shortcomings of Amick & Stead
  - Consider other factors
- Guidelines for New Spaces

Microscopes Used in Study

- Nikon Eclipse E400
  - 40x, 100x, 400x
- Nikon Optiphot
  - 1000x

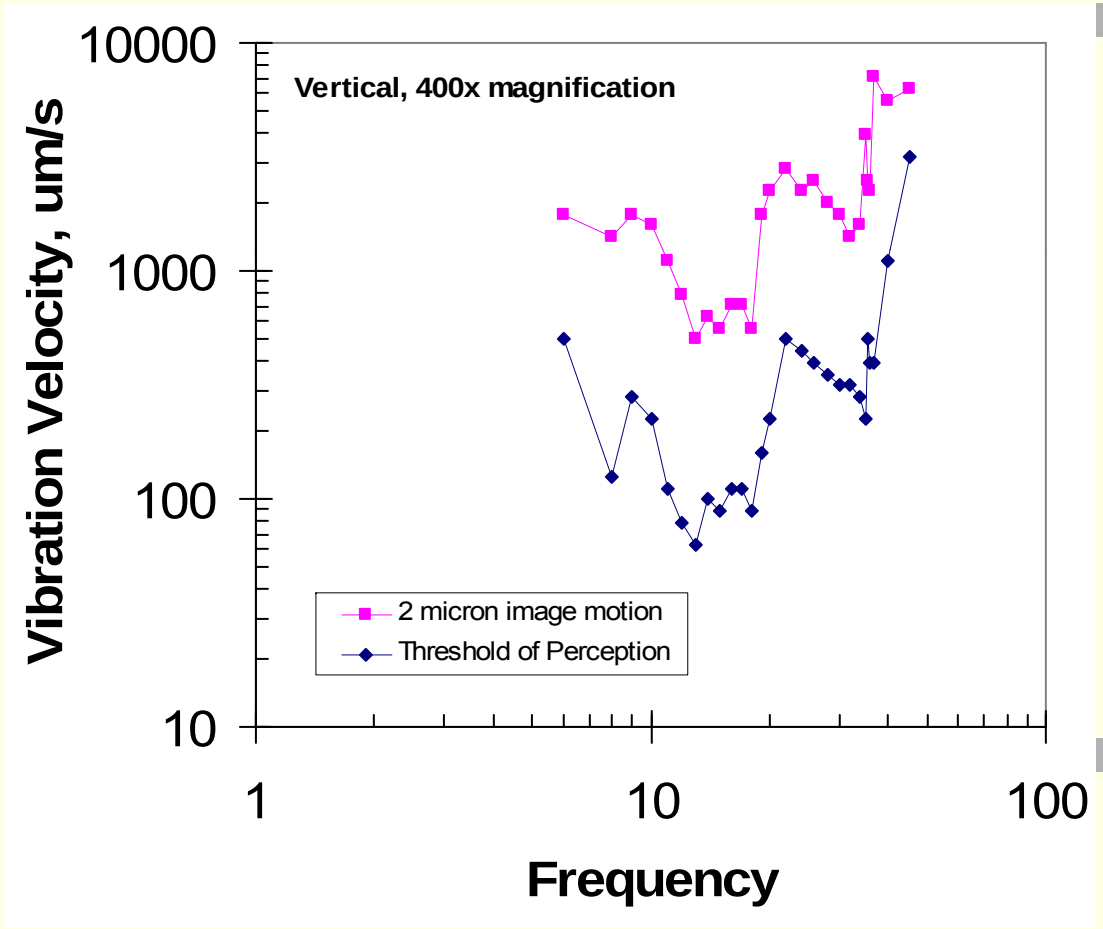
Hal Amick & Michael Stead, "Vibration Sensitivity of a Laboratory Bench Microscope," Presented at the First Pan-American/Iberian Meeting on Acoustics; 144th Meeting of the Acoustical Society of America, 2-6 December 2002, Cancun, Mexico

Basic Test Configuration



- Microscope and shaker on "springy" table
- Graduated Calibration Standard for gauging motion
- Tested in three directions:
  - ***Vertical*** – Microscope placed midspan, shaker placed behind it, vertical force
  - ***Fore-and-aft*** – Microscope placed at one end on centerline, shaker placed on table behind it with force axis through microscope (Position "B")
  - ***Side-to-side*** – Microscope placed at middle of edge on long side, shaker on table behind it above long'l axis of table (Position "A")

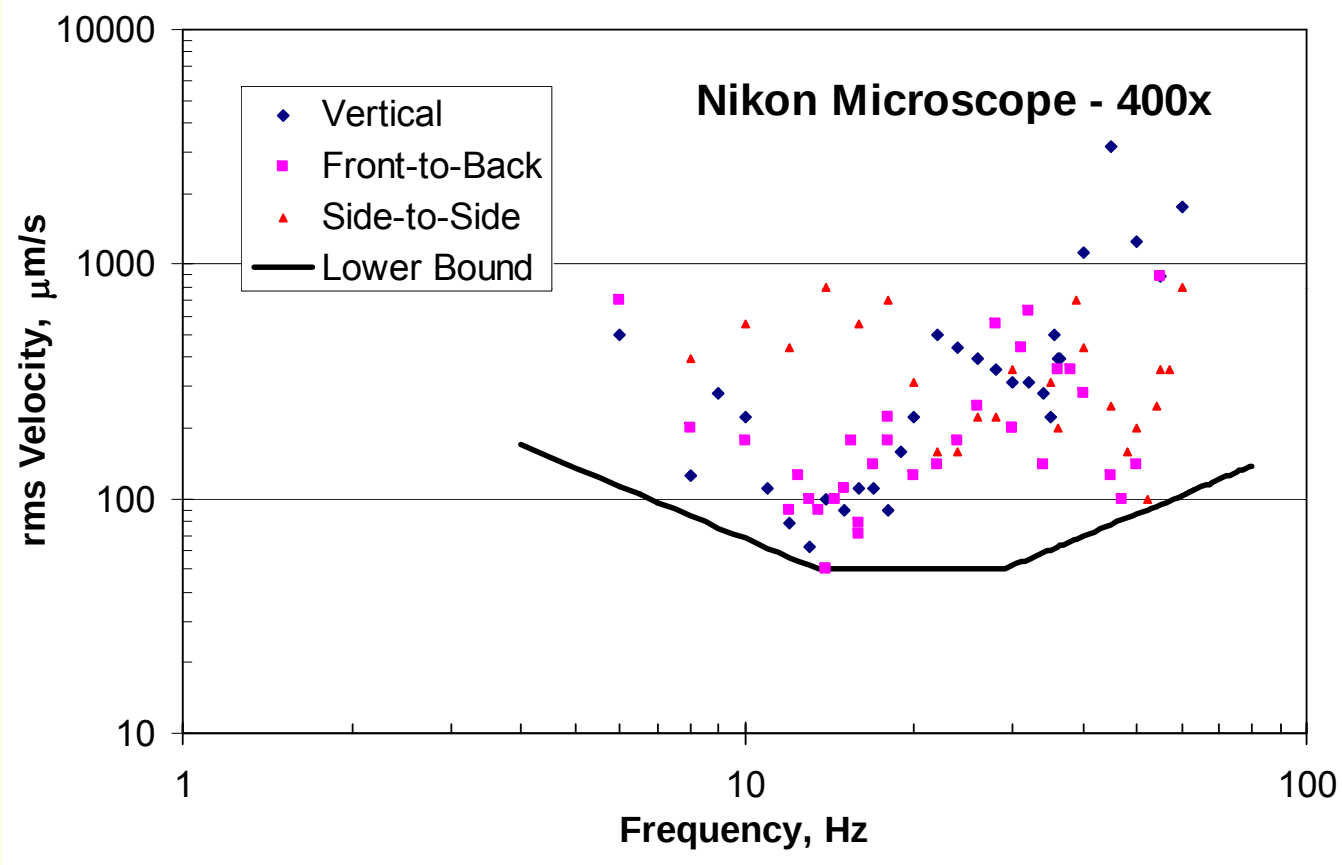
Results from Individual Test



- For each frequency …
  - Identify amplitude at initial perception threshold
  - Identify amplitude at loss of visual acuity
- Repeat for each direction

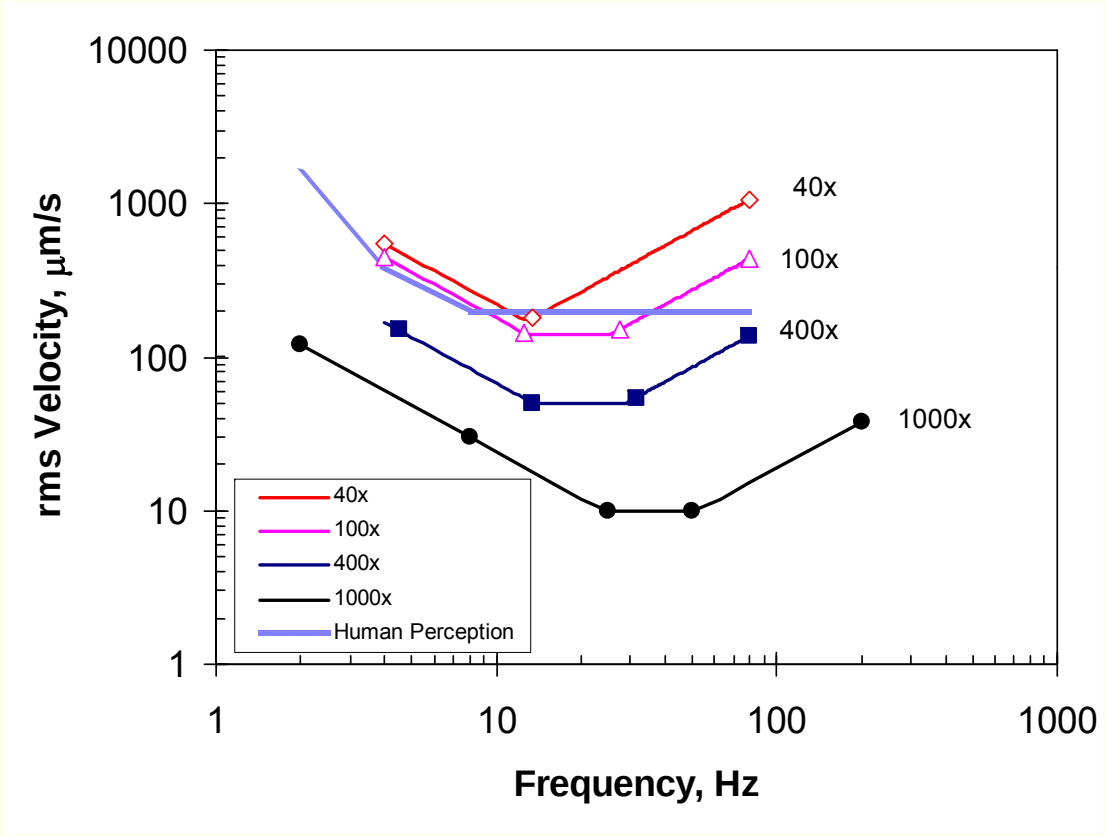
Lower Bounds – 1

(Omnidirectional)



Hal Amick & Michael Stead, "Vibration Sensitivity of a Laboratory Bench Microscope," *Sound & Vibration*, v. 41, No. 2, pp. 10-17 (February 2007)

Compare Thresholds (by magnification)

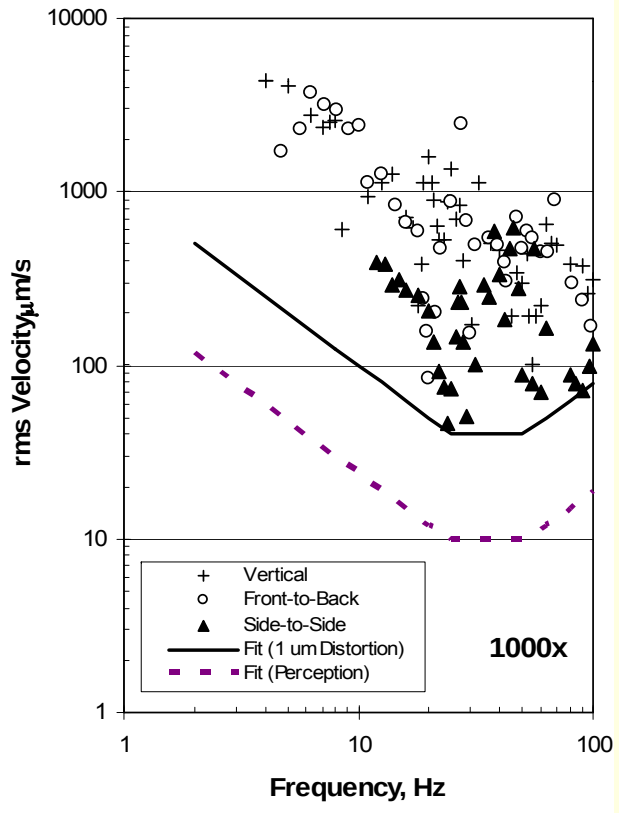


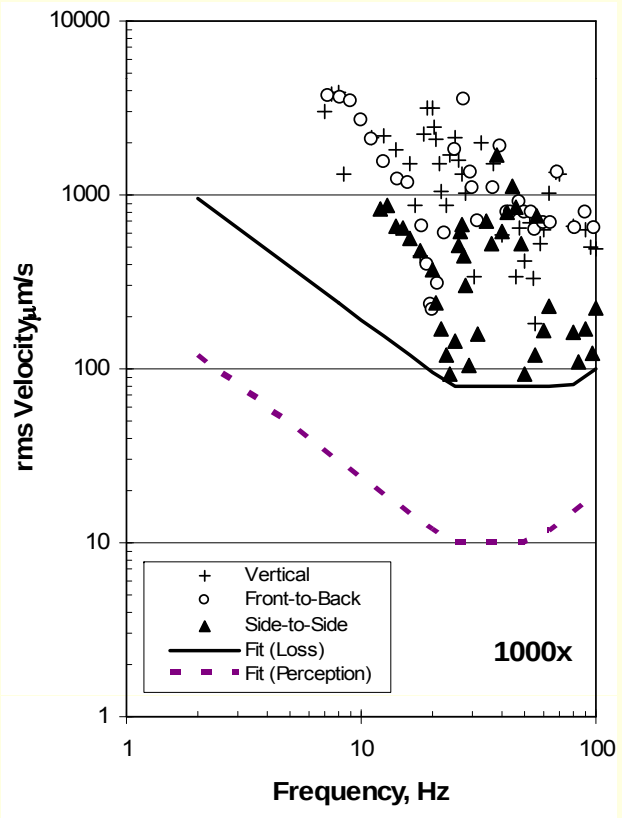
- Barely perceptible vibrations okay for 40x and 100x
- Perceptible vibrations problematic for magnification ≥200x

Hal Amick & Michael Stead, "Vibration Sensitivity of a Laboratory Bench Microscope," *Sound & Vibration*, v. 41, No. 2, pp. 10-17 (February 2007)

Thresholds of Distortion – 1000x

	Perception Threshold	1 µm Image Distortion	Image Loss
Displacement, µm $63 \leq f \leq 100$ Hz	0.03	0.125	0.16
Velocity, µm/s $25 \leq f \leq 50$ Hz	10	40	80
Acceleration, µm/s^2 $2 \leq f \leq 20$ Hz	1500	6300	12100





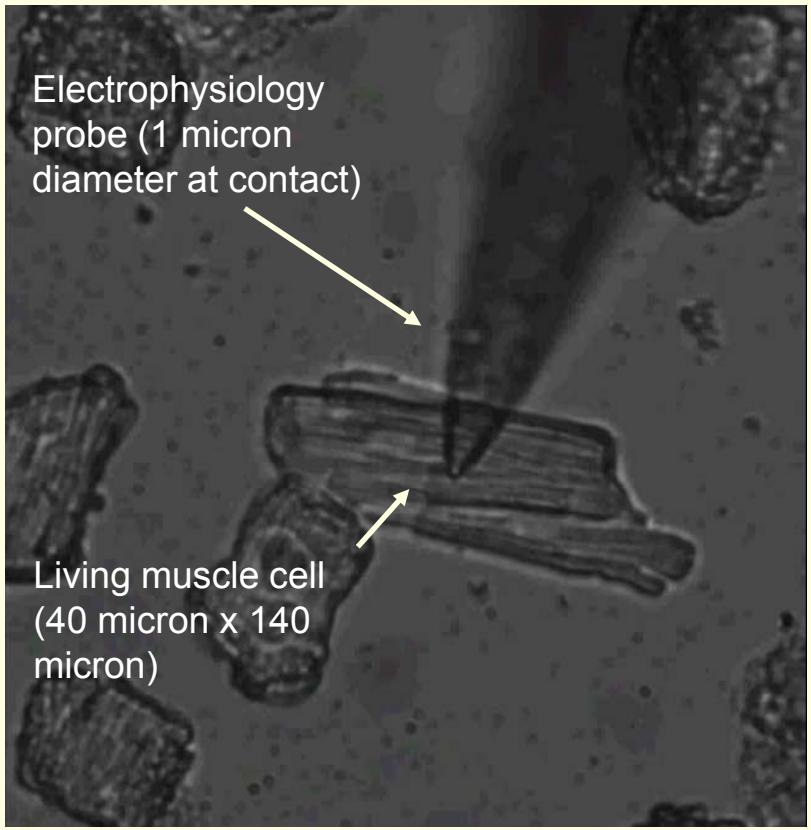
Thoughts Regarding Further Experiments

- Shortcomings of Amick & Stead
  - Applicable only to visual observation
    - Subjective
    - Could be tainted by “learning”
  - Study did not address common modification to generic microscopes
    - Electrophysiology rigs
    - Image processing
    - Confocal microscopy
    - Bioluminescence
    - Etc.

Thoughts Regarding Further Experiments

- Consider other Factors
  - Electrophysiology
    - Manipulation problems
    - Effects on experimental data
    - Cell death
  - Image processing
    - Image clarity (e.g., blurring)
    - Superposition
    - Drift
  - Etc.

Vibration can Kill



- Excessive vibration can damage (and thus kill) cells during electrophysiology experiments.
  - Image at left shows a typical setup.
  - Movie shows cell death after horizontal tap of isolation bench.
- Excessive vibration can prevent important discoveries from happening
  - Researchers may incorrectly assume a process “just doesn’t work” and not realize it didn’t work because of vibration

Image and movie courtesy Dr. Harold Spurgeon, NIH

Specific Suggestions from Tony Havics

- Examine small spheres of known size (latex or styrene) around known resolution limits, varying vibration to find shifts in resolution
  - Where d = resolution
    - d = (2*L)/NA
    - L = wavelength of light
    - NA = numerical aperture
- Take a known spatial frequency pattern (lines vertical and horizontal) and obtain digital images, converting the images with a Fourier or power transform and
  a) looking at them in these domains for frequency shifts (pretty accurate compared to the human eye), or
  b) subtracting different ones in Fourier space to provide a value of spatial shift (equivalent to displacement in classical vibration)
- Examine a pattern of "noise" in a micro-FTIR (with varying vibration) to see if shifts are detectable
  - Consider synthetic fibers of 10 µm diameter (generally the limit of resolution on FTIR)

Guidelines for New Spaces

- Be familiar with your own needs
  - Magnification
  - Type of work
- Insert yourself into the design process
  - Don't do the design work, but *make sure your needs are known*
  - Sanity checks where possible
    - Experience of design team
    - Criteria being used
    - Walker speeds
    - Structural spans
- Be willing to be involved, answer questions

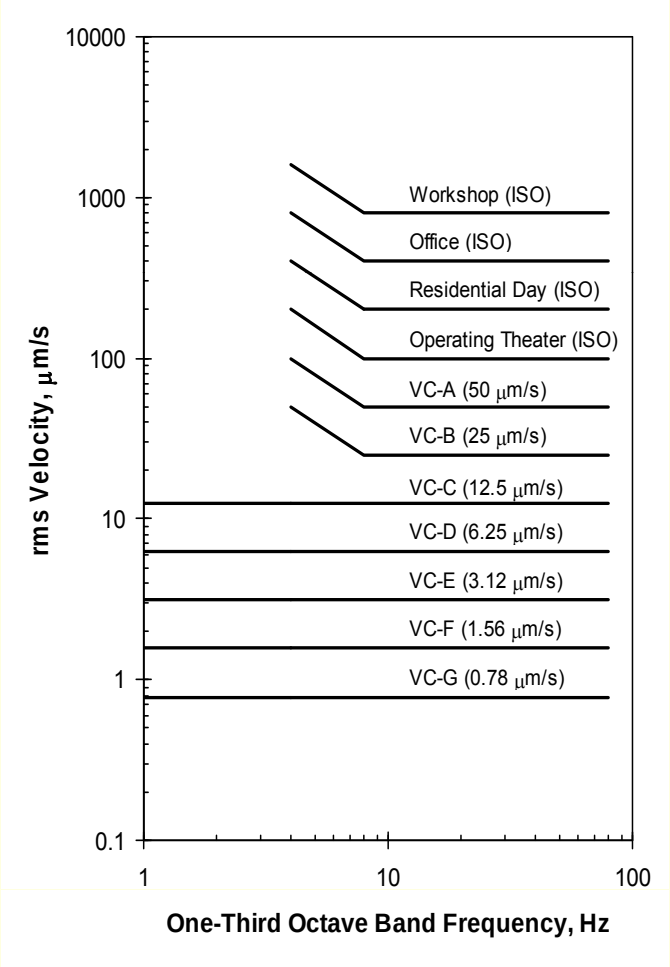
Inserting Yourself into the Design Process

- *Making sure your needs are known*
  - Many design teams (and vibration consultants) are unaware of the more sophisticated microscopy tasks
    - Electrophysiology
    - Confocal microscopy
  - If you're working beyond conventional microscopy, let it be known
  - Do the legwork and talk to the equipment manufacturers
    - Where have they seen problems?
    - Disregard "Vibration-free" type of requirements, no matter who you get them from

Sanity checks

- Experience of design team
  - Not all design teams are equally qualified
  - Ask what labs they've designed
- Criteria being used
  - IEST VC curves (see subsequent slide)
  - ASHRAE curves
  - BBN curves
- Walker speeds
  - Context driven is best (see subsequent slide)
- Structural spans
  - Be wary of spans greater than 32'
  - Be wary of major walkways at the middle of long spans

IEST VC Curves



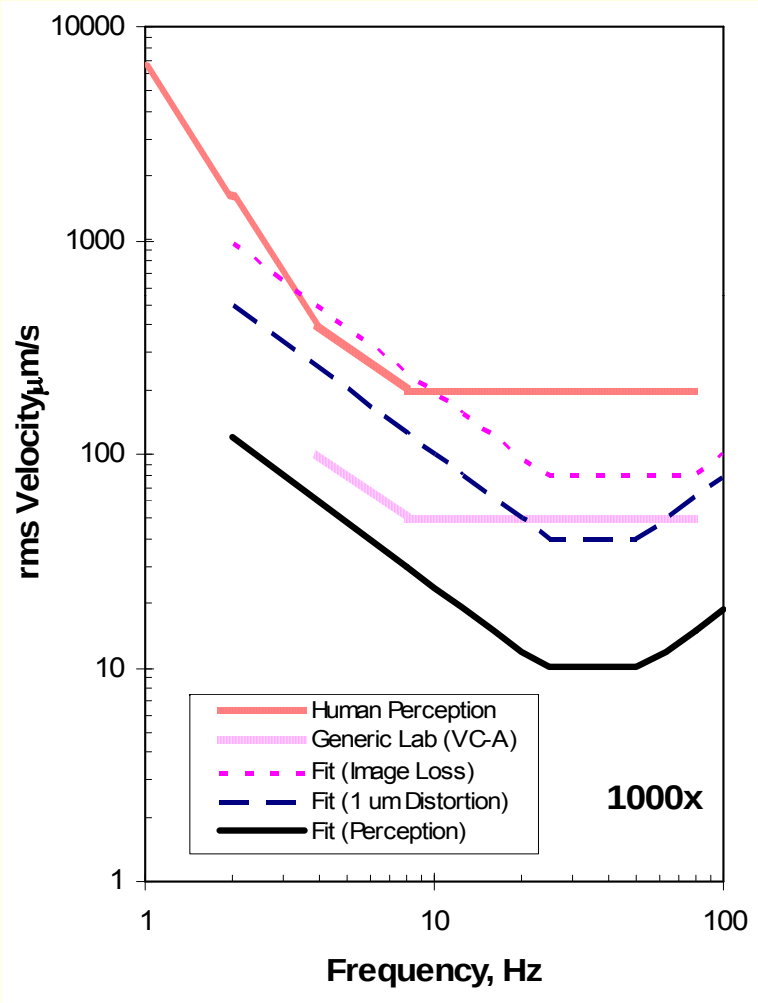
Criterion Curve	Amplitude[1] µm/s (µin/s)	Detail size[2] µm	Description of use
Workshop (ISO)	800 (32 000)	N/A	Distinctly perceptible vibration. Appropriate to workshops and nonsensitive areas.
Office (ISO)	400 (16 000)	N/A	Perceptible vibration. Appropriate to offices and nonsensitive areas.
Residential day (ISO)	200 (8 000)	75	Barely perceptible vibration. Appropriate to sleep areas in most instances. Usually adequate for computer equipment, hospital recovery rooms, semiconductor probe test equipment, and microscopes less than 40x.
Operating theatre (ISO)	100 (4 000)	25	Vibration not perceptible. Suitable in most instances for surgical suites, microscopes to 100x and for other equipment of low sensitivity.
VC-A	50 (2 000)	8	Adequate in most instances for optical microscopes to 400x, microbalances, optical balances, proximity and projection aligners, etc.
VC-B	25 (1 000)	3	Appropriate for inspection and lithography equipment (including steppers) to 3 µm line widths.
VC-C	12.5 (500)	1 – 3	Appropriate standard for optical microscopes to 1000x, lithography and inspection equipment (including moderately sensitive electron microscopes) to 1 µm detail size, TFT-LCD stepper/scanner processes.
VC-D	6.25 (250)	0.1 – 0.3	Suitable in most instances for demanding equipment, including many electron microscopes (SEMs and TEMs) and E-Beam systems.
VC-E	3.12 (125)	< 0.1	A challenging criterion to achieve. Assumed to be adequate for the most demanding of sensitive systems including long path, laser-based, small target systems, E-Beam lithography systems working at nanometer scales, and other systems requiring extraordinary dynamic stability.
VC-F	1.56 (62.5)	N/A	Appropriate for extremely quiet research spaces; generally difficult to achieve in most instances, especially cleanrooms. Not recommended tor use as a design criterion, only for evaluation.
VC-G	0.78 (31.3)	N/A	Appropriate for extremely quiet research spaces; generally difficult to achieve in most instances, especially cleanrooms. Not recommended for use as a design criterion, only for evaluation.

[1]As measured in one-third octave bands of frequency over the frequency range 8 to 80 Hz (VC-A and VC-B) or 1 to 80 Hz (VC-C through VC-G).

[2]The detail size refers to line width in the case of microelectronics fabrication, the particle (cell) size in the case of medical and pharmaceutical research, etc. It is not relevant to imaging associated with probe technologies, AFMs, and nanotechnology.

The information given in this table is for guidance only. In most instances, it is recommended that the advice of someone knowledgeable about applications and vibration requirements of the equipment and processes be sought.

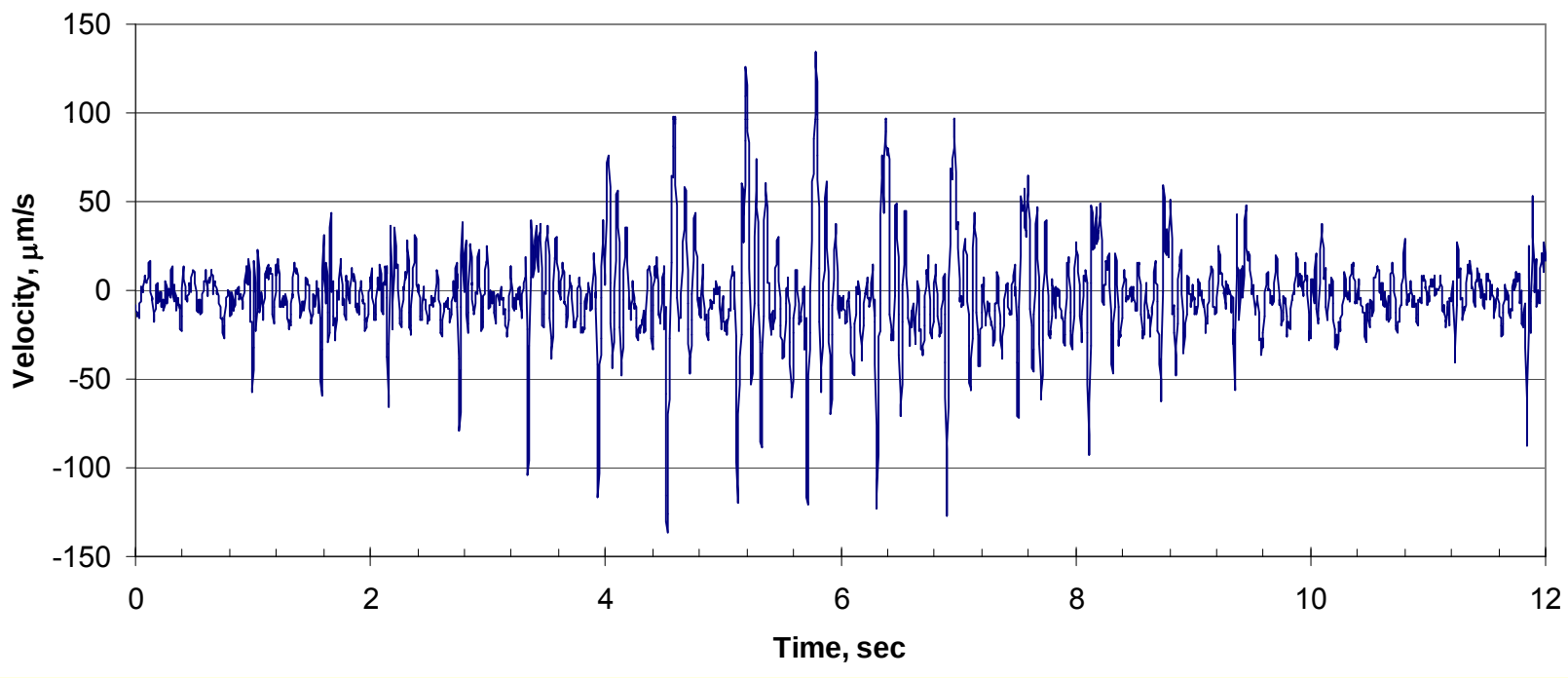
Compare Thresholds to Criteria – 1000x



- Generic lab criterion (VC-A) allows some jiggle or blurring with 1000x
- Perceptible vibration will render 1000x microscope useless

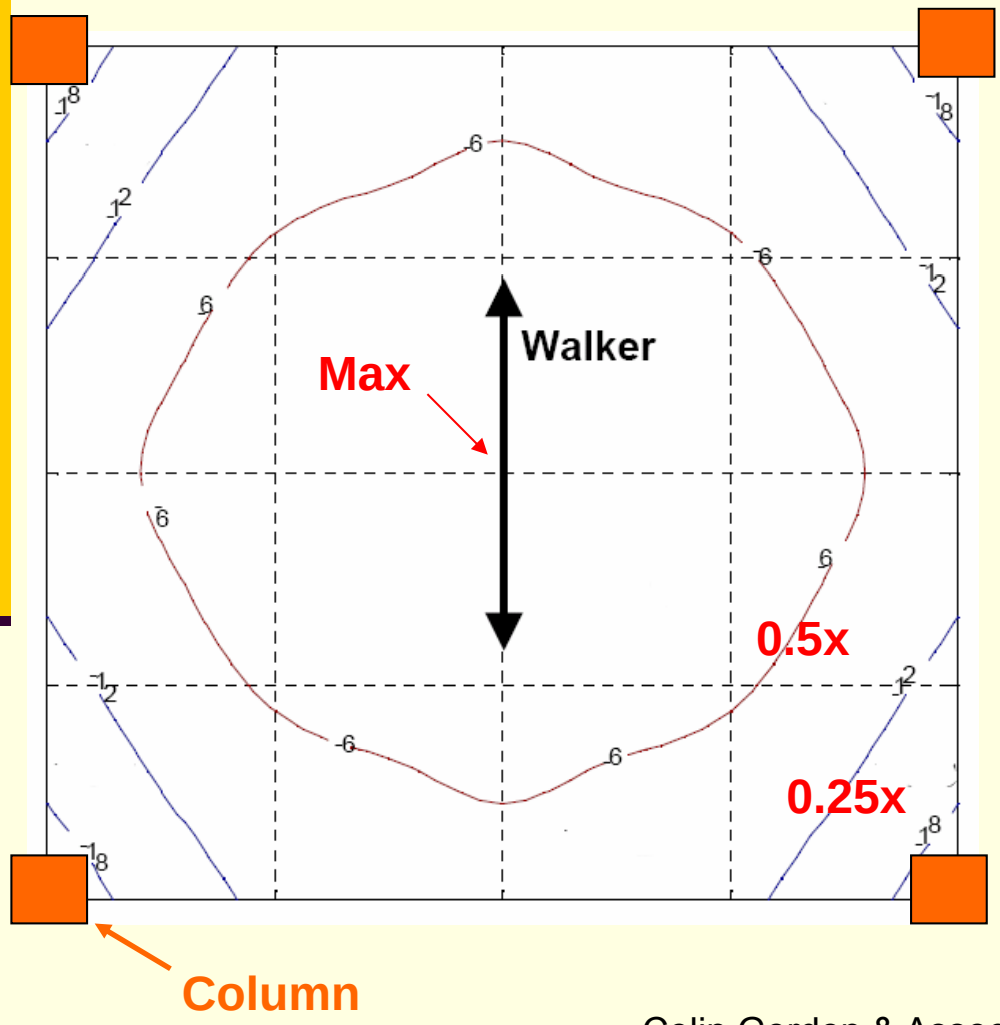
Typical Walker-Generated Floor Vibration (Vertical)

Enters bay → Walker passes through midbay → Leaves bay



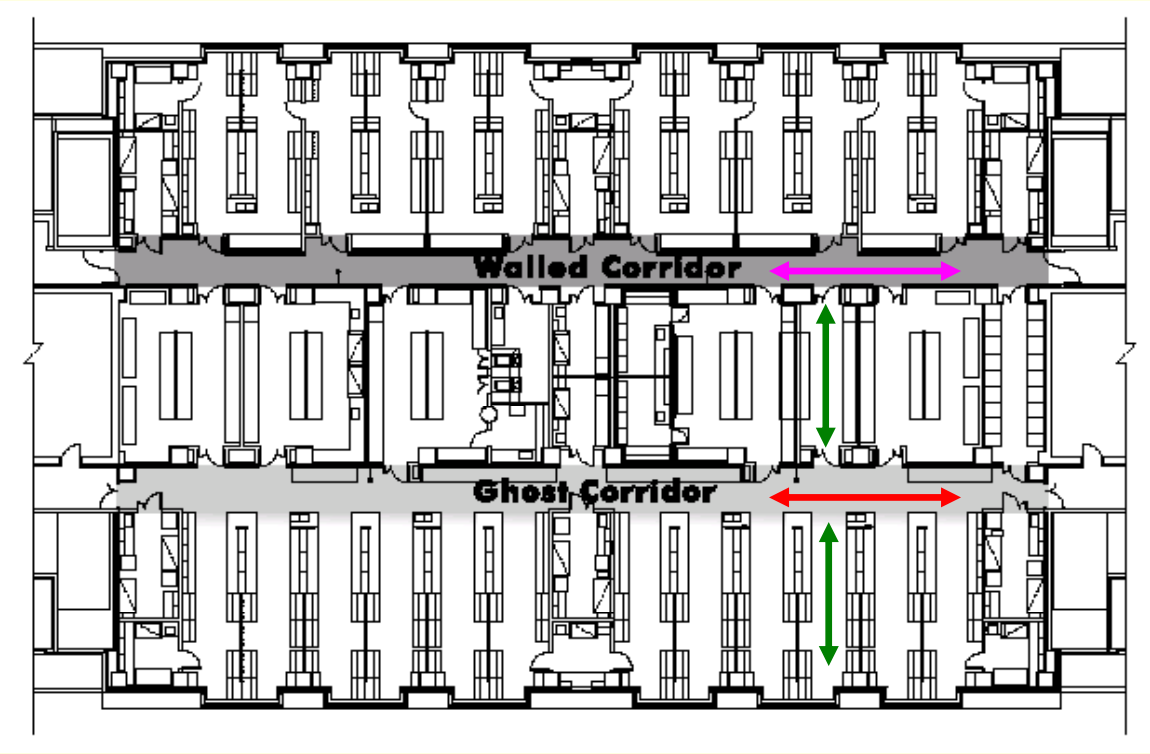
From Hal Amick & Michael Stead, "Vibration Sensitivity of a Laboratory Bench Microscope," Presented at the First Pan-American/Iberian Meeting on Acoustics; 144th Meeting of the Acoustical Society of America, 2-6 December 2002, Cancun, Mexico

Distribution of Amplitude in Bay



- Maximum is at center of bay (“midbay”)
- Amplitude decreases toward columns
  - Reduced by 50% halfway to column
  - Reduced by 75% three-fourths of the way
- “Nodal line” extends into neighboring bay

Walker Rate Related to Path

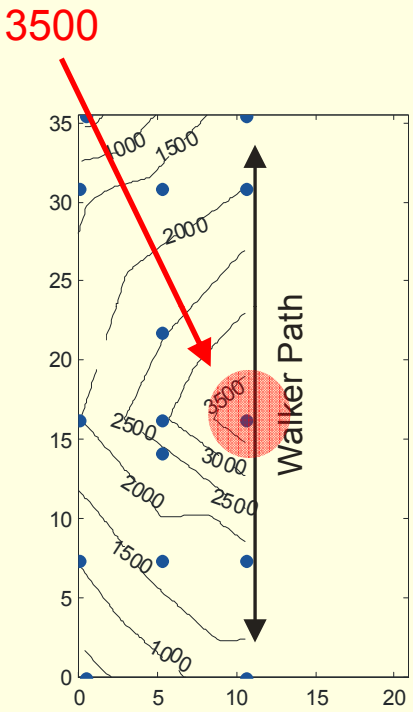


Corridor Types

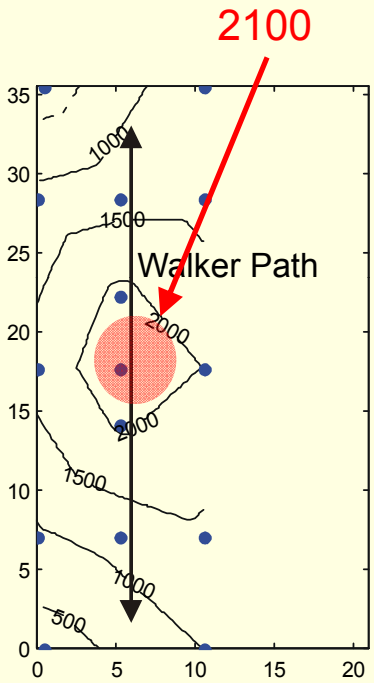
- Walled or Closed Corridor
  - 100 paces/min
- Ghost or Open Corridor
  - 85-90 paces/min
- Cross Corridor or Lab Aisle
  - 75-80 paces/min

Effect of Walker Path

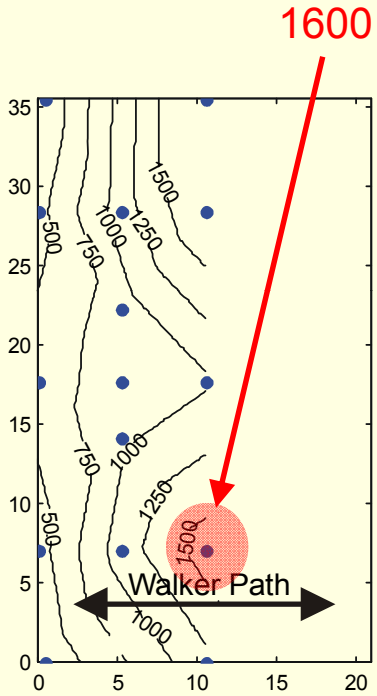
(Example Measured Contour "Maps")



Walker at midspan



Walker at 1/3 span



Walker along column line

Conclusion - 1

- Experimental Study by Amick & Stead
  - Threshold of perception
    - Frequency dependent
    - Magnification dependent
  - Image degradation
    - Ditto re frequency and magnification
    - “Headroom” between 3:1 and 8:1 w.r.t. threshold
  - Visual threshold similar to human threshold at 40x, 100x
- Further Experiments are Warranted
  - Examine multiple systems (multiple observers, multiple scopes)
  - Address Amick & Stead shortcomings
    - “attachments” and processes like electrophysiology
    - photography and image processing

Conclusion - 2

- Guidelines for New Spaces
  - Be involved!
  - Watch for …
    - Unrealistic vibration criteria
    - Long spans
    - Long walker paths along the middle of a long span
    - Unrealistic walker pace rates (designing for < 70 ppm)
  - Consider where you're placing your instrument within the bay (mid-bay is worst)

COLIN GORDON & ASSOCIATES

Thank you …